I want to look at visualization of the primary outflow pathway for aqueous humor. I’ll look briefly at visualization and then some quantification of Schlemm’s Canal measurements, oh sweet, I can see from here. We will look both at differences in Schlemm’s Canal size in healthy and glaucoma subjects, we will also look at how rapidly Schlemm’s Canal changes in cross-sectional area and how we might be able to account for that fluctuation in size. As you all know primary outflow pathway for aqueous humor outflow we saw gorgeous images of Schlemm’s Canal from Dr. Ishikawa a few minutes ago, distal to that we can see in this corrosion casting Schlemm’s Canal has been filled with neoprene rubber and the rubber pushed through numerous collector channels and aqueous veins and finally through a scleral vein. You can see that it’s a complex system with overlapping vessels. It’s a complex system with overlapping vessels, in some cases there are meshwork shapes, there is a number of features that we can see within this virtual casting.

We are doing this with OCT imaging, we are using a – this 4 by 4 mm scan of the limbus region approaching from 90 degrees to the surface to give us the best penetration into the tissue. And as we saw earlier the outflow structures appear as openings in an otherwise opaque region in the limbus. We can see some larger vessels, we can see Schlemm’s Canal, all of these are openings. What you get doing this – it’s possible to do this this afternoon with commercially available OCT systems. This is from a Cirrus scan and you can see possibly the region of Schlemm’s Canal, what we are doing is flying through a 4 mm region of the limbus in radial fashion as we saw on the previous slide. And you can see these openings as they move around throughout the tissue. We are tracing those different overlapping vessels. With some image processing it’s possible to enhance visualization, enhance visualization of the openings clearing, clarifying the boundaries between the
openings and the surrounding tissue letting us see Schlemm’s Canal. Yeah, go ahead and play this if this one was still a video, it may not be. I got it from here then. Thank you.

Going to a magnified view of this region near the angle adjacent to the trabecular meshwork I’ve taken a series of slices, these slices again are radial slices through the limbus, they are separated by only 30 microns from slice to slice. We can see two things happening as I go through here. Right now I believe this region to be Schlemm’s Canal although this may also be part of it. The other thing I want to look at are these openings up above. As I trace through these slices frame by frame I can see a couple of things happening. That region that I thought to be Schlemm’s Canal down here, here is a branch coming out of it and it’s joining this other structure. And I go sampling cross-sectionally through this region I can see a transition into a very clear region of a well defined Schlemm’s Canal structure here. So what I want you to take away from this is we are looking at a structure that is not an inner tube, it’s not a continuous round opening through the limbus, the location changes, we saw it. And again this is separated by maybe 90 microns from a point where it was down here to now it’s gone and contain a little bit more anterior. What we can also see as we go through these regions we can track coming here as different aqueous outflow vessels course through the limbus come to branch points, what have you.

So what we have is something dark against something light. Applying further image processing you can reverse this, give yourself something light against something dark and remove the background so that now all of my openings appear light. From here just by enhancing contrast I’m able to isolate cleanly the openings within the limbus. And what that allows me to do - if you would please play
this, this is another video. This is noise but this region is Schlemm’s Canal and I can see here a channel coming up, joining, here I have a bit of a small meshwork structure and I have various outflow vessels here. This is from a person with glaucoma.

The beauty of this, two things, this is a living eye. This is a functioning glaucomatous outflow system and we can now look at the morphology of that outflow system. And we now look at the morphology of that outflow system and we can look at it in 3-D, not just through slices because as you saw with the previous slices a single slice will show you a snapshot but that changes rapidly as you go through the limbus one location to the next.

This is from a dataset from a Bioptigen scanner, this is in a cadaver eye in a perfusion model. By perfusing the system and taking numerous adjacent radial scans we can assemble them to get an overall view of the outflow system. And again we see these meshwork structures, maybe during a break we can discuss what they might be. We can see regions where large vessels are receiving outflow for a region. To compare and to validate that this is outflow this is an image from a microsphere, fluorescent microsphere tracer study. This is from Darryl Overby, her performed this, I know it’s a cadaver, I study obviously. But we can see that when you give a tracer into the anterior chamber and light up the outflow system you see the same kind of meshwork structures throughout. So we believe that what we can see noninvasively in a flow model corresponds very well to what you see when you paint and isolate the outflow system.
This is impressed now in ophthalmology, another nice thing about having this 3-dimensional data is coming down and isolating a visualization of Schlemm’s Canal. This is two different images. The asterisks here and the asterisks here are marking Schlemm’s Canal in an eye, one is from the temporal, one is from the nasal side. The arrows are pointing to collector channels, we can actually see locations of ostia where the collector channels are coming off of Schlemm’s Canal and again what you can appreciate from a 3-D visualization is that there are places where suddenly Schlemm’s Canal appears to be pinched off, other places where it becomes very wide and the course is not like a smooth arc, again like we saw in those slices jumping around we can see it moving throughout the limbus.

This is a single slice. It’s possible to actually do cross-sectional measurements of Schlemm’s Canal to measure area. Within here because of the variability again in size and location I’ve taken a 1 mm sample along Schlemm’s Canal which is comprised of 32 cross-sectional images and in normal healthy eyes those cross-sectional areas have a distribution centered around 4,000 sq. microns. In an early manuscript, I think 2010, we had looked at Schlemm’s Canal where it’s very easy to see. The temptation when you are doing this kind of imaging is to survey the tissue, there it is, it’s huge, I can see it, you grab that image and you measure it. When you subjectively find a location on Schlemm’s Canal to measure you are going to measure something out here at about 10,000 sq. microns. If you want to be able to see it and measure it that’s fine, this was the location on Schlemm’s Canal. But if you wanted to describe Schlemm’s Canal, if you want to be able to say this is how big it is on average, these are the distribution of sizes a single measurement isn’t going to be sufficient.
Again this is from Cirrus data, commercial available scanners can provide this for you. Hopefully in the future in your practices you’ll be able to assess Schlemm’s Canal size with the changes. Again at break we can talk about all you can do with it, 10 minutes, I’ll try and stick to it. But both the mean and the median of the distribution of sizes is around 4,000 microns, so if you are subjectively going and trying to find a single slice odds are you are going to be drawn to a location that is in no way representative of Schlemm’s Canal cross-sectional area.

Comparing healthy and glaucoma subjects, this was 10 healthy subjects and 6 glaucoma subjects. The worst mean defect in this group was about -6, there was relatively mild disease from MDs of -2 to -6. And you can see that in the glaucoma groups the range of Schlemm’s Canal size is skewed to the left to smaller sizes and this was statistically significant. So we can see that glaucomatous eyes do present with reduced outflow morphologies for structures of reduced size.

To address how much Schlemm’s Canal is changing, as I had said I had a 1 mm sample with repeated measurements. We could take the absolute difference in size and look at that as well. And what we find is for the most part from one slice to the next radial section of Schlemm’s Canal you get a change under 1,000 sq. microns; however the change in size one slice to the next was as big as 20,000 sq. microns from a large opening to being immediately pinched off, this is in normal healthy eyes. So again if you want to do a repeat follow-up measurement you can’t just take a slice, set it down say I’ll come back to that unless you have a very solid landmark. For instance a collector channel ostia marking that location because within 30 microns you can have Schlemm’s Canal size change anywhere from less than 1,000 to more than 20,000 sq. microns.
So the take home, 3-D visualization is just plain cool. It’s amazing to be able to see in a living eye a functioning aqueous humor outflow system. It’s wonderful to be able to quantify some of the sizes, I’m starting with Schlemm’s Canal of all the different things here that’s the low hanging fruit. And indeed you see a reduced Schlemm’s Canal cross-sectional area in glaucoma. I have no idea when this presents, it’s possible that this has always been there, maybe this is pre-parametric, maybe this is something that comes after the disease like my blood flow days, it’s going to be a wonderful chicken and egg problem. What I need to do is get some young family members, people at risk and start to compare. But OCT visualization of the active living outflow system is going to open up a whole new area of research, but these kind of measurements are available right now from commercially available systems.

Again I’d like to recognize the fantastic people, the group that make the research as fulfilling as it is, and the funding that keeps the door open. And thank you for your attention.