



EXAKT

FROM FRESH TISSUE TO THE STAINED THIN SECTION

TASK – PREPARE MULTIPLE THIN SECTIONS OF COMPLEX MATERIAL COMBINATION FOR MICROSCOPIC DETERMINATION

What you have:

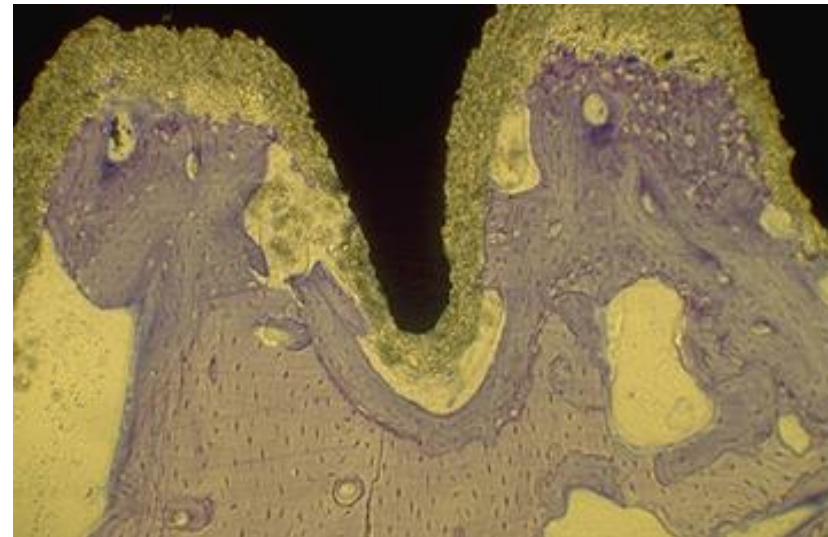
native fresh sample



e.g. jaw bone with tumour

What you need:

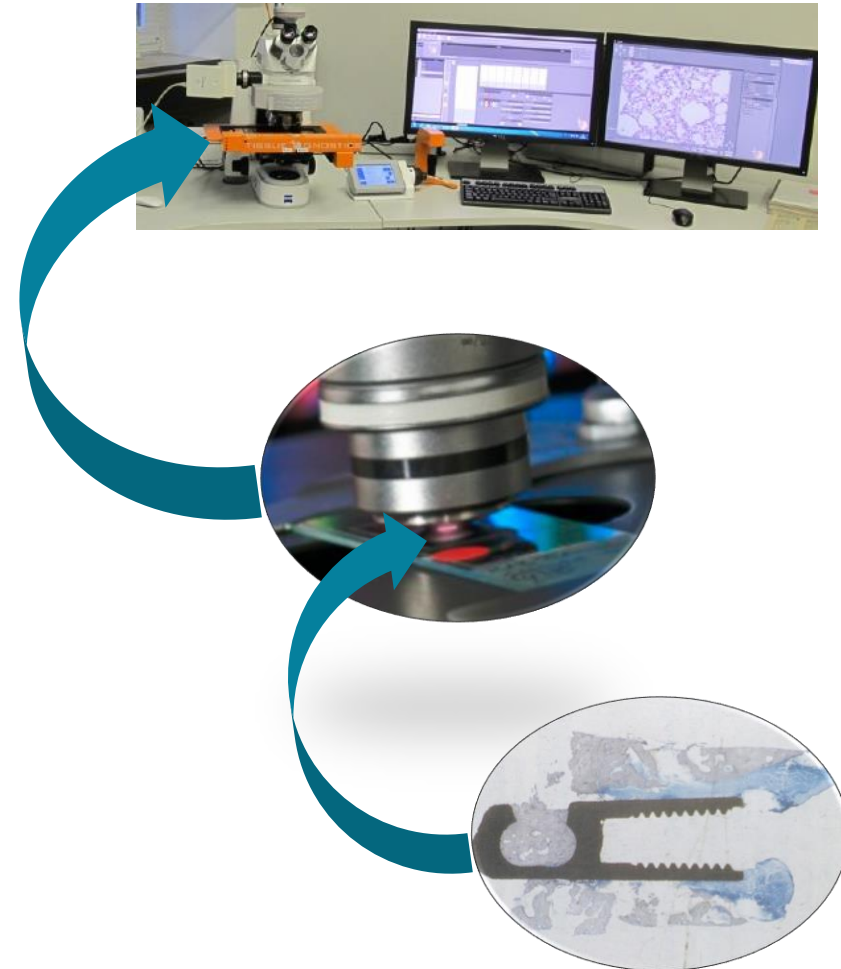
plastic embedded stained thin section
down to a thickness of 10 μ m



e.g. thin section with CoCrMo implant
with hydroxyapatite coating

REQUIREMENTS FOR SAMPLE PREPARATION AND MICROSCOPIC DETERMINATION

- › Smearing, edges or any kind of artefacts must be avoided to ensure good results at light microscopic determination
- › Section has to be precisely coplanar
- › The „real“ specimen thickness must be measurable throughout the entire process
- › Thickness down to 10 μm is possible (material depending)
- › No deformation on the finish sample
- › Minimum loss of material



THE SOLUTION: EXAKT THIN SECTION PREPARATION PROCESS

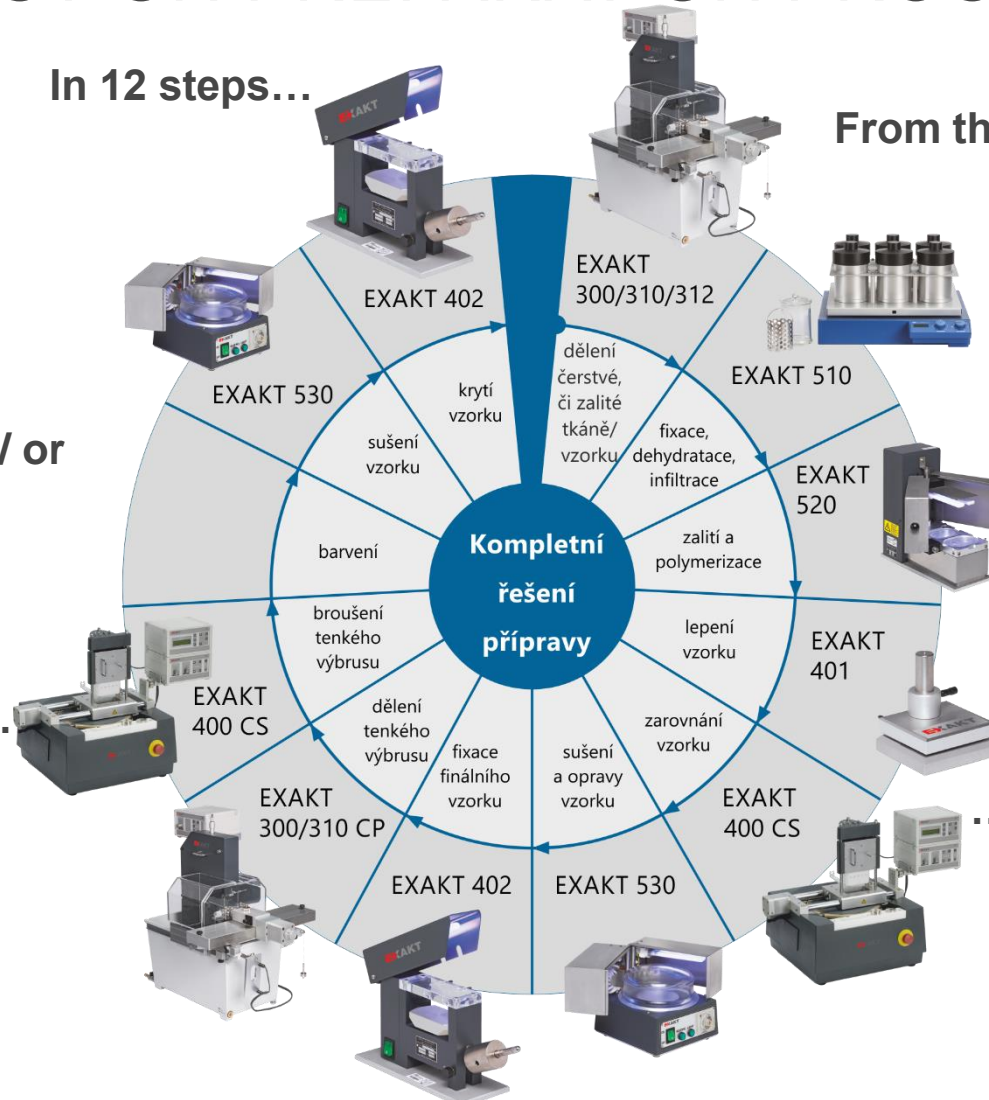
In 12 steps...

From the fresh tissue sample...

Followed by staining and / or
IHC

...to precise thin section.

...in a documented process...





THE EXAKT PROCESS 12 STEPS TO THE STAINED THIN SECTION

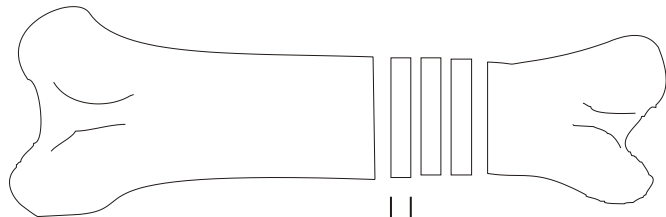
STEP 1: SECTIONING OF FRESH TISSUE

Prepare the sample:

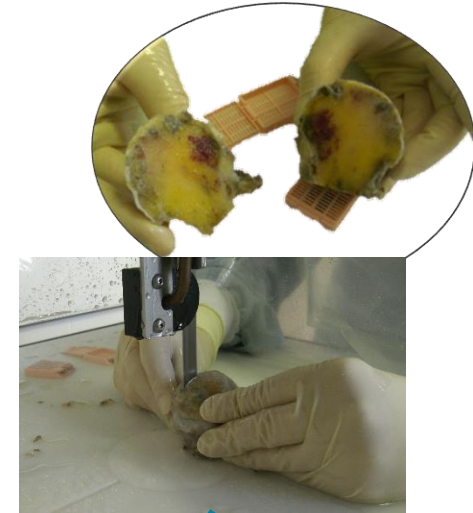
- › Preparation of slices in a thickness of ideally 1mm up to 5mm as a maximum
- › The thinner the slices, the better/faster the fixation, dehydration and Infiltration with resin in Step 2

EXAKT 300/310/312 Diamond Band Cutting Systems

- › Precise and safe cutting of fresh tissue, bones, implants, etc



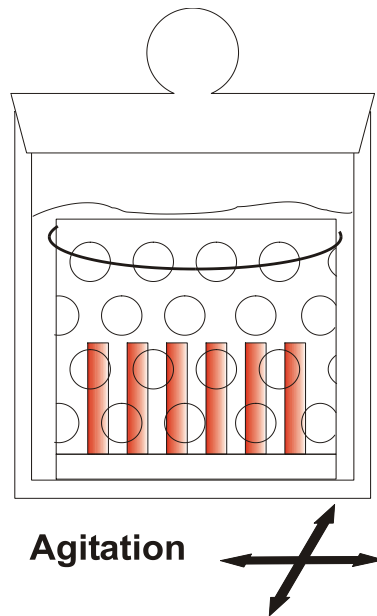
S max = 2 .. 5mm !!



STEP 2 – DEHYDRATION & INFILTRATION

The key for good infiltration:

- › biggest possible surface of the pre-sectioned specimen
- › agitation decreases infiltration time by approx. 50%



EXAKT 510

Dehydration and Infiltration System

- › 6 steps simultaneously in a decreasing serial dilution
- › Possibility of vacuum function for the finish step



Dehydration

ascending alcohol /
water dilution series:

- › 60%
- › 80%
- › 96%
- › 100%
- › 100%

STEP 2 – DEHYDRATION & INFILTRATION

Main aspects for a good sample preparation

- › Sample structure has to be preserved
- › Polymerisation
 - › Has to be sensitive
 - › Low temperature increase
 - › Homogeneously
 - › Time efficient
- › It has to be the right resin in regard to
 - › Compatibility
 - › Hard enough for the process
 - › Safe for operator handling

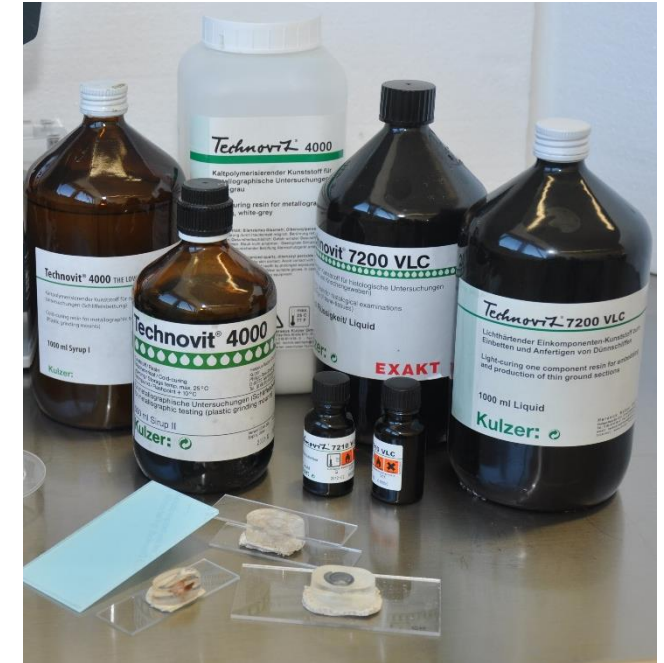
Heraeus Kuzer Technovit 7200 VLC

- › One bottle
- › No mixture process
- › Save handling

Infiltration

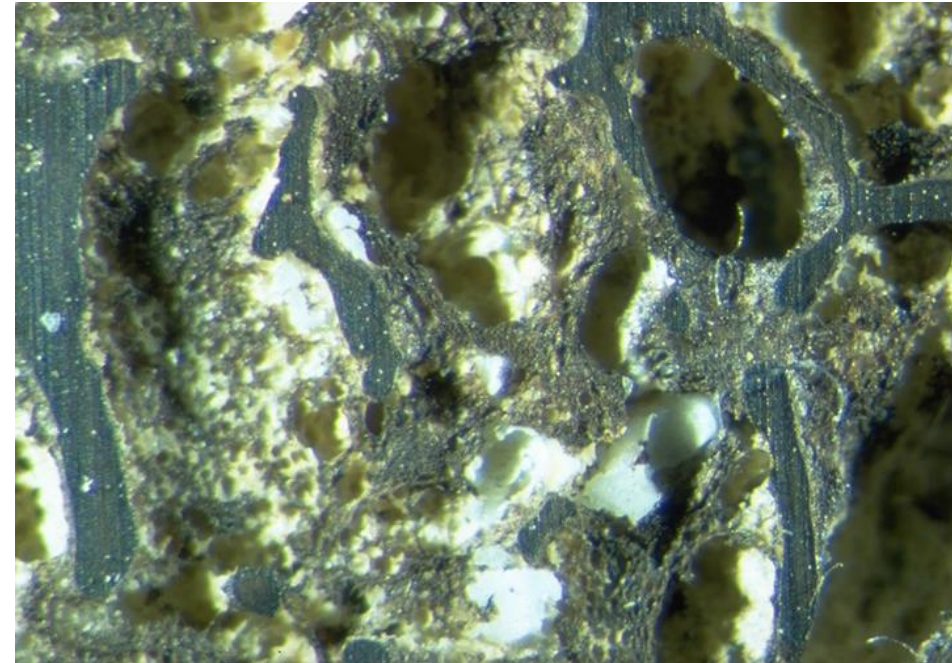
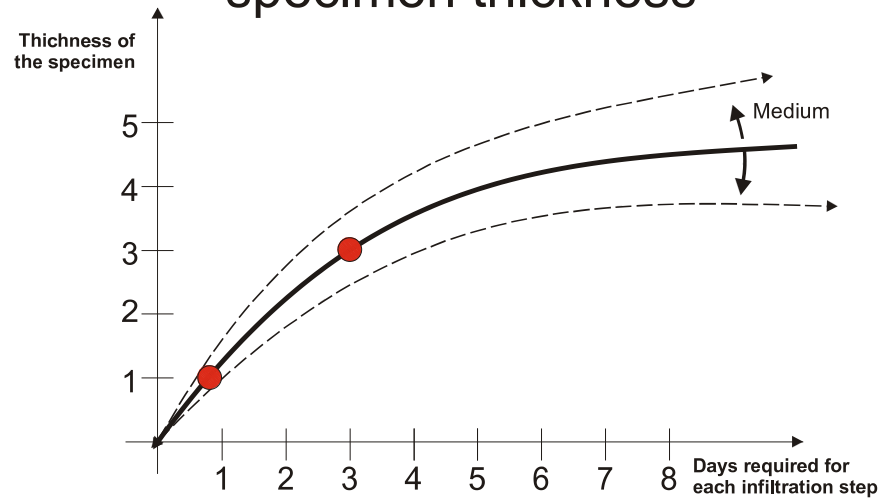
ascending resin/
alcohol dilution series :

- › 30/70%
- › 50/50%
- › 70/30%
- › 100%
- › 100%



STEP 2 – DEHYDRATION & INFILTRATION

Diffusion time
as function of
specimen thickness

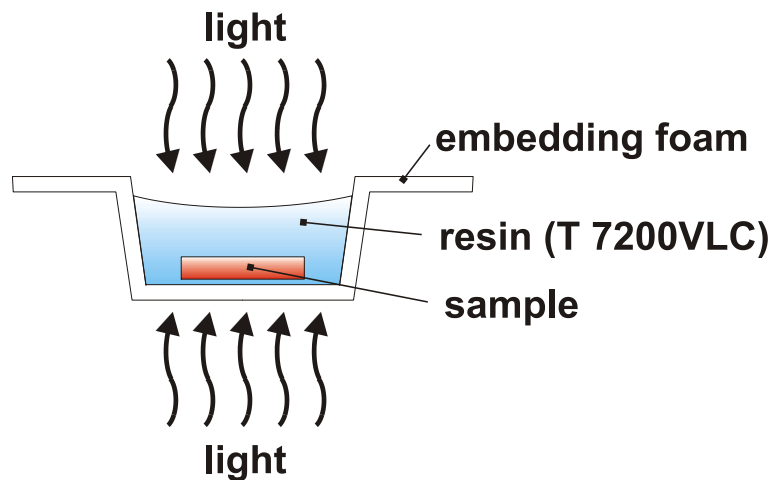


Most Errors during preparation occur because of too thick specimen and bad infiltration

STEP 3 – EMBEDDING & POLYMERIZATION

Embedding in Technovit 7200VLC

- › Controlled polymerization in 2 steps by different wave length (white / blue light)
- › Avoid polymerization artefacts like cracks or overheating
- › Ready embedded sample in just 8 hours



EXAKT 520 Light Polymerization System

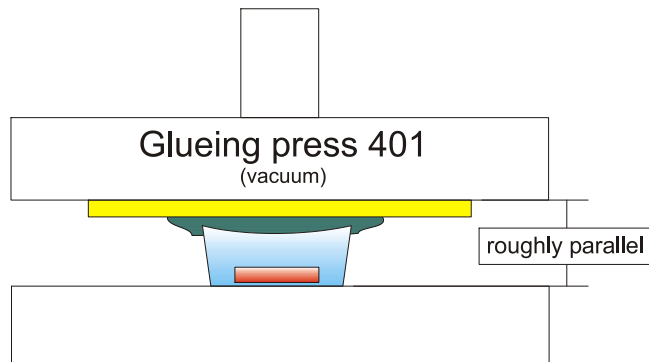
- › 4 different types of embedding moulds (shape / size)
- › water cooling feature
- › Variable adjustment of the light position






STEP 4 – SAMPLE MOUNTING

Precise & durable

- › Sample block is mounted onto the plastic slide (Slide A)
- › uneven backside of the resin block is eliminated with the filling glue



-  plastic slide (Slide A)
-  Filling glue (Technovit 4000)
-  sample block

EXAKT 401

Vacuum Adhesive Press

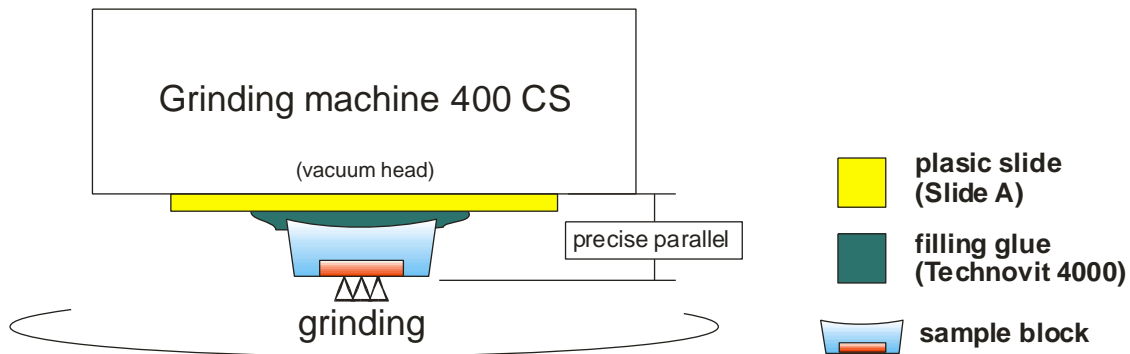
- › Mounting of small and big samples
- › Simple handling and high precision of 1µm



STEP 5 – SAMPLE FACING

Sample has to be grinded to:

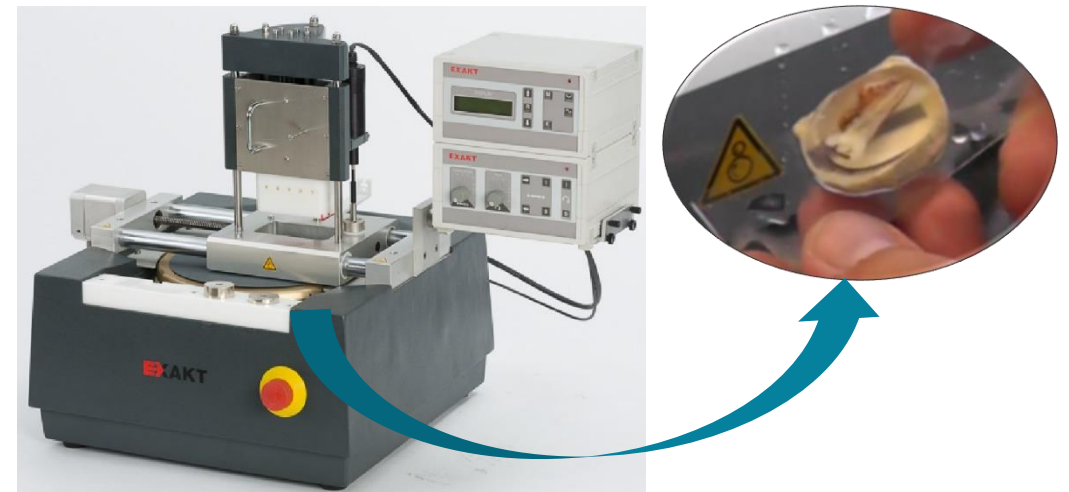
- › Remove embedding plastic to open sample
- › Produce a very even sample surface coplanar to the slide
- › Trim the surface of kindlings



EXAKT 400 CS

Micro Grinding System

- › Even surface patterns by oscillation
- › Grinding accuracy μ -precise
- › Vacuum fixation of slides
- › Precise grinding method in tolerances $< 5\mu\text{m}$



STEP 6 – DRYING OR REPAIR

Drying, heating and vacuum :

- › Process sample quicker after grinding
- › Stability of difficult application samples
- › Filling material for embedding
- › Repair sample in case of embedded air bubbles and failures

EXAKT 530

Block Drying & Post Infiltration

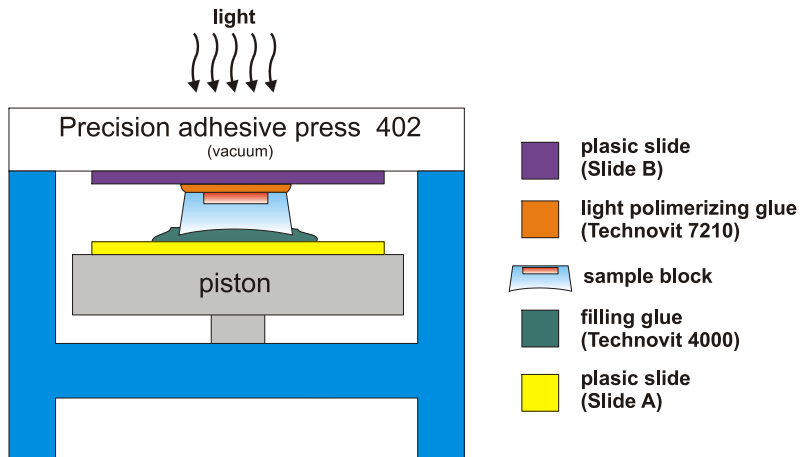
- › Blue light for polymerisation
- › Heating plate of 40°C
- › Vacuum option
- › Flexible handling of different specialities



STEP 7 – MOUNTING THE FINAL SLIDE

Reproducible Precision

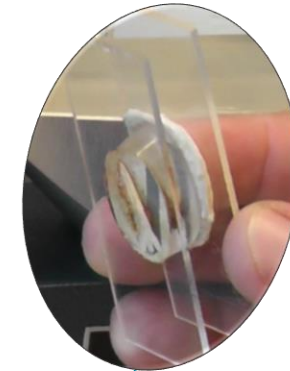
- › Both Slides must be as coplanar as possible
- › Avoid air bubbles to get maximum adhesion
- › Calculation of the adhesive layer by measuring the different pieces with micrometre



EXAKT 402

Precision Adhesive Press

- › Transparent Perspex vacuum block
- › Blue light for polymerisation
- › Adjustable weight
- › Precise up to 1µm

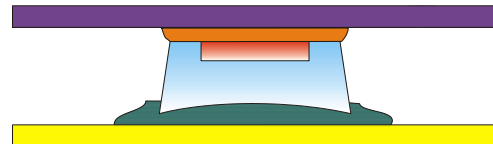


STEP 7 – MOUNTING THE FINAL SLIDE

Calculation of the glue layer

- › The correct thickness of the “sample”
- › The thinner the glue layer the merrier the focus on the microscope
- › Low risk to los the sample in the next steps
- › No shrinking of the glue
- › Slow polymerisation for correction of the sample
- › **Heraeus Kulzer Technovit 7210**

Calculation of the glue layer

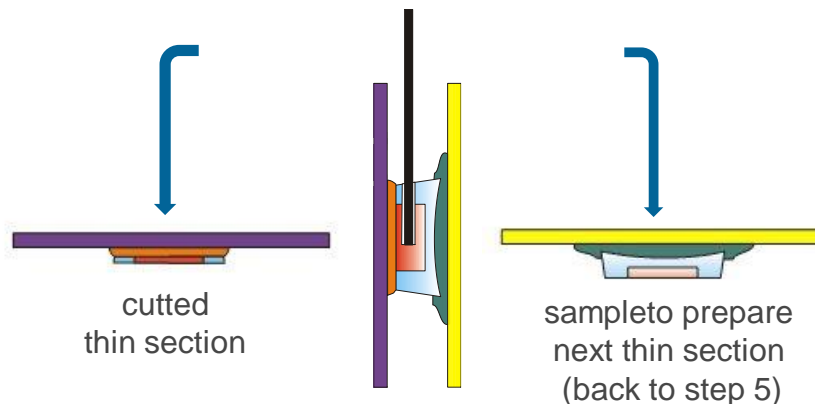


$$\begin{array}{r} 8020\mu\text{m} \\ - 1500\mu\text{m} \\ - 6500\mu\text{m} \\ \hline = 20\mu\text{m} \text{ Glue layer} \end{array}$$

STEP 8 – CUTTING THE THIN SECTION

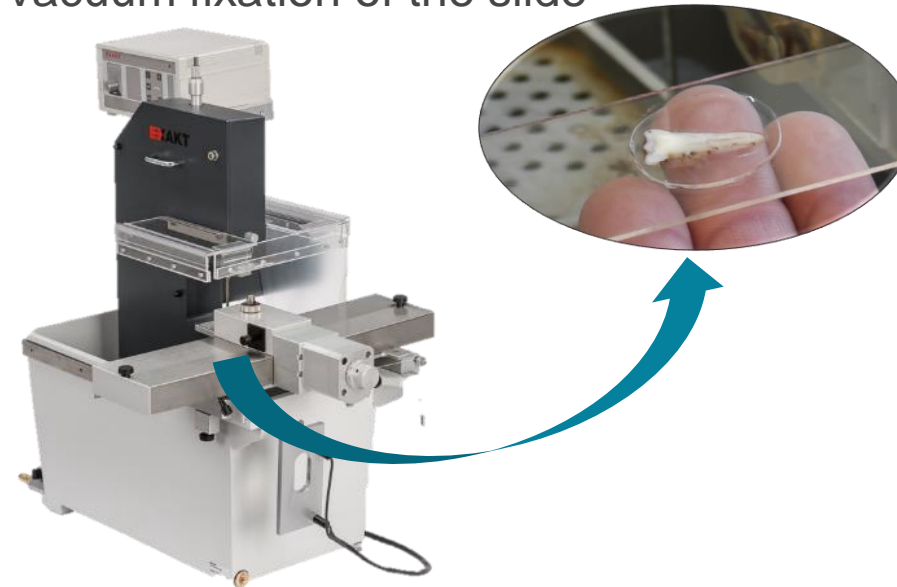
Cutting the thin section

- › A thin section as thin as below 100 µm can be cut (sample material depending)
- › Low stress for the embedded sample
- › Minimum material loss
- › The remaining section (embedded block sample) will be re-used to gain the next thin section



EXAKT 300/310 CP (Contact Point) Diamond Band Saw

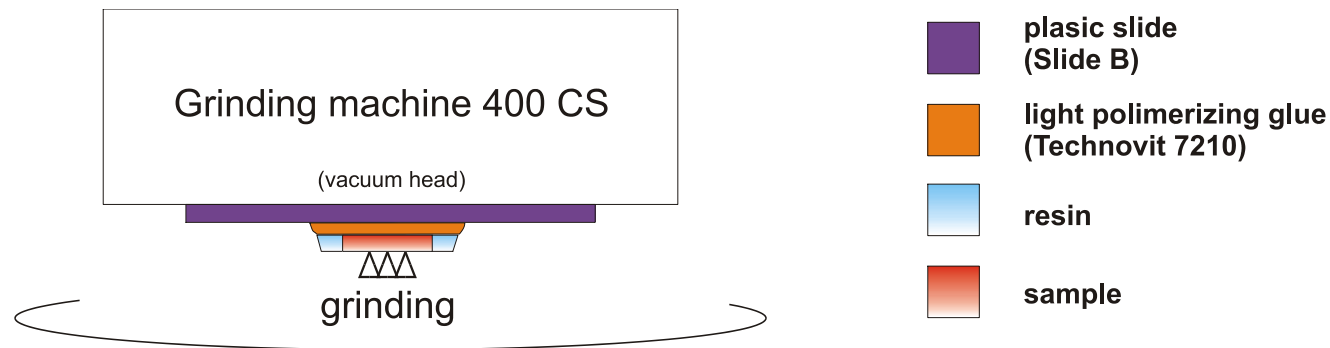
- › EXAKT Parallel Control System and micrometre measuring
- › Even and smooth surface
- › Perfect sample and diamond band cooling
- › Vacuum fixation of the slide



STEP 9 – GRINDING THE THIN SECTION

Perfect finishing

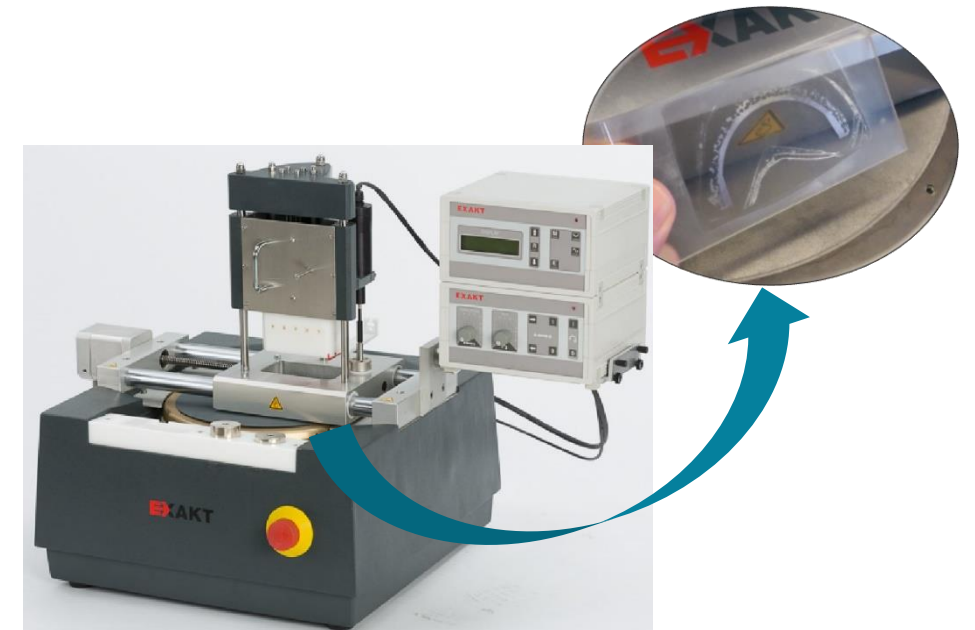
- › Sample is grinded to the final thickness
- › Low material stress
- › No scratches on the surface
- › Pre-grinding to the polishing with different grinding/polishing papers
- › The precision of the entire system is designed to prepare a thin section down to 10 µm



EXAKT 400 CS

Micro Grinding System

- › Diamond coated plate for ceramic samples
- › Grinding paper fixation by water adhesion
- › Use of plastic slides in different sizes



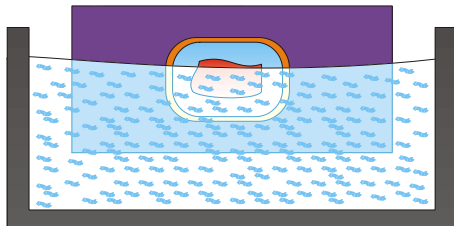
STEP 10 – STAINING / DRYING / COVER SLIPPING

Staining Process

- › Etch slightly (samples embedded in Technovit 7200)
- › Different staining methods can be processed
- › Make sure the staining method is qualified for Technovit 7200

Cover slipping

- › Possible with Technovit 7200VLC and coverslip glass



Staining Workstation

e.g. Leica



Drying and Cover slipping



EXAKT 530
Blockdrying and
Infiltration device



EXAKT 402
Precision Adhesive Press

EXAMPLES FOR STAINED THIN SECTIONS

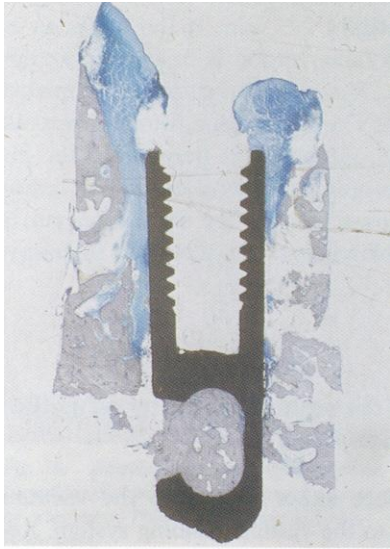


Fig. 22: IMZ-Implant with deep pocket formation and direct bone contact in the lower hind. (Thin section. Toluidine blue stain.)

Toluidine blue

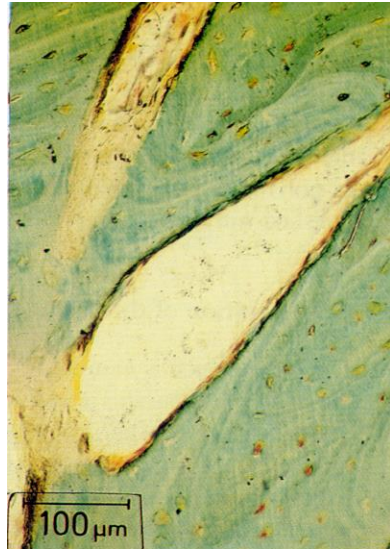


Fig. 24: Peri-implanted bone with infiltration of corrosion products in the cytoplasm of the osteocyte and within the area of mineralization. Thin section. Masson-Goldner stain.

Masson-Goldner



Fig. 35: Edentulous mandible (red) and overlapping alveolar mucosa. Thin section, Van Gieson stain.

Van Gieson



Fig. 31: Interdental region with horizontal section of teeth. The calculus on the teeth extends subgingivally. Thin section, Giemsa stain.

Giemsa

EXAKT



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