THE FOURTH HEART CENTRE EUROPEAN
SYMPOSIUM ON ULTRASOUND CONTRAST IMAGING

CORRIE EEFTING

ABSTRACTBOOK

The Fourth Heart Centre Symposium on Ultrasound Contrast Imaging

Folkert J. Ten Cate, MD

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David O. Cosgrove, MD

ICIN

to be held on *January 21 - 22, 1999*
Rotterdam The Netherlands

4th HEART CENTRE EUROPEAN SYMPOSIUM ON ULTRASOUND CONTRAST IMAGING. 21 AND 22 JANUARY 1999, Rotterdam, The Netherlands.

WEDNESDAY **20 January 1999**

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

THURSDAY **21 January 1999**

08.00 - 09.00 **Registration**

09.00 Opening address by N. Bom

09.05 - 10.35 **CARDIAC AND OTHER APPLICATIONS** ..... *Chairpersons: F.J. Ten Cate and P. van der Wouw*

10.35 - 11.00 **Intermission**

11.00 - 12.45 **TECHNOLOGY I** ..... *Chairpersons: V. Uhlendorf and J. Powers*

Short presentations:

12.45 – 14.00 **Lunch**

14.00 - 15.45 **RADIOLOGY** ..... *Chairpersons: F. Moriyasu and D. Cosgrove*

15.45 – 16.15 **Intermission**

16.15 – 17.45 **YOUNG INVESTIGATOR AWARD** ..... *Chairperson: P. Burns*

19.00 - 22.30 **SOCIAL EVENT "Bubbles on the river" (incl. Dinner)** 96

4th HEART CENTRE EUROPEAN SYMPOSIUM ON ULTRASOUND CONTRAST IMAGING. 21 AND 22 JANUARY 1999, Rotterdam, The Netherlands.

FRIDAY	**22 January 1999**	
08.00 - 08.30	**Registration**	
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P.P. Chang	Acoustic cavitation in the presence of microbubble contrast agents	78
J.M. Gorce	Experimental and simulated acoustic properties of Sonovue™: predicted behavior in fundamental and harmonic imaging modes	80
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R. Yamamoto	Physical principles of ultrasound-directed drug delivery	84
S. Kaul	Delivery of colloidal microparticles and red blood cells to tissue through microvessel ruptures created by microbubble destruction with ultrasound: a novel method of local drug and gene delivery?	85
P.A. van der Wouw	Electrophysiologic effects of ultrasound contrast imaging	86
A. Bouakaz	Non-invasive pressure measurement in a fluid-filled cavity	87
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P. Nihoyannopoulos	Myocardial perfusion using a different group of ultrasound imaging agents	89
G. Rocchi	Myocardial perfusion in recent myocardial infarction using Power Doppler contrast echocardiography	91
C. Algermissen	Signal amplification in Transcranial Doppler Sonography by a sulphur hexafluoride containing ultrasound contrast agent (SonoVue™)	92
M. Claudon	Changes in renal blood flow depicted with contrast enhanced harmonic imaging during acute urinary obstruction	93
F.J. Ten Cate	Example of DMR (Direct Myocardial Revascularisation)	95
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15.30	**Adjourn**	

First announcement 2000

LEVOVIST DURING STRESS ECHOCARDIOGRAPHY: METHODS AND CLINICAL USEFULNESS

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Current protocols for stress echo rely on the assessment of wall motion abnormalities for diagnosis of abnormal perfusion. Adequate delineation of endocardial borders is the prerequisite for this approach. In patients with poor acoustic windows contrast was used to enhance LV border delineation. Using fundamental two dimensional echocardiography two small studies showed a benefit of Levovist in the delineation of the lateral wall (4 chamber view) or anterior wall (2 chamber view), but complete and intense opacification of LV cavity cannot be obtained in fundamental mode. In harmonic 2 D echo even low amounts of Levovist result in strong LV contrast and may be used for improved LV border delineation. However, the strong contrast signals in the LV cavity may obscure endocardial border delineation. Further studies are required to define the reliable protocols for clinical routine. Harmonic power Doppler or harmonic angio (H-PDI) have been shown the best methods to define endocardial borders in difficult to image patients, but they suffer from a limited frame rate which impairs wall motion analysis especially at high heart rate. However, measurements of end-diastolic and end-systolic volumes are reliable and LV ejection fraction can be obtained at rest and during exercise.

In addition to improved delineation of endocardial borders it has been shown that visualization of myocardial perfusion (MP) is feasible using H-PDI which is superior to harmonic 2 D echo. The assessment of perfusion defects by means of the H-PDI amplitude is limited due to attenuation and bubble destruction. Therefore the time domain is more promising than the spatial domain in the evaluation perfusion abnormalities. Constant infusion of the contrast agent is necessary for this approach (e.g. Levovist flow rate of 8 ml/min) Intermittent end-diastolic H-PDI frames are obtained at trigger intervals varied from every cardiac cycle up to every 10^{th} cardiac cycle. Following image alignment regions of interest can be evaluated using a calibrated software tool. Refill kinetics (RK) following bubble destruction within the myocardium have been evaluated in patients with flow limiting LAD stenoses at rest. Preliminary findings suggest that refill kinetics may be a valuable tool for the assessment of myocardial perfusion. The prerequisite of reliable H-PDI recordings is the elimination of wall motion artifacts. During physical or dobutamine stress wall motion artifacts increase. Therefore dipyridamole stress should be preferred for contrast stress studies to evaluate stress induced myocardial ischemia.

THE POTENTIAL OF MYOCARDIAL CONTRAST ECHOCARDIOGRAPHY TO EVALUATE CHANGES IN MYOCARDIAL PERFUSION.

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There are many clinical and diagnostic scenarios where the ability to evaluate rapid changes in myocardial perfusion would be advantageous. These situations include acute ischaemia, before and after thrombolysis, during angioplasty and during stress perfusion studies. The ability to make repeated contrast injections, with ultrasound destruction of residual contrast agent between injections and the real-time nature of echocardiographic imaging does make contrast echo a potential perfusion imaging tool to document acute changes.

Studies have now been performed to demonstrate that the acute ischaemia and perfusion defect induced by angioplasty balloon inflation may be evaluated by IV myocardial contrast echo. Furthermore, reperfusion immediately following balloon deflation may also be seen. This implies that contrast echo may be used to study reflow/no-reflow following PTCA and thrombolysis. In addition, it should be possible to evaluate the presence and importance of collaterals and microvascular integrity, as has been previously demonstrated using intracoronary contrast injections.

The investigation of stress induced reversible ischaemia also requires a technique able to document changes in perfusion. Contrast echo seems ideally placed to perform this. Several investigators have now compared stress echo contrast perfusion studies using dobutamine, dipyridamole and adenosine with equivalent nuclear studies and coronary angiography. Concordance between the techniques appears promising, however it is important to understand that echo, nuclear and angiography provide different insights into the pathophysiology of ischaemic heart disease. It is likely that echo contrast and nuclear techniques will emerge as complementary rather than competitive imaging techniques.

Quantification of absolute and relative changes in perfusion requires processing of non-video, linear echo signal amplitude data. Together with an understanding of the effect of attenuation and other artefacts which can confound any analysis. There is a large spatial variation in segmental contrast signal amplitude at rest and stress following IV injection of a contrast agent. This spatial variation can make visual analysis very difficult and also requires compensation during quantitative analysis. Analysis of the reflow rate of contrast into individual segments following ultrasound destruction is

possible. This may be achieved by varying the triggering interval between individual ultrasound frames and does appear to be a promising technique.

In conclusion, myocardial contrast echo does appear to be able to document changes in perfusion, which may occur during various clinical scenarios. This will confirm the technique's position both as a major diagnostic and monitoring tool in patients with ischaemic heart disease.

LEVOVIST: ITS USEFULNESS IN AN OUTPATIENT SETTING

[1,2]Jarosław D. Kasprzak, [1]Guido Rocchi, [1]Folkert J. Ten Cate, [1]Youssef F.M. Nosir, [2]Jarosław Drożdż, [2]Michał Plewka, [2]Maria Krzemińska-Pakuła

[1]Heart Centre, Erasmus University Rotterdam and [2]Department of Cardiology, Medical University of Łódź, Poland

Recent advances in contrast echocardiography expand the clinical applications of the method by improving quality of technically difficult studies and introduction of new indications such as perfusion studies. Better understanding of echo contrast and ultrasound physics allows optimalization of imaging protocols and efficient use of registered, commercially contrast agents in the new areas. Major indications for contrast echocardiography in an outpatients clinic include:

1. Shunt detection
2. Enhancement of weak Doppler signal (old but not obsolete indication)
3. Enhancement of endocardial border in resting and stress studies
4. Myocardial perfusion studies (work in progress).

With help of novel strategies such as application by continuous infusion, and advanced contrast detection techniques, all these aims can be efficiently addressed in outpatients with registered agents such as Levovist™ (Schering AG).

Three case studies will be shown to illustrate the benefits of contrast used in:

1. 61 year old female with calcifications of aortic valve and poor imaging window, refusing TEE. The true transaortic gradient of 120 mmHg could be recorded only after the injection of Levovist.
2. 50 year old male with syncope and mild systolic and diastolic dysfunction of the left ventricle. Normal perfusion pattern was detectable with greyscale harmonic and harmonic power Doppler during an infusion of Levovist. The diagnosis of early stage cardiomyopathy was supported by normal coronary angiogram.
3. 47 year old male with LBBB, resting and stress-induced perfusion defects by sestaMIBI SPECT who underwent Flash Echo Imaging with a bolus of Levovist with a normal perfusion pattern. Conflicting clinical data were judged in favor of echocardiography by normal coronary angiogram.

*Conclusions:*Appropriate use of bloodpool enhancing contrast agents merged with novel imaging approaches such as greyscale harmonics, harmonic Power Doppler or Flash Echo Imaging expand the diagnostic potential of echocardiography through significantly improved quality of diagnostic information and the potential for direct perfusion imaging.

HARMONIC GREY-SCALE IMAGING OF THE BRAIN PARENCHYMA USING A NEW PERFLUOROBUTANE BASED ULTRASOUND CONTRAST AGENT

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Purpose: Using the new perfluorobutane based ultrasound contrast agent BR14 (Bracco Research, Geneve) we analized the change of acoustic brain parenchyma density after bolus injection as a tool for visualization of brain perfusion.

Method: Six sedated beagle dogs were insonated transtemporally using harmonic B-mode (1.8/3.6 MHz sector transducer, transient responds imaging, HP SONOS 5500). After intravenous bolus injection (0.2 ml/kg BW) 60 images (samples) were stored on a MOD for off-line analysis. Mean acoustic density was calculated for three different regions of interest (m. masseter ipsilateral [1cm depth], parietal brain parenchyma, [3cm depth], contralateral scull [7cm depth]).

Results: The increase of normalized mean acoustic density in the brain parenchyma and the muscle were 24% and 20% respectively. In the contralateral skull we found a decrease of acoustic density up to 29% (figure).

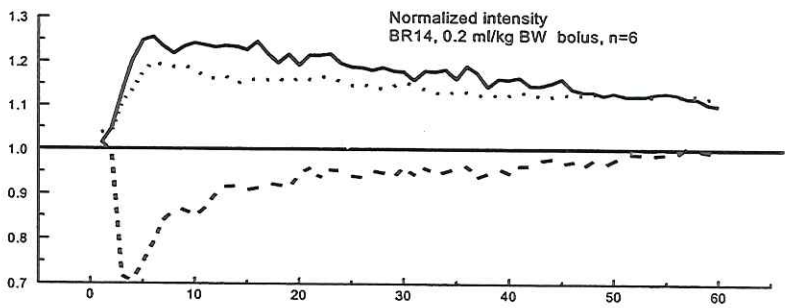

Figure. *Normalized mean acoustic density after injection of 0.2 ml/kg BR14 i.v. (n=6).*

Conclusion: BR14 leads to a strong increase of acoustic density in the brain parenchyma. As a result of this, the acoustic density of underlying structures like the contralateral skull is reduced („shadowing"). This visible enhancement of brain parenchyma is a marker of brain perfusion.

COMPARISON OF FUNDAMENTAL M-MODE AND B-MODE HARMONIC IMAGING AND CONTRAST FOR LV MASS WALL THICKNESS AND LEFT VENTRICULAR MASS CALCULATION

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Left ventricular (LV) mass calculated from M-mode is widely used as an indicator of LV hypertrophy. However, M-mode images may be suboptimal in up to 20% of subjects. B-mode harmonics (H) and contrast injection (Optison™) may improve the quality of the suboptimal images obtained by fundamental M-mode (F). We recorded LV dimensions in 9 patients(P) using F and H imaging without and with contrast (CF and CH) to validate these techniques in assessing the LV mass. Mean value for septal (S) and posterior wall (P) thickness, and LV diastolic dimension (DD) for each patient and each technique was calculated from three measurements. Subsequently, the mean value for 9 P was computed for each technique. Results are shown in the table.

Conclusions: LV dimensions measured by different echo modalities appear comparable suggesting that use of contrast with fundamental or harmonic technique may provide adequate measurements of LV dimensions when fundamental images are suboptimal.

	F Mean±SEM	H Mean±SEM H-F	CF Mean±SEM CF-F	CH Mean±SEM CH-F
S (mm)	9.4±0.6	9.6±0.7 0.6*	9.7±0.7 0.9*	9.7±0.7 1.1*
P (mm)	9.1±0.5	8.8±0.5 0.9*	9.0±0.6 0.7*	8.9±0.6 0.8*
DD (mm)	54.9±1.2	54.7±1.6 1.3*	53.7±1.9 3.0*	55.3±1.7 2.6*
M (gm)	192.9±12.6	188.5±13.6 13.6*	190.2±17.4 16.4*	197.0±16.6 19.1*

* indicates mean of absolute differences between the measurements obtained by each echo technique (H, CF, CH) compared to F.

PREDICTIVE VALUE OF CONTRAST IN STRESS ECHOCARDIOGRAPHY

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Objective: The purpose of this study was to evaluate the impact of echocardiographic contrast agent (Optison™) usage plus contrast harmonic imaging on the positive predictive value of stress echocardiography versus fundamental imaging alone.

Methods: Data were gathered from existing echocardiographic and angiographic databases at our institution, spanning the periods 2-9/97 for the non-contrast group (Group A) and 4-10/98 for the contrast/harmonic imaging group (Group B), recognizing that routine contrast usage for stress echocardiograms began in 1/98 at our center. Retrospective data analysis yielded 48 positive dobutamine or treadmill stress echocardiograms with accompanying recent coronary angiographic data in the Group A and 39 such studies in Group B. Contrast usage at peak stress was confirmed in the appropriate patients by review of the original echocardiographic studies. A stress echocardiogram was considered positive if regional wall motion abnormalities consistent with ischemia, infarction, monophasic viability or biphasic viability were present in one or more left ventricular segments. Data were analyzed using angiographic criteria for significant coronary artery stenoses of $\geq$50% severity in one or more native vessels.

Results: Angiographic criteria of >50% stenosis yielded a positive predictive value of 85% (33/39) for Group B versus 73% (35/48) for Group A for detection of significant coronary artery stenoses.

Table I

Imaging Mode	**Coronary Stenoses $\geq$ 50%**
Group A, n=48 (Fundamental imaging)	35/48 = P.P.V. of 73%
Group B, n=48 (Harmonic imaging with contrast)	41/48 = P.P.V. of 85%

Conclusions: The use of intravenous echocardiographic contrast at peak stress in conjunction with contrast harmonic imaging improves the positive predictive value of stress echocardiography for the detection of significant coronary artery stenoses.

IS THERE AN OPTIMAL METHOD FOR MYOCARDIAL PERFUSION IMAGING WITH ULTRASOUND CONTRAST AGENTS?

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The major objective of microbubble contrast agent research has been to optimize methods for detecting and imaging myocardial perfusion. Today, two distinct phenomena are exploited to detect microbubbles in high dilution and in the presence of moving solid tissue. First, nonlinear oscillation of the bubbles produces nonlinear echoes which can be detected using such methods as harmonic or pulse inversion imaging. Second, bubble disruption gives rise to echoes which decorrelate over time at one location in space. These echoes also contain strong nonlinear components. In a given clinical setting, both of these phenomena coexist, rendering the optimal choice of detection method a considerable challenge. In practice, the nature of the agent and the objectives of the imaging examination offer different solutions for different applications.

Harmonic greyscale methods represent a compromise between resolution and contrast sensitivity, one which becomes more acute at low signal levels. For this reason, it is often used at high MI, even though contrast is limited by tissue harmonics. However, bubble disruption at high MI is optimally detected using decorrelation techniques such as power Doppler. If intermittent imaging is acceptable, this is often the best choice, especially with agents containing highly diffusible gases such as air. However, the power dependence of bubble destruction places a considerable demand on transmit field uniformity, which may be best met by using a separate destruction pulse. Motion suppression can still be a challenge. Is it therefore possible to detect bubbles in the microvasculature without destroying them? Because of the inescapable compromises of RF domain filtering, we are left with multipulse methods, such as pulse inversion Doppler, which are able to detect both decorrelation and nonlinear scattering without necessarily reducing bandwidth.

Ultimately, all detection methods depend on bubble behaviour, and innovation in the acoustic response of bubbles will surely lead to further improvements in detection methods.

PULSE INVERSION IMAGING: IN SEARCH OF A BUBBLE DETECTION SCHEME

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From the early days of ultrasound contrast imaging it was understood that microbubble specific imaging modalities would have to be invented in order to utilize the contrast effect. The two main characteristics of contrast microbubbles that have defined today's ultrasound modalities are: 1) microbubbles are highly nonlinear scatterers that generate harmonic signals and 2) they exhibit a very transient response, i.e., they are easily destroyed by ultrasound. Harmonic imaging addresses the former and harmonic power Doppler the latter.

The improvements of harmonic imaging in contrast research equipment resulted in the invention of a new tissue imaging modality known as Tissue Harmonic Imaging (THI) [1,2]. Even though the quality of harmonic images has improved considerably in the recent years due to THI, the tissue harmonic component is competing with the microbubble harmonic component and is a limiting factor. Since harmonic imaging is a single pulse scheme the transient nature of bubbles is not utilized. Harmonic power Doppler is utilizing the transient nature of the microbubbles. It does not image the tissue but the blood (with the bubbles). It is a Doppler scheme applied to the harmonic components of the backscattered signal. The fundamental component of the backscattered signal from the bubbles is discarded. Tissue motion (flash artifact) is a problem here which may be addressed by various types of wall filters.

Pulse inversion imaging is the newest of modalities to be added in the list of bubble detection schemes. This method is effectively a Doppler technique that combines some of the characteristics of harmonic imaging as well, and it is thus imaging both tissue and blood. It is currently commercially available as a two pulse scheme presented in gray scale. Pulses of opposite polarity are transmitted along a line and the backscattered signals are added and detected.
Odd harmonic components (fundamental, 3rd, 5th, etc.) cancel and the even (2nd, 4th, etc.) add as a result of this method. Both tissue and bubble harmonic signals are detected. In the absence of bubbles this would be equivalent to THI with bandwidth improvements. The transient nature of bubbles causes incomplete cancellation of the fundamental signals, a fact which is also utilized. The received spectra from tissues perfused with bubbles have two distinctive components: a fundamental component due to mainly the bubble changes from pulse to pulse, and a harmonic component due to the nonlinear scattering of bubbles.

In cardiology, where tissue motion is present the two pulse scheme has limited success but offers a definite improvement over harmonic imaging [3] by including transient information in addition to nonlinear scattering. Another advantage is the improved resolution. In harmonic imaging narrow band processing is required in order to separate the harmonic component from the fundamental. In pulse inversion this limitation is overcome because the fundamental signals are considerably reduced before any filtering is applied. Preliminary studies with pulse inversion with more than two pulses suggest that tissue motion problems are reduced. In radiology, where tissue motion is not as serious as in cardiology pulse inversion works well even in the two pulse scheme. The improved spatial resolution is beneficial for liver tumor detection.

In conclusion, pulse inversion is a Doppler technique aimed at microbubble detection that separates the linear from the nonlinear scattered components and improves lateral and axial resolution. The currently available two pulse scheme is more sensitive than a two pulse scheme harmonic power Doppler. The tissue generated harmonic component and tissue motion continue to be the limiting factors. The n-pulse scheme currently under investigation will address these issues.

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ACOUSTIC CROSS-SECTIONS: IMPLICATIONS OF STEADY-STATE AND TRANSIENT MODELS FOR EXPERIMENTS WITH CONTINUOUS-WAVE AND PULSED ULTRASOUND

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There are two common ways of defining acoustic cross-sections with respect to bubble-based ultrasonic contrast agents. The first is empirical, and simply normalises the scattered intensity invested in the n^{th} harmonic to that scattered at the fundamental frequency. As such, this allows a description of certain nonlinear characteristics of the bubble, such as the generation of harmonics; and it can readily be calculated from measurements. However it contains no information about the fundamental properties of the agent, and cannot be interpreted in terms of the underlying bubble dynamics. The dimensions of area are introduced by the somewhat artificial multiplication of the intensity ratio by the geometrical cross-sectional area, which has very little acoustical significance when the conditions are near resonance.

None of these limitations are true of the second method of defining the acoustic scattering cross-section, which is from the ratio of the power scattered spherically by the bubble to the intensity of a plane wave incident upon it. An equivalent acoustic extinction cross-section can similar be defined using the total power loss from the beam. This loss results from all the scatter and absorption which the target causes. Scatter and extinction cross-sections are extremely useful, since they can be interpreted in terms of the effective 'target area' which a gas body presents to an ultrasonic beam. However to date the only expressions for these cross-sections have been analytical solutions based on linear, steady-state bubble pulsations. As such they are applicable in the main to the response of gas bodies when subjected to continuous-wave fields of low amplitude. Bubble-based contrast agents are, however, usually subjected to high amplitude, pulsed fields. This paper describes a scheme for calculating the extinction and scattering cross-sections of gas bodies using a nonlinear model of their pulsations, and demonstrates how the 'efficiency' of the scatter (i.e. the backscattered proportion of the energy contained within the incident pulse) can be enhanced by modifying the pulse length, frequency, and amplitude.

SYNCHRONOUS OPTICAL MONITORING OF THE MICROBALLOONS UNDER PULSED AND FOCUSED ULTRASOUND IRRADIATION

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Abstract: Submicrosecond pulsed laser diode stroboscopic synchronous illuminator combined with microscopic CCD TV camera is devised as dedicated visualization system to monitor microbubble/ microballoon contrast agent under pulsed insonification. It successfully monitored cracking and dying process of the microballoons.

Introduction: Although microbubble and microballoon contrast agents are already an established diagnostic means in our community, their precise sonic behavior in terms of general safety is yet only modestly understood. To know more and better, its shell cracking and gas release process is one of major concern. In spite of previous researchers successful visualization of "before and after" of cracking by gated high-speed CCD TV camera (1), we moved the snapshot function to illumination side, with pulsed visible (end-of-red) laser diode.

Taking advantage of ordinary CCD camera chip*s integration capability, all the system timing including insonifier*s transmission gate are TV frame synchronous. Timing of the laser diode firing, single or double exposure pulse, is met to sonic pulse propagation to reach to the visualization site including the subject microballoon. It successfully monitored cracking and dying process of microballoon.

Electronic shutter or pulsed illuminator: On-chip electronic shutter of the CCD imaging device can gate even 10 microsecond with reasonable accuracy for nearly 1000 frame per second image taking, however, no shorter E-shutter and higher frame rate device is available. Dedicated high speed camera of this sort is relatively expensive and inflexible. We switched The gating function to illumination side and put the entire system to a semi-darkroom environment. Laser diodes are nowadays capable to transmit GHz or more high frequency pulse train, submicrosecond gated illuminator is an easy task for them. Problem may be yield of optical power to CCD imaging plate.

Here cooling down of CCD plate is of no meaning because we try to preserve pulsed light energy image only down to perform entire frame readout. Rather, absolute quantum efficiency of the entire system will be of primary concern. After several generation trial and error we successfully used

Olympus Optics CK40-21PHP-Sp "upside down" microscope with Hitachi HL6734FM 680 nanometer visible light CW laser diode (used in pulsed mode, out of specification) and Sentech STC-400 CCD TV camera. All the other devices including laser pulser and insonifier transducer/transmitter are hand-made.

Single or double pulsed illumination of 200 nanosecond light pulse images the entire of about 100 micron square area of the sample chamber located at the bottom of water vessel, with reasonable S/N of snapshot image. A 30mm diameter 2.0MHz 95mm radius concave PZT transducer is pulsed maximum for 200Vpp 16 wavelength burst wave to give maximum about 2MPa in situ Isptp (in water) insonification for the sample chamber in the test vessel. All the system timing is TV frame (not field) synchronous (=30Hz, EIA standard).

Experimental findings: First of all, for system verification, slightly larger size microballoon than real contrast agents is used. Expancel* model 551-DE20 is the selected microballoon for this feasibility study which has about 15 to 25 micron diameter shell size. Shell material and hollowness factor is not exactly disclosed but supposed similar to known mimicking agent (2). Its water-glycerin suspension is injected to test chamber, about 1mm height and 10mm diameter for synchronous monitoring under insonification. Glycerin water dilution is used to suppress too much streaming to prevent "escaping" of the subject out from maging/ insonifying spot.

The instance of cracking is yet very hard to capture because of many unsolved problems, mainly system timing accuracy. However, some "continuous" snapshot pictures recalled from video tape suggest they may include the instance of shell breakage. However, frame by frame recall-reviewing of long videotape is very tough business. On the other hand, one pulse per one frame cinematography takes up consistently the total process of microballoon breakage, shell debris emission and total dissolution.

It shows that, so far in case of Expancell 551-DE20, shell cracking follwed by gas and sphere fragment debris release seems all at once to happen after some numbers of pulsed insonification. However, total dissolution of the gas bubble takes still minimum 50 to 100 more pulses to be experienced. During this timeframe, the gas bubble "dances" dizzily and chaotically.

Although it needs further verification, such chaotic dance of the gas bubble may serve another cause of echogenicity scintillation to show it chromatically in color (and power) Doppler modes.

Discussion: Although one may not be able to define a "safe way" for shell cracking and gas+debris dissolution, to understand its process will be an important step to find a way for total safety management of this family of contrast agent. We will further make effort to go ahead for such way.

The first aid finding of this project is sufficiently encouraging. Since the test sample microballoon used in this experiment is far larger in size (15 to 25 micron) than possible real agents (3 to 5 micron, as known), it would be valid only qualitatively rather than quantitatively.

Acknowledgment: Author thanks Dr. M. Natori, chairman of this working group, and all the WG members, for their general cooperation for this project, including basic policy making. Author also thanks for Japan Fillite Co. for their sample supply of the Expancel* microballoon, and Hitachi Semiconductor Corp. for their extra sample supply of selected pulse drive durable high power visible CW laser diodes.

This work is supported by JSUM (Japan Society of Ultrasound in Medicine) and a part of activity of its safety committee. A preliminary form of this report has been given in IEEE International Ultrasoincs Symposium, Oct. 1989, in Sendai Japan.

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AN IN VITRO STUDY OF THE ACOUSTICAL RESPONSES OF AI-700.

Charles C. Church

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AI-700 is a new ultrasound contrast agent currently in clinical development for continuous, real time imaging of myocardial perfusion. AI-700 was designed to address the limitations of other contrast agents, such as high attenuation, low echogenicity and gas leakage. AI-700 comprises a low solubility gas within an encapsulating shell composed of the synthetic polymer, poly-D,L-lactide-co-glycolide with a hydrophobic compound embedded therein. The agent has a number-mean diameter of ~2.2 μm at a concentration of ~2.2×10^9 microparticles/mL. *In vitro* acoustic measurements of attenuation coefficient and integrated backscattered power (IBP) were performed using focused, pulsed ultrasound beams similar to those produced by diagnostic machines operating in fundamental imaging mode. The frequencies (1.0 – 5.0 MHz) and powers (MI = 0.5 and 1.1) employed span the range used in most echocardiographic examinations. Samples of AI-700 and Optison™ (used as a reference material) were diluted at ~1/1100 in sample chambers containing normal saline at 37 °C and degassed to 90% of saturation; chambers were rotated at ~15 rpm to maintain the agents in suspension; 50 pulses of ultrasound (PRF = 2 Hz) were directed into the suspensions through an acoustically transparent window in the chamber; backscattered signals were received, amplified and processed to yield values for attenuation coefficient and IBP (relative to background) at 6 time points up to 10 minutes following sample dilution. These data were then combined in a calculation of echogenicity, the net relative power returned from a fixed depth (2.5 cm in this case) in the diluted agent. It was found that values of both attenuation coefficient and IBP for AI-700 were essentially independent of the time of measurement while the corresponding values for Optison decreased markedly with increasing time. For example, measured values of attenuation coefficient for AI-700 generally were much less than those for Optison at the early time points (8.7 vs. 14.9 dB/cm at 3.5 MHz, MI = 0.5), but the values were comparable at later time points (8.8 vs. 10.0 dB/cm). Similarly, values of IBP for AI-700 were generally somewhat (5 – 10 dB) less than those for Optison at early time points but greater (5 – 10 dB) at later time points. These results indicated that the Optison microbubble size distribution was changing with time while the gas content of AI-700 microparticles was stable. In addition, values of IBP for AI-700 increased approximately linearly with acoustic power while the values for Optison increased approximately as the square of the power. Comparison of this result with recent computations of nonlinear scattering from free bubbles tends to confirm the conclusion that Optison was disrupted during the pseudo-imaging, measurement process while AI-700 was not. Finally, values of echogenicity were consistent with the strong visual shadowing often seen with Optison and the

much lower attenuation produced by AI-700. Taken together, these results indicate that AI-700 is able to provide strong, long-duration image enhancement with low visual attenuation.

SUBHARMONIC IMAGING OF ULTRASOUND CONTRAST AGENTS

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Introduction: Recently several investigators have studied the posibility for subharmonics imaging of ultrasound contrast agents (Lotsberg et al 1996; Shankar et al 1998; Forsberg and Shi 1998). Lotsberg et al.(1996) observed subharmonic scattering for Albunex. It was concluded that the acoustic pressure for the onset of subharmonics was lower then expected from the theory, as developed by Eller and Flynn (1969) where they corrected for the presence of the shell of the Albunex microspheres. The authors gave a possible explanation by assuming that because of the acoustic pressure the encapsulating shell brakes and the free air bubbles appear and will oscillate with subharmonics at a lower sound pressure. Shankar et al. (1998) described the advantages of subharmonic imaging over conventional harmonic imaging concerning agent-to-tissue ratio. As function of pressure this ratio increases for the subharmonic, whereas for the second harmonic decreased. This is probably due to the nonlinear propagation effects in tissue where second harmonic components are generated, especially at higher values of the acoustic pressure. Also, attenuation is lower for subharmonic compared to second harmonic components.

Simulations: To demonstrate the presence of subharmonics, simulations were performed by using the Rayleigh-Plesset equation as a free gas bubble model. Damping and surface tension was included. Fig 1 (top) shows the bubble wall velocity as function of time of a single microsphere with a diameter of 4 μm, in response to a 3.38 MHz sine burst of 150 periods with amplitude of 200 kPa. The growth of the subharmonic component is shown by the 3 different time stages (A-C) of the bubble wall velocity, with their corresponding scatter spectra (D-F).

The applied frequency of the sine burst was set to 3.38 MHz because this is twice the resonance frequency for a 4 μm microsphere. At this frequency, according to Eller and Flynn (1969) the acoustic pressure threshold should be minimal for subharmonic generation. From Fig 1 D-F it can be concluded that mainly the subharmonic components increase in time.

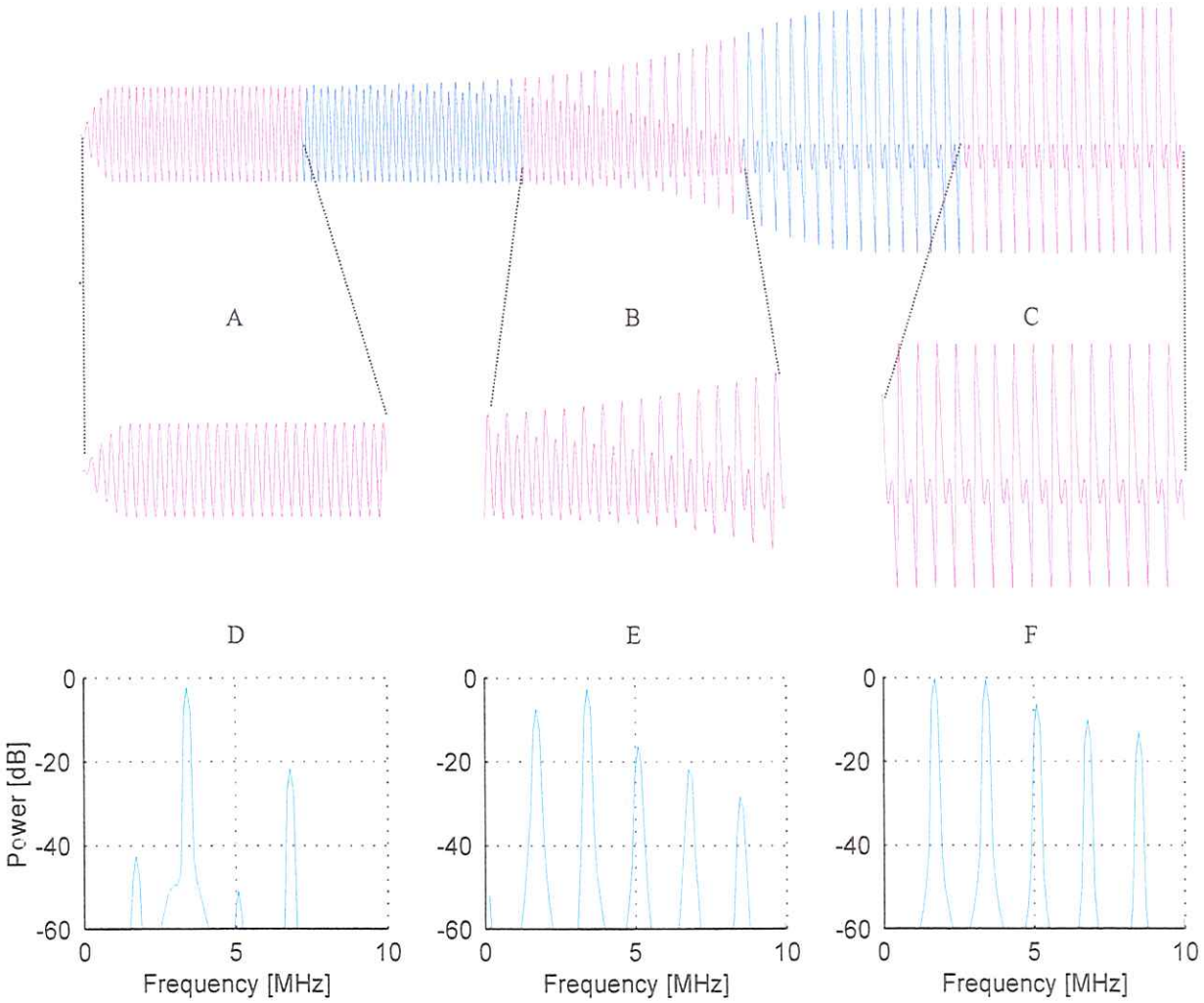

Figure 1. Growth of subharmonic in time for a 4 μm air bubble, driven at 3.38 MHz at 200 kPa.

Measurements: Subharmonics have been observed with Sonovue™ (Bracco, Geneve). Figure 2 shows the in-vitro response of Sonovue™ when insonified with a burst of 40 periods at a frequency of 3.5 MHz. The applied peak negative pressure is 75 kPa. The figure shows the fundamental peak at 3.5 MHz, the subharmonic at 1.75 MHz. Furthermore, second harmonic and ultraharmonics are observed.

Discussion: Although the harmonic nature of oscillating gas bubbles has been exploited extensively, new opportunities arise by applying the subharmonic component of an oscillating gas bubble. However, subharmonics are currently not used in diagnostic ultrasound. The characteristics are such that the axial resolution of subharmonic imaging is low as shown in figure 2. A new transducer design and imaging strategy will be discussed where the increased sensitivity of the subharmonic, over the second harmonic, will be used and that will circumvent the limited resolution.

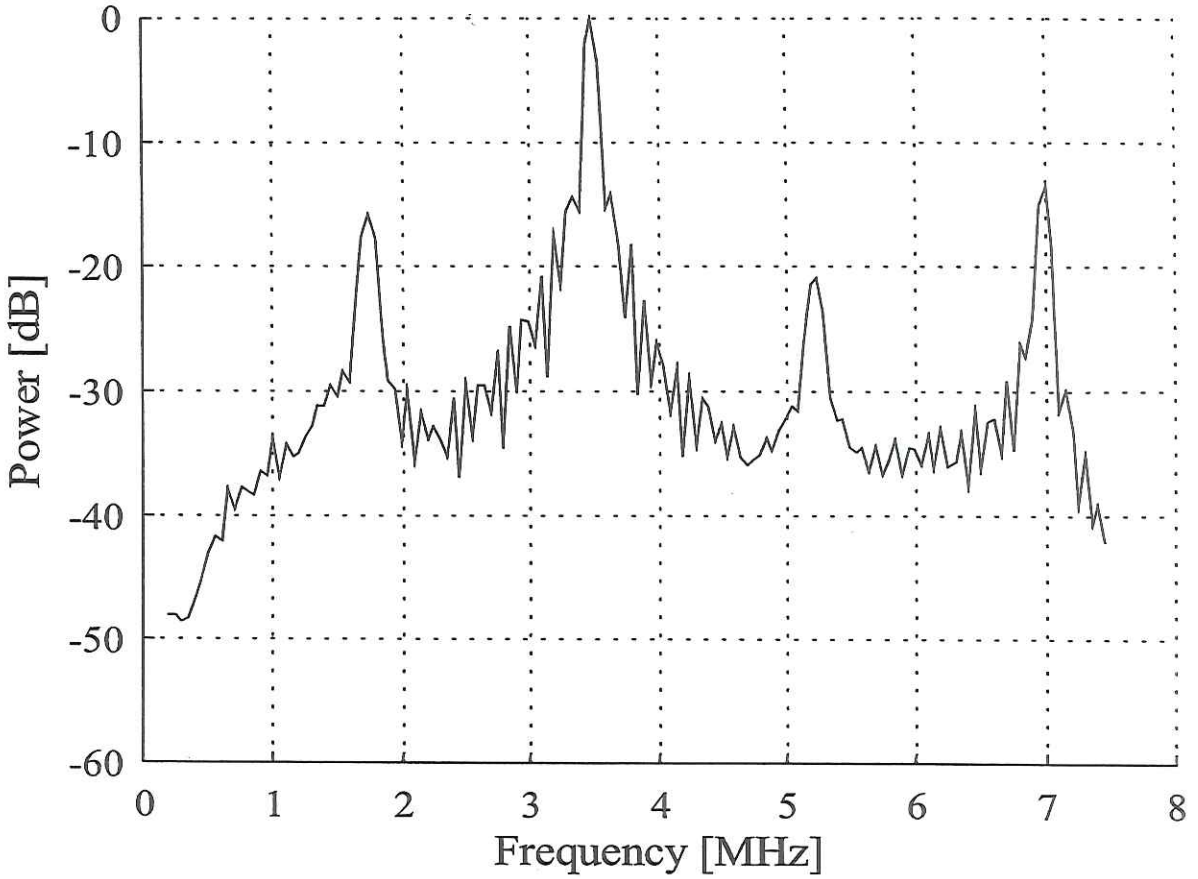

Figure 2. Scatter spectrum of Sonovue™. The transmitted burst has a frequency of 3.5 MHz, 40 periods and the peak negative acoustic pressure is 75 kPa.

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SYSTEM PERFORMANCE FACTORS IN LOSS OF CORRELATION IMAGING

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Multiple techniques have been developed to take advantage of various acoustic properties of ultrasound contrast agents. Of those currently in use or active investigation, wave shaping techniques separate transmit and receive spectra and pulse cancellation techniques eliminate the fundamental acoustic energy through addition of two transmit pulses, each an inverse of the other. Loss of correlation technique takes advantage of selective bubble destruction during the contrast. Each technique has distinct advantages in certain applications, although enough disadvantages exist to preclude any one from being the universal solution to contrast agent imaging challenges. All of these technologies take advantage of specific contrast agent properties.

A new technology has been developed that improves performance in fundamental target acquisition. Called plano-concave (PC) transducer design, it allows software control of the elevation slice thickness, reduces slice thickness side lobes, and extends –6dB round trip fractional bandwidth by as much as 120%. Since transducer spatial and temporal characteristics have a predominant effect on image quality, the PC technology has a visible impact on both images generated with and without contrast agents.

The transducer array design is based on varying the single element thickness in the elevation direction with axial symmetry around the azimuthal imaging plane. Performance data clearly show increased bandwidth without loss of sensitivity, ability to control elevation aperture slice thickness by controlling just the excitation frequency, and extended transmit zone range. In addition, all that is accomplished without additional hardware system channels or additional cables.

Experimental data shows that the longer transmit zone shows promise as a means of enhancing loss of correlation imaging. Thus in addition to obvious benefits in 2-D and 3-D imaging, the PC technology should have a very beneficial effect in contrast agent imaging.

IN VIVO BEHAVIOR OF MICROBUBBLES OBSERVED USING HARMONIC GRAY SCALE IMAGING

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Microbubble contrast agents are recognised as particles while they are administered intravenously and observed using real time harmonic grey scale harmonic imaging. In this paper behaviour of microbubbles in the vascular space and parenchymal circulation is described based on real time observation using harmonic grey scale imaging.

Methods and materials: Optison™ (MBI, San Diego) was used in human study and administered intravenously by bolus injection. NC100100 (Nycomed, Stockholm), Sonovue™ (Bracco, Geneva) and Levovist™ (Shering, Berlin) and OUC82755 (Kyoto University, Kyoto) were used for animal studies. The equipment used was a second harmonic imaging system, PowerVision 7000 and 6000 (Toshiba, Tokyo) and a pulse inversion harmonic imaging system, SONOLINE Elegra (Siemens, Issaquah). The patients with various liver tumour were examined. Benign tumours included hemangioma, regenerative nodule, abscess and focal nodular hyperplasia (FNH). Malignant tumours were early and advanced hepatocellular carcinoma (HCC) and metastasis. The animals used were rabbits and dogs. VX2 tumours were implanted to the rabbit liver two to four weeks prior to the study.

Results: The images were obtained using a wide-band transducer with central frequency of 7.5 MHz in animal studies and 3.5 MHz and7.5 MHz transducers were used for human liver imaging in deeper and shallow portions respectively. Microbubble contrast agents were observed as "staining" when the blood concentration was higher and as "each particle" when the concentration became lower. The microbubbles observed as each scatterer were categorised into two patters regardless of kind of agents. One is small and so fragile that they were easily destroyed during one to several scanning frames. The other is larger one and so resistant to ultrasound exposure that they were observed in many frames. Sometimes obviously huge bubbles were observed in the portal vein and they were trapped in the periphery of the hepatic portal system. Existence of microbubbles enabled us to observe hemodynamics of blood in vascular space such as portal vein, IVC and aorta. Blood steam was understood well at real time in the branching vessels, confluence of venous system and pulsatile flow in the aortic cavity. In large portal veins, such as the trunk and its main branches, the huge sized microbubbles are often observed. The sizes of these bubbles were large enough to embolize the peripheral intrahepatic branches of the portal vein. The size of these large microbubbles do not seem

to be original ones because these bubbles can be seen with conventional B mode and they are not destroyed by continuous exposure of ultrasound at the maximum MI value. It is suspected that these bubbles are regenerated from the microbubbles in the capillary and/or venous channels. The lifetime of microbubbles and bubbles are varied from one frame to many frames, indicating variety of fragility to ultrasound exposure. Behaviour of microbubbles in the tumour parenchyma has a potential for tissue characterisation of liver tumours. In benign tumour represented by FNH, microbubble are flowing regularly and at a steady speed, indicating benign (well-regulated) vascular structure. On the other hand, microbubbles flowing in the malignant tumour indicate irregular and tortuous blood flow. The irregularity means anatomical tortuosity, distribution of vascularity and flow speed of microbubbles.

Conclusions: Microbubbles administered intravenously can be changed in the flowing blood. They are changed in the size and fragility to ultrasound. Observation of microbubble behaviour in the tumour tissue has a potential for a new diagnostic criterion in clinical settings.

ULTRASOUND CONTRAST APPLICATIONS IN RADIOLOGY

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Background: Regardless of the tissue characterization and image contrast capability of an imaging modality, contrast media are needed to increase the information yield of the technique. Besides their ability to increase contrast between pathologic and background tissues, any agent whose pharmacokinetics is understood will provide meaningful physiologic information as its rate of entry, rate of elimination, and degree of accumulation are monitored over time in a region of interest. Although the ability of ultrasound to quantitate echogenicity is limited, significant strides have been made to quantitate contrast concentration in blood. The objective of this presentation is to describe the current status of the ultrasound contrast field in Radiology from the agent as well as the instrumentation perspective and highlight some of the possible uses of this new capability.

Contrast agents have played an important role in diagnostic radiology. Aside from angiography and nuclear medicine techniques that are, by definition, contrast examinations, contrast agents have added significantly to the diagnostic capabilities of all imaging modalities. Sonography is the last technique that has not gleaned the benefits of contrast media administration. Ultrasound contrast agents have been sought since the advent of medical US with currently 2 approved agents in the USA and few more in Europe. All of these approved agents enhance Doppler signal and one agent, Optison, can in addition alter tissue echogenicity. Although most efforts are to carry the existing agents through the approval process, research is focusing on targeted agents and contrast specific instrumentation to optimize image quality, improve vascular imaging, increase the conspicuity of pathologic tissues, and quantitate tissue perfusion.

Despite the many potential radiologic applications that were described for the liquid perfluorocarbon (PFC) emulsions [1], the development of such agents has halted in favor of the new bubble-based agents. The latest generation of microbubbles that provides greater blood concentration in the systemic circulation has allowed for the rebirth of the many radiologic applications described for the liquid PFC emulsions.

Potential Applications: Although each agent has its unique properties, vascular dwell time, and sensitivity to ultrasound pressure, all the PFC-based microbubbles share common characteristics. They all fill the cardiac chambers with echoes for several minutes at minuscule doses (0.1 to 0.5 mL total volume). They all enhance spectral, color, and power Doppler imaging throughout the body at the

same or slightly larger dose for many minutes. For the agents capable of enhancing the B-mode signal, a higher dose (3-10ml) is required for gray-scale enhancement of tissues.

As microbubbles are too large to escape the capillaries, they remain intravascular to enhance the blood pool. Agents not visible on gray-scale enhance Doppler signals, thus increasing the visualization of smaller and deeper vessels that could not be imaged before. These agents could then be used to either measure velocity or detect the presence of vessels in regions of interest. Some investigators have emphasized quantitation and have shown distinct time/intensity curve characteristics in benign and malignant lesions as well as normal and diseased tissues.

Most of the PFC-based agents are visible on gray-scale, providing new capabilities and potentials that are not possible with the Doppler agents such as high-resolution imaging of the intravascular space and tissue enhancement. It should be noted that gray-scale filling of vessels is more powerful than filling the vessel with Doppler signal. This is because Doppler information is being used to fill vessels to provide anatomical detail. Although this method is reasonable in a significant number of situations, flow and structure no longer overlap in conditions when flow is disturbed by plaque, aneurysm, clot, or vascular tortuosity. Further, regions of slow flow or small volume flow such as channels in reanalyzed clots or very tight stenoses, may be invisible with Doppler but will be depicted with B-mode imaging [2]. The interior and the exterior arterial wall are then clearly depicted, allowing the accurate assessment of plaque and atherosclerotic disease. In fact, this technique may become the standard method to assess carotid disease, rather than estimating stenosis because of abnormal velocities. In a study comparing gray-scale, color and power Doppler for the assessment of atherosclerosis in vitro and in animals, gray-scale proved to be superior to the other techniques because of its high spatial and time resolution [3]. The filling of the lumen with echoes allowed the visualization of plaque that was not seen precontrast [4]. When combined with Wideband Harmonic Imaging, it was possible to visualize the lumen through the plaque, to allow the direct measurement of the lumen, and to demonstrate turbulence [5].

The B-mode technique should also be more powerful for solid organ imaging than Doppler imaging. Because the latter is dependent upon loss of correlation that is caused by motion or bubble destruction, it is susceptible to instrumentation and tissue variable that are difficult to predict. Further, blooming and flash artifact and either poor spatial or temporal resolution limit the technique's ability to adequately assess tissues. B-mode on the other hand, detects the presence of all microbubbles, even if they are stationary, with high spatial and temporal resolution. Since capillary volume makes up a significant fraction of tissue blood volume, and since capillaries are not detectable with Doppler, the B-mode technique is more informative than the Doppler techniques. It is this author's belief that the assessment of solid organs for the detection of perfusion defects and tumors will likely become an important role for contrast media.

Complete diffuse enhancement of normally perfused tissues occurs with B-mode imaging including myocardium and placenta [6, 7]. Partial renal infarction was highlighted as non-enhanced wedge of tissue. The defect on gray-scale was similar in size and appearance to the gross specimen [8]. The hypervascular rim of the Vx2 tumor enhanced significantly on gray-scale and increased tumor conspicuity [9, 10]. Several tumors not seen pre-contrast became apparent post contrast [10]. The kidney as well as liver enhanced on gray-scale relative to the less vascular or necrotic tumor center that appeared hypoechoic [11]. With this capability, the recognition of vascular from non-vascular tissue (cystic versus solid) will be easily accomplished.

Because of the high frame-rate, sonography demonstrates the passages of microbubbles through the circulation. When imaging liver tumors a triple phase enhancement pattern was observed as bubbles traversed the arterial and then portal venous system and finally accumulated in the parenchyma. The initial phase was a shimmering enhancement of the tumor's vascular portions relative to the surrounding hepatic parenchyma and the tumor center, which corresponded with the arrival of the contrast agent through the hepatic arterial system. When the contrast agent reached the portal vein 10–20 seconds later, the liver parenchyma enhanced. During this phase, although the tumor-rim enhancement and shimmering were still observed, the progressive enhancement of the surrounding hepatic parenchyma caused the echogenicity differential between the liver and the vascularized portion of the tumor to fade. During the final enhancement phase, the liver parenchyma continued to enhance, surpassing all regions of the tumor and vessels converting the lesion into a hypoechoic focus. The parenchymal enhancement phase continued for several minutes. The ability to visualize the pattern of enhancement of lesions should aid significantly in lesion characterization.

It is not clear whether the increased liver echogenicity during the late phase when vessels were devoid of echoes is due to the entrapment of bubbles in the sinusoids or to phagocytosis of the microbubbles by the reticuloendothelial cells. When bubbles are trapped in the liver in the late phase, liver enhancement lasts for 1 or 2 or several frames as the transducer is swept across the bubble-filled liver dependent upon the sensitivity and/or the concentration of the agent in the liver. When enhancement is lost quickly, it is important to freeze the images and review them in memory one frame at a time. When enhancement lasts for several frames, enhancement is lost from the near to the far field allowing the serial assessment of the liver. Because space occupying lesions lack sinusoids and reticuloendothelial cells, they appear hypoechoic relative to the enhanced background of the liver. The disadvantage of this technique is the creation of destruction waves whose front is dependent upon beam profile, side-lobes, and variable tissue attenuation creating pseudo-lesions. Further, vessels have the potential to mimic small lesions.

Bubble destruction: Microbubble systems are susceptible to ultrasound pressure [12, 13]. Bubble destruction is dependent upon the characteristics of the agent and beam intensity. Tissue enhancement decreases more rapidly as the sensitivity of the agent to ultrasound increases, as transmit power or frame rate increases, as bubble transit time through the tissue decreases, and as the influx of new unexposed bubbles into the field decreases. The loss of microbbubles to ultrasound has both advantages and disadvantages.

The major disadvantage to bubble destruction is the shorter enhancement period. Should tissue enhancement be the goal, then imaging with low power settings and intermittent imaging at slow frame rates becomes important. We found that at 1 frame/second, there was optimal liver enhancement while allowing the sonographer the ability to maintain anatomic orientation [13].

The major advantage is the faster elimination of bubbles from the near field decreasing attenuation and allowing improved visualization of the far field and the ability to manipulate image contrast. It is possible to decrease tissue enhancement (slow transit time) to promote vascular visualization (fast transit time) or create image contrast between tissues with slow transit time (hypoperfused) vs. fast transit time (normal or hyperperfused) allowing the assessment of relative perfusion differences. In a pilot study, we showed that once bubbles were destroyed, the tissue with faster transit time (normally perfused) enhanced faster than tissue with slow transit time where the artery was stenosed (hypoperfused). We also showed that with manipulation of the inter-frame delay, it was possible to construct an exponential growth curve whose time constant is dependent upon blood flow and whose plateau is dependent upon blood volume. When stenoses were created in the renal artery both blood flow and blood volume decreased [14].

Contrast specific instrumentation: Equally important to the development of contrast media is the development of specific instrumentation aimed to take advantage of the unique physical properties of microbubbles. The most important advance to date has been the implementation of harmonic imaging.

The added benefit of harmonic imaging is the increased contrast between vessels and tissues and tissue with and without bubbles. We showed that tissue contrast between areas containing bubbles (normally perfused tissue) and areas lacking bubbles (infarcted tissues) increases with harmonic imaging by 40% and lasts twice as long as with standard imaging [8]. In B-mode imaging the Wideband Harmonic Imaging technique is the imaging tool of choice because of its effective suppression of tissue signals and its high spatial resolution. We showed that echogenicity with this technique is not only dependent upon bubble concentration allowing the visualization of less perfused tissues, but because it is a multi-pulse technique, contrast is also dependent upon bubble velocity and bubble destruction. With this high-resolution harmonic imaging, the arteries appear brighter than veins and arteries and veins brighter than tissues. Tumor arteries are easily imaged and distinguished from veins. In fact, we

showed that this technique was superior to color and power Doppler imaging which either suffered from poor spatial or temporal resolution or were unable to distinguish arteries from veins [15].

Conclusions: The future of ultrasound is exciting with many new and highly effective agents on the horizon. When combined with contrast specific instrumentation, the clinical practice will undoubtedly be affected. We expect higher sensitivity and specificity when assessing vessels, and solid organs.

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LATE PHASE LIVER AND SPLEEN IMAGING WITH MICROBUBBLES

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Some of the newer microbubble contrast agents, such as SHU 563A (Sonovist) , are known to have a liver-specific phase seen after the agent has cleared from larger vessels [1,2]. Recently, however, it has been recognised that Levovist is also a targeted agent with a strong late liver phase when it can be imaged in the liver and spleen [3,4]. At this time, the agent is stationary or moving only very slowly and so does not produce conventional Doppler signals; non-linear effects such as colour Doppler stimulated acoustic emission (SAE) or pulse inversion imaging must be used to detect it [5]. In an evaluation of 5 healthy volunteers given single injections of Levovist 2.5g and imaged using an intermittent SAE technique, liver uptake lasted for over 30 minutes. It is widely distributed throughout the parenchyma of the liver and spleen and is weak or absent in most small focal lesions, notably in metastases.

Colour Doppler picks out the microbubbles as a characteristic, transient coloured mosaic similar to electronic noise. Because the SAE effect is transient, particularly with Levovist, intermittent or triggered scanning works best. Use of a high pulse repetition frequency suppresses the conventional Doppler signals from flowing blood and simplifies the images. SAE is very dependent on the amount of acoustic power deposited and often cannot be elicited at depths beyond about 12cm or deep to attenuating structures, even when a high transmit power is used. For the same reason, good skin contact is essential and use of a low Doppler frequency improves penetration. The transmit focus should be set near to the depth of interest as the effect is maximal at this level. Thus SAE can be elicited with any conventional colour Doppler scanner.

Using scanners modified to provide second harmonic grey-scale imaging reveals the stationary microbubbles as transient grey-scale signals in the liver and spleen. The transience of the effect means that triggered modes of scanning, such as the Flash Echo method (Toshiba Medical Systems) can be useful (Fig. 2). Pulse-Inversion imaging has the important advantage of combining high sensitivity with excellent spatial resolution (identical to that of the system in conventional grey sale). It allows very small lesions of a few millimetres' diameter to be detected as grey scale defects; such small lesions are not always well visualised even with state of the art CT and MR.

All these methods are subject to limitations, however, including the transience of the effect (although this is less severe with newer agents), fall-off in the far field and artefacts, such as shadowing.

We have performed a series of studies of focal lesions in the late phase after Levovist administration in which we obtained pairs of registered grey-scale and colour SAE images so that subjective and objective comparisons of conspicuity could be made. Liver metastases are seen as defects on SAE imaging and in a series of 16 patients SAE improved their conspicuity in all cases using both objective and subjective assessments. In several cases, lesions that were invisible or difficult to recognise were revealed as defects on SAE. Early data suggests the same is true of pulse inversion scanning [6]. Thus these methods may improve the sensitivity of liver ultrasound.

In recent studies we have examined the specificity of the late liver phase of Levovist. Based on a subjective impression that benign lesions show more late phase uptake of Levovist than malignancies, we compared videotaped sequences of SAE within and around different types of liver lesion at 5 minutes after an injection of Levovist 2.5g. Two blinded observers compared 15 subjects with metastases and 8 with benign lesions (haemangiomas, FNH, focal fat and regenerating nodules). Both observers scored more SAE within all benign lesions than metastases. Presumably this is because of the presence of functioning liver tissue in conditions such as FNH, focal fat and regenerating nodules. In FNH, the signals are often stronger than in normal liver, a finding reminiscent of the way they may concentrate radio-labelled sulphur colloid. The reason for the stronger signals from hemangiomas than metastases is not clear; the very slow flow rate in hemangiomas leading to delayed clearance of blood pool is a possibility.

Other uses of the late phase effect include splenic imaging and as a novel method of measuring liver attenuation [7].

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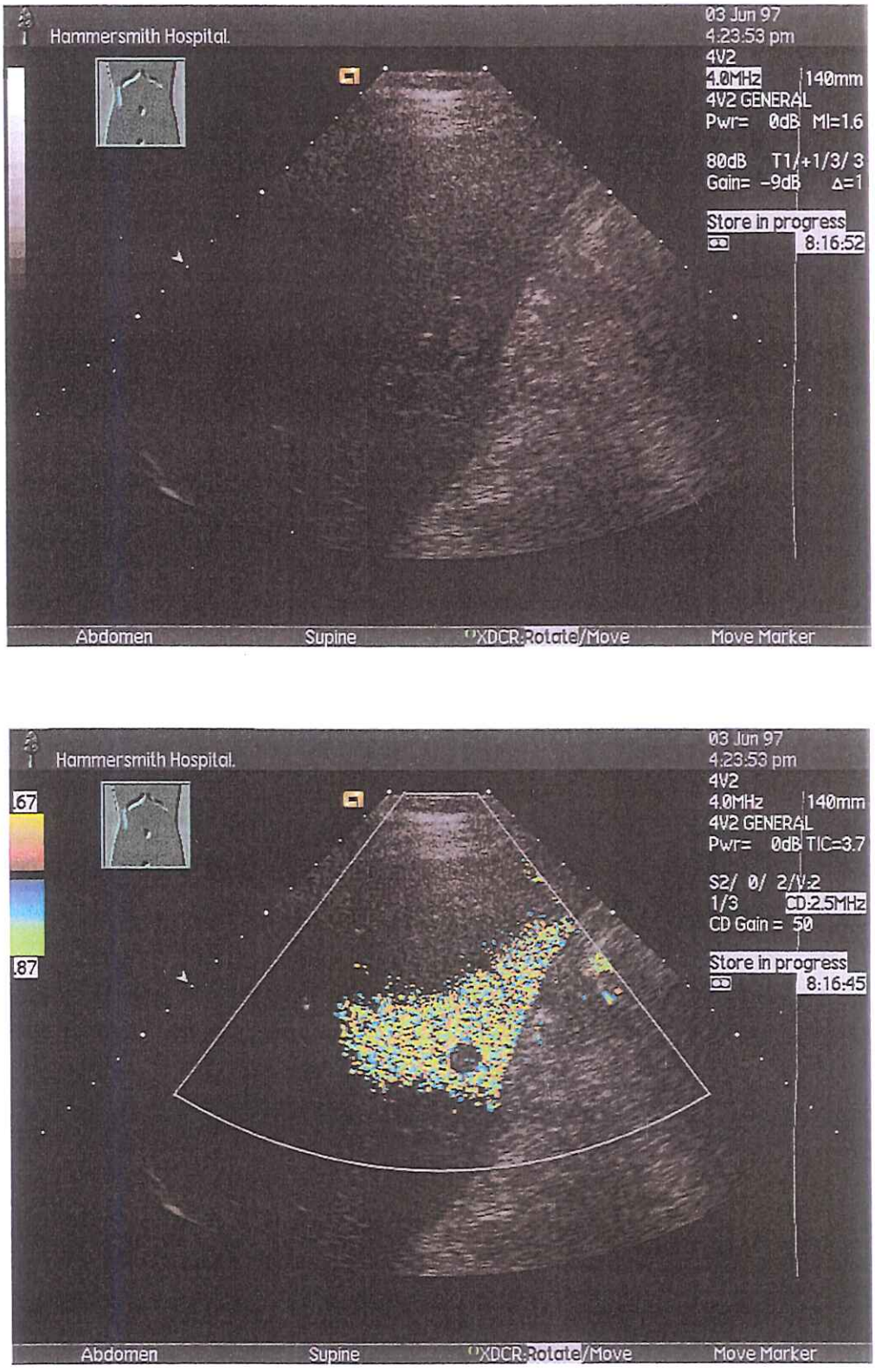

Figure 1: SAE in the late phase of Levovist. A small echogenic lesion can just be discerned in the control image (upper panel); it is much more clearly seen when colour Doppler is switched on to elicit stimulated acoustic emission (lower panel).

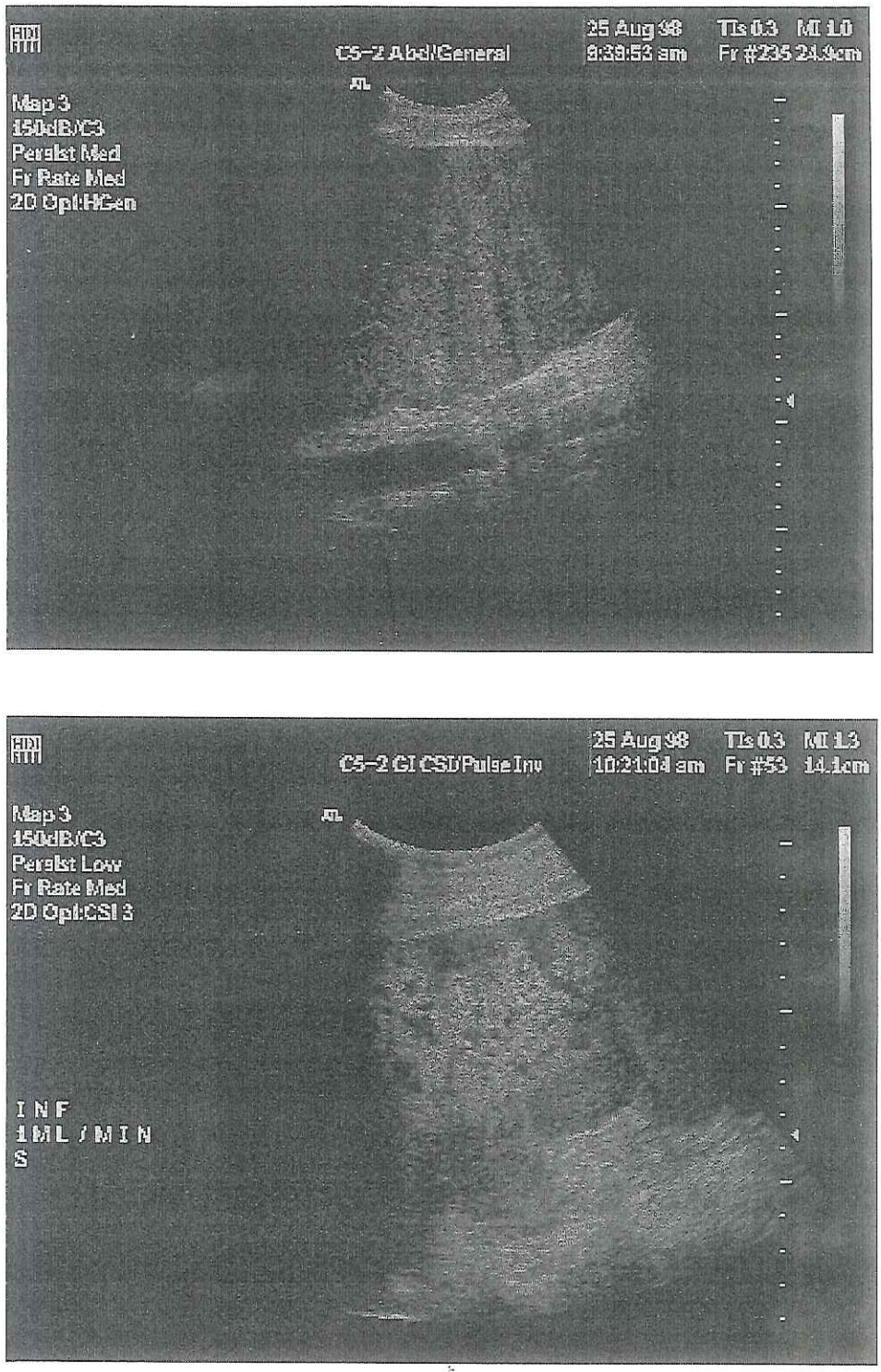

Figure 2: The conventional scan (a) was normal in this patient suspected of having neuro-endocrine metastases to the liver. Using pulse inversion in the post vascular phase after Levovist injection (b), multiple small defects are picked out. Though not proven, these are assumed to be multiple metastases.

VISUALIZATION OF INTRAVASCULAR THROMBOSIS: PRACTICAL IMPLICATIONS

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Background: Vascular thrombosis is the major cause of death in Western industrialized countries. In the United States each year, 3.4 million patients die of myocardial infarction and stroke, and usually vascular thrombosis is the primary causative factor. In myocardial infarction this is usually due to thrombus formation on plaque in the coronary arteries. In stroke, the causative event is usually embolus of thrombi from a source in the extracranial circulation or the heart resulting in occlusion of intracranial vessels. Pulmonary emboli kill 200,000 patients each year in the U.S. The most common source of pulmonary emboli is from deep venous thrombosis (DVT); each year 5 million patients in the U.S. suffer from DVT.

Prompt diagnosis and treatment of vascular thrombosis is necessary to improve patient survival and to decrease morbidity. The tests used for diagnosing vascular thrombosis and treatment vary depending upon the anatomic site of the disease. In myocardial infarction, acute MI may be diagnosed by EKG and enzymes and confirmed with angiography and treated in many centers with intra-coronary arterial administration of thrombolytic therapy. In stroke the diagnosis is usually made clinically and CT scan is performed to exclude intracranial hemorrhage. Patients with non-hemorrhagic stroke may then be treated with thrombolytic therapy. Intracranial arterial catheter administration of thrombolytic therapy (e.g. t-PA) may improve restoration of cerebral blood flow. In MI and stroke, prompt diagnosis and treatment is paramount. Several hours may be available as a time window for treatment of MI and restoration of blood flow. As MI is often associated with chest pain this may lead patients to seek treatment and effective thrombolytic therapy may be administered within a time window sufficient to save much of the myocardium at risk from the ischemic event. In stroke, there is even less time to save regions of the brain at risk from ischemia. Stroke is also often a silent event, e.g. the patient wakes up the next morning with a neurological defect such as hemiplegia.

One common source of emboli causing stroke is from the left atrial appendage in patients with atrial fibrillation (A fib.). There are 2 million patients in the U.S. with A fib. It is important, particularly, to exclude thrombus in the left atrial appendage in A fib patients prior to cardioversion. Detection of left atrial thrombosis is problematic. Presently the best test is probably trans-esophageal echo (TEE). TEE is expensive compared to trans-thoracic echo (TTE). TEE is also an imprecise test and picks up only about 15% of cases of left atrial appendage thrombosis.

Thrombus in the left ventricle in patients with trans-mural MI is another important source of emboli in stroke. Trans-thoracic echo is effective in few of these cases. Evidence suggests that the smallest thrombi, i.e. the most difficult to detect, may also be the ones most susceptible to embolize and cause stroke.

Pulmonary embolus (PE) is diagnosed by several different tests. A patient presenting with signs of DVT and PE is usually studied first with ultrasound to evaluate for thrombosis in the lower extremities. Since there is a high prevalence of DVT in association with PE, patients with clinical suspicion of PE, but without definite DVT are still often evaluated first with ultrasound. When DVT is confirmed, patients are usually treated with heparin and then anticoagulated with warfarin. The sensitivity and specificity of ultrasound for detecting DVT in the thigh are reported to be greater than 90%. While ultrasound does a good job of detecting DVT in the thigh, it does a poor job of detecting DVT in the calf veins, the pelvis and inferior vena cava. X-ray venography, using iodinated contrast media, is referred to as the *Gold Standard* for diagnosing DVT in the extremities, pelvis and IVC. X-ray venography is invasive and is associated with a small risk (2-3% incidence) of phlebitis as a complication of the procedure. Also X-ray venography requires contrast media and therefore carries a small risk of contrast media induced nephropathy. Each year more than 1.5 million ultrasound procedures are performed in the U.S. for DVT while less than 220,000 X-ray venograms are performed and the number of venograms is decreasing as ultrasound is increasingly utilized.

In patients with negative ultrasound studies and clinical suspicion of PE there are several possible tests. These tests include radionuclide lung ventilation/perfusion (V/Q) scans, spiral CT, MRI and pulmonary angiography. All of these tests are more expensive than ultrasound. Nor is the equipment for these tests always available in smaller clinics and rural areas, while it is increasingly common for all urgent care settings to have an ultrasound unit. Pulmonary angiography, although referred to as the *Gold Standard* for diagnosis is the most invasive test and there is some mortality associated with the procedure. When pulmonary embolus is diagnosed it is usually treated similarly to DVT. It is important to note that heparin therapy does not generally dissolve clots but prevent propagation of additional pulmonary emboli. Complications of DVT include phlebitis and leg ulcers. For patients who survive PE there is often an incidence of pulmonary hypertension which may lead to cor pulmonale (right heart failure).

To decrease complications from DVT, thrombolytic therapy has been instituted with urokinase (UK) via catheter administration in the involved veins. While this appears to lower the incidence of phlebitis from DVT, it is expensive to administer enough UK and the procedure is invasive. It often requires mechanical fragmentation of the clot in addition to thrombolytic therapy administered via catheter to achieve sufficient thrombolysis.

To the extent that new or improved diagnostic tests can more rapidly detect thrombi in MI or stroke this may improve survival and quality of life for survivors. In the case of stroke it is probably even more important to have a test which will detect thrombi (e.g. in the left atrium) before they embolize into the intracranial circulation. If a diagnostic test (viz ultrasound) costs less than other tests and this cheaper test can be improved, the use of the improved test in lieu of other more expensive tests may decrease health care costs for diagnosing vascular thrombosis. If this diagnostic test can be coupled with a minimally invasive and effective thrombolytic procedure this then has potential to improve quality of medical care for vascular thrombosis. One example would be to use ultrasound contrast agents to improve visualization of blood flow or clot with ultrasound and then to titrate thrombolytic therapy rationally on the basis of findings on contrast enhanced ultrasound with serial imaging performed during thrombolytic therapy. Another way of improving thrombolytic therapy with ultrasound and contrast agents is to use the physical interactions of microbubbles with ultrasound to effect clot lysis.

Basis for ultrasound contrast agents in the diagnosis of vascular thrombosis: Current microbubble based ultrasound contrast agents such as Difinity (DMP 115, DuPont Pharmaceutical Company, Billerica, Mass.) and Optison (Mallinckrodt Medical, St. Louis, MO.), as well as others, function as blood pool agents. These agents increase the backscatter within the blood and may serve to define vascular thrombosis as *cold* or low signal regions within the *hot* or high signal intensity vascular structures. More work needs to be done to define the potential of these agents in detecting vascular thrombi, but pre-clinical and early clinical experience does indicate that these blood pool agents may improve the detection of vascular thrombosis in at least some anatomic regions. Blood pool agents have clearly shown utility in detecting portal vein thrombosis where, the detection of flow and its direction (e.g. hepatopedal versus hepatofugal) and the presence of collateral vessels are perhaps primary factors in the diagnosis rather than visualization of actual thrombosis per se. Using current blood pool contrast agents, thrombi will appear as *black spots* or *cold* regions within a bright vessel.

An alternative to non-specific blood pool agents is to develop targeted tissue-specific contrast agents for detecting vascular thrombosis. Our group has developed such an agent, MRX-408, which is in advanced stages of pre-clinical development. MRX-408 consists of phospholipid coated perfluorobutane microbubbles bearing oligopeptides targeting the activated GPIIb/IIIa receptor of platelets. Preclinical imaging in various animal models allows us to conclude the following about this agent. Within seconds or at most a minute or two following IV injection, the agent enhances clots in the veins, arteries, left atrium and left ventricle. Clots appear as *hot* spots or high signal regions within low signal intensity *black* vascular structures. Clots as small as 3 mm in size have been shown with MRX-408. Sub-chronic clots, 6 weeks old, were identified more clearly in arteriovenous grafts with MRX-408 by radiologists who interpreted ultrasound images for presence of vascular thrombosis. In several of the models, a blood pool contrast agent, MRX-113 a perfluorobutane microbubble without

targeting ligands, was injected IV and the findings compared with MRX-408. In all cases the *hot spot* contrast agent, MRX-408, was superior in showing the vascular thrombi.

It is well known that ultrasound destroys microbubbles. In general a peak negative pressure of 1.0 MegaPascals at diagnostic frequencies of ultrasound is more than sufficient to destroy microbubbles. Interestingly, however, when MRX-408 is bound to clot, the bubbles become resistant to ultrasound mediated rupture and are stable to 1.0 MegaPascals. Stabilization of bound bubbles to ultrasound has been described by other groups with other models. Destruction by ultrasound of free microbubbles circulating in the blood, leaving bound microbubbles intact, may be an important mechanism in improving the *hot spot contrast* of a targeted ultrasound contrast agent such as MRX-408.

Endothelial cell dysfunction is an important counterpart to vascular thrombosis. Various integrins have been identified on endothelial cells in association with atherosclerosis, restenosis following angioplasty and other vascular procedures as well as thrombus platelet aggregation on the surface of endothelial cells. In vitro models we have shown that MRX-408 binds to damaged endothelial cells but not to normal endothelial cells and not to normal nor damaged epithelial cells or hepatocytes. In experimental animal imaging following damage of a vessel wall, e.g. by catheter, guidewire or balloon, we have seen enhancement of the vessel wall by MRX-408. It is not yet clear in vivo whether this represents microbubble adherence to damaged endothelial cells or to small aggregates of platelets adherent to endothelial cells. More work needs to be done to assess the potential of MRX-408 and other agents in diagnosing endothelial cell dysfunction, but it is possible that targeted ultrasound contrast agents might be used to detect endothelial cell dysfunction before significant thrombus formation. This might open a new potential in medicine for the diagnosis and prophylactic treatment of endothelial cell dysfunction before thrombosis occurs.

Basis for ultrasound contrast agents in the treatment of vascular thrombosis: Several researchers have studied the potential of microbubbles for treating vascular thrombosis with ultrasound. Microbubbles lower the cavitation threshold for ultrasound. Cavitation is an important mechanism underlying the enhancement of clot dissolution with ultrasound mediated sonothrombolysis. Ultrasound application to clot increases the spacing between fibrin polymers in clot and likely increases the permeation of thrombolytic agents such as urokinase into clot to increase the rate of thrombolysis. Experimentally it has been shown that microbubbles increase the rate of thrombolysis from ultrasound. Ultrasound with microbubbles works synergistically with thrombolytic agents to decrease the requisite dose of thrombolytic agent as well as to decrease the amount of time necessary for clot lysis. Catheters can be used to deliver concentrated ultrasound to thrombosis along with microbubbles or the ultrasound may be delivered transcutaneously. In a rabbit model of bilateral, femoral arterial, occlusive clots working with Dr. Siegel at Cedars-Sinai Hospital we have shown that transcutaneous low frequency ultrasound can completely dissolve clots without the use of thrombolytic agents. It appears that effective clot lysis

can be achieved with transcutaneous ultrasound and IV administration of bubbles as a minimally invasive procedure without damaging overlying tissues and without catheterizing the vessel.

The ideal frequency of ultrasound and pulsing regime for ultrasound for sonothrombolysis have not yet been identified. It does appear that lower frequencies of sound, e.g. 100 kilohertz, are more effective that higher frequencies of sound, e.g. 3 megahertz. Of note the propensity to cavitation is roughly proportional to the mechanical index and the mechanical index is roughly proportional to 1/Ö frequency. In other words, lower frequencies of ultrasound than we commonly use for diagnostic imaging may have advantages for sonothrombolysis. Lower frequencies of sound are better also for penetrating the ribs and the skull. For example at 40 kilohertz, Å50% percent of ultrasound energy penetrates the ribs and at 300 kHz >33% penetrates the skull. The use of microbubbles with external ultrasound may afford a rapid, minimally invasive new treatment technique for effectively treating vascular thrombosis. This technique could have applications in MI, stroke, venous and peripheral arterial thrombosis. There also may be a role for ultrasound delivery via catheter where concentrated high levels of ultrasound energy can be delivered directly to the clot.

We have incorporated thrombolytic agents into microbubbles (e.g. MRX-521 which contains urokinase). Targeting ligands may also be incorporated into the drug carrying vesicles (viz MRX-522). The potential therefore exists to create a *smart* thrombolytic agent which is also a contrast agent. Thrombolytic agents may then be targeted, released and even activated at the site of thrombosis. This affords the potential to increase the rate of clot lysis as well as to decrease the requisite dose of thrombolytic agent. These agents are currently under pre-clinical development.

Practical implications of microbubbles for diagnosis and therapy of vascular thrombosis: Blood pool agents have applications in improving ultrasound for diagnosis of vascular thrombosis. Even more promising however are targeted tissue-specific ultrasound contrast agents such as MRX-408. *Hot spot* ultrasound contrast agents may improve the accuracy of ultrasound for detecting venous thrombosis, particularly in the calf, pelvic veins and IVC. Pre-clinical imaging shows that MRX-408 also facilitates visualization of central pulmonary emboli as well. Targeted ultrasound contrast agents may ultimately allow us to detect thrombi in the left atrium, left ventricle, intracranial circulation as well as peripheral arteries. These applications have the potential to extend the reach of ultrasound into areas where the technique is not currently used, supplanting other more expensive and invasive techniques as well as to improve the overall accuracy of ultrasound within its existing domain.

Additionally the potential to detect endothelial cell dysfunction with ultrasound and contrast agents opens a whole new possible frontier in medicine. On the basis of imaging findings it may be possible to intervene and institute preventive therapy prior to thrombosis and then to follow-up with ultrasound to assess response to therapy.

Even more exciting than the potential of diagnostic ultrasound with targeted ultrasound contrast agents is the therapeutic potential to treat vascular thrombosis. Convergence of ultrasound with microbubbles and drug delivery affords the opportunity to treat thrombosis more quickly, cheaply and less invasively than hitherto possible. Extensive pre-clinical and clinical testing is necessary to bring microbubble mediated sonothrombolysis to its full potential. The field is moving rapidly, however, and the physics of bubbles with ultrasound hold much therapeutic promise.

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FUNCTIONAL IMAGING WITH MICROBUBBLES

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(i) Why quantify? Perhaps the most obvious application is in pharmacological research, where quantification may offer considerable advantages over subjective assessment. For example, there is often a need to objectively compare different agents or delivery regimes (e.g. infusion versus bolus [1]).

Secondly, and more importantly, there are strong grounds for believing that quantitative echo-enhancement could, in itself, be an important diagnostic tool. In recent years, the cross-sectional imaging techniques have been increasingly appreciated as ways of imaging function as well as anatomy. By observing the changes produced by an injection of a contrast agent through vessels and tissues, much useful physiological information can be obtained and correlated with anatomy. Magnetic resonance (MR) and computed tomography (CT) have shown great promise as non-invasive techniques of assessing the microcirculation. By comparison, ultrasound has lagged behind the other modalities owing to the lack, until recently, of effective contrast agents and the difficulties of quantifying ultrasound displays. This is unfortunate as ultrasound compares very favourably in cost-effectiveness, safety and availability. Quantitative echo-enhancement may prove of great value in the myocardium or kidney, where the measurement of tissue perfusion would have an immediate and obvious value. The measurement of such indices may also be important in other applications, such as in tumour imaging.

*(ii) What aspect of ultrasound can we quantify?*In principle any ultrasound imaging mode can be used to perform some degree of quantitative assessment, but some important principles need to be considered. The first of these is the complexity of the interaction between the ultrasound beam and microbubbles. Somewhat simplistically (the interested reader is referred elsewhere for a full discussion of this complex subject [2]) a division will be drawn between "passive" and "active" enhancement mechanisms for most microbubbles. At lower sound pressures, the dominant enhancement mechanism is a (relatively) simple resonance phenomenon, which is "linear" in the sense that a proportionate relationship can be expected between relative microbubble concentration and the increase in the intensity of the backscattered signal. At higher sound pressures, however, microbubble disruption occurs, associated with the production of various non-linear or transient signals. These form the basis of many recent imaging techniques which rely on microbubble disruption or destruction such as transient response imaging [3], related intermittent harmonic grey scale [4,5] and stimulated acoustic emission imaging with Doppler. [6-10]. These techniques offer many potential advantages, including increased sensitivity and specificity to the presence of microbubbles and the ability to detect very slowly moving or stationary microbubbles. They may also offer the potential for a unique type of physiological

measurement as refilling of a tissue bed can be observed and measured after a destructive pulse of ultrasound. On the other hand, however, their destructive nature may create severe problems for accurate quantitation as the imaging process may destroy that which it is trying to detect.

The division between these "active" and "passive" mechanisms is actually somewhat blurred - for example microbubble destruction may still occur at low sound energies. The important point to grasp, however, is that there are two alternative approaches to quantitative imaging with microbubbles: on the one hand, aiming deliberately to disrupt microbubbles with sound, and on the other hand striving to minimise such effects. This leads to different considerations in the choices of scanner mode and machine settings.

For passive modes, the aim is to detect microbubbles in the tissue bed of interest whilst keeping disruption to a minimum. In general, therefore, low transmit power settings should be used. Both B-mode and Doppler techniques are useful, although B-mode imaging has the advantage of generally requiring lower sound pressures. This may be outweighed, however, by the much greater sensitivity of Doppler techniques, particularly in radiology, where motion artefacts are less of a problem. These can make the use of Doppler modes difficult in some situations such as the rapidly beating heart. Of the two two-dimensional color imaging modes, conventional (velocity) color Doppler and power (or energy) color Doppler - hereafter referred to as color Doppler and power Doppler respectively - the latter is the more logical choice here. This is because the relationship between the color display and microbubble concentration is much closer in power Doppler. From a practical point of view, the power Doppler map approximates to a log-compressed map of the number of moving reflectors. Thus, in principle regional microbubble concentration can be estimated after appropriate corrections as is discussed later in this chapter. In a conventional color Doppler mode, by contrast, the relationship between microbubble concentration and color display is much more complex and less intuitive: here, microbubbles produce enhancement by augmenting low amplitude Doppler signals, which were previously below the noise floor of the system, causing them to be displayed. If only a small sample volume (such as a single vessel) needs to be studied, spectral Doppler may offer advantages as very high temporal sampling rates are possible, whilst color modes are more appropriate for larger regions of interest.

A dedicated software package has been developed, for the analysis of colour Doppler and power Doppler images. This segments out colour pixels from the grey-scale background, and quantifies the colour data. This is done using the colours within the displayed colour bar to generate a look up table, assuming that the colour bar is a linear map of the colour dynamic range, which assumption does seem to hold for many ultrasound systems. This system, originally described for conventional colour Doppler [10] has been subsequently adapted for power Doppler and includes a correction for log-compression in power Doppler images. It has been successfully employed to measure relative microbubble numbers in vitro and in vivo even when videotaped data is used [12,13].

Separate considerations apply to the use of active modes. Here, in general, high sound pressures should be used - although still, of course, within the limits permitted for diagnostic imaging. 2-D Doppler techniques may have special advantages here. The reason for this is that the autocorrelations systems used in almost all Doppler systems rely on comparisons between sequential pulses sent down an acoustic line. The sudden disappearance of a reflector is interpreted as a random frequency Doppler signal [14-16]. Autocorrelation based colour Doppler systems are known to be particularly sensitive to SAE [17]. If B-mode imaging is used, harmonic techniques such as second harmonic imaging and pulse inversion techniques should be considered as they are more microbubble specific and microbubble disruption is known to be associated with the production of harmonic signals [1].

(iii) What can we use our processed data for? If relative microbubble numbers have been calculated as described, for example to calculate a time-enhancement profile for a vessel, organ or mass a variety of measurements can be made. For example, we can measure indices such as peak enhancement or the are under the time- enhancement curve, and this may be of value in tumour imaging. A variety of temporal indices can also be measured. The width of the curve is easy to calculate using indices such as full-width, half-maximum. Another index which is relatively easy to calculate is the rise time or the time to peak enhancement. A further technique is to try to calculate the "centroid" of the curve. This index is a very useful and objective way of estimating the "average" enhancement time of an organ. It can be used to show a longer duration of enhancement in some cancers, compared to controls for example.

Useful information may also be obtained from the "shape" or regularity/irregularity of the time-concentration curve. There is some evidence that this may be especially helpful in tumour imaging and data using subjective assessments suggests that cancers enhance longer than benign lesions. Recent work, using quantitative power Doppler imaging of breast tumours with EchoGen suggests that cancers may have a particularly irregular washout phase [18].

Fractional vascular volume and perfusion can also in principle be calculated using echo-enhancement; they are the proportion of tissue occupied by blood and the blood flow per unit volume (or mass) respectively. The practical importance of these indices is that they are measures of the physiological 'vascularity' of a region. Perfusion, whether measured as flow per unit volume or mass, is a fundamental measure of blood flow at the level of the microcirculation - essentially it is blood flow normalised to the amount of tissue under consideration. For an intravascular tracer, such as a microbubble, perfusion can be found simply by dividing the fractional vascular volume by the true mean transit time [19.]

All these indices can be calculated on a region of interest basis, but can also be mapped on a pixel by pixel basis to produce "functional images." The potential value of this will be illustrated.

Another relatively simple technique, which may turn out to be very powerful has been recently described using spectral Doppler data [20, 21]. This is to analyse the time-intensity curve of a hepatic vein after a bolus injection of a microbubble. In a normal subject this will increase relatively late, typically 30s or later from injection. In cirrhosis and many malignancies, it is much earlier. A simple analysis of the rise time has proved both sensitive and specific in pilot studies for cirrhosis, and malignancy, although it is likely that more sophisticated temporal indices will be developed as the technique evolves.

Active modes: Two types of approaches to the exploitation of active scanning modes mode have been made. The first is based on using varying scanning parameters to alter the amount of microbubble destruction during an infusion and making physiological measurements from this. The second uses active methods to image static microbubbles in tissue specific phases and attempt tissue characterisation.

The first method is based on in vitro observations made with microbubbles while they are being infused in phantom models. Microbubble destruction, observed as reductions in reflectivity, can be shown with a variety of agents. It is more marked with higher sound energies and is not seen at very low energies. When flow is slowed, microbubble destruction increases because microbubbles are exposed to sound for longer periods. [22,23] Thus, in principle, flow velocity in the microcirculation could be measured by using pulsed ultrasound and varying the pulsing interval. The relationship between echogenicity and pulsing interval should be a measure of tissue flow.

This principle was extended to the measurement of myocardial blood flow in a dog model by Wei and colleagues [24]. They infused the microbubble MRX-115 while scanning in an intermittent mode and observed the relationship between pulsing interval and echogenicity in a phantom and then a dog model. They observed an exponential relationship between pulsing interval PI and video intensity VI

$$VI = VImax(1-e-\_.PI)$$

where VImax is the maximal video intensity, seen at longer pulsing intervals. The initial upslope of this curve will be proportional to microbubble velocity in the area being insonated. Wei and colleagues observed a good correlation between changing flow rates and this value in vitro and in vivo.

These exciting results suggest that regional flow can be measured in the microcirculation directly, although it is not clear how easy the technique would be to perform in clinical practice, particularly in rapidly moving tissues such as the myocardium.

A second, quite different type of quantitation attempts to make static measurements of microbubble numbers in different regions of tissue using non-linear imaging modes. In an early published description of the stimulated acoustic emission (SAE) effect, whereby microbubble disruption is associated with a sudden transient acoustic response, using the polymeric agent SH563A, Uhlendorf and Hoffman suggested that the effect could be used to count individual microbubbles [25]. As each microbubble is disrupted, a signal is produced that, in principle, could be detected and counted. SAE does not rely on reflector motion and instead detects the presence of microbubbles. The principle is thus similar to radionuclide scintigraphy: indeed the term "sonoscintigraphy" has been proposed [8]. One way of attempting to count the number of SAE effects would be to scan in color Doppler mode, in which SAE characteristically produces a mosaic like effect. In early work, an attempt has been made to use this effect to study the late liver phase of the agent Levovist using SAE. Color pixel densities were compared within and without metastases, to show that the effect increases the conspicuity of metastatic lesions (which show reduced SAE) [9]. In another study, an attempt was made to demonstrate the liver uptake of Levovist quantifiably. It was also used to see if differences in late phase Levovist uptake could distinguish between patients with and without cirrhosis, although no difference was seen [10]. It is likely that this type of quantitation will become very important with the newer agents such as SHU563 (Sonavist) and NC100100 in clinical trials, which have both strong liver-specific and SAE effects.

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ULTRASONOGRAPHY OF THE RENAL VASCULATURE: CLINICAL BENEFITS OF ULTRASOUNDCONTRAST AGENTS

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One hundred and fourteen contrast-enhanced ultrasound scanning of 66 kidneys in 64 patients were prospectively evaluated to determine the additional value and tolerance of ultrasound contrast agents (USCAs). USCAs were administered intravenously, following a non-conclusive Doppler examination according to 2independent radiologists in controlled trials (EchoGen®, Sonus Ph., USA/Sonovue® Bracco, Italy/ Levovist®, Schering, Germany). Diseases included renal or perirenal masses (21) with renal vein thrombosis in 6 cases, suspicion of renal artery stenosis (21), hypoperfusion in native kidneys or renal transplants (17), arteriovenous malformations (AVM 3) and false aneurysms (FA 2). All cases were correlated to gold standard imaging except in case of chronic renal failure.

USCA improved the detection of intratumoral vessels and helped in delineating the lesions and evaluating the venous extension of carcinomas. USCA improved the detection and visualization of main and supernumerary renal arteries and increased the signal to noise ratio on spectral mode. USCA provided accurate assessment of cortical ischemic disorders and improved detection of AVM and FA. Nineteen minor and transient adverse events were reported. No renal toxicity was detected.

HIGH SPEED OPTICAL EXPERIMENTAL ANALYSIS OF MICROBUBBLE DESTRUCTION, SUPPORTED BY THEORETICAL DEVELOPMENT

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Introduction: This work provides the first optical measurements of expansion and contraction and non-spherical oscillations of ultrasound contrast agents on the time scale of nanoseconds. The resulting optical data demonstrating radial fluctuations and wall velocity may improve the understanding of shell properties and mechanisms of destruction in encapsulated microbubbles. In order to accomplish these goals, a novel imaging system was developed with the capability to image on a time scale of nanoseconds and a spatial extent of micrometers.

Received echoes from contrast agents have been observed to disappear within microseconds. Optically we have observed three mechanisms of microbubble destruction: fragmentation, acoustically-driven diffusion, and static diffusion. The time scale of bubble destruction for diffusion is on the order of milliseconds. Fragmentation produces a very rapid decorrelation of the received echoes from a bubble, on the order of microseconds, allowing detection of microbubbles in the vasculature. With the use of a high power laser, images of microbubble activity were captured with an effective shutter speed of 20 nanoseconds. We have observed a mechanism of fragmentation being the inception of non-spherical oscillation due to high pressure ultrasonic excitation. The non-spherical oscillations result in a pinching-off of bubble fragments from the original bubble.

Methods: Novel methods are presented in this work that allow the ability to image a small spatial region of interest with high temporal resolution. The use of a high power laser provides enough light to adequately illuminate a microbubble, generating light for a very short amount of time. A CU10 Copper Vapor Laser (Oxford Lasers, Inc.), with a flash pulse of duration 20 nsec and peak power of 100 kW is used to illuminate the bubble. A precise timing system was developed to step through the expansion and contraction of an insonified microbubble corresponding to the center frequency of the ultrasound system. The position of the microbubble, which is critical to the timing of capturing bubble activity on the nanosecond scale, is isolated by a micropipette that holds the bubble with a vacuum. The bubble can also be positioned and released before insonation. The micropipette and bubble position is controlled by a micromanipulator. The bubble was imaged with an inverted microscope.

MP1950 (Mallinkrodt, Inc.), a phospholipid encapsulated contrast agent, was observed while being insonified. A 2.25 MHz spherically-focussed transducer (Panametrics, V305) was mutually aligned with the tip of the micropipette and the microscope objective. The transducer was excited by a five cycle sine wave produced by a arbitrary function generator (Tektronix AWG 2021) and amplified by an RF amplifier (ENI 325LA). A high speed camera operating at 30 frames per second (Kodak Motioncorder Analyzer model 1000) was used to record optical images of the bubbles during insonation. The timing of the laser illumination flash was scanned through the insonation cycle of the bubble by using a scanning delay generator (EG&G model 9650A).

Results: Preliminary work with the laser system yielded accurate measurements of the change in bubble radius during insonation. Figure 1 shows the radius of a lipid-shelled bubble throughout a set of cycles of pulsed ultrasound. The initial bubble diameter is approximately 6 μm, shown in Figure 1a. Figures 1b and 1c show the extreme compressional and rarefactional phases of the bubble while being insonified. In the compressional phase image of Figure 1b the bubble diameter is 3.7 μm while in the rarefactional image the bubble diameter is 7.4 μm. In Figure 1d, non-spherical oscillations were observed. The four major peaks observed were radially symmetric, appearing to be modes of higher order oscillations.

Figure 2 shows the destruction of an individual bubble of MP1950. In Figure 2a the original bubble diameter is approximately 3.5 μm. During insonification, non-spherical oscillations were observed. Figure 2b shows the bubble during insonification appearing as a three-lobed bubble. The bubble then split into three bubble fragments, shown in Figures 2c and 2d. In Figure 2c, the first bubble fragment was released from the original bubble. The original bubble appeared to be two-lobed. The second fragment is released in figure 2d, appearing in a similar position to the first fragment. The first fragment is further from the micropipette (above the "A" in "PLAY"). The two bubble fragments were released from the micropipette vacuum and quickly left the field of view due to buoyancy and radiation force acting to move the bubble up and away from the transducer.

The time required for echo decorrelation has been determined from experiments to be on the order of microseconds. Optical experiments show that fragmentation may be the mechanism. The alternative destruction mechanism is dissolution. The time for complete dissolution of a high-molecular weight core contrast agent was modeled. In the presence of surface tension and neglecting shell effects (which would decrease the diffusion rate and therefore increase the time for dissolution), a bubble of resonant size requires on the order of one hundred milliseconds. Acoustic pressure increases the rate of diffusion but still requires multiple pulses of insonation to destroy the bubble, requiring milliseconds for the destruction of a bubble with clinically utilized ultrasound pulse repetition frequencies. With the

laser imaging system, high resolution images of non-spherical oscillations and fragmentation were observed to occur on the order of nanoseconds.

Conclusion: In an attempt to recognize bubbles while being pulsed with ultrasound, novel high-speed optical analysis of individual bubbles was conducted. This work has characterized the mechanisms of microbubble destruction, as well as provide pertinent information on the dynamics of bubble shell and gas core properties. These properties may play an important role in the modeling of contrast agents.

While being insonified with low transmitted pressures, bubbles oscillate spherically. The mechanism of bubble destruction in this scenario is diffusion of the gas core into the surrounding medium. The time required for the bubbles to be destroyed is on the order of milliseconds, or multiple pulses of ultrasound at clinically utilized pulse repetition frequencies. Very fast detection of a bubble through correlation analysis may be accomplished via rapid destruction of the bubble from one pulse of ultrasound. Fragmentation is a mechanism of rapid destruction of a bubble. In order to optically capture fragmentation, a laser imaging system with effective time resolution of 20 nanoseconds was developed. The resulting images capture non-spherical oscillations of bubbles at high-transmitted pressures. As predicted in unshelled bubble analysis, non-spherical oscillations of the contrast agents studied resulted in the pinching-off of smaller bubble fragments. Further analysis of fragmentation with varying bubble parameters and ultrasound parameters may lead to optimized shells and optimized bubble detection strategies.

FIGURE 1: Expansion and Contraction of MP1950

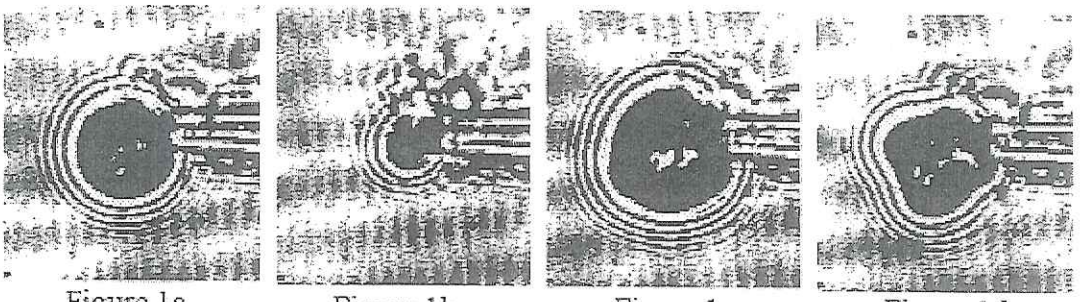

Figure 1a Figure 1b Figure 1c Figure 1d

FIGURE 2: Fragmentation of MP1950

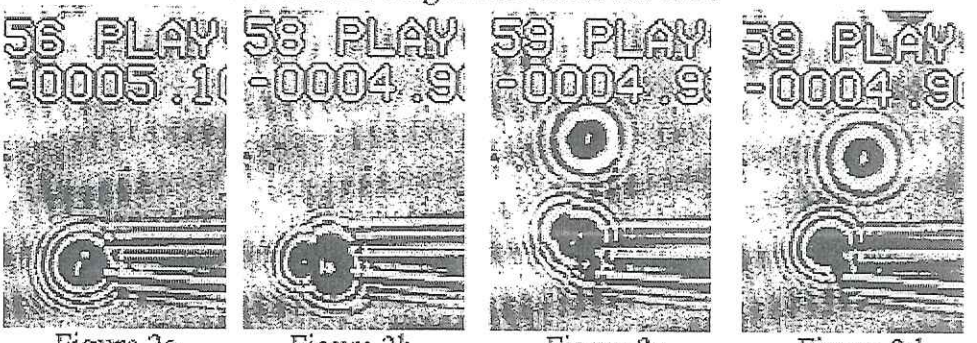

Figure 2a Figure 2b Figure 2c Figure 2d

IMPROVING THE SENSITIVITY OF POWER-DOPPLER FOR ULTRASOUND CONTRAST IMAGING BY USING A HIGH POWER RELEASE-BURST

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Introduction: Second Harmonic Power-Doppler (2HPD) is currently known as one of the most sensitive techniques for detecting ultrasound contrast agents. It is demonstrated that acoustic pressures used in diagnostic ultrasound imaging can destroy contrast bubbles [1]. Power-Doppler imaging utilises the apparent Doppler shift in echoes from collapsing contrast bubbles. This means that the transmitted pulses must destroy contrast agent in order to get sufficient power-Doppler sensitivity. However, the most optimal destructive pulses are not necessarily the best for imaging resolution and clutter removal, and a trade-off between sensitivity and resolution must be made.

Frinking et al. [2] proposed a new contrast detection strategy where different pulses are used to destroy and detect the contrast bubbles. The idea is based on measurements showing that contrast agents release free gas bubbles when the shell is broken and that the released gas bubbles can be detected for several milliseconds after the release [3]. This means that a single high-power release-burst (RB) can release the gas, and the transient increase in backscatter caused by the free gas bubbles can be detected using high-resolution, low-power pulses afterwards. The approach circumvents the trade-off sacrificing either sensitivity or resolution. As an extension to the idea, we have included the concept of a release-burst in power-Doppler.

Colour Doppler images are calculated by transmitting multiple pulses in the same direction. For each range sample, the temporal samples are processed to remove the stationary clutter signal. The power-Doppler signal is found as the power of the remaining signal after the clutter has been subtracted. One of the main issues of power-Doppler imaging with contrast agents is to find the best way of estimating the clutter component.

Methods: A model for backscattered signal from tissue with contrast is proposed. The signal model has 3 signal components, a clutter part containing the backscatter from tissue, a contrast component and a white noise component. The clutter component is a temporal low-pass Gaussian process. The contrast component is a zero-mean complex Gaussian process with variance varying with time. The variance of

the contrast component depends on the amount of gas bubbles in the sample volume, and can thereby be shaped by release- and detection pulses.

Based on the model, a method for estimating the clutter signal by polynomial approximation is suggested. To get the best possible estimation of clutter, the part of the signal with presumed lowest contrast variance is selected and used as a clutter reference signal.

Experiments: The proposed method is verified on RF-data collected in vitro with three different contrast agents, Quantison™ (Andaris Ltd, Nottingham, UK), Levovist™ (Schering AG, Berlin, Germany) and an experimental agent from Bracco (Geneve, Switzerland).

The experimental setup (Figure 1) consisted of a 200 μm diameter fibre (C) placed inside a cubic phantom (A) made from agar mixed with carborundum (SiC) particles to give a background scattering level. The fibre was continuously flushed with diluted contrast using a roller pump (E). A Vingmed System Five ultrasound scanner with a 2.5 MHz phased array transducer (B) was used to transmit the detection pulses. The pulse-triggering signal from the scanner was used to synchronise the RB transmitted by a focussed 1 MHz single element transducer (D). The RF-signals for the detection pulses were recorded digitally by the scanner and transferred to a Pentium computer. All processing of the data was done using Matlab.

The detection pulses were 1.7 MHz minimum-length pulses with mechanical index (MI) 0.4, corresponding to a peak negative pressure of about 0.5 MPa. The detection pulses were band-pass filtered for the second harmonic component. The RBs were 1 MHz, 10 cycle pulses with peak negative pressure of 2 MPa. 16 detection pulses were fired in each direction with a repetition frequency of 4 kHz. The RB was synchronised to the seventh detection pulse, giving an RF-dataset with 6 pulses before and 9 pulses after the RB.

Results: Signal profiles from the phantom were calculated from the RF-data for all three agents using the new method and existing 2HPD methods. 2HPD signals were calculated from the last 6 pulses before and the first 6 pulses after the RB (first and second column in Figure 2). In the 2HPD calculations we used zero-order regression filters [4] to remove clutter. The third column shows signals computed with the new method combining 3 pulses before and 3 pulses after the RB and with zero-order polynomial clutter approximation. The results from the different contrast agents are arranged row-wise with Quantison™ on top, Levovist™ in the middle and the Bracco agent at the bottom.

The intensities of the signals are plotted in dB vs. depth in centimetres. The fibre was located at about 9 cm depth, and can be seen as a peak in the profiles. For each plot, we extracted the peak value from

the depth corresponding to the fibre, and computed a reference background level by averaging over a 1 cm section of the phantom. The respective values are shown as horizontal lines in the plots, with peak-to-reference ratio indicated in dB as a measure of sensitivity.

The curves presented in Figure 2 are from a single experiment, but summarise the observed results:

- Quantison™ gives almost no signal with 2HPD before the RB (a), but shows a well-defined peak after the RB (b). The new method improves the sensitivity by more than 20 dB (c).
- Levovist™ gives a defined peak before the RB (d), but the peak drops dramatically after the RB (e). The new method (f) gives a small (0 to 5dB) increase in sensitivity over 2HPD without RB.
- The Bracco agent shows strong peaks in 2HPD both before (g) and after (h) the RB, but 5-15 dB additional sensitivity (i) is obtained with the new method.

Discussion and conclusion: The results show that the new method gives improved sensitivity over 2HPD for all the agents, but the sensitivity increase depends on the agent. The differences can be explained by the properties of the agents:

- Quantison™ consists of relatively large bubbles with a strong shell made from human albumin. The stiffness of the shell makes the bubbles unable to oscillate freely unless the shell is cracked. Our findings indicate that Quantison™ needs a RB to be detected.
- Levovist™ is made from galactose (milk sugar) stabilised by palmitic acid. When the sugar dissolves in blood, air trapped in the molecules is released. Because the gas bubbles do not have a rigid shell, a strong RB is not needed to detect it. However, in a situation with more attenuation, the benefit of the RB might be larger, because the detection pulses will be less destructive.
- The Bracco agent has a flexible liposome shell, which allows the particles to oscillate even if the shell has not been broken. This explains the nice peak seen without RB. It also seems that the RB introduces additional variations that can be used to enhance the power-Doppler signal.

The different response for the different agents suggests that an optimal sequences of detection pulses and release-bursts must be designed for each agent. The frequencies, amplitudes and pulse-lengths of the detection pulses and RBs should also be tuned to the agent. The sequence and pulses used in our experiments seems to be best suited for Quantison™.

Our experiments have limitations. We have an *in vitro* situation with little attenuation and no motion. The next step in verifying our method is to implement it on an ultrasound scanner to get more realistic *in vivo* data. This will enable us to compare the sensitivity and resolution of our method to the existing 2HPD methods. We also believe that the method can be further improved, for instance by including the pulse-inversion technique [5] for the detection pulses. In summary, we see the new method as a potentially very sensitive detection modality for contrast agents.

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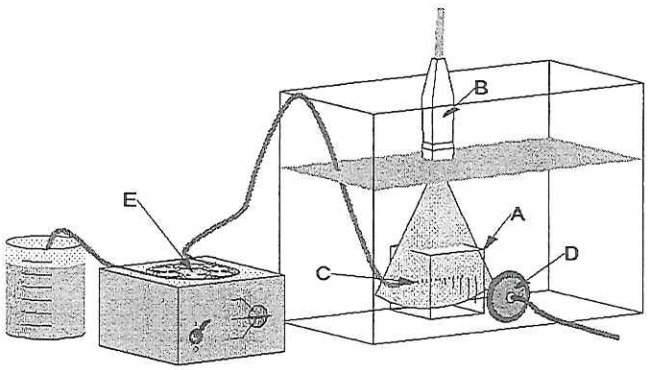

Figure 1. Experimental set-up

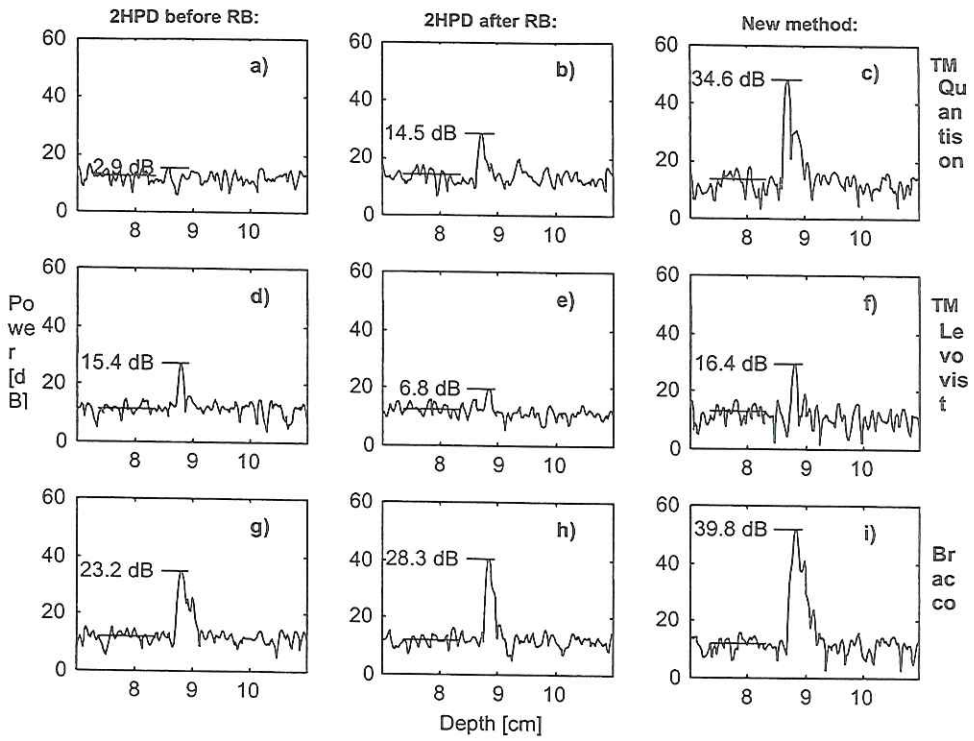

Figure 2: Signal profiles from the phantom using different contrast agents: Quantison™ (top row), Levovist™ (middle row) and Bracco agent (bottom row).

A METHOD FOR DETECTING ECHOES FROM CONTRAST AGENTS BASED ON TIME-VARIANCE

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Although microbubble contrast agents improve the sensitivity of Doppler ultrasound by increasing the echogenicity of blood, there are still limitations to the imaging of small blood vessels, and to the quantification of perfusion. In this presentation, a new approach to time-variance imaging (TVI), a contrast specific high resolution imaging technique, will be proposed.

Sequences (ensembles) of broadband transmit pulses are transmitted. The insonification can cause shrinking, growing, splitting, combination, and disintegration of microbubbles. Therefore, the acoustic properties of insonified microbubbles may change significantly, causing the amplitude and the spectra of the echo signals to change accordingly. In order to reduce motion artifacts and to separate between time-variant effects resulting from normal dissolution and time-variant effects that are due to the insonification, the interval between pulses is minimized. The time between sequences should allow some reperfusion of the insonified area and the approach of a new equilibrium.

From the echo data, two different parameters are extracted. The first one is the amplitude A, where the −20 dB bandwidth of the echo signal is considered. The second one is the difference D in mean amplitude of two frequency bands within the receive bandwidth. The imaging techniques corresponding to the parameters A and D are A-TVI (Amplitude-Based Time-Variance Imaging) and S-TVI (Spectrum-Based Time-Variance Imaging), respectively.

For either parameter, the following algorithm quantifies the time-variance and suppresses artifacts that are due to noise:
In order to quantify time-variance, the standard deviation σ of the set of measurements corresponding to a given parameter and position within the image plane is determined. The size of this set of measurements equals the size of the ensemble. The set of measurements can be considered the course in time of the given parameter. We evaluate this time signal by means of a spectral analysis. Processes that alter or destroy bubbles exhibit characteristic features in the spectrum. Assuming that there are no severe motion artifacts, a process that alters or destroys a bubble e. g. should not repeat within an ensemble. By combining the standard deviation and the result of this spectral analysis, a high

resolution, high contrast image can be formed showing the distribution of contrast agent within the image plane.

The two images based on *A* and *D* may be superimposed applying non-linear and adaptive filters in order to further improve the performance of TVI.

In vitro images will be presented showing the microbubble distribution within a vessel phantom and a perfusion phantom, respectively. Using a 7.5 MHz linear probe, a lateral resolution of 0.5 mm and an axial resolution of 1-0.25 mm was achieved. The sensitivity to motion artifacts is low, and no minimum flow velocity is required for detecting the microbubbles. Furthermore, sequences of TVI images allow the assessment of perfusion rates, since the intensities in the TVI images approach an equilibrium determined by the local destruction rate and reperfusion rate. The combination of B-mode and TVI images employing a 2-dimensional colormap provides the full information of both imaging modalities, which will be useful for clinical applications.

Experimental Results

(Example)

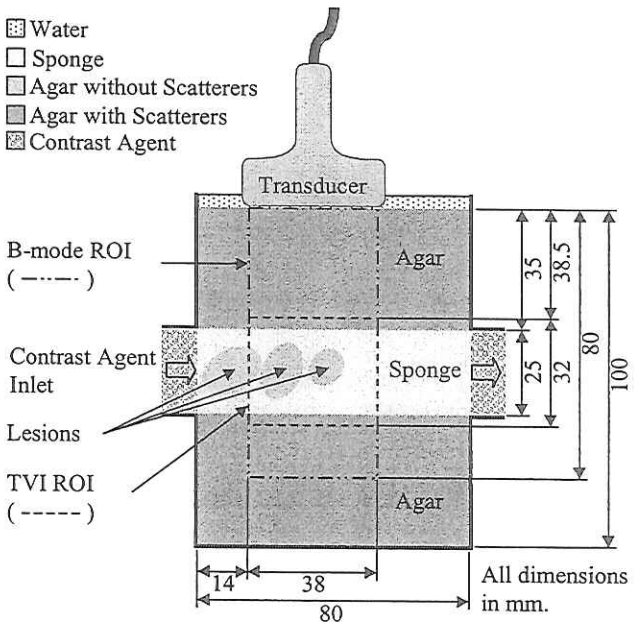

Figure 1: Experimental Setup.

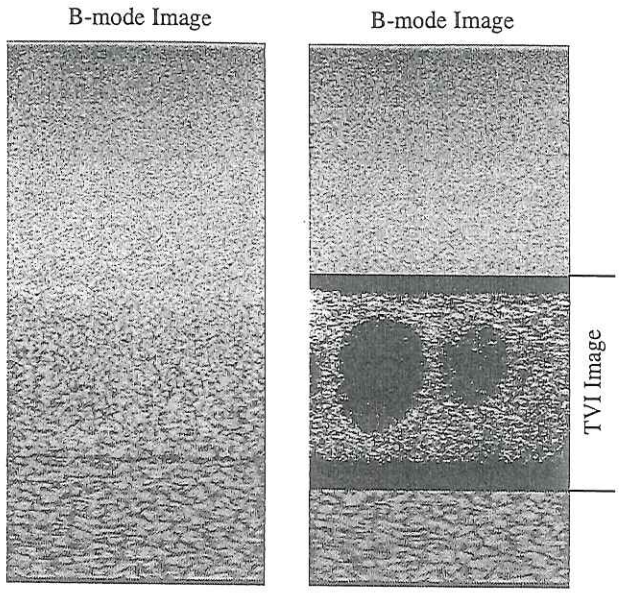

Figure 2: Comparison of B-mode image and TVI image.

Experiments were conducted using a perfusion phantom as illustrated in Figure 1. The concentration of agar was 30 g/l. The reflectivity of the agar results from silica gel at a concentration of 10 g/l, which provides solid particles having a mean diameter of 15 µm. The cylindrical sponge, which has a reflectivity similar to the surrounding agar, contains unperfusable lesions. The lesions were made by injecting agar without additional scatterers into the sponge. The ultrasound machine was a Siemens Sonoline® Elegra with a 7.5 MHz linear array. Contrast agent was pumped through the sponge at a mean velocity of 2 cm/s. The concentration of Levovist® was ~0.05 g/l. The contrast agent did not change the B-mode intensity by more than 1 dB. Figure 2 shows a result. In the B-mode image, which has a dynamic range of 55 dB, the lesions in the cylindrical sponge are barely visible and the brightness of the sponge is similar to the brightness of the surrounding agar. The TVI image, which combines an A-TVI image and an S-TVI image by means of a non-linear adaptive filter that works on neighborhoods of 3-by-3 pixels, clearly shows the lesions, and the sponge-agar interface is well defined.

ECHOSCINTIGRAPHY – A NEW IMAGING MODALITY FOR IMPROVED ASSESSMENT OF VESSEL DIAMETERS USING HARMONIC-POWER-DOPPLER-IMAGING

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High concentrations of contrast media usually are needed to visualize intramyocardial vessels using intravenous administration of contrast. However, shadowing and blooming artefacts limit further quantitative assessment. The aim of this study was to evaluate a new imaging method (echoscintigraphy), which is performed with low concentrations of contrast. To enhance the weak contrast signals of single frames, summation of consecutive frames was done.

Methods: In a pulsatile flow phantom tubes of 6mm diameter were insonated during infusion of four different concentrations (2.250, 22.500, 250.000, 875.000 microspheres/ml) of SH-U 563A (Schering AG, Berlin, Germany). The recordings were performed by means of an ATL ultrasound system (ATL-HDI 5000, Bothell, WA, USA) in the intermittent Harmonic-Power-Doppler-mode (HPD). The receive gain was gradually increased from 50% to 80%. The signals of 60 consecutive frames were added by means of a newly developed imaging software. The cross-section area and signal-intensity of the summation frames (Fig. 1C) were assessed and compared with single-frame recordings obtained at high concentrations.

Results: At lowest concentration summation of the frames provided complete display of the vessel diameter above a gain of 71% (Fig. 1C). Further increase of receive gain resulted in an overestimation of the real vessel diameter of up to 9%. Using single frames at high concentration (Fig. 1A) the overestimation at corresponding gain levels was up to 21% ($p<0.01$).

Conclusion: Summation of contrast Power Doppler signals is feasible. Using very low concentrations of ultrasound contrast agents echoscintigraphy allows the visualisation and quantification of HPD-signals reducing blooming or shadowing artefacts.

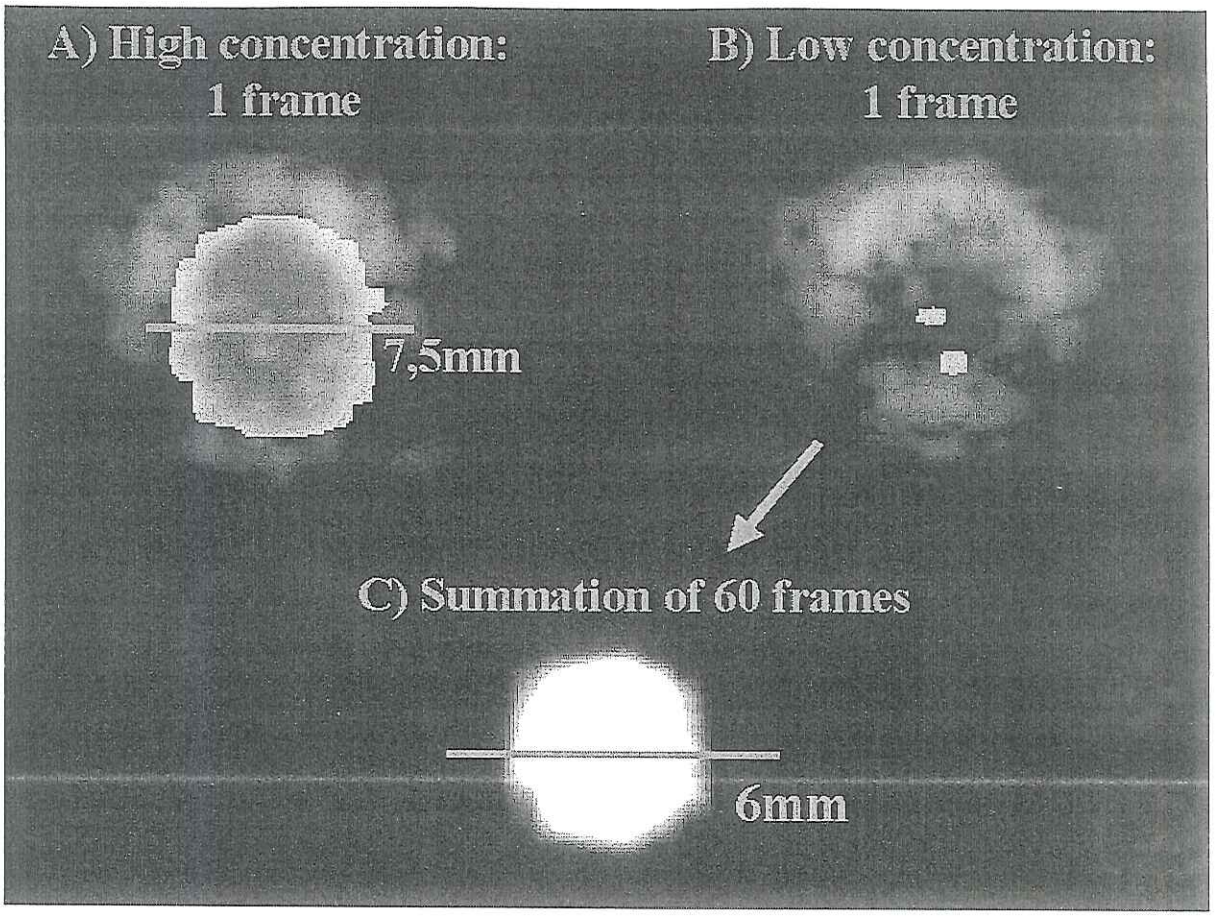

Figure 1: The size of HPD-signals at variable concentrations of SH-U 563A insonating an artificial vessel (∅ 6mm). A: At highest concentration blooming artefacts lead to an overestimation of the vessel diameter. B: Lowest concentration reduces artefacts but the contrast is insufficient. C: After summation of 60 frames at lowest concentration the correct vessel diameter is imaged.

NEGATIVE BOLUS INDICATOR DILUTION MEASUREMENT OF MICROVASCULAR BLOOD FLOW IN SKIN AND SKELETAL MUSCLE USING ULTRASOUND CONTRAST AGENTS

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Motivation: The need for accurate and reliable blood flow measurement in the diagnosis and treatment of cardiovascular and cerebrovascular diseases is well recognized. However, quantitative flow detection can also provide essential information for other medical and surgical specialists, in particular, for the plastic and reconstructive surgeon. The pre-operative planning and post-operative monitoring of tissue-transfer procedures, used in cancer, burn or trauma reconstruction, hinge on the availability and maintenance of adequate blood supply to transplanted tissue. A clinical, real-time flow measurement system would allow optimal pre-operative tissue selection, provide post-operative monitoring of flap ischemia, and as a research tool, would further our understanding of the physiology of replantation, ischemia-reperfusion injury and pharmacological optimization of tissue survival. Existing real-time flow detection techniques, whether MR, conventional Doppler ultrasound or laser Doppler, are based on velocity measurements and can provide only relative estimates of volume flow rate. Detecting flow in the microcirculation is further complicated by very low flow rates and low blood volumes which are often well below the resolution limits of these techniques. Indicator-dilution, however, is a well established, velocity-independent method for measuring blood flow that can be applied on both a macrovascular and microvascular scale. To carry out blood flow measurements with indicator-dilution, one needs a suitable tracer, a method for injecting it into the region of interest as a bolus, and a non-invasive means of detecting and quantifying its concentration. The volume flow rate in the region of interest is then given as:

$$Q = \frac{M_0}{\int_0^\infty c(t)dt}$$

where Q is the flow rate, M_0 is the total mass of indicator injected and $c(t)$ is the concentration of indicator (mass/volume) measured at the outflow[i].

Performing indicator-dilution measurements in-vivo, however, is often impractical due to the impossibility of placing an injection catheter precisely at the vascular inflow to the region of interest,

while bolus injection into a peripheral vein results in severe bolus spreading and measurement error. We propose to address this fundamental problem by using microbubble contrast agents as indicators.

The negative bolus indicator-dilution method: Several properties of microbubble contrast agents make them an ideal choice as indicators: Microbubbles are disrupted when exposed to high power ultrasound pulses, a phenomenon that can be observed on most commercial scanning systems. This allows the injection of a bolus to be replaced with selective destruction of tracer by an ultrasound transducer placed over the region of interest. Following intravenous infusion of ultrasound contrast agent, a negative bolus confined within the scan volume of an ultrasound transducer is created by acquiring a single, high MI image frame. A commercial ultrasound scanner in Power Doppler mode is then used to track the wash-out phase of the negative bolus.

To implement this technique in-vivo, microbubble-specific imaging modalities are necessary to maximize the detection of contrast agent signal and overcome the clutter due to tissue motion. Non-linear imaging techniques, such as Harmonic Doppler and more recently Pulse Inversion Doppler, allow real-time tracking of contrast agent flowing slower than and in vessels smaller than the resolution limits of conventional ultrasound. Since high peak pressures (MI > 1.0) are needed to maximize microbubble detection, the agent is destroyed as it is being imaged, and it is not possible to follow bolus wash-out with continuous imaging. Instead the wash-out curve must be reconstructed in a piece-wise fashion from a sequence of intermittent Power Doppler images. The creation of a negative bolus is repeated several times, with each bolus separated from its predecessor by an increasing time interval. The Doppler image is used both to quantify agent concentration within the region of interest and to create the next negative bolus. The time intervals used depend on the anticipated flow rates in the tissue under study.

In-vitro testing and validation of the technique was carried out on a large vessel phantom immersed in a water bath and early in-vivo demonstration has been carried out in canine thigh muscle. Imaging was performed with an HDI 5000 commercial scanner with image analysis and quantification software provided by ATL Ultrasound (Bothell, WA).

Results: Using the large vessel phantom, it was shown that Doppler power is linearly proportional to contrast agent concentration (figure 1). It was also demonstrated that negative boluses of agent could be created with a commercial scanner and subsequently tracked with a two transducer setup (figure 2).

In parallel with the in-vitro work, it was established that steady in-vivo microbubble concentrations could be maintained using slow intravenous infusion in a canine model (figure 3).

Using the thigh-adductor muscles in the same canine model, it was also demonstrated that contrast agent within microvessels could be detected and bolus wash-in and wash-out tracked from the skin surface (figure 4).

We are currently conducting experiments to validate the technique in-vitro and in-vivo. In addition to the large vessel phantom, a second phantom consisting of 100 μm diameter vessels will be used to test and optimize detection under very low flow conditions. In-vivo testing will be carried out in the canine thigh muscle model, while actual validation will require the use of a previously established porcine myocutaneous flap model. This model allows isolation of the blood supply to a surgically raised unit of muscle and overlying skin, making external metering as well as mechanical and pharmacological manipulation of blood flow possible.

Discussion and significance: The three requirements for performing indicator-dilution flow measurement are: the availability of a suitable indicator, a means of delivering a bolus of indicator and a sensitive method of tracking indicator concentration post injection. With this work we have established the suitability of ultrasound contrast agents for use as indicators by satisfying all three requirements: (1) Steady indicator concentrations can be achieved and maintained through IV infusion, (2) Destruction of agent can be used to generate negative boluses of indicator and (3) Agent concentration can be tracked accurately using existing ultrasound imaging techniques both in-vitro and in-vivo. Furthermore, several advantages inherent to this technique become evident: (1) By creating a negative bolus directly in the region of interest, bolus spreading - a significant source of error in positive bolus methods - is eliminated. (2) Infusion of indicator at a peripheral intravenous site allows thorough mixing of agent throughout the vascular volume, reducing errors due to non-uniform tracer distribution, which can amount to as much as 100-200%[ii]. (3) The sensitivity of the method to flow rate is determined by the interval delay between the imaging sequences. In other words, when longer time delays are used, agent flowing in large vessels with high flow rates has sufficient time to return to baseline concentration levels between consecutive images, which means these large vessels would not contribute to the indicator wash-out curve. Varying the initial time delay between imaging frames could therefore theoretically select out a population of blood vessels for study, based on size and flow rate. Such physiologic selectivity has not been possible with other flow estimation techniques to our knowledge.

While this method was designed for and tested in skeletal muscle and skin, the principles involved are not tissue specific, and should allow measurement of microvascular blood flow in other organ systems including the heart, liver and kidneys.

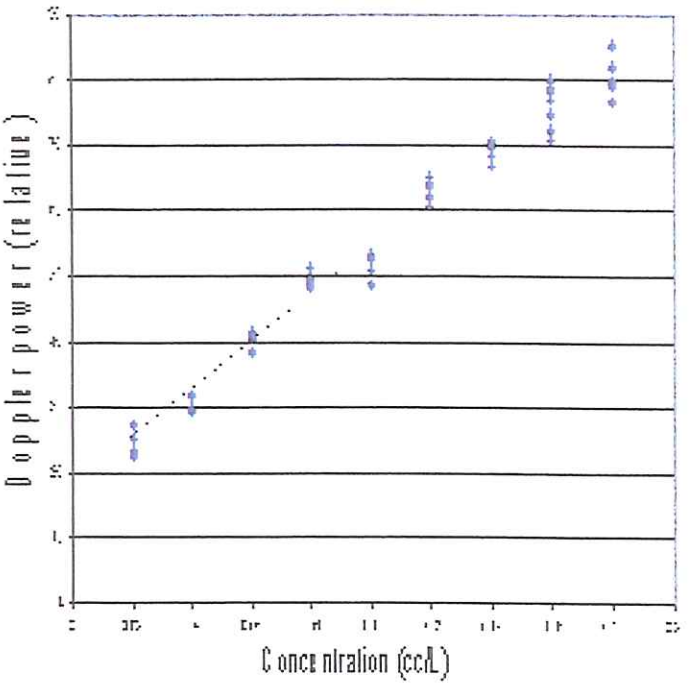

Figure 1: Contrast agent concentration can be measured from Doppler signal power.
Relative Doppler power vs agent concentration for DMP 115 (DuPont-Merck) in a latex tube.
Power = 359·Concentration + 18.3 (r = 0.990)

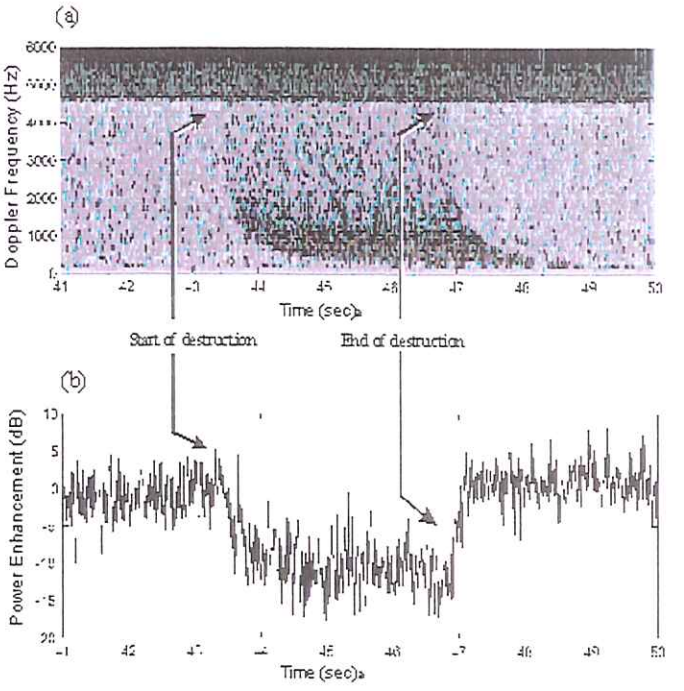

Figure 2: A negative bolus of DMP 115 created and tracked in-vitro using a two transducer setup.
(a) Spectral Doppler display (10MHz centre frequency) and (b) Integrated Doppler Power vs time, demonstrating negative bolus wash-in and wash-out over 5 seconds. Arrows mark period of active microbubble destruction.

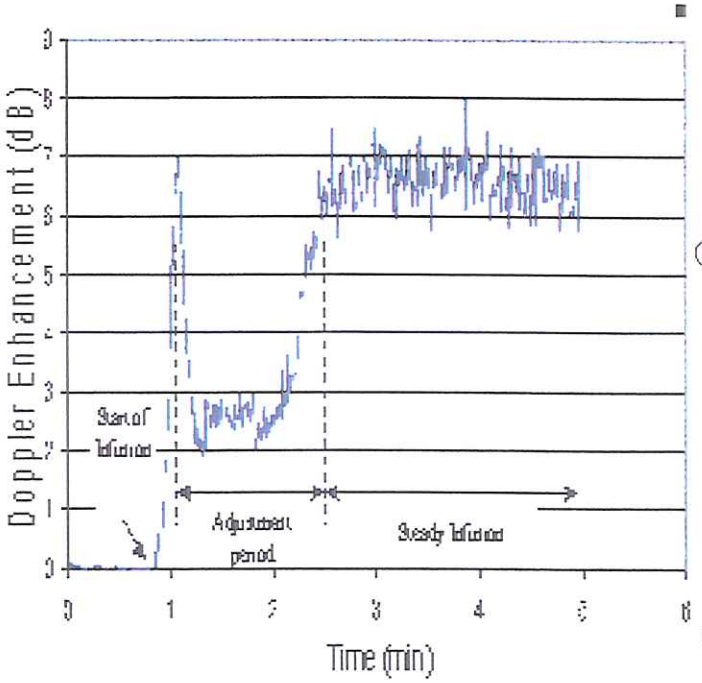

Figure 3: Slow intravenous infusion of contrast agent produces steady intravascular concentration in-vivo. Doppler power enhancement in the femoral artery of a dog is plotted against time during administration of DMP 115 (1cc of agent in 50cc saline, infused over 15 minutes.) The infusion rate was adjusted over a 2 minutes period, followed by stady enhancement.

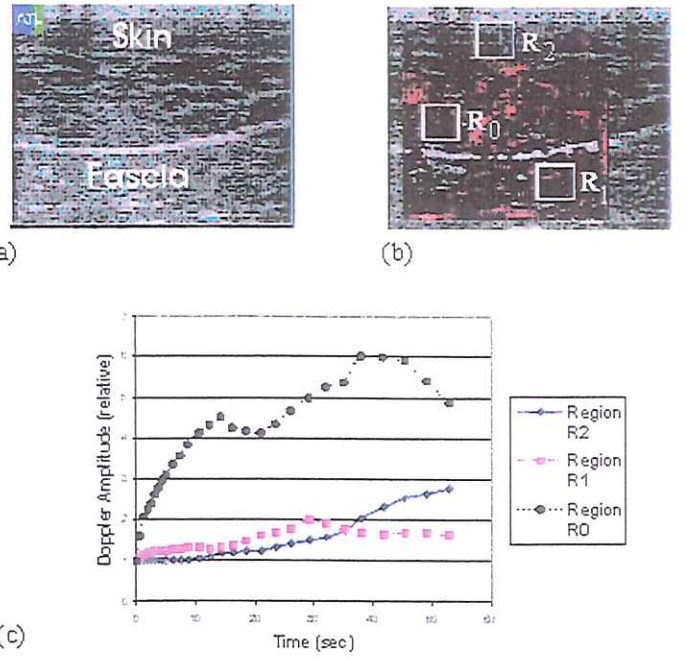

Figure 4: Contrast agent can be detected in the micro-circulation of skeletal muscle in-vivo. A positive bolus of DMP 115 was injected intravenously while intermittent Power Doppler images of canine thigh muscle were taken. (a) Baseline image of adductor muscles prior to contrast injection. (b) Post-injection Power Doppler image showing parenchymal enhancement. Three regions are drawn for quantitative analysis. (c) Mean Doppler signal amplitude is plotted against time for three regions of interest, showing different wash-in rates and overall enhancement.

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HARMONIC POWER DOPPLER TECHNOLOGY

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In recent years equipment manufacturers have developed new imaging techniques for ultrasound contrast agents. Some of these techniques have become commercially available (Harmonic imaging, Harmonic Power Doppler, Pulse Inversion Imaging). One of the more sensitive imaging modalities has proven to be Harmonic Power Doppler.

Power Doppler is a imaging modality that has been available in vascular/abdominal imaging for a few years but is relatively new to cardiac imaging. It is similar to Color Doppler in that a packet of pulses is transmitted along an acoustic line instead of the single pulse transmitted in B-mode. However, it is also similar to B-mode in that the intensity of the acoustic signal is displayed instead of the velocity. In vascular /abdominal applications Power Doppler is used to look at slow flow in smaller vessels. There is an apparent gain in dynamic range in these situations over standard Color Doppler, even though the dynamic range in the system is identical. This apparent gain comes about from the fact that intensity is displayed instead of velocity thereby allowing receiver gains to be higher and the PRF's (scales) to be lower. Thermal noise generated in the system from a higher gain and aliasing from a low PRF are no longer objectionable in Power Doppler as they are in Color Doppler because low level signals are mapped to low intensities.

When using Power Doppler with ultrasound contrast agents, there is a signal-to-noise improvement over B-mode imaging. This is true for some of the same reasons that Color Doppler has a signal-to-noise improvement over B-mode without an ultrasound contrast agent (i.e., more than 1 pulse per packet). However, when imaging contrast agents there are additional reasons Power Doppler is more sensitive.

Power Doppler processing, very similar to Color Doppler processing, is not actually based on the Doppler method at all. It is a correlation technique and therefore, it looks for changes between pulses of a packet. The objects being imaged only need to appear different between pulses of the packet for Power Doppler to detect the signals. These differences can be either in terms of a phase shift between pulses of a packet or in intensity changes between pulses of a packet. Phase differences can arise from movement, such as blood flow with red blood cells or myocardial movement from contraction or respiration. With contrast agents there are other potential sources of change. It has been shown that

ultrasound energy can destroy a contrast agent. This specific signature can be used to distinguish microbubbles from tissue. This is what Power Doppler imaging of contrast agents attempts to do.

The first couple of pulses of the packet rupture or damage the shell of the agent, with the first pulse doing most of the destruction. This destruction allows the gas inside to become a free bubble (i.e., no shell). Free bubbles have vastly different resonance and backscatter characteristics than encapsulated microbubbles. These free bubbles then dissolve over time, at a rate that depends on the density of the gas and its diffusivity in blood. Throughout the length of the packet, each time the system "looks" (transmits a pulse of the packet) it measures changes from the previous pulse and produces changes in the microbubbles for the next pulse to "see". The system detects differences in backscattered intensity, in backscattered frequency (resulting in phase shifts) or from microbubble movement (phase shifts). These changes produce a Power Doppler signal.

The increase in sensitivity of Power Doppler comes at the price of an increase in artifacts from tissue motion. How can the system distinguish microbubble changes from tissue movement? First of all, a 10dB gain can be obtained in bubble-to-tissue ratio by using Harmonic Power Doppler instead of Power Doppler. Secondly, by using a high PRF (i.e., shooting the pulses as quickly as possible) to minimize tissue movement, bubbles can be selectively detected and tissue can be filtered out. For example, on the SONOS 5500, using a PRF of 3000 attenuates motion of 10cm/sec about 20 dB (a factor of 100 in intensity) relative to a PRF of 700. However, tissue motion artifact since it is such a strong signal, may still make it to the screen.

Shooting two consecutive frames can further help artifact reduction. If the trigger point is selected such that cardiac motion is minimal between the two frames (end-systole or end-diastole during isovolumic relaxation), then the 2^{nd} frame can be used as a means to verify the quality of the data by discriminating motion artifact from contrast agent destruction. The assumption made here is that the microbubbles become acoustically invisible before the 2^{nd} frame is fired (about 50msec later). If this is the case, a "baseline" image (no bubbles in the myocardium) is created from the 2^{nd} frame. This multi-frame technique will be successful with most but not all contrast agents. Agents that are difficult to destroy and take a long time to dissolve in blood will not gain an advantage from Multiple Frame Trigger.

In summary, Harmonic Power Doppler is a more sensitive technique to detect microbubbles. However, great care needs to be taken to eliminate motion artifacts that are not present in B-mode imaging (Pulse Inversion has motion artifacts as well because it is a multi-pulse technique).

POWER HARMONIC™ QUANTIFICATION

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Most quantification of contrast agents has been done using some form of temporal analysis of the brightness of greyscale images. Kaul has published extensively on this subject, especially on the use of background subtraction. There has been considerable skepticism as to whether Power Harmonic Imaging™ would be quantifiable, since it depends on the destruction of microbubbles, and has more complex signal processing involved than does greyscale imaging. It has also been said that the limited dynamic range of Power Harmonic Imaging™ would limit its application for quantification. This paper will show that Power Harmonic Imaging™ is quantifiable and that the reduced dynamic range is an advantage, rather than a limitation.

Prior to the development of harmonic imaging, background subtraction used the fundamental images that were available at the time. When harmonic imaging was developed, it was assumed that tissue was linear, and that a perfect harmonic system would image only contrast agent. It was thought this would provide "realtime background subtraction", since it would show only contrast. However all harmonic systems had a residual tissue image, which was later shown to be due to nonlinear propagation in tissue, rather than imperfect system design, as was originally thought.

Even with harmonic imaging, background subtraction is still necessary to remove the residual tissue image and show only the contrast. Since background subtraction occurs after image acquisition, the display must capture the entire signal range. Even though the absolute change in contrast intensity may be only 20dB, it might be added to a background signal from 0dB to 40dB, requiring the entire 60dB to be displayed and stored. In order to capture this on videotape or even digital images requires compression, then the images should be decompressed prior to analysis. All of this argues for the highest dynamic range and linearity in the signal path all the way through to display and storage.

If background subtraction can be performed in realtime prior to display and storage, the requirements are significantly reduced. If the contrast intensity change is only 20dB, then that is all the dynamic range required to display it, even if the raw signal had a much higher dynamic range. This is precisely how Doppler processing works. A spectral Doppler display is only about 40dB, as that is all the information in the Doppler signal. However a good Doppler processor requires 80 – 100dB or more prior to the wall filter because of the huge tissue component that is always present.

Like harmonic B-mode, Power Harmonic™ Imaging was developed to provide realtime background subtraction, but it has more degrees of freedom for optimizing the discrimination between contrast and tissue. It uses not only the harmonic response from the bubbles, but their motion, instability, and destruction as well. Multiple pulses are transmitted down each ray line and the returned signals are subtracted from each other, highlighting change from one pulse to the next, in effect realtime background subtraction. Since the Doppler signal path is used, the full linear signal of 100dB or more dynamic range is maintained up through clutter rejection, or background subtraction.

After the tissue signal has been removed, the dynamic range may be reduced for display. Now, though, instead of having to compress the full signal dynamic range for storage and display, only the contrast signal itself is displayed, resulting in a basically linear signal which can be displayed without compression.

This talk will show that Power Harmonic Imaging is linear with contrast concentration over two orders of magnitude, making it ideal for contrast quantification. It will also show how Power Harmonic Imaging may be used for perfusion measurement using the microbubble destruction / reperfusion method.

ACOUSTICAL BEHAVIOR OF SINGLE CONTRAST AGENT BUBBLES: EXPERIMENTAL AND THEORETICAL OBSERVATIONS

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For the first time we are able to compare echoes recorded from a single contrast agent bubble with those predicted by a model for a shelled bubble. By examining a combination of experimental and theoretical echoes, we can develop new signal processing techniques to differentiate between bubbles and tissue, taking advantage of unique properties of the bubble. In particular, we have used these tools to examine the effect of transmitted phase on the echo received from a single contrast agent bubble.

Based on theoretical predictions and experimental recordings, we have developed a new phase inversion technique that exploits specific differences in the response of bubbles to the phase of transmission. This technique differs from the phase inversion technique developed by other researchers in that it uses a frequency shift between the echoes received when a pair of pulses with opposite phases is transmitted. This frequency shift is unique to a bubble and could be used to distinguish between tissue and bubbles. A benefit of a technique based on this frequency shift is that it is not degraded by nonlinear propagation through tissue. Following transmission of a pair of 2.25 MHz pulses with opposite phases, the received echoes demonstrate a shift of approximately 0.9 MHz in mean frequency [1]. The ability to record echoes from single contrast agent bubbles is achieved by using a low concentration of contrast agent solution flowing in a 200 um diameter cellulose vessel.

Our experimental results are supported by new theoretical predictions for the bubble echoes. In order to predict the received echo from a single bubble, a modified version of the Herring model is employed [2]. This model incorporates the effects of shell friction and shell elasticity in a manner similar to that developed by de Jong [3]. The modified Herring equation predicts fluctuations in radius and wall velocity in response to a given driving pulse. We then predict the received echo based on the resultant radius and wall velocity using $p \propto \dot{\Phi}$, with $\Phi \propto R^2\dot{R}$. The predicted echoes accurately demonstrate the effect of transmitted phase on the time domain signal and correctly predict the frequency shift observed in the experimental echoes. Furthermore, the model can be used to analyze the effect of shell parameters on the bubble echoes. Specifically, by optimizing the shell elasticity and shell friction parameters, the frequency shift in the predicted echo can be maximized.

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NONLINEAR SCATTER FROM THE NC100100 ULTRASOUND CONTRAST AGENT: COMPARISON OF THEORY AND EXPERIMENT

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Based on elasticity theory, we have developed a full nonlinear model for the oscillation of a shell-encapsulated gas-bubble in an acoustic field, obtaining a Rayleigh- Plesset like differential equation. This model has been used to investigate nonlinear scatter of ultrasound from the contrast agent NC100100 (Nycomed Amersham). The two unknown parameters of the encapsulating shell, elasticity and viscosity, are estimated from ultrasound measurements in the linear range, and used as input into the nonlinear model. Time domain simulation of the resulting ordinary differential equation gives bubble radius and radiated sound as function of time, as response to specified incoming ultrasound pulses.

Experimentally, we measured nonlinear sound scatter by transmitting ultrasound pulses from one transducer into samples of diluted NC100100. Scattered sound was received with a separate transducer of higher nominal frequency, detecting signals at the harmonics of the transmit frequency. Typical transmit pulses were 20 cycle sine wave bursts enclosed in a Hanning window, ensuring little sound energy outside the main frequency lobe, and good separation between the harmonics. Pressure amplitudes were varied between 0.03 and 0.9 MPa. Received spectra were corrected for the frequency response of the receiving transducer. In signals sampled by this method, we found frequency components up to the 9th harmonic of the transmit frequency. Power at the harmonic frequencies increased more strongly with transmit power than power at the transmit frequency. We found good agreement between experimental results and predictions from the shell-encapsulated bubble model. Theories based on gas-bubbles without shell predict higher harmonic levels than we measured, and give poor agreement between theoical and experimental results.

We conclude that the scatter of sound from NC100100 is highly nonlinear at sound amplitudes used in medical imaging, and that a model describing this nonlinear oscillation and sound scatter must include the effect of the encapsulating shell.

ACOUSTIC CAVITATION IN THE PRESENCE OF MICROBUBBLE CONTRAST AGENTS

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Current diagnostic ultrasound imaging systems can work in the fundamental, second harmonic, color Doppler and power Doppler modes. Ultrasound contrast agents have been used in conjunction with each of these imaging modes to enhance the backscatter signal from blood. The images are formed using a pulse-echo technique with a diverse combination of transmit acoustic pressure amplitudes, pulse repetition frequency (PRF), and number of acoustic cycles. Because of these different imaging parameters and ultrasound contrast agents that are now available, it is usually not clear whether the main contribution for the increased backscattered signal is due to non-inertial or inertial cavitation effects. Although several research groups have investigated the threshold for inertial cavitation of microbubbles to assess the potential of gas carrier agents to induce bioeffects, little is known about the consequences of the destruction of contrast agent bubbles on the ultrasound image.

In the present work, an experiment was designed where a 1.1 MHz/ 8 cm therapeutic transducer was used to insonify a cloud of contrast agents subject to a variety of ultrasound imaging parameters. The effect of this acoustic irradiation on Albunex® was monitored by a 5 MHz transducer operating in pulse-echo mode and also by an HDI-1000 ultrasound imaging system using a C4-2 scan head (ATL Ultrasound, Bothell, WA). We measured the threshold for the disappearance of echo-contrast from the imaging system (pressure P1), and the threshold for the onset of inertial cavitation (P2), as a function of the concentration of Albunex®, PRF, and number of acoustic cycles in both Isoton® II and whole blood solutions.

The results show a direct relationship of P1 with the concentration of the agent and PRF. These pressure values were within diagnostic ultrasound range. The threshold for P2 was inversely proportional to the concentration and independent of the PRF. The thresholds P1 and P2 increase as the number of acoustic cycles per pulse decreases. By using a high intensity ultrasound transducer (with a certain combination of imaging parameters) and an HDI-1000 imaging system, we showed that it is possible to regenerate image contrast from Albunex® microbubbles once they have ceased to provide adequate echo-enhancement in conventional imaging modes.

Determination of the thresholds P1 and P2 as a function of ultrasound parameters should improve the design of bubbles used in stimulated acoustic emission mode, and also monitor more efficiently the release of drugs from microbubbles used in drug delivery. [This work has been supported by ONR-DARPA].

EXPERIMENTAL AND SIMULATED ACOUSTIC PROPERTIES OF SONOVUE™: PREDICTED BEHAVIOR IN FUNDAMENTAL AND HARMONIC IMAGING MODES.

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Sonovue is an ultrasound contrast agent (UCA) from Bracco Research SA, containing encapsulated microbubbles. The shell of these microbubbles is a phospholipid monolayer and contains SF_6 gas. Acoustic properties such as backscatter coefficient, attenuation, and harmonic response levels allow to characterize the potential efficacy of any UCA for practical clinical use.

We assess these acoustic properties from in-vitro experiments. The general setup for these measurements is as follows:
A plane steel reflector is used to produce reference wideband echo signals. It is arranged as a movable piston defining the back wall of a measuring cell. Backscatter signals are first acquired from within the UCA solution, with the piston in a "far" position. The steel piston is then brought *in situ* to a "near" position, allowing acquisition of reference echo signals at exactly the same position where the UCA signals were acquired. In this way, we are able to remove diffraction and other system effects according to the theory developed by Chen [1]. Moreover, compensation of any attenuation is directly included in this process since the reference echoes are acquired within the solution.
The actual attenuation coefficient may be measured from reference echoes in the near and far positions, or by the use of blank measurements in saline.

The measurements for assessing harmonic responses are performed by replacing the one-transducer by a side by side two-transducer setup, and applying narrower-band excitation. In this case, diffraction and system corrections are performed separately. Since different transducers are used in transmit and receive modes, the required one-way frequency responses of these transducers have been obtained from calibrated-hydrophone measurements. Diffraction correction is performed by adapting the theory of Chen to the two-transducer setup, taking into account the different radiation patterns in transmit and receive modes due to the specfic geometries of the transducers and the different frequencies (f_o in transmit and $n \cdot f_o$ in receive). Particular attention was paid to a careful distinction between harmonic components generated by the UCA from those caused by non-linear acoustic propagation in the water bath or by electronics components, as well as to avoid excessive acoustic pressure resulting in possible microbubble destruction.

Another direct interest of these acoustic measurements is the possibility to assess the physical properties of the microbubble shells, primarily stiffness and viscosity. The simulation model proposed by de Jong et al. [2-3], adapted from the Rayleigh-Plesset equation to the specificity of encapsulated bubbles, provides the theoretical basis for estimating stiffness and viscosity coefficients of the shell. Several transmission and scattering measurements performed on narrow-distributed decanted suspensions of Sonovue provide experimental data from which the shell properties can be estimated in a sensitive way, from the best fits of the simulation model. These estimates are made in conjunction with Coulter Multisizer™ distributions of the narrow-distributed suspensions of microbubbles. Once the stiffness and viscosity parameters of these suspensions are found, the global attenuation and backscatter coefficients are computed for the case of the native Sonovue size distribution.

The results obtained in this way show that Sonovue is well adapted in the frequency range of usual medical applications in both fundamental and harmonic modes.

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TOXIN AND GENE TRANSFER INTO CELLS BY EXTRACORPOREAL SHOCK WAVES:

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Shock waves are therapeutically used for lithotripsy. Shock wave application to suspended cells in vitro causes a transient increase of the permeability of the cell membrane which does not lead to cell death. Molecules of any size and solid particles can be transferred into the cytoplasm. It was hypothesized that shock waves might be a new method of transferring therapeutic agents directly into cells. To test this, biological effects resulting from the acoustical transfer of proteins and nucleic acids into cells were examined.

Protein transfer was examined with the ribosome inactivating proteins gelonin and saporin. They inhibit the cellular protein synthesis in minute concentrations and lead to cell death. Dose response curves were established with three tumor cell lines in the presence and absence of shock waves. Compared to the controls, shock waves enhanced the action of gelonin and saporin from 300 to 40.000 fold. The encouraging result prompted to perform in vivo experiments with a fibrosarcoma mouse tumor. It was found that systemic administration of the ribosome inactivating protein together with shock waves had a clear influence on the tumor growth. Complete remissions were observed in a fraction of the animals. It was concluded that the ribosome inactivating protein had been acoustically transferred into tumor cells in vivo.

Gene transfer by shock waves was examined in vitro with a number of cell lines by plasmid vectors carrying standard reporter genes. Transfer succeeded in many cases yet the transfection efficiency was lower than with other established methods of gene transfer. In vivo experiments were performed in animals carrying subcutaneous tumors. Gene transfer into the tumor in vivo was observed after shock wave exposure. The transfection efficiency was slightly above the mechanical gene transfer observed in control animals without shock wave application. Further experiments established that gene transfer by shock waves was as good as an optimized mechanical method of gene transfer. It was concluded that shock wave mediated gene transfer has to be improved to surpass other established methods of transfer.

In summary shock wave permeabilization is a new method for tumour therapy and gene transfer.

DETRIMENTAL EFFECTS OF LOW FREQUENCY 20 KILOHERTZ TRANSTHORACIC ULTRASOUND ON CORONARY ARTERY NEOINTIMAL HYPERPLASIA FOLLOWING BALLOON INJURY: 30 DAY ANGIOGRAPHIC, HISTOLOGIC, AND ANGIOGRAPHIC OBSERVATIONS

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Background: Low frequency ultrasound (LFUS) can significantly enhance the intravascular deposition of intravenous (IV) injected antisense oligonucleotides (AO) when they are delivered bound to perfluorocarbon containing microbubbles (PESDA). LFUS may have significant bioeffects, however, because of a lower cavitation threshold. Purpose. The purpose of this project was to determine whether neointimal hyperplasia (NH) and vascular remodeling (VR) could be inhibited by transthoracic LFUS targeted deposition of AO to the protooncogene c-myc bound to IV PESDA.

Methods: Nineteen pigs underwent left anterior descending and/or left circumflex balloon injury followed by randomization to either IV AO bound to PESDA in the presence of 20 kilohertz LFUS (PES-US) versus LFUS + IV AO without PESDA (US alone) versus control (no ultrasound, no PESDA). Intravascular ultrasound (IVUS) measurements of reference (RS) and injury site total vessel area (TVA), intimal area (IA), as well as quantitative angiography (QA) measurements of lumen diameter (LD) and histologic measurements of intimal area (IA) were made at baseline and day 30. Results are shown (*p<0.01 versus control)

	TVA (mm2)	Change in LD (mm)	IA (%stenosis)
PES-US	7.7±2.2	-0.4±0.5	27±14%
US alone	6.6±2.0	-0.7±0.5*	33±11%
Control	6.7±2.4	0.0±0.4	21±7%

LFUS alone induced greater stenosis formation (p=0.08) and also a greater decrement in lumen area by IVUS compared to the RS (p=0.05).

Conclusions: Transthoracic LFUS stimulates both NH and VR following coronary balloon injury. Although suppression of NH did not occur in the US targeted group with PESDA, the reason for this may have been the detrimental effects of LFUS. The optimal ultrasound parameters for the non-invasive targeted delivery of DNA with microbubbles remains to be determined.

PHYSICAL PRINCIPLES OF ULTRASOUND-DIRECTED DRUG DELIVERY

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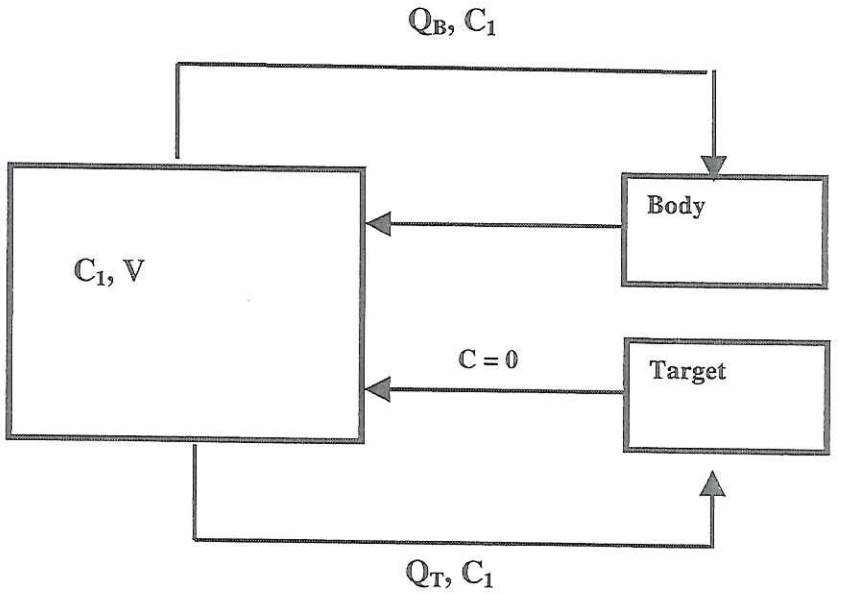
The use of external ultrasound to localize the delivery of drugs in the body has been an area of interest to medical researchers. Recent advances in ultrasound systems and ultrasound contrast agent technology have stimulated new approaches to ultrasound directed drug delivery. In particular, our research group is investigating the use of a dual walled microsphere (biSphere™) which is freely circulating in the blood stream and can be triggered to rupture and release drug upon certain ultrasound conditions. The fragility of the dual walled microspheres is adjusted by the properties of the inner structural wall. The localization of free drug by triggering release from the circulating microspheres could provide a simple physical means to localize drug treatment. The advantages to both pharmacokinetics and pharmacodistribution of a drug locally released in the blood pool can be described in a simplified mathematical model. The use of such a system to treat heart disease is of particular interest with drugs whose pharmacology can be significantly improved by changes in localized concentration.



Simple Microbubble Circulation Model

DELIVERY OF COLLOIDAL MICROPARTICLES AND RED BLOOD CELLS TO TISSUE THROUGH MICROVESSEL RUPTURES CREATED BY MICROBUBBLE DESTRUCTION WITH ULTRASOUND: A NOVEL METHOD OF LOCAL DRUG AND GENE DELIVERY?

Sanjiv Kaul, Richard Price, Danny Skyba,, Thomas Skalak

University of Virginia, Charlottesville, Virginia

Background: We have previously shown that the application of ultrasound to encapsulated microbubbles flowing through small microvessels (<7 μm diameter) produces vessel wall ruptures *in vivo*. Because many intravascular drug and gene delivery vehicles are limited by the endothelial barrier, we hypothesized that this phenomenon could be used to deliver drug bearing vehicles to tissue.

Methods & Results: An exteriorized rat spinotrapezius muscle preparation was used. Intravascular fluorescent red blood cells RBC and colloid microparticles (205 and 503 nm diameter) were delivered to the interstitium of rat skeletal muscle through microvessel ruptures created by exposing microbubbles to ultrasound *in vivo*. On intravital microscopy, the mean dispersion areas per rupture for RBC, 503 nm and 205 nm colloid microparticles were 14.5 x 10^3 μm^2, 24.2 x 10^3 μm^2, and 27.2 x 10^3 μm^2, respectively. The colloid microparticle dispersion areas were significantly larger than the mean dispersion area for RBC ($P<0.05$).

Conclusions: Microvessel ruptures caused by insonification of microbubbles *in vivo* may provide a minimally invasive means for delivering colloidal microparticles particles and engineered RBC across the endothelial lining of a targeted tissue region.

ELECTROPHYSIOLOGIC EFFECTS OF ULTRASOUND CONTRAST IMAGING

Poll A. van der Wouw[1], Annette C. Braun[1], Sarah E. Bailey[2]

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Background: Although ultrasound at diagnostic levels is generally believed to be safe and virtually without side effects, the addition of contrast agents during ultrasound imaging might lead to new side effects because of lowered cavitation thresholds. In a phase I volunteer study using continuous infusion of a contrast agent for myocardial perfusion assessment, premature ventricular contractions (PVC's) were observed. Further investigations into the relationship of this phenomenon to both ultrasound energy and contrast agent administration mode and dose were carried out during a subsequent bolus versus infusion study in healthy human volunteers.

Methods and results: two open label studies in healthy male human volunteers were performed. The initial study was a dose response study in10 subjects comparing three infusion rates. Each volunteer received three continuous infusions with different infusion rates of the contrast agent for either 10 (n=6) or 20 (n=4) minutes. End-systolic triggered imaging with a mechanical index (MI) of 1.5 was used throughout this part of the study. The second study compared bolus injection with a continuous infusion in 9 volunteers, using a single dose level but with different imaging modalities: end-systolic and end diastolic triggered imaging at MI's of both 1.1 and 1.5. Spontaneous baseline PVC's were uncommon: 10 in 344minutes (0.03 PVC/min, maximal 1 PVC/min) of baseline imaging. During end-diastolic triggering no increase in PVC's was seen, irrespective of MI.A significant increase to 1.06 PVC/min ($p<0.001$) was seen during end-systolic imaging with an MI of 1.5, but not with an MI of 1.1. The increase in PVC rate was dose dependent in the initial study.

Conclusion: imaging of contrast agents with high acoustic pressures can cause PVCs if end-systolic triggering is used. This effect is related to both the dose of contrast agent and acoustic pressure. It does not occur during end-diastolic triggered imaging. Precautionary measures would include using lower mechanical indexes or end-diastolic triggering.

NON-INVASIVE PRESSURE MEASUREMENT IN A FLUID FILLED CAVITY

A. Bouakaz[1], P.J.A. Frinking[1], N. de Jong[1-2].

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Non-invasive blood pressure measurements do not provide reliable and reproducible blood pressure values. The complications involved and the conflicting results yielded to the development of various alternative methods for blood pressure monitors. The aim of this study is to provide a new method for non-invasive pressure measurement based on the use of micro sized free gas bubbles used as a pressure sensor. An ultrasound contrast agent, consisting of encapsulated gas bubbles, is used as a vehicle to transport the free gas bubbles to the desired region where the pressure is to be measured.

The small free gas bubbles are generated from the encapsulated gas bubbles when they are exposed to a high acoustic pressure amplitude burst in the region of interest (e.g. heart chambers). These released gas bubbles persist for only a few milliseconds and dissolve in the liquid, depending on the gas, the liquid characteristics and ambient parameters, like temperature and pressure.

In the fluid of interest, encapsulated air bubbles (Quantison™, Andaris Ltd., Nottingham, UK) are injected with optimal concentration. A low frequency, high acoustic amplitude burst (0.5 MHz, 1.6 MPa, 10 μs) is transmitted to excite the microspheres and to generate free air bubbles. The disappearance rate of the released air bubbles is determined by using a high frequency, low acoustic amplitude, broad band pulses (10 MHz, 100 kPa, 100% at the -20 dB level, PRF of 1.6 kHz). Reproducible results show significant differences between persistence of bubbles as function of ambient pressures (50, 100, 150 and 200 mmHg).

This new method is compared to existing methods employing the resonance frequency shift. The measurements are compared to corresponding theoretical models.
In conclusion, encapsulated gas bubbles can be used as carriers from which free gas bubbles can be released in a desired region and at desired times. These released gas bubbles can subsequently be used for non-invasive acoustic determination of the ambient pressure.

CLINICALLY REPRODUCIBLE CONTRAST ECHOCARDIOGRAPHY AND PERFUSION STUDIES

Steven B. Feinstein, M.D., FACC, James E. Macioch, Philip R. Liebson, Joanne Sandelski, Tracy Ostoic, Ken Krueger, Bobi Jensen, Mickey Callis, Mahala Johnson, Faye Kuperman

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Beginning, on February 11, 1998, we have performed over 250 routine echo-contrast myocardial perfusion studies. Perfusion imaging was acquired in patients undergoing the following routine echocardiogram procedures (along with acquiring a routine 2-D, Doppler study): (1). Resting perfusion image, (2). Resting and peak stress perfusion images, (3). Pre and Post intervention (i.e. PTCA, Stent placement, thrombolytics, Laser Therapies – PMLR, TMLR) and (4). Screening perfusion (emergency room triage). Typically, the amount of Optison™ used per injection was 0.1 to 1.0 ml i.v. bolus. The ATL HDI-5000 with Power Harmonic™ Doppler imaging was used. The images were acquired using every 4th frame ECG gating. The efficacy and safety profiles were excellent.

Summary: (1) Myocardial perfusion imaging with contrast echo techniques is feasible in routine practice in all patients (2) The resting perfusion abnormalities correlated to cardiac catheterization data in > 75% of the cases (a stenosis of > 75% was considered significant)

(3) contrast echo studies can be used clinically to identify regional microvascular perfusion at rest, during stress, pre and post intervention and for screening patients for significant occlusive coronary artery disease.

MYOCARDIAL PERFUSION USING A DIFFERENT GROUP OF ULTRASOUND IMAGING AGENTS

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The most exciting prospect of transpulmonary contrast agents is to opacify the myocardium. If the contrast agent is a flow tracer, that is to be distributed proportionally to the blood flow distribution, when present in the myocardium causes scattering of impinging ultrasound waves, thus providing information on myocardial perfusion in healthy and diseased myocardium. Contrast echocardiography has been used after intracoronary or intra-aortic injections to assess myocardial perfusion defects, areas at risk, coronary flow and coronary flow reserve but only recently has been used in patients using intravenous injection (1). At present however only improved endocardial border delineation has convincingly been proven to be effective after intravenous contrast injection (2).

There is no doubt that the majority of transpulmonary agents improve endocardial border delineation and we have tested this with agents such as Infoson^{TM}, Sonovue^{TM}, Echogen® and NC100100 (Nycomed Imaging, Norway). However, a number of recent studies have also demonstrated that the simple utilisation of 2nd harmonic imaging with state-of-the-art ultrasound equipment is also in the position to improve endocardial border detection without the need of contrast. It is therefore essential to demonstrate additional myocardial opacification at rest and during stress.

We tested EchoGen^{TM} (Sonus Pharmaceutical, USA) to assess endocardial border delineation and myocardial opacification after intravenous administration in 22 healthy subjects. EchoGen^{TM} emulsion is a liquid-in-liquid dispersion which contains, as the dispersed phase, a liquid, dodecafluoropentane (DDFP) which has a boiling point below body temperature (28.5^{0}C). The dispersion contains emulsion droplets with a diameter of approximately 0.3 mm. Following intravenous administration, the liquid dispersion becomes a dispersion of microbubbles of DDFP with an average diameter of 2-3 mm providing a strong backscatter during echocardiographic imaging. Echocardiography was performed in short axis parasternal view. The opacification of the left ventricle and the endocardial delineation was considered as diagnostically useful in 21 subjects (95.5%).

However, the most important aspect was the detection of myocardial perfusion as assessed by videodensitometry. There were no side effects. In 13 subjects (59%) the contrast was visualised in the myocardium and lasted for 133±81 seconds. Myocardial opacification was graded by video densitometry (HP) with the use of software package for videodensity analysis based on a level of 256

grey scale unit (GSU). Grey scale intensity was measured in the septal and inferior walls in each volunteer and expressed in GSU before and at the peak of contrast enhancement. The percent enhancement in each region was calculated as the difference between grey scale intensity at peak- and pre-injection normalised to the pre-injection multiplied by 100. The density of at the septum increased by 35.9% (from 65.3 to 88 GSU, $p<0.01$) and inferior wall by 34.6% (from 63.1 to 85 GSU, $p<0.01$).

QW 7437 (Sonus Pharmaceutical, USA) is an other agent, similar to EchoGen^{TM} but with a negative charged surfactant that prolongs its persistence and stabilisation of the bubble. Following hypobaric activation, the solution becomes a dispersion of microbubbles that circulate within the intravascular space providing a strong backscatter image which can be quantified. Twenty healthy volunteers were studied to assess the efficacy of myocardial opacification with, using both fundamental and 2nd harmonic imaging in the standard apical 4- and 2-chamber views as well as parasternal long- and short-axis and quantitated using acoustic densitometry with linear analysis. Myocardial opacification was visualised by a blinded reviewer in both fundamental and harmonic imaging. QW7437 opacified the myocardium better with harmonic imaging in all coronary vascular beds but endocardial border detection was better using fundamental imaging. Similarly, compared with EchoGen^{TM}, QW7437 provided a better myocardial opacification but EchoGen^{TM} showed a the best endocardial border delineation in fundamental imaging.

It would therefore appear that new contrast agents such as QW7437 that are specifically designated for evaluating myocardial opacification may not be as effective in endocardial border delineation.

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MYOCARDIAL PERFUSION IN RECENT MYOCARDIAL INFARCTION USING POWER-DOPPLER CONTRAST ECHOCARDIOGRAPHY

Guido Rocchi, Folkert J. Ten Cate

Heartcentre, Erasmus University, Zuiderziekenhuis, Rotterdam, Netherlands

Power-Doppler imaging is a new modality that selectively evaluates the backscatter signal coming from ultrasound contrast agents. Therefore, we hypothesized that it could provide a better assessment of myocardial perfusion.

We performed a myocardial contrast echocardiography in thirty patients after a recent myocardial infarction. The imaging was acquired both gray-scale harmonic and harmonic power-Doppler during continuous intravenous infusion of contrast agent. Different acquisition intervals were used (1:1, 1:3 and 1:5 cardiac cycle). The results were compared to coronary artery angiography and to ^{99m}Tc-sestamibi single-photon emission computed tomography (SPECT). An echocardiographic follow-up was performed to determine whether late recovery of dysfunctional myocardium was present, and whether it was related to the myocardial perfusion. A 16-segment left ventricle model was used to relate perfusion with coronary artery territories.

Results: Myocardial perfusion was detectable in all the patients. Using coronary angiography as end-point test, the longest acquisition interval (1:5 beats) had the best results: Power-Doppler MCE and SPECT had both high and comparable accuracy for detection of infarct-related artery. Power-Doppler MCE imaging had a significantly higher sensitivity compared to gray-scale MCE imaging. Follow-up results indicated that the assessment of perfusion by power-Doppler imaging yields prognostic information regarding short-term cardiac events and late recovery of function. Some clinical cases will be shown. MCE power-Doppler imaging will be compared to MCE gray-scale imaging, to SPECT imaging and to coronary artery angiography.

SIGNAL AMPLIFICATION IN TRANSCRANIAL DOPPLER SONOGRAPHY BY A SULFUR HEXAFLUORIDE CONTAINING ULTRASOUND CONTRAST AGENT (SONOVUE™)

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[2] Bracco SPA, Italy

Background: Insufficient ultrasound penetration through the temporal bone is a serious limitation of transcranial ultrasound diagnosis. In a Phase-I-Study, we studied safety and ultrasound enhancing potentials of SonoVue™ (generic: BR1, Bracco Research, CH-Geneva), a new transpulmonary ultrasound contrast agent consisting of sulfur hexafluoride microbubbles stabilized by a phospholipid shell.

Methods and Results: Twelve healthy volunteers randomly received four different doses of SonoVue™ (5mg/ml) by i.v. bolus injection [0.3 ml (A), 0.6 ml (B), 1.2 ml (C), 2.4 ml (D)]. Pharmacokinetic characteristics of SonoVue™, duration and strongness of spectral enhancement were measured by Transcranial Doppler Sonography and analysed via TCD8-software (DWL, Multidop X_4). Safety and tolerability were monitored during the study and for 24 hours following contrast agent administration.

Duration of spectral enhancement (signal intensity 5 dB over baseline) was observed dose related ($p < 0{,}0001$; Friedman-Test) for (inject. A) 136 ± 63,4 s, (B) 191 ± 63,3 s, (C) 314 ± 88 s, (D) 434 ± 168 s (mean ± SD). Dependent on dosage the peak signal amplification in TCD was significantly different ($p < 0{,}001$; Friedman-Test): (A) 24.5 ± 2.0 dB; (B) 26.0 ± 1.6 dB; (C) 27.6 ± 2.2 dB; (D) 28.4 ± 2.2 dB (mean ± SD). Pharmacokinetic analysis during elimination of SonoVue™ (by linear regression analysis) revealed no dose related differences. Serious adverse events were not observed during the study.

Conclusions: This investigation describes the ultrasound enhancing potential of SonoVue™ in the intracranial cerebral circulation. SonoVue™ proved to be well tolerated and provided a long lasting ultrasound contrast enhancement that supports an optimal transcranial ultrasound diagnostic.

CHANGES IN RENAL BLOOD FLOW DEPICTED WITH CONTRAST ENHANCED HARMONIC IMAGING DURING ACUTE URINARY OBSTRUCTION

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1. Hôpital de Brabois, University of Nancy, France.
2. Children's Hospital, Harvard Medical School, Boston, MA.

Previous animal and human studies have shown that complete obstruction of the ureter is associated with a reduction in global renal blood flow by up to 45%. Despite initial promising results, several authors have reported a lack of sensitivity of renal Doppler sonography in showing the vasoconstrictive response of kidney to urinary obstruction.

The purpose of this study was to evaluate the use of contrast-enhanced grey-scale harmonic imaging and Doppler hemodynamics for evaluation of regional renal blood flow during acute urinary obstruction.

In 12 anesthetized piglets, the distal ureter was obstructed for 60 minutes using a balloon catheter, followed by IV furosemide injection. In 6 piglets, ureteral pressure was further elevated to mean arterial pressure by gravity saline infusion, and in 6 other piglets, ureteral obstruction was released. Serial harmonic grey-scale imaging was obtained before, during and after injection of Imagent (AFO150, Alliance Pharmaceutical Corp., San Diego, CA) at a dose of 0.1 mg/kg. The right kidney was imaged in situ using a curved transducer transmitting at 4.0 MHz and receiving at 8.0 MHz (Sequoia Systems, Acuson Corp., Mountain View, CA). Images were digitized and analyzed for changes in mean pixel intensity. A bolus injection curve was constructed by plotting mean pixel intensity versus time, and the area under this normalized curve was compared to renal blood flow. In addition, interlobar resistive index (RI) was measured and renal blood flow were determined using a radiolabeled microsphere technique, at baseline and during each experimental condition.

Results first showed that ureteral obstruction and high ureteral pressure reduced cortical renal blood flow to 88 % and 66 % of baseline values respectively, and that ureteral pressure and proximal diameter progressively increased during obstruction and subsequent diuresis. Contrast administration resulted in marked, homogeneous enhancement of renal cortex, with a mean cortical pixel enhancement from 67.8 intensity units before contrast injection, to 136.6 at peak contrast effect ($p<.0001$). Peak medullary enhancement was later and significantly less than peak cortical enhancement. Both peak cortical enhancement and the area under the curve diminished during acute ureteral obstruction, furosemide injection, and high ureteral pressures ($p<.0006$). There was a significant correlation between the area under the curve and mean cortical blood flow ($p<.0003$). RI

progressively increased with obstruction, furosemide, and high ureteral pressure, and decreased during release. RI correlated well with renal perfusion pressure, but correlated weakly with renal vascular resistance, and poorly with changes in renal blood flow.

In conclusion, contrast-enhanced harmonic imaging can depict changes in renal blood during acute urinary obstruction. Interlobar RI is a good predictor of renal perfusion pressure during acute obstruction but not of changes in renal blood flow.

EXAMPLE OF DMR (DIRECT MYOCARDIAL REVASCULARISATION)

F.J. ten Cate

Heart Centre, Rotterdam, The Netherlands

There are a number of patients with angina pectoris, who could not undergo PTCA or CABG and have many complaints using maximal medical treatment.

A new modality is making small (< 100 mm) holes from the endocardial side using specifically designed catheters. These catheters either use direct puncture by a needle or laser therapy. Although patients generally improve symptomatically, there are no data whether flow to the myocardium has really improved.

A case will be presented before and after DMR, using Levovist Power Doppler harmonic imaging showing improvement of perfusion.

Congres Location: Inntel Hotel
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- only three stations with the underground (Central station-Stadhuis-Churchillplein-Leuvehaven)
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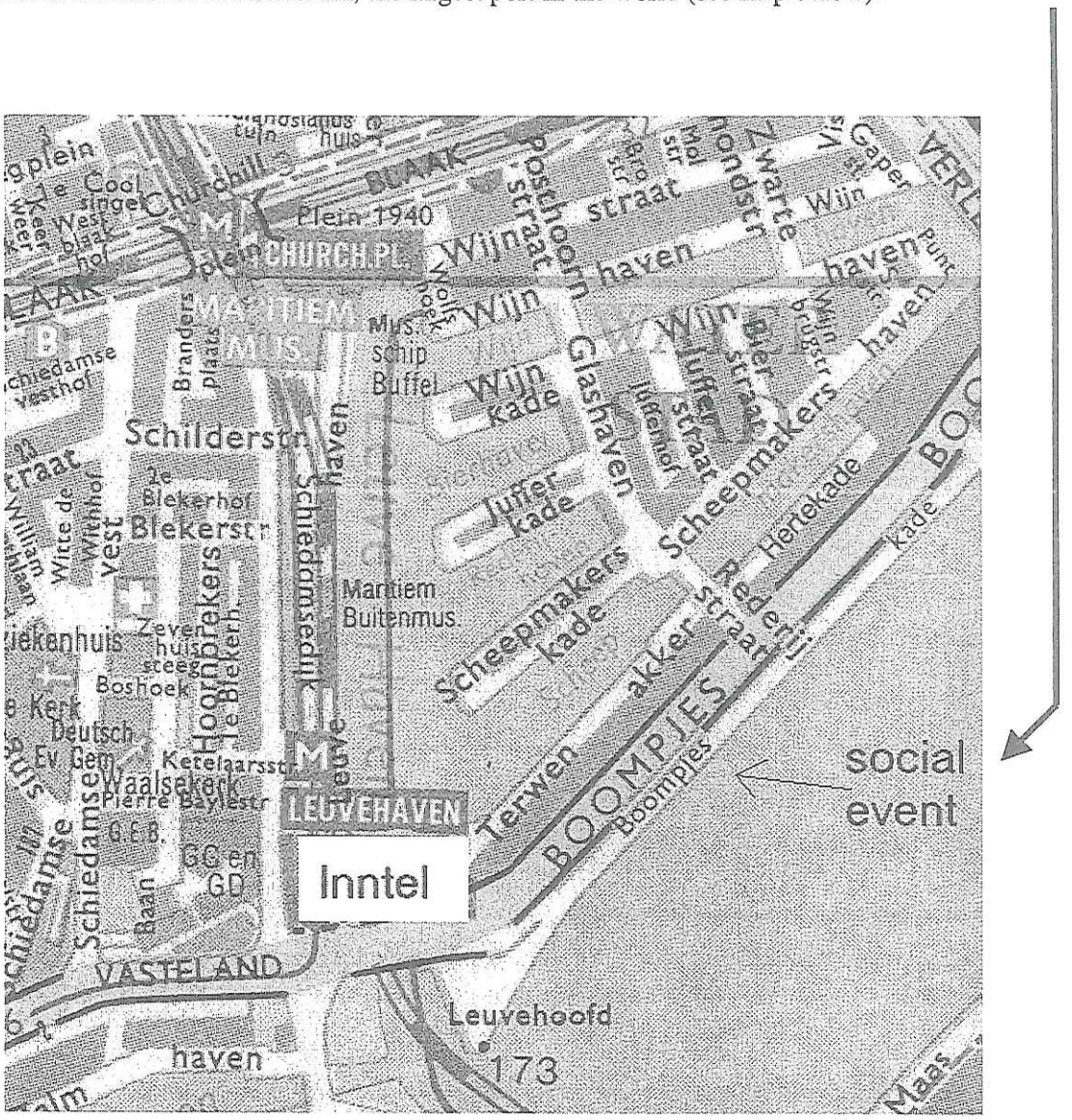
Bubbles on the River
Social event: Five minutes walk from the Hotel to ship "RIVERSTAR" for a Boat tour on the River Maas to the Harbor of Rotterdam, the largest port in the world (see map below).



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Rotterdam, January 1999

5 TH ULTRASOUND
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19, 20 AND 21 JANUARY 2000

During the Conference, Peter Frinking will defend his
Ph. D. thesis before a Committee of Erasmus University Rotterdam

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Question to the pharmaceutical companies

- Do we need further improvement of the machinery

Question to clinicians

- How to change current practise

- Where goes LIDDD.

- Quest to 2D-machine manufacturers

~~Why are~~

Is LICA imaging a MAin target within the company

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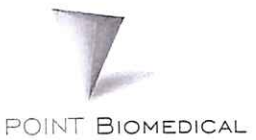

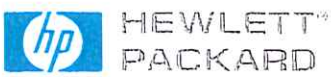

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