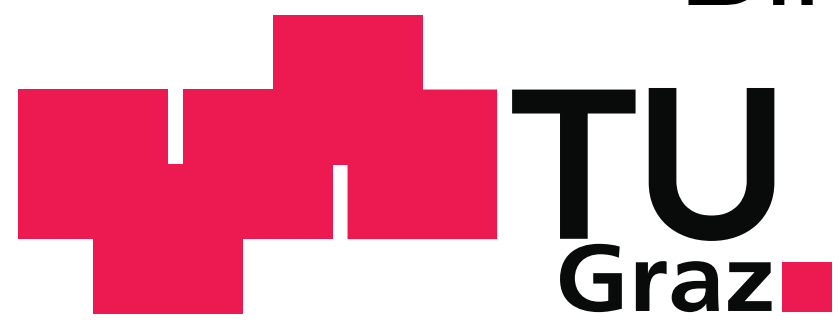


# Oxygen Imaging in Microfluidic Devices with Integrated Sensors Applying Color Cameras

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## Introduction

Three different designs of optical luminescent sensors for monitoring dissolved oxygen in microfluidic devices are developed in our group. These sensors can easily be integrated into microfluidic devices while signal readout is accomplished off chip by imaging techniques with a fluorescence microscope. In preliminary studies, sensor layers and nanosensor particles are integrated into microfluidic chips and readout with lifetime imaging or ratiometric imaging using the color channels of a CCD-camera. Model culture systems are used to demonstrate monitoring of cell viability.

## Integration of Sensors in Microfluidic Devices

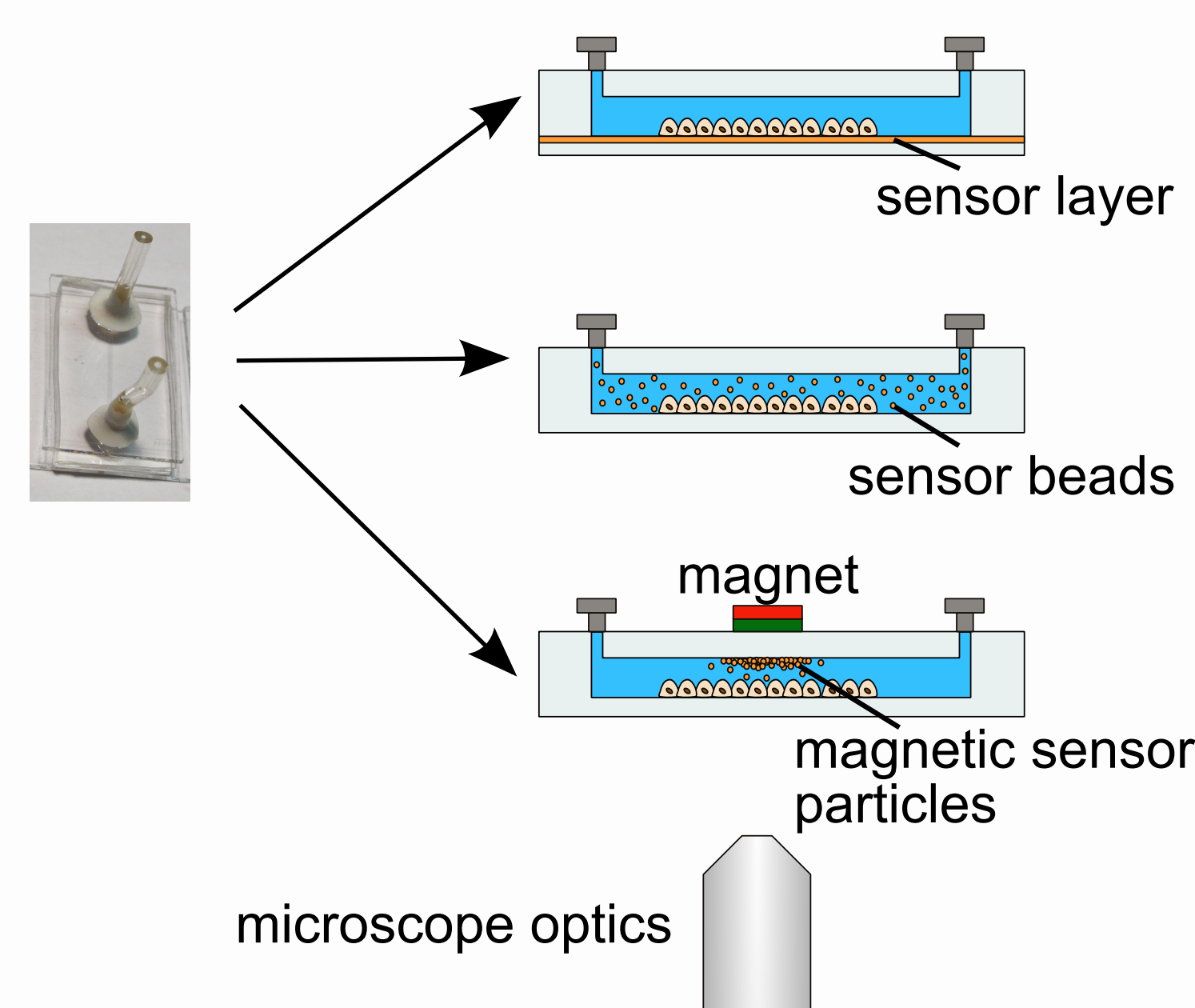


Figure 1: Different sensor designs for application in microfluidic devices. Optical sensors are integrated into microfluidic devices by sensor layers, stained PSPVP-nanoparticles and magnetic optical nanosensor particles. Ultrabright indicators [1] and the concept of light-harvesting [2] were applied to improve signal intensities.

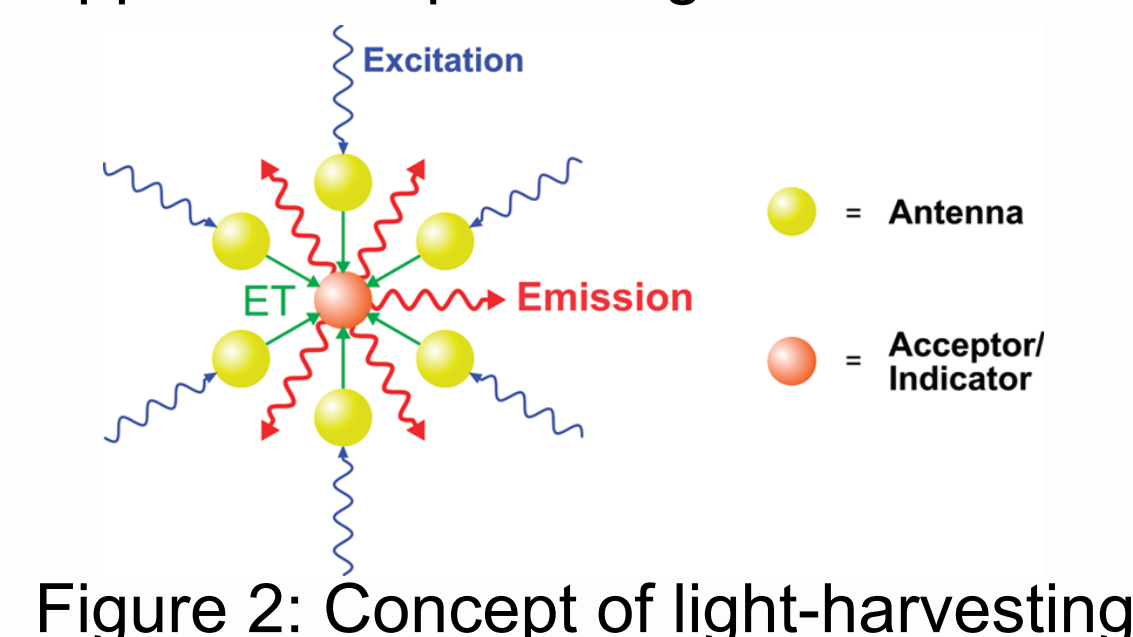


Figure 2: Concept of light-harvesting

## Referencing Schemes

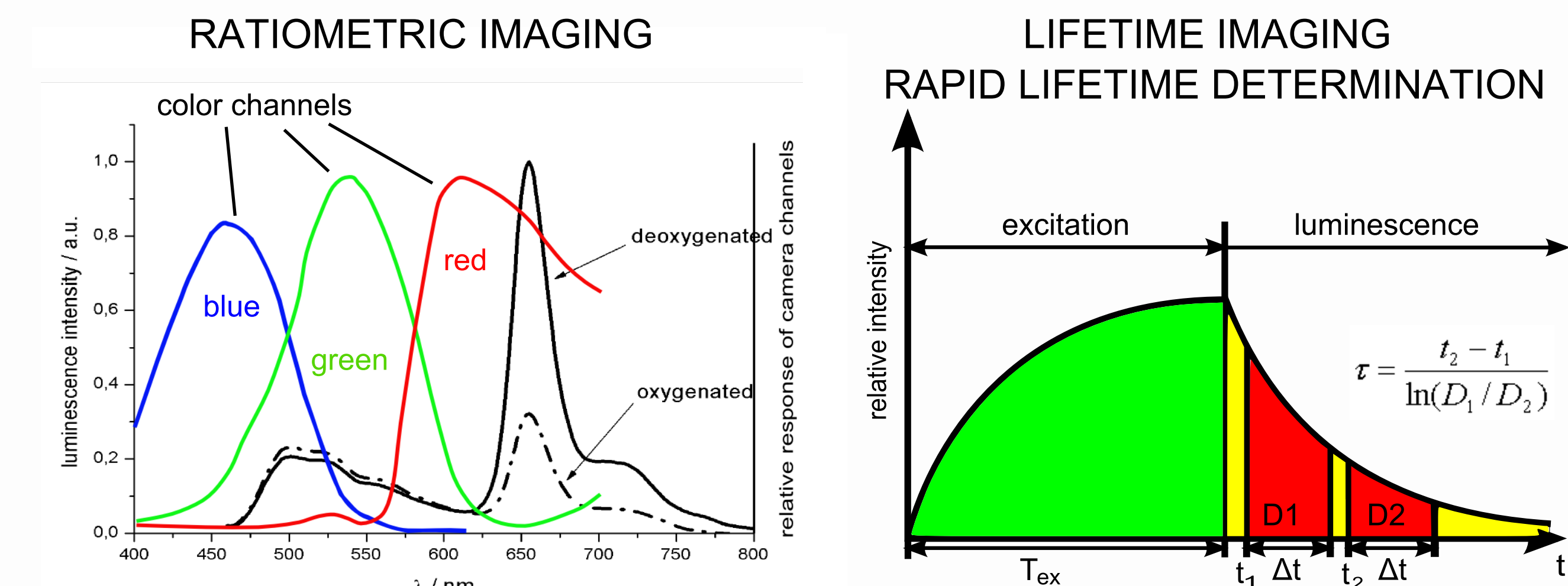


Figure 3: Referencing schemes for ratiometric (left) and lifetime (right) imaging. In ratiometric imaging the red channel of the color camera detects the oxygen-sensitive emission of the indicator dye. The green channel serves as constant reference signal. Lifetime imaging was accomplished using rapid lifetime determination (RLD).

## Ratiometric Imaging

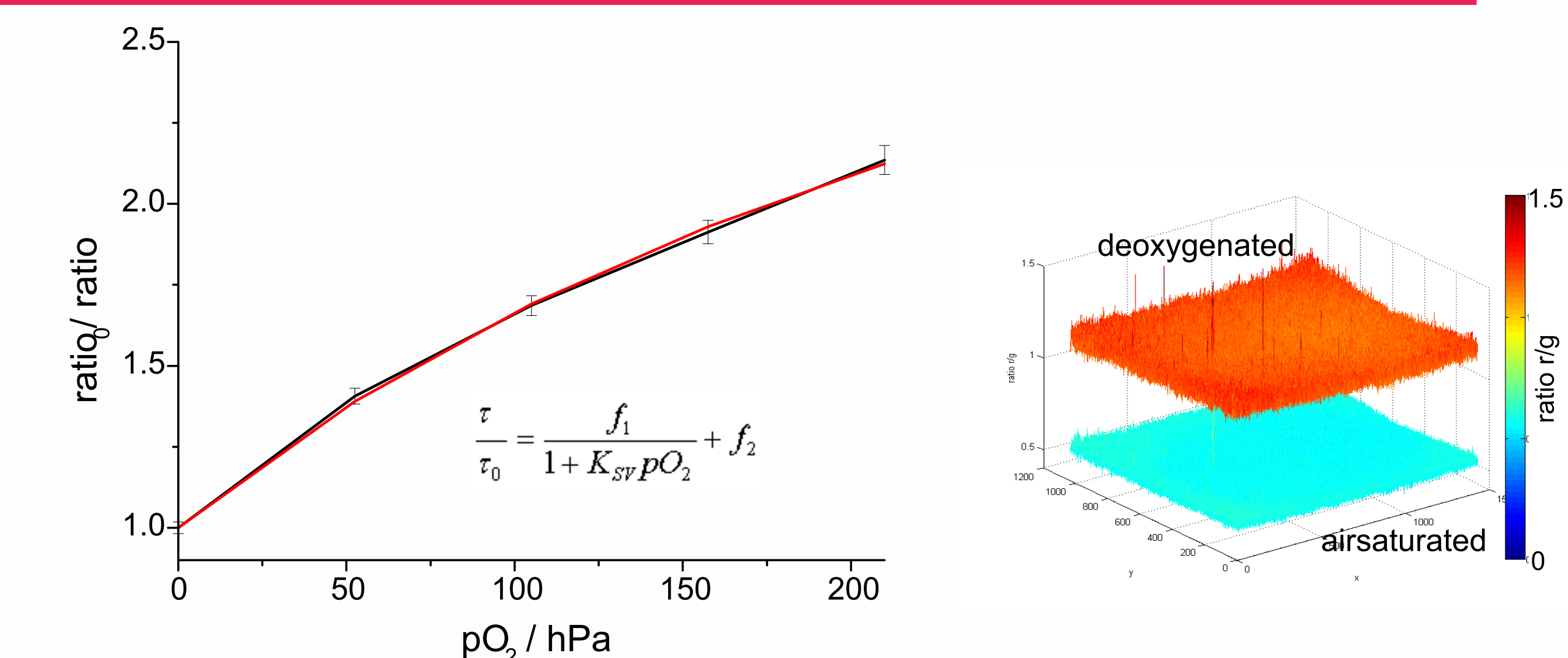


Figure 4: (A) Stern-Volmer plot of the calibration data and (B) ratiometric surface plots of deoxygenated and air-saturated sensor layer obtained via ratiometric imaging using a color camera. The sensor layer contains 4% Macrolex Yellow (antenna dye) and 0.5% PtTFPP (oxygen-sensitive indicator dye). Standard deviation was calculated over the whole imaged area.

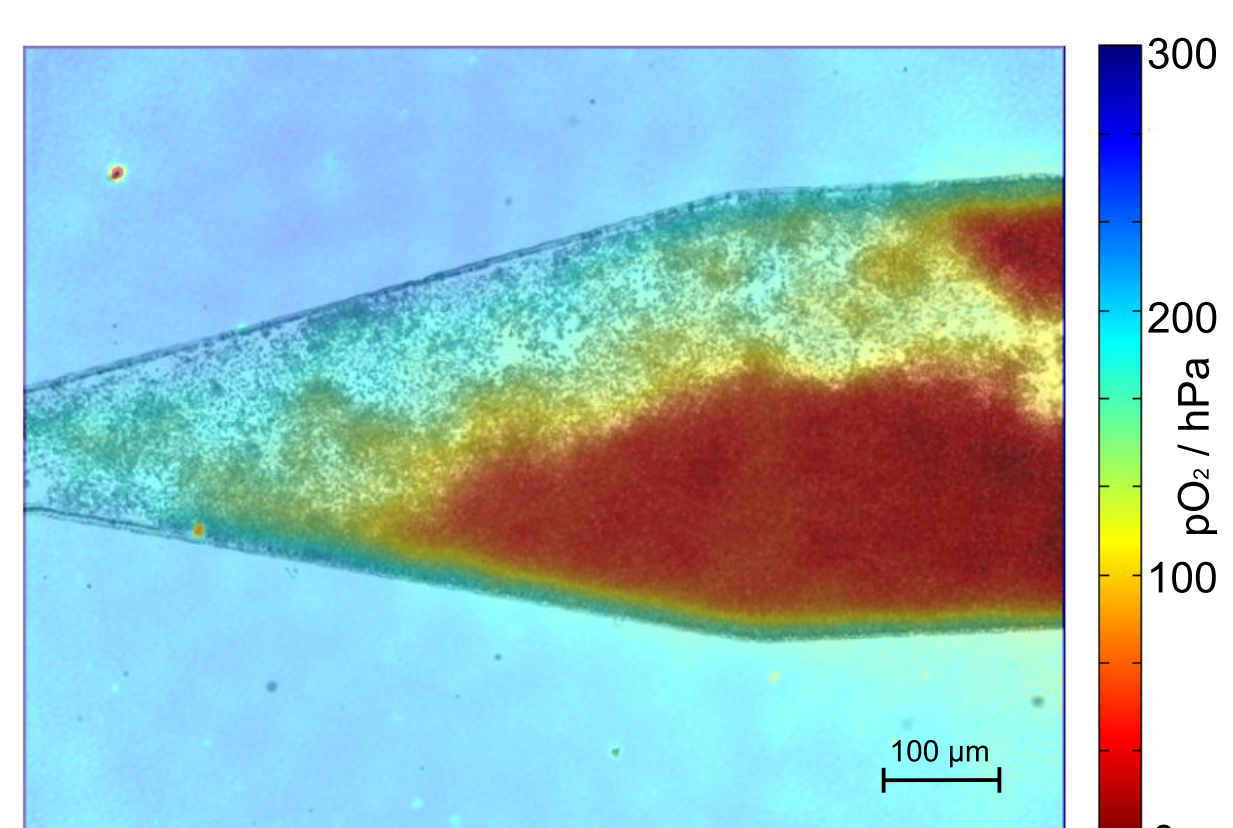
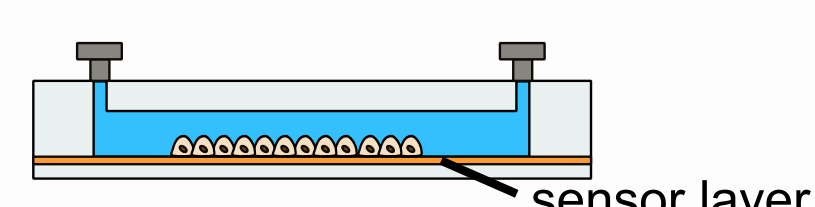


Figure 5: Overlay of transmitted light image and ratiometric image of *Pichia pastoris* in a microfluidic chip with integrated sensor layer. Highest oxygen levels can be observed exactly at the highest microorganism density.



## Lifetime Imaging

Lifetime imaging was accomplished on an epifluorescent microscope via rapid lifetime determination applying a gateable monochrome camera (PCO Sensicam).

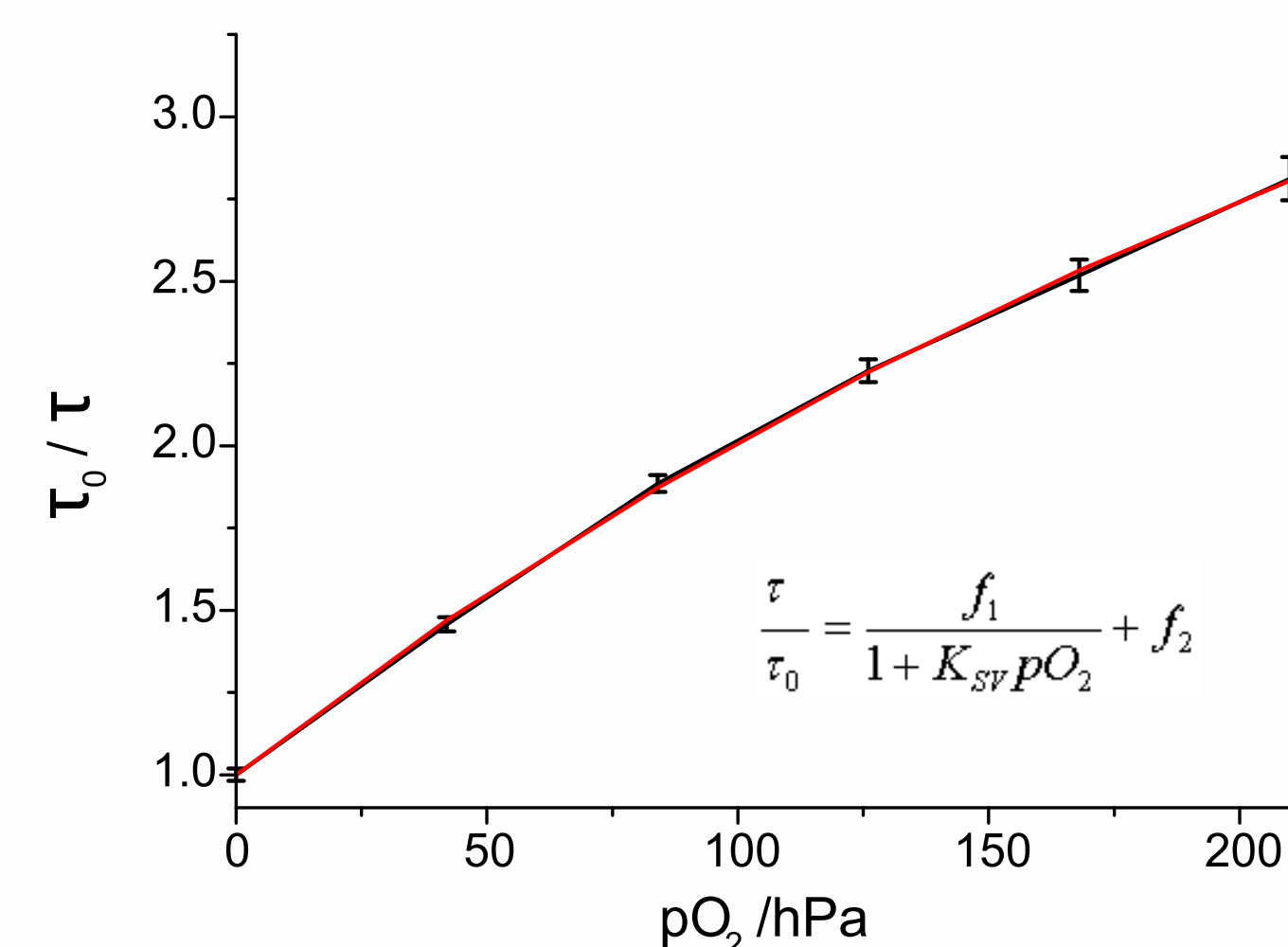


Figure 6: Stern-Volmer plot of the calibration data obtained via rapid lifetime determination applying a gateable monochrome camera. The sensor layer contains 4% Macrolex Yellow (antenna dye), 0.5% PtTFPP (oxygen-sensitive indicator dye) and 30% titaniumdioxide. Standard deviation was calculated over the whole imaged area.

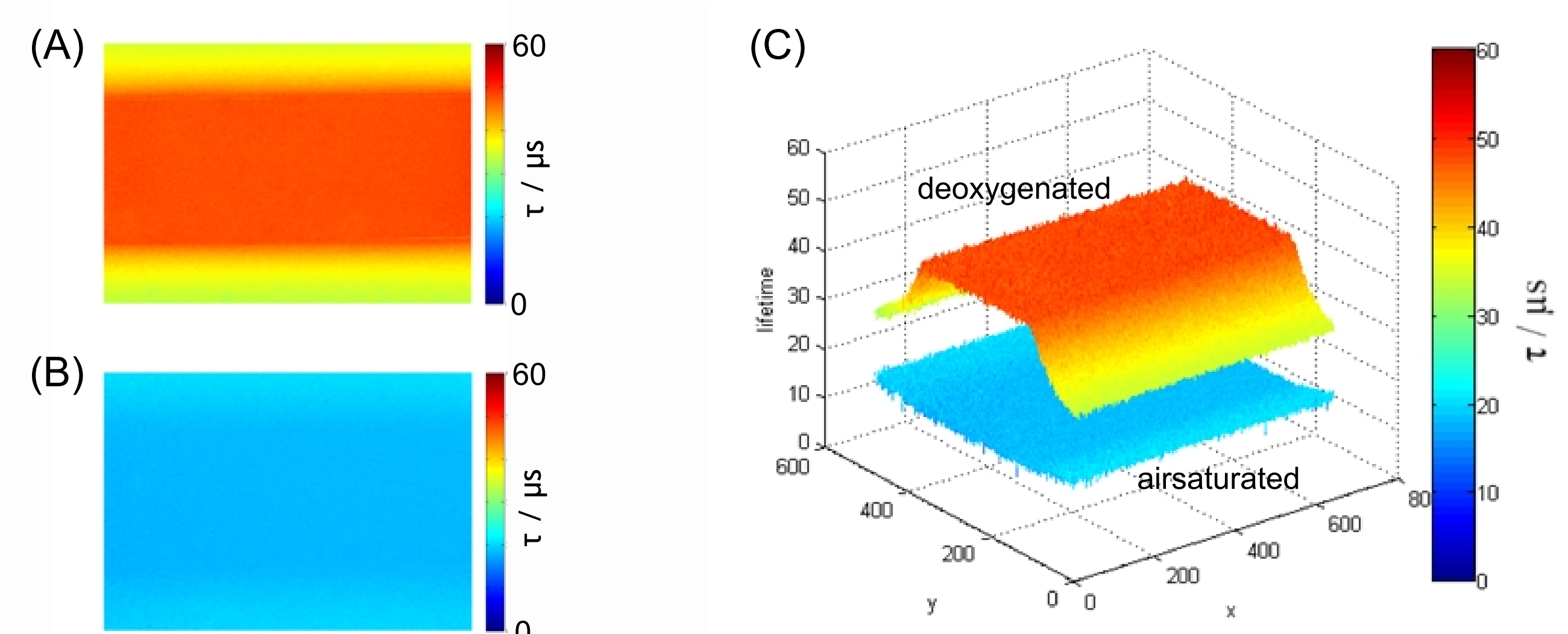


Figure 7: Lifetime images of a microfluidic channel with an integrated sensor layer flushed with (A) nitrogen and (B) synthetic air. (C) Surface plot of (A) and (B)

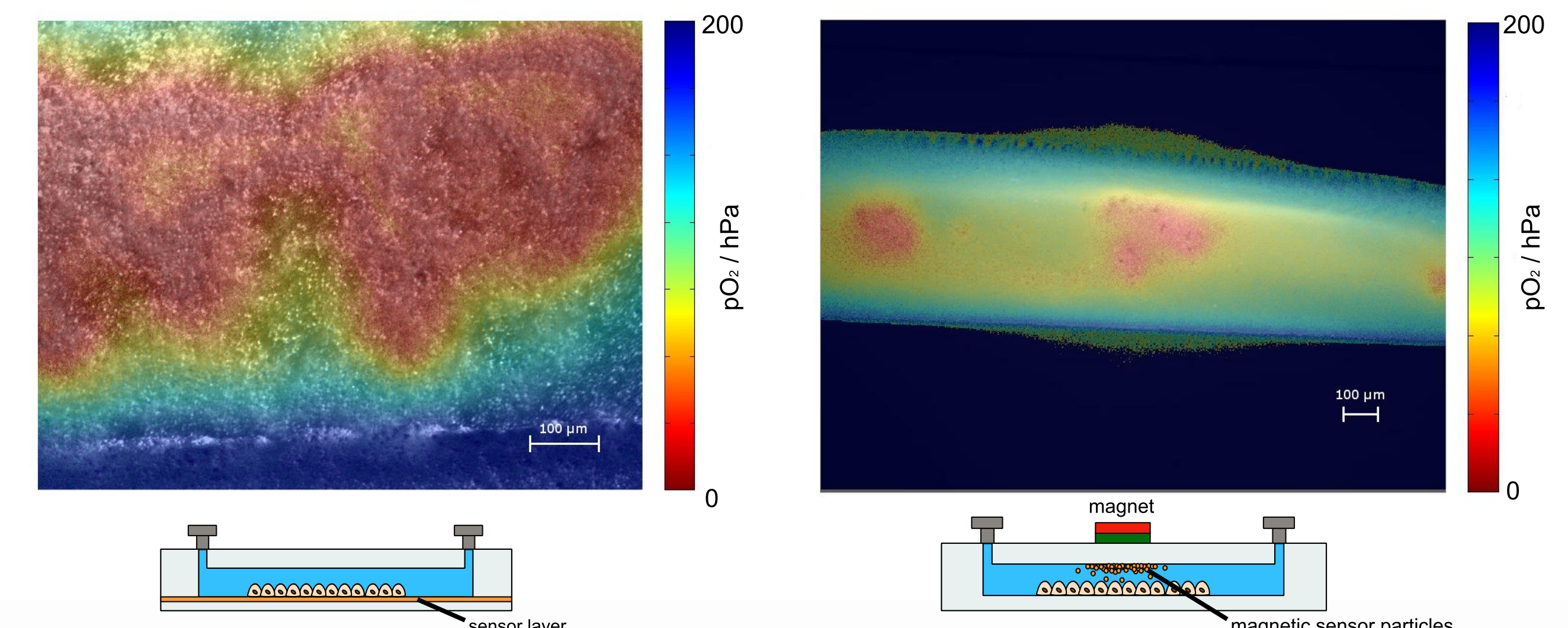


Figure 8: Overlay of transmitted light image and lifetime image of *Candida albicans* in a microfluidic chip with integrated sensor layer.

Figure 9: Overlay of fluorescence intensity and lifetime image of *E. coli* in a microfluidic chip employing magnetic nanosensor particles.

Lifetime images showed the respirometric activity of the microorganisms. Lowest oxygen levels were observed at high densities of microorganisms.

## Additional Notes

Application of PSPVP-nanoparticles turned out to be more difficult than expected. During experiments in the microfluidic chip we noticed a dye transfer from the particles to the lipophilic chip material (PDMS).

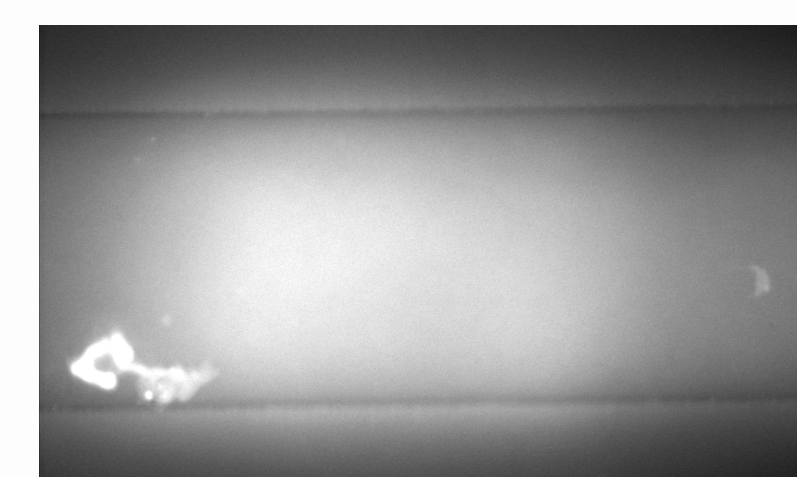


Figure 10: Dye transfer from PSPVP nanosensor particles to the surrounding PDMS. Even outside the channel high luminescence intensities can be seen. These intensities are due to the dye migration.

## Conclusion

The presented sensor designs can easily be integrated in microfluidic devices. Application of these sensors yields highly homogeneous lifetime and ratiometric images with low standard deviations.

Nanosensor particles facilitate a maximum flexibility for sensing in microfluidic systems. It was possible to monitor respiration of biofilms. This could lead to a better understanding of biofilm behaviour.

## Acknowledgement and References

Peter Ertl, Austrian Institute of Technology



- [1] S. Borisov et al., Analytical Chemistry, 2007, 79(19), 7501-7509  
[2] T. Mayr et al., Analytical Chemistry, 2009, 81(15), 6541-6545