Lien vers project Proposal :
https://docs.google.com/document/d/1-zwgUUmswFLc1M6NGqPrPLSwb89mPJKqguFFqY7JhRM/edit

Lab Notebook :

25/01/2017
Choice of a project : how *Caenorhabditis elegans* react to an electric field.

- Does the electric field change the way *C. elegans* move ?
- Is *C. elegans* able to orientate through the field ?

Remarks from Tamara :

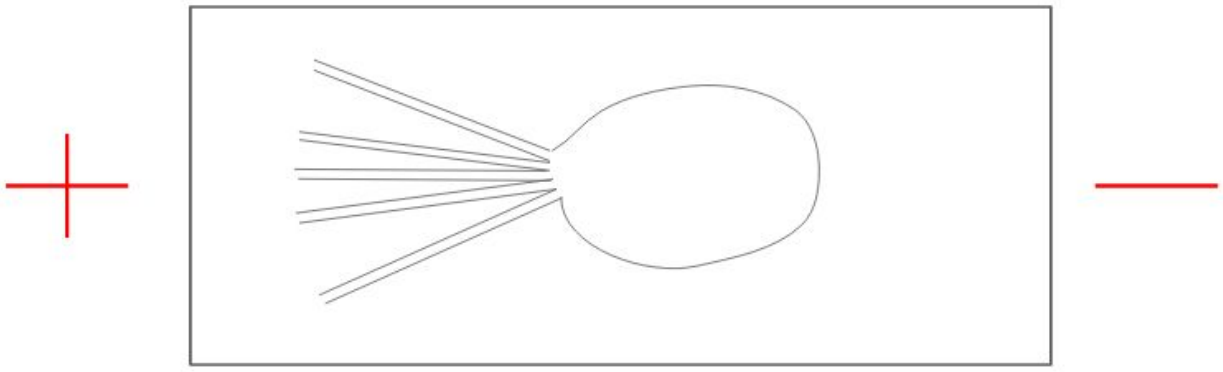
- We should work with electrophoresis and turn the gel where *C. elegans* are
- Print in plastic, above the gel, channels and use them to count the worms per direction.
- We can't use a voltmeter it's not precise and adapted for the project, we should use an electric field sensor made with antenna and transistor.

Choice of the set up:

- Average speed = 400 µm/s
- Size of *C. elegans* = around 1 mm
- Straight microchannels (5 cm long and 80 mm deep) with varying widths of 2 mm, 1 mm, 500 mm, 300 mm, and 150 mm with electrodes instrumented in their reservoirs (extracted from *Electrotaxis of Caenorhabditis elegans in a microfluidic environment†*)
- About the microscope :

Observations / Quantifications :

- Speed (measuring the distance travelled / time) => ImageJ
- Type of movements (?)
- Orientation (counting how many C. elegans are in a channel, cf. draw 2)



- About the sensor : quantify the intensity, the orientation, the current
- About the electric field : AC/DC, Voltage, Current, Signal shape (square, triangle, …)

Pre-experiment :

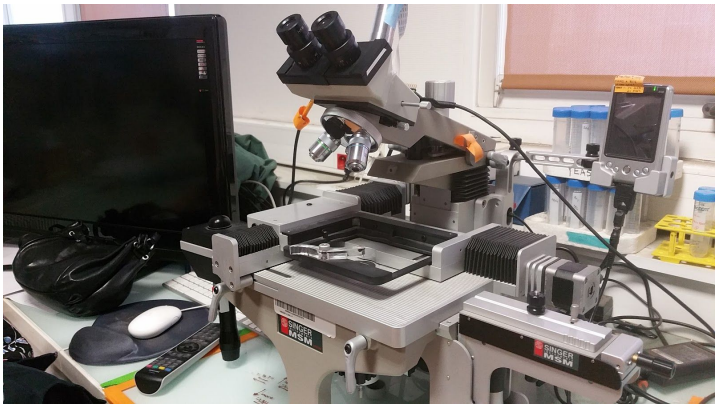
- We checked the singer microscope to do our experiments.
- We will use a 96 wells plate box (without the wells of course) to realise our experiments, it is possible to see 8cm long, yet we only need to see 5cm long.
- We can see the C. elegans quite well and can record with the video. We can move the video to track the C. elegans.
- We found by calculation that since a plate radius is 4,5cm and we generally must poor 20mL of agar to fill it, then the depth of the medium is 3mm.
- Optimal frequency : 1mHz to 100mHz (between 100mHz and 1kHz it's not working very efficiently)
- Shape of signal is not supposed to have influence on the *C. elegans* mvmt
- 20°C
- Circle = 2,5cm away from each electrode

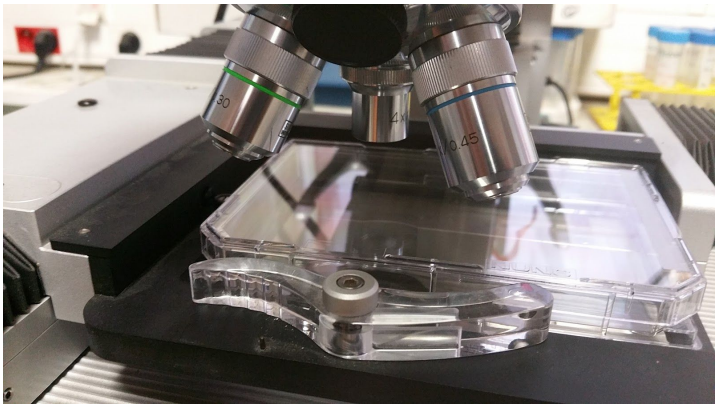
Media for the “electrophoresis”:

“The agar gel was composed of: de-ionized water, 2% of Bacto-Agar, glycerol (3.7 mL of glycerol 60% for 1 L), NaCl (0.250 mmol/L) as previously described in [16]. The gel was cast by pouring a first layer of agar and adding a PDMS (polydimethylsiloxane) block onto it so that it will shape the future cavity where nematodes will crawl (6×8 cm). A second layer of gel was then poured around the PDMS block. Once solidified the PDMS block was removed. The resulting agar pad was then placed in an electrophoresis box filled with a buffer. It was composed of de-ionized water, glycerol (3.7 mL of glycerol 60% for 1 L) and NaCl (0.250 mmol/L) as previously described in [16]”

To prepare the plates where *C. elegans* will be observed, we prepare media for 10 plates. We measure that 60 mL of water is needed to fill the plates with 3 mm height which . We take into account that the structure printed will also reduce the volume by half.

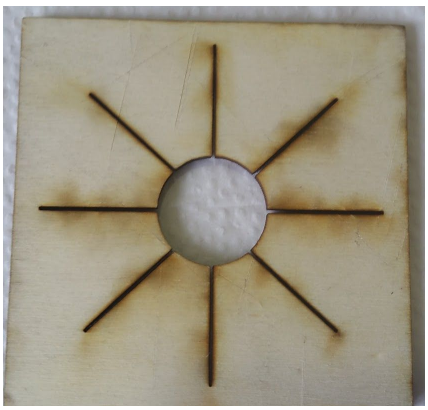
About the set up :

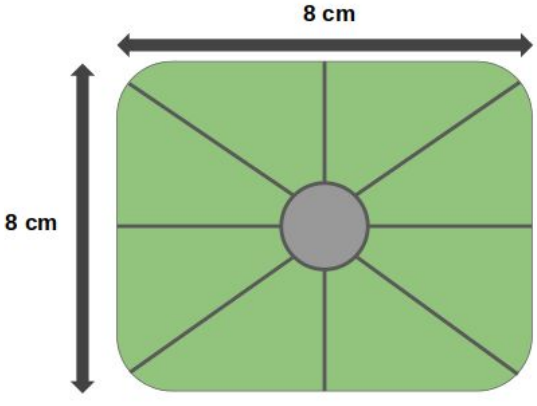


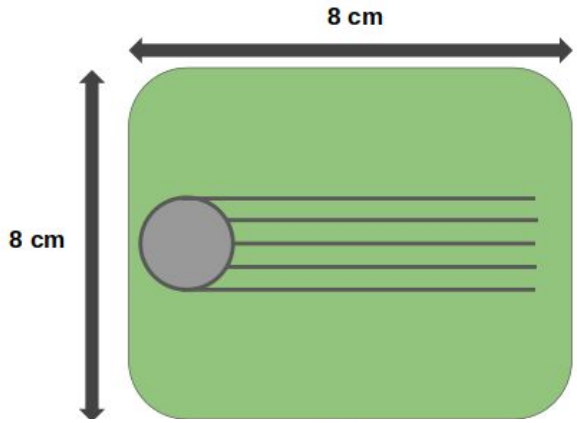


First design of the channels, in the center we put the worms and then let them go in one of the eight channels. The purpose is to see both speed and direction of the worms depending the frequency/amplitude/orientation of the electrical field.

There are more structure to come

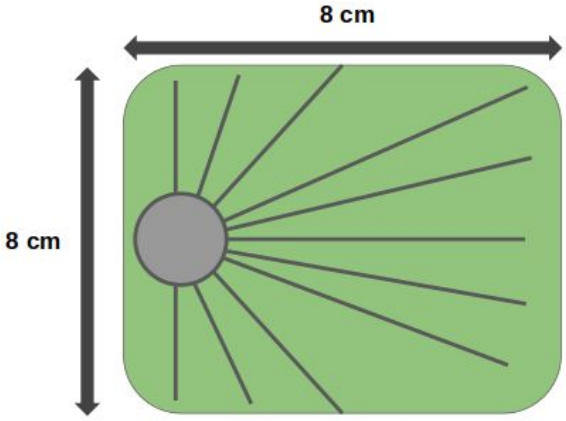






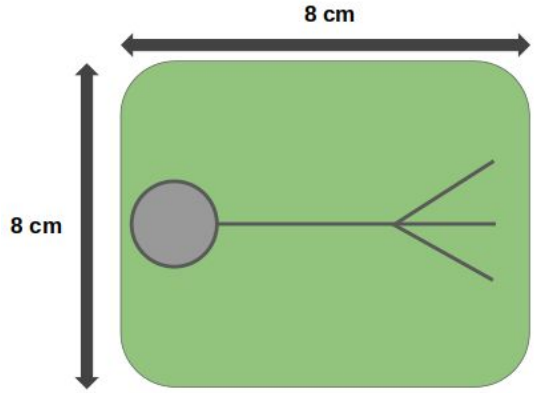
This chip will be used to see if the average speed of *C. elegans* changes with frequency/amplitude of the electrical field.

Settings : frequency - amplitude - AC/DC
Measurements : speed by measuring distance and time
What is fixed : orientation of the field



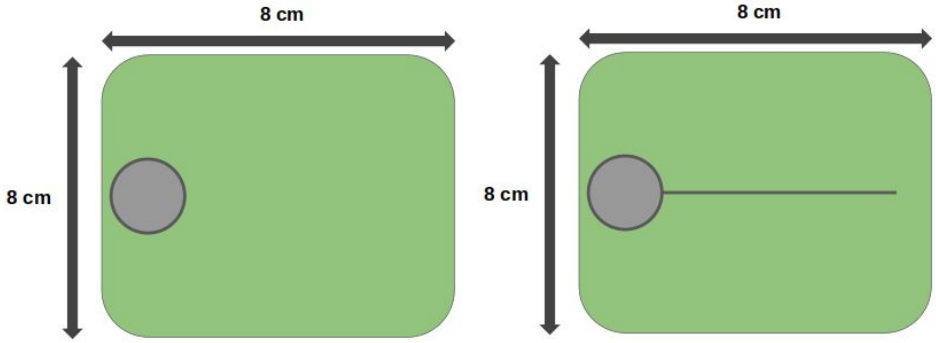
This chip will be used to see if the orientation of lines of the electrical field induces the worms to change their direction.

Settings : orientation of the field
Measurements : number of individuals in each channel
What is fixed : frequency and amplitude

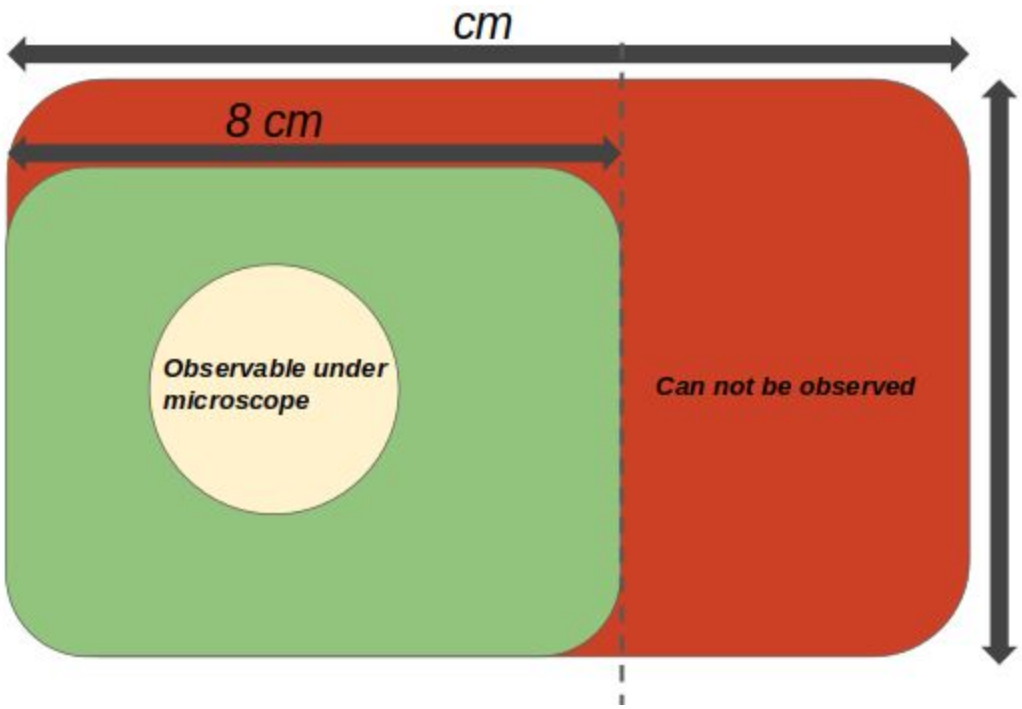


This chip will be used to see the sensitivity of the C. elegans to the location of the electric field source.

Settings : orientation of the field
What is fixed : frequency - amplitude - AC/DC
Measurements : speed by measuring distance and time

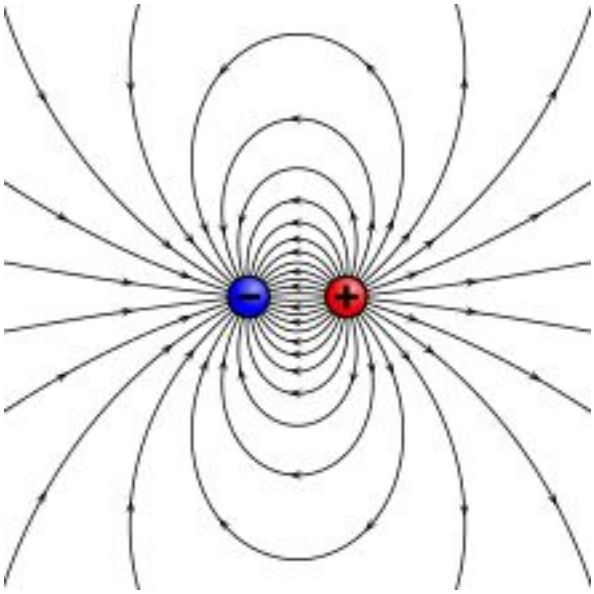


These are the **two controls** used : the first, the negative one, prevent worms from moving and the second one makes them go strait .

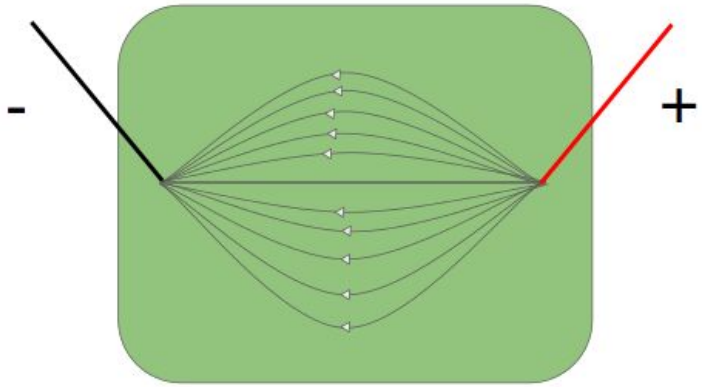


The chip is put inside the square plate and recovered partially by the medium.

Remarks : In each experiment, the current must be fixed around 50 mA.



We expect the lines of field to be as shown in this picture (***from wikipedia***)



Source of power :

Digital Dual channel DDS Function Signal Generator MHS-2300A 5MHZ

from aliexpress

Here is the datasheet :
http://blog.pennybuying.com/down/fb/FB170A.pdf

Medium preparation

Before the experiment we prepare the medium for the “electrophoresis”. We use a variant of the recipe see before.

<u>For 500ml of media:</u>

osmosis water : 500ml
Glycerol 60% : 1,85ml
NaCl : 0,125mmol → 0,025g for 500ml
Agar (2%) → 10g

Pre-experiment:

We poured this medium, on our square plate, with our models in wood with the channel of 2mm and 1mm.
After a battery of tests with the models, we concluded that the medium can go in all the channels, therefore we decided to start the experiment with the C.elegans on Monday.

29/01/17

We decided to use a new electronic sensor : a Movuino, able to detect a magnetic field and therefore an electric field.
The python code is almost finished.

30/01/17

Experiment:

During our experiment we try to observe the C.elegans with the microscope for yeast (micromanipulator), but this microscope is too precise, because we can't see the totality of the channel. We choose to change the experiment. We just cut a little piece of agar and we put this piece under the binocular magnifying glass, who can take the totality of the piece, and see the C.elegans.
We change the power device too, at the beginning we use Digital Dual channel DDS Function Signal Generator MHS-2300A 5MHZ, but he doesn't work, so we try to use a battery of 9V, but it doesn't work too, and at the end we choose use the device […].
But during this day we could manipulate the C.elegans, begin to observe their behaviour, movement etc and adapt the way to observe them.

31/01/2017

The totality of our expectation about the reaction of C.elegans in function of electric field are dead.
We try differents things with the agars, the power supply and the microscope and nothing work.
We try to take one by one the C.elegans and transfer a part of 1,5 cm X 2cm (the maximum size of viewing of our microscope), but we don't have the material to do this right, and we can't manipulate them. We try to 'glue' the two part of agar, the virgin part of agar without C.elegans and the other with the 'colony' of C.elegans to permit to C.elegans to go in the virgin part. And after 'glue' put the electric field with the negative point in the virgin part, wait 5minute, and see how many C.elegans are in the ex-virgin part. But we can't really 'glue' the two part and the C.elegans can't passed.
We try to put few C.elegans in the virgin plate to scrapping the first plate thanks to a trombone.
This technique work, but at the end we can't capture the picture, and we don't observe any special behaviour.
To have data, we try to change the experiment, and do the same things, but with Pyrocystis fusiformis a bioluminescent algae.
[...]

At the end of the day, we don't have any data or new experiment, and more we don't have new experiment.

Therefore we decided to test the electric field on all the organisms that we could use in the lab.

- One of the protocol involved dinoflagella, organisms that react to movement by luminescence. We found litterature about how electromagnetic fields would influence the luminescence of this organisms.
  Therefore we tried to put a plate containing 10mL of media and organisms, and put the place on a vortex.
  The idea was to induce movement with the vortex and apply an electric field to influence the luminescence. Sadly, despite this experiment being amazing, the luminescence could hardly be observed, nor measured.

- We also tried to open and close carnivorous plants, *dionaea*, by inducing an electric field between the two parts of it, with the idea that since the signal is an electric signal, we could force the signal into the plant.
  Unlucky us it didn't work quite as planned, however it still might be due to a low intensity of the power supply.

- We then tried to do the same experiment of the C. elegans, with *Chironomus plumosus* and *Artemia salina*. In order to do this we placed them in water, each in different plates of course. We then applied the electric field and took videos of each plates.

Our results are based on the study of the videos of *Chironomus plumosus*, compared to the study of the sensitivity of movuino.