# Compression in ultrathin sections

Helmut Gnaegi

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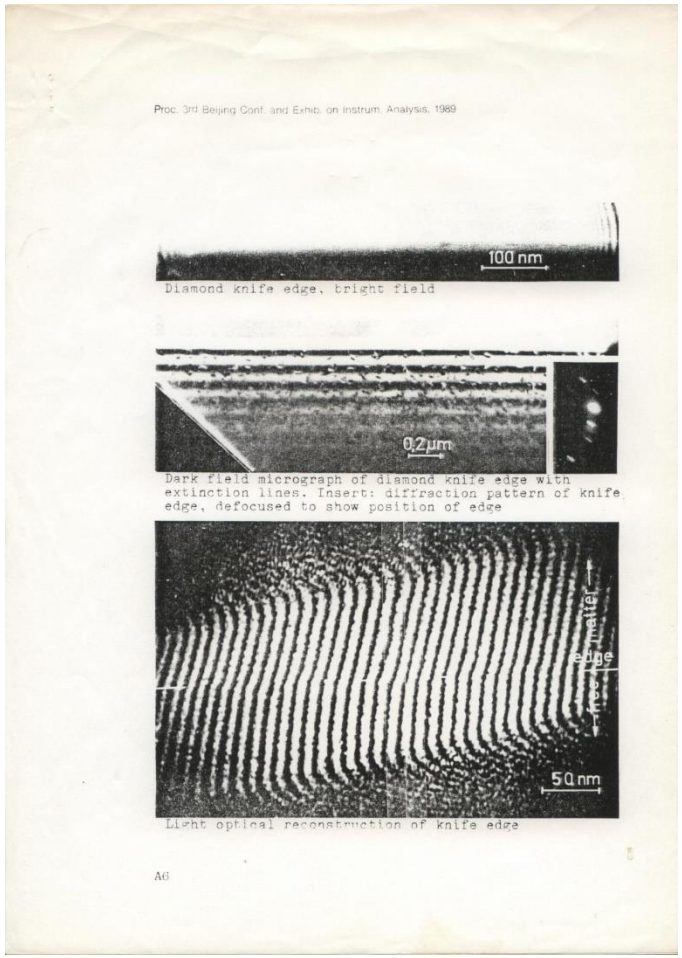
The precision of the diamond knife cutting edge

Compression in room temperature sectioning

Diamond knife cleaning

# The precision of the diamond knife cutting edge



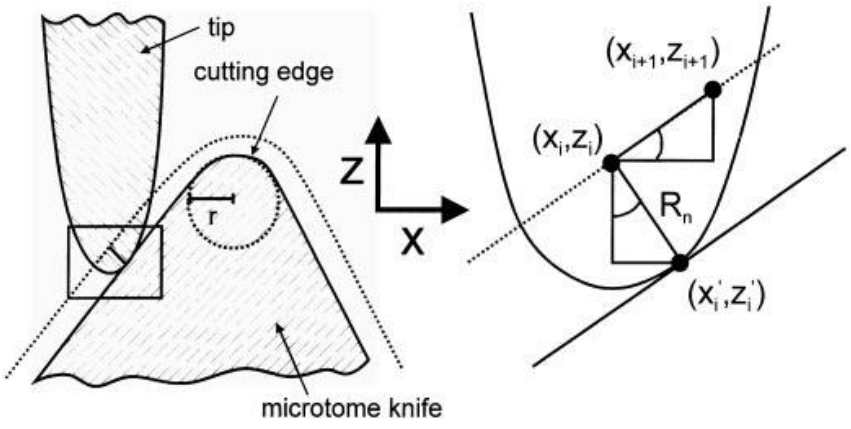

In holographic studies the radius curvature of a diamond knife is found to be around 2nm

Proc. 3rd Beijing Conference and Exhibiton on Instrum. Analysis. 1989

Electron optical and holographic studies on a diamond knife edge

R. Lauer, G. Ade, K.H. Lickfeld, H. Gnaegi

# The precision of the diamond knife cutting edge




Journal of Microscopy 2003

Characterization of the cutting edge of glass and diamond knives for ultramicrotomy by AFM using cantilevers with a defined tip geometry. Part II.

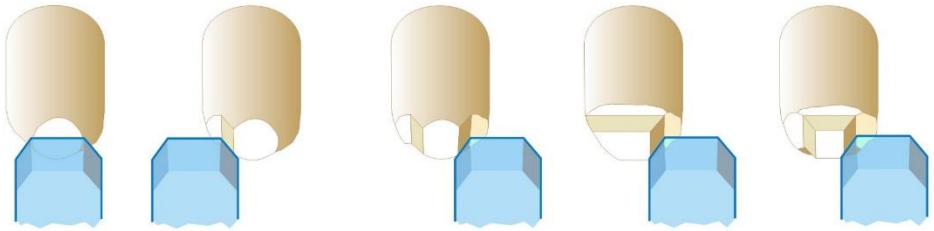
T.R. Matzelle, H. Gnaegi, A. Ricker and R. Reichelt

# The precision of the diamond knife cutting edge

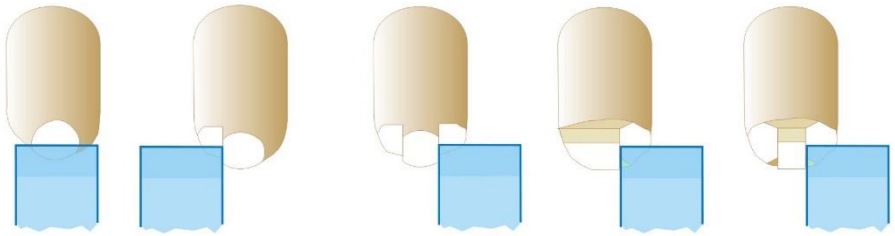
The cutting edge looks perfect in the optical microscope (reflecting mode, Nomarski contrast, 750x)

- Sections are cut over the entire cutting edge, according to the knife cutting range
- Sections are free of compression
- The sample block is examined in the optical microscope and found free of knife marks

# Room temperature sectioning

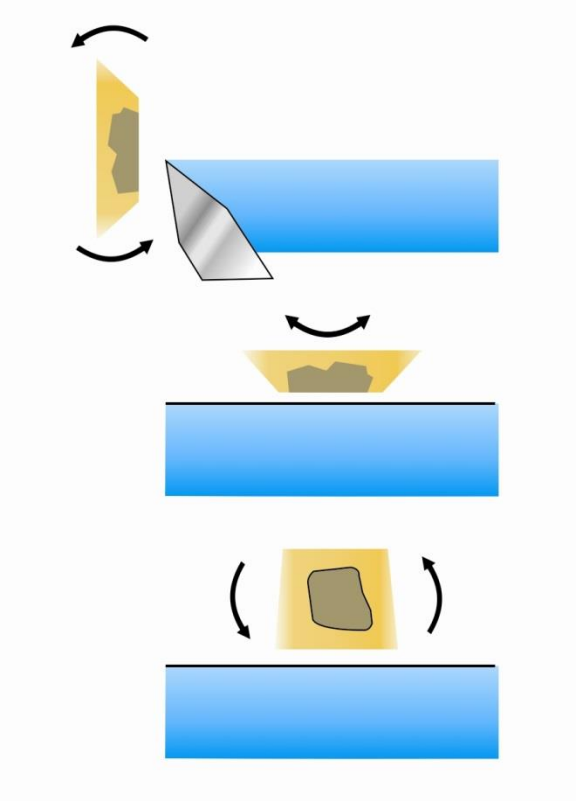


Routine trimming with trim 45



Trimming with trim 90 for FIB, sectioning in the SEM (3View, Volumescope)

# Room temperature sectioning



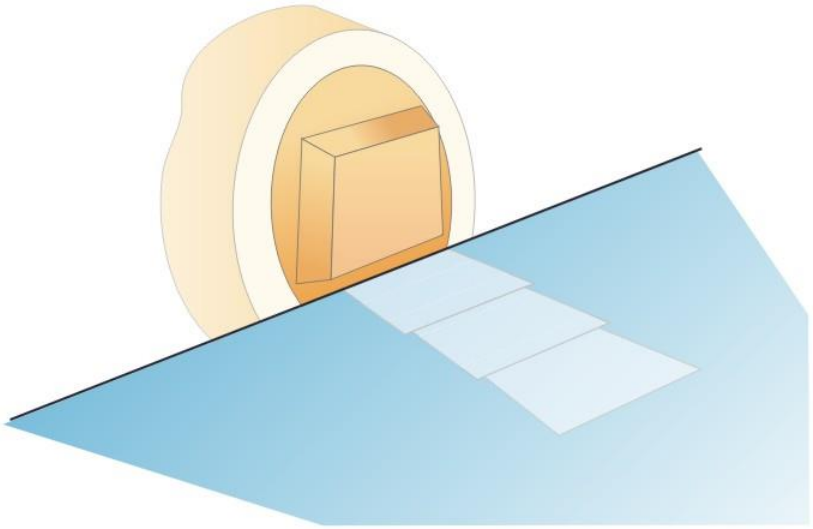
The alignements

# Room temperature sectioning


Using an antistatic device helps when:

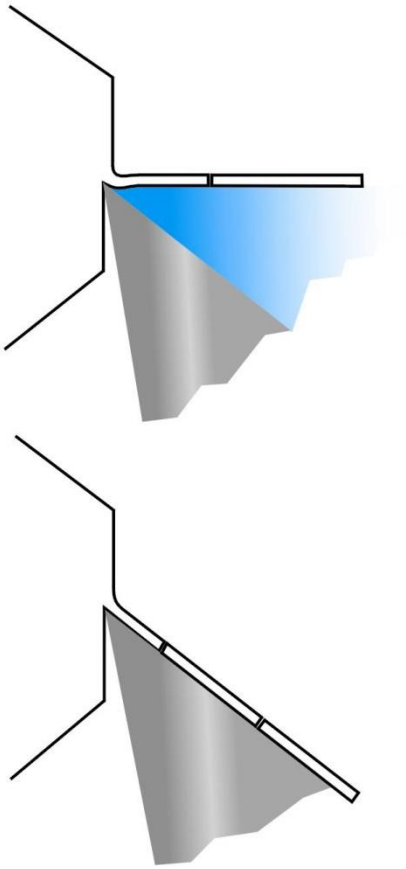
- the water in the boat jumps to the sample block
- the sections beeing pulled back to the sample block
- the sections beeing distored instead of gliding flat.

# Compression in room temperature sectioning



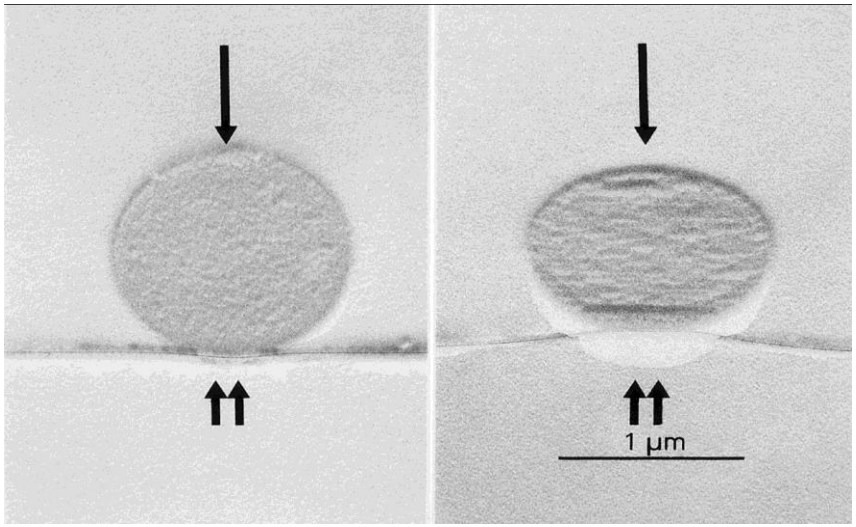
Compression: the section is shorter as the height of the sample block

# Compression in room temperature sectioning



The sectioning process

# Compression in room temperature sectioning




Sectioning of a polystyrene sphere embedded in epoxi resin

Left: sectioned with a 15° knife

Right: sectioned with a 45° knife

J.C. Jésior, Journal of Ultrastructural Research 1985

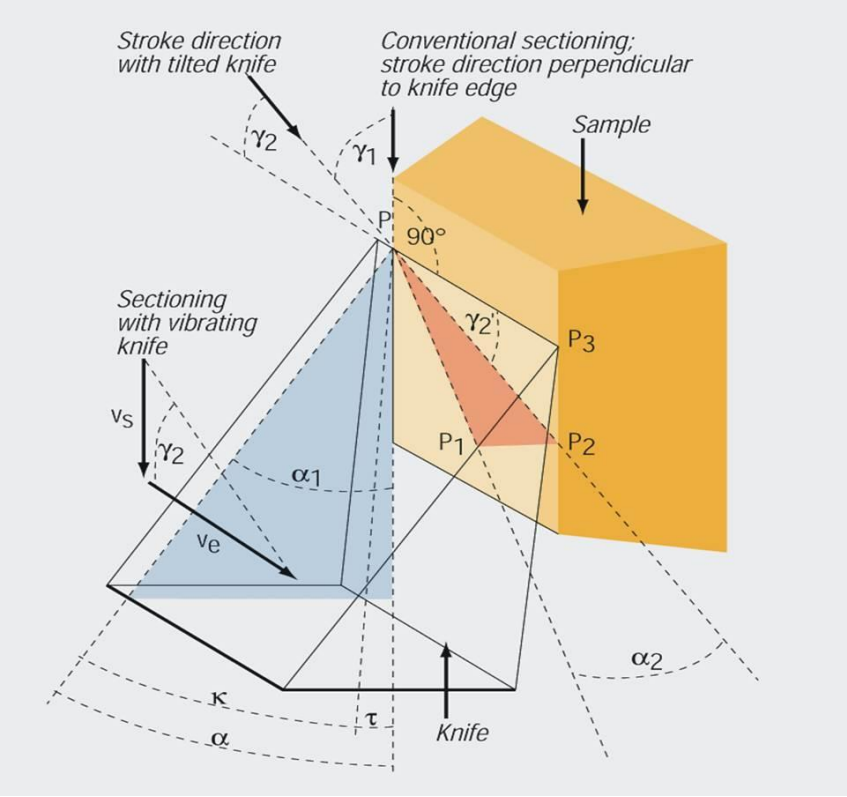
# Compression in room temperature sectining



An oscillating diamond knife for improved structure preservation

Studer et al., Journal of Microscopy 2000

# Compression in room temperature sectioning



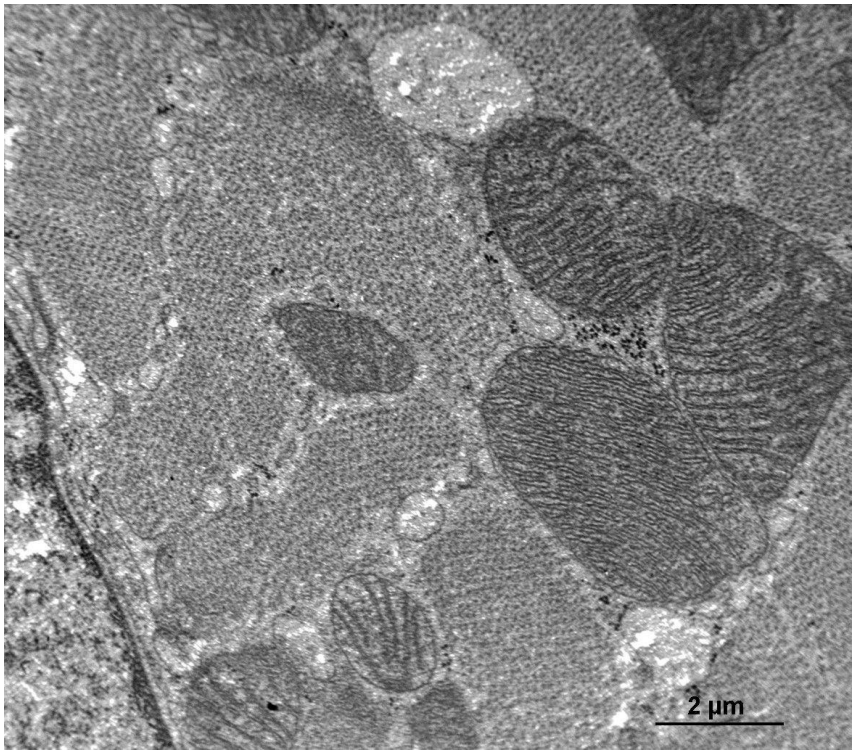

An oscillating diamond knife for improved structure preservation

Studer et al., Journal of Microscopy 2000

# Compression in room temperature sectioning

| Knife type | Compression factor | |
|---|---|---|
| | 50nm section | 30nm section |
| Glass knife | 30 - 40 % | 50 - 60 % |
| ultra 45° knife | 25 - 30 % | 40 - 50 % |
| ultra 35° knife | 15 - 20 % | 30 - 40 % |
| ultra sonic knife | 0 - 5 % | 5 - 10 % |

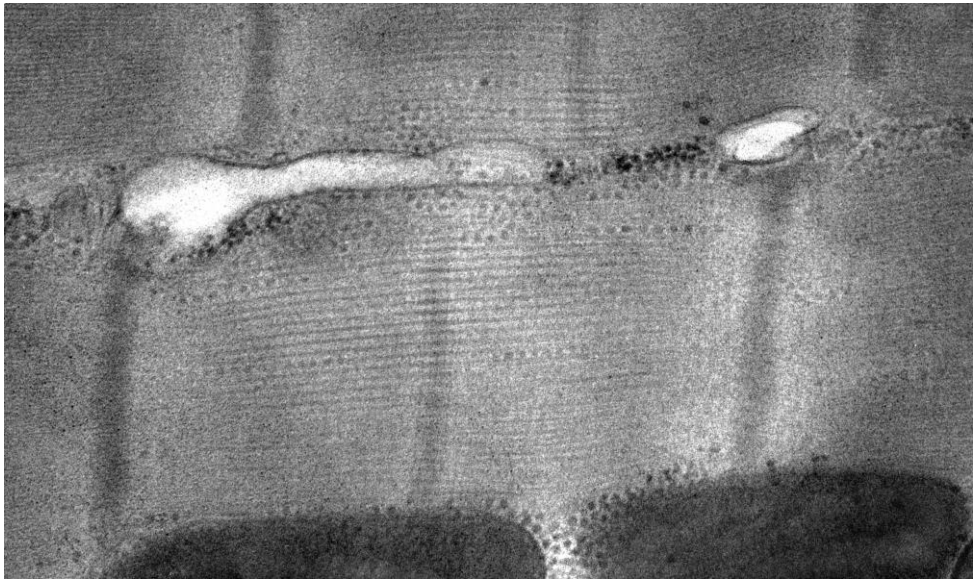
# Compression in room temperature sectioning




Rat heart, unstained, feed 20nm, imaged in a LVEM at 5kV.

Jana Nebesarova
Laboratory of Parasitology
Ceske Budejovice

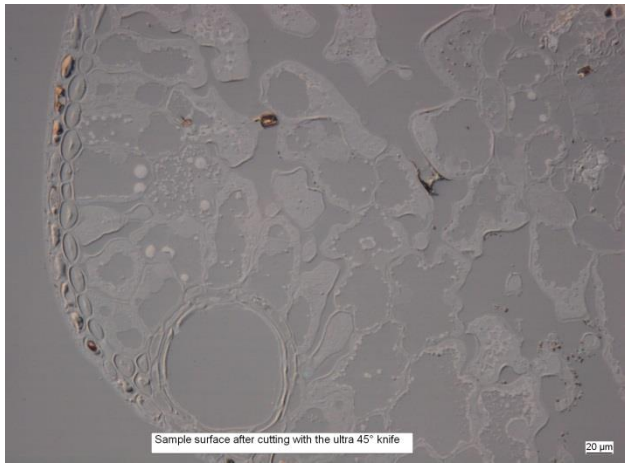
# Compression in room temperature sectioning

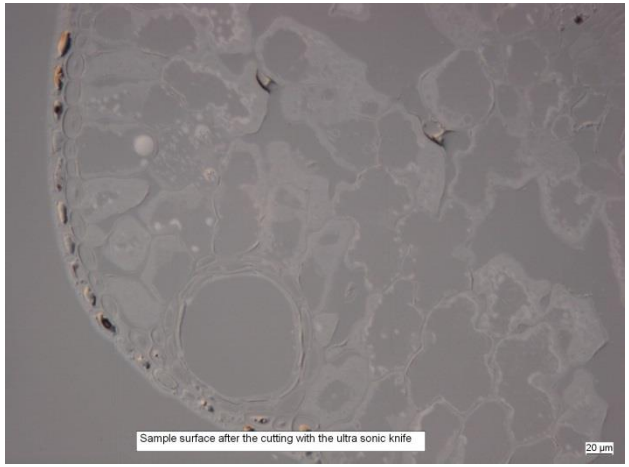


Single cultured rat myocytes,
sarkomer, feed 20nm

Ludwig Edelmann
University of Homburg

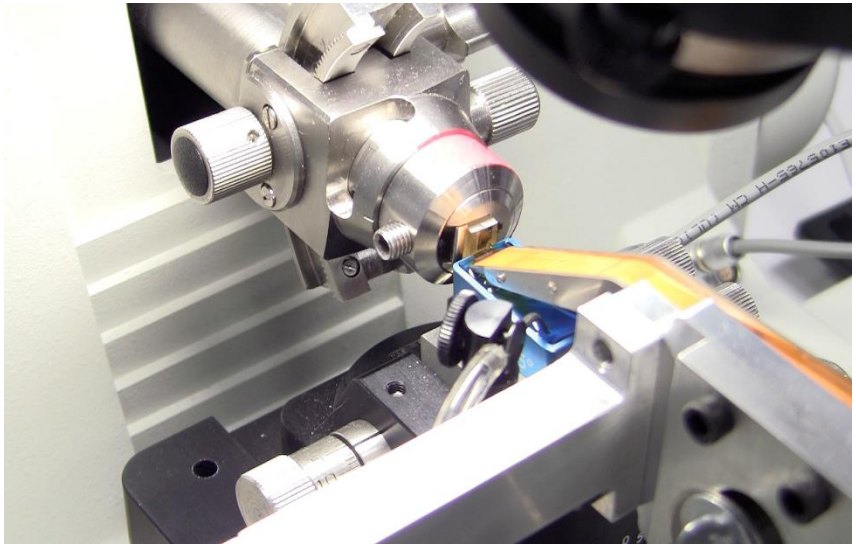
# Compression in room temperature sectioning




Sample block surface of a spruce needle after sectioning with an ultra 45° knife (above) and an ultra sonic knife (below)

# Compression in room temperature sectioning

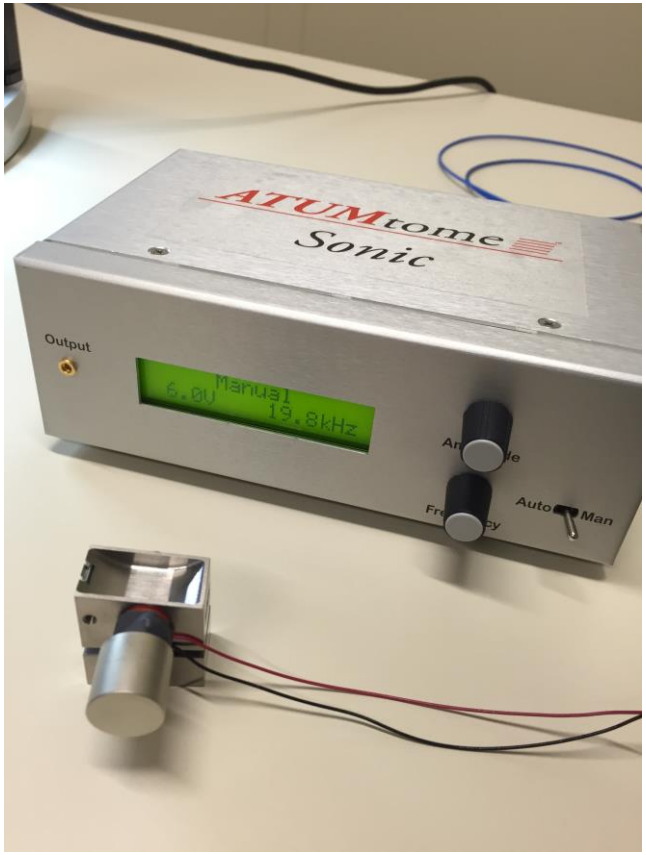


Collecting sections on a tape for imaging in the SEM

R Schalek, N Kasthuri, K Hayworth, D Berger, J Tapia, J Morgan, S Turaga, E Fagerholm, H Seung and J Lichtman

Microscopy and Microanalysis 2011

# Compression in room temperature sectioning



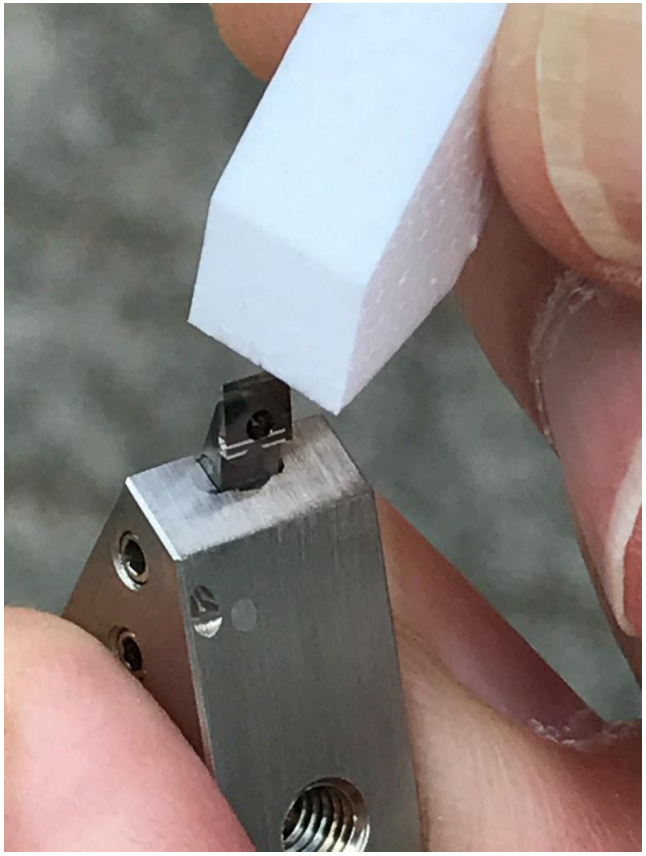

The RMC sonic for the use in the ATUMtome

# Diamond knife cleaning


Knife cleaning: use polystyrene sticks and ethanol

Use perfectly cleaned razer blades only!

# Diamond knife cleaning



Cleaning the cryo immuno knife:
Ethanol 50%, keep wet

# Diamond knife cleaning

Drying the cryo immuno with a dustblower