

Ice Nucleation Controller

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Abstract

Ice Nucleation Controller is a system used to perform high precision ice nucleation experiments. System temperature range is from +10°C to -30°C. A specified temperature gradient is held throughout experiments using a cooling circuit consisting of a closed water loop and a PID controlled pair of peltier elements. Temperatures are measured using a FLIR A655sc thermal camera and run-time calibrated using a thermistor in a fixed calibration cavity. Freezing temperatures for each sample are estimated using extracted temperature gradients and can be used for further processing.

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1 Safety Information

1.1 General safety instructions



DANGER

Danger to life or serious injury can occur when live parts are touched. Do not touch or modify electrical installations without proper training or guidance.



WARNING

Danger to life, serious injury, or equipment damage can occur due to incorrect handling of equipment. Only trained personnel may service or modify system.



CAUTION

Risk of skin irritation or burn when handling gallium. Always handle with care in both solid and liquid form.



CAUTION

Risk of adverse health effects from long-term use and high humidity build-up if not ventilated. Always operate system in properly ventilated room.

NOTICE

Risk of damage to sensitive equipment or loss of calibration due to incorrect handling. Always handle equipment marked SENSITIVE with care.

NOTICE

Risk of damage to camera lens or calibration due to incorrect handling. Under no circumstance touch or affect camera lens. Always transport camera with lens-cover attached.

NOTICE

Risk of damage to cooling base fittings and seals due to tube-connection strain. Always be cautious when manipulating tubes and cooling base.

2 Overview

2.1 Components

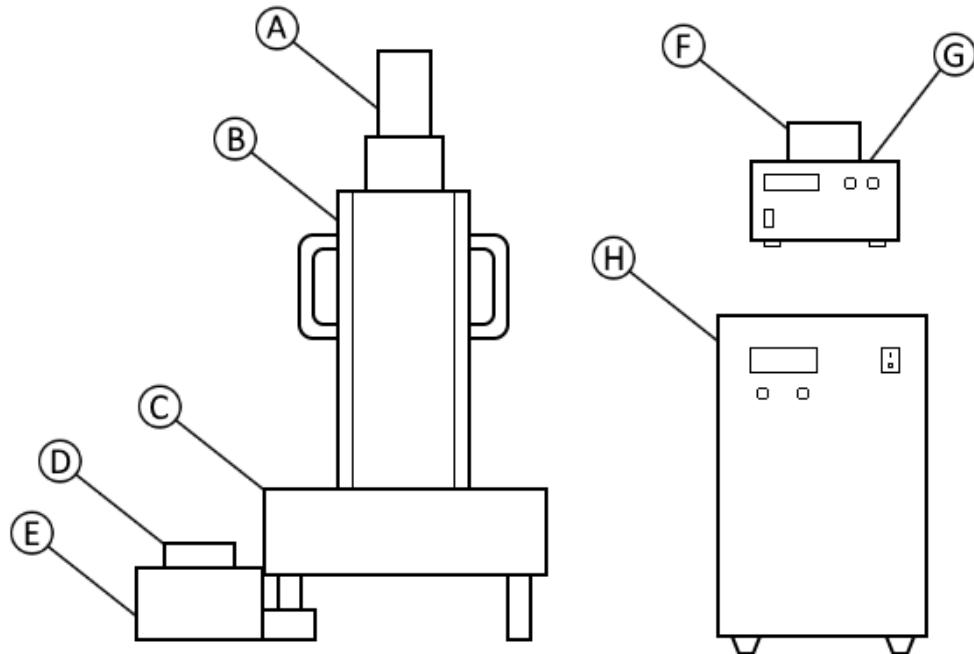


Figure 1: System Components.

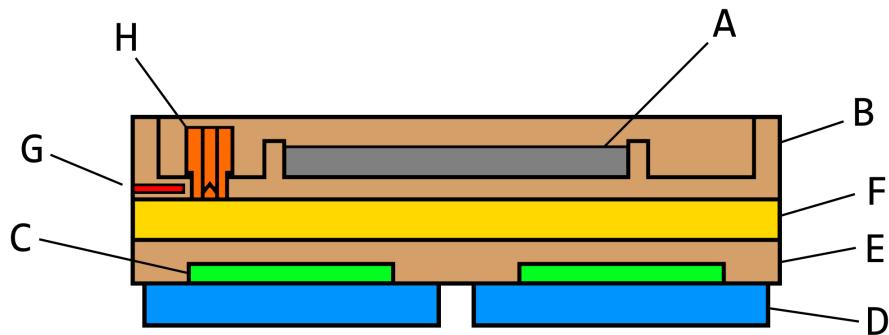
2.2 Description

Component	Description	Notes
A	Thermal Camera	FLIR A655sc (SENTIVE) [1]
B	Camera Tower	-
C	Cooling Base	Protruding Sensor Cables (SENTIVE)
D	cDAQ Module	NI 9219 and cDAQ-9171 [4] [5]
E	Sensor PCB Housing	Protruding Sensor Cables (SENTIVE)
F	Peltier Power Switch	MCU and 4x SS Relay [9]
G	Power Supply	PeakTech (P1580) [7]
H	Water Cooler	Alphacool Eiszeit 2000 Chiller [6]

3 System Description

3.1 Cooling Base

One primary component of INC is the Cooling Base. Temperature controlled gallium (**A**) contained in the Top Copper Base (**B**) houses the PCR plate. Temperature and gradient is controlled with a pair of PID regulated Peltier Elements (**C**), coupled with a Water Cooler Base Plates (**D**), both are mounted in the Bottom Copper Base (**E**). A Vapor Chamber (**F**) ensures a minimal distribution gradient. Control and calibration temperature is measured with a Thermistor Probe (**G**) inserted into the Top Copper Base. A Fix-point Cavity (**H**), used for run-time Thermal Camera calibration, is mounted directly in contact with the Thermistor Probe into the Top Copper Base.



Component	Description	Notes
A	Gallium	Above 99.99% purity
B	Top Copper Base	-
C	Peltier Elements	QuickCool HighTech 270W [3]
D	Water Cooler Base Plate	Aqua Computer cuplex kryos NEXT sTRX4 Full Cover [6]
E	Bottom Copper Base	-
F	Vapor Chamber	-
G	Thermistor Probe	TE Connectivity 2.252 kOhm [2]
H	Fix-point Cavity	-

3.2 Cooling Circuit

Temperature control is achieved with PID regulated peltier elements and a Water Cooling Loop. A water cooling pump circulates 5°C water (T_{In}) through a pair of Water Cooler base plates mounted against the peltier elements.

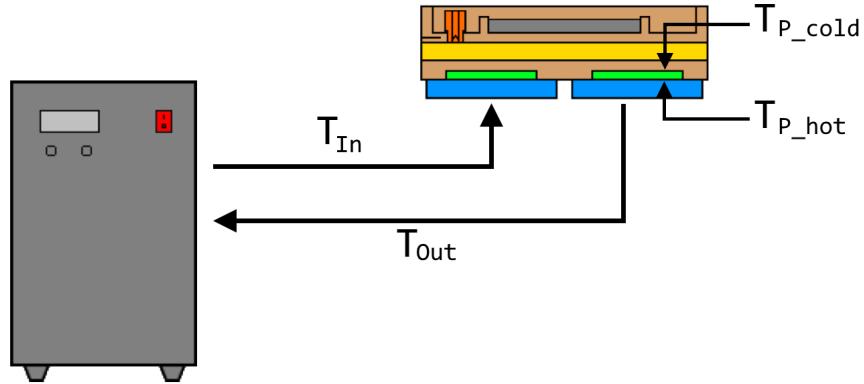


Figure 2: Cooling Circuit.

By creating a voltage potential over peltier elements a temperature difference is achieved between top and bottom (T_{P_cold} and T_{P_hot}). An increased voltage potential increases temperature difference.

Temperature difference between T_{P_hot} and T_{In} creates energy flow out of the Cooling Base, heating up the return water T_{Out} . T_{P_cold} reduces top temperature of the Cooling Base, lowering Gallium and PCR plate temperature.

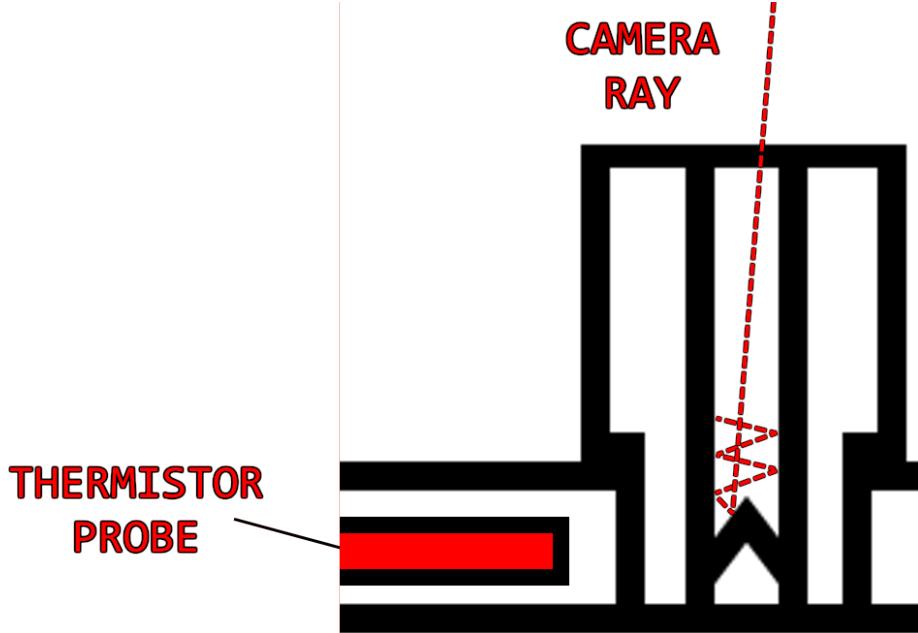
3.3 Temperature Measurements

Two temperature measurement devices are present in INC. A Thermal Camera (FLIR A655sc) and an NTC Thermistor. The thermistor acts as primary system temperature measurement and is the input for PID regulated peltier elements. Secondary use is for Thermal Camera reference calibration.

The Thermal Camera has a low precision ($\pm 2^\circ\text{C}$), but a high pixel-wise relative accuracy. To increase precision a fix-point cavity is used. Using the calibrated thermistor precision and accuracy, a reference calibration can be achieved for every image.

3.3.1 Fix-point cavity

The Fix-point Cavity is a black painted copper tube cavity with an angled bottom. The angled bottom bounces Thermal Camera rays inside the cavity. This ensures temperature measurements are not reflected and accurately measure the cavity temperature.



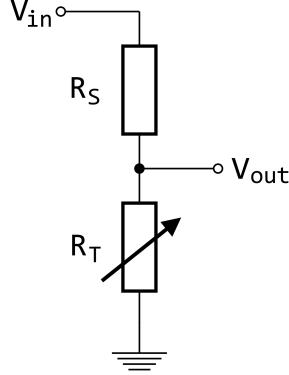
Thermistor probe is positioned directly against the Fix-point Cavity and is used as reference temperature. Offset between Fix-Point Cavity temperatures measured by thermistor T_{Fix_therm} and thermal camera T_{Fix_cam} is applied globally to Thermal Camera measurements as a direct offset.

$$T_{Cam} = T_{Cam} + (T_{Fix_therm} - T_{Fix_cam}) \quad (1)$$

3.3.2 Thermistor

A TE Connectivity 2.252 kOhm thermistor [2] is used for reference temperature measurements. A voltage divider circuit is used to measure change in thermistor resistance due to temperature changes. A Universal Analog Input Module (NI 9219[4] and cDAQ-9171[5]) measures voltage potential in the circuit.

Electrical Circuit consists of a 12VDC power supply, a 10 kOhm low variation, low thermal change fixed resistor [10] and 2.252 kOhm thermistor [2].



Measuring V_{out} and V_{in} allows for calculating thermistor resistance using Beta parameter equation. (Steinhart-Hart equation with $a = 1/T_0 - (1/B) \cdot \ln(R_0)$, $b = 1/B$ and $c = 0$) [8].

$$B = \frac{T_2 \cdot T_1}{T_2 - T_1} \cdot \ln\left(\frac{R_1}{R_2}\right) \quad (2)$$

$$R_{Therm}(T_2) = R_{25} \cdot e^{-\frac{B}{T_1}} \cdot e^{\frac{B}{T_2+273.15K}} \quad (3)$$

from equation (2) and (3) a Temperature-Resistance Conversion can be derived

$$T_{Therm}(R_{Therm}) = \frac{B}{\ln\left(\frac{R_{Therm}}{R_{25} \cdot e^{-\frac{B}{T_1}}}\right)} \quad (4)$$

From the voltage divider circuit the following equations can be derived

$$V_{out} = V_{in} \frac{R_T}{R_T + R_S} \quad (5)$$

$$R_{Therm}(V_{out}) = \frac{R_S \cdot V_{out}}{V_{in} - V_{out}} \quad (6)$$

From equation (4) and (6) a Temperature-Voltage Conversion equation can be derived

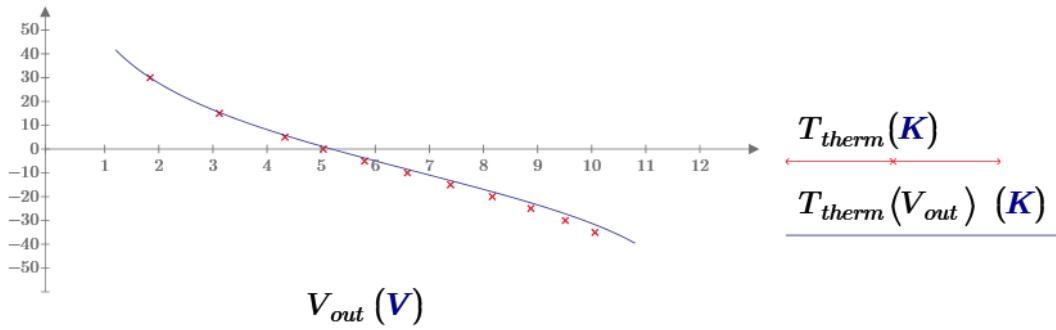
$$T_{Therm}(V_{out}) = \frac{B}{\ln\left(\frac{R_S \cdot V_{out} \cdot e^{\frac{B}{T_1}}}{R_{25} \cdot (V_{in} - V_{out})}\right)} \quad (7)$$

Calibration Procedure to calculate thermistor parameters B and R_{25} , is performed with an AMETEK Reference Temperature Calibrator (RTC-157) [11].

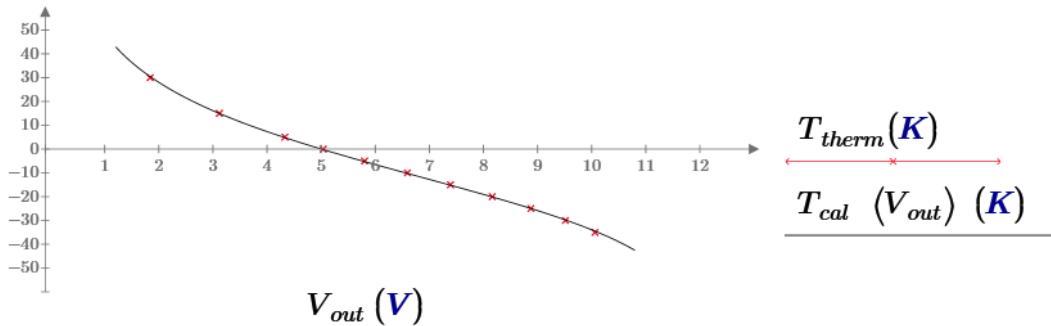


Thermistor is submerged in ethanol inside RTC calibrator. Temperature measurements are made at 30°C, 15°C, 5°C, 0°C, -5°C, -10°C, -15°C, -20°C, -25°C, -30°C, -35°C. Calibration starts at 30°C when a steady-state of +/- 0.001°C has been held for 5 minutes, data is sampled for 5 minutes. After a data point has been logged the next temperature is set and once steady state for 5 minutes is achieved, a new data point is logged. The full temperature range is measured 3 times.

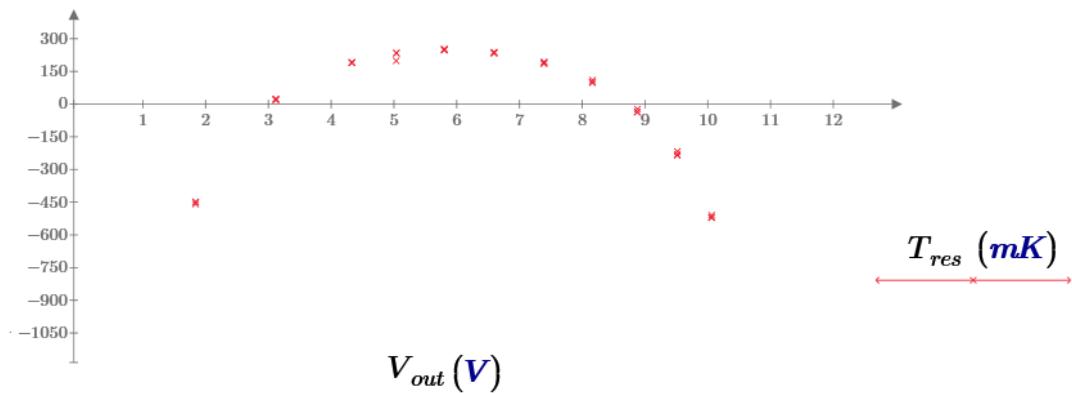
Fitting Parameters Manufacturer supplied parameters have a large variation and plotting measurements from calibration experiments against manufacturer supplied parameters result in a significant error.



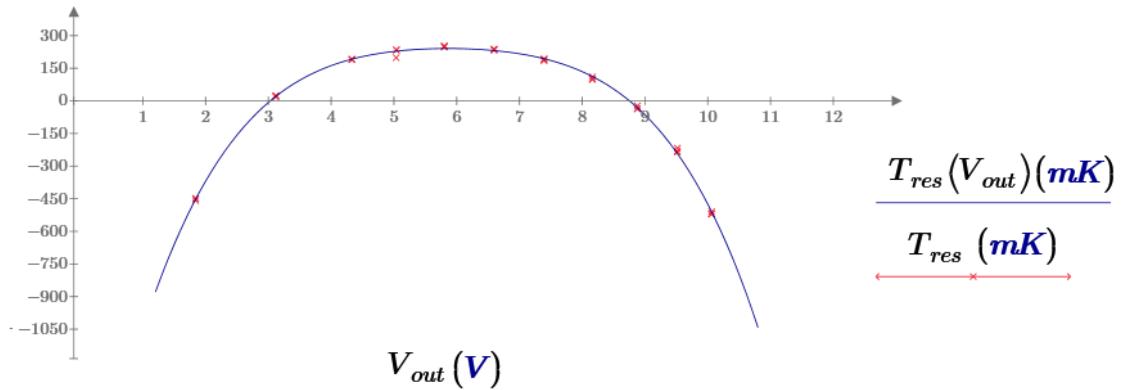
Fitting measured results to calibration data and solving for thermistor parameters B and R_{25} in equation 7 results in a better fitting curve.



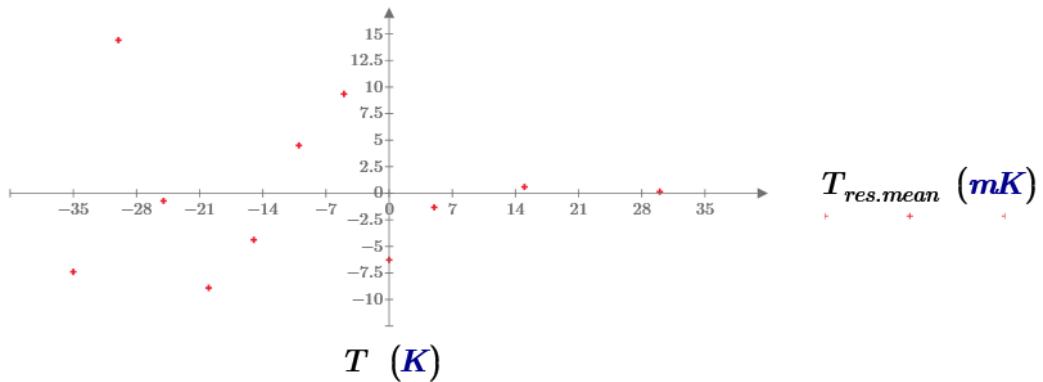
Plotting remaining residual errors as a function of the measured voltage shows a fourth order residual, which can be explained by the lower order Steinhart-Hart equation. Residuals range up to 450 mK.



A fourth order polynomial can be fitted to compensate for a large part of the residual error.

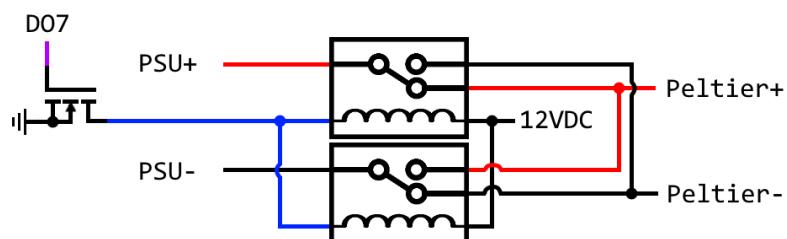


Applying the fourth order polynomial compensation results in a final residual mean error of +/- 15 mK from thermistor calibration measurements, within +30°C to -35°C.



3.4 Peltier Switching Circuit

Switching Peltier Element polarity alters which side heats up and cools. This is utilized to melt and freeze gallium to the PCR plate before experiments to improve thermal contact. A polarity switching circuit allows for software-controlled polarity changes. An MCU (Arduino Uno) sends a digital signal (D07) to an N-Channel Enhancement Mosfet (ZVN2110A) which switches peltier polarity making top side of peltier hot allowing for melting gallium.



4 Procedure

4.1 Starting the system

1. **Plug in Main Power Cable** 230VAC mains voltage.



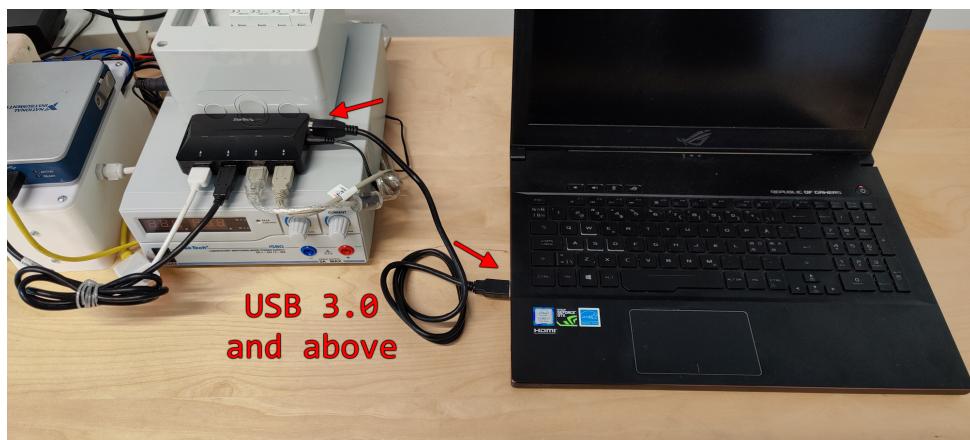
2. **Start Water Pump** It should beep once, if continuously beeping see 7.4.



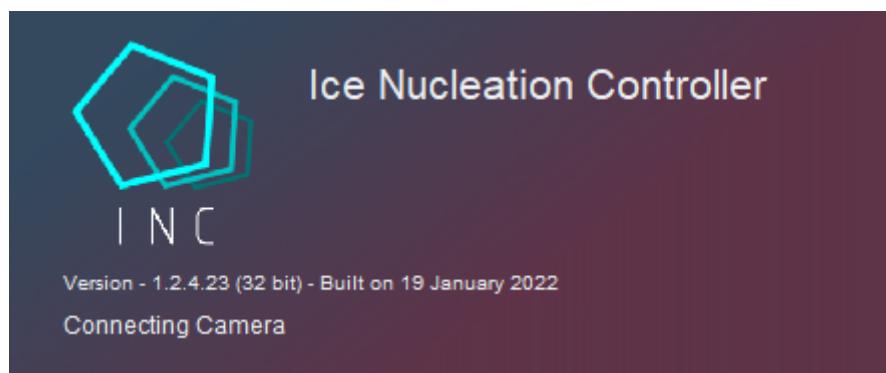
3. Start Power Supply It will write a sequence of start messages, once completed it should say either [O P OFF] or a low voltage and current (0 to 3 V).



4. Connect PC to USB-hub Only use USB 3.0 and above.

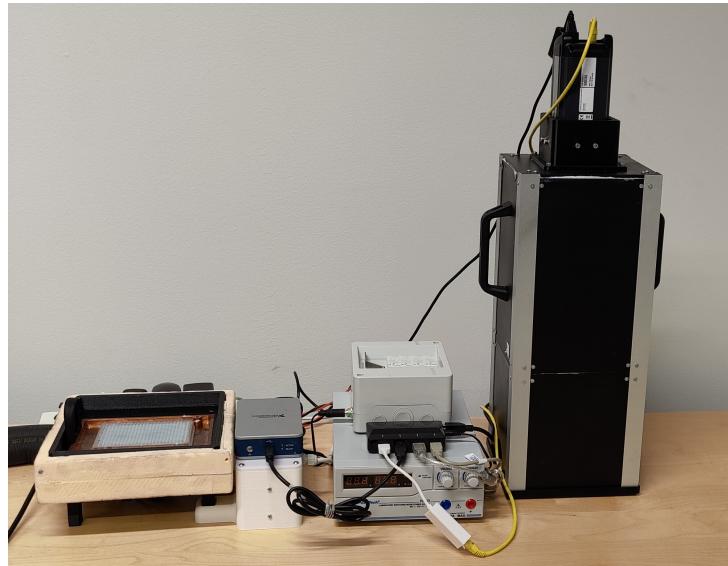


5. Start INC Software If everything is correctly setup no errors should appear.

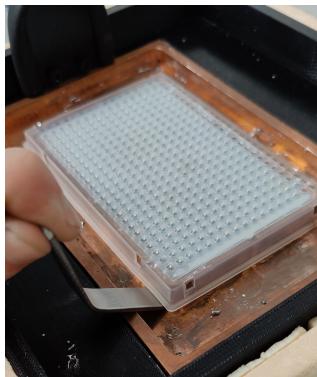


4.2 Preparing Sample

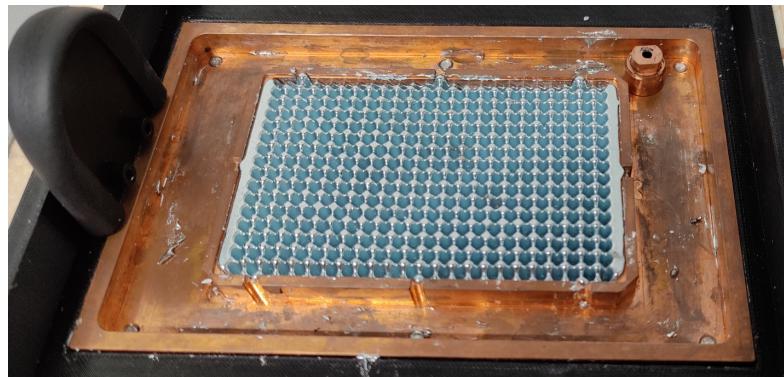
- 1. Remove Camera Tower** place to the side. Camera Tower fit is tight, wiggle slightly as you pull to loosen.



- 2. Remove Old PCR Plate** use crowbar tool to remove previous PCR plate if present.

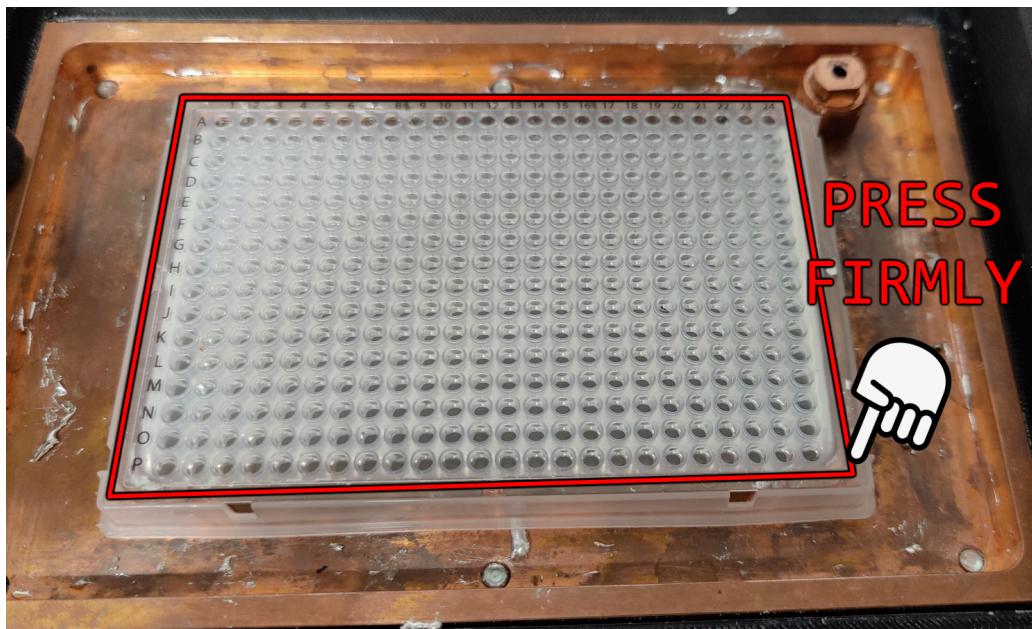


(a) Crowbar Tool

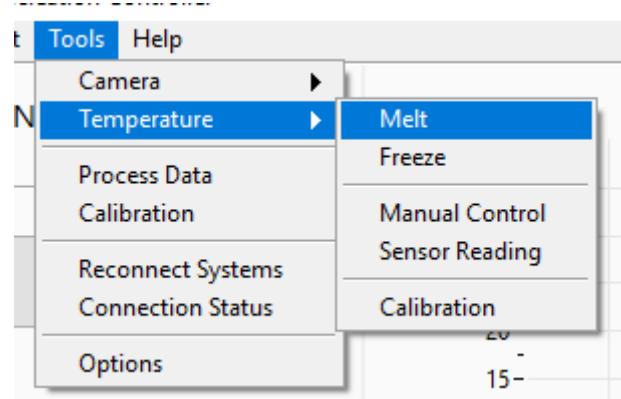


(b) PCR plate removed

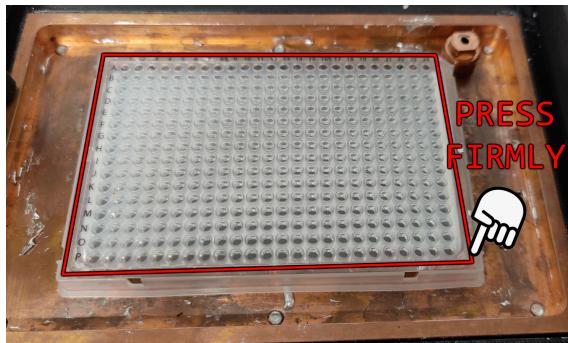
3. Insert New PCR Plate STERILE, make sure to press firmly on every corner and sides.



4. Melt Gallium with new PCR plate inserted [Tools] > [Temperature] > [Melt]. Wait until melting process is complete to continue.



5. When Gallium is melted, firmly press on every corner of PCR plate make sure PCR plate is firmly secured, pressing on every corner and side. Make sure to not press center of PCR plate as liquid gallium can spill out. See 7.2 in case of gallium spill.



(a) Press along edge

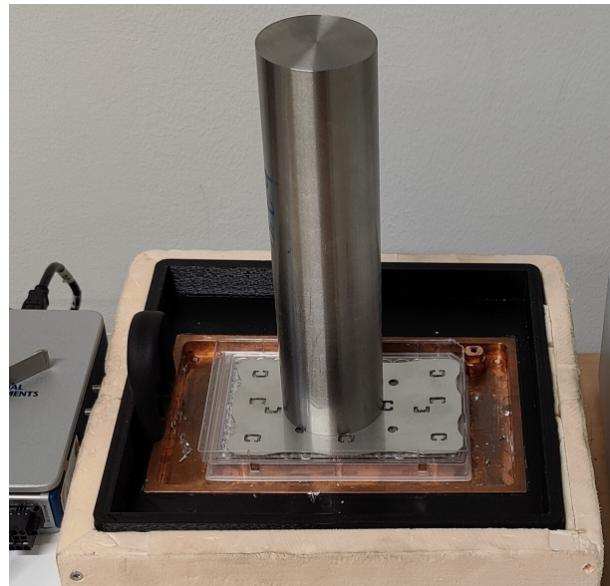


(b) DO NOT PRESS CENTER

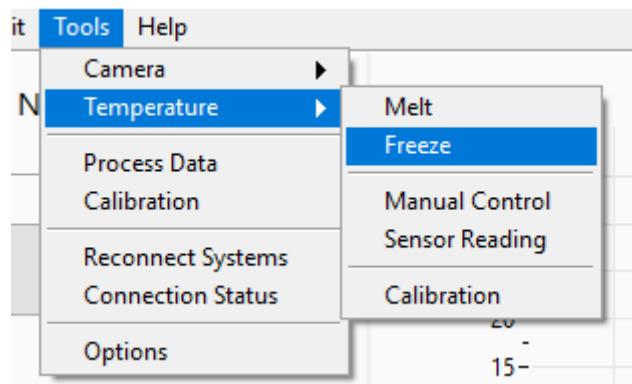


DO NOT PRESS CENTER

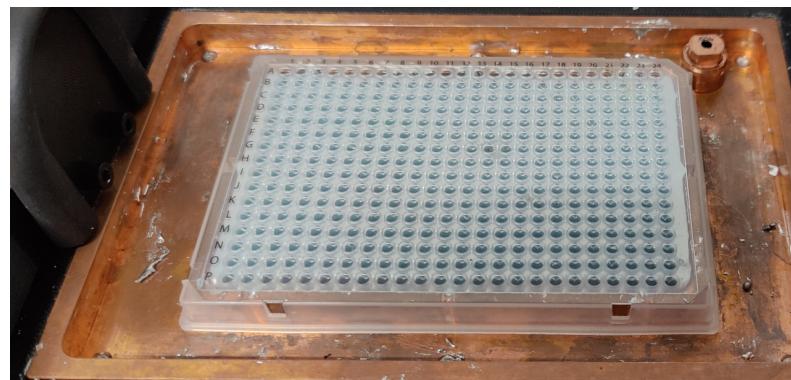
6. Place Lid, Plate and Weight STERILE on top of PCR plate to weigh down PCR plate during cooling process.



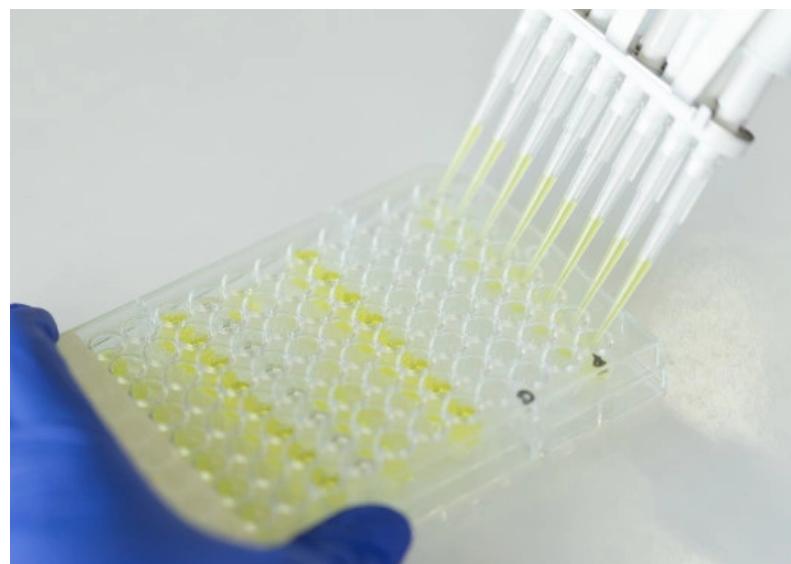
7. Freeze Gallium with **Tools** **Temperature** **Freeze** to ensure ideal thermal contact.



8. Remove Lid and Weight once freezing process is complete.



9. Fill PCR wells with desired content.

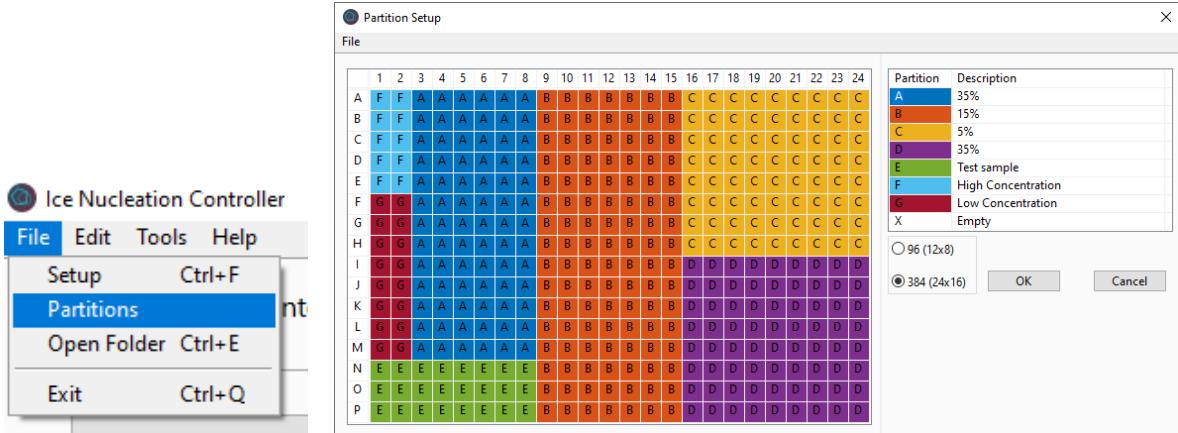


10. Place Camera Tower on Cooling Base make sure Camera Tower is firmly secured and pressed onto Cooling Base.



4.3 Configure Partitions

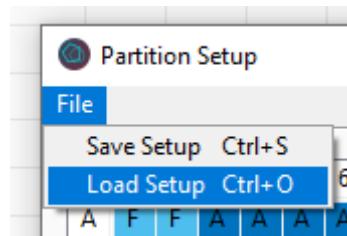
Open partition settings **Files > Partitions**.



(a) Open Partitions

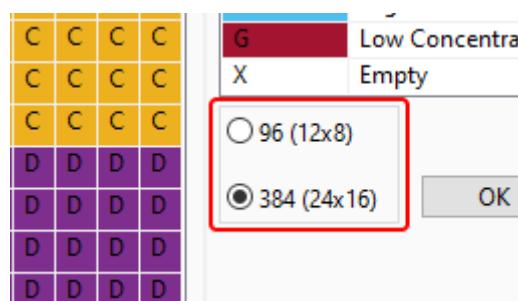
(b) Partition Menu

Load Partition (Optional) Load and edit previously created partition setup. If none are available, create a new Partition Setup.

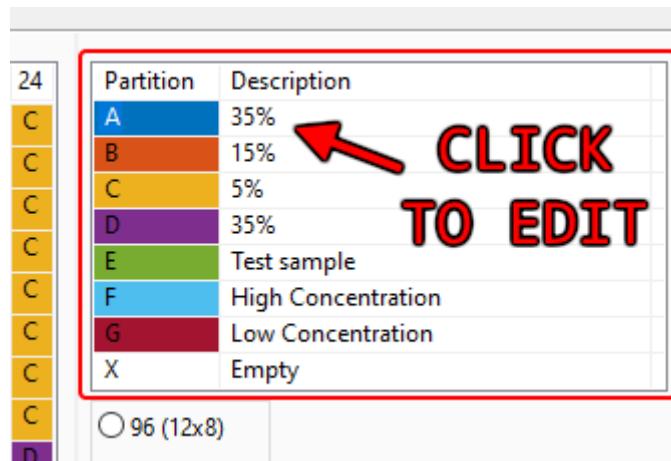


4.3.1 Creating a new Partition Setup

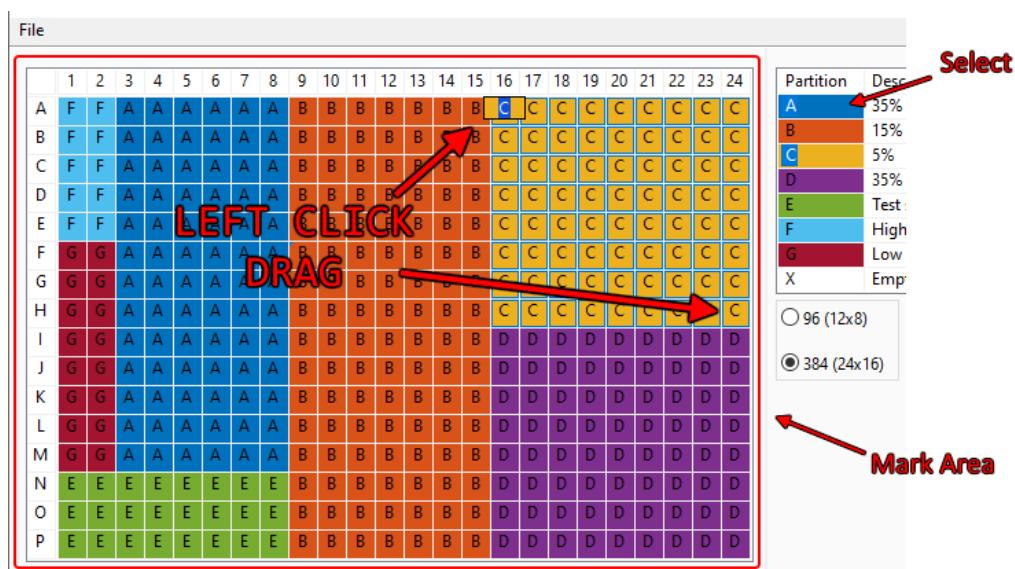
1. Select PCR size Select between 96 and 384.



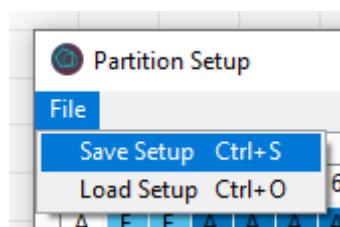
2. Configure Partitions Select partition and left click description to edit contents. Fill out as required.



3. Mark Partitions Select a partition (A, B, C, etc.). Mark an area of the PCR plate containing contents by left click and dragging. Fill out as required.

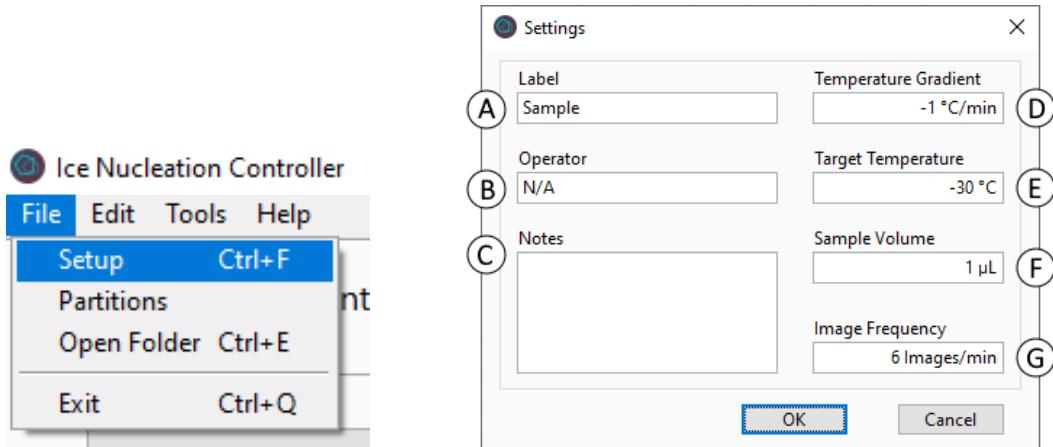


4. Save Partition (optional) Save created partition setup for future uses.



4.4 Configure Setup

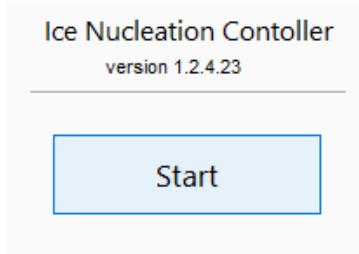
Open Setup settings **Files > Setup** and fill out.



Description	
A	Title of Experiment
B	Initials of operator
C	Any additional information
D	Temperature gradient of experiment
E	Ending temperature of experiment
F	Volume present in each PCR well
G	Camera and data output rate

4.5 Start Experiment

Once previous steps are completed press the **Start**-button to start the experiment. Accept and/or edit Partition- and Setup-settings.



CAUTION

Avoid shaking or moving any parts of the setup while experiment is running.

5 Process Data

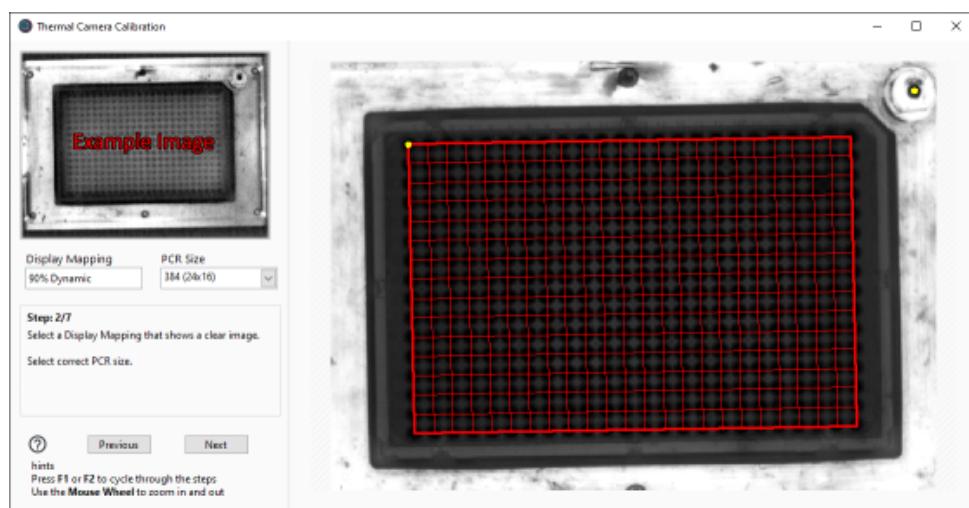
5.1 Thermal Camera Calibration (TCC)

System setup is susceptible to minor movement, which can affect camera feed positions. For every experiment a camera calibration must be performed. To perform a calibration open TCC **Tools > Calibration**.

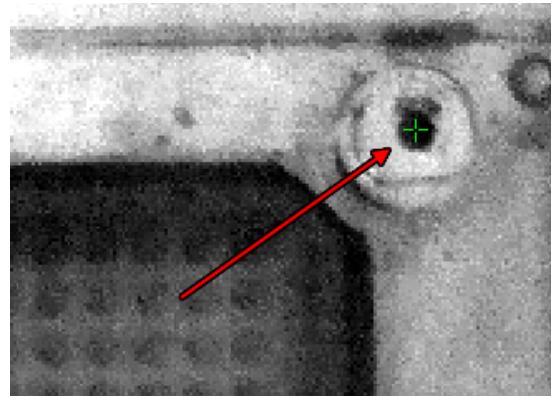


1. Load Calibration Image navigate to experiment folder and select any image (.../Data/Experiment_Label/Images).

2. Image Settings select PCR size and modify Display Mapping until a clear image shows.

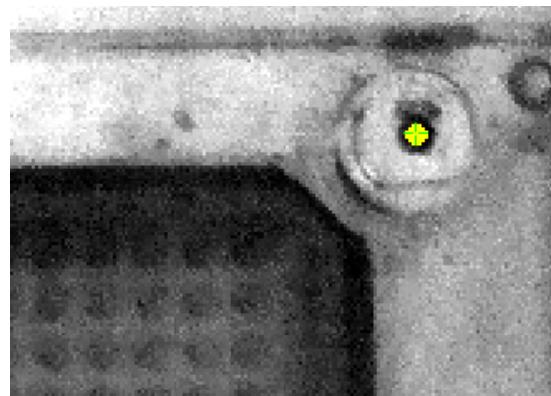


3. Select Fix-point Zoom in with mouse-wheel and left-click to mark the fix-point cavity.

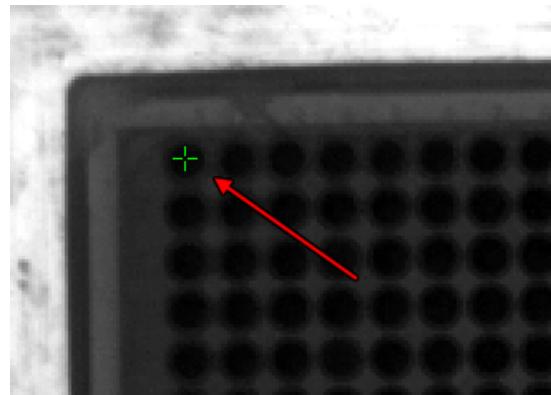


hint: position can be moved by left click and dragging pointer.

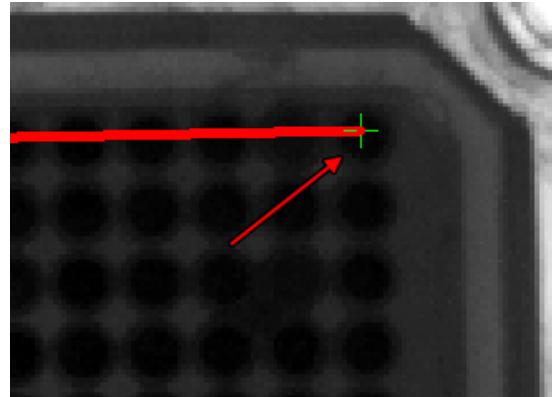
4. Fix-point Radius increase size until cavity is sufficiently covered by yellow pixels while still within cavity.



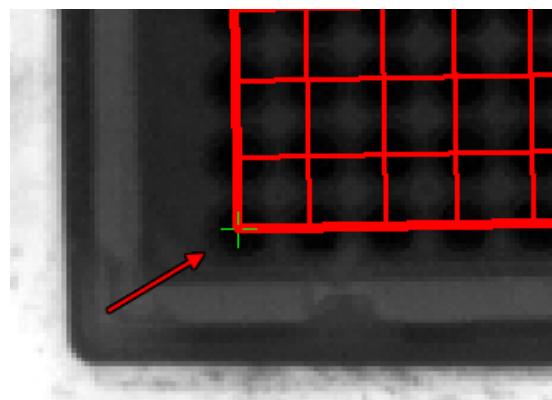
5. Mark Top Left Corner of PCR plate, zoom in with mouse wheel and left click on the top left well.



6. Mark Top Right Corner of PCR plate, zoom in with mouse wheel and left click on the top right well.



7. Mark Bottom Left Corner of PCR plate, zoom in with mouse wheel and left click on the bottom left well.

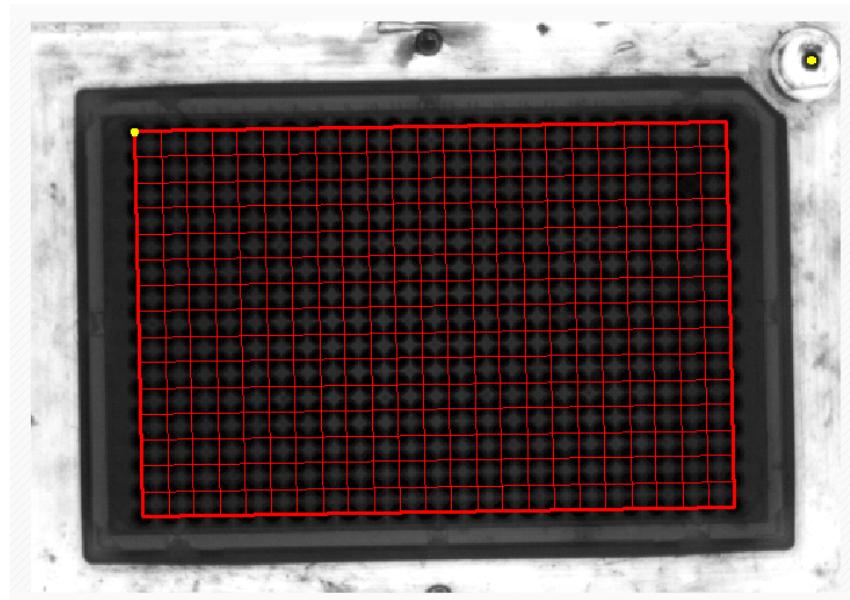


8. Well Radius increase size until well is sufficiently covered and within well boundaries. Check all grid points are within correct well position.



hint: enable Show All Points to check measurement area of all wells.

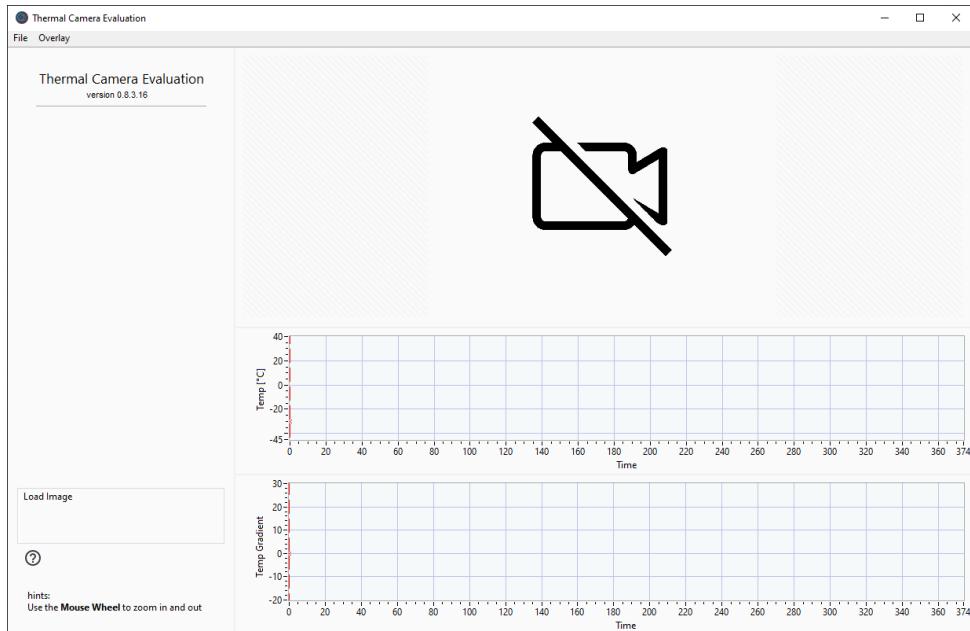
9. Verify All Positions are correct, navigate back to previous steps to alter positions until satisfied.



10. Save Calibration once satisfied with the calibration, save and exit software.

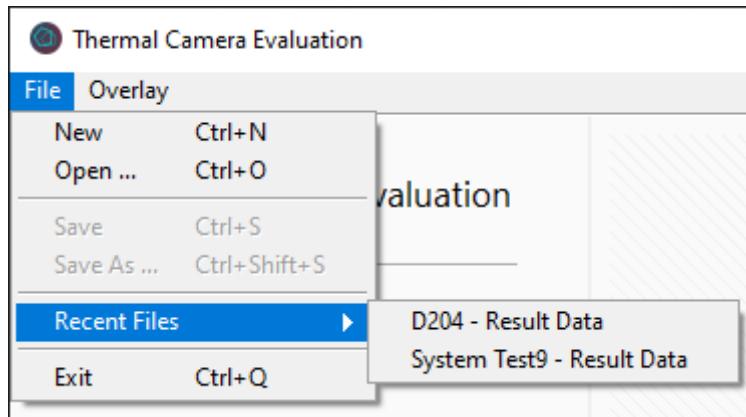
5.2 Thermal Camera Evaluation (TCE)

To process experiment data open TCE (**Tools** \gg **Process Data**).



5.2.1 View Processed Data

To open already processed data **File** \gg **Open** and navigate to an experiment folder containing processed data. Alternatively use **File** \gg **Recent Files** $\gg \dots$ and select a previously opened experiment.

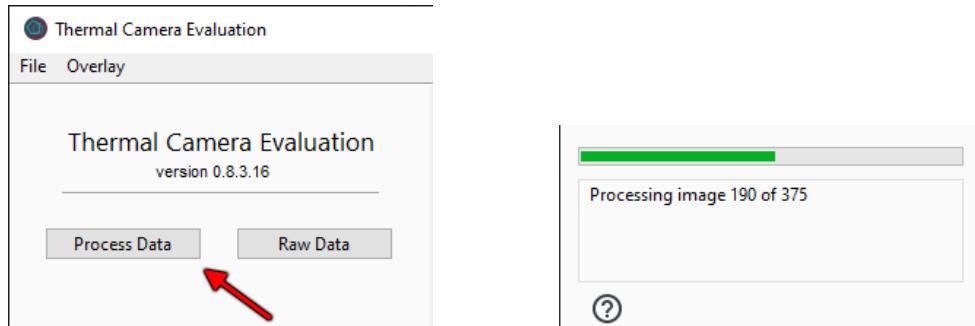


5.2.2 Process New Data

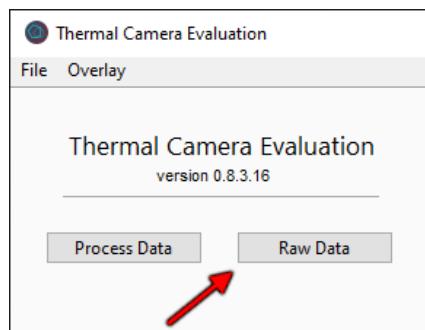
To process new data **File** \gg **New** and navigate to an experiment folder.

1. Select Calibration File system will auto-load calibration file if correctly saved. Alternatively navigate to a calibration file.

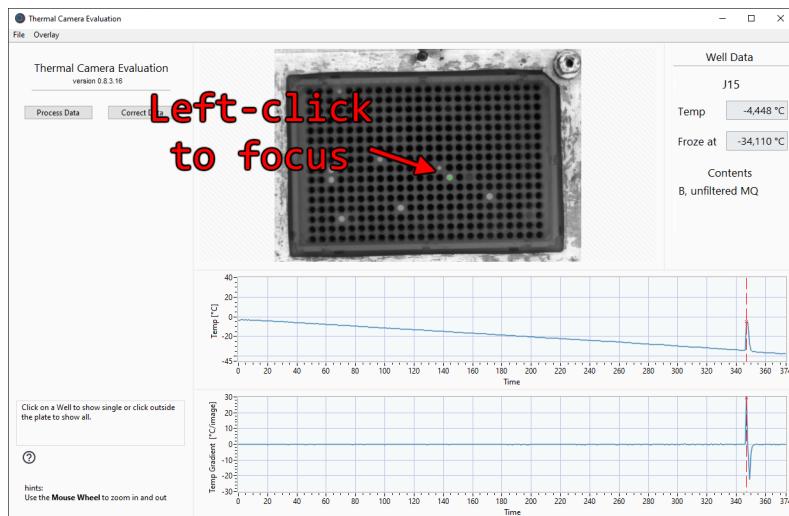
2. Process Data press **Process Data** and wait until progress bar finishes.



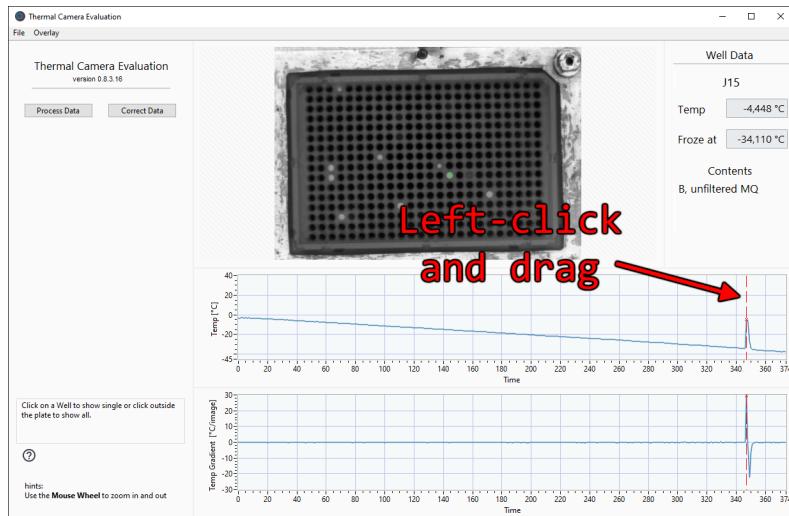
3. Evaluate Results Press **Correct Data**/**Raw Results** to switch between thermistor compensated results or raw thermal camera results.



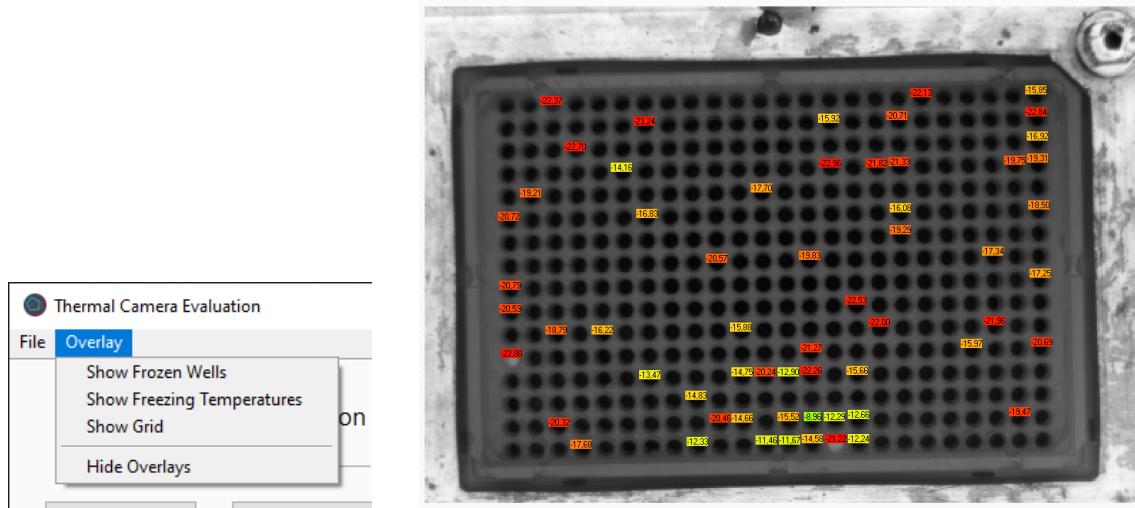
Left-click on any well to focus, displaying contents and temperature measurements. Click outside well area to un-focus.



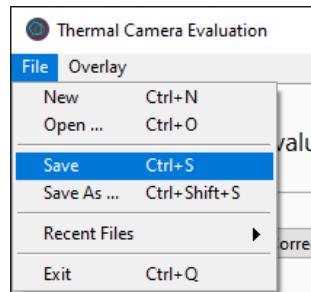
Left-click and drag red marker on graph to navigate timeline.



Use **Overlay** to display different overlays (Calibration Grid, Freezing Temperatures, etc.). Wait for overlay to complete then navigate timeline by left-click and dragging red marker to observe different overlays.



4. Save Results **File** **Save** results saved in .csv format, to use in further post-processing.

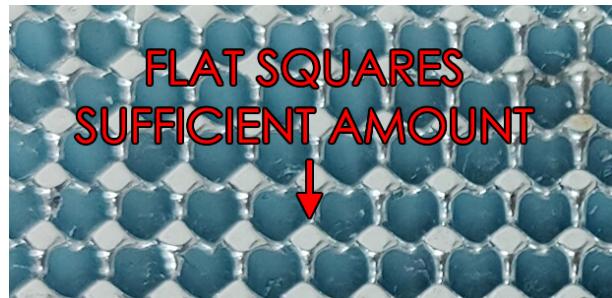


6 Advanced Procedures

6.1 Re-leveling Gallium

Re-leveling procedure is performed if gallium levels have become uneven, or if a significant amount of gallium has been drained or spilled from Cooling Base.

1. **Evaluate Gallium Amount,** remove PCR plate with gallium frozen. Gallium should form small flat surfaces between each hole, where contact is made between PCR plate and gallium.

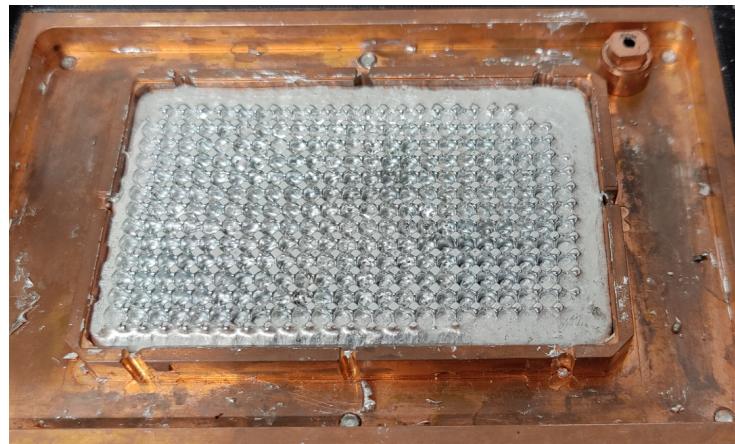
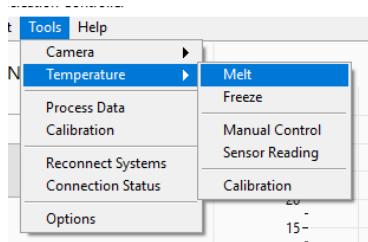


2. **Evaluate Gallium Leveling,** size of gallium squares should be even across entire PCR plate.



3. **Refill Gallium** if gallium amounts were insufficient add additional pure gallium. This can be added either as molten gallium (40°C) or in smaller amounts as solid gallium.

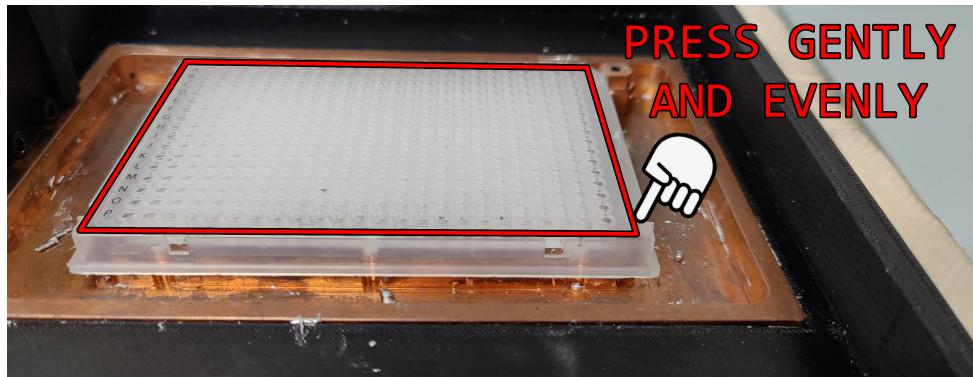
4. Melt Gallium, without PCR plate attached [Tools](#) [Temperature](#) [Melt](#) and wait for all to melt.



5. Homogenize Gallium using a STERILE tool to gently stir out surface-tensioned deformities. Make sure gallium is sufficiently melted, re-run [Tools](#) [Temperature](#) [Melt](#) if temperature drops below 35°C.



6. Gently Insert new PCR plate make sure to slowly insert every part evenly. This step is key to a perfectly level gallium. Slowly insert PCR plate little by little, by applying gentle and even pressure on every corner and side.

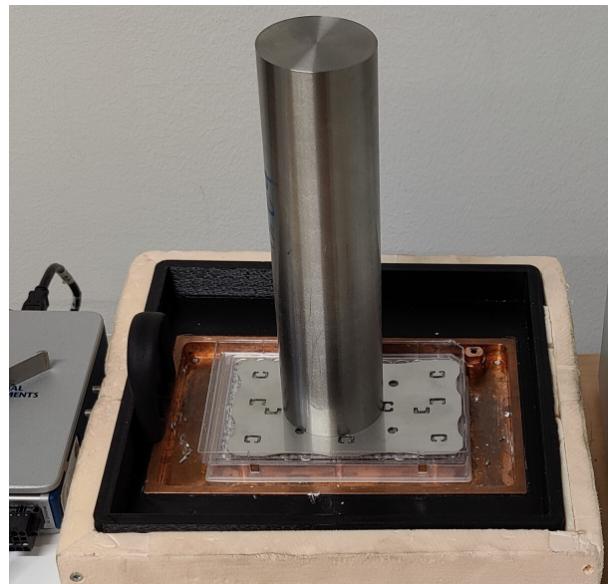


Make sure not to apply any pressure to center of PCR plate.



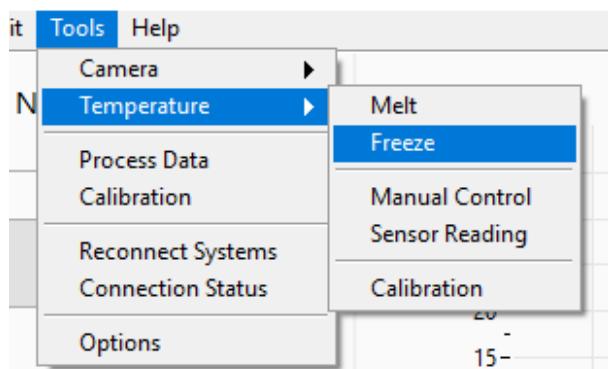
DO NOT PRESS CENTER

7. Place Lid, Plate and Weight on top of PCR plate to weight down during cooling process.



8. Turn on Cooling Pump if not already turned on.

9. Freeze Gallium with **Tools** **Temperature** **Freeze** to set gallium.



10. Evaluate Gallium Level remove Weight, Plate, Lid and PCR plate and evaluate levels again. Perform re-leveling procedure until satisfied.

7 Common Errors

7.1 Thermal Camera

Error	Cause	Fix
Camera Connection Error	Camera Starting Up	Reconnect Systems Tools > Reconnect System
	Bad Cable Connection	Check Ethernet and Power Cable Connection
	Connection Blocked	Disconnect and reconnect Power, then reconnect systems.
Camera Feed Dark	Camera out of Focus	Focus Camera Tools > Camera > Auto Focus
	Camera Lens Cover blocking	Check and remove lens cover

7.2 Cooling Base

Error	Cause	Fix
Gallium Spill	Center pressure while gallium molten	Remove spill with pipette (if liquid) avoid touching center while molten
	Gallium Level too high	Remove excess gallium and do re-leveling procedure
Uneven Gallium Level	Poor Gallium distribution	Perform re-leveling procedure
	Low Gallium Level	Refill gallium and perform re-leveling procedure
Water Leak	Loose Connector	Tighten loose connector(s) Contact Service
	Broken Seals	Replace faulty connector(s) Contact Service

7.3 NI cDAQ Module

Error	Cause	Fix
cDAQ Connection Error	cDAQ Starting Up	Reconnect Systems Tools ➤ Reconnect System
	Bad Cable Connection	Check USB and Power Cable Connection
	Connection Blocked	Disconnect and reconnect Power and USB, then reconnect systems.
No Temperature Measurement	cDAQ Initialization Error	Reconnect Systems Tools ➤ Reconnect System
	Power Cable Missing	Check Power Cable Connection
	Sensor Cable Missing	Check Sensor Cable (DSUB-9)

7.4 Water Pump

Error	Cause	Fix
Continuous Beeping	Disconnected Water	Check Water Line Connections
	Temperature Too High	Room or Water Temperature too high, power off PSU and let water temperature drop.
	Low Water Level	Check Water Level Indicator and refill to between MAX and MIN. Contact Service
Water Temperature stays above 8°C	Room Temperature too high	Lower Room Temperature or reduce temperature gradient
	Temperature Gradient set too high	Lower Temperature gradient of experiment
Water Leak	Loose Connectors	Tighten loose connector(s) Contact Service
	Broken Seals	Replace faulty connector(s) Contact Service

7.5 Power Supply Unit

Error	Cause	Fix
PSU Connection Error	PSU Starting Up	Reconnect Systems Tools ➤ Reconnect System
	Bad Cable Connection	Check USB and Power Cable Connection
	Connection Blocked	Disconnect and reconnect Power and USB, then reconnect systems.
No/Low/Static Output	Connection Error	Reconnect Systems Tools ➤ Reconnect System
	Mode Error	Verify backside MODE is set to NORMAL.
	Cable Disconnected	Check output cable (Red and Black).

7.6 Micro Controller Unit

Error	Cause	Fix
MCU Connection Error	MCU Starting Up	Reconnect Systems Tools ➤ Reconnect System
	Bad Cable Connection	Check USB and Power Cable Connection.
	Connection Blocked	Disconnect and reconnect Power and USB, then reconnect systems.
No Output	Connection Error	Reconnect Systems Tools ➤ Reconnect System
	Cable Disconnected	Check USB and power Cable.

References

- [1] <https://www.flir.eu/products/a655sc/>
- [2] <https://dk.rs-online.com/web/p/termistor-ic/8937234?sra=pstk>
- [3] <https://www.conradelektronik.dk/p/quickcool-qc-241-16-150m-hightech-peltier-elemen>
- [4] <https://www.ni.com/da-dk/support/model.ni-9219.html>
- [5] <https://www.ni.com/da-dk/support/model.cdaq-9171.html>
- [6] <https://www.alphacool.com/shop/durchlaufkuehler/geraete/21410/alphacool-eiszeit-2000-chiller-black>
- [7] <https://www.elfadistrelec.dk/da/laboratoriestromforsyning\ -32v-30a-960w-justerbar-peaktech-p1580/p/11095172>
- [8] <https://en.wikipedia.org/wiki/Thermistor>
- [9] <https://www.matronics.dk/kategori/el-materiel/afbrydere-kontakter/230v/produkt/arbejdsrelae-din>
- [10] <https://dk.rs-online.com/web/p/hulmonterede-modstande/2019905>
- [11] <https://www.ametekcalibration.com/products/temperature/temperature-calibrators/rtc-series-reference-temperature-calibrator>