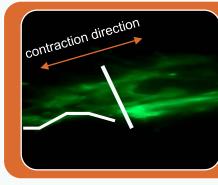
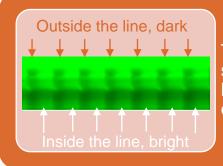
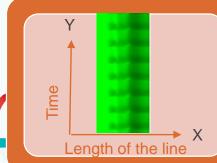
Understanding line scanning (1)



You basically image/scan a line drawn in your sample/live window. When drawing the line, it is recommended to go across the sample's movement direction (note: not the same direction as the movement direction) or along the signal edge area/line (you just need to try out which line direction works out best to detect movement).



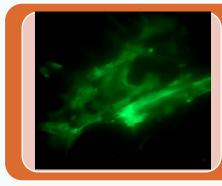
The major point of lice scanning is, if there is a frequent movement of the testing sample, the signal along the drawn line will be moving in a repetitive pattern like this: bright (when the line-covered cells/areas are still staying within the line)-dark (when the cells/areas previously drawn the line on have moved outside the line)-bright-dark-bright-dark...



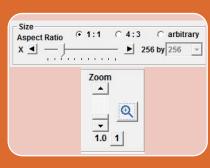
Line scanning is an XT experiment. The X axis is the drawn line (can be short/long, or straight/curve, or flat/angled) area of the sample. There is no Y position/location/depth in line scanning compared to normal scanning (this is because you always scan one single line not an XY area). In the final data/image, your X is the single line of your sample (from left to right along the length), your Y is time (made of time points)

hope.

Understanding line scanning (2)



Line scanning is a good imaging technique to measure high-frequency biological processes such as fast heart beating. The expected data/result to expect is more about the frequency of the fast movement i.e. heart beat rate rather than an image. However, this does not mean no need to acquire a time course image to show vision data on heart beating.



Line scanning normally requires high speed scanning to match fast moving processes in a live sample. To achieve high speed, you can try these in the acquisition software:

1. Low pixel size (its okay to go low pixel numbers as you don't care about image quality/resolution since you are aiming to get frequency rather than an image)

2. Zoom in (its okay to zoom in a bit again you don't care about resolution as your final data is not a normal image but frequency data)



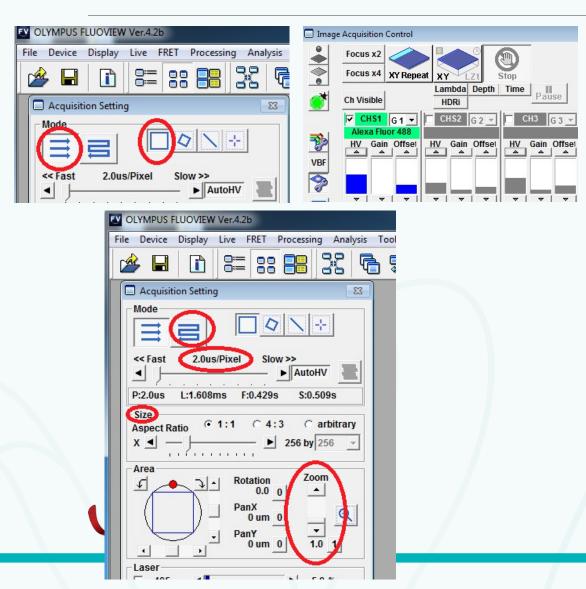
3. Draw a shorter line over your sample

4. Choose dual scanning direction mode for line scanning (not the single direction mode)

2

hope.

Step-by-step procedure



1. Set up channels (focus, saturation etc) as usual.

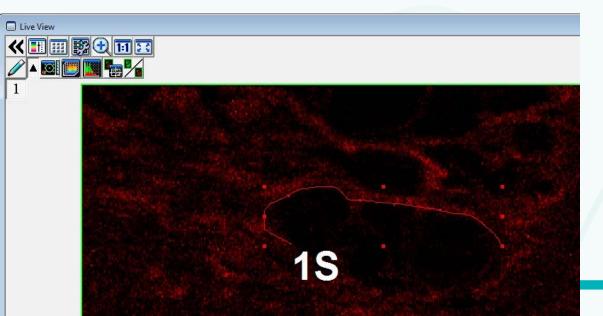
2. Calculate the speed required to match biological speed so you can figure out pixel size, zoom in ratio, dual direction or single direction) etc

Finding cures. Saving lives. Giving hope.

3

Step-by-step procedure

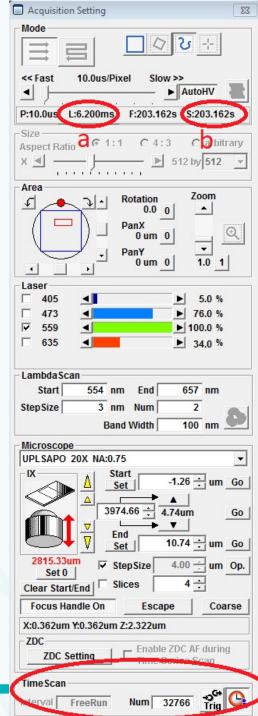
V OLYMPUS FLUOVIEW Ver.4.2b
File Device Display Live FRET Processing Analysis
Acquisition Setting
<
P:2.0us L:1.608ms F:0.429s S:0.509s



3. Choose line scan mode. Note: if you hover the mouse over the "line" icon, it will pop up with additional "freehand drawing" option to draw a line.

4. Draw a line on the sample in the live window. Its recommended to draw the line in this direction: across sample movement direction (not the same direction as its movement direction) or along the signal area as shown in the left screenshot.

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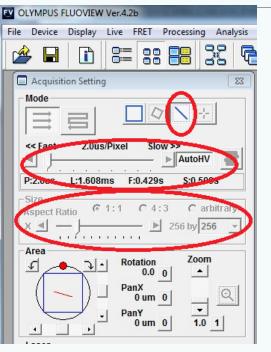
Step-by-step procedure

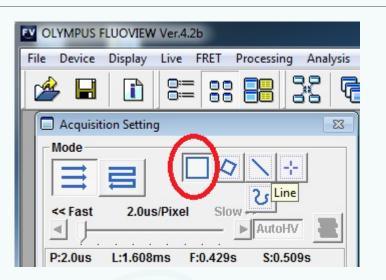
5. In "Acquisition settings, set up your time course (at the bottom). Normally you would go for "freerun" in "interval and just decide how many cycles, i.e.:

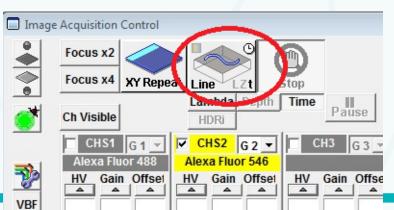
- a. Current scanning speed set to 10us/pixel
- a. Total scanning time based on the current speed and total cycles of 32766

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Notes





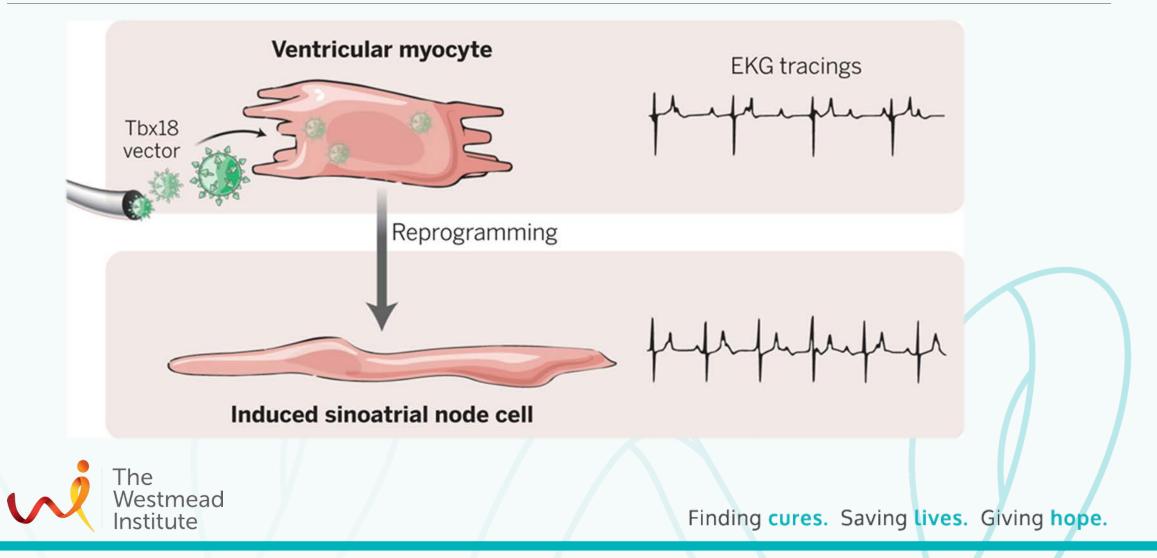


 Better to calculate your required scanning speed before clicking "line" scanning mode. Because once Line scanning mode is turned on, you are not able to change scanning speed nor pixel size to adjust final imaging speed any longer. However, you can always toggle back to full scanning mode to reactivate scanning speed & pixel size adjustment options

2. Once, you choose "Line" scanning mode, your image acquisition becomes automatically to Line/T (not XYT as there is no Y info acquired).

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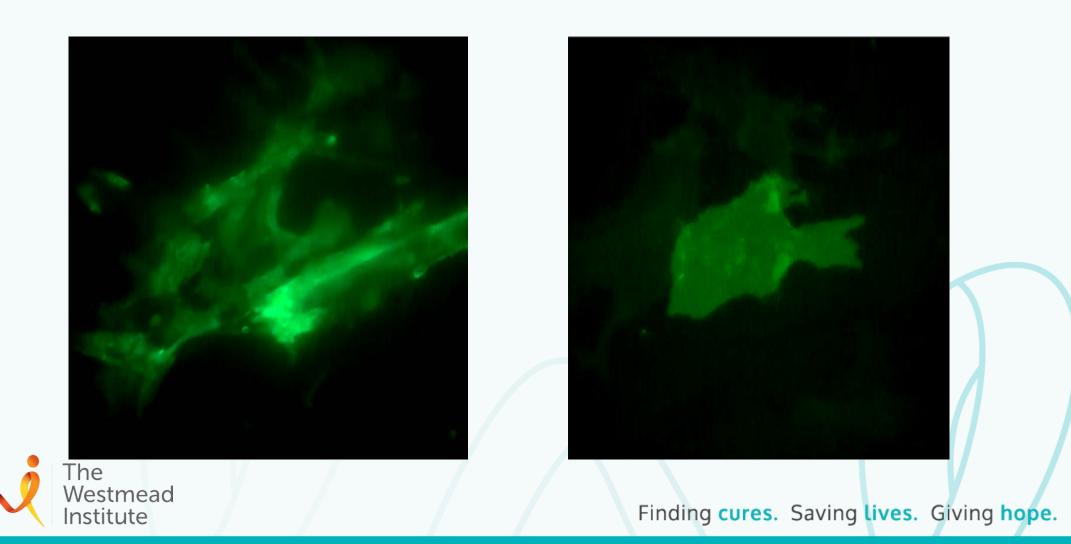
Line scanning example: DEVELOPMENT OF BIOLOGICAL PACEMAKER USING GENE THERAPY



7

Science, 345 (6194): 268-269

VISUALISING BEATING HEART CELLS



8

ANALSYING THE DATA



9