## **Iterative Time-Reversed Ultrasonically Encoded Light Focusing in Backscattering Mode**

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## **System setup**

A full schematic diagram of the iTRUE system is shown in Supp. Fig. 1. The iTRUE system consists of four major modules: a light source regulation module, a DOPC module, a sample operation module, and a quality assurance module. In the light source regulation module, a pulsed laser beam is split into a reference beam and probe beam. The reference beam is frequency-shifted so that matches frequency of the ultrasound modulated light. Both beams are polarized in the horizontal direction by adjusting two half-wave plates (WP3 and WP4), and spatially filtered by two polarization-maintaining single mode fibres. These two beams are then collimated and sent to the next stage: the DOPC system. The intensity of the probe beam is much stronger (a factor of  $\sim 10^4$ ) than the reference beam, as only a small portion of the back scattered probe beam is detected by the camera.

The DOPC module's camera is used to record the wavefront of light emerging from the target, and it creates an amplified phase conjugated wavefront using its SLM. The pixels of the camera and the SLM need to be precisely aligned so that the phase-conjugated copy of the measured wavefront can be precisely reproduced by the SLM. A plate beam splitter, rather than a cube beam splitter, is used to couple the camera and the SLM (surface reflection of the cube beam splitter is considerable with strong probe beam intensity). A beam compensator with the same thickness as the plate beam splitter is used to compensate for wavefront distortion caused by the finite thickness of the plate beam splitter. In order to maximize the intensity of the back scattered light reaching the camera, a beam splitter with 90% transmission is used to combine the reference beam and the back scattered light.



**Supplementary Figure 1 | Schematic diagram of iTRUE.** 

In the sample operation module, the lens L3 (Supp. Fig. 1 blue box) is placed approximately one focal length away from the ultrasound focus, which maximizes the light delivered to the sample and collected by the camera. The aperture AP2 at the back focal plane of the lens L3 sets the speckle size for the DOPC system.

The quality assurance module monitors the performance of the DOPC system, which is very sensitive to alignment. Typically, this module runs when system calibration is needed, e.g. before an iTRUE experiment. While running, a branch of the probe beam is directed to the sample via mirror M3 and beam splitter BS2. The wavefront of the transmitted light through the sample is measured by the DOPC system. In order to measure the wavefront, the AOM needs to be driven by an appropriate frequency so that the beating frequency between the reference beam and the probe beam are locked to the laser pulse train's frequency (e.g. the AOM is set to 50 MHz when laser pulse repetition rate is 20 kHz). If the DOPC system is well aligned, the phase conjugated light is able to trace its original path back to beam splitter BS2. Therefore a collimated beam can be obtained and creates a focus on the camera CAM2 for quality monitoring purpose.

## **Signal diagram**

A signal flow diagram of the iTRUE system is shown in Supp. Fig. 2. There are two clock sources in the iTRUE system. The first one is used to trigger the laser and ultrasound pulse as well as synchronise the AOM signal. This clock source, which is generated by a digital delay generator (DG), outputs a trigger signal at a repetition rate of 20 kHz for the laser (Supp. Fig. 2, signal I) and trigger signal for the ultrasound pulse. Each ultrasound pulse is phase-inversed from the preceding pulse (Supp. Fig. 2, signal II). An appropriate delay (41.7 µs in our experiment) is added to ensure each ultrasound pulse coincides with a laser pulse at the focus of the ultrasound transducer. The digital delay generator synchronises the function generator AFG2, which sends a sinusoidal signal (Supp. Fig. 2, signal III) to the AOM. This synchronisation guarantees phase-locking between the reference light and ultrasound modulated light. The second clock source is used to generate a timing sequence for the 4-phase stepping digital holography

setup. This timing sequence is generated by a computer-controlled digital acquisition (DAQ) board. The DAQ generates voltage-stepping signals (Supp. Fig. 2, signal IV) to modulate the phase of the AOM signals (Supp. Fig. 2, signal III). At the same time, the DAQ also triggers the camera with a synchronised pulse signal (Supp. Fig. 2, signal V).



**Supplementary Figure 2 | Signal flow of the iTRUE system.** Abbreviations: Digital delay generator (DG), Data acquisition board (DAQ), Arbitrary function generator (AFG), RF power amplifier (AMP), Ultrasound transducer (UST), Acousto-optic modulator (AOM).

In an iTRUE experiment, both the ultrasound modulated light and the unmodulated light are back scattered to the camera. In order to wash out the interference pattern due to the inference between the unmodulated and reference light, the frequency of the AOM signal needs to be carefully selected. With a 20 kHz pulsed laser, the beating frequency at its harmonic frequencies (20 kHz, 40 kHz, 60 kHz …) will be locked by the pulsed laser. Therefore, we cannot use 50 MHz for the AOM although the nominal

central frequencies of both the AOM and ultrasound transducer are 50 MHz. Instead, a 50.010 MHz sinusoidal signal is input to the AOM and the pulse signal for the ultrasound transducer is alternately inverted. This locks the ultrasound modulated light while the unmodulated light averages out resulting in a DC background. While running the quality assurance module, however, the AOM signal is set to 50 MHz, so that the beating signals between reference light and signal light is locked to the 20 kHz laser pulse train.