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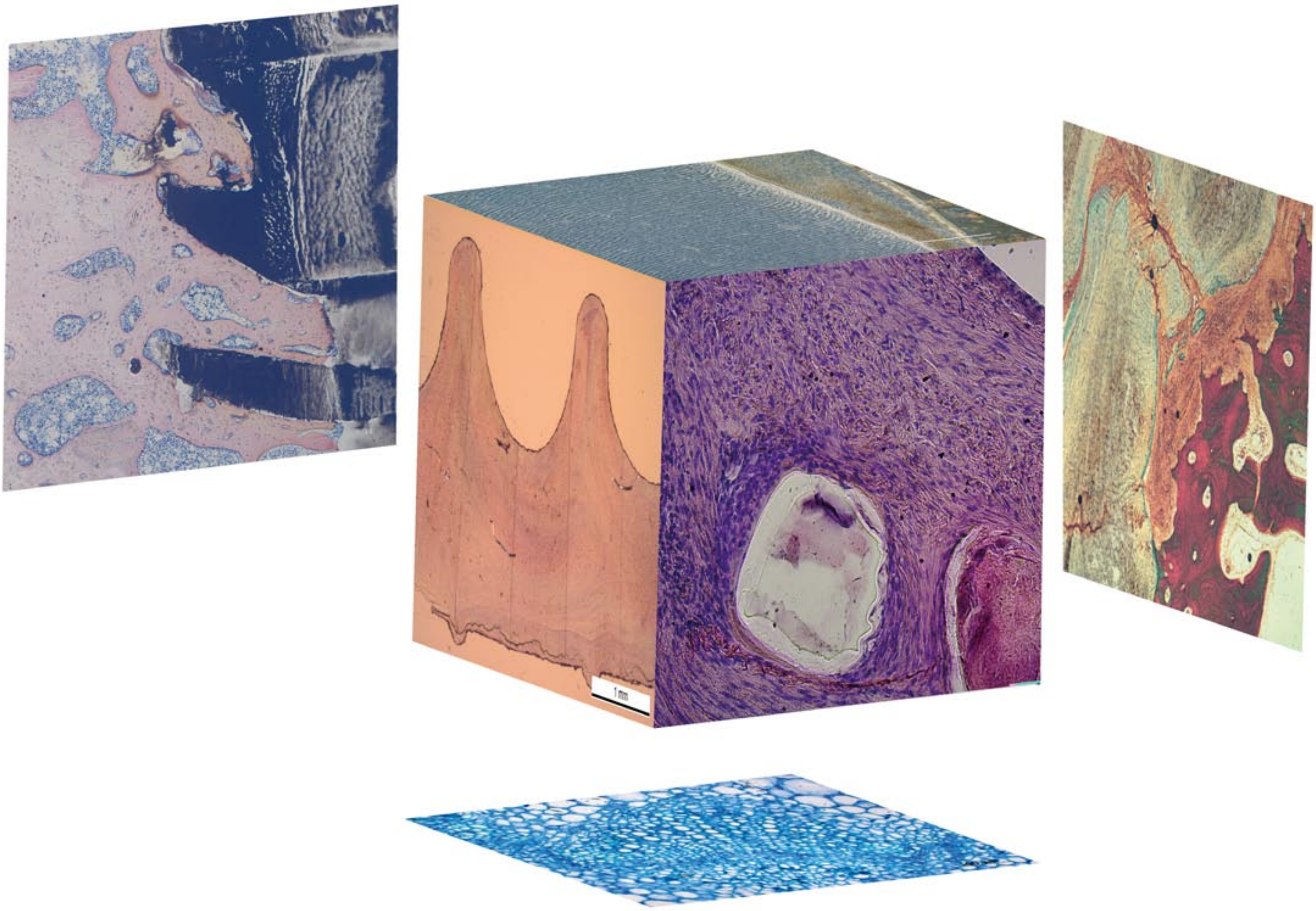
LaserLabSolutions

TissueSurgeon

Cut In Three Dimensions



More than a Microtome: Laser-Based Preparation of Biological Tissue and Materials

Laser-Based Imaging, Navigation and Sectioning

TissueSurgeon is a multi-purpose system designed for sectioning, micro-structuring and imaging of biological tissue and various non-metal materials, such as ceramics, polymers and resins. The TissueSurgeon overcomes the limitations of common methods like microtomy, ground sections 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.

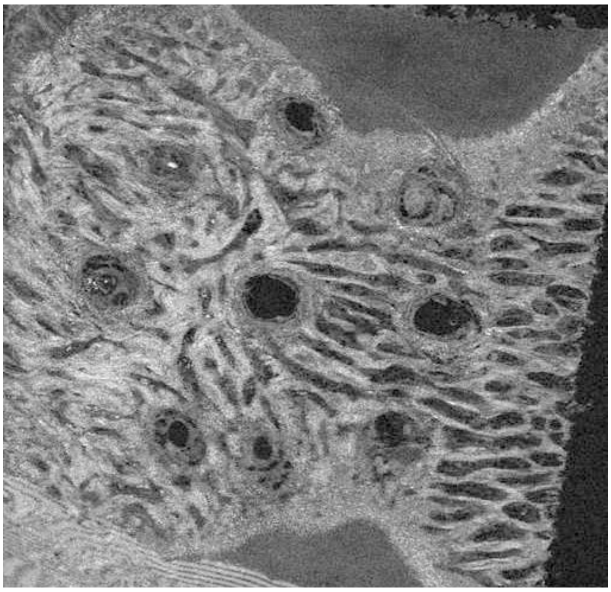
TissueSurgeon Saves Time, Material and Manpower

- Contact free cutting of tissue avoids artefacts like compressions, scratches or cracks
- No more decalcification: sectioning of calcified hard tissue
- No more ground sections: serial sections of non-decalcified hard tissue and tissue with implants (i.e. medical devices in cardiovascular research) with minimum loss of material
- Image-guided cutting allows for defined 2D-cutting and quality control
- Image guided 3D sample extraction of native hard and soft tissue allows biochemical analysis of native tissue

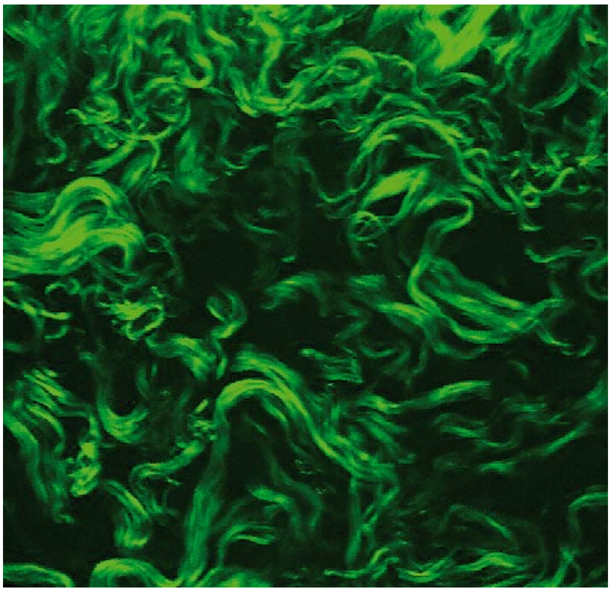
Navigation and Imaging Modules

TissueSurgeon is optionally equipped with different imaging and navigation modules: **Optical Coherence Tomography (OCT) and Multiphoton Microscopy (MPM)**. Laser microtomy with integrated imaging enables a unique combination of 3D imaging and cutting, facilitating analysis and dissection of samples. Both imaging modules can be used for navigation and full control of cutting process by defining shapes, assessment of cutting quality or measurements of distances.

OCT uses an infrared light source which creates an image out of differences in the index of refraction, whereas the MPM generates contrast by (auto-) fluorescence, SHG or THG. Resolution of OCT is about 10µm which allows distinguishing between different tissues, whereas the resolution of MPM (1 µm) allows imaging on a cellular level.



OCT of human bone



MPM imaging in SHG mode of aorta

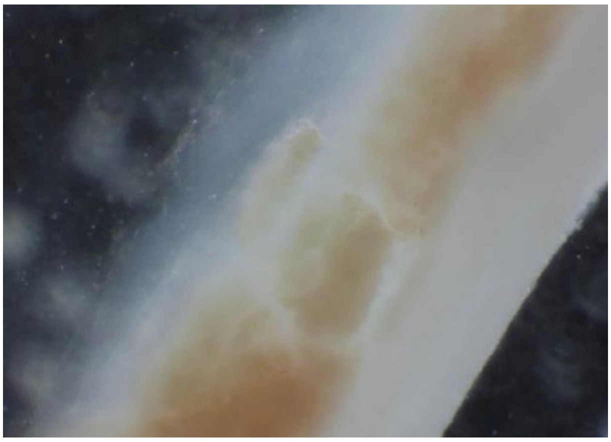
Benefits

- Imaging into the depth of the sample
- 2D-imaging and monitoring of thin sections
- 3D-imaging of cutting shapes
- Measurement of sample dimensions
- Structural information with resolution of 10 µm (OCT) up to 1µm (MPM)
- Differentiation of tissues and structures for guided cutting
- Imaging of fluorescence labelled tissue
- Imaging without staining by using autofluorescence e.g. SHG or THG

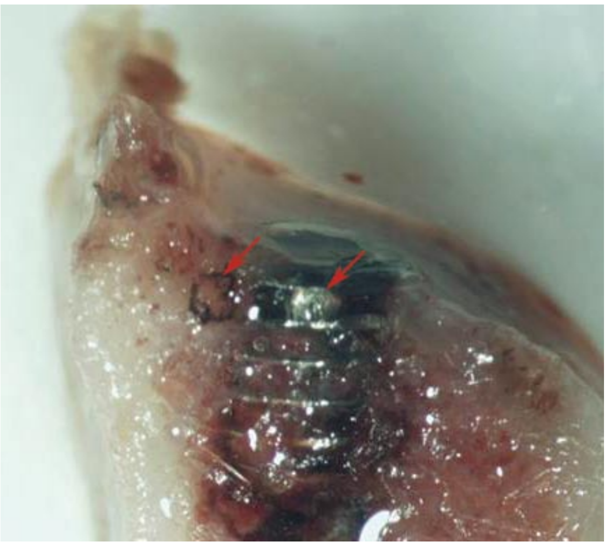
3D Cutting

The site-specific analysis of areas inside a sample requires a very exact method of extraction. At present the collection of samples from a tissue for biochemical analysis is performed by cutting thin sections of embedded or frozen samples with a microtome. Out of these sections, the area of interest is isolated by Laser Microdissection.

However, this method suffers from taking considerable time to collect enough material, and harsh chemical treatment impairing analytical results. Recent publications show that this method does not really work for hard tissue. Especially if implants are involved, it is nearly impossible to cut the sample with a knife.



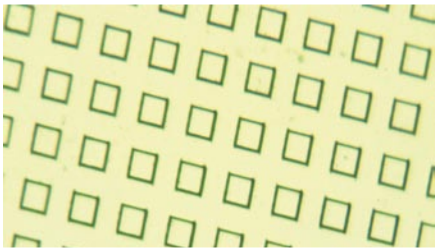
3D section of bone marrow in rat femur



Sample collection along medical implant out of rat femur (arrows). Omar O. Lenneras, M. Richter, H. Thomsen P.: (2012) Laser microtome for site-specific sectioning of the interface and subsequent qPCR analysis. 4th International Symposium Interface Biology of implants (Poster Contribution).

TissueSurgeon enables a new way of sample collection for biochemical analysis. Not only does it allow for two-dimensional sectioning for histology but also for three-dimensional cutting and cell isolation. In a single-step procedure the area of interest in a fresh tissue sample can be identified by the imaging capabilities of TissueSurgeon and then cut with the laser. Thus, the system offers a new approach to collect cell material out of fresh, even calcified tissue by cutting a 3D-shape around the area of interest. This method is fast and works without chemicals harming the tissue. Biochemical analysis is much more efficient and specific as comparison to common methods show. Preservation of e.g. RNA can be supported by cutting the sample in RNA-Later® solution.

Further applications can for example be the contact free extraction of material samples from polymers for i.e. spectrographic analysis or the contamination-free trimming of sample blocks for analysis i.e. in a synchrotron or cutting of channels into polymers (lab on a chip). The TissueSurgeon is a versatile instrument for life and material science.



Precise micro structured grid cut out of polymer block

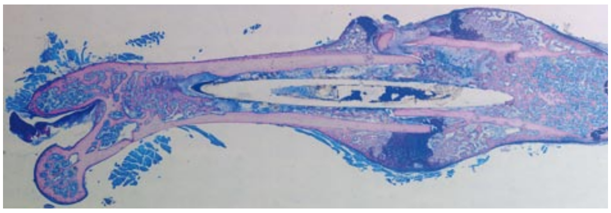
Contact and contamination free excision of Zebrafish embryo from resin block for Transition Metal Mapping with a synchroton microprobe (Bourassa D, Gleber S-C, Vogt S, Yi H, Will F, Richter H, Shin C H,. Fahrni C J (2014) **3D Imaging of Transition Metals in the Zebrafish Embryo by X-ray Fluorescence Microtomography**. Metallomics 2014, 6, 1648-1655)

Applications at a Glance

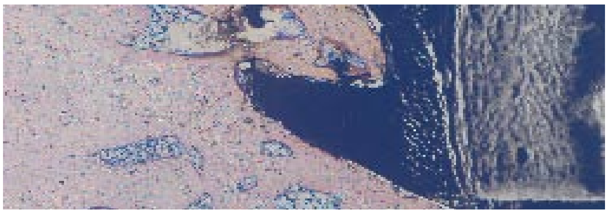
- Histology of soft tissue, even delicate samples (e. g. brain)
- Histology of non-decalcified hard tissue
- Serial sections of hard tissue for histology
- Sectioning of fluorescence labeled tissue
- Implant-tissue interface histology by serial sections (e.g. dental screws, cardiovascular stents)
- Gentle isolation of site-specific samples with 3D-sections (e. g. along the implant-tissue-interface dental screws, skin implants)
- Preparation of contamination and contact-free samples for (bio-)chemical analysis
- Sections of thick tissue samples for bio-mechanical tests and electrophysiological parameters (e.g. heart valves, brain, bone)
- Cutting of biomaterials for tissue engineering (e.g. scaffolds, teflon, hydrogels)
- 3D-microstructuring of tissues, matrices and materials
- Image-guided cutting by OCT or MPM
- 3D-imaging via OCT and MPM

This list gives an excerpt of potential applications. Has tissue and material processing with your conventional methods been in vain? Microtomy with ultrafast laser may be your solution.

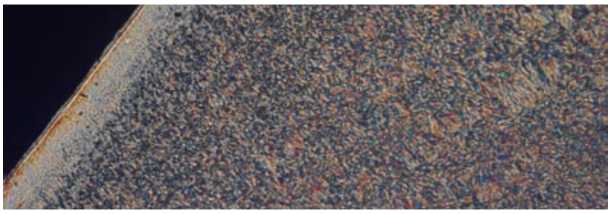
Think about the impossible. Challenge us!



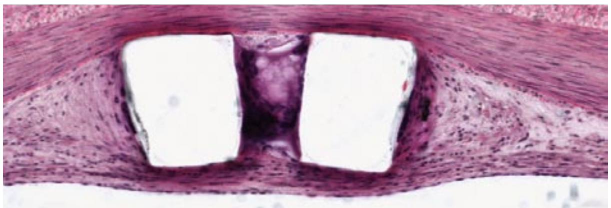
Mouse femur with fracture (10 µm). Healing was supported by intramedullary pin. Sandersons Rapid Bone Stain and van Gieson



Cortical bone of rabbit femur with dental screw (10 µm), Sanderson Rapid Bone Staining and van Gieson staining



Polyoxymethylene (POM, 10 µm) no embedding



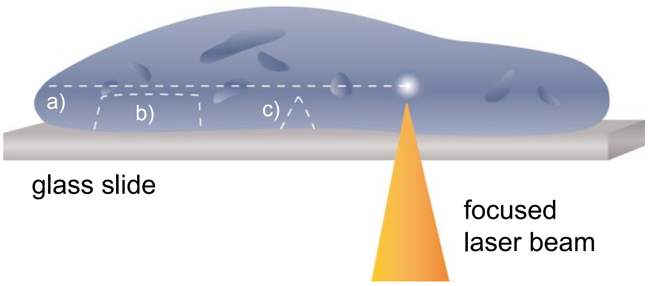
Artery containing Nitinol stents (10 µm), Hematoxylin & Eosin-Image is courtesy of Med Institute

Principle of the TissueSurgeon

Cutting-Edge Laser Technology

Main component of TissueSurgeon is a near infrared femtosecond laser. To perform a cut, the laser beam is tightly focused into the specimen by a high numerical aperture objective. Very high photon density inside the laser focus induces non-linear optical processes, which lead to material separation. This effect is limited to the very small focal volume, thus allowing cuts with micrometer precision.

To prepare tissue sections the laser beam and the specimen are moved simultaneously - the laser beam by a fast scanner and the specimen by a three-axis piezo-driven positioning stage. Depending on the material being processed, slice thicknesses from 10 µm to 100 µm are feasible. The method is not only suited to prepare thin slices but 3D-sections as well.



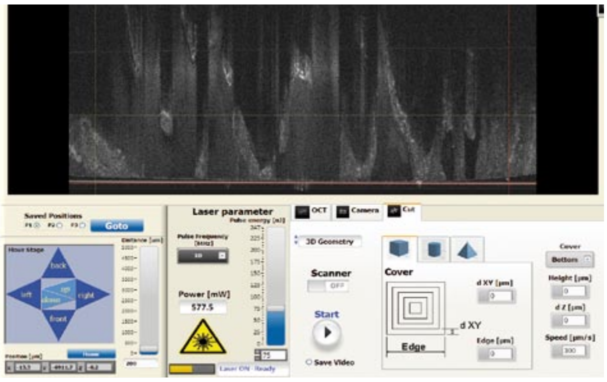
Principle of TissueSurgeon: The focused laser beam penetrates the tissue through a glass slide and performs (a) planar, or (b) and (c) threedimensional shaped cuts

Software User Interface

The TissueSurgeon software provides an easy-to-use, intuitive Graphical User Interface (GUI). The software includes functions for sectioning, three-dimensional cutting and data storage. It also includes distance measurement tools and different imaging features (OCT and MPM). Sample positioning, self-adjustment of the system and the cutting process itself are monitored by an integrated ccd-camera. The software is capable of generating 3D-OCT images. That simplifies analyzing the internal structure of samples, and assessing the quality of three-dimensional cuts for the user.

Benefits

- Intuitive graphical user interface
- Online control of the cutting process
- Automated self-adjustment of system
- Functions for sectioning and 3D-cutting
- Distance measurement tools
- Catalogue of cutting parameters for a list of tissue and material types
- Easy-to-use and self-explaining set up of parameters



Screenshot of TissueSurgeon Software Graphical User Interface

TissueSurgeon Specifications - Technical Data

Features	System No. 1	System No. 2	System No. 3
Soft Tissue	o	o	✓
Bone	✓	✓	✓
Teeth	×	×	✓
Calcification in other tissues (arteries, heart valves)	×	o	✓
Stented Vessel	✓	✓	✓
Polymers	o	o	✓
3D Cutting	×	×	✓

Imaging Modules	System No. 1	System No. 2	System No. 3
OCT	✓	✓	✓
MPM	✓	✓	✓

Key:
✓ (Yes)
× (No)
o (limited numbers of application tested)

Technical Information

Cutting Depth	Minimum 10 µm, Maximum 70 - 100 µm (depending on sample)
Cutting Dimension (x/y)	Sample size of 40 x 40 mm
Cutting Time	Average cutting speed of 1mm^2/s

LLS ROWIAK LaserLabSolutions offers customized solutions and its expertise in application development.

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