Study of the robustness of Mean Transit Time with respect to Gain and Dynamic Range changes in ultrasound scanners

Master's Thesis

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Abstract

Pulmonary blood volume (PBV) has become a crucial indicator for cardiologists in order to assess the gravity of a cardiovascular disease. Indicator dilution theory has become a "gold-standard" in order to calculate perfusion parameters to obtain PBV. The improvements of contrast enhanced ultrasound technology have allowed the clinical community to avoid invasiveness in PBV calculations. However, some settings in the ultrasound scanners can influence perfusion parameters such as mean transit time (MTT). Software for modeling the influence of gain and dynamic range settings in ultrasound images was developed, and an in-vitro experiment was carried out.

We propose a solution to reduce the influence of gain and dynamic range on mean transit time. Gain normalization in log-compression functions in the ultrasound machine makes a smart adaptation of the scanners to different dynamic ranges and avoids saturation of the images and the indicator dilution curves obtained. With this new technique and with a dynamic range set above 40 dB, cardiologists could work safely knowing that their measurements in MTT will be reliable with very low errors (0.25%).

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1. Introduction

Pulmonary blood volume (PBV) is an important parameter in cardiology, anesthesiology and intensive care, due to the fact that it enables monitoring of the circulatory system and improves diagnosis of cardiac diseases.

Indicator-dilution theory has been a useful non-imaging based technique to measure cardiovascular parameters like PBV, cardiac output (CO), ejection fraction (EF) or stroke volume (SV) [1-2]. An indicator is a substance injected into the circulatory system that is easy to trace while it is passing through the body. In the case of thermo-dilution, a double catheter insertion was needed to detect the indicator concentration versus time (indicator dilution curve, IDC) of a contrast bolus [3]. Therefore, PBV measurements performed with this technique are considered very invasive.

Since recently, the development of contrast enhanced ultrasound (CE-US) allows a minimally invasive application of IDC in which ultrasound contrast agents (UCAs) are used as tracers and the catheters are replaced by an external US probe. UCAs are microbubbles (1µm - 10µm) that reflect ultrasound signal and respond with a shape change. They can easily be detected and their signal can be caught by a transducer, in order to get the desired IDC: the passage of the UCA through a region of interest (ROI) can be detected versus time in B-mode [4], which converts acoustic intensity into 8-bit grey level.

PBV is calculated as the multiplication between the CO and the mean transit time (MTT) of the indicator between the right ventricle (last cardiac chamber before entering the pulmonary circulation) and left atrium (first chamber after the pulmonary circulation) [4].

CO is computed from the product of Doppler-derived stroke volume and heart rate (HR) [5]. SV is estimated using ultrasound Doppler to measure the volume of blood moving to the ascending aorta (coming from the LV) during systole (contraction of the heart). MTT is defined as the time that the indicator takes to cover the distance between the injection and the detection sites and can be derived easily from fitting models of the IDCs [4].

To date, gaps exist in our understanding of how the images obtained by CE-US, and therefore IDCs and MTT, are influenced by different settings in the US machines. For example, it is known that the use of a high mechanical index (MI, ratio of peak negative pressure used in transmission and the frequency of the transmitted signal) can disrupt the bubbles [6] and cause other bio-effects. Currently the FDA (Food and Drug Administration) has ruled that a maximum MI of 1.9 may be used by US scanners [7].

Others have stated that a gain applied to linearized log-compressed images can produce errors in perfusion parameters while working on a low dynamic range (<45dB), which is the range clinically available [8-9].

The aim of this study was to assess how dynamic range and gain variations in the ultrasound scanner can influence perfusion quantification parameters like MTT. To this

end, a simulation model was built: log-compression, linearization and gain were applied to a simulated IDC and the error on MTT was estimated by comparing those parameters to the ones introduced in the IDC during simulation.

2. Methodology

2.1. Software

A script in Matlab (MathWorks, Natick, NA) was developed in-house, in order to simulate a sequence of images similar to the one the user obtains from the US machine (multi-frame DICOM file). The sequence was created assuming the transducer was set in a transverse position (90º angle) scanning the passage of an UCA through a single cross-section of a tube:

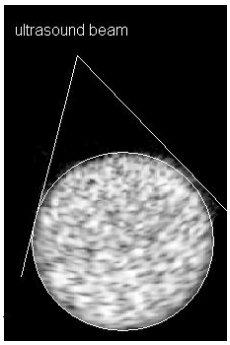

Fig. 1 Cross-section of the tube. Ultrasound image after setting the transducer in a transverse position

2.1.1. LDRW model

The passage was simulated based on the local density random walk (LDRW) model, which provides the most accurate IDC interpolation and volume measurements as well as a physical representation of the dilution process [4] [10]-[13] (Fig. 2). It assumes a Gaussian spatial distribution of the bubbles that travel with the fluid. LDRW models the UCA concentration $C(t)$ as a function of time:

$$C(t) = \frac{m}{Q} e^{\lambda} \sqrt{\frac{\lambda}{2\pi\mu t} e^{\frac{-\lambda}{2}\left(\frac{t}{\mu}+\frac{\mu}{t}\right)}} \qquad (1)$$

where perfusion parameters can be easily derived: m is the injected mass of the contrast agent, Q is the volumetric flow, λ is a parameter related to the diffusion constant of the system (skewness of the curve), and μ is the average time the contrast takes to go from the injection to the detection site (MTT).

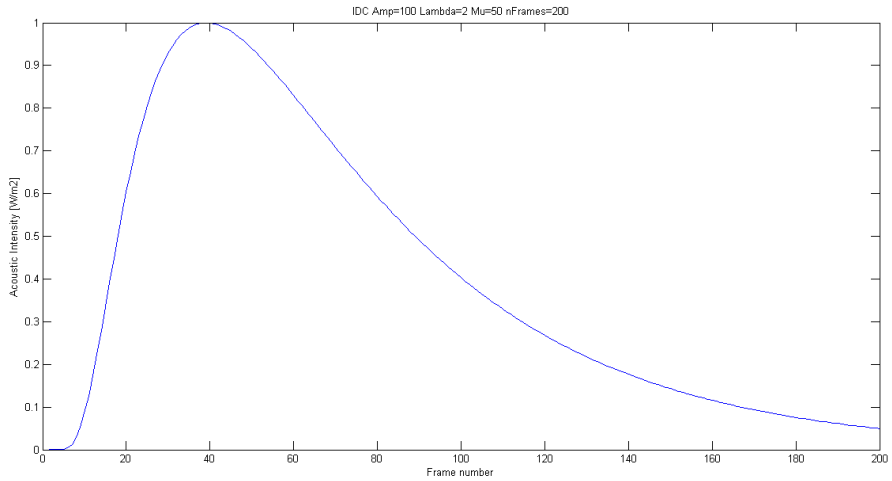

Fig. 2 IDC generated with the LDRW model. λ=6, μ=50, nr. Points=200.

2.1.2. Dynamic Range

The dynamic range is the range of signal echoes that can be displayed by the echocardiography system. After demodulating the echoes received, the US machine converts pressure measurement into voltage, which will be quantized and represented as grey-levels by a logarithmic compression [8]:

$$QL(V) = uint8\left[(2^8 - 1)\frac{20dB}{LC_{DR}} log_{10}\left(\frac{V}{V_{max}}\right) 10^{\frac{LC_{DR}}{20dB}}\right] \quad (2)$$

followed by a linearization [8]:

$$EP(QL) = \left(V_{max} 10^{\left(\frac{QL}{2^8-1}-1\right)\frac{LC_{DR}}{20dB}}\right)^2 \quad (3)$$

where V is the echo-amplitude amplified by the gain $(s(t) \cdot G)$, $Vmax$ is the maximum echo-amplitude of the signal in the selected ROI, LC_{DR} the dynamic range of log-compression, expressed in dB, and $uint8$ represents the 8-bit unsigned integer quantization typecast operator.

Similarly, log-compression (Eq. 2) and linearization (to reverse log-compression) (Eq. 3) were applied to the sequence of images.

A graphics user interface (GUI) for the program was designed. Several parameters that influence the intensity of the signal in the sequence of images can be changed by the user: parameters of the IDC (λ, μ, amplitude), number of frames of the sequence, number of horizontal and vertical pixels of the images, different types of noise and its variance, (parabolic) velocity profile and velocity dependent spatial distribution of the bubbles, and different values for gain and dynamic range (log-compression).

2.2 In-vitro

In-vitro images were acquired in order to observe the influence in MTT of changing gain and dynamic range settings in the machine. The in-vitro system is shown in Fig. 3. A reservoir of water is connected to an occlusive roller pump via a ½ inch tube. The pump is set to maintain a constant flow rate. The output of the pump is connected to a variable volume network (consisting of 4 diverging tubes that merge again in a single tube), in order to be able to vary the volume of the system and to ensure adequate mixing of the UCA with the water in the system between the injection point and the detection point. The ultrasound transducer is placed just above a soft polyurethane tube in a small basin filled with water for image acquisition. An electromagnetic flowmeter is set after the basin, in order to get the precise flow at any time. The hydrodynamic circuit is closed.

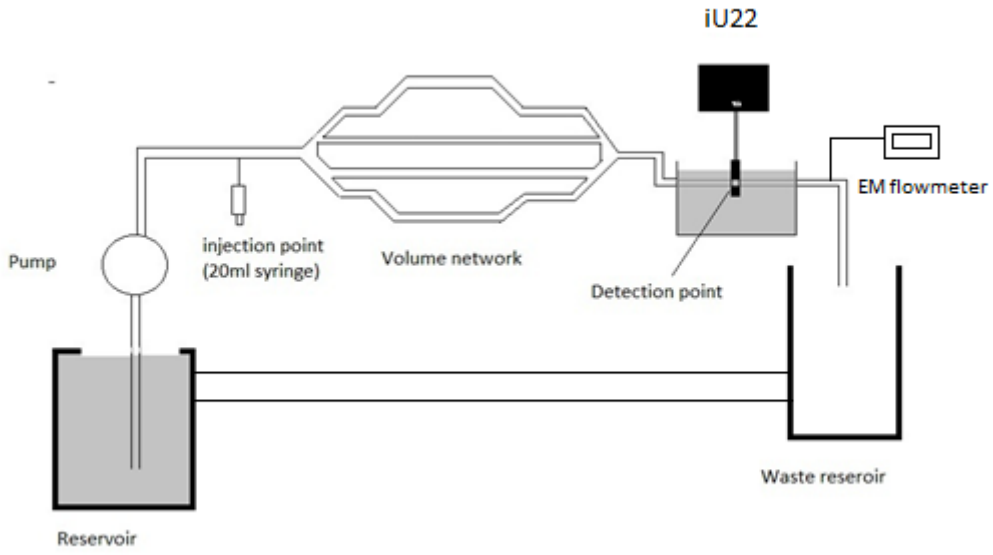

Fig. 3 Schematic representation of the in-vitro setup

3. Experiments

3.1. In-silico

A sequence of images simulating the passage of bubbles through a circular tube was generated. The change of intensity and bubble concentration are following an IDC based on the LDRW model (see Fig.4). The amplitude for the IDC selected was equal to 100, λ was equal to 6 and μ was equal to 50 frames. The sequence was designed to have a length of 200 frames and a size of 400x400 pixels per frame. A parabolic velocity profile of the bubbles and Gaussian noise with a 0 mean and a σ^2 of 0.04 were added to the

signal. Gain and log-compression values ranging from -10 to 50 dB and from 10 to 70 dB were chosen respectively.

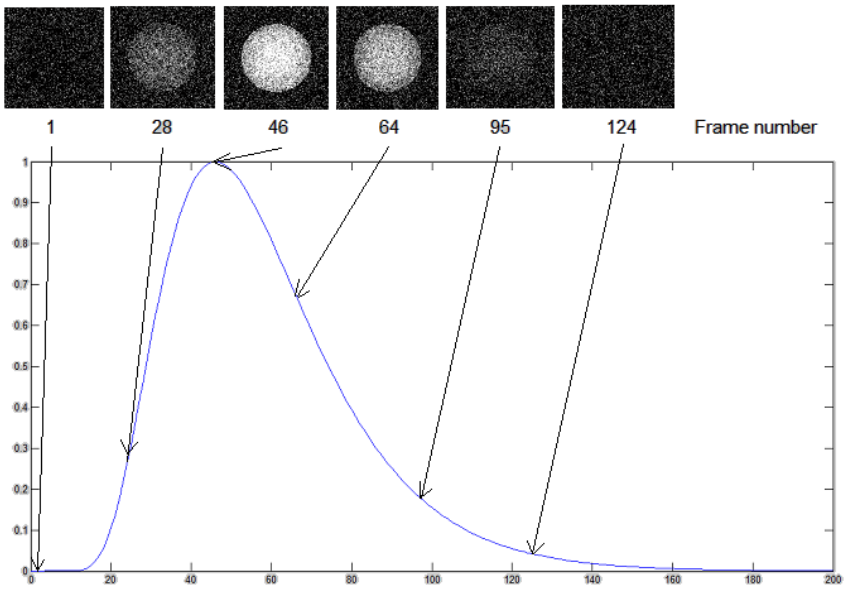

Fig. 4 Six different frames from the sequence of images generated following the IDC above.

A ROI (Fig. 5) was drawn to include the whole circle that represents the tube. Thus, a measured IDC was obtained by taking the average in the ROI for all frames subsequently and a fit was made using the LDRW model, in order to obtain the perfusion parameters of interest. This process was repeated for each gain and dynamic range combination.

Relative errors ε_μ of the estimated μ as a function of LC_{DR} and different values of gain were computed by comparing the estimated μ with the one introduced in the input curve:

$$\varepsilon_\mu = \frac{\mu_{est} - \mu_{inp}}{\mu_{inp}} \; x \; 100\% \qquad (4)$$

3.2 In-vitro

12 injections were performed into the system, one for each combination of gain and dynamic range. Gain values of 25, 50, 75, and 100% and dynamic range (compression) values of 36, 55 and 70 were used. The UCA was made by mixing 25 mg of SonoVue® lyophilised powder with 5 ml of saline. Images were acquired with a Philips C5-1 Transducer set at 90º (transverse position) from the tube, and connected to a Philips iU22 ecocardiography system. Subsequently, an IDC was quantified from a ROI that includes the whole tube with Philips QLAB. The IDC was fitted following the LDRW model with an in-house script in Matlab (MathWorks, Natick, NA) in order to obtain what we want to evaluate: that MTT may depend on combinations of settings for gain and LC_{DR}.

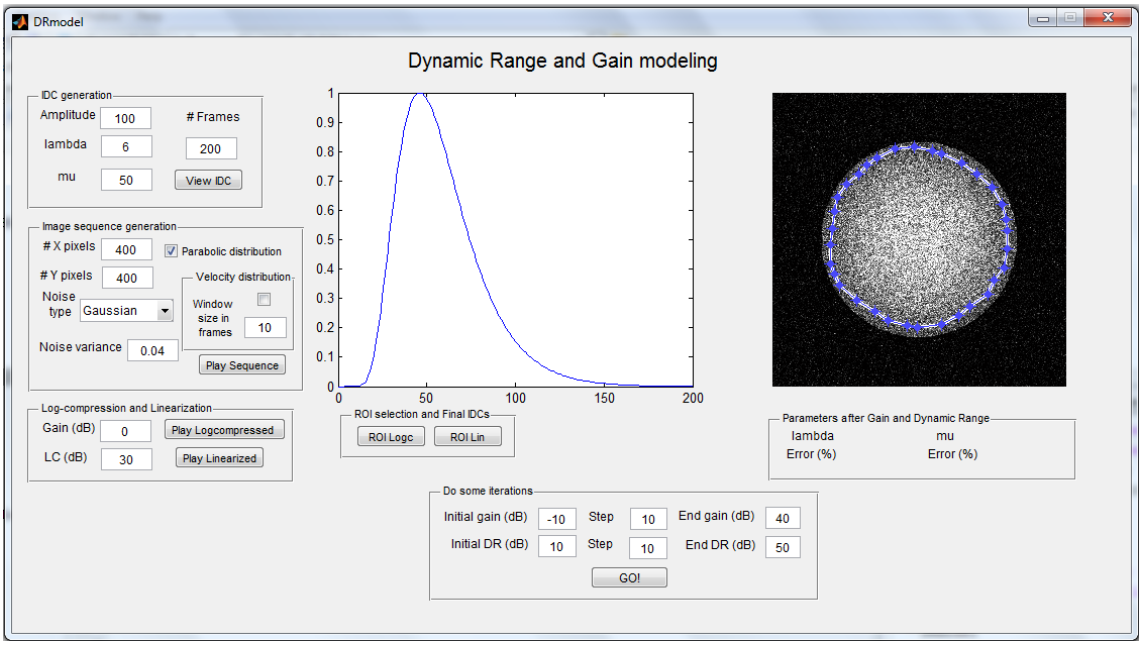

Fig. 5 Graphics User Interface of the software.

4. Results

4.1 Software

Results are shown in Fig. 6. A color map for the relative error in μ for each combination of gain and dynamic range has been plotted. The darker colors mean low error, and the brighter colors mean high error. It can be appreciated that ε_μ is quite stable and low (0.06% - 5%) until a gain value of 10 dB. Then, the error rises abruptly to more than 75% above 20 dB gain. This is due to the fact that when increasing the gain above a certain value, the curve gets clipped, as it can be observed in Fig. 7. Clipping affects the parameters extracted from the fitted curve and the quality of the fit. Until now, it is not clear that a solution is needed.

What we propose to avoid the clipping is to normalize the fraction in Eq. (2), viz. $\frac{V}{V_{max}}$, by a gain factor. This means that $Vmax = max_{ROI}[s(t) \cdot G]$, $s(t)$ being the sequence of images, in a way that when V is maximum the fraction will always be 1. Thus, log-compression does not depend on the gain anymore but on the dynamic range.

The effect of using the new value for V_{max} instead of a fixed value independent of the gain is shown in Fig. 8, where the log-compressed and linearized IDCs obtained do not suffer from clipping. Relative errors in μ with the new formula are shown in Fig. 9. For dynamic ranges larger than 30 dB, μ errors become stable and low (0.16% - 0.25%). This is also shown in Fig. 10, where ε_μ is plotted as a function of dynamic range since is not dependent on the gain anymore.

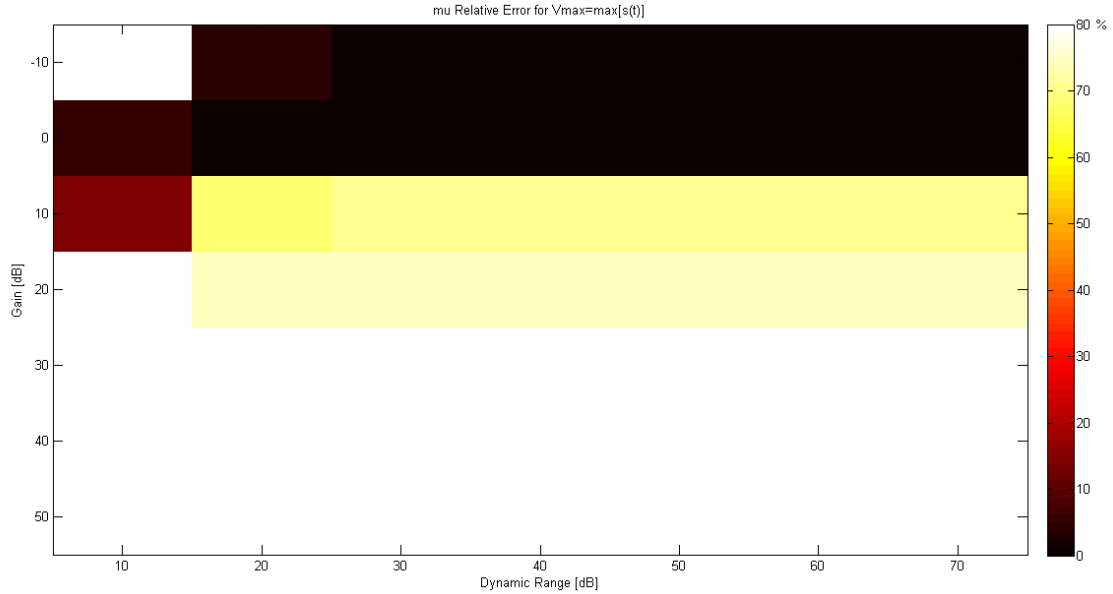

Fig. 6 µ relative error for V_{max}=max[s(t)].

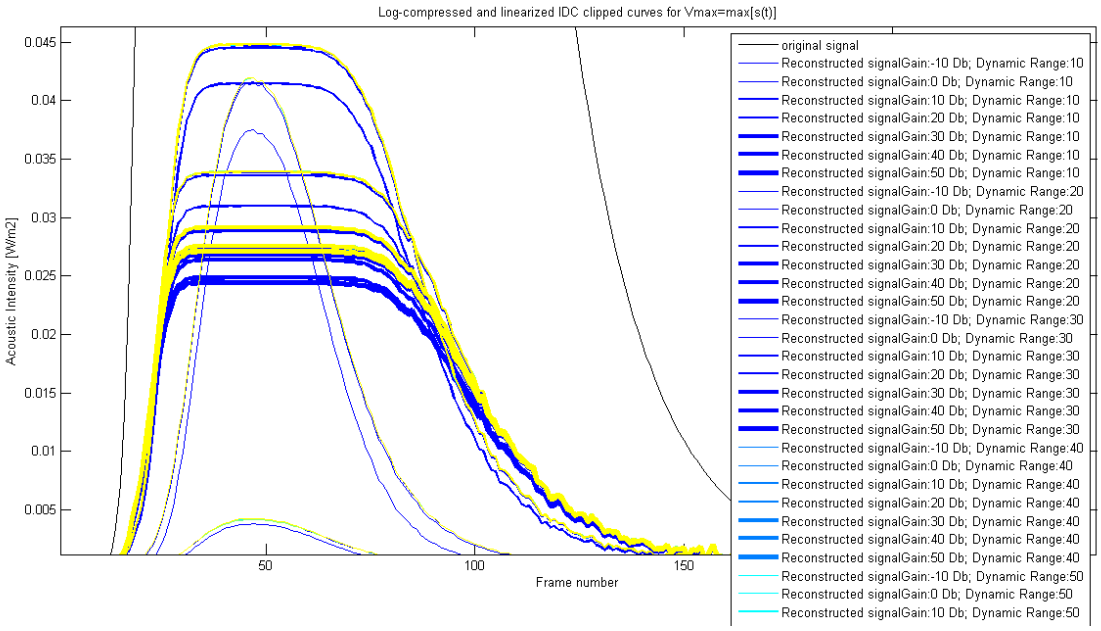

Fig. 7 Log-compressed and linearized IDC clipped curves after 20 dB gain for V_{max}=max[s(t)]

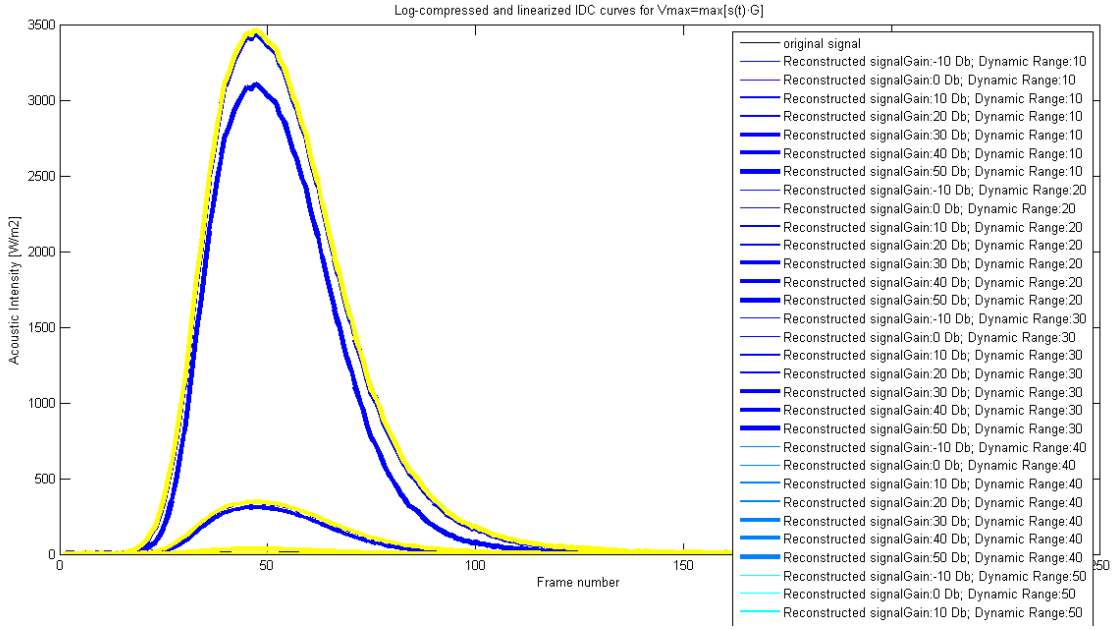

Fig. 8 Log-compressed and linearized IDC curves for Vmax=max[s(t)·G]

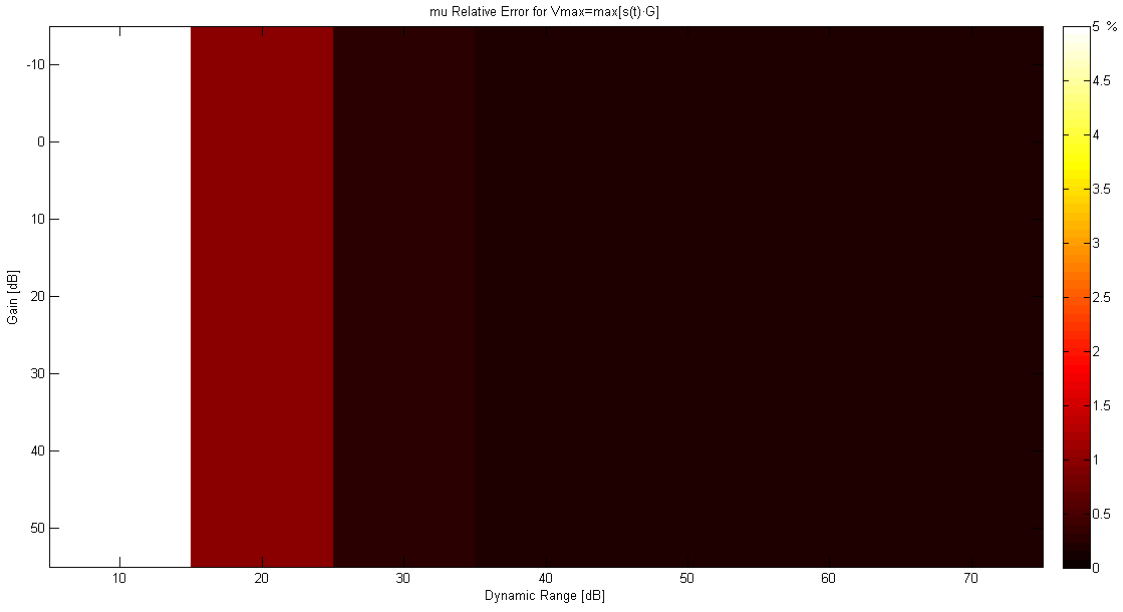

Fig. 9 µ relative error for Vmax=max[s(t)·G]

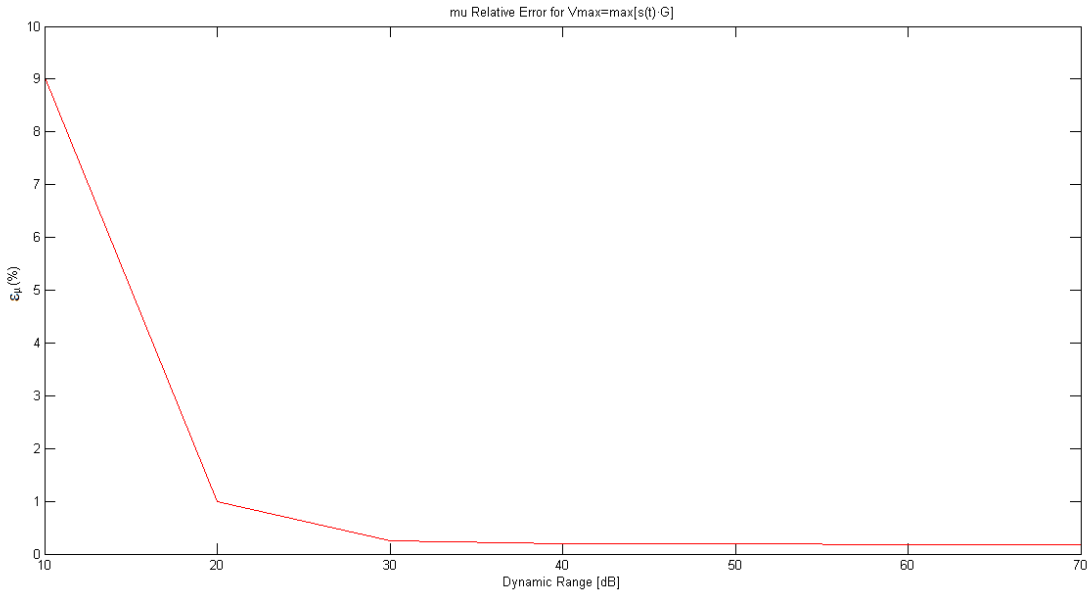

Fig. 10 μ relative error as a function of dynamic range.

4.2 In-vitro

Results of the in-vitro experiments can be seen in Fig. 11. A color map for MTT values (8 – 14 s) for different combinations of gain and dynamic range has been drawn. The resulting curve from 25 dB gain and 36 dB dynamic range was not plotted because the values are so low that no bubbles appeared on screen. It appears that for higher gains, MTT increases, there are outliers and we do not see a pattern.

In Fig. 12 we can observe that this is probably produced by the gain: for 25 dB gain the curve is really weak in intensity; when higher values of gain are reached (75-100 dB), the curves get spread, clipped and they don't look like an IDC anymore. We also want to note that a huge dynamic range (70 dB) combined with high gains can damage the shape of the curve. The solution again would be to normalize the log-compression function of the machine so MTT becomes independent of the gain.

Fig. 11 MTT values for in-vitro setup

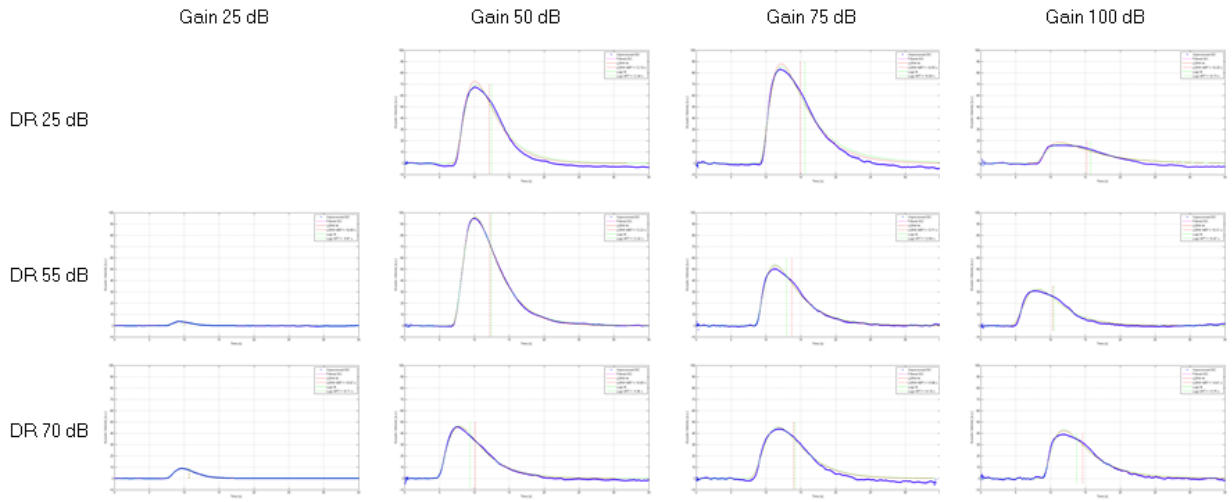

Fig. 12 In-vitro IDCs for different gain and DR combinations

5. Conclusions

We found that different settings in ultrasound scanners can affect perfusion parameters and, therefore, derived cardiac indicators like PBV or EF. Log-compression functions in the actual machines give saturation problems and this can influence in perfusion parameters calculations. Directly, highly varying values of MTT are obtained as seen in-vitro.

However, the solution by normalization shows that gain should not influence MTT at all, but only dynamic range should. Beyond a certain value of dynamic range we did not observe large errors (0.25%), so a recommendation would be to always set the dynamic range above 40 dB.

Therefore, another suggestion would be to take V_{max} as the maximum of the ROI drawn instead of a fixed value. With this feature, the machines could show the correct adjustments for gain and dynamic range to be changed by the user in order to work in an optimum way.

6. References

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Collection of descriptions and results of the experiments performed at the Catharina Hospital

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Introduction

Ultrasound Indicator Dilution Curves (IDC) can be used to analyze several cardiovascular parameters, like cardiac output (CO) or pulmonary blood volume (PBV) [1-2]. IDC technique has become very useful in order to analyze cardiovascular dysfunctions.

In the late 70s, cardiologists needed the use of invasive techniques like catheterization of the patient: firstly, a contrast agent (cold saline-thermodilution or dyedilution) was injected at a concrete time and was detected with a catheter at some point in the circulatory system [1] [2]. Nowadays, with technology progress, invasive techniques can be avoided with the use of echography machines and ultrasound contrast agents (UCAs) [3]. UCAs are microbubbles (1μm - 10μm) that reflect ultrasound signal and respond with shape change. They can be easily detected and processed by a transducer, in order to get the desired IDC [4] [5]: the passage of the UCA through a region of interest (ROI) can be measured versus time in B-mode [6], which converts acoustic intensity into a 8-bit grey level.

With parameters like Mean Transit Time (MTT) or Area Under the Curve (AUC) derived from IDC, cardiologists, anaesthetists, and intensivists can assess how the heart is functioning.

However, there is uncertainty about the effect of several situations on the behavior of the bubbles (settings of the echomachine, concentration of injected UCA, peripheral resistance, etc.) unlike in the past IDC techniques, like dye-dilution, where dye-concentration in the blood was measured, or thermodilution, where temperature change was measured over time.

The objective in this project is to have an idea of how much MTT and AUC can change due to the circumstances and try to predict these variances. In order to convert this technique into a reproducible system, we tried to prove our different hypotheses with in-vitro and in-vivo experiments, although calculations in-vitro are made with water instead of blood (different flow regime). In this way, measurements will be more precise, and information on the heart will be more reliable for cardiologists.

Experiments

I. Pump speed and flow calibration

- **Introduction and hypothesis**

  In order to obtain good and reproducible results, sometimes it is needed to go to the basics. The first thing to do is to get in touch with the system with which the measurements will be performed. This one is the in-vitro system, which is described in Appendix A.

  One basic thing to check in the hydrodynamic system is how the flow is affected by the speed of the pump. The hypothesis is that the flow will increase linearly with the speed of the pump.

- **Methodology**

  The PiCCO-meter (Appendix A) can detect temperature variation and give a flow measurement in l/min, as well as it will be given by the Electromagnetic (EM) flow-meter.

  4 injections of cold saline (4°C) were injected to the system at 5 different speeds of the pump to ensure reproducibility of the measurements. The volume chosen for the injections was 20 ml, due to the fact that the PiCCO-meter cannot work with less volume injections.

  Since the PiCCO-meter and the EM flow-meter give different values, another experiment was performed in order to measure the flow of the system: in a certain pump speed, the output of the system was collected in a volume measuring jar during 1 minute. Then the amount of water accumulated in the jar was measured using the jar's scale.

  Calibration was performed until 110 RPM, because farther than that the soft tube gets damaged/explodes due to high pressure.

- **Results and recommendations**

  Results are shown in Table 1.

Table 1. Results on the calibration of flow – pump speed

RPM	EM Flow (l/min)	PiCCO Flow (l/min)	Volume jar Flow (l/min)	AUC (linear s)
30	1,24	1,83	1,25	2,24
	1,25	1,77		2,29
	1,24	1,68		2,38
	1,26	1,64		2,44
50	2,07	2,81	2,00	1,45
	2,02	2,69		1,53
	2,01	2,60		1,56
	2,06	2,67		1,50
70	2,95	3,71	2,85	1,06
	2,91	3,58		1,10
	2,96	3,64		1,11
	2,90	3,53		1,12
90	3,68	4,80	3,68	0,86
	3,67	4,64		0,88
	3,67	4,56		0,89
	3,68	4,67		0,87
110	4,53	6,33	4,45	0,63
	4,53	5,88		0,71
	4,57	5,65		0,74
	4,53	5,60		0,74

As expected, flow is increasing linearly with pump speed (see Fig.1), whereas AUC given by the PiCCO-meter is doing exactly the opposite thing.

The average of the 4 results was taken for each pump speed in order to show the graphs on Fig. 1 – 3.

Figure 1 shows EM and PiCCO flow measurements as a function of pump speed. Although an overestimation (higher values than the ones measured with the jar) made by the PiCCO-meter can be appreciated respect to the volume jar flow, in Fig. 2 is shown that the pump works linearly with the flow. Correlation coefficients are very high in both methods.

Flow can influence volume, defined by Eq. 1 [Mischi]. Therefore in a same volume, if flow increases, MTT should decrease. If the MTT of the IDC decreases then AUC should do it as well. In Fig. 3 this effect can be observed, though we plotted 1/AUC to see that the linearity of the pump gets confirmed.

$$V = Q \cdot MTT \qquad (1)$$

One of the main problems in this experiment was that with every cold injection the temperature of the system decreased. So a recommendation is to use an immersion heater to stabilize the temperature.

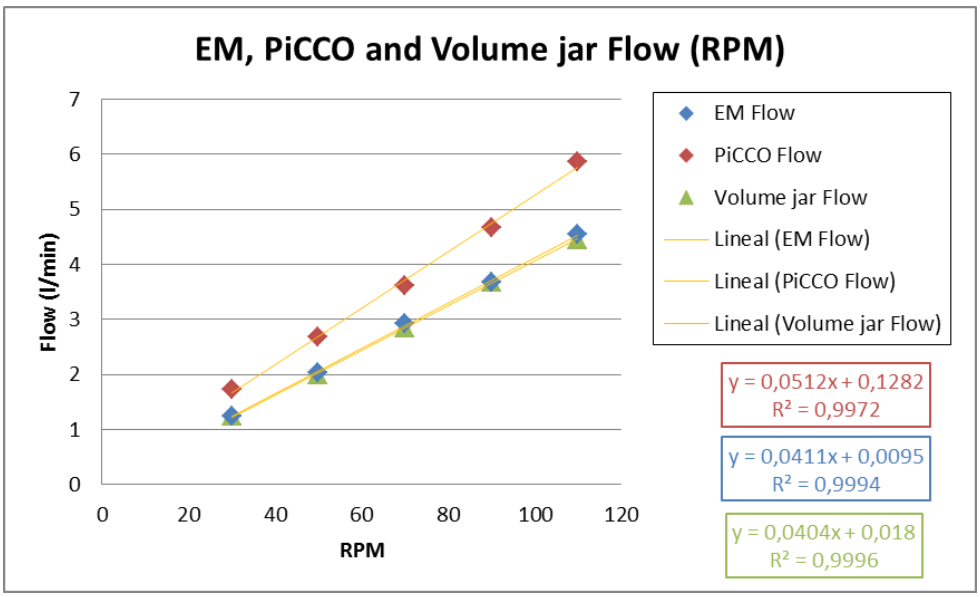


Fig. 1 EM Flow and PiCCO Flow as a function of pump speed

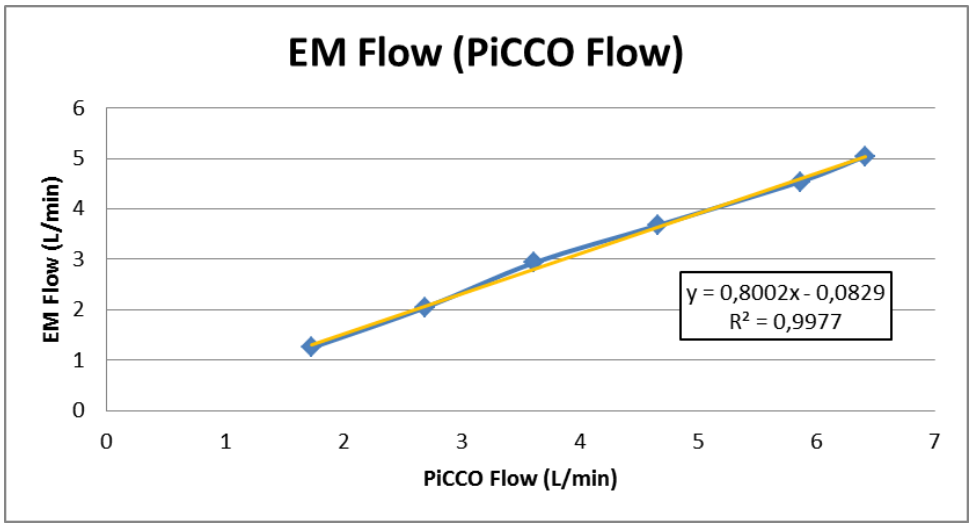


Fig. 2 EM flow as a function of flow given by PiCCO

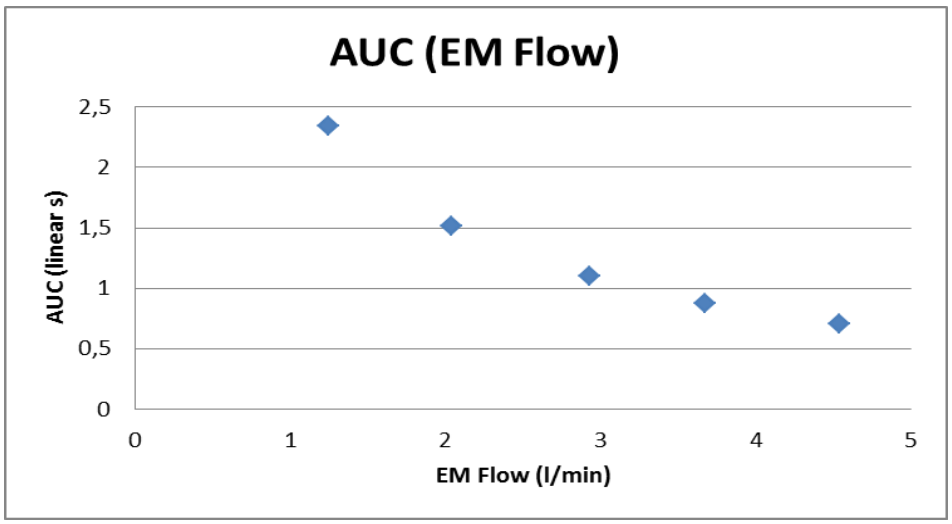


Fig. 3 Area Under the Curve as a function of flow

II. Reliability of QLAB

- **Introduction and hypothesis**

  When making experiments and processing the data with a program, its reliability must be checked. We want to test how the size, shape and position of the drawn regions of interest (ROI) and other different settings of Philips QLAB software affect MTT. A ROI is a drawing that can be made with QLAB and can detect the passage of an UCA from a DICOM Image Sequence.

- **Methodology**

  The experiment consisted of obtaining IDCs from several patient DICOM sequences, made with a Philips iE33 Ecocardiography System. These data were obtained by means of transthoracic echocardiography (TTE), that means with the transducer set in direct contact with the body, after aproval of the local ethical committee.

  - **Size, shape and position of the ROIs**

    In order to test the position of the ROIs, 6 ROI squares of $5mm^2$ were placed on top of each other in the image, inside the left ventricle (LV). One bigger ROI was drawn covering the whole LV, as shown in Fig. 4 (maroon line). Thus, 7 IDCs were generated and processed with an in-house script in MATLAB called cardioGUI (see AppendixB) to obtain MTT. The same test was made in the RV.

    ROIs should always be drawn such that they don't touch any wall of the heart chambers and cover the space where the bubbles pass by.

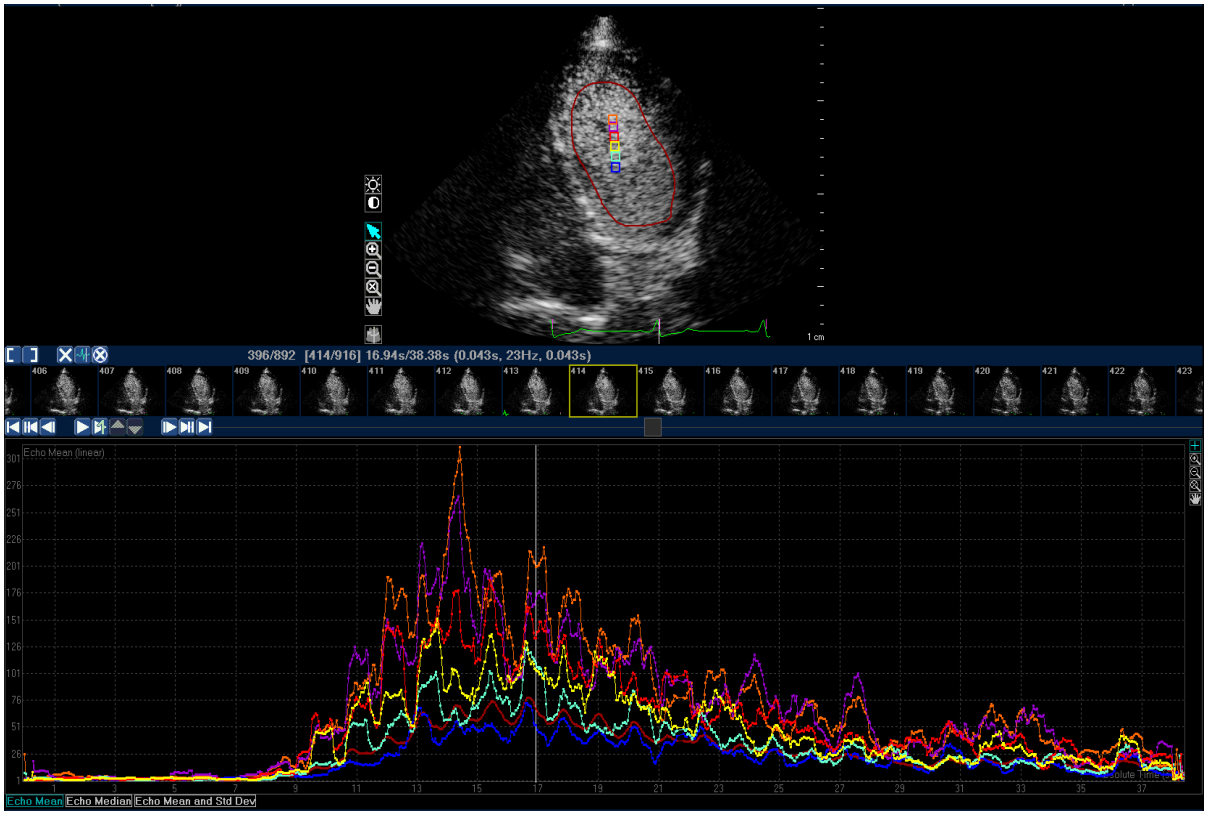


Fig. 4 ROIs and IDCs generated with QLAB on RV

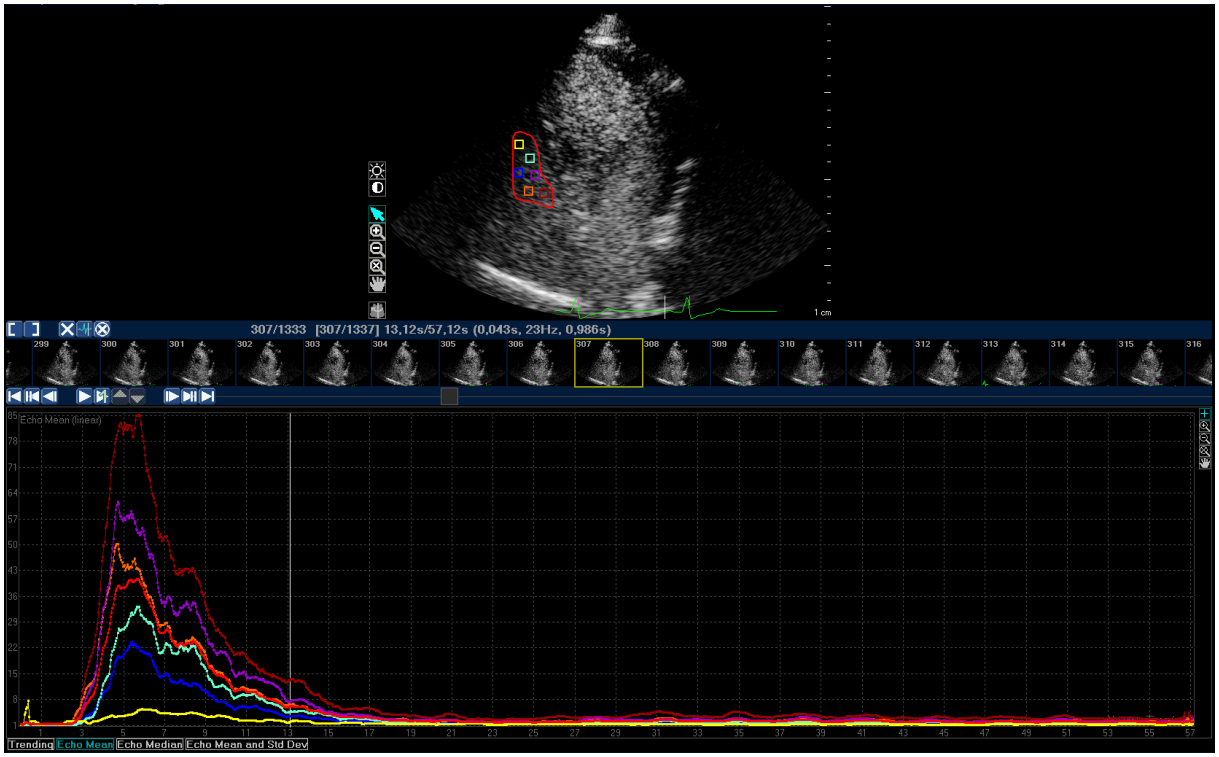


Fig. 5 ROIs and IDCs generated with QLAB on RV

- **ECG mode ON/OFF and Data Smoothing ON/OFF**

  If electrocardiogram (ECG) mode is ON, QLAB only takes into consideration those frames that are synchronized with the contractions of the heart taken from the DICOM Images.

  Data Smoothing is a feature based in an algorithm that captures similar patterns on the IDC and reduces the noise (Fig. 6) [7]. It increases the signal-to-noise ratio and allows the signal characteristics (peak position, height, width, area, etc.) to be measured more accurately, especially when methods of locating and measuring peaks are being employed.

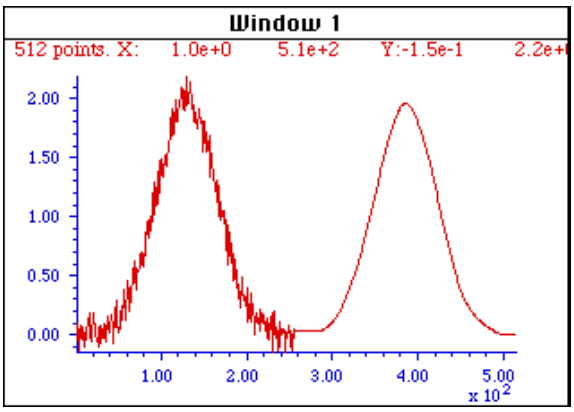


Fig. 6. Data Smoothing on a curve. Left curve is an original noisy curve, right curve is a curve with Data Smoothing applied.

We tested all combinations of Data Smoothing and ECG Mode settings by placing a ROI in the LV and another one in the RV, then obtaining the MTT with cardioGUI.

- Results and recommendations

- **Size, shape and position of the ROIs**

  Results are shown in Tables 2 and 3: although the ROIs are in different positions and have different shape, MTT only changes a 2,61% (coefficient of variation is defined as standard deviation/mean or how far are the values from the mean of all of them) for the LV, and a 4,86% for the RV.

Table 2. MTT comparison in the different ROIs, placed on LV

ROI	MTT (s)
Square 1	19,69
2	19,33
3	18,66
4	18,61
5	18,75
6	19,81
Whole	19,38
mean	19,18
stdev	0,50
c.v. (%)	2,61

Table 3. MTT comparison in the different ROIs, placed on RV

ROI	MTT (s)
Square 1	6,55
2	6,92
3	7,60
4	6,85
5	6,65
6	6,91
Whole	6,87
mean	6,91
stdev	0,34
c,v, (%)	4,86

- **ECG mode ON/OFF and Data Smoothing ON/OFF**

The results in one patient data are shown in Tables 4, Fig. 7, 8 and 9. In most of them the curves in ECG mode could not be fitted by cardioGUI.

As stated in the Methodology section, it is better to have activated the Data Smoothing feature if the signal will be subsequently processed by an algorithm that can be adversely affected by the presence of too much high-frequency noise in the signal (LDRW) [7].

Thus, a recommendation to get better results is to work always with Complete Framing and Data Smoothing ON.

- MTT in QLAB and cardioGUI

While developing this experiment we observed that there were differences between the MTT values given by QLAB and cardioGUI. Trying to understand why, we reached the conclusion that cardioGUI gives MTT from 0 and QLAB from the beginning of the curve. A first recommendation would be to always use cardioGUI in order to calculate MTT values. This is shown too in Fig. 7.

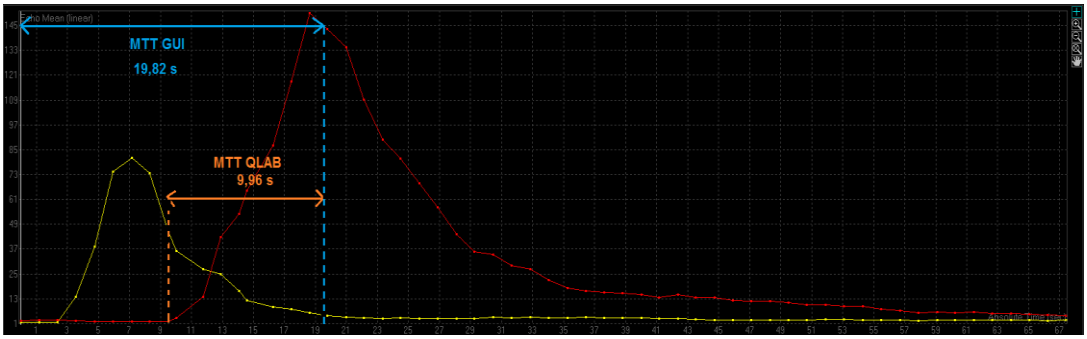


Fig. 7 ROI in RV (yellow) and in LV (red) with ECG mode ON

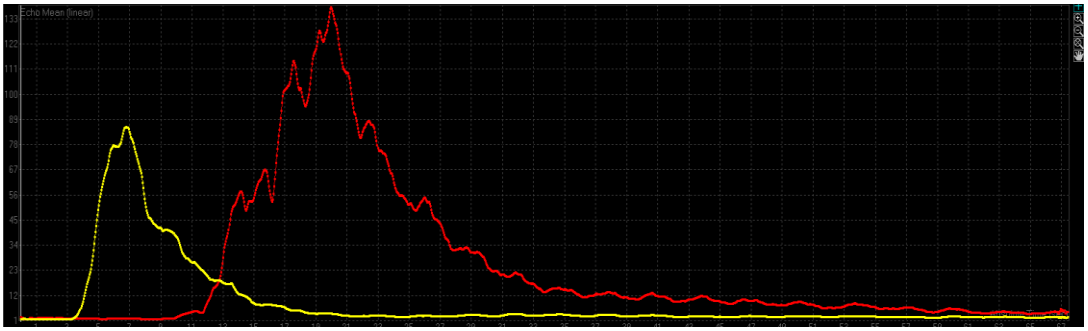


Fig. 8 ROI in RV (yellow) and in LV (red) with ECG mode OFF and Data Smoothing ON

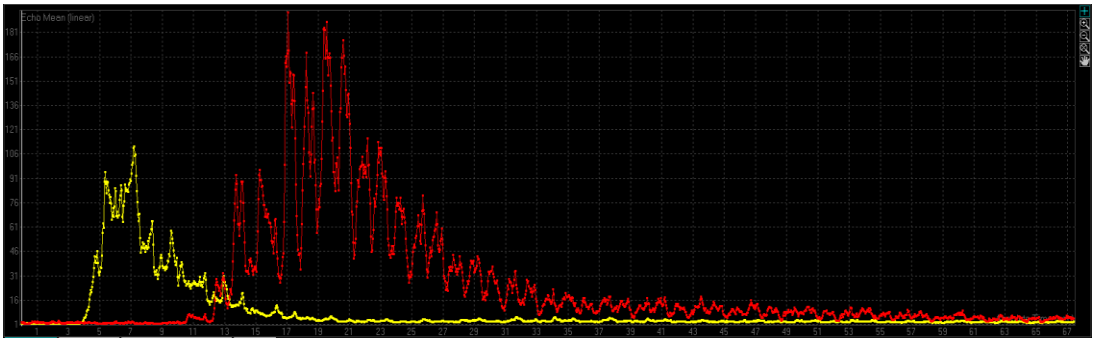


Fig. 9 ROI in RV (yellow) and in LV (red) with ECG mode OFF and Data Smoothing OFF

Table 4. MTT results on the different settings

ECG	DS	MTT RV (s)	MTT LV (s)	ΔMTT (s)
ON	OFF	7,11	19,19	12,68
ON	ON	7,32	19,82	12,50
OFF	OFF	6,76	19,54	12,78
OFF	ON	6,85	19,71	12,86

III. "Guyton" and Polynomial fittings

- **Introduction and hypothesis**

  Since QLAB was returning unexpected values for MTT, the values given by cardioGUI had to be validated as well. The approach was to build our own scripts in MATLAB using the basic concept of MTT found in "Cardiovascular Function, Principles and Applications" [18].

  In the book, MTT is described as the average of the time that a particle needs to pass from one point to another in a vessel.

  For example, let's assume that 10 particles are injected at the same time into a flowing system and mix evenly in it. At a subsequent point all the particles are collected again with different transit times which are:

Numbers of particles	Transit time (sec)	Number multiplied by time
3	7	21
1	8	8
2	9	18
1	10	10
3	11	33
Total : 10		Total : 90

  Mean transit time = 90/10 = 9 seconds, following Eq.1, stated in the book:

$$\frac{\int_0^\infty t \cdot C(t)dt}{\int_0^\infty C(t)dt} \qquad (1)$$

  Besides, another method came up using the *polytool* function from MATLAB. It fits the curve with a polynomial function, depending on the degrees the user inserted.

  The expectations are that after writing our own programs to fit IDCs and calculate MTT the results will be similar to the ones given by cardioGUI. QLAB is also added to prove that the values of MTT obtained with this program are wrong, as seen in Experiment II.

- ## Methodology

Since the programs had to be user-oriented, we needed to learn how to use GUI (Graphics User Interface) from MATLAB to create an easy interface for our scripts.

Seven different DICOM Images obtained from patients with heart failure (TTE, as in Exp. II) were used. Two ROIs were drawn; one in each ventricle, so ΔMTT could be computed. Then, IDCs obtained were processed with the four programs: cardioGUI, *"Guyton"*, *"Polytool"* and QLAB.

"Guyton" program

In [18] there is a program written in BASIC programming language that fits IDCs approximating the curve by a decaying exponential and calculates CO, MTT and AUC. The user has to input a maximum of 255 values of the curve. Then, to fit the curve, the program finds its peak value. Thus, it tries to find a downslope ratio and when it finds it, it replaces the remaining points of the curve by an exponential decay. To calculate the AUC, it uses a trapezoidal formula.

This script was translated into MATLAB language in order to obtain the results. We calculated MTT after fitting the function and AUC with the function *trapz(x,y)* [21] from MATLAB.

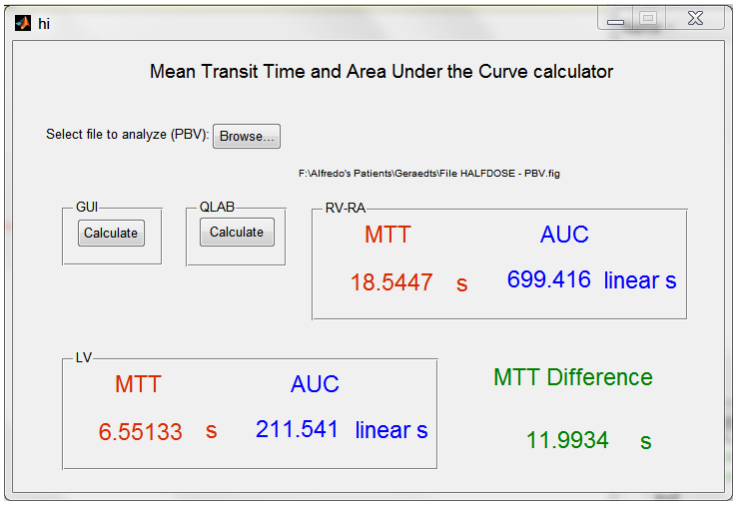


Fig.10 User interface of "Guyton" program

Polytool

Several values for the degrees of the polynomial function were tried. We found that with 10 degrees the function fitted the IDC precisely. With lower degrees the function was not fitted very well and with higher degrees the coefficients were close to zero. AUC was also calculated with *trapz(x,y)* function.

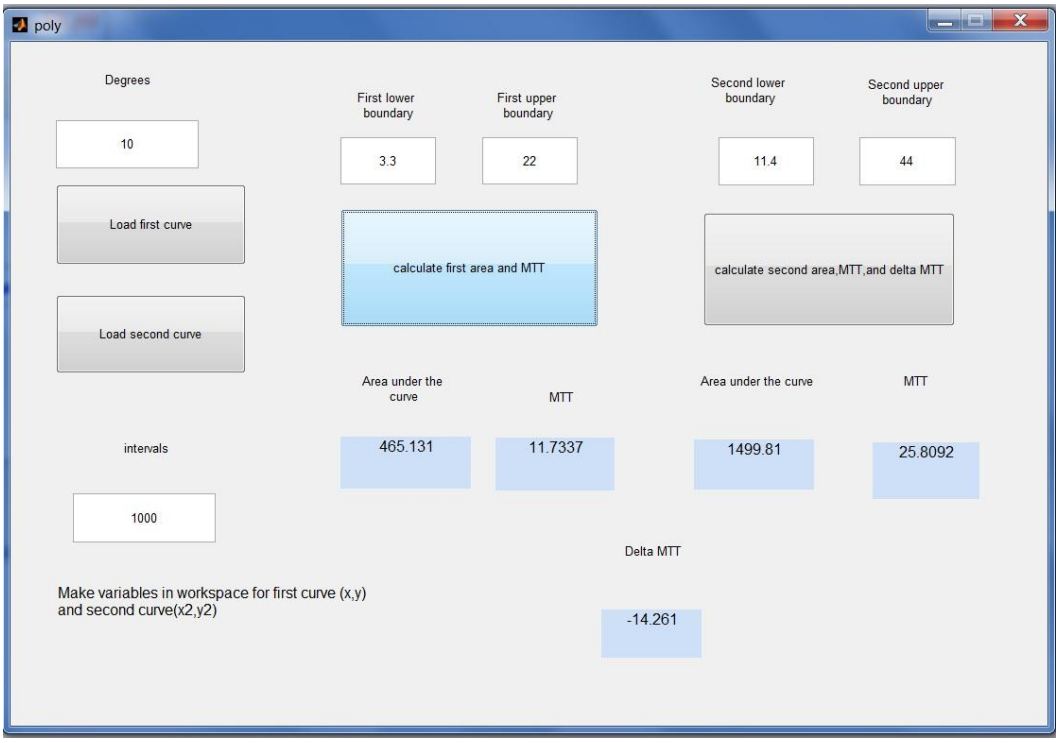


Fig.11 User interface of "polytool" program

- ## Results and recommendations

In Table 5 the similarity of the ΔMTT values given by *cardioGUI*, *Guyton* and *Polytool* programs can be observed. This difference is defined as ΔMTT = μ2 − μ1, where μ1 and μ2 are the first order moments of the RV and LV IDCs model fit (LDRW), respectively. By calculating the mean and standard deviation of the values, it was proved that QLAB's values are incorrect: standard deviation becomes slightly reduced when QLAB's values are ignored. Besides, some of the values of MTT are completely different.

Table 5. ΔMTT different values for each program

ΔMTT (s)	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
cardioGUI	6,79	12,37	5,99	12,08	11,29	9,95	13,98
Guyton	7,28	13,03	6,25	11,46	9,74	9,45	13,77
Polytool	7,08	12,12	6,28	11,85	11,52	9,96	14,36
QLAB	2,52	6,43	0,97	6,62	5,59	6,21	9,71
mean with QLAB	5,92	10,99	4,87	10,50	9,54	8,89	12,96
stdev with QLAB	2,27	3,06	2,60	2,60	2,75	1,80	2,18
mean without QLAB	7,05	12,51	6,17	11,80	10,85	9,79	14,04
stdev without QLAB	0,25	0,47	0,16	0,31	0,97	0,29	0,30

AUC values can be found in Tables 6 and 7. The values seem to be reliable in all four programs.

Table 6. AUC in RV different values for each program

AUC RV (linear s)	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
cardioGUI	57,46	93,78	924,40	222,80	335,60	98,09	497,80
Guyton	52,29	87,62	873,28	207,95	316,10	88,43	464,40
Polytool	70,77	122,80	1058,39	250,98	386,67	126,77	865,13
QLAB	78,21	121,03	1022,40	226,25	334,73	127,52	616,18
mean with QLAB	64,68	106,31	969,62	227,00	343,28	110,20	610,88
stdev with QLAB	11,91	18,21	85,62	17,85	30,30	19,96	181,58
mean without QLAB	60,17	101,40	952,02	227,24	346,12	104,43	609,11
stdev without QLAB	9,53	18,79	95,60	21,86	36,44	19,94	222,35

Table 7. AUC in LV different values for each program

AUC LV (linear s)	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
cardioGUI	125,40	283,00	2590,00	853,20	1286,00	378,40	1628,00
Guyton	114,15	263,54	2345,97	796,45	1125,81	341,14	1495,38
Polytool	148,53	316,86	2710,28	906,28	1267,04	397,49	1499,81
QLAB	136,13	306,25	2899,57	927,02	1280,58	445,84	1608,82
mean with QLAB	131,05	292,41	2636,46	870,74	1239,86	390,72	1558,00
stdev with QLAB	14,71	23,88	231,82	58,47	76,45	43,57	70,21
mean without QLAB	129,36	287,80	2548,75	851,98	1226,28	372,34	1541,06
stdev without QLAB	17,53	26,98	185,62	54,93	87,53	28,66	75,32

MTT values obtained by our own scripts were quite similar to cardioGUI. Results given by QLAB are quite different, so the conclusion we obtained in Exp. II (that QLAB is a software not recommended in order to obtain reliable MTT values) gets ratified.

However, it seems to be that in terms of AUC, all four programs give similar results.

The recommendation is to keep using cardioGUI in order to get precise parameters, and use the new scripts if needed.

IV. Intra and inter observer reliability in ΔMTT

- **Introduction and hypothesis**

  Before making any kind of experiments with UCAs (bubbles), reliability and reproducibility of the results we want to obtain must be checked. In order to do so, a study to check *inter* and *intra* observer variation was realized.

  - Intra-observer variation is the amount of variation (different results) one observer gets when observing the same material more than once. In other words, if a same experiment is going to be repeated by the same person, we must check how much the results are going to be similar.

  - Inter-observer variation is the amount of variation between the results obtained by two or more observers examining the same material.

  The goal of this study is to show that the ΔMTT calculated from ultrasound IDCs will be the same regardless of who does the measurement.

- **Methodology**

  - **Participants**

    15 different patients with one or more heart diseases from various ages were chosen randomly in the same way as in Exp. II. For each patient a DICOM sequence of 25mg/L of SonoVue in 10ml injection was used. The measurements were performed using Philips iE33 Echocardiography System (Philips Medical Research).

- **Study Procedure**

These 15 images were examined by 5 doctors and 5 engineers. An instruction manual was given to the observers before commencing the measurements. Each person first measured all 15 images of the patients in a certain order. In QLAB, one ROI in the RV and another one in the LV were placed, so two IDCs were generated.

After a certain time, the same images were measured again but in a randomized order so that the observers did not remember the shape, size and position of the ROIs they made before.

Finally, all the IDCs obtained were processed with cardioGUI in order to obtain MTT in the LV, RV and the ΔMTT.

- **Data Analysis**

The reliability assessments were appraised by calculating Intraclass correlation coefficient (ICC)[]. Standard error of measurements (SEM) was calculated to complement ICC[], and the data spreads of the observers were visualized by modified Bland and Altman plots[]. ICC has 6 formulas according to shrout and fleiss []. For intra rater reliability one way random, ICC (1,1) was calculated[]. While the inter rater was evaluated by two way random, ICC(2,1) [].

$$ICC(1,1) = \frac{MS_B - MS_W}{MS_B + (k-1)MS_W}$$

$$ICC(2,1) = \frac{MS_S - MS_E}{MS_S + (k-1)MS_E + \frac{k(MS_T - MS_E)}{n}}$$

In order to be considered for clinical use, the value of ICC must be above 0,90 []. SEM were calculated to determined individual score test and it is a better indicator than ICC because it has same unit as the measurements [].

$$SEM = SD\sqrt{1 - ICC}$$

The ICC and SEM values were calculated using IBM SPSS 20 Statistics software and the modified Bland and Altman plots were made in Microsoft Excel.

V. Dose of UCA influence in ΔMTT

- **Introduction and hypothesis**

  There is uncertainty about the effect of the ultrasound contrast agent dose in MTT [22]. In order to convert this technique into a reproducible system, we tried to prove our hypothesis that the dose of the UCA applied to the system does not affect time difference (ΔMTT) between an IDC in the right ventricle (RV) and one in the left ventricle (LV) [22]. This difference is defined as $\Delta MTT = \mu 2 - \mu 1$, where $\mu 1$ and $\mu 2$ are the first order moments of the RV and LV IDCs model fit (LDRW), respectively.

- **Methodology**

  1. **In-vivo**

     14 different images from patients with one or more heart diseases from various ages were chosen randomly in the same way as in Exp. II. For each patient a DICOM sequence with a certain dose of SonoVue (see Appendix E) in 10 ml injection was used. The measurements were performed using Philips iE33 Echocardiography System (Philips Medical Research).
     Three different doses of UCA were used in each patient: 12.5, 25 and 50 mg/L in 10 ml injections respectively

  2. **In-vitro**

     The in-vitro setup was built as described in Appendix A, except that the circuit was open at the end in order to prevent bubbles recirculation.

     The UCA was made by mixing 25 mg of SonoVue [8] lyophilised powder with 5 ml of saline, which produce bubbles with very high concentration (5 mg/ml).

     In order to make the concentration lower, a certain volume of UCA, shown in Table 8, was injected into a bag of saline to dilute. To create further dilution, the contrast agent was mixed once again with saline in a particular proportion (Table 8) in a 20 ml syringe. Then the contrast agent was infused into the system at the injection point.

     For the measurements, 3 injections of 11 different concentrations of SonoVue were used. After each injection, 30 seconds of the images acquired by the iE33 were recorded so they could be

processed the same way as in-vivo measurements (QLAB and cardioGUI).

Table 8. Dilution distribution of SonoVue in saline bags and syringe

volume of saline bag	Volume of injected 5mg/ml sonoVue into the bag	20ml syringe proportion (injection)		injection concentration
		Diluted SonoVue (from the bag)	Saline	
100ml	1ml	20ml	0ml	50mg/l
		10ml	10ml	25mg/l
		5ml	15ml	12,5mg/l
500ml	0,6ml	20ml	0ml	6mg/l
		10ml	10ml	3mg/l
		5 ml	15ml	1,5mg/l
		2,5ml	17,5ml	0,75mg/l
	0,4ml	20ml	0ml	4mg/l
		10ml	10ml	2mg/l
		5ml	15ml	1mg/l
		2,5ml	17,5ml	0,5mg/l

- ## Results and conclusions

  - ### In-vivo

    Results are shown in the chart in Fig. 12. The X-axis indicates the number of the patient and the Y-axis indicates ΔMTT value as stated in the Methodology section above. The three points in each patient represent the three different doses.

    As can be appreciated on the graphs, in most of the patients the three estimations are quite similar measurements for the ΔMTT. The mean of the three different ΔMTT values obtained was calculated, as well as standard deviation.

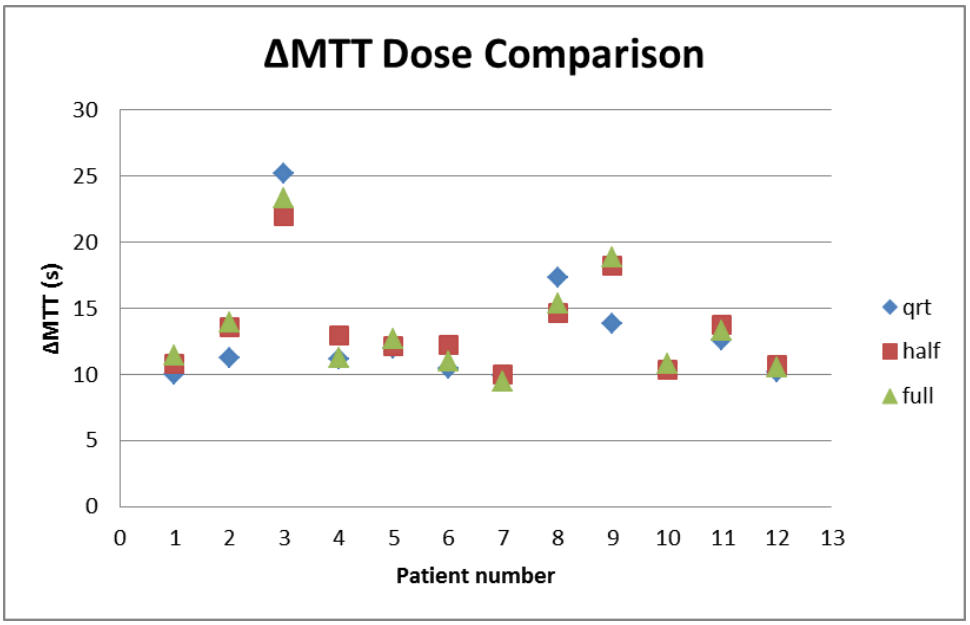


Fig 12. ΔMTT value for each dose in each patient

- In-vitro

Average values of the three MTT injections as a function of concentration of SonoVue are shown in Fig. 13. Although the time of injection was not the same in every experiment, cardioGUI is able to find it when it has the IDC. Different colors in Table 9 and Fig. 12 indicate different saline bags that were used for dilution.

As displayed in Fig.13, MTT values from dilutions made with the same bag were similar. Again, we have a mean of MTT of 12.36 sec and standard deviation of 1.47 sec (12%) so the values are quite close to the mean, which indicates that the difference in the doses does not affect MTT values.

A recommendation would be to repeat the measurements in order to understand better how the fact of making a dilution in a new saline bag can affect the behavior of the bubbles.

Table 9. Results in different concentration of Sonovue

conc	MTT (s)	mean	stdev	AUC (linear s)	Mean	stdev	dose
0.5 mg/l	14,26	13,06	1,70	874,00	640,05	330,86	0,4 ml sonovue in 500 ml bag
	11,85			406,10			
0.75 mg/l	11,16	11,96	1,04	824,50	747,47	92,41	0,6 ml sonovue in 500 ml bag
	11,58			772,90			
	13,14			645,00			
1 mg/l	12,37	13,97	1,53	215,10	459,90	220,41	0,4 ml sonovue in 500 ml bag
	15,41			522,00			
	14,12			642,60			
1.5 mg/l	13,10	12,17	0,84	865,10	712,90	212,36	0,6 ml sonovue in 500 ml bag
	11,92			803,30			
	11,48			470,30			
2 mg/l	9,87	10,06	0,29	189,50	207,83	71,78	0,4 ml sonovue in 500 ml bag
	10,40			287,00			
	9,92			147,00			
3 mg/l	12,97	13,00	0,40	706,10	877,70	152,08	0,6 ml sonovue in 500 ml bag
	13,41			931,20			
	12,61			995,80			
4 mg/l	10,64	10,36	0,48	393,50	365,13	25,12	0,4 ml sonovue in 500 ml bag
	9,80			345,70			
	10,63			356,20			
6 mg/l	11,55	11,81	0,30	1377,00	1058,97	289,10	0,6 ml sonovue in 500 ml bag
	12,14			987,80			
	11,74			812,10			
12,5 mg/l	12,82	13,41	0,57	2344,00	2320,67	86,40	1 ml sonovue in 100 ml bag
	13,95			2393,00			
	13,45			2225,00			
25 mg/l	12,66	13,19	0,90	2813,00	2690,00	573,48	1 ml sonovue in 100 ml bag
	12,67			3192,00			
	14,23			2065,00			
50 mg/l	11,46	13,28	1,64	4048,00	3479,33	503,97	1 ml sonovue in 100 ml bag
	14,63			3302,00			
	13,76			3088,00			
Mean	12,37			1251,24			
Stdev	1,47			1087,49			

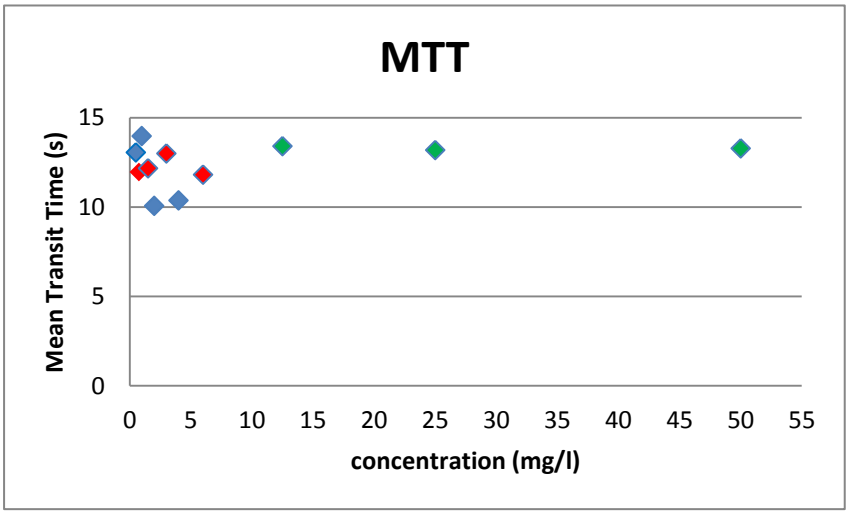


Fig 13. MTT mean values for each concentration

VI. Two echo-machines (volume measurement)

- **Introduction and hypothesis**

The main goal of this study is to be able to measure the volume between two detection points with Contrast Enhanced Ultrasound.

To do so, we made three in-vitro experiments with two echo-machines (Philips iU22 Echocardiography system) in two different detection points, separated by 1 meter of distance. Thermodilution (PiCCO) was added as well, to compare results. ΔMTT is defined by MTT in the machine 1 – MTT in the machine 2.

Besides, we want to show that this method is very robust: since both of the scanners detect the same errors, they should be nullified. Moreover, others [9] have stated that Dynamic Range and Gain settings in the iU22 affect considerably MTT values. Our aim is to show that this is not true.

- **Methodology**

The in-vitro system is the same as explained in Appendix A, but with the addition of another echo-machine (Fig. 14) and a battery powered pulse generator. The pulse generator device is used to indicate the injection time in two echo machines so the injection time in the two machines is exactly the same (Fig. 15). 20 ml of diluted SonoVue in cold saline were injected. The PiCCO detection point was set just under the probe of the first machine.

- **1st experiment: changing flow**

  Observation of how changes in flow affect volume measurement outcome. Flow was set to vary from 1 to 4 L/min. 2 injections were made at each flow, to check for reproducibility.

- **2nd experiment: changing contrast volume**

  Deliberate errors were made in the injection volume, injecting from 14 to 22 ml. 2 injections were made by each flow, to check for reproducibility.

- **3rd experiment: changing system volume**

  Testing how sensitive the method is to detect changes in volume: the first transducer was set on the end of the tube in the first box. Then, the second transducer was set on the beginning of the tube in the second box, and at 10, 20 and 30 cm from this point. The

flow was changed from 1 to 4 L/min in each point, and 2 injections were used to test reproducibility.

Dynamic Range and Gain settings in the iU22 were changed in 5 measurements with a flow rate of 3 l/min, in order to observe how this affects MTT.

Images were processed with QLAB (ROI), and MTT and AUC parameters were obtained with cardioGUI.

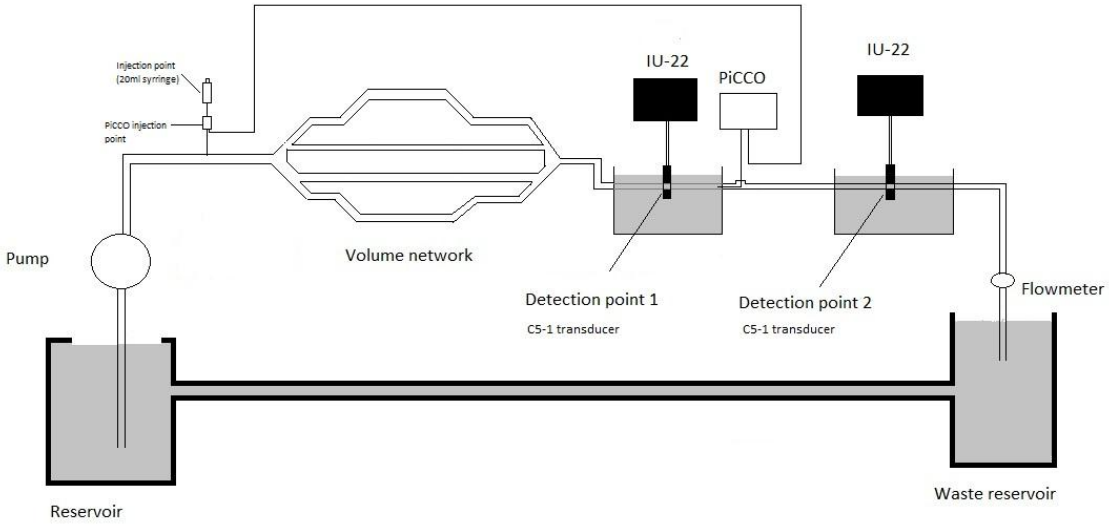


Fig. 14 In-vitro system setup

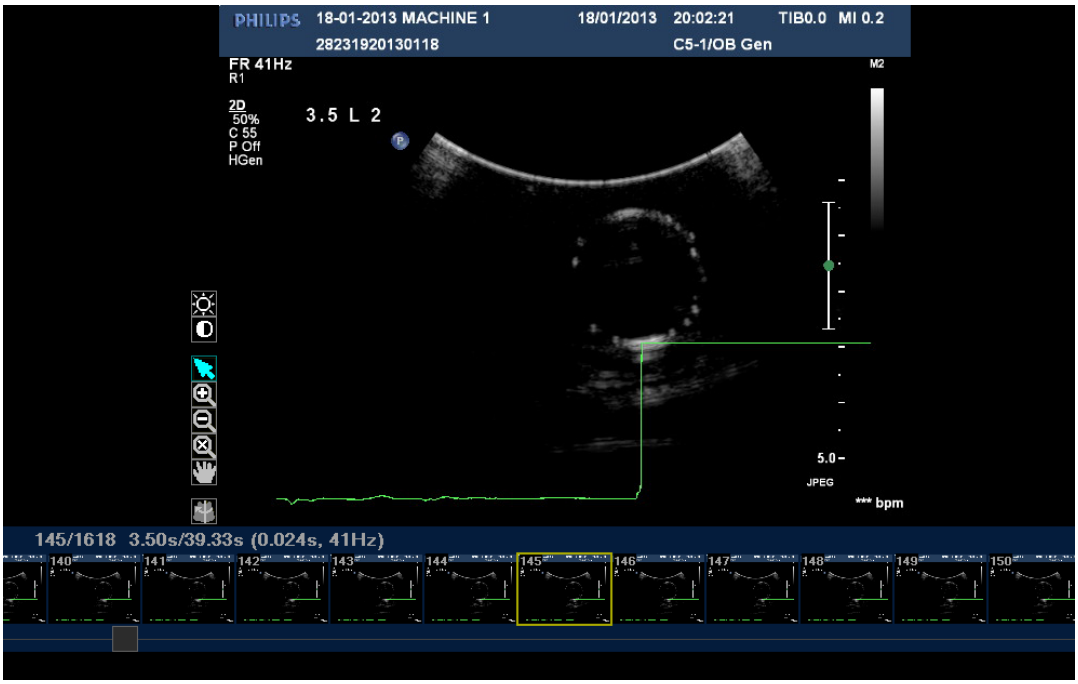


Fig 15. DICOM image from machine 1 with sychronisation pulse at injection time

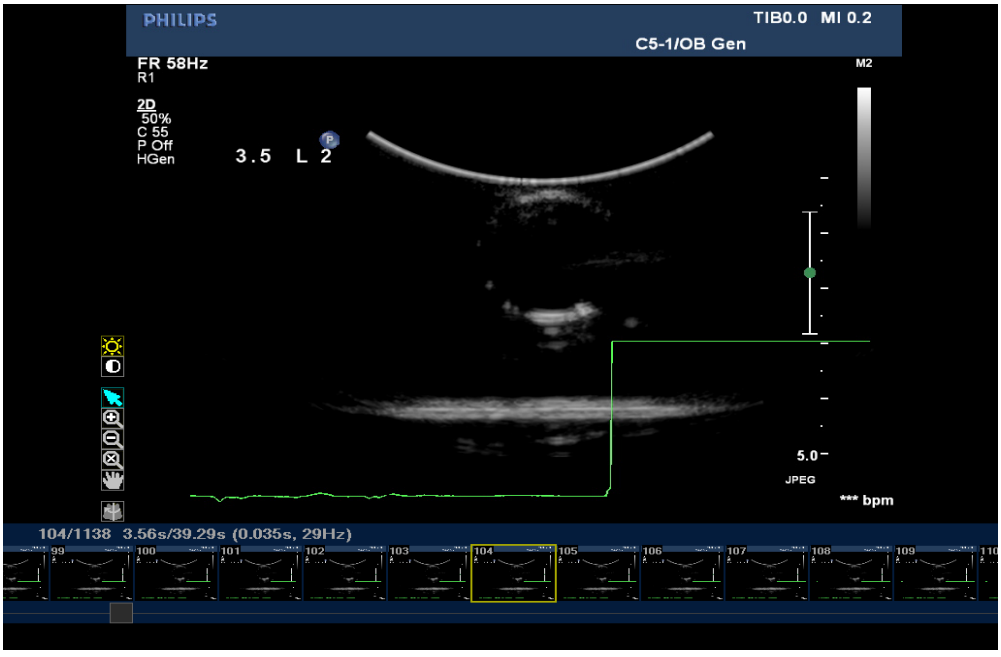


Fig 16. DICOM image from machine 2 with sychronisation pulse at injection time

- Results and recommendations

  - 1st experiment (changing flow):

    Results on changing flow are shown in Table 10 and Fig. 17. We can observe that MTT decreases with flow. Thermodilution ratifies this in Fig. 18, where the decreasing MTT in machine 1 (only one PiCCO-meter available) is shown. On the other hand, volume increases (Fig. 19) because with higher flow the soft tubes where the detection points are set inflate in a 1mm diameter. AUC in machine 2 is much smaller than in machine 1 because the first transducer is disrupting bubbles, in a way that the acoustic intensity received in the second machine is lower.

Table 10. Results in changing the flow

Flow (L/min)	PiCCO MTT (s)	Machine 1 MTT (s)	Machine 2 MTT (s)	ΔMTT (s)	Volume (ml)	AUC Machine 1 (linear s)	AUC Machine 2 (linear s)
1	20,93	38,54	45,06	6,52	108,67	2448	1235
1	20,14	38,54	45,04	6,5	108,33	2412	1131
1,5	17,63	25,19	30,75	5,56	139,00	3934	1240
1,5	17,43	25,35	31,26	5,91	147,75	3828	1169
2	16,12	19,51	23,88	4,37	145,67	1297	718,6
2	16,19	19,16	24,04	4,88	162,67	1405	793,4
2,5	15,41	14,92	18,79	3,87	161,25	1873	1059
2,5	15,52	15,46	19,01	3,55	147,92	1825	1084
3	14,89	12,63	15,97	3,34	167,00	817,4	470,3
3	14,9	12,83	16,16	3,33	166,50	975,1	597
3,5	14,51	10,61	13,66	3,05	177,92	1108	681,9
3,5	14,6	10,68	13,75	3,07	179,08	1104	636,9
4	14,3	9,99	12,64	2,647	176,47	622,2	279,5
4	14,21	9,55	12,31	2,757	183,80	782,4	461,9
mean	16,199	18,783	23,023	4,240	155,144	1745,079	825,536
std dev	2,129	9,844	11,187	1,388	24,083	1069,360	322,194

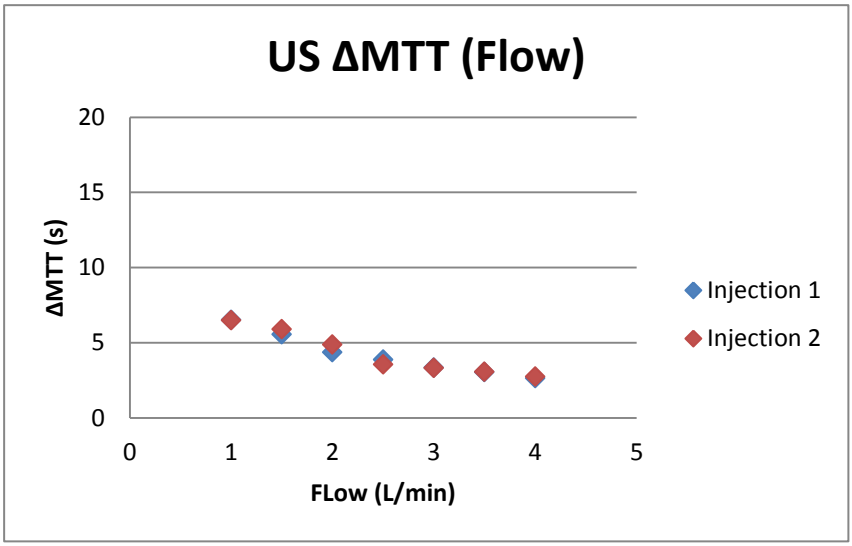


Fig. 17 ΔMTT as a function of flow

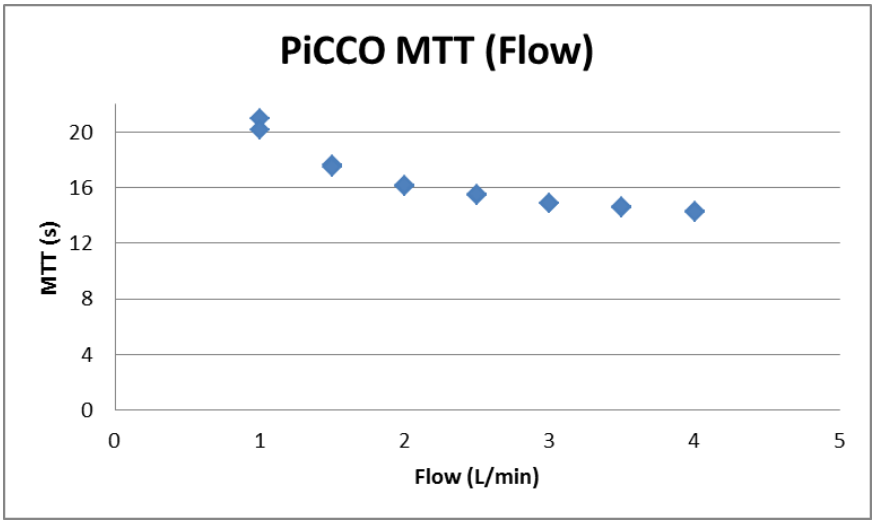


Fig. 18 PiCCO MTT as a function of flow in machine 1

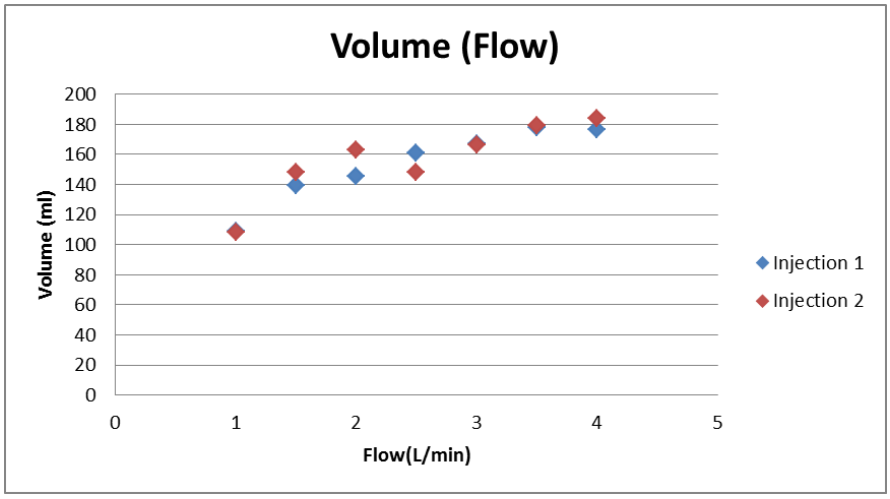


Fig.19 Volume as a function of flow

- **2nd experiment (changing contrast volume):**

  Concerning the deliberated errors (2nd experiment), results are shown in Table 11 and Fig. 20, 21, 22, and 23. Differences in ΔMTT change a 15%, as the MTT measured by PiCCO remains constant.

Table 11. Deliberated errors in syringe volume results

dose volume (ml)	PiCCO MTT(s)	MTT machine 1 (s)	MTT machine 2 (s)	AUC machine1 (linear s)	AUC machine2 (linear s)	ΔMTT (s)	flow (L/min)	volume (L)
14	14,79	11,88	15,56	616,4	276,4	3,68	3	0,18
14	14,82	11,94	15,25	586,9	246	3,31	3	0,17
16	14,82	12,53	16,42	677,1	278	3,89	3	0,19
16	14,9	12,58	16,03	667,4	274,6	3,45	3	0,17
18	14,98	12,36	16,15	642,5	214,9	3,79	3	0,19
18	14,92	12,01	15,42	457,9	208,2	3,41	3	0,17
20	14,98	11,74	15,17	580,3	292,9	3,43	3	0,17
20	14,96	12,62	15,96	393,7	168,6	3,34	3	0,17
22	15,06	13,06	16,27	492,1	215,1	3,21	3	0,16
22	14,99	12,35	15,77	299,1	162	3,42	3	0,17
23	15,04	12,59	16,06	274,3	113,6	3,47	3	0,17
23	15,03	12,56	15,99	152,6	67,7	3,43	3	0,17
mean	14,94	12,35	486,69	15,84	209,83	3,49		0,17
stdev	0,09	0,39	173,27	0,40	70,70	0,20		0,01
c.v. (%)	0,61	3,13	35,60	2,55	33,69	5,74		5,74

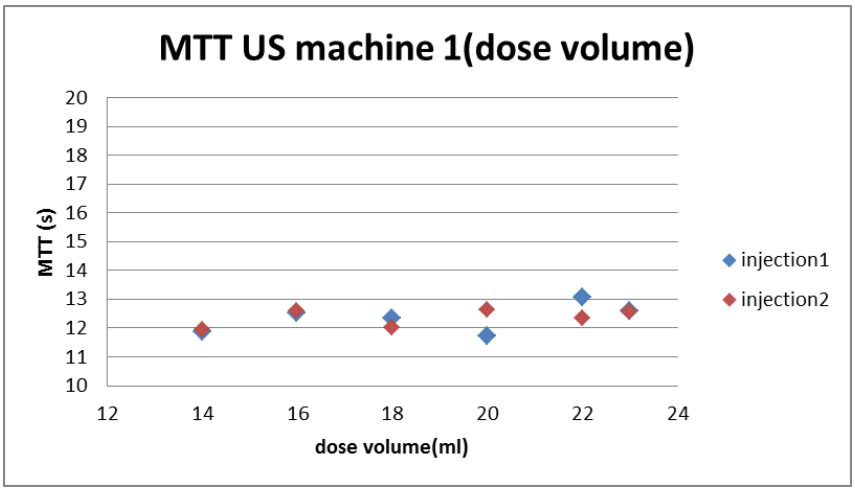


Fig. 20 MTT in machine 1 as a function of dose volume

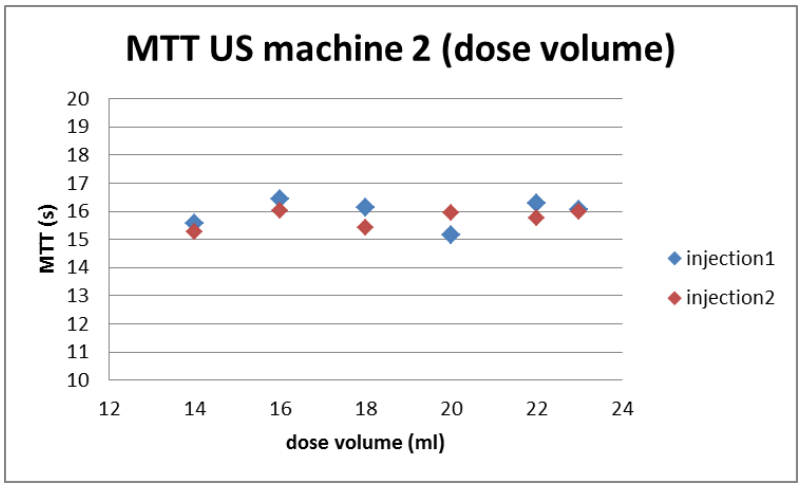


Fig. 21 MTT in machine 2 as a function of dose volume

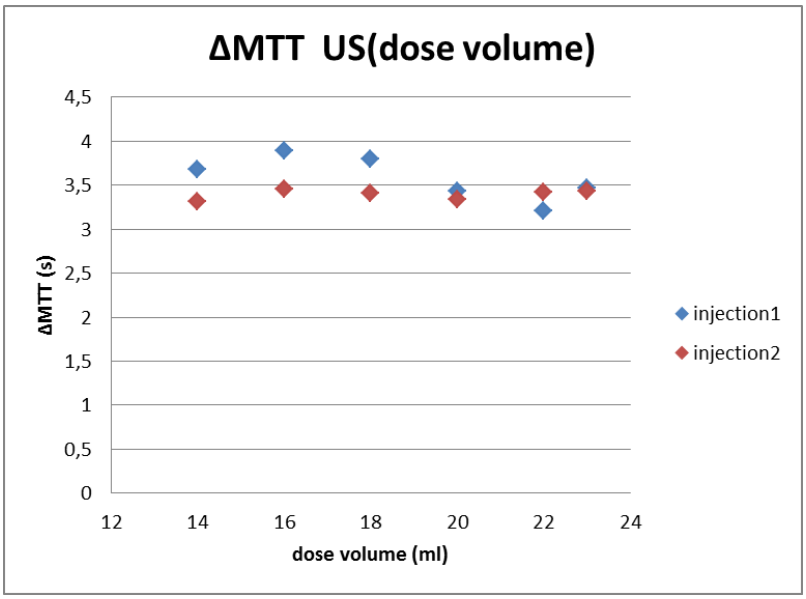


Fig. 22 ΔMTT as a function of syringe volume

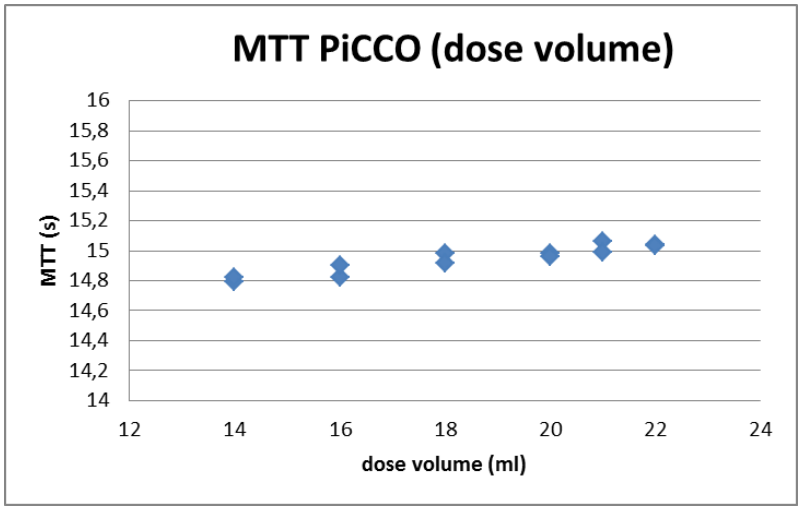


Fig. 23 PiCCO MTT as a function of dose volume

- 3rd experiment (changing the system volume)

Table 12. Results on changing the distance of second detection point

Flow (L/min)	Distance (cm)	ΔMTT (s)	Volume (ml)	Mean volume (ml)	Stdev volume (ml)
2	0	2,68	89,33	84,67	6,60
2	0	2,40	80,00		
2	10	3,83	127,67	133,67	8,49
2	10	4,19	139,67		
2	20	4,57	152,33	156,83	6,36
2	20	4,84	161,33		
2	30	6,60	220,00	225,83	8,25
2	30	6,95	231,67		
3	0	2,10	105,00	103,00	2,83
3	0	2,02	101,00		
3	10	2,71	135,50	151,25	22,27
3	10	3,34	167,00		
3	20	3,99	199,50	204,25	6,72
3	20	4,18	209,00		
3	30	4,78	239,00	238,75	0,35
3	30	4,77	238,50		
4	0	1,76	117,33	119,67	3,30
4	0	1,83	122,00		
4	10	2,43	162,00	161,00	1,41
4	10	2,40	160,00		
4	20	3,27	218,00	224,23	8,82
4	20	3,46	230,47		
4	30	3,83	255,33	254,00	1,89
4	30	3,79	252,67		

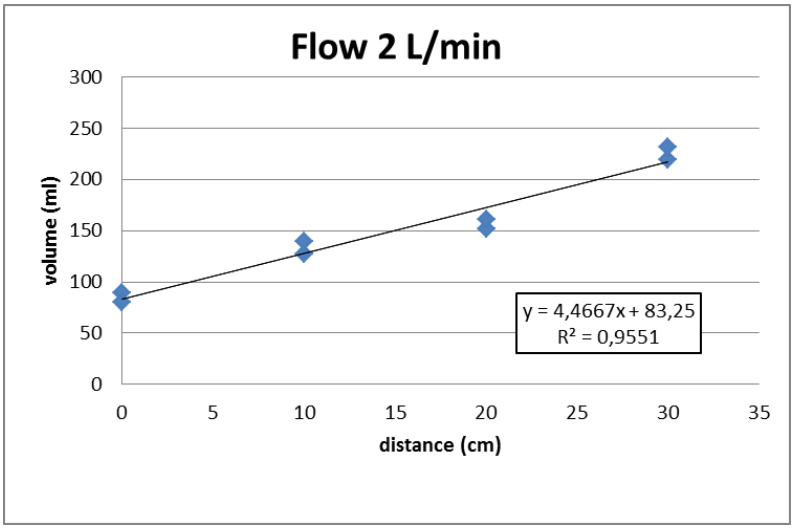


Fig 24. Volume as a function of distance of second detection point

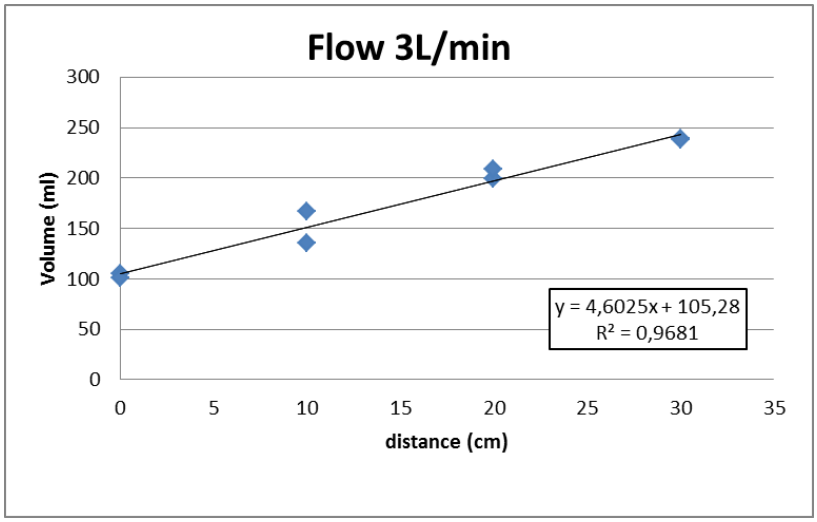


Fig 25. Volume as a function of distance of second detection point

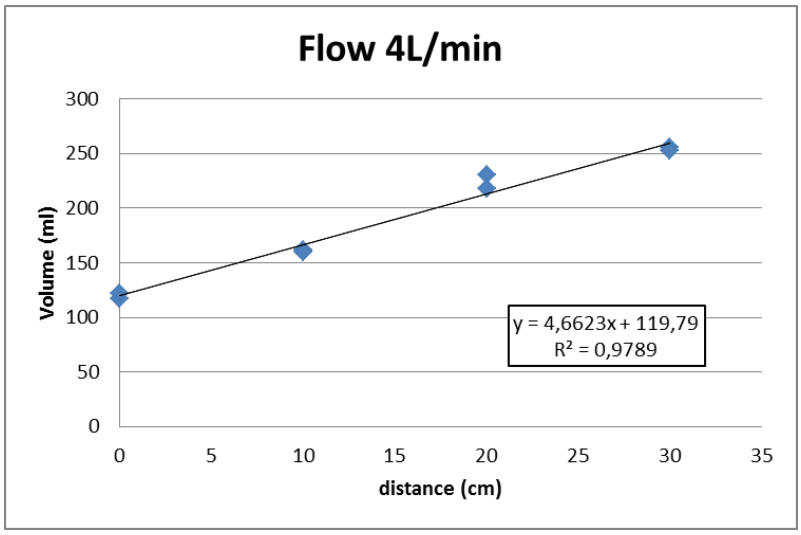


Fig 26. Volume as a function of distance of second detection point

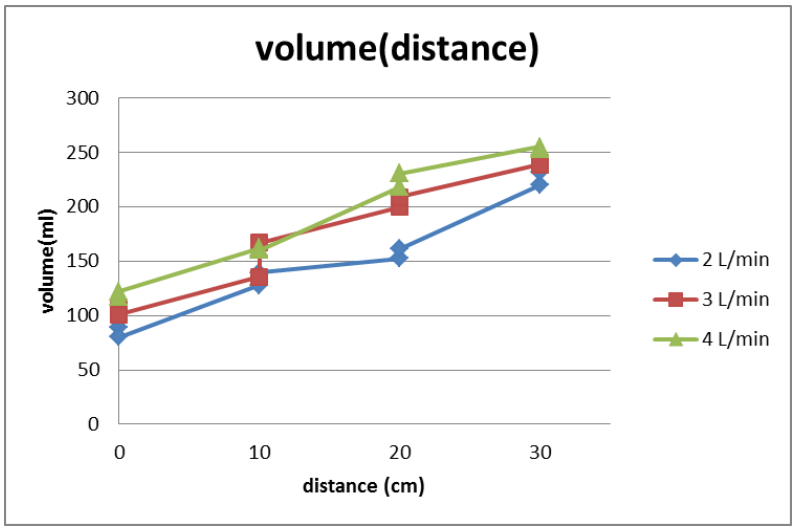


Fig 27. Volume as a function of distance of second detection point (all flow)

VII. Total Peripheral Resistance effect in IDC

- **Introduction and hypothesis**

Total Peripheral resistance (TPR) is the force against blood flow that ejects from the heart in a systemic circulation. It is defined by the change of pressure across the systemic circulation divided by cardiac output, thus, the pressure difference is proportional to the TPR. There are three factors that affect the pressure difference in laminar flow: the diameter of vessel, the total length of vessel, and the blood viscosity, as described in Poiseuille Law (Eq. 2):

$$\Delta P = \frac{8\mu L Q}{\pi r^4} \quad (2)$$

Where ΔP is the change of pressure, μ is the dynamic viscosity, L is the total length of the vessel, Q is the volumetric flow rate and r is the radius of the vessel. [19]

It is proved that peripheral resistance can affect the measurement of some several cardiac parameters like Cardiac Output [16].The goal of this study is to observe how peripheral resistance will affect interesting parameters we normally use from Indicator Dilution Curves (flow, Mean Transit Time, Area Under the Curve, Skewness – shape of the curve).

To do so, we made in-vitro experiments, varying the pressure in the system and acquiring the results using thermodilution (PiCCO) and one echo-machine (Philips iU22 Echocardiography system) at the same time, in order to see the differences between the two methods as well.

- **Methodology**

The setup for the in-vitro experiment is displayed in Fig. 28. It's the same as explained in Appendix A, but after the echo-detection the water gets into a small basin of 6L capacity, that will be filled until it overflows into another basin connected to the waste reservoir.

This small basin will be placed at 3 different heights in order to observe different peripheral resistances. The increase of height in some points of the system will increase the pressure in other points of the system which have lower height referred to the Bernoulli's principle.

The hydrodynamic circuit is closed and kept a constant temperature of 35ºC with an immersion heater.

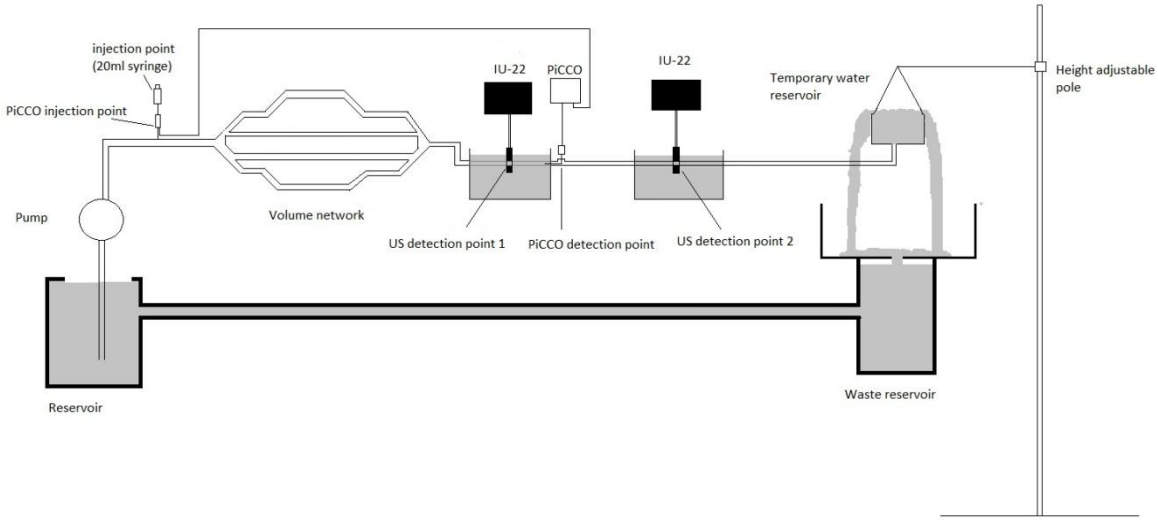


Fig 28. Setup for the in-vitro experiments

For the measurements, 2 injections at medium height of the temporary water reservoir, 2 at low height and 2 at high height were made. After each injection, during 45 seconds images were acquired by the iU22. At the same time, the PiCCO detector was recording the changes in temperature.
We processed the images with QLAB and cardioGUI. We placed one ROI that covers the whole tube.
We processed the temperature recordings obtained by the detector with the PiCCOWin (Appendix C) software, which gathers the data from the curves in order to be processed in cardioGUI.

- **First Results**

Results are shown in Table 13. MTT is almost the same (only changing a 0.3%) for different TPRs. Lambda changes with height, so it means that the peripheral resistance affects the skewness of the curve. Flow also changes and becomes lower while height increases (more resistance).

height	Lambda	Mean(lambda)	MTT (s)
normal1	118,3		14,46
normal2	176,6	147,45	14,48
low1	128,1		14,57
low2	123,9	126	14,5
mid1	117		14,56
mid2	116,7	116,85	14,53
high1	132,7		14,58
high2	119,3	126	14,56

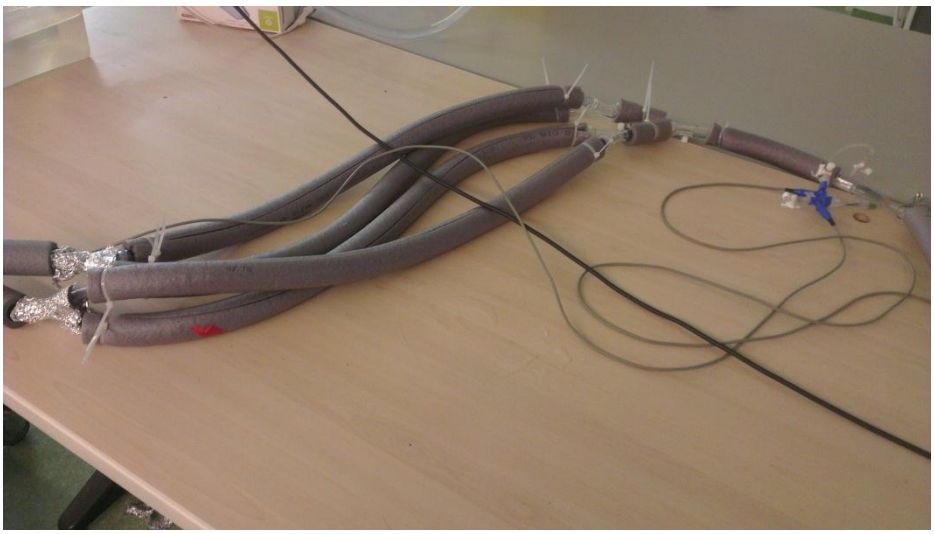
Table 13. Results on TPR

Appendix A: Equipment used in the in-vitro setup

- **Centrifugal pump**

  Also known as peristaltic pump, it is used for pumping a variety of huge fluids. A flexible tube with the fluid inside is set inside the circular pump casing. The pump has a rotor with wipers that compresses the tube, forcing the fluid to move. The speed is adjustable from 0 to 256 RPM.

- **½ inch tubes**
- **Mixing network**
  4 diverging plastic tubes that converge again in a single tube.



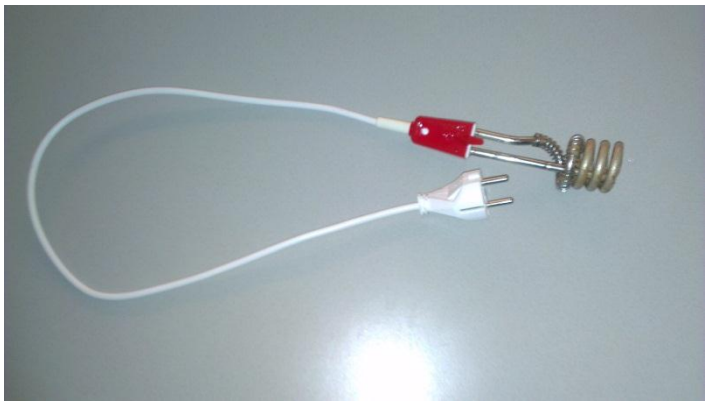
- **Box with soft tube**

A small basin filled with water with a soft tube inside, where the transducer is scanning.

- 60 L Basins

- Small electric heater

An immersion heater consisting on a resistor working on the principle of Joule heating: an electric current through a resistor converts electrical energy into heat.



- **Thermo controller**

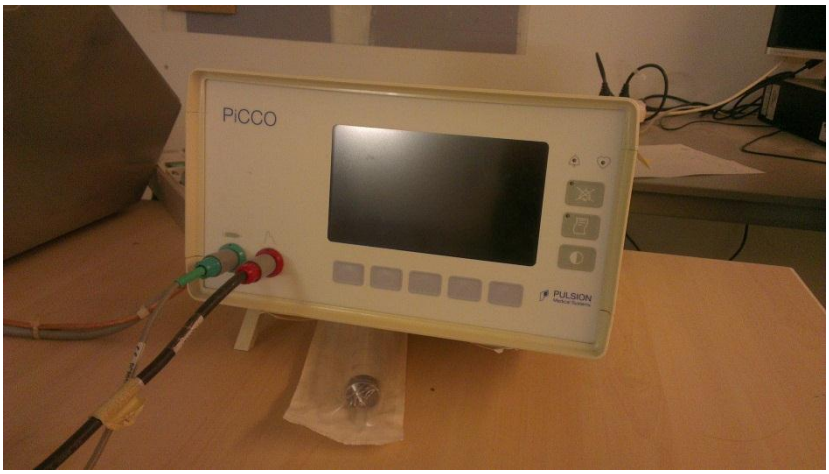
  A digital thermostat with a temperature range from 0 to 50°C. Heaters of up to 1200W total can be connected through a coupling (electric socket). The external sensor is water-proof and can measure the temperature in water and air. In addition, it has an alarm function which warns when the desired temperature is reached (possibility to set it before).

- **Electro-magnetic Flow-meter**

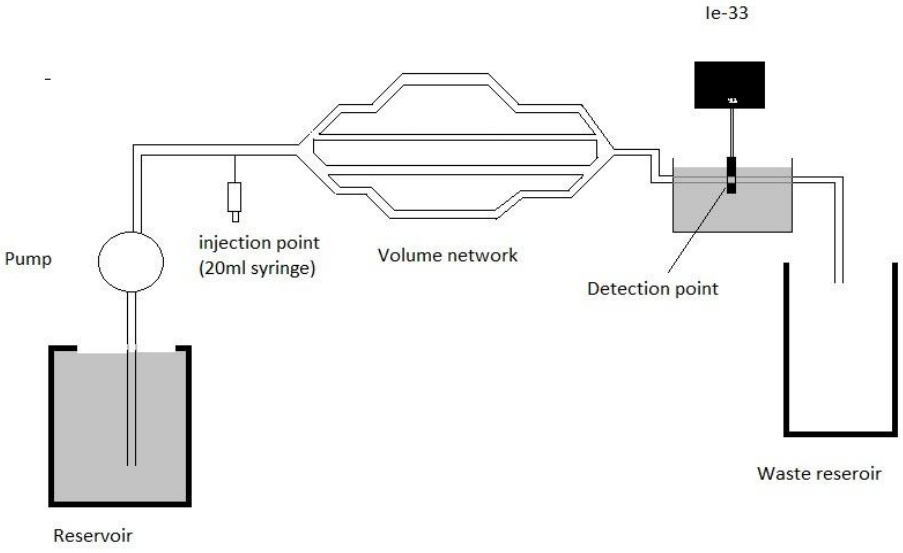
  A device able to measure the flow of a fluid passing through a sensor.

- **PiCCO machine**

  The PiCCO machine is used as an essential part of the whole system. It will measure the IDC and calculate CO out from the IDC after the cold bolus passing by the PiCCO catheter.



A reservoir of water is connected to an occlusive roller pump via the ½ inch tube. The pump is set into a constant flow rate. The output of the pump is connected to a volume network (consisting of 4 diverging tubes that converge again in a single tube), in order to increase the volume of the system and ensure adequate mixing between the injection point and the detection point. The ultrasound transducer is scanning a soft polyurethane tube in a small basin filled with water. An electromagnetic flow-meter is set after the basin, in order to get the precise flow at any time. The hydrodynamic circuit is closed.



Appendix B: Software

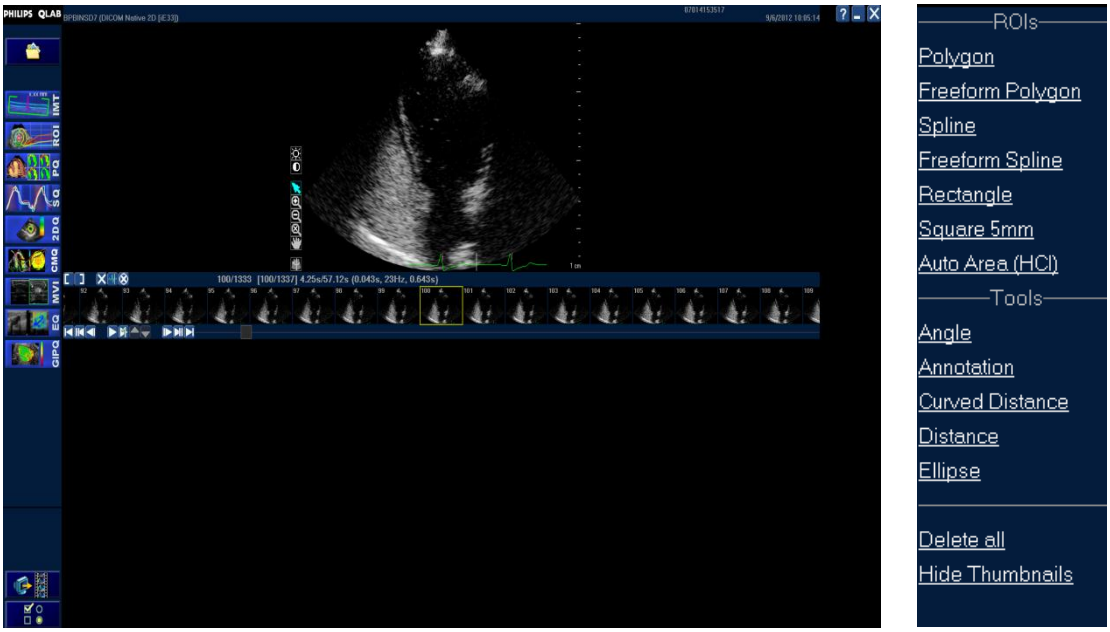
- **QLAB (Philips Medical Research)**

Philips QLAB Advanced Quantification software helps to provide useful data by extending and complementing the extensive on-cart tools provided by Philips ultrasound systems.

QLAB is multifunctional. It offers many workflow advantages and allows investigators to perform analysis either at the time of the study or at a later time. This flexibility is further enhanced by the ability to save and export all or any selected portion of the QLAB analysis screen.

The resultant images may be stored as single or multi-frame DICOM sequences thereby making advanced analysis data available for later review as part of the patient electronic record, which is the basic method for us to study and analyze.

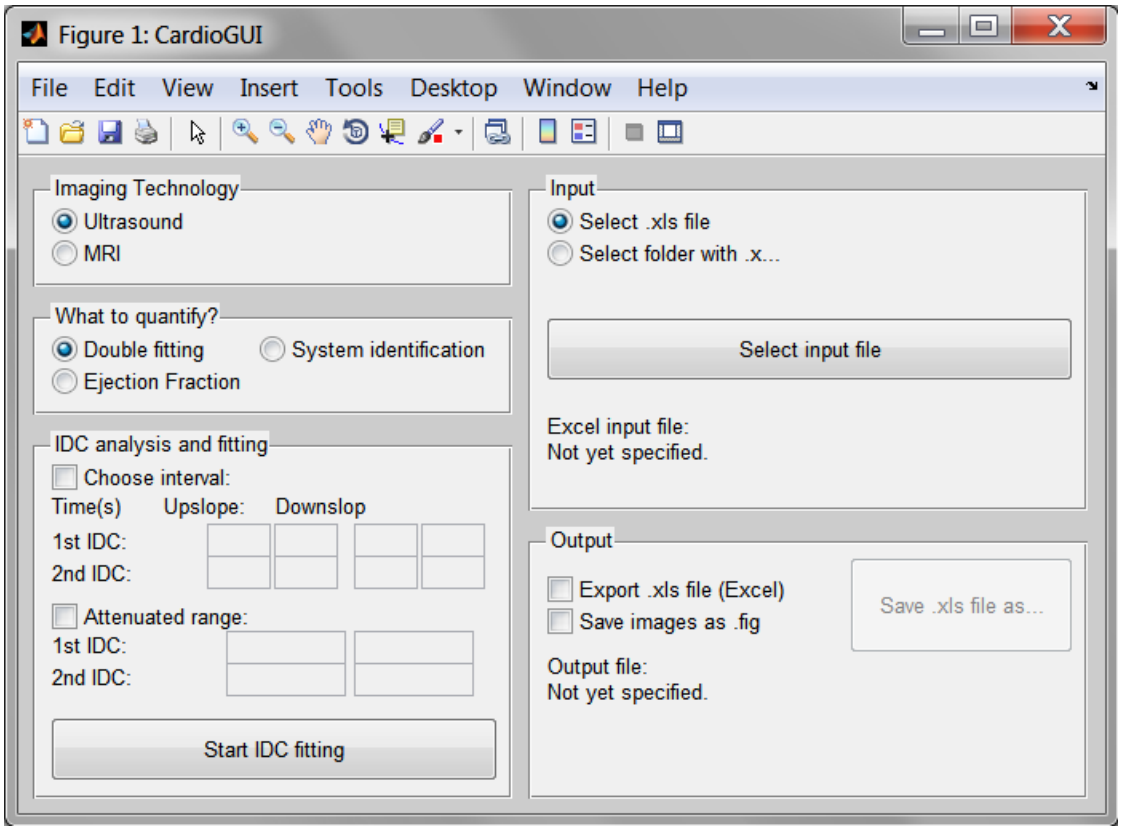
The quantification result can be seen directly in the software and can be exported into excel compatible spreadsheet format. Below the user-interface of the program is shown:



As we can see in the picture, ROI section allows us to draw a Region Of Interest of the size, shape and position we want.

- **MATLAB (Mathworks) - cardioGUI**

Data processing software in form of Graphical User Interface program, created by the Signal Processing Systems department of TU/e. It is used to calculate indicator dilution curve's parameters such as MTT and AUC .



As we can see, the program has a lot of possibilities. It allows us to select a single excel file or a whole folder, to go faster. We can also choose the interval where we want the LDRW fitting, and select the range which is attenuated so the program does not take it into account.

- **PiCCOWin**

A software developed by Pulsion Medical Systems, Munich, Germany. With the help of this software and RS-232 cable, a Windows-computer can be connected and obtain IDC data that calculated by the PiCCO machine.

Appendix C: IDC Theory and LDRW fitting

The basic version of this theory applies to a stationary flow system with one inlet and one outlet where:

1. A small amount M of indicator is injected at time t = 0 at the inlet (injection)
2. The indicator mixes with the bloodstream (mixing and dilution)
3. The concentration of the indicator is determined downstream (detection)

The (constant) flow ∅ through the system can be calculated by use of the known amount of indicator, M, and of the indicator concentration-time curve c (t) recorded at the outlet. The resulting formula, referred to as Stewart-Hamilton equation gives the measurement of the mean flow ∅:

Since the measurements are influenced by several noise sources, we need to use an IDC model to fit the curve, since filtering the noise is very complex. Another reason to use a model is that it gives access to useful parameters like the λ and the AUC, which are simultaneously estimated by the curve fitting. The model we chose is the LDRW, which provides the most accurate IDC interpolation and volume measurements as well as a physical representation of the dilution process [6] [11]-[14]. It assumes a Gaussian spatial distribution of the bubbles that travel with the fluid. LDRW models the UCA concentration as a function of time is modeled as shown in Eq. (C.1), where **m** is the injected mass of the contrast agent, **Q** is the volumetric flow, **λ** is a parameter related to the diffusion constant of the system (skewness of the curve), and **μ** is the average time that the contrast takes to go from the injection to the detection site (MTT). To fit the curves and obtain the interesting parameters, we used an in-house script in MATLAB (Mathworks, Natick, MA) on a personal computer.

$$C(t) = \frac{m}{Q} e^{\lambda} \sqrt{\frac{\lambda}{2\pi\mu t}} e^{-\frac{\lambda}{2}\left(\frac{t}{\mu}+\frac{\mu}{t}\right)}$$

Appendix D: Philips iU22 Echocardiography System

Philips iU22 is an ultrasound system that has advanced imaging capabilities for general imaging. Philips iU22 ultrasound system can be used by radiology and vascular labs and can be used in OB/GYN and perinatology applications. Below are the settings we used in our in vitro experiments.

Imaging mode	2D grayscale
Mechanical Index (Output power)	0,2
Gray map (gray map to be used for image display)	2
Depth	5 cm
2D option (transducer frequency settings)	Harmonics general
Persistence (Color Power Angio adjustment used to select the level of smoothing or frame averaging for the image display)	Off
Pulse repetition frequency	Low
SonoCT (Philips real-time compound imaging technology)	Off
XRES (Philips adaptive image processing technology)	Off
Dynamic resolution system (a macro control that provides adjustment of fast frame rates or optimal image quality)	R1

The iU22 produced DICOM images which are processed with QLAB software.

Philips C5-1 Ultrasound Transducer

Is a broadband curved array transducer with 5 to 1 MHz operating frequency rate. It can be used for general purposed abdominal, obstetrical, gynecological and interventional applications. The setting we used for our in vitro experiments is OB general.

Appendix E: SonoVue

SonoVue is the new ultrasound contrast agent produced by Bracco Diagnostics, formerly known as BR1. SonoVue is a suspension of stabilized sulfur hexafluoride (SF_6) micro bubbles, coated by phospholipid monolayer membrane shell encapsulating the gas. It have a mean radius of around 2,5 µm and concentration up to 5108 bubbles/ml with a favorable size distribution. SF_6 is a gas that has low solubility and diffuses slowly in blood, therefore SonoVue will abide in the blood stream. Due to the small size of the micro bubbles, it is capable to pass through the pulmonary and systemic capillary network and will not trapped in the capillary vasculature [17].

The bubble provides strong echogenicity over the entire medical frequency range (1±10 MHz) and can be used in both destructive and conservative contrast bubble specific imaging methods. In lyophilisate state, the bubble suspension is easily reconstituted by addition of saline.

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