Developing an Arduino-powered cryostage

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Goals

Measuring antifreeze activity is classically done by quantifying thermal hysteresis. This measurement is sometimes biased and it is always time consuming.

The goal of this project was to create a fast, unbiased and user-friendly method for detecting antifreeze activity. To this end, detection of recrystallisation inhibition, *IRI*, was chosen over thermal hysteresis. The effect is easier to detect, see *Fig. 1*, and works at much lower protein concentrations than thermal hysteresis.

TH measurements are typically performed on a nanoliter osmometer, and much of the inconvenience inherent to the method stems from this instrument.

The Clifton Nanoliter Osmometer, see *Fig. 2*, is an instrument that has been unrivaled for decades in terms of temperature precision. This extreme precision is an absolute must for TH quantification, but certainly overkill for IRI measurement, which just requires a stable temperature for extended time periods.

A new design for a much smaller and less costly cooling solution was therefore proposed as a direct result of this.

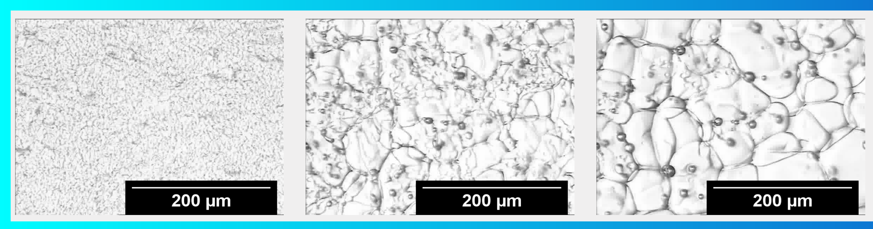

Figure 1
Recrystallisation in action: Frozen 300 mOsm PBS after 1, 5 & 15 minutes, respectively.

Figure 2
Left: Clifton control unit with monitor connected
Right: Clifton cryostage under the microscope
Unrivaled precision, but cumbersome and prone to bias.

Platform

The Clifton Nanoliter Osmometer consists of

I: a small temperature controlled stage
II: a temperature control unit.

Replacing it therefore logically requires two new components that work well together. To this end, the Arduino open hardware platform was chosen for development. More specifically the Arduino Uno R3 was used, owing to its low price and popularity, see *Fig. 3*.

The Arduino controls a larger power supply which powers a Peltier unit. This Peltier unit functions as a small solid-state heat pump, moving heat from one side to the other. The "hot" side is kept at a constant 5°C and the sample is placed on the cold side. Any current running through the system will essentially cool down the sample and stopping the current will increase the temperature towards 5°C. See *Fig. 4*.

In order to actually measure the effects of recrystallisation, a capturing scheme was set up using a microscope with a connected camera and PC. While this basic system is adequate for simply observing crystal growth, or lack thereof, a solution for quantifying said phenomena was also needed.

Using a FIJI (1), a variant of the popular ImageJ software package, a macro function was created to handle the large amounts of data captured by the camera, see *Fig. 5* for details.

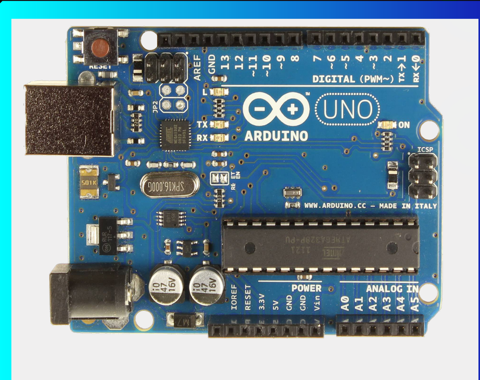

Figure 3
Arduino Uno. Sits at the heart of the control unit.

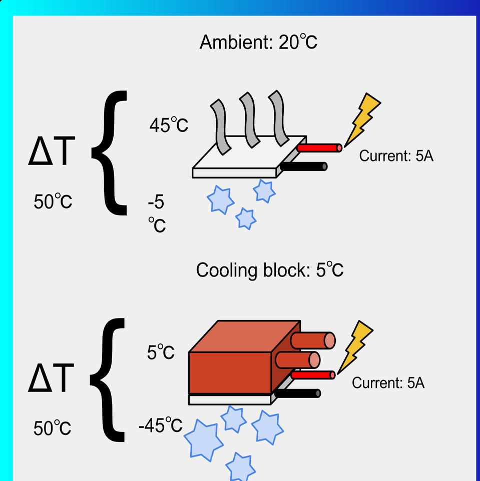

Figure 4
Peltier-unit schematic overview. The hot side is kept at a constant temperature, thus allowing for a much lower temperatures on the opposite side, compared to ambient.

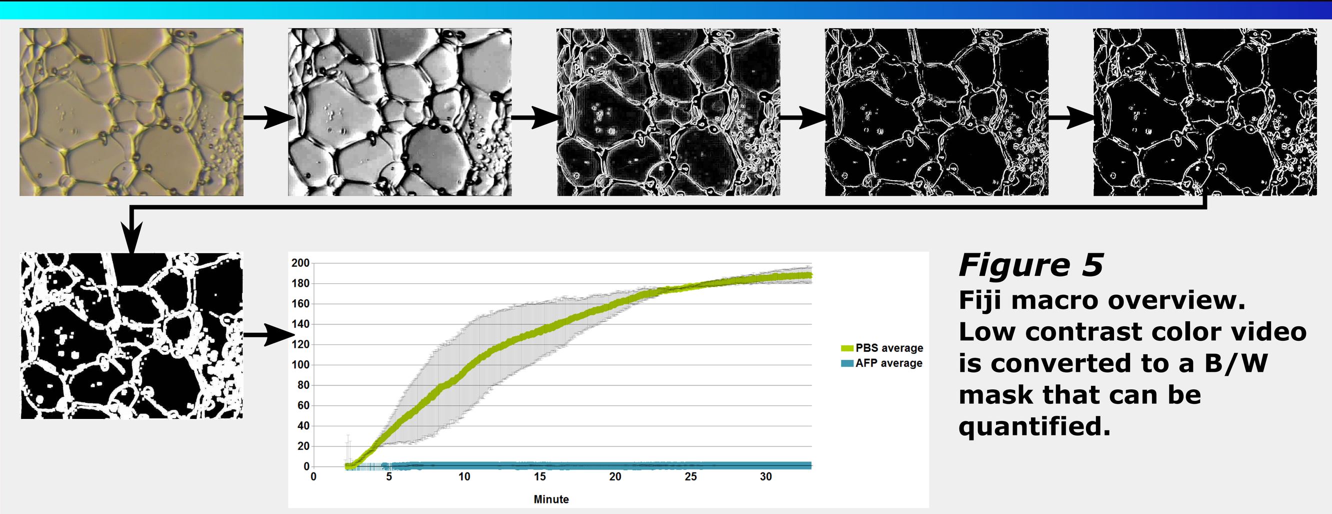

Figure 5
Fiji macro overview. Low contrast color video is converted to a B/W mask that can be quantified.

The product

The temperature control component of the cryostage is divided into a software part and a hardware part.

The software is a simple Arduino sketch which incorporates an open source PID algorithm, all of which can be freely accessed and copied from my online repository (2). The software is by no means static and can be improved upon when the need arises.

The hardware component consists of the control circuitry, which was printed in the Arduino Uno R3 shield form factor, see *Fig. 6A*. Not all I/O pins were used in this project, so the shield supports future sensory and communication upgrades.

The Arduino powered cryostage seen in Fig. 6A+B wins over the Clifton by a large margin when it comes to the physical space it occupies. It must be stated that the temperature control is only accurate to ~0.1°C, which means that the Clifton wins by a factor of 100 in this department. The extremely high accuracy is however not needed for IRI assays, and that was part of the original motivation for developing this solution.

Fig. 7 shows the method I developed for loading IRI samples into the cryostage.

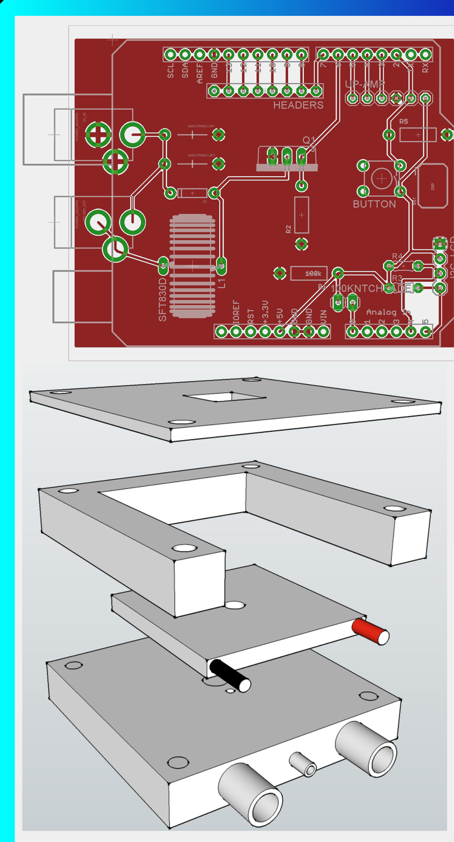

Figure 6
Cryostage version 3

Top:
Temperature control PCB made with EAGLE(3). Form factor is that of an Arduino Uno R3 shield.

Bottom:
3D sketch of cryostage. All parts made from aluminium. 2D output from SketchUp Make (4).

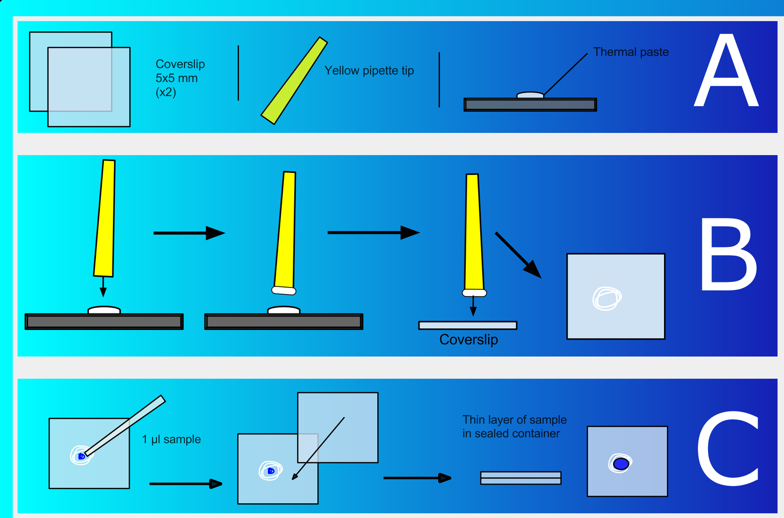

Figure 7
Overview of "Glass sandwich" loading method.

A: The components of the method.

B: Using the wide end of a yellow pipette tip, silicone paste is applied to a coverslip.

C: Sample is added inside the silicone mold and glass is added to create the seal.

Conclusions

Modern hardware- and software development is often an iterative process, and this project has been no exception. Versions 3 and 2 of the cryostage, seen in Figures 6 and 8, respectively, are a testament to this fact. Version 2 accurately holds a temperature setpoint, but is prone to condensation. Version 3 addresses this issue by allowing dry air to pass the sample chamber. Version 3 also has a powerful LED built in to provide light for bright field microscopy.

One of the biggest advantages of this instrument is the transparency of components. Not literally, as it is mostly made from aluminium, but every aspect of the design is freely available online. Even the software used to create the designs is all freeware. Anyone is able to copy, modify and improve upon the hardware- and software design. In fact they are invited to. To the author's knowledge, this is the only project of its kind.

The estimated cost of this cryostage with Arduino, excluding metalwork is around $150.

Future versions may include lenses and camera sensors in order to make the solution truly compact, self contained and inexpensive.

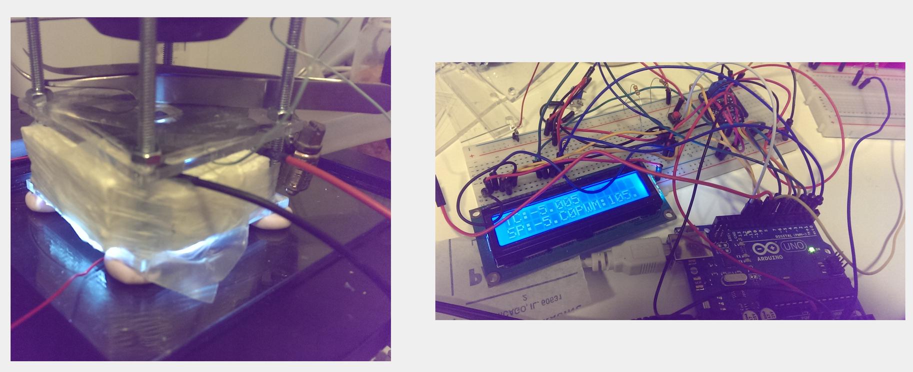

Figure 8
Cryostage v 2

Left: Cryostage with peltier mounted on cooling plate.

Right: Temperature control circuitry. Notice how the PCB has not been implemented yet.

1: Schindelin, J. *et al.* (2012), "Fiji: an open-source platform for biological-image analysis", Nature methods 9(7): 676-682
2: https://github.com/pechano/cryostage - GitHub repository containing everything related to the cryostage.
3: http://www.cadsoftusa.com/ EAGLE PCB Design Software
4: http://www.sketchup.com/ SketchUp Make 3D Modelling