A Quick Guide for Using the Olympus iX80/CARV CONFOCAL MICROSCOPE

1 – Switch on Both power strips

There are two power strips controlling the Confocal microscope components. The strip on the right (near floor) controls the Olympus fluorescent and Nikon Chroma (both Mercury) burners. The power strip on the left (near floor) controls all the equipment located on the rack below the microscope and listed below.

- Olympus iX80 inverted microscope
- Carv confocal/camera device (Control box is on table behind isolation Crypt)
- Switch box (toggle on back)
- Hamamatsu Orca camera (beige box)
- Photonics QEM camera (black box), also power switch on top of camera.

Switch on both power strips. If you do not need fluorescence, turn on only microscope power strip (left).

2 –Start Metamorph, Camera Illumination and Acquisition

Wake up computer and double click MetaMorph (2) icon. It should open with no error messages. Press Camera > Configure Camera (CC) and Acquisition (A) setup (extreme right of monitor)

3 – Select 10 - 20X Condenser stop 1

Make sure 10x or 20x objective (bottom of left monitor) is selected and is centered in the opening on the stage. Adjust the condenser Selector (on body of microscope above stage) to 1 (open position).

3 – Insert slide, Select DIC Orca, monitor). Live.

Place slide sample side down (may need a coverglass) onto microscope stage. Select DIC Orca (bottom of left monitor) in drop down list. Push Live (right column on right

4 – Focus.

On microscope push light path select (front panel top left) to binoculars. Move specimen around to find a good location. Push button on front panel top right to select coarse (green) focus. Focus specimen.

5 – Kohler illumination

Set Kohler illumination (optimizing the condenser). Although this is for brightfield imaging, it is a good way to begin imaging for any mode and allows quick searching for good areas. Can only be done at 10x or 20x.

A - Close field iris to image the 8 sided iris aperture.
B - Focus edge of iris aperture (not specimen) with knob on column behind microscope.
C – Use centering knobs on condenser to center the field iris image in the field of view.
D – Open the field iris most of the way.

6 – Select Monitor view, Set exposure 1 msec, Set Iris diaphragm for contrast.

Set light path from binocular to monitor. For most samples the specimen z (bottom right of view monitor) will be around 950-1050 microns. In Acquire menu the exposure should be 1 or 2 msec. Try different exposures to maximize the spread of the grey scale indicator just to left of the image. If either upper or lower gray scale marker is off the scale then the exposure is not set correctly. Thick samples, higher mags or fluorescent modes will require more exposure (into 100s of ms) which take longer to acquire. Use the iris diaphragm (left side of condenser body) to darken slightly and increase contrast.

7 – BF All objectives, Phase 10, 20, 40x,

With the condenser selector set to 1, brightfield (BF) imaging can be carried out with any of the objectives (including water or oil immersion). With the 10x, 20x and 40x objectives, brightfield or phase contrast modes can be imaged. For Phase Contrast

DIC 60 water, 100 Oil. Follow Table.

imaging with 10x or 20x objective, the condenser selecter should be set to 2. For Phase Contrast imaging at 40x, set the condenser selecter to 3. For DIC using the 60x water immersion lens, use condenser select 4 and DIC using the 100x oil lenses condenser selector should be set to 5.

Quick Guide to Setup for Microscope Modes (Brightfield, Phase, DIC, fluorescence)

	Objective	Condenser	Polarizer	DIC	Olympus/Carv	Exposure (msec)-Orca
Brightfield	All	1	Out	Out	Out	1-2
Phase Contrast	10x, 20x	2	Out	Out	DIC Binoc	1-5
	40x	3	Out	Out	Dic Binoc	10
DIC	60x (water)	4	In	In	Dic Binoc	50-100
	100x (oil 1.3;1.4 NA	5	In	In	Dic Binoc	50-100
Fluorescence						
Olympus	All	as above		as above	Olym Source	100-500
Nikon	All	n/a		n/a	Carv Source	100-500
Confocal	All	n/a		n/a	Confocal (Carv)	100-1000

8 – Aquire. Save

Press Acquire. To save use Save As. The .tif images are 10 or 12 bit and can be hard to open. The easiest for routine imaging and display is to save as .jpg.

9 - Select camera, Rotate camera lever.

If more sensitivity (low fluorescence) is needed switch to the QEM camera (mounted above Carv unit). In Camera:Configure Camera menu, select QEM camera and press OK. Manually move lever (left side of Carv behind Orca camera) to Up position.

10 - 60x -water 100x – oil

To move to a water or oil objective, remove slide, select objective (60x or 100x). If 60x place a drop of water on face of objective. For 100x (either the 1.3 or 1.4N.A.), place a drop of supplied immersion oil either onto the objective or onto the desired location on the underside of slide. Carefully lower slide over objective to avoid entrapping bubbles.

11 time lapse

12 z stack