Standard Operating Procedure

SOP details

Title	Imaging of TopoChip with Nikon Ti2 microscope
Description	This SOP describes how to scan a whole TopoChip with the Nikon Ti2 system
Author	Phani Krishna Sudarsanam
SOP number	3.1
Version number	1

	Name	Date	Signature
Prepared	Phani Krishna Sudarsanam	20-03-2021	
Reviewed	Jan de Boer	17-4-2023	
Reviewed	Nikita Konshin	04-04-2023	
Reviewed	Jan de Boer	17-4-2023	

Version changes

Version	Name	Date	Changes made
1	Phani Krishna Sudarsanam	20-03-2021	Made in TU/e
2	Jan de Boer	16-4-21	Using track changes
3	Phani Krishna Sudarsanam	15-10-21	Addressed changes made
4	Nikita Konshin	04-04-23	Added binning information. Several binning modes used for the same imaging results in stitching error.
5			

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1 Purpose

This SOP describes how to use the Nikon Ti-2 inverted microscope for scanning of TopoChips.

2 Principle

The Nikon Ti-2 inverted microscope is a microscopy system which can rapidly scan slides at high resolution and converts them to high definition digital images for subsequent analysis. This allows the digitization of TopoChips stained with multiple fluorescent markers.

3 Before You Start

- For easier analysis of the images, the TopoChips should be mounted in such a way that the unpatterned corner TopoUnit is in the top right corner.
- Slides should be put on the microscopy stage upside down (coverslip facing down; flip glass along the longitudinal axis) in such a way that the unpatterned corner TopoUnit is in the bottom right corner (row-col 66-66). To ensure this can be easily recognized, mark the coverslip with a marker on the bottom right corner. Alternatively, the TopoChip can be marked by clipping off a corner.
- When using the slide holder make sure to use glass slides that fit in the holder.
- When mounting make sure the chips are aligned as straight as possible on the object glass for better alignment by the software. Mount with hardening mounting medium (Mowiol) on object glass and use nail polish for the cover slips.
- Get an introduction the microscope from the Superuser.

4 Required materials

4.1 Workplace

This SOP is performed in the Ceres scale/e lab. Follow the safety protocols instructed by the lab managers and perform the experiment accordingly in allocated locations in the lab.

4.2 Equipment and disposables

- Nikon Ti2 microscope with all below functional components:
  - Light source: Spectra x
  - Color camera: Photometrics prime 95B camera (25 FOV)
  - Jobs software for acquisition of complicated commands in simple steps.
  - Triggered Piezo stage controller
  - PC HP Z4 or equivalent of r high data handling capacity.
- Stained and mounted TopoChips

4.3 Reagents

- None needed.

5 Procedure

5.1 Working procedure

1. Turn on the system in the following sequence:
    - Camera (wait for 3-4 minutes)
    - Light source (black box below)
    - Microscope (white box below)
    - Stage
    - PC (it is always on, so restart)
    - Software NIS- Elements
2. The PC is always on in Ceres lab because of automatic data back up overnight and hence has to be restarted in the sequence above every time.
3. Ensure there is enough storage space on the data drive (10 TopoChips is about 20 GB).
4. Place the slide to be imaged in the stage slide holder with the coverslip facing down. Make sure the unpatterned corner TopoUnit is in the bottom right corner.
5. Open the software *NIS Elements* by clicking the shortcut on the screen.
6. Set up the correct optical configuration (OC) for each channel to be acquired.
    - Select the OC panel as you see below in the red highlighted box and find the right channel (for eg: DAPI : Nuclei, TRITC: Cell cytoskeleton, FITC: cell marker of interest) that are intended for imaging TopoChip (see Figure 1 below).
    - All channels that will be used for imaging MUST have EQUAL values selected for “Bit Depth” and binning “Format” (Gray square, Figure 1). Use binning 2x2 to average values of 4 adjacent pixels into 1 (reduces quality and file size). In case staining signal is oversaturated at lowest exposure time, try no binning, 16-bit.

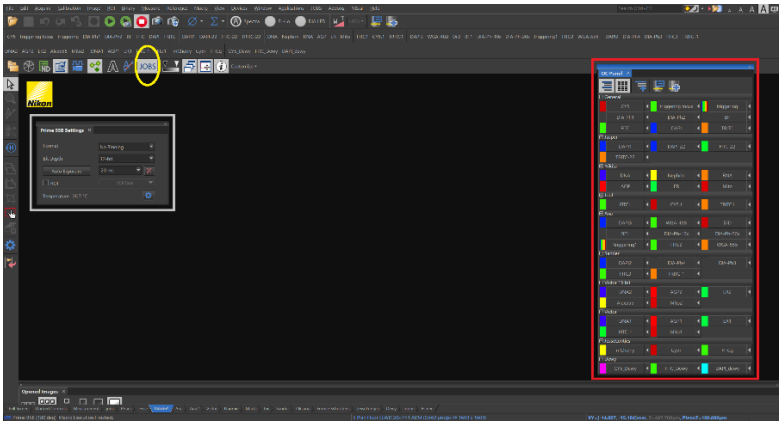


Figure 1: The home page of the Nikon elements software to select the OC panel

7. Once the exposure settings are defined, click the JOBS (yellow ellipse, Figure 1) tab. This opens a tab JOBS explorer (Figure 2) from where the protocol for the image acquisition can be defined.
8. JOBS is a user interface which specifies the acquisition settings of the TopoChip by creating a set of modules.Drag and drop modules to achieve specific sequence of your desire as you see in figure 3. In case action sequence was set up in a wrong way (auto focus module is not set or set AFTER the imaging channel is selected), script will not be executed.
9. Use TopoChip_FS which you see in Figure 2 as a basic structure to customize a protocol for any protocol that you want to make for your own job.

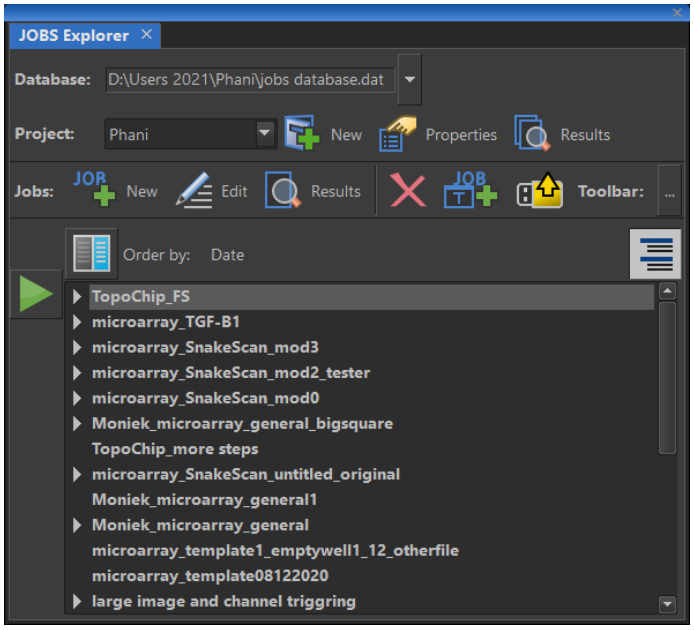


Figure 2: JOBS Explorer

10. Double click on *TopoChip_FS* which opens a window with all the mandatory modules needed to perform an imaging job for the TopoChip, which you see in the Figure 3
11. A basic JOBS protocol to image the TopoChip should have all the basic modules as mentioned below:
    - autofocus settings
    - Capture definition
    - Stage Area
    - LIScanHolder1

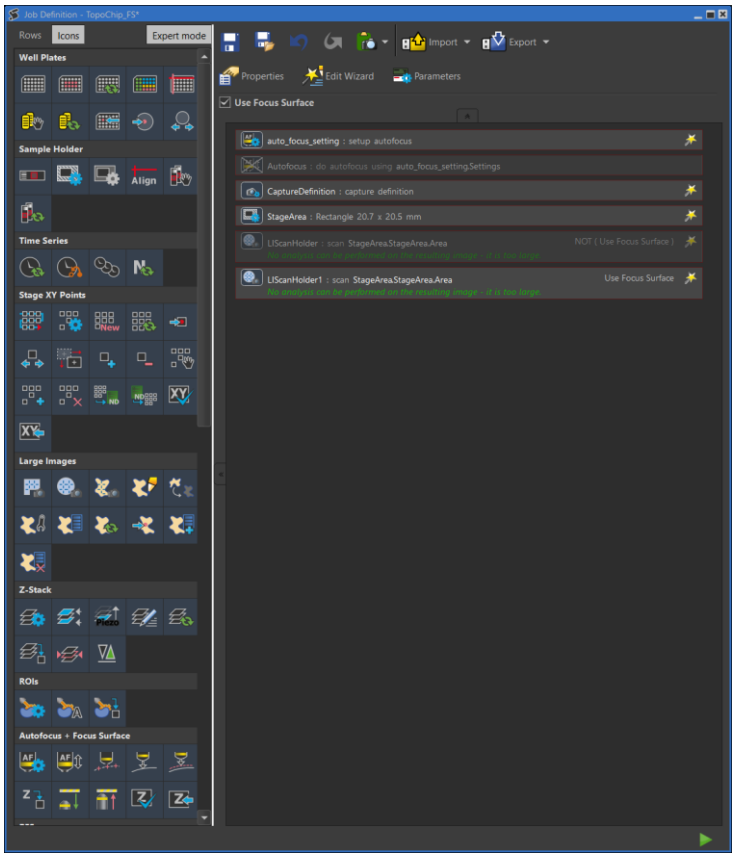


Figure 3: TopoChip Jobs protocol overview

12. Each module in Jobs defines the optimal configurations to acquire images from the TopoChip.
13. The first module defined in the jobs protocol is the *Auto focus settings* which you can see in Figure 4
    - Z drive: fastest (default)
    - Criterion: should be on "Fluorescence" when TRITC staining is used.
    - Range: start at 50 µm and optimize based on your sample. This changes with sample thickness.
14. The next module is the *capture definition* where channels to be imaged can be defined, which depends on your staining protocol. For instance: we have defined to image the sample with DAPI for nuclei and TRITC for cell cytoskeleton and FITC for M1 macrophage and cy5 for M2 macrophage in the figure 4 below.
15. The exposure settings are derived from the OC setting which was done before. Make sure these settings are saved in the capture definition module.

16. Next module is the *stage area*, in which we define the position of the TopoChip on the stage using three corners of the TopoChip. Make sure that all TopoUnits of the TopoChip are in the defined stage area.

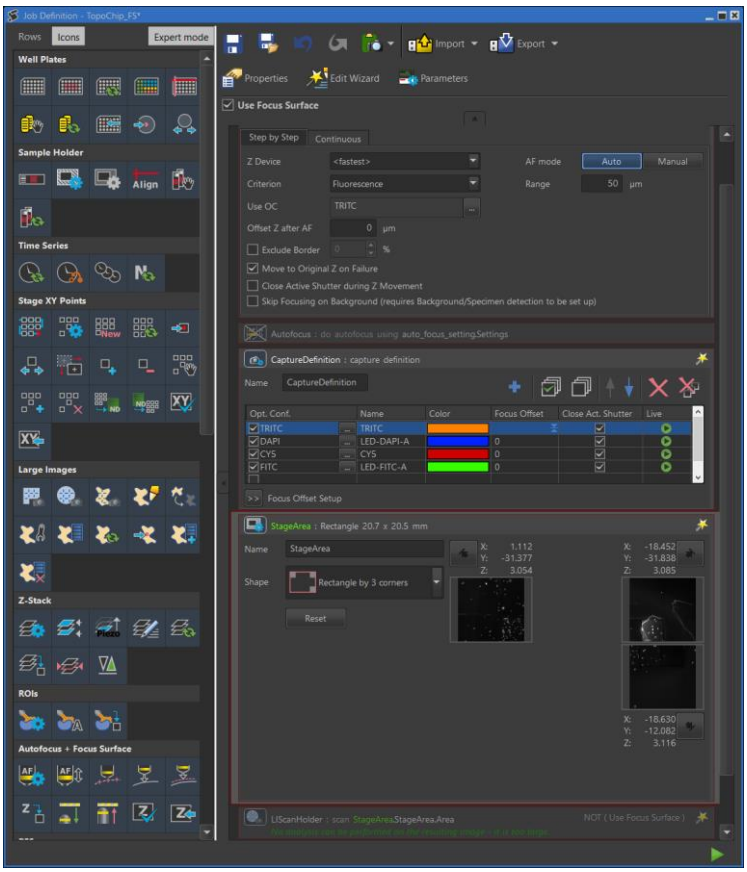


Figure 4: Definition of each module in the Jobs protocol

17. In the next module *LIScan Holder*, all the above tested modules are loaded to perform the job. Check that the module are specified in the module.
18. The scan is done using the focus surface which is defined manually across the TopoChip as you see the figure 6. The focus surface is needed to get the whole TopoChip acquired with optimal focus for each TopoUnit as this scan is intended to be done for the whole area of the 2 x2 cm^2 in a single scan.
19. Once this is done, the jobs protocol is run by pressing the green Play button at the right end of the wizard which is rounded in red circle in the figure 5 below.
20. The file is stored in the jobs default folder where the job protocol is saved after the Nd2 file is exported in a tiff file for each channel if this scan is to be used for further analysis.
21. This is to be stored in the F drive in users folder which is created by the superuser of the microscope for you after the introduction as this data drive is backed up every week by the ICT services.

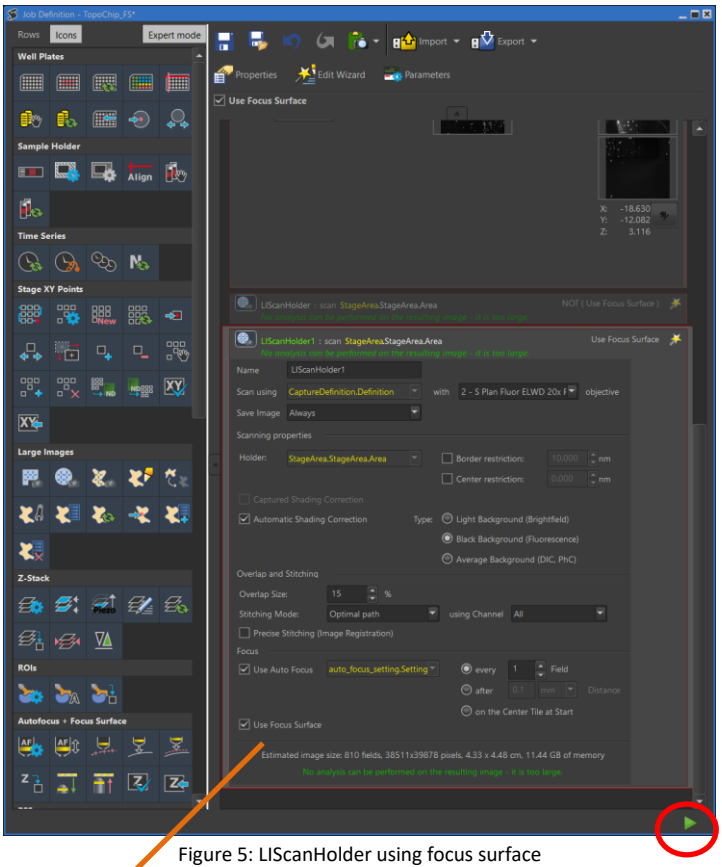
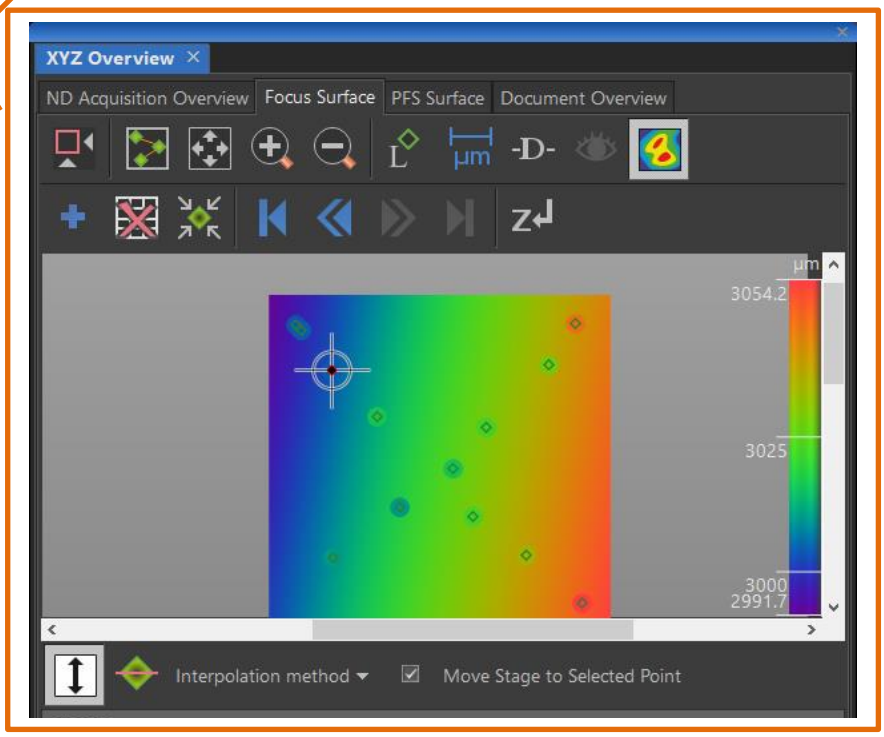


Figure 5: LIScanHolder using focus surface



5.2 Checklist of the TopoChip screening

- Mounted Topochips with unpatterned surfaces at the right bottom corner when flipped for the imaging.
- When mounting make sure the chips are aligned as straight as possible on the object glass for better alignment by the software.
- Check if enough storage space is available on the data drive and set JOBS to run from the storage location
- Open the JOB *Scan TopoChip*
- Open JOBS Wizard
- Complete all the modules in the JOBS wizard
    - Autofocus: Test autofocus before confirming the default settings
    - Job parameters
    - Capture definition: select OCs to be acquired
    - Stage area: Align correctly
    - Select wells
- Create the focus map using the objective intended for the image acquisition.
- Run JOBS protocol

5.3 Safety.

Work in the scale/e lab in Ceres according the safety regulations. Follow the instructions given in the lab introduction by the lab managers and the super user of the microscope

6 Waste

When working in the Scale/e lab, handle waste according to guidelines which are labelled at the waste disposal based on its categories as given below in the table 1

7 References

SOPnr	Title
2.5	Immunofluorescence staining of TopoChip_v2_SPK
2.3	Cell seeding for TopoChips

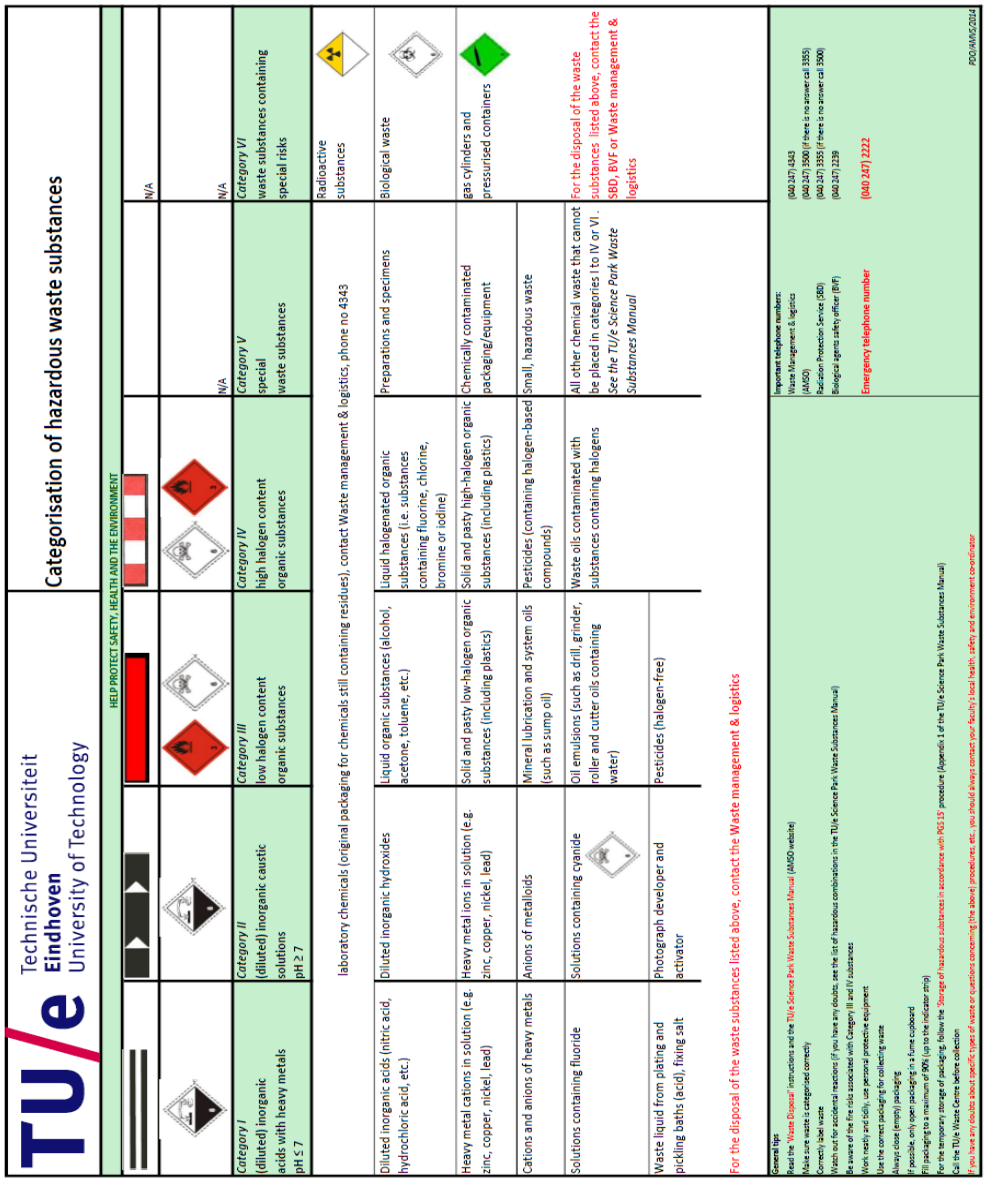


Table 1: Categories of hazardous liquid waste