GALAHAD project workshop

Dr. Juergen Schnekenburger
Edinburgh, 08.01.2020

New applications of OCT

BIOMED TECH
BioMedizinisches Technologiezentrum

GALAHAD

medizinische fakultät
Westfälische Wilhelms-Universität Münster

Westfälische Wilhelms-Universität Münster

Biomedical Technology Center (BMTZ)

BIOMED TECH
BioMedizinisches Technologiezentrum

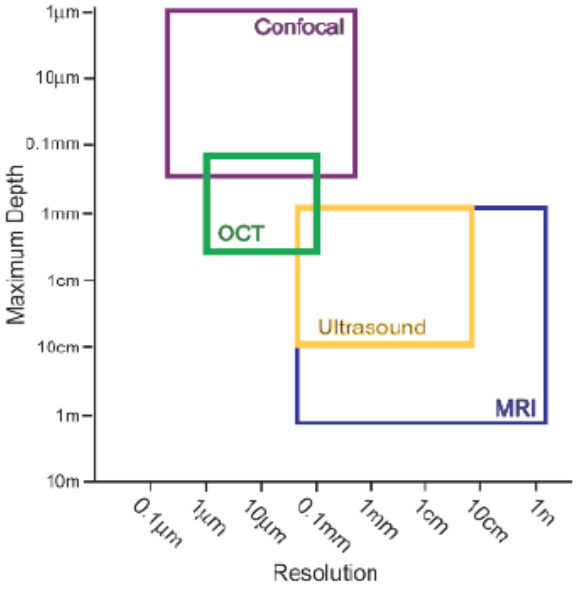
Optical Technologies/Biophotonics	Application Fields
• **Quantitative Phase Imaging (QPI)** • Flow Cytometry	• label-free analysis of living cells and tissues and toxicity testing
• **Holographic Tomography** • Fluorescence microscopy • Optical Cell Stretcher	• cellular biophysics, characterization of physiologic processes
• midIR spectroscopy • RAMAN spectroscopy	• vascular- and tumor biology • non destructive testing, high resolution surface inspection
• **Optical Coherence Tomography (OCT)**	• …

Optical technologies as OCT can be combined for specific cell and tissue analysis

OCT - Optical Coherence Tomography

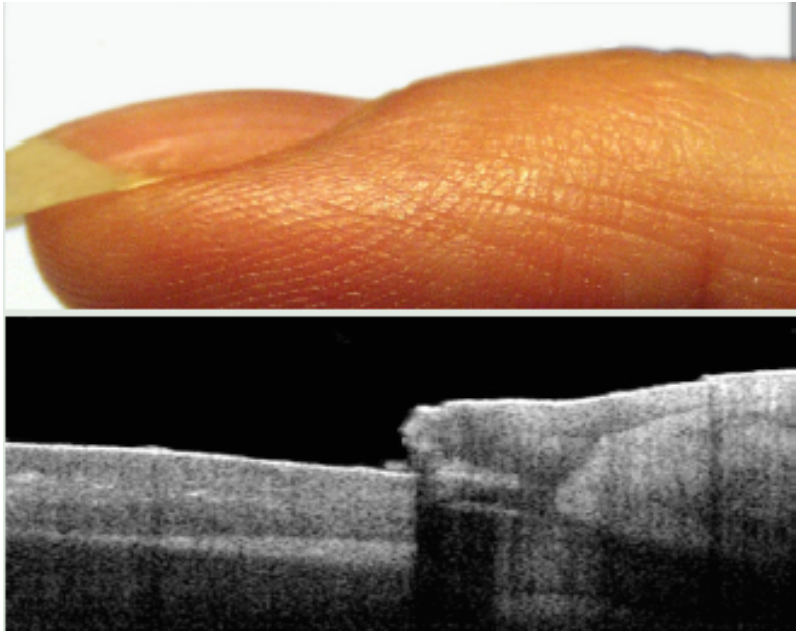
BIOMED TECH
BioMedizinisches Technologiezentrum

- Fast, real- time, high-resolution cross-sectional medical images of tissue with a micron resolution
- comparable to ultrasound imaging with the difference that OCT has a resolution at least a factor of one hundred higher
- the near- infrared light used in OCT penetrates human tissue typically only millimeters before absorption and scattering become too high.
- ultrasound has a penetration depth of more than ten centimeters while OCT has an imaging depth of only a few millimeters



OCT- Optical Coherence Tomography - Criteria for biomedical applications

- Thin structures (cells, spheroids, tissues and organisms up to 2 mm
- Regular structure, optimal are defined layers
- Stable for the time of a scan
- µm scale resolution required
- Measurement depth and resolution should cover an area that is not covered by other imaging methods



Material dependent penetration depth (up to 11 mm in air, ca. 2 mm in tissues)

OCT Optical Coherence Tomography - Biomedical Applications

- Ophthalmology - most OCT systems used today are in the field (e.g., retina or cornea examinations)
- Gastrointestinal endoscopy - commercial systems and clinical applications established
- Cardiovascular medicine - commercial systems and clinical applications established
- Dentistry - dental examination of oral tissue, dental cracks, oral cancer, prototypes available
- Dermatology – skin examination, under development
- Environmental OCT – biofilm detection

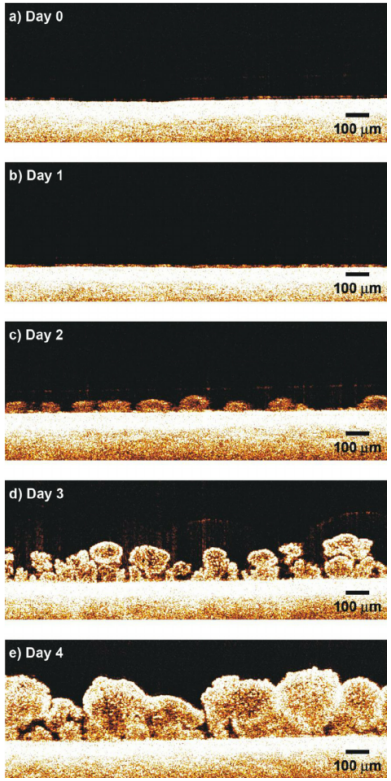
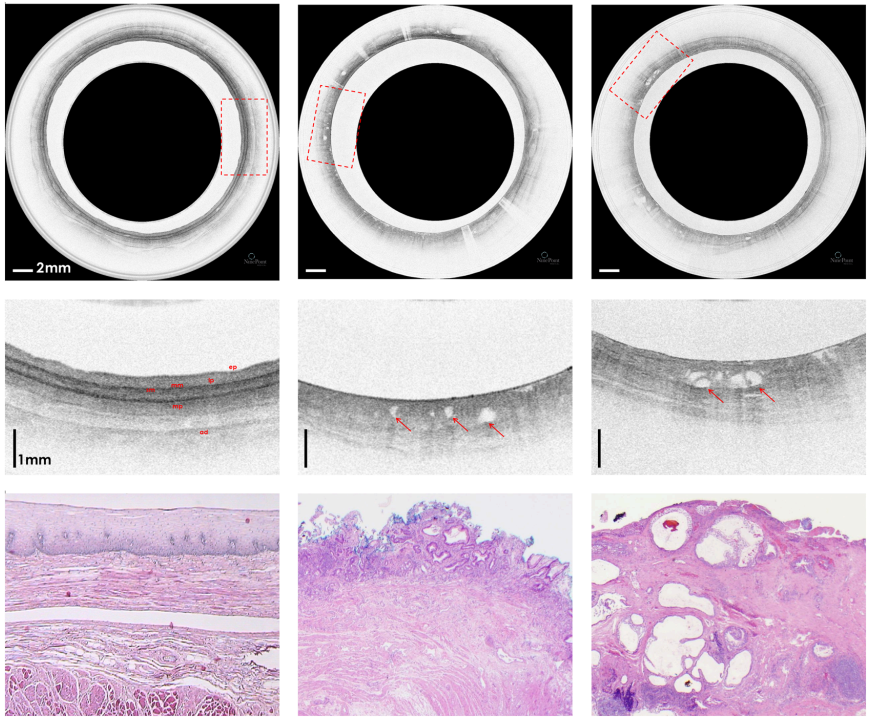


Image: Dreszer, Claudia & Wexler, Adam & Drusová, Sandra & Overdijk, T & Zwijnenburg, Arie & Flemming, Hans-Curt & Kruithof, Joop & Vrouwenvelder, J.S. (Hans). (2015). In-situ biofilm characterization in membrane systems using Optical Coherence Tomography: Formation, structure, detachment and impact of flux change.

OCT medical applications - gastroenterology

NinePoint MEDICAL

Tsung-Han Tsai, Cadman L. Leggett, Arvind J. Trindade, Amrita Sethi, Anne-Fré Swager, Virendra Joshi, Jacques J. Bergman, Hiroshi Mashimo, Norman S. Nishioka, Eman Namati, "Optical coherence tomography in gastroenterology: a review and future outlook," J. Biomed. Opt. 22(12) 121716 (19 December 2017)



(a) (b) (c)

Representative VLE circumferential (top), magnified (middle), and histological (bottom) images in the esophagus:
(a) normal esophagus shows well-defined layered structure;
(b) dysplastic BE shows loss of layering and irregular glands;
(c) esophageal cancer shows loss of layering with septate cribriform glands. Arrows indicate irregular glands. Images provided by NinePoint Medical.

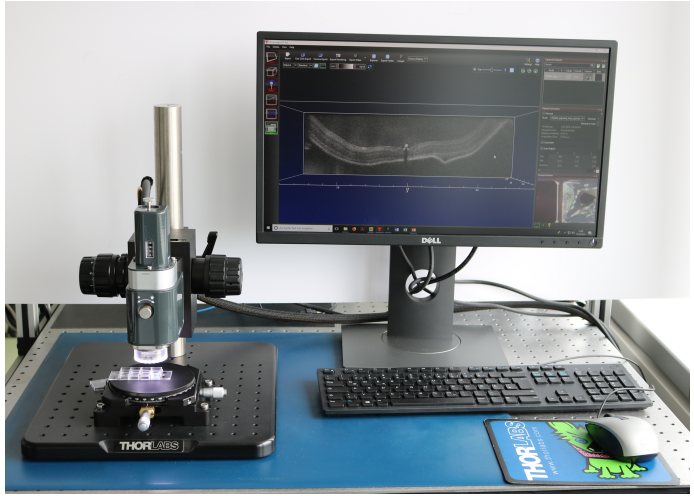
Optical Coherence Tomography at BMTZ

BIOMED TECH
BioMedizinisches Technologiezentrum

Specifications

Spectral Domain OCT

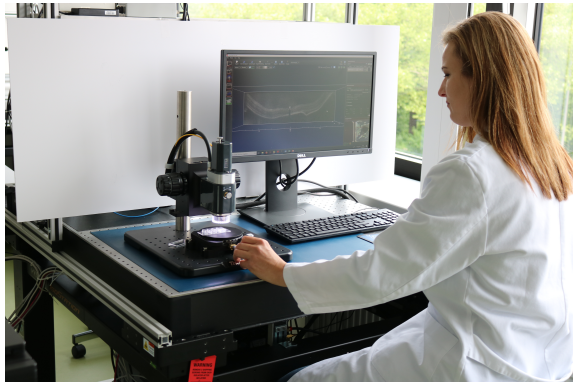
- Central wavelength: 900nm
- Spectral width ≈ 170 nm
- Axial resolution: ≈ 3 µm
- Measurement depth: ≈ 1.9 mm
- Measurement speed: 36kHz (adjustable)



Flexible probe head

Flexible magnification

- General-Purpose Scan Lens KitL (SM03-BB)
  - Lateral Resolution: 8µm
  - Working Distance: 25.1mm
  - Field of View: 10 x 10 mm²
- High-Resolution Scan Lens Kit (LSM02-BB)
  - Lateral Resolution: 4 µm,
  - Working Distance: 7.5 mm
  - Field of View: 6 x 6 mm²
- UHR-Resolution Lens (20x, OCT-LKM20-SP1)
  - Lateral Resolution: ≈ 2 µm

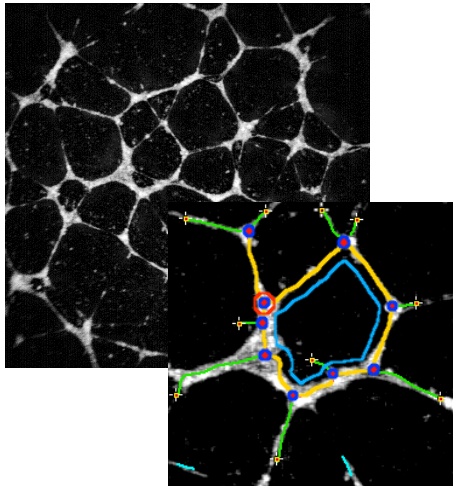


Optical Coherence Tomography of cells, tissue and animal models

BIOMED TECH
BioMedizinisches Technologiezentrum

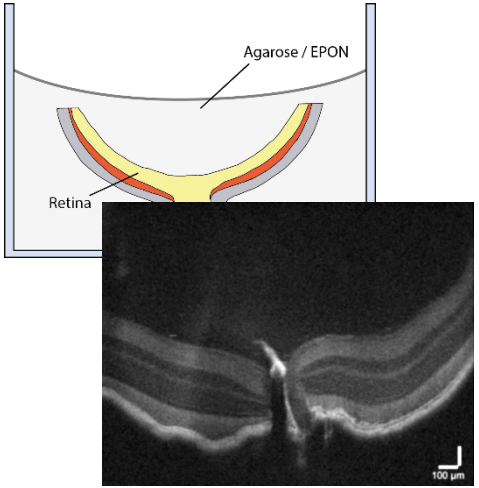
1. Single cell level

HUVECs cells



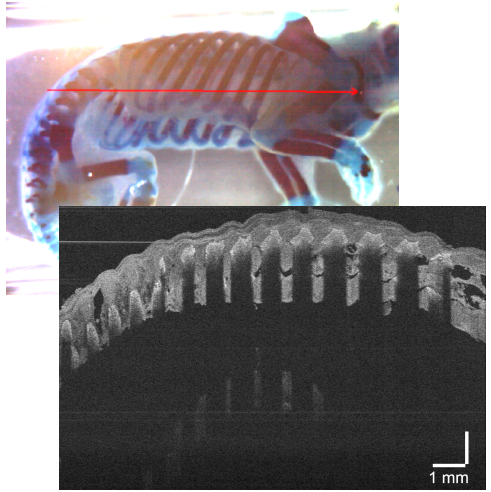
2. Tissue models

Murine retina tissue



3. Animal models

Rat skeleton

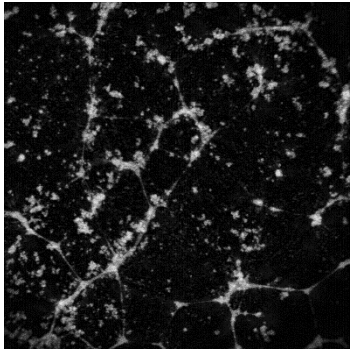


Cell differentiation - tube formation assays with Optical Coherence Tomography

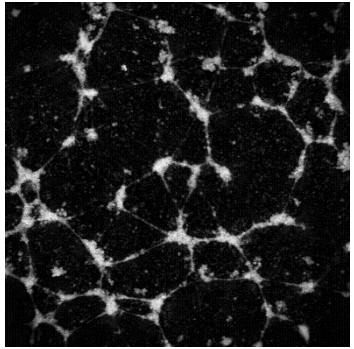
BIOMED TECH
BioMedizinisches Technologiezentrum

- Angiogenesis assay on coated plates with matrigel matrix
  - Cells cultivated 24 hours in 96-well plate

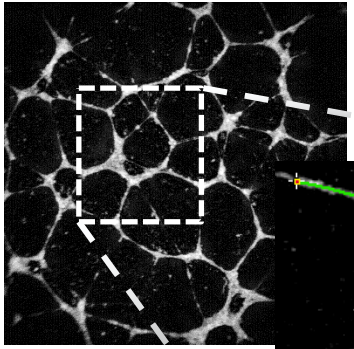
Cancer cells MDA-MB- 231 (no tube formation)

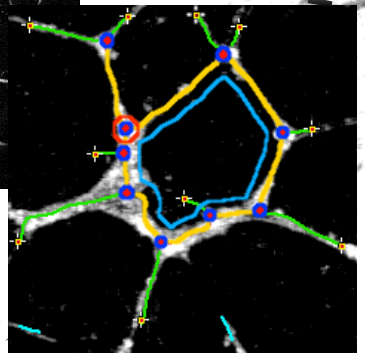


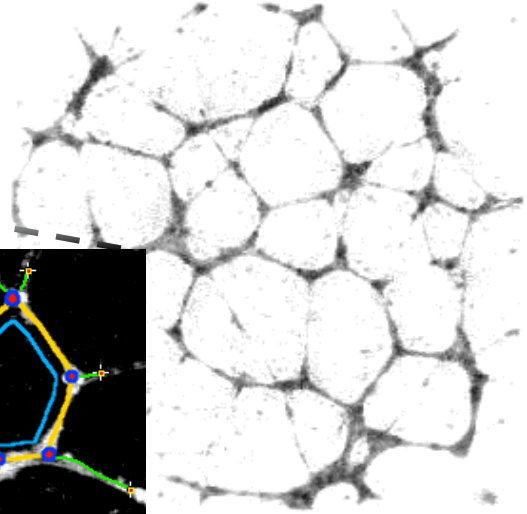
miR1-treated cancer cells (MDA-MB- 231) & HUVECs cells (no tube formation)



cancer cells (MDA-MB- 231) & HUVECs cells (tube formation)







- Quantitative 3D analysis [1]
  - Tube length, number of nods and meshes…

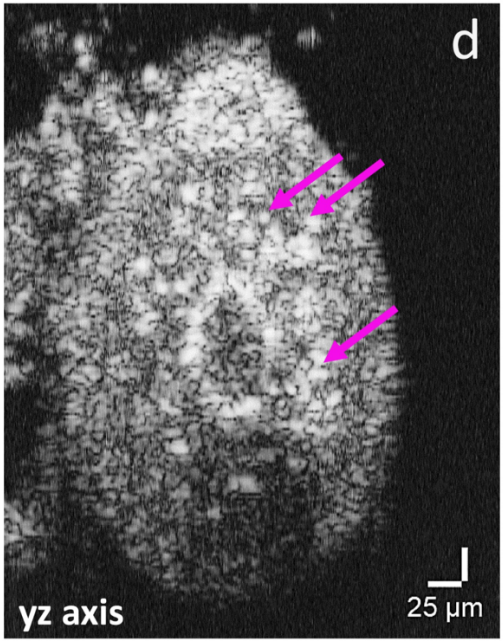
*[1] Eglinger, J, . et al.. Inflammation and Regeneration **37(2)**, 2017*

OCT as a novel intravital method for human hair follicle morphological analyses

BIOMED TECH
BioMedizinisches Technologiezentrum

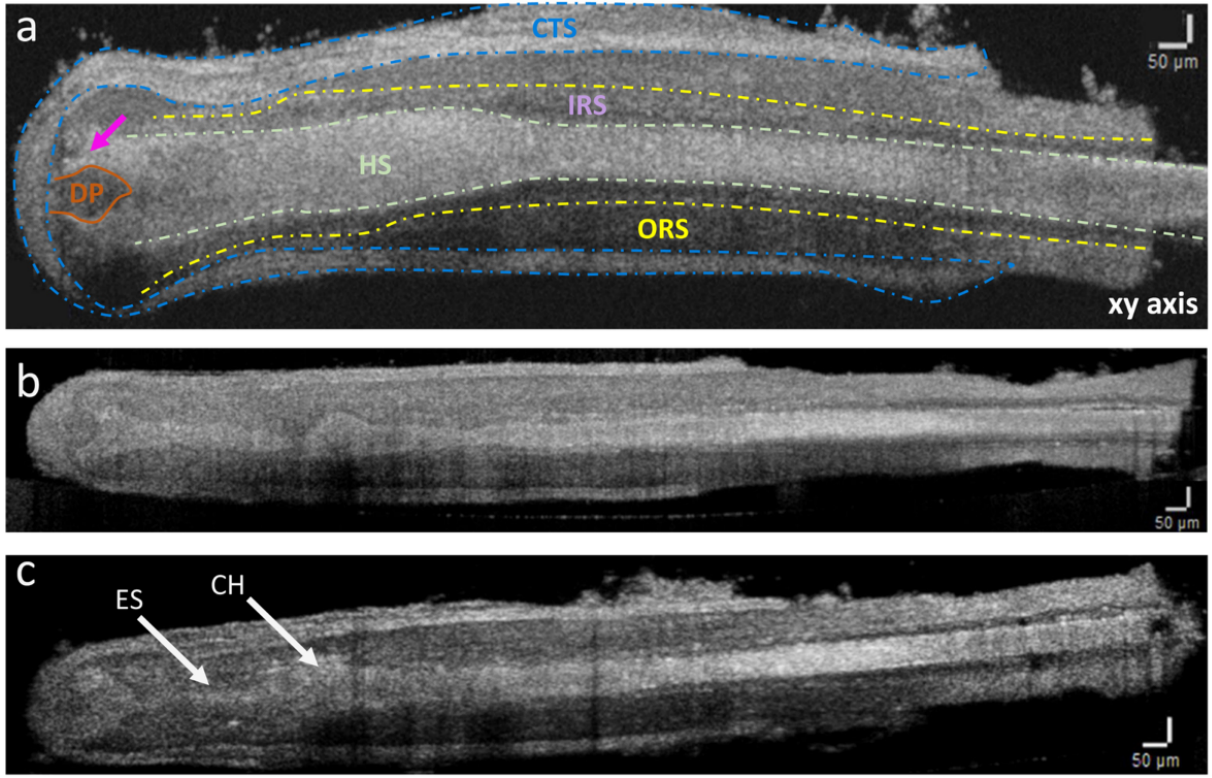
- HFs have to be fixed for histology (and thereby killed) to discern their anatomy in sufficient detail.
- OCT was used as an **intravital method** for obtaining **2D or 3D high-resolution images** of viable human microdissected HFs at a previously unreported level of anatomical detail.

- OCT allows **stress-free temporal analysis** of **morphological HF changes.**
- Key HF compartments (dermal papilla, outer root sheath, inner root sheath, connective tissue sheath, hair shaft) and even distinctive cell populations, such as HF pigmentary unit melanocytes, can be visualised in live HFs.
- OCT is a powerful intravital method to obtain important first indications on whether a specific substance affects growth, and possibly pigmentation or hair shaft structure, in treated live HFs *ex vivo*.



→ : Melanocytes?

Intravital hair cycle staging using OCT

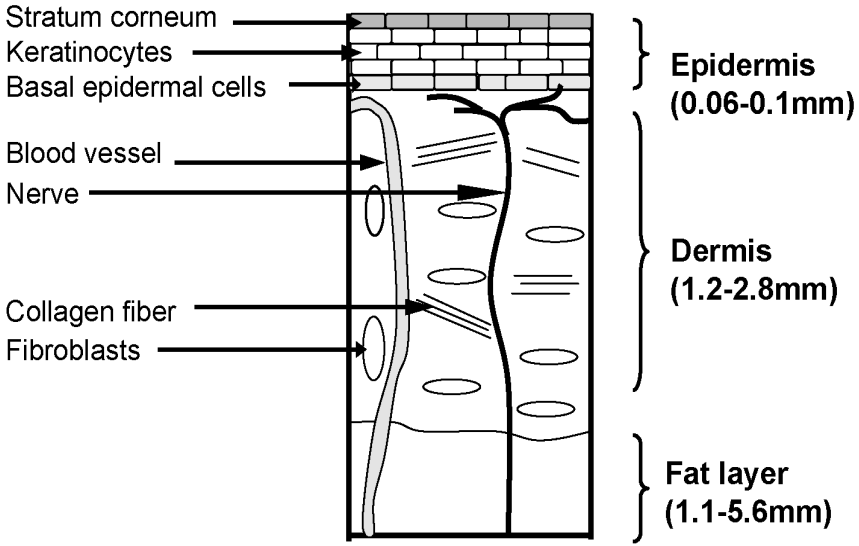


Organ-cultured **hair cycle stages** anagen (a) and catagen (b, c) HFs, i.e. appearance of brush-like club hair (c), opening of the hair matrix tips (b, c).

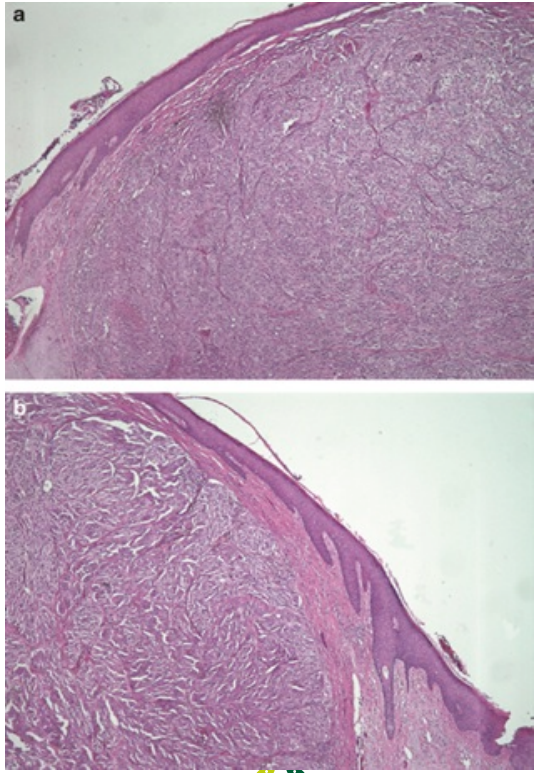
DP, dermal papilla; ORS, outer root sheath; IRS, inner root sheath; CTS, connective tissue sheath; HS, hair shaft; ES, epithelial strand; CH, brush like club hair; pink arrows: melanocytes.

Using OCT, it is possible to distinguish macroscopically each HF stage, i.e. anagen, early, mid or late catagen, in live microdissected HFs.

OCT - Skin examination

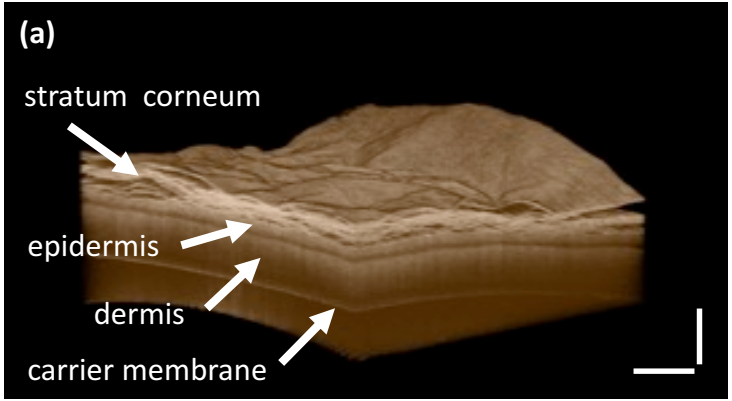


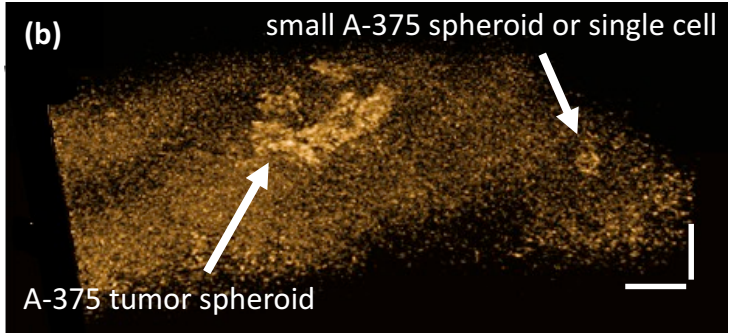
Scheme of human skin layers. From Nacer Chahat, Maxim Zhadobov and Ronan Sauleau. Antennas for Body Centric Wireless Communications at Millimeter Wave Frequencies. DOI: 10.5772/58816 in Progress in Compact Antennas", Ed. Laure Huitema, ISBN 978-953-51-1723-0, September 10, 2014.



Melanoma nodal tumor. These two photomicrographs demonstrate the sharp circumscription that characterizes nodular melanoma. A large, well-circumscribed proliferation of atypical melanocytes is seen growing throughout the dermis. From: Bruce R Smoller. Histologic criteria for diagnosing primary cutaneous malignant melanoma. Modern Pathology (2006) 19, S34–S40

OCT - Skin morphology

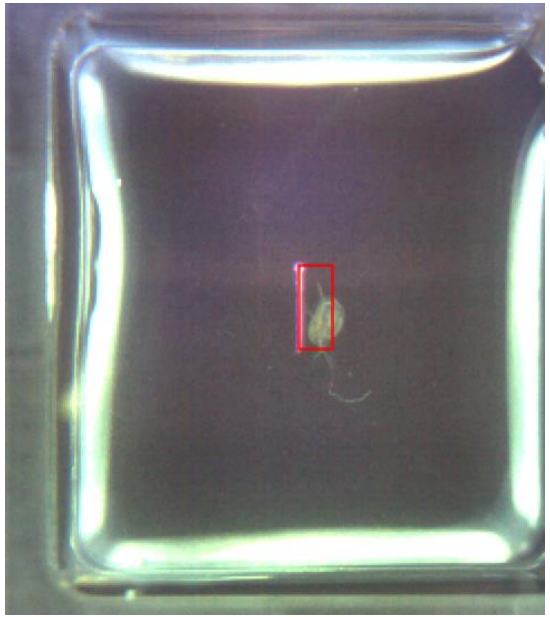




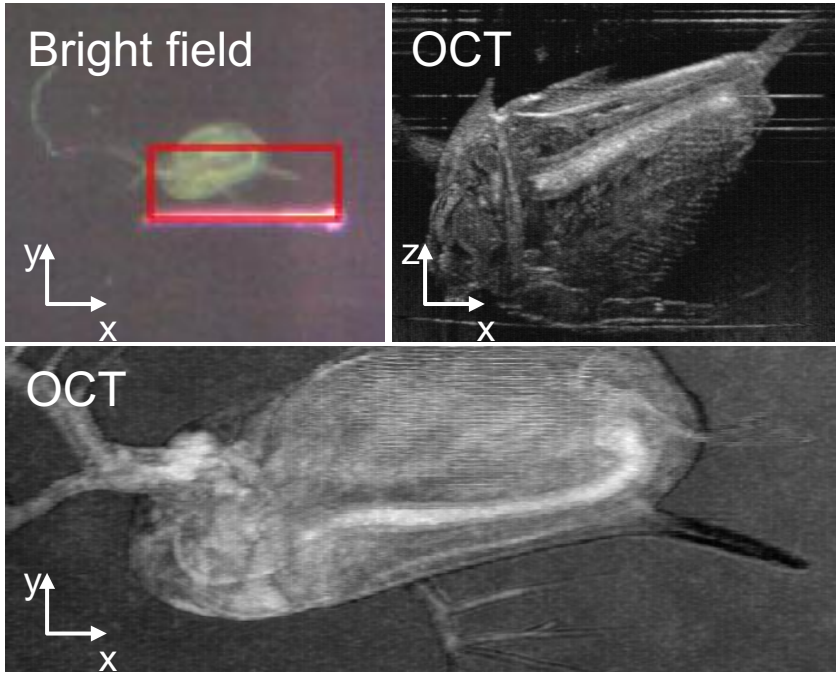
Representative optical coherence tomography image of a 3D skin equivalent. The different tissue layers (stratum corneum, epidermis and dermis) and the carrier membrane are clearly resolved. Scale bars are equal to 250 µm;

(b): Representative optical coherence tomography image of 3D tissue models with included tumor spheroids. Scale bars are equal to 50 µm.

OCT - Microplastic particle detection in Daphnia magna

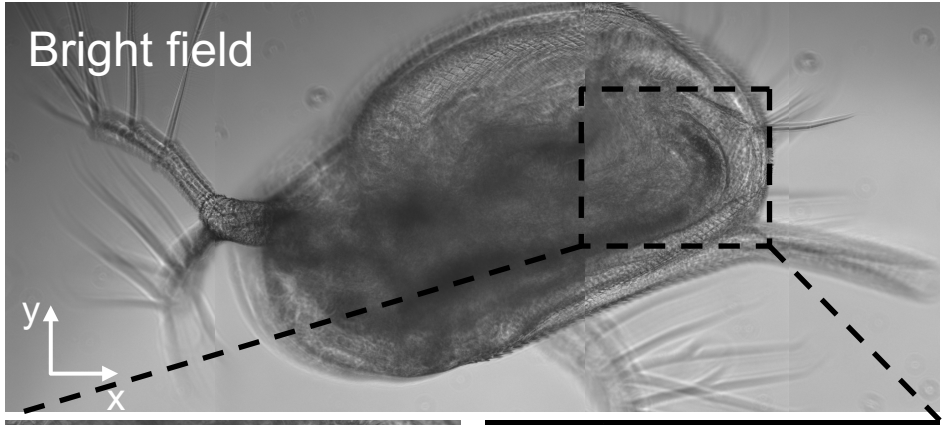


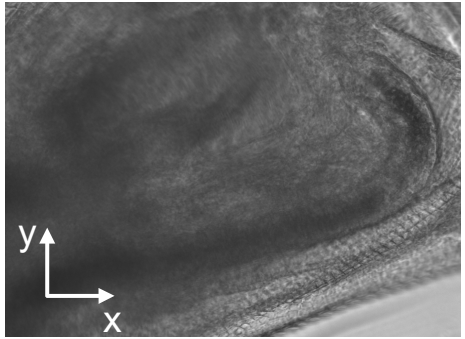
Live *Daphnia magna* were embedded in agarose

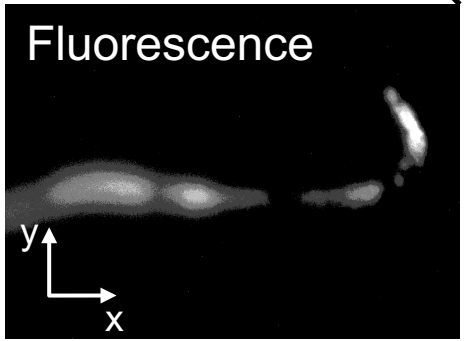


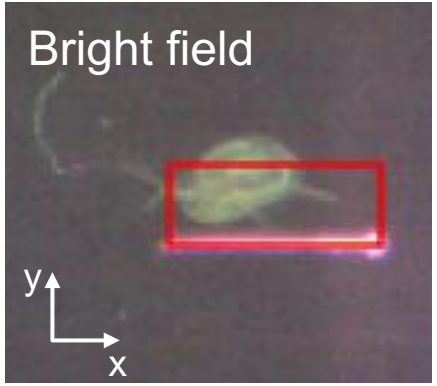
Comparison of fluorescence microscopy and OCT of Daphnia in solution with 8µm fluorescent PMMA beads

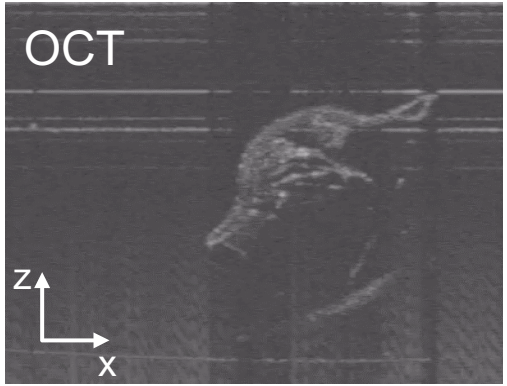
OCT - Microplastic particle detection in daphnia magna intestines







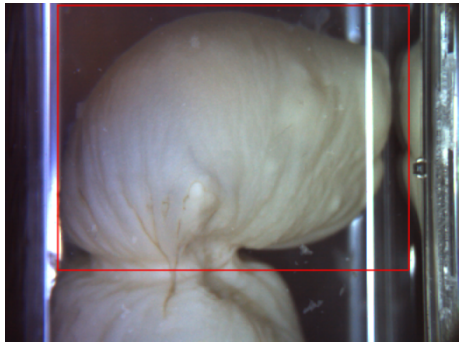


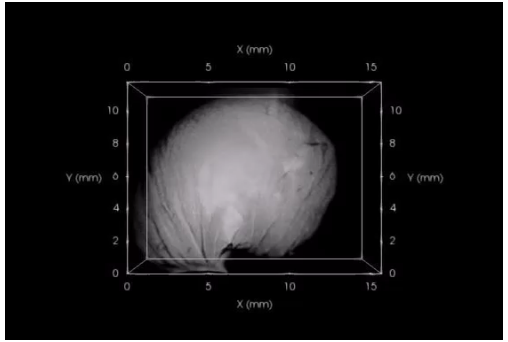


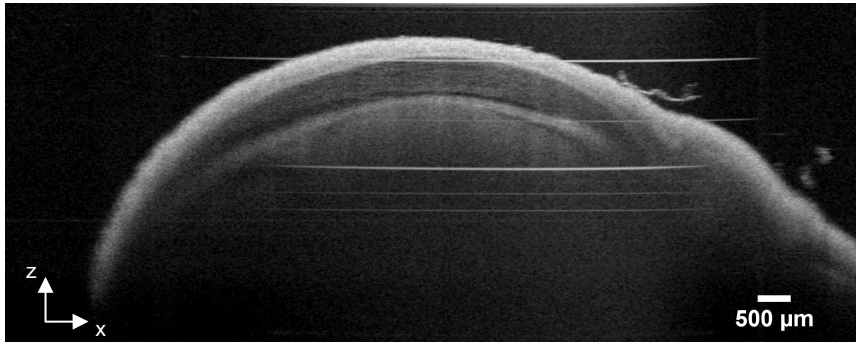
Investigation of animal models for regulatory toxicity tests of chemicals and drugs with OCT

BIOMED TECH
BioMedizinisches Technologiezentrum

- Fixated rat embryo embedded in glycerol





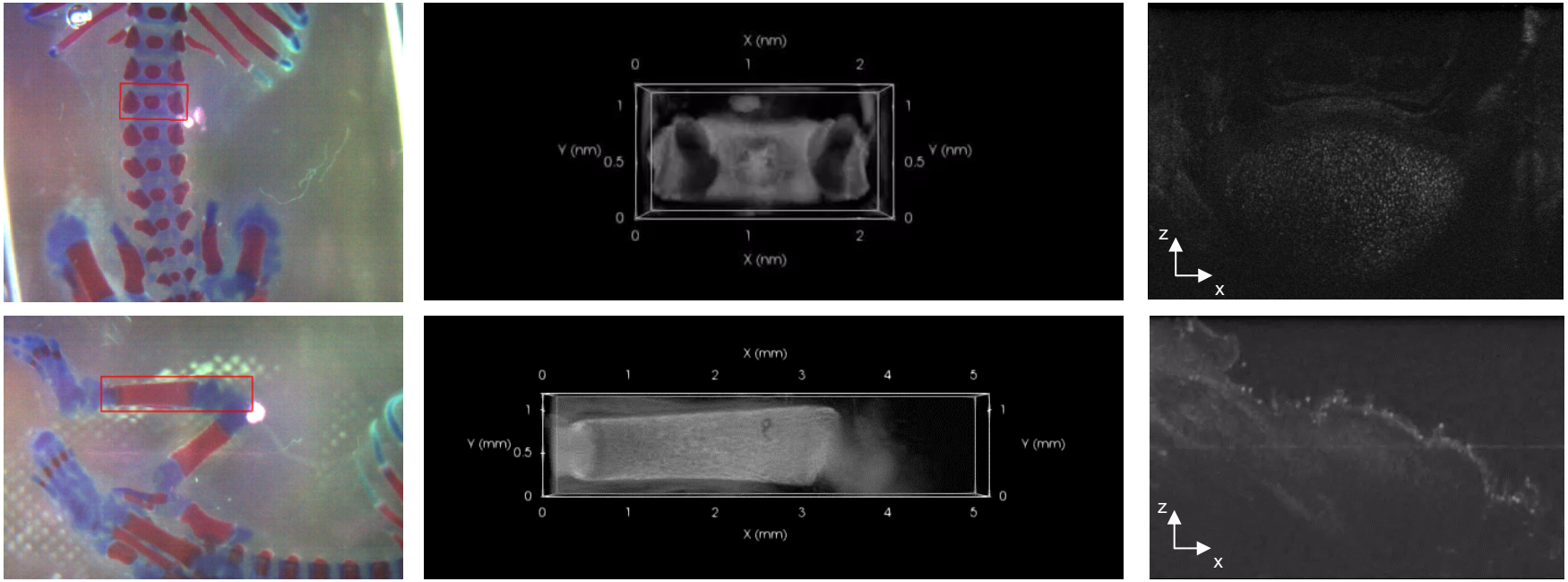


- Regulatory toxicity testing for REACH or EMA/FDA approval of chemicals and drugs requires a variety of animal testings including teratology testing.
- A high number of animal samples and tissue slices need examination.
- Demand for new imaging and AI technologies for sample analysis.
- Evaluation of OCT application in veterinary pathology.

Investigation of animal models with Optical Coherence Tomography

BIOMED TECH
BioMedizinisches Technologiezentrum

- Skeleton of rat embryo embedded in glycerol (vertebra and leg)



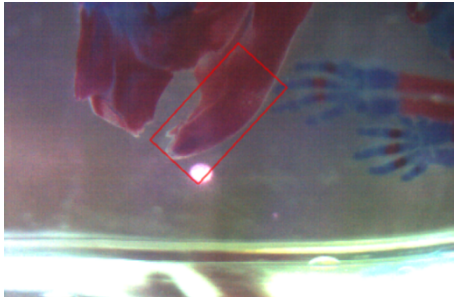
medizinische fakultät
Westfälische Wilhelms-Universität Münster

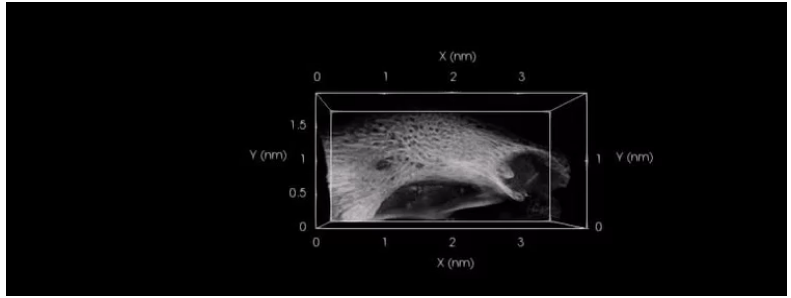
Westfälische Wilhelms-Universität Münster

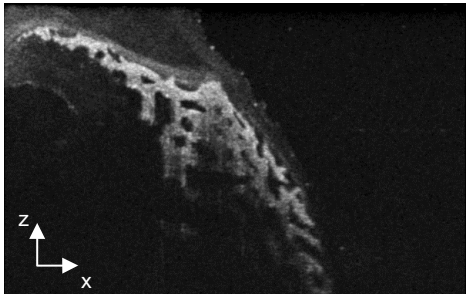
Investigation of animal models with Optical Coherence Tomography

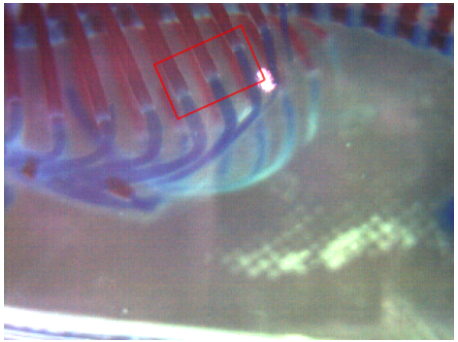
BIOMED TECH
BioMedizinisches Technologiezentrum

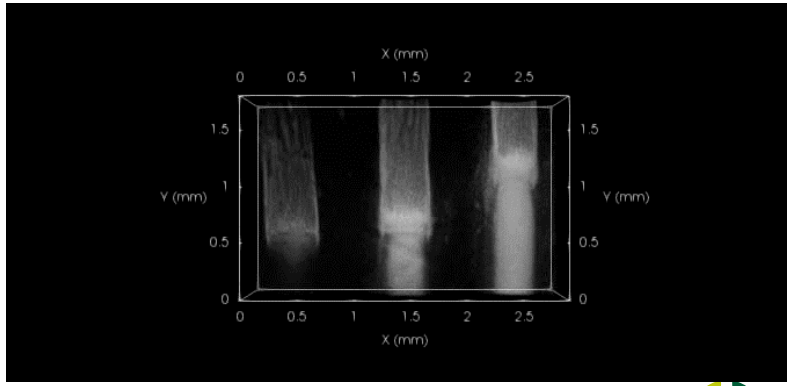
- Skeleton of rat embryo embedded in glycerol (jaw and ribs/cartilage)

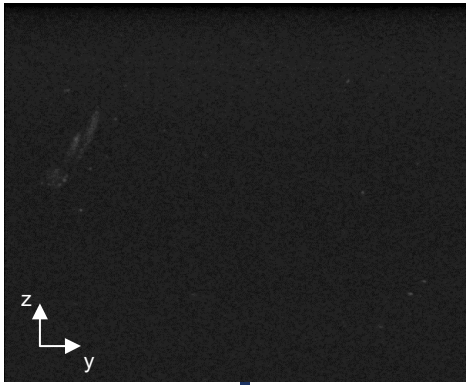












Conclusions

BIOMED TECH
BioMedizinisches Technologiezentrum

OCT can address various processes and topics in cell, tissue and whole animal analysis by label-free detection and quantification of:

- growth / proliferation of structures
- morphology of tissues and tissue alterations
- **Main challenges for further establishing the techniques are**
- Increasing lateral and axial resolution
- AI based image analysis and data extraction

⇒ **OCT is a multifunctional biomedical tool for the analysis of 3D *in-vitro* cells and *ex-vivo* tissues and animal samples**
⇒ **OCT has a broad application field – just try!**

In collaboration with

Biomedical Technology Center, University of Muenster, Muenster, Germany
Björn Kemper
Steffi Ketelhut
Alvaro Barroso Pena
Lena Kastl

Department for Ophthalmology, University Hospital, Muenster, Germany
Peter Heiduschka

BASF SE, Ludwigshafen, Germany
Robert Landsiedel
Sibylle Groeters
Steffen Schneider

Thorlabs GmbH, Lübeck, Germany
Martin Krah
Sebastian Schäfer
Martin Thunert
Matthias Pues

Monasterium Laboratories
Janine Lehmann

Wessling GmbH
Jens Reiber
Stefan Grass

BioMed Tech
BioMedizinisches Technologiezentrum

medizinische fakultät
Westfälische Wilhelms-Universität Münster

Westfälische Wilhelms-Universität Münster

Acknowledgements / funding

NMWP.NRW
Cluster Nano Mikro Werkstoffe Photonik

GIF
German-Israeli Foundation for Scientific Research and Development

Bundesministerium für Ernährung und Landwirtschaft

SEVENTH FRAMEWORK PROGRAMME

BiophotonicsPlus
European Competition for Collaborative R&D Funding

Bundesministerium für Bildung und Forschung

Horizon 2020
European Union funding for Research & Innovation

GALAHAD

Dr. Juergen Schnekenburger
Biomedical Technology Center
Mendelstr. 17
48149 Muenster
Germany
schnekenburger@uni-muenster.de

Thank you for your attention !

BioMed Tech
BioMedizinisches Technologiezentrum

medizinische fakultät
Westfälische Wilhelms-Universität Münster

Westfälische Wilhelms-Universität Münster