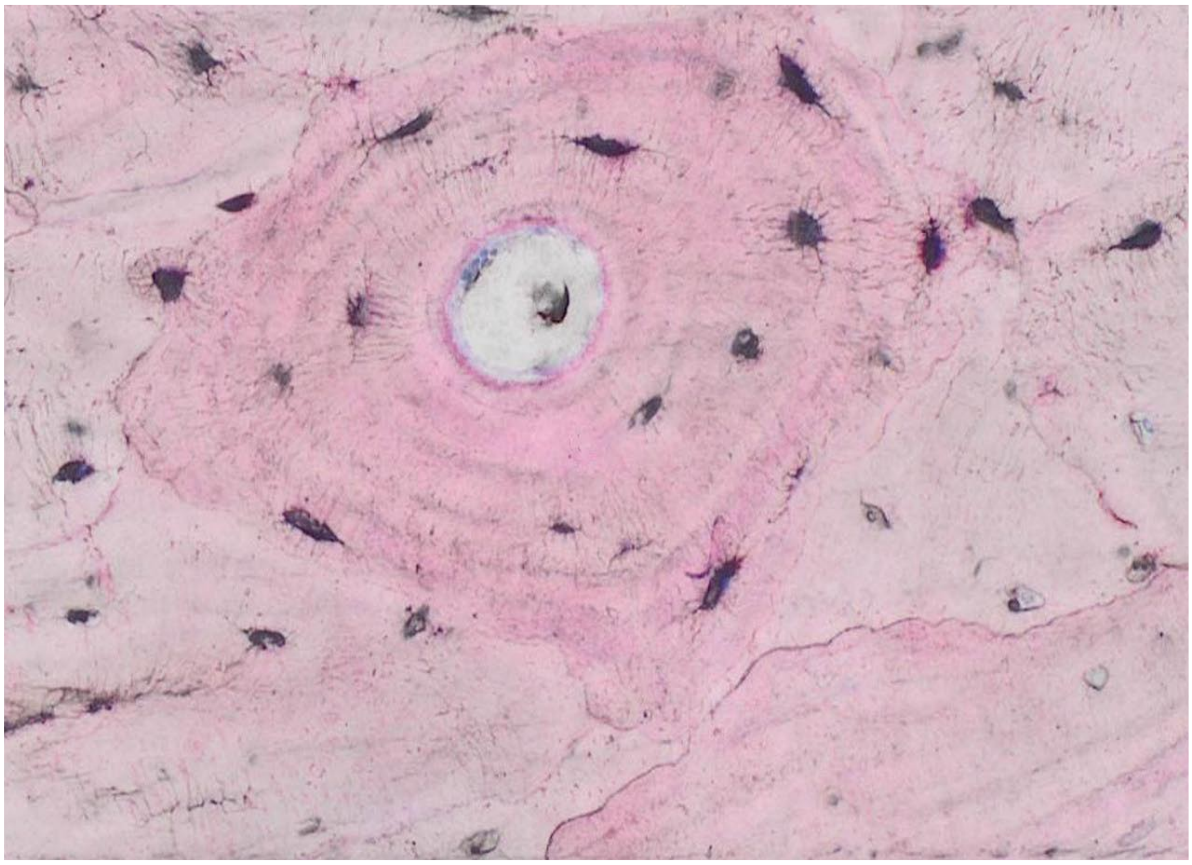
LLS ROWIAK LaserLabSolutions

TissueSurgeon

Hard Tissue Applications



Laser Based Histology

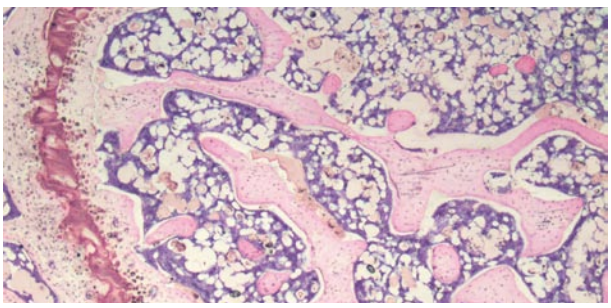
Hard Tissue Sectioning with TissueSurgeon

TissueSurgeon is an excellent tool for easy, fast and flexible sectioning of hard tissue.

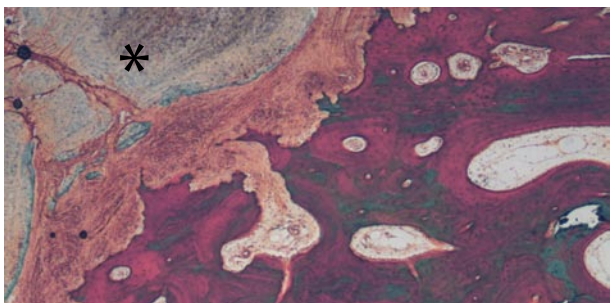
Native hard tissue histology is unique in its requirements, complexity and processing. If decalcification is no issue, sledge microtomes or ground sectioning are usually applied. But these methods are prone to cause artifacts as the knives or diamond-studded blades contact the sample directly. Common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

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.

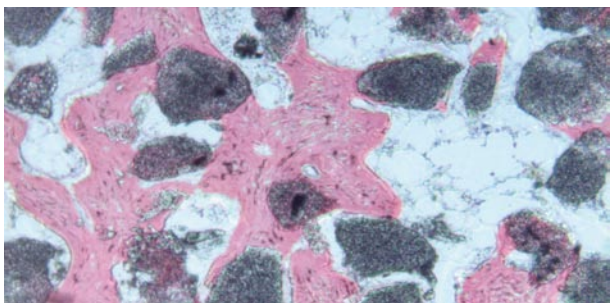
TissueSurgeon Surpasses Common Methods



Rat femur joint (10 µm), Sanderson Rapid Bone Staining and van Gieson staining



Rat Tibia with polymer* implant (10 µm), Masson Goldner Trichrome stain



Rat femur containing TCP particles for bone regeneration (10 µm); Sanderson Rapid Bone Staining and van Gieson staining

Traditional sample preparation for non-decalcified hard tissue samples requires time and material consuming methods: Cutting plastic embedded hard tissue with a sledge or rotary microtome is either impossible or can only be achieved with compromises in quality. Large hard tissue samples are impossible to cut with a blade and require ground section technology. With ground section systems serial sections are impossible – several hundred microns of material are lost between two sections – and minimum sample thickness is approx. 20-30µm. TissueSurgeon can generate nearly serial sections of plastic embedded hard tissue of 10µm thickness. TissueSurgeon works with nearly all common plastic embedding media available – it is not necessary to change your lab protocols.

TissueSurgeon is very time effective compared to ground section technology. The output of sections per day produced is about 4 times (large samples) and eight times (small samples) higher than output with ground section technology. Semi-automated set up of the TissueSurgeon supports efficiency.

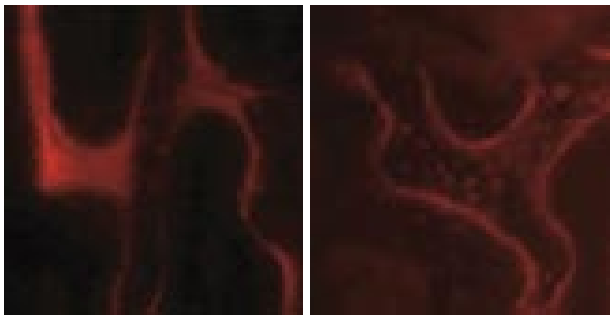
The software can be upgraded with a tool for consistent documentation. Every step from sample reception to labeling a finished stained slide can be controlled and documented by integrated software tool. This allows integration into GLP environment.

Cover: Osteon in cow vertebra (10 µm) Levai Laczko stain (image courtesy of Mag. Stefan Tangl, Head of Karl Donath Laboratory for Hard Tissue and Biomaterial Research, University Clinic of Dentistry, Vienna)

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.

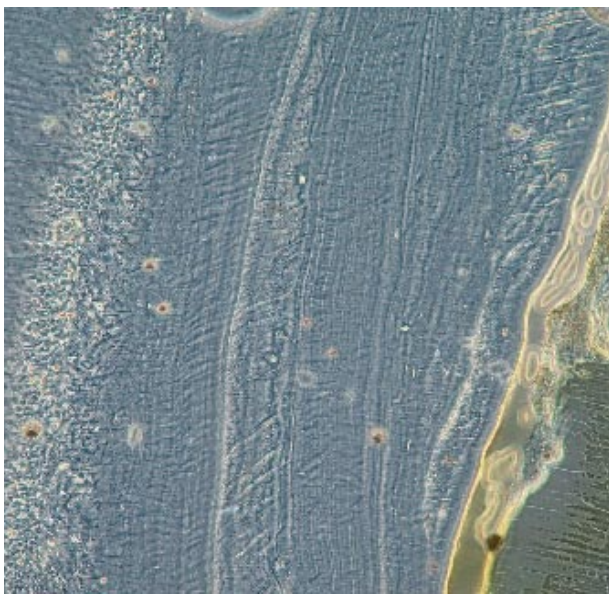
Labeling bone with fluorochromes in vivo is an important technique in bone research. Fluorochromes are sensitive to light, bleaching is a common problem. TissueSurgeon works with a near infrared laser (1030 nm). Fluorochromes that are excited with shorter wavelengths are not bleached by the laser of 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 approximal 30 µm.

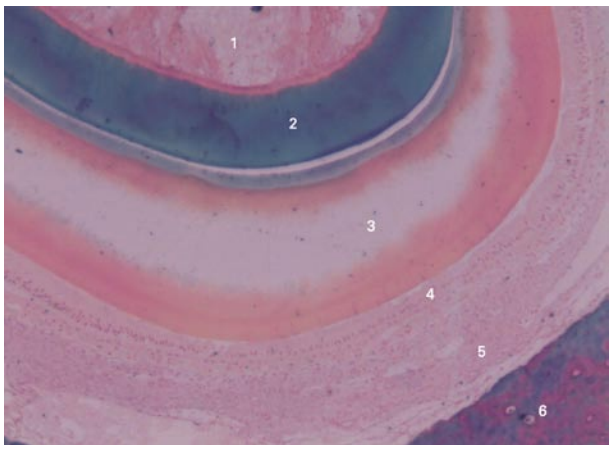
TissueSurgeon is an easy-to-use device. Handling the system is very intuitive and simple. Compared to ground sections, the loss of material per section is also extremely reduced, as only in the focus of the laser (<5 µm) tissue gets lost by the cutting process. TissueSurgeon allows cutting of up to ten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

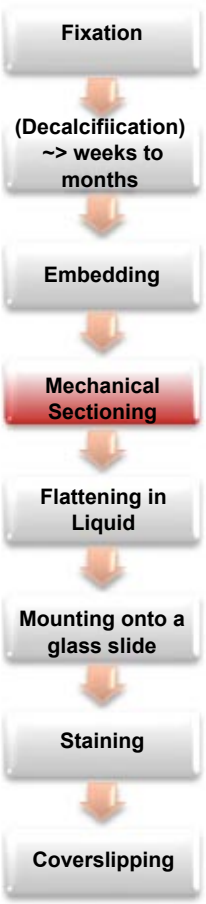
This laser based method enables new ways of analysis: Hard tissue does not have to be decalcified, so structures like growth lines or pathologic effects manifested in the hard tissue can be analyzed without overlay effects in very thin sections. Also processes of odontogenesis or bone remodeling can 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 possible.



Bucco-lingual section of mouse mandible (10 µm), Masson Goldner Trichrome stain. (1) Pulp, (2) Dentin, (3) Enamel, (4) Ameloblasts, (5) Enamel Organ, (6) Mandibular bone

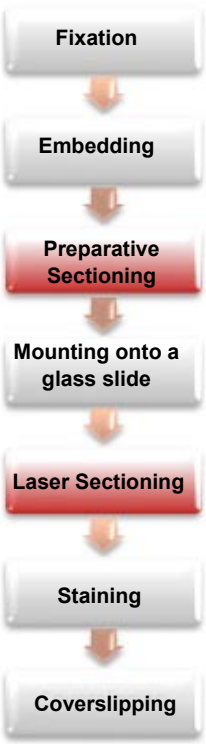
Comparison of Sectioning Methods

Common hard tissue processing methods compared to TissueSurgeon sectioning



Standard Microtome

Ground Sectioning



TissueSurgeon

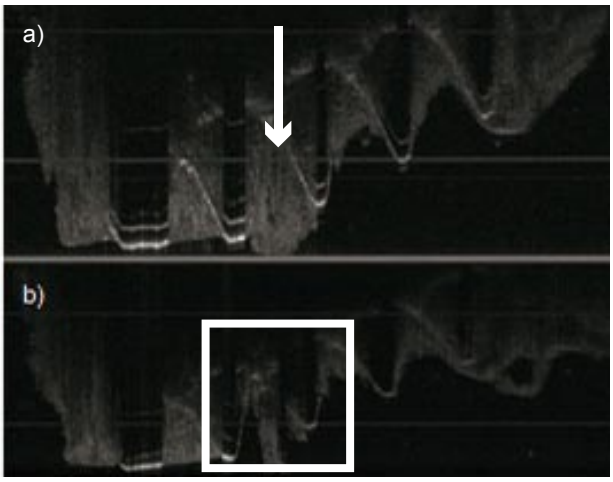
3D-Cutting

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.

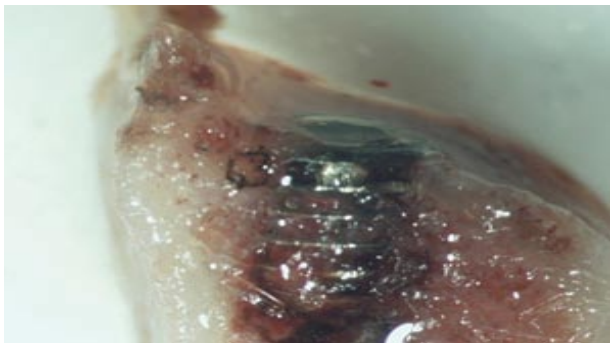
However, this method suffers from taking considerable time to collect enough material, and harsh chemical treatment impairing analytical results. Recent publications show that this method does not really work for hard tissue. Especially if implants are involved, it is nearly impossible to cut the sample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

Site Specific Sample Extraction

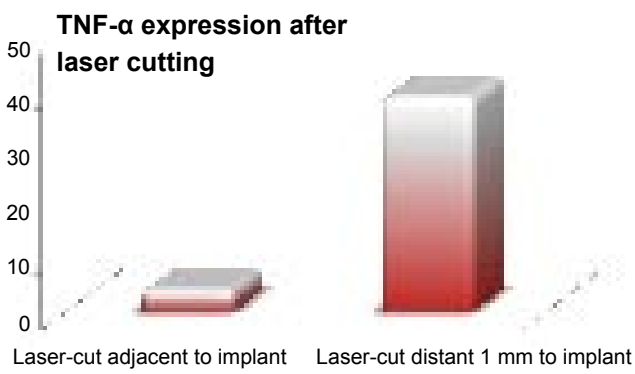
TissueSurgeon enables a new way of tissue processing for biochemical analysis. Not only does it allow for two-dimensional sectioning for histology but also for three-dimensional cutting and cell isolation. In a single-step procedure the area of interest in a fresh tissue sample can be identified by the 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. Preservation of e.g. RNA can be supported by cutting the sample in RNA-Later® solution.

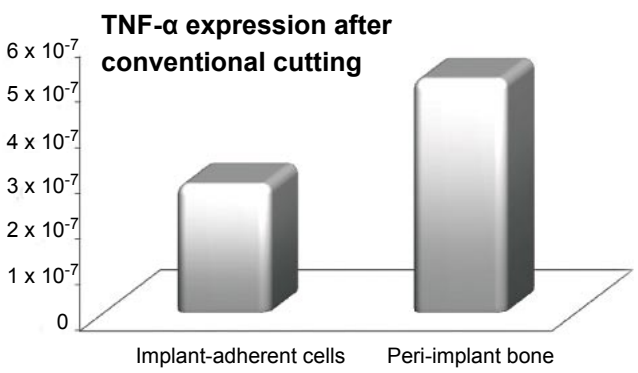


OCT-image of rat tibia with titanium screw implant. a) Note the bone formed after 21 days (arrow) b) a cuboid shape was cut around new formed bone, sample is ready for extraction



Titanium screws were implanted 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.

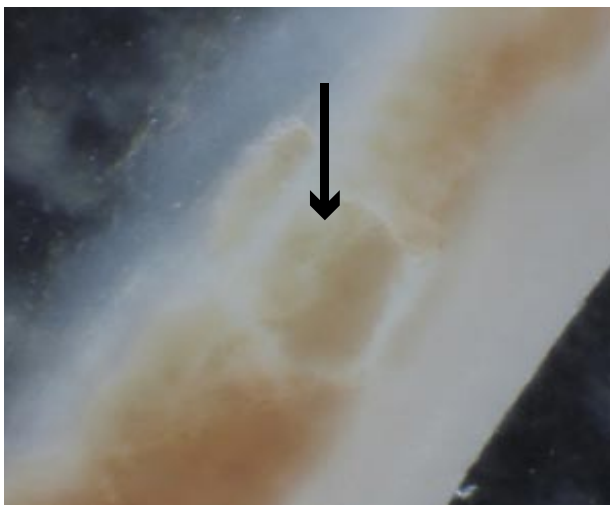




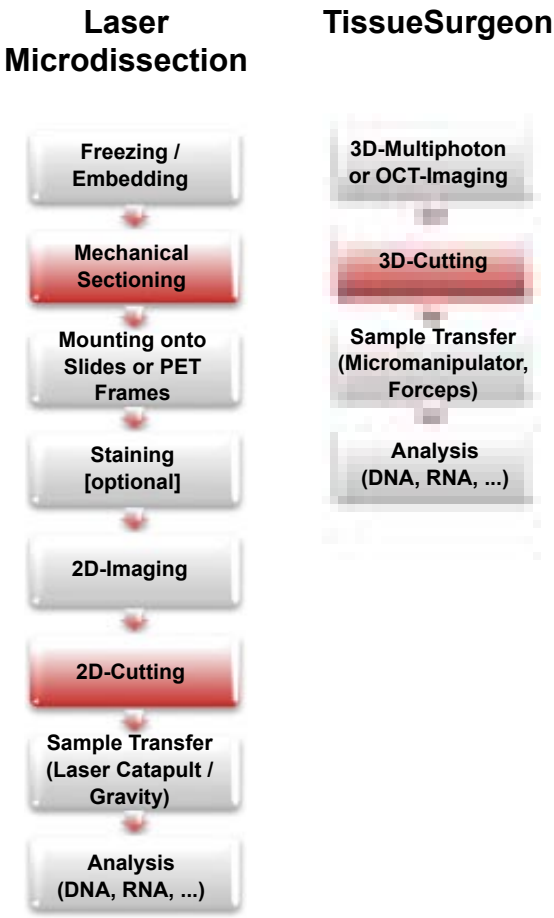
The relative gene expression of samples cut with TissueSurgeon (left) is one hundred million (10^8) times higher than expression of samples cut by mechanical methods (right).

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.



Advantages

- No decalcification necessary
- High throughput of non-decalcified samples
- 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 sectioning
- Sections can be prepared thinner than ground sections (min. 10 µm)
- Compared to ground sections almost no material is lost
- Structure of the sample is preserved very well, (bio-)chemical information (i.e. RNA) is preserved better compared to mechanical methods
- Fluorochromes in hard tissue are stable
- 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 hard tissue for common microscopy methods (transmitted light, phase contrast, polarized light, fluorescent microscopy)
- Site specific 3D-extraction of fresh samples for biochemical analysis

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