

Portable Temperature-Controlled Microplate for Protein Crystallization

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Introduction

The physic-chemical parameters that affect protein crystallization are vast, and include: pH, salt type, concentration, additives and temperature among others. Although the importance of temperature has long been recognized, not many crystallographers consider it when setting up their experiments. Currently the crystallography community is restricted to use fridges/incubators and temperature controlled rooms, however these do not always offer the best solution. Problems arise when the samples are taken out of the fridge/incubator for inspection under the microscope or when fishing out the crystals. At this point the temperature control is lost, which in some cases can affect the system in a negative manner (dissolution of the crystal, protein denaturation, destabilization of the system). Even when the unintended temperature fluctuation result in the formation of a crystal, without knowing anything about this temperature fluctuation, the results are often difficult to reproduce.

The use of temperature as an additional crystallization variable has many advantages: it is easy to control, it can modify the solubility and super saturation of the sample in a reversible manner and more rapidly than by changing other crystallization parameters¹; using it together with other crystallization variables can increase the probability of finding better crystallization conditions; it helps to prevent denaturation of temperature sensitive proteins and improves the reproducibility of results.

A careful use of temperature can aid in controlling crystal nucleation, growth and dissolution of defects on the surface of the crystal^{2,3}. It has also been reported to be important in phase separation in detergents solutions, which are used in the crystallization of membrane proteins⁴.

In an attempt to solve these problems, Centeo has designed an electronic portable temperature controlled microplate. This work describes the system and its performance. Finally it presents some crystallization results achieved with the system.

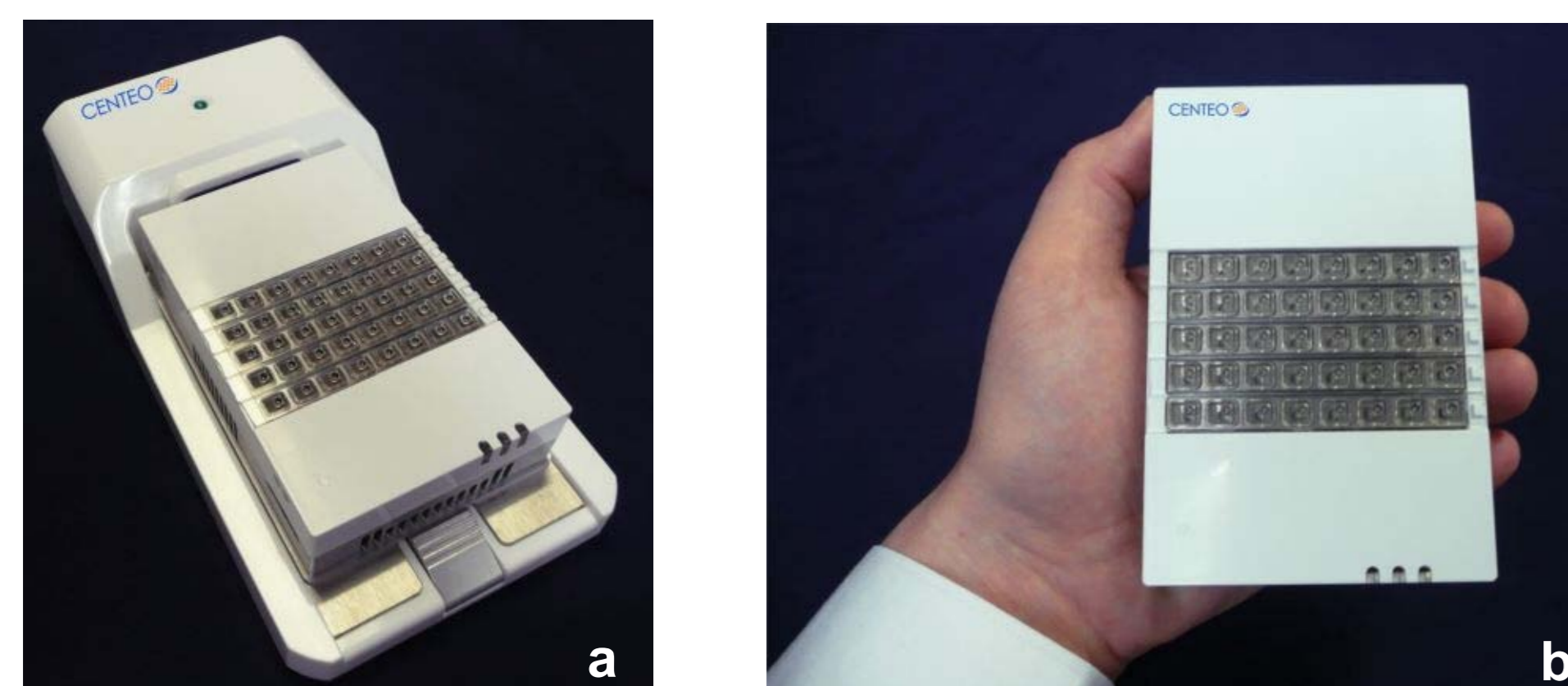
The System

In order to address the above mentioned problems, the system was developed with 4 key objectives in mind:

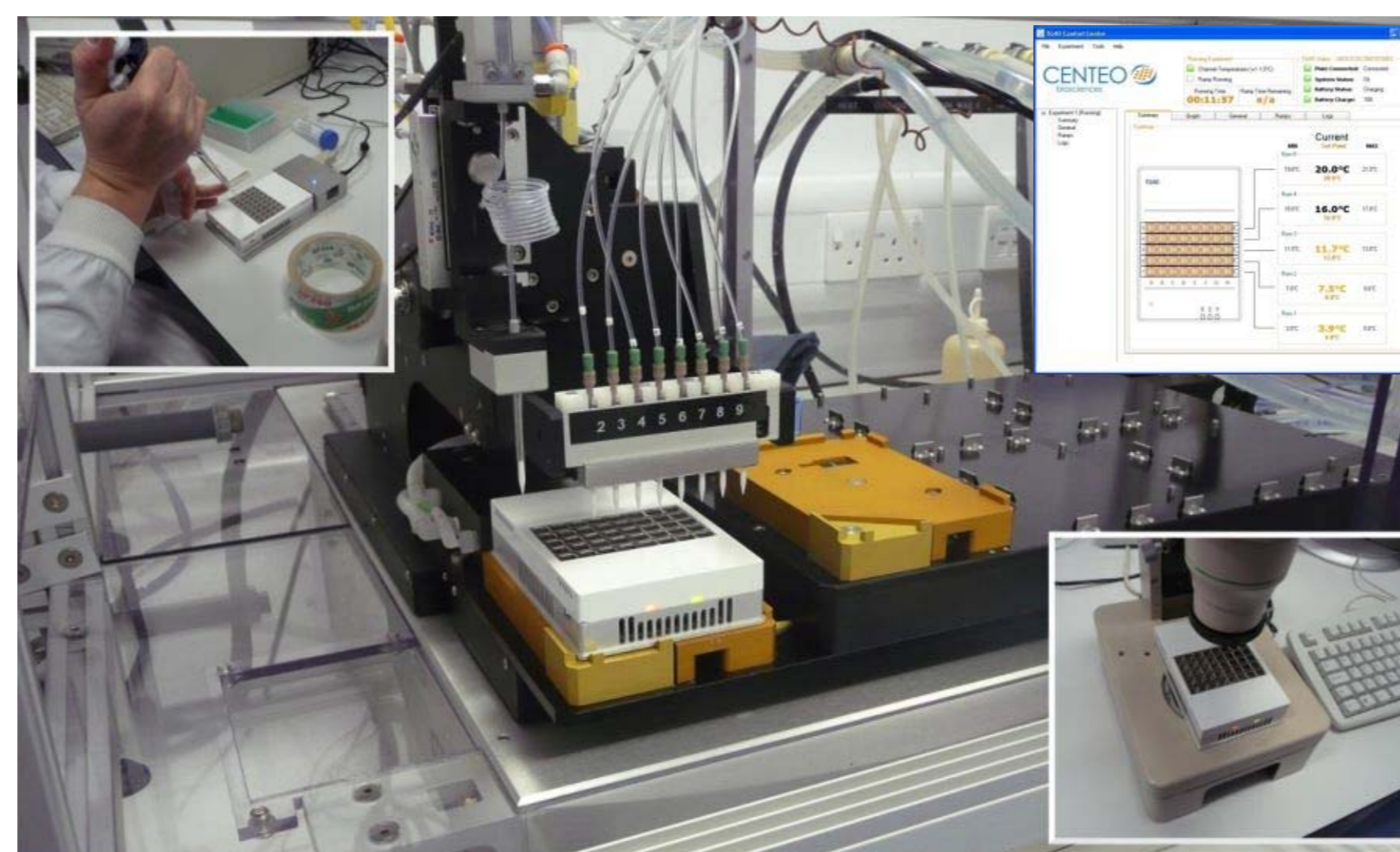
1. Compatibility with other laboratory instruments
2. Portable
3. Programmable
4. Accurate

The system was designed with the footprint of a standard 96 well microplate while using a maximum height of 31mm, which is less than the height of a deep-well storage plate. The device has 5 rows with 8 wells per row. Each row can be independently controlled allowing to screen up to 5 different temperatures from 4°C to 60°C. The rows are made of copper which has a thermal conductivity of 400 W/(m K) which provides better and faster thermal transfer compared to aluminium which has a thermal conductivity of 255 W/(m K).

When in use, the microplate resides in a docking station (Figure 1a), from which it can be removed for portable, battery powered operation (Figure 1b).



Because of its SBS footprint and portability, the system can maintain the set temperatures even when moved to a microscope, or when dispensing the sample manually or robotic system (Figure 2).



The docking station is connected to a PC from which the temperatures can be programmed into the plate. Once the plate is programmed, the system can be disconnected from the docking station without losing temperature control.

Figure 3 shows the thermal performance of the system when connected to the docking station and running of an external power supply. At 100s the automatic temperature control system is activated by removing the plate from the docking station. The plate was previously programmed to maintain the temperature of the rows at: 10°C, 14°C, 18°C, 22°C and 26°C.

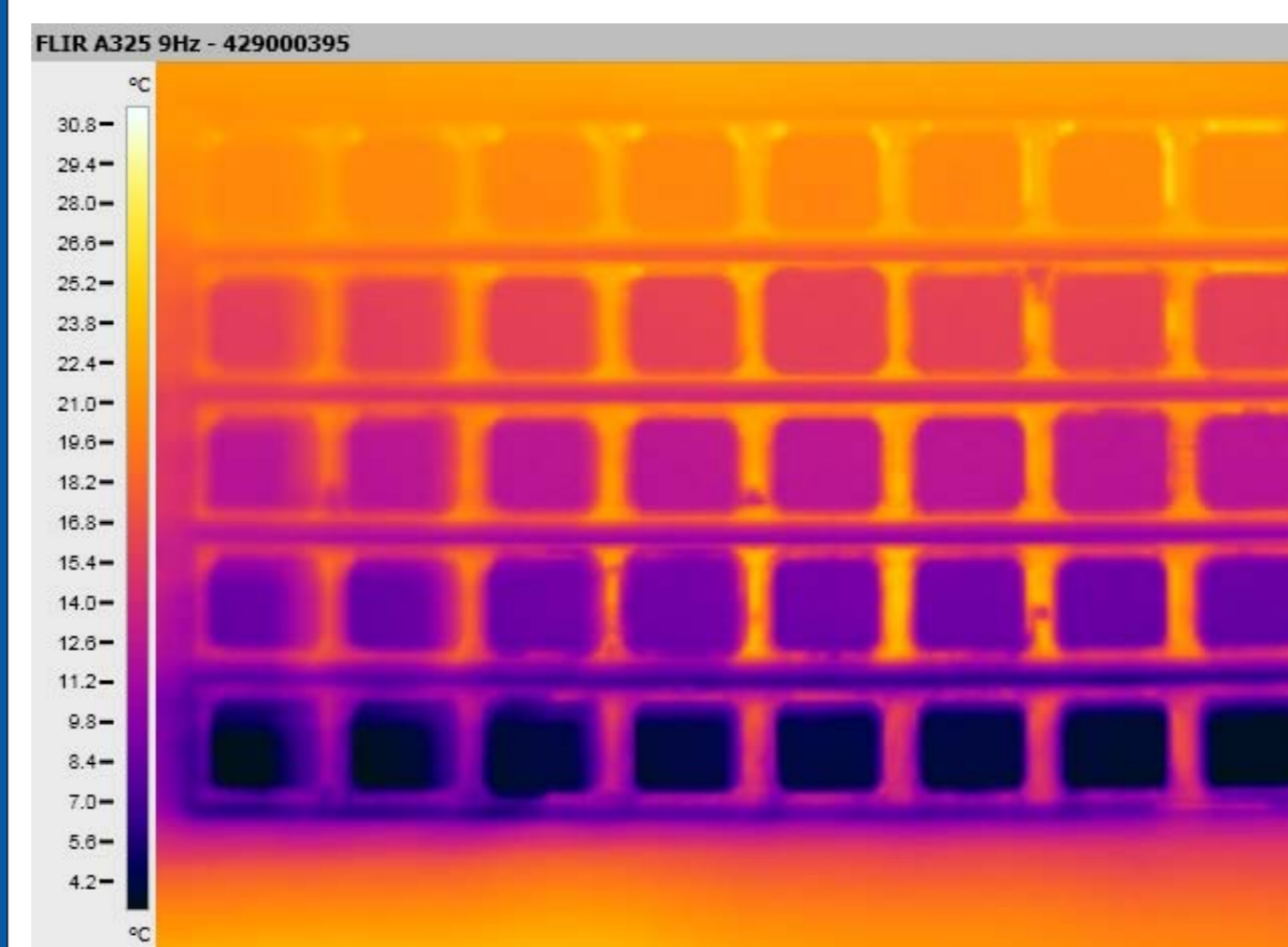
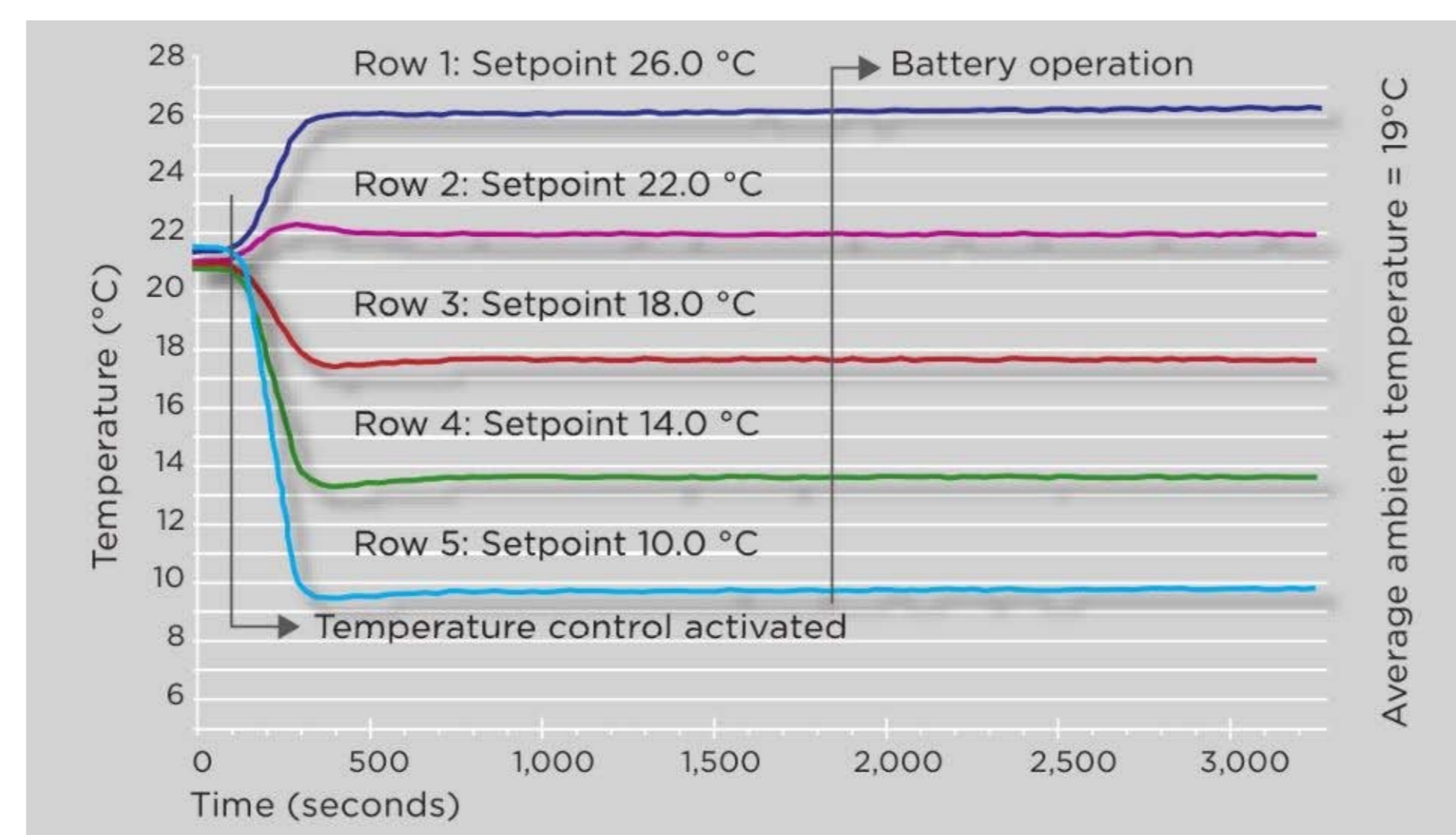
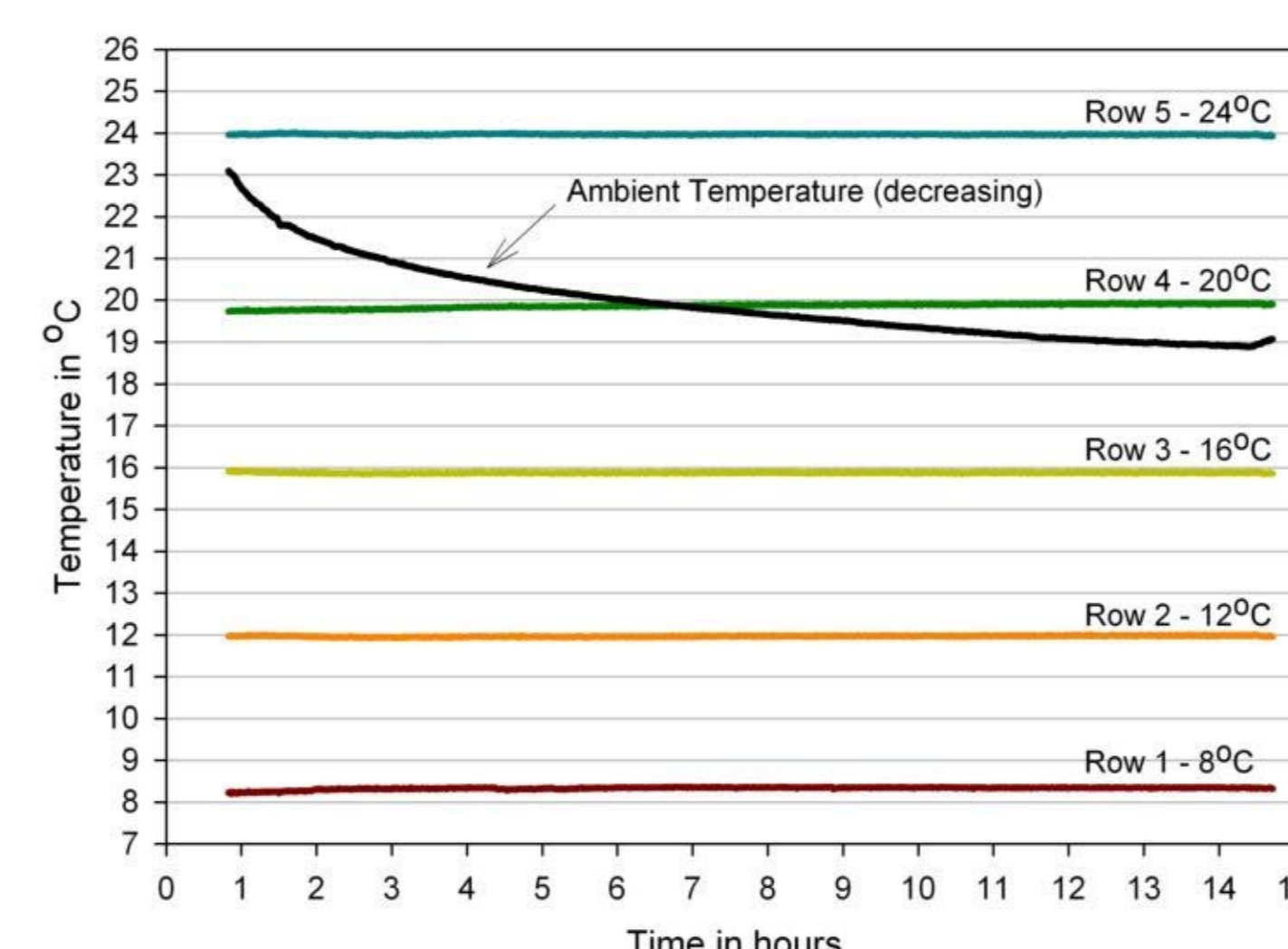


Figure 4 shows the temperature uniformity across the copper rows. The measurement was made using a FLIR A325 thermal imaging camera. The wells of the system were filled with isopropanol, which was then imaged after the temperatures were set. Isopropanol was chosen because it can be removed without leaving residue inside the metal blocks. It is important to note that it is difficult to get accurate temperature readings of the metal blocks directly due to reflections.

Figure 5 shows the stability of the temperature control system. The rows of the microplate were set to temperatures between 8°C and 24°C degrees in 4°C increments.



The temperature of each row is controlled by an independent digital PID controller, which measures the absolute temperature of each block.

The graph illustrates typical ambient temperature fluctuations that can occur in any laboratory over night. It can be seen that as the ambient temperature decreases, the temperature control system of the microplate maintains the setpoint temperatures of the rows.

The temperature of each row was measured using a type K thermocouple with a quoted accuracy of $\pm 0.6^\circ\text{C}$ over the temperature range shown. A thermocouple was placed in well 4 of each row in direct contact with the metal surface. No plastic insert was used.

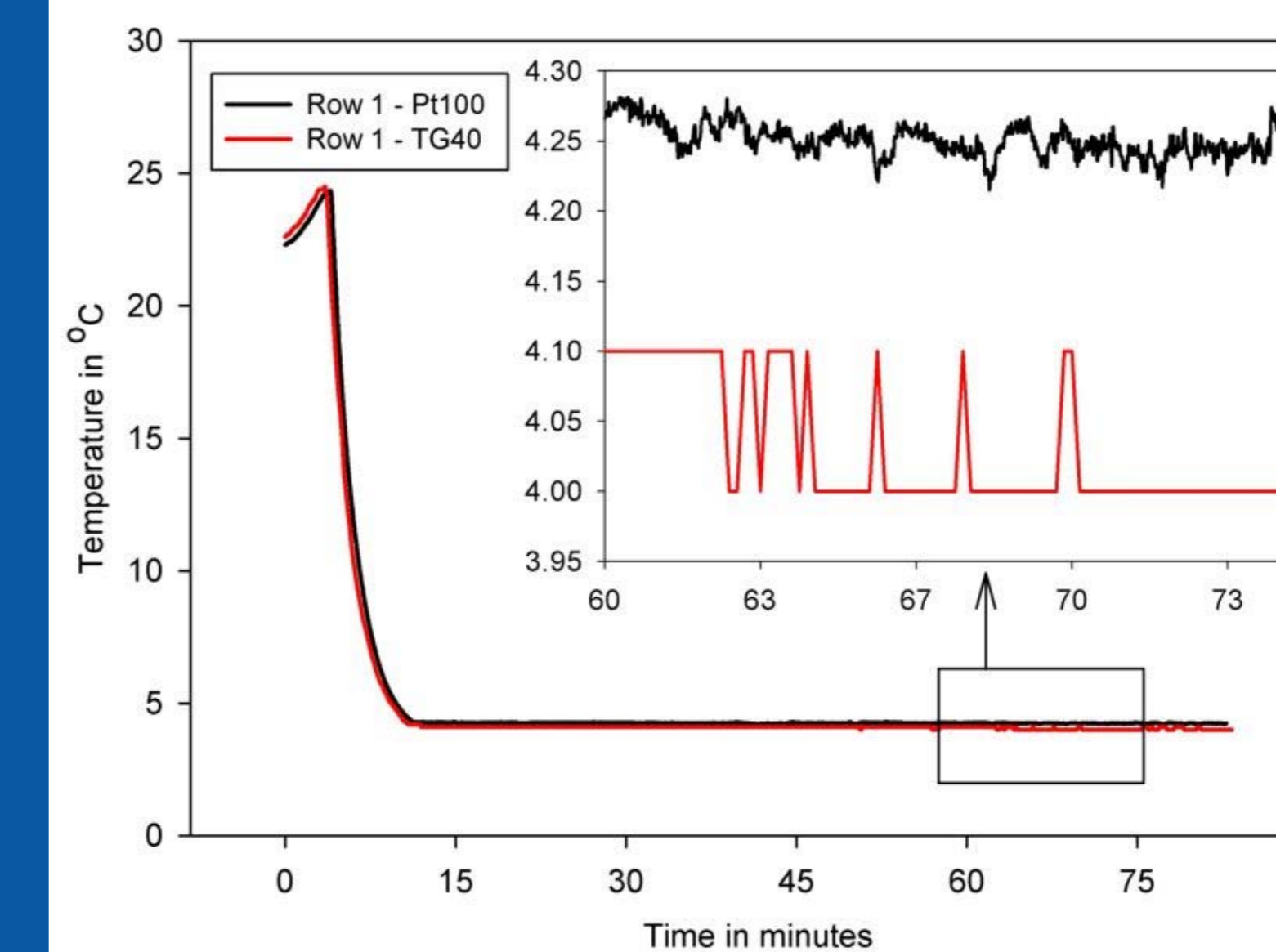


Figure 6 shows the typical accuracy of the system at 4°C is much better than $\pm 1^\circ\text{C}$. The red trace shows the temperature reading for row 1 as reported by the built-in temperature measurement system of the microplate. The black trace shows the temperature as recorded by a Pt100 sensor attached to well 4 of row 1. Note that the internal sensor of our microplate is located between well 4 and 5.

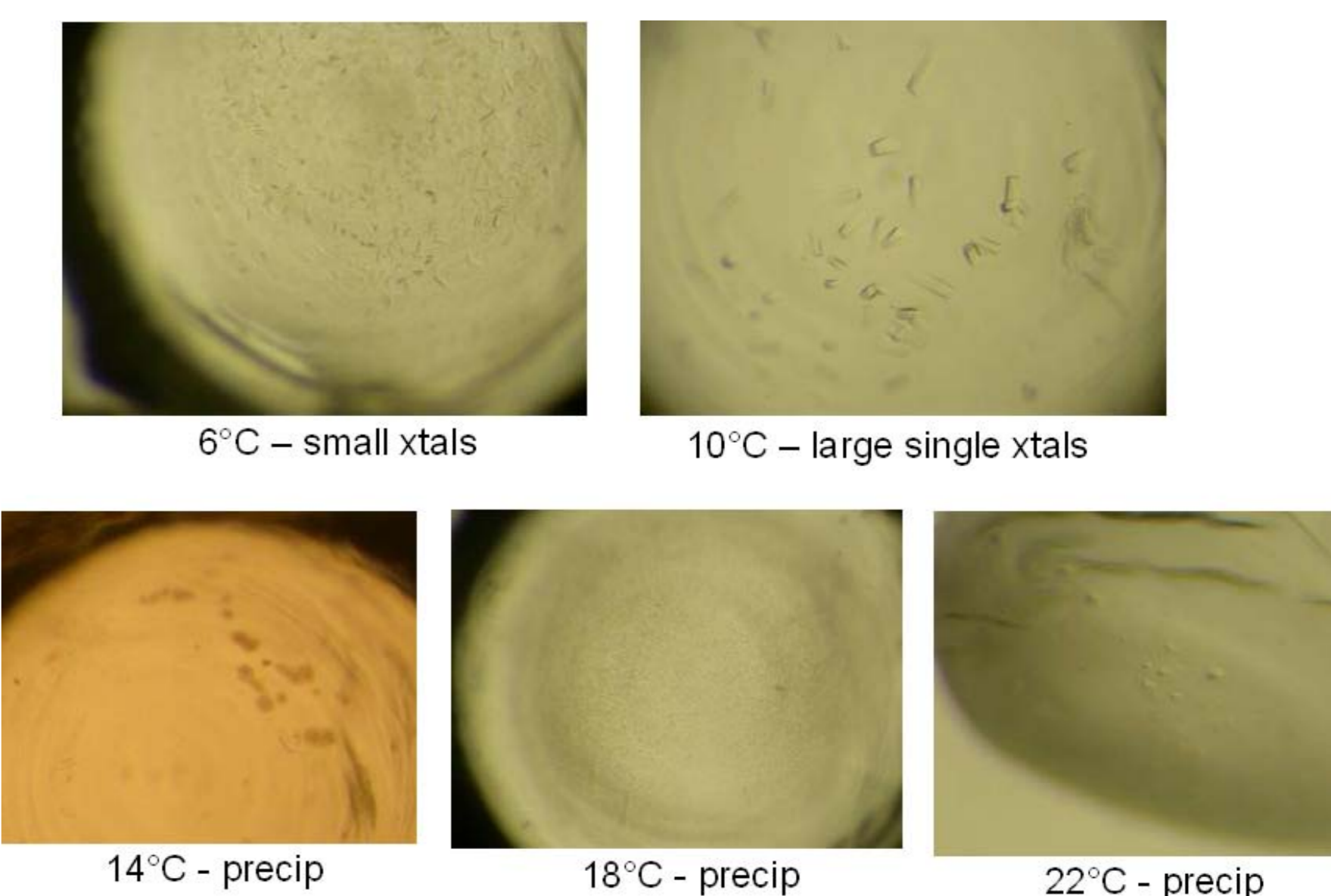


Figure 7 shows crystallization results where no crystals were obtained at standard temperatures of 4°C or 20°C. However after using this system to screen other temperatures, single crystals were obtained at 6°C and at 10°C. The latest being larger crystals.*

Conclusion

We have developed a portable temperature controlled system for protein crystallization and crystal optimization. The portability allows uninterrupted temperature control throughout the entire experimental procedure, while screening up to 5 different temperatures simultaneously. The system therefore offers advantages over the traditional temperature control methods.

References

1. Christopher, G. K., Phipps, A. G. and Gray, R. J. 1998. Journal of Crystal Growth, vol. 191, no. 4, pp. 820-826.
2. Long, M. M., Bishop, J. B., Nagabhushan, T. L., et al. 1996. Journal of Crystal Growth, vol. 168, no. 1-4, pp. 233-243.
3. Astier J.P. and Veessler S. (2008) Cryst. Growth Des., vol 8 no. 12, pp 4215-4219
4. Garavito, R. M. and Picot, D. 1991. Journal of Crystal Growth, vol. 110, no. 1-2, pp. 89-95.

*These experiments were carried out by Andrew Bent at a leading pharmaceutical company, due to the nature of this research no information can be provided on the protein and crystallization conditions.