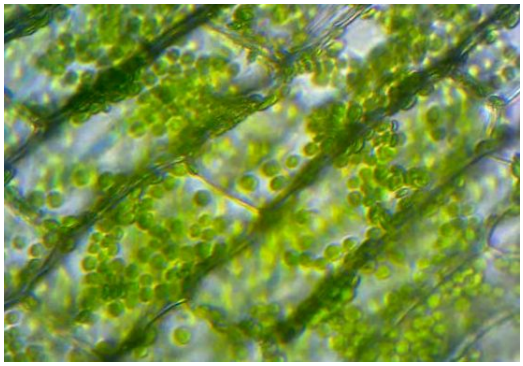
Lab Notebook

19th January 2017 - Day #3

Project : Observation of chloroplast movements according to different intensity of light.

To deal with our project, we bought *Egeria densa* - aquatic plants. They produce photosynthesis with their chloroplasts. We would like to understand better the relationship with the intensity of light and the movement of chloroplasts inside of the cell.

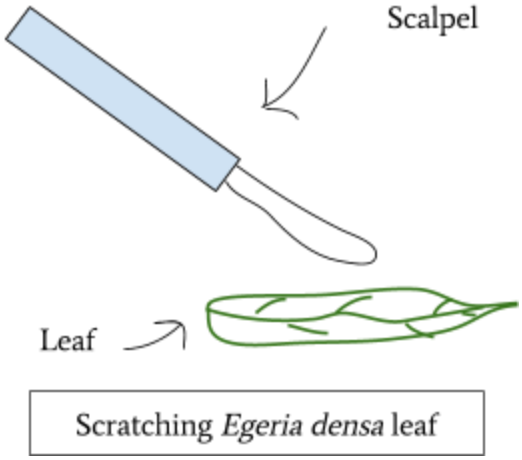


Chloroplasts
https://www.youtube.com/watch?v=jYg8-ZjGe9g

Manipulations:

- **With leafs** :

We buy two *Egeria densa* bouquets, in Truffaut - each bouquet contains four stems, about 15 centimeters height. It costs us 4,10€ per bouquet.
We put these bouquets in a box of water, to provide them enough light and water to let them grow.
To observe *Egeria densa* chloroplasts inside of leaves, we use a scalpel to scrape them and try to have just one layer of cells. The idea is to have the more precise image of chloroplasts, so not be bother by others cells below (multiple layers of cells).



Scratching *Egeria densa* leaf

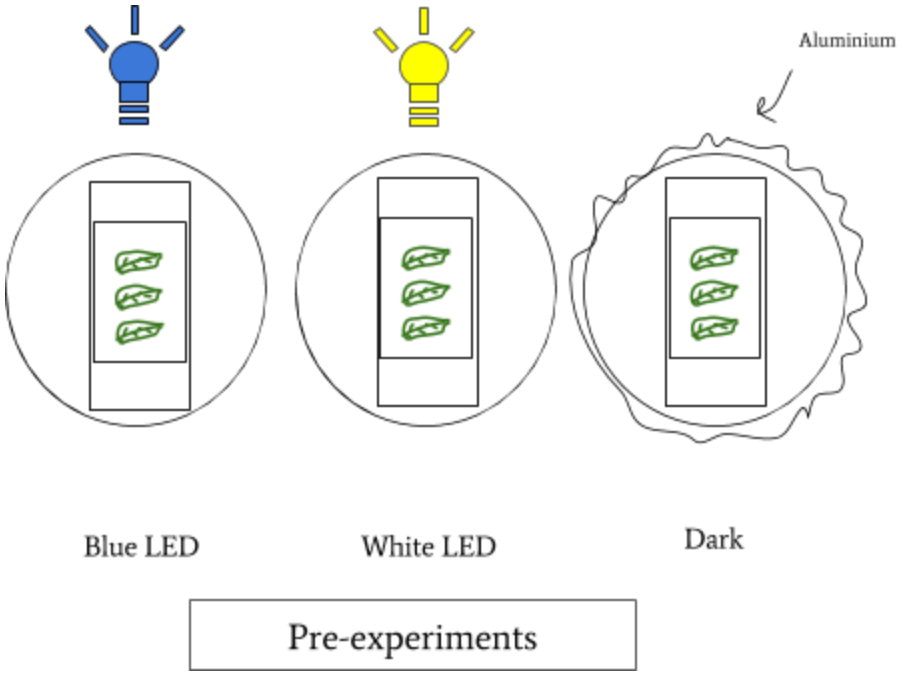
Egeria densa stem

This operation is not that easy, the leafs are very dense and difficult to separate.
After having obtained a single layer of cells, we are putting the leaf on a slide, a drop of water and a cover slide.

Pre-tests :

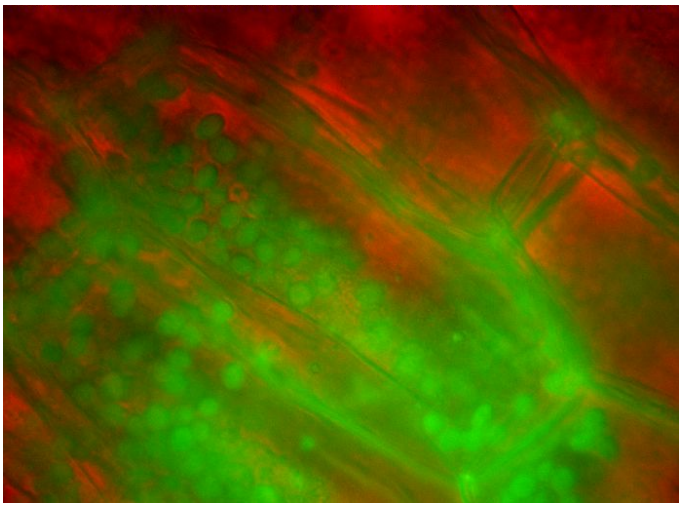
We would like to test different conditions of conversation of leafs (dark, blue light and white light) and after observed them on the microscope, to see the different positions of chloroplasts.

We prepared three slides, with three scratched leafs of each. We put each slide on a petri dish empty.
We conserved one petri dish in a closet with only a white light (Arduino LED), another in a closet with only blue light (Arduino LED), and the last one packed with aluminium, to have complete dark around the slide.



- **With macroscope**

We learn how to use the microscope ZeissAxioPlan2-Imaging. It allows us to observe chloroplasts with fluorescence filter, and observe their movement.
To our first utilisation of it, we use the slide of leaves which comes from the white LED petri dish .
The microscope has three objectives x10, x,25 and x63. We learn how to make observations with a simple filter, with fluorescence. Also, with color combined option, we can this kind of image, which could help us to better observe chloroplast movements.



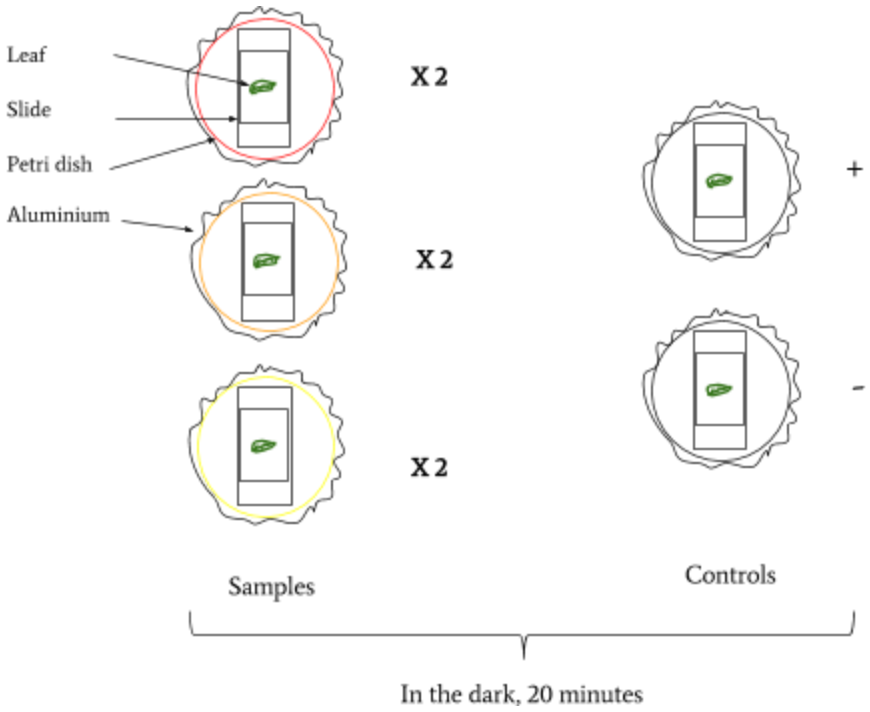
20th January 2017 - Day #4

Recording data :

- Leafs on petri dish

Today, during the morning lab session, we record chloroplasts movements. The protocol was :

- Preparing a slide with a scratched leaf (to obtain only one layer of cells),
- put this slide on a petri dish with drops of water around the slide to keep the leaf wet;
- the petri dish was after that packed in aluminium, to keep the leaf into the dark during twenty minutes.



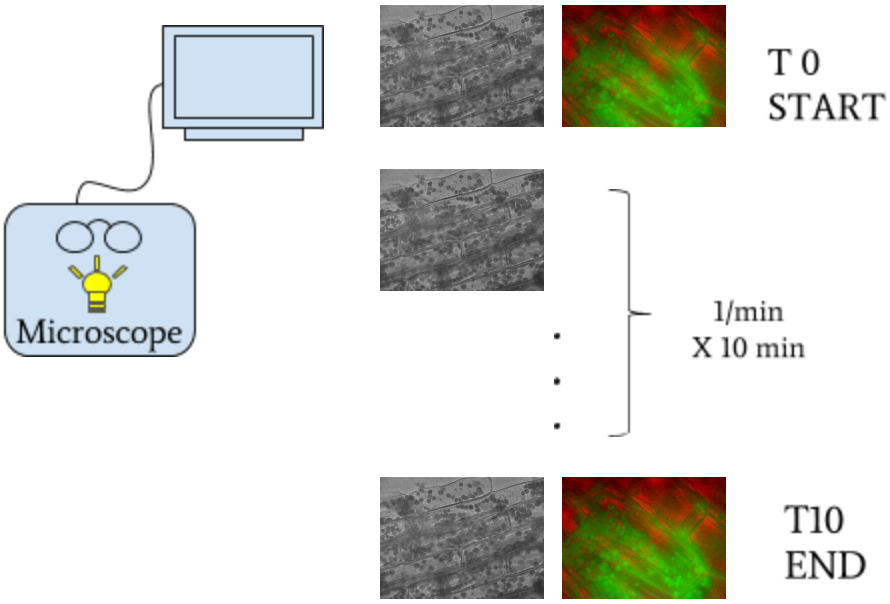
- Microscope observations :

After twenty minutes, we observe the leaf on the microscope. We use as yesterday the microscope ZeissAxioPlan2-Imaging.

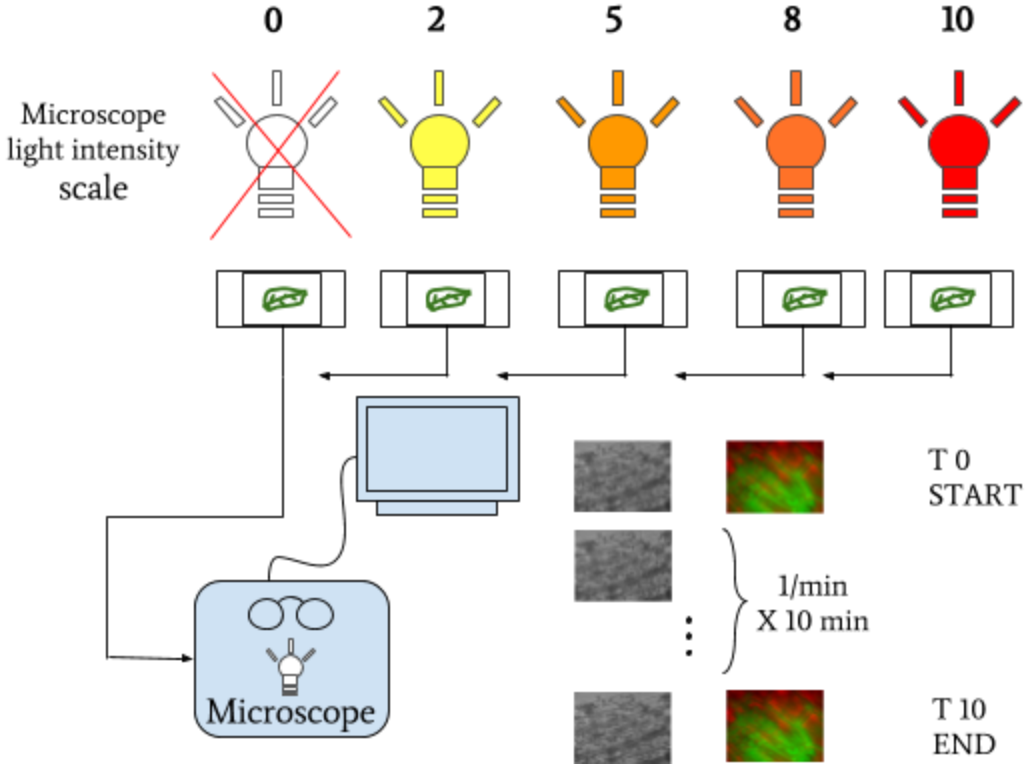
During 10 minutes for each slide, we observe a cell a the leaf, x63, to recorde chloroplasts movements. We test here the influence of light to chloroplast movements. So, we test 3 intensity of light. We use the microscope light, and we change it on a scale between 0 and 10 (10 is the brighter intensity) on each observation.

We have 5 different conditions : a positive control (with the microscope light at 10), a negative control (light at 0), a high intensity (8), a medium intensity (5) and a low intensity (2).

We did two replicates of each condition except the replicates.



On each observation, we focus a cell containing chloroplasts, we took a picture with the simple camera of the microscope, of these chloroplasts every minute during 10 minutes. For each observation, we also take a fluorescent picture at GFP wavelength a the cell on the beginning and at the end of the observation.

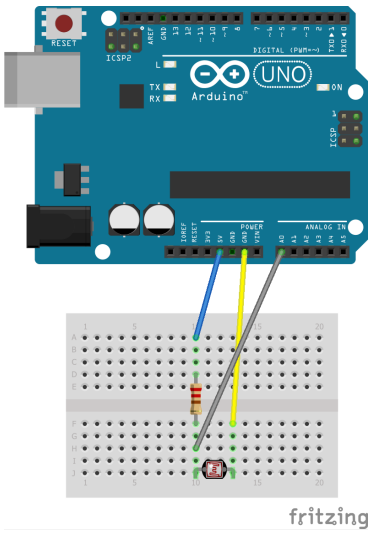


To use the microscope, we made the focus with the objective x40, then put some oil on a slide (because the x63 objective touches the slide, so to avoid breaking it, oil is necessary).
Then, we are looking for a cell where there is only one layer of cells. The goal is to be able to observe chloroplasts of only one cell, to not be confusing with others ones.
This part was one of the most difficult. The technique to extract one layer of cell from a leaf was not optimum.
For almost every sample, the leaf was not flat. So it was difficult to observe only one cell with the microscope.

How to use the fluorescence microscope :

- Turn on the camera, microscope, lamp (at least 20 minutes before) and computer
- Open the software “MetaMorph” on the computer.
- Click on the icon below “Records”; it will open a window allowing you to control the light.
- To use the normal light, click on the button “Turn on/off” (command the normal light).
- Put the slide on the microscope.
- To change the objective there are buttons behind the coarse focus.
- Begin with the red objective (x10), then the yellow one (x40) to focus on the sample. For that, use the coarse focus then the fine focus (which are inside on the microscope). Use the joystick outside of the microscope to move on the slide.
- On each objective, there are a “Ph x” writing on it. For each objective a philter is associated. To change the philter, turn the adjuster just in front of the sample. For example, x63 objective corresponds to “Ph3”.
- To use the blue objective (x63), put a drop of oil on the microscope before putting the objective.
- To take a picture with fluorescence, turn of the light, turn on the GFP filter (on the computer). In the bottom left-hand corner, there is a pull-down menu : for the normal light picture, the 100ms-trans section has to be on. For the fluorescence, put the 0.1s or 1s fluo. And to take the picture click on “Acquire”.
- To save the picture, close the picture and say “Yes” to “Save ?”. Name the picture as “Name-condition of culture-100mstrans OR 1s fluo … -x??-Ph?.

Electronic receptor : Arduino arrangement.



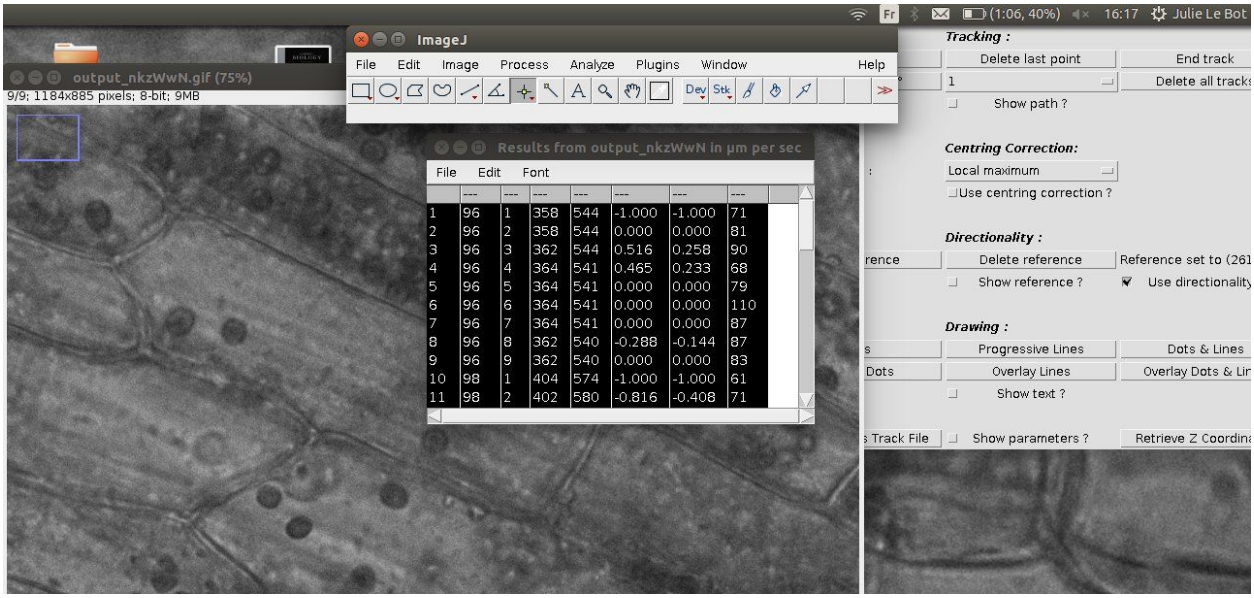
We made the arduino and light dependent device following the plan (as it is made in the figure). (LDR = Light Dependent Resistor).
The arduino use is a leonardo and the resistance is a 10kΩ.
We put the arduino device at the same place than the microscope to measure its response to different intensity of light. We tested three different intensities (10 lux, 158 lux, 756 lux) and we made two replicate. In addition we made one positive control (4 lux) and one negative control (1512 lux).

We made an error because we didn't take the same filter than the filter we used for the leaves. But we will normalize the data afterwards to compare rather the tendance than the absolute value.

21rst January 2017 - Day #4

Analysing data :

For each intensity, we had 10 pictures x 2, except for the replicates (only 10 pictures).
Clément transforms the picture into GIF format. It allows us to have a "video" of each intensity to observe movements of chloroplasts.
For each GIF, we use Image J software to "track" 6 chloroplasts. We record the position of each chloroplast according to a reference point. The reference point was the bottom left-hand corner of the cell (and it was updated for each GIF).



1	96	1	358	544	-1.000	-1.000	71
2	96	2	358	544	0.000	0.000	81
3	96	3	362	544	0.516	0.258	90
4	96	4	364	541	0.465	0.233	68
5	96	5	364	541	0.000	0.000	79
6	96	6	364	541	0.000	0.000	110
7	96	7	364	541	0.000	0.000	87
8	96	8	362	540	-0.288	-0.144	87
9	96	9	362	540	0.000	0.000	83
10	98	1	404	574	-1.000	-1.000	61
11	98	2	402	580	-0.816	-0.408	71

Data provided by Image J were : X and Y position, Distance and Velocity.
We use distance data, and make mean for every chloroplast by intensity.
We know that the intensity of the microscope camera is :

- for Ph2

0 = 4lux, 2 = 10 lux, 5 = 158 lux, 8 = 756 lux , 10 = 1512 lux.

- For Ph3 :

0 = 4 lux, 2 = 4lux, 5 = 25 lux , 8 = 127 lux, 10 = 249 lux.
It allows us the plot a graph of the mean of chloroplast distance according to intensity.

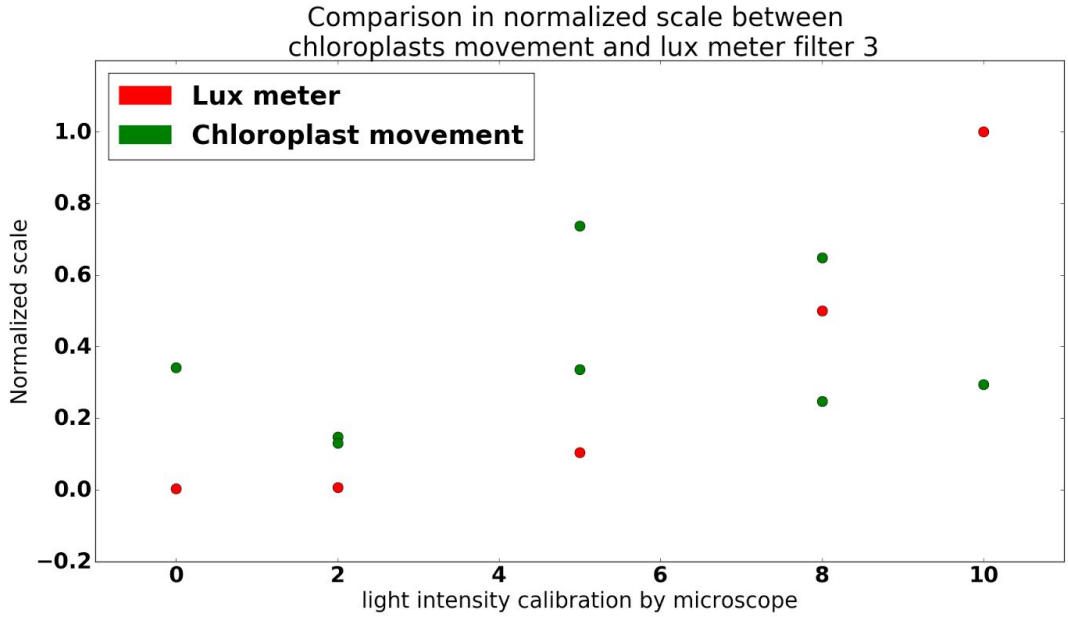
23rd January 2017 - Day #6

Today we plot our data before analyzing it with python and matplotlib module.

Here is the code to analyse the LDR rough data :

```
#!/usr/bin/env python
# -*- coding: utf-8 -*-
#Packages needed
import matplotlib.pyplot as plt
import matplotlib.patches as mpatches
import pylab as pltt

#definition of the label size
font = {'weight' : 'bold',
        'size'   : 25}
plt.rc('font', **font)

#Rough data
LDR = [0.4736842105, 201.8947368, 276.8947368, 844.5789474, 811.7368421, 945.3684211,
929.5789474, 967.9473684]
luxmeter = [0.003, 0.007, 0.104, 0.5, 1]
lightss = [0, 2, 5, 8, 10] # intensity of the microscope light
lights = [0, 2, 2, 5, 5, 8, 8, 10] # intensity of the microscope light for duplicate

#normalize the LDR output value
normLDR = []
for i in LDR:
    a = (i * 1)/(967.9473684) #noramalize between 0 and 1
    print(a)
    print(i)
    normLDR.append(a)

#Making the graph
red_patch = mpatches.Patch(color='red', label='Lux meter')
green_patch = mpatches.Patch(color='blue', label='Arduino Output')       # legend
plt.legend(handles=[red_patch], loc='upper left')
plt.plot(lightss, luxmeter, "o", color="red", markersize=18)             # curve
plt.plot(lights, normLDR, "^", color="blue", markersize=18)
plt.xlim(xmin=-1)
plt.xlim(xmax=11)                    # set size of the graph
plt.ylim(ymin=-0.2)
plt.ylim(ymax=1.2)
plt.ylabel('Normalized scale')
plt.xlabel('light intensity calibration by microscope')
plt.title("Comparison in normalized scale between \n Arduino output and lux meter filter 3")
plt.show()
```

We have this results as graph :



This graph shows that most of the chloroplasts don't move after 10 minutes of light exposure. Or at least doesn't seem to change anything in their movement.

We imagine two hypothesis to explain this non-movement :

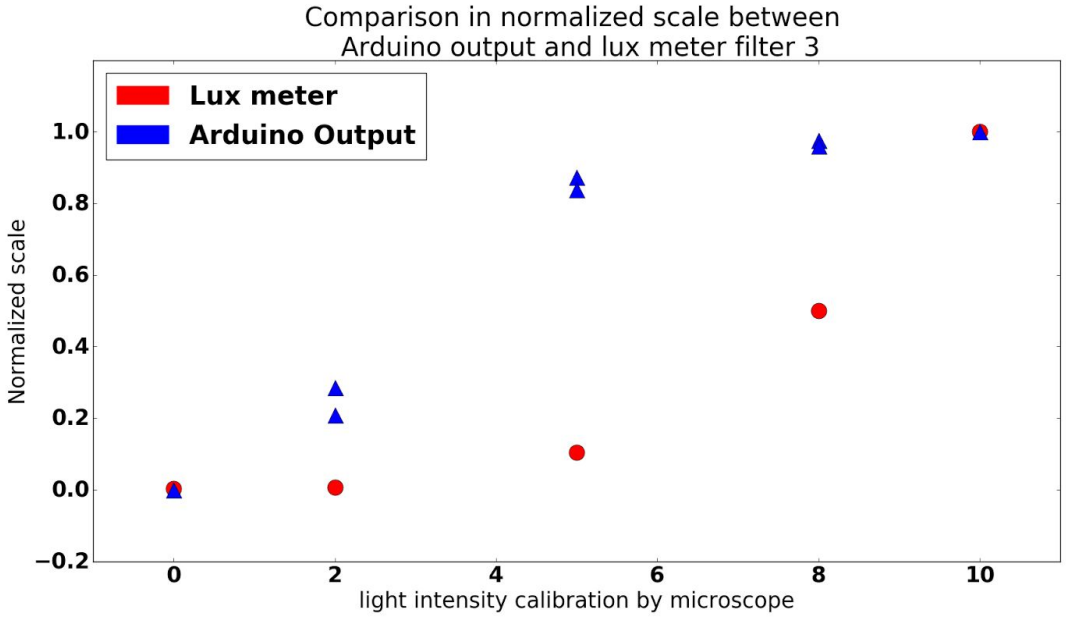
- the time response of chloroplasts is very slow, so no movement was able to be seen in only 10 minutes.
- The light was not enough intense to activate the movement mechanism.

Here is the code to analyse the Chloroplasts movement :

```
#!/usr/bin/env python
# -*- coding: utf-8 -*-
#Packages needed
import matplotlib.pyplot as plt
import matplotlib.patches as mpatches
import pylab as pltt

#definition of the label size
font = {'weight' : 'bold',
        'size'   : 25}
plt.rc('font', **font)

# data for the lux meter
luxmeter = [0.003, 0.007, 0.104, 0.5, 1] # normalize measurement
lightss = [0, 2, 5, 8, 10] # list of the intensity given by the microscope
label = [4, 5, 25, 127, 249]

#parcing the image data
a = open("Raw.csv", "r") #open the datafile
```

```
distances = [] #keep all the distances
numeros = [] #keep all the name of the chloroplasts
light = [] #keep the intensity of the light
vitesses = [] #keep all the velocities
temps = [1, 2, 3, 4, 5, 6, 7, 8, 9, 10] #time in minutes
lights = [0, 2, 2, 5, 5, 8, 8, 10] # x axis to make the point cloud
numero = [] # initalise the backup of chloroplast name
distance = [] # initalise the backup of chloroplast movement
vitesse = [] # initalise the backup of chloroplast speed
light = 0 # counter of the movie
moy_distances = [] # mean of all the distances
for i in a: #read each line
    data = i.split(",") # make a list of each line, one data between each comma
    moy_distance = [] #initalizing

    if data[0] == "": # if it is a new video
        light += 1 # iterate the number of video
        moy_distances.append(sum(distance)/len(distance)) # upgrade of the mean of movement
        numero = [] # reinitlizing
        distance = [] # reinitlizing
        vitesse = [] # reinitlizing
    elif float(data[1]) == 1: # ignore the first movement
        coucou = "busous" # joke
    else: # treat the line
        numero.append(data[0])
        distance.append(((float(data[2]))**2)**(0.5))
        vitesse.append(data[3])
moy_distances.append(sum(distance)/len(distance)) #add the last video

#building the graph
red_patch = mpatches.Patch(color='red', label='Lux meter')
green_patch = mpatches.Patch(color='green', label='Chloroplast movement')
plt.legend(handles=[red_patch, green_patch], loc='upper left') #make the legend
plt.plot(lightss, luxmeter, "o", color="red", markersize=12)
plt.plot(lights, moy_distances, "o", color="green", markersize=12)
plt.xlim(xmin=-1)
plt.xlim(xmax=11)
plt.ylim(ymin=-0.2)
plt.ylim(ymax=1.2)
plt.ylabel('Normalized scale')
plt.xlabel('light intensity calibration by microscope')
plt.title("Comparison in normalized scale between \n chloroplasts movement and lux meter
filter 3")
plt.show()
```

We have this results as graph :



This graph shows that the electrical LDR response is extremely sensitive at very low light intensity. However, the LDR sensor seems to be saturated in high intensity of light. In addition, we made two replicates of the measurement. We noticed that the two were quite different, up to 20% of variation.

We also have received a email from our very nice mentor M. Masamitsu Wada.
He says “
I am not sure what you want to ask me and whether I will be able to answer you questions.
In any way, you can ask me that you want to know, of course.

As for the chloroplast movement that is my expertise, however, the chloroplast movement of Egeria dens is different from that of land plants that we work on, so that I am afraid that the questions might not be possible to answer you from my experience.

In the cases of chloroplast movement in seed plants and also in fern gametophytes, the movement is completely independent of cytoplasmic streaming, so that it is no need to think about the noise (or influence) of cytoplasmic streaming on the chloroplast movement. This must be most crucial difference between the chloroplast movement in land plants and that of Egeria densa where chloroplasts move along the cytoplasmic strand. I do not understand what do you mean by 'the noise of cytoplasmic streaming' in your system.”

We checked in the literature and it is clearly said that the light has an impact on the cytoplasmic streaming. We considered from the beginning that the cytoplasmic was more a noise than something to measure.

At the very end of our project we advice the next research to measure the streaming rather try to avoid it.

Cf : “Induction of Cytoplasmic Streaming and Movement of Chloroplast Induced by L-Histidine and Its Derivatives in Leaves of Egeria Densa | Plant and Cell Physiology | Oxford Academic.” Accessed January 23, 2017. https://academic.oup.com/pcp/article/32/2/253/1840268/Induction-of-Cytoplasmic-Streaming-and-Movement-of.
We also believe to let the leaves more time in the dark and under the light of the microscope should be done.

You can see our other document in the Github of Biosensors :
https://github.com/learningthruresearch/Biosensors2017