# High frequency nanobubble nonlinear scattering and array-based imaging in phantoms and *in vivo*

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## Background, Motivation and Objective

Nanobubble (NB) imaging has been reported at high concentrations (>$10^{10}$ $mL^{-1}$) via B-mode or contrast mode at low frequencies (3-12 MHz). However, none of these studies directly examined NB signatures, nor accomplished imaging in a systematic manner based on physical behavior. We recently reported the first direct evidence that encapsulated NBs can initiate pressure-dependent nonlinear scattering that persists over repeated exposures in vessel- and tissue-mimicking environments at clinically relevant low frequencies and concentrations (Pellow *et al, PMB* 2018). Here we investigate multipulse modulation schemes at high frequencies with single-element and array transducers to improve sensitivity to NB nonlinear behavior for high resolution imaging applications.

## Statement of Contribution/Methods

We investigated behavior of beads (1 µm) and NBs (0.2-0.4 µm; $10^6$ $mL^{-1}$) in a 1.5 mm diameter vessel phantom. A 25 MHz PVDF transducer was used to assess agent response to 12.5, 25, 30 MHz pulses (for enhanced 2nd harmonic, fundamental, or subharmonic signals) of varying types (sine, cos-tapered, gaussian) with 5 cycles, 1 kHz PRF, 100 bursts, over a range of 0.1-1 MPa. Multipulse schemes utilized the same parameters with pulse-inversion (PI), amplitude-modulation (AM), and PI-AM approaches. The Vevo2100 (256-element linear array) was custom programmed at 21 MHz in AM for analogous preclinical system acquisitions in a channel phantom (1.5 mm diameter, in agar doped with $SiO_2$) and *in vivo* in a CT26 tumor mouse model.

## Results/Discussion

The results demonstrate that NBs exhibit pressure-dependent activity at high frequencies with a threshold for a substantial scattering increase by 20 dB between 200-400 kPa. Comparatively, beads demonstrated a monotonic increase in power with pressure. With multipulse imaging, bead signals were suppressed to the noise floor, while NB nonlinearities were preserved. While multipulse methods are conventionally used to enhance harmonic components of bubble behavior, here we demonstrate maintenance of the fundamental frequency based on threshold behavior. With a linear array, CTR in the phantom in B-mode was 5 dB, improved to 17 dB with AM. Contrast enhancement is further demonstrated *in vivo*. These fundamental single-element studies supplemented by preclinical system acquisitions demonstrate the potential of NBs for imaging applications.

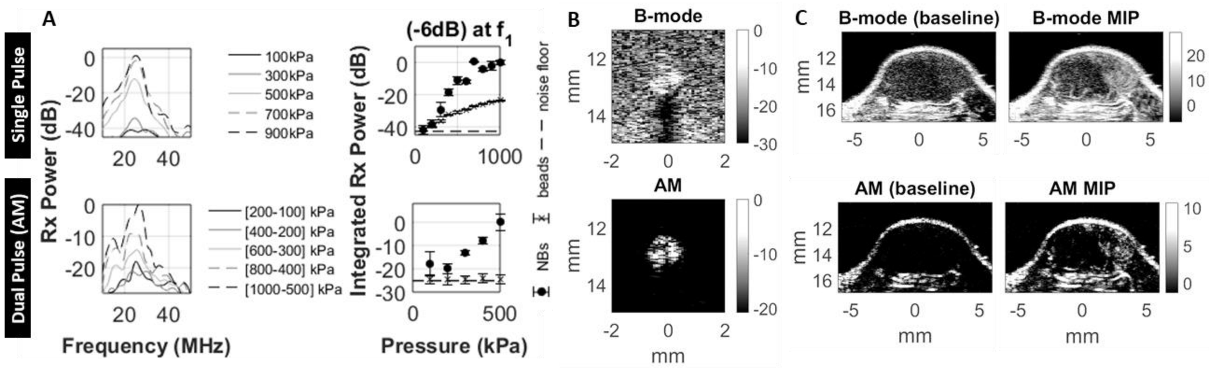


**Figure 1. (A)** Single and dual pulse benchtop results at 25 MHz transmit frequency with NBs in a channel phantom, displaying received power as a function of frequency, and integrated (-6dB of fundamental frequency) received power as a function of transmitted pressure. Bead scattering is reduced to the noise floor with amplitude modulation, while the fundamental frequency amplitude of NBs is maintained, notably increasing upon reaching the threshold pressure. **(B)** Channel phantom images with injected NBs acquired with a linear array transducer, with a 5 dB CTR in B-mode and 17 dB CTR in AM-mode. **(C)** B-mode and AM images acquired *in vivo* of intravenously injected NBs ($10^8$ $mL^{-1}$) in a subcutaneous CT26 tumor model in mice, with baseline (pre-injection) and maximum intensity projection (MIP) images following agent injection.