Influence of ultrasound image acquisition setup and region of interest size on the grey-scale echo intensity values of ultrasound images using a muscle phantom

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Abstract— **The influence of ultrasound equipment setup and region of interest (ROI) size on the grey-scale brightness values of ultrasound images was investigated using a muscle phantom. The effects of focus, gain, depth, zoom and ROI size were verified. The Echo Intensity (EI) was estimated using ImageJ software. No significant differences were found in average brightness when changes were made to focus and zoom. EI rose linearly with an increase in gain and decreased linearly with increasing depth of penetration selected on the equipment. EI decreased logarithmically with the increase in size of the ROI. We propose dividing EI values by respective gains as an option to limit the influence of gain changes on EI. Other alternatives must still be investigated regarding the influence of depth on EI. This work is intended to help researchers make decisions based on parameters that may be influenced by their EI measurements, such that valid interpretations can be made.**

Keywords— **Brightness, Ultrasound Parameters, Phantom.**

I. Introduction

B-mode ultrasound has been used to investigate muscle changes in people suffering from different pathologies such as low-back pain (LBP) [1–3]. Image grey-scale mean values have been used to distinguish between normal and pathologic muscles (e.g. fibrosis or fatty infiltration) that have increased echo intensity (EI) [4], while Wallwork et al. (2008) used ultrasound imaging to investigate individuals with and without LBP [5]; however, many of these ultrasound measurements depend on the image acquisition system and its settings [6].

When ultrasound images are analyzed quantitatively, it is crucial that all system settings are known in every measurement as the EI can change when one of these parameters is varied [7]. This fact makes it difficult to visually assess whether changes in grey-scale values are physiological and altered due to pathology, or if they are due to ultrasound equipment settings.

This work aimed to investigate the influence of ultrasonic parameters on the grey-scale brightness values obtained from ultrasound images using a custom-made phantom with ultrasonic properties similar to human muscle.

Materials and methods

A. Phantom

The phantom was prepared by heating a solution with 200 ml of PVCP (M-F manufacturing Co, INC®, Fort Worth, TX, USA) and with 5 g of commercial vanilla powder (used as ultrasonic scatterer to produce the brightness patterns that characterize image texture) in a microwave oven. This material was chosen due it possessing acoustic properties very close to those of human skeletal muscle. The phantom had ultrasound speeds of 1501.5±0.7 m/s and 1331.8±0.3 m/s, attenuation coefficients of 0.46±0.03 dB/cm and 0.94±0.09 dB/cm, shear velocities of 8.4±1.2 m/s and 1.7±0.8 m/s [8], and density of 1.00 ± 0.04 g cm^{-3} at 20°C and 45°C, respectively [9]. Moreover, it is inexpensive, is easy to mold, and has stable properties over time. The phantom was molded to a final block of size 6.5 x 6.5 x 3.5 cm (Fig. 1 - Left).

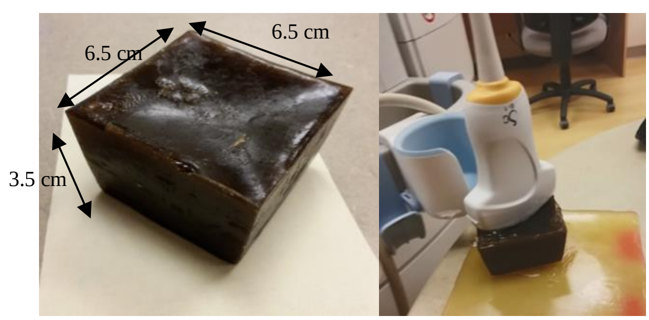

Fig. 1 Left: Phantom with its dimensions. Right: Ultrasound Transducer on the phantom for image acquisition.

B. Ultrasound Image Acquisition

For the acquisition of images, an ultrasound system (Aixplorer V.9, Supersonic Imaging, Aix-en-Provence, FRANCE) with a convex transducer (Single Crystal Curved XC6-1, Number of elements: 192, Bandwidth: 1-6 MHz) was used. The transducer was positioned on the upper surface of the phantom, and the phantom was laid over a plate of pure PVCP to reduce reflections from the surface below. Ultrasonic coupling gel was used between the transducer and phantom and between the phantom and the PVCP base (Fig.1 – Right).

Each image acquisition was changed throughout minimum and maximum settings in which it was possible to clearly see the phantom image. The following parameters were verified:

1. Focus – ranging between 1.5 and 5 cm.
2. Gain – ranging between 20 and 100%.
3. Depth – ranging between 2 and 10 cm.
4. Zoom – ranging between 90 and 150 %.
5. Size of Region of Interest (ROI) – from 0.4 to 25 cm^2.

The explored parameters (yellow arrows) and phantom image can be seen in Fig. 2. The uniformity and isotropic characteristics of the phantom image were important at this stage to reduce variability that would be seen *in vivo*. All images were saved and transferred to a personal computer for analysis.

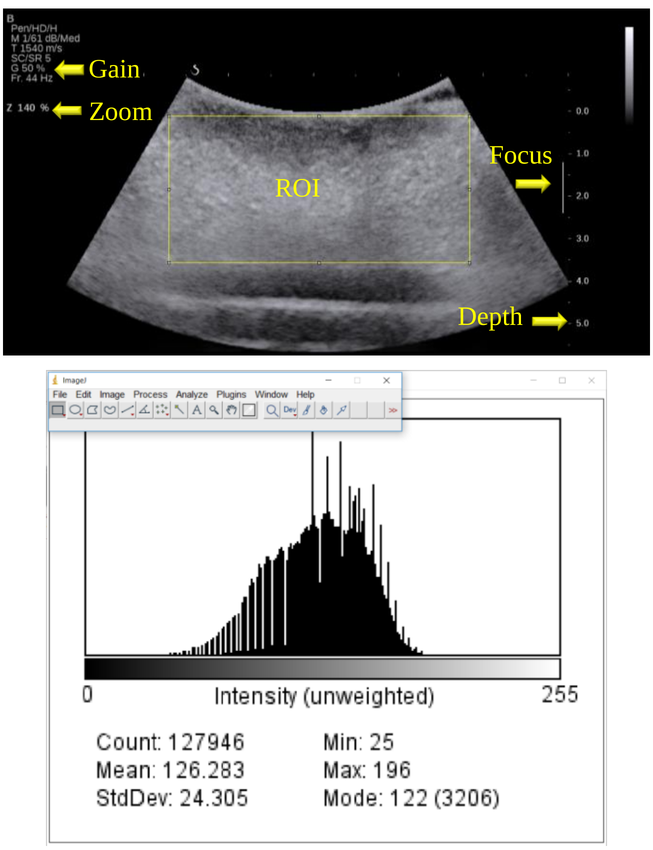

Fig. 2 Top: Ultrasound image of the phantom. Yellow arrows point to parameters changed during analyses, whereas the selected ROI can be seen in the centre of the image. Bottom: ImageJ software can be seen at the top left of the image. The histogram tool in ImageJ can be seen at the bottom.

Images of the lumbar multifidus were also acquired to compare with phantom brightness. The transducer was placed over the spinous process and moved laterally to find the muscle. Initially three longitudinal images of multifidus at the levels of the L3 to L5 spinal processes were taken bilaterally. After that, the examiner rotated the transducer 90 degrees, to a transverse orientation, and repeated the same process bilaterally.

C. *Image Analyses*

The EI was estimated using ImageJ software (version 1.52e, National Institutes of Health - NIH, USA) where histograms were plotted to identify the mean brightness within the ROI (Fig.2).

II. Results and Discussions

The results are presented below according to the parameters that were altered during acquisition.

A. *Focus*

As the phantom has 3.5 cm of thickness, only three different foci were considered (1.5, 3 and 5 cm) when measuring the brightness values. During these analyses, gain was kept at 52%, depth 5 cm, and zoom 120%. At each focus 3 images were obtained, and a mean image was calculated to compensate for measurement variation. In each image, a ROI was selected comprising as much of the phantom as possible. There were no statistical difference between the average brightness based on changes in focus (Table 1).

Table 1 Variations of Echo Intensity (Brightness) with different values of focus.

Focus (cm)	E.I. Average (SD)	ANOVA
1.5	129.4 (0.1)	
3.0	132.6 (0.1)	p = 0.32
5.0	132.8 (0.2)	

B. *Gain*

During these analyses focus was kept 1.5 cm, depth was 5 cm and zoom 120%. It was expected that the gain would influence EI since it increases the brightness on the screen. However, our goal is to understand and document the exact association between EI and gain. Fig. 3 shows that EI rises linearly with increasing gain. A linear regression between EI and gain was generated, which had a high coefficient of determination ($R^2 = 0.97$). It is important to note that the level of brightness of the phantom is quite similar to the human multifidus muscles tested in 10 subjects imaged with the same transducer oriented in both the longitudinal and transverse planes (Fig. 3).

To investigate a way to minimize or eliminate the influence of gain on brightness, EI values were divided by each respective gain. The result can be seen in Fig. 4. In the phantom, the value of EI/Gain remained reasonably stable, between 2.2 and 2.4 when the gain was over 60%. The typical gain used within clinical settings range from 60% to 80% [4], therefore in clinical settings, this scale factor may be applied to minimize for the influence of gain on EI output values.

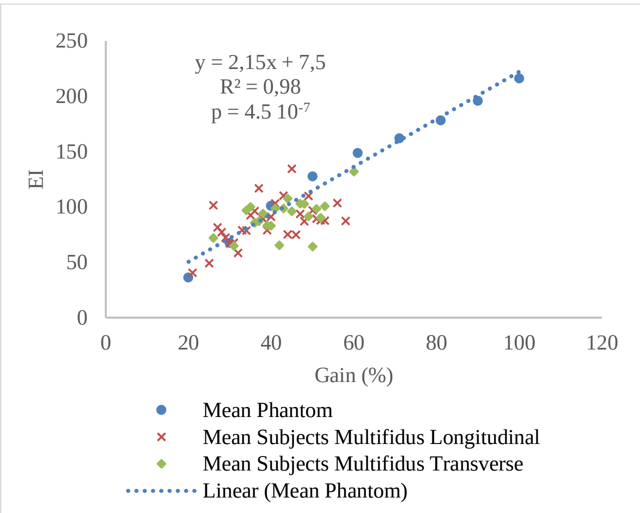

Fig. 3 Plot of Gain (%) versus Echo Intensity in the phantom and in human (subjects) multifidus muscles imaged in both the longitudinal and transverse planes.

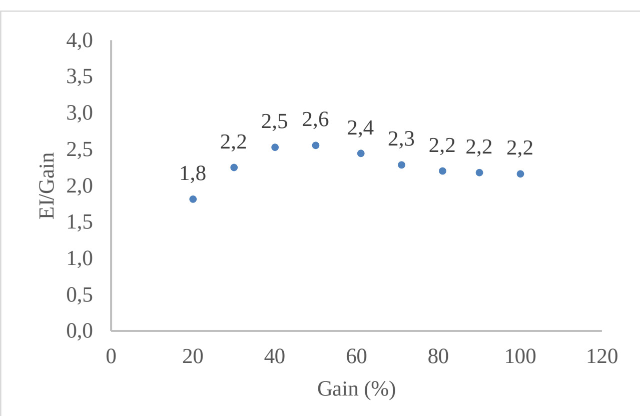

Fig. 4 Plot of Echo Intensity/Gain versus Gain (%) based on images acquired from the phantom.

C. Depth

It was expected that increasing the depth would influence EI since the same energy released by transducer is distributed over a larger area on the screen. Attenuation increases with depth, so less energy will decrease the level of brightness and therefore EI average values should diminish too. Sometimes image databases have been used in research and the depth is not always optimized for the muscle of interest, therefore it was necessary to investigate the influence of depth on the brightness in a region of interest. As expected, EI decreases linearly with increasing depth (Fig. 5). During these analyses, the gain was 50% and zoom 120%. The focus changed from 1.5 to 3 cm automatically with the depth increase.

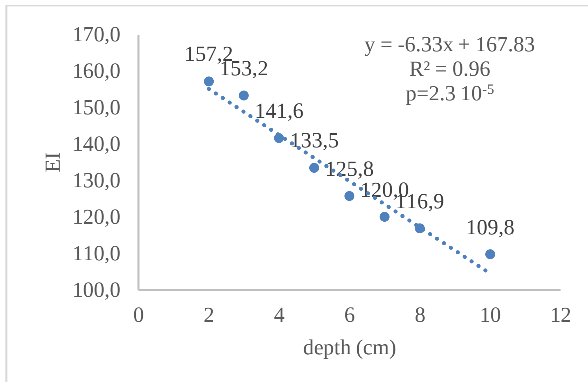

Fig. 5 Plot of Echo Intensity vs. depth (cm).

D. Zoom

Alterations in zoom changes the visualization on the screen, but it is not clear how this variation might change the value of EI as the number of pixels inside the same selected ROI change. A linear regression resulted in a slope value near zero (Fig. 6), and thus zoom does not appear to have and important influence on EI.

E. Region of Interest

There is no consensus about what would be the best size of the region of interest (ROI) to use in texture analysis. Some authors use 4 cm^2 [10,11] in the muscle region, while others chose to select the largest area possible [12]. Therefore, the influence of ROI size on EI was investigated. The central region of the image was chosen as the starting point, and after that, the area selected was increased to its maximum. EI decreases logarithmically with increase in ROI size (Fig. 7). This relationship reaches an asymptote near ~15cm^2.

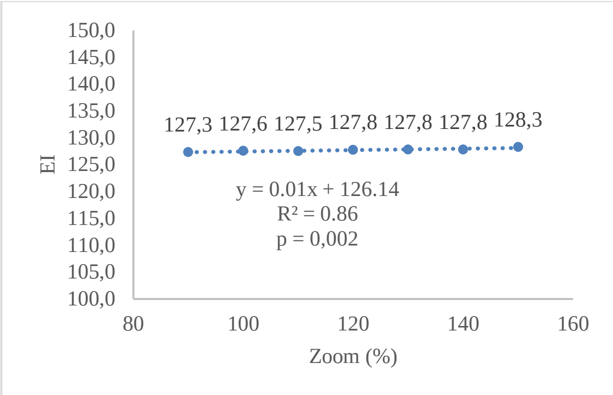

Fig. 6 Plot of Echo Intensity versus Zoom (%) on images of the phantom.

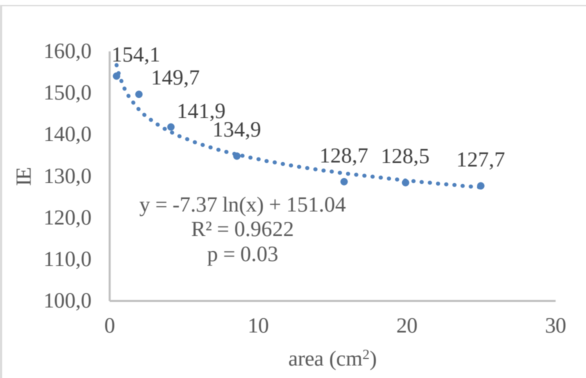

Fig. 7 Plot of Echo Intensity versus ROI size in images of the phantom.

III. Conclusions

The influence of ultrasound acquisition setup and ROI size on the grey-scale echo intensity values on ultrasound images were investigated. There were no important changes in average brightness with increases in focus and zoom. The EI was influenced by gain, depth and ROI area. We propose dividing the EI by gain as a means of reducing the influence of gain changes during acquisition, particularly when gain is higher than 60%. A similar normalization may be possible to limit the effect of depth but this requires further investigation.

This work explored the relationships between EI and parameter settings of ultrasound machines. The main purpose was to develop tools to define possible common standards and help researchers decide on which parameters can influence their measurement, thus allowing clearer data interpretation.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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