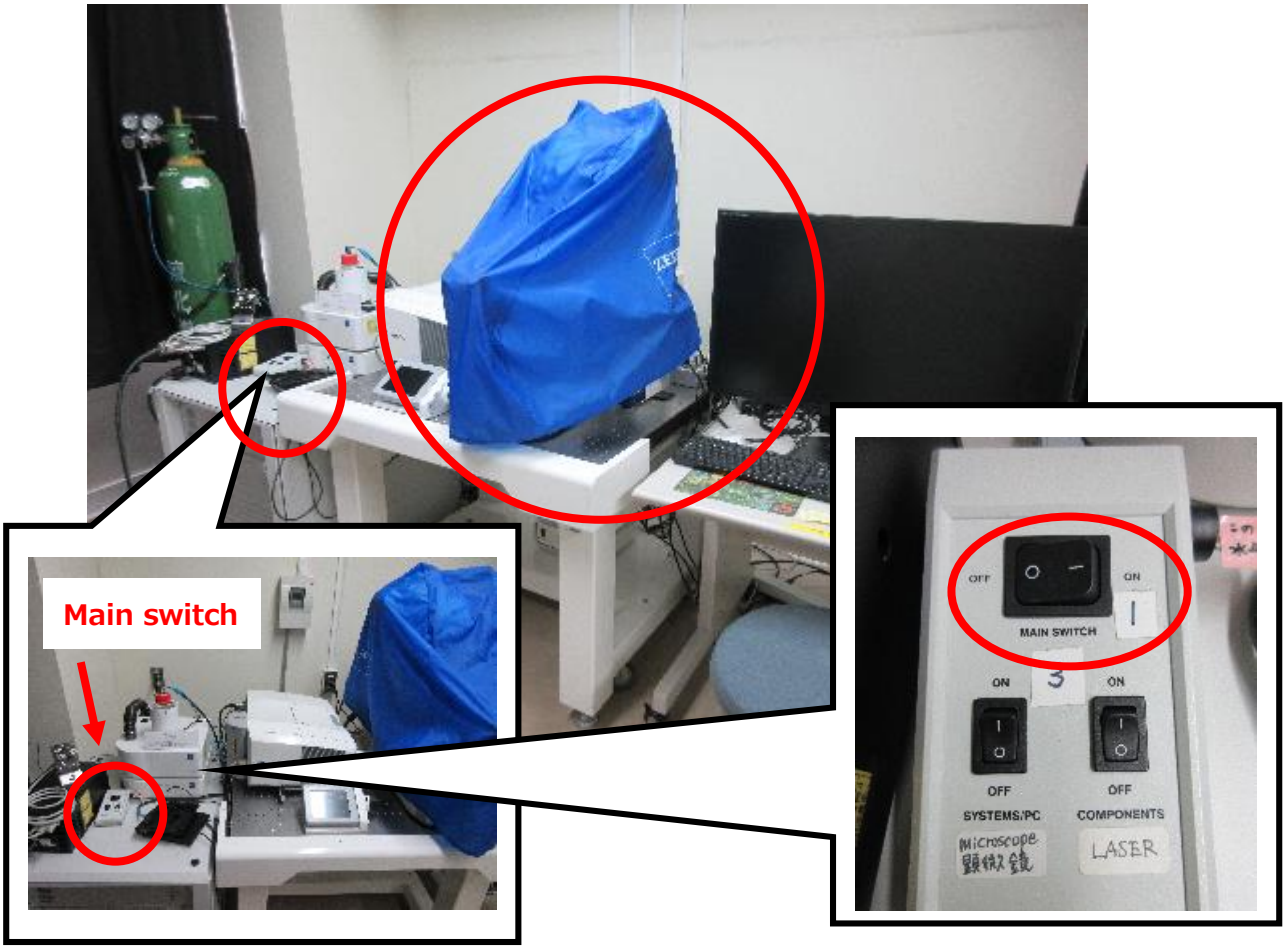
Confocal laser scanning microscope LSM710 Basic operation

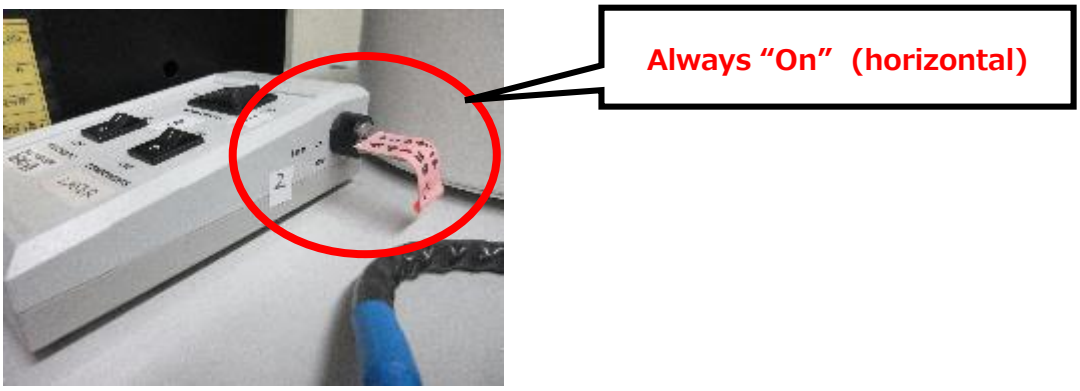
2021.5.18

<Start up the system>

1. Remove the **blue cover**. Turn on the **main switch** 1

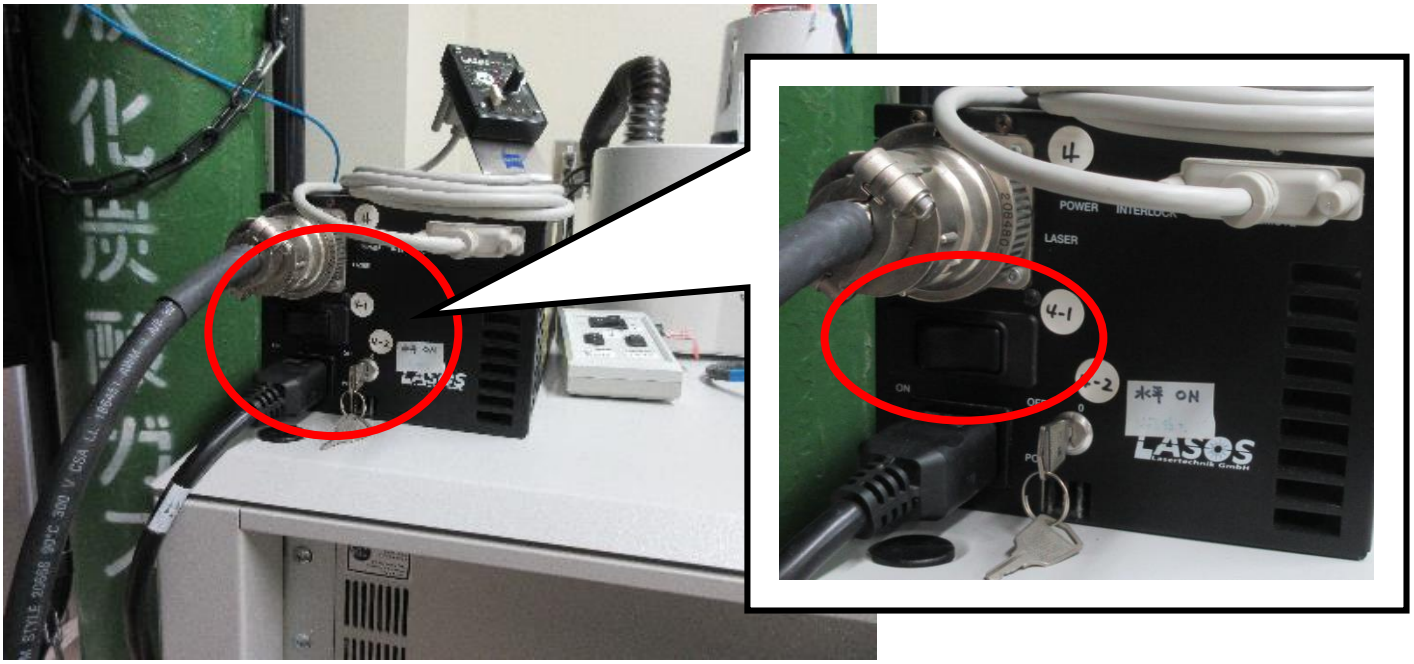


2. Confirm the **horizontal position** of the **power key** 2 (You don't have to turn it.)



3. Turn on **two sub switches** 3

4. Turn on the **laser box** (4-1)



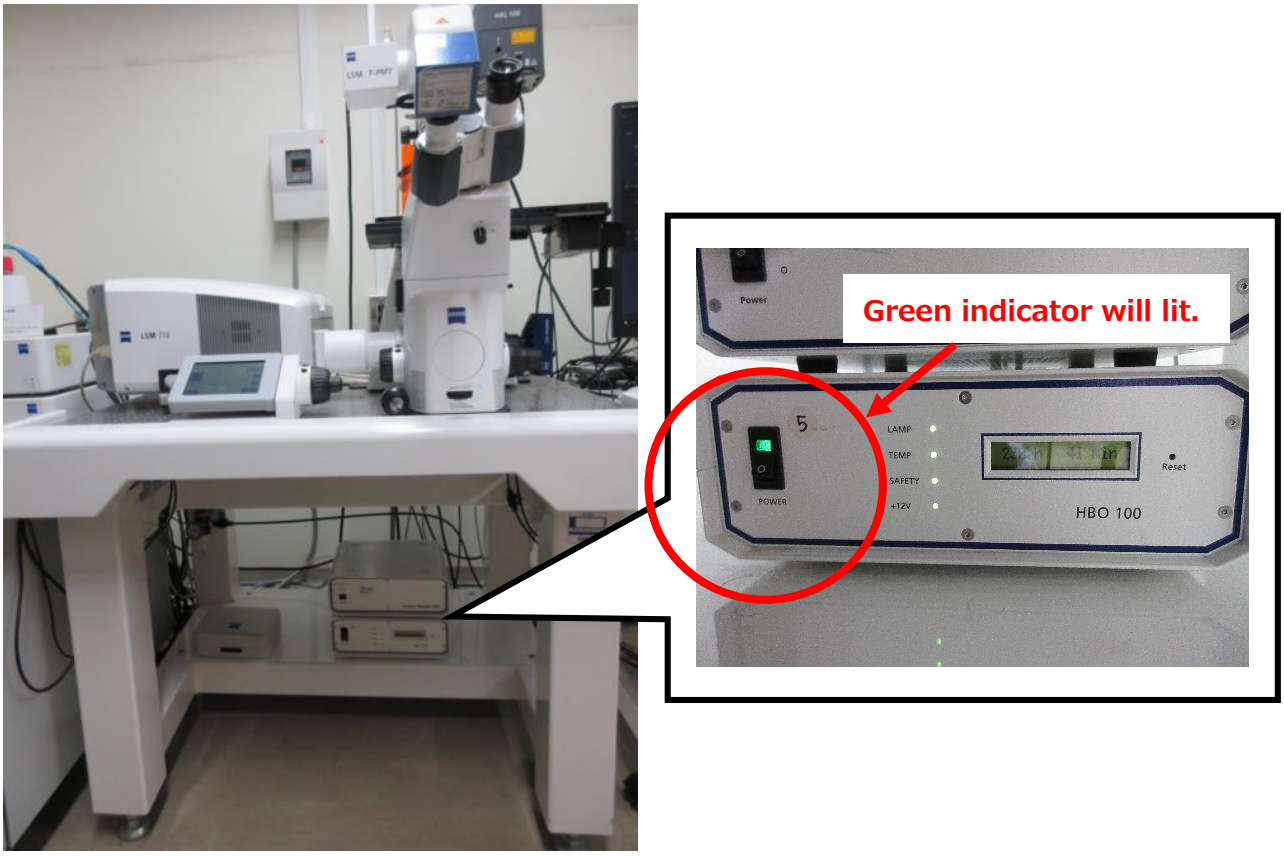
5. Turn the **laser key** (clockwise to **horizontal**) of the box (4-2) to the on position.

6. Wait about **5 min** to warm up the laser.

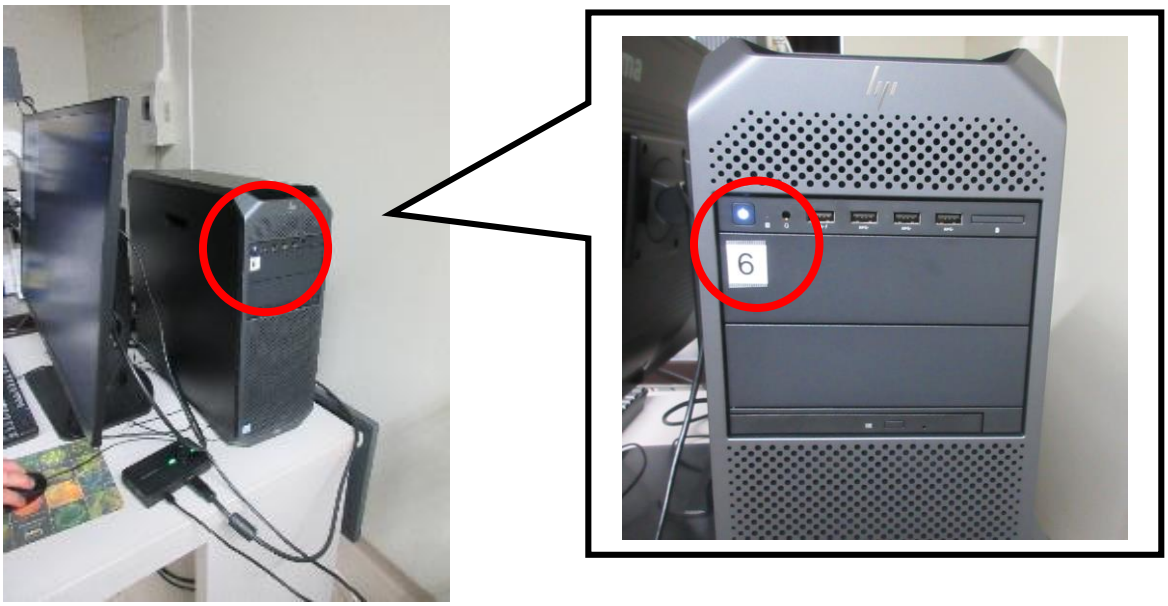
※ You can proceed below while waiting.

The laser does not lit unless wait enough.

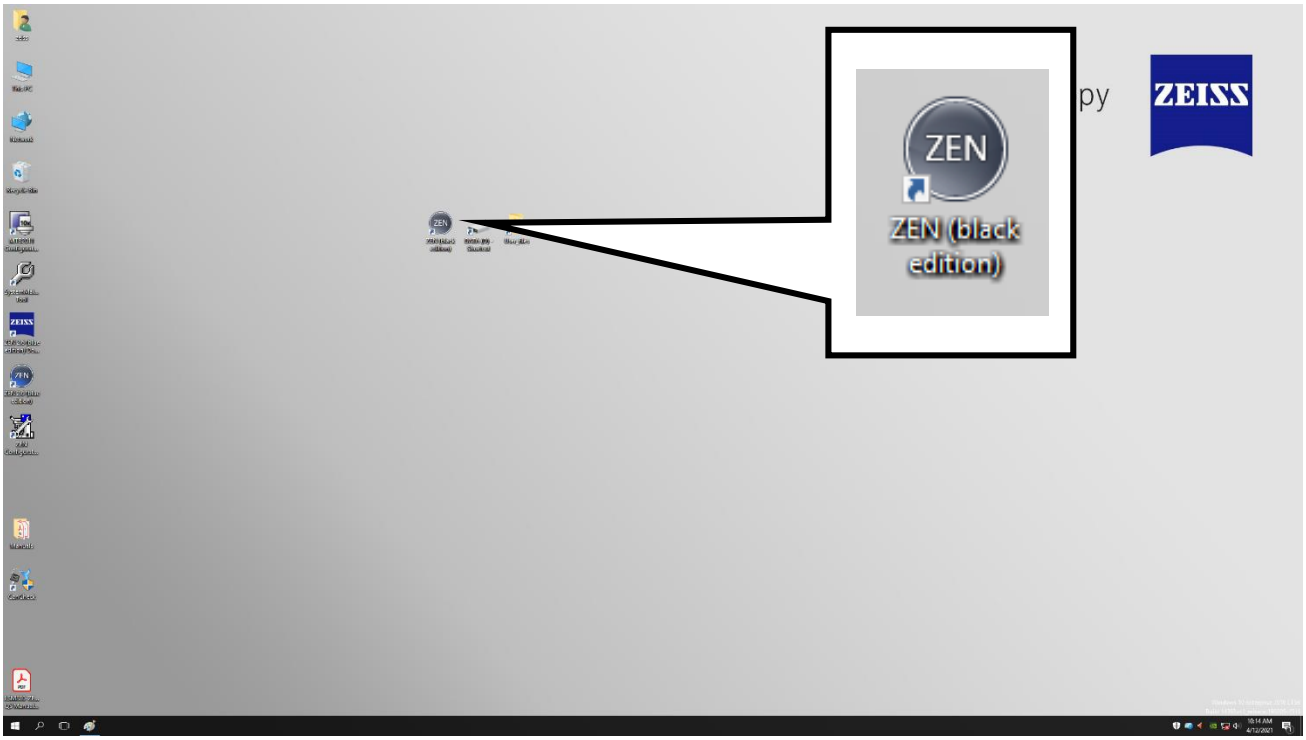
7. Turn on the **fluorescent lamp** 5



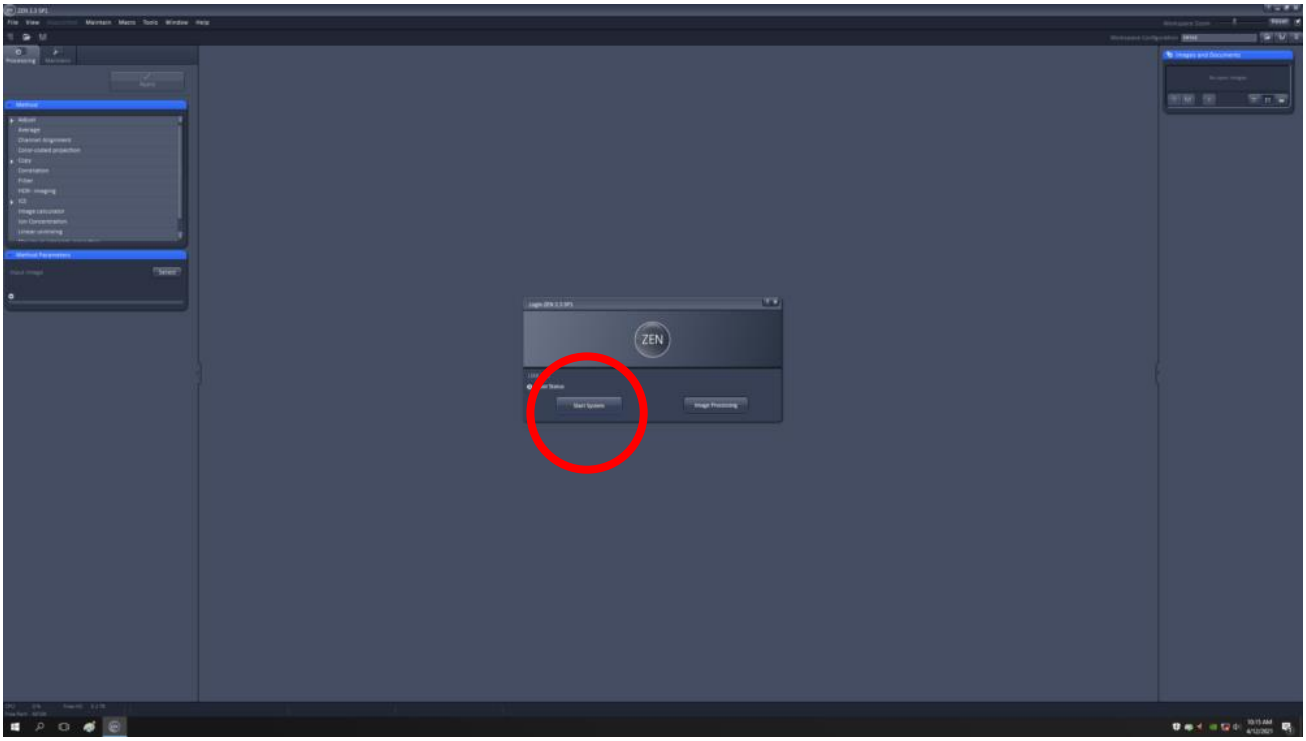
8. Turn on the **computer** 6



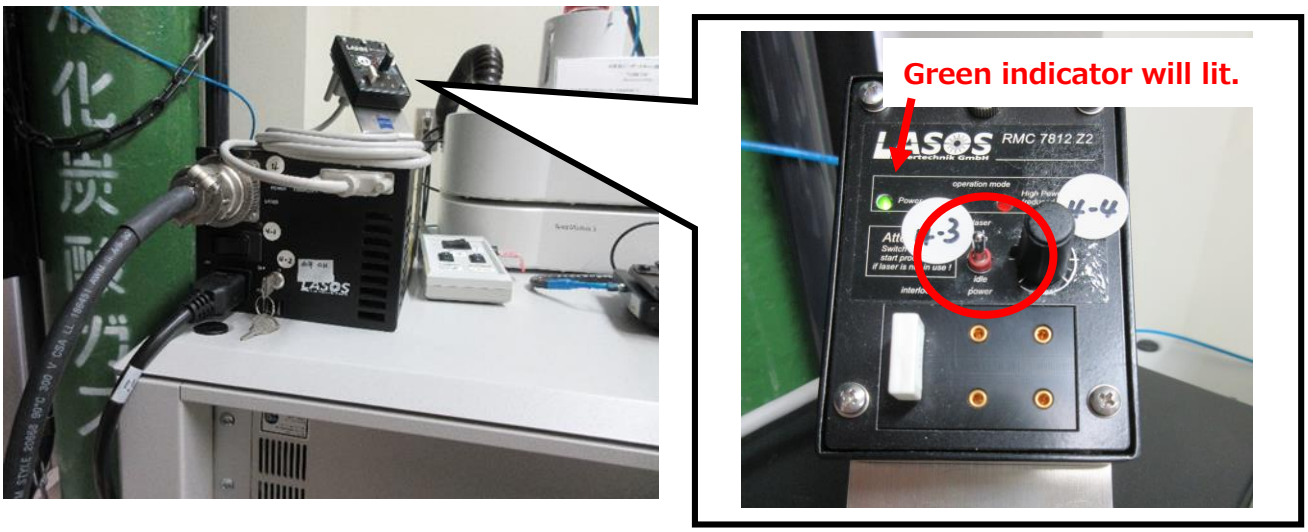
9. Click **ZEN (black edition)**



10. Chose **Start System**



11. Turn on the **laser switch** (4-3) after waiting **5 min** (page 3, step 6).



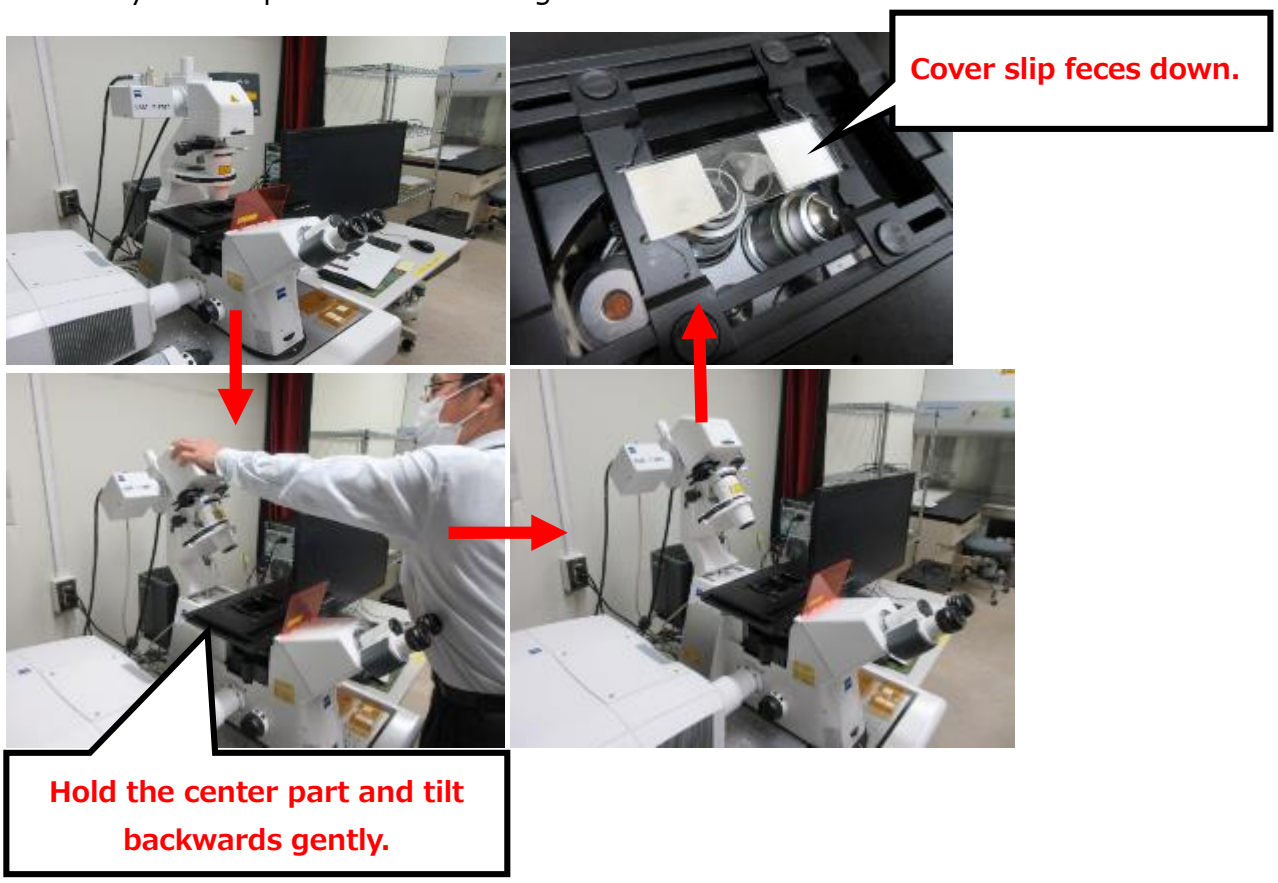
12. Turn the **power knob** (4-4) very slowly (**clockwise**) to increase the laser power. When you see a red right turned on, it means you over-turned the knob so reverse the knob until the red light goes out.

※When the lamp is not warm enough, the green light will go out when you increase the power (by turning the knob). If this happens, please stop turning the knob until you see the green light catches up.

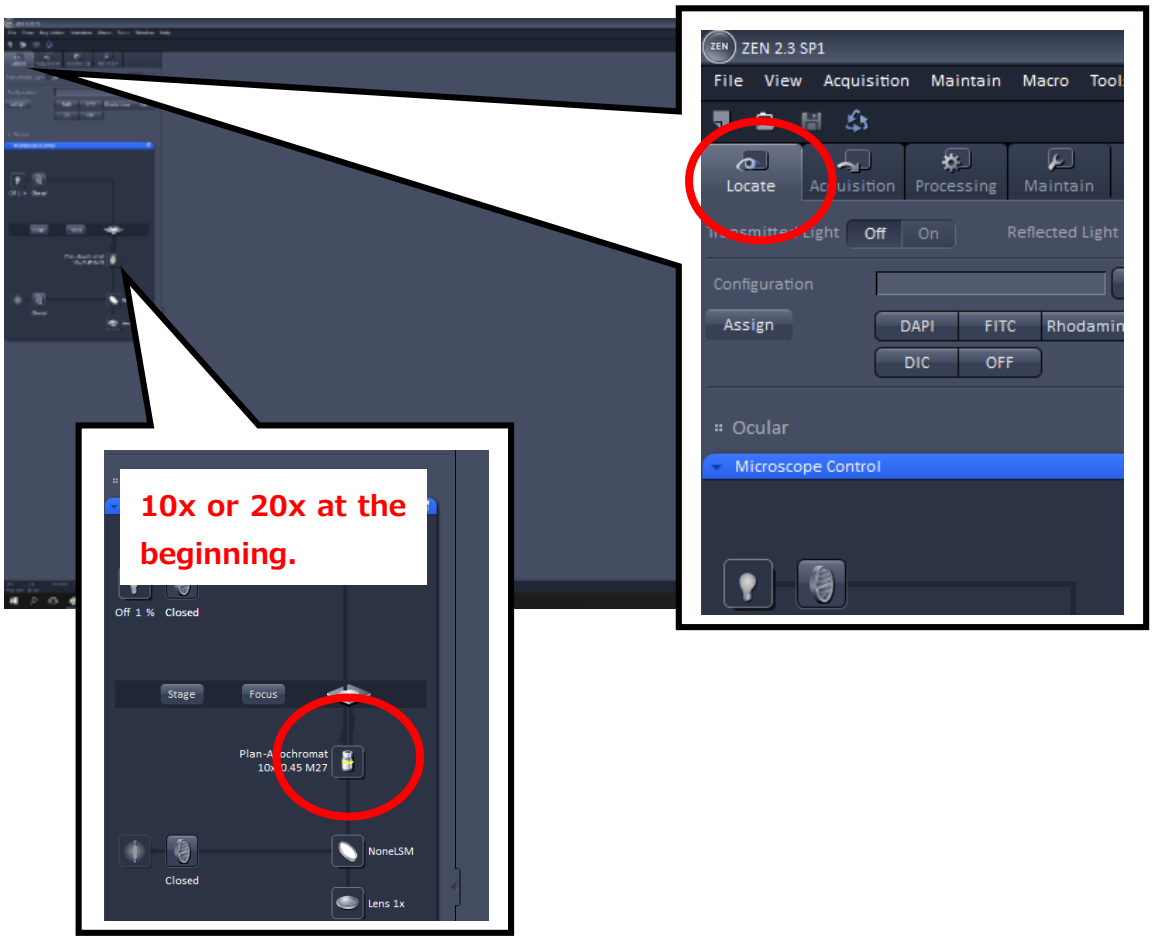
<Basic operation>

1. Press **Lord position** on the touch screen.

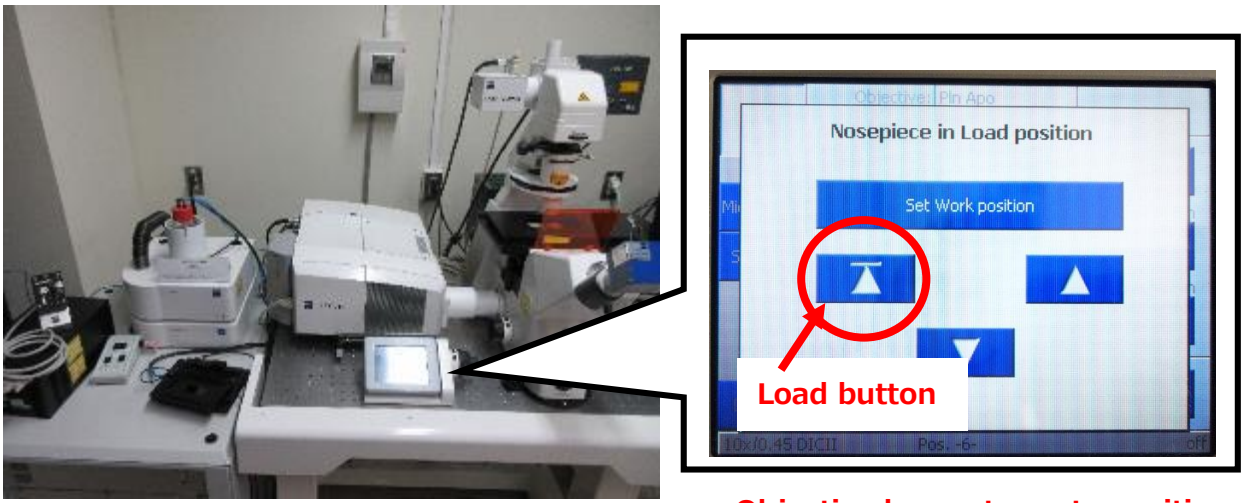
2. Place your sample slide on the stage.



3.Click **Locate tab**. Chose **10x** or **20x objective lens** by clicking the lens icon.

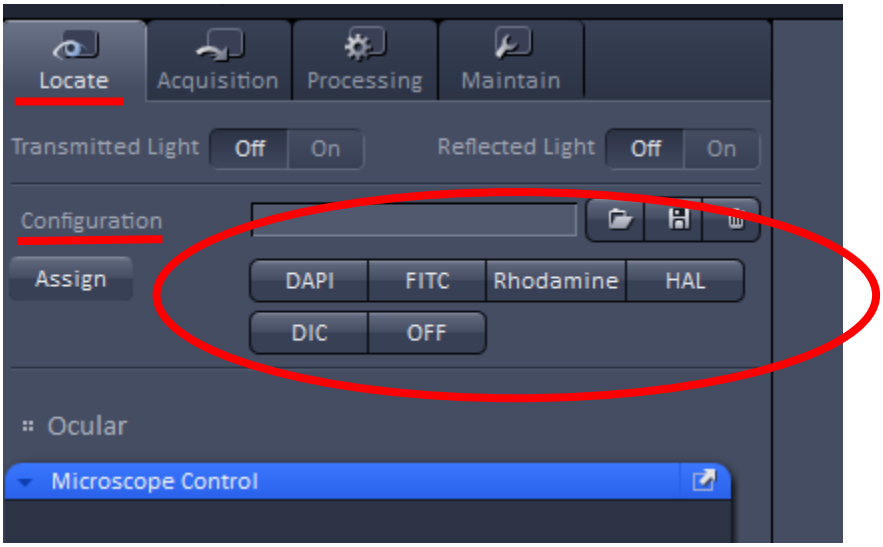


4. Press **Load button** (a barred triangle) on the touch screen.



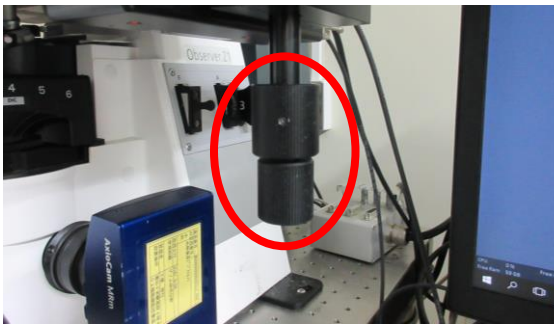
Objective lens returns to position.

5. Choose desired **fluorescent filter** from the configuration section in **Locate** tab.



6. Focus on your sample through **eyepieces**

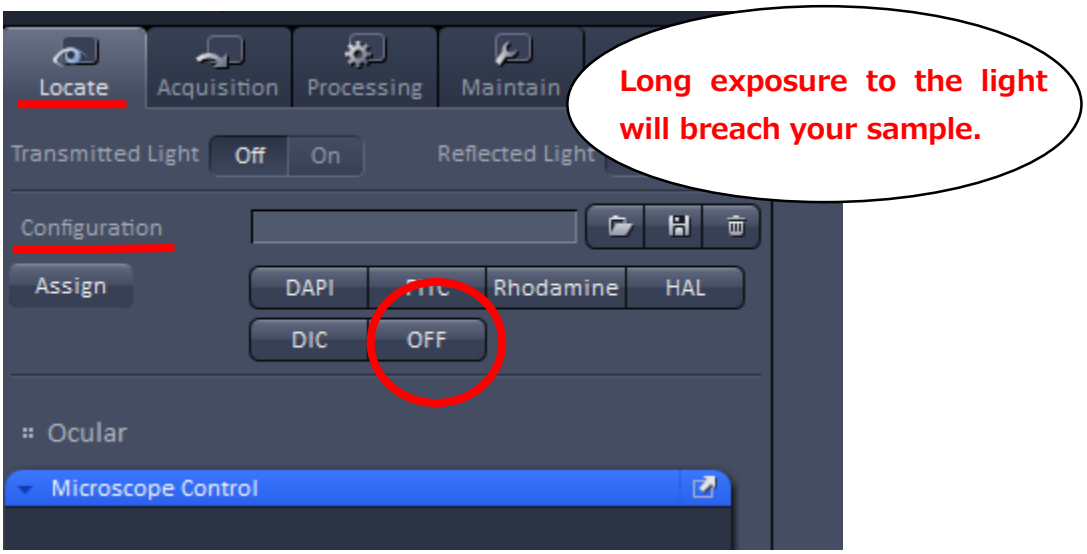
Planner adjustment (x-y direction) of the stage



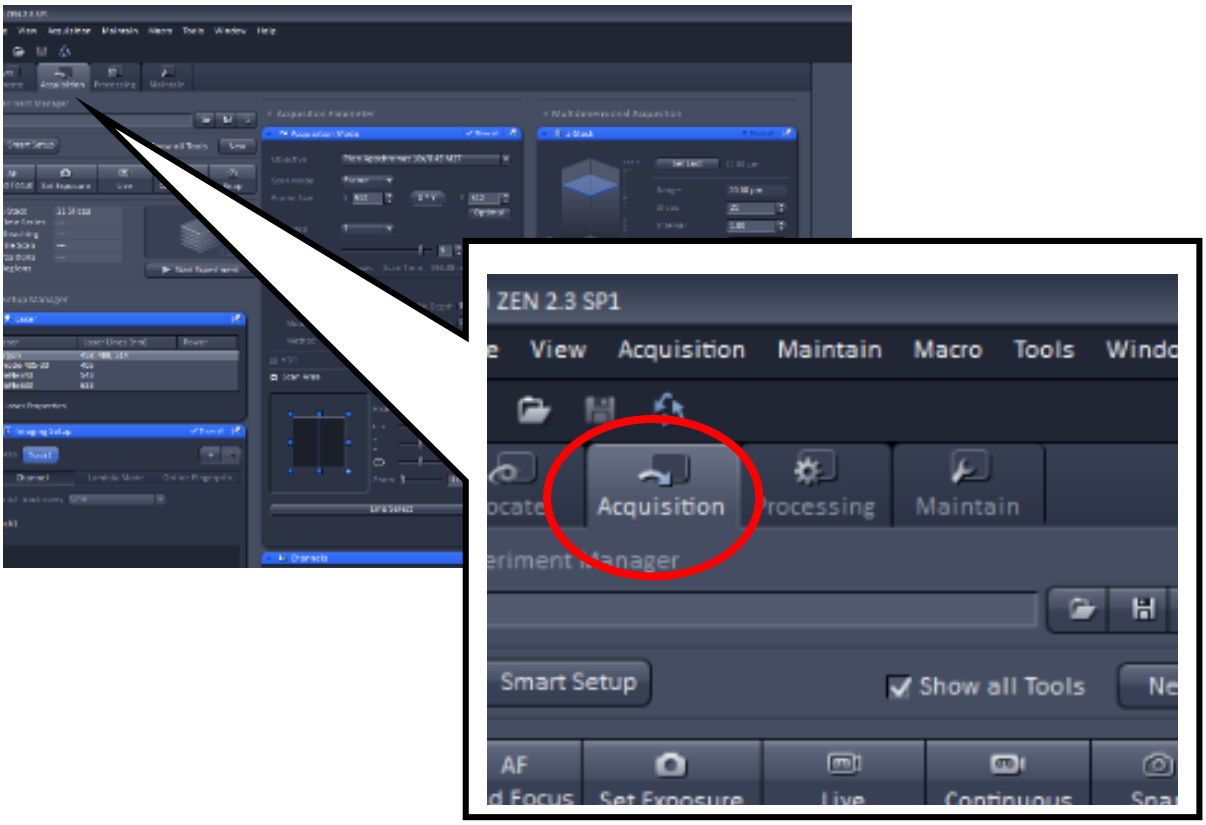
Lens up and down

Use fine adjustment (outer) handle.

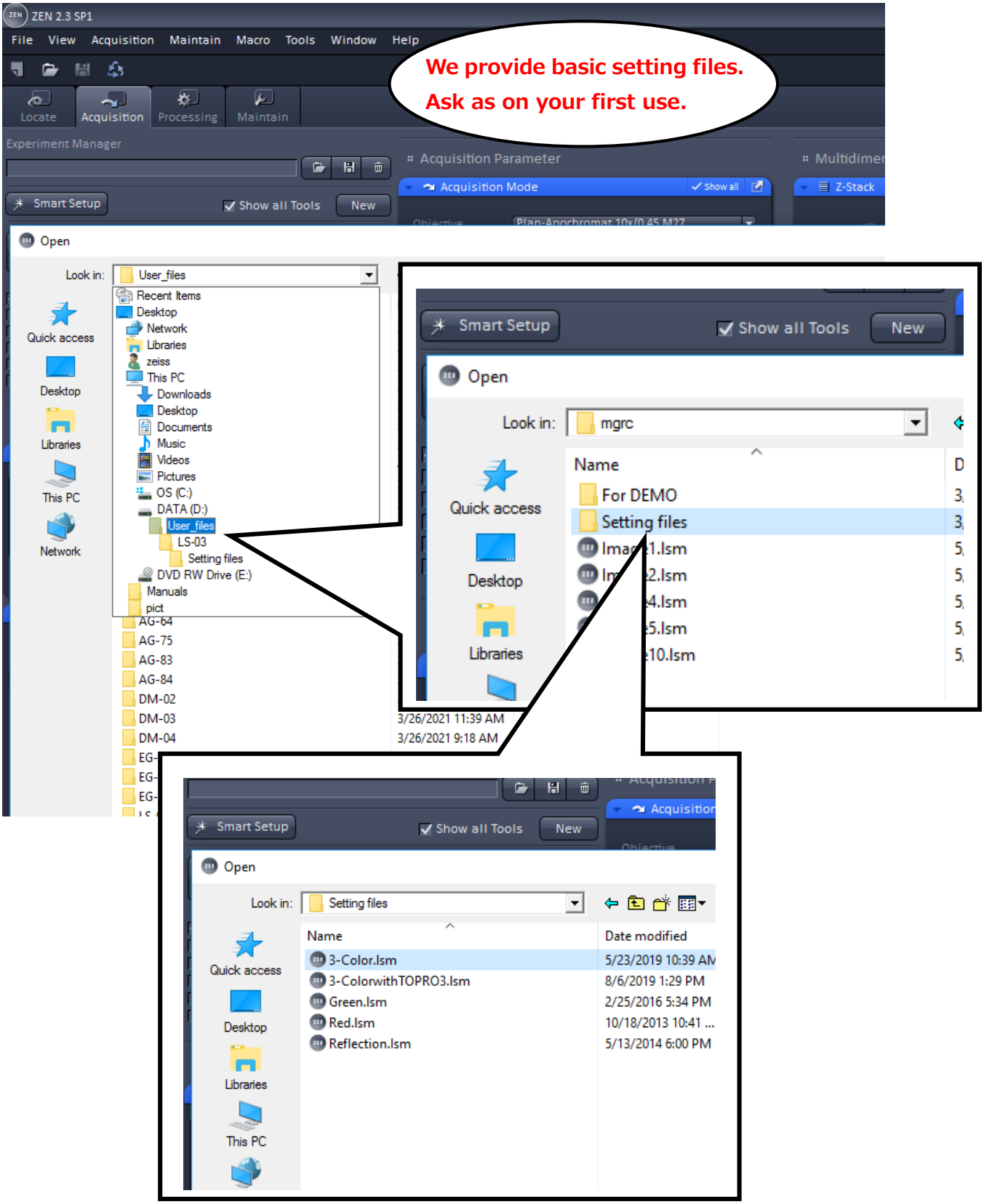
7. Press **OFF** button from the **Configuration** section in **Locate** tab



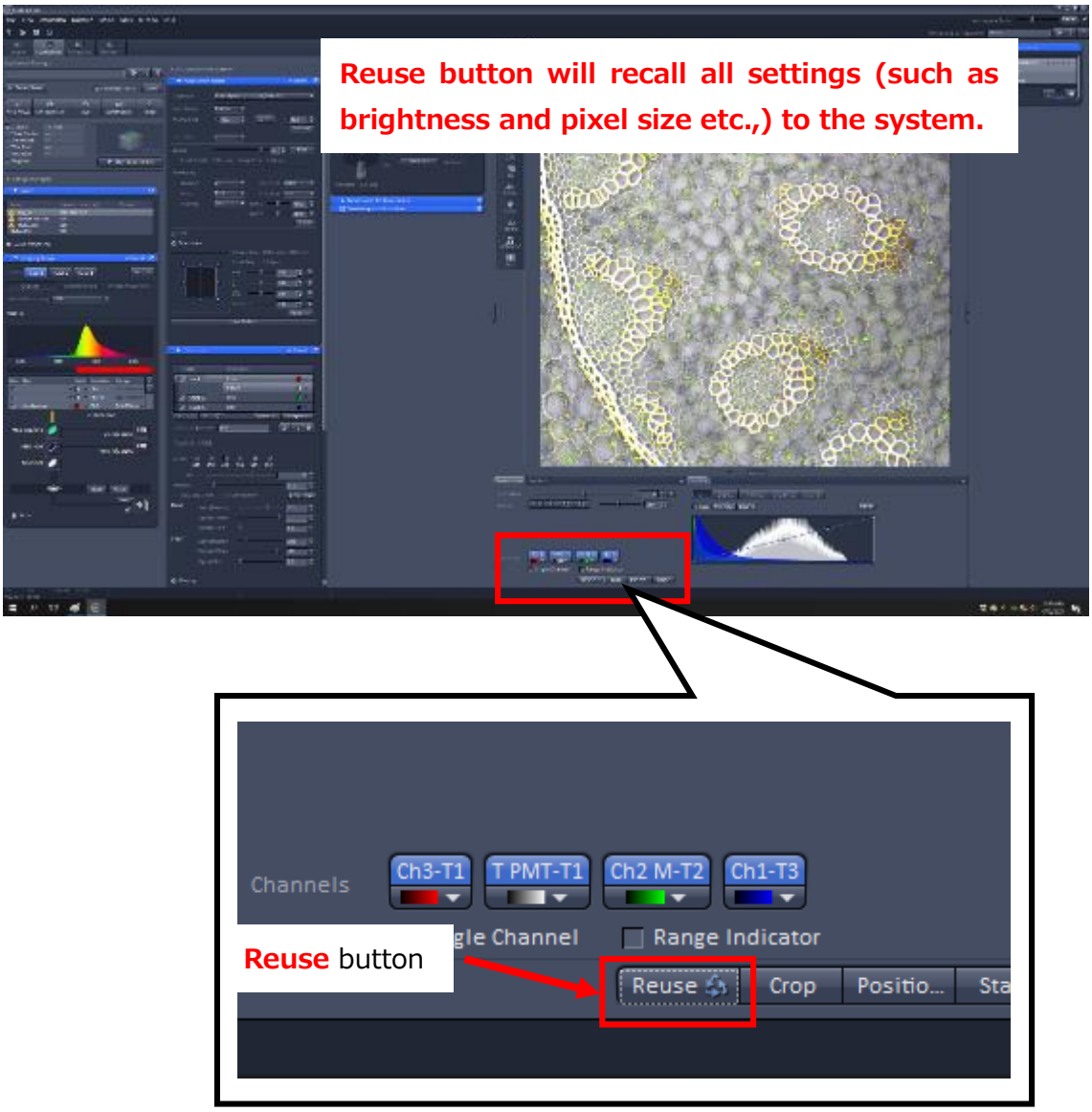
8. Chose **Acquisition** tab for laser imaging.



9. **File** → **OPEN** → **D: drive** → **User Files** → **Your lab's folder** → Chose adequate **Setting file** for your sample.



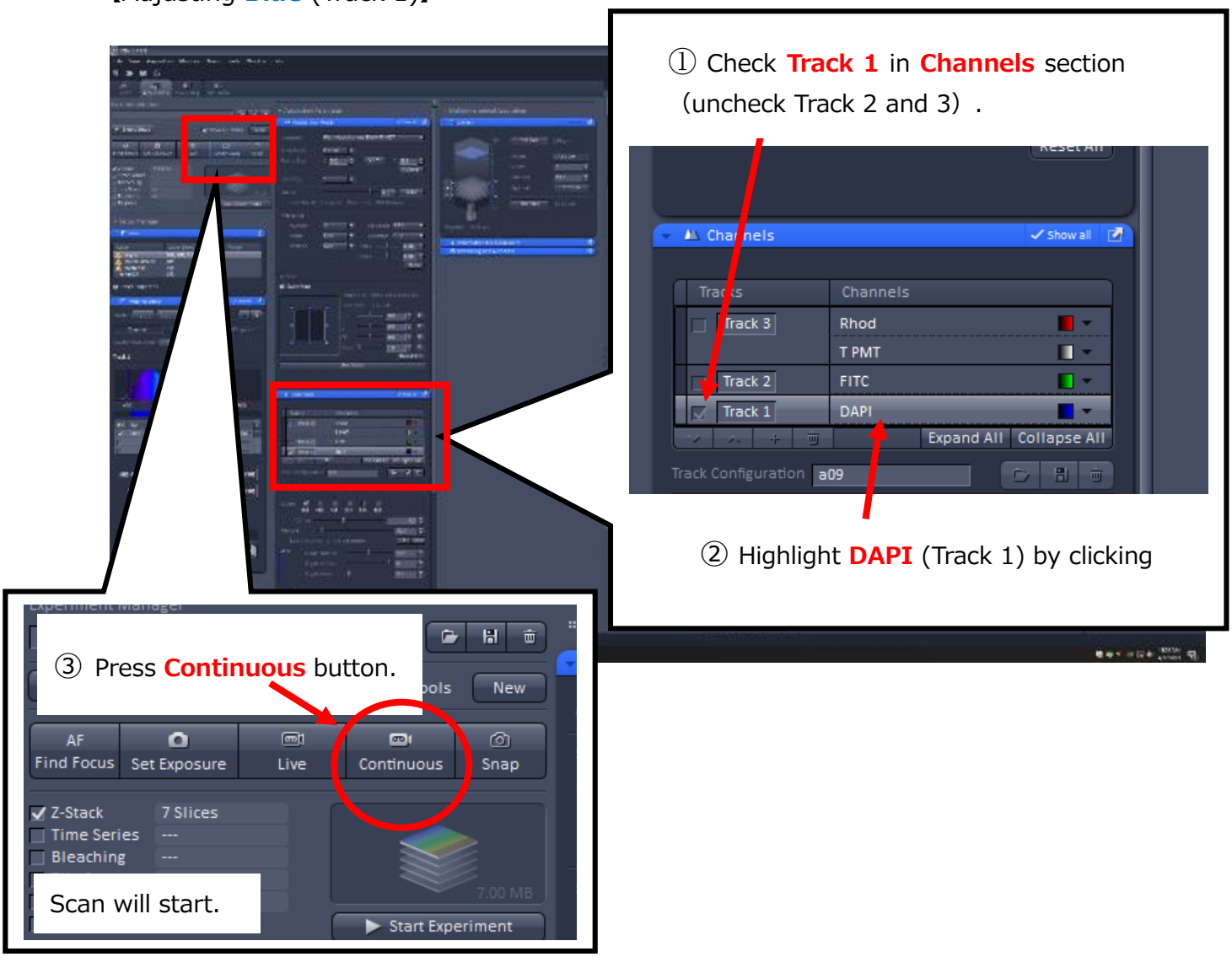
10. After opening the setting file, click **Reuse** button.



Now the initial settings for fluorescent images and a transmission image (if chosen) are ready.

11. Adjust parameters of each color channel (blue, red, green, transmission) as below.

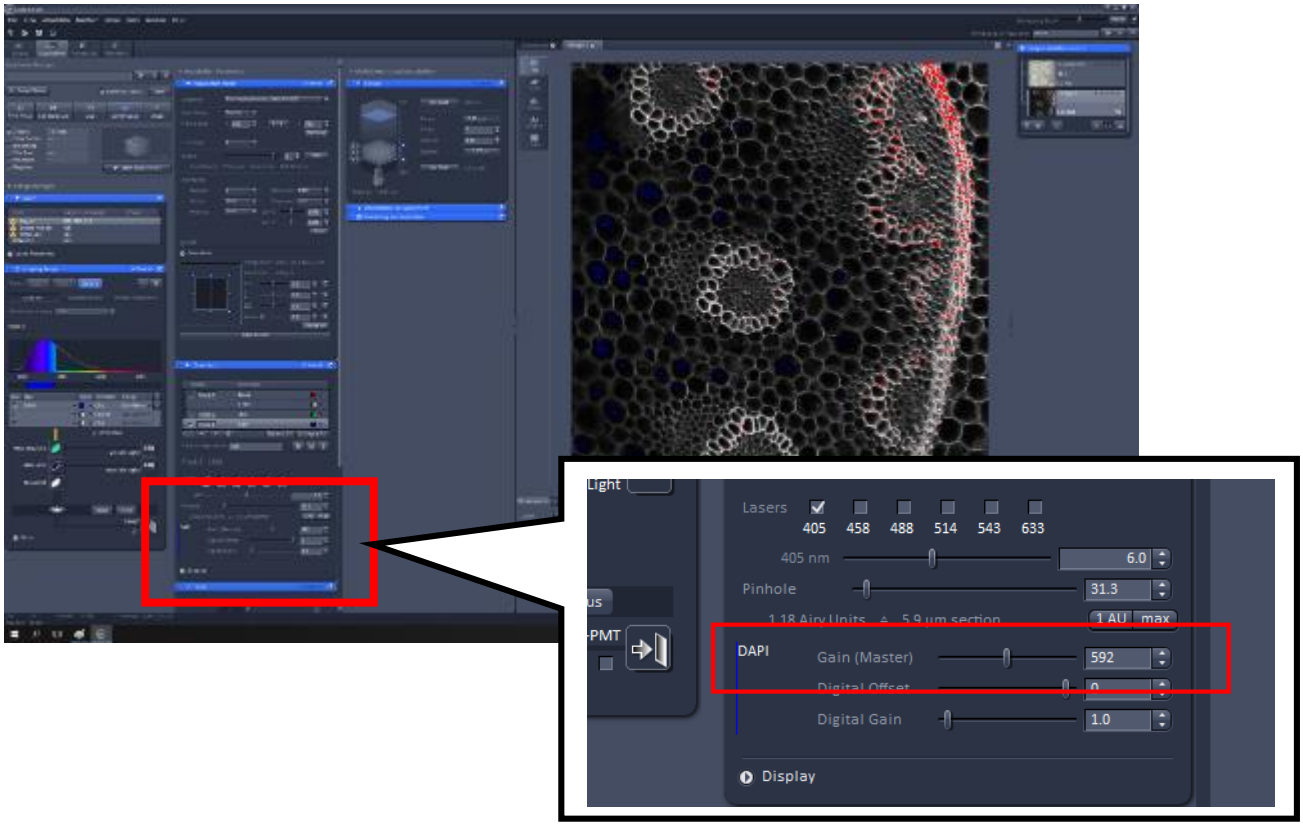
【Adjusting **Blue** (Track 1)】



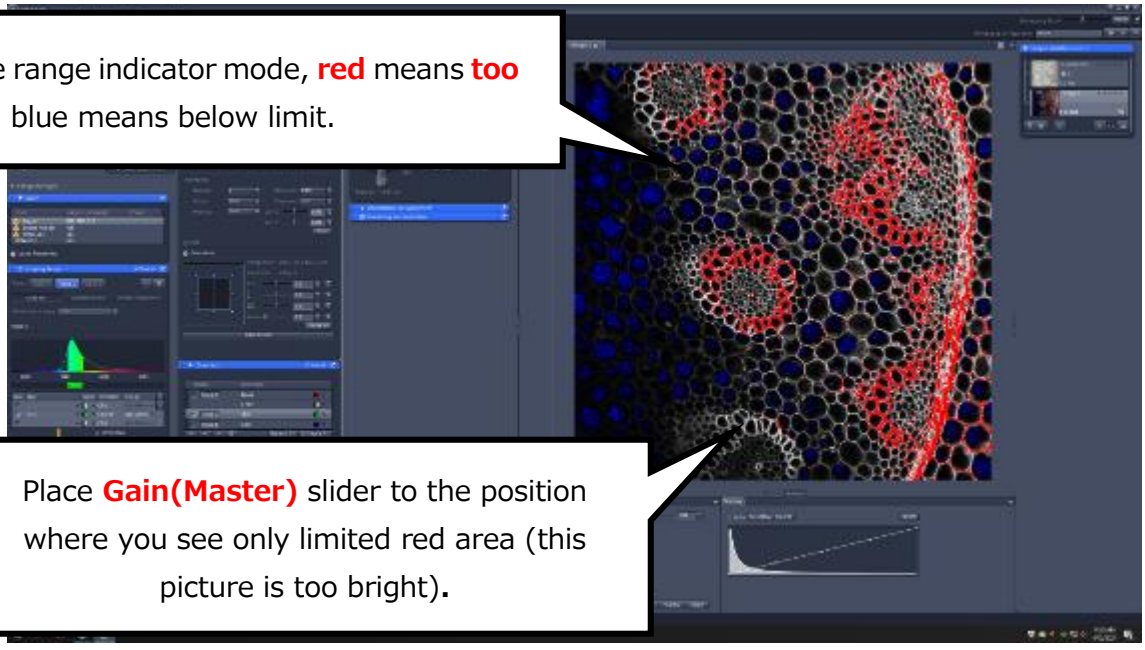
④ Check **Range Indicator**.

【Adjusting **Blue** (Track 1) continued】

⑤ Move the **Gain (Master)** slider of DAPI (**Channels** section) to adjust the detector gain.



※How to adjust※

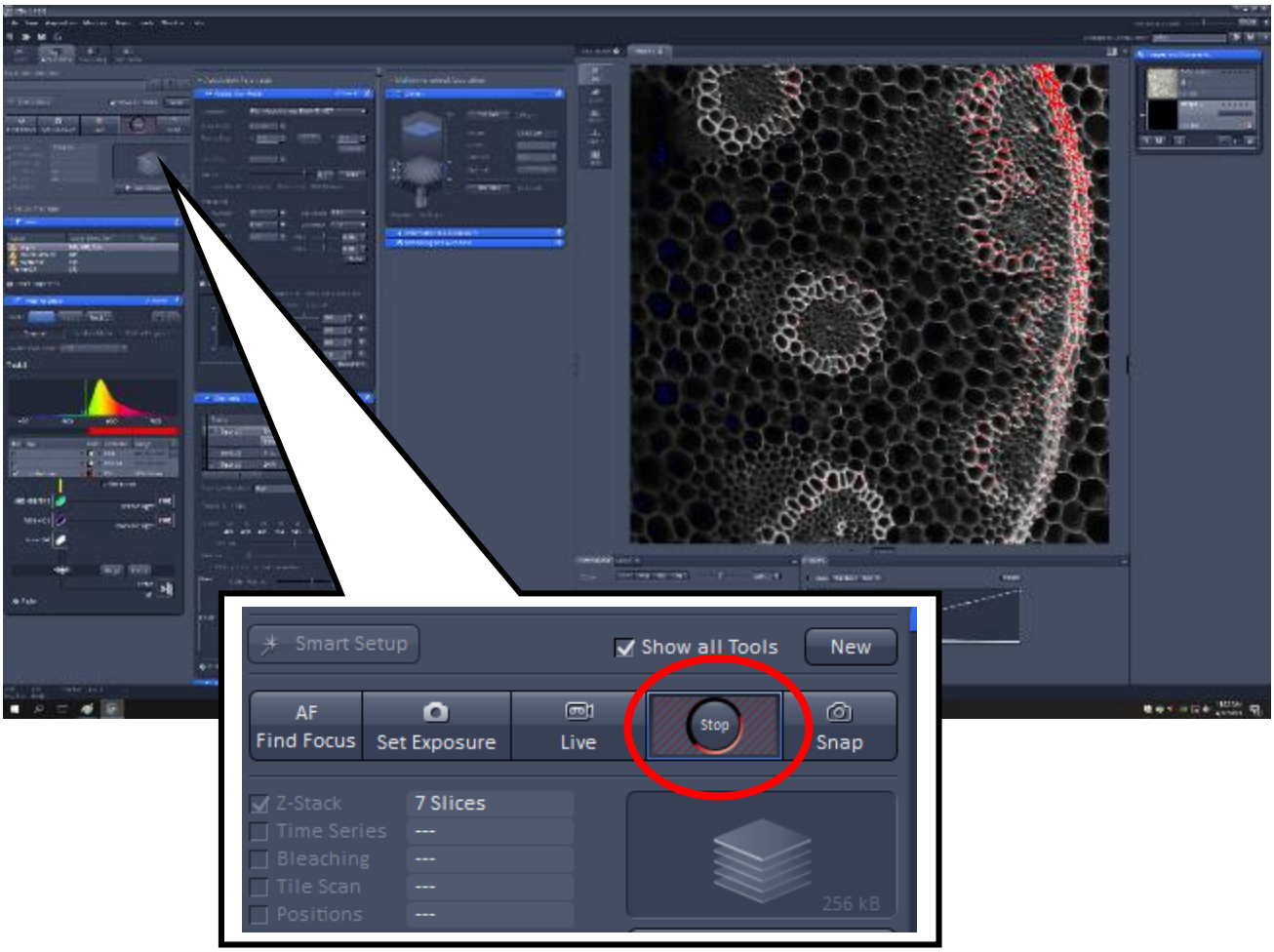


In the range indicator mode, **red** means **too high**, blue means below limit.

Place **Gain(Master)** slider to the position where you see only limited red area (this picture is too bright).

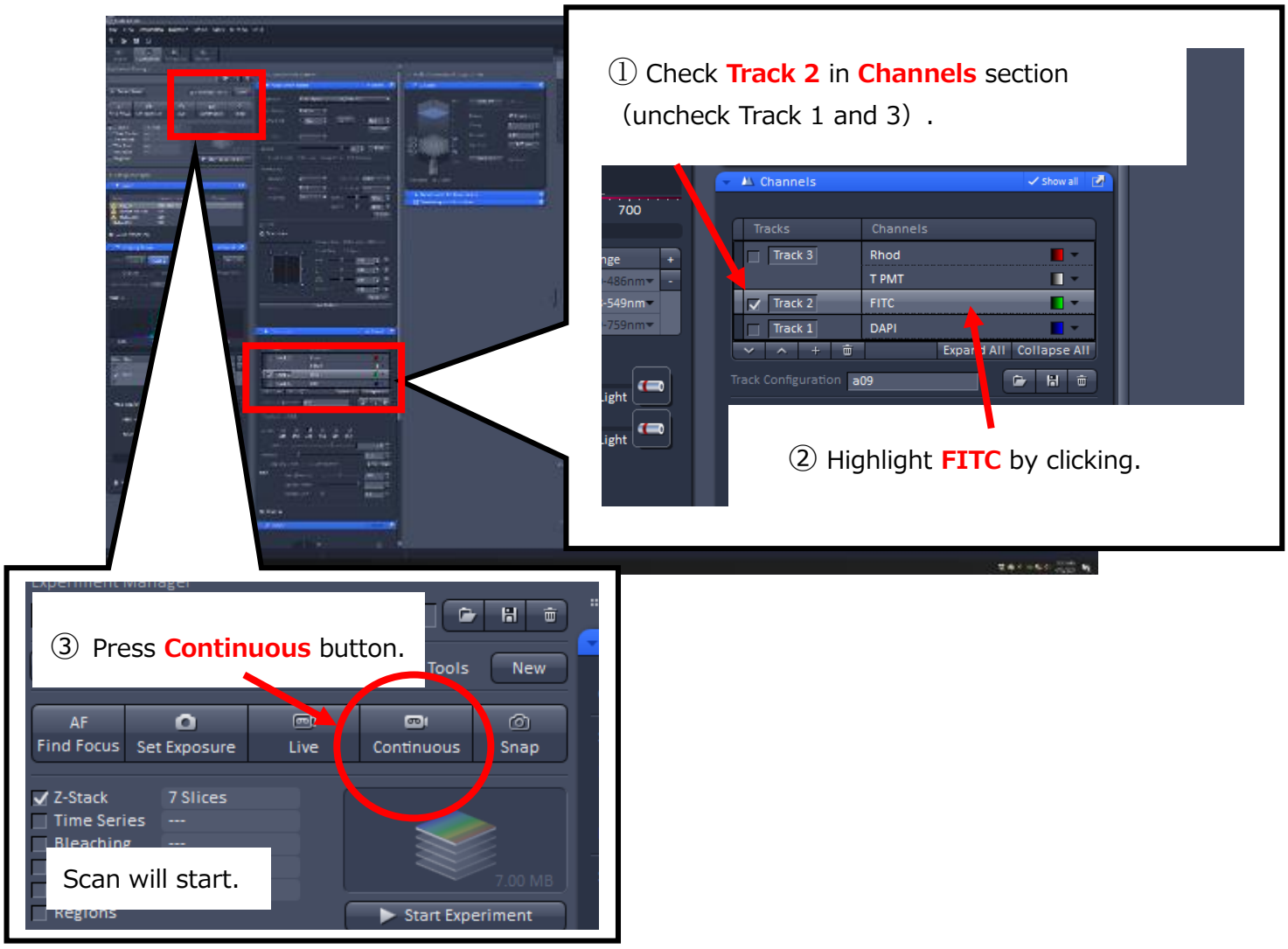
【Adjusting **Blue** (Track 1) continued】

⑥ Press **Stop** button after adjustment.



Now blue is OK.

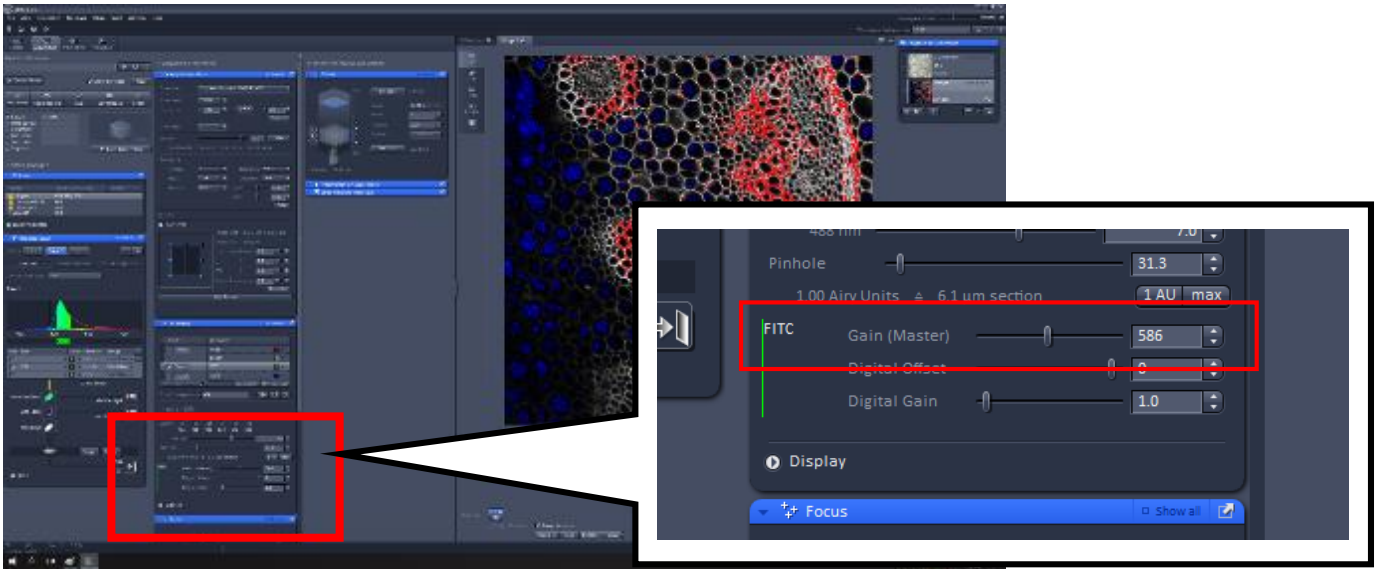
【Adjusting **Green** (Track 2)】



④ Check **Range Indicator**.

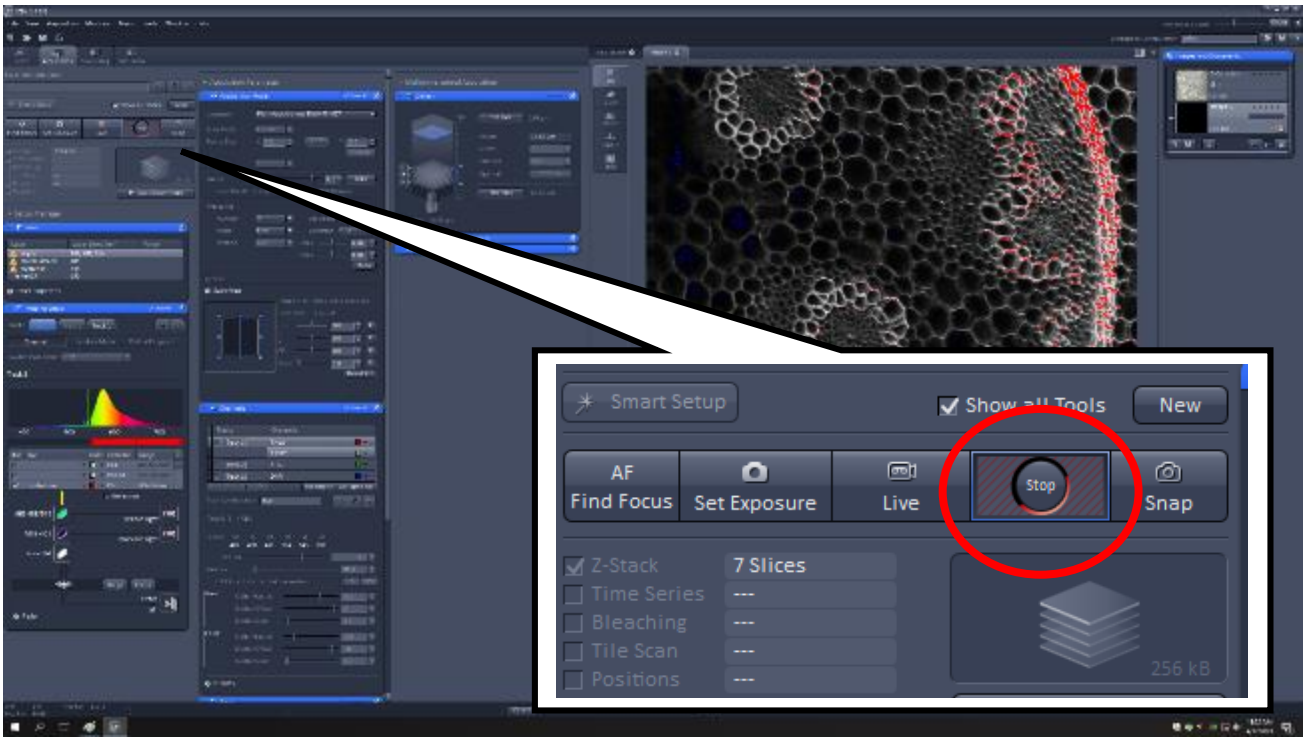
【Adjusting **Green (Track 2)** continued】

⑤ Move the **Gain (Master)** slider of FITC (**Channels** section) to adjust the detector gain.



Refer to ※How to adjust※ on page 13 for optimal adjustment.

⑥ Press **Stop** button after adjustment.



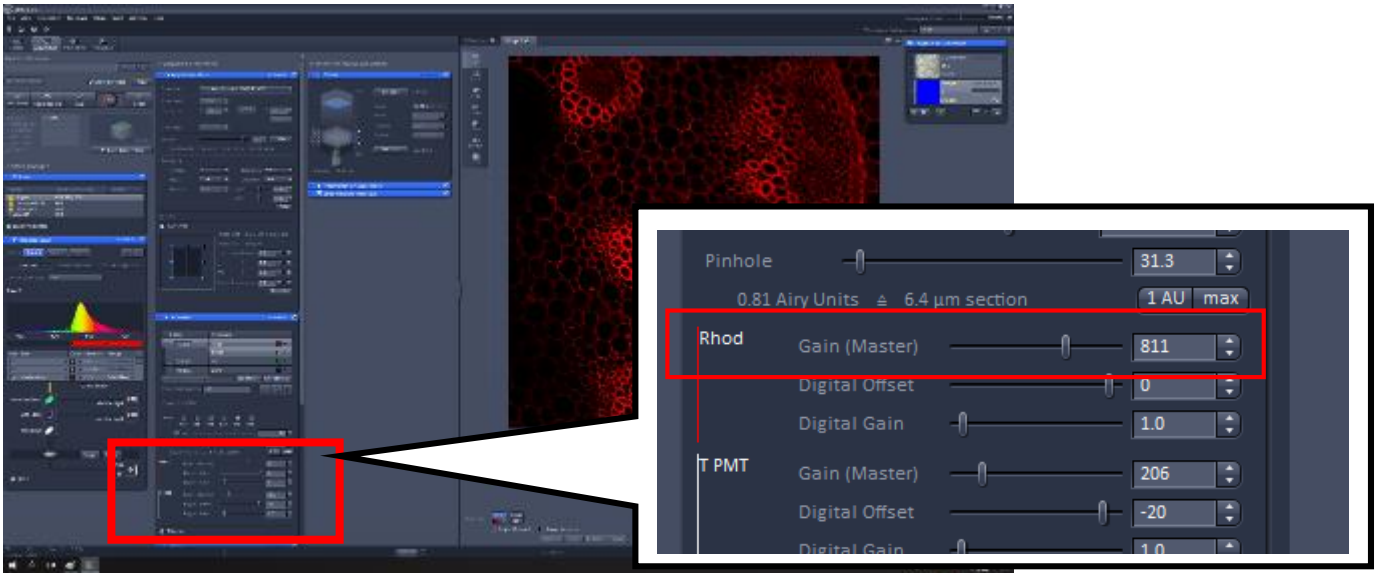
Now green image is OK.

【 Adjusting **Red** and **Transmission** (Track 3)】

<Adjusting **Red** (Track3)>

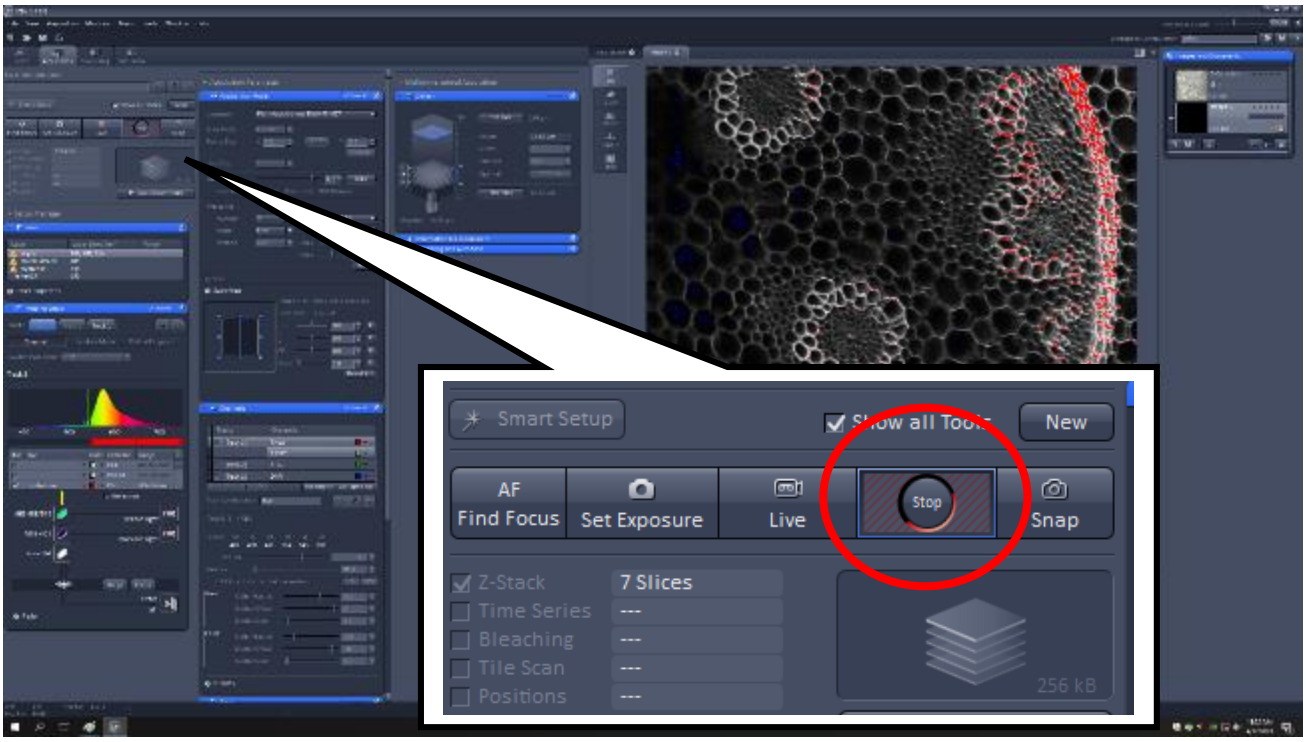
＜ Adjusting **Red** (Track 3) continued＞

⑥ Move the **Gain (Master)** slider of Rhod (**Channels** section) to adjust the detector gain.

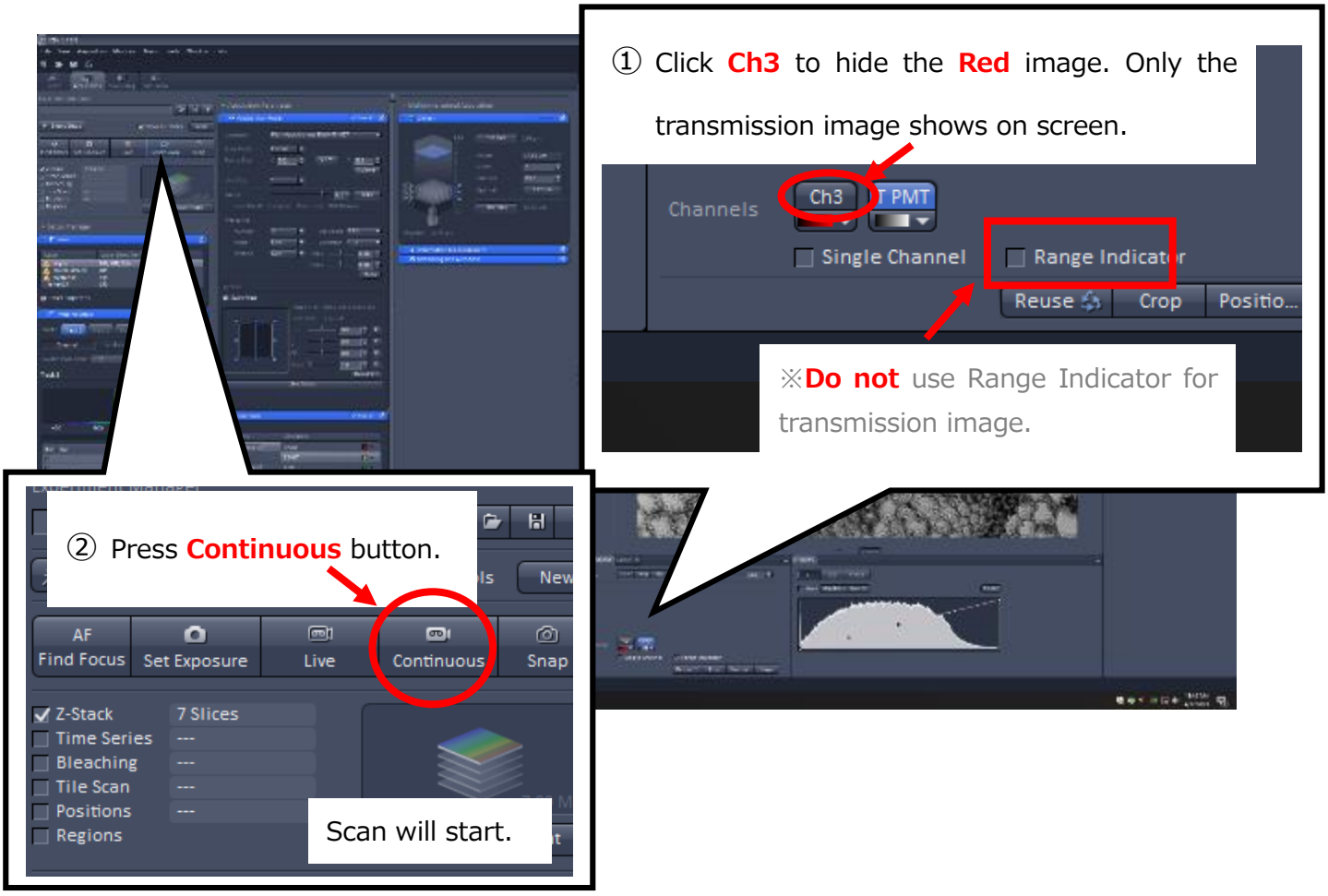


Refer to ※How to adjust※ on page 13 for optimal adjustment.

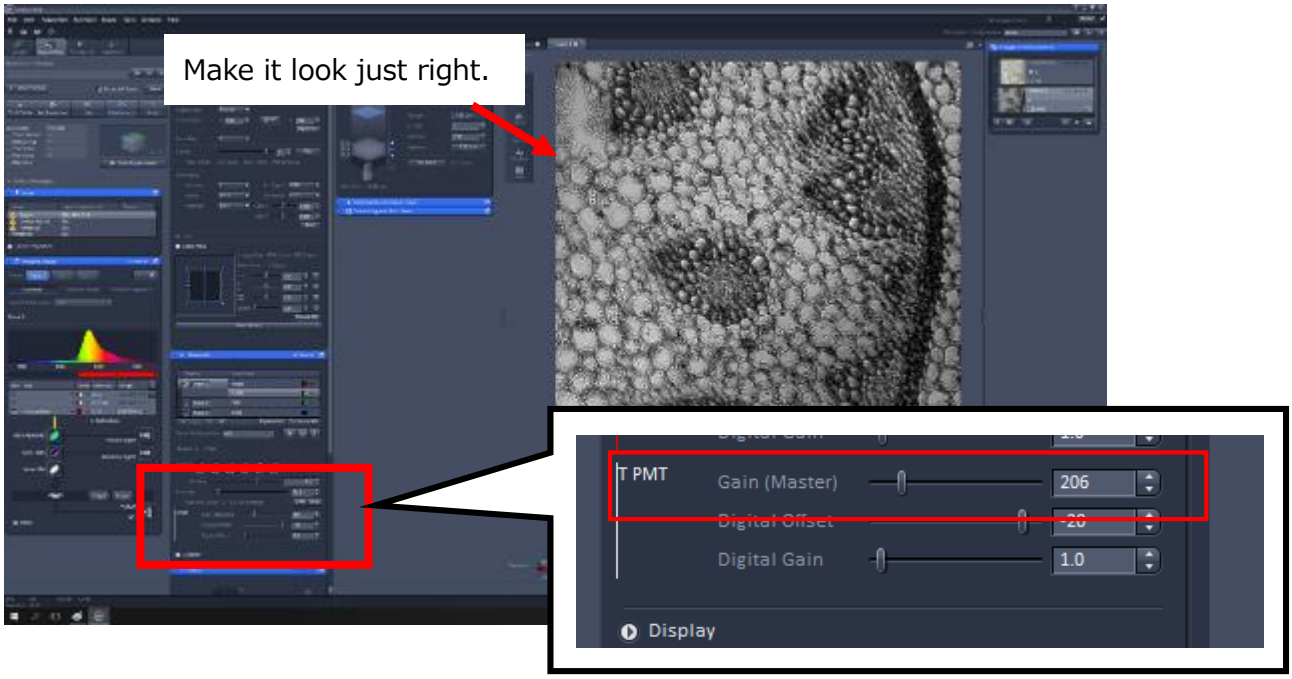
⑦ Press **Stop** button after adjustment.



<Adjusting **Transmission** image (Track 3)> ※Skip this part if unnecessary.

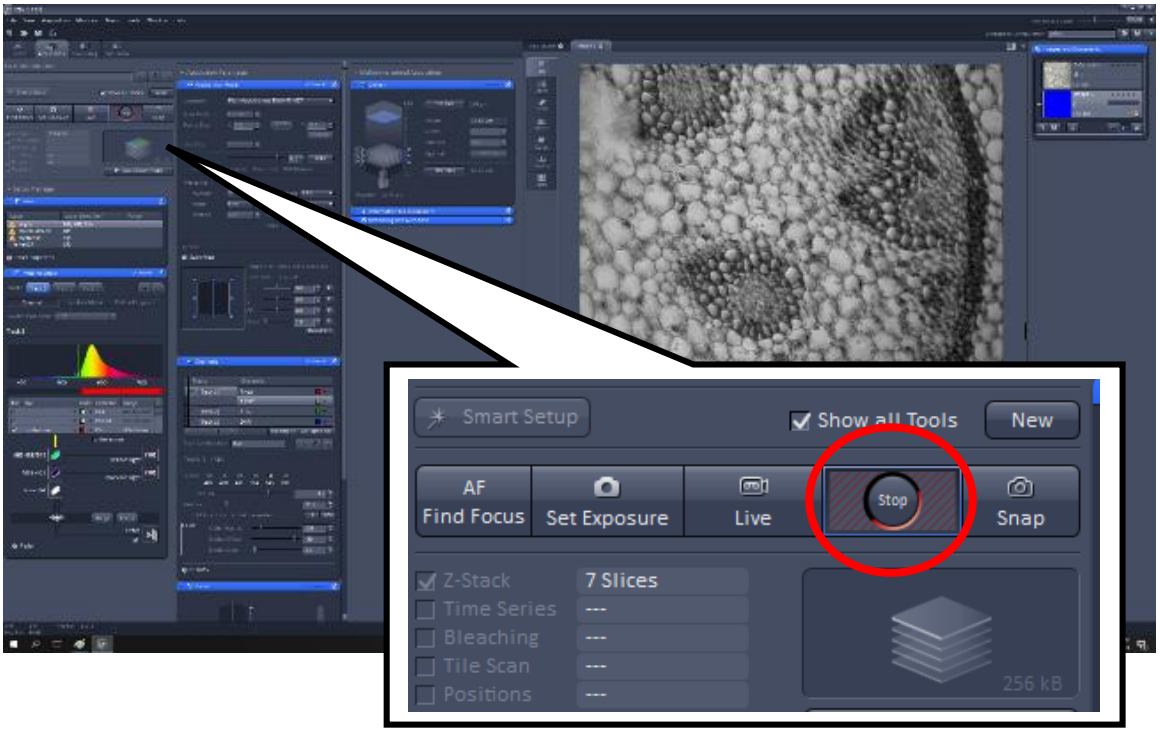


③ Move the **Gain (Master)** slider of T PMT (**Channels** section) to adjust the detector gain.



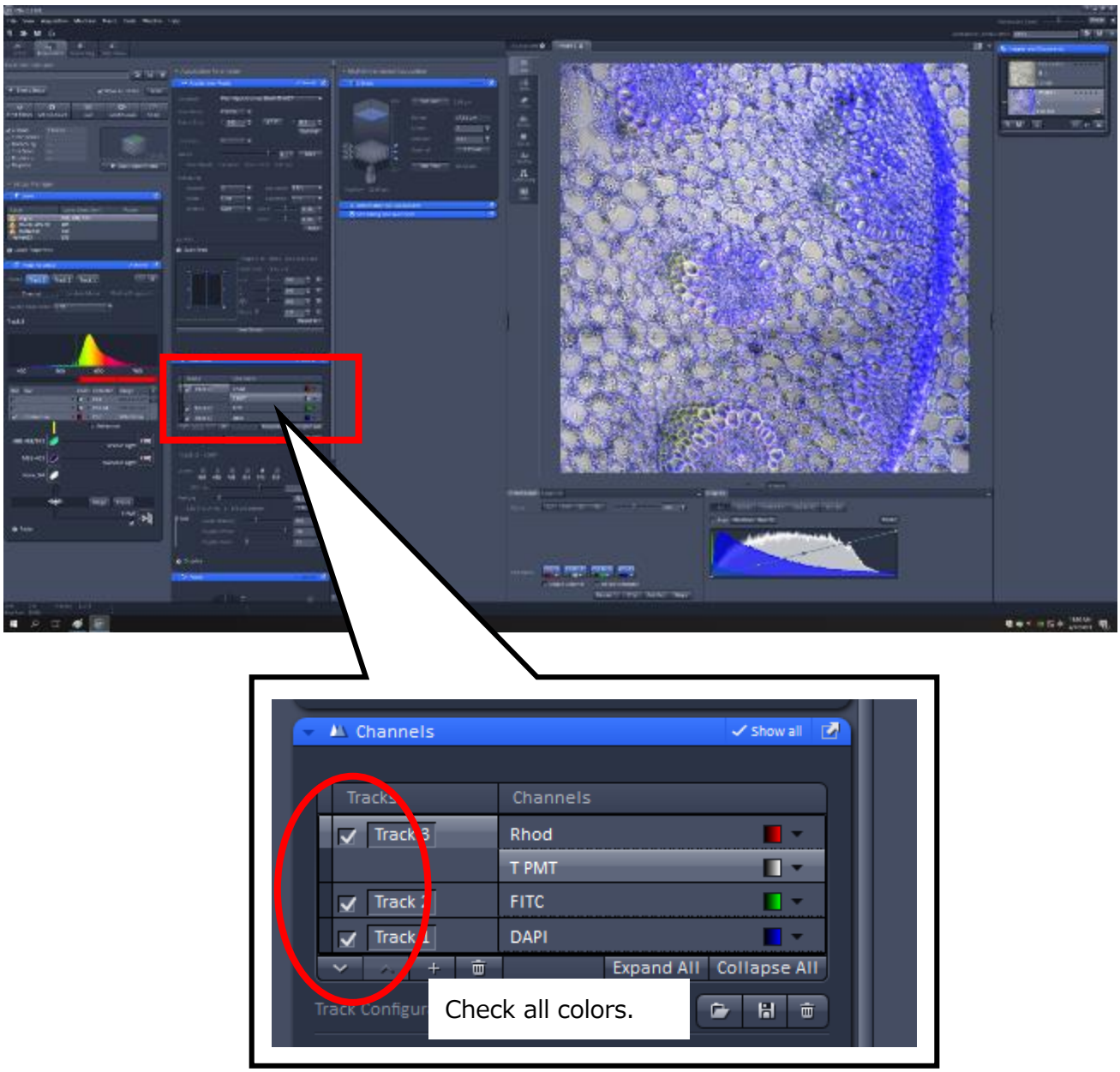
<Adjusting **Transmission** image (Track 3) continued>

④ Press **Stop** button after adjustment.

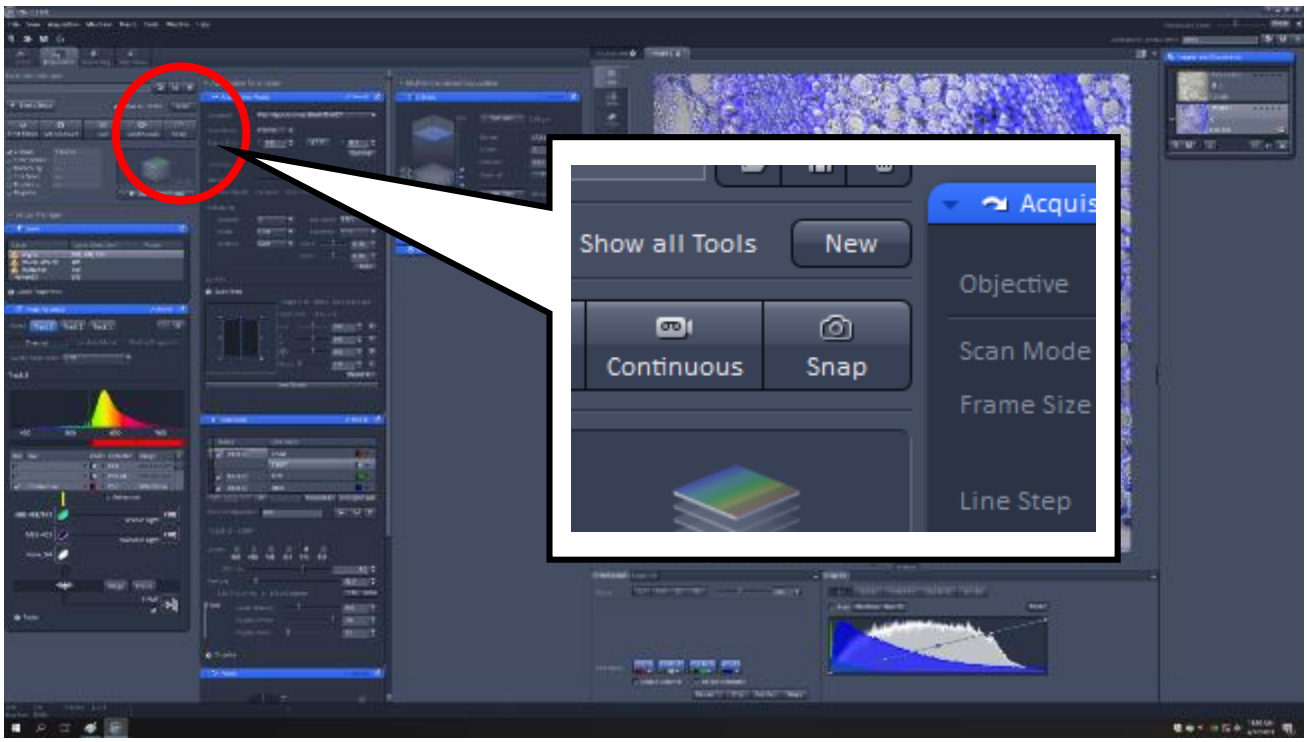


Now red and transmission images are OK.

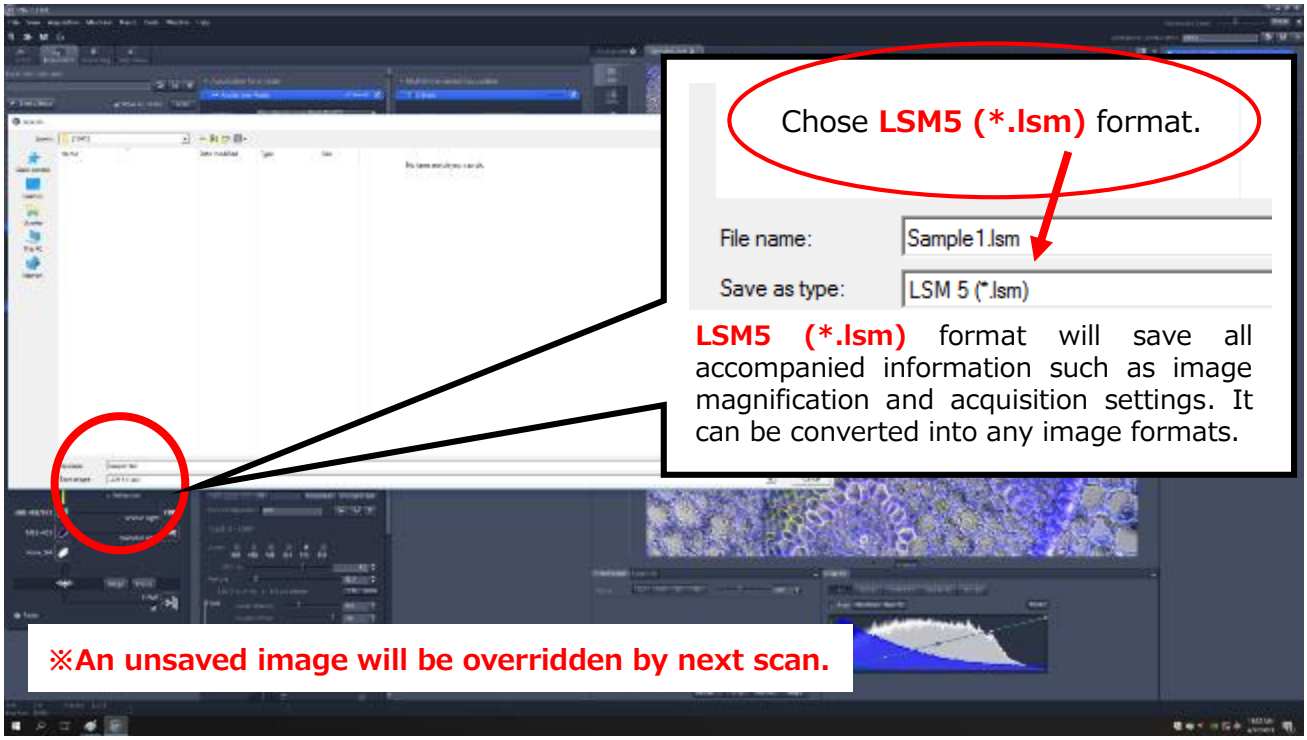
12. After adjusting all colors, check all tracks of **Channels** section.



13. Press **Snap** button to acquire the final image.



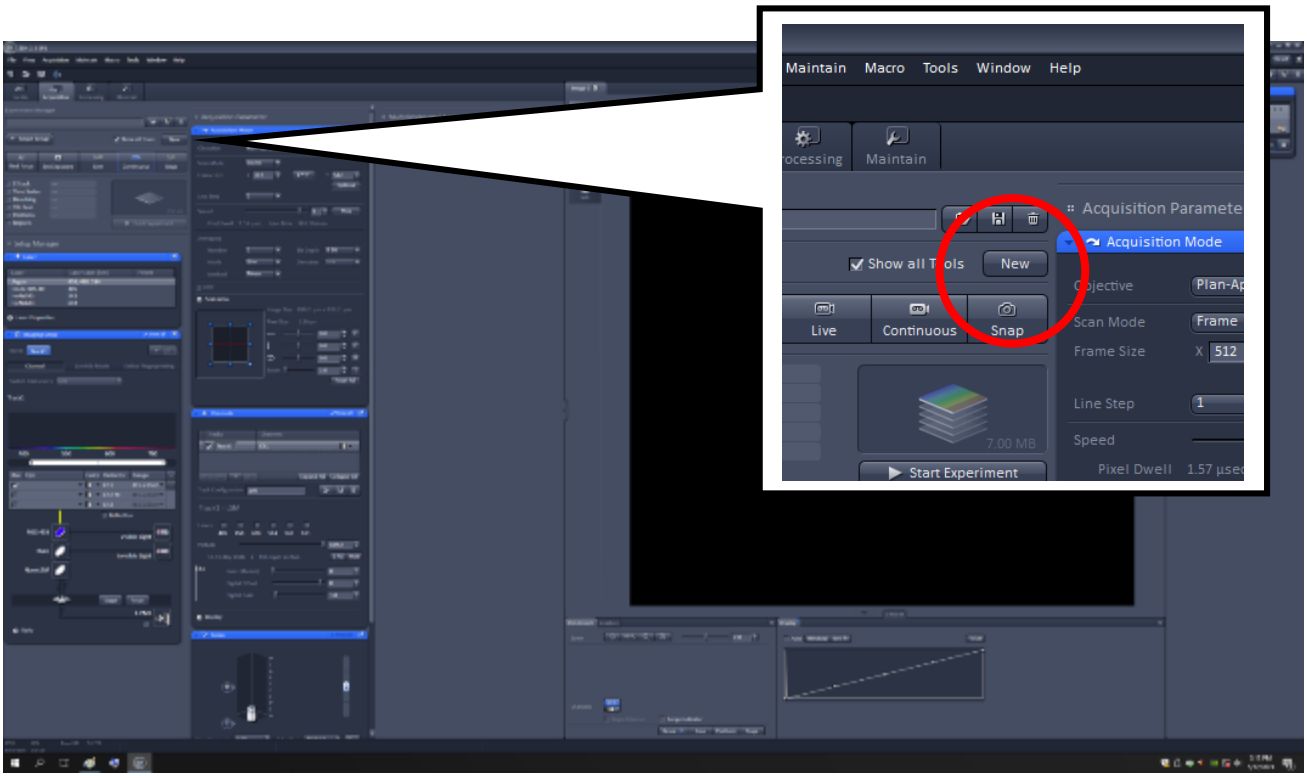
14. **File** → **Save As** → **Your lab's folder** → Save your file there.



- You can analyze your image by a free version of ZEN software. It can be downloaded from Carl-Zeiss web site (only for Windows).
- ImageJ is also compatible for LSM5 (.lsm) format. You can download ImageJ from NIH web site (http://imagej.nih.gov/ij/).

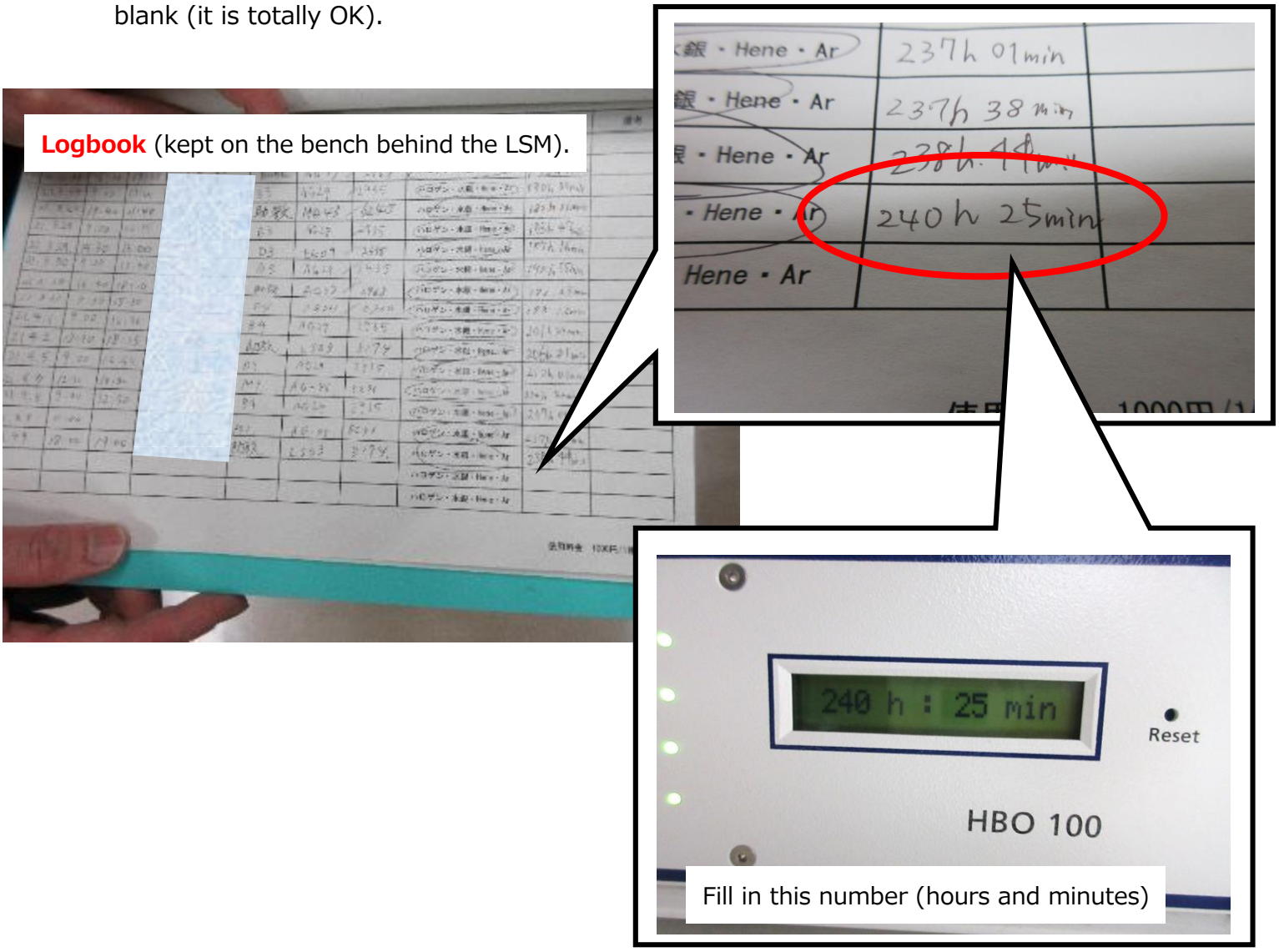
15. Click **New** to open another image window for next scan.

※ If you saved the previous image, pressing **Continuous** button will automatically open a new image window.



<Shut down the system>

1. Close ZEN window (or File→Exit)
2. Shut down the computer.
3. Check the indicator on the fluorescent lamp box [5] and fill the lamp hour in the logbook (see below). Turn off the lamp box.
   ※ If you forget to check the indicator before turning off the lamp box, please **avoid turning it on again**, or the lamp will be damaged. Just leave the part of the logbook blank (it is totally OK).



4. Turn the **power knob** (4-4) counter-clockwise to decrease the laser power (please do not overturn).
5. Turn of the laser (4-3)
6. Turn the **laser key** (4-2) to the off position (counter-clockwise to **vertical**).
7. **Wait** about **5 min** until the fan in the laser box stops.
8. Turn off the **laser box** (4-1)

9. Turn off the two **sub switches**. 3

10. **Power key** is always in the **horizontal position**. 2
(you don't have to turn the key).

11. Turn off the **main switch** 1

12. Cover the microscope by the **blue cover** (a slit will be back side).

13. Fill in the logbook.

※ Please wait at least **15 minutes** until turning on the laser and the fluorescent lamp again.

※ If there is a next user after you within 30 minutes, please leave the system on for the next user.