Project Proposal

Aim : We want to observe the sensitivity to perceive an electric field for a biological sensor, *Caenorhabditis elegans,* and an electronic sensor, a homemade electric field sensor.

Question : Which sensor from C. elegans and an electric field sensor can sense an electric field ?

Biological Sensor : We chose to work with *C. elegans* because of its sensitivity to electric field that induces movement towards the positive side of the field. All of the studies we found were made using electrophoresis[1],[2].
Indeed, experiments were conducted using either microfluidic chips or electrophoresis kits, and directing the electric field on different part of the setup.[3]

Electronic Sensor : We chose to build our own sensor to detect electric fields because voltmeters were not adapted to our experiment. Here is how we would want to build it : Cool stuff (hyperlink)

Parameters : We want to study different parameters with the two different sensors, in order to compare them in the most precise and exhaustive way we can.

<u>Biological :</u>

- Movement (speed, ?) according to intensity(amplitude), frequency
- Direction of the movement according to the location of the source of the electric field

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[1] « Electrotaxis for C.elegans ». Consulté le 24 janvier 2017. https://sci-hub.ac/10.1039/B917486A.

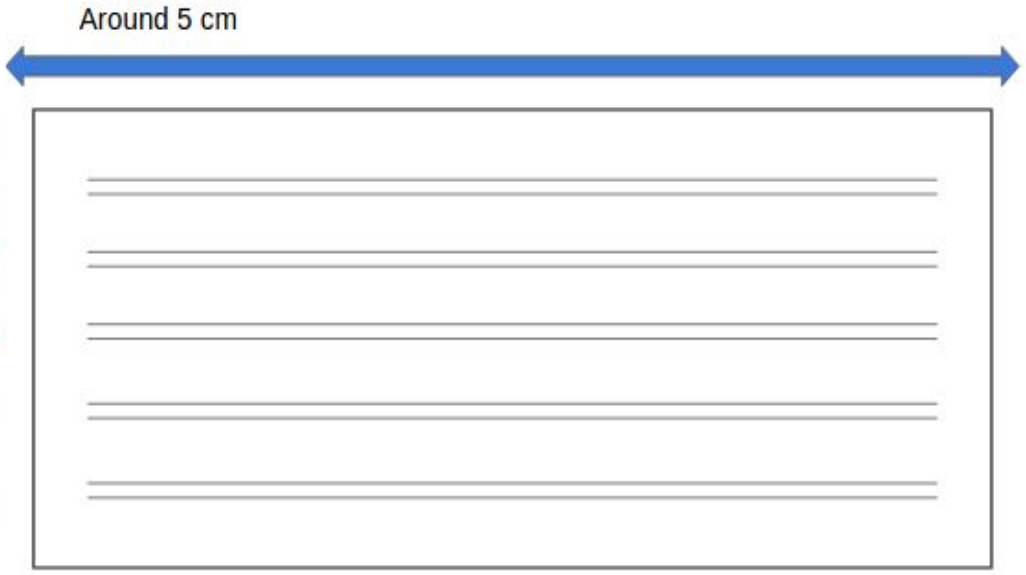
[2] Manière, Xavier, Félix Lebois, Ivan Matic, Benoit Ladoux, Jean-Marc Di Meglio, et Pascal Hersen. « Running Worms: C. elegans Self-Sorting by Electrotaxis ». *PLOS ONE* 6, nº 2 (4 février 2011): e16637. doi:10.1371/journal.pone.0016637.

[3] Running Worms: *C. elegans* Self-Sorting by Electrotaxis

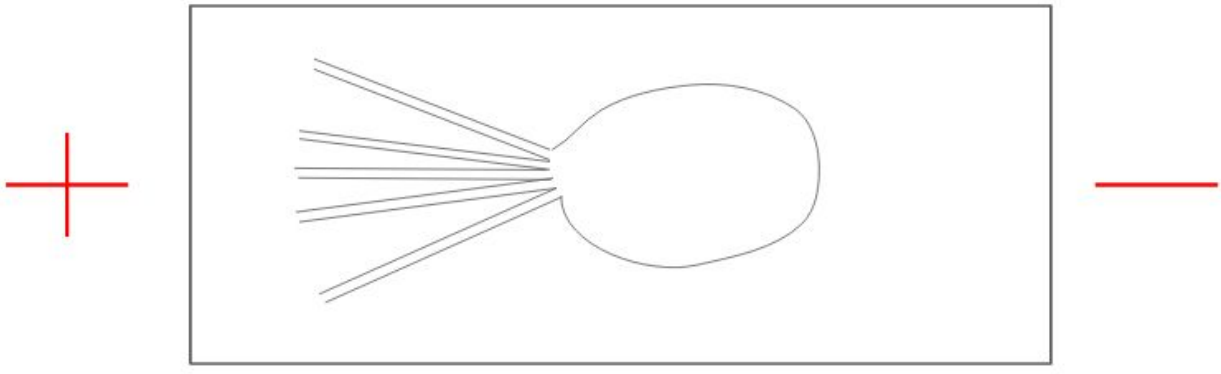
Electronic :

- Precision, compare input and output value
- Accuracy, compare replicates and repetitions to check for constancy
- Sensitivity to the direction of the source of the electric field

Setup number 1 :



- We decided to use an electrophoresis gel to realise our experiments, because we can create and control an electric field easily. We will put it inside a tank and supply the power with another device than an electrophoresis tank, in order to control the voltage, amplitude, frequency, type of wave. Moreover we can add nutritive media to the gel assuring that our C. elegans fella will survive and be healthy and active.
- In order to test the direction of the source of the electric field, we will 3D print barriers that will act as separation for different “pathways” that the worms will be able to take, and then count the number of worms per pathway.



Material :

- 1 tank
- Electrophoresis gel
- 2 metal rods
- 2 pliers
- C. elegans, N = ?
- 1 arduino (and its cables)
- 1 resistor
- 1 antenna
- 1 transistor
- Microscope plugged to a camera
- Video taken via the microscope
- Light from the microscope (problem cause white, maybe filter ?)

Object	Where ?	quantity	Price (for 1)
96 wells tank	lab	1	? (*free)*
Electrophoresis gel	lab	1	? (*free)*
1 arduino	Openlab / shops	1	20€ (free)
1 resistor	Openlab / shops	1	0,50€
1 antenna	openlab	1	free
Microscope	Lab / singer	1	A lot

Protocol :

- Print channels with the 3D printer
- Sink the Electrophoresis gel above it.
- Plant the electrodes in the gel and link it them to the generator
- Put *C. elegans* in the chip
- (Open the gates)
- Start taking pictures while measuring time
- Change the intensity, the frequency and the shape of the signal

Controls

Positive:

- (direction) Only one pathway to go to, *following a line of the electric field*
- (frequency) According the literacy : 1.5 to 1.9 Hz
- (amplitude) around 5V/cm

Negative:

- (direction) put them into a closed square
- (frequency) f = 0 Hz
- (amplitude) A = O V or

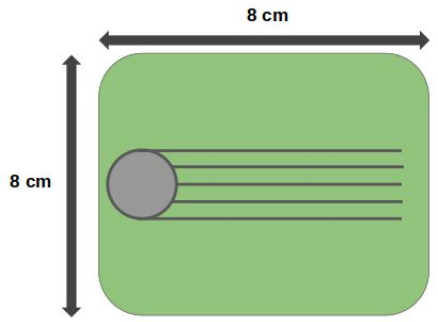
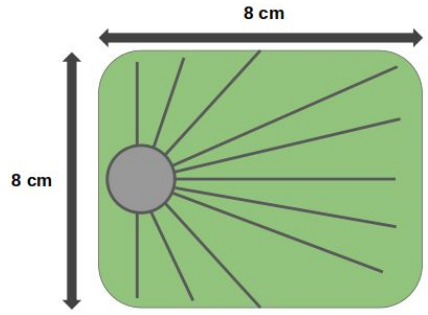
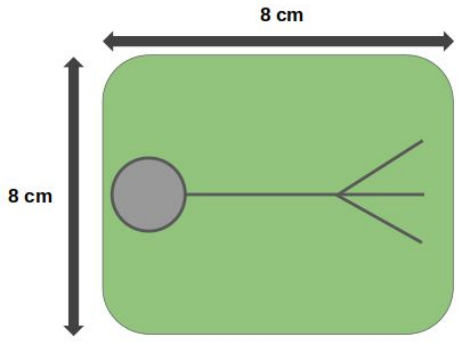
Repetitions/Replicates

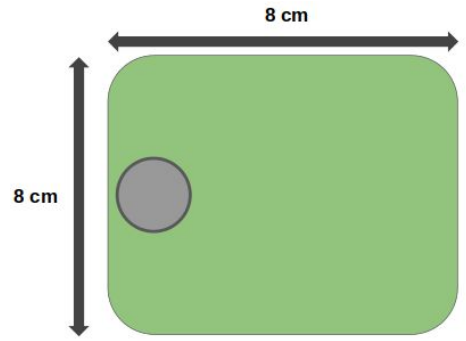
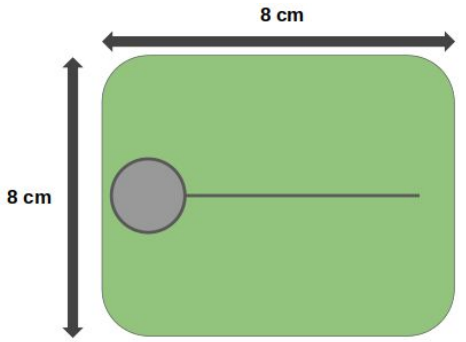
Preliminary test :
Compare DC and AC current effects on *C. elegans*

- Orientation : 3x (n > 30 & 5 min) = 10 times
- Frequency : 3x (from 1.7 to 2.6Hz with a step of 0.2 Hz = 5 times) = 15 times
- Amplitude : **(1 to 10 V, step = 0.5 V, AC and DC = 40 times)**
- Controle + : 3 times
- Controle - : 3 times

Around 60 tries …

Data analysis

Characteristic	Media	Method	Chip
Speed	Video + photo	Move tracking with ImageJ	
Direction of the field	Photos / video	Counting individuals in a specific channel	
Movement quality	video	Still unknown	
Direction of one of the electrical field			

Controle -			
Controle +			

We create four models (all the precedent, except the one with the direction of the field) with a plate of wood of 5mm, and we choose the channels size 2mm.

Setup number 2 :

Due to numerous technical problems :

- The microscope was zooming too much and thus not allowing us to study the whole environment of the plate. → We decided to use a binocular that will allow us to see a bigger part of the plate and therefore capture more C. elegans at the same time.
- The power supply did not have an intensity that was big enough to even be measured with an amperemeter, and to stimulate the C. elegans. → Therefore, we chose to switch to another power supply, a bigger one, allowing us to control the amperage, the voltage and the frequency.
- Moreover, after giving up on the arduino electromagnetic field detector that gave us random values and was totally unstable, as it was described in the instructions we found to build it, we decided to use another sensor. → We will use a magnetometer that is directly attached to a Movuino, allowing us to pull the data more easily than an usual magnetometer.

Protocol :

- Take a sample of C. elegans from a plate and put it on another agar filled plate.
- Plant the electrodes in the gel and link it them to the generator
- Put the plate under the binocular
- Start taking pictures while measuring time
- Change the voltage and the frequency.

Controls

Positive:

- (direction) Only one pathway to go to, *following a line of the electric field*
- (frequency) According the literacy : 1.5 to 1.9 Hz
- (amplitude) around 5V/cm

Negative:

- (direction) put them into a closed square
- (frequency) f = 0 Hz
- (amplitude) A = O V or

Preliminary test :
Compare DC and AC current effects on *C. elegans*

- Frequency : 3x (from 1.7 to 2.6Hz with a step of 0.3 Hz = 3 times) = 9 times
- Amplitude : **(1 to 10 V, step = 1 V, AC and DC = 20 times)**
- Controle + : 3 times
- Controle - : 3 times

Ethical question/problem:

Our problem is that because we have a limit of time and of material, we don't really know how we can keep our C.elegans alive and recover our materials.
Because we can recover the plate and the models if we clean all the things with water, but the C.elegans died.
And at the same time, if we keep the C.elegans in the plate, with the models etc they don't have food in their media, so they die too.

Setup number 3 :

<u>Due to another set of problems we had to change the protocol again, and again, and again :</u>

- The power supply could not deliver enough intensity for the electric field to be sensed either by the movuino or by the organisms.
- The method we thought would work to study the C. elegans was not really effective, since getting rid of the chips forced us to study 1cm / 2cm cubes of agar which contained too much organisms, that above all, would not react to the electric field.

Therefore, we decided to study most of the organisms that were present in the lab :

	C. elegans	*Chironomus plumosus*	*dionaea*	*Artemia salina*	*dinoflagella*
Electric field reaction	Non responsive	Non responsive	Non responsive	Non responsive	Non responsive

All these non response might be due to the fact that the intensity of the power supply was not strong enough, and therefore the electric field was not strong enough.
It can also be due to the agar that seemed to be quite resistant, and that reduced the voltage of the electric field induced.