CHRIS CHEN: My pleasure to introduce Rohit, and he'll tell you about what he's working on.

ROHIT BHARGAVA: Thank you so much, Chris. I'd like to thank the organizers for giving us the opportunity to be here.

Today, I'll talk to you about something that is actually rather new in imaging itself, and there aren't very many reports of this being used in tissue engineering, so I hope this is really a great opportunity to get some feedback from you as well and tell you what we've been up to.

I'll talk to you a little bit today about an emerging technique that we call chemical imaging, but, before that, I'll give you a flavor as to what we're trying to do.

So this is a piece of tissue that many of you might be familiar with. We section it, stain it with HME, and, most commonly -- Sorry, this is not tissue engineering. This is actually cancer, and this is pretty much the diagnostic standard in cancer that you would stain a tissue like this. A pathologist would look at it under a microscope and then circle regions.

Here’s a tumor. Here’s a tumor. Here’s some normal tissue.

What we’re trying to do in our lab is actually provide views like this in which we say, Here are the cell types that are relevant. In this case, this is a piece of prostate tissue, so the green are all epithelial cells where most of the prostate cancers arise. The pink is all the other cells we just lump into stromal.

When we try to do disease diagnosis, we sort of forget about the stromal for a second and just focus on epithelial cells. The ones that we label as tumor, then we can color code differently, and the ones that aren’t we sort of leave off as green itself.

So this is much about sort of a development state. This is sort of a snapshot in time. It’s more relating structural changes in the tissue to something that we can color code and make accessible rather easily.

We’ve taken this a step further in the last few months or so, roughly, because what one does normally, at least in the cancer domain, is actually to get functional information, take a variety of stains. So

what is it that the tissue is actually doing? How aggressive is the tumor, for example? You would actually look at molecular markers of things.

And what we have done is looked at the same molecular markers, at least try to predict the expression levels and the spatial distribution of the same molecular markers, except this panel on the right is done without using any stains. This is without using any dyes. This is without using any exogenous agents.

What we are doing here is capturing the intrinsic molecular composition of the material non-destructively using an optical technique that I'll talk to you about in a second and trying to reproduce the functional and sort of developmental state as well as the histology of tissue that we actually see.

And here's a quick sort of relevant slide here. This is actually a physically-stained specimen with HME. This is a computationally-stained specimen. This was done in about 30 minutes. This took about three minutes to do.

But for this audience, I think more importantly, what this does is it sort of removes the intermediate steps. You remove the staining. You remove the

manual-recognition process, and you have a much closer relationship with your data.

So as you get pieces of tissue out, you can start to visualize what is the molecular content and the structure detail in your data without needing any external help. So this could perhaps speed up the process in tissue engineering. It could perhaps improve the amount of information that you get.

So on one section, now, you don't need multiple sections to stain and look at under microscope. On a single section, we can provide many, many different levels of sort of molecular expression that you can see. And I'll give you more examples of that.

So to summarize, what we're really doing is we're not using any stains. We're not using any dyes, and, hence, as opposed to molecular imaging that you'll hear a lot about, we call our technique chemical imaging.

There are no manual decisions in this process. So we collect a lot of chemical data [unintel.] in a couple of slides and actually use computation on the back end. So we have no choice in this case. We are using

computation sort of built in, and models can get progressively more complex.

But, really, what we're placing our bets on is that by combining chemistry and structure both at the same time we're somehow doing better than just structural analysis alone. In fact, we're doing perhaps better than just looking at ground-up chemistry or sort of average chemistry alone, because we can provide the distribution of both techniques.

So this seems like magic, and there are two aspects to remember. There's a fair amount of technical background in this, and there's obviously a price to pay. So in molecular imaging, what you have to do is recognize which molecule you want to label, design a probe against it that survives and gives you the right readouts and then go image it.

But, in this case, since you don't have any probe, you have to be very, very careful about what is it that your data mean. You can record data quite easily, as I'll show you, but then there's a lot of information in it or there's a lot of work to be done to extract the right information, make the positive correlations and make sure that you're doing this in a robust

manner and so on. So we have a few challenges that I'll also point out to you as well.

So, again, just to recap, the principle of the process is that we're using the intrinsic chemistry present in the materials to actually make decisions. Our particular content, in our lab, we have a particular in two forms of spectroscopy to do this, infrared absorption or FDIR imaging that I'll talk to you a little bit about, and Raman scattering spectroscopy. Both of these essentially monitor the fundamental vibrational modes and molecules and then one can extract out correlative sort of expressions in larger scales.

So these are very active fields, and there are a variety of flavor of these. I'll try to give you a sort of overview as to where many of these technologies are going and what the state of the art is.

Of course, you can keep doing this for a variety of other fields. You can go to neuron Fred [phonetic] or visible. But then what you do is get overtones and combination modes of the exact same information that you have here. So perhaps that's a little diluted and

the specificity of that information becomes a little bit less.

But with neuron Fred [phonetic], again, you can penetrate much deeper, so you have this tradeoff, not just in space and time and coverage, but also in the molecular specificity of things that you can actually see.

Of course, you can keep going and do MR spectroscopic imaging, which allows you to penetrate much, much deeper, but then you can only see a handful of metabolites and not a whole lot of structural [unintel.].

So, again, there are a variety of tools available, but then you have to choose what level of detail, molecular, spatial, and what level of coverage and what depth you require your data and perhaps choose the right tool to go with it.

So our particular -- I'll discuss infrared spectroscopy a little bit in the beginning. The technology actually is rather old. This is the same interferometer that Michelson used about 100 years ago to prove that we all live in vacuum, not in ether, perhaps. And so we use actually essentially the same

technology, take light out from the interferometer, pass it through the sample and go to a spectrometer.

Again, this is nothing new. About 100 years have passed where people have made correlations on sort of the measles-scale chemistry, not molecular-level understanding and biologics, but more on the sort of level of classes of molecules. How much carbohydrate is there? How much protein is expressed? What is the structure or secondary structure of the typical proteins? How much nucleic acid is there? Where is it distributed? And so on.

So all of these are available in the so-called spectrum in which you have peaks over here that indicate very specific molecular classes of things, and then you have broad backgrounds that actually give you structural information, scattering or how light transports through tissue, for example.

What we have been able to do over the last 15 years or so is actually change this from a one-spectrum-at-a-time technology by putting in new detectors here, by collecting data differently and now turning it into this sort of data cube.

So what you have is XY information, which gives you the spatial information in a sample, but at every pixel, you have a spectrum like this. So for every image, you actually have 2,000 images or so, roughly, that go beyond that. And, certainly, you can imagine that our datasets run into tens and hundreds of gigabytes without any effort whatsoever.

There's not much interest in collecting data. I only want to give you one sort of important sort of idea of the progress in this field. When I started with this technology as a grad student, to image one centimeter by one centimeter would take about seven years of data-acquisition time.

Today, in our lab, thankfully, we are not at the same stage. Today, we can do that in about three or four seconds, roughly, give or take a second.

So, really, the instrumentation, the speed and coverage have come a long, long way and overnight they've changed. Only took 15 years or so.

Really, the challenge today is how do we convert this data or this massive amount of data to knowledge? How do we extract out specific molecular or specific functional changes in tissue from this mass of data

that we are actually extracting? And how do we take out overlapping and remove things that might be non-specific to the problem that we understand?

So we've adopted a variety of approaches to do this. It involves both a combination of cell and tissue engineering at a very rudimentary level, as well as fundamental optical theory and certainly modeling of chemistry.

So I'll step you through. The next two-three slides are showing what we're doing in that regard.

So the first thing we can do is take a very bottom-up approach. We can say we know what molecules we have. We know what cell states we have, and we simply record the data.

So if you go back to your Chemistry 101, it tells you here if you have a small molecule, you know, each and every vibrational peak can be modeled and actually assigned to some molecular vibrational motion. That's obviously not possible in large complex molecules.

So what we do in cell types is actually we stimulate a variety of conditions. We culture cells. We coculture cells. We give them different kind of

stimulants. And we actually record data in the spectral domain that tell us if we do this, this is what the spectral [phonetic] look like. So this is sort of a bottom-up approach.

Now, you can take a top-down approach in which you can take multiple conditions of the same type. So you might have reactors that you tune slightly differently and experience the whole range of things that can happen.

So in our case we do this by tissue microarrays, especially since we're looking at cancer diagnostics. And the idea is quite simple is that if you can induce all sorts of possibilities that you can possibly have in your sample, then you have a variety of conditions that you can then image and correlate all kinds of information to actually give you data. So this is going again from all known conditions back to the spectral data and so on.

We've also done a fair bit of work trying to relate what the origin of the variability as well as the signal is. So it's not just the signal that's important in these samples, as you might imagine. It's also the variability. And using statistical models, again, with these datasets, we can actually

now pinpoint exactly how variable one cell is from another, whether that variability arises from its optical properties or its chemical properties and so on. So there's a fair bit of understanding in this way.

When we put all of this together, we can take fundamental modeling. We can take our known tissue constructs. We can take this variability. And then what we can do is take the optical understanding of the data. So all you saw previously was sort of approaching this from the biologic side. We've also spent the last three-four years actually modeling our process or modeling our data-acquisition process starting from Maxwell's equations from the source to the detector entirely. And, now, we can understand exactly what our optics means.

So, again, combining optics, combining biology and combining statistics gives us an idea of what our data possibly are doing.

I just want to leave you with some quick sort of updates as to what's going on in the field. This is the best state-of-the-art commercially. Previously, we were at a resolution of about five micron or so per pixel, because of throughput considerations, because

of sampling considerations and so on. Just last year we showed that we can actually go down to about a half a micron per pixel and these are what the data look like.

So, now, in our lab, we can actually look at fairly complex tissues. Of course, again, there's a tradeoff when you go smaller and smaller and try to become more specific in a faster time. You lose signal to noise ratio and you have to pay a price for that.

So we develop instruments to take care of that, and we can now actually make images that look something like this. So this is colon tissue, and here's a little focus of five or six cells. In the commercial state of the art, you can't actually see these, but, again, in the lab, we can actually look at individual cells and even examine subcellular domains within infrared.

We've also spent a fair bit of time actually extracting information from this. So this is a combination both of basic knowledge, sort of the forward modeling as well as the statistical pattern recognition. So in the same sense that you'd look at gene-expression data and try to recover a functional state from this, you can look at optical expression data and try to recover a functional state.

So at this end, we take our recorded data and come down to a smaller dataset that we actually understand from our models from the optical modeling as well and from the pathology side or from our tissue-engineering colleagues. We say, What is the question that you want to ask? And then we go around and build an actual algorithm. So for every single case, we actually build a different piece of software that does a very specific function.

So if you would like to figure out what is the molecular expression of these four, five or six things in your samples, then we would build a whole framework off the algorithm, off the underlying statistical analysis of the underlying variability, off the underlying theory as well to predict what your accuracy will be and what your results are likely going to look like. So there's a fair amount of computational modeling that goes on at the back end. But at the end of the day, then, we're ready to do some of the tasks that you'll probably see in the next few slides.

So here are some examples of what you'll actually see. We started off trying to do some very simple things. Here's mainly work that shows you that you can find

all 10 cell types in prostate tissue that you would like to predict clinically. So, in this case, in the clinic, you would perhaps label them with HNE or molecular markers and then look at different slices of tissues. Here, we can do this all at once.

The same thing can be done for colon tissue. We can keep going on and on, for lung, for breast and so on, but I don't want to bore you with those details. I just want to give you two pieces of information. One is we have a very precise way of saying that if we label a particular cell as one cell type or as a functional, you know, up-regulated or down-regulated cell in a certain molecule, we can give you statistics on that. So, statistically, we would tell you how accurate we are likely to be.

And in this case, what we are trying to give you is the sensitivity and specificity tradeoff in looking at identifying individual cells and colon tissue in clinical biopsies that we have not really controlled, other than just getting them to our lab.

We've also validated these in well over 800 patients now in the colon, for example; 2,500 in the prostate and 1,250 or so in the breast. So there's a fair

amount of rigorous validation of these things that you will see.

We can also look at many different cell types, as you saw in the previous case, and this is actually particularly important in the field of cancer, because, now, the understanding is evolving from just one cell type being an active player to multiple cell types interacting. So the question is can we see multiple cell types? Can we see their transformations? Can we localize where they are spatially and give a quantitative analysis of all that?

So, certainly, we can localize cell types. What we've been working on is expanding this list of things that are sort of the non-epithelial compartment and tissue. So perhaps in tissue engineering you can see some of the parallels that we look at our clinical imaging data and start to see that we can actually look at many of these things at the same time.

One of the things that we've become very, very interested in looking at is trying to go back to single molecules, trying to go back to a genomic basis and receptors and trying to see if we can -- how far can we push this technology. Can we actually start

seeing coexpressions of things and individual expressions? And so I'll talk to you a little bit about that.

But before we go there, I just wanted to give you a few quick examples for where this technology is going. As I mentioned, the speed has become now quite rapid. So we can actually image fairly large sections, and this is an attempt to go in operatively where we can actually start to see now actually cancers there as well as their molecular expressions.

Again, our ROC curves compare rather favorably with what is seen in the clinic, and we valid it again on roughly 800 patients in this case.

So, certainly, for clinic, where we're pushing in that direction quite a bit, we haven't quite pushed as much on the molecular engineering side or on the cell-and-tissue-culture side, but, intrinsically, as I showed you a few sides ago, that has been an effort in the lab. We really need -- We have no choice, if we want to develop a clinical assay, but to go to the lab and actually start to culture more and more realistic microenvironments that mimic what happens in clinical tissue as cancers drive forward. So we've done a fair bit of that.

But, again, before that, I wanted to interject a couple of slides that our colleague Marcus has been kind enough to provide me. And what we have not been about to do with infrared is get these very high-resolution views of cells in subcellular changes, and Marcus has been a leader in this. So he has used Raman spectroscopy and, more commonly, or at least in his lab, non-linear approaches to Raman spectroscopy. And what one can do now is actually look at individual cells, compare them with conventional sort of molecular staining and use mathematics now to both recover accurate data as well as to sublabel or segment out different parts of the cell.

And, again, this thing can be taken down to a science, and what you can do is actually look at all kinds of differentiation. In this case, Marcus' group has looked at stem-cell differentiation. Again, what would perhaps result in more adipocyte-like cell lines would have more lipids in them, what would be more osteogenic would perhaps have mineralized components or at least the early evidence of those. And those are spectroscopically actually quite easy to separate out.

So if you can get fast enough data like their group can and you can actually implement a little bit of math of the background, which now we can do routinely, you can actually start to make decisions, start to see right away, without any help, what your cells and tissues are doing down in the samples that you have.

So, again, continuing sort of in this trend, what we have shown is that we have a molecular technique here, and we're trying to make sort of tissue-level decisions or cell-level imaging from those decisions. And the question to ask is can we actually take cell biology and integrate sort of this molecular, cellular and tissue levels all into this one package that makes it easy for somebody to do studies to measure data and gather information from.

So what we've been doing in this regard is actually a very rudimentary -- and I apologize to my tissue-engineering colleagues. This is actually very simple sort of stuff that we started in our lab, but it might be trivial for you.

We started looking at actually skin models and looking at melanoma and seeing how melanoma would progress and whether we can measure that. So this is actually well established in the clinic, the non-commercial sort of

skin replacement, tissue-engineered constructs available.

So we collaborated with one such group. We seeded melanoma cells, and we asked the question that could we measure changes in melanoma cells, could we track them as well as measure changes in the surrounding stromal?

And, again, to cut a long story short, this project evolved into not just a tumor-progression study, which we were originally interested in, but as a way to characterize tissue, as a way to get quick histology out, as a way to look at drug diffusion in these constructs and compare them with human cells, as a way to actually model transformations in cells. For example, how do fibroblasts change when you input TGF beta in three dimensions and how do they model?

So the back end of this is actually mathematical models to look at, drug diffusion to look at, cell transformations and all that. And those are very easily integrated into the computational models that we have.

I just want to leave you with a quick result from some of these studies, and the quick result is that when we

look downstream or spatially offset a little bit from the growing melanoma lesion, we actually can monitor changes in the surrounding collagen. We don't know what these mean. We can just say that this part of the collagen is different from another part of the collagen, and that's about the first level of information that you can get.

The second level is, of course, then you model the [unintel.] to go deeper and deeper. But, perhaps, a more interesting strategy would be to look at some other techniques which can give you really detailed information on the collagen, except, now, we can pinpoint where you need to look.

And it turns out, in these models, at about 30 days, you only need to look at about 100 microns around the growing tumor. After that, the effect of the tumor sort of dissipates very, very quickly.

So this was an initial attempt many years ago. Since then, we've really extended this quite a lot in the lab. And, now, we've made fairly more sophisticated models, so we start from one D-cell culture to 2-D, in which we have actually a lair of collagen on which cells are sitting, but they're still flat to do the tissue that you just saw in the previous slide, to a

sandwich [phonetic] assay in which we can peel off layers of cells, put cocultures together and take them off to actually [unintel.] cocultures.

And the idea here is not so much as replacement organs or to understand [unintel.], but it's to take these cultures and drive tumors to grow based on the stromal control. So there's a whole science behind this.

And what we are really, really interested in doing is looking how these spheroids interact with fibroblast in the very initial stages. Can we take changes in the stromal and start to drive these arrested-growth spheroids to become a more proliferative phenotype than is there possible.

And if we can do that, then perhaps we can take the same signatures we actually focused for a variety of reasons in the protein signature of this interaction and can we then translate this into the clinical datasets that we've already measured with our spectroscopic studies in the past?

So this is sort of the overview of where this is going. I'll try to give you, in the remaining two minutes, a couple of results with what we've done.

So this is a particular system in which we've taken two well-known cell lines, one is more of a tumor mimic, the other is a normal cell line, and tried to drive the normal growth-arrested spheroids into a more proliferative phenotype.

And here's the evidence for that. What we are actually doing is activating the fibroblasts and hope that that activates the MCF [unintel.] that are cultured there. And as we are doing this, we can take imaging and measure what's going on.

We can actually capture the degree and quality of proliferation, but then we can go back and start profiling. This is through protein arrays, so we actually know the particular proteins that we're looking for and we can start to see which ones are up-regulated and down-regulated.

So in this particular case what we were trying to do is figure out if there are early stromal-epithelial interactions, which would give us clues to Tomoxifen resistance later on in breast cells. And we actually came up with a list of 17 proteins.

The interesting part is each one of them by themselves is only moderately sort of predictive, but when you

put it altogether and actually go back to clinical data, we see a slightly more -- a chance of prediction.

And so this is our initial attempt at doing this. We're trying to refine our methods a little bit more. But, nevertheless, the idea is that if we can measure a particular protein phenotype, then we can perhaps quickly go and try and figure out if it has clinical import.

We can actually monitor people in real time by building probes that can go inside the body and make these measurements without too much trouble.

So we've done a fair bit of correlative science, again, with this. We've gone back to the gene and protein level and tried to predict what we see in the optical domain. So, again, this has to be very specific and there's a fair bit of work behind this, but, nevertheless, in some cases, we're able to predict if there's a molecular change, if there's a phenotypic change in the cells, we can pick it up optically, and, in some cases, we're not able to do this. So we can pick and choose what we would like to do.

So this is interesting. What it does is it gives us a sort of phenotypic state. It doesn't give us an exact molecular level of understanding. If you said is PSA up-regulated or is the estrogen receptor alpha up-regulated or down-regulated in this case, I can't perhaps give you a very definite answer. I can give you on-an-average answer and can give you that perhaps it's regulated to this precision or that, but I can't image where it is. I can't image the quantitative nature of this expression.

So we started asking this question: Could we use spectroscopy, which has a lot of molecular discriminatory power, to actually look at molecular discrimination in cells and tissues?

And, again, this is a sort of general question that a lot of people have looked at, and the idea is that could you build an assay which would give you single-molecule sensitivity, which would allow you to profile tens or hundreds or thousands of molecules at the same time and also perhaps give you spatial localization? Could you use the same thing in vivo? Could you use the same thing ex vivo? Could you get heaven and earth essentially in the same platform? And how far can we push this?

So, again, this is a fairly long effort in the lab. We've done two ways in which we can push this. We've used surface-enhanced [unintel.] and spectroscopy. The first one being structured surfaces where we actually have to extract out our lysis cells and then you can start looking at antibody sort of conjugated expression levels on this.

The second is perhaps more interesting for this audience, and what we've worked on is actually making nanoparticles that use conventional conjugation chemistry to actually go target things.

So the chemistry, the biology is nothing new. What is new is this signal transduction mechanism. So we are taking a molecular expression, turning it into an optical signal, enhancing that optical signal by surface-enhanced [unintel.] and trying to get both single molecule and molar concentrations in the same time.

And the particular flavor of probe that we're using is a sort of onion-like structure, and what it does is it consists of metal and dielectric alternating layers. So there's a metal gold shell. There's a dielectric with a dye loaded in it, a metal shell and so on.

And the challenge here was predicting how far we can go with this. Can we actually get single molecule sensitivity or not?

So, again, we've done a fair bit of modeling in this. So we started from basic electromagnetic modeling. We were able to predict -- So these are predictions only. This is not proven yet. We are able to predict that you actually don't need more than six layers if you use this onion-like structure. You have to structure the layers correctly, but you don't -- you need about 100 nanometer particles, six layers and a resonant dye, and then you can perhaps get down to single-molecule level.

And, of course, by tuning the dye, by changing the layers and so on, you can tune different sensitivities. You can tune the dynamic range of your assay. You can tune the molecular identification of your assay and so on.

Of course, making these particles is very, very challenging, and we've been trying for about three years now. We've gone up to four layers where we've shown that we can get a reliable million-fold or 10-million-fold enhancement in some dyes, but we have another six-seven orders of magnitude to go and we

need two more layers that have been very, very tough to synthesize so far.

So this is where it's at, and, with that, I think my time's up. I'd just like to acknowledge the support of both NIH and NSF.

One thing that perhaps didn't come up here and I think should be is we really need this integrated support from both technology as well as the medical side, both NIH and NSF to work together. Otherwise, these tools would not actually have functioned.

So -- and this is my group and collaborators. Thank you so much for having me here. I'll take a few questions. [Applause].

MALE SPEAKER: Can you say a word, maybe, about [unintel.] versus your approach [unintel.] and what are the advantages or disadvantages? How would they compare and perhaps also how expensive are these technologies?

ROHIT BHARGAVA: So the question was how does this technology or these technologies compare to mass spec imaging and what are sort of the benefits and

disadvantages of each? And what would sort of win out at the end, I guess, is the question.

So with mass spec, of course, you get protein information. So you're focusing down on a specific class of molecules in your tissue. You're destructive. So you can't monitor that in real time.

One of the things I didn't emphasize was one direction for this field is we're trying to become faster and faster and try to go in vivo, so that you can actually see tissues in real time. You can start to see everything I showed you sort of as it's happening, which is not possible with mass spec.

I think the perhaps best answer to this question lies not so much in the competition of these technologies, but in the integration of these technologies.

So could you use these technologies to pinpoint where we should look at with mass spec? Rather than profile large pieces of tissue with mass spec, could we say that if we focus down on this particular area, for example, there are only a few cells. We can recognize now epithelial to mesenchymal transition very easily in culture, you know, spheroids using spectroscopy.

So could we pull out these three, four, five cells and say these are the ones to look at without labeling and these are the ones to track as we go forward?

So I think perhaps that's the answer is the combination of these things, because they offer different advantages and different benefits.

FEMALE SPEAKER: [Unintel.].

ROHIB BHARGAVA: So the limitations of this technology, so with -- Right now, the limitations are speed, number one. A lot of groups -- Marcus, Sunny She [phonetic] at Harvard, for example, and others -- are trying to push Raman spectroscopy to become faster and faster using long [phonetic] linear techniques. Of course, the cost goes up and you won't be able to afford a lot of these techniques quite easily.

There's also theoretical problems with things. So there's actually technology that limits.

We're trying to push infrared spectroscopy to do three-dimensional things, and the theory isn't easy, so we've resolved that.

I think we're not able to do 3-D in the ease and nice way in which confocal microscopy can do today. We certainly cannot label things with the specificity and the sensitivity that sort of fluorescent labels can do. We cannot introduce a diversity of where we want to measure things. The size of the probes that you see will never go down to the level of a fluorescent probe.

But then the advantages are that you don't have to label sometimes. The advantages are that you measure a lot more things -- You can measure the polymer scaffold, look at the structural changes, even the orientation of the polymer scaffold and the cells and the different kinds of cells and their cellular activation all at the same time.

So, for certain things, I think this technique is worth considering, for certain things that are already existing techniques.

FEMALE SPEAKER: Hi, I just wanted to ask about the state of the tissue and the cells you were looking at. For example, you early on showed some very nice of your digital historiography. Were those cells in the hydrated state? Because, typically, with FTIR, you

have issues with large water absorption bounds [phonetic] interrupting with the spectra.

So in our lab, we're using Raman spectroscopy to -- when we've seen the neuro stem cells and we can phenotype them into neurons and gleocells [phonetic]. We've also looked at specifying collagen within inginate skin.

The other question is wonder what your light source was because typically vibrational bounds are very broad and it's non-trivial to interpret these bounds, and I wondered if your light source was a syntechtron.

And the third question is with regards to in-vivo imaging, do you think this is restricted to an endoscopy approach due to the sort of limited penetration of light into tissue?

ROHIT BHARGAVA: Sure, that's -- Let me take those questions one at a time.

So the first question was that can you -- Some of the data I showed you were mostly fixed cells. So these are ex-vivo fixed samples as if you would do histology on them, the sort of standard sample preparation.

We have done these in wet tissue and so on. There are two ways to do it. The first is to limit the intraction [phonetic] field with water, so, obviously, you don't get the absorption.

Water absorption is actually not a problem. The problem is our source is not bright enough. So the source that we use is a heated wire, because we actually need broadband light to come through.

A syntechtron is indeed possible. You can do life-cell imaging and certainly wet tissue with syntechtrons, but there are only so many syntechtrons in the country and it's pretty expensive to maintain one.

We have now, collaboratively with a company, developed quantum cascade lasers in our lab and we're testing those out. Those actually have enough flux for us to image a little bit deeper. So we can image easily millimeters through water, no problem with that.

But, again, when you image deep tissue, the data coming back or the data that you record are non-trivially related to the structure, and so that's where we're at right now. We're trying to work through that problem. Did I answer everything there?

FEMALE SPEAKER: Yes, thank you.

ROHIT BHARGAVA: Thanks.

MALE SPEAKER: I think maybe we should get ready for lunch. It’s down the hallway on -- That way at the very end on the right. [Applause].