MODERATOR: So, our next speaker is Lihong Wang from Washington University, and he'll be talking about photoacoustic imaging.

8:30 a.m. – Photoacoustic Tomography: Ultrasonically Breaking through the Optical Diffusion Limit

DR. LIHONG WANG: Thank you, Marc. Let me also thank Ralph and Gordana for inviting me to present our work.

I'm here to talk about photoacoustic tomography. It's an emerging technology. Our goal is to ultrasonically break through the optical diffusion limit. I'm required to disclose my financial interest with two companies which are commercializing photoacoustic tomography. We're funded primarily by NCI and NIBIB. Starting from the motivations and challenges, I'll be covering photoacoustic CT and photoacoustic microscopy and close with a discussion and summary.

Why optics? First of all, it's very safe to use light. We're talking about non-ionizing radiation, a non-carcinogenic form of energy. Ralph showed his EM spectrum yesterday. There's a tiny portion of that very vast spectrum for light, but that's the only portion that allows you to interact with molecules directly, whereas x-ray interacts with electrons and nuclei. That means by using light, we can probe essentially all molecules, including nucleic acids, carbohydrates, lipids, proteins and others.

Light can provide functional, metabolic and even molecular contrasts. Examples include concentrations of hemoglobin, oxygen saturation of hemoglobin, cell nuclei, blood flow, even the metabolic rate of oxygen, and biomarkers through targeted contrast agents and reporter genes. In addition, light can provide controlled activation for manipulation purposes, such as optogenetics and nerve stimulation. Light is also good for therapeutic purposes, with very low systemic

toxicity. PDT is one example. Photothermal therapy is another example.

There’re many reasons why light is so critically important, and it’s unique, but we face two fundamental challenges. Diffraction as a wave phenomenon limits the spatial resolution of all ballistic imaging modalities. Recently, it has been overcome for super-resolution imaging, as reviewed yesterday. Diffusion as a scattering phenomenon limits the penetration of all ballistic imaging modalities to about a millimeter in the skin. We use photoacoustic tomography to overcome this limitation to achieve what’s called “super depth imaging.”

This movie shows you how photons propagate in biological tissue. Within about a millimeter, the photon propagation transitions from the early ballistic regime into the later diffusive regime. This transition point is defined as the diffusion limit, that correlates with one transport mean

free path as the mean distance between equivalent isotropic scattering events.

There’re two very important decay constants. These are 1/e decay constants. If you were to use ballistic photons as your signal source, your decay constant is about 100 microns. So, in practice, you can penetrate ten times that number, so that means you can penetrate about a millimeter. To penetrate much deeper, you have to depend on diffuse photons (multiply scattered photons) because the decay constant is up to about ten millimeters. So, in theory, you can penetrate about ten centimeters if you were to use diffuse photons.

We’re going to focus on the diffusion problem today. Alexander Graham Bell first reported photoacoustics as part of the idea of a photophone instead of the telephone over a hundred years ago. He wanted to encode sound into a light beam, then propagate the light beam in space, convert light

back into sound again through the photoacoustic effect. When light is first absorbed, it generates a temperature rise. Due to thermoelastic expansion, you generate acoustic emission.

Bell was much more interested in the audible frequency range. We are much more interested in the ultrasonic frequency range, because we want to shorten the wavelength, broaden the band for higher spatial resolution. Tomography, remember, was not even in the lexicon in Bell's days, so we're combining this very old physical phenomenon with a very modern concept.

If you were to use a very short laser pulse, you generate a temperature rise right after the laser pulse, and the pressure rise is proportional to the temperature jump, which is in turn proportional to the optic absorption coefficient. So, by detecting the pressure, we're able to quantify the optical absorption properties, and

the spatial dependence of this parameter gives you an optical image; so, you know, we are listening to an optical structure, as opposed to looking at one.

The simplest analogy I can think of to photoacoustic tomography is the triangulation of a single sound source, like a thunderbolt. The time difference between seeing the lightning and hearing the thunder is recorded as time delay t1. Multiply that time delay by the speed of sound, you will define a radius for a spherical shell on which lightning took place. If you have three such spherical shells, the triangulation will give you the source location. It'll pinpoint the source. So, this is photoacoustic CT in a nutshell. So, it's extremely straightforward – except, in reality, instead of having three detectors, we have to have a lot more detectors because we're dealing with not just one point source; we're dealing with a volumetric, 3-D source.

Let me start with photoacoustic CT in a circular geometry. Again, starting from this unknown 3-D source – a 3-D object – we expand a laser beam, make sure we stay within the ANSI safety limit. We will allow photons to wander around, because that's the only way to penetrate deep into biological tissue. Any photons are useful here. We don't reject any scattered photons, but when a photon is absorbed, it generates some heating. Every milli-degree temperature rise gives you roughly 8 millibars, which is detectable already using standard ultrasound transducers.

All we have to do is to place a lot of ultrasound transducers outside the tissue, typically hundreds of detection positions. The rest is a mathematical problem. The ultrasound scattering is about a thousand times weaker than optical scattering, so that allows you to form a very clear image. However, the contrast comes from light absorption. So, that's the difference in

this hybrid modality. You're measuring all the good things light can provide you – the contrast at the molecular level – but you're using the transparency of ultrasound to give you the high definition of images.

This is the first set of functional photoacoustic images, which were acquired totally noninvasively. We allow light to go through the skin and skull. We depilate the head, so the hair is removed, but the light has to wander through a scattering layer of tissue to reach the brain cortex. We're looking at whisker stimulation. You can see the contralateral activation of the brain, as indicated by the red signals here. These are due to the hemoglobin activation.

This was not possible using the standard confocal or two-photon microscopy. This work really galvanized the growth of this field; as you can tell. Since 2003, the field doubled in size every three years. So, this is an exponential growth.

Since 2009, the conference on this topic as part of Photonics West became the largest. So, of course, before ’03, it was one of the smallest; and now it’s the largest by far. And Photonics West has 20,000 attendees.

One of the unique advantages of photoacoustic tomography is the multi-scale imaging capability in vivo with the same contrast mechanism, because we measure optic absorption as the contrast. This is a plot of penetration in tissue versus the spatial resolution, and you can see here photoacoustic tomography allows you to image from organelles through cells, tissues all the way to organs, using the same contrast.

Now, this slope is important. It tells you, in practice, how many pixels you can resolve in the depth direction. You know, as Ralph mentioned, it’s not possible to get the organelle type of resolution at organ depth. There’s always a tradeoff, but the number of pixels, the relative

resolution stays roughly the same. So, 200 is a good number to work with, because whenever you have 200 pixels, you get a decent image.

Well, in current practice for organelles and cells, you use optics for imaging. For tissues and organs, you use no optics. You use MRI and whatnot. So, you're dealing with different contrast mechanisms. There's a huge gap between the cell and tissue levels. Photoacoustic tomography allows you to bridge that gap and provide a continuum. Why is that important? You know, the applications could be numerous, but I think it'll include (1) to enable systems biology research at multiple length scales (In fact, this might be very applicable to tissue engineering as well.) and (2) to accelerate translation of microscopic lab discoveries to macroscopic clinical practice. Each bar here represents an experimental system that has been implemented in our lab. I'll go through some of the systems today.

In ’03, we used a single-element transducer. We scanned around an animal. That took 20 minutes to acquire a dataset. In collaboration with Prof. Zhu at U. Conn., now we have this 512 element array, that allows us to acquire data in parallel. As a result, we can acquire the full dataset for a 2-D image within a couple of seconds. So, depending on whether you want to image the head or the trunk, you can use top elimination for the head, or side elimination for the trunk. It’s very versatile.

This is a full-body image acquired, as shown in this movie. We basically scan along the trunk, and we also concentrate on the kidney region. You can see organs, for example, like the colon. As the colon gets in focus, you can see how well we can delineate the corss sections of the structure. This is all endogenous contrast, so there’s optical contrast for you to see. In a way, we’re basically detecting what you see with the naked

eye if you cut open the animal. So, we're looking at the same set of contrast as our vision will give you.

The photoacoustic signals travel essentially in all directions, so that means the geometry is very flexible. In addition to the circular geometry, it comes in the linear geometry as well, which is highly compatible with the clinical ultrasound machine. In collaboration with Philips, we've adapted this clinical ultrasound system for concurrent photoacoustic tomography. This is a handheld linear ultrasound array with usually 128 or 256 elements. We flank this linear probe with two fiber bundles for light delivery. With a single laser shot, you illuminate the volume underneath the linear probe, and you acquire all the data in parallel. All the data will arrive at transducers within, say, a hundred microseconds. Intrinsically, this is a very fast imaging modality – very different from MRI.

This system operates around this point, multiple centimeters in penetration, hundreds of microns up to about a millimeter or so in resolution.

We can provide reporter gene imaging, as shown here, at a depth up to about 5 centimeters. Unlike GFP imaging, if you depend on fluorescence, you have very poor spatial resolution when you're at this kind of depth. So, this is a demonstration using lacZ gene. You can see the reporter gene product quite well.

One of the key questions is how deep we can penetrate. Ex vivo, we demonstrated about 7 to 8 centimeters already, and in vivo, this is the first breast patient that we imaged. You can see features up to a depth of six centimeters. You can see the breast tumor right here very well, and there're tumor boundaries overlaid with these ultrasound images. This is one advantage as well. Because we use the same ultrasound machine for

both ultrasound imaging and photoacoustic imaging, automatically the images are co-registered.

The real application we're after is for breast cancer staging using this device. The standard procedure right now, as you know, is a surgical procedure. You've got to cut open the axilla area. You dissect the central lymph node. But instead, we want to replace that surgery with a needle biopsy procedure. The first task is to pinpoint the first draining node – namely, the sentinel lymph node. Then we send a needle there. Eventually, we want to biopsy some tissue out of that node for histology or cytology analysis; but for now, we just guide a needle using photoacoustics to place a clip. After the patient goes through the standard surgery, we radiograph this lesion and see if we can find a clip for validation purposes.

And this movie shows you part of the process. You can see how well we can see both the needle and

the sentinel lymph node through this methylene blue accumulation in the first draining node.

Photoacoustics can be scaled. So, now we're talking about a microscopy level, pushing for higher resolution, but by sacrificing the penetration requirement. I'll cover first the acoustic resolution version. Unlike in photoacoustic CT, where we use unfocused ultrasound transducers and look at the object from all different angles. Then we use math to reconstruct an image. So, we're counting on a digital computer to do the reconstruction for you. But in photoacoustic microscopy mode, we're counting on physics to form an image. So, we're going to use a focused ultrasound transducer to form an image directly.

Assuming there's an optically absorbing target in a piece of tissue. We fire a laser pulse. You generate a photoacoustic wave. As we see by this focused ultrasound transducer, you get this time

trace, which can be converted into an envelope by doing the Hilbert transformation. And you see this spike corresponding to this absorbing target. Of course, when you have an unknown set of targets distributed randomly, you will see a set of spikes, and that will give you a 1-D image. The focusing action will give you transverse resolution. So this acoustic lens serves as an analog computer. Essentially, that does the image formation for you.

This 1-D image is called an A-scan image, analogous to the OCT A-scan, or ultrasound A-scan. You can scan across the tissue to get a 2-D B scan image. You can raster scan to get a 3-D image.

This is a photograph of the first 3-D photoacoustic microscope. Start from the laser. We route the laser beam through the fiber. You see the solid beam coming out of the fiber. That’s converted into a hollow cone beam through this key component, a conical lens; and then you

see this close-up of this component right here. The hollow beam is refocused into the tissue. On the tissue surface, you have this donut beam. We made the core dark just to minimize the surface interference. And the ultrasound detection is confocal with light elimination just to maximize the signal to noise ratio.

Again, a single laser shot will give you a 1-D image in the depth direction. This head is scanned in this water tray. Through the membrane window right here, we couple light and sound to the tissue placed underneath.

This system operates around this point. It gives you about 3 millimeters penetration and tens of microns in resolution.

This is one of the first images we acquired on a melanoma. You see a little bump right here, but you don’t see the depth of the melanoma. You don’t see the surrounding blood vessel structures,

and we can see both by tuning the laser wavelength at two, different wavelengths; because the melanoma has much increased melanin concentration. Melanin is a great absorber. You can detect it, and the blood vessel has lots of hemoglobin molecules, which will give you absorption at a different wavelength. And you can see how high the contrast is. If you were to use x-ray CT, the soft tissue contrast is 1 percent or so.

We're trying to apply this in the clinic, working with our dermatology chief. This is the first melanoma patient we imaged. You can certainly see a lot of features right here. The depth seems to be adequate, and we're recruiting a lot more patients now to get a much more correlated study with histology.

And this is a normal volunteer. This image shows the blood vessel structures, again, just by using intrinsic contrast to provide label-free imaging.

This is a B-scan image showing you some of the standard structures in the skin. So, one of the key parameters we're trying to identify is the depth of the melanoma. Right now, we have to use histology to identify it – to measure it.

Molecular imaging is possible with organic dyes targeting alpha-v-beta-3 integrin, but this example targets MSH receptors, melanocyte-stimulating hormone receptors, which are unique to melanoma. These are nanocages – gold nanocages – which are very strongly light-absorbing, so they provide excellent contrast. By targeting MSH receptors, we can increase the contrast by threefold in comparison to non-targeted versions.

Gordana mentioned that we need to do both imaging and intervention, or therapy, and this is very much conducive for that purpose because the cages are hollow. We can load the cages with drugs and wrap the cages with polymer. When you heat up the cages, the polymers will shrink. It'll open up

the pores at the corners of the nano cages. That allows you to release drugs. And very interestingly, when you cool the nanocages, the polymers will reseal the nanocages. So you can come back and control the therapy at any time. You can release the drug again at a later time; and, of course, photoacoustics can be used to monitor the entire process.

The probe can also be miniaturized for endoscopy. One of the key components is right here, where this mirror surface reflects both light and sound. Light is delivered through this fiber. It's reflected by the mirror toward the tissue, which generates a photoacoustic wave. The wave comes back to this mirror, gets reflected toward this ultrasound transducer. So, this mirror has to be rotated. It's driven by this micro motor.

Ultrasound propagation does not like air, so this whole chamber is liquid-filled, but the micro motor doesn't like liquid, so this side is

hermetic. We separate these two chambers and transfer the torque using this pair of magnets.

You can see the device in action right here. As we pull back, this is a set of images acquired in vivo in a rabbit esophagus. You can see this hemoglobin contrast. In fact, we can tune the laser wavelength to quatify concentrations of both oxy- and deoxyhemoglobin, from which you can quantify the oxygen saturation. At the same time, we can acquire ultrasound images.

One of the key advantages is the penetration capability. We’re talking about up to 7 millimeters in penetration, so this could allow you to identify some deeper lesions that would otherwise be missed using standard optical endoscopy.

We can pull back further within the optical diffusion limit, so now our penetration goal is about a millimeter or so in scattering biological

tissue; but we want to push the absolute resolution as far as possible. So, within a millimeter penetration, you can focus light optically, and it allows you to get light-defined, or optically defined transverse resolution.

The key component is right here, which is a light-sound combiner, because we want to make the optical and acoustic axes coaxial. This component is made of two prisms with a gap in between. The gap is filled with liquid. Along the optical axis, the optical refractive indices are highly matched, giving you a very strong light transmission. But at this interface, the acoustic impedences between the solid in the prism and the liquid in the gap are highly mismatched. As a result, the ultrasound will be reflected toward this ultrasound transducer. With a single laser shot, again, you get a time-resolved ultrasound image that gives you a 1-D A-scan image; and you can raster scan to get a 3-D image. The only difference between this version – optical

resolution version and the previous, acoustic resolution version is now the transverse resolution – XY resolution – is determined by the optical focus, where in the previous version, everything is determined by the acoustic focus.

This device operates around this point, in fact, penetrates about 1.2 millimeters. It gives you single-digit micron resolution.

This is a version working at 2.6 micron transverse resolution. You can see how well it can resolve blood vessels. You can see pretty much every blood vessel in a mouse ear in this case. You have to zoom into a smaller area and see capillary beds, single capillaries, single red blood cells.

We can tune the laser wavelength to quantify, again, the concentrations of both oxy- and deoxyhemoglobin molecules, from which we compute the oxygen saturation of hemoglobin. So, you can see here we’re essentially doing single-cell

oximetry, very different from pulse oximetry, which is volume-averaged, arterial only. Here, we can image both arterial blood and venous blood.

This is possible for human applications. We imaged our own finger cuticles. You can see a dense collection of capillaries. These are hairpin-line capillaries. In fact, we can quantify the oxygen saturation of hemoglobin. We can zoom into one of the capillary loops. You can see here on one side it's highly oxygenated, and the other side has a much lower oxygenation, which means the sharpest gradient is right here at the tip of the capillary. That means most of the oxygen is released at the tip of the capillary.

Brain imaging is of interest for the neurology department, and this is what we see with the naked eye. And the skin is scattering enough to basically destroy all the image information, and we can't see anything in the cortical region. And this is what we see at this moment using the

photoacoustic CT device. You can see some of the big blood vessel structures. Once you remove the scalp – you keep the skull intact, in fact. Now you can compare. The skull is translucent enough that you can see some of the structures, so you can see how well we can reveal the big vessel structures noninvasively.

At this moment, if you bring in the optical-resolution version, you say, “Wow. You see a lot more details.” You can see a lot of the small vessels that we would otherwise miss, even in this invasive manner. So, keeping the full thickness of the skull is an important process in neurology studies, because once you open up the skull, you generate a lot of artifacts.

Another very important development is the metabolic imaging. We can image the metabolic rate of oxygen, because we can quantify not only the total concentration of hemoglobin – the oxygen saturation – but also the blood flow, and it

allows you to compute metabolism, and you can see this in early-stage tumor. We see both hyperoxia and hypermetabolism. This is a really bad combination. We can potentially use this for early cancer detection.

We can also image single cells. This is a voice coil running at 20 Hz, but we can push it to 200 Hz, and it allows you to get a volumetric image in single red blood cells as they bifurcate in this capillary loop. By tuning the laser wavelength, we can monitor the oxygen release out of a single red blood cell.

This is a demonstration of in vivo histology label-free. We're imaging DNA and RNA directly, and in fact we can compare with standard HE staining. So, we're thinking of using this for tumor demarcation in the OR.

Let me close with a few sample images in tissue engineering and finish up very quickly. So, we

can image live melanoma in a scaffold, again, because the increased melanin concentration gives you endogenous contrast. You don't have to really label the melanoma cells. And this is a demonstration of vessel imaging in a scaffold – and you can do it in vivo, in fact. These were implanted. And we can also label the scaffold, because the scaffold doesn't really absorb in the visible or in the IR wavelength regions naturally. So, we label the scaffold. That allows you to monitor the degradation of the scaffold.

There's another recent development which allows us to focus light much more deeply into a scattering medium, and we call that "time-reversed ultrasound encoded optical focusing." So, we're trying to break through this optical diffusion limit in a different way. And I'll skip some of the details in the interest of time.

Here's a quick summary. Photoacoustic tomography comes from light absorption and ultrasonic

detection. We broke through the optical diffusion limit. Single capillary, cells, or even organelles can be resolved in vivo. Multi-scale imaging is possible by scanning the depth and resolution.

Photoacoustic detection is background-free. It gives you really high sensitivity to absorption. We can image either non-fluorescent or fluorescent pigments. Multiple chromophores can be resolved spectrally. Functional imaging comes from endogenous contrast. Molecular imaging comes from targeted contrast. Reporter genes can be imaged as well. Doppler imaging is possible. The data acquisition is very fast. There’re no speckle artifacts, and only non-ionizing radiation is used. The costs are relatively low.

There are remaining challenges, or some disadvantages to photoacoustic tomography. We have trouble with gas cavities and very thick bones. The lung parenchyma is hard to image.

There’re potential solutions, of course. Ultrasound attenuation by adult skulls. We’re actually pushing in that direction as well. It’s very insensitive to optical scattering, making this modality highly complementary to OCT. Light delivery at even greater depth. You know, because we’re still pushing that direction, we know scattering really doesn’t destroy photons.

So, I talked about the first two constants already, but there’s a third one which is extremely important. If we can overcome light scattering, light attenuation gives you a 1/e decay constant of ten centimeters. Multiply that number by 10. That means we can potentially image about a meter. So, we’re talking about full-body imaging, if anyone can do that. For now, that’s just a dream, but the TRUE optical focusing – I mentioned as the time reversal approach – may be one step in that direction.

Thank you very much.

[APPLAUSE.]

MODERATOR: We have time for one or two, quick questions.

Q: [Unintelligible] – one thing that you didn't mention in your – [unintelligible] – was that, you know, there are issues that you have – [unintelligible] – issues that can reflect sound or dampen sound, you know. So, you've got to – [unintelligible] – is it fair to say that it is more applicable to more image soft tissue than surfaces at this point and also small – [unintelligible]? Because it looks like you have to know kind of where to get the information, you know.

DR. WANG: Well –

Q: Is that a fair enough assessment?

DR. WANG: -- we're imaging the breast, so that's a pretty large field of view, but nobody's using this for whole-body, human imaging yet. So, if you define "whole-body" as a large field of view, yeah, we're – right now, we're talking about a smaller field of view. But we're talking about – for some animals, it's whole-body already, and we're pushing human brain imaging as well. We're imaging ex vivo. We got some very encouraging data. And so there're ways to overcome some of the problems, even the human skull.

For some animals, as you can tell, we can penetrate some animals' skull. That is not a problem, because it's relatively thin – [unintelligible] – for us to overcome that problem.

Q: Hi. I had a question about – [unintelligible] – microscopy, and in particular, the image reconstruction with the A-scans. Is that based on the time of light? And if so, is the imaging and

the spatial information based on a homogenous ultrasonic speed of sound? So, you highlighted difficulties with the gas cavity and the bone, and I wondered what sort of level of errors that that introduced.

Secondly, in our lab, we're sort of using the reverse effect of ultrasound-modulated tomography of modulating the optical signals with ultrasound and using sort of fluorescence emission and modulating that. And I just wondered your thoughts on a comparison of the two approaches.

DR. WANG: So, on the first problem, which is the ultrasound heterogeneity, in soft tissue that's a very small variation. We're typically talking about 5 percent or so. In our lab, we worked on mapping the acoustic heterogeneity and then took that into account in the image reconstruction. So, the correction is moderate. It's not a big enough problem for us to worry about – until you get a very thick bone, like the human skull. For

that, we have to take that into account, and in fact, what we're doing right now is to use CT data to figure out the ultrasound speed distribution; and then we can correct for that operation. You will improve the image dramatically.

Regarding the second question, both are very exciting interactions. In fact, we're working on both as well. So, the time-reversed ultrasound encoded, or TRUE optical focusing, is based on that. I think the next – [chuckles] – if I want to call that a revolution – could come from that direction, because if anyone can overcome the scattering problem, we are talking about whole-body, full-human imaging; and that – you know, it's a very wild dream for now because, you know, who would ever think of that possibility ten years ago, even? But right now, it looks possible.

Q: Thank you.

DR. WANG: Thank you.

MODERATOR: One more quick one.

Q: So, questioning also. For melanoma imaging, where in the clinical decision process are you thinking about doing this? Are you thinking about replacing a diagnostic biopsy? And how do you differentiate melanoma versus just a benign mole?

DR. WANG: So, our dermatology chief – she's interested in replacing the – [unintelligible]. Right now, that's basically one slice. You cut one slice out of the volume of a melanoma, and that may not sample the deepest region of the melanoma, so that may misrepresent the true significance of that lesion. And so she wants us to image the 3-D morphology of the melanoma. And we can also add potentially functional information, even metabolic information, so that will give you much better diagnosis or even prognosis.

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

[APPLAUSE.]