THE EIGHTH EUROPEAN SYMPOSIUM ON ULTRASOUND CONTRAST IMAGING

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The Eighth
European Symposium
on Ultrasound
Contrast Imaging

to be held
on January 23-24, 2003
Rotterdam,
The Netherlands

Erasmus MC
Universitair Medisch Centrum Rotterdam

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ABSTRACTBOOK

January 23-24, 2003

8th EUROPEAN SYMPOSIUM ON ULTRASOUND CONTRAST IMAGING
23 -24 JANUARY 2003, Rotterdam, The Netherlands

WEDNESDAY, 22 January 2003

Pre-congress

THURSDAY, 23 January 2003

8th EUROPEAN SYMPOSIUM ON ULTRASOUND CONTRAST IMAGING
23-24 JANUARY 2003, Rotterdam, The Netherlands

FRIDAY, 24 January 2003

07.30 - 08.00 **Registration**

07.30 - 09.00 **POSTER DISCUSSION** ..... *Moderators: F.J. ten Cate and N. de Jong*

09.00 - 10.30 **TECHNOLOGY II** ..... *Chairpersons: Ayache Bouakaz and Mark Monaghan*

Discussion

10.30 - 11.00 **Intermission**

11.00 - 12.30 **DRUG DELIVERY II** ..... *Chairpersons: Folkert Ten Cate and Thomas Porter*

Discussion

12.30 - 13.45 **Lunch**

CONTRAST ECHOCARDIOGRAPHY – CURRENT CLINICAL APPLICATIONS

Mark J Monaghan

Cardiology Department, King's College Hospital, London.

Virtually every cardiac imaging technique utilises contrast agents, yet the adoption of ultrasound contrast agents into the routine practice of echocardiography has been slow. The commercial and clinical promise of contrast echo has not yet been fulfilled and we are truly standing at the crossroad between success and failure of the technique.

Patent issues, cost, reimbursement, difficult regulatory issues in the USA and a smaller market than originally envisaged has led a number of contrast agent manufacturers to review their position. Some agents have been withdrawn from development and some put on hold. Instrument manufacturers have been reflecting upon their substantial investment in contrast imaging technology. The road to failure was clearly signposted.

Contrast Echo – the Threats

- Myocardial perfusion imaging still research rather than clinical
- Cost
- Reimbursement
- Slow progress on licensing
- Patent issues
- Improvements in image quality – reduce need
- IV line required
- Lack of education

Contrast Echo – the Opportunities

- Enhance Doppler studies
  - TR, AS, PV
- Enhance endocardial definition
  - Difficult patients
  - Stress Echo – major role
- Delineate anatomy
  - Thrombus
  - Fistulae etc
- Myocardial perfusion
  - Near future
- Drug delivery
  - Far future
- Pressure measurement
  - Far future
- Now reimbursed
- New agents becoming available
- Equipment progressing fast

Fortunately, a number of key issues have occurred which indicates a far more optimistic future for contrast echo. These issues include agreement on reimbursement for the use of contrast in the USA, resolution of some patent disputes, new technology, which makes myocardial perfusion imaging realistic and more of a consensus on what is needed to make contrast echo successful.

Contrast Agents Licenced in the UK

- Levovist Schering) polysacharide + air
- Optison Mallinkrodt Albumin+ octofluoropropane
- Sonovue – Lipid + Sulphur Hexafluoride

In many countries there are currently three licensed contrast agents. Although all three have a licence for left ventricular opacification and Doppler enhancement, improvements in imaging technology, particularly 2nd harmonics, have paradoxically reduced the need for contrast in routine studies.

However, figure 1 illustrates an example of how useful contrast left ventricular opacification can be. This patient had had a large anterior infarct and was due to have CABG. His routine (harmonic) transthoracic echo had suggested a large apical thrombus, which the surgeons wanted to remove. Unfortunately, he was a poor echo subject and a definite diagnosis was required. Therefore a contrast study was performed which confirmed the presence of a thrombus, although it was much smaller and not in exactly the same location as the routine echo had suggested.

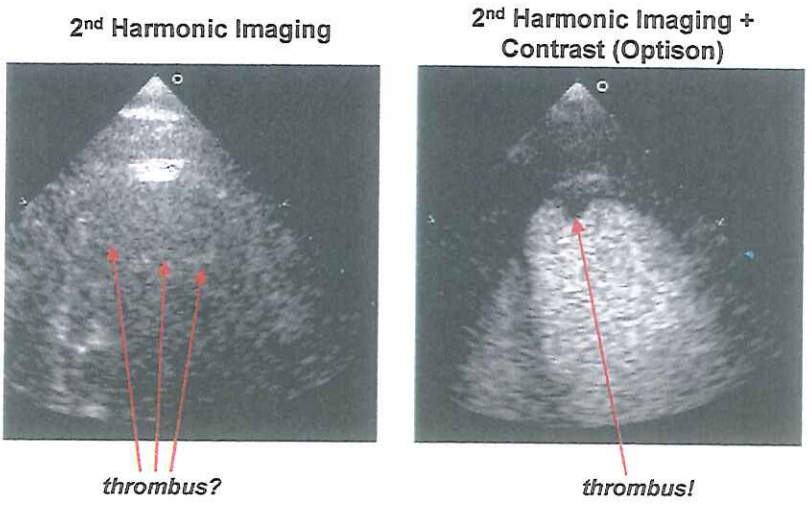

Figure 1 *- Zoomed apical 4 chamber views in a patient who had suffered a large anterior infarct and was due to have CABG. His routine (harmonic) transthoracic echo, seen on the left, had suggested a large apical thrombus which the surgeons wanted to remove. As shown, he was a poor echo subject and a thrombus could not be definitely confirmed. Since the presence or absence of a thrombus would affect the surgical approach, a contrast study with 2nd harmonic imaging was performed and is shown on the right. This confirmed the presence of a thrombus, although it was much smaller and not in exactly the same location as the routine echo had suggested. The structure seen on the non-contrast image was in fact an anomalous chord near the apex.*

Stress echocardiography remains an area when contrast can and should make a significant contribution. Stress echo is an operator dependent technique with poor image quality, especially at peak stress, being the limiting factor. Despite the clinical value of stress echo, it's application is often limited by the lack of time and staff available to perform the studies plus the steep learning curve and the undoubted fact that the images can be difficult to interpret, especially at peak stress. Contrast can help overcome this and should become routine practice in stress echo examinations.

Why should you use Contrast during Stress Echo

- Makes it easier to perform and interpret
- Reduces variability
- Increases Diagnostic confidence
- Relative cost is small

There are many studies showing a significant improvement in endocardial definition and interpreter confidence when contrast is used in stress studies. In our own department, contrast is used in at least 90% of stress patients. It adds little additional inconvenience to a pharmacological stress test since an IV line is already in place, and in terms of the real cost of performing a stress echo, the additional

factor of contrast is not that significant. A stress echo study with contrast is still cheaper than a stress Sestamibi scan and the techniques have similar sensitivity and specificity for detection of reversible ischaemia and myocardial viability. One major indication for stress contrast studies in our own department is the evaluation of the physiological significance of known coronary lesions prior to proposed revascularisation. If the technique can help us make better management decisions over which patients would benefit from CABG or PTCA, then the additional cost of contrast is a small price to pay for increased diagnostic accuracy. This is especially true when you consider the cost of revascularisation. One saved PTCA or CABG would probably pay for a whole year's supply of ultrasound contrast media! Furthermore, it is possible, although not recommended by the manufacturers, to make one vial of some contrast agents last for up to three stress echoes, providing they are all performed within the same session.

Contrast Imaging Methods

- Harmonic Power Doppler (Angio) – Contrast Destruction Technique
- Grey scale harmonics (power pulse inversion, ultraharmonics) – Non linear response technique.
- Real time contrast imaging (Power modulation, Power pulse inversion, Coherent imaging) low output power, non-destructive techniques.

The major market for contrast echo will occur when we can make myocardial perfusion imaging during rest or stress studies a reality. This has been described as the "Holy Grail" of Contrast Echo. The development cycle for contrast agents is much longer than that for ultrasound instrumentation. Therefore, most of the advances in this area have been in terms of increasing the sensitivity of the imaging technology to detect contrast micro bubbles in the small quantities with which they occur in the myocardium. The contrast imaging technologies can be divided into three distinct groups. All of which rely upon the fact that contrast agents backscatter ultrasound in a different way to tissue. This issue is critical, since the technologies are trying to separate out noise *from* tissue *from* contrast micro bubbles. However, the method of separating noise *from* tissue *from* contrast is different with each group of techniques.

Harmonic Power Doppler (Power Angio) is based upon a Doppler technique for detecting moving red blood cells. Multiple, high power, pulses are transmitted down each scan line and the ultrasound system analyses any difference in the backscattered signal from each pulse. Red blood cells, which have moved between subsequent pulses, cause a difference in the backscattered signal, which is detected by the ultrasound system. In tissue, red blood cells move too slowly to be detected by this method. However, if contrast micro bubbles are present, they will be destroyed by the ultrasound pulses. This means that the ultrasound system still detects a difference in the backscattered signal caused by the destruction and disappearance of the contrast microspheres. This technique has proved to be very successful for the detection of contrast. Especially agents, which have a fragile shell and a gas which dissolves quickly in blood. Levovist is an example of an agent, which works particularly well with this technique. Unfortunately, because the technique relies upon contrast agent destruction,

this means that after one harmonic power Doppler imaging frame, there is insufficient contrast left within the myocardium to provide further perfusion images. Therefore, it is necessary to suspend imaging for a number of cardiac cycles to allow new contrast microspheres to reperfuse into the imaged myocardial segments before another perfusion imaging frame can be obtained. This technique is known as intermittent imaging and it is usually performed by triggering the image acquisition to the ECG, so that perfusion frames are collected at either end-systole or end-diastole on every 2^{nd}, 3^{rd} or 4^{th} cardiac cycle etc.

Variations on this triggering technique include collecting 2 sequential frames together at the desired triggering interval. The first frame should demonstrate contrast in the left ventricular cavity and in the myocardium. The second frame should only show contrast in the cavity because the myocardial contrast will have been destroyed during the first frame. This technique is useful for detecting artefacts caused by myocardial movement or excessive gain etc. It is also possible to vary the triggering interval from every cardiac cycle to up to every 10 cardiac cycles during a contrast infusion. If the contrast intensity is measured from a myocardial region of interest, then the signal intensity is directly proportional to the number of contrast microspheres within that region of interest. A lower intensity will occur at shorter triggering intervals because contrast will have had less time to reperfuse the myocardium between each imaging frame. A graph can be constructed of signal intensity against triggering interval. The slope and plateau of the graph can be used to calculate myocardial blood flow – in theory. This has been shown to work well in animal models.

Greyscale techniques exploit the non-linear characteristics of contrast microspheres and the fact that they generate much stronger harmonics than tissue. These techniques rely on fairly high output power in order to provoke microbubble oscillation and the generation of bubble harmonics. Therefore, intermittent imaging is also necessary with this type of technique. In its simplest form, filters are used to detect the second harmonic signal and separate it from the fundamental signal. The contrast to tissue ratio is much greater at the second harmonic than it is at the fundamental frequency. This makes it easier to separate out the weak contrast signal from the strong tissue signal. However, as we know, tissue also causes ultrasound harmonics to be generated and these will be detected alongside the contrast harmonics. Ultraharmonic techniques partially overcome this by detecting the third harmonic signal. Tissue has virtually zero third harmonic components whereas the contrast signal is still relatively strong at this frequency and this allows it to be detected with higher sensitivity. Broad bandwidth transducers are required to allow the third harmonic to be detected. Some manufacturers have developed transducers with frequency characteristics and beam profiles especially suited to contrast imaging.

Pulse inversion is another greyscale technique that uses a novel method to separate out the contrast harmonic from tissue. Essentially, two pulses are fired down each scan line. Each pulse is 180^0 out of

	Harmonic Power Doppler	Greyscale Techniques	Real-time perfusion Imaging
Advantages	Very sensitive. No background subtraction needed.	Good dynamic range. Works with most agents. Reasonable sensitivity.	Wall motion and perfusion information. Easier to perform.
Disadvantages	Intermittent imaging, Requires fragile contrast. Wall motion artefacts.	Intermittent imaging. Poor visual effect. Background subtraction needed.	Requires robust contrast. Software not yet optimised.

phase with the other and the received echoes from each transmitted pulse are summed within the ultrasound scanner. Echoes from essentially linear reflectors such as tissue will be received 180^0 out of phase and will cancel when summed. Whereas echoes from non-linear contrast micro bubbles will be distorted in a different way, depending on the phase of the transmitted pulse. The received echoes from microbubbles will not cancel each other when summed and it can be shown mathematically that the remaining signal is effectively the bubble harmonic. This methodology is very sensitive although still requires intermittent imaging and can be sensitive to movement artefacts. It works well with most types of ultrasound contrast agents.

From a purely practical point of view, intermittent imaging is a tiresome, difficult technique and no wall motion information is available. Therefore, some real-time perfusion methods have been developed. In order to work in real-time, both robust contrast microspheres and very low output power must be used, in order to minimise contrast destruction. Low output power means that the returning contrast signal will be very weak and highly sensitive detection methodology is required. Power pulse inversion is one such method and uses multiple pulses down each scan line. Each pulse is 180^0 out of phase with the preceding one (like pulse inversion). Using multiple pulses, rather than just two, allows further discrimination between the contrast and tissue, especially at low power. Power modulation, is fairly similar except that the multiple pulses are in phase but every other pulse is half the amplitude of the preceding one. Again this technique exploits the non-linear characteristics of contrast. Coherent imaging also uses multiple pulses, however they are fired down sequential scan lines and this technique utilises the fact that scan lines do in fact overlap each other slightly. Since multiple pulses are not fired down the same scan line, coherent imaging leads to slightly higher frame rates than the other techniques.

Potential Clinical Roles for Real-time Perfusion Imaging

- Acute chest pain syndromes
- Viability assessment ?hibernation
- Endo/epi perfusion ratios
- Stress perfusion echo studies
- ?Quantification of myocardial blood flow
- Evaluation of success of thrombolysis
- Evaluation of success of PTCA/CABG

As previously mentioned, real time perfusion imaging techniques require contrast microspheres with robust shells that are resistant to ultrasound destruction at reasonably low power continuous imaging. A few frames of high output power imaging can be used to destroy contrast

within the myocardium. It is then possible to record the contrast reperfusing each myocardial segment and analyse the rate of reperfusion, in order to try and calculate myocardial blood flow. Again, this technique has so far only been validated in experimental settings.

Real time perfusion imaging has many potential advantages. The technique is relatively easy to use, many artefacts can be avoided and wall motion information is obtainable alongside perfusion. The ability to supplement wall motion information with perfusion will make this technique particularly invaluable during stress echocardiography.

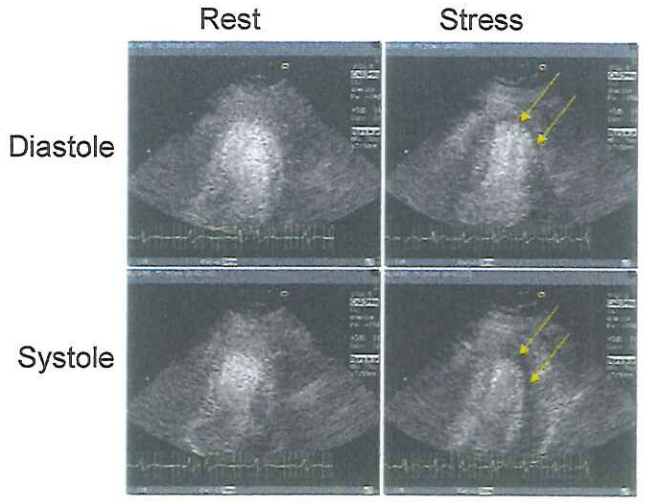

***Figure 2** - This illustrates an example of real-time (Coherent) contrast imaging during Dobutamine stress echo. Simultaneous wall motion and perfusion information is obtained. An apical and lateral perfusion defect (arrowed) is seen in both systole and diastole at peak stress in this patient with circumflex disease. A stress induced wall motion abnormality was also seen in the same territories during the real-time study. The use of contrast during stress echo enhances endocardial definition and may provide very useful simultaneous perfusion data. This will almost certainly improve the interpretation and accuracy of the technique.*

Figure 2 illustrates an example of real-time (Coherent) contrast imaging during Dobutamine stress echo. An apical and lateral perfusion defect is seen in both systole and diastole at peak stress in this patient with circumflex disease. A stress induced wall motion abnormality was also seen in the same territories during the real-time study. Clearly the ability to supplement wall motion data with perfusion data will increase the power of contrast stress echo. There are obviously many other chronic and acute clinical scenarios where real-time perfusion imaging, at the bedside, would have a tremendous impact upon patient management. One example is the use of contrast to detect myocardial viability. If contrast is visible within the myocardium, this implies an intact microvasculature. The microvasculature will only remain intact if there are viable myocytes to supply. Several studies have recently demonstrated that the presence of myocardial contrast in akinetic segments can predict functional recovery and may also give useful information about the potential for re-modelling post MI.

Clearly this technique would have several potential advantages over low-dose Dobutamine studies, including speed and the fact that it would be a truly bedside technique.

Another emerging role for contrast echo is in combination with automatic boundary detection systems (ABD). ABD technology has been available for several years but has been hampered by the need for good image quality in order to facilitate endocardial detection. In particular, this has made it an impractical technique to use during stress echo. However, some instrument manufacturers have modified this technology so that it will work with contrast and utilise the excellent endocardial definition that provides. This technique now works well in most patients and can provide more objective, real-time evaluation of LV volumes, EF and regional wall motion. The latter technique has been named Contrast Colour Kinesis. A European multi-centre study (Euconet) has recently commenced to evaluate the role of this technology to provide automatic and almost simultaneous assessment of wall motion and perfusion. This should, for example, make stress echo more objective and easier to perform and interpret.

Conclusion

The clinical applications for Contrast in Echo are now well defined. The indications for Contrast in routine Doppler enhancement, Thrombus delineation and left ventricular function studies are limited, given the quality of modern echocardiography systems. However, if you are performing stress echo, Contrast is your friend and I believe that you will want to use it in the majority of studies. In the very near future, we will be using Contrast for myocardial perfusion studies and to enhance automated left ventricular function analysis systems. The far future holds the promise of drug delivery and intracardiac pressure measurement by ultrasound contrast agents. So, there is no doubt in my mind, that the role of contrast agents in echo imaging will be as important as contrast is in virtually every other medical imaging modality.

MICROVASCULAR IMAGING OF THE BREAST

Jeff Powers, Seth Jensen, Cyrile Villet, Paul Kalman, David Hope-Simpson
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Background: There has long been the belief within the breast imaging community that blood flow in breast lesions should play a major role in differentiating benign from malignant lesions. However, conventional color flow imaging suffers from limited sensitivity to the very low flow velocities in breast lesions and has never proven to add a great deal of diagnostic information to complement a normal B-mode exam. MR angiography of breast cancer has been shown to be highly diagnostic, especially when time-intensity curves are produced. Consequently there has been great interest in the use of ultrasound contrast agents to provide more diagnostic information.

While blood flow is helpful in diagnosing breast cancer, the presence or absence of blood flow to a lesion is not. For example, benign fibroadenomas can be hyper-vascular, while some breast cancers are hypo-vascular, and often have a high interstitial pressure that reduces blood flow within the lesion. What is common to most cancers, though, is the process of angiogenesis. Blood vessels grown to supply nutrients to a rapidly growing malignant lesion tend to be chaotic, with a randomly branching structure, and arteriovenous shunts. To date, however, no modality has been able image these small vessels with adequate resolution to properly characterize the morphology for a confident diagnosis.

Early in our breast contrast work it was observed that individual bubbles could often be seen moving slowly through the smallest vessels in many breast lesions. However, at the very low flow rates it was difficult to make out the vessel morphology due to the few bubbles seen traversing the lesion. This led to a scintillating effect with no distinct vessels visible.

MicroVascular Imaging (MVI) captures the tracks of the individual bubbles as they pass through the microvasculature, making the vessel morphology much more apparent. During MVI imaging, a dual display is used showing the images being acquired in real-time alongside the integrated images. In the event of too much contrast being captured or a motion induced artifact, the image can be cleared and integration resumed. Following the exam, both the real-time and the processed images can be reviewed in a dual cineloop format or stored for offline review or analysis.

ADVANTAGES OF ULTRASOUND CONTRAST AGENTS IN EVALUATING CAROTID ARTERY PATHOLOGY

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The value of contrast agents as adjunct in a wide range of radiological imaging procedures has long been recognized. Many of the same principals that allow for improved diagnostic confidence and reliability in using contrast agents in radiological imaging, also apply for applications in ultrasound imaging.

Among the many advantages of contrast, increased diagnostic confidence, improvement in cost effectiveness, reduction in the need for additional exams, decrease in physician and sonographer time commitment, and expanded applications are all important. The first generation agent Levovist, has enjoyed the longest clinical application as an aid to ultrasound studies, especially in cardiac applications. The second-generation agents, including Optison, Definity and SonoVue are in current clinical use in countries throughout the world. Expanded uses for ultrasound contrast agents are now being investigated.

Particular advantages for contrast agents in vascular applications could be in the detection of flow in vessels that are deep to the skin surface, studies with difficult windows, and in the targeting of small vessels. Other potential imaging improvements could be seen in areas of slow flow through vessels, or in the evaluation of tissue perfusion, e.g. renal parenchymal perfusion.

Evaluation of transcranial studies could be improved due to the significant number of patients with difficult windows. Better visualization of intracranial pathology such as aneurysms and A V malformations could be accomplished. Early investigational work in brain perfusion and evaluation for cerebral ischemic disease is underway. In the area of duplex scanning of the peripheral arterial tree, contrast improves visualization of lumen pathology and can aid in detection of flow. The better definition of the adductor canal and calf regions, in the evaluation for collateral vessels, and in the determination of subtotal versus complete occlusion are additional further benefits.

Potential for carotid arterial definition in both intimal medial thickness determinations and in the evaluation of carotid wall pathology are important applications. Difficult cases involving deep and tortuous branch vessels would be most significantly improved. Determination of carotid plaque morphology, presence of ulcerated lesions, diameter percent stenosis and length of lesions could be

done. More recently, the diagnosis and follow-up of patients who have undergone carotid endarterectomy or carotid stent placement could be more reliably undertaken.

In summary, the above discussion outlines many potential applications for contrast agents in peripheral arterial examinations. Research in this area may define new areas of work as the field of duplex ultrasound expands. The beneficial impact on cardiac and radiological diagnoses portends for an exciting future with this mode of imaging.

DIFFERENCES IN DEFINITY AND OPTISON MICROBUBBLE DESTRUCTION RATES AT THE SAME MECHANICAL INDEX WITH DIFFERENT REAL TIME PERFUSION SYSTEMS

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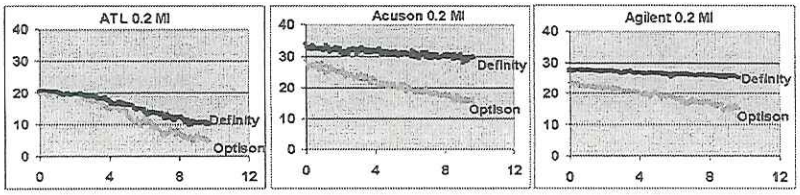
Background: Different pulse sequence schemes (PSS) have been invented to induce non-linear behavior (NLB) from microbubbles at a low mechanical index(MI). These PSS either send pulses of alternating polarity (PID; Phillips HDI 5000), alternating amplitude (PM; Phillips Sonos 5500) or both alternating polarity and amplitude (CPS; Siemens Acuson Sequoia). In addition, CPS analyzes both fundamental and harmonic NLB, PM analyzes fundamental NLB, and PID analyzes even order harmonic NLB. We hypothesized that these differences would result in significant differences in contrast intensity (CI) and microbubble destruction rates (MDR) at the same MI when using either FDA approved albumin coated (Optison) or lipid encapsulated (Definity) microbubbles.

Methods: A tissue mimicking phantom was created which had imaging chambers at 3 (near field) and 9 (far field) centimeters from the diagnostic transducer face. Optison or Definity microbubbles were injected into the chamber which was then insonified with either PM, CPS, or PID at the three different MI (0.1, 0.2, and 0.3), using the same frame rate (25-30 Hertz), and frequency ranges (1.5-1.7 megahertz). CI was measured in decibels (dB) and corrected for both noise and NLB from tissue by subtracting phantom intensity at each MI. Peak negative pressures (PNP)for each transducer at each MI were measured with a needle hydrophone.

Results: PSS which analyzed fundamental NLB(CPS and PM) had significantly higher initial CI ($p<0.05$ compared to PID). Optison microbubbles were already destroyed in the near field with PID at an MI of 0.1 ($p<0.05$ compared to PM and CPS). Definity was more resistant to destruction than Optison, but was also destroyed at a lower MI with PID (0.2) than with PM or CPS (Figure). Summed PNP was higher with PID at an MI.



Conclusions: These data indicate that at the same MI, real time perfusion systems that send PSS which alternate amplitude are less destructive than PSS that send alternating polarity. Increased contrast sensitivity at the same MI is seen when both fundamental NLB is analyzed. These differences must be taken into account when deciding an optimal MI for real time perfusion imaging with
Different PSs.

OVERVIEW OF CONTRAST IMAGING METHODS

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Perfluorocarbon contrast agents with soft, pliable shells are now becoming widely available in Europe and Canada and present new opportunities for the diagnostic use of contrast agents in radiology, cardiology and oncology. Whereas air-based agents like Levovist are in practice only capable of being detected by means of their disruption, most perfluorocarbon bubbles can be driven into non-linear oscillation which lasts over seconds or even minutes, creating a steady stream of harmonic echoes. Because such echoes are coherent (that is, in phase with each other) over many pulses sent by the ultrasound transducer, a new set of imaging methods becomes available for their detection. In these methods, a sequence of pulses is sent and the same bubble responds to each pulse. Unlike tissue structures, which give rise to echoes that simply mirror the incident pulse, bubbles respond differently to the phase and amplitude of each pulse they experience. It is therefore a simple matter to make an imaging method that differentiates between tissue and these bubbles: one simply sends a sequence of pulses of differing phases and amplitudes. On receiving the echoes from this sequence, the scanner combines them in a way that ensures that the 'mirror-like' echoes from tissues combine to zero. What is left is then some combination of the non-linear components of the bubble echo. Precisely what non-linear components are produced by a sequence of pulses can be determined mathematically. For example, keeping the amplitude of the pulse constant but changing the phase of alternate pulses by 180 degrees (known as *pulse inversion* imaging) produces an echo with even order harmonics. On the other hand, keeping the phase constant and changing the amplitude (known as *power modulation* imaging) detects odd and even order non-linear components, though at some cost to the signal-to-noise ratio. Combinations of amplitude and phase are also possible. Almost all manufacturers now use some form of this multipulse modulation processing (1) in their contrast-specific imaging modes. As long as the MI is kept low and the bubble is not disrupted by the pulses, real time imaging of perfusion can be achieved in many organ beds, including the myocardium, liver, skin, thyroid and breast.

One obvious problem with changing the phase of the pulse transmitted into tissue occurs when the tissue moves. Movement of tissue causes successive echoes to be phase shifted slightly because the distance from the transducer has changed (this is the familiar Doppler effect). Thus the processing that is relied upon to cancel the tissue echoes fails and we see a 'flash' artifact from, for example, the heart wall using pulse inversion imaging. However, if the speed of movement is constant, the phase shift can be predicted from a short series of successive echoes from a moving target. The echoes can then be adjusted by shifting their phase back and allowing them to cancel each other.

This process is already built into colour Doppler imaging systems as the so-called 'wall filter'. With a little modification, it allows multipulse non-linear (known generically as *pulse inversion Doppler*) methods to suppress moving tissue while still detecting the non-linear bubble echoes. This is the reason that several pulses are necessary to achieve a real time myocardial perfusion image without motion artifact, and the frame rates are always lower than those possible with conventional B-mode imaging. Looking more closely at the result of the detection process, we see that pulse inversion Doppler maps the non-linear components of the bubble echoes into a spectrum of Doppler shift frequencies. The same filters used to suppress motion can now be turned to eliminate other unwanted components of the echoes in contrast examinations. One of set of such components is that produced by non-linear propagation of the ultrasound pulse, the *tissue harmonic*. Tissue harmonics generally produce an artifact in the contrast examination that reduces the visibility of perfusion and limit the MI that can be used (tissue harmonics are more prominent as the peak negative pressure increases). These filters can be used to reduce the tissue harmonic, allowing pulse inversion Doppler methods to be used for high MI contrast imaging, as well as the low MI imaging with which they are more commonly associated.

Of the emerging areas of technical development in bubble imaging, one of the most intriguing is the challenge of imaging *angiogenesis*. Angiogenesis is the term used to describe the development of a new microvascular supply in solid tissue; it could be the result of revascularisation of a myocardium injured by ischaemia or, in the case of cancer, the transformation of a harmless, avascular colony of cells into a malignant tumour. Although known for many years, it is only recently that advances in the understanding of angiogenic transformation, and particularly the potential to modulate it, have propelled this field into the forefront of both cardiological and cancer research. Angiogenesis in breast cancer, for example, has implications for diagnosis, prognosis and treatment. Identifying those breast cancers which have new blood vessels may help identify those which are exhibiting malignant progression and presumably need to be treated more aggressively. For many tumours, it is been shown that the number and density of blood vessels provides prognostic information which is independent of clinical staging. Furthermore, a burgeoning number of new treatment strategies target the proliferating vasculature of a developing cancer, including drugs specifically designed to inhibit (or, in the case of ischemic injury, promote) the angiogenic transformation itself. The ability to provide imaging information on the status of blood supply to a cancer is of great clinical significance. Direct imaging of the angiogenic circulation is extremely challenging, as the vessels are beyond the resolution limit of conventional radiological imaging. However, the pathological characteristics of these vessels, which influence both their morphology and the dynamic properties of blood flowing within them, offer several possibilities for identification by non-invasive means. Micro bubble destruction-reperfusion may be able to show changes in the volume of tumour tissue occupied by blood and changes in overall flow rate at the microvascular level. These are being applied successfully to the quantitative monitoring of antiangiogenic therapies (2). However, many

cancers have a similar bulk flow rate and volume as normal tissue. What is needed is some indication of the *morphological* changes to the vasculature brought about by malignant transformation. Two further contrast methods might be capable of offering morphological information about the tumour vasculature: one based on fractal modelling of reperfusion, the other on maximum intensity processing of the ultrasound echo. Initial clinical images will be shown.

References

1. Hope Simpson D, Burns PN. An Analysis of Techniques for Perfusion Imaging with Microbubble Contrast Agents. IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control 2001, 48, 6, 1483-1494.
2. Foster FS, Burns PN, Hope Simpson D, Wilson SR, Christopher DA, Goertz DE. Ultrasound for the visualization and quantification of tumor microcirculation. Cancer and Metastasis Reviews 2000, 19, 131-138.

EXPANDING CONTRAST INTO NEW DIMENSIONS: LIVE 3D LVO AND TEE MCE

Pat Rafter

Philips Medical Systems, Andover, MA, United States

Over the last several years, increased understanding of the interaction between ultrasound and micro bubbles has fueled equipment advancements. Improvements in transducer technology, new detection and acquisition techniques, and improved usability of these techniques have allowed Myocardial Contrast Echo (MCE) to reach the critical threshold allowing for clinical validation against gold standards such as angiography. Hopefully, over the next few years this will be followed by FDA (and other regulatory bodies) approval and routine clinical adoption. Very recent advancements in transducer and system technology promise to open even more opportunities, extending ultrasound and contrast into exciting new areas.

Live 3D and Contrast

We have reached a point where transducer and system technology are making real-time 3D beam forming and rendering a reality on standard ultrasound equipment. Philips Medical Systems has recently developed and introduced Live-3D technology on the SONOS 7500 based on these tremendous breakthroughs. New transducer technology allows dicing a transducer into a matrix such that all 3000 elements are utilized. Electronics inside the transducer performs beam forming. This leads to much improved 3D image quality when compared to prior sparse matrix solutions. Philips' "x4" matrix transducer uses technology that offers extremely wide bandwidth and thus enables harmonic imaging. In addition to the x4, system improvements now allow real-time volume rendering and processing of the 3D image. Images can be easily rotated and cropped to obtain different views from the same dataset.

This new 3D technology has many clinical possibilities, one of the most exciting of which is the combination with contrast. The ability to quickly acquire a full 3D volume opens the possibilities for accurate LV volume calculations with echocardiography. Preliminary results with harmonic imaging and contrast have shown that just like in 2D imaging contrast can help immensely in the discrimination of the border. Additionally, the ability to transmit and receive data in arbitrary directions with arbitrary apertures and focuses has very important implications for improving contrast detection techniques for MCE

Despite the superb acoustic windows available with TEE imaging it has proven a challenge to image contrast agents from this window. The decreased bandwidth and the higher frequencies of standard TEE transducers relative to transthoracic transducers have made development of contrast detection techniques a challenge. A new TEE has been developed and just introduced to the market, with increased bandwidth and harmonics capabilities to address these issues and open new possibilities. Preliminary results indicate TEE perfusion is feasible with this transducer opening up several possible new applications, such as in the operating room.

Analysis of Acoustical Responses of Microbubbles for the Optimization of Phase- and Amplitude-Coded Pulse Sequences

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Introduction

Our group has previously introduced an algorithm for the design of optimized receive filters that are used with phase- and/or amplitude-coded transmit pulse sequences to enhance the contrast between 2 media [1]. The N echoes that result from the N transmit pulses are convolved with N filters and then summed up to a receive signal $r(t)$. The optimization of the FIR filters is based on sample data from the 2 media. Any filter length J can be chosen, where longer filters tend to provide better contrast at the cost of bandwidth. The optimization process yields $N \cdot J$ sets of filters representing a global maximum and local maxima of the contrast between the 2 media. The aim of the present study was to find out what pulse sequences are best suited for contrast agent imaging and how the contrast can be further improved without a loss in bandwidth.

Optimization Criteria

Contrast

The achievable contrast defined as

$$c = \frac{\int_t \left[{}^1r(t)\right]^2 dt}{\int_t \left[{}^2r(t)\right]^2 dt}, \qquad {}^1r, {}^2r: \text{ medium 1, medium 2} \tag{1}$$

was chosen to be the criterion of highest priority with respect to the effectiveness of a pulse sequence.

Effective Bandwidth

The effective bandwidth B is defined as the minimal bandwidth, which may be split in an unlimited number of sub-bands, that covers half of the total energy of a signal. To calculate B, we compute the discrete power spectrum of ${}^1r$ and sort the samples in descending order. B is then derived from the K sorted samples p_i, each representing the power within the bandwidth Δf as

$$\sum_{i=1}^{J} p_i \overset{!}{=} \frac{1}{2}\sum_{i=1}^{K} p_i, \quad B = J \cdot \Delta f. \tag{2}$$

${}^2r$ represents the suppressed signal and is, therefore, not considered with respect to image resolution. It is also important to note that a pulse compression filter may be required to make use of the resolution in B.

Media Separability

Contrast as defined in (1) is well suited for optimization problems. It is, however, not an accurate measure of media separability in ultrasound images, especially if echo amplitudes are not Rayleigh distributed. Nonlinear imaging does not show Rayleigh distributed echo amplitudes so that a different measure was chosen, i. e. the classification error ε. A threshold optimally divides echo amplitudes into two groups (2 media). The ratio of misclassified echo amplitudes to all classified echo amplitudes will be referred to as the classification error ε.

Optimal Pulse Sequences

A setup was built, where single scatterers or ensemble of scatterers were insonified in a water-filled chamber with a broadband transducer. A second transducer received the echoes that were digitized with an oscilloscope. An arbitrary function generator and a power amplifier were used to accurately reproduce a phase- and amplitude-coded pulse sequence. 4 phases (0°, 90°, 180°, 270°) and 2 amplitudes (full amplitude: H, half amplitude: L) were combined. The maximum pressure for full amplitude

was about 0.7 MPa. To investigate time-variant effects, the total sequence consisted of 16 pulses at a prf of 10 kHz, where all pulses occur twice, denoted by 1 and 2:

270°H1	180°H1	90°H1	0°H1	270°L1	180°L1	90°L1	0°L1
270°H2	270°L2	180°H2	180°L2	90°H2	90°L2	0°H2	0°L2

The complete sequence is repeated 4 times with pauses of 18.5 ms in between to further investigate decorrelation. Three types of scatterers and combinations thereof were investigated: linear scatterers, Levovist® microbubbles, and free gas bubbles, produced by breaking the polymer shell of an encapsulated microbubble (experimental agent).

Data Evaluation

All combinations of 2, 3 and 4 out the 16 pulses were analyzed with respect to energy ratio (contrast) c and effective bandwidth. Thus, pure decorrelation sequences (e. g. 270°F1, 270°F2) were also tested. Contrast and effective bandwidth were determined for the following pairs of media:

- Levovist® / linear scatterers
- Free gas bubbles / linear scatterers
- Levovist® / free gas bubbles

For all combinations of pulses and media, optimal receive filters were generated and applied, where 1-tap filters (optimal weighted superposition) and 16-tap filters were considered.

Results

In brief it can be stated that

- contrast increases with sequence length (2, 3, 4),
- 90°, 270° pulses outperform 0°, 180° pulses,
- 2-pulse sequences require full amplitudes for acceptable contrast,
- longer sequences require full amplitudes or amplitude modulation (full/half, less contrast),
- longer filters emphasize higher harmonics and improve contrast by about 10 dB,
- energy ratios of about 31 dB can be achieved with 4-pulse sequences and 16-tap filters.

NONLINEAR FREQUENCY COMPOUNDING

Frequency compounding improves the SNR of B-mode images by averaging images taken from different frequency bands of the receive spectrum, thus showing decorrelated speckle. In the case of nonlinear imaging, partly decorrelated images can even be generated from the same frequency range, because different spectral features (e. g. harmonics) share frequency bands but are separable due to their amplitude and phase response to multiple coded transmit pulses.

The filter optimization discussed in [1] yields $N \cdot J$ complete sets of filters for a sequence with N pulses per sequence and J-tap FIR filters. Each set of filters represent a global or local maximum with respect to the optimization of c. The best maxima may give images of comparable contrast c and effective bandwidth but with partly decorrelated speckle, so that averaging these images improves the SNR and, hence, improves media separability.

Data Acquisition

Data were acquired from a contrast agent phantom using a 3.5 MHz probe (see [1]). A 4-pulse sequence with $\varphi_i = [0°, 120°, 180°, 240°]$ at $\omega_0 = 2.0$ MHz was used. Echoes from the contrast agent Definity® and tissue were taken from a depth range of 6.25 – 7.25 cm covering a lateral span of 1 cm.

Results

Fig. 1 – Fig. 4 show normalized histograms, segmentation images based on optimal thresholding, and gray scale images (55 dB dynamic range) for different processing techniques.

Fig. 1 reveals that contrast agent and tissue cannot be distinguished in B-mode. Optimal receive filtering with a 16-tap filter clearly improves media separability (classification error $\varepsilon = 19\%$, effective bandwidth $B = 0.71$ MHz), Fig. 2. Averaging the best 4 images based on 16-tap filtering greatly improves image separability ($\varepsilon = 8.2\%$, $B = 0.64$ MHz) by reducing speckle, i. e. by narrowing the histograms of the two media, Fig. 3. In comparison, a single 64-tap filter provides a somewhat better separability but substantially reduces the effective bandwidth ($\varepsilon = 6\%$, $B = 0.4$ MHz), Fig. 4.

CONCLUSIONS

The absolute phase is an important parameter in the design of pulse sequences, especially for short and low MI (mechanical index) sequences. Mean image brightness is not useful to quantify image contrast. Nonlinear frequency compounding, for example, significantly improves media separability but leaves the mean brightness unchanged.

ACKNOWLEDGEMENT

The work was carried out by the Ruhr Center of Competence for Medical Engineering (KMR Bochum), BMBF (Federal Ministry of Education and Research, Germany) grants 13N8017 and 13N8079.

REFERENCES

[1] W. Wilkening, B. Brendel, H. Jiang, J. Lazenby, H. Ermert, "Optimized receive filters and phase-coded pulse sequences for contrast agent and non-linear imaging," Proceedings of the 2001 IEEE Ultrasonics Symposium, 1F-4.

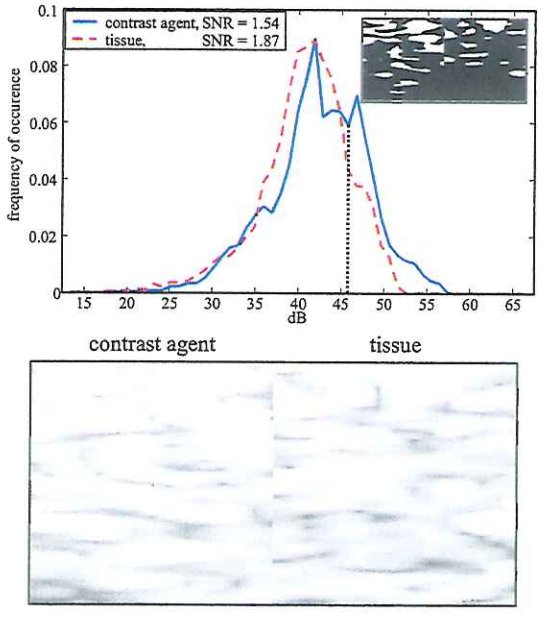

Fig. 1: B-mode processing.

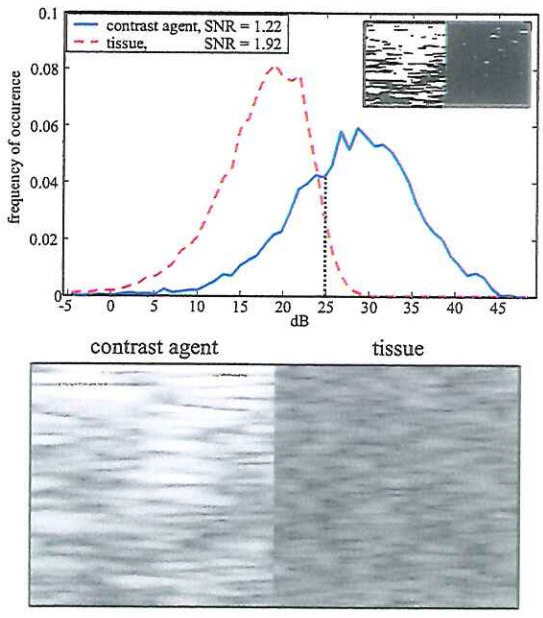

Fig. 2: 16-tap optimal filtering.

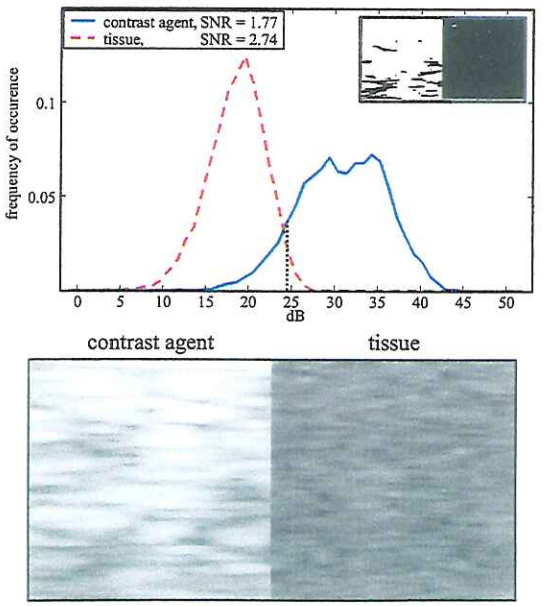

Fig. 3: Nonlinear frequency compounding, 16 taps.

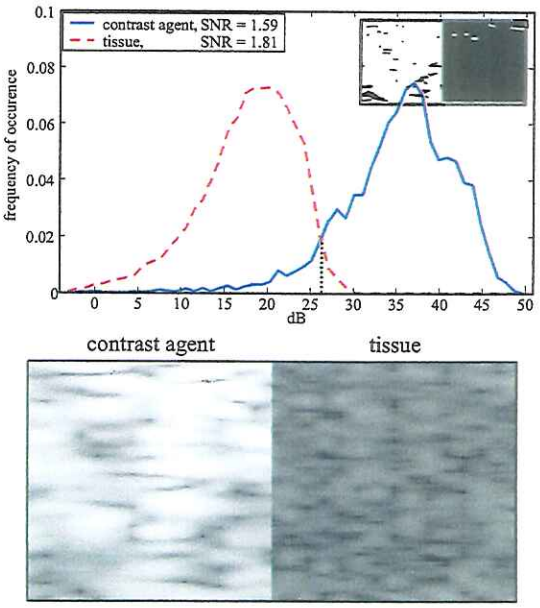

Fig. 4: 64-tap optimal filtering.

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 Dayton[1], Takeuchi[2], de Jong[3], Kodama[4], and Postema[5], 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"[6] 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[7]. 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).

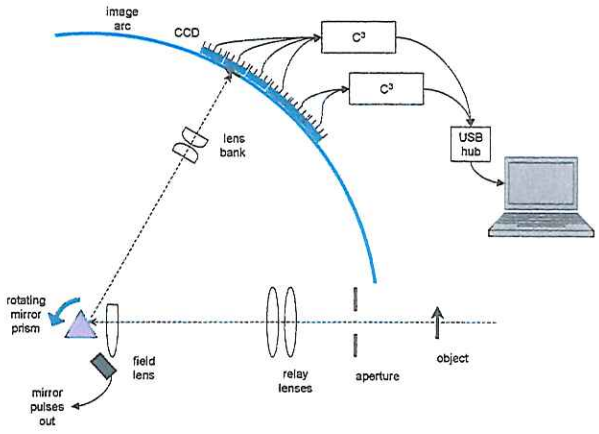

Figure 1. General camera setup.

The recorded image is transferred through flexprint cables to a CCD Controller Card (C^3). Four CCD's are controlled by each C^3 and 32 C^3'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 C^3 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 C^3 (see figure 2). It contains a Field Programmable Gate Array (FPGA) that was programmed to perform a number of time-critical tasks. The C^3 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 C^3's.

The C^3 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 C^3 has been created for the Matlab programming language. A graphical user interface and analysis toolbox is currently in development.

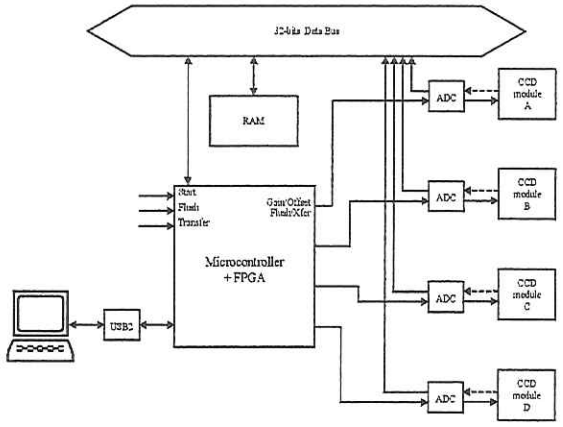

Figure 2. Schematics of CCD Controller Card (C^3)

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.

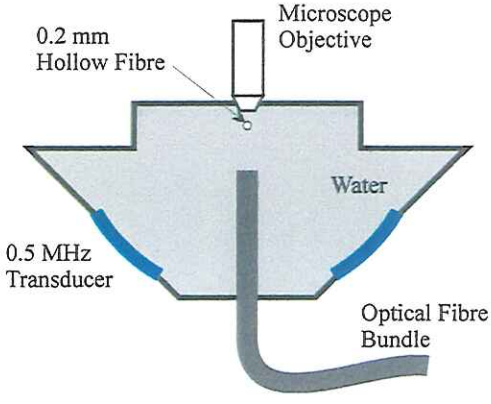

Figure 3. Experimental setup for bubble ultrasound interaction studies

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.

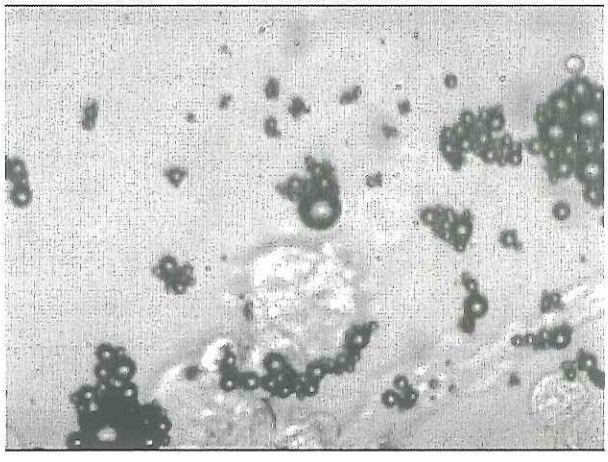

Figure 4. A typical frame from the Brandaris 128, the magnification was 120×, the FOV was 89 μm × 68 μm.

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 Mpfs, and demonstrated a group of bubbles undergoing repeated coalescence, finally becoming a single large bubble. Figure 5 shows a 100×100×36 portion.

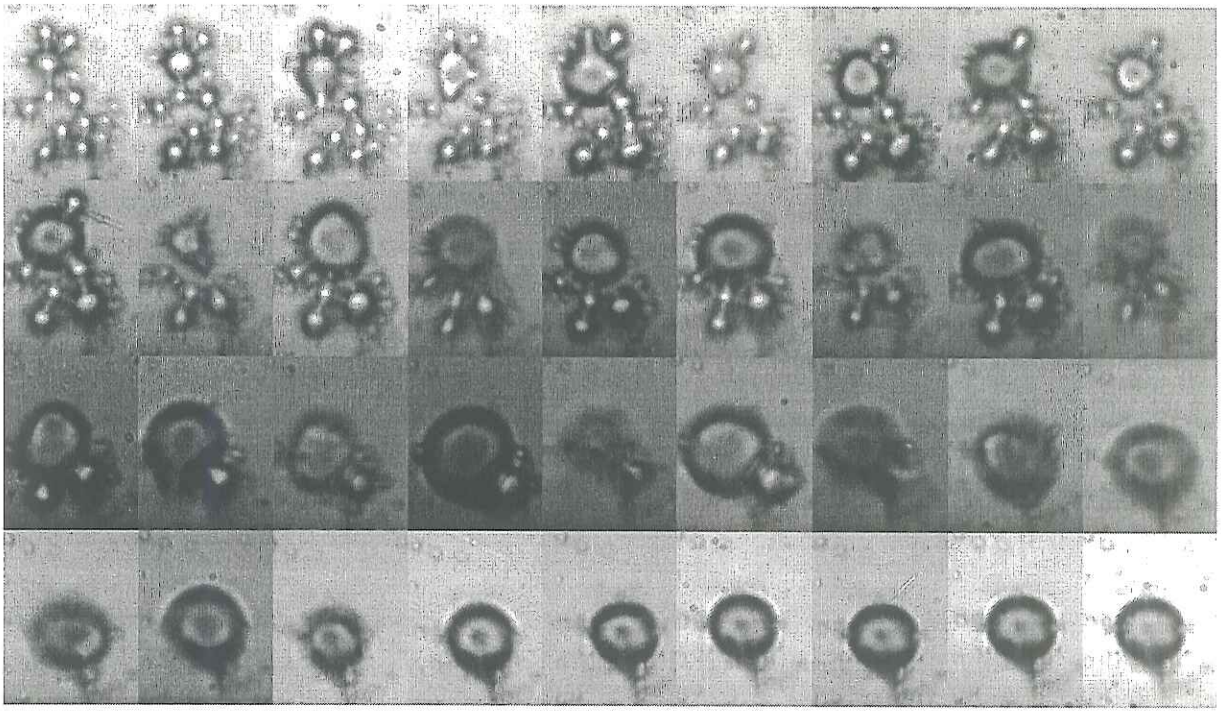

Figure 5. A portion of a recording (100 pixels × 100 pixels × 36 frames) showing multiple coalescence of a bubbles aggregate in a 0.5 MHz sound field. The frame rate was 1.05 Mfps.

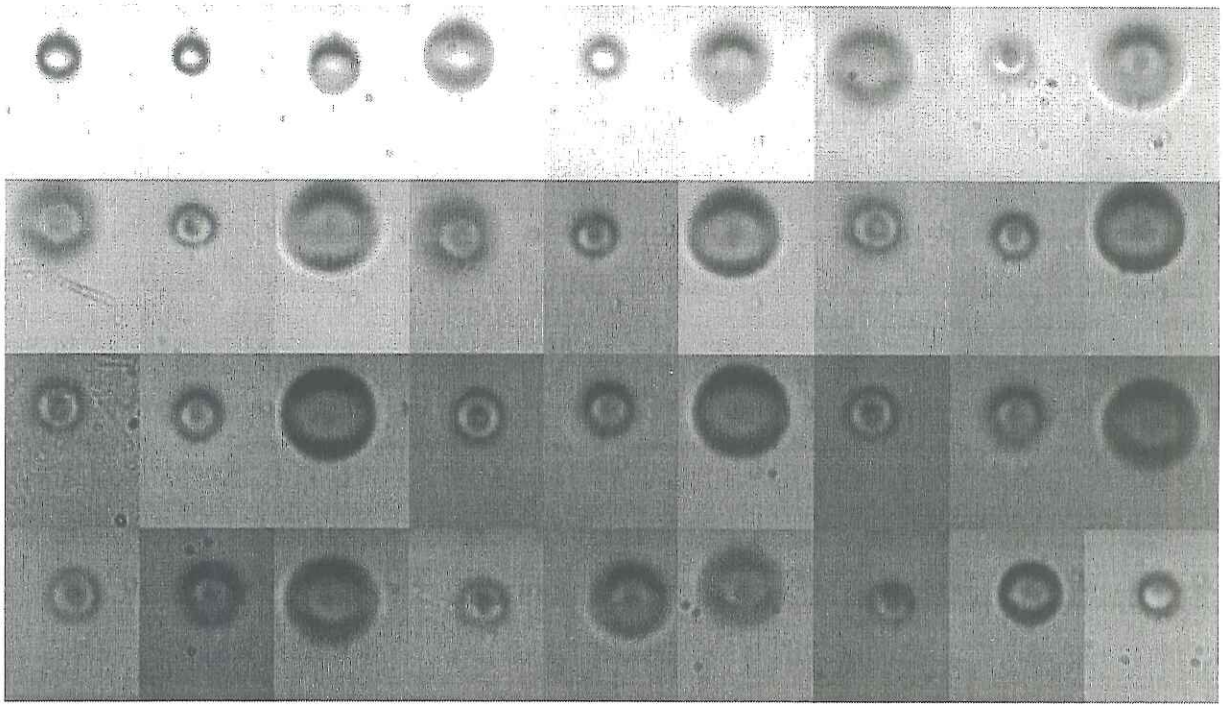

Figure 6. A portion of a recording (80 pixels × 80 pixels × 36 frames) showing a probable micro-jet formation in a bubble in a 0.5 MHz sound field. The frame rate was 1.5 Mfps.

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 80×80×36 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 Mpfs. 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

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References:

1. Dayton P, Morgan M, Klibanov A, *et al.* IEEE Trans Ultrason Ferr Freq Contr, 1999; 46(1): 220-32.
2. Takeuchi Y. IEEE Ultrason Symp, 1998: 1645-9.
3. De Jong N, Frinking PJ, Bouakaz A, *et al.* Optical imaging of contrast agent microbubbles in an ultrasound field with a 100-MHz camera. Ultrasound Med Biol. 2000; 26(3): 487-92.
4. Kodama T, Tomita Y, Appl. Phys. B 2000, 70: 139-149.
5. Postema M, Bouakaz A, Chin CT, and de Jong N. IEEE Trans Ultrason Ferrorelect Freq Contr, in press.
6. Named after a lighthouse on the island of Terschelling, The Netherlands.
7. Igel EA, Kristiansen M. Rotating-Mirror Streak and Framing Cameras: SPIE Press.

NONINVASIVE VOLUME MEASUREMENTS IN FLUID FILLED CAVITY

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The purpose of this study is to suggest a new approach to measure noninvasively the volume of a body organ using ultrasound waves. Accurate measurement of organ volume is important to assess disease state, response to therapy and growth patterns. For these reasons, we selected the urinary bladder as a target organ. Indeed, determination of bladder volume is important clinically for patients suffering from bladder dysfunctions. For example, for patients where sensations that normally alert individuals to void are either reduced or totally absent, or in cases of retention of urine in the bladder, which puts the patient at risk of infection. Accordingly in some situations, an accurate determination of the volume is indispensable, sometimes however just an indication of the volume is sufficient. For example after surgery, it is important to know if the bladder filling is above a certain level so that voiding is necessary. Presently such measurements are carried out with for instance the Bladderscan™. These factors provide a high motivation for a noninvasive and a fast measurement alternative to avoid unnecessary catheterization. The purpose of our study is, in a first approach, to develop a simple method that is able to provide an indication whether the bladder volume has exceeded a certain threshold volume. Second, the possibility of accurate volume assessment will be studied. The approach takes advantage of the difference in harmonic properties of liquids (urine) and tissues. We know from literature that ultrasound waves undergo different degrees of harmonic distortion depending on the propagation medium. These nonlinear effects occur most strongly when ultrasound propagates through liquids with relatively low acoustic attenuation such as water, amniotic fluid or urine. Indeed, acoustic propagation in liquids gives rise to extreme nonlinear effects at diagnostic settings. However within soft tissues, nonlinear processes are modified as a result of different acoustic characteristics, most notably their high acoustic absorption. The tendency for wave distortion to occur in tissue is hence limited. At diagnostic settings, wave distortion can be up to thirty folds higher in liquids than that of tissue.

To take advantage of these harmonic differences, a wide acoustic beam is needed in order to encompass and surround the full bladder volume and to limit aiming problems. Echo signals returning from a large distance beyond the posterior wall of an average filled bladder are then analyzed for harmonic contents.

Phantom measurements were carried out with a single element transducer transmitting a wide acoustic beam. The wide beam was created by the defocused surface of the transducer. The transducer had an aperture of 30 mm and a center frequency of 3 MHz. The bandwidth was estimated to 85 %, allowing thus 2nd harmonic tests. Phantoms containing different volumes were constructed. The transmitted acoustic signal contained 7 cycles with an acoustic pressure high enough to induce wave distortion.

The measurements showed that the harmonic generation at a fixed depth increases when the liquid volume crossed by the ultrasound beam is larger. Further hydrophone measurements demonstrated that higher harmonics up to the 4^{th} harmonic were generated for the largest volumes.

These preliminary results demonstrate the feasibility of the approach. However improvements (transmitted power, transducer size, transmit frequency, frequency bandwidth and sensitivity) are still needed to refine the accuracy of the volume measurements.

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FUNCTIONAL "TRANSIT TIME" TESTS IN LIVER ULTRASOUND: READY FOR WIDER APPLICATION?

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Diffuse liver is a major worldwide problem: viral hepatitis alone afflicts about 8% of the world's population. A non-invasive method of characterising diffuse liver disease would be a major clinical advance as biopsy is often necessary, with its attendant hazards and costs. Ultrasound, which is widely available throughout the world (1 in 4 imaging examinations are US) is often the first line investigation but it, in common with other imaging methods, is unsatisfactory as an accurate means of grading disease severity, An ultrasound based method, relying on the measurement of vascular transit times has been developed. This relies on administering a bolus of a microbubble into an antecubital fossa vein and observing the arrival time in a hepatic vein. The resultant time-intensity profile will reflect transit times sequentially through the heart and lung and the splanchnic vascular bed. This "hepatic vein transit time: HVTT", which can be measured by computer-aided analysis of the audio output, in a hepatic vein is significantly shorter in cirrhosis. The main reasons for this appear to be the arterialization of the liver blood supply and the presence of both intrahepatic and intrapulmonary shunts. In a pilot study [Albrecht T, Blomley MJK, Cosgrove DO, Taylor-Robinson S et al. Non-invasive diagnosis of hepatic cirrhosis by transit time analysis of an ultrasound contrast agent. Lancet 1999;353(9164):1579-83] all subjects with cirrhosis showed early arrival times (HVTT <24s) while all controls showed late enhancement (HVTT >30s). The method also shows great promise in detecting early "occult" micrometastatic disease; also this will not be the main theme of this presentation.

This presentation will provide an overview of our follow up studies to date, in over 150 additional subjects with diffuse liver disease or controls. The main aims have been:

- to assess the method in a larger series of patients with a variety of biopsy characterized disease and controls
- to evaluate the methods in a single disease entity (Hepatitis C)
- to investigate reproducibility
- to see if allowing for the effect of cardiopulmonary transit differences (for example by looking at the delay between carotid or hepatic artery and HVTT) improve the method still further
- to see if another contrast agent, notably SonoVue, gives similar results
- to see if simpler, subjective, analyses are as reliable.

Key findings include the observation that:

- Early enhancement (under 21-24s) is consistently seen in cirrhosis and this is a sensitive test
- Some patients with fibrosis also show early enhancement: this may reflect undersampling by biopsy or the underlying physiological changes.
- Both in a mixed population and in HCV, the method can grade diffuse liver disease and shows great promise as a longitudinal method of following patients.
- The method appears reproducible provided care is taken and pitfalls such as hepatic venous reflux and technically poor studies eliminated from analysis
- Allowing for cardiopulmonary differences does not appear to be necessary in this class of patient
- Using a more potent agent, such as SonoVue, enhancement is more reliable and subjective analysis facilitated.
- However, in a series of normal volunteers, SonoVue showed consistently earlier enhancement in the hepatic veins compared to Levovist (although cardiopulmonary transit was identical). We believe this reflects the marked liver-specific properties Levovist has. This therefore means that data obtained for Levovist may not be directly carried over to agents such as SonoVue. The implications for this will be discussed.

DETECTION OF LIVER LESIONS

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Conventional ultrasonography (US) is relatively insensitive in detecting liver metastases and HCC. In studies with intraoperative correlation, US has been shown to have a sensitivity of only 50 – 76% (1-3). This is mainly due to the often relatively poor contrast between the signal in the lesion and the surrounding liver. Furthermore, reliable characterisation of a single lesion on US as a metastasis is usually not possible. Conventional US is therefore inferior to CT and MRI for imaging liver metastases.

This situation has changed with the advent of contrast agents, which lead to a marked increase in liver-to-lesion contrast and thus dramatically improve detection of metastases (and HCC).

High MI techniques with Levovist have been shown to be very effective in improving detection of metastases when the liver-specific late phase is exploited. However, high MI-techniques are limited by the extreme transience of the contrast effect and thus require special examination techniques such as "sweeping" or intermittent imaging, thus loosing the real time nature of US.

Newer contrast agents such as Sonovue, BR14 (both Bracco SPA, Milan, Italy) or Sonazoid (Amersham Medical, Oslo) have a strong harmonic response even at low acoustic pressure, thus giving strong signal enhancement even at low MI without substantial bubble destruction. Low MI imaging with these agents is therefore ideally suited for continuous real time imaging of the liver during the arterial, portal venous and delayed sinusoidal/liver phase. During the liver phases of these agents, continuous real time imaging of the liver can be performed in multiple planes looking for metastases without requiring unusual examination techniques and almost free of time restrictions.

Assessment of the pattern of dynamic enhancement of a lesion during the three phases is extremely valuable for characterisation of metastases. "Hypovascular" metastases (e.g. clorectal) show a variable degree of mainly peripheral ring-like enhancement during the arterial phase. "Hypervascular" metastases (e.g. melanoma) show marked arterial enhancement of the entire lesion. Both types of lesions show no or little enhancement during the portal venous and delayed sinusoidal phase. As with Levovist, this latter phenomenon leads to a marked increase in liver-to-lesion contrast and is most useful for improving lesion detection.

Detection of HCC is also improved with contrast agents. Two approaches can be used: a survey of the liver during the arterial phase to look for hypervascular lesions and/or scanning of the liver during the liver phase looking for enhancement defects in the otherwise enhancing liver, although not all HCC show a lack of enhancement on late phase imaging.

References:

1 Wernecke K, et al. AJR Am J Roentgenol 1991;157:731-739.

2 Clarke MP, et al. Surgery 1989;106:849-855.

3 Ohlsson B, et al. Eur J Surg 1993;159:275-281.

LIVER LESIONS CHARACTERIZATION: INCIDENTAL FINDINGS

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Incidental lesions are one of the most common diagnostic issues in liver imaging. They are usually discovered during an US examination of the abdomen in patients with neither history of malignancy nor chronic hepatic disease. While most of these findings are benign lesions - such as cysts, hemangiomas, or focal nodular hyperplasias - incidental detection of unsuspected hepatic malignancies may occur. US may correctly characterize cysts and most hemangiomas with typical hyperechoic pattern. However, in all the other cases, US findings are non-specific, as there is enough variability and overlap in the US appearances of benign and malignant liver lesions to make a definite characterization unreliable. Spiral CT or MR imaging are commonly used to clarify questionable US findings and to provide a more confident diagnosis. The introduction of US contrast agents and the development of contrast-specific US techniques that produce images based on non-linear acoustic effects of US interaction with micro bubbles have opened new prospects for US imaging of the liver. Real-time analysis of lesions contrast enhancement patterns has become feasible owing to second-generation agents and low mechanical index (MI) scanning. We therefore undertook a prospective clinical trial aimed at investigating the usefulness of contrast US in characterizing focal liver lesions of incidental detection. A series 37 focal lesions incidentally detected - but not characterized - by baseline US in 31 patients were examined with contrast US by using continuous, low MI (0.01-0.04), real-time scanning (Contrast Tuned Imaging, Esaote Biomedica) after bolus injection of 2.4 ml of SonoVue (Bracco). All patients also underwent spiral CT (n=6), MR imaging (n=14), or both (n=11). Videotapes of contrast US studies were reviewed by three blinded readers. Final diagnosis was based on combined results from clinical, laboratory, and imaging findings. Four patients without a definite diagnosis were submitted to US-guided biopsy for histologic assessment. Final diagnoses included hemangioma (n=14), focal nodular hyperplasia (n=18), hepatocellular adenoma (n=2), metastasis from unknown primary cancer (n=2), and angiosarcoma (n=1). Twelve hemangiomas showed peripheral globular enhancement in the arterial phase followed by progressive fill-in during the portal venous and delayed phases; two small (less than 1.5 cm) hemangiomas showed homogeneous enhancement in the arterial phase and were slightly hyperechoic in the portal and delayed phases. All of the focal nodular hyperplasias and the two hepatocellular adenomas showed rapid and homogeneous enhancement in the arterial phase and appeared isoechoic in the portal and delayed phases. The two metastases did not show any intratumoral enhancement in the arterial phase and were hypoechoic in the portal and delayed phases. The angiosarcoma showed inhomogeneous arterial-phase uptake and was hypoechoic in the portal and delayed phases. The three blinded readers differentiated benign from malignant

tumors in 37 of 37 lesions (100%). Correct lesion characterization was achieved 30 of 37 lesions (81%). Although further investigation is needed to confirm these encouraging preliminary results and to prove the cost-effectiveness of a diagnostic work-up based on the contrast US study, it can be foreseen that, with continuous improvement in US technology and the optimal use of contrast agents, contrast US will soon challenge CT and MRI for incidental liver lesions characterization.

CONTRAST ENHANCED ULTRASOUND IN ABDOMINAL BLUNT TRAUMA

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Objective: To perform a preliminary evaluation of microbubble enhanced ultrasound of abdominal organs in blunt and penetrating trauma.

Methods: 14 patients who suffered abdominal trauma were scanned after iv SonoVue, a phospholipid coated microbubble at a dose of 1.2-2.4 mL, using low MI, bubble-specific modes using Esaote and Sequoia systems. The liver, spleen and both kidneys were studied over the 3-5 minutes duration of parenchymal enhancement. If necessary, the contrast injection was repeated once. In 5 CT was available for comparison and 2 patients went to laparotomy.

Results: On the unenhanced scan no parenchymal lesions were confidently visualised. Excellent enhancement of the parenchyma was obtained in all cases with the kidneys showing intense increase in echogenicity in the arterial phase (form 15-30 sec post injection), fading after about 60 sec, and the liver and spleen enhancing later, staring at about 30-40 seconds post injection and persisting for 3-5 minutes.

Lesions were clearly depicted in the liver in 5, spleen in 5 and kidneys in 4 patients as defects in the enhanced parenchyma. The position and size of the lesions corresponded well with the CT and surgical findings in 7 patients.

Conclusion: In this limited series, SonoVue improved detection of liver, spleen and kidney trauma by revealing the damaged tissue as non-enhancing regions. If confirmed in larger series, this simple method could be deployed within A&E departments and expedite management of trauma patients.

CONTRAST HARMONIC IMAGING AT LOW MECHANICAL INDEX ALLOWS FOR BIOPSY OF HEPATIC LESIONS INVISIBLE OR DIFFICULT TO DETECT ON FUNDAMENTAL B-MODE ULTRASOUND

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Purpose: Biopsy of hepatic lesions for diagnostic analysis and therapeutic decisions is a widely accepted procedure in interventional ultrasound (US). Recently, contrast-enhanced US at low mechanical index (low MI) has been introduced for the detection and characterization of hepatic lesions. With CHI at low MI it is possible to detect and evaluate lesions which are invisible on conventional B-mode US. Here we addressed the question, whether CHI allows for biopsy of otherwise undetectable hepatic lesions.

Materials and Methods: We evaluated 6 patients with hepatic tumors or abscesses, which were difficult to analyze and puncture under fundamental B-mode guidance. All examinations were performed with the Siemens Sonoline Elegra®. The second generation ultrasound contrast agent BR1 (Bracco, Italy) was used for detetction and characterization of the hepatic lesions under contrast harmonic imaging (CHI) conditions with phase inversion at low MI. Puncture was performed with standard true cut and Chiba needles.

Results: Patients with metastases from the following cancers underwent hepatic biopsy: 2 colonic cancers, 1 pancreatic cancer, 1 cholangiocellular cancer and 1 patient with hepatic infiltrates of malignant histiocytosis. The sixth patient suffered from occult hepatic abscesses. Biopsies had to be performed in two steps. The first injection of 2.5 ml of the 5 ml BR1 vial served for the detection of the lesion, for the infiltration of the skin with a local analgesic and for the optimized settings needed for the following biopsy. After the second 2.5 ml BR1 the most intensive tissue to lesion contrast ratio was awaited, followed by the biopsy. In all but one patient biopsy under CHI conditions was successful, as proven by histology. The patient with malignant histiocytosis was extremely incompliant and therefore we interrupted the attempt to obtain material. The needles were visible in all cases.

Conclusions: Our investigations show that biopsy of liver lesions under real-time continuous CHI at low-MI is feasible. It is the first imaging technique, in which biopsies can be routinely performed under contrast-enhanced conditions.

ADJUNCTIVE TUMOUR TREATMENT

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Purpose: Radionuclides have shown to be effective in tumour therapy. However, the side effects determine the maximum deliverable dose. Recently, it has been demonstrated that cells can be permeabilised through sonoporation using ultrasound and contrast microbubbles. The use of sonoporation in treatment of tumours may increase the anti-tumour efficacy of radionuclide treatment. The mechanisms as well as the effects sonoporation in tumour treatment strategies are still not understood. The purpose of this study is to determine the effects of ultrasound and contrast microbubbles on the internalisation of the radionuclide ^{111}In-DOTA-Tyr^3-octreotate in tumour cells. For that purpose we investigated the uptake by tumour cells at different ultrasound setting.

Method: 1] To find the optimal ultrasound settings for ultrasound adjunctive tumour therapy we incubated Rat-pancreatic CA20948 tumour cells with two dyes (MW 40 and 70 KDa) and the uptake levels were compared with cells treated with ultrasound and contrast microbubbles. Ultrasound settings were varied in terms of duty cycle, and total treatment time. Lipid-shelled microbubbles at different concentrations were used. Acoustic frequency (1 MHz), acoustic pressure (MI 0.42), contrast agent (lipid-shelled) and temperature (37°C) were constant. 2] The estimated optimal settings were then used whereby Rat-pancreatic CA20948 tumour cells were incubated with ^{111}In-DOTA-Tyr^3-octreotate.

Results: 1] The highest molecular uptake was found with addition of contrast microbubbles (ratio of 10 bubbles to 1 cell) and with the ultrasound setting: duty cycle 0.0013, MI 0.42, and treatment times of 30 and 60 minutes. 2] These settings were used to enhance the internalisation of ^{111}In-DOTA-Tyr^3-octreotate. For 30 minutes incubation, the internalisation of ^{111}In-DOTA-Tyr^3-octreotate increased by 65% when adjunctive treatment with ultrasound and contrast microbubbles was used compared to control (only ^{111}In-DOTA-Tyr^3-octreotate) (figure 1).

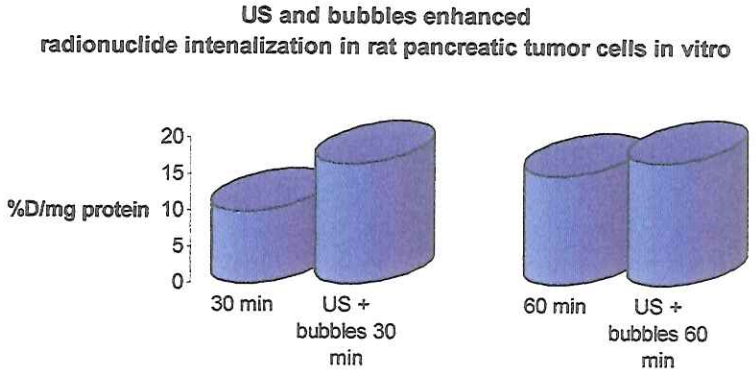

Figure 1: US and bubbles enhanced radionuclide internalisation in rat pancreatic tumour cells in vitro.

Conclusions: These results demonstrate the feasibility of adjunctive tumour treatment with the radionuclide ^{111}In-DOTA-Tyr^3-octreotate and ultrasound contrast micro bubbles. When using adjunctive ultrasound contrast micro bubble treatment, a lower radionuclide doses are required to reach the same anti-tumour effect.

We gratefully acknowledge the financial support from the Dutch Technology Foundation STW (Grant RKG 5104) and the Dutch cardiology institute ICIN.

PARAMETERS INFLUENCING ULTRASOUND-MEDIATED GENE DELIVERY : PHYSICAL CONSIDERATIONS

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Introduction: Ultrasound has been used for several decades to perform diagnostic imaging in medicine. Recently, the introduction of ultrasound contrast agents (UCA) has given new tools to ultrasonography (e.g. perfusion imaging and blood flow quantification). These contrast agents are gas-filled micro bubbles, typically 2-8 μm in diameter, stabilized by an outer shell composed of lipids, denaturated albumin or polymer. When exposed to ultrasound, the stabilized gas bubbles give strong echoes. Although UCA micro bubbles were designed for diagnostic improvement of ultrasound imaging, recent studies have shown that they present interesting features for gene delivery as well. When exposed to ultrasound, gas micro bubbles may promote micro streaming in the vicinity of an interface and can collapse violently. In the presence of cells, this may form discrete pores in the plasma membrane, a process called sonoporation, and can enhance cellular uptake of genes, for example. The success of this novel technology is largely dependant on a better understanding of the physical parameters required for an efficient sonoporation/gene delivery.

Purpose: We have studied which are the parameters affecting gene delivery mediated by ultrasound and contrast agents (Sonovue™, Optison™, BR14...). The influence of acoustical parameters such as duty cycle, pulse repetition time, ultrasound transmit frequency and pressure were investigated to optimize gene transfer.

Methods: In vitro transfection of MAT B III cells with a plasmid encoding for the green fluorescent protein (GFP) was performed with a rotating tube system. Analysis of GFP expression was carried out by flow cytometry. Prior to measurement, propidium iodide (PI) was added to the tube to determine cell viability. GFP was excited with a 488 nm line of an Argon laser, and emitted light collected at 520 nm (green fluorescence) and 575 nm (PI, red fluorescence) to enable correction for autofluorescence by diagonal gating.

Results: Transmit frequencies ranging from 1 to 3 MHz were the best suited for gene delivery. We found that duty cycle can, in some conditions, dramatically affect the level of GFP expression, *ie* for low duty cycle transfection efficiency can drop to 20% or less. High levels of expression were achieved for acoustic pressures ranging from 300 to 600 kPa.

Conclusion: Efficient gene delivery *in vitro* (30% of GFP-positive cells) can be achieved with relatively mild conditions (0.4 MPa, 4% duty cycle, 50 ms pulse repetition time). In the future, we will assess the potential of ultrasound for mediating gene delivery *in vivo*.

VASCULAR GENE TRANSFER USING ULTRASOUND-ENHANCED DESTRUCTION OF PLASMID-LOADED ALBUMIN MICROBUBBLES

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Gene therapy is a promising tool for the treatment of several diseases, but current clinical application is hampered by the development of safe and efficient systems for local gene delivery to a specific tissue or organ. Non-viral gene delivery can be performed by the direct injection of DNA, but such approaches are generally associated with a low transfection efficiency and transient expression of the gene product. Viral vectors significantly increase the efficacy of transfection, because of the specific viral machinery, which has specifically evolved to introduce foreign DNA into mammalian cells, but viral proteins elicit an immune response within the targeted host/tissue.

Recently, ultrasound-induced microbubble destruction has been proposed as a new technique for local delivery of drugs and genes to specific target tissues, including the heart. Ultrasound can cause transient non-lethal perforation of the capillary and cell membrane by cavitation effects and thereby improve transfection. Ultrasound has also been shown to up regulate the activity of several cell repair genes which also facilitate transfection. Most of the ultrasound-enhanced transfection techniques use microbubbles to encase an expression vector until the site of transfection is reached, thereafter an ultrasound probe is used to burst the bubbles, thus distributing material in a specific area of interest. The microbubble approach has been previously used to deliver colloidal particles to tissues following microvessel rupture. The ultrasound-induced destruction of albumin-coated microbubbles containing an adenoviral transgene has been shown to significantly increase myocardial gene expression in rats, as well as to enhance cationic lipid-mediated gene transfer into primary tumors. However, it was unclear whether or not ultrasound can be used to facilitate transfection with pure plasmid DNA. We therefore investigated whether or not ultrasound-induced destruction of plasmid-loaded micropheres results in efficient transfer of a gene to the vascular wall of coronary arteries without impairing the functional activity of the endothelial cell layer.

Gas-filled microbubbles (3.0±1.2 μm) were created by sonication of 2 mL of 5% human albumin in the presence of 20 μg plasmid DNA encoding for LacZ , eNOS S1177D or an empty vector. Porcine coronary arteries (PCA) were perfused with pcDNA-loaded microbubbles and exposed to diagnostic ultrasound (System V, GE Vingmed, 2,2 MHz, MI 1.2, 172.9 frames/s, 5 s). After a further incubation period of 18 to 20 hours, intense blue staining, indicating expression of LacZ protein, was detected in the region of the vessel segment targeted by ultrasound. Segments perfused with microbubbles carrying an empty vector but otherwise treated identically showed no blue staining. β-galactosidase

was expressed in endothelial cells and histochemical analysis revealed that more than 90% of the endothelial cells were positively stained whereas only sporadic staining of subintimal smooth muscles cells was detected. The function of the β-galactosidase introduced into the arteries was quantified by measuring the enzymatic activity. The combination of LacZ-loaded microbubbles and ultrasound treatment markedly increased the β-galactosidase activity measurable in PCA homogenates compared to arteries perfused with LacZ-plasmid but in the absence of microbubbles and arteries perfused with the LacZ-loaded microbubbles but not exposed to ultrasound respectively. The expression of β-galactosidase was flow- and concentration-dependent and greatest at the highest dose tested.

The LacZ-transfection had no effect on the bradykinin-induced endothelium-dependent relaxation of rings cut from the transfected vessels. Relaxation of prostaglandin (U46619) pre-contracted PCA rings was similar in untreated control vessels and vessels exposed to microbubbles and ultrasound. Ultrasound exposure did not induce any histologically detectable alteration of the vessel wall. Furthermore, trypan blue staining of the endothelium and measurement of LDH activity in the perfusate excluded a cytotoxic effect of the treatment.

To determine whether or not the ultrasound-enhanced destruction of microbubbles carrying a gene construct leads to alterations in coronary artery function, PCA were transfected with the endothelial nitric oxide synthase (eNOS) S1177D-construct. The eNOS-construct simulates activation of the eNOS by phosphorylation of the amino acid Ser 1177 resulting in an enhancement of the basal activity of the enzyme without the need for an increase in the intracellular concentration of calcium.

Western blot analysis of the vessel segments targeted by the ultrasound revealed a pronounced increase in the expression of eNOS protein. To assess whether or not the introduction of S1177D into endothelial cells was associated with an increase in NO production, we assessed the contractile response to the application of the specific NOS-inhibitor L-NA in vessels contracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Compared to the control group, $PGF_{2\alpha}$-induced contraction was significantly impaired in PCA rings prepared from segments transfected with eNOS S1177D. In addition, there was an approximately two-fold increase in the L-NA-induced contraction in eNOS S1177D transfected vessel segments compared to vector transfected vessels. These results indicate that the ultrasound-enhanced delivery of the eNOS S1177D construct to PCA significantly enhanced vascular NO production.

Ultrasound-mediated destruction of plasmid DNA-loaded albumin microbubbles is a feasible and efficient method for vascular gene transfection. Indeed, the application of ultrasound to vessels perfused with albumin microbubbles carrying plasmids encoding either β-galactosidase or eNOS resulted in level of endothelial cell transfection significantly greater than that observed in vessels exposed to the DNA-loaded microbubbles in the absence of ultrasound. Moreover, the transfection of

endothelial cells with the eNOS S1177D construct was associated with enhanced NO-mediated responses, indicating an increased NO synthesis within transfected vessel segments.

Taken together, ultrasound-induced destruction of albumin microbubbles coated with a naked plasmid-DNA is an efficient technique for local gene delivery to the vascular wall and does not impair the functional integrity of the endothelium.

INCREASED SUPPRESSION OF INTRACORONARY C-MYC PROTEIN SYNTHESIS WITHIN THE STENT OR BALLOON INJURY SITE USING AN INTRAVENOUS MICROBUBBLE DELIVERY SYSTEM CONTAINING ANTISENSE TO C-MYC: COMPARISON WITH DIRECT INTRACORONARY INJECTION

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Background: Although perfluorocarbon containing albumin microbubbles (PESDA) can bind large quantities of antisense (AS) to the c-myc protooncogene (anti-c-myc) which promotes intimal hyperplasia, it is unknown how much c-myc synthesis within the intracoronary (IC) stent or balloon injury is actually suppressed by this intravenous (IV) targeting technique in the early period following vascular injury. To examine this, we performed high phase liquid chromatography of AS to c-myc uptake and Western Blot studies of c-myc protein synthesis in coronary arteries from eight pigs 90 minutes following IC stent and balloon injury (two vessels per pig). Pigs were treated with either direct IC anti-c-myc (4 milligrams), or the same dose of anti-c-myc IV bound or unbound to PESDA. IV PESDA containing anti-c-myc was given in the presence or absence of transthoracic 1 megahertz ultrasound (TTU) (pulsed wave at 0.6 W/cm^2).

Results: C-myc protein synthesis in the injured coronary arteries (normalized for control vessels) was significantly lower when pigs were given IV anti-c-myc bound to PESDA irrespective of whether TTU was concomitantly delivered (TABLE). Suppression of c-myc synthesis was comparable to direct IC injection.

Conclusion: These data confirm that simply binding anti-c-myc to IV PESDA is a non-invasive method of targeting therapeutic genes to selective sites of IC balloon or stent injury and suppressing the formation of the c-myc protooncogene which mediates intimal hyperplasia and restenosis.

*p<0.05 compared to other groups (ANOVA)

	Direct IC AS	IV AS/PESDA	IV AS/PESDA+ TTU	IV AS + TTU
c-myc proteinratio	0.94±0.26	0.88±0.11	0.89±0.28	2.11±0.28*
anti-c-mycUptake (nanograms)	13±16	24±3	31±6	29±38

ENHANCED MEMBRANE RUPTURE WITH BUBBLE-PARTICLE DOUBLETS

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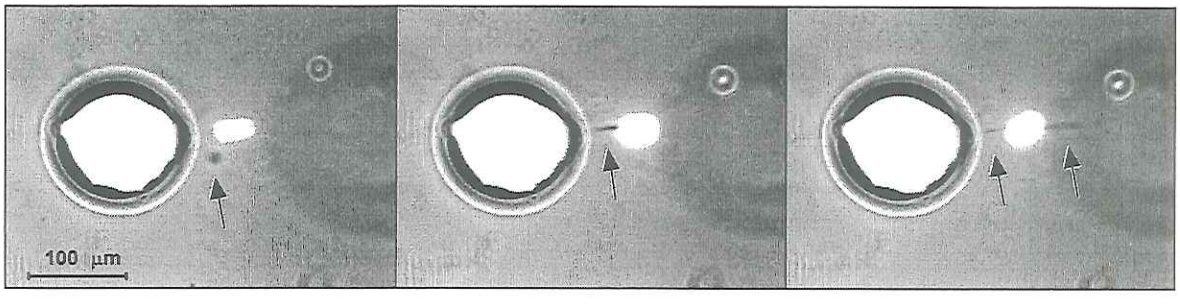
Recent therapeutic applications of ultrasound include sonoporation, the permeabilisation of the cell membrane for drug molecules or DNA. This process is tremendously enhanced in the presence of micro bubbles, such as ultrasound contrast agents. Many physical mechanisms may contribute to the poration or rupture of the cell wall.

In our experiments, we focus on the dynamics of a single bubble near a boundary, allowing for quantitative investigation and modeling of the forces exerted by the bubble on soft objects such as cells or lipid vesicles. We have shown earlier that the bubble oscillation generates steady a streaming flow which is well controlled and quantitatively understood. If it is intense enough the flow will lead to strong deformation and rupture of the lipid membrane.

The effect of micro streaming can be further enhanced by the introduction of a solid micro particle. The flow field and the stresses generated by the *doublet* consisting of a micro bubble and a particle are significantly stronger than those due to the bubble alone. In this fashion, particle assisted vesicle rupture is achieved.

Moreover, the flow field symmetry is broken by the introduction of the particle, giving rise to directed transport from the bubble towards the particle and beyond. Arrays of bubble-particle doublets can thus be used to transport and guide micrometer sized objects without using channels. During transport, controlled shear forces can also be applied.



Rupture of a lipid vesicle (arrows) in the combined flow field of the bubble (large bright object on the left) and a quartz particle (small bright object on the right).

HIGH FREQUENCY NONLINEAR MICROBUBBLE IMAGING

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The last decade has seen the rapid development of micro bubble contrast imaging techniques for medical ultrasound operating in the 1-10 MHz range. Nonlinear scattering from resonant bubble populations has been exploited to implement novel detection methods, which have led to improved sensitivity to flow in small vessels and suppression of tissue signals. A variety of contrast agents have been developed using different gases and shells, but they are typically designed to have a population of bubbles with diameters in the 3-10 micron range in order to exhibit resonant behavior at conventional medical ultrasound frequencies. With the recent development of flow imaging techniques in the 20-60 MHz range, it is interesting to consider the possibility of extending micro bubble contrast techniques to higher operating frequencies. Theoretical considerations suggest that at these frequencies the resonant diameters of bubbles will be on the order of 1-2 microns and below, depending on shell properties. We previously demonstrated that it is possible to produce substantial amounts of nonlinear scattering at high transmit frequencies (14-32 MHz) using Definity™, an agent known to have a large sub-population of small bubbles (<1-2 microns). Clear evidence of subharmonic and ultraharmonic scattering was shown, while the observation of second harmonic nonlinear scattering was complicated by the presence of second harmonic signal arising from nonlinear propagation.

In this study, nonlinear micro bubble B-scan and flow imaging is investigated using Definity™ in a frequency range relevant to high frequency ultrasound instrumentation. We first present the results of agent characterization experiments, which show that nonlinear scattering occurs for the bandwidths and pressure levels employed in this study. A prototype system for high frequency nonlinear bubble imaging is then presented, along with *in vitro* results for a 1 mm diameter wall-less vessel flow phantom in subharmonic, ultraharmonic, and second harmonic imaging modes using transmit frequencies of 20 and 30 MHz. Both subharmonic and ultraharmonic imaging modes achieved suppression of the tissue signal to below the noise floor, though the SNR in subharmonic mode was higher than that for ultraharmonic mode (~ 5-10 dB, depending on imaging conditions). These effects were explored over a range of transmit bandwidths (2-10 cycles) and pressures. Second harmonic imaging did not result in notable improvements in tissue signal suppression, due to the substantial amounts of nonlinear propagation present under the conditions that were employed. *In vivo* results are presented that show the nonlinear detection of micro bubbles in the micro vessels of a rabbit ear, and

in the left ventricle of a mouse heart. In both cases subharmonic imaging mode was employed for a 20 MHz transmit frequency. In the third component of this study, we investigated nonlinear micro bubble velocity imaging. A prototype system is described and we demonstrate its function *in vitro* and in the rabbit ear using the subharmonic of a 20 MHz transmit pulse. These data suggest that coherent nonlinear scattering is occurring rather than simply a single pulse disruption mechanism giving rise to broadband acoustic emissions.

The results of this study have demonstrated the feasibility of nonlinear bubble imaging at high frequencies, which may have implications for high frequency flow imaging applications in ophthalmology, dermatology, and small animal imaging.

Acknowledgements. This work was supported in part by the National Cancer Institute of Canada and Bristol Myers Squibb, who provided the Definity™.

DEPENDENCE OF CELL PERMEABILITY AND VIABILITY ON ULTRASOUND PARAMETERS

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Beyond their diagnostic application, the combination of ultrasound and micro bubbles may offer therapeutic potential in delivering drugs or genes to cells non-invasively. Central to this prospect is the phenomenon by which cells exposed to ultrasound appear to change their membrane properties, principally shown by an increase in permeability to large molecules. These changes can be reversible, leaving the cell viable. It is also possible that they are enhanced by the presence of micro bubbles, though there is conflicting evidence on this. In this study, we attempt to determine the dependence of membrane permeability and viability of Chinese hamster ovary cells in suspension using fluorescent markers attached to molecules of varying sizes.

Chinese hamster ovary (CHO) cells were exposed to ultrasound of varying acoustic parameters in the presence and absence of micro bubbles. Membrane permeability and cell viability were measured using two different fluorescent markers. The first, FITC-dextran, is attached to a large sugar molecule; the second has a different wavelength and is attached to propidium iodide, which enters the nucleus of a dead cell. The ultrasound exposure system consists of 3.5 MHz transducer excited with a 4.3 μs pulse (15 cycles sine wave) at 2.0 kHz PRF, with calibrated pressures of 250 kPa and 2250 kPa in water at the focus. The focal beam was aligned with the centre of the exposure chamber. The cells were suspended at 1.5×10^6 cells/ml in a volume of 1.5 ml and stirred gently with a magnetic stirrer during ultrasound exposure. The FITC-dextran and microbubbles (Optison, 5% concentration) were added to each sample just prior to transferring the cells into the exposure chamber. After exposure, all samples were placed on ice for 15-30 minutes before being analyzed for fluorescence by means of flow cytometery. Controls were obtained using the same protocol but without application of ultrasound. The time required to destroy most bubbles within the chamber was tested and kept consistent (3 minutes).

Intracellular uptake is monitored using FITC-dextran (fluorescein isothiocyanate-dextran). The FITC-dextran used has a molecular weight of 70 kDa, which is unable to penetrate intact cell membrane. FITC-dextran is composed of a fluorescent dye and a sugar molecule that comes in different molecular weights. Cell viability is monitored using PI (Propidium Iodide), which stains only dead cells.

Preliminary results demonstrate the uptake of FITC-dextran in ultrasound treated CHO cells. As long as the ultrasound peak pressure is not too high, cell viability following exposure is good (>92% for 1500 kPa peak-to-peak pressure). It is thus possible to change reversibly cell membrane permeability using ultrasound and micro bubbles. Future work will continue to characterise the effect of different exposure parameters and bubble combinations.

HOW VALUABLE IS REAL-TIME MYOCARDIAL CONTRAST ECHOCARDIOGRAPHY TO ASSESS MYOCARDIAL VIABILITY? COMPARISON WITH ^{18}FLUORDEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY AND DOBUTAMINE STRESS ECHOCARDIOGRAPHY.

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Background: Myocardial contrast echocardiography (MCE) using intravenous contrast administration is a novel modality for assessing myocardial perfusion. The aim of our study was to investigate its feasibility of evaluating myocardial viability and to compare its diagnostic value to dobutamine stress echocardiography (DSE) and ^{18}Fluordeoxyglucose positron emission tomography (^{18}FDG-PET).

Methods: Real-time MCE was performed in 35 patients considered for coronary revascularisation, who underwent low-dose DSE and ^{18}F-FDG-PET imaging. Myocardial contrast intensity was evaluated visually and assessed quantitatively (A = peak signal intensity, β = slope of signal intensity rise) in 16 myocardial segments. Follow-up echocardiograms were obtained in 30/35, including all 28 revascularised patients. Improvement of regional function on follow-up served as standard reference for myocardial viability. Twenty patients without CHD underwent MCE and served as a control group for assessment of normal values for myocardial perfusion.

Results: PET, DSE and MCE were feasible in 100%, 93% and 87% of myocardial segments respectively. Recovery of function was observed in 132/235 (56%) hypokinetic and 32/86 (37%) a- or dyskinetic of 448 revascularised segments. The sensitivity of PET, DSE and MCE were 94%, 86% and 91%, the specificity 61%, 83% and 60% respectively. The positive predictive value of PET, DSE and MCE were 82%, 90% and 79%, the negative predictive value 69%, 77% and 71%, respectively. Myocardial segments with an uptake <50% in nuclear perfusion imaging had a mean A of 0,56 ± 0,61 dB and a mean β of 0,13 ± 0,15 dB, segments with an uptake >50%, a mean A of 2,1 ± 13 dB and a mean β of 0,38 ± 0,15 dB ($p<0.001$ vs. <50% for A and $p=0.02$ for β). Segments with normal perfusion (control group) had a mean A of 3,9 ± 1,7 dB and a mean β of 0,42 ± 0,29 dB ($p<0.001$ vs. segments with an uptake >50% for A and ns for β).

Conclusions: Real-time myocardial contrast echocardiography is useful for assessing myocardial viability. Wall motion analysis during inotropic stress is more specific suggesting to gatherer information from both echocardiographic techniques.

ULTRASOUND-MEDIATED GENE DELIVERY: KINETICS AND MECHANISM

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Purpose: Sonoporation is a promising approach for drug and gene delivery. This technique, using ultrasound and gas microbubbles, has been studied for several years [1], but its fundamental mechanism is not yet elucidated. The purpose of the present study is to investigate how microbubble collapse affects the transport of DNA into the cells.

Methods: Rat mammary adenocarcinoma cells (MAT B III) were sonoporated with a rotating tube exposure system in presence of phospholipid-stabilized micro bubbles (BR14, Bracco), under the following conditions: transducers of 1.15MHz (0.4MPa negative peak pressure) and 2.25MHz (0.57MPa negative peak pressure), 20% duty cycle with 10s exposure time. MAT B III cells were sonoporated with a fluorescent marker (FITC-Dextran) or a plasmid encoding for the GFP (Green Fluorescent Protein). The kinetics of plasmid internalization were studied by labelling with the YOYO-1 intercaling fluorochrome (Molecular Probe). Evaluation of pore size was studied using FITC-Dextrans of MW ranging from 70 to 500kDa and calibrated fluorescent nanospheres. The positive cells were quantified by flow cytometry and observed with a confocal microscope. In some experiments, sonoporation was compared to lipofection (Lipofectamine 2000).

Results: More than 90% of the micro bubbles were destroyed under our experimental conditions. Compared to lipofection, the kinetics of GFP expression were faster for sonoporation. We observed very different fluorescent patterns between sonoporation and lipofection: sonoporation induced a more homogeneous diffusion of the plasmid into the cytoplasm. Flow cytometry measurements confirmed that sonoporated cells were able to internalize molecules of 37nm in diameter [2] and confocal microscopy showed evidence of solid particles of 70nm in diameter (polystyrene beads) within the cytoplasm.

Conclusion: The destruction of micro bubbles is evident and seems to be related to sonoporation efficiency. It was also demonstrated that sonoporation causes a direct plasmid transfer into the cytoplasm, unlike lipofection, which requires endocytosis. Moreover, particles of 40nm can be easily sonoporated into the cells. Further studies are needed to understand in more detail how sonoporation can improve gene delivery.

Reference List:

1. M. W. Miller. Gene Transfection and Drug Delivery. Ultrasound Med.Biol. 26[1], 59-62. 2000.

2. H. R Guzman, D. X NGuyen, A. J McNamara, and M. R Prausnitz. Equilibrium Loading of Cells With Macromolecules by Ultrasound: Effects of Molecular Size and Acoustic Energy. J.Pharm Sci 91, 1693-1701. 2002.

TRANSCRANIAL CONTRAST DIMINUTION IMAGING OF THE HUMAN BRAIN - A PILOT STUDY ON HEALTHY VOLUNTEERS

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Background and Purpose. Analysis of contrast diminution kinetics after bubble destruction is a new aspect in harmonic imaging. Purpose of this study was to investigate this approach to human cerebral perfusion.

Methods. 12 healthy volunteers were investigated transtemporally (Philips SONOS 5500, S4-probe, 1.8-3.6 MHz, 10 cm) at two ultrasound contrast agent (UCA) infusion rates (0.5 and 1.0 ml/minute of Optison™). After achieving a steady state, a set of 12 ultrasound pulses (6.67 Hz, MI 1.0-1.6) was applied. Time-intensity plots of three regions of interest (thalamus, white matter and cortex) were analyzed, using an exponential curve fit ($I_{(t)} = I_0 e^{-\beta t} + B$).

Results. 20/20 successful investigations showed a signal decrease after pulsed ultrasound application. In all cases, it was possible to generate exponential time-intensity curves. Half life [$T_{1/2} = \ln 2/\beta$] and baseline intensity [B] showed a significant dependence on infusion rate ($p=0.01$). At 1.0 ml/min, $T_{1/2}$ also depended on investigation depth ($p=0.01$).

Conclusions. It is possible to assess contrast diminution kinetics in human cerebral microcirculation. This new approach may provide additional information on cerebral perfusion within a short investigation time.

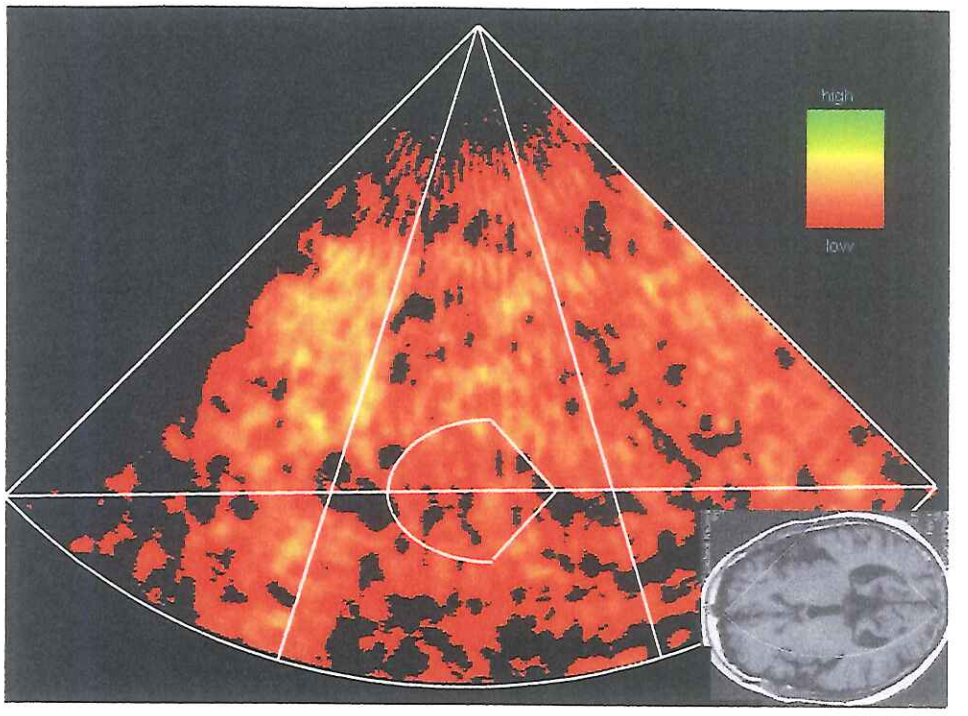

Figure 1 -Colored digital subtraction image (Scion Image for Windows, beta 4.0.2, Scion Corporation, Frederick, MD, USA) of image 1 at 0 ms and image 4 at 600 ms representing the UCA within the cerebral microcirculation (Wiesmann and Seidel 2000).

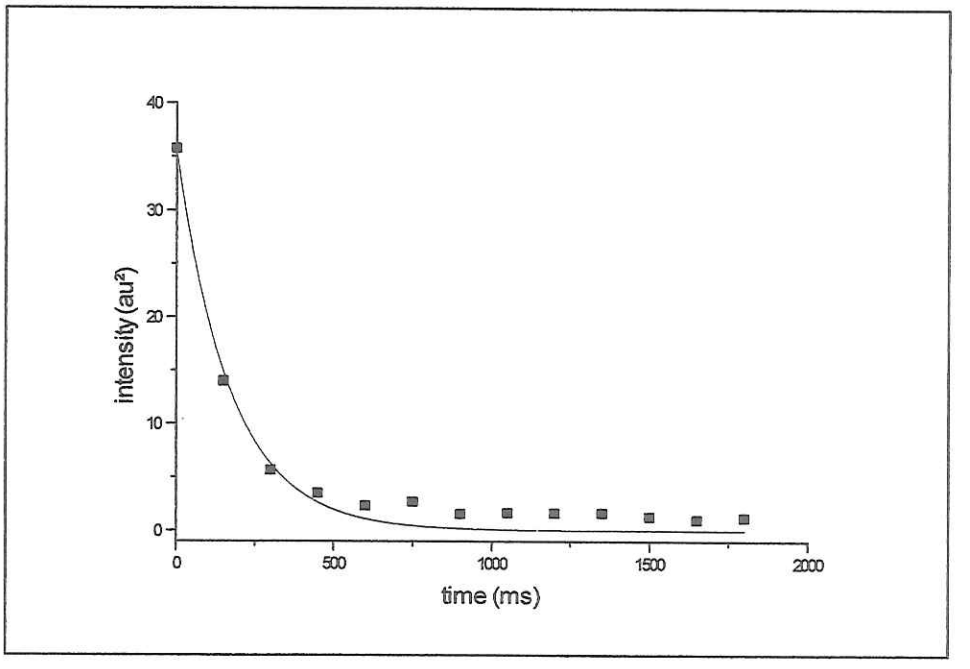

Figure 2 -Exponential decay curve fit of subject CB at 0.5 ml/min of Optison™. The region of interest was located in the ipsilateral thalamus.

ASSESSMENT OF CARDIAC PARAMETERS BY ULTRASOUND CONTRAST AGENT DILUTION

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A fast and objective measurement of the main cardiac parameters, such as cardiac output (CO) and ejection fraction (EF), as well as the intra-thoracic blood volume (ITBV), would be an asset in both the operating room and the intensive care unit. Nowadays, the indicator dilution methods, such as thermodilution and dye dilution, are the most accurate techniques that are employed for these measurements. However, the disadvantage of these techniques is their invasiveness, since they require cardiac catheterisation.

This study shows that ultrasound contrast agent (UCA) dilution is suitable for the assessment of CO, EF, and ITBV. The use of echocardiography leads to a substantial reduction of the invasiveness. Moreover, it allows the simultaneous measurement of CO, EF of both the ventricles, and ITVB. In fact, after an intravenous injection of a single UCA bolus (in this study SonoVue^{TM}), indicator dilution curves (IDC) can be measured simultaneously in all the cardiac chambers. Each IDC is measured by videodensitometric analysis of the B-mode video output of an ultrasound scanner. The video-intensity-versus-time curves, which are measured in automatically defined regions of interest (one in each chamber for instance), are transformed (calibration) into UCA-concentration-versus-time curves, referred to as IDCs. Each IDC is then fitted by a proper model in order to overcome low signal-to-noise ratio and indicator recirculation issues. Eventually, CO, EF, and ITBV can be easily derived from the fitted model.

The adopted model is the Local Density Random Walk model, since it is reported to provide the best fit of the IDC when applied to thermo and dye dilution. Furthermore, this model has a tight relation with the physics of the dispersion process. In fact, it is solution of the diffusion with drift equation.

The calibration issue is very complex due to many factors that influence the relation between video intensity and contrast concentration. Two different experimental approaches are used: the video analysis of different UCA concentrations (static calibration), and the comparison of measured UCA dilution curves with reference IDCs (dynamic calibration). Reference IDCs are obtained by lithium dilutions.

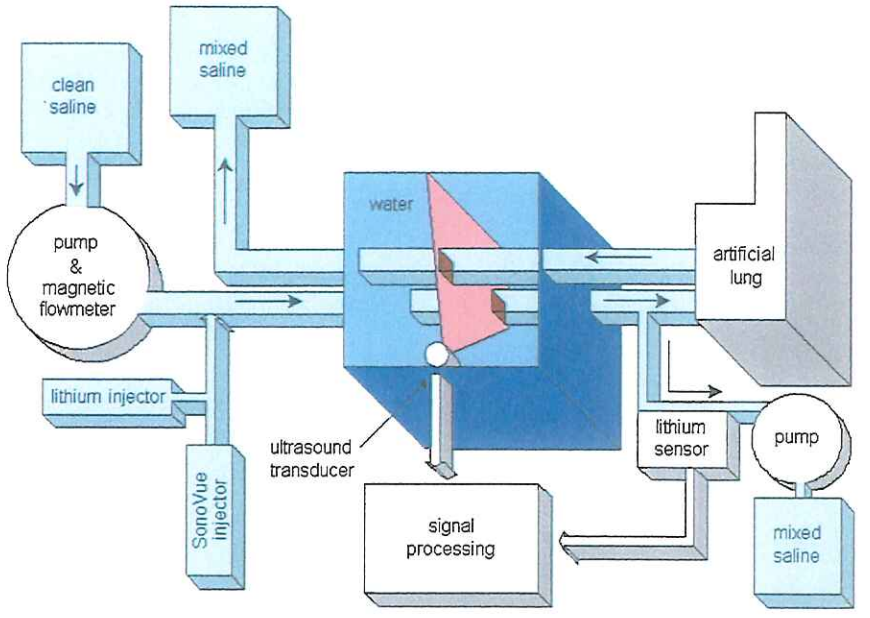

Figure 1 -Experimental set-up for flow and volume measurements.

The experimental set-up of Fig. 1 is used for both the dynamic calibration and system validation. The UCA IDCs before and after an artificial lung allow flow and volume measurements, while a third lithium IDC is used for calibration purposes. The flow measurements (from 0.5 L/min up to 4.5 L/min) show a determination coefficient higher than 0.99 with respect to the magnetic flowmeter measurements, while the volume measurements performed with the same flows have a standard deviation smaller than 10%. If a pulsed flow is generated, the fitted model can give the EF estimation too.

Reference: M Mischi, AACM Kalker, HHM Korsten, "*Videodensitometric methods for cardiac output measurements*," accepted for publication, EURASIP Journal on Applied Signal Processing, special issue on *Advances in Modality-Oriented Medical Image Processing*, 2nd quarter 2003.

ALIASING ARTIFACTS AS A RESULT OF MOVING BLOOD FLOW IN THE RECONSTRUCTION OF THE BLOOD FLOW PATTERNS IN THE PROSTATE USING THREE-DIMENSIONAL CONTRAST ENHANCED POWER DOPPLER IMAGING

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Introduction and objective: Three-dimensional contrast enhanced power Doppler (3D-CE-PD) provides additional information to traditional gray-scale B-mode scanning for the detection and localization of prostate cancer. Power Doppler information is correlated to the speed of moving blood particles, and therefore, 3D-CE-PD is related to the blood flow in the tissue. This information can be used to distinguish malignant from benign areas. The acquisition of a 3D-CE-PD volume takes about 100 seconds, and therefore the interpretation is hindered by an aliasing effect caused by the changing blood flow during the heart cycles. A possible solution to this problem is addressed.

Material and methods: A Voluson 530 (Kretz Technik AG, GE Austria) was used during in-vivo measurements on patients scheduled for radical prostatectomy. Contrast agent Levovist 2.5 (300 mg/ml.) is administered over 30 seconds. A 3D volume (256x256x256 voxels) scan is acquired 1 minute after the start of the injection. This volume is created from a collection of 2D-PD scans. Scanning the volume takes about 100 seconds, depending on the quality setting. The acquired volume is than post-processed by thresholding and surface rendering to produce a 3D interpretation of the blood flow.

Results: The 3D-CE-PD volumes of the patients show a 'blobbing' effect, instead of a blood flow pattern that resembles the vascular structure in the prostate. Only the well-perfused areas show connectivity, whereas the less perfused areas show only separated spots of blood flow.

Discussion: As mentioned before, the collection of a 3D volume takes about 100 second. Assuming for simplicity that every second 256/100=2.56 2D-PD scans are acquired, and assuming a heart rate of 60, this results in 2.56 images every heartbeat (for higher heart rates this would be even lower). From sampling theory it is known that for reconstruction of a simple sine wave 2.56 samples are not enough and thus an aliasing effect will occur. Since the blood flow cycle will be more sophisticated, an even higher sampling frequency is required compared to the simple sine. Furthermore, because the vascularity of the prostate is very complex and the blood flow is never in a straight line, the aliasing effect will be further enhanced. These effects influence the interpretation of the blood flow pattern and the distinction of benign from malignant areas.

1.5 HARMONIC IMAGING – A NEW METHOD FOR HIGH MI CONTRAST IMAGING

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One of the major problems in high MI contrast harmonic imaging is the separation of bubble echoes from interfering tissue harmonic components. A number of methods like sub-harmonic and higher harmonic imaging have been suggested to reduce both fundamental and harmonic tissue component. Especially the effective bandwidth of current transducer technology is a practical limitation for these methods.

We have developed 1.5 Harmonic Imaging, a contrast imaging technique that uses the intermediate frequency domain between fundamental and harmonic component. By applying dedicated transmission settings with controlled transmit spectrum and suppressed fundamental leakage, the tissue echo spectrum is well separated into fundamental and second harmonic components. In the intermediate region tissue echoes are very weak. On the other hand, due to bubble destruction, the spectral broadening of bubble components is maintained. Thus a high bubble/tissue ratio can be obtained using 1.5 Harmonic Imaging.

We compared the bubble/tissue ratio of this new method with conventional high MI second harmonic imaging in vitro using a 2.5 MHz cardiac sector probe and a tissue mimicking phantom with Levovist (Schering) pool. Our experimental results reveal an increase of 20 dB in bubble/tissue contrast in case of 1.5 Harmonic Imaging. In patient studies we were able to confirm the results of these in vitro experiments.

ULTRASONIC CONTRAST AGENT DESTRUCTION MECHANISMS REVEALED BY OPTICAL OBSERVATIONS

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Introduction: Ultrasonic contrast agent (UCA) consists of liquid containing gas bubbles with diameters of a few micrometers, either free or encapsulated by a shell. UCA bubble destruction behavior has been under investigation for its potential application in high MI imaging techniques, noninvasive pressure measurements, neovascularization, and in drug and gene delivery. The success of such new diagnostic and therapeutic techniques is directly related to our understanding of UCA microbubble destruction.

We investigated UCA destruction mechanisms by subjecting two different contrast agents to high-amplitude ultrasound, and optically recording their behavior with a fast-framing camera. Optically observing ultrasound insonified UCA micro bubble with a high-speed camera is a promising method for analyzing micro bubble oscillation and destruction behavior.

Methods: The fast-framing camera was capable of recording an optical image sequence of eight frames per experiment and operated at a speed of three million frames per second. Each image sequence was taken during one cycle of ultrasound. We recorded 482 image sequences with an experimental UCA (Bracco Research SA, Geneva, Switzerland), freely flowing through a capillary cellulose tube. The microbubbles had a median diameter of 2 μm. The shells were phospholipid monolayers with an estimated thickness of 10 nm. We also recorded 57 image sequences with Quantison™ (Upperton Ltd., Nottingham, UK), poured into a container and covered with a microscopic slip. The microbubbles had a mean diameter of 3.2 μm. The shells consisted of human serum albumin (0.2–0.3 μm).

In all experiments we transmitted 10 cycles of ultrasound with a center frequency of 0.5 MHz. Peak negative acoustic pressures corresponded to mechanical indices (MI) between 0.9 and 1.2.

Results: The optical image sequences revealing destructive behavior were classified as follows.

Coalescence – the fusion of two or more bubbles. This mechanism was only observed while microbubbles were expanding. We recorded 133 image sequences showing experimental UCA

microbubble coalescence. The coalescence mechanism was found to be analog to mechanisms described for larger free gas bubbles.
We included bubble coalescence as a UCA microbubble destruction mechanism, since the total number of bubbles in the UCA decreases due to coalescence.

Fragmentation – the fragmentation (fission) of one or more bubbles into smaller bubbles. This mechanism was only observed, as suggested from cavitation bubble theory, around maximum compression. We recorded 83 image sequences showing experimental UCA microbubble fragmentation.

We observed successive coalescence and fragmentation in 12 image sequences.

Sonic cracking – the formation of a bubble shell defect causing gas escape (Takeuchi, 1999). We recorded 17 image sequences showing Quantison™ microbubble cracking. The number of cracking bubbles compared to the number of bubbles visible in the image sequences is low.

We recorded 15 image sequences demonstrating destruction mechanisms that could not be classified into the categories coalescence, fragmentation, and sonic cracking, for example asymmetric collapse without fragmentation. Jet behavior was clearly observed twice. One of these events was previously published (Postema, 2002). Apparently liquid was propelled into the freely flowing microbubble, in the direction of the capillary wall nearby.

Discussion and conclusions: Coalescence we observed in 28% of our high-MI experimental events with a lipid-shelled UCA, when insonifying at 0.5 MHz. We proposed a coalescence mechanism of UCA microbubbles, based on a mechanism of colliding free gas bubbles and the so-called stalk-pore hypothesis. Bubble coalescence was only clearly observed with the experimental UCA, and not with Quantison™. For the bubble concentration used, the spacing between the Quantison™ bubbles was much larger than a maximal excursion, and therefore bubble collision during the expansion phase may not have occurred.

Bubble fragmentation was only observed with the experimental UCA, and not with Quantison™. We conclude that its bubble wall velocity and acceleration are too low to induce instabilities big enough for fragmentation.

The repetitive coalescing and fragmenting behavior recorded suggests that the destruction behavior of contrast microbubbles is more complex than previously assumed (Chomas, 2001).
We showed that sonic cracking is feasible for the hard-shelled contrast agent Quantison™. Tiny flaws in the bubble shell may account for the reason why certain bubbles cracked and others stood intact.

At before-mentioned acoustic frequency and pressures, when not taking into account coalescence as a real destruction mechanism, we found that fragmentation is the primary destruction mechanism for the experimental UCA, in case of destruction during the first cycles of ultrasound insonification. Since we were only looking at early cycles of the ultrasonic waves, this limits our conclusion.

The destruction mechanisms presented have potential clinical applications in high-MI imaging, noninvasive blood pressure measurements, neovascularization, and targeted drug and gene delivery.

Acknowledgment: This work has been supported by the Technology Foundation STW (RKG.5104).

References:

Chomas JE, Dayton P, Allen J, Morgan K, Ferrara KW. Mechanisms of contrast agent destruction. IEEE Trans Ultrason, Ferrorelect, Freq Contr 2001; 48:232-248.

Postema M, Bouakaz A, de Jong N. A sequence of microscopic image frames of a freely flowing contrast agent microbubble. IEEE Trans Ultrason, Ferrorelect, Freq Contr 2002; 49(3):cover page.

Takeuchi Y. A sequence of microscopic image frames collected during insonification of a hard-shell model contrast agent. IEEE Trans Ultrason, Ferrorelect, Freq Contr 1999; 46(4):cover page.

A BUBBLE DYNAMICS MODEL INCLUDING HEAT AND MASS DIFFUSION AND CHEMICAL REACTION: WHAT DO WE LEARN FROM SONOLUMINESCING BUBBLES FOR ULTRASOUND DIAGNOSTIC BUBBLES?

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Phase diagrams for single bubble sonoluminescence (SBSL, [1]) are calculated. The employed model is based on a set of ordinary differential equations and accounts for the bubble hydrodynamics, heat exchange, phase change of water vapor, chemical reactions of the various gaseous species in the ubble, and diffusion/dissolution of the reaction products in the liquid [2,3]. The results of the model are compared in detail to various phase diagram data from recent experimental work [4,5], among which are air-water systems as well as systems with a xenon-nitrogen mixture as the saturated gas. Excellent quantitative agreement is found for all considered cases. Moreover, we find that the onset of SBSL is hysteretic. When starting with air typical temperatures before onset are 5500K and 15000K thereafter.

We also explore the possibility to apply this model to bubbles under ultrasound diagnostic conditions.

References:

[1] M.-P. Brenner, S. Hilgenfeldt, and D. Lohse, Rev. Mod. Phys. 74, 425 (2002).

[2] R. Toegel and D. Lohse, J. Chem. Phys. 118, 1863 (2003).

[3] B. D. Storey and A. J. Szeri, Phys. Rev. Lett. 88, 074301 (2002).

[4] J. A. Ketterling and R. E. Apfel, Phys. Rev. Lett. 81, 4991 (1998).

[5] J. A. Ketterling and R. E. Apfel, Phys. Rev. E 61, 3832 (2000).

STUDY ON THE MECHANISM OF CELL DAMAGE CAUSED BY MICROBUBBLES EXPOSED TO ULTRASOUND

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Recent studies of sonoporation have shown that ultrasound contrast agent gas bubbles activated by diagnostic ultrasound exposure can cause damage to cell membranes [1, 2]. However, the mechanism of this adverse effect on cell membranes is not yet clear. In this study, arterial endothelial cells were exposed to ultrasound with and without microbubbles, and resulting cell damage was evaluated using fluorescence microscopy [3].

An observation chamber was filled with microbubble-suspended culture solution supplemented with a fluorescent dye, propidium iodide (PI). Two types of microbubbles, PVC (polyvinylidene chloride-acrylonitrile)-shelled microbubbles and Levovist® were used in the experiments. Bovine endothelial cells were cultured on a 0.16-mm-thick cover glass, and the cover glass was attached to an observation chamber upside down so that microbubbles could make contact with the cells. The observation chamber was then exposed to pulsed ultrasound of 1 MHz in center frequency and 0.6 MPa in peak-rarefactional pressure. The damage to the cells was evaluated by fluorescence microscopy. PI is a membrane-impermeant fluorescent dye that stains nucleic acid and therefore enables detection of cell membrane damage.

In the cell viability test using PI, the percentage of viable cells that had been exposed to ultrasound with bubbles was significantly lower than that of cells that had been exposed to ultrasound without bubbles. Furthermore, close-up observation of cell-bubble behavior during ultrasound exposure showed that the punctured sites of damaged cell membranes were consistent with the locations where bubbles collapsed. These findings suggest that bubble collapse is responsible for cell membrane damage. In our previous study, it was shown by high-speed observation that bubble collapse is due to the action of a small stream generated by nonuniform bubble contraction and that this stream can cause mechanical stress to act on a cell [4]. Based on these results, we concluded that the cell damage was mainly caused by mechanical stress induced by the bubble collapse.

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References

[1] D. L. Miller et al.: Sonoporation of monolayer cells by diagnostic ultrasound activation of contrast-agent gas bodies. Ultrasound Med. Biol. 2000; 26:661-667.

[2] D. L. Miller et al.: Lysis and sonoporation of epidermoid and phagocytic monolayer cells by diagnostic ultrasound activation of contrast agent gas bodies. Ultrasound Med. Biol. 2001; 27: 1107-1113.

[3] N. Kudo et al.: Study on mechanisms of cell damage caused by microbubbles exposed to ultrasound. IEEE UFFC Ultrasonics Symposium Proc. 2002; in print.

[4] N. Kudo et al.: Optical observation of cell-bubble behavior using a high-speed camera. 7th European Symposium Ultrasound Contrast Imaging Proc. 2002; 70-71.

OPTICAL AND ACOUSTICAL INTERROGATION OF SUBMICRON CONTRAST AGENTS

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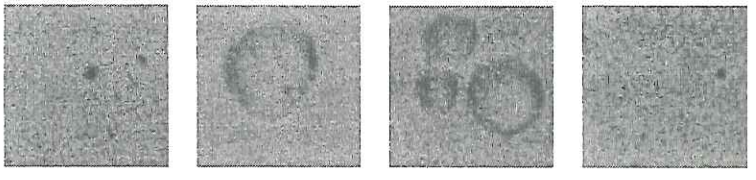
Conventional ultrasound contrast agents have a diameter of several microns. In this research, we explore the behavior of a new class of contrast agents that have bubble diameters ranging from 0.3 to 1.3 μm. These sub-micron agents are gas-filled, double-walled microspheres.

We have used two experimental systems combined with model predictions to assist in understanding the response of these unique agents to a range of signal transmission parameters. Contrast agent expansion is evaluated as a function of acoustic pressure, pulse length, and center frequency using a high-speed camera system. The optical images demonstrate an order of magnitude expansion in radius during the pulse rarefaction, where the expansion magnitude is dependent on the transmitted pressure and frequency.



(a) (b) (c) (d)

Sub-micron contrast agent before (a), during (b and c), and after (d) insonation with a 2.25 MHz, 10-cycle pulse at a peak negative pressure of 1.7 MPa. The resting diameter of the agent is approximately 0.5 μm; during pulse rarefaction it expands to many times its initial size.

Simulations using a modified Rayleigh-Plesset model predict an increasing relative expansion for bubbles with increasing pressure and decreasing initial radius.

In addition, acoustically-recorded frequency spectra reveal nonlinear behavior of these agents for a range of transmitted pulses. Narrowband insonation by an eight-cycle pulse results in echoes with both harmonic and subharmonic frequency components. Micro bubbles may also be differentiated from tissue by echo amplitude changes in response to identical sequential pulses, or frequency shifts in response to phase-inverted pulses. We hypothesize that these agents are activated by high-pressure ultrasound pulses, which disrupt the agent wall and allow for oscillation of the gas-filled bubble followed by diffusion over a time scale of milliseconds.

The small size and long-term stability of these contrast agents enable their use in a new set of clinical applications. In-vivo results from a normal canine model reveal marked contrast enhancement of first order lymph nodes following subcutaneous injection of sub-micron contrast into the distal extremities, demonstrating uptake across the lymphatic endothelium. Ultimately we hope to develop an alternative to present intra-operative procedures for sentinel node detection.

In this talk, we will summarize the results of optical and acoustical experiments, propose signal-processing strategies for detecting sub-micron contrast agents, and present our preliminary in-vivo results.

TECHNICAL MODELING OF ULTRASOUND CONTRAST BEHAVIOUR

John Allen

Ultrasound contrast agents are encapsulated bubbles 1--5 microns in radius developed for diagnostic ultrasound. Reviewed are the theoretical models for ultrasound contrast agents with the inclusion of the encapsulating shell highlighted. Early models offered good intuitive insights into the shell behavior and later models put the formulation on a more rigorous footing using a generalized Rayleigh-Plesset equation. Still limitations exist in describing elastic materials and associated deflation instabilities. New formulations for elastic shell contrast agents based on the governing or field equations used for solid mechanics rather than fluid mechanics are discussed. These offer new insight into contrast agent destruction and potential manufacture. While the theoretical models have been for a single bubble or agent, multiple agents are used in practice and paramount to the efficacy of incipient drug delivery applications. The previous theoretical treatment of multiple agents has been limited to independent scattering approximations. Discussed are consideration of the non-linear coupling between agents in close proximity and non-linear resonance effects unique to multi-degree of freedom systems.

THEORETICAL PREDICTION AND MEASUREMENT OF THE NON LINEAR PARAMETER DISPERSION IN CONTRAST AGENT

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With the introduction of harmonic imaging in sonographic imaging, there have been more and more investigations in the field of non-linear propagation of finite amplitude waves. Amplitude of the received signal in these new imaging modalities depends on the non-linear parameter B/A of the propagating medium. In the particular case of ultrasound contrast agent it is well known (de Jong et al. Ultrasonics 1994, Higher harmonics of vibrating gas filled microspheres) that the non-linearity is dramatically increased due to non-linear resonance oscillation of their gas bubbles.

In this work, we present for the first time, to our knowledge, a theoretical and experimental study of the changes of the non linear parameter B/A of contrast agents with frequency. An analytical model for second harmonic propagation in ultrasound contrast agent is proposed in the parabolic and quasilinear approximations. This model, based on a Gaussian beam superposition, takes into consideration attenuation, dispersion, diffraction and non-linear effects together with the finite aperture of the receiver. The "effective" attenuation, phase velocity and non linear parameter of the contrast agent introduced in this model are calculated considering the encapsulating shell properties and the size distribution of gas bubbles. The non-linear parameter presents two resonances: the higher one occurred when the incident acoustic wave frequency coincided with the bubbles resonance frequency, and the lower one occurred when the second harmonic frequency matched this resonance frequency. With a volume fraction of gas of 10^{-5}, B/A value as high as 250 has been found near the lower resonance, two orders of magnitude higher than biological tissues. Moreover in the low frequency range, under the lower resonance, the non-linear parameter remained higher than 100, which can be explained by a mixture law for the acoustic parameter B/A. In this frequency range, non-linear distortions will be greater due to the fact that attenuation and dispersion effects are weak.

Experimental measurements of the non-linear parameter B/A have been made using SonoVue^{TM} (Bracco) contrast agent between 1 and 10 MHz with a second harmonic insertion / substitution method. Attenuation and phase velocity needed to the determination of B/A from second harmonic measurements have also been measured with the same apparatus. Adequate quantitative agreements have been found between experimental and theoretical results.

ECHOGRAPHY-AIDED GENE THERAPY: BUBBLE OR BURST

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Gene therapy is the introduction of foreign DNA or gene sequence into host somatic cells. Gene transfer does not only serve as a research technique to study gene expression and regulation in animal models, but also shows immense potential as a novel therapeutic tool, striking the disease at the site of the causative genetic defect. Recombinant genes can be introduced into somatic cells of patients to treat a disorder through de novo synthesis of a missing or defective gene product. This approach permits the introduction of new gene products and functions to eukaryotic cells, and allows the elimination of gene function by anti-sense oligonucleotides, ribozymes, solubelized receptors and "decoy" oligonucleotides technology. In the last 10 years gene therapy has evolved through a proof of principle phase to initiation of clinical protocols. Gene therapy may be amenable to many cardiovascular diseases ranging from restenosis to ischemic heart disease.

The delivery and entry of recombinant material into target cells is facilitated by use of vectors. DNA can be directly transferred to somatic target cells by viral vectors, such as retroviruses and adenoviruses, and non-viral methods, such as cationic liposomes, liposome viral conjugates, and polymers. However, the non-viral approaches are hampered by the low transfection efficiency and do not target intimal and medial cellular components, whereas viral vector mediated gene transfer raises biosafety concerns due to the infectious complications presented by the first generation viral vectors. The requirement for local high-level exposure at the target site, which is clinically not feasible by systemic administration, and the theoretical risk for inadvertent transgenic expression or mutagenesis in germ cell lines, led to the development of local drug delivery systems for genetic vectors. As such, micro bubbles ensure high titer delivery locally to the desired vessel segment, potentiate the gene transfer efficiency and contribute to improved safety by directing gene transfer to the coronary circulation by local destruction of coated micro bubbles, thereby reducing the risk of systemic toxicity and lowering the total dose to which the patient is exposed. Initial studies of cardiovascular gene transfer have shown that ultrasound/micro bubble mediated gene-vector delivery is safe and effective and may represent a new avenue in cardiovascular gene therapy.

PERSONAL EXPERIENCE

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On the fall of 1981, my father presented results from his first experiments combining thrombolytic agents and ultrasound in Stockholm, Sweden (1). Ever since, I have been involved with him in most of the experiments that later evolved over the years into various fields. Ultrasound was used with transdermal insulin delivery (2), cancer chemotherapy, activation of photosensitive drugs(3, 4) and currently for gene therapy. It was in 1995 that I published a study in CIRCULATION (5) that first demonstrated that ultrasound contrast agents can enhance thrombolysis in vitro. Since then, the role of ultrasound contrast agents have increasing widened other than merely enhancers for sonography. Ultrasound contrast agents are now being developed to specifically bind to target tissue, encapsulate drugs and produce microjets that can inject practically anything into the cells. It has now become a very exciting period were the number of researchers are rapidly increasing with new ideas coming from outside the territory of sonographers or acoustic scientists. Recent reports have even suggested combining microbubble, ultrasound and electroporation for gene therapy (6). Meanwhile, ultrasound instrumentation have advanced greatly, revolutionary technologies and devices are constantly being introduced. Hopefully this year, the first therapeutic ultrasound/drug release catheters for stroke therapy will be released in the market in Europe. My father and I both hope that this year will mark the beginning of a new era for ultrasound/drug delivery therapy.

Historical background and future prospects will be introduced in this presentation.

References:

1) Tachibana S: Ultrasonic vibration for boosting fibrinolytic effect of urokinase. Thromb Haemostas 46(1): 211, 1981

2) Tachibana K, et al:Transdermal delivery of insulin by ultrasonic vibration. J Pharm Pharmacol 43: 270-271, 1991

3) Tachibana K, et al: Enhancement of cell killing of HL-60 cells by ultrasound in the presence of photosensitizing drug: Photofrin II. Cancer Letters 72: 195-199, 1993

4) Tachibana K, Uchida T, Hisano S, Morioka E: Eliminating adult T-cell leukaemia cells by ultrasound. Lancet 349: 325, 1997

5) Tachibana K, et al: Albumin microbubble echo contrast material as an enhancer for ultrasound accelerated thrombolysis. Circulation 92: 1148-1150,1995

6) Yamashita Y, et al: In vivo gene transfer into muscle via electroporation-sonoporation. Human Gene Therapy 13: 2079-2084, 2002

MICROBUBBLE ENGINEERING AND APPLICATIONS FOR DRUG DELIVERY.

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In order to prepare micro bubbles capable of performing their *in vivo* functions properly, basic bioengineering concepts are applied. Micro bubbles are prepared from non-toxic biodegradable materials. They can be coated with a brush layer of poly(ethylene glycol) to ensure enhanced stability on storage and to reduce complement activation and deposition when administered in the bloodstream. Lipid monolayer-coated bubbles prepared in such a manner can circulate in the bloodstream for many minutes. Targeting ligands can be attached to the bubbles to ensure selective binding to the targets (e.g., on activated endothelium). Micro bubble surface can also be modified to carry positive charge, to attract and immobilize plasmid DNA. For the purpose of drug delivery, drug-carrying nanoparticles or liposomes can be attached to the micro bubble surface. Upon the ultrasound irradiation of the target tissue, drug (or DNA)-carrying bubbles are destroyed, and the materials released and deposited in the tissue (outside of the vasculature). Specific engineering designs of such microbubble constructs will be discussed.

DETACHMENT AND SONOPORATION OF ADHERENT HELA-CELLS BY SHOCK WAVE INDUCED CAVITATION

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The interaction of lithotripter-generated shock waves with adherent cells is investigated using high-speed optical techniques. Shock waves permeabilize adherent cells in vitro through the action of cavitation bubbles. The bubbles are formed in the trailing tensile pulse of a lithotripter-generated shock wave where the pressure drops below the vapor pressure. Upon collapse of cavitation bubbles a strong flow field is generated which accounts for two effects: First, detachment of cells from the substrate and second, the temporary opening of cell membranes followed by molecular uptake due to shear stress.

MICROBUBBLES: TOOLS FOR VESICLE BIOMECHANICS

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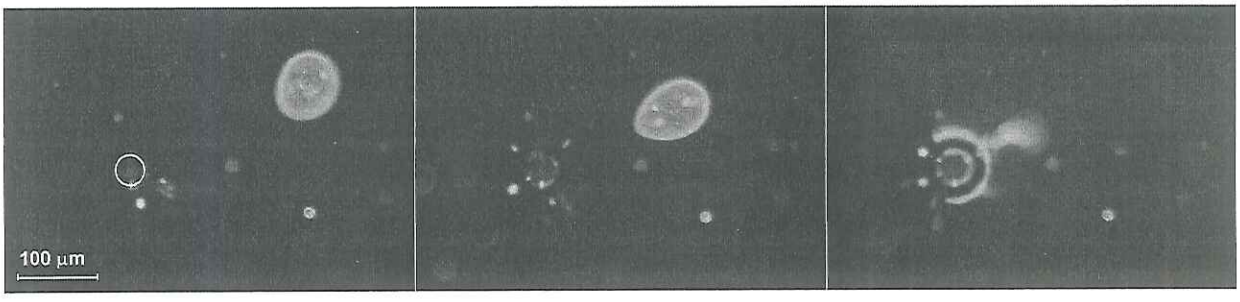
When subjected to ultrasound, micron-sized bubbles oscillate, giving rise to significant ultrasound scattering and increased echographic contrast. It has been reported that this excitation can also alter nearby cell membranes [D. L. Miller and J. Quddus, Ultrasound in Med. & Biol. 26, 661-667 (2000)], and increase the cell wall permeability for drug delivery or gene transfection purposes in various therapeutic applications. Many physical processes could be behind this phenomenon, all related to the concentration of acoustic energy onto small scales that is characteristic of small bubbles. It is unknown which process is dominant under which experimental conditions, and extremely difficult to control the collapse of strongly driven bubbles in those experiments.

We have therefore developed a new line of experiments to elucidate the possible mechanisms of ultrasound-driven cell wall permeation (sonoporation) in a well-controlled setup. Micron-sized bubbles attached to a wall are excited in a liquid containing lipid vesicles. These vesicles are commonly used to mimic cell membranes, with the advantage that their mechanical properties are well-known. In contrast to previous experiments, we observe the motion and deformation of *single* vesicles directly, rather than *a posteriori*.

The effect looks dramatic under the microscope: the vesicles are periodically accelerated towards and repelled from the bubble. In this "bouncing" motion the vesicle is subjected to a shear stress that is reflected in its elongated shape. As this deformation increases (upon increasing driving pressure or adjusting material parameters) the break-up of vesicles is also observed (see figure).



Lipid vesicle deformation and rupture (the vesicle membrane is fluorescence-marked) in the acoustic streaming flow generated by the bubble (white circle).

We interpret the motion as acoustic streaming induced by the bubble oscillations, a nonlinear effect creating a steady flow with closed streamlines from periodic driving. A quantitative theory of acoustic streaming is available, enabling us to directly model the streaming flow, vesicle transport, and vesicle deformation. Moreover, because the bubble oscillation amplitudes are actually small, it is possible to control these processes with great accuracy, and improve vastly on current methods relying on inertially collapsing bubbles.

EVALUATION OF INTESTINAL PERFUSION OF INFLAMED BOWEL BY HIGH RESOLUTION WIDEBAND PHASE-INVERSION CONTRAST HARMONIC IMAGING (CHI)

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Background: Imaging of intestinal inflammation is routinely performed by B-Mode- and power-Doppler-sonography. Severity of inflammation positively correlates with the intensity of Doppler signals. However, estimation of Doppler signals can be disturbed by tissue motion artifacts and intramural enteric vessels may be below the detection threshold of Doppler-sonography. Contrast harmonic imaging (CHI) at low Mechanical Index (low MI) allows for more sensitive detecion of flow even in very small vessels at low perfusion velocity. We evaluated whether CHI at low MI is suitable for the estimation of perfusion of the intestinal bowel wall.

Methods: We performed intestinal sonography with a 7.5 MHz scanner plus CHI (7.5L40; Sonoline Elegra®; Siemens, Germany) on five patients with inflammatory bowel diseases. The ultrasound contrast agent Sonovue® (Bracco, Italy) was used at low MI (0.1-0.2) with or without the daylight mode Photopic®. The following settings were proven to give a satisfactory result of bubble visualization and bowel perfusion: gain 60dB, MI 0.1, CHI mode plus Photopic® at a frame rate of 33/s. Grey scale values before and after injection of Sonovue® were calculated by using a public domain Java image processing program (image-j). Basic gray scale values in an area of interest (AOI; defined number of pixels) were compared to those at the time of estimated maximum of contrast enhancement.

Results: The bowel wall was not visible at baseline low MI settings. Therefore, the daylight mode Photopic® had to be used. After i.v. injection of 5 ml Sonovue we were able to visualize the perfusion of the highly inflamed bowel without any additional artifacts with a B-mode technique at the above mentioned settings. The arterial inflow of contrast agent from the mesenteric vessels was detected in all cases. Due to higher resolution the vessel architecture of the bowel wall could be discriminated much better than on power-Doppler. The strongest signal was observed in the submucosa in all cases. The signal of the Sonovue® bolus lasted over 5 minutes. Gray scale values within the AOI were calculated by image-j and showed a mean basic value of 38 (26-50). At the time of estimated maximum of enhancement the mean was 59 (43-75). Contrast-enhancement occurred in all cases, however, interindividual differences were observed.

Conclusion: While CHI of organs at emitted frequencies around 2 MHz has been widely described, the visualization of bowel perfusion with a 7.5 MHz scanner at the CHI mode has not yet been reported. Furthermore, the daylight mode Photopic® can be used to better visualize the intestinal wall prior to injection of the contrast agent. Calculation of B-mode gray scale values can possibly be used to quantify perfusion. As to our knowledge this is the first report of high frequency contrast enhanced bowel ultrasonography with CHI at low low MI.

PERFUSION HARMONIC IMAGING (PHI) AFTER SONOVUE™ BOLUS INJECTION IN ACUTE ISCHEMIC STROKE

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Background and Purpose. With transcranial perfusion harmonic imaging (PHI) and ultrasound contrast agent (UCA) bolus injection, it is possible to display cerebral perfusion deficits in acute stroke. Using SonoVue™, a second generation UCA, we performed a prospective patient study to investigate this approach.

Methods. 15 patients suffering from acute ischemic stroke, were investigated with PHI (SONOS 5500) after UCA bolus injection. Inclusion criteria were sufficient acoustic window for transcranial duplexsonography (TCCS) and onset of symptoms no longer than 12 h before PHI. Results. 15 patients (7 w, 8 m, median age 67 years, median NIH-SS 14 pts) underwent PHI (median 5h30' after symptom onset).

Pathologic findings (TCCS) were middle cerebral artery (MCA) occlusion (n=8) and stenosis (n=5) or occlusion (n=3) of the ICA. Lesion patterns (CCT) were 14 MCA infarctions and one infarction in the anterior cerebral artery (ACA) territory. In all patients, SonoVue™ led to an increase in signal intensity of the brain parenchyma. Corresponding to the area of infarction (CCT), in 13/15 patients a perfusion deficit could be detected by PHI. In two patients (one ACA-infarction, one small lenticulostriate infarction), no hypointensity was found. In all 13 patients we found a core area of total contrast deficit in the center of the infarction. 3/13 patients had a surrounding region with relative signal reduction (>50%). All three patients showed a delay in time to peak intensity of >4s within this area.

Conclusion. We could demonstrate that SonoVue™ bolus injection with PHI technique is a promising approach for the assessment of perfusion defects in acute stroke. It may be possible to identify "tissue at risk" with critical hypoperfusion as areas of relative contrast reduction.

REAL-TIME PERFUSION IMAGING: A NEW ECHOCARDIOGRAPHIC TECHNIQUE COMBINING MYOCARDIAL PERFUSION AND CONTRACTION

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Myocardial contrast echocardiography (MCE) with high acoustic energy and triggered harmonic imaging is the best-established ultrasound technique to date for the assessment of myocardial perfusion. However, non-invasive real-time assessment of myocardial perfusion and function simultaneously after intravenous injection of micro bubbles is the ultimate goal of MCE. Recent technological advances have enabled myocardial opacification to be visualized during low-energy real-time imaging. By this approach, high-energy ultrasound bursts can be periodically transmitted to produce bubble destruction, after which consecutive frames delineate the restoration of contrast intensity. Micro bubble replenishment rate and peak intensity after bubble destruction, as assessed by real-time imaging, provide excellent parameters of regional microcirculatory flow. This review will introduce the modalities used for real-time perfusion imaging with focus on power pulse inversion imaging (PPI) and quantitative analysis using dedicated software (the HDI Lab). With the advent of real-time perfusion imaging, wall motion and myocardial perfusion can be assessed together, without the need of presently time-consuming combination of different imaging modalities. It has important clinical implication in the identification of coronary artery disease, quantification of coronary stenosis severity, assessment of myocardial viability, determination of infarction size, and evaluation of reflow and no- or low-reflow after acute myocardial infarction (AMI).

DIAGNOSIS OF NON-COMPACTION CARDIOMYOPATHY USING CONTRAST ECHOCARDIOGRAPHY

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Non-compaction cardiomyopathy is a rare disorder characterized by excessive and prominent trabeculation of the left ventricle (LV) with deep intertrabecular recesses. Diagnosis is often missed or postponed, although echocardiography has shown a greater awareness of this rare disorder. We describe a case with prospected non-compaction cardiomyopathy, which was confirmed using contrast echocardiography showing the connection of intertrabecular spaces and the LV cavity.

THE CLINICAL USE OF ULTRASOUND CONTRAST AGENTS IN ECHOCARDIOGRAPHY: WALL MOTION, THICKENING AND PERFUSION

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Background: Cardio-vascular related diseases remain as the number one and two causes of overall mortality in the United States. The uses of newer non-invasive technologies have improved the early detection of ischemic heart disease and, consequently, have become critical components in the management of cardiac patients.

While there are several different approaches to screening patients for ischemic heart disease, the exercise or pharmacologic stress test is a mainstay of today's approach to the assessment of patients with documented or suspected cardiovascular disease. The usual sequence of diagnostic management consists of a history, physical, and a risk factor assessment. Following the initial intake examination, often a functional or physiologic stress test is ordered. Typically, if the patient is physically capable of walking (or bicycling) an exercise test is ordered. In the setting of a patient with an underlying abnormality in the resting ECG, or clinical conditions that have been associated with a high rate of false positive studies, an additional imaging modality will be utilized. In order to enhance the sensitivity and specificity of stress examinations, an echocardiogram or a nuclear imaging will accompany the stress test.

Non-Invasive Testing: In choosing the appropriate adjunctive imaging modality to be used with stress testing, Scheinmann et al. (1) reported on the results of a Meta-analysis that included forty-four previously published articles on the clinical utility of screening patients for heart disease using stress echocardiography or SPECT imaging. Their conclusions emphasized that both modalities (echo and SPECT) shared equally in the overall diagnostic sensitivity, however stress echo was found to provide increased specificity over SPECT, and accordingly, stress echo was found to have a higher clinical utility. (Of interest, the earlier published reports, which served, as the basis for the Meta-analysis, did not include two substantial technical improvements in stress echocardiography; specifically harmonic imaging and ultrasound contrast agents).

In the overall management of patients with cardiac disease, the issues of additional testing must be evaluated with respect to the benefit accrued to the patient. The use of additional non-invasive testing used to determine the presence or absence of occlusive coronary artery disease should be based upon evidence-based medicine. Consequently, the prognostic value of performing a stress echocardiogram for the detection of ischemia in patients is of paramount interest. To address these issues, Marwick et

al. (2) and Poldermans et al., (3) have published results on the prediction of clinical outcomes in patients undergoing exercise or pharmacologic stress echocardiography.

In Marwick's study, they used traditional twelve lead ECG's along with the stress echocardiogram. It was determined that the yearly mortality of patients without intermediate risk was 2-7%. Therefore, an ischemic outcome from a stress echo is an important predictor of outcome. Similarly, Poldermanns et al. identified the natural history of patients that were identified as having a "positive" dobutamine stress echocardiogram. In the mean follow up period of thirty-six months in 1,737 patients, the number of ischemic events was predictive for late cardiac events; whereas, a normal dobutamine stress echo carried a relatively good prognosis for late cardiac events.

Advances in Non-Invasive Testing: The technologic advances of harmonic imaging and ultrasound contrast agents have substantially improved the diagnostic yield and accuracy of echocardiography stress testing. Previous reports have documented the importance of using contrast agents to enhance the endocardial surface of the cardiac chambers: (4) Feinstein SB, Cheirif J, ten Cate FJ; et al., Safety and efficacy of a new transpulmonary ultrasound contrast agent: initial multicenter clinical results. J Am Coll Cardiol 1990;16:316-24), (5) Crouse LJ, Cheirif J, Handy DE, et al., Opacification and border delineation improvement in patients with suboptimal endocardial border delineation in routine echocardiography: Results of a phase III Albunex multicenter trial. J Am Coll Cardiol 1993;22:1494-1500, (6) Porter TR, Feng X, Kricfeld A, Chiou A, et al., Improved endocardial border resolution during dobutamine stress echocardiography with intravenous sonicated dextrose albumin. J Am Coll Cardiol 1994;23:1440-3, (7) Yvorchuk KJ, Sochowski RA, Chan K-L, Sonicated albumin in exercise echocardiography: Technique and feasibility to enhance endocardial visualization. J Am Soc Echocardiogr 1996;9:463-9, (8) Reilly JP, Tunick PA, Timmermans RJ, et al., Contrast echocardiography clarifies uninterpretable wall motion in intensive care unit patients. J Am Coll Cardiol 2000;35:485-90).
All these reports emphasize the importance of using ultrasound contrast agents to improve in the yield and accuracy of detecting the cardiac endocardial borders, wall motion, and wall thickening during stress echo.

An important report by Rainbird et al.(9), in 2001, assessed the clinical utility of using Optison™ in 300 hundred consecutive patients studied at Mayo Clinic in Rochester, Minnesota. The authors concluded that ultrasound contrast agents enhanced the endocardial definition of all patients and was particularly useful in those patients that had poorer quality images. Their conclusion resonated with the data that has been consistently reported; that is, the use of ultrasound contrast agents with harmonic imaging substantially improves the clinical diagnostic yield when performing non-invasive stress testing.

Results from our echocardiography laboratory (10) are consistent with the observations that of Rainbird et al., (9) and those findings of the earlier reports (4-8). Initially, we determined the positive predictive value (true positives determined by coronary angiography divided by all positives) of two groups of patients undergoing stress echocardiography. In the initial data set, we retrospectively examined six months of stress echo studies that did not use ultrasound contrast agents versus an additional six months in which 100% of the consecutive studies used ultrasound contrast and harmonics. Both groups consisted of forty-eight patients with documented positive stress tests and subsequent confirmatory coronary angiography. For the patients in the first group, the positive predictive value was 73% with a technical difficulty rate of 9%.

In the second group of patients, the positive predictive value was 85% and an associated technical difficulty rate of <1%. Subsequently, we have analyzed the results of over 5,000 stress echo examinations with the use of harmonic ultrasound contrast agents. The results are consistent with our conclusions generated from our initial observations, that is, ultrasound contrast agents when used with stress echo testing improve diagnostic yield and clinical utility. Overall, our clinical results continue to support the widespread use of ultrasound contrast agents to improve the positive predictive value of diagnostic stress testing.

After further analysis, our positive stress echo "call rate" was found to be statistically lower with the use of ultrasound contrast agents than in the group of patients that did not receive contrast agents. However, our positive predictive rate was substantially and statistically higher in those instances when contrast agents were used. These data appear to be paradoxical, however, if the positive predictive rate is more accurate with ultrasound contrast, and there is a decrease in the positive "call rate"; then by inference, there must be an increase in the true positive rate while reflexively, a decrease in the false negative rate (not directly confirmed). Further, the yield of technically "adequate" studies was substantially improved with the use of ultrasound contrast agents and, as such, has profound cost-efficient implications for stress testing.

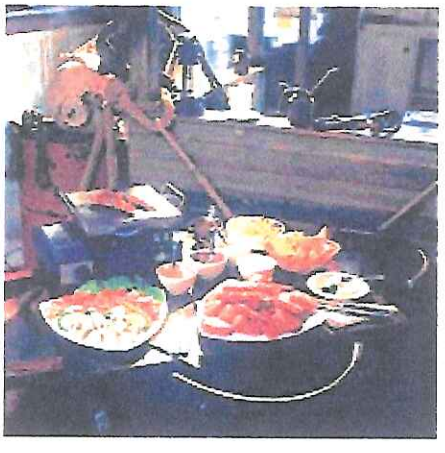
Future: Finally, we suggest that through the continued utilization of ultrasound contrast agents and the associated ultrasound equipment improvements, accurate quantitation of myocardial perfusion will be achieved. Oft times reported as "just around the corner", the use of myocardial perfusion as an adjunctive aid in the diagnosis of ischemic cardiac tissue may, in fact, is coming of age. Although, not yet a practical addition to our clinical diagnostic armamentarium, myocardial perfusion with contrast echo techniques ultimately may prove to be a sensitive indictor of tissue ischemia when used with pharmacologic vasodilators or in conjunction with wall motion and wall thickening. Further, the most recent clinical trials have correlated stress echo perfusion studies with complementary nuclear imaging techniques. The comparative results have been promising and have been recently published. The exciting topic of myocardial perfusion with stress echo techniques is the subject of the subsequent reports in this supplement.

Conclusion: The non-invasive diagnostic testing of patients suspected of having ischemic heart disease has been substantially enhanced with the use of ultrasound contrast agents and harmonic imaging. There exists a substantial database to support an evidence-based approach to routinely using ultrasound contrast agents with non-invasive stress testing. Currently, wider application of these ultrasound contrast agents with stress testing is supported by the existing literature based upon numerous clinical studies. Most recently, Tardif et al. published their results of the economic impact of using contrast stress echocardiography to diagnosis and treatment of patients with suspected coronary heart disease. They stated in their conclusion…

"Contrast echocardiography has a similar success rate to nuclear perfusion imaging in diagnosing CAD, but has a 28% lower cost and has the potential to of additional cost savings through the elimination of further diagnostic tests (11)."

References:

1. Exercise Echocardiography or Exercise SPECT Imaging? A Meta-analysis of Diagnostic Test Performance. Fleischmann KE, Hunik MGM, Kuntz KM, Douglas PS. JAMA 1998, Vol 280(10):913-920.
2. Marwick TH, Colin C, Vacey C, et al., and Prediction of Mortality by Exercise Echocardiography: A Strategy for Combination with the Duke Treadmill Score. Circuation 2001; 103:2566-2571.
3. Poldermans D, Fioretti PM, Boersma E, et al., Long-term prognostic value of dobutamine-atropine stress echocardiography in 1737 patients with known or suspected coronary artery disease: A single-center experience. Circulation 1999 Feb 16;99(6):757
4. Feinstein SB, Cheirif J, ten Cate FJ, et al., Safety and efficacy of a new transpulmonary ultrasound contrast agent: initial multicenter clinical results. J Am Coll Cardiol 1990;16:316-24.
5. Crouse LJ, Cheirif J, Handy DE, et al., Opacification and border delineation improvement in patients with suboptimal endocardial border delineation in routine echocardiography: Results of a phase III Albunex multicenter trial. J Am Coll Cardiol 1993;22:1494-1500.
6. Porter TR, Feng X, Kricfeld A, Chiou A, et al., Improved endocardial border resolution during dobutamine stress echocardiography with intravenous sonicated dextrose albumin. J Am Coll Cardiol 1994;23:1440-3.
7. Yvorchuk KJ, Sochowski RA, Chan K-L, et al., Sonicated albumin in exercise echocardiography: Technique and feasibility to enhance endocardial visualization. J Am Soc Echocardiogr 1996;9:463-9.
8. Reilly JP, Tunick PA, Timmermans RJ, et al., Contrast echocardiography clarifies uninterpretable wall motion in intensive care unit patients. J Am Coll Cardiol 2000;35:485-90.
9. Rainbird AJ, Mulvagh SL, Oh JK, McCully RB, et al., Contrast dobutamine stress echocardiography: Clinical practice assessment in 300 consecutive patients. Am Soc Echocardiogr 2001;14(5):378-85.
10. Nathan S, Lee J, Sandelski J, Almeda F, Weiss K, Macioch J, Liebson PR, Parrillo J, Feinstein SB. Improvement in Positive Predictive Value with Routine Use of Echocatrdiographic Contrast and Harmonic Imaging in 4,865 Patients Undergoing Stress Echocardiography. Circ 2001;104(17):II-674.
11. Tardif J-C, Dore A, Chan KL, et al. Economic Impact of Contrast Stress Echocardiography on the Diagnosis and Initial Treatment of Patients with Suspected Coronary Artery Disease. J AM Soc Echocardiogr 2002;1335-45.



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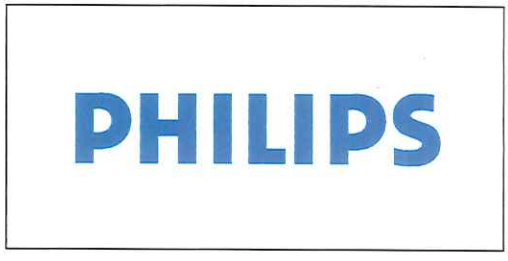

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