10.1117/2.1201308.004937

Focusing light into tissue

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A novel technique uses ultrasonic encoding and time reversal to break the diffusion limit and enable optical imaging, manipulation, and therapy at greater depths.

A variety of applications that use light to excite or trigger specific biological events require light focusing or delivery deep into tissue. In medicine for instance, light can be used to image inside the body (e.g., fluorescence tomography), for non-invasive measurement of blood-hemoglobin's oxygen content (oximetry), to control neurons (optogenetics), stimulate nerves, or for photodynamic therapy or photothermal therapy. However, within ~1mm in human skin, multiple scattering of light randomizes the light propagation directions, preventing direct light focusing beyond this depth. This makes it difficult to deliver light to specific internal sites.

Various approaches have been explored to overcome the depth limit. Optical clearing[1] artificially reduces the scattering by introducing agents that match the refractive indices of the scatterers with the background. However, it also alters the optical and physiological properties of the medium. Optical phase conjugation (OPC) reverses the propagation direction and phase variation of light, so that can exactly retrace its path through a scattering (i.e., turbid or foggy) medium.[2] However, its usefulness is limited to focusing light through, not inside, a diffuse medium. Finally, wavefront shaping can focus light inside[3] or through[4] a scattering medium, but its feedback mechanism relies on the presence of a guide star—a luminous point—for the incident light to focus on. This limits the technology where arbitrary, dynamic, or real-time light focusing is desired.

To overcome these restrictions, we extended the guide star concept, and invented a method we call time-reversed ultrasonically encoded (TRUE) optical focusing.[5] It uses ultrasonic modulation and OPC of multiply-scattered coherent light to dynamically focus light into a scattering medium in a two-pass configuration. The signal is modulated by ultrasound waves, which are not significantly scattered in soft tissues and can be focused at significant depths. The diffuse light gets strongly modulated at the ultrasound focus in the tissue as it passes through. When it emerges from the other side of the sample, a phase-conjugate mirror (PCM) reverses the signal's propagation direction and phase variation so that it exactly retraces its path through the sample, propagating towards the ultrasonic focus.

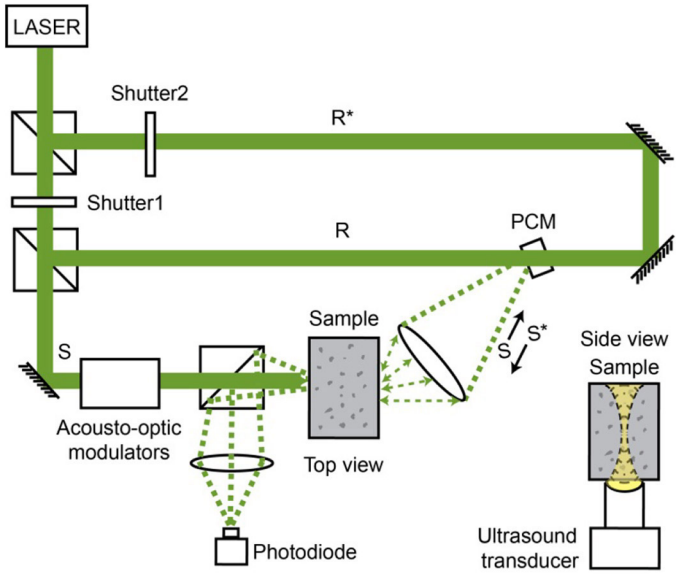

Figure 1. *The time-reversed ultrasonically encoded (TRUE) optical focusing setup. With shutter 1 open and shutter 2 closed, the wavefront of the sample beam S can be holographically recorded onto the phase-conjugate mirror (PCM) in the presence of the reference beam R. Then shutter 1 is closed and shutter 2 is opened, and the reconstruction beam R* illuminates the PCM to generate a phase-conjugated copy S* that converges to the virtual source. The photodiode detects S* after it transmits through the sample.*

Experimentally (see Figure 1), the light from a long-coherence length laser is split into a sample beam S and two mutually conjugated reference beams R and R* that counter-propagate and retrace each other's beam profile. S is spectrally tuned to $f_S = f_0 - f_a$ by acousto-optic modulators, before propagating diffusively through the medium (f_0 is the laser frequency and f_a the frequency shift). A focused ultrasonic wave of frequency f_a traverses the medium and modulates the diffused light. The ultrasonically modulated light, having frequencies $f_+ = f_0$ and $f_- = f_0 - 2f_a$, can be regarded as emanating from

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a virtual source, defined by the ultrasonic focus. Outside the medium, the diffused light is holographically recorded onto a PCM. The stationary hologram that is recorded results from the interference between R and S(f_+). Then, the hologram is read by R* to generate a time-reversed copy of S(f_+), denoted as S*(f_+). By reversibility, S*(f_+) back-traces the trajectory of S(f_+) and converges to its virtual source, thereby achieving optical focusing into the scattering medium.

Direct visualization of TRUE focusing is not possible when the focus dwells completely inside a turbid medium, and so we could not test our system directly. Instead, we tomographically imaged a 10mm-thick 'phantom slab' that mimics tissue and contained, at its midplane, three 1mm-wide blocks: two absorbing and one strongly scattering. Using TRUE to focus light at the midplane, we were able to image the three targets with submillimeter resolution. Moreover, we determined that if the light did indeed converge to the ultrasonic focus, then the theoretical resolution should be $1/\sqrt{2}$ of the ultrasound focal width, a result of the light passing through the virtual source twice.

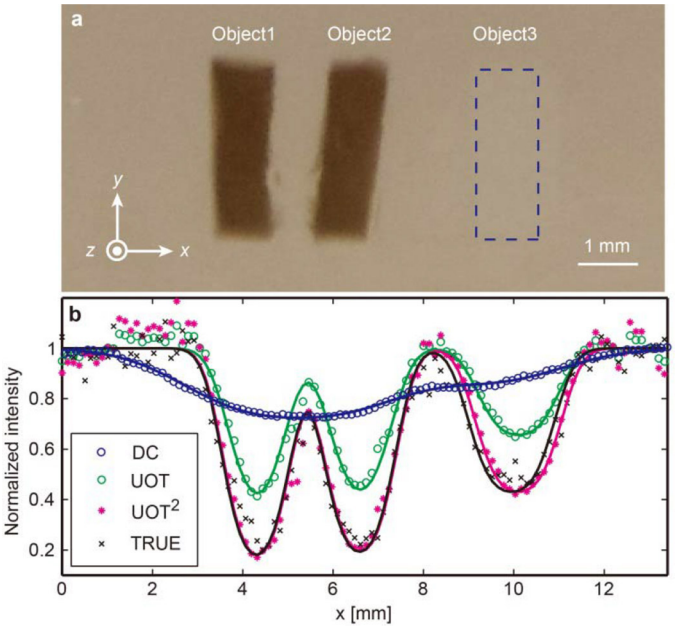

Figure 2. *Results from imaging experiments validating TRUE focusing. (a) Photograph of the imaged sample dissected at the midplane containing one scattering and two absorbing objects. (b) Comparison of the normalized transillumination (DC), ultrasound-encoded optical tomography (UOT) and TRUE images. As light is focused to the virtual source, the TRUE signal is proportional to the square of the UOT signal. The full widths at half-maximum of the point-spread functions were 0.89mm (UOT) and 0.63mm (TRUE), whose ratio is 1.4 ($\approx \sqrt{2}$).*

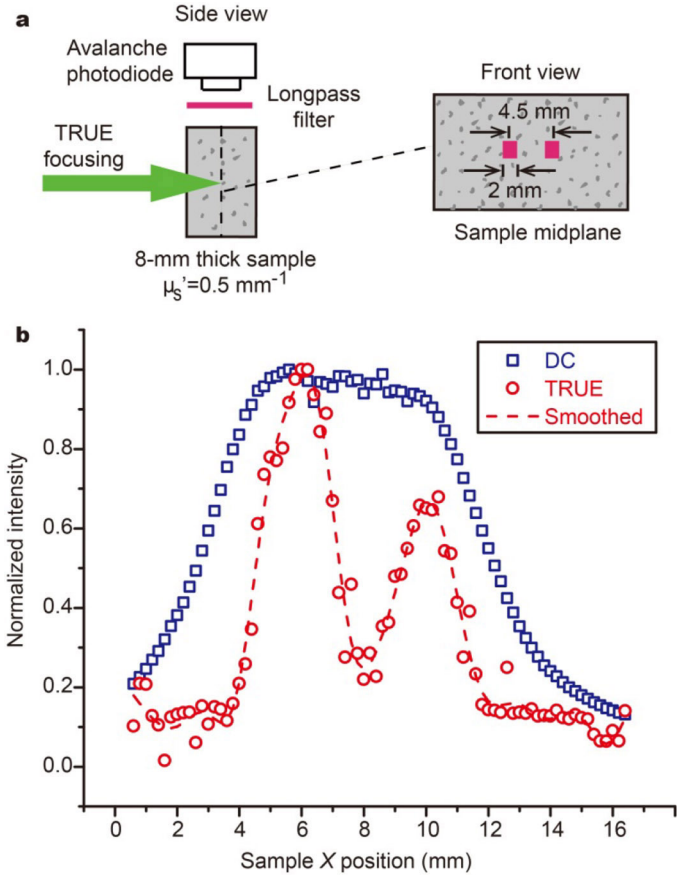

Figure 3. *Fluorescence imaging with TRUE focusing. (a) Imaging setup. Two fluorescent objects were embedded in the midplane of a thick turbid layer. (b) Comparison of fluorescence images excited by DC and TRUE-focused light.*

We confirmed this prediction by comparing the TRUE image with that acquired directly from the ultrasonically encoded light S(f_+), a technique known as ultrasound-encoded optical tomography (UOT). In UOT, light passes through the virtual source just once, and the imaging resolution is determined by the ultrasound focal width. Therefore, in theory, the TRUE signal should be roughly the square of the UOT signal. Experimental data also supports the square law: see Figure 2.

An immediate application of TRUE focusing is fluorescence imaging at greater tissue depth.[6] Figure 3 shows tomographic images of an 8mm-thick turbid layer with a fluorescence-labeled structure at its midplane. With conventional fluorescence imaging, diffusion of the excitation light results in poor imaging resolution and the two dyed objects cannot be distinguished.

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In contrast, TRUE can focus the excitation light at depths greater than the diffusion limit, and elucidate the fine structure that is otherwise inaccessible.

As a diffuse optical method, TRUE is not very sensitive to the optical thickness of a turbid medium, and could potentially reach focal depths beyond ten centimeters in scattering-dominant media. A variety of applications that require light focusing or delivery deep into tissue, e.g., fluorescence tomography, oximetry, optogenetics, nerve stimulation, photodynamic therapy, and photothermal therapy, can benefit from TRUE focusing. We are now working to improve the speed, resolution, and time reversal gain of the technique.

This work was partly supported by National Academies Keck Futures Initiative grant IS 13 and National Institutes of Health grants DP1 and EB016986 (NIH Director's Pioneer Award).

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