Scintica INSTRUMENTATION

Questions & Answers

WEBINAR: *In Vivo* Photoacoustic Tomography in Assessing Acute Systemic Vasoactivity: A Noninvasive Systemic Myography Tool

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Questions and answers from the February 29, 2024 webinar titled "*In Vivo* Photoacoustic Tomography in Assessing Acute Systemic Vasoactivity: A Noninvasive Systemic Myography Tool"

This document includes questions we received and answered during the webinar, as well as those that we did not have time to address.

1. **Did you do any comparisons with more traditional myography techniques? To really validate this model of using imaging versus something more traditional. Did you do any comparisons there to see how they line up?**

Dr. Kristie Huda: We have not compared our imaging system with the traditional myography techniques. Instead, we have solely compared imaging systems, specifically, ultrasound versus photoacoustic tomography.

Dr. Lawrence Yip: Do you think it would be beneficial to conduct such a comparison? Have you come across any studies demonstrating differences?

Dr. Kristie Huda: These are two different conditions because traditional myography has already been performed. It's more of an *ex vivo* measurement, akin to an isolated condition. In contrast, our approach involves *in vivo* measurements. Therefore, I believe it is appropriate to assess these changes under in vivo conditions. This is why we have opted for the ultrasound technique.

2. **What is the depth that photoacoustic imaging can reach. And why did you have to place the animal in water?**

Dr. Kristie Huda: Great question. I tend to skim through the imaging, to quickly go through the slides. So, the first question is, what is the depth? Currently, photoacoustic imaging reaches about 1.5 cm to 2 cm with the traditional photoacoustic imaging system. For mice, this depth is sufficient because the placentas are our targeted organs, and in the placenta, the arteries are very close to the skin. Additionally, the skin is essentially transparent at these wavelengths. Thus, we can easily visualize the internal vasculature. We needed to place the animal in the water tank because we require a coupling medium for the signal generated by the laser, which is ultrasound. Ultrasound requires a coupling medium to travel, hence the use of the water tank.

Dr. Lawrence Yip: And to add onto that, this system does allow for whole-body small mouse imaging, penetrating all the way through the body due to the system's design.

3. **Did you do any fluence correction when estimating the placental oxygenation?**

Dr. Kristie Huda: Yes, we did. We normalized the volumes by the energy levels. However, we did not implement a model-based fluence correction if that makes sense.

Dr. Lawrence Yip: So you corrected for the variation in energy levels, the shot by shot variation from the laser itself, but didn't correct for any attenuation as penetration increases, which may have occurred.

4. **Okay, what's the average penetration depth of a laser in soft tissue?**

Dr. Lawrence Yip: That can range from a centimeter to many centimeters.

Dr. Kristie Huda: Again, if you're using... So, the main benefit of this work is that we're not using any contrast agents. Unlike other angiography imaging techniques such as CT or MRI, which require contrast agents to visualize blood vessels, or ultrasound, which needs microbubbles. In photoacoustic imaging, we rely on the blood itself to create signals, making it easier for us to visualize arteries without

worrying about the dosage level of the contrast agent - whether it's enough, too much, or affecting vascular responses.

Dr. Lawrence Yip: Exactly. And to add to that, in terms of the average penetration depth of the laser, that's why a lot of this type of imaging tends to be done in the 600-1000-nm range, known as the optical window, where light penetrates quite deeply due to reduced absorption.

5. **Does the vascular reactivity of arteries change between the 2 techniques. *In vivo* tomography versus *ex vivo* pressure wire myography?**

Dr. Kristie Huda: Yeah, definitely. It will require consideration of the dosage level you are using, such as the doses of this drug, or even the time response. In both cases, it will vary because for traditional myography or isolated vessels, you're directly applying those drugs to the vessel. However, when we inject through the catheter tube, it disperses throughout the body. It's not targeted for one single thing; it goes everywhere. So, again, whatever dosage level we're using, it's going to be divided by the total blood volume as well as the time it takes to reach different parts of the body, among other factors. For example, the placenta has a lower blood flow, so it would take a longer time compared to the arteries, which receive blood much faster.

6. **In this publication, photoacoustic tomography assesses the vascular reactivity of various arteries, such as the iliac, superficial EPA, internal thoracic artery, and uterine arteries. It is clearly demonstrated that vascular endothelial function of resistance arteries is altered in cardiovascular disease such as hypertension, atherosclerosis, and type 2 diabetes. Does this technique assess the vascular response of tiny, small resistance arteries such as cerebral skeletal arteries or cremaster in male or epigastric arteries in the female mouse or small mesenteric arteries or not?**

Dr. Kristie Huda: So, one aspect of this system that we always must consider is the spatial resolution. The spatial resolution for this system ranges from 150 microns to 200 microns. If the vessels are within this range, we can observe them clearly.

However, if they are smaller than that, we may lose spatial resolution and be unable to detect changes in these tiny arteries. But we were still able to perceive an increase in photoacoustic signal intensity in the placenta, for example, with tiny arteries below the spatial resolution limit. The placenta's arterial network resembles a tree with numerous small branches. Instead of focusing on individual arteries or groups of arteries, we examined the entire volume to determine if there was an overall increase. This approach still provided valuable information regarding the increase in blood volume, which correlates with the increase in photoacoustic signal.

7. **How do you know that signal intensity is the best way to assess these changes. As this may not account for changes in morphology or geometry, or potentially other aspects?**

Dr. Kristie Huda: Yeah, I addressed that earlier, but when vasodilation occurs, there is an increase in blood flow and blood volume. So, our hypothesis is that if we observe an increase in photoacoustic signal intensity, it indicates a higher amount of hemoglobin in that area, which causes the bump in our photoacoustic signal intensity. However, if you're examining individual arteries, which are within the spatial resolution of the system, then you can observe changes like diameter changes of the vessels. That's what we compared for the major arteries.

8. **What is the frequency range of the photoacoustic signals being generated?**

Dr. Kristie Huda: So, this is the arc array, the photoacoustic detector array with a 6-MHz central frequency. However, photoacoustic signals, photoacoustic waves, are broadband signals. This means they cover a wide range of frequencies, depending on your targeted depth. If you choose a higher frequency detector area, you are limited to observing the skin, vasculature, or shallow depths. Conversely, if you opt for a lower frequency, you can observe more structures in greater depth. This represents a trade-off in what you wish to visualize. Therefore, I believe the 6-MHz frequency works better for visualizing the entire abdomen rather than just the skin vasculature. Additionally, you can select different wavelengths to observe different vasculature. For instance, if you were to use a 532-nm wavelength, which has a smaller wavelength, it would only show the skin vasculature because it cannot

penetrate further. By selecting different light wavelengths, you can also choose to observe different depths of the volume.

9. **Do you know the pulse repetition rate of the laser you used? It seems to them it's a pretty high energy rate per pulse. Could this system be used for humans?**

Dr. Kristie Huda: Yeah. So, the pulse repetition rate is 10 hertz. It's very slow, which makes it perfectly safe if you account for the energy used. We have ensured its safety for human use. We calculated the fluence on mouse skin, considering that the laser travels through water in the tank, which absorbs light. We accounted for the wavelength we used, 808 nanometers, in calculating the fluence, which is about one to two mJ/cm^2. The maximum permissible limit from the ANSI threshold is around 20 mJ/cm^2, well below what we're using now. If our laser could generate higher energy, we could utilize higher energy levels, but for this laser, we are limited to the energy I presented.

Dr. Lawrence Yip: Perfect. I'd like to clarify that the system Kristie used doesn't use the laser that normally comes with the TriTom system. The actual laser system that usually ships with this TriTom system has about three times the energy level and operates at 20 Hz instead of 10. However, the system isn't designed for human use; it's specifically designed for small animals. Nonetheless, photoacoustic imaging as a technology can indeed be used for humans.

10. **Does photoacoustic tomography measure or reflect the level of calcium influx or changes post vasodilation?**

Dr. Kristie Huda: I don't have a clear answer for that. Could we? I don't know. But at this scale, I don't think it can detect such tiny changes. Maybe if we used microscopy, specifically photoacoustic microscopy, it could be possible. However, if there are any calcifications in the tissue or placenta, or structures, we might observe the absorption of light in those areas. We conducted some studies before, for example, on human placental tissue, and we observed some changes. But this was before the COVID pandemic, and that project didn’t proceed.

Dr. Lawrence Yip: Yeah, I imagine this might be something we could investigate further with a photoacoustic contrast agent targeted to bind to specific components that could potentially highlight these changes.

11. Compared to other near-infrared or purely optical techniques. For instance, with two-photon imaging, it's traditionally limited in depth to around 1 millimeter. However, we can achieve depths of 1.5 centimeters or even deeper.

Dr. Kristie Huda: Yes. Here, we utilize a high-energy laser, emitting photons with high energy, which can penetrate deeper. In traditional optical imaging, where light signals are transmitted and received, the signal attenuates significantly as it travels through tissue, often resulting in no signal. However, in photoacoustic imaging, we generate ultrasound signals instead of light signals. Ultrasound waves have much higher penetration depth and lower attenuation compared to optical or photon signals. This allows us to visualize much deeper into the tissue.

Dr. Lawrence Yip: And to add to that, in pure optical imaging, if you aim for high spatial resolution, you're limited to the ballistic regime of photons. If you aim to go deeper and let the photons diffuse, you sacrifice spatial resolution. In photoacoustics, our resolution is determined by the acoustic resolution of the ultrasound waves. Thus, we get the benefit of both high resolution and deep penetration.