

UC2 workshop

Instructions sheet

Workshop content:

2-4 hours (probably 2 hours), 6-8 participants, three times in the week

1. Short introduction (10 min)
2. The participants can play with the lenses, e.g. try the telescopes (10 min)
3. Build the incubator microscope (with the sketch and all parts are labelled), acquire an image of a test probe: 20 min > experiment 1 (E1)
4. Smartphone microscope: 20 min > experiment 2 (E2)
5. Light sheet microscope: 20 min > experiment 3 (E3)
6. Participants can image their own samples with any of these 3 setups: left time

Notes instructions:

Introductory notes: there are 3 electronic components that are controlled via the Raspberry Pi (RasPi): the LED array, the focusing stage ("Z") and the sample stage ("X"). They each have their own micro-controller (ESP32) and the command codes should be already uploaded. They are controlled wirelessly by the RasPi but get their power supply through USB (typically, a HUB plugged to the RasPi). Each box contains its own set of components, they are not interchangeable. Therefore, before starting, check that the ID of each component is matching to that of the box. This is also true for the keyboard.

Procedure to set things up before the workshop starts:

- 1) Plug the router, wait a few minutes
- 2) Plug the camera in the RasPi BEFORE turning ON
NB: in case a hard reboot of the RasPi is needed, just disconnect the power and plug it in again (no button)
- 3) Start the RasPi by connecting it to the power socket (password: youseetoo). Insert the dongle of the keyboard
- 4) The RasPi should connect automatically to the WIFI router, check by clicking on the icon (the name of the WIFI is typically UC2_wifi_some number (password: _lachmannUC2)
- 5) Open the command window and type python main.py
- 6) The GUI opens after some time
NB: On the RasPi, one can use either the mouse (integrated in the keyboard) or the display as a touchscreen
- 7) Check that the camera is working by clicking on "preview"
- 8) Connect the USB HUB and then power each ESP32 by connecting it to the HUB via USB.
NB: there are typically only 2 micro-USB cables per box because no experiment requires more than 2 of the components at the same time
- 9) Connect the LED array. It should boot and show the beginning of UC (the C is cut)
- 10) In the GUI, choose LIGHT, and try the different functions. With CUS (custom), you can choose which LED to turn ON. Check that this is working
- 11) Connect the Z-stage. Some blue LED should blink on the ESP32
- 12) In the GUI, choose MOTOR and Z. You can choose the step size in um (16 um minimum, 160 maximum) and then push on the double arrows in one direction or the other. When doing

so, some red LED should turn ON on the side of the cube of to the stepper motor (this shows that the command was transferred) + if you observe the screw you should see it moving.

The Z-stage is mostly used for focusing (E1 & E2)

13) To go out of the Z mode, click again on Z

14) Connect the X-stage. Depending on the version, it may be a red LED labelled "ON" which turns on once powered. The principle for moving this motor is the same as for step 12, only under "X".

NB: the distance in X is not calibrated (but the relations are correct, i.e. a step size "32" will be twice more than a step size "16"! The distance in Z is approximately calibrated correctly.

The X-stage is mostly used for scanning the sample in the light sheet (E3)

There is no absolute zero for the motors, so they will not remember their position before and after boot.

NB: don't leave the motors ON unnecessarily long (e.g. coffee break) because they get warm

15) GUI > start experiment enables you to acquire a time series (XXX more information missing),

16) GUI>start experiment> custom turns the previously defined custom LED pattern on. Click then on SNAP to acquire a single image frame (saved automatically)

NB: it is necessary to turn on the LED array for acquiring an image, even for the light sheet which actually uses the laser

17) **Test building the incubator microscope (E1).**

The sketch can be found in [UC2-](#)

[GIT/CAD/APP_Incubator_Microscope/IMAGES/UC2_fullBOX_incubator_EN.pdf](#)

18) Good samples for the incubator microscope: pretty much all of the Betzold boxes. The fly head is a good sample for showing what happens when you replace the 10x objective with a 4x objective (bigger FOV)

19) **Test building the smartphone microscope (E2)**

20) XXX (here comes more about the smartphone microscope)

NB: there are not as many phones as boxes, so some participants will have to use their own phone. The phone will only be used for its camera and not as a controller through the app

21) **Put up the light sheet experiment (E3):** insert the laser pointer in its cube, turn it on using the clamp, check alignment/align with hex key. Then comes the cube with the beam expander and collimator: check that the beam is approximately collimated. This consists of a smartphone lens and a 25 mm focal length lens. The smartphone lens can be slightly moved back and forth for collimation. Then comes the cylindrical lens which typically is at the very end of its cube. The objective lens is taken out of the 4x objective (which contains actually only 1 lens). The detection unit (two cubes objective + motor) has to be slightly modified for E3 with respect to E1 and E2: carefully remove the objective and put it back in the other way around

XXX Sketches and/or pictures of the optimal position of the objective in its holder will be inserted here!

NB: if one turns the cylindrical lens of 90°, it is possible to observe the light sheet in the FOV of the camera and for example measure its thickness

NB: the light sheet experiment is more sensitive to stray light than others

22) Sketch for the light sheet can be found under [UC2-](#)

[GIT/CAD/APP_LIGHTSHEET_Workshop/IMAGES/UC2_fullBOX_lightsheet_EN.pdf](#)

23) Alignment of the light sheet setup:

- The laser has a line which should be consistent to the direction of the light sheet i.e. vertical
- Check that the laser spot is collimated (its size doesn't change): if needed, move the smartphone lens
- Check that the last spot is homogeneous: use the 4 screws that hold the laser in place. They should be in contact with the plastic mount, not directly on the laser pointer
- The cylindrical lens typically should be as far as possible in its cube. The flat side is towards the mirror, and as close as possible to the mirror
- The 4x objective (illumination objective) typically should be as far as possible in its cube. For its orientation, imagine the mounting (which was removed) of the objective: the threading is towards the mirror, the front lens towards the sample
- Adjust the kinematic mirror so that light goes through the illu. objective nicely
- Typically it may be at this point that the produced light sheet is too low and everything has to be raised up (the laser pointer, the collimator, and the illu objective can be slightly lifted up)
- Releasing the screws on the cube of the illu objective of 2 turns also will lift the objective slightly up
- Now we must align the detection part. For this we use the alignment sample made of lens cleaning tissue with a marker pen inside of the special holder
- put the flash light behind the sample with the camera ON
- Move the sample by hand until you focus on some fibres. Try to bring the focus in the middle of the field of view
- Make sure the sample is approximately at the centre of its cube (on the x-stage)

Important: the laser pointer is strong! If it is turned on while there is no sample, this is a real laser hazard!

24) For looking at acquired images: close the GUI, click on the file manager icon, go to UC2/RASPlapp-py3/data where there will be a new folder which name is a date (this date might be wrong, just choose the newest date). Inside of this folder, there is a first empty sub-folder. All saved images are in the next one (in .jpg format and with an automatic name).

Important: if the GUI crashes, after restart the new images will be named with the same convention starting from 1 again and therefore will overwrite images saved before the crash. Therefore, in case of a crash, rename or move your images!

25) Shut down procedure: Raspberry symbol > shut down

For a box where the phone is the master (e.g. Aurélie's box):

Follow the instructions 1-5.

6) in the terminal, write `raspistill -k` ENTER: this opens the screen

7) turn on the phone. Activate the hotspot (UC2_wifi01)

8) open the UC2 controller app and press GO: it should connect

9) Connect the LED and the motors to the USB hub: all should be recognized (the camera gets connected as step 1)

10) `raspistill -o ImageName` saves an image under Desktop/ImageName.jpg

11) `raspivid -o` saves a video under Desktop/VideoName.h264

NB: It is possible to change the format to mp4, see Eda's email