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Experimental Pathology Laboratory Services

Advanced Laser-Based technology for the preparation of biological tissues and materials in plastic embedded sections for a histopathological evaluation

Methodological Aspects of an Advanced Technology purchased by Patho-Logica (supported by the Israeli Innovation Authority).

By Dr. Emmanuel Loeb

February 18, MDI meeting.

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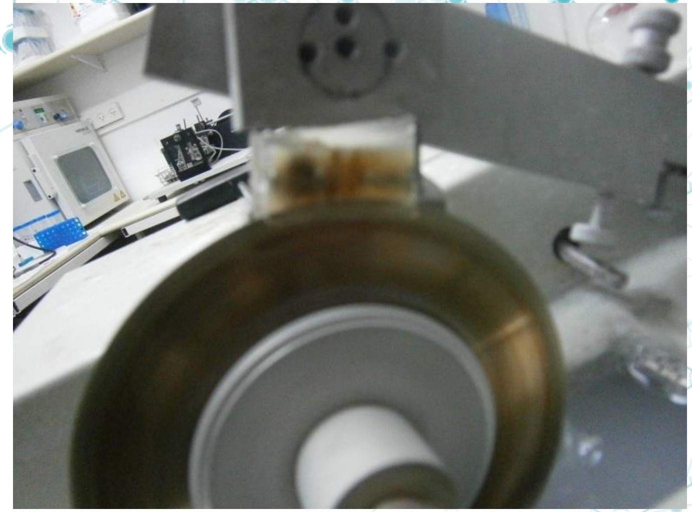
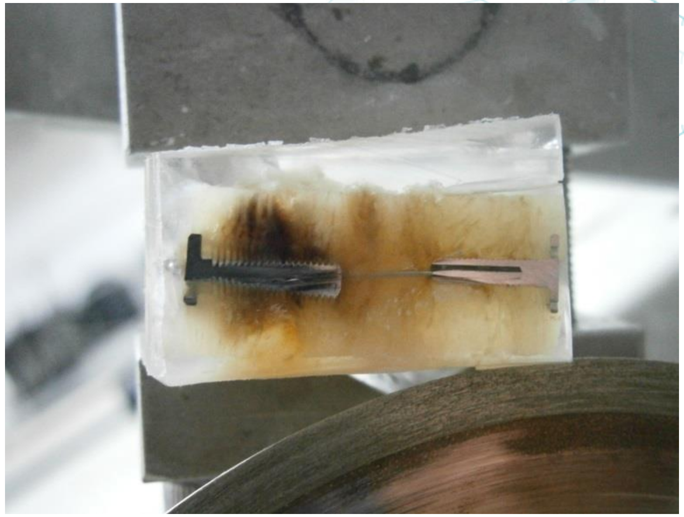
Background: Medical device histopathological evaluation

- Plastic embedding was originally designed for bone histology, to avoid the decalcification process and later it was adjusted to solid and implanted medical device research.
- The analysis of the interaction of invasive medical device with the surrounding tissues in animal models is crucial for safety and efficacy evaluation.
- Bone tissue and hard, solid implants can not be dissected in routine paraffin embedded sections, therefore plastic embedding was introduced to solve this problem.

The classical method (ground sections):

- Ground sections (<u>cutting-grinding technique</u>) was introduced in the early Eighties:
- Cutting-grinding technique produce thick sections of 30-100 microns depending on the material. Thinner sections need years of experience.
- Results: Moderate quality of stained slides, difficult to see details in high power fields, labor intensive and loss of material, 0.5mm between two sections (no serial sections), many artifacts by some implants





An example of an orthopedic medical device implant in bone tissue processed in Ground section method.

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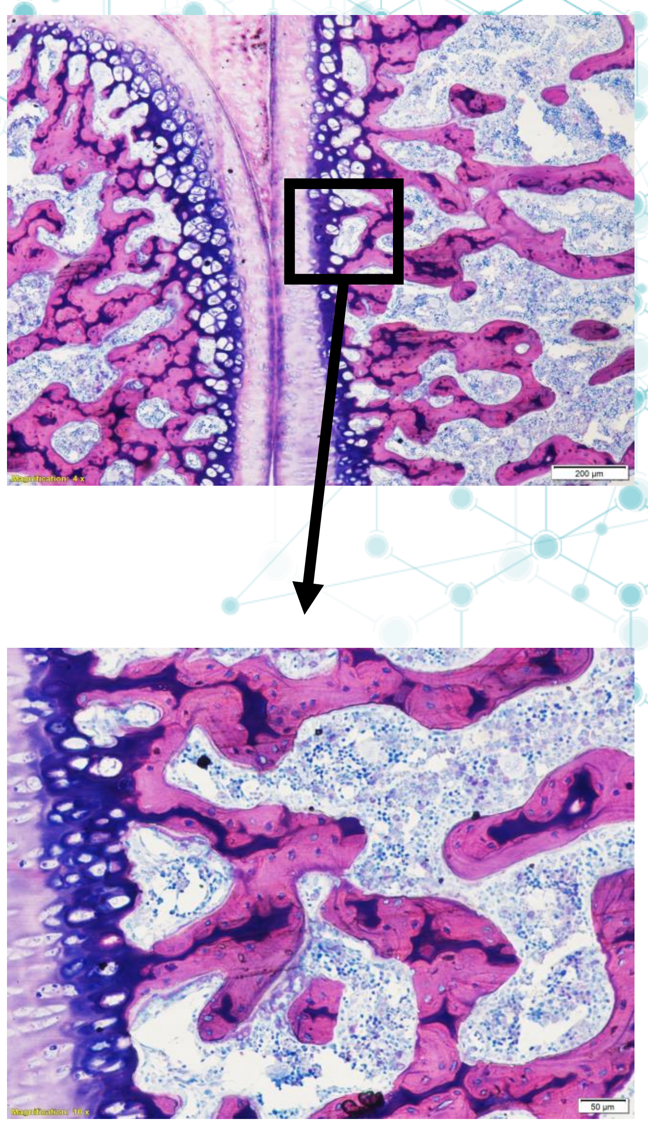
The advanced **Laser Microtomy** dissection

Novel Laser Microtomy raised Plastic Embedded Histology to a new level:

- Designed in 2007 by LLS Rowiak, Hanover, Germany.
- Laser microtomy overcomes limits of classical (hard tissue) microtomy and ground sectioning technology, required for histological analysis of bone tissue, medical device and implant development.
- Optic table enables an **accurate dissection** of cross-sections along the subject glass.
- Clean **laser dissection** with no temperature increase during the process (femtosecond laser)
- Relatively **thin sections** (8-15 micron). **High quality, detailed,** sections on high magnification!
- Enabling high number of sections without loss of material (**serial sections** is possible).
- **Results:** Fine structure with clear details in high magnifications, high quality slides for documentation, crucial for regulatory product evaluation.
- **A wide range of applications:** Bone pathology, cardiac stents, tooth implants, orthopedic hard devices, regenerative medicine, tissue engineering (implants, scaffolds).



Knee joint of a rat: Note the slide quality in a high magnification X10. H&E

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The process:

- Tissue fixation and embedding in MMA or Spurr plastic medium
- First cross section using diamond saw
- Section surface treatment and glued on a subject glass
- Optic table measurements for the laser dissection using Optical Coherence Tomography
- Laser dissection with accurate cross section parallel to the glass
- Removal of the plastic block
- Deplastinization of the slide
- Staining phase
- Stained slide is ready to analyze / scanned as for digitalization.

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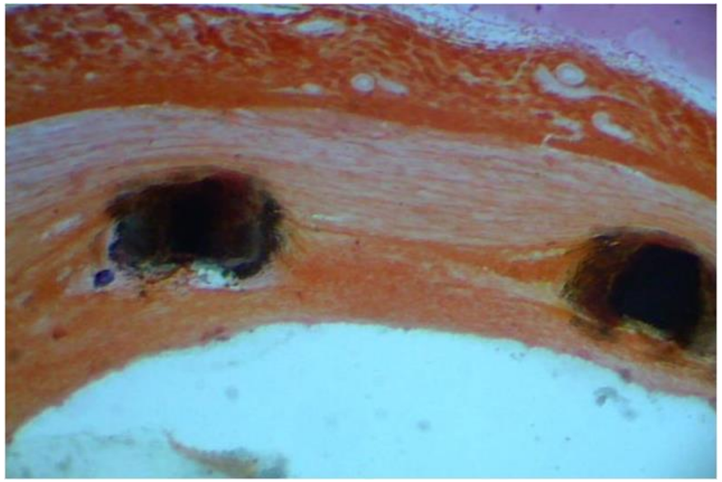
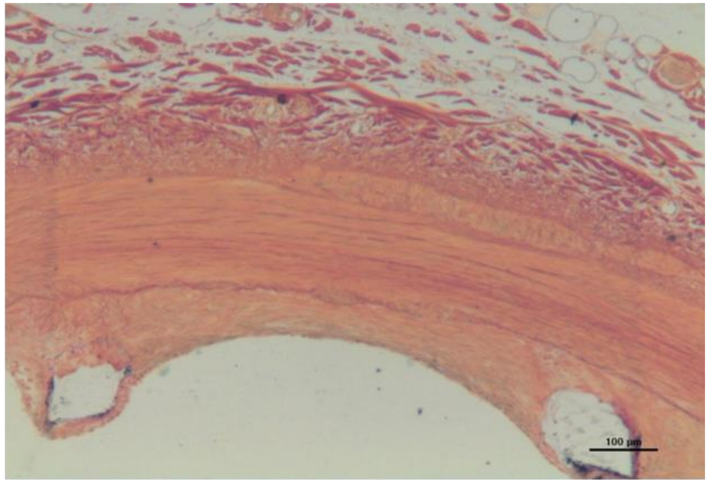
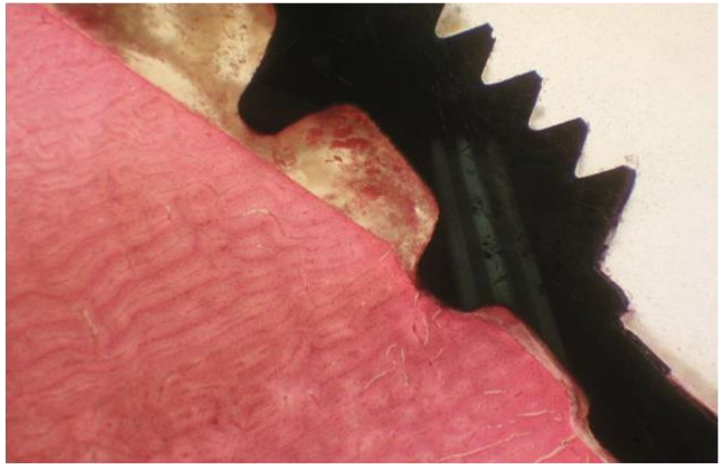
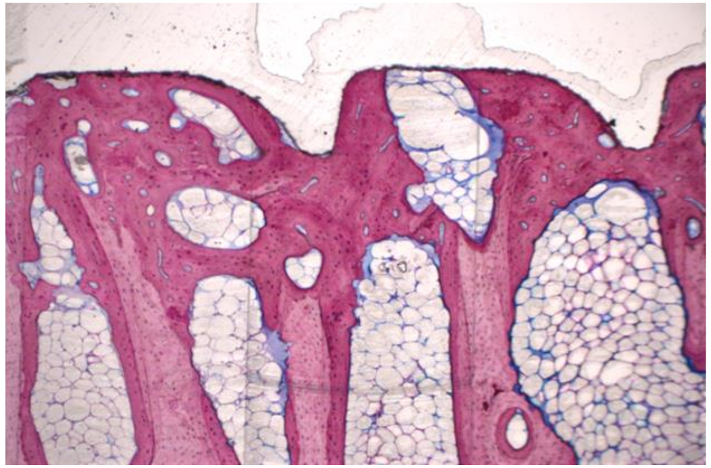
Plastic embedding: Ground sections saw vs. Laser microtomy









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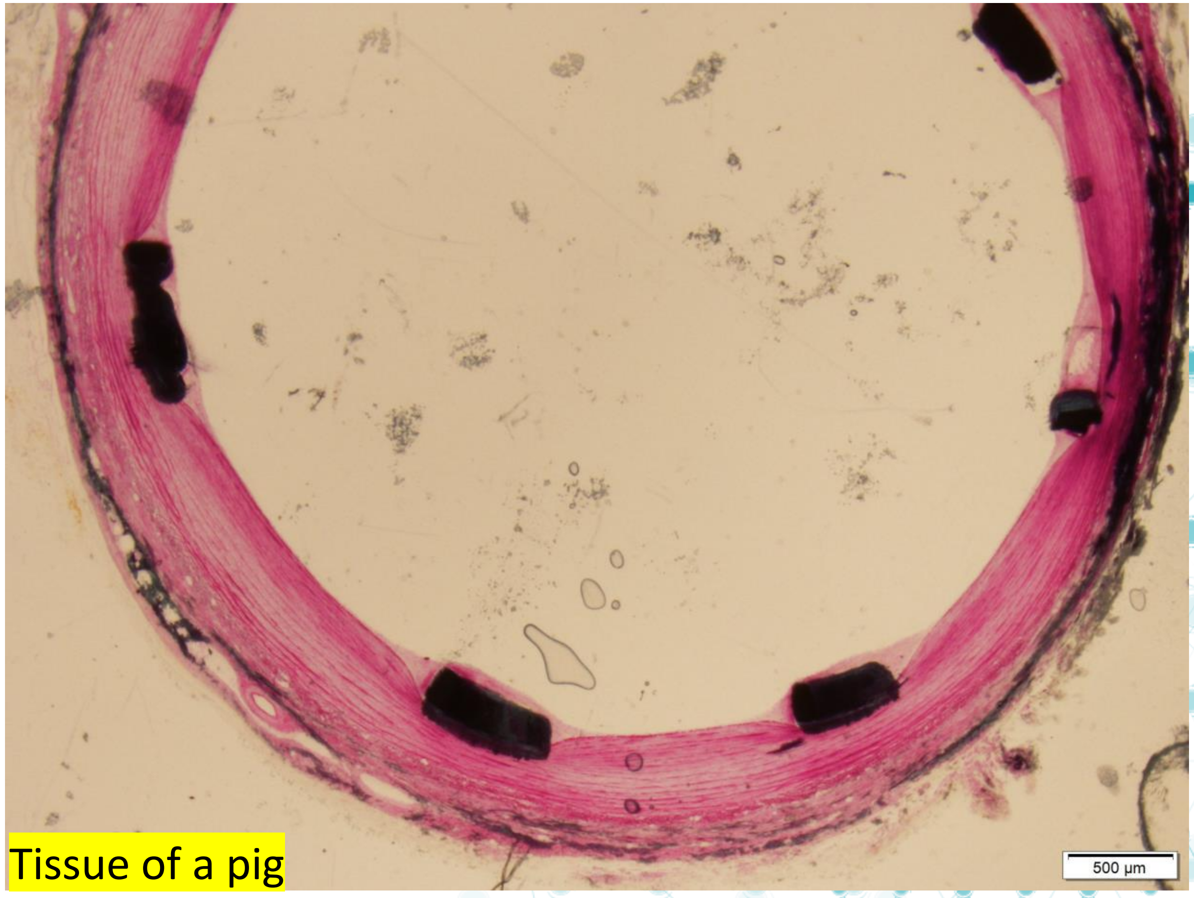
Histopathological results: Laser microtomy vs. Ground sections.



Vessels stents: Low magnification. Left: Laser microtomy; Right: Ground section, X1.25. H&E
Description: Same information on small magnification from both methods.

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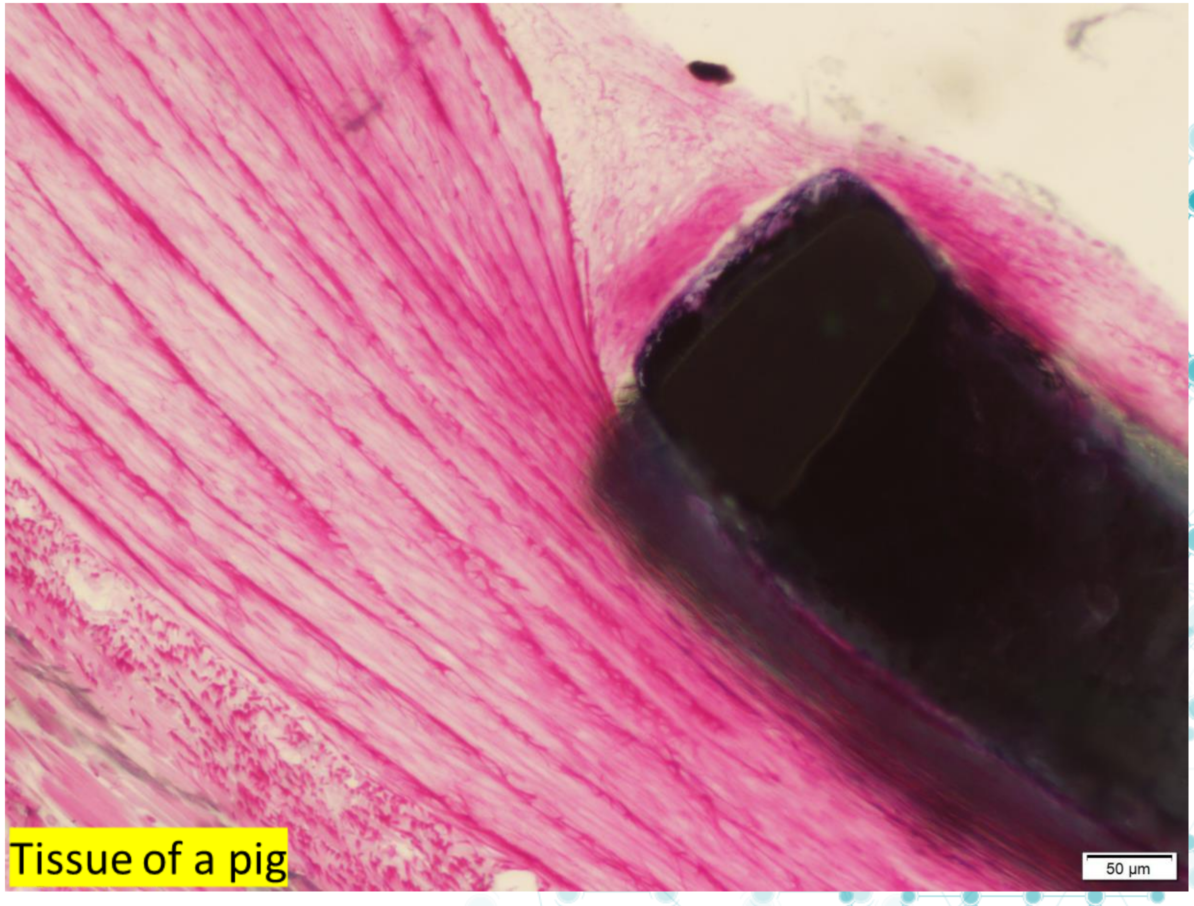
Histopathological results: Laser microtomy vs. Ground sections.



Vessels stents: Higher magnification. Left: Laser microtomy; Right: Ground section. X10, H&E.
Description: In the Laser microtomy method more details (arrows) on a higher magnification.

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Histopathological results: Laser microtomy vs. Ground sections.

Vessels stents: Higher magnification. Left: Laser microtomy; Right: Ground section. X20, H&E.
Description: In the Laser microtomy method more details (arrows) on a higher magnification. Yellow arrows inflammatory cells, Red arrows show macrophages with hemosiderosis, Blue arrows show smooth muscle, Green arrows show connective tissue.

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Classical ground sections versus novel laser microtomy

Ground sectioning method	Laser microtomy method
Metal device is present	Metal device is absent
Poor quality on high magnifications	Fine quality on high magnifications
Labor intensive	Rapid and simple to perform
Poor quality of tissue details (30-100 microns thick) and More artifacts	High quality of tissue details (10 microns thick) and less artifacts
High material loss between two sections	Nearly serial sectioning is possible
Expensive	Cost effective

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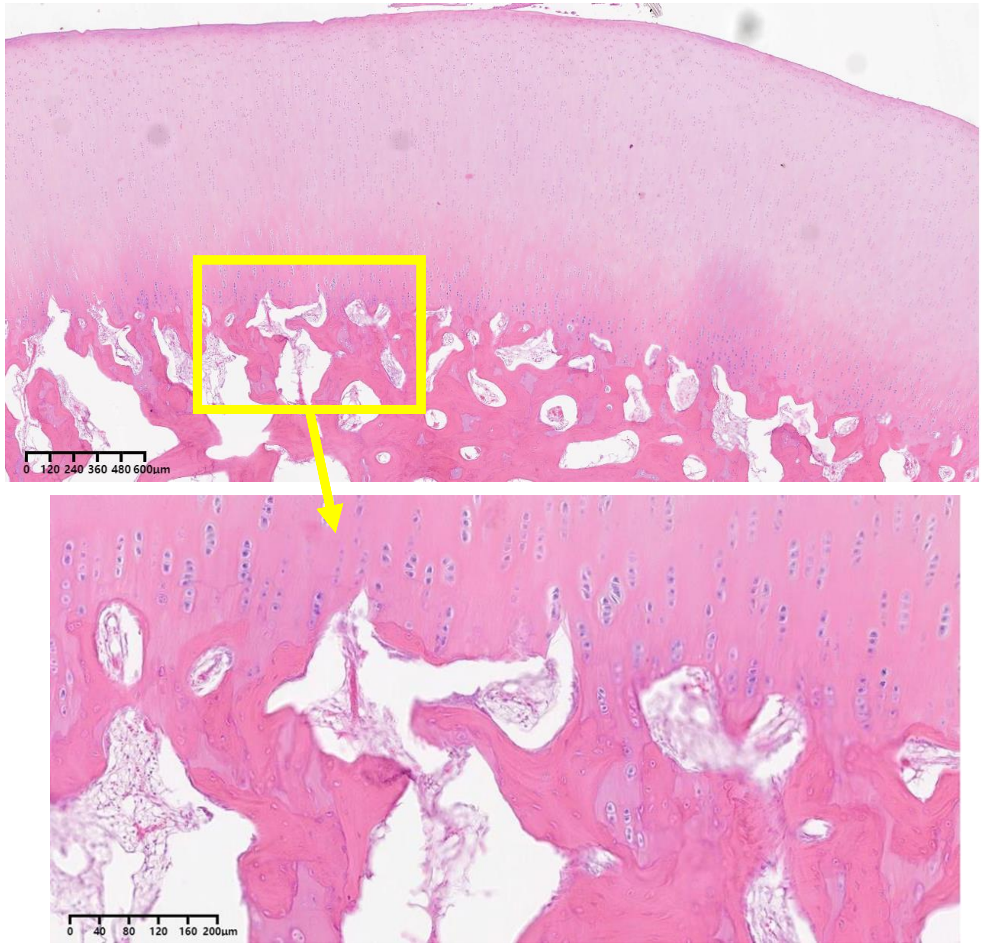
H&E stain of paraffin sections after a decalcification process compared to MMA (methyl Methacrylate) sections using our novel Laser Microtome. X2.5 and X10 Magnifications

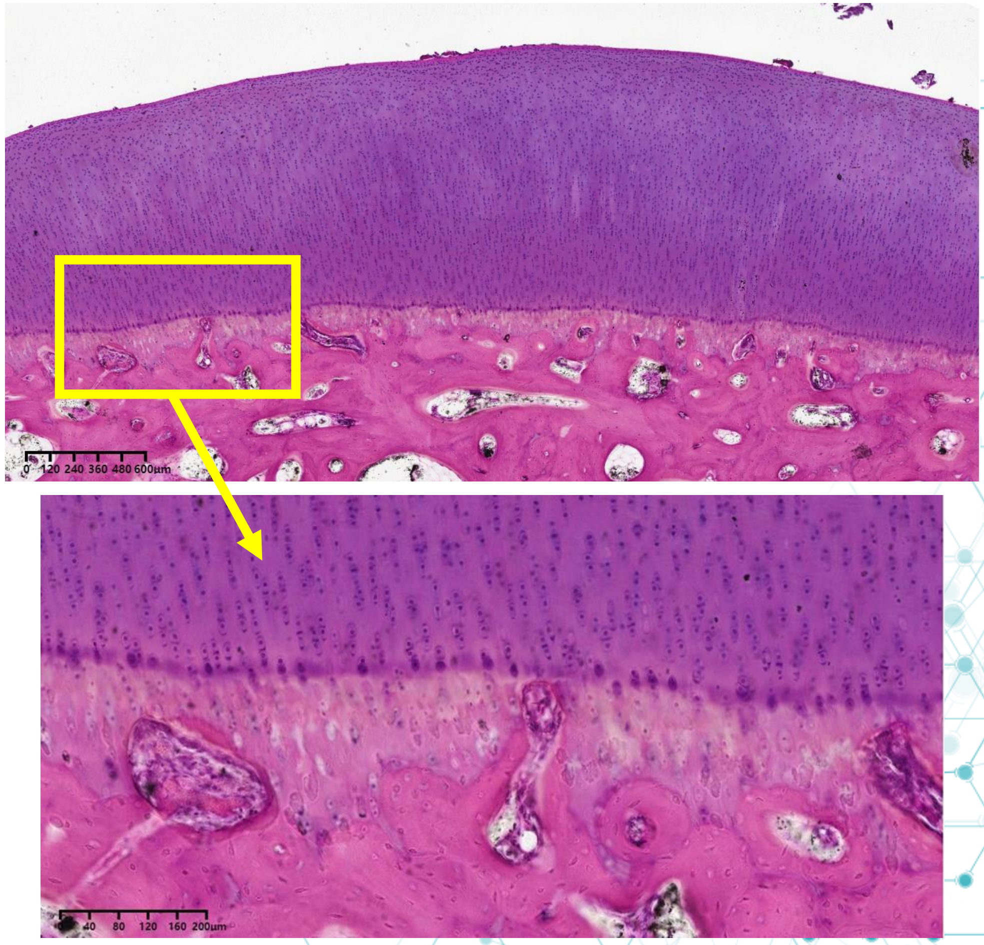
Paraffin sections after decalcification process



Clear differences between the tissues, note the sharp interface Between the bone and cartilage.

Note the cellular details Of the cartilage and bone parts

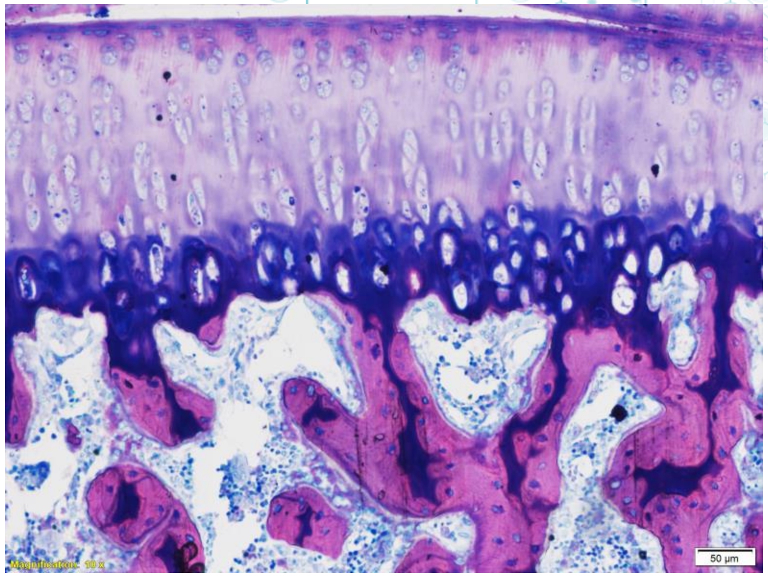
MMA sections using the Laser microtome



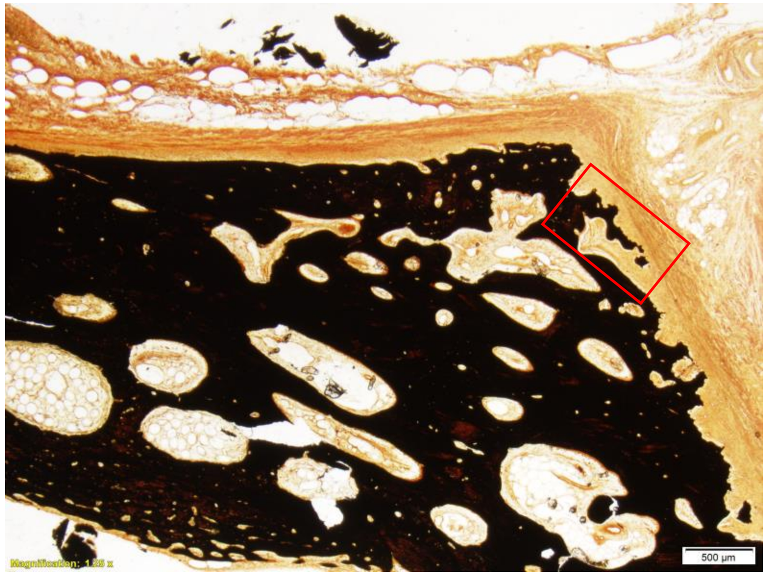
Examples

A

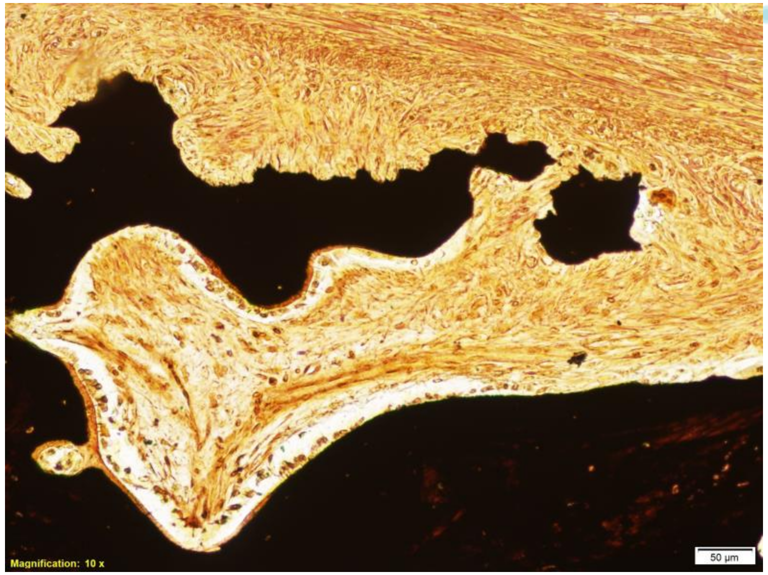
B



C



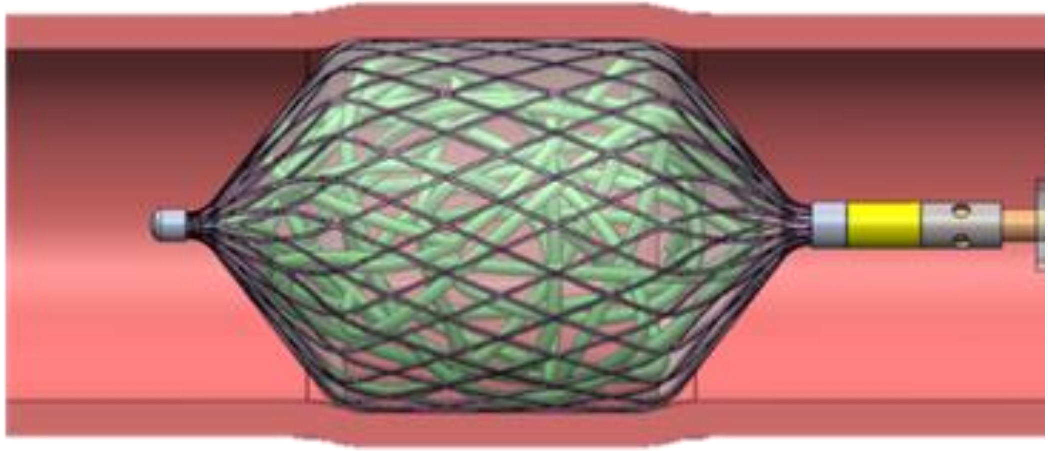
D



Upper left (A): Rat, knee, H&E X1.25. Upper right (B): Rat, knee, H&E X10. Lower left (C): Rabbit knee with polymer implant, Von Kossa stain X1.25. Lower right (D): Rabbit knee with polymer implant, Von Kossa stain X10.

Case report: Evaluation of histopathological changes in goat vein model following Implant + Filler stent implantation, using the laser microtome

Scheme of the device



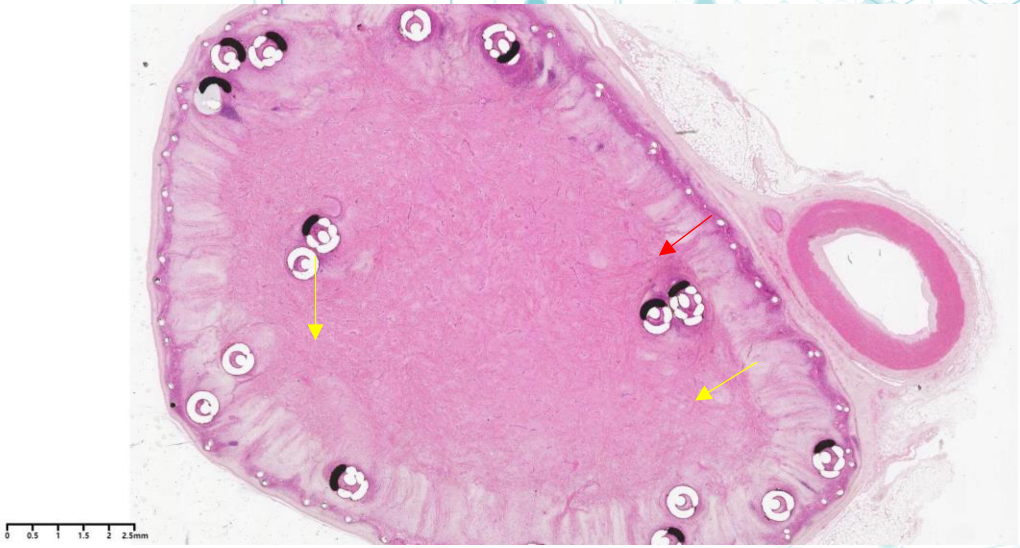
The device: A vascular embolization device, intended to control hemorrhaging due to aneurysms, vascular tumors and arteriovenous malformations. Note the two components black and green.

Gross picture of the sample after fixation

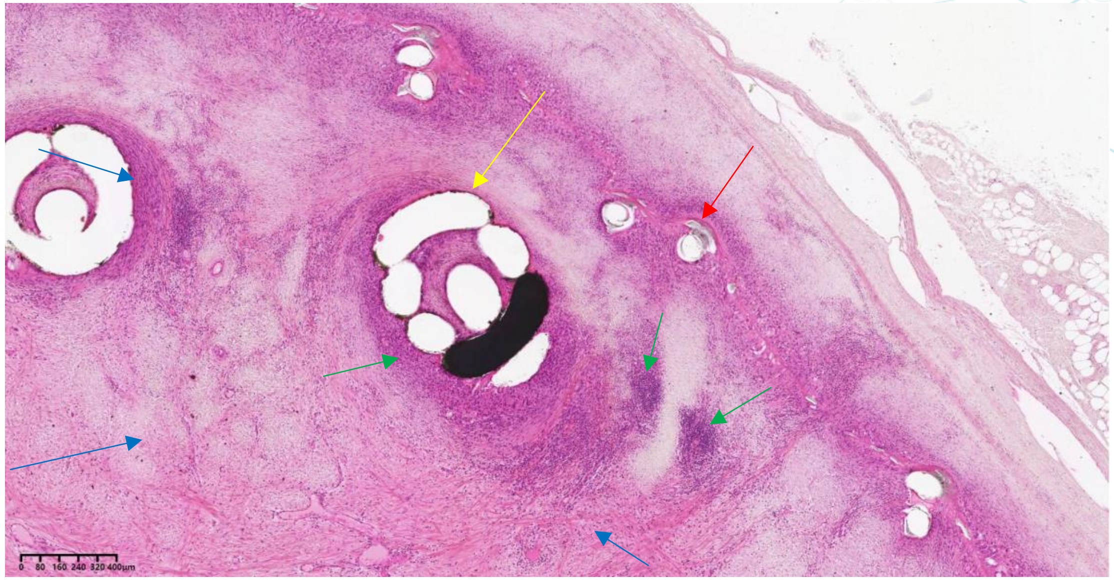
Gross: A vein with an obstruction of 1.5 cm of an implant and no apparent other pathological changes.

Case report:

A cross section within the vein device, showing the device frame (red arrow), coils (yellow arrow) and tissue ingrowth. X0.9, H&E stain.



A cross section within the vein device, In a larger magnification, showing the device frame (red arrow), coils (yellow arrow), granulation tissue ingrowth (blue arrows) and a mild inflammation (green arrows). X2, H&E.



Histopathological analysis shows a clear understanding of the tissue ingrowth and reactions. Thanks to the high quality of the tissue dissection!

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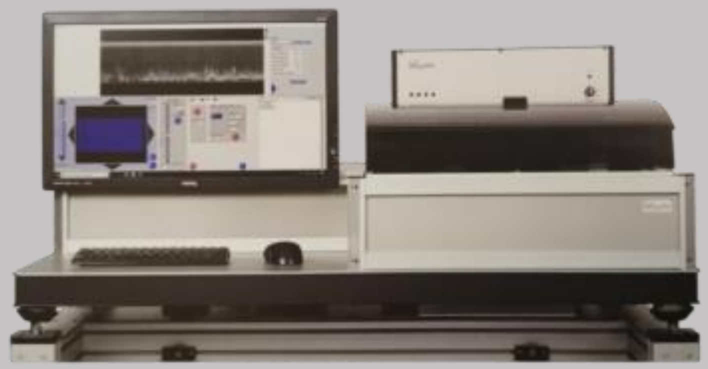
Summary

- **Fast and easy** cutting of **undecalcified** hard tissue and a broad range of implants and biomaterials.
- **Semi-serial sections,** based on minimal material loss
- Minimal sectioning artifacts, due to **contact free cutting**
- Preservation of the implant-tissue interface
- **Quality control** of sectioning, via Optical Coherence Tomography



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