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MOM#2: HIGH RESOLUTION MAGNETIC RESONANCE MICROSCOPY (MRM)

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Introduction. Although magnetic resonance imaging (MRI) is usually considered part of the armamentarium of radiologists rather than pathologists, recent advances have raised the possibility that it will soon be possible to achieve resolution at the cellular level. This will allow MRI to provide the same kinds of views that we are used to in H&E sections. For many years it was thought that physical limits imposed on both field induction and spin detection would make this impractical, but new approaches have led to devices capable of resolving a single protein. Cellular structures occupy a middle ground between molecules and animals, and so it is appropriate to speculate on the future of this modality in pathology.

General principles of MRI. Unlike the use of light and optics in conventional imaging, MRI is based on the magnetic properties of subatomic particles and their perturbation by external fields. The magnetic effects associated with both electrons and nuclei at the atomic level are due to a quantum property known as “spin.” Spin is not rotation in the classical sense, but rather is an intrinsic angular momentum of elementary particles; thus it does not imply that these particles are actually spinning. Regardless, one can think of these particles as behaving like tiny magnets. These can be aligned by an external magnetic field and can be perturbed by an applied force (radiofrequency waves) to create oscillations. These oscillations will gradually decay due to retarding forces resulting from interactions between nearby nuclei and between nuclei and electrons (“coupling”). A good visual analogy is a compass that is aligned with the earth’s magnetic field. If you perturb it by tapping it gently, it will oscillate around its equilibrium position. In this case, friction provides the retarding force, and thus the oscillations will slowly decrease until the needle is stationary again.

In clinical magnetic resonance imaging, the major contribution to the signal comes from protons (hydrogen nuclei) in water molecules, although other more complex molecules can generate signals as well. In MRI, a powerful magnet aligns the protons, analogous to the earth’s magnetic field aligning the compass needle. However, in the case of MRI, the perturbing agent is not a tap, but rather consists of short bursts of radio waves. These knock the protons out of alignment. When the radio pulses are turned off, the protons realign, generating endogenous signals (free-induction decay, FID) that are then detected and Fourier transformed from the time domain to the frequency domain, creating the classic nuclear magnetic resonance (NMR) spectral peak. Because of coupling effects, realignment is influenced not only by intrinsic spin but also the surrounding environment in tissue. Thus, the signals provide information not only about location and intensity, but also about different tissues.

Two of the measurable parameters associated with this “relaxation” process are:

T1: longitudinal relaxation time, (a measure of the time required for the displaced nuclear magnetic moments to return to equilibrium), and
T2: transverse relaxation time (the time of the FID response signal to decay).

These signals are processed via complex algorithms that display the information in visual form; i.e., computer generated images. Thus, MRI is a good example of computational imaging, where what we are looking at are virtual images based on intensities and contrasts, rather than images created by optical lenses. General principles and the underlying physics of MRI are discussed in greater detail in (1,2).

Improvements in Resolution. In conventional MRI, a superconducting magnet generates the aligning field. The injected radiofrequency pulse(s) are detected by a small number of sensors. Increasing resolution requires more powerful external magnets, higher intensity of radiofrequency pulses, and smaller and more numerous detectors, all of which seem to preclude the use of MRI for pathologic examination of tissue. Typical clinical devices achieve resolutions of about 300 microns with magnet strength in a range of about 1.5 to 3 Tesla. Resolution of 100 microns has been defined as magnetic resonance microscopy. For research purposes, there are now high strength (21 Tesla) commercially available devices that will achieve resolution of less than 30 microns. However, even this is clearly inadequate for revealing histologic detail at the cellular and subcellular level.

Because of these limitations and the relative weakness of signals from small samples, signal augmentation via the use of magnetic labels has been utilized. This technique is known as magnetic particle imaging and was introduced by Phillips in 2005. For example, using conventional instruments, one study (3) was able to detect 100 iron-labeled cells *in vivo* and the fate of those cells could be monitored over time as they developed into tumors.

High Resolution Magnetic Resonance Microscopy. From the above, it is clear that we are far from the goal of obtaining *in vivo* MRI images at the same level of resolution that we can achieve by viewing a stained histological slide through a light microscope, let alone at confocal levels of resolution, in order to achieve Star Trek technology. However, a number of new technologies are now firmly in place, and these will ultimately make it possible to approach that goal. In fact, in clearly focused (pun intended) experimental settings, it is now possible to resolve detail at nanometer scales, far beyond what is needed for cellular and subcellular detail. Basically, this technology involves using physically small high frequency radiofrequency emitters and, most importantly, multiple highly sensitive submicroscopic detectors to overcome the need for more massively powerful magnets compared to the relatively small number of conventional detectors used in typical clinical and translational research settings. This is achieved by taking advantage of the recent observations that tiny defects in diamond films, called nitrogen-vacancy (NV) centers, can serve as magnetic field sensors at the nanometer scale. In 2015, three groups (DeVience, et al, Haberie et al, and Rugar, et al) independently reported the use of these detectors for magnetic resonance studies (reviewed in 4). In one such study, an organic polymer sample was scanned past the NV center detector. It was found possible to achieve a special resolution of 12 nanometers (4). Recently, another group (5) was able to do single cell imaging, using similar techniques. Because of

the relative weakness of endogenous signals from the cell, the cells had to be doped with magnetic nanoparticles. This is the first example of the combination of diamond-based MRI and magnetic particle imaging.

Summary and overview. Conventional NMR spectroscopy, and therefore MRI, requires high magnetic fields, large samples of people, organs, or small animals, and sources of strong radiofrequency pulses, as well as limitations in the number of detectors. This all conspires against MRI of *in vitro* samples with resolution of histological and cellular detail, let alone the ability to do so *in vivo*. However, the recent discovery that small defects in diamond films can serve as atomic sized magnetometers has made it possible to achieve resolutions that are orders of magnitude better than this. Within the past several months, the nuclear magnetic resonance signal and its spectral analysis have been described for a single 3 protein (6), way beyond the resolution needed for cellular morphology. Thus, there is ample proof of principle for ultra-high resolution MRI to evolve into a useful tool for clinical, translational, and basic pathology. The remaining technical challenges appear resolvable. Therefore, there is every reason to be optimistic that in the future, MRI will prove to be as useful to the pathologist as it has been to the radiologist. The first step will be magnetic resonance imaging of *in vitro* biopsy specimens at these high levels of resolution. This will be followed by endoscope-based probes for *in vivo* virtual biopsies, and ultimately truly 3D non-invasive *in vivo* 3D imaging of an intact organism. We may be only a decade or two away from true “tricorder” technology.

Further Reading:

1. “Biophysics: An Introduction”, Cotterill, R., John Wiley and Sons, 2005, pp.93-98.

2. Badea, A., and Johnson, G.A. Magnetic Resonance Microscopy, in “Biophotonics in Pathology”, Cohen, S., editor, 2013, IOS Press, pp.153-184.

3. Foster, P.J. et al. Cellular Magnetic Resonance Imaging: *In vivo* imaging of melanoma cells in Lymph nodes of mice. Neoplasia, 10: 207-216,2007.

4. Doerr, A.N. Diamonds for MRI, Nature Methods, 12: 176-8, 2015.

5. Glenn, D.R. et al, Single Cell Magnetic imaging using a Quantum Diamond Microscope. Nature/Methods, 12: 736-738,2015.

6. Lovchinsky, A.O. et al, Nuclear magnetic resonance detection and spectroscopy of single proteins using quantum logic. Science, 351, 836-841, 2016.