

TissueSurgeon

Hard Tissue Applications



Laser Based Histology

Hard Tissue Sectioning with TissueSurgeon

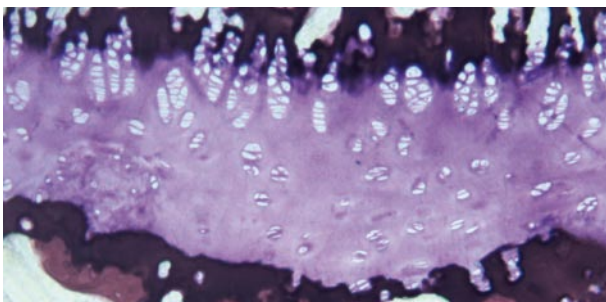
TissueSurgeon has been designed and developed for easy, fast and flexible sectioning of hard tissue.

Native hard tissue histology is unique in its requirements, complexity and processing. If decalcification is no issue, sledge microtomes or ground sectioning are usually applied. But these methods are prone to cause artifacts as the knives or diamond-studded blades contact the sample directly. Common processing of hard

tissue is time-consuming and needs skilled and experienced staff.

TissueSurgeon as ultrafast laser system enables precise, non-contact processing of specimens. With its wide range of applications the preparation of different samples for histological, molecular or biochemical analysis is much more efficient than conventional procedures. TissueSurgeon outplays the limits of microtomy, ground sections or laser microdissection.

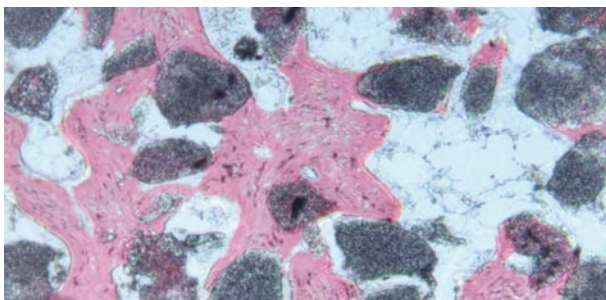
TissueSurgeon Surpasses Common Methods



Cross section of cow tail (15 μm); SRS & VG staining



Bone-implant interface* (10 μm); McNeal staining



Rat femur containing TCP particles for bone regeneration (10 μm); SRS & VG staining

Traditional sample preparation for non-decalcified hard tissue histology requires time consuming methods. For good embedding results, fresh hard tissue needs to be fixed and properly dehydrated afterwards. The embedding process itself can last up to one week. Afterwards the resulting blocks need to be trimmed and pre-sectioned. For ground sections, the surface resulting from pre-sectioning has to be polished and is then mounted to a microscope slide.

In most cases, the time consuming and laborious preparation steps are unnecessary when using TissueSurgeon. Fixed or even fresh hard tissue is mounted on a microscope slide with special glues designed for hard tissue. This mounted sample is cut plan-parallel to the surface of the slide and the section is ready for staining. The whole process only takes about five minutes per sample. Bone for example can be cut between min. 10 μm and max. 100 μm section thickness.

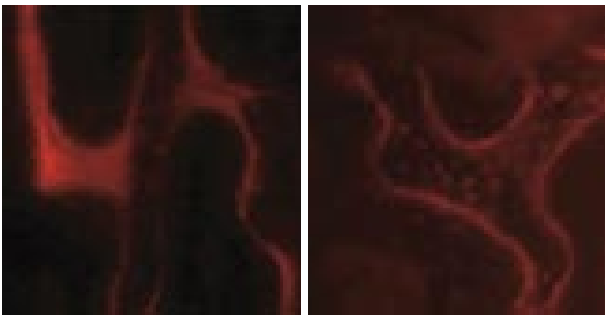
Very brittle or delicate samples, like archeological bone or teeth, sometimes need embedding; protocols are the same as for ground sections. Post processing of resulting sections is similar to established methods.

High Quality - Fast Processing

As the TissueSurgeon is a contact free method, artifacts from knives or grinding and polishing are avoided. For polarized and phase contrast light microscopy, scratches or uneven samples complicate imaging. TissueSurgeon achieves results that can be compared with very accurately prepared ground sections, but with far less effort

and lower risk of rejections. Sample preparation is adapted from ground section methods. The sample is mounted on a microscope slide and cut parallel to the slide surface by focusing the laser into the sample. Exact mounting of samples on the slide can be controlled by integrated Optical Coherence Tomography (OCT).

Bone Labeling Preserved



In vivo fluorescent labeled bone (Tetracycline) before (left) and after (right) sectioning with TissueSurgeon.

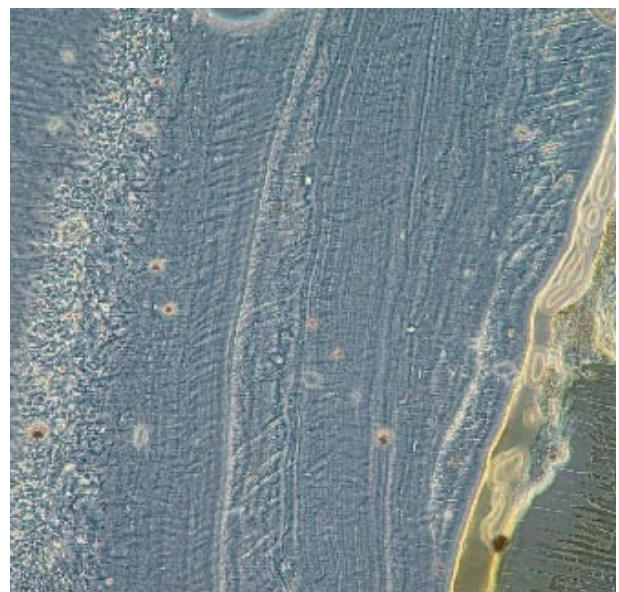
Labeling bone with fluorochromes *in vivo* is an important technique in bone research. Fluorochromes are sensitive to light, bleaching is a common problem. TissueSurgeon works with a near infrared laser (1030 nm). Fluorochromes that are excited with shorter wavelengths are not bleached by the laser of the TissueSurgeon.

This allows fast and easy sectioning of bone from *in vivo* labeled mammals to monitor bone formation after certain time of fluorochrome injection.

Serial Sectioning

The great disadvantage of conventional methods is the high loss of material. The preparation of serial sections of hard tissue is exceedingly tough. Sections prepared with a tungsten-carbide knife and a sledge microtome come close to serial sections but the number of rejects is quite high. Ground section methods lose up to 500 μm per section as lots of cutting, grinding and polishing has to be performed. The section thickness using a ground section method is limited to a minimum of 30 μm .

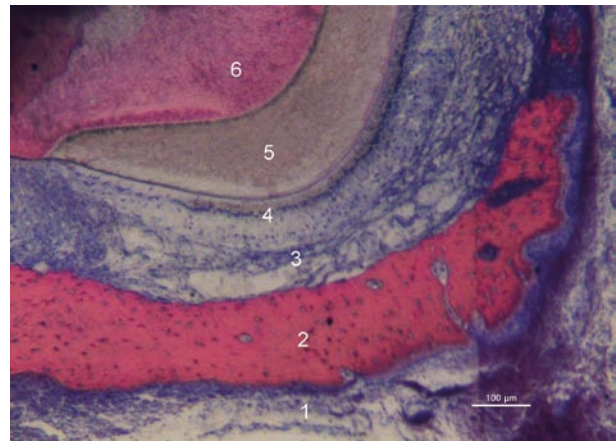
TissueSurgeon is an easy-to-use device. Handling the system is very intuitive and simple. Compared to ground sections, the loss of material per section is also extremely reduced, as only in the focus of the laser (<5 μm) tissue gets lost by the cutting process. TissueSurgeon allows cutting of up to ten ready-for-staining sections per hour.



Detail of cementum, layered structure of the cementum (15 μm); phase contrast

Soft and Hard Tissue Sectioning In One Step

This laser based method enables new ways of analysis: Hard tissue does not have to be decalcified, so structures like growth lines or pathologic effects manifested in the hard tissue can be analyzed without overlay effects in very thin sections. Also processes of odontogenesis or bone remodeling can be observed without losing information of neither hard nor attached soft tissue. As the method is contact free and therefore works without contamination, new analyses (e.g. carbon content of hard tissue) are enabled.

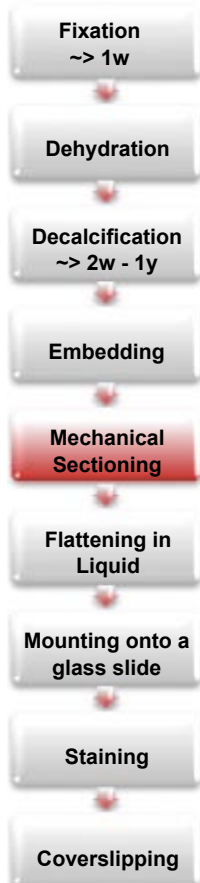


Bucco-lingual section of rat mandible (15 µm section thickness), (1) M. mandibulae; (2) Os mandibulare; (3) Enamel Organ; (4) Ameloblasts; (5) Enamel; (6) Dentin; SRS & van Gieson stain

Comparison of Sectioning Methods

Common hard tissue processing compared to TissueSurgeon sectioning: Time-consuming preparational steps like fixation, decalcification or embedding are not mandatory, they are optional with TissueSurgeon.

Standard Microtome



Ground Section



TissueSurgeon



3D-Cutting

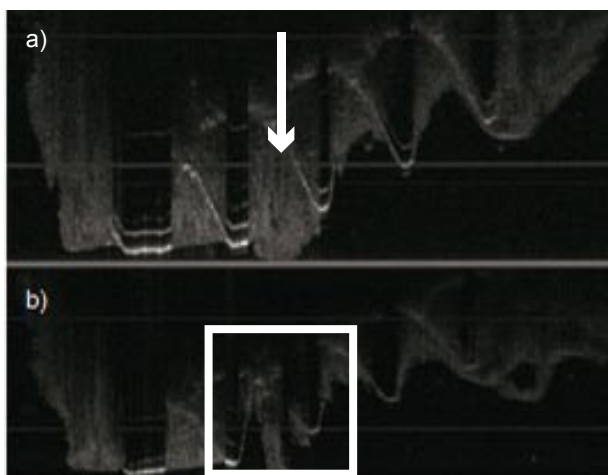
The site-specific analysis of areas inside a sample requires a very exact method of extraction. At present the collection of samples from a soft tissue for biochemical analysis is performed by cutting thin sections of embedded or frozen samples with a microtome. Out of these sections, the area of interest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

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 work for hard tissue. Especially if implants are involved, it is nearly impossible to cut the sample with a knife.

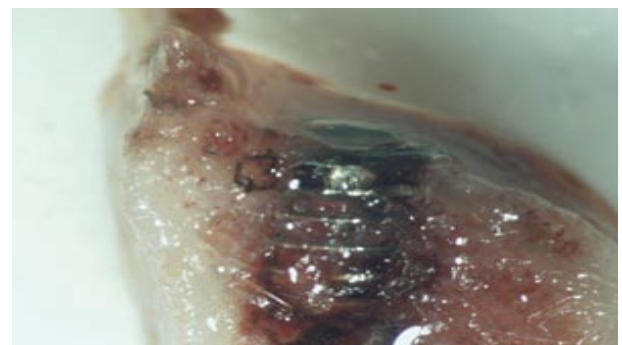
Site Specific Sample Extraction

TissueSurgeon enables a new way of tissue processing 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

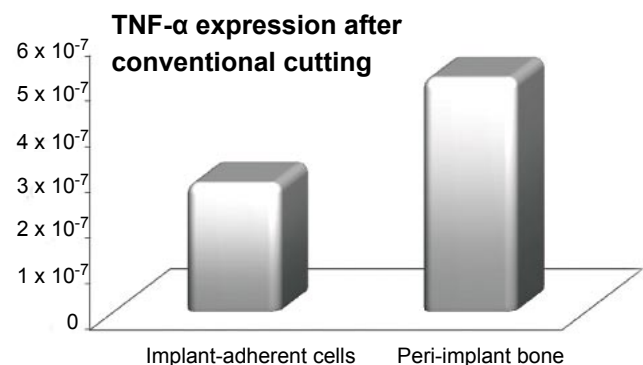
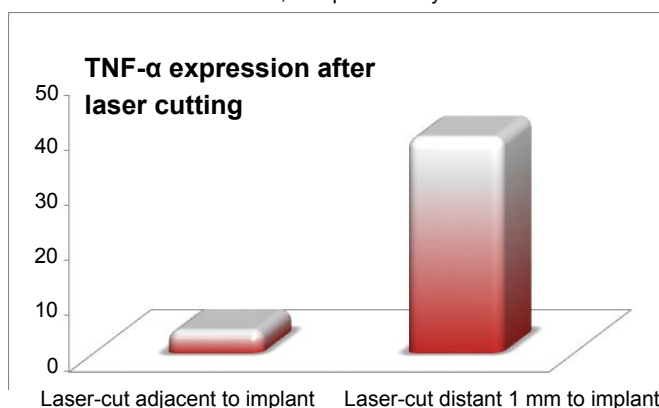
to collect cell material out of fresh, even calcified tissue by cutting a 3D-shape around the area of interest. This method is fast and works without chemicals harming the tissue. Biochemical analysis is much more efficient and specific as comparisons to common methods show. Preservation of e.g. RNA can be supported by cutting the sample in RNA-Later® solution.



OCT-image of rat tibia with titanium screw implant. a) Note the bone formed after 21 days (arrow) b) a cuboid shape was cut around new formed bone, sample is ready for extraction



Titanium implants were screwed into the tibia of rats *in vivo*. After 21 days of bone formation, rat tibiae were extracted. With a dental saw a cut along the implant was performed to get a plain surface. For analysis and cutting, samples were transferred into chamber slides filled with RNA-Later. Areas of interest at the tissue-implant interface and from the surrounding cortical bone were located via OCT and cut out afterwards. Samples were transferred into mercaptoethanol for further analysis of RNA.



The relative gene expression of samples cut with TissueSurgeon (left) is one hundred million (10^8) times higher than mechanical methods (right).

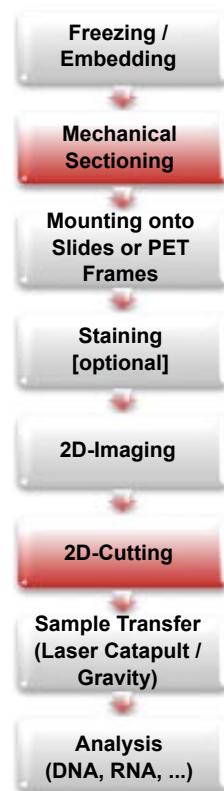
Laser Microdissection Compared to TissueSurgeon

The TissueSurgeon reduces time consuming steps of preparation. The site-specific 3D-cutting of sample material is possible for hard and soft tissue with minimum preparation and maximum results.

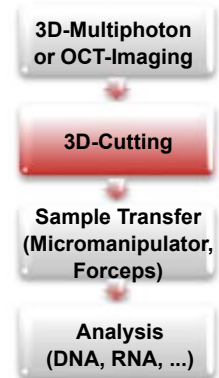


Fresh rat tibia was fleshed and cut lengthwise with a diamond blade and transferred into a chamber slide. The area of interest was located via OCT and a cuboid shaped 3D-cut was performed with the TissueSurgeon. The sample was collected with forceps and transferred into tubes for RNA analysis.

Laser Microdissection



TissueSurgeon



Advantages

- No fixation, freezing and decalcification necessary
- High throughput of non-decalcified samples
- Embedding is not necessary for cortical bone
- No artifacts as method works contact free
- No contamination: elemental analysis (i.e. carbon content) on cut surface is possible
- Common staining protocols are suitable for TissueSurgeon sections
- TissueSurgeon supersedes laborious ground sections
- Structure of the sample is preserved very well, (bio-)chemical information (i.e. RNA) is preserved better compared to mechanical methods
- Sections can be prepared thinner than ground sections (min. 10 µm)

- Fluorochromes in hard tissue are stable
- Compared to ground sections almost no material is lost
- 3D-section and extraction of samples is very gentle, fast and site specific

Applications

- Sections of non-decalcified hard tissue in a range between 10 µm and 100 µm
- Sample preparation for histology, biophysical test and (bio-)chemical analysis
- Serial sections of non-decalcified tissue
- Sections of fresh hard tissue without embedding
- Sections of hard tissue for common microscopy methods (transmitted light, phase contrast, polarized light, fluorescent microscopy)
- Site specific 3D-extraction of fresh samples for biochemical analysis