Needed Materials:

- Proper clothing (especially if using with cells)
    - Lab coat
    - Gloves
    - Laser protective goggles
- Samples
- Baseline sample (suspension liquid)
- Objective lens oil
- Objective lens wipes

Step-by-step Process:

1. Turn on the laser microscope normally following the "Laser Microscope Procedure".
2. **Ensure that the objective lens stage is in the lowest position.**
3. Screw the 100x objective lens into the lens stage.
4. Wrap the 100x objective lens with the objective lens warmer.
5. Use the oil dropper and add an oil drop to the 100x lens.
    - DO NOT touch the lens directly.
    - DO have a drop suspended from the oil dropper and have the drop touch the lens. Make sure no air bubbles.
6. Use an appropriate sample plate and load the sample into it.
7. Load the sample plate onto the stage.
8. Move the stage using the controller so that the objective lens is in the center of the glass.
    - The glass is the circular portion at the bottom of the petri dish.
    - The glass is approximately 4 mm in diameter.
9. Use the coarse objective focus knob to slowly bring the objective lens up to the bottom of the petri dish.
    - The goal is to have the oil disperse on the bottom of the plate without having the lens touch the dish.
    - **AGAIN do not leave the radius of the glass portion of the dish.**

10. Focus the white light so that proper contrast is achieved.
    - While doing this ensure that the white light is toggled on in the computer software.
    - To do this on the left toolbar under illumination, click "White light".
    - Then in the same section on the computer enable the illumination.
11. Put on the laser protective goggles.
12. Take pictures of sample with white light, with laser, and a cube image.
    - Ensure the pictures are in focus.
    - Change exposure time for laser depending on the sample.
    - When taking pictures with the laser, ensure the white light is turned off.
    - You can use sequences found in **Aidan 04 > Sequences** to make the process quicker.
13. Save pictures to a proper folder on Google Drive.
14. Remove sample plate from stage.
15. Move the objective lens stage to its lowest position.
16. Once done taking pictures with a single sample wipe the objective lens of oil using the wiping paper.
    - **If you are going to take pictures of another sample restart this procedure from step 5.**
    - If you are done with the microscope turn off the microscope by following the "Laser Microscope Procedure".
17. Remove samples and return them to the actual incubator if cells as soon as possible.
18. Make sure to take pictures of blank petri dish samples for proper data.
    - **Just like taking pictures of another sample you must restart the procedure from step 5 to take pictures of the blank sample.**
    - For this fill a petri dish with the suspension liquid of the samples to the same volume.
    - Find a surface bubble and go just below it for blank imaging.
    - Take a blank image with BB ON and OFF.
    - Take a white light image with the white light ON and OFF.

IF USING STAGE INCUBATOR:
(ie if imaging live cells)

on OKOlab box - adjust CO2 levels to 0.03 and air levels to 0.6

make sure to seal off all open holes in bottom plate of incubator

For Blanks:

need Z stacks of Cubes (laser ON) and BB (laser ON)) and one dark cube and dark BB

run Matlab code : “SearchCubeBackground” for background subtraction