Expanding the toolbox of in cellulo transient absorption spectroscopy

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1. Main Text

Biologically relevant chromophores are ubiquitously employed in sensing, imaging, and therapy. Certain organic fluorophores as well as metal coordinated compounds find application as photoactive functional colorants. They exhibit photobiological activity due to their tunable excited state and electron or energy transfer processes.[1]
While the target site of application for these small functional molecules is the human body, there exists a gap in current state of the art, in elucidating the photophysical processes that they undergo in biologically relevant surrounding. A step in this direction is to measure the photophysical properties of these dyes and relevant functional molecules in 2D cell layers as well 3D models. Previous works in the group address these concerns for fluorophores, and for photosensitisers in live human cancer cells. [2,3]
In this work, "dark" but "loud" photoacoustic agents[4] were measured in cellulo, thus obtaining temporal information environment specific information for non-emissive molecules. The outlook is to use optical imaging to probe the spatial information about the localisation of these molecules, which will complement the kinetic data. Optical imaging becomes especially relevant, for these "dark" molecules, which are intended to have eventual applications in optical coherence tomography.

2. Methods and results

Transient absorption spectra were obtained with a home-built setup.[5] 800nm pump pulse of ca. 110 fs pulse duration with 500 Hz repetition rate and a white-light with 1 kHz repetition rate are focused on the sample position. The polarization between the pump and probe beam is set at the magic angle (ca. 54.7°). The solvent samples were measured in 1 mm quartz cuvettes.

For the *in cellulo* experiments, to decrease the acquisition time, kinetic traces are recorded at single wavelengths values. This is achieved by using an optical parametric amplifier, and looking at spectral features of interest, obtained from the white light TA data collected. Moving from a solvent setup to a cellular setup to measuring tissues with a certain thickness involves challenges caused by scattering of light through the thickness of the sample.

Fig: *in cellulo* transient absorption setup and a schematic figure of the setup

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4. References

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