Review Article

Review of advanced imaging techniques

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Abstract

Pathology informatics encompasses digital imaging and related applications. Several specialized microscopy techniques have emerged which permit the acquisition of digital images (“optical biopsies”) at high resolution. Coupled with fiber-optic and micro-optic components, some of these imaging techniques (e.g., optical coherence tomography) are now integrated with a wide range of imaging devices such as endoscopes, laparoscopes, catheters, and needles that enable imaging inside the body. These advanced imaging modalities have exciting diagnostic potential and introduce new opportunities in pathology. Therefore, it is important that pathology informaticists understand these advanced imaging techniques and the impact they have on pathology. This paper reviews several recently developed microscopic techniques, including diffraction-limited methods (e.g., confocal microscopy, 2-photon microscopy, 4Pi microscopy, and spatially modulated illumination microscopy) and subdiffraction techniques (e.g., photoactivated localization microscopy, stochastic optical reconstruction microscopy, and stimulated emission depletion microscopy). This article serves as a primer for pathology informaticists, highlighting the fundamentals and applications of advanced optical imaging techniques.

Key words: 2-photon microscopy, 4Pi microscopy, advanced imaging, confocal microscopy, digital microscopy, optical coherence tomography, optics, photoactivated localization microscopy, spatially modulated illumination microscopy, stimulated emission depletion microscopy

INTRODUCTION

Microscopy is important for diagnostic pathology as well as biomedical informatics research. Transmitted light microscopy has been the main technique for diagnostic pathology for over a century. Recently, several specialized microscopy techniques emerged.¹¹⁻⁴ Table 1 lists several new microscopy modalities and compares them with conventional transmitted light microscopy. These techniques have great potential for clinical diagnostic applications, as they permit imaging to be performed at much higher resolution. Many contemporary instruments not only offer imaging at higher resolution, but allow users to study single molecules and better image live cells. The advent of these new techniques will likely alter the role of pathologists in future diagnoses. Not only may tissue biopsies be replaced by “optical biopsies,” but pathologists may be called upon to acquire, manage, and possibly interpret these high resolution digital images. Therefore, a better understanding of these techniques.
Table 1: Spectrum of specialized imaging microscopy modalities (adapted in part from reference[120])

| Imaging modality                        | Microscopy principle                                                                 | Achievable resolution       | Pros                                                                                                           | Cons                                                                                      |
|-----------------------------------------|---------------------------------------------------------------------------------------|----------------------------|----------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| Phase-contrast microscopy               | Refractive index differences are used to provide contrast to view unstained microscopic specimens. Small phase shifts in light passing through a transparent specimen get converted into amplitude or contrast changes in the image. | Lateral resolution ~200 nm  | Can observe living cells/organisms in a natural/unstained state; can provide more information than specimens that need to be killed, fixed or stained to view under a microscope; high-contrast, high-resolution images | Not ideal for thick organisms or particles; Halo effect, where images are often surrounded by bright areas, might obscure details along the perimeter of the specimen. |
| Fluorescence microscopy (wide-field)    | Fluorescence microscopy uses fluorescence instead of transmitted or reflected light.   | Lateral resolution ~200 nm  | Can provide high contrast, high-resolution images; versatile in choosing difference fluorophores and ability to visualize multiple fluorophores simultaneously. | Not ideal for thick organisms or particles; needs to stain/process the sample to emit fluorescence; fluorescence signals are usually weak. |
| Confocal microscopy                     | Confocal microscopy increases optical resolution and contrast. By using a scanning platform where the light source is imaged through a pinhole to block out-of-focus photons, it is possible to reconstruct 3D structures from images. | Lateral resolution ~200 nm  | Able to provide 3D sectioning by reducing the out-of-focused light; high-resolution images can be acquired rapidly and noninvasively. | Relatively high cost and smaller field of view; image depth limited to a few hundreds of microns. |
| Two-photon microscopy (TPM)            | Based on non-linear excitation of fluorophores, this mode of microscopy offers submicron depth-resolved imaging without using a pinhole. | Lateral resolution ~300 nm  | Wide separation between excitation and emission spectrum ensures rejection of excitation light; deeper image penetration than confocal microscopy, suitable for thick specimens; elimination of confocal pinhole minimizes signal loss. | Relatively lower resolution compared to confocal as longer wavelength is used; usually require introducing fluorophores into specimens for imaging. |
| Optical coherence tomography (OCT) and microscopy (OCM) | OCT enables micron scale imaging based on low-coherence interferometry. OCM combines confocal microscopy with OCT to achieve depth-resolved cellular resolution imaging | Lateral resolution ~1000 nm | Can observe living tissues in a natural/unstained state; deeper image penetration than confocal microscopy, suitable for thick specimens; high-speed imaging to image 3D volume in vivo. | Relatively lower molecular contrast compared to fluorescence imaging. |
| 4Pi microscopy                          | Using two oppositely-aligned objective lenses effectively doubles the angular aperture, permitting greater resolution to be obtained. | Lateral resolution ~100 nm  | Higher resolution than confocal microscopy. | Limited resolution improvement compared to STED or PALM/STORM. |
| Structured illumination microscopy (SIM) and saturated SIM (SSIM) | SIM uses laser illumination of samples from which coarse barcode-like interference patterns (Moiré fringes) are emitted and processed to generate high-resolution images. | Lateral resolution ~100 nm  | Compatible with 100 nm for SIM wide-field microscopy and ~50 nm for SSIM | Limited resolution improvement compared to STED or PALM/STORM; needs data processing. |
| Stimulated emission depletion (STED)    | STED uses two beams, one for excitation and the other for quenching. The difference between these two point-spread-functions gives a smaller effective area for excitation. | Lateral resolution <10 nm  | High resolution; fast for small field of view; no need for data processing. | Fluorophore limited. |
and their potential uses will be beneficial for the pathology informatics community. This article reviews several advanced microscopy modalities and highlights some of the emerging applications using these optical imaging techniques.

**CONFOCAL MICROSCOPY**

Confocal microscopy increases optical resolution and contrast. Laser scanning confocal microscopy uses a point light source that is imaged on the sample, with a pinhole placed in front of the detector. The use of the pinhole rejects out-of-focus photons from reaching the detector. Confocal microscopy enables the reconstruction of three dimensional (3D) structures by stacking individual 2D en-face images at different depths. Microscopy can also be performed with parallel imaging approaches, including slit scanning or tandem scanning. Confocal imaging using dual-axis configuration enables imaging at an even deeper penetration.

The scanning laser ophthalmoscope used to examine the retina was the first device utilized for in vivo confocal microscopy. Reflectance-mode confocal microscopy (RCM) has also been translated into clinical applications for noninvasive high-resolution imaging of human skin in vivo. Confocal laser endomicroscopy (CLE) is a relatively new endoscopic tool that creates high-resolution images of cells and tissues in vivo. CLE essentially provides in vivo histology-level images during ongoing endoscopy. Confocal endomicroscopy can also be performed through fiber bundles or a rigid gradient-index (GRIN) rod lens (special rod-shaped optical component that can relay images).

**TWO-PHOTON MICROSCOPY**

Two-photon microscopy (TPM) provides high-resolution (submicron) imaging with lower phototoxicity and deeper tissue penetration than confocal microscopy. In the two-photon process, a molecule simultaneously absorbs two photons whose individual energy is only half of the energy state needed to excite that molecule, and then releases the energy to an emission photon. To achieve reasonable excitation/collection efficiency, typical TPM systems use the half of the wavelength of the incident photons, can provide direct visualization of anisotropic biological structures such as collagen. TPM can image conventional fluorophores or fluorescent proteins such as green fluorescent protein (GFP). It can also image endogenous fluorescence molecules such as flavin adenine dinucleotide (FAD), and keratin, among others. In addition, second harmonic generation (SHG), an energy-conserving scattering process which also absorbs two incident photons at once and release all the energy to the emission photon (at the half of the wavelength of the incident photons), can provide direct visualization of anisotropic biological structures such as collagen. Intravital TPM has provided unprecedented anatomical, cellular, molecular, and functional insights into host-tumor interactions as well as immune cell dynamics. Such dynamic intravital microscopy is transformative as it can reveal cellular behavior in vivo. It was recently demonstrated that TPM can also excite fluorescence
Typical TPM systems use an excitation wavelength at \(~800\) nm. To further improve the penetration depth of TPM, longer wavelength excitation at \(~1300\) nm can be directly from hemoglobin,\(^{29,30}\) allowing investigators to directly visualize microvasculature. The rich biological information provided by TPM therefore makes it an attractive tool for biomedical imaging applications.

Traditional TPM image formation uses 2D raster scanning (line-by-line scanning), which could be slow for applications requiring high temporal resolution. Hence, other imaging approaches have been developed, including random-access scanning using acousto-optic deflectors (AODs), a device that can change beam deflection angle by tuning the input electric frequency applied on the acoustic crystal,\(^{31}\) and parallel scanning with multiple beams.\(^{32}\) More recently, alternative scanningless TPM using temporal focusing has been developed.\(^{33}\) Instead of allowing laser pulse traveling through the optical system with constant pulse duration, temporal focusing broadens the pulse duration along with the propagation path, and reaches the shortest pulse duration only at the focal plane of the objective. This approach, also called plane-projection multiphoton microscopy, enables video-rate wide-field optical sectioning of live tissues.\(^{34}\) Rapid 3D imaging of living cells has been achieved using novel Bessel beam plane illumination microscopy.\(^{35}\) Bessel beam has a relatively long focusing waist compared to a conventional Gaussian beam. Coupled with structural illumination and/or two-photon excitation, this method provides isotropic (in all directions) resolution of \(0.3\) µm and rapid imaging speed of 200 planes per second, thereby enabling 3D imaging of subcellular features \(in\) \(vivo\). With this technology the dynamics of mitochondria, filopodia, membrane ruffles, intracellular vesicles, and mitotic chromosomes in living cells have accordingly been visualized in real-time.\(^{35}\) Figure 2 shows an example of Bessel plane two-photon excitation imaging of chromosomes during mitosis.\(^{35}\)

![Figure 1: Jablonski diagram for single photon (a) and two-photon (b) excitation](image1.png)

![Figure 2: High-speed volumetric imaging of chromosomes in mitosis using scanned Bessel beams in conjunction with two-photon excitation. The images shown demonstrate 3D tracing of two chromatids (green and purple) in a living cell over a series of eight image planes. (Reproduced with permission from reference 35.)](image2.png)
used. To further extend TPM imaging for subsurface tissues, several two-photon microendoscopes using GRIN rod lens or fiber bundles have been developed. In those designs, the GRIN rod lens or fiber bundles relay the light from the proximal end of the endoscope to the distal end (in the tissue). Alternatively, Wu et al., developed a 2.4-mm-in-diameter all-fiber-optic endomicroscopy using a piezoelectric transducer (PZT) scanner (a device that can deform its shape based on the applied voltage) at the distal tip. Two-photon microendoscopy enables numerous applications such as imaging neuronal function and the gastrointestinal or reproductive tract.

OPTICAL COHERENCE TOMOGRAPHY AND MICROSCOPY

Optical coherence tomography (OCT) is analogous to ultrasound imaging, except that the imaging is performed using light rather than sound. By measuring the echo time delay and intensity of back-reflected light, OCT can reveal tissue microstructure with micron-level resolution and 1-2 mm penetration depth, approaching those of standard excisional biopsy and histopathology, but without the need of tissue removal. Since the speed of light is much faster than that of sound, the echo delay time in OCT is detected using a technique called low coherence interferometry. Figure 3 shows a schematic diagram of a Michelson interferometer. OCT uses a broad bandwidth light source; therefore interference signal is only observed when the path lengths of the reference and sample arms are matched to within the coherence length (the length over which the phase of optic wave oscillation is correlated) of the light. By scanning the reference mirror position, the magnitude and echo time delay (equivalent to depth) of the reflected light from the sample can be measured. The optical beam can be scanned across the tissue surface to form 2D or 3D images. The contrast in OCT comes from the difference in light scattering properties in various tissue layers, and as a result, OCT can reveal tissue microstructure without the use of contrast agents (i.e., label-free imaging).

The axial resolution of OCT is determined by the coherence length of the light source: \( D_z = \frac{2\ln 2}{\pi} \left( \frac{\pi}{2} \right) \left( \frac{l}{D_l} \right) \), where \( D_l \) is the full-width-at-half-maximum of the source spectrum (the difference between the two wavelength values at which the power is equal to half of its maximum value) and \( l \) is the center wavelength of the source spectrum. From this equation, broadband light sources can achieve high axial resolution. Typical OCT systems can achieve axial resolutions of \( \sim 10-15 \) µm. More sophisticated broadband light sources can generate a broadband spectrum and hence result in ultrahigh-resolution OCT imaging. The transverse resolution of OCT is the same as that in optical microscopy, and is determined by the diffraction limit of the focused optical beam: \( D_x = \frac{4l}{\pi d} \), where \( d \) is the beam size on the objective lens and \( f \) is its focal length (the distance from the lens to the focus spot). Fine transverse resolution can be obtained by using a large numerical aperture (NA) objective that focuses the beam on a small spot size. The combination of a very broad bandwidth light source and Bessel beam has culminated in the realization of micro-OCT with 1-2 µm isotropic resolution in 3D.

Optical coherence microscopy (OCM) combines confocal microscopy with OCT to achieve cellular resolution imaging in the en-face plane (the plane parallel to the surface of the sample). The combination of coherence and confocal gates enhances rejection of unwanted scattered light, thereby allowing improved imaging depth compared to confocal microscopy alone. Figure 4 shows representative OCM images of human colon specimens ex vivo. Representative histology images in this figure at the corresponding depth in this figure show good correlation with the OCT images. Detailed structures such as crypt lumens and translucent mucin-containing cells can be clearly visualized. Also shown in this figure is the 3D isosurface view of two central colonic crypts. Various types of organs have been shown to be amenable to ex vivo study using full-field OCT. As high-resolution OCT images are able to recapitulate the main histological features in tissues, this technique looks promising in performing fast histology, especially in intraoperative procedures. In addition, OCM has been demonstrated to provide high-resolution images of renal pathology in real time without exogenous contrast medium or histological processing. High sensitivity and specificity was achieved using OCT to differentiate normal from neoplastic renal tissues, suggesting possible applications for guiding renal mass biopsies or evaluating surgical margins.

Using fiber-optic and micro-optic components, OCT can be integrated with a wide range of imaging devices such as endoscopes, laparoscopes, catheters, and needles to enable imaging inside the body. Endomicroscopic
OCT provides unique advantages for evaluating diseases present within the epithelial surface of hollow organs as well as buried in deep solid organs. Clinical applications of endoscopic OCT/OCM include the detection of pre-malignant lesions, identification of disease below the tissue surface, assessment of depth of tumor invasion, localization of cancer margins, evaluation of effectiveness of therapy, and reduction in the number of biopsies and frequency of surveillance.\(^{26,27,78}\) Figure 5 shows an example of a forward-viewing OCT imaging needle that can be used for neurosurgery guidance. The OCT image obtained can clearly identify anatomic landmarks that can be used in stereotactic surgery.\(^{79}\) Simultaneous OCT and Doppler OCT (DOCT) imaging is also possible. DOCT measures the frequency shift of the back-scattered photons caused by the motion of samples (e.g., red blood cells). This technique has been used to quantify the blood flow velocity in biological samples.\(^{78}\)

The development of endoscopic OCT greatly facilitates imaging of certain conditions. For example, OCT has been shown to be successful at detecting intestinal metaplasia in patients with Barrett's esophagus patients\(^{80,84}\) and transmural inflammation in those who have inflammatory bowel disease.\(^{85}\) OCT also has the potential to distinguish hyperplastic from adenomatous polyps in the colon.\(^{86}\) Of particular interest, is the fact that OCT may be useful in identifying high-grade dysplasia, such as in Barrett's esophagus. However, additional work in this area is still required. Evans et al. reported a sensitivity of 83% and specificity of 75% for detecting high-grade dysplasia and intramucosal carcinoma with blinded scoring of OCT images from 55 patients.\(^{87}\) Isenberg et al. reported a sensitivity of 68% and a specificity of 82%, with an accuracy of 78% for the detection of dysplasia in biopsies from 33 patients with Barrett’s esophagus.\(^{88}\) Employing computer-aided diagnosis, Qi et al. found an increased sensitivity of 82%, a specificity of 74%, and an accuracy of 83% in 13 patients.\(^{89}\) It is anticipated that further improvements in spatial resolution will hopefully result in better diagnostic capability.

OCT/OCM can perform high-resolution imaging of tissue structures in situ and in real time. Images are available immediately without the need for excision and histological processing of a specimen. The development of high-resolution and high-speed OCT technology, as well as OCT endoscopic devices, will soon provide clinicians with more diagnostically relevant information in increasing clinical applications. Potential applications of OCT/OCM in pathology might include enhanced specimen grossing, reduced need for frozen sections during intraoperative consultation,\(^{90}\) and direct scanning of tissue blocks to produce 3D histology images, potentially bypassing certain steps involving glass slide workflow.\(^{91}\)

**SUPER-RESOLUTION MICROSCOPY**

Super-resolution microscopy refers to a suite of techniques that pushes or breaks the diffraction limit (\(-200-300 \, \text{nm}\) in the lateral direction and \(-500-700 \, \text{nm}\) in the axial direction) to achieve a spatial resolution down to \(-8-25 \, \text{nm}\).\(^{92}\) thereby allowing the observation of subcellular structures that are not visible in conventional light microscopy. One category of methods overcomes the diffraction limit by using patterned excitation to sharpen the point spread function (PSF) of the imaging system, including saturated structured illumination microscopy (SSIM)\(^{93}\) and stimulated emission depletion (STED) microscopy.\(^{94}\) The other category uses single molecule localization techniques (i.e., statistically sample the point source to locate the center of the Airy disk) to accurately identify the object position, such as photoactivated localization microscopy (PALM),\(^{95}\) fluorescence photoactivated localization microscopy (FPALM),\(^{96}\) and stochastic optical reconstruction microscopy (STORM).\(^{97}\) Structured illumination microscopy (SIM) illuminates the sample using a sinusoidally modulated stripe pattern with...
a spatial frequency (i.e., how often the sinusoidal structure repeats per unit of distance). Each spatial frequency from the sample interferes with this illumination frequency, resulting in a coarse beat frequency (moire fringes), which can be resolved by a conventional microscope. Hence, structured illumination increases the highest detectable spatial frequency, or equivalently, increases the spatial resolution of conventional optical microscopy. SIM extends the resolution by a factor of 2 (~100 nm).

Saturated SIM can generate harmonics with spatial frequencies that are multiples of modulation frequency. Less than 50 nm resolution has been demonstrated using SSIM. SIM can also be extended to generate 3D views by mixing three beams. 3D SIM images demonstrate much improved lateral and axial resolution over conventional wide-field microscopy. Since SIM uses wide-field illumination, it can achieve a high imaging speed for live cell imaging in vivo. Recently, video imaging at 11 Hz with 100 nm resolution has been demonstrated.

In contrast, STED overcomes the diffraction limit by employing stimulated emission to “turn off” the fluorophores in the focal region, except those located at the center. In STED, two beams are used, one as in conventional fluorescence microscopy to excite the fluorophores from the ground state to the excited state and the other beam (STED beam) to stimulate the excited state to spontaneously revert back to the ground state (a process called stimulated emission) where no fluorescence photon is emitted. By making the STED beam a “donut” shape using a phase mask, much smaller PSF can be achieved. STED can provide an xy resolution of ~50 nm. The axial resolution initially attained was ~100 nm with a single lens and can be improved to ~30-40 nm when using 4Pi geometry. STED exhibits 10-fold higher resolution (20-30 nm) to visualize biological structures more clearly. Furthermore, by applying pulsed excitation together with time-gated detection (where the detection event is triggered by the excitation pulse), sharper images with 35% enhancement in resolution can be obtained at 2-3 times lower average power.

Another category of super-resolution microscopy relies on single (or sparse) molecule localization, where the localization of the fluorophore can be estimated by statistically sampling to find the center of the fuzzy Airy disk. The accuracy of single molecule localization can reach nanometer accuracy despite the broad PSF (>200 nm). PALM and FPALM use photoactivatable fluorescence proteins (FAP, FPs that can be turned on and off by a UV laser, for example EosGFP). Initially, the fluorophores are in an inactive state (nonfluorescence). When shining with a flash of UV light, the dye is photoactivated into a fluorescing state. Since the photoactivation process is stochastic, only a few, well-separated molecules are “turned on” at a given time. The position of those molecules can be located by single molecule localization techniques described above. After those fluorophores photobleach, another flash of light is applied to activate another random set of fluorophores. This process is repeated many times, and the final image is the summation of the individual images. Because the image is built up molecule by molecule at different times, the final image resolution can be much higher than the diffraction limit. PALM is hence able to visualize detailed mitochondria structures with comparable resolution as TEM. Furthermore, dual-color PALM enables direct visualization of molecular interactions at the nanometer level (resolution ~20 nm). One limitation of the single molecule localization technique is the long data acquisition time (several hours). However, a recent form of PALM decreased the acquisition time to ~25 s per frame with 60 nm resolution, therefore allowing live-cell PALM imaging of nanoscale adhesion dynamics. Recently, Pavani et al. used an engineered PSF to form

Figure 5: (a) A 21-gauge needle OCT probe overlying the human basal ganglia (PUT = putamen, GPe = globus pallidus externa, GPi = globus pallidus interna). Above it is the full-track reconstructed expanded OCT image that was created ex vivo. (b-d) Simultaneous OCT and Doppler OCT (DOCT) imaging of an anesthetized sheep brain in vivo showing real-time monitoring of vessel compression by the OCT probe. (Reproduced with permission from reference 79)
a double helix pattern in 3D, whose angular orientation rotates with the axial (z) position of the emitter.\[^{10}\] Each z position was associated with a unique orientation of two spots that could be resolved by a diffraction limited detector, thereby allowing precise single molecule localization in 3D.\[^{10,11}\] This method can be used for traditional wide-field fluorescence microscopy, as well as super-resolution microscopy such as PALM.

Another localization-based technique called STORM uses a pair of different organic dyes (called the “activator” and “reporter”) at close proximity (a few nanometers) to achieve photoswitching,\[^{97,112}\] and forms super-resolution images using a similar algorithm to that used in PALM. The reporter is often a cyanine dye (Cy5, Cy5.5, or Cy7), and the activator is usually a shorter wavelength cyanine dye (Cy2 or Cy3). The advantage is that different combinations of dye pairs can be simultaneously imaged. Recently, the Zhuang group extended high-resolution STORM to an axial dimension with ~50 nm resolution by using a cylindrical lens.\[^{113}\] The depth (z) information can be inferred by the ellipticity of the measured PSF. Live-cell 3D STORM imaging [Figure 8] with ~30 nm lateral resolution, ~50 nm axial resolution and 1-2 second temporal resolution (with several independent snapshots) has been achieved.\[^{114}\] In Figure 8 STORM images clearly discriminate transferrin and clathrin, which appeared as completely overlapping puncta in the conventional images. As demonstrated in this image, the morphology of the clathrin coat enclosing the transferrin cluster was resolved in this nanoscopic cellular structure.\[^{114}\]

**Spatial-Domain Low-Coherence Quantitative Phase Microscopy**

Spatial-domain low-coherence quantitative phase microscopy (SL-QPM) is a novel optical microscopy technique based on the light interference effect (i.e., superimposition of a reference wave and a sample wave), developed to detect subtle changes in cell architecture as small as 0.9 nm, a scale much smaller than the resolution of a light microscope.\[^{115-118}\] SL-QPM quantifies optical refractive index distribution by physical parameter of optical path length (OPL), associated with the macromolecular density or organization within a...
single cell or a subcellular component (e.g., nucleus). Importantly, this technique is suitable for analyzing the nanoscale structural changes of cell nuclei in original, unmodified cytology and histology specimens prepared using standard clinical protocols, without any additional processing or modification.

The instrument used to perform SL-QPM is shown in Figure 9. Briefly, parallel white light is focused onto the sample by a low-NA objective. The reflectance-mode signals are projected by a lens onto the slit of a spectrograph coupled with a charge couple device (CCD) camera, which is mounted on a scanning stage. The detected backscattering spectrum is the result of superimposition of the reference wave (i.e., reflection from the glass) and backscattered sample waves with a shared common light path. Due to the intrinsic variation of stain levels, a correction model has also been developed to minimize the effect of stain-induced variations on the measured OPL. The nanoscale-sensitive architectural parameters provide a comprehensive quantitative assessment of nuclear structural characteristics. For example, the average OPL of the cell nucleus is associated with the average nuclear density; while the intranuclear standard deviation of OPL or entropy quantifies the chromatin texture of the cell nucleus.

The SL-QPM system has shown great potential for improving the detection of malignancy even in cells morphologically labeled as “normal” or “indeterminate” by expert surgical pathologists and/or cytopathologists that were confirmed at surgery to be malignant; or in histologically “normal” appearing cells from uninvolved tissue distant from the primary cancer site. Figure 10 shows an example of analyzing the original unmodified histology specimens of breast biopsies processed with a standard clinical protocol (formalin fixed, paraffin embedded and stained with hematoxylin and eosin) using the SL-QPM system. Figure 10a shows the representative histology image and the corresponding OPL map of cell nuclei from three groups of cells, including “normal” cells from patients undergoing a reduction mammoplasty, “uninvolved” cells (i.e., histologically normal cells located at a distance from a nearby malignant tumor), and “malignant” cells. The average OPL from the cell nuclei in this figure shows a progressive increase in normal, uninvolved and malignant cells. More importantly, although both normal and uninvolved cells both appear to be morphologically “normal” by histopathological diagnosis with a light microscope, the average nuclear OPL is significantly elevated in the uninvolved cells of the cancer specimen when compared to normal cells from the reduction mammoplasty.

CONCLUSION

Advanced microscopy techniques have shown great promise for imaging biological structures at the cellular, subcellular and molecular level. These tools have increasingly enabled the visualization of cellular and molecular interactions in situ at the submicron to nanometer scale, thereby furthering our understanding of the fundamental biological and pathological mechanisms involved in many diseases. With further development in technology, these microscopy techniques will likely provide novel tools beyond what is currently available in the arsenal of pathologists. It is important that pathologists therefore understand these advanced imaging techniques and the impact they have on pathology.
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