Advances in human brain imaging technologies are critical to understanding how the brain works and the diagnosis of brain disorders. Existing technologies have different drawbacks, and the human skull poses a great challenge for pure optical and ultrasound imaging technologies. Here we demonstrate the feasibility of using ultrasound-modulated optical tomography, a hybrid technology that combines both light and sound, to image through human skulls. Single-shot off-axis holography was used to measure the field of the ultrasonically tagged light. This Letter paves the way for imaging the brain noninvasively through the skull, with optical contrast and a higher spatial resolution than that of diffuse optical tomography.

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Imaging through highly scattering human skulls with ultrasound-modulated optical tomography

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Advances in human brain imaging technologies are critical to understanding how the brain works and the diagnosis of brain diseases. Magnetic resonance imaging (MRI) is the most widely used approach in functional brain imaging. However, the equipment is costly and non-portable and it cannot image subjects with implanted metal or electronic devices (for example, pacemakers). Positron emission tomography is also costly and non-portable; more importantly, it uses ionizing radiation. Pure optical imaging technologies do not have the aforementioned drawbacks, but they suffer from the poor spatial resolution caused by the scattering of light in biological tissue [1,2]. Compared to photons, ultrasound is much less scattered in tissue, but when it is used for brain imaging, ultrasound is attenuated and distorted by the round-trip propagation through the skull. Therefore, ultrasonography is suited for use in pediatric brain imaging before fontanelles close. However, its use in adult human brain imaging remains challenging. In addition, the primary contrast mechanism in ultrasound imaging is acoustic impedance contrast. Therefore, it cannot be used to collect biochemical information, and the low acoustic impedance contrast of soft tissue further limits the specificity.

Hence, hybrid technologies that use both light and sound such as photoacoustic tomography (PAT) [3] and ultrasound-modulated optical tomography (UOT) [4–6], have been regarded as potentially promising modalities for adult human brain imaging. They combine the advantage of functional molecular (e.g., hemoglobin) contrast provided by optical imaging with the advantage of minimal tissue scattering by ultrasound. In addition, the ultrasonic wave transmits through the skull only once in these methods as opposed to twice in ultrasound imaging. Therefore, the signal should be less attenuated and distorted than the echo signal in ultrasonography.

While PAT through ex vivo monkey and human skulls has been demonstrated [7,8], UOT (or acousto-optic imaging) through human skulls has not been reported. In UOT, light passing through an ultrasonic focus undergoes a frequency shift by multiples of the ultrasonic frequency [9]. By detecting the frequency-shifted light (i.e., ultrasonically tagged light) as a function of the ultrasonic focus position, ultrasound-defined resolution can be achieved in UOT [10–16].

As UOT is fundamentally different from PAT in the way ultrasound and light interacts, the impact of measurement parameters on the detectable signal level and resolution can differ for the two methods. Unlike PAT, which is sensitive mainly to the optical absorption property of tissue, UOT is sensitive to both the optical absorption and scattering properties [17]. Therefore, the UOT signal or signal change can potentially monitor a larger range of physiological changes in the human brain. Specifically, UOT can potentially measure blood oxygenation changes in the brain by using the spectral differences between oxygenated and deoxygenated blood (absorption contrast). UOT can also potentially measure blood perfusion changes by honing in on the scattering changes as the relative volume of the blood flowing through a brain region changes (scattering contrast). Additionally, a numerical simulation study has shown that one type of UOT, based on spectral hole burning (SHB), has the potential to achieve a larger imaging depth in human bodies compared with PAT [18]. One caveat is that SHB experiments require expensive, non-portable, and cumbersome equipment such as cryostats.

In this Letter, we demonstrate for the first time, to the best of our knowledge, imaging through highly scattering human skulls with UOT. Our method does not require cryogenic cooling and has the potential to be implemented as a head-mountable device. We used a single-shot off-axis holography method [19] to detect the UOT signal which, to the best of our knowledge, has not been demonstrated in the UOT field. The large pixel count of a camera enables parallel detection of multiple speckle grains within a single shot, which is crucial for this type of
application where the signal-to-noise ratio (SNR) is low, and the speckle decorrelates rapidly due to physiological motions such as blood flow [20–22]. In previous work [23], multiple frames were recorded to reconstruct the UOT signal, making the method vulnerable to the rapid speckle decorrelation in living tissue. Single-frame UOT measurements have previously been reported [24–27]. However, some methods require special lock-in cameras in which each pixel is an analog lock-in detector [25–28]. Although off-axis holography has been used in heterodyne holography-based UOT, multiple frames were required to filter out the local oscillator beam [29].

In our experiments, we first evaluated the extent by which a human skull distorts the ultrasonic field. A hydrophone (HNR-0500, ONDA) was used to measure the ultrasonic field distribution on the focal plane of a spherically focused single-element ultrasonic transducer (A303S, Olympus; central frequency = 1 MHz, focal length = 15.2 mm, element diameter = 12.7 mm) with and without the presence of a human skull (3–5 mm thick, human parietal bone, SHN-46, Skulls Unlimited International Inc.). Figure 1(a) shows a photo of the setup, and Fig. 1(b) shows the normalized ultrasonic field distribution. The full width at half-maximum (FWHM) focal spot size was measured to be 3 mm when the skull was absent. When the skull was present between the transducer and the hydrophone, depending on the location of the ultrasonic transducer relative to the skull, the FWHM focal spot size varied from 3 mm to 6 mm, and the ultrasonic pressure at the focus was attenuated to 10–20%. Although the focus was distorted and broadened, we observed that it was still achievable through the human skull for 1 MHz ultrasound—a result that is consistent with previous literature findings [30].

Next, we built a camera-based UOT system (schematically shown in Fig. 2) to demonstrate the feasibility of imaging an absorptive object buried between two pieces of highly scattering human skull. The output of a continuous-wave (cw) laser (671 nm, MSL-FN-671-S, CNI Optoelectronics Tech Co.; ~35 mW on the sample) passed through an optical isolator, a variable attenuator composed of a half-wave plate (HWP1), and a polarizing beam splitter (PBS1), before it was split into a reference beam (R) and a sample beam (S) by a polarizing beam splitter (PBS2). After passing through a neutral density (ND) filter, the reference beam was expanded to a diameter of 1” by two lenses (L1 and L2), and reflected by a 90:10 (TR) non-polarizing beam splitter (BS) before it illuminated a camera sensor. The sample beam passed through a half-wave plate (HWP3), two acousto-optic modulators (AOM1 and AOM2, which shifted the frequency of the sample beam by 50 MHz and −49 MHz sequentially), and a beam expander, before it illuminated the human skulls. An ultrasonic transducer focused 1 MHz ultrasound through the skull, and the focal pressure amplitude is ~0.34 MPa. A portion of the light passing through the ultrasonic focus was tagged by the ultrasound, and its frequency was shifted to the same frequency as the reference beam. The ultrasonically tagged light and untagged light passed through an absorptive object (a strip of 2 mm wide and 3 cm long black tape, transmittance <0.1%, attached on the skull), a second human skull (1.5 cm away from the first skull), a 4f system (with an iris in the pupil plane to adjust the speckle size), a polarizer, and the BS before they interfered with the reference beam (~45° with respect to the x and z axes) and detected by a camera (pco.edge 5.5, PCO-TECH; global shutter, 2560 × 2160 pixels, 6.5 μm pixel size). Because the ultrasonically tagged light had the same frequency as that of the reference beam, its interference pattern was stable on the camera. In contrast, the interference pattern formed by the untagged light and the reference beam was a 1 MHz beat, and thus was averaged out during the camera exposure time (5 ms).

To reconstruct the field of the ultrasonically tagged light, we first took the two-dimensional (2D) Fourier transform of the recorded interferogram. An example of the resulting spectrum is shown in Fig. 3(a). The two faint circles along the 45° diagonal are the spectra corresponding to the interference pattern...
In our experiment, we used a single-element transducer to generate an ultrasonic focus through the human skull. Better focusing quality (e.g., smaller focal spot and higher focus to beam widths at out-of-focus planes are larger than that at the focal plane). Therefore, it can achieve a higher spatial resolution than that of diffuse optical tomography [20]. Currently, the resolution along the acoustic axis direction is poor, because we used a cw laser (and thus a long burst of ultrasound) in this experiment. The axial resolution can be improved by using a pulsed laser and a single cycle of ultrasonic pulse [31]. This can also improve the lateral resolution and image contrast, because the tagging volume is much bigger in the cw case, and the beam widths at out-of-focus planes are larger than that at the focal plane.

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The expression of the SNR can be derived and written as:

\[ SNR = \frac{N_{\text{p}} N_{\text{T}}}{\sqrt{\text{Var}(N_{\text{T}}) + 1}}, \]

where \( N_{\text{T}} \) is the number of photons per pixel, \( N_{\text{p}} \) is the pixel count of the camera. In deriving Eq. (2), we assumed that the number of reference beam photons per pixel equals the average number of tagged photons per pixel, and both numbers are much larger than \( N_{\text{T}} \), which is commonly achieved in experiments; we also assumed a quantum efficiency of 1. and a rectangular iris is employed. Assuming \( N_{\text{p}} = 10^8 \), Eq. (2) shows that the SNR is above one as long as \( N_{\text{T}} > 1/\sqrt{N_{\text{p}}} = 10^{-3} \).

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