Delta Vision Troubleshooting

Hello Dan,

My name is Jessica Hill and I am a new postdoc in Dr. Nishimura’s lab. I am emailing in regards to the DeltaVision Elite within our lab.

I have just started using it and am imaging whole C. elegans, which requires me to image in panels and then use the stitching feature. However the alignment of these panels after stitching is off and apparently this has always been an issue with the microscope (per Dylan and Robs comments, other lab members). Do you have any thoughts as to what the problem might be and how this can be remedied?

Thanks!

Hi Jessica,

OK. I’m putting some stuff together for you. Could be 2 things, camera needs a bit of rotation and/or the objective/pixel calibration may be a bit off. You should be able to make these adjustments yourself with the right docs at hand. Also, a stage micrometer (~$20 on amazon). I will send you the docs as soon as i can.

One question to start with: are the images crooked or just not spaced correctly? Or both?

Have a great weekend!

My best,

Dan

Ok great thank you!

The images are misaligned, I don't know if I would say crooked. They aren't spaced apart.

So they are overlapping a bit too much?

Sorry I worded that poorly. The panels aren’t overlapping or spaced apart. They just seem to be shifted up and down.

I am attaching a stitched image as example.

OK, these images are pretty out of focus but i think i see a bit of both, rotational and    that pixel shift. Maybe. Do you have any images more in focus? Is this a processed or raw image? A max projection or single slice?

Thanks!

Dan

Hi Daniel,

Just checking touching base.

I believe I correctly checked the objective/pixel calibration for the 60X objective that I am using and used for the stitched image I sent you. For the 60X objective, it is 0.1072 microns/pixel, and then I also checked the log file for the stitched image and it was 0.10718 microns/pixel.

Hope this helps!

Hi Jessica,

So, I’ve been looking into it and the pixel calibration is fairly easy to do/check. You just need one of these: [https://www.amazon.com/Microscope-Micrometer-Calibration-Multifunctional-Calibrating/dp/B088R1SCK2/ref=sr\_1\_2\_sspa?crid=X1TRCITY6J5P&dchild=1&keywords=stage+micrometer+calibration+slide&qid=1607637795&sprefix=stage+micrometer%2Caps%2C161&sr=8-2-spons&psc=1&spLa=ZW5jcnlwdGVkUXVhbGlmaWVyPUEyRjFYSFdQSEFDRlVCJmVuY3J5cHRlZElkPUEwMTc3Njc4NjhaNUU4OEVSTTNTJmVuY3J5cHRlZEFkSWQ9QTAxMzQ3MTJTS0pEWVJIR1JSNjAmd2lkZ2V0TmFtZT1zcF9hdGYmYWN0aW9uPWNsaWNrUmVkaXJlY3QmZG9Ob3RMb2dDbGljaz10cnVl](https://nam01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.amazon.com%2FMicroscope-Micrometer-Calibration-Multifunctional-Calibrating%2Fdp%2FB088R1SCK2%2Fref%3Dsr_1_2_sspa%3Fcrid%3DX1TRCITY6J5P%26dchild%3D1%26keywords%3Dstage%2Bmicrometer%2Bcalibration%2Bslide%26qid%3D1607637795%26sprefix%3Dstage%2Bmicrometer%252Caps%252C161%26sr%3D8-2-spons%26psc%3D1%26spLa%3DZW5jcnlwdGVkUXVhbGlmaWVyPUEyRjFYSFdQSEFDRlVCJmVuY3J5cHRlZElkPUEwMTc3Njc4NjhaNUU4OEVSTTNTJmVuY3J5cHRlZEFkSWQ9QTAxMzQ3MTJTS0pEWVJIR1JSNjAmd2lkZ2V0TmFtZT1zcF9hdGYmYWN0aW9uPWNsaWNrUmVkaXJlY3QmZG9Ob3RMb2dDbGljaz10cnVl&data=04%7C01%7CJessica.Lynn.Hill%40colostate.edu%7C93be0afacdee4c2f598e08d89d5db191%7Cafb58802ff7a4bb1ab21367ff2ecfc8b%7C0%7C0%7C637432373037569957%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C1000&sdata=wiHeXcmaztJFGiTDmTGteT%2B8u8%2BirPIRgMIia0ZwfNU%3D&reserved=0)

The camera rotation is much trickier and likely will require a service visit although I know that Keith DeLuca is pretty handy with the DVs. Perhaps you could ask him if he’s done it before?

I got your second, more in focus stitched pic. I still think both issues are in play here and have asked a few colleagues if they concur. Let me know if Keith is up for camera rotation or no.

My best,  
Dan

Hi Jessica,

So, I’ve done a bit of a deeper dive on your situation with the *C. elegans* and stitching images on the DV.

First, I wonder if you might try using the other DeltaVision in the lab, the Ultra. The reason I suggest this is that the camera in that system has a bigger chip in it and can collect a larger field of view (FOV). This *may* let you collect single images and capture the whole worm, depending on the size of the worm and the lens you are using. Just a thought.

Secondly, I am attaching a document that talks about stitching on the DV. I see that a certain amount (up to 1-2 degrees or so) of rotational bias can be compensated for in the post-processing software! Also, while imaging, you can increase the percentage of image overlap, which can also assist in getting rid of other alignment issues. Anecdotally, binning the images can also help. One of the key things to pay attention to is if you see a black square where the images meet, increase the overlap during imaging. Overall, using the post-processing and imaging settings is really quite a trial and error process, that may take a little time and is almost an art. The good news is that when you successfully get the desired result, those settings should be reproducible. Aa a general rule of thumb, try tweaking the post-processing settings first and see how good you can make the stitching before you make changes to the imaging settings. Finally, if the edges of the images seem “weird” compared to the more central parts, use the cropping feature, post-processing, which may require increasing the overlap during imaging to make sure all of the sample is seen.

Please let me know how it goes and don’t hesitate to reach out for more advice and help!!

My best,

Dan

Hi Dan,

So Erin (my lab PI) is ok with me performing the pixel calibration, but doesn’t want to do the camera rotation as she is concerned it might disrupt current work and data being collected.

We have a calibration slide, so I will do that and then utilize the materials you sent for post-acquisition correction.

Thank you for your help!

Absolutely, Jessica,

As per my last email, i think you should play with the rotational correction settings in the post processing software, not by physically manipulating the camera.

Good luck!

Yes will do.

Before I forget, do you happen to have instructions for this particular microscope in regards to pixel calibration? I’m sure its in the manual, but thought id ask.

So i recall you found the number in the config file. So what you want to do is measure the actual pixel size using the calibration slide (do it multiple times and take the average) using the measurement tool in the software. Then edit that file to reflect the (hopefully) more accurate value.

Let me know if you have issues.

Ah. That’s a mirror slide. I would use tour new one. This one you use orange ex and orange em. No markings. Get in focus and take a pic. Zoom until you can see individual pixels. Use measurement tool and consult the excel spreadsheet for the correct size. I doubt you have that spreadsheet. Try with the new slide and ill look for the sheet.

Dan

Hi Jessica,

OK, I found it the document that addresses the pixel calibration stuff. Please read this document (attached) and see how much of it is applicable to your situation.

Lots of stuff here, including how to use our slide for pixel calibration. With this new info, you may find it easier than using the new slide from Amazon (or wherever you got it from. Your call.

As always, reach out to me with any questions.

My best,

Dan