Peter E. Andersen
DTU Health Tech
Technical University of Denmark
peta@dtu.dk

Biophotonic Imaging: Early Disease Detection and Guidance

About My Professional Life

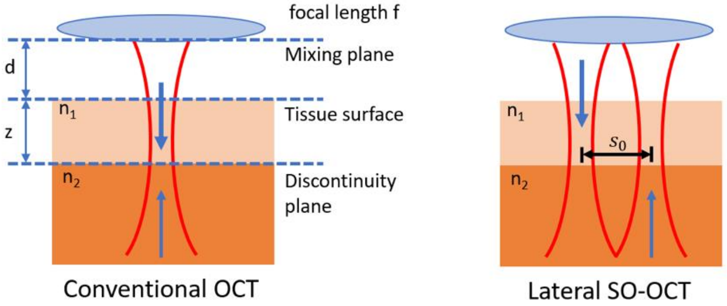
- Key points
  - Obtained my MSc (1991) and PhD (1995) in optical materials and nonlinear optics from DTU
  - After my PhD, I explored new territory by switching to **biomedical optics** in my postdoc (3 years)
  - Since 2000, I have been leading research groups in biomedical imaging techniques and applications at DTU and Lund University (Sweden).
  - Since 2006, I have coordinated and led several European research and education programmes
  - In 2014, I co-founded Norlase (medical device manufacturer)

- Research area: Biomedical optics and imaging
  - Multimodal biophotonic imaging; nonlinear/multiphoton microscopy, optical coherence tomography
  - Light-tissue interactions
  - Optical imaging systems development and clinical/biomedical applications within dermatology, ophthalmology, and endoscopy

- Appointments (Editorial Board Member)
  - Journal of Biophotonics (2010-)
  - Journal of Biomedical Optics (2013-)
  - Light: Science and Application (Nature, 2020-)

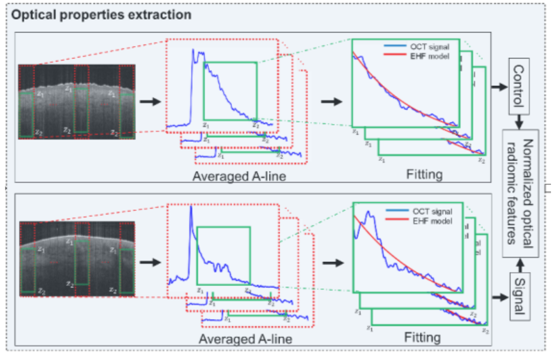
- Community Contributions
  - Co-founded The International Graduate Summer School on Biophotonics (2003 – onwards)
  - Diversity & Inclusion Task Force installed at CLEO

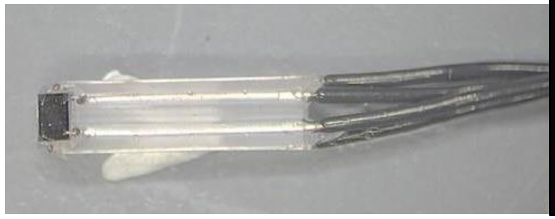
Biophotonic, Functional Imaging

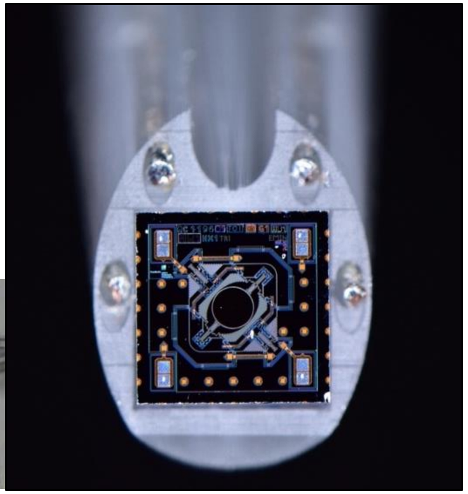


Multimodal imaging concepts combining structural high-resolution imaging and functional information through

- optical coherence tomography/microscopy
- multi-photon tomography/microscopy & light sheet microscopy
- tissue optics and modelling





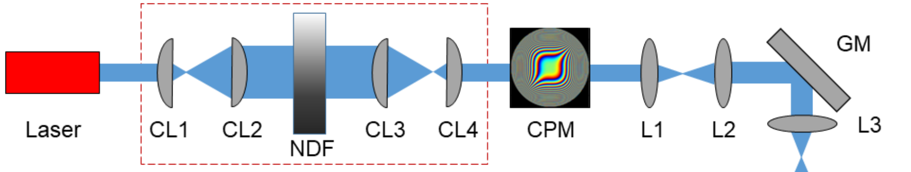


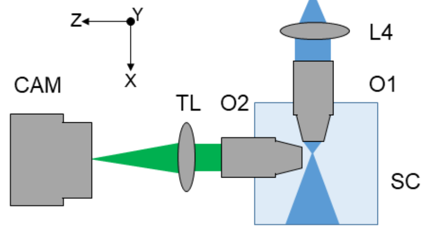
Current research challenges

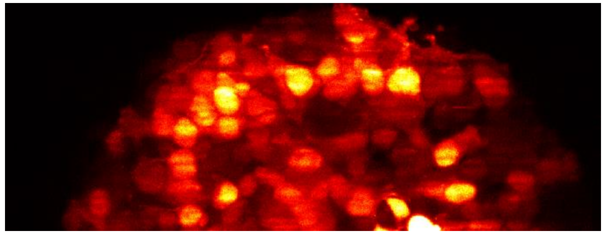
- integration of multimodal imaging into probes
- understanding optically measured biomarkers and their diagnostic potential impact
- tissue optics, light propagation and modelling for diagnostic and clinical applications

Funding sources (selected current/recent)

- MIB (Coordinator, RIA; H2020); *systems and endoscopy (light delivery)*
- FBI (Coordinator, ITN, MCSA); *sources and multimodal systems*
- T-SPIF (DFF-FTP); *light delivery and light sheet microscopy*
- CAG-IGCS; *Image-guided cancer surgery*
- PROSCOPE (Coordinator, RIA; H2020); *endoscopy and colo-rectal cancer diagnosis*







MIB-H2020.eu
Innovation transforming healthcare

FBI

PROSCOPE

Biophotonic Imaging Group (09-2024)

Faculty

- Peter E. Andersen

Postdocs & PhD Students

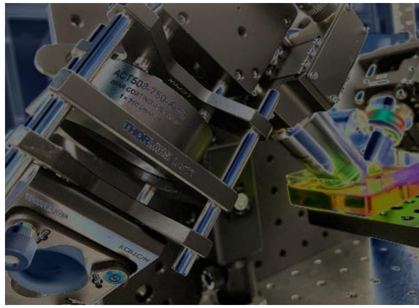
- Madhu Veettikazhy (postdoc)
- Gavrielle Untracht (postdoc)
- Anja Lykke Borre (graduated 09-24)
- Freja Høier
- Setareh Malekdoust (assistant)
- Helton Ciriaco (tech. assistant)

Master students (Summer/Autumn 2024)

- 7 MSc students
- 4 students BSc
- 5 project students

Alumni (2019-)

- Iliana Petridi (MSc, 2024)
- Adéle Hamon (MSc, 2024)
- Jirapa Limsuriwong (MSc, 2024)
- Matina Georgiou (MSc, 2024)
- Johan Barfoed (MSc, 2024)
- Louise Frost (MSc, 2024)
- Maja Johannesen (BSc, 2024)
- Mette-Sofie Johansen (BSc, 2024)
- Anne-Mette Meijburg (BSc, 2024)
- Othilia Wagner (BSc, 2024)
- Anna Victoria Vejlsby (BSc, 2024)
- Theofanis Angelis (MSc, 2024)
- Konstantinos Karageorgos (MSc, 2024)
- Maria Pedersen (MSc, 2024)
- Stefan Mark Jensen (MSc, 2024)
- Anna Arregui (2024)
- Sascha Siri Dahl (2023)
- Dominika Melczer (MSc, 2023)
- Lars Lindvold (faculty, 2023)
- Sofie Degn (BSc, 2023)
- Lasse Bo Mortensen (BSc, 2023)
- Josephine Schwarz (BSc, 2023)
- Ali Mohebi (visiting PhD student March-August 2022)
- Magnus Nymann (2022)
- Monika Justyna Kupska (MSc, 2022)
- Oriol Vidal Casasayas (MSc, 2022)
- Jeremie Sobel (MSc, 2022)
- Keqing (Sunny) Dai (MSc, 2022)
- Dominik Marti (faculty, 2022)
- Michael S. N. Madsen (BSc, 2022)
- Merle Loop (Dipl. Thesis, 2022)
- Peter Groth Stounbjerg (MSc, 2022)
- M. Tahir Jamal (postdoc 2021)
- Rasmus Tue Nielsen (MSc, 2021)
- Kristian Moltved (MSc, 2021)
- Joana Kira Besecke (MSc, 2021)
- Vasiliki Koulianou (MSc, 2021)
- Morgane Zimmer (MSc, 2021)
- M. Tahir Jamal (PhD, 2020)
- Lærke Krøjer (MSc, 2020)
- Adrianna Rokosa (MSc, 2020)
- Mahmoud Tawfieq (postdoc, 2020)
- Björn-Ole Meyer (PhD, 2020)
- Ida Videcrantz (MSc, 2019)



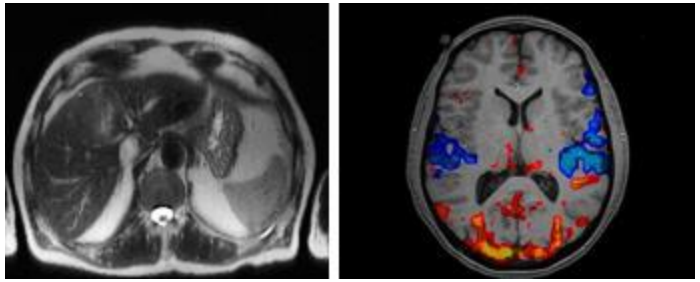
Towards Early Diagnosis of Colo-Rectal Cancer

PROSCOPE: Point-of-care instrument for diagnosis and image-guided intervention of Colo-Rectal Cancer

Peter E. Andersen, Technical University of Denmark

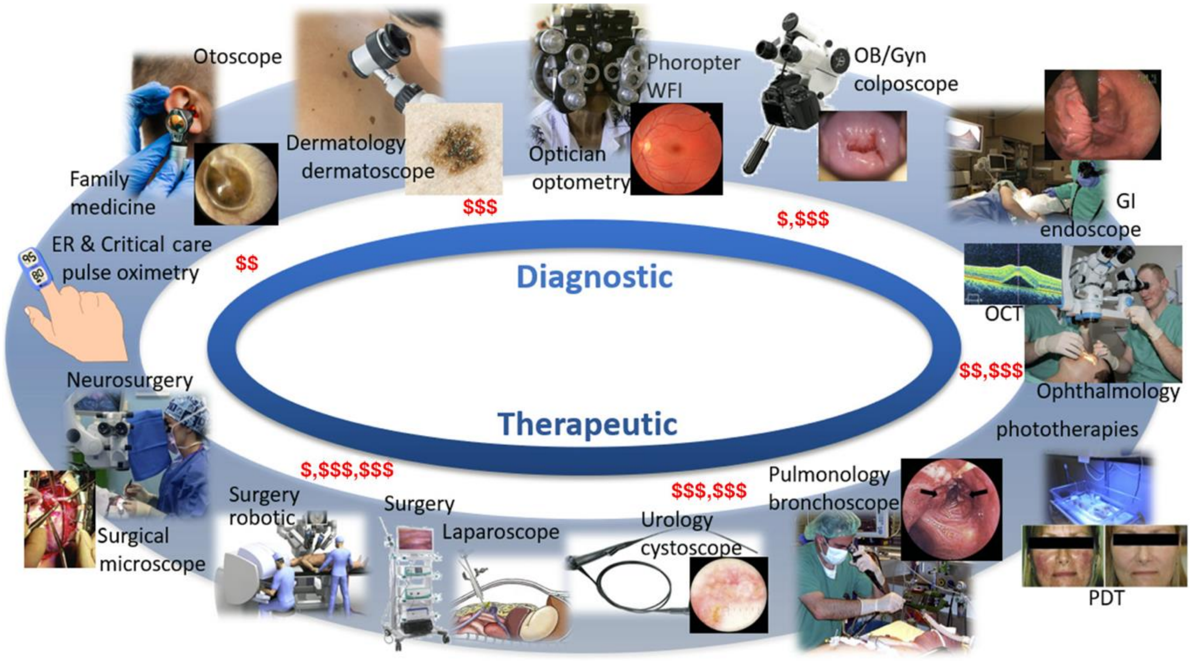
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 871212

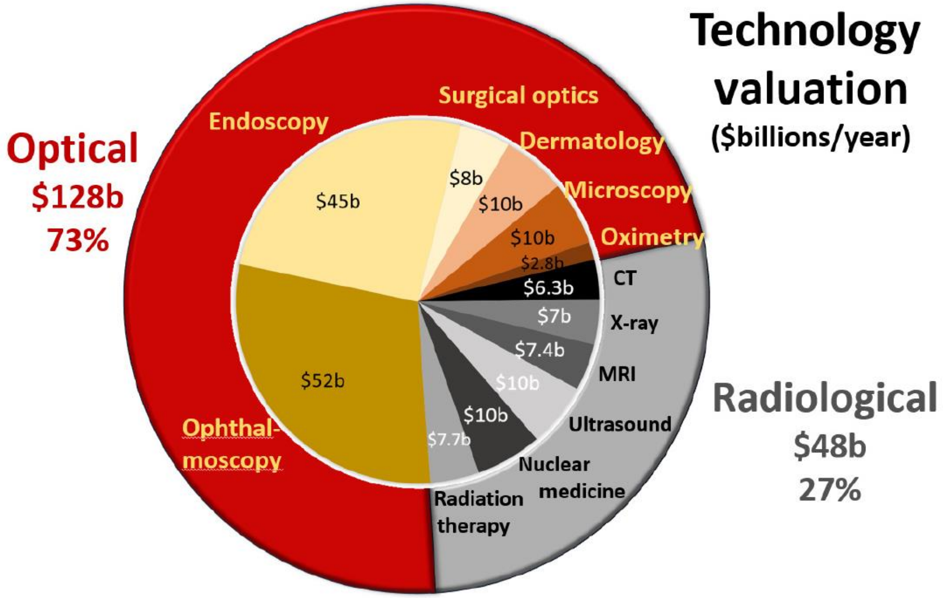
Diagnostic Imaging: WHO Website



- Conventional radiography
- Fluoroscopy
- Angiography
- Mammography
- Computed Tomography
- Ultrasound and Ultrasound/Doppler
- Magnetic resonance Imaging
- Nuclear Medicine
  - involves radioactive substances in the diagnosis (and treatment) and images function
  - single photon emission computed tomography (SPECT) and positron emission tomography (PET) scans most commonly know
- **Optical Imaging**
  - is it relevant for diagnostic imaging?

Optics Plays a Dominant Role in Medical Imaging Market





Global market valuation of six radiological areas is summarized above totaling $48 billion/year for 27% of the global device market. The ***optical technology areas*** *that were largest are also summarized* ***totaling $128 billion/year for 73%*** *market share.*

"More broadly, the largest societal impacts would be in earlier detection via screening technologies."

https://spie.org/news/spie-professional-magazine/2018-january/optics-of-medical-imaging?SSO=1

BW Pogue. Perspective on the optics of medical imaging. JBO (2023). DOI: 10.1117/1.JBO.28.12.121208

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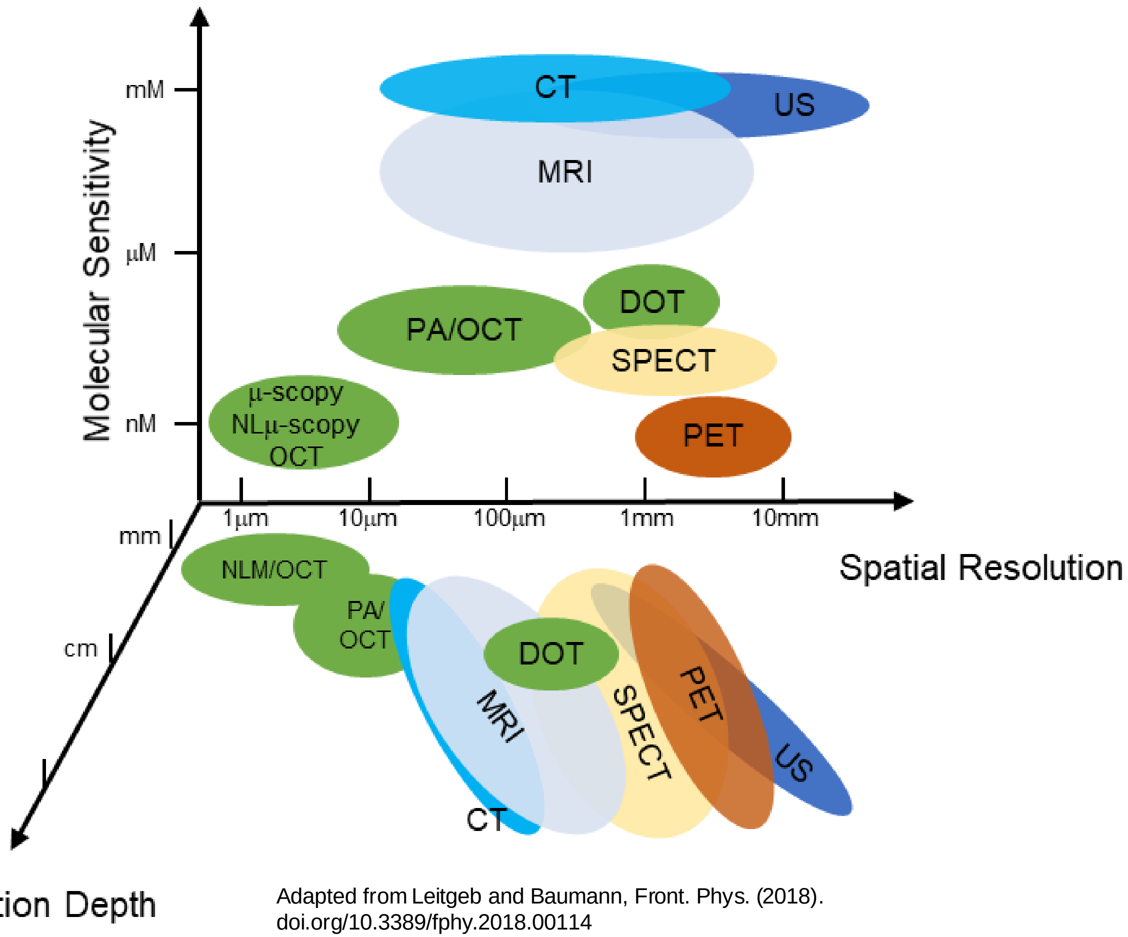
Grant Agreement No. 871212

Optical Imaging Provides Biomarkers *In Vivo*, Real Time Suited for Longitudinal Studies

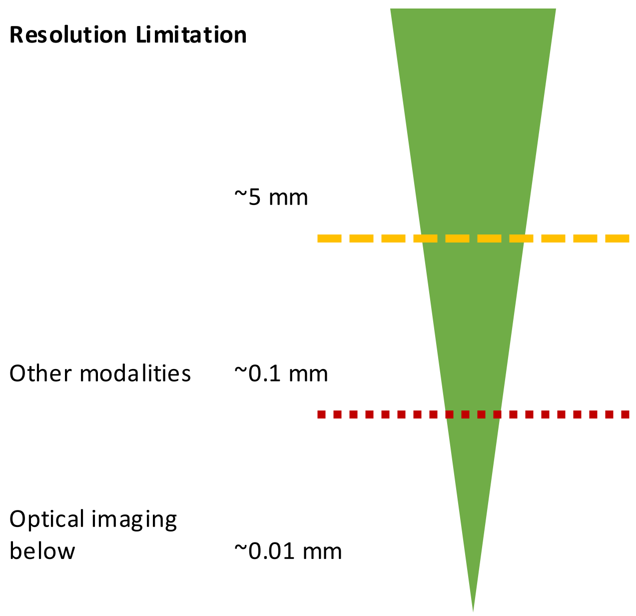
PROSCOPE

Established Biomarker	Existing methods		Photonics	
	Excisional biopsy (ex vivo)	Imaging (in vivo)	Optical modality	Optical modality provides (**in vivo/real time, longitudinal**)
Morphology				
Structure	Specific staining, processing and microscopy-based evaluation	▪ Ultrasound ▪ MRI ▪ CT	▪ OCT ▪ Multiphoton: SHG, THG	▪ OCT: Morphology up to 2 mm depth with subcellular resolution measuring tumour borders/demarcation ▪ MPM: Cellular level morphology (~1 mm depth), providing information on infiltration; contrast from lipids/collagen
General cell markers				
DNA, proteins, lipids, collagen	Specific staining, processing and microscopy-based evaluation	▪ fMRI	▪ Raman spectroscopy (CARS) ▪ Multiphoton: SHG, THG	▪ Label-free molecular information from DNA, lipids, proteins and collagen ▪ **Grade classification**
NADH/FAD (metabolites), cell nuclei vs cell size ratio (pleomorphism), cytoskeleton	Specific staining, processing and microscopy-based evaluation	▪ fMRI ▪ PET	▪ MP fluorescence microscopy ▪ MP fluorescence life-time imaging	▪ Biochemical/metabolic information from NADH/FAD and pleomorphism with sub-cellular resolution imaging ▪ **Grade classification and metabolic information**
Micro-angiography	Micro vessel density (CD31 immunohistochemistry)	▪ CT / µCT ▪ fMRI ▪ 1-P fluorescence microscopy	▪ OCTA ▪ Laser Doppler ▪ Opto-Acoustics	▪ OCTA: Micro-angiogenesis additional functional parameter ▪ Opto-acoustics: Oxygenation mapping

Medical Imaging Technology Landscape



Adapted from Leitgeb and Baumann, Front. Phys. (2018).
doi.org/10.3389/fphy.2018.00114



Optical imaging provides biomarkers in vivo, real time suited for longitudinal studies

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The Challenge: Optical Biopsy

(wikipedia.org/wiki/Endomicroscopy)

Optical biopsy

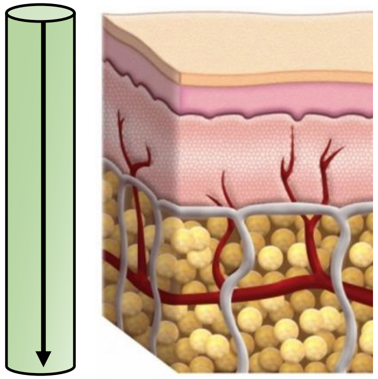
Any technique that provides imaging of tissue morphology <u>without</u> the need for excision of the tissue

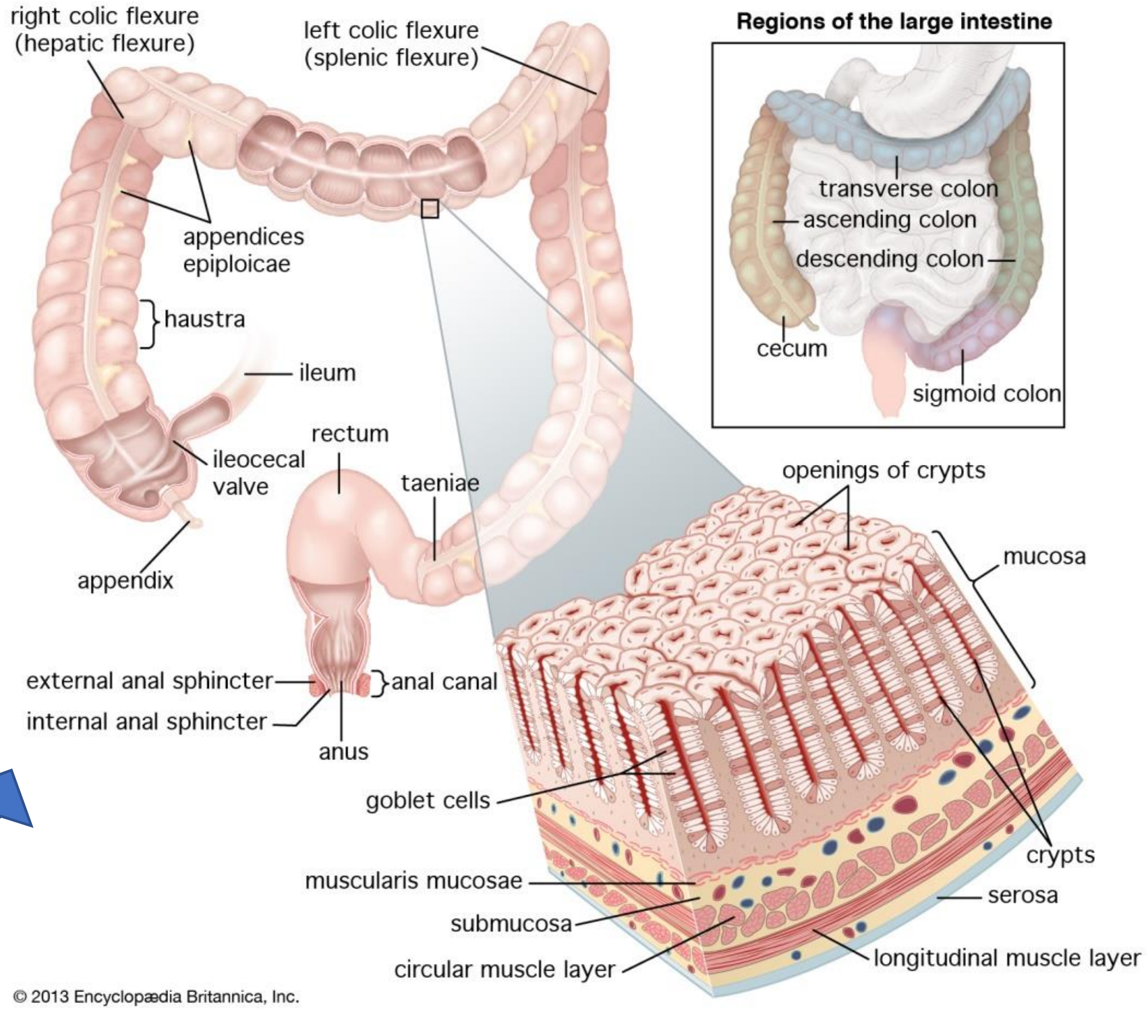
- Example: optical coherence tomography
- Allows longitudinal studies

Advancing the concept

- functional information (angiography / flow)
- metabolic information
- molecular information (specific to disease)

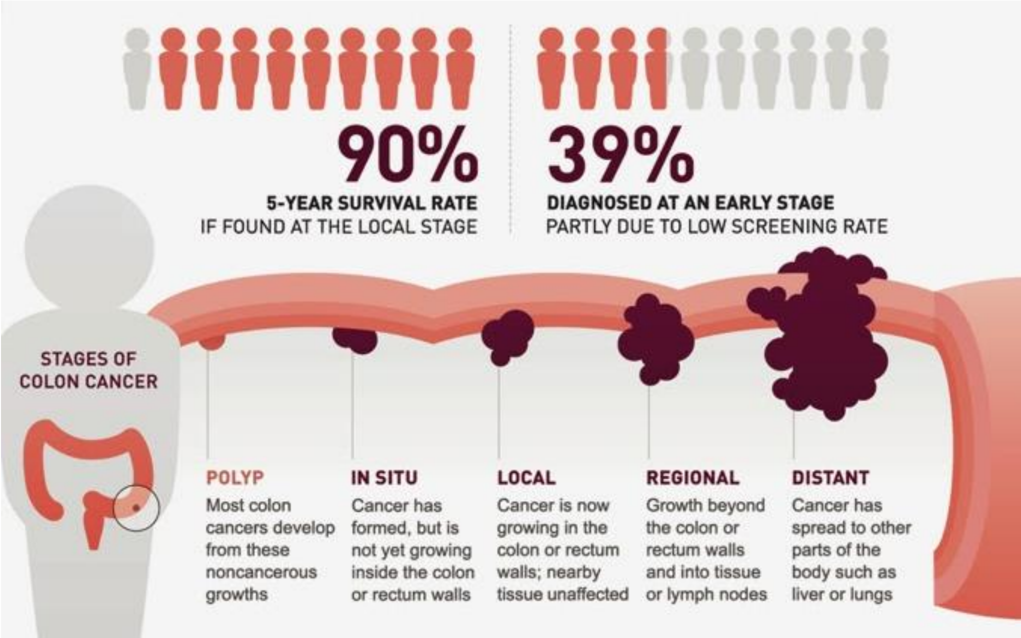
3D volumetric multimodal imaging: accurately representing the morphology and function of tissue



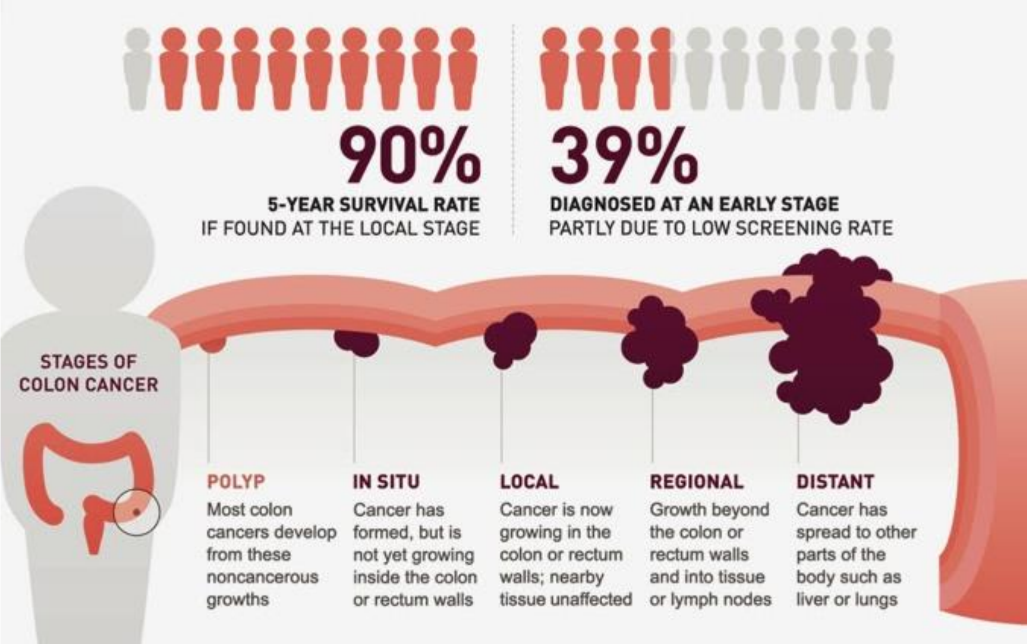


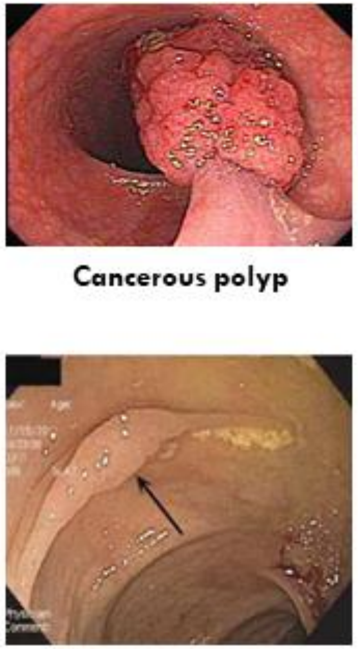
Early Diagnosis of Colo-Rectal Cancer

- CRC: Europe's 2nd most prevalent cancer (European Cancer Info System, 2021)
  - 341,419 cases/year
  - 156,105 deaths/year
  - rising incidence due to diet, obesity, age
- Economic Costs in Europe = €19bn/year
  - €9.5bn in direct costs (primarily healthcare systems impact)
  - €9.5bn in indirect and informal costs
- Early diagnosis is critical
  - 80,000 lives p.a. could be saved if Stage I diagnoses increases from 14% of cases to 50%
    - Stage I diagnosis: 90% 5-year survival
    - Stage IV diagnosis: 10% 5-year survival
- Early diagnosis and treatment has major economic impact
  - early diagnosis: €4,000 / case
  - late diagnosis: €40,000 / case
  - Lancet Oncology (03-2020): "compelling evidence" of positive economic benefits of early cancer diagnosis and treatment
  - Lancet Oncology (2019): scaling up of imaging alone would (for all cancers) save 354,000 lives in Europe and deliver major economic benefits in the period up to 2030

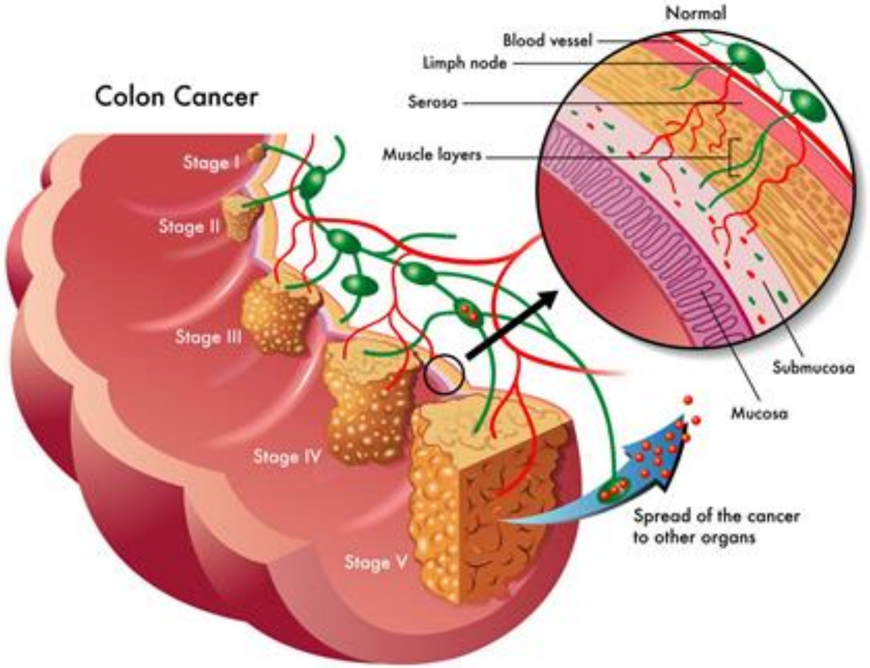


The Problem




Cancerous polyp

Flat/serrated lesion



Colorectal cancer arises from epithelial precursor lesions

Current endoscopic criteria for differentiation of lesions are based on

- size and shape,
- surface pattern (crypts), and
- vessel architecture

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Assessing ‘Polyps’

Ideally gastroenterologists would like to determine if it is

hyperplastic or neoplastic (Adenoma)?

– If neoplastic:
  - Adenoma (with grade of dysplasia), or carcinoma?

– If carcinoma:
  - Differentiation (“grading”), submucosal infiltration, infiltration of lymphatic or blood vessels

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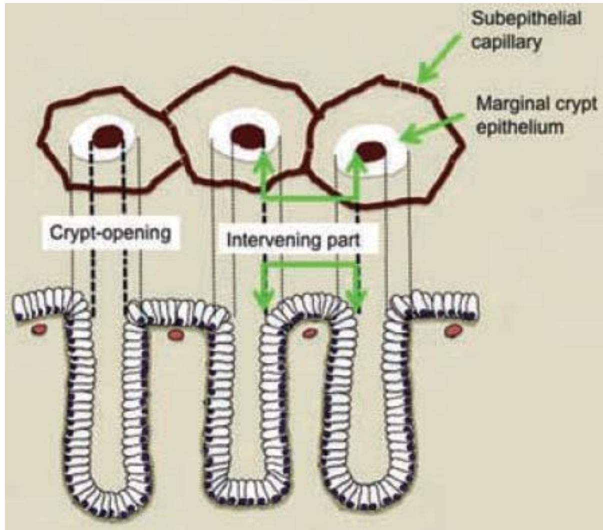
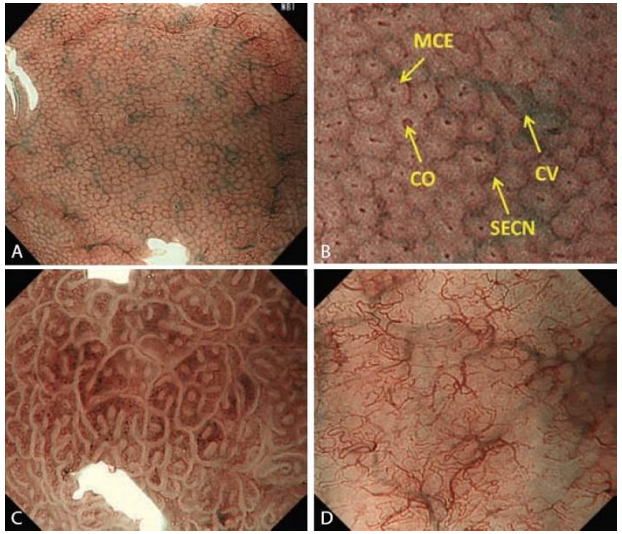
Adapted from
Prof. Dr. A. Schmidt

Gastroenterologist’s View

The gastroenterologist currently views and inspects

- surface, crypt-opening and vessels



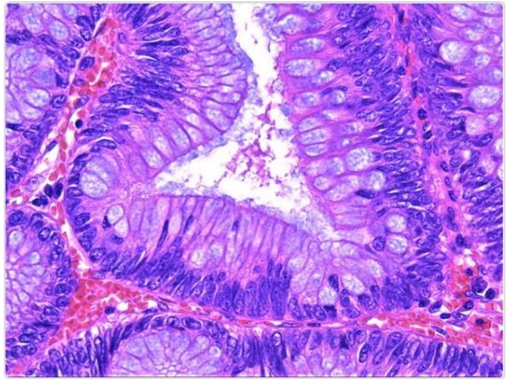


The gastroenterologist would like to view

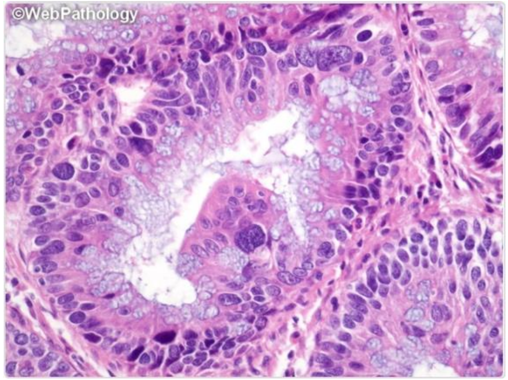
- cells
- nuclei

The gastroenterologist would like to view this information *in vivo* at depth (similar to histo-pathology)

www.webpathology.com



Adenoma with low grade dyspasia / IEN

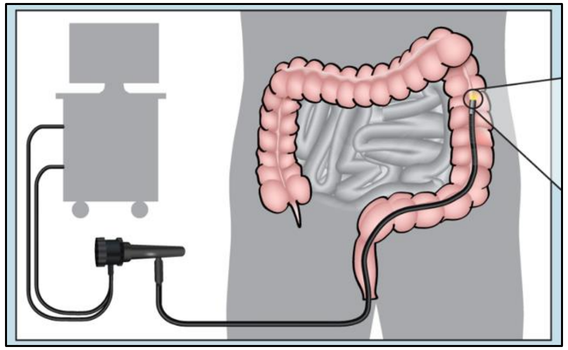


Adenoma with high grade dysplasia IEN (Tis)

Adapted from
Prof. Dr. A. Schmidt

PROSCOPE Concept: Multimodal, Functional Optical Imaging

- Combination of non-invasive optical imaging modalities providing complementary biomarkers targeting endogenous chromophores (label-free)
  - optical coherence tomography (OCT) and OCT angiography ➔ morphology and micro-angiography
  - two-photon light-sheet microscopy ➔ metabolic information
  - wavelength modulated Raman spectroscopy ➔ molecular specific information



- Google Map-like ‘See-Zoom’ imaging concept
  - lesions are detected and diagnosed in situ in one procedure
  - lesions are demarcated providing image-guided biopsy-taking or surgery

- <u>Project Goal</u>
  - translation of imaging concept (optical biopsy) into clinical applications targeting CRC
  - improve prognosis due to earlier detection and therefore earlier onset of effective treatment

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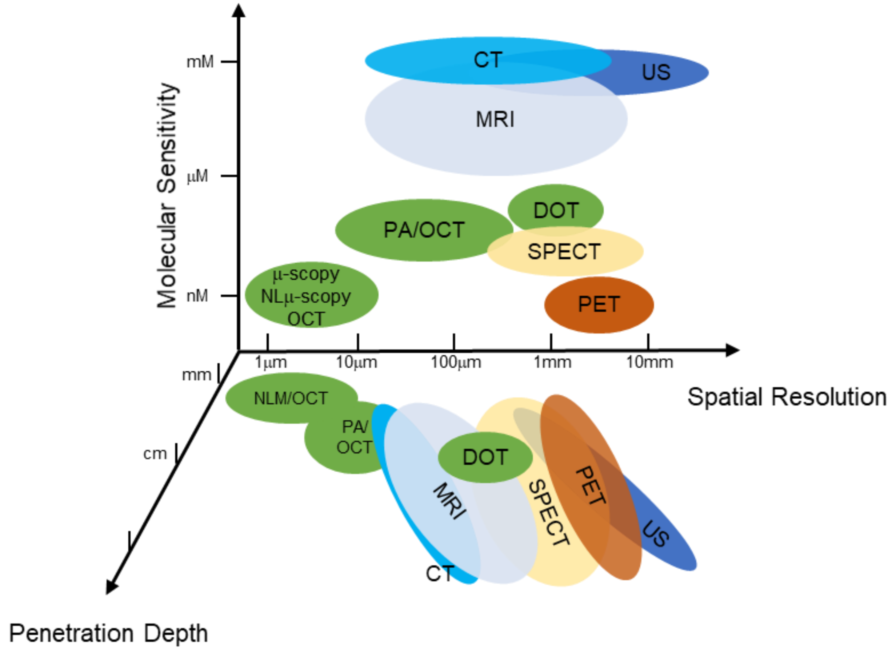
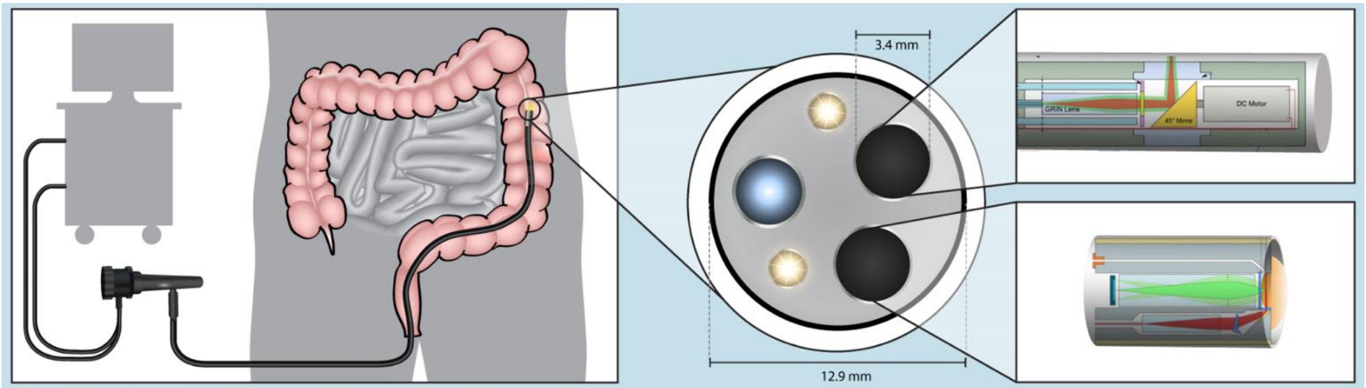
PROSCOPE Methods

- Optical Coherence Tomography (OCT) and OCT angiography (OCTA)
  - morphology and micro-angiography
- Two-Photon Light-Sheet Microscopy (TPLSM)
  - metabolic information
- Wavelength Modulated Raman Spectroscopy (WMRS)
  - molecular specific information



Upper right: OCT/WMRS probe head
Lower right: TPLSM probe head



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Two-photon Excited Fluorescence (TPEF)
Contrast

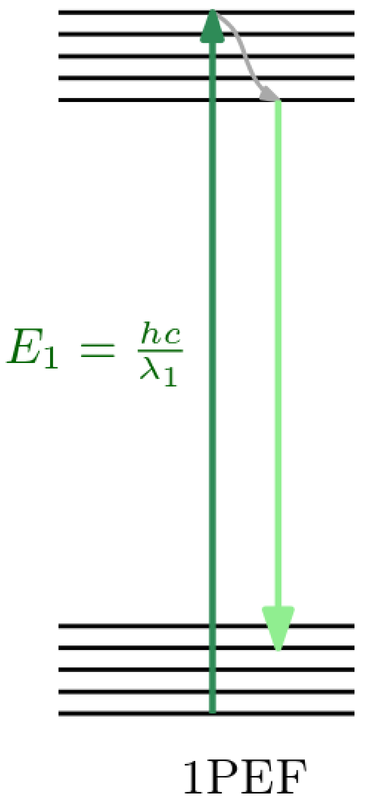
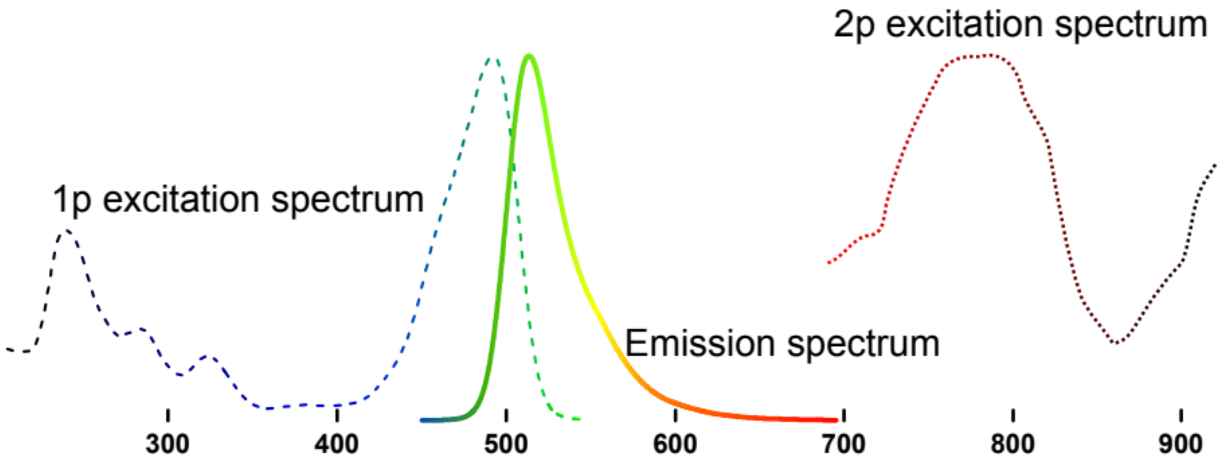


Image by Steve Ruzin and Holly Aaron, UC Berkeley



Principles of Fluorescence Spectroscopy.
https://doi.org/10.1007/978-0-387-46312-4

Light-sheet fluorescence microscopy (LSFM)

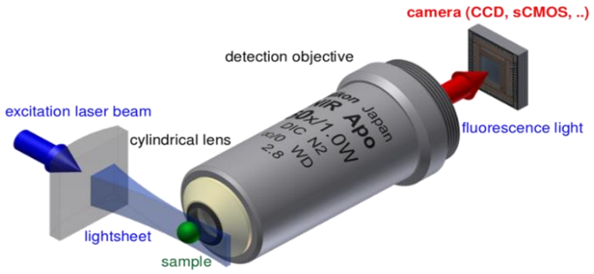
Modality
- Gaussian LSFM
- Contrast: **Two-photon excited fluorescence (TPEF)**

Samples
- Porcine colon tissue
- Tissues stained with uPAR (Rigshospitalet)
- Other *ex vivo* bulky biological samples

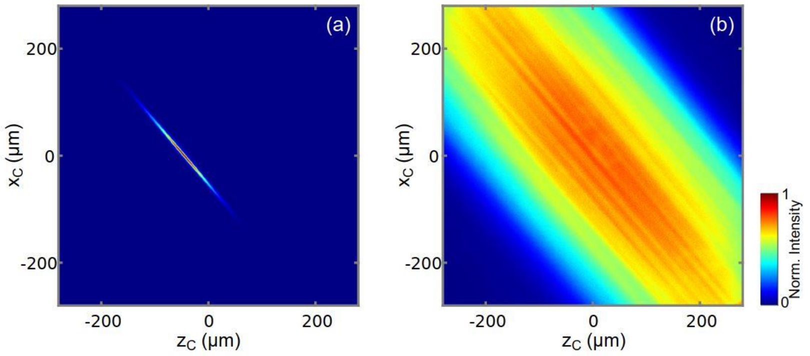
Methods
- Femtosecond laser for TPEF excitation
- Cylindrical lens forms the Gaussian light-sheet

Results
- Two-photon fluorescence from Coumarin solution
- Volumetric TPEF stacks of defrosted porcine colon

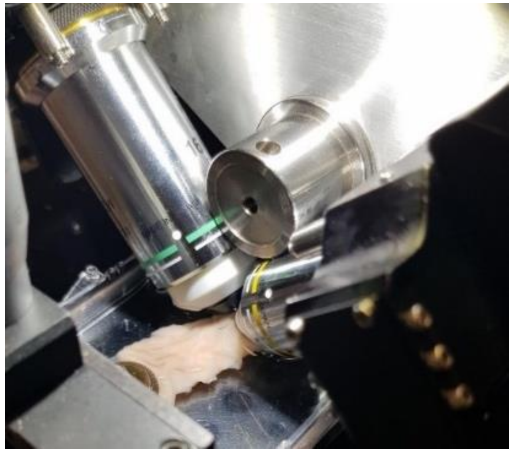
Outlook
- Hyperspectral LSFM
- Translation to lensless endoscopy

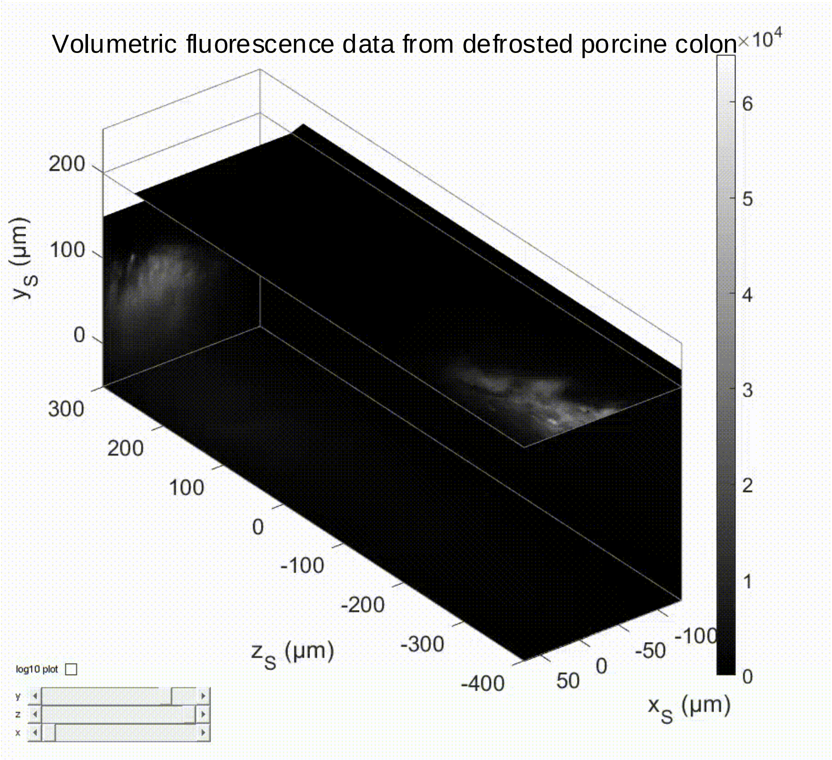


Gaussian Beam -> Gaussian Light-sheet

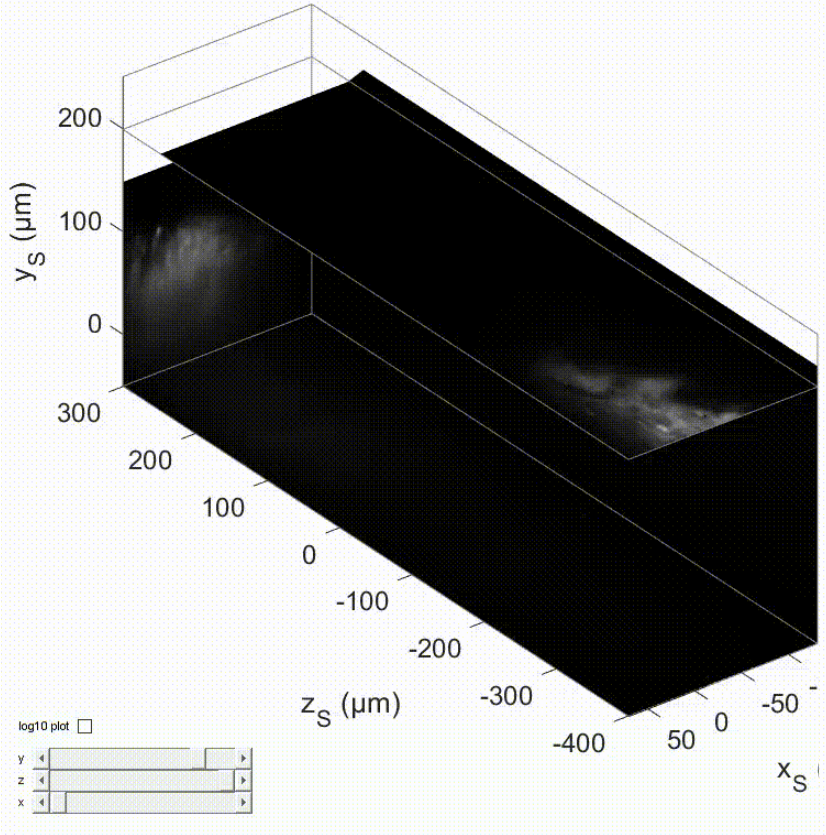


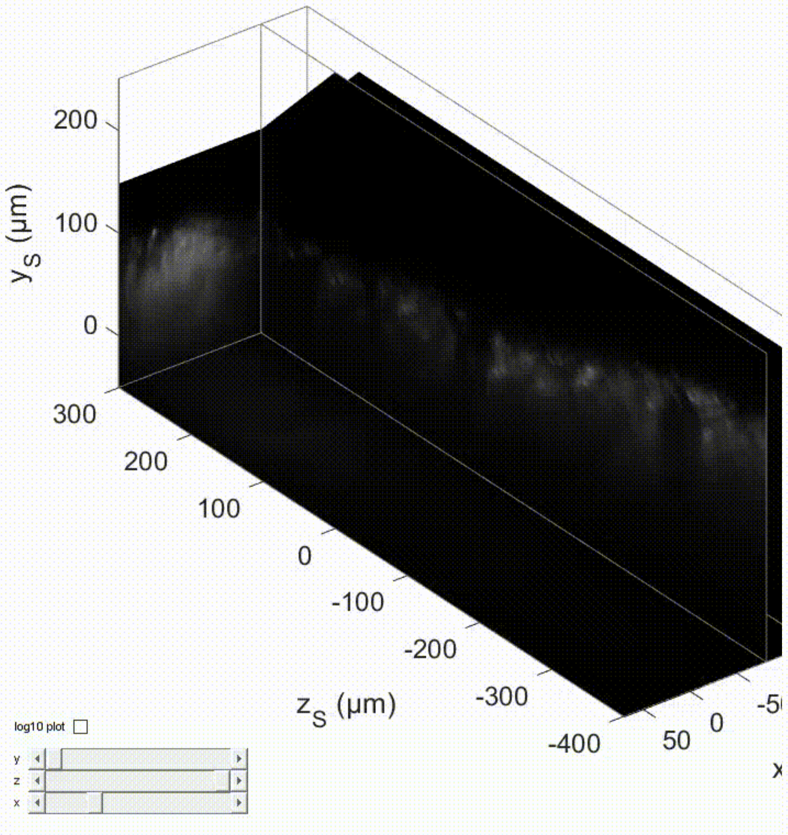
Porcine colon under G-LSFM

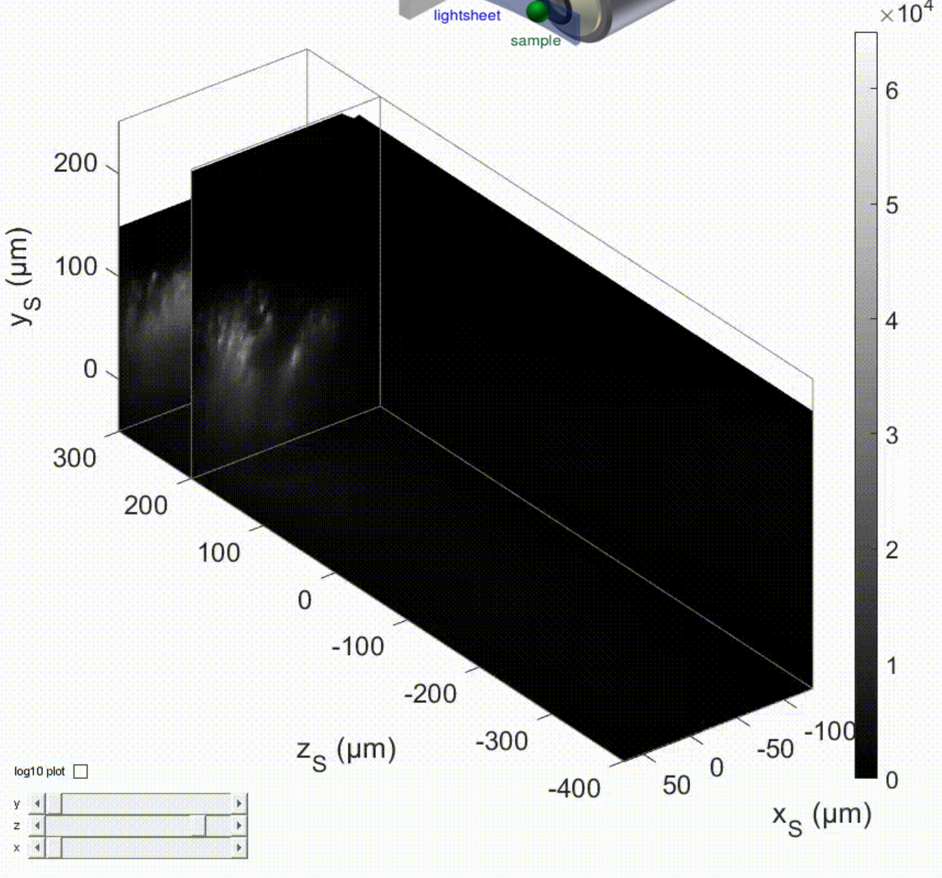




Two-photon Lightsheet imaging of porcine colon



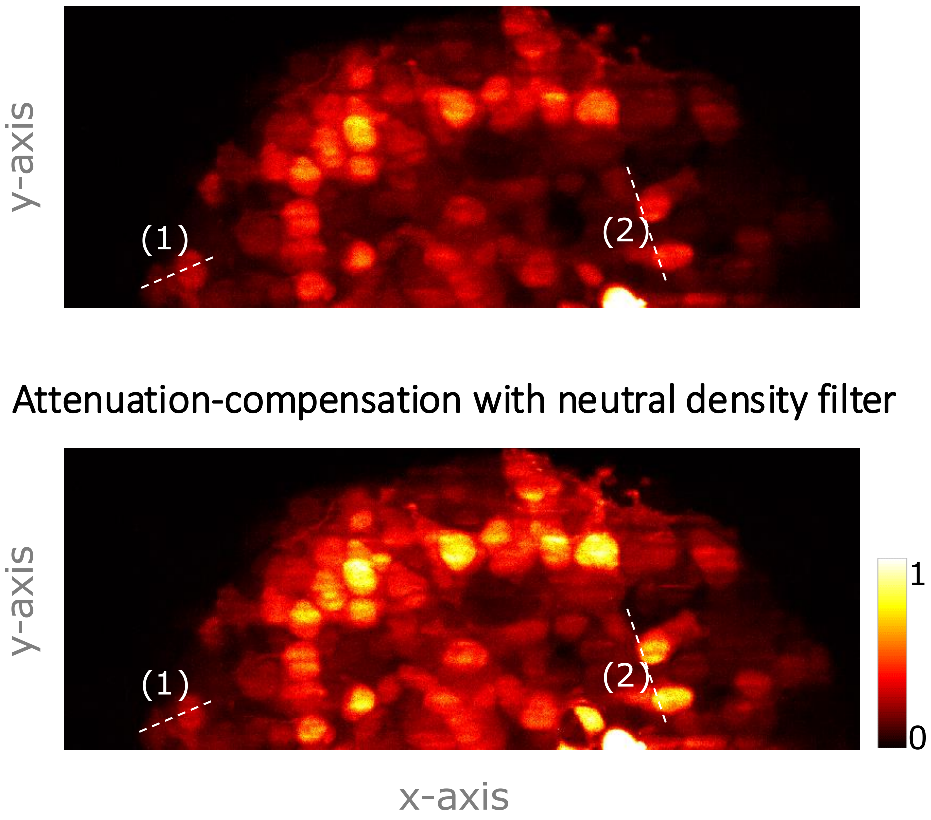




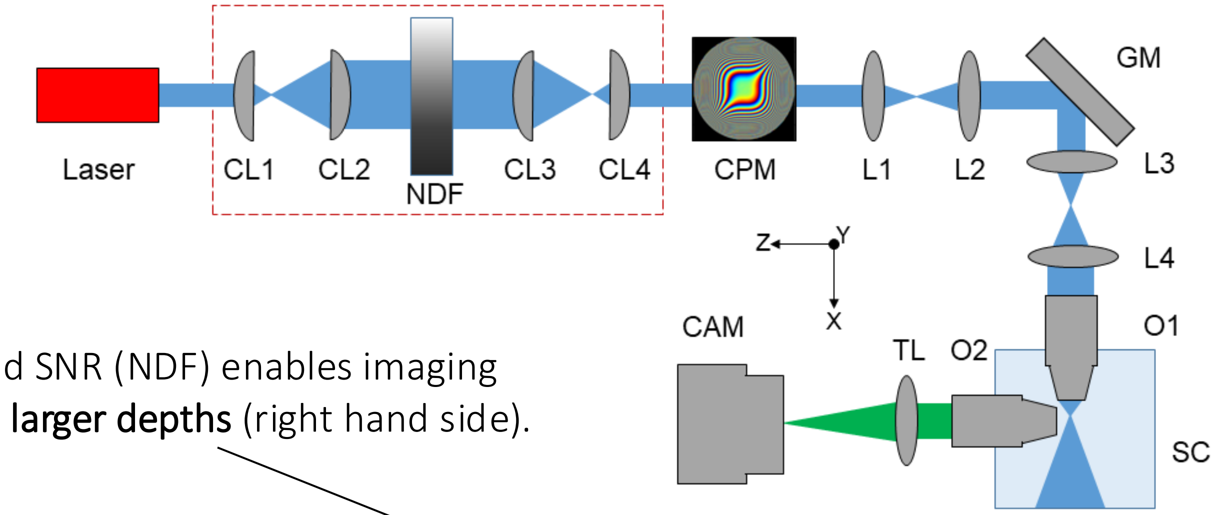
Two-photon Airy Lightsheet Fluorescence Microscopy

Enlarging imaging depth using simple attenuation compensation

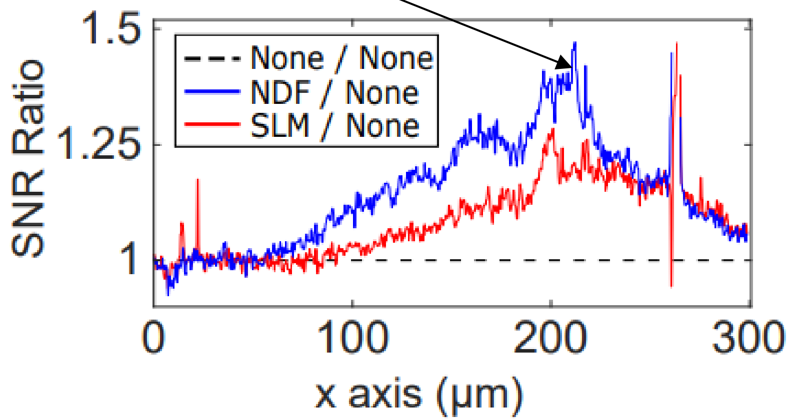
No compensation



Two-photon Airy lightsheet imaging of **HEK-293 Spheroid**



Improved SNR (NDF) enables imaging at much **larger depths** (right hand side).



nature SCIENTIFIC REPORTS

M. Veettikazhy, K. Dholakia, et al., "Multi-photon attenuation-compensated light-sheet fluorescence microscopy," Sci Rep **10**, 8090 (2020)

Colon Structures and Mucosal layer

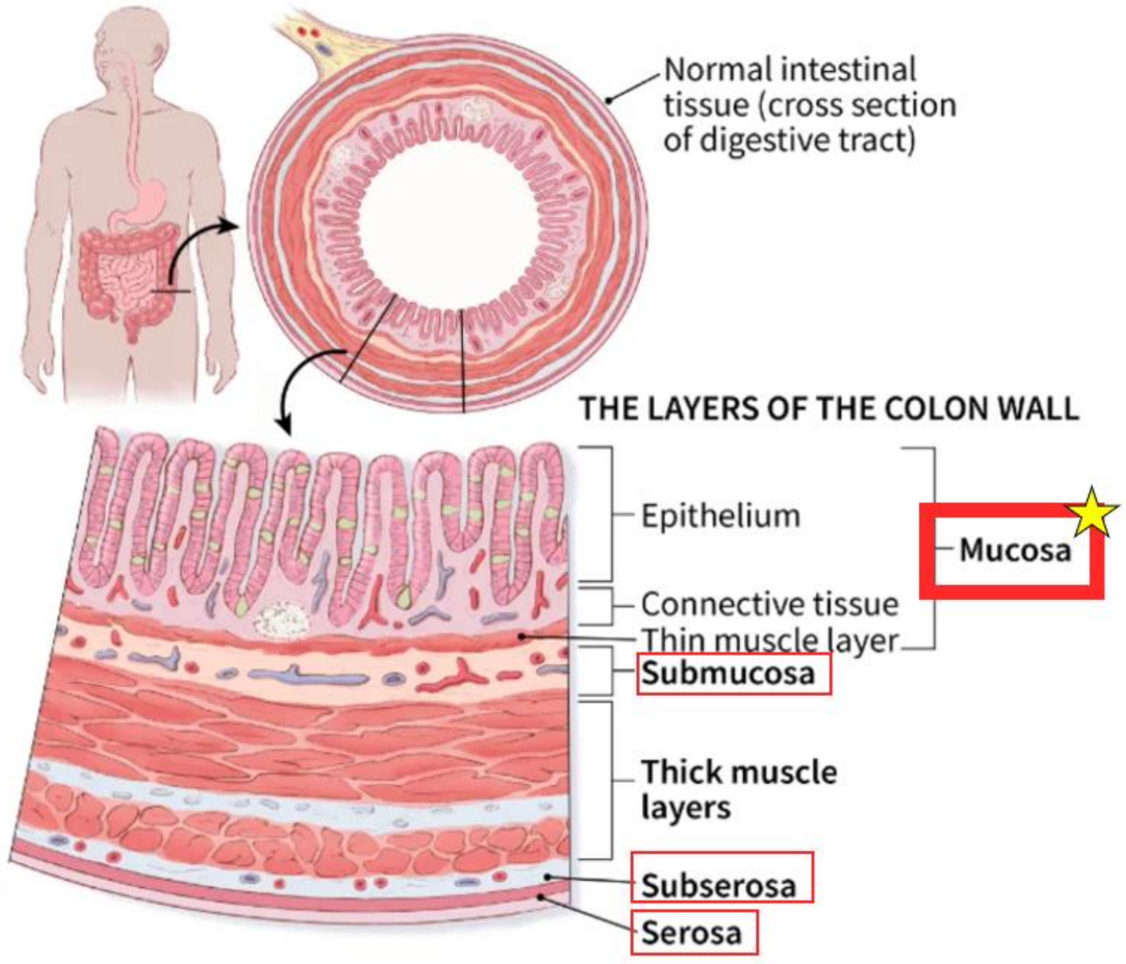


Figure adapted from: American Cancer Society

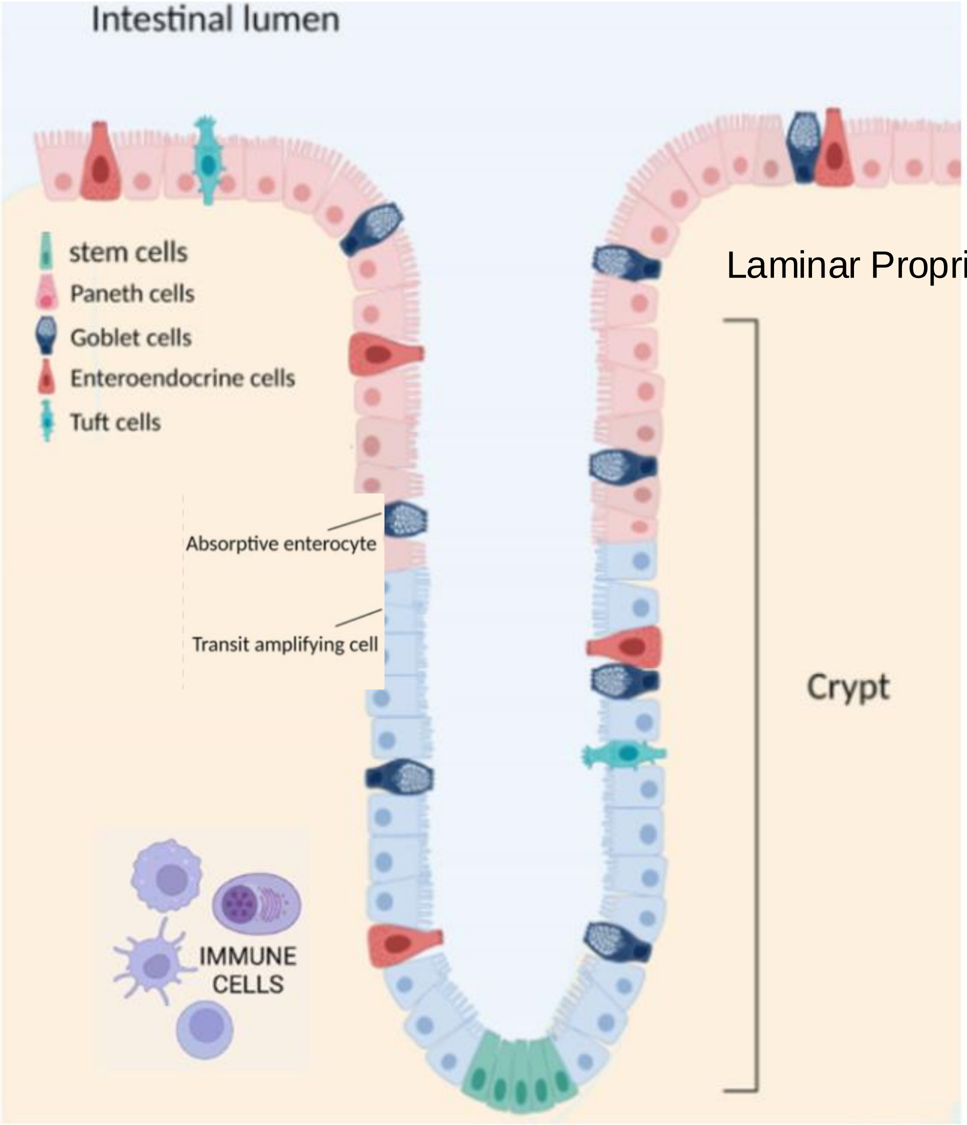


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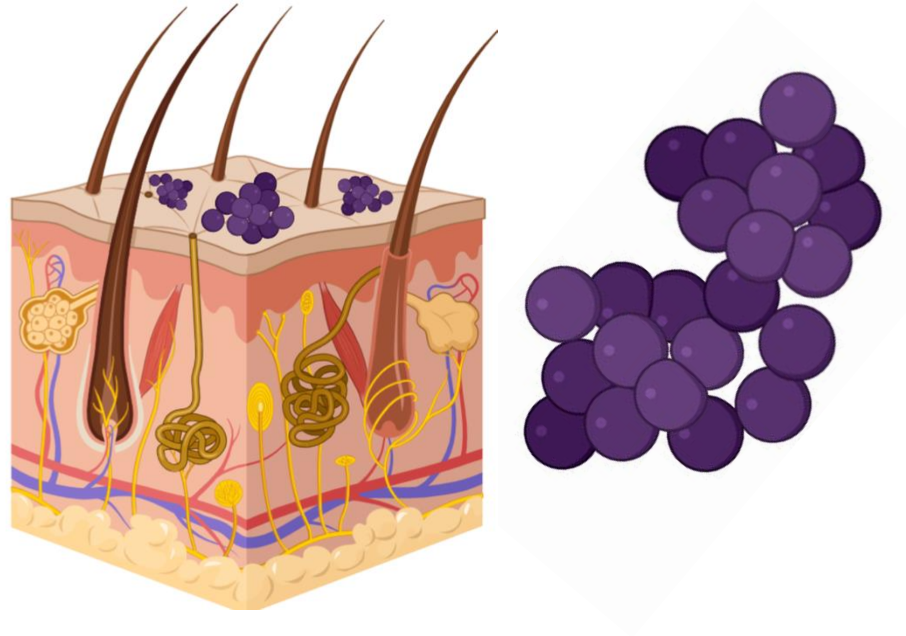
Figure adapted from: Lubkowsk et al, 2021

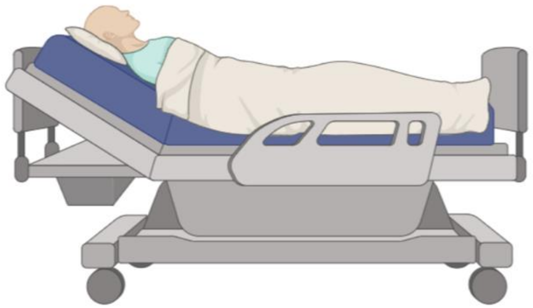
Early diagnosis of S. epidermidis infected colon

- Staphylococcus epidermidis
  - significant impact on infants and is a leading cause of neonatal sepsis
  - particularly in newborns with hospital-acquired infections

- Screening
  - Histopathology/Tissue biopsy: Gold-standard

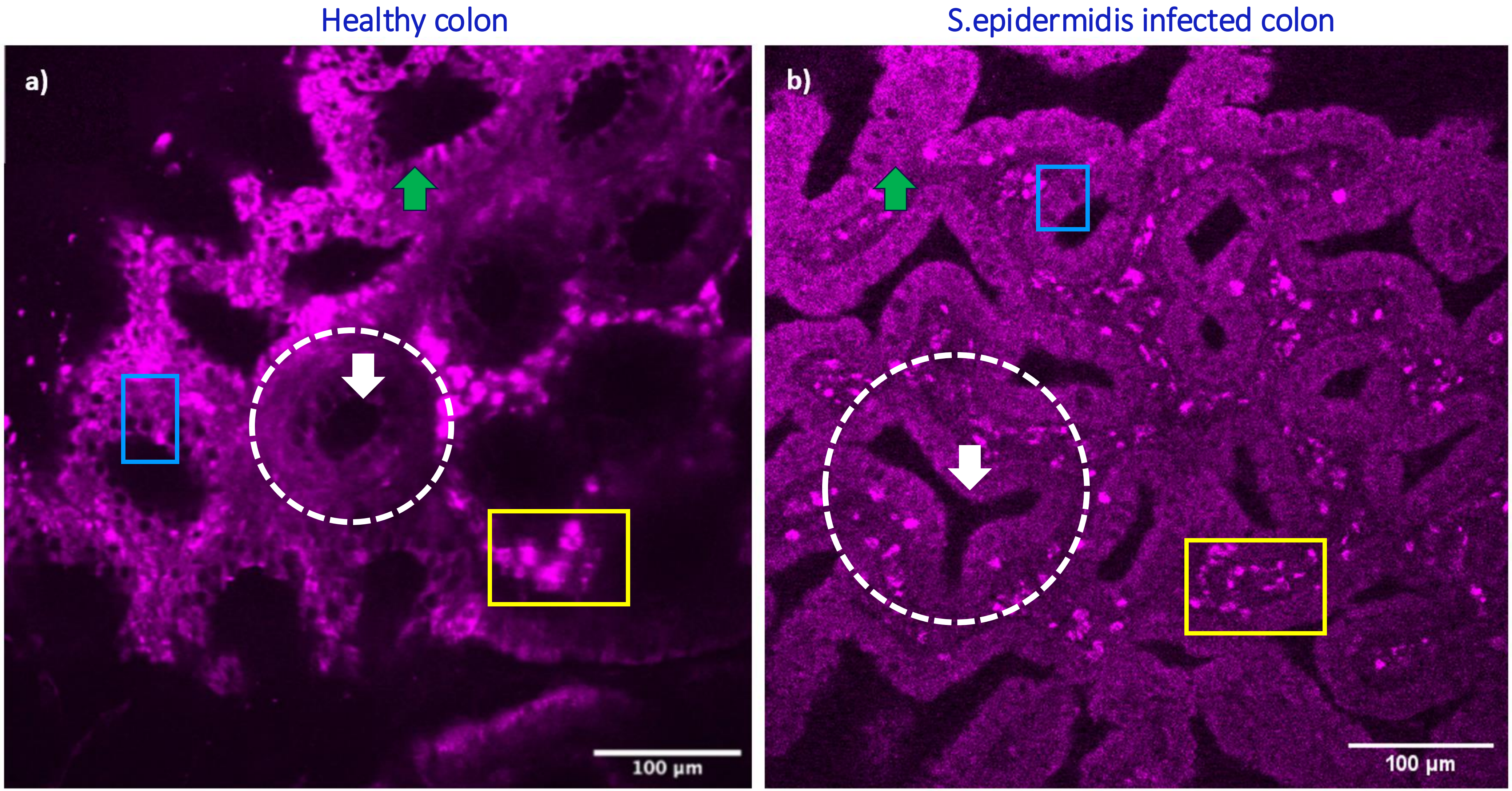
- Imaging technique
  - Multiphoton Microscopy
  - Contrast: 2-photon excited fluorescence
  - Rastor scanning of high-power focused laser beam and subsequent fluorescence collection





Healthy vs infected colon imaged using 2PM

2-photon excited fluorescence scanning microscopy



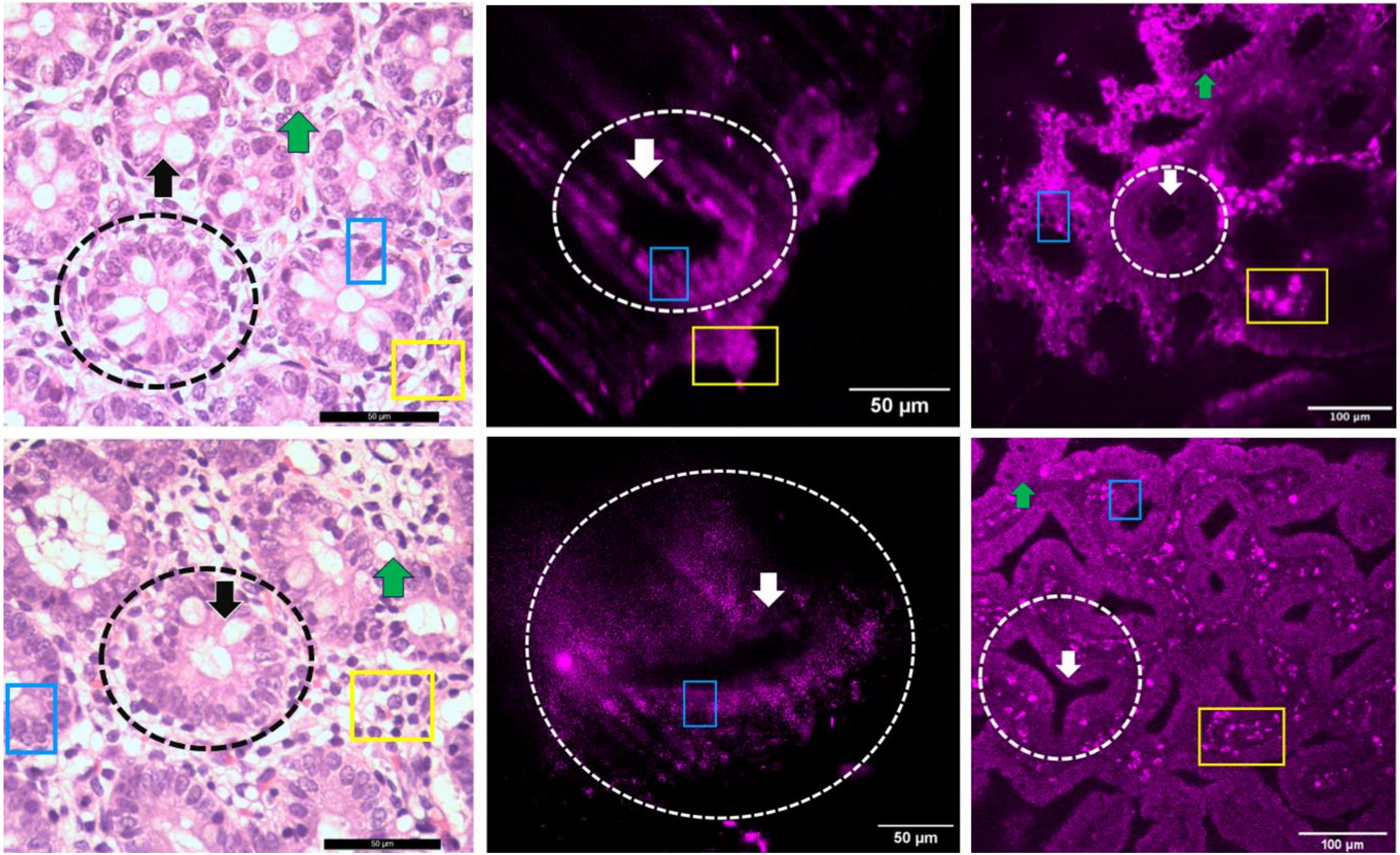
Unpublished results: Keqing Dai, Jirapa Limsuriwong, DTU, 2024

Results overview

Histology | 2P Lightsheet Microscopy | 2P Scanning Microscopy

Healthy colon

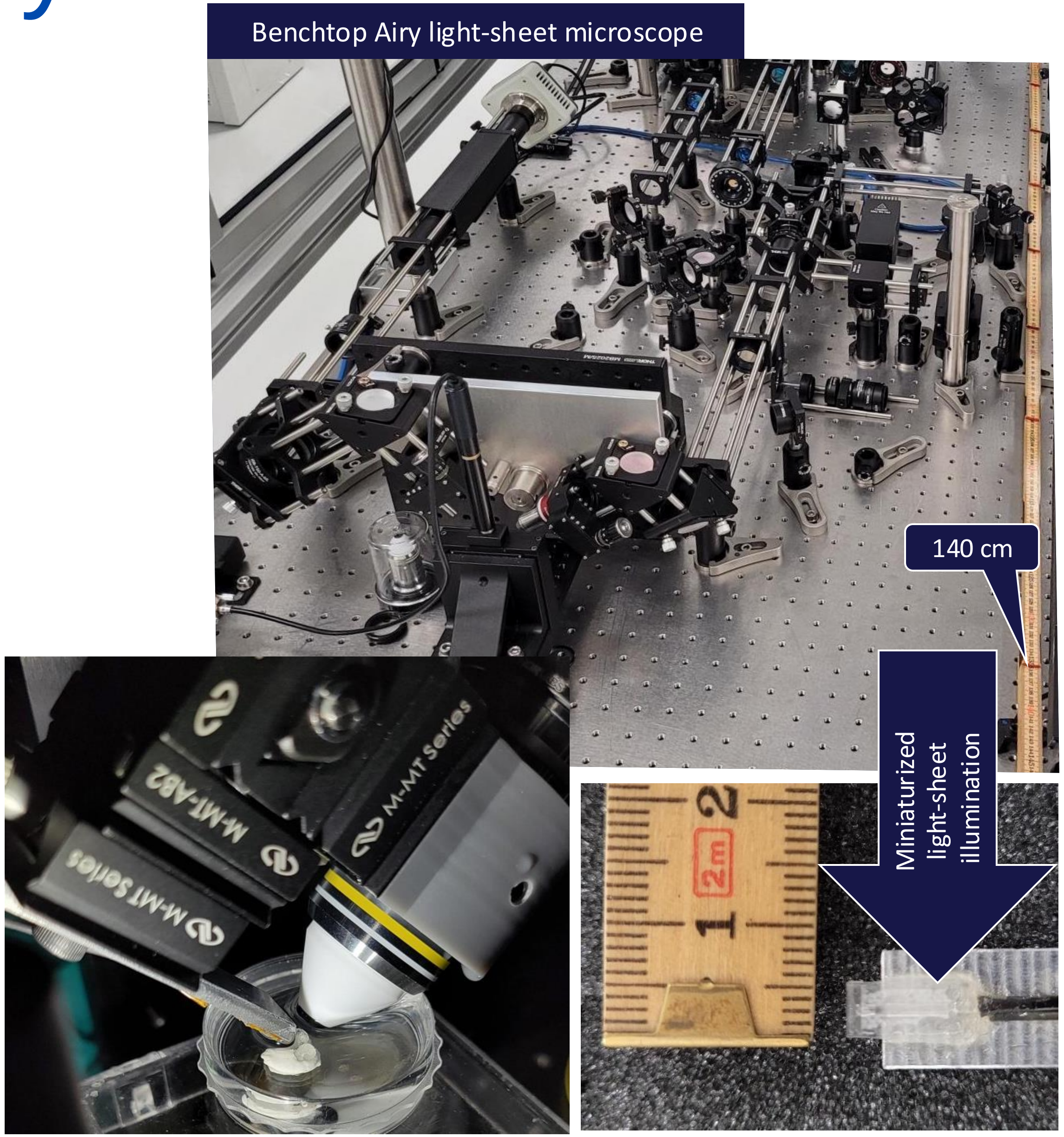
S. epidermidis Infected colon



PROSCOPE

Light-sheet microscopy in fibre-optic probes

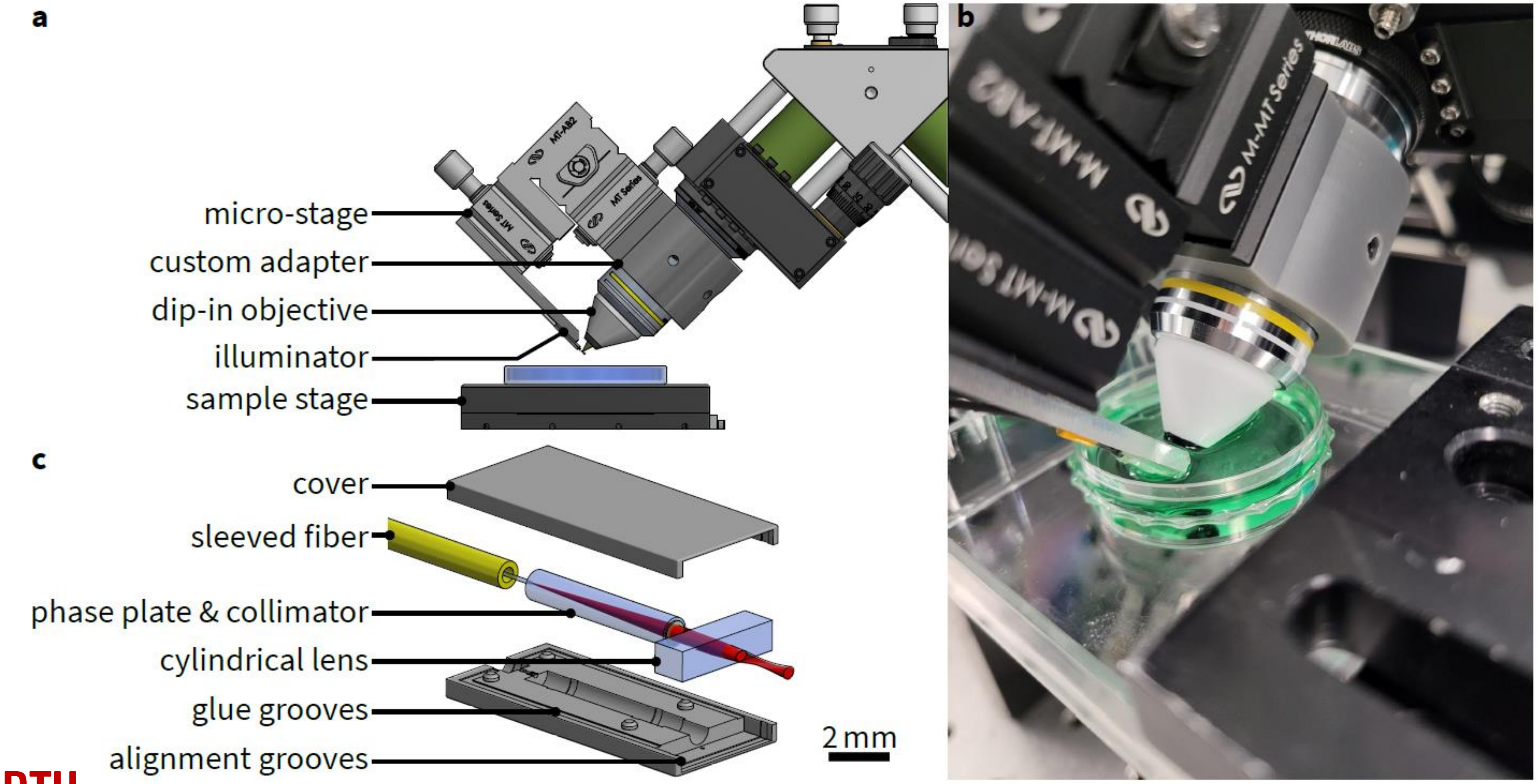
- Miniaturization of Airy light-sheet microscope including
  - complex beam shaping and focusing
- Can convert any conventional microscope into a two-photon Airy light-sheet microscope
- Fibre-optics enable clinical translation

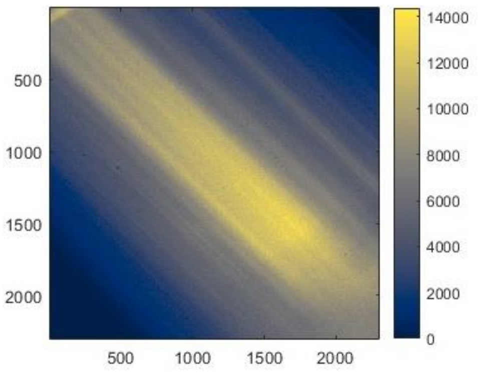


PHOTONICS21
PHOTONICS PUBLIC PRIVATE PARTNERSHIP
Grant Agreement No. 871212

The Airy illuminator

PROSCOPE





GRINTECH Gradient Index Optics Technology

DTU

ALBERT-LUDWIGS-UNIVERSITÄT FREIBURG

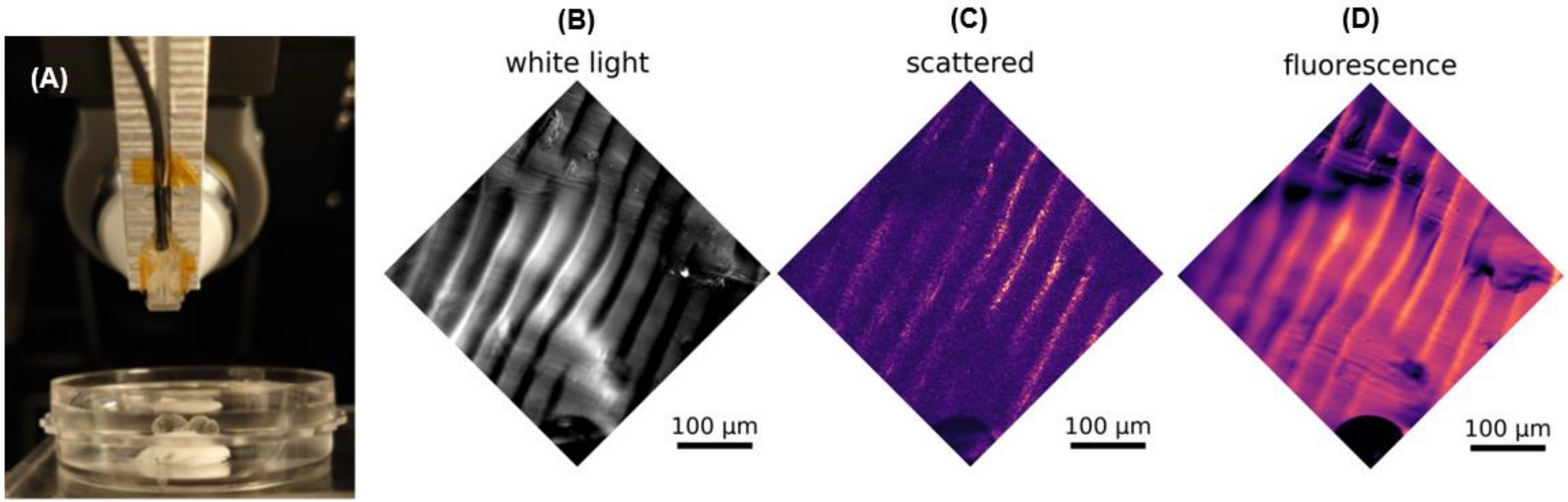
PHOTONICS21 PHOTONICS PUBLIC PRIVATE PARTNERSHIP

Grant Agreement No. 871212

First Tests on Phantoms

1-photon fluorescence

- Experiments
  - beam profiling in fluorescent solution (ICG)
  - brain model made from infused resin (novel NIR fluorescence marker)

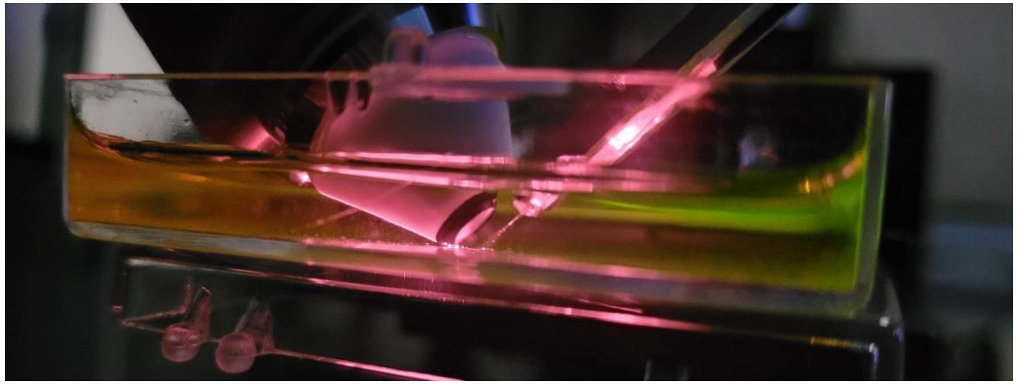


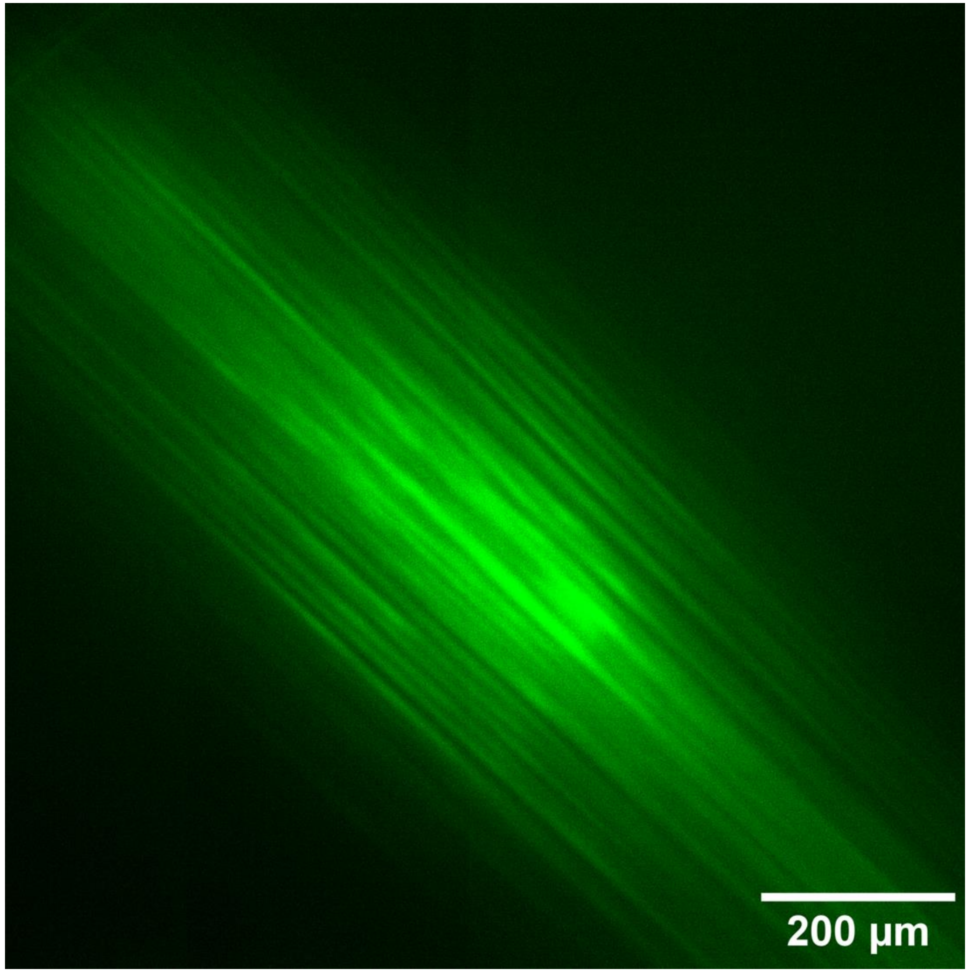
https://fluoguide.com/technology/upar/

Grant Agreement No. 871212

First Test – 2PEF

- First probe test for 2PEF





2-photon fluorescence from Airy lightsheet

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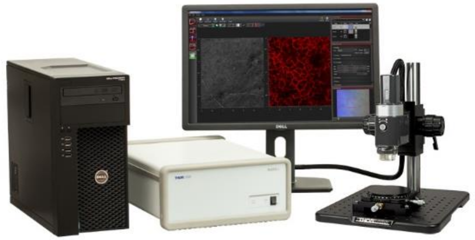
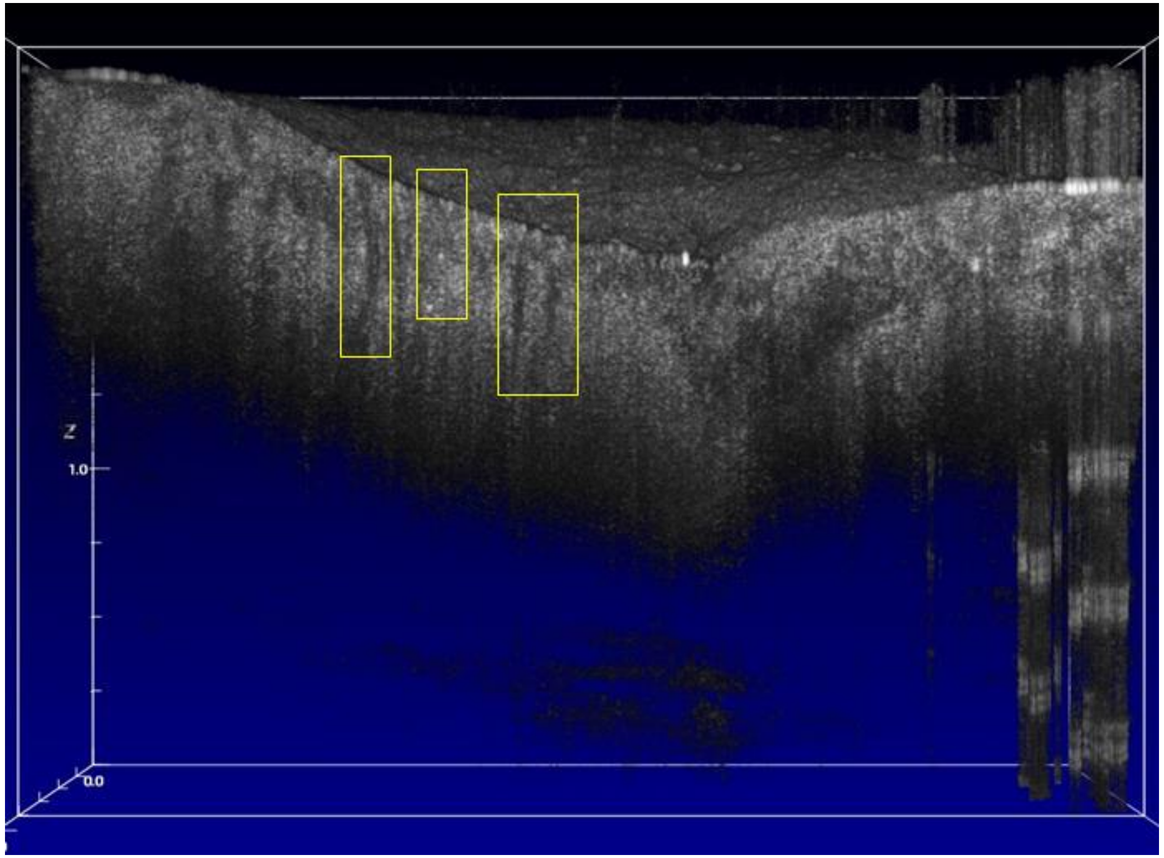
PHOTONICS PUBLIC PRIVATE PARTNERSHIP
Grant Agreement No. 871212

OCT imaging of porcine colon

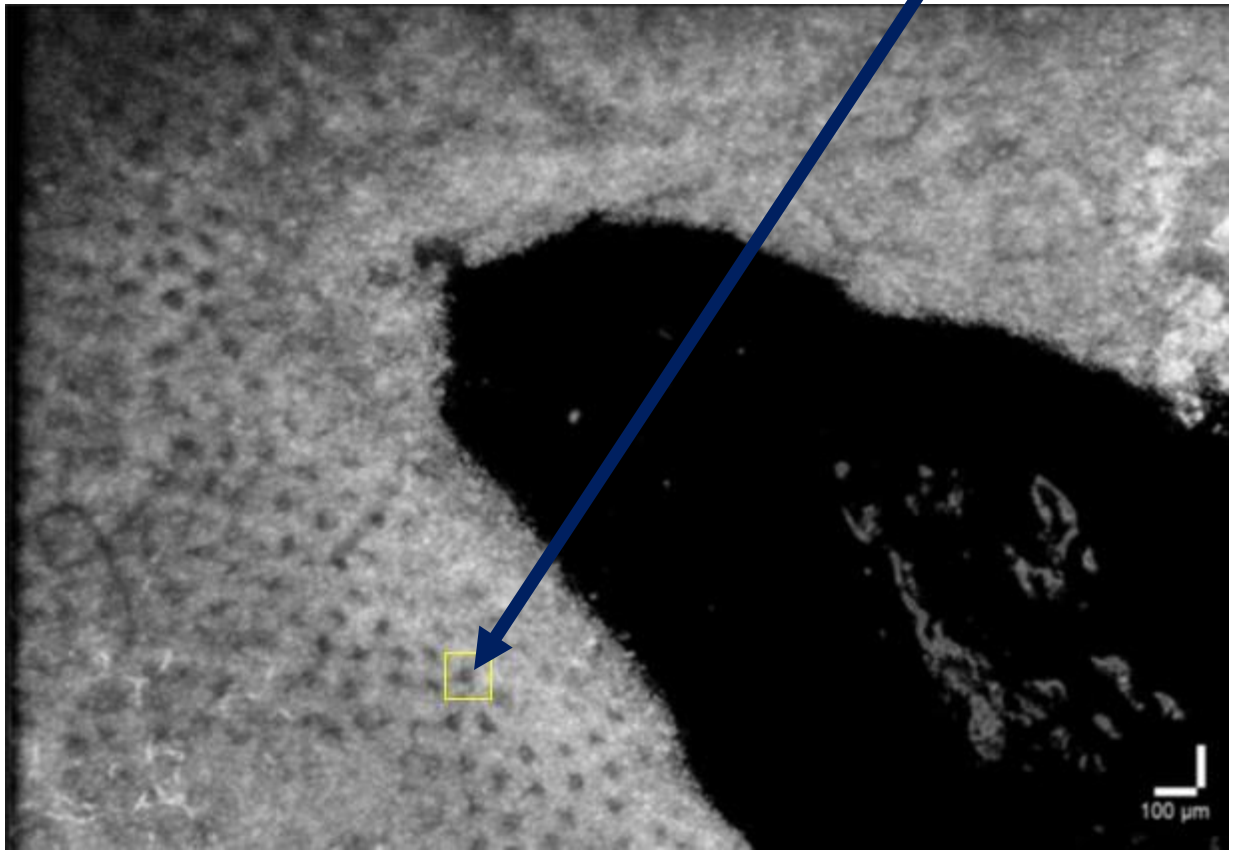
Thorlabs Telesto – Commercial system



Colonic crypts



Side view

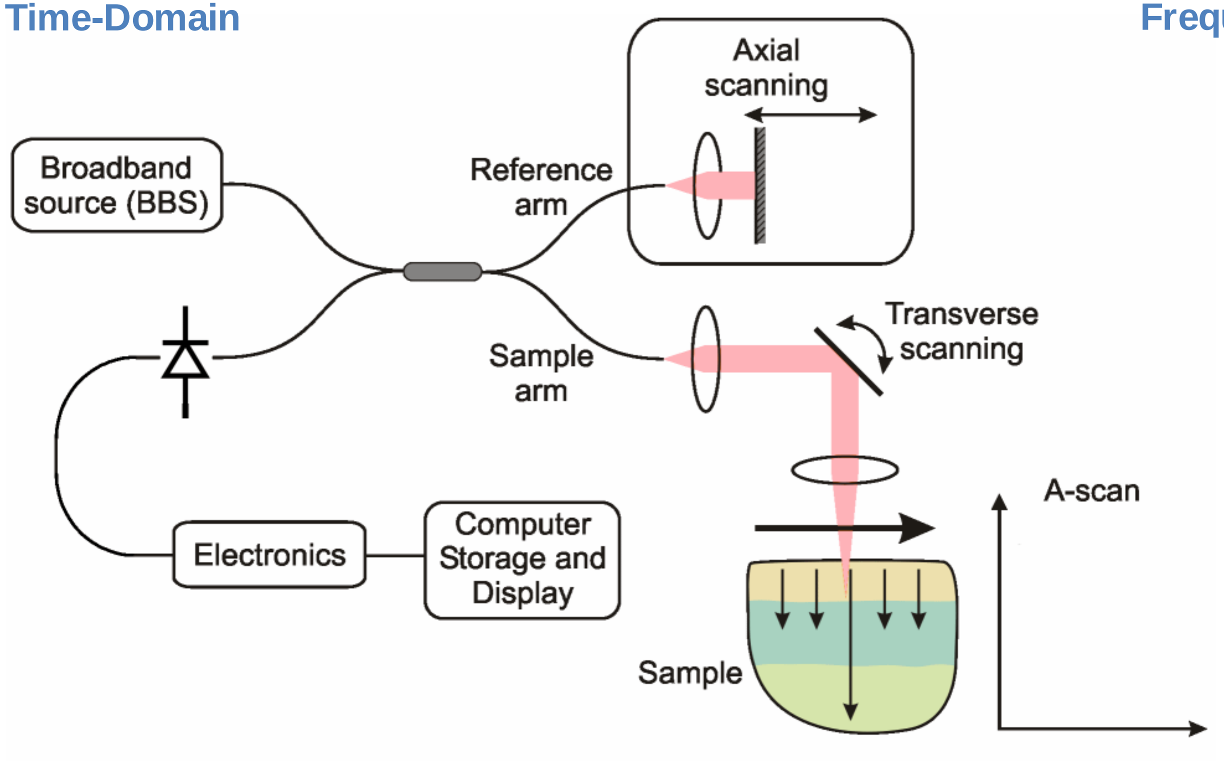


Top view

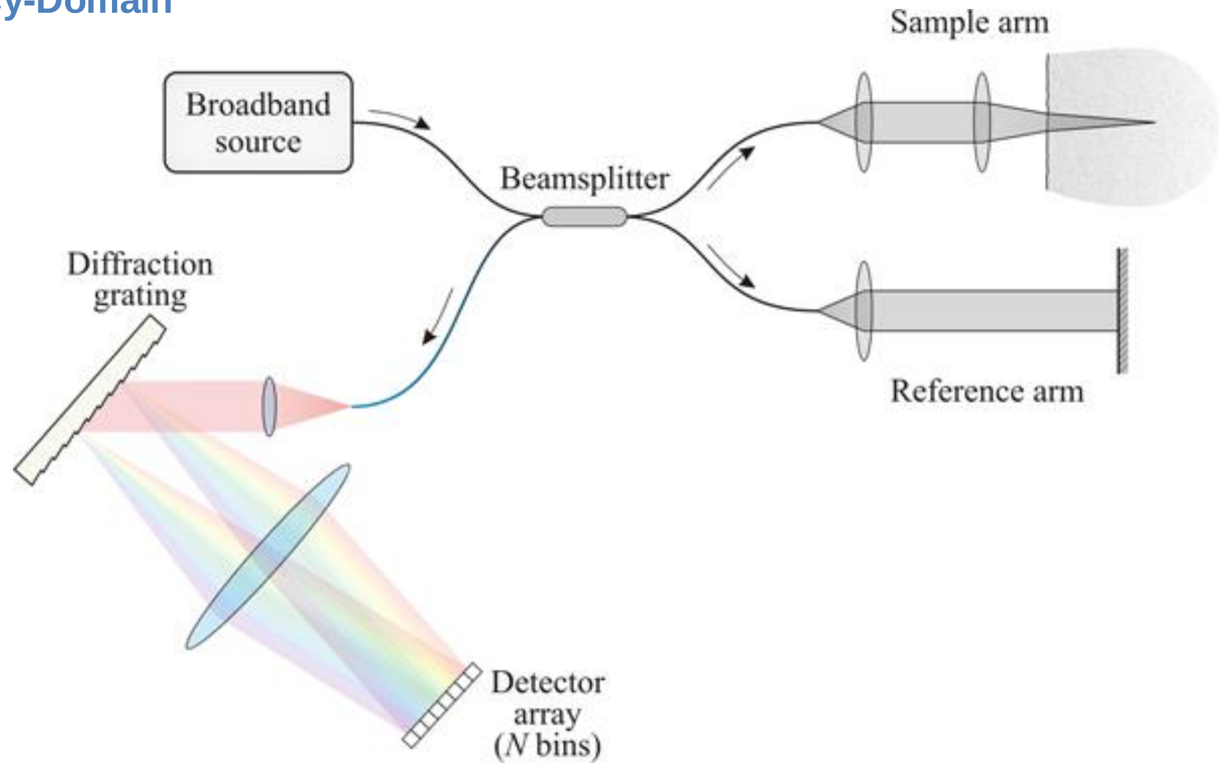
Optical Coherence Tomography

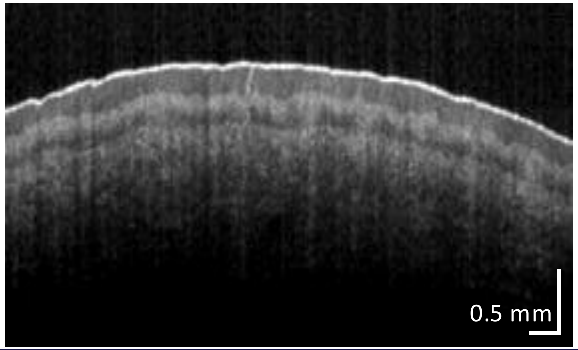
Huang *et al*., Science, 1991:
30th anniversary in 2021!

Time-Domain



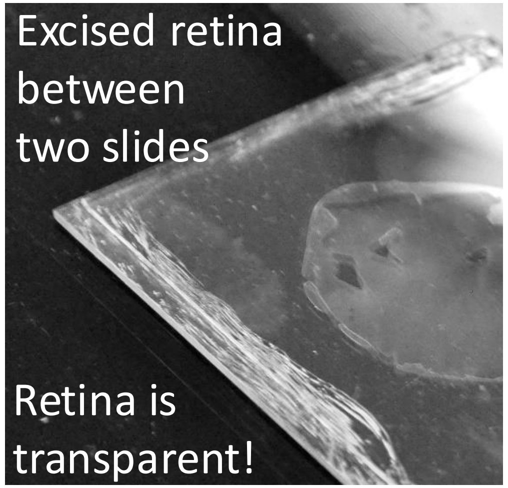
Frequency-Domain

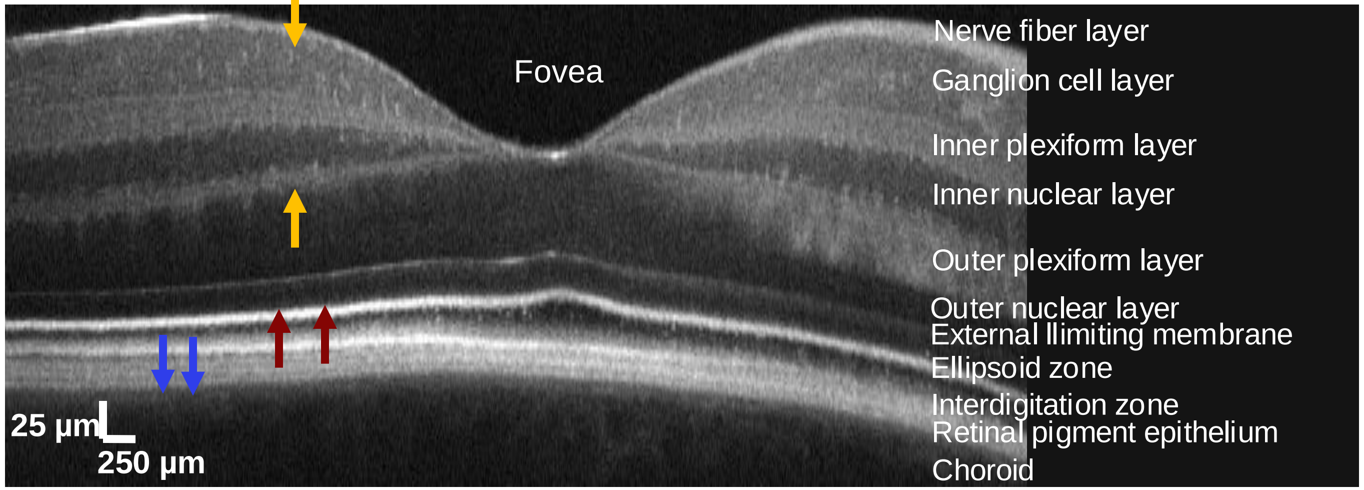




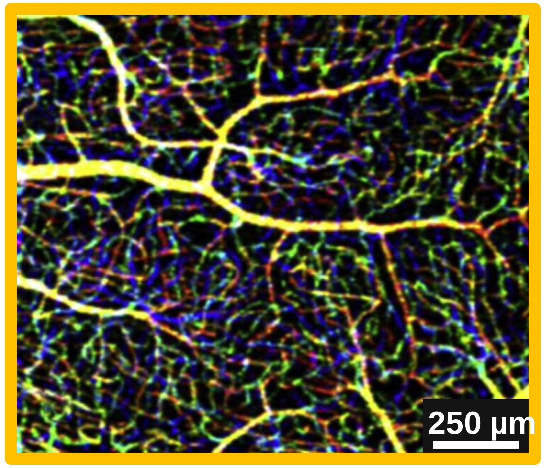
OCT in the human retina – a transparent organ

M. Szkulmowski *et al.*, *Optics Express* 20(20), 2012



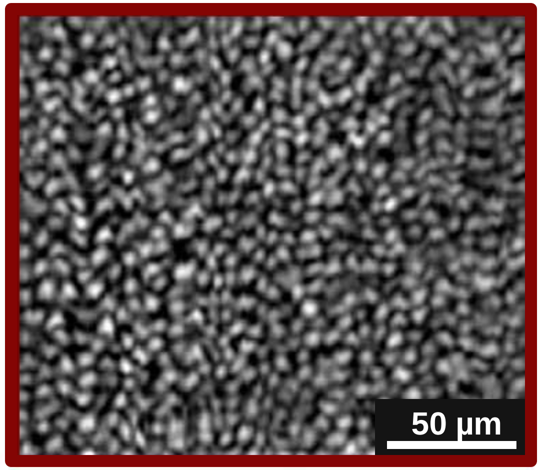


Retinal microvasculature



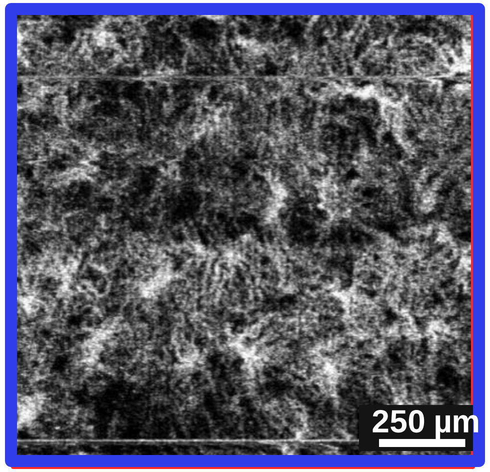
D. Schwartz *et al.*, *Ophthalmol*. 121(1), 2014

Cone photoreceptor cells



R. Jonnal *et al.*, *IOVS* 57(9), 2016

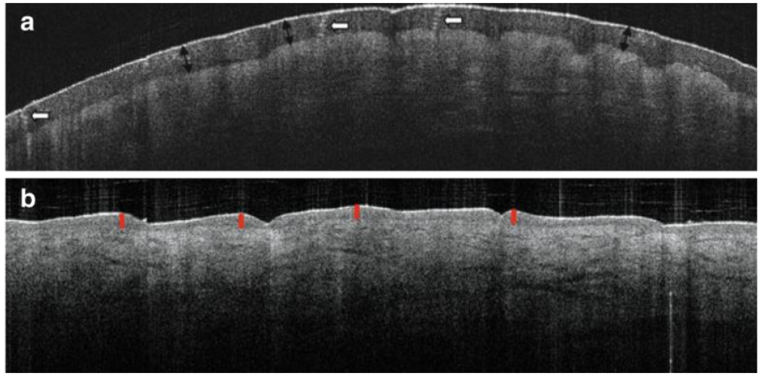
Choriocapillaris



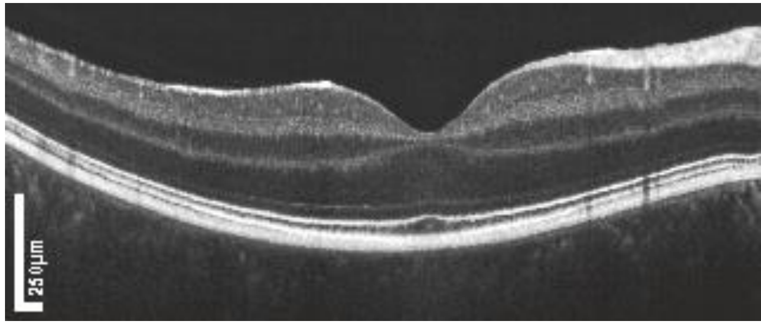
J. Migacz *et al.*, *BOE 10*(1), 2019

Optical Coherence Tomography – Overview

skin, healthy volunteer



retina, healthy volunteer

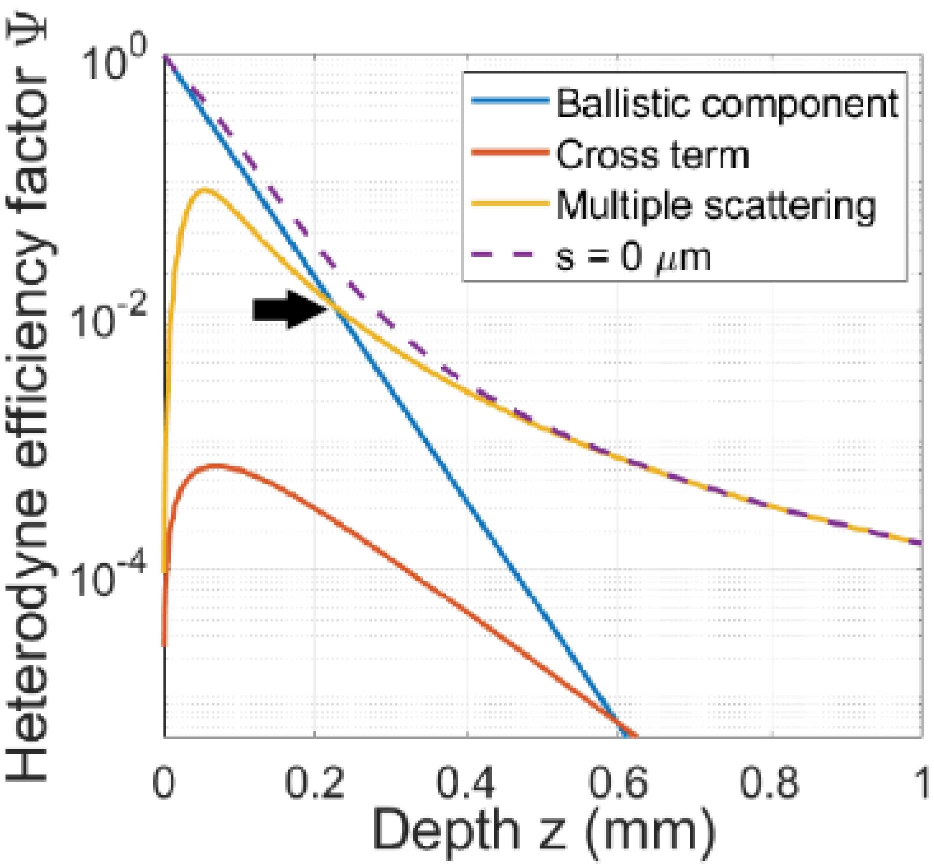


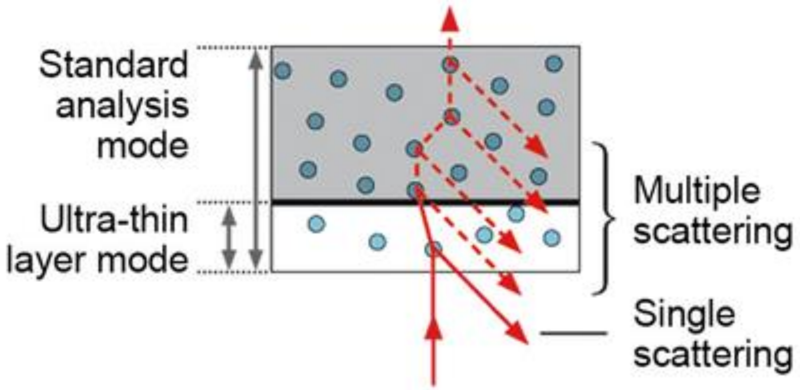
- Cross-sectional (2D, 3D) high-resolution imaging in tissue
  - – 1-2 mm penetration depth
    - determined by source centre wavelength
  - – from 3 micrometer axial resolution
    - source spectrum determines axial resolution
  - – transverse resolution determined by diffraction
    - focusing optics/objective
  - – real-time, video-rate (high definition) image acquisition

- Integration into catheters & endoscopes

- Next generation technologies
  - – OCT-A: Angiography
  - – OCE: Elastography
  - – SO-OCT: Deep tissue imaging

Modelling the OCT signal



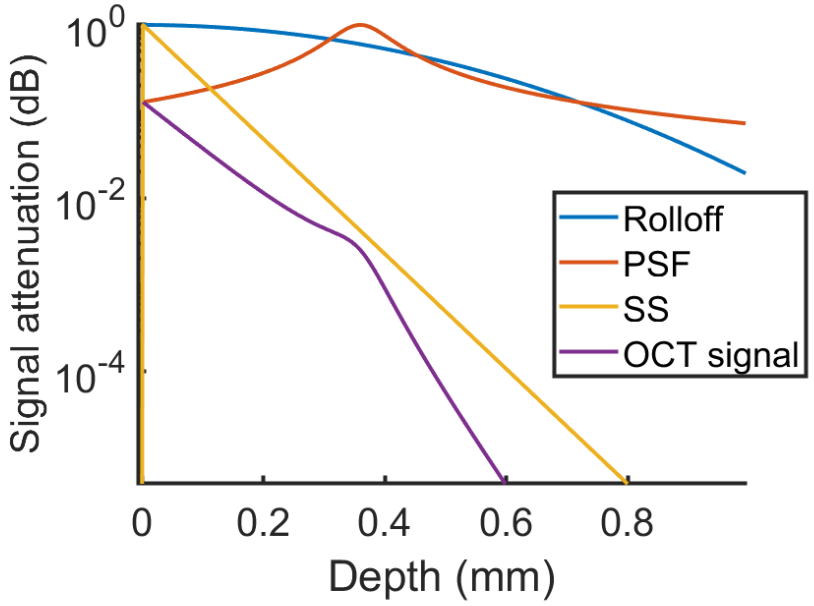


Based on the EHF model, ballistically scattered photons have the largest influence on the signal close to the surface – the signal from deeper in the sample is dominated by multiply scattered light!

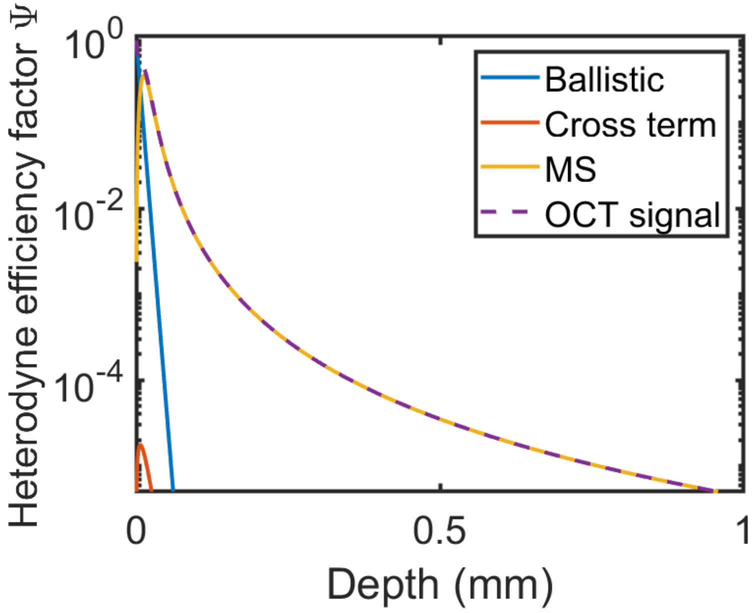
Gavrielle R. Untracht et al., Sci. Adv. **9**, eadh5435(2023).
DOI:10.1126/sciadv.adh5435

Modelling the OCT signal

Fitting OCT data to a model can be used to calculate the optical properties of the sample

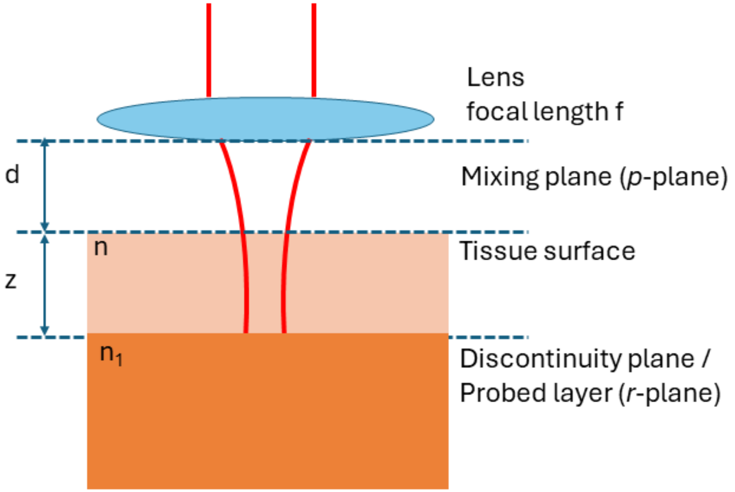


Single scattering model – based on exponential decay (Lambert-Beer law)



Multiple scattering model – based on Extended Huygens-Fresnel principle and derived from Maxwell's equations

OCT A-scan signal model
(two-parameter model)



The mean square heterodyne signal current (lens plane)

$$\langle i^2(z)\rangle = \frac{\alpha^2 P_R P_S \sigma_b}{\pi w_H^2}\left[e^{-2\mu_s z} + \frac{4e^{-\mu_s z}(1-e^{-\mu_s z})}{1+\frac{w_S^2}{w_H^2}} + (1-e^{-\mu_s z})^2 \frac{w_H^2}{w_S^2}\right]$$

$$w_S^2 = w_0^2\left(A-\frac{B}{f}\right)^2 + \left(\frac{B}{kw_0}\right)^2 + \left(\frac{2B}{k\rho_0}\right)^2 \qquad w_H^2 = w_0^2\left(A-\frac{B}{f}\right)^2 + \left(\frac{B}{kw_0}\right)^2 \qquad \rho_0 = \sqrt{\frac{3}{\mu_s z}}\frac{\lambda}{\pi\theta_{rms}}\left(1+\frac{nd}{z}\right) \qquad g = \cos\theta_{rms}$$

Thrane *et al*., J. Opt. Soc. Am. A **17**, 484 (2000)
Andersen *et al*., Chp. 4 in Optical Coherence Tomography: Technology and Applications, (eds. W. Drexler and JG Fujimoto), pp. 95-140 (Springer, 2015)

OCT A-scan signal model
(three-parameter model, including absorption)

- Efficiency:

$$\psi_{SA}(z) = e^{-2\mu_a z}\left[e^{-2\mu_s z} + \frac{4e^{-\mu_s z}[1-e^{-\mu_s z}]}{(1+\mu_a \Delta z_D)\left(1+\left(\frac{w_{SA}^2}{w_H^2}\right)\right)} + \frac{(1-e^{-\mu_s z})^2 w_H^2}{(1+\mu_a \Delta z_D)^2 w_{SA}^2}\right],$$

- Irradiance radius

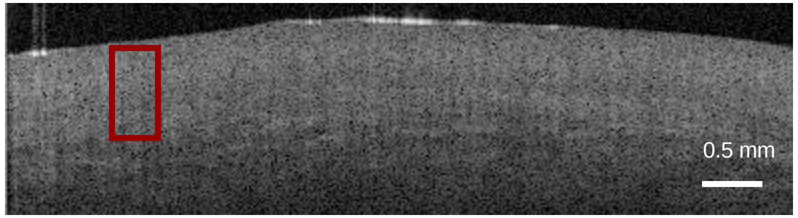
$$w_{SA}^2 = (1+\mu_a \Delta z_D)^{-1}\left[w_0^2 (A - \frac{B}{f})^2 + {\frac{B}{kw_0}}^2 + (\frac{2B}{k\rho_0})^2 (1+\mu_A \Delta z_N)\right],$$

- Optical paths

$$\Delta z_N = \frac{z(w_0^2 + \frac{\rho_0^2}{2})}{4n_R^2 B^2} \text{ and } \Delta z_D = \frac{z}{2n_R^2}\left[(\frac{w_0}{f})^2 + (\frac{1}{kw_0})^2 + (\frac{2}{k\rho_0})^2\right]$$

D. Levitz, MSc Thesis, 2004
Z. Turani et al. Cancer Res **79**(8), 2021-2030.
DOI: 10.1158/0008-5472.CAN-18-2791

Procedure for obtaining the optical scattering properties

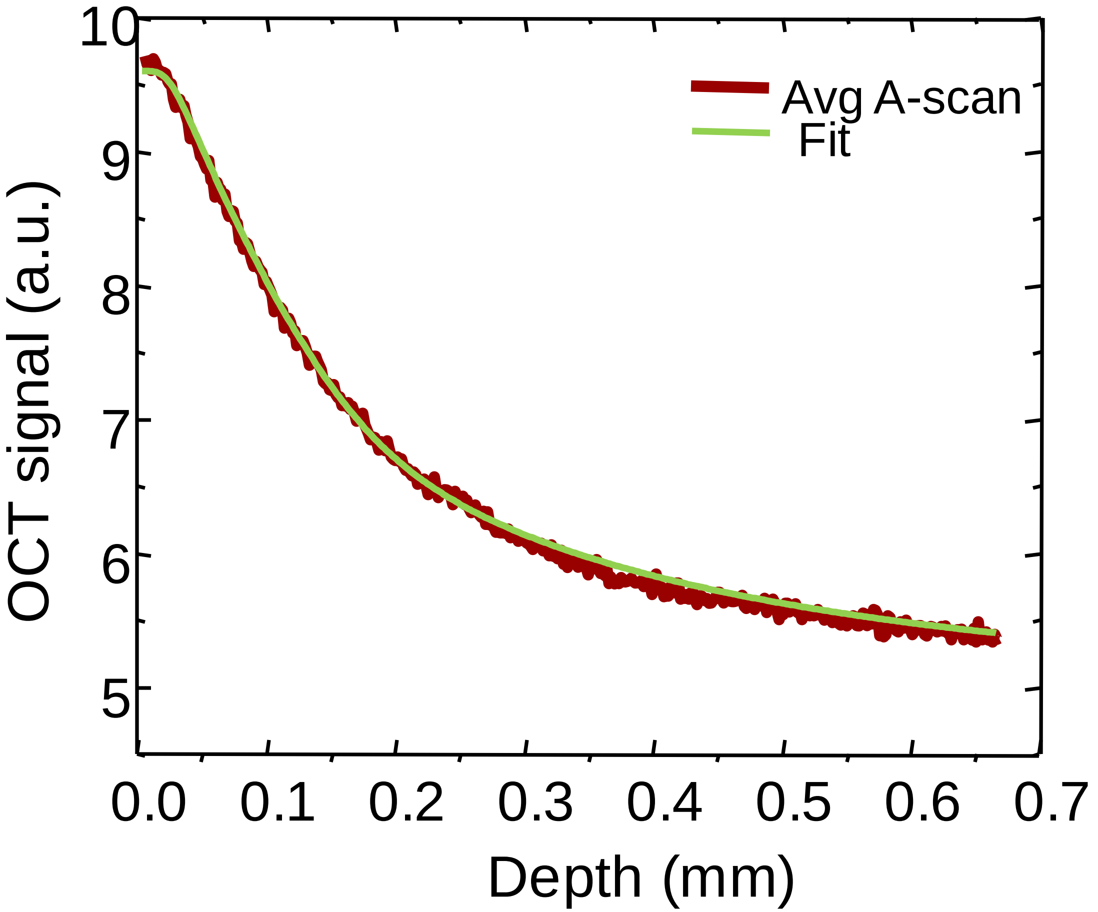


Known input parameters
- wavelength,
- focal length of sample arm lens,
- beam properties.

From region of interest, averaged A-scans are obtained

Curve fitting to expression from model
- in this case, fitting outputs scattering coefficient and anisotropy

Relate optical properties to lesion
- input to systems for classifying lesions



Optical Properties of Healthy, Benign Nevi and Melanoma Skin

Major tissue optical properties

Absorption coefficient	μ_a	[cm^{-1}]
Scattering coefficient	μ_s	[cm^{-1}]
Anisotropy factor	g	[-]

Some statistics about tissue optical properties:

– Scattering and absorption coefficients increase with concentration of melanocytes:
  - Healthy: 14 μm^{-3} $\pm$ 3 %
  - Benign nevi: 18 μm^{-3} $\pm$ 3%
  - Melanoma: 71 μm^{-3} $\pm$ 11%

– Anisotropy factor increases with cell size:
  - Healthy: 6 μm $\pm$ 0.4 μm
  - Benign nevi: 7 μm $\pm$ 0.4 μm
  - Melanoma: 16 μm $\pm$ 3 μm

– Tissue disorder increases from healthy to melanoma, due to cellular displacement.

Therefore, there are significant, measurable characteristic differences between benign nevi and melanoma

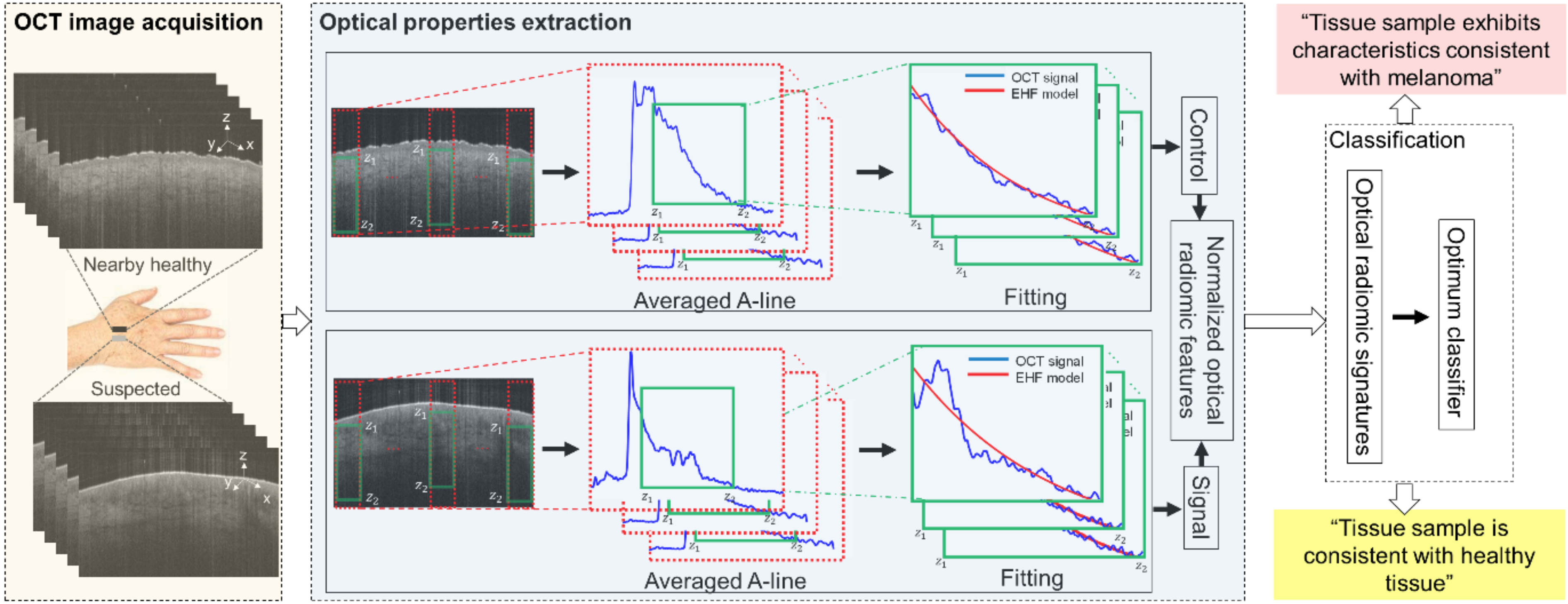
O. Markowitz *et al*., J. Clin Aesthet Dermatol., 2015

Optical Radiomic Melanoma Diagnostic Assistant Algorithm:

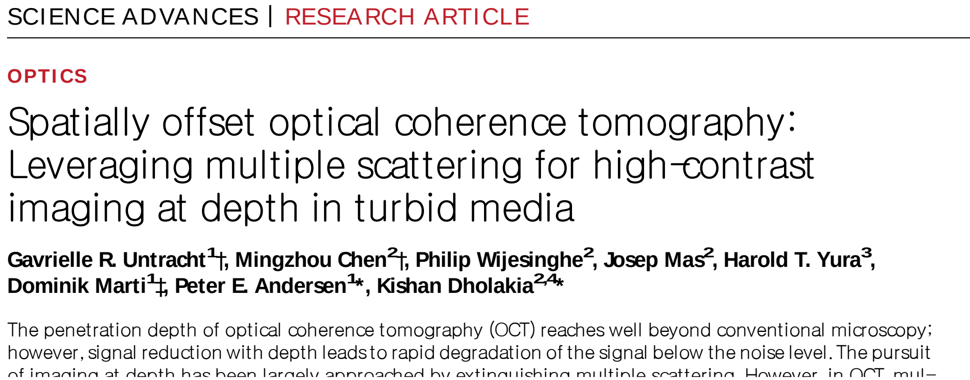
Achieves sensitivity/specificity **96%/95%**
in a study with **+150 patients** (updated 2023)

OCT imaging of suspect and nearby healthy skin → Optical radiomic features extraction → Normalization → Classification → Recommendation

95% diagnostic accuracy



Z. Turani et al., "Optical Radiomic Signatures Derived from OCT Images to Improve Identification of Melanoma," Cancer Res **79**(8), 2021-30 (2019); DOI: 10.1158/0008-5472.CAN-18-2791
K. Avanaki and P. E. Andersen, US20200359887A1 (2020)
Avanaki, K and Andersen, PE 2021, Optical Coherence Tomography for Melanoma Detection. in New Technologies in DermatologicalScience and Practice. 1 edn, CRC Press.

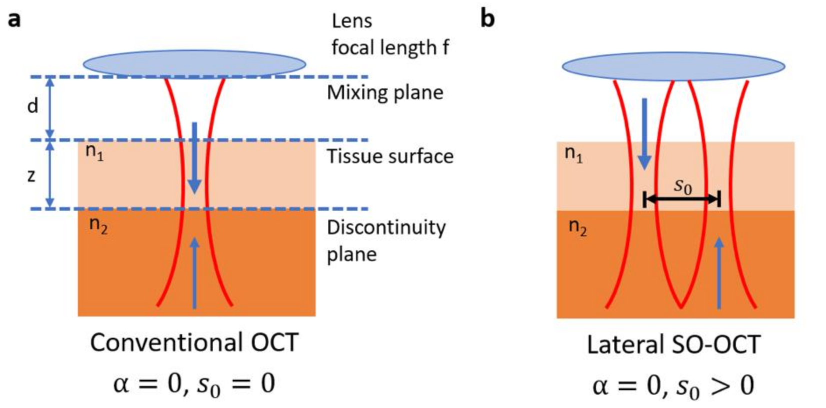

SCIENCE ADVANCES | RESEARCH ARTICLE

OPTICS

Spatially offset optical coherence tomography: Leveraging multiple scattering for high-contrast imaging at depth in turbid media

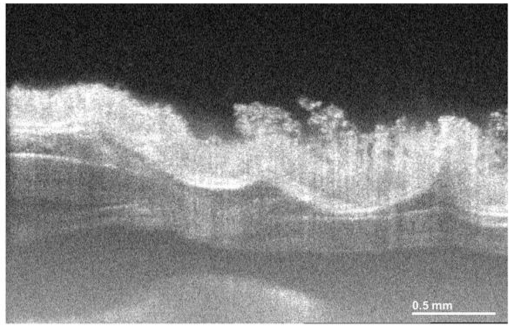
Gavrielle R. Untracht[1]†, Mingzhou Chen[2]†, Philip Wijesinghe[2], Josep Mas[2], Harold T. Yura[3], Dominik Marti[1]‡, Peter E. Andersen[1]*, Kishan Dholakia[2,4]*

The penetration depth of optical coherence tomography (OCT) reaches well beyond conventional microscopy; however, signal reduction with depth leads to rapid degradation of the signal below the noise level. The pursuit of imaging at depth has been largely approached by extinguishing multiple scattering. However, in OCT, mul-

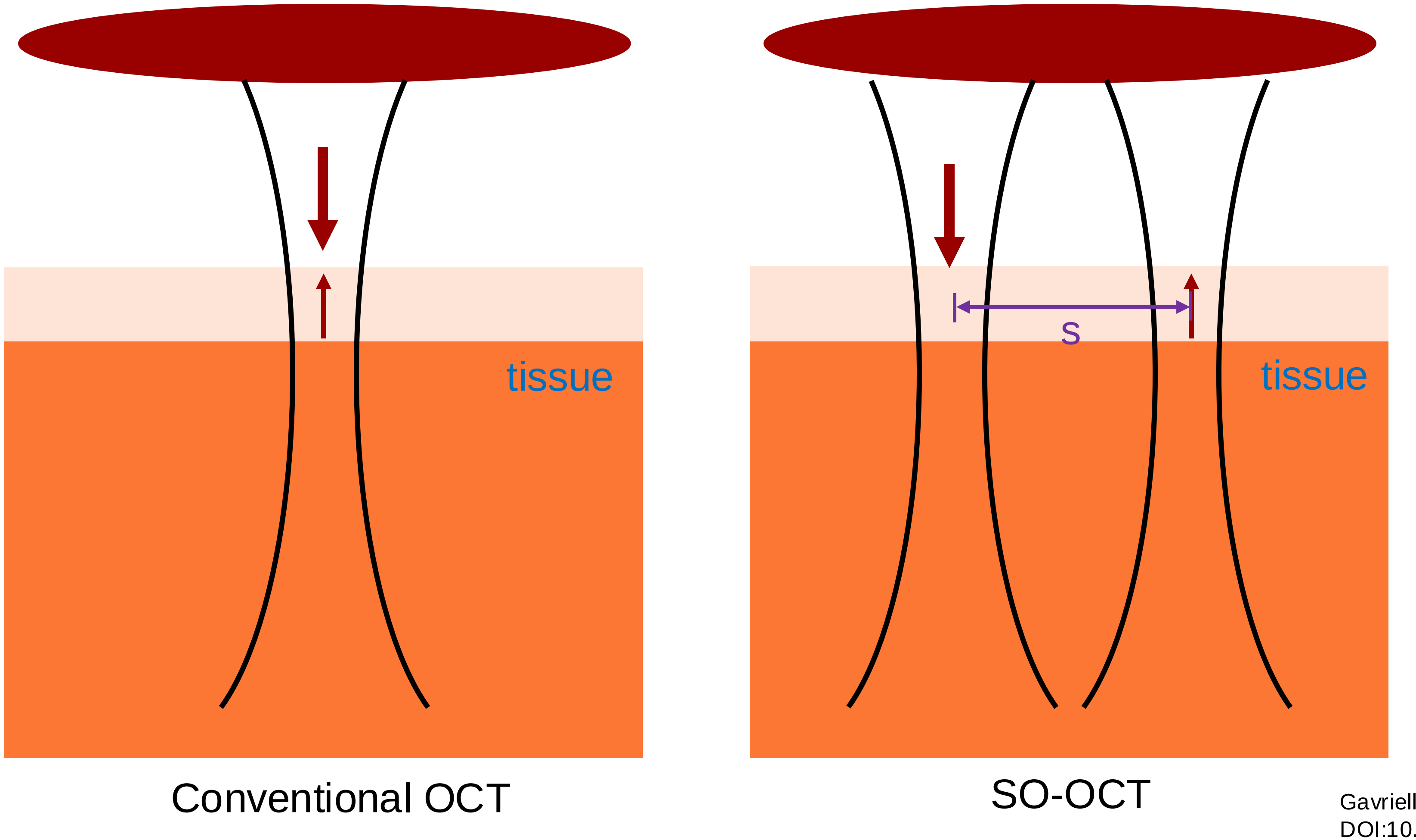


Novel Approach in Optical Coherence Tomography:

GOING DEEP USING MULTIPLY SCATTERED LIGHT



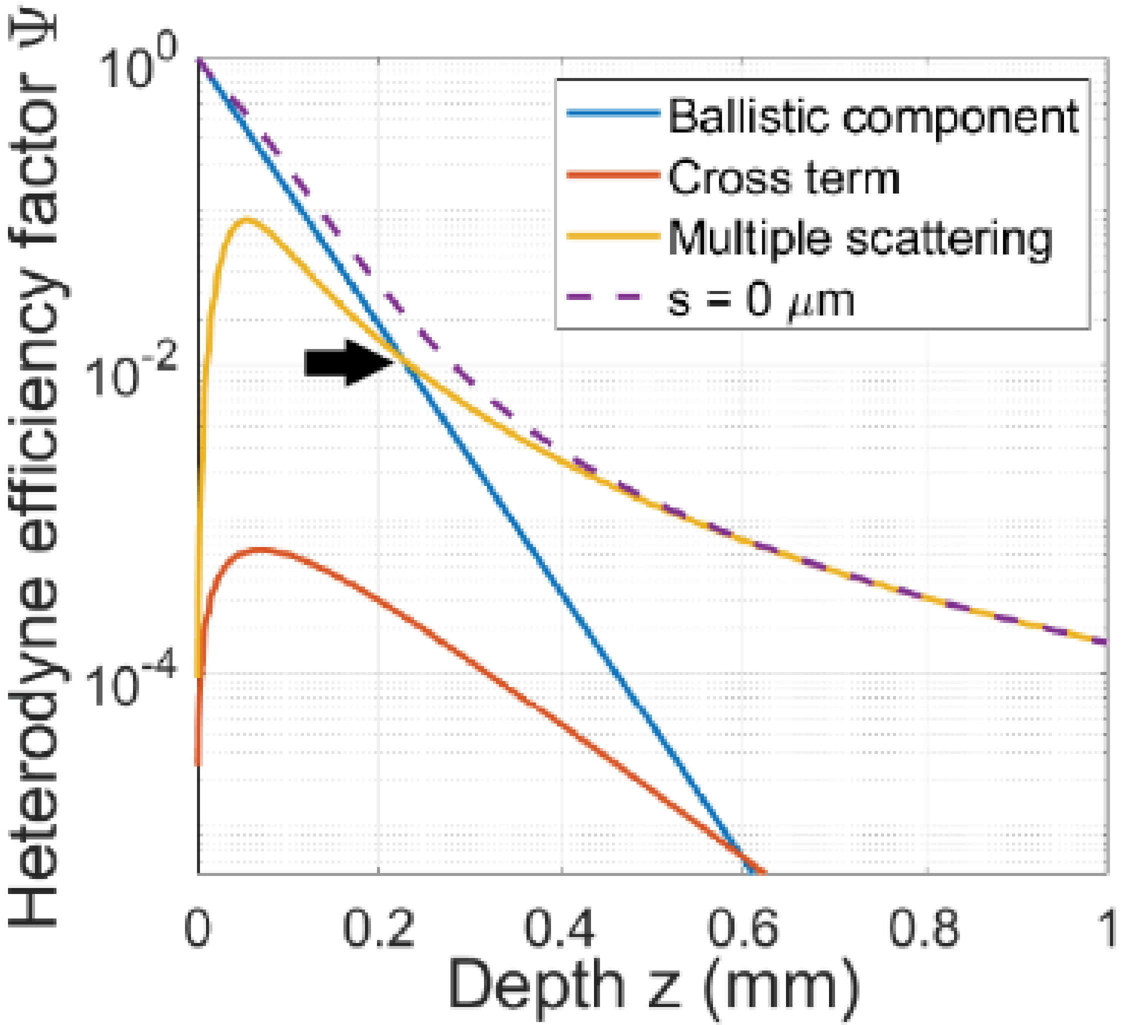
Spatial Offset OCT – a New Approach to Leverage the Role of Multiply Scattered Light in Image Formation



Offset between illumination and collection path allows selective collection of multiply scattered light from deeper in the sample

Gavrielle R. Untracht et al., Sci. Adv. **9**, eadh5435(2023).
DOI:10.1126/sciadv.adh5435

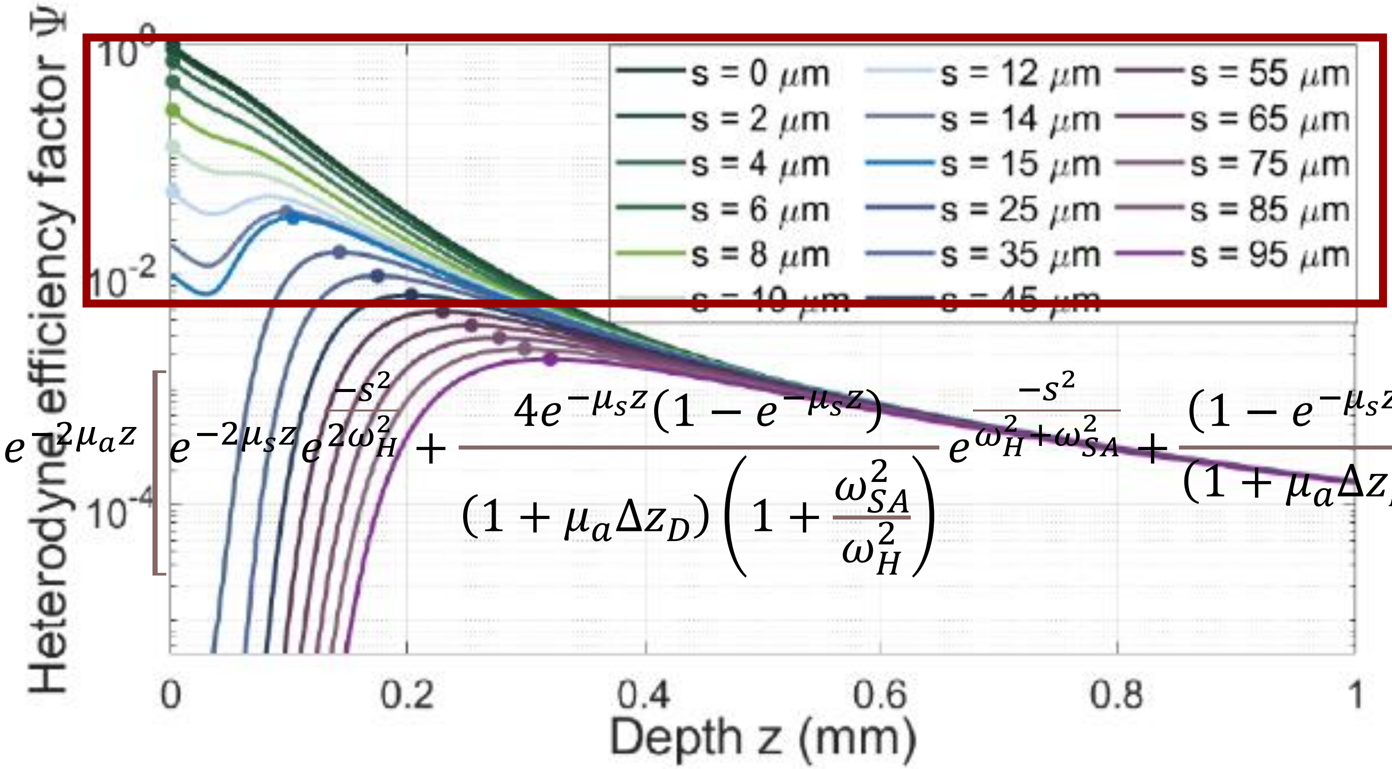
Multiple scattering in OCT



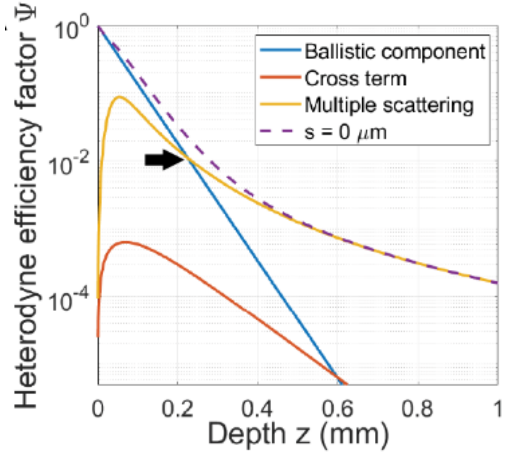
Gavrielle R. Untracht et al., Sci. Adv. **9**, eadh5435(2023).
DOI:10.1126/sciadv.adh5435

Multiple scattering comes mainly from depth... and dominates the signal at depth!
So, we need to image deep in order to understand the effect.

Modelling framework

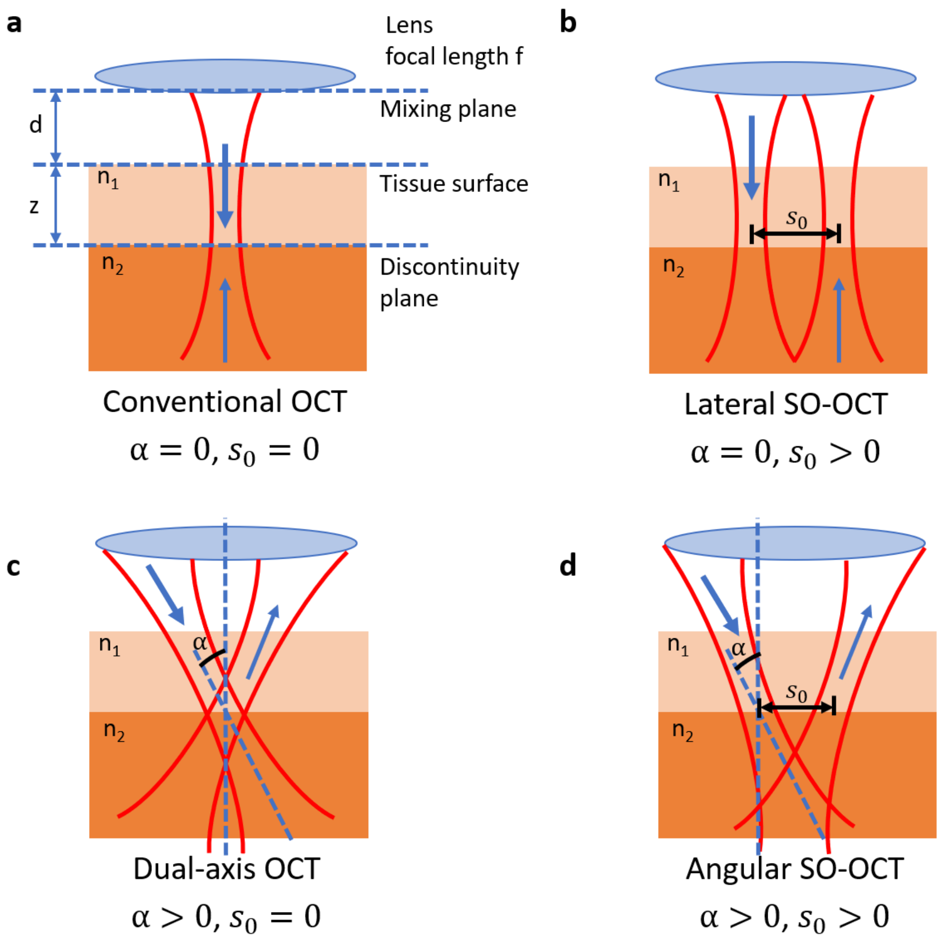


$$\Psi(z,s) = e^{-2\mu_a z}\left[e^{-2\mu_s z}e^{\frac{-s^2}{2\omega_H^2}} + \frac{4e^{-\mu_s z}(1-e^{-\mu_s z})}{(1+\mu_a \Delta z_D)\left(1+\frac{\omega_{SA}^2}{\omega_H^2}\right)}e^{\frac{-s^2}{\omega_H^2+\omega_{SA}^2}} + \frac{(1-e^{-\mu_s z})^2\omega_H^2}{(1+\mu_a \Delta z_D)\omega_{SA}^2}e^{\frac{-s^2}{2\omega_{SA}^2}}\right]$$



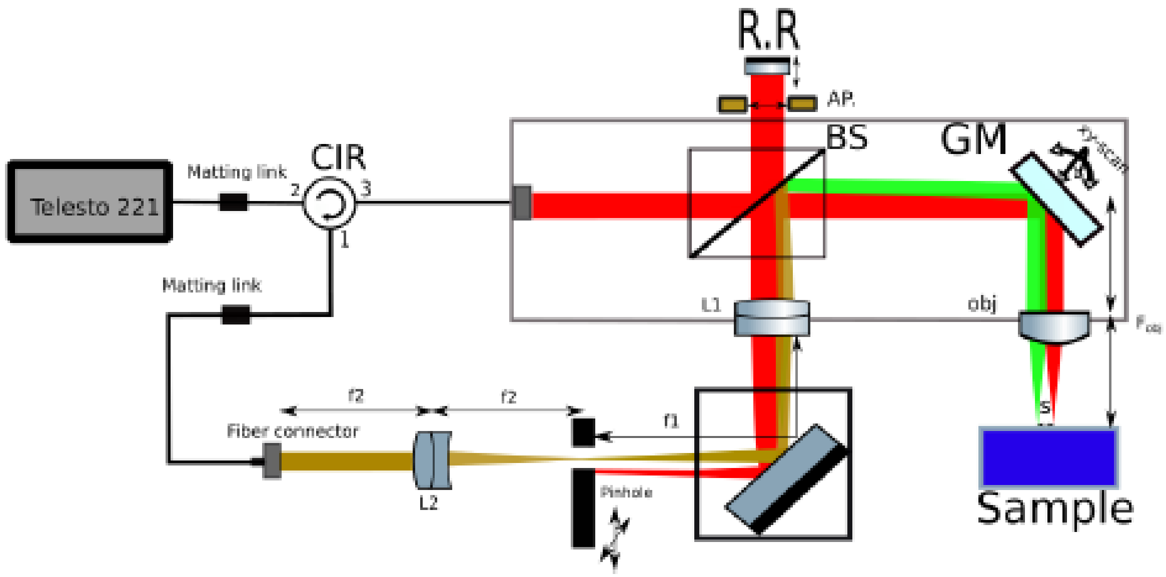
Gavrielle R. Untracht et al., Sci. Adv. **9**, eadh5435(2023).
DOI:10.1126/sciadv.adh5435

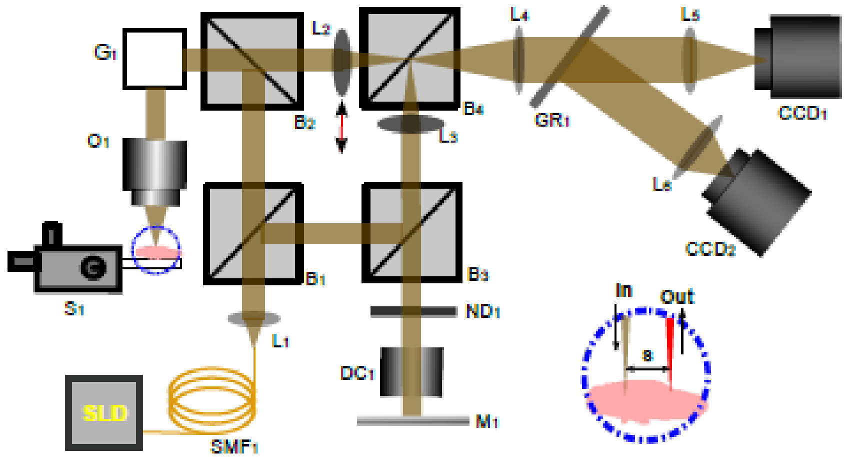
EHF: Generalised Wave-Based Model



$$\Psi(z, s_0, \alpha) = e^{-2\mu_a z}\left[e^{-2\mu_s z} e^{\frac{-(z\alpha+s_0)^2}{2\omega_H^2}} + \frac{4e^{-\mu_s z}\left(1-e^{-\mu_s z}\right)}{\left(1+\mu_a \Delta z_D\right)\left(1+\frac{\omega_{SA}^2}{\omega_H^2}\right)} e^{\frac{-(z\alpha+s_0)^2}{\omega_H^2+\omega_{SA}^2}} + \frac{\left(1-e^{-\mu_s z}\right)^2 \omega_H^2}{\left(1+\mu_a \Delta z_D\right)\omega_{SA}^2} e^{\frac{-(z\alpha+s_0)^2}{2\omega_{SA}^2}} \right]$$

SO-OCT: two implementations

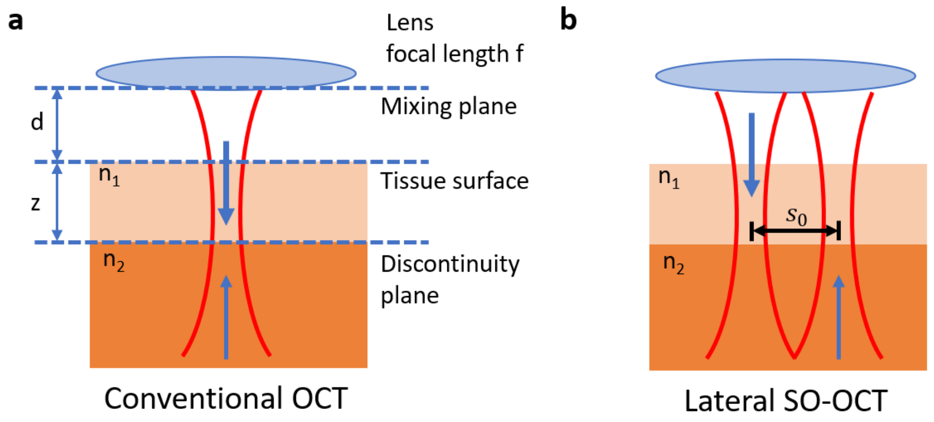




1300 nm excitation, add-on to a commercial system (Thorlabs)

800 nm excitation, home-built system (St. Andrews)

Spatial Offset OCT: A New Approach for Enhancing CNR at Depth in Scattering Tissue



SCIENCE ADVANCES | RESEARCH ARTICLE

OPTICS

Spatially offset optical coherence tomography: Leveraging multiple scattering for high-contrast imaging at depth in turbid media

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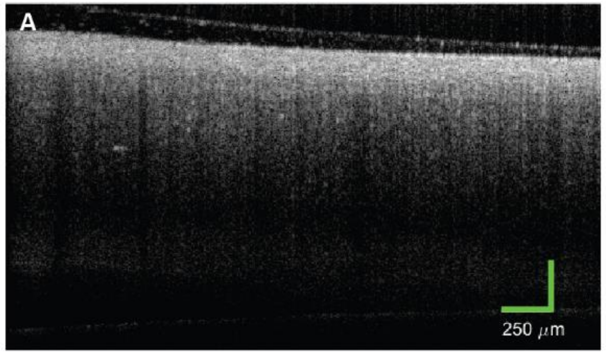
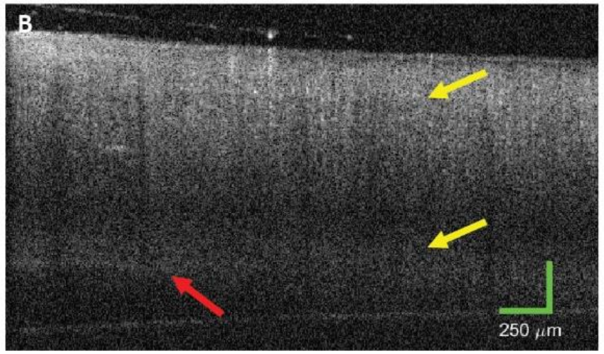
The penetration depth of optical coherence tomography (OCT) reaches well beyond conventional microscopy; however, signal reduction with depth leads to rapid degradation of the signal below the noise level. The pursuit of imaging at depth has been largely approached by extinguishing multiple scattering. However, in OCT, multiple scattering substantially contributes to image formation at depth. Here, we investigate the role of multiple scattering in OCT image contrast and postulate that, in OCT, multiple scattering can enhance image contrast at depth. We introduce an original geometry that completely decouples the incident and collection f elds by introducing a spatial of set between them, leading to preferential collection of multiply scattered light. A wave optics–based theoretical framework supports our experimentally demonstrated improvement in contrast. The ef ective signal attenuation can be reduced by more than 24 decibels. Notably, a ninefold enhancement in image contrast at depth is observed in scattering biological samples. This geometry enables a powerful capacity to dynamically tune for contrast at depth.

DOI:10.1126/sciadv.adh5435

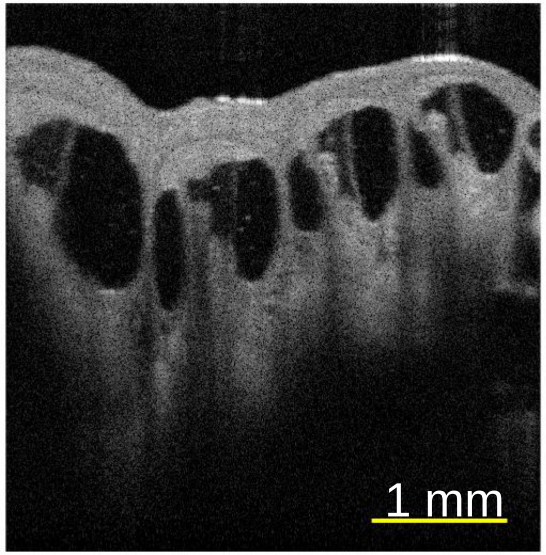
Conventional OCT



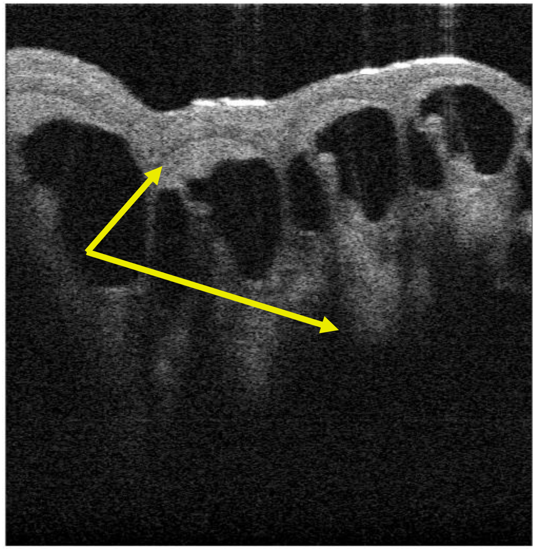
40 μm offset



Conventional OCT

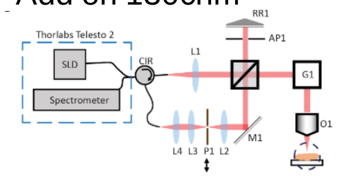


50 μm offset



G. Untracht et al., 2022; unpublished

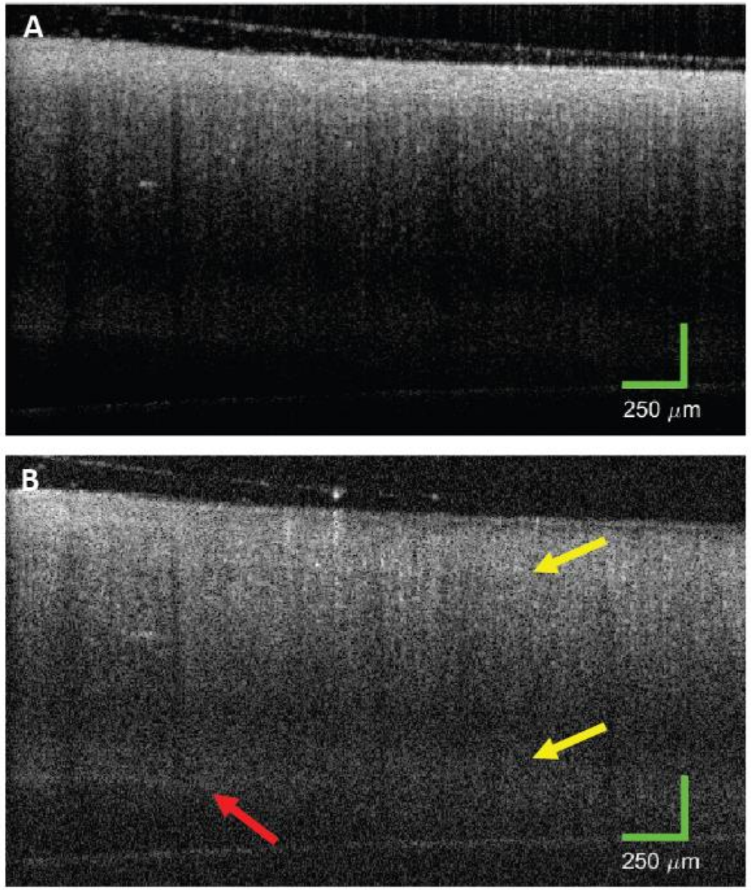
Add on 1300nm

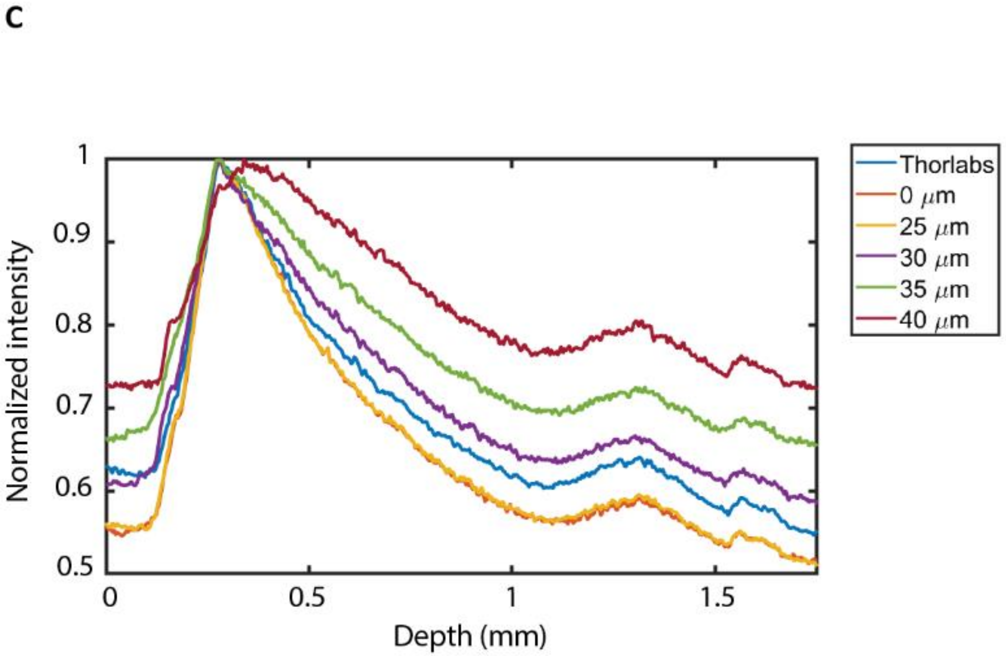


SO-OCT in Hard Tissue: Ex Vivo Mouse Femur

0 µm
offset

40 µm
offset



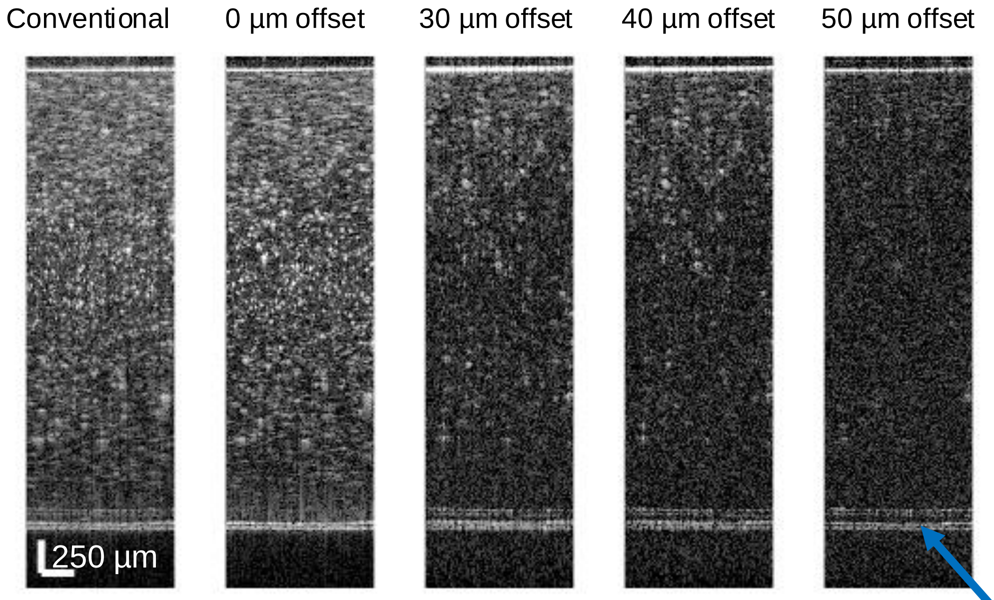


Improved CNR at depth **reveals hidden features** like the bottom surface of the bone and the dish below.

Gavrielle R. Untracht et al., Sci. Adv. **9**, eadh5435(2023).
DOI:10.1126/sciadv.adh5435

Results in (controlled) phantoms

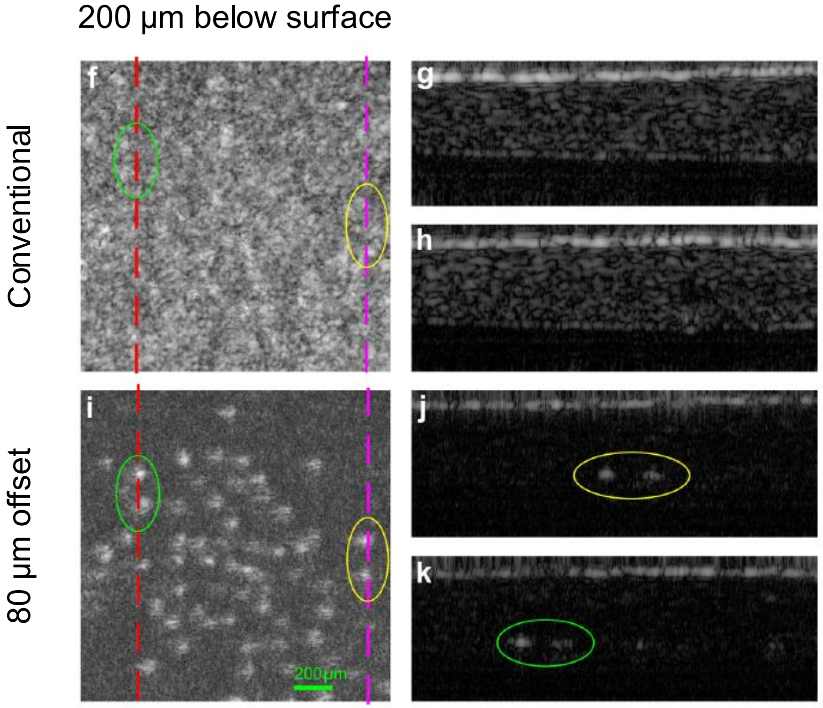
Background scattering is suppressed, hidden beads are revealed!



Enhanced contrast at depth!

0.1% w/w TiO_2 (<5 µm) in silicone

G Untracht et al., unpublished, 2022



100 µm polystyrene microspheres embedded in butter

M. Chen et al., unpublished, 2018

Investigation of Improved Layer Segmentation using SO-OCT

Samples

- Tongue and colon samples from healthy mice

Methods

- Images were acquired of the superior and inferior sides of tongues/colons from healthy mice.
- Postprocessing done with MATLAB using averaging and superimposing techniques.

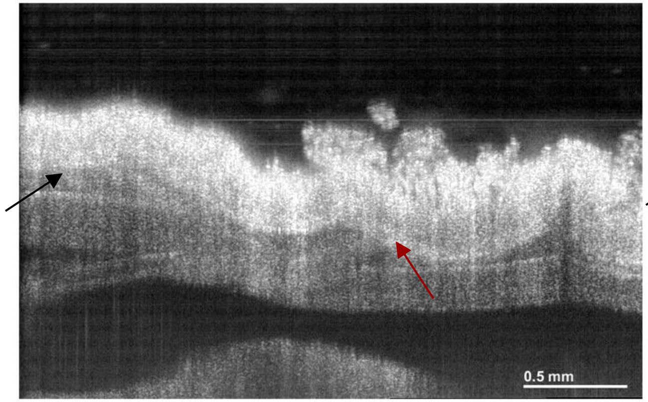
Results

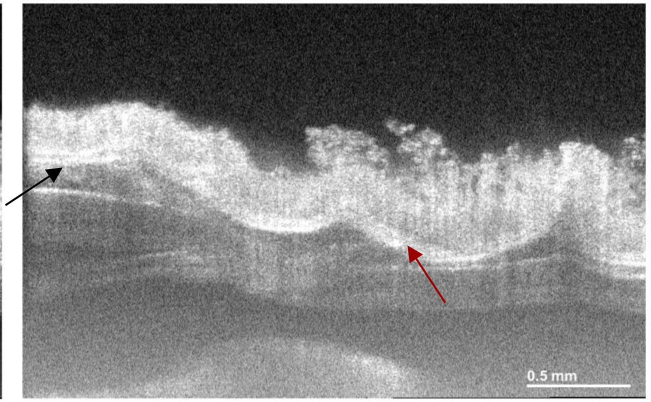
- We can segment better between the layers of the tongue and colons compared to conventional OCT images

Outlook

- These results are the initial steps of a study investigating new biomarkers for tumour detection based on layer segmentation

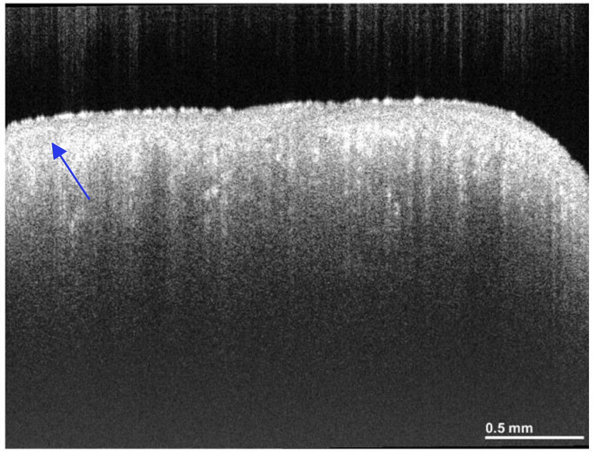
Distal colon

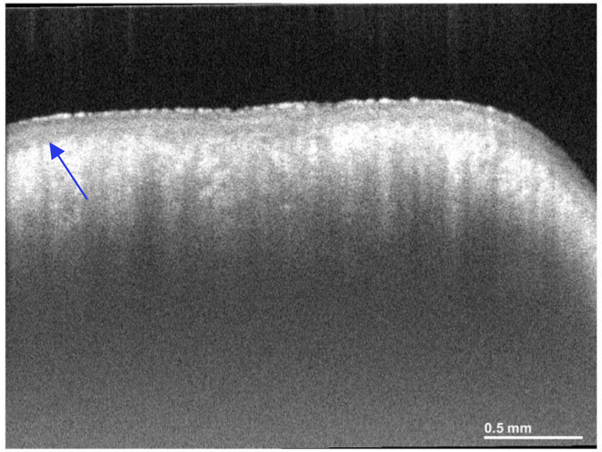




Conventional OCT

Spatial Offset OCT

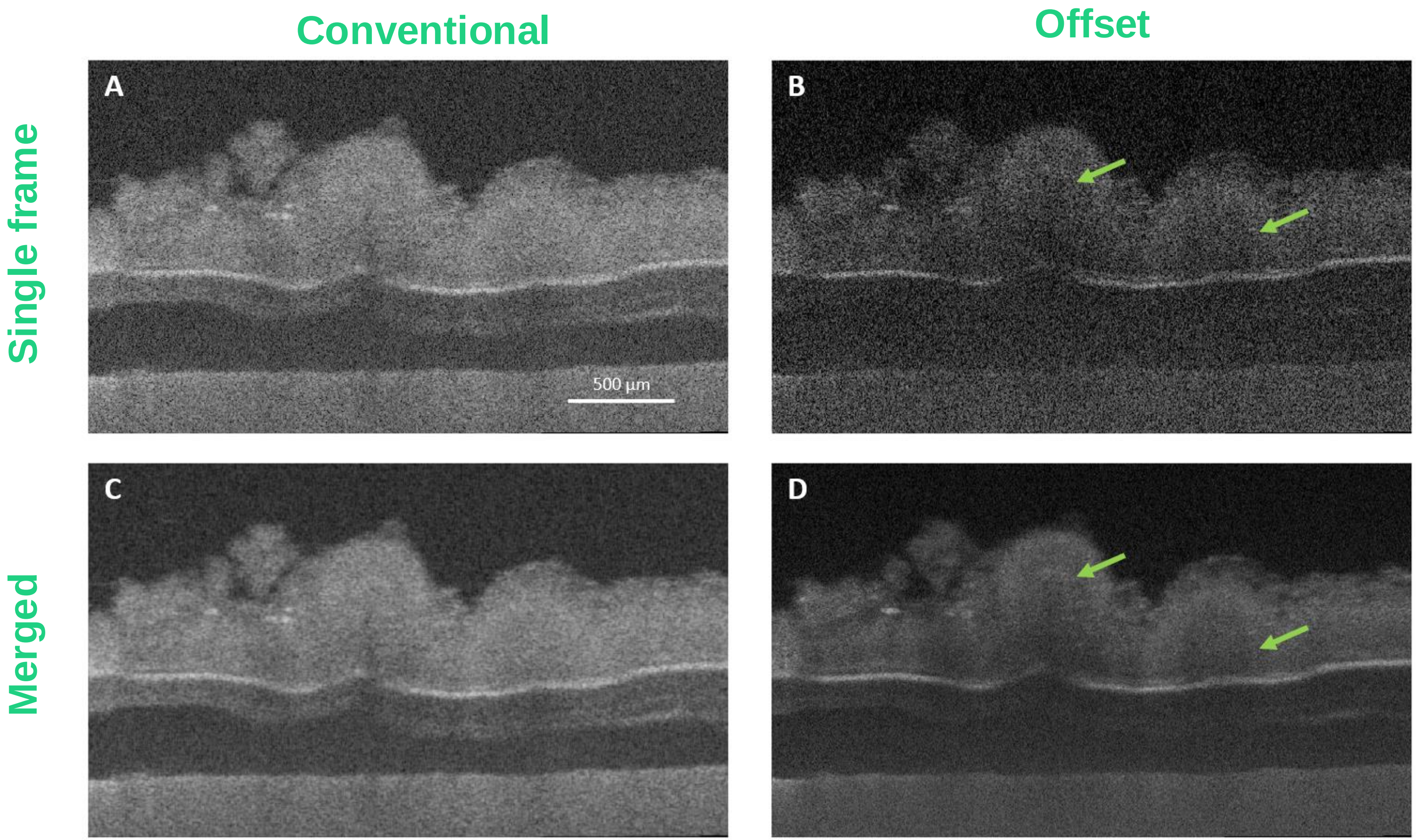


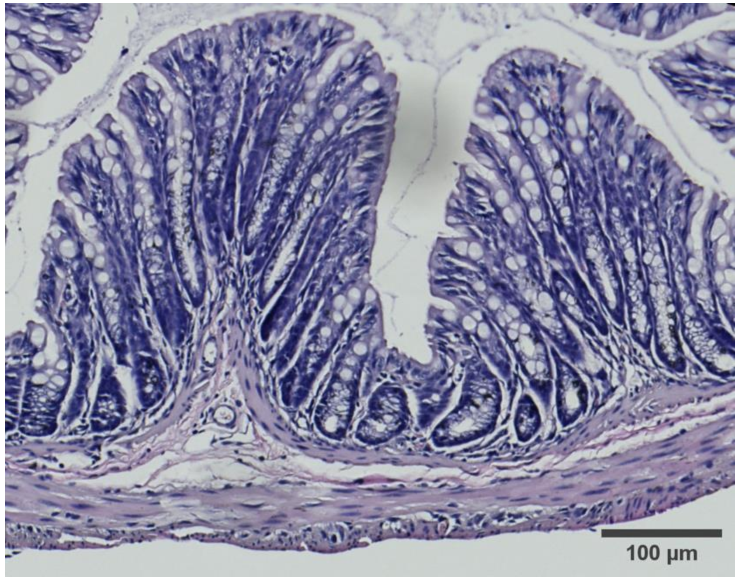


Inferior side of a tongue

F. Høier et al., 2024; *in preparation*

Improved differentiation between tissue layers based on SO-OCT





F. Høier et al, 2024, unpublished

Investigation of Tumour Demarcation using SO-OCT

Samples

- Mice tongues with tumours in the muscle layer

Methods

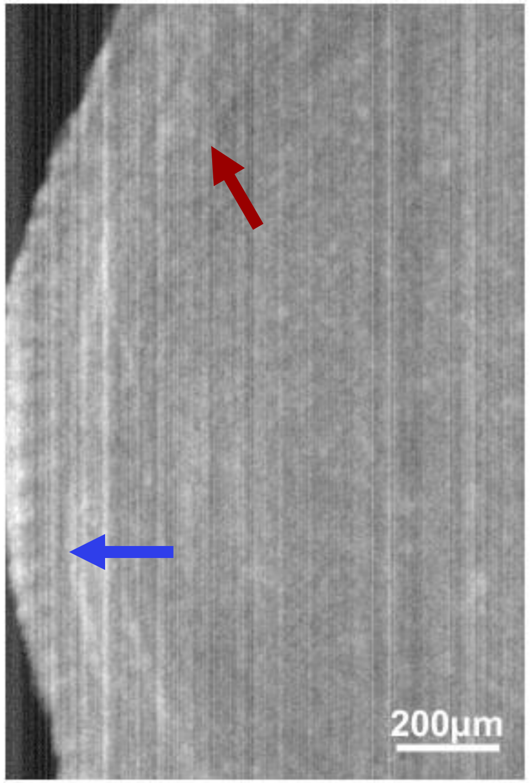
- Images were acquired along the cut
- Postprocessing happened with MATLAB using averaging and superimposing techniques

Results

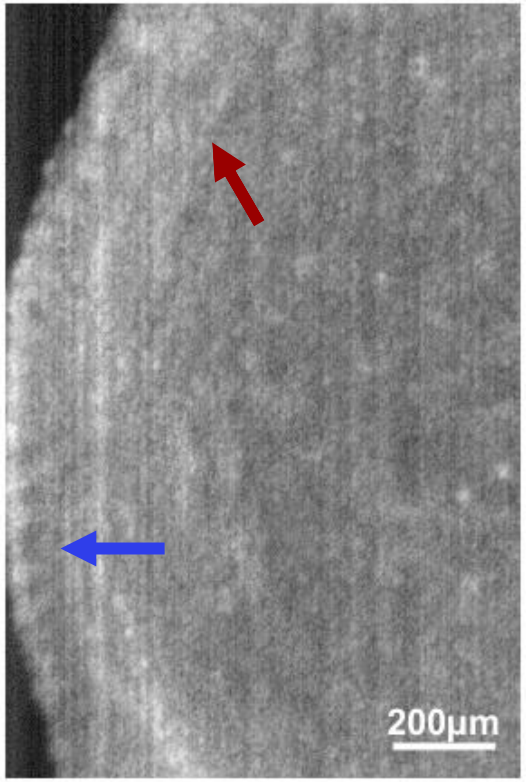
- We can segment better between the borders of the tumour with SO-OCT compared to OCT
- The images show similar information as the HE-stain histology image

Outlook

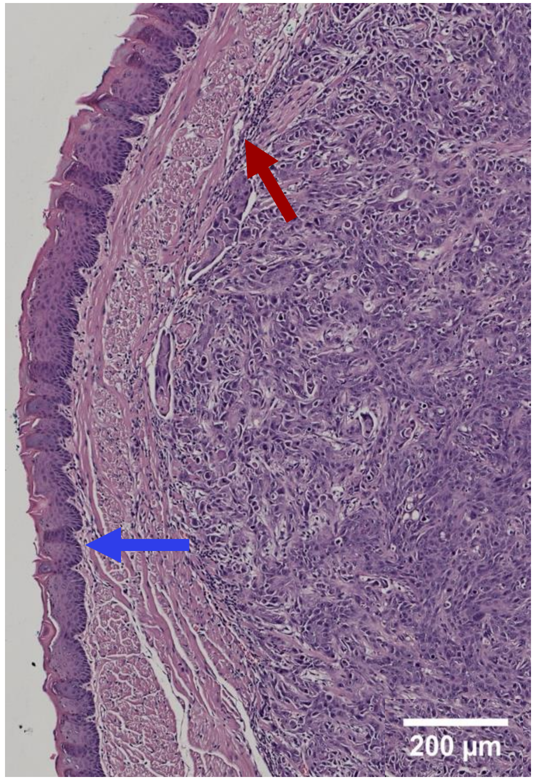
- These results are the initial steps of a study investigating tumour detection based SO-OCT.



Conventional OCT



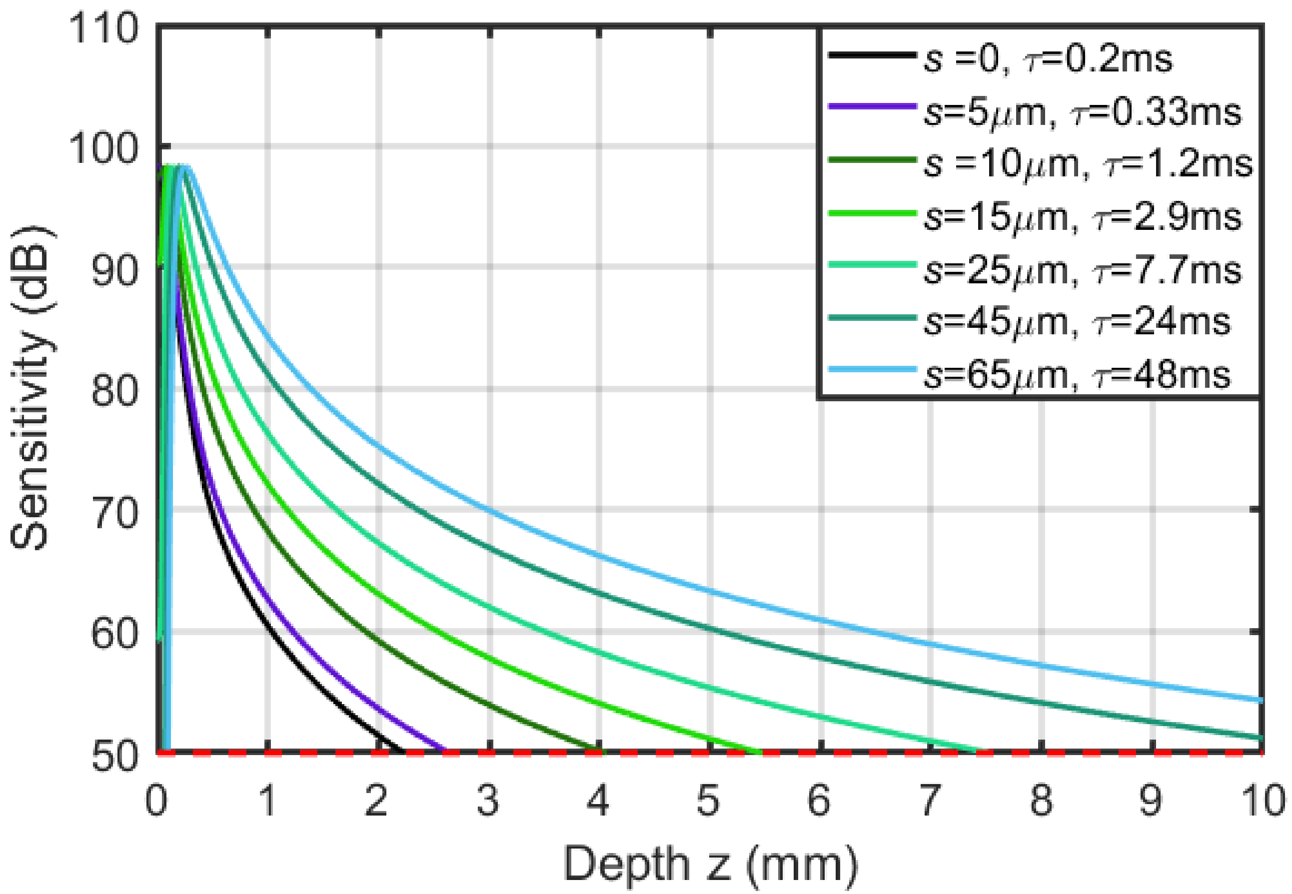
Spatial Offset OCT

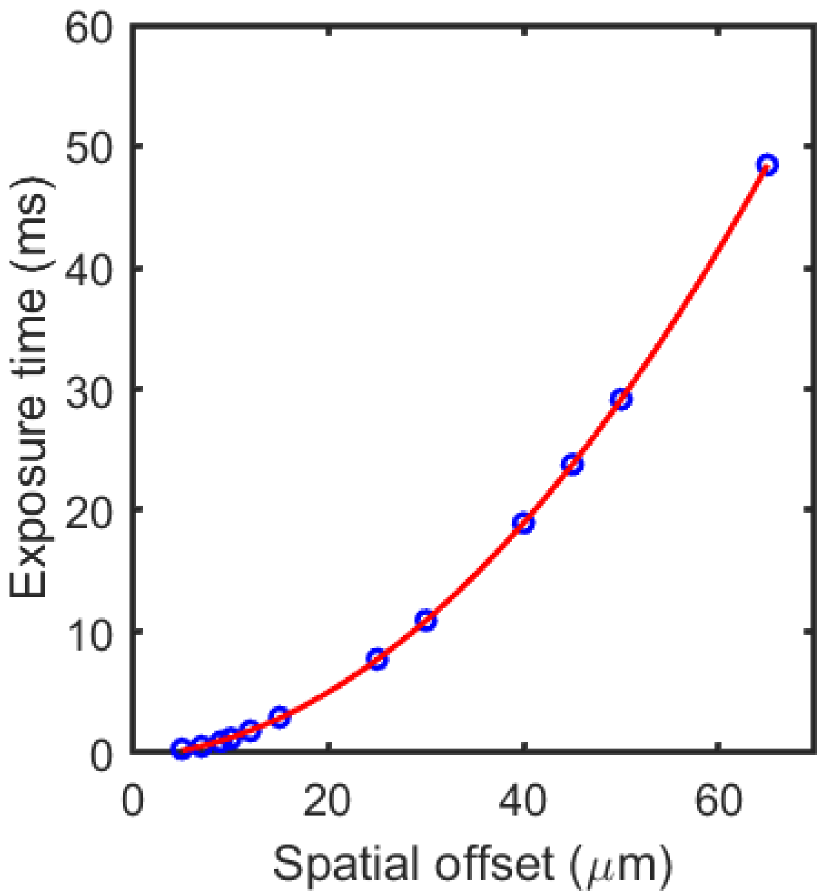


Histology (HE-stain)

F. Høier et al, 2024, unpublished

Estimating Maximum Probing Depth





S. Georgiou et al, 2024, submitted

Going deep with SO-OCT

- Optical coherence tomography
  – scattering being the key contrast
  – light-tissue interaction can be modelled using EHF

- New OCT concept: Spatial Offset OCT
  – multiple scattering plays a dominant role (generally in OCT)
  – enables deep tissue imaging ➔ allows several mm imaging depth

Summary

Colorectal cancer arises from epithelial precursor lesions

- current endoscopic differentiation of lesions is based on size and shape, surface pattern (crypts), and vessel architecture

PROSCOPE: CRC diagnosis will improve from accurate *in vivo* multimodal optical imaging providing **morphology**, **angiography**, **molecular** and **metabolic** information of deeper layers

PROSCOPE – endomicroscopy in general – holds huge potential to

- provide early diagnosis,
- demarcate lesions *in vivo* (thereby reduce recurrence rates),
- guide surgery,
- avoid under/over treatment of patients,
- improve survival rate and quality of life.

PHOTONICS PUBLIC PRIVATE PARTNERSHIP

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PROSCOPE

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