OPTICAL IMAGING OF ULTRASOUND CONTRAST BUBBLE MOTIONS
AT 25 MILLION FRAMES PER SECOND

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Introduction: Applications of the nonlinear scattering of contrast microbubbles in diagnostic ultrasound are well documented. However, there is still a need to investigate the fundamental aspects of bubble-ultrasound interaction. In the emerging areas of molecular-specific targeted imaging as well as acoustic microbubbles for therapeutic use, it is believed that asymmetric motion, especially asymmetric collapse of the bubbles, is crucial in effecting in-vivo endpoints. Therefore, direct observation by optical imaging of individual bubbles in an ultrasound field is highly desired. However, investigators face a number of special difficulties. The optical resolution must be better than 1 μm and the temporal resolution must be better than 0.1 μs. Several groups, notably Dayton¹, Takeuchi², de Jong³, Kodama⁴, and Postema⁵, have successfully made images of oscillating or collapsing bubbles in recent years. Some of these studies are performed at normal TV frame rates of 50 or 60 frames per second (fps) with or without stroboscopic synchronization; some studies were performed at high frame rates of 1000 to 100,000 fps. At ultrahigh frame rates of more than one million fps (Mfps), existing cameras are limited to 1-D frames (streak cameras) or eight 2-D frames.

For our study of nonlinear oscillation and transient collapse of microbubbles in ultrasound (ultrasound frequency at 0.5-5 MHz), an ultrahigh speed 2-D camera is required. The number of frames limits the recording time. With a maximum frame rate of 25 Mfps and 128 frames, e.g., one can make a recording with eight frames per ultrasound cycle and for a continuous duration of more than ten ultrasound cycles. This paper reports our development of a new camera, called the “Brandaris 128” and preliminary results from ultrasound microbubble studies.

Equipment: Brandaris 128 combines the superior flexibility and sensitivity of electronic CCD detectors with the ultrahigh frame rate and high number of frames available in rotating mirror cameras. The working principle of such cameras is based on the Miller principle for high-speed cinematography. A real image (or real object) positioned on the object plane is relayed as an image on a rotating mirror prism. The mirror prism redirects the light beam to successive lens pairs in the lens bank, which refocus the image on CCD’s arranged on a circular image arc (see figure 1).
The recorded image is transferred through flexprint cables to a CCD Controller Card (C³). Four CCD’s are controlled by each C³ and 32 C³’s are connected via eight USB hubs to a PC. In this way, 128 CCD’s are controlled directly by a single PC.

The imaging frame rate is proportional to the rotation speed of the mirror prism. A specialized turbine driven by high-pressure helium or air spins the prism at a maximum rotation speed of 20,000 revolutions per second, resulting in frame rate between 1–25 Mfps. The gas flow is controlled by a mass flow controller, which in turn is controlled by a PC. An infrared laser-photodiode pair mounted near the mirror prism generates three mirror pulses per turbine revolution. Since a rotating mirror camera cannot be triggered externally, the mirror pulses are used as master timing triggers for the target event and light source.

A commercially available CCD was chosen for its combination of resolution, light sensitivity, price and availability. This inter-line video chip produces 500×292 pixels with an approximate dynamic range of 48 dB, and was specified with a sensitivity of 0.03 lux. The C³ are custom designed for driving the CCD electronics, digitizing the raw CCD signals, storing multiple images in RAM memory and data transfer to a PC. Using hubs, the camera can be completely controlled with a laptop PC.

A microcontroller is the main component of the C³ (see figure 2). It contains a Field Programmable Gate Array (FPGA) that was programmed to perform a number of time-critical tasks. The C³ architecture controls each CCD individually, allowing for operation in the normal mode capturing 128 images in a sequence or in a segmented mode in which multiple sequences can be captured at very high repetition rate. To synchronize the detectors, three trigger inputs (start, flush and transfer) are provided to all the C³’s.

The C³ is programmed to perform individually the following tasks: flush, charge transfer, readout and RAM dump. By individually controlling these four tasks, a great deal of flexibility is achieved. For example, while readout requires a fixed duration of about 20 ms, a faster repetition time can be
achieved by dividing the 128 detectors into multiple groups. Repetition time as short as 16.7 μs is possible when the turbine is ran at maximum speed. The on-board RAM buffer allows six images per detector to be stored before RAM dump, this allows better usage of pressurized helium and the limited lifetime of the turbine. For example, six experiments totaling 768 frames can be acquired within a fraction of a second. The total data set, of about 120 megabytes, can be transferred to the PC in less than 5 seconds, so that a large number of experiments can be performed quickly.

A USB 2.0 device driver has been written for the Windows 2000 platform. An instruction set that provides complete access to all the functionality of the C³ has been created for the Matlab programming language. A graphical user interface and analysis toolbox is currently in development.

![Figure 2. Schematics of CCD Controller Card (C³)](image)

**Experimental Methods:** An experimental contrast agent from Bracco Research SA (Geneva), was studied. An Olympus microscope with a 60× high-resolution water immersion objective was mounted in front of Brandaris 128 so that a real image is formed on the object plane. The combined Olympus-Brandaris system has an optical resolution better than 0.6 μm.

![Figure 3. Experimental setup for bubble ultrasound interaction studies](image)
Figure 3 shows the experimental setup under the microscope, a special water tank was constructed, on which is mounted a 0.5 MHz, ultrasound transducer and a 0.2 mm hollow fibre which pass through the focal zone of the transducer. An optical fibre bundle conducts light from a modified xenon flash illumination unit to the hollow fibre. The contrast agent was very gently introduced into the hollow fibre by a syringe. The transducer was a single element f/2 transducer with a spherical-focus at 75 mm. Ten cycles of 0.5 MHz ultrasound was transmitted to the hollow fibre. Only one burst of ultrasound is transmitted per recording, and between recordings the experimenter has the option to introduce fresh bubbles by gently pressing on the syringe. For these preliminary studies, 64 image frames per ultrasound firing were recorded at a relatively slow rate of about 1–2 Mfps. Therefore the total recording time was about 64 µs, allowing for the observation of the bubbles before, during and after the ultrasound burst.

Results: Figure 4 shows a typical frame demonstrating the appearance of bubbles at rest. In this case the bubbles are placed in a culture dish mounted upside-down in the water tank. Attached to the surface of the dish is a mix of vascular endothelium and smooth muscle cells. The contrast bubbles float upwards and rest against the surface of the dish next to the cells. Bubbles and some organelles of the cells are visible in the frame. The field of view was 89 µm × 68 µm. The largest bubble near the centre of the image was about 6.6 µm diameter.

Figure 4. A typical frame from the Brandaris 128, the magnification was 120×, the FOV was 89 µm × 68 µm.

Figure 5 and 6 show portions of two 64-frames recordings of the behavior of ED-14 bubbles in a 10 cycles, 0.5 MHz ultrasound field at about 0.7 MPa (peak negative amplitude). Each sequence was cropped from the original 500 pixels × 292 pixels × 64 frames recording.

The first sequence was recorded at 1.05 Mfps, and demonstrated a group of bubbles undergoing repeated coalescence, finally becoming a single large bubble. Figure 5 shows a 100×100×36 portion.
Figure 5. A portion of a recording (100 pixels × 100 pixels × 36 frames) showing multiple coalescence of bubbles aggregate in a 0.5 MHz sound field. The frame rate was 1.05 Mpfs.

Figure 6. A portion of a recording (80 pixels × 80 pixels × 36 frames) showing a probable micro-jet formation in a bubble in a 0.5 MHz sound field. The frame rate was 1.5 Mpfs.

It can be seen clearly bubble coalescence occurred during the compression phase in several distinct steps over several cycles of ultrasound.
The second sequence was recorded at 1.5 Mfps, and shows a single bubble oscillating in the sound field. Figure 6 shows a $80 \times 80 \times 36$ portion. The small feature in the center of the bubble during the compression phase may be a recurring micro-jet directed upwards towards the wall of the hollow fibre, which is parallel to the image plane. Micro-jet and micro-streaming are being investigated as possible mechanisms for the enhanced uptake of drugs or genetic materials of cells in the presence of micro bubbles and ultrasound.

**Conclusion:** The camera offers a unique combination of ultrahigh frame rate, extended recording time, very high light sensitivity without employing image intensifiers, and the ease of processing of digital imaging. Imaging frame rate can be set at 1–25 Mfps. Full sequences of 128 frames can be captured at repetition frequencies up to 50 Hz. In the segmented mode, higher repetition frequencies (up to 60 kHz) can be achieved by a trade off in frame number. For example, four sequences of 32 frames can be recorded at a PRF of 8 kHz, which is typical in colour Doppler mode. This camera is especially suited for studies of diagnostic and therapeutic uses of contrast micro bubbles and cavitation bubbles.

For more information, visit the website:  [www.brandaris128.nl](http://www.brandaris128.nl)

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