

Good morning, thank you for coming and... I appreciate the opportunity to be able to speak here or to be allowed to...I don't know if I'm able to, but you can decide that later, and thanks for being here on the last day. I see the number in the audience is quickly decreasing, but, if you have any questions during the talk, feel free to interrupt me. With so few people here that, I think that's just fine. I'm going to talk about ultrasound contrast agents, I assume that you know something about physics but otherwise, maybe a little bit about ultrasound but I'm explaining most of it, I hope and so maybe you get something out of this, maybe can take something away with you. The objectives are as listed as here, I wanta show how ultrasound contrast agents work, how they enhance ultrasound images and, what techniques are currently implemented on, ultrasound

scanners and explain you the physics of the agents so that you understand how they work and if, if you do something on a machine like you go away all the way up in the power and a contrast goes away that you know why that is. As a clinical background, actually I should have found this on the web page of MSM, they are, one of the manufacturers of ultrasound contrast agents. There are about 135 million ultrasound scans per year in the U.S. and only .5% of those actually use ultrasound contrast agents. One reason is that there not many, products that, are certified by the FDA yet and, currently they are all used in cardiology. Non-FDA approved contrast agent is actually, available for radiological applications. In, so contrast agents are pretty, together with ultrasound, pretty inexpensive technique to get very nice images, as I can hopefully show you. This

is, a movie supplied by, MRX, one of the manufacturers of ultrasound contrast agents, that's, a vessel/artery, there's a needle coming in, you see the red blood cells, through the needle you will see, ultrasound contrast agent coming in here. It's flowing through the blood stream, so, ultrasound contrast agents are vascular agents and you see it, it's going through the capillaries. He does his tissue. Those are ultrasound waves that are hitting the contrast agent and in this case actually it's... a therapeutic agent where when the agent is, exposed to ultrasound, it's rupturing the, the wall. Now, there can be two reasons, one is that this was a bio-effect or the other reason is it's therapy that you actually wanta do. This is a movie supplied by, Moriatsu from Japan. You see ultrasound contrast agent and yesterday... gosh I forgot his name, from, a gentleman

from GE was showing movies with ultrasound contrast agents and this is very similar. You see actually individual gas bubbles going, flowing through here and that's, that's, that's pretty, pretty nice, pretty new that you can see individual bubbles going through the capillaries. I'm trying to figure out how I can find this in time. Here we go. So, you see, and you see this flash going on, I will explain you later what, what actually happens here, but you see that after the flash it seems that vessels refill and you see individual bubbles. There are about six sections in my talk, I will explain to you again what new modes are out there. They're not really called ex-modes, I just made that up. Before a typical mode was a B-mode for brightness mode and the other ones have fancy names such as so I just replaced with bio-X. Then I tell you about the physical principles, is just basically

physical principles about ultrasound. Then, how those contrast agents are made, what they are composed of and some modeling. Then we go into imaging modes and I have some examples in there, so I will show you the mode, what the physics of it or how it works and a cartoon. Then I will show you some clinical pictures and last but not least, all bio-effects to show you what can go wrong and, how you can avoid it. The classical application's are b-mode and m-mode for those who don't know, b-mode is brightness mode, so you see those typical gray pictures where the, the gray scale indicates the intensity of the backscatter ultrasound and, backscatter ultrasound. M-mode is a streak mode, you look at the single line as a function of time, a vertical line in the ultrasound

image. Then there are various... applications to measure flow that are called spectral Doppler colorful and power Doppler. Spectral doppler is basically what you know from before you transfer me sending a tone burst, you listen for the, for the echo and see what the frequency shift is and that's proportional to the speed, except that you don't know, usually you don't know what the flow direction is. So, and the machines, they allow you to dial in a direction and you can align it with a vessel if you can, then you get accurate speed unless the vessel is perpendicular to the noise. In color flow, it's not the really Doppler. Color flow is actually just a phase shift for anything that is moving without even producing a doppler shift that sends, will show up in color flow. New modes are comprised of harmonic imaging, I'll just tell you the names here and then I'll tell you

what they actually are. Pulse inversion or harmonic, in harmonic modes and then something called microvascular imaging or flesh contrast. Now you see this is actually going off the screen and so it wasn't gonna preview that thanks to the software company. So how does it work? How do you enhance contrast? The definition, definition of contrast is that you have two different scenarios or two different tissues. What's the intensity difference relative to one of them, so if you put, if you introduce contrast agent into the body and now you have intensity I_2 as greater, then your contrast is defined as this when I_1 was the contrast before introduction of contrast agent. Now, the role of ultrasound contrast agents is to increase the backscatter amplitude and I'll show you why that actually works, and maybe, to create something that is different from what you send

in that you wouldn't get just with tissue and it has to do with frequencies. The physical principles behind ultrasound contrast agents are rooted and as I just said having bubbles in there, ultrasound contrast agents are all made out of bubbles, gas bubbles and their acoustic response to the, to the incoming field and it can respond basically in three different ways. They can show a greater amplitude, they can show a phase shift that you didn't see before or maybe generate frequencies that you didn't have before and as interesting, relationship that's the Minert frequency relationship and it allows you to select your parameters like the size of the gas bubbles that you have as ultrasound contrast agent and it will give you the frequency and we are lucky that like a three micron gas bubble has a one megahertz resonance frequency. We are lucky because blood

vessels and the capillaries are at max, six or eight micron and you want to operate in

megahertz frequencies in the human body. If you are lower, you don't have resolution, if you're much higher you don't have good penetration. So it just works out that having gas bubbles that are the size of red blood cells actually have resonance frequencies that are in the megahertz ultrasound range. If that wouldn't be the case, there wouldn't be ultrasound contrast agents or maybe a different type. Now, how does ultrasound work in general? You have an incoming wave and then you have a boundary layer. You have two different impedances, the impedance and acoustics is defined by the, acoust... I meant the mass density times the speed of sound and if there is a change in impedance as given here in the formula, you have a reflection that is proportional to the difference in the impedances then you have a reflection. If you have a perfect reflector here vacuum then

everything will be coming back, otherwise you have some transmission. Now ultrasound contrast agents are based partly on that in the sense that the surrounding medium is water and the, the, unit for impedance is rayl, and so the, the wal... impedance for water is 1.5 MRayl. The impedance for air is 0.3. So, you see that there is a pretty nice difference and based on, because of that difference you can actually see ultrasound contrast agents if they are made out of gas. Now, ultrasound contrast agents are basically, oscillator. You have an incoming wave which is an oscillating elastic wave, a compressional wave and in that wave field ultrasound contrast agents oscillate as was depicted by this sphere here and they oscillate and that oscillation is described or governed by the pressures, cuz you have a gas bubble and the gas bubble can become smaller or become larger, depending on

what the pressure field is, depending on also what surface tension is, so at the low pulse pressure or the internal vapor pressure is, what the static pressure is, and all those variables go into equation that comes later, sorry. Ultrasound contrast agents are dominated or are described as **Rayl** scatterers and that is because, if you think of the wavelength and the object size, if you have a one megahertz acoustic wave in the body, it's about 1.5 millimeters wavelength. Compared to ultrasound scatterer, like a gas bubble of three micron, that's huge. So it's kind of interesting that you actually still see it and how well you can see it and that's because ultrasound contrast agents are, great scatterers and that's because you have this monopoles term in here where you have the difference in compressibility and you see that this, that's a pretty large number and water

is virtually incompressible and air, it's pretty much compressible, so this makes a great scatterer as the monopoles scatter and with a frequency dependence that is discarded by **Rayl**. Now, there's one other thing that I wanted to tell you, it's not necessarily important for ultrasound contrast agents but for gas bubbles. If you take those gas bubbles and you have them in a strong acoustic field, you can produce temperatures that are in, the excess, at least ten thousand, but, I guess that's one million. You can have a million degrees inside of gas bubbles and inside of a gas bubble. So, you can actually do sonoluminescence and people have studied this for a long, long time and newer models are there now and they show that it's actually about a million degrees Celsius and people propose to use this for chemical reactions. The nice thing is, it was, it was first

discovered in Germany which I'm proud of and but they thought, it's not very interesting

and they dropped it and then Larry Crumb in, now in Seattle did a lot of work on sonoluminescence and related activities. Yeah! **(question or comment) Question: There recently was a lot of enthusiasm about the possibility of nuclear reaction. Is it still alive?** Uh huh. Uh, huh. It's still alive, but... I thought... I haven't seen a major energy source...but yeah, people are doing research on this because you have those insane temperatures and you think you could do, you could use them to do diffusion. Now, modern contrast agents are, **are** let me start where, what, what very old contrast agent is and that is I think Gramiak and, shaw or sham... I forgot, I'm sorry. They saw the first time ultrasound contrast enhancement I think in '68. What they did was, they had a

catheter and arterial catheter in the body and injected saline and they did ultrasound and injected saline and they saw that when they injected saline really rapidly, that they saw enhancement in the vascular system. Now, what they saw was cavitation because if you inject a fluid through a catheter and it comes out of catheter tip it sees a pressure drop and because of that pressure drop you can produce small gas bubbles and those gas bubbles were lightening up on the ultrasound and that, that was the first ultrasound contrast agent. The problem with that was that it was just gas that was released... stored in a liquid and it would immediately dissolve again so you had a contrast enhancement for a very short time, like a couple of seconds. But that's, that's basically what you need, just a gas bubble. Now, to make modern contrast agents as in the slide here, I don't know if you

can see this, this is ten micron, the bar down here. You have contrast agent that I have a sophisticated shell and complex interior gases. A sophisticated shell is as it says on the slide, they're to reduce the diffusion of the interior gas to the liquid to the blood and examples are albumin or lipid based and you can also have targets on that shell. So some people actually... claim or show that they have ultrasound contrast agents that attach to certain types of cancer. That would be great, because you inject the contrast agent, you do ultrasound, you see where they accumulate and you say that's my, my site. The interior gas is defined by, or a good one, is defined by having low diffusion and low solubility and that makes sense, because if it's, if it diffuses low then it stays inside, you have your contrast agent for a long time and if the solubility is low, then you build up a

gradient very quick...or no you get rid of the gradient and you have saturation on the local fluid layout on your bubble and your bubble is more stable and one example is for fluorocarbons which are very popular lately as interior gases. This is a selection of ultrasound contrast agents. Here is the manufacturer, how what they are called, they are the interior gases and you see a lot of them, say, perfluoro, perfluoro, perfluoro, butane, pentane, hexane, you name it. Then shell materials are typically, lipid based, some phospho lipids, some albumin and in the last column I showed what or I showed what the statuses of them and some of them are approved in the U.S. but only a very few, but a lot of them are in clinical trials and then there's also a big difference, I put this asterisks on status, it's, it depends on the proposed application. As I said before, they're non-

approved for radiological purposes, only for cardiology. So even if, if, tw...three are

actually approved, they're only approved for cardiology. Now come back to the physical and, uh, mathematical modeling, I said it's, it's, a gas bubble and there are pressures and with that you can write an equation of motion and look for example it's sample at the harmonic response. A very basic and very often used, equation is the **(15:35)** equation. It's a very simple equation; even so, if you first look at it, it's not that simple, but otherwise it wouldn't be fun, if it would be too simple. Now, you have a surface tension in here, you have viscosity in here, so if the bubble is oscillating and the risk is medium of course, that should influence the, the motion. You have your static pressure in here, you're, you're Ambient static pressure, you have your sound pressure, you have a vapor pressure, you have the restoring force that's the, the internal gas pressure as it was at

equilibrium and you have some inertial terms. That's it. They...this is all assuming ideal gas law. If you plug it in, I like using mathematic, uh, which you might recognize from, from those pictures. If you plug that in and you let it run, you solve the differential equation, you see it, but nothing goes on, which this is a three-micron gas bubble as a function of time. That's good, that means your boundary conditions are right and the bubble is just resting in the liquid. There are no diffusion terms in these equations and the bubble should just be stable. Now, if you play a few tricks, if you change the boundary condition and you say the bubble is larger than it should be at the beginning, like you stretch it and you let go, you see that this thing oscillates like a rubber band, same is true if you, if you compress it. Now, if you, if you do acoustic excitation of this

which is actually the interesting thing, if you use ultrasound and you hit the bubble with the single pulse as depicted here, just one cycle, then you see that the bubble starts oscillating and it rings down and of course this ring down is described by or dominated by the Ambient viscosity. If you do a con...continuous wave excitation or a long burst you will see that the bubble start oscillating too, it's going through some transient initial response and then it steady stayed and those initial responses are typically four or five cycles. Yeah. **(comment) Audience: Obviously this is very, very slightly forced.**

Speaker: Yeah. (continues) Audience: You must raise the (inaudible (17:38) slightly. What is realistic to really get you to ten thousand degree temperatures? Is that normal? Speaker: The ten thousand degrees are actually, when you basically

collapse the bubble **(continued comment) Audience: Inaudible (17:51) Speaker: and (continues) Audience: But it's not part of the resident process? Speaker: you...you, well, yes and no because, no because it's not something that is ongoing, but yes because obviously you want a bubble at it's present infrequency to have maximum transfer of air acoustic energy into the bubble, but at some point the inertial forces that were in the very placid equation are becoming so dominant that the bubble once it's under compressional phase is collapsing under the force of the water, Ok? And that's something that you typically would not have in the clinical application. The sound pressures are too low for that. (comment) Audience: When it collapses, what happens to the gas? Speaker: It dissolves in the, in the, in the liquid. (comment) Audience: It**

just continues? Speaker: I don't want to use that word, but, yeah. I mean nothing is

instantaneously, but more or less. Yeah. Now, what else do I have here? I have different, I show different excitations, this is 0.5 kilopascal, so that's, that's very little. It's less than, a percent radius change. You see that the bubbles are non-linear. Ok? This is the fundamental frequency at one megahertz, that's the excitation and you see there's a peak at two megahertz. Those bubbles are all non-linear. There's no threshold for it, there's a threshold for detecting it, but they're always non-linear and the reason why that is, is actually very easy to understand, if you think of a harmonic oscillator usually has a constant mass, ok? You have, you have a weight and it's bouncing back and forth on a string, that's a, that's a very simple oscillator. Those bubbles don't do that

because the oscillating mass is the mass of water that is oscillating with them at a given point in time. Well, that mass changes all the time, cuz if the bubble is really big, then a radius change in ΔR is more water than if the bubble is really small and that's the reason that the bubbles are non-linear and of course if you drive em harder, then it's easier to see the second harmonic and higher harmonics. I'm dri...I'm exciting it more at five kilopascals and fifty kilopascals and we see how those harmonics come up and they are beautiful because they allow you to detect frequencies that should not occur otherwise. So you transmit at F_0 and you, you receive your backscatter signal that has F_0 and $2F_0$ and you say well, that's because of bubbles. That's exciting, it's not the, the whole story because tissue does something similar. Almost everything in nature

becomes non-linear at some point, I assume. **(comment) Audience: So, you're saying lets just use the video, simple basic physicist equation on mega (inaudible 20:26)**

Speaker: Yeah, yeah. Well, I don't use that equation, I use, uh, did I confuse the power point, nah, that's, that's the equation that I use. **(continues) Audience: However your (inaudible 20:38) Speaker:** Oh, yeah, yeah, sure, yeah, yeah, yeah, yeah. Absolutely. Oops, that was too fast. Yeah, at the, at the hundred kilopascals which is pretty high for a free gas bubble, this is all for free gas bubbles, this has no shell, no special gas, it's just ideal gas and showing you what the basic physics are of, of the bubbles. You see that if you drive it really hard, you have higher harmonics but you see the other peaks coming up and that is because, and I have a slide later... the bubble becomes really non-linear

and it becomes chaotic too. Let me see where the...oh, and, and if it's chaotic, you know you have period, doppling, period, doppling corresponds to sub-harmonic frequencies. Now you have sub-harmonics and higher harmonics and they are all mixing. So, you, you have the sub-harmonics and then you have ultra-harmonics, which are multiples of sub-harmonics, so you basically get the whole frequency spectrum at some point. Umm, yeah, so as I said before, this was all for a free bubble. For a shell bubble, one of the, the models and I didn't reference him, Charlie Church, it's, he, he, did a lot of work in, in shell bubbles and that's his equation. He modeled elastic layer around the gas bubble and he described how, umm, ultrasound contrast agents in, spe...specifically albu...what is it? Alunex can be described by this equation and he puts something in like a viscosity

in the, in the shell and a compass ability of the, of the, or elastic module is off the shell

material. Then you have two surface tensions because you have, a outer surface and then you have your shell, so you have a inner surface again, but actually it's a pretty small contributions and everything else is pretty much the same. Now if you do that, you see that you have the same response but you need to hit the bubble harder because, because of the shell right now, the bubble does not want to oscillate as much as freely as a free gas bubble, but it's more stable in the blood stream and that's the reason that you need to use it and you see that you have harmonics of those bubbles too. Now, this is a little cartoon, a little movie, you see this is the acoustic excitation. This is the bubble response and the bubble starts oscillating in the field and we see how non-linear this is because the bubble in the compressional phase is very fast. It doesn't want to stay compressed and that's producing all the, the higher harmonics in, in, in the scattered field and it's, that's a

free gas bubble fairly high... some pressure. This is what I was talking about before. You have chaotic oscillations in gas bubbles you're probably familiar with this type of plot that you have a system and you have a parameter and if you increase this parameter then you have bifurcates points and this becomes pretty much chaotic and this is, this is for a gas bubble, this is from Andrea **Prosperetti** who did a great deal of work in, in, modeling, gas bubbles in acoustic fields. So this is radius of the gas bubble and then if you drive it hard enough you see that at some point the bubble starts oscillating and it, it bifurcates in two, so this is your first period doubling here and then it keeps going from there, but in, in, a lot of applications you would probably not see this because you destroyed a bubble. You need to be in very good ideal circumstances to actually keep

that bubble going. Now, it's, I come to the imaging modes, which is probably ok, we're halfway through. The imaging modes listed here again are a whole bunch, but they're all based on non-linearities. oh,yeah, it's, it's good to have large backscatter, it's important if you're in the, in the capillaries because you have only very, very few bubbles and the larger the backscatter of the single bubble the less bubbles you need in you volume in your, in your, what is it called? In your focals to actually see a single one and see where the vasculature is. That's what ultrasound contrast agents do. They do not en...just enhance tissue. They don't enhance tissue at all. The only thing that they show is where they are and they are where the blood is. If you have, if you have leakage, sure, then they get closer to the tissue, but otherwise they are only in the blood stream. They are in the

heart, they are in the liver, they are in, in, in the liver and, and a lot of places, it's because blood goes through a lot places, but they are only in the blood stream. And on ultrasound scanners, you see the mechanical index and actually Christy **Howen Holland** and Robert **Apfel, Apfel**, developed or introduced this quantity, it's a measure for the, the intensity of the cousti...yeah of the acoustic field in, in situ that, uh, tells you if there is a likelihood for cavitation or not. Because that is one of the bio-effects of ultrasound. You can cavitate liquid, you can cavitate the blood or tissue in the body and you wanta avoid that. You don't wanta create any free radicals even though it has been shown that it's actually beneficial for cancer treatment, but if you wanta do diagnostics anywhere and having any one of those. So the mechanical index is defined as the peak negative

pressure in megapascals divided by the square root of the frequency in megahertz and

then on machines you see that the FDA limited the MI to avoid cavitation and limiting, Doppler limit as 1.9. Now, there is a kind of a lower limit or, it's a...the lower limit is interesting too of course the lower limit is 0, but the lower range is interesting because if you scan ultrasound contrast agents you wanta be very low, cuz if you're too high, you will actually rupture the bubbles and I hear a little bit of feedback here from the loudspeaker, maybe hit it even more. Yeah, **(comment) Audience: Is there anytime in physical meaning when you get to the MI? I know you have a subsequent (inaudible 26:52) Speaker:** It's telling us when, when the liquid fails. In **(?) Holland and Apfel** saw that if you apply acoustic field to a liquid, the liquid will fail at some point, it will

just rupture the liquid. You will pull the molecules apart, ok? Ultrasound can be very, very intense. I mean you can use it for industrial applications to tear things apart...well actually, in medicine you do it. If you have lithotripsy, right? And, at some other meetings, you see, it talks about how to get rid of gall stones and it's amazing, I mean it's like sandblast...blasting and that's invivo. Now, there is a frequency dependance to that and that frequency dependance is described as in this equation. So, if you're at different frequencies, if you're at one megaher...or they say you're at a hundred kilohertz or you're at ten megahertz, you will have a different threshold for rupturing or that the liquid fails. In that threshold, it would be the peak negative pressure cuz the compressional phase doesn't do anything, it's the peak negative...it's the refractional

pressure. **(comment) Audience: So does the, does the tearing apart pressure increase with it's proven frequency? Speaker:** At higher frequencies, well, yeah...yeah, yeah right, right. So, if the MI is constant, then and you are at the, the threshold, then this formula tells you what the frequency would be. Yeah. **(comment) Audience: And the MI tells you how close you are to the rupture sutures. Speaker:** Yeah. Ok, this is the first, mode, harmonic imaging and I have, as I have shown, a few slides ago, bubbles have a non-linear response, so tissue in general, I'll tell, I will tell you right now, tissue is not linear, but tissue is more linear than gas bubbles are. So, I'd say in the first approximation for relatively low sound pressures, tissue is linear, you send in frequency at...of one and that's what you get back, but if you have a gas bubble, or actually I say

non-linear responsive tissue, if, if you, if you rev up the pressure, you see that you have a non-linear responsive tissue too. You have to be over eight parameter for different tissues and it tells you how much the contribution is in the second harmonic. Now, gas bubbles do that too, but gas bubbles have a larger backscatter at the, the second harmonic. So if you threshold your image, you can still see the bubbles better than the tissue, but that depends on the concentration of the bubbles. Now, if you, if you're in the capillaries and you have only a couple of bubbles in your volume in you, in your focus, then you might not see em or they're not, you might not be able to distinguish them from the surrounding tissue. In the same slides as before, you have the, the, the sound pressure that hits the bubble, you have the radial response and I apologize that I didn't describe

this more than I didn't point out what those axis' are, but it's, it's labeled, so hopefully

you saw it. This is the incoming wave at one megahertz and you see that the bubble response is pretty bright and at higher harmonics. This is a slide that shows you how them what is the, this is the mechanical index now and this is the, the pressure at the fundamental response and at the second harmonic response and you see it's always there. It just becomes very small, but you also see that at the MI of point one or close to that, the second harmonic has its maximum because at that point, you actually have a whole bunch of other harmonics that are coming up third and fourth and fifth and so on that you don't see it because they get attenuated in the tissue so you can't really use em much, but they exist and then later you also see, and that's not in the simulation, but in, in experiments you see that the bubble bursts. OK. The, the next imaging mode would be coded excitation which was described actually, I have too many, I, I'm so sorry, that I

forgot his name, he, he gave the talk yesterday about, vascular imaging and he described coded excitation. It's basically a way of overcoming attenuation and it's kind of easy to understand hopefully after you saw those slides if you don't know it already. Umm, coded to excitation it's the opposite of sending in a single pulse. This is now time, before it was frequency, now it's time. Sending in a single pulse is nice, it gives you great spacial resolution. The shorter the pulse the better spacial resolution, but a single pulse can get buried in tissue, in, in, in the noise. It's better to send in a whole pulse train and maybe have different amplitudes in there or this is a coded pulse train, or this here would just be a longer tone burst and harmonic coded excitation would be the same thing, but now we are back in the frequency space, that, sending something in that has some

amplitude modulation but also some, frequency some, some kind of band width and that way you can make use of the bubbles second harmonic response and you can have a mixture of having a non-linear contribution and a unique fingerprint, acoustic fingerprint that you send in and it was the example I was waiting for myself. This is the single pulse, this is a lot of noise, this is eight pulses and the same amount of noise and of course, I mean if you see it down here, you say ah, well here's you, you're pulse, but if you just see this plot by itself, it's not as easy to see that there is actually a single pulse in there, as down here and that's what coded excitation is. It gets a little bit more tricky and I explain that in the next two slides. There are two types or at least two types of coded excitation, one is a chirp that's just changing your frequencies, so you see, you started low

frequency, maybe four and a half kilohertz and you go up to, one point four megahertz and you sent that in and people have shown that you can get twelve DB or four times increase in backscatter signal or in detectability. Another type of coded excitation is, uh, Barker codes or, (?) **Golay** codes and they sent in the same frequency, well, I can't say the same frequency, cuz if you look at this, they are basically two, frequencies in here and they make use of some technology that has been know for a long time in radar and as almost everything in ultrasound, or medical ultrasound, a lot of it comes from radar, because both of them are coherent imaging modalities and a lot of it was already, was developed by, for radar cuz of military purposes or civilian aviation and it's transferred to ultrasound and it works very well. Yeah. **(comment) Audience: Is coded pulse train**

applicable both to the standard oversounding and a contrast or are we talking

specifically like contrast command? Speaker: I, I'll tell you, I would say it's both depending on what type of code you use, cuz if you have a linear scatterer and use a code that is based on a non-linear response, it will not work and that's, that's the example that I have down here. Yeah, this, this is, this is basically uncoded, how those, the, the, the **(?) Barker and Golay** codes work is that you send in a, a tone burst, certain length tone bursts and then you have a matched filter and you filter your ink, your backscattered signal and you have point spreaded function or a axial focals that is relatively narrow. It's not necessarily as long as your tone burst because you have that matched filter, but if you play some games and you allow two transmits. So if you look on one line and you

say what's, what's my, what's my echo genicity down here and you have the luxury of firing twice. You can fire two different types of codes, you can fire this code and this code, have the matched filter, have, compute the result and subtract them from each other and if you do that, you have a relatively narrow response or impulse response. So you can have longer bursts to gain, gain signal to noise and can still retain a, a appreciable amount of, axial resolution. And that is then, there's another example, this is stolen from, **(?) Novicky** in, in ultrasonics just last year and these are, these are actual sequences and you see the same thing there. You have the, the, the, the filter, you add em up and it doesn't matter if you add em or subtract em. It just depends on the phase that you send in originally and you get a very nice, uh, temporal response. I might be running close on

time, here. These are example that, for those, for the coded excitation that you still have, this is, chirp and, and a single pulse, this is for chirps this is, from **(?) Nico De Jong** 2004, some current literature that you have actually increased in, in the spectral response and you also see that, do I have the simulator there too, now, they also show that you have a pretty good spacial resolution. Now these are two images, clinical images and in, in the gall bladder and you see that there is a improvement in, penetration, using those codes and they can be directly used with ultrasound contrast agents even so they didn't, so that answers your question too. Depending on what type of code you use, you can use it in regular B-mode imaging too. Now, the next example is pulse inversion, which is actually pretty simple. If you have a linear response and you send in a wave that goes

this way and then you send in a wave that is a hundred degrees out of phase and you add em up, you end up with zero. So, that's how people propose to suppress tissue. If you're at low enough amplitude, you do this everything will be black on the machine, on the screen except ultrasound contrast agents because, and, this is not a very nice way of showing it, but I basically set the first one as, as a normal response which is not necessarily the case, but I wanta point out that there is a difference between, a positive phase first and a negative phase and then you get some finite response and I, I, simulated it with the ___ equation and there are two pulses in the outer phase on the eighty degrees. In the linear system, or, no, no, in at, at, at low sound pressures where the bubble is more linear you see that there is very small, a very small response or very small

difference in the backscatter but if you're at fifty times the sound pressure which is still

kind of low, you get a finite response and they're the same scale and this is the, the, the frequency distribution and in the backscatter signal, so you see that in, in, in the signal here which we just had there is, there are higher harmonics in there, so you could actually do a pulse inversion and harmonics at the same time and this is... example from, that I got from, (?) **Mike Aveque 38:23** in ultrasound quality and this is, both, both images use ultrasound contrast agents as it says here, this is regular B-mode imaging and this is pulse inversion imaging and you see how the ultrasound contrast agent has, is much brighter because it's more non-linear than tissue is. There is still tissue, but you, but that can be useful too, but because the, the radiologist wants to see the tissue and the contrast obviously, the contrast agent. Now power pulse inversion is a very nice way of, how should I say, of suppressing tissue that moves because if you do pulse inversion and you

have tissue that is moving, then that will give you a phase shift and that phase shift will not cancel the signal perfectly and so you will have a finite amplitude backscatter even from something that is linear. Let's assume you have you have a breathing motion and you move two centimeters per second and these are the other parameters, you would have a phase shift of about five degrees and as simulated as here having zero, five and ten degrees. If you take the zero degree, though that's the initial pulse and you take the, the pulse that was sent at the third position and you, you add em up. You get an average signal and that average signal should be the same as at five degrees. If you take the five degree signal and you subtract it from the average of zero and ten degrees, you, you get something that should be zero and that's how you cancel no...that's how you cancel

linear motion, ok? Now, if your backscatter signal is not, uh, from a linear scatterer that just moves in a linear fashion, then this would not cancel. So with power pulse inversion you'd see non-linear objects and you suppress linear objects that move linearly. This is again, how it, how it works, just as equation and these are the simulated... pressures and you see that it cancels for tissue and for a non-linear bubble it doesn't. These are examples clinical images and you see the enhancement. It's very nice. The next modality is called flash echo imaging and if you have a bubble in acoustic field, they oscillate, but if you have a field that is too strong, then...then the bubble bursts and that can be very useful because if you have contrast all over the place, you might not be able to see what you want to see and that is wh...what the flow actually is, where, which vessel

is feeding which region for example and so it would be good to actually turn the contrast off, turn it on again, turn it off, turn it on again, whenever you want to and with flash echo imaging you can do that. If you, if you increase, the acoustic field that you ruptured the bubble, everything in your scan plane is gone. Then if you, if you go down again with your, with your pressure, you allow the bubbles to flow into your plane again in your, in your field of view and you can image them and you can see them coming in. That's the beauty of ultrasound, being a real time imaging modality. Now, umm, umm, uh Kathy (? **41:59**) is a (? **41:59**) a part of her group did a whole bunch of work on the destruction of ultrasound contrast agents, this is published in Applied Physics letters and those movies are taken or just those pictures are taken with, uh, very nice camera. (?) **six**

Imacon 486, that's, a solid state camera that can take, a hundred million pictures a

second. The army uses that a lot, to monitor, missiles. They fly pretty fast, I didn't quite need a hundred million, but if, well, if you want to see how a missile impact, ok, that, that's a very, very short time range and that's where you need lots of temporal resolution, but the same is true in, with ultrasound contrast agents. If you have a five-micron bubble and you expose it to a very large sound pressure, you see that that bubble actually is ruptured...torn apart, ok? Down here, it is the B-mode or the streak mode, so you take the center line here of, of an image and you plot that as a function of time and then you see that at first, the bubble is at rest, that's this frame, and then the bubble goes through violent oscillations and at some point it's just torn apart and then it disintegrates, because

the bubble is so small here, and this is a different, uh, umm, magnification. Bubbles so small that the Laplace pressure dominates, cuz it's **(?)two sigma over R** so if R is very small then the pressure is so large that the, the gas just diffuses in the liquid. So, you, you, can with a single pulse, you can destroy all your contrast agents. Obviously that depends on the sound pressure, but I actually I've seen in observations that even...even if your pressure's a little lower, you hit it twice or three or four or five times and then your contrast agent is gone. This is a clinical example ultra from Ever Q, it's showing a cardiac application, it's, to show the cardiac perfusion and this is actually not approved by the FDA yet. I, I said cardiac is approved but it's only to outline the border in the heart, it's not to measure perfusion, but Ever Q shows here that it actually works, here is

the heart, this is the, the heart chamber, it's full of blood, a lot of blood, so it, there's a lot of contrast agent, that's the reason that it's so bright and then you have the heart muscle around here like upside down u and you see, you see some contrast in there, but much less because the number density of gas bubbles is much less. Then you increase the mechanical index to destruct all of the ultrasound contrast agent, but there are a couple of reasons that this is so bright; one is that if you liberate all those gas bubbles from their shells, then they're free gas bubbles and I've shown you before that if you have a free gas bubble, it likes to oscillate a lot more than a contrast agent. It's not as stable though, but that's why we actually want to, we want to destroy em. The other reason is that the machine might actually have a lack in compensating the received gain, so if you ram up

the acoustic pressure, the machine actually does it by itself in this mode. The received gain might not change and therefore you're overcompensating and you are basically flashing the display in all the, the, I forgot what I want to say. Now, right after that flash, all your contrast agent is gone in the heart muscle. It's there in the, in the chamber, but that's because in the, in the chamber of the heart, blood moves in and out very quickly, ok. It's one of the fastest places where you have, flow, but in the muscle you don't have ultrasound contrast agent yet because it needs to get there, ok, and that, that's what you see on the, on them on the, refill curve. This is the contrast amplitude is a function of time and so you're, right after the flash there's no contrast agent, that's what you see here and then sometime afterwards, you see that it comes back and so here you can basically

measure the perfusion in that tissue. You see how well, how well is your heart muscle

profuse and you can do this for every region in here. That's, beautiful movie from, Moriatsu from Japan, showing flash echo imaging and look at this, how the vessels fill in. So, here you have your major vessels coming in major arteries and then afterwards you see the contrast agent, appearing in all the capillaries. So, this was the flash and I'll see how it comes back. Yeah. (comment) **Audience: Because of fresh blood coming in? Speaker: Yeah. (continuing)** **Audience: Because of bubbles reforming? Speaker:** No, they're not reforming, they are basically shipped in with the blood. So you, you, you're basically destroying all the gas bubbles in your, in your field of view and it's empty. Blood is there but the gas bubbles are all gone or at least you don't see them. They could still exist. The frequency response of gas bubbles is pretty sharp. So, if they

are too large or too small, you don't see them and so, you need to wait until new bubbles come in that are of the appropriate size or at least existent or not at least but and existent. I'll quickly tell you what micro-vascular imaging is. It's, very similar to what I have shown you before, it's just that people start combining things. So the problem in the capillaries is that you have slow velocity. So, if you do Doppler, you don't have much of a Doppler shift. You have a few bubbles because, the capillaries are very small, however there are a lot of em, but still, depending on where you are in the body you don't have many capillaries. You have a lot more capillaries in your fingers than you have in the remainder of your hand. Then you have large scatter from tissues, you can have tendons, bones, other structures that might scatter much more than the bubbles do. The solution is

to combine techniques, to combine Doppler with harmonic response and maybe do some thresholding. Now, if you think of Doppler, if you, if you do Doppler on a one megahertz signal and you do Doppler on a two megahertz signal, we will see, twice the phase shift on the two megahertz signal and that's what people use in combining those. If you insonify a gas bubble that is a harmonic oscillator and scatterer and you do a Doppler shift on there, you will see a signal that is twice the phase shift in the second harmonic relative to the first harmonic and if you sample at the right frequency, you will have a phase wrap and that twice phase shift will actually show up at zero, ok, I'll show you this in this picture here. If you samp...if you do your Doppler frequency and you're sampling in the right way, then you can have ultrasound contrast agent showing a

frequency shift at half your sampling frequency, ok, which is the maximum that you detect, but because those bubbles are non-linear they actually get phase wrapped back to zero and now your harmonic agent, not your fundamental agent, your linear response, but your second harmonic response, is showing up at zero, ok, that's how we differentiate moving bubbles from moving other things, ok. Now, if you also apply a threshold on there, then you can separate the harmonic agent from the harmonic tissue and that's how people pro...prod...uh, propose to actually image agents in the micro-vasculature and this, this is a example, this is, the Doppler frequency and this is the, the, the, the, the, RF of your original carrier and you see that your harmonic flow shows up at zero with a phase shift because it's moving, but tissue is not moving, so it's over here, so, if you, if

you, you do some filtering on here, you will see only your bubbles, but you're suppressing the fundamental, your suppressing, in, in, in, for flowing objects and for not, for, for stationary objects and you get only your bubbles that move. Now there are some more in vivo examples, I'm almost up with the time, what time is it, yeah. This is also from Moriatsu and you see how it builds up the amount of contrast and there's the flash, ultra flash echo imaging and you see that it's, it's not just flash echoing imaging, it's flash ech...echo with a max hold afterwards, so where ever there was a gas bubble before, it's showing up for a long time and you see that there is a tumor that is not profuse. There is another example, they see all those traces of the gas bubbles. It's very

nice. Let's jump ahead a little bit here, cuz I don't wanta go over in time. Last chapter, everything is flashing, bio-effect it's the important part. If you wanta do this just in the lab, almost who cares, but if you wanta do this on patient's everybody cares a lot. Cavitation is the most important part. You have inertial cavitation, which is also called transient cavitation. That's when you have a collapsing bubble, say if the bubble in the acoustic field, the acoustic field is too strong, the bubble collapses, you have cavitation and I, I show you later what, what it actually, what happens. In, in, in, clean water, you have a theoretical threshold for cavitation or inertial cavitation, it's a hundred megapascals, will not have that in the body, ever, ever, but in the body you have, dirt or you have particles that might trap gases and those gas pockets here are sources for

cavitation. So, if you have acoustic field and one of those gas pockets is around, the gas pocket might actually grow if it's beyond the so-called blank threshold and then you would have a bubble and that bubble could cavitate. This, actu...this, this, this, cartoon depicts a kind of the, one of the first ultrasound contrast agents called ligovist and I might actually say, yeah, it's, this is the mod...modem, I don't know, model for Levovist, you can, it's a, it's a, a sugar-based particle and it has air trapped in it and that air actually is visible with ultrasound and that's why it's a contrast agent. Now this is the collapsing bubble, this is a famous picture by Larry Crumb, it's a awesome picture. This is a bubble that collapses and it's, umm, uh, creating a jet. You see this inside column? This bubble is collapsing and then there is a jet going this way into the mid-surface and the other way,

ok, and that's what you have on let's say ship propellers cavitation in, in, in a marine application. Ship propellers all have, it's, it's called pitches or, I don't know what it's called, little indents on it, ok, that's because the propeller is moving away from the water all the time and that's creating a under-pressure and that's creating gas bubbles, and those gas bubbles collapse and when they collapse they create a jet and that jet is taking off material out of the metal and so the same thing can happen in the body, you can have ultrasound contrast agent in the large acoustic pressure field next to a cell, it collapses, it has a, it creates a jet, that jet is rupturing the cell. That's a big bio-effect. Now, if that is only one cell out of your, I don't know, ten to the whatever cells we have, it's not a big deal and that's what, what I show now, umm, there is, umm, I apologize for this, this was

not, I, I, haven't see this before, uh, by Pang Lee and Doc Miller and they were looking in, in, uh, Armstrong, they were looking at, bio-effects in vivo and they saw that there are three types of bio-effects. Micro-vascular permanization, which I just said that you

rupture a cell, then bleeding and pre-ventricular contractions which I might get if I don't finish soon. Now, they tested three ultrasound contrast agents that are approved. Optison, Definity and imaging and that's where all the bio-effects are, as here, if you change the acoustic pressure, and you look at, what kind of how, how much bleeding you can count in, in a tissue slide, or size of tissue, you see that if you increase the rare fractional pressure then, you have more bio-effect. That's, that makes sense, because you give more bubbles the opportunity to actually cause a bio-effect and this is an, an, I, the,

the, the pressure, because of the frequency works out to be very close to the mechanical index, but if you're at the upper end of the mechanical index then you will have more bio-effect. If this is significant or not is a completely different story. If this is something that is very localized and is in conjunction with a therapeutic treatment or you need to do this diagnostic procedure it doesn't matter, but it's something to think about, that you should not have your acoustic amplitude all the way up for two reasons. You will destroy the agent, ok, if you want that then it's a positive thing, but otherwise you destroy the agent and you cause bio-effect. If you can get the same image quality at the lower acoustic output that would be your choice and here is, what is that, the pre-ventricular contractions, same thing. If you are at a, MI of less than 0.8, then there are no

observations, ok, and then there are differences in contrast agents. Actually, if you, as you see here, it looked like that Optison performed the best in terms of this is, not a agent dose plotted. I'm almost done, this is second to last slide. If you do a particular count, you see that, if you have a very low dose, you have no bio-effects. If you have a high dose, you have more bio-effects. It makes sense too, because again you give more bubbles the opportunity to actually do something and this is the same for pre-ventricular contractions. These are my, acknowledgements and references, as you have seen, this is, this is not all my research. I copied a lot from the literature and that's outlined here and I got the beautiful movies from, Moriatsu in Japan and, I appreciate that you listened so patiently and are still with me even though I'm a little bit over and if you have any questions, I'm happy to address them. Thank you!