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# Title: New calibration setup for quantitative DCE-US imaging protocol: towards standardization.

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## **ABSTRACT:**

**Background:** The DCE-US (Dynamic Contrast-Enhanced Ultrasonography) imaging protocol predicts the vascular modifications compared with Response Evaluation Criteria in Solid Tumors (RECIST) based mainly on morphological changes. A quantitative biomarker has been validated through the DCE-US multi-centric study for early monitoring of the efficiency of anti-angiogenic cancer treatments. In this context, the question of transposing the use of this biomarker to other types of ultrasound scanners, probes and settings has arisen to maintain the follow-up of patients under anti-angiogenic treatments. As a consequence, radiologists encounter standardization issues between the different generations of ultrasound scanners to perform quantitative imaging protocols.

**Purpose:** The aim of this study was to develop a new calibration setup to transpose the DCE-US imaging protocol to the new generation of ultrasound scanners using both abdominal and linear probes.

**Methods:** This calibration method has been designed to be easily reproducible and optimized, reducing the time required and cost incurred. It is based on an original set-up that includes using a concentration splitter to measure the variation of the harmonic signal intensity, obtained from the Area Under the time-intensity Curve (AUC) as a function of various contrast-agent concentrations. The splitter provided 4 different concentrations simultaneously ranging from 12.5 to 100% of the initial concentration of the SonoVue™ contrast agent (Bracco Imaging S.p.A., Milan, Italy), therefore, measuring 4 AUCs in a single injection. The plot of the AUC as a function of the four contrast agent concentrations represents the intensity variation of the harmonic signal: the slope being the calibration parameter. The standardization through this method implied that both generations of ultrasound scanners had to have the same slopes to be considered as calibrated. This method was tested on two ultrasound scanners from the same manufacturer (Aplio500™, Aplio900™, Canon Medical Systems, Tokyo, Japan). The Aplio500™ used the settings defined by the initial multicenter DCE-US study. The Mechanical Index (MI) and the Color Gain (CG) of the Aplio900™ have been adjusted to match those of the Aplio500™. The reliability of the new setup was evaluated in terms of measurement repeatability, and reproducibility with the agreement between the measurements obtained once the two ultrasound scanners were calibrated.

**Results:** The new setup provided excellent repeatability measurements with a value of 96.8%. Once the two ultrasound scanners have been calibrated for both types of probes, the reproducibility was excellent with the agreement between their respective quantitative measurement was at the lowest 95.4 and at the best 98.8%.

The settings of the Aplioi900™ (Canon Medical Systems) were adjusted to match those of the Aplio500™ (Canon Medical Systems) and these validated settings were for the abdominal probe: MI=0.13 and CG=34dB; and for the linear probe: MI=0.10 and CG=38dB.

**Conclusions:** This new calibration setup provided reliable measurements and enabled the rapid transfer and the use of the DCE-US imaging protocol on new ultrasound scanners, thus permitting a continuation of the therapeutic evaluation of patients through quantitative imaging.

**KEYWORDS:** Dynamic contrast-enhanced ultrasound (DCE-US), Methodologies of ultrasound calibration, Ultrasound contrast agent, phantom

## INTRODUCTION:

Oncology has major advances in recent years with the development of treatments, in particular those targeting tumor angiogenesis to limit the vascularization of cancerous tissues. Anti-angiogenic treatments induce lesion necrosis<sup>1</sup> without modifying the initial tumor size. In this context, conventional techniques such as the Response Evaluation Criteria in Solid Tumors (RECIST) based on morphological changes, are unsuitable to predict vascular modifications<sup>2</sup>, although the tumor necrosis reflects the effectiveness of the anti-angiogenic treatment. Dynamic Contrast-Enhanced Ultrasonography (DCE-US) imaging opened the path for much more accurate real-time functional perfusion imaging. DCE-US made it possible to quantitatively assess solid tumor micro-vascularization, and monitor its evolution throughout the treatment. This imaging modality proved to be a very attractive tool in the context of personalized therapeutic strategies, allowing the rapid selection of the most effective treatment for the patient, by limiting i) the side effects; ii) duration of the therapy and iii) cost of treatment. Thus, the use of contrast-enhanced ultrasound for vascular imaging has increased over the past two decades<sup>3-5</sup>. In 2005, the first single-center studies were performed in patients with gastrointestinal stromal tumors<sup>2</sup> and metastatic kidney cancer<sup>6</sup>. A qualitative analysis of contrast intake showed a statistically decrease in perfusion ( $p \leq 10^{-4}$ ) as early as on the 7<sup>th</sup> day after the initiation of treatment in good responders. Thus, DCE-US imaging allowed early prediction of tumor response to treatment, based on vascular changes even before morphological changes became apparent, i.e., RECIST criteria change<sup>2</sup>. Today, this approach is widely used and described in the literature<sup>7-10</sup>. However, the question raised by this imaging modality was the quantification of the associated biomarker, i.e. the physical harmonic signal measured, which allowed validation of the therapeutic effect. For this purpose, Lassau et al.<sup>11</sup> conducted a French multicenter study that included 539 patients treated with antiangiogenic drugs on different solid tumors, from 19 different centers (2007-2010). This study highlighted the existence of a biomarker defined as the Area Under the enhancement Curve (AUC) of the harmonic signal from contrast agents extracted within the tumor region of interest during the 3 minutes of image acquisition. More precisely, it was shown that a decrease of more than 40% of the AUC after one month of treatment was correlated with a positive response to treatment, to progression-free survival ( $p=0.005$ ) and to overall survival ( $p=0.05$ )<sup>12</sup>.

DCE-US imaging is the only method with a quantitative biomarker<sup>13</sup>, used on all metastases, to assess response to antiangiogenic therapy earlier than conventional methods. This led to the publication of the European guidelines by the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) and international guidelines the World Federation for Ultrasound in Medicine and Biology (WFUMB), defining validated pathological indicators and methodologies to be respected<sup>14-16</sup>, in order to facilitate the standardization of the DCE-US technique.

However, the predictive value of this biomarker identifying a good responder after 30 days was validated on the condition of implementing a particular quantitative imaging protocol for acquisition, i.e. with specific settings and on a single type of ultrasound scanner. This was a major obstacle in monitoring patients at different radiology departments and conducting multicenter clinical research studies, as all centers do not necessarily have the same ultrasound scanner. This led to the issue of protocol being used on the new generations of ultrasound scanners and preventing the adaptation of the method despite the international recommendations<sup>17,18</sup>. Beyond the DCE-US imaging, inter-machine standardization of imaging protocols was an international challenge for the use of biomarkers in multicenter studies, widely explored by the Quantitative Imaging Biomarkers Alliance (QIBA, Oak Brook, IL, USA).

Today, the challenge is to consider the variety of ultrasound and instrumental developments while maintaining the predictive values of therapeutic response established in clinical trials. In this context, Pitre-Champagnat et al.<sup>19</sup> have already developed an original method to calibrate two devices regardless of their references in order to apply standardized protocols. The calibration method was based on the variation of the harmonic signal intensity obtained from the area under the Time-Intensity Curves (TICs) according to different concentrations of contrast agent realized by manual dilution. The slope of this measured curve was the calibration parameter. This method is innovative,

but the dilution of the contrast agents was responsible for the variability of the measurements, implying a long experimental time over a week and a high cost.

The aim of our study was to develop a new and reliable setup further improving the ultrasound calibration method in order to move towards a fast, simple and inexpensive process with the use of a single vial of SonoVue™ (Bracco). The upgraded method was applied to calibrate the dynamics of the Aplio500™ (Canon Medical Systems, Tokyo, Japan) on a new generation ultrasound scanner from the Gustave Roussy Cancer Campus: the Aplioi900™ and to use the DCE-US imaging protocols to monitor patients undergoing antiangiogenic therapy through new ultrasound scanners. This study was completed by the characterization of the new calibration setup to establish the distribution of the concentrations obtained simultaneously and to evaluate its reliability in terms of repeatability and reproducibility of measurements.

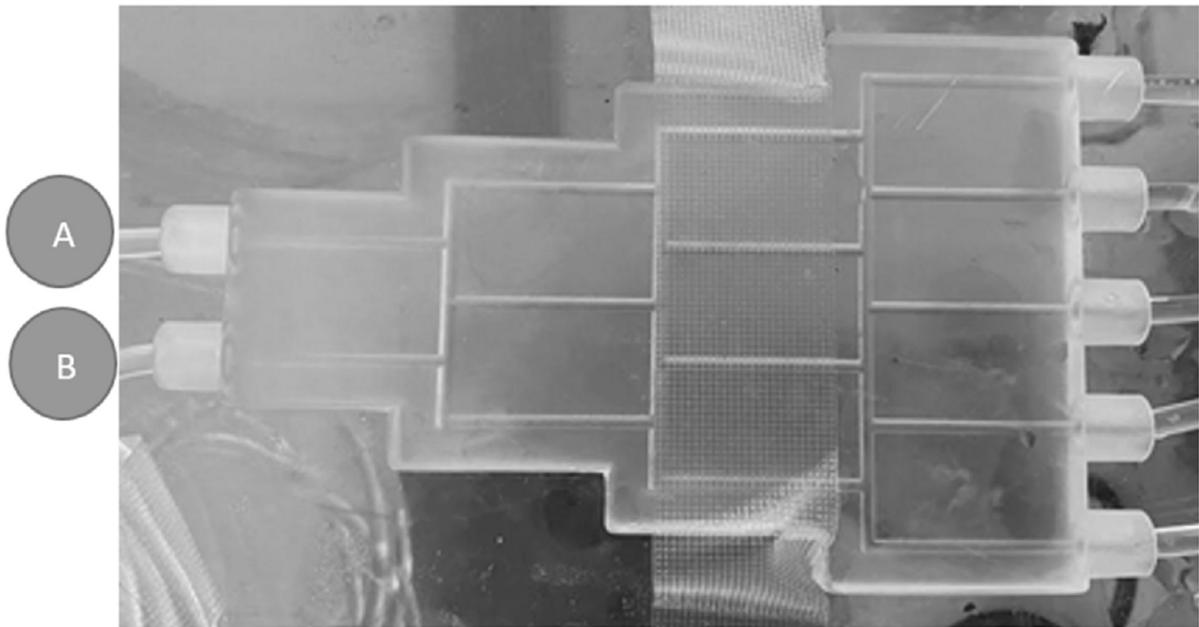
## **MATERIALS AND METHODS:**

### *Calibration Setup*

To perform the calibration of ultrasound scanners in the DCE-US mode, the variation of the harmonic signal was considered as a function of the concentration of the contrast agent<sup>20</sup>. The calibration procedure was based on matching the slopes extracted from the plot of the Areas Under the time-intensity Curves (AUCs) measurements as a function of concentration. The first method<sup>19</sup> previously developed by the team consisted in preparing four different concentrations of ultrasound contrast agent from manual dilutions into vial. In this present study, this step was replaced by a novel concentration splitter presented in Figure 1 and allowing a set of several concentrations to be obtained simultaneously from a single injection. The concentration splitter was based on the principle of the mixed microfluidic gradient generator with a flow splitter design. The concentration splitter<sup>21,22</sup> relied on a system with two inlets, one for each fluid, from which a succession of interconnected bifurcations derived. At each additional T-shaped bifurcation, the fluids split with a concentration distribution inversely proportional to the hydraulic resistances involved at each bifurcation. Mixed concentrations of the two fluids were obtained at each level of bifurcation. Three levels of the concentration splitter have been chosen to have 5 concentrations simultaneously ranging between 0 and 100% of the initial concentration. The 4 non-zero concentration values thus collected are used to extract 4 AUCs using a single injection of the contrast agent. This concentration splitter is optimal to mixing miscible fluids with a high diffusion coefficient. In the case of microbubbles fluid with a very low diffusion coefficient (about  $1 \cdot 10^{-8} \text{m}^2 \text{s}^{-1}$ ), what dominates mass transport and mixing is the convection phenomenon. In other words, the microbubbles remain in their velocity field line and do not move. This effect implies a partial mixing of the contrast agents in water which results in a poor distribution of the initial concentration, with the final values of the collected concentrations too close to each other. The mixing of the contrast agents in the splitter can be improved by establishing a large difference in flow velocity, and therefore flow rate, between the 2 fluids at the inlets of the setup. Considering Poiseuille's law which gives the pressure drop in an incompressible and Newtonian fluid in laminar flow in a cylindrical tube, it is possible to modify the flow rate of a fluid which is inversely proportional to the length of the tube, if all the other parameters are identical (radius of the tube, and pressure difference). In the case of this study, the lengths of tubes at the inlets of the splitter were fixed at 2300cm for the one reserved for the microbubbles flow and at 11,5cm for the other one with water only. Thus, this difference in length implies a difference in flow velocities of the two fluids which are inversely proportional, and 10 times faster for water.

In addition, and as stated in the previous study<sup>19</sup>, this long length made it possible to artificially mimic the temporal distribution of microbubbles in clinical conditions, i.e. to have a transit time similar to

those measured in the study of 539 patients (mean transit time of 20 s and range: 0–180 s). The geometry of the splitter concentration was made with: i) circular sections of 3mm diameter; ii) 3 levels of bifurcations permitting 5 different concentrations of contrast agents at the output. The final distribution of the 5 different concentrations of contrast agent have been determined experimentally. The concentration splitter was manufactured by 3D printing using stereolithography (silex3Dprint, France). The process allowed to obtain a transparent device for the smooth flow of the microbubbles. The transparency was an essential parameter to verify the smoothness of the flow for different concentrations of the contrast agent.



**Figure 1:** The novel flow splitter allowing the mixture of 2 fluids in input A and B, with respective concentrations  $C_A$  and  $C_B$ . Here, A was water and B was water with SonoVue™ (Bracco).

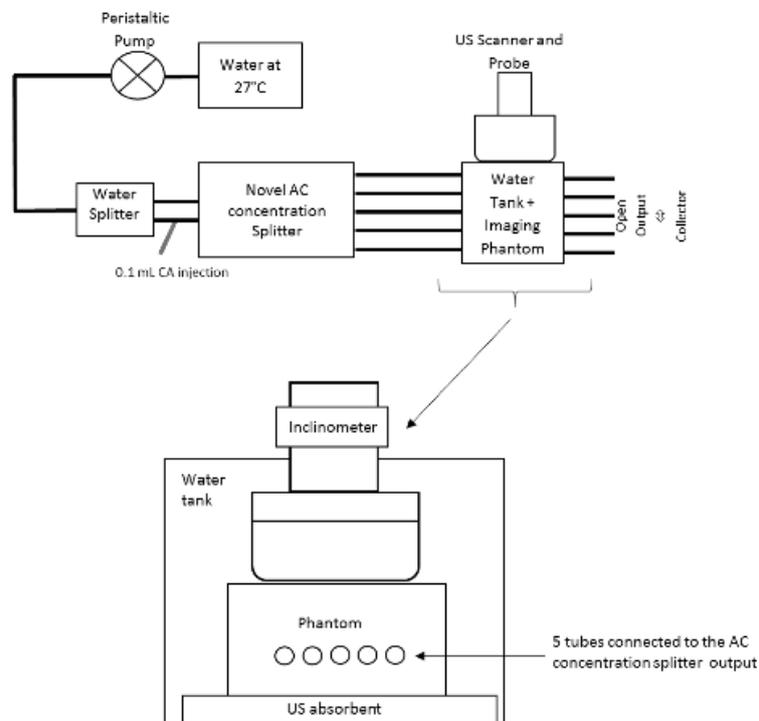
Figure 2 presents the concentration splitter placed within an open-circuit flow model with 2mm diameter perfusion tubes. The fluid was non-degassed water at  $27^{\circ}\text{C}^{23}$  and was driven by a peristaltic pump (PumpDrive PD 5201, Heidolph Instruments, Schwabach, Germany), providing a non-pulsatile flow at 1.59 mL/s to mimic the physiological vascular condition. The contrast agent used was SonoVue™ (Bracco)<sup>24,25</sup>, which is widely used in clinical routine. A 0.1 mL of contrast agent was injected in one of the two inlets of the splitter. To be as close as possible to the physiological concentration used in the clinical examinations, i.e. 4.8 mL of SonoVue™ (Bracco) for 5L of blood in the patient, the total volume of flow circuit was 143.7 mL. An insulin syringe (BD Micro-Fine™ +, BD Medical - Diabetes Care, Becton Dickinson France S.A.S) was used to provide the bolus accuracy of SonoVue™ injected. Finally, in order to simulate clinical ultrasound images, the output tubes of the concentration splitter passed through a phantom made of a material that mimics the acoustic properties of tissue<sup>26</sup> and was placed in a water tank to ensure the transmission of ultrasound waves. In practice, two phantoms were designed with different thicknesses to match the ultrasound frequencies of the probes: phantom #1 dedicated for imaging with the abdominal probe, and the second phantom #2 with the linear probe shown in figure 3.A.

Phantom #1: 2.5 cm thick and 4.5 cm width; tube centers spaced 1.3 cm apart and located 0.5 cm from the top border.

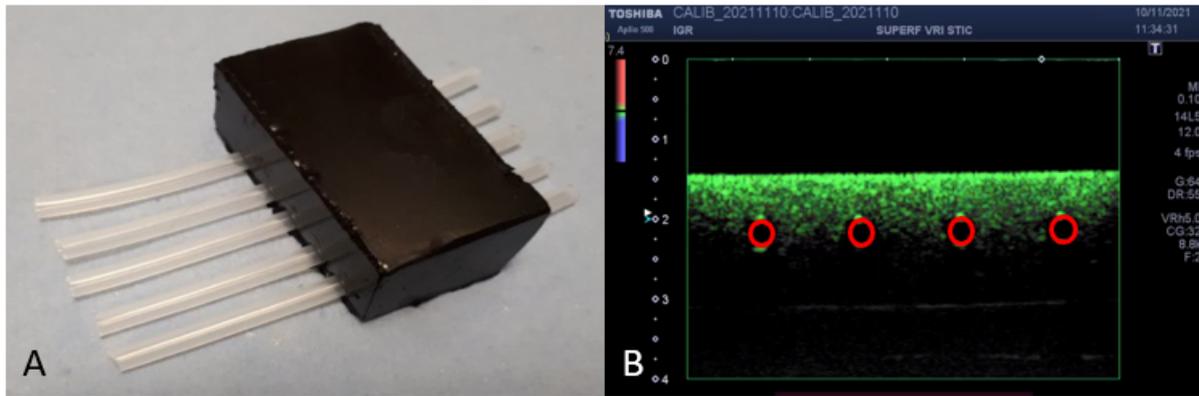
Phantom #2: 5 cm thick and 4.5 cm width; tube centers spaced 1.3 cm apart and located 1.7 cm from the top border.

The material mimicking tissue was based on agar gel<sup>19</sup> and was composed of i) 82,6% distilled water with 3% of agar agar gel; ii) 6% glycerol which allowed to obtain the average celerity acoustic properties of  $1548 \pm 5 \text{ m.s}^{-1}$ ; iii) 8% of carbon powder that was used to modify the attenuation, with a linear frequency dependence (an acoustic attenuation of  $0.5 \pm 0.01 \text{ dB cm}^{-1}\text{MHz}^{-1}$ ) and the scattering of the ultrasound waves in order to imitate the tissue and iv) 0.4% preservative which allowed the phantoms to be preserved for several months<sup>26,27</sup>.

The probe was immersed in the tank in front of the phantom and its positioning was controllable. Its inclination, conditioning the detection area, was set by a digital inclinometer with a magnetic base (AUTOUTLET, EFUTL341-DESA) at fixed with the vertical axis. In addition, the optimal field of view of the probe away from edge effects was centered on the 4 tubes with a non-zero concentration of SonoVue™ to ensure homogeneous signal measurement. The tank had external dimensions of  $275 \times 105 \times 100 \text{ mm}^3$  and Ecoflex® walls to limit the reflections of the US waves on the Plexiglas walls of the box.



**Figure 2:** Experimental design depicting the circulation of water. The experimental set-up including i) a peristaltic pump providing the vascular flow at the entrance ii) a water splitter providing two equal water deviations iii) the concentration splitter in its center providing the 5 contrast agent concentrations and iv) the water tank containing the phantom and the probe.



**Figure 3:** Image of the superficial phantom A) with the tubes passing through; B) in VRI mode, with four regions of interest (ROI) drawn on tube sections.

### *Ultrasound scanners*

In this study, we calibrated the new Aplioi900™ (Canon Medical Systems) ultrasound scanners by comparing them with the settings already validated on the Aplio500™ (Canon Medical Systems). Acquisitions were performed using the contrast-imaging mode used for quantitative imaging protocol, i.e. the Vascular Recognition Imaging (VRI) mode developed by the manufacturer. This VRI mode was driven by two main parameters: the Mechanical Index (MI) and the Color Gain (CG).

The Aplio500™ (Canon Medical Systems) was previously calibrated for clinical DCE-US quantification applications<sup>19</sup> and used in our study to validate the performance of our setup. The Aplioi900™ (Canon Medical Systems) ultrasound scanner needed to be calibrated in this study. The search for settings to ensure similar performance between the two-ultrasound scanners was performed with four probes: the abdominal curvilinear probe PVT-375 BT (3.5 MHz) and the linear probe PLT-1005BT (10 MHz) for the Aplio500™ (Canon Medical Systems); the abdominal curvilinear probe PVI-475BX (4 MHz) and the matrix linear probe PLI-1205BX (12 MHz) for the Aplioi900™ (Canon Medical Systems). The calibration consisted of adjusting the two ultrasound scanner parameters linked to the VRI mode: MI and CG. The MI conditioned the volumetric amplitudes of the microbubbles and contributed directly to the collected harmonic signal. To avoid destruction of the microbubbles, the MI had to be strictly < 0.2. Therefore, the choice not to exceed an MI of 0.15 was made. The CG modifies the gain of the analogue amplifier of the probe and acts on both the collected signal and the noise. All settings are summarized in Table 1. The settings of the Aplioi900™ (Canon Medical Systems) were adjusted in two steps: 1) determination of the optimal MI with adjustments in the interval [0.12 - 0.14] for the abdominal probe (PVI-475BX / i8CX1) and the interval [0.09 - 0.11] for the linear probe (PLI-1205BX / i18LX5) with a CG to find a slope value in the range of the previous ultrasound scanner; 2) determination of the optimal CG with the value of the previous MI fixed by adjusting the CG in the interval [34 – 36] for the abdominal probe and in the interval [37 – 39] for the linear probe.

	Aplio500™		Aplio900™	
	3,5MHz (PVT-375BT) Abdominal 6C1	10 MHz (PLT-1005BT) Linear 14L5	4 MHz (PVI-475BX) Abdominal i8CX1	12 MHz (PLI-1205BX) Linear i18LX5
MI	0,15	0,10	To be adjusted	To be adjusted
CG (dB)	35	32	To be adjusted	To be adjusted
VRh (MHz)	3	5	3,5	6
Focal mode B (%)	72%	48%	72%	48%
AP	0,75	1	0,53	2
Focal VRI (%)	75%	50%	75%	50%
VRI window	<b>Full VRI window size</b>			
Depth (cm)	7	4	7	4

MI=Mechanical Index; CG=Color Gain; VRh=Vascular Reception harmonic frequency (MHz); AP=Acoustic Power; VRI=Vascular recognition imaging

**Table 1:** Ultrasound scanners settings

### Data acquisition and analysis

Raw data from the 100 seconds acquisition were recorded after the SonoVue™ (Bracco) injection. The quantification processes were performed with the CHI-Q software (Canon Medical systems). Circular Regions Of Interest (ROI) shown in Figure 3.B. were manually drawn respectively on each of the 4 core tubes with sizes: 3 mm for the abdominal probe and 2 mm for the linear probe. The extracted time-intensity curves were plotted according to a robust mathematical model, developed and patented by the team (N° PCT/IB2006/003742)<sup>28</sup> allowing the determination of the corresponding AUC for each ROI.

The calibration method proposed in this article was based on the study of the dynamics of the enhanced signal as a function of different concentrations of the contrast agent<sup>19</sup>. Thus, the measurement of the AUC  $x_i$  as a linear function of the concentration  $C_i$  is defined as follows:

$$x_i = a + bc_i \quad (1)$$

To establish the AUC calibration curve as a function of the concentrations, the average AUC was established from a single experiment consisting of six successive acquisitions.

The AUC was plotted as a function of the four contrast agent concentrations, representing the variation in intensity of the harmonic signal, the slope  $b$  being the calibration parameter. The intensity variation obtained with settings on the Aplio500™ (Canon Medical Systems) was considered as the reference, since it was the one used during the multi-centric DCE-US study<sup>12</sup>. The calibration method implied that the new ultrasound scanners had to have the same slope to be calibrated.

The accuracy of the AUC measurements obtained from the new setup in terms of repeatability and reproducibility was evaluated in 3 different configurations. The first being within a single experiment consisting of 6 successive acquisitions, the second, the repeatability of two sets of experiments performed on the same day but with different vials of SonoVue™ (Bracco) and finally, the reproducibility of two sets of experiments at different days involving a new vial of SonoVue™ (Bracco) and disassembling the measurement setup.

Data analysis consisted in evaluating the agreement between the measurements obtained during the different experiments defined according to the following statistical model for the AUC measurement  $x_{ijk}$ , at concentration  $c_i$ , for acquisition  $j$ ,  $1 \leq j \leq 6$  and experiment  $k$ ,  $k=1$  or  $2$ :

$$x_{ijk} = a + bc_i + \beta_k c_i + \epsilon_{ijk} \quad (2)$$

In the above equation (2):

- $(a + bc_i)$  is the fixed-effect linear regression model, such that  $E(x_{ij}) = a + bc_i$  which gives the “baseline” measurement
- $\beta_k c_i$ , the effect of the condition k, consider as proportional to the concentration
- $\epsilon_{ijk}$ , the residual term

It implies that:

$$E\left(\frac{x_{ijk}-a}{c_i}\right) = b + \beta_k \quad (3)$$

The agreement between measurements  $\alpha$  was defined as follows:

$$\alpha = 1 - \frac{|E((x_{ijk}-a)/c_i \mid k=1) - E((x_{ijk}-a)/c_i \mid k=2)|}{E((x_{ij}-a)/c_i)} \quad (4)$$

$$\alpha = 1 - \frac{|\beta_1 - \beta_2|}{b} \quad (5)$$

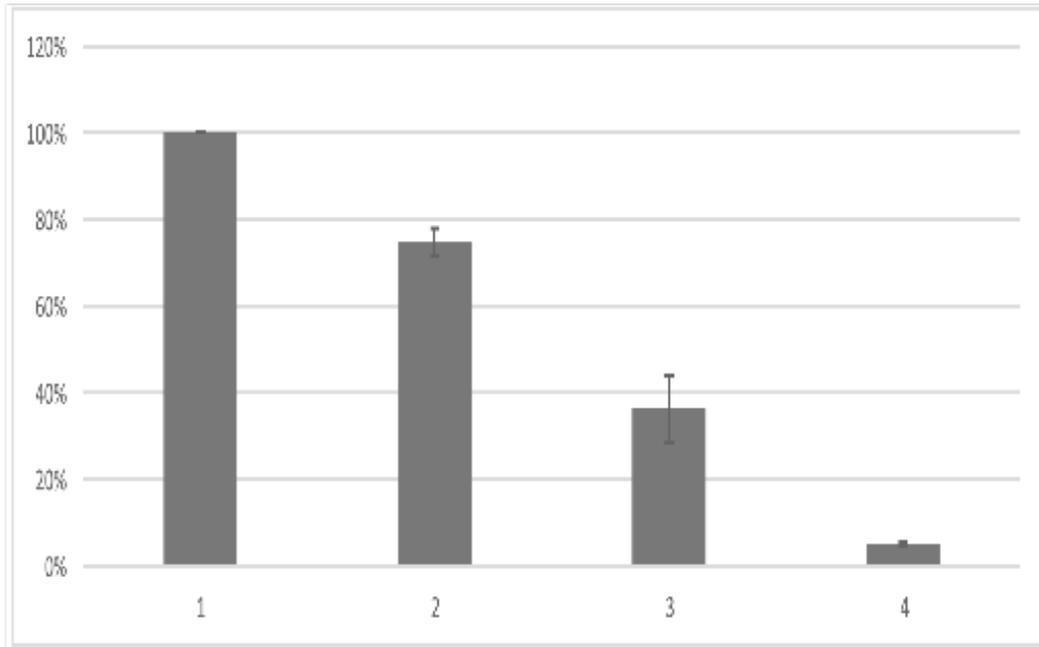
In case of a perfect agreement,  $\alpha=1$ , and  $\alpha \leq 0$  if the difference in the  $\beta$  variation due to the experiment is of the order of magnitude of the main effect. This equation (5) was also used to determine the agreement between the measurements on the two Aplio™ (Canon Medical Systems) scanners after the calibration process.

Finally, the accuracy of the measurements was estimated from the coefficient of variation (CV %) of AUC (CV=Standard deviation/mean) for each acquisition.

## RESULTS:

### *Characterization of the calibration setup: concentration distribution and reliability of measurements*

Figure 4 shows the SonoVue™ (Bracco) relative concentration at the outlet of the setup which decreased as a function of the splitter outputs. The relative measurement of the harmonic signal at the output of the setup was estimated over 3 experiments to reflect the concentration of the contrast agent. On the 18 measurements, the average percentages of the relative concentrations were 100% $C_0$ , 68±2.3%  $C_0$ , 36±7.5%  $C_0$  and 5±1.1%  $C_0$  with a small coefficient of variation of 3 to 20%, the maximum deviation being for the lowest concentration.



**Figure 4:** Bar chart depicting the distribution of relative concentration (%) as a function of the splitter outputs #1, #2, #3 and #4.

Table 2 summarizes the values obtained in an experiment with the linear probe on the Aplio500™ (Canon Medical Systems) with 6 successive injections. The coefficient of variation varied from 5 to 10% depending on the relative concentrations of SonoVue™ (Bracco), which remained the same for all measurements done, regardless of the probe and ultrasound machine used.

	Concentration 1	Concentration 2	Concentration 3	Concentration 4
<b>Injection #1</b>	153952	101940	51277	11883
<b>Injection #2</b>	137458	96805	44564	12605
<b>Injection #3</b>	147870	85376	51921	13636
<b>Injection #4</b>	140251	97903	44830	14663
<b>Injection #5</b>	145063	106771	42759	11359
<b>Injection #6</b>	137618	88490	45819	13775
<b>Mean AUC (a.u.)</b>	<b>143702</b>	<b>96214</b>	<b>46862</b>	<b>12987</b>
<b>SD (a.u.)</b>	6514	8056	3805	1254
<b>CV (%)</b>	<b>5%</b>	<b>8%</b>	<b>8%</b>	<b>10%</b>

*AUC = Area Under the Curve; SD = Standard Deviation; CV = Coefficient Variation*

**Table 2:** Summary of AUC measurements performed on the Aplio500™ (Canon Medical Systems) with the linear probe during six successive acquisitions with an MI 0.10 = and a CG = 32dB. The concentrations 1,2,3 and 4 correspond respectively to the 4 relative concentrations measured at 100%, 68 %, 36% and 5%.

The reliability of the calibration setup was defined by the notion of agreement, between the AUC measurements obtained during two separate experiments, for a given concentration.

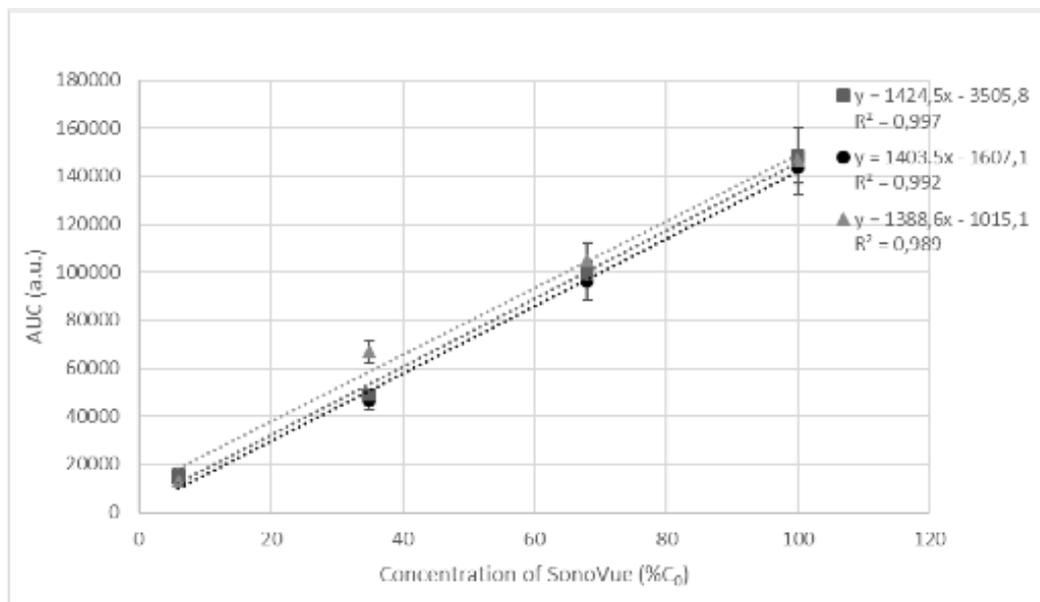
When the series of experiments was performed on the same day on the Aplio500™ (Canon Medical Systems), the device provided an excellent agreement between the measurements with a value of

96.8%. This agreement on the measurements when the experiments were carried out on different days was between 93.4 and 96.7 %.

### Calibration results

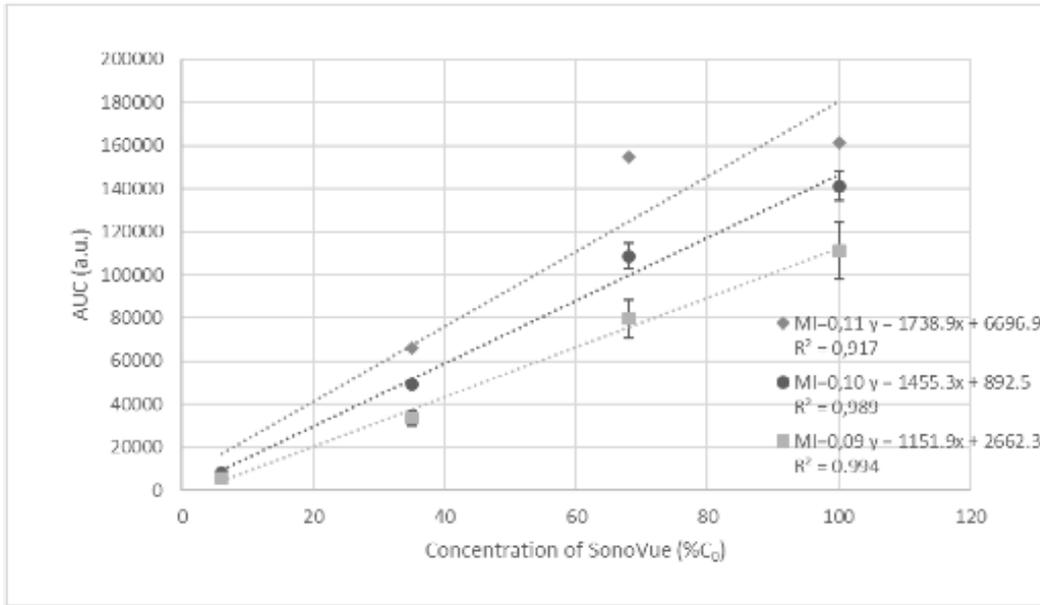
#### Linear probe results

The reference curves obtained with the linear probe, on the Aplio500™ ultrasound scanner (Canon Medical Systems) obtained from 3 distinct experiments are presented in Figure 5. The corresponding slopes were close to each other, ranging from 1388 a.u. to 1424 a.u..

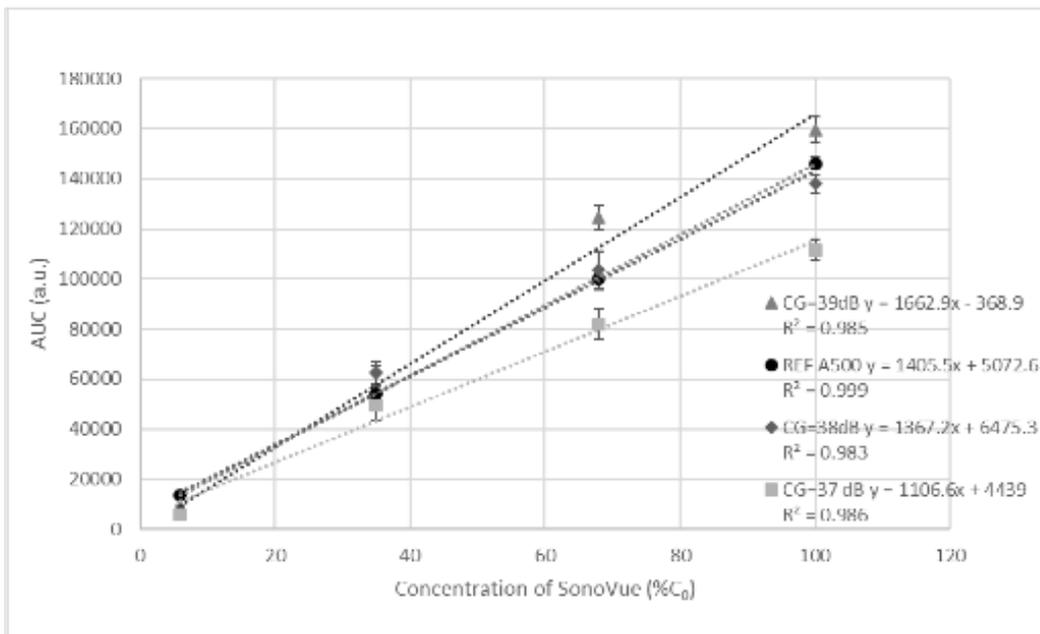


**Figure 5:** Graph representing three reference curves on the Aplio500™ (Canon Medical Systems), with the linear probe PLT-1005BT, established from 3 different experiments. The settings used were MI=0,10 and CG=32 dB, same as the multi-centric study. Mean AUC values were plotted in function with the relative concentration of SonoVue™ (Bracco). AUC: Area Under the Curve; MI=Mechanical Index; CG=Color Gain.

The determination of the MI setting on the Aplioi900™ (Canon Medical Systems) was done by using the reference values previously measured with the Aplio500™ (Canon Medical Systems). The curves obtained for the MI at 0.09, 0.10 and 0.11 with a CG set at 38dB are shown in Figure 6 and the slopes measured were 1151 a.u., 1455 a.u. and 1738 a. u. respectively. This first series of experiments allowed to retain MI=0.10 as one of the two parameters to be calibrated. Using this MI, different experiments were performed with CG ranging from 37 to 39 dB. Figure 7 presents the results obtained and shows the mean reference slope of 1405 a.u.. Therefore the gain of 38 dB with a slope of 1367 a.u., the closest to that of the reference, is the CG to be used for the Aplioi900™ (Canon Medical Systems). Thus, the settings validated on the new ultrasound scanner Aplioi900™ (Canon Medical Systems) were MI=0,10 and CG=38dB for the linear probe.



**Figure 6:** Variation of the mean AUC with 3 MI (0.09, 0.10 and 0.11) on Aplioi900™ (Canon Medical Systems) using the matrix linear probe PLI-1205BX, VRh 6.0 and CG=38 dB – Mean AUC values were plotted as a function of the relative concentration of SonoVue™ (Bracco). The acquisitions were not repeated for MI=0.11 with the linear probe due to signal saturation making the quantitative analysis obsolete. VRh=Vascular Reception harmonic frequency (MHz); CG=Color Gain; MI=Mechanical Index



**Figure 7:** Variation of the mean AUC with 3 CG (37, 38 and 39) on Aplioi900™ (Canon Medical Systems) using the matrix linear probe PLI-1205BX, VRh 6.0 and MI=0.10 – Mean AUC values were plotted in function with the relative concentration of SonoVue™ (Bracco). VRh=Vascular Reception harmonic frequency (MHz); CG=Color Gain; MI=Mechanical Index

For the abdominal probe, the same steps were followed. The slopes obtained with the abdominal probe on the Aplio500™ (Canon Medical Systems) ultrasound scanner were close, ranging from 2419 a.u. to 2560 a.u.. The AUC varied linearly with the concentration of SonoVue™ (Bracco).

The first measurements performed with the Aplioi900™ (Canon Medical Systems) aimed to determine the MI that would approach the reference values previously measured with the Aplio500™

(Canon Medical Systems). The slopes of the calibration curves were deducted to be 1119 a.u., 2314 a.u. and 3298 a.u., with the CG fixed at 35 dB and different MI of 0.12, 0.13 and 0.14, respectively. These first series of experiments allowed to retain MI=0.13 as one of the two parameters to be used on the Aplioi900™ (Canon Medical Systems). Using this MI, different measurements were made on varying CGs to reveal a slope close to that of the reference line for a gain of 34 dB. Thus, the settings validated on the new ultrasound scanner Aplioi900™ (Canon Medical Systems) were MI=0,13 and CG=34dB for the abdominal probe.

Finally, once the two-ultrasound scanners were calibrated for both types of probes, the agreement between their respective quantitative measurements ranged from 95.4 and 98.8%.

## **DISCUSSION:**

The advent of quantitative imaging, radiomics<sup>30,31</sup> and associated artificial intelligence methods<sup>32-34</sup> have paved the way for the biomarkers research as objective criteria for conducting personalized medicine. Nevertheless, the use of these new biomarkers in clinical routine is hampered by a lack of standardization of imaging systems with very uneven and uncalibrated instrumental performances. This is particularly the case with DCE-US ultrasound, which has a wide range of applications, particularly in oncology<sup>29</sup>, since the clinical validation of AUC as an early imaging biomarker correlated with overall survival. This biomarker determined from the same ultrasound machine and the same settings is not generalizable to date neither to other machines nor to other updates. After having proposed and validated a new method of ultrasound calibration<sup>19</sup>, the objective of the present study was to develop a new setup in order to make possible the transfer of the DCE-US imaging procedure with the application of AUC with reliability and rapidity.

Our study proposes a concentration splitter to calibrate ultrasound scanners based on the simultaneous measurement of several concentrations of contrast agent. Its application is focused on two models from the manufacturer Canon Medical System with the VRI acquisition mode, which required the modulation of two acquisition parameters, to standardize the measurement of AUC, the validated imaging biomarker. The calibration method<sup>19</sup> was proposed in 2017 by the team with the measurement of the signal as a function of four contrast agent concentrations obtained from 4 dilutions of SonoVue™ vials. Once this dilution was achieved, the measurements were reliable with a coefficient of variation of 12%, slightly higher than that obtained with the new setup which was between 5 and 10%, this maximum being obtained only for the lowest concentration of SonoVue™, mathematically implying a greater fluctuation in values. However, it was very difficult in practice to obtain identical dilutions, because the process led to more or less outgassing of the solutions. It was then necessary to check the quality of the dilutions by preliminary measurements and if necessary to start again the process. This required a large number of measurements, a large quantity of Sonovue™ with several dozen vials, and a long experiment time. In this study, the development and implementation of the concentration splitter made it possible to simultaneously obtain 4 different concentrations of microbubbles with a single injection of the contrast agent. The relative concentration measurements obtained at the outlet of the setup were particularly stable despite convective mixing of the fluids and were 100% C0, 68±2.3% C0, 36±7.5% C0 and 5±1.1% C0. The concentration splitter reduces both the number of vials used for calibration and the sources of variability related to the manual injection method. The repeatability and reproducibility of the calibration system were defined as the agreement between the AUC measurements obtained in two separate experiments. The agreement between the measurements was excellent, ranging from 93.4 to 96.8% depending on whether the experiments were conducted on the same ultrasound machine on the same day or on different days. This good agreement between the measurements was illustrated in Figure 4 where it appears, for three series of experiments, similar calibration lines with close slope values, whose maximum relative difference was 2%. The sources of variability attributed to the daily dismantling and moving of the measurement bench were minimized in part by the implementation of

the inclinometer facilitating the precise repositioning of the probes. These excellent results confirm the robustness of the measurements performed with this new setup, a key criterion to easily extend the calibration method to imaging departments in different hospitals or centers. The calibration on Aplio series™ (Canon Medical Systems) was easily performed and it was quickly possible to obtain the optimal settings for the department's new ultrasound scanner: the Aplioi900™ (Canon Medical Systems).

The calibration method using the concentration splitter successfully determined the equivalent setting of the two ultrasound systems, the Aplio500™ and i900™ (Canon Medical Systems), to provide the same dynamic range of harmonic ultrasound signal. The calibration method allowed differentiation of each variation of the acquisition parameters, MI or CG, with a relative difference between the slope values of about 20%. The settings obtained with the abdominal probe were MI=0.13 and CG=34dB, and with the linear probe: MI=0.10 and CG=38dB. The choice was made to use the lowest MI and a slightly lower CG. Indeed MI is favored over color gain because it adds noise to the image. Besides, it is important not to anticipate a discontinuation of this therapy by giving the patient a margin. The agreement between the two ultrasound scanners once calibrated to measure AUC was also excellent, varying between 95.4 and 98.8% depending on the day.

This calibration method applied to two specific ultrasound scanners is a universal method applicable to all ultrasound systems. In particular, from this method, it would be possible to transpose the validated biomarker, AUC, according to three methodological variables often reported by the international community: i) the pulse subtraction (PS) mode; ii) the quantity of injected microbubbles, iii) the use of another contrast agent. In the case of an extension of this study in PS mode, it is necessary to be able to identify a referential biomarker value that does not currently exist. Moreover, the PS mode is particularly sensitive to the coupling between the variation of the focal depth and the variation of the MI. An additional positive point of this calibration method is that it is based on the raw data, therefore the radiologists can change the image gain their own way without altering the measurements involved in the signal enhancement, which is essential for radiologists in their clinical routine.

From a practical point of view, one of the difficulties encountered when calibrating ultrasound scanners was the variation of the ultrasound signal, both transmitted and recovered, with respect to the focal depth. Thus, to ensure good image quality, the focal depth automatically conditions the characteristics of the transmitted ultrasound beam, i.e. the acoustic power (AP) and the initially set MI. In order to avoid this, six sub-presets were defined by fixing AP and MI according to 6 ranges of lesion depth covering successively 2 cm intervals, after consultations with the constructor. Moreover, in the near field of 0-4 cm, the abdominal probe (PLI-1205BX, Canon Medical Systems) offered excellent spatial performances, a wide field of exploration and at 3MHz a more efficient detection of the harmonic signal of the contrast agent. Thus, in this superficial zone of exploration, its use competes with that of the linear probe.

Finally, one of the limitations of our robust calibration method is the manufacture of the agar-agar gel phantom which is perishable by definition with a several months life span. In this context, during the implementation of this study, several phantoms were manufactured and used. Their replacement involved a change in the slope values but in a proportional way, which allowed the identification of the settings to be adjusted. However, it would be interesting to improve this part of the assembly with another material. In a possible context of dissemination of the method, a possible improvement to gain more robustness would be to obtain a mono-block system including the splitter, the phantom and the whole tank, cast in gel in order to avoid readjustments of the probe in relation to the phantom. This would considerably reduce the mobilization time of the ultrasound scanners.

## **CONCLUSIONS:**

This new calibration setup is robust and has enabled the DCE-US imaging protocols to be used on the new ultrasound scanners, the Aplioi900™ (Canon Medical Systems), and to continue therapeutic evaluation by quantitative imaging of patients.

Moreover, this original setup based on a novel concentration splitter is a further step towards the standardization of the calibration in terms of robustness, time taken and the cost of the experiment. The next new challenge is to take into account the diversity of ultrasound scanners, with their different technologies, in order to extend the use of DCE-US imaging while maintaining the predictive values of therapeutic response established in clinical studies. This step would allow the dissemination of the DCE-US imaging protocols throughout the medical community regardless of the ultrasound scanner being used.

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