zIncubascope: long-term quantitative imaging of multi-cellular assemblies inside an incubator

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1 Parts list

We provide in the table below, a detailed list of the parts required to assemble the zIncubascope.

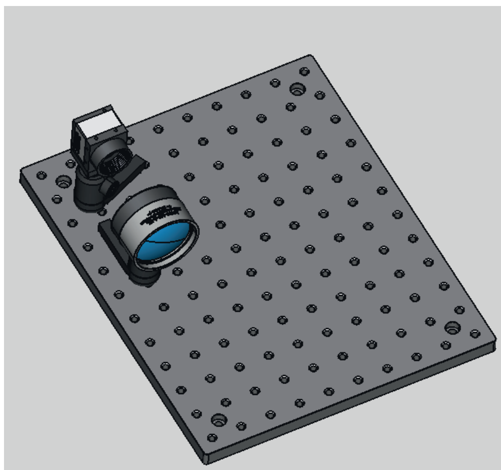
Component	Product code	Supplier	Quantity	Cost per unit (€)	
USB monochrome camera	acA5472-17um	Basler	1	550	
Arduino / DAQ board	Arduino Uno	Arduino	1	20	
625 nm mounted LED	M625L4	Thorlabs	1	200	
LED drivers	LEDD1B	Thorlabs	1	294	
Microscope objective	Olympus RMS10X	Thorlabs	1	380	
Tube lens	AC508-150-A-ML	Thorlabs	1	135	
Collimation lens	AC254-030-A-ML	Thorlabs	1	103	
Mirror	PF10-03-G01	Thorlabs	2	50	
Breadboard	MB2530/M	Thorlabs	1	130	
XYZ Translation stage	LX30/M	Thorlabs	1	1500	
Right-angle mirror mount	KCB1C/M	Thorlabs	2	140	
Various optomechanical components		Thorlabs	1	300	
				Total	**4000**

Table 1. Detailed list of the components of the *zIncubascope* and their prices.

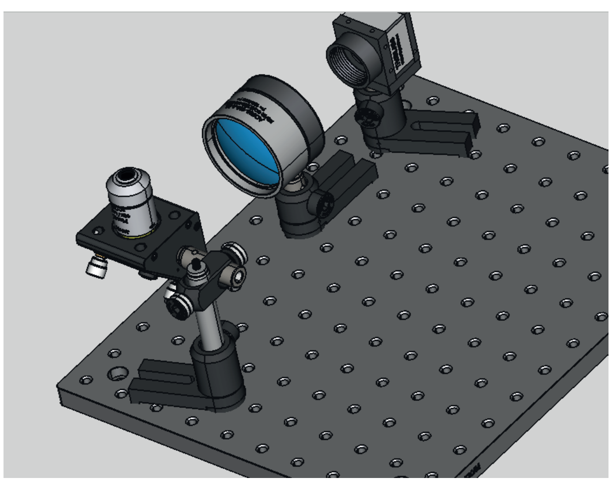
2 Instructions on how to build a zIncubascope

In this section, we present detailed instructions to build your own version of the zIncubascope. These instructions are illustrated by images of the 3D model made with Freecad sofwtare.

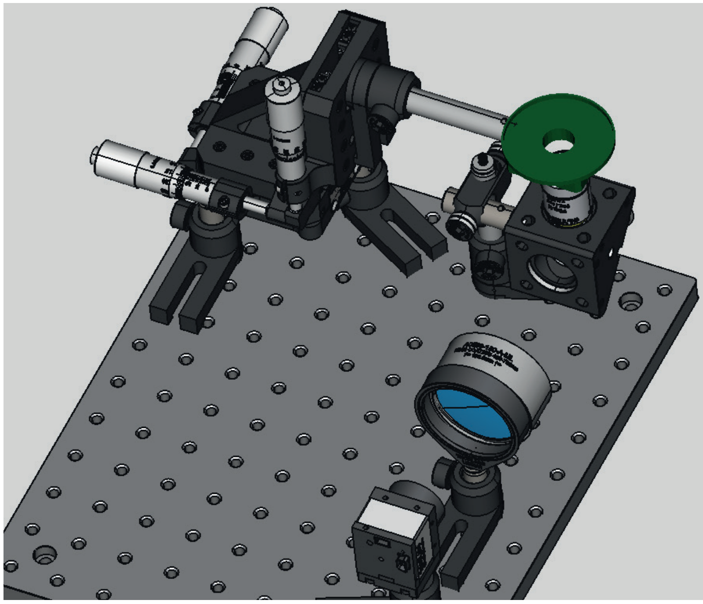
Step 1 : First attach the CMOS camera on an optical post and insert it inside a post holder. The bottom of the camera is placed at a height of around 5 cm. The CMOS is placed in a corner of the breadboard, 3cm away from the sides. Place the tube lens on a 2 inches mount attached onto a small optical post. Insert the post inside a post holder placed at roughly 15 cm from the camera sensor. Observe a scene far away (ideally from a window) and adjust the position of the lens in order to obtain a sharp image on the camera using the manufacturer software.



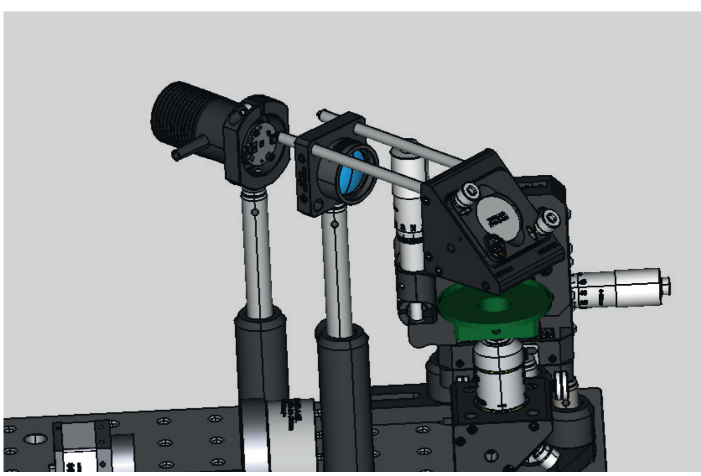
Step 2 : Install the microscope objective directly to the right-angle mirror mount using an adapter (SM1A3, Thorlabs). With two posts and two right angle clamps (RA90/M, Thorlabs), install the objective in the vertical position, roughly 12 cm away to the tube lens (15cm including the reflexion on the mirror). Make sure the height of the right-angle mount is similar to the one of the camera and tube lens.



Step 3 : Now, attach two small posts (TR30/M, Thorlabs for instance) under the XYZ translation stage. Inster these two posts inside small post holders (UPH30/M, Thorlabs for instance). Screw the post in the corner to the opposite of the camera. Then, screw another post holder with a post and the 3D printed sample holder attached on it. Adjust the height in order to have a distance between the sample and the objective in the order of the working distance of the objective.



Step 4 : Attach the LED to the LED holder (SM1RC, Thorlabs). Screw it on a large post (TR75/M, Thorlabs) and insert it inside a post holder (UPH75/M). Do the same with the collimation lens and lens holder (CP33/M, Thorlabs) with the same post and post holder. Use two rods (ER3, Thorlabs) to attach the right-angle mirror mount to the lens mount. Place this assembly on top of the sample holder and screw it to the breadboard. Then, place the LED at one focal distance of the collimation lens in order to obtain a collimated beam in the illumination arm. Then, attach the LED post holder to the breadboard.



Step 5 : Now,you can perform smaller adjustement, both in the illumination arm and the detection part. In the illumination, you can use the knobs of the right-angle mirror to finely adjust the illumination angle and position. Do the same in the detection part in order to have an homogeneous image on the camera sensor.

3 Instructions on how to install and use the code to control the zIncubascope

In this section, we present detailed instructions to install and run the code we developped in Python that allow to easily control the parameters of the zIncubascope such as the exposure time, axial position, illumination power, time between frames, *etc.*

Step 1: Install Python on your computer. In our case, we used Miniconda on a Windows 10 computer for this project.

Step 2 : From our Github page, download the python code (.py) or jupyter notebook file (.ipynb), the logo picture (logo1.jpg) and the requirements text file entitled **requirements.txt**.

Step 3 : From the Python console, install the required libraries using the following command : **pip install -r requirements.txt**.

Step 4 : Update the COM port of the Arduino and the pin numbers that connects the Arduino to the LED controller and the motor.

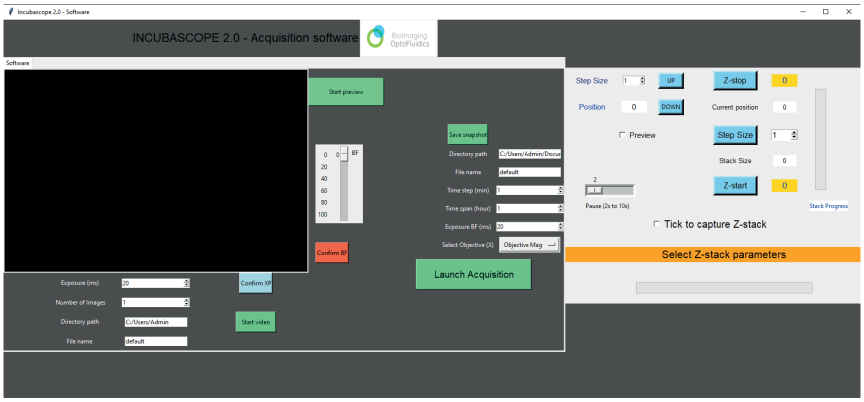
```
carte = Arduino('COM17')
sortie1 = carte.get_pin('d:3:p')
#servo = carte.get_pin('d:10:s')
pin=10
carte.digital[pin].mode=SERVO
```

Step 5 : Update the directory path of the BiOf logo to the directory where you placed the image.

```
frame0 = Frame(root, width=1450, height=80, background="gray32")
Title=Label(frame0, text='INCUBASCOPE 2.0 - Acquisition software', background="gray32")
Title.config(font=('Arial', 18))
Title.grid(column=0, row=0, rowspan=1, columnspan=1)
test0 = Image.open('C:\\Users\\BiOf\\logo1.jpg')
test0=test0.resize((150, 70), Image.ANTIALIAS)
photo0 = ImageTk.PhotoImage(test0)
label0 = Label(frame0,image=photo0,height=70,width=150)
label0.image = photo0
label0.grid(column=1,row=0,columnspan=3)
frame0.grid(row=0, column=0, rowspan=1, columnspan=6)
```

Step 6 : From the Python terminal, change the working directory to the folder where the code is placed using the following command : **cd 'new working directory'**.

Step 7 : From the Python console, run the Python code using the following code : **"python zIncubascope.py"**.The graphical user interface will appear shortly. If you obtain an error, check the Arduino port is correctly defined and that the camera is connected.

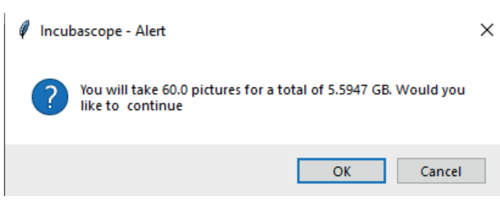


At first, the LED is off so the screen is black. Adjust the LED power with the slider and click on red button ”Confirm BF’. The black window now displays the image of the sample. Each time you click on this button or on the ”Confirm XP” that confirms the new exposure time value, the displayed image is refreshed. If you click the ”Start preview” button, a new window will open with live images from the camera. Press ”Escape” button on your keyboard to close this window.

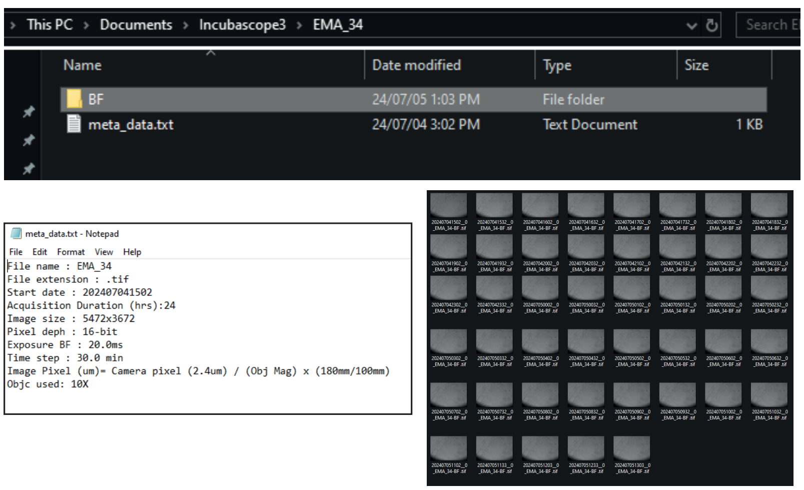
Step 8 : If you want to perform a z-stack acquisition during the time lapse, you need to configre the right panel. From the software set the position of the motor to **0**. Now set the z-position of the sample such that the lowest plane of the 3D object is in focus. To acquire images of the entire object, set the **Z-start=0** and **Z-stop=180** with suitable **Z-step** to acquire a stack. In the motor control panel, the ’position’ indicates the angle of rotation of the motor (in translation, 0° gives 0 µm and 180° gives 304 µm, a step size of 5 gives 10 µm.

Now check the ”Tick to capture Z-stack’ button.

Step 9 : Enter the parameters you want for the acquisition on the right part of the left panel (exposure time, time step, time span, file name and file directory, objective magnification). Then click on the ”Launch Acquisition” button. A small pop up window should be displayed. It gives an estimation of the number of images it will captures and how much memory it will take on your computer. Click ”OK” to start the acquisition.



Step 10 : Now, a new folder with the name you provided shoud appear in the directory folder. Inside, you can find a .txt file that contains the acquisition parameters. There is also a subfolder named ”BF” and that contains all the individual images captured with a time stamp in the file name.



This PC › Documents › Incubascope3 › EMA_34
Search E
Name
Date modified
Type
Size
BF
24/07/05 1:03 PM
File folder
meta_data.txt
24/07/04 3:02 PM
Text Document
1 KB
meta_data.txt - Notepad
File Edit Format View Help
File name : EMA_34
File extension : .tif
Start date : 202407041502
Acquisition Duration (hrs):24
Image size : 5472x3672
Pixel deph : 16-bit
Exposure BF : 20.0ms
Time step : 30.0 min
Image Pixel (um)= Camera pixel (2.4um) / (Obj Mag) x (180mm/100mm)
Objc used: 10X