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A perspective on digital and computational pathology

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Abstract

The digitization of images has not only led to increasingly sophisticated methods of quantitating information from those images themselves, but also to the development of new physics-based techniques for extracting information from the original specimen and presenting this as visual data in both two and three-dimensional (3D) forms. This evolution of an image-based discipline has reached maturity in Radiology, but it is only just beginning in Pathology. An historical perspective is provided both on the current state of computational imaging in pathology and of the factors that are impeding further progress in the development and application of these approaches. Emphasis is placed on barriers to the dissemination of information in this area. The value of computational imaging in basic and translational research is clear. However, while there are many examples of “virtual diagnostics” in Radiology, there are only relatively few in Pathology. Nevertheless, we can do cellular level analysis of lesions accessible by endoscopic or catheterization procedures, and a number of steps have been taken toward real-time imaging as adjuncts to traditional biopsies. Progress in computational imaging will greatly expand the role of pathologists in clinical medicine as well as research.

Key words: Computational imaging, confocal-based imaging, pathology-radiology convergence, super-resolution, virtual biopsy

INTRODUCTION

This discussion will examine the evolution of digital pathology to embrace all forms of computational imaging, with a consideration of possible impediments for the availability of this technology for diagnostic and experimental pathology. One such impediment is a lack of awareness by pathologists of many of the newer modalities that are emerging in this field. Another is a lack of integration among the many relatively small societies that serve as conduits for association, dissemination, and advocacy in the field. A third is the relative lack of journals that embrace sophistication both in the development of new technologies and the use of those modalities to solve experimental problems, with content accessible to both biomedical investigators and the engineers, physicists, and mathematicians that are essential for synergistic progress. Many authors gravitate toward engineering journals, which are not usually read by pathologists or, for that matter, most biomedical investigators. Often, the pathobiological application shown is a proof-of-concept demonstration rather than a sophisticated investigative study. Papers that appear in pathology journals have the opposite problem; they are sketchy about the physical/engineering side of the work, and do not attract many readers from the engineering and physics community. Perhaps the simplest way of summarizing these interrelated issues is by...
EMERGENCE OF COMPUTATIONAL IMAGING IN RADIOLoGY AND PATHOLOGY

As seen above, digitization of images opens up a whole new world of computational analysis. The converse is also true, namely that computational analysis opens up a whole new world of digital imaging. Photonic interactions with a biological specimen involve a small number of parameters, namely transmission, absorption, reflection, secondary emission, diffraction and scatter. The power of computational imaging is that it can optimize the imaging potential of these parameters while minimizing deleterious effects (for example diffraction).

From an historical perspective, it is clear that the emergence of magnetic resonance imaging (MRI) and computer-assisted tomography (CAT) have revolutionized radiology. Conventional tomography, or planography was developed in the 1950s and was used extensively in clinical medicine through the early 1970s. In this technique, a sectional image is made by moving an X-ray source and film in opposite directions during the exposure. This makes subjects in the focal plane appear sharper while structures in other planes appear blurred. This approach was of limited utility at the time. However, with the advent of powerful computers, it became possible to obtain data from multiple directions and use reconstruction software to create extremely sharp images in multiple planes, which may be viewed as two-dimensional slices or 3D reconstructions.[6] In many ways, this use of computer processing to sum multiple images was the ancestor of computational imaging. MRI, in contrast (pun intended), makes use of a strong magnetic field to align proton spin (primarily but not always) in the hydrogen atoms of water molecules. A right angle radiofrequency (RF) pulse is then used to perturb this state. The return to equilibrium, when the RF stops (relaxation) is accompanied by an induced RF signal from the nuclei. Measurement of these signals at multiple points in the subject is used to reconstruct an image. In this technique, then, the computer is used both to convert an induced RF to a visible signal and to analyze and manipulate the multiple signals to construct an image.[6] This represents a higher level of computational analysis than CAT.

In pathology, one of the earliest forms of computational imaging was confocal microscopy, and in particular, confocal laser scanning microscopy (CSLM) reviewed in.[5] Traditional widefield epifluorescent microscopy is hampered in its ability to provide fine detail, because of limits imposed by diffraction, and is also hampered in its ability to provide 3D information because of light scattering. In a CSLM, the specimen is excited by laser light focused to a diffraction-limited spot within that specimen. The emitted light from the spot is separated from the exciting
laser light and then passed through a confocal pinhole that rejects light generated outside the focal plane as well as scattered light. The image is created by scanning the diffraction-limited spot in three dimensions. The emitted light is detected, and the resulting dataset is reconstructed to create an image by computer, utilizing similar reconstruction algorithms to those described above.

Another well-established technique is optical coherence tomography (OCT) which saw initial use in ophthalmology and is now a tool for radiologists and pathologists, and directly by clinical interventionalists. OCT uses light to capture images from within optically scattering media such as tissue. It uses relatively long wavelength (near-infrared) light to allow penetration and utilizes backscatter to obtain information. Micrometer resolution is obtained but only for a depth of a few millimeters into the tissue.

RECENT ADVANCES IN CONFOCAL LASER SCANNING MICROSCOPY AND OPTICAL COHERENCE TOMOGRAPHY

Just as confocal microscopy evolved from traditional fluorescence microscopy and computed tomography evolved from crude superimposition of image planes, these modalities themselves have rapidly evolved. As this communication is meant to provide a broad overview and perspective on this rapidly emerging field rather than a scholarly review per se, with a few exceptions, no primary references are cited. Instead, reference is made to monographs and/or websites where appropriate. Some examples of advances in confocal microscopy include spinning disk, multi-photon, total internal reflectance, and lateral sheet illumination microscopy. Confocal based techniques allow super-resolution microscopy via various techniques (APLM STORM, STED, etc.) Light sheet illumination microscopy illluminates the tissue with a laser beam that is projected on the sample as a thin sheet of light parallel to the focal plane. This allows for high-resolution reconstruction of the sample in three dimensions. These techniques are described in. Another recent technique is spatial light interference microscopy that combines phasor contrast and holography with topographic accuracy comparable to that of atomic force microscopy. There have also been advances in OCT such as Angle Resolved Low Coherence Interferometry, which allow quantitative measurements of size and texture of subcellular structures as well as ultra-high resolution spectral domain OCT and Doppler tomography. OCT has also been combined with other modalities, such as photoacoustic tomography.

RAMAN IMAGING

Although we usually consider light scatter the enemy of visual observation and resolution, light scattering can be our friend as well. This is best seen in Raman spectroscopy and Raman imaging. When a beam of photons transverses a specimen, the bulk of light scattering is via Rayleigh scattering where the scattered photons have the same energy as the incident photons. Raman scattering, in contrast, results from inelastic interactions with vibrating molecules (most intensely in the region of double bonds) and results in a small number of scattered photons with a frequency different (usually lower) than the incident photons. The resulting Raman spectra provide characteristic molecular fingerprints and allow chemical analysis of intact cells. Recently, Raman information has been used to construct images through computational approaches analogous to those used in the modalities described above, the most useful being intensity maps of specific molecular species in a cell in three dimensions enhanced by mathematical deconvolution.
nanometer resolution (!) using ATF probes as antennas and doped diamond lattices as detectors. The tricorder used by the crew of the Enterprise in Star Trek may not be that far out of reach in the near future. However, the question remains as to whose hand will hold the tricorder. It seems clear, from the various examples presented above that the disciplines of radiology and pathology are converging toward a new specialty, with a head start for radiology. Unless pathologists accelerate the pace of embracing new imaging modalities to provide better diagnostic and prognostic data for clinical use, this new entity will be called “Radiology” and anatomic pathologists will become part of a radiological subspecialty called “cellular imaging.” This may or may not be a bad thing in terms of hospital support. However, we would rather see an entire new discipline, possibly called “diagnostic imaging” emerge, which would allow radiologists and pathologists to retain their professional identities within it. In either event “proof of concept” for convergence can already be seen in the increasing ability of radiologists to perform molecular imaging and the pathologist’s ability to image and detect molecular interactions.

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