Spinning Disc microscope

Nikon Eclipse T*i*

Principal:

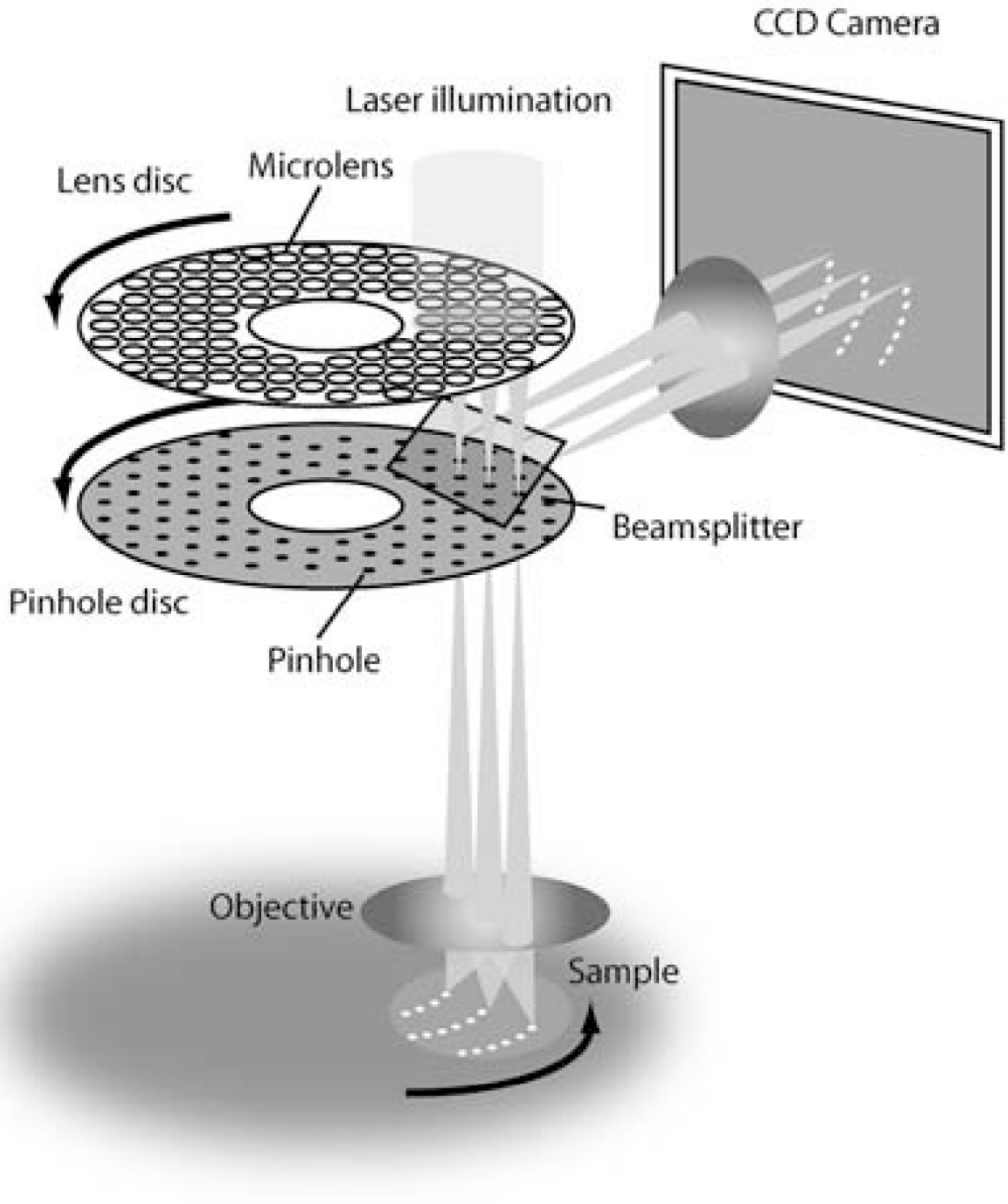
- Fast rotating discs are mounted to each other
- A Laser is projected on the first disc with micro lenses
- The focused and split beams will pass the pinholes in the next disc
- The beams rotate at high speed to excite fluorophores in the specimen in de field of view.
- Out of focus signals from your specimen will be blocked by the pinholes in the second disc
- The dichroic mirror projects the confocal-like-image on a camera

Benefits:

- High speed confocal-like imaging
- Lower bleaching rate due to speed of laser intensity

Downside:

- Fixed pinhole size



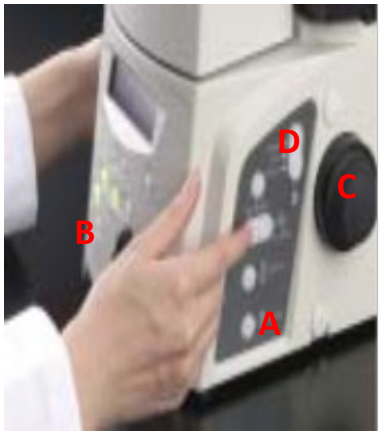
Start system

- Switch on the power by the power socket (on the floor under the table)
- Switch the mercury lamp on and then ignite it.
  - If not needed don't turn the mercury lamp on
  - The unit on the shelf closest to the door
- Switch on all devices with a button on the shelf and the table.
  - Camera and laser box are automatically enabled
- Next switch on the devices with key (Spinning disc unit and Laser control panel)
  - SPD unit has a shutter, press the little button. A red LED will go on
- Start the computer and start the program Metamorph.
  - Select suited user account.

Live Cell imaging:

- When imaging at 37°C:
- Place heating unit in microscope table before heating the device
  - Don't use any force if the unit is already heated before installing in microscope table
  - Wait till the unit is cooled down before installing the heating unit
- The heating device to maintain your cells uses water.
  - Fill reservoir with demineralized water if it runs empty
- Allow the system to warm up for ~30 minutes
- Settings for the heating unit are printed and at the wall

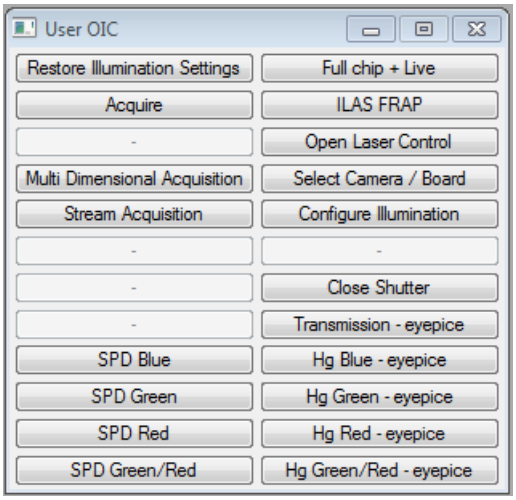
Finding focus



- Put the objective in the lowest position using button A
  - Lowest position is ~500 um
- Choose the objective with the preferred magnification
- Put a little drop emersion oil on the objective
  - Only if it's an oil emersion objective
- Place the metal ring in the middle of the heating chamber
  - Secure with the metal clips
- Centre you cells above the objective with the joystick
  - Button on top of joystick will change speed, S (slow) or F (fast)
- Turn focus wheel until the oil is in contact with the coverslip
- In MetaMorph choose transmission button in the Taskbar to activate transmission light
- With button D you can use the fine adjustment control knob
  - course, fine or extra fine
- Finding appropriate fluorescence cells can be done with the mercury lamp
  - Buttons are in the taskbar
  - Increase or decrease brightness by changing the ND filters
  - Located at the right back side of the microscope
  - ND 4, 8 will block intensity, they can be combined
- To change coverslips, bring objective down with lowest button A. Bring objective back to previous height with highest button A

Perfect Focus System (PFS)

- If focus is found a LED (E) will turn orange
  - Focus can be kept using the Perfect Focus system
  - Press the green blinking button E
- The focus of the PFS is adjustable with an extra focus wheel module
- The turntable navigate the lens up and down as shown and with the blue button toggles between fine (pressed) and coarse (out)
- Focusing with perfect focus should be viewed through the camera



Metamorph - Taskbar

- Metamorph functions from a Taskbar and an Acquire window
- You can restore illumination settings with the button: "Restore OIC settings"
- Open the Acquire window with Acquire
- Access Acquisition modes with: MDA & Stream
- Select preferred illumination settings
  - Left side: lasers for camera,
  - right side: mercury lamp or transmission to eyepiece
- Open FRAP or laser console
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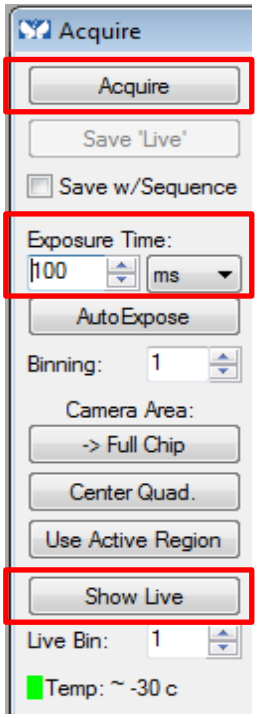


Metamorph - Acquire window

Sidebar left:

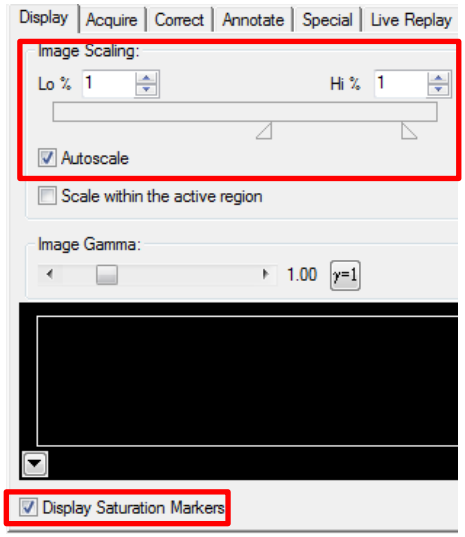
- Exposure time
  - Minimum interval time at full chip is 90ms, quad chip is 50ms
  - Exposure time can be lower than interval time
- Full Chip, Quad Chip, Use Active Region
  - Use full chip, middle quadrant, or region selected by ROI
- Live bin & bin
  - Combine pixels to increase readout speed
  - 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

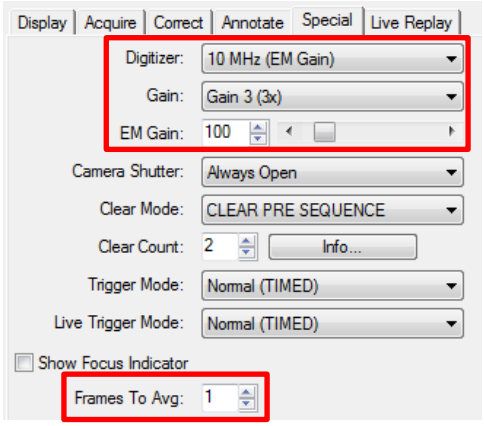


Tab Acquire:

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

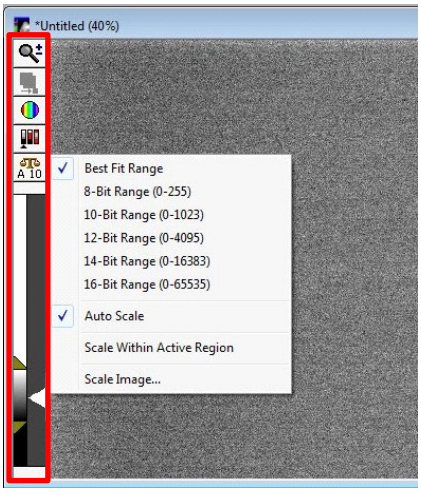
Tab Special:

- Gain options:
- 10 MHz (EM gain), gain3 3x is standard setting
- Control gain between 0 and 1000
- Use "Frames To Avg" option to average images / or use longer exposure time

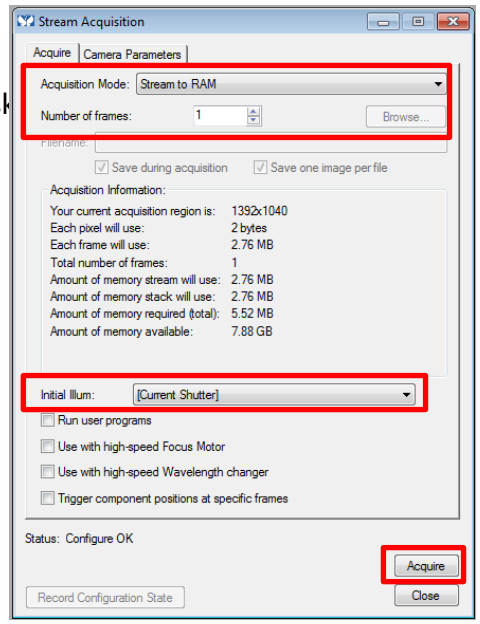


Live image window

- Magnifying glass
- Display image in other LUT
  - Grayscale, Color 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 task

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

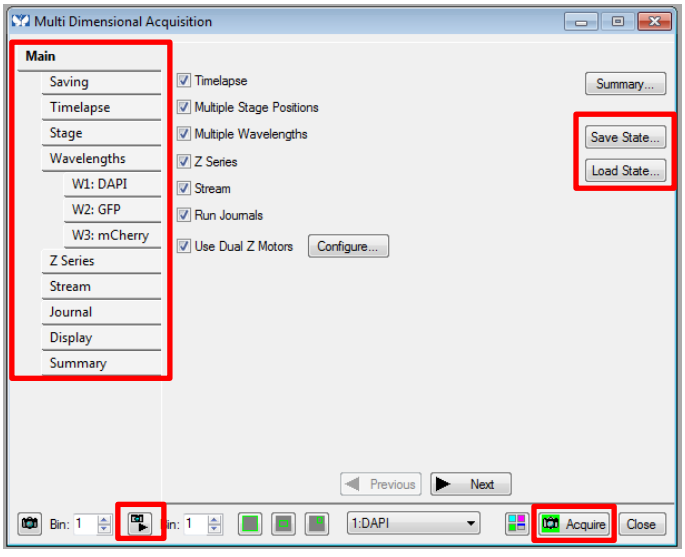
Tab Acquire:

- 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

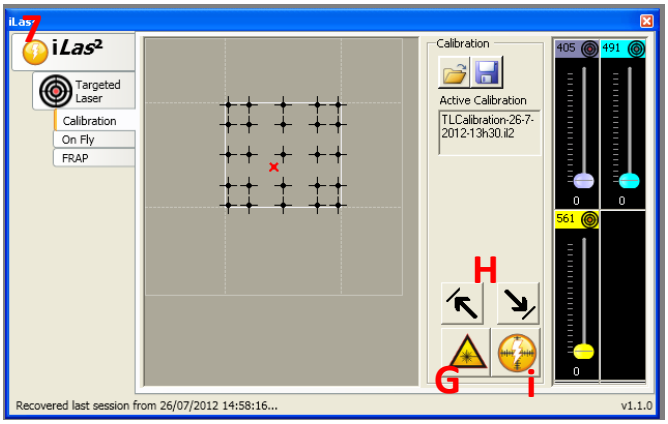
MDA: Z-stack

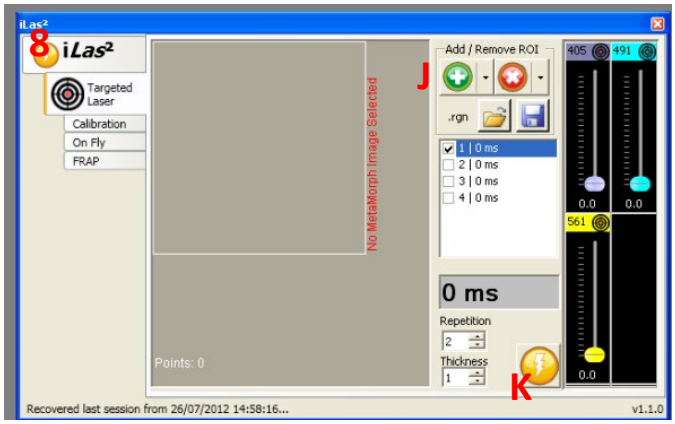
- Activate Z-stack option in MDA's main window
  - Activate use dual Z motor
  - Set Z-stack motor to: *Piezo*
  - Set all other options to: *TiZ*
- Select option: Center around current
  - Height of objective is shown between -50 µm and 50µm
  - Not the same as display microscope , different Z-motor
- 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

* For extensive multi-position, -wavelength, -z-stack experiment see the manual on the OIC website, www.erasmusoic.nl

FRAP on the Fly Calibration

Before you can use FRAP the FRAP unit must be calibrated:





- Choose illumination setting suited for FRAP, e.g. “SPD_FRAP G”
- Find an empty piece of glass without cells/fluorescence
- Set the exposure time around 100 - 200ms
- Open ILAS2 with button [F]
- Open calibration tab [7] and put the power of selected laser to 100% (in ILAS2 window)
- Activate the laser [G] and press “ Show Live”
- Place laser in upper left corner by moving the red cross, press [H]
- Repeat for lower right corner
    - If the spot isn’t visible or too big and bright change power and/or exposure time
    - view is rotated, so move laser in other direction
    - keep in mind to move red cross into left and right corners of the calibration screen
- Calibrate with button [i]

- To check if your calibration was successful open “ on the fly” tab
- Change time to 3000
- Increase the power of your laser and press “ Show Live”
- Click in the image and check if laser pulse in at the position of your mouse pointer

FRAP with ROI

- Start live or acquire one image
- Draw a ROI with the desired dimensions on the image
    - ROI drawing elements in top bar of Metamorph
- Open tab targeted laser and add the ROI with the green "+" button
    - Setup the desired iterations and check if the time it take to bleach corresponds with your desired experiment setup
    - Set the desired laser power
- Go to the FRAP tab and setup the actual frap experiment
    - Choose pre bleach frames, post bleach frames
- For the actual recording
    - Click on setup MDA
    - Check the MDA if the correct components are selected
        - Timelapse selected, correct save directory and base name & correct filter set.
    - Press acquire button in the MDA window
- When you repeat the experiment, you can click on the setup MDA and acquire button in ILAS2

FRAP live bleach / "FRAP on the Fly"

- Open Ilas2
    - Yellow icon in top bar Metamorph
- Got to tab "On Fly"
    - Set duration to desired units (3000 is ~1sec)
    - Set desired laser power
- Open the acquire window, tab special
    - Choose journal FRAP2Ilas.jnl
    - Set desired pre and post bleach frames
- Start live or acquire one image
    - Wait for buffer (pre bleach frames) to be filled (countdown visible in acquire/special tab)
    - Press F11 button to start recording and very quickly thereafter click in your image to bleach the desired positions.
- Wait for the recording to finish
    - If your bleach failed, press the cancel button as fast as possible in the lower right corner of the Metamorph window. Waiting longer can cause Metamorph to crash
- Save the acquired stack before closing the window.

Troubleshooting - FRAP

<u>FRAP calibration error</u>

- Notification: Calibration canceled (exceeded 15 bits)

Possible errors

- Emitting fluorophore or auto fluorescence
    - Ensure that the area of the slide really empty
- Too much laser power or exposure time
    - Reduce laser power in the ILAS2 window [7] and/or exposure time [C]

Shut down system

- 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 box
- Save data to OIC network storage (O:\ drive)
  - To collect the data from your own pc, connect to network storage drive 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, the network-drive is only for transport
  - Extended manual about network storage can be found on the OIC website www.erasmusoic.nl Facility>Manuals>OIC network drive

- **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

Contact

- Martijn de Gruiter Be-343 tel. 31105
- Gert-Jan Kremers Be-346 tel. 43578