WEBINAR:

Exploring a Novel NIR-2 Photoacoustic Agent to Improve Image Contrast for Deep Tissue Applications

Questions and answers from the May 3, 2023, webinar titled "Exploring a Novel NIR-2 Photoacoustic Agent to Improve Image Contrast for Deep Tissue Applications"

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

[Lawrence Yip]
Early on, you had a comparison slide between ICG, the gold nanorods and copper sulfide, and their pros and cons.

And you mentioned that there are concerns about clearance with even copper sulfide, for example. **Now, you mentioned that the Bornite that you're using here doesn't have those issues. How are they different? Is bornite not a type of copper sulfide?**

[Vinoin Devpaul Vinceley] It is. I think the composition is what's unique in our particular bornite particles that are used. Again, this is still an ongoing study; so, I do want to remind everybody that we are looking to do a little bit more of an extensive biodegradation study. The preliminary results that we've looked at with a specific set of bornite particles showed up to a 70% clearance of these in the initial dose that was given in an animal model. That's essentially what gave us the confidence to try this and I think what what's different between the copper sulphides and this. What we're using right now has a very unique composition that I think just degrades more quickly. And I'm not very keen on or know a lot about the chemistry there, but we're working on extending those studies to make sure we're confident in those results as well. But this is just taken from a preliminary study that seemed pretty confident in using these particles.

[Lawrence Yip] Thank you. Then another question here regarding the porcine imaging that you do with the 2D imaging on that phantom—So just to understand

the question correctly, I think it is saying you had the two-centimeter, three-centimeter, four-centimeter kind of stacked. **Did you adjust the laser and the probe height along that, to get the different thicknesses? Is that what you did?**

[Lawrence Yip] Now shifting gears a little bit, there's a question about the ICG concentration. I believe it's because you mentioned you used a very high ICG concentration when you were doing the studies right here. In fact, this is the same slide here, but we know that with ICG, the peak wavelength tends to change with the concentration used, so **did you account for any shifts in that peak wavelength absorption wavelength due to the amount of ICG used?**

[Vinoin Devpaul Vinceley] That's actually a very good question, but not in this case, we haven't really accounted for this. We really wanted to use ICG as just as a control for seeing how deep we can image them. I mean, not just how deep we can image them, but we also just wanted to see how the photoacoustic concentration performs. So, the depth study, we really just wanted to have it for completeness, so we haven't really accounted for that here because as it is, like with the similar mass concentration results shown just in the previous slide, here we observed that bornite just outperforms ICG. Then moving it to the *in vivo* studies, since we're imaging at 1064 nm for the gold and bornite, it really wouldn't be a fair comparison to do it with ICG as well, so we really didn't account for it there, but it's a good question.

[Lawrence Yip] So I think that kind of answers the next question I have here. Basically, the question was **whether ICG was then tested in the mouse model as well for comparison or control or was that not done?**

[Vinoin Devpaul Vincely] It wasn't done again because we felt like we would be outperformed by both gold nanorods and the bornite.

[Lawrence Yip] We do have a question here more about the system itself, which I'll answer quickly. Basically, **can we image the brain of the mouse without drowning the mouse?** Absolutely yes. So, in this system, even when you're doing abdominal imaging, the head of the mouse is underwater. The way the system

works, delivering anesthesia and air, there's a bubble at the top that maintains that constant flow and so there's no problem at all with the mouse drowning, whether you're doing brain imaging or abdominal imaging.

[Lawrence Yip] **There is a question here about the accuracy level of the imaging and how you evaluated that.** I'm not quite sure if they mean how quantitative it is potentially, I think that's what I'm understanding from here.

[Vinoin Devpaul Vinceley] Since we are just looking at the raw photoacoustic signal. We really haven't done any quantitative measurements in it, at least through this study. Let's just say we haven't evaluated blood oxygenation or actually done any spectral unmixing to obtain any concentration estimate. So, it's just a raw measurement of the photoacoustic signals that's being generated by the respective contrast agent that's being imaged. We think that's where the SNR, I think this CNR comes to play. We see a really good contrast compared to the background noise, so we know we're actually seeing photoacoustic signals and not conflating with noise. Hopefully that answers the question.

[Lawrence Yip] Another question now**, can the bornite be targeted specifically through some molecular or physiological processes and maybe can it be diffused out of the blood capillaries in some cases?** For example, if you're trying to image cancer, so I know you mentioned placental imaging, do you know how well it could be targeted to various applications?

[Vinoin Devpaul Vinceley] Not off the top of my head, but I think since it has a smaller particle size, is maybe. This is just my hunch that you could definitely target it. For example, let's say if you wanted to image past the maternal fetal barrier. I think we definitely can, and that's again because these particles are much smaller, and they can actually pass through the capillaries. And that's again one of the ideas that we have with using bornite. That's sort of one of the future directions that we are thinking of doing. But I still have to look at it more and see what specific applications we can, you know, really use bornite for.

[Lawrence Yip] And then something more about just the experimental setup with the TriTom. **What is the energy level that you're able to achieve using the**

existing OPOTEK laser you're using? And would you have benefited if you had the laser supplied with the system. If you had used, for example, the one mentioned at the beginning?

[Vinoin Devpaul Vinceley] No, I I think especially since I'm interested in deep tissue applications, I would say we would have really benefited if we had the EKSPLA laser. Because I think the OPOTEK currently, the one that we have in house, it's probably capable of generating up to 100 mJ at the current state. So, we really don't see a lot of the deeper vasculatures. And even when imaging at 1064 nm. So, we would really like to have the higher laser that the Explorer has. And yeah, especially when you're doing NIR-2 imaging just to combat the absorption of water. So yeah, it would be very beneficial.

[Lawrence Yip] Absolutely. Yeah. So, I guess as context, cause the laser typically supplies the system would be providing about three times that power at 1064, so. And then one other question is, **what is the path length of the laser light through the coupling medium? For this system.**

[Vinoin Devpaul Vinceley] So I think the path length is roughly and correct me if I'm if I'm wrong on this. But for our imaging system with like a pregnant mouse, I think our path length was roughly around like, I want to say 1 cm or so before we hit the top surface of the skin of the animal. But if you're looking at the phantom setup, there's a little bit more path length of roughly 2 centimeters. Looking at it from where my tubes are to where the optical excitation is at.

[Lawrence Yip] I think it might be a bit further than that in terms of actual distance, probably a few centimeters. The optical coupling medium for the TriTom system is just water unless you're adding scattering media to it, the lights are just going straight through and hitting your target, so you're getting a lot of light diffused through the area and it's quite a nice even strong illumination.

[Lawrence Yip] One last question. It's regarding the porcine phantom again. And it's the question of at 780 nm, which you use, **what would be attenuating your signal since there shouldn't be much hemoglobin left in the tissue since it's not *in vivo*.**

[Vinoin Devpaul Vincely] Right. So, our porcine tissue has both a layer of subcutaneous fat and then we have a muscle layer that still has a lot of myoglobin in it. On top of that, you have higher scattering at 780nm with adipose tissues, so you know more attenuation that is what I'm guessing is going on.