MODERATOR: So, our last speaker of the workshop is Sam

     Wickline, and he will be talking about ultrasound.

DR. SAM WICKLINE: Everybody, take a big breath. It's

     almost – *sotto voce*, at least. [Breathes deeply.]

     So, I'm going to talk about some stuff that would be

     potentially applicable to tissue regeneration, both on

     the lab or on the bench top and potentially in the

     clinic. And Ralph asked me to focus on ultrasound,

     which we do quite a bit of. Our lab does a fair

     amount of other stuff related to nanotechnology for

     therapeutics and also imaging, using many different

     imaging modalities; but we're really – our real goal

     is to get something into the clinic, and that includes

     the imaging technologies that might be translatable,

     you know, from the bench to the bedside. And if it

     doesn't work like that, we're a *little* less interested

     in it – not completely, obviously, but it *is* one of

     our driving themes. So, I'll point out some of the

     disclosures here, as I'm required to do.

     So, the topics that I'm going to talk about are

     molecular imaging. So, the way this is set up [is]

I'm going to do the Ghost of Christmas Present, Past

and then Future, basically. [I'll] talk about stuff

that we're doing now with molecular imaging, which

we've been doing for 15 years or so, in ultrasound;

and I'll diverge a little bit into MRI, because I

think it's important for some of the applications to

vascular growth and so forth, that folks here are

interested in. And then I'm going to talk about

native tissue classification with ultrasound – in

other words, using ultrasound machines that already

are out there in the clinic without having to modify

them, or make new machines, or add things to them –

just taking the RF data that you can get, and what can

you get out of those data.

And so what we're really interested in is extracting

quantitative indexes [sic] of tissue structure,

organization, physical properties and function. And

I'll show you how we do that. That's the Ghost of

Christmas Past. And we'll talk about tissue

characterization as we've practiced it, and others

practice it, and as it is being practiced today in

both academe and in commercial entities. And,

finally, the transition from classical tissue

characterization to what is a work in progress for us

using new signal processors to extract even more

information out of the RF data that you get out of

clinical imagers or machines that you can roll up to a

patient that weigh[s] 400 pounds and, if they roll

over your foot, like they did with mine one time and

break your toe, it energizes you to make very small

machines that are held in your hand so that you never

have to roll anything again. And I'll talk about

that, too.

[CHUCKLING.]

DR. WICKLINE: Here's an example of some application of

tissue characterization that exist. Meat on the hoof.

Meat grading on the hoof – this is done all the time.

You can look this up. There's tons of other things

like this, but here's a guy – or, a gal with a cow

here in a gantry of sorts, and here's an ultrasound

machine, and they're pointing at the steaks and the

flanks and all of that. This is done routinely to

figure out how marbled tissue is, and so you already

know that this is being useful. This is not pie-in-

the-sky. This is today. It's being used today. And

I have another example of that later in the medical world. What you ate last night at the little get-together was graded by a technique *exactly* like this.

[CHUCKLING.]

DR. WICKLINE: But what you want to get to maybe for clinical use – because some of these applications for tissue regeneration, or stem cell whatever are going to have to be looked at in people who are alive with some kind of technique that doesn't scare them, like getting into a large tube, or using ionizing radiation five or ten times a week in order to image whatever you're going to image.

Now, we do that, too, so I'm playing devil's advocate a little bit. But nevertheless, here's an example of something we're working on that's a complete ultrasound system in a handheld gizmo right here. It has all the acoustic elements: the pulser[?], receiver, electronics, amplifier, digitizers, USB interface. There's nothing in here that requires a 400-pound machine to be rolled anywhere. All you do is you take your laptop computer, and all the

computing power is in here, and you['ve] got this
little connector. That's it. Power runs off of this
– off of your computer.

And so there's tradeoffs here, obviously. This is
small machine. The other ones have more bells and
whistles, but the tradeoffs – and this is a single-
element transducer that works by the wobbler
technology – a toothbrush scanner kind of thing, if
you will.

Compared to these expensive array-based systems, which
are very closed architecture made by ultrasound
companies – which is really unfortunate, but true –
there are some drawbacks to such a handheld system,
including signal noise and so forth. And my question,
and the question that our group sometimes faces is,
"Can these be overcome by combined use with advanced
signal processing?" And so advanced signal processing
is going to be something that would provide the
ability to use these cheaper, more portable
technologies in the future and offer enhanced,
quantitative and signal noise capabilities.

So, what we've been working on lately with ultrasound is targeted ultrasound imaging. There're several different kinds of constructs that you can use to target nanoparticles or other sorts of particles that will be responsive to ultrasound energies. The ones that we've been using are liquid profluorocarbon nanoparticles. I'll have more to say about that in a moment. This is just imaging a clot on a piece of paper, if you will. There're epigenic liposomes. There are bubbles that have been functionalized for targeting liggins[?]. This is actually fairly simple to do. And most people do this to image – well, here's targeting inflammation by Jonathan Linder and – [unintelligible] – Nueves[?] targeting P-selection[?] here and so forth. These are 2 to 4 to 5, maybe up to 50 microns, given the distribution of sizes; and most people use these because they're easy to use and they can use them. And because most ultrasound systems are not sensitive to nanostructures like this, which is why – at least at the frequencies that we use for clinical medicine, and so that's why the extra signal processing oomph is required to see things like this. And I'll get into that.

So, here's an example of one of the first things that we used for ultrasound imaging, in fact, which is a fluorinated nanoparticle that has a lipid monolayer wrapper on it. You can functionalize this with targeting liggins, drugs, other imaging agents like gatalinium[?], or radionuclides and so forth. This comes together spontaneously in about 50 nanoseconds. We've modeled this with molecular dynamic simulations. You can put it in a bottle, and it'll stay there for a long time on the shelf. And here's an example of it binding to fibrin, which came up as an interesting concept earlier – yesterday, I think.

And this is a fibrin clot, and you can see this binding with an antibody targeted to fibrin here on this SEM.

And so we invented the first one of these for ultrasound imaging in 1995 and published this. And here's an example of a clot that you can't see, in the neck of a dog, made by a nodal current. Hard to see with regular ultrasound, but when you add these contrast agents that target fibrin, it sort of brightens up, and you can see where the clot is now.

And so this was the first molecular imaging tool for ultrasound, reported in 1995.

You can also image other sort of interesting things, like tissue factor, which is responsible for clot formation. It's basically thromboplastin. You can see it on the surface of endothelial cells here, on fo-[unintelligible]. And here's a vessel that's been disrupted with a balloon, and it's expressed on smooth muscle cells and up-regulated in a few hours and ultimately causes clotting to occur. This is the approximate cause of clotting. You can image it over here. So, tissue factor imaging – and this works for a lot of different sorts of molecular epitopes as well.

Seen with ultrasound here at 30 MHz – or, 30 to 40 MHz. Higher frequencies see smaller things better. Lower frequencies don't, and so there comes the signal-to-noise issue that I'm going to talk about at the end of the talk.

We've also worked with Lee Hong Wang on looking at various kinds of nanostructures for photoacoustic

imaging. Here's one that's imaging sentinel nodes.
It has a copper core, and you can see over a period of
time this sentinel node lights up after an injection
quite nicely, seen by photoacoustic imaging here.

Here's another one that has a gold core. This one's
targeted to alpha-V beta-3 integrins, or angiogenesis,
if you will. You can see initially in this model –
it's a matri-gel[?] model – where vessels have come
into this, growing under the influence of growth
factors. Over time, when you inject this, it's a
little hard to see; but these little, tiny vessels
start showing up. You can't see them early on, even
with photoacoustic imaging. They're *so* small, but
these gold absorbers actually work quite well to show
up the very slow diffusion into these near-blind
alleys that comprise the growing angiogenic features
of this model. So, some advantages there with
photoacoustic imaging in terms of sensitive and,
certainly, resolution, making it quite a nice picture
for looking at these structures that are expressing
very early signals for angiogenesis – like alpha-V
beta-3 integrins.

Okay. I'm going to diverge a little bit into MRI, and so one thing that we did quite early around the same time was to put gatalinium[?] on this, and we were probably the first group to take this kind of a construct and put about 100 to 200,000 gataliniums on this 100- to 200-nanometer structure. And that provides a really huge bang for the buck in terms of relaxivity when you're looking at a contrast agent of the order of 10^6, or a million, or 2 million or so in terms of R-1, if you will, for those of you who know what that means. It's very bright, in other words.

And you could use this to image angiogenesis if you're targeting alpha-V beta-3 integrins. Take a rabbit. Feed it cholesterol. Vessels grow. Here's a micro CT showing these vessels. It targets them. You can take a picture with a 1.5 Tessla scanner. Another example of using conventional imaging equipment without having to modify that. And I'm sure Ralph talked about this yesterday. Again, showing the vasculature even when there's no obstruction, representing now inflammation in these vessels, seen, again, at 1.5 Tessla. It takes about 30 minutes to develop that signal.

Detecting tumors – here's a tumor. Same story here.

Angiogenesis imaging. Here's the tumor in Gray Scale,

cut out from the computer. Here's the vasculator, now

mapped in three and four dimensions, actually. And

you can map this over time. Here's exactly where all

the angiogenic capillaries are forming a placenta-like

unit to feed this tumor. And so after you treat

these, you could actually use this time and time again

to look at the growth or regression of angiogenesis –

as is done here in this vascular panel, showing that

if you treat these with a drug – which, actually now

is on these nanoparticles – an anti-angiogenic

compound. A week later, you can do the same imaging

and watch these vessels kind of melt away. And then

you can watch them recur, if you want, over time.

This is after a single dose of this material,

fumagillan [phonetic], on a nanoparticle, which is an

anti-angiogenic compound. So, imaging angiogenesis

with MRI – this is now in clinical trials through one

of the companies that we formed, and we're looking

forward to a successful phase 1, at least, in this.

But the interesting thing about this compound – this material – is that it has fluorine in the middle of it. You can actually image and detect that fluorine with fluorine spectroscopy – not with hydrogen or proton spectroscopy, which is usually used. And not only can you get a unique signature from these particles, but it's also quantitative in terms of the amount of fluorine that's deposited related directly and linearly to the number of nanoparticles that are in the site where you're imaging. And so now this makes quantitative MR possible. And also unique, no background MR, and you can do a multi-color experiment with multiple profluorocarbons in the core as well.

Here's just an example of imaging the fluorine that these fibrin-targeted particles have attached to on this human carotid endarterectomy specimen, which the last speaker showed when they break, they form a crust of fibrin here. And now it's color-coded in nanomolar[?] of particles – an MR image coded in nanomolar[?] of particles.

To do this clinically, you have to fix your scanner so it registers not only the protons, but also the

fluorine signatures. You['ve] got to transmit at two
frequencies and receive at two frequencies. Phillips
helped us do that. And when you do that, you can make
a picture of tumors, here in green, which is the
fluorine, with no background; and the whole animal,
which is a rabbit, with a VX2 tumor implanted in its
leg – again, coded in millimolar of fluorine this
time. And so you're looking at tumor angiogenesis
here with a conventional 3T imager that's been fixed
to be able to image fluorine and proton signatures
simultaneously, actually.

Well, the point of this story is to get to this slide.
One thing that's interesting about this is that these
nanoparticles can be used to study barrier function,
and we did this in a series of rabbits, published last
year, where if you feed these rabbits cholesterol for
a long time – say, a year; it doesn't take quite a
year, but six or seven months – what happens is these
particles will actually traverse the entema and go all
the way through the plaque passively, with no
transport required. This doesn't require ATP. I
won't tell you how we know that, but – actually, I
will. We did it by poisoning these in a Petri dish.

You get this diffusion through here, and this kind of a picture develops after a few months.

Here, you can see the excised vessels with a fluorine scan showing intimal penetration of these. Here's a side view, or a sagittal view showing where this plaque is with penetration. It doesn't occur early, like three months of feeding; but after a while, it occurs to a great extent. And it goes through little holes. This vessel endothelium is like a Swiss cheese, and so if you guys were growing vessels and making new vessels, you really want to know if the barrier function is working, or operable. This is about the right size particle to do this in vivo, because small particles would go through anywhere. Large particles wouldn't go through anywhere; but medium-size particles, like 200-nanometer particles, are about right. And if you can register the fluorine signal, which has no background and is quantitative, you could actually probably calculate a diffusion metric for that.

Anyway, look this up. It's kind of interesting. It doesn't go into the parts that are normal. It only

goes into the plaques that are old, and so here's a way to sort of detect disrupted barriers, if you will. And this may be important for those you who are developing vascular grafts and so forth, to look at the integrity of the vascular barrier, or the endothelium covering them.

All right. Let's go through ultrasound tissue classification. And as I said, we're going to use the native radio frequency information. And the motivation to do this is it's easy to do and learn. It's fast and cheap. It's portable. There's no ionizing radiation. It's multi-functional, including molecular imaging and therapy and guidance. There's a huge installed base worldwide – order of magnitude over everything else except regular x-ray. And there's a marked advantage ultimately as a clinical cost center under CMS bundled payment paradigms, and so they're not going to be a profit center. No imaging technology will be a profit center. They will all be cost centers; and so, therefore, cost containment will be absolutely critical to doing any of this stuff in real patients.

This is already adopted. This came out of the "St. Louis Post Dispatch" a few days ago. I'm *scared* of this, but it's actually true. Focused aspiration of soft tissues. Here you've got a surgeon who's poking a needle, and this imaging – he's got an imaging transducer here and a needle that sort of vibrates and sucks out the *bad* tissue – whatever that is – and he's got the ultrasound thing over here. But it said in the article – and this is FDA-approved – that he's identifying the tissue that needs to be sucked out by the ultrasound signal. Okay. Whatever that is. It seems to work. It's been approved; and so, therefore, it must be good. The FDA wouldn't make a mistake.

All right. Anyway, so this is being done by surgeons. They don't need a cardiologist, or a rheumatologist, or radiologist or anything – apparently – in order to do this. So, this has already penetrated clinical medicine.

Now, the reason that ultrasound is really interesting is because ultrasound depends on acoustic impedance mismatches, which depend on material properties and density. So elastic properties – speed of sound, if

you will – and density govern acoustic impedance mismatches between one tissue and the other, and this is what produces reflections when these are different enough. As Lee Hong Wang noted, for compressional waves, these differences are actually fairly small, but they're big enough to do tissue characterization with, as it turns out. And so these acoustic impedance mismatches are what we're registering when we look at typical back-scattered ultrasound.

But there's a lot of other things that you can do in the lab. Probably, some of these you can do elsewhere, like you can calculate a whole bunch of elastic stiffness constants, and these would comprise de Young's[?] modulus. It's anisotropic. [I] won't go through how you do this, but you can see if you're pointed along the direction of the fibers, de Young's[?] module is an attendant[?]. Our[?] myocardium is one thing. It's high here and low here, and you can make these nice anisotropy plots of[?] myocardium intendin[?]. This should be a lot longer, but we had to compress it to get it on the picture. But anyway, this is the kind of stuff that you could get out of some tendon that you're growing or some

piece of tissue that has well-oriented materials that you want to know what the orientation is, and you want to know what the strength is with nondestructive testing. This is classical sort of materials testing stuff.

This also applies to hearts. Here's the classical Streeter diagram. The fibers that precess through about 150 degrees from the endocardium to the epicardium. When you're perpendicular to them, you get big scatter. Parallel, low scatter. You can take out a chunk and do this and twirl this around in a water tank. You can see that your peaks at the epicardium, mid-myocardium and endocardium occur at different places, but the fiber angle shift measured by ultrasound and histology is about the same. So, you can get anisotropy out of this easily. Notice. 1991. This is old stuff. Never been applied to tissue regeneration or stem cell whatever as far as I can tell, but any of your local ultrasound physicists can do this, if they really had the will to do it.

It's also apparent in clinical images. Here's a simulation. Here's the real thing. High scattering

here, low scattering here, high scattering here, low

scattering here. We actually developed something

called "later game compensation" for clinical imagers

to take care of this, to boost the signal here so it

was equal to the signal there in normal tissues. So,

there's actually technology out there that can help to

do this in vivo.

But one thing ultrasound cannot do is it can't tell

you anything about sheet structure. Sheets are the

higher-order structure[s] that are present in hearts

and responsible for about 50 percent of the

contractile function, and you can only get this – as

far as I know right now, although I could call myself

a liar in another couple of weeks – by MRI – through

diffusion tenser MRI. And these sheets change their

orientation as the heart contracts from diastole to

systole, and they actually squeeze the endocardium out

so that the blood pumps out of the heart. So, this is

MRI. Ultrasound can't yet do this.

But another interesting structure that, if you're

growing a heart, you need to take advantage of some

technique that'll tell you are you getting sheets *and* fibers, and are they all lined up correctly.

This also recapitulates itself after infarction. Perpendicular and parallel in infarct. Happens exactly the same as in normal tissue because the tissues line up exactly the way they used to be, because the forces pulling on them laterally cause them to do that.

And I'm going to run through this more quickly. There's also – it's also clear that the collagen matrix, which you can get in our case a long time by digesting a piece of heart with sodium hydroxide, also manifests anisotropy, and it's due to the collagen matrix. The absolute value changes because you change the materials, but the anisotropy still exists in this collagen-isolated matrix. So, the collagen in the matrix is important not only for the fiber orientation, but the sheet orientation as well, as it turns out. You can get this out of ultrasound as well.

Skip these two just to get on to the next.

So, the issues for quantitative use in vivo of these kind[s] of technologies are lack of a robust calibration standard. There're some machine dependencies, if you wanted to do this in real people, that have to be taken into account. This can be done. Inability to measure and account for attenuation is a little tougher problem. There are ways of combating this, including time reversal, as Lee Hong mentioned. There's confounding specular echoes that get in the way, create noise. Low contrast between soft tissue types, because there's weak scattering for compressional waves, as I've shown you.

But finally, one of the reasons that we wanted to move into the next phase of this was molecular imaging of targeted nanoscale structures at clinical frequencies is very difficult. They're very small. They don't show up very well.

And so a work in progress here, although we've published about ten papers on this in the last five to seven years; high-contrast imaging using entropy quantities. And so we're interested in constructing

and using information images – and I'll describe that in a minute – to look at tissue organization and physical properties. The way clinical ultrasound is handled analytically in a clinical ultrasound machine has not changed in over 50 years. You're basically getting a Hilbert transform. You're getting a sum of squares of energies. It's all an energy measure, or an envelope measure, and that's all that people have been able to use.

Now, those are all convoluted with various signal processing schemes and modification to the signal by knobs that you turn on a machine, but you don't know what they're doing. But these theoretic detectors are immune to some of that, and their applications are in sonar, radar communications, image post processing; and we're using them for compressed sensing – I won't go into that – for ultrasound.

The benefits are the entropy information computes a single value for each pixel in a moving window that gives you subresolution feature detection, noise suppression. Specular echoes are cut out by the way the processing is done. And for molecular imaging,

it's more sensitive at lower frequencies for nano-size

constructs.

So, a simple example of what entropy is is you take

the word "daffodil," and you code it. There's a

couple of d's, a couple of f's and so forth and so on.

You add this all up, and you get a number for this.

When you fly this around, there's another thing over

here and another thing over here. It's like bit

dithering, and you can get subresolution imaging

features out of that, but basically entropy is

basically this mathematical function on a voxile-by-

voxile basis. Typically, if you do the established

approaches that I just talked about, you take a whole

bunch of RF waves. You sample them, and you get an

ensemble probability distribution function. Here

you're taking one RF line. You're getting a single

probability distribution function with a very

different look, including some singularities, which

are handled mathematically in a very complicated way

that I won't go into, but [which] produces an

interesting result.

And so in the materials applications, what you get out of this is if you have a flaw stuck into, for example, a graphite epoxy composite that you can't see with energy, you can pull this out quite nicely with entropy signals and see this very easily because it's not susceptible to the ring-down[?] on the front. You wipe this out with the kind of probability density function that you derive using these entropy measures. And so very sensitive flaws or things hidden in the noise is what this will enable.

Here's just an example of this used in muscular dystrophy in mice, published not too long ago, showing the muscular dystrophy being treated. Here's the normal tissue. Here's dystrophic tissue, and here's prednisone in dystrophic tissue. And you can see the entropy measures. This little glob right here is the bicep. You know, here's a normal. Here's an abnormal, and here's a treated one looking quite different. The ones that have the energy measures don't discriminate between the treated and the controls, but the ones that have the entropy measures do quite nicely. So, this is much more sensitive to things that change on a very small scale.

For contrast agent detection, here's just an example.
I'll just run through this quickly. This is a mouse.
Here's an ultrasound imager at 40 MHz – mouse imager.
It develops squamous carcinomas that have
angiogenesis. If you use the entropy measures, you
can see nice development after a little bit of time as
entropy changes, versus the control, which doesn't
change much. These are very sensitive. And not only
do we have entropies like Shannon entropy, but
Raney[?] entropy and many other entropies. So,
there's a whole family of these entropies that show
different things, allowing for different kinds of
contrast to be developed.

Another kind of scan showing, again, differences in
different kinds of entropies – ones that are not so
long ago and ones that are even more recent, with
alpha-B beta-3-targeted nanoparticles in tumors.
Again, we're running through a whole host of these
entropies – this whole family – and we have, in fact,
automated this now. And even though Allan said – what
did he say? If somebody says "automated
thresholding," don't believe them, or don't buy land.

Next year, I might have some land to sell you, because
this actually – because this is a nice probability
distribution function, it can be auto-threshold. And,
in fact, when you do that, you get a baseline or a
reference scan, which provides information about what
the normal tissue is. And so our goal is to use this
to classify normal tissues, with these entropy
measures to serve as a dictionary, if you will.

And so – now, this will all be automated on a Phillips
machine very shortly, and it'll be able to be done in
a very short period of time.

Let me just go back. Like, this is the real-time one.
You can do this in minutes. These used to take days
of computational time. Now it takes minutes, and
there's some mathematical tricks that are used to get
that done.

But the reviewer question that has come up in the past
couple of days. So, we got one of these grants that
said R1. The problem of general tissue classification
is not particularly significant.

[CHUCKLING.]

DR. WICKLINE: Normal tissue – we don't need to know

anything at all about normal tissue. This was what

this guy said. Well, we've got some next steps. We

don't agree with this. We think it's absolutely

critical to know what normal is before you can decide

what abnormal is. But there are very promising ways

we're using model-based interpretations of these

entropy metrics, factoring in size, shape, material

properties in these scatter ensembles which we're

working on now, to show how these seemingly

statistical measures relate to real stuff that's in

human hearts and other organs.

We want to categorize normal tissues based on the

native RF, and we will develop a set of reference wave

forms by looking at various normal tissues, so that we

can compile the data that says we know what normal is.

And, finally, we're working now on the implementation

on this on commercial clinical scanners – a Phillips

machine which will be used not only for noise

reduction, as it is in signal processing for

telephonic communications, but for compressed sensing

and for tissue classification, as the next thing to do

over the typical tissue classification schemes.

Here are half of the people who've done all the work.

The other half are on the other side of the picture.

You can't see them. But anyway, this continues to be

a combined effort of our group and commercial

entities, like Phillips Healthcare now, among others,

to get this material out into the clinical space.

So, thank you for your attention.

[APPLAUSE.]

MODERATOR: We have a few minutes for questions.

Q: So, these entropy methods – as I recall, this was –

    the statistician that first proposed these lived in

    sort of the '40s or something like that – right?

DR. WICKLINE: Shannon.

Q: Right, and so there was this long, long argument

      between him and some other big figures in statistics.

      But that's over, and has been, I think, for 20 years.

DR. WICKLINE: Right.

Q: So, why has it taken this long? I mean –

DR. WICKLINE: God, that's a good question that you're

      going to ask!

Q: [Chuckles.]

[CHUCKLING.]

DR. WICKLINE: [Chuckles.] I'd better bite my tongue.

Q: No, please.

DR. WICKLINE: Well, you know, I think – since we work –

      I'll give you an example. Since we work in the MR

      arena and the ultrasound arena, I'll tell you it's a

      lot more fun to work with companies in the MR arena

      than it is in the ultrasound arena because they're a

lot more amenable to open architecture, to innovation;
and it just seems to me like the ultrasound community
has not been open, not only – well, the academic
community is fine, but they're hobbled by getting
innovations into machines that have very, very closed
architecture.

And so you have to do it on your own. We've done
this. This has been done or – this really has been
done for ten years – or, at least eight years – at
least the initial part. But getting it into a machine
and convincing someone they should try something new
simply by creating a new ASIC [phonetic] or something
like that – it's almost impossible. You know, you
have to do it yourself and demonstrate that it's done.
But it's very hard to do it on a machine, so really –
you know, we can do this 'til the cows come home in
the lab, but until you get it onto a clinical machine
– which is not that hard to do, and there're many
other examples of that – then it becomes very
challenging to penetrate clinical medicine.

Q: So, that's not because of the clinicians who don't –

DR. WICKLINE: They don't even *have* it.

Q: -- [crosstalk] – it's just the companies that aren't –
      [crosstalk].

DR. WICKLINE: Yeah. Well, I mean that's part of it, of
      course. And then there's always resistance to
      changing the way you do things. Everybody has that
      proclivity, but I think – yeah. I mean, you know,
      classical tissue characterization ran its course and
      was pronounced dead ten or 15 years ago; but it'll, of
      course, come back – and particularly when it has a new
      need, like this, I think; because these things can be
      done quite readily.

      So, this is the next phase of that, and it's – it's
      the newness of signal processing to other folks who,
      you know, have their interest in certain other ways of
      signal processing that, I think, partly keeps it out.

      Okay.

MODERATOR: Sounds like coffee. Remember to come back….

[INFORMAL COMMENTS.]