Laser Microtomy

Opening a New Feasibility for Tissue Preparation

The laser microtome (LMT) has been designed to slice biological tissue and various materials with high precision. The cutting process is performed by ultra short laser pulses in the near infrared region, inducing an optical breakdown below the surface of the material. Biological Tissue can be processed in its native state without prior fixation and embedding. This is of great importance for many biological applications such as immunohistological investigations.

THE AUTHOR

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Prof. Dr. Holger Lubatschowski has studied physics at the University of Bonn. After his PhD he moved to Hanover and became Head of Medical Laser Group at the Laser Zentrum Hannover (LZH). In 2001 he made his Habilitation for Physics at the University of Hanover and became the Head of the Biomedical Optics Department at the LZH. In 2003 he founded Rowiak GmbH as a spin off company of the LZH which develops ultrafast laser systems for applications in life sciences.

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A microtome is an instrument used to prepare thin sections of biological tissues for microscopic examination. Usually sectioning of biological specimens is performed by mechanically working microtomes. In order to process a tissue sample, a range of pretreatments such as fixation, dehydration and embedding in resin or paraffin, or freezing are needed. These treatments can cause changes inside the biological material, which may lead to artefacts.
A laser microtome is a new sectioning device, which cuts tissue or other material with the help of photons instead of steel blades. The method is contact-free and enables to cut tissue in its native state. Special preparation techniques are not required.

Cutting by optical breakdown

At the heart of the laser microtome is a femtosecond laser, emitting light in the near infrared range. Due to the rapid development of commercial available turn key laser systems during the last years, the use of ultra-fast laser technology has become much easier. It has opened up new applications in the field of life science.
Laser light in the near infrared range around 1000 nm is well suited for the processing of biological material since most biological tissues have a very low absorption coefficient at this wavelength. Thus, manipulation of tissue is not only limited to the surface, but can even be done inside the material. This significant advantage is at the basis of laser microtomy.
To perform a cut, the laser beam is tightly focused into the specimen by a high-numerical objective. Due to the extreme intensities up to 1 TW/cm^2 inside the focus, multi photon absorption causes ionization of the tissue. This process is called optical breakdown and leads to the formation of a plasma. The fast expansion of the plasma causes disruption of the tissue and is responsible for the cutting process (Fig. 1).
If the pulse duration is sufficiently short (100 - 400 fs) and the diameter of the focal spot is diffraction limited (1 µm), there is only very low pulse energy of somewhat 10 nJ needed to induce the optical breakdown. It limits the interaction range to diameters below one micrometer. The material separation only takes place within the focal region. Outside this region no thermal or mechanical damage can be detected.

The laser microtome at a glance

The laser microtome is designed as a stand alone device controlled by a separate touch

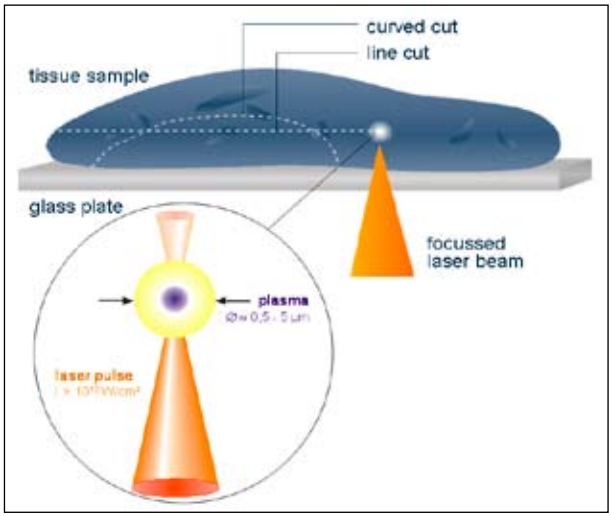

FIGURE 1: **Working principle of the laser microtome. A laser induced plasma is used to cut the tissue without any mechanical forces applied. The near infrared radiation of the laser penetrates the tissue up to one millimetre.**

FIGURE 2: **The Laser Microtome as a stand alone system.**

screen (Fig. 2). Main component of the laser microtome is a high power fs-oscillator emitting ultrashort laser pulsing with a wavelength of 1030 nm, a pulse duration of about 300 fs and a pulse repetition rate of 10 MHz. The maximum average power of this laser system is 2,5 W. A custom made objective with high numerical aperture delivers pulse energies of up to 100 nJ to the sample.

For the cutting process, both the laser beam and the specimens are moved simultaneously. The laser beam is deflected by a fast scanner whereas the specimen on top of a standard glass plate is moved by a 3D piezo driven positioning stage. Parameters, such as pulse overlap, slice thickness and the size of the cutting area, can be set by the user. Typically an area of up to 14 x 14 mm can be processed. The cutting speed depends on the properties of the sample, whereas 1 mm^2/s is typical. At present slices of 5 to 100 µm thickness have been generated.

FIGURE 3: **10 µm thin slices of Teflon were cut with the laser microtome.**

Thinner slices are also possible, but handling of such tissue slices following removal of the sample bulk is more difficult. For light microscopic examinations a thickness of 5 to 10 µm is in most cases sufficient. However, thicker slices are of interest for e.g. primary culture, neurobiological experiments or pretreatment of samples for ultra microtomy.

Preparing and sectioning the tissue samples

Because no mechanical forces are applied to the sample, tissue can be processed in its native stage and does not need prior fixation, embedding or freezing. The tissue sample is placed on a conventional microscopic glass slide. In order to achieve good results the tissue surface has to be plane with a good optical contact to the glass slide. If needed, a small drop of liquid is helpful to assist index matching. Saline solutions are as good as most of the media used for cell culture.

The software allows three cutting modes: cutting of the whole field (maximum size and maximum time), of a defined rectangular field (e.g. 5x6mm) or of a customized field by setting position markers. According to the precision of the z-axis, the thickness of the slices can be controlled with an accuracy of better than three microns.

During the cutting process a live video of the sample, i.e. the cutting plane, is shown on the control screen. This allows the user to observe whether the cutting procedure is successful or not. A fast growing field of micro bubbles is typical for the occurrence of photodisruption and therefore a sign of correct cutting. After the cutting process the tissue section is separated from the bulk with tweezers and placed on a glass slide. Now it can be stained and covered if necessary.

Tissue samples

In principle every soft tissue with the exception of melanin containing tissue can be processed, since the 1040 nm radiation is barely absorbed by tissue chromophores. Even plastic material has been cut, as long as it has a bright colour (Fig. 3). Hard tissue like bones or of dental origin is also suitable for cutting, however the cutting parameters have to be optimized.

In Fig. 4 sections of kidney tissue, lung tissue as well as cartilage tissue is shown as an example for laser processed sectioning. The samples show no thermal or mechanical alterations and are well suited for further light microscopic examination. The preparation of lung tissue was somewhat challenging to put into practise because of the large amount of air inside the lung. This leads to reflection and scattering losses at the interfaces between tissue and air cavities and deteriorates the laser focus. However, by filling the lung with nutrition solution or with agar

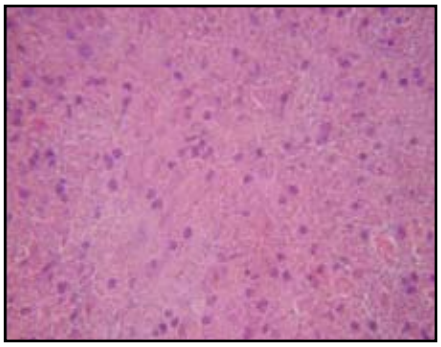

FIGURE 4A: **Kidney tissue (pig) 10 µm thickness, (40x Objective).**

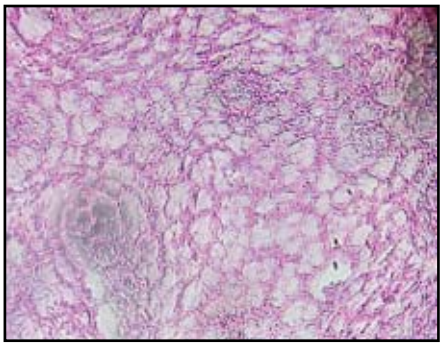

FIGURE 4B: **Lung tissue (mouse, 20x objective).**

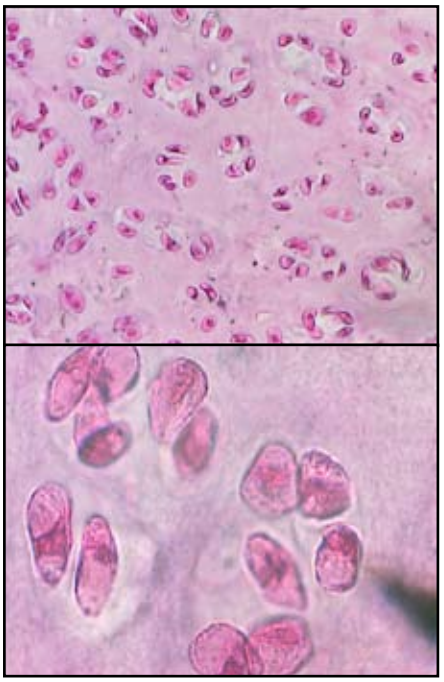

FIGURE 4C: **Cartilage tissue (pig) 15 µm thickness, 20x objective and 100x oil objective.**

gel as an index matcher the number of scattering events is reduced and cutting of even thicker slices than 10 µm is possible.
Preparation of thin histological sections for light microscopy is not the only application for the laser microtome. Because the cutting process does not apply any shear forces to the sample, morphological analysis of brittle or very soft tissue is also made feasible.
In Fig. 5 a longitudinal cut of a human hair is shown. At the lower end of the laser cut, a second cut, performed with a mechanical knife, is shown for comparison. As demonstrated on the SEM, the laser does not destroy the original structure of the hair. The knife cut, however, due to the mechanical forces applied, leaves a flat and smooth surface what prevents the investigator to gain any information about the structure of the tissue behind.

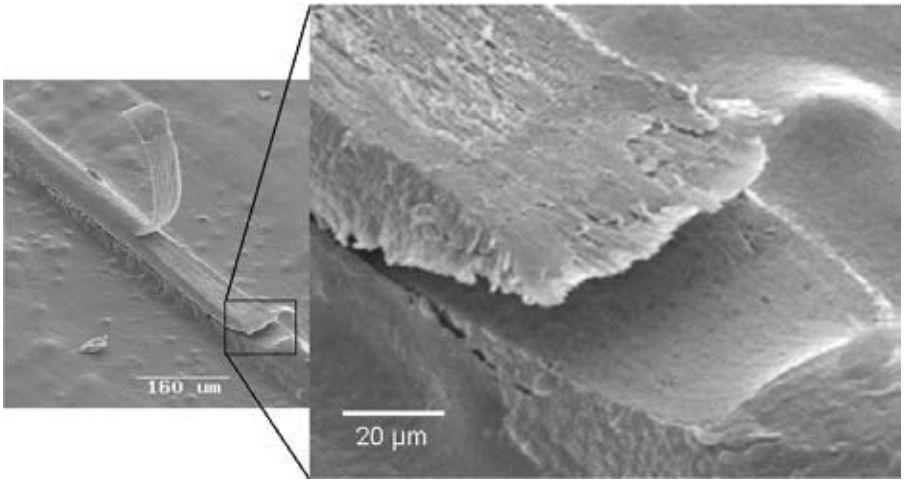

FIGURE 5: **Longitudinal cut of a human hair in comparison with a smaller cut by a mechanical knife.**

See what you cut: OCT controlled 3D processing

Cutting of flat sections by femtosecond laser technology is only the first step into a new direction in laser microtomy. For the next step, OCT imaging will be implemented into the system. Optical coherence tomography (OCT) is a method for imaging different layers of transparent or scattering tissue by scanning the area of interest with a low coherent light source. The measuring principle is similar to ultrasound imaging. As a typical light source, superluminescent diodes or even the fs laser pulses themselves with a broad emission band and a corresponding low coherence length can be used. The penetration depth of the NIR radiation is of up to 2 mm into the tissue. Due to differences in their optical path lengths, photons reflected from different layers inside the tissue, can be distinguished by interference measurements with an external reference plane. Fig. 6 shows an OCT image of a finger tip, where some perspiratory glands can be seen as spiral pattern.
Having such a tomographic imaging system implemented, real 3D cutting near the surface of tissue sample is possible, targeting specific areas or volumes of interest.

Not for routine but for special cases

Of course the Laser Microtome is not a device for the routine pathology, as the procedure of tissue separation is too slow. However, the Laser Microtome has its advantages over standard procedures when preparing tissue in its native state. For investigations based on immunostaining it can be desirable, to keep tissue alive. Furthermore there are several applications which are, at present, difficult or impossible to realize with conventional sectioning methods. This includes the cutting of hard tissue, plants, wood and other materials. The Laser Microtome might be a good option for these applications. It has the potential to solve different problems in various research fields from plant biology to regenerative medicine.

THE COMPANY

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Rowiak GmbH was founded in November 2003. The company designs and manufactures laser based instruments, suited for applications in life and materials sciences. The product portfolio includes instruments for the biological and medical research or the clinical diagnostic.
Ultra short laser technology is the key for the realization of innovative solutions for precise laser surgery, non invasive diagnostics as well as cell surgery. This still young laser technology uses non linear effects to enable real 3D processing with micrometer or even nanometer precision.

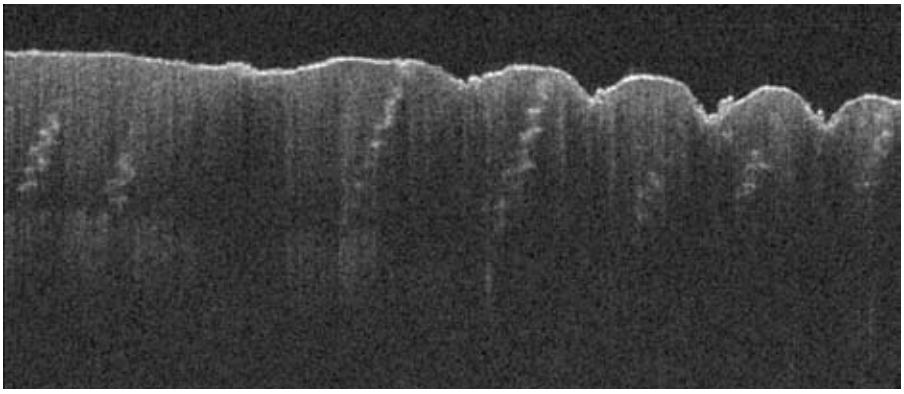

FIGURE 6: **OCT Image of a finger tip. The spiral pattern are some perspiratory glands coming up to the surface of the skin.**