Ultrasound Imaging of Single Cells Using Acoustic Reporter Genes and BURST Reconstruction

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Background, Motivation and Objective

The recent development of acoustic reporter genes (ARGs) enables biomedical ultrasound to image gene expression in engineered cells [Bourdeau 2018, Nature]. ARGs encode air-filled protein nanostructures known as gas vesicles, or GVs, which function as physically stable contrast agents producing detectable ultrasound contrast in engineered bacterial cells at concentrations on the order of 10^8 cells/ml. However, many applications would benefit from the ability to detect smaller numbers of cells and resolve single cells in the imaging plane. To make this possible, we present Burst Ultrasound Reconstruction with Signal Templates (BURST), an imaging paradigm that exploits the ability of GVs to collapse under acoustic pressures above a genetically defined pressure threshold (**Fig. 1a**).

Statement of Contribution/Methods

By applying a step increase in pressure during a series of image frames, we obtain a time trace for each pixel, which we decompose into a linear combination of three template time traces corresponding to GVs, linear scatterers, and noise (**Fig. 1b**). This allows us to isolate the GV collapse signal by linear "spectral" unmixing. We implemented BURST on a programmable Verasonics Vantage ultrasound system with a 128-element linear array at 6 MHz using half-cycle pulses. To test the *in vivo* specificity and robustness of BURST, we imaged probiotic ARG *E. coli* Nissle cells in agarose gel within the colon of a mouse at 10^7 cells/ml. To validate single-cell detection, we imaged in degassed liquid buffer a range of concentrations of ARG *E. coli*, on the order of 10^2-10^3 cells/ml, as well as pre-collapsed controls. We detected individual point sources in each frame, which we counted and compared to ground truth estimates based on fluorescence microscopy. A similar approach was used to test the ability of BURST to detect individual GVs, with ground truth quantification performed using electron microscopy.

Results/Discussion

The superiority of BURST (**Fig. 1d**) over amplitude modulation (**Fig. 1c**) in visualizing ARG-expressing cells was clearly demonstrated *in vivo*, improving CTR from -20 dB to 10 dB. In *in vitro* experiments, the number of sources in suspensions of both cells and GVs closely tracked the expected number (**Fig. 1e-g**). These results demonstrate the ability of BURST imaging to detect single cells and protein nanostructures, representing a sensitivity improvement of more than 10^5-fold.

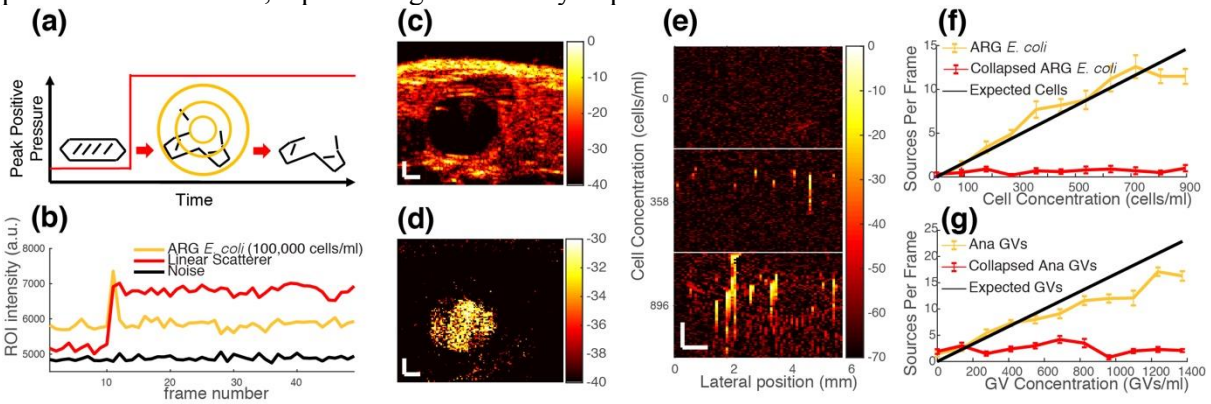

FIG. 1. The BURST imaging paradigm. **(a)** Illustration of GV collapse in response to high acoustic pressures. **(b)** Mean pixel intensity over successive ultrasound image frames for GV, scatterer, and noise regions. **(c)** AM ultrasound image at the moment of collapse of a mouse colon filled with *E. coli* expressing ARG (10 million cells/ml). **(d)** BURST image separates the GV collapse signal from the tissue background with high specificity. **(e)** BURST images showing point sources originating from single ARG-expressing *E. coli* in liquid buffer at different concentrations. **(f)** The number of point sources counted in BURST images of ARG-expressing *E. coli* in liquid buffer at varying concentrations. **(g)** The number of point sources counted in BURST images of Ana GVs in liquid buffer at varying concentrations. Error bars: SEM. N = 10. All images are dB scale. Scale bars: 1 mm