FLIM Turn ON





Switch on the pulsed 488 laser



Turn on FLIM computer



Click on 'FLIM' remote desktop icon



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login to FLIM computer USERNAME: microscope PASSWORD: microscope

Windows Security				
Enter your credentials These credentials will be used to connect to mb229.				
	microscope Þassword			
	Use another account			
Remember my credentials				
	OK Cancel			



Start SPC64 software

Ε



FLIM (I)

Click on Lightpath diagram icon

Set the lightpath manually so that it looks like this:



▶ AutoHV ◄ Set scan speed to 'Fast' 2.0us per pixel P:2.0us L:2.116ms F:1.109s S:1.109s Size • 1:1 04:3 O arbitrary Aspect Ratio Set 'Aspect Ratio' to 512 by 512 X 🖣 512 by 512 -. Sequential Untick sequential scanning Filter Mode ⊙ Line O Frame Untick 'Kalman' averaging mode Kalman 4 + Focus x2 Click 'XY Repeat' scan button Focus x4 XY Repeat RepeatStop

<< Fast

2.0us/Pixel

Slow >>



FLIM (II)

An initialization window appears Click 'OK'

Three windows should appear:

SPC-150 is the main display window

DCC-100 control the detector Predefined setups





DCC-100 - - -Main Parameters Exit Connector 1 Connector 2 Connector 3 Cooling On ■ +12V ■ +5V **+**12V **_**+12∨ Curr Imt +5V _ +5∨ __-5∨ __-5∨ _-5∨ 2 Amps 📕 b7 100% 100% **b**6 OVLD OVLD ____ b5 ľ 1.69 ۲ ۲ 📕 b4 Ê 5 **b**3 ____ b2 Volts 📕 b1 n 0 📕 b0 \$ 76.87 \$ 76.87 3.87 Gain / HV DigOut Gain / HV Cooler Settings from auto.set Enable outputs

🋐 Predefined Setups	8
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FLIM (II)

An initialization window appears Click 'OK'

Three windows should appear:

SPC-150 is the main display window

DCC-100 control the detector Predefined setups

Click 'Enable outputs' to enable the detector (the Gain / HV should be roughly 80%)



Settings from auto.set Outputs disabled Enable outputs



FLIM (IV)



Scan clocks green light: The FLIM computer has the correct timing from the confocal

FLIM Turn OFF





Close SPC64 software

Log off FLIM computer





Switch off the pulsed 488 laser



Tick 'Kalman' averaging mode Tick Sequential scanning

Filter Mode	€ Line	C Frame 2	
Sequential			
• Line	Frame		

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Return lightpath to standard 3 colour imaging **IMPORTANT** - make sure last DM is in the '**MIRROR**' position.

