LEXT 3D Measuring LASER Microscope

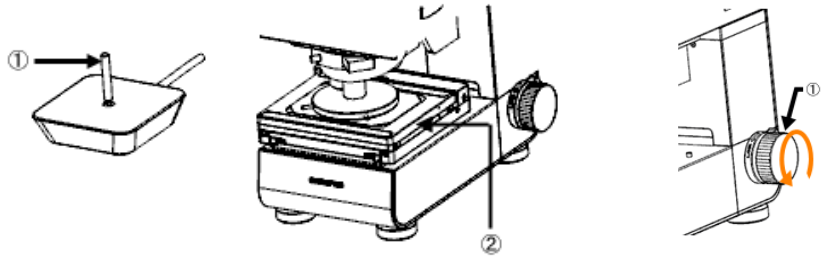
<u>Warning:</u> This instrument may only be operated by those who have been trained by AAF staff and have read and signed the AAF laboratory policies.

A) STARTUP

1. Computer should be on [User: **LEXT** Password: **olympus**]
2. Open Software: OLS4100 (Shortcut on Desktop)
3. Logon: Click OK with following credentials -
   a. User ID: **ADMIN**
   b. Password: **olympus**
   c. Language English (Default)
4. Click OK to the prompt for checking safety around sample, object lens and stage. (The stage should traverse in the X-Y)
5. Click OK for the prompt asking if you need to move the Z reference position (The lens should move to its equilibrium Z position ±5mm)
6. Alternate computer adjacent to Steve H.'s Office {AAF User:**DB2USER** Password:**DB2USER2015**}

B) PLACEMENT OF SPECIMEN

1. Place sample (0.3kg Max) on the stage center. Sample rotating top plate can be removed if not needed. Do not take 3D images < 20X.
2. With the joystick, move the motorized stage to the position under the microscope. Turn the rough focus adjustment knob in the arrow direction to lower the observation tube. To lock the focus drive, switch the focus lock knob in the arrow direction



C) OBSERVATION OF IMAGE

1. You should be in the "Imaging" Tab 

in the software (Top menu bar)
   - Left side is the Image display screen with bottom status bar displaying image size, Magnification, z-coordinate (relative to Z-reference origin), image center coordinates. You can also toggle between the Laser microscope tab and roughness tab on the extreme left (of the live screen)
   - Right side has the Toolbar, Control Panel, Map screen, Data list panel etc.
   - Center has the Focus tab, Brightness tab and the Accessory tab

1. Top menu
2. Toolbar
3. Laser microscope/Roughness tab
4. Image display screen
5. Focus tab
6. Brightness Tab
7. Accessory tab
8. Control Panel
9. Map Screen
10. Data list panel
11. Status bar
12. Wizard button

2. Click 'Color' button  under 'Microscope'  button on the (top) right toolbar. A live image should appear.
3. Click 'Laser' button  under 'Microscope'  button on the (top) right toolbar. A live laser observation should appear.
4. You can switch off the light be clicking `color` again.

D) FOCUSING

1. Coarse Focus
    a. If the focus knob (on instrument) is locked, unlock it by turning it clockwise
    b. Adjust Rough Focus by turning the rough focus adjustment knob
    c. Switch the focus lock knob to locking position by rotating it anticlockwise (in the arrow direction)
2. Fine Focus: Adjust Fine focus by one of the three ways -
    a. Adjustment with mouse wheel button Z revolver is raised/lowered by rotating the wheel forward/backward. Switch between coarse/fine mode by right-clicking on live image and selecting the desired mode.
    b. Adjustment with move focus button Under focus tab the following actions can be performed with the given buttons

Button	Description
	Raises the Z revolver coarsely.
	Raises the Z revolver finely.
	Lowers the Z revolver finely.
	Lowers the Z revolver coarsely.

    c. Auto Focus Under the focus tab, click the  (AF) button OR press the joystick of the stage controller

- If adjusting the focal point is difficult for laser observation, carry it out first for color observation for which focusing is easier. Then switch to laser observation. This process will enable you to adjust the focal point smoothly.
- For observation with a high power objective lens, first adjust the focal point with a lower power objective lens for which focusing is easier. Then switch to the high power objective lens. This process will enable you to adjust the focal point

3. Adjust the brightness: During color observation, clicking 

<Auto> button once always adjusts the brightness automatically.

E) STAGE MOVEMENT

1. Stage movement can be controlled by following ways:
    a. By clicking on the controller button on live screen (bottom right)
    b. Single click on the square results in smaller steps on highlighted axes. Clicking 5 times would give you an option to input a distance to traverse in XY plane.
    c. User can also use joystick to move sample.
2. Green scale lower right of live image can measure x, y and an angle (use snipping tool to save image)

F) IMAGE ACQUISITION

1. HDR (High dynamic Range) correction Highlight to turn on. Laser brightness adjust can be weird if on.
2. The live white light image can be transferred (at any time) to the Map screen by clicking Camera Icon tab (used for stitching 3d images see section E)
3. In Acquisition Setting, leave color box checked to save true color white light "image" on 3D laser image. Click on Laser Icon to turn laser on
    a. **Auto Mode -** For new users and quick tests, best mode to use. - Z slices are fixed and set as Fine (200nm at 20X and 60nm at 50X and 100X. Auto automatically finds top and bottom ("range") of sample, and adjusts to the "best" laser intensity. Auto range sometimes not the best? Typical 20X 3d scan time 3-5 minutes. CLICK Acquisition button to start 3d image
    b. **Manual Mode -** Fine will give same Z slices as Auto - Step allows users to adjust Z slices down to 10nm. The total number of Z steps depends on range {top/bottom} and pitch {Z slice distance}. Collection time proportional to the total number of steps.
        i. First find/click on top and bottom of sample to be imaged In focus region, click up arrow to find top (image region goes dark – increase laser brightness to make sure)
        ii. Next find/click on bottom of sample
        iii. Does distance (top/bottom make sense?)
        iv. Choose pitch in Fine or Step
        v. While "focusing" from top to bottom, adjust laser brightness manually so that it does not saturate (red areas or profile tops out)
        vi. Click Acquisition to start imaging. (3-10min?)

G) IMAGE ENHANCEMENT

1) After preforming imaging, click Measurement Icon (top)  and icons beneath allow user to see image and measure/change some 2D and 3D parameters.
2) Noise reduction (objective >20X have much less noise). One –shot filter Noise. Try first four (one at a time) to get best noise reduction. If not the best, click Img.Cor. icon and try a few Noise removal options. The undo button in one-shot filter undos last operation.
3) Surface tilt correction, 1st try one-shot surface correction. If not best, click Img.Cor. icon to Surface correction and try a few options (inclination, 3 point inclination on image…).
4) Intensity/Height scale settings can be changed by left clicking Intensity, Color, Height small image to see in main image display area. Right click to see Display settings. Adjust as needed.

H) ROUGHNESS MEASUREMENTS (LINE AND SURFACE)

Method I: Single Line Roughness (compare Profilometer)

1. Click on Roughness Tab to the left of main window
2. Focus sample (10X or 20X)
3. Pick Objective Lens of interest (20X or 50X)
4. Advanced Settings, then advanced settings again
5. Data Acquisition
6. Roughness Objective lens (choose 20X or 50X)
7. Choose Evaluate Length (2mm??)
8. Pre/post travel (10%)
9. Acquisition
10. Click top Measurement Tab
11. Can "correct" profile tilt, noise or define measurement range in Profile correction Region
12. Analysis Parameter (R ok) But check other options if needed
13. Pick Cutoff parameters [see help files, search key word: "cutoff" for more detail]
    a. Roughness a high-range filter (cutoff value λc) is applied to the primary curve and the long wavelength portion is excluded
    b. Waviness this is the curve after a bandpass filter (cutoff value λf/ λc) is applied to the primary curve. The cutoff value λf excludes the long wavelength portion, while the cutoff value λc excludes the short wavelength portion. Parameters calculated from the waviness curve are called "waviness parameters", and their parameter symbol is "W".
    c. Linear Roughness Parameters http://www.olympus-ims.com/en/knowledge/metrology/roughness/2d_parameter/
14. Calculate
15. Move results to data sheet and save.

Method II: 3D Laser Image sample as usual (20X or 50X)

1. Measurement Tab
    a. Line Roughness (see above D1)
    b. Surface Roughness

2. One-shot filter (noise, level,…)
3. Analysis Parameters (R-Height or choose)
4. To mark out an ROI. Must start in 2d view. Click Rectangular ROI and draw out. This Area/Volume will be used for Analysis
5. Pick Cutoff values
6. Calculate http://www.olympus-ims.com/en/knowledge/metrology/roughness/3d_parameter/
7. Move Results to Datasheet and Save

I) STITCHING

1. Take Screen shot at a lower Objective Mag
2. Increase Mag 20X, 50X, 100X
3. Green Box in Map screen is area to be imaged (update map screen with camera button at top right of map)
4. Click Stitching icon (upper right)
5. Stitching setting - choose area tab
6. Areas to be stitched together are visible as yellow area. Changed by picking # of pieces 1X2, 2X3…..
7. Stitched areas moves to right and down.
8. Now go to main screen acquisition setting.
9. Easiest to use Auto Mode and check auto for each stitched area. Manual can be used but user must monitor laser intensity.
10. Click start stitch.

J) AREA/VOLUME MEASUREMENTS

1. Take 3d Image as usual
2. Do any corrections needed (noise, surface…)
3. If want ROI, user must pick in 2D (any shape)
4. Pick Specifying threshold (peak, valley…)
5. For Valley (profile) can see Threshold 1 value (0-100%) In profile region, Threshold is seen as blue line. Measured valley is pinkish color. If threshold value is too high volume numbers are wrong (try). On image the pink area is what is measured. ROI is see as yellow (can pick as many as desired). To get believable volumes/ surface area can be tricky (practice)
6. Shift results to datasheet and save.

K) APPENDIX A: IMAGING ENHANCEMENT MODES

i. High Resolution Setting (XY)

a. Normally xy is saved in 2000X2000 pixels.
b. High Resolution doubles this to 4096X4096 (~15nm for 20X objective).
c. Advanced Setting (upper right icon)
d. Advanced Setting (next row down)
e. Data Acquisition – Acquisition mode
f. Check enable high resolution box (de-check when finished)
g. Low or High Reflectivity samples
h. Advanced Settings – Advanced Settings
i. Auto Acquisition High, Standard, or Low Reflection Sample _ Pick best
j. Return to Standard Sample when finished

ii. Brightness Switch

a. Five levels of Laser brightness can be user chosen throughout scanning range.
b. Advanced Setting – Advanced Setting – Data Acquisition – Brightness switch
c. Check enable brightness switch
d. User can pick up to 5 laser brightness throughout the Z range.
e. Set position and set brightness.

L) APPENDIX B: TRANSPARENT FILM THICKNESS MEASUREMENTS

1) Imaging tab
2) Under acquisition setting, change from “normal” to “film thickness”
    a. Find bottom, then top of sample as usual.
    b. Press acquisition
3) Go to measurement
    a. Go to “film thickness” at top far right.
    b. Specify measurement line by dragging line on image.
    c. Select appropriate correction (refractive index) value.
        i. If new type, click register button to input new refractive index value.
    d. Specify peak positions (start point and end point).
    e. Click “export to datasheet” and save.