

METABOLOMICS BASED ON RAMAN SPECTROSCOPY

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The main goal of this study is to overcome methodological and technical limits of Raman imaging in detecting small organelles and specific molecules within cells by harnessing physics, chemistry, medicine, pharmacology, biology, engineering, and data analysis, to use these advanced technologies in life sciences. The combination of specificity and sensitivity of the Raman probes with information obtained from spontaneous Raman spectroscopy and SRS/CARS on living cells gives diagnostic potential to these methods. They can support classic approaches in tracking cellular processes, studying chemoresistance, and characterizing drug-cell interactions.

The use of molecular probes in Raman imaging is a relatively new technique in subcellular research, however, very fast and dynamically developing. Compared to the label-free method, it allows for a more sensitive and selective visualization of organelles within a single cell. Directly visualizing biological structures and activities at the cellular and subcellular levels remains by far one of the most intuitive and powerful ways to study biological problems.

For hyperspectral detection and imaging of living cells, it is very desirable to use probes with strong and unique Raman vibrations in the biological silent region ($1800 - 2800 \text{ cm}^{-1}$). Here it is shown a biorthogonal chemical imaging of cells to track biochemical changes associated with mitochondrial function at the cellular level in an in vitro model. Both commercially available and newly synthesized highly sensitive Raman probes for selective imaging of mitochondria in live cells is presented. In addition to spontaneous Raman microscopy, nonlinear techniques were used for rapid cell imaging.

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References

- [1] K. Brzozowski et al. A unique Tandem Raman Microscopy (TRAM) integrating spontaneous and coherent Raman scattering offers integrated data analysis to improve optical biosensing, *Optica*, vol 12 (1), 11-23, 2025.
- [2] A. Pieczara et al. A new highly sensitive and specific Raman probe for live cell imaging of mitochondrial function, *ACS Sensors*, 9,2, 995–1003, 2024.
- [3] A. Pieczara et al. Modified glucose as a sensor to track the metabolism of individual living endothelial cells - observation of the 1602 cm^{-1} band called “Raman spectroscopic signature of life”. *Biosensors and Bioelectronics*, 230, 115234, 1-9, 2023.
- [4] B. Radwan et al. EdU sensing: the Raman way of following endothelial cell proliferation in vitro and ex vivo. *Biosensors and Bioelectronics*, 15, 114624. 2022.
- [5] Matuszyk, E. et al.. Multiplex Raman imaging of organelles in endothelial cells. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* 255, 119658, 2021.
- [6] W. Korona, B. Orzechowska, K. Siąkała, A. Nowakowska, A. Pieczara, S. Buda, R. Pawłowski, J. Młynarski, M. Barańska, Bioorthogonal Raman and IR probes for live cell metabolomics: A library, *Sensors and Actuators B Chemical*, 430 (2025) 137363, 2025.