Proceedings of 20th International Congress on Acoustics, ICA 2010

23-27 August 2010, Sydney, Australia

Ultra high-speed fluorescence imaging of ultrasound contrast agents for imaging and therapy

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PACS: 43.35.Ud, 43.25Yw

ABSTRACT

Given the poor optical contrast of bright field imaging, fluorescence imaging is required to image ultrasound microbubbles in stratified vascular flows and to visualize drug release from loaded capsules. Fluorescence recordings using a combination of a high power CW laser and the Brandaris 128 ultra high-speed imaging facility give unique insight into the physical mechanisms of local intravascular drug delivery. Recordings of fluorescently labeled phospholipid-coated contrast agents show an excellent delineation of the bubble wall at frame rates of up to 5 million frames per second. This allowed us to reveal the time-resolved dynamic distribution of the shell material, including lipid shedding. Oil-filled polymeric microcapsules with a high dye concentration mixed in the hexadecane liquid core demonstrate a profound photo-acoustic effect when excited with a laser intensity of 1 MW/cm^2 or higher. The dye molecules absorb the laser light leading to intense heating of the liquid core. A rapid phase change leads to an impulsive thermal expansion. The resulting vapor bubble dynamics imaged using fluorescence imaging at a timescale of 100 nanoseconds revealed a typical oscillation frequency of 220 kHz. The generated acoustic pressure is in the order of 100 Pa at a distance of 2.5 cm from the capsule.

INTRODUCTION

Microbubbles are routinely used as contrast agents for organ perfusion imaging in ultrasound diagnostics. Their behavior under influence of ultrasonic excitation has been studied with the use of high speed imaging to visualize bubble dynamics. Recently these microbubbles have been adopted as probes for molecular imaging and active targeted drug carriers for intravascular therapy. Fluorescence imaging can be used to visualize microbubbles in stratified vascular flows in intravital microscopy and drug release from microcapsules, drug flow and resulting transfection through the cell membranes.

Also more detailed information can be gained on dynamic phospholipid shell morphology. However, the nanoseconds timescales at which bubble oscillations and rupture take place make time-resolved fluorescence imaging extremely challenging.

Here we present the results of a comparison of microbubble dynamics in bright field imaging and in fluorescence imaging. Furthermore we show the time-resolved break-up of an oil-filled microcapsule.

MATERIALS AND METHODS

Experimental setup

Figure 1 shows a schematic drawing of the experimental microscope setup. A 5W CW laser with a wavelength of 532 nm provides the fluorescence illumination. An acousto-optic modulator prevents over-exposure of the sample by gating the laser and an adjustable beam expander lets us control the spot size at the sample level. Before the laser light is focused by a 60X objective, it enters the microscope and is reflected by a dichroic mirror. Just above this mirror a notch filter is placed to avoid any excitation light from entering the camera.

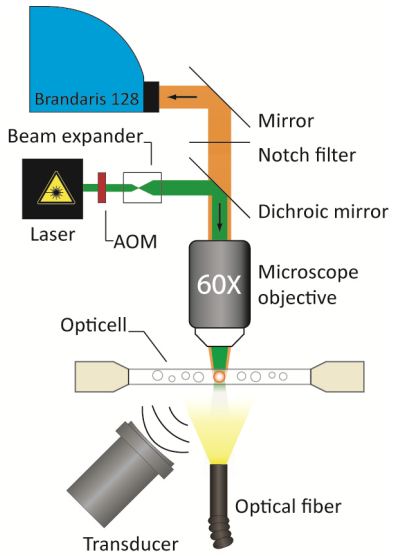

Figure 1. Schematic view of the experimental setup.

The fluorescently labeled ultrasound contrast agents are contained in an Opticell, which is submerged in a water-filled perspex container. A focused ultrasound transducer with a center frequency of 1 MHz and an optical fiber are located at the base of the container. The latter is connected to a xenon flash source for bright field illumination.

The Brandaris 128 high speed camera [1] can record 6 consecutive recordings of 128 frames at a maximum frame rate of 25 million frames per second with a 100 ms delay in between recordings. Each recording can be controlled to contain a fluorescent recording, a bright field recording or a combined recording, through full control over the laser and the xenon flash trigger. All experiments described in this paper consist of alternating bright field and fluorescence recordings.

Ultrasound contrast agents

Two different fluorescently labeled ultrasound contrast agents were studied with the setup described above. The first consists of phospholipid coated microbubbles, produced in-house following a method described by Klibanov et al [2]. The fluorescent dye DiI (1,1'-dioctadecyl-3,3,3'3'-tetramethyl-indocarbocyanine perchlorate) is incorporated into the shell through mixing. The microbubbles were insonified at an ultrasound frequency of 500 kHz with driving pressures varying from 50 to 500 kPa. Figure 2 shows a typical bright field image and the corresponding fluorescence image of a microbubble.

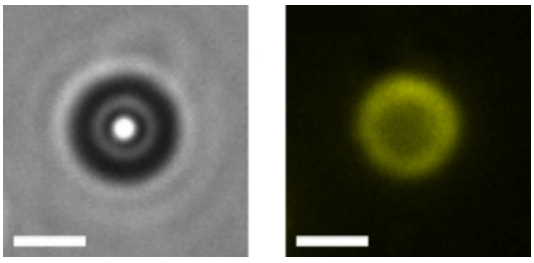

Figure 2. Bright field and corresponding fluorescence image of a phospolipid microbubble. Scale bar indicates 2 µm.

The second agent studied here consists of oil-filled polymer capsules made by Philips Research (Eindhoven, The Netherlands) [3]. The capsules contain a high concentration of the fluorescent dye Nile Red dissolved in the hexadecane core. Due to the rigidity of the thick polymer shell, the ultrasound did not yield any radial oscillations. Therefore no ultrasound was applied during these experiments.

RESULTS

Phospholipid coated microbubbles

The radial oscillations of a phospholipid-coated microbubble in bright field imaging and in fluorescence imaging are compared in figure 3. The bubble was insonified at a frequency of 500 kHz with an amplitude of 50 kPa for a period of 10 cycles and recorded first in bright field and 100 ms later in fluorescence at a frame rate of 1.8 Mfps.

The two imaging techniques show similar overall behavior of the bubble. However, the initial radius and the amplitude of oscillation are smaller for the fluorescence recording. One cause can be an incorrect focal position for the bright field recording. A slight focusing error can lead to substantial deviations of the measured radius compared to the radius in fluorescence. Also, gas loss as a result of lipid shedding due to the oscillations can explain the decrease in measured amplitude.

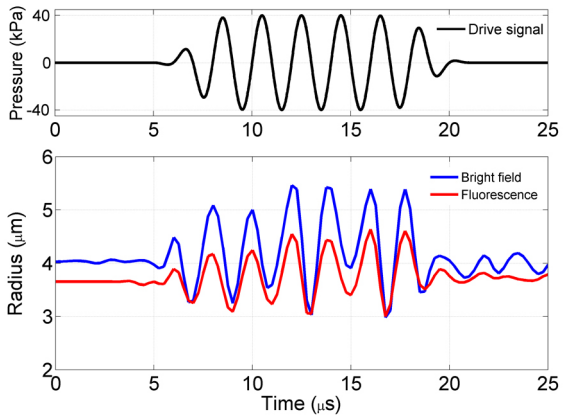

Figure 3. Radius time curve of an oscillating microbubble, recorded first in bright field (blue curve) and with a delay of 100ms in fluorescence (red curve).

To visualize the dynamical dye distribution along the bubble wall, a pair of microbubbles depicted in figure 4a was insonified at a frequency of 500 kHz and a pressure of 500 kPa. Figure 4b shows a fluorescence image at peak negative pressure during oscillation. The red arrows point to regions of high fluorescence intensity indicating high dye concentration at the bubble wall of the left bubble as it coalesces with the bubble on the right. Figure 4c shows the fluorescence residues after the bubbles have moved away from the field of view, due to primary acoustic forces. The remaining fluorescence signal indicates dye and possibly lipid shedding from the bubble shells.

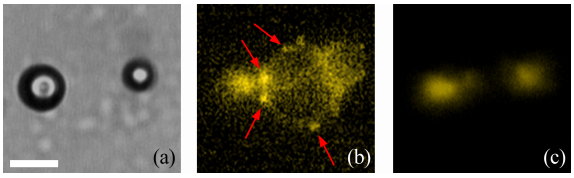

Figure 4. (a) Bright field image from the first recording before ultrasound. (b) Fluorescence image from the second recording at -500kPa, red arrows indicating high dye concentration at bubble wall. (c) Fluorescence image of dye residue at initial bubble positions from the sixth recording. Scale bar indicates 5 µm.

Oil-filled polymer capsules

Laser intensities of 1 MW/cm^2 and higher cause extensive heating of the oil due to absorption of excitation light by the large concentration of dye molecules inside the hexadecane core and at the inner wall of the polymer shell. Rapid vaporization leads to volume expansion and oscillations of the vapor bubble.

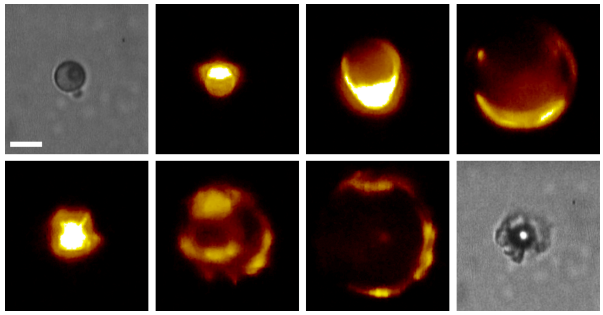

Figure 5. Frames from a fluorescence recording of vapor bubble oscillations due to high intensity laser illumination. The bright field images show the microcapsule before and after the laser-excited oscillations. Scale bar indicates 5 µm.

Figure 5 shows several frames of the fluorescence recording. It also shows bright field images taken before and after the fluorescence recording. This particular recording was taken at a frame rate of 3 Mfps. The maximum frame rate for the fluorescence recordings with the microcapsules is limited to 25 Mfps i.e. the maximum frame rate of the Brandaris 128 high speed camera.

The equivalent radius time curve of the oscillating vapor bubble is plotted versus time in figure 6a. The red profile at the bottom represents the time trace of the applied laser intensity. Initially the vapor bubble grows shortly after the laser was switched on. This is followed by large volumetric oscillations which end abruptly when the laser is switched off again. The frequency of oscillation is near 220 kHz.

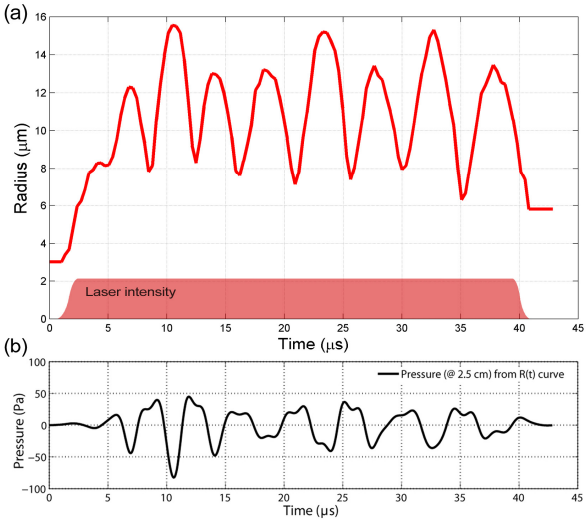

Figure 6. (a) Radius time curve of a microcapsule illuminated with a laser intensity of 1 MW/cm^2. (b) Predicted pressure field of the vapor bubble at 2.5 cm.

The volumetric oscillations of the vapor bubble cause sound emission in the far field. From the conservation of mass and momentum it follows that the emitted pressure wave is a function of the second derivative of the volume of the bubble [4].

$$P_S(r,t) = \frac{\rho}{r}\left(R(t)^2\,\ddot{R}(t) + 2\dot{R}(t)R(t)^2\right) \qquad [1]$$

The photo-acoustic effect generates pressures in the order of 100 Pa at 2.5 cm which is depicted in figure 6b. This effect can be measured using a sensitive ultrasound transducer.

Experiments where ultrasound was applied simultaneously with the laser pulse showed vapor bubble oscillations under influence of the ultrasound field after the laser light had been switched off.

CONCLUSION

By combining a gated high power CW laser system and the Brandaris 128 high speed camera, fluorescence imaging of a fluorescently labeled phospholipid microbubble is possible at frame rates up to 5 million frames per second. For oil-filled polymer microcapsules fluorescence imaging is possible up to 25 Mfps with this setup. This allows for the visualization of the instantaneous dynamics of coated microbubbles. Even shell morphology can be made visible at the nanoseconds time scale of bubble oscillations. The improved contrast of fluorescence over bright-field imaging is highly relevant for combined optical and acoustical characterization of coated microbubbles, as their dynamics is governed by the non-linear behavior of the shell material.

We have shown that a photo-acoustic effect can be generated by oil-filled capsules through high intensity continuous laser illumination. The high amplitude oscillations can be measured in the far field. This behavior could be implemented into PFC nanodroplets as a thermal activation mechanism.

Ultra high-speed fluorescence imaging may also be applied to other multiphase flow visualization such as microfluidic devices using μPIV or to turbulent reactive flows, e.g. in combustion diagnostics.

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