Stereo 2 – Nikon SMZ18

Quick-start Guide

Location: Room 2.136

(Updated: 31/8/2021)

Acquisition Software:
NIS Elements AR

Available Objective Lenses: 0.5x and 1.0x lens plus Magnification Range of 0.75 – 13.5x

Be mindful of what you are doing at ALL times: The microscope and the lenses are delicate and very expensive and should be treated with care.

Sample Cleaning and Preparation:

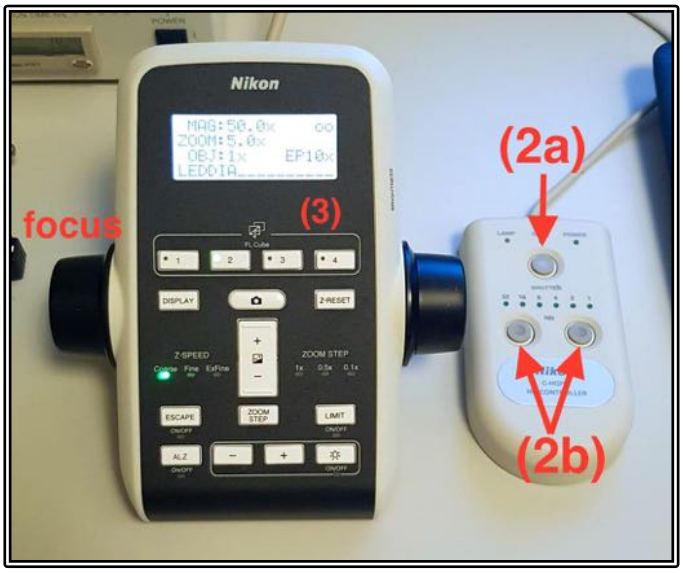
- Make sure your sample container (e.g. your Petri dish) is scrupulously clean and dry before placing it onto the microscope
- Use an appropriate sized sample holder for your sample to minimise spill risk
- Be careful not to over-fill sample containers as the microscope must be kept dry

Start-up Procedure

- Check the microscope and table are clean before you start doing anything else
- Rotate the desired objective into place and set the zoom to the lowest setting
    - Line the objective up for the required mode of imaging – a single white dot indicates monocular while dual white dots indicate stereo mode
- Check the eyepieces to be sure that the Dioptre adjustment on both eyepieces are set to zero and that the eyepieces are a comfortable distance apart
- Pull the eyepiece/camera rod all the way out to send 100% of light to the binoculars **(1)**
- Log in to the computer using your IMB username and password
- If you are planning on doing Fluorescence, click the **Lamp ON** Icon on the desktop
    - If the lamp won't turn on, check the control box to the left of the scope – if the yellow light is on then you will need to wait for it to turn off before starting the lamp
- Double-click the NIS-Elements icon on the desktop – this will turn on the microscope hardware, initialise the Z-Drive for the motorised focus and launch the acquisition software
    - Make sure to let this finish completely before proceeding, failure to do so can lead to problems with Z-stack settings later in the acquisition process
- Place your sample on the stage and centre it below the objective
- Focus on your sample through the eyepieces using the focus wheel on the Nikon Remote Controller – adjust the speed with the button on the side to the rear of the focus controller

Light Path Settings

- To use the fluorescence light source, open the shutter using the puck shutter button **(2a)**
    - Adjust the intensity of the fluorescence light using the ND selection buttons **(2b)**
        - 1 is the brightest, 32 is the dimmest
    - To change the fluorescence filter cube, select the 1, 2 or 3 buttons on the Nikon Remote Controller **(3)** – 1 = DAPI; 2 = GFP; 3 = RFP



- To use the white light source for Brightfield imaging, turn on the lamp in the base by switching on the power at the front-left of the microscope base
    - To adjust the intensity of the white light, rotate the control dial to the front-right of the microscope base

Saving Your Data

Save your Data to C:\UserData** and create yourself a folder if it doesn't already exist. **DO NOT** save directly to the network, only transfer your files at the end of your session.

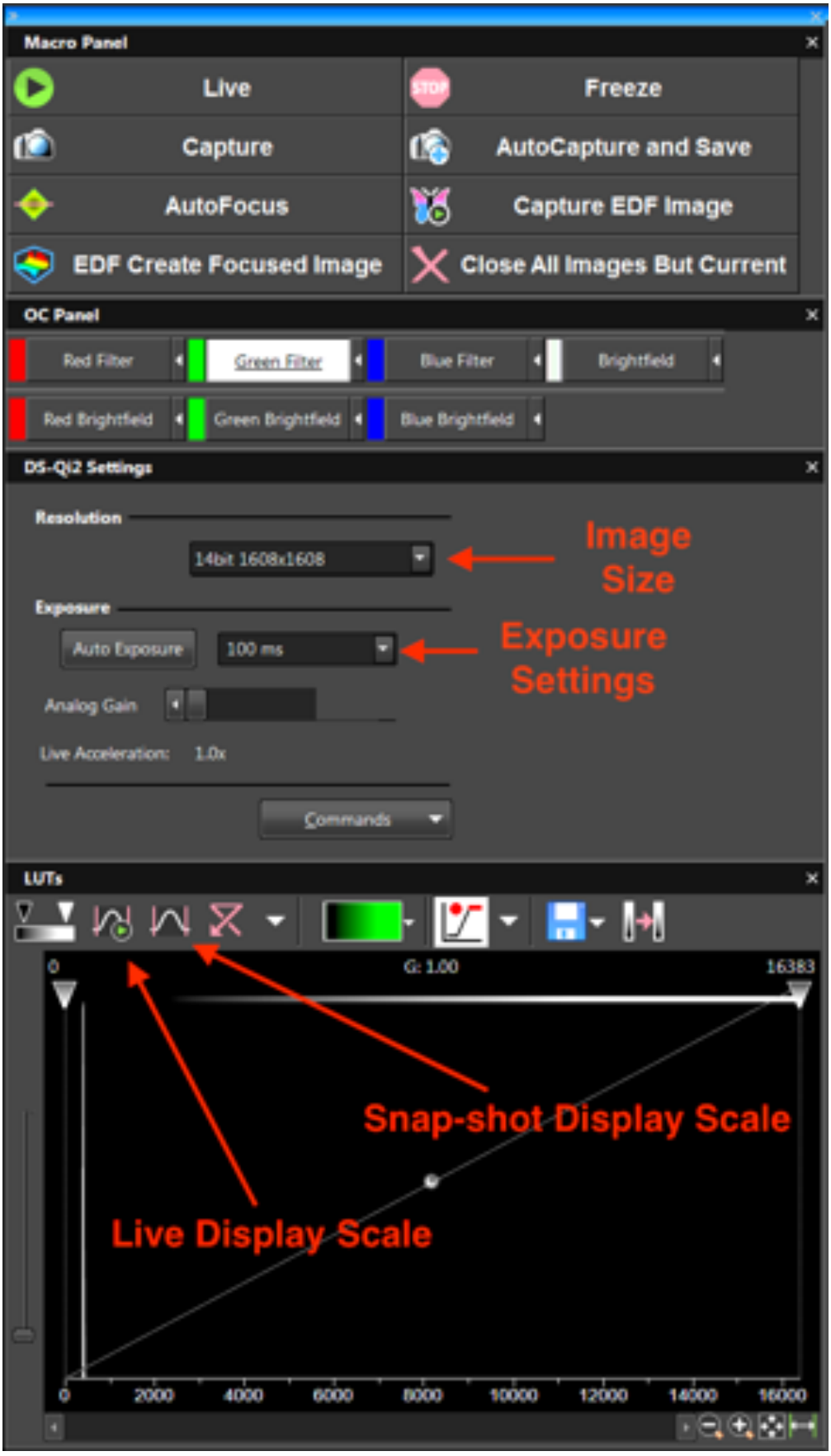
Connecting to Network attached Storage

- Data you acquire on this device should be backed up to a server for future retrieval and use.
- The IMB has access to a number of servers both internally managed, as well as UQ-wide resources, think carefully where you would like to store your data.
- Internal IMB Server address which will be UNAVILABLE by end of 2020, include:
    - **IMB Share**
        - Backed up
        - Group shared server
        - \\imbshare.imb.uq.edu.au
    - **IMB Transfers**
        - Not backed up
        - Deleted every 7 days
        - \\transfers.imb.uq.edu.au
    - **Objective Server**
        - Microscopy only
        - Not backed up
        - \\objective.imb.uq.edu.au
- UQ-wide resources:
    - **UQ RDM**
        - Project based shared drive
        - Can be cached locally (\\data.imb.uq.edu.au)
        - For more information see rdm.uq.edu.au

- Note this uses UQ-SSO credentials (type uq*username*)

Basic Image Capture

- Push the eyepiece/camera rod all the way in **(1)** to send the light to the camera
- Select the required colour channel under the OC Channel tab
    - To acquire fluorescence channels, ensure the brightfield light is off and the shutter is open on the puck
    - To acquire brightfield images, close the fluorescence shutter using the puck and turn the brightfield light on
- Click Live to show a live view of the camera image
    - Adjust the resolution as needed (**Cam Rez**)
    - Change the Exposure time if too dark/bright or click Auto Exposure to have the computer adjust the exposure time automatically (**Exposure Settings**)
    - You may also adjust the display curve at the bottom of the software window to increase the image brightness or use the auto-display adjust button to do this on the fly or as a one-off snap-shot adjustment
- Click Freeze if you want to pause the live image
- Click Capture to capture the current channel, this will need to be manually saved
- Click AutoCapture and Save to capture an image of the current channel and save it automatically


Macro Panel
Live
Freeze
Capture
AutoCapture and Save
AutoFocus
Capture EDF Image
EDF Create Focused Image
Close All Images But Current
OC Panel
Red Filter
Green Filter
Blue Filter
Brightfield
Red Brightfield
Green Brightfield
Blue Brightfield
DS-Qi2 Settings
Resolution
14bit 1608x1608
Image Size
Exposure
Auto Exposure
100 ms
Exposure Settings
Analog Gain
Live Acceleration: 1.0x
Commands
LUTs
0
G: 1.00
16383
Snap-shot Display Scale
Live Display Scale
0
2000
4000
6000
8000
10000
12000
14000
16000

Advanced Image Capture

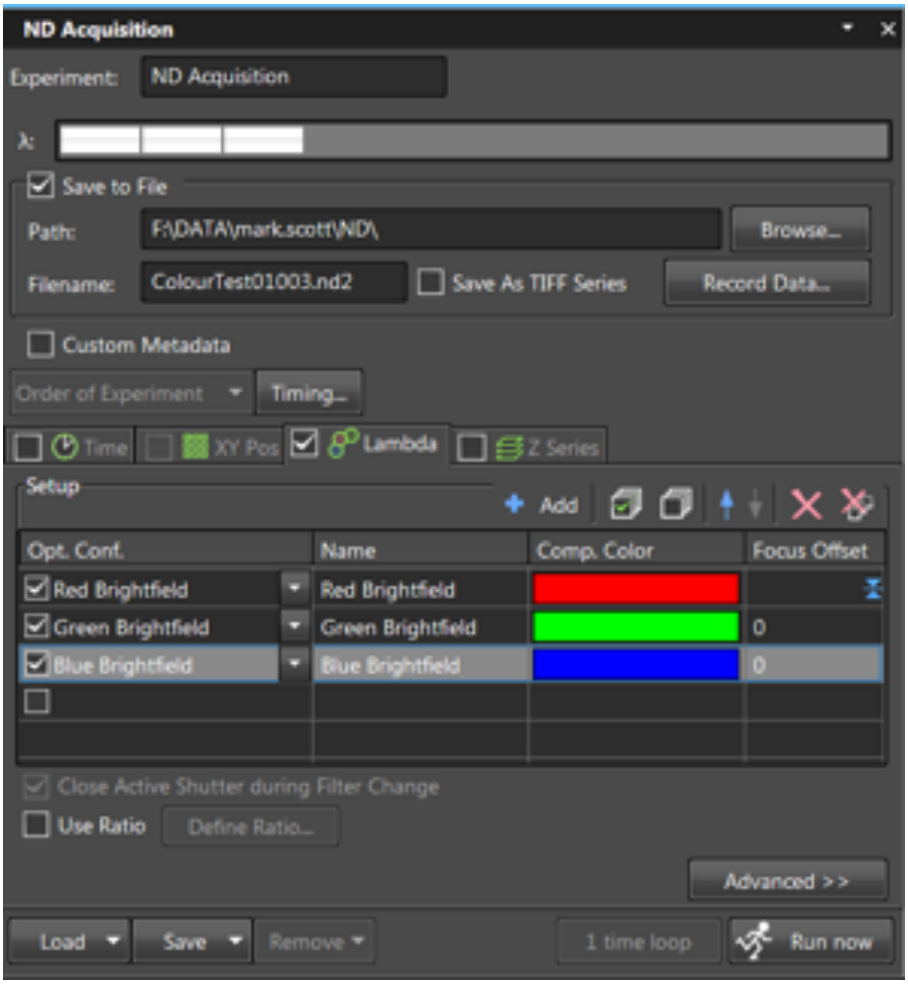
ND Acquisition

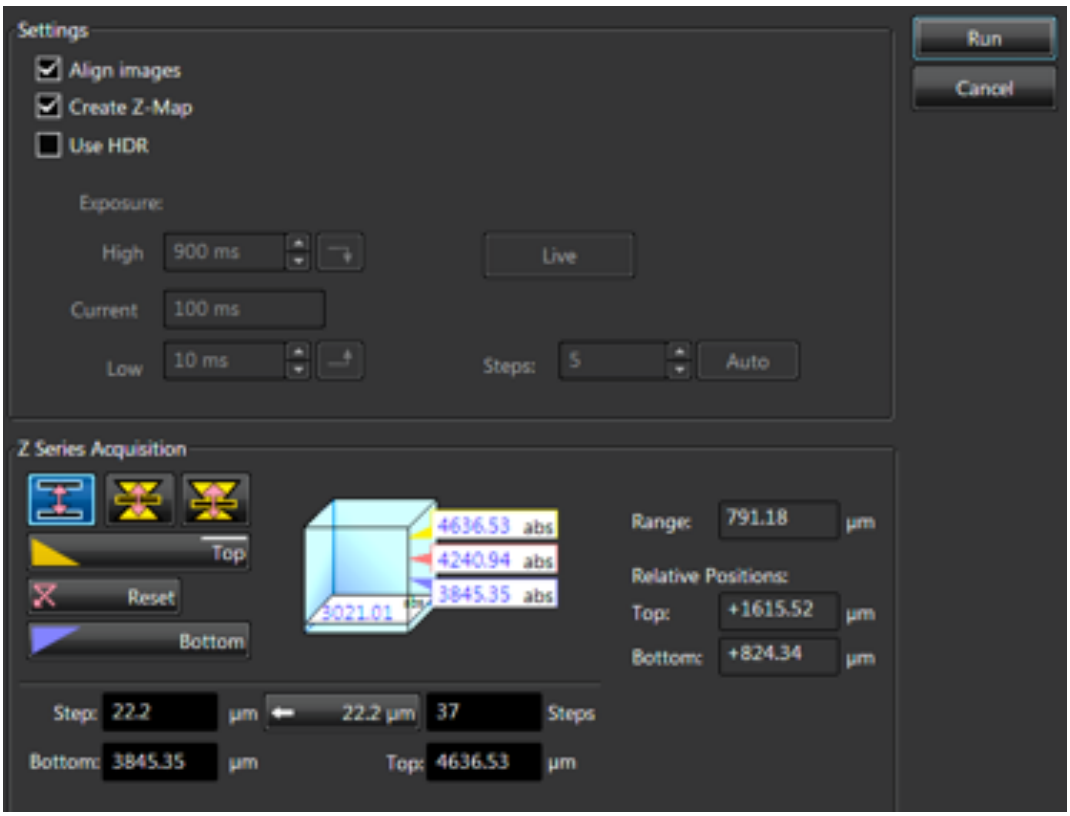
- Set up your individual channel settings as for a basic channel setup above, these settings will be imported into the experiment acquisition setting
- Set your Experiment name at the top of the ND Acquisition window
- Ensure "Save to File" is ticked and set both your Path for the AutoSave as well as the Filename to save as
    - While you can save as a TIFF series it is recommended to always save as a *.nd2 file format
    - The filename will have an auto-incrementing number placed at the end of what you assign so you may capture multiple files without fear of overwriting previous ones
- To add time-lapse settings, tick the "Time" tab
    - Add in a new time series line and set the interval and length
    - You can add multiple time-series for complicated experimental setups
- There is no multi-position available as this system does not have a motorised stage
- To add a 3d stack tick the "Z Series" tab
    - Set the mode of z-stack (i.e. top-bottom, centre-point or offset centre-point)
    - Set the Top and Bottom Coordinates or the Centre point as required
    - Set the z-step size or the slice number
- Add channels to the experimental setup by using the "Lambda" tab
    - You must add at least one channel to your experiment to make it valid
    - Add multiple channels if so desired
- Click **Run Now** to begin acquiring the experiment

EDF Acquisition

Extended Depth of Field acquisition allows users to acquire 3d stacks and process the data to produce a single image with all areas in focus, this is perfect for embryos and larger samples that would traditionally be hard to achieve a single image with everything in focus.

- Click the Capture EDF Image button
- Set the Z-stack settings to cover the 3d area of interest
- Untick the Use HDR button as this slows the process down massively
- Click Run to begin the automated capture
- Once finished, click the EDF Create Focussed Image to create the single plane EDF image


ND Acquisition
Experiment:
ND Acquisition
λ:
Save to File
Path:
F:\DATA\mark.scott\ND\
Browse...
Filename:
ColourTest01003.nd2
Save As TIFF Series
Record Data...
Custom Metadata
Order of Experiment
Timing...
Time
XY Pos
Lambda
Z Series
Setup
Add
Opt. Conf.
Name
Comp. Color
Focus Offset
Red Brightfield
Green Brightfield
Blue Brightfield
Close Active Shutter during Filter Change
Use Ratio
Define Ratio...
Advanced >>
Load
Save
Remove
1 time loop
Run now


Settings
Align images
Create Z-Map
Use HDR
Exposure:
High
900 ms
Live
Current
100 ms
Low
10 ms
Steps:
5
Auto
Run
Cancel
Z Series Acquisition
Top
Reset
Bottom
4636.53 abs
4240.94 abs
3845.35 abs
3021.01
Range:
791.18
µm
Relative Positions:
Top:
+1615.52
µm
Bottom:
+824.34
µm
Step:
22.2
µm
22.2 µm
37
Steps
Bottom:
3845.35
µm
Top:
4636.53
µm

Shut Down Procedure

- Clean any spills within the working area and ensure the microscope is left in a clean and tidy state
- Exit the software and turn the microscope hardware off with the desktop icon
- Turn the Brightfield lamp off if you have used it
- Turn the Fluorescent lamp off via the desktop icon
- Log out of the computer – **DO NOT** shut the computer down

System Hardware Summary

Microscope Stand: Nikon SMZ18 Stereo Microscope

Objectives: 0.5x and 1.0x interchangeable lenses

Camera: Nikon Ds-Qi2 CMOS Camera

Fluorescent Filters: DAPI/GFP/RFP – Other filters are available through Imaging Facility staff.