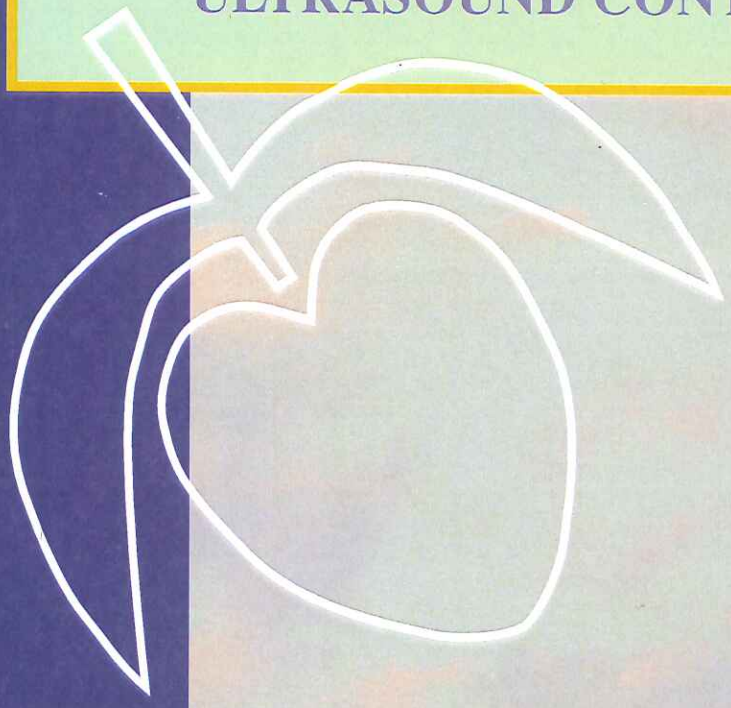
THE SEVENTH EUROPEAN SYMPOSIUM ON ULTRASOUND CONTRAST IMAGING



The Seventh
European Symposium

on Ultrasound
Contrast Imaging

Rotterdam
The Netherlands

Folkert J. Ten Cate, MD
Nico de Jong, PhD
David O. Cosgrove, MD

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ABSTRACTBOOK

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January 24-25, 2002

7th EUROPEAN SYMPOSIUM ON ULTRASOUND CONTRAST IMAGING
24 - 25 JANUARY 2002, Rotterdam, The Netherlands

WEDNESDAY, 23 January 2002

18.00 - 20.00 **Registration - Welcome Drinks - Posters** ..... **Inntel Hotel**

THURSDAY, 24 January 2002

08.00 - 09.00 **Registration**

09:00 - 09:05 **Opening address by D.O. Cosgrove**

09.05 - 10.35 **CARDIAC AND OTHER APPLICATIONS**

..... *Chairpersons: S. Feinstein and F.J. Ten Cate*

Discussion

10.35 - 11.00 **Intermission**

11.00 - 12.30 **TECHNOLOGY I** ..... *Chairpersons: N. de Jong and M. Versluis*

Discussion

12.30 – 14.00 **Lunch**

14.00 - 15.45 **RADIOLOGY** ..... *Chairpersons: D.O. Cosgrove and M.J. Monaghan*

Discussion

15.45 – 16.15 **Intermission**

16.15 – 17.45 **FUTURE DIRECTIONS** ..... *Chairpersons: F.J. ten Cate and P. Burns*

Discussion

18.45 - 22.30 **SOCIAL EVENT (incl. Dinner buffet)** 80

FRIDAY, 25 January 2002

THE ROLE OF CONTRAST DURING STRESS ECHOCARDIOGRAPHY

Mark J. Monaghan

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Contrast now has a well-established role for enhancing endocardial borders during routine stress echo studies. Unfortunately, sub-optimal image quality is the Achilles heel of this technique and leads to poor inter and intra observability and a lack of diagnostic confidence. The use of contrast in patients with sub-optimal stress images has been shown to help address these problems. More recently, low MI, contrast specific imaging modalities have been developed to facilitate real time myocardial perfusion imaging. However, these imaging modalities may also be used for endocardial border enhancement, since they provide excellent contrast sensitivity, with minimal contrast destruction and at frame rates greater than 25 Hz which makes them applicable during stress echo studies. These new methodologies have significant advantages over conventional harmonic imaging for contrast left ventricular opacification studies. In addition, they may be used with automated endocardial border detection techniques to assist with the evaluation of regional wall motion. New software has been developed which will also facilitate quantification of wall motion during stress using this technology. This should further enhance reproducibility of this diagnostic technique.

The classical ischaemic cascade demonstrates that myocardial perfusion abnormalities occur before wall motion and thickening abnormalities during stress induced ischaemia. Contrast echocardiography has a potential to demonstrate myocardial perfusion during stress and therefore, in theory, this should enhance the sensitivity for the diagnosis of reversible ischaemia, if this can be performed in a reliable and reproducible manner. Two alternative approaches are currently utilised for stress perfusion imaging.

High MI, destructive imaging modes such as Harmonic Power Doppler (Angio) utilise contrast micro bubble destruction to facilitate detection of myocardial perfusion. This imaging modality requires intermittent imaging and therefore wall motion information is not available simultaneously with perfusion information. However, with certain contrast agents, this is an extremely sensitive technique and does appear to work well when coronary vasodilators such as Adenosine or Dipyridamole are used as stress agents. Several studies have demonstrated the ability of this approach to document both areas of reversible and irreversible ischaemia. Since this technique is entirely perfusion based, it is best applied utilising a coronary vasodilator rather than an inotrope such as Dobutamine, which mainly induces wall motion/thickening effects and may also produce wall motion artifacts. Although this

destructive approach is a very sensitive technique for detecting myocardial perfusion, it does have a number of disadvantages, these include difficulties in setting the triggering point to avoid wall motion artifacts, the need to adjust the triggering interval during stress, difficulties in maintaining a consistent scan plane during intermittent imaging, the presence of artifacts and, as previously mentioned, lack of wall motion information. Despite these limitations, this technique is undergoing extensive clinical validation at present.

As previously mentioned, low MI, contrast specific imaging modalities such as power modulation, power pulse inversion and cadence imaging have been developed in order to permit evaluation of myocardial perfusion during real time imaging so that wall motion can be assessed simultaneously.

These techniques appear to have slightly lower sensitivity for contrast than the destructive imaging modalities. In particular, myocardial segments in the far field are more difficult to demonstrate perfusion in because of poor penetration of the low MI signal. However, early studies have shown the ability of this technique to demonstrate changes in myocardial perfusion, or more correctly, myocardial blood volume, during stress-induced ischaemia. Furthermore, since these techniques also provide excellent contrast endocardial border enhancement, wall motion information is not lost and perfusion data may be considered complimentary. The improved spatial resolution of the latest versions of this technology allows differentiation of sub endocardial from transmural perfusion defects. Sub endocardial ischaemia will occur at an even earlier phase in the ischaemic cascade and should further enhance the sensitivity for detection of reversible ischaemia.

Contrast agents may be administered in 2 different ways during stress. A steady contrast infusion has a potential advantage of providing more reproducible results and allows for flash reperfusion imaging. During this technique a small number of high MI frames are generated. These frames destroy contrast within the myocardium and then the reperfusion rate may be monitored in individual segments. This potentially allows quantification of myocardial blood flow and should also facilitate the differentiation of sub-endocardial from transmural ischaemia. However, many contrast agents are not suitable for infusion and/or suitable infusion pumps are not available. In addition, infusions are less convenient to set up than bolus injections and require critical adjustment to ensure that enough contrast is administered to allow myocardial perfusion to be detected and at the same time avoiding contrast attenuation within the cavities by administering too much agent. Bolus contrast injections are easier, quicker and more economical to administer. However they have the significant disadvantage of a continuously changing contrast concentration. Nevertheless, this technique can be used by observing the contrast wash out phase during which, concentration is decreasing. At a critical point on the wash out phase, below the concentration at which the myocardium becomes saturated, it is possible to differentiate the transmural extent of perfusion. Early studies suggest that this is relatively easy to

apply during stress echo studies and may provide a convenient and sensitive marker of sub endocardial ischaemia.

Low dose Dobutamine stress echocardiography is widely used to evaluate myocardial contractile reserve, as a substrate of myocardial viability. The demonstration of myocardial perfusion using contrast echocardiography also has the potential to provide information on myocardial viability, by showing the integrity of the coronary micro-vasculature. Recent studies have demonstrated that this technique may be used in a number of different clinical settings, including post myocardial infarction, to predict functional recovery post MI or following revascularisation. In addition, the demonstration of micro-vascular integrity may enhance the sensitivity of low dose Dobutamine stress echo for detecting viability, by showing segments that may not have contractile reserve, but are viable and therefore may not be subject to remodelling following. A technique which could predict functional recovery and also absence of remodelling would be a very powerful tool in the management of chronic ischaemic conditions. The combination of myocardial contrast echo +/- low dose Dobutamine stress echo may provide that ability.

The use of contrast during stress has clearly moved from simply enhancement of endocardial borders to evaluation of myocardial perfusion and viability. Early studies suggest a significant role for contrast echo in this direction and multi centre studies are currently being planned which should help define future potential of this exciting technique.

MYOCARDIAL PERFUSION IMAGING USING AI-700

Jiefen Yao

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AI-700 is an ultrasound contrast agent that has been designed specifically for echocardiographic imaging of both wall motion and myocardial perfusion. AI-700 consists of perfluorocarbon gas within synthetic biodegradable polymer (PLGA) microspheres, engineered to have a mean particle diameter of approximately 2 microns to allow transpulmonary passage and to have a high degree of mechanical strength. AI-700 is less sensitive to the cavitational effects of ultrasound, resulting in prolonged myocardial enhancement. *In-vitro* and *in-vivo* results indicate that AI-700 has the potential to provide strong image enhancement with low visual attenuation that is superior to other widely used ultrasound contrast agents. The acoustic properties of AI-700 contribute to its ability to provide added value over non-contrast echocardiography in coronary artery disease (CAD) patients with a wide range of acoustic window quality.

Phase I and II clinical trials for safety and efficacy analysis of AI-700 have been completed in over 200 healthy volunteers and patients with CAD. The majority of adverse events observed following AI-700 administration were mild and transitory; no serious adverse events were observed. With a single bolus injection, AI-700 demonstrates prolonged myocardial enhancement of up to 5 minutes duration, allowing a thorough examination of multiple views from both apical and parasternal windows while using different imaging techniques. Myocardial perfusion has been demonstrated in subjects administered various doses of AI-700 using both low-power, real-time perfusion imaging and medium-power, triggered harmonic imaging techniques.

In an initial phase II trial, 18 healthy subjects and 35 patients with fixed myocardial perfusion defects by SPECT imaging were studied. Using SPECT imaging as the standard of truth, the majority results from 3 blinded reviewers of AI-700 contrast echo showed sensitivity, specificity, overall agreement and chance-adjusted agreement (kappa) values of 84%, 88%, 85%, and 0.68, respectively, for detecting myocardial perfusion defects. In a subgroup of subjects imaged with a dose of AI-700 optimized for each specific imaging technique, the sensitivity, specificity, overall agreement and kappa value were 94%, 86%, 92% and 0.80, respectively. The results also demonstrated that myocardial perfusion and real-time wall motion echocardiography with AI-700 is superior to non-contrast echo when wall motion alone is observed. AI-700 contrast echo imaging was also examined in a later phase II trial in patients with not only fixed perfusion defects, but also inducible myocardial ischemia. These results are not yet published.

CONTRAST AGENTS IN CAROTID ARTERY ULTRASOUND: DIAGNOSTIC AND PROGNOSTIC ROLE

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Introduction: Contrast agents have played a valuable role for many years in the multiple imaging applications of Radiology and Nuclear Medicine with established clinical utility in a wide range application. Virtually all imaging modalities use a contrast agent to provide additional anatomy or physiology information of the target organ. Ultrasound imaging, now no exception, has commercially available contrast agents that provide additional pertinent information to the clinician. These applications include intravascular arterial and venous use, as well as intracardiac and intracoronary arterial applications. Recently, the commercial approval of advanced generation albumin and lipid based ultrasound contrast agents composed of high molecular weight gases established the widespread clinical application of contrast ultrasonography. The advanced hardware and associated software technologies of the ultrasound imaging equipment permitted major advances in the delineation of myocardial endocardial borders, improved left ventricular opacification, and myocardial perfusion.

Extra-Cardiac Applications: These new ultrasound contrast agents that provide exquisite cardiac border definition are equally applicable to non-cardiac imaging including the lumenal borders of the vasculature throughout the arterial and venous system. An impressive compilation of scientific work accumulated over recent years directly applied these ultrasound-based technologic advances to the non-invasive assessment of atherosclerosis. These techniques provide a surrogate marker for cardiovascular risk and, as such, predict the likelihood of future cardiac events.

Carotid IMT Applications: Specifically, carotid artery intima-media thickness (IMT) measurements and left ventricular mass used as surrogate markers have been extensively validated. Furthermore, the therapeutic modification of associated cardiovascular risk factors in patients can be reliably monitored, thus, leading to improvement in the general quality and longevity of life for patients. The carotid artery wall thickness, specifically the intimal medial thickness, has been identified as an important predicator of future cardiovascular events. Ranges of normalcy and disease progression for carotid IMT, adjusted for age, have been published. However, the earlier described limitations that made the accurate measurement left ventricular endocardial borders in the heart difficult are equally observed when defining of the lumenal surface of the carotid arteries. The additional factors that limit the current accurate interrogation of the carotid IMT includes body habitus, difficult imaging windows,

tortuosity of vessels, patient cooperation and time frame limitations in acquiring data. Ultrasound contrast-enhanced carotid artery examination with advanced generation micro bubbles and improved software has greatly improved the sensitivity and specificity of these precise IMT measurements.

Physiologic properties and the direction and force of blood flow into the carotid system have been investigated as playing a role in the determination of IMT. Bioengineering models along with histopathologic studies of vascular rheology and accompanying wall stress revealed differential effects on location and thickness of the atherosclerotic process on the IMT. The ability to accurately delineate the IMT located at both the near and far wall of various segments of the carotid arterial system is critical in accurately assessing the associated risk factors. The individual importance of each arterial segment, including common carotid, carotid bulb bifurcation, and internal carotid artery have different expressions of importance in each study. The Plac-II investigators using statin therapy (Pravastatin^{R}) showed an important correlation of regression of maximal IMT over time; stressing the importance of accurate and reproducible measurements.
With regard to the validation of measurements of IMT based on therapeutic interventions, beta-blockers have long been known to have a favorable effect on long-term prognosis in post myocardial infarction patients. These agents, in general, have been shown to slow the progression of IMT. Other classes of pharmacologic agents such as statins, anticoagulants, calcium channel blockers, and diuretics have similarly shown limitation on the progression of atherosclerosis in the IMT; thus, further supporting the need for accurate measurement techniques.

Several studies have investigated topics to include stabilization and regression of IMT with interventions highlighting the benefit of medical therapy and atherosclerotic risk factor modification. Important clinical correlations have been established between the measurements of the carotid IMT and differing therapeutic approaches. As an example, the efficacy of medical therapy versus invasive coronary arterial angioplasty has been identified in patients with established coronary artery disease. The unresolved role of estrogen replacement therapy on IMT in the carotid arteries of postmenopausal women requires additional investigation with accurate IMT determinations. It is in these particular areas that the use of contrast-enhanced IMT may provide a substantial clinical and scientific benefit.

Summary: The importance of providing an accurate and reliable measurement of intima-media thickness (IMT) of the carotid artery as a surrogate marker of atheroscleosis for predicting cardiovascular events is increasingly recognized as critical to the advancement of this field. The use of the newer contrast agents adds confidence, reliability and reproducabilty to these measurements, and consequently, expands their usefulness for the area of carotid artery imaging.

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ASSESSMENT OF MICROVASCULAR TISSUE PERFUSION CHANGES USING THE FLASH-REPLENISHMENT CONCEPT AND CONSTANT INFUSION OF SONOVUE TM: A FEASIBILILTY STUDY

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Today the development of micro bubble contrast agents and new imaging modalities allows the spatial assessment of microvascular perfusion in different organs (mainly myocardium, liver and kidney). Quantitative assessment of regional microvascular perfusion abnormalities could provide important clinical insights into possible pathologies and further orientate patient management.

A major step forward has been achieved by the development of the "flash replenishment concept" which can be used to quantify the two specific components of microvascular blood flow: flow velocity and microvascular blood volume. The method of blood flow velocity quantitation uses a constant venous infusion of micro bubbles, followed by a rapid destruction of micro bubbles by ultrasound, and subsequent assessment of the bubble replenishment rate into the microvascular bed within the ultrasound beam elevation. In the same vascular bed, assessment of steady state video intensity provides a measure of capillary blood volume.
However, this technique requires a constant micro bubble concentration in the microvascular bed during the process of quantification. This can be achieved by a constant infusion of a second-generation contrast agent such as SonoVue™ using a novel syringe pump developed by Bracco.

Our first objective was to optimise the imaging parameters with SonoVue™ both during the destruction phase (number of destruction frames at high MI in function of SonoVue™ concentration in the microvascular bed) and during the replenishment phase (optimum low MI, frame rate and interval between two flash sequences) in real time imaging. This optimisation was performed with an ATL HDI5000, in Power Pulse Inversion mode (myocardial perfusion in minipigs) or in B-mode Pulse Inversion (kidney perfusion in rabbits or MATBIII implanted breast tumours in rats).

We then altered microvascular tissue perfusion either by provoking a stenotic situation in the myocardium (see figure I) or by infusing a vasoactive agent (Angiotensin II), which reduced kidney cortex blood, flow (see figure II) or increased tumour blood flow.

Our results showed that alterations due to stenotic conditions in the minipig myocardium and Angiotensin II in the rabbit kidney and rat tumour can be clearly demonstrated with the "flash-replenishment concept" under SonoVue™ infusion. The detection of ischaemic or infarcted areas, the monitoring of tumour growth and the detection of alterations in kidney blood flow are all areas of intense clinical and research interest and are offering new exciting opportunities in diagnostic imaging.

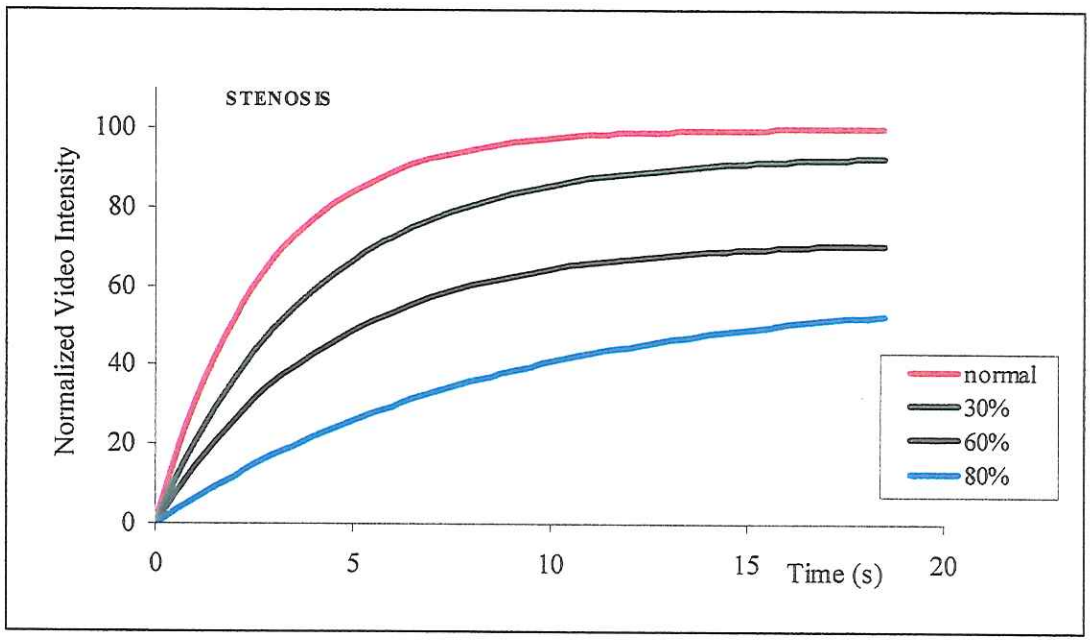

Figure I: myocardial replenishment curves under different stenotic conditions

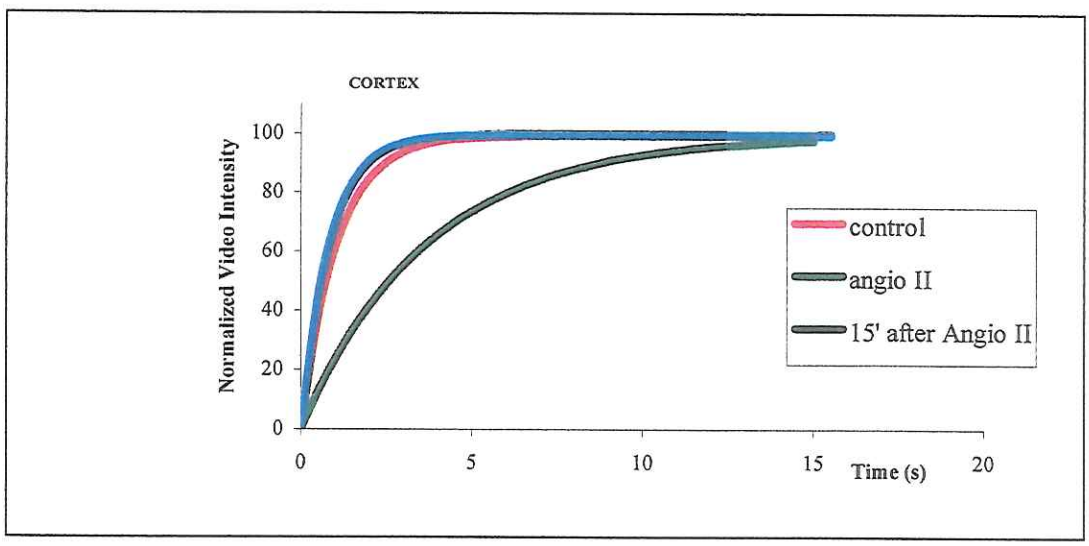

Figure II: Evolution of the kidney cortex replenishment curves under Angiotensin II infusion.

MYOCARDIAL PERFUSION WITH HARMONIC POWER DOPPLER AS CLINICAL ROUTINE

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In just a couple of years, the development of myocardial contrast echocardiography (MCE) has revealed to numerous methods to determine regional myocardial perfusion. The most important of these is Power Doppler Harmonic Imaging (PDHI). In experimental series, it could be demonstrated that qualitative, semi qualitative, and quantitative analysis of regional myocardial perfusion with PDHI is possible. The transfer from bench to clinical routine, however, has not yet occurred. The primary reasons for the delay are the current restricted diagnostic profits in the presence of relevant additional costs due to contrast agent usage, as well as the lack of standardized methodological instructions for the documentation of regional myocardial hypoperfusion using objective criteria. Using a standardized positive bolus injection technique or a standardized continuous infusion technique it was shown that a semi quantitative detection of regional hypoperfusion in clinical practice is possible according to an objective approach using MCE with PDHI. The main parameters to detect regional myocardial hypoperfusion with PDHI and MCE were the time course of Doppler intensity (DI) wash-in, the peak maximum DI-value, and the time course of DI wash-out using bolus injection protocol as well as the DI wash-in rate and the maximum plateau value using continuous infusion protocol. To determine input function and to avoid inconstant input function into the myocardium, an inverse bolus technique is normally performed in modern MCE methods, especially during real-time perfusion imaging. Several disadvantages, however, have to be considered if the replenishment curves are calculated in clinical practice. The variations of the DI upslopes are large, the amount of contrast agent for the infusion procedure is more expensive, additional staff is needed. In summary, the positive bolus technique using PDHI with MCE becomes more important due to high image quality in nearly all commercially available equipments and very low amounts of contrast agent in comparison to other new methods of myocardial perfusion measurements with MCE. The positive bolus application comes into fashion again, appears easier to handle than infusion technique in clinical practice and, especially during stress tests, numerous repetitive investigations are possible.

Conclusion: MCE using PDHI is suitable to detect regional myocardial hypoperfusion in clinical practice. Thus, PDHI seems to be one of the first objectively evaluated methods in contrast echocardiography and is at the threshold for the usage in clinical settings at bedside.

METHODS FOR PERFUSION IMAGING: A BRIEF REVIEW

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Power Doppler was the first, and remains the most popular method for the imaging of myocardial perfusion with ultrasound. While many of its shortcomings have been overcome by the advent of newer techniques such as pulse inversion and pulse inversion Doppler, these methods have not rendered power Doppler obsolete. Indeed, there are still situations where power Doppler is the imaging method of choice. Power Doppler methods arose from the observation that high MI ultrasound is capable of disrupting bubbles. When the shell is broken, free gas is released which is driven to resonance by the sound field, producing a strong, highly nonlinear echo of brief duration. These gas bubbles are unstable, and within a very short time diffuse into the surrounding medium. How long this takes is crucial to the efficacy of power Doppler: the shorter the better. Although the physics is too complicated for us to resolve a precise picture of the process, we know that bubbles of highly diffusible, highly soluble gases (such as air) disappear more quickly than those containing lower solubility gases such as perfluorocarbons, and we know that exposure to more intense, lower frequency (i.e., higher MI) ultrasound also speeds bubble dissolution.

The major challenge in myocardial perfusion imaging is to suppress the rather strong echo from the myocardial tissue (1). Because the echo from a disrupting bubble is rich in harmonics, harmonic imaging might seem the obvious detection method (2). However, at the high MI necessary to disrupt the bubbles, propagation (or "tissue") harmonic produces an intense echo from the muscle itself. It is therefore well known to be necessary to use some sort of subtraction procedure to image myocardial contrast agent. This requires registration of a baseline image with post contrast ones, a procedure that is time consuming, operator dependent and never completely effective. In essence, power Doppler performs this subtraction in real time (3). A series of two or more pulses are transmitted in rapid succession into tissue, in much the same way as they are for conventional colour Doppler. The first pulse begins bubble disruption, so that the echo from the second pulse is much weaker; typically the bubble and its echo have gone by the third or fourth. Successive echoes are subtracted, so that power Doppler detects the difference between echoes. For stationary tissue, power Doppler gives nothing, for a disrupting bubble a large signal is produced. The image thus produced is really an image of 'decorrelation', that is, the degree of change in the echoes between pulses. Because the entire bandwidth of the signal is used, the method can produce images of very high resolution. Because the echo from a bubble disrupting is stronger than that which it gives when not disrupting, it is a very

sensitive, in fact at present the most sensitive, way to detect bubbles in high dilution when hidden by tissue. The method is typically implemented as an overlay to a greyscale image, which might be in fundamental or harmonic mode.

There are, however, problems. For perfusion imaging, this is not a real time method. Once bubbles have been destroyed in the microcirculation, it takes a number of seconds for new bubbles to reperfuse the imaged area, so triggering is necessary. If the tissue moves between pulses, its echo decorrelates (which is, in fact, the Doppler effect), producing a signal seen as a flash artifact. The key is to arrange the bubble echo to decorrelate faster, so that the sequence of pulses can be sent before the tissue has had time to move. This requires a high pulse repetition frequency and is one reason that air-based agents work so well with power Doppler. It is also possible to use harmonic filtering to help with tissue motion suppression, though this is limited by the same tissue harmonic effect described above. Practical expedients to subdue motion artifact in the myocardium include careful attention to the trigger timing, for which the onset of the t-wave is a good starting point. It often appears that power Doppler images have a lower dynamic range than greyscale ones, but it should be appreciated that this is not a fundamental consequence of the method, but rather a reflection of the way in which it is implemented in many scanners. The power Doppler dynamic range should be placed against that of the final, subtracted image when comparing it to greyscale harmonic imaging. Perhaps the most fundamental physical limitation of the method is the requirement that bubbles must be disrupted in all regions of the field of view in order to see perfusion in all the imaged segments. The transmit field is not, however uniform, the peak negative pressure (and therefore the MI) decreasing with depth and lateral location in the sector. New transducer and transmitter technology is required to address this problem effectively; it is not clear to many manufacturers that this investment would currently be justified.

Power Doppler performs best when sensitivity to contrast, rather than real time imaging, is the major objective. It is also best with air-based agents and when tissue motion is not too severe. It is heavily dependent on the uniformity of the transmit field, something that a new generation of beamformers may be able to help with. It is the method of choice for destruction-reperfusion imaging when accuracy is valued over speed. It remains the most popular approach to perfusion imaging because of its intuitive and, compared to other methods, relatively consistent performance. It has many proponents, who keep it in reserve for whenever low MI, real time methods fail.

Greyscale harmonic imaging forces an inherent compromise between image resolution and contrast that limits its sensitivity to nonlinear signals. Overlap in frequency between the fundamental and harmonic echoes results in linear echoes being detected in the harmonic signal, reducing contrast. Narrowing both the transmit and receive bandwidths reduces these effects, but at the expense of image

resolution. This compromise limits both the resolution and contrast of harmonic imaging, and is especially significant at low transmit pressures when harmonic echoes are weak. The loss of resolution that accompanies adequate tissue suppression effectively rules this method out for radiological applications.

Pulse inversion imaging overcomes these limitations of harmonic imaging by detecting nonlinear echoes over the entire transducer bandwidth (4). It exploits the fact that second harmonic nonlinear echoes from both microbubbles and tissue are caused by an asymmetric response to regions of high and low pressure in the transmitted sound. In pulse inversion, two ultrasound pulses are transmitted down each line of sight, with the phase of the second pulse inverted. When the corresponding echoes are added together, the linear component cancels but the nonlinear even harmonic components reinforce to produce a strong signal. By exploiting differences between echoes rather than within a single echo, pulse inversion imaging removes the fundamental component (and other odd harmonics) even when the fundamental and second harmonic overlap, thus overcoming the limitations of harmonic imaging. In particular, it allows microbubbles to be detected with high resolution at low transmit intensities, making possible realtime contrast perfusion imaging. At higher transmit pressures, pulse inversion imaging also offers benefits for tissue harmonic imaging.

Target motion between pulses results in incomplete removal of the fundamental echoes, introducing a fundamental component into the pulse inversion signal that is approximately proportional to target velocity. While motion sensitivity aids in the detection of moving or disrupting microbubbles, it may introduce artifacts from moving tissue in cardiac applications. The principles of pulse inversion imaging may be extended by transmitting more than two pulses of alternating polarity along each line of sight, a generalisation which we call pulse inversion Doppler (4). Doppler frequency filters can now be applied to the detected echoes to provide improved suppression of moving tissue compared to the two-pulse method. Filters can be tailored for specific applications, such as contrast perfusion imaging, tissue harmonic imaging or bubble disruption imaging. At low incident pressures pulse inversion Doppler has provided the first real time perfusion images of the myocardium (5).

It is likely that current nonlinear contrast methods only scratch at the surface of what is possible. The major focus is on the controlled induction of stable nonlinear oscillation of the bubbles and the detection of the echoes which result. Both are strongly influenced by the exact form of the bubble excitation, which is likely to be the focus of more work in the immediate future. Whereas the majority of current methods for detecting nonlinear bubble oscillation use a two or more phase or amplitude modulated pulses, a general understanding of the way in which nonlinear echoes result from a sequence of pulses with both forms of modulation is just emerging. Combining phase and amplitude modulation using Doppler methods offers the opportunity to explore higher order bubble harmonics,

and new means to separate tissue harmonics from bubble echoes. The simple combination of amplitude and phase modulation in a 4-pulse inversion Doppler excitation allows the detection of odd-order harmonic energy at the fundamental, which at low MI can offer about 5dB additional perfusion signal. The effective tissue-to-bubble contrast can be increased further by the refinement of the traditional segmentation algorithms used in colour processing. Finally, great gains stand to be made in the manufacture of bubbles. It is remarkable that we have done so well with bubbles that were designed with nothing but the crudest notion of how they would behave acoustically. For example, bubbles that produce continuous nonlinear echoes without disruption at reasonably high MI's (say, 0.5) would offer a significant improvement in image signal-to-noise ratio in perfusion imaging.

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MAKING CONTRAST CLINICALLY USEFUL: TRIGGERED REPLENISHMENT IMAGING

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Summary: A new contrast imaging technique called Triggered Replenishment Imaging (TRI) has recently been introduced. The method combines low emission pulse inversion imaging with triggered imaging and Flash. The result is improved sensitivity over Real-time Perfusion Imaging, faster and easier data acquisition compared to intermittent triggered imaging, immediate review of perfusion images on the ultrasound machine, and faster and easier data transfer for storage and off-line perfusion analysis.

Abstract: Low mechanical index Pulse Inversion ultrasound has made real-time perfusion imaging a clinical reality. The previous technique of high mechanical index intermittent triggered imaging (ITI) required the clinician/sonographer to capture a series of "snapshots" (single frame) of contrast enhancement over a staggered series of cardiac cycles. Advantages of the technique include high signal to noise ratios, the ability to image deep cardiac segments and difficult to image patients, and images which are time gated with respect to the cardiac cycle for ease of post-processing. Although the validity of ITI has been established both experimentally and clinically (Wei, et al; 1997; Wei, et al; 2001), the method has not been widely adopted for routine clinical use. The most commonly cited reasons include the long amount of time required to collect the image data (>1 minute), contrast agent consumption, artifacts due to patient and respiratory motion, and sonographer skill required to maintain a steady scan plane during long triggering periods. Additionally, he method exposes the patient to significant durations of high mechanical index ultrasound with micro bubbles prior to the procedure, and between views. Recent experimental research has confirmed that it is prudent to maintain ultrasound emission levels "as low as reasonably allowed" due to the potential of micro bubble expansion and microvascular rupture. (Skyba, et al; 1999)

Real-time Perfusion Imaging (RTPI) allows the clinician to visualize contrast at frame rates up to 30 Hz. After infusion of contrast agent, the sonographer simply scans the required views in the usual way. A steady scan plane must be maintained, however, image feedback at a high frame rate allows the sonographer to do this easily. When coupled with a Flash, micro bubbles are predominantly destroyed during the high MI flash, and replenishment can be visualized at low MI in real-time. This reduces the time required to collect data for a particular view from greater than 1 minute to

approximately 15 seconds. A short breath-hold by the patient ensures that respiratory and other movements are minimized during the critical image collection period. Experimental and clinical studies have shown that Flash-RTPI yields results comparable to intermittent triggered imaging and is generally regarded as being easier to use (Tiemann, et. al.1999, Lafitte, et. al. 2001)

Despite the success of Flash-RTPI, one significant drawback confounds the method: low emission power yields a low signal to noise ratio, and micro bubbles are destructible even at low emission power. Since sensitivity of detection of signals from contrast micro bubbles is paramount in obtaining perfusion information from difficult to image patients and from deep myocardial segments, the steady-state concentration of micro bubbles must be preserved. Real-time imaging may cause a decrease in the concentration of the micro bubbles both before and during the time course of the image collection, therefore decreasing the signal available for perfusion visualization. Another drawback is that the amount of image data collected for perfusion analysis is large (~20 frames/sec * 15 sec * 3 views * 5 stage stress protocol = 4500 frames). The trend of cardiac echo labs is moving toward digital streaming and storage of ultrasound data. Since most perfusion analysis is performed off-line, this amount of digital data becomes cumbersome to transfer and store.

A hybrid method of contrast imaging called Triggered Replenishment Imaging (TRI) has recently been introduced. The goals of TRI are: 1) to improve contrast sensitivity, 2) decrease micro bubble destruction, 3) improve ease-of-use, 4) provide immediate replenishment visualization and 5) ease off-line post-processing for replenishment analysis. TRI allows the user to image intermittently, as in ITI, but at one frame per cardiac cycle. This allows the user to easily establish the imaging plane and maintain a steady scan head position. Initial imaging is at low MI, thereby further conserving micro bubbles and maximizing sensitivity. One button press fires a series of high MI Flash frames to destroy the contrast agent in the scan plane. Replenishment is immediate, and can be easily visualized at the 1 image per cardiac cycle rate. Similar to real-time perfusion imaging, the full myocardial replenishment can be observed in about 10-15 frames (~10-15 sec). Table 1 compares typical cineloop acquisition times for ITI, RTPI and TRI. Both TRI and RTPI allow the user immediate review of the image series in the ultrasound system memory. However, an additional advantage of TRI is that the perception of perfusion is not confounded by cyclic variations in contrast intensity occurring over the cardiac cycle.

Table I. Typical transfer times for Flash replenishment cineloop images from an ATL HDI5000 ultrasound system to a magneto-optical disk (MO) using ResearchLink. Transfer time from MO disk to an off-line PC is not shown, but may be estimated by doubling the values shown.

Acquisition Mode	Notes	Total Number of Frames	Scanning Time (sec)	Data Transfer Time (sec)	Data Storage Size (Mb)
ITI	3 frames each at 1:1, 1:2, 1:3, 1:4, 1:8, 1:10, and 1:12	18	130	40	2.0
RTPI	15 cardiac cycles @ 20 Hz	300	15	255	20.7
TRI	15 cardiac cycles @ 1:1	15	15	20	1.8

Off-line analysis software such as HDILab can be used to more easily quantify myocardial flow and volume after TRI. Table 1 shows typical transfer times and storage requirements of digitally stored images from an ultrasound system memory to magnetic optical media (HDI5000, Philips Ultrasound, L10.4 software) using ITI, RTPI, and TRI. A cineloop acquired using TRI contains only the essential information required for perfusion analysis. Trimming the cineloop requires only setting the first and last frames and possibly cutting a single spurious frame. Background subtraction is only required if the Flash frames did not fully destroy the contrast agent in scan plane. Figure 1 shows the Time Strip and image sequence of a typical TRI dataset. Figure 2 shows the associated replenishment curve created by setting a ROI in the apex.

Finally, since TRI images can be quickly reviewed in their entirety on the ultrasound system prior to data transfer, poor data may be deleted and reacquired immediately during the examination. This makes TRI particularly attractive over techniques such as ITI or RTPI and it is hoped the method will be adopted for routine clinical use.

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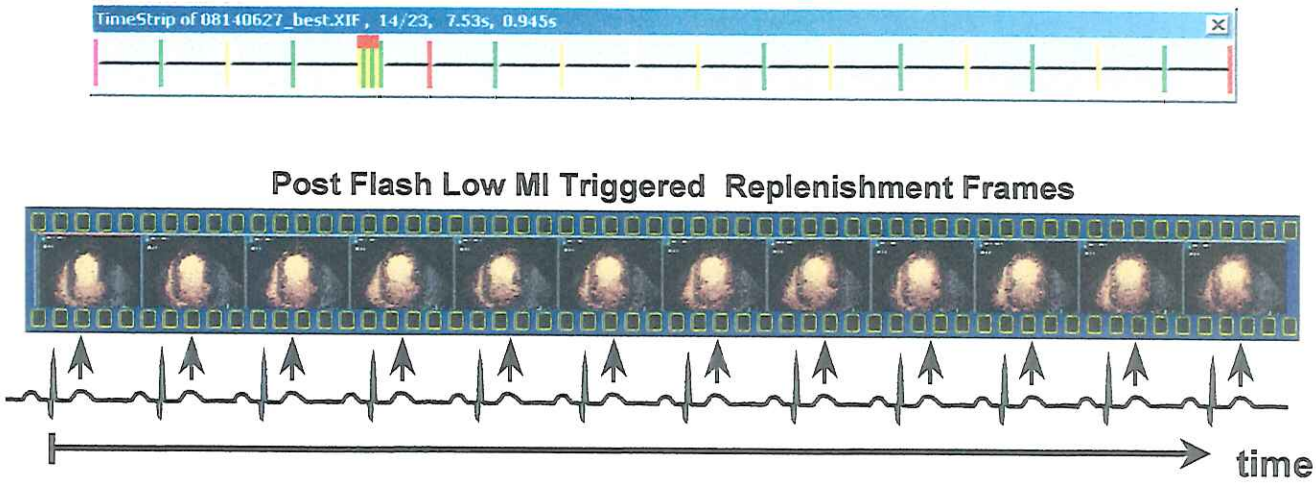

Figure 1. A Triggered Replenishment Imaging (TRI) cineloop showing timing information in the HDILab Time Strip (above). The time strip and ECG indicate that only one image is acquired to cineloop memory per cardiac cycle. In this example, the time strip shows 4 low MI triggered frames followed by a Flash sequence consisting of 4 high MI frames at a real-time frame rate. Following the Flash, low MI triggered frames at a 1:1 triggering interval capture the contrast replenishment. For analysis the user has edited the loop by selecting the first post-flash low MI frame as the first frame. Note that full replenishment has occurred by the 12th frame (set as the last frame).

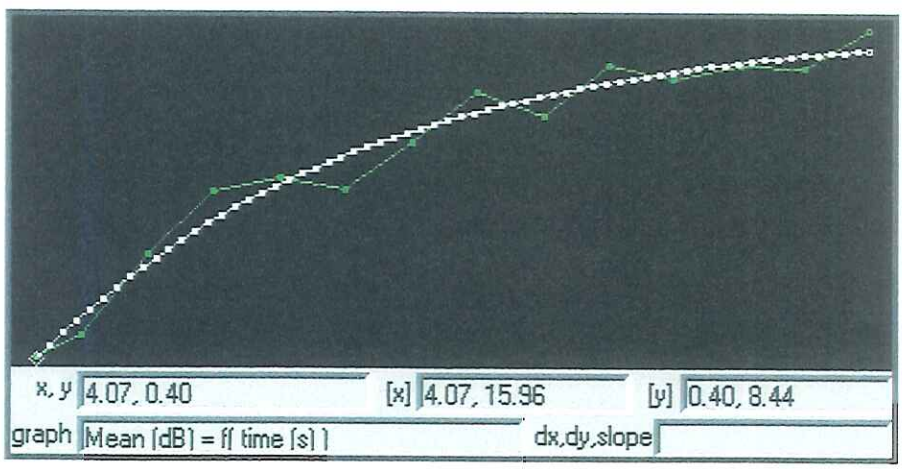

Figure 2. A typical Triggered Replenishment Imaging replenishment curve. The edited data from the cineloop in figure 1 is plotted. A one-minus-exponential curve (white) is fit to the original data (green).

REAL-TIME MYOCARDIAL CONTRAST ENHANCEMENT WITH ULTRAHARMONIC-MODE

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Background: Levovist® was one of the first Ultrasound Contrast Agents crossing the pulmonary capillary bed and reflecting echo signals from the left ventricle and whole vascular system. It also belonged to the first agents providing diagnostic myocardial perfusion echocardiography (MPE) images in human. However, during MPE micro bubbles are destroyed by high amplitude ultrasound waves and the slow blood flow in arterioles and capillaries is not sufficient to refill them within the short frame-to-frame interval. To overcome that hurdle, MPE was first carried out in intermittent imaging with Harmonic Power Doppler, with which impressive diagnostic results in human were achieved.

However, intermittent imaging may cause a lack of intermediate image information and creates difficulties with keeping the transducer position during patient breathing and movement. Additional problems with equipment settings have limited the reproducible clinical use of MPE to few experienced investigators.

The current method to assess myocardial perfusion is observation of the contrast replenishment in the myocardium after initial destruction of almost all of the agent by a short high energy pulse. Comparing neighboring segments or the same segment at rest and stress, changes in the time course and intensity of replenishment indicate reduced perfusion. Recently, first clinical results on continuous myocardial perfusion imaging with the Philips Sonos 5500 system and Levovist® infusion were reported. The ultraharmonic-mode is specifically designed to improve the signal to noise ratio of USCM and provides MPE with Levovist® even in continuous imaging mode.

Method: In an orientating dog study (n=9), we assessed the potential of the ultraharmonic-mode for MPE with Levovist®. The influence of different device-settings on myocardial contrast enhancement was determined and particular device-settings to achieve good real-time MPE images in animal were established.

Results: Sufficient real-time MPE images were achieved with both, ultraharmonic and harmonic-power-angio-mode, even with high MI in all dogs. The perfusion signals clearly differed between smaller and smallest coronary vessels and well-distinguished regions of "normal" replenishment from areas of delayed and reduced replenishment. The identification of effective device-settings was less complicated as with other devices and agents previously tested in our laboratory.

Conclusion: During the recent years, we perceived Levovist® to be less suited for continuous imaging than newer agents. This is our first report on surprisingly good continuous MPE results with Levovist® and the ultraharmonic-mode of the Philips Sonos 5500 device in animal. We are optimistic that a translation into clinical practice will overcome part of the obstacles to the broader use of MPE. Based on less limiting device-settings, we are also optimistic, that more investigators can achieve better comparable and reproducible results in more patients.

According to the 2001 revision of the ACC/AHA Guidelines for Percutaneous Coronary Intervention (PCI), PCI in comparable subjects leads to similar results than CABG surgery. The two exceptions are the better survival of diabetic patients with CABG and the higher need for revascularisation with PCI. A mono-centric clinical study is planned to investigate the combination of Levovist® with the Agilent ultraharmonic-mode. MPE data of the effects of macro vascular stenosis and microvascular flow impediment on infarct size and reflow may predict the recovery of regional myocardial function and outcome of diabetic AMI patients after PCI.

IMPROVED CONTRAST IMAGING WITH A 1.5 D ARRAY TRANSDUCER

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Conventional phased array transducers are focussing in the scan plane (azimuth direction) by adding delays to the different elements which are distributed evenly in a 1-dimensional row. Beam focussing in the elevational direction (perpendicular to the scan plane) is done acoustically by a fixed lens with variable thickness and elevation focus is hence fixed to one depth. As a compromise between near- and far-field the fixed elevation focus is normally placed in the centre of depth of interest of the given probe. When the users are adjusting focus only the azimuth focus is actually changed. Dynamic focussing on receive is also limited to one dimension for the same reasons.

The 1.5 dimensional transducers recently introduced in cardiac imaging has several elements in the elevational direction as well as in the scan plane direction, and is therefore referred to as *matrix array transducers*. By adding delays to these elements both in azimuth and elevation direction, focussing on transmit as well as dynamic focussing on receive is possible in two dimensions.

The major advantage for contrast imaging with this type of transducer is considered the more homogenous pressure fields. Since bubbles are extremely pressure sensitive a more homogenous beam leads to a significant improvement in contrast imaging. Dynamic aperture and apodization in the elevational plane are other features only possible on 1.5 D array transducers. These in turn will contribute to thinner scan slices and lower side lobe levels, of which the latter may reduce artificial myocardial signals originating from the cavity.

A paper will be presented that will describe and illustrate the advantages of 1.5 D array transducers compared to conventional phased array transducers used in contrast imaging.

SUPER-LOW MI REAL TIME IMAGING AND REPLENISHMENT IMAGING OF THE LIVER

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Introduction: There are two categories of ultrasound contrast agents, high MI agent and low MI agent. Levovist is belonging to the former category and Optison, SonoVue, Definity, and Imavist are the latter. Sonazoid can be recognized as an intermediate one.

Optimized modes of contrast imaging for Levovist have been developed recently; such as Coded Harmonic Angio (CHA from GE), Agent Detection Imaging (ADI from Acuson) and Advanced Dynamic Flow (ADF from Toshiba). These methods can be called as Doppler-like multi-pulse imaging. The signals derived from these methods are from destruction of micro bubbles, which are flowing in the blood and accumulating in the sinusoidal space.

Super low mi real time imaging: Optimized method for low MI agents can be called as "super-low MI imaging". Super-low acoustic pressure in this case means that the mechanical index (MI) is less than 0.1. The acoustic pressure should be controlled between the thresholds of resonance and destruction according to the characteristics of each agent. For example, the optimal MI is 0.05 for SonoVue.

Tissue harmonic signals from the liver under such low MI are so small that phase (pulse) inversion harmonic imaging can make the signal to noise ratio increased, because the acoustic pressure is so low that almost no tissue harmonic component is produced. Signals derived from super-low MI imaging are originated by resonance of micro bubbles but not destruction. Therefore, micro bubbles can emit non-linear signals continuously during many frames and therefore the frame rate can be increased. Small number of echo pulses can make the frame rate increased because Doppler like sequence is not necessary.

Clinical application of super-low MI imaging in the liver is detection of space occupying lesions of the liver. The whole liver can be searched using this method to detect the space occupying lesions as higher intense areas or lower intense ones than the normal liver tissue, that is, hyper-vascular lesions can be recognized as higher intense area and hypo as lower.

Replenishment imaging: Characterization of the lesions which were detected with low MI real time imaging can be performed by using "replenishment imaging". This is composed of low MI imaging and manual insertion of high MI pulses to destroy the micro bubbles occupying the capillary or sinusoidal vascular beds in the scanning plane. Low MI pulses following destruction of micro bubbles with high MI makes replenishment again with fresh micro bubbles in the scanning plane.

We can observe vascular imaging over and over by using replenishment method after or during infusion of contrast agent. Morphological changes of tumor blood vessels can be evaluated repeatedly. Replenishing speed might be different between tissues which blood is supplied from hepatic artery or portal vein. Quantification of the slope of the replenishment curve can help differential diagnosis from above mentioned mechanism.

MULTIPULSE TECHNIQUES

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Low MI multipulse approaches provide the opportunity to image microbubble contrast agents in real-time both in the microcirculation as well as the larger vessels. They work because at low MI the bubbles are not disrupted. In addition, at these low powers the response of the bubbles is still non-linear while the tissues behave in a linear manner.

The key to differentiating between the tissue and the microbubbles is the preferential detection of the non-linear bubble echoes and the cancellation of the background tissue signals. By improving this separation, multipulse techniques will be applicable to a wider range of clinical problems involving deeper tissues and smaller regions of blood flow.

The results from a series of in-vitro experiments will be used in this presentation to demonstrate the differences between established multipulse techniques such as Pulse Inversion (PI) and Amplitude Modulation (AM) and compare these with combined phase and amplitude modulated sequences.

In these experiments the microbubble agent Definity (Dupont, Boston, MA) was diluted to physiological concentrations in degassed water. The suspension was flowed slowly through a flow cell in a water bath. The bubbles were exposed to ultrasound pulse sequences in which the phase and amplitude of the excitation was changed from one pulse to the next. Echoes from these pulses were digitised, weighted and combined to provide suppression of linear echoes while non-linear echoes were preserved or enhanced. The acoustic power, transmit frequency and pulse length were varied for a progression of pulse sequences with both phase and amplitude modulation. Each experiment comprised a stream of 1000 pulses transmitted over a period of 1s. In this way the effects of any bubble disruption by the ultrasound could be detected. Between each experiment the bubbles within the flow cell were replenished. Experiments were repeated 10 times. Control experiments were performed using degassed water, graphite particles or a single fixed scatterer.

The results of the experiments show that the two-pulse PI and AM approaches performed similarly providing 14±1 dB of enhancement compared to the echoes from the linear scattering control

experiment. A two-pulse combined phase and amplitude sequence achieved an additional 4±1 dB of enhancement. This improvement is the result of improved preservation of the 2nd and 3rd order harmonic signals while maintaining the suppression of the linear signals.

By using precisely defined pulse sequences and specific arithmetic combination of the returned echoes it is possible to optimise the extraction of the non-linear echo component of microbubble echoes. These results were obtained at very low acoustic power (MI<0.1), well below the threshold of microbubble destruction, so that they are suitable for real time perfusion imaging. Continued development of real-time tissue perfusion imaging with ultrasound and microbubble contrast agents is likely to rely on multi-pulse methods with both phase and amplitude modulation.

QUANTITATIVE ANALYSIS OF ULTRASOUND CONTRAST AGENT'S EFFICACY: DOPPLER INTENSITOMETRY, RENAL CORTEX AND TUMORS ENHANCEMENT USING PULSE INVERSION

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Purpose: To quantify the Doppler signal enhancement following the injection of two different ultrasound contrast agents (USCAs) in humans, the Levovist® (Schering SA, Germany) and Optison® (FSO69, Mallinckrodt, USA). The efficacy of contrast-enhanced sonography of renal masses using a FSO69 (Optison®, Mallinckrodt, USA) was evaluated using quantification of the contrast intensity in real time harmonic and pulse inversion imaging.

Materials: Twenty-four patients received 2 bolus injection of Optison® (1, 2, 3, and 4 ml, randomized dose) and 1 injection of Levovist® (2.5 g, 5ml, 400 mg/ml). The continuous Doppler signals from the radial artery were digitized in phase quadrature to calculate the mean duration of the enhancement, the peak enhancement and the area under the time-intensity curve (AUC). In 3 patients, the Doppler signals suffered from wrist movement artifact and could not be analyzed.
The 24 renal masses were studied using harmonic imaging (HI) and pulse inversion imaging (PII) at baseline and following a bolus injection of FSO69 (1, 2, 3 and 4 ml, randomized dose, 6 patients per dosage group, ATL HDI5000, C5-2 probe). Cineloops were transferred to a PC for quantification with HDI Lab. The cortical and solid mass enhancement was calculated for each modality as the difference between the signal intensity of a region of interest located upon the normal cortex and the mass before and after injection (dB). The final diagnosis was obtained by CT, MRI and/or surgery (adenocarcinomas =16, complex cysts =5; hamartomas =3).

Results: Doppler intensitometry: the duration of the contrast enhancement was respectively (mean ± standard deviation) 259 ± 63 sec for the Levovist® and 313 ± 86, 334 ± 77, 291 ± 79 and 341 ± 66 s for each dose of Optison® (1, 2, 3, and 4 ml). The peak enhancement was respectively (mean ± standard deviation) 18.2 ± 5.2 dB for the Levovist® and 20.5 ± 3.4, 21.2 ± 2.6, 21.9 ± 3.2 and 22.5 ± 2.1 dB for each dose of Optison® (1, 2, 3, and 4 ml). The AUC was respectively (mean in linear units) 7966 for the Levovist® and 7283, 10664, 13521 and 14560 for each dose of Optison® (1, 2, 3, and 4 ml). A linear relationship was found between the dose of Optison® and the enhancement (peak enhancement and AUC, $r > 0.97$). The enhancement obtained with the same dose of Levovist® exhibited a large variability between patients.

HI and PII results: cortical enhancement was correlated with the dose (r = 0.98) for each modality and was consistently greater when the mechanical index was lower than 0.4. The detection of normal and atypical cysts was improved and was correlated with CT and MRI features. Renal mass delineation was improved except when the enhanced mass signals matched with the normal surrounding cortex (2 cases). The visibility of the necrosis within the lesion was comparable to the CT and MR appearance. The contrast enhancement of solid renal masses was greater for PII compared to HI. However, a large variability was observed within the lesion due to the heterogeneous perfusion of the masses. The contrast between the renal mass and the adjacent cortex was also greater with PII. The peak enhancement in PII was: 9.6 dB ± 4.0dB for the cancers, 5.8 ± 3.5dB for the hamartomas and 0.8 ± 0.5dB for the complex renal cysts.

Conclusion: The enhancement (peak and AUC) was correlated with the dose of Optison®. Doppler intensitometry allows objective comparison between dose and linear response of USCAs in clinical situation. This measurement may be helpful in predicting enhancement of conventional Doppler and linear imaging. However, it does not reflect the efficacy of the agents with non-linear imaging. Following FSO69 injection, the contrast enhancement of the renal cortex and the solid renal masses was significantly greater using PII than with HI. PII of renal masses improved the detection and the characterization of renal masses, particularly in small tumors and complex cysts.

LOW-MI AND HIGH-MI AGENT-SPECIFIC IMAGING TECHNIQUES FOR RADIOLOGY AND CARDIOLOGY APPLICATIONS

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The dynamics of contrast agent behavior change with incident acoustic pressure and the differences suggest a clinician's imaging tools will include two different imaging techniques: a low-MI technique and a high-MI technique. While excellent agent-to-tissue specificity is achieveable with both techniques, there is a trade-off between spatial resolution and frame rates. The best imaging techniques for cardiology and radiology applications are the same even though the clinical problems are often different, since the underlying bubble characteristics are similar.

For imaging at high mechanical indices the dominant bubble behaviors are disruption and destruction. Techniques that receive and process single acoustic pulses per image line are unable to efficiently detect bubble disruption and therefore rely on harmonic oscillations for detectability. While these harmonic techniques can generate images with noticeable contrast enhancement, the agent-to-tissue specificity is sub-optimal for second harmonic detection due to tissue propagation nonlinearities and is also sub-optimal for higher harmonic detection due to decreased transducer sensitivity and increased tissue attenuation. Techniques that receive and process more than one acoustic pulse per image line are preferred since they detect bubble disruption. For currently available agents, transmitting identical pulses and looking for differences between returned signals to detect the presence of bubbles is a preferred approach. All frequency components originating from slowly moving, or stationary, tissue can be suppressed and detected differences between pulses will not be due to system imperfections in the transmit signal pathway. Thus, high-MI imaging techniques which use identical transmit pulses can offer excellent specificity, sensitivity, spatial resolution, and tissue flash suppression. Broadening of the received frequency spectra beyond the transmitted bandwidths, due to the complex signature and numerous disruption mechanisms of bubbles, offers resolution consistent with the best second harmonic images. Despite the ability of optimal high-MI implementations to obtain high frame rates, the depletion of agent during insonification forces users to reduce frame rates so fresh agent can replenish the imaging plane. The reduced frame rates drive the need for low-MI, minimally-destructive techniques that can maintain high frame rates but still achieve excellent sensitivity even with significantly less transmitted power.

For imaging at low mechanical indices the dominant bubble behavior switches from disruption, or destruction, to harmonic oscillations. Thus preferred low-MI techniques should be most sensitive to

the oscillatory bubble responses. Techniques that receive and process single acoustic pulses per image line partially satisfy the clinical needs, however second harmonic imaging suffers from poor spatial resolution and third harmonic imaging suffers from poor sensitivity as well as poor spatial resolution. Techniques that receive and process more than one acoustic pulse per image line provide the opportunity to change transmit characteristics between pulses, and improve spatial resolution and sensitivity. Continuous improvements that correlate with increased sophistication incorporate not only transmit phase, but also transmit amplitude, and the combination of transmit phase and amplitude. Due to the significantly smaller bubble oscillations at low MIs, the bubble responses do not appear to offer the broadened received spectra seen with high-MI insonification, so while some loss in spatial resolution is seen with low-MI techniques the frame rates can remain high.

In this presentation fundamental principles will be outlined that support two successful imaging techniques, both incorporating multiple received pulses per image line, one for low MI imaging and one for high MI imaging. Experimental in-vitro results and results from human clinical studies will be presented supporting the use of two different techniques for both radiology and cardiology applications.

DEFINITY ENHANCED ULTRASOUND OF FOCAL LIVER MASSES: A COMPARISON WITH CONTRAST ENHANCED CT/MR SCANS

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Objectives: To compare vascular imaging with Definity enhanced ultrasound (DEUS) with vascular imaging on CT and/or MR scan.
To determine the predictive value of ultrasound findings on DEUS for the differential diagnosis of specific liver lesions.

Methods: 100 patients with liver lesions had DEUS and contrast enhanced CT and/or MR scan. There were 24 hemangiomas, 26 FNH, 35 HCC, 8 metastases and 14 others (total 107 lesions). 3 readers blinded to the results of the other imaging studies, will independently answer standard questions regarding lesional vascularity on each study to determine concordance of results. Combinations of findings on DEUS will then be used to establish algorithms to predict diagnoses of specific liver lesions.

Results: Preliminary evaluation of 25 of the 100 studies has been performed with 2 readers. Detection of arterial phase enhancement (APE) was concordant on DEUS with enhanced CT/MRscan in 24 of 25 (96%) cases for both readers, for the pattern of APE in 92 and 84 %, and for the degree of APE in 92 and 88% respectively. Portal venous phase enhancement (PVPE), poorly assessed in the first half of our study, was concordant in only 13(52%) and 14 (56%) of 25 cases. Morphologic vessel assessment was superior on DEUS to CT and MR scan, showing discernible vessels with features contributory to diagnosis.

Statistically significant parameters for liver lesion diagnosis on DEUS include peripheral nodular enhancement and centripetal progression in hemangiomas; profuse vascularity, positive enhancement in the arterial phase, with rapid washout in the portal venous phase in HCC; and profuse vascularity, positive enhancement in the arterial phase, with sustained enhancement into the portal venous phase in FNH. Stellate vascularity, a nonenhancing scar, and tortuous feeding arteries also had an association with FNH.

Conclusion: Arterial phase vascular imaging with DEUS is highly concordant with CT /MR scans. Superior vessel morphology is shown with DEUS and contributes to lesion diagnosis.

INHIBITION OF CORONARY ARTERY INTIMAL HYPERPLASIA WITH INTRAVENOUS ANTISENSE TO THE C-MYC PROTOONCOGENE BOUND TO PERFLUOROCARBON EXPOSED SONICATED DEXTROSE ALBUMIN MICROBUBBLES

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Background. Two unique properties of perfluorocarbon exposed sonicated dextrose albumin (PESDA) microbubbles could potentially be used to improve the effectiveness of gene therapy. First they bind to antisense oligonucleotides which inhibit neointimal hyperplasia (anti c-myc). Secondly, they increase the vascular uptake of this antisense following venous injection, even in the absence of ultrasound targeting. However, this method of delivery still has not been shown to inhibit intimal hyperplasia in the coronary artery following balloon injury.

Methods and Results. The number of anti c-myc molecules which bind to each PESDA microbubble was determined with isothermal titration calorimetry. In order to test whether increased vascular uptake of the antisense with PESDA inhibits coronary stenosis formation, forty two pigs then underwent both left anterior descending and left circumflex balloon injury, and were subsequently randomized to receive one of the following three treatments: (1) transthoracic ultrasound (1 megahertz continuous wave) following intravenous (IV) anti c-myc bound to PESDA; (2) IV anti-c-myc bound to PESDA without ultrasound; or (3) a control group which consisted of either IV anti-c-myc and ultrasound without PESDA, IV sense sequence to c-myc bound to PESDA with ultrasound, or no treatment. At 28 days following balloon injury, intravascular ultrasound of both vessels was performed, followed by histologic measurements of intimal hyperplasia and severity of balloon injury.

Results. By isothermal titration calorimetry, $>10^{7}$ anti-c-myc molecules bound to each PESDA microbubble. In vessels with more severe vascular injury (≥grade II), maximal intimal thickness and intimal area were significantly reduced in pigs treated with IV anti-c-myc bound to PESDA alone ($p < 0.005$ compared to control). The addition of 1 megahertz continuous wave ultrasound eliminated the effectiveness of antisense bound to PESDA.

Conclusions. Intravenous anti-c-myc, when administered bound to intravenous PESDA microbubbles, may be a non-invasive method of preventing intimal hyperplasia following intimal injury.

NOVEL DUAL-USE TARGETED AGENTS FOR DIAGNOSIS AND NON-INVASIVE THERAPY

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At least two kinds of targeting are possible with ultrasound contrast/therapeutic agents. In the first kind of targeting, ultrasound is applied to a tissue region and the localized application of sonic energy is used for targeting. In this form of targeting the focal zone of the ultrasound energy defines the treatment region as microbubbles cavitate within the zone of insonation. In the second form of targeting, the microbubbles themselves are modified for selective targeting. Targeting ligands are bound to the surface of microbubbles, enabling these blood pool agents to be used as cell-specific agents. Following IV injection, targeted microbubbles accumulate at their target tissue or organ and act as beacons to absorb the ultrasound energy within localized tissue regions. Dual use agents combine therapeutic materials into the targeted microbubbles for localized delivery.

Genes are macromolecules which pose significant challenges to therapeutic delivery. For gene therapy in mammals, genes must be transported to the cell nucleus and transcribed within this subcellular compartment. Ultrasound in concert with acoustically activated carriers can be used for specific delivery and highly efficient transgene expression. Ultrasound in concert with dual-use targeted agents presents a new paradigm for drug delivery.

Recent Advances in Ultrasonic Contrast Imaging Technology

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There is an ever growing number of reports describing the clinical value of ultrasonic contrast imaging for an enormous number of clinical indications. Sensitivity, specificity and ease of use are important factors to support contrast echography moving from the research field to clinical routine use. In this paper we present major new developments that are especially designed to meet these requirements. We introduce two new contrast imaging methods based on Advanced Dynamic Flow:

- Tissue Signature Imaging, a high-MI technique used to detect contrast agent perfusion in an especially high spatial resolution.
- Vascular Recognition Imaging, an ultra-low MI imaging method to visualize and characterize both vascularization and perfusion at the same time.

Advanced Dynamic Flow

Advanced Dynamic Flow (ADF) is a wide-band Doppler technique that features high spatial resolution and sensitivity combined with superior real-time characteristics and ease of use.

In contradiction to conventional Color Doppler techniques ADF makes use of a similar transmit/receive bandwidth as usually applied in B-mode imaging, of a reduced packet size and of an increased PRF. This results in a substantial increase in axial resolution and frame rate while the velocity range of clutter components decreases with regards to the Nyquist limit.

To compensate the Doppler sensitivity loss caused by the high bandwidth the Digital Image Optimizer (DIO), a waveform shaping technology that is normally applied in B-mode to optimize axial resolution and sensitivity, is used. Since ADF does not require precise velocity information, DIO technology can be applied to ADF as well. The wide-band Doppler also causes an increased overlap of flow and clutter components due to spectral broadening. To separate the overlapping components, a special signal processing algorithm called Doppler Digital Image Optimizer (Doppler DIO) is introduced.

Finally, Adaptive Image Processing (AIP) is employed to combine ADF and B-mode information in one ultrasonic image. AIP analyzes and compares the structure of ADF and B-mode signals and generates a composite image from both components. Thus, residual clutter can further be eliminated and overpaint is minimized.

ADF does not detect THI signals, a major source of clutter in many other contrast imaging techniques.

As a result ADF features an extraordinary spatial and temporal resolution, a high sensitivity, minimized blooming and a low artifact level.

Tissue Signature Imaging

Tissue Signature Imaging (TSI) is a high-MI technique performed with ADF. TSI is used as a lesion detection method featuring an especially high spatial resolution due to the high bandwidth applied. Thus, even small lesions can be depicted accurately.

Since ADF employs fundamental and harmonic components, the sensitivity of TSI is excellent. Even very small bubble populations can be detected. Although a part of the bubbles are collapsed during insonation, TSI can be operated as a quasi-real-time technique during the vascular phase. The replenishment of small amounts of contrast agent is sufficient for continuous imaging. In the late phase residual microbubble populations can be detected sensitively.

Vascular Recognition Imaging

Vascular Recognition Imaging (VRI) uses ultra-low MI to visualize and characterize both vascularization and perfusion at the same time. Due to the low MI setting the contrast agent is not destroyed and VRI works as a real-time technique.

The use of Doppler technology enables the detection of flow velocity and direction of the microbubbles. In contradiction to all other known contrast imaging methods VRI can color-code the direction of flow like done in normal color Doppler imaging. Additionally, vascularization and perfusion can be distinguished by analyzing bubble movement velocity and concentration. Next to the known velocity presentation in red/blue the perfusion is displayed as green in the same image. Fig. 1 shows the principle of this tri-color display method.

Fig. 1: Tri-color bar of VRI

The simultaneous display of vascularization and perfusion is demonstrated in fig. 2. The upper left image shows the pre-injection phase of a liver study. Vascularization, here mainly coded in red, is visible 18 sec. p. i. in the upper right image. The early perfusion phase is nicely depicted as green after approx. 1 minute (lower left). In the late phase, approx. 6 ½min p. i., perfusion of the contrast agent is still visible like demonstrated in the lower right image.

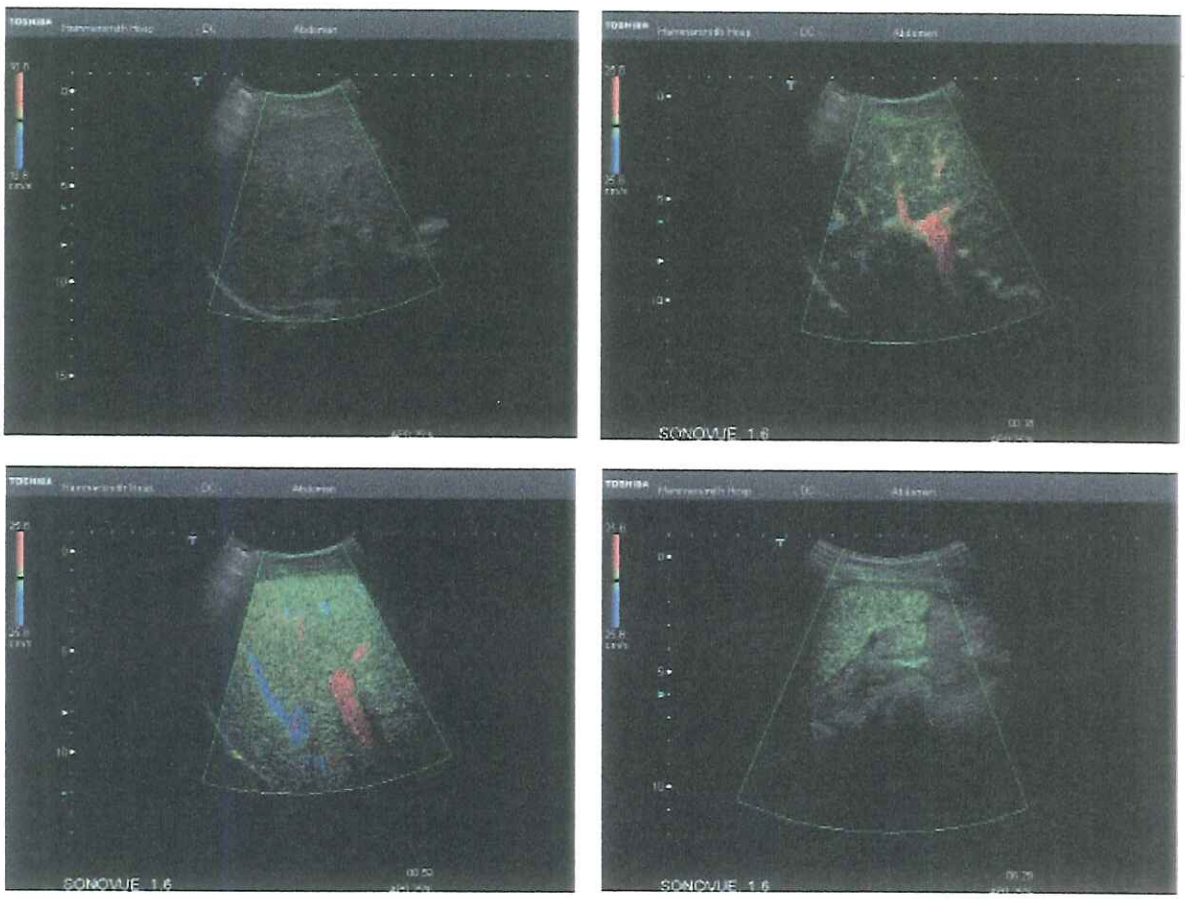

Fig. 2: Different phases of bubble infusion (images courtesy of D. Cosgrove, Hammersmith Hosp. London, UK)
upper left: pre-contrast
upper right: early vascular phase (18 sec p.i.)
lower left: perfusion phase (59 sec p.i.)
lower right: late phase (389 sec p.i.)

TSI and VRI do not require excessive user interaction or system setting control and the infusion of contrast agents can be controlled from the ultrasound system. This results next to the high resolution and sensitivity in easy-to-handle methods suitable for clinical routine use.

SUPERHARMONIC TISSUE IMAGING

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Improvement in image quality is achieved with second harmonic tissue imaging compared to fundamental imaging. This is attributed to different mechanisms: Less reverberations, better sidelobe and grating lobe suppression and less haze and clutter. With the selective use of the 2nd harmonic frequency signal, there is a sacrifice however of a certain amount of dynamic range. With the current machinery settings (MI, frequency), it is know that in most extreme situations, the amount of 2nd harmonic energy returning from tissue is much less than that reflected at the fundamental frequency. Thus, there must be excellent sensitivity and dynamic range in the ultrasound receiver to display the harmonic energy without an unacceptable amount of thermal noise. In addition, to increase the sensitivity of a 2nd harmonic based system; the spectral overlap between the fundamental frequency and the 2nd harmonic frequency has to be diminished, which deteriorates the imaging resolution. Consequently, a trade-off is mandatory between the resolution and the sensitivity.

We show using acoustic measurements and simulations in water and in tissue medium as well as phantom measurements in combination with a dual frequency probe that, for high excitation pressures (MI >0.5), the levels of the generated harmonic components are substantially high and that significant amount of energy has been converted and transferred from the fundamental frequency to the harmonic frequencies up to the 4th and the 5th harmonic components. For a transmitting frequency of 1.7 MHz and an MI of 0.5, the 2nd harmonic component is 10 dB below the fundamental component and the 5th harmonic is only 20 dB down. The higher harmonic components represent and additional information, which is more relevant and with characteristics superior to the 2nd harmonic alone. An elegant way to take advantage of the higher harmonics and to bring all the information together is to combine and incorporate all the multiple higher harmonics into a single component that we call Superharmonic component. We show that the extra information that is brought by the superharmonic compared to the 2nd harmonic alone is translated into different advantages for ultrasound imaging. At the transducer surface, the 2nd harmonic energy is for example 35 dB below the fundamental energy whereas the superharmonic energy is 70 dB down. Even though the artifacts due to reverberations at the chest wall are reduced when imaging at the 2nd harmonic frequency, they will be entirely eliminated and removed at the superharmonic component. The second advantage of superharmonic component is that even

though it was much lower than the 2nd harmonic component at the transducer surface (35 dB lower), it builds up so fast that at imaging distances (eg. focus), enough superharmonic energy has been regained back to yield a significant superharmonic component. The superharmonic showed to be higher than the 2nd harmonic energy around the focal point, which gives a higher SNR. Another major advantage of the superharmonic component is the complete removal and elimination of off-axis echoes by suppressing the side lobes and grating lobes. The measurements showed also that the superharmonic generation is mainly confined to the strongest part of the fundamental beam, more than the 2nd harmonic. The measured beamwidth at the superharmonic frequency showed to be half of the transmitted fundamental beamwidth, whereas the 2nd harmonic beamwidth is only 30% narrower of the fundamental beamwidth. This means that the lateral resolution is improved by a factor of 2. Moreover, superharmonic imaging does not suffer from the resolution- sensitivity trade-off and since it operates over a wide frequency band, the axial resolution is thus further increased.

Consequently, the combination of multiple higher harmonic components into a single superharmonic component is an elegant way of increasing the amount of energy compared to the 2nd harmonic energy alone and therefore the SNR, while reducing the multi-path reverberations and clutter even more. The superharmonic brings back the lost energy due to distortion into a usable and valuable information. For tissue nonlinear imaging, the superharmonic beam offers better sensitivity, higher lateral and axial resolution and improved SNR compared to 2nd harmonic imaging. Phantom images show that superharmonic imaging is a wide-band harmonic technique that provides exceptional image clarity without the use of contrast agents. The phantom images show dramatically cleaner and sharper contrast between a fluid filled cavity and the surrounding wall. Thus cavity will appear much darker than at the 2nd harmonic frequency making contour detection for example much easier.

EFFECTIVENESS OF TRANSCRANIAL AND TRANSTHORACIC ULTRASOUND AND MICROBUBBLES IN DISSOLVING INTRAVASCULAR THROMBI

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Objectives: The purpose of this study was to examine the effectiveness of 1 megahertz and 40 kilohertz ultrasound with microbubbles alone in fragmenting thrombi in attenuated conditions.

Methods: An *in vitro* transcranial model examined the ability of these frequencies to fragment thrombi in the presence or absence of perfluorocarbon exposed sonicated dextrose albumin (PESDA) microbubbles. Secondly, an *in vivo* transthoracic model tested the effectiveness of these same frequencies with intravenous PESDA in fragmenting left circumflex coronary thrombotic occlusions.

Results: In the *in vitro* model, both transcranial 1 megahertz and 40 kilohertz ultrasound were effective at fragmenting thrombi only in the presence of microbubbles. In the *in vivo* model, 1 megahertz ultrasound and intravenous PESDA alone angiographically recanalized only 4 of 14 occlusions, but were consistently effective at improving myocardial blood flow to the risk area even in the absence of angiographic recanalization. Both 40 kilohertz and 1 megahertz ultrasound with PESDA improved regional wall thickening and electrocardiographic abnormalities ($p<0.05$ compared to control or ultrasound alone).

Conclusions: These data indicate that transcranial and transthoracic ultrasound, in the presence of intravenous microbubbles, can improve flow to ischemic regions, and should be considered as a supplement to current pharmacologic therapy.

DIAGNOSIS ACCURACY OF ULTRASOUND CONTRAST TUNED IMAGING METHOD

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Ultrasound is the first imaging modality to be done in liver or kidney disease but due to patients' limitations or technical limitations the sensitivity of conventional sonography remains poor and generally lower than that reported with other modalities such as CT or contrast-enhanced MR.

The recent contrast agents present marked non linear signals which are easily detected by the use of modern scanners with different software adapted to exhibit their response to an ultrasound pulse. This is highlighted by a perfect adaptation of the characteristics of the machine in terms of pulse emission and reception to the agent used. In the present collaborative study, scanning was performed by experienced radiologists using Esatune* scanner (ESAOTE, Italy) with a curved or a phased 3,5 MHz and a 7.5 MHz linear center frequency transducers using CnTI technology. CnTI is adapted to maximize contrast-to-tissue ratio with minimal tissue generated echoes and minimal electronic noise. This was done using narrow transmission signal and narrow receiver bandwidth focused on second harmonic for Sonovue* (Bracco, Italy).

After completion of the baseline scanning, Sonovue* (2.4 ml) was injected IV as a bolus followed by a 5 ml normal saline flush. Real-time contrast-enhanced scanning using very low MI (< 0.1) was started as soon as contrast agent was injected and was terminated within 5 min. A single focal zone located at the bottom of the scanned area was used. Images were recorded simultaneously on DAT for a secondary interpretation.

The purpose of this study was to assess if Esatune* with Sonovue* improves the detection and characterization of lesions in comparison with conventional unenhanced B-mode sonography.

Subjects and Methods: One hundred eighty eight patients out of 269 patients (130 women and 139 men ; mean age, 63.1 ± 14.4 yrs) who participated in this prospective study were finally included based on the availability of a reference imaging examination (CT or MR imaging or biopsy).

The target organs were: liver (*n*=117), kidney (*n=12*), breast (*n=24*), spleen (*n=2*), pancreas (*n=6*), lung (*n=2*) and various in 25 cases. The patients' underlying lesions were: focal liver lesions (*n=117*), tumors (*n=41*), vessels (*n=8*), and others (*n=23*).

Results: No adverse events were reported. In all patients a marked enhancement was observed during arterial phase with a perfect assessment of lesion and/or organ perfusion whatever ultrasound frequency used.

Vascular features of the detected lesions were noted as hypervascularized when contrast enhancement was observed during arterial phase, hypovascularized when no enhancement was observed during arterial phase and increased contrast enhancement when a progressive enhancement was observed with time.

The agreement with reference method was improved for overall population from 36% using conventional sonography to 85% with CnTI* and Sonovue*. This is marked for focal liver lesions with a dramatic increase from 32 to 88% especially for hypervascularized lesions (focal nodular hyperplasia, hepatocarcinoma, hypervascularized metastases), as well as for kidney lesions from 42 to 92%. This leads a change in patient management in 79% out of patients based on an increased confidence in detection (21%) and moreover in characterization in 79% entailing an increased confidence in diagnosis in 75% of cases.

Conclusion: The use of very low-mechanical-index techniques allowing real-time sonography improves dramatically our confidence in characterization and thus in diagnosis with marked changes in patient management. This study demonstrated that Esatune* using CnTI sofware and Sonovue*, which was very simple to use, is suitable for routine application with a high diagnosis value.

CARDIAC OUTPUT MEASUREMENTS BY DILUTION OF ULTRASOUND CONTRAST AGENTS: MODELING AND IN-VITRO EXPERIMENTATION

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Cardiac Output is clinically measured by invasive indicator dilution techniques, which are usually based on dye or cold saline injection. Recent developments of stable Ultrasound Contrast Agents (UCA) could lead to new non-invasive opportunities for dilution-method principles. However, several problems concerning the interpretation of dilution curves as detected by ultrasound transducers have arisen.

A new method for blood flow measurements based on UCA dilution-curves is presented. Dilution curves are measured by real time densitometric analysis of the video output of an ultrasound scanner and are automatically fitted by a Local Density Random Walk (LDRW) model. A new fully automatic fitting algorithm based on multiple linear regression has been developed. Preliminary in-vitro results using SonoVue® contrast show an accurate dilution curve fit and flow estimation (see Figure) with determination coefficient greater than 0.99. Applications of this method also include the assessment of ejection fraction and intrathoracic blood volume.

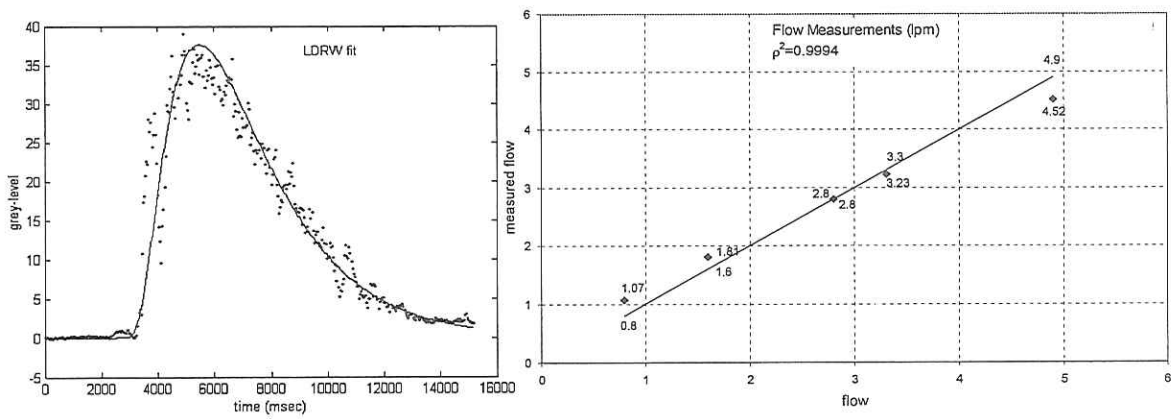

Figure: On the left side, LDRW fit of an in-vitro SonoVue® contrast dilution curve is shown. On the right side, preliminary in-vitro flow-measurement results are shown. The 45° line represents the flow as measured by magnetic flowmeter while the points are the IDC flow measurements. The determination coefficient is 0.9994.

GRAPHICAL USER INTERFACE SIMULATING ULTRASOUND CONTRAST IMAGING

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Introduction: The goal of this work is a conception of graphical user interface (GUI) simulating ultrasound contrast imaging. This tool has been developed with MATLAB software. Fixing parameters such as probe geometry, bubble physical parameters, concentration of ultrasound contrast agent (USCA), biological tissue characteristics and ultrasound scanner settings, it is possible to quantify the 'image quality'. This tool allows evaluating probe and bubbling parameters influence on the image. The numerical phantom geometry is a cavity, within blood streams, surrounded by two biological tissue layers.

Simulation method: The simulation is based on spatial impulse response of a probe. This last can be a circular transducer or a phased array probe. The spatial impulse response is provided by FIELD II software [1]. A scatter point, denoted by $\vec{r}_1$ in the field, has an electrical received signal from the probe formulated by a set of temporal convolutions (denoted $*$):

$$p(\vec{r}_1,t)=\frac{1}{c^2}e(t)*imp_{EA}(t)*h_t(\vec{r}_1,t)*\alpha(\vec{r}_1,t)*f(\vec{r}_1,t)*h_r(\vec{r}_1,t)*\alpha(\vec{r}_1,t)*imp_{AE}(t)$$

where $e(t)$ is the transducer excitation. imp_{EA} and imp_{AE} are the electro-mechanical impulse responses during transmission and reception of the pulse, respectively. h_t and h_r are the spatial impulse responses for the transmitting and receiving apertures, respectively. f is the diffusion function which is analytically calculated [2] for the bubble case, and it is a Dirac delta function for the tissue case. α is the attenuation function defined of the bubble cloud [3] or the tissue. c is the speed of sound. The algorithm is based of the summation of each individual bubble response in order to predict the bubble cloud response (Born approximation). The bubble model [2] includes shell and gas parameters. The size distribution of a bubble cloud is assumed to be normally distributed. The tissue is described by its backscatter and attenuation coefficients. The spatial distribution is arbitrary generated and may be saved so as to simulate several images with others settings. A lungs filtering may be taken into account in the simulation [4].

Conclusion and perspectives: This GUI is an appropriated tool to quantify the contrast image quality, in terms of a sensitivity criterion, provided by an ultrasound scanner and a specific USCA. For instance, a used sensitivity criterion is Agent to Tissue Ratio (ATR). The Figure 1 shows a screen

copy of the interface. The GUI has been designed to implement the non-linear behaviour of bubble response. Therefore, it will allow us to assess specific imaging modalities.

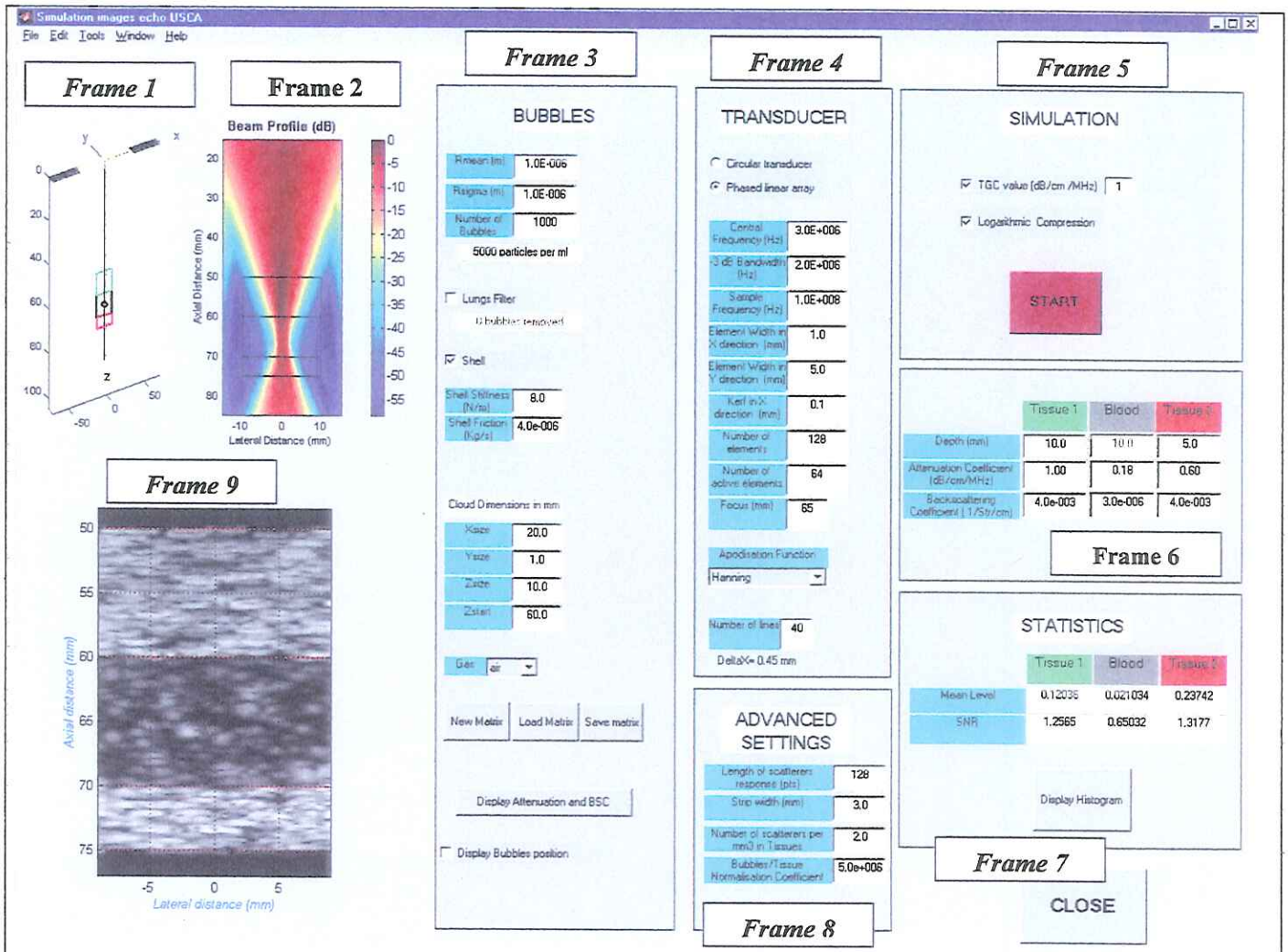

Figure 1: Screen copy of the graphical user interface simulating ultrasound contrast imaging. Frame 1: Global schematic including transducer and three medium locations, Frame 2: Probe beam profile (maximum negative pressure distribution), Frame 3: Ultrasound contrast agent parameters, Frame 4: Probe settings, Frame 5 : Post-processing, Frame 6 : Tissue and blood characteristics, Frame 7 : Statistics toolbox, Frame 8 : Advanced settings, Frame 9: simulated image.

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HARMONIC RESPONSE VS. ATTENUATION IN REAL-TIME ULTRASOUND CONTRAST IMAGING: THEORETICAL AND EXPERIMENTAL STUDIES WITH SONOVUE™ AND OPTISON™

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Real-time perfusion imaging with an ultrasound contrast agent is becoming a reality, thanks to basic research on the physical interaction between ultrasound waves and micro bubbles. A crucial criterion for this technique to be successful is that the micro bubbles need to be stable during the imaging procedure, i.e. undergoing minimal bubble destruction. This means that the mechanical index (MI) should remain below the threshold causing substantial bubble destruction. Moreover, the bubbles need to be able to generate significant signals at these low MI's to be detected by the ultrasound machine. Using high-performance, second-generation contrast agents and sensitive detection techniques, such as pulse/phase inversion or power modulation, harmonic bubble signals at low MI can be detected. In addition, if the MI can be reduced considerably (e.g. 0.1 typically), propagation in tissue generates very low harmonics, allowing a good separation between contrast agent and tissue echoes, resulting in a high agent-to-tissue ratio (ATR) making real-time perfusion imaging possible.

To obtain sufficient signals from the bubbles at a low MI, the concentration of bubbles could be increased. However, ultrasound waves are attenuated as they traverse regions filled with bubbles, such as the left ventricular cavity. In many instances, this attenuation is a dominant factor in limiting the imaging depth. Therefore, the overall performance of an ultrasound contrast agent for real-time imaging at low MI strongly depends on its ability to generate harmonics at low MI with minimum attenuation. The relative importance of these aspects has been studied, both theoretically and experimentally, for the cases of SonoVue™ and Optison™.

SonoVue™ (Bracco) and Optison™ (Mallinckrodt) are commercially available second-generation ultrasound contrast agents. SonoVue™ micro bubbles are made of hexafluoride (SF_6) gas stabilized by a phospholipid shell. Optison™ micro bubbles are made of octafluoropropane (C_3F_8) gas stabilized by an albumin shell. These formulations result in agents with good stability following intravenous injection (persistence of several minutes), and bubbles with a flexible shell.

The performance of both agents was investigated in an *in vitro* setup. Radio frequency (RF) echo signals were acquired from a phased array probe using a Megas ultrasound system (EsaOte, Florence,

Italy), interfaced to a digital RF-grabber (FEMMINA, University of Florence, Italy). By analyzing harmonic responses at two depths, in relation with echoes from a tissue-mimicking phantom, important differences were observed in the ATR and attenuation of SonoVue™ and Optison™ at normalized concentrations. These differences may be explained theoretically using non-linear bubble modeling, on the basis of the two-agents' bubble size distributions and their viscoelastic shell parameters. The results will be discussed in conjunction with the experimental observations.

In summary, the shell material properties and size distribution of contrast agent micro bubbles are critical parameters for achieving efficient harmonic contrast imaging at low MI with minimal attenuation. These characteristics could play an even larger role in the future when considering contrast imaging techniques that exploit higher harmonic (3^{rd}, 4^{th}, etc.) response of the bubbles, such as super harmonic imaging.

DOES PRESSURE AMPLITUDE AFFECT RESONANCE FREQUENCY? A NUMERICAL INVESTIGATION

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The analysis of the dynamical response of gas filled cavities surrounded by elastic capsules, acting as contrast agent microbubbles, in ultrasonic fields is of immense importance in elucidating the full diagnostic use of ultrasound imaging. When the frequency of the external acoustic field is at or near the natural frequency of the gas bubble/contrast agent microbubble, resulting in resonance, larger amplitudes of oscillation than would otherwise occur are observed; enhancing the backscattered signal. It is therefore clearly beneficial to medical ultrasound imaging to be able to accurately determine the complex resonant behaviour of the bubble oscillation motion.

In our work we consider the Keller-Herring model of Prosperetti and Lezzi (1985), which under certain parameter regimes reduces to either a Keller-Miksis, Herring or Rayleigh-Plesset type equation. It will be shown through appropriate variations in the cavity (bubble/microbubble) diameter, frequency and amplitude of the insonating field how computer-based simulations of bubble/micro-bubble response can be employed to determine resonance frequencies that are directly influenced by the driving pressure amplitude. Anderson and Hampton (1980) describe a hierarchy of mathematical models, of varying complexity, that exist for calculating resonance. However, to the best of our knowledge all the existing formulae for obtaining f_0 fail to take into account the effect of the amplitude of the incident acoustic field.

For example, we demonstrate how with the general Keller-Herring model formulated as a Keller-Miksis type equation simulating a $1\mu m$ gas bubble driven at $10kPa$, $50kPa$ and $100kPa$ the respective resonance frequencies (f_0) are identified as $3.8MHz$, $3.7MHz$ and $3.2MHz$. In comparison applying either Minnaert's formula (1933) or Miller's formula (1977) results in a value of $f_0 = 3.9MHz$ for **all three** pressure amplitudes. Furthermore, it will be shown that different models can result in variations in the calculated value of f_0 for a given bubble size. In support of our numerically derived resonance values a scattering cross-section will be calculated and it will be shown how, in general, maximum scattering occurs close to the calculated f_0 value – thereby providing some verification for our approach.

THE LOCALIZATION OF PROSTATE CANCER BASED ON DYNAMIC CONTRAST ENHANCED POWER DOPPLER ULTRASOUND EXAMINATIONS

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Introduction & objectives: The development of Prostate Cancer (PCa) is associated with changes in perfusion. This abstract describes the localization of PCa based on differences in perfusion dynamics. Perfusion is imaged using contrast-enhanced power Doppler transrectal ultrasound (CE-PD-TRUS) imaging.

Material & methods: 29 Patients with proven prostate malignancy, scheduled for radical prostatectomy, underwent an ultrasound examination prior to surgery. A bolus injection of contrast agent (Levovist®) was administered intravenously. The delivery of the contrast agent to the prostate was imaged using CE-PD-TRUS and the examination was recorded on video. The video recording was digitized and the Doppler signals were separated from the B-mode image. The enhancement data of the first circulation of contrast agent was fitted to a dilution curve, thus obtaining a parametric description of the Time-Intensity Curve (TIC). This procedure was performed for the whole prostate, the left and right lobe, the dorsal and ventral part, and the four quadrants of the prostate. The parameter-set consists of Time to Start (TtS), Time to Peak (TtP) and Peak Value (PVal). Furthermore, the Rise time (TRise) of the enhancement was calculated. Signal quality was evaluated prior to processing. The patients were divided into 3 categories (I,II & III) based on the TIC of the whole prostate. I and II showed clear enhancement. III showed no appreciable enhancement and was excluded (N=6). First circulation could clearly be distinguished in category I (N=11). The calculated parameters of the left and right lobe were compared to identify the major malignant area. This procedure was repeated for the dorsal and ventral sides of the prostate and for the quadrants of the prostate. The results were compared to the pathological findings.

Results: The minimal TtP (minTtP) proved to be the most predictive parameter for selecting the major malignant area in the comparison of the left and right lobe of the prostate. 78% of the patients were diagnosed correctly (N=23). For category I only, this improved to 91% (N=11). Minimal TtS provided an accuracy of 65% (N=23), Minimal TRise an accuracy of 70% (N=23) and Maximal PVal resulted in 43,5% accuracy. MinTtP based selection of the major malignant area in either the dorsal or ventral side of the prostate resulted in 35% accuracy (N=23). Selection of the most affected quadrant resulted in 26% accuracy (N=23). MinTtP based comparison of the left and right ventral quadrants resulted in

an accuracy of 77% (N=13). Comparison of the left and right dorsal quadrants resulted in 73% correct diagnoses. Comparing respectively the left-ventral to the left-dorsal (N=16) and the right-ventral to the right-dorsal quadrants (N=19) resulted in both cases in an accuracy of approximately 40%.

Conclusions: TICs obtained from CE-PDU examinations have a high predictive value for localizing malignancies in either the right or the left lobe of the prostate. The lack of accuracy in the comparison of the Dorsal and Ventral areas and the quadrants is most likely due to the anatomical differences between the compared areas, which make the hemodynamic behavior incomparable. The results indicate that the development of PCa is associated with changes in the hemodynamics of the prostate and that these changes can be measured using contrast enhanced ultrasound techniques.

Optimized Receive Filters and Coded Pulse Sequences for Contrast Agent Imaging

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Introduction

Phase- or amplitude-coded pulse sequences are known to be useful for imaging nonlinear scatterers. It can be assumed that the N pulses of such a pulse sequence have the same envelope and carrier frequency but different carrier phases and/or amplitudes. The pulses are transmitted along the same beam line. A weighted summation of the received echoes can cancel out the part of the signal that results from linear scattering and propagation, i. e. the fundamental. Depending on the carrier phases used, harmonics or sub-harmonics are enhanced. Amplitude coding may be introduced to image frequency components in the fundamental frequency range that are due to e. g. a third order nonlinearity. Commercial scanners reproduce the desired transmit pulses inaccurately. Thus, the suppression of the fundamental is incomplete. This problem can be solved by linear receive filters, where N different filters are assigned to the N transmit pulses. Design criteria are an optimal suppression of the fundamental or optimal echo energy ratio between two media (contrast agent / tissue) after summation. Due to various nonlinear effects, the two criteria are not identical, and the latter is of greater practical interest. Since convolution and summation are linear operations, the problem can be described and solved by linear algebra. Based on training data, optimal coefficients for FIR filters can be calculated by solving an Eigenvalue problem.

The derivation of the optimization problem can be found in [1]. The training data that is needed for the optimization is taken from 2 sample regions within the same depth range, where one region represents contrast agent and the other one represents tissue. An example for the positioning of the sample regions is shown in Figure 1.

Experimental Results

To acquire in vitro data, a tissue-mimicking phantom with a cylindrical hole containing a contrast agent was imaged with a Siemens Sonoline® Elegra. The hole has a diameter of 2.5 cm and is positioned at an imaging depth of 5 cm.

4-pulse sequence at 2.0 MHz

The first experiment was conducted using a 3.5 MHz curved array, where the pulse sequence consisted of 4 pulses with $\varphi_i = [0°, 120°, 180°, 240°]$ and with a carrier frequency of $\omega_0 = 2.0$ MHz.

The contrast agent filled space can easily be identified in Figure 1 as an irregularly filled circular region that causes shadowing because of the fairly high concentration of the contrast agent Definity™. To further analyze the discrimination between contrast agent and tissue, normalized histograms for the 2 media were calculated. The histograms in Figure 2 show a significant overlap, which is determined by the reflectivity of the tissue and the reflectivity, i. e. the concentration, of the contrast agent.

A coherent weighted summation of the 4 echoes per beam line, where the weights are optimized with respect to contrast agent imaging, leads to the image shown in Figure 3. The weights represent a set of 4 1-tap filters. The corresponding histograms, see Figure 4, show a reduced but still substantial overlap. The poor result is due to the fact that a 1-tap filter cannot correct for broadband, frequency dependent amplitude and phase errors. The weighted summation partly suppresses the fundamental and, therefore, most of the echo energy, so that the noise floor becomes evident in the image.

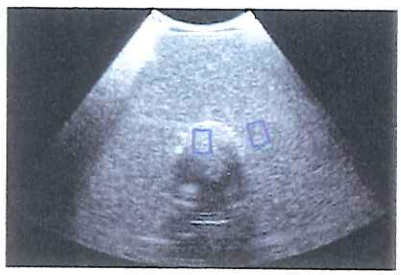

Figure 1: B mode image of the contrast agent phantom. Dynamic range: 55 dB. Boxes: sample regions for filter optimization (left: contrast agent, right: tissue).

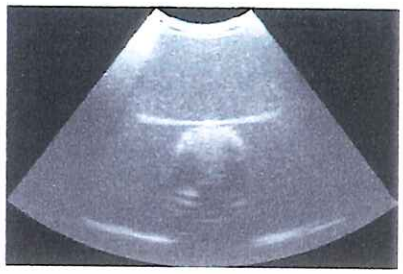

Figure 3: Demodulated image after optimized 1-tap filtering. Dynamic range: 55 dB.

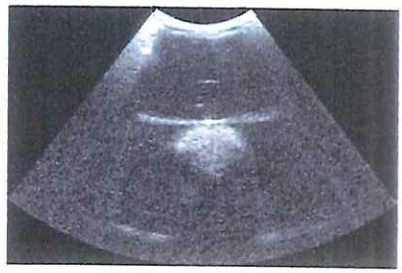

Figure 5: Demodulated image after optimized 64-tap filtering. Dynamic range: 55 dB.

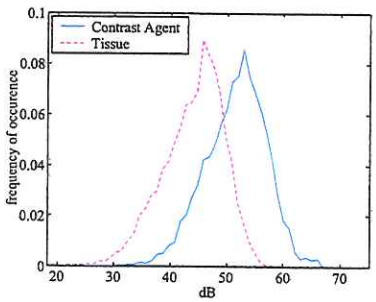

Figure 2: Normalized histograms for the representation of contrast agent and tissue in the image shown in Figure 1.

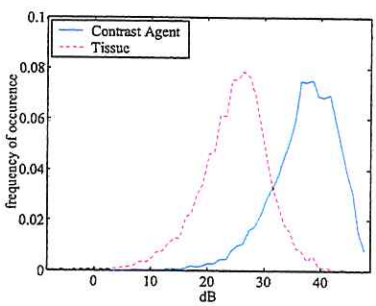

Figure 4: Normalized histograms for the representation of contrast agent and tissue in the image shown in Figure 3.

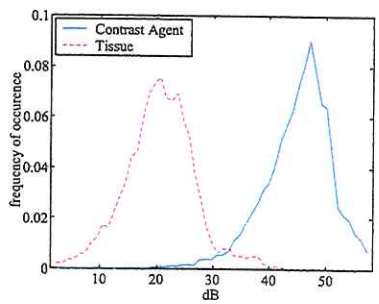

Figure 6: Normalized histograms for the representation of contrast agent and tissue in the image shown in Figure 5.

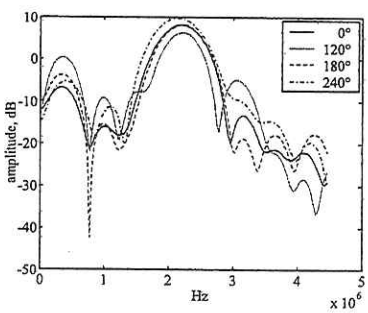

Figure 7: Amplitude spectra of the 64-tap filters.

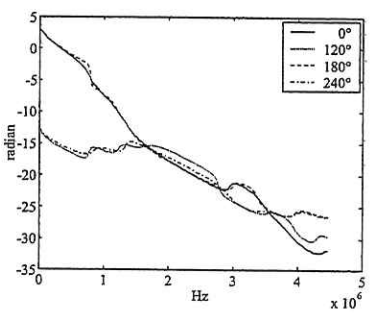

Figure 8: Phase spectra of the 64-tap filters.

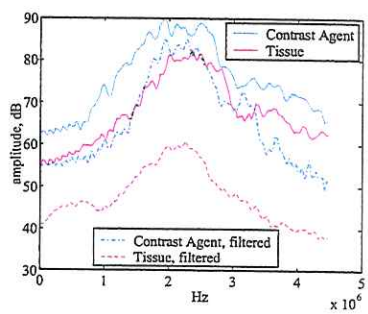

Figure 9: Amplitude spectra for tissue and contrast agent before and after filtering and summation.

The improvement achieved by extending the filter length to 64 taps is illustrated in Figure 5. The visual impression is confirmed by the histograms given in Figure 6. The histograms confirm an almost complete separability of the 2 media. Setting an optimal threshold at 33 dB results in a total classification error of less than 3.5 % in the depth range of the sample regions (B mode, threshold: 48 dB, error: 24.5 %).

To better understand the filtering process, the amplitude and phase spectra of the filters are shown in Figure 7 and Figure 8, respectively. The filters for the echoes corresponding to the 0° and 180° carrier phase are very similar, since a bipolar transmitter can achieve a phase shift of 180° most accurately, while other phase shifts require more advanced pulse shaping capabilities than most commercial scanners offer. Thus, the filters for the 120°- and the 240° differ from the other two significantly and do not show a perfect symmetry. This is espe-

cially noticeable in the phase spectra. The filters correct for errors in amplitude and phase by introducing a frequency dependent amplitude weighting and phase shift. Furthermore, the sub-harmonic frequency range and the frequency range that matches the transducer bandwidth best are emphasized while others are suppressed. The preferred frequency ranges are those that enhance the contrast between the media. It is important to note that these frequency ranges are not necessarily those that should be used for single transmit harmonic imaging, because enhancement and suppression of harmonics is predominantly achieved by the summation due to the phase relationships within the multi-pulse sequence. It is interesting to note that the original echoes contain significant energy in the frequency range of 3 – 5 MHz. The filters suppresses this frequency range, see Figure 9, indicating that this part of the spectrum does not allow the discrimination of the 2 media.

5-pulse sequence at 3.6 MHz

Another experiment was conducted with Levovist® using a 7.2 MHz linear array, where the pulse sequence consisted of 5 pulses with $\varphi_i = (i-1)\cdot 72°$ and with a carrier frequency of $\omega_0 = 6$ MHz . In this case, the separation of the 2 media less than 1 %. 10 different subsets of 3 out of 5 echoes per beam line were processed to form 10 demodulated A-lines. These A-lines were than averaged to give one line of the image. This procedure, but with a simple weighted summation instead of a summation after optimal filtering, was proposed in [2,3]. Alternatively, all 5 echoes were filtered, summed and demodulated to form a line of an image. In the former case, the resultant image showed less speckle noise. In the latter case, the axial resolution was slightly better. The spatial resolution was very poor in both cases. Further analysis revealed that the filters limited the frequency range to sub-harmonics (0 – 2 MHz). Due to the broadband excitation of the transducer, the transmitted spectrum showed a center frequency of approximately 5 MHz. This frequency is higher than the resonant frequency of the insonified microbubbles. Consequently, the generation of higher harmonics is unlikely. Further experiments will be conducted to explore the use of 5-pulse sequences at lower frequencies.

CONCLUSION

The proposed optimal receive filters have the potential to greatly improve nonlinear contrast agent imaging using multi-pulse sequences. Phase and amplitude errors on the transmit side of ultrasound scanners can be compensated by the optimal receive filters.

Since the optimization process is not only applicable to contrast agent and tissue but to any two media that differ in terms of non-linearity or frequency dependent backscattering or attenuation, the same approach can be used to differentiate between different types of tissue.

Further experiments will be conducted to explore the benefits of combined phase- and amplitude-coded sequences.

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THE EFFECTS OF ULTRASOUND PARAMETERS ON LYSIS AND SONOPORATION OF CELLS

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Introduction: The success of drug and gene delivery is limited by inability of those components to cross biological barriers like the cell membrane. To overcome this barrier, ultrasound has been shown to increase the permeability of cell membranes [1]. However, the mechanisms as well as the effect of ultrasonic parameters are still not fully understood. More inside in this mechanism will give a better understanding how ultrasound can be used as a local drug delivery system. The purpose of this study is to determine, in an *in-vitro* set-up, the effects of different acoustic parameters on cell membrane permeability and viability/lysis of cells exposed to ultrasound. We examined particularly the effects of pulse length, PRF, exposure time, MI and frequency.

Experimental set up: The acoustic set-up contained a single element transducer operating at 1 MHz. The acoustic pressures used corresponded to MI ranging from 0.2 to 1.4. The US exposure was varied by changing the pulse length, the PRF and total exposure time. To study the US effects we used Green Fluorescence Protein (GFP) transfected Chinese Hamster Ovary cells (CHO) cultured on a membrane. Fluorescein-labeled dextran was used as a marker to study the cell membrane permeability. Dextran was chosen because of its high molecular weight and therefore its inability to enter a cell without further aid. The permeation or sonoporation of the cells is indicated by the presents of dextran inside the cell. Fluorescein is visible as the colour red. Therefore, in our experiment red stained cells contain dextran.

Results: Figure 1 shows the percentage of cells surviving the ultrasound treatment. The decreasing number of viable cells is correlated to the exposure time. Longer the exposure times result in less viable cells. Also is the number of viable cells negatively correlated to the MI. Higher MI's result in fewer cells.

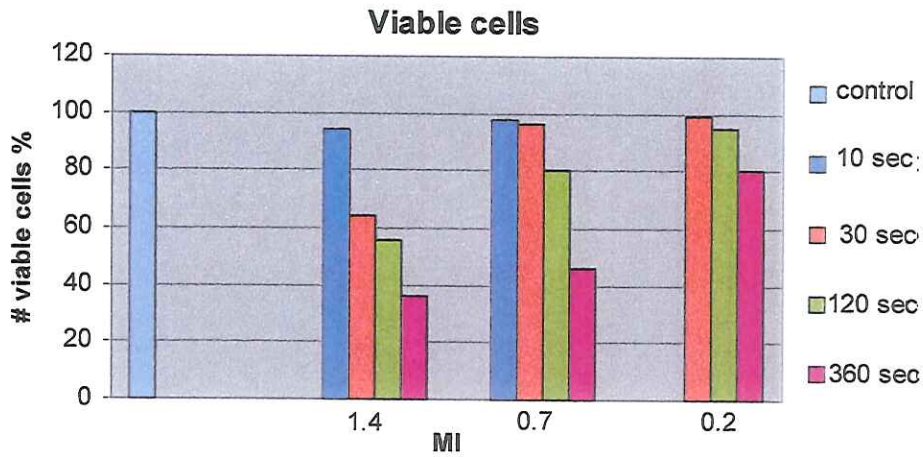

Figure 1. *Viable cells.* GFP transfected CHO cells exposed to ultrasound during various exposure times (seconds) at various MI's. Relative number of cells surviving compared to the control (=100%)

Figure 2 shows the percentage red stained cells of the viable number of cells. When CHO cells are exposed to an MI of 1.4 for 30 seconds, 42% of the viable cells are stained red and therefore sonoporated. When 6 minutes exposure to ultrasound, almost no cells are sonoporated. When cells are exposed to ultrasound during 120 seconds, the number of sonoporated cells seems to be negative correlated to the MI.

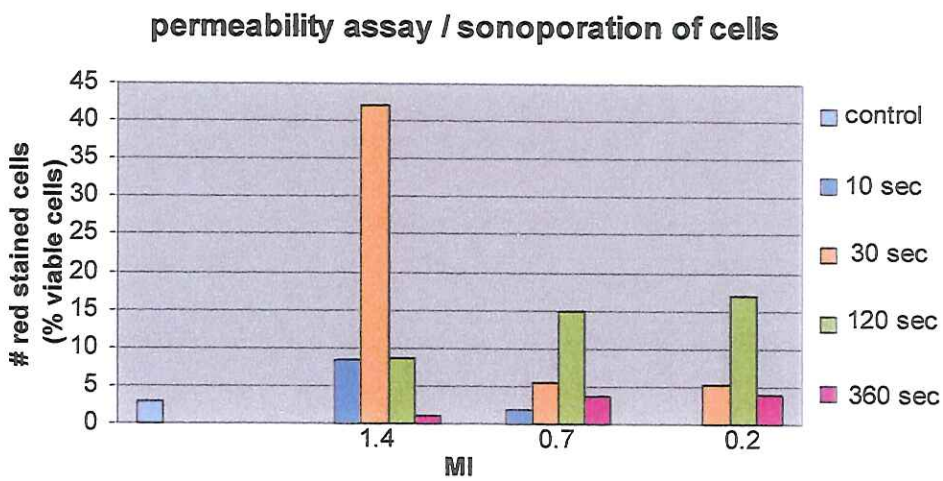

Figure 2. *Permeability assay / Sonoporation of cells.* The permeation of fluorescein-labeled dextran through the membrane of GFP transfected CHO-cells, which were exposed to ultrasound during various exposure times (sec) at various MI's. Number of red stained cells, as a result of Fluorescein-labeled dextran uptake, per hundred viable cells.

Conclusion: These preliminary results show that we are able to sonoporate cells. When higher MI's and longer exposure times where used, cell lysis is evident. Low MI's result in a low number of sonoporated cells. The number of sonoporated cells is exposure time dependent but longer exposure times does not necessarily mean more sonoporated cells. The number sonoporated cells after ultrasound treatment is depended on the MI and total exposure time. Thus, US parameters MI and exposure time are important sonoporation parameters. More detailed studies are needed to understand the effects of different acoustic parameters on cell membrane permeability and viability/lysis of cells.

Literature

1. Tachibana K, Uchida T, Ogawa K, Yamashita N, and Tamura K. Induction of cell- membrane porosity by ultrasound. *Lancet* 1999;**353**:1409.

TARGETED ULTRASOUND CONTRAST AGENTS

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Future clinical applications of contrast-enhanced ultrasound are likely expand beyond the assessment of microvascular perfusion. One promising direction includes the development of techniques for molecular or cellular imaging. This new application requires novel "site-targeted" contrast agents that do not behave as flow tracers and, instead, are retained within diseased tissues. Since micro bubbles are pure intravascular tracers, the pathophysiologic processes to be targeted must be characterized by specific ligands that are expressed within the vascular compartment. Accordingly, development of site-targeted contrast ultrasound imaging has focused on disease states where the intravascular molecular and cellular mediators have been well-characterized, such as inflammation. We have recently employed 2 different strategies to target micro bubbles to regions of inflammation.

The first strategy involves the chemical alteration of the micro bubble shell. We have previously demonstrated that lipid-shelled micro bubbles attach, via serum complement, to activated leukocytes adherent to the venular endothelium in regions of inflammation. We have markedly increased the avidity of micro bubbles for activated leukocytes by incorporating phosphatidylserine (PS) into the lipid shell, which results in greater complement activation. We have recently used PS micro bubbles to temporally assess myocardial inflammation in response ischemia and reperfusion. Myocardial contrast echocardiography (MCE) with intravenous administration of PS micro bubbles was performed following 90 min of LAD or left circumflex artery occlusion and reperfusion. The region of myocardial inflammation by MCE was greatest immediately following reflow and encompassed the entire risk area. The spatial extent of inflammation by MCE decreased over 2 hours but still involved the non-infarcted risk area. At 1-2 hours after reperfusion, the spatial extent of inflammation by MCE correlated well with radionuclide imaging of a technetium-labeled agent targeted to the neutrophil leukotriene-B_4 receptor (^{99m}Tc-RP517). The severity of inflammation by MCE, determined by background-subtracted acoustic signal from retained PS micro bubbles, correlated with quantitative ^{99m}Tc-RP517 data and tissue myeloperoxidase activity. These results indicate that MCE with leukocyte-targeted micro bubbles may provide a means to assess new therapeutic strategies that have been developed to attenuate post-ischemic inflammatory responses.

The second strategy for contrast ultrasound imaging of inflammation involves the conjugation of ligands that recognize endothelial or leukocyte adhesion molecules to the micro bubble surface. We

have developed micro bubbles targeted to inflamed endothelium by conjugating monoclonal antibodies (mAb) against P-selectin to the surface of lipid micro bubbles (>30,000 per 2-3 □m bubble). Intravital microscopy of the cremaster muscles of mice revealed that microvascular retention of P-selectin-targeted micro bubbles in inflamed tissue was much greater than control micro bubbles. Enhanced retention of P-selectin micro bubbles resulted in a much greater signal than control micro bubbles during contrast-enhanced ultrasound imaging of the kidneys of mice following renal ischemia-reperfusion injury. Enhanced retention of P-selectin-targeted micro bubbles in inflamed tissue, assessed by intravital microscopy and by ultrasound imaging, was abolished in P-selectin-deficient mice. These results indicate for the first time that molecular processes can be assessed by contrast ultrasound imaging following intravenous administration of site-targeted micro bubbles.

ACOUSTIC FORCES ON BUBBLES IN PIPES AND IN THE CAPILLARY SYSTEM

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Experiments in order to study the effect of acoustic forces on individual bubbles in pipes are presented. In the system that we have used, the competition between acoustic and fluid dynamical forces results in a spiraling bubble trajectory. This dynamics is modeled by expressing the balance between Bjerknes and hydrodynamic forces in terms of an ODE model, to which a separation of time scales is applied. The success of this model shows that the simple force balance approach is still meaningful when bubbles are subjected to sound fields.

In the second part of the lecture we present theoretical estimates to downscale the phenomenon towards bubbles in capillary system. In particular, we show how cavitating bubbles can be used as a micro pump in the capillary system

HIGH SPEED OPTICAL IMAGING FOR BUBBLES

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Introduction: Ultrasound contrast agents have been established experimentally to enhance cardiological and radiological images. Nonlinear imaging methods such as harmonic imaging and pulse inversion, which exploit the nonlinear scattering of the microbubbles, are used regularly. Other imaging methods such as triggered imaging and flash imaging adapt to or exploit the fact that microbubbles can be liberated or destroyed by ultrasound. Other areas being aggressively pursued by many groups are the targeting of microbubbles to pathologic tissues and controlled release of drugs transported by microbubbles. In both diagnosis and therapy, a detail understanding of the acoustic and mechanical response of the bubbles under ultrasound is required.

So far, most of the experimental studies on bubble dynamics are limited to two approaches: optical and acoustic. Optical imaging is an established technique for individual macroscopic (> 100 μm) bubbles. For microbubbles, bulk acoustic measurements are far more common. These measurements are ultimately important for development of acoustic detection methods, however, bulk measurements convey limited information about the dynamics of microbubbles. Currently, researchers interested in *seeing* the dynamics of individual microbubbles face a number of physical and technical challenges. These include frame rate, number of frames, size and illumination.
In this project, we are interested to understand nonlinear oscillation and acoustic disruption of microbubbles. To this end, we are developing a 2-D imaging system combining high image resolution, high frame rate, high number of frames and high light sensitivity.

Method: A number of dynamic optical studies can be found in the literature, these can be categorized as: single frames, streak and multiple frames. An image intensifier based on an MCP (multi-channel plate) is used as a high speed shutter to obtain single frames. Single frame systems can be used to reconstruct bubble motions for a continuous wave experiment by shifting through different phase of the ultrasound cycle. Streak imaging is similar to ultrasound M-mode imaging and can provide high speed realtime information, however, the bubble must be located precisely along a 1-dimensional line focus. Multi-frame (2D) imaging can provide spatial information and only requires the bubble to be located on a 2-dimensional focal plane.

Broadly high-speed multi-frame imaging systems are based on either beam-splitters or rotating mirrors. A beam-splitting system employs a prism to split the incoming light into a number of parallel channels, each ending with an imaging detector; high shutter speed and light sensitivity are achieved with a MCP in each channel. Existing beam-splitter systems are limited to eight channels (frames) and can achieve a minimum exposure time and inter-frame time of 5 ns.

Rotating mirror systems employ three mirrors mounted on a rotating prism to direct the incoming light to different channels (frames). More than 100 frames can be recorded on a strip of photographic film. However, photographic films are not sensitive enough for our high-speed, high-magnification application. The "NedCor" system incorporates high-sensitivity CCD cameras into a state-of-the-art rotating mirror device made by Cordin (Salt Lake City, USA). Custom electronics developed in the Netherlands control each CCD camera, digitize the images and interface with a PC.

As illumination, a Xenon flash tube is used. This device can produce more than 1 kW of optical power for over 20 μs, which has been proven to be sufficient for imaging contrast microbubbles at an effective exposure time of 20 ns.
The completed NedCor system will combine high resolution (0.4 μm), high frame rate (25 MHz) and high number of frames to provide previously unavailable 2-D optical imaging capability. With a fully completed system we will be able to measure, for example, the 3rd harmonic oscillation of a bubble in 3 MHz ultrasound. With 128 frames, it's also possible to record the transient oscillation during the first and last few cycles of a 12-cycles burst.

Result: The first phase of the "NedCor" system was recently completed with 16 functional frames. As demonstration, we operated the camera at 2–6 MHz frame rate, the formation and extinction of an electric arc in a Xenon flash tube was recorded. The dynamics of Sonovue™ microbubbles under 0.5 MHz ultrasound was imaged at 4 MHz.

Future: With a functional prototype in place, we are currently developing the final version of the controller electronics for full 128-frames experiments. Our custom-designed control electronics allow each channel to be activated independently. This will allow us to sub-divide the 128 frames into multiple segments for multi-pulse experiments.

MOVING MCE INTO CLINICAL MAINSTREAM - THE PHILIPS APPROACH

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Over the last few years, there have been multiple advances in the area of contrast echocardiography. A major area of research has been in understanding the interaction between ultrasound and micro bubbles. This increased knowledge has fueled equipment manufacturer's progress and has resulted in the invention and introduction of new imaging modalities, at low-MI's as well as high-MI's. New nonlinear detection techniques (e.g., Pulse Inversion, Power Modulation) focused on improving the signal/noise ratio (i.e., contrast/tissue ratio) at low-MI's has resulted in real-time visualization of ultrasound contrast agents within the myocardium. Additionally, improved understanding of the mechanisms of micro bubble destruction has allowed for new high-MI B-mode imaging techniques with high resolution (Ultraharmonics) as well as improved sensitivity of Harmonic Power Doppler (Harmonic Angio).

Philips Medical Systems offers three imaging techniques on the SONOS 5500 for MCE research; Power Modulation, Ultraharmonics and Harmonic Angio. Each technique has it's own strengths. Power Modulation offers function assessment simultaneously with myocardial opacification and easy image acquisition due to its real-time nature. Ultraharmonics is a unique high-MI technique that offers very high resolution, high sensitivity, and excellent tissue suppression, is easy to set up, and has minimal motion artifacts. Harmonic Angio offers the highest sensitivity and superior tissue suppression with Multiple Frame Trigger to discriminate between signal and artifact. In combination with the s3 transducer, which was designed to produce a more uniform power field, these modalities offer a major advancement in contrast echo over our previous offerings. However, despite these improvements and the advancements by other equipment manufacturers, a perfect technique does not yet exist. Low-MI techniques lack the superior sensitivity of high-MI techniques and High-MI techniques lack the ease of image acquisition of low-MI techniques. Also, Stress packages have not been able to fully incorporate MCE images into the protocols making image acquisition of MCE images during Stress much more difficult and time consuming than wall motion images. These factors have dramatically slowed the uptake of MCE into clinical mainstream.

To address the sensitivity issue of Low Mi techniques a new low-MI technique HiRes Power Modulation has been developed. This technique uses the grayscale imaging path in the system rather

than the color flow imaging path. Using the grayscale path significantly increases the resolution and frame rates over using the color path. The grayscale imaging path allows for an increase in dynamic range through the use of more bits, operation of TGC's to optimize signal-to-noise with depth, and lateral gain controls to optimize signal-to-noise at angles to the transducer where signals are weaker. These advancements significantly improve low-MI imaging of myocardial contrast.

In order to improve the image acquisition of high-MI techniques, two big issues have been be addressed. First of all, there is the requirement of maintaining the image plane with long trigger intervals. Secondly, the length of time it takes to generate a replenishment curve by manually changing the trigger interval makes the exam time consuming and difficult. The new SONOS 5500 UltraPerformance has new features to deal with these issues, Monitoring Mode and AutoBeat Sequence. Monitoring Mode is a very low MI image that allows real-time visualization of the heart so that the user can easily maintain the imaging plane. AutoBeat Sequence allows user-controlled programmable triggering sequences upon acquiring the image and vastly increases the speed at which triggered images can be obtained. These techniques dramatically improve the ease of acquistion of triggered high-MI imaging.

Another major step on the SONOS 5500 UltraPerformance for moving MCE into clinical mainstream is the added flexibility to the Stress package. The new UltraDynamic Stress package on the SONOS 5500 fully integrates MCE. With UltraDynamic Stress it will be possible to acquire triggered loops (with Monitoring Mode), long loops (up to 20 beats), end-systolic frames only (e.g., loop/ECG feature) as well as wall motion loops. All other benefits of the Stress package apply including Gain Save, Compare, and Shuffle. With the Gain Save feature, the system will remember the settings from baseline for each view. The Compare feature allows for visualization of previously acquired views to help the user acquire the same plane. The Shuffle feature allows for side-by-side comparisons of MCE images and wall motion images – either separate or together to aid in the reading of these images.

These new features on the SONOS 5500 UltraPerformance go a long way into getting MCE into the clinical mainstream.

TRIGGERED IMAGING FOR DETECTION OF MYOCARDIAL HYPO-PERFUSION IN PATIENTS

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Detection of coronary artery disease (CAD) by assessing myocardial perfusion has been seen as the most attractive indication for ultrasound contrast agents. Development has been ongoing for several years, but so far without regulatory approvals for this indication. In order to understand the challenges, a definition of the requirements in different patient groups is needed. After the introduction of perfluorcarbon-based agents and non-linear imaging modes, it has been easy to observe microbubbles in the myocardium. Their presence indicates the existence of perfusion and this can be used to show viable tissue in patients after an acute myocardial incidence.

The next technical challenge is to estimate the amount of contrast agent in the myocardium, which is proportional to the amount of blood. Ultrasound is a single-view tomographic technique and thus image intensity is not only related to the concentration of bubbles present, but also to the attenuation in the ultrasound beam pathway. In a limited region, however, the intensity in the image is approximately proportional to the number of bubbles and to the blood volume. Blood volume is, however, of limited clinical interest.

Perfusion is related to the blood flow in the myocardium. Techniques involving destruction of bubbles and measurement of replenishment have, therefore, been designed. Provided that imaging conditions are kept constant, the time constant of the intensity increase in the image can indicate how well perfused the myocardium is. It is clinically relevant to obtain this information, during stress tests, for patient with CAD.

Sonazoid™ has been tested in a clinical study, using an ATL HDI 5000 scanner, in 60 patients with suspicion of CAD. In this study high MI imaging was used and the length of the trigger intervals were manually changed to give an impression of replenishment for an observer. The results showed that with the use of dipyridamole stress, sensitivity varied between 77% and 86%, depending on the observer, and specificity between 20% and 75% for detection of disease. Disease was defined as more than 50% stenosis by coronary angiography as the truth standard. The study was technically complex to perform, and this may have influenced the results.
A similar study was done with Sonazoid and an Agilent Sonos 5500 scanner in 15 patients with suspicion of CAD. Manual changing of trigger intervals with high MI imaging and real time imaging

with low MI after contrast destruction were employed to observe the replenishment of contrast in the myocardium. Disease was defined as more than 50% stenosis by coronary angiography as truth standard. The triggered method had a sensitivity of 92% and specificity 100% for detection of disease. The real time method had a sensitivity of 36% and specificity 33%. In this study, real-time imaging was not shown to be useful in detection of CAD in patients with moderate degree of stenosis (≥50%). These poor real-time results may be due to destruction of bubbles, which depend on energy at power levels below and MI of 0.5 and the real-time framerate implies higher energy exposure to the bubbles. The triggered techniques, on the other hand, show promising results, but user-friendliness needs to be improved.

Several new automated trigger techniques are under development. One approach is to automatically perform the trigger interval stepping. This has recently been improved with a monitoring mode between the longest trigger intervals where a live image is shown side-by-side with the triggered images (Agilent). Another approach is Triggered Replenishment Imaging (ATL), where an initial burst is followed by imaging every heartbeat. Clinical test in a few patients with Sonazoid have shown promising results, but improvements are desirable. Only future clinical studies can show if these methods are good enough to reliably detect hypoperfusion in patients with CAD.

THERAPEUTIC CONTRAST AGENTS

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Recently, there has been incipient work in using a new type of therapeutic contrast agent as a drug delivery device. These agents would provide localized delivery since drugs contained in their shells would remain inert until they reached a specific site. These agents differ from traditional ultrasound contrast agents in that they are constructed with a thick oil shell typically 500 nm in size. The destruction or fragmentation of these agents by ultrasound is necessary to allow the release of the suspended drugs in the shell. This drug delivery method has the advantage of reducing the toxic exposure and increasing efficacy. Particularly for either direct action on endothelial cells or sub-endothelial targets, local release in greater concentration is of considerable interest. Recently, the ImaRX Corporation has developed an oil shelled ADDA known as MRX-552. The drug Paclitaxel, which is resistant to solubility in water, may be suspended in the oil shells. Paclitaxel has been employed as a chemotherapeutic drug to combat breast, lung, and other types of cancers. High-speed camera (Imacon 468, DRS Haland) studies have visualized the different destruction scenarios at 1.0 and 2.5 MHz at pressure amplitudes 1.0-3.0 MPa for pulses 5-10 cycles. A pinch-off mechanism is visualized as result of surface waves on the agent's shell. A generalized Rayleigh-Plesset equation has been developed to account for a thick deformable viscous fluid shell. For a 4.0 micron bubble, with a 500 nm shell of viscosity of 28 cP, insonified over 5 cycles, a maximum relative expansion of 2.2 times the equilibrium radius is predicted at 2.25 MHz and 3.1 times at 1.5 MHz. Predictions from this model on the convergence velocity of the inner and outer radii have been correlated with fragmentation.

POTENTIAL OF AUGMENTATION OF GENE THERAPY USING ULTRASOUND /MICROBUBBLE COMBINATIONS IN GENERAL RADIOLOGY

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This presentation will briefly review from a general imagers perspective some of the potential of sonoporation and micro bubble strategies for drug delivery, particularly of genetic material.

More than a decade after the first approved patient study of gene therapy, and despite a large number of trials in progress (over 579 protocols are currently listed by the Wiley webpage http://213.80.3.170:80/trials/index.html) development of gene therapy to give clinical benefit has been frustratingly slow, although some successes are now apparent. Arguably the biggest single barrier has been safe delivery of genetic material to the target cells. Both viral and non-viral methods have been employed. Non-viral delivery, using plasmid DNA, has theoretical and practical advantages, including ease of preparation, a relative lack of immunogenicity and inflammatory side-effects, improved safety profile and the ability to carry relatively large DNA sequences. Unfortunately, gene transfer efficiency with naked DNA is poor and cell-specific targeting is difficult, even when complexed with carrier liposomes or other vehicles. Viral methods offer better transfer efficiency, but all vectors have problems including immunogenicity (especially with adenoviruses), cytopathic effects (for example with herpes viruses), undesirable viral tropisms, and limitations on the length of the DNA sequence that can be carried. In addition many viruses will only deliver DNA effectively into actively dividing cells. Viruses that have non-mammalian hosts such as baculovirus (which has liver selectivity) may offer advantages, but complement-mediated reactions can be a problem.

Ultrasound is cheap, widely available and most parts of the body can be relatively easily insonated. There are several ways in which ultrasound can be used to improve transfer efficiency and targeting.

Ultrasound on its own can induce cells to take up larger molecules through the mechanism of acoustic cavitation, in which small bubbles are produced and act to focus the energy in the ultrasound beam. It has been known for several years that this can open transient nonlethal pores in cell membranes. This process, termed "sonoporation", has recently started to be used to enhance gene transfection with highly encouraging results without significantly affecting cell viability.

Microbubbles can greatly enhance the sonoporation process, because they are highly efficient promoters of cavitation. Recent work suggests that the additional increase in efficiency produced by applying microbubbles with ultrasound by an order of magnitude. In addition, microbubble disruption can produce microvessel ruptures, which may further potentiate drug delivery across endothelial barriers. This could be of obvious value where extravascular targeting is planned. A crucial advantage of using microbubbles is that there is some evidence sonoporation can be achieved with acoustic powers within diagnostic limits. Thus microbubble-enhanced sonoporation could potentially be performed while imaging a target tissue using conventional imaging equipment. Another advantage of a combined sonoporation-microbubble strategy is that the ultrasound could be applied at a particular phase of the microbubbles kinetics.

Targeted/preloaded microbubbles may be used as drug delivery vehicles, as they can carry a payload of genetic and other material. Methods of incorporation include direct binding, particularly with albumin-based agents, and the production of bespoke liposomes with agents incorporated into their surface membranes. Although some of these technologies are relatively complex, it is known that locally made albumin-based microbubbles, such as perfluorocarbon-exposed sonicated dextrose albumin: PESDA can bind genetic material such as oliogonucleotides. In principle, microbubbles can thus be relatively easily engineered in-house, as gene therapy vehicles.

Although a number of preliminary studies have shown promise, much of the work in this area has focussed on cardiovascular gene therapy. This is not surprising, given the ease of access to both microbubbles and genetic material, but it is noteworthy that 69% of patient studies to date have involved cancer, compared to only 1.7% with vascular diseases. Furthermore 35% of patient studies have involved direct intratumoral injection, compared to 9% with intravenous injection. In other words, the potential of microbubble/sonoporation applications in oncology and organ-specific use (eg the liver) and for potentiating direct injection strategies have been underexploited. In response to this, we have recently started to explore microbubble/sonoporation delivery strategies at this institution, using as a model marker gene transfer to skeletal muscle in a mouse model using a variety of ultrasound energies in the diagnostic and physiotherapy ranges and will present some preliminary results.

SUBHARMONIC PHASE INVERSION CONTRAST ENHANCED ULTRASOUND AS A METHOD TO MONITOR TUMOR VASCULARITY IN A RAT MODEL

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A new technique for destruction-reperfusion imaging of tumor blood flow will be described, together with preliminary results for the application of this technique in a rat tumor model. In this mode, a set of destructive pulses is swept through a two dimensional frame, followed by a set of non-destructive pulses. Destructive pulses use a low center frequency and pressure on the order of 2 MPa to destroy the contrast agent. A non-destructive pulsing sequence is then applied, utilizing 32 transmitted pulses with a minimum time gap of 100 msec and a maximum observation time of 34 seconds. The non-destructive imaging mode combines subharmonic frequencies and phase-inversion, and data are processed using new algorithms to estimate flow parameters. Microvascular flow velocities below the threshold of traditional Doppler and colorflow techniques are estimated. The flow of the contrast agent into a given region is modeled by a rising exponential with parameters of slope, initial value, peak value and time delay. Sonographic evaluation is performed using a 5.0 MHz linear transducer and modified Siemens Sonoline Elegra system.

R3230 rat mammary adenocarcinomas are implanted within the subcutaneous tissues of the thigh. Rats are first imaged 28 days following implantation, and then are serially imaged every 3-4 days until day 42. Measurement of maximum tumor height, width and length is obtained with standard B-mode imaging. A constant rate infusion of ultrasound contrast media is delivered intravenously. The contrast is diluted to 100 ul/ml and delivered at 10 ml/hour for a maximum volume of 3 ml's. Imaging commences 2 minutes after the onset of infusion using the destruction-reperfusion mode. On day 42 immediately following the ultrasonic examination, CT images are obtained pre contrast and post contrast at 2-second intervals for 1 minute. The CT imaging planes mimic the sonographic planes. In both modalities, regions of interest (ROI's) are placed throughout the tumor, including regions that are representative of total tumor vascularity. Rats are then euthanized and tumor tissues excised. Tissue is preserved in 10% formalin and sections are obtained in the same orientation as the ultrasound and CT images. Sections are prepared with H & E stain to evaluate overall morphology and factor VIII to highlight tumor vasculature.

Serial contrast-enhanced ultrasound images reveal a progressive change from a diffuse vascular network in the earlier stages to a peripheral distribution of vessels. Ultrasound-generated maps of time

to 80% perfusion estimate flow velocities over the range of expected values. Perfusion maps closely approximate viable tumor area based on H&E histologic sections and contrast-enhanced CT images. In conclusion, serial contrast-enhanced subharmonic destruction-reperfusion ultrasound imaging of subcutaneously implanted tumors provides an accurate assessment of regional tumor viability. Tumor vessels with slow flow velocities appear to be well visualized.

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IN VIVO ULTRASOUND IMAGING OF THROMBI WITH TARGET-SPECIFIC CONTRAST AGENT

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Objective: To detect thrombi in vivo by echography using a target-specific ultrasound contrast agent

Methods: A ferric chloride-induced model of thrombosis was developed in the rabbit abdominal aorta. The receptor of activated platelets was chosen as one of the most abundant and accessible targets as determined by histological studies. Avidin-conjugated microbubbles were injected intravenously thirty minutes after the administration of a biotinylated antibody specific of GPIIbIIIa (CD41). In other experiments microbubbles bearing a platelet-specific peptide were injected intravenously, after formation of the non-occlusive thrombus. Intermittent as well as real time pulse-inversion B mode ultrasound imaging at low MI was used to follow the accumulation of the targeted microbubbles onto the thrombus.

Results: A significant opacification of the thrombus was obtained allowing a clear delineation of the lesion as soon as 10 minutes post contrast administration. Progressive signal enhancement representing microbubbles accumulation was detected up to 20 minutes at low acoustic power. Control experiments were performed with unconjugated bubbles or injecting saline instead of the biotinylated antibody. In both cases, no significant enhancement of the thrombus was observed.

Conclusion: We have demonstrated the feasibility of in vivo thrombus detection by echography using either platelet-specific microbubbles or a specific antibody to selectively label the target followed by avidin-conjugated microbubbles injection.

Optical observation of cell-bubble behavior using a high-speed camera

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Introduction

It has recently been reported that activation of ultrasound contrast agent gas bodies by ultrasound exposure can cause damage to a cell membrane.[1-2] To elucidate the mechanism, we have developed an optical observation system using a high-speed camera and observed the behavior of gas bubbles exposed to ultrasound.[3] In this study, we investigated the mechanisms of bubble collapse and cell damage from the results of observation of the behavior of microbubbles and cells.

Method

A system consisting of a high-speed camera (Ultranac, NAC Imaging Technology, Japan) and an inverted microscope (IX70, Olympus, Japan) was used for observation of PVC-shelled microbubbles of 3-5 microns in diameter and Levovist.® The microbubbles were exposed to 1-MHz short-burst ultrasound, and bubble behavior was photographed in 24-frame sequential images at the maximum frame rate of 8 million fps.

To determine whether rapid motion of a bubble causes cell damage, the behavior of microbubbles placed around a cell exposed to ultrasound was observed. Bovine arterial endothelial cells cultured on a cover glass of 0.16 mm in thickness and peritoneal exudate macrophages collected from a mouse were used in the experiments, and these cells were immersed in saline with PVC-shelled microbubbles.

Results and discussion

Bubble collapse

Bubble collapse was observed in a rapid contraction phase of bubble oscillation,[4,5] and nonuniform bubble contraction followed by generation of a small stream was also observed in this phase. Furthermore, deformation of a bubble from a spherical into a doughnut shape was observed, indicating that the small stream had made a hole in the bubble. Based on these observation results, we concluded that a microbubble can be crushed into fragments by a small stream generated by nonuniform contraction of the bubble.

Mechanical stress acting on cells

In the interaction of the cells with the PVC-shelled bubbles, deformation of a cell beside a bubble was observed. Since the cell deformation was obviously related to the bubbles behavior, it was thought that this deformation was also caused by the small stream. In the experiments using macrophages, it was observed that a macrophage was stretched out at the speed of about 10 m/s. Further study on the biochemical effects of this mechanical phenomenon is needed.

Conclusions

To elucidate the mechanisms of bubble collapse and cell damage, the behavior of microbubbles of several micron in diameter exposed to 1-MHz ultrasound was observed using a high-speed camera at the maximum frame rate of 8 million fps. Based on the observation results, we concluded that bubbles collapse 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.

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CONTRAST ECHOCARDIOGRAPHY IN ACUTE CORONARY SYNDROME

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The pathophysiology of acute coronary syndrome is known to comprise, beyond atherosclerotic plaque rupture and superimposed thrombosis of an epicardial coronary artery, microvascular obstruction to flow due to vasoconstriction, microembolisation and adherence of activated blood cells to microvascular endothelium. Therefore, myocardial perfusion imaging can add important information to coronary angiography in patients with acute coronary syndrome. The comparison between myocardial contrast defect sizes on venous contrast echocardiography before and early after reperfusion therapy in patients with acute coronary syndrome allows for evaluation of myocardial salvage. Assessment of the magnitude and spatial extent of residual myocardial contrast defect several hours after reperfusion therapy allows the prognostication of functional recovery or future presence of contractile reserve within the initial myocardium at risk. In addition, in the group of patients with acute coronary syndrome without ST-segment elevation, the detection of myocardial contrast defects on venous contrast echocardiography may contribute to the early diagnosis of the condition.

HIGH POWER CONTRAST IMAGING RISK OF ECTOPIC BEATS

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Current imaging of myocardial perfusion uses intermittent "high power" ultrasound to destroy the microbubbles. There has been some concern whether these acoustic power levels induce arrythmias in the presence of microbubbles. Therefore we retrospectively reviewed our database, which includes all digitally stored recordings of myocardial contrast echocardiography (MCE) of the last 3 years. MCE was performed using Harmonic Power Doppler performed with Levovist, SonoVue and Optison at a mechanical index of ≥ 1.0 (group 1), Power Pulse Inversion Imaging using a MI of < 0.2 for real-time imaging with Optison, Definity and SonoVue (group2) and Power Pulse Inversion Imaging with intermittent "high power" flashes (MI > 0.5) to clear the myocardium (group3). In those patients who had arrythmias during the recordings, the patients notes were reviewed. The data of an interim analysis will be presented at the meeting. In those patients, who had ventricular ectopic beats during MCE, most had ectopic beats before application of contrast or arrythmias were more frequent in previous ECGs and 24 hour ECG recordings.

Conclusion: These preliminary data suggest that the risk inducing of ventricular arrythmias is minimal when standard protocols are used with one of the approved agents.

REAL-TIME MYOCARDIAL CONTRAST ECHOCARDIOGRAPHY FOR EVALUATION OF REGIONAL MYOCARDIAL PERFUSION: COMPARISON WITH 99MTC-SESTAMIBI-SPECT

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Background: Real-time myocardial contrast echocardiography (MCE) is discussed as a useful method to evaluate myocardial perfusion.

Aim: We tested agreement between real-time Power Pulse Inversion imaging and 99mTc-Sestamibi-SPECT in evaluating myocardial perfusion on a segmental level.

Methods: MCE (Optison, 8-10 ml/h) was performed at rest and during peak dipyridamole stress in 40 unselected patients (mean age: 59 years; 33 m, 8f, 15 anterior-, 6 inferior infarcts) with angiographically proven coronary artery disease undergoing SPECT imaging for clinical reasons. From apical four- and two chamber views and comparable SPECT views 12 myocardial segments were scored for regional opacification/uptake by two pairs of blinded observers (0= absent, 1= mildly reduced, 2 = severely reduced, 3= normal). Ischemic segments by either method was defined as the difference of 1 grade between stress and rest images.

Results: Of 480 segments 62 were inadequate for reading by MCE and 58 segments by SPECT, mostly confined to basal segments. Interobserver variability was good for MCE (kappa=0,71), and SPECT (kappa =0,72). Overall agreement between the two methods was poor (kappa=0,19) when including unreadable segments but good (kappa=0,69) when restricting analysis to readable segments (concordance in 76% of fixed defects, 75% for reversible defects and 83% for normally perfused segments).

Conclusion: Real-time MCE agrees reasonably well with 99mTc-Sestamibi-SPECT in the evaluation of myocardial ischemia. However, limited feasibility to evaluate basal segments is still a critical limitation of the current method.

EVALUATION OF THE NO-REFLOW PHENOMENON IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION USING INTRAVENOUS MYOCARDIAL CONTRAST ECHOCARDIOGRAPHY

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To investigate whether myocardial contrast echocardiography could be used for the evaluation of presence and extent of myocardial perfusion defects in patients with acute myocardial infarction (AMI), and more specifically, to evaluate the ability of MCE to detect the no-reflow phenomenon, and how and when to assess the no-reflow phenomenon during AMI. Examples will be presented together with a current review of the present literature. Thus, there are important prognostic and potential therapeutic implications of the assessment of no-reflow.
It seems that MCE is superior to 12-lead ECG to assess the no-reflow phenomenon

SONOGRAPHIC ASSESSMENT OF A CEREBRAL PERFUSION DEFICIT IN ACUTE ISCHEMIC STROKE USING PERFUSION HARMONIC PARAMETER IMAGES

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Abstract: Transcranial Perfusion Harmonic brain imaging (PHI) is a new ultrasonographic technique for assessing cerebral perfusion. Using ultrasound contrast agent (UCA) bolus injection, it is possible to generate time-intensity-curves comparable to those acquired by perfusion magnetic resonance imaging (MRI) (Federlein et al. 2000; Seidel et al. 2000).
In a case report comparing PHI and MRI and a cohort of 22 patients comparing PHI and follow up CCT we evaluate the potentials and limitation of parameter images based on PHI wash-in curves after UCA bolus injection.

A 31-year-old woman was referred to our center, 4 hours after acute onset of left-sided hemiparesis. An initial CCT scan (75 min after onset of symptoms) had shown no pathology. As the patient improved spontaneously (NIH-motor score: 2), we refrained from thrombolytic therapy. Magnetic resonance imaging (MRI, Siemens Magnetom Symphony) was performed 7 hours after onset of symptoms. Whereas diffusion weighted imaging showed only minor changes within the right hemisphere, Perfusion-MRI displayed a significant delay in perfusion located in the anterior middle cerebral artery (MCA) territory (A, C).
Transcranial color-coded duplex sonography (8 h after onset) confirmed an occlusion of the right MCA (F).

Given informed consent, we started PHI 12 hours after onset of symptoms. Each hemisphere was investigated after bolus injection of 5 ml Levovist™ at a concentration of 400 mg/ml (Schering). PHI was performed with a SONOS 5500 ultrasound system and a 1.8/3.6 MHz (S4) sector transducer (Philips). The investigation was performed in an axial diencephalic plane with a maximum depth of 10 cm (focus on 8 cm).

Using the software NIH Image 1.62 (National Institutes of Health, Bethesda, MD, USA) on a conventional portable personal computer (Macintosh PowerBook G3, Apple Computer, Cupertino, CA, USA), pixelwise peak intensity (PPI) and time-to-peak-intensity (TTP) parameter images were calculated from the data sets as described previously (Wiesmann and Seidel 2000). If the total blood

flow in the infarcted area is reduced, the signal of PPI images will decrease. Delayed perfusion, as found in areas perfused by collateral vessels can be identified as signal decrease on TTP images. The PPI image showed no area of reduced peak signal intensity (B), but the TTP image revealed a clear delay of contrast enhancement in the anterior MCA territory (brown to red area) corresponding to the area of delayed perfusion as displayed with perfusion MRI (C, D). A CT scan after three days confirmed an infarction in the right anterior MCA territory (G).

In addition to the case report we present our first results using parameter images of the PHI wash-in curves (Levovist 400 mg/ml 5 ml bolus) in a cohort of 22 patients suffering from ischemic stroke. The conclusion of our study is that PHI gives reliable information about cerebral macro- and microcirculation which is important in the management of patients with ischemic stroke. The method is especially helpful for the short time follow up in a stroke unit or ICU (no patient transport needed) and for hospitals without 24 hour access to MRI technology.

Figure legend: 31-year-old woman suffering from acute stroke due to middle cerebral artery (MCA) occlusion. A Diffusion MRI, 7h after symptom onset. No major diffusion deficits can be shown B Pixelwise peak intensity (PPI) image (PHI, 12h after symptom onset). C Perfusion MRI (7h). A severe delay in perfusion of the anterior MCA-territory is detected (white area). D Time-to-peak (TTP) imaging (PHI) in the same area shows a signal delay (red/brown area). E Magnetic resonance angiography (7h) and F Transcranial color coded sonography (TCCS, 12h) confirm an MCA occlusion (1. missing MCA flow signal lateral fissure, 2. anterior cerebral artery (ACA), 3. posterior cerebral artery (PCA), 4. brainstem). G A CCT scan, three days after stroke, confirms the definite area of infarction.
(For presentation, MRI and CT scans have been turned and twisted 90° to the left. PHI-investigation planes are highlighted as white sectors, B, D)

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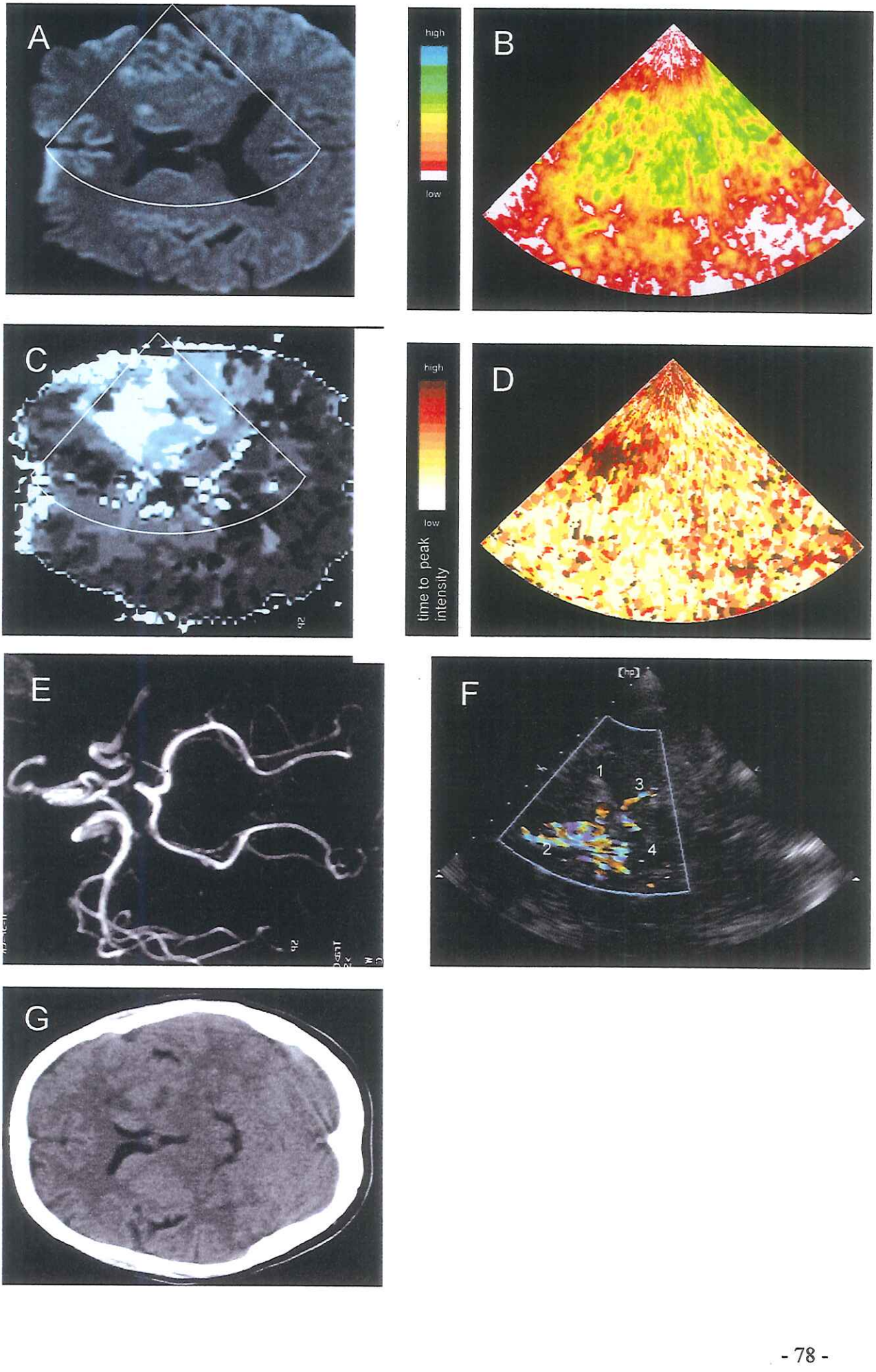
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MYOCARDIAL CONTRAST ECHO FOR DIFFERENTIATION OF MYOCARDIAL MASSES: SOLUTION, CONFUSION OR BOTH

Folkert J. Ten Cate

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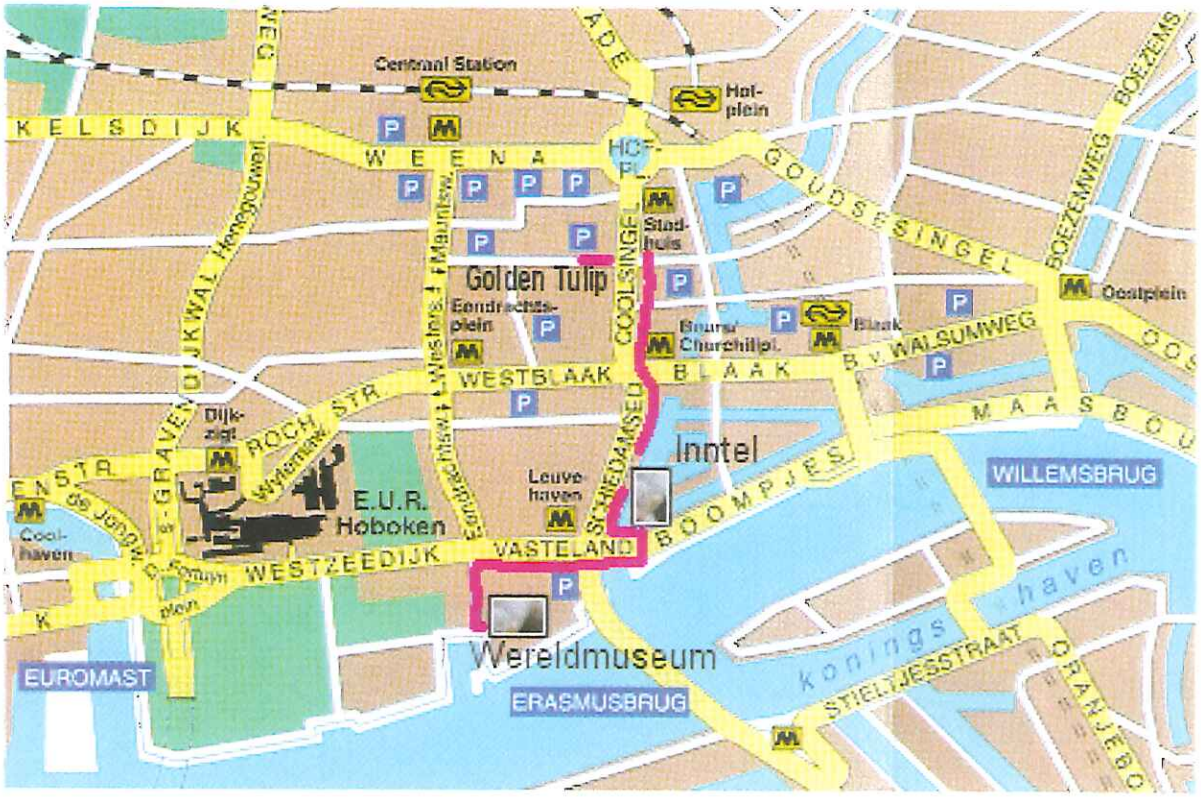
Case: A 50 yr old woman was referred to the clinical department of cardiology for analysis of systemic thrombo emboli and ischemic heart disease. She has been ill since 4 months. Her first clinical presentation was intermittent claudication. In the analysis an abdominal aneurysm with thrombus was found. In the pre-operative evaluation elsewhere a cardiac catheterization for coronary anatomy (Sones technique) was performed complicated by acute closure of several left finger arteries resulting in finger necrosis of her 2nd an 4th finger.

Echocardiogram: This showed reasonable good LV function with hypokinesis of posterior wall. Two moving masses were found in the LV cavity and two on the LV walls. A real time MCE showed perfusion of the wall and these masses making an left ventricular thrombus unlikely. Patient was operated and the masses resected. On gross examination the cardiac surgeon believed the masses were thrombus. Pathologic examination at the time of operation confirmed thrombus diagnosis. Detailed pathologic examinations for vessels in the specimen are not ready yet. Is this a case of false positive contrast echo or is it really a perfused LV mass or thrombus?



This year the Conference Dinner Buffet will be at the Wereldmuseum (World Museum).

Departure from the Inntel is planned between 18:00 and 18:30 on Thursday, January 24th.
As is obvious from the above map, the Wereldmuseum is in the close vicinity of the Inntel. In fact it is a ten-minute's walk. For those who prefer transportation, a coach will be available to take them to the Wereldmuseum and bring them back to the Inntel by 23:00.

After the welcome drinks, the Wereldmuseum will open to us three treasure chambers, which will show to us contrast in civilizations from all over the world and echoes from the past.

We hope you will enjoy your stay.

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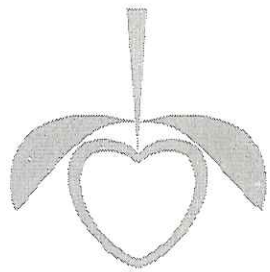

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