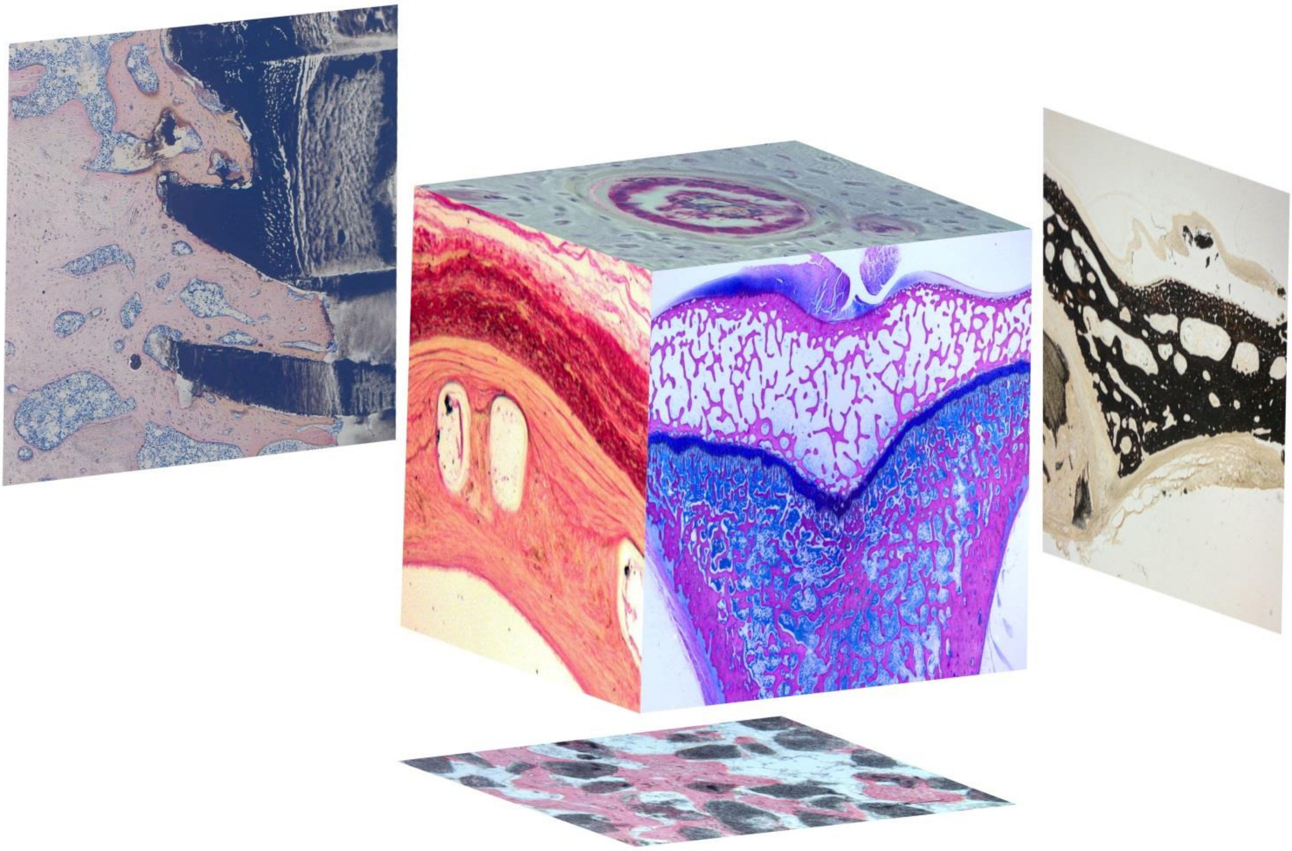
LLS ROWIAK LaserLabSolutions

TissueSurgeon

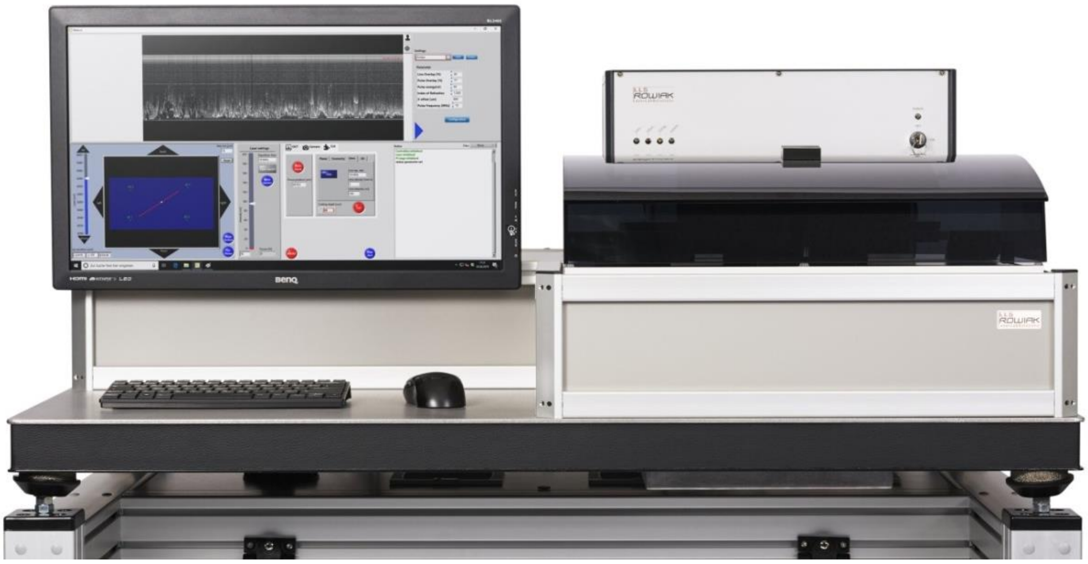
Image Guided Laser Microtome



Histology and more: Laser-Based Preparation of Biological Tissue and Materials

Laser-Based Imaging, Navigation and Sectioning

The laser microtome TissueSurgeon is a multi-purpose sectioning instrument, which enables precise and contact free cutting of biological samples and a broad range of (bio-) materials. Based on femtosecond laser technology, it can be used for sectioning, structuring or gentle extraction of samples and materials in 2D and 3D for analysis. Fundamental limits of mechanical tissue preparation can be overcome for cutting of hard tissue, implanted tissue or difficult to cut materials, such as adipose tissue, ceramic scaffolds or polymers. The TissueSurgeon surmounts major limitations of common methods like mechanical microtomy, ground sectioning or laser-microdissection (LMD). In addition, in combination with imaging modules the TissueSurgeon does not only allow for guided two-dimensional sectioning but also for three-dimensional cutting. This provides great flexibility and enables novel approaches.

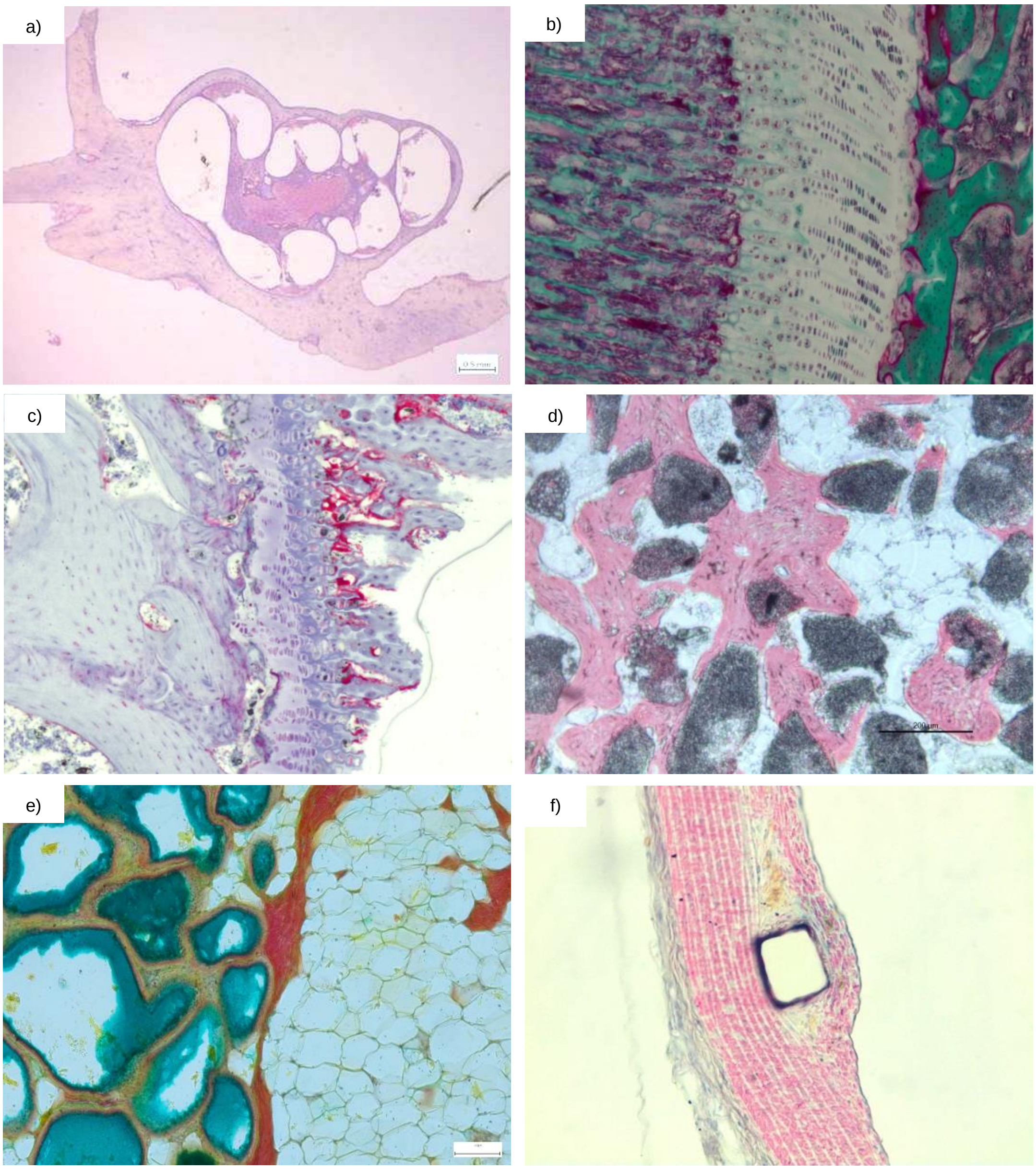


Benefits

- Nearly serial sections of non-decalcified hard tissue (e.g. bone) with minimal loss of material
- Histology of hard and soft tissue, even delicate samples (e. g. cochlea, adipose tissue)
- Sectioning of biomaterials for tissue engineering (e.g. scaffolds, teflon, hydrogels)
- Implant-tissue interface histology (e.g. dental screws, cardiovascular stents, scaffolds)
- Contact free laser cutting of tissue avoids artefacts like compressions, scratches or cracks
- Gentle isolation of site-specific samples by image guided 3D-sectioning (e. g. along the implant-tissue interface of dental screws) for biochemical analysis
- 3D-microstructuring of tissues, matrices and materials

Laser Microtomy – Fields of Application

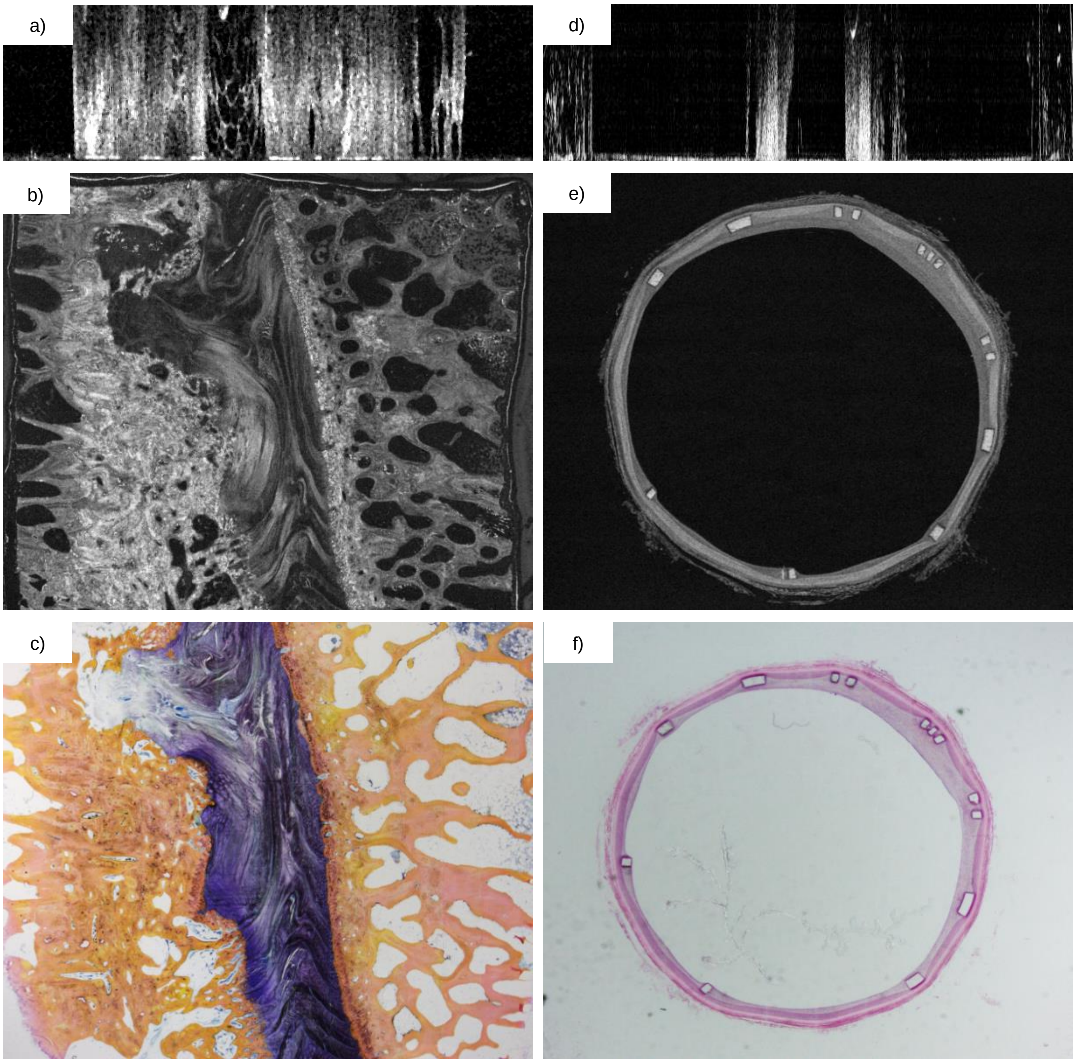
- Orthopedics/ Trauma and Osteology
- Oro-facial and Dental Medicine
- Oto-laryngealogy and Audiology
- Cardiology and Cardiovascular Research
- Regenerative Medicine and Tissue Engineering
- Soft tissue difficult to cut
- Preclinical studies from small to large animal models
- ...



Examples of histological, histochemical and immunohistochemical stainings from resin embedded laser microtome sections. a) Brightfield image of guinea pig cochlea with cochlea implant with central wire (longitudinal section) Haematoxylin & Eosin staining (H&E) b) Masson Goldner Trichrome staining (MG) of rat knee growth plate c) Tartrate-Resistant Acid Phosphatase (TRAP) staining of rat femur d) Sandersons Rapid Bone Stain/van Gieson (SRS/VG) of rat femur with tricalcium-phosphate scaffold a-d) sections of undecalcified bone e) Movat's pentachrome (MP) staining of pig belly adipose tissue with milk gland f) Immunohistochemistry of Smooth Muscle Actin (SMA) of rat stented vessel.

Guided Cutting by Optical Coherence Tomography

For navigation and imaging of samples or quality control of cutting, the TissueSurgeon is equipped with Optical Coherence Tomography (OCT). This provides a unique combination of two- and three-dimensional cutting and imaging, facilitating dissection and analysis of samples. Beyond, simple Multiphoton Microscopy (MPM) is an option to be integrated into the laser microtome for deeper tissue imaging.



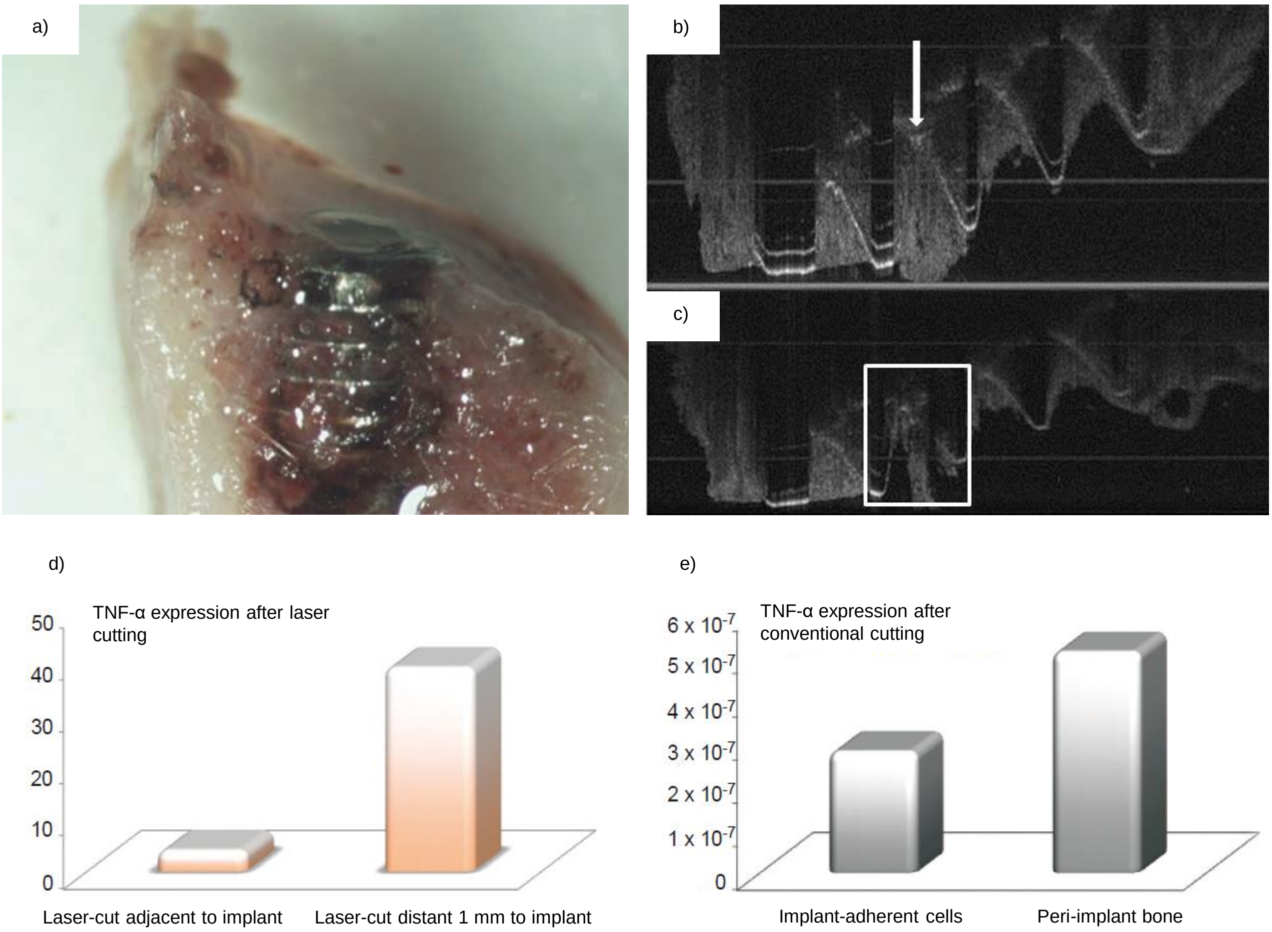
Examples of Guided Cutting by Optical Coherence Tomography a) OCT lateral view bone for quality control before cutting b) OCT 2D reconstruction bone c) Sanderson Rapid Bone staining (SRS) after image-guided cutting by OCT d) OCT lateral view stented vessel for quality control before cutting e) OCT 2D reconstruction stented vessel f) Haematoxylin & Eosin staining (H&E) after image-guided cutting by OCT, all samples are MMA-embedded

Benefits

- Image-guided cutting allows for defined 2D-cutting and quality control
- Measurement of sample dimensions and layer thickness
- Differentiation of tissues and structures for guided cutting
- Structural information with resolution of approx. 10µm (OCT) up to approx. 1µm (MPM)
- Image guided 3D sample extraction of native hard and soft tissue allows biochemical analysis of fresh tissue

3D-Cutting for site specific tissue preparation

Beyond laser-based sample preparation for histology the laser microtome enables 3D-cutting, a new way of sample collection, for e.g. biochemical analysis. The integrated optical coherence tomography allows identification of regions of interest in tissue samples and optimal positioning for cutting and isolation. This opens new dimensions of analysis in different fields such as cell biology, molecular biology, biomechanics or biochemical tissue analysis in regenerative medicine.

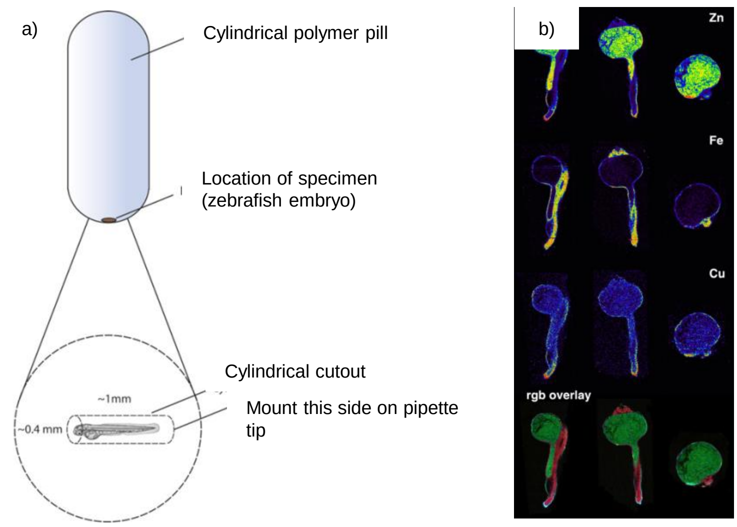


3D-sample collection for molecular analysis along implant-tissue interface. a) A titanium screw was implanted into a rat femur. After 21 days of bone formation, rat tibiae were explanted. With a dental saw a cut along the implant was performed to get a plain surface. b) Covered with RNAlater, areas of interest at the tissue-implant interface and from the surrounding cortical bone were located via OCT (note the new formed bone formed (arrow)). c) A cuboid shape was defined around new formed bone and cut out afterwards with the laser microtome. Samples were processed for further of RNA analysis. d) Relative TNF-α RNA-expression in samples cut with the laser microtome and e) extracted mechanically.

Omar, O. Lenneras, M. Richter, H. Thomsen, P.: (2012) Laser microtome for site-specific sectioning of the interface and sub-sequent qPCR analysis (4th International Symposium Interface Biology of Implants, Poster Contribution).

Customized specimen preparation

The TissueSurgeon is a versatile instrument for life and material sciences and allows for customized sample preparation. Applications beyond histology can be the contact free sectioning of material samples from polymers for i.e. spectrographic analysis. Contamination-free trimming of sample blocks for analysis i.e. in a synchrotron or cutting of channels into polymers (lab on a chip) are among further applications.



Contamination free reduction of polymer block around zebrafish embryo for metal analysis by synchroton based X-ray Fluorescence Microtomography a) Scheme of sample preparation b) Metal ion mapping with a synchroton microprobe (Bourassa D, Gleber S-C, Vogt S, Yi H, Will F, Richter H, Shin C H,. Fahrni C J (2014) 3D Imaging of Transition Metals in the Zebrafish Embryo by X-ray Fluorescence Microtomography. Metallomics 2014, 6, 1648-1655)

Principle of Laser Microtomy

In contrast to mechanical microtomy laser microtomy is a contact free cutting method for preparation of tissue sections. Main component of the TissueSurgeon is a femtosecond laser, tightly focused into the specimen by a high-numerical aperture objective. The high intensities inside the focal volume lead to nonlinear absorption processes and finally to the disruption of the illuminated material. This effect is limited to the focal diameter of the laser pulse of approx. 1 - 5 µm. The whole area of the sample is scanned by the pulsed laser to perform the cutting process with no damage of the surrounding tissue. Depending on the material being processed, section thicknesses from approximate 10 - 100 µm are feasible. The method is not only suited to prepare thin sections but also 3D-sections.

Cutting Conditions

Cutting Thickness: approx. 10 – 100 µm
Cutting Speed: approx. 1 mm² /s
Sample Size: max. 40 x 40 mm