Bruker TruLive User Guide

Version 1.1

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Contents

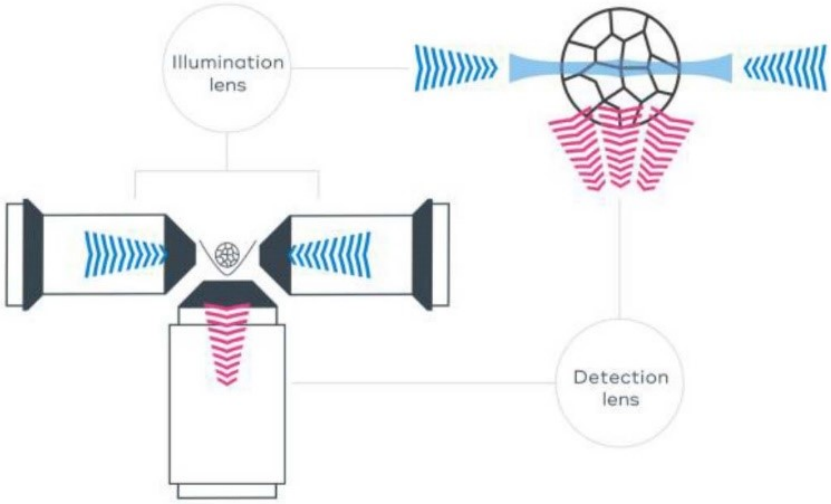
Introduction

The Bruker TruLive Lightsheet is made specifically for small samples in aqueous solutions. The sample chamber is filled with ddH_2O. Therefore only samples in ddH_2O, PBS, and cell culture media are compatible with the system. Phenol red in media will cause autofluorescence and poor imaging quality.

Cleared samples are not appropriate.

Disclaimer: This user manual was compiled using Bruker "LuxBundle" software version xx on the system that is installed in Sherman Fairchild. Luxendo changes software versions often, so screenshots and wording may vary.

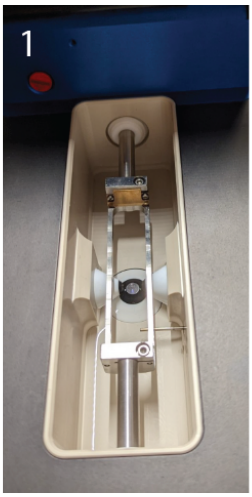
Fig. 1: TruLive 3D set-up:

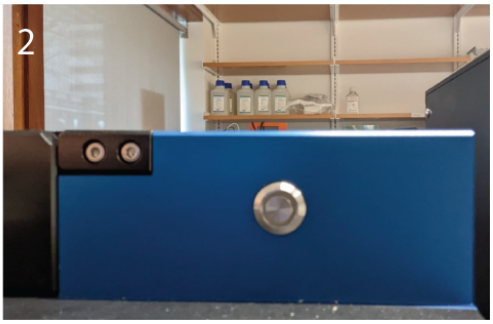


1 Starting System

1. Fill the basin with ddH_2O until the front lens is fully immersed (e.g. above the black ring around the glass on objective).
   (a) There is a squeeze bottle of water near the microscope for filling the chamber
   (b) Do this 30 minutes before you plan to start imaging so that the water has the time to equilibrate to room temperature.

2. After closing the sample chamber press the interlock button on the back of the blue box.
3. Turn on the microscope, cameras (2), and turn on the monitor with remote control. No specific order is necessary for the proper start-up.
4. Turn on environmental controls if necessary
    (a) Luxendo gas mixer
    (b) ThermoCon temperature control
    (c) Open gas valve
5. Open Lux Bundle





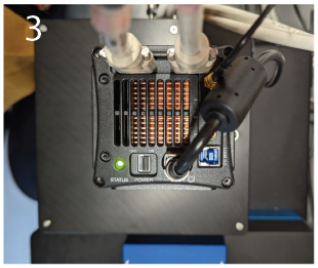


Figure 1: Starting System

2 Calibrate System

Sample should not be in beam path, so no need to load it yet if the sample is sensitive. Alternatively, if you are only using one side of a trough, you may use the empty media-filled trough for calibration. The trough can either be moved physically within the sample holder or using the "X" stage control in the software.

1. Click "Calibration" on the left side menu.
2. On top of the right software box, press the "connect all streams" button.
    (a) Cameras will appear on the screen
3. Click "Start Live" on top of the software
4. Turn on LED to check that sample is not in the light path if it has been put in the system. Turn it off once confirmed.
5. Turn on 488 laser to 100%
6. Set Detection to LP488, LP488 with a dichroic of LP560
7. In the Scanner calibration window, unlink by deselecting “Link” (by doing this, each light sheet will be controlled individually)
8. "Park" front sheet and turn the back sheet to "off."
9. Lightsheet alignment window has:
    (a) Y (Controls how far the beam is from the center axis)
    (b) Z (Controls shape of the beam)
    (c) Tilt X (Controls angle of the beam)
    (d) Tilt Z (Controls pivot of the beam)
10. Unfocus Z, set beam expander in Illumination window to 2.0 m and then use TILT Z to expand beam until it looks like a ruler
11. Set beam expander to 2.0 m and refocus “Z”
12. Adjust "Y" until the center of the sheet is on tick mark one from center
13. Adjust "Tilt" until the hourglass shape is parallel to the x-axis of the image
14. Turn the back light-sheet to park and the front light-sheet off

15. Store positions
16. Repeat steps 9-15 for the back light-sheet.
17. Put both light-sheets in park
18. If sheet waists are not the same up and down on the screen then use “Offset.” This will be on the order of +/- 0.002
19. Run line calibration. If calibration fails return to step 10.
20. If both sheets are not crisp, adjust using the camera alignment tool, store
21. Re-link light-sheets and select "autoscan"

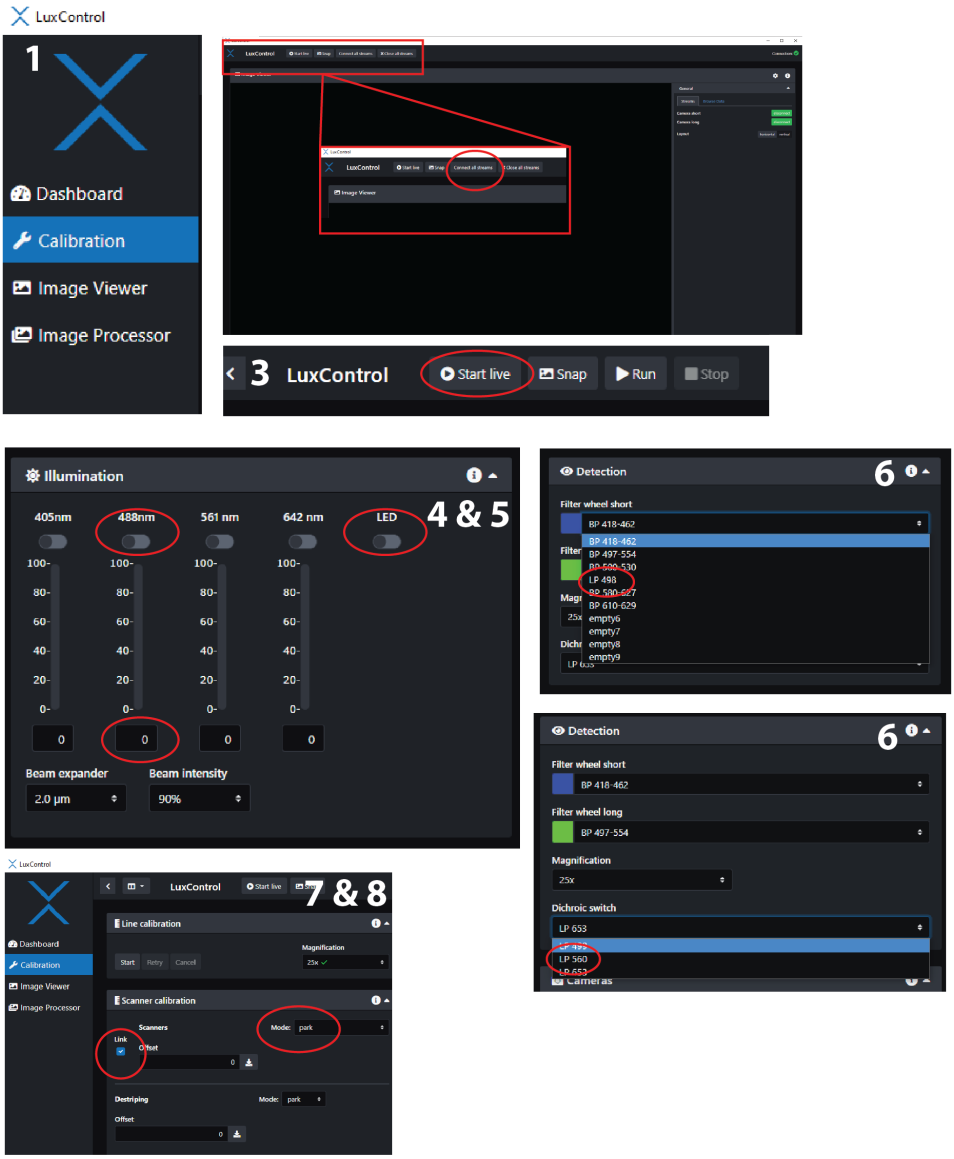


Figure 2: Calibration Part 1

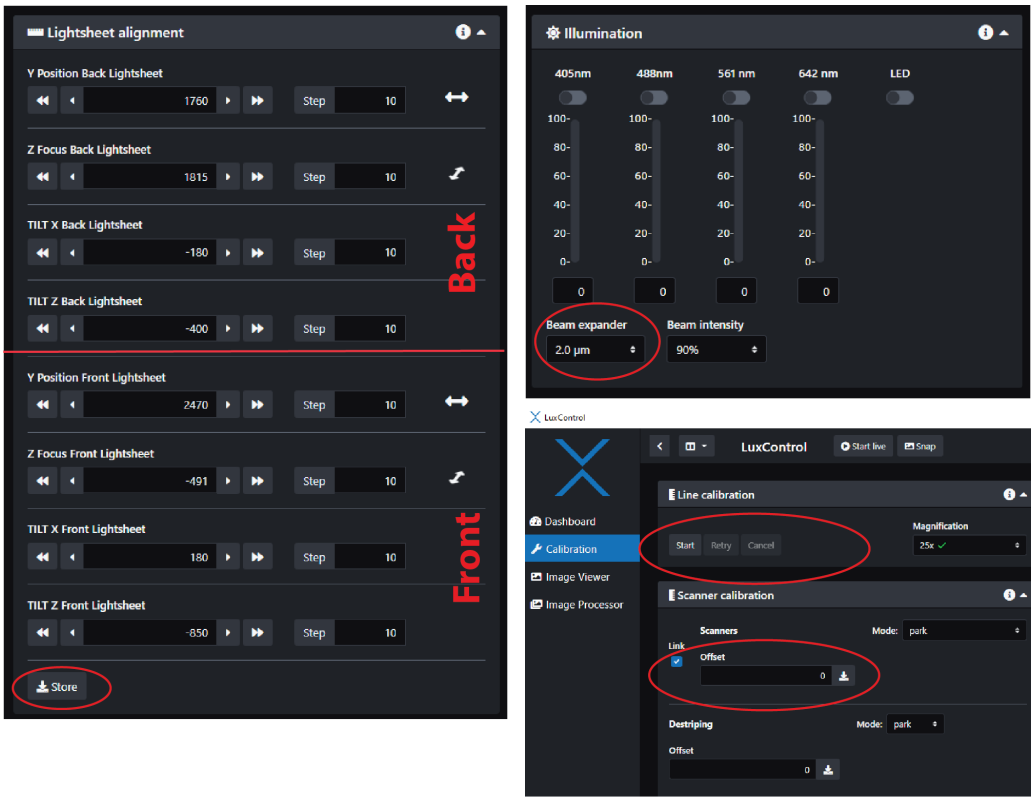


Figure 3: Calibration Part 2

3 Finding your Sample

3.1 Using LuxBundle Software

1. If not already done, place the sample into the sample chamber and close the lid
2. Press the interlock button on the back of the blue box to re-engage the laser
3. Click on Dashboard tab on the left side of the software
4. In Detection Window, change magnification to 12.5x to give as wide a field of view as possible
5. Turn on LED (far red, so most gentle on live samples)
6. In Cameras window select area for both long and short cameras.

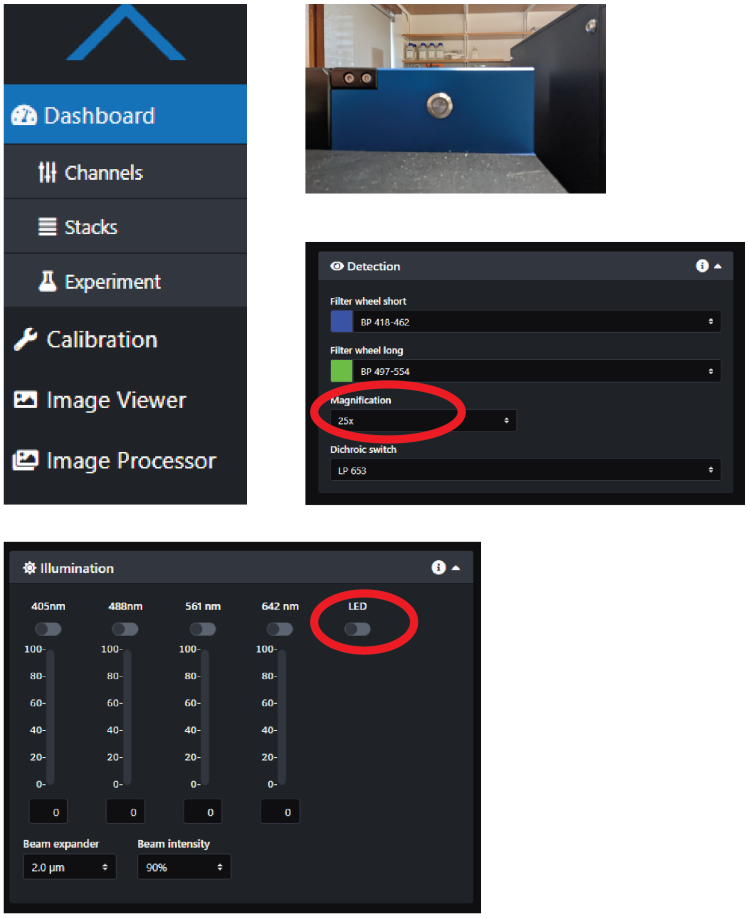


Figure 4: Finding Your Sample

7. Find the bottom of the trough by typing in -500 in the Stage Control Z location.
8. Use the X-stage control scan back and forth for your sample
9. Use the Y stage control to focus your sample
10. If you can't find your sample, use one of your fluorescence channels to aid in your search but turn it off as soon as you have found your sample to prevent bleaching and/or phototoxicity.

3.2 Using SpinView

SpinView is a separate software program designed to give you a larger field of view than you can in the LuxBundle software. In SpinView, what appears to be the y-axis in Luxendo, acts like Z in SpinView

1. Connect camera up to system
2. Place window on the right hand side of screen
3. Open LuxBundle on the left side of your screen. It will not work if both aren't visible at the same time.
4. Use the same steps as LuxBundle 5-9.

4 Experimental Setup

4.1 Basic

1. Adjust emission filters and dichroic beam splitter
   (a) Beam splitter should be between the windows of the two emission filters
   (b) GFP/RFP dual experiment would be:
      i. Filter wheel short = BP 497-554
      ii. Filter wheel long = BP 580-627
      iii. Dichroic switch = LP560
2. Turn on appropriate lasers for excitation and adjust laser power
3. In the cameras box, select exposure time and either area or line mode.

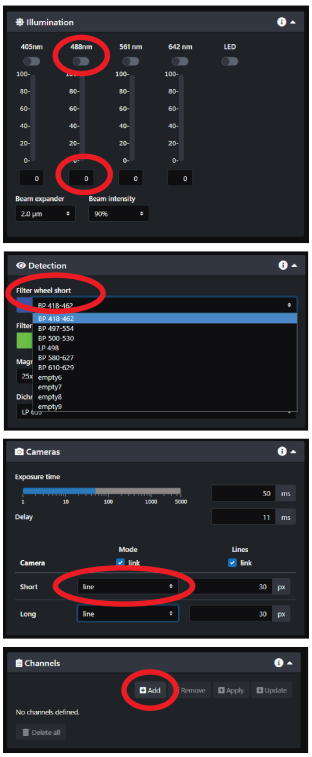


Figure 5: Channel Setup

4. Add channel. If more than two colors are required, multiple channels for imaging may be set up.
5. Add Stack
   (a) Adjust range by clicking the arrow in front of the start for the first frame
   (b) Move to the end of your region of interest and click end
   (c) XY is also saved for the location, so tiles are set up in the same fashion in the stack box.
   (d) Delta is the step size, nyquist sampling can be found in Delta Settings figure
6. Experiment = Events + Task + Trigger
   (a) Add an event that will be composed of a task and a trigger
   (b) Add a task and pick stack and channels
      i. If you need more than one channel or are doing more than one position, add an additional task here

(c) Trigger

i. No time series: Start- 2 seconds

ii. Time-series: Set up how often to acquire an image and how many times

7. Run experiment

8. There is a progress bar but it will not estimate the time of the experiment

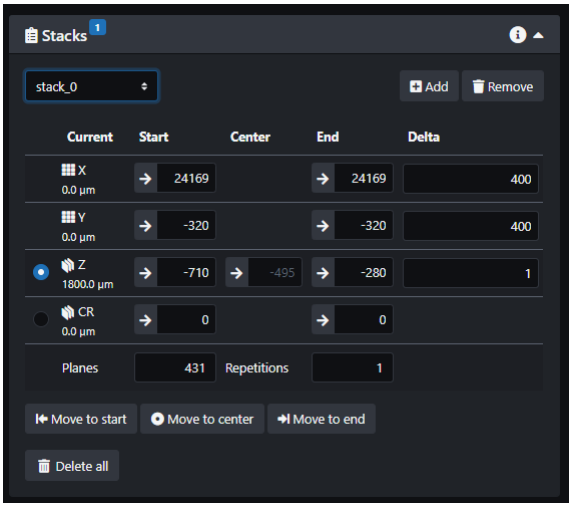


Figure 6: Stacks Section

25x/1.1 Objective with Hamamatsu Flash 4 V3			
Final Manification	FOV	Lateral	
		10%	20%
12.5	1065	958	852
25	532	479	426
37.5	355	319	284
50	266	240	213

Axial Nyquist (2um)	
2µm Beam	6µm Beam
0.152	0.249
0.165	0.286
0.174	0.331
0.179	0.331

Figure 7: Delta Settings

4.2 Advanced

4.2.1 Rapid Time Series

It is not possible to image faster than 1 fps using the trigger function. Therefore this workaround has been developed for 142 fps imaging.

1. In Calibration tab:
   (a) Set beam expander to the desired size (note: smaller sizes have more pronounced waists)
   (b) Scanners- Mode: Autoscan
   (c) Destriping- Mode: resonant, amplitude 3
   (d) TAG lens enabled with amplitude of 35%
2. In Dashboard tab:
   (a) Set exposure time to 6ms with time delay of 11ms
   (b) Narrow ROI to 512 (W) x 512 (H), 768 (L), 768 (T)
   (c) Focus sample and add "stack" to experiment
   (d) Adjust stack repetitions to 2000
3. Experiment: Use steps 5-8 of the basic experiment
4. Visualizing Data: Data will appear as a 2000 slice z-stack and will be very hard to visualize, so follow these instructions
   (a) Export data using standard protocol
   (b) Open FIJI
   (c) Find .lux file and drag into FIJI one at a time
   (d) Bio-format importer window will pop up– select "Swap dimensions"
   (e) Image should now have Z- and T- swapped
   (f) Once both are imported, go to Image⇒ Color ⇒ Merge channel
   (g) Check "keep source images"
   (h) Set images to appropriate channels
   (i) Go to Image ⇒ Adjust ⇒ Brightness/Contrast (CTRL + Shift + C) to adjust

5 Exporting your Data

1. Click image processor on left-hand column
2. Add task: Select file to process
3. In "Task Configuration" select these options unless instructed otherwise
    (a) Input: Add MTF file to make pixel perfect alignment of long and short cameras
    (b) Output: Depth non-isotropic, BigTiff
4. Click "Run all"
5. File will be saved in same folder as raw data but in a folder called "Processed"

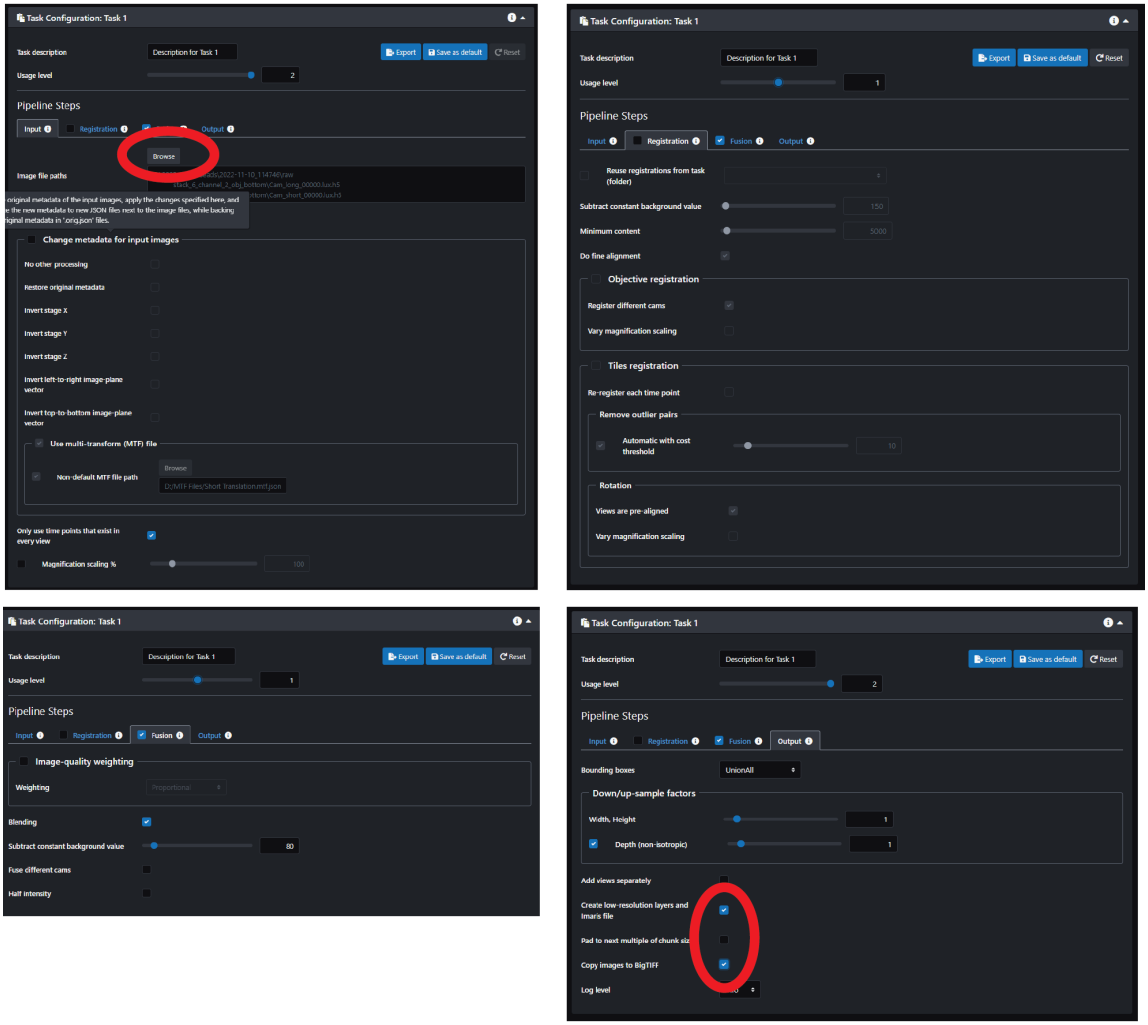


Figure 8: Export Parameters

6 Shutdown Procedure

1. Remove sample from the basin
2. Remove water from the basin with syringe
   Note: Clip tubing to self when your remove syringe so water doesn't spill onto the floor
3. Shutdown software
4. Turn off microscope and cameras
5. Turn off monitor with remote control

7 Trouble Shooting

7.1 Alignment

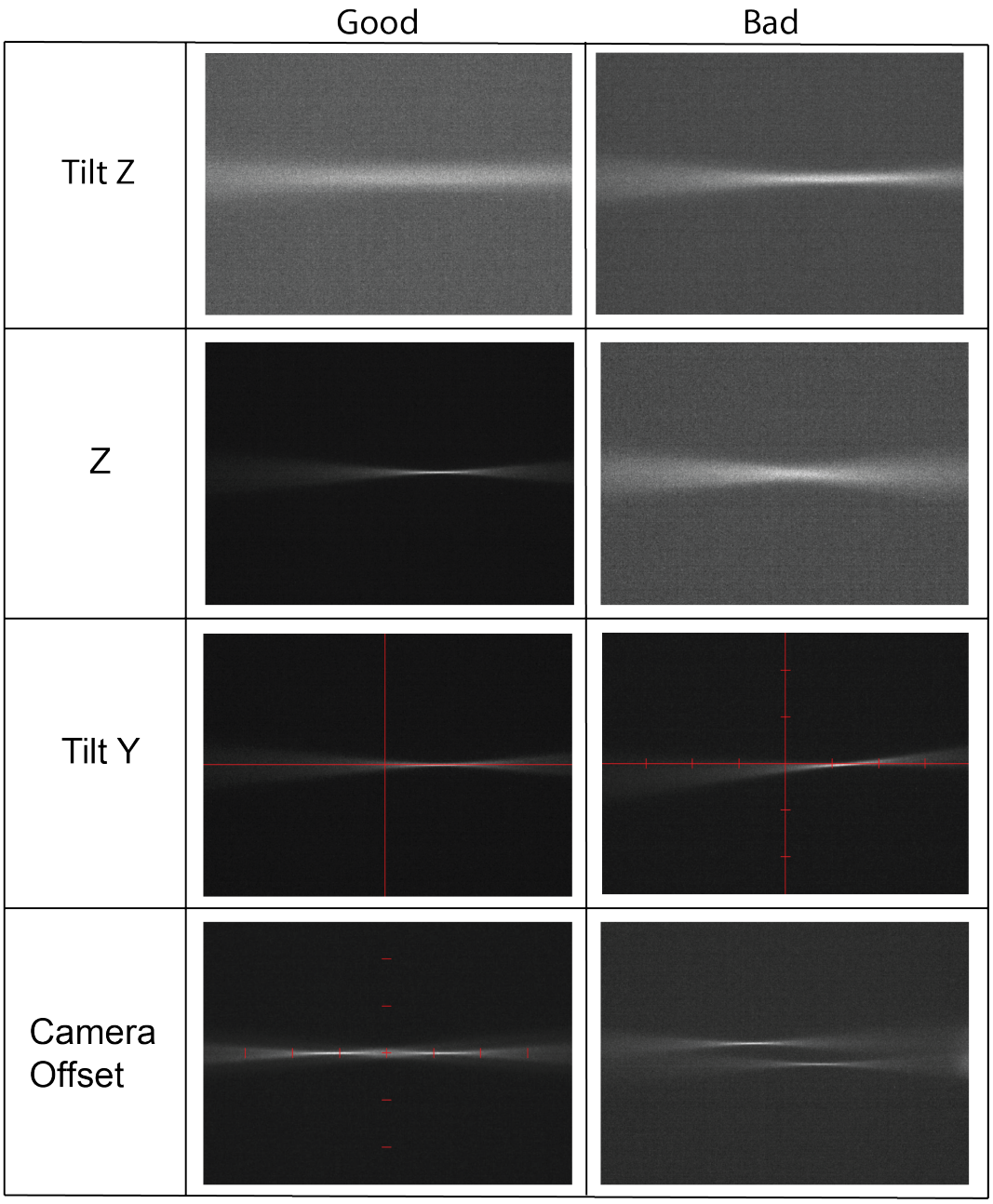
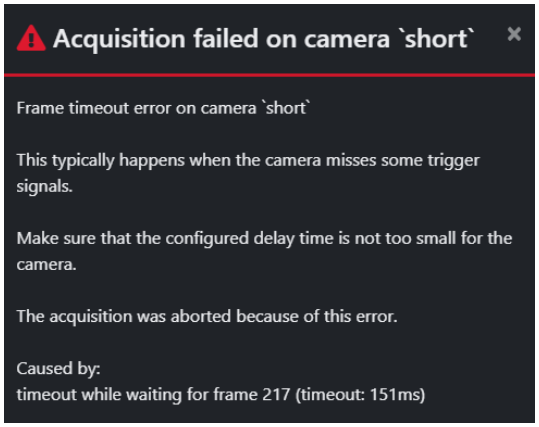


Figure 9: Correct Alignment

7.2 Time Series Errors

Camera times out: Set delay to >10ms



7.3 Cannot See Sample

Generally setting the "Z" to -500 and moving the X back and forth should get you close to your sample. If you don't at least see debris when you are scanning back and forth please check these areas:

1. Has the button on the backside of the blue box been pressed after the sample chamber was opened?
2. Are the lasers switched to ON? Laser power alone does not trigger them.
3. Are the emission filters and dichroic beam splitters in the correct configuration?
4. Does only a line appear? Check that lightsheet is on autoscan and not park.