Journal of Southeast University (English Edition) Vol. 26, No. 1, pp. 82 – 86 Mar. 2010 ISSN 1003—7985

A system for evaluation of ultrasound contrast agent's enhancement effect

Chen Ping[1] Yang Fang[1] Fang Kun[1] Gu Aiyuan[2] Qian Zhuoyu[3] Wang Peng[4] Gu Ning[1]

([1] Jiangsu Key Laboratory for Biomaterials and Devices, Southeast University, Nanjing 210096, China)
([2] Belson Imaging Technology Co., Ltd., Wuxi 214092, China)
([3] Imaging Science and Technology Laboratory, Southeast University, Nanjing 210096, China)
([4] Jiangsu Province Institute of Metrology and Testing Technology, Nanjing 210007, China)

Abstract: A system for *in vitro* investigation of ultrasound contrast agent's enhancement effect is presented and evaluated. It includes the digital B-mode ultrasound scanner Belson3000A, the tissue-mimicking ultrasound phantoms and the software which is used for image quantitative analysis. The linear range, optimal settings and repeatability of the system are assessed and explored by scanning the ultrasound phantoms with different reflective intensities. The measurements are performed under an acoustic power from 4. 8 to 12. 3 mW, the scanner centre frequency is 3. 5 MH and the gain setting is 50 dB. Both a self-made surfactant encapsulated microbubble and a commercial ultrasound contrast agent are scanned. The results show that the pixel intensity of ultrasonic images increases with the increase in the sound power, and for the stronger reflective phantoms of more particles, the increasing trend is much more evident. The system is optimal for evaluating the microbubble contrast agents' enhancement effects. It presents a simple, effective and real-time means for characterizing the enhancement ability of microbubbles.

Key words: microbubble; ultrasound phantom; enhancement imaging; optimal settings

In recent years, encapsulated microbubbles as ultrasound contrast agents (UCAs) have aroused much interest because of their excellent characteristics for enhancing the contrast effects of US imaging[1] and for being a novel drug delivery carrier[2–6]. Compared with the traditional medical ultrasound imaging, the application of ultrasound contrast agents can significantly improve the detection of vascular and microcirculatory lesions, especially for the heart, liver, kidney and other functional organs. Therefore, creating microbubbles, characterizing their properties and exploring their behaviors under ultrasound fields have become a primary research focus in the medical ultrasound field[7–9]. Establishing a system for in vitro evaluation of ultrasound contrast agents is the basis for further related research work.

Tissue-mimicking ultrasound phantoms is originally developed for performance testing and the optimization of medical ultrasound equipment as well as for medical training projects[10–12]. In particular, some reports show that the phantom is useful for the study of ultrasound contrast agent microbubbles. Caskey et al. [13] explored the relationship between microbubbles and phantoms under ultrasound exposure. Demitri et al. [14] observed microbubbles in the pipeline within the phantom which simulates the micro vessels in vitro.

The backscatter ability would be deduced from the changes in pixel intensity under a suitable setting. So the microbubble contrast agents' enhancement effects can be known by studying the pixel intensity of their images. However, the suitable setting of imaging equipment plays an important role during the imaging process and changes for different reflective ability imaging objects. The reflective intensity of the phantoms can be controlled and diversified by adding reflective particles, so the phantoms with reflective particles are ideal imaging objects for scanning to explore the most suitable setting of the system. They are not expected to simulate the backscattering properties of microbubbles.

The phantom provides a better background for microbubble ultrasound imaging than water. When the solution is introduced into the pipeline in the phantom, just like the vessel in the tissue, the region of interest(ROI) is located within the pipeline. The activity state of all microbubbles can be observed through real-time ultrasound imaging. This cannot be achieved when introducing the sample solution into a sink for scanning.

In this paper, a platform used for evaluating the enhancement effects of ultrasound contrast agent microbubbles is constructed. The phantom has two roles in the study, one for exploring the suitable setting of the imaging system and the other as the pipeline for the sample. Both the commercial contrast agent microbubbles and the surfactant microbubbles are scanned and their images grayscales are quantified. The results indicate that the system has good accuracy and repeatability. It is an effective and intuitive method for the evaluation of ultrasound contrast agents.

1 Materials and Methods

1.1 Preparation of ultrasound phantom

The procedures for the preparation of a phantom is identical to those described in other works[15]. The agar powder (Sunshine Bio-Technology Co., Ltd., Nanjing, China), glycerol(Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), metal particles and distilled water are used to prepare the ultrasound phantom. The ratio of agar, glycerol and distilled water follows the mass ratio 3 : 4 : 90, and the metal particles, as the sound reflectors, influence the backscatter character of the phantom. The mixture is heated to a temperature of 90 ℃ and held there for more than half an

Received 2009-11-16.

Biographies: Chen Ping (1984—), female, graduate; Gu Ning (corresponding author), male, doctor, professor, guning@seu.edu.cn.

Foundation items: The National Basic Research Program of China (973 Program) (No. 2006CB933206), the National Natural Science Foundation of China(No. 50872021, 60725101).

Citation: Chen Ping, Yang Fang, Fang Kun, et al. A system for evaluation of ultrasound contrast agent's enhancement effect[J]. Journal of Southeast University (English Edition), 2010, 26(1): 82 – 86.

hour until the mixture becomes viscous and transparent. During the whole process, the suspension is continuously stirred with a glass rod to prevent aggregation. Then the solution is poured into a mould container, where it is cooled down at room temperature and takes on an ideal and solid form. Small air bubbles which get mixed into the solution during preparation must be eliminated.

The container in which the mixture is to cool down is a mould made of plexiglass. Medical silicone tubes(6 mm × 9 mm) are previously set in the mould. After the phantom is formed, the silicone tubes are removed, leaving the channel in the phantom. At the bottom and the wall of the container, there is a layer of sound-absorbing sponge to absorb the echo and avoid interference. The channels with a diameter of 9 mm, curving downward into an arc are located in the phantom. The distance between the pipeline's deepest point and the upper interface of the phantom is 40 mm. This layout is beneficial for fixing the sample solution to ultrasound imaging. The schematic diagram of the system is shown in Fig. 1. The system includes the phantom, the scanner and the image processing PC. Microbubble suspension is injected into the pipeline in the phantom.

Besides, phantoms with different reflection properties are prepared in a column (The diameter is 3.5 cm, and the height is 2 cm) for the experiments. The concentration of metal particles in a phantom is 0, 0.02 and 0.04 g/mL, respectively.

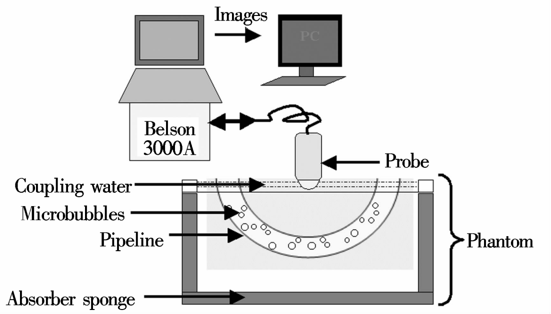


Fig. 1 Schematic diagram of the system

1.2 Preparation of microbubble

The surfactant-encapsulated microbubbles are prepared by acoustic emulsification according to Refs. [16 - 17]. Span 60 (1.48 g, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), NaCl(1.5 g, Guangdong Xilong Chemical Co., Ltd., China), Tween 80 (1 mL, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and phosphate buffered saline (PBS, pH = 7.4 ± 0.1, 50 mL) are mixed and grinded with a pestle in a mortar for 5 min. Then the solution is stirred, heated to a temperature of 60 ℃ and held there for 20 min. Next, the cooled solution is probe-sonicated in a 250 mL beaker continuously at 110 W constantly purging a steady stream of nitrogen gas for 90 s. The final solution has three distinct layers. The experienced microbubble samples are collected from the middle layer. The upper larger bubble samples and the lower residue are discarded. When the microbubble solution is used for ultrasound imaging, it needs to be diluted (approximately 10^7 bubbles/mL) with PBS(pH = 7.4 ± 0.1).

The commercial contrast agent is prepared half an hour before ultrasound imaging according to the manufacturers' specifications. When imaging, it also needs to be diluted to the same concentration as the self-made microbubbles.

The mean diameter of the bubbles are characterized by a microtrac S3500 particle size analyzer(Microtrac Inc, US). The size distribution of the self-made surfactant-encapsulated bubble is shown in Fig. 2. The diameters of 90% of the bubbles are less than 30 μm, and those of 50% of the bubbles are less than 10 μm. A small amount of bubbles' sizes are greater than 30 μm as a result of aggregation. Optical microscopy is also employed to observe the morphology of the self-made microbubbles and the commercial contrast agent microbubbles. The images are displayed in Fig. 3.

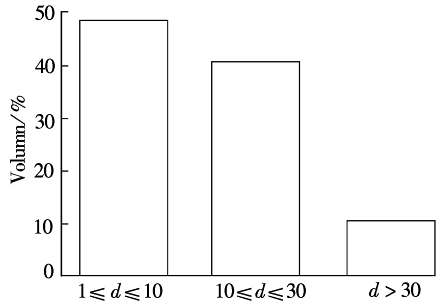


Fig. 2 Size distribution for surfactant microbubbles

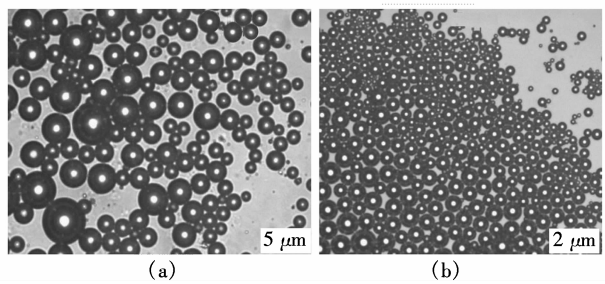


Fig. 3 Optical microscope photographs of the microbubbles. (a) Self-made microbubbles; (b) Commercial contrast agent microbubbles

1.3 Scanner and software

The imaging equipment used in the experiment is the digital B-mode diagnostic ultrasonic instrument Belson3000A (Belson Imaging Technology Co., Ltd., Wu Xi, China) with a 3.5 MHz R60 convex array probe and a 7.5 MHz linear array probe. The sound power can be varied by changing the source voltage which controls the excitation pulse amplitude. And it is also influenced by the settings of detection depth and focus position. The scanner's output is calibrated by the portable BCZ100-1 microwatt-level ultrasonic power meter (Langfang Institute of Measurement and Testing, Hebei province, China) when the detection depth is set to be 98.3 mm and the focus position is located at 80 mm before the probe. Tab. 1 shows the measurement results of the sound power changing with the source voltage.

The software used for image processing and quantified gray-scale is developed based on Microsoft Visual C++6.0 (Microsoft). When a pixel point is selected within the region of its valid range, the gray value of this local point will be acquired. Pixel intensity within a selected area ROI can be calculated. The accuracy of the software is validated by the commercial software Photoshop (Adobe).

Tab. 1 Calibration of the sound output

Source voltage/V	Sound power/mW	
	3.5 MHz	7.5 MHz
40	4.8 ±0.1	3.4 ±0.1
45	5.8 ±0.1	4.3 ±0.1
50	7.1 ±0.1	5.0 ±0.1
55	8.2 ±0.1	5.9 ±0.1
60	9.3 ±0.1	7.2 ±0.1
65	11.2 ±0.1	8.1 ±0.1
70	12.3 ±0.1	9.0 ±0.1

2 Ultrasound Imaging

2.1 Phantom scan

The aim of scanning the phantom is to find suitable and ideal setting parameters of the overall gain and the sound power, as the overall gain setting and the sound intensity of the system are the most important factors for the results of the imaging. Different reflection property phantoms are immersed into the degassed water for imaging. In this paper, ten different gain settings from 10 to 100 dB are used, and measurements are performed under seven levels of acoustic power when the frequency is 3.5 MHz (see Tab. 1).

The final acquired image is a complex result that comes from the object and the equipment settings such as the overall gain, time gain compensation (TGC), dynamic range, focus position, detectable depth and so on. So it is more practical and efficient to assess the linear range in terms of the average pixel intensity of the final image.

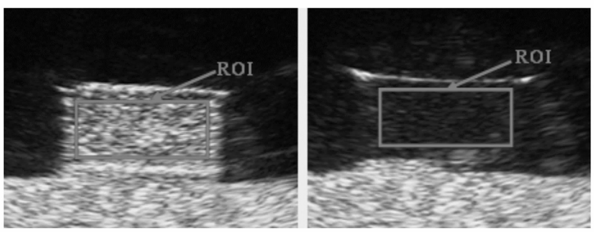
2.2 Microbubbles scan

The diluted agent solution (3.5 mL) is introduced into the arc pipeline in the phantom. The sound power used in this experiment is 4.8 mW corresponding to a source voltage of 40 V. Once the sample solution is introduced, the images are stored every 10 s. Then the ROI (approximately 2 700 pixels) is located within the pipeline in the phantom excluding the walls of the channel, and the average pixel intensity of the ROI is calculated. The measurements of the two kinds of microbubbles are repeated 3 times.

3 Results and Discussion

3.1 Ideal setting of the system

During the process of scanning the phantom, there is an absorber sponge at the bottom of the sink to prevent multiple reflections. The images are shown in Fig. 4 when the overall gain is 50 dB and the source voltage is 40 V. Fig. 4(a) corresponds to the phantom with a particle concentration of 0.04 g/mL and Fig. 4(b) corresponds to the phantom with a particle concentration of 0 g/mL. The light region at the lower position is the sponge; the black background is degassed water and the rectangular brighter region in the center is the vertical section of the cylindrical phantom. For quantitative analysis, the ROI is placed in the interior of the phantom and the strong reflection interfaces are not included. The ROI is approximately 12 000 pixels.



(a) (b)

Fig. 4 Images of phantom with different concentrations of metal particles. (a) Phantom with the particle concentration of 0.04 g/mL; (b) Phantom with the particle concentration of 0 g/mL

Fig. 5 is a plot of the average pixel intensity over the ROI of the phantom against the overall gain setting when the sound power is 4.8 mW. The concentrations of reflective particles in the phantom are 0, 0.02 and 0.04 g/mL, respectively. For the lowest reflective intensity phantom, the pixel intensity has no significant change when the gain is below 50 dB; for the highest reflective intensity phantom, the pixel intensity has no significant change when the gain setting is beyond 70 dB. For the phantom with a particle concentration of 0.02 g/mL, it changes with a consistent trend for the whole gain setting range. And the corresponding intensity of the pixel is between 40 and 175 (for 256 grayscales). The trends are greatly consistent when the source voltage is changed from 40 to 70 V except that the grayscales are brighter when the voltage is higher.

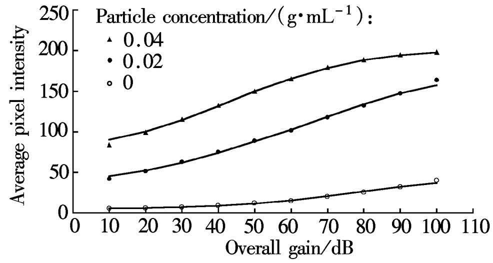


Fig. 5 Changes of average pixel intensities of phantom vs. overall gain setting

In Fig. 6, the graph presents the reflective ability of the phantom against sound intensity for a gain of 50 dB. The concentrations of reflective particles in each phantom are 0, 0.02 and 0.04 g/mL, respectively. For the three objects, their pixel values increase with the increase in the sound power. But the more obvious fact is that the pixel intensity increases much faster with the increase in the sound power for the stronger reflective phantoms with more particles. This can also be seen from the fitting function; the slopes of 0.809 1, 0.616 5 and 0.157 7 are calculated for the phan-

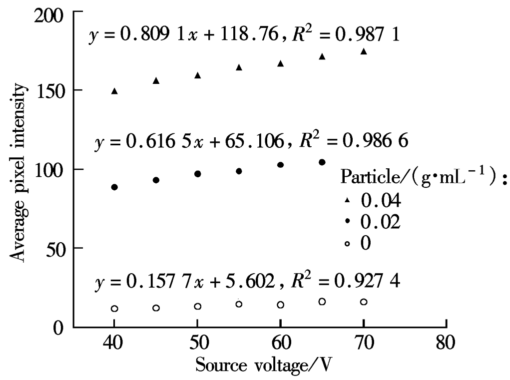


Fig. 6 Changes of average pixel intensities of different phantoms vs. overall gain setting

toms with particle concentrations of 0. 04, 0. 02 and 0 g/mL, respectively. Besides, a much better linear relationship between the average pixel intensity and the sound power is found for the phantoms with particle concentrations of 0. 02 and 0. 04 g/mL.

A better linear relationship between the sound power and the average pixel intensity can be achieved by increasing the concentration of the reflector in the phantom. But for the stronger reflective phantoms, the change trends of the pixel intensity are different between the high overall gain setting and the low overall gain setting. The result is an important guidance for the following microbubble ultrasound imaging. For a certain kind of microbubbles, the concentration is the main factor influencing the relationship between the pixel intensity and the backscatter properties of the sample solution. A suitable concentration of a microbubble solution should be obtained when it is used for ultrasound imaging to deduce the backscatter properties of microbubbles from the pixel intensity of an image, thus avoiding the nonlinearity for the low concentration and the non-consistent change trend for the high concentration.

3. 2 Enhancement effect of microbubbles

The feasibility of the platform can be further tested and verified by the study of the self-made surfactant microbubbles and the commercial contrast agent microbubbles. The concentration of microbubbles should keep the system working within a linear range. This means that the relationship between the pixel intensity and the overall gain is linear(see Fig. 5). Fig. 7(a) refers to the degassed water in the pipeline, which almost has no echo from the solution; Fig. 7(b) refers to a lower concentration(approximately 10^5 microbubbles/mL) suspension of microbubbles; Fig. 7(c) refers to an appropriate concentration (approximately 10^7 microbubbles/mL) with a brighter region within the pipeline comparing with the background of the phantom; Fig. 7(d) refers to the highest concentration(approximately 10^8 microbubbles/mL) solution which results in the low border becoming fuzzy. The sound power is 4. 8 mW and the gain is 50 dB.

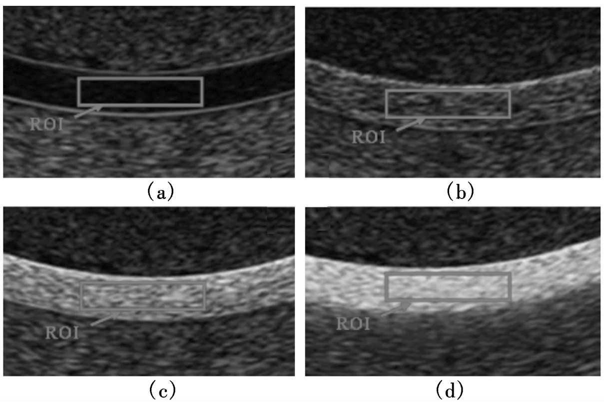


Fig. 7 Ultrasound images of microbubble suspensions

The plots of the average pixel intensity over ROI vs. time for self-made surfactant encapsulated microbubbles and the commercial contrast agent microbubbles are shown in Fig. 8 (The sound output is 4. 8 mW and gain is 50 dB). The results show that both the bubbles can enhance the contrast for 1 min at least. The brightness of the ROI in the centre of pipeline reduces over time during the first 40 s. When the solution is gently stirred at the time point of 50 s, the pixel intensity of the contrast imaging can be resumed with only a little decrease. But the total reflective effect of the ROI drops down with the time again. The stability and enhancement trends of the self-made microbubbles are almost the same with those of the commercial contrast agent microbubbles.

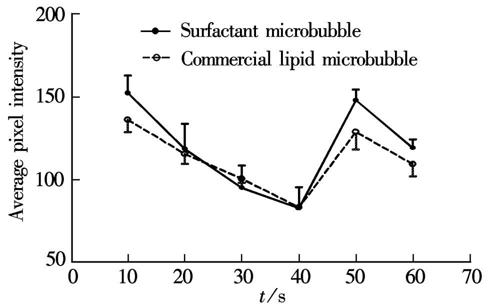


Fig. 8 Changes of average pixel intensities for two types of microbubbles vs. time

The pixel intensity for the surfactant bubbles and the commercial contrast agent bubbles displayed in the graph shows that the system setting is appropriate for the concentration of the microbubble solution. The pixel intensity changes from 70 to 160 and this means that the assessed results are accurate and reliable within the linear range of the system. The reflective abilities of the surfactant encapsulated microbubbles and the commercial contrast agent microbubbles reduce with time. The reduction trend can be attributed to the aggregation of microbubbles because of the buoyancy and adsorption. The bubbles cluster at the upper wall of the pipeline. The pixel intensity at the time point of 50 s is a little lower when compared with the pixel intensity at the time point of 10 s. This is likely to be caused by the destruction of small amount bubbles under ultrasound exposure.

4 Conclusion

An experiment system for *in vitro* study of contrast agent microbubbles is constructed and evaluated, which includes ultrasound scanner, phantom and quantitative software. The linearity range for the system is assessed with phantoms with different reflective properties. The stability of the self-made surfactant encapsulated microbubbles is explored. At the same time, based on the system, the ultrasound imaging enhancement effects are also studied. The results show that the phantom based system is feasible and reproducible. It can be used as the evaluation platform for ultrasound contrast agents microbubbles. Of course, under such a system the other detailed influence factors, such as the concentration, size, and membrane shell of bubbles, sound power and so on, will be further studied in forthcoming research. The particle size distribution of the self-made surfactant encapsulated microbubbles is wide. Some larger microbubbles are needed to be removed for further detailed research.

References

[1] Voigt J U. Ultrasound molecular imaging [J]. *Methods*, 2009, **48**(2): 92 -97.

[2] Stride E, Porter C, Prieto A G, et al. Enhancement of microbubble mediated gene delivery by simultaneous exposure to ultrasonic and magnetic field[J]. *Ultrasound in Med and Biol*, 2009, **35**(5): 861－868.
[3] Tinkov S, Bekeredjian R, Winter G, et al. Microbubbles as ultrasound triggered drug carriers[J]. *Journal of Pharmaceutical Sciences*, 2009, **98**(6): 1935－1961.
[4] Zhao S K, Kruse D E, Ferrara K W, et al. Acoustic response from adherent targeted contrast agents [J]. *Journal of the Acoustical Society of America*, 2006, **120**(6): EL63－EL69.
[5] Tartis M S, Kruse D E, Zheng H R, et al. Dynamic micro-PET imaging of ultrasound contrast agents and lipid delivery [J]. *J Control Release*, 2008, **131**(3): 160－166.
[6] Yang F, Li Y X, Chen Z P, et al. Superparamagnetic iron oxide nanoparticle-embedded encapsulated microbubbles as dual contrast agents of magnetic resonance and ultrasound imaging [J]. *Biomaterials*, 2009, **30**(23/24): 3882－3890.
[7] Soetanto K, Chan M. Fundamental studies on contrast images from different sized micro bubbles: analytical and experimental studies [J]. *Ultrasound in Med and Biol*, 2000, **26**(1): 81－91.
[8] Sboros V, Moran C M, Anderson T, et al. Evaluation of an experimental system for the in vitro assessment of ultrasonic contrast agents [J]. *Ultrasound in Med and Biol*, 2000, **26**(1): 105－111.
[9] Yu T H, Xiong S H, Mason T J, et al. The use of a microbubble agent to enhance rabbit liver destruction using high intensity focused ultrasound [J]. *Ultrasonics Sonochemistry*, 2006, **13**(2): 143－149.
[10] Zell K, Sperl J I, Vogel M W, et al. Acoustical properties of selected tissue phantom materials for ultrasound imaging [J]. *Phys Med Biol*, 2007, **52**(20): N475－N484.
[11] Surry K J M, Austin H J B, Fenster A, et al. Poly(vinyl alcohol) cryogel phantoms for use in ultrasound and MR imaging[J]. *Phys Med Biol*, 2004, **49**(24): 5529－5546.
[12] Chiarelli P, Lanat A, Carbone M. Acoustic waves in hydrogels: a bi-phasic model for ultrasound tissue-mimicking phantom [J]. *Materials Science and Engineering*, 2008, **7**(36): 1－9.
[13] Caskey C F, Qin S, Dayton P A, et al. Microbubble tunneling in gel phantoms[J]. *J Acoust Soc Am*, 2009, **125**(5): EL183-EL189.
[14] Demitri C, Sannino A, Conversano F, et al. Hydrogel based tissue mimicking phantom for in-vitro ultrasound contrast agents studies [J]. *J Biomed Mater Res Part B: Appl Biomater*, 2008, **87**(2): 338－345.
[15] Landry A, Fenster A. Theoretical and experimental quantification of carotid plaque volume measurements made by three-dimensional ultrasound using test phantoms[J]. *American Association of Physicists in Medicine*, 2002, **29**(10): 2319－2327.
[16] Oeffinger B E, Wheatley M A. Development and characterization of a nano-scale contrast agent [J]. *Ultrasonics*, 2004, **42**(1/2/3/4/5/6/7/8/9): 343－347.
[17] Wheatley M A, Forsberg F, Dube N, et al. Surfactant-stabilized contrast agent on the nanoscale for diagnostic ultrasound imaging[J]. *Ultrasound in Med and Biol*, 2006, **32**(1): 83－93.

一个用于体外评价超声造影剂显影效果的系统

陈　平[1]　杨　芳[1]　房　坤[1]　顾爱远[2]　钱卓宇[3]　王　鹏[4]　顾　宁[1]

([1] 东南大学江苏省生物材料与器件重点实验室，南京 210096)
([2] 贝尔森影像技术有限公司，无锡 214093)
([3] 东南大学影像科学与技术实验室，南京 210096)
([4] 江苏省计量科学研究院，南京 210007)

摘要：构建并评价了一个用于体外研究超声造影剂显影效果的系统，该系统包括一套数字化 B 型超声诊断仪 Belson3000A、仿组织超声体模和用于图像量化分析的软件. 制备了具有不同反射强度的仿组织超声体模，通过对这些体模材料超声成像，研究了 B 超系统的线性范围、参数设置和系统的重复性等. 超声成像设置为：输出声功率为 4.8～12.3 mW，探头的中心频率为 3.5 MHz，总增益为 50 dB. 利用该系统，对自制备的表面活性剂微气泡和一种商用造影剂进行了显影成像. 结果表明超声图像的灰度值随声功率的增加而增大，强反射特性的超声体模成像时，这种增加趋势更明显. 该系统适合用于体外评价超声造影剂的显影效果，为体外表征超声微气泡造影剂的显影能力提供了一种简单、有效、实时的评价方法.

关键词：微气泡；超声体模；显影成像；优化设置
中图分类号：R445.1