Biomedical Ultrasound: Fundamentals of Imaging and Micromachined Transducers

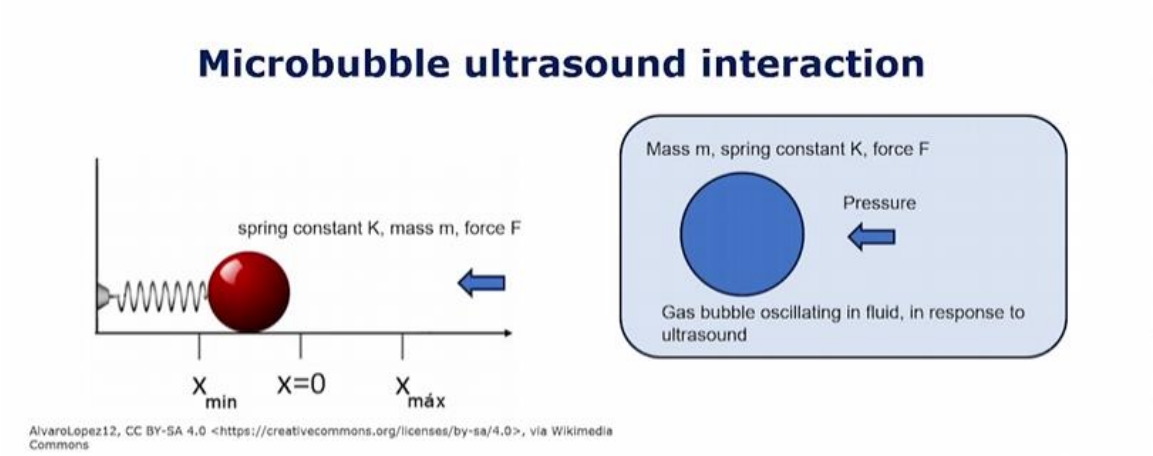
Prof. Karla P. Mercado-Shekhar, Prof. Himanshu Shekhar, Prof. Hardik Jeetendra Pandya

IIT Gandhinagar, IISc Bangalore

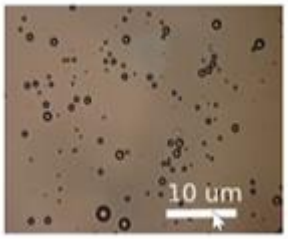
Lecture: 38

Contrast-enhanced imaging

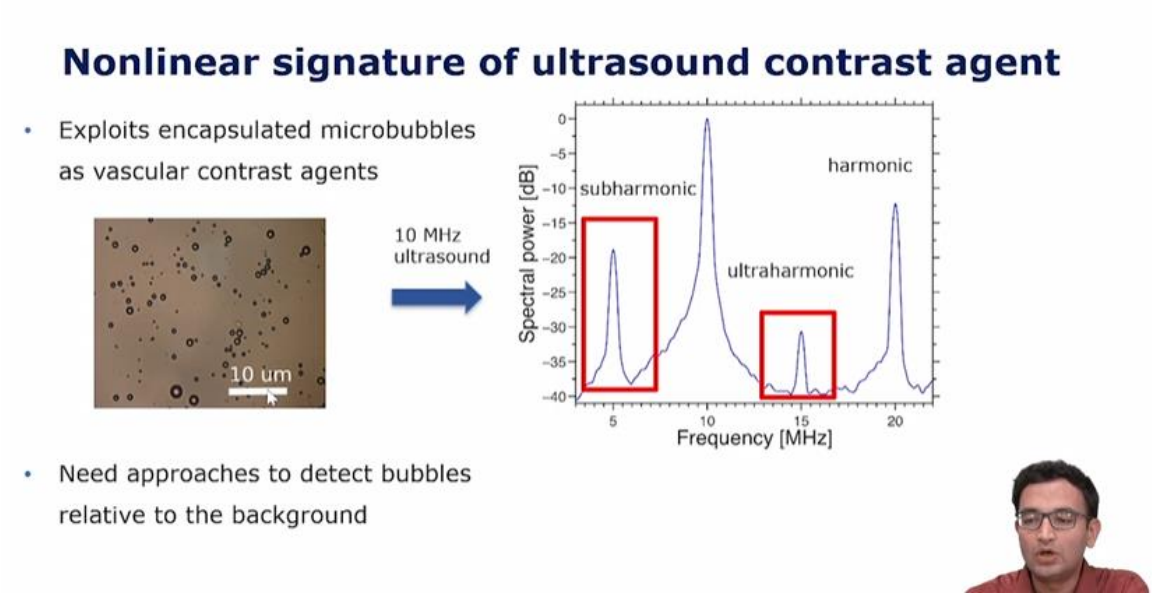
Hello and welcome to the lecture on contrast enhanced imaging. So you will recall from the last lecture, where we discussed the physics of ultrasound and microbubble interaction. So here is the spring mass system. You have a spring constant K, a mass M, and a force, which is a sinusoidal force being applied. Similarly, you have a microbubble, you have the mass of the surrounding liquid, The bubble has gas. So if you compress the gas, it provides resistance. So in that sense, it has a spring constant. And then we have the force. The acoustic pressure is analogous to the force, which is being applied in the case of the spring mass system.



So if you recall this, now let's see how we can make use of this for imaging. So here is a bright field microscopy image of the micro bubbles that was taken in our lab.



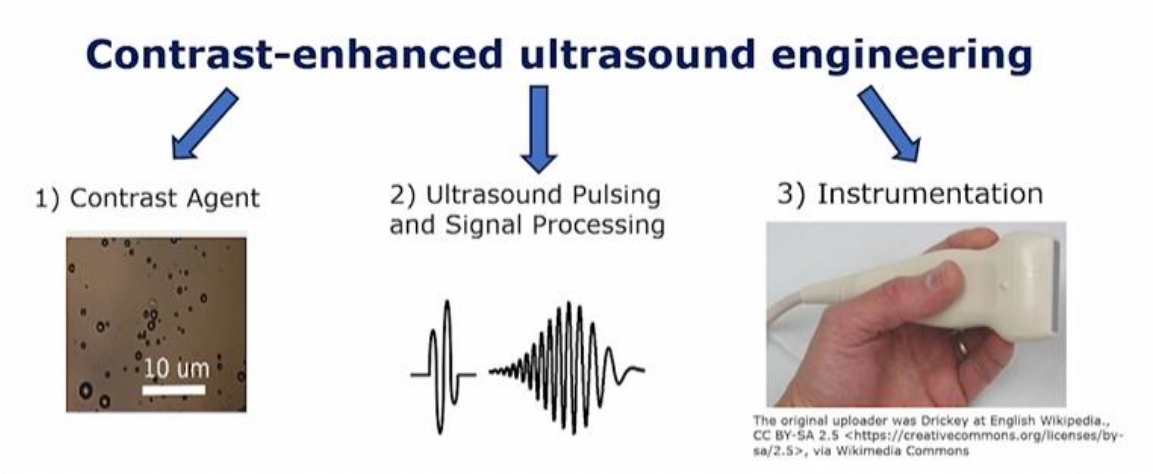
So you can see the scale bar is 10 micrometers. The size of red blood cells is about seven to eight micrometers. So most of these bubbles are very small; much smaller than two or three micrometers. And then when you excite these bubbles to ultrasound, let's say, we send out a 10 MHz ultrasound, then what kind of frequencies will the bubbles scatter? So intuitively thinking in the linear regime, we would think that if we send a 10 MHz ultrasound to excite these bubbles, then you would get back 10 MHz, and that is true. You do get back a 10 MHz signal. So on the figure below, on the right, we are looking at the spectral power in relative terms. So this is normalized. So the decibel is a relative unit. And here is the normalized power, and you have the frequency on the x-axis.



So you do see a peak at 10 MHz, which is the strongest. But you also see some other peaks. So you see a peak at 20 MHz, you do see a peak at 5 MHz and 15 MHz. So these are non-linear frequencies. These were not present in the signal that was sent. So in other words, these are frequencies that are specific to the bubble because these frequencies are not generated to a large extent from the surrounding tissue. It turns out, particularly the sub-harmonic and the ultra-harmonic frequencies are highly specific to the micro-bubbles. They are not generated in tissue. Harmonic signal is generated in tissue. We will talk about it in a later lecture, but not to the same extent as in the microbubbles. And therefore, by using these nonlinear signals, we can actually preferentially visualize the microbubbles and suppress the tissue background. And that is what we need to develop to be able to use these microbubbles as contrast agents. We need approaches that will enhance the visualization of the bubbles and suppress the background.

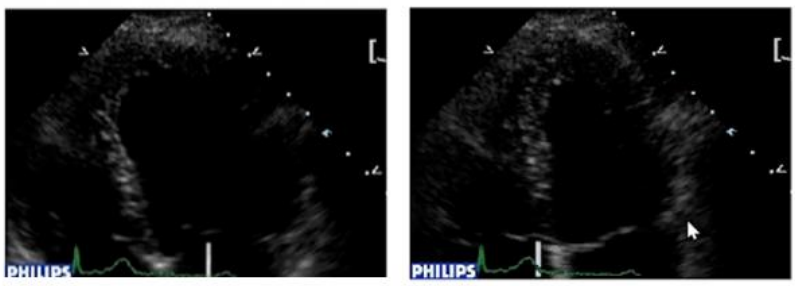
So now, if we talk about the field of contrast-enhanced ultrasound engineering, making this happen, there are three different aspects to it.

The first aspect is the contrast agent. You need to engineer the contrast agent itself. What kind of lipid would you use? What would be the size of the microbubbles? What kind of gas? How you would stabilize the microbubbles, etc. And also understanding the chemical engineering aspect of microbubble engineering.



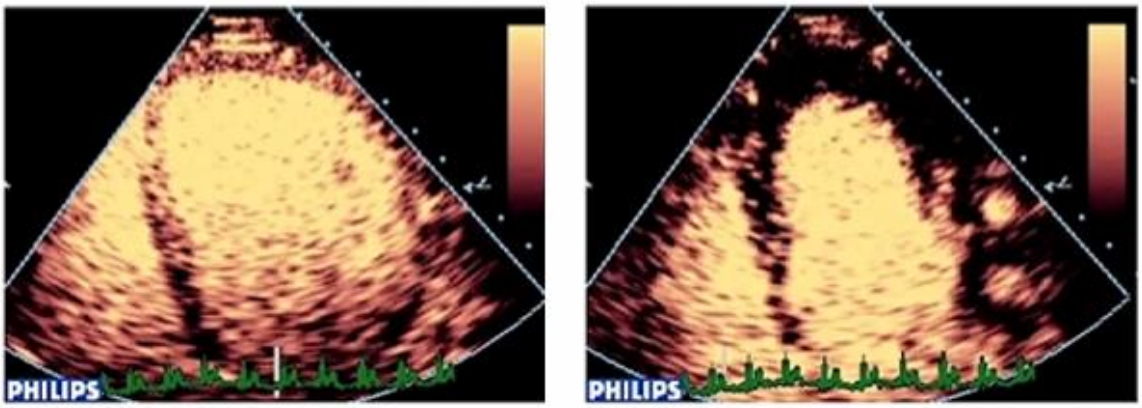
Similarly, there is ultrasound pulsing and signal processing. What kind of pulses you are using to excite these micro bubble contrast agents, what would be the acoustic pressure, how you would match the resonance frequency, etc. These are things which fall in the domain of pulsing and signal processing. Thirdly, there are instrumentation aspects to it. So what kind of instrumentation, would be needed to optimize the performance of contrast enhanced ultrasound imaging These methods are slightly different from the traditional ultrasound. These are some aspects that can be looked at and are active areas of research.

So here, I am showing you one application of contrast enhanced ultrasound. We are looking at a video of the heart chamber and the heart is beating, the chamber is expanding and contracting but because of poor signal, it is a little difficult to discern the boundary of this heart chamber and typically this is used to measure the volume of blood that is ejected.



Now to measure the volume of blood that is being ejected, we would need a very good estimate of the size of this chamber and we would need to segment the walls of the

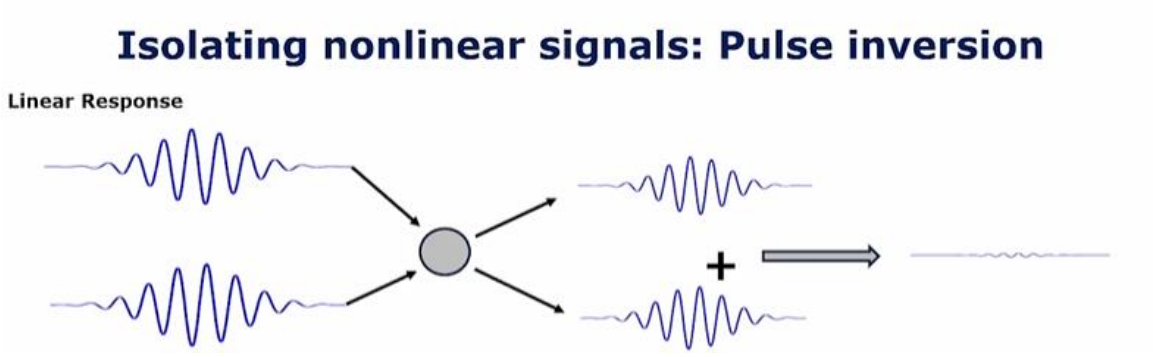
chamber. Now that is not possible here in this case because there is some ambiguity with respect to the boundaries of the heart wall. But now we inject the ultrasound contrast agent microbubbles and we are showing in a slightly different color map.



But you see this kind of inverted U shape on the top, which is actually the wall and the whole area which was dark initially because there is only blood, now lights up when blood comes in. This is because blood is inherently hypoechoic and is not a very strong scatterer of ultrasound. With the microbubbles, thy scatter more ultrasound, and now you can see this inverted U essentially the boundary of the left ventricle very clearly. Now this can be used to calculate the ejection fraction and get a proper estimate. This is one of the oldest applications which were approved for ultrasound contrast agents.

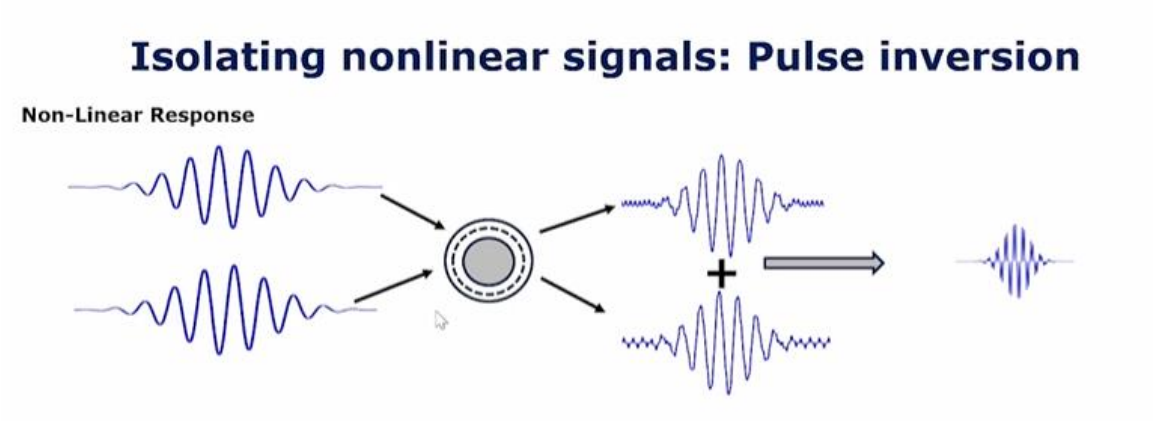
Now, if we want to sensitively visualize the micro-bubbles while suppressing the background, then we need approaches which will help isolate non-linear signals. So you would say that these bubbles are very strong scatterers, so why really we need non-linear signals? Why can't we simply rely on linear signals? And the bubbles would scatter much more than the background tissue and hence be differentiated. But it turns out that although bubbles are very strong scatterers, they are located in low concentrations in the blood. If you put more bubbles than blood cells in the blood, it will affect the rheology of blood flow and it will be unsafe. And therefore typically you will not have a very high concentration of these bubbles in the blood. And because of that, you will not get a very strong signal in the linear mode. Therefore, nonlinear modes are used in which the signal is coming largely from the bubbles and not from the surrounding tissue. And therefore, you can preferentially isolate and visualize organs where bubbles are present, which are blood filled organs in this case.

So now there is a technique which is commercially approved called pulse inversion, uses a very simple principle of superposition.



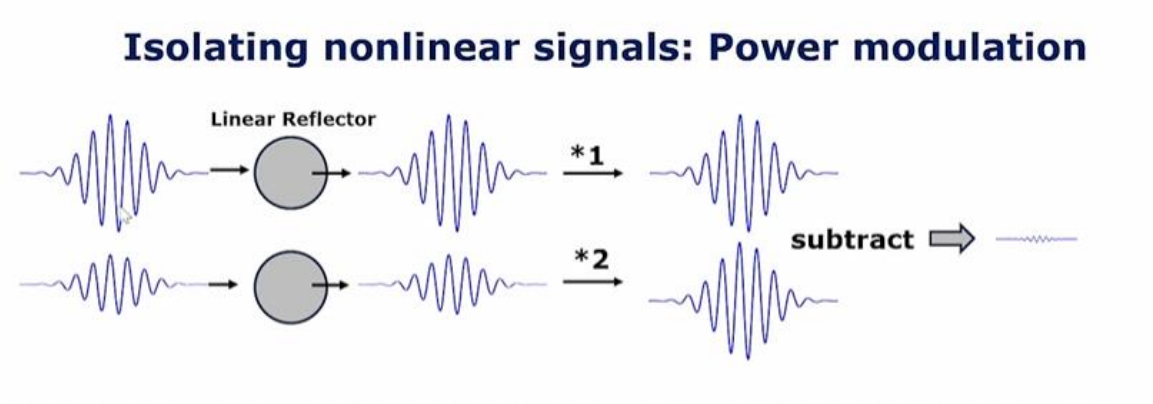
If you have studied signals and systems, you would have studied the concept of superposition. So if a system is linear, then it follows the principle of superposition. So consider a target which is linear. Now we are going to fire two pulses in quick succession at this target. The first pulse and the second pulse, if you look carefully, have the opposite phase. They are inverted in phase. So if I were to just take these signals and sum them up, they would cancel each other. Now when I get the output from the first pulse and I get the output from the second pulse, because of the linearity property, the output should also be phase reversed. And when I sum them up, I will get almost zero output. Now in practice, the output will not be exactly zero because the pulses may not be exact replica of each other. There may be some noise or there may be some motion in between the pulses. But nonetheless, the signal that you get in the end is largely suppressed.

Now consider a bubble which is oscillating in the field. Now, when you give two pulses, both phase inverted and you get the output. It turns out that the bubble can be modelled as a non-linear system so the principle of superposition does not hold. Even when you sum up these outputs they don't fully cancel each other and you get a residual signal which is the non-linear signal ,but the surrounding tissue signal will be canceled. So thus now you are able to visualize the bubbles, but suppress the surrounding tissue.

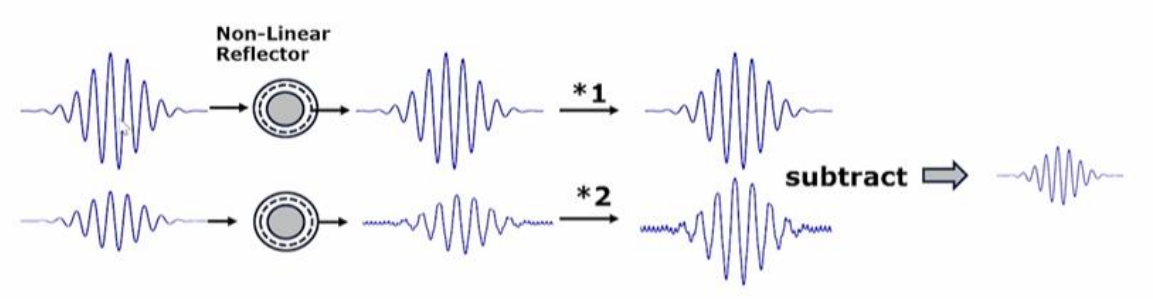


And this is how you would be able to detect the micro bubbles in a highly specific manner.

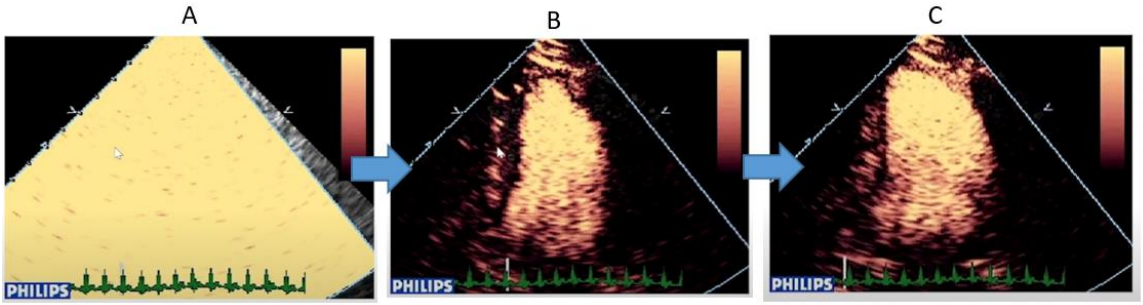
There's another technique which is a variant of this, which is called power modulation. in which you send two signals, the signals are identical except they are different in amplitude. So one would be a high pressure signal, the other would be a relatively lower pressure signal. Now if a linear reflector is there, once you get the outputs, the outputs would have the same scaling ratio as the inputs. And if you multiply, let's say the inputs are in the ratio of 2 is to 1. So if you scale up the inputs to match each other, then the outputs would also be identical. Now if you subtract them, the result will be almost 0.



However, if you have a nonlinear reflector, if you give it a high amplitude pulse, it will oscillate highly non-linearly. And at the low amplitude, it will probably not oscillate so non-linearly. And when you take a difference after scaling, you will still have a residual signal, which will help differentiate the bubble region and suppress the background.



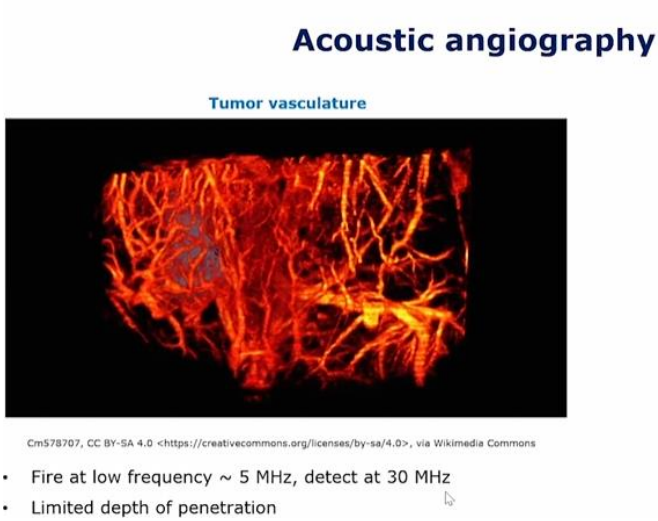
So these are two examples, where using the principles of signals and systems cleverly, we conceptually explained how microbubble imaging can be performed. Now there's another technique called flash contrast imaging. So let me show you what happens here.



If you pay attention, you will see a flash (fig A). This flash is a high amplitude ultrasound pulse which destroys the bubbles. Now the bubbles get destroyed and they start filling again. So what you will see, look at this U-shaped wall which actually represents the heart wall. And in the center are the chambers which are filled with bubbles. But the heart walls also have blood vessels and microcirculation so bubbles are also filling up the heart walls. Now you will see immediately after the flash the heart walls will appear dark (fig B). But they are slowly increasing in intensity (fig C).

So what is going on here? After the flash, most of the bubbles in the heart wall are destroyed, but they slowly fill up and if it doesn't fill up in a certain region, then we can be sure that, that region is either dead or not receiving enough blood flow. So this can give similar information to more advanced and expensive modalities such as PET. And ultrasound is non-ionizing, safe and cheap. And therefore, this technique can be used to look at the functional properties of the heart walls. Now, this will tell you whether a region is ischemic or infarcted. If a region is ischemic, it is getting low blood flow. If a region is infarcted, it is not getting any blood flow. And these informations actually help a clinician decide on what kind of therapy to pursue.

There is another very interesting technique called acoustic angiography, which relies on the nonlinear response of contrast agent, in which you fire at low frequencies, let's say about 5 MHz and you detect at 30 MHz. So this is some high frequency signal, which is generated because of nonlinearity.



Since the signal is at high frequencies, it generates very high resolution. So as you can see on the figure above, the blood vessels which are very small are being visualized with great resolution and that is the advantage of acoustic angiography. Now the challenge with this technique is that because it uses high frequency in the receive mode, the depth of penetration is limited. But nonetheless, this technique has been employed for imaging some superficial organs and also for small animal imaging, where the penetration depth requirements are low. We recently published a review article, Bisht et al., in which we compiled and contrasted the different techniques to detect microbubbles. You may find this interesting, "Pulsing and Detection Strategies for Contrast-Enhanced Ultrasound, a Narrative Review". And as you can see, there are a variety of techniques which are categorized into different modes, and you will probably find this interesting. We are unable to discuss all these topics in today's lecture, but I hope you will look this article up.

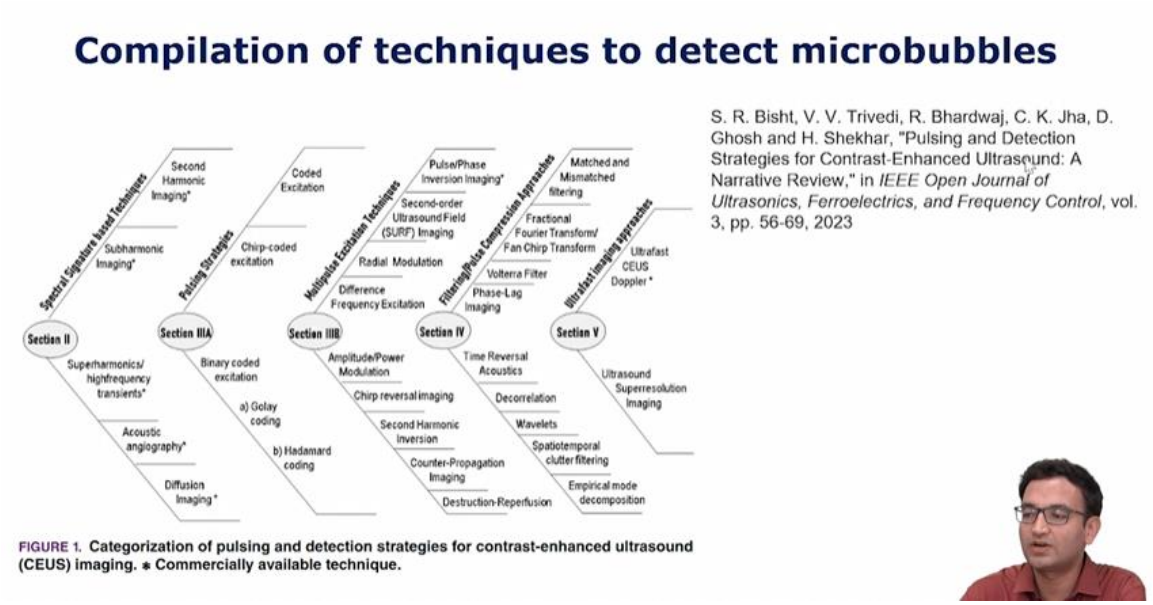
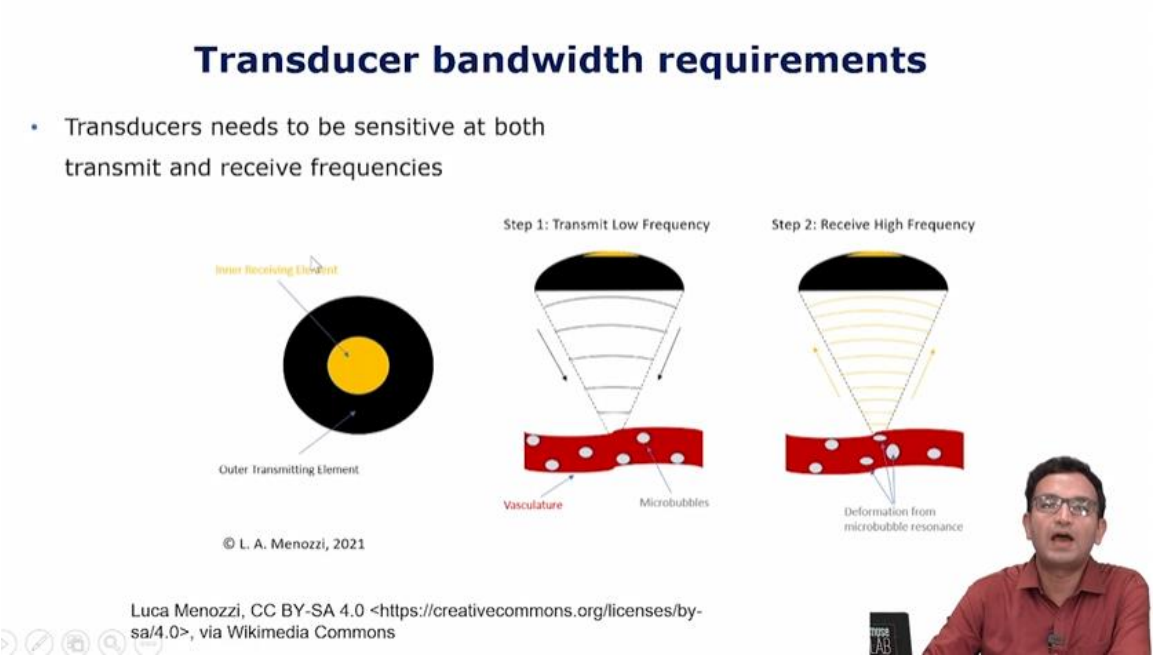


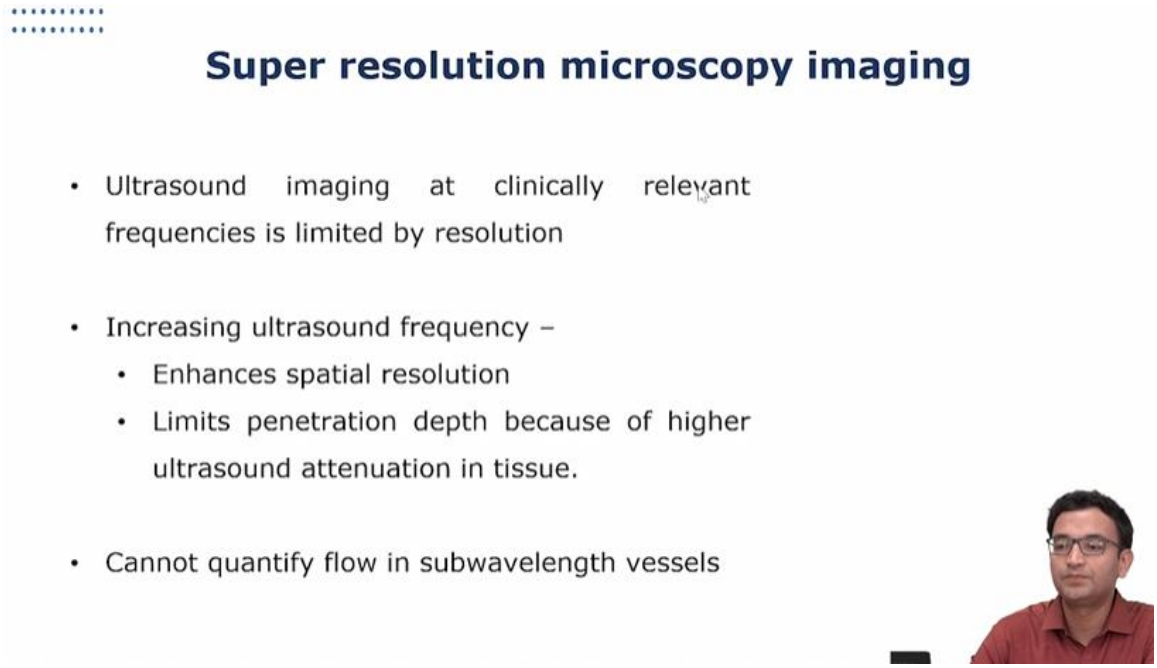
FIGURE 1. Categorization of pulsing and detection strategies for contrast-enhanced ultrasound (CEUS) imaging. * Commercially available technique.

Now, let's discuss from the point of view of the transducer. When you want to perform non-linear imaging, let's say second harmonic imaging, in which we are firing at a particular frequency f_0, but we are receiving at a frequency of $2f_0$. So in that case, you would need a transducer which is sensitive not only at f_0, but also at $2f_0$. So the transducer would have to be broadband, and in some cases it can be challenging. In such cases, researchers have developed specialized transducers in which there are dual frequency elements. One element is capable of transmitting a low frequency and the other element, the inner element is receiving at high frequency.



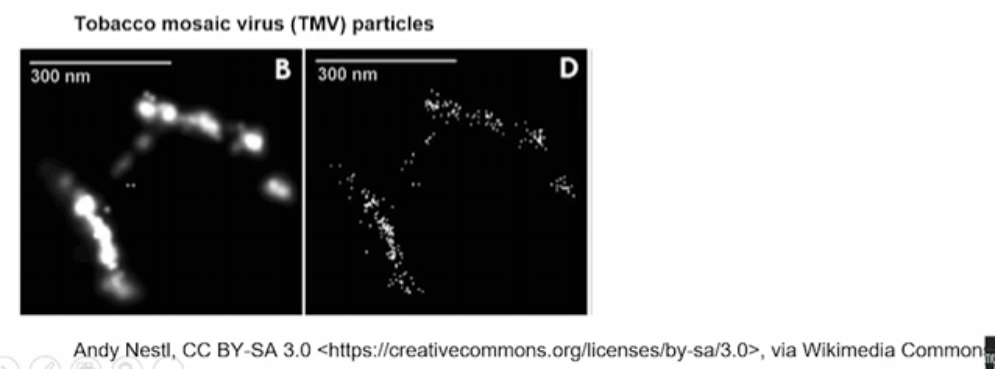
So this is an example from Manzoni et al. What is interesting here is that you need to have micro bubbles which are carefully fabricated, you need to have the right signal processing approach and you also need to have the right hardware and instrumentation to be able to do micro bubble imaging or contrast enhanced imaging.

Now let us look at a very exciting area of super resolution microscopy imaging, which is really developing in the last 5-7 years. So ultrasound imaging at clinically relevant frequencies is limited by resolution. Suppose I perform imaging at 1-2 MHz, the resolution will be approximately of the order of 1 mm. But now, if I want to get better resolution, particularly to visualize the microcirculation in the body, one option that I have is to increase the ultrasound frequency. For a given number of cycles, if I increase the frequency of ultrasound, then the spatial pulse length decreases. Hence the axial resolution improves. Similarly, the focusing that you can perform is limited by the wavelength. For higher frequencies, the wavelength decreases and thus you would get better focusing as well. So as you can see, increasing the frequency enhances the spatial resolution.



However, it penetrates less in the body as you increase the frequency, because of higher attenuation. So therefore, we cannot enhance the resolution if we need to achieve adequate penetration depths and there is a trade-off between resolution and penetration depth. So now if we want to quantify flow in sub-wavelength vessels at a certain depth, we would have to break this trade-off and that is what super-resolution microscopy imaging does. This technique improves the resolving power of ultrasound imaging. It helps us break the diffraction limit, which is about $\lambda/2$.

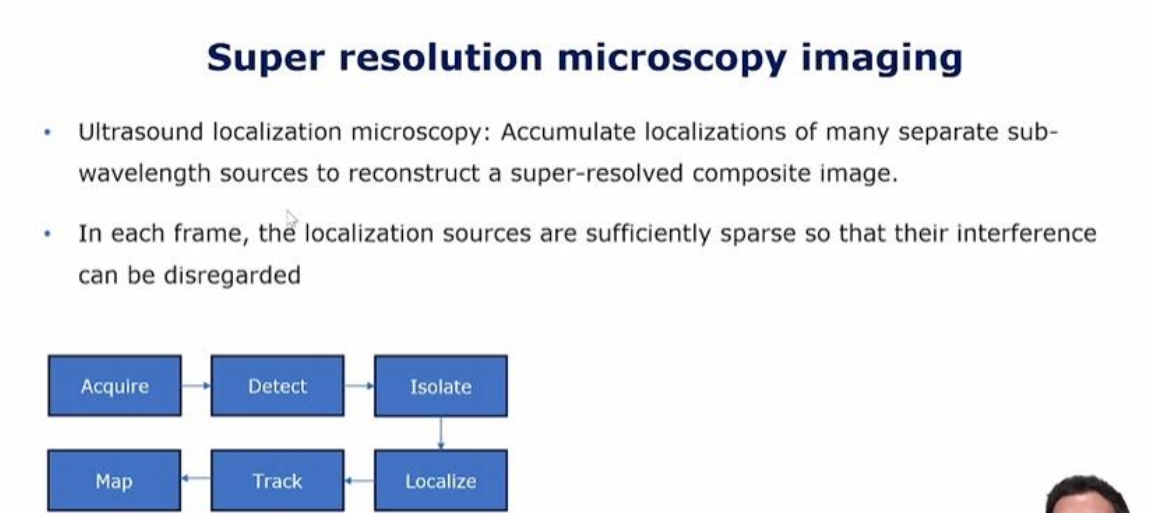
Typically you cannot image at a better resolution than the diffraction limit because it is physically limited. But The ultrasound super resolution microscopy imaging technique separates the echoes coming from sources, that are closer than that classical diffraction limit. And this technique is inspired from an optical technique of super resolution fluorescence microscopy, which was awarded the Nobel Prize in Chemistry in 2014. So here is a super resolution microscopy image of tobacco mosaic virus. On the left is a conventional imaging in which you see the virus particles appear blurry. But on the right, you can see the virus particles at a higher resolution.



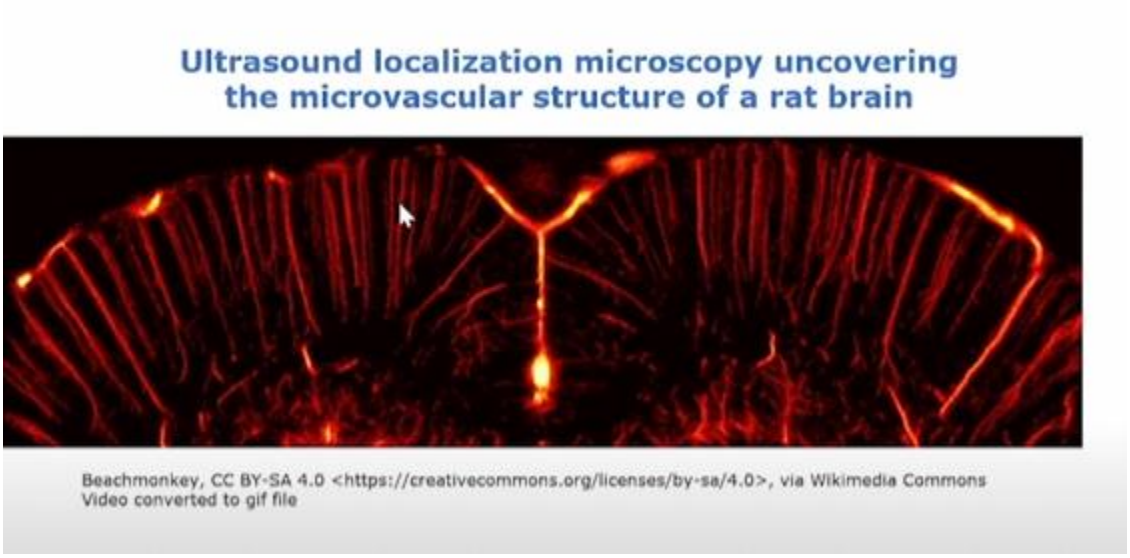
So how is this done? In ultrasound localization microscopy, we accumulate the localizations of many separate sub-wavelength sources to reconstruct a super-resolved composite image. So for example, These blurry patches can represent the point spread function of your imaging system. If we can localize the maximum intensity points and replace them with points, then we can get better resolution. So this is the general principle. Next, let's discuss super resolution microscopy imaging with ultrasound. In this technique, we exploit microbubbles as sub-wavelength sources. Now these microbubbles, as we discussed, can be about 1.5 or 2 micron in diameter. And the wavelength that is being used in clinical ultrasound can be ranging from several hundred of micrometers to about a millimeter.

Therefore, microbubbles are an excellent sub wavelength source. Now, because they are subwavelength sources, they act like an ideal point object and they create a blurred response, which is actually the point spread function of the imaging system. Now, if we can look at the point spread function and localize the microbubble by looking at the pixel which has the highest intensity and then accumulate the localization of these pixels to reconstruct a super resolved composite image, then we can visualize microcirculation. So in this case, what we need to do is that we need to have a sparse distribution of microbubbles flowing through the vessels, so that we don't confuse multiple clustered microbubbles as a single object.

So in summary, what is done in this approach is, first you acquire the data in which you have microbubbles flowing in the microcirculation and other vascular organs. Then you detect the microbubbles. These microbubbles will be visualized as blobs because they represent the point spread function of the object. You can find and isolate microbubbles. If there are multiple microbubbles, then that case can be discarded. Then you can localize the microbubbles by pinpointing the highest intensity pixel in the point spread function. And then you can track these micro bubbles over multiple frames. And finally, you can map the location of the microbubbles, which will become a surrogate for the blood vessel itself.



This is the ultrasound localization microscopy of rat brain. And as the bubbles are flowing through these vessels and they are being localized and tracked, we are able to form this nice, very high resolution map of the rat vasculature, which is unprecedented actually.



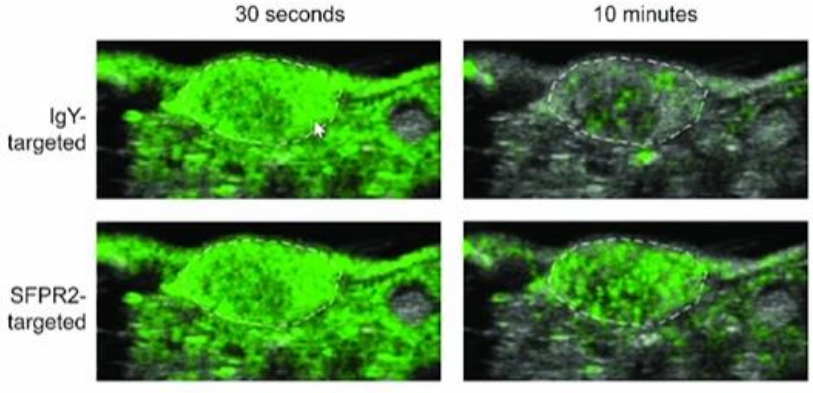
Now let us discuss ultrasound molecular imaging, which is a different type of contrast enhanced imaging. Now, imaging can be divided into different categories. Imaging can be anatomical imaging, physiological or functional imaging, and molecular imaging. And by molecular imaging, we typically refer to the visualization, typically a non-invasive visualization and assessment of biological processes at the molecular or cellular level in living systems. So that is where microscopy in a petri dish of some ex vivo tissue will not qualify as molecular imaging.

Ultrasound molecular imaging

- Imaging: Anatomic, Physiologic (Functional), and Molecular
- Molecular Imaging: Visualization (typically non-invasive), and assessment of biological processes at the molecular or cellular level in living systems
- Traditionally conducted using PET, MRI, and CT
- Targeted microbubbles: Ultrasound molecular imaging
- Ligand – antibody, protein, aptamer
- Affinity to the target of interest

Now traditionally the modalities which are best known for molecular imaging are PET, MRI and CT. And for ultrasound, we can also do molecular imaging but we need to use targeted microbubbles. These are microbubbles which have a ligand and they may have an antibody, a protein or an aptamer which has affinity to the target that we want to image. Now this target could be something like a marker for angiogenesis or blood vessel formation. It could be a marker for inflammation and many other markers which have biological significance. So here I am giving one example from a published paper where these micro bubbles were injected and these micro bubbles were either targeted to this IgY which is a non-specific ligand or SFPR2 which is a marker for inflammation and then these micro bubbles were let to adhere with the tumor.

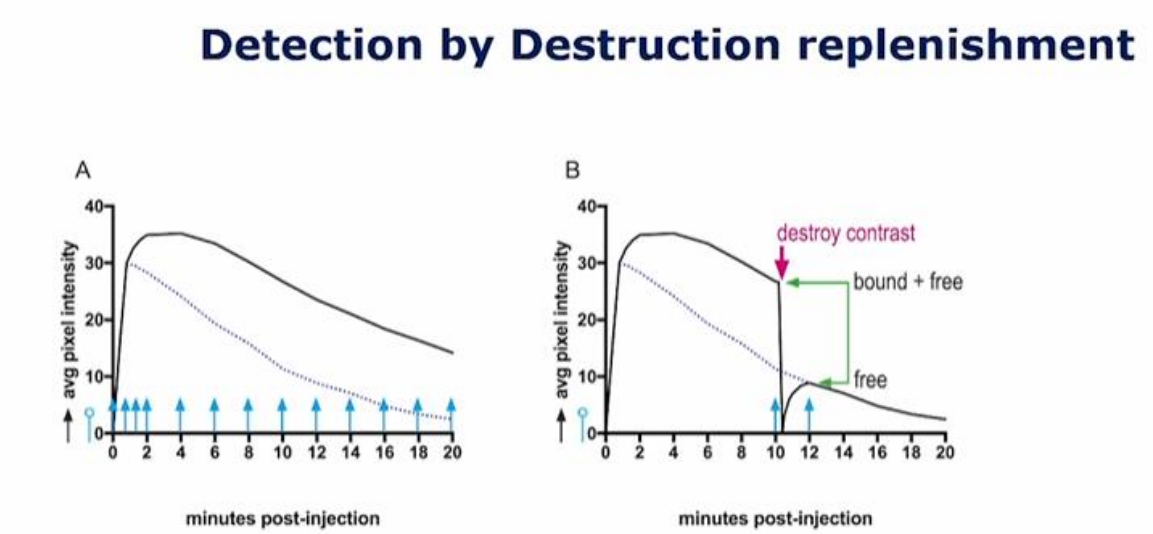
Detection by delayed imaging



Tsuruta et al. Plos One 2017 (open access)

So after waiting for about 10 minutes, what was seen that the non-specific antibody targeted microbubbles cleared off mostly from the space. As you can see, there's not much signal originating from the tumor. But the microbubbles which were targeted to the tumor, they remained there. And then by measuring the intensity of the targeted microbubbles, we can get an understanding of how much expression of the molecule that we are interested in, is present at the target.

So this is what molecular imaging does. And in this case, we were able to differentiate between targeted and non-targeted bubbles by just waiting it out. But it turns out in some cases just by waiting we are also losing some of the targeted bubbles, because of the short half-life in the body. And therefore there are other approaches such as detection by destruction replenishment.



You can use a flash pulse to destroy the micro bubbles. So initially the signal that you have contains bound and free micro bubbles, and then you destroy the bubbles; all of them get destroyed. The free micro bubbles quickly come back into the field of view, but the bound micro bubbles will take time to bind and therefore are not present yet. If you take the difference, then you can find the intensity of the bound microbubbles initially. So this is another approach to detect bubbles that are already adhered to a surface.

So let me summarize the contents of this lecture. I hope I have convinced you that ultrasound contrast agents are a powerful tool for imaging. We discussed the nonlinear scattering from the contrast agent and approaches to bubble detection, including some very simple approaches such as pulse inversion, power modulation, flash contrast agent imaging, etc. We discussed the transducer and system bandwidth requirements for contrast enhanced imaging. We discussed the exciting fields of super resolution and molecular imaging. Now, taken together, there are a large number of applications that are already approved for contrast enhanced ultrasound and numerous new applications are in

clinical or preclinical stage in different countries in the US, European and Asian markets. As the instrumentation, modeling, and analysis softwares are improving, this field will grow further and probably have a bigger clinical impact in the future. So with that, I would like to close this lecture. See you in the next lecture. Thank you.