Start-up system

- Switch on the power socket located on the floor
- Switch devices on in the order of:
  - Mercury lamp control-unit — switch on and ignite
  - Halogen lamp
  - Incubator control-box
  - Microscope stand — located at the right, behind the serial plug
  - Stage controller — located at the back of the device
  - Shutter controller
  - Camera — via power supply located on the floor
- Wait approximately 5 seconds before you start the computer
  - Log in with user: nikon01 password: nikon01
- Start imaging software Metamorph
  - Select "User" account

Using Tokai Hit Incubator + CO_2

- Fill reservoir with demineralized water up to level of the CO_2 tube
- Place lens heater around desired objective (adjust the Velcro to fit around lens)
  - For Oil lenses only
- Settings for the heating unit are:

	Dry objective	Oil objective
Top heater	39.0 °C	41.5 °C
Bath heater	36.5°C	38.5 ° C
Stage heater	36.5°C	38.5 ° C
Lens heater	-	37.0 ° C

- Open the valves of the CO_2 gas cylinder
  - Turn the valve on top of the cylinder ¼ open
  - Turn the tap at the pressure meters open
  - Pressure at the left meter should be 1-2 bar
- Enable on CO_2 mix unit of the incubator control-unit
- Percentage is set to 5%, mixing gasses takes about 5-10 minutes

- Elements of the incubator can be switched on/off individually
  - Use this is when lens heating is not needed
- Allow the system to warm up for ~30 minutes

Focus your cells

- Choose your objective
  - 100x , 60x , 40x **oil** or 20x **air**
- Put the objective in the lowest position
  - By using the focus knob, or button Escape (right side lowest button)
  - Lowest position is ~500 um
- Put a little drop emersion oil on the lens
  - Only if it's an oil emersion objective
- Centre you coverslip/well/cells above the objective with the joystick
  - Button on top of joystick will change speed, S (slow) or F (fast)
- Focus the objective till the oil is in contact with the coverslip
- In Metamorph for normal halogen light choose:
  - for 20x: Taskbar button: DIC Binocular
  - use filter "N2" in microscope head
  - for 100/60/40x: Taskbar button: DIA Binocular
  - use filter "A" in microscope head
- Adjust precision of focus wheel
  - course, fine or extra fine Buttons are located left and right on the stand
- The display of the microscope shows the height of the objective
- Buttons L100 and EYE at the front of the microscope stand display the direction of the light path (left -> camera, up -> eyepiece)

Hardware autofocus: Perfect Focus System (PFS)

- When imaging with just a coverslip, hardware autofocus is possible
  - Orange LED will light up if coverslip is within reach
  - Enable PFS with button "Focus" located at the front of the microscope stand
  - Focussing is done with external focus device
    - Button at the front changes precision of focus wheel
  - Focusing with PFS should be viewed through the camera, it's to precise to see with your eyes

Controlling intensity mercury lamp

- Fluorescence can be detected with use of the Mercury lamp
  - Choose illumination settings according to your fluorophore or dye
  - By pressing a button in the taskbar
- Intensity of the light can be controlled with neutral density (ND) filters.
- These are located between the actual lamp (right) and the microscope stand.
  - Press inwards to reduce intensity by 4 or 8 times (ND4, ND8)
  - Most right filter will block all light

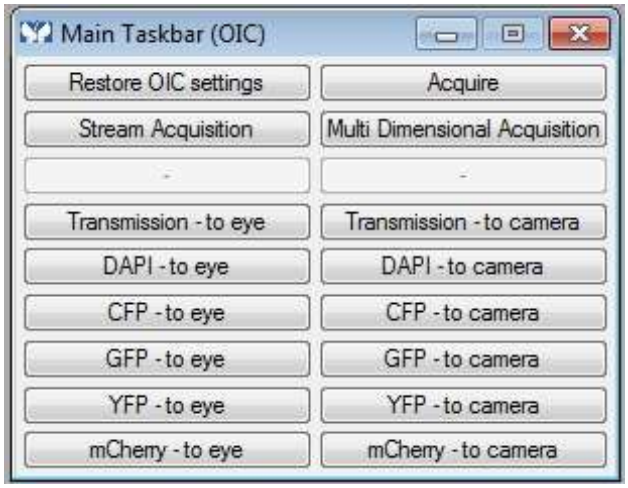
Metamorph - Taskbar

- Metamorph functions from a taskbar and an Acquire window
- When the taskbar is not visible
  - Load the taskbar via: Journals>Taskbar>Load Taskbar
  - Location is C:\MM\TASKBARS\
- You can reset the illumination settings with the first button: "Restore OIC settings"
- Select preferred illumination with buttons
  - Left column to eyepiece
  - Right column to camera with live mode

Restore illumination settings

Open stream window

Select illumination setting to eyepiece

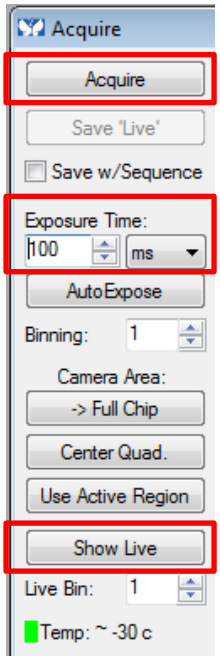


Open acquire window

Open MDA configuration

Select illumination setting to camera and start Live mode

Metamorph - Acquire window



Sidebar left:

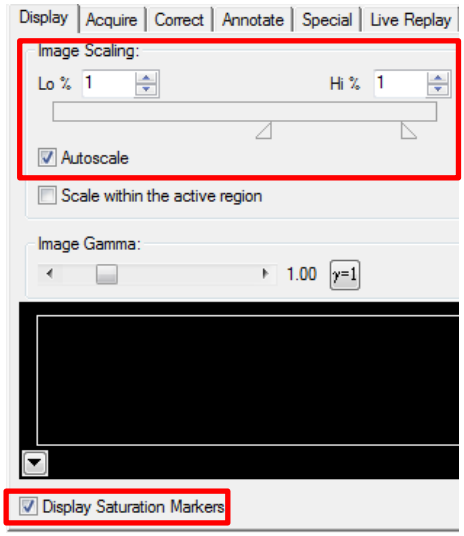
- Exposure time
  - Minimum interval time at full chip is 90ms, quad chip is 50ms
  - Exposure time can be lower than interval time
- Full Chip, Quad Chip, Use Active Region
  - Use full chip, middle quadrant, or region selected by ROI
- Live bin & bin
  - Combine pixels to increase readout speed
  - Use only for live viewing or real recording
  - Normal state = 1
- Start Live/Stop Live
  - Start/stop live mode

Acquire images

- To acquire live mode press "Acquire" button
- Separate images can be recorded as separate images or in stack

Tab: Display:

- Auto scale option
  - Enable Auto scale or change custom auto scale
  - Full bit-range is saved to file, this is only for visualization in the software
- Visualize saturated pixels

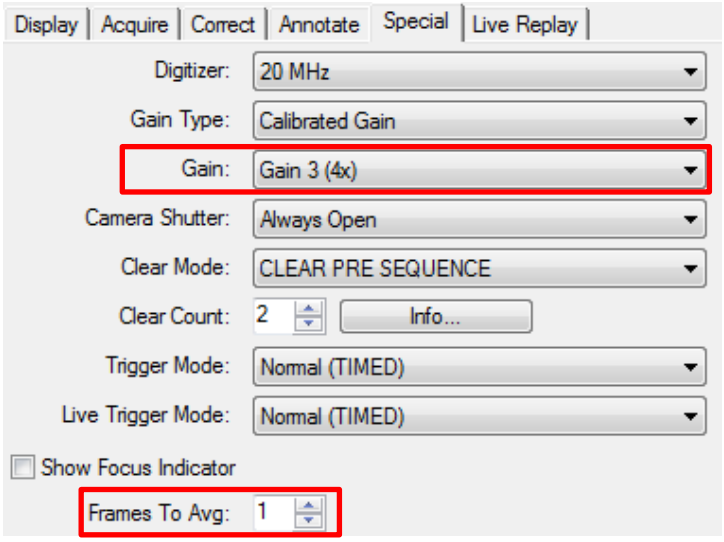


Tab Acquire:

- Be sure to set the Illumination choice to "Current shutter"

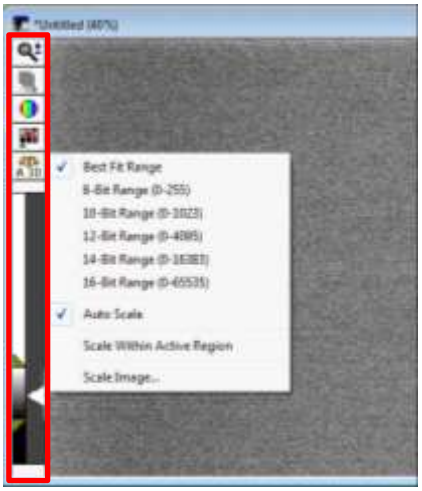
Tab Special:

- Adjust digital gain
  - Calibrated gain: 1, 2, 3
  - Gain 3 (4x) is standard option
- Use Visual Gain to control gain between 0 and 580
- Use "Frames To Avg" option to average images / or use longer exposure time

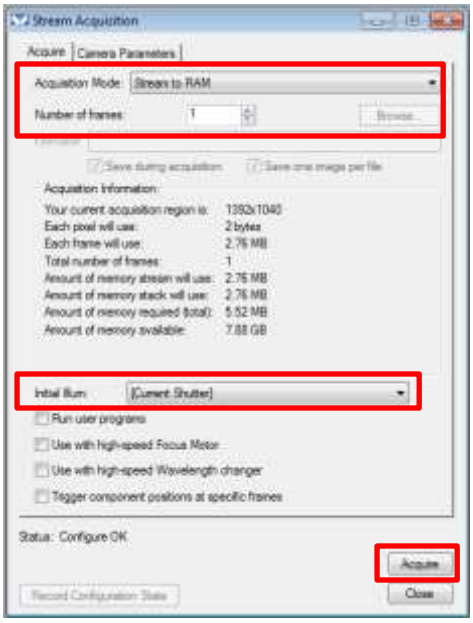


Live image window

- Magnifying glass
- Display image in other LUT
  - Grayscale, Colour by wavelength
- Histogram of intensities
  - Manual scaling can be done with the orange arrows
- Auto scaling options
  - Best fit range, 8/12/14/16 bit
  - Auto scale on/off
  - Scale within region



Streaming imaging



Open the Stream Acquisition window with the button in the

- Start recording with Acquire button
- Check if chosen configuration is ok

Tab Acquire:

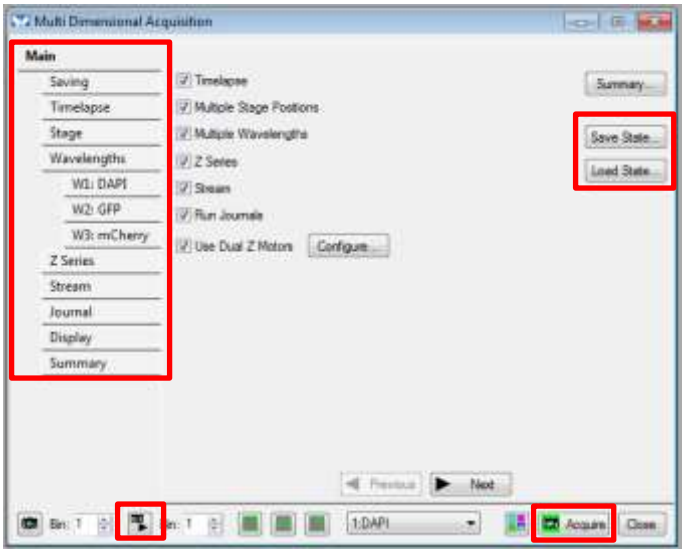
- Acquisition modes:
  - RAM save later to hard disk
  - HD choose name and location to save
    Save as on file or separate TIF files
- Enter the desired number of frames
- Select "Initial illumination": *Current Shutter*

Tab Camera Parameters:

- Recording options:
  - Average frames
  - Number of frames to skip, during acquisition
  - Don't show recording (faster when exposure time < minimal interval time)
- Camera readout default options should be:
  - Camera state: HALT
  - Shutter mode: OPEN PRE SEQUENCE
  - Clear mode: CLEAR PRE SEQUENCE

Multi-Dimensional Acquisition (MDA)

Combine multiple tasks: z-stack, timelapse, streaming, multiple positions, multiple wavelengths, use of journals.



- Open the MDA window using Taskbar
- Select acquisition mode(s) in *Main*
- Continue with the tabs shown left:
  - Saving: select filename and folder
  - Select illumination settings
  - … options for acquisition modes
- When setup is done press acquire

- Save or load settings of experiment setup, with Save/Load State buttons
- Live mode can be started from MDA window

MDA: Time lapse imaging

- Activate the time-lapse option in MDA's main window
- Set the desired interval and time
- Choose the desired illumination settings in the wavelength tab
  - Or multiple when the option multiple wavelengths are selected
- Start recording by clicking on Acquire

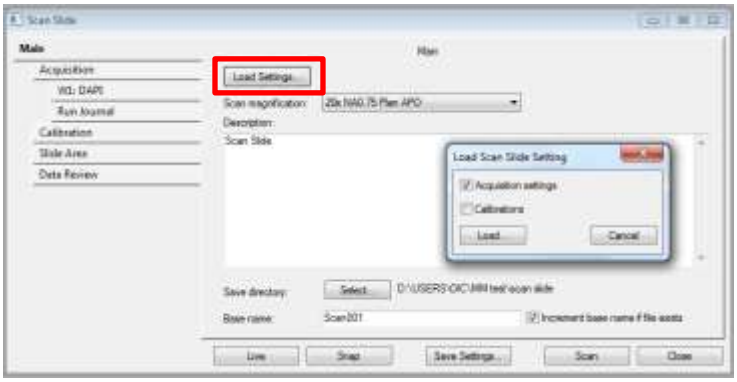
MDA: Z-stack

- Activate Z-stack option in MDA's main window
  - Activate use dual Z motor
  - Set all options to: *TiZ*
- Select option: Center around current
  - Height of objective is shown (same as display microscope)
- Give range, step size (in um), number of steps
- Choose the desired illumination settings in the wavelength tab
- Start recording by clicking on Acquire
- Don't use PFS during Z-stack
  - Activate "Run Journal" in MDA's main window
  - Choose PSF off before Z-stack and PFS on after Z-stack

Scan Slide (a.k.a. Tile scan)

Use this to make images of a larger area and stitch them together

- Access via Apps > Scan Slide



Main:

- Load Settings for re-use purposes
- Load calibration for used magnification

Acquisition:

- Select and configure desired wavelength(s)

Slide area:

- Navigate to upper left region and click on "set to current"
- Navigate to lower right region and repeat

Data review:

All images are saved, last image in sequence is stitched image.

- Stitch file in Data review tab
  - Choose overlap and channels

Shut down system

- Save data to OIC network storage (O:\ drive)
  - Connect to network storage with your own PC via the address: \\oic-station\oic
    user: guest, password: guest
  - When the hard disk of the microscopes pc gets full, data will be deleted
  - You are responsible for your own data
- For live cell imaging:
  - Remove Tokai Hit incubator from the stage and place it on the table
  - Close the Tokai Hit incubator with a plastic well
- **Clean objective carefully**
  - Remove oil with lens paper:
  - Hold lens paper tight between your fingers and wipe over objective
  - Clean lens with 2-propanol on your lens paper
- Cover stage with lens paper cleaning box

- **If you are the last user of the day**
- Shut down all devices
  - Shut down Mercury lamp at last
  - Camera power is switched at the adapter box at the floor
- Switch off the power socket located at the floor

OIC contact information:

- Martijn de Gruiter Be-341a tel. 42205
- Gert-Jan Kremers Be-346 tel. 43578