Instructions on how to build an Incubascope

In this document, we present detailed instructions to build your own version of the Incubascope. These instructions are illustrated by images of the 3D model made with Freecad software. The model is available on our github page.

Step 1 : First, attach the CMOS camera on an optical post and insert it inside a post holder. Install the kinematic mirror mount in the top right corner of the optical breadboard (see Fig. 1). Adjust the height of the elements at 8 cm (for the center). Place the post of the camera 15 cm away of the kinematic mirror mount. Then place the 2 inch lenses on a post holder at roughly 5 cm from the kinematic mirror. Observe a scene far away (ideally at infinity) and adjust the position of the lens in order to obtain a sharp image on the camera using the manufacturer software. Usually, we observe a scene farther than 100 m through a window of the office or the lab.

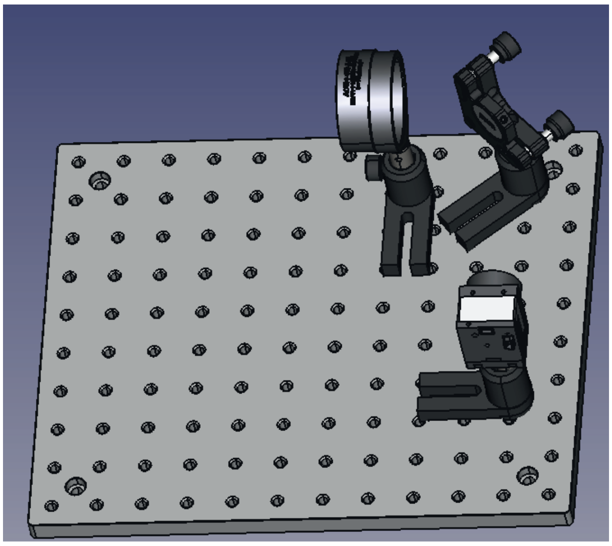

FIGURE 1 – Setting up the detection.

Step 2 : Install the microscope objective on a post holder using an optical post and a lens mount. Place this component 20 cm away from the 2 inch lens. Check carefully the height of the objective. Then install the 45° mirror mount in front of the objective. As the objective has a 56.3 mm working distance, we recommend to place the center of the mirror at half of this distance. The same distance will separate the sample from the mirror which will be practical to easily install the sample mount and the sample itself.

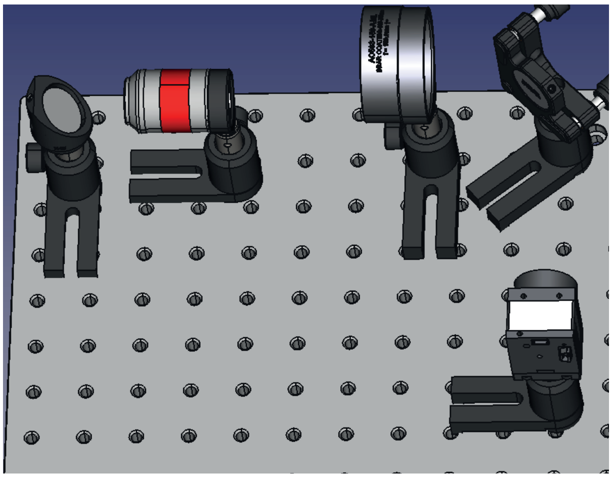

FIGURE 2 – Setting up the microscope objective.

Step 3 : Install the red LED for the transmission mode with the LED mount and an optical post on a tall post holder. Place it in the opposite corner of the kinematic mirror mount (top right on figure 3). The center of the LED should be 18 cm high in relation to the breadboard. Install a small focal length lens on a lens mount compatible with cage systems. Place it also on an optical post and a tall post holder. Adjust the distance between the lens and the LED in order to have a collimated beam after the lens. This will provide the widefield illumination.

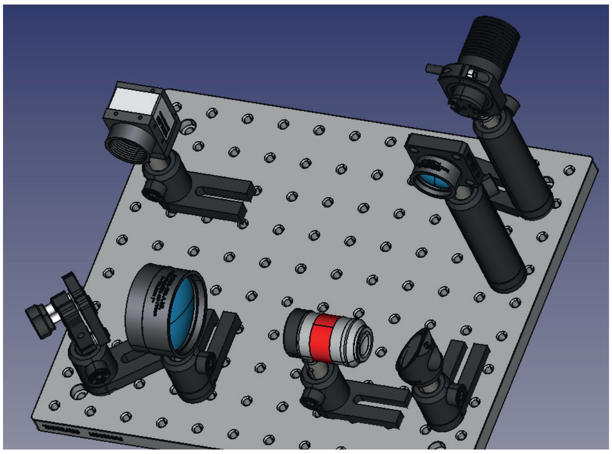

FIGURE 3 – Setting up the illumination, part 1.

Step 4 : In order to redirect the collimated beam to the mirror and microscope objective, place two or four rods in the lens mount with the right angle kinematic mount attached on them. Adjust the distance to redirect the maximum of the illumination light to the microscope objective. On the CMOS, the beam should cover most of the sensor area and a bright and almost homogeneous image should be obtained.

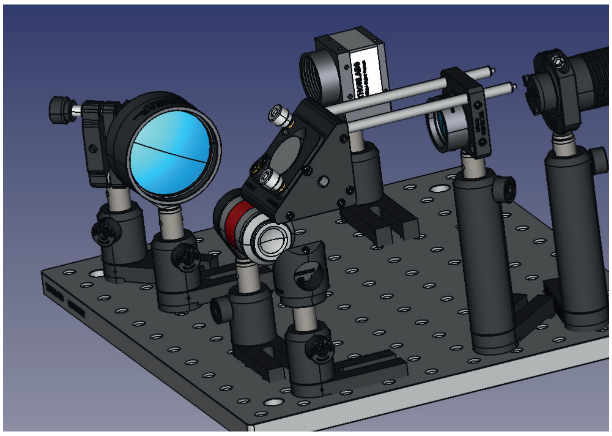

FIGURE 4 – Setting up the illumination, part 2.

Step 5 : Install the sample holder in between the right angle kinematic mount and the 45°mirror mount. In our case we used a simple lens mount for cage system attached to an optical post and a translation stage to adjust the focus (not shown on figure 5). A sharp bright field image of the sample can be obtained on the CMOS when the focus is correctly adjusted.

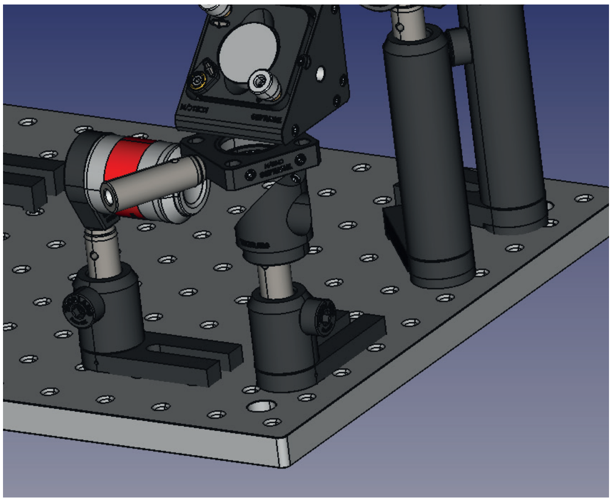

FIGURE 5 – Setting up the sample mount.

Step 6 : We now aim to setup the second LED for the epifluorescence mode. In between the microscope objective and the tube lens but 10 cm away (see Fig. 6) place the blue LED with its mount, optical post and post holder. Then, place the small focal lentght lens few centimeters away from the LED. Here, we do not want a collimated beam, we will adjust the position of the lens later.

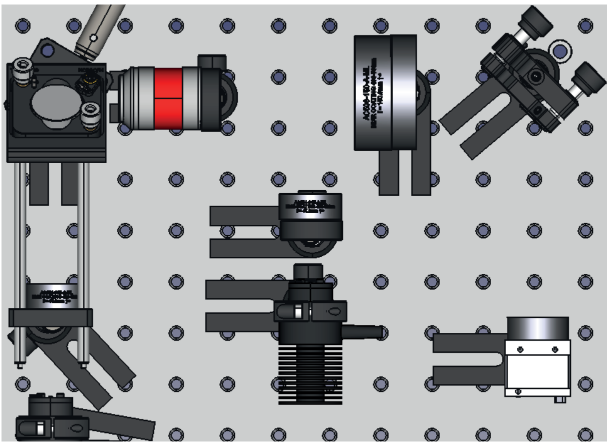

Figure 6 – Setting up the fluorescence illumination, part 1.

Step 7 : Then, we need to place the dichroic mirror. Put it on its mount on top of an optical post and post holder, then place it at 45° in between the microscope objective and the tube lens. Visually, you should see the blue light in the focal plane of the microscope objective with the naked eye. Ideally, an homogeneous fluorescent sample should be placed in the sample plane. Change the orientation of the dichroic in order to illuminate the center of the field of view imaged with the CMOS. You can now add the emission filter in front of the CMOS to collect only the fluorescent light. You can now change the position of the lens in front of the blue LED such as the illuminated area corresponds only on the imaged FOV.

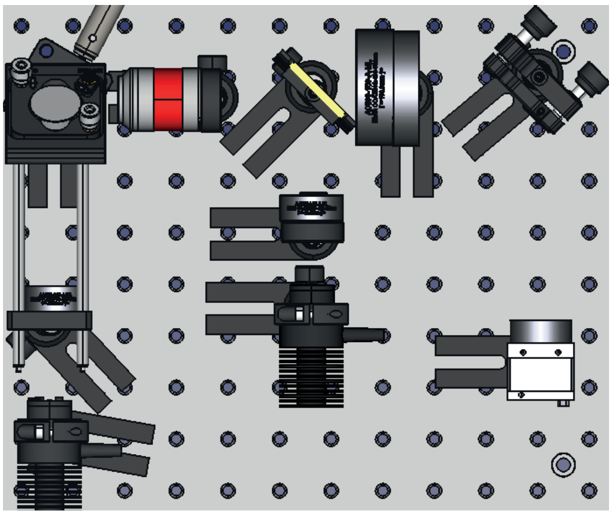

Figure 7 – Setting up the fluorescence illumination, part 2.

Step 8 : Connect the Arduino to the computer using an USB cable and install the Arduino software. To allow communications between Python commands on the computer and the Arduino we use the pyfirmata library. First, install it on your computer using the command *pip install pyfirmata* in the python terminal. Then, open the Arduino software, go to file, examples, firmata and select StandardFirmata.

You can now connect the Arduino to the LED controllers following figure 8. Each controller is connected to the Arduino using a test BNC cable with a BNC port on the controller side and two cables on the Arduino side. These two cables are plugged onto a ground output and an digital output to digitally control the output of the LEDs inside the incubator. Connect the controller 1 (epifluorescence mode) to the digital output 3, and the controller 2 (brightfield mode) to the digital output 6.

Note that we used the Arduino Uno which only has digital outputs. It means that the LED illumination power can be modulated only digitally which can cause the appearance of dark and bright stripes on the image if the exposure time of the camera is of few milliseconds only. This can be avoided by using Arduino with true analog outputs, for instance the Arduino MKR.

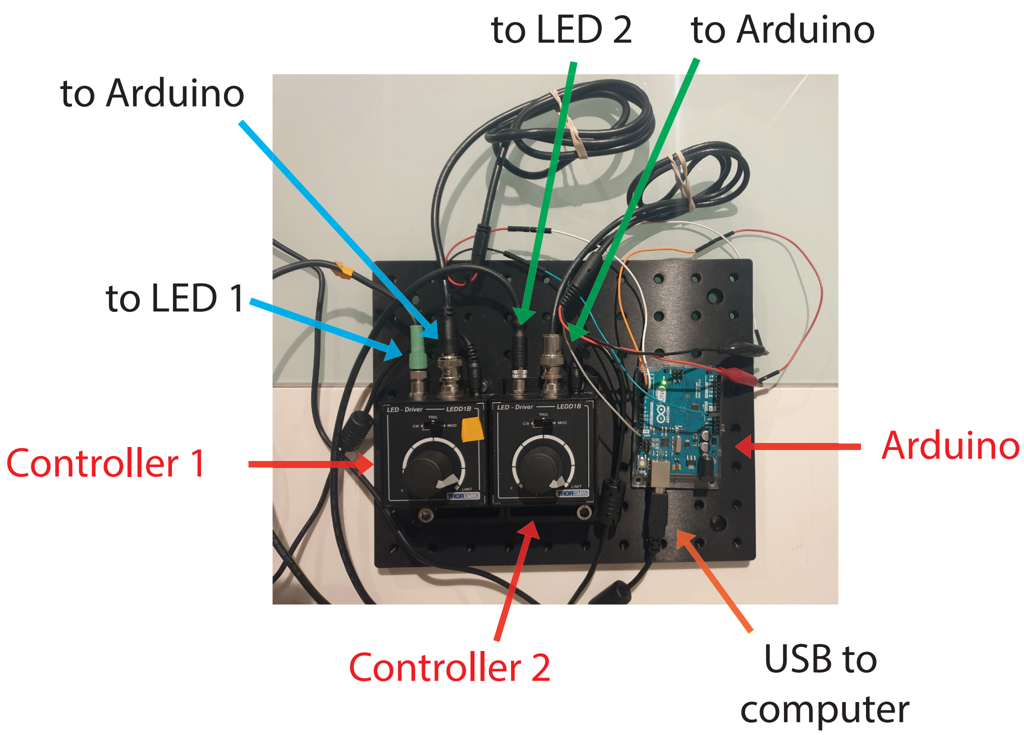

FIGURE 8 – Arduino and controllers cables.