M.5: Development of a multimodal microscopic imaging system capable of hyperspectral and multi-wavelength time-lapse imaging for biomedical applications

Optical microscopy techniques play a crucial role and have been widely employed in biomedical research. Fundamentally, these techniques exploit various aspects such as morphological feature, transmittance, fluorescence, scattering, and phase, enabling a deeper insight of the intricate structures and functions in the biological systems at the cellular and subcellular level. Although conventional microscopy enables real-time visualization of cells, tissue, and extracellular matrices, it often relies on chemical-staining or fluorophore tagging. Moreover, the information is typically limited to colour contrast, which lacks sufficient spectral specificity to distinguish biomolecules with closely overlapping spectral signatures. Such difficulties can be addressed by integrating spectral information into the microscopic images, yielding rich spatio-spectral content and thereby elucidating more precise biochemical characterization of biological specimens. In addition, integrating time-lapse imaging facilitates temporal-spectral image acquisition, enabling visualization and analysis of dynamic biological processes. Since temporal morphological changes are the aftermath of the intrinsic biochemical alternations, hence, temporal-spectral imaging is crucial for comprehensive monitoring of dynamic biological phenomena.

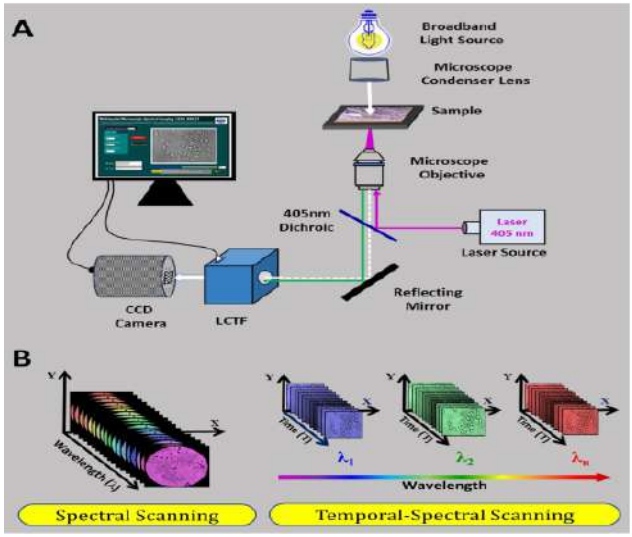

Fig. M.5.1: (A) Schematic diagram and (B) schematics of Hyperspectral data cube generation through Multimodal Microscopic Spectral Imaging system.

Recently, we have developed an imaging system (fig. M.5.1) employing liquid crystal tunable filter (LCTF) as spectral decomposition element that offers flexible waveband selection without compromising spatial resolution. A Graphical User Interface (GUI) software was developed for hardware control of the system to offer user-friendly spectral data acquisition and analysis platform. The main GUI of the system comprises six dedicated modules: configuration settings module, single wavelength spectral image acquisition module, multi and hyper-spectral image acquisition module, multi-wavelength time-lapse image acquisition module, spectral image analysis module, temporal image analysis module (fig. M.5.2). Developed system was calibrated and systematically characterized for all domains: spatial, spectral, and temporal. Further, multi-wavelength time-lapse fluorescence images of resected breast tissue were acquired and analyzed.

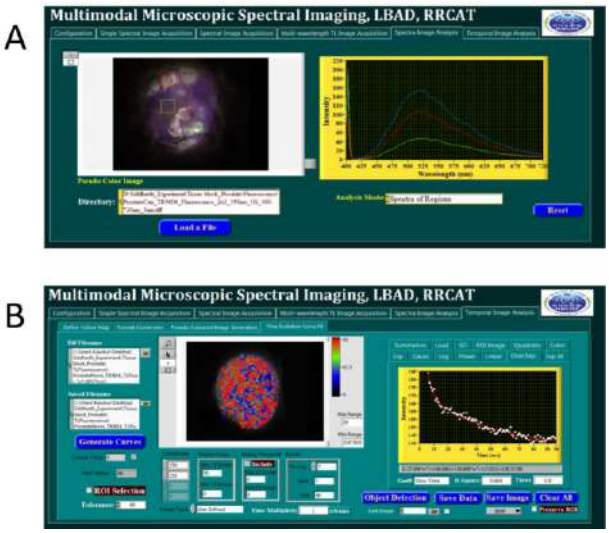

Fig. M.5.2: GUI for spectral image analysis: (A) Hyperspectral and multispectral image analysis, (B) time-lapse spectral image analysis

Multimodality of the developed imaging system was demonstrated through Transmission Hyper-Spectral Imaging (THSI) and Fluorescence Hyperspectral Imaging (FHSI) as well as spectral and temporal-spectral scanning mode. Systematic calibration and characterization of the system in the spatial and spectral domains ensured its accuracy, reliability, and reproducibility. The linearity of its response to exposure time and emission intensity further confirmed its suitability for quantitative imaging. Validation experiments conducted on real biological samples and model systems highlights its potential for a wide range of biomedical applications. The performance of the system was evaluated for delineation of cancer margin in resected tissue samples. The fluorescence photobleaching characteristic measured via time-lapse fluorescence imaging at multiple wavelength bands was found to be a potential biomarker in breast tissue characterization. Further, the supremacy of state-of-the-art machine learning algorithms over the conventional parametric model-based approach were established in semantic image segmentation of interrogated samples.

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