Frozone Protocol

Introduction **3**

Software Information **3**

Build Information **3**

Setup **4**
- Global setup 4
- Hardware 5
- Software 6
- Sample preparation 6

Thermal Hysteresis Assay **7**
- Cycle 1 7
- Cycle 2 7

Aprifreeze

Introduction

Software Information

The EDNA software uses the Python VISA library to communicate with the power supply and the multimeter via USB. To use it, NIMAX must be installed on your computer. Python with all the necessary libraries as well must be operational on your computer.

Build Information

The design is pretty homemade.

What you will need:

- Peltier cell with a heatsink or commercial thermoelectric cooler (we used the Wakefield Thermal 40W Direct-Air Thermoelectric Assembly - TEMA-AP-40-12)
- Acrylic plate for laser cutting the vacuum chamber
- Plexiglass for the top plate
- Rubber pad for laser cutting the vacuum seals
- Vacuum outlet we used a festo QS-1/2-12
- A small Copper plate (1cm x 1cm)
- ⅛ Screws
- A power supply (600W powerful one) (we used Sorensen DLM6010) with GPIB0 interface
- A digital thermometer, we used a thermocouple linked to a multimeter

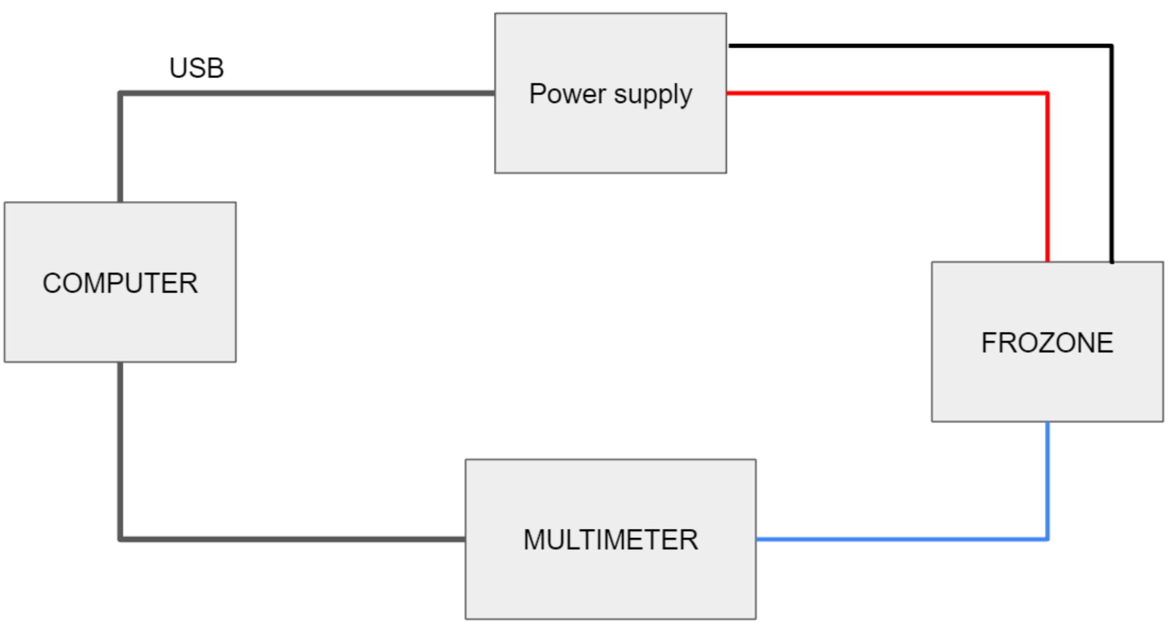
Step by step:

- Use the template you can find here (https://2021.igem.org/wiki/images/4/4f/T--UNILausanne--PRINT_Acryl.pdf) to cut acrylic and glass plates with the laser cutter.
- Cut rubber vacuum seal parts with the same template as step 1.
- Glue the two layer of Acrylic together
- Glue rubber to Acrylic parts.
- Screw the complex to thermo electric cooler

It should look like this:

Setup

Global setup



Hardware

1. Turn on the multiplug.
2. Plug the USB cables from the power supply and the multimeter to the computer. The blue USB should be plugged to the middle input if you don't want to code later on.
3. Switch on the DLM6010 (the black power supply box, the switch is on the left). The LCD should be turned on and indicate 0 V and 0 A.
4. Switch the multimeter to the temperature preset by turning the knob to the 🌡️ position and pressing the shift button.
5. Turn the light source on and center the beam onto the sample holder

Software

1. Open the NIMAX software. Under *devices and interfaces* select the last one. Next scan instruments. *DLM6010* should appear under *devices and interfaces*.

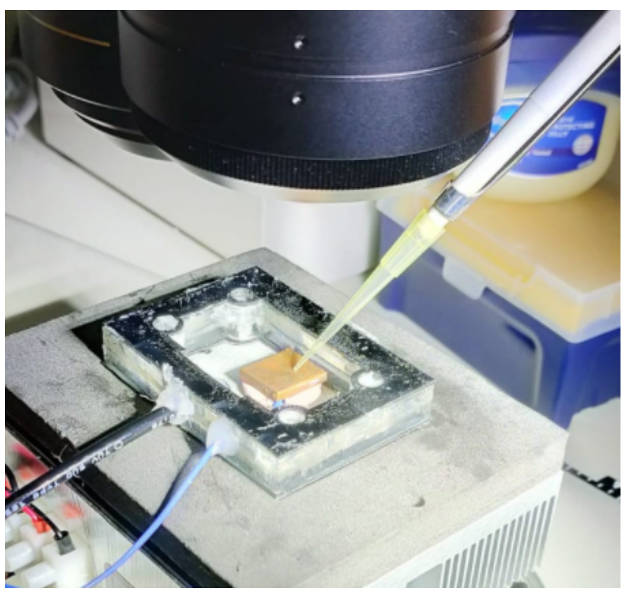
2. Open *OBS* (screen recording software) an *NIS elements* (microscope software)

3. Open the windows powershell and type **conda activate igem2021 (or your python environment)**. (To open powershell type *powershell* in the search bar). Next type **spyder**. Open and run *frozone-propre.py* once samples are ready.

Sample preparation

1. Add a 10 µl drop of your sample on the copper sample holder (at 5°C to avoid degradation). Make sure there are as few bubbles as possible.

2. Add vaseline to the joint and the window. Turn on the pump and place the window on top of the device or desiccant gel.



Thermal Hysteresis Assay

Cycle 1

A first cycle is conducted to determine the freezing point of the sample in absence of ice nucleating centers (other than the copper surface) and to get an idea of the melting point of the sample in order to save some time.

1. DON'T FORGET TO RECORD THE SCREEN
2. Insert -20°C as the target temperature.
3. Insert -6°C once the sample is completely frozen. Note the melting temperature.
4. If the sample has not yet melted, insert 0°C.

Cycle 2

Now that we know approximately at which temperature the sample freezes and melts, we can reconduct a cycle with more precision.

1. Insert -20°C as target temperature.
2. Insert 1-2°C **higher than the previously noted melting temperature**.
3. Use the arrows to reach the melting temperature aka isolate a single crystal.
4. Get the crystal to be stable.
5. Gradually increase the temperature until burst or growth. The growth may be slow depending on the AFP.
6. Repeat this step as often as you wish.

Once your essay is completed save the output *Temp.xls* file as *afp_name-concentration-attempt.xls*. Do the same for the video and upload them to the drive.