FTIR Imaging as a New Tool to Characterize Immune Cells in Various Biological Tissues: Focus on Secondary Lymphoid Organs and Breast Tumor Infiltrating Lymphocytes

M. Verdonck[1], S. Garaud[2], L. Buisseret[2], C. Desmedt[2], R. de Wind[2], C. Sotiriou[2], K.Willard-Gallo[2] and E. Goormaghtigh[1].

[1]Laboratory for the Structure and Function of Biological Membranes, Center for Structural Biology and Bioinformatics, Université Libre de Bruxelles, Brussels, Belgium;
[2]Institute Jules Bordet, Université libre de Bruxelles, Brussels, Belgium.

It is now widely recognized that the immune system plays a dual role in cancer progression, promoting or inhibiting tumor growth and metastases.[1-3] In human breast cancer, particular spatially organized Tumor-Infiltrating Lymphocytes (TILs) have been associated with an effective therapeutic response and favorable clinical outcome.[4-6] This study investigates the potential of InfraRed (IR) imaging to identify and characterize infiltrating lymphocytes in breast tumors, focusing on T CD4+, T CD8+ lymphocytes and B lymphocytes (CD20+). Over the past few decades, Fourier Transform InfraRed (FTIR) spectroscopy coupled with microscopy has been recognized as an emerging and potentially powerful tool in cancer research and diagnosis.[7,8] IR imaging enables to probe the chemical composition and molecular structure of complex systems such as cells and tissues with spatial resolution at the cell scale, providing a global and unique signature of all cellular components. Compared to the gold standard used for the study of immune cells in tissue sections, *i.e.* immunostaining, IR imaging shows numerous advantages, including minimal sample preparation, high data throughput, standardization, automation and no requirement for staining. We therefore suggest that IR imaging could provide additional and complementary information to study immune infiltrates in solid tumors. In this study, infrared spectra of lymphocyte subpopulations (CD4+, CD8+ and CD20+ cells) were recorded on both lymphocytes present in FFPE tissue sections of secondary lymphoid organs, *i.e.* tonsils, and breast tumors. Samples were deposited on a BaF_2 window, the spectroscopic imaging data were acquired in transmission mode, using a Hyperion imaging system (Bruker) equipped with a 64x64 MCT (Mercury-Cadmium-Telluride) FPA (Focal Plane Array) detector. Each IR image covered an area of 182 x 182 µm² and contained 4096 spectra, each one being the average of 256 scans. All spectra were preprocessed using baseline and water vapor corrections, normalization and quality filters. The areas comprising the specific lymphocyte subpopulations were determined by Hematoxyline & Eosin (HE) and Immuno-Fluorescent (IF) stainings of adjacent tissue sections. Principal Component Analyses (PCA) together with Partial Least Square-Discriminant Analyses (PLS-DA) indicate that IR spectra obtained from subpopulations of CD4+, CD8+ or CD20+ lymphocytes recorded on tonsils, each show significant differences and can be identified relative to the other subpopulations. These results indicate that FTIR imaging is able to identify specific lymphocyte subpopulations based on their spectral features. Our current studies are investigating this hypothesis on immune infiltrates in breast tumors and these data will be presented at the meeting. In conclusion, this exploratory study shows the ability of FTIR imaging to distinguish and identify CD4+, CD8+ and CD20+ lymphocytes from secondary lymphoid organs without staining requirement. These results highlight the potential use of IR imaging as a new tool for studying immune cells and pave the way for its use to identify and characterize lymphocytes infiltrating breast tumors.

[1] R.D. Schreiber et al., *Science* 331(6024), 1565-1570 (2011).
[2] X. Liu et al., *Trend Immunol.* 34(3), 120-128 (2013).
[3] S. Demaria et al., *InTech*, ISBN: 978-953-307-714-7, Chapter 22, pp. 469 (2011).
[4] L. Martinet et al., *Cancer Res.*, 71(17), 5678-5687 (2011).
[5] K. Willard-Gallo and C. Gu-Trantien, *Ann Oncol* 24(suppl 3), iii38 (2013).
[6] C. Denkert et al., *J. Clin. Oncol.* 28(1), 105-113 (2010).
[7] G. Bellisola and C. Sorio, *Am J. Cancer Res.* 2(1), 1-21 (2012).
[8] J. Nallala, *J. Biomed. Opt.* 17(11), 116013 (2012).