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Quantification of Contrast-Enhanced Ultrasound

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Quantification of Contrast-Enhanced Ultrasound

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Quantification of Contrast-Enhanced Ultrasound

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Abstract

The aim of this experiment was to investigate the effect of manipulating ultrasound scanner settings on time-intensity curve parameters in a tube perfusion phantom system using contrast-enhanced ultrasound imaging. Imaging was performed using a Philips LOGIQ E9 ultrasound scanner equipped with a C1-6VN transducer and utilized two different microbubble contrast agents: Definity and Lumason. The ultrasound scanner settings manipulated included: gain, dynamic range, and frequency. Additionally, relative microbubble concentration, microbubble type, and perfusion flow rate were manipulated. Four time-intensity curve parameters (time to peak, area under curve, gradient, peak intensity) were measured from linearized pixel data. Time to peak was the least impacted time-intensity curve parameter by manipulation of ultrasound scanner settings or the tube perfusion phantom system. Dynamic range and perfusion flow rate manipulation resulted in moderate variation in area under curve, gradient, and peak intensity. Gain, frequency, and relative microbubble concentration manipulation resulted in a high degree of variation in area under curve, gradient, and peak intensity. Both microbubble contrast agents demonstrated similar effects when manipulated. The tube perfusion phantom system contained a small degree of built-in variation, which was incorporated into all variation measurements. Contrast-enhanced ultrasound offers a novel way to quantify microvasculature perfusion. However, variability caused by manipulation of ultrasound scanner settings is still a challenge that hinders the clinical application of contrast-enhanced ultrasound quantification. Standardization practices can be used to limit some of the observed variation. Further research is warranted to investigate how variability in contrast-enhanced ultrasound affects the clinical assessment of microvasculature perfusion.

Introduction

The advancement of diagnostic imaging instruments has enhanced medical professionals' ability to accurately diagnose, treat, and monitor disease progression to improve the clinical outcomes of their patients. One of the most widely used diagnostic imaging modalities is ultrasound imaging. Unlike other imaging modalities like magnetic resonance imaging (MRI), ultrasound is relatively inexpensive, safe, portable, and offers fast real-time imaging (Hangiandreou, 2003; Saini & Hoyt, 2014). The discovery of microbubble contrast agents has further enhanced the versatility of ultrasound imaging (Tang *et al.*, 2011). Contrast-enhanced ultrasound (CEUS) imaging provides a novel way to view and quantify real-time tissue perfusion, which can improve the clinical assessment of diseases/conditions with changes in blood perfusion. However, while CEUS imaging has many advantages, it still faces many technical challenges that could significantly influence its reliability and application as a clinical diagnostic imaging modality. One challenge to the use of CEUS imaging as a clinical tool is the variability caused by the manipulation of ultrasound scanner settings. Previous studies have shown that the manipulation of ultrasound scanner settings can lead to significant variations in the clinical assessment of certain hemodynamic parameters such as area under the curve (AUC) and peak intensity (PI) (Gauthier *et al.*, 2012). CEUS imaging can be utilized in longitudinal studies, which measure small variations over time making it important that no other variation impacts the clinical assessment of these studies (Fröhlich *et al.*, 2015). The aim of this paper is to investigate the effect of manipulating specific ultrasound scanner settings on time-intensity curve (TIC) parameters within a tube perfusion phantom system and to identify possible TIC parameters that could be used for the clinical assessment of tissue perfusion.

Table 1. Common abbreviations of contrast-enhanced ultrasound terminology. *Common terminology was abbreviated as stated.*

Abbreviation	Terminology
CEUS	Contrast-enhanced ultrasound
AUC	Area under the curve
PI	Peak Intensity
TIC	Time-intensity curve
ROI	Region of interest
TtoPk	Time to peak
MI	Mechanical index
MTT	Mean transit time
Grad	Gradient
CV	Coefficient of variation

Ultrasound Imaging Basics

The process of ultrasound imaging works by sending ultrasonic mechanical waves through a tissue region of interest (ROI) and recording the echoes reflected by the tissue structure. The general mode of ultrasound imaging is brightness-mode or B-mode (Hangiandreou, 2003). A transducer sends ultrasound pulses through the tissues and the detected echoes are transmitted into luminance signals. The transducer interconverts mechanical ultrasound vibrations into electrical signals via piezoelectric rods within the transducer. After many pulse-echo cycles, an image is formed from the conversion of electrical signals into a pixel matrix (Hangiandreou, 2003). These images can then be looped together to form "cine" videos, which can replay prior actions in real time. Common abbreviations used in CEUS terminology can be found in Table 1.

Contrast-Enhanced Ultrasound Imaging

CEUS imaging relies on the use of microbubble agents to enhance ultrasound signal intensity (Pecere *et al.*, 2018). Microbubble agents consist of gas filled bubbles encapsulated in a coating of phospholipids or proteins (Saini & Hoyt, 2014; Tang *et al.*, 2011). Depending on the microbubble manufacturer, the bubbles are filled with fluorocarbons, sulfur hexafluoride, or air (Tang *et al.*, 2011). Each microbubble is roughly the size of a red blood cell making them ideal for imaging perfusion in the tissue vasculature. Gas filled bubbles amplify ultrasound signal intensity by having the ability to resonate when impacted by ultrasound waves (Yeh *et al.*, 2003). This resonation increases the echo signals received by the transducer and therefore enhances the luminescence of the image (Tang *et al.*, 2011). There are two main injection methods of microbubble agents: bolus injection and constant infusion with destruction-replenishment. Bolus injection of microbubble agents consists of quickly infusing a short burst ("bolus") of microbubble solution into the blood stream and focusing the ultrasound scanner onto the specific tissue ROI. The bolus injection is the most common injection method used clinically (Dietrich *et al.*, 2012). However, in some clinical cases constant infusion of microbubble agents is preferred. In this injection method, microbubble agents are continuously injected at a constant rate over a period of a few minutes (Tang *et al.*, 2011). Constant infusion is usually accompanied by the process of microbubble destruction and then observing how the microbubbles replenish/refill the ROI (Dietrich *et al.*, 2012). Microbubble destruction is accomplished by using high power ultrasound waves to rupture the microbubbles followed by low power ultrasound waves to view tissue replenishment (Dietrich *et al.*, 2012).

Time-Intensity Curves

Upon impaction by ultrasound waves, microbubbles begin to oscillate in a nonlinear fashion (Gauthier *et al.*, 2011). The consequence of the nonlinear behavior of bubbles is the backscattered echoes have a range of frequencies (harmonics) including the frequency of the original ultrasound wave. The harmonic frequencies can be separated out from the incidence frequency to create enhanced contrast images focusing on microbubble perfusion, separate from the B-mode tissue image (Tang *et al.*, 2011). The enhanced contrast images can then be used for quantifying tissue perfusion. Enhanced contrast images can be used to form TICs, a quantification of image intensity versus time (Yeh *et al.*, 2003). From the TICs, a variety of

hemodynamic parameters (i.e., area under the curve) can be extracted and clinically assessed (Fröhlich *et al.*, 2015).

Theoretical Curve-Fitting Models

CEUS TICs are primarily broken down into two main parts: a wash-in phase and a wash-out phase (Dietrich *et al.*, 2012). The wash-in phase starts at the first incidence of microbubble signal and ends at peak microbubble intensity. The wash-out phase then begins at the peak microbubble intensity and goes until no microbubble signal is detected (Supplementary Figure 1). Due to microbubble perfusion, TICs usually contain some amount of "noise", which can be reduced using theoretical curve-fitting models (Supplementary Figure 2). These models use various interpretations of indicator-dilution theory and are summarized nicely by Strouthos *et al.* (2010). Indicator-dilution theory attempts to determine the amount of microbubbles traveling through an ROI per unit time. At low microbubble concentrations one can calculate blood flow rate and blood volume in terms of AUC and mean transit time (MTT), the average time each microbubble spends in the ROI, using:

$$F = m \times (AUC)^{-1}$$

$$V = F \times MTT$$

where F, m, and V represent blood flow rate, indicator amount, and blood volume, respectively (Strouthos *et al.*, 2010). Additional assumptions of indicator-dilution theory can be found in Strouthos *et al.* (2010). There are multiple theoretical curve-fitting models that can be used for quantification of parameters that deal with hemodynamics; however, for the practicality of this experiment only the Gamma Variate fitting model was used. The derived Gamma Variate fitting model used to suppress the "noisy" TICs in this experiment can be expressed by the following equation:

$$Y(t) = At^{C}\, exp(-kt) + B$$

Here, Y(t) is the backscattered intensity at a specific moment in time. A is the intensity from the contrast agent in Acoustic Units (AU) or decibels (dB). B is the intensity from the tissue at baseline (AU or dB). The variable k represents a time constant (1/s) while the variable C makes the equation into a power function dependent on time. The wash-in phase is represented by the t^C values and the wash-out is represented by the exp(-kt) values. If the signal intensity increases quickly before the peak, then c will increase in value. If the signal intensity decreases quickly after the peak, then k will increase in value. Larger A, B, C, and smaller k values increase PI. Time to peak (TtoPk), the measure of time it takes for PI to be reached upon bolus arrival, equals C/k (GE Healthcare, 2011). Curve-fitting models act as probability density functions that can be used to extract hemodynamic parameters from the CEUS TICs (Strouthos *et al.*, 2010). Common time-intensity curve parameters are shown in Figure 1.

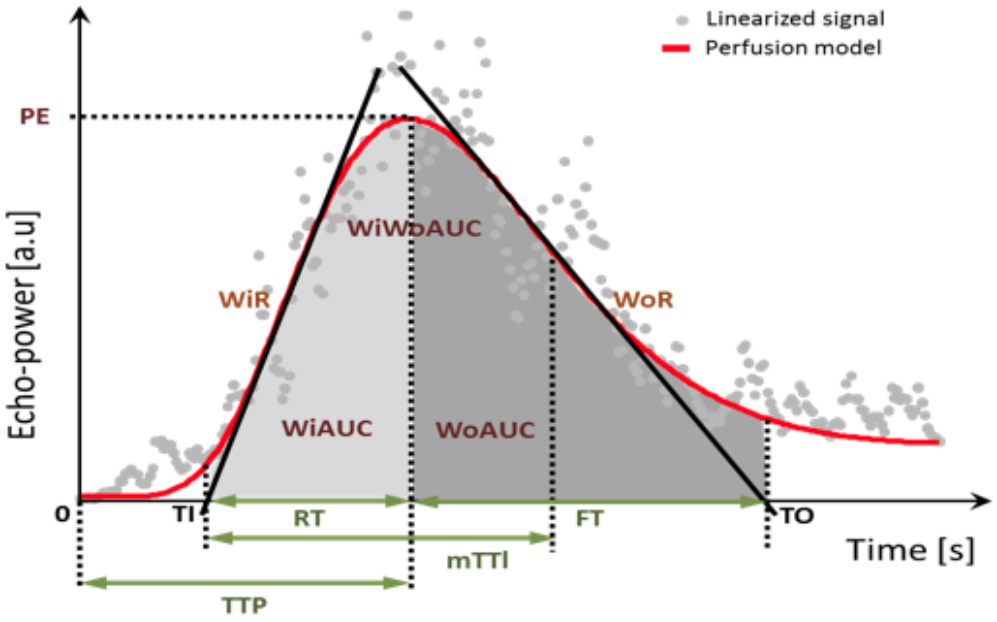

Figure 1. Common time-intensity curve parameters. *TTP (TtoPk): time to peak; PE (PI): peak intensity; AUC: area under curve; WiR (Grad): gradient of wash-in; RT: rise time; MTTI: mean transit time; WoR: gradient of wash-out; FT: fall time. Figure adapted from Bracco quantification software.*

Phantom Model Use in Contrast-Enhanced Ultrasound Testing

Imaging phantoms are a fundamental way of testing multiple imaging modalities including CEUS. They can range in composition, size, and function and are usually designed to mimic a specific type of tissue. Perfusion phantoms can vary from simple tube phantoms to more complex dialysis cartridge models and even 3D printed tissues (Gauthier *et al.*, 2011). This experiment was originally designed to use a dialysis cartridge model; however, due to technical issues with shadowing and bubble interference a simpler tube phantom system was constructed instead (shadowing and bubble interference are shown in Supplementary Figure 3). Tube perfusion phantoms can be composed of rubber, plastic, and various other materials ranging in diameter and wall thickness (Gauthier *et al.*, 2011). There are usually two reservoirs connected to the tube system: one input reservoir contains a specific liquid (i.e. degassed water or blood mimicking solution) located at the beginning of the tube system and one output reservoir to collect the liquid at the end of the system preventing recirculation. A scanning window is set up in between the two reservoirs to record perfusion. The scanning window can be submerged in a water bath to allow the mechanical ultrasound waves to reach the tubing and act on the microbubbles within the tubing (Gauthier *et al.*, 2011). Perfusion can be generated via a peristaltic pump (Gauthier *et al.*, 2011; Gauthier *et al.*, 2012). Microbubbles can be injected directly into the tubing system through the tube wall or via a three-way stop valve apparatus.

Tube systems may have variations in perfusion liquid, tube system set-up, and injection method depending the variables being tested.

Ultrasound Scanner Settings

Ultrasound scanners are equipped with a variety of onboard settings, control knobs, buttons, sliders, and tracking balls (Supplementary Figure 4). These onboard controls allow the user to adjust the echo detection capabilities of the ultrasound scanner and optimize image quality by modulating ultrasound signal processing. Received echoes are amplified by the transducer and can also be amplified by a user-controlled knob (Hangiandreou, 2003). This amplification of echo signal is called gain, and determines the brightness of the image. A higher gain results in a brighter image. Echo signal amplification can also be controlled at different tissue depths. This is referred to as time-gain compensation (TGC). Echo signals from deeper tissues may need to be amplified whereas echo signals from surface tissues may need to be suppressed due to tissue attenuation. Attenuation is a phenomenon that causes ultrasound pulse/echo intensity to decrease as the ultrasound waves travel through tissue. The tissue causes the reflection and scattering of the ultrasound pulse, which decreases the intensity of the pulse (Hangiandreou, 2003). The contrast of the image, the difference in shading between light and dark tissues, can also be altered by adjusting the dynamic range, the range of the largest and smallest signal levels that can be detected. A larger dynamic range causes the image to have low variations of gray in the ultrasound image (Dietrich *et al.*, 2012). Ultrasound pulse frequency can also be adjusted to focus on specific tissue depths to limit tissue attenuation (Hangiandreou, 2003). The general frequency preset, Gen, is the default frequency setting and is used for short and medium tissue depths. Other frequency presets like Res and Pen are used for superficial and deep tissue depths, respectively.

Variability in Contrast-Enhanced Ultrasound Quantification

The accuracy and reliability of quantifying CEUS is essential to its use as a clinical tool. Unfortunately, there are many factors that can significantly impact the quantification of CEUS. Previous experimentation has shown that all of the following can lead to variation in the quantification of CEUS: composition of microbubble agents, log compression of cine videos, mechanical index settings, focal depth settings, dynamic range settings, gain settings, frequency settings, blood pressure of patient, bubble interaction with human tissues (i.e., lung filtration), tissue motion (i.e., patient breathing), tissue attenuation, microbubble size, microbubble injection method, microbubble concentration, and the model of the ultrasound machine (Tang *et al*., 2011; Vinke *et al*., 2017; Pitre-Champagnat *et al*., 2017; Gauthier *et al*., 2012). This paper focuses specifically on CEUS variability in regards to dynamic range, gain, and frequency settings in addition to microbubble concentration, microbubble type, and perfusion flow rate. All of the other sources of CEUS variability were mitigated (i.e., used same ultrasound machine) or not relevant (i.e., used tube system so bubble interactions with human tissues is not relevant). Gauthier *et al.* (2012) demonstrated that varying bolus volume, transducer type, gain, mechanical index (MI), focal depth, pulse center frequency, and pulse sequence can cause variations in hemodynamic parameters such as rise time (similar to TtoPk), AUC, MTT, and PI. They found coefficients of variation ranging from 2% all the way up to 126% depending on what variable was being altered and what hemodynamic parameter was being measured (Gauthier *et al.* 2012).

Time-dependent parameters (i.e., MTT and rise time) tended to have smaller coefficients of variation compared to volume-dependent parameters (i.e., PI and AUC). Gauthier *et al.* (2012) used a dialysis cartridge perfusion phantom monitored by a Philips iU22 ultrasound scanner and did not investigate the effects of varying dynamic range settings, microbubble type, or perfusion flow rate on CEUS quantification variability.

Methods

Tube Perfusion Phantom System

Water at ambient temperature was pumped using a Cole-Parmer Instrument peristaltic pump at 220 mL/min (unless stated otherwise) through ~25 ft. of ½ in. coiled tubing as shown in Supplementary Documentation 1. The input of the tubing was placed in a 1 L input reservoir and the output of the tubing was connected to a 1 L output reservoir. A scanning window was chosen between the two reservoirs and submerged in a 5 L water bath. The system was discontinuous to avoid the recirculation of microbubbles past the scanning window. At the scanning window, a Phillips C1-6VN transducer was placed connected to a Phillips LOGIQ E9 ultrasound scanner. The scanning window was submerged ~ 10 cm into the water bath kept at ambient temperature. The transducer was placed in the transverse plane of the scanning window and held in place by a clamp. A reflection dampening material was placed underneath the scanning window to suppress echoes reflected off of the bottom of the water bath.

Contrast Agents

Two different contrast agents were used in this experiment: Lumason (more widely known as SonoVue) and Definity. 0.5 mL bolus injections of contrast agent (unless stated otherwise) were applied over an average of 1 sec. durations using an 18-gauge needle inserted into the tubing wall upstream of the scanning window. Injections were made at the same location by the same operator to ensure reproducibility. Vials of contrast agent were refrigerated at 1.6 °C. Prior to injection, the contrast agents were allowed to acclimate to room temperature and were agitated to ensure a homogeneous injection. The exact concentration of the microbubble solutions was unknown.

Scanner Settings

Initial scanner settings were adjusted to the onboard Abdominal Preset settings (a complete list of Abdominal Preset settings can be found in Supplementary Info X). The tube perfusion system was tested for reproducibility under the Abdominal Preset settings (gain was set to 10 dB) using Definity. Subsequently, one scanner setting was varied at a time to assess its effect on CEUS quantification parameters: TtoPk, AUC, Grad (rate of microbubble wash-in measured in Acoustic Units/sec), and PI (Figure 1). The following variables were investigated: relative microbubble concentration (0.25X dilution, 0.5X dilution, 1X solution), gain (4, 10, 16 dB), dynamic range (57, 69, 84 dB), frequency (2.5, 9, 12 MHz), and perfusion flow rate (140, 185, 220 mL/min). After bolus injection, cine image loops were recorded and linearized data was acquired. Each condition was replicated at least three times. Both contrast agents were tested in

the relative microbubble concentration, gain, and dynamic range variations. Definity alone was used in the frequency and perfusion flow rate variations. For each condition (i.e., gain), the same vial of unknown microbubble concentration was used.

Image Analysis

The cine image loop linearized data was analyzed using the Phillips LOGIQ E9 onboard TIC analysis feature. An ROI was drawn over the tubing within the scanning window and motion corrected. TICs were then fit with the Gamma Variate function (as stated above) and the hemodynamic parameters (TtoPk, AUC, Grad, PI) were recorded. The traces were subsequently exported offline for further analysis. Trials for each variable condition were averaged and coefficients of variation (CV) were calculated using the following equation:

$$Coefficient\ of\ Variation\ (CV) = 100\ \times \frac{\sigma\ of parameter}{\bar{X}\ of\ parameter}$$

The results were graphed with the manipulated target variable on the x-axis for all four TIC parameters of interest (TtoPk, AUC, Grad, and PI) graphed on the y-axis. Regression analysis was performed to determine if the slopes of the lines of best-fit were statistically significant to identify correlations between parameters. From the regression analysis t-scores, degrees of freedom, and p-values were determined. A significant $\boldsymbol{\alpha}$ was designated as < 0.05.

Results

Tube Perfusion Phantom Systems Contain Built-In Variation

Reproducibility trials (n=7) of the tube perfusion phantom resulted in CV's of 16%, 19%, 21%, and 19% for TtoPk, AUC, Grad, and PI, respectively. This "built-in" variation resulted from no deliberate changes to the ultrasound scanner settings or the tube perfusion phantom system. The variation resulting from subsequent deliberate changes to the ultrasound scanner settings or the tube perfusion phantom system will contain this built-in variation. An example of the linearized TIC reproducibility data before curve-fitting analysis is shown in Figure 2.

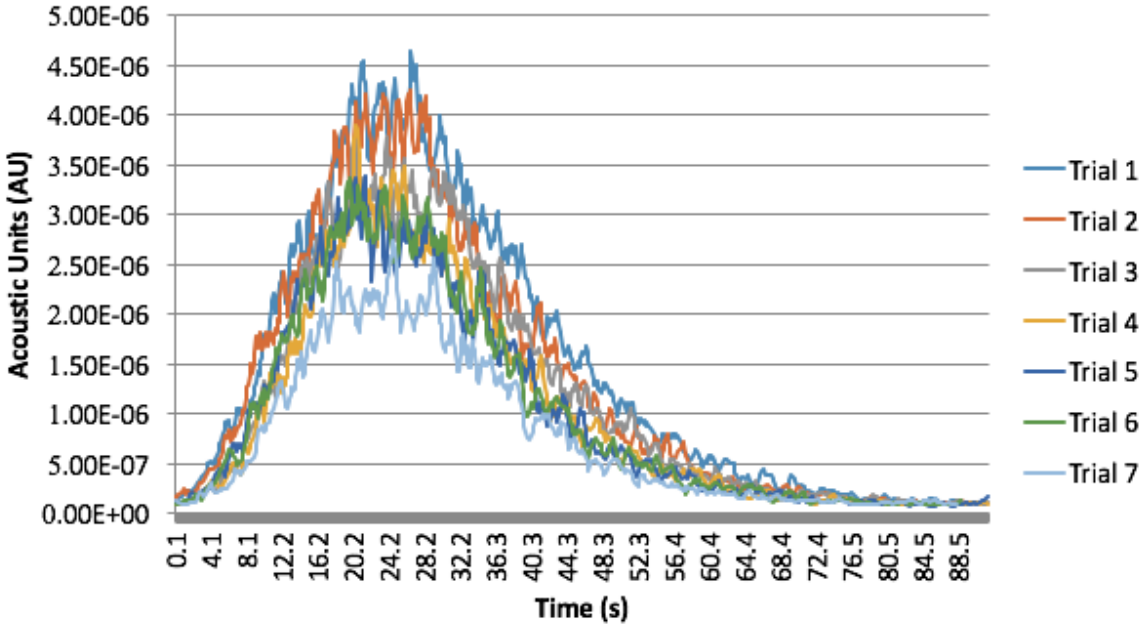

Figure 2. Example of raw linearized time-Intensity curves of seven reproducibility trials in a tube perfusion phantom using Definity. *Data acquisition was carried out using a Philips C1-6VN transducer connected to a Phillips LOGIQ E9 ultrasound scanner. Linearized data was obtained using the Phillips onboard TIC analysis program. Measurements are in arbitrary acoustic units (AU). n=7*

Manipulation of Relative Microbubble Concentration, Gain Settings, and Frequency Settings Induces High Degrees of Variation in Time-Intensity Curve Parameters

Manipulation of the relative microbubble concentration resulted in CV's of 97%, 82%, and 90% (Definity) and 118%, 97%, and 108% (Lumason) for AUC, Grad, and PI, respectively (Table 2). As the relative Definity microbubble concentration increased, a corresponding significant positive correlation regarding AUC, PI, and Grad was observed (Table 3; Figure 3). A similar significant positive correlation in AUC, PI, and Grad in addition to TtoPk was observed using Lumason (Figure 4). Likewise, high coefficients of variation resulted from the manipulation of the relative Lumason microbubble concentration (Table 2).

Manipulation of gain settings resulted in CV's of 111%, 115%, 110% (Definity) and 74%, 83%, and 83% (Lumason) for AUC, Grad, and PI, respectively (Table 2). Similar to the manipulation of relative microbubble concentration, there was a significant positive correlation observed between gain setting and AUC, PI, and Grad for both Definity and Lumason (Tables 5 & 6; Figures 5 & 6). There also was a significant negative correlation in TtoPk for gain manipulation and Definity (Table 5).

Manipulation of frequency settings using Definity resulted in CV's of 94%, 94%, and 93% for AUC, Grad, and PI, respectively (Table 2). Unlike manipulation of relative microbubble concentration and gain settings, manipulation of frequency settings resulted in a significant negative correlation between frequency and AUC, PI, and Grad (Table 7; Figure 7).

Table 2. Coefficients of variation of time-intensity curve parameters after manipulation of five variables in a tube perfusion phantom using Definity and Lumason microbubbles. *Data acquisition was carried out using a Philips C1-6VN transducer connected to a Phillips LOGIQ E9 ultrasound scanner. Scanning was done using the Abdominal Preset, except gain was set to 10 dB. Perfusion velocity was set at 220 mL/min. Each ultrasound setting was changed between trials.*

Variable Manipulated	Microbubble Brand	Coefficients of Variation of Time-Intensity Curve Parameters (%)			
		Time to peak (TtoPk)	Area under curve (AUC)	Gradient (Grad)	Peak Intensity (PI)
Relative Microbubble Concentration	Definity	11	97	82	90
	Lumason	16	118	97	108
Gain	Definity	19	111	115	110
	Lumason	10	74	83	83
Dynamic Range	Definity	11	22	17	36
	Lumason	7	60	57	63
Frequency	Definity	9	94	94	93
Perfusion Flow Rate	Definity	9	30	20	27

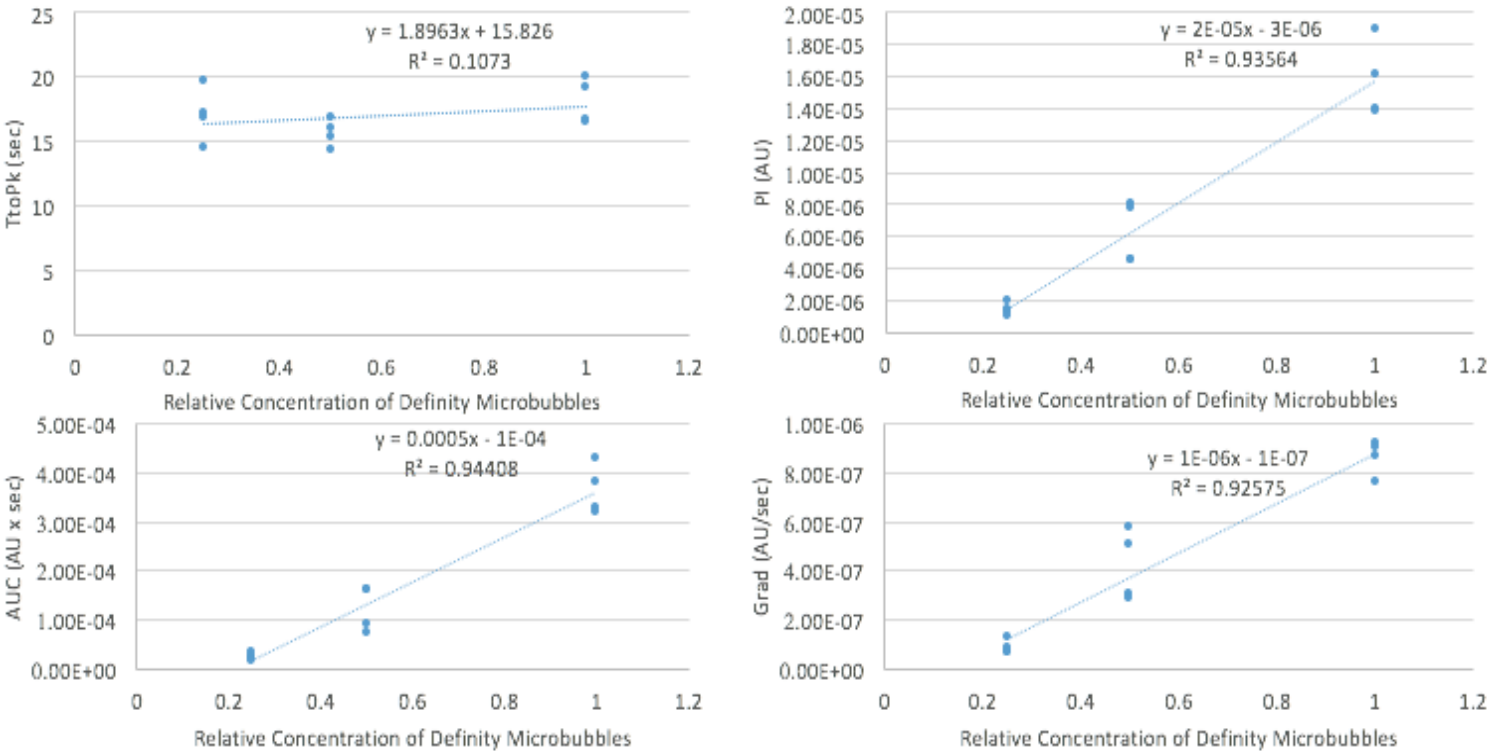

Figure 3. Impact of varying relative microbubble concentration on time-intensity curve parameters in a tube perfusion phantom using Definity. *Data acquisition was carried out using a Philips C1-6VN transducer connected to a Philips LOGIQ E9 ultrasound scanner. 2.5 mL of 1X microbubble solution was diluted in 2.5 mL of water to make the 0.5X dilution. 2.5 mL of the 0.5X dilution was further diluted in 2.5 mL of water to make the 0.25X dilution. TtoPk: time to peak; PI: peak intensity; AUC: area under curve; Grad: gradient of wash-in; AU: acoustic units; sec: second. n=4*

Table 3. Regression analysis of best-fit lines after varying relative concentrations of Definity. *Linear lines of best-fit were equated for each TIC parameter at 0.25X, 0.5X, and 1X relative Definity concentrations. Regression analysis was performed to determine if the best-fit lines had significantly different slopes from zero. α= 0.05. *=significant p-value.*

Time-Intensity Curve Parameter	T-score	Degrees of freedom	p-value
TtoPk	1.09	10	0.29
AUC	12.99	10	1.4E-07*
Grad	11.17	10	5.7E-07*
PI	12.05	10	2.79E-07*

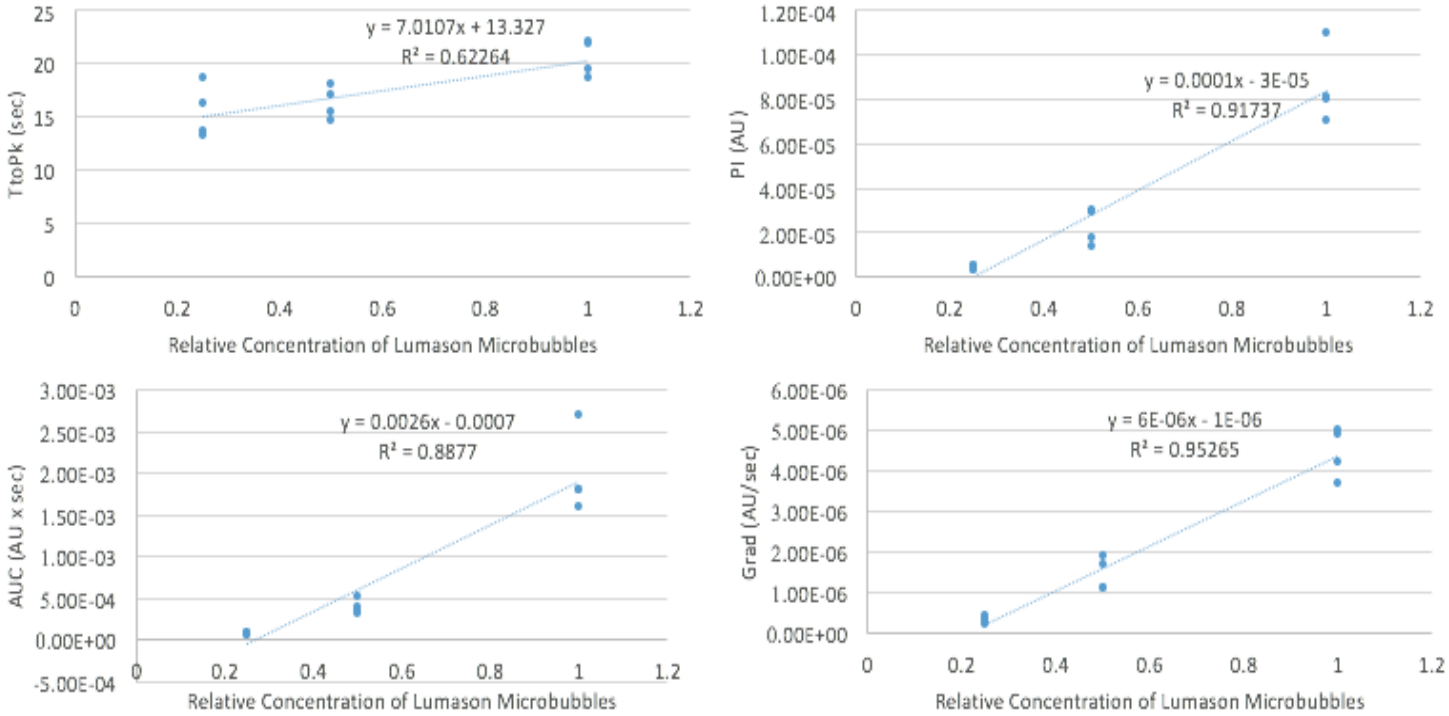

Figure 4. Impact of varying relative microbubble concentration on time-intensity curve parameters in a tube perfusion phantom using Lumason. *Data acquisition was carried out using a Philips C1-6VN transducer connected to a Philips LOGIQ E9 ultrasound scanner. 2.5 mL of 1X microbubble solution was diluted in 2.5 mL of water to make the 0.5X dilution. 2.5 mL of the 0.5X dilution was further diluted in 2.5 mL of water to make the 0.25X dilution. TtoPk: time to peak; PI: peak intensity; AUC: area under curve; Grad: gradient of wash-in; AU: acoustic units; sec: second. n=4*

Table 4. Regression analysis of best-fit lines after varying relative concentrations of Lumason. *Linear lines of best-fit were equated for each TIC parameter at 0.25X, 0.5X, and 1X relative Lumason concentrations. Regression analysis was performed to determine if the best-fit lines had significantly different slopes from zero. α= 0.05. *=significant p-value.*

Time-Intensity Curve Parameter	T-score	Degrees of freedom	p-value
TtoPk	4.06	10	2.1E-03*
AUC	8.89	10	4.6E-06*
Grad	14.18	10	5.9E-06*
PI	10.53	10	9.8E-07*

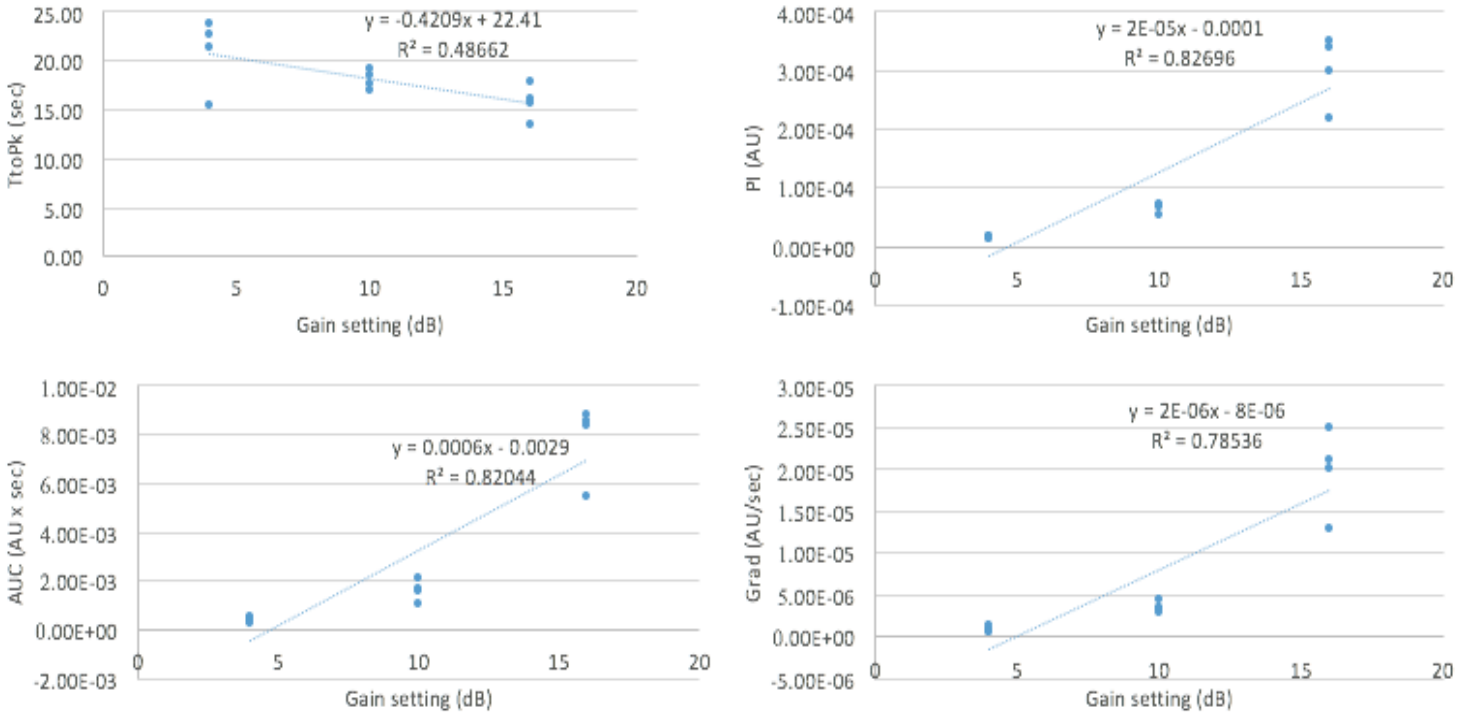

Figure 5. Impact of varying gain on time-intensity curve parameters in a tube perfusion phantom using Definity. *Data acquisition was carried out using a Philips C1-6VN transducer connected to a Philips LOGIQ E9 ultrasound scanner. TtoPk: time to peak; PI: peak intensity; AUC: area under curve; Grad: gradient of wash-in; AU: acoustic units; sec: second; dB: decibels. n=4*

Table 5. Regression analysis of best-fit lines after varying gain settings using Definity. *Linear lines of best-fit were equated for each TIC parameter at 4, 10, and 16 dB gain settings. Regression analysis was performed to determine if the best-fit lines had significantly different slopes from zero. α= 0.05. *=significant p-value.*

Time-Intensity Curve Parameter	T-score	Degrees of freedom	p-value
TtoPk	-3.08	10	0.01*
AUC	6.76	10	4.9E-05*
Grad	6.04	10	1.2E-04*
PI	6.91	10	4.1E-05*

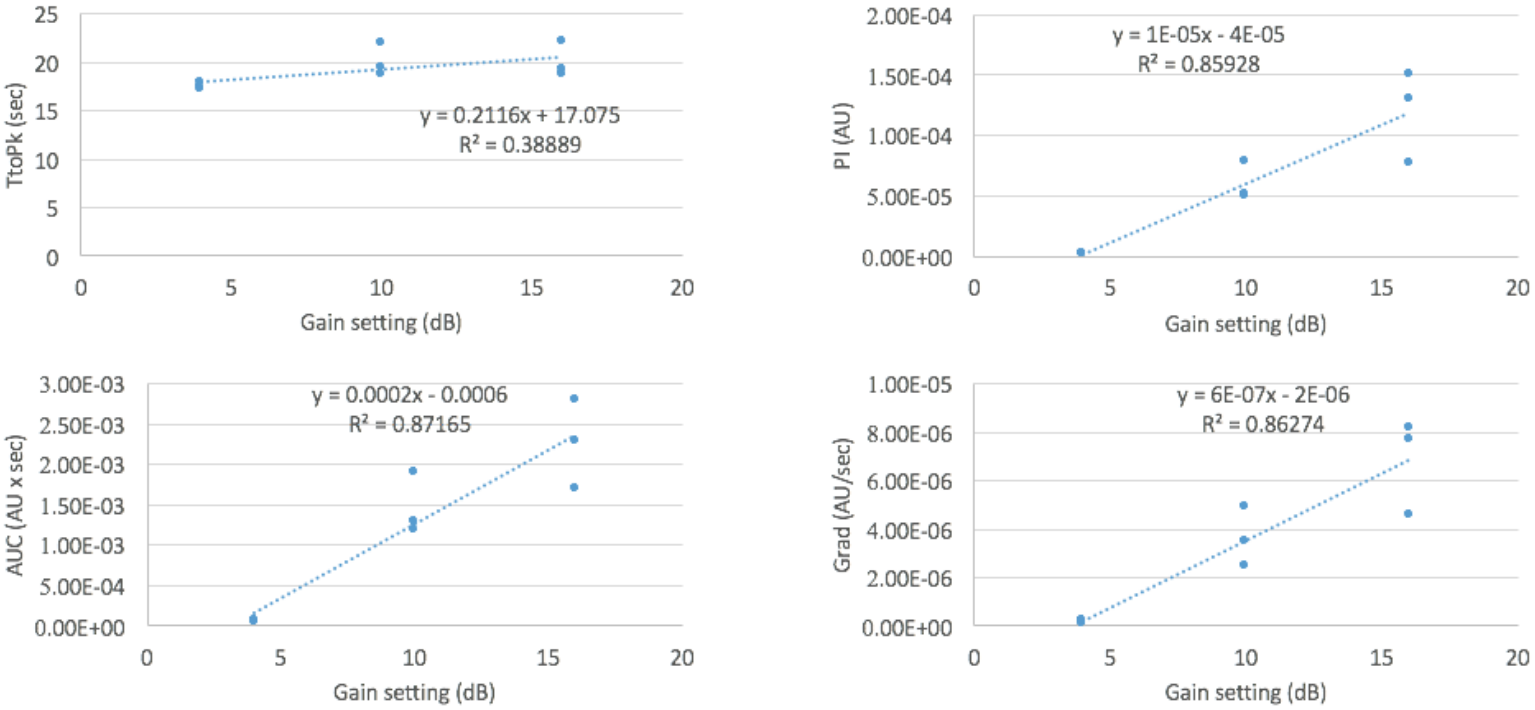

Figure 6. Impact of varying gain on time-intensity curve parameters in a tube perfusion phantom using Lumason. *Data acquisition was carried out using a Philips C1-6VN transducer connected to a Philips LOGIQ E9 ultrasound scanner. TtoPk: time to peak; PI: peak intensity; AUC: area under curve; Grad: gradient of wash-in; AU: acoustic units; sec: second; dB: decibels. n=3*

Table 6. Regression analysis of best-fit lines after varying gain settings using Lumason. *Linear lines of best-fit were equated for each TIC parameter at 4, 10, and 16 dB gain settings. Regression analysis was performed to determine if the best-fit lines had significantly different slopes from zero. α= 0.05. *=significant p-value.*

Time-Intensity Curve Parameter	T-score	Degrees of freedom	p-value
TtoPk	2.11	7	0.07
AUC	6.89	7	2.3E-04*
Grad	6.63	7	2.9E-04*
PI	6.53	7	3.2E-04*

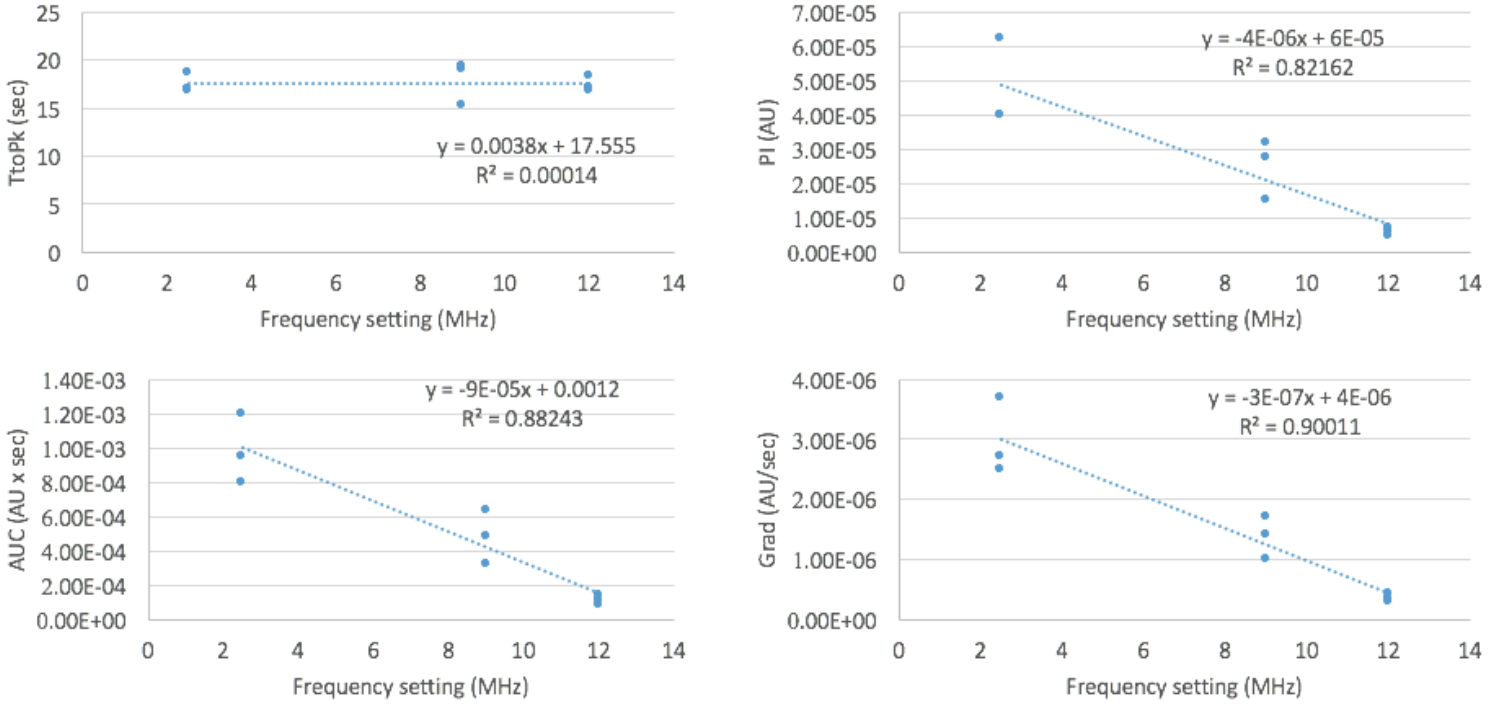

Figure 7. Impact of varying frequency on time-intensity curve parameters in a tube perfusion phantom using Definity. *Data acquisition was carried out using a Phillips C1-6VN transducer connected to a Philips LOGIQ E9 ultrasound scanner. TtoPk: time to peak; PI: peak intensity; AUC: area under curve; Grad: gradient of wash-in; AU: acoustic units; sec: second; MHz: megahertz. n=3*

Table 7. Regression analysis of best-fit lines after varying frequency settings using Definity. *Linear lines of best-fit were equated for each TIC parameter at 2.5, 9, and 12 MHz frequency settings. Regression analysis was performed to determine if the best-fit lines had significantly different slopes from zero. α= 0.05. *=significant p-value.*

Time-Intensity Curve Parameter	T-score	Degrees of freedom	p-value
TtoPk	0.03	7	0.98
AUC	-7.25	7	1.7E-04*
Grad	-7.94	7	9.6E-05*
PI	-5.68	7	7.5E-04*

Manipulation of Dynamic Range Settings and Perfusion Flow Rate Induce Moderate Degrees of Variation in Time-Intensity Curve Parameters

Manipulation of dynamic range resulted in CV's of 22%, 17%, and 36% (Definity) and 60%, 57%, and 63% (Lumason) for AUC, Grad, and PI, respectively (Table 2). However, while moderate degrees of variation were observed, there was no significant correlation observed between varying levels of dynamic range and AUC, PI, and Grad for either microbubble brand (Tables 8 & 9; Figures 8 & 9). A similar observation resulted with Definity from manipulation of perfusion flow rate regarding AUC and Grad (Table 10; Figure 10). Manipulation of perfusion flow rate resulted in CV's of 30%, 20%, and 27% for AUC, Grad, and PI, respectively (Table 2). A significant negative correlation was observed between perfusion flow rate and both PI and TtoPk (Table 10; Figure 10).

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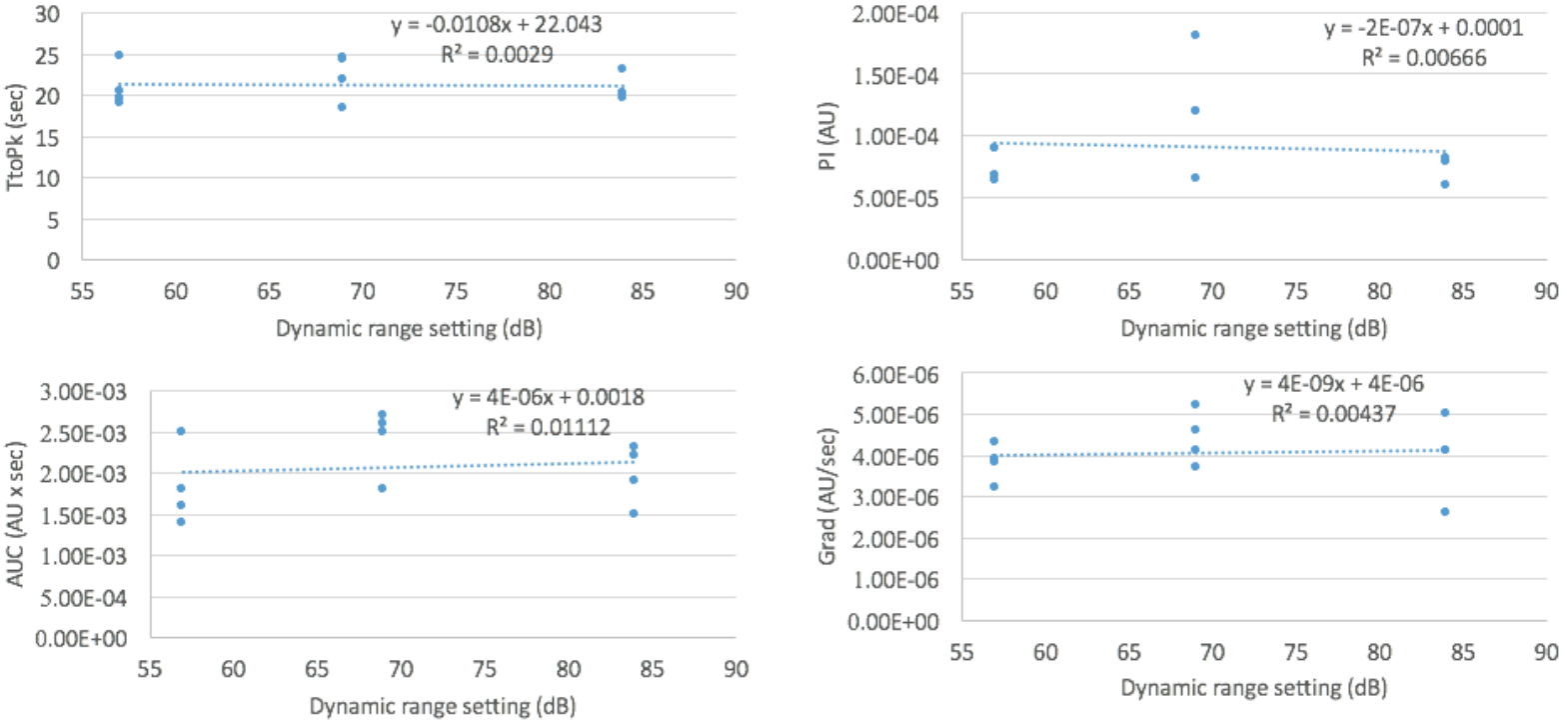


Figure 8. Impact of varying dynamic range on time-intensity curve parameters in a tube perfusion phantom using Definity. *Data acquisition was carried out using a Philips C1-6VN transducer connected to a Phillips LOGIQ E9 ultrasound scanner. TtoPk: time to peak; PI: peak intensity; AUC: area under curve; Grad: gradient of wash-in; AU: acoustic units; sec: second; dB: decibels. n=4*

Table 8. Regression analysis of best-fit lines after varying dynamic range settings using Definity. *Linear lines of best-fit were equated for each TIC parameter at 57, 69, and 84 dB dynamic range settings. Regression analysis was performed to determine if the best-fit lines had significantly different slopes from zero. α= 0.05. *=significant p-value.*

Time-Intensity Curve Parameter	T-score	Degrees of freedom	p-value
TtoPk	-0.17	10	0.87
AUC	0.33	10	0.74
Grad	0.21	10	0.83
PI	-0.26	10	0.80

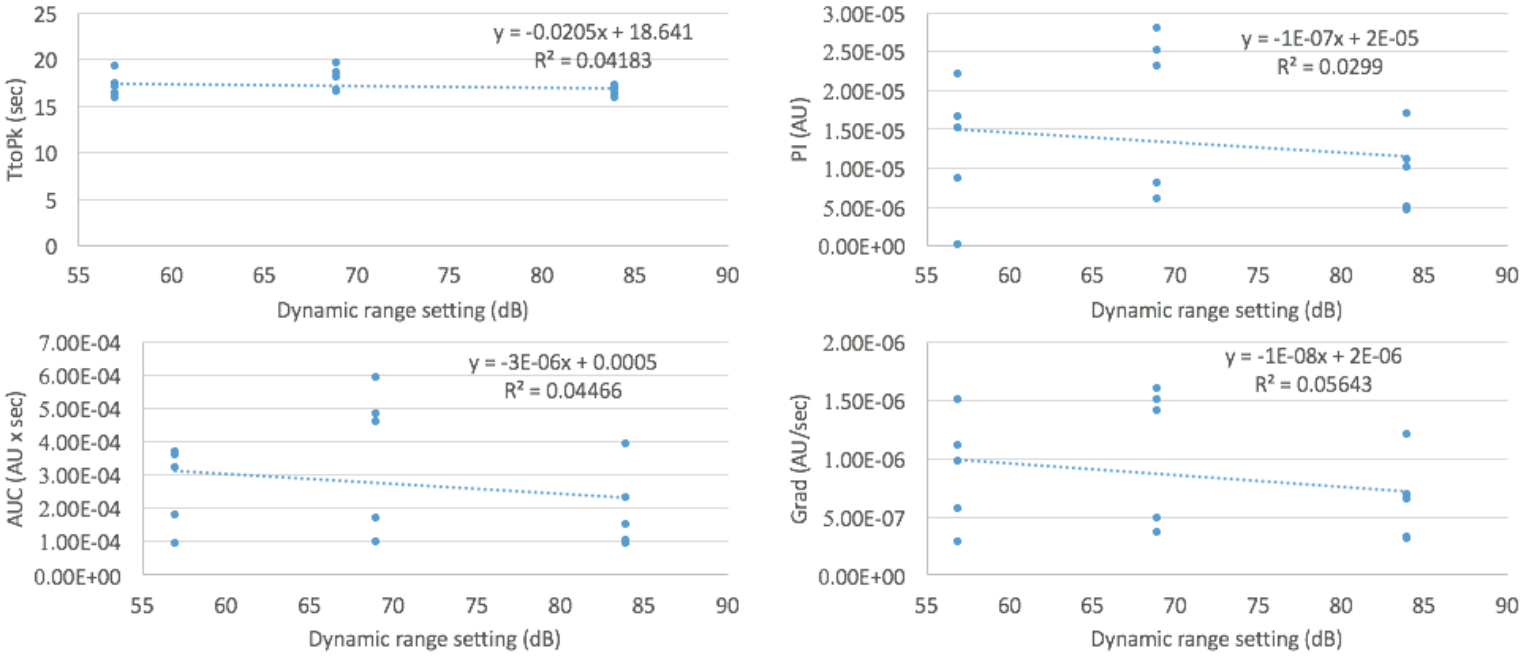


Figure 9. Impact of varying dynamic range on time-intensity curve parameters in a tube perfusion phantom using Lumason*. Data acquisition was carried out using a Philips C1-6VN transducer connected to a Philips LOGIQ E9 ultrasound scanner. TtoPk: time to peak; PI: peak intensity; AUC: area under curve; Grad: gradient of wash-in; AU: acoustic units; sec: second; dB: decibels. n=5*

Table 9. Regression analysis of best-fit lines after varying dynamic range settings using Lumason. *Linear lines of best-fit were equated for each TIC parameter at 57, 69, and 84 dB dynamic range settings. Regression analysis was performed to determine if the best-fit lines had significantly different slopes from zero. α= 0.05. *=significant p-value.*

Time-Intensity Curve Parameter	T-score	Degrees of freedom	p-value
TtoPk	-0.75	13	0.46
AUC	-0.78	13	0.45
Grad	-0.88	13	0.39
PI	-0.63	13	0.54

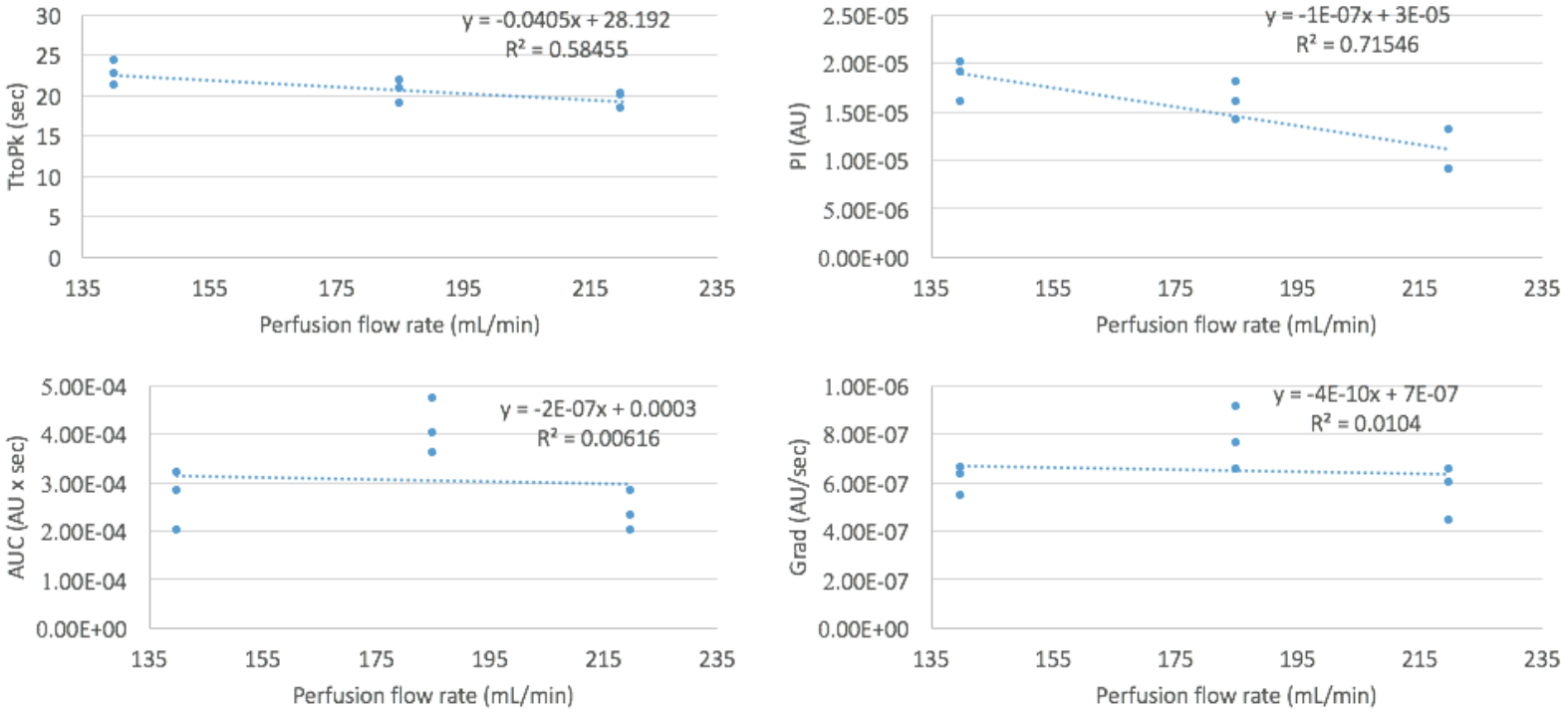


Figure 10. Impact of varying perfusion flow rate on time-intensity curve parameters in a tube perfusion phantom using Definity. *Data acquisition was carried out using a Philips C1-6VN transducer connected to a Philips LOGIQ E9 ultrasound scanner. TtoPk: time to peak; PI: peak intensity; AUC: area under curve; Grad: gradient of wash-in; AU: acoustic units; sec: second; mL: milliliters; min: minute. n=3*

Table 10. Regression analysis of best-fit lines after varying perfusion flow rate using Definity. *Linear lines of best-fit were equated for each TIC parameter at 140, 185, and 220 mL/min. Regression analysis was performed to determine if the best-fit lines had significantly different slopes from zero. α= 0.05. *=significant p-value.*

Time-Intensity Curve Parameter	T-score	Degrees of freedom	p-value
TtoPk	-3.14	7	0.02*
AUC	-0.21	7	0.84
Grad	-0.27	7	0.79
PI	-4.19	7	4E-03*

Time to Peak Represents Potential Time-Intensity Curve Parameter for Contrast-Enhanced Ultrasound Quantification

The lowest CV values from the manipulation of ultrasound scanner settings or the tube perfusion phantom system occurred in the TtoPk TIC parameter (Table 2). The CV for TtoPk during reproducibility trials was 16%. Accordingly, the CV's for TtoPk were: 19%, 10%, 9%, 11%, and 7% for gain manipulation (Definity & Lumason), frequency manipulation (Definity), and dynamic range manipulation (Definity & Lumason), respectively. Likewise, there was almost no correlation observed between TtoPk and many of the manipulated variables (Tables 3-10). Low variation in TtoPk makes it a potential TIC parameter for CEUS quantification.

Discussion

The aim of the tube perfusion phantom was to mimic blood perfusion in the body's vasculature (e.g., liver lesion, a common target of contrast-enhanced ultrasound). From there, different ultrasound scanner settings (i.e., gain) or tube perfusion phantom system adjustments (i.e., relative microbubble concentration) were manipulated to investigate their impact on TIC parameters. This investigation stems from the fact that many of the variables tested (all except perfusion flow rate) do not directly affect perfusion. For example, gain is the amplification of echo signals to enhance image brightness. Gain does not affect perfusion or the microbubbles directly, yet as shown in Figures 5 & 6 it affects TICs (a model of perfusion) and TIC parameters. The perfusion never changed, yet the quantification of the perfusion changed. This problem is at the heart of CEUS quantification. Linearized data (the raw echo data) is used to try and prevent this problem; however, it is unclear at this point in time how onboard ultrasound settings affect the raw echo data. Standardization of certain ultrasound techniques could limit this problem to a degree (Pitre-Champagnat *et al.*, 2017). However, standardization may at times limit image quality and/or the qualitative analysis of the perfusion. Overall, both microbubble types (Definity and Lumason) elicited similar changes when different variables were manipulated (Figures 3-8).

Manipulation of gain settings resulted in large CV's for AUC and PI, similar to previous CEUS quantification experiments (Gauthier *et al.*, 2012). Additionally, time-dependent parameters (i.e., TtoPk) were less affected by variable manipulation than amplitude-related parameters (i.e., AUC and PI) (Table 2). The reproducibility trials demonstrated that the tube perfusion phantom system had built-it variation (Figure 2). This variation may have been caused by a variety of factors such as air-bubble accumulation in the phantom, small variations in the scanning window, and fluctuations in the peristaltic pump motor settings.

Manipulation of the relative microbubble concentrations for both Definity and Lumason saw positive correlations between relative microbubble concentration and AUC, PI, and Grad, respectively (Figures 3 & 4). These correlations were expected according to indicator-dilution theory. Having a greater microbubble concentration results in more reflection of the ultrasound pulses and greater echo signals. This results in a larger A value in the gamma variate fit of the TIC resulting in a taller TIC, and a taller TIC increases AUC, PI, and Grad. However, previous experiments manipulating microbubble concentrations have shown that at high microbubble concentrations attenuation caused by the microbubbles themselves tends to lessen this correlation

(Gauthier *et al.*, 2012). A limitation to this experiment was microbubble concentrations were unknown (because the microbubbles were previously mixed and donated). The microbubble solutions were all diluted to ensure low microbubble concentrations to avoid extra attenuation.

The frequency setting also caused high variation in multiple TIC parameters (Table 2). Gauthier *et al.* (2012) demonstrated that frequency can cause moderate CV's in AUC and PI; however, they used a max frequency of 2.3 MHz on a curvilinear transducer whereas this experiment used three very different frequencies. High frequencies can increase the mechanical index (measure of ultrasound's bioeffects on tissue), which can rupture the microbubbles. This effect is utilized for destruction-replenishment bolus injections. The frequencies used in these experiments were kept below the level that ruptures microbubbles.

Dynamic range manipulation caused only moderate variations in TIC parameters (Table 2). However, the effect of low dynamic range (< 40 dB) on variation in TIC parameters is unknown. Usually, high dynamic ranges are used to maximize the range of signals received and increase contrast between the microbubbles and the surrounding tissue.

Perfusion flow rate additionally caused moderate variations to TIC parameters (Table 2). As noted above, there was an observed negative correlation between perfusion flow rate and PI (Figure 10). One explanation for this observation is that increasing perfusion flow rate "spreads" the bolus of microbubbles out faster thus at any given moment in time a lower concentration of microbubbles will be flowing past the scanning window.

This experiment utilized a Phillips LOGIQ E9 ultrasound scanner. However, it has been observed that using different types of ultrasound scanners can cause variation in TIC parameters (Pitre-Champagnat *et al.*, 2017). This makes comparing variability between different studies utilizing different ultrasound scanners difficult. Each scanner can vary in onboard curve fitting algorithms, linearized data acquisition, ultrasound settings, and ROI selection. Standardization of ultrasound scanners is crucial for the advancement of CEUS quantification.

This experiment demonstrated variation in TIC parameters caused by manipulation of ultrasound scanner settings or the tube perfusion phantom system. However, it is unknown if these variations are clinically relevant. For example, Medellin *et al.* (2017) used CEUS quantification to help determine severity of Irritable Bowel Disease (IBD). Analyzing bowel wall blood perfusion (using log-compressed data instead of raw linear data, hence the shift to dB as opposed to AU), Medellin *et al.* (2017) were able to classify IBD into four different categories based off of PI measurements: inactive (0-15 dB), mild (15-18 dB), moderate (18-23 dB), and severe (>23 dB). If varying a variable (i.e. gain) in this experiment caused a variation in PI of 2 dB then it would have low impact on the clinical assessment of IBD. However, if varying the variable caused a variation in PI of 20 dB then it would have a high impact on the clinical assessment of IBD. It is hard to determine the full effect of varying ultrasound settings on the clinical assessment of perfusion diseases/conditions due to the variety of clinical parameters, diagnostic methods, and procedures.

TtoPk demonstrated the lowest CV's when scanner settings or additional variables were manipulated (Table 2). The low variation makes TtoPk a potential target for CEUS quantification studies. The TtoPk parameter has been used effectively in multiple quantification studies evaluating perfusion (Wouters et *al.*, 2017; Kundi *et al.*, 2017). However, the use of summary perfusion parameters like TtoPk in other quantification fields has been challenged. TtoPk does not take into account the arterial input function (concentration of contrast agent entering the ROI) or the residual fraction (fraction of contrast agent remaining in the ROI at a specific point in time), yet both arterial input function and residual fraction are known to strongly affect TtoPk

in contrast MRI studies (Perthen *et al.*, 2001). It has been suggested to use summary hemodynamic parameters such as TtoPk with caution and to account for arterial input function and residual fraction during quantification (Perthen *et al.*, 2001). Further studies are warranted to elucidate the effectiveness of using TtoPk in the assessment of perfusion.

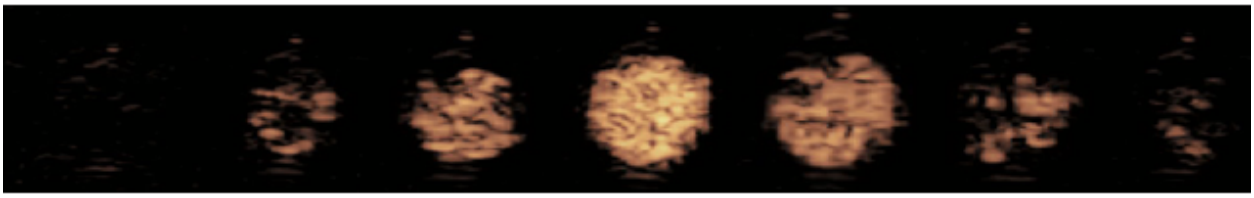
Conclusions

TtoPk was the TIC parameter least affected by manipulation of ultrasound scanner settings or the tube perfusion phantom system. Further CEUS quantification studies are warranted to determine what CV's are large enough to impact clinical assessment of perfusion. Standardization of CEUS scanners and procedures is crucial for limiting variability in CEUS quantification.

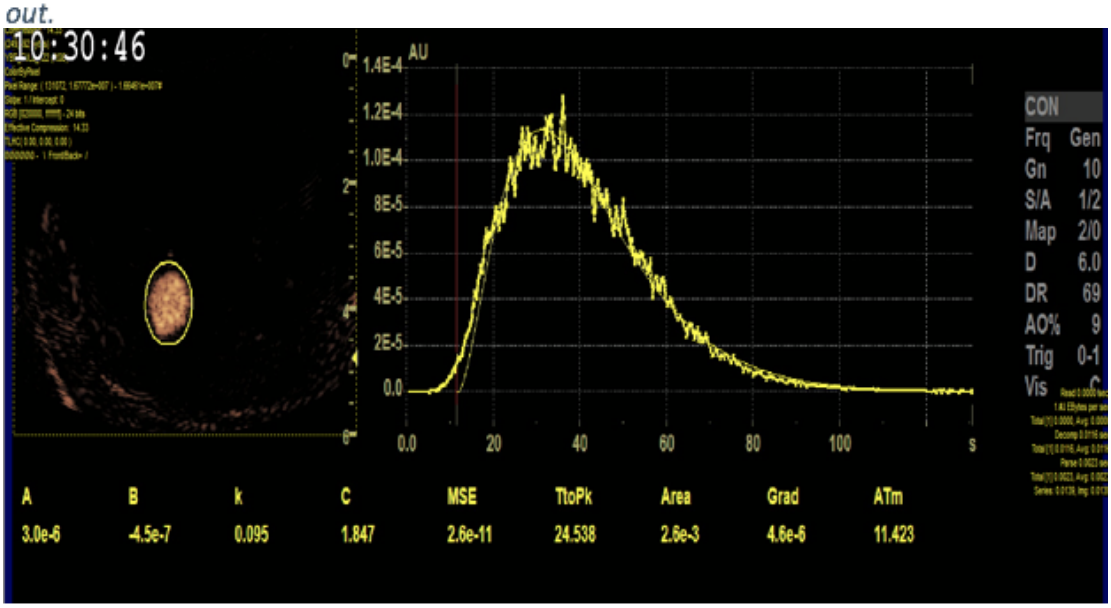
Acknowledgements

The contrast agents were generously gifted by the Ultrasound Research group at the Mayo Clinic, Rochester, MN. I would also like to thank Dr. Nicholas Hangiandreou, Dr. Zaiyang Long, Donald Tradup, and Scott Stekel for their generous contributions to this project.

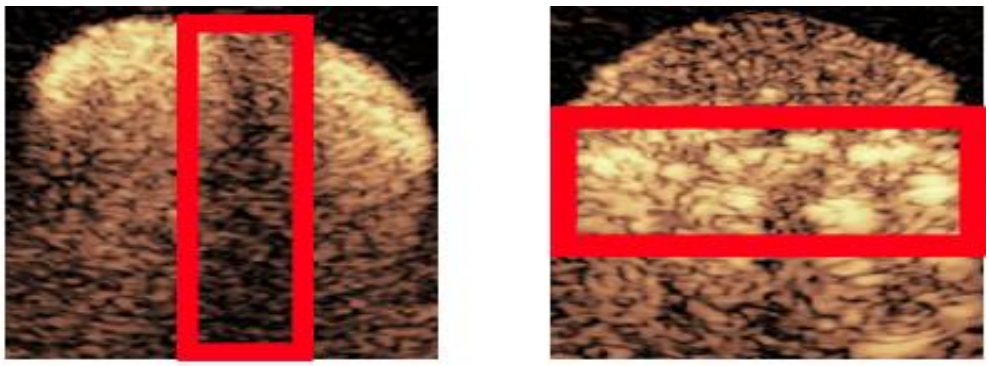
Supplementary Figures and Documentation



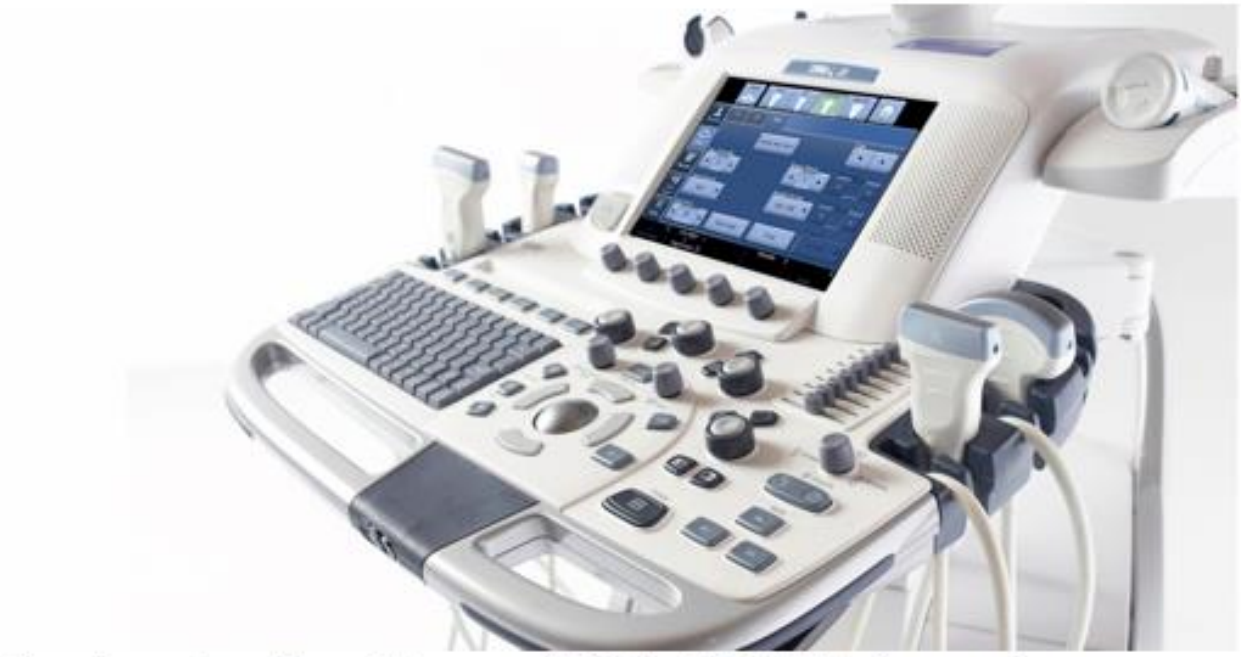
Supplementary Figure 1. Example of microbubble wash-in/wash-out in a tube perfusion phantom. *Data acquisition was carried out using a Phillips C1-6VN transducer connected to a Phillips LOGIQ E9 ultrasound scanner. These images represent snapshots of cine footage. Microbubbles are shown as golden spheres (individual microbubbles not shown). Left: represents wash-in; Middle: bright image represents peak intensity; Right: represents wash-out.*



Supplementary Figure 2. Example of raw linearized time-Intensity curve with Gamma Variate fit. *Data acquisition was carried out using a Phillips C1-6VN transducer connected to a Phillips LOGIQ E9 ultrasound scanner. Linearized data was obtained and fitted with a gamma variate curve fitting function using the Phillips onboard TIC analysis program (right). An ROI was selected and contains the entire tube diameter (left).*



Supplementary Figure 3. Example of shadowing and bubble interference in dialysis cartridge perfusion phantom. *Data acquisition was carried out using a Phillips C1-6VN transducer connected to a Phillips LOGIQ E9 ultrasound scanner. Shadowing (left) occurs when something obstructs the scanning window. Bubble interference (right) occurs when bubbles get trapped inside the phantom system.*



Supplementary Figure 4. Image of Phillips LOGIQ E9 ultrasound scanner dashboard and display. *Dashboard and display settings vary among different manufactures and models.*

Supplementary Documentation 1

Tube Flow Phantom System Manual

Joseph Pathoulas

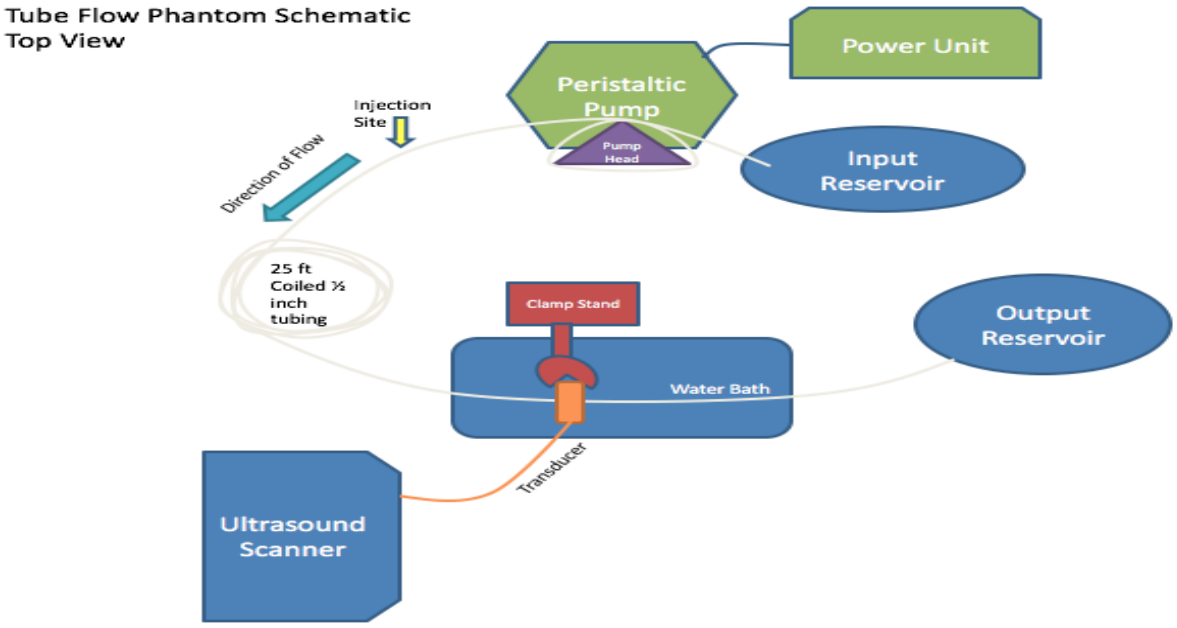
08/09/18

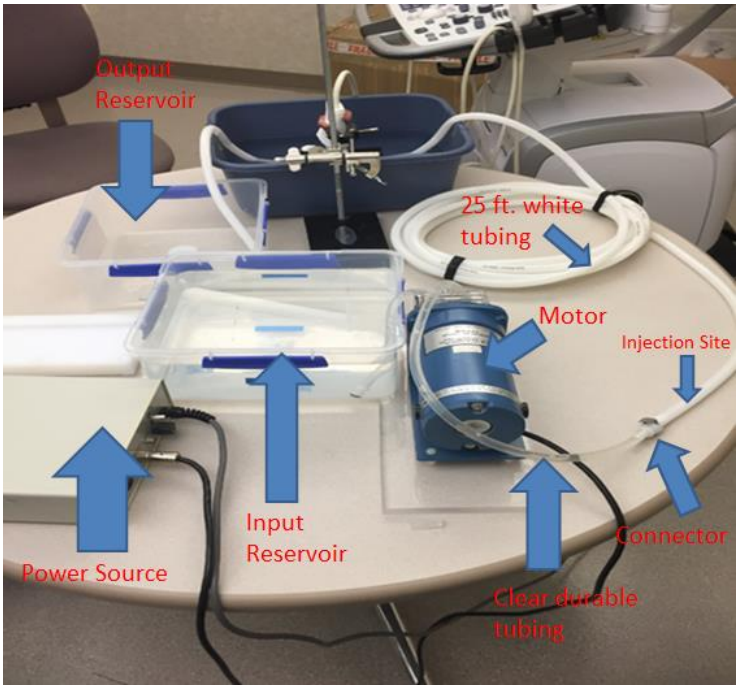
Table of Contents (pg. 1)

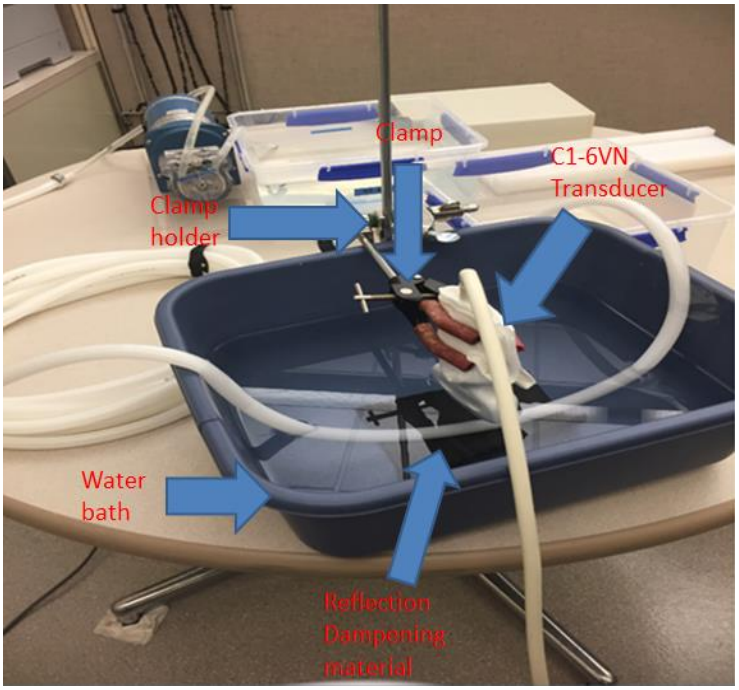
Materials List (pg. 2)

- ~25 ft 1/2 in. white tubing
- ~2 ft 3/8 in. clear durable tubing (goes through motor)
- 1 tube connector (connects white tubing to clear tubing)
- 1 Cole-Parmer Instrument peristaltic pump w/power source
- 3 plastic tubs (2 for water reservoirs, 1 for water bath)
- Clamp holder w/clamp
- Reflection dampening material (place under scanning window)
- C1-6VN Transducer
- 18-gauge needle
- Tape
- Water source (preferably degassed)
- Contrast microbubbles

Tube Flow Phantom Set-up (pg. 3)







Steps for setting up tube flow phantom (pg. 4):

- First, remove motor head from motor
- Place clear, durable tubing in the motor head and clamp motor head shut
- Reattach motor head to motor
- Connect clear, durable tubing to 25 ft white tubing via a connector keeping white tubing coiled
- Fill water bath with water and set-up clamping apparatus
- Submerge part of the white tubing in the tube and tape it down so it doesn't move
- Place tube endings in respective input/output reservoirs
- Fill input reservoir with water
- Turn on power source and twist knob to start the motor
- Pump out as much air as possible; remember to keep input reservoir full at all times and to empty output reservoir as needed
- Turn on ultrasound scanner and tightly clamp transducer positioned over submerged tubing
- The set-up is ready to run

Notes:

- Air bubbles will form, make sure that none are too close to the scanning window
- Make sure there are no air bubbles at the injection site when injection is imminent
- Tape down the clear, durable tubing to the input reservoir; the motor likes to "eat" the tubing, which will pull the tubing out of the input reservoir introducing air bubbles to the system

Default Scanner Settings (pg. 5)

All contrast imaging using this tube flow phantom system had been done on the **Abdominal Preset**.

The default scanner settings for the **Abdominal Preset** are:

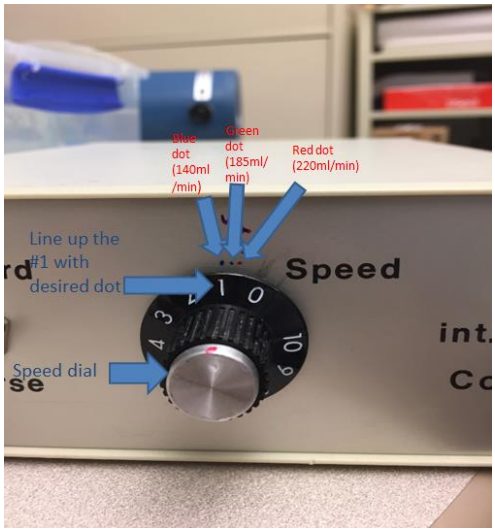
- MI: 0.13
- Tls: 0.0
- Frq: Gen
- Gn: 24
- S/A: ½
- Map: 2/0
- D: 15.0
- DR: 69
- AO%: 9
- Trig: 0-1
- Vis: C

For all standard tube flow phantom trials:

- The **Abdominal Preset** was selected
- **Gn was set to 10** (reduces background noise)
- Depth was set between 4-6, depending on how submerged the white tubing was in the water bath

Pump Settings (pg. 6)

The pump is controlled by its power source. The pump can move in the forward and reverse directions and has a speed dial that is numbered 0 to 10. There is no specific indication of what the actual velocity of the pump is, so three colored dots were marked above the dial for reference. Line the #1 on the dial up with each dot to obtain the corresponding flow rate (Blue dot=~140ml/min, Green dot=~185ml/min, Red dot=~220ml/min). If the tube flow phantom system set-up is correct, then the motor should be set to pump in **reverse** (pumping fluid forward through the tube). The pumps control setting should also be set to internal.



Contrast Injections

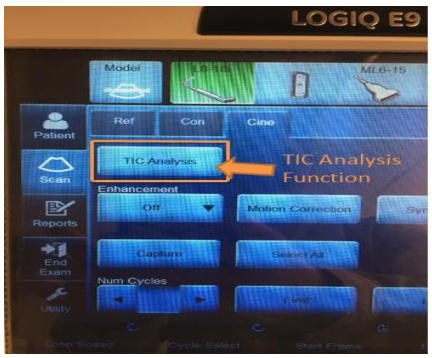
Injections of contrast agents were made at the injection site near the connector that connects the clear, durable tubing to the white tubing. 0.5ml bolus injections were applied over an average of 1 second. Prior to injection, the contrast agent was allowed to acclimate to room temperature and was then agitated by rolling it back in forth. Injections were given using an 18-gauge needle, which was poked through the wall of the white tubing.

Step-by-Step Instructions for Running Flow Trials (pg. 7)

1. Set up tube flow phantom as described above in **Tube Flow Phantom Set-up**
2. Power on the scanner and motor power source
3. Remove contrast agent from the fridge and allow it to acclimate to room temp.
4. Fill input reservoir and pump out as many air bubbles as possible
5. Open a new exam on the scanner and select the C1-6VN probe and **Abdominal Preset**
6. Switch **gain to 10** and adjust depth accordingly
7. Select the contrast function on the scanner (dual-view is sufficient)
8. Set pump to desired velocity, see **Pump Settings**
9. Once ready, agitate the contrast agent and then inject contrast agent at the injection site using a 18 gauge needle, see **Contrast Injections**
10. **Do Not** start the cine loop clip right away (the bolus takes some time to go through all the tubing depending on the velocity; the clips are very large)
11. For the first trial, measure the time it takes for the bolus to arrive then start the cine loop when signal first appears (use the initial time the bolus takes to arrive in subsequent trials to know when to start the cine loops)
12. Once the signal fades back to black (or close to black), approximately 120 seconds, select P1 to save cine loop
13. Keep the pump running to let all of the contrast agent run through scanning window (note: this may take a while; increase velocity and power to break the bubbles and have them run out faster)
14. Repeat steps 1-13 for subsequent trials

On-Board Curve Fitting (pg. 8)

The GE LOGIQ E9 scanner has an on-board curve fitting function called TIC Analysis. Once a cine loop is selected, the TIC Analysis button can be selected on the dashboard.



Once the TIC Analysis function is activated, the pointer can be placed on the cine loop to select an ROI (drag cursor over ROI and press the right trackball key to select). The shape and size of the ROI can be changed using the dashboard. After an ROI has been selected, move the cursor over the graph and press the left trackball key. This will activate a pop-up menu. On the pop-up system menu select **Vertical Unit**, and then **Acoustic units** (if preferred). The lines can be smoothed by selecting smoothing iterations (i.e. 7-sample average) by clicking on the **Smoothing** button on the dashboard. A data set can then be fitted by selecting the **Curve Fitting** button on the dashboard and selecting the fitting function of choice. There are three fitting options on the GE LOGIQ E9: Gamma Variate, Wash-in, and Wash-out; Gamma Variate being the only function that can fit the data completely.

To fit data to a Gamma Variate function, select **Gamma Variate**. This will then prompt the user to manually select a start and end time. This can be done by twisting the third (start point) and fourth (end point) knobs that are right below the dashboard. The on-board manual selection of a start and an end point are not very robust and would ideally be done offline.

Exporting TIC Traces

The GE LOGIQ E9 scanner allows for manual (USB) removal of trace data. To export a trace, select the ROI, smooth and fit the data as needed, and select the **Export Traces** button on the dashboard. It will then prompt the user to select a device to export the trace to, which will likely be a USB drive. Traces are exported as txt files and any smoothing will carry over in the trace along with the fitting parameters

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