Clinical Translational Laser Spectroscopy for an Improved Cancer Diagnosis and Therapy

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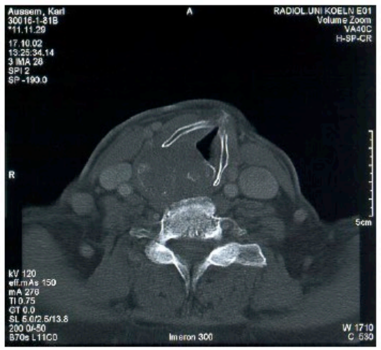
2) Leibniz Institute of Photonic Technology Jena a member of the Leibniz Research Alliance Leibniz Health Technology, Germany

Today‘s standard diagnostics

- Clinical examination
- White light endoscopy and microscopy
- **Medical Imaging** (Ultrasound, CT, MRT, SPECT, PET etc.)



MRT = Magnetic resonance tomography
MRS = Magnetic resonance spectroscopy
CT = Computer tomography
SPECT = *single photon emission computed tomography*
PET = Positron-Emissions-Tomography

Images courtesy Prof. Dr. Orlando Guntinas Lichius

Pre-, intra-, post-operative imaging

Needs in all scenarios: screening, therapy, follow-up

- Better **visualization** of the tumor
- Better **discrimination** from normal surrounding
- Better discrimination of cancer from precancerous lesions and chronic inflammation
- **Detection** in **earlier** stage
- **Less sampling error**
- **Online guidance** during surgery
- **Monitoring** during therapy
- Help during follow up

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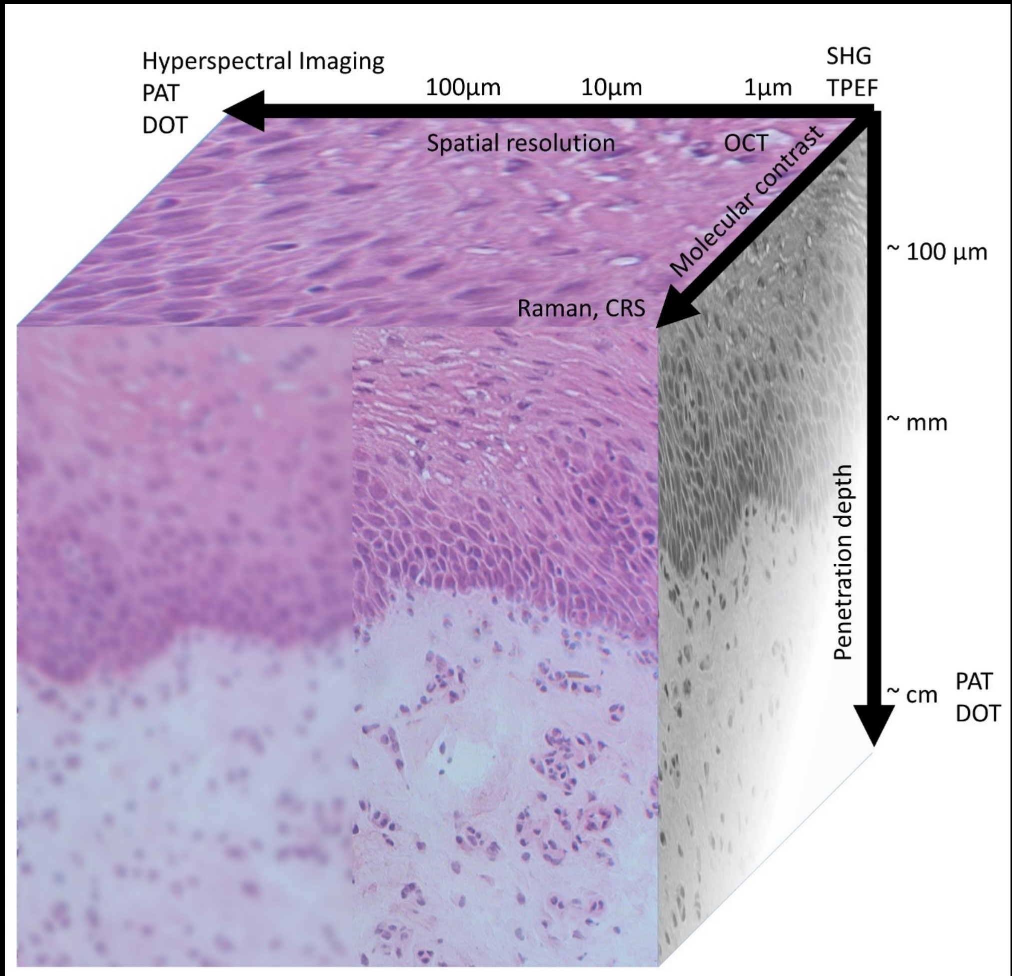
Label-free multimodal / multispectral imaging



- Properties of most important biophotonic imaging techniques with respect to:
  - **Penetration depth**
  - **Spatial resolution**
  - **Molecular specificity**
  - **Speed**

⇨ Various methods are necessary in order to achieve multivariate molecular contrast as well as morphological information

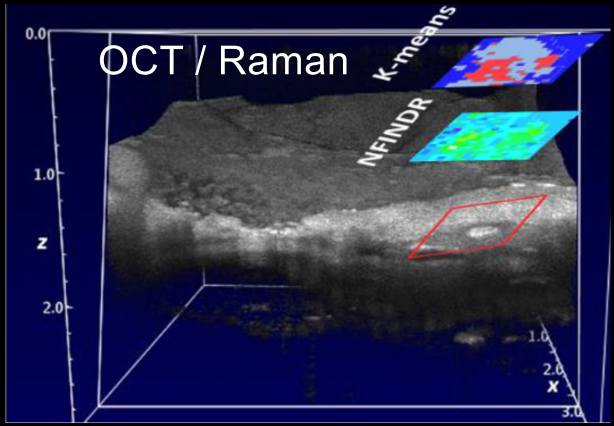
⇨ **Multimodal imaging**

CRS: coherent Raman scattering
PAT: photoacoustic tomography
OCT: optical coherence tomography
DOT: diffuse optical tomography
SHG: second-harmonic generation;
TPEF: two-photon excited fluorescence
ETC.

Biophotonic imaging approaches – where do we stand?

1. **Combination of fast imaging techniques (e.g. FLIM, OCT) with slow but molecular specific approaches e.g. Raman**

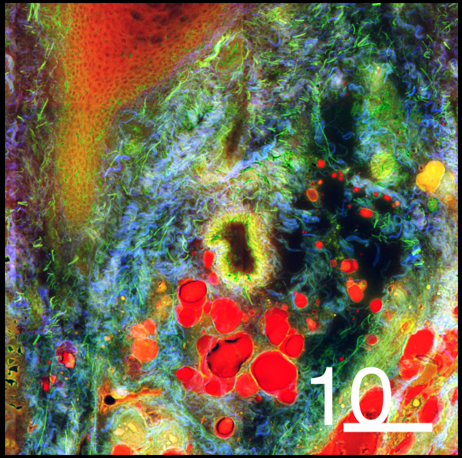
   ⇨ linking a large field of view of morphological information with a richness of molecular detail of selected points or confined areas



Egodage *et al.* COC, 2017, 15, 090008.

2. **Multi-contrast imaging utilizing imaging approaches with similar image acquisition times**

   ⇨ Combination of imaging modalities requiring similar experimental equipment: e.g. CARS, SRS , TPEF, SHG, FLIM



CARS
TPEF
SHG

Heuke *et al.* Head &Neck, 2016, DOI 10.1002/hed.24477

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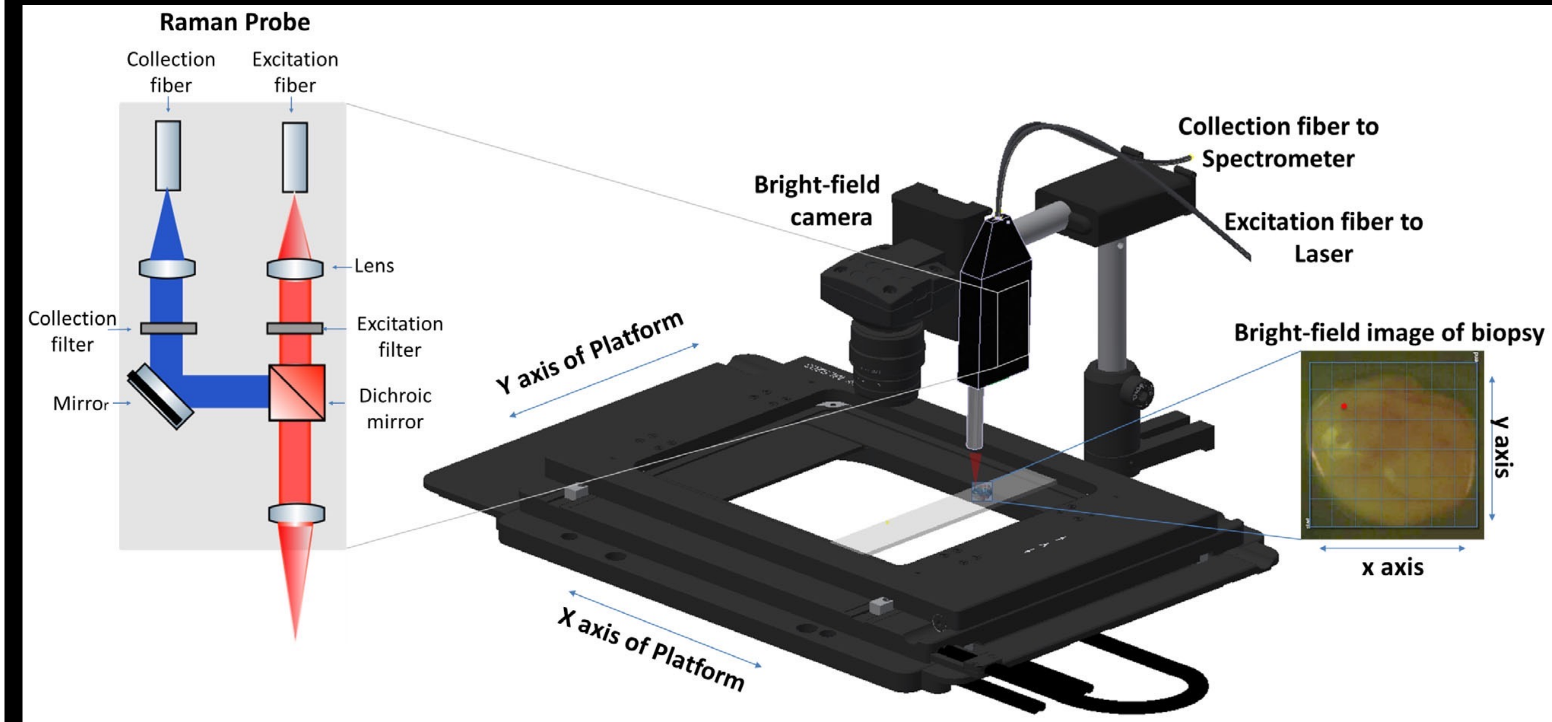
Bladder Cancer – Intraoperative Staging and Grading

Compact fiber probe-based setup for Raman mapping



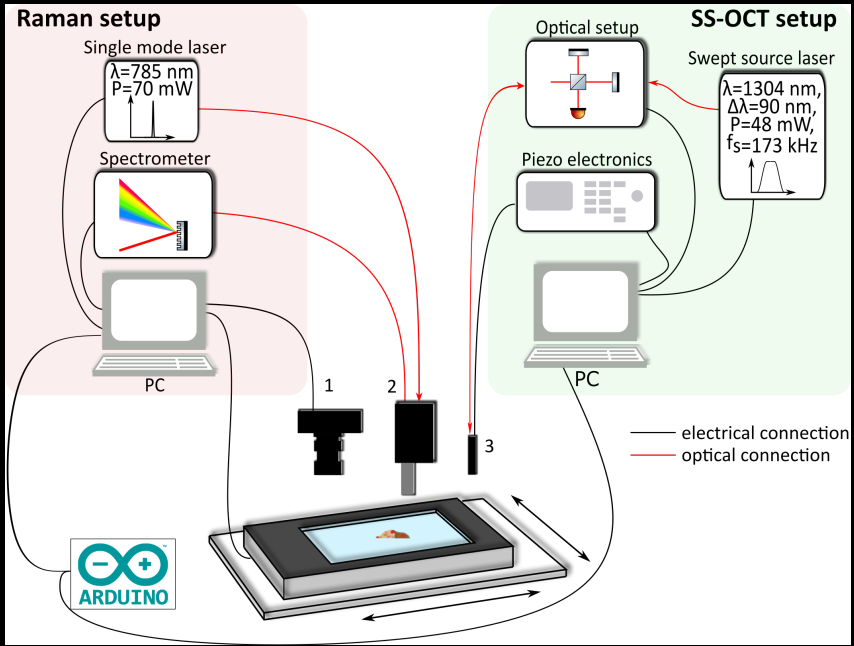
Clinical information

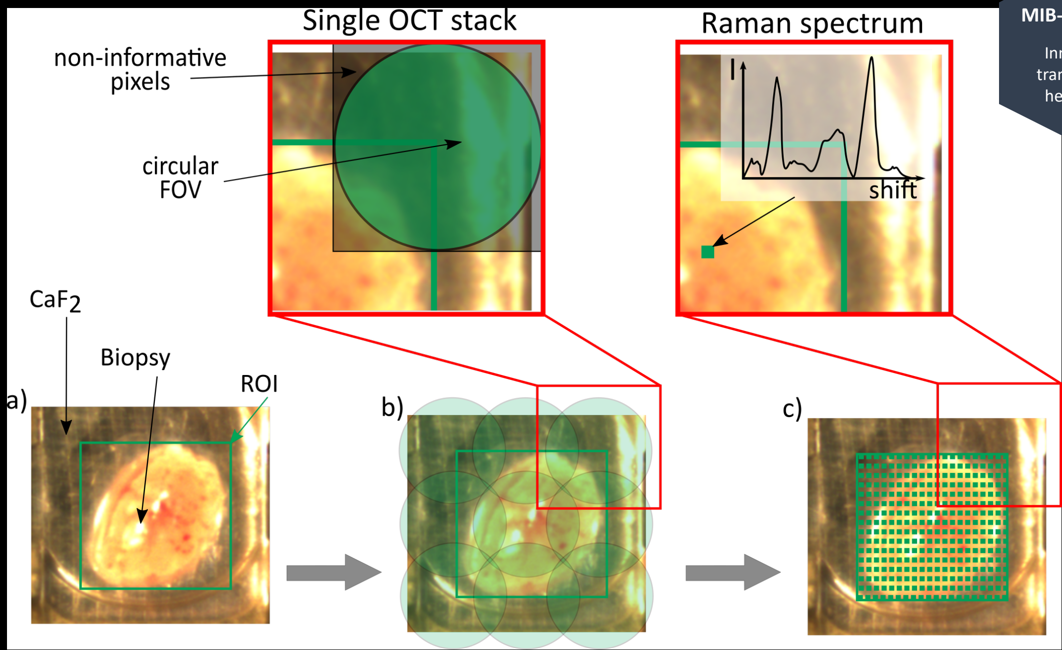
- **25 patients** in total provided fresh biopsies
- 3 patients provided frozen biopsies
- **66 Biopsies** were measured
- 6 Biopsies SERDS were performed
- 9 frozen biopsies and 61 fresh biopsies

Experiment parameters

- Integration time **3 sec**
- Power **200 mW**
- Avrg. scanned area 2.6 x 2.6 mm 20 by 20 pixels
- SERDS were performed the last day
- High fluorescence background observed in the prostate biopsies

Imaging of bladder cancer using OCT and Raman spectroscopy

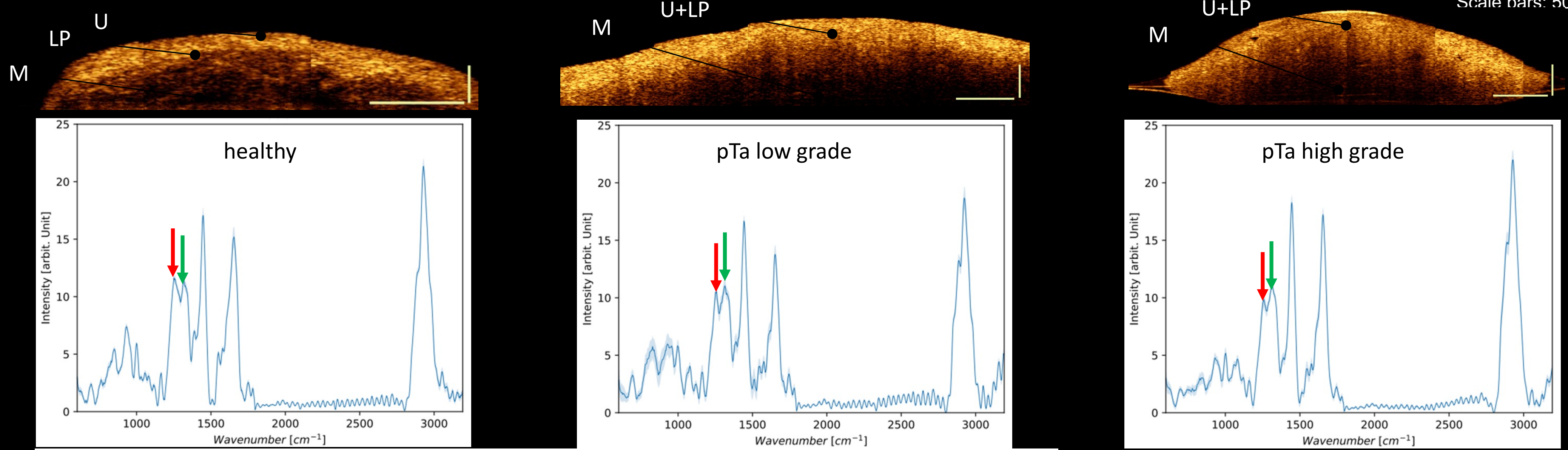




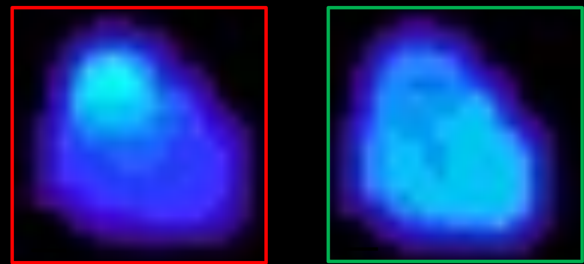
Placzek et al, Analyst 2020, 145, 1445–1456.

Combination of OCT and RS for bladder tumor characterization

Scale bars: 500 µm

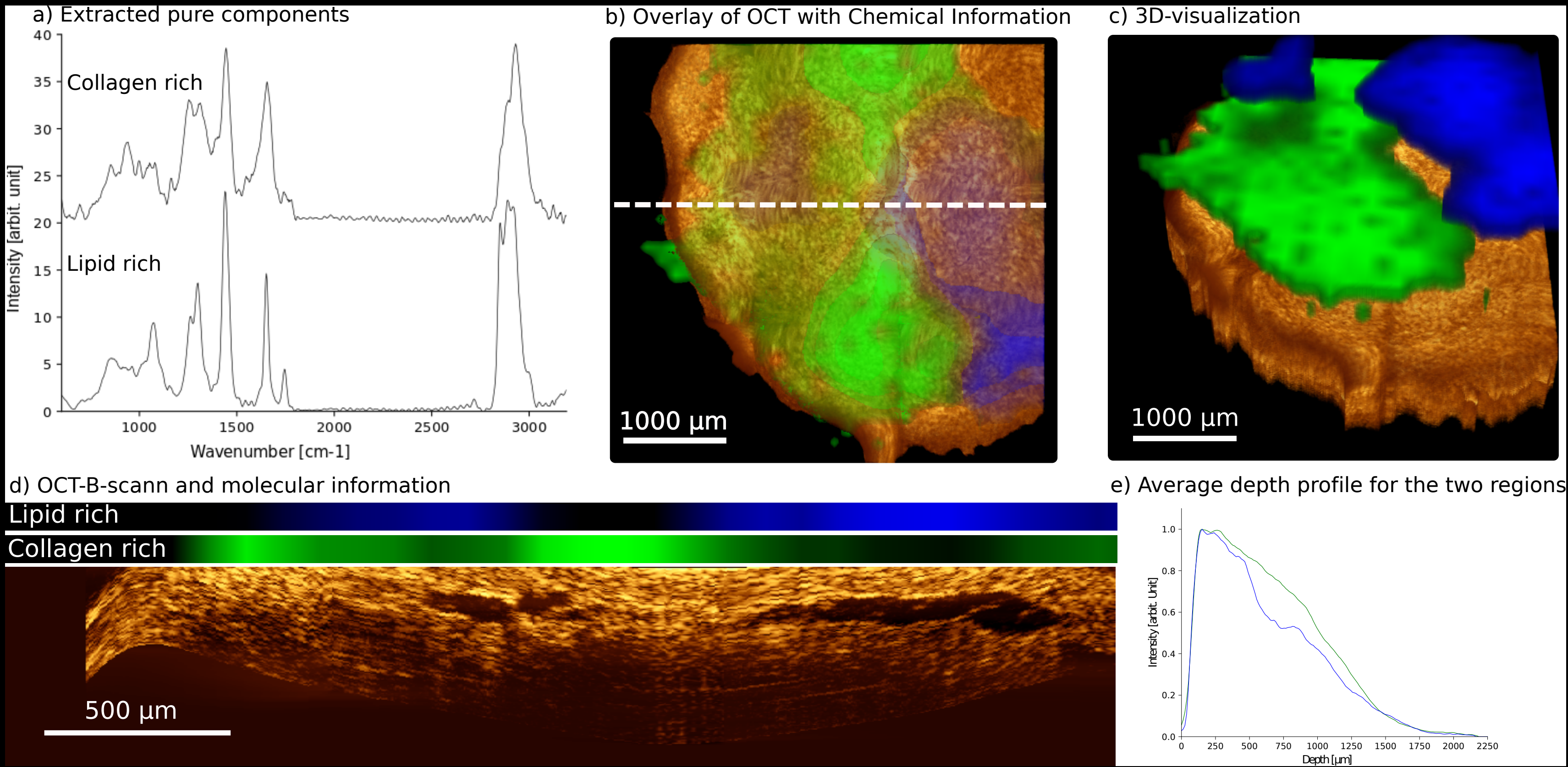


	OCT (%)	RS – T/NT (%)	RS – HG/LG (%)
Accuracy	73.4	92	77
Sensitivity	78	95	81
Specificity	69	88	68
Confidence interval	(72.9–73.9)	(92.2–92.6)	(73–81)



Placzek

Modalities provide comprehensive information and enable to understand the underlying origins



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Innovation transforming healthcare

Placzek et al, Analyst 2020, 145, 1445–1456.

Translation to *In-Vivo I*nvestigations.

Plug-and-Play Raman-probe for clinical applications

- Mechanically robust, medical grade components and sterilizable
- No alignment
- Bending radius 1,5 cm
- Preparation of documentation according to MDR2017/745 for an in vivo clinical investigation

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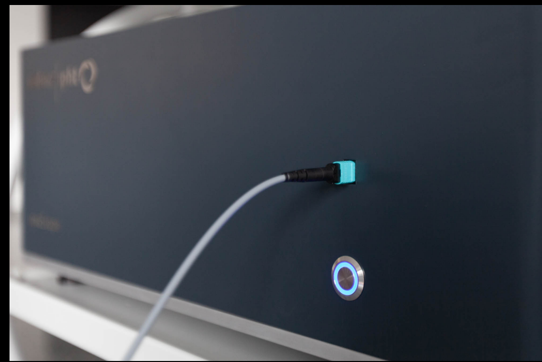
Raman-invaScope: a device for label-free non-destructive in vivo diagnostics

- Designed for the clinical application and high and reproducible performance
- Build on a medical cart for easy access to the surgery room
- Medical-graded computer and interface components designed for clinical applications
- Acquisition is simply performed by pressing a food switch
- Emergency button allows to shutdown the device immediately
- Excitation power is always monitored to ensure a safe working environment and to prevent overexposure of the patient
- Intuitive software to control the data acquisition and visualization
- All important information rapidly at hand
- User can be trained in a short amount of time



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Ex-Vivo and In-Vivo Tumor Border Detection or Disease State Activity by Multimodal Non-Linear Imaging

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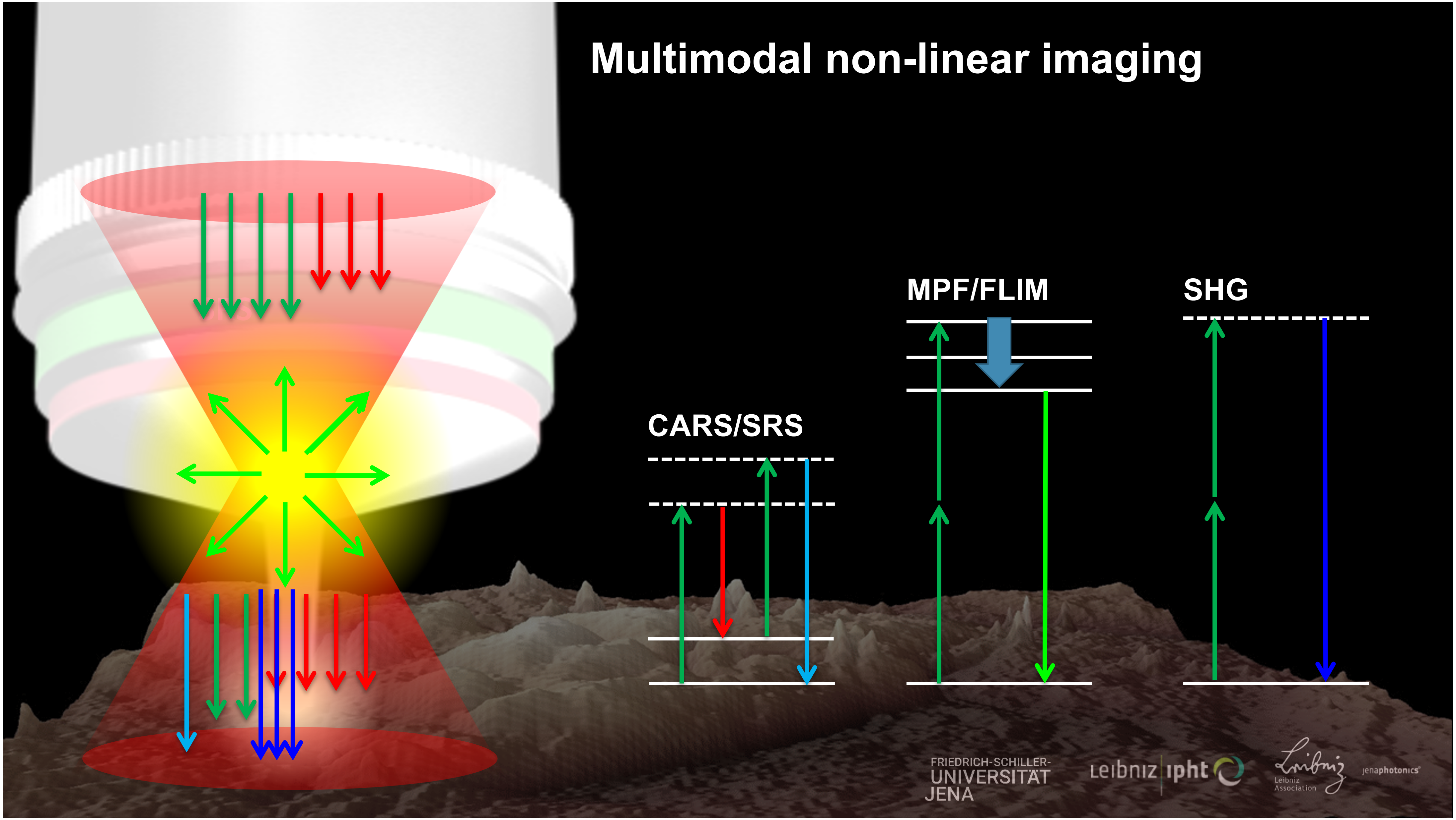
Multimodal non-linear imaging
CARS/SRS
MPF/FLIM
SHG
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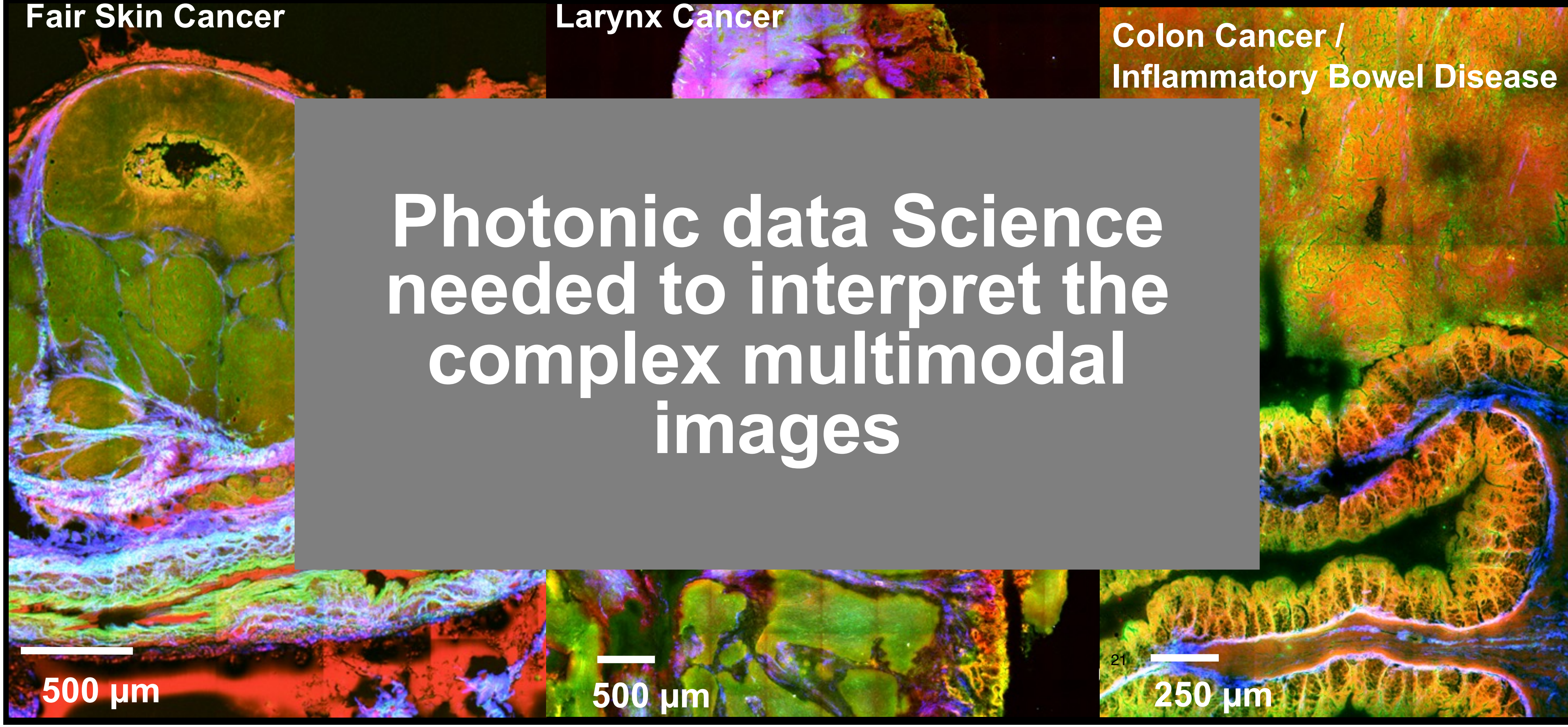
Non-linear multimodal Imaging

TPEF SHG CARS

Heuke *et al*. British Journal of Dermatology, 2012, 169, 794.
Heuke *et al*. Healthcare, 2013, 1, 64.

Meyer *et al*. Head & Neck, 2013 35, E280.
Heuke *et. al*. Head & Neck, 2016 DOI 10.1002/HED.

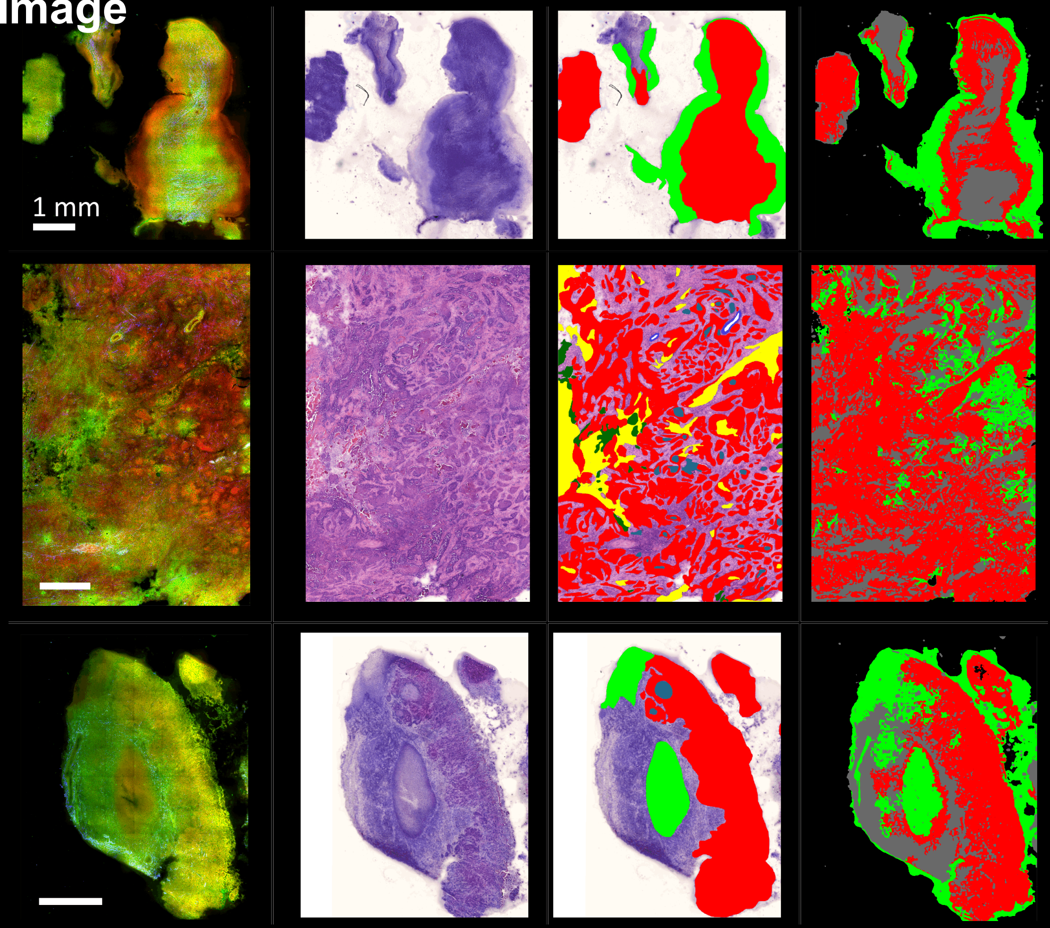
Bocklitz et al. BMC Cancer, 2016, 16, 534.
Chernavskaia et al. Scientific Reports, 2016, 6:29239.



Head and Neck Cancer

Prediction of tissue type by linear discriminant analysis

Examples of correct predictions of cancer



Prediction Groups

- Cancerous Tissue
- Epithelial Tissue
- Background
- other

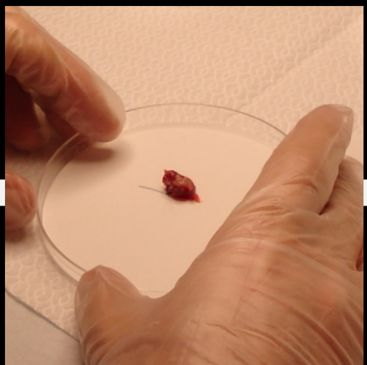
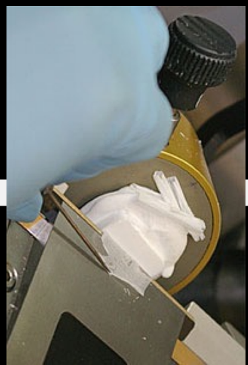
Diagnosis Groups

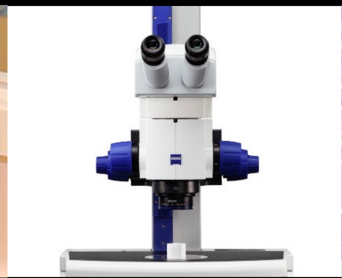
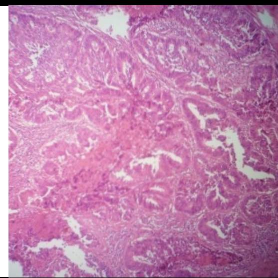
- Inflamed Area
- Grandular Tissue
- Adipose Tissue
- Vascular Tissue
- Keratinisation
- Necrosis
- Muscle Fibre Bundles
- Cartilage

Heuke et al. Head & Neck 2016, DOI 10.1002/hed.24477

Multimodal intraoperative cryosection diagnosis in head and neck surgery

Standard Cryosection

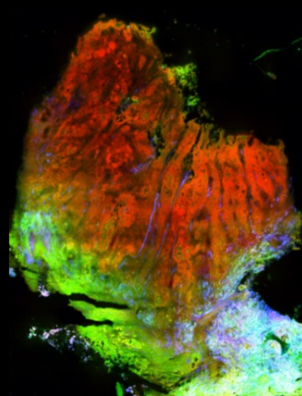



- HE section in bad quality
- Needs an experienced pathologist
- Long time in operative theater
- Huge workload

Multimodal Cryosection





- Automatic prediction of tissue types / disease
- Shorter time in operative theater due to instant feedback
- Smaller workload due to automatization

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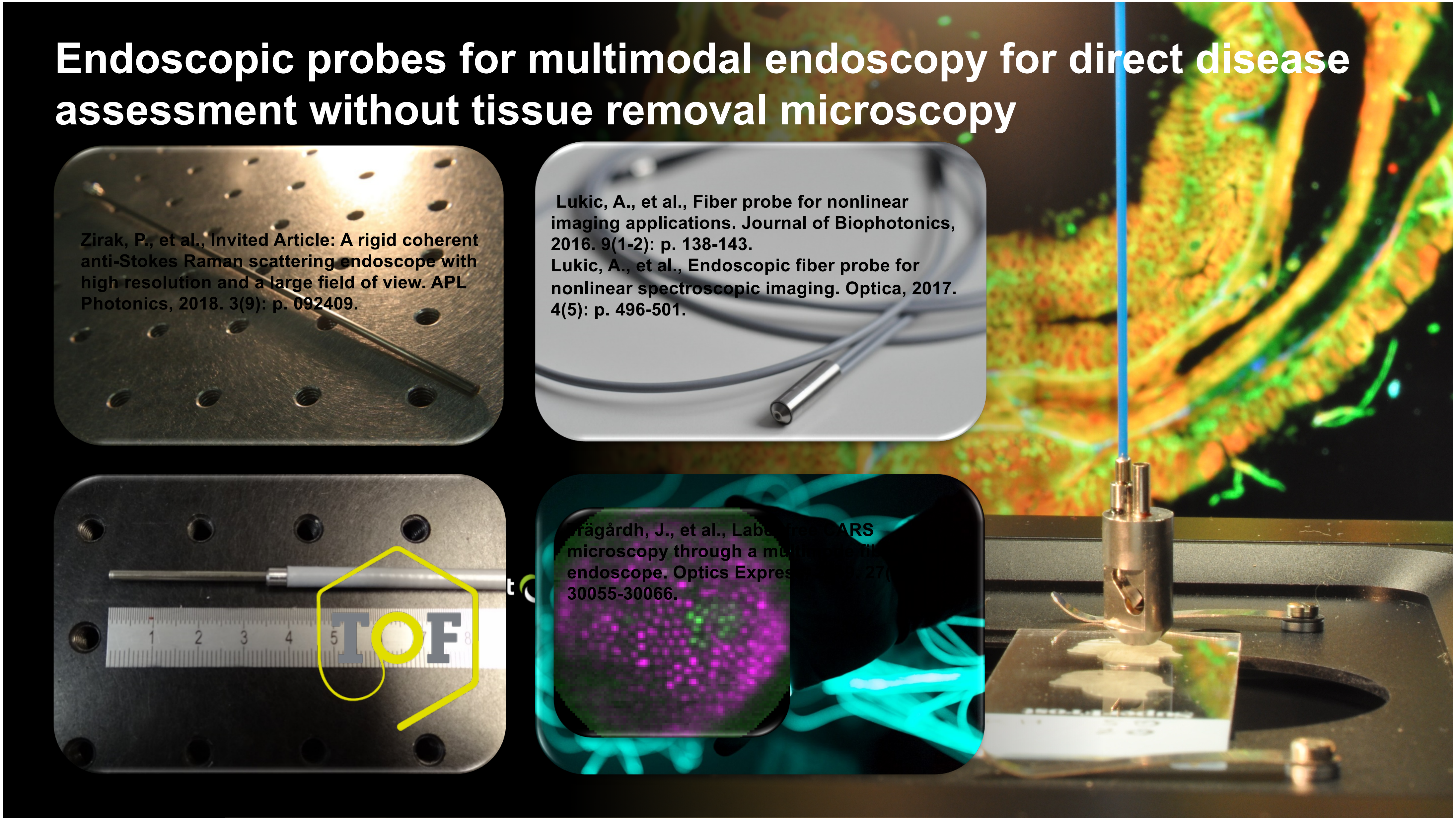
Translation to *In-Vivo I*nvestigations.


Endoscopic probes for multimodal endoscopy for direct disease assessment without tissue removal microscopy
Zirak, P., et al., Invited Article: A rigid coherent anti-Stokes Raman scattering endoscope with high resolution and a large field of view. APL Photonics, 2018. 3(9): p. 092409.
Lukic, A., et al., Fiber probe for nonlinear imaging applications. Journal of Biophotonics, 2016. 9(1-2): p. 138-143.
Lukic, A., et al., Endoscopic fiber probe for nonlinear spectroscopic imaging. Optica, 2017. 4(5): p. 496-501.
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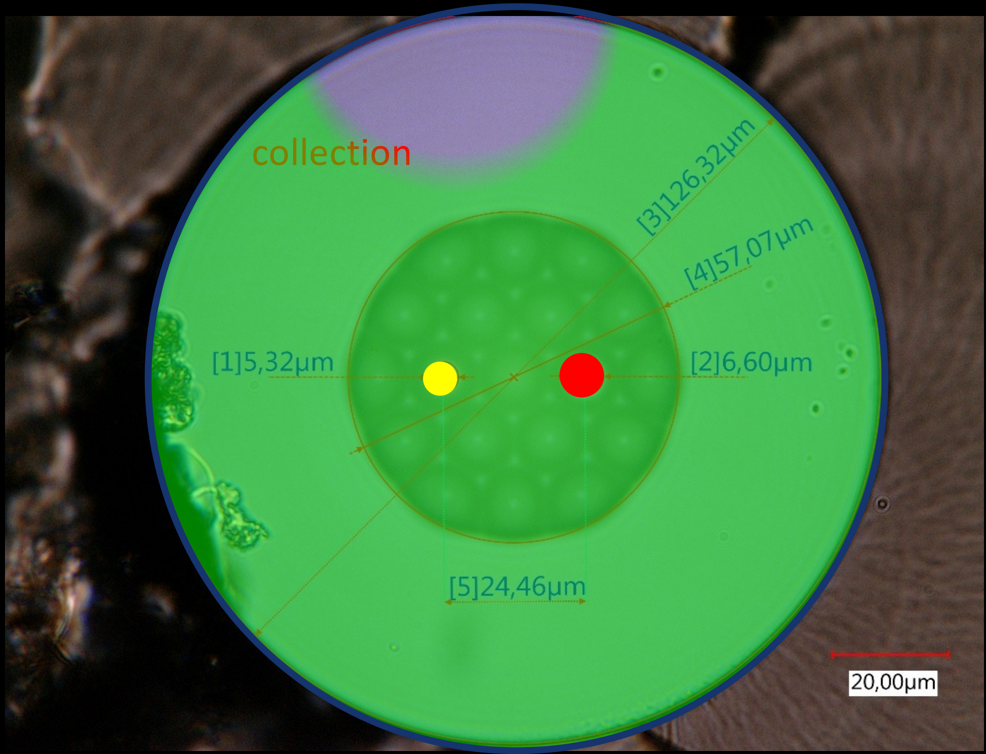
DCDC-Fiber for laser delivery and signal collection

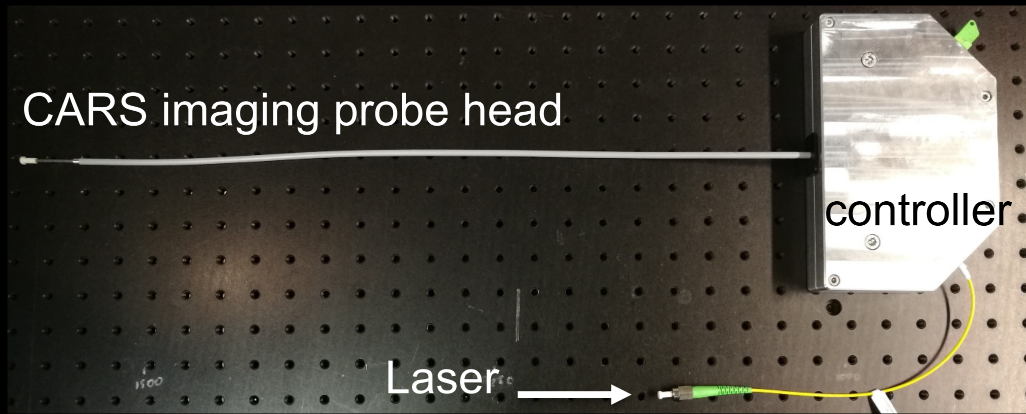


Pump ~800nm

Stokes ~1030nm

Double clad signal collection

Grating is used to overlay pump and Stokes



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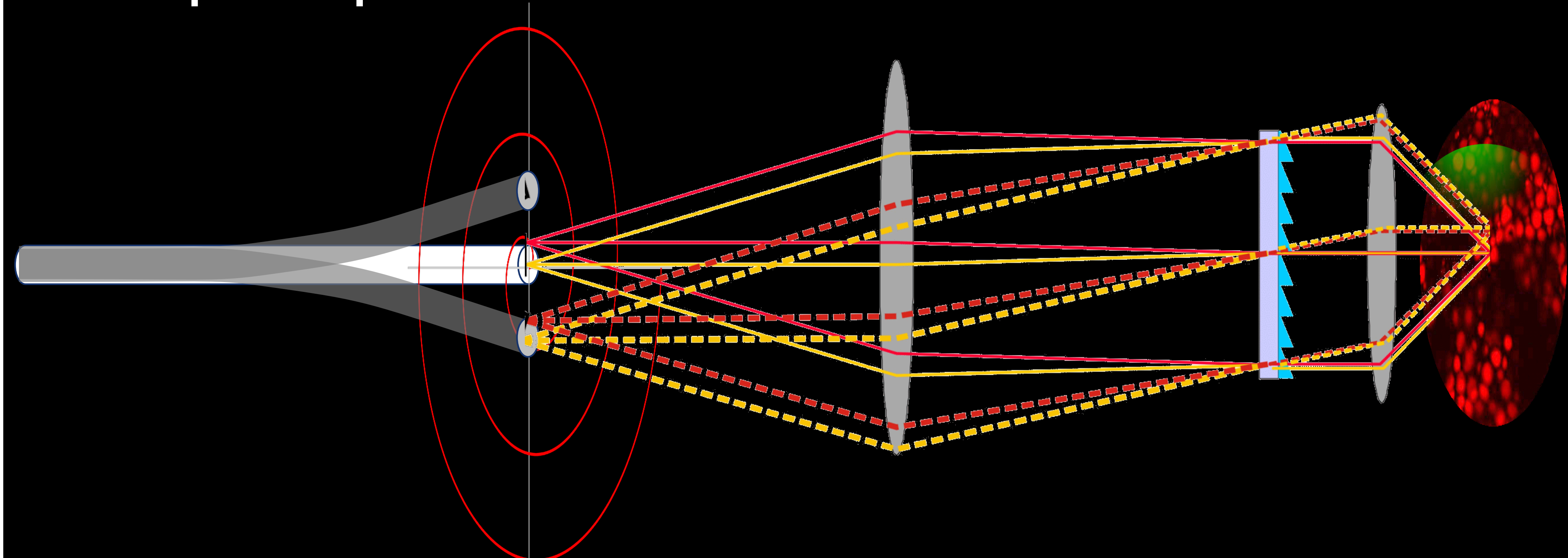
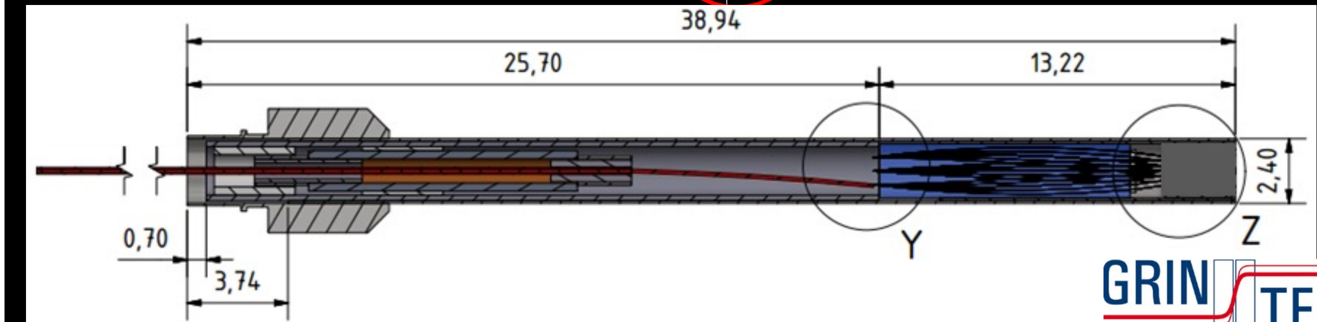
Principle of operation





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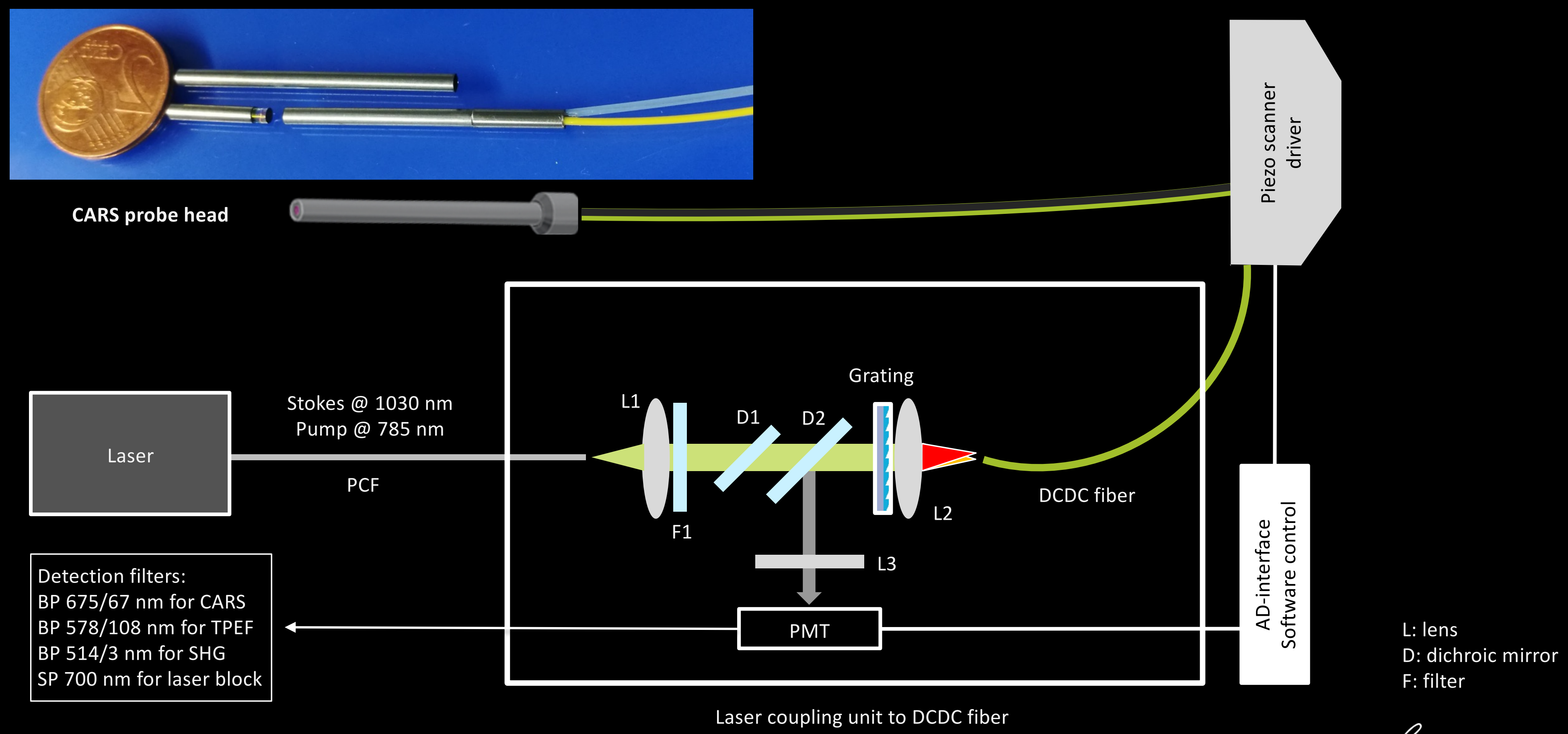
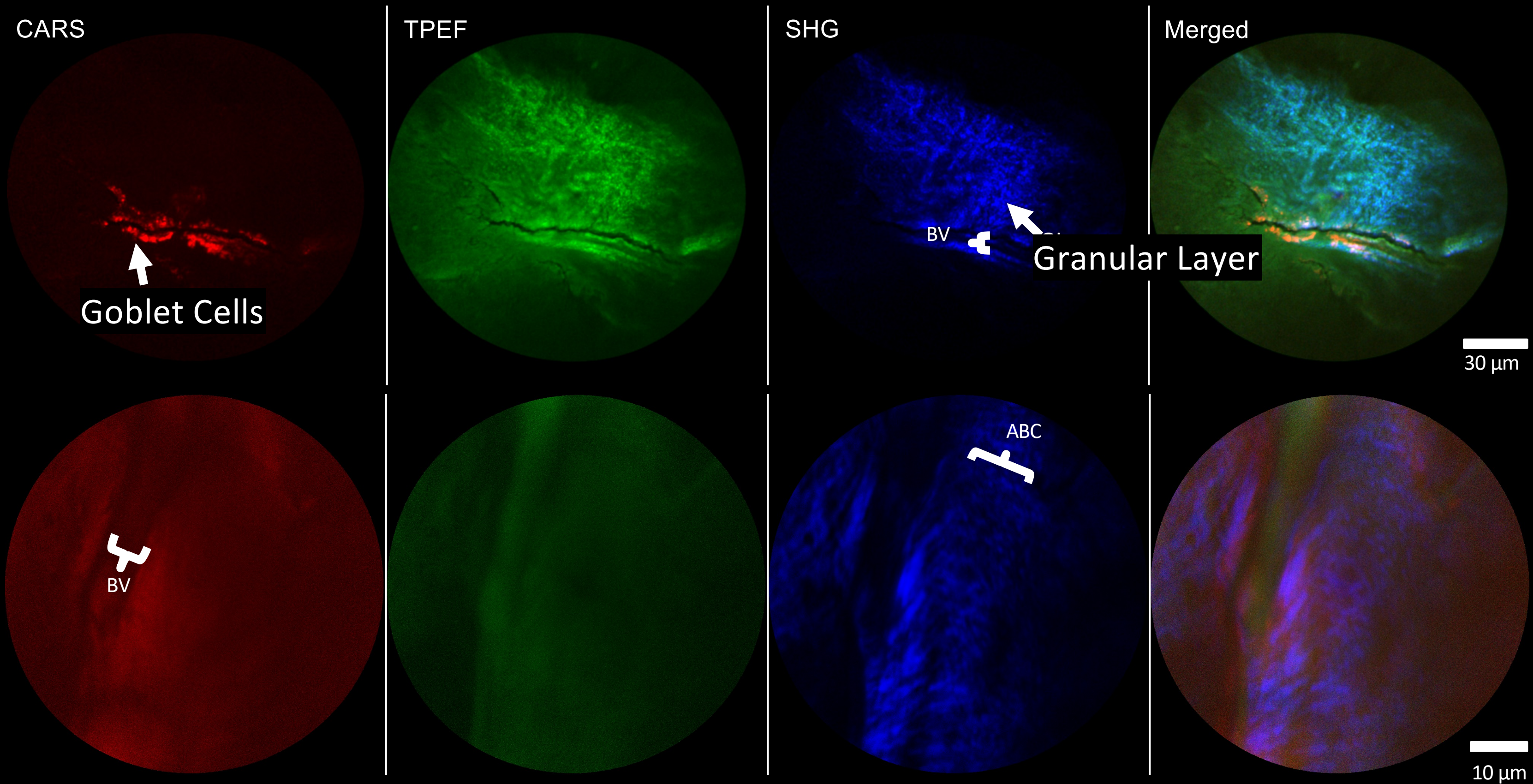
Integrated endoscopic fiber probe in coupling unit



Multimodal images of pig brain and sheep dura



Pig brain tissue

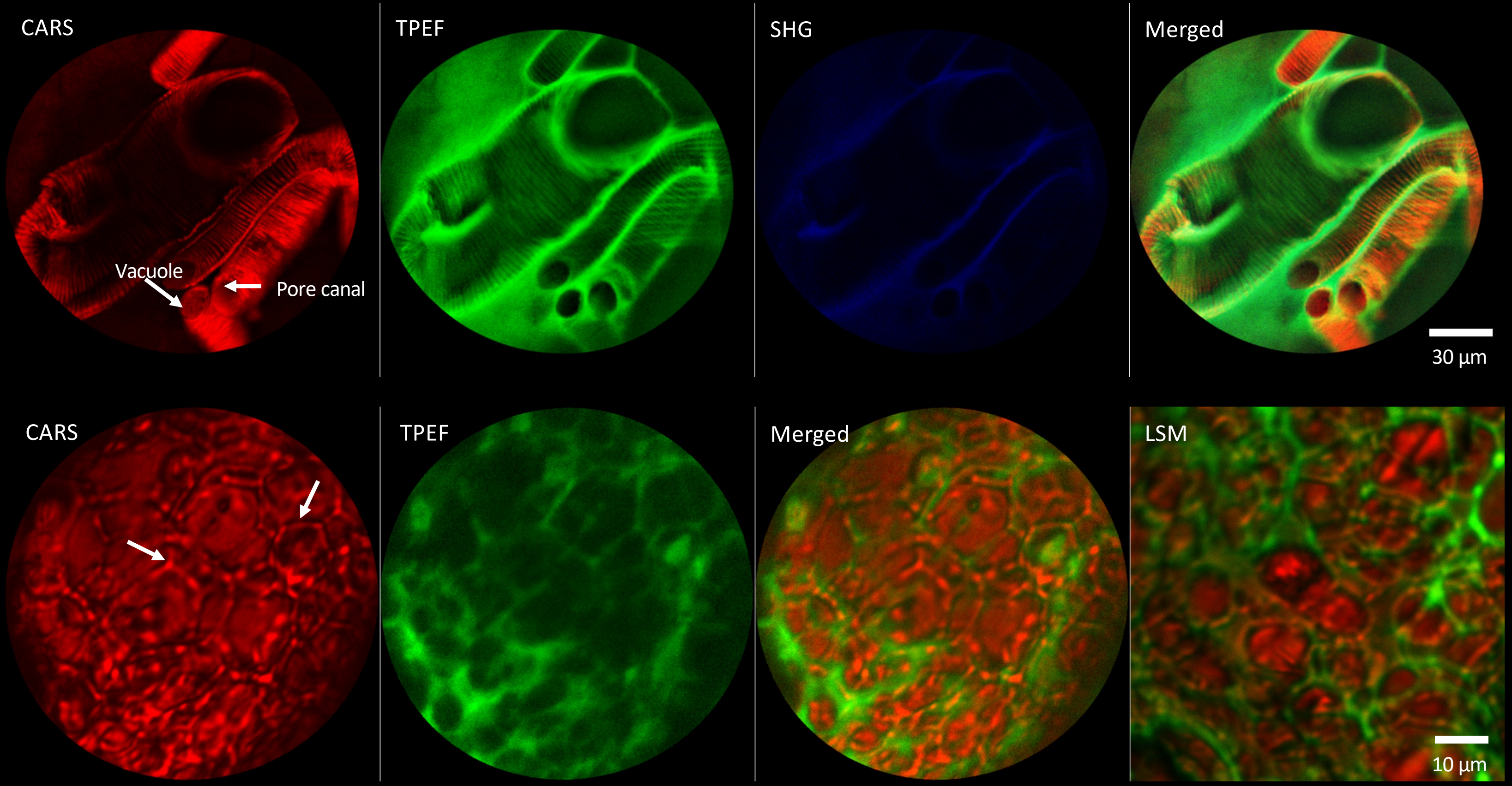
Sheep Dura tissue

Blood vessels (BV)

arachnoid barrier cell (ABC)

Pump (795 nm, 20 ps, 1 MhZ) 10 mW and Stokes (1030 nm, 60 ps, 1 MHz) 32 mW @ sample

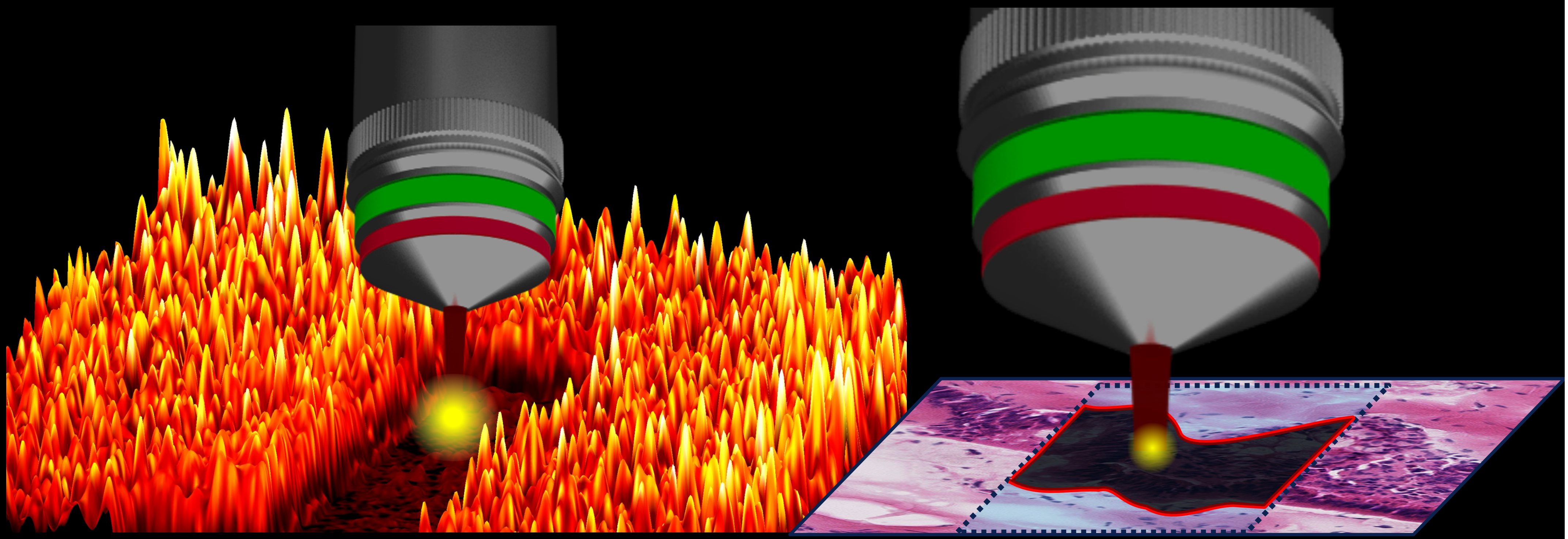
Multimodal images of Galleria mellonella Larvae worm



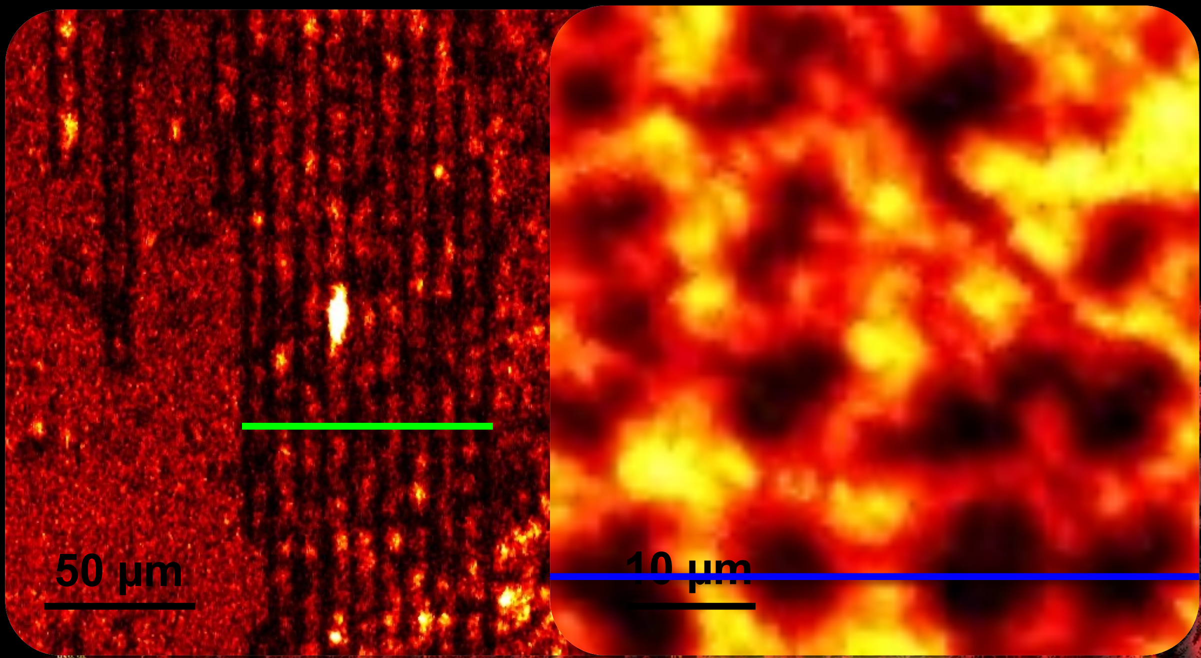
Pump (795 nm, 20 ps, 1 MhZ) 10 mW and Stokes (1030 nm, 60 ps, 1 MHz) 32 mW @ sample

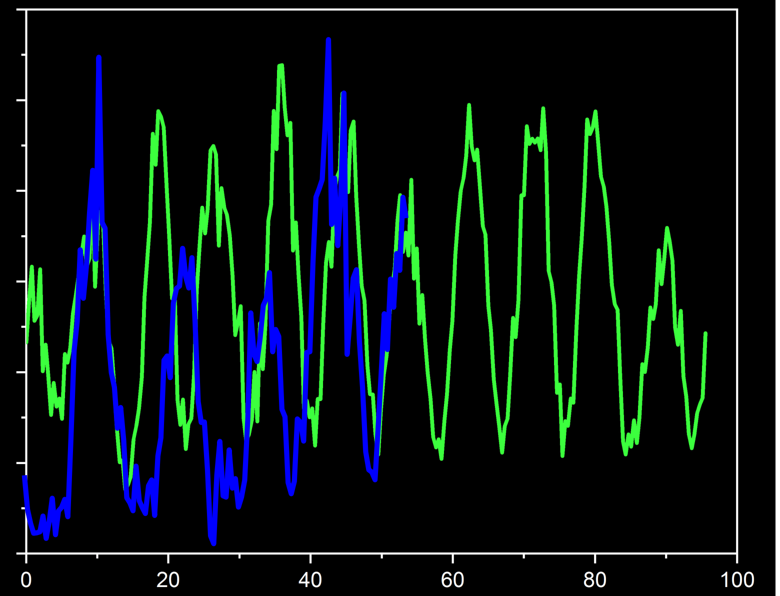
Image Guided Laser Surgery

Combining multimodal nonlinear imaging & fs-laser ablation for image guided surgery



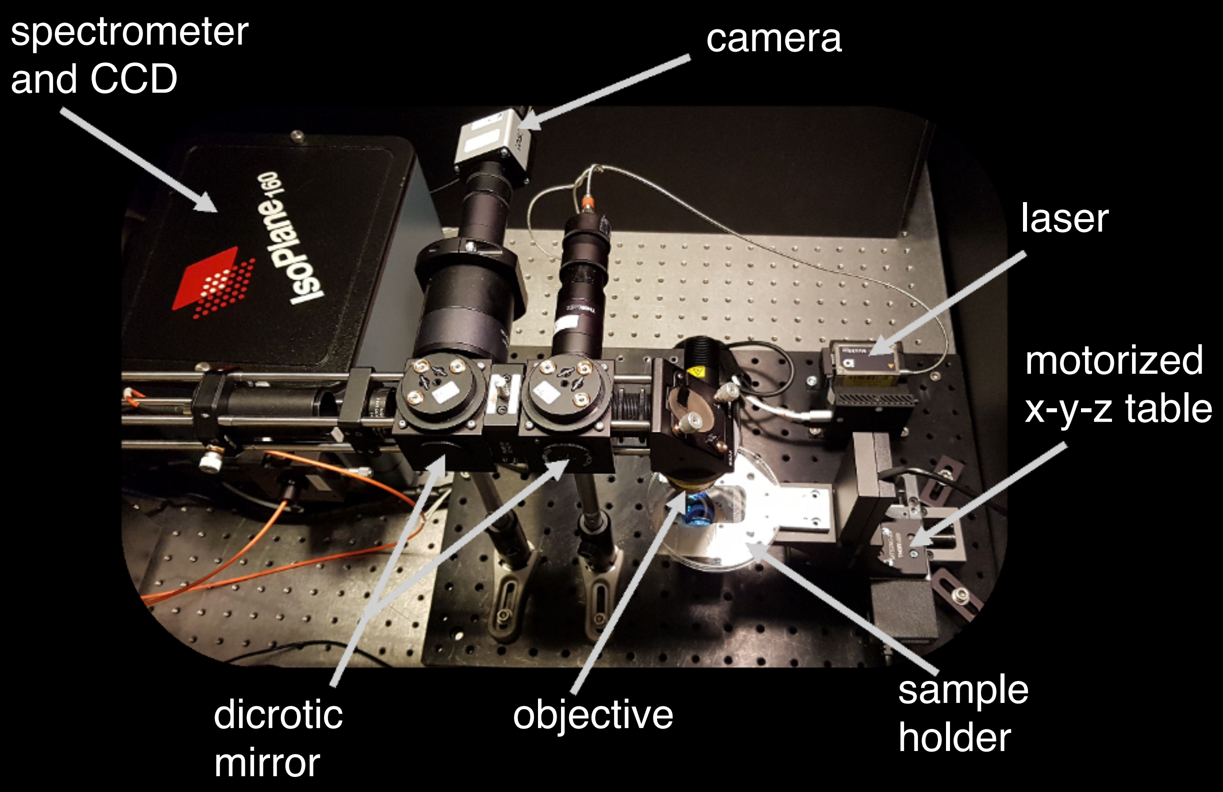
Spatial resolution of the fs-ablation process





Cancer Drug Monitoring on a Single Cell Level

High-content Raman Setup for Automated Single Cell Analysis



Features:

- 785 nm excitation
- Changing between upright and inverted microscope in 30 min.
- Conventional Raman Imaging
- Line-Raman Imaging
- **Rapid Mean Spectra Acquisition**
- **Automated Data Acquisition**
- Low-Resolution Raman Spectroscopy

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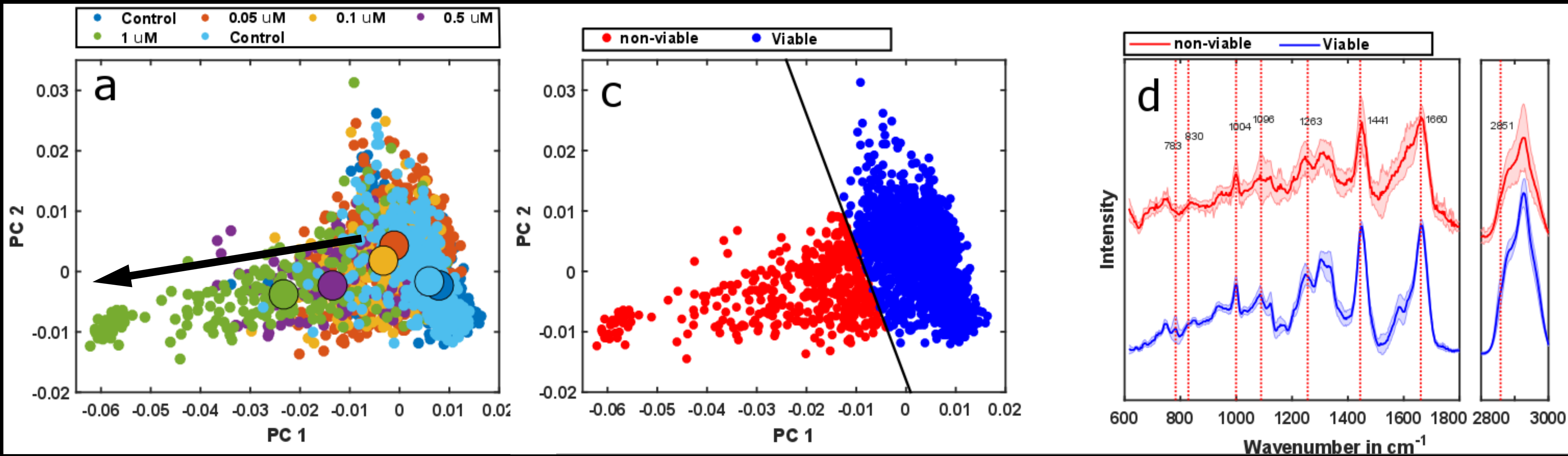
Development of a Raman-based viability assay

Test system: Doxorubicin (DOX)14 and THP-1 cells (monocytic acute myeloid leukemia cell line)



- Changes can be observed in a drug-dependent manner
- Principal component analysis combined with support vector machine (PCA-SVM)
- Decision boundary indicates the difference between viable and non-viable cells

A. S. Mondol et al. *Scientific Reports,* 9, 12653 (2019) .

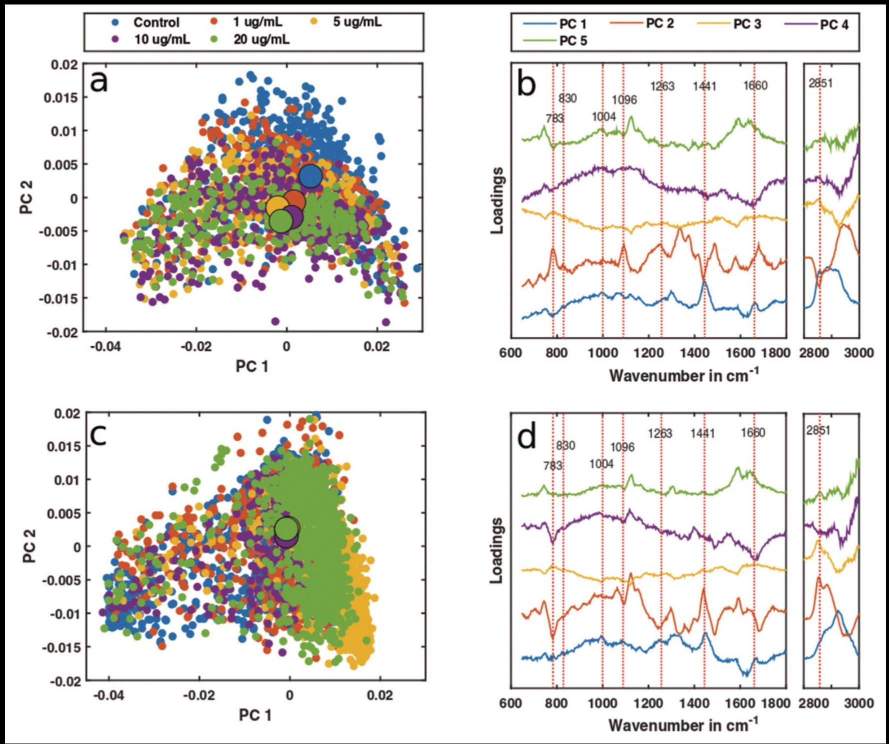
Comparison Raman-based and trypan blue based prediction of viable cells

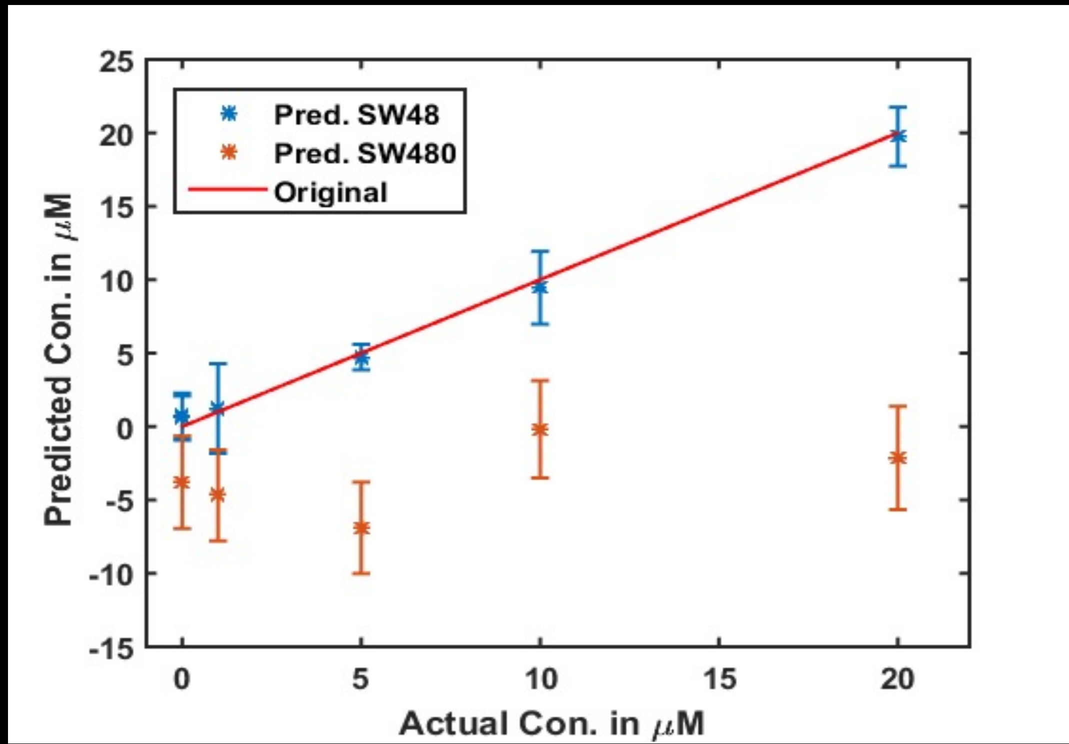
	Batch 1		Batch 2	
	Raman Results in %	**Clinical Results in %**	**Raman Results in %**	**Clinical Results in %**
Control	83	82	98	99
0.05 um	73	78	69	70
0.1 um	70	69	52	59
0.5 um	62	59	30	32
1.0 um	52	47	21	13
Control	82	87	96	95

- Raman provides comparable results for cell-viability as conventional approaches
- Other drugs, e.g. panitimumab (monoclonal antibodies), Dithiothreitol (DTT), erlotinib, nanoparticle induced cytotoxicity

A. S. Mondol et al. *Scientific Reports,* 9, 12653 (2019) .

High content Raman spectroscopic analysis of Panitumumab exposed to colorectal cancer cells





Mondol et al., Analyst, 2019, 144, 6098.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from Ideas to Instruments
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Thanks

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Die Förderbank.

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Thank you.