Chamelogator: A software tool for Chameleon data analysis

Ivan Fong[1], Ouliana Panova[1], Miriam Weckener[1], Paul Thaw[1], Michele Darrow[1], Silvia da Graça Ramos[2], Mark Basham[2]

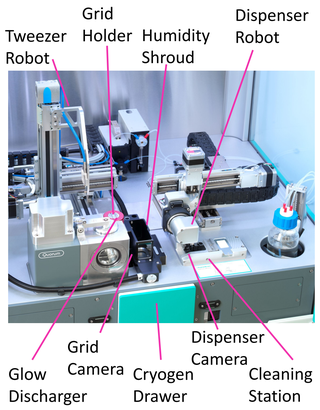
[1]SPT Labtech LTD., Melbourn Science Park, Hertfordshire, UK - [2]Rosalind Franklin Institute, Artificial Intelligence and Informatics, Harwell Campus, Didcot, UK

Introduction to chameleon

The Resolution Revolution was triggered by great technological advances in cryo-electron microscopes, from their electron source down to new electron-counting detectors. Automated data-collection protocols and better software altogether helped make molecular cryoEM the method of choice for high-throughput structure determination. However, sample preparation – a key determinant in the success of any cryoEM structural study – has not seen much innovation since the introduction of commercial plunging devices, which still rely on paper-blotting and suffer from various unwanted side effects caused by the slow freezing process.

Current sample preparation and optimization workflows are still of the "guess-and-check" type, using the electron microscope to determine the outcome. The chameleon instrument and self-wicking grids represent a paradigm shift towards a routine, automated, fast-plunge future for sample optimisation.

Here, we report best-practices from early adopters of the chameleon technology, including experiences and lessons learned through recent case studies. The self-wicking grid manufacturing and quality control process play a central role in controlling the wicking process. The chameleon requires an update of the freezing strategy, and we will share our experience and offer recommendations for future users.



The chameleon system is a blot-free, pico-litre dispense instrument for quickly and robustly freezing samples for use in cryoEM [1]. The chameleon system was developed from Spotiton [2,3] and uses self-wicking copper nanowire grids to form the thin sample film [4]. This process occurs 'on-the-fly' as the grid passes in front of the dispenser on its way to the cryogen bowl, resulting in a stripe of sample across the frozen grid.

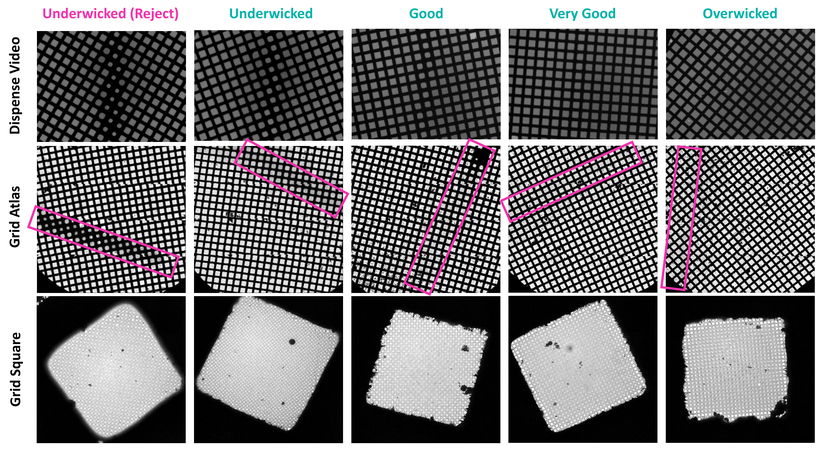
chameleon provides many benefits:

- Blot-free high-speed plunging
- Automated grid handling
- Grid screening based on film thickness
- Intuitive automated workflows
- Sample tracking and recording
- Cryogen feedback and control

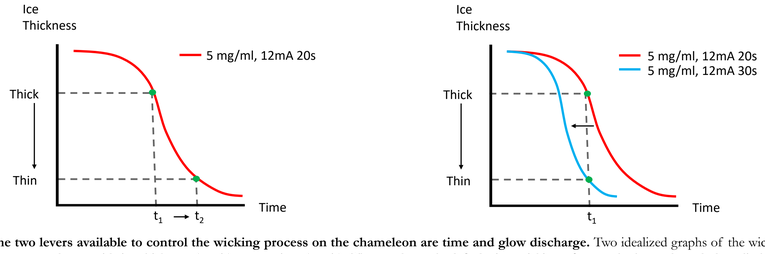
Importantly, by varying the plunge time during grid preparation, the chameleon system can be used to understand sample specific behaviours and overcome sample specific AWI effects. Together with walk-up usability, the chameleon enables opportunities for cutting-edge research despite poorly behaved cryoEM samples.

Controlling wicking to target film thickness

A continuum of film/ice thicknesses are shown below to demonstrate the correlation between the dispense video on the chameleon (top row), the atlas (middle row), and square level (bottom row) images on an electron microscope. As the grid becomes more wicked, the stripe becomes thinner and more difficult to find in the atlas; at the square level, more empty holes are observed around the outside edge of the square.



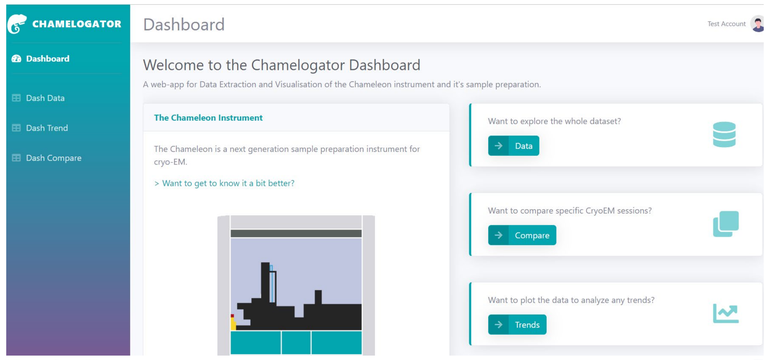
Controlling wicking to target film thickness, cont.

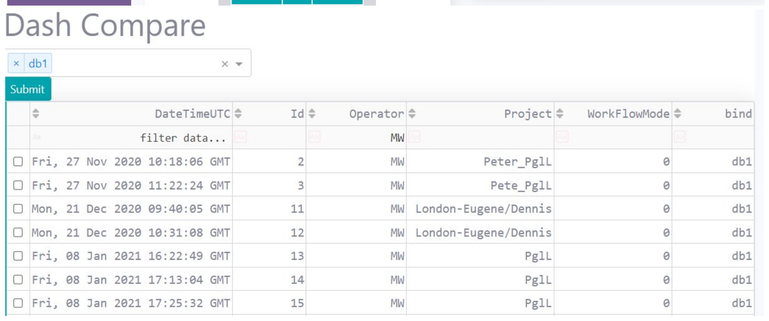


The two levers available to control the wicking process on the chameleon are time and glow discharge. Two idealized graphs of the wicking process are shown with ice thickness (y-axis) versus time (x-axis). The graph on the left depicts wicking of a standard sample and glow discharge parameters, with ice thickness controlled by modulating the dispense-to-plunge time with longer times leading to thinner ice. The graph on the right modulates the wicking process by adding extra glow discharge and holding the dispense-to-plunge time constant.

CHAMELOGATOR – data mining to inform and optimize freezing outcomes

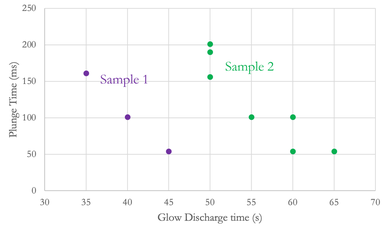
We are developing a new browser-based software that **aggregates anonymized freezing data** from users across the world to **glean relevant correlations and generalizable behaviours across samples**. Cryo-EM facilities and individual users can download a local version onto their platforms to manage their freezing sessions separately from the global database and tailor it to their samples.

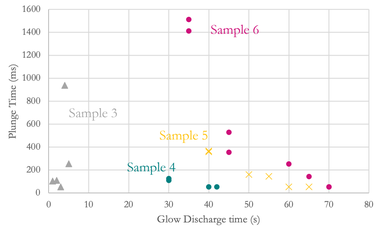




	DateTimeUTC	Id	Operator	Project	WorkFlowMode	bind
	filter data...		MW			
☐	Fri, 27 Nov 2020 10:18:06 GMT	2	MW	Peter_PglL	0	db1
☐	Fri, 27 Nov 2020 11:22:24 GMT	3	MW	Pete_PglL	0	db1
☐	Mon, 21 Dec 2020 09:40:05 GMT	11	MW	London-Eugene/Dennis	0	db1
☐	Mon, 21 Dec 2020 10:31:08 GMT	12	MW	London-Eugene/Dennis	0	db1
☐	Fri, 08 Jan 2021 16:22:49 GMT	13	MW	PglL	0	db1
☐	Fri, 08 Jan 2021 17:13:04 GMT	14	MW	PglL	0	db1
☐	Fri, 08 Jan 2021 17:25:32 GMT	15	MW	PglL	0	db1

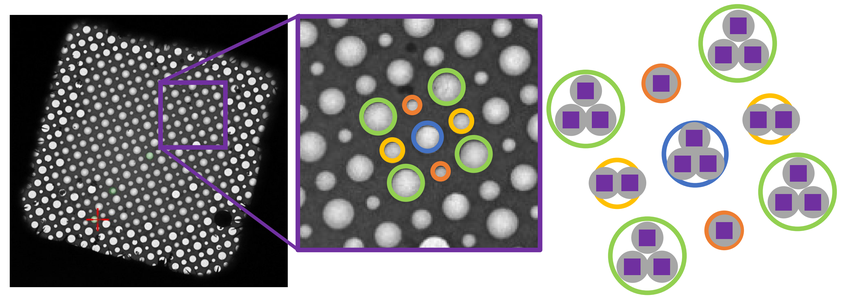
The data can be filtered by any of the parameters collected by chameleon: concentration, buffer composition, detergent additive, particle size, etc. Trends and correlative plots can be generated that can inform freezing strategies for subsequent runs of the same sample, or first runs of new samples.



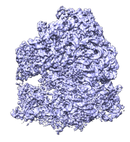
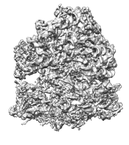
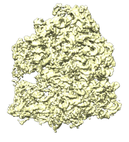
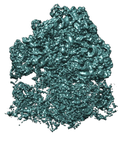
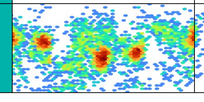
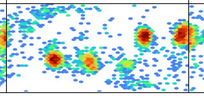
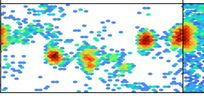
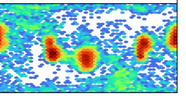


Alternative film supports: Multihole Gold

Alternative film materials and geometries allow for a wider array of freezing conditions to be evaluated at once. We have developed a film with four distinct hole diameters arranged periodically over a gold or carbon foil over the grid. At the outset of the freezing event, each hole diameter will exhibit different ice thicknesses and particle distributions.
This allows for a fast screening and targeting of particle ice preference: In one grid, four ice thickness and particle distribution conditions can be sampled, and optimal targets established, without having to screen additional grids. If only one target condition is needed, a collection can be set up on a specific subset of the holes; if the particle shows preferred orientation with ice thickness or other related tendencies, a collection can be set up on all the holes and pooled together for reconstruction.



TEM image of a square on a multihole gold film. The pattern is divided into 4 separate hole sizes, and a potential collection setup is schematized on the right. Some hole sizes allow for several micrograph acquisitions.
We collected a dataset on NEB E. Coli Ribosome to investigate the dependency of particle quality and quantity on hole size and ice condition. Data was collected in SerialEM using the pattern delineated above and processed with Relion 3.1 and CryoSparc. The dataset was split by hole size; results are summarized in the table below:

Hole Size (um)	0.8	1.2	1.6	2.0
Number of movies	250	500	345	1,500
Number of particles on micrograph	118,165	180,571	178,091	753,723
Number of particles after 2D	31,149	37,657	22,585	114,195
Final number of particles	8,761	8,731	6,113	28,689
Resolution of reconstruction (Å)	**3.9**	**4.4**	**4.2**	**3.6**
Reconstruction map				
Orientation distribution				

Ribosome reconstructions show thathet angular distribution of particles depends on the character of the ice in which they are found. The reconstructions show a preference for a specific set of orientations, but we see that which of these orientations are sampled depend on the hole size. The largest and smallest hole sizes show additional orientations that are not seen in the medium hole sizes.

Conclusions

There are as of yet no optimization workflows specific to sample type. Only a consensus that optimization is required for most samples and that approximately ten different methods will be attempted in combination before improvement is seen. Additionally, none of the currently available methods can be determined to work in advance of sample preparation. [6]
The variation of grid geometries and support films have shown better outcomes in final map reconstructions. IceBreaker studies have also established the strong dependency of particle distribution, orientation, and quality as a function of ice thickness.
As samples become more and more varied and less and less standard, tools such as the multihole grids and support films offer great advantages to sample optimization on the chameleon.

References

[1] MC Darrow, et al. *Microscopy and Microanalysis, 25 (2019)*, p. 994-995
[2] I Razinkov, et al. *Journal of Structural Biology* 195 (2016), p. 190-198
[3] V Dandey, et al. *Journal of Structural Biology* 202 (2018), p. 161-169
[4] H Wei, et al. *Journal of Structural Biology* 202 (2018), p. 170-174
[5] T Levitz, et al. *Journal of Structural Biology* 214 (2022)
[6] B. Carragher, et al. *J. Microsc* 276 (2019), p. 39-45.