Supplementary

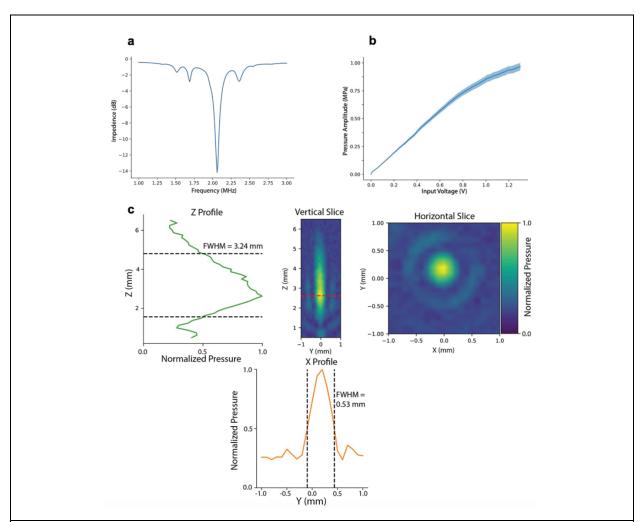


Fig. S1: Transducer characterization. a, Transducer heigh-mode resonant frequency at 2.1 MHz. **b,** Calibration of function generator driving voltage to pressure amplitude. **c,** 3D mapping of the transducer pressure profiles. The red dashed line in the vertical slice image denotes where in the focus the horizontal slice image was taken from and the location of the two-photon focal plane.

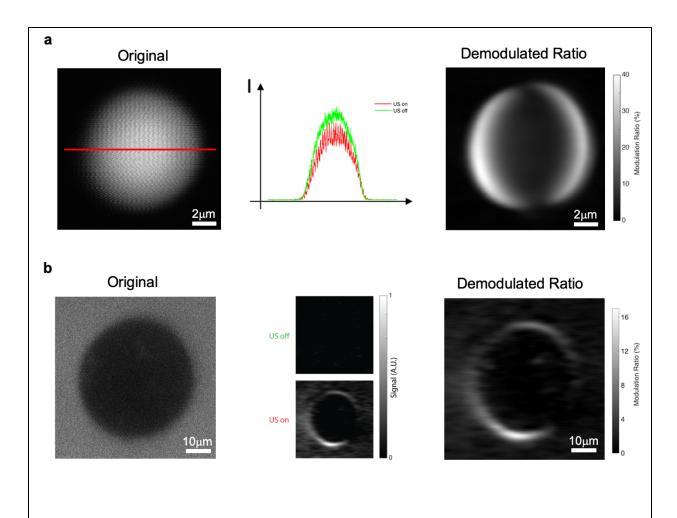


Fig. S2: Bead Demodulation Results. a, Left, a single original, raw frame of fluorescent bead under CW US modulation. The fringe pattern is noticeable across the bead surface. Middle, single modulated and unmodulated line profiles of the representative line in the original image. Right, an example demodulated ratio image, as calculated by dividing the filtered demodulated image by the original image (24 frames averaged). The enhancement of bead edges is evident in the image representation. **b, L**eft, single frame of a 45 μ m non-fluorescent polystyrene bead in fluorescein-laden agar undergoing AOM. Middle, he AOM signals are observed in demodulated images when the US is on. Right, a similar demodulated ratio image as in **a**. The edge enhancement effects are readily observed here as well.

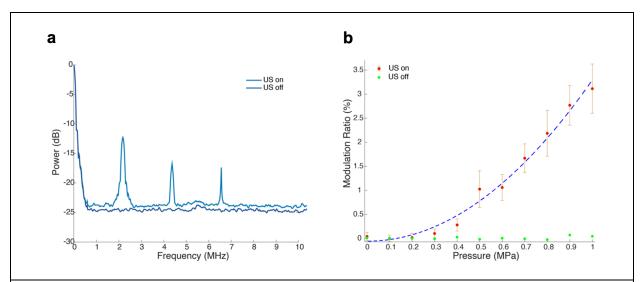


Fig. S3: AOM signal harmonics. a, Example power spectrum of an AOM fluorescent bead image. **b,** Second harmonic modulation ratio pressure dependence for 10 μ m fluorescent beads. Blue dashed line represents a quadratic fit. [The (linear) dependence of the first harmonic signal is characterized in Fig. 1d].

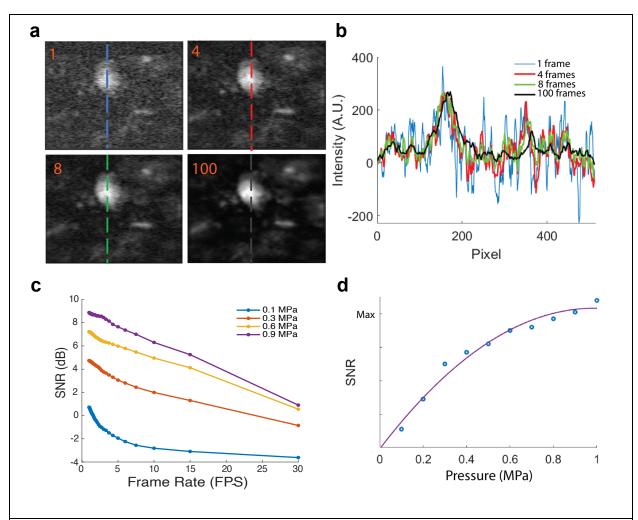


Fig. S4: Pressure and SNR. a, The averaged demodulated AOM frames of eGFP neurons over 1, 4, 8, and 100 frames. All images were taken at 1 MPa. **b,** Plotted signal traces for the lines depicted in **a. c,** SNR for different pressures by varying frame rate. **d,** Improvements in SNR by increasing the US pressure (100 frames averaged each).

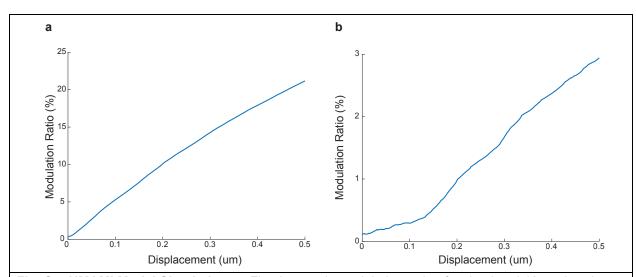


Fig. S5: UMAMI Model Simulation. a, First harmonic modulation ratio of a simulated 10 μ m bead using the demodulation procedure described the methods. **b,** Second harmonic modulation ratio of the same bead as in **a**.

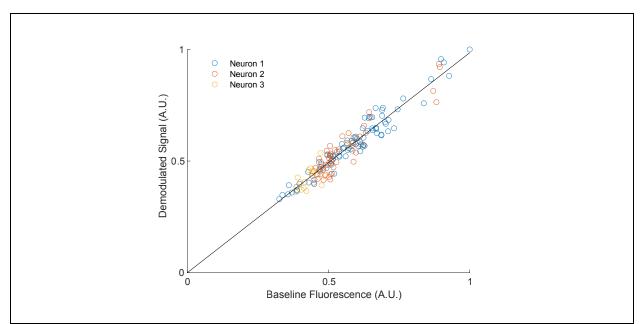


Fig. S6: Baseline and Demodulated GCaMP Signal Correlation. The fluorescent signals across three neurons showed strong correlation between the unmodulated baseline signals with demodulated signals. Pearson correlation coefficient, r =0.96.

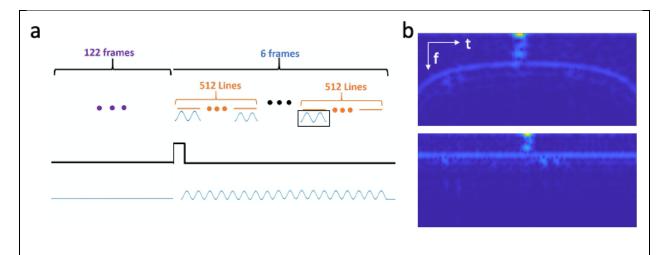


Fig. S7: Triggering and Resonant Scanner correction. a, Depiction of the US triggering scheme used in the experiments. Each raster scanned frame is comprised 1800 pixels, fast axis, by 512 pixels, slow axis. **b,** The effects of adaptive pixel binning turned on (top) and turned off (bottom). Distortions at the edges of the acquired signal are present when the pixel binning is on.