Instructions for using Leica DCM3D confocal microscope

Startup
1. **Turn on power to microscope** (power unit is located at your feet, next to the computer. Press the front-facing button. Will light up red when on)
2. Check to make sure **anti-vibration table is on** (power switch in the back of unit)
3. **Open LeicaSCAN software** (username: advanced, password: adv1234)
   a. See reference at end of instructions for main screen layout
4. Click **< RECIPES** in the right workflow column. **Single Measurement -> LOAD**: 5X_Default.SMR to start. Allow Nosepiece movement to place objective "OK".
   a. This step loads pre-set default parameters into place that give you a place to start regardless of the previous user's final settings

Sample loading:
5. **Stage -> Load Sample** from the menu in the upper left. This sets the absolute position of the head up away from the stage (Z height) and X,Y to allow for the sample to be mounted. "OK" will initiate the move. There will be a second prompt that will pop-up. Do not click "OK" on this second prompt until sample is loaded.
6. **Place sample on stage**. For the most part, the sample just needs to be placed in the center of the stage. There is generally not a need to further secure it with most material science applications. Make sure the sample will not collide with the objective lenses when the stage moves back into place. This should only be a concern with VERY tall samples.
7. **Click "OK"**. Stage will move back to previous position. You should be able to see white-light illuminating the center of the stage atop your sample. If this illumination is not on your sample, move the stage (X,Y virtual controls on left side of LeicaScan main screen, double-clicking with the mouse to center the screen, joystick, or T/F/L/R buttons to the right of joystick. See reference joystick diagram for more information) until this is true.

Finding Focus:
8. Ensure that **Image(BF)** and **Field Zoom(1X)** are selected in the menu toolbar.
9. Click **AUTO** under **< LIGHT SOURCE**. This automatically adjusts the amount of illumination. Red dots on your screen are saturated pixels. Some saturated pixels is acceptable. If too much of the screen is red, the detector is oversaturated and another illumination correction is required (click AUTO < LIGHT SOURCE again). [F3 quick-key]
10. **Adjust Z height to find rough focus**. (Z virtual controls on left side of LeicaScan main screen, or +/- buttons in the upper right of joystick) Lower the head (at the fastest speed using the virtual controls or + button on joystick) until features on the surface of your sample become visible.
    a. NOTE: you will likely have to adjust illumination a few times as you lower the head (AUTO < LIGHT SOURCE).
    b. On a thin flat surface, ~Z: -27 mm

11. Click **Autofocus icon** in the menu toolbar to refine the focus. [F2 quick-key]
    a. NOTE: Autofocus has a limited search range and requires a rough focus to be successful. Also, for uneven samples, the center of the screen is used for determining focus (though this can be adjusted in AF options).

Topography (Studiable) Acquisition:

As a general rule of thumb, working your way down the acquisition parameters workflow on the right side of the LeicaScan main screen will enable you to acquire your dataset (termed "Studiable" in LeicaScan & LeicaMaps). A topography studiable is a stack of confocal images that is reconstructed into a 3D volume that very accurately generates a roughness map (Z). (Stage step resolution = X,Y: 140 nm. Z: 100 nm. 1 nm interpolated Z resolution).

12. Ensure that **Image(BF)** and **Field Zoom(1X)** are selected in the menu toolbar.
13. **Move stage to region of interest**.
14. Adjust **< OBJECTIVE** to desired magnification (5X, 10X, 20X, 50X, 150X).
    a. NOTE: 10X Mirau MR-L is the Interferometry objective and has a separate set of instructions. Used ONLY for VERY smooth samples (<4 µm roughness variation).
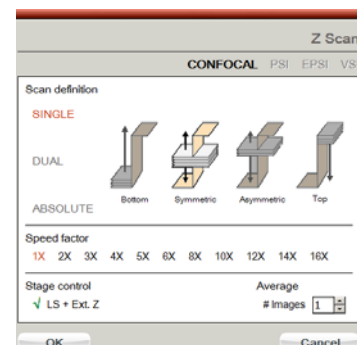15. Confirm **< AREA** to scan. Default 768x576 pixels utilizes entire scan-able range.
16. Adjust **autofocus [F2]** and **illumination [F3]** at desired magnification
17. Adjust **< Z SCAN** to probe thickness.
    a. If at focus, collecting "symmetric" data is appropriate.
    b. Otherwise, user can manually adjust focus to above or below the focal plane and alter the scan direction.
18. **THRESHOLD** should be at 3.0% (as per the 5X Default recipe)
19. Change **Image(BF)** to **Confocal: Image (Co)** in the menu toolbar. This switches from Bright Field mode to Confocal mode. Since confocal microscopy only captures information in a single focal plane at a time, most of the screen will be dark.

20. Switch light sources from white light to the blue LED by clicking on:
21. **AUTO < LIGHT SOURCE** to adjust **illumination [F3]** under the blue LED.
22. Click **ACQUIRE** to collect the dataset ("studiable").
23. If **LeicaMaps** was not already opened, you will get prompted to open it before continuing the acquisition. The shortcut can be found on the taskbar, desktop or through the Start menu. LeicaMaps icon looks like a pair of snow-capped mountains.
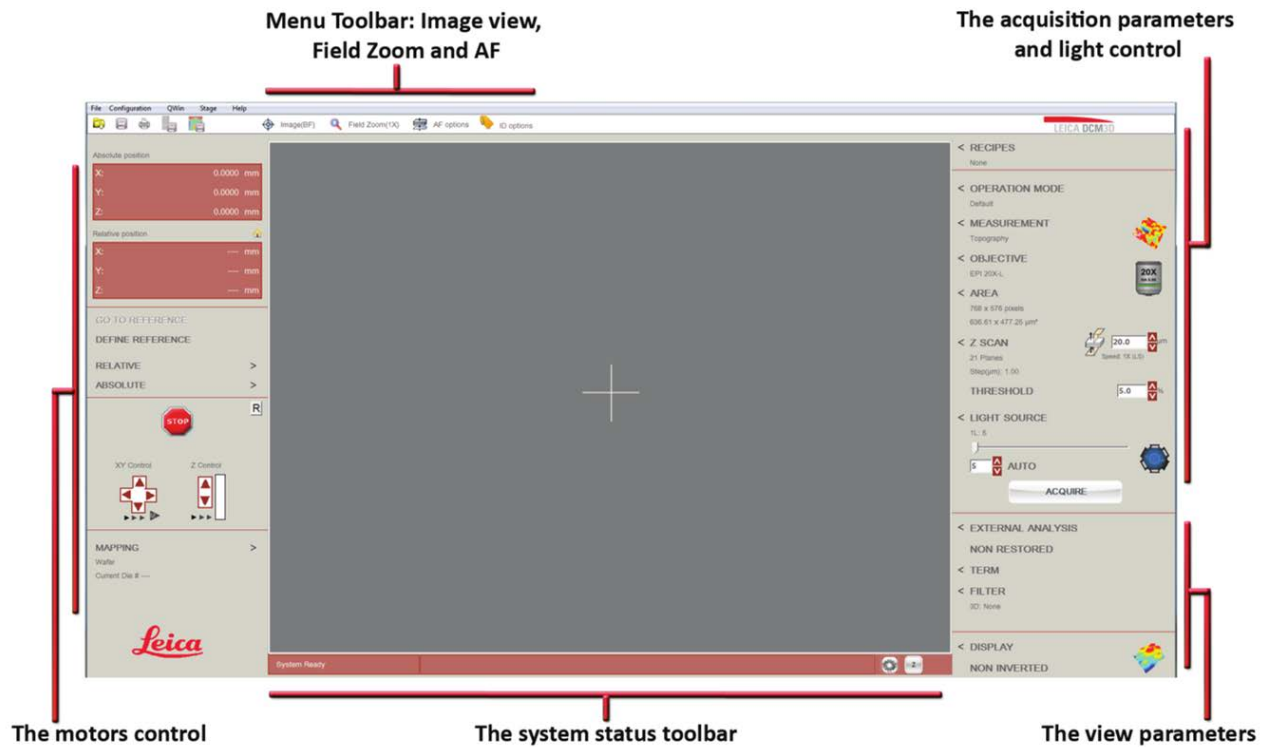24. See **LeicaMaps instructions** for data processing.
25. **New Acquisition** when done with active studiable (see reference section. Main screen after collection). Save if desired [make your own folder for data storage]. Repeat for each region of interest.
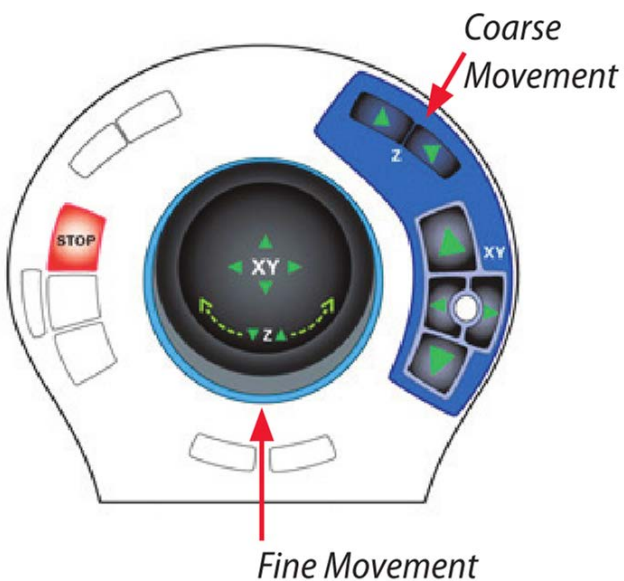
Shut-down procedure:

26. **Stage -> Load Sample** to recess the head and stage to remove the sample. "OK"
27. **Remove sample and cleanup.** "OK"
28. Reset X,Y stage through **ABSOLUTE >** located on left side of the main screen to = X: 0.0, Y: 0.0 mm.
29. Close **LeicaScan, LeicaMaps.**
30. **Power down microscope** (glowing red button on Leica power unit at your feet)
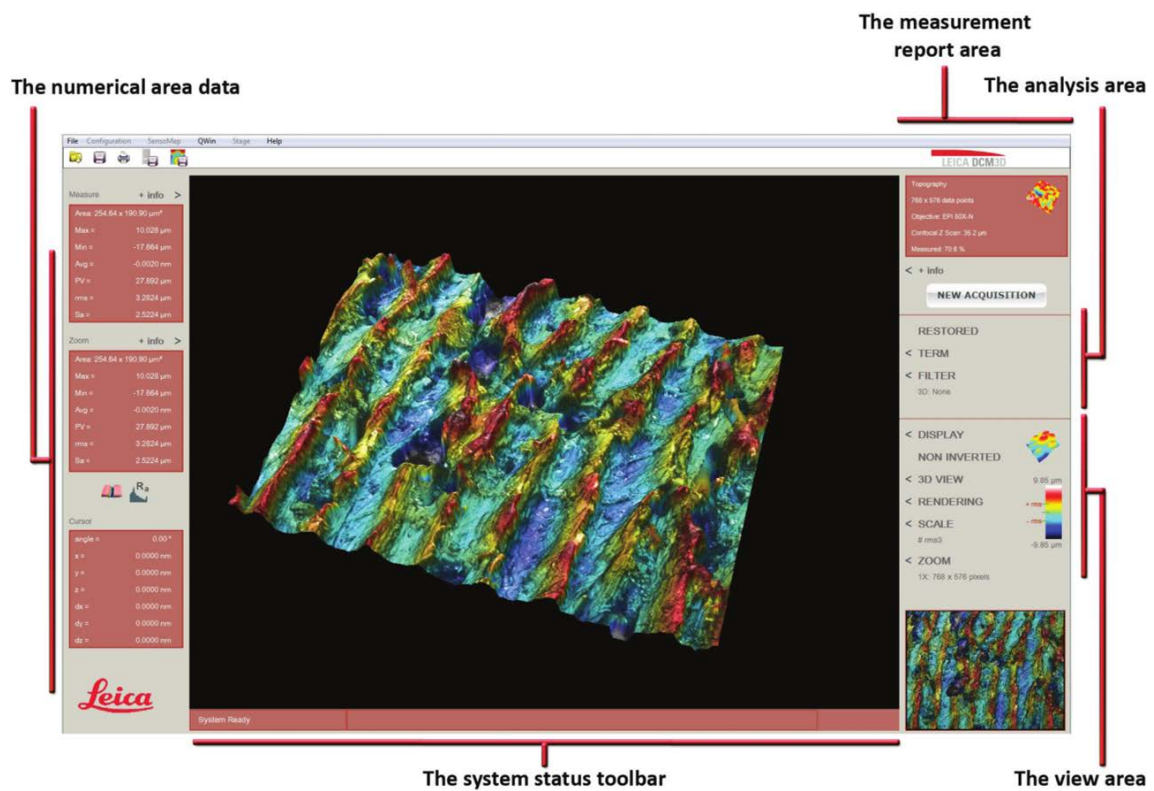31. **Cover microscope with red dust cover**

Reference:

Main Screen Layout:

Joystick Diagram:

Main screen after data collection:

The measurement report area

The analysis area

The numerical area data

The system status toolbar

The view area

Instructions for using LeicaMaps

NOTE: If you are ever curious what something does, make sure the thing you are curious about is selected and click **Help -> Reference guide**. LeicaMaps will bring you immediately to the relevant entry in the manual.

Opening a studiable:
When a studiable is collected in LeicaScan, LeicaMaps should open up, allowing manipulation of the studiable (represented by a frame/object within the LeicaMap document). If not, or if using a saved studiable, **Studiable -> Load Studiable**.

Applying a level:
- Make sure the studiable is selected (i.e. click on the frame/object within the LeicaMaps document that represents your data).
- **Operators -> Level**
  - Least-squares method applies a level according to a mathematical algorithm that maximizes what is flat within your scanned area
  - Define 3 points creates a triangular plane that the user defines as "this is level".

Height measurements via 'Extract Profile':

- Make sure studiable is selected (if already leveled and you wish to apply the measurements to the leveled dataset, make sure the leveled frame/object is selected)
- **Operators -> Extract Profile**
  - A window will open up allowing you to define the line you want to extract the Z-height profile from.
    - NOTE: Take a screenshot of this window as the profile graph will not give you reference information about where you drew the line.
  - After hitting okay, a Z-height graph will be generated. You can right-click on the graph to adjust axis information and how the height is calculated
  - Below your graph, paste the screenshot and crop as necessary.
- To export your 'Extract Profile' data, right-click on the extract profile step in the history workflow on the right side. There, you can save your data as a .txt (two-column ASCII of (x,y) information of the extracted graph).

3D visualization of a topology studiable:
Either:
- Right click on the studiable in the Analysis Workflow. **Minidocs -> Visualization 3D**
Or
- **Minidocs -> Visualization 3D**

Minidocs are macros that perform a series of operators all in a row. Each operator generates a new study, displayed as a frame within your document.

NOTE: Analysis Workflow acts as an interactive history that can be manipulated.

Emergency Information:

Medical Emergencies: Contact 911 and Public Safety (609) 258-1000
Room / facility emergencies: Contact Public Safety (609) 258-1000
Issues related to the instrument:
1. Contact IAC Staff.
2. Leave system as is.
3. Try to shut off system (computer & red button).
Audible/Siren Emergency Alerts:
Follow previous steps 2 & 3 and leave the building.

Emergency Contact Information:

Nan Yao: Office (609)258-6394; Cell (908) 922-2236 Email: nyao@princeton.edu
John Schreiber: Office (609)258-0034; Cell (215) 431-4670 Email: js51@princeton.edu
Paul Shao: Office (609)258-3851; Cell (847) 721-086 Email: pshao@princeton.edu

How to calculate surface roughness via Leica Map

1. Open up Leica Map.
2. Load a desired studiable (*.plu file obtained from LeicaSCAN DCM3D). See **Figure 1**.
3. Define an area of interest to perform the roughness calculation.
   - *Operators > Extract area*. See **Figure 2**.
   - Define the area of interest and click OK. See **Figure 3**.
   - *Studies > Parameters table*. See **Figure 4**.
   - The statistical data about the area is then generated in the current document. See **Figure 5**.
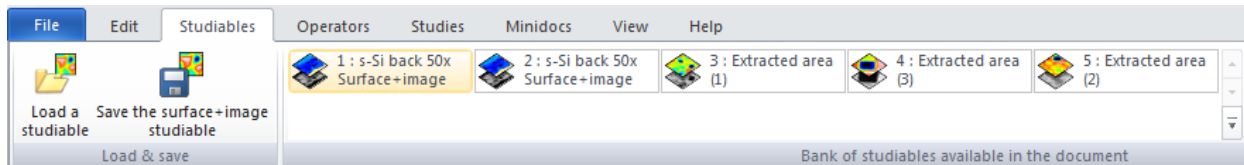

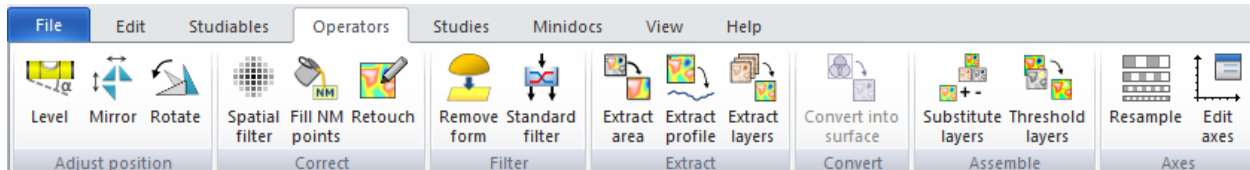**Figure 1**: Load a studiable under *Studiables* on the ribbon tool bar.

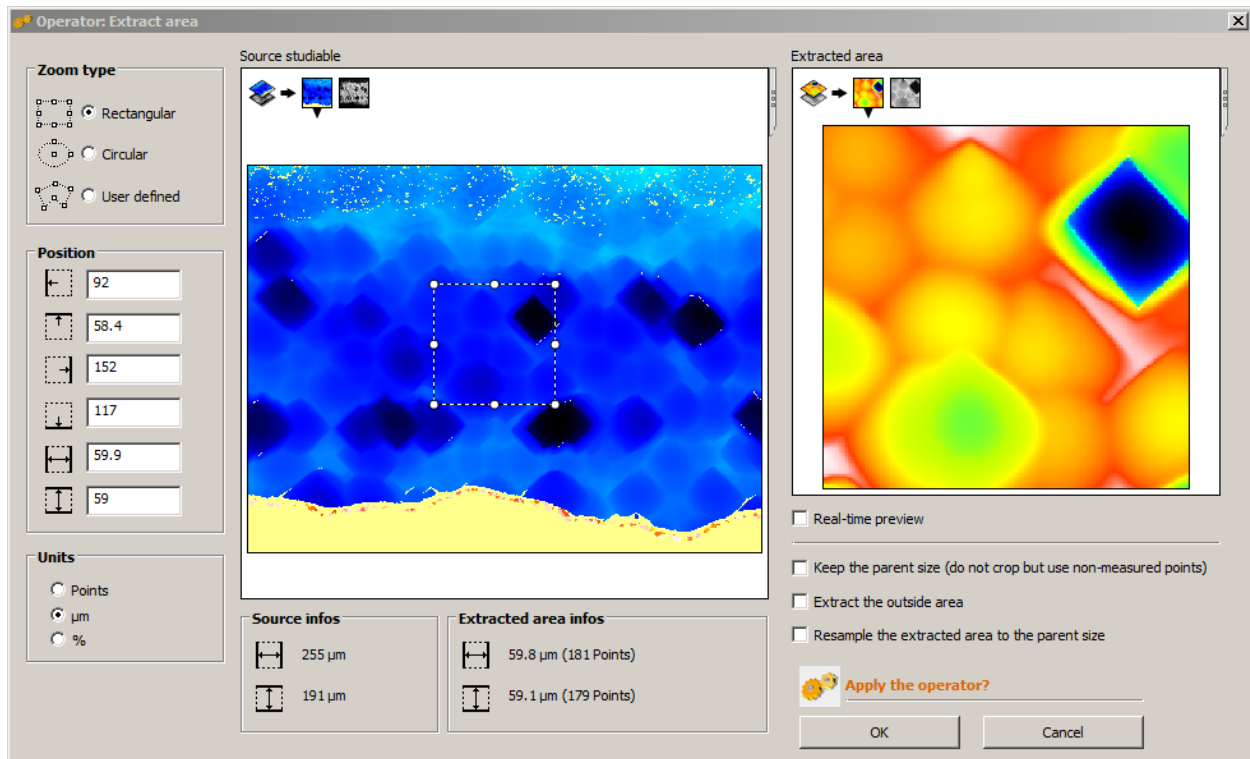**Figure 2**: Extract an area of interest with Operators > Exact area.
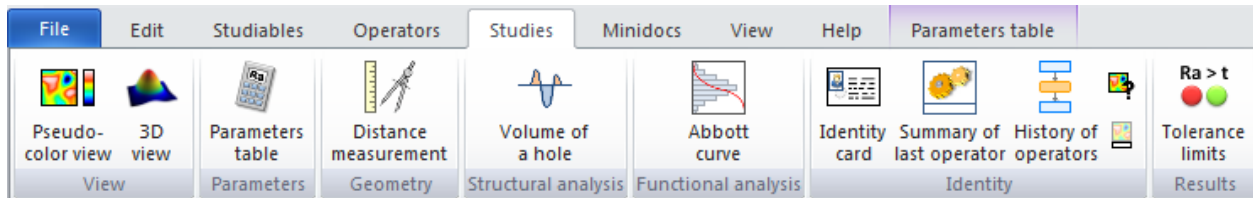

Figure 3: Define an area of interest.

Figure 4: Statistical information can be generated by *Studies > Parameters table*.

Figure 5: Statistical information of the selected area.

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