MID-INFRARED OPTOACOUSTIC MICROSCOPY (MiROM) AS A TOOL FOR LABEL-FREE METABOLIC IMAGING OF LIVING CELLS AND TISSUES

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Label-free chemical imaging in living cells and tissues is a very attractive but still challenging task. Existing high-resolution label-free imaging techniques, including near field imaging, electron and atomic force microscopy, impose strong limitations on the imaged sample and are often unsuitable for the study of live biomedical objects. Optical methods are especially well suited for this purpose as they are non-invasive. Intrinsic autofluorescence from endogenous fluorophores is typically too weak for reliable imaging with high signal-to-noise level, which led to development of exogenous labels and wide use of fluorescence microscopy. Although significant progress has been made in fluorescence imaging, including explosive development of various super-resolution methods reaching single-digit resolving power, the need of labelling imposes an inevitable question of label's influence on the target molecule and its behavior.

Chemically specific vibrational imaging modalities based on Raman Scattering, including Coherent Raman Scattering and Stimulated Raman Scattering microscopy, have enormously extended the range of possibilities for endogenous biomolecular imaging. However, spontaneous Raman scattering is relatively weak and requires relatively high light irradiances and elaborate pump-probe measurements schemes using well synchronized short pulses.

Direct vibrational excitation in the mid-IR range, on the other hand, provides cross-sections up to eight orders of magnitude larger than Raman methods, leading to higher sensitivity – which manifests in low illumination irradiances and much lower detection limits down to low-micromolar concentrations. In MiROM, as read-out mechanism for mid-IR imaging we use acoustic detection of pressure waves resulting from the optoacoustic transient thermoelastic expansion due to thermal dissipation of energy. The positive-contrast detection scheme of MiROM strongly benefits from low attenuation and low scattering of acoustic waves.

We showcase the unique label-free biomolecular contrast capabilities of MiROM [1] in living cells by monitoring the spatiotemporal distribution of carbohydrates, lipids, and proteins in 3T3-L1 cells during lipogenesis as well as monitoring the lipid-protein dynamics in brown and white adipocytes during lipolysis.

[1] M.A. Pleitez; A.A. Khan; A. Solda; A. Chmyrov; J. Reber; F. Gasparin; M.R. Seeger; B. Schätz; S. Herzig; M. Scheideler and V. Ntziachristos, "Label-free metabolic imaging by mid-infrared optoacoustic microscopy in living cells"; *Nature Biotechnology*, (2019) doi:10.1038/s41587-019-0359-9