Abstract

Various analogs of NMR and MRI are now technically possible in optics; specifically, high-resolution laser-pulse shaping and complex pulse sequence generation with well-defined phase shifts has been demonstrated. Here we summarize this technology and discuss the potential for these methods to enhance optical functional imaging, competing with (and surpassing?) what is possible by functional MRI. © 2003 Elsevier Inc. All rights reserved.

1. Introduction

Many of the spectacular achievements of twentieth century science followed the same simple paradigm. As new directions in basic atomic or molecular physics matured, they were adopted by chemists and applied physicists; this work in turn enabled applications in biologic, clinical, and environmental science. For example, 50 years ago, magnetic resonance was at the forefront of esoteric physics research; measuring nuclear spin properties had no conceivable application. Today, NMR is the single most important spectroscopic method for chemists, anatomic MRI is a mainstream diagnostic tool, and functional magnetic resonance imaging (fMRI; literally watching people think) promises to revolutionize neuroscience and neurology.

Coherent spectroscopy—the use of radiation fields with well defined amplitude and phase modulation to extract information about atoms and molecules—began around 1950 with demonstration of the spin echo in nuclear magnetic resonance [1a,1b]. Generation of complex pulse sequences of precisely shaped (phase and amplitude modulated) pulses, with precisely controlled interpulse phases, coupled with phase-sensitive detection of the spin evolution, were absolutely critical to the development of modern NMR applications. In addition, the simple theoretical framework embodied at first by the Bloch equations, and later by the density matrix formalism, made it possible to predict what classes of pulse sequences would prove useful.

For many years, magnetic resonance could have been described as the only coherent spectroscopy. However, in 1956, Feynman, Vernon and Hellwarth published a landmark paper [2] pointing out that all two-level systems are mathematically identical, and that if coherent light fields were ever created, it would be possible to use these same methods on optical transitions. Not surprisingly, then, the invention of the laser in 1960 was followed quickly by demonstration of the “photon echo” [3]—the optical version of the spin echo. In the ensuing decades, literally hundreds of different NMR pulse sequences have been developed and are now in routine use by chemists to unravel molecular structure. In contrast, the inherently fast (subpicosecond) relaxation and dynamics in electronic transitions made it impossible until very recently to do experiments with the same level of pulse sequence sophistication that was routine decades ago in magnetic resonance (where relaxation times are usually seconds).

The past decade has seen revolutions in our ability to manufacture and control optical fields. This is now leading to optical experiments that are more easily understood by NMR spectroscopists than by traditional laser spectroscopists! For example, we recently published the first experimental demonstration of two-dimensional optical spectroscopy with collinear pulses and well-defined phase shift between pulses, and even showed that phase cycling could...
be used to select specific coherence transfer pathways [4].

Fig. 1 looks like an NMR two-dimensional spectrum, but it is not: the range of frequencies on each axis is 20 Terahertz, and the peaks in color are the echo peaks, which are the only ones to survive a specific 16-step phase cycle. Femtosecond laser pulse shaping has also come of age: thousand-point approximations to an arbitrary waveform, with pulse peak powers of hundreds of megawatts [5a,5b,6], are now possible.

Our goal in this short article is to explain what analogs of NMR and MRI are now technically possible in optics, and to specifically provide a perspective on the prospects for optical experiments to compete with what is possible by functional MRI.

2. Optical imaging of tissue: an overview

Absorption [7], reflectance [8], Raman [9], fluorescence [10a,10b,11], fluorescence lifetime [12,13], and backscat-
tering [14a,14b] measurements have been shown to be useful in a wide variety of clinical applications where the target is near a surface (even an internal surface such as the esophagus [15]. For example, fluorescence microscopy readily locates metabolic co-factors (NAD(P)H, FAD), structural proteins (collagen or elastin crosslinks), and aromatic amino acids. Because of its inherently high sensitivity, fluorescence has traditionally been the technique of choice for optical detection of trace-level analytes (at the femtomole level or lower). For high-quantum-yield fluorophores, the effective fluorescence cross-sections can be as high as $10^{-16}$ cm$^2$/molecule. However, the exciting UV light typically penetrates only a few microns around 300 nm (to excite tryptophan or tyrosine), or a few tens of microns in the near-UV. The most common solution is to switch to chromophores which can be added (dyes) or expressed (green fluorescent protein and its variants [16], and can be excited at visible wavelengths, but this is not always possible in live tissue.

Of course, people are opaque, which means visible light does not penetrate. Greater penetration depths can be achieved in the near-infrared (NIR, Fig. 2). Near-IR imaging (ca. 800 nm) can detect oxygenation state with very modest energies [17,18] ($\ll 1$ mW) because of the low tissue absorption coefficient and differences between hemoglobin and deoxyhemoglobin in this spectral region [19]. Detection through 5 cm of tissue with modest powers (less than the power of a laser pointer), even through the skull, is easily achieved, both with pulsed lasers and with diode arrays [17-19]. Thus, the same factors which generate the fMRI signal (blood oxygenation and blood volume changes) can be detected by near-infrared absorption and transmis-

Optical deep-tissue imaging has obvious potential advantages over other imaging methods; for example, a skull cap is much less restrictive than an MR magnet bore and far less expensive, and near-IR lasers can be focused to 10-μm spots, comparable to single neurons in size. Even the power of a typical laser pointer (5 mW) corresponds to over $10^{16}$ photons per second, so photon-counting methods should be able to achieve extremely high temporal resolution. Unfortunately, the best functional imaging performances reported to date (4 mm spatial resolution, 150 ms temporal resolution) [18] hardly correspond to “microscopy”—and those results represent “hero experiments” rather than routine achievements (as would be the case with fMRI). The fundamental problem is light scattering. At 800 nm, photons are typically scattered 30-50 times before they are absorbed, leading to a characteristic “banana-shaped” range of sampled optical paths. In fact, the transmitters are not even “pointed at” the receivers; there is no advantage to doing this, or to focusing the transmitters. Ultrafast pulses and time gating can be used to partially eliminate this problem, since the first photons to go through the tissue (“ballistic photons”) are the unscattered ones [20]. However, ballistic photons are generally overwhelmed by scattered light, and short pulses are temporally broadened through scattering (detected light arrives for over 100 ps.) [21] Scattering decreases at longer wavelengths (approximately as $\lambda^{-4}$), but absorption increases dramatically as shown in Fig. 2. [22] and the “water windows” around 1.06 μm and 1.3 μm have relatively little endogenous contrast.

Over the last decade, two-photon fluorescence microscopy has evolved as a partial solution to these problems. Molecules can absorb two photons in the near-IR (e.g., at 800 nm) to access states at energies that would require UV photons for direct absorption [23a–23d] (and would only penetrate a few tens of microns). In addition, since the signal is proportional to the square of the intensity, scattered light induces minimal two-photon absorption. The absorption is detected by fluorescence, typically in the 500- to 600-nm wavelength range, although contrast agents which fluoresce at other wavelengths can be added. Because of these advantages, multiphoton imaging has evolved from a laboratory curiosity into commercial products with significant performance advantages over more traditional methods such as confocal microscopy (most notably in penetration depth, which is usually hundreds of microns). The current record is 1-mm depth imaging of stained vasculature and GFP-labeled neurons with exceedingly high amplified powers [24]. However, it still has some significant limitations. Only species that fluoresce are visible (thus, for example, this approach has not been used to measure hemoglobin oxygenation); the fluorescence still has to get out of the sample; and unwanted higher-order multiphoton processes commonly cause cell damage.
3. Exploiting lessons from magnetic resonance: high resolution optical pulse shaping and optical pulse sequence generation

In magnetic resonance, pulse sequences always start out as pieces chopped out of a continuous sine wave. This same approach is possible in optics—Warren and coworkers did such experiments nearly two decades ago [25a,25b]—but the method is not very general, partly because continuous lasers are restricted to relatively low powers (1 Watt might be typical), and partly because optical modulators are “slow” (tens of picoseconds or longer) compared to subpicosecond relaxation times of condensed phase samples such as tissue. The speed and power issues are solved by pulsed lasers: for example, commercially available titanium-doped sapphire amplifiers typically produce 1-3 mJ pulses centered between 775 and 810 nm, with pulse durations of approximately 100 fs (peak power \( \approx 10^{10} \) W), at a repetition rate of 1-3 kHz. The modern revolution in solid-state laser technology has made it possible to produce “ultrafast” lasers (the record is now less than 1 fs) with high powers at virtually any interesting wavelength from the deep-UV to the mid-infrared.

The delay between firings (1/repetition rate) of even the fastest amplified lasers is much longer than \( T_2 \), so in practice a single femtosecond pulse is used as the input to create a pulse sequence. In the traditional approach, this was done by splitting a single pulse into multiple replicas, sending them down different delay lines (300 microns of delay in air corresponds to 1 ps), and recombining them. Unfortunately, this process makes it exceedingly difficult to control and stabilize the relative phase of the recombined pulses, since the 1 ps delay illustrated above is approximately 500 optical cycles in the visible. In fact, in most optical experiments, the pulse are forced to propagate noncollinearly to avoid issues with relative phase; in this case, the relative phase between two pulses depends on position in the sample. Here we focus instead on the direct analog of NMR: collinear pulses with well-defined and controlled phase shifts.

Sequences of collinear, phase-controlled pulses are manufactured from a single laser pulse by spectral pulse shaping [26,27]—the laser pulse spectrum is spatially dispersed with a grating, the individual color components are separately modulated, and then the spectrum is recombined with another grating. In other words, we calculate the Fourier transform of the pulse sequence we want, and then encode the appropriate phase and amplitude modulation on the spectrum of the input laser pulse. The time-bandwidth uncertainty principle implies that 100 fs pulses have approximately 7-nm bandwidth; thus, the shaped pulses can have the same bandwidth, and will by necessity be longer than the original pulse. Fig. 3 shows a method invented by Warren’s group that has an intriguing technological similarity to magnetic resonance technology: the key technology is the use of an acousto-optic modulator (AOM) to control the shape of high power femtosecond laser pulses using shaped, modest power (1 W peak) microsecond radiofrequency pulses. An ultrafast laser pulse is bounced off a diffraction grating, then collimated with a lens to spatially disperse the different colors in the laser pulse. The pulse...
then passes through an acousto-optic modulator. A shaped rf pulse drives the AOM’s transducer, which creates an acoustic wave traveling through the crystal and diffracts a portion of the beam. The undiffracted beam passes out of the system, and the spectrum recombines in the grating. This approach has advantages of rapid update rates (MHz), commercially available components, simplicity (phase and amplitude modulation is achieved with a single RF pulse), and extremely high resolution. For example, the commercially available TeO$_2$ AOM used in our 800 nm work has a 40 mm clear aperture and 7 ns rf rise time. This rise time translates into spatial variations of the acoustic grating in 30 $\mu$m, so a waveform with (40 mm/30 $\mu$m) $\approx$1300 independently adjustable amplitudes and phases is possible.

Complex shaped pulses are possible and sometimes useful. For example, we have manufactured amplified laser pulses with modulation $e^{i\phi} \times \text{sech}(at)$ [1+2i] for adiabatic molecular excitation [28]; this is the exact same waveform commonly used in MRI experiments to give clean $\pi$ pulses from a surface coil or other inhomogeneous rf field. There have been many other applications in fields such as high-speed telecommunications and coherent control of chemical reactions [26]. We have also designed novel laser systems that give tunable, amplified, shaped pulses through the visible [29] and the near-infrared, with peak powers as high as $10^8$W and bandwidth as high as 100 nm.

Much of our work has focused on applications that have some similarity to magnetic resonance experiments. For example, it is possible to detect the (rapidly oscillating) “optical free induction decay” induced by a fs laser pulse, if a second pulse with a well-defined phase shift is used to probe this polarization [30] (Fig. 4), and as shown in Fig. 1, optical two-dimensional experiments are possible as well.

4. Combining nonlinearity with pulse shape control to improve optical imaging

As noted above, two-photon excitation discriminates strongly against scattered light, because the probability of excitation is proportional to the square of the intensity. Of course, both single-photon absorption and scattering decrease the total power in the laser beam, and whichever one dominates determines the maximum penetration depth. For the vast majority of two-photon studies to date, scattering causes the coherence to disappear before absorption sets in and limits the penetration depth. Thus, the longer wavelength water windows (around 1.1 microns and 1.3 microns respectively) are very attractive for reduced scattering; however, there is virtually no endogenous fluorescence after two-photon excitation at such wavelengths.

Detection of nonlinear processes in the form of two-photon absorption would open up new spectral windows for deep tissue penetration. For example, two-photon absorption from 1.3 microns would excite directly into the hemoglobin band (Fig. 2) and would have significant oxygenation contrast; the lower scattering would also permit penetration depths about a factor of (1.3/0.8) [4] $\approx$7 greater than 800 nm excitation with the same power. The fundamental difficulty is that far fewer photons are lost from the beam by two-photon absorption than by scattering or other linear effects, thus the detection is exceedingly difficult. There is thus a tremendous dynamic range issue. Of course, MRI also has a tremendous dynamic range in principle, but in practice it is solved by the diode, which can be strategically employed to significantly reduce the coupling from the transmitter to the receiver. There is no optical equivalent of a diode (which would also be very useful, for example, for making laser safety glasses that protect the eyes while allowing weak signals to penetrate). Traditional approaches to two-photon absorption thus involve very high laser powers, which would be unacceptable in tissue imaging applications.

We have demonstrated that low-power pulses can be used to measure the very onset of two-photon absorption, if we use the wavelength or frequency structure of the optical pulses (or pulse trains) to detect nonlinear processes with high sensitivity. Fig. 5 shows one example [31]. A mode-locked laser produces a high-stability pulse train with well-defined frequency components in the power output. If relatively slow amplitude modulation is also imposed, two-photon absorption creates sidebands in the transmitted light that would not be generated by linear processes. These sidebands permit very sensitive detection of the small nonlinear signals.

Reference [31] showed detection of the two-photon absorption cross-section using much less than 1% of the pulse.
energy needed by other methods. It also showed the fundamental detection limits, dictated by laser shot noise (Fig. 6), are very favorable. For example, for 1 Hz noise bandwidth (1 s acquisition time) TPA of $10^{-7}$ of the input energy can be detected with pulse energies of only a few picojoules. Also, while reference [31] focused on measurements of transmitted light, scattered light would work just as well (as long as the scattering takes place after the region where TPA is induced).

A second, complementary approach is to manufacture shaped laser pulses to reduce or eliminate the shot noise. This takes advantage of the fact that nonlinear processes can create new frequency components in a laser pulse, but linear processes (such as scattering or absorption) cannot do this. Hence it is possible to combine shaped-pulse excitation with wavelength resolved detection to essentially eliminate the resonant laser background light. We have demonstrated this at Princeton on test samples (dye solutions).

As a simple illustration of this effect, consider a laser pulse with a "hole" in the center of its spectrum (Fig. 7, top left). By the definition of the Fourier transform, such a pulse has zero area in the time domain—that is to say, it consists of a short and intense pulse on top of a weak, negative going, longer pulse. If the pulse bandwidth is less than the width of the two-photon spectrum, two-photon absorption is proportional to the square of the intensity, hence the intense pulse

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**Fig. 5.** Using a laser with repetitive pulse spacing and relatively slow amplitude modulation, two-photon absorption creates sidebands in the transmitted light that would not be generated by linear processes. These sidebands permit very sensitive detection of the small nonlinear signals. Adapted from Ref. [31].

**Fig. 6.** Limits of detection of TPA using the technique in Fig. 8. The black lines represent the signal intensity from the shot noise limit; the blue lines represent the signal from molecular absorption under a variety of assumptions. Note that two-photon absorption of about $10^{-7}$ can be detected with only a few pJ pulse energy. Adapted from Ref. [31].
is attenuated more than is the weak pulse. The consequence of this is that after two-photon absorption, the area is no longer zero, and the hole tends to refill. This means that frequency resolved detection can find the weak TPA signal on top of a very low background.

Preliminary results show that realistic test samples do refill spectral holes, although the picture in Fig. 7 is oversimplified (Fig. 8). In this experiment, we observed refilling of a 10 nm wide spectral hole, and the refilling varied with sample content (even though none of the components in the sample absorbs near this wavelength). The pulse energy was well under 1 μJ, and no sample damage was observed. This refilling must originate in nonlinear processes, including TPA and self-phase modulation. However, this preliminary result shows complex dynamics that are not included in the naïve argument above. We find similar results at longer wavelengths. Fig. 9 shows pulse shape modification from pulses centered around 1.1 microns, and shows major changes when DCM (which absorbs two photons at that wavelength) is added. Finally, we note that the amount of spectral refilling is sensitive to the pulse shape, including the phase modulation.

5. Summary

Today, functional magnetic resonance imaging gives better spatial resolution than optical techniques, but a strong case can be made that this situation will be changing in the near future. Coherent techniques in optical spectroscopy are just now beginning to come of age, spurred on by novel technologies for femtosecond lasers, femtosecond-shaped pulse generation, and collinear pulse sequence generation. For example, two-photon absorption creates new frequency components, and that the absorption depends on pulse shape. In large molecules virtually all one-photon allowed electronic transitions are also two-photon allowed, so the known linear tissue absorption spectra very likely provide excellent guidance.
The advantage of longer wavelengths is greater penetration. Two-photon microscopy with 800-nm lasers is limited to penetration depths of about 400 μm, limited by scattering. The longer-wavelength water windows (1.05 μm and 1.3 μm) have reduced scattering, and TPA is possible at both wavelengths; for example, TPA at 1.3 μm is dominated by hemoglobin. At 1.3 microns, typical scattering lengths and absorption lengths will both be 3-5 mm, and thus much greater penetration is possible. However, there is little endogenous fluorescence associated with these wavelengths, which has restricted their clinical utility. Thus the development of direct detection of TPA will open new spectral windows for deep tissue imaging. The experiments in Fig. 9 are a first step in this direction—the sample thickness was 5 mm.

This method holds out the prospect, in the foreseeable future, for optical brain imaging with sufficient penetration depth for functional imaging, and with scattering effects suppressed (thus permitting micron scale resolution). High repetition rate laser pulse trains can investigate many different spots per second, with x and y position determined by beam steering and z position determined by focusing. The “holy grail” would be imaging of a very large number of neurons simultaneously, with temporal resolution sufficient to resolve electrical signals, and there seems to be no physical limitation to preclude this.

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References