Label-free multimodal nonlinear optical microscopy for intraoperative brain cancer detection

Gabriel Giardina[1], Arno Krause[1], David Reichert[1], Rainer Leitgeb[1], Wolfgang Drexler[1], Adelheid Woehrer[2], Georg Widhalm[3], Angelika Unterhuber[1], Marco Andreana[1]

[1] Center for Medical Physics and Biomedical Engineering, Medical University of Vienna
[2] Division of Neuropathology and Neurochemistry, Department of Neurology, Medical University of Vienna
[3] Department of Neurosurgery, General Hospital and Medical University of Vienna

Objective

State of the art **intraoperative diagnosis for brain cancer** is based on time consuming and labour intensive **histopathological assessment** of tumour biopsies[1]. This method requires many sample preparation steps leading to longer surgery times and higher risks for the patient. There is a clear need for **faster and more labour efficient strategies** for intraoperative cancer diagnosis.

Our goal is to create a **label-free multimodal optical microscope** to be used as an alternative to the time consuming histology. By observing a combination of **intrinsic metabolic**[2], **morphological** and **chemical biomarkers**[3], we remove the need for staining and reduce the time required to obtain a diagnosis[1].

Methods

The system uses a single titanium sapphire laser at 805 nm to simultaneously excite **two photon fluorescence lifetime imaging (2γFLIM)**, **second harmonic generation (SHG)** and, combined with light generated at 1050 nm by a photonic crystal fibre, **stimulated Raman scattering (SRS)**.

Modality	Contrast	Use for diagnosis
SRS	Molecular vibrational levels of C-H_2 and C-H_3 bonds[4]	Lipids vs. protein content change depending on the pathological status[3]
2γFLIM	Fluorescence lifetime of flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NADH)	FAD and NADH lifetimes change depending on the metabolic state of the cells (aerobic vs. anaerobic) linked to pathological status[2,5,7]
SHG	Spatial distribution and orientation of collagen fibres	The morphology of the extracellular matrix is disturbed by the presence of a tumour[3,5,6]

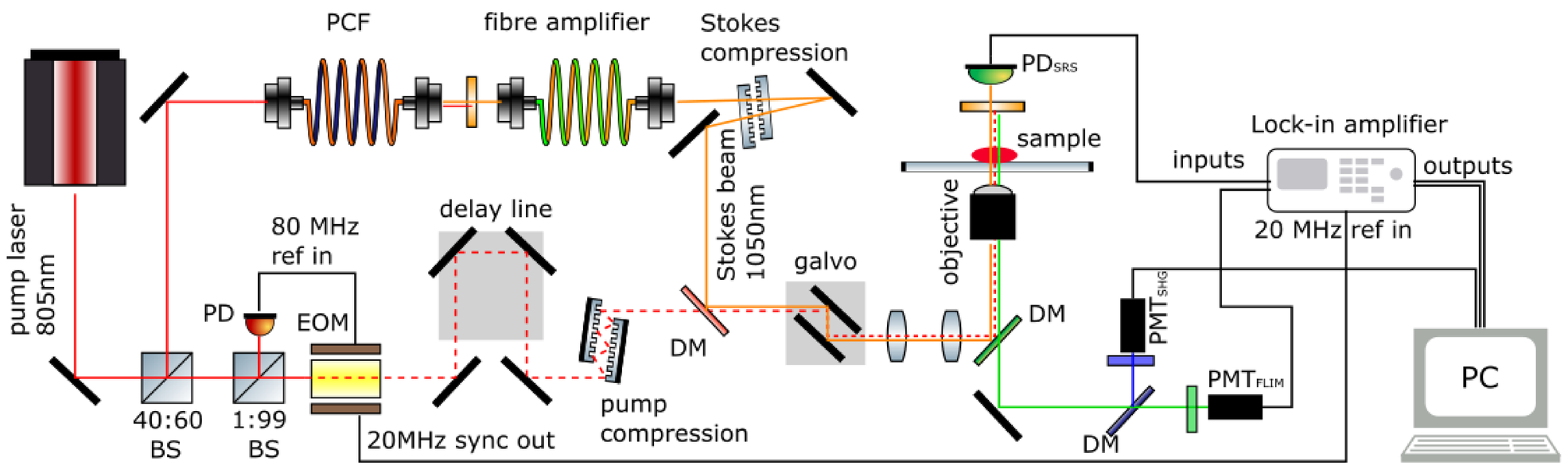

***Figure 1**: Sketch of the microscope. DM: dichroic mirror, BS: beam splitter, PD: photodiode, PMT: photomultiplier tube, PCF: photonic crystal fibre, EOM: electro-optical modulator.*

Two Photon Fluorescence Lifetime Imaging (2γFLIM)

- Visualizes changes in **cell metabolism**[3,5]
- Targets endogenous **FAD** & **NADH**
- **Fluorescence lifetime** influenced by preferred metabolic pathway (**aerobic** vs. **anaerobic**)

Second Harmonic Generation (SHG)

- Visualizes **morphological** changes in the extracellular matrix[3]
- Contrast mechanism specific to **collagen** in tissue[5,6]
- Presence of tumor **disrupts** the extracellular matrix

Stimulated Raman Scattering (SRS)

- Vibrational **spectroscopy** of **C-H_2 (proteins)** and **C-H_3 (lipids)**[3,4] bonds
- Relative concentrations of proteins and lipids in cells shown to be a **biomarker for cancer**[3,4]

Preliminary Results

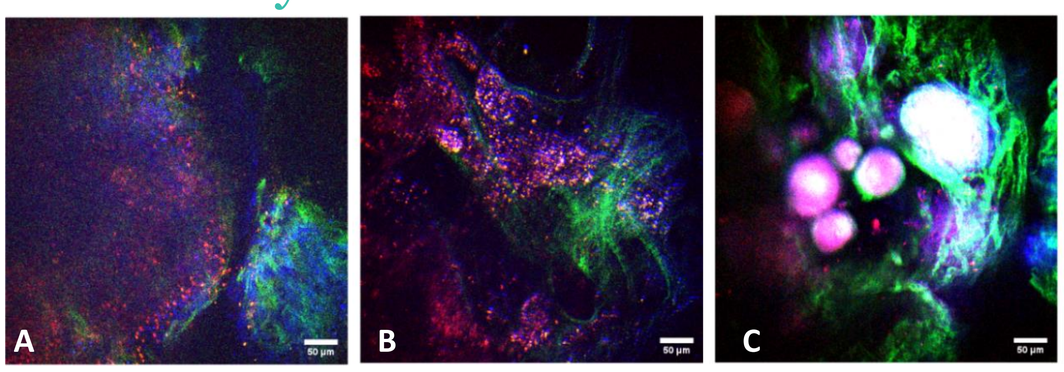

***Figure 2:** Nonlinear microscopy images of a healthy pituitary gland (A), meningioma (B) and pituitary gland adenoma (C). In blue NADH fluorescence, in red FAD fluorescence and in green SHG of collagen.*

Preliminary images of biopsies of **pituitary gland** and **cranial tumours** using the label-free multimodal nonlinear optical microscope showing the potential for this tool to be used as an alternative to histology in the future.

Conclusion

This **label-free multimodal nonlinear microscopy** approach is capable of **3D optical sectioning** at **micrometre resolution** and delivers contrast based on **endogenous biomarkers**. It requires almost **no sample preparation** and is thus both **faster** and **less labour intensive** than histology. The microscope is based on a **single laser system** and thus the images are intrinsically **co-registered** on the same field of view and multiple modalities are **acquired simultaneously**. Taken together, this approach has the potential to unlock **intraoperative timescale** for diagnosis.

In the future, we plan to explore the diagnostic potential of this multimodal approach by feeding the **complementary metabolic, morphological and biochemical** information to **A.I. classification algorithms** to increase the sensitivity and specificity of cancer diagnosis and reduce the workload of pathology specialists.

References

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