

## Editorial

# The coming paradigm shift: A transition from manual to automated microscopy

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## Abstract

The field of pathology has used light microscopy (LM) extensively since the mid-19<sup>th</sup> century for examination of histological tissue preparations. This technology has remained the foremost tool in use by pathologists even as other fields have undergone a great change in recent years through new technologies. However, as new microscopy techniques are perfected and made available, this reliance on the standard LM will likely begin to change. Advanced imaging involving both diffraction-limited and subdiffraction techniques are bringing nondestructive, high-resolution, molecular-level imaging to pathology. Some of these technologies can produce three-dimensional (3D) datasets from sampled tissues. In addition, block-face/tissue-sectioning techniques are already providing automated, large-scale 3D datasets of whole specimens. These datasets allow pathologists to see an entire sample with all of its spatial information intact, and furthermore allow image analysis such as detection, segmentation, and classification, which are impossible in standard LM. It is likely that these technologies herald a major paradigm shift in the field of pathology.

**Key words:** Automated light microscopy, high-throughput, mixed reality, three-dimensional imaging, virtual reality

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## A BRIEF HISTORY OF THE ROLE OF LIGHT MICROSCOPY IN PATHOLOGY

During the mid-19<sup>th</sup> century, the use of light microscopy (LM) in pathology grew exponentially, driven primarily by improved optics, reduced costs, and increased availability.<sup>[1]</sup> To this day, LM has remained the quintessential tool for pathologists around the world. The practice of surgical pathology is primarily interested in the analysis of tissue and cellular structures (and alterations thereof) and begins with a careful “gross” examination of the excised tissue using the naked eye.<sup>[2]</sup> Gross examinations are followed by a more exhaustive examination of histologic preparations of tissue sections using a compound light microscope.<sup>[3]</sup> Ancillary tools, such as immunohistochemistry, flow cytometry, and

electron microscopy, are variably deemed necessary for the diagnosis of certain diseases; however, the histologic findings are usually the most important indicator of pathologic dysfunction, and LM remains the single most used instrument and method of investigation in the arsenal of pathology tools.<sup>[4-7]</sup>

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Regardless of the degree or index of clinical suspicion, a diagnosis of cancer cannot be definitively established, and definitive therapy should not be undertaken, in the absence of a tissue diagnosis.<sup>[2]</sup> Most hospitals, health networks, and private pathology practices have policies or regulations in place supporting this practice, and they are regularly monitored by hospital tissue committees or accrediting agencies.<sup>[8]</sup> Thus, a pathologist's primary tool (i.e., compound light microscope), technique (i.e., histologic tissue examination), and general workflow (i.e., rendering a definitive diagnosis based primarily on LM examinations of glass slide-mounted tissue sections) have remained essentially unaltered since the second half of the 19<sup>th</sup> century.<sup>[1]</sup> As such, the analog realm of pathology appears superannuated compared to contemporary medical counterparts (i.e., radiology, surgery, and hemato-oncology), which have witnessed radical, technologically-driven transformations.

However, pathologists may soon be able to catch up with their tech-savvy medical colleagues, thanks to advancements in LM methods, which are already being used for the mapping of point-to-point connectivity between all anatomical regions in animal brains.<sup>[9-11]</sup> This emergent field, commonly referred to as connectomics, is leveraging automated LM instruments and computational methods in a race to complete a connectivity map of a whole-mouse brain, in hopes of providing the larger scientific community with an online atlas for viewing the entire anatomical datasets.<sup>[9]</sup> It has already been shown that current, automated LM methods are fully capable of creating high-resolution, high-throughput anatomic models.

## ADVANCED IMAGING TECHNIQUES

Traditional LM techniques utilize "linear" (i.e., one-photon) absorption processes for contrast generation, and are therefore limited to areas at and near the tissue surface for high-resolution imaging. At tissue depths >100  $\mu\text{m}$ , the effects of light scattering begin to limit the resolution and the images produced become blurry.<sup>[12]</sup> In recent years, advanced imaging techniques and novel microscopic technologies have emerged as potential alternatives to LM.<sup>[13-17]</sup> These advanced imaging modalities, which include diffraction-limited (e.g., confocal, multi-photon, and 4Pi microscopy) and subdiffraction techniques (e.g., photo-activated localization, stochastic optical reconstruction, and stimulated emission depletion microscopy), are advantageous because they enable nondestructive, high-resolution, and/or ultra-sensitive imaging, down to the molecular level.<sup>[18]</sup> Many of these imaging methods are capable of producing high-resolution, three-dimensional (3D) datasets from sampled tissues.<sup>[12,13]</sup>

More recently, additional computational imaging methods have been developed for microscopic analysis, including lens-free digital holographic, Fourier ptychographic, and tomographic techniques.<sup>[19-22]</sup> All these methods have the capacity to exceed diffraction limits that traditionally hamper LM, and can thus produce high-resolution images. Theoretically, some of them can be combined to create low-cost imaging systems for clinical pathology; however, none currently exist in commercial form and none of the aforementioned methods are adequately designed or optimized for high-throughput automation.

## AUTOMATED LIGHT MICROSCOPY FOR THREE-DIMENSIONAL IMAGING

Within the context of automated LM methods for 3D imaging, there are several approaches that can already be applied to clinical pathology. They are divided into two categories, based on the method of tissue clearing: Block-face/tissue-sectioning versus chemical clearing.<sup>[23]</sup> Chemical clearing methods are beyond the scope of this review; however, the former approach is quite similar to traditional histologic techniques, and we shall briefly review two bright-field, tissue-sectioning instruments that are currently in use.

Knife-edge scanning microscopy (KESM) and micro-optical sectioning tomography (MOST) are both designed to perform imaging and sectioning in a single, simultaneous process: bright-field, line-scan imaging sensors and diamond-knife embedded ultramicrotomes are utilized for automated sectioning and 3D imaging of resin-embedded tissue. The former is capable of generating 155 terabytes of data per day, while the latter has demonstrated an ability to generate >8 terabytes of data in approximately 10 days.<sup>[24]</sup> MOST performs imaging in reflection, which requires the use of *en bloc* reflective stains (i.e., Golgi stain) that are highly specific. Furthermore, reflective stains mark numerous features that are below the diffraction limit and cannot be resolved with the standard light microscopes using visible light. KESM, on the other hand, uses transmission illumination to perform imaging, and thus it is not reliant on reflective stains. In addition, KESM's utilization of the diamond knife as both sectioning and optical instrument provides greater illumination near the knife edge, improving imaging speed and boosting the signal-to-noise ratio.<sup>[25]</sup> Finally, KESM is already available as a commercial service, whereas MOST has not yet been adapted for commercial utilization.

## THE POTENTIAL ROLE OF THREE-DIMENSIONAL IMAGING FOR PATHOLOGY

Automated LM methods for 3D imaging are intriguing for clinical pathology because it can generate comprehensive,

high-resolution, volumetric datasets of cellular architecture and morphology in a high-throughput fashion. Furthermore, many, if not all, of these 3D imaging modalities are heavily reliant on advanced cognitive technologies such as machine/computer vision and machine learning. These advanced technologies, derived from decades of cutting-edge artificial intelligence research, are optimally suited for deployment within the realm of digital pathology because they enable algorithmic approaches to image analysis, which have been shown to be beneficial in numerous contexts (e.g., detection, segmentation, and classification).<sup>[26-28]</sup> If the resolutions, throughput speeds, and data processing pipelines of automated LM 3D imaging techniques continue to improve over time, it also holds true that these techniques will approach the levels of efficiency currently offered by contemporary histopathology and traditional LM. Sometime, in the not-so-distant future, tissue biopsies and tissue blocks from resected organs may be altogether replaced by whole-specimen or whole-organ imaging, thereby transforming the field of pathology. The implications of automated, high-throughput, quantitative 3D LM analysis of tissue specimens will likely culminate in a major paradigm shift for the practice of pathology.

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