HANDBOOK OF LASER SYNTHESIS & PROCESSING OF COLLOIDS

SECOND EDITION

S. Barcikowski, V. Amendola, M. Lau,
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Cover & Internal Design: Sasha Ediger // designmodus.com

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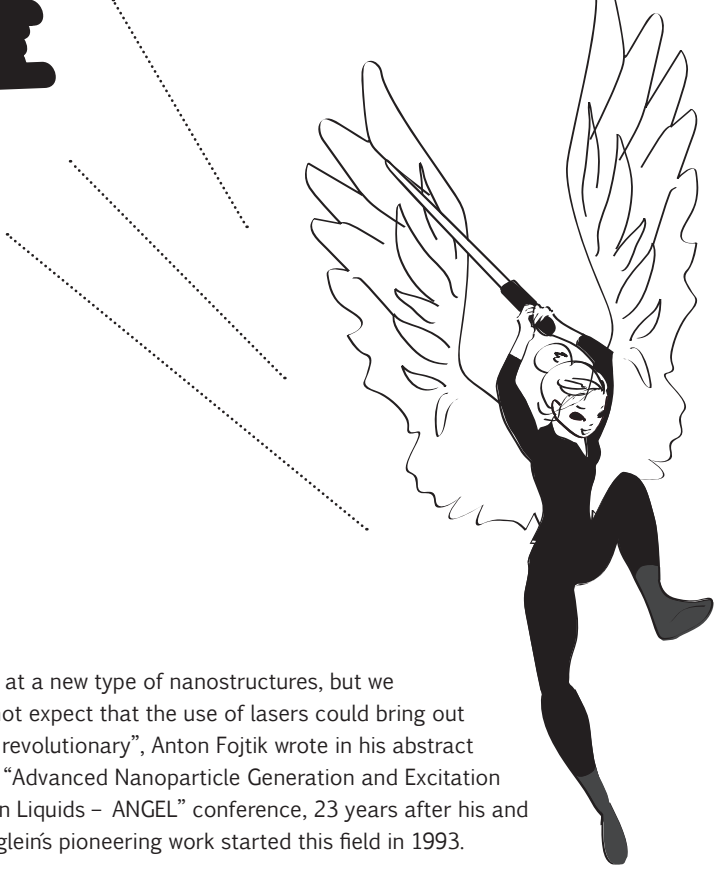
PREFACE



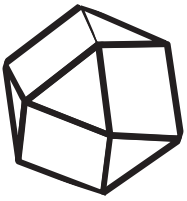
”We aimed at a new type of nanostructures, but we really did not expect that the use of lasers could bring out something revolutionary”, Anton Fojtik wrote in his abstract for the 4th “Advanced Nanoparticle Generation and Excitation by Lasers in Liquids – ANGEL” conference, 23 years after his and Arnim Hengleińs pioneering work started this field in 1993.

Today, the field of laser synthesis and processing of colloids (LSPC) has expanded to all over the world, so that whenever a researcher enters in this field, she/he has to fumble her/his way forward amidst a mass of scattered papers, focused reviews and Ph.D. theses all of which are not always educational. This is why it seemed to us that one coherent presentation in the form of a handbook might help newbies (in the following referred to as “you”) with this predicament.

The main purpose of this book is to introduce you to this interdisciplinary topic, which contains elements from chemistry, physics, engineering, and sometimes even biology. While LSPC is pretty simple to apply, it is not as easy to understand, in part caused by its interdisciplinary nature. And to be honest even among the experts in the fields there are many disagreements on how LSPC really works. Although by now there is quite a collection of publications on LSPC, none of them treats it in a way suitable for beginners, unravelling the secrets of experimental setups, reproducibility, colloidal stability, and yield.

Instead of aiming for a scientific and structural approach, which would first establish the theory and properties of LSPC and then try to view the consequences of these, we decided to arrange the book by phenomenological "steps" that you will need to take to be successful in our field. In order to visualize what really matters we tried to give instructional examples. Since there is no need for you to make the same mistakes that we did (yes, we can tell you!), we will also explain what you should avoid during your journey in the world where ultra-small particles meet giant laser intensities. Of course, it would be unreasonable to refrain from any theory if this really makes things simpler; hence we limited the fundamentals to a minimum and refer to the scientific reviews that are out there, e.g. Chemical Reviews (2017).

It was our intention to write all chapters of the book in a sufficiently leisurely style, while only minor preliminary knowledge is required to read it. After "getting started with setting up the experiments" in Chapter I, you may want to learn how to "get more" colloidal nanoparticles in Chapter II by some gimmicks. General strategies of quick laser treatment of the yielded liquid will be explained in Chapter III, whereas specific case files are presented in the hands-on Chapter IV "tuning the sizes", six examples that cover most wishes you may have for a given product property. Of course, once having finished synthesis and product quality tuning, "characterization" (Chapter V) of your particles needs to be done with high accuracy. These high-yield, high-quality (but naturally metastable) nano-products benefit from rules and recipes to "keep your colloids" stable (Chapter VI), while managing to "keep you alive" during your contact with lasers and chemicals in Chapter VII. Having your final colloid proudly in your hands, the legendary nanofuntionality potential of such colloids demand for their integration into application scenarios, often increasing the impact of research, and happiness. As laser synthesis is conquering attractive applications fields with proven advantages, we added 3 case files in Chapter VIII as "cooking recipes" to show how easy catalysis, biomedical and additive manufacturing applications can be demonstrated, often in less than 2 days. Last but not least, we would like to thank all of the scientists who contributed to this work by words and quotes, and the many emails we received from all around the world whose comments we tried to embed in the current revision of the book. We hope you enjoy reading this book as much as the authors enjoyed writing it!



Stephan Barcikowski and Bilal Gökce

CONTENT

INTRODUCTION

Two decades after the first colloidal nanoparticles were generated with a laser, more than 350 institutes all over the world are actively involved in the synthesis and processing of nanoparticles by laser ablation. If 3 students per institute would work in this field we would have a total of 1000 students that are eager to make their way into academia or industry using this laser-based method.

Different terminologies such as “pulsed laser ablation/fragmentation/melting in liquids (LAL/LFL/LML)” or “Laser Ablation Synthesis in Solution (LASiS)“ are used to describe the field that this book is about. If you want to know more about LASIK (Laser-assisted in situ keratomileusis or simply laser eye surgery), then you are wrong here and should consider reading another book. But if you want to know more about the practical aspects of the exciting field, which we call overall “laser synthesis and processing of colloids (LSPC)” then you are at the right address.

The first chapter of this book starts as you would start your first days in the laboratory, you would first read about the basics and then about the specific experiment you plan to perform. Since the flexibility of this laser-based method is very high you have a huge set of parameters that you need to choose from and with each of these variations, you will get a different result. If you don’t want to waste expensive gold or platinum targets for your first “walking steps” you might want to start with a cheaper and more abundant metal such as silver or iron. What do you do next? You need to choose the liquid that you want to use to collect your nanoparticles. However, for reactive metals such as iron, the liquid medium isn’t just a collecting medium it also defines the nature of your synthesis product. If you use water you will get a mixture of iron oxide(s), if you use an alcohol you will additionally obtain some iron carbide. You see

that the choice is crucial. Let's make a simple calculation to demonstrate the number of possible outcomes: the periodic table of elements tells us that there are 91 metals, you can imagine that the number of liquids can't even be numbered, estimates range from 10^{18} to 10^{200}. But the motto of this book is "keep it simple", so let's limit our consideration to solvents. Solvents can be categorized into inorganic solvents such as water or ammonia and organic solvents, which additionally are divided into, oxygenated solvents (e.g. ethanol), hydrocarbon solvents (e.g. hexane), and halogenated solvents (e.g. chloroform). If we now want to try five liquids from each of these categories for each metal in the periodic table, we will have almost 2,000 possible combinations and possible outcomes. There is also the laser, which will not just have an impact on the physical parameters of your nanoparticles but might also change their chemistry. You see, you have to be smart about choosing the right target, liquid, and laser.

Let's say you chose to synthesize iron oxide nanoparticles in water. If you use a nanosecond-laser and perform your ablation in a beaker for one hour you will get micrograms of nanoparticles. This might be sufficient for UV-vis spectroscopy, but if you want to analyze your nanoparticles properly, e.g. the crystal structure, or even apply them in a real-world application you have to perform a lot better.

Chapter II will show you how you can boost your productivity by changing your ablation chamber, laser parameters, fluidics, or your target shape. You don't necessarily need to buy the most expensive laser in order to get more nanoparticles, good news for your boss.

Sometimes, powders suspended in liquid are the starting material of choice, so Chapter III introduces two tools for either downsizing or surface-smoothening such powders. After having learned the synthesis basics, the next brief chapter will give best-practice examples of how to fine-tune particles sizes.

After you followed the steps in Chapter II-IV and obtained, let's say, 100 mg of size-tuned nanoparticles after one day of ablation, you have to analyze your product(s). Just by looking at your colloid you won't be able to tell much, especially if you ablate iron. How big are your particles? What is it? What is on its surface? These are just some of the questions that immediately arise when you have a colloid of unknown properties.

Chapter V summarizes the most useful methods that are out there to analyze nanoparticles or colloids. We will also mention practical aspects such as how much nanoparticles you need or how you prepare your particles for the analysis with each method.

You might not need to be a chemist in order to be successful in this field, but you definitely won't have an impact on the international community without basic knowledge of chemistry. Especially colloidal chemistry is essential for reasons such as to keep your colloids stable. You need stability for colloidal characterization but also for further processing of your particles. If you have aggregated particles on the bottom of your ablation chamber and the pure liquid above, you won't be happy with this outcome. So follow the steps in Chapter VI and be happy!

We not only want you to be happy but also to be safe during your journey that you've just started. The interdisciplinarity of laser synthesis of colloids is a big advantage but from a safety point of view there are additional dangers, you have to be concerned not only with chemistry but also with laser safety. If you don't want to perform unintended LASIK by your own – check out Chapter VII. Many things can happen during laser ablation in chemicals, you have to be extremely careful. As you might imagine, flammable liquids don't go well with powerful lasers.

After having proudly made our stable, well-defined colloid in a reasonable amount, you may think of "publish or perish". As having a pinch of the application included in your manuscript may give access to higher impact journals, having a look at Chapter VIII may let you feel even better and provide a nice endpoint to your research journey.

We additionally included many "lab stories" from students, post-docs and even professors that are evenly distributed over the whole book to help you to realize what might get wrong during your experiments.

CHAPTER I

1 GET STARTED

You probably know what it is like to start something completely new. You are highly motivated and at the same time know that all beginnings are hard. Exactly here, this handbook comes into play. It will help you with tips and tricks and will let you avoid typical beginners' mistakes. You will not just learn how to dodge the mighty cavitation bubble and tune your experiment but also to study the exciting world of laser-generated nanoparticles. In order to start your first experiments on the synthesis of nanoparticles by laser ablation in liquids, you have to be well prepared. Thus not just experimental preparation is crucial but also theoretical knowledge is of fundamental importance for a successful work. At the beginning of this chapter the basics of lasers and laser ablation in liquids are summarized. Then, the most important equipment and setup you can choose will be described and you will learn how to manage your first steps of the practical work when starting with your laser ablation experiments.

STEP 1: LEARN ABOUT THE BACKGROUND

Did you know that gram of bulk gold costs about 40 €, but in its colloidal form, the sales price increases by a factor of about 500? If you want to create a tremendous added value, just make it smaller. But take care, the product, on the one hand, gets quite reactive and on the other hand, it has to be stable for a reasonable period of time. Compared to metal nanoparticle colloids, the world sales of quantum dots or other fancy materials like single-walled carbon nanotubes is negligible. This is driven by demanding applications in real life, such as lateral flow assays (for pregnancy or early cancer diagnosis tests) or chemical catalysis. Nanoparticles are indispensable in trying to solve the worlds energy demands or global water purification problems. But macroscopic function requires handling of the nanoparticles in a way their function (mainly their surface) is not compromised or even "buried" in a heap. Hence, the dispersion of nanoparticles in the colloidal form is known to create the highest functionality. Not only colloidal stability during handling and downstream processing of the particles is important, but also the accessibility of each individual nanoparticle surface determines its real benefit for application. Moreover, this surface purity demand comes from many textbook equations and theories as well, the purer the colloid is the better it fits the theories. Have you heard of Sir George Stokes? The famous laws Stokes created, including the one with Einstein, are based on the assumption of a "hard sphere", that is, a naked particle in a solvent. Hence, purity is another big value, both for comparing prediction with an experiment of particle behavior, as well as for application (less poisoning or cross-effects). Methods to create naked colloidal particles are rare. But luckily, we have this mighty tool directly in front of us. When size and purity matters, lasers come into play.

It all started with a joke: "A laser is a solution seeking for a problem", this is how the first working laser was described in 1960 by skeptics who wanted to make fun of Theodore Maiman's invention. Maiman made the world's first laser operate in his laboratory at Hughes in Malibu. The laser was based on an optical pumping of a ruby crystal using a flash lamp that generated pulsed laser radiation at 694 nm. As you know every beginning is difficult, the same happened to the father of optical lasers. Many researchers just did not realize the significance and the dimension of his invention. After his first report on the ruby laser was rejected by reviewers of the journal *Physical Review Letters,* Maiman turned to *Nature* where his paper was published on August 6th 1960.

Even though the relevance of his discovery was not clear, Maiman believed in his device's potential. Nowadays lasers are everywhere and their applications have become an indispensable part of our daily life. Just have a closer look at your surroundings: applications can be found everywhere from research laboratories to the steel industry, from dentistry to medical clinics, from discotheques to pointing devices. For example, lasers can be applied everywhere an object has to be quickly identified without direct contact. Cashiers only need to bring a barcode close to the scanner without the need of typing the article number by hand. Isn't it great to have more time for shopping? At least for all women, this is a great invention. Lasers are used for online shopping when you use a computer mouse. In an optical computer mouse, a laser can identify the direction of the movement. In addition, all optical discs drivers like DVD or Blue-ray use laser diodes. Next, to everyday products, pulsed lasers have gained huge importance in medical applications such as eye surgery or photothermal laser resection of organs. Huge industries have found multiple ways to make money from laser technology including laser entertainment shows, cosmetics (laser tattoo and hair removal), and measuring large distances by lasers, to name just a few things. There is no car or airplane built without laser technology, either for steel welding or for drilling the gasoline nozzles. In addition, pulsed laser structuring is essential to build mobile phones and tablets. The skeptical colleagues of Maiman must have looked dumbfounded when they realized that indeed, a lot of problems have been solved by the laser.

Laser Ablation in Liquids (LAL)

Lasers are powerful tools that can lead to an astonishing outcome if the laser beam is directed with high energy density onto a surface. You irradiate a piece of gold in water and obtain a red-colored liquid. As a result of irradiating a target, material detachment can take place, which can be exploited by scientists for particle synthesis. What a brilliant idea, isn't it? Particularly because the technique was originally used to structure the surface of the target and the particles were considered "just waste" for a long time. Focusing a laser beam in a liquid environment, enabled to catch the ablated materials in the solvent

directly. This makes particle synthesis safer, because no fine dust (aerosols) or particulate matter is released in the surrounding workplace, avoiding health risks, in particular, respiratory diseases. But most important, colloids are easier and safer to process into valuable products than clumped nanopowders filtered off the gas phase. The liquid is the key to quality and value of the synthesis. Fabrication of nanoparticles by laser ablation in liquids has become an interesting and important technique for many applications. Laser-generated particles are not only very useful for fundamental research, but also for their widespread use in application-oriented development of medical devices, photonic materials, catalysts, and more.

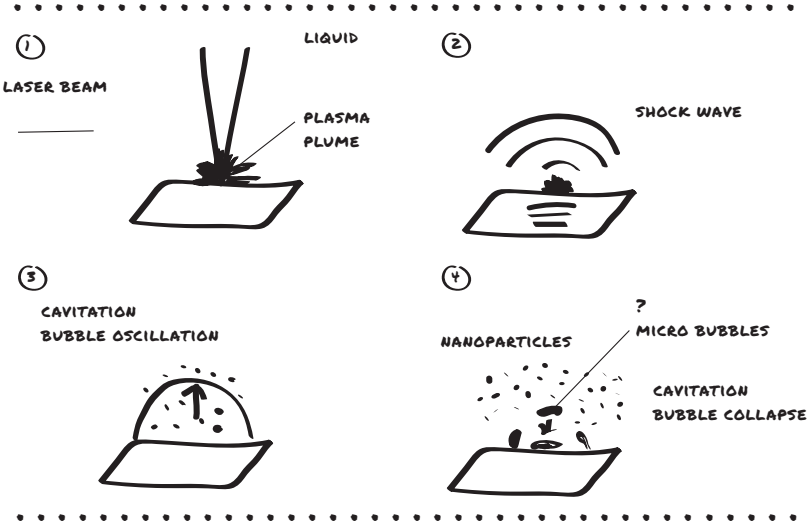


Figure 1: Sketch of what happens during LAL of a bulk target, just after the laser hits the target. A plasma with ionized or atomic species is generated on the surface (1), followed by a shockwave (2) and an oscillating cavitation bubble (3). After the cavitation bubble collapses, nanoparticles are released and dispersed in the liquid (4).

Considering the principles of laser ablation in liquids, nanoparticle formation may take place by various mechanisms and may be influenced by different laser parameters (such as pulse duration, wavelength, energy, repetition rate) and materials involved. When a laser beam irradiates a solid-state material in a liquid medium, the energy provided by the laser pulse can be absorbed by the target and leads to the formation of an expanding plasma plume containing the ablated material (Figure 1). This is accompanied by the emission of a shockwave that releases energy in the surrounding liquid. When the plasma cools down, it releases its heat to the liquid, which is transformed into hot vapor. This leads to the formation of an oscillating cavitation bubble containing both the ablated matter and dominantly the liquid's vapor, where particle formation takes place.

After the collapse of the cavitation bubble, another shockwave is generated and the particles are released into the solvent. In case of aqueous solutions and materials such as gold or zinc, electrostatic stabilization of the particle is then achieved due to partially or fully oxidized surfaces, respectively, where ion adsorption takes place and forms an electrostatic double layer (see Chapter VI). By this method, a variety of nanoparticle materials can be obtained in different solvents, which opens a wide range of applications.

Of course, to fully understand the basics, you would need to go into more detail by reading one of the specific reviews (i.e., Chem. Rev. 2017) that are out there.

Producing particles by LAL is quite simple. For the easiest setup, you just need a laser, a vessel filled with the solvent and a target, which is immersed into the liquid and appropriate focusing optics (Figure 2). The most expensive part of this setup is the laser itself which costs at least 30.000 € if you want a reasonable nanosecond laser. Maybe you share a laser with another lab, or look for used ones. All the other costs are almost negligibly small, but also depend on the target you use. Talking about laser ablation of gold in water the noble metal with about 40 €/g (purity of 99.99 %) mostly influences the price. The remaining equipment is quite cheap (a glass beaker or chamber, distilled water, lens). In total, the costs for consumable materials amount to less than a hundred Euros for many weeks of fabricating colloidal nanoparticles. Search on the internet, you will find that buying only some 100 ml colloid is often more expensive. Note that for laser ablation not the whole target can be transformed to nanoparticles, but the remains can be recycled by molding a new target. By laser ablation of a target, you multiply the value of your material by three orders of magnitude, it́s like printing money. For example, a liter of a colloid with 100 mg/L laser-generated gold nanoparticles in water is marketed at least for 1.000 €, which makes 10.000 €/g. Moreover, wet chemically gold nanoparticle synthesis requires expensive gold salt that is mixed with a reducing agent. Note that for each gold atom reduced to build up the nanoparticle, one citrate molecule undergoes oxidation, so that ketones, esters and other by-products are produced in excessive amount. These organic reactants and by-products are well known to stick to the gold nanopartilcés surface. Depending on the application, you would then need to remove the ligands on the particles' surface, which will again cost time, energy, and money and is never complete. You see, purity and simplicity of LAL have multiple values.

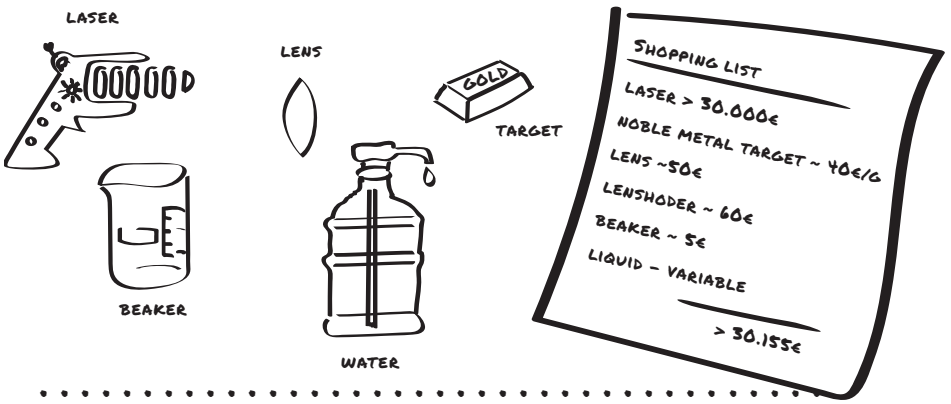


Figure 2: Basic elements needed for laser ablation in liquid and their approximate prices

STEP 2: CHOOSE THE TARGET MATERIAL

Before starting with any experiments you should be well prepared. Take time to think about the material you want to ablate and about the suitable solvent for your research and application.

One of the main advantages of laser ablation in liquids is the possibility to choose a variety of materials. So if you realize that for instance gold is not the right material for your study, you just change the target and synthesize other nanoparticles, maybe put the gold in an oven with silver and go for ablation of alloys. Other chemists synthesizing nanoparticles would dream of it, because simplicity and robustness of LSPC experimental setup makes the research much more effective and more fun. Typically there are two different types of materials that are mostly used to generate nanoparticles by laser ablation in liquids: metals and oxides. But also other types of nanomaterials such as sulphides, nitrides or carbides were successfully fabricated by LAL.

We recommend to let the product you want determine the selection of your target, if it's zinc oxide or titania, it's easier to start with the oxide rather than going for metal ablation and hope for controlled oxidation in water. In the latter case, it is likely that you will end up with nanoparticles which contain a mixture of defect-rich crystal phases (and sometimes amorphous phases) that are not always easy to reproduce.

Laser ablation in liquids does not just allow the synthesis of pure materials such as metallic or oxide nanoparticles, but also alloy nanoparticles possessing solid solution, segregated or core-shell structures are accessible depending on the experimental parameters (target, solvent, solutes, etc.) you use. The importance of nano-alloys lies in the changed properties of the materials compared to their single elements.

Multi-elemental nanoparticles often have multifunctional purposes. You can combine the prospective features of both metals such as their surface plasmon resonance and magnetic properties, as it is the case for AuFe nanoparticles. Furthermore, many other benefits can be derived from different material properties. It's up to you and your innovative spirit. Indeed, while they are often referred as "nano-alloys", these nanoparticles can have a complex structure requiring a more rigorous classification. Luckily, this handbook is here to make your life easier, so let's try to put an order in the "zoo" of multi-elemental nanoparticles. First consider the simple case of nanoparticles containing only two different elements, i.e. those composed of two metals. First of all, the multimetallic particles can be grouped as homogeneous or segregated. In these particles we can further arrange the two elements in the following ways: Let's first start with homogeneous alloy nanoparticles. In these HOMOGENEOUS particles we can arrange the two elements in the following ways (see Fig. 3, left i - iv)

i) in a "substitutional" alloy, i.e. an ordered crystalline lattice in which the two elements are homogeneously distributed (either randomly or with a precise ordering);

ii) in an "interstitial" alloy, i.e. an ordered crystalline lattice of only one element, where the second one is present as an interstitial defect (or dopant);

iii) in an amorphous structure, which has a random arrangement of the elements but no ordered crystal structure (i.e. a glassy metal)

Homogeneous alloy nanoparticles are formed when there is at least some (room temperature) solubility at the given concentration. The random atom distribution in this binary alloy lattice makes it called SOLID SOLUTION nanoparticle. A prominent example of a solid solution easily made by LAL is AgAu. Depending on the size of the mixed atoms, a solid solution can be a substitutional (i) or an interstitial (ii) alloy. In an interstitial alloy, the solute atoms are far smaller (~<60%) than the radius of the solvent atom, so that it fits in between the solvent atoms ('interstitial'). By contrast in a substitutional alloy, the solvent atom is substituted by a solute atom. In the case of solid solutions, the two different atoms are randomly distributed and the lattice type is that of the matrix (solution atoms).

In some cases, when there is an ordered arrangement and the lattice type is different from that of the matrix material, these homogeneous nanoparticles are termed INTERMETALLIC nanoparticles. A classic example is CuZn.

A special case occurs when the two elements are clearly “SEGREGATED” within the same nanoparticle, such as in

iv) core-shells

or

v) heterostructures.

Segregated alloys nanoparticles are formed when the solubility limit is exceeded, so two phases have to be matched in one particle. Resulting alloys appear mainly in two different forms. One is CORE-SHELL nanoparticles (iv), often marked as C@S, such as Fe@Au. LAL enables the synthesis of core-shell particles, in particular when a metallic and a non-metallic compound can form, and they usually consist of an inorganic core (i.e. metal, oxide, nitride, carbide) and an inorganic or organic shell. However, bimetallic core-shells composed of Au-Fe alloys with high yield were also obtained by LAL.

The second type of segregated nano-alloy is a JANUS nanoparticle (v-a), having two distinct faces. The name comes from the Roman god, who is usually depicted having two faces since he looks to the past and the future. Some materials, such as Ag-Cu may form both, Ag-Cu-Janus and Cu@Ag particles in parallel. If you are not sure what you have created via LAL of an alloy target made of AB or have a mixture of ordering types, just term it nano-alloy, and write it as “A-B nanoparticle” or “A-B alloy nanoparticle”. By a clever choice of different materials for the synthesis of core-shell nanoparticles, you can combine the material properties and maybe synthesize a god-like structure.

However, heterostructures may be also phase segregated nanoparticles (v-b), which consist of a fine mixing of two crystalline phases, each one containing only one element, but on a size scale smaller than the single nanoparticle, so that these two phases are randomly distributed inside the single nanoparticle (or phase A is randomly distributed in a matrix of phase B like raisins in a panettone). Obviously, intermediate conditions may also exist, such as heterostructures of alloys (v-c): for example, phase 1 with 80% of element A and 20% of element B, and phase 2, with 75% of element A and 25% of element B, randomly distributed inside the single nanoparticle.

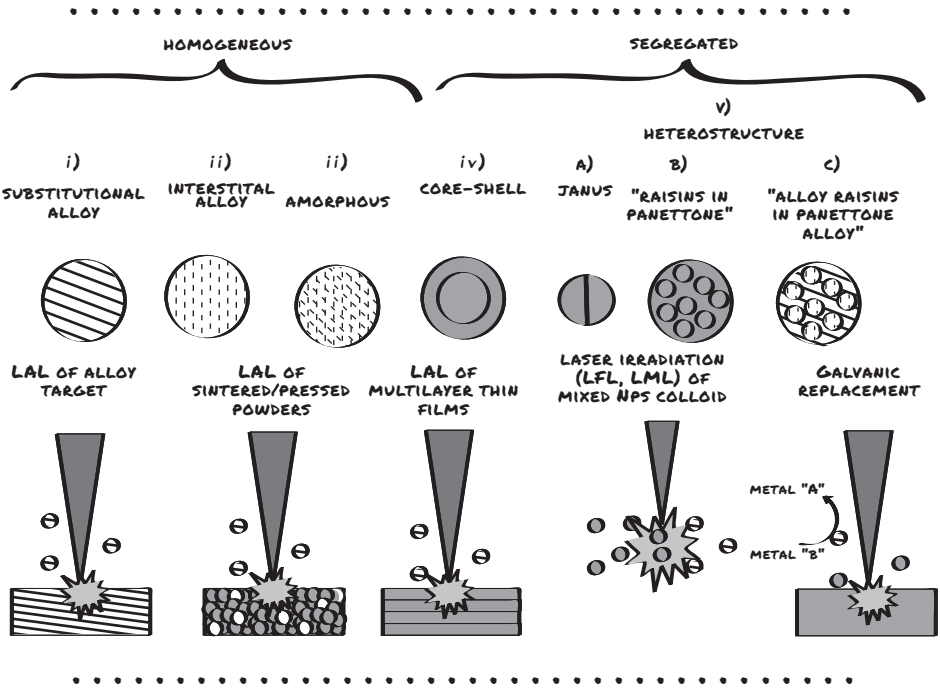


Figure 3: Ways to obtain alloy nanoparticles by an appropriate choice of the target for laser synthesis of colloids, and terminology of the resulting particles.

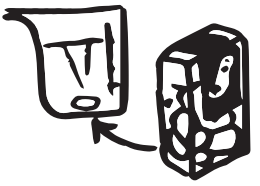
Can you imagine how many possibilities exist for mixing different materials? The really amazing thing of laser synthesis is that, in several cases, you can achieve all of them starting from the same multi-element target. The less amazing part of the story is that sometimes you have all of them contemporarily in the same sample, and all your talent is required to refine the synthesis conditions and to focus only on one structure among the many possible ones. This can be done, by using the appropriate target. In fact, after you have decided to synthesize nanoparticulate alloys, the simplest choice is that you can just ablate an alloy target. Try a series of AgAu, it gives a beautiful color sequences, where the color changes with composition, which you can follow by the naked eye or by UV-Vis spectroscopy. The composition of the targets determines the (starting) composition of your nanoparticles. However, you should carefully choose your solvent, which strongly influences the formation and thus the mixture and structure of the particles (see Step 3 for it). But where to get such an alloy target? For coinage or noble metals, you may simply go to the goldsmith or jewelry around the corner and ask them to prepare it. We get our Ag-Au alloys and even Au-Pt there as well. This may even be far cheaper than buying from a fine chemicals company catalog.

Another fancy approach for alloys is to use a self-made target. You can mix different powders and press them to form mixed powder targets. Depending on the compressibility of the materials and stability of the targets you can sinter the pellets by heating them up subsequently (this also avoids breaking of the consolidated powder targets). In case the diameter of your laser beam is larger than the grain size of your powder, you now will synthesize alloy nanoparticles. We recommend micropowders <50 micron in diameter. The same works with other composites, such as mixing silver powder with an oxide. The key to reproducibility is an intense mixing of the powder in a mortar. Inspect color and gloss of the pressed target (by the naked eye or in a side-illuminated "dark field" microscope): is it the same on both sides or has the particle density and size difference caused inhomogeneities? This micropowder-based preparation method is a simple approach to achieve alloys or doped oxides on the nanoscale without much effort. It's up to you which composition you would like to take because you prepare the educts as you want and it could be much cheaper compared to bulk alloy targets, which are usually only available in certain compositions from alloy distributors.

In addition to taking a solid bulk or consolidated-powder material for LAL, other forms of a target material can be used. For some materials (e.g. oxides), powders are more easily available and you can use a suspension of that powder directly as the educt. This process is then called laser fragmentation in liquid (LFL, at high laser fluence) or laser melting in liquid (LML, at low laser fluence), see Chapter III). The main disadvantage here is that after irradiation of the

Ina came desperately to me and said that the magnetic stirrer isn't working any more. After testing several others we found that the magnetic stirring bar isn't magnetic and is changing its appearance. After smelling a minty taste we realized that this is not a magnetic stirring bar but a tic tac. Imminently the question raised who was willing to sabotage our experiments by putting this tic tac into the box with stirring bars. After detailed investigations and having several suspects we found out that Galina lost a tic tac several days ago and Ina finding it was wrongly identifying it as a stirring bar, thus placing it in the box for the stirring bars. The lesson is clear: tic tac's should get a new shape!

Marcus Lau and Ina Haxhiaj, Essen



powder you need to separate it from the colloidal nanoparticles, this is called educt-product-mixing and can be quite nasty. E.g., the determination of the particle concentration (the product without the educt) is long-winded compared to bulk materials, where you just need to weight the target before and after ablation.

In case of powders you always need to separate them from the liquid and it's sometimes difficult to fully dry them before weighting. Sieve the powder before use, to remove everything larger than 50µm, facilitating dispersion and homogeneous ablation.

In case of powders, you always need to separate them from the liquid and it's sometimes difficult to fully dry them before weighing. Sieve the powder before use, to remove everything larger than 50µm, facilitating dispersion and homogeneous ablation.

Two more starting material types need to be mentioned if you want to create alloy nanoparticles. First is the mixing of two colloids (e.g., Au and Ag) and irradiating them. Femtosecond pulses focused into the liquid so that they cause white light are efficient for that. Or fuse them together by LML. The second variant needs a metal salt dissolved in the liquid and a target. A nice effect is now created during LAL when the target is less noble than the metal element dissolved in its salt form in the liquid, e.g. LAL of Co in Pt salt. This is called reactive-LAL, as there will be a redox reaction between the two species, called galvanic replacement. Note that its quite difficult to have high yields, so a sequence of cleaning steps by etching-washing will be required. As a newbie, start with solid targets.

The following case study will describe what to keep in mind when you need alloy nanoparticles with a specific composition (molar fraction) and structure (solid solution or core-shell)

Case study: Harvest alloy or core-shell particles

Let's try to get a feeling on how alloy and core-shell nanoparticles may be tuned in composition and size. A nice way to obtain alloys or core-shell nanoparticles is from ablating an alloy target already exhibiting the required composition (e.g. a Ag:Au target in 50:50 composition). However, these are usually expensive which is why you may want to bring some joy to your bosses pocket and just buy a metal powder of each alloy component (e.g. a five micron Au powder and a 10 micron Ag powder). The price of those powders is often about one order of magnitude less than the similar weight of a finished alloy target. Additionally, you can tune the composition freely by just mixing different amounts of powder, e.g. 1 g Au powder and 2 g Ag powder – to get a Au:Ag composition of 33 wt%:66 wt%. Usually, alloy stoichiometry is given in molar fractions (written as Ag_xAu_y, so the density needs to be taken into account, hence, that mixture will

yield AgAu with 50% molar fraction (x=y=1). If you have a ball mill around you can further decrease the particle size while deagglomerating the often sticky micropowder aside also mixing the powder homogeneously. Do not take too small powders as they tend to form larger islands, anyway. Using an isostatic press, a powder pellet can be obtained and used for laser ablation. Hereby, it is important to make sure that the grain size is at least 10 times smaller than the laser-spot-size. Additionally, sintering of the pellet at 1000 °C is usually a good strategy to further increase pellet integrity, its mechanical stability (against the shockwaves), and thereby maintain a homogeneous ablation.

When planning to ablate the target, it is important to employ fluencies as high as possible to make sure that plasma temperature is very high and hence the different ablation threshold values of each individual material species in the pellet are not as important (e.g. ablation rate of Au is about two times higher than of Ag). Maybe factor 3 above the ablation threshold. Now, if you rather want to get an alloy nanoparticle, vigorous oxidation of the less noble component should be avoided. Hence, in the case of present ignoble materials, alloys are usually obtained when ablation is conducted in organic solvents with slight reducing chemical potentials like isopropanol or acetone. In the case of alloys from noble metals, the situation is much easier as the ablation can also be performed in water, due to the resilience of noble metals against complete oxidation.

In summary, you can generally tune your nanoparticle composition by ablating a pressed (and sintered) powder pellet being obtained from a mixture containing chosen amounts of different metal components. Solid solution or formation of segregated structures is mainly tailored by the solubility of the involved elements. Generally, you can easily deduce from the phase diagram that elements which show low miscibility in the solid state tend to form segregated or core-shell particles (e.g. Fe and Au at high Fe content), while elements with good miscibility form solid solution particles (e.g. Ag and Au). Let's see if you understand the following examples while looking at the phase diagrams on the internet: For the Au-Fe system, it switches from solid-solution to core-shell particles when increasing the iron content in the gold matrix, with segregation setting in at molar iron contents above 50%, better around 80-90%. In contrary, AuAg will always be a solid solution. Pt_3Cu or Pt_9Ir also forms nice solid solution particles in acetone. Same with FeNi. Did you know that FeNi was the first alloy humans have seen and used? It's called meteorite iron. So as you can see, changing the nanoparticle composition by laser based methods is not that hard. It's far more easy than by chemical co-reduction. You may even think of ternary elements. Or even beyond: the quinary, noble-metal-free alloy CoCrFeMnNi can easily be fabricated by picosecond LAL in ethanol. It's difficult to synthesize such high-entropy alloys in colloidal form by conventional chemical methods; with laser synthesis, just mix the 5 powders in equimolar ratio and shoot at it. So there are two main advantaged using LAL for alloy nanoparticle synthesis:

the convenience to fabricate alloys just from the bulk, and the quick access to molar fraction series. Such series are highly interesting both in biomedical research (e.g. Ag-Au) and catalysis (Pt-X, Pd-X, Fe-X, Ni-X,...). However, watching people climbing the Mount Everest in TV also doesn't look that hard so keep in mind that this was just the tip of the real iceberg. Hence, in case you plan to start your own journey of alloy or core-shell nanoparticle preparation you should also expect a few periods of bad weather during your trail.

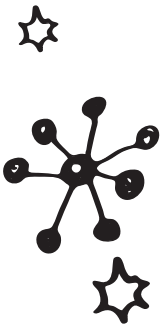
But there's one more thing to say. We always try to make our research convenient, and as characterization of the nanoparticles after synthesis consumes most of our time, we select the material always also by this criterion. It's a pity if you wait a long time for TEM or XRD data and it was the wrong colloid that was analyzed: It's easy to make 20 samples a day, but which to select for in-depth analysis? It is very convenient if the material you ablate leads to nanoparticles that have a color since in this case, you can immediately tell if you had success or not. Examples include the metal nanoparticles (red gold, bright yellow silver, brown copper) or the doped oxides, such as ruby. Colored, doped oxide targets you may find in gemstone shops. Also, crystal defects may give color, such as titania which turns nicely blue if titanium is ablated in water.

STEP 3: CHOOSE THE LIQUID

The liquid environment plays an important role in synthesizing nanomaterials by LAL since it influences the nature of the materials you will get. Basically, you should have your application in mind. For instance, if gold nanoparticles are needed for biomedical applications, ultra-pure water (and maybe the addition of some albumin for stabilization in the saline biological media) is ideally qualified. For stabilization tricks, you may use in water against aggregation, see Chapter VI.

As you know, less noble metals such as copper may lead to oxidation, hence alternatives are required if you want to obtain non-oxidized metallic particles. Materials, which are oxidation-sensitive, can be ablated in the presence of reducing agents or in solvents such as ethanol, propanol, acetone, toluene, etc. For example, acetone works well with copper. However, you have to keep the application in mind and you should be aware that some solvents (such as toluene, and even sometimes acetone) lead to a formation of a thin carbon layer on the particles' surface. Inside the bubble created by the laser ablation, there is a huge excess of solvent molecules surrounding the hot ablated matter, so even coinage metals will quickly (and in water often quantitatively) react with water, and carbon-affine elements like titania or iron will form carbides instead of staying in its elemental form. Working with organic solvents, which have a low flash point and evaporate already at low temperatures, may cause flames if applying a laser beam on the solvent vapor. Then a dust particle crossing the laser beam in the vapor could be enough to ignite the mixture. Ethanol or acetone is often the first choice when the nanoparticle is sensitive to oxidation.

Some degassing by nitrogen bubbling prior to LAL helps. Note that organic liquids like ethanol, or even worse, the glycols, may cause far lower productivities because of their high viscosity that hinders bubble removal. Mainly in case of non-noble materials the pH value can influence the structure and the composition of your material. If you look at the Pourbaix diagrams, which show the redox-potential and pH-dependence of a certain material, you will note which oxides can be formed at a certain pH. Furthermore, the pH strongly influences the colloidal stability (as will be seen in Chapter VI). As a rule of thumb, stabilization of (noble) metals is achieved at high pH, and oxides often go well at neutral pH (not too low, to avoid dissolution). Some oxides (that tend to dissolve at low pH can also be stabilized at very high pH, e.g. 11 for manganese. Check the isoelectric points of the product in the web (already the value for the bulk gives a good hint), and keep the pH away from it. If you run into stability problems and notice aggregation of particles the next day even though you have tried different pH, you may like to read the chapter on particle stability. Just one word on that: add a trace of soap or protein. More precise, add a pinhead piece of curd soap or add albumin (works with almost everything). Of course, never use tap water, only distill led or desalinated water.



As mentioned in the previous part, the liquid environment you use also influences the formation of alloy nanoparticles or core-shell structures. This can get complicated. For instance, laser ablation of an alloy target with an oxidation sensitive material such as iron and noble metal gold (equimolar Au:Fe) leads to the formation of a non-oxidized, elemental iron core with a gold shell in organic solvents. In contrast to this, LAL in water forms a gold core surrounded by an iron oxide shell. This happens because iron is only partly miscible with gold, so that segregation has to occur, and you have to make sure that the iron does not see oxidative species created from solvent photolysis. Just to name a few of other approaches for particle synthesis with a core-shell structure: a quite less investigated method is the ablation of a target which is already immersed in a colloidal solution with particles. But also a combination of LAL and chemical reduction is possible, as mentioned above. In that case, you can synthesize your particles and add a precursor solution or you directly ablate a target in the presence of a precursor, this is then called reactive laser ablation in liquid. Try ablation of a less noble element in a metal salt solution of a nobler element, the ablated species will reduce the precursor and form a composite nanoparticle. That's advanced colloidal nano-redox-photochemistry done in 1 minute (and maybe understood after 1 year). No worries, in this handbook we will keep it simple and most rules apply as well to the more complex material-liquid combinations. Take PtAu, PtNi, or PtCu alloys as an example: they will not segregate into core-shell particles and give well defined solid solution alloy nanoparticles (e.g. interesting for catalysis application) if you ablate them in an organic solvent (avoiding oxidation of the less noble element). In this way, it is quite simple to fabricate an alloy series (that is, a molar fraction series) by laser synthesis, by far more simple than trying the same by conventional chemistry.

STEP 4: CHOOSE THE LASER

The literature is full of papers dealing with laser ablation in liquids and often many different laser parameters are applied, so that for beginners, it can be confusing and you might wonder why exactly that laser with that parameter was used. But the truth is, mostly the laser, which is often the only one available in the lab, is used while the parameters are optimized for this given laser. But if you have an option to choose a certain laser you could pay attention to some general details. Therefore we would like to give a brief overview of what one might consider when choosing the laser for ablation.

It is mostly established to use a wavelength of 800-1064 nm for laser ablation in liquids, but also green light with 532 nm is often used to ablate a material. However, if you want to prevent two different mechanisms to take place simultaneously, the wavelengths in the near infrared are is more appropriate to synthesize nanoparticles by laser ablation in liquids. For instance, if you ablate a gold target with 532 nm in a vial, where the concentration of the particles increases and forms an optically dense liquid, the particles will be re-irradiated.

At this wavelength e.g. gold nanoparticles will absorb a high amount of the laser energy because of their intense plasmonic resonance band at about 520 nm (depending on particle size). Hence, ablation of the target, as well as re-irradiation of the particles, take place simultaneously, compromising reproducibility. In addition, the colloidal solution efficiently swallows the laser energy and your productivity will quickly decrease (as will be addressed later in Chapter II). To avoid this, ablation synthesis at wavelengths where the particles show minimal absorption of the laser light is advisable for better control and understanding of the mechanism that takes place during ablation. In contrast, if you hunt for smaller particles, short wavelengths and long irradiation times may shift the process balance from ablation to colloid excitation, at the expense of productivity.

... everyone was quickly leaving the building, and heavily dressed fire workers seeking the fire source. Smoke sensors pointed at my laser lab, and the surprised firefighters found my guest scientist (a renowned guest professor) smoking. He later said he was blowing the cigarette smoke over in order to visualize the laser beam during its alignment on the optical table.

Stephan Barcikowski, Essen

Before talking about laser power, energy, and intensity it is useful to understand what these terms refer to. The power of a laser is measured in Watts. This refers to the mean power output of the laser. The definition of the mean power output P_{mean} is simply calculated as the Energy E_{mean} released during the operation time t_{op} ($P_{mean}=E_{mean}/t_{op}$). For instance, as shown in Figure 4, if a continuous wave (cw)-laser emits light with an energy of 3,000 J in 10 minutes, the laser has a mean power output of 5 Watts (3,000 J / (10 · 60 s)). When using pulsed lasers the situation is much different as there are two separate definitions of the laser power possible. Remember that a pulsed laser releases its energy in packages (laser pulses) during the operation time t_{op} with some repetition rate (e.g. 20000 pulses per second) having a given pulse duration t_{pulse}. If the pulsed laser fires 20,000 (= $2 \cdot 10^4$) pulses per second having the energy amount of e.g. 250 µJ (= $2.5 \cdot 10^{-4}$ J) per pulse, its mean output power simply equals to 5 W ($2 \cdot 10^4$ [pulse/s] · $2.5 \cdot 10^{-4}$ J [J/pulse]).
That's the same average power output as the cw-laser before. But the cw one will not even make the target hot whereas the pulsed one is already quite productive in colloid synthesis. Accordingly, due to the compression of the energy into pulses of a specific pulse duration t_{pulse} (e.g., 1 ns or 1 ps), the stream of single pulses is by far more efficient in removing matter from a solid surface. You wonder why? Imagine two situations: you are the target getting irradiated with a 250 µJ laser pulse of A: 1 ns pulse duration and B: 1 ps pulse duration. In case A you absorb 250 µJ of energy within 1 ns which means that the laser is irradiating you with a peak power of 250,000 W ($P_{peak} = E_{pulse}/t_{pulse} = 2.5 \cdot 10^{-4}$ J / ($1 \cdot 10^{-9}$ s) or in another unit 0.25 MW. This peak power P_{peak} is comparable to the added power of about 20 single-family houses. Now it's better to stop imagining being the target as we get to case B. In case of a 250 µJ pulse having a pulse duration of only 1 ps the peak power (or pulse power) impacting the target equals 250 MW! This is the amount of energy a small power plant produces. Practically, nanosecond lasers often have similar peak powers than picosecond lasers, as ns lasers provide mJ pulse energies and ps laser pulses are in the µJ range. E.g., 200 mJ @ 10 ns or 200 µJ @ 10 ps will both shoot with 20 Megawatt peak power. But a typical femto laser with 1 mJ @ 100 fs will allow you to fire with 1 GW, allowing to disintegrate every solid (with the risk of vaporizing the liquid before reaching the target).

Of course, this peak power only affects the target for a very short time, though with a tremendous effect we call ablation. But do not underestimate the target's defense mechanisms, which mainly are light reflection and energy dissipation. In order to crack the defense mechanisms of the target, you need to increase the penetration to use the full force of your single pulse. To do so you need to decrease the lateral extension of the pulse, which basically is the beam area. To achieve this, lenses are used to focus the laser beam. As depicted in Step 5, the other option is to use a telescope of which two telescope types namely the Kepler and the Galilei telescope are especially famous. The advantage in using telescopes is that the light wave still

propagates in the same direction (parallel beam) while a focusing lens will result in a diverging beam. The telescopes are limited by the destruction limit of the lens material, which is why they can't be used in cases where very small spot areas and high pulse energy lasers are being used. Now, upon decreasing the beam area, two parameters of the laser light called "intensity" and "fluence" will increase.

Intensity is simply defined as the power (either mean power or, in our community, the peak power in Watt) per beam area (in cm^2). On the other hand, laser fluence is defined as the beam energy (in J) per area (cm^2) or speaking in formulas:

$$F = \frac{E_P}{A} = \frac{\bar{P}/\dot{N}}{A}$$

Hereby F depicts the desired laser fluence, A the laser beam area and E_P represents the energy of a single laser pulse. The pulse energy E_P can be obtained by dividing the mean output laser power measured by the number of laser pulses per second $\dot{N}$.

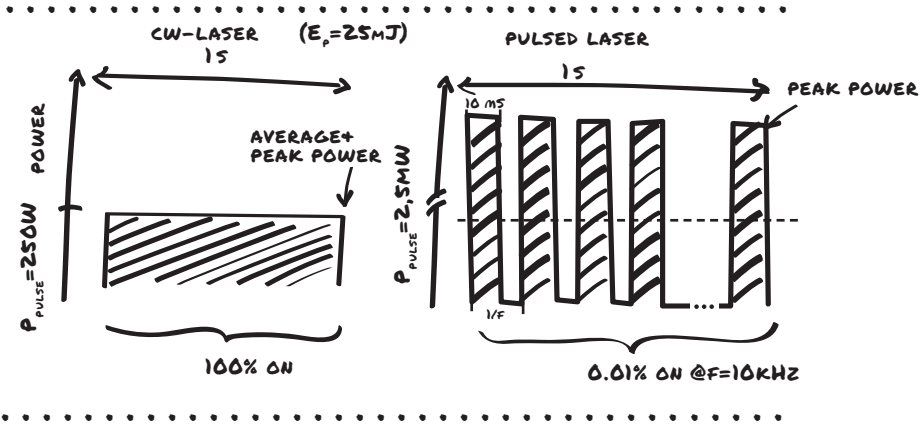


Figure 4: Comparison of laser power of a continuous wave (cw) laser with a pulsed laser beam with 25 mJ. The average power of both is the same so that the peak power of the pulsed laser is far higher.

The definition of laser fluence: only applies to pulsed lasers as it is based on the pulse energy as shown above. The laser fluence is a key parameter for all types of laser processing of materials in liquids: for laser ablation, laser fragmentation, and laser melting. The fluence rules the productivity during laser ablation, sets the smallest size you can achieve by laser fragmentation and is the main parameter to control particle size by pulsed laser melting in liquid. Hence, it is of utmost importance for the sake of reproducibility to measure and control this parameter "laser fluence" as precisely as possible. And if you want to be kind to people that like to follow your footsteps, you should say how you measured it. But this requires to measure the pulse energy and the spot

area. Then both values can be set into ratio to calculate the applied fluence. The pulse energy can be determined by a power meter (e.g. at the beam outlet) that gives you the output power. Dividing the power by the repetition rate gives you the pulse energy. A better position for measuring the power is as close to the ablation chamber as possible (e.g. behind the last mirror or just before the focusing optics). This gives you a precise value as energy losses accumulate at every optical element (so the deviation of output power from your laser to the applied power on your target increases with each optical element). Forget about the values that are written on the laser (or in the manual) or in the last Ph.D. thesis that used this laser. Pumping lamps or diodes naturally degenerate, so that the emitted laser power decreases continuously. Please also consider that for most lasers their power will be different directly after turning them on or after one hour after laser operation because they need to "warm up".

We recommend measuring the laser power 30 minutes after the laser resonator is on. Now you need to find out what the "spot area" definition on the target (for laser ablation) or the liquid entrance (for laser fragmentation/melting) is. Don't wonder if you will find very different values in the literature regarding the laser fluence for the same setup (e.g. same laser, same focal length) because measuring the fluence is done differently. In principle, there are 3 ways of doing it:

1) The "theoretical fluence" or "geometric fluence" you get by simple calculation based on focusing law (input parameters are raw beam diameter, wavelength, and focal length). We recommend then to measure at least the raw beam diameter before the lens as it often deviates from the diameter the system manufacturer provides. For this, you take a beam detector card and hold it into the beam (read the chapter on safety before). If you don't have a beam card, (thermosensitive) telefax paper or thin black paper does a good job, too. Wetting the telefax paper before shooting on it is a good way to avoid non-representative big holes and smoke if you have high pulse energy.

2) The same you can do to acquire the "measured fluence at target plane" at the distance from the lens where the target is going to be placed. Maybe you put the paper on top of the target. Test how many pulses you need to see a hole. But this will not always work in liquid.

3) The most suitable way to determine the fluence is to measure the spot on the target in liquid, that's the closest to the really applied fluence. In order to obtain the spot diameter based on this setup, you will need to set a limited number of pulses (e.g., start with 10 and 100) and measure the average crater diameter with a microscope. A larger heat affected zone surrounds the crater very often, but for calculating the fluence value, we look for the ablated crater diameter. To make it clear, you can only make sure you get similar fluence after disassembling the setup. So, you can't really know the effective fluence until

ablation is finished. To get reproducible results, your positioning system, sample thickness and distance of the chamber to the lens have to be very (!) accurate. Maybe you always use a personal rod of defined length that calibrates the distance of the target surface to the lens. But once you know the ablation rate, in particular, the target thickness loss per time, you will know when you run out of the fluence window and have to re-adjust the sample position towards the lens. But as lab lasers use to vary in power and beam quality during the day, you are on the safe side when measuring the fluence as described above (and listen to the noise, as sound change is a good indicator for focal shift).

In case of continuous laser beams, the impact of a laser can only be measured as a function of laser intensity, which was introduced above prior to the fluence. If the laser intensity is high enough, it is possible to ablate a target with continuous waves, however as already indicated while a 5 W pulsed laser can create peak powers of, e.g. 250 MW (in case of 1 ps pulses with 250 µJ), the continuous wave laser will still only operate at 5 W. That's why in cw laser manufacturing like laser welding or plate cutting, kilowatt laser output power is standard today, whereas for pulsed laser machining tens of watt are often enough. As hinted before, the target has several "defense mechanisms" one being the energy dissipation. Against continuous wave lasers, this works perfectly as the energy is continuously delivered it can be continuously dissipated resulting in slow heating and eventually melting of the target. But this can change when pulsed lasers are used to irradiate surfaces or particles.

Reason for this is that different heat exchange mechanisms come into play when irradiating the target. In general, one can distinguish between thermal and non-thermal ablation of the target. Thus the pulse duration of the laser has an important influence. Nanosecond pulses cause thermal ablation because the pulse length is much longer than the time that the electrons need to heat the atom lattice of the irradiated material. But this can change for ps pulses and fs pulses because the pulse length is in the timescale that the electrons need to heat up the lattice of the material. Therefore, cold ablation processes (phase explosion) can take place.

For instance, if you operate with ultrashort pulsed lasers, the ablation rate is so fast that the solvent even does not realize if the target is being hit by the laser (no heat transfer) and stays cool, which enables laser ablation in solvents with low boiling point. Furthermore, because of energy losses to the environment in case of ns pulses, the ablation threshold is higher compared to ps pulses.

The "ablation threshold" is also called "threshold fluence", and is the value of the minimum fluence at which ablation takes place (a noticeable material removal, not only surface melting or roughening). The lower the better.

The threshold fluence decreases with pulse duration because of negligible energy dissipation by thermal conduction, and thus leads to higher ablation efficiencies. Below a pulse duration of 100 ps (for some materials < 10 ps), the ablation threshold is constant, and starts to increase towards the nanosecond scale. Above several ns pulse duration, it is you mainly create heating than ablation. 2 ps may be short enough to be on the "cold side" of efficient ablation for most materials. That's the theory, but in practical application, also ps and fs laser ablation will heat up the target slightly for longer ablation time, caused by the accumulation of minimal residual heat that every pulse leaves at the target. It is not much if you use a 10 Hz or 1 kHz laser, but noticeable for high repetition rate lasers, in particular, those approaching the MHz regime.

Even though the ablation threshold is smaller for ultrashort pulses, these may cause an optical breakdown, namely a strong local ionization of a medium (the liquid) due to energy absorption. For better insights look up in Chapter II, which describes the effect of the pulse duration in more detail and nicely illustrates these phenomena.

Recently at the TEM I could observe the presence of impurities in nearly all of my colloids consisting of an organic solvent. According to EDX measurement they were made of silicon compounds and were present in a comparable concentration to the nanoparticles. The impurities interacted with the electron beam of the microscope and covered up the particles, no possibility to get nice images. Different sources like air dust, dirty grids or production additives of the solvents stood in suspicion. Then a mass spectroscopy analysis of different organic solvents revealed high amounts of polysiloxanes in a p.a. grade acetone I normally used for synthesis.

Friedrich Waag, Essen

Ns lasers are usually available with high pulse energy and high power at low or moderate repetition rates and could be more productive. But another important point influences the ablation efficiency, namely the cavitation bubble. This bubble that is caused by ablation shields the target locally at the point of irradiation for several tens to hundreds of microseconds. As a result, following laser pulses in that time scale will be scattered at the cavitation bubble and therefore less material can be ablated. The lifetime and the size of the cavitation bubble depend on the pulse energy, which is usually smaller for ps and fs lasers. But typically the bubble has a lifetime in the (hundreds of) microseconds regime,

while its size is in the (hundreds of) micrometers to (single) millimeter regime. Hence, the laser-cavitation bubble interaction takes place only at high (> kHz) repetition rates, where the temporal pulse distance reaches values of the bubble lifetime. To prevent that the cavitation bubble interferes with the laser beam, a spatial or temporal pulse separation by bypassing the bubble is needed in order to get higher productivities. If you use a < 100 Hz laser, you will definitely not have to worry about any cavitation bubble shielding.

However, in case you think about ablation of a pressed powder target, mild conditions are favorable to prevent mechanical removing of the initial microparticles by huge shock waves. High pulse energies (typical for low repetition rate lasers) are more likely causing partial disruption of a pressed pellet. At given laser power, high repetition rate lasers have lower pulse energy, allowing gentler ablation at similar yield. As you see, the laser parameters strongly depend on your interests and the materials or liquids you use.

Table 1: Pros and Cons of different laser pulse lengths for pulsed laser ablation in liquids

Laser pulse duration	Pros	Cons
ns	· more and cheaper high power lasers available (i.e. high productivity) · high power at moderate repetition rates · supports alloy formation from powder targets (first pulse melts and sinters the target surface)	· strong heating of liquid due to pronounced heat transfer (check temperature and avoid boiling!) · less efficient compared to ps and fs
ps	· more efficient due to less heat loss · low liquid heating due to insignificant heat transfer · compromise between efficiency and productivity/costs · high power systems available	· optical breakdown at high pulse energies · high power requires high repetition rates (bubble shielding) · high power more costly than ns
fs	· 'gentle ablation' · very efficient ablation per pulse · insignificant heat transfer to liquid	· high power requires high repetition rates (bubble shielding) · optical breakdown (liquid ionization competes with ablation) · high power more costly

STEP 5: USE THE RIGHT OPTICS

Working with a laser requires at least basic knowledge about laser optics, since they need to be chosen carefully. The main items are lenses and mirrors. The area of optics is a perfect playground and you can tumble-around especially with the variety of lenses. Starting from the simplest lens, common lenses to focus or to spread the laser beam are shown in Figure 5.

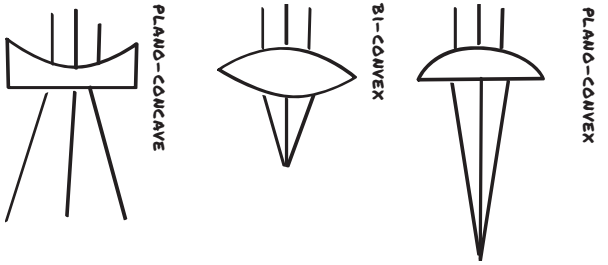


Figure 5: Types of lenses: Plano-concave, bi-convex and plano-convex

Two main orientations should be kept in mind, namely convex and concave. A convex lens is curved outwards and a concave lens inward. In order to memorize the geometric structures you can help yourself by thinking on a flat surface, which is curved inward, to make a little cave (concave) or outward to make humps like a T-Rex (convex). To focus the laser beam and thus concentrate the entire laser power to a small spot a convex lens is used. You can decide whether you want to bundle the beam at long distances by using a plano-convex lens or at shorter distances if working with a biconvex lens, which is symmetrical on both sides (collecting lens). However focusing a laser beam with a plano-convex lens leads to reduced spherical aberrations ("sharper" focusing) and is mostly used for laser ablation, whereby the curved surface of the lens is faced opposite to the direction of the laser beam (Figure 5). Concave lenses can be used to expand the laser beam or to increase the focal lens in an optical system (diffusing lens). For instance, if you want to change the laser fluence (remember that this is energy per area and the entrance key to any laser synthesis castle!) you can change the spot diameter by using such a lens. Now you may ask, how can the beam be expanded to keep the beam parallel? For this, a beam expansion system can be assembled by combining two lenses. You can build a beam telescope by displacing the concave and convex lens against each other. There are two main assemblies. A Galilei telescope consists of a collecting and a diffuse lens, whereas a Kepler system is build up with two collecting lenses (Figure 6). How much the beam is expanded depends on the focus of the lenses.

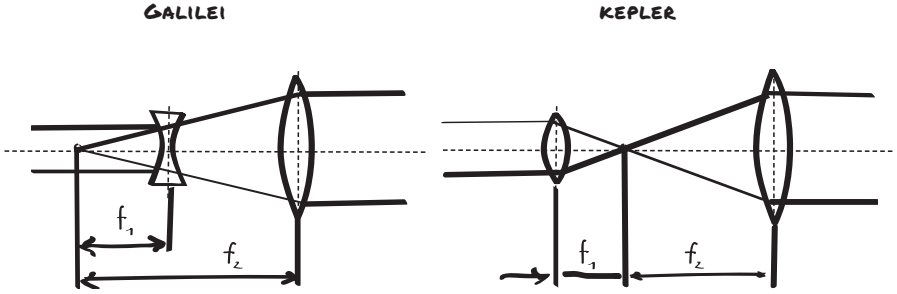


Figure 6: Systems for parallel beam expansion: Galilei (left) and Kepler (right)

As you can see in Figure 6 using a Kepler telescope a focal point is created between the two lenses. If the laser is strong enough even the air can be ionized and forms a plasma in that point, which can be clearly observed as a bright white-purple spark of light. To avoid losses of laser power, Galilean telescopes are more frequently used (Figure 6, left). Of course, you can use it in both directions, making a raw beam wider or smaller, keeping it parallel before and after the telescope. Now think that you want to increase the laser fluence. A plano-convex lens will sharply focus the light to the highest fluence (e.g., to increase productivity during laser ablation), and the fluence will be higher the larger your raw beam diameter is (better focusability). And the shorter the focal distance (the supplier sells focal lenses with fixed focal distances, very typical are 50, 60, 100, and 300 mm) the sharper the spot. But sharpness also goes along with divergence around the spot, which is high fluence gradient before and after the focal plane. This will increase the required precision of target positioning at a defined distance and would make re-adjustments needed once the target gets thinner. Very often, we are quite happy with 60 or 100 mm for laser ablation synthesis of colloids. When you read papers on laser fragmentation or laser melting, often very long focal distances (e.g. 300 mm) are used in order to minimize fluence deviation (sharpness) along the beam path in the liquid. Hence, the thicker the liquid (e.g., 10 mm) you pass during laser fragmentation or laser melting of suspended particles in liquids, the longer the focal distance of the lens should be to allow similar condition everywhere along the irradiated volume. For this situation, the Galilei telescope is a good deal, as it allows to conveniently adjusting the fluence while keeping the beam non-divergent so that the fluence is always constant no matter where you put the cuvette.

Compared to a 'standard' lens as described above which focus a laser beam on a spherical area an f-theta lens allows focussing on a larger planar surface (Figure 7).

This enables a steady focusing in one plane independent of the incoming position of the beam on the lens. For instance, in case of LAL a focal point will be always on the flat target and the same energy density reaches the target. This is very useful if using a scanner system, where the beam is always scanned and thus moves in several positions on the lens. This leads to a minimized fluence deviation when scanning the beam by a galvanometric laser scanner, in particular at short (< 100 mm) focal distances and large (> 1 cm²) target areas.

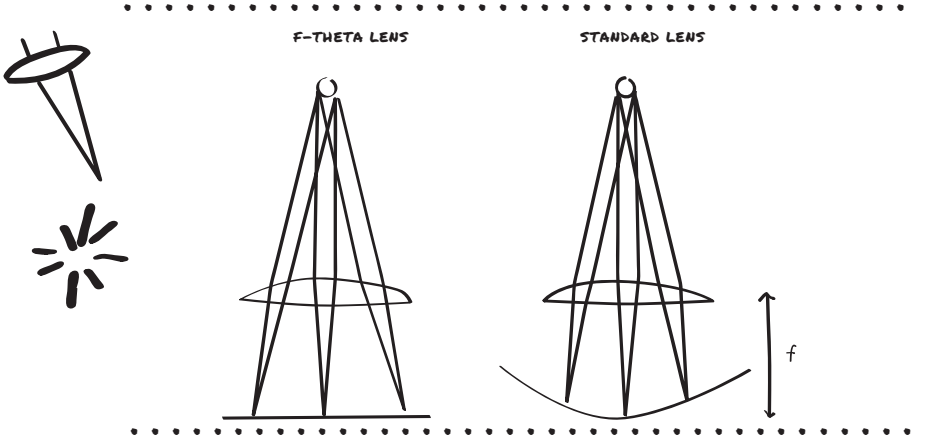


Figure 7: Comparison of the focal position using an f-theta (left) and a standard lens (right).

If you are working with lasers, you cannot avoid using mirrors. Mirrors are useful to reflect the laser beam in a desired direction without changing the position of the laser beam source. But, here you have to note a few things when working with mirrors for instance in combination with high-energy lasers. In all cases, you need to make sure that your lens and mirrors are suited for the working conditions you want to apply. Important questions you need to answer before using the optical component device by checking the manufacturer specifications:

- Is the wavelength you are planning to work at suitable for the optical component?
- What is the damage threshold of the optical component at the wavelength and pulse duration you plan to work at?
- Does the intensity you plan to work with exceed the damage threshold stated in the optics catalogue of the supplier?
- Are the optics suitable only for CW or pulsed lasers as well?

The story behind these questions is that optical devices are sealed with an optical coating, which is optimized on a specific wavelength regime. Hence, it is

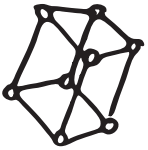
important to check whether the devices are suited for the given wavelength to make sure they even perform properly. Secondly although the optical devices are made of glass (e.g. BK7 or Quartz glass) a little amount of the laser energy (<0,5%) will be absorbed, depending on the wavelength and the type of glass. Therefore, there is a laser intensity (peak power per raw beam area irradiating the mirror or lens) at which the glass will get damaged which is specified by the manufacturer as the damage threshold. Making sure that the working conditions are in the specification of the optical devices is essential for a successful ablation (ask the provider for information on suitable wavelength, maximum raw beam diameter, and threshold fluence).

You can differentiate between two main different types of coatings. Shiny metals such as gold, copper or bronze have been used as mirrors since ancient history. Nowadays glass is coated with a tiny layer of metals such as silver or alumina. The reflectivity of metallic coated mirrors is determined by the physical properties of the metal. Using mirrors with a metallic coating are quite cheap but less resistant to high laser energies, thus dielectric coatings are mostly used to synthesize particles by LAL, where strong laser beams are common.
By using dielectric coatings defined optical interference between the reflected and transmitted light can be set and the reflectivity can be precisely adjusted by the thickness of the coating layer. Each layer reflects the beam and weakens the intensity that reaches the next lower layer. You already know that absorbing laser energy lead to ablation of a substrate, the same can happen to glass.
To avoid this, always use anti-reflectance coated glasses. We tested some glass windows in our laser setups by measuring the laser power transmitted through an empty chamber sealed by 2 glass windows. Depending on laser pulse energy, the power loss was 10-50%. Imagine this. Not only the productivity is compromised by the power loss, but also there will be dangerous multiple laser light reflections propagating in the lab! In the best case, only the window heats up and will break after a while (hoping that there is not inflammable liquid behind). Coatings for windows and mirrors (look for the label “HR-coating”) are standard supply today in any optics and laser component shop.

My supervisor wanted me to maximize the productivity of PLAL by using a high repetition rate with the highest possible laser power. Soon I realized that the ablation sound becomes quitter, which is an indication of low ablation rate. That was strange because I was expected to achieve the highest productivity ever. After turning the laser off I’ve noticed that the lens of the scanner looked opaque. Obviously, the laser-induced threshold damage was exceeded and the expensive lens was now broken.”

Toshimitsu Sato

Are you then on the safe side? Yes, but only when you avoid dust particles to settle on the optics. A dust particle is an absorbance center and will cause spikes at the optics that again act as even more effective collecting centers. When you shut the laser down, put coverings (simple plastic bags) over each mirror. When you notice that laser power is lost after a while, it's very often the optics that need to be cleaned. Imagine a single mirror "consumes" only 5% and you have three of them aligned, what you get at the end is the cumulative loss, mathematically 0.95 x 0.95 x 0.95 = 0.85 energy yield. Small thing, big effect, and easy to avoid. The thickness of the layer is in the nanometer range and thus the coatings are sensitive to any scratches. You need to be careful not to destroy the layers if you want to clean the mirrors. For this purpose, specific optical cleaning supplies are commercially available. These will be useful for many years for the whole laser lab. For instance, extremely soft tissues can be wetted with very pure methanol and glided carefully over the lenses or mirrors without scrubbing it. If you just need to remove only the dust on your optics air duster that blows the dust away are suitable.



If you are using lasers with high energy per single pulse for your experiments, such as nanosecond lasers in the 1-100 Hz regime, another danger exists for your beloved optics, that are back reflections. These occur especially with metallic targets, or when placing glass windows on the cell, and are due to the fact that the focus of the reflected beam can be close to the focusing lens or to the mirrors. You have two ways to reduce the problem. That are using a lens with shorter focal distance and tilting the optics so that the reflections will spontaneously extinguish in air, instead of ablating your optics. According to some laser manufacturers, this is also indispensable to avoid irreversible damaging of your LAL best friend, the laser itself, by creating holes in the laser crystal.

For this day laser fragmentation was scheduled. So I started with the setup of the funnel and the lenses. Funnel, done. Cylindrical lens, done. Damn it... which way round do I have to set up the telescope again? Keeping my supervisor's advice in mind I remember being able to tell if the telescope is set up the right way by looking at the laser beams' shape during the alignment. After turning the laser on it suddenly became very noisy for a moment. I was startled and realized that I didn't verify the laser parameters and shot with high power. I readjusted the parameters and turned the laser back on. But why wasn't any beam getting out? After briefly looking for the cause I noticed that one of the telescope's lenses was broken. Discovery: the telescope was the wrong way round. Thus my workday came to an end early.

Christoph Schmidt, Essen

STEP 6: CHOOSE YOUR ABLATION SETUP

Besides the simplest setup described in Step 1, several experimental configurations are possible to perform particle synthesis by laser ablation in liquids. To ensure uniform mixing of the dispersed particles in the solvent, a magnetic stirrer can be added. For a high utilization of the target, the vessel or a target holder can be moved during laser ablation. Some examples of the setups with a different kind of sample holder are sketched in Figure 8.

Of course, it is also possible to penetrate a vessel with the laser beam and for instance to work with closed setups with flammable solvents. In that case, you need to be careful not to damage the glass. You should carefully choose the distance of the laser focus, which is not to close to the glass so that high fluences are reached only in the proximity of target and not on the glass window. For a higher stability, it is recommended to use quartz glass (like a cuvette) or a self-designed chamber with a coated window that enables to minimize surface reflections and thus any loss at the optical surfaces due to reflections.

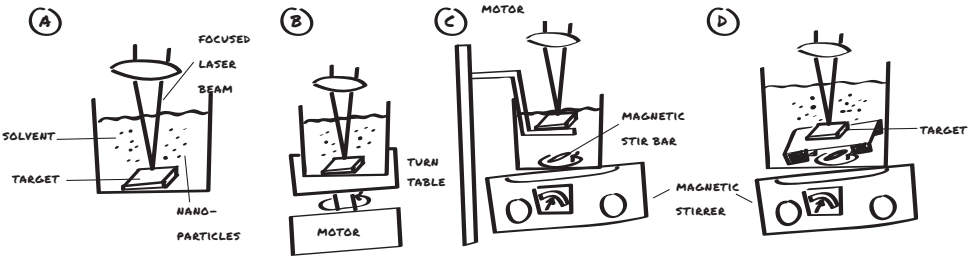


Figure 8: Pulsed laser ablation in liquids using different setups in a batch operation mode.

Thinking about long-term particle production, where a large amount of particles and solvent are required, a continuous ablation process can be quite useful.

Sounds complicated, but in fact, it makes multi-milligram scale synthesis easier and more robust. Here, the solvent flows over the target and carries the just formed particles (and the nasty bubbles) away from the ablation zone and collects the product in a storage vessel (Figure 9). More detailed setups are shown in Chapter II. As long as the target is not perforated you will have a continuous ablation process, however, if you need to change a thin target often this can take longer than in a batch process. Most importantly, it enables

a so-called steady-state, where concentration does not change with time in a given volume everywhere downstream. You may imagine that steady-state synthesis conditions favor steady state particle growth conditions, improving reproducibility. A gold plate could feed a laser ablation process for many hours, and all you have to do manually is the taking the vessel at the end, it is like tapping nanoparticle colloids (*see: http://youtube.com/nanofunction*). Since time is money, and being lazy is linked to creativity (in particular for sunshine-affine Italian guest scientists), researchers also study how to optimize the process by wire ablation.

This technique enables a fully continuous synthesis of nanoparticles. For LAL the wire may be either just dipped and fed into an ablation chamber or run without a chamber being surrounded by a concurrent liquid jet. In the setup, a motor enables to move the wire continuously through a cannula (a tip broken from a syringe) with a desired velocity (Figure 9). The setting of the feeding speed then should be adapted for the entire wire thickness is ablated. By this method, a higher productivity can be achieved due to different cavitation bubble dynamics compared to the processes involved during LAL of a bulk target. The mass that is ablated can be easily pre-calculated and pre-set by knowing the length of the wire that is ablated. Note that many metals are available in wire shape at no extra costs, and a roll of silver wire can run for a long time. A disadvantage of the ablation of a wire is that process stability is crucial. For example, large micro to millimeter nuggets can be removed from the wire if the feeding rate is too high, and thus not the whole material can be converted into a nanoparticle colloid. Hence using this setup is more convenient for experienced operators, because you have to optimize your experimental setup very precisely.

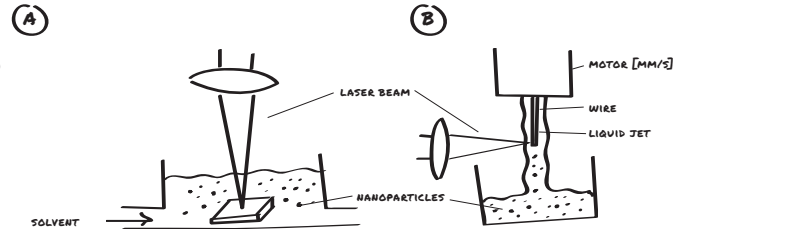


Figure 9: Pulsed laser ablation in liquids using a continuous flow system (A) with a wire (B).

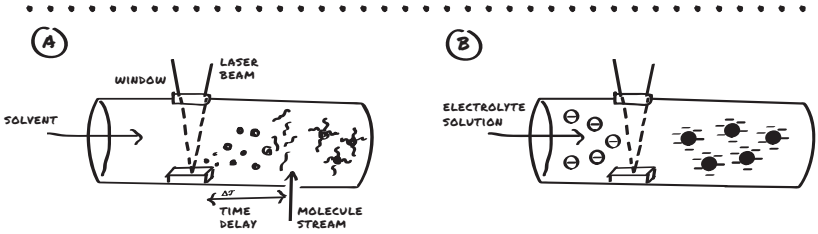


Figure 10: Continuous ablation with the possibility of particle property control by (A) delayed conjugation and (B) in the presence of electrolytes in a flow-through chamber.

For many applications such as biology or catalysis, small nanoparticles are desired. In case you want to do laser ablation in a continuous operating mode and tuning the particles' properties by adding molecules or specific ions, you can construct a chamber with inlets. There you have a reservoir where you add, e.g. surfactant, albumin, or salts. When pristine particles are synthesized by LAL in liquid flow, they tend to grow on in the stream. In case a quencher molecule is added, this growth stops immediately and the particles become smaller.

This can be utilized by using a flow-through reactor where quencher molecules are added after certain time delays. The time delay within the reactor can be controlled by the flow rate and by the distance between the point of particle generation and the point of quencher addition (for more detailed information please check Chapter II). Then the size can be controlled by the downstream position of the delayed injection (Figure 10 A) or in situ by adding electrolytes or stabilizers to the feed (Figure 10 B).

Overall, for experiments where you only need to do ablation for a couple of minutes or need to change the target often, the batch chamber is in most cases the best choice. It is always worth to add a mixing effect (magnetic stirrer, fast rotating target, ...). If you have to run laser ablation longer and have thicker targets, add a pump to create a continuous flow. Depending on the concentration you want, the flow is easily switched to run recirculated or run in one path. One path flow creates a steady state for particle formation, increasing reproducibility.

STEP 7: FIND THE FOCUS

You may imagine that irradiating something with a laser beam e.g. such as a laser pointer, does not automatically lead to ablation of a material. The criteria for doing so include an adequate intensity of a beam. Using a high-energy laser and focusing a beam on the target enables a high local fluence, due to the bundled energy in a tiny spot area. In this manner, the ablation threshold can be overcome and the solid can be vaporized. To focus the beam onto the target a convex lens is required. The focal position can be found by moving the target or the lens, which has a certain focal length. But how does it work, to 'find' the focal position? In the field of laser materials processing many parameters are used to describe the laser beam, focal position, etc.. Since we do not want to complicate the first steps, let's keep it simple.

You knew that you have to use your hands and your eyes for pulsed laser ablation in liquids, now you learn that you can use your ears as well! The probably simplest and fastest possibility to position the focus onto the target is by hearing the intensity of the sound, which a laser causes if ablating a material. If the laser beam hits the target with an appropriate intensity, it starts to ablate the material and makes noise. At the position where you hear the loudest noise, the highest ablation rate can be affirmed due to the highest effective fluence at the focal position. The sound amplitude depends on the bubble cavitation. And the larger the bubble the louder its noise. It is useful to know that as ablation rate is proportional to laser fluence, the cavitation bubble volume is proportional to laser fluence as well, in particular at high fluences. Hence, the sound amplitude is a good measure of productivity. So probably, your boss won't hear a sound, while you will hear more precisely high-frequency noise just because you are younger than he or she is. If you don't believe it, just test how old your ears are by hearing to several frequencies on the internet. You'll find a lot of different self-tests at YouTube, simply by typing 'how old are my ears'.

Hence, the loudness but not the tone of the laser music is important while monitoring if everything is running stable and the target is still in the focal plane.

Figure 11: Sketch of someone, who is listening to "laser music".

To find the focus in a way independent of your ears, a microphone can be used. The magnitude of microphone signal linearly correlates with the ablation rate. You don't need a special microphone system if you want to keep your approach simple. In case you want to make sure the productivity is high, you can easily do that by using a basic 'SingStar' microphone e.g. coupled with a readout software.

I planned to do some UV-Vis measurement and I washed a cell. After that, I wanted to dry it as fast as possible so I used a nitrogen blower. Since I didn't think of the gas pressure and hence I underestimated it so that the cell blow up in my face and broke. It was really high pressure!

Mizutaru Tsukasa, Tsukuba

Back to other possibilities for an appropriate focal positioning, you can use an indirect but very result-oriented method to find the properly focal distance to the target. That is by measuring the productivity. You can provide a series of ablation experiments, where the distance between the lens and the target is varied. The obtained colloids, which are synthesized at several positions of the lens to the target, can then be analyzed by UV-Vis spectroscopy (see Chapter V). Oftentimes (depending on the material that is ablated) the extinction of the spectra at a certain wavelength correlates with the particle concentration. E.g., in case of gold, the extinction at 380 nm increases with a higher production rate. If there is no specific peak, use the intensity of the shortest wavelength in the spectrum where the solvent does not absorb. Or integrate the whole spectrum. By varying the position of the target relative to the lens, you can find the maximum of the extinction and by this way the focal distance. Alternatively, just weighing the target before and after ablation works as well, which is more appropriate for several materials. An important thing to note is, before doing any laser ablation tests, the laser spot should be located only on the target and not e.g. on the ablation chamber or the window. For this, first position the laser beam with a low energy or use a positioning laser, which is directly integrated into some laser systems.

Once you know which materials you need and how to find the focal position, you should be able to synthesize nanoparticles by pulsed laser ablation in liquid. What do you think will happen if you perform laser ablation of the target for a while - let's say 5 minutes (note that the time depends on the repetition rate, pulse duration, fluence and so on)? You'll be able to efficiently drill a hole into your target, however, this is not what you call an efficient use of material and you will also ablate the material that is behind the target and get impurities in the colloid. In order to avoid a fast hole-formation and thus contaminations, two approaches are possible. On the one hand, the target can be mechanically moved

e.g. by circular movement of the whole chamber. By this way, you may exploit a larger surface of the target that makes it possible to use it for longer ablation time without the need of replacing the target.

A much more elegant but also more expensive solution is the continuous shift of the laser beam on the target, either by using a motor stage moving the chamber or by using a rapid scanner system moving the beam. By using a scanner system (it's always faster than most axis systems) the cavitation bubble can be spatially bypassed and thus a higher ablation rate can be achieved since the laser beam is not shielded from the bubble. A scanner system can create different ablation pattern. The simplest form of scanning patterns are stripes (Figure 12 B). However, if one strip is scanned to the end, the mirrors cause a certain delay time by moving back to start the next strip. During this period, no pulse is coupled and thus the productivity is influenced. For example, in case of a 50 mm long strip and a scan speed of 1 m/s, the scanner needs 0.05 s to move this distance back. By working with a repetition rate of 5 kHz about 250 pulses cannot be coupled in this time. Second, unless you have an advanced "on the fly" scanner, the software will accelerate and decelerate the beam guidance mirrors at the beginning and at the end of the line, respectively. This will cause deeper ablation there, and again the target is not ablated homogeneously in the whole area, making early target replacement necessary. Thus it is more efficient to move the laser beam continuously. For example, by moving the beam 50 mm to the right, 50 mm downwards and then 49 mm to the left generates a rectangular spiral pattern (12 C). However, in that case, only one mirror is moving in each direction, which again causes a delay before the other mirror reacts (and again, deeper ablation at these turning points, and it's the same with motor stages). Alternatively, scanning the laser beam in an Archimedean spiral pattern with 'rounded corner' has proved worth and preferable shape, which enables to exploit as much as possible of a target per time and assures maximal target use (12 D). Also here at the end of the spiral one del ay occurs if the scanner had to move to its initial position. The highest utilization rate then would be by using a Fermat's spiral pattern, which is unified within itself and allows a continuous movement of the laser beam (12E). Anyway, for an optimal utilization of the target material, the target needs to have the form of the scanning pattern, e.g. in case of stripes a rectangular target is the best and a round target shape can be used with a Fermat's spiral. A set of annex spirals can mimic a rectangular shape.

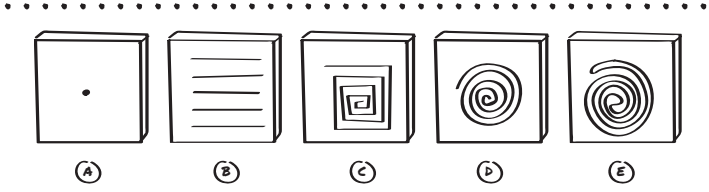


Figure 12: Scan pattern on the target for laser ablation in liquids: (A) Without scanning, (B) Stripes, (C) Rectangular spiral, (D) Archimedian spiral, (E) Fermat's spiral.

STEP 8: SPICE UP YOUR NANOPARTICLES

A huge advantage of laser-generated nanoparticles is the exclusion of toxic substances or by-products that adsorb onto the particles' surface, since chemical precursors are not needed. Depending on the laser parameter, pulsed laser ablation in pure water very often leads to polydisperse particle sizes or even multimodal size distributions. To spice up your nanoparticles you can use (either micromolar saline solution for noble metals, or) several post-treatment methods. Centrifugation is such a method to play with the size distribution (compare Chapter V). The cut- off diameter is the particle diameter value that defines the intended fractionation size level between supernatant and sediment. By using a combination of Svedbergs' equation and Stokes-Einstein relation (sounds smart, isn't it?), a pre-defined cut-off can be calculated, which is practically quite useful:

$$\Delta t = \frac{9 \cdot \Delta h \cdot \eta_0}{2 \cdot r_p^2 \cdot (\rho_p - \rho_0) \cdot RCF \cdot g} \quad \text{with } RCF = \frac{4 \cdot \pi^2}{g} \cdot r_c \cdot n^2$$

Δt = time

Δh = height or filling level of the centrifuge tube with the colloid [m]

η_0 = dynamic viscosity of the solvent (for H_2O ~ 0.001 Pa·s at 20°C)

r_p = particle radius [m] at which the two size fractions shall be separated

ρ_p = density of the material to be centrifuged [kg/m^3]

ρ_0 = density of the solvent [kg/m^3]

RCF = relative centrifugal force (about 4 x r_c x n²)

g = force of gravity, 9.81 m/s^2)

r_c = centrifuge radius [m]

n = turning speed in rounds per second [s^{-1}]

A software for calculating this can be found here:

http://wwwdisc.chimica.unipd.it/vincenzo.amendola/pubblica/sedtrj.zip

Using this formula, the centrifugation time can be calculated if parameters such as particle size (intended cut-off), nanoparticle material density (take the value of the bulk), viscosity of the solvent, rotation speed of the centrifuge and dimension parameter of the centrifuge are known (rotor type that holds the vials). Due to the faster sedimentation of larger particles compared to smaller ones, different fractions can be separated into desired particles sizes by taking advantage of centrifugal forces. In this manner, after centrifugation, the largest particles or agglomerates are located at the bottom (sediment or pellet) and

small particles (supernatant) at the top of the centrifugation tube shown in Figure 13. LAL of gold in water (with some sodium chloride for size quenching) is a good example. It yields quite a good particle size distribution centered at 10 nm in diameter, but there is always a larger fraction around 20-50 nm that you may want to get rid of. Considering the parameter in Fig 13 for gold nanoparticles with a cut-off diameter of 15 nm (radius 7.5 nm!) you would need to centrifuge for ~9 minutes. This is calculated exemplary for gold in water, and a standard tabletop centrifuge with 18,000 rounds per minute (300 per second), a rotor with a radius of 8.5 cm and a liquid layer of 3.7 cm.

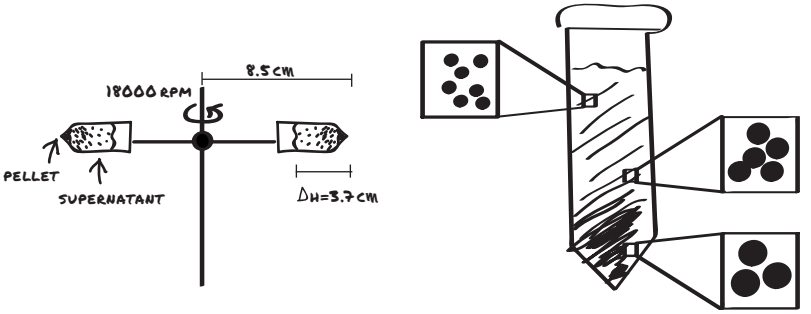


Figure 13: Particle size separation by centrifugation.

Even though size separation by centrifugation is a quite simple method, one main disadvantage is the loss of mass if only one fraction is needed (the pellet cannot always be redispersed). Typical loss of a bimodal colloid of laser-generated noble metal nanoparticle dispersion in water, if the pre-set cut-off is in the range of 10-20 nm (to get rid of the large fraction by-product of LAL), is about 30-50% of the mass. It will take only 5-10 minutes with typical benchtop centrifuges (far longer centrifugation times should be avoided anyway, unless you have a centrifuge or ultracentrifuge that cools down the colloid). Finally, you will end up with a monomodal, often monodisperse colloid, free of any additive!

The size of nanoparticles can be also tuned by re-irradiation of a colloid with pulsed lasers. At high laser fluence, post-irradiation of nanoparticles leads to fragmentation of nanoparticles and thus to smaller sizes and narrower size distributions. This Laser Fragmentation in Liquids (LFL) generally benefits from a wavelength at which the particles can absorb significant light, however laser fluence should be still efficient. As a rule of thumb: the product of absorption at the laser wavelength (measured in the UV-Vis spectrometer) and the pulse energy at that wavelength should be maximal.

For very small nanoparticles, surfactants can be used during laser fragmentation. But also re-irradiation of nanoparticles in totally ligand-free environments or micromolar saline solution facilitates size reduction by high laser fluence laser fragmentation. For noble metals in water, maybe adding 0.1-0.3 mM NaOH or carbonate buffer (there is CO_2 in water, anyway) could be a worth a try.

Re-irradiation of a colloid can be followed in different setups. The simplest approach is laser fragmentation in a batch chamber, where a colloid is irradiated from above or from the side (Figure 14, left). Similar to this, post-irradiation can proceed in a continuous operation mode, in which a colloid is operated in a flow-through mode and is irradiated by a pulsed laser light (Figure 14 middle and right).

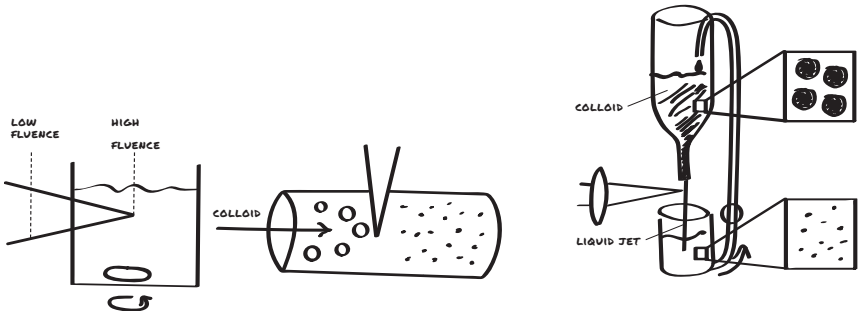


Figure 14: Set-ups for pulsed laser post-Irradiation of nanoparticles in liquids.

In Chapter III you can find more details about choosing the right parameter and set-up for laser-induced downsizing or melting of powder suspensions. How to influence the particle size by using ions or other additives such as biomolecules will be explained in Chapter VI.

Here described strategies for controlling particle size distributions are basically post-processing methods, where size distributions are changed after the laser process. However, there are also in situ methods where particle size distributions are changed during the process. Here, emphasis will be on in situ methods which are easy to implement during basic laser ablation synthesis.

A) Influence of ablation time: Particle size may be changed by altering the ablation time. Even though the effects are not very strong (usually changes of a few nm can be achieved) this strategy is very easy to use. As a rule of thumb, the particles become larger with increasing ablation time. The reason for this is pretty obvious. The final particle size is driven by

ripening and growth processes in the solution. In case the concentration of particles is higher due to long ablation times, these growth processes are more pronounced, yielding larger particles (unless you use high repetition or high-intensity ultrashort-pulsed lasers that cause downsizing by laser fragmentation during laser ablation). Also, high concentration at prolonged ablation time attenuates the laser beam, bringing the fluence above of the target into the melting regime. If you want to avoid concentration supersaturation, simply use liquid flow and drain the colloid off the chamber.

B) Influence of the solvent: laser ablation experiments can be conducted in multiple solvents. The critical parameter controlling particle size, in this context, is the in situ stabilization of nanoparticles by the liquid. The stronger the interaction, the smaller the particles you obtain. For example, the ablation of gold in acetone yields smaller gold nanoparticles than in water, probably due to an efficient adsorption on the particle surface of molecules present in the liquid.

C) Influence of pH, ionic strength and stabilizing ligands: as a general rule of thumb, whatever stabilizes your nanoparticles in solution usually also induces a size quenching effect. As described previously, stabilization can be efficiently done by adding ligands and low salinity electrolytes with highly polarizable anions. Furthermore, it may be concluded that a pH far from the isoelectric point is good for stability. So you can simply use the same approach to efficiently reduce both the width and the average size of your particle size distributions. Typical additive concentrations are in the sub-millimolar range for size quenching.

That's enough for a brief overview start, as further insights are given in the specific chapters on size control, as well as in the case study examples.

STEP 9: NOW, JUST DO IT

You are entering the lab and see this 'magic box', which is more powerful than it may seem. You don't need to be afraid of powerful lasers, but you should treat them with respect. You need to take the safety aspects into account, which are described in detail in Chapter VII ("Stay alive"). By this, you'll have good precondition to safely operate with lasers.

The day should start with all preparation steps for the set-up. Sometimes experiments can fail, if only a screw is missing, thus it is worthy to make a list with the required preferably on the afternoon before you actually intend to use the laser (Figure 15). It may be satisfactory having everything waiting for you in the lab and get very productive already right after your morning coffee.

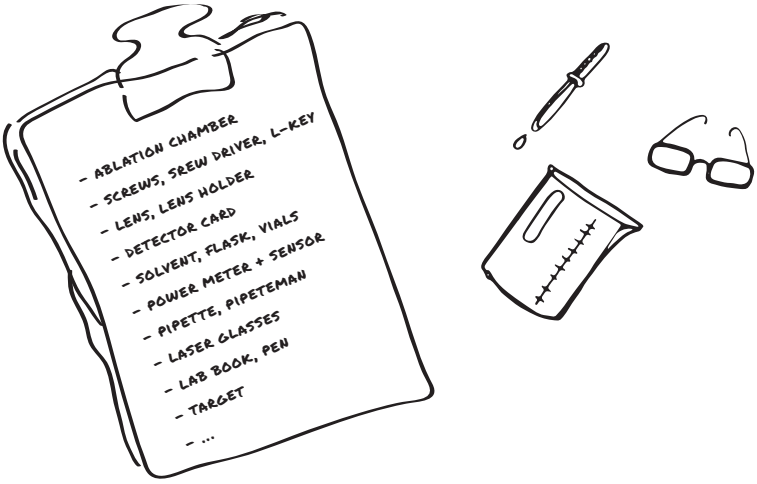


Figure 15: Check-list for preparing pulsed laser ablation in liquids in the afternoon before your day in the laser synthesis lab.

You need to build up the optical components to align the laser beam to the desired position. All optic compounds should be free of any defects and dust, to ensure a high yield of light reaching the target. To locate the laser beam, a detector card can be used (as your laser is invisible through safe glasses), where a photosensitive region allows viewing the light spot. Before use, such a card requires "charging" with visible light, and for optimal brightness of the beam, one must move the card around because the emission from the card is not persistent. If you use a laser scanner, align the laser beam with a low intensity into a scanner optic and then onto a thermal laser power sensor, which is connected to a power meter. Note, that you should measure the power only in the raw beam or out of the focal plane to avoid a destruction of the sensor.

Point out that you have to check the fluence of the raw beam directly at the source and – most relevant for the synthesis - behind all mirrors (right before the focusing lens) before you start in order to avoid potential fluctuations in the laser fluence. Once you have directed the raw beam spot on the sensor, you can slowly increase the laser intensity and measure the power that you intend to use for laser ablation.

Wait a couple of seconds for the thermal equilibrium of the sensor (and remember, many lasers need a warm-up period of 20-30 minutes to reach a steady state of output power).

Using the example of laser ablation in a flow-through chamber, the preparation steps are briefly described. First, the mass of the target should be determined, hence the ablated particle mass and with it the colloidal mass concentration can be concluded by weighting the dry target before and dried target after ablation. The ablation chamber should be prepared by embedding the target and screwing all component parts. After placing the chamber in front of the scanner in a distance of the focal length, very often, because of liquid refraction, the point of maximal productivity is behind the focal plane in air (or the focal distance the lens supplier has provided), e.g., at 1-2 mm longer distance. Tubes should be connected with the chamber and the pump. A certain volume of the solvent should be filled in a flask and the tubes should be dipped into the solvent. Before starting ablation, the liquid should be passed through the chamber and recycled back to the flask until no bubbles can be observed to prevent ablation in air or bubble scattering. Be sure, that no solvent is leaking during the flow.

The target can be ablated in a continuous process, where the solvent is pumped through one flask to another collecting vessel. If you want a highly concentrated colloid, quasi-continuous ablation (so-called semi-batch) can be carried out by cycling the solvent during laser ablation in the same flask. It should be noted, that by this mode of concentrating operation particles can be re-irradiated more intensely by the laser beam. In most setups, you will have some post-irradiation, and no flow setup is fast enough to cope with kHz repetition rates. But flow brings everything in a controlled steady state.

Now you can align the laser beam through a scanner optic onto the target (or directly on a moving target) by directing a laser beam with a lowest possible energy. Some lasers have integrated an additional positioning laser, which helps

Once we performed damage threshold experiments on silicon in water with femtosecond pulses. First, we fired two markers of hundreds of shots using output power maximum and we have not seen any flash! We believed that ablation in water is hard to see and especially when you take goggles with only 10% light transmission. We decreased the power and continued with pulse shooting. We made about 40 spots using pulse energies near expected damage threshold. Then I wanted to block the beamline for safety reasons before going to the microscope and I saw that the beamline is already blocked!

Marek Stehlík, HiLASE Centre, Dolní Břežany, Czech Republic

to adjust the beam on a target object. Oftentimes your laser is equipped with a shutter, which can be controlled by a software or opened and closed manually. After you have found the optimal set up parameter, and you are sure that the beam hits only the target, you can start to pump the solvent and increase the laser energy.

Now the time has come when you can use your knowledge on finding the focal position and continue to perform systematic studies on laser synthesis, size-tuning, and stabilization of colloids.

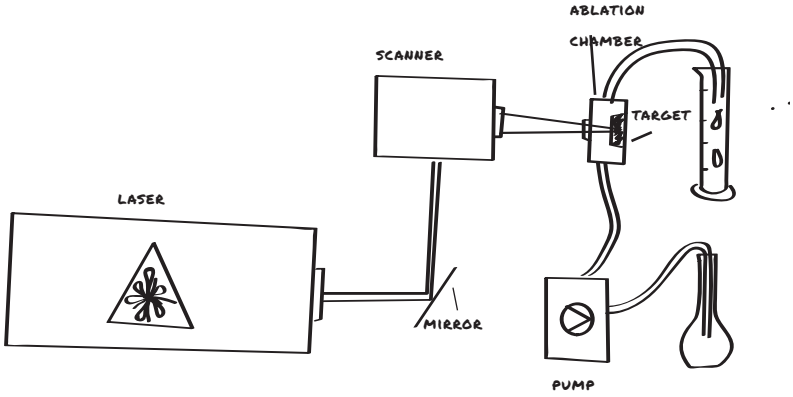


Figure 16: Optimized experimental set-up for pulsed laser ablation in liquid using a flow-through chamber and scanner.

In the beginning, many mistakes can be made and of course, you learn from all of them. However, there are some typical mistakes that can be avoided. The list of lessons learned by us may help you to prevent unnecessary waste of time and difficulties shown in the following list:

NOT-TO-DO LIST

- Forgetting to weight the target
- Working without laser goggles
- Leaving shutter closed, when trying to find the beam
- Aligning the beam with maximum laser energy
- Leaving shutter open, before pump was started
- Adjusting the beam out of focal position
- Ablating till hole formation at the target causing ablation of the chamber material
- Setting optics incorrectly (maligned optics or covered with dust)
- Sealing ablation set-up (chamber, pump) insufficient
- Irradiating in open flammable solvents or leaving combustible material near laser set-up

You can find all the things mentioned above in the following that can help you to synthesize your first particles by laser ablation in a liquid:

PREPARATION

- ✓ Do your checklist with all necessary objects for your set up
- ✓ Bring all the things together the day before you start synthesis
- ✓ Check your optics and clean it if necessary
- ✓ Weight your target
- ✓ Prepare the solvent

SET UP

- ✓ Build up your set up (optics + chamber)
- ✓ Remove all flammable items near the laser beam (solvents, paper, packaging, ...)
- ✓ Put your laser goggles on
- ✓ Check laser alignment, use detector card and low power
- ✓ Allow laser warm-up and measure the laser power (raw beam!) after all positions (behind the laser source, lenses and mirrors)
- ✓ Position the chamber and fill it with solvent

LASER ABLATION

- ✓ Align the beam on the target
- ✓ Find the focus
- ✓ Start to produce your particles
- ✓ Enjoy

Good luck and may the force be with you!

CHAPTER II

GET MORE: PRODUCTIVITY

II

In the previous chapter, we used the power of light to produce very pure and tiny nanoparticles by pulsed laser ablation of different materials in liquids. With this fancy setup, you will be able to delve down into the amazing playground of nano-sized objects. However, exploring this small universe doesn't necessarily mean being content with tiny quantities. In case we want to characterize or even apply our nanoparticles in some novel material combinations, it is not enough to end up with small amounts. Therefore, let's grow the scales and make small things huge – not just in size but also in quantity.

STEP 1: HOW TO DEFINE THE NANOPARTICLE PRODUCTIVITY?

To start with our scale-up procedure it is important to initially think about how we want to measure productivity and consequently find a proper definition for the expression "nanoparticle productivity". Does "yield", "efficiency" and "productivity" have the same meaning? Yield refers to the product, e.g. 80% after centrifugation or "100 mL with 1 mg monodisperse gold". Efficiency needs something to refer to, so that there is often the effort you put in for the synthesis in the denominator, e.g. mass you obtained per laser energy (mg/J). Productivity has the unit of time in the denominator, e.g. mg/h. The meaning of productivity seems obvious, but once we look closer in this topic there will be several possible ways to define the nanoparticle productivity.

Let's begin with the most apparent choice and define the productivity as the total amount of ablated target mass per unit time. It can be obtained by simply weighting the target before and after an ablation interval. The precision of this gravimetrical technique is only dependent on the chosen time of ablation and the scale that is being used. Evidently, the significance of your results will be enhanced, if an extended timespan is selected. In general the ablation time should be chosen such that a single ablation experiment will last at least 5 minutes while the minimum amount of ablated target mass should not fall below 3-5 mg. The latter limitation is necessary in order to keep the relative weighting error lower than 5% (following a typical error of about 0.1 mg for a standard analytical balance). Additionally it should be kept in mind that most lasers do need about 20-30 minutes pumping to reach a stationary power output (new lasers maybe 2-5 minutes). Consequently, the laser beam should be aligned into a beam trap or onto a power meter before starting laser synthesis, until the stationary power output has been reached in order to maximize reproducibility.

Working with intense laser radiation is a task, which requires concentration due to the risks of burning ones skin and eyes. Still, there are easier ways to get hurt. When checking lenses and mirrors one should make sure not to bend over in a way that leads to hitting the edge of a safety screen and suffering of a bleeding ear afterwards.

Alexander Letzel, Essen

Now you may have yielded 10 mg in 15 minutes, which gives a productivity of 40 mg/h. Sounds good. But is it better than literature? Is it what you expected? Of course, productivity will depend on the power of your laser. So productivity values shall be communicated along with the laser output power employed to make it. The unit for this "power-specific productivity" is mg/hW, which allows better literature comparison. A value of around 10 mg/hW is quite good. Note that productivity scales with density, so Platinum or Gold will give about the double productivity compared to Silver.

As easy and convenient as the gravimetrical approach may seem, it always includes interrupting the ablation process making time-resolved productivity studies impossible. However, in case some knowledge about the temporal alteration of the productivity is required, the second definition of productivity may be introduced. In contrast to the "gravimetrical productivity", let's additionally define a "visible productivity" (or "extinction productivity") considering the amount of colloid being detectable e.g. by optical measuring techniques. You may be thinking – shouldn't those two definitions be the same? Bear in mind that nanoparticles may not be stable and precipitate or even dissolve in the liquid. It may also be required to exclude generated micrometer-sized particles from the productivity definition as they often aren't the desired product. Therefore, it is obvious that both definitions may differ significantly depending on the chosen metal-liquid system and the required nanoparticle specifications.

In order to quantify the "visible productivity," the nanoparticle concentration may be monitored by UV-VIS absorbance spectroscopy over time. With this method, the concentration of a colloid can usually be calculated considering the extinction of incident light over a given path length. More about this type of detection will be given in Chapter V. As the ablated mass always gets converted into particles with 100% yield, both the "gravimetrical" and the "detectable" definition are equal as long as the particles are sufficiently stable over some period of time. For the ablation of most noble metals, this statement holds,

however when ablating metal oxides the “detectable productivity” may be lower than the “gravimetrical productivity” as nano-/ microparticle precipitation is often observed in those systems.

The final question about which of the two definitions is to be chosen may arise. To answer this question: it depends on what you’re interested in! The gravimetrical approach is easy to measure (and does not need material-specific calibration curves) but lacks temporal resolution and ignores precipitation and dissolution whereas the “visible productivity” definition gives rise to a well-defined time-resolved productivity profile while requiring more effort and knowledge about the used nanoparticle system. The latter will, however, become a very powerful tool when thinking about long term continuous ablation methods. However, it requires a calibration curve, to be done by simply diluting a colloid and find out which UV-Vis wavelength gives best correlation coefficient in the linear fit (don’t force it to go through zero) of the diagram concentration vs intensity. By knowing the different ways to measure productivity we are now fit to generate our first nanoparticles.

STEP 2: CROSS THE ABLATION THRESHOLD

Well, maybe this chapter should be called crossing the information desert without some refreshments in you back-pack but unfortunately, our sleeves will still remain unwrapped until we haven’t thought about the basic idea behind the laser synthesis of colloids. In fact, to generate some of our desired midgets it is required to reach the so-called ablation threshold already introduced in Chapter I Step 7. This threshold depends on a manifold of different properties. Next, to material properties, it is defined by laser parameters such as laser wavelength, laser fluence, and the pulse duration. Keep in mind - laser synthesis of colloids is like performing martial arts – so let’s consider the aforementioned parameters from the angle of e.g. Muhammad Ali. Let’s say the laser wavelength

I entered the laser lab and found the surface of the power meter significantly damaged, with a distinct ablation spiral. Obviously one of the students tried to measure the energy of the laser close to the focus during operating and scanning the ablation spiral.

Sebastian Kohsakowski, Duisburg

represents your striking precision, the laser fluence creates your punch virtue and the pulse duration is your quickness. Analyzing your opponent's weaknesses first, you want to precisely strike where it hurts most. For an ablation target, you'll find its weaknesses in its wavelength dependent light absorption. Choosing the right laser wavelength will significantly increase your impact. But maybe you can't choose a weak spot (i.e. fixed laser wavelength of your laser) on your own, it is obvious that you can always strike harder (increase laser fluence) to make up for that. Of course, your striking power is limited so you have to make use of your quickness (pulse duration) as well. A fast strike with a laser pulse of very short duration will surprise the target making it "bleed" and lose nanoparticles:

$$F_{theshold}: femtosecond\ pulse \leq picosecond\ pulse < nanosecond\ pulse$$

resulting in different time scales for the successively occurring heat exchange mechanisms. Basically, the target material will just be struck faster than it is able to react. This situation is roughly summarized in Figure 17. As you can see, there are mainly two different extreme cases: one when using a nanosecond pulse, and the other with a femtosecond pulse. The reason for the two extreme cases is a property of materials, which is the so-called electron-phonon-relaxation time. When the laser beam starts being absorbed the electrons get excited.

It's the same as if Muhammed Ali struck his opponent: in the first second after striking his opponent, he doesn't feel any pain. However once the brain of his maltreated victim realizes there was a strike, his quarry collapses due to KO. The same happens in the material when the excited hot electrons start to interact with the cold lattice, heating up the material. The delay in this heating process is the aforementioned electron-phonon-interaction time. The usual timescale of these is in the picosecond regime for metals. Subsequent on this interaction delay the target temperature increases while the electron temperature decreases until both, lattice and electron temperature are roughly the same. Now if you consider the first extreme of, e.g., a 10 nanosecond laser pulse, the pulse duration is far longer than the time required for the electron-phonon relaxation (Figure 17, middle). Therefore electron-phonon relaxation (several picoseconds) is already occurring, thus heating the target in the irradiated area, while the laser pulse is still continuing. Due to the increasing temperature gradient between target and liquid as well as inside the target itself, an additional heat transfer kicks in after a few nanoseconds, leading to heat conduction into the target and heat transfer (via the plasma plume) into the liquid while the laser pulse is still ongoing (Figure 17, middle). Now, from this argument, you may already imagine that this condition results in deep heating, finally resulting in vaporization and plasma formation, which is why this case is called hot ablation. The laser is still on when the plasma arises into the liquid, hence the incoming light is shielded and consumed by the plasma, lowering ablation efficiency (ablated mass per pulse or ablated mass per joule pulse energy) compared to ultrashort pulsed (often called "ultrafast") picosecond and femtosecond lasers. Going back to boxing, this nanosecond technique is similar to Nikolai Walujew

standing in the ring. Due to his slow punches, he needs more force (energy) to knock his opponent out which is why we may get the impression nanosecond pulses are rather less desirable. Therefore, let's think about a very short laser pulse in the femtosecond regime. In this extreme case, the laser beam duration is already finished before the electron-phonon-interaction is over with the result that the whole energy gets stored by the electrons and suddenly released into the lattice, creating a sudden expansion and explosion of the material.

As no heating occurs, this ablation is often called cold ablation (in practice, there is a small portion of heat deposit in the target that is often not noticed when working in liquid unless you go for very high repetition rates). It is obvious that this technique creates much less heat loss which is why a higher fraction of energy is used for the real ablation process.

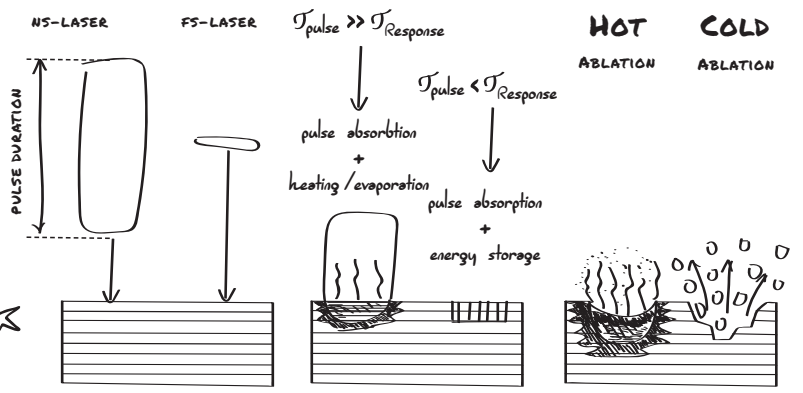


Figure 17: The effect of pulse duration on the ablation mechanism.

Hence, when using nanosecond pulses, a higher fluence is needed to cross the ablation threshold. Of course, from this behavior you may infer that femtosecond or picosecond pulses should be highly favored especially because the ablation is more energy efficient. Yet we're only at the beginning of our journey and we will see that high efficiency also comes with a cost, creating additional efforts and therefore rendering inefficient brute force is the easier option in some situations...

STEP 3: MAXIMIZE THE ABLATION EFFICIENCY

With the gained knowledge about the ablation threshold, especially about its dependence on the pulse duration, it is time to discuss how we can increase the ablation efficiency without varying the pulse duration of the laser beam. That will become your routine work, because pulse duration is fixed at most laser systems. You may already smell the rat – in order to increase the fluence we have two options: either increase the pulse energy or decrease the beam area. Before working on practical approaches on how to achieve either of these alternatives, we may split our ideas into cost-intensive and non-cost intensive measures. It is obvious that you will always endorse yourself by using a non-cost intensive approach saving lots of money. Did you already figured out which of the two given approaches may embody the trail towards success and popularity amongst your boss? If your choice fell upon maximizing the pulse energy you probably chose the sledgehammer to crack a nut. It is almost certain that initially following this approach you will sooner or later end up buying a new expensive laser because the old one seems too feeble. Of course, the laser manufacturers will be pleased by this approach however your budget may not be amused. Also, very high pulse energies don't make you happy once you've seen how huge pulse energies damage your optical components or ablation chamber windows. To save some of your money, initially the non-cost intensive approach of simply changing the beam spot area should be chosen. In order to do so the laser beam can be focused using a collecting lens. This is one of the easiest actions available to significantly increase your ablation efficiency and should always be your first priority when thinking about scale-up measures. With the collecting lens introduced into our setup, your first scale-up action is to optimize the distance between lens and target as shown in Figure 17. When ablating in gaseous media, the highest ablation rate will usually be found when the beam focus is aligned with the target surface as the fluence reaches a maximal value within the focal spot. Having a target immersed in a liquid – say water - interactions of the laser beam with the liquid have to be considered. One of those interactions is the refraction of the laser beam at the gas-liquid phase boundary, shifting the focal spot further away from the lens. The absolute shift depends on the chosen liquid height. This shift can easily be calculated using linear optics for the known liquid height. Depending on the setup, laser and liquid applied, you may, however, want to discard the calculation of the refraction (considering the manifold of possible disturbances to be addressed soon) and simply choose an experimental approach following Figure 18. Here, the displacement between lens and target is being varied until the highest productivity has been found. As a general rule of thumb, the initial distance between target and collecting lens should equal the working distance of the collecting lens given by the manufacturer of the lens (Figure 18), after which you increase the distance.

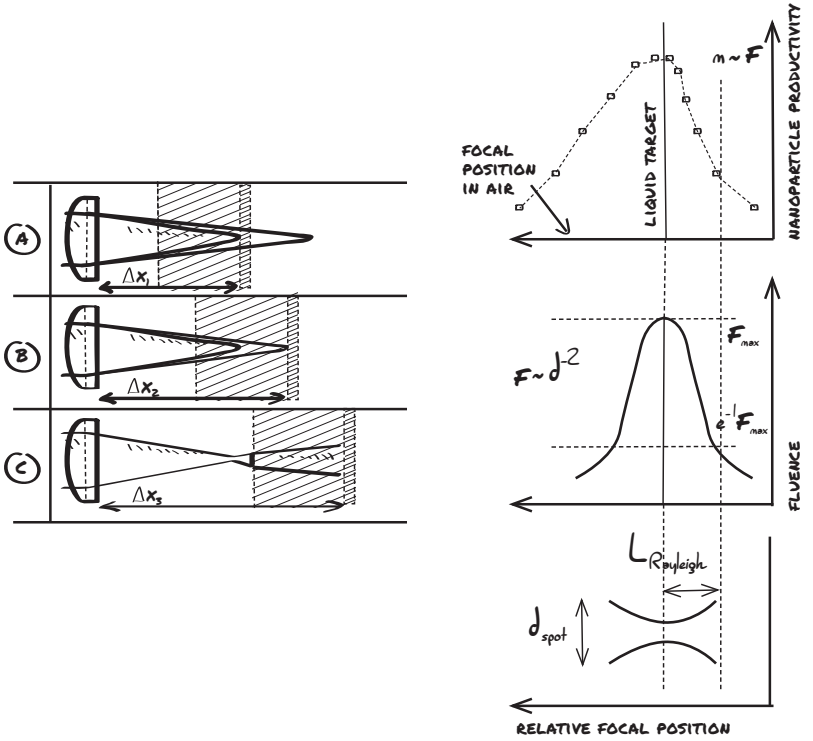


Figure 18: Optimization of productivity by changing the distance between the collecting lens and the target relative to the focal position in air. The productivity optimum is reached when the beam waist is located on the target surface and the maximum fluence is applied. Starting at this point, a shift in the distance leads to a decrease in productivity caused by an increased spot size.

The productivities shown in Figure 18 may be determined by the gravimetrical or the visible approach (or sound amplitude) discussed earlier. Once approaching point B, the productivity reaches a desired maximal value as the beam area at the target surface becomes minimal (fluence is maximized) with the focus located on the target surface. A further increase of the distances should be avoided. Above the optimal distance your laser focus will be located in the liquid, typically forming a plasma within the liquid leading to severe absorption, local boiling, and bubble formation, significantly decreasing the LAL's nanoparticle productivity. In case the ablation is done in an ablation chamber where the fluid is confined by a quartz window a focus placed on the used window should be avoided. Otherwise damaging of the window and termination of the experiment (maybe even inflammation of the liquid, e.g. when using acetone or ethanol) due to a bursting window may be happening. Therefore, it is highly recommended to only conduct this optimization in between the range of

A and B while starting at point A until B is reached. Note that ultrashort-pulsed laser beams may undergo self-focusing (see Step 4) so that the focal plane is shifted to shorter distances towards the lens.

This routine of "focus finding" is one of the main basics when trying to maximize productivity. It should always be repeated if the setup (e.g. liquid height, target thickness, different lens) or the liquids (refractive index) were changed in the meanwhile. You do not need to do this with the precious target material reserved for your flagship experiments series. Instead, you may take another material with the same thickness. Avoid using high reflective materials for finding the focus (such as silver) if you have no idea where the focus is, as it may back-reflect the partly focused laser light (Figure 18) and inscribe or break the window from the inner side.

However, it was already insinuated that there are numerous biasing effects that may still decrease the productivity or even avoid finding a proper productivity maximum. The next section will address those interferences and prevent you from spending too much of your precious lifetime while waiting for your nanoparticles being generated.

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STEP 4: AVOID FLUID BREAKDOWN AND NONLINEAR EFFECTS

The previously described basic method will always be a starting point of your optimization. But as soon as the productivity starts rising, the previous simplicity becomes a rare luxury. When applying high laser fluencies and/or very short pulse durations, a whole series of stumbling blocks may be evoked on your way towards higher productivities. Let's shed some light on how to evade those obstacles and make sure we boost our productivity to the maximum.

The first "banana peel" may possibly occur when focusing your laser beam into an open beaker filled with the target immersed in e.g. water. Let's consider the worst-case scenario of high laser intensities, lenses with a long focal distance and a low liquid layer thicknesses being applied. Once exceeding a given fluence threshold in the liquid, you risk causing a significant fluid vaporization followed by severe bubble generation as shown in Figure 19 – Case A. This effect will drastically reduce your nanoparticle productivity, due to severe scattering of light at the bubble interface, rendering all effort of finding the best focal position inefficient and pointless. In order to avoid liquid vaporization when using lenses with long focal distance (small focusing angle α_f, see Case A and B), you will be forced to work at rather high liquid layer thicknesses (or smaller pulse energies). However, increasing the liquid layer thickness (or decreasing the pulse energy) will again cause a substantial depletion of our desired productivity, as laser light might be scattered by possible gas bubbles (from solute gas) or absorbed of already produced nanoparticles.

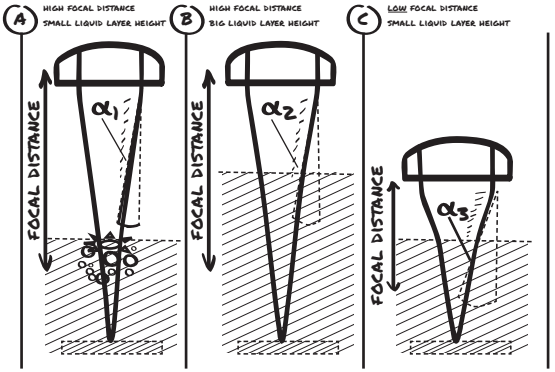


Figure 19: Influence of focal distance and liquid layer height on the generation of an optical breakdown at high pulse energies (high peak power). Case A: High focal distance and small liquid layer, resulting in a small beam area (high fluence) at the phase boundary causing in optical breakdown. Case B and Case C: Fluence at phase boundary doesn't exceed breakdown threshold → no optical breakdown occurs.

To avoid the aforementioned disadvantages it is feasible to refrain from using collecting lenses with a very long focal length. The application of shorter focal distances should be favored as those lenses possess higher focusing angles (short Rayleigh length) and therefore a bigger beam area at the liquid surface even at small layer height (Figure 19 – Case C). A smaller focal length will additionally result in a smaller beam waist at the focal spot leading to higher fluencies or, generally speaking, higher productivities. A big downside of lenses with short focal distance ($f \ll 100$ cm) is the limited action window. In this context, the action window refers to the spacing of the plateau at Point B in Figure 18 shown in Step 3. The shorter the focal distance the smaller this plateau, resulting in a stronger sensitivity of productivity on changes in the focal distance when using this type of lenses. One effect causing constant variation of the focal distance is caused by the ablation itself. Let's consider ablating a target, which implies removing material. It is obvious that a crater of increasing depth will be forming with ongoing ablation, continuously increasing the distance between the target surface and the focal spot position. Considering a lens with short focal length, you'll find a fast drop in productivity with increasing crater depth (due to the small action window / short focal distance) in case you're not constantly adjusting the distance between target and focusing lens. Therefore working with lenses of short focal distances increases short term productivity which on the contrary quickly drops if the distance is not constantly adjusted (e.g., every 15 minutes) increasing the working effort. On the other hand, a long focal distance ($f > 100$ cm) will increase the operating window but strongly limits the productivity due to the higher beam waist area. Hence, a suitable compromise between these opposing tendencies is necessary. A proper

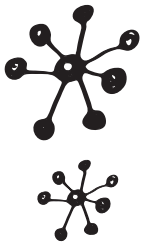
trade-off was usually found when using f-theta lenses with a focal distance of around 60 mm (and liquid layer thickness of around 4-6 mm).

In my laser lab there is also a micro Raman spectrometer, that often needs long measurement sessions by my colleagues. For this reason, I'm sure to obtain some terrible curses by them when I tried laser ablation in iodo-benzene. Perhaps this solvent has not a scaring datasheet, but it has a persistent and really unlovable smell!

Of course I realized that a sort of "technological limit" stays in front of me: lasers cannot be positioned under laboratory extractor fan, and the least cannot be moved over the entire optical bench. So the solution was a sort of little portable extractor fan, linked with a flexible tube and equipped with some metal shields that allow to fit on my laser ablation set up. The next Raman users sincerely appreciated my set-up improvement!

Lucio Litti, Padova

Now, since we discussed what may happen when working with high laser fluencies, there is another extreme we didn't address until now – ultrashort pulse durations. As already discussed in Step 2, using ultrashort pulse durations in the femtosecond regime will result in nearly no heat losses and therefore high ablation efficiencies (based on energy input). In contrast to the mean power being released by the laser while switched on, the peak power is much closer to the apparent ablation process. From the example given in Chapter I, Step 4 it is obvious that an ultrashort pulse duration will cause a very high peak power during the pulse, causing a more efficient ablation (as discussed in Step 2). However, this peak power will also have a tremendous effect on the liquid in which the target is immersed. As soon as the peak power exceeds a threshold that depends on the liquid nature, events like self-focusing and optical breakdown will be the result.



Self-focusing in liquids comprises a nonlinear effect usually occurring at peak powers in the order of GW. Mostly these high peak powers can only be reached with femtosecond pulses. The basis of self-focusing is the so-called Kerr effect which describes a dependence of the refractive index on the laser intensity. In normal applications, the refractive index of a liquid is only dependent on the wavelength of light. However, in case of such an intense electromagnetic field strength like in a laser pulse with a peak power of several GW, the positive Kerr effect results in an increase of the refractive index with increasing laser intensity. This results in a lensing effect where the medium acts as an additional collecting

lens significantly shortening your focal distance. Therefore when working with femtosecond pulses in liquid, the focal position may be found even above the geometrical focal plane (closer to the lens). You will notice self-focusing in liquid by a white-light filament, like a straw of light. It causes some energy loss, but benefits from tight focusing.

The other effect called optical breakdown mainly happens when a laser beam of sufficient peak intensity enters a liquid out of air. And it will strongly diminish productivity. The intensity threshold for optical breakdown to occur was found to be $2{,}2 \cdot 10^{13} \frac{W}{cm^2}$ in case of water (800 nm and 120 fs) while the thresholds of organic liquids are often even lower. To get an idea about the scale of this value, let's just consider an example. In typical femtosecond ablations setup, 100 fs and a focal diameter of 100 µm are common. If you consider the fluence threshold given above, the optical breakdown will already occur when a pulse energy of about 170 µJ has been exceeded. However, this actually is a typical pulse energy when working with ultrashort pulsed lasers. Therefore, optical breakdown and reduced productivity are very likely when femtosecond lasers are being used at high pulse energies. Unfortunately, your only option to avoid optical breakdown of the liquid is to increase pulse duration or lower the fluence. Moreover, in many cases, even this measure does not let you escape, because colloidal nanoparticles, in particular, plasmonic nanoparticles are very effective "sensitizers" for filamentation. In practice, unless you have created severe optical breakdown and splashing of the liquid, filamentation can still be handled. Here, maybe picosecond lasers are the better choice. Many groups work with fs and ps lasers in liquids since decades and are quite happy with that. Maybe productivity is not always optimal for laser ablation synthesis, but there are no better laser tools for efficient fragmentation (downsizing) of colloids than ultrashort pulsed lasers.

Also keep in mind that during the "cold ablation" where the pulse duration is shorter than the electron-phonon-relaxation time (Figure 17), the fluence threshold for laser ablation and therefore the productivity (assuming the same laser fluence and wavelength) is less dependent on the pulse duration, rendering 100 fs - 10 ps laser ablation with similar efficiency. The shorter pulse duration is more efficient at the ground but disturbed to a higher extent before reaching it. Increasing the beam area may be a proper alternative in these cases. However, when applying pulse durations significantly longer than the electron-phonon- relaxation time (that is, nanosecond lasers), the ablation efficiency forces you to bring out the earlier called sledgehammer to crack the productivity nut. In this context, the focusing conditions are crucial for nanosecond lasers.

Wrapping up the take-home message of Step 4, if you try cutting target chunks using a scalpel (femtosecond laser) make sure to be gentle (limit pulse energy) and easygoing (prefer ps pulses) or you may spook the solvent (liquid break down) while carving out the desired target areas. In case you prefer using the machete (nanosecond laser) avoid wielding it like a claymore (using inappropriate lenses

with f > 300 cm) while also not begin fencing it (going too short for f < 50 cm). Nanoseconds are the working horses of LAL, and with ultrashort-pulsed lasers running "colder" ablation you are on the safer side when working in organic liquids. This in mind let's move on to the next step trying to outrun some bubbles.

STEP 5: DODGE THE CAVITATION BUBBLE

In Chapter I, the concept of cavitation bubbles occurring during laser ablation was already introduced and discussed. These cavitation bubbles are a significant issue for productivity as they operate as a lateral limited protection shield of the target, deflecting the strikes of subsequent laser pulses during bubble lifetime. Consequently, we need to think about how to handle this shielding wall in order to stop it from limiting our desired productivity.

We actually suggest two different ways – the temporal and the lateral evasion of the cavitation bubble. The idea of temporal evasion technique is to successively reduce the repetition rate until the subsequent laser pulse hits the same ablation spot only after the cavitation bubble is gone. Hereby the ablation efficiency (mass per laser pulse) can be significantly increased once the temporal spacing between the pulses is longer than the lifetime of the cavitation bubble. A typical cavitation bubble lifetime is 200 µs which would equal a maximal "undisturbed" repetition rate of 5 kHz. Hence, this technique is only at all feasible for lasers operating in the lower kHz regime. Not a big issue if you work with a laser from the 90ies that delivers only 10 Hz. But, if you are lucky to run a kHz laser with more power, you may already sense another flaw in this idea. When the repetition rate (number of pulses per second) is being decreased, the mean laser power output may decrease as well depending on the type of laser and its characteristics. Even if the amount of ablated mass per pulse increases by the temporal bypassing, decreasing the number of pulses (due to a lower repetition rate) may still cause the overall productivity to stagnate or only slightly increase. Therefore, the temporal evasion is a robust strategy for an increase of productivity but has physical limits. Again, the bubble size and lifetime scales with the pulse energy. If you are allowed to select a nanosecond laser from scratch, 5-10 kHz is enough at high (>> 10 mJ) pulse energies, maybe 100 kHz at low pulse energies (<< 1 mJ) may still get to the ground undisturbed by cavitation bubbles.

Still following the idea of a peaceful solution, the second and probably most generally applicable idea is the lateral evasion of the cavitation bubble. In order to achieve this goal it is mandatory to use a scanner or displace the target during the ablation (e.g. by spinning the target). For simplicity, let's only consider using a scanner at this point. One of the most efficient patterns applied during ablation is the use of a Fermatian spiral (closed spiral) as described in Chapter I. In this case, the laser follows this spiral, homogeneously ablating the target. The curved nature of the path makes sure the mirrors in the scanner are constantly moving, whereas the adoption of a path with corners would result in sudden operation of one mirror and consequent introduction of mapping errors. Back to the cavitation bubble, in case you plan to work at high repetition rates, e.g. >> 5 kHz, there may be an interaction of subsequent laser pulse and cavitation bubble even when the target displacement is applied. Yet if you manage to guide the second laser pulse towards a space next to the cavitation bubble fast enough, no interaction between pulse and bubble occurs. Consequently, the ablation efficiency and, as the repetition rate remains unchanged, the overall productivity will increase significantly. Note that this also makes sure that every shot gets the same energy to the ground, increasing reproducibility. To manage the previously said you simply need to increase the lateral interpulse distance by increasing the scanning speed. Measuring the productivity after a stepwise increase of the scanning speed (interpulse distance), an optimal interpulse distance can be found. A good starting point for this optimization is to start at a rather low interpulse distance of e.g. 5 $\frac{\mu m}{Pulse}$ (which equals a scanning speed of 50 $\frac{mm}{s}$ at 10 kHz). The idea of the technique is shown in Figure 20.

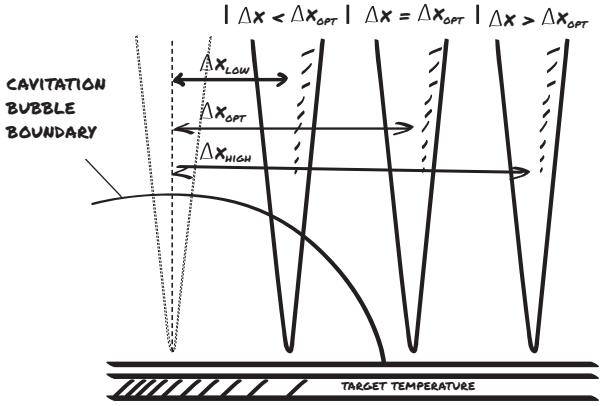


Figure 20: Variation of the interpulse distance Δx in order to avoid scattering of a subsequent laser pulse at the boundary of a cavitation bubble generated by the preceeding laser pulse. Therein Δx_{opt} represents the optimal interpulse distance. On the bottom of the picture, a scheme with dashed lines (close lines depict higher temperatures) depicts the temperature evolution in the target considering residual heat accumulation.

In case of too small interpulse distances, the laser pulse is likely to be scattered at the phase boundary of the cavitation bubble. In this case, your precious productivity will be low unless you increase the interpulse distance. Reaching the optimal interpulse distance Δx_{opt}, you will just bypass the prior cavitation bubble increasing your overall productivity. When exceeding this optimal interpulse distance, heat diffusion and preheating of the subsequently ablated area needs to be considered. Consequently, it is feasible to ablate this already preheated spot and limit the interpulse distance to an upper value. In case of e.g., 10 kHz, the time between two pulses is 100 µs, which is more than enough to have heat conduction into several hundreds of µm around the previously ablated spot (in particular for ns laser). A thorough examination of the optimal ablation distance should be made in this case. Typical galvanometric scanners easily provide lateral scan speeds of 1 m/s, allowing to separate 10 kHz pulses laterally by 100 µm, maybe already enough if you work at moderate pulse energies (causing moderate bubble sizes).

STUDENT: THE DEVICE ISN'T WORKING, I'VE TRIED EVERYTHING.

ASSISTANT: IS IT PLUGGED IN?

STUDENT: OH

ALEX HEINEMANN, ESSEN

However, in case the scanner being used has high scanning speed of up to 100 m/s (e.g. a polygon scanner), the previously mentioned upper interpulse distance limit will be rather irrelevant when working in the MHz laser pulse repetition rate regime. The reason for this statement is the short interpulse delay of only several tens of nanoseconds being shorter than the heat diffusion timescale, rendering it impossible to use the residual heat from a previous laser pulse. Simply speaking, the timescale for heat diffusion to take place between two subsequent laser pulses in MHz regime is just too short. Therefore, when you're working with MHz-repetition rates (as modern high-power pulsed lasers often provide) while having a sufficient scanner at hand, you don't need to worry about working at too high interpulse distances and rather run your scanner at maximum speed. However, in case you have a scanner with a scan speed of << 10 m/s, even your highest scanning speed may not be sufficient to fully bypass the cavitation bubble in case you work with repetition rates of several hundreds of kHz. This again is strongly depending on the cavitation bubble size and lifespan which again is based on applied pulse energy, pulse duration, etc. As you may realize, due to the huge number of different possible parameters

depending on the setup chosen, it is hard to find a general rule directly giving the optimal scanning speed (interpulse distance) for obtaining maximal productivity. Therefore, following the previously explained technique, the optimal working conditions need to be determined exclusively for the used setup, target material and liquid you are working in. To keep it simple, when you work at 100 Hz or below, you do not have to worry about the bubble.

Approaching the kHz regime may render faster movement of the beam relative to the target a good choice to improve both productivity and reproducibility. Approaching the MHz regime, polygon scanners of the latest generation are demanded to bring every pulse to an undisturbed site at the ground.

STEP 6: KEEP IT THIN

During the remarks made in Step 4, it was already mentioned that scattering and absorption of laser light by nanoparticles is a possible reason for the reduction of productivity. Actually, this is another big issue when working with a batch chamber. There are three ways to compensate this effect – either decrease the ablation time such that the nanoparticle concentration remains low or work with a non-circulating liquid flow or decrease the liquid layer height so that the laser beam has a very short path through the liquid containing nanoparticles. All approaches aim at minimizing absorption and scattering losses due to nanoparticles or bubbles. Those vapor bubbles should not be underestimated. In order to maximize productivity and avoid disturbances of the laser beam during its path through the liquid, it is therefore feasible to minimize the final concentration and additionally decrease the liquid layer height. When working in an open environment with the laser beam passing a liquid-air boundary, it was already stated in Step 4 that there is a lower limit value for the liquid

We once designed an innovative setup for wire ablation in a flow-through chamber. However, it had one downside. For evaluating the wire position you had to put your head directly on the table and look through the optics. I had a student who worked with this setup for months. One day he felt a slight stinging on the back of his head while putting his head in. Apparently the laser was still on. From this we can learn 1) Accidents happen particularly when everything is routine 2) Do not put your head into the path of the beam, EVER, but put safety first during design of optical setups.

Christoph Rehbock, Essen

layer height to avoid liquid vaporization. Using the setup shown in Figure 20 the case is a little different. In this setup, the laser passes a glass-liquid phase boundary (like in a cuvette irradiated from the side or a glass-covered chamber). Due to both the similar refractive index liquid and the (quartz) glass window and the high damage threshold of glasses (higher than liquid vaporization threshold), no direct ablation of the window is expected at its entrance side. However, window damage is often observed if the liquid layer is too thin. With the setup using a quartz-glass window, the lower limit of liquid layer thickness is mainly determined by the dimensions of the cavitation bubble and the range of its mechanical ablation by outward jets ejected from the bubble during the collapse. Usually, lowering the distance between target and glass window below 2-3 mm will result in the abrasion and consequently in the destruction of the window.

Therefore, it is recommended to not reduce the liquid layer height below 3mm when using glass windows (air/glass/water boundary) instead of an air/water phase boundary for the laser to enter the liquid. For maximal LAL productivity, of course, the liquid layer should be as thin as possible (4 – 6 mm). And liquid flow always helps.

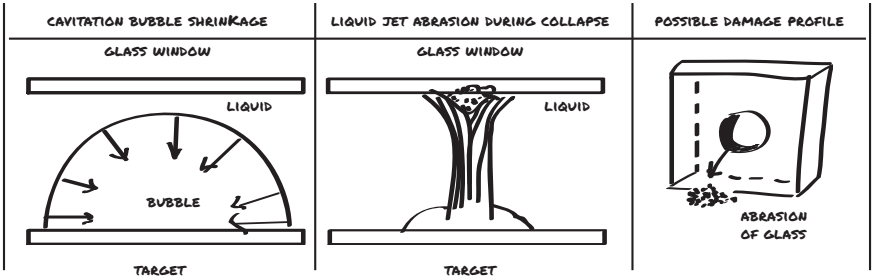


Figure 21: Typical glass damage scenario during ablation with a too low liquid layer height. To the right, a typical picture of a glass cover is shown after abrasion occurred.

STEP 7: SCALING IT UP – FROM BATCH WISE TOWARDS CONTINUOUS PROCESSING

Since we already learned how to find the best operating conditions and geometry for an ablation setup, it is now time to think about optimizing the whole setup concept. Of course, the widely used batch process is good for a "quickie", but will not be very efficient. Due to the previously mentioned accumulation of nanoparticles during ablation, productivity and nanoparticle yield per pulse will decrease during the process. Additionally, when thinking by economical means, emptying the batch chamber after a specific concentration is reached, re-adjustment and setting it up for another run will consume crucial and "precious" working hours. In order to circumvent the problem about the nanoparticle accumulation and the high workload of a scientist, it is time to think about changing to a more compatible, continuous nanoparticle production using a flow chamber. A simple and very effective setup to achieve a temporally continuous ablation process is given in Figure 22.

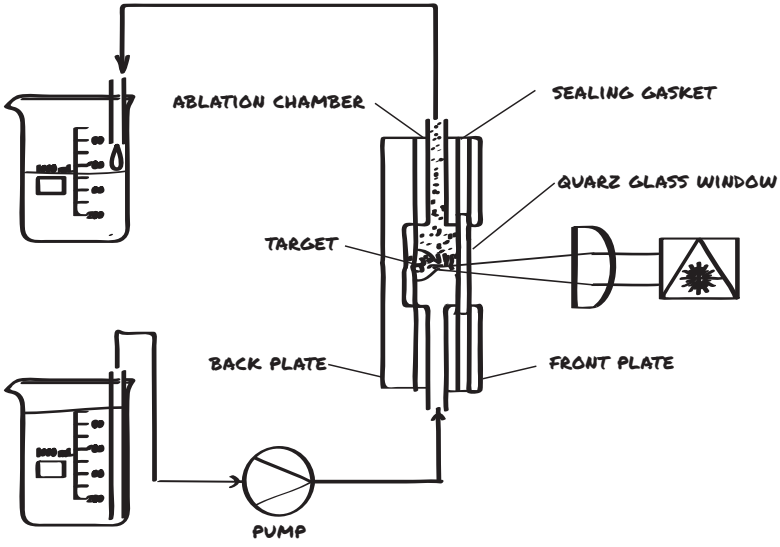


Figure 22: Scheme of a continuous flow processing including a flow chamber cross-section view.

The design is held simple and chambers can be built with basic workshop equipment. It is recommended to use aluminum plates as a basis material as it is light and easy to handle when mechanically working it. As basic shape a cube with 40mm x 40mm x 5mm dimension for each chamber part is convenient. The chamber is built of 3 basic parts:

- BACK PLATE (HOLDING THE TARGET)
- MIDDLE PLATE (ABLATION CHANNEL FOR LIQUID PERFUSION AND PARTICLE TRANSPORT)
- FRONT PLATE (FIXING THE QUARTZ WINDOW)

Additionally the middle piece should be equipped with suiting hose connection in order to connect tubes to your chamber.

In order to assemble the 3 chamber parts, four drilling holes in the corner of each part and slid bolds may be used as depicted in Figure 23. The different plates should be separated by some gaskets preventing leaks. In this context, 0.5 mm Teflon or Nylon slabs are recommended. In case of persistent leaks, Teflon paste applied onto the gaskets proofed to be a handy tool to prevent leaks occurring after assembly. The chamber may be fixed on a breadboard attached to a 1 axis or 2 axis translation stage using a suitable chamber holder, facilitating chamber positioning towards the focal plane. With this chamber, you are now able to easily perform a continuous nanoparticle production.

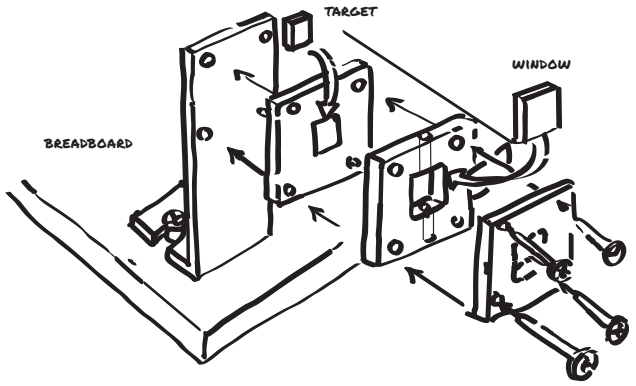


Figure 23: Assembly of a simple, but fine working flow chamber to achieve continuous ablation conditions. Liquid flow is directed upwards. Use Teflon sealings with additional Teflon paste between the parts.

Of course, the target needs to be changed once it is completely ablated. In general, chambers shall be made of an inert polymer or aluminum (never glass, as it may break, or steel at it leaches ions). Yet, before starting your first experiments, it is highly recommended to anodize all aluminum chamber parts to make sure the surface is covered by an aluminum oxide layer. Without passivation, aluminum may dissolve into e.g. the water pumped through the chamber. The dissolved aluminum ions will significantly destabilize your produced colloids leading to agglomeration (yes, we suffered from this experience). More details on the stability of nanoparticles will be given in Chapter VI; however, keep in mind that a healthy aluminum oxide layer is very important. Even when the chamber is made out of Teflon, it needs to be cleaned. We use Aqua Regia for that (be careful, check the safety instructions!), and found out that colloidal quality decreases after 3 ablations without rigid cleaning. Now, before we start looking into more detail about the performance of the new ablation process, let's address some issues you should be considered in the first place:

- avoid pH < 4 and pH > 9 in order to keep the aluminum oxide layer intact. For corrosive liquids, use Teflon or polyamide
- do not use tubes and seals made of silicone and other materials that have considerable porosity, to minimize nanoparticle losses and contamination carryover due to deposition inside the pores
- check solvent compatibility table for all seals, tubes or plastics in contact with the solvent you plan to ablate your nanoparticles in. Recommended tube materials when using solvents like acetone are Polyamide (PA12) or Teflon or PVC.
- consider leaching of plasticizers from your tube (i.e. from cheap PVC) and seals material, especially when ablating in organic liquids like acetone, in order to avoid impurities (one example siloxanes leached from silicones and seals immersed in acetone – siloxane impurities will adsorb onto nanoparticles, even after several cleaning steps!).
- make sure the pump is clean and specified for the liquids you plan to work with (e.g. solvent). Be aware that pump oil is transparent, not dark-brown!
- clean the chamber.

According to the previously listed points, silicone should be avoided and Teflon (or high-quality PVC) based tubes and seals should be preferred. Those, however, may provide less efficient sealing properties and some tinkering to attach the tubes to the hose connector resulting in leaks during liquid perfusion. To avoid leaks consider using Teflon paste additionally to the already used gaskets and tubes. Teflon is thermoplastic, so it tightens best only during the first mounting.

With your finished sealed setup, you're ready to perfuse liquid through the chamber. Pump flow has to go upwards, and the chamber is mounted vertically, with laser beam horizontally. Prior to pumping make sure the inlet is attached to the lower hose connector following the sketch in Figure 22. In doing so you make sure that all gas bubbles either sucked in by the pump or generated during ablation are leaving the ablation chamber at the top without accumulating. In case you decide to connect the inlet to the top you may end up with a chamber full of gas bubbles.

Finally, you are ready to start your new continuous production line tapping nanoparticles. This setup helps in minimizing reassembling times as the interruption of the process is only necessary when the target is consumed. Considering the reassembly time as a timespan where no nanoparticles are produced the productivity of your process will be drastically increased when using this setup as a guide. And you will benefit from reproducibility. In order to examine the continuously produced nanoparticle, the setup shown in Figure 22 may be upgraded downstream with a fiber UV-VIS set up probing a flow cuvette. As every LAL process has a tune-in period of a few minutes, we recommend to waste the first 3-5 min of the colloid stream and then relax while your setup is doing the work. A batch process also suffers from this tune-in period, but here extraction of that fraction is impossible.

STEP 8: LOWER THE RESIDENCE TIME USING 3D-PRINTING TECHNIQUES

In the previous step, a very flexible chamber and setup design was introduced. The chamber is easy to access and can be manufactured with a simple tool kit. However, this design is still not optimal when it comes to consider the flow profile inside the cell. Now before thinking about better chamber designs exhibiting a better flow profile, we may first think about the impact of liquid flow on the productivity of nanoparticles.

In the previous step, a very flexible chamber and setup design were introduced. The chamber is easy to access and can be manufactured with a simple tool kit. However, this design is still not optimal when it comes to consider the flow profile inside the cell. Now before thinking about better chamber designs exhibiting a better flow profile, we may first think about the impact of liquid flow on the productivity of nanoparticles.

In previous sections, the issue of nanoparticle accumulation was already addressed several times, stating that the enrichment of nanoparticles within the beam pathway needs to be avoided in order to minimize absorption and scattering losses. Within the batch process, we found that our only degrees of freedom are the liquid layer height and nanoparticle concentration. Using continuous flow setup we created a new degree of freedom to handle "laser shielding" minimizing absorption and scattering by already produced particles

(and some drifting bubbles). But chamber volume geometry has a big effect and needs not much effort to be optimized. Figure 24 displays unwanted geometries on the left and a laminar flow pattern on the right with minimized back-flow. As you can imagine, if some particles are moving into the flowing direction away from the ablation spot, while others are surfing on eddies into the opposite direction, right back into the ablation spot, large channel width (and a steep change in channel opening diameter) may not be the best solution. In case of the chamber design presented on the left in Figure 24, strong levels of re-mixing are to be expected due to the small liquid entrance/outlet and the quick widening of the channel. On the other hand, a more tube-like shape with smooth geometry changes results in a more homogeneous flow pattern. In order to get an idea of the flow pattern inside the chamber, you can simply use software tools like "open foam" or "ANSYS fluent" in order to create a rough CFD (computational fluid dynamics) simulation similar to the sketch in Figure 24. A good is to choose a design where the liquid is directly guided towards the drain avoiding obstacles like corners. A proper design to assure a laminar flow pattern would be that of a standard pipe having no surface roughness. However, a tube is far from feasible to act as an ablation chamber holding a planar target. Yet if we cut off two sides of the cylinder such that one side can be covered with the quartz glass and the other with the target, a laminar flow pattern without too much re-mixing is likely to appear. Note that laminar flows have the highest flow rate in its center, exactly the place where the laser ablation happens and nanoparticles need to be effectively drained off.

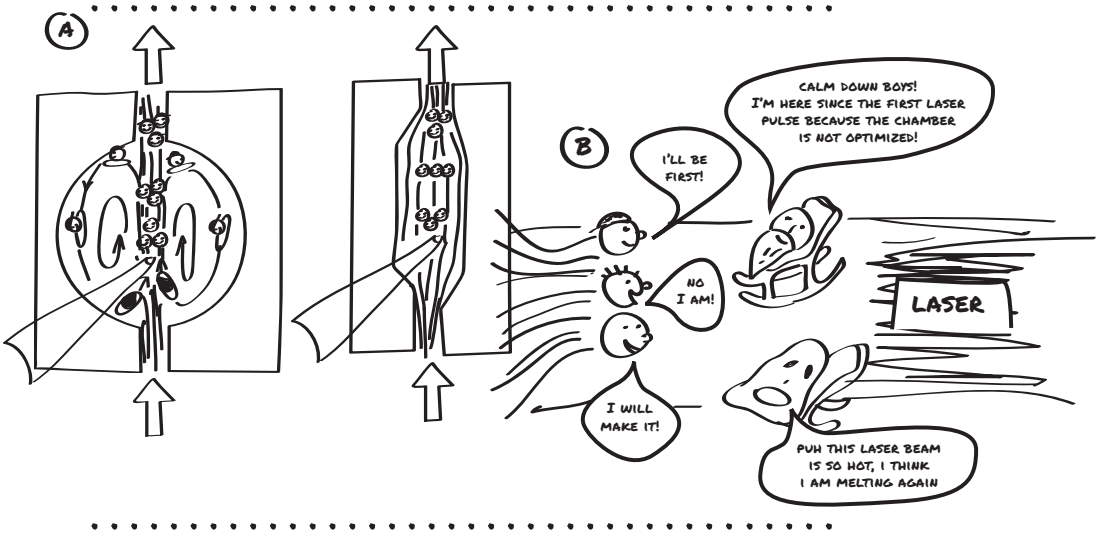


Figure 24 (A): Flow pattern inside a steep channel geometry change in the chamber volume (left) and the optimized chamber design (right). (B): Sketch of fresh particles arriving, while previously produced old ones are still present.

With this new concept in mind, you should, however, avoid attending your workshop manager in case you want to leave the workshop without bruises. Actually, the optimized chamber concept, especially the gradual diameter reduction shown in Figure 24 (left) is not that simple to manufacture. Yet once you are able to consider yourself to be a proud owner of a 3D printer you're able to simply print this chamber within a couple of minutes. Obviously, the 3D printer won't complain whatever design you have in mind. In case you decide to use a 3D printer it is, however, crucial to choose the right material, especially when working with organic solvents. A good choice for a printable polymer material with good acidic, basic and solvent stability would, in this case, be nylon. By following all previously given steps (focusing, scanning, liquid level) and using an ablation flow chamber with optimized flow properties like the one in Figure 24 (right) included into the flow concept presented in Figure 22 you will be able to maximize the nanoparticle productivity towards hundred mg nanoparticle per hour with tens of Watt laser power.

One day I went into the lab to produce some gold nanoparticles immersed in acetone. I was a bit worried as acetone is flammable it was by far not my intention to burn down the lab. I decided to use the flow chamber – which is a good choice as it is sealed and ones filled with acetone, its interior is free of oxygen. I remembered a short lesson about safety issues stating that a burnable liquid can only burn if both oxygen and and ignition source are present. Now as the oxygen was ruled out in the sealed flow chamber I considered this experiment quite save. While I was ablating using a high power laser that I know likes to kill the glass cover of the ablation chamber I still was a little cautious about the whole process. But thinking – no risk no colloid! I started the acetone flow and the laser – surprisingly without any accidents ... well at least in the first place... I was nearly finished when I heard some strange deviations in the ablation sound which hasn't been there before. Now I thought – whatever – eyes shut and go for it ... just a little more ... but this sound ... where does it come from? So I looked a little closer not being aware of that leaky little hole in the glas cover. Suddenly the laser ignites this ejected jet of acetone forming a pretty big flame making my heart jump. Lucky for me – as soon as the flame appeared it was gone again as the acetone jet burned off instantly and couldn't continue burning as the acetone flow through the leak was too slow. To avoid further fire spectacles I directly turned off the pump followed by the laser. Except of a little shock not much had happened however nowadays my fingers do not leave the turn-off-button of pump and laser control in order to turn everything of directly when I hear something suspicious.

Sven Reichenberger, Duisburg

STEP 9: MOVE BETWEEN DIMENSIONS

Since you already became an expert regarding optimizing the laser ablation of a two-dimensional target, it is time to finish this chapter on productivity maximization with some additional novel concepts regarding the laser ablation technique. Until now, the presented ablation concept concluded in a continuous process which significantly decreased assembly times and outages due to target or liquid change, compared to the initially presented batch process. However, to think big scale a fully continuous automatic setup is required. Therefore, the last obstacle to overcome is to get rid of time-consuming target replacement steps. Now how can we achieve something like that?

The answer to this question may be counter-intuitive to our goal but as we are already in the predicament of going big scale with the small scale one additional bafflement shouldn't be too hard to swallow. So prepare yourself! It is time to cut off unnecessary target dimensions.

Let's begin with the 2nd dimension and get rid of this large area of metal but instead consider a very long metal strip of rather small thickness (e.g. several tens or hundreds of microns, as thin as the focused laser beam). Therefore let's decrease the dimensions move to a 1D shape which would be a simple target wire. Wire ablation has been studied in the literature for a while and can be considered as one of the ablation techniques with the highest potential to be applied in fully automated, continuously driven ablation processes.

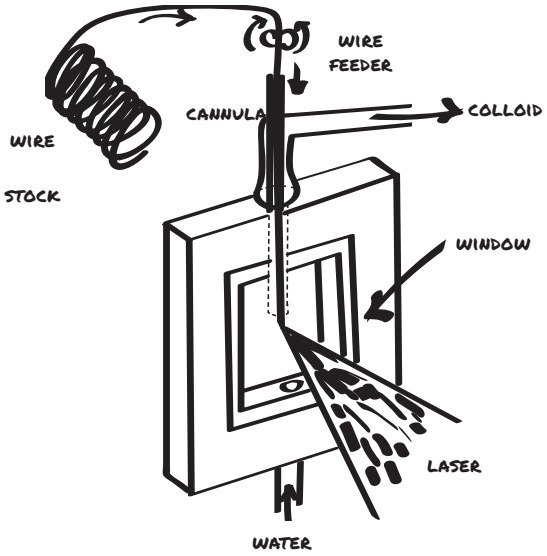


Figure 25: Sketch of a fully continuous wire ablation setup.

As depicted in Figure 25, wire ablation is done in the same ablation chamber presented in Figure 23 only with the exception that the back plate holding the target is exchanged by a second window and a second front plate such that the laser can pass through the chamber. The wire is to be fed into the ablation chamber using a cannula inserted at the top. Using a wire conveyor the wire may be pushed through the cannula while the laser spot position needs to be adjusted such that the wire tip is being ablated. By controlling the feeding speed and choosing a wire diameter that is slightly thinner than the focal spot diameter of the laser, the wire can be constantly ablated over a long period of time. Not all wire materials work. They shall be flexible (to be compatible with a feeder) and not be too ductile, as the mechanical force during LAL may bend the tip of the wire, so that's out of focus. Nickel, for example, works very well. The ablation time is only limited by the chosen feeding speed and wire length which can be chosen to be a couple of 100 m if needed. When properly adjusted the wire is ablated with 100% material yield without any non-ablated metal waste. Using the feeding speed set for the wire conveyor, the productivity can directly be set. And isn't a continuously fed target a perfect match to continuous flow and continuously provided laser pulses?

Obviously, wire ablation already is a very suitable concept and we've seen that with decreasing target dimensions more and more issues disappear. Maybe it is time to think about cutting off the last dimension as well and move towards a zero-dimensional target. It may sound paradox – in the mathematical definition a zero-dimensional target would be represented by a point in space with infinitely small diameter taken in absolute units. Naturally, this is not possible in reality but we may converge when considering decreasing target dimensions up to the point where the target particle diameter is considerably small compared to the laser spot diameter in the working area. This assumption is valid for most commercially available micro-powders. Using the same setup suggested for the wire setup, probably leaving the cannula, it is possible to form nanoparticles from irradiating micro-powders dispersed into a liquid (e.g. water) under continuous flow conditions (Figure 25).

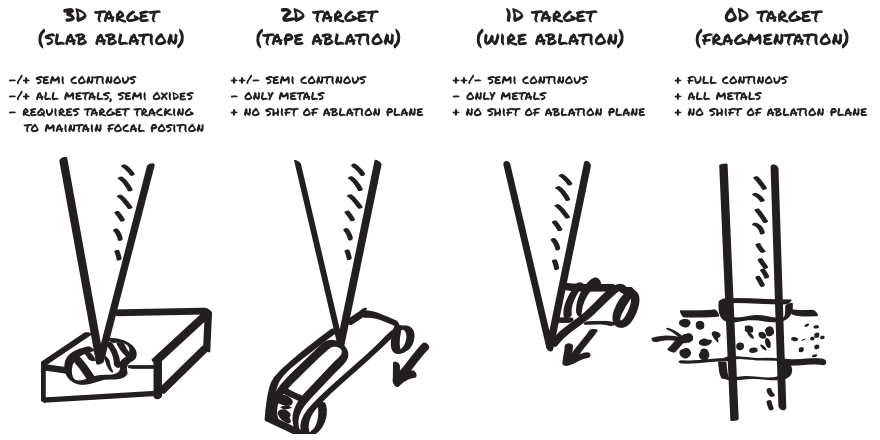


Figure 26: Comparison of different ablation geometries including an overview of the major up- and downsides.

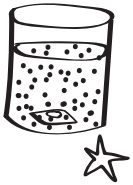
Applied to micro- or nanoparticles this technique is commonly discussed as laser fragmentation in liquid (LFL) as already mentioned in Chapter I and mainly used to produce particles with downsized particle diameter. Obviously, by changing the target dimensions down to 1D or even 0D (Figure 26) we gracefully managed to pass the last obstacles and introduced a final concept for a 100% continuous process with 100% nanoparticle yield, 0% waste and high productivity potential.

Concluding, much effort is necessary during the adventure of climbing the productivity mountain. The path may be cloudy and every herein discussed step needs to be taken with diligence and caution in order to make sure not to fall off the steep cliffs appearing on your way up to the top. However, by understanding and avoiding the obstacles and barriers presented, you may soon be sitting on the peak of the mountain enjoying the view over the nanoparticle ocean.

II

After conducting a series of measurements for different calibration curves at the UV/Vis spectrophotometer, nothing but noise could really be extracted from the data. After reassuring that the samples were prepared correctly, we expected a major defect of the instrument. However, upon opening the lid, it became apparent that only the cuvette changer was not calibrated correctly so that the light beam could neither reach the sample nor the detector. Lesson learned: check the instrument setup and question your results directly before continuing with 100 more samples and wasting your time.

Carmen Streich, Essen



PULVERIZE IT: LASER-INDUCED DOWNSIZING OR SPHERIZATION OF PARTICLES IN SUSPENSION

An alternative approach to nanoparticle generation from macroscopic target materials is the laser irradiation of suspended particles. In case the particle's diameter is significantly smaller than the laser spot size, we can consider this as a zero-dimensional target, as already described in Chapter II. At high laser fluence, downsizing (reducing the particle size) takes place, whereas melting (particle recasting into a spherical shape) requires far lower laser fluence.

The basic principle of laser processing of colloids (LPC) is simple, just irradiate a suspension of particles in the nanometer to a few micrometer diameter ranges. But looking at the details, you will be suddenly faced by even more parameters to be set in LPC compared to LAL. For example, for LAL you start from a clear liquid in which the nanoparticles are harvested during the ablation process and by ablation time you can control the nanoparticle concentration (or total nanoparticle amount). The target is your reservoir, and the mass of this reservoir just has to be larger than what you want to yield, more is not a problem. For irradiation of suspended particles, in contrast, you disperse your particles into a liquid and irradiate them. But how much powder should be suspended? From a first estimation, probably it should be not too little so that as many particles as possible will be irradiated to have a sufficient yield. But can you add as much as you want? Probably not. This will reduce the process efficiency because at high particle concentrations, increased attenuation of the laser beam occurs, according to Lambert-Beer law. This light attenuation reduces the volume illuminated with sufficient laser energy density for the desired particle processing effect and thus makes the process less efficient. Note that Lambert-Beer tells that this attenuation is an exponential effect, both with concentration

Once we focused with a green Nd:YAG laser into a cylindric glass vial (one of the cheap ones with the snap-on lid) from the side for laser post-irradiation of a platinum colloid. Of course, we did not see the beam because we were wearing safety glasses, but we had a camera on and looked at the pictures later. There was a meter-sized laser light wing reflected into the half lab, on half body height. Luckily, the laser was not on full power that day and no one was too close to the vial.

and distance. This would in principle mean that the concentration of the colloid should be just as low as possible (but still relevant for your application) and that the beam path through the liquid should be as short as possible (but still thick enough to get some throughput). Hence, both parameters – concentration and liquid thickness, have a distinct optimum.

But which energy density (fluence [J/cm²], see also Chapter I – Step 4) value do you need to apply in order to cause fragmentation or melting of your particles? For particle fragmentation, you mostly need to focus a pulsed laser beam to reach the required laser fluence. But how do you ensure to irradiate all particles with the same fluence when you focus the laser beam into the particle suspension? For laser melting, you will melt your particles only in the liquid volume elements that are irradiated with sufficient laser energy density. But in all other illuminated volumes that are reached by less light intensities no interaction occurs. Well, that can be true. But in case of laser fragmentation, due to light attenuation caused by the particles, some particles can also pass volume elements that are irradiated with laser energy densities probably not high enough to cause fragmentation but still high enough to cause melting. Therefore, precise control of laser fluence during melting and fragmentation is the key.

Another important role plays the solvent that you use. You can influence the chemical composition of the formed particles.

In brief, you have a parameter matrix consisting of the laser parameters (pulse length, wavelength, and energy density) that mainly determine the mechanism of particle-light interaction (fragmentation – that can be subdivided into two path-ways – or melting) and of colloid parameters (educt particle size, particle material, particle concentration and the type of liquid that surrounds your particles). Unfortunately, most of them have a cross-correlation and an influence on the yield, product particle properties and the interaction mechanisms that take place. It's better to explain it if we use examples.

VARIANT 1: FRAGMENTATION (LFL) BY HIGH INTENSITY

Let's start with some theory about laser fragmentation of particles in liquids. When the particle is (distinctive) smaller than the laser beam diameter and wavelength used for particle irradiation, we can define the particle as a zero-dimensional target. Now, you might say that if you use the laser raw beam with a few mm in diameter with sufficient pulse energy, the particle can be in the mm regime as well. Well, it might be true that you still can talk of a 0-D target, but

most likely you will only ablate small parts from the surface of your particle so that the effects are similar to LAL. We have made good experience (with high-intensity lasers) when the particle size does not exceed a few µm. On the other hand, there is also a lower size limit, as smaller particles are more difficult to heat (in detail, their optical cross-section decreases, and at the same time heat losses by the particle transferred to the surrounding gets worse, as the specific surface scales with the root square of decreasing particle size). For example, it was almost impossible to downsize gold nanoparticles smaller than 13 nm. Only at a laser fluence larger than 2 J/cm² (intensity of 1.6 TW/m²), it was possible to further downsize such small particles. Here you can also learn about the importance of the tremendous value of laser intensity compared to laser fluence. The picosecond laser at 0.03 J/cm² easily reaches the same intensity as the nanosecond laser (ran at 50 times higher fluence), with identical laser fragmentation products.

III

In general, there are two pathways known to cause particle fragmentation. Hence, the laser fluence thresholds for particle fragmentation is usually three orders of magnitude lower using picosecond pulse lengths compared to nanosecond pulse lengths, due to the difference in the reached peak (pulse) intensity. But LFL is limited to a maximum intensity determined by the optical breakdown threshold of the liquid (see earlier chapters). If you exceed this threshold you will ionize and severely vaporize the liquid rather than the particles. You will see a track of bubbles in the beam path. When working with a liquid jet stream, splashing will occur, similar to jam up a spoon below a water tab. Working below this threshold avoids a haze shower to the optics and workplace.

Another interesting effect that can be caused during LFL is the possible change of the particle's chemical composition. For example, copper-oxides or copper nitride can be fully reduced to elemental copper nanoparticles in organic solvents (e.g., ethyl acetate), whereas oxidation happens in water. High-intensity LFL of gold particles increases their surface potential (their surface charges), as a sign of increased oxidation. This is beneficial, as it stabilizes them against coalescence. During the milder LML, also chemical composition change is observed, with a higher extent at increased fluence (intensity). If you want to start with some general rules: for LFL, work at a highest intensity just below the optical breakdown, but still irradiate the whole liquid cross-section. For LML, there is an optimal fluence, lower or higher fluences will yield two particle size populations instead of one. Better slightly too low than too high. And – if you are good in maths – calculate how many pulses a volume sees, i.e. if it flows, and allow about 3-5 pulses per volume, as LML is not a single pulse event. Same is practically true for LFL (formally, LFL only needs one pulse pert volume element, but in practice, you need 2-3 to catch them all).

VARIANT 2: LASER MELTING IN LIQUID (LML) BY LOW INTENSITY

Laser melting of particles in liquids (LML) is known as a particle re-shaping and/or fusion process. What does that mean? The product will always be a sphere, but the starting material can be different. Either it is a submicron, usually edgy, crystal (e.g. from chemical synthesis), or it is an agglomerate/aggregate of small particles (e.g. from gas phase or laser ablation synthesis). The nature of LML is that it delivers spherical, crystalline (often multi-domain) particles in the submicron size range, typically around 200-300 nm in diameter. If you irradiate particle agglomerates or aggregates, the primary particles that make up this aggregate will fuse together, building up a single sphere. For this, fluences from around 50 to 200 mJ/cm² generated with UV nanosecond-pulses are known to cause LML. For gold nanoparticles, a green laser wavelength is also appropriate to cause melting (of course below the threshold for fragmentation). As you want to heat up particles, interband excitation, and heat transfer is what you want, so UV nanosecond lasers are preferred, e.g. at 355 nm wavelength where many solvents are still transparent. Hence, LML is an interesting method to fabricate monodisperse, spherical particles, that are often in the size regime of a few hundreds of nanometers. This is quite interesting not only for optical applications (phosphors, plasmonics) but in particular for hard materials, such as boron carbide. There is no other method that can fabricate such particles. Always good to have a unique selling point, also in science.

CHOOSE THE RIGHT PARAMETERS AND SET-UP

It's important to choose the right parameters and an appropriate experimental set-up for good and reproducible experimental results. Therefore, we want to discuss which parameters need to be considered and set. We have divided this depiction into three steps: 1 suspension parameters, 2 laser parameters, and 3 experimental design, as described in the following.

STEP 1: PREPARE YOUR PARTICLE SUSPENSION

If you have a powder and identified a liquid (make sure that your particles are not dissolved in the liquid, potential stabilization agents can be added in the formulation as well, and the liquid should, of course, be transparent for the laser) that you want to use, you will need to choose a particle mass concentration. We found that you can achieve good results well below 0.1 wt% (start with 100 mg/L). At higher concentrations, the process efficiency will vanish with respect to the high amount of non-processed particles (due to significant

attenuation of the incident laser light).

STEP 2: CHOOSE THE RIGHT LASER SOURCE AND PARAMETERS

Next, you need to choose a laser source. In most cases, you won't have too many options, depending on the institution where you work. But if you can choose between different pulse lengths, wavelengths and/or laser powers you will need to think of what you want to do with your particles. Downsize them (fragmentation) or fuse them (melting)? In case of melting, UV-laser light (e.g. 355 nm, 3rd harmonic of Nd:YAG lasers) and nanosecond lasers are a good choice due to the sufficient heating of the particles. For fragmentation of your particles, the intensity is more important than the wavelength. Of course, shorter wavelengths are better (if you still have enough pulse energy at the given wavelength). Try to be in the Terrawatt per square meter range for a good yield. At given pulse duration, laser fluence is crucial to control if you cause fragmentation or melting. Melting is well studied for many materials with 355 nm nanosecond laser pulses. It turned out that for fluences from 50 mJ/cm² to 150 mJ/cm² (or 200 J/cm²), many particulate materials can be molten and/or fused. At higher fluences, fragmentation of your particles with 355 nm nanosecond pulses starts to set in, contaminating your molten submicron spheres with satellite nanoparticles.

"A strange smoky smell comes from laser lab!", told me Fabrizio. Few seconds later I realized for the first time that a little aspiration chamber is not enough to avoid the ignition of solvents during laser ablation in alcohol! The holders of lenses and mirrors (made of plastic) were melt by a flame developed over the ablation flask. From that time I always performed laser ablation in sealed cells, in cases of flammable solvents...

Lucio Litti, Padova

STEP 3: BUILD UP YOUR EXPERIMENTAL DESIGN

Another bottle-neck for achieving good results is the experimental set-up. There are probably hundreds of ways how you can spice up your set-up, but we want to introduce here two main approaches, along with their advantages and disadvantages.

One possibility (the most widely used approach) is to irradiate your particle suspension in a vessel. This experimental batch-chamber approach can be compared to a stirred tank reactor. Unfortunately, the reaction does not take place everywhere in the reactor but only in the laser irradiated zone. This convenient way to irradiate your suspension is illustrated in Figure 27 a) and b). For this set-up, you just need a vessel, e.g. a simple beaker, and use the focused or unfocused (depending on the fluence you want to apply) laser beam to irradiate your particles. You can do this via irradiating the suspension directly from the top of an open vessel (Figure 27 a) or from the side (Figure 27 b) irradiating through the laser light transparent (quartz) glass wall. The former has the advantage that the glass cannot be damaged, the latter allows better definition of the beam path length (think of Lambert-Beer) in your work in a cuvette. That cuvette may be the one you use later directly inside particle analysis instruments). But both set-ups suffer from two distinctive drawbacks that make this process difficult to be controlled. One is that often a large volume fraction of the liquid is not irradiated so that particles pass the laser irradiated zone just randomly. If your particles pass the beam randomly you will hardly know how often you have irradiated a particle. Some might already be hit by several (thousands) of pulses while other particles haven't been hit by the light. The other drawback is that light attenuation takes place while passing through the suspension, with an exponential decay of intensity. This can easily result in different mechanistic regimes (complete and incomplete LFL, or LFL and LML, in parallel) within the same vessel. But if you want to minimize the aforementioned drawbacks, you might use the experimental set-up as shown in Figure 27 c). Basically, this set-up is just like a funnel. In detail, it is a standing flask with a capillary outlet (around 1 mm in diameter) at its bottom. At the end of the capillary, a free-falling liquid jet is formed. The main advantage of such a liquid-jet reactor is, that the laser fluence gradient in beam propagation direction is minimal, as the liquid layer is very thin. The geometry of the reactor forming a liquid jet allows a tight focusing of the laser beam in a thin volume, so that each volume element, and thereby each particle is reached by (almost) the same laser fluence. Furthermore, even at high light intensities, there is no risk to damage any optical element like windows or cuvettes. We often observed window breaking during LFL at high intensities or prolonged processing time. Probably it's not the direct ablation on the glass, but rather jets from collapsing bubbles that

destroy the window, or particles sticking to the inner side of the laser entrance into the liquid. Like in the horizontal cuvette set-up, you can also place a laser power-meter behind the liquid jet to quantify the energy input. But there is a big difference between the cuvette and the liquid jet. In the liquid jet, you exactly know the amount of irradiated volume (as no particle can escape passing the beam). Hence, the energy input per volume element, and from this the laser energy input per particle mass can be calculated. This is great for fundamental studies, but also to extract scaling factors. If the reservoir is empty, you have finished one cycle (called passage) with defined cumulative energy input and you can see if the colloid meets your demands. If not, just refill and go for the second passage. Hence, another advantage of the passage-style processing in the liquid jet is that particle properties can be gradually tuned quite finely.

The simple funnel-like setup benefits from a small upgrade. The outflow speed changes significantly (some tens of percent) during the process because hydrostatic pressure changes when the reservoir gets empty. A workaround is quite simple: use the concept of a Mariottés bottle (see Wikipedia, it is just a perforated plug on top, with a pipe or straw through it that is dipped deep into the flask) to minimize the temporal decrease in volume and particle mass flow passing the beam. You can even calculate easily the number of pulses that you have per particle (PPP-value) in one passage by measuring the liquid flow velocity, determining the laser spot diameter on your liquid jet and taking the repetition rate of the laser referring to particle number concentration per volume. For this set-up, kHz-Lasers are beneficial to have enough pulses per particle on your free falling liquid jet. If the laser has a lower repetition rate, just take lower particle concentration, e.g. 10 mg/L at 100 Hz. This brings us to the drawbacks of the liquid jet method. You need a high repetition rate laser. 100 Hz is minimum, and still then you will have to work with diluted colloids. Note that also LML needs about 3 pulses per volume element. 10 kHz and more is fine. For LFL, having more pulses than required is not a problem (with > 2 PPP you are on the safe side), but for LML you precisely have to adapt concentration to repetition rate, in order to avoid over-heating of the spheres and their vaporization. Second, although the liquid jet setup is quite convenient and probably the best choice for systematic experimental series, you should keep an eye on it. The liquid is free, so there are two safety issues. First, errors or shifts in focal adjustment may cause liquid splashing around (optical breakdown), second, liquids with high vapor pressure flowing freely at a high speed will permanently emit gases into the workplace, bearing a risk of inhalation. In the case of flammable liquids, the risk is even higher. In this case, build a housing around (a big bottle) that is flooded and permanently supplied by the inert gas shield.

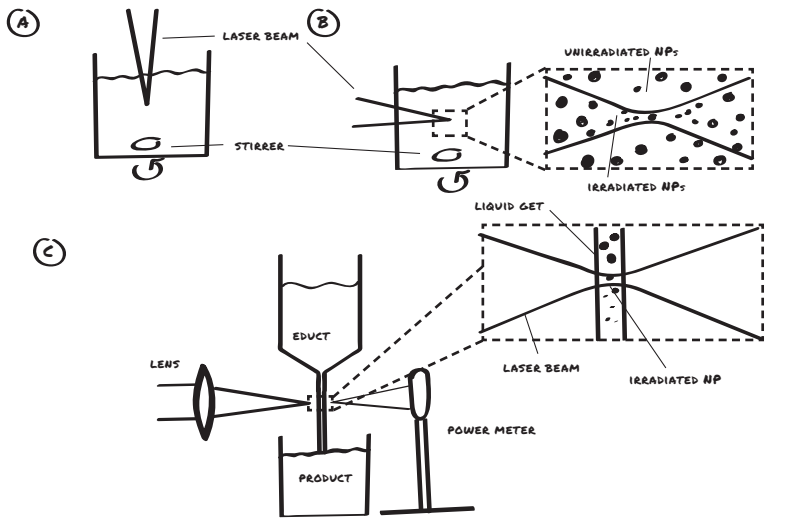
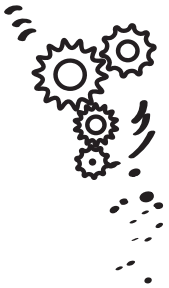


Figure 27: Set-ups for laser irradiation of suspensions; A) irradiation of the suspension from the top into an open vessel, B) irradiation of the suspension from the side, C) liquid-jet flow reactor design for improved particle irradiation.

BE AWARE OF ANALYTICAL CHALLENGES

There are some challenges, compared to LAL, you additionally might face when you want to characterize your laser-irradiated particle suspensions. You always need to consider that there can be educt particles present, that were not (or only partially) laser-processed. These particles can strongly affect your analysis result if you think of scattering or absorption methods that are usually (far) more sensitive for larger than for smaller particles. Keep in mind that you have the possibility to remove larger (possibly your educt) from smaller (possibly your product) particles, e.g. by centrifugation (Chapter I, Step 8). In the next chapter, you will also learn how to size tune your particles in several case studies to get a better feeling for what you have learned so far.

CHAPTER IV



TUNE IT: SIZE CONTROL STRATEGIES

IV

Based on the previous chapters you are well equipped to fabricate colloids by ablation (LAL) and try some downsizing (LFL) or melting (LML). However, what will you do in case your boss, your project partner or in the worst case the nasty reviewer of your beloved manuscript draft are not satisfied and criticize your particles are either I) too big, II) too small, III) show particle size distributions which are too broad or IV) contain different particle modes? To counter these unpleasant naggers, we would like to teach you simple strategies by which you can either adapt your particle formation kinetics "in situ" to produce particles with a different particle size distribution or directly alter the particle size distributions of your laser-fabricated colloids by post-processing. Often, only the interplay of both approaches can give you the maximum efficiency in size control. Furthermore, you need to keep in mind that not all strategies are applicable for all sorts of materials in the same way, e.g. approaches useful for metals may not always be applicable to oxides. Hence, this chapter is subdivided into 6 typical case studies (or duties), which will give you handy solutions for typical size control problems, destined to occur during your studies on laser-generated nanoparticles. Hence, these case files are far more specific than the previous methodological chapter.

CASE 1 - Harvest smallest particles: Your gold or platinum nanoparticles in water are too big (average diameter 10-30 nm) and you need to reduce the average size down to < 10 nm:

As briefly introduced in Chapter I, one easy way to produce noble metal nanoparticles (like AuNP, PtNP or PdNP) with lower average particle diameters is the "salt quenching trick". Herein you use very low amounts (micromolar concentration) of electrolytes e.g. table salt (NaCl) added into your stock solution of water before LAL synthesis. Be aware that only one tiny grain of salt per liter is sufficient. Higher amounts will already be counterproductive because they will significantly reduce the colloidal stability and initiate aggregate formation (see Chapter VI), called salt screening. The mechanism of size quenching is based on anion adsorption onto the nanoparticle surface, which increases surface charge and inhibits particle growth during nanoparticle formation by electrostatic repulsion. If the salt concentration is low (micromolar, typically 100 micromole), then this stabilizing effect of the anions is higher than the destabilization induced by the cations (of the salt) present as well. However, not all salts are evenly well suited. Hence, you should preferably use anions that are electrostatically "soft" like Cl-, Br- , carbonate or phosphate buffer, as they are big enough with high polarizability and therefore do adsorb very effectively onto non-polar sites on the noble metal nanoparticle surface, thereby increasing the charge (density) of the nanoparticle (surface) and its colloidal

stability. On the other hand, you should avoid "hard" anions (e.g. F^-) having low polarizability and very small size. These anions with a highly localized charge can hardly interact with the hydrophobic metal gold surface and are therefore unsuitable for stabilization. Another big "no-go" are multivalent cations (e.g. Ca^{2+} or even worse Al^{3+}) because they shield the stabilizing nanoparticle charge much more efficiently than monovalent cations like Na^+ and therefore strongly favor aggregation. Furthermore, you have to be aware that your size-controlling salt needs to be compatible with the application, e.g. in catalysis, you often can't use halides (like Cl^-, Br^- etc.). Here you should switch to using phosphates, hydroxides or (hydro)carbonates for size control, which in our experience work very well. A closely-related but evenly simple approach is tuning the pH of your ablation medium to the alkaline regime. In this case deprotonation of surface groups initiates a higher surface charge density, which inhibits particle growth and leads to smaller particles. With carbonates or phosphates, a buffer system is easily created, so that these anion types catch two birds with one stone.
The addition of organic stabilizer molecules, e.g. short thiols (i.e., lipoic acid or mPEG) or sulfates (i.e., dodecylsulfate) to your stock solution (before ablation) works in a similar way and can be highly efficient for size quenching, however, this strategy should be avoided in case ligand-free colloids are your aspired products. Here, anions are the most elegant way, still providing a highly active (accessible), naked nanoparticle surface.

During my research internship I was working with centrifugation of colloids to separate big particles from smaller one. To avoid unbalances I filled my tubes all equally to a certain volume and inserted them into the tube holder. For saving the time I chose the highest rotation number, which was 5000 RPM. Once I started the centrifugation, everything went so fast. During the acceleration the sound was slightly different than commonly and suddenly the centrifuge itself started to dance on the table. A moment of shock came into me and my heart beats strong and fast. Immediately after a short second of rigidity I pushed the button to stop the centrifuge. Fortunately the speed was just at about 100 RPM and the centrifugation stopped before something worse happened. I opened it and realized that one of the tube holder was interlocked and thus didn't have the possibility to float freely which caused the unbalances. The machine seemed so monstrous to me already only at very low speeds, so that after this happening I always checked everything at least twice with care. In the meantime we purchased a new centrifuge, where the holder are already in the right position and no interlocking is feasible. However this experience always makes me wait until the full speed is reached before dedicating to other work.

Galina Marzun, Duisburg

Alternatively to in situ size quenching, you may also post-process the colloid by laser fragmentation, maybe with a UV nanosecond laser at 1-2 J/cm². And do not forget to add the anions (or surfactants) as well prior to LFL, as the product would otherwise quickly ripen back to larger sizes.

CASE 2 - Harvest medium-sized particles: Your gold or platinum nanoparticles in water are too small (average diameter 10-30 nm) and you need to increase your average diameter to >30 nm:

Generally, increasing particle size by altering the particle formation mechanism in situ is far more difficult than decreasing it. A suitable initial approach in this context is to go for a rather high fluence, or even better, high intensity. As a rule of thumb, you can memorize that the mass share of bigger particles becomes higher with increasing intensity, so go for picosecond pulses.

Hence, in case you have the possibility to tune your pulse duration laser pulses in the regime of a few picoseconds or less should be favored. Due to this very short pulse durations a “phase explosion” occurs (see Chapter II – productivity), usually yielding a very distinct bimodal particle size distribution with a < 10 nm fraction and a > 20 nm fraction. For silver, this is quite prominent, for gold a bit less. Now, as you intend to maximize the large fraction, do not add anions, but work in deionized water instead.

On the other hand, a nanosecond laser pulse yields only one peak in the particle size histogram, termed monomodal size distribution. However, this peak maximum is usually only a few nm larger than the first size mode (the small fraction) of the picosecond LAL product. Consequently, nanosecond pulsed lasers usually produce a lower yield of particles larger than 20 nm and a pulse duration of a few picoseconds should be favored in this specific case hunting for medium-sized particles.

Now, in order to extract the >30 nm fraction from the bimodal distribution produced by picosecond laser pulses, you will have to resort to colloid post-processing techniques. Laser melting would not work here, as the sizes will get far too large. The simplest strategy, in this case, would be centrifugation. A bench centrifuge is available in almost every lab. Hereby it is recommended to have a cooling unit attached to the centrifuge in order to work below room temperature (e.g. 7°C) and minimize particle aggregation. To use centrifugation properly, you first have to calculate the centrifugation parameters (rotation speed, centrifugation time) associated to the required particle size cut-off (here > 60 nm) by using the Svedberg equation (see Chapter I, Step 8). Once you perform the centrifugation, keep in mind to be accurate about these parameters as too long centrifugation times (as well as too high rotation speeds) will result in an increasing amount of smaller nanoparticles being present in the pellet forming at the bottom of the centrifugation tube and containing your

desired fraction. On the other hand, too weak centrifugal force or time will result in incomplete separation of the nanoparticle fractions. Remember that centrifugation time affects the cut-off diameter by a root function, whereas the rotor's speed effect is stronger (linear). Also, it is clear that vials with smaller height (or filling) are finished faster. Once you have finished the centrifugation (Figure 28 B), you just need to carefully separate the supernatant (avoid swirling up the pellet) (Figure 28 C), refill the tube containing the pellet (of big nanoparticles) with fresh water and redisperse the particles using an ultrasonic bath (Figure 28 D). To completely redisperse and dismantle all aggregates, it is also highly recommended to use a sonotrode setup in addition to standard ultrasonic bathes as the latter are usually not sufficient enough to disrupt smaller aggregates. If your particle fraction doesn't redisperse at all, even when using a sonotrode, then the applied centrifugation speed was too high. Usually, we would recommend not to exceed a centrifugal force of 3000 g in case you want to retrieve the particles from the pellet. If your particle fraction after redispersion is still too broad, you have to repeat the method several times to selectively extract your desired nanoparticles (Figure 28 E-G).

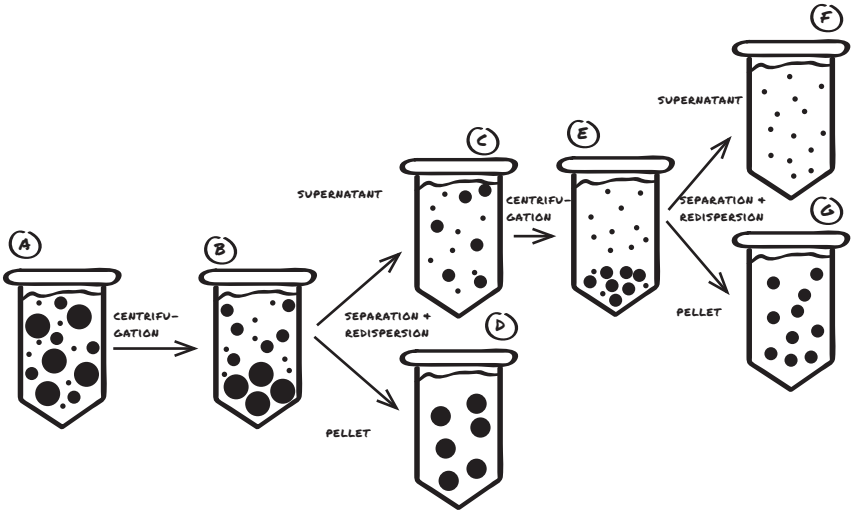


Figure 28: Sequential particle separation steps to fractionate a multimodal or polydisperse sized colloid.

Basically, you will have to perform at least two centrifugation runs, one at low speed/low time to get rid of big particle fractions and then one at a higher speed to separate the smaller particles from your desired nanoparticles of 30-60 nm. Please keep in mind that during the first step, your desired product is in the supernatant, while in the second step the nanoparticles you want are in the pellet and need to be redispersed. The primary down-side of centrifugation is that you will always lose a significant mass fraction of your synthesized particles, as aggregation of the particles in the pellet may occur.

CASE 3 - Harvest particles of strictly one size class: Your gold or platinum nanoparticles are bimodal with one mode at 10 nm which is your desired size and a second mode at 30-50 nm which has to be removed:

The formation of bimodal particle size distributions is a pretty common phenomenon during LAL, particularly when working with ultrashort pulsed lasers like picosecond pulse lengths. Even during nanosecond-pulsed LAL, we often see a wing of larger particles in the size histogram. Many applications only tolerate a single mode. As these broad distributions are often not completely avoidable, your chances of fully preventing the second mode in situ are not very good. Even though the "salt trick" (compare Case I) may reduce the second mode of larger particles, it will not completely avoid it. Now, in order to receive a monomodal colloid with a particle size of less than 10 nm, you basically have two options. The first one would be size separation by centrifugation (compare Case II). Hereby the big particles are being removed by hard centrifugation, however, you will definitely lose a significant amount of precious nanoparticle mass. Now applying the pulsed laser fragmentation technique (compare Chapter III) seems to be a good strategy to achieve the predetermined quality goal (<10 nm). Lucky for us - laser fragmentation in liquids usually exhibits the most efficient size reductions with nanoparticles in the starting size regime of 30 nm – 100 nm. Hereby, smaller nanoparticles (i.e. < 10 nm, maybe < 20 nm) will basically remain unaffected – of course depending on the laser parameters that you use. As usual, the whole story is somewhat more complicated as it always depends on the type of nanoparticle material, the liquid, and the available maximal laser parameters. As a rule of thumb the higher the laser fluence, the shorter the pulse duration and the shorter the laser wavelength the lower the particle size threshold that will be fragmented. We are also lucky that the smaller ones are not affected by LFL so that once a fraction is converted into < 10 nm ones, it is left alone not being excited again. Hence, LFL physics are somewhat auto-selective for the intended product. Good for us. For more details, you should, however, check Chapter III. The big advantage of using the laser fragmentation which in theory may be a little bit more complicated in theory compared to simple centrifugation is that you won't lose many of your little soldiers. However, it was also found that as more of the soldiers are still alive, nanoparticle colloids obtained from laser fragmentation processes are "living systems". The latter expression hereby summarizes that fragments which may only be a few nanometers in size are often subjected to minor nanoparticle growth and ripening processes hours to days after the laser fragmentation, probably caused by the formation of metal atom clusters during fragmentation. Hence, your particle size distributions could shift to larger average diameters with time, a phenomenon you need to be aware of and monitor closely when resorting to laser fragmentation for size control. Hence in case you need these very little soldiers of only a few nanometers size obtained by laser fragmentation you should either directly use them in combat (experiment) within

the first hours to days or entrench them on to the battlefield by supporting them onto a desired carrier (see Chapter VIII). The latter strategy (deposition onto a carrier material) was found to be very effective in preventing nanoparticle growth. However, in case of AuNP or PtNP, the ripening does not go on forever but stops when the nanoparticles smaller than 3 nm are gone. Hence, in case nanoparticle sizes in the range of 4 nm – 10 nm are sufficient just storing the colloid after fragmentation for a few days until ripening has stopped is recommended in order to ensure reproducibility of subsequent experiments. This ripening can be suppressed to some extent (but not totally) by the addition of soft anions prior to LFL.

CASE 4 - Harvest large-sized particles: Your gold or platinum nanoparticles need to be 100 nm or bigger:

Some applications like (bio-imaging or surface-enhanced Raman spectroscopy) require submicrometer spheres (SMS) like Au-SMS. However, as already addressed in the previous cases, LAL usually yields nanoparticles with sizes in the sub 10 nm and nanoparticles with sizes of several tens of nanometers depending on the ablation parameters used. However, in order to grow giants like SMS from these nanoparticle dwarfs, some scientists started to become laser-wielding nano-architects inventing a new post-processing technique which the proudly call pulsed laser melting in liquid (LML). In Chapter III you have already learned, that in principle the LML-setup and strategy are very similar to laser fragmentation, however, two main differences can be addressed:

- In LML, the laser fluence should not exceed the fluency range of 10 mJ/cm^2 – 100 mJ/cm^2 in case of AuNP or PtNP and 532 nm laser pulses, in order to only induce melting but not cause evaporation. However, in case of shorter laser wavelength where most materials absorb more light, lower fluencies may be required.

- The main prerequisite to induce LML is the presence of agglomerated or aggregated instead of colloidally stable nanoparticles. As a rule of thumb, the spherical volume of the resulting SMS will roughly equal the random volume of the agglomerated nanoparticles before laser melting.

Hence, to start generating your SMS you first need to use all your knowledge about how to get a stable nanoparticle colloid, reverse it and use this reversed knowledge to destabilize your nanoparticles. Of course, to gain rather homogeneous agglomerates, the primary nanoparticles you plan to destabilize should be as monodisperse as possible. To induce agglomeration, the slow addition of salt just enough to slightly destabilize your colloid is a suitable strategy. As you may remember from Case I where it is said that cations with high valency like Ca^{2+} or even Al^{3+} are a big "no-go", here they may actually be a big "go-go" as they efficiently destabilize even at low ionic strength.

For example, if you plan to destabilize your colloid with NaCl (Na^+) the ionic strength needs to be somewhere in the range of several 1000 µM. In case of $CaCl_2$ (Ca^{2+}), several 100 µM are already enough and in case of $AlCl_3$ (Al^{3+}) even several 10 µM are sufficient to destabilize a common AuNP colloid. Hereby, as a rule of thumb, higher cation valency usually yields a more distinct and controlled agglomeration. Tuning the pH may be another suitable strategy. Once the size of your agglomerates is sufficient (which you may want to monitor online via fiber UV-VIS), it is time to set the laser into cuddling mode (low laser fluencies < 50 mJ/cm_2) releasing some "I never wanna dance again" tuned laser photons towards the agglomerates so they begin melting together in passion and desire. Choosing the tunes right is the big trick. While too low laser fluencies won't get the bunched up nanoparticles fired up enough to start a relationship and melt together, too high laser fluencies may already get them heated up so hard that they already decide to vaporize and go separate ways meaning they undergo laser fragmentation already. As already said earlier, as a rule of thumb for AuNP fluencies in the range of 10 mJ/cm^2 to maybe 50 mJ/cm^2 are already suitable in case of 532 nm laser pulses are used. If the absorption of your nanomaterial at the available laser wavelength is however less than the absorption of AuNP at 532 nm (which is true for most materials), you may need to employ higher laser fluencies. Hereby using the Mie-Theory to calculate the absorption cross-section of your nanomaterial at the given laser wavelength can give you a good hint whether you need to increase or decrease romantic potential of the laser photons (fluence) that you plan on using.

CASE 5: Harvest monomodal oxides: You need oxide particles such as (doped) Fe_3O_4 or rare-earth doped Y_2O_3 for biological or optical applications, in which case monodispersity is of great importance. The polydispersity index (PDI see also Chapter V, Step 2) is often used to demonstrate the colloidal monodispersity. A PDI value of < 10% is excellent but very difficult to obtain. A PDI in the range of 10 ~ 30% means good dispersion but bears some broadness in size distribution, accessible by the methods mentioned below. When σ is larger than 30%, the particles require post-treatment to be utilized in most applications and even their characterization by many methods e.g. luminescence is greatly impaired by polydispersity. Broad size distributions with PDI >30% are commonly found during LAL of oxide materials in the absence of organic stabilizers that suppress particle growth. One issue that needs to be considered is the LAL target selection. In case you wish to generate oxide nanoparticles it is often more feasible to use an oxide target as educt material. Even if you use the metal target of a non noble metal like titanium, and you are sure that it will oxidize in water, you can never be sure that your oxidation is quantitative and that you get exactly the oxide you want. In the worst case, the application of a metal target may result in the formation of metal@oxide core-shell particles and potentially also oxides with mixed oxidation states and most likely, not the pure

oxides that you may desire.

We found that oxides are often more difficult to obtain with particle size distributions as narrow and well controlled as in metal nanoparticles. In general, monodisperse oxide particles can be achieved after particle synthesis (ex situ) and during particle formation (in situ). For the former strategies, centrifugation of the particles at high speed (as their density is usually far lower compared to noble metals) to extract the small ones can be a useful approach, similar to metal colloids. This method requires a high-speed centrifuge with a rotating speed of 18000/min or higher. As stated above, note that using centrifugation will definitely result in a significan mass loss of colloidal oxide particles compared to the fresh colloids. Hence, repeating the same procedures several times is indispensable while you want to gain a high amount of monodisperse oxide particles.

If you want to save your time and skip centrifugation of the colloids, inhibition of oxide particle growth by adding additives in the solution or fragmenting the synthesized oxide colloids provide two options. One of the good additives that has realized brilliant monodispersity of doped oxides (e.g., Y_2O_3:-Eu^{3+}, Gd_2O_3:Eu^{3+}, and $Y_3Al_5O_{12}$:Ce^{3+}) is 2-[2-(2-methoxyethoxy)ethoxy]acetic acid (MEEAA), in which case polyether chain of MEEAA anchors on the surface of the oxide particles to limit the particle growth and sharpen the size distribution. The additive concentration should be optimized since lower concentration is insufficient to inhibit the growth of all colloids while high concentration additives will lower the productivities and the decomposition of additives may pose a risk of sample contamination. Maybe a good start is something slightly below the critical micelle concentration (a value that is well documented for most surfactants). Special attention should be paid to the chemical reactions during the synthesis of oxides, such as ZnS while doing LAL of Zn in SDS solution. Even though with the help of surfactants to inhibit the particle growth, some large particles may also be generated because of the difference in the local concentration of the surfactants near the particles. In this case, successive fragmentation of the obtained colloids is still recommended to obtain fully monodisperse oxide particles. You will probably be happy working with pulsed UV lasers for oxide LFL.

UNDERSTAND IT: OPTICAL AND SOLID MATTER CHARACTERISATION

Imagine you synthesized one-of-a-kind nanoparticles and spiced them up. What would you do next? Show them to the world by putting it on your website? Make a video of them and put it on YouTube (e.g., *http://youtube.com/nanofunction*)? Or maybe you would write a manuscript about your particles and submit it to "Nature"? You should first hold your horses and imagine the following: someone gave you a shiny diamond ring and disappeared. What would you do with this ring? Run to the love of your life and propose to her (sorry this story only works if "I" am a man and "you" are a woman)? You know that this might backfire if the ring is fake. The obvious step would be characterizing it or having it characterized. Is it a gold ring? Is the diamond real? What is its clarity, color grade, shape, Carat weight? All these questions need to be answered for a proper estimation of its value and if it's suitable for the love of your life. Same thing goes for your nanoparticles. You need an exhaustive characterization to fully identify and confirm the nature of your products.

The questions that usually arise in front of a sample of nanoparticles with unknown nature (no matter if obtained by laser ablation or by other methods) can be summarized as follows (Figure 29):

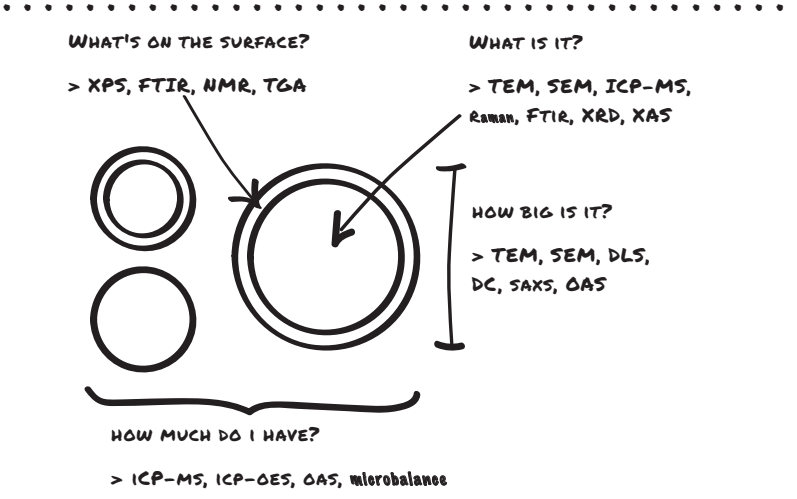


Figure 29: Illustration of nanoparticles and questions that arise while trying to understand them. Once this is done, function (e.g. optics, catalysis, biomedicine) can be attributed to the structure. This structure-function-relationship is exactly what everyone is hunting for.

To answer each of these questions, you can use the different characterization methods in a step-by-step approach, as described in the following paragraphs and summarized in Figure 29. Of course, depending on the material and synthesis objective, the sequence of these questions can change from case to case. In fact, characterization of nanomaterials is often non trivial and Ancients would have said that we must proceed "cum grano salis" (Latin for "with a grain of salt", i.e. use your brain before acting!) to avoid misinterpretation of results and exponential growth of the research work. In particular, for each technique, it is important to have very clear in mind the answer to the following questions:

- A. What do I see?
- B. How does it work?
- C. Which amount of sample is needed?
- D. How do I prepare the sample for analysis?
- E. Are there any tips/ warnings/hazards?

Let's see how the answer to the above questions can help us to achieve a perfect knowledge of our laser-generated nanoparticles.

STEP 1: PROVE THAT YOU ACTUALLY HAVE "NANO" PARTICLES

Try to ask any Italian who was abroad: he/she will confirm that not enough to have pasta, tomatoes, salt and water to make a genuinely Italian style pasta... The same goes for the synthesis of nanoparticles by laser ablation: it is not necessarily true that shooting the laser at an object immersed in a liquid is enough to collect a colloidal nanoparticle solution (or make a paper on such "shoot and run" experiment and ruin your reputation). Therefore, first of all, we need direct evidence on the existence of nanoparticles in our sample.

The "Queen" of characterization techniques for nanoparticles is, without any doubt, transmission electron microscopy (TEM), because of its unmatched spatial resolution and completeness of achievable data. Though with lower spatial resolution, scanning electron microscopy (SEM) permits in most cases the direct visualization of nanoparticles with size larger than ~ 5 - 10 nm (and larger particles often may be a mass-dominant side product). A great advantage of these electron microscopes is that they can analyze a single nanoparticle at time. A relevant consequence of this peculiar ability is that electron microscopes are the only methods allowing real and direct "number weighted" analysis (i.e. by counting nanoparticles one by one), contrary to all the other techniques that only give volume-weighted results (i.e. measuring signals which are proportional to the x-the power of the volume of each nanoparticle, with $x \geq 1$).

However, TEM and SEM are not easily or immediately available, and analysis of one particle by one may be time-consuming without the support of appropriate software (e.g. the freeware ImageJ). Also, EM techniques provide bad statistics (< 1000 particles of millions in a drop) and tend to over-estimate small particles. In this case, the quickest and simplest way to check the existence of nanoparticles in a liquid medium is the optical absorption spectroscopy (OAS). In fact, visible (vis) and ultraviolet (UV) light interact in a very peculiar way with objects of nanometric size homogeneously dispersed in a transparent matrix. Besides, when dealing with nanoparticles dispersed in a liquid matrix, OAS does not need any sample preparation. A great advantage of OAS is that it can be performed also in real time during laser ablation or irradiation experiments, especially if the absorbance is monitored at a single wavelength, by coupling a photodiode with a laser beam crossing the sample chamber.

In a limited number of cases, depending on the properties of nanoparticles under investigation, fluorescence spectroscopy (FS) can be a viable technique for immediate characterization of nanoparticles which emit light. Indeed, emission bands are usually located in a smaller spectral range than absorption bands for the same compound (i.e. fluorescence bands are more specific and easier to recognize). Besides, the position and intensity of fluorescence bands in semiconductors are distinctive of their size. Let's look at the features of these techniques and what we can learn from each of them.

STUDENT: I THINK THE LASER DOESN'T WORK ANYMORE. FOR THREE SAMPLES I DIDN'T ABLATE ANYTHING EVEN AFTER 15 MINUTES.

ASSISTANT: DID YOU REMOVE THE PROTECTION CAP OF THE SCANNER?

STUDENT: YES.

ASSISTANT: DID YOU PRESS „START" AT THE LASER? STUDENT: YES.

ASSISTANT: DID YOU TURN ON THE SCANNER?

THE STUDENT MADE A THUNDERSTRUCK FACE AND MOVED BACK TO THE LASER WITHOUT ANY FURTHER COMMENTS.

NINA MILLION, ESSEN

1.1. Optical absorption spectroscopy (OAS).

A. What do I see?

By OAS, we see if nanoparticles are present in the liquid medium. In the large majority of cases, OAS is performed with bench-top spectrometers, giving an averaged information on all the nanoparticles dispersed in the solution, clearly meaning that it is not a single particle technique.

In case of nanoparticles with well defined absorption bands, OAS gives the absolute certainty of their presence. If the absorption band is related in some way to nanoparticles size and shape, this information is also achievable, such as in case of plasmonic (Figure 30, left) and semiconductor nanoparticles, although the precise interpretation of the results requires an accurate elaboration of the experimental spectrum with complex theoretical models.

Some polymer, oxide or wide bandgap semiconductor nanoparticles may not have well-defined absorption bands in the UV-visible range. In that case, OAS can still detect the presence of nanoparticles due to light scattering effect, whose intensity scales with the -4th power of wavelength ($\propto \lambda^{-4}$, the so-called Rayleigh scattering curve). Once you've seen this characteristic curve (Figure 30, right), you can be sure to have submicro or nano inside.

B. How does it work?

The principle of OAS is simple: a light beam with well-defined wavelength (λ) is transmitted through the sample, typically contained in a cuvette or on a transparent substrate. A light intensity detector is placed behind the sample. The procedure is repeated in a range of wavelengths predetermined by the operator. At each wavelength, the ratio between transmitted (I) and incident light (I_0), called transmittance (T), is measured. More useful is the absorbance (Abs) defined as the

$$Abs = -log_{10}(T)$$

because it is proportional to the concentration (C) of absorbing objects according to the Lambert-Beer law:

$$Abs = \varepsilon b C$$

where b is the length of the path travelled by light in the sample ("optical path", it depends on the sample geometry), and ε is the coefficient of molar extinction (it depends on sample nature, being higher for highly absorbing objects).

It is worth to stress that two distinct phenomena contribute to the decrease of I/I_0 light "absorption" and "scattering". Absorption means that photons are annihilated and their energy transferred to the absorbing object, whereas scattering means that photons just change their travel direction, and will not reach the light intensity detector behind the sample, without releasing energy

to the scattering object. In case of "real" absorption, it is often possible to recognize distinct peaks in the spectrum (Figure 30, left). In case of scattering, the spectrum shows a typical $\sim\lambda^{-4}$ trend, without a clearly identifiable absorption peak (Figure 30, right).

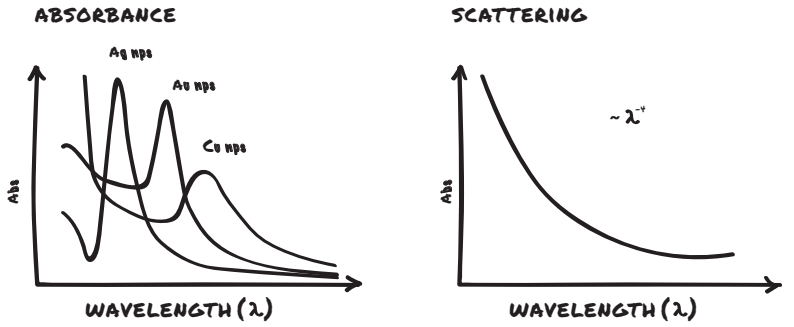


Figure 30: Typical OAS spectra of purely absorbing (left) or scattering (right) nanoparticles.

c. Amount of sample needed?

A great advantage of OAS is that, with the use of appropriate cuvettes, 100 µL of solution may be enough, although typically 0.5 – 3 mL of liquid is required. However, the detection of samples depends on the combination of three factors in the Lambert-Beer law (b, ε and C). When nanoparticles are present at very low concentration in the liquid solution, the simplest way to improve the signal intensity is to increase the optical path (b) of the light beam passing through the sample. This can be done using cuvettes with 1 cm or even longer optical path. In ordinary spectrometers, the optimum interval of absorbance is between 0.5 and 2.5. Obviously, ε is a material property and cannot be modified. Most spectrometers are not linear anymore above absorbance of 2, dilution is a simple workaround.

d. How do I prepare the sample for analysis?

In the majority of cases, you just need to fill the cuvette with the solution. However, if nanoparticles are not stable in the liquid phase for the time required from the analysis (< 5 minutes), they can be included in a polymeric matrix (e.g. add PVP) and/or deposed on a transparent substrate (i.e. a microscope slide).

e. Tips / warnings / hazards?

The simplest way to stabilize an aqueous colloid is: add a pinhead-sized piece of soap (the cheap, solid one, not the colored and perfumed). Of course, any organic additive will screen the UV part of the spectrum, same with plastic cuvettes compared to quartz cuvettes. In water, sometimes sodium hydroxide

helps for stabilization (adjust pH around 10 or 11), keeping the UV range free for analysis.

You must consider that absorption and scattering are phenomena strongly correlated to nanoparticles volume. In particular, absorption scales linearly with the volume of the nanoparticle, whereas scattering scales with the 2nd power of the volume. As a consequence, the information obtained by OAS is the volume (or volume^2) weighted average of the nanoparticles in the sample.

Store and handle it cold (fridge is enough, don't freeze) to minimize sedimentation.

1.2. Transmission electron microscopy (TEM) imaging.

A. WHAT DO I SEE?

TEM allows the direct visualization of a single nanoparticle with nanometric or subnanometric spatial resolution. In the high-resolution modality (HR-TEM), atomic planes and rows within the single nanoparticle are visible. This gives straightforward access to a series of information such as nanoparticle size, size distribution, structure homogeneity or, alternatively, phase segregation, formation of shells, crescents and heterostructures, crystalline order, lattice defects.

B. HOW DOES IT WORK?

The principle is the same as an optical microscope, with the remarkable difference that a monochromatic electron beam is transmitted through the sample, instead of a photon beam. Electrons are scattered (sometimes also absorbed) by nanoparticles, with an efficiency which depends on the square of the atomic number (z) of elements composing the nanoparticle, and on their crystalline order and orientation. This generates a contrast between electron dense and not dense regions (i.e. an inorganic nanoparticle and a thin carbon film acting as substrate), which is at the basis of image generation. As in the optical microscopy, the spatial resolution depends on the wavelength of electrons, which is determined by their kinetic energy according to the De Broglie equation (corrected for relativistic effects due to the high speed of electrons in a TEM):

$$\lambda_e \approx \frac{h}{\sqrt{2m_0E\left(1+\frac{E}{2m_0c^2}\right)}}$$

where h is Planck constant, m_0 electron mass, c light speed and E the energy of the accelerated electron. The consequence of the De Broglie equation is that, usually, nanometric spatial resolution is possible with TEM operating at 100 kV of accelerating voltage, whereas subnanometric resolution is possible only with acceleration voltages of 200 kV or higher.

c. Amount of sample needed?

Being a technique with single nanoparticle capability, virtually 1 nanoparticle is enough. Obviously a statistic analysis of each sample is possible only by considering more than N~500 nanoparticles each time. In practice, 1 – 10 μL of solution are always enough.

d. How do I prepare the sample for analysis?

Nanoparticles must be deposed on TEM grids, which host a thin film with high transparency to the electron beam, such as nanometric thick carbon films or Si_3N_4 membranes. A drop of solution can be casted on the grid and left drying in air (TEM operates in vacuum to avoid interference of atmospheric gas with the electron beam, therefore samples must be dried prior to analysis). If there are non-volatile organic residues in the sample, prior plasma cleaning (standard TEM sample preparation equipment) may be required to improve the contrast.

e. Tips / warnings / hazards?

The electron beam deposits a considerable amount of energy on the nanoparticles, and this energy increases with increasing magnification (the energy density per unit area increases). Therefore, care must be used when observing materials which undergo modifications under electron beam illumination (especially organic nanoparticles and semiconductors). Even noble metals or refractory materials can undergo modification, melting, segregation, regrowth, and etching when exposed to an electron beam. This is especially true for laser-generated nanoparticles, whose surface is clean, uncoated and very reactive, and whose composition can be metastable. Nanoparticle modification can usually be observed in real time during TEM imaging and can be reduced only by lowering the magnification (i.e. decreasing the energy density for unit area), the exposure time and the TEM operating voltage. Another workaround is dilution. If you see peanut-shaped gold particle twins, they often are artifacts from fusion caused by the electron beam.

He checked out his setup and examined all the optical parts on the table. He started the laser and frightened because of a machine gun-like regenerated noise. After he switched off the laser he examined what happened. It took him several minutes to find out the reason but he realized that his laser had drilled some holes in the coating of the laboratory door. Obviously a loose mirror had twisted independently.

Sebastian Kowsakowski, Duisburg

1.3. Scanning electron microscopy (SEM) imaging.

A. What do I see?

It gives the size and morphology down to the single nanoparticle level, for objects larger than about 5 – 10 nm. Due to the spatial resolution of ~1-10 nm, morphological information is reliably extracted only for larger objects (> 10 nm).

B. How does it work?

A focused electron beam is scanned over the sample area, and a detector collects the electrons emitted from the sample. There are two types of electrons emitted from the sample under electron beam illumination, and both can be used for image formation in a SEM: backscattered and secondary electrons. Backscattered electrons are high-energy electrons of the incident electron beam, reflected from the specimen by elastic scattering interactions with sample atoms. Secondary electrons are extracted from atoms in the specimen by an inelastic scattering process promoted by the interaction with the electron beam. Each type of electrons is collected by a distinct detector, which works pretty much like a photocamera that collects the light reflected by a shiny three-dimensional object. Therefore, SEM images contain information on surface topography of the sampled area and show the typical "light and shade" effect due to the directional imaging.

Backscattering is more intense with high – z elements, providing complementary information to secondary electron imaging in samples where high – z compounds are deposed on low – z substrates. In this imaging modality, heavier atoms appear brighter.

Note that today's SEMs provide transmission mode as well, it's called "STEM" (ask operator if the instrument is equipped with this option, it's also not too expensive to buy later for modern instruments, because it's essentially just a sample holder), and before said for TEM is true as well for this STEM technique.

C. Amount of sample needed?

Same as TEM, few μL of solution is enough.

D. How do I prepare the sample for analysis?

Same as TEM, a drop of solution can be casted on a substrate and left drying before analysis (also SEM operates in vacuum). The substrate should be as flat as possible (in this way, topographical information will enhance nanoparticles over the flat surface), conductive, and should allow z-contrast with your nanoparticles. For instance, carbon tape for electron microscopy is suitable for oxides, and doped Si is very well suited for metals and almost all types of nanoparticles with elements heavier than silicon.

e. Tips / warnings / hazards?

Specimen topography is greatly affected by any impurity or solute (i.e. salts, surfactants, chemicals, synthesis by-products) eventually present in the solution and forming a matrix embedding nanoparticles in the dried sample. The smaller the nanoparticles are, the higher must be the purity of the liquid.

You get nice pictures from your particles if you tilt the sample to the maximum possible: in this way you will get nice 3D appearance. Note that this modus it is not accurate to measure height, it's just to make it beautiful.

1.4. Fluorescence spectroscopy (FS).

a. What do I see?

Only a limited number of materials shows appreciable fluorescence: in these lucky cases, FS easily allows the detection of these compounds in your sample. In case that fluorescence bands are size-dependent, direct proof of the presence of nanometric particles is achievable at the same time!

b. How does it work?

In FS, photons are emitted by the sample as a consequence of a light absorption process. Therefore, monochromatic light is extracted from a lamp and conveyed to the sample, where it is absorbed by the fluorescent compound (fluorophore), whose electronic structure passes from the ground state to an excited state. Fluorophores have the ability to release an appreciable fraction of the absorbed energy by emission of other photons, which have longer wavelength (lower energy) compared to the absorbed ones. The photons emitted by the sample are dispersed by a diffracting optic and counted by a photodetector. The fluorescence spectrum reports fluorescence intensity (in arbitrary units) versus photon wavelengths. Absolute fluorescence efficiency (defined as the ratio of emitted to absorbed photons and called "quantum yield") is achievable only by comparison with a standard with known quantum yield in the same spectral range of the sample.

c. Amount of sample needed?

Typically 2 – 3 mL of liquid solution with absorbance < 0.15 in the excitation and emission spectral regions. The solution is placed in a 1 cm x 1 cm cuvette.

d. How do I prepare the sample for analysis?

As in OAS, you just need to fill the cuvette with the solution. However, if nanoparticles are not stable in the liquid phase for the time required from the analysis (< 5 minutes), they can be included in a polymeric matrix and deposed on a transparent substrate (i.e. a microscope slide). The slide can be analyzed with a ~30° or ~60° geometry to the direction of excitation and of the collection

(which are at an angle of 90° one each other). Avoid placing the film at 45° to minimize reflection of the excitation beam toward the detector.

E. Tips / warnings / hazards?

In principle, fluorescence intensity is proportional to the concentration of fluorophores. However, self-absorption takes place already at very low absorbance. Therefore care must be used in the choice of the absorbance of the sample for analysis. Absorbance higher than ca. 0.15 in the absorption and emission spectral regions usually results in artifacts due to self-absorption. Run calibration curves to find a robust concentration range of linearity.

STEP 2: MEASURE THEIR SIZE

Nanomaterials take their name from their size, hence sizing the nanoparticles of our sample is an obvious step. Therefore, an obvious question arising about your colloid is "What is the size of your nanoparticles?". Pulsed laser ablation generally yields not one defined particle size but a whole "zoo" of particles composed of different sizes. So a more meaningful question in this context would be: "How many particles of which size can be found within your colloid ?". This correlation between the frequency of particles in a colloid and the individual particle sizes is called a particle size distribution. The main points raised in this paragraph will be how to interpret particle size distributions, how to measure them and finally how to alter them by simple methods during laser ablation in liquids.

When measuring the zeta potential of my colloids, the values fluctuated quite a bit. When I saw the measuring cell, I also knew why: There was no liquid left in the cell. The measuring cell was not resistant to the solvent I used. Furthermore the solvent leaked in the device. So I spent some time cleaning the device. Always remember to check if the lab equipment withstand your solvent, so you'll save the subsequent cleaning!

Jasmin Paduska, Duisburg

Expressing particle size distributions

Particle size distributions can give a lot of interesting information about your colloid. In this context, however, you need to keep in mind that there are at least three meaningful ways to express particle size distributions in colloidal science, which require weighting based on number, volume, and surface. In a number weighted size distribution, you basically count the number of particles in your sample associated to a certain size class, e.g. all particles with a diameter of 20 nm and plot this number against the respective particle size. If you repeat this procedure for all particle sizes in your sample, you will end up with your number weighted size distribution as illustrated in Figure 31 A. Number weighted size distributions are generally highly relevant as they are the direct result of size distributions obtained from electron microscopy. And this is the standard in the literature. Unfortunately, for most "nano-functions", this statistic is less meaningful (and sometimes without any meaning), as most functions are proportional to either the volume or mass (e.g., ion release, optical effects) or the surface (catalysis, bio-response, ...) of the particles. In a volume-weighted size distribution, on the other hand, you basically plot which volume is filled by all particles of a certain size class, e.g. which is the combined volume of all particles with a diameter of 20 nm, and plot this against the particle size (Figure 31 C). Volume weighted size distributions are highly relevant as they are generally the data obtained from light scattering measurements. In a surface weighted particle size distribution (Figure 31 B), the particle surface area found in a certain size class is displayed. In case you know the geometry of your particles, conversion of the different size distributions is pretty straightforward. During laser ablation, you are generally lucky as most nanoparticles can be considered spherical and $V_{sphere}=4/3\ \pi\ r^3$ and $A_{sphere}= 4\ \pi\ r^2$ are the only equations you will need. When you compare the particle size distributions depicted in Figure 31 you will notice that volume

The person prepared his sample and measurement device for the automatic pH dependent zeta potential measurement, to find out the isoelectric point of his sample. He left the device to go to lunch. After one hour he came back with his colleague, examines the measurement and immediately began to grumble on the device. The zeta potential was only measured for one pH-value. His colleague examined the device and started laughing. His college forgot to switch the pH electrode from buffer solution into the device.

Sebastian Kohsakowski, Duisburg

weighted size distributions generally over-represent larger particles, while number weighted size distributions are shifted to smaller particles and surface weighted size distributions are located in the middle. This is a universal rule and it is important to consider this correlation when further interpreting particle size distributions.

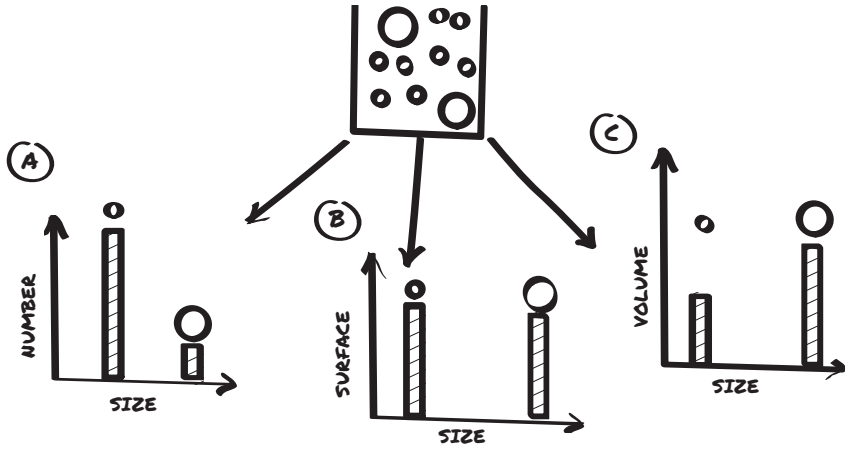


Figure 31: Illustration of particle size distributions for a typical example of laser-generated particles with 2 different particle sizes (bimodal): (A) Number weighted, (B) Surface weighted, (C) Volume weighted particle size distribution. It's the same sample, and all results are "true".

As you have now learned about the different types of particle size distributions you may now pose the question: "Which of these distributions do I use and when?". In general, all particle size distributions carry the same information and are equivalent. However, in some cases one or the other may be physically more meaningful. For example, when you want to support metal nanoparticles on metal oxide carriers (e.g., for heterogeneous catalysis), this is usually expressed as mass load and hence volume distributions should be used as volume is directly proportional to mass. On the other hand, in many relevant areas of colloidal science e.g. toxicity, binding of surface ligands and catalytic reaction rate, the surface area is the driving factor and surface weighted size distributions should be used in this case. So there is no simple answer to this question as it highly depends on the addressed application, this is something you can choose. Once chosen, clearly name it, as "size" is not clear. For example state "number-weighted average particle diameter measured by TEM".

Ups, we smuggled the term “average” into that quote. Is that the same as “median” or “mean”? Hence, the next thing you may want to learn is how to interpret particle size distributions.

Interpreting particle size distributions

In all cases, nanoparticles will not be identical to each other, and the average size and the size distribution can be used to describe our sample. By measuring the size of a reasonable large number of particles (N), generally larger than 300, a size histogram can be generated. When particle size distributions are fitted with an appropriate mathematical model, its interpretation is much more straightforward. Nanoparticles obtained by laser ablation usually show lognormal size distribution $f_{LN}(d)$:

$$f_{LN}(d) = \frac{1}{\sqrt{2\pi}(wd)} exp\left[-\frac{1}{2w^2}\left(log\left(\frac{d}{d_{max}} \right) \right)^2 \right]$$

where d_{max} is the point of maximum and w is the parameter determining the width of the distribution. Conversely, a Gaussian size distribution $f_G(d)$ is often observed in nanoparticles obtained by laser irradiation (e.g. after laser fragmentation):

$$f_G(d) = \frac{1}{\sqrt{2\pi}\sigma} exp\left[-\frac{(d - \langle d \rangle)^2}{2\sigma^2} \right]$$

where $\langle d \rangle$ is the mean size and σ its standard deviation.

In general, you have multiple options to present the most abundant (average) particle size of a size distribution fitted by a Gaussian or Log-normal curve. The first and most obvious way would be to look at the peak maximum (= mode) and to pick the value with the highest abundance and to blank out all other particles. Other common ways of presenting average values in particle size distributions are the mean and the median value. While the mean represents the sum of all particle sizes, divided by the number of all particles (that is the average), the median or D_{50} represents the value where exactly 50% of the particles are larger and 50% are smaller. Generally,

mean (average) > median (D_{50}) > mode (peak)

is a rule of thumb. The difference between these values is illustrated in Figure 32.

Indeed, the most general parameters for identification of nanoparticle size (derived from Gaussian distribution) is the average size expressed as the arithmetic mean $\langle d \rangle$ and its standard deviation σ, expressed as

$$\langle d \rangle = \frac{1}{N} \sum_{i=1}^{N} d_i$$

$$\sigma = \sqrt{\frac{1}{N} \sum_{i=1}^{N} \left(\langle d \rangle - d_i \right)^2}$$

The advantage of $\langle d \rangle$ and σ are twofold: 1) they are of immediate interpretation for the comparison of different samples with different size distributions, and 2) they can be always extracted from any dataset. The disadvantage is that only the size histogram tells you all the truth on the polydispersity and mono or multimodality of a sample. Therefore, additional useful information are the size range (i.e. minimum and maximum size observed) and the width of the size distribution, obtained by fitting the size histogram with lognormal or Gaussian curves.

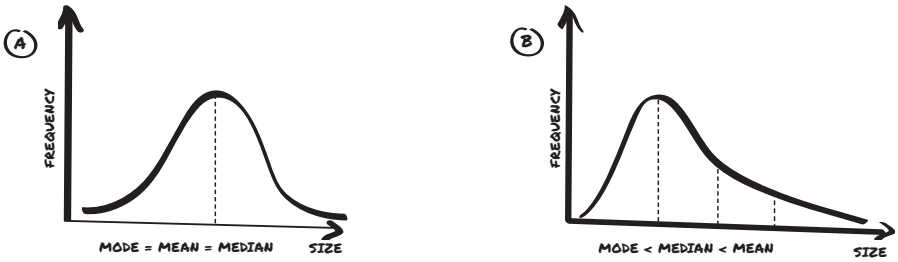


Figure 32: Illustration of mode, median and mean values during the interpretation of (A) Gauss and (B) log-normal fitted particle size distributions.

Based on what you have learnt so far, you can definitely say that the presentation of an average particle size is far from trivial. You need to consider the type of particle size distribution as well as the mathematical model. Consequently you will have to be extremely careful when presenting and particularly when comparing average values from different studies. And if someone tells you about the size he made: ask how it was weighted and how the maximum was found. Meaningless: “size of 3 nm”. Precise: “Number-weighted, lognormal mode particle diameter measured by TEM” or “Surface-weighted Gaussian mean diameter of 3 nm +/- 1 nm, as measured by XY”.

The second point which needs to be addressed about particle size distributions is that you need to differentiate between the modality and the dispersity. Modality indicates whether there are two or more distinct populations of

particles within a particle size distribution, which can be clearly differentiated. The clearest indication is a disruptive size distribution with multiple peaks. Based on this, a colloid can be characterized as monomodal (having one peak/most abundant size) or bi-, tri-, multimodal (having more than one peak/most abundant size). Interpretation of multimodal size distributions usually necessitates fitting of every mode with an individual fit function, an endeavor which may be tricky, particularly in case there is a certain overlap between the different modes.

CH4

My student and I were performing a fragmentation experiment. I was just checking in on him when I scented a funny smell. After some time I realized the bright spot on my sleeve originating from some laser reflection. It was a mirror that some other student had muddle headedly put in the wrong box.

V

Sven Reichenberger, Duisburg

On the other hand, dispersity indicates how broad your particle size distribution is. Dispersity is generally used to characterize monomodal size distributions or individual modes within a multimodal distribution. The difference between dispersity and modality is illustrated in Figure 33. So within a multimodal size distribution, each mode can be characterized concerning its dispersity. Suitable ways to characterize this value are the standard deviation and the variance. Both can be taken directly from your fit function (mostly log-normal) and give you a rough idea about to what extent the different particle sizes scatter around the average value, generally the higher the variance and the standard deviation, the broader the size distribution. Based on these values one can define the polydispersity index (PDI), calculated via: PDI = variance/mean^2. In this formula the Gaussian "mean" may be substituted by the Lognormal mode (x_c). Note that variance is usually displayed in units of nm².

E.g. a diameter of "5 +/- 2 nm" tells: mean of 5 nm and standard deviation is 2. But 2 nm is not the variance! Depending on the software you use, you might not get the variance or standard deviation. For instance, OriginPro gives you the width (w) of your Gaussian fit, which equals to two times the standard deviation. The variance (that you need for a PDI calculation) in turn is obtained by squaring the standard deviation (in case of Gaussian distribution). Hence, 5 nm +/- 2 nm is monodisperse (PDI = 0.16), but 5 nm +/- 3 nm is not (PDI = 0.36).

Based on their PDI, particle size distributions can be characterized as monodisperse (having a narrow size distribution and a PDI<0.3) or polydisperse (having a broad size distribution PDI>0.3). However, you need to keep in

mind that these definitions are by no means fixed within the nanoscience community and can greatly differ based on what PDI values are achievable by the corresponding standard technique. For example, you may proudly call your laser-generated colloid monodisperse based on the above-mentioned definition, however a chemist synthesizing oligoatomic clusters with atomic precision or a physicist requiring very narrow size distribution e.g. for optical application may tend to strongly disagree with your definition even laugh at you or even worse, reject your beloved manuscript during peer review.

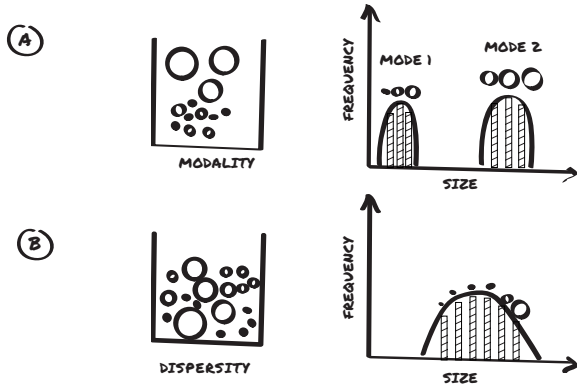


Figure 33: Modality and dispersity: (A) Illustration of a bimodal particle size distributions. (B) Illustration of a monomodal, polydisperse particle size distributions.

Note that it is easier to achieve monodispersity with number-weighted histograms than with volume-weighted histograms. If you hunt for very high quality, rate your own colloid by volume-weighted criteria. The other statistics will always be better so that you're on the safe side.

So up to this point it can be concluded that asking for "nanoparticle size" is a huge simplification and needs to be expanded to the question of the particle size distributions within the colloid. Furthermore, you have learned how to express and term a particle size distribution weighted by number, surface or volume and how to analytically interpret them based on the average value, modality, and dispersity. Now you know about the interpretation of particle size distributions, a suitable follow-up should be how to measure them.

Measuring particle size distribution.

Here you can basically differentiate between four types of methods: electron microscopy, dynamic light scattering, analytical disc centrifugation and small angle x-ray scattering.

According to what was stated in the previous paragraph, TEM (and SEM in case of particles larger than 5 – 10 nm) allows direct imaging of nanoparticles and consequent identification of their shape and size distribution. However, laser synthesis frequently gives a sol, i.e. a colloidal dispersion of solid particles in a liquid matrix. Although the sol can be stabilized by the presence of surface charges on particles surface, the formation of agglomerates of nanoparticles with final size larger than that of its single constituting "monomers" is very frequent in laser-generated nanomaterials (as will be discussed in Chapter VI). Due to spontaneous soft agglomeration or hard aggregation of nanoparticles on a substrate during solvent evaporation, the size of these assemblies in the liquid phase is not accessible by SEM. It can be accessed by TEM only using cryo-microscopy (a type of microscopy carried out below the freezing temperature of the solvent, which is very time consuming and expensive!), or by embedding the nanoparticles in a polymeric matrix which can "artificially" freeze the aggregation state in solution (you will find literature reports about how to do it in detail).

V

We had a student who has been working on the laser for a couple of days. One morning he was working within the laser lab with a colleague being present. After several ablations and breaks he put on his laser safety goggles and started the laser for the next ablation. However, he didn't make sure that everyone else in the lab was wearing their laser safety goggles. The colleague noticed the sound of the ablation and was able to react right away. When this break in safety procedure was addressed the student tried to make excuses stating that the colleague was wearing his goggles some time before. However, it is the responsibility of the laser operator to ensure that everyone within the lab wears the personal equipment prior to turning on the laser every single time.

Elisabeth Maurer, Essen

The size (d) measured by electron microscopy is the geometric size, also called Feret diameter (Figure 34), and it is defined as the distance between the two parallel lines tangential to the object, as if you use a caliper to measure particle diameter.

In comparison to all other techniques, electron microscopy has a couple of striking advantages. The main point is that it will give you a direct insight into the shape of your nanoparticles and tell you the exact size of the metal core (Feret diameter). However, electron microscopy also suffers from some drawbacks when it comes to particle size distributions. First, unless you use cryo-microscopy or a trick such as inclusion in a polymeric matrix, it works on dried samples, which means you can obtain no information about your particles in the colloidal state. For example, information, whether your particles are agglomerated in the colloid, are very difficult to verify with electron microscopy as particle agglomerates you see in the images could also form during sample drying. The next drawback you have to face with electron microscopy is limited statistics. When you look at typical TEM images of nanoparticles, you usually find, depending on the resolution, up to a few hundred particles per image.

Just for comparison, 1 mL of a gold colloid with a mass concentration of 50 mg/L (that's already reddish in color) and a particle size of 10 nm will contain about $5{\cdot}10^{12}$ particles, which means you will have characterized only ~10^{-8} % of your sample with TEM. As a result of this, proper TEM image interpretation necessitates that your sample is very homogeneous and thorough mixing prior to loading your sample on the carrier is absolutely vital. In addition, even this characterization is very labor intensive because you usually have to measure all particles by hand. Even though there are algorithms for automated analysis of TEM images, they usually fail for non-uniform particles or overlapping particles, which are unfortunately quite common in laser-generated colloids. So your will have to get used to spending hours staring at your computer screen. But the public domain software ImageJ is for TEM image analysis, as its very useful plugin "ParticleSizer" has been made available recently. Great news, it can now handle even aggregates, as it differentiates overlapping particles, and it shows you an image of the red-circled particles in your TEM micrograph so you can check if you want to trust that automated analysis run. We are quite satisfied with it. And it runs a set of images in a folder automatically in a batch.

Other techniques exist that can measure nanoparticles size directly in the colloidal solution, without the need for solvent evaporation or vacuum. One of these is small angle X-ray scattering (SAXS), which is able to simultaneously extract the size of aggregates (secondary particles) and of monomers (primary particles) contained therein, although at the price of complex data analysis.

Student: It takes ages to transfer always 1.5 mL with the 1 mL pipette. I have always to change between 0.5 and 1.

Assistant: Just put it on 0.75 mL.

Alex Heinemann, Essen

In addition to the Feret diameter, also the hydrodynamic size is important for nanoparticles in a liquid solution. The hydrodynamic size, also called Stokes-Einstein size, is defined as the size of a hard sphere that diffuses in the liquid medium at the same rate as that of the object (i.e. the size of the sphere with the same diffusion coefficient D of the object, Figure 34). There are two techniques which are sensitive to the hydrodynamic size: dynamic light scattering (DLS) and disc centrifuge (DC). Remarkably, DLS and DC also give information on the hydrodynamic size of nanoparticles coated by a layer of stabilizing molecules (the typical example is that of organic ligands grafted on the surface of noble metal nanoparticles). The shell of organic ligands has lower electronic density than the inorganic core, which makes its detection with electron microscopy or by SAXS uncertain. Besides, the ligand shell often has a "soft" structure, meaning that the size in the liquid phase is generally different than after drying of the sample for TEM/SEM imaging. It is worth to observe that also in case of charged ligand-free nanoparticles, the hydrodynamic size may differ slightly from the Feret one, due to the presence of an electrochemical double layer slowing Brownian motion or sedimentation in a centrifuge (mimicking smaller primary particles as would be expected).

Therefore, in comparison to electron microscopy, DLS and DC can characterize nanoparticles in their colloidal state. This has a few nice advantages: DLS and DC are generally much faster, do not need sophisticated sample preparation and possess far better statistics, as they are prone to characterize a huge collection of particles. Also, most colloids are tested for functionality in liquid state, to DLS and DC assess a size that is often more close to the colloids in operando state. However, you need to be careful when interpreting the results because size distributions from electron microscopy and light scattering are fundamentally different:

I) Light scattering characterizes colloidal particles consisting of the metal/hard matter core as well as a shell of counter ions or potential organic ligands. This sum of core diameter and shell is called hydrodynamic diameter, which of course is larger than the Feret diameter obtained from electron microscopy. So as a rule of thumb you can memorize that particle size distributions from light scattering are always larger than those from electron microscopy. This difference is usually negligible when completely ligand-free particles are evaluated, however, differences by a factor of two or three can be found in case bulky ligands like proteins or polymers are used.

II) Light scattering can usually not differentiate between single particles and agglomerates/aggregates dispersed in solution. This can lead to huge differences between particle size distributions from electron microscopy and light scattering. Therefore, whenever you encounter discrepancies between electron microscopy and light scattering techniques, which are too big to be explained by a hydrodynamic shell (in most cases the hydration shell is even less than a nanometer, and ligands or surfactants have typical length of only a few nanometers), agglomeration/aggregation in the dispersion is the most probable cause.

While electron microscopy gives direct access to the particle size, light scattering yields indirect results which originate from an approximation by a mathematical model. In case you do not properly understand these underlying models, data can be easily misinterpreted. Hence it is mandatory that you critically evaluate all data obtained by light scattering.

It is now clear that the definition of nanoparticle size may be tricky, unless you don't have in mind that it is important to discriminate between:

i) the Feret diameter of the objects physically surrounded by the liquid environment in the sample (which often is an aggregate of "monomers"),

ii) the Feret diameter of constitutive units ("monomers") in the aggregates,

iii) the hydrodynamic size of the aggregates (or of monomers if no aggregation occurs), which also contains the contribution of the ligand shell if present.

Depending on which size we are interested in, we must use the appropriate investigation technique, and none of the techniques is generally better or worse for colloid analysis, but they bring complementary information. So it is very useful to verify your particle size distribution always (!) with at least two different methods. It is very unlikely that 2 methods give the same value. Instead, by the difference in diameters, you will learn a lot about your sample and may give impressively precise statements in your thesis or manuscript.

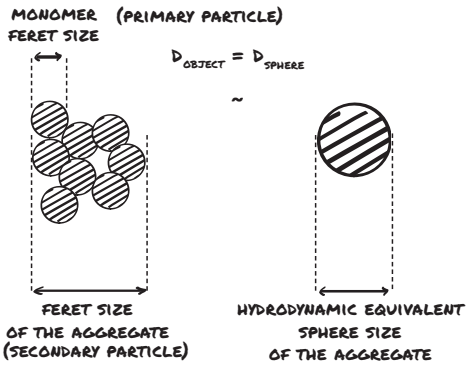


Figure 34: Understanding nanoparticle "size" coming out from different techniques.

V

2.1. Dynamic light scattering (DLS).

A. What do I see?

The total (core + ligand shell) average hydrodynamic size of all the objects dispersed in the sol, and an estimation of their size distribution. DLS cannot differentiate between an agglomerate, aggregate or solid sphere. Instead, it sees the hydrodynamic equivalent diameter. Typically, size distribution can be weighted on the volume or on the number of nanoparticles. However, DLS is not applicable to very small nanoparticles (i.e. below 5 nm).

B. How does it work?

The physical principle exploits here the fact that the diffusion coefficient of a particle is size dependent, i.e. small particles diffuse faster than large particles. DLS exploits a coherent monochromatic polarized laser beam (typically in the red) to collect the light scattered by the nanoparticles in the sample. The scattered beam forms a speckle pattern on the detector area, i.e. a pattern with an inhomogeneous density of photons due to the occurrence of constructive or destructive interference in different parts of the detector area. In fact, the scattering objects have different position in the sample, and light scattered from each object will have a different phase on the detector surface. By repeating the measurement at different time intervals, the time evolution of scattered patterns can be correlated to the Brownian motion of the scattering objects (i.e. to the random walk of particles suspended in a liquid, resulting from their collision with the liquid molecules or with other particles). So the detector sees a flickering light, called intensity fluctuations. It is intuitive that scattering signals coming from smaller particles change/fluctuate much more quickly than those stemming from larger particles. Based on this, the instrument acquires an

SAXS

autocorrelation function, indicating how quickly signal intensity changes and based on this calculates diffusion coefficients and particle sizes.

The use of complex mathematical functions can be applied to the time-dependent scattered pattern to extract the diffusion coefficients D of the scattering objects, and their relative distribution. Finally, the size of the objects is extracted from D by using the Stokes – Einstein equation:

$$D = \frac{k_B T}{6\pi\eta R_S}$$

with T the temperature, k_B the Boltzmann constant, η solvent viscosity and R_S the equivalent sphere radius.

However, no worries: the mathematical analysis is done automatically by the instrument, and the operator just needs to set parameters, place sample and launch analysis with the mouse. The underlying software for data interpretation is designed to work even for unskilled personnel. The main advantage of DLS is that measurements are very fast and results can usually be obtained after a few minutes. In fact, DLS is the standard technique for the characterization of size distribution in colloids, and this type of instrument is frequently found in research labs.

However, the technique has one striking disadvantage which can be particularly critical when analyzing colloids from laser ablation experiments, where you may be faced with polydisperse or even polymodal size distributions: DLS measurements, the scattering intensity is generally proportional to the sixth power of the particle diameter! Even though the underlying algorithm considers this correlation, the light emitted from small particles in the presence of larger ones may be too weak to be detected (the detector is dazzled). Just to illustrate this point, let us assume you intend to analyze a sample collective containing 5 nm and a 50 nm particle with DLS. In this case the light intensity from the 5 nm particles is a million times lower. Based on this it can be concluded that DLS often fails in characterizing small particles in the presence of large ones. Note that the widely used Malvern DLS instrument always puts out artificially symmetric Gaussian distribution around a calculated peak value, independent on how asymmetric your sample size distribution looks like.

c. Amount of sample needed?

Approximately 1 – 2 mL of solution are enough, at a concentration which allows scattered light to cross the sample without considerable re-absorption. Actually, the use of high concentration and high absorbance solutions is not compatible with DLS: in these cases dilution of the sample until convergence of DLS results

is suggested. Obviously, excessive dilution will result in a weak scattering signal and noisy/not reproducible measurements: the presence of few dust grains in solution can completely alter the results in this case!

→ D. How do I prepare the sample for analysis?

You just need to fill the DLS cuvette with the colloidal solution, as in OAS.

If you don't see the small particles, try again after dilution. Typical concentration threshold (e.g. for gold) is 20 mg/L, better 50 mg/L. Use a simple syringe dead-end-filter (e.g. with 400 nm pores, often called virus filter) to quickly remove the dust. Repeat measurement after 1-2 days. The temperature has a double effect on the basic equation used to calculate the size (on the kinetic energy of the numerator and in the viscosity of the denominator of the above formula), so it is very important to have the sample measured at a known and constant temperature. Which temperature will you set in the DLS instrument software for a vial taken out of the 8°C fridge, carried in your 37°C hot hand into the 20°C lab where the instrument stands?

V

→ E. Tips / warnings / hazards?

In case of samples with a wide distribution of size, the largest size will dominate the scattering response, and the only reliable size distribution is the volume weighted one. In this case, DC can give more reliable information because the sample is sorted in size during the analysis.

For the same reason, the presence of large objects in the liquid, such as dust grains, must be avoided. So always cover your sample, a foil is enough.

2.2. Disc Centrifuge (DC)

→ A. What do I see?

DC gives the full-size distribution of the sample and is compatible with polydisperse and multimodal colloids. In case of high-density nanoparticles, DC is not applicable for sizes below 5 nm, and this limit increases with decreasing particle density.

→ B. How does it work?

In a DC, particles are separated by size using centrifugal sedimentation in a liquid medium. The basic principle is the separation of particles of different sizes in a gravitational field, generated by a rotating disk. Naturally, larger particles will sediment more quickly than small ones. The sedimentation is promoted by the centrifugal force generated by the rotation of a discoid cell containing the sample (in the inner part) and a liquid phase with increasing viscosity from

the center to the extremity of the disc (Figure 35). A sucrose initiated density gradient within the disk ensures that individual size fractions of particles uniformly move to the edge of the disk. The cell and the liquid phase are optically clear so that, when particles approach the outside edge of the rotating disc, where an optical detector is located, they are detected by absorption or scattering of a light beam passing through the disc at a fixed position. The change in light intensity recorded at a fixed wavelength over time is converted by the software into a particle size distribution, because the time required for nanoparticles to cross the light beam is a function of the hydrodynamic particle size and medium viscosity. In order to correlate sedimentation time and particle size, a calibration standard needs to be added prior to each measurement. Based on the known speed of the disk and an external calibration standard, the instrument "knows" which particle size fraction is meant to pass the detector and which time. The scattering intensities for each size fractions can then be recorded transformed into a particle size distribution. Due to this measuring principle, analytical disk centrifugation is particularly well suited to characterize polydisperse and polymodal particle size distributions, predominantly formed during laser ablation experiments. Main disadvantages, however, are that measurements can be highly time-consuming, particularly for very small particles or particles with low density (measurement time ~ $(\text{diameter})^{-2}$).

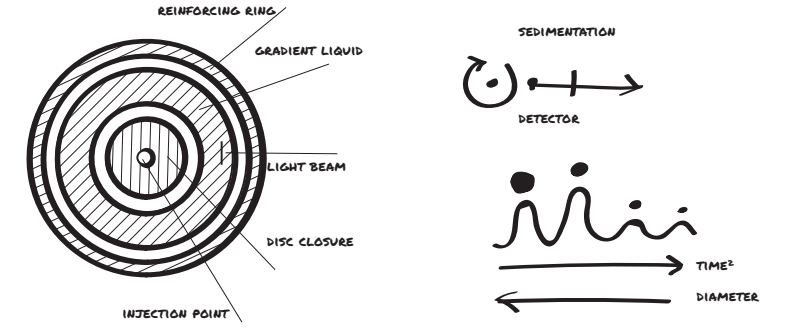


Figure 35: Sketch of the DC disc, sedimentation principle and chromatogram.

→ c. Amount of sample needed?

Only 0.1 mL of solution is required, at a concentration that allows detection by the light beam, corresponding to 10^{-8}-10^{-6} g of sample in the best cases.

→ d. How do I prepare the sample for analysis?

The sample just needs to be injected in the center of the DC discoid cell, after an appropriate build up of the viscosity/density gradient. Look at the

baseline. Does it not reach zero for maximal size and/or does it give negative values (after baseline subtraction) at smaller sizes? This is a clear indication of either the sucrose gradient has to be refreshed (after a hand full of samples), or the disk is dirty.

The disk shall make a constant, high-frequency noise. If the sound starts to get wavy (toning up and down), immediately shut down and call service, as the bearing may be close to its end of lifetime.

→ E. Tips / warnings / hazards?

DC works under two main assumptions:

i) particles will have the same hydrodynamic size in the viscous medium as in the original solution.

ii) absorbance of nanoparticles with different size is well reproduced by the instrument software (the software uses a correlation function to consider absorbance of different sizes, that is why optical parameters of measured particle species need to be added).

Obviously, these assumptions should be checked before use with independent techniques. Concerning assumption *i)*, you must verify particles aggregation or disaggregation for instance by mixing the nanoparticles solution with the viscous solution and performing DLS, or by crossing DC analysis with TEM imaging, at least the first times the analysis is performed.

Assumption *ii)* is more complicated to be verified (think for instance to aggregates of plasmonic nanoparticles, whose absorption is strongly related to size and shape), and actually requires a solid a priori knowledge of the sample. But even for unknown absorption property, not the peak intensity but its hydrodynamic diameter will be analyzed correctly.

General comment on DLS and DC:

The software of both methods, DLS and DC often prints out the “z-Average” or “intensity distribution”. Both are useful for quick pre-inspection, to see if you have strong scatterers in the sample that may have screened the small ones, but are scientifically meaningless and need to be converted into volume or surface or number weighted statistics!

We have made round-robin tests and compared many instruments under different conditions. Although instrument suppliers may claim different, you cannot reproducibly measure particle diameters < 4 nm. We always measure down to ≥ 3 nm diameter and take everything seriously until 3-4 nm. For the smaller fraction, you will need the help of TEM (or, even better, the Analytical Ultra Centrifuge that is a mighty tool, and maybe even the best analytical method for ultrasmall particles, but far less common). Also, you will need to

put the density or an optical parameter into the software before measurement (e.g. material refractive index or absorbance at a given wavelength). A big gold particle property will not be influenced largely by a monolayer of ligand in density or extinction, so you take the value of gold. But for lighter particles or bulky ligands, this may have an effect. Choose the material parameter of the core and disclose this in the experimental part of your thesis or manuscript. Now you know why a hydrodynamic diameter measured by DC may be counter-intuitively even smaller than a diameter measured by TEM, and larger by DLS (even if it does not aggregate). A ligand has two effects. It may reduce the particle's density, to make it appear smaller. But most importantly, it adds a drag force to the particle's mobility. Thereby it is diffusing slower mimicking a bigger particle in DLS, and sedimenting far slower, mimicking a smaller particle in DC. That's great to know since it provides evidence for successful grafting a ligand on a colloidal particle.

2.3. Small angle X-ray scattering (SAXS)

→ A. What do I see?

Size and structure of particles in the 3 – 150 nm range can be measured.

→ B. How does it work?

SAXS is based on the elastic scattering of monochromatic X-rays by atoms in a sample. Collection of scattered X-ray intensity takes place in a very small angle interval (0.1° - 10°, Figure 36) from the beam propagation direction (forward scattering), where the scattering events are more probable and diffractive effects are nearly absent (see V.3.3). Although the theory behind SAXS is not simple, the main concept is that X-ray scattering depends on the

We had a student who has been working on the laser for a couple of days. One morning he was working within the laser lab with a colleague being present. After several ablations and breaks he put on his laser safety goggles and started the laser for the next ablation. However, he didn't make sure that everyone else in the lab was wearing their laser safety goggles. The colleague noticed the sound of the ablation and was able to react right away. When this break in safety procedure was addressed the student tried to make excuses stating that the colleague was wearing his goggles some time before. However, it is the responsibility of the laser operator to ensure that everyone within the lab wears the personal equipment prior to turning on the laser every single time.

Elisabeth Maurer, Essen

discontinuity of electron density in the sample, when it occurs on a length scale of 1 – 100 nm. Consequently, the scattering pattern at small angles contains information on the typical size and shape of the scattering objects with high electronic density immersed in the matrix with low electronic density. It can measure polydisperse samples, but intensities are dominated by the high mass fraction of the sample (the large particles). And size differentiation of overlapping modes is almost impossible (e.g. differentiation of 7 nm and 10 nm particles).

The typical SAXS plot reports X-ray intensity versus angle, and it is fitted with mathematical models which includes the size and shape of the scattering object as fitting parameters. Contrary to DLS, these mathematical models must be applied by the operator, making SAXS data analysis a non-trivial task. Besides, SAXS instrumentation is not available in most labs.

On the other hand, the advantage of SAXS compared to DLS is that it can be used to characterize polydisperse samples and it may provide additional information on the particle shape. In addition, SAXS is the only method suitable for monitoring processes in optically opaque media e.g. inside the laser-induced cavitation bubble.

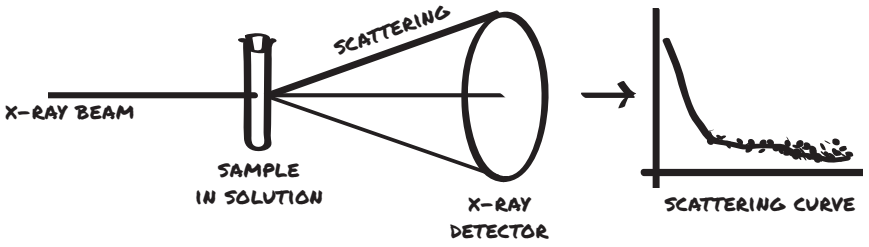


Figure 36: Sketch of the working principle of SAXS.

c. Amount of sample needed?

The technique requires ~1 mL of sample with a concentration of the order of 0.1 – 0.5 mg/mL (depending on the z of the elements in the scattering object).

d. How do I prepare the sample for analysis?

Stable liquid dispersions of nanoparticles are fine.

e. Tips/ warnings/hazards?

If the colloid is not stable, the viscosity of solution can be increased to grant stability for the duration of the analysis, which may require up to several days to achieve acceptable signal-to-noise. To this purpose, you can use glycerol or high concentration polymer solutions.

Table 1: Summary of properties, advantages and limitations of the principal methods for the characterization of particle size distribution.

	Electron Microscopy	**Dynamic Light Scattering (DLS)**	**Small Angle X-ray Scattering (SAXS)**	**Disk Centrifugation (DC)**
Type of particle size	Feret (core)	hydrodynamic	hydrodynamic	hydrodynamic
Acquisition of particle size	direct	modelling	modelling	modelling
Sample processing	very slow	fast	very slow	average
Skills required for data interpretation	low	low	very high	low
Raw data output (size distribution)	number	intensity	volume	volume
Statistics (number of analyzed particles)	~10^{-8} %	up to 100%	up to 100%	up to 100%
Information on colloidal state	generally no (unless using specific tricks)	yes	yes	yes
Information on ligand conjugation	generally no (sometimes from interparticle distance)	yes	no	yes
Characterization of single particles	yes	no	no	no
Characterization of aggregates	generally no (unless using specific tricks)	yes	yes	yes
Polymodal and polydisperse particles	yes	limited	yes	yes
Particle morphology	yes	no	yes	no

STEP 3: IDENTIFY THEIR COMPOSITION

Imagine you're in Italy, on a sunny day, but well arranged under a vine-covered pergola in the porch of a restaurant from which you can admire green hills planted with dozens of different types of fruit and vegetables. You're tasting a wonderful pasta with a red flavorful tomato sauce as you did not know that it existed. The question you put to the waiter at the first opportunity will be "what are the ingredients of this dish?" (or maybe it will be your second question, just after asking him "Can I have some more?"). With our beloved nanoparticles it is the same: the time comes when we have to exactly know about their ingredients. Therefore, once you are sure that nanoparticles are present in our sample, and you know their size distribution, the next step is to clearly identify their elemental composition and phase.

The quickest way to qualitatively identify elemental composition is the energy dispersive X-ray spectroscopy (EDS). Most TEM and all SEM are equipped with EDS, with the obvious difference that TEM allows elemental analysis with nanometric resolution, while SEM only allows resolution of the order of 10 nm or higher in ordinary conditions. Quantitative information on the elemental composition by EDS is also possible, but it suffers from matrix effects and becomes reliable only on isolated clusters of nanoparticles.

Alternative techniques such as inductively coupled plasma assisted (ICP) mass spectrometry (MS) or optical emission spectroscopy (OES) gives reliable quantitative information on particles ensemble, with sensitivity as low as part per trillions (ppt) in best cases.

Elemental composition still does not tell us the phase of the nanoparticles, as solid matter can be either amorphous or ordered, and every combination of elements can be achieved in a multitude of phases. Just to give an example, iron and oxygen can form magnetite, maghemite, hematite, wustite, goethite and amorphous iron hydroxide, and all of them are achievable by laser synthesis.

The student switched on the high power laser system to perform the proper adjustment of the laser. During the adjustment he noticed a burning smell and switched off the laser again. After examining the setup and lab, he realized some black spots in the windows curtain. An inadvertently movement of one of his mirror with his elbow was the trigger for this incident.

Sebastian Kohsakowski, Duisburg

Ordered phases are accessible by diffraction techniques, which for nanoparticles are X-ray diffraction (XRD) or electron diffraction (ED). XRD is accurate and easier to apply, and it can also give reliable quantitative information on the weight fraction of all phases constituting the sample, but these are averaged information over all particles in the specimen. ED is possible with TEM and can give information on a single nanoparticle at time, but its quantitative application is often not possible.

For some classes of materials such as oxides and semiconductors, qualitative identification of ordered phases is possible by vibrational spectroscopy, such as Raman and Fourier-transformed infra-red (FTIR).

In the most complicated cases, where complex, disordered or unknown phases are obtained, the above investigation methods may be not enough to precisely identify the nature of the sample. In these "desperate" cases, you can refer to a group of "magic" techniques which is applicable to any material: the group of X-ray absorption spectroscopies (XAS), including X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS). Although the XAS group gives average information on the sample, these information are complete and cover the composition, the chemical state of each element and the composition and arrangement of nearest neighbor (NN) and next nearest neighbor (NNN) atoms. This is very useful for multielement materials, such as alloys or doped oxides. Unfortunately, the payback is high because XAS analysis requires a synchrotron radiation facility and at least one dedicated expert for data collection and analysis (which is absolutely not trivial!).

3.1. Energy dispersive spectroscopy (EDS)

A. What do I see?

EDS allows the detection of elements with z larger than Be, although sensitivity increases with z and depends on matrix composition. Quantitative information can be obtained easily when matrix effects are negligible.

B. How does it work?

EDS is based on the fact that i) X-rays are produced by the interaction of a high energy electron beam with matter, and ii) each element has a unique atomic structure allowing unique set of peaks on its X-ray emission spectrum. In particular, the collision of the electron beam with sample atoms promotes the kick off of electrons from inner atomic shells, leaving an empty energy level which is filled by electrons of the outer shells. The energy difference is released as a photon with frequency typical of X-rays. Since the electronic structure is specific of each element, the spectroscopy of X-rays produced by the sample allows the identification of elements contained therein. X-rays are measured by

an energy dispersive spectrometer exposed on the sample and compared to a database for element identification. The typical EDS spectrum reports X-ray counts (in arbitrary units) versus their energy (in keV, Figure 37). Integration of peaks and comparison with a standard or a database allows the extraction of quantitative information.

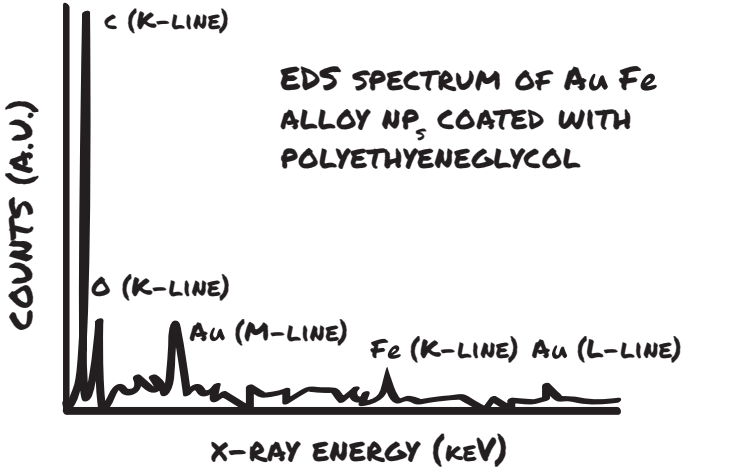


Figure 37: Typical EDS spectrum of AuFe alloy nanoparticles coated with polyethylenglycol.

EDS is based on the fact that i) X-rays are produced by the interaction of an high energy electron beam with matter, and ii) each element has a unique atomic structure allowing unique set of peaks on its X-ray emission spectrum. In particular, the collision of the electron beam with sample atoms promotes the kick off of electrons from inner atomic shells, leaving an empty energy level which is filled by electrons of the outer shells. The energy difference is released as a photon with frequency typical of X-rays. Since the electronic structure is specific of each element, the spectroscopy of X-rays produced by the sample allows the identification of elements contained therein. X-rays are measured by an energy dispersive spectrometer exposed on the sample, and compared to a database for element identification. The typical EDS spectrum reports X-ray counts (in arbitrary units) versus their energy (in keV, Figure 37). Integration of peaks and comparison with a standard or a database allows the extraction of quantitative information.

c. Amount of sample needed?

See in V.1.2.c (TEM) and V.1.3.c (SEM).

d. How do I prepare the sample for analysis?

See in V.1.2.d (TEM) and V.1.3.d (SEM).

e. Tips / warnings / hazards?

Avoid thick or crowded agglomerates of nanoparticles to minimize matrix effects and to increase the reliability of quantitative results, which is best in isolated nanoparticles. Don't worry about carbon, it's always there, and sometimes you will find silicon (or silicates), which is an additive in seals and are found everywhere in the lab and sample holders, in particular where organic solvents were used for cleaning by you or your colleagues. Of course, you will not use any equipment made of metal (spatula etc.) during synthesis or sample handling.

3.2. Inductively coupled plasma assisted (ICP) mass spectrometry (MS) and optical emission spectroscopy (OES).

a. What do I see?

All the elements in the sample can be detected and precisely quantified, excluded hydrogen. It will also tell you the amount of dissolved elements (e.g. analyzing the supernatant after centrifugation).

b. How does it work?

The measurement takes place in two stages: the atomization of the sample and analysis of atomic species. The inductively coupled plasma serves for the atomization of the sample. The ICP is alimented by an electromagnetic coil which has the function of inductively heating the plasma, which is composed of a highly ionized and electrically conductive argon gas. The liquid sample is introduced in the plasma with a nebulizer, immediately reaching the same temperature and ionization conditions.

In ICP-MS, detection takes place by conveying the atomized sample to a mass spectrometer, where charged particles are separated by a mass selector which exploits the Lorentz force, and counted by a charged particles counter, to obtain signal intensity versus mass for a unit charge.

In ICP-OES, the plasma is coupled to a diffraction grating and a photodetector (typically a photomultiplier or a CCD), to measure the intensity of light emitted at the wavelength characteristic of highly ionized atomic species. Atomic species emit a set of very sharp bands, which clearly are related to the electronic structure of the emitting element.

Quantitative analysis requires calibration with a standard in both MS and OES methods.

c. Amount of sample needed?

Detection limits of ICP-MS are impressively low (usually below parts per billions, ppb), thus requiring few µL of solution. ICP-OES has higher detection limits, but fully compatible with a typical sample containing laser generated nanoparticles.

d. How do I prepare the sample for analysis?

For quantitative analysis, sample preparation requires the complete dissolution of the solid phase into ions (sample digestion), which typically requires the use of acids or bases at high temperature. This means that a colloidal solution is not suitable for analysis without appropriate sample preparation if reliable quantitative results are desired.

Several days after my first experience with high intensity lasers, I began to feel more confident in handling these machines. However, once after a short break I went to the laser lab and continued my experiments, when I observed increasing smoke formation I realized that I forgot to remove the cap of the focus lens.

Sandra Jendrzej, Essen

e. Tips / warnings / hazards?

Sample digestion is critical for quantitative measurements: check literature for the most appropriate procedure for your sample (for instance aqua regia, which is a 1:3 mixture of HNO_3 and HCl, works very well in dissolving gold nanoparticles, but cannot be used with Ag nanoparticles because it can form the insoluble AgCl precipitate). For contamination with C, Si, metals, see (V.3.1.e).

3.3. X-ray diffraction (XRD)

a. What do I see?

The ordered phases can be identified, and the volume-weighted average size of ordered domains in the sample can be evaluated. By applying the Rietveld analysis, quantitative information on the mass fraction of each crystalline phase can be achieved. Hence, crystal diameter can never be smaller than the particle

diameter. But particles (e.g. larger metal particles) may consist of several crystals (e.g. twins) even if they are spherical, so that combination of XRD with TEM of DC/DLS may already tell a lot about the sample. XRD is "blind" for amorphous materials, whereas SAXS sees both, crystalline and amorphous scattering objects.

B. How does it work?

X-rays are scattered by atoms. When atoms form ordered arrays with well-defined translation periodicity on a length scale comparable to X-ray wavelength (~0.1 nm), the X-rays are scattered only at well-defined angles (Θ) typical of the crystalline structure. In this way, a diffraction pattern is obtained by the measure of the intensity of scattered X-rays versus the scattering angle. The XRD pattern (intensity and diffraction angle of all peaks) is related to the atomic position and interplanar distances in the crystal, as described by the Bragg's law (Figure 38):

$$n\lambda = 2l\sin\theta$$

where n is any integer, λ is X-ray wavelength, l is the distance between reflection sites (typically interplanar distances d), and Θ is the angle between the reflected beam and the plane containing the sample.

The comparison of the XRD pattern with a database allows the identification of the compound. The volume-weighted average size (τ) of ordered domains in the sample can be measured by using the Debye-Scherrer formula

$$\tau = \frac{K\lambda}{\beta\cos\theta}$$

where K is a constant (typically ~ 0.9), λ is the X-ray wavelength and β the full width at half maximum (FWHM) of the diffraction peak located at the diffraction angle Θ. Hence, XRD tells you the type of crystal and its size. Sharp, high peaks indicate large particles. But take care by using the right diffraction peak value in the Scherrer formula, as most instruments deliver spectra with "2Θ" in the y-axis.

In case of multiple compounds, the Rietveld analysis is used to extract information on the relative mass abundance of all species involved. By this, lattice strains from doping, defects or alloying can be attributed. It's good to have Rietveld fits for a phospors or alloy nanoparticle series.

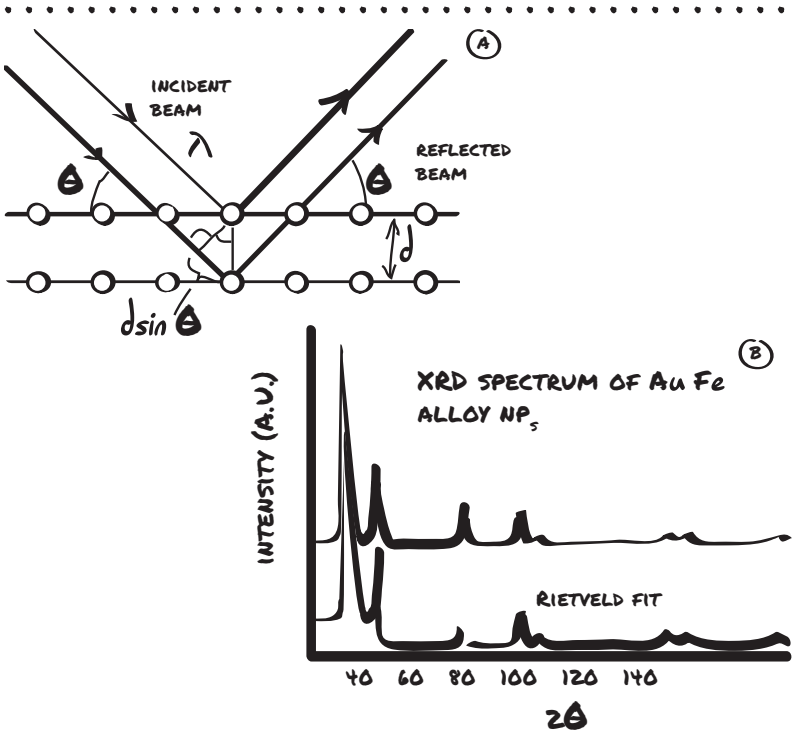


Figure 38: (A) The principle of Bragg's law and (B) a typical XRD spectrum of AuFe alloy nanoparticles.

c. Amount of sample needed?

At least 2 – 10 mg of nanoparticles are required. Yes, you might need upscaling (Chapter II)

d. How do I prepare the sample for analysis?

Nanoparticles are deposed on XRD substrates (for instance quartz plates).

e. Tips / warnings / hazards?

It is important to avoid heating of sample during deposition, in all those cases where oxidation, segregation or phase transition may occur, such as with iron oxides or metastable alloy phases. Because of the volume-sensitivity of the method, minute amount of larger particles will screen the information (crystal structure and size) of the small fraction. First calculate crystal size by Scherrer equation, if it's far larger than expected (e.g. sharp, high peaks are not nanocrystals) maybe filter or centrifuge the sample and repeat.

3.4. Electron diffraction (ED)

a. What do I see?

Ordered phases can be detected and identified by ED, with the possibility to perform the analysis at the single nanoparticle level.

b. How does it work?

The principle is the same as XRD (V.3.3.b), with the sole difference that here the diffraction pattern is produced by scattering of a monochromatic high energy electron beam instead of an X-ray beam.

c. Amount of sample needed?

See V.1.2.c (TEM).

d. How do I prepare the sample for analysis?

See V.1.2.d (TEM).

e. Tips / warnings / hazards?

Measurement of scattering intensity is not simple with ED, because of its dependence on nanoparticle orientation and on the limited number of nanoparticles probed. Therefore, ED is more suited for qualitative identification of nanoparticle structure, while quantitative information on the ordered phases are preferably obtained by XRD.

Everybody knows that one should wear laser protection glasses when the laser is running. So what to do, when you notice that your colleague does not wear glasses? For sure, do not slap him into the face while trying to protect his eyes by hand. It's better to tell him: Please wear your protection glasses.

Lisa Gamrad, Essen

3.5. Vibrational spectroscopy (Raman, FTIR).

A. What do I see?

Several compounds (mostly oxides and semiconductors) can be identified based on the presence of peaks ascribable to well-defined chemical bonds.

B. How does it work?

In both cases, the energy of vibrational levels is probed. In Raman spectroscopy, the sample is irradiated with a monochromatic visible laser beam, and the portion of backscattered light is collected. The backscattered light has a main component due to elastic scattering, which is discarded, and a low-intensity inelastic scattering portion originated by Raman scattering. The Raman scattered light is dispersed on a detector to measure intensity versus photon energy. The energy difference between inelastic scattered photons and incident photons (the Raman shift) is the energy of vibrational levels in the sample. Raman spectra are reported as intensity (counts) versus wavenumber or Raman shift (expressed in cm-1, Figure 39). The position of each peak is distinctive of specific functional groups in a compound. However, a certain level of crystalline order is required to observe appreciable Raman bands from crystalline solids, which typically happens only for sizes above ~5 nm.

In FTIR, vibrational levels are probed directly by measuring the transmittance of the sample as a function of infrared radiation energy. The term Fourier transform infrared spectroscopy originates from the fact that a Fourier transform of the transmitted beam is used to obtain the final spectrum (don't worry the instrument software will do it for you). FTIR spectra are reported in transmittance (T) versus wavenumber (in $cm.^{-1}$, Figure 39).

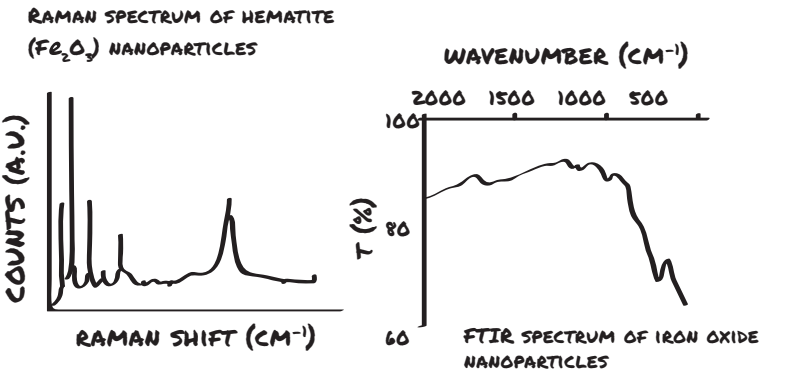


Figure 39: Typical Raman and FTIR spectrum.

c. Amount of sample needed?

Raman spectroscopy only requires a few μg of material, when coupled to a microscope (micro-Raman). FTIR requires more than 0.5 – 1 mg of material.

d. How do I prepare the sample for analysis?

The solvent must be removed for the analysis, and nanoparticles must be deposited on a substrate with negligible background. For Raman spectroscopy, this can be a metal foil or a glass slide. For FTIR, specific crystals which are transparent to infrared radiation must be used (such as CaF_2 or KBr windows).

e. Tips / warnings / hazards?

Often Raman and FTIR gives complementary information about inorganic materials, hence the two techniques give their best when used together.

3.6. X-ray absorption spectroscopy (XAS).

a. What do I see?

Composition, chemical state, type of NN and NNN and their location in space.

b. How does it work?

XAS principle is pretty much like OAS, being based on the measurement of X-ray absorption bands from the sample. However, the physics behind the absorption of X-rays is rich and strongly related to the chemical nature of the sample. Contrary to OAS, that typically shows absorption peaks, in XAS you find absorption edges. These edges can be divided into three portions: the pre-edge, the rising edge and the extended edge structure (Figure 40). The pre-edge contains the information on the element, being determined by the electronic structure of the atom absorbing X-rays. Absorption intensity is related to the abundance of the absorbing element by the same Lambert-Beer law exploited for OAS (see V.1.1.b).

X-ray absorption is followed by the emission of a photoelectron (photoelectric effect). The great point of XAS is that photoelectrons undergo a scattering process with surrounding atoms, and this generates a modulation of the X-ray absorption intensity in the region of the rising edge and the extended edge. In particular, the rising edge reflects the interaction of the photoelectron with the NN, and it's the subject of XANES. The extended edge reflects the interactions with NNN and other atoms, and it's the subject of EXAFS. The XANES and EXAFS portions of the X-ray absorption edge can be fitted with complex mathematical models which account for the relative position and composition of NN, NNN and so on. In this way, you can find the average composition and atomic disposition around the absorbing element. In particular, XANES is more sensitive to the oxidation state and to the atomic site symmetry, while EXAFS can give the full atomic coordination number, the chemical nature of the surrounding atoms and the interatomic distances.

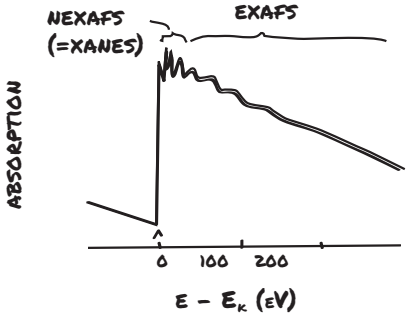


Figure 40: Sketch of a XAS edge and its three parts.

V

c. Amount of sample needed?

XAS is very sensitive, thanks to the high performance of synchrotron light: the amount of material needed is the same necessary to cover a small area of few mm^2 on the sample holder.

d. How do I prepare the sample for analysis?

Simply by drop casting on the XAS substrate, followed by solvent evaporation. It can also run wet samples

e. Tips / warnings / hazards?

Anyone working with nanoparticles sooner or later needs a friend expert of XAS. The tip is...be nice to any XAS expert!

STEP 4: QUANTIFY THEM

In Chapter II you already obtained good information about how to evaluate the productivity of your set up. In addition, it is good to know that the best way for the quantification of matter in a sample is ICP-MS or ICP–OES, as discussed in section V.3. Sometimes, you may be tempted to use a microbalance to quantify the mass of a sample, but this procedure can be error prone for amounts lower than ~5 mg, and you must always account for solvent residuals and solutes when drying the colloid. On the other hand, the weight of the target can be altered by the formation of micrometric fragments which are not part of the colloid.

In case of absorbing nanoparticles, OAS can be used for quantification thanks to the Lambert-Beer law, which simply relates absorbance to nanoparticles concentration (see V.1.1.b). This method works well with metals and oxides

whose size and optical properties are already known, and especially benefits of start-up calibration with an independent and more reliable technique such as, for instance, ICP-MS.

It is worth to stress that the most common unit for the quantification of a sample of nanoparticles is mass (e.g., mg) or mass per volume (e.g., mg/mL). However, the comparison of laser ablation yield among nanoparticles with different composition is possible only by considering the number of atoms in your sample, expressed as mol or mol/L (or M). The reason can be easily understood considering that a sample with a concentration 0.001 mol/L corresponds to 0.197 mg/mL if composed of Au, and to 0.063 mg/mL if composed of Cu (Figure 41).

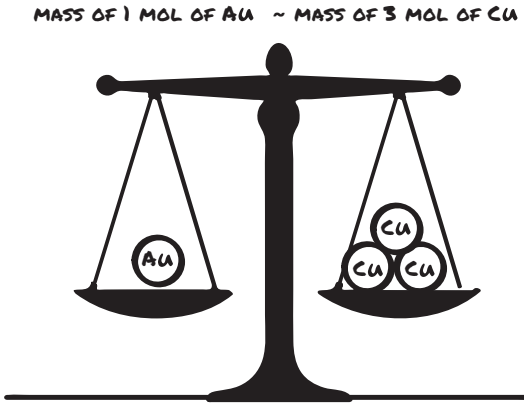


Figure 41: Comparison between the mass of 1 mol of gold and 1 mol of copper.

STEP 5: ANALYZE THEIR SURFACES

How many times you said, "You can't judge a book by its cover"? Sorry, but this is not the case of nanoparticles: since the proportion of surface atoms to volume atoms increases, while decreasing the size of condensed matter, the surface has special importance in nanomaterials. For instance, approximately 1 atom every 10 is a surface atom in a 10 nm Au nanoparticle.

Surface atoms have different reactivity than bulk ones, due to incomplete saturation of their atomic bonding capability. In case of laser-generated nanoparticles, which are often obtained in the absence of any stabilizer or coating agent with a highly reactive surface, this is even more true. For this reason, surface stoichiometry and oxidation state are typically different than

in the nanoparticle core. The consequences for electronic, optical, catalytic or biomedical properties are huge, and these justify the need to obtain a thorough knowledge of nanoparticle surface, with the same dignity of the "body" of nanoparticles.

In addition, the surface of nanoparticles has crucial importance when it is coated with specific functional molecules, such as in noble metal nanoparticles conjugated with biopolymers, dyes, DNA or antibodies.

The "queen" technique for surface studies is X-ray photoelectron spectroscopy (XPS), which gives complete information on the chemical nature of the sample. However, in case of nanoparticles coated with organic molecules, other techniques can be more informative such as FTIR, nuclear magnetic resonance (NMR) or thermogravimetric analysis (TGA).

5.1. X-ray photoelectron spectroscopy (XPS).

V

→ A. What do I see?

It tells you about the composition of the nanoparticle surface and its oxidation. Surface elements with $Z \geq 3$ (Li) are detected, and their oxidation state identified. Although in principle quantitative information on the surface abundance of each element is achievable, in case of nanoparticles these information are averaged between the surface and the inner layers, due to the complex matrix effects and geometry of the sample. Hence it is good for seeing oxidation trends and roughly knowing what composition you have created.

→ B. How does it work?

Monochromatic X-rays are used to extract electrons from the sample (photoelectrons). The energy difference between the kinetic energy of electrons and the energy of X-rays is related to the binding energy of the electrons in the atoms of provenience, being therefore characteristic of each element. Besides, the energy resolution of XPS is high enough to be sensitive to minimal energy modifications which take place when an atom establishes chemical bonds with other species, thus giving information on the oxidation state and chemical environment of each element.

Since photoelectrons of the nanoparticle surface have a very low mean free path in condensed matter, only the first 1 – 10 nm are probed (depending on the electronic density of the sample and the used angle between sample and detector).

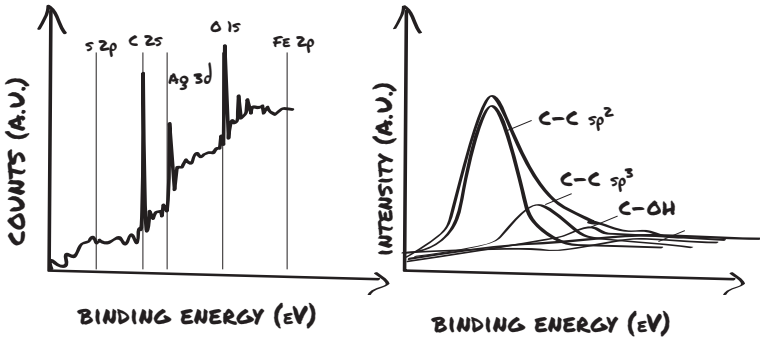


Figure 42: Typical XPS spectrum (left) and magnification of one of the peaks (C peak for example, right), showing that it can be deconvoluted in different components corresponding to different oxidation states and/or chemical environment.

A typical XPS spectrum reports the number of electrons detected (as counts) versus their binding energy (in eV, Figure 42), and each element produces a characteristic set of XPS peaks at characteristic binding energy values.

V

c. Amount of sample needed?

XPS is only sensitive to the first few nm of a sample, therefore a monolayer of nanoparticles spread over an area of ~1 cm^2 is enough.

d. How do I prepare the sample for analysis?

Sample must be dried before use (XPS is performed in ultrahigh vacuum), and can be prepared by drop casting on a clean and flat substrate which has no interfering peaks with those of our sample. Note that calibration is required for each substrate.

e. Tips / warnings / hazards?

Due to the high surface sensitivity of XPS, contaminants like salts or synthesis byproducts can shield the signals from the nanoparticles. In principle, the topmost layer can be removed by ion sputtering in the XPS chamber, but sometimes this can alter the stoichiometry of the surface. Sputtering also significantly changes the oxidation state of the remaining species making it hard to trace the oxidation state before sputtering.

In case the material of interest being shielded e.g. by a carbon layer – the oxidation state can be accessed if the sputtering time is adjusted so that the covering carbon layer is not fully removed. However some uncertainty about the oxidation state remains when sputtering is used. Note that the holder itself has an effect on the peak shifts, e.g. carbon needs different calibration than silicon.

Peak deconvolution (e.g., to extract the degree of oxidation) is very tricky, please

ask a trained person to help you at least when you do it the first time, you will see that it makes a big difference how you deconvolute (deconvolution is "dissectioning" a shouldered peak into two or more Gaussian peaks, Figure 42).

5.3. Nuclear magnetic resonance (NMR).

➞ A. What do I see?

The typical NMR spectroscopy is performed on protons (^{1}H nuclei), and it is therefore sensitive to their presence and chemical environment. The NMR pattern tells us about which organic molecule is present in a sample, and its relative abundance.

➞ B. How does it work?

Some nuclei (such as ^{1}H, ^{13}C or ^{14}N) have a non-zero nuclear spin. In the presence of an external magnetic field, the nuclear spin acts much like a small magnet and can assume a collinear (lowest energy in ^{1}H) or anti-collinear (highest energy in ^{1}H) orientation with respect to the direction of the external field. Electromagnetic radiation can promote the transition from one state to the other. Therefore, in NMR spectroscopy, an external magnetic field is exploited to produce a splitting of spin energy levels in ^{1}H) nuclei, and the absorption of electromagnetic radiation by the sample is measured as a function of its wavelength (pretty much like in absorbance spectroscopy). Since the energy gap in ^{1}H) nuclei is very small, radiofrequency radiation (10^{2} MHz) is used instead of visible light. The energy gap in ^{1}H) nuclei is sensitive to the chemical environment because electron density exerts a diamagnetic shielding effect on the external magnetic field, which depends on the distance and number of electrons in proximity of the hydrogen nuclei. Besides, the spin of ^{1}H) nuclei interacts with each other (when sufficiently close), generating a splitting of the energy levels. For these reasons, NMR spectroscopy gives information on the structure of the molecule, and thus the NMR spectrum is a fingerprint characteristic of each molecule.

➞ C. Amount of sample needed?

NMR typically requires 0.1 – 1 mg of organic matter. However, signal intensity is sensibly damped when short molecules are bound to nanoparticles, and in these cases up to 10 mg of organic matter can be needed.

➞ D. How do I prepare the sample for analysis?

Samples for NMR are in liquid phase. The only requisite is matching the concentration required for appreciable results.

➞ E. Tips/ warnings/hazards?

Since the signal of molecules bound to nanoparticles is greatly damped,

sometimes it is simpler and quicker to remove the ligands from nanoparticles surface and analyzing only the organic component. With noble metal cores, this is performed by addition of strongly interacting thiols (such as dithiothreitol), which replace the original ligands on a timescale of 1-2 days. Then nanoparticles can be removed by centrifugation or the supernatant can be collected by dialysis for NMR analysis.

5.4. Thermogravimetric analysis (TGA).

➞ A. What do I see?

In case of organic ligands which undergo complete oxidation into volatile compounds (CO_2, H_2O, NO_x, etc.), the mass fraction of the organic component can be easily and precisely quantified. This is possible only when the nanoparticle (inorganic) core is chemically inert at the combustion temperature of the organic component. TGA can also be run with inert gas. So you may find out the amount of ligand that is covering the nanoparticle.

➞ B. How does it work?

TGA basically consists of a microbalance and of a chamber with controlled temperature and atmosphere. While temperature is increased, the microbalance registers any weight increase or decrease. In case of organic compounds, combustion in the presence of oxygen is detected by weight decrease. The temperature ramp can be continuous or step by step, and weight can be reported as a function of temperature or time.

➞ C. Amount of sample needed?

In micro-TGA, few milligrams of oxygen-reactive matter are enough.

➞ D. How do I prepare the sample for analysis?

Solvent must be removed and the dried sample deposed on the TGA holder.

➞ E. Tips / warnings / hazards?

In the first run, take a slower heating program. To exclude chemical modification of the inorganic core, run TGA also on uncoated nanoparticles as a reference measurement to exclude background contributions, and on a known amount of ligands without nanoparticles. Plot the first derivative of the signal. Do you see a shift of the ligand's temperature? This indicates ligand binding (immobilization), in particular for polymers grafted on nanoparticles. TGA is very powerful if coupled with calorimetry (so-called, TGA-DSC), then you also know if mass loss is endotherm (e.g. degassing or drying) or exotherm (oxidation).

STEP 6: ANALYZE THEIR PROPERTIES

The reason for generating nanoparticles is that they have some properties of interest for scientific or technological applications. Obviously this opens up a range of different possibilities for completing the investigation of our product, and each nanomaterial requires a specific technique or a group of techniques. However, no matter what is the property of interest, it will be always related to the structure of the nanomaterial, and therefore all the previous steps are starting pillars for the functional study or the direct application of our laser-generated nanoparticles.

Conclusions

In summary, the range of techniques available for the characterization of our laser-generated colloids is wide, but they should be used "cum grano salis" to save resources and obtain reliable results. As the last step to guide the characterization of our samples, it is useful to conclude the chapter with a sketch where all the techniques previously described are classified on the basis of:

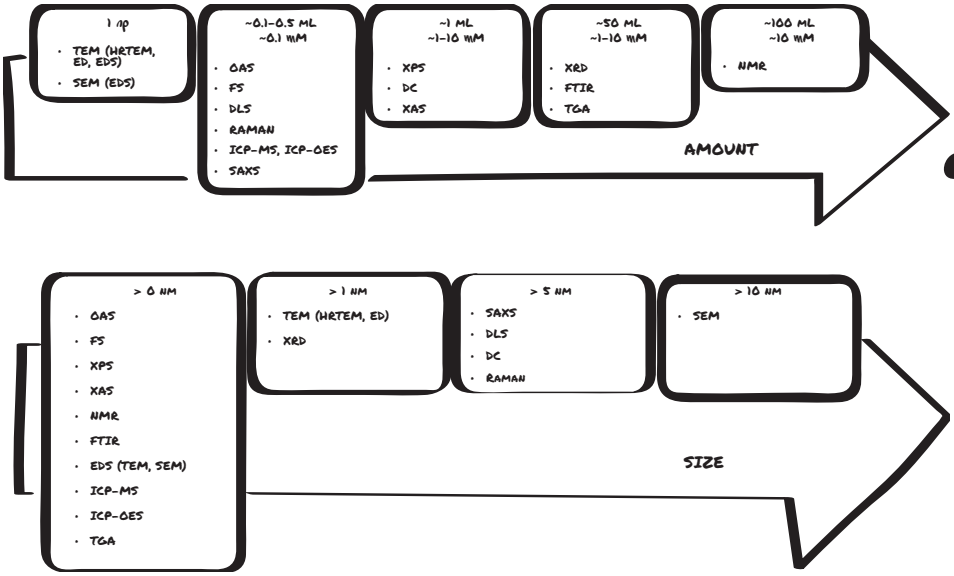
i) the amount of matter required for the investigation, starting from the single nanoparticle techniques up to the most demanding;

ii) the smallest size detectable.

The power of colloid characterization comes from combining the methods. 2 methods may already be enough to extract nice insights. Examples :

- XRD gives smaller sizes than other methods: you have multi-crystalline particles
- DC gives smaller particles than TEM or XRD: you have successfully conjugated the particle
- DLS gives the volume-weighted diameter of 50 nm, but TEM tells 6 nm. It's aggregated, you may want to add surface charge by anions or pH, or add a surfactant or polymer for stabilization
- You have ablated elemental Pt but find 40% of its surface is oxidized in XPS. Nice to know for catalysis. Maybe try Zeta potential measurement (see Chapter VI) to see this surface charge in liquid as well?
- You ablated an alloy but the XPS or EDX composition gives a different molar ratio compared to the educt. Try ICP to see what has been dissolved in the liquid
- XRD crystal structure does not fit to elemental composition. Try SAXS or HR-TEM to learn about amorphous fraction.

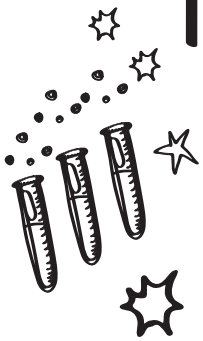
- You know the above table but have no time and your supervisor needs results by tomorrow: start with UV-Vis and DLS/DC. Collect arguments (e.g., present different histogram statistics) to ask for more. Tell that always 2 independent analytical methods are needed to validate results ready for publication with her/his highly reputed name on it.
- You tried everything but are lost. Send an email to the authors of this book.



Sketch 1: Described techniques classified by the amount needed and smallest detectable size.

CHAPTER VI

KEEP IT: STABILITY



After your first days in the laser lab you will probably have produced your first samples of laser-generated colloids and you will be able to proudly present them to your boss with a full set of structural characterization data. At this stage, two questions will inevitably come to mind, if not to yours then definitely to your boss's:

How stable are the nanoparticles? And how can stability be influenced during or after laser ablation?

These will be basic questions sticking with you up to the end of your life in the world of laser-generated colloids and nanoscience. Guided by these fundamental questions, this chapter will give you a basic understanding of how to define "stability" in the field of colloidal science, how to measure its value, how to interpret the results and how to alter the corresponding parameter using simple modifications of the laser ablation process.

VI

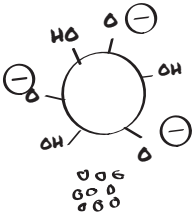
How stable are the nanoparticles?

You will certainly have to experience the hard way that particle stability is a critical issue during synthesis and handling of laser-generated colloids. After you have spent an entire day in the laser lab generating beautiful colloidal samples, the next day you may be in for quite an unpleasant surprise. You could find them either containing large soft flocks or situated as an ugly, brown or black precipitate on the bottom of your vessel or almost irreversibly fused with your container's wall. Another phenomenon you may encounter (and we often did) could be that some of your samples are perfectly stable while others precipitated, even though you are sure you treated them exactly the same way. At this point you will certainly, depending on your level of stress and your personality, either think or cry out: "Instability sucks". In order to avoid or at least minimize such pitfalls, this paragraph will give you a step-by-step overview of how to define stability, how to control it during laser ablation in liquids and how to measure it.

STEP 1: DEFINE STABILITY

The definition of this term in colloidal science is not always universal, however a straightforward answer could be that stability actually defines to what extent your sample changes with time. When specifying the word stability you have to differentiate between chemical stability and colloidal stability. Chemical stability defines whether your colloid is subject to chemically-induced changes caused

by the environment, in most cases by the solvent. The most relevant processes are oxidation and dissolution of your solid material in the respective solvent. While chemical stability is a very specialized phenomenon strongly depending on the used material, colloidal stability is universally applicable to all colloidal systems. It is, furthermore, particularly critical in laser-generated colloids which are generally completely ligand-free and hence only "meta-stable" without any external stabilizing agents. Consequently, this paragraph will focus on the aspect of colloidal stability. Colloidal stability indicates to what extent your nanoparticles are resistant to return from the dispersed (finely distributed within the solvent) state to the bulk state. From a thermodynamic point of view, all colloids are unstable since a reduction of their surface area is energetically favored. However, this process can be kinetically inhibited, which could lead to colloids stable in solution for centuries (as the gold colloid made by Faraday 150 years ago). So in order to understand the concept of colloidal stability you need to basically understand the nature of attractive and repulsive forces in a colloidal system. This knowledge can be provided by the DLVO theory named after its inventors Boris Derjaguin, Lev Landau, Evert Verwey and Theodoor Overbeek. Based on this theory, the interactions between colloidal particles are driven by 2 main forces: 1) the van der Waals attraction and 2) the electrostatic repulsion. Van der Waals forces are caused by permanent or induced dipoles between uncharged but polar or polarizable materials (actually any material is polarizable!) and are much stronger on a short distance. Electrostatic forces, on the other hand, are caused by the so-called electrical double layer around a charged particle and are usually more pronounced at longer distances. Based on this, a potential curve can be constructed (Figure 43 A), which gives you the sum of all the potential energy in correlation with the distance between two particles in the colloids. Stability means keeping a large distance. Looking at this graph you can observe that there are two minima of potential energy.



Our laser lab has sheets covering the window. On a Monday I entered the room in the morning and instantly got the feeling that something was different. After some time I realized the sunlight coming in through holes in the sheets covering the windows. I also saw some straight lines in some covering boards leading towards the holes. Actually the last student that had worked with the laser on a Friday was dithery adjusting the laser beam while the laser was running on high power settings thereby unintendedly optimizing the sunlight incidence into the laser lab...

Sven Reichenberger, Duisburg

The first, the so-called primary minimum, is located at a lower distance and indicates a state where two nanoparticles are irreversibly fused, this process is called aggregation and the resulting materials are hence named aggregates. In addition, there is a second minimum found at greater distance between the particles. Here the particles are more loosely bound, predominantly in a reversible fashion. This process is called agglomeration and the resulting materials are termed agglomerates. For the stability of the laser-generated colloids you can conclude that there are two main processes, which could interfere with the colloidal stability of the particles, being reversible "loose" agglomeration and irreversible "tight" aggregation. While agglomerates may generally be re-dispersed easily e.g. by intensive shaking or short ultrasonication, aggregates are generally permanent and you may need to adapt your synthesis parameters in order to avoid it. In the potential curve diagram (Figure 43 A), a stable colloid needs particles kept at a distance of > "min 2" or better even further away from each other.

To enhance the general understanding of colloidal stability you now need to take a closer look at the main stabilizing force (that is, the repulsive force) in ligand-free colloids, which are surface charge and the consequent formation of the electrical double layer. The first fundamental question relevant in this context should be why laser-generated colloids, e.g. composed of metal, possess a surface charge at all. A pure metal surface in a solvent should not be charged and hence the particles should be subject to immediate aggregation. E.g. elemental gold is hydrophobic, insoluble in water and loves to bind to other particles by van der Waals force, and once the solids are in contact, inter-particle atom diffusion diminishes particle boundaries causing irreversible fusion. However, the laser process generally yields a partial surface oxidation of the colloidal metal nanoparticles, and partial reduction of oxide nanoparticles.

Hence, the surface of laser-generated nanoparticles carries defects (which are very valuable for both, application and colloidal stability), and the defects attract surface adsorbates. These surface adsorbates are of course countercharged. Coming back to elemental metal nanoparticles with electronic defects at its surface (e.g. a few Au+ and Au3+ sites at the surface of a sphere dominated by Au(0) atoms): These oxidized surface atoms can then attract hydroxyl anions from water and form a pH-dependent equilibrium between M-OH and $M\text{-}O^-$ (M=metal) groups, which generally yield a partially negative surface charge in laser-generated colloids. Hence, the particle became an acid, ready to exchange protons with water. Yes, it even has a defined value where the charge flips towards positive charge ($M\text{-}OH_2^+$) if more protons are taken up at low pH. E.g., for laser-made gold nanoparticles, the flipping point, the so-called isoelectric point is reached at a pH of around 2, which shows that ligand-free gold nanoparticles are very acidic.

In case these charged nanoparticles are dispersed in an electrolyte like water, they attract counterions and form an electrical double layer. It consists of

two parts with different potential drops as specified by Stern. (Figure 43 B): i) An inner layer termed Helmholtz layer where ions are densely bound to the nanoparticle surface (e.g., M-OH-) and we have a linear drop of the potential with distance from the surface. ii) A diffuse layer of loosely bound, more or less mobile counter ions (the beneath negative Helmholtz layer attracts cations, which in turn bring anions, and so forth). The most critical criterion for colloidal stability is, whether the electrical double layer extends far enough to keep the nanoparticles at an appropriate distance and prevent aggregation or agglomeration. This can be described by the Debye screening length (κ^{-1}), which indicates the distance at which the electrostatic potential has dropped by 1/e. This means that the E_{pot} at the Debyé's distance has dropped by about 67% of its original value.

For oxides, the same rules apply, just the charge sign is turned, with oxygen defects resulting in a positive Helmholtz layer, attracting negative counterions, etc. E.g. ZnO made by laser ablation in water is a base with oxygen defects (oxygen vacancies) and (slightly) positive surface charge at neutral pH.

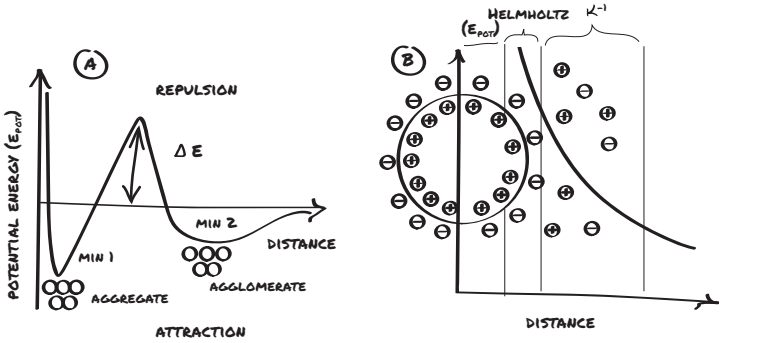


Figure 43: Stay away: DLVO theory and electrical double layer. (A) Potential energy-distance curve in a colloidal system indicating the primary minimum (Min1), secondary minimum (Min2) and activation energy (ΔE). (B) Illustration of the electrical double layer around a spherical particle. Indicated is the potential energy curve, the Helmholtz layer and the diffused layer. κ^{-1} illustrates the extension of the Debye screening length.

Now you know how to define the term "colloidal stability" and you have learned that electrostatic repulsion (good) and van der Waals attraction (bad) are the main forces at work. Based on this you have learned the physical origin of surface charge as well as the electrical double layer in a completely ligand-free laser-generated colloidal system. As a follow up, it would be interesting to know how you can affect colloidal stability.

STEP 2: INCREASE COLLOIDAL STABILITY...

In this context, it is essential to know practically how to affect colloidal stability by external parameters like temperature, particle concentration, pH, ionic strength, impurities and surface ligands.

...BY TEMPERATURE:

The impact of temperature on colloidal stability is straight forward when you look at the potential energy curves derived from the DLVO theory. From this graph, you can clearly see that agglomeration and aggregation processes are generally inhibited by a barrier of potential energy (ΔE in Figure 43). The only way for a certain particle to cross this barrier is when it collides with another particle: only when the kinetic energy of the two surmounts this energy barrier, the barrier will be surpassed. And it's our intention in a stable system to avoid that this happens so that particles keep their distance.

It is well known that the mean kinetic energy which corresponds to the movement of particles (k_BT, with k_B being the Boltzmann constant and T the absolute temperature) increases with temperature. And this movement is the cause of collisions between two particles. This means that at higher temperature the number of particles exceeding the necessary energy barrier for agglomeration and aggregation is elevated. As a result, aggregation and agglomeration are more pronounced at higher temperatures. To clarify this point let's assume you have a collective of 6 particles with 1 particle possessing the necessary kinetic energy to induce agglomeration or aggregation, each of them exposed to one collision event within the observed timeframe (Figure 44 A). Let us further assume that elevated temperature doubles the number of particles with the necessary kinetic energy to 2. Consequently, the probability of agglomeration or aggregation due to a collision with a particle of sufficient energy would increase (Figure 44 B). Based on this, you should consider keeping the temperature of your colloids as low as possible to enhance stability. Concluding this thought, storage in the fridge is recommended, 8 degrees Celsius is fine. However, you should keep in mind that freezing a ligand-free system should be avoided.

STUDENT: THE CONTACT ANGLE MEASUREMENT IS NOT WORKING.

ASSISTANT: THE COVER IS STILL ON THE CAMERA.

SVEN KOENEN, ESSEN

There is another negative effect of high temperature: the liquid's viscosity decreases exponentially with the temperature. That is a pity because the diffusion constant will in turn increase by the same factor. Knowing that diffusion is also proportional to kinetic energy (T_k, see above), means that the speed of a particle that approaches collision with another particle is proportional to $T \cdot e^T$. Hence, every degree of temperature is your foe in the lab. Note that your fingertips are permanent 36°C heaters, and a small 1 mL vial taken freshly from the 8°C fridge has nothing to defend itself while you carry it to the DLS machine measuring aggregation.

Cooling is the most simple, effective, and contaminant-free way to preserve a colloid's stability. Everybody in a cool lab is chilled, so are the samples.

... BY CONCENTRATION:

While the impact of temperature on colloidal stability is pretty obvious, the effect of particle concentration is less plain. You learned that a lower mean kinetic energy of the particles is beneficial in order to not exceed the activation barrier of aggregation and agglomeration. In addition to this, not only the energy of the corresponding particles can enhance the probability of aggregation but also the frequency of collisions, which, of course, is elevated at higher particle number concentration. Let's take the example from the previous paragraph: a collective of 6 particles, 1 exceeding the activation barrier, one collision each. Let's now assume that an increase in particle concentration would double the number of collisions (Figure 44 C). Hence, collision scales with the square of the particle number concentration. Consequently, the probability of agglomeration or aggregation would also increase. Based on this, it can be assumed that colloidal stability is generally favored in less concentrated colloids. So to make it simple: dilute your colloid in order to enhance colloidal stability. You will often need to dilute it for analysis, anyway. We have had good experience keeping gold at 100 mg/L stable for months stored in the fridge. But ligand-free 300 mg/L or even 500 mg/L gold stored in at room temperature is a lucky or bad shot, maybe depending on the degree sunshine on the day of synthesis.

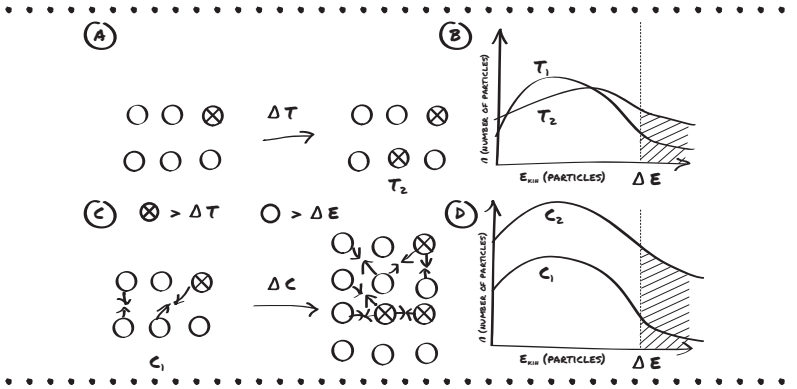


Figure 44: Impact of temperature and concentration on the energy distribution of colloidal particles: (A) Cartoon illustrating an increasing number of particles with a kinetic energy (E_{kin}) > activation energy for aggregation (ΔE) and (B) energy distribution of the corresponding particles showing a higher number of particles with E_{kin} > ΔE at higher temperature. (C) Cartoon illustrating an increasing number of particles with increasing particle concentration as well as an elevated collision probability (indicated by a higher number of arrows). (D) Energy distribution of the corresponding particles showing an increasing number of particles with E_{kin} > ΔE at increasing particle concentration, due to the higher number of particles and due to elevated collision probability.

VI

pH

... BY PH VALUE:

Laser-generated metal colloids in aqueous medium are at least partially oxidized and carry a portion of M-OH_2^+/ M-OH/ M-O^- (M=metal) groups on their surface. Again, this is true for most oxides as well, resulting in cationic defects (oxygen vacancies), e.g., positively charged $ZnO_{(1-x)}$. Hence, noble metals are often acidic nanoparticles, and oxides are often alkaline.

It is obvious that alterations in the pH-value could lead to a protonation and deprotonation of these surface groups, which can critically influence surface charge and colloidal stability. Consequently, there is a certain pH-value where all surface groups, in sum, are in an uncharged state. This value where the charge flips sign is termed point of zero charge or isoelectric point (IEP). Naturally, this point highlights the regime of lowest colloidal stability and should be avoided during fabrication and storage of laser-generated colloids. A general rule for achieving maximum stability should be to adjust your medium pH to be as far as possible from the isoelectric point (but still avoiding dissolution) and, with the medium being about one pH unit away from it, you are often already on the safe side. This concept is illustrated in Figure 45 A, with the pH of the medium increasing from the left to the right, crossing the IEP in the middle picture. Knowledge on the isoelectric point can be either obtained from the literature (already Wikipedia has a short list at the entry “isoelectric point”), in case you are working with a well-established material, or determined by titration

experiments. In the latter case you titrate your colloid by adding acid or base and the point of zero charge is reached when your particles aggregate and precipitate. This procedure is ideally probed by zeta potential measurements (the concept of zeta potential is described later). But the aggregation point is often so easy to see that IEP can be found simply with the naked eye and a pipette of acid or base.

... BY IONIC STRENGTH:

The impact of ions on the colloidal stability of laser-generated colloids is rather ambiguous. But once you understood it you will never want to miss this mighty tool, in particular for laser synthesis in water. In our lab, the salinity-trick is used on a daily basis and basically entails adding low salt concentrations to increase stability. But let's first understand the basics. The first and foremost thing you need to be aware of in this context is that high ionic strengths critically reduce the Debye length (κ^{-1}) and a low Debye length is actually a stability death sentence. The underlying physical phenomenon is a screening of repulsive surface charges by an overabundance of counterions (Figure 45 B). To put it simply: With a lot of counterions around, the particles can no longer "feel" the repulsion from neighboring particles and hence they aggregate due to attractive forces. This correlation between the Debye screening length and the ionic strength (I) in water at 25 °C is given by the following equation:

$$\kappa[nm^{-1}] \approx 3.3 \cdot (I[M])^{1/2}$$

Based on this you could calculate a Debye screening length (κ^{-1}) of 30 nm in the presence of a 100 µM electrolyte, however this value is reduced to only 0.7 nm in a 200 mM electrolyte, completely annihilating colloidal stability and initiating aggregation under these circumstances. As a rule of thumb you can conclude that high ionic strengths e.g. > 2 mM should be avoided when synthesizing and handling laser-fabricated colloids. In addition, you need to keep in mind that the destabilizing effect is intensified in case multivalent ions $M^{Z+/-}$ like Ca^{2+} or SO_4^{2-} are present. So the utilization of these ions should be totally avoided during laser ablation in water. Whereas the concentration screens stability by $c^{0.5}$, the valence of the ion contribute additionally by $c^{Z \cdot 0.5}$ to kill stability.

In contrast to the above mentioned well-known fact of oversalting (or "salting-out-effect"), recent studies have concluded that the page is turned at a minute amount of salt! This is an effect that is very specific and can be very beneficial for ligand-free particles, as those are the ones we make by laser ablation or laser fragmentation in water.

The *in situ* presence of certain anions at micromolar salinity during the particle synthesis process can be beneficial for colloidal stability. In situ means that the salt has to be dissolved in the liquid before you switch on the laser. In this way, it is most effective. This effect has been reported by the authors of this handbook

to work best with highly polarizable anions (e.g. Cl^-, Br^-, I^-) and only on noble metal nanoparticles with low oxidation tendencies (Au, Pt, Pd). Phosphate and carbonate work as well. For gold, also an equimolar mixture of NaOH and NaCl works quite nicely, in particular for LFL.

Using this method, the colloids are far more stable and the size distribution is narrowed. That's two birds with one stone. But how does it work? These effects were attributed to specific anion adsorption to the nanoparticle's surface during the particle formation process. Sodium chloride works well, also carbonate or phosphate works in our lab. Best friends are sodium bromide and sodium hydroxide at 100 – 300 mg/L. So, during your noble metal particle synthesis, you may add low salinity electrolytes to enhance colloidal stability. Make your first try with dissolving 200 micromole table salt. Note that this is only 0.1 mg per liter, a bit less than a grain of salt.

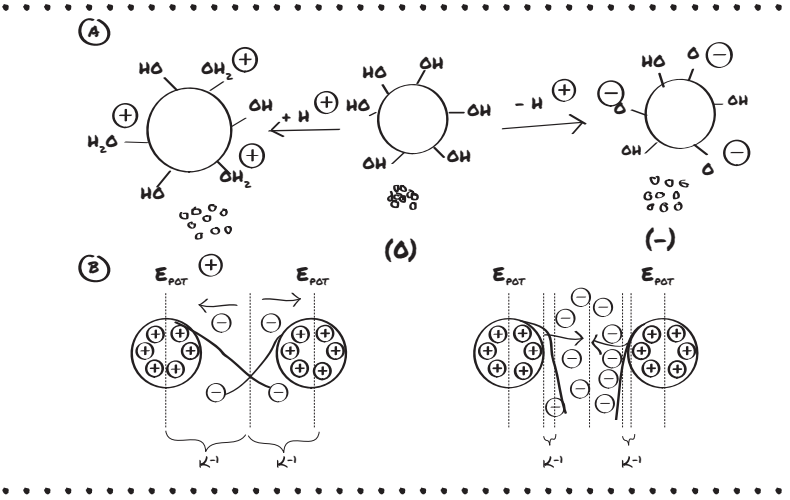
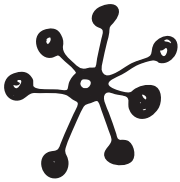


Figure 45: Neutral is worth nothing. Stay in charge. Add a grain of salt. Effect of pH and ionic strength on colloidal stability: (A) Cartoon illustrating the effects of stable protonation at acidic pH (left) and stable deprotonation at alkaline pH (right) of a particle at the colloidal instable isoelectric point. (B) Cartoon illustrating the potential energy (E_{pot}) of two particles in the presence of low counterion concentrations (left) and high counterion concentrations (right) as well as the extension of the Debye length (κ^{-1}) as a measure of repulsive stabilization. Arrows indicate repulsive forces between the particles (left) and attractive forces (right).

...BY IMPURITIES:

Sometimes colloids aggregate during your laser ablation experiments, while others remain perfectly stable, even though your synthesis conditions seem completely the same. This lack of reproducibility can be highly frustrating and is in most cases because you have worked dirty, so in other words, it is attributed to

impurities in the sample. These can be simply dust particles from the surrounding atmosphere (often partly soluble in liquid), residual particles remaining in your chamber from previous ablation experiments as well as multivalent ions leaking from your glass vessel or metal chamber. Hence, if you want to precipitate a colloid (of your nasty colleague in order to keep him/her in good mood): add calcium chloride, or make sure to deposit some salty fingerprints. Also, metal containers, stainless steel spatula, and chambers deliver multivalent ions even after using them for years. If you need to work with metal in contact with the liquid, use anodized metals (mechanical workshops do that easily, or use so-called Piranha liquid), or passivated metals such as aluminum chambers, or just Teflon. But even teflon needs to be harshly cleaned by aqua regia after every 3rd to 5th synthesis batch.



Other impurities may come from the target material, in case it is not of highest purity. A noble metal even at 99.9% purity naturally contains silver and, even worse, copper, easily dissolving from the target or the particles after ablation and destabilizing the colloid via charge screening. So in conclusion, etch before use, and buy highest grade purity.

VI

I once asked a student to perform laser ablation experiments in saline solutions, while I gave her a concentration regime of 5 – 500 millimolar. She discovered some interesting effects on size quenching, however, we later discovered that she made a mistake in her dilution series and she actually examined 5– 500 micromolar concentrations. This is how we discovered that salinity quenching occurs even at very low ionic strengths.

Christoph Rehbock, Essen

In addition, the liquid itself is a well-known source of impurities. Organic solvents always contain unknown impurities, whose concentrations can be way higher than your colloid mass concentration. Let's assume a typical colloid mass concentration of 50 mg/L and technical grade acetone with 99.9 % purity. In this case the amount of undefined dirt (0.1 %) would still sum up to 800 mg. So in total you have a 16 times higher mass of dirt in your sample compared to the mass of your particles. As a consequence, the only way to avoid interference from impurities is to work in an environment as clean as possible. But even very high commercial solvent purities still will have enough contaminants inside to cover the surface of all particles you've fabricated. So you can't be lazy, you need to clean your solvents on your own by distillation and checking purity with gas chromatography is the only way out if 100% purity is what you want. But very often, in our lab, simply the solvents used for cleaning have been detected

as source of contamination. Once the solvent bottle is unsealed, the solvent takes up softeners and additives from the seal, the tubings, your protective wear, etc. And this is retarded in the equipment and found as traces even during the aqueous synthesis post cleaning. Practical advice you should adhere to is:

I) Thoroughly clean all your glassware (or use disposables), your chamber as well as your target. Use the solvent you work with later for cleaning as well.

II) Get target materials at maximum purity

III) Purify your solvents e.g. by distillation

IV) Preferably do not use the same chamber for different materials, never use non-anodized metal chambers or spatula

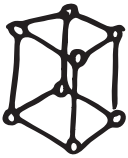
V) Use a chamber composed of a material that can be easily cleaned by the strongest possible oxidants like “aqua regia”. We like Teflon.

VI) In the case of aqueous solutions: use plastic containers instead of glass vessels for long time storage, however in case of organic solvents, interactions between solvent and container may render glass more suitable.

VII) For the tubings, silicon is nicely flexible but the worst you can do because it retards everything in its pores. Better go for Teflon or something similar that is resistant to solvents and has a smooth surface.

VI

... BY LIGANDS:



The most important characteristic of laser-generated colloids is their purity due to a completely ligand-free synthesis process. However, some applications e.g. utilization in a salty biological environment may necessitate the presence of stabilizing or biofunctional surface ligands. Once you have effectively grafted stabilizers to your surface, in particular bulky ones, your colloid will survive everything, even freezing and repeated freeze-drying. It́s wonderful to see a freeze-dried, golden pellet to eject a perfectly rubin-coloured liquid once you put a drop of water on it.

These stabilizers can either just be serum proteins, present in a biological environment anyway, chemical polymer ligands, as well as functional ligands used in bioactive nanobioconjugates. Basically, conjugation processes can either be conducted in situ or ex situ. During in situ conjugation, the ligands are present during the laser ablation or laser fragmentation process. The main advantages, in this case, are generally a more narrow size distribution as well as a very high surface coverage due to an instantaneous reaction (surface adsorption) between the just formed nanoparticle surface and the ligand. The downside of this procedure, however, is also pretty obvious. Firstly, organic ligands are present during the laser ablation process. This may go along with

a significant degradation of conjugates by post-irradiation events by the laser beam, a problem which necessitates careful adjustment of the applied laser fluence during ablation (Chapter II). Another impairment originates from the fact that the nanoparticle-to-ligand-ratio cannot be properly controlled in situ. While the ligand concentration in the ablation medium is fixed, the particle concentration increases during the laser synthesis process. As a consequence, the ligand-to-nanoparticle-ratio decreases with time and uniform conjugation is unlikely. Ex situ conjugation, on the other side, entails a two-step process where the ligands are added to the previously generated colloids. This process allows for a precise adjustment of the ligand-to-nanoparticle-ratio and a well-controlled conjugation process. This approach, however, requires alternate strategies for size control, e.g. salinity-induced size quenching (see above). As a rule of thumb, single-digit micromolar ligand concentrations already work (if ligand has a high particle affinity).

The presence of surface ligands has a significant impact on the stability of colloids as surface charge density, in this case, will be dominated by the properties of the ligand as well as by those of the metal core. Based on the nature of the ligand, stabilizing effects can either be electrostatic, steric, or electrosteric (illustrated in Figure 46 A), and the steric is always the most robust.

Working with substances in the micromolar concentration range often includes the necessity of using volumes below 10 µL which is in fact not more than a droplet. In this case, never trust a physicist doing bioconjugation and telling you to dip the tip with your biomolecule into your colloid. You will never get nearly reproducible results, in particlular concerning the surface coverage.

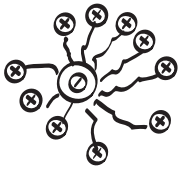
Lisa Gamrad, Essen

Electrostatic stabilization usually occurs when small highly charged ligands (e.g. citrate or short peptides) are used. When these ligands covalently or electrostatically attach to the particle surface, their net charge is added to the particle's surface charge. In case the charge of the particle and the charge of the ligand carry the same sign, e.g. negatively-charged citrate on negatively charged gold nanoparticles, this may lead to a significant stabilization of the colloid. However, in case the charge of the particle and the charge of the ligand carry opposite signs, an effect commonly referred to as charge-balancing occurs (illustrated in Figure 46 B). As a consequence, colloidal stability in oppositely charged systems is generally reduced. On the other hand, steric stabilization is basically found when nanoparticles are coupled to bulky uncharged ligands

e.g. hydrophobic polymers (bound by van der Waals force). The main stabilizing force at work here is Born repulsion, that is, the ligands prevent the particles from reaching the necessary distance required for aggregation and hence keep the particles dispersed in the solvent. Amphiphilic polymers are the best for metal nanoparticles, since they may bind to the particle's hydrophobic surface, and at the same time provide hydrophilic groups keeping the particle dispersed in water. Such a polymer widely used in laser synthesis in literature is polyvinylpyrrolidone (PVP) or polyethyleneglycole (PEG). If you want to go Bio, a typical serum protein like albumin is another amphiphilic macromolecule that provides steric stabilization, and is present in most biofluids and cell culture media, anyway.

A hybrid form of stabilization termed electrosterical stabilization, is found in case particles are conjugated with bulky, charged surface ligands e.g. proteins or oligonucleotides or classical surfactants. How big shall the ligand be? As a rule of thumb, 12 carbon bond lengths are required to add enough steric character to the electrostatic stabilization. Maybe you heard about sodium dodecylsulfate? Same works in non-polar solvents, e.g. dodecane-thiol is a gold standard of capping agent in hexane (but does not work in water). But if the ligand gets too bulky, its diffusion coefficient is lowered, decreasing the effectiveness of reaching the particle's surface during its growth (in situ) or before aggregation starts (ex situ).

The final thing you want to optimize is the tightness of binding to the nanoparticle surface. The stronger it binds the more is the equilibrium shifted towards the nanoparticle, and you will need less excess of ligand for grafting a monolayer. In ideal cases, such as thiolated peptides or thiolated oligonucleotides, almost 100% conjugation efficiency is achieved. Decorating the surface of laser-generated nanoparticles with surface ligands usually requires a strong interaction between the ligand and a metal core, which is usually achieved by electron donor moieties like amines or thiols, which strongly interact with the metal surface atoms. It is an old rule of conjugation chemistry: the soft Lewis acid (noble metal nanoparticle) has the highest affinity to soft Lewis base (disulfide > thiols > thioethers > carboxy, amines). For noble metals, thiols work very efficiently, so that this is the gold standard for the fabrication of gold nanoparticle bioconjugates. For the peptides it is most simple, just add a terminal cysteine amino acid. Same works with polymers, gold conjugated to mercapto-polyethyleneglycol (m-PEG) has survived the highest salinities among several stabilizing agents we have tested. And for metals that oxidize or oxides? Carboxy groups have been reported to work well to be anchored on oxides, and you will need a carbon chain pointing outwards to contribute to steric stabilization. Without going into detail, you will need to have the right pH adapted to both the molecule (deprotonation) and particle (IEP). Maybe the molecule MEEAA (see Chapter IV – Case 5) or other complexing agents are a good start.



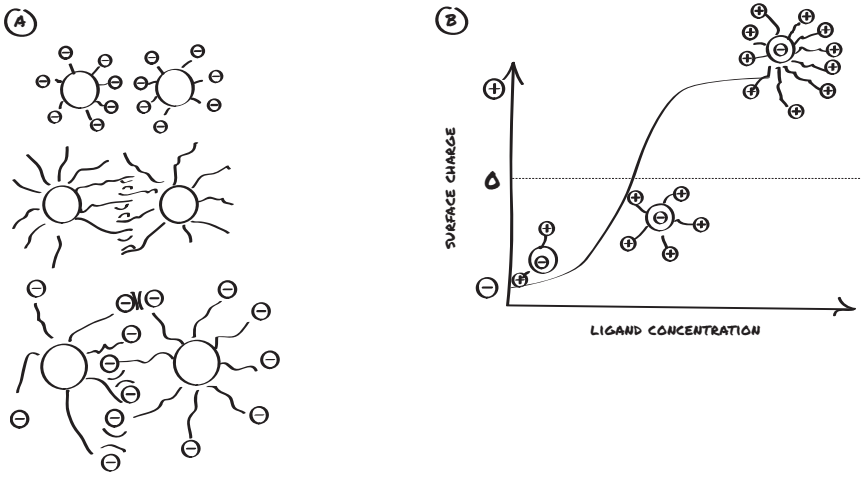


Figure 46: Decorate. Stability delivered by ligands: (A) Cartoon illustrating electrostatic (top), steric (middle), and electrosteric (bottom) stabilization of nanoparticles. The electrosteric stabilization by surfactants is often a double-layer with the charges pointing both inwards and outwards. (B) Scheme illustrating charge compensation while adding positively charged ligandsto a negatively charged colloid.

In the previous part of this chapter, you learned how to define the term colloidal stability and how it can be correlated with surface charge as well as properties of the electrical double layer. In addition, you learned how colloidal stability changes when temperature, pH, ionic strength are altered and when impurities, as well as different surface ligands, are present. The final step to a unified understanding of colloidal stability is how to measure it.

STEP 3: MEASURE COLLOIDAL STABILITY...

In order to measure colloidal stability, you need to monitor to what extent the properties of your colloid changes over time. In this context, you need to keep in mind that visible aggregation, like discoloration of your colloid and formation of precipitates, are only the tip of the iceberg, more subtile effects are far more frequent. A popular and very powerful tool to monitor colloidal stability are UV-Vis spectroscopy and zeta potential measurements, which are explained in the following subsections.

... BY UV-VIS ABSORPTION SPECTROSCOPY

UV-Vis spectroscopy is a fast and easy to use standard method available in most laboratories. The basic principle entails shining light through your

sample while the attenuation (extinction) of your light intensity is observed in comparison to a standard, e.g. the pure solvent. When this is done for all wavelengths you acquire a spectrum were light extinction (typically expressed as absorbance) is plotted against wavelength. More information on how UV-Vis spectroscopy works and what else it can be used for is included in Chapter V where all relevant characterization methods are listed. Almost all colloidal nanoparticles show a distinctive extinction spectrum, the most obvious examples are plasmon resonant metal nanoparticles like Au, Ag or Cu. While the details of spectra interpretation are explained in Chapter V, this paragraph focuses on how this technique can be used to evaluate colloidal stability. The simplest way to utilize this method is to measure whether or to what extent your spectrum changes with time. In this case, the simplest rule of thumb is: I) The spectrum does not change with time = stable colloid; II) The spectrum changes = unstable colloid. This approach is admittedly rather crude but gives you general information about your colloid and it is generally applicable for all colloids that possess any kind of light extinction in the UV-Vis. A proper follow-up could be to use either the extinction at a certain wavelength or even better the sum (integral) of all extinction values in a certain wavelength regime to quantify your stability, e.g. the loss with time (Figure 47 A). The decrease of extinction with time is the most straightforward phenomenon, as precipitation is meant to occur in case colloidal stability deteriorates. Maybe a good definition is that the integral of extinction does not change by more than 5% in 4 weeks.

Unfortunately, it is not always that easy. Sometimes you may encounter that your extinction even increases with time, would this mean that particles get more stable? Most certainly not! This observation would be a clear indication that there is more going on in your sample (it is still “aging”) like chemical changes e.g. the formation of reaction products with different optical parameters or change of particle concentration due to particle growth from precursors with time. In this case, the aforementioned simple stability criterion fails and more detailed investigations e.g. of particle size need to be added (compare Chapter V). To give one example, after laser fragmentation (post-irradiation) of a platinum colloid is terminated, the extinction still drastically increases as there are still atom clusters that undergo coalescence and ripening to particles, increasing the colloid’s extinction. This is happening in real time, you can follow it in the UV-Vis directly after laser fragmentation. So better wait until it is calmed down, in order to probe and report reproducible properties.

However, UV-Vis spectroscopy can do more than just measure the total change in extinction. In many materials it can be used to monitor the aggregation or agglomeration process as larger particles generally scatter light at a longer wavelength. As a consequence, an increase in infrared extinction can be an indicator to monitor particle aggregation. A commonly used parameter is the ratio of primary particles to aggregates = the Primary Particle Index (PPI). For gold nanoparticles, this value is already well established and is defined by

dividing the extinction at the gold interband extinction (@λ=380 nm) by the extinction of the aggregates or agglomerates in the NIR regime (@λ=800 nm). A high PPI value goes along with a low number of agglomerates and consequently with a good colloidal stability (Figure 47 B). A similar PPI value can also be used for semiconductor nanoparticles e.g. ZnO where the extinction at the bandgap energy in the UV can be divided by the NIR aggregate/agglomerate scattering. So concerning the PPI, you can memorize that it is a suitable way to quantitatively evaluate aggregation and colloidal stability. As a rule of thumb, you should keep in mind that for ligand-free gold colloids a PPI < 4 should be avoided in order to prevent precipitation of your colloid. A PPI of > 10 sounds stable.

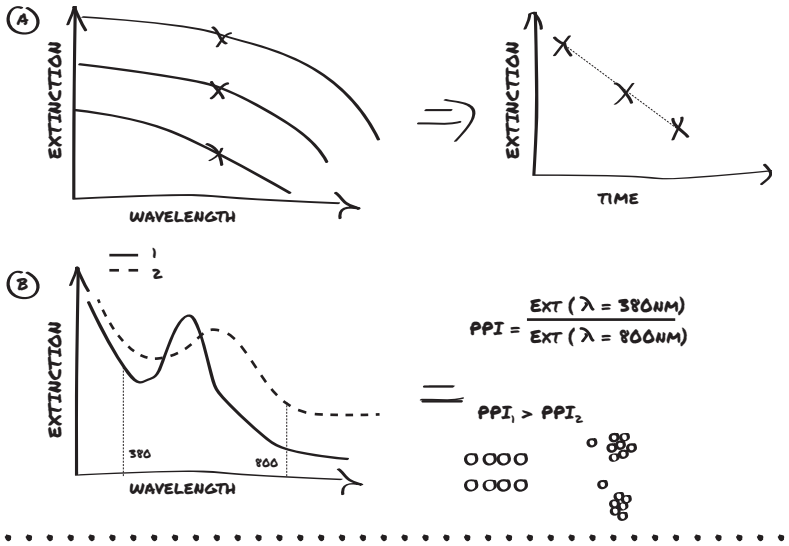


Figure 47: Stay with the color. UV-Vis spectroscopy to evaluate particle stability. (A) Illustration of how to use time-resolved changes in the UV-Vis extinction to evaluate the loss of colloidal stability. (B) Illustration of the primary particle index (PPI) in gold colloids evaluating colloid 1, with a high PPI, to be more stable than colloid 2 with a lower PPI.

... by Zeta Potential:

Many works use zeta potential measurements to evaluate colloidal stability, but information obtained from this parameter can tell you much more. Therefore, the first question which needs to be addressed is: "What is the zeta potential?". The zeta potential (ζ) or electrokinetic potential is closely linked to the above-mentioned surface charge and it is a powerful value for the characterization of the electrical double layer. In case charged colloidal particles are exposed to

an external electric field, these particles will move within the field; e.g. in case they have a negative surface charge, they will drift to the positively charged electrode. The tightly bound ions in the Helmholtz layer will follow the movement of the particle, those situated in the diffuse layer cannot and will slip off when a certain potential is reached (because they cannot cope with the speed of the particle core and are peeled off by the drag force). This potential at the slipping plane is called the zeta potential and can be considered an indicator of the surface potential caused by surface charge. Naturally, it requires more energy (a higher potential) to remove ions from a highly charged particle in comparison to a particle with a lower charge. However, you need to be really careful when interpreting zeta potential measurements. On the particle you will basically have 3 values of relevance: 1) The surface potential caused by charged surface atoms, 2) The Helmholtz potential caused by tightly bound counter ions and 3) the zeta potential at the slipping plane (Figure 48 A). The zeta potential is the only one of these potentials which is experimentally accessible in liquid, but during all your interpretations you always need to keep in mind that it is not necessarily equivalent to surface potential/surface charge. The surface charge/oxidation state of the inorganic core can be measured by XPS in the dry state.

VI LAB STORY

A weakness when working with LAL is the glass of the chamber. Once when working with ethanol in a flow chamber the glass cracked and ethanol was pumped out of the chamber. The organic solvent was pumped with 250 ml/min right into the laser beam. And so the inevitable happened: the organic solvent was ignited before the laser and the pump could be stopped. After the ignition source was eliminated the flames were easily extinguished, but only due to a timely response.

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The next question which needs to be addressed is: "How can you measure the zeta potential?". The zeta potential cannot be measured directly but it is usually calculated from the drift velocity of the charged particles in an AC electric field. Measurement of the particle's drift velocity (v) is usually conducted via light scattering techniques. The drift velocity itself, however, is not a suitable value as it is linearly dependent on the external electric field (E). In order to obtain a measured value which is independent of the external field, the electrophoretic mobility (μ) is usually used, which can be obtained via:

$$v = \mu \cdot E$$

The electrophoretic mobility (μ) is linearly correlated with the zeta potential and can be calculated by approximations made by Smoluchowski and Hückel. You can choose between the models in the instrument software. The most important rules of thumb you have to be aware of are:

I) Smoluchowski: Valid for high ionic strengths and larger (i.e. > 300 nm @ 1 mM) particles

II) Hückel: Valid for low ionic strengths and smaller (i.e.< 20 nm @ 1 mM) particles

III) Transformation: $\zeta_{Hückel} = 1.5 \cdot \zeta_{Smoluchowski}$

As there is a factor of 1.5 between the values, you need to state in your measurement protocol or manuscript if the zeta potential value refers to the Smoluchowski or Hückel regime.

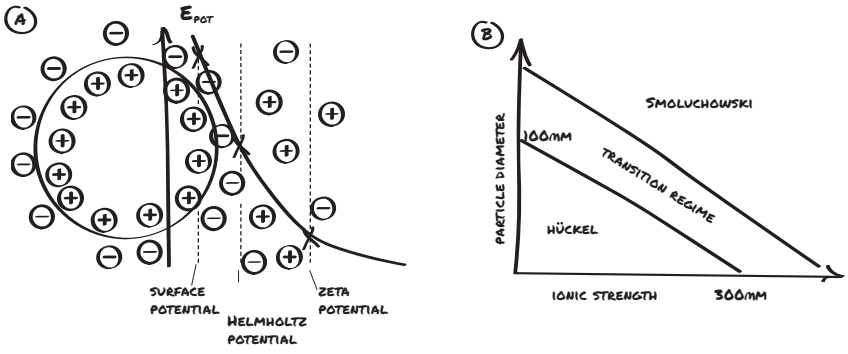

Figure 48: Measure the charge. (A) Illustration of surface potential, Stern potential and zeta potential in the electrical double layer of a ligand-free nanoparticle. Dashed lines represent (from left to right): the surface charge, the Stern layer and the slipping plane. Ions located outside the slipping plane are stripped off during electrophoretic mobility measurements. (B) Representation of the validity of the Hückel and the Smoluchowski regime for different ionic strengths and particle diameter.

Based on what you have learned so far, you basically know what the zeta potential is and how to measure it. As a logical follow-up, it has to be addressed what practical uses the zeta potential has for evaluating the properties of your colloid, or in other words: “What can you learn from the zeta potential ?”. The most straightforward answer is that the zeta potential can give you a general idea on the surface charge of your colloid. The sign of your zeta potential is a good representation of whether you find a positive or a negative surface charge. In addition, the value of the zeta potential gives you a rough indication on the

electrostatic stability of your colloidal system, as a high value usually goes along with a high surface charge and hence a high stability.

This correlation may, in some cases, even be used to calculate the surface charge density. A general rule of thumb often found in literature is that colloidal stability can be found in case $\zeta > \pm$ 30 mV. However, you need to keep in mind that this value is not universal. It was calculated based on the potential energy required to surmount a kinetic energy k_BT found at room temperature (25°C). Hence, 30 mV means that the average number of particles have a kinetic energy that is too low to cross the potential barrier during their random walk. However, if you look at the Maxwell-Boltzmann-Distribution of speed at a given temperature, you will note that a large fraction of particles will be fast enough to undergo agglomeration or even aggregation. And even at doubled zeta potential, there will always be some particles that are faster than the average. So aggregation always happens, colloids are living systems, it is only about to slow it down. As good as you can.

As a hands-on summary, here are some important points you need to consider when correlating the zeta potential with surface charge and colloidal stability:

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I) Effects of temperature and concentration: As previously mentioned, high temperatures and high particle concentrations can impair colloidal stability. This is usually not represented by appropriate changes in zeta potential. Consequently, you need to figure out individual stability criteria in case these values dramatically deviate from standard conditions. And only measure the zeta potential at the temperature that is relevant for the stability information you want. Most instruments have thermostats, but you need to wait at least 10 minutes for your sample to equilibrate to the temperature set in the instrument's software.

II) Effects of particle size distributions: The effect of particle size is generally considered while evaluating whether the Smoluchowski or the Hückel regime are used. Some difficulties may arise in case you try to determine zeta potentials of particles with a very broad size distribution (see Chapter V on Light scattering DLS). These problems, however, do not originate from the modeling but are rooted in drawbacks of the corresponding measurement of the electrophoretic mobility via light scattering. As the scattering signal from bigger particles is significantly higher than that of small particles, the signals from small particles may not be properly detected. As a consequence, you only determine the zeta potential of your large particles while the smaller ones remain invisible (at least significantly underestimated) to your method.

III) Effects from specific ion adsorption: When laser ablation is carried out in the presence of micromolar salinity electrolytes, the particles are prone to specific anion adsorption. This can yield a significant electrostatic

stabilizing effect, which does not necessarily show in the zeta potential as the adsorption may occur in the Helmholtz layer behind the slipping plane. Hence these effects would not be detectable by zeta potential measurements (but by the PPI in the UV-VIS, and in the XPS).

IV) Effects from ligands: In the presence of ligands, particularly when they are bulky, zeta potential can no longer be considered a reliable indicator of surface charge and stability. This drawback basically originates from the measuring principle. During measurements of the electrophoretic mobility, you determine the movement of charged particles in an electric field. If these particles are now covered with surface ligands, these ligands will induce a drag force which slows them down. Consequently, the correlation between electrophoretic mobility and zeta potential based on simple models like Smoluchowski and Hückel are no longer valid and the displayed zeta potentials are too low. This reduction of the zeta potential was frequently observed in case nanoparticles were surrounded by a protein corona when exposed to a biological medium and has nothing to do with changes in surface charge. In addition, the correlation between colloidal stability and zeta potential also fails in ligand-capped systems as the underlying theory is based on electrostatic effects and does not consider steric stabilization effects. A polymer-stabilized particle often has a zeta potential around zero, although it is very stable.

After reading this chapter you can even impress your supervisor about basic insight into colloidal stability. You know how to define stability, how to measure it and how to affect it via different physical and chemical parameters. Aren't that great prerequisites?

During fragmentation experiments of an oxide in ethanol smoke emerged from the flow chamber used. First it was thought that the organic solvent was a problem. After a while it was realized that the chamber itself was the reason. The chamber was custom-made by 3D-printing of nylon. Due to scattered radiation the chamber was heated up, melted and charred. Therefore it is important to choose the right chamber and to monitor

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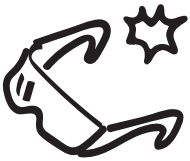
CHAPTER VII

STAY ALIVE: LASER SAFETY



After reading the former chapters, you must be very confident and excited to do some experiments on your own, maybe you are now planning which materials and liquids to use. Maybe you are also astonished by the convenience and simplicity of this technique: just one-step for such nanoproducts. And laser synthesis of colloids is an emerging field, growing since decades. How amazing is that?

For experimental operation, in fact, you do not need a master or Ph.D. degree. But is it really that simple and more important, is it really always occupational safe for you and your environment? This depends on the precautions you take and if you minimize the (potential) hazards during your experiments: a small mistake will cause a severe damage. You can compare laser ablation of a target to a "nanomine" you dig, where the laser is your mining tool and nanoparticles are the minerals you extract (Figure 49). In the newspapers, television news, broadcasts, etc. lots of reports cover mining accidents, some disastrous ones even causing peoples' death. The average lifetime of the miners who worked tens of years' ago was less than 40 years.

From this fact, you should get a glance at the danger of hiding behind traditional mining. Since the technique of laser ablation in liquid is an interdisciplinary field of chemistry, physics, material science, optics and engineering, the dangers during mining of nanomaterials by laser digging in liquids are summarized from different subject perspectives (e.g., chemistry, material and optics), in order to show you the appropriate precautions to prevent the dangers of being poisoned or injured. In detail, the contents of this chapter follow the sequence of Laser risk → Precaution → Experimental operation → Sample Labeling → Nanowaste disposal → Nanocolloids Storage. Note that the main purpose of this chapter is to provide some introductive information about experimental workplace safety specific to laser synthesis and processing of colloids. It is not intended to replace the mandatory chemical and laser safety training from your institution. Perhaps you get inspired by this chapter to prepare yourself with questions for the safety training day!

ASSISTANT I: YOU CAN'T WALK AROUND HERE WITH YOUR COAT.

ASSISTANT II: SURE – AS IF THIS COAT EVER SAW CHEMICALS

ALEX HEINEMANN, ESSEN

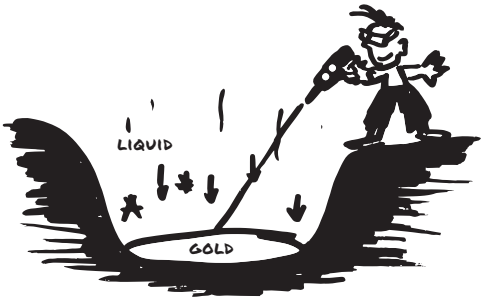


Figure 49: Nanomining in liquid using a laser gun

STEP 1: LEARN THE RISKS

Maybe, till now you have never had a chance to use a laser but most of you are familiar with the scenes of lasers. They have become an important element in science fiction movies, laser guns in Terminator 2 and Star Trek or lightsabers in the Star Wars universe. Although your interaction with lasers won't be as lethal as in those movies, the consequences can still be so severe. In the following part, four sections related to laser risks are introduced, including laser classification (Section 1), laser damage on human eyes (Section 2) and skin (Section 3) and some typical accidents that have already happened before.

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1.1 Laser Classification

Lasers are generally classified into four groups (Class 1-4) according to the U.S. Food and Drug Administration (FDA) laser regulations and international standard IEC 60825, as shown in Table 2. Laser beams of Class 1 lasers are safe under normal operation. Even direct exposure to eyes will not induce any damage.

For Class 1 classification, the maximum laser power threshold is very low and depends on the wavelength. For example, the often mentioned Nd:YAG laser system has a laser safety threshold that is only 0.6 mW at a fundamental wavelength of 1064 nm, at the second harmonic wavelength (532 nm), this threshold is only 0.39 μW, and at fourth harmonic (266 nm), this threshold is further lowered to 0.8 nW. You should be aware that even for Class 1 lasers a potential hazard still exists when the laser beam is enhanced or refocused by a telescope or microscope with a large aperture.

When a laser is in the range of Class 2-4, a label should be put on the door of your laser lab (clearly visible, e.g. at the height of eye) to warn of the potential risk of "laser exposure" as illustrated in Figure 50. Thanks to the self-defense mechanism of human eyes, Class 2 lasers are also considered to be safe. The so-called "blink or aversion response" of your body limits the continuous exposure to less than 0.25 s when a bright light enters your eyes. Nevertheless, if you intend to suppress your eye blink reflex under this condition, eye injury will occur.

Most laser pointers at 1 mW fall under Class 2. But note that they are only eye-safe for healthy, awake people. After 6 hours working in the lab or listening to 8 hours of chemistry classes, your reflex will be far slower and even these "supermarket lasers" are not eye-safe for you and your colleagues anymore.

When the laser scales up to Class 3, especially 3B with a medium laser power in the range of 5-499 mW, eye hazard appears, whereas skin hazard is not so likely. The degree of eye damage depends on the laser power. The higher the power, the less time it needs to cause irreversible retinal damage. Therefore, eye protection by eyewear is indispensable and a note of the potential eye damage risk should be put close to the label of "laser exposure" warning. The most dangerous are Class 4 lasers because they can cause severe skin burn and permanent eye damage. The laser power is also high enough to ignite flammable/combustible materials/gases, such as paper, ethanol, and hydrogen. As a result, it can trigger explosions when flammable gases are present in high density. If you want to make a BBQ outside the lab and you don't find a match, Class 4 lasers are good candidates as fire-starters. Note that lasers used for laser synthesis and processing of colloids – your lasers – are usually in this class.

Lasers used to weld a car roof have the same class. Do you think the power of your laser is lower? No, it's only the average output power that's lower. See Chapter I, pulsed lasers often create pulse powers and intensities that are even higher than those used in the steel industry. And a single nanosecond pulse may be enough to cause severe irreversible damage to the eyes. Note that by using special laser safety encapsulation, you can reduce the class of your laser system from Class 4 to Class 1. This needs to be done only by certified personnel and you will know this when getting a certificate and plate installed at the laser. But note that it's only safe when the enclosing is shut. Once you open the door to install a chamber or sample, it's a Class 4 laser again. In this situation it is dangerous when the electrical power is on, even the beam shutter is closed, since it fails sometimes. Hence, before you remove the casing on a Class 1 laser, everyone in the lab has to wear laser glasses and at the lab entrance and a signal has to sign laser operation.

To help researchers handling the risks of Class 4 lasers in the lab, a brief summary on "workplace safety standards" should be also posted near the laser warning label. (Figure 50). The summary should include the parameters of the laser systems (e.g., maximal power, laser wavelength, pulse duration, repetition rate, etc.) as well as the correct operating procedures. For the sake of the researchers' security, a lamp to signify the laser current status (on or off) should be also assembled in a prominent place outside the laser lab.

Figure 50: Warning label for laser in Class 2 and higher.

Table 2: We are in a high class. In laser synthesis, you will have Class 4 laser setups according to the official Laser Classification Table.

Class	US:FDA/CDRH	IEC60825 (Amendment 2)	Example
Class 1	· No known hazards during to eye or skin during normal operation · Note: Service Operation may require access to hazardous embedded lasers		· laser printers · CD players · DVD devices
Class 1M	N/A	· No known hazards to eye or skin, unless collecting optics are used	· geological survey · equipment · laboratory
Class 2a	· Visible lasers not intended for viewing · No known hazards to maximum exposure time of 1000 seconds	N/A	· visible continuous wave Helium-Neon lasers · laser pointers · laser scanners
Class 2	· Visible lasers · No known hazards with 0.25 seconds (aversion response)		
Class 2M	N/A	· No known hazard with 0.25 seconds (aversion response) unless	
Class 3a	· Similar to Class 2 with the exception that collecting optics cannot be used to directly view the beam · Visible only	N/A	· HeNe lasers above 1 milliwatt but not exceeding 5 milliwatts radiant power
Class 3R	N/A	· Replaces Class 3a (with different limits) · 5x Class 2 limit for visible · 5x Class 1 limit for some invisible	
Class 3B	· Medium-powered (visible or invisible) · Intrabeam and specular eye hazard · Generally not a diffuse or scatter hazard		· Spectrometry · stereolithography · entertainment light shows
Class 4	· High powered lasers (visible or invisible) · Acute eye and skin hazard intrabeam, specular and scatter conditions · Non-beam hazard (fire, toxic fumes, etc.)		· **(all!) LAL, LFL, and LML lasers**

1.2 Eye Damage

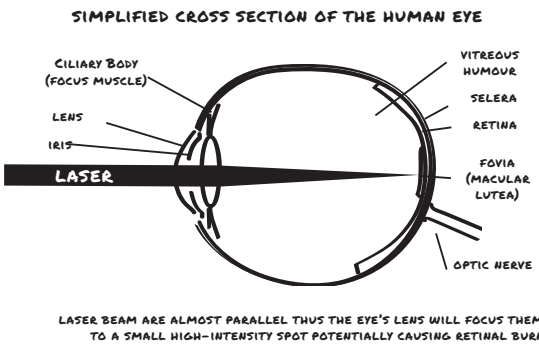

Figure 51: Laser focus when it enters the human eyes.

Figure 51 illustrates the cross section of an eye with an incident laser beam. The human eye mainly contains the cornea on: the transparent front part being responsible for protecting eyes, the iris serving as a light modulator for controlling the light volume inside the eyes, and the lens which focus the light on the retina. The retina has many photoreceptors that detect the light information and convert it to electrical signals which then are carried to the brain through optic nerves. Your friends think one Watt is nothing, because the power is less than a light bulb? For a better understanding of the effect of laser light, you can do a small experiment by yourself by using the sun light as a reference. When you look directly into the sun (when you are in Australia please do it only short), the power that arrives at the retina is about 10 W/cm^2. In that case, you cannot stand a long-period of exposure and may people get dizzy afterwards. In comparison, a 1 Watt laser beam can be focused to 100,000 W/cm^2 (!) at the retina, four orders of magnitude higher than sun exposure, no matter if the laser beam is unfocused or diverging before reaching your eye, because it will be focused anyway on your retina! Therefore damage may immediately occur to the eye structures and cause permanent loss of function. The pathological effects (e.g. cataract, retinal or corneal burn and photokeratitis) towards eyes (e.g., retina, eye lens and corneal) are laser wavelength dependent due to the different photon energies, as summarized in Table 3. Lasers with wavelengths from 400 – 1400 nm are much more dangerous than other wavelengths. Therefore this regime is particularly defined as a "Retinal hazard region". When the retina is damaged, the vision will be dramatically reduced. Such vision loss is normally permanent with little chances to be repaired. Lasers with wavelengths of 315 ~ 400 nm cause injury toward eye lens and trigger the photochemical

After her accident, Anne H. said she would not have expected that she did not notice immediately that a part of her retina has got irreversibly damaged. A part of her vision angle was lost, and she only noticed it later the day when she drove a car and got headache. The doctor did the right diagnosis, but couldn't do anything anymore. She can still see with both eyes and drive car again (after some months her brain needed to compensate the vision angle on her upper right vision hemisphere that was lost), but always has to keep in mind having a "dead angle".

She was trying to remember how that could have happened. She was wearing a clock on her arm while adjusting an invisible 1 watt femtosesond laser with a beam viewer card, and she thinks the glass or housing of the arm clock might have reflected the beam towards her eye.

cataract. Near infrared (near-IR) and infrared (IR) laser (i.e. those with wavelengths in the region of 0.78 µm – 1.4 µm and 1.4 µm – 1 mm, respectively), both lead to a corneal burn.

If only the corneal epidermis is partially burned, the vision can recover in two or three days. But if deeper parts of the corneal are damaged resulting in corneal scars, you will become incurably blind. Set safety first: you and all other persons in the lab, including your professor, have to wear laser goggles to lower the risk for your eyes to be exposed by a laser beam.

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But are you really safe with the certified laser goggles? Yes, if you use it as protection within its specifications. This means that it has to be suitable for your laser source. If you have more than one laser source the goggles might not be certified for both lasers. They are made to stand some seconds of direct laser radiation but are not built to stare directly into the beam for more than a minute. Unfortunately, a laser break through happens suddenly, due to so-called incubation effects. There is no pre-warning when the safety goggles are going to break from the intense laser irradiation.

In our lab, we only use fully closed safety goggles. You will not look like Keanu Reeves or Lara Croft with that decoration, but it has several advantages:
None will borrow or steal it for a party; You can wear eye glasses below;
And dangerous side reflections (from chamber holders, misalignment, back reflection, walls, etc.) are captured.

Table 3: Pathological effects towards eyes and skin of laser exposure at different wavelength (as summarized by some institutes and universities for safety guidance.

Wavelength range	Pathological effect towards	Pathological effects towards skin
180–315 nm (UV-B, UV-C)	photokeratitis (inflammation of the cornea, equivalent to sunburn)	Erythema (sunburn), Skin cancer, Hyperpigmentation, accelerated skin aging
315–400 nm (UV-A)	photochemical cataract (clouding of the eye lens)	Hyperpigmentation, Erythema
400–780 nm (visible)	photochemical damage to the retina, retinal burn	Photosensitive reactions, Erythema
780–1400 nm (near-IR)	cataract, retinal burn	Erythema
1.4–3.0µm (IR)	aqueous flare (protein in the aqueous humour), cataract, corneal burn	Erythema

1.3 Skin Damage

Besides being harmful to eyes, laser beams are also detrimental to the skin after long time exposure originating from either photochemical reaction (mainly from UV lasers) or thermal reaction (mainly from IR lasers). The laser power threshold for skin injury is almost the same as eye damage except in the retinal hazard region (400–1400 nm). Due to the large surface area of skin compared to eyes, it is highly vulnerable when the operators are not dressed properly. Similar to eye damage, the way of skin damage also depends on the laser wavelength due to different wavelength dependent skin penetration depth.

In general, the harmful effect is associated with the laser exposure period. When skins are exposed to laser for several nanoseconds, only superficial evaporation happens, while in the range from 100 μs to several seconds, thermal effects are predominant, longer exposure than 100 s can induce a photochemical effect. Normally, the symptom of erythema (e.g., burn) originates from thermal effects. Similar to the blink self- defense mechanism of eyes at visible radiation (not for the invisible radiation), skin also has a warning signal when it feels the danger of too intense laser exposure from almost all lasers except for some high-power far-infrared lasers.

Also a professor is not impeccable: everybody who works with anti-reflex coated windows knows: „never touch the coating". But sometimes, when a hardworking student presents proudly his new chamber to his professor he of course touches the window and damages its coating.

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When the skin absorbs laser energy, its temperature increases and the sensation of warmth response makes you get rid of further laser exposure. Any laser with a power larger than 0.1 W/cm^2 can give a warmth warning. A temporary skin pain from a short exposure period at low or medium laser power will heal very soon and not cause severe injury. But this may be different when the skin is exposed to high power laser irradiation for a long period.

There are three degrees of skin burn according to the injury degree. The first degree only affects the epidermis like the sunburn and causes pain and erythema. Erythema often heals within a few days without the risk of leaving a skin scar. If all layers of the epidermis (the upper 50-150 μm of the skin) and part of the dermis (below the epidermis with varying thickness from 0.3 to 3 mm) are affected by laser irradiation, erythema scales up to the second degree. When the epidermis, dermis, and part of the lower subcutaneous tissue are affected, third-degree erythema forms what usually leads to the formation of an irreversible skin scar. Lasers with wavelengths less than 200 nm or larger than 1 μm are mostly absorbed in the epidermis layer, UV-A can reach the dermis layer, while visible and near IR (700-1200 nm) can easily reach the subcutaneous tissue.

Compared to other laser wavelengths, UV lasers, in particular, UV-B lasers (280 nm - 315 nm) can only induce the second degree of erythema, however,

they are much more harmful because of their induced long-term carcinogenic effects leading to skin cancer. Besides the carcinogenic effect, long-term exposure to UV lasers is also able to accelerate skin aging and gives rise to hyperpigmentation (pigment-darkening effect), erythema and blistering.

Although exposure of large skin areas is unlikely to happen in normal laser work, precautions are required to prevent these adverse effects, especially summer time when people dress less and do not work with chemicals (colloids) that require to wear a lab coat and gloves. Hence, protect your skin in the laser lab, even when working with gold and water only.

Ultrashort pulses may not hurt in a classical sense, and photochemical damage may feel "just itchy" with red skin. But skin damage by lasers is quite complex and it may even promote cancer or be underneath the skin, therefore: always seek the doctor's advice when you feel that you got hit.

1.4 Accidents: Little Mistakes with Big Consequences

The danger of laser exposure towards both eyes and skins has been discussed above. In the following, we will talk about accidents that happened showing you what can happen if you make similar mistakes. Please consider this as a warning to be very careful to circumvent similar risks from the static "laser weapon" in your lab. Even if you have many years of laser experience, you still need to be very careful because you are an endangered group of people who might neglect the rules and take your experiments for granted, maybe because in your lab nothing bad has happened so far. The following accidents as happened should help to bring the safety concerns back into your mind if you feel too safe because of several years of laser lab experience.

In March 2004, a Berkeley graduate student was subject to a serious injury at his left eye from an Nd:YAG laser. In the beginning, he carefully wore eyewear during his experiments. But when he came back from dinner he thought there was no danger for laser exposure any more since the system was aligned. Then the disaster happened to him when he leaned over the optical table and adjusted the power meter because the laser beam was reflected into his eye. This accident also affected his professor. Since then, his funding was significantly cut. Even though the student's vision was impaired only a little, he must have regretted about his misconduct and the negative consequences for his professor. Wearing the eyewear would have prevented this accident. Similar accidents are likely to occur in other laser labs as well, even though some of the personnel have many years of laser experience.

For example, in October 2001 an Argonne scientist with over 15 years of laser experience suffered from a serious eye injury caused by laser beam alignment (reflection from a mirror) of a 800 nm beam from a Ti: sapphire laser (invisible, femtosecond). The consequence was disastrous: his vision was reduced to near-

blindness.

Another example is a Chinese scientist who burned his retina and lost some sight of his left eye because he removed his goggles to check the crystal he used. And a Dutch scientist was hit by secondary-reflection of laser light from a cylinder of liquid what caused a central foveal lesion with vitreous hemorrhage. These are some painful lessons from experiments, from which the laser safety training is strongly reinforced in every country nowadays. The fact that the power of the fabricated laser sources increases year by year, any mistake can be more disastrous. Even 1 watt is too much for our eyes. Generally, the fear of unknown or possibly dangerous things usually makes people follow the rules as they are taught, especially the young students. But when they become more experienced and see that nothing bad happened so far, they may decline his/her vigilance level and deliberately challenge the rules, hence the risk of accidents raises and may be triggered by a small mistake.

Figure 52: A scenario of eye damage by laser when you do not wear goggles.

Also, non-beam related hazards should need to be considered. High power laser systems are typically high voltage devices. Most of them are equipped with a high-pressure water cooling system which may also be a risk if there is something wrong with pressure control. The “laser accident database” (http://www.rli.com/resources/accident.aspx) lists several accidents based on the researchers’ misconducts or a laser malfunctioning. There you find different scenarios that ended in noticeable accidents.

The laser lab can bear additional issues for safety, especially if the laser lab has glass windows. Chemical labs often have a window for safety reasons, but laser labs shall not. Make sure that all windows are covered or non-transparent for the laser irradiation to avoid any irradiation leaving the lab. Your laser lab will also need a double door system where people can put on their safety

goggles. The best and cheapest way is a safety curtain behind the door (it's a heavy curtain that can be simply installed and cut in any length needed), anyone who is entering is protected against direct laser light and can communicate with the people in the lab. That's also the place where the laser safety goggles should be placed (in front of the lab is often not wanted, and inside they do not provide optimal use for people entering the lab in case the laser is already on).

STEP 2: TAKE PRECAUTIONS

Since your nanoparticles are synthesized in liquids, both optical and chemical precautions are indispensable. Here are some precautions tips (Table 4) leading you to the shining way that at least can keep yourself healthy and alive if no natural disaster happens. Let's discuss how you should dress in the laser-chemistry-lab. The principle of clothing is to reduce the chance for you to be exposed by both chemicals and laser exposure. Your shoes should be resistant to the chemical solution permeation. No flip flops! Your pants should be as long as possible to cover most of your skin, in which case those with long-sleeve and no cuffs are preferred. Another choice is to use professionally protective clothing, one is for chemical protection containing garments, gloves, boots, and coveralls. Experimental clothes cannot be repeatedly used in different experiments and cross used by different persons. Each individual should have his/her own protective clothing and one closet for clothing storage. Gloves, especially the chemical resistance gloves are generally selected according to the solutions/ solvents you want to use. Take DMSO for example, when it gets in touch with human skin, it may induce allergic reactions or headaches, even worse symptoms if it brings about the aggregation of contaminants and toxins on human skin. To prevent the penetration of DMSO to human skin, butyl rubber, fluoroelastomer gloves should be adopted rather than nitrile ones because they may be degraded by DMSO. The ventilation system is also suggested to be established in the working places to reduce the concentration of volatile flammable gases (e.g. ethanol or acetone) or to remove poisonous substances generated during laser ablation.

Table 4: Different protective equipment during nanoparticle synthesis by laser ablation or processing.

Type	General precautions
Dressing	· closed-toed shoes made of a low permeability material · long pants without cuffs, a long-sleeved shirt · gauntlet-type gloves or nitrile gloves with extended sleeves · wear splash goggles · watches and jewelry (e.g., bracelet, ring, etc.) are not allowed
Laser	· Put on eyewear fitting your laser system · Do not wear the gloves outside the lab · Cover your hair under the clothing or bundle it if they are long · Check the wavelength and power fitting between optics/ scanner and laser beam · Check the stability of every optical elements along the laser path
Tools	· Check the cracks and deformities of tubes and bottles before use · Ensure the containers are dry and clean. · Never overfill the tubes (threshold of 3/4 volume).
Environment	· Remove nearby flammable/combustible materials to limit fuel in the case of fire
Others	· No food or drink inside

If you get married, you might wear rings to demonstrate your marital status. Do you know that some of your favorite watch/jewelry (e.g., bracelet, ring, etc.) may endanger you during your laser experiments, especially during the laser alignment? The surface of watches/jewelry are usually smooth enough to reflect laser light, which may bear risks to your skin or eyes or other researchers or devices nearby. Such reflection can cause skin/eye damage or device destroying.

For instance, watches have shiny metallic housing and a glass window. When you put your own hands on the optical bench and the laser beam is reflected by your watch it's propagating in random directions. Necklaces are more subtle because you will hardly realize when they hang in unknown areas of the optical bench when you lean on it to manipulate optics. And a nicely reflecting edge of a necklace bouncing directly below your chin can reflect directly into your eye bypassing the safety goggles (but this risk is minimized if you have fully closed goggles. Nevertheless: take off your shiny jewelry when working with lasers!

For your own security, already take them off before you enter the laser lab. Additional attention should be paid to long hair, which may catch fire by laser expose when hanging in the laser beam line. So fix your long hair under the lab clothing or use a hair tie.

Once upon a time, a student (not named here) used UV laser to do some UV laser fragmentation using ethanol flowing out of a funnel. A fire started at the beginning of interaction between laser pulses and the falling down ethanol, it immediately expanded to the beaker which was used to collect the ethanol. Some of the splashed ethanol even dropped out and burned the laser table. Fortunately, the student responded very quickly. He stopped the laser system and moved his notebook away. After about one minute, the fire extinguished naturally without causing serious consequences. Just image if there is some flammable solution nearby, the fire may expand very quickly without control, and the whole lab may be destroyed, in particular the laser beam source which is very sensitive to aerosol contaminations.

Dongshi Zhang, Essen

Safety goggles only protect in specific wavelengths and have a maximal threshold of laser power: check the applicability of your goggles. Safety goggles for all wavelengths at one time do not exist, or maybe they exist but you cannot see directly through them (therefore useless if you need your eyes).

Something you should also consider: NEVER sit in the laser lab with your head at the same height as the optical beam, especially when someone else is operating on it. If the optical table is equipped with a PC, move it (in particular the screen) higher than the optical bench, so that it has to be used while standing and make sure the operators head is higher than the beam line (for example by sitting on a stool).

Precautions should also be taken to the optical elements/scanners (other than the ones belonging to the laser system itself). First, check all the optical elements and mount them during the light alignment. Another suggestion for the optical elements is to arrange in a good order, neither too crowded nor confusing beam lines and crossing another experimental set up will square the risk. Optical elements are better kept away from the edge of the laser table to give your arms some free space to move.

Attention should also be paid to the wavelength/power fitting range of the optical elements/scanners. If they are not fit well, the optics and scanners will be damaged, which means you will lose thousands of euros and have to postpone your experiments to wait for their substitutes to come back.

VII

In order to make your experiments reproducible, all the tools you use, should be clean, including the ablation chamber, glassware, etc. This is because you are not sure whether your colloids will interact with the pollutants and whether the pollutants or the reaction products are poisonous. Meanwhile, as already addressed in Chapter VI, from the viewpoint of nanoparticles reproducibility, it is also strongly recommended that all of the tools you use are pure, if possible as pure as laser-generated NPs

Do not store large volume (only the volume for 1-day consumption) of inflammable solvents in the lab, such as ethanol, methanol, etc. because once there is a fire, these solvents are good fuels for fire expansion. In some cases, they may also cause explosions. During experiments using inflammable solvents, you need to be particularly careful, especially when the laser energy is high.

Before you start your experiments, gather information about the materials you use and that can be formed. Take the corresponding precautions according to the potential risks you may face.

To help you work occupationally safely we have drawn together a safety questionnaire where you can quickly check step-by-step if you will work safely:

Once a friend was running towards me screaming fire, fire and asked me to have a look in the laser lab. I realized that the solvent might have caught fire since I knew he was working with acetone. I just started running towards the laser lab since I was really upset that he left the flame just like that in the lab. But fortunately, the flame was not spread meanwhile, and I could immediately put it off. I couldn't believe that he just left the flame like that for a few minutes knowing how dangerous it could be!

Jacob Johny, Mexico

SAFETY QUESTIONNAIRE

1. Do you have the correct safety goggles for the laser you are going to use? (Check the wavelength, the intensity and the laser mode)

   ☐ Yes: Ok! ☐ No: Find appropriate goggles!

2. Are there any other lasers operating in the lab that the goggles are not sufficient for?

   ☐ Yes: Find appropriate goggles ☐ No: Ok!

3. Do you need additional safety wear because of chemicals you use?

   ☐ Yes: Identify what you need (e.g. lab coat, gloves, etc...) and wear it! ☐ No: Ok!

4. Is your skin covered appropriately? (In particular when you work with short wavelengths and set-ups that scatter the laser light)

   ☐ Yes: Ok! ☐ No: Cover it!

5. Do you wear any rings, watches, bracelets, or other reflecting things that you can accidentally reflect the beam with?

   ☐ Yes: You should take them off! ☐ No: Ok!

6. Do you work with optical mirrors or lenses compatible with the used laser specs?

   ☐ Yes: Ok! ☐ No: You should use other ones or take the risk of damaging the optics and the laser!

7. Will your experiment release potential toxic dust or fume?

   ☐ Yes: Use a fume hood! ☐ No: Ok!

And one more thing: if you close your shutter or put the laser into pause mode always double check if there is really no laser irradiation coming out anymore (use a sensitive detector card) to make sure before you take off your goggles!

If you consider these important aspects you will be on a good way to keep your vision and health unaffected from the lab work.

STEP 3: TURN ON THE LASER

When all the materials and the tools are ready for your experiments and you have taken all precautions as described in the previous section, the time has come for you to do experiments. When you enter the laser lab, do not forget to close the safety curtain properly behind you, to give your colleagues a safe space to put on the goggles.

Here some steps that can easily be forgotten are summarized.

1) Before you start the laser system, check if all caps or dust coverages are removed from the optical components you will use.

2) When you switch on the laser system, you have a responsibility to inform other colleagues working in the lab (the need to put on appropriate goggles as well). Never check if the goggles are working by putting them into the laser beam. The goggles only stand the beam for a limited time. After sufficient incubation, they do not block the laser light anymore and anyone using them is at high risk.

3) You should also know how you can turn off the laser in the fastest possible way in case of an emergency, so check for emergency-buttons. During the break, of course, leave gloves and protective clothing in the lab.

When I first arrived at my current lab and did some experiments using flowing liquid, due to inappropriate connection of the chamber, some water flowed out and then fell down on the ground. I did not recognize this was a big problem, but afterwards when it became a pool, even enough for a frog to swim. I suddenly saw some electrical wires and a socket were floating on the water, "oh, how stupid I am and how dangerous it is!" Then I stopped the liquid and then start cleaning the floor, and luckily nothing happened at time. But the optical table now has rust spores.

Dongshi Zhang, Essen

LAB STORY

Since I am an engineer I only had a limited chemical background. Before I suffered from an accident I hardly knew anything about chemical and nanoparticle toxicity. One day, with the aim to do some experiments with cobalt oxide powders, I had to first mechanically press them into a pellet. But unfortunately, before I started experiments, I did not look up the toxicity information so that I forgot to wear gloves (That is a bad habit for me! Maybe also for you!). I touched the powders with my hand. Even though I washed my hand afterwards, I still had a severe skin allergy at that night (see Figure 51), I did not know the reason. The first thing that came to mind is that there should be something wrong with the food. But that day I just ate a döner which I have ate tens of times before. One hour later, it came to my mind that I had done some experiments with cobalt oxide. Then I quickly searched from the internet and found skin touching would cause skin allergy. From this event, I learned that some materials are really dangerous. Something happened later taught me that even laser ablation in liquid is one of the purest synthesis method but not totally safe for your health. After pressing these cobalt oxide palates, I did some experiments with gloves. But at that night, I still had a skin allergy and much severe than last time which should be related to the water evaporation during long period of laser ablation, containing cobalt oxide or cobalt ions inside. They were airborne and were inhaled inside my body during my breath. When I went to the hospital, even the doctor did not know the toxicity of cobalt oxide. You see, I became a knowledgeable person from my accident. If you want to try something else and test the ability of the doctors in your country, you can do your own experiments carelessly like me. But I can not promise you are so lucky enough to just have the symptom of skin allergy.

Dongshi Zhang, Essen

Figure 53: Skin allergy induced by exposure of cobalt oxide colloids and powders.

In Chapter II you have learned how to yield large amounts of nanoparticles. For this purpose sometimes long laser ablation time is needed. When you plan to do long-period laser ablation, liquid evaporation is another issue to be taken into account. Organic solutions are volatile, as the ablation proceeds, the liquid thickness gradually decrease. Once they are unable to immerse the target underneath, you are performing laser ablation in air, in which case the ejected particles will no longer be confined in liquid, but haunt in the air. As a result, it is easy for you to inhale them and receive toxic effects after inhalation. Another concern is the flammability of solvent vapor. Once the environment is rich of this flammable gas, the laser will then become the matchstick to ignite it and an explosion may also happen. By law, it is not allowed to combine an ignition source (a pulsed laser) with an inflammable liquid. So how to do laser ablation in ethanol or acetone, as widely reported in the literature? To answer these questions expands the scope of this handbook. In general, you need to write a risk assessment scenario together with your safety officer, taking into account what may happen and which safety measures can be appropriate. In our lab, we focus a 500 watt picosecond laser in a flow chamber pumping large amounts of inflammable liquid. For that, we have built an active safety chamber around it with gas sensors and valves that automatically open and flood the chamber with nitrogen, and shuts the beam and the liquid pump. This is the upper end. For laser ablation of gold in a 20 ml ethanol batch, the safety measure will be less extensive, but still, you will have to take into account what may happen in the worst case. Consult your safety staff before working with organic liquids and lasers.

STEP 4: BE ORGANIZED

When you collect colloids, what you have to do is labeling your sample. Sample labeling is a good habit for you to know the information after many days. In China, there is a famous saying “good memory is inferior to sodden ability to write”. Even if you have a memory like a computer and can search for the information whenever you want, it is still better for you to categorize the samples. The sample label should at least give information on its composition, production date, and name (e.g., “gold-silver-alloy in acetone, 28.4.2014, S. Yusuf”). The processing parameters (e.g., laser power, repetition rate, scanning speed, wavelength, pulse duration, processing period; liquid volume, liquid thickness, liquid composition), etc. you should note in your lab book. Really important, from the security point of view, is to check whether your colloidal solutions have one or some of the following hazards (Figure 54), e.g., exploding bomb, corrosion, flame over circle, gas cylinder, environment, skull & crossbones, exclamation mark, health hazard and flame. The exploding bomb and the gas cylinder are very rare for your nanoproducts received from laser ablation. All other marks are likely for your products.

Figure 54: Labels for different kinds of chemicals.

The danger of corrosive substances (e.g., acids, alkalis, organic halides) towards other substances (e.g., metals, organic, living tissue) during contact should be kept in mind. If there is little chance to form corrosive substances after laser ablation, even if you are not sure, please label them using this tag. Oxidizing materials, like hydrogen peroxides (e.g., H_2O_2), that can supply oxygen or other oxidizing substances, or react chemically to oxidize combustible materials to increase the risk of a fire or explosion should be used very carefully. Attention to acute toxicity during the synthesis of arsenic-based compounds should be paid since rapid occurrence of adverse effects often takes place within 14 days. “Flammable chemicals” means that they are very easy to catch fire. The most frequent flammable chemicals are organic solvents you use for particle generation by laser ablation, including ethanol, methanol, isopropanol, etc. Carcinogenic colloids (cobalt-, nickel- and copper-based nanoparticles) or solutions that can cause cancer are the most dangerous substances towards the human. Reproductive toxicity is related to your offspring because it influences your sexual function and fertility. Since oocyte cell of women can be endangered by DMF’s exposure leading to birth defects, women should be very careful with DMF or replace it with other solvents.

STEP 5: DISPOSE OF YOUR WASTE

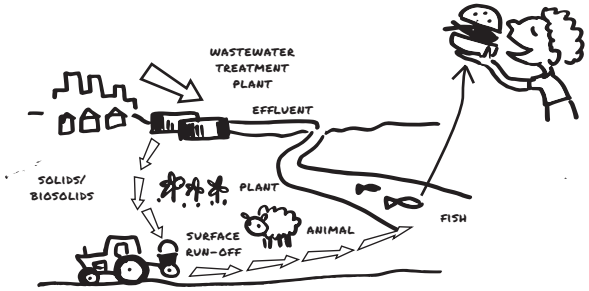


Figure 55: Nanoparticle contamination in soil, water and are retaken by human through eating.

Not every laser shot is successful in generating nanoparticles. Hence, some of the colloids become trash during your focus search or rechecking the reproducibility. How to dispose these colloids? The easiest way that might come to your mind is to dump them in the sewage, like you do with water after you boiled eggs in it. Its only mg of nanoparticles in standard liquid, isn't it? Yes, this is the most convenient way, but it is not a wise choice. Some solids are biopersistent and are taken up during plant growth, and then eaten by the animals. Once these animals or fishes or vegetables are eaten by humans (Figure 55), you can image that what you have done to the environment came back to you. So do not pour the colloidal trash in the sink! Store them in the specific waste containers for professional disposal. Organic and aqueous solutions should be stored separately.

While I was collecting my laser generated colloid in acetone, I noticed an abnormal smell coming from the solvent. I went back to check the bottle from which I took it. When I read the name on the bottle clearly, I understood instead of acetone, acetonitrile was used. Since both names start with "aceton" I misread it as acetone in a hurry!

Jacob Johny, Essen

CHAPTER VIII

FIND AN ENDPOINT

So far you have learned how to prepare nanoparticles by LAL and LFL in a controlled manner, how to experimentally design your set-up, how to characterize them and how to handle with them carefully. This chapter now will guide you through three exciting applications, where laser-generated nanoparticles may have a promising future.

APPLICATION 1: CATALYSIS

In order to perform meaningful catalytic tests, the first and most important question you need to ask yourself is: Which application is my prepared catalyst suited for in future? This question seems trivial but infers a whole load of further issues to determine beforehand. Do you plan to apply it in an oxidation reaction at elevated or low temperatures in basic environment? Or do you rather prefer to develop a good catalyst for the reduction of oxidized species? Do you want to apply it in liquid-based reactions or do you intend to optimize your catalyst for gas phase reactions? Maybe you plan to use your catalyst in photocatalysis using photons to split water or degrade pollutants? Or do you rather prefer electrocatalysis like in fuel cells to provide electricity e.g. using the oxygen reduction or in electrolysis for hydrogen evolution reaction? Is it most important for you that the catalyst is only highly active without being selective or do you have the premise of having high selectivity and yield for only a specific reaction? And what's about the durability of the catalyst? As you see, there is a manifold of questions to answer beforehand determining the setup you need to use to perform the catalytic tests. Not for nothing does the field of heterogeneous catalysis employ a whole fraction of scientists. Due to the complexity of heterogeneous catalysis, being an interdisciplinary field of research reaching from surface science and applied chemistry into thermodynamics, reaction kinetics, and computational studies, the following section can and will only address a tiny piece of the big catalytic research cake. Hence, the following section intends to give you some impulses for designing catalytic experiments using heterogeneous catalysts, specifically addressing the unique features of laser-generated or laser-processed nanoparticles.

VIII

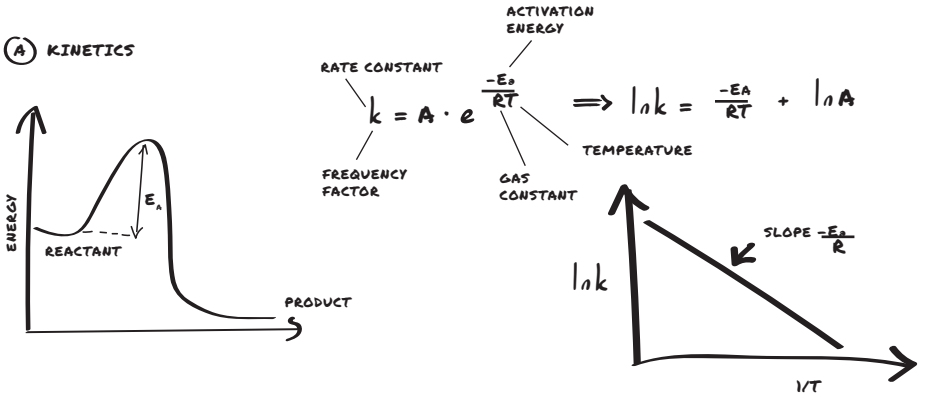
IMPORTANT CATALYTIC PARAMETERS

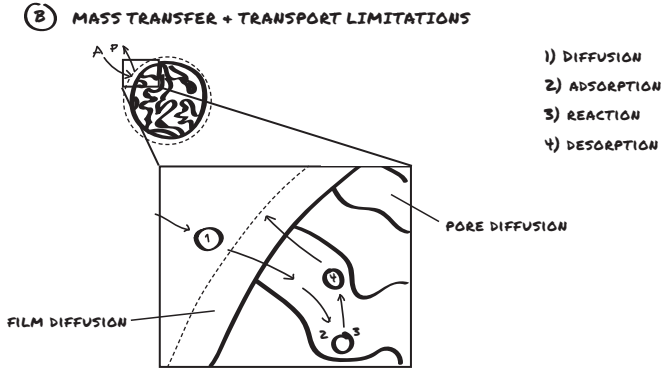
In every catalytic test you plan to perform special catalytic parameters need to be measured or calculated in order to evaluate your catalyst activity and compare the activity of your catalyst with other catalysts from other groups. Especially when you are new in heterogeneous catalysis be aware that it

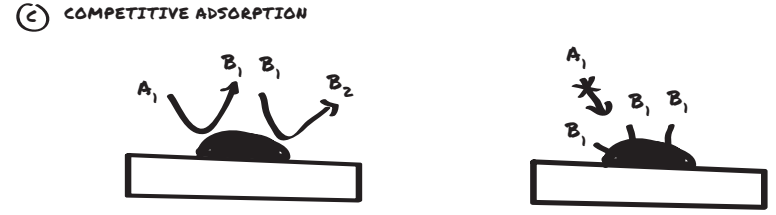
is important to make sure that you use comparable reaction conditions (temperature, pH, educt concentration, etc.) like other groups working in this field. If you do not pay attention you may find yourself being ripped apart by the reviewers when you try to publish your awesome results.

In case you start your catalytic experiments the most important parameter to look out for is called **conversion**. The conversion hereby describes by the ratio of the reacted educt compared to the initial amount of educt before reaction. This very basic and important parameter will be your guide, informing you about the stage your reaction is in. Hereby this parameter is heavily dependent on the reaction time as well as the ratio of the catalyst-amount used compared to the provided educt concentration. Hence, make sure to always add the same ratio of catalyst and educt prior to your experiment (as well as choosing amounts comparable to other experiments from literature) to avoid the wrath of the reviewers.

In case you are able to track the change of educt and/or product concentration during your reaction, the conversion will be an additional tool for you to receive a reaction kinetic profile when plotting conversion against reaction time. Of course, the conversion will hereby increase with the reaction time.
If you are interested in determining the reaction kinetics or the energy of activation (Figure 56 A) present during your reaction it is important to make sure having enough data points at low conversion best far below 10% of conversion. Keep in mind that the reaction kinetics, as well as the kinetic constant, is derived by the derivative of your conversion-time profile. In case you've been clever enough to choose an elementary reaction (e.g. the oxidation of carbon monoxide, called CO-conversion) as your model reaction, the unknown kinetic constant as well as the reaction order (if it is not known) can be derived by fitting the kinetic data using the rate equation of an elementary reaction of "n"-th order which can be found in any textbook or online reference database. However, if you chose an equilibrium reaction, consecutive chain reaction or enzymatic reaction, other kinetic laws need to be applied following the reaction mechanism. But keep in mind, do not forget to only take conversions into account which are significantly lower than 10%. You may wonder why? The answer is easy – at higher conversion, different phenomena like transport limitation (Figure 56 B), competitive adsorption of educts and products (Figure 56 C) or catalyst (de-)activation (Figure 56 D) become more and more pronounced, phenomena which are not respected within most reaction rate equations. In case you are further interested in the activation energy, the kinetic experiments should additionally be performed at different temperatures determining the kinetic constants. When having measured at least five rate constants at different temperatures ranging over at least 50-100 K, the Arrhenius equation shown in every textbook on catalysis or physical chemistry can be employed to determine the energy of activation (Figure 56 A). The latter will give you valuable information about the activity of your catalyst.







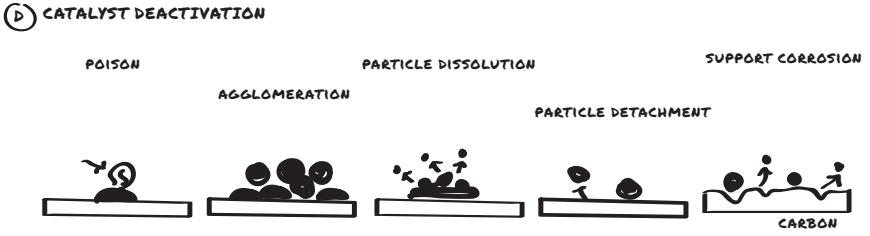


Figure 56: Important elements of heterogenously catalysed reactions: (A) Reaction kinetic principles; (B) Mass transfer and Transport Limitations inside porous materials; (C) Competitive reactand adsorption at catalytically active sites; (D) Catalyst deactivation mechanism;

Another important parameter within catalytic research is the **selectivity**. You may be able to develop an unselective highly active shredder machine dismantling every educt into its basic elements but are you also able to design a catalyst only oxidizing the α-carbon of an allylic terpene like β-pinene not harming the fragile C=C-bond forming flavors like pinocarvone or trans-pinocarveol? Determining if the desired product has been produced when formation of several products is possible, it is usually done by calculating the selectivity of a catalyst. Hereby the selectivity is defined as the ratio of the desired product divided by the total amount of product formed. From this definition you may already smell a rat - a highly selective catalyst does not necessarily need to be a very active one. Actually according to selectivity - selective catalysts are very often quite inactive in terms of conversion. Hence, to holistically evaluate this, a catalysis scientist usually employs the catalyst yield as well as the (gold standard called) turn-over-frequency (TOF) of the desired product as their basis for evaluating the catalyst activity. Hereby, the yield is defined as the amount of desired product generated in relation to the amount of educt added initially to the experiment. Note that again, TOF is only valid at low conversion! Now in case, your catalyst shows a high yield for a specific product during a very short reaction time while having a reaction with several possible products, your catalyst is obviously very feasible due to its high space-time-yield which is an important industrial rating parameter for catalysts.

EXEMPLARY CATALYTIC TESTS

With all the parameters defined in the previous part, the time to wrap up the sleeves and go for some catalytic testing has come. In the following short section hints, tips and suggestions for simple straight forward catalytic testing comprising of CO-oxidation, reduction of nitrophenol, the photocatalytic pollutant degradation and finally the electrocatalytic oxygen reduction reaction will briefly be presented.

One of the most famous techniques employed with nanoparticle - functionalized catalysts is the **oxidation of CO to CO_2** in the gas phase, which has a big importance in exhaust gas catalysis e.g. inside cars. Hereby the usually inert metal gold became very famous when Masatake Haruta demonstrated that nanometer-sized gold particles (AuNP) with a size of less than 5 nm were actually extremely active in the oxidation of CO to CO_2 even at temperatures of -76 °C. However, the authors did also show that the carrier material also plays a big role in the catalytic activity of the AuNP. Hereby oxides like Co_3O_4, Fe_2O_3, and TiO_2 were usually the best material of choice at that time. The strange activity of AuNP in the oxidation of CO has since then often been explained by the preferential CO adsorption on AuNP. The oxidation reaction then occurred at the contact zone of AuNP and oxide carrier with the latter activating the oxygen (Figure 57 inset). This contact zone called “perimeter site” is often rather

defect-rich while especially oxygen vacancies are highly feasible candidates for high activity also in another reaction like the selective oxidation of alcohols using TiO_2 functionalized with AuNP. The catalytic test is usually quite simple and state of the art. Hereby the nano powder Au/TiO_2 is granulated to gain a feasible grain size of about 50 µm. The grains are loaded into a U-shaped tube and fixed with some quartz wool to prevent the catalyst granulate from being carried away when applying the gas flow e.g. of CO (Figure 57). The typical required catalyst amount is (some) 100 mg. Before or during the application of CO flow, also containing some amount of O_2 the tube needs to be heated up to maintain a desired constant reaction temperature. The gaseous mixture can then be analyzed, e.g. by gas chromatography or mass spectroscopy. By varying the reaction temperature, the gas flow and composition, all required catalytic tests that you need to benchmark your catalyst can easily be performed. This gives you the possibility to determine the reaction kinetics, energy of activation or the turn over frequency. Keep in mind that granulating is quite important to minimize the pressure drop over the fixed bed. A high pressure drop when using non-granulated nano-sized powder will completely deny any flow or even worse cause short-cut-flow at the walls or within the powder bed minimizing the contact area of the catalyst and gaseous educt. Therefore, a conscientious pretreatment of your powder will increase the reproducibility of your catalytic tests.

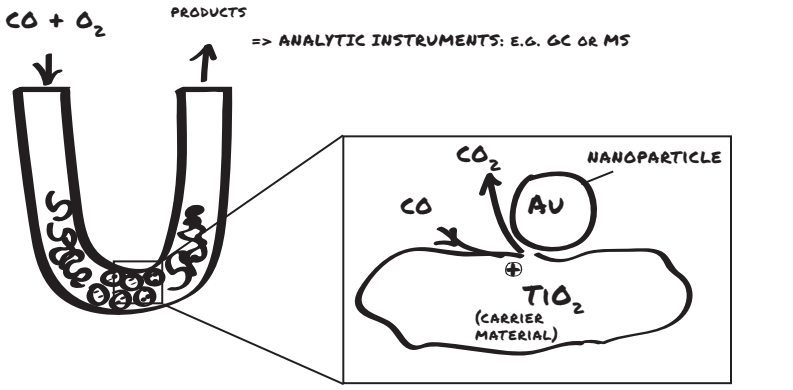


Figure 57: Principle for a catalytic CO conversion on gold nanoparticle-loaded titania.

Another easy-to-perform experiment is the **reduction of nitrophenol** (NIP) to amino phenol (AmP) in liquid phase e.g. with $NaBH_4$ as a reduction agent. This reaction follows the well established Langmuir-Hinshelwood reaction and additionally represents an elementary textbook reaction with several subsequently generated intermediates (Figure 58). The basic reaction kinetics can easily be tracked via online UV-VIS techniques e.g. by using a commonly

used fiber spectrometer setup. Hereby the NIP has a very pronounced absorption around 400 nm wavelength disappearing with an ongoing reaction while the AmP shows an absorption around 320 nm increasing when AIP is formed during the reaction. The reaction can be performed with a manifold of nanoparticles with literature ranging from standard noble metal nanoparticles like Au, Ag, Pt, Pd or Cu to their alloys like PtAu, AgAu, PtCu or PdCu, without the usage of any carrier materials. Hence, the NIP reduction is an easy tool to characterize the activity of nanoparticles with a low effort required.

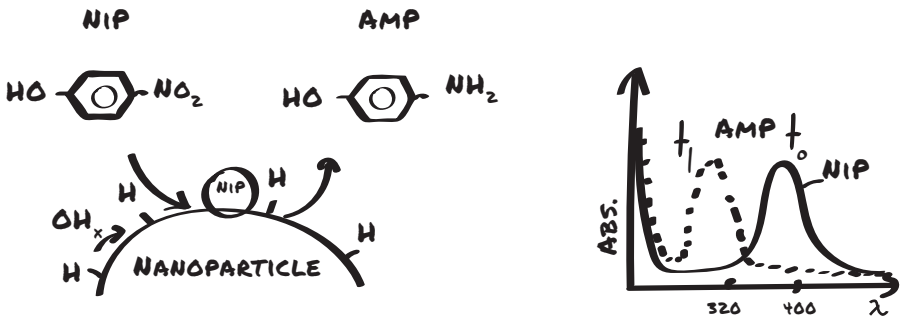


Figure 58: Reduction of nitrophenol (NIP) to amino phenol (AmP) over nanoparticles can be easily tracked by UV-Vis spectroscopy, from t_0 towards t.

If you are interested to work in the field of photocatalysis, another widely applied reaction which is also easily tracked by UV-VIS extinction is the **degradation of pollutants and dyes**. Examples would be the degradation of phenol or of Rhodamine B by UV-light in the presence of photocatalysts. Commonly investigated photocatalysts are nano-sized transition metal oxides like TiO_2, $BiVO_4$, Fe_2O_3 or ZnO which are often additionally doped with cations of higher (p-doping) or lower (n-doping) valence or functionalized with nanoparticles. Doping is usually used to decrease the band gap as well as to shift the energy of conduction and valence band. On the other hand, the functionalization with nanoparticles is conducted in order to increase the lifetime of electron-hole pairs (exciton) which are the reactive species to induce the reduction of e.g. oxygen (caused by electrons) on the one side and the oxidation of the pollutant or dye (caused by electron-holes) on the other side. The main idea of how to test a photocatalyst is to employ a rather simple photocatalytic setup. The setup obviously requires a light source to excite electron hole-pairs on the surface of the photocatalyst. Commonly Xenon arc lamps emitting light similar to sunlight are being used, however they also emit much heat what needs to be considered when setting up your experiment (cooling). Alternatively, rather cheap diode-stacks can be employed which only emit at a specific wavelength (i.e. selectively UV or Vis). The latter is actually a

big advantage when you try to understand your photocatalyst in terms of basic research but it's disadvantageous when you plan to focus on applied research which usually needs to be conducted with light sources mimicking the spectrum of the sun. To apply the light, a quartz glass vessel containing the catalyst and the dye or pollutant as well as a stirrer is the best choice. The lamp can either be dipped into the solution or fixed outside of the vessel. However, depending on the power of the lamp you plan to use make sure that the lamp doesn't heat the whole batch like an immersion heater, so consider cooling the whole vessel. Further, make sure to set up your experiment in a way that you can set up an energy balance to determine how much energy you are actually applying. This will be very important for the discussion of your results. Once you set it up, it is again very easy to gain kinetic details like the amount of pollutant or dye is easily accessible via UV-VIS. Another research topic that hasn't been mentioned but also takes up a big chunk of research endeavors on photocatalysis is the photocatalytic water splitting. In this case, only water, light, and the catalyst are your ingredients to generate hydrogen and oxygen from water. As you can't detect hydrogen by UV-VIS, the analytics and hence the setup requires a bit more effort. Hydrogen sensors or a gas chromatography are good candidates for proper balancing the amount of hydrogen produced within some time unit.

If you are more interested in the fundamentals of photocatalytic activity you may also consider measuring the photocurrent using a PC-controlled potentiostat setup combined with your photocatalytic setup. In this case, you don't measure how much hydrogen is generated but how much electrons or holes are formed when the lamp is switched on. To do your needs, coat an electrode, e.g. an indium tin oxide plate (ITO), with your photocatalyst e.g. by spin coating or doctor blading and subsequent drying at about 100°C.

VIII

Once I wanted to ablate a coin and a magnet. My partners told me: "You mustn't do it. You do not know what can go wrong, and maybe our directors do not like you are playing".

When I told our director what I was going to do, he replied: "OH great!!! Actually, I already have done that, nothing special happens.

It was so boring and disappointing. But go ahead do it and get disappointed you too" and we laughed a lot.

David Muneton Arboleda

The coated electrode called working electrode will then be connected to the potentiostat and immersed into the water containing the reaction vessel. Additionally, a counter electrode (e.g. a platinum net) and a reference electrode (e.g. chloride saturated Ag/AgCl electrode similar to often used pH-electrodes) are to be added as well. When applying a positive potential (e.g. 0.5 V) between the working and the counter electrode while switching on the light, photo-electrons are detected. On the other hand, working with a negative potential between working and counter electrode (e.g. -0.3 V), the current of photo-induced electron-holes is detected. As the current of holes and electrons is proportional to the catalytic activity, different catalysts can qualitatively be compared assuming no differences in the electric conductivity (including the thickness) of the catalyst layer. Hence, these experiments require careful catalyst preparation and analysis of the properties of the prepared layer.

As the last (but not least) example you may also perform an electro-catalytic analysis or **electrocatalysis**. Yes, it́s both at the same time, a catalytic application and an analysis method. You can call it a catalytic spectrometer. In this case, you can basically use the same setup as it has been explained in case of the photocurrent but without the lamp and another working electrode material. The working electrode is a glassy carbon electrode that is coated by the material you are interested in, (e.g. PtNP or alloy NPs as well as carbon supported nanoparticles) and further immersed into water including a reference and counter electrode (Figure 59). Following a strict cleaning protocol is extremely important when you plan to perform cyclovoltammetric analysis, as any dirt or pollution will significantly influence your results. There are many papers available presenting suitable cleaning protocols. The basic principle of cyclovoltammetric measurements is to apply and scan the potential (voltage) between the working electrode (coated with your electro-catalyst) and the reference electrode (usually a chloride saturated Ag/AgCl electrode or saturated mercury sulfate electrode). In this context, scan means to linearly increase and then again decrease the potential in an alternating way, performed in the given potential range (e.g. -0.2 V to 1.2 V when using a Ag/AgCl reference electrode). The scan rate is usually beneath 0.5 V/s and needs to be set according to common literature. By measuring the current between working and counter electrode, the material- and activity-specific current-voltage-spectra are being generated. Basic information you gain from this technique are: the electrochemical active surface area (ECSA), the overpotentials of several reactions (i.e. oxygen reduction reaction (ORR) in fuel cells, or hydrogen evolution reaction (HER) in electrocatalytic water splitting), the kinetics of mass transfer phenomena, the capacitance of your electrode or the stability of the catalytically active centers, to just name a few. With cyclovoltammetry, you can also probe the **durability** of your catalyst, which is of similar interest for industrial application as the activity and selectivity.

The durability can be checked by re-running the cyclovoltagramm after harsh aging or mimicking aging by, let's say 1,000 cycles and see how the peak areas drop. It is always good to have a reference at hand, e.g. a standard purchased from a chemical supplier. With that reference, you may also find out about the (hopefully) superior properties of laser-made catalysts.

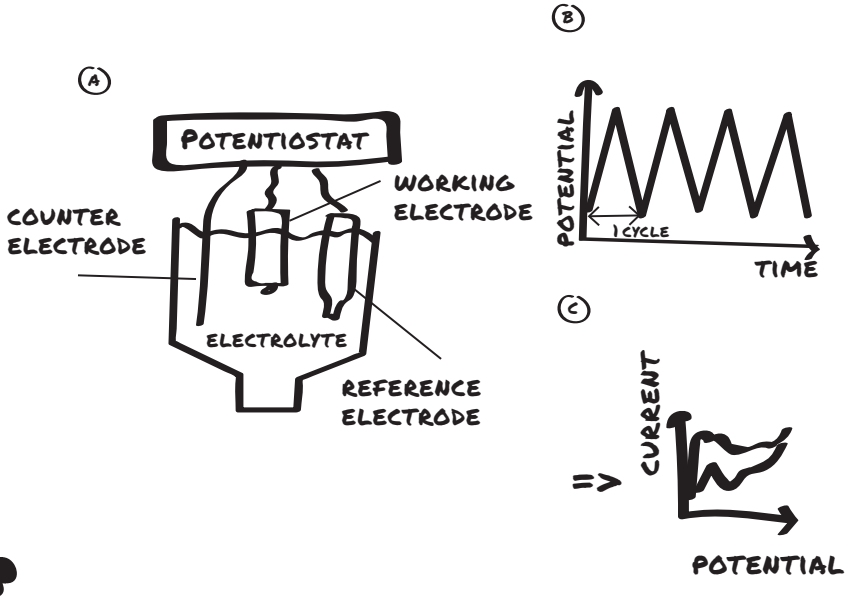


Figure 59: Experimental setup (A) for electrochemical investigations of a catalyst and the applied potential-time diagram (B) to obtain a cyclovoltammogramm (C).

In summary, you may already notice that the field of heterogeneous catalysis and application of NPs in catalysis is very broad and complex. However, don't be discouraged by the vast possibilities the field of heterogeneous catalysis offers, but thrive on the many opportunities for catalyst design and application the pulsed laser processing technique is offering you! Just pick one of the introduced variants and take a deep dive.

RECIPE 1: PT-LOADED CARBON (PT/C) AS A FUEL CELL CATALYST (FOR OXYGEN REDUCTION REACTION ORR)

Ingredients

- Pulsed Laser (ns or ps, 1064 nm)
- If available: pump, scanner
- Balance, Stirrer
- Ablation chamber or a beaker
- Pt target (>99.99%), Carbon black powder
- distilled water, KOH
- Centrifuge
- Ultrasonic bath
- pH-meter
- UV-Vis spectrometer or ICP-OES/ICP-MS,
- Measuring system for zeta potential

Instructions

Usually, for electrocatalytic applications, small nanoparticles are desired. As described in Chapter IV, there are several ways to decrease the particle size during (in situ size quenching) or after (ex situ particle centrifugation or fragmentation) laser ablation in liquids. In this recipe, both approaches will be used complementarily. We assume you already determined the optimal focal distance as it was described in Chapter I.

Nanoparticle Synthesis:

S1) First, to increase the yield of small nanoparticles (< 10 nm), prepare a KOH solution with a molarity of 0.1 mM. It is easier to prepare a stock solution of a 1 M KOH solution and then dilute it to 0.1 mM KOH, especially if you work only with small volumes. (E.g. prepare a 1 M KOH solution from the solid KOH. Take 100µL with a suitable pipette, mix it with distilled water and fill it up to 1L).

S2) Weight the target on your scale and fix it in the ablation chamber (or place it in your beaker).

S3) Fill the chamber (or beaker) with e.g. 100 mL (or another volume of your preference) of the as-prepared KOH solution. Write down the exact volume of liquid in your lab journal since you will need it later.

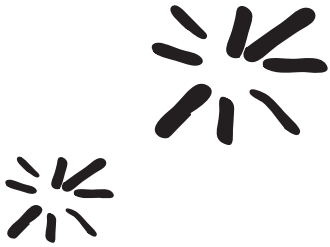
S4) Perform pulsed laser ablation in the KOH solution while using a ns- (or S2) ps-) laser at 1064 nm. Remember, as described in Chapter II, productivity strongly depends on the power of your laser. Therefore, the ablation time can vary between ~ 1-60 min or more to obtain a reasonable particle concentration (~100 mg/L). If you don't know your ablation rate, measure ablation time using a clock and stop ablation when the colloid has a dark brown color. Weight the ablated and dried target on the scale and substract the final from the initial weight. Use this weight difference together with ablation time and liquid volume used to calculate the ablation rate and colloid concentration. Continue ablation until a particle concentration of 100 mg/L – 200 mg/L has been reached.

S5) Measure the UV-VIS extinction spectrum of your colloid. Make sure to dilute the colloid in case the extinction is above 1 in the range of 300 nm – 800 nm. Note the dilution ratio (e.g. 1 mL colloid with 3 ml KOH solution → dilution ration of 4) in your lab book.

S6) Measure the particle size distribution following one of the methods described in Chapter V.

Processing by centrifugation:

If your mass-weighted particle size analysis shows that the colloid contains substantial amounts of nanoparticles with diameters above and below 10 nm at the same time, a centrifugation step is required to remove the large particle fraction. In case mainly particles above 10 nm are found check if you applied the correct working distance, laser parameters, and salt concentration and optimize these.



A laser fragmentation step in line with the instructions from Chapter III may be helpful. When using dynamic light scattering as a technique to measure particle size, keep in mind that a big particle fraction may mask existing small particle fraction completely (Chapter V). Analytical disc centrifuge will be the better choice for particle size analysis. To remove larger particle fractions, a centrifugation step is the easiest option to go with.

C1) Calculate the centrifugation time according to Svedberg's equation (Chapter I, step 8). Fill the colloid in a centrifugation tube (e.g. ≈25 mL ≙ 5.5 cm fill height) and perform centrifugation for 213 minutes at 5000 RPM (e.g. RCF≈3856).

C2) Separate your supernatant from the sediment carefully.

C3) Measure UV-VIS extinction of the supernatant and compare with the measurement you performed initially after laser ablation. Remember to dilute the colloid if the extinction is too high. Use both UV-VIS measurements (consider the dilution factor if dilution was conducted!) to estimate the nanoparticle concentration using the extinction signal at 300 nm (for Pt), see Fig. 60 Please refer to Chapter V for a more detailed description especially when applying this recipe for other materials! If available to you, use ICP-MS (Inductively Coupled Plasma Mass Spectrometry), ICP-OES (Inductively Coupled Plasma Emission Spectroscopy) or X-ray fluorescence (XRF) analysis for more accurate analysis of the nanoparticle mass concentration.

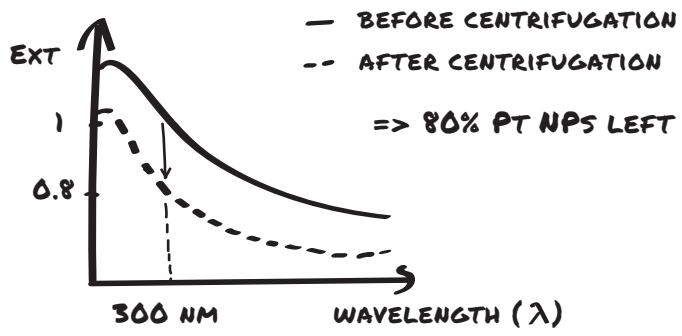


Figure 60: UV-vis extinction spectra of colloidal platinum nanoparticles before (solid line) and after (dashed line) centrifugation.

C4) Measure the particle size of your supernatant. If it still contains unwanted size distribution repeat the centrifugation starting with step C1.

Particle adsorption:

The colloid is ready to be adsorbed on the support material. For an electrostatically driven particle adsorption, you should know the isoelectric points (IEP, Chapter V) of both your support powder and your nanoparticles. Successful particle adsorption can be performed if your pH is between both IEP's and not too close to the IEP itself (see. Figure 61).

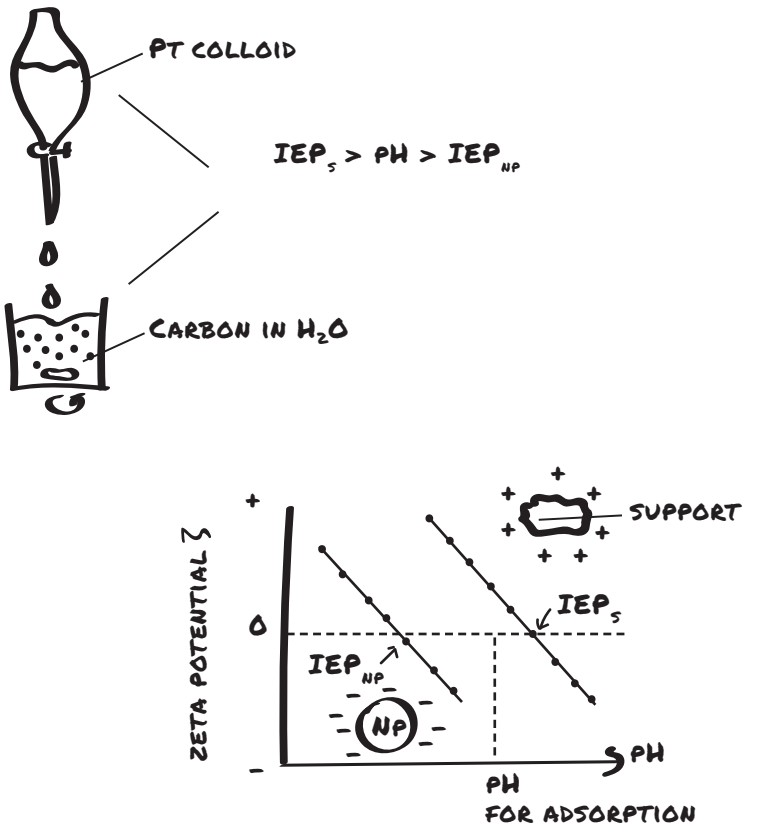


Figure 61: Experimental set up for particle adsorption (left) and zeta potential curves and isoelectric points of colloidal particles (IEP_{NP}) and the support (IEP_S) to find a suitable pH range for particle adsorption.

A1) Presumed your Pt colloid has a concentration of 80 mg/L after centrifugation. For a catalyst with a Pt loading 20 wt% and a total catalyst mass of 1 g, you will need 2.5 L colloid (= 200 mg Pt) and 800 mg carbon support. Weigh the corresponding amount of carbon black and disperse it in water.

A2) Measure the pH of your carbon black (use catalysis grade quality) dispersion and adjust the pH to an increment of one below the IEP of carbon black (e.g. pH=4 if IEP of your carbon black is at pH=5).

A3) Use a supersonic bath or finger for 10 minutes to disperse the carbon black particles homogenously.

A4) Measure the pH of your platinum colloid and adjust it to an increment of one above IEP of platinum (e.g. pH=4 if IEP of Pt NP is at pH=3).

A5) Add a magnetic bar to your pH-adjusted dispersion containing the carbon support and place the vessel onto a magnetic stirrer. Turn it on and adjust the stirring speed until a small vortex is visible. Insert baffles when available.

A6) Carefully add the platinum colloid dropwise (~10 ml/min) to the carbon dispersion under constant mixing (Figure 61).

A7) We recommend stirring overnight to make sure the particles are completely adsorbed on the support. Sometimes, the catalysts already sediment when adding the colloid. In this case, you can already check if the supporting was quantitative by observing the supernatant. If no brownish color stemming from platinum is visible, chances are high, that the supporting is already complete. In this case, you can easily remove the remaining water and analyze for remaining platinum using UV-VIS or ICP analytics. If no precipitation of the support is observable, carefully increase the pH value towards the IEP of the support to initiate precipitation. Note that the IEP of the nanoparticle-loaded support shifts towards the IEP of the pure nanoparticle colloid with increasing weight loading!

A8) After the supernatant is removed and quantitative nanoparticle adsorption is verified, we recommend rinsing the final powder two times with 500 ml of desalinated water to remove additional ions stemming from pH-adjustment. We recommend using filtration or centrifugation to remove the water used for the rinsing procedure.

A9) Dry the final catalyst to obtain a powder that can be used for further processing steps to investigate a fuel cell membrane. The as-prepared heterogeneous catalyst can be dried in air or using an oven at mild condition (<60°C). If available in your lab, it is recommended to use a freeze-dryer to obtain a 'fluffy' catalyst which can be easier redispersed in a liquid, when processing further electrocatalytic investigations.

A10) Run your first laser-generated fuel cell test. And check the durability (performance after aging) compared with a reference Pt/C catalyst at same Pt loading.

APPLICATION 2: BIOMEDICINE

Nanoparticles from laser-based synthesis may be particularly useful in biomedicine, as they can be generated without any potentially toxic organic stabilizer and side effects from unnecessary chemicals may be kept to a minimum. In the majority of cases, nanoparticles for diagnostic and therapeutic purposes should be conjugated with specific **functional biomolecules,** forming an organic corona around the inorganic core: these inorganic-organic hybrid materials are termed **nanobioconjugates**. For instance, a direct bond between the metal core and the biomolecule is usually established by gold-thiol chemistry, e.g. with cysteine residues in peptides and proteins. This looks exotic and complex, but laser ablation allows to make it very easy, especially in case of noble metal nanoparticles! The following paragraph will provide you with basic recipes on how to synthesize and characterize nanobioconjugates starting from laser synthesis, and how to assess their biochemical functionality and specificity. You will see superior durability.

VIII

Once we had to exchange a H2/N2-bottle in the lab for a fresh one, but I couldn't be there to assist in exchanging it. So I told a student to disconnect the empty bottle and give it to the supplier the day they came to collect it. The day arrived and I got a desperate message from him saying: "This is not possible, I have tried everything in my power, but the connection won't come off! I asked around and no one was able to assist!" So knowingly I asked if he knew that gas bottles containing flammable gasses have a left-hand thread and he told me he knew.

So expecting the worst I showed up a day later to inspect the problem myself. Usually, threaded connections come off like the water bottle you know from home, that's a right-hand thread. Flammable gasses have connections turning the other way (left-hand) to prevent accidents. I went to the gas bottle, armed with caution (it's still a gas bottle) and expecting a jammed thread. I turned the connection just the other way you would turn any usual water bottle and surprise surprise: It opened magically!

Simon Siebeneicher

STEP I: WHAT INGREDIENTS DO I NEED?

To make nanobioconjugates by laser synthesis, you don't have to change your set up that much. Of course, you also need the biomolecules, for instance, DNA or peptides. These biomolecules can be custom made and purchased from several commercial suppliers, once you or your bio-expert colleague have established which sequence of nucleotides or amino acids you need, i.e. which biological function your nanobioconjugate should perform. Don't forget to always add a thiol group to one terminus of your biomolecule to allow coupling with the noble metal nanoparticles. You may also include a fluorescent label for ligand tracking and proof of ligand binding to the nanoparticles by fluorescence spectroscopy (see Chapter III.1.4). In this context you need to make sure that your designed biomolecule is water soluble prior to your purchase - so when designing e.g. your peptide ligands, watch out for the portion of hydrophobic amino acids.

When delivered, the biomolecules usually come as a lyophilized powder, which is to be taken up by an appropriate buffer to make a highly concentrated solution. Furthermore, you need to closely watch the ionic strength in your liquid: On the one hand, it needs to be low for your nanoparticles not to aggregate, while on the other hand some biomolecules require a minimum ionic strength to retain their functionality. From our experience, diluted buffers < 2 mM are generally fine for most biomolecules and colloids during synthesis. In addition, the purity of your ligand is important, customized peptides usually come with a purity of $> 95\%$, which is usually sufficient for basic applications. After you have gathered all your ingredients, the next step would be to choose an appropriate synthesis route.

Basically, you have two choices: "in situ" or "ex situ" conjugation.

STEP II: IN SITU OR EX SITU CONJUGATION – SPEED VS. CONTROL

As the name already implies, in situ conjugation entails that the laser synthesis is carried out in the presence of the thiolated biomolecules. It is a fast one-step procedure in which the biomolecules immediately bind to the forming nanoparticles. This method achieves a high surface coverage and also goes along with a size quenching effect yielding nanoparticle cores with diameters < 10 nm. However, there are also some disadvantages. Firstly, nanobioconjugates post-irradiated by laser pulses are prone to biomolecule degradation, so you need to make sure to determine a threshold fluence up to which no biomolecule degradation is found and minimize the permanence time of the nanobioconjugate in the ablation chamber. Secondly, during in situ conjugation, it is difficult to control the coating with multiple different ligands or the formation of nanoparticle

cores with large size. In this case, your alternative is ex situ conjugation, where you first generate nanoparticles and you can use any size control and characterization methods applicable for ligand-free nanoparticles you know from the previous chapters. Afterward, you just have to mix the educt particles with defined amounts of the desired biomolecules. Here, the main advantage is that you are controlling exactly the ligand/nanoparticle ratio. Ex situ conjugation is well compatible with multifunctional conjugation, in addition, you can choose if adding different ligands simultaneously or consecutively. Basically, you can memorize that ligand mixtures can be used in case the biomolecules have similar molecular masses (diffusion coefficients) and surface charges. In the case at least one of those is significantly different, you may want to go for consecutive addition, by taking care that the amount of the first ligand is low enough to avoid saturation of nanoparticles surface before the addition of the second ligand, and so on.

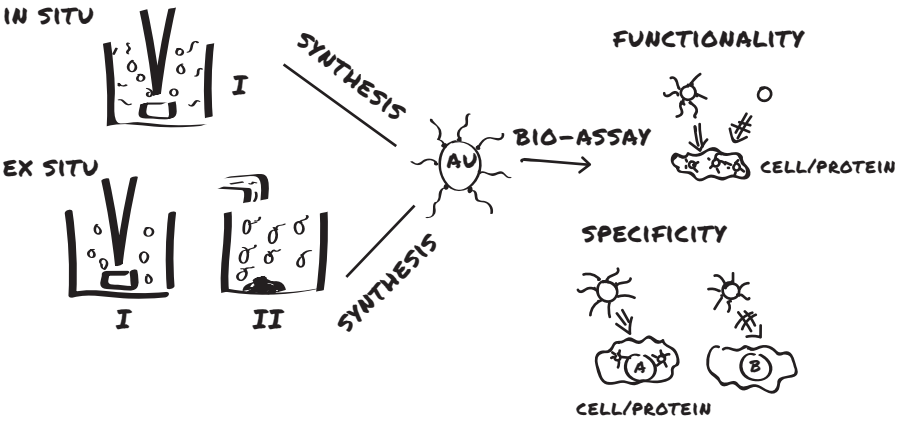


Figure 62: In situ and ex situ bioconjugation of nanoparticles and its bio-assay: functionality and specificity

STEP III: HOW ARE THEY? – SURFACE COVERAGE AND STABILITY

After obtaining the colloidal solution of nanoconjugates, of course, you will ask yourself whether and to what extent the conjugation (binding of biomolecules) succeeded. The first thing to check is how many ligands are bound to your nanoparticle surface, or in other words- what is your surface coverage and conjugation efficiency. The easiest way to measure surface coverage is to pull down your nanoconjugates by centrifugation and then determine the amount of unbound ligands in the supernatant. In case you know the exact amount of your ligands you originally deployed, which you usually do, you can easily calculate

the number of bound ligands from the difference. Ligand concentrations can be measured either by optically active amino acids in peptides and proteins, like tryptophan, or by fluorescent labels previously added to the biomolecules.

The next thing you need is to make sure that your conjugates are still in the colloidal state during the desired bio-application. Aggregation of your conjugates may reduce the number of available functional groups and is generally bad for the overall performances. To probe for these effects, you have to test your nanoconjugates in the corresponding biofluid, e.g. blood serum, for several days, while you can use optical absorption spectroscopy (see Chapter III.1.1) for monitoring the stability versus time. In case your conjugates aggregate and precipitate under real-life conditions, you may have to start from scratch, e.g. adapt surface coverage or add additional stabilizer molecules.

STEP IV: WHAT CAN THEY DO? – BIOLOGICAL ACTIVITY AND SPECIFICITY

Next, of course you will be eager to find out whether your conjugates actually do what they are designed for- exercise a defined biological function. In this context the conjugates need to fulfill two important characteristics which are I) functionality (= make sure a biological effect is caused by the conjugate) and II) specificity (= the conjugate predominantly reacts with one defined target). These features can be probed by a large variety of assays, while only two examples will be discussed here.

I) The interaction of nanobioconjugates with a specific protein

Let's assume you have a monofunctional gold-nanobioconjugate, carrying a specific peptide, prone to bind to proteins with e.g. a high β-sheet content (relevant for Alzheimer disease diagnosis). A β-sheet is a special protein morphology which in this case is your target site. To design an appropriate assay, you will first require a suitable readout. A commonly-used strategy in this context is based on fluorescence quenching. Here, the target protein is labeled with a fluorescent dye, whose fluorescence intensity is quenched (=reduced) when binding to the nanoconjugate. However, even if you see a reduction in fluorescence in your assay, this does not necessarily proof that your conjugate is functional. Fluorescence quenching may also be caused by the gold nanoparticle core itself by unspecific effects. So you have to perform controls with unconjugated gold nanoparticles and only in case, your conjugates can outperform the controls you have successfully demonstrated the conjugate functionality. Another vital aspect is specificity- what good comes from a conjugate that binds to every protein in the same way? So you need to perform control experiments with another relevant protein (without β-sheet structure), such as albumin to make sure that your conjugate is not only functional but also specific for your target site.

II) Cellular uptake and transfection

A frequent case related to cell culture experiments consists of bifunctional gold-conjugate exposing on the surface both, a short peptide and a plasmid DNA. In this case, the nanoconjugates must be in the cytoplasm to exert their function, meaning that cellular uptake is a primary criterion. Here, cationic peptides are useful. Uptake can be monitored by confocal microscopy, where a scattering signal from the gold cores cross-correlated with a fluorescence signal from the fluorophore-tagged peptides is measured. When the plasmid DNA of the nanoconjugate reaches the cytoplasm, it implements a specific genetic code, a process referred to as cellular transfection. A convenient mean to know that transfection occurred, is to implement the instructions for the production of a fluorescent protein called green fluorescent protein (GFP) into the DNA. Hence functionality of the conjugate is indicated by the onset of the GFP fluorescence. In this case, the efficiency of the nanoconjugate compared to the free DNA can be easily probed by GFP fluorescence intensity, as well as specificity of the nanoconjugate can be proven with control experiments using other cell lines.

RECIPE 2: EX-SITU NANOBIOCONJUGATE

I) Make the required colloid with diameters < 10 nm

Place the target in the ablation chamber, fill it with the low salinity electrolyte solution and perform laser ablation for 5-10 minutes. The colloid is ideal when its mass concentration is around 100 mg/L and when it exhibits a deep red color and has a PPI higher than 20, so it is not aggregated.

II) Do the conjugation

Pipet an aliquot of your colloid (500 – 900 µL) into a smaller container like an Eppendorf tube (you will want to use small volumes as biomolecules can be damned expensive) and then add a few microliters of the biomolecule at an appropriate concentration. Usually, you will aim for final concentrations of 0.5 – 10 µM. Then all you have to do is vortex the solution for a few minutes and just let it stay at room temperature for about 5-10 h to let the reaction take place. Depending on the used ligands, the solution may turn slightly more violet, this is fine as long as it is not completely blue and you have no black precipitates.

III) Get rid of residual ligands

Spin down your nanobioconjugate with an ultracentrifuge (remember to set the temperature below room temperature to avoid aggregation). Then redisperse the pellet in a low salinity buffer solution by using ultrasonication and also keep the supernatant. Repeat the process 3-5 times to make sure your conjugate is clean from residual unbound ligands.

Be careful not to get nanoparticles into your supernatants. In case the separation is not working well, better leave more liquid on top of the pellet and do more washing steps. Finally analyze all supernatants with UV-Vis spectroscopy to determine the amount of unbound ligands, which will give you surface coverage.

IV) Make it ready for use

Now transfer the nanobioconjugates into a relevant cell culture medium. This is best done during the last redispersion step. Simply use your medium here, instead of the buffer, to take up your pellet. Do not worry too much about high salt concentrations in the medium as it usually also contains serum proteins which sterically stabilize the conjugates. Just to be on the safe side concerning colloidal stability, leave your colloid in the cell culture medium for 24 h before use and check with UV-Vis whether it changes. If the PPI is higher than 5 and no black precipitate is found, you are fine. Finally, your nanobioconjugate is ready for use and can be served with specific proteins or cells.

APPLICATION 3: 3D-PRINTING (NANOPARTICLE-DOPED ADDITIVE MANUFACTURING POWDER)

There are many techniques of 3D printing, however, only some of them have industrial relevance. **Laser powder bed fusion** (LPBF) is one of these methods. LPBF of metals and polymers are relevant for technically sophisticated components, in particular for the automotive, aerospace, biomedical and electronics industries.

For instance, the brake caliper of the Bugatti Veyron (a car with a price tag of 3 million euros (!)) was recently manufactured by LPBF. A current challenge in LPBF-research is the limited amount of available powder materials that can be successfully processed by LPBF. One way to tackle this problem is by introducing nanoparticles to change the crystal structure or the microstructure of the 3D-printed part. For what purpose? It's quite simple and effective for both metal and polymer LPBF. In LPBF, the powder is molten and resolidifies after fusion along the scanned tracks. The nanoparticles added in quite a low amount (ideally less than a weight percent) now strongly affect the crystal growth. They act as heterogeneous crystallization seeds, resulting in finer and more uniaxed grains, which is good for mechanical stability and isotropy of properties. Also, nanoadditives that do not contribute to grain refinement are interesting, such as oxide nanoparticles which hinder dislocation movement during creeping of steel.

However, when introducing nanoparticles onto a micro powder, a sufficient dispersion plays an important role. Conventional additivation methods such as ball milling or melt compounding often lead to agglomeration of the

nanoparticles. Therefore, sufficient surface doses are typically achieved by high additive loads, which in turn promotes agglomeration. And you don't really want agglomeration. By increasing the dispersion of nanoparticles on the surface you would significantly reduce the nanoparticle weight dose. Here, laser-generated colloidal nanoparticles and their adsorption on powders come in to play. The adsorption (supporting) happens directly in aqueous solution by adjusting the electrostatic interaction between the nanoparticle and the microparticle surface through pH variation as described previously for catalysis (Figure 61). This route is described in the figure below. The polymer or metal micro powder is dispersed in the colloid and the laser-generated nanoparticles are adsorbed on the polymer surface by pH-controlled electrostatic adsorption. After the supporting process, the composite is separated from the liquid phase, e.g. by filtration, and is dried and sifted to get ready for the LPBF-process.

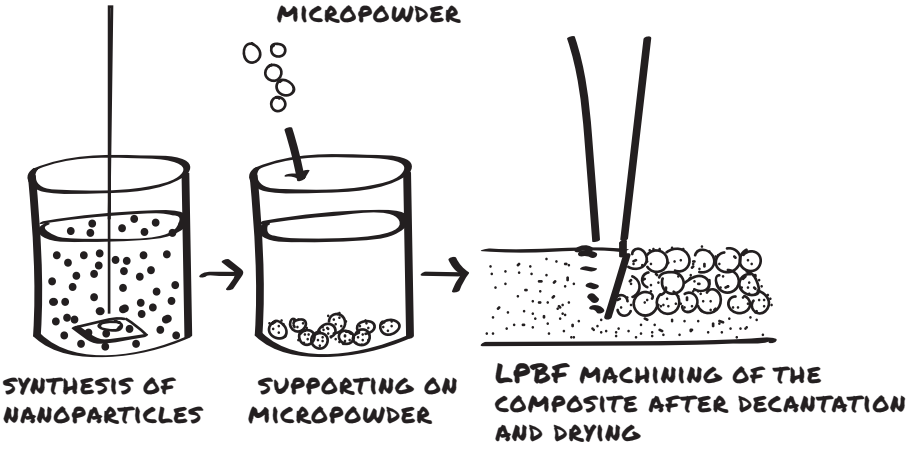


Figure 63: Schematic illustration of the process steps to support laser-generated nanoparticles on polymer or metal powders, from left to right: Laser synthesis of nanoparticles, mixing of micro powder with the colloid and LPBF machining of the composite micro powder

It is obvious that composite powders obtained by ball milling show strong agglomeration, whereas colloidal deposited nanoparticles lead to significantly less agglomeration and a homogeneous distribution of the nanoparticles. This quite simple route has recently shown a huge potential in the development of new powder materials for Laser 3D printing.

RECIPE 3: NANOPARTICLE-DECORATED MICROPOWDER FOR LASER POWDER BED FUSION:

I) Prepare the required colloid

Depending on the application you might need a different nanoparticle size. In general, smaller size <10nm is aimed at in order to be able to deposit nanoparticles with a large specific surface. Actually, the volume of the nanoparticles is more relevant in additive manufacturing rather than the mass fraction. The volume fraction of nanoparticles that you will have deposited on the micropowder will depend on the concentration of your colloid. How to change the concentration of your colloid you know by now. Also note, that as usual, we want to have a big effect with a small amount of nanoparticles, this also indicates that smaller sizes make more sense. Please refer to the chapter on size manipulation and choose one of the ways described there to prepare the colloid you want. Also, the upscaling chapter is of particular relevance here, as usual, LPBF batches require about a kilogram of powder, so that 0.1 wt% requires already one gram of nanoparticles, just to run a single LPBF build job.

II) Adsorb the nanoparticles on the micropowder

To adsorb your generated nanoparticles on the micropowder you mix the micropowder with the colloid at a given pH. Mixing can be done for instance by a magnetic stirrer for approximately 2 hours. Why is the pH important? This is nicely explained above in the catalysis recipe ("particle adsorption") and Figure 61. The pH has to be between the isoelectric point of the nanoparticles and the micropowder material. For instance, steel has an isoelectric point around pH 3, while oxide nanoparticles are around pH 7, hence you have to work with a pH between these points. During mixing you will see if the adsorption is successful or not, since the liquid-phase will lose its color if the nanoparticles are deposited on the powder. To know for sure how much of the nanoparticles were adsorbed by the powder you can (and probably should) perform ICP-MS/OES or XRF. While in the catalysis case this efficiency is quite high, for metal powders it could be the case that you only can adsorb 50%-60% of your nanoparticles. This is not a big issue, as no precious metals are required. Cheaper nanoparticle materials already do a good job as crystal seeds or dispersion strengthening.

III) Dry the powder

For laser powder bed fusion you will need a (completely) dry powder. Hence drying is the next step. After decantation of the clear liquid from (II), you need to find a method of drying. Putting it in an oven with mild condition (<60°C) seems to be the best way of achieving this goal, even for 1 kg of powder.

Of course, other options are also possible such as microwave drying or spray drying. However, you have to be careful not to undo the wonderful things that you have already done. Spray drying of polymer powder can, for instance, lead to agglomeration of the nanoparticles and also aging of the micropowder. If you heat your powder too much during drying and do not work in an inert atmosphere, you may unintentionally change the composition of your steel powder. These are points to be carefully considered when drying your powder.

Once you have the dried powder you are ready to go. It is always good to compare characteristics of the powder such as its flowability with a reference powder which does not contain nanoparticles. If you decreased its flowability or the powder agglomerates this might be an indication that your supporting procedure or the drying process was not very successful.

VIII

FINAL STEP

After reading this book you hopefully realized that the force is strong with you. Now, it's time for you to take your laser, spread your wings, dive into the liquid and start synthesizing nanoparticles that will make a difference in science and technology.

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DOI: https://doi.org/10.17185/duepublico/70584

S. Barcikowski, V. Amendola, M. Lau, G. Marzun, C. Rehbock, S. Reichenberger, D. Zhang, and B. Gökce. Handbook of Laser Synthesis of Colloids & Processing, DuEPublico, 2019.

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DOI: 10.17185/duepublico/70584
URN: urn:nbn:de:hbz:464-20190930-220335-6

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