HYPERSPECTRAL POLARIMETRIC IMAGING FOR MULTISCALE LABEL-FREE CHARACTERIZATION AND FUNCTIONAL DIAGNOSTICS OF BIOTISSUES

Alexander BYKOV[1,*] Oleksii SIERYI[1], Ivan LOPUSHENKO[1], Anton SDOBNOV[1], Viktor DREMIN[2], Igor MEGLINSKI[2]

[1] *Opto-Electronics and Measurement Techniques, University of Oulu; Oulu, Finland;*
[2] *Aston Institute of Photonic Technologies, Aston University, Birmingham, UK*
* *Correspondence: alexander.bykov@oulu.fi*

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Bulk tissue polarimetry exploits the vector nature of light to provide intrinsic contrast based on tissue microstructure, while hyperspectral imaging reveals functional and biochemical information. Combining these two modalities enables a multidimensional characterization of biotissues at different spatial scales, providing a versatile tool for functional imaging of *in vivo* tissues [1] as well as label-free, non-destructive characterization of histological tissue blocks [2], with the potential for rapid automated segmentation of malignant regions.

Two types of systems have been implemented and compared. The first one is based on full Stokes polarimetry. This system utilizes a supercontinuum fiber laser coupled with an acousto-optic tunable filter to select probing wavelengths between 450-650 nm with 5 nm accuracy. Illumination is right circularly polarized via a quarter-wave plate and focused on the sample using a 45 mm lens. Diffusely reflected light is collected by a 100× objective, passed through a 100 μm pinhole for spatial filtering, and analyzed by a polarimeter providing Stokes parameters at each pixel and wavelength. This configuration achieves a spatial resolution better than 5 μm and enables scanning of areas ranging from cm to μm scales, approaching single-cell-level resolution. Optimal source-detector separations of 60-100 μm were identified: smaller separations decreased cancer sensitivity, while larger ones increased depolarization noise. Specific experimental results demonstrated that the system could clearly demarcate cancerous regions within formalin-fixed paraffin-embedded breast tissue blocks. The obtained degree of polarization maps showed high contrast between cancerous, fibrotic, and adipose tissue areas, with strong correlation to standard histopathological ground truth. The second system is a compact handheld assembly incorporating a hyperspectral camera based on a microtunable Fabry-Perot filter. It achieves a spectral resolution of 6-10 nm across the 510-900 nm range, utilizing broadband illumination from a halogen lamp delivered via a fibre-optic ring illuminator. Hyperspectral images were captured over an area of 8 × 8 cm² with a megapixel resolution. For polarization measurements, a rotating linear polarizer enabled acquisition of co-polarized and cross-polarized images, from which the Degree of Linear Polarization (DOLP) spectra were calculated in each spatial pixel. *In vivo* skin measurements, as well as Monte Carlo simulations incorporating a multilayered skin model with realistic optical properties, demonstrated that tissue scattering changes had a greater impact on polarization alterations than absorption variations, confirming the sensitivity of the DOLP to microstructural changes, such as collagen fibre density and organization. In conclusion, the integration of hyperspectral imaging and polarimetry provides a powerful approach for label-free, non-invasive functional imaging of biological tissues. Full Stokes polarimetry excels in multiscale structural characterization of histological tissue blocks with high spatial resolution, while camera-based hyperspectral polarization imaging is better suited for in vivo assessment of tissue structure and function due to its compactness, robustness, and rapid data processing. Combined, these complementary approaches have the potential to advance diagnostic histopathology and functional tissue imaging towards automated, rapid, and objective assessments in clinical workflows.

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