MODERATOR: So, our next speaker is Bret Bouma, and he will be telling us about OCT.

DR. BRET BOUMA: Okay. Good afternoon. Thank everybody for, first, sticking with the conference this long. Second-to-the-last talk. So, I apologize for this, but my institution requires that I tell you that I have patent royalties, sponsored research; and I consult for companies whose products are related to the work that I going to talk about. I want to point out right away that several of my collaborators have contributed significantly to this work, and I'll get to that at the end as well.

So, the technology that I'm going to be talking about is optical coherence tomography, and I won't spend much time on the principles or the technique, but instead will try to get across some of the highlights of capabilities.

But just one slide on the background. So, what OCT does is measure the electric field cross-correlation of light back-scattered from a sample, and the objective is to have a very narrowly constrained

spatial illumination so you have transverse resolution and, for each lateral location, acquire[?] one reflectance scan as a function of depth – and, again, by measuring the electric field cross-correlation. So, if we take that data, and we map it to a Gray scale, or a Falls Color Look-up table, and then just display each of those A lines vertically, we generate an image.

So, OCT was originally applied to ophthalmology in the ’90s, and has become a standard for diagnosing especially retinal pathologies, but OCT was limited to a few images per second, and that really constrained the clinical utility. Fortunately, about 2002, we came up with an alternative way to collect that same electric field cross-correlation. So, the same data, same spatial resolution, but acquired with a different paradigm. That was the use of a narrow-band wavelength swept[?] laser, keep our reference arm fixed. And what that does is the interference encodes depth information on frequency, so that the beat[?] tone that we receive in the photodector is proportional to the distance between the reference arm

of the sample arm[?]. So, if we 4A[?] transform that data, it gives us back our standard A line from OCT.

So, the benefit to that approach is that we get something like 100 or a thousandfold improvement in our sensitivity, and that allows us to acquire many hundreds of images per second instead of just a few, while actually increasing the range of the image without compromising any of the resolution or contrast that we appreciated in OCT.

So, some of the previous speakers talked about mechanism for overcoming attenuation of ballistic propagation of light within tissue. Photoacoustic is a very elegant solution that lets us get down to several centimeters. Implantable approaches are just being explored. Some very good promise there.

Our approach with OCT has been to use catheters and endoscopes and narrow-gauge needles to bring the delivery point and the collection point of light to the lumen, or the organ, or to the imaging site. Fortunately, with OCT, we deliver light, and we collect light through a single-mode fiber. So, that

can be as small as about 80 microns, so it's very easy to make very narrow-diameter, very flexible catheters. In fact, we've found ways to do that. They're all monolithically integrated and can be made with a standard fiber fusion splicer. So, it doesn't even require micro optic alignment or fabrication. And that type of a device can be embedded in a conventional catheter scheme and then wedded to the standard configuration – interferometer configuration – to do scanning in a helical or rotational paradigm.

So, this is just a representative image of a probe that we use for imaging the esophagus or a large luminal organ in the body. And you can see there's a balloon for centering the optics, and we've slowed down the rotation, and we've coupled through a visible light to show the rotational property of this. So, each time it does one rotation, we get a cross-sectional image because the interferometer is measuring the reflectance as a function of depth at each A line. And then while that inner core is pulled back through there, it maps out a helical scan. So, in most of the tissues that we're talking about today,

we image with light that's about 1.3 microns, so that's in the infrared.

So, this is data from a human subject at the esophagus, pulling back this helical scan in real time, and the clinical value of this is that now we can see the full esophageal wall. This is the outermost layer of the esophagus. We can see the epithelium and the muscularis mucosae here, where the – these are vessels, and there're lymphatics as well in that layer.

So, the goal of endoscopic screening and surveillance is to detect diseases within this very superficial layer, but to be able to see that through the entire wall into the muscularis propria.

So, once we've acquired that full helical scan, then we have, perhaps, 100 gb of data that is very powerful in terms of how we can represent that. So, we can look at, you know, the full cylindrical structure of the esophagus. We can look at it from the inside out, the outside in. We can do cross-sectional images longitudinally, or in the circumference. And, again,

we preserve the architectural morphology, which correlates quite well with histology in terms of seeing the discreet layers within the wall of the esophagus.

We can also do maximum-intensity projections of the data to see features such as the vasculature. This is quite convenient if we want to look at multiple time point structures. We can use that vascular map to navigate by at each successive time point.

Another presentation we've found convenient is to take that cylindrical volume and slice it longitudinally and open it, look at it from the inside out. And what we see very easily is regions such as these, where the nuclear-to-cytoplasmic ratio is enhanced through surface maturation, indicating dysplasia, which is a disease that we'd really like to detect at an early stage, rather than waiting until it becomes intermucosal cancer.

So, in our early clinical application of this, the paradigm was do this volumetric scan, inspect the entire esophagus, because what biopsy and endoscopy is

capable of and is normally used for is to sample just a few, discreet locations within this whole volume. At most, the pathologist will see something like .1, or 1 percent, of the entire volume. So, they'd be sampling tiny bits, looking for these focal regions of disease onset.

What we can do now is map that full area and volume out and then direct biopsy back to confirm and to let the endoscopist have[?] his biopsies where that information is most relevant. So, we want to take this dataset and now direct the endoscopist to biopsy. So, what we do is we leave that same balloon in place. We identify our regions, perhaps by the presence of curiform glands, and then we deliver light of a slightly longer wavelength, where the water absorption is strong, with a slightly higher power so we can deliver enough energy to coagulate the tissue just at the epithelium; and it leaves a nice, little, white tattoo so that when we pull our probe out, the endoscopist can go back and then sample those locations exactly.

So, the clinical significance of that is that we've recently shown that we can actually perform this exam without conscious sedation, without Versid, and without endoscopy. So, now you've transformed a screening procedure that was previously too expensive to apply to the patients in the U.S. with refractory reflux disease into something that could potentially be done in the outpatient setting. So, you've enabled screening that couldn't be applied to a large patient population previously. And also, we've changed the concept or the paradigm for surveillance from being biopsy and point sampling to being comprehensive evaluation of the full esophagus.

So, the status of this technology now is that recently the company, 9 Point Medical, has a device with FDA approval and a reimbursement code, and they're just releasing their product now.

Other sites within the GI tract that are of interest for especially the realm of cancer is in the biliary tree. You have the stomach here, and at the duodenum there's a small tube that leads from the duodenum up into the common bile duct and that also leads to the

pancreas. There are reasons why we would like to screen for disease in these areas as well; and, fortunately, there's an endoscopic procedure. You can see the endoscope here, coming down through the stomach into the duodenum, and it places a "daughter" scope, or a probe, from that endoscope up into the common bile duct so it can look for, in particular, colangiocarcinoma of that organ.

And it's very easy to make a device, an OCT or OFDI[?] probe that can go up in that channel and image, and in this case we see these features which correspond very well to the histopathologic features of colangiocarcinoma in this case.

What about pancreatic cancer? Incidental findings of cysts in the pancreas are becoming more common, and the dilemma is what to do. What physicians would like to do is to distinguish between cirrus and mucinous cysts. One is cancerous, and one benign. And the instrumentation of the pancreas through the common bile duct is very risky because of the risk of pancreatitis. So, instead, endoscopists have come up with a procedure where they take the endoscope into

the stomach, and they pass a needle through the stomach wall and into the pancreas body, guided by ultrasound.

And what we've done is to make a very narrow-diameter imaging catheter that can go down the bore of that needle and perform that same type of imaging directly through that biopsy needle. So, that's just with a 250-micron diameter catheter. And our colleagues Nick Iftimia [phonetic] and Dr. Brugge [phonetic] have demonstrated now that they can distinguish between those two different, very important clinical features of cirrus and mucinous cysts.

These same devices, the endoscope and the needle, can be used in the lung. One of my colleagues, Melissa Suiter [phonetic], is just beginning that clinical investigation now, and her focus is on two, separate issues. One is in guiding the location of biopsy in the central airway through bronchoscopy, and the other is by guiding the placement of a needle in terms of transferasic biopsy of nodules. And she's already shown that the wall of the lung is very easy to image with OFDI; and, in fact, we can see well down into the

cartilage surrounding the lung. And, again, this ability to image over a relatively large volume can guide the biopsy procedure.

Another area where OFDI has made – well, is *starting* to make an impact clinically is in coronary imaging. This is an angiogram, and you can see stenoses at different locations. But what would be really ideal is to know the pathology, the specific composition of that narrowing in order to guide the response.

So, one challenge for virtually all optical imaging is that blood attenuates the propagation of light through very strong scattering. So, one strategy that we've taken is to perform our imaging while the vessel is being profused with a contrast agent that's used for the angiogram. So, through the injection of that contrast agent, which is transparent to the infrared radiation, we have our helical scan. Now you can see the probe starting at the distal end and working its way back. We scan at about 8 centimeters of vessel in just a few seconds.

So, this is just a glimpse of the data that one gets in real time. It's actually slowed down by about a factor of 2 for display. But now, once we have that volumetric data, we can do segmentation. And as the previous speaker mentioned, not all segmentation can be done robustly in an automated fashion, but certainly the identification of artery wall, macrophages and stents can be done in a very automated way; and then the identification of lipid and calcium can be done in a guided fashion and propagated from frame to frame, using criteria that were established and validated in histopathologic correlative[?] studies.

And that allows us to generate volumetric images like these, where we can see the prosthesis, or the stent. You can see side branches. You can see calcium and macrophages and generate fly-through perspectives like these that, you know, obviously are not essential for diagnosis, but kind of enhance the ability to visualize the morphology of the vessel.

And this technology is now being used for many different, important, clinical observations, providing

better understanding of atherosclerosis and acute myocardial infarction. And this is just one case example, basically, of a plaque ruptured during a patient's time on the cath lab table. So, the first pullback image showed one region of the vessel where there was a lipid-rich plaque with a relatively thin cap. Fifteen minutes later, that same vessel was reimaged, and that same anatomical location now shows a dissected cap and, in fact, was reimaged immediately then; and just with a three-minute interval, you can see that a portion of that plaque now has been extruded into the lumen. In this case, the patient is on heparin, and there is no clot; but this would normally be the case, which would trigger a cascade of clot formation and blockage of the vessel. And then 42 minutes after the procedure, the wall has already started to close off.

So, the clinical significance for the cardiovascular application is now the vessel and the plaque characterization can be performed in advance of stenting to, perhaps, guide stent selection and its placement, and then to follow up with a confirmation and look for correct placement of the stent, correct

deployment of the stent – whether there's poor apposition, or if there's over expansion of the stent. And the current status of the technology in the cardiovascular field – the intravascular field – is that this is now FDA-approved, and there are products by two companies, St. Jude Light Lab and Torumo [phonetic], that are available for clinical use.

So, follow-on technologies or capabilities for the clinical cardiovascular case include the integration of fluorescence imaging directly with the structural or morphology provided by OFDI with the hope that we can gain insight into molecular features. And fortunately, the optical catheter of OFDI can very easily be modified with, instead of a single-mode fiber, the use of a double-clot fiber so we can simultaneously collect the OFDI image as well as the fluorescence image, using that same sort of monolithically integrated optical element at the end of the probe.

This can be made even stronger through the beacon concept, for example, where the enzymatic activity of macrophages can be mapped. And this is just an

example here where, in the OFDI image we can macrophage presence in this rabbit model, and then through the molecular beacon we can actually distinguish between regions of activated macrophages and those that aren’t.

So, another unique aspect of biological tissues that we can get at with OFDI is measurements of birefringence. Birefringence is a property of highly structured materials that have, typically, a high aspect ratio. That means that as light propagates through that media, eigenstates of polarization propagate with different velocities and, therefore, accrue a relative phase delay between those two, which we can measure very sensitively. And a few years ago, Smith and Carney in our group at Mass General demonstrated that there’s a very nice correlation between this measurement of biorefringence and the presence of both collagen and smooth muscle cells. So, the idea is that we can now use this intrinsic characteristic of the plaque itself to get at those molecular components and cellular components of the tissue.

So, fortunately, in OFDI, the implementation of the strategy for birefringence mapping is very, very straightforward. We can, instead of illuminating the sample with a single optical beam, we can illuminate with two. Those two beams both have the same wavelength, and they're both simultaneously delivered. However, they're frequency-encoded to two, different polarization states. So, this allows us to map the birefringence simultaneously without sacrificing any of the other characteristics of the instrument. And we're currently using that now to look at the composition of different plaques in different states and trying to understand some of the origins of features such as these – in this case, of a stent that has undergone instant restenosis, clinically.

So, what about resolution? So, this is an OFDI image that looks very different than the one I showed before. That's because I've blown it up to an extreme where this is 100 microns now. And we see some features which – this is the corresponding histology. You might say there's something there, but we're really not getting at it. This is a new approach for OFDI or OCT where we tailor the delivery of light so

that it has a very high transverse resolution over a large depth of field, using a technique called apodization. And we enhance the depth resolution by controlling the wavelength spectrum of light in illuminating the sample.

So now, instead of having something like 10 micron by 10 or 20-micron resolution, we have something like 1- to 2-micron resolution in three dimensions. So, it really transforms the ability to see the very smallest structures within the wall. And right now, we're applying this ex vivo. We haven't refined this technique to be compatible with intravascular use yet.

But these are just some glimpses of human cadaver plaques that we've acquired, ex vivo.

I'm running a little bit behind, so I'm going to go just a little bit quicker. This is a calcification. We've seen these features many times. Until we processed them in the transverse plain, we didn't realize that that was actually a cholesterol crystal. Smooth muscle cells show up just by their morphology, which corresponds directly to histology now.

Individual leukocytes, pseudopods and, in fact, transmigration of monocytes.

The perspective we gain on stents now is fundamentally different. This is a bare metal stent in a cadaver artery. This is a drug-lu-[unintelligible] stent. We were curious what these features were initially, and we finally resolved that that was actually the polymer coating the stent; and in many cases, we can see that that polymer has, in fact, separated or lifted up from the stent wall. We can do that same, very high-resolution imaging – huh. Huh. Sorry. That's a movie. Well. No, can't show you the movie.

So, we can do that same, very high-resolution imaging and look at dynamic functions as well by speeding up the acquisition. So, in this case, we could see the function of human epithelial cells in the case of wild-type and cystic fibrosis and see a dynamic difference.

Andy Yun [phonetic], a collaborator of ours, has developed a technique that he's referring to as "vibrometry," or "vibrography" now, where he

synchronizes the acquisition to a repetitive dynamic event, thinking of looking at the vocal folds in the tympanic membrane. And in the laboratory, they've taken this very nice, simple model of a moving sample – in this case, this is a battery-powered toothbrush with a gel-like phantom on it – and shows that, by triggering properly and reconstructing the image, you get a very nice, dynamic view of that moving sample.

So, angiography's been discussed from several different perspectives. Multiphoton microscopy has been the mainstay of angiography in small-animal models. This is a mosaic of, I think, 64 different stacks of multiphoton microscopy. Fantastic resolution. You know, the ability to see down to single blood cell-size capillaries. But you also see the effect of dye leakage, so you're marking the endothelium with a dye. You're not looking at the functional status of the vessel.

By comparison, we can use OFDI in kind of a Doppler technique, if you will, to image that same tumor, that same volume, all in one go. So, with our collaborator Rakesh Jain's[?] multiphoton microscope, this took

over an hour to acquire. Again, the stack – in this case, we can acquire the same tumor volume in just about a minute with OFDI. You see the same, exact vessels here, and you can see our resolution isn't *quite* as good; but we are seeing down to the single – the small capillaries, and now we have a very large field of view. We're doing this contrast-free. We don't have to inject any dyes. Therefore, we don't have leakage. We don't have integration, and we can repetitively image over time. And it gives us, in fact, a much deeper penetration. We can image down to about 2 millimeters.

This is a mouse brain in one of these cranial window models. You can see a glioblastoma in the upper left, a very good delineation of the vessels. It's a three-dimensional projection where the superficial vessels are color-encoded toward the yellow and the deeper vessels toward the red.

Again, I mention that the resolution of OFDI isn't quite as good on the finest scale, and if we look at the correlation between the quantitative diameter measurement with OFDI versus multiphoton, there's a

very good correlation above about 12 microns. Below that, we still see the vessels, but we misrepresent their size. But it's very nice that you can do this type of quantitative assessment systematically over the full volume of the tumor. And, in fact, you can do time series images – these angiograms – over a sequence of hours or days. This is a tumor in a mouse that's growing over time; and, in fact, this was a case of VEG-[unintelligible] blockade, so you can see changes in the morphology of the vessels inside the tumor and the periphery of the vessel over that time course as well.

A capability we were surprised to find was an insight into cell or tumor viability. So, if we quantify the scattering properties of the tissue within the tumor, we can actually get a measure of the viability of those cells; and we tested that with a model that uses a human tumor in a murine model. And through the administration of diphtheria toxin, you attack the tumor cells only and not the surrounding host tissue, and we can show a very good correlation between our measurements and the death of that tissue.

Another feature – if you look at this on – [unintelligible] – progression down, you see that stripe came up. We didn't understand what those features were until we looked more carefully and realized that's actually the lymphatics. So, we not only see the angiogram and the tissue microstructure throughout the tumor, but we also can map out the lymphatics – again, without needing to inject an exogenous contrast.

So, for the intravital microscopy portion, this is a technique [that] provides unique capabilities that augment those of multiphoton microscopy and confocal in the sense that without an exogenous contrast agent, we can perform lymph[?] angiography and get an insight into tumor viability over large fields of view and deep into the tumor in just minutes. So, unique capabilities for tumor biology studies.

But the question that we've been struggling with was, how do we best disseminate this capability? How do we make this available broadly? One of the things we've been able to do is to make a few of these instruments in our academic laboratory and put those in the mouse

colonies of our collaborators, but we really can't sustain that for a larger community. And, yet, I'm not sure whether this type of capability would represent a commercial opportunity. I kind of doubt that.

So, one of the things that we've done is to establish an IBIB biotech resource through a P-41 funding mechanism that allows us to reserve the capacity to respond to inquiries regarding collaboration. But I still think that there need to be other models for how we disseminate technologies like this. This is not a simple technology. I don't think that we can train people to replicate the instrument in just a few months. I think it's possible that we could set up other mechanisms for dissemination that allow instruments to be brought into the facilities of biologists, but we really need to explore that. How do we disseminate, how do we make available these new technologies?

So, just in summary for OFDI in general, we've really, you know, focused on its capability to image over large fields of view and, in the clinical case, whole

organs or large portions of organs, providing 3-D microscopy via minimally invasive access.

In the GI case and in the intravascular case, we have a clinical pull and a real commercial need that has driven the uptake of those systems, and we haven't needed investment. We haven't needed to worry about dissemination, because companies are very willing to do that. There's a profit in the end for the company.

I think there are ways to enhance that capability to integrate molecular capacity along with the maybe functional and architectural capability of the OFDI technology. But the thing in the context of this meeting that's quite exciting to think about is, what are the new opportunity areas where we can dedicate some of these resources and perhaps modify them and adapt them to applications in tissue engineering?

So, I thank you.

[APPLAUSE.]

MODERATOR: Do we have questions?

Q: How did you do the angiography images? I think you mentioned – [crosstalk] –

Q: The angiography images looked excellent. If you're doing Doppler, don't you have a cosine data problem? Or, is there some other way that you're recording the angiography images?

DR. BOUMA: So, many of these images and in the outset, we used the Doppler technique. And in the case of angiography, where we just want to see the vessels – see the regions of flowing blood – we don't care about quantifying flow. So, the cosine data factor becomes less relevant. We just set a threshold on the acquisition for a Doppler signature. When we see that – you know, there's very rarely going to be a case where there isn't some perpendicular component to the flow. Even if the capillary's coming, you know, directly perpendicular to the beam, you still have some turbulent flow. You still have a rolling of the vessel. There's always going to be some transverse component to the velocity.

However, what we've found more recently is that the Doppler technique is really not the optimal way to do the angiography, and instead what we look at is speckle decorrelation. So, we're looking at the very fine-scale decorrelation of the image from frame, or portion of a frame to portion of a frame and using that as a signal for flow. And that actually turns out to be robustly calibrated – or, calibratable to flow. So, we can, in fact, now quantify flow that we weren't [able to] before.

Q: So, I have a question that's probably stupid, but is there something as an equivalent as an objective? When you do your microscopy application, like, can you change magnification?

DR. BOUMA: Yes. So, in this sort of a prototype, we have designed our own objective, if you will. And because we have almost monochromatic illumination, rather than over the full visible spectrum, we don't have to worry about chromatic aberration. We don't have to worry about spherical aberration and flatness of field to the extreme that an Olympus microscope microscope objective would have. So, with just a few,

simple elements and then apodization through a phase mask, we can achieve, you know, micron-size focal volumes.

So, it *is* an objective, if you will; but it's just a few elements, rather than maybe ten or 12 elements, as you'd have in a conventional objective. And to achieve, you know, a 2-micron focal spot, the NA doesn't need to be extremely high – right?

Q: Right. You mentioned at the end of your talk, you know, availability of instrumentation and commercialization, so I know that Four[?] Labs, for example, has got these, I think, fairly inexpensive OCT setups.

DR. BOUMA: Yes.

Q: Nothing like what you've got in your lab.

DR. BOUMA: That's the problem.

Q: [Chuckles.] Okay. So, that's my question. What kind of resolution can you get with these? Is it – I mean it's sub-millimeter – right?

DR. BOUMA: What type of resolution can you get from the commercial system?

Q: Yeah.

DR. BOUMA: The resolution is fine. That's easy. And if you have a laboratory with very sophisticated image processing skills, you can go a fair distance down the road with those systems, but they're never going to have the capacity to do this type of precision angiography, for example.

Q: Right.

DR. BOUMA: And we've gone and visited Thorne[?], to their laboratories, their production centers; and we've seen their research capability. And the problem is they're completely divorced from the tumor biology community. They're not listening. They're not out there looking at the needs of that community, so they're not adapting – they're not focusing their capabilities toward that application.

And, similarly, if you look at the companies that are making cardiovascular systems, you can't simply take their instrument and use it for GI. It's optimized for cardiology and, you know, the cardiology companies, in order to make a profit, must focus on their product. They can't deviate. You can't go into a startup company that's making a GI product and say, "Can you make this for imaging the bladder?" or the cervix.

So, I think that, you know, there's a real need for pushing the innovation. There's a real need for mechanisms to effectively disseminate what's already possible.

Q: So, there's always an SBIR – right?

DR. BOUMA: I think – you know, the SBIR model has huge merit in many cases, but the problem is you have to have a viable commercial vision – right? And I'm not sure that there's a viable commercial vision for this type of microscopy. I'm not sure it would be profitable. I mean there should be a mechanism for dissemination that doesn't care about profit – right –

that cares about advancing science and making tools available to researchers. So, I think that there need to be some other mechanisms that help with that.

MODERATOR: Okay. Let’s thank our speaker again.

[APPLAUSE.]