

Chemical Histopathology with Practical Infrared Spectroscopic Imaging Instrumentation and Algorithms

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Abstract

Chemical imaging (CI), especially infrared (IR) spectroscopic imaging, enables label-free biomedical analyses while achieving expansive molecular sensitivity. In conjunction with classification algorithms, these instruments can provide objective and automated evaluations to aid pathologists improve diagnostic accuracy. Current industry standard spectroscopic imaging microscopes can take days or weeks to image a full spectrum at every point from a tissue sample. The quantum cascade laser (QCL), with high intensity and narrowband emission, allows for a viable discrete frequency approach, enabling drastic increases in imaging speeds with superior spatial and spectral resolution. We present a mid-IR microscope design that simultaneously provides high throughput recording, low spectral noise, and high spatial resolution for imaging whole slides. The bottom-up design of its compound optical train enables dual-axis galvo laser scanning of a diffraction-limited focal point over large fields of view with its interchangeable, infinity-corrected, high numerical aperture (NA), refractive objectives. QCL illumination allows for rapid chemical mapping of full spectral ranges or only the discrete spectral frequencies necessary for analyses. We demonstrate whole-slide, speckle-free imaging in ~3 min per discrete frequency with a 2 mm pixel size using our 10X / 0.4 NA configuration, and high resolution 1 mm/pixel capability with its 20X / 0.8 NA counterpart, both offering spatial quality at their respective theoretical limits with minimal optical distortion or speckle while maintaining high signal to noise ratios (>100:1). The data quality enables superior applications of modern machine learning, leading to a suite of capabilities not previously feasible – 3D reconstructions using serial sections, comprehensive assessments of whole model organisms, and

histological assessments of disease in a time comparable to clinical workflows. Distinct from conventional stained approaches that focus on morphological investigations or molecular immunostaining techniques that target specific proteins or genetic alterations, this development makes all-digital, label-free imaging of minimally processed tissue practical.