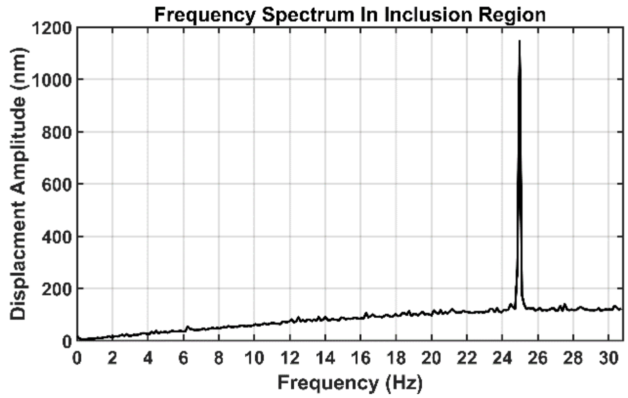
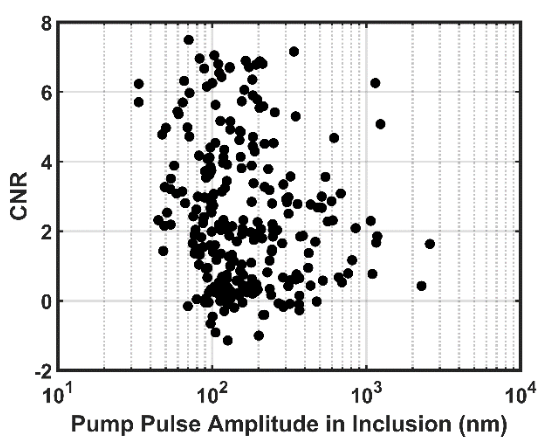
SUPPLEMENT I: *PUMP-INDUCED VIBRATION AMPLITUDE DOES NOT CORRELATE WITH CNR*

The peristaltic pump operates on a clamped piece of soft tubing that is repeatedly squeezed by a rotating head consisting of four metal rollers. The result is pulsatile flow, with a pulse frequency of 4 times the frequency of pump head revolution. A pulse dampener is placed in series with the pump to decrease (but not to eliminate) the magnitude of the pulsatile motion, as without the dampener, pump-induced vibrations are large enough both to rupture the Pebax 35, 20% $BaSO_4$ tube, and to mechanically damage the gelatin phantom. While this study demonstrated that MMUS Signal and CNR did not have any significant dependence on pulsatile flow *rate*, it is interesting to consider whether any dependence on the *magnitude* of the motion existed. To this end, frequency spectra were obtained for each image, and the height of the peak at the pump pulse frequency was used to calculate the amplitude of pump-induced vibrations. An example spectrum from an image which exhibited a high displacement amplitude is presented in Supplemental Fig. 1. Note that while the frequency- and phase-locking algorithm was designed to measure motion at the exact magnet modulation frequency, the frequency steps obtained from the Fourier transform do not in general align perfectly with the various pump pulse frequencies used. As such, only an estimate of the pump-induced vibration amplitude is possible.

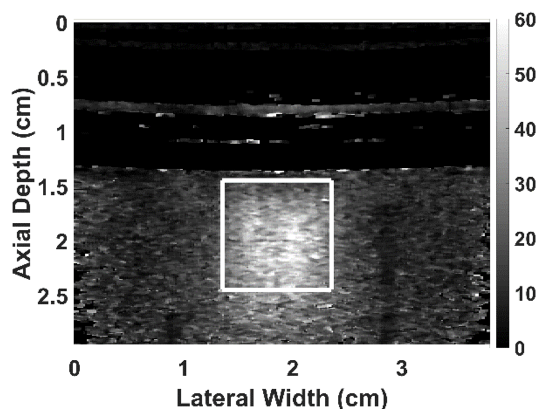


Supplemental Fig. 1. B-off displacement amplitude frequency spectrum within inclusion region of a gelatin blood vessel-mimicking phantom with a 5 kPa elastic modulus, 1 ml inclusion. A sharp peak at the 25 Hz pump pulse frequency (corresponding with a flow rate of 275 ml/min) is observed. The corresponding MMUS image is shown in Supplemental Fig. 3.

For all phantoms which contained SPIO-laden inclusions (control phantoms were excluded for the purposes of this supplementary analysis), the pump-induced vibration amplitude within the inclusion region was extracted from every B-off image for which the pump was running. Supplemental Fig. 2 shows a plot of CNR as a function of pump-induced vibration amplitude in in the inclusion, where the correlation coefficient is -0.09. It is clear from this plot that these quantities are independent. As an example of high CNR in the presence of a great deal of vibration, Supplemental Fig. 3 displays an image with a CNR of 6.3, an MMUS signal of 31 nm, and a pump-induced vibration amplitude of 1100 nm – two orders of magnitude higher than the magnetically-induced vibrations.

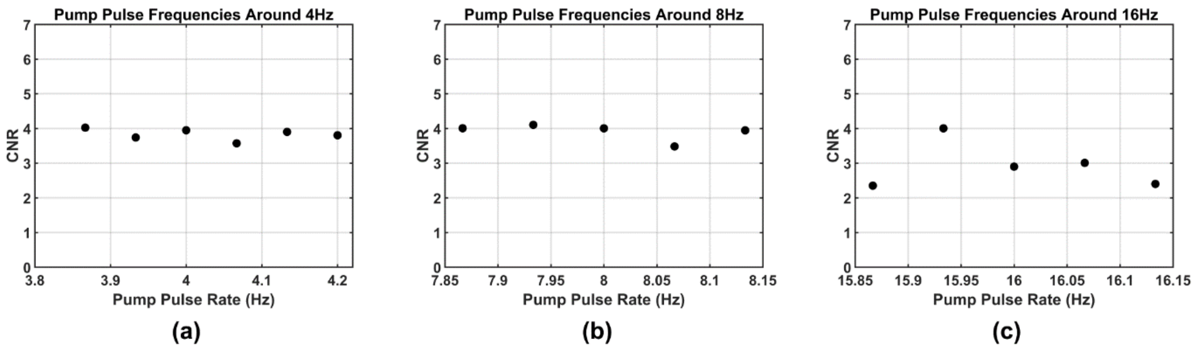


Supplemental Fig. 2. CNR of each image for which pulsatile flow was present in phantoms containing SPIO-laden inclusions plotted as a function of pump-induced vibration amplitude in the inclusion region. The correlation coefficient is -0.09 indicating no significant relationship.



Supplemental Fig. 3. MMUS image corresponding to the frequency spectrum in Supplemental Fig. 1. This is a gelatin blood vessel-mimicking phantom with a 5 kPa elastic modulus, and a 1 ml inclusion in the presence of 275 ml/min (25 Hz) pulsatile flow. The pump-induced vibration amplitude in the inclusion region is ~1100 nm. Despite the high background motion compared to the 31 nm MMUS signal, this image demonstrates a CNR of 6.3.

SUPPLEMENT II: *PUMP FREQUENCIES AT MAGNET MODULATION FREQUENCY HARMONICS DO NOT CONFOUND MMUS*

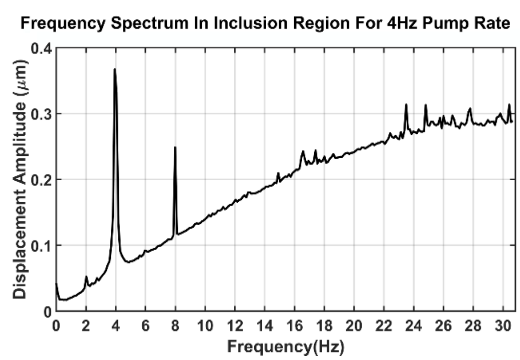


Supplemental Fig. 4. CNR measurements vs. peristaltic pump pulse frequency in the neighborhoods of three harmonics of the 2 Hz magnetic driving frequency. The data show that CNR does not significantly decrease when the pump rate is scanned across harmonic frequencies of 4, 8, or 16 Hz.

As described in Supplement I, the peristaltic pump produces pulse frequencies at four times the pump head revolution rate. For the data reported in Fig. 3, MMUS images were collected at 10 flow rates corresponding to 10 pulse frequencies. Initially it was assumed that it would be necessary to avoid pulse frequencies at the magnetically-induced vibration frequency of 2 Hz and its harmonics in order to avoid motion artifacts leaking through the 2 Hz bandpass filter used to process MMUS data. After collecting the data reported in Fig. 3, another flow phantom was constructed with the express purpose of determining the magnitude of the negative impact on CNR associated with pumping at 2 Hz harmonics. This phantom was identical to the ones used previously, with 5 kPa background gelatin, and a 1 ml, 5 kPa SPIO-laden inclusion. MMUS images were obtained in the following three pulse frequency ranges:

a) 4 Hz neighborhood: Data was collected at 6 integer rpm values ranging from 58 – 63 rpm, corresponding to the following pulse frequencies: 3.87, 3.93, 4.00, 4.07, 4.13, and 4.20 Hz. Two images were obtained for each rate, CNR values were calculated for each image and averaged, and the results are plotted in Supplemental Fig. 4a.
b) 8 Hz neighborhood: Data was collected at 5 integer rpm values ranging from 118 – 122 rpm, corresponding to the following pulse frequencies: 7.87, 7.93, 8.00, 8.07, and 8.13 Hz. Two images were obtained for each rate, CNR values were calculated for each image and averaged, and the results are plotted in Supplemental Fig. 4b.
c) 16 Hz neighborhood: Data was collected at 5 integer rpm values ranging from 238 – 242 rpm, corresponding to the following pulse frequencies: 15.87, 15.93, 16.00, 16.07, and 16.13 Hz. Three images were obtained for each rate, CNR values were calculated for each image and averaged, and the results are plotted in Supplemental Fig. 4c.

In each case, the blood vessel-mimicking tube was observed to contract and dilate at the frequency of the peristaltic pumping action. This motion created displacements at 2 Hz harmonics within the surrounding phantom with pump-induced vibration amplitudes at least an order of magnitude larger than the MMUS signal (see Supplement I). Supplemental Fig. 5 shows a representative frequency spectrum for axial depths spanning the extent of the inclusion with a pump pulse frequency of 4 Hz, and the magnetic field modulated at 2 Hz. Despite the fact that the peristaltic pump-induced motion dwarfs magnetically-induced vibration, CNR was still high as shown in Supplemental Fig. 4a. These data suggest that pump pulse frequencies at 2 Hz harmonics are not associated with a drop in CNR. This in turn indicates that the frequency and phase-locking algorithm is working as expected. The greater variability in Supplemental Fig. 4c is attributed to the increasingly violent motion of the pump at higher rpms, and not necessarily indicative of a deficiency in the MMUS imaging system.



Supplemental Fig. 5. Representative B-on frequency spectrum of motion at inclusion depth, with 4 Hz peristaltic pump pulse frequency, and 2 Hz magnetic driving force. Despite the larger amplitude of displacement induced by the pump in the imaging region, the frequency and phase-locking algorithm successfully suppressed this noise, leading to a CNR of 4.0.