



Spectral Confocal - C1si

Do NOT use this microscope unless you have been properly trained by Kurt or Sebastian.

Start Up Procedure -

1. Turn on arc lamp (EXFO) to the right of the microscope table
2. Turn on the lasers:
 - a. 405nm (turn key behind laser – green light behind laser should be on)
 - b. 488nm (turn key to on position and press green button)
 - c. 561nm (turn key to on position)
3. Turn on controller box (located underneath laser launch)
4. Turn on z-stepper motor
5. Turn on halogen lamp if you want to image using brightfield
6. Open EZ-C1 software and begin imaging

Shut Down Procedure -

1. If no one is signed up 2 hours after your session is over:
 - a. Raise objectives fully up and move up the focus
 - b. Clean off oil objectives with lens paper
 - c. Turn off focus knob
 - d. Turn off halogen lamp
 - e. Turn off the lasers: 405nm, 488nm, 561nm
 - f. Turn off arc lamp
2. If someone is signed up after you:
 - a. Turn off EZ-C1 software
 - b. Leave everything else on

Overview

The C1si is a Confocal Laser Scanning Microscope with three lasers for red/green/blue imaging, a spectral detector for doing more advanced unmixing, and a transmitted light detector for doing DIC. It is on an FN1 upright microscope and can do imaging from low magnification tissue slices to high magnification spectral unmixing. It can focus up to 100-200 um into a sample depending on the opacity.

Objectives

#	Objective	Resolution limit (nm)	Depth of field (um)	Suggested step size (um)	Transmitted light	Comments
F1	10x/0.45 na air Plan Apo	678	6.42	2.57	DIC N1/10x	
F2	20x/0.75 na air Plan Apo	407	2.31	0.924	DIC N2/20x	
F3	40x/0.95 na air Plan Fluor	321	1.44	0.576	DIC N2/40x I	
F4	10x/0.30 na W Plan Fluor	1017	14.4	5.76	DIC N1/10x	Water Dipping
F5	40x/0.8 na W NIR Apo	381	2.03	0.812	DIC N2/40x III	Water Dipping
F6	60x/1.0 na W NIR Apo	305	1.3	0.52	DIC N2/60x I	Water Dipping
F7	40x/1.30 na oil Plan Fluor	235	0.77	0.308	DIC N2/40x II	
F8	60x/1.4 na oil Plan Apo VC	218	0.66	0.264	DIC N2/60x I	
F9	100x/1.4 na oil Plan Apo VC	218	0.66	0.264	DIC N2/100x I	

Brightfield Contrast Methods

Köhler Illumination – How to focus the condenser to get uniform illumination

1. Open the EPI illumination shutter
2. Focus your sample in brightfield
3. Close down the field diaphragm
4. Focus the condenser until you see a small hexagon of light on your sample
5. Center the condenser using the two centering screws
6. Open the field diaphragm until the hexagon of light just fills the field of view

Note: You may need to tweak the illumination when switching objectives

<http://microscopyu.com/tutorials/java/kohler/index.html>

DIC – Differential Interference Contrast Microscopy

For DIC you need 2 Wallaston prisms and 2 polarizing filters, the light path is as follows:

1. *Lamp* – Turn it on
2. *EPI Shutter* – Open it
3. *1st Polarizing Filter* – Slide the polarizer in and twist it to modify contrast
4. *1st Wallaston Prism* – Turn condenser wheel to either N1 or N2 depending on the objective
5. *Condenser Lens* – Focused using Köhler Illumination
6. *Sample* – Make sure you are looking at something
7. *Objective Lens* – Focused on your sample
8. *2nd Wallaston Prism* – Sliders located above objective in nosepiece
9. *2nd Polarizing Filter* – Also called Analyzer

<http://microscopyu.com/articles/dic/>

Fluorescence Microscopy

Filters for the Eyes	
Fluorophore	Cube
DAPI	1. UV2E/C
GFP	2. GFP LP
YFP	3. YFP
Rhodamine	4. G2E/C
D/F/R	5. D/F/R
Confocal	6. Empty

Confocal Lasers	
Laser Lines	Emission Filters
405nm	450/35
488nm	525/50
561nm	605/60
brightfield	n/a

The Spectral Confocal has:

- 3 lasers for doing traditional 3 color (red/green/blue) Imaging
- A transmitted light detector
- A spectral detector that can image the whole spectrum in 32 channels
 - This is useful for spectral un-mixing, 4 or 5 color imaging, and other applications

There are 5 filters in the filter turret to visualize your specimen and focus on an ROI with your eyes.

Troubleshooting

- The z stepper motor is not working:
 - ✓ Make sure that the knob is pulled up on the rear left of the microscope
- If you cannot see anything in the eyes follow this checklist:
 - ✓ The arclamp is turned on
 - ✓ The shutter above the nosepiece is open
 - ✓ Number 1-5 is selected on the filter wheel
 - ✓ The eyepiece/scanhead changer is pushed in
 - ✓ You are focused on your sample
- If you cannot see anything on the computer follow this checklist:
 - ✓ You are focused on your sample
 - ✓ The filter wheel is set to 6
 - ✓ The eyepiece/scanhead changer is pulled out
 - ✓ Both the detector and laser are turned on so they are colored
 - ✓ The detectors that you want are moved to 6.5 or higher
 - ✓ The Lasers that you want are slid on
- Your sample is getting photo-bleached too quickly:
 - ✓ Try using less laser power and more detector gain
 - ✓ Try focusing using single frames instead of live
- Your image is too noisy:
 - ✓ Try increasing laser power and decreasing detector gain

- Optimizing to get higher resolution:
 - ✓ Use a higher NA objective
 - ✓ Zoom in by clicking on the cube in the top right corner of the screen (it will tell you when this has been reached when the NON OPTIMAL PIXEL SIZE disappears)
 - This happens when you get Nyquist sampling of $(0.61 \cdot \text{emission}) / \text{Numerical Aperture}$ so that you have 2.7 pixels per diffraction limit.