Submitted to Warsaw Summer School on Advanced Optical Imaging 2024

Comparative analysis of multispectral imaging of T and B cells in murine spleen utilizing LDIR, FTIR, and OPTIR spectroscopy techniques

A. Shydliukh[*a], S. Deinhardt-Emmer, J. Popp[a,b], C. Krafft[b]

[a] *Leibniz Institute of Photonic Technology (IPHT), Member of the Leibniz Center for Photonics in Infection Research, Albert-Einstein-Straße 9, 07745 Jena, Germany*

[b] *Friedrich Schiller University Jena, Institute of Physical Chemistry and Abbe Center of Photonics, Member of the Leibniz Center for Photonics in Infection Research, Jena, Germany*

*shydliukhartem@ipht-jena.de

**Artem Shydliukh*

Introduction

Multispectral imaging techniques based on infrared (IR) spectroscopy have become pivotal in biomedical research, offering insights into the spatial distribution and chemical composition of cells within biological tissues. A previous FTIR imaging study already identified vessels, red pulp, white pulp, and B and T lymphocytes in spleen tissue sections[1, 2]. In 2019, new generations of IR instruments were introduced that were coupled to quantum cascade lasers (QCL) namely Laser Direct Infrared (LDIR, Agilent) and Optical Photothermal Infrared (OPTIR, Photothermal Inc.) spectroscopy. A comparative application of these three IR approaches for spectroscopy and imaging to murine spleen tissue sections with and without infection by influenza virus A will be presented.

FTIR microspectroscopic imaging with focal plane array detection is well-regarded for its precise chemical analysis via molecular vibrational frequencies offering high specificity and providing a full spectrum for each image pixel (900–4000 cm^{-1}). LDIR spectroscopy is notable for its rapid scan speed at discrete wavenumbers, making it a preferred choice for time-sensitive imaging tasks, but it's restricted to operating in reflection mode and a narrower spectral range (975–1800 cm^{-1}). OPTIR spectroscopy combines excitation by infrared laser pulses followed by induction of a photothermal effect which is probed by visible light, and the infrared absorption is obtained by lock-in detection schemes. Main OPTIR advantage is submicron resolution which enables to identify single cell nuclei.

Methods and results

To compare the distribution of white pulp and red pulp in both infected and non-infected spleen tissue on IR-transmissive CaF_2 and reflective MirrIR (Kevley Technologies) slides, FTIR and OPTIR data were collected both in transmission and reflection modes while LDIR data could only be recorded in reflection. Similar than in reference [1], the FTIR spectra were normalized to the intensities to the amide I band of proteins followed by k-means cluster analysis in the spectral range 990-1340 cm^{-1} that contains the most intense nucleic acid bands.

Figure 1 shows the microscope image and the color-coded chemical image of IR intensities from 990 to 1340 cm^{-1}. White pulp (yellow to red) is clearly separated from red pulp (blue to cyan). The fine structure is further visualized in the cluster membership map where cyan and green clusters are assigned to red pulp, red cluster to follicle margin (T lymphocytes) and the light blue and beige clusters are assigned to the follicle center (B lymphocytes).

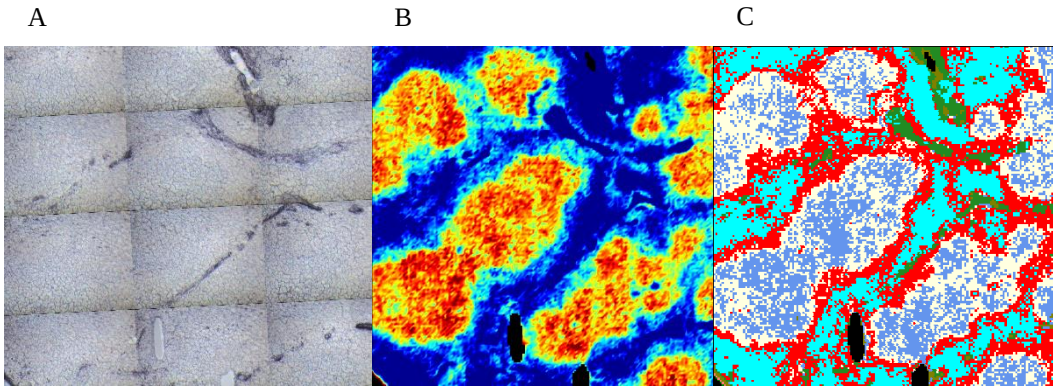

Fig. 1. A) Microscopic image, B) FTIR image of non-infected spleen on CaF_2 at 990-1340 cm^{-1} C) K-means cluster image

Similar contrast can be obtained by IR imaging of discrete wavenumbers using LDIR and OPTIR. This comparative analysis sheds light on the strengths and weaknesses of each spectroscopy technique, elucidating their suitability for various research applications. By providing an in-depth understanding of these techniques, this study equips researchers in the field of multispectral imaging with the knowledge to make informed decisions tailored to their specific experimental requirements. This analysis serves as a valuable resource for selecting the spectroscopy method that best aligns with their research objectives.

Funding

This work is supported by BMBF and is integrated to QUANCER project (FKZ 13N16439)

References

[1] C. Krafft, R. Salzer, G. Soff, M. Ern, M. Meyer-Hermann. Identification of B and T cells in human spleen sections by infrared microspectroscopic imaging. Cytometry A, 2005, 64A, 53-61

[2] Feng, Z., Jensen, S. M., Messenheimer, D. J., Farhad, M., Neuberger, M., Bifulco, C. B., & Fox, B. A. Multispectral Imaging of T and B Cells in Murine Spleen and Tumor. Journal of immunology, 2016, 196(9), 3943–3950.