The ”PEMpire State Building“ - An Automatic Plunge Freezer for Cryogenic Electron Microscopy

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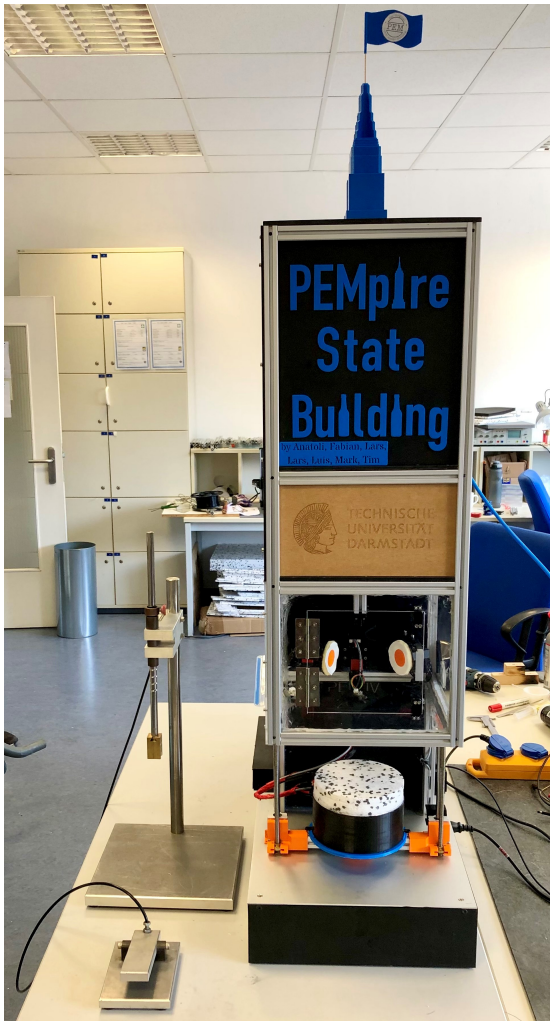

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1 Introduction

Hello and thank you for purchasing the revolutionary PEMpire State Building! We are happy to welcome you as a new customer. As this product is still in its prototyping phase, we kindly ask you to be indulgent when any errors occur and report it to us immediately. Thank you for beta testing our new product.

In order to safely operate this device please read the following instructions carefully.

The purpose of this document is to inform about the automatic plunge freezer, which was designed and built during the 2021 PEM IV project at TU Darmstadt. The main focus is on the explanation of the functionality of the machine and how to operate it safely. Therefore, it serves as a manual for the product.

1.1 Key Features

- Temperature controlled cryo bath containing liquid nitrogen and ethane
- Heated and moisturized air in upper chamber (37 °C, 100% humidity)
- Automatic blotting and plunging process
- Custom Windows application for controlling the machine

1.2 Scope of Delivery

- ”PEMpire State Building“ plunge freezer
- Power cable
- Custom side tray for putting the cryo bath
- ST-Link
- Various spare parts

1.3 Requirements for Operation

The plunge freezer needs a constant pneumatic connection to a source of compressed air, e.g. a compressor. It requires a minimum of 2 bar and maximum of 10 bar for operation.
For the blotting process, at least one blotting paper and one teflon paper is needed. Both of these papers need to have a circular shape and a hole in the middle.
Additionally, a large amount of liquid nitrogen should be available to the user, so that the cryo bath can be filled and refilled if needed.
The plunge freezer itself should be cleaned regularly! The cleaning should at least include the upper chamber and the climate chamber to ensure a long-term operation. Therefore, it is useful to position the plunge freezer on a large desk, that allows for access to the front and the back of the machine.

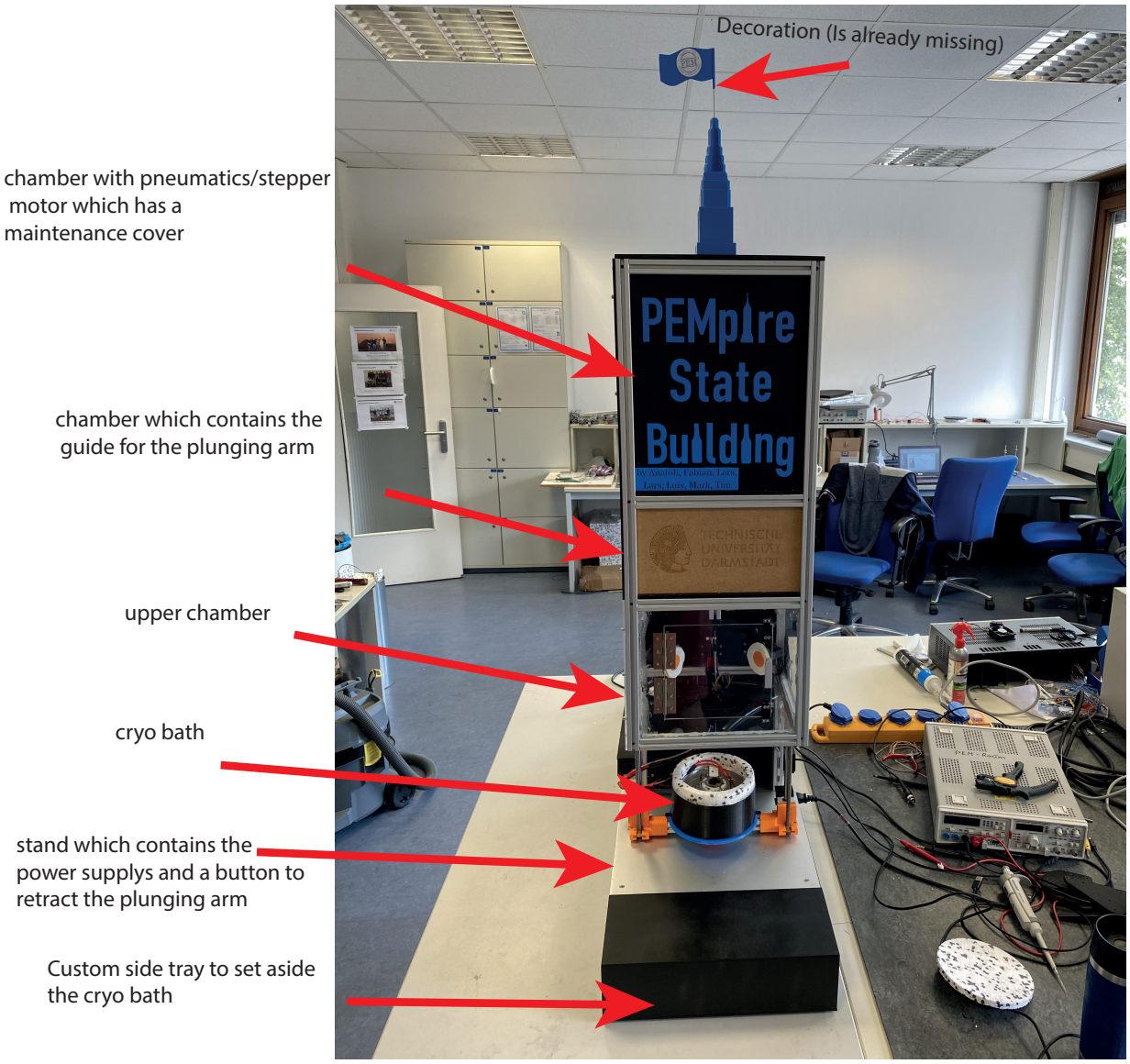

Figure 1: Overview of the PEMpire State Building.

2 Safety Instructions

This product contains various moving parts, as well as heated and freezing components. Therefore, it must be handled with care at all times!
The water in the climate chamber (in the back of the machine) is heated during operation. The heating element gets **very hot**, so make sure that the water level is always sufficient to spread the heat. The cover of the climate chamber should be closed while the machine is active!
Contrary to the climate chamber, the temperature in the cryo bath is below -180 °C due to the liquid nitrogen. This requires a specific training to ensure the safety of the user and all persons involved.

 It is necessary to wear protective gloves when working with liquid nitrogen!

The vertical plunging arm in the middle of the machine is attached to a pneumatic cylinder and a stepper motor. The stepper motor is only used for slow movements, but the pneumatic cylinder ”shoots” down with high speed, if it gets triggered. Because of this, it should be avoided to have fragile parts directly below the plunging arm.
Even if the compressor is not connected to the PEMpire State Building, the plunging arm can still damage components inside the upper chamber, because the pneumatic cylinder does not maintain its fixed state.

 Do not operate the plunge freezer without connecting the pneumatic pipe to the compressor!
This can damage the tweezers and the sample!

3 Operating Instructions

This chapter describes how to operate the PEMpire State Building and use the PC application.

3.1 Cryo Bath

The cryo bath is used to freeze the sample. Liquid nitrogen in the outer aluminum and the inner copper cup is used to cool down the whole bath. After the liquid nitrogen in the copper cup is gone and the temperature is low enough, ethane gas can be filled in the copper cup to get liquid ethane. The temperature control ensures that the ethane remains liquid to freeze the sample.

3.2 Upper Chamber

The upper chamber is used for the blotting process. The tweezers can be mounted on the plunging arm in the middle of the chamber using a screw. The sample can then be applied to the tweezers through the hole in the right wall of the chamber.

The blotting is done by two blotting arms, which remove excess water from the sample. For this step, the user has to apply the blotting paper on one of the arms and the teflon paper on the other arm. These papers are held by magnets and can be clipped to the circular mount.

⚠ Keep the window in the front closed during the entire operation! This ensures the safety of the user and keeps the air inside hot and moisturized.

3.3 GUI

The application is written in Python and can be executed on any computer with an ”Windows” operating system. It is not necessary to have Python installed for using the application.
In fig. 2 you can see the main window of the application. In the center contains information about the current status of the process. The progress bar shows how much of the process is completed.

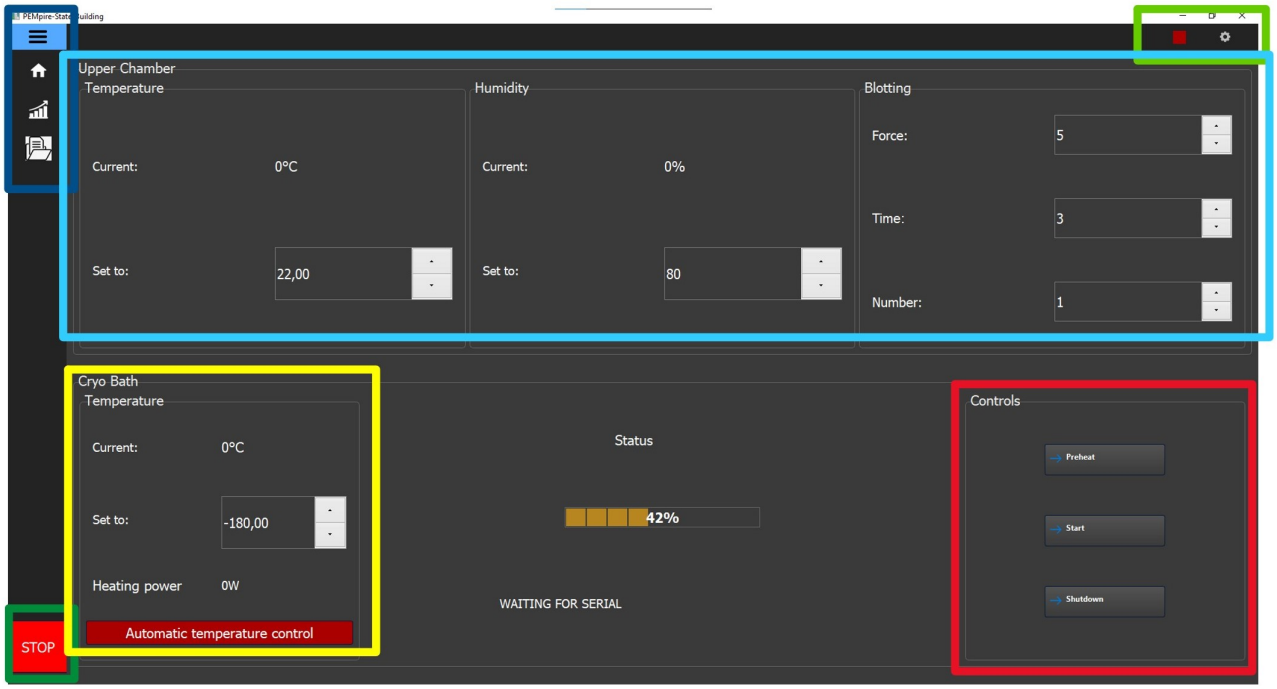

Figure 2: Main window of the application.

On the top left in the dark blue box you see the ”hamburger menu” to navigate to the different tabs of the program.
The tabs are: ”Home”, ”Plots” and ”Data”. In the green box, there is a USB connection indicator and the settings button. The connection indicator turns green when a connection to the PC application is established.
The cyan box called ”Upper Chamber” contains the settings for the temperature and humidity in the upper chamber and the settings for the blotting process. The blotting force influences the pressure of the blotting arms on the sample during the blotting phase. ”Time” contains the blotting time in seconds and the field ”Number” sets the number of blotting phases, that shall be executed before freezing the sample. The yellow box ”Cryo Bath” contains the settings for the cryo bath. The Button ”Automatic temperature control” activates the PID controller for the cryo bath. It should be activated after the cryo bath is cooled down with liquid nitrogen. In the dark green box is the emergency stop button. You need to restart the device after an emergency stop! The red box ”Controls” includes buttons for preheating, starting the blotting and plunging process and for the shutdown of the device.

In the image below, you see the settings page of the application (fig. 3).

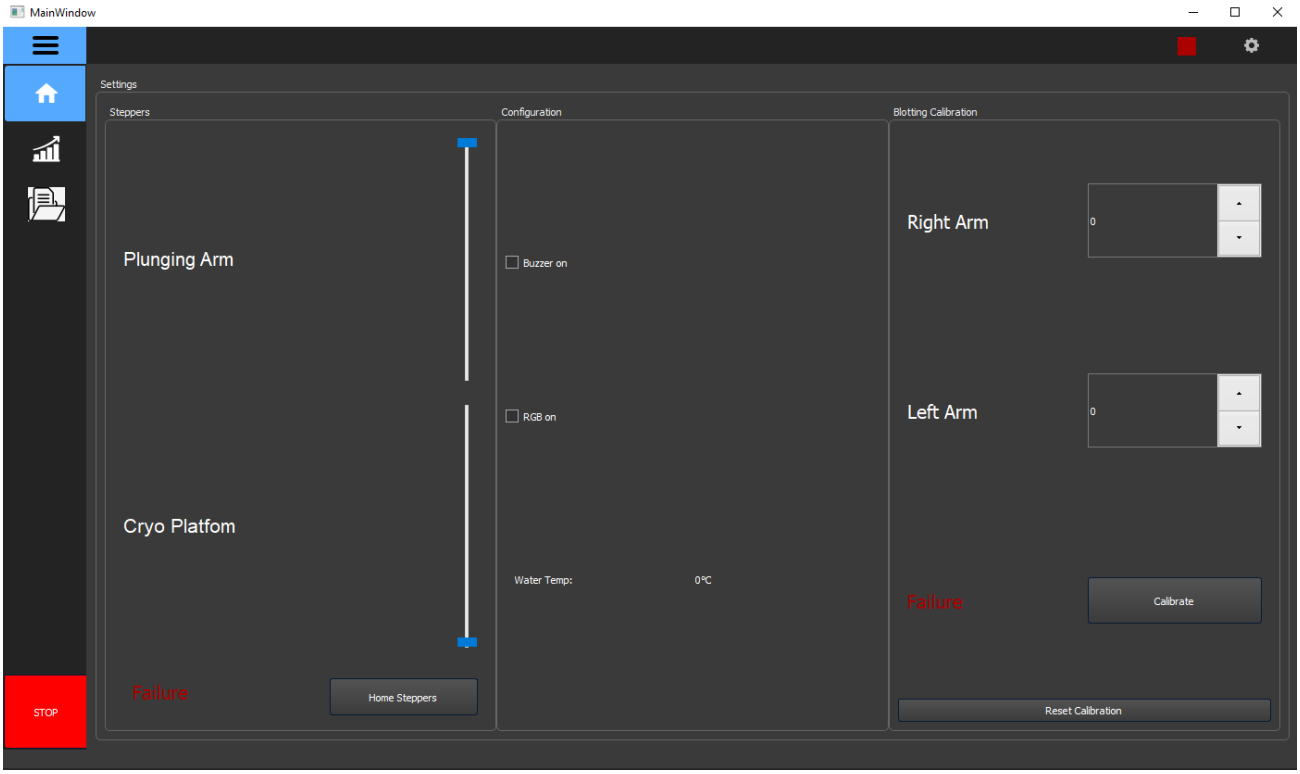

Figure 3: Settings page of the application.

The box ”Steppers” contains sliders to manually move the plunging arms and the platform of the cryo bath. Move the stepper motors with caution.
Reminder: You can always press the ”STOP” button, if something unexpected happens.

On the bottom right is the ”Homing” button for the steppers, which sets the current position as the new ”Home” position.

⚠ Move the steppers with caution, especially when the tweezers are installed!

On the right you see the box for the calibration of the blotting arms. To calibrate the arms, place the tweezers in its mounting and move it to the blotting position using the slider. Next, increase the calibration offsets for the left and right arm until they barely touch the tweezers. There should be no one-sided pressure acting on the tweezers. After the positioning is finished, press ”Calibrate”. To reset the current calibration press the ”Reset Calibration” button at the bottom.
The next image shows the ”Plots” tab, which contains the sensor readings of the temperature and humidity in the upper chamber and the temperature in the cryo bath.

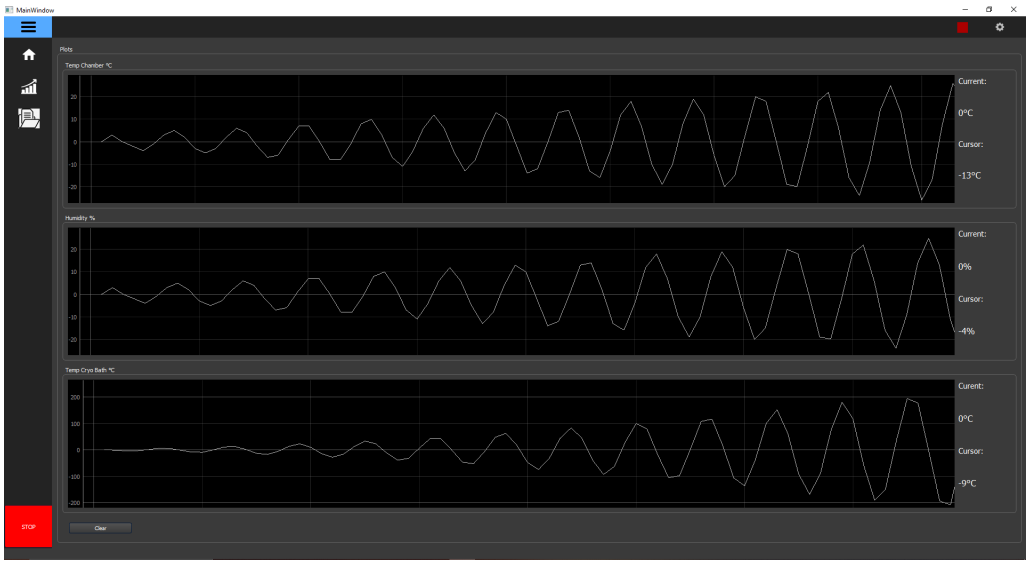

Figure 4: Plotting window of the application.

You can adjust the plots dynamically by zooming or moving them with the mouse. The axis can be adjusted automatically to fit the current plot. To activate this automatic axis adjustment after manual zooming, press the ”A” in the bottom right of the plot.
You can clear the current state of the plots using the ”Clear” button.

3.4 General Procedure

1. Activate the PEMpire State Building and connect the GUI via USB to your computer.
2. Connect the compressor to the pneumatic pipe.
3. Check that the water level in the climate chamber is sufficient.
4. Preheat the upper chamber.
5. Fill the cryo bath with liquid nitrogen.
6. Apply the grid to the tweezers and the tweezers to the plunging arm.
7. Wait until the correct temperatures in upper chamber and cryo bath are reached.
8. Activate the automated temperature control in the cryo bath.
9. Fill the ethane in the copper cup.
10. Apply the sample to the grid using the pipette.
11. Start the blotting & plunging process.
12. Wait for the operation to finish.
13. Remove the grid from the tweezers and store it in the storage box inside the cryo-bath.
14. Push the ”Restart” button in the GUI.

Use the styrofoam lid to better maintain the temperature of the cold bath, if the bath is not used for several minutes.

⚠ Make sure to take the lid off again before starting the next freezing process!

4 Theory of Development

This chapter describes the theory and thought process behind certain decisions in the design of the PEMpire State Building.

4.1 Electronics

The main part of the electronics is the main PCB (fig. 5). The main component on this PCB is a STM32L462 microcontroller. It is responsible for controlling all of the electronics and communicating with the GUI. Furthermore, you can find the headers and ICs for several sensors and actuators on this PCB. The names and pins for the project in the STM32CUBE IDE can be found in figure 5.

In order to operate reliably, a 24 V/10 A and a 5 V/3 A power supply is required. The inputs are properly buffered and fused.

In order to program the microcontroller, a ST-Link device is required (included in the delivery). Just plug in the JST header and connect the ST-Link to your computer. An external power supply is required.

The heater elements are supplied by an external PCB (fig. 6). This PCB requires an external 24 V/18 A power supply which is different from the other 24 V power supply on the main PCB. The heaters are controlled by the main PCB.

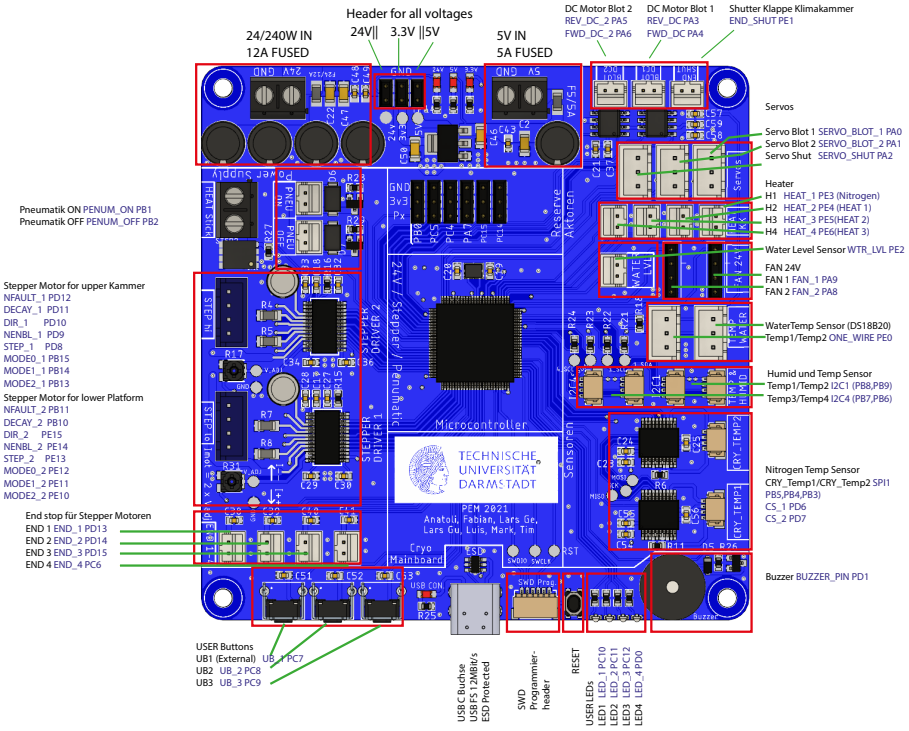

Figure 5: Description of the main PCB. The corresponding names and pins in the STM32CUBE IDE of each feature are shown in blue.

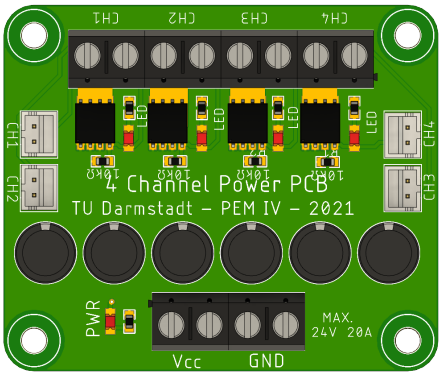

Figure 6: The Power PCB. CH1 = Nitrogen heater. CH2 - CH4 = Water heater.

4.2 Cryo Bath

The idea for the design of the bath comes from [1]. To achieve reasonable temperature control while maintaining low heat and infrequent refilling of liquid nitrogen, the copper and aluminum cups are lined with styrofoam and cooling copper arms extend from the copper cup into the liquid nitrogen of the aluminum cup. A heater and temperature sensor are contacted with the bottom of the copper cup. Unlike in [1], four cooling arms are needed to provide enough cooling power to the copper cup. This is because silver was used as the solder in [1]. The thermal conductivity of silver is higher than that of copper. In this work, indium was used, which has a lower thermal conductivity than copper.
A good freezing temperature is near the melting temperature of ethane [1], which is -182.75 °C. For this reason, the ethane in the copper cup is controlled to a temperature of -181.0 °C.
In addition, the design is modular. This makes it easier to clean the cups. The two cups are additionally grounded (see the red crocodile clips in fig. 7) because the ethane is highly flammable.

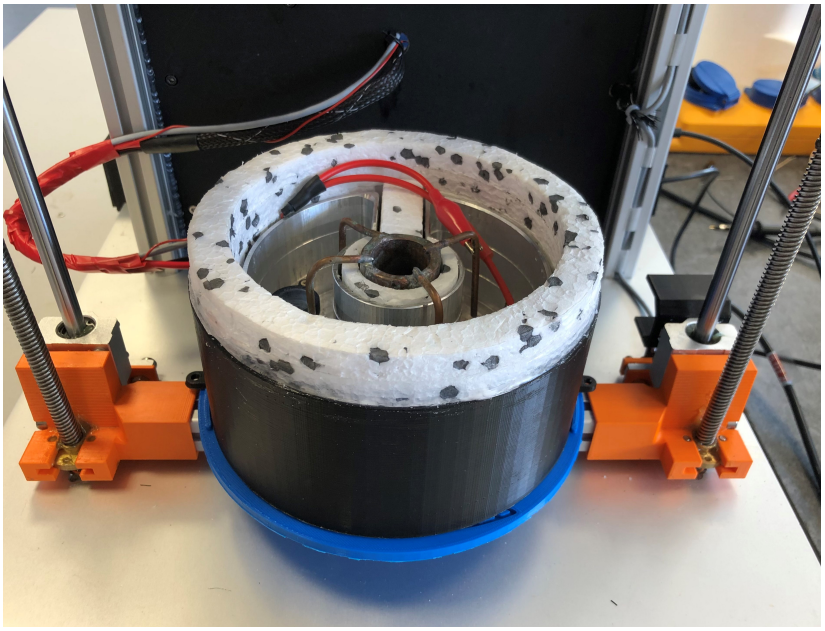

Figure 7: Close-up view of the cryo bath showing the copper cup with its four attached arms. The outside of the bath is isolated with styrofoam. The styrofoam on top of the bath keeps the nitrogen gas inside the bath, which forms an isolating atmospheric layer.

4.3 Upper Chamber

The air in the upper chamber is heated and heavily moisturized by the climate chamber. Therefore, it is imminent to seal all edges and corners with silicon. Because of the high humidity, the wooden material inside the chamber needs to be painted with weather-resistant lacquer. The electronic contacts are isolated with silicon as well.
The height of the upper chamber and the height of the whole PEMpire State Building are chosen to enable the tip of the plunging arm to move from the ceiling of the upper chamber as the highest point to the bottom of the cryo bath in the lowest position. This is necessary to perform the proper plunging mechanism.
The shutter at the bottom prevents the cold air from the cryo bath below to mix with the warm air in the chamber. This will keep the temperature at the tip of the tweezers, where the sample is located, mostly constant and reproducible for multiple measurements. When the shutter opens, it triggers a mechanical switch whose output signal is used to ensure that the shutter is actually open.
On top of the shutter is a temperature sensor attached to a tilted ”roof”. This is supposed to keep the water from dropping down directly into the sensor.
If maintenance in the chamber is required, the left side of the chamber can be removed to allow easy access to the inner components.

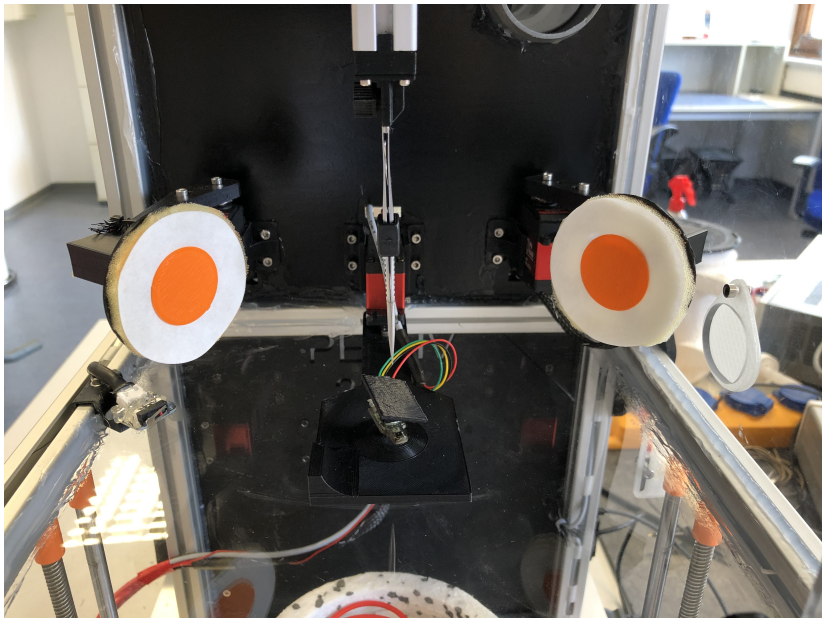

Figure 8: Close-up view of the upper chamber showing the two blotting arms, the shutter at the bottom and the tweezers in the middle. The blotting papers are held in place by the orange clips, which attach magnetically to the blotting arm.

4.4 GUI

The application is written in Python 3.8 and the graphical user interface is created using the ”QT Designer”. It comes packaged as an ”.exe” file, so that the user does not need the development tools to execute it. However, for further development of the GUI, the installation of the QT Designer, as well as Python 3.8 (or newer) is required. The source code is well documented and has been uploaded to a Git repository. This enables the user to modify the application easily.

5 Warranty & Support

The developers assume no liability for any damage caused by incorrect usage of the product.
If you have further questions or problems, which could not be solved by reading this document, you may contact one of the developers:

tim.maier@stud.tu-darmstadt.de
luis.durillo@stud.tu-darmstadt.de

References

[1] Christopher J. Russo, Steve Scotcher, and Martin Kyte. “A precision cryostat design for manual and semi-automated cryo-plunge instruments”. In: *Review of Scientific Instruments* (2016). DOI: 10.1063/1.4967864.

List of Figures