Fluorescence lifetime imaging and spectroscopic analysis combined in a surgical microscope for optimized fluorescence-guidance in brain tumor resection

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Objective

- About 25,000 malignant brain tumors are prognosed for 2020 in the United States, ~ 50 % of those glioblastoma with a median survival of ~ 8 months[1]
- Extent of resection is a key prognostic factor in the treatment of brain tumors
- Protoporphyrin IX (PpIX) fluorescence guidance intraoperatively visualizes tumorous tissue and has shown to increase the extent of resection[2]

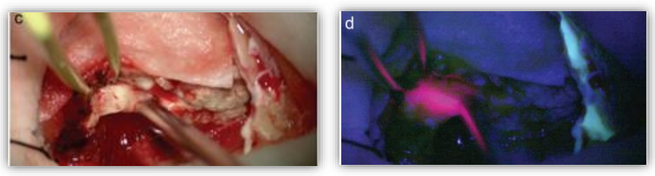


Figure 1: White-light *(left)* and fluorescence image *(right)* of a conventional surgical microscope. Courtesy: Georg Widhalm (AKH Wien)

- Weak PpIX concentrations, as found e.g. in low-grade glioma (LGG) or infiltration zones of high-grade glioma (HGG), cannot be visualized due to spectrally overlapping autofluorescence

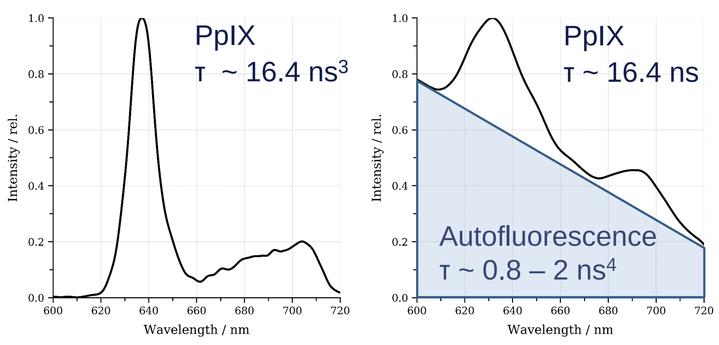


Figure 2: Emission spectra of PpIX

Left: strong PpIX fluorescence*; Right:* weak PpIX fluorescence on an autofluroescence background

τ = fluorescence lifetime

Objective → Sensitive PpIX detection through a surgical microscope by measuring the fluorescence lifetime τ for improved brain tumor resection

Materials & Methods

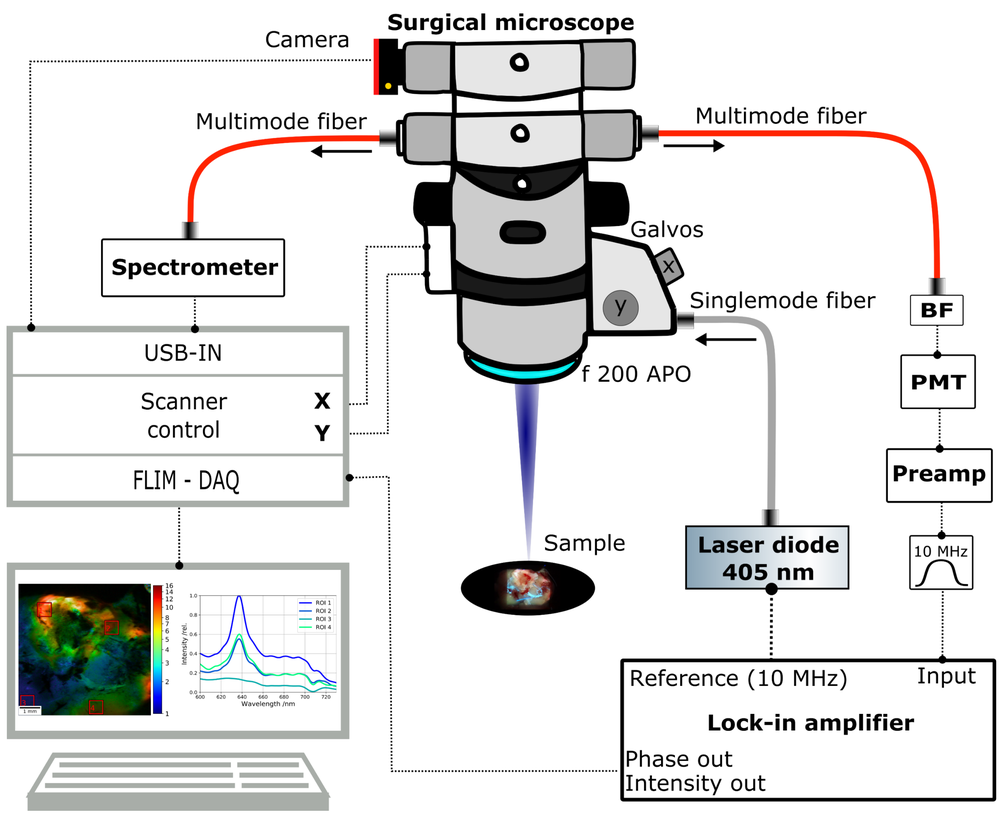


Figure 3: Laboratory setup, BF: bandpass filter, 590 – 740 nm; PMT: photomultiplier tube

- Raster-scanning frequency domain fluorescence lifetime imaging was integrated in a surgical microscope together with a spectrometer
- FLIM acquisition time about 16 seconds at a spatial resolution of 40 µm
- Spectroscopic information can be acquired on selected regions of interest (ROI)
- Brain tumor samples were imaged ex vivo within 1 hour after resection

Funding

This project has received funding from the Austrian Christian Doppler Research Association as well as from the innovation board of the Carl Zeiss Meditec AG.

Results

Figure 4: High-grade glioma - strong visible fluorescence during surgery
- Fluorescence lifetime increased up to 16 ns
- Typical peaks of PpIX at 635 and 705 nm in spectra

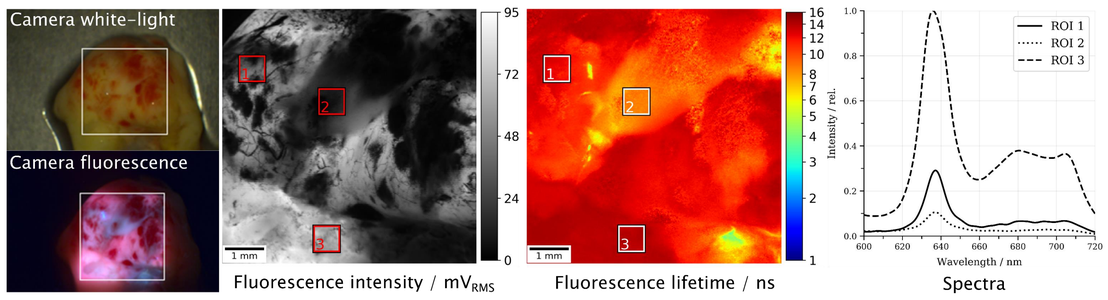
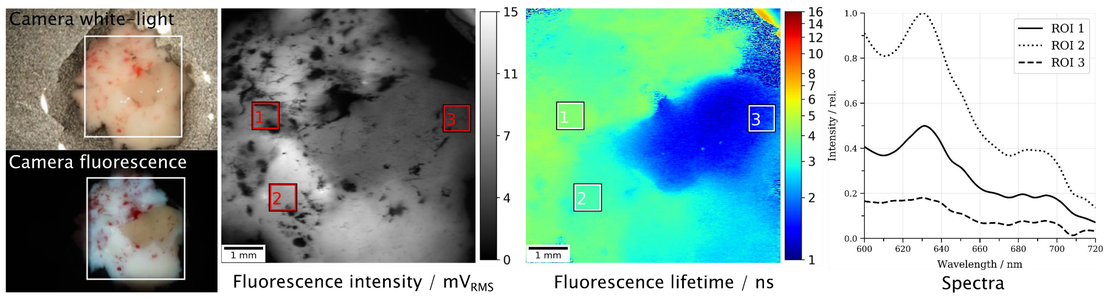


Figure 5: Low-grade glioma - no visible fluorescence during surgery
- Weak PpIX signal on an autofluorescence background (ROI 1 & 2) increased the fluorescence lifetime up to 5 ns and could be contrasted from an area with pure autofluorescence (τ < 2 ns, ROI 3)



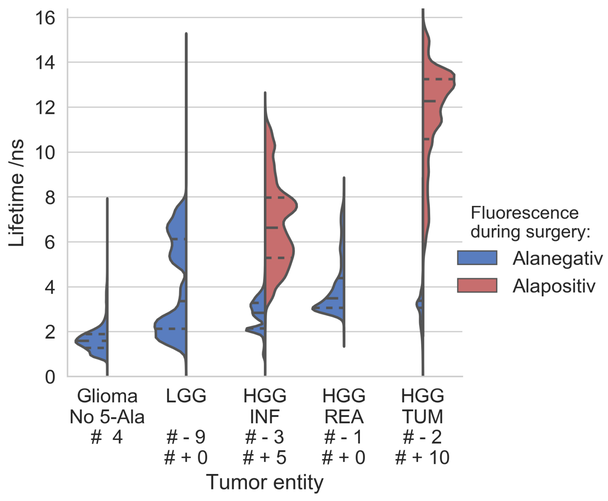
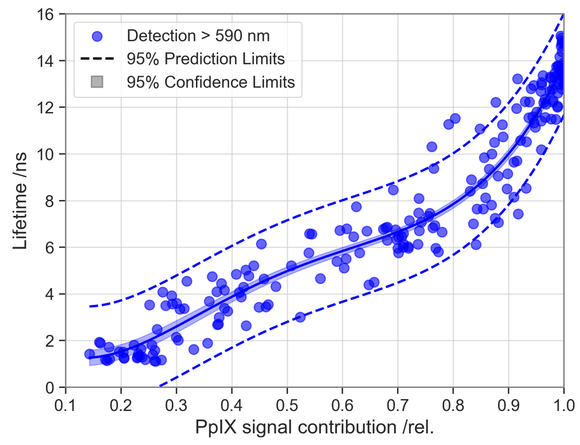


Figure 6: Violin plots with the lifetime distribution for LGG & HGG (INF: infiltration zone, REA: reactive tissue, TUM: core tumor)

Alanegativ: no visible fluorescence during surgery
Alapositive: visible fluorescence during surgery

Median and quartiles are indicated by solid and dashed lines

Figure 7: PpIX vs. autofluorescence signal contribution at 635 nm plotted as a function of the fluorescence lifetime measured at the respective ROI.
Higher PpIX signal contributions entailed increased lifetimes when measured with frequency domain FLIM.



Conclusion

FLIM enables the delineation of weak PpIX signals from an autofluorescence background. Integrated into a surgical microscope, this technology potentially improves the extent of resection and therefore patient outcome.

References

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