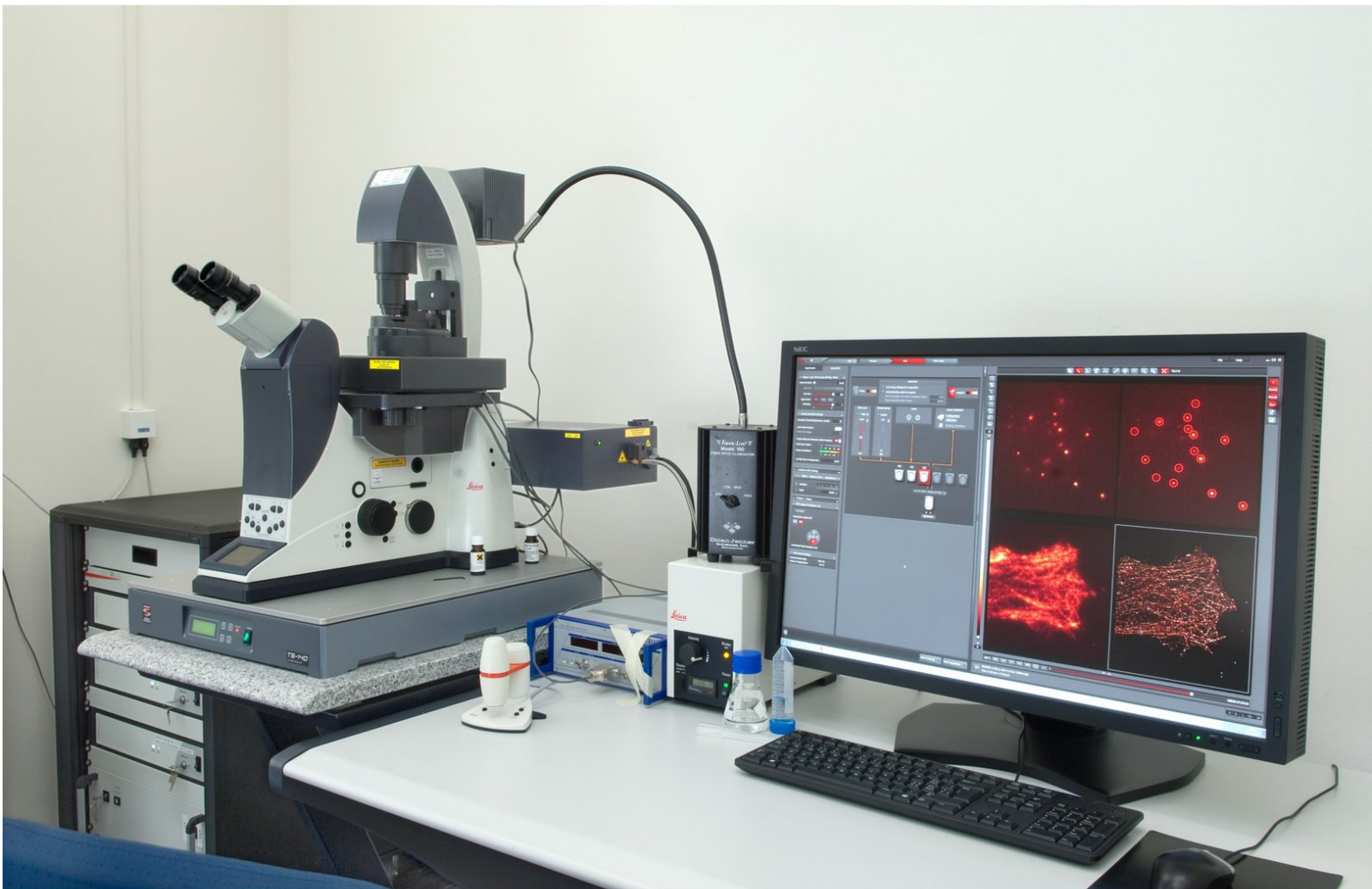


# Leica SR GSD 3D TIRF (Irchel)

How to start up and mount a sample on the Leica SR GSD 3D TIRF microscope.

Written By: Jana Döhner

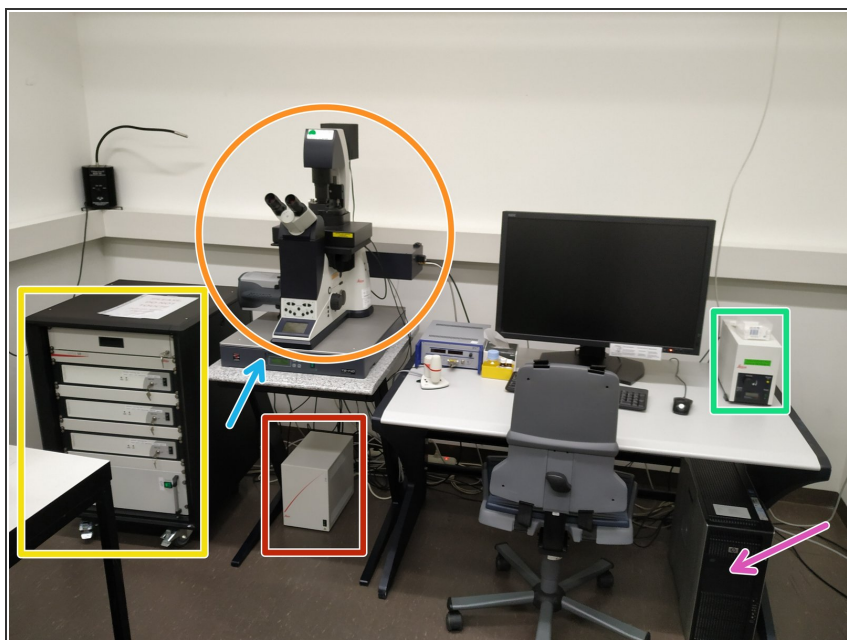


## INTRODUCTION

In this guide of the Center for Microscopy and Image Analysis we show how to start up and mount a sample on the Leica SR GSD 3D TIRF microscope.

Please find more information about the system [here](#).

## Step 1 — Microscope hardware



- Laser Rack
- Microscope body ("DMI6000")
- Microscope control box ("CTR box")
- Fluorescence lamp
- Isolation Table
- Computer

## Step 2 — Switch ON microscope



**i** The control box is always kept ON to maintain steady temperature of the microscope components.

- **Switch OFF** the **CTR box**, as lasers must be turned ON **BEFORE** the CTR box.
- At the **laser rack**:
  - **Turn ON** the main power switch.
  - Turn the laser key for the **405 nm** laser to "**I**" in order to enable "LASER Emission".
  - **Switch ON** all other **lasers**. Do NOT turn the keys yet.
  - The LEDs ("SHG Ready") light shortly up in green and turn then orange.
  - Once **continuously** green, **turn laser keys** to "LASER ON".

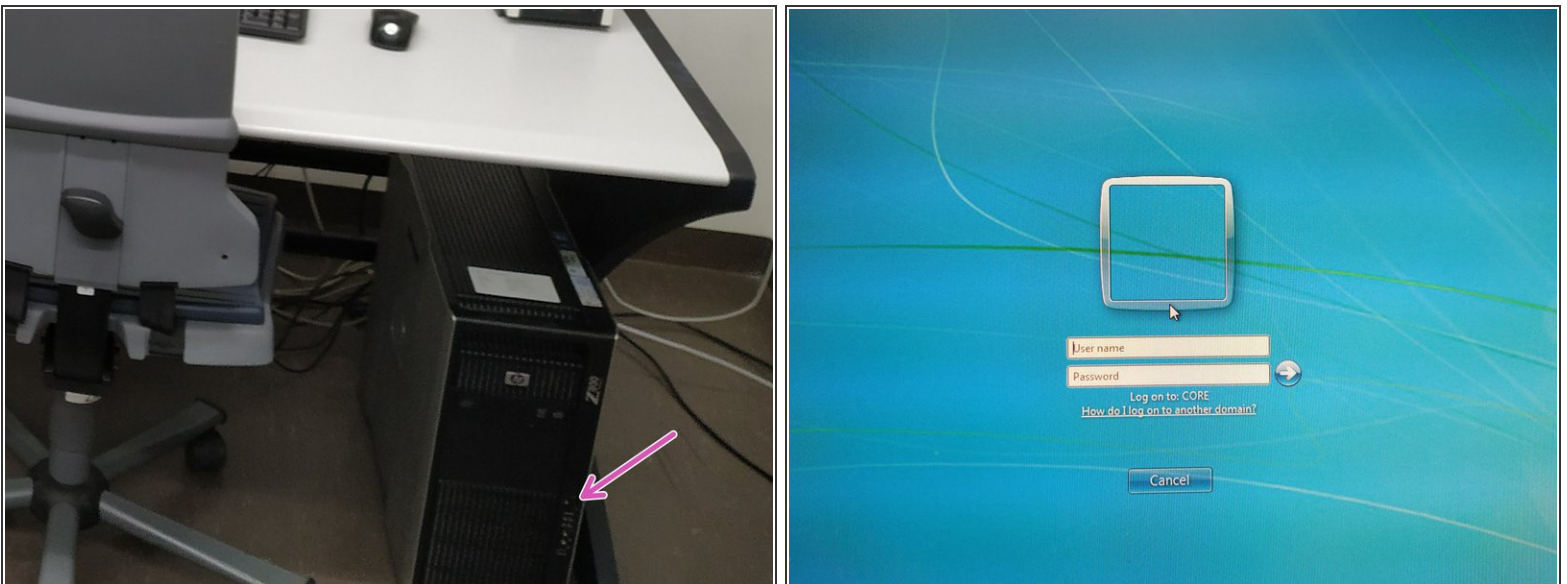


### Step 3 — CTR box, EL6000 and isolation table



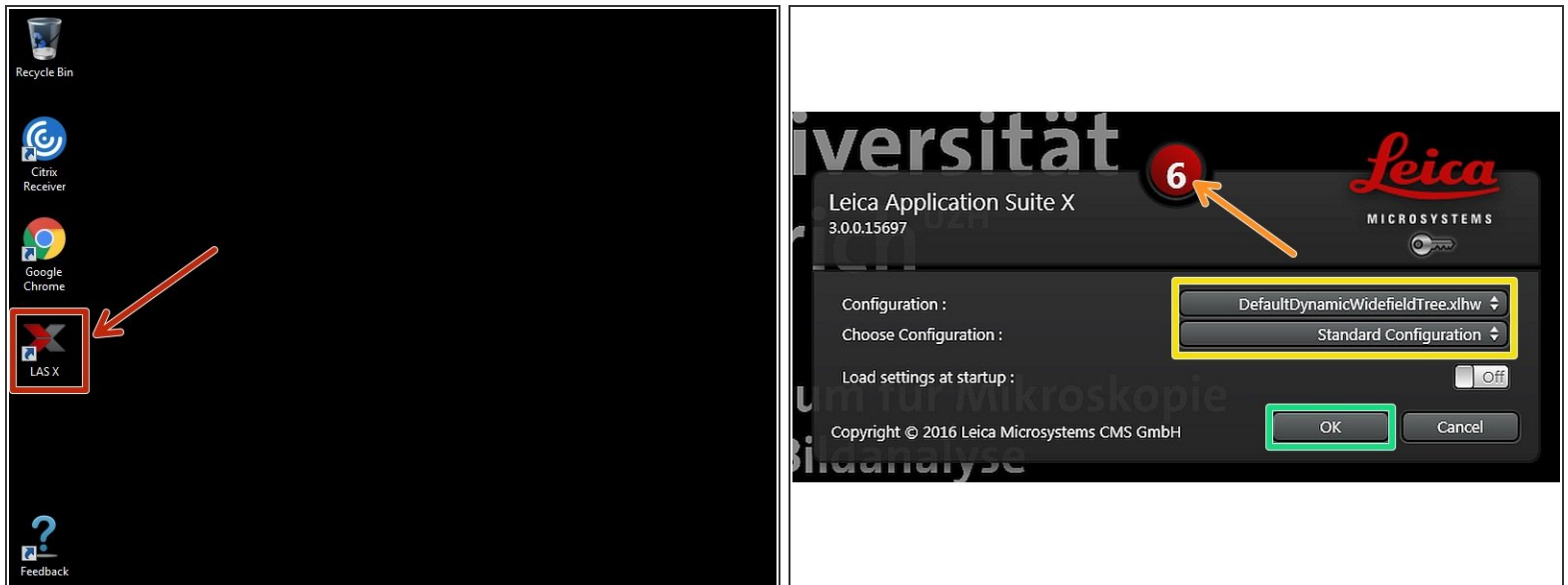
- Switch ON the **control box**.
- Switch ON the **fluorescence lamp**.  
 ⚠ *Once turned on, the lamp should stay on for at least 30 min.*
- Switch ON the **isolation table**.
  - Press "E" in order to **enable isolation** (indicated by the red LED "ISOL. ON" and display).

### Step 4 — Turn on computer and sign-in



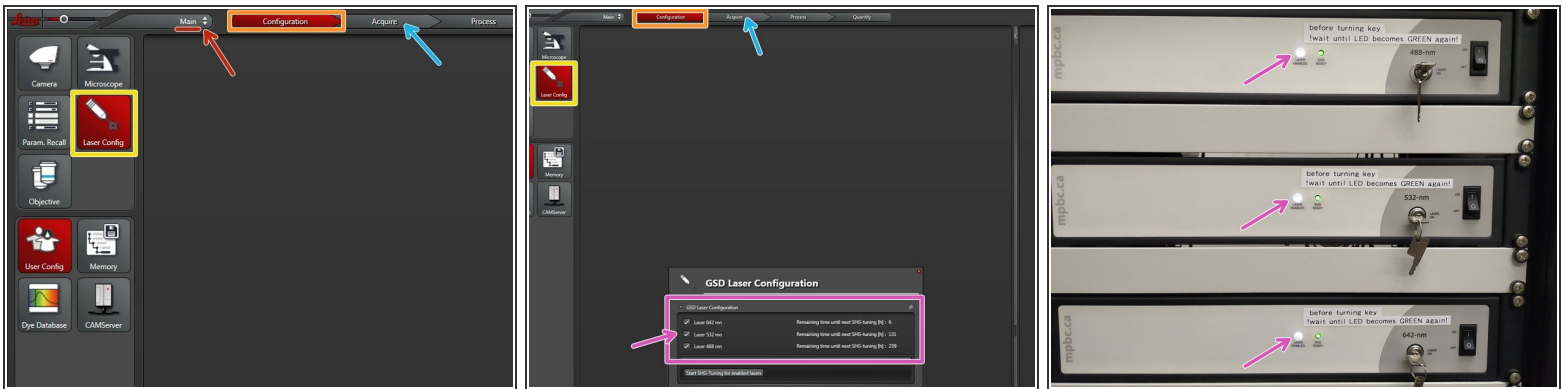
- Turn ON the computer.
- Sign-in with your **ZMB core** credentials.

## Step 5 — Start the "LAS X" software



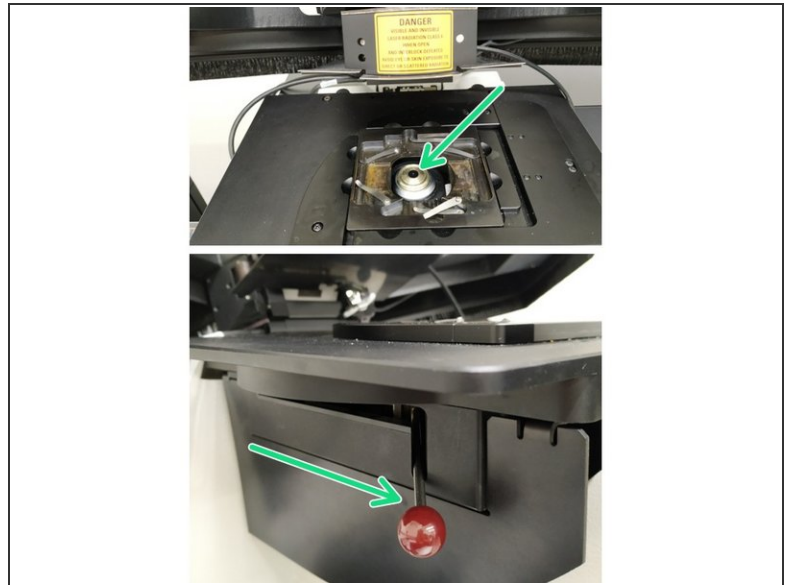
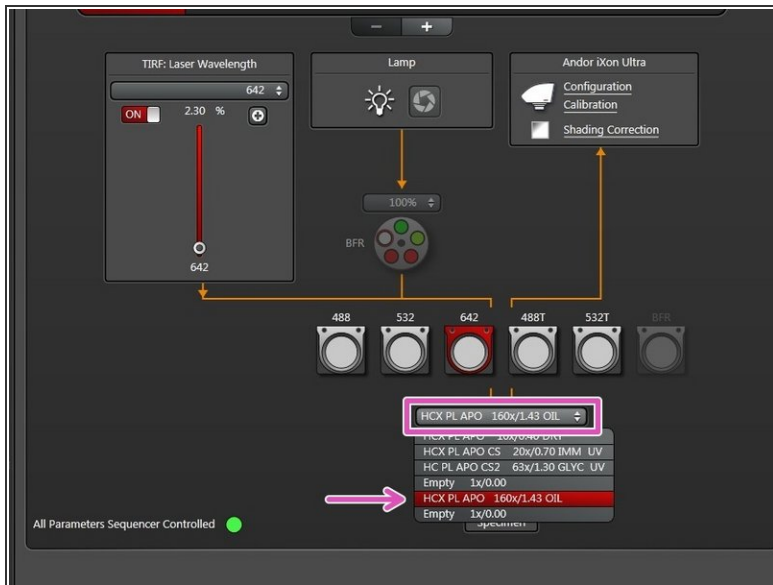
- Start the "**LAS X**" software via the desktop icon.
- Stop the countdown by clicking on it.
- Make sure "**DefaultDynamicWidefieldTree.xlhw**" and "**Standard Configuration**" is selected.
- Click "**OK**".

## Step 6 — Switch ON the lasers



- Make sure you are in the **"Main" module**.
- Go to **"Configuration"**.
- Select **"Laser Config"**.
- **Switch "ON"** the lasers you need.
  - The "LASER ENABLED" light should turn white.
- Go back to **"Acquire"**.

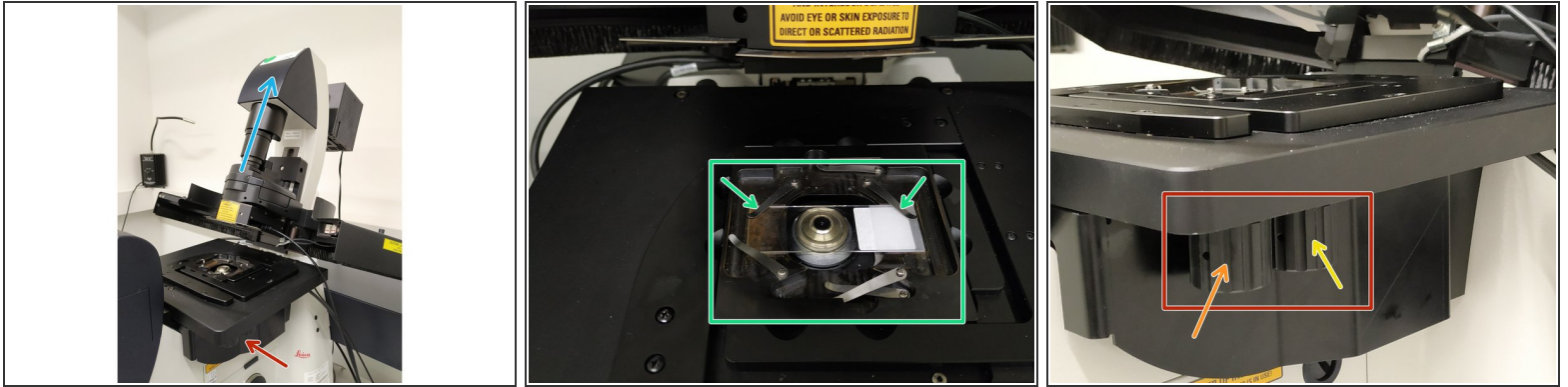
## Step 7 — 160x / 1.43NA oil objective



- Select the **160x/1.43** objective in the "LAS X" software.
- ❗ The **160x/1.43** objective is the only suitable objective for **GSD imaging**.
- Double-check if the objective is correctly inserted (clicked in).
- ❗ *The GSD objective is directly connected to the manual stage (SuMo-Stage) in order to reduce drift.*

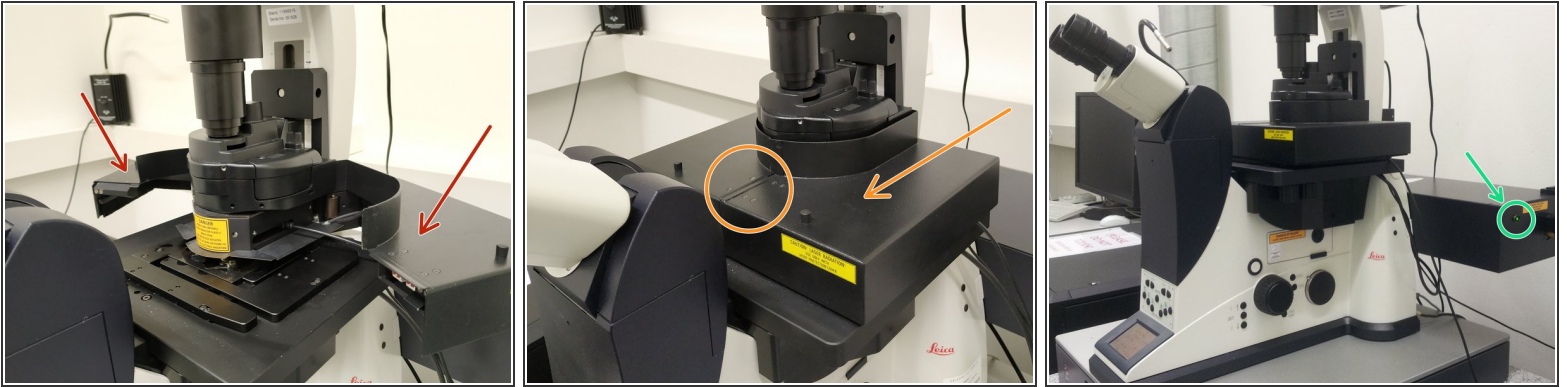


## Step 8 — Mount a sample



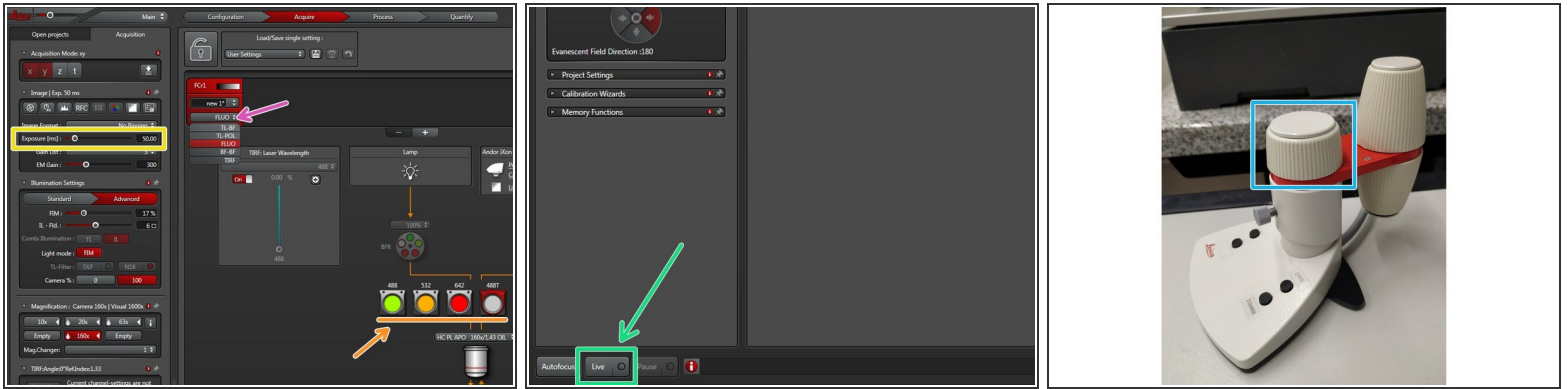
- **Push** the condenser arm to the **back**.
- Apply **"Type F" immersion** to your sample or onto the objective (*do not touch the lens with the applicator*).
- Insert a sample with the **coverslip facing down** and fix it with the two springs.
- **Move** your sample over the objective by using the manual stage knobs.
  - Movement in y-direction: *clockwise moves the stage towards the back*.
  - Movement in x-direction: *clockwise moves the stage to the right*.
- Pull back the condenser arm.

## Step 9 — Laser protection cover



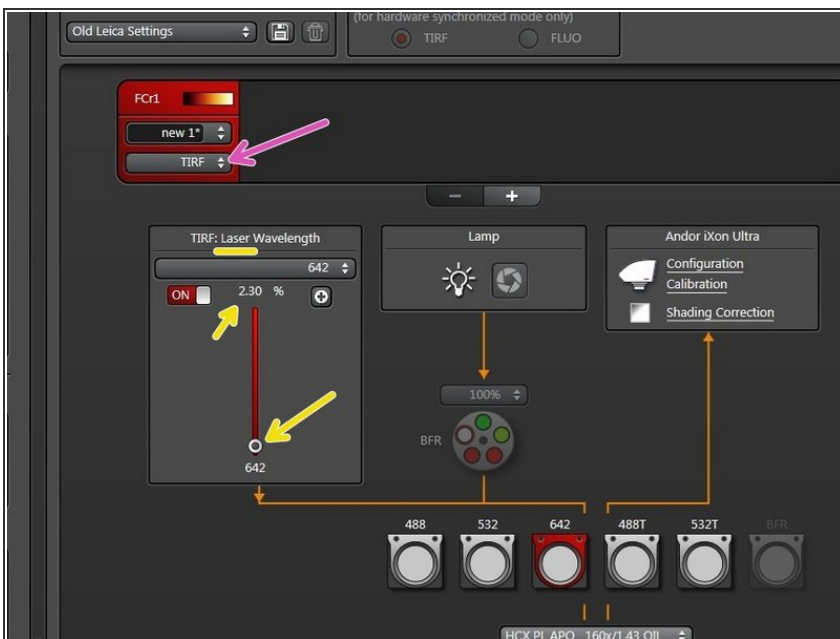
- i** An interlocked "**Laser Protection Cover**" is attached to the condenser arm to protect from the strong laser illumination needed for imaging.
- This cover has to be **opened** for accessing the sample.
  - For engaging the lasers during imaging the **cover** has to be **closed**.
  - **Proper closure** is confirmed by a **green LED** on the back of the microscope.

## Step 10 — Focus your sample in epi-fluorescence mode



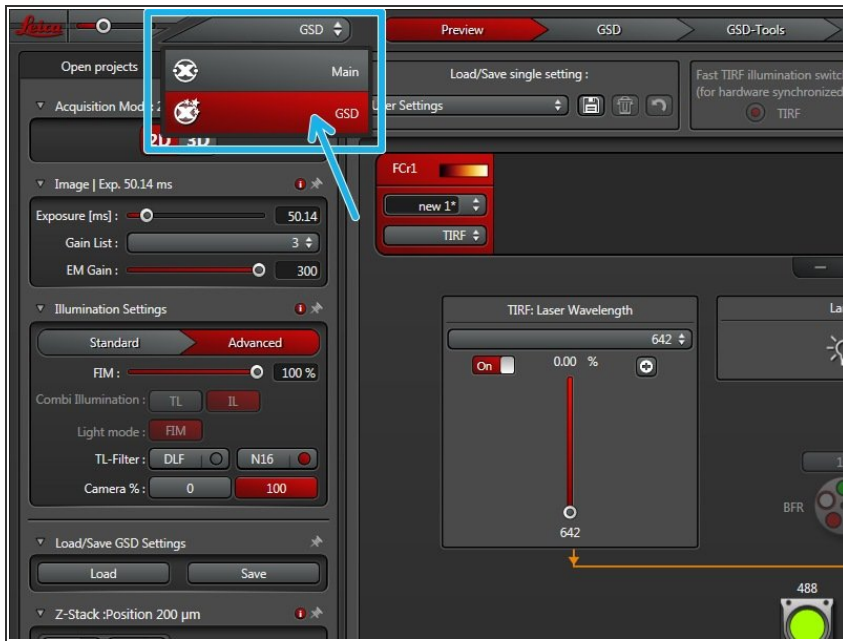
- In the "**LAS X**" software:
  - Choose **FLUO** to enable widefield illumination.
  - Choose an **appropriate filter cube** for your fluorophore.
  - Set the camera **exposure time**.
  - Click "**Live**".
  - Use the external controller to focus your sample (*max. travel range 400 um*).

## Step 11 — Focus your sample - continued



- Switch to "**TIRF**" mode to enable laser illumination.
- Click "**Live**".
- Adapt **laser power** while being in "Live" mode.

## Step 12 — Starting the "GSD" wizard



- After finding the focus and proper laser and exposure settings switch to the **"GSD" wizard**.
- GSD operation will be explained in another ZMB guide.