3D imaging procedures of descSPIM

(ver. 230520)

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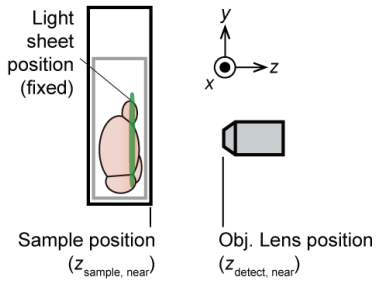
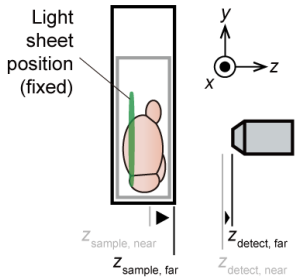
3D imaging (bioRxiv 2023; Supplementary Fig. 4)

1. Check the position of focus of both actuators near the sample ($z_{sample,\ near}$, $z_{detect,\ near}$)



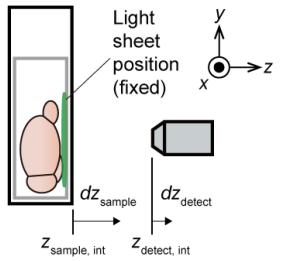
2. Check the position of focus of both actuators on the far side of the specimen ($z_{sample,\ far}$, $z_{detect,\ far}$)



3. Calculate relative velocity (v) of actuators
(v_{detect} = ($z_{detect,\ far}$ - $z_{detect,\ near}$)/ ($z_{sample,\ far}$ - $z_{sample.,\ near}$) × v_{sample}

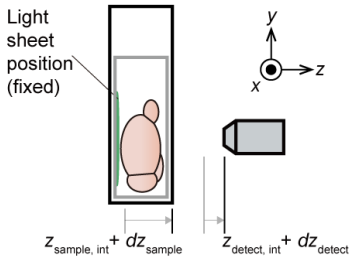
4. Set the velocity and acceleration (a) of both actuators ($a \gg v$; v_{sample} : v_{detect} = a_{sample}: a_{detect}) in the software KINESIS™ (Thorlabs)

5. Set the required initial positions ($z_{sample,\ int}$, $z_{detect,\ int}$) and scanning ranges (dz_{sample}, dz_{detect}) in KINESIS™

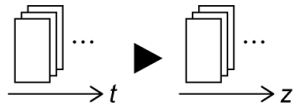


6. Start capturing time-lapse images with the required exposure time (t_{exp}) using software ThorCam™ (Thorlabs), and continuously move the actuators using KINESIS™

7. Stop the measurement process in ThorCam™ after scanning is complete



8. Convert acquired *xyt* images to *xyz* images ($z_{interval}$ = v_{sample} × t_{exp})



For other channel measurements

1-4 can be omitted if you use the synchronous speed correction value (the relative velocity of two actuators) estimated from ideal refractive index value as v_{detect} = ((n-1)/n) × v_{stage}

Detailed procedures about 5-8 are described in following 2 (3) pages

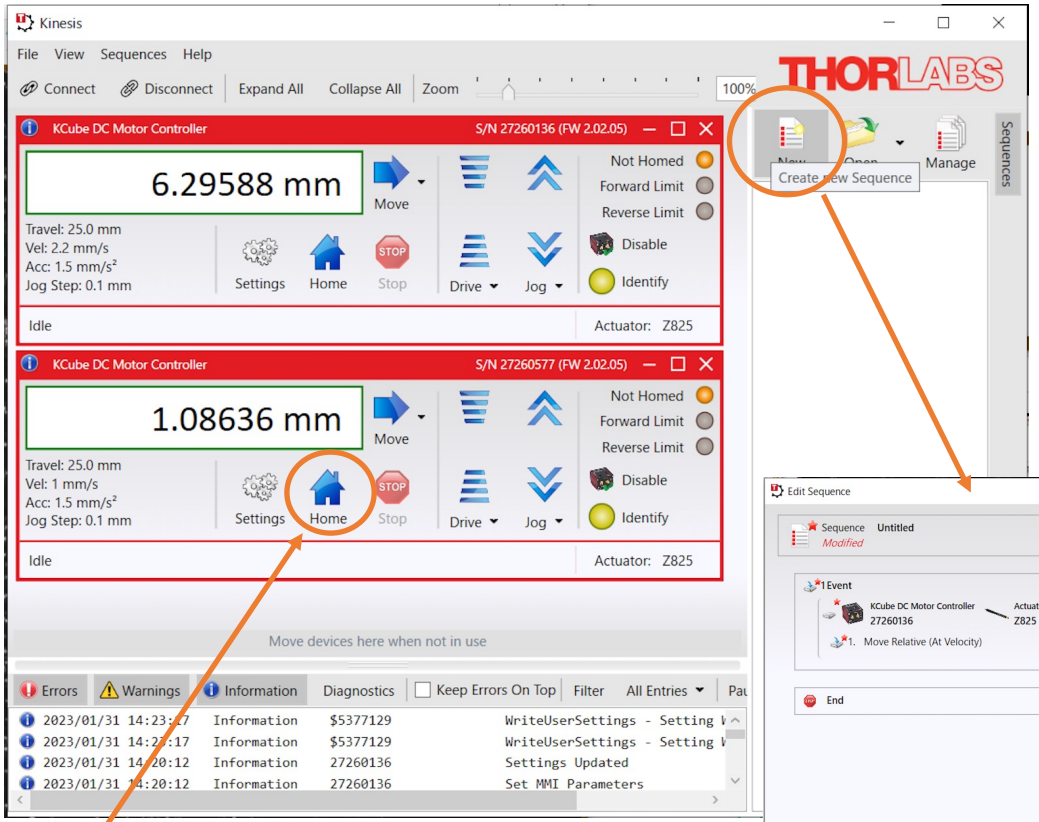
3D imaging

Acquire continuous time-lapse images utilizing ThorCam™ *1, while moving two motorized stages by "Sequence" function of Kinesis™ *2. And convert acquired xyt image dataset to xyz image dataset *3.

*1 Camera and software are exchangable, if they can capture continuous time-lapse images and save them as tif format

*2 Manual jog operation of the speed wheel enables the same job.

*3 Original program to control the whole process is also under construction.



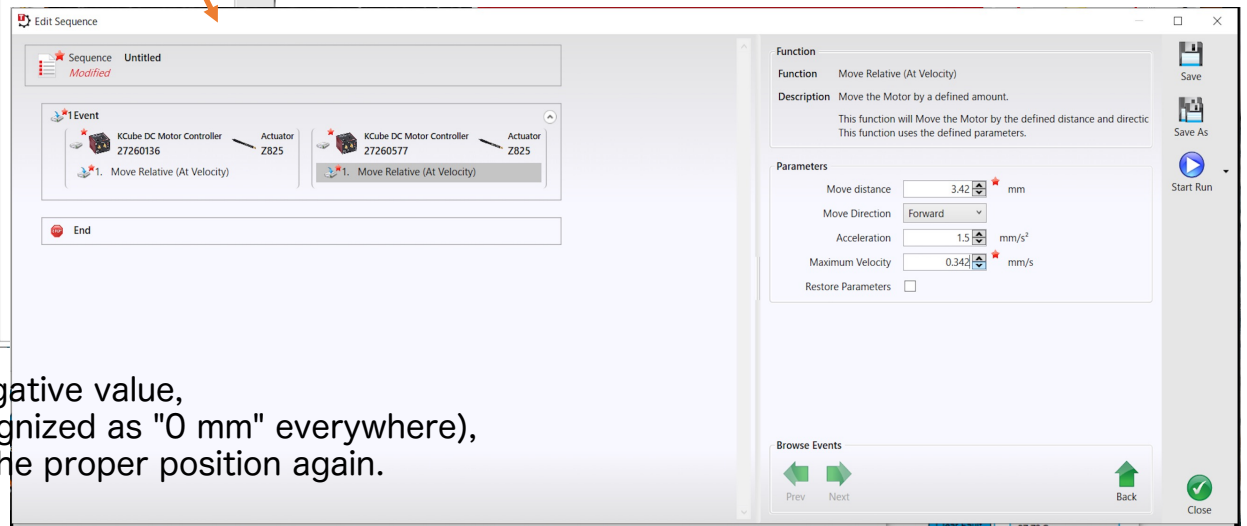
↓Create a new sequence from the sequence tab in Kinesis™

↓Insert→Device event→Edit→Add
Select connected DC Motor Controller

↓Edit→Insert→Action→Move Relative (At Velocity)

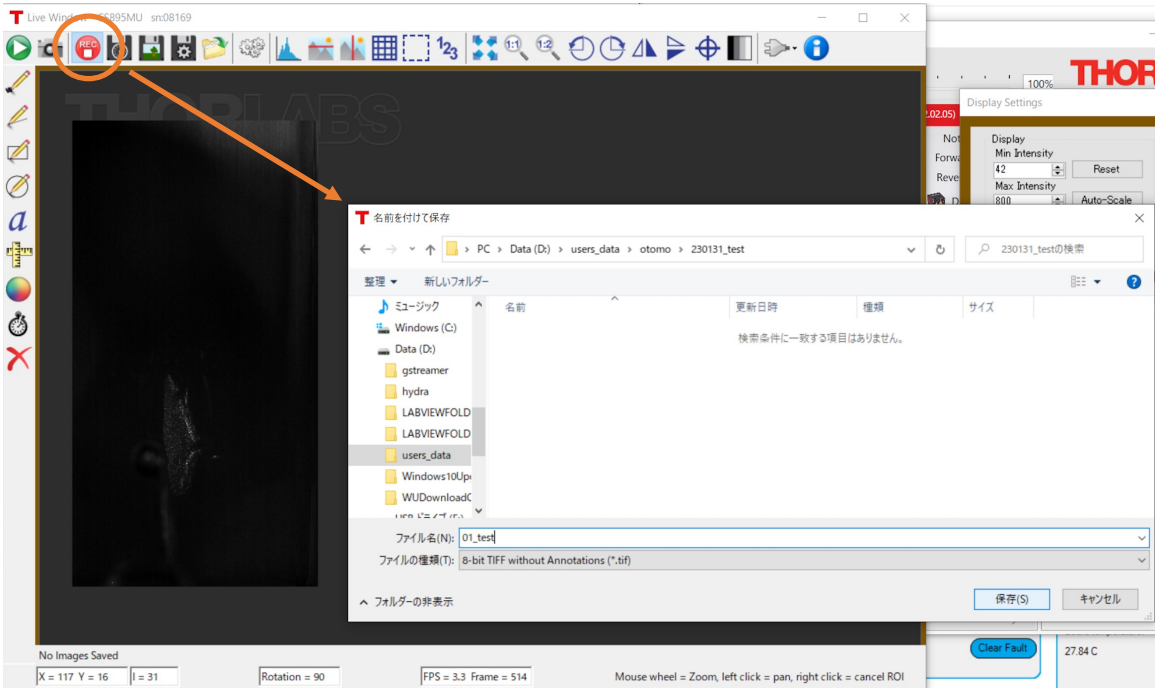
e.g.	Sample stage	10 mm movement Acceleration 1.0 mm /s Max velocity 0.1 mm /s
	Detection optics	3.42 mm movement Acceleration 0.342 mm /s max velocity 0.0342 mm/s

↓"Run" just after time-lapse imaging started with ThorCam™



*Since "sequence" function does not accept negative value,
at the time of launch (the initial position is recognized as "0 mm" everywhere),
set it to the home position and then return to the proper position again.

3D imaging



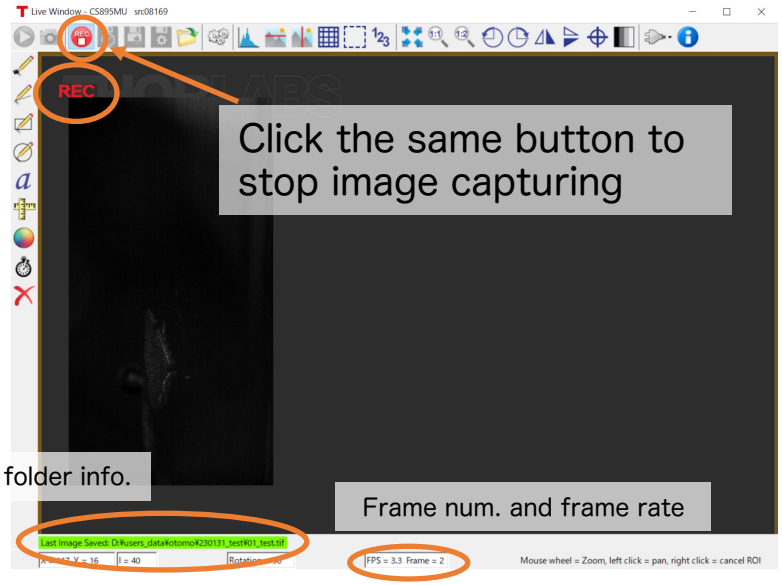
↓Click the "Rec" button in ThorCam™.

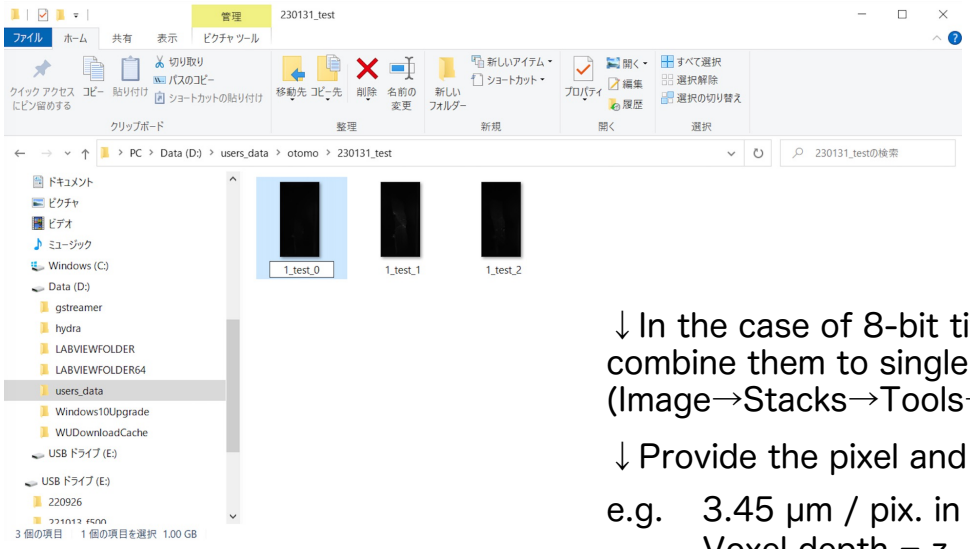
↓Convert the file format from the default avi to tif, and set the file name and folder in appeared saving panel.

↓Click "Save" to start capturing video.

＊After a slight time lag, "REC" appears on the GUI, then click "Start Run" in Kinesis.

↓Do not forget to click "Rec" to end the imaging





File name and folder info.

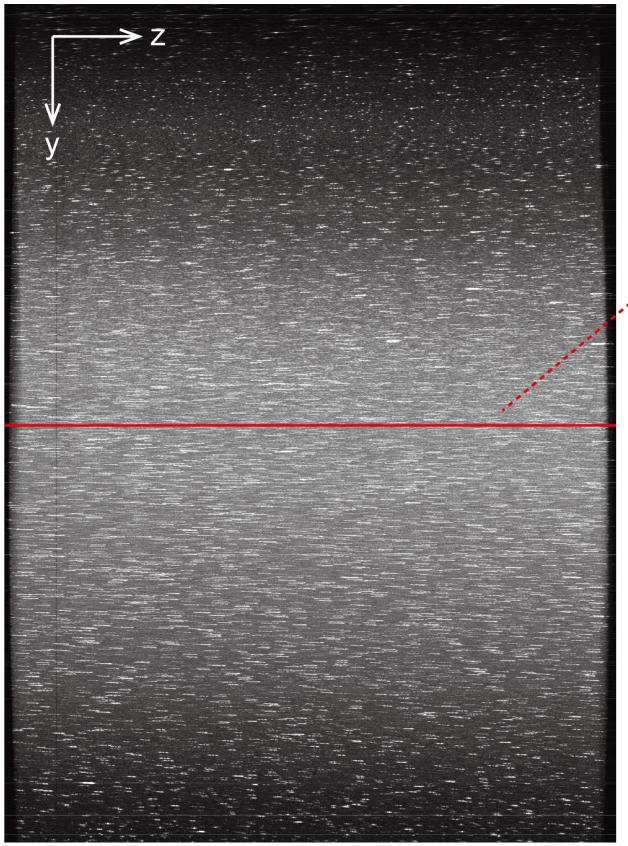
↓In the case of 8-bit tif and no binning, a tif file is saved every 122 images (1 Gbyte). To combine them to single stack, use the concatenate function in ImageJ (Image→Stacks→Tools→Concatenate).

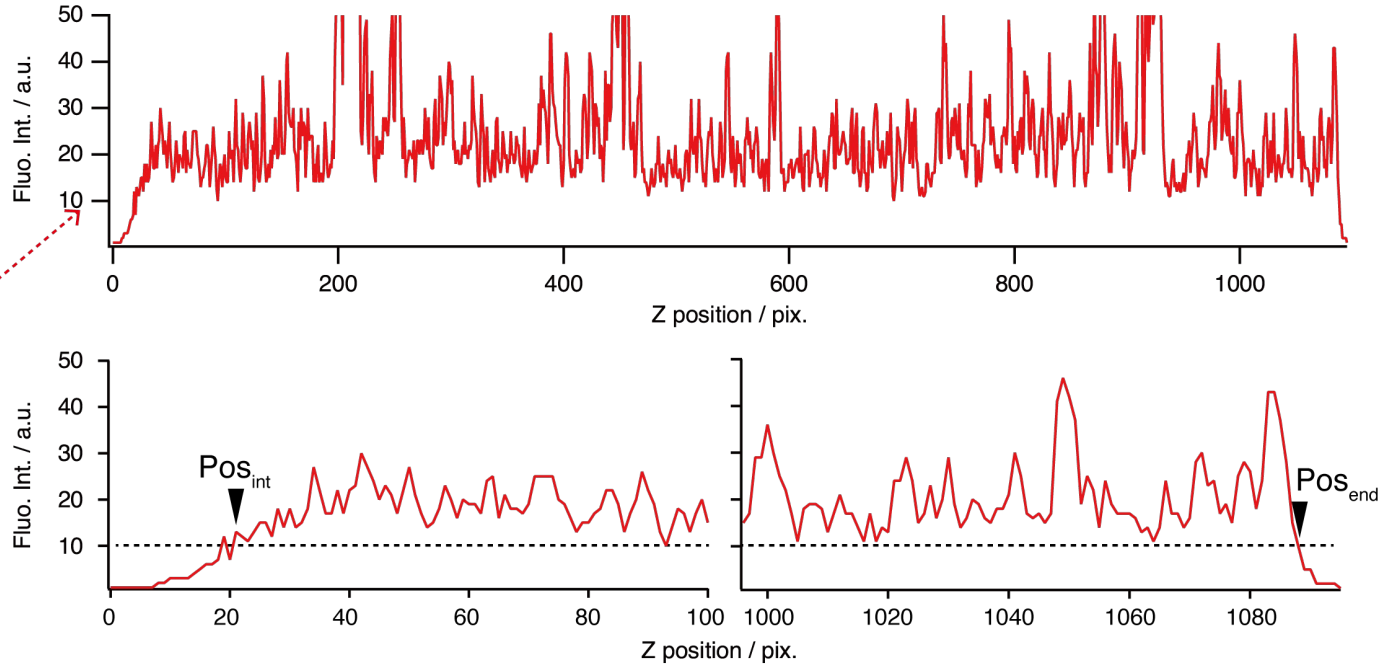
↓Provide the pixel and voxel info. to the image-stack. (ImageJ: Image→Properties)

e.g. 3.45 μm / pix. in default setup (CS895MU, no binning, TL2XSAP, $f_{tubelens}$ = 100 mm)
Voxel depth = $z_{interval} = v_{stage} \times$ exposure time

z-interval normalization (if required)

The z-interval (voxel depth) is estimated by the product of v_{stage} and exposure time. However, the set maximum velocity value may be different from actual due to the load capacity of the stage, specifications of the PC, and other unknown factors. Just in case, normalization of the z-interval values using actual measurement data is recommended.





$$\text{Opt. path length} = \text{z-interval} \times (Pos_{end} - Pos_{int})$$

$$\text{z-interval} = \text{Opt. path length} / (Pos_{end} - Pos_{int})$$

*Pos_{int} and Pos_{end} were determined as the initial and end point of a range of which fluorescent intensity does not fall below a certain value (in this case 10 a.u.)

↓ Measure the z-stacked images of gel-embedded fluorescent beads in a cuvette with a 10-mm light path
↓ Estimate pix. numbers corresponding to the distance between the inner gel-cuvette interfaces
↓ Estimate *z*-interval as the optical path length divided by the number of pixels