Instructions for Olympus Wide-field microscopy

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Start the system - 1

1. Turn on the Arc Lamp.

NOTE: This step can be skipped if the arc lamp is not use.

**If you turn off the Arc Lamp and for turn it on again, you have to wait for 1 hour to cool down.

2. Turn on electronic controls by switching on the power outlet extension strip.

- 3. Turn on the computer. Log into the computer with your email account.
- 4. Start the Internet Explorer web browser and log into the 'Record Usage Time' page at the KCCI website with your KCCI account.

4. Stat the Metamorph for Olympus Basic software on the desktop.

Start the system - 2

5. In MetaMorph Software, open the "Configure Acquisition..." window from the menu "Acquire->Acquire..." or

the "Configure Acquisition" icon.

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- 6. In the "Configure Acquisition..." window, click the 'Special' tab to change the Digitizer from '14 bit' to '12 bit'.
- 7. Close Metamorph software.
- 8. Restart Metamorph software.

NOTE: Steps 5-8 are required because of a communication problem between the electronic and the Metamorph software.

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Shutdown the system

- 1. Close the software (Metamorphosis).
- 2. Start to copy the acquired images to the K drive for your data transfer.
- 3. Click 'Set Current Time' for your ending time on the time recording page and click 'Submit' to record your usage time.
- 4. Turn off the computer if data is copied to the K drive .
- 5. Turn off electronic controls by switching off the power outlet extension strip.
- 6. Turn off the arc lamp.
- 7. Clean the objective lens and working bench area

Select the objective lens



Turret to hold maximally 6 objective lens – you need to manually rotate the turret to select an objective lens.







Make sure to read and understand the labels on an objective lens and put the correct immersion for it. Clean the objective lens before and after use.

For dry lens, although no immersion is required, cleaning is still needed at the beginning to remove any dirt.

Follow instruction given by the center staff for cleaning an objective.

Moving Stage and Focus Manually or by Software

Manual Control





Coarse Focus

Stage XY Movement

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Focus Control

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The software shows the stage and focus current positions. You can move stage and focus by clicking the 'arrow buttons'. Each click moves in a certain steps, which can be defined in 'Devices->Stage->Move Stage to Absolute Position' 'Device->Focus'



Each XY step is 0.2µm. Each Focus step is 0.01µm.

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Load the imaging method



Make sure to have the correct cube in the cube turret (upon your imaging and the method set up for you). Verify this with the KCCI staff if you are not sure.

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In the "Configure Acquisition..." window,
Go to the 'Acquire' tab and choose the imaging method.
NOTE: To enable the method, you have to click the 'Live' or 'Acquire' button at least once.

NOTE: Get trained by the KCCI staff for using a method. Do NOT create a new method by yourself. Ask the KCCI staff to create it for you.

To achieve the desired image intensity,

- Adjust the camera exposure time.
- >Adjust the illumination intensity (explained below).

Brightfield Light Control



Brightfield light HAL lamp house. Brightfield shutter (software controlled). Field Diaphragm. Condenser.

Brightfield light on/off switch button Brightfield light intensity adjustment bar

In the software, two methods are created for brightfield imaging:

- 1. 'brightfield' (after chosen and enabled by clicking the 'Live' or 'Acquire' button once) allows the brightfield shutter open all the time.
- 2. 'brightfield_wshutter' only allows the brightfield shutter open for imaging when the 'Live' or 'Acquire' button is clicked.

NOTE: For brightfield imaging, the Köhler illumination alignment should be checked. Get trained by the KCCI staff for the Köhler illumination alignment.

Fluorescent Light Control

- Adjust Arc lamp output intensity. NOTE: Typically it is set up for its maximal and should not be changed.
- -In the external excitation control box,
 - > 'Neutral Density (ND)' filters are installed on a filter wheel.
- 'Excitation (Ex)' filters are installed on a filter wheel.
- Shutter is installed.
- In the external emission control box,
 - 'Emission (Em)' filters are installed on a filter wheel.
- Shutter is installed.

All of them are controlled by the software.

Typically, the Ex. and Em. filters are defined by the imaging method you select, and the shutters are open when only imaging is enabled through the 'Live' or 'Acquire' button. NOTE: make sure to have the correct cube in the cube turret. Adjust the excitation intensity by selecting a proper ND filter -

- **Go to 'Devices->Device Control'** in the menu.
- Click 'Component Control' in the 'Device Control' window.
- Choose 'Ludi Wheel 1' for 'Component'.
- Select the 'ND' filter from the dropdown list The higher number the less excitation intensity. 'Blank' means no filter and the slot is empty.



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Acquire an image

Click 'Show Live' to see a live image on the screen.



Note: Remember to switch to the **'SP'** position (for camera) from the **'EYE'** position (for ocular) on the microscope right side.



Adjust the focus and then click 'Acquire' to take an image.

Note: Enabling 'Autoscale' will have the software automatically adjust the contrast.

Save an acquired image

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-Zoom in/out the image digitally, you can also do this by right clicking the image

Assign a color LUT to the image

Define the dynamic range for display

the histogram

Untitled (50%)

Adjust the contrast by sliding the two marks

Click 'Save' icon to save your image. By default, the image will be saved as a greyscale TIFF image.

Save images into the folder in the D drive. Do NOT save images to the server (K drive).

After finishing imaging, copy the acquired images to the K drive for your data transfer.

Widefield Objective Lens Calibration

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Calibrate by Region.

Load from File ...

Apply To All Open Images

Save to File.

Close

All the widefield objective lenses are calibrated for the pixel resolution without and with the 1.5X additional zoom enabled. You can check the calibration by going to the menu – ` 'Measure->Calibrate Distances...' **NOTE:** Not all objective lenses are installed on the scope and verify it with the KCCI staff.



Pull out to get an additional 1.5X zoom.

Acquire single-channel timelapse and z-stack

Time Lapse

Z-stack



NOTE: Make sure to have the correct cube in the cube turret and set up the proper ND filter for fluorescent imaging.

Acquire multi-channel timelapse

Get trained by the KCCI staff for using the 'Multi Dimensional Acquisition'.



NOTE: If there is focus drift during time course, you can pause the acquisition, click 'Live' to re-adjust focus and then continue the time course.

Go to the menu – 'Acquire-> Multi Dimensional Acquisition ...'

This powerful interface will allow you combine –

- Timelapse
- Multiple stage positions
- Multi imaging channels
- Z-stack

Check the corresponding label(s) to activate the functions to be applied.

For multi-channel timelapse,

Follow the setup steps to

- Choose the folder to save your images in a given name pattern.
- > Define time interval and duration.
- Define the number of imaging channels and load the imaging method for each one.