LABEL-FREE METABOLIC MONITORING OF LIVING CELLS BY MID-INFRARED OPTOACOUSTIC MICROSCOPY

Miguel A. Pleitez,[a,b] Asrar Ali Khan,[c,d,e,f] Alice Soldà,[a,b] Andriy Chmyrov,[a,b] Josefine Reber,[a,b] Francesca Gasparin,[a,b] Markus R. Seeger,[a,b] Benedikt Schätz,[a,b] Stephan Herzig,[c,d,e,f] Marcel Scheideler,[c,d,e,f] and Vasilis Ntziachristos[a,b]

[a] Institute of Biological and Medical Imaging (IBMI), Helmholtz Zentrum München, 85764-Neuherberg, Germany [b] Chair of Biological Imaging (CBI), Center for Translational Cancer Research (TranslaTUM), Technische Universität München, 81675-München, Germany[c] Institute for Diabetes and Cancer (IDC), Helmholtz Zentrum München, 85764-Neuherberg, Germany [d] Joint Heidelberg-IDC Translational Diabetes Program, Heidelberg University Hospital, 69120-Heidelberg, Germany [e] Molecular Metabolic Control, Medical Faculty, Technische Universität München, 81675-München, Germany [f] Germany Center for Diabetes Research (DZD), 85764-Neuherberg, Germany

E-mail: francesca.gasparin@tum.de

KEY WORDS: living cells, optoacoustic, label free imaging

Imaging modalities based on vibrational spectroscopy, as Raman scattering or mid-infrared absorption, have demonstrated high chemical specificity for different biomolecules in a label-free manner. Coherent Raman Scattering imaging can detect lipids, proteins, and nucleic acids in living cells and excised tissues, but its sensitivity, especially in the fingerprint region, is limited to target concentrations above 1 mM, which is inadequate for live-cell analytical imaging where, mostly, µM to nM concentrations need to be assessed. Conventional Mid-IR methods with high sensitivity in the fingerprint region, on the other hand, have been limited mainly to dry tissues and fixed cells due to the strong mid-IR absorption of water and due to the negative-contrast detection scheme of conventional mid-IR imaging.

Here, we introduced a label free bond-selective mid-infrared optoacoustic microscopy (MiROM) for metabolic imaging in living cells based on positive contrast. MiROM achieves low-micromolar concentration sensitivity with negligible cell photodamage using up to three orders of magnitude less laser power than other vibrational-spectroscopy imaging modalities, such as Raman imaging. We monitored the distribution of biomolecules such as carbohydrates, lipids, and proteins in adipocytes during lipogenesis and we followed the lipolysis dynamic in brown and white adipocytes monitoring protein and lipid. For the first time, we visualize carbohydrate patterns in early-stage adipocytes revealing, over time, an initial spread throughout the adipocyte body, followed by a co-localization with lipid droplets upon adipocyte maturation. MiROM yields unique label-free metabolic imaging abilities for a broader range of bioanalytical studies in living cells, showing additionally its potential application for analytical histology in fresh/unprocessed tissues. [1]

[1] Pleitez, M.A., Khan, A.A., Soldà, A. *et al*. Label-free metabolic imaging by mid-infrared optoacoustic microscopy in living cells. *Nat Biotechnol* (2019) doi:10.1038/s41587-019-0359-9