

This guide obtains just brief information. In case you need detailed instructions, read the Olympus OLS4100 OEM manual. It is recommended to read the **Safety Instruction and Basic Troubleshooting** manual prior to your work.

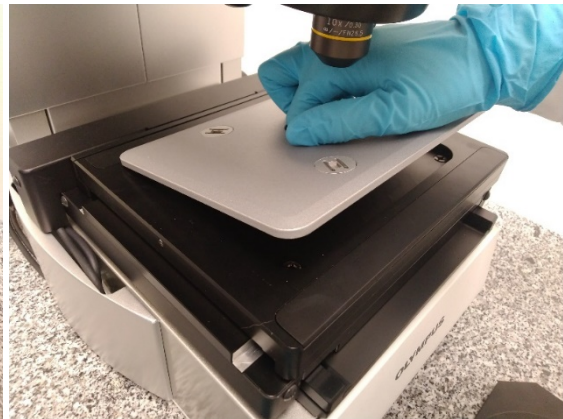
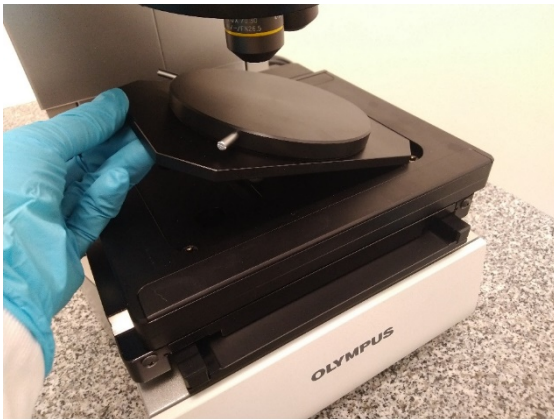
## OLYMPUS LEXT OLS4100

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### Turning on procedure

1. **OPTIONAL:** Replace the revolver head with lenses (refer to “**Replacing the revolver head**” section of this manual).
2. **OPTIONAL:** Replace the stage feature. The stage feature is simply lying on the stage, lift it up and replace with a different one.



3. Turn on the control unit (main switch in the back and long press of the button in front).

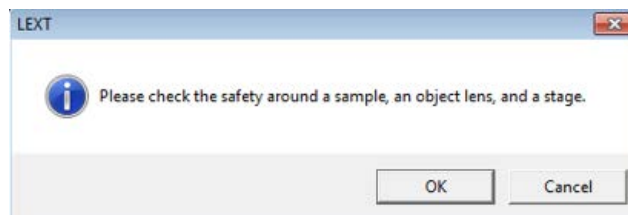


4. Turn on the CLSM computer (it is the right one).

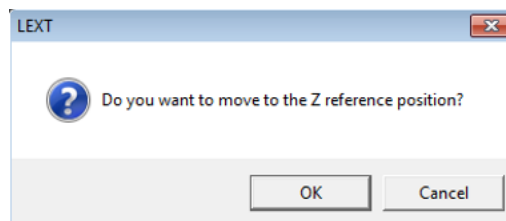
5. Start the OLS4100 software. Log in as Guest, no password needed.



6. **Make sure that the stage is safe to move in all directions (x and y) with no risk of collision!** Then confirm the prompt. Stage movement initialization will proceed.



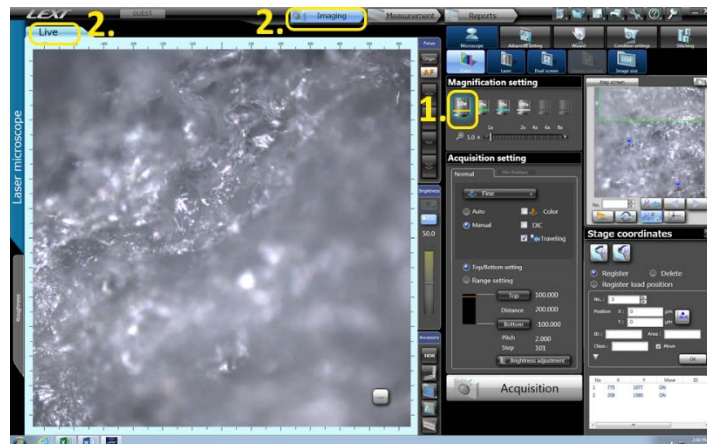
7. **Make sure that the revolver head is safe to move in the z-direction with no risk of collision!** Then confirm the prompt. Revolver head will move from the parked position to the z reference position.



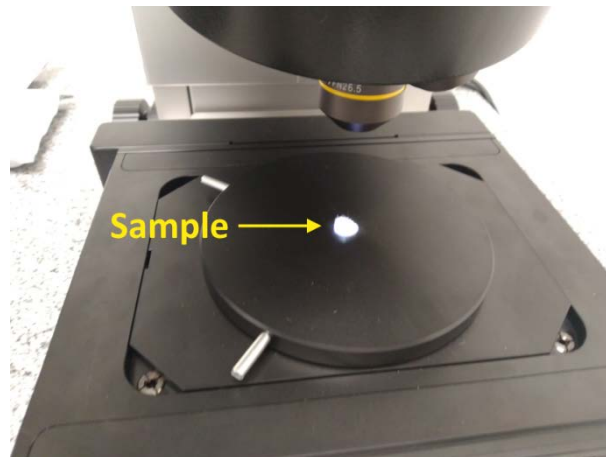
8. The instrument is ready for use.

## Collecting images

1. Select the lens with the lowest magnification.
2. Switch the microscope to Live view.



3. Place your sample on the stage.



4. Bring the head down into focus.

Untighten the manual height adjustment knob. **Keep your hand on the black knob to prevent the head from spontaneous downslide due to its own gravity!** The knobs on the right and the left are interconnected. Use them to bring the head down into focus on the sample (keep eye on the **Live view** window in Lext software, see the image at the step 2), then **tighten (lock) the height adjustment knob again!**



**NOTE:** For most samples, you cannot focus on the whole observed area at once due to low Depth of Focus. Generally, higher magnification lenses have lower depth of focus so smaller area of your sample will be focused. Do not worry about this, you can use motorized fine focus and compose the image from multiple layers using the **confocal principle**.

5. Select the spot of interest.

You can either use a joystick, click in the Live View window, or manually reposition the sample/stage (be careful not to touch the lens neither with hand nor your sample). Fine focus on the sample using SW buttons or mouse roller.

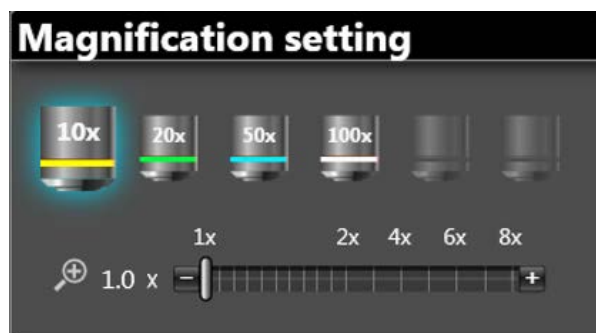
**Avoid contact of the lenses with the sample when focusing, moving the stage or selecting another lens!** Be especially careful with the short working distance lenses on the Revolver 1.

**NOTE: Fine focus is moving the head in the z-direction.** The range of fine focus is high enough to bring the lens from focus into contact with your sample. **Keep eye on the lens while focusing**, especially with short working distance lenses!

**OPTIONAL:**

Select different lens.

Available magnifications are 2.5x, 5x, 10x, 20x, 50x, 100x (Revolver 1) and 10x, 20x, 50x, 100x (Revolver 2). It is always advised to start with low magnification lenses and continue to higher magnification. Always refocus after you change lens even if you are not taking any image. It helps focusing at the next magnification and lowers the risk of hitting the sample with lenses.



**NOTE:** Revolver head with lenses is motorized and moves both ways in the shortest possible path. Lenses have different sizes, 100x lens is the physically largest one, thus, it is also most likely to crash into your sample/stage. If you are not sure, change the lenses in several steps in the order of decreasing magnification. You can safely select the 100x lens if you are focused on the top of a flat sample, but the **risk of a crash increases for uneven**

**and rough samples and/or when focused elsewhere than on the topmost spot of your sample.** For such samples, consider using the revolver head with long working distance lenses (see **Standard procedures** manual).

- Choose the type of image and click “Acquire” to collect the image.

More information about the imaging options is provided below. Note that some samples may require manual setting of brightness and/or z limits to obtain a good quality CLSM image.

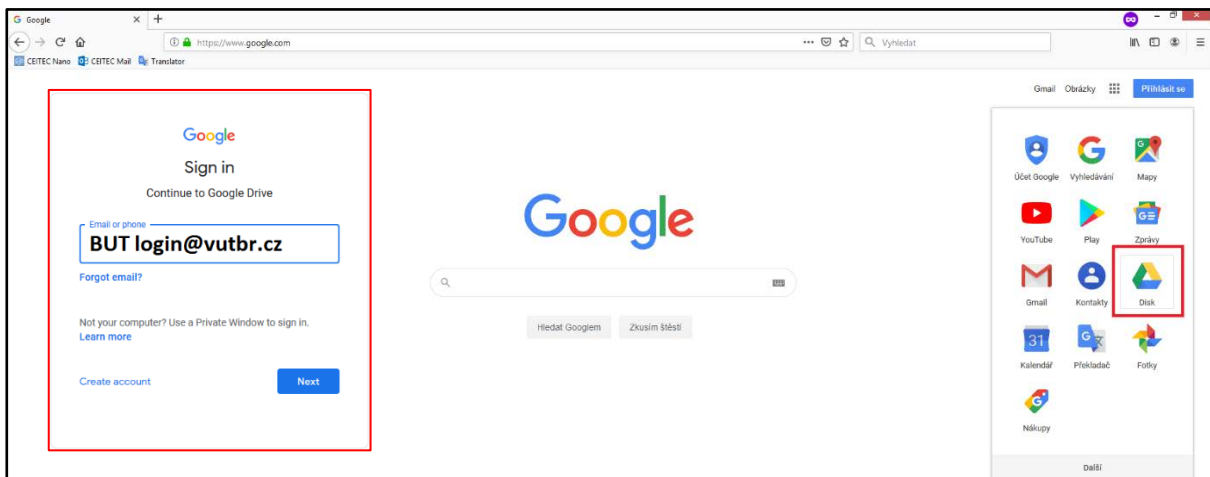
- Save and Export your images.



It is recommended to always export your data in *txt* format to store all acquired data. However, this format is not standardly supported by common graphical software. You can additionally export in *jpg/png/tiff* format to have your images directly available. You can store your data in “C:\Users\olympus\Documents\CLSM users” in your folder.

**It is forbidden to use any type of USB drives!**

Use the internet for alternative data storage. For example, every BUT student/employee has access to his/her own Google drive with large storage capacity via BUT login and BUT password. You can access the drive from anywhere and download your data. Data can be shared with your colleagues. Non-BUT users with no access to Google drive please use alternative services (e.g. DropBox, OneDrive, e-mail, FTP server, etc.) to transfer your data via the internet.





## Turning off procedure

1. Select the lenses with the lowest magnification.

**Avoid contact of the lenses with the sample when focusing, moving the stage or selecting other lenses!**

2. Rise the head.

Untighten the manual height adjustment. **Keep your hand on the black knob to prevent the head from spontaneous downslide due to its own gravity!** The knobs on the right and on the left are interconnected. Use them to bring the head up, then **tighten (lock) the height adjustment again!** The head does not need to be in the topmost position – ca 15–20 cm gap between the stage and the revolver head is sufficient to ensure safe and easy manipulation.

3. Turn off the software.

The head and the stage will park into a safe position.

**Avoid contact of the lenses with the sample when focusing, moving the stage or selecting other lenses!**

4. Turn off the control unit (first by long-pressing the button in the front, then by the main switch in the back).
5. If you have replaced the revolver head, please return the Revolver 1 into position.
6. Turn off the computer.

## Imaging options

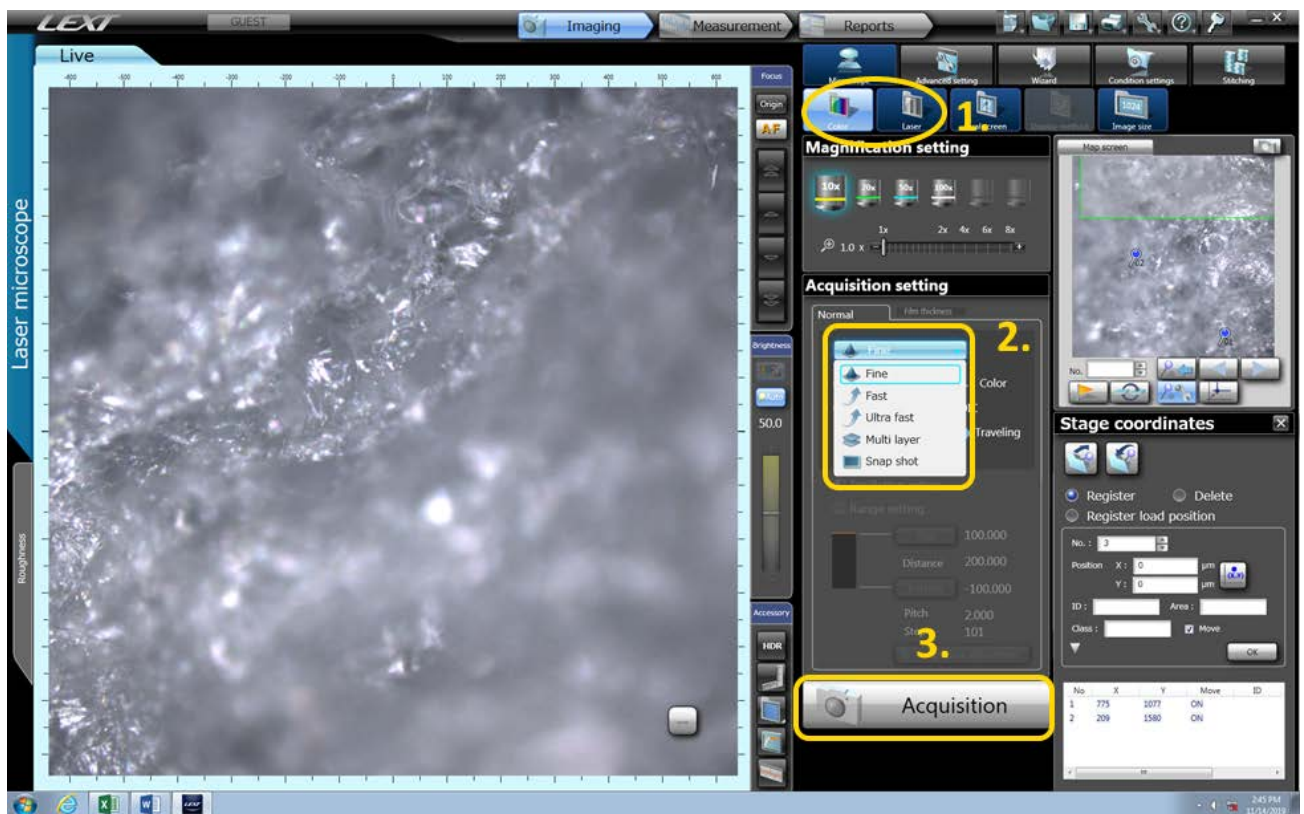
### Optical mode (OM)

Captures a full-colour image “as it is”. Practically limited by low depth of focus.

### Confocal laser scanning mode (CLSM)

Light coming from above and/or below the plane of focus is blocked out using a spatial pinhole. Layer by layer, the surface of a sample is scanned by a laser, then the lens is refocused (moved in the z-direction) and the next layer is scanned. Layers are composed into a 3D image.

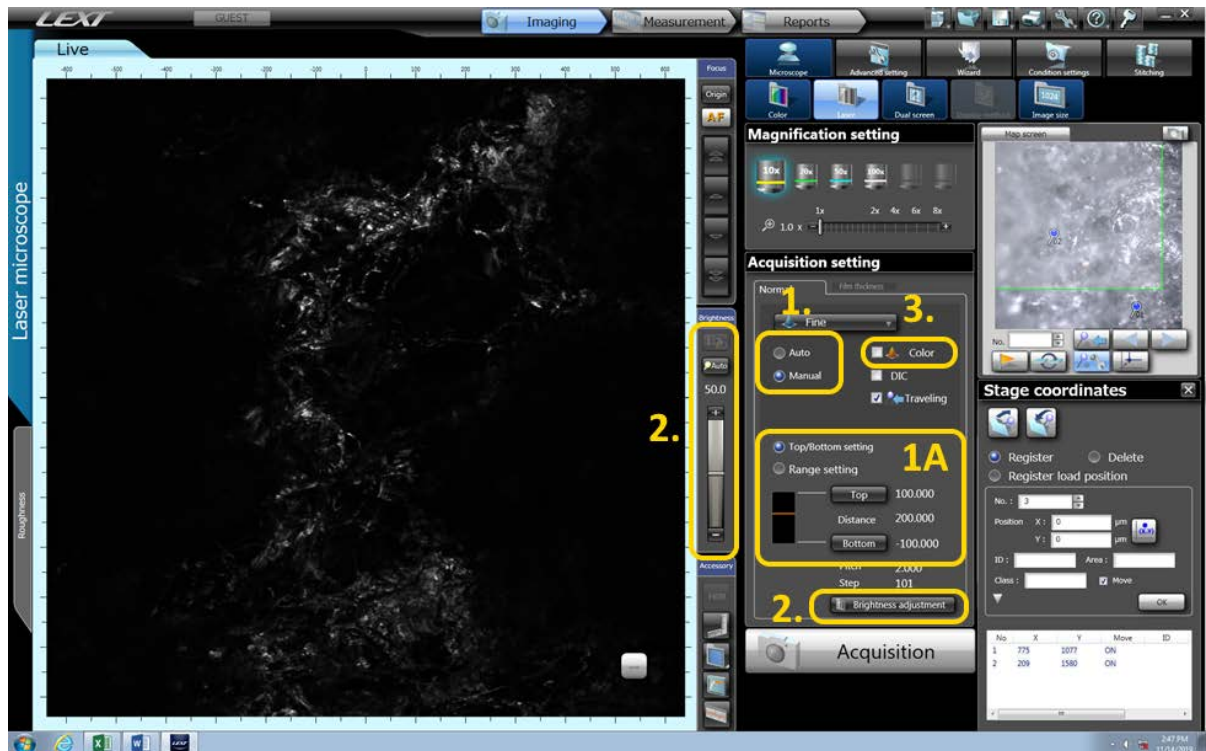
1. Switch the live view between laser scanning mode and optical (colour) mode in the menu.
  2. Select the type of acquired image.  
 CLSM – Fine, Fast, Ultrafast, Multi layer  
 OM – Snap shot
- NOTE:** The selected live view mode is not connected to the type of acquired image.
3. Acquire image.



## Acquisition parameters:

### 1. z range (CLSM only)

select the vertical range of scanning. The z-step is controlled by the scanning quality (Fine, Fast, Ultrafast) and varies for different lenses. If *Auto* is ticked (default setting), the z range will be evaluated automatically. Otherwise, manually select the bottom and the top plane of scanning (1A).

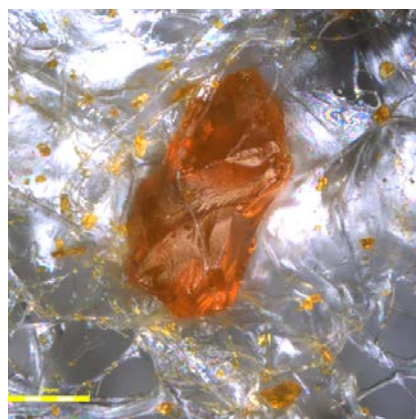


### 2. Brightness

Adjust brightness. For confocal mode, one setting applies to all planes. **Avoid oversaturation of the detector.**

### 3. Colourized confocal laser scanning mode (CLSM only)

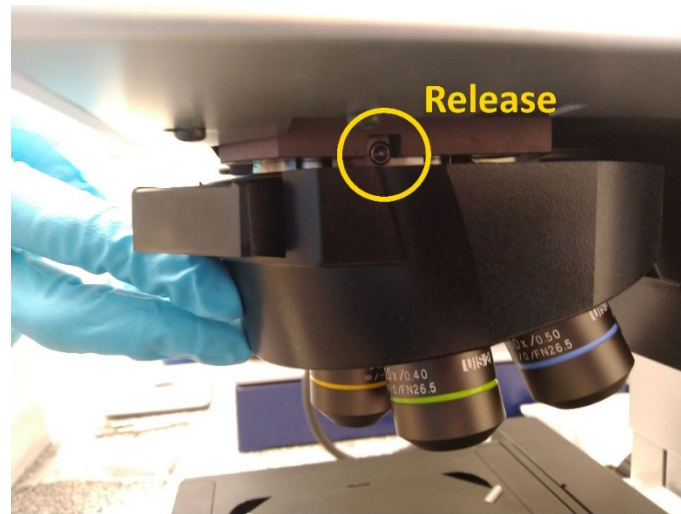
A combination of CLSM and OM provides a full-colour 3D image. Also allows to make full-colour images with high depth of focus at high magnifications.



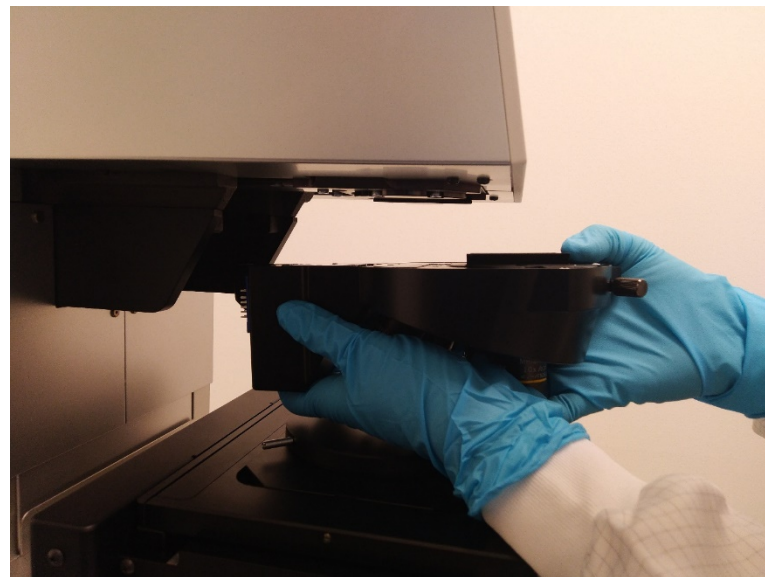


## Replacing the revolver head

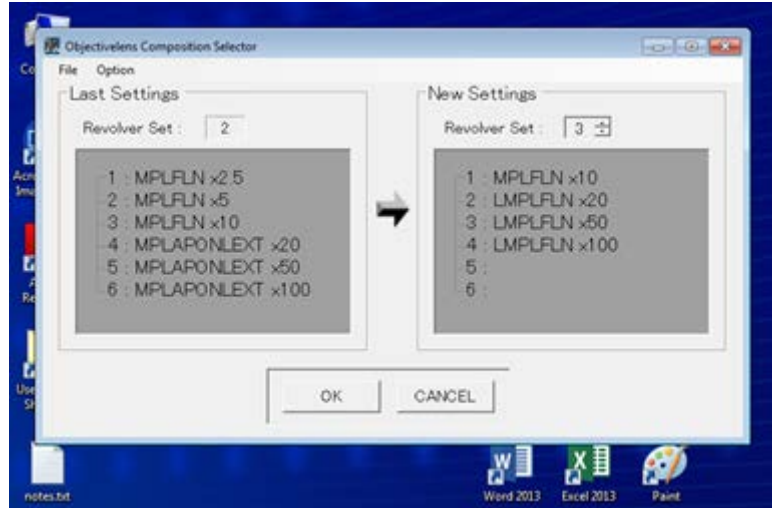
1. Make sure the microscope control unit is switched off. Computer might be running (but Lext software is closed).
2. Untighten the fixing screw on the right side of the head mount with a hex key (“imbus key”).



3. Wearing gloves, gently pull out the head towards you. Listen to a loud “click” as the connector disconnects.



4. Put the head to a safe and stable position (e.g. on a desk) on a **tissue upside down**.
5. Reverse the procedure to install a new head. Gently pull in the head, hear a loud “click” as the connector connects, tighten the fixing screw on the right side of the head mount.
6. Turn on the control unit.
7. Open the **Objective lens Composition Selector** Software (on a Desktop) and select the appropriate set of lenses. Note: There are two heads available, with set 2 and set 3 configurations. Confirm your selection.



8. Microscope is ready for use. Open the Lext software and check that the available lenses have been updated.