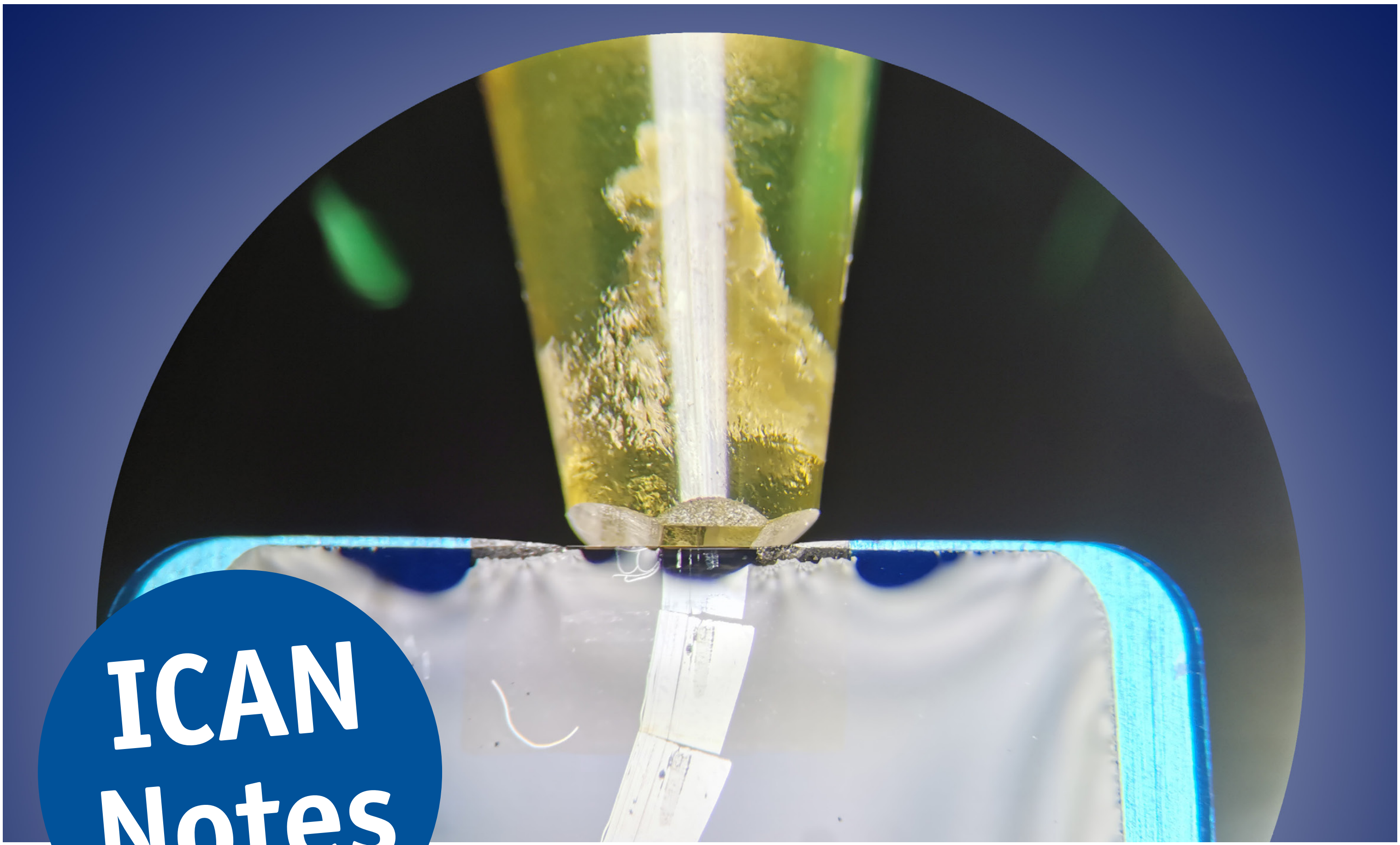

ICAN Notes

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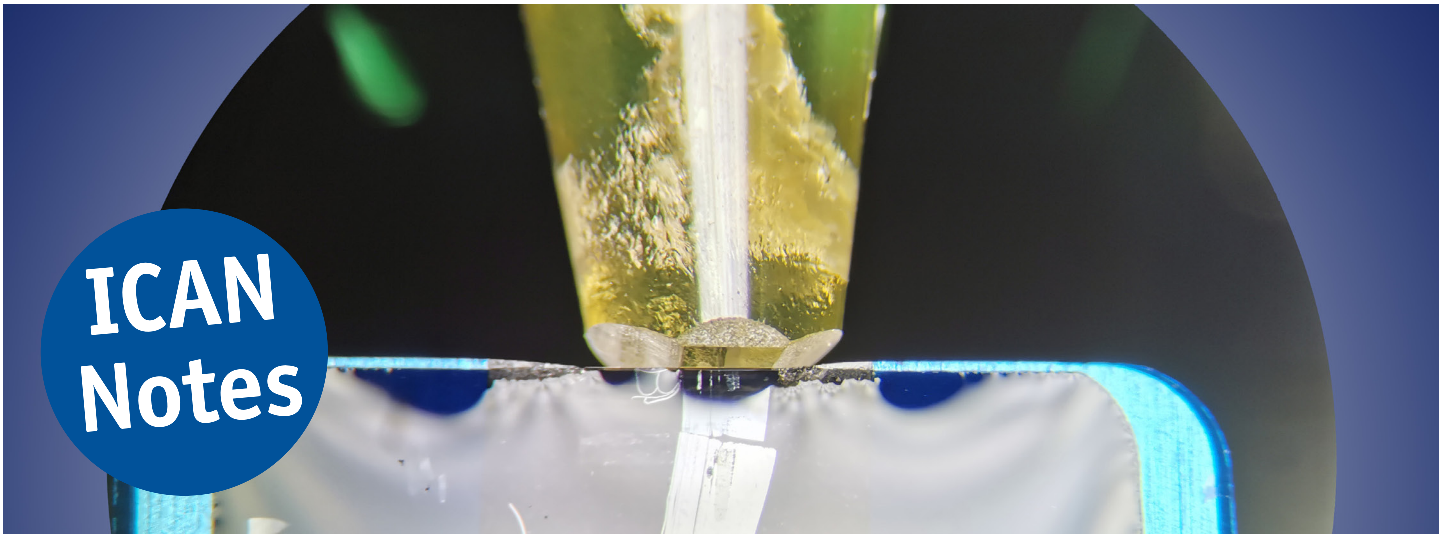

The ultramicrotome as a tool for the preparation of ultra-thin samples for TEM investigations

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Interdisciplinary Center for Analytics on the Nanoscale (ICAN)

Transmission electron microscopy (TEM) is a powerful tool for the characterization of a wide variety of materials on an atomic scale. Since the electrons which are used for imaging have to pass through the material, however, the limitations regarding the specimen thickness are quite strict. Depending on the material and the TEM's accelerating voltage, samples with a thickness of 50 to 100 nm are still electron-transparent, whereby the attainable spatial resolution decreases with increasing thickness of the sample. Compared to other microscopy methods, such as optical microscopy or scanning electron microscopy, electron-transparency often requires complex sample preparation procedures. Furthermore, the necessary preparation depends on the specifics of the material. In the case of hard materials like metals or ceramics, the preparation path involves sawing, grinding, polishing and finally thinning with an ion beam. If the area of interest itself needs to be selected with high precison targeting, Focused Ion Beam (FIB) methods can be used. For soft materials like polymers or biological specimens, these methods can cause severe thermal stress or even destruction of the specimen material. To avoid this, the preparation technique of choice would be the use of an ultramicrotome. The ultramicrotome creates ultra-thin sections (usually tenths of nanometers) from the specimens mechanically and with as little stress as possible by using glass or diamond knives. Particularly sensitive samples can be nitrogen cooled to avoid damage during cutting. This note describes the preparation of a TEM sample by ultramicrotome cutting with the ultramicrotome (EM UC 7 with EM FC 7 module for cryo applications, Leica Microsystems) in the ICAN.

In order to meet the ultramicrotomes size requirements, the specimen has to roughly fit into a (5x5x20) mm^3 block. Depending on the original size of the specimens, they can be trimmed using razor blades and a scalpel. Alternatively, the trimmer (EM TRIM 3, Leica Microsystems) in the ICAN can be used.

For small, very soft, or thin specimens (i. e. like foils) it is beneficial to embed them in epoxy resin. The resin fixates these specimens for the actual cutting. Without embedding, the specimen would move during the cutting process and an uneven cut would be the result. Fig. 1 shows different casting molds for embedding. The molds a) and b) in Fig. 1 are embedding molds for flat samples. These are well suited for thin specimens such as foils. Alternativelly an Eppendorf container (see Fig. 1c) can also be used for embedding samples.

The embedded samples are cured in the vacuum drying oven (VT6060, Thermo Scientific) at 80°C for approx. one hour, or alternatively at room temperature for at least 24 hours in case the specimens are temperature sensitive. In the ICAN, usually Epoxy 3000 from Cloeren Technology GmbH is used.

Once the epoxy resin has been cured, the specimen has to be removed from the mold and than trimmed into the shape of a truncated pyramid shape with the EM TRIM3 trimmer before ultramicrotome cutting. Fig. 2a shows the epoxy pyramid with embedded sample shows the epoxy pyramid with embedded sample fixed into the specimen holder of the ultramicrotome after the initial trimming. The surfaces of the pyramid are still rough.

The truncated side of the pyramid should be as flat as possible so that the pressure from the ultramicrotome blade cuts evenly through the specimen. Therefore, the fine trimming of the pyramid is accomplished with a glass trimming knife, or a diamond trimming knife in the ultramicrotome. Fig. 2b shows the epoxy pyramid fixed into the specimen holder of the ultramicotome after completed fine trimming.

Note that the knives used for trimming are different from the

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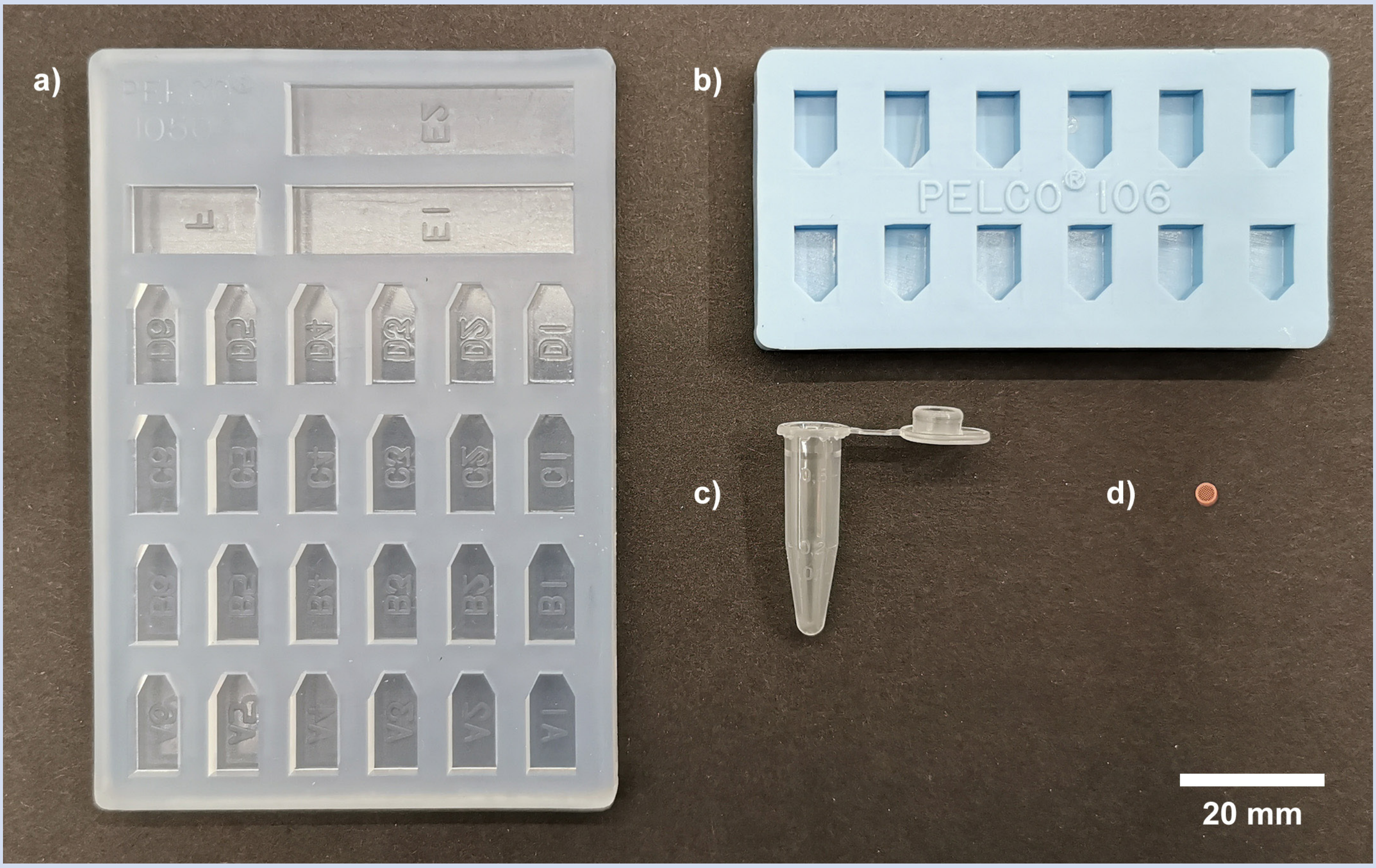

Figure 1: Different embedding molds with a TEM grid (d) for size comparison.

knives used for ultramicrotome cutting. Using the ultramicrotome knife for trimming is also possible, but it would take more time and would wear out the knife.

Re-trimming with the glass trimming knife shown in Fig. 3a leads to very smooth surfaces which later on facilitate approaching the knife for the ultra-thin cuts to the specimen via the reflection of the knife at the tip of the pyramid. Looking through the microscope, it is difficult to see how close the blade is to the block. This can be asseessed with the help of the optical reflection on the trimmed smooth surface. Moving the knife to the block, the distance between the real blade and the mirrored blade is reduced.

After fine trimming the sample is ready for the ultramicrotome cutting. Fig. 3b and 3c show knives for the ultra-thin cutting. Both knives have integrated basins (tubs) that can be filled with distilled water. The knife shown in Fig. 3b is made of glass while the knife in Fig. 3c is made of diamond.

Even though diamond knives are very hard, they eventually wear out with repeated use. Since re-sharpening is very expensive, it is advisable to first try a freshly broken glass knife with tub for cutting the sample. These are cheaper and they are sufficient for soft materials. If, however, the glass knife shows cutting marks after the first cut, it is recommended to switch to a diamond knife.

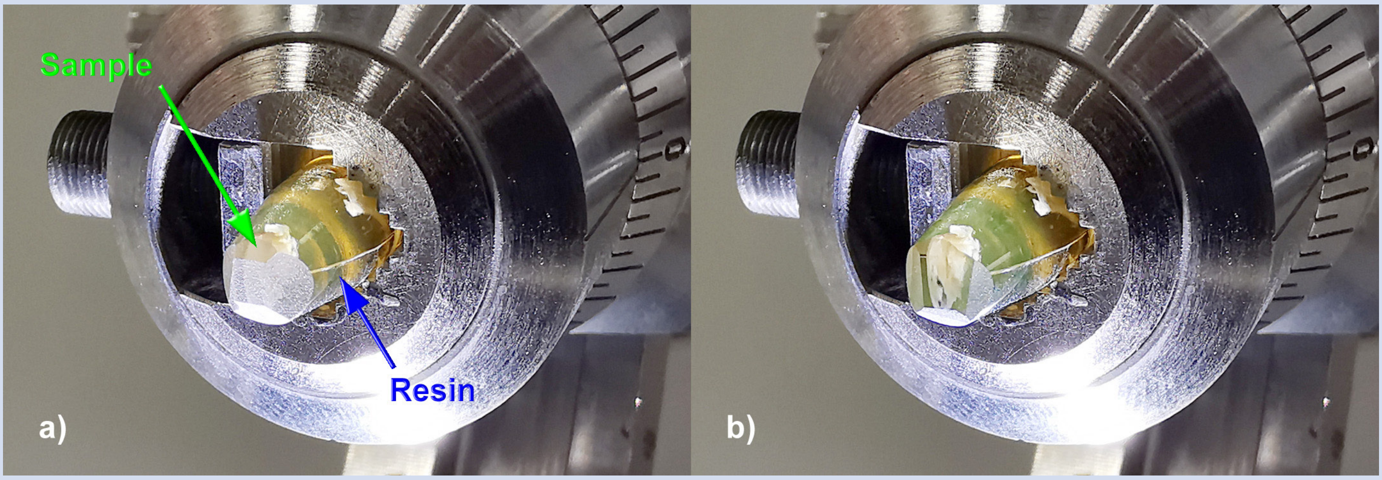

Figure 2: Truncated pyramid located at the ultramicrotome sample holder a) after trimming with trimmer and b) after fine trimming with glass trimming

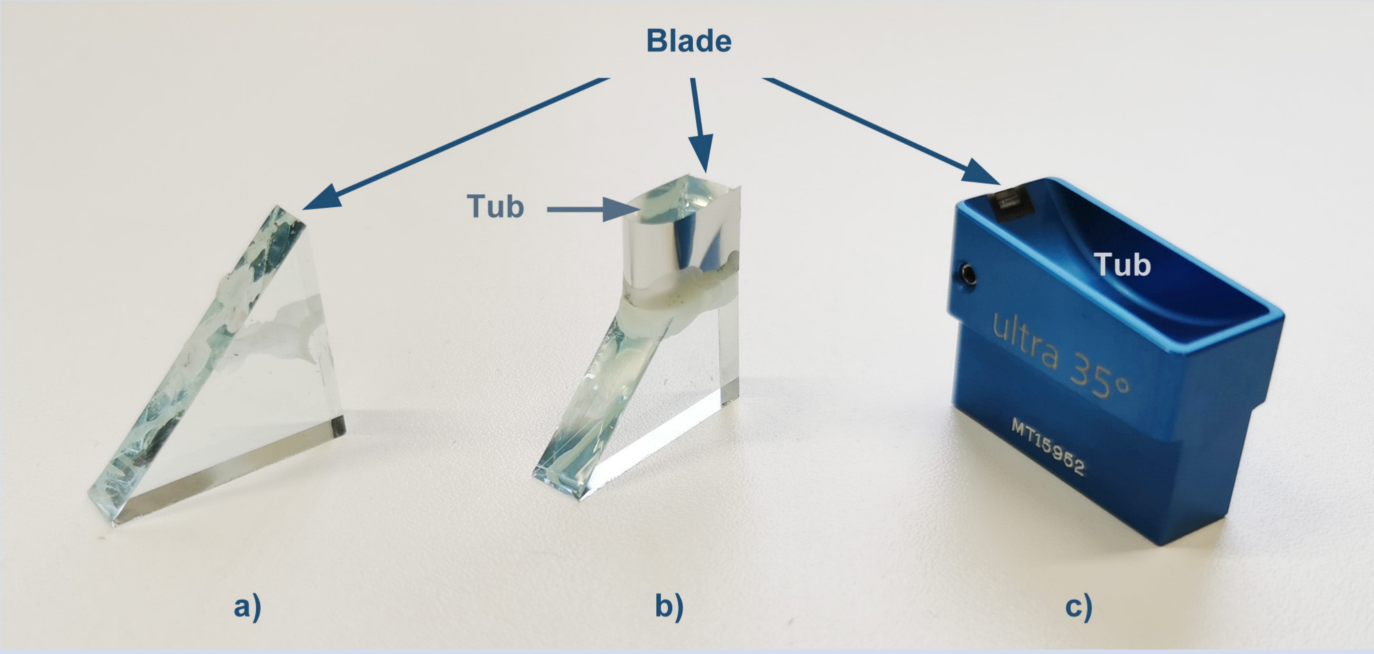

Figure 3: a) Glass knife for trimming; b) Glass knife with tub; c) Diatome 35° Ultra with blue metal tub with diamond blade.

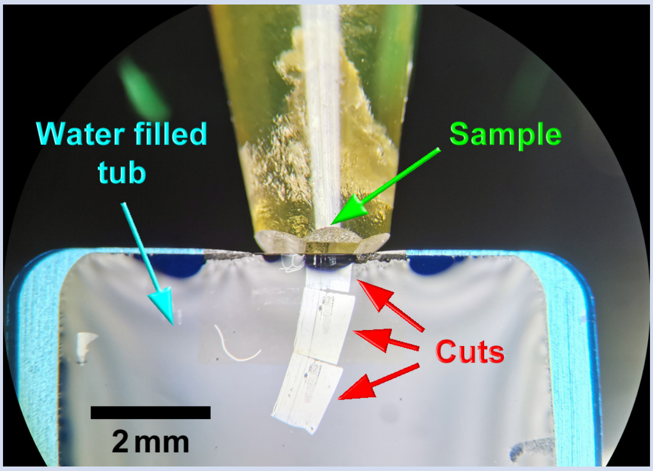

Figure 4: Floating Slices.

Once the cutting parameters (requested thickness, cutting speed) are set and the tub has been filled with distilled water, the cutting process can be started. During the cutting process, the ultra-thin cuts slide into the tub onto the water surface. Fig. 4 shows three slices floating on water that have been cut from the embedding pyramid at the upper half of the figure. Floating slices are picked up with a TEM grid and dried in air at room temperature. The final result can be seen in Fig. 5.

After this procedure the sample is ready for TEM measurements.

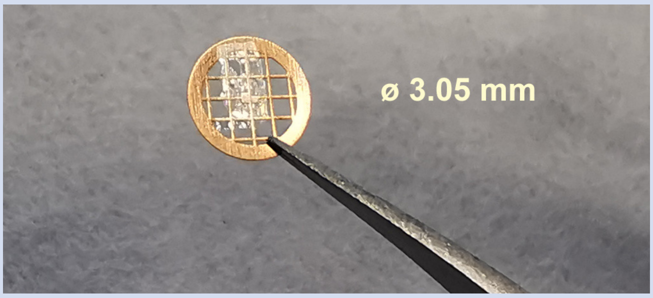

Figure 5: Air dried slice on a TEM grid.

In case of a problematic charging up of the specimen is observed during irradiation with the electron beam inside the TEM, the sample may be additionally coated with a nanometer thin layer of carbon in a sputter coater as a final preparation step prior to the TEM investigation.

In summary ultramicrotomy is most suitable for the preparation of soft materials and biological samples. Unlike conventional methods including FIB, where only one electron-transparent sample can be prepared at a time, the ultramicrotome is capable of creating several electron-transparent samples in a sequence during the preparation process. In addition, the remaining block with the embedded sample can also be used for analysis of cross-sections, for example by using SEM/EDX or SAM.

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Cover image: View on the cutting process in the ultramicrotome.

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