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MoM#5: X-RAY AND TERAHERTZ (THz) IMAGING

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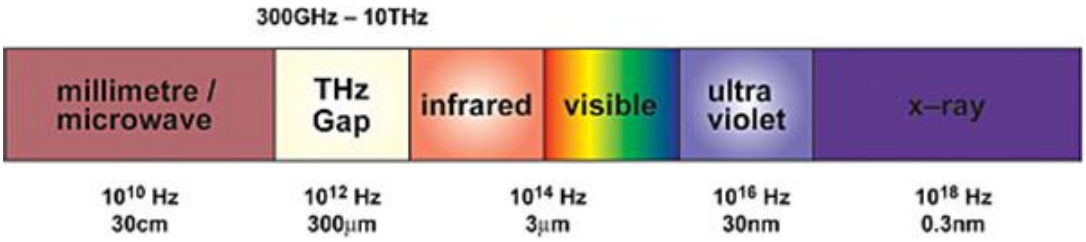
Introduction

In its most general form, the visual observation involves using light as a probe to interact with an object to create an image. In general, microscopy relies upon transmission, reflection, refraction, and diffraction of visible light. Even in fluorescence microscopy, where ultraviolet rather than visible light is utilized, we nevertheless achieve visualization through secondary emission within the visible light spectrum. In either case, the interaction between object and observer is mediated by energy carried by photons within a small part of the electromagnetic spectrum. Even when we confine ourselves to visible light there are limitations both in the human eye and imaging sensors. Examples include making distinctions between fine gradations of color and precise quantitation of the various features within an image. We also visualize specimens over a narrow range of frequencies. However, in recent years there has been the development of various techniques for hyperspectral imaging. These have the common feature of providing full spectral information at every pixel of the image, with subsequent computational "unmixing" and quantitating these signals to provide false color or heat map images for visual observation and interpretation [reviewed in (1)].

In previous MoMs, we have seen how technology has gone beyond photonic interrogation of our specimens. We are now able to visualize samples on the basis of proton spin, atomic mass, and other physical properties. Also, as we have seen, we can find loopholes in the laws of physics to achieve levels of resolution far greater than those allowed by the diffraction limit. Because of the diffraction limit, resolution increases as wavelength decreases (i.e., as frequency increases), which is in part why super-resolution schemes are based on UV methodologies. Modern super-resolution can achieve resolutions of 10-15 nM (compared to 200 nM for visible light microscopy).

Another approach is to extend the range within the electromagnetic spectrum to shorter wavelengths (higher frequency higher energy) up to and including X-ray radiation, to achieve resolution theoretically beyond that of conventional super-resolution. There are also attempts to harness the lower end of the spectrum in the region beyond infrared, known as terahertz (THz) radiation. Here the goal is not to improve resolution (typically in the 10-20 micron range) but for other potential advantages that these wavelengths bring. These include better resolution than can be obtained by ultrasound-based methodologies and the fact that, unlike X-rays, it is non-ionizing, thus avoiding damage to biological materials, such as living patients.

The spectral range is shown below:



X-ray imaging has the potential of exceeding super-resolution ranges. Electron microscopy, on the other hand, offers even higher resolution than X-ray imaging, but with many limitations, such as long imaging times and intensive sample preparation, including difficult chemical fixation, staining and sectioning — all of which can lead to artifacts. X-ray microscopy offers advantages in the ability to visualize unsectioned, frozen-hydrated whole cells using natural contrast and short imaging times. Also X-rays are attenuated considerably less than are electrons, and so thicker specimens can be imaged, thus obviating technically difficult thin sectioning. Further, X-ray scattering varies greatly, from material to material, whereas electron scattering varies only slowly with atomic number thus making X-ray spectroscopy feasible.

General Aspects of X-ray Microscopy

For a historical perspective, see (2). In order to move X-ray imaging from radiology to the pathology laboratory, focusing optics had to be developed and optimized. Since 1980 there have been advances in compound refractive lenses and reflective optics for X-ray imaging, as well as the development of Fresnel zone plates. Advances in nanofabrication capabilities including electron beam lithography and thin-film deposition have enabled these changes, and thus X-ray imaging at the cellular and subcellular level is now available. [See reviews (2), (3)].

If zone plates are used for focusing, the spatial resolution is set largely by the outer zone width of the zone plate. Thus, although X-rays can yield theoretical resolution of fractions of a nanometer, practical considerations limit microscopic resolution to about 10-15nM. Improvement in both the resolution and efficiency of X-ray focusing has been accomplished by using zone plates with multilayer coatings that can be polished to an effective outermost zone as small as 4 nm (Laue lens). By way of comparison, for reflective optics, a typical resolution that can be achieved is about 10 nm.

X-ray energies range from <1keV to >100keV. X-rays are often characterized as “soft” and “hard.” X-rays with energies about 5-10keV (below 0.2 to 0.1 nM wavelength) are called hard X-rays and used in medical radiography. Lower energy (soft) X-rays have wavelengths around 1 nM. For purposes of this discussion we will ignore this distinction. X-ray microscopy can be performed on specimens about 10 microns thick. Contrast, dose, and depth-of-field limit the effective resolution, especially for tomographic imaging of thicker specimens.

Implementation of X-ray microscopy

Full Field Transmission X-ray Microscopy (TXM) is analogous to a visible-wavelength bright field microscopy. The sample is illuminated with X-rays via a condenser optic, and X-rays retransmitted from the sample are focused by an objective zone plate onto an imaging sensor. Phase contrast techniques can be employed. 3D imaging is obtained by acquiring a series of 2D images at different sample angles and then applying tomographic reconstruction techniques (3). It is also possible to focus the radiation to a small spot on the sample. The sample is then raster-scanned in two dimensions to form an image (STXM).

It is also possible to map chemical states and thereby obtain molecular information from X-ray data. As one example, micro-X-ray fluorescence spectroscopy is performed by spectrally analyzing emitted secondary photons as the sample is raster-scanned in front of a small X-ray focal spot. The incident photons knock out the core electrons from the various atoms present in the irradiated spot. The atoms then "relax" as an electron from an upper energy state jumps into the vacancy with resulting emission of a fluorescent photon of characteristic energy for the element being interrogated. Other techniques for obtaining spatially localized spectral fingerprints at the subcellular level include atomic force microscopy and Raman imaging.

Biomedical applications

X-ray microscopy lends itself to the study of the structural pathology of subcellular features in diseased cells. For example, Schneider et al (4) performed tomography on a mouse adenocarcinoma cell line using TXM at 510 eV. Because the cell was grown on a flat substrate, a full tomographic dataset was not obtained, a limitation of electron microscopy as well. Nevertheless, high-resolution 2D slices from a reconstructed tomogram revealed subcellular structure, including mitochondria, lysosomes, endoplasmic reticulum, vesicles, nuclear pores and a 29 nm double-layer nuclear membrane (4). The example provided by the study by Schneider et al (4) demonstrates the power and potential of X-ray microscopy, as do many other contributions by other investigators.

Terahertz imaging as a contrast to X-ray imaging

THz imaging, because of its low resolution (by pathology standards) has been utilized most extensively in radiologic research. However, it has a number of properties that make it of potential interest to our discipline, and it provides a nice contrast (pun intended) to X-ray imaging, because of the difference in behavior of radiation at the relatively low and high ends of the electromagnetic spectrum.

As THz radiation falls in between infrared radiation and microwave radiation in the electromagnetic spectrum, unlike visible light, it can penetrate through a variety of non-conducting materials, including clothing. Since it is non-ionizing, unlike X-irradiation, it can interact with tissue without hazard, and so it is of interest as an imaging modality. As indicated above, due to its longer wavelength, images made using THz are in the 10-20 micron range and thus not suitable for imaging of cellular detail. However, it has a number of properties that make it of interest. THz radiation is strongly attenuated by water and very sensitive to water content. THz waves can thus provide better contrast for soft tissues than X-rays. The presence of cancer often

changes blood supply to affected tissues, thus increasing local water content. This acts as a natural contrast mechanism in the THz imaging of cancer. Structural changes at cancer sites also contribute to THz image contrast. It is also possible to employ phase contrast techniques; in this regard Rong et al (5) have demonstrated imaging of the absorption and phase-shift distributions of 3.2 mm × 2.3 mm × 30-μm-thick human hepatocellular carcinoma tissue by continuous-wave terahertz digital in-line holography. Gold nanorods can be used to further improve image contrast in THz imaging of cancer (6). Additionally, THz radiation can be used to interrogate vibrational modes and thus can yield spectroscopic data. For example, one can detect mutations in DNA by shifts in resonance frequency. For these reasons, there have been many studies of cancer utilizing this modality [reviewed in (7)].

SUMMARY AND OVERVIEW:

X-ray and THz imaging extend the spectrum by which we are able to examine a specimen. They provide tools not only for visualization but for obtaining molecular information about the various components and biological compartments of the specimen at the cellular and subcellular level. Both these aspects are critical. It is extremely important to be able to define the spatial relationships involved in biological interactions, as location is as significant as the quantitative aspects of a reaction. A simple real world analogy involves two houses that are otherwise identical in every property and parameter that goes into making a house and that in overall aspect look identical. Now imagine that in one, the microwave is in the bathroom, and the bathtub is in the kitchen – clearly a case of functional disruption. Quantitation at the local level is also important for function; in addition to overall under-expression or over-expression of gene products, we have to be able to identify local concentrations of these. To continue the house analogy, a refrigerator is good to have and two might even be better. Put ten of them in the kitchen and surely kitchen function is degraded, over and above refrigerator-dependent activities. This is a major reason that molecular biological analysis and next generation imaging must go hand in hand. By extending the range of observation, increasing resolution, and correlating visual data with molecular, functional, and biophysical information, evolving imaging science sheds new light on biomedical phenomena. In so doing, it reaffirms the central role of the human eye in pathology with a little help from our silicon-based friends (to be addressed in a subsequent MoM).

REFERENCES:

1. Levenson R, Beechem J, and McNamara G. Spectral Imaging in Preclinical Research and Clinical Pathology, in Biophotonics in Pathology, Cohen S, ed, pp. 43-75, IOS Press, 2013.
2. Richards KS and Myring WJ., X-ray microscopy. J Zool 1990, 221: 683-687.
3. Kihara H. 3D Imaging of X-ray Microscopy. In 2nd International Symposium for Science of Firm, Ishizaka S, ed, pp. 105-114, Scientific Publishers, Tokyo, 1990.
4. Schneider G et al. 3D cellular ultrastructure resolved by a partially-coherent X-ray microscope. Nat Methods (in press).
5. Rong L et al. Terahertz in-line digital holography of human hepatocellular carcinoma tissue. Scientific Reports 5, Article number: 8445 (2015)
6. Oh SJ, et al. Nanoparticle-enabled terahertz imaging for cancer diagnosis. Opt Express 2009, 17: 3469–3475.
7. Calvin Y et al. The potential of terahertz imaging for cancer diagnosis: A review of investigations to date. Quant Imaging Med Surg 2012, 2: 33–45.