



# Real-time optical imaging of individual microbubbles in an ultrasonic field

Michiel Postema, Ayache Bouakaz, Chien Ting Chin, Nico de Jong

## ► To cite this version:

Michiel Postema, Ayache Bouakaz, Chien Ting Chin, Nico de Jong. Real-time optical imaging of individual microbubbles in an ultrasonic field. 2001 IEEE Ultrasonics Symposium. Proceedings. An International Symposium (Cat. No.01CH37263), 2001, Atlanta, United States. pp.1679-1682, 10.1109/ULTSYM.2001.992044 . hal-03193431v2

**HAL Id: hal-03193431**

**<https://hal.science/hal-03193431v2>**

Submitted on 13 Apr 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# REAL-TIME OPTICAL IMAGING OF INDIVIDUAL MICROBUBBLES IN AN ULTRASONIC FIELD

Michiel Postema\*, Ayache Bouakaz, Chien Ting Chin and Nico de Jong  
Dept. of Experimental Echocardiography, Erasmus University Rotterdam,  
P.O. Box 1738, zip code 3000 DR, Rotterdam, The Netherlands

*Abstract* – In this study we analyze the behavior of individual experimental ultrasonic contrast bubbles, insonified by 500 kHz ultrasound, at acoustic pressures between 0.06 and 0.66 MPa. The oscillations were observed under a microscope with a fast framing camera.

It is concluded that apparently identical bubbles can expand to different maximal diameters.

## I. INTRODUCTION

We investigated an experimental ultrasonic contrast agent (UCA), supplied by Bracco Research SA, Geneva, Switzerland. It consists of phospholipid-encapsulated sulfur hexafluoride gas bubbles ranging in diameter from 1 to 6  $\mu\text{m}$  with a median of 2  $\mu\text{m}$ . The acoustic behavior of the contrast agent was modeled and described by [1].

In this study we did optical observations, recorded with a fast framing camera, of UCA bubbles insonified by 500 kHz ultrasound at peak negative acoustic pressures between 0.06 and 0.66 MPa. The observations were done with a two-dimensional fast framing camera, as described by [2].

## II. OPTICAL CONSIDERATIONS

The optical observations show oscillating contrast bubbles. The oscillating behavior was studied by measuring bubble sizes from the optical images. The optical system was studied, to determine the relation between the observed bubble size and the true bubble size.

An image observed through a microscope is the convolution of the true object image and the point-spread function of the optical imaging system. Our optical system had an objective lens with a focal length of 0.8 mm and an aperture of 1.5 mm. Its

point-spread function was computed using the Rayleigh integral[3], for a wavelength of 500 nm.

The theoretical threshold to be used in automatic bubble segmentation for size measurements is 50% of the intensity value between foreground (bubble) and background. This was validated for bubbles larger than 0.4  $\mu\text{m}$  by measurements on simulated images, obtained by convolving the point-spread function with different shapes.

The intensity gradient between foreground and background is steepest (the contrast between foreground and background is highest) when in focus, as was expected from forward modeling.

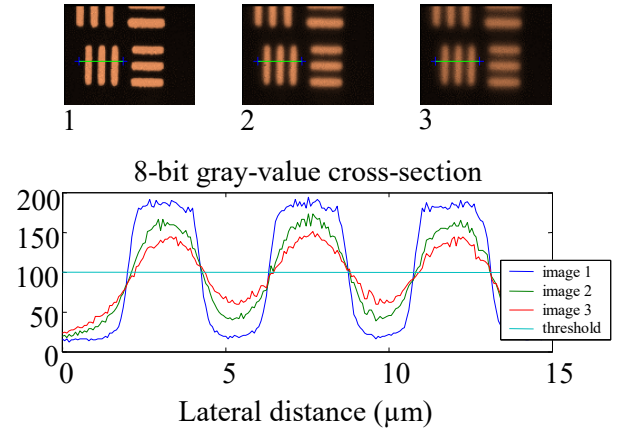


Figure 1: Intensity profiles in focus (1), 2  $\mu\text{m}$  above focus (2) and 4  $\mu\text{m}$  above focus (3)

For a CCD image with a certain gain and dynamic range, the chosen threshold is still valid if the object is out of focus, as demonstrated by figure 1: Three images of a 228.0 cycles/mm grid on a negative 1951 USAF glass slide resolution target were taken with a black & white CCD video camera through a microscope with a 100 $\times$  objective lens. Image 1 is in focus, i.e. it has the maximal intensity gradient. Image 2 and 3 were taken 2  $\mu\text{m}$  and 4  $\mu\text{m}$  respectively proximal to the focus.

### III. SETUP

The contrast bubbles were insonified using a single-element transducer, spherically focused at 75 mm, transmitting 10 cycles of 500 kHz. The amplitude of the signal was adjusted by attenuators and a linear power amplifier. Acoustic pressures applied were measured with a calibrated hydrophone at the site of interest.

The transducer was mounted in a Perspex container at an angle of 45° relative to the top of the container, as shown in figure 2. This container was filled with water. Into the container a Ø 200 µm capillary fiber was placed in the focus of the transducer.

Contrast fluid (5ml of a 0.9% NaCl dilution added to a 25 mg vial) was inserted through this capillary fiber.

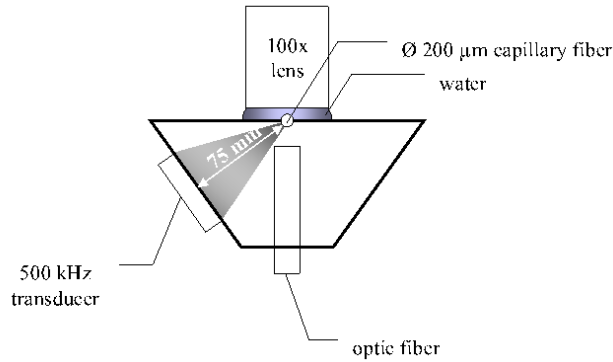


Figure 2: Front-side view of container

The Perspex container was positioned beneath a microscope with a 100× objective lens. On top of the microscope a 100 MHz fast framing CCD camera was placed.

The camera was computer controlled. The first frame was taken a few microseconds before ultrasound arrival. The other seven frames were taken during ultrasound insonification, with 330 ns interframe time for 500 kHz ultrasound, spanning a full ultrasound cycle. Frame exposure times ranged from 10 ns to 70 ns.

### IV. SEGMENTATION AND ERROR CALCULATION

Bubble diameters were measured with a semi-automated segmentation program, using the 50% threshold between foreground and background described in the previous section.

From each binary-segmented image, the size of an enclosed area representing a bubble was measured. From this area the bubble diameter was calculated.

The dependence of bubble diameter measurement on light intensity, choice of region of interest, channel and multi channel plate amplification, was calculated by measuring UCA bubble diameters from image sequences without ultrasound insonification and by measuring  $1.069 \pm 0.008$  µm polystyrene latex calibration spheres, using different light intensities and selecting different regions of interest. The standard deviation determined in diameters was 0.16 µm.

Based on the measurements with two-dimensional grids, we assume that diameters from bubbles that were slightly out of focus could be correctly measured with the threshold used. Bubbles that were clearly out of focus were not taken into account in this study.

### V. RESULTS

In this study, 583 insonified bubbles were subjected to an extensive analysis. Peak negative acoustic pressures applied to these bubbles ranged from 63 kPa to 856 kPa. The maximal diameters measured were normalized by the initial bubble diameters:  $(d_{max}/d_{initial}) - 1$ , indicating diameter growth.

Data from events at relatively low pressures are presented in figure 3. Relative diameter growth is plotted as a function of initial bubble diameter. The figure shows that bubbles of the same size appear to have different maxima by factors over 10.

This phenomenon was separately investigated at high peak negative pressures of 659 kPa. An example is demonstrated in figure 4, where three apparently identical UCA bubbles were selected. The initial bubble diameters as shown in the first frame are 4.11 µm, 4.12 µm and 4.26 µm, respectively. The measured amplitudes in frame 3 are 9.14 µm, 10.0 µm and 10.7 µm, respectively. The difference in maximal excursion between bubble 1 and bubble 3 is therefore roughly 30% against a 3.6% difference in initial diameters.

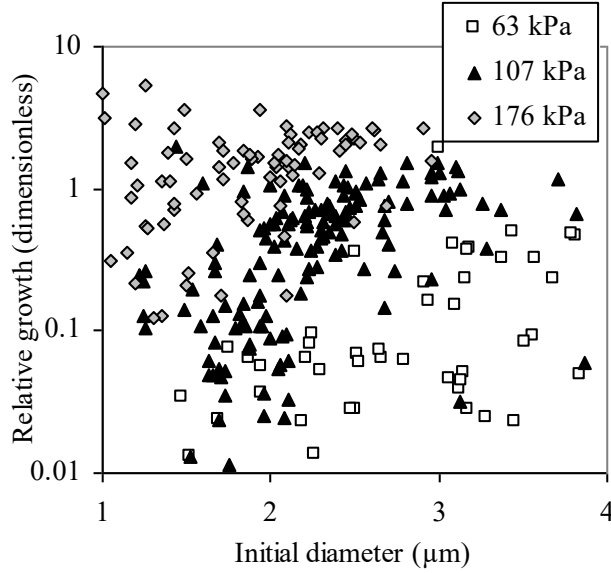


Figure 3: Relative bubble growth at three different peak negative acoustic pressures

Figure 5 gives an overview on a linear scale of nine events analog to this situation. The maxima of the events indicated by ‘x’, ‘+’ and ‘o’ are quite close, whereas excursion differences of 100% are found at the other events.

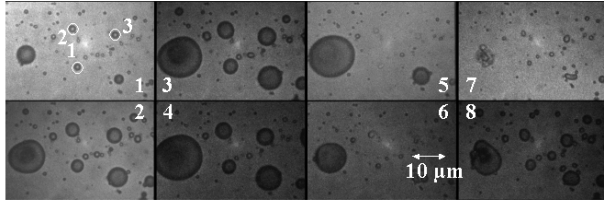


Figure 4: Optical image of 659 kPa insonified contrast– frame 1 taken before ultrasound arrival

At acoustic pressures  $>0.4$  MPa the phenomena of bubble rupturing and bubbles merging were observed.

## VI. DISCUSSION AND CONCLUSIONS

This was the first optical investigation of a large UCA data set with a 500 kHz transducer. No results of acoustic data have yet been published for this frequency applied to this contrast agent.

The random errors caused by our optical system and segmentation process are within 8% of the initial bubble sizes measured for the median UCA bubble diameter of 2  $\mu\text{m}$ .

We checked the representativity of the bubbles analyzed for the whole agent, by comparing the initial size distribution with a SonoVue™ distribution measured with a Coulter instrument[4]. The median diameter of 2  $\mu\text{m}$  is confirmed by these measurements, but bubbles over 4  $\mu\text{m}$  are hardly observed in the optical data.

We conclude that our optical observations are representative in the diameter range 1  $\mu\text{m}$  to 4  $\mu\text{m}$ .

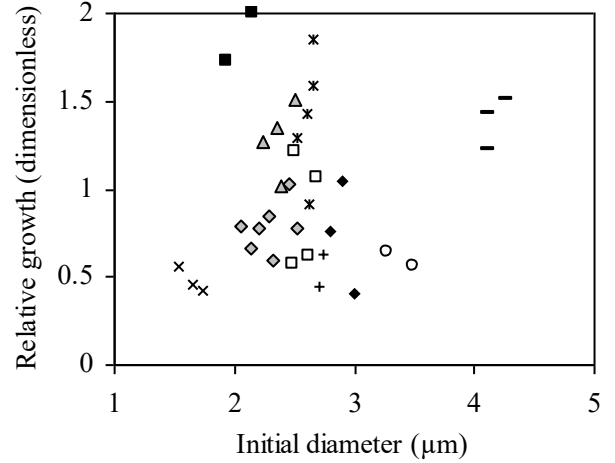


Figure 5: Relative bubble growth at 659 kPa, where identical markers indicate bubbles from the same image sequence

Because the image exposure times are low relative to the interframe times, the measured maxima do not have to correspond to the true maximal bubble expansions. In worst case, if the bubble expands according to a sine, the maximal growth measured can be off the true value by half an interframing interval, which equals a twelfth expansion cycle, i.e.  $\cos(2\pi/12) \cdot 100\% = 87\%$  of the true excursion.

Since the bubbles in the experiments shown in figure 5 are closer to each other than 1% of the acoustic wavelength, acoustic differences due to location are negligible in this situation.

From the above results it is concluded that identical bubbles can have different oscillating behavior. The differences in the oscillation maxima observed can be explained by differences in shell properties of individual bubbles. Asymmetric oscillations were regularly observed at high acoustic pressures.

Mean bubble growth is plotted as a function of acoustic pressure in figure 6, from 134 bubbles with

diameters between 1.8  $\mu\text{m}$  and 2.2  $\mu\text{m}$ . The plotted theoretical curves were calculated numerically from the models by De Jong[5] and Morgan[6] for a  $\varnothing$  2  $\mu\text{m}$  bubble and based on the parameters of the constant thickness model[1]. Although there is no clear fit, the Morgan model describes the maximal growth better.

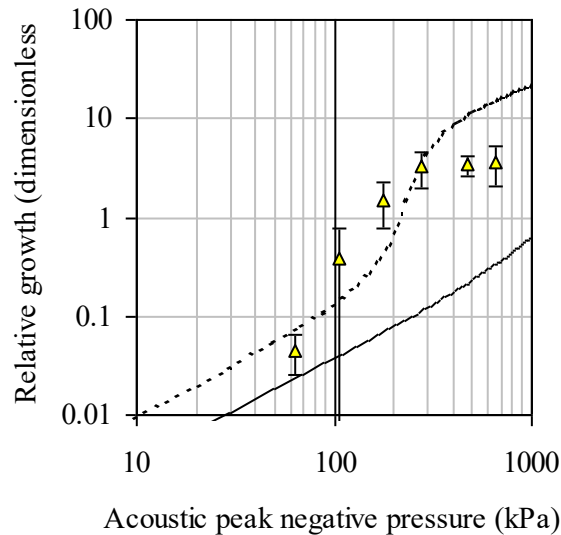


Figure 6: Mean relative growth as a function of acoustic pressure applied, for bubbles with diameters ranging from 1.8  $\mu\text{m}$  to 2.2  $\mu\text{m}$ , compared to Morgan model (--) and De Jong model (—)

## VII. ACKNOWLEDGMENTS

We thank Leo Bekkering for making the capillary fibers and doing the mounting adjustments. This work has been supported by the Technology Foundation STW (RKG.5104) and the Interuniversity Cardiology Institute of the Netherlands.

## VIII. LITERATURE

- [1] J.-M. Gorce, M. Arditi and M. Schneider, "Influence of bubble size distribution on the echogenicity of ultrasound contrast agents: A study of SonoVue™," *Invest Radiol*, vol. 35, pp. 661-671, 2000.
- [2] N. de Jong, P. Frinking, A. Bouakaz et al., "Optical imaging of contrast agent microbubbles in an ultrasound field with a 100 MHz camera," *Ultrasound in Med & Biol*, vol. 26, pp. 487-492, 2000.
- [3] J.A. Jensen and N.B. Svendsen, "Calculation of pressure fields from arbitrarily shaped, apodized, and excited ultrasound transducers," *IEEE Trans Ultrason Ferroelec Freq Contr*, vol. 39, pp. 262-267, 1992.
- [4] M. Schneider, M. Arditi, M.-B. Barrau et al., "SonoVue™: A new ultrasonographic contrast agent based on sulfur hexafluoride-filled microbubbles," *Invest Radiol*, vol. 30, pp. 451-457, 1995.
- [5] N. de Jong, R. Cornet and C.T. Lancée, "Higher harmonics of vibrating gas filled microspheres: Part one: simulations," *Ultrasonics*, vol. 32, pp. 447-453, 1994.
- [6] K.E. Morgan, J.S. Allen, P.A. Dayton et al., "Experimental and theoretical evaluation of microbubble behavior: Effect of transmitted phase and bubble size," *IEEE Trans Ultrason Ferroelec Freq Contr*, vol.47, pp. 1494-1509, 2000.

\*Michiel Postema e-mail: [postema@tch.fgg.eur.nl](mailto:postema@tch.fgg.eur.nl)