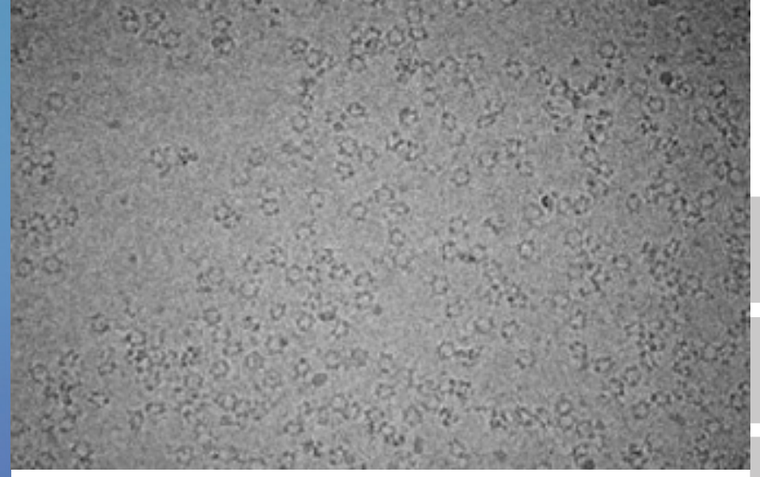
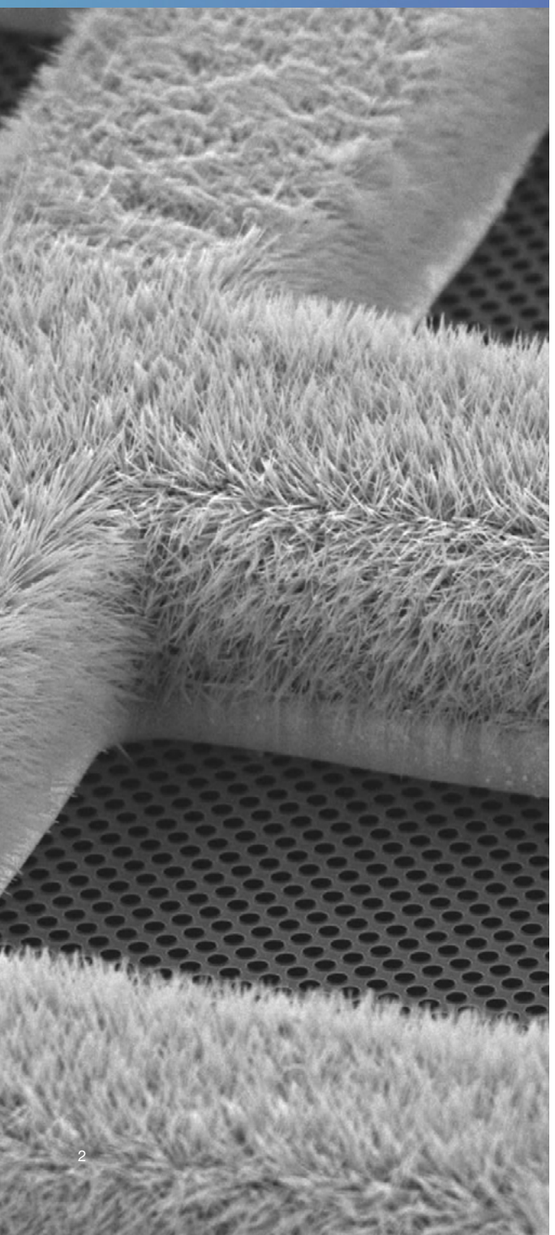
chameleon

A paradigm shift in sample preparation for cryo-EM

sptlabtech

a rapid and efficient path to quality frozen grids for cryo-EM





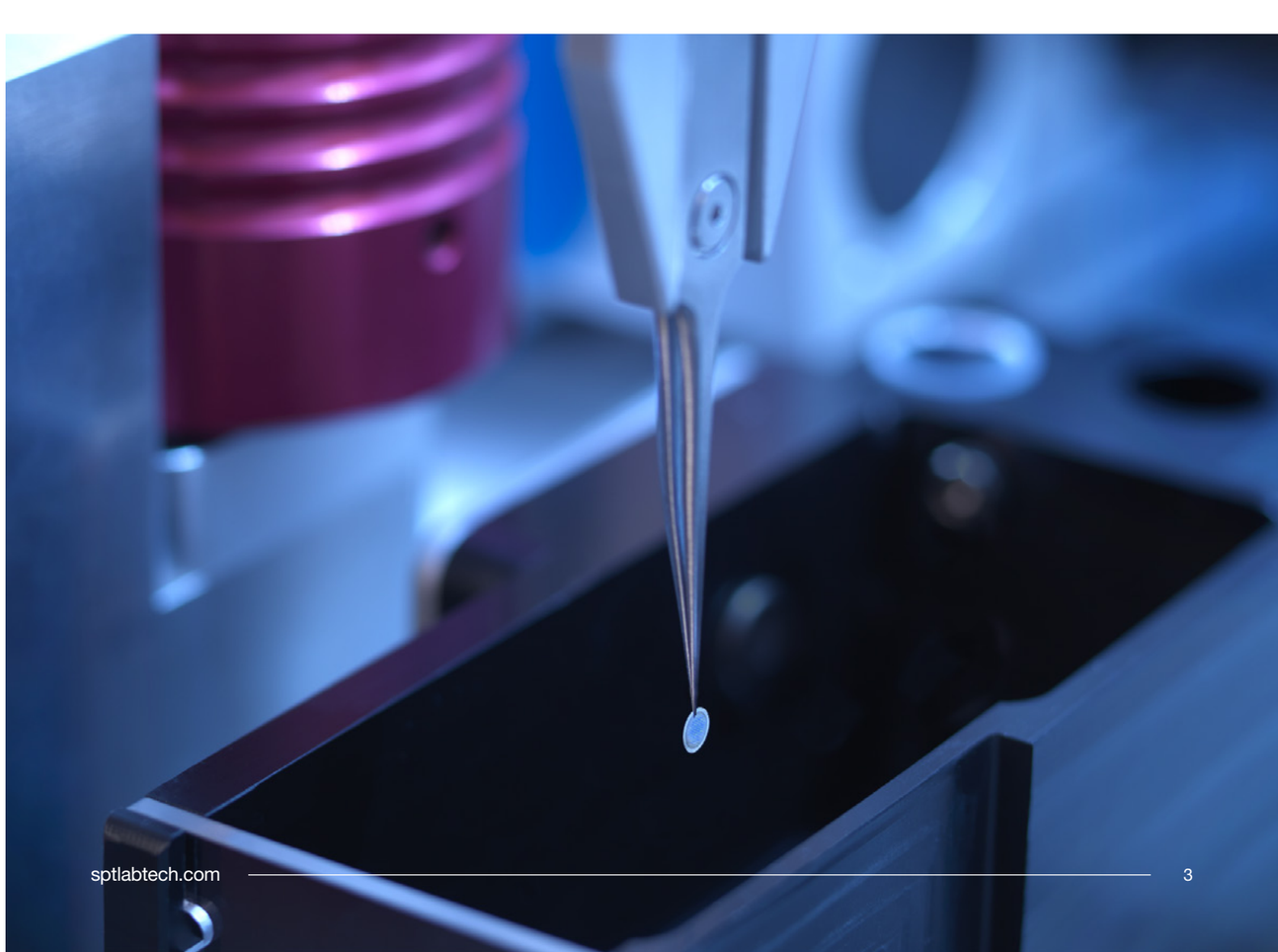
Recent advances in cryo-EM have led to an explosion of interest in the technique for challenging structural biology projects.

Producing quality frozen grids remains one of the major bottlenecks in the structure determination process. Currently, highly skilled manual handling is required; causing a significant learning curve. Feedback on ice quality requires screening on an electron microscope, time which could be used for more productive data collection. chameleon's aim is to enable automated, rapid progress to the perfect ice thickness for a range of samples.

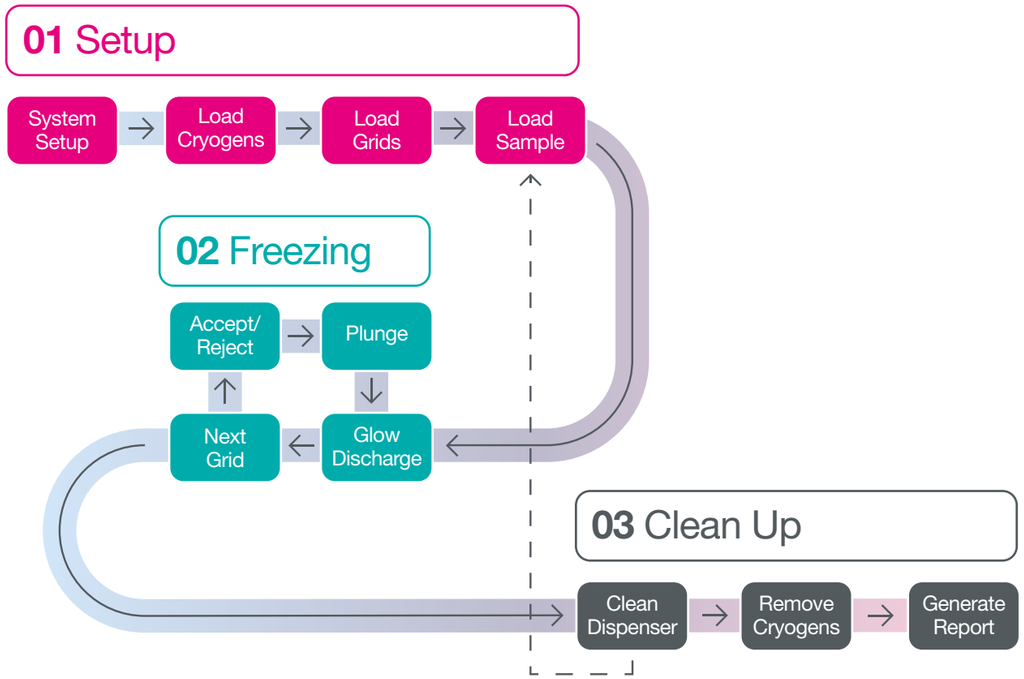
The Future of Sample Preparation for Cryo-EM

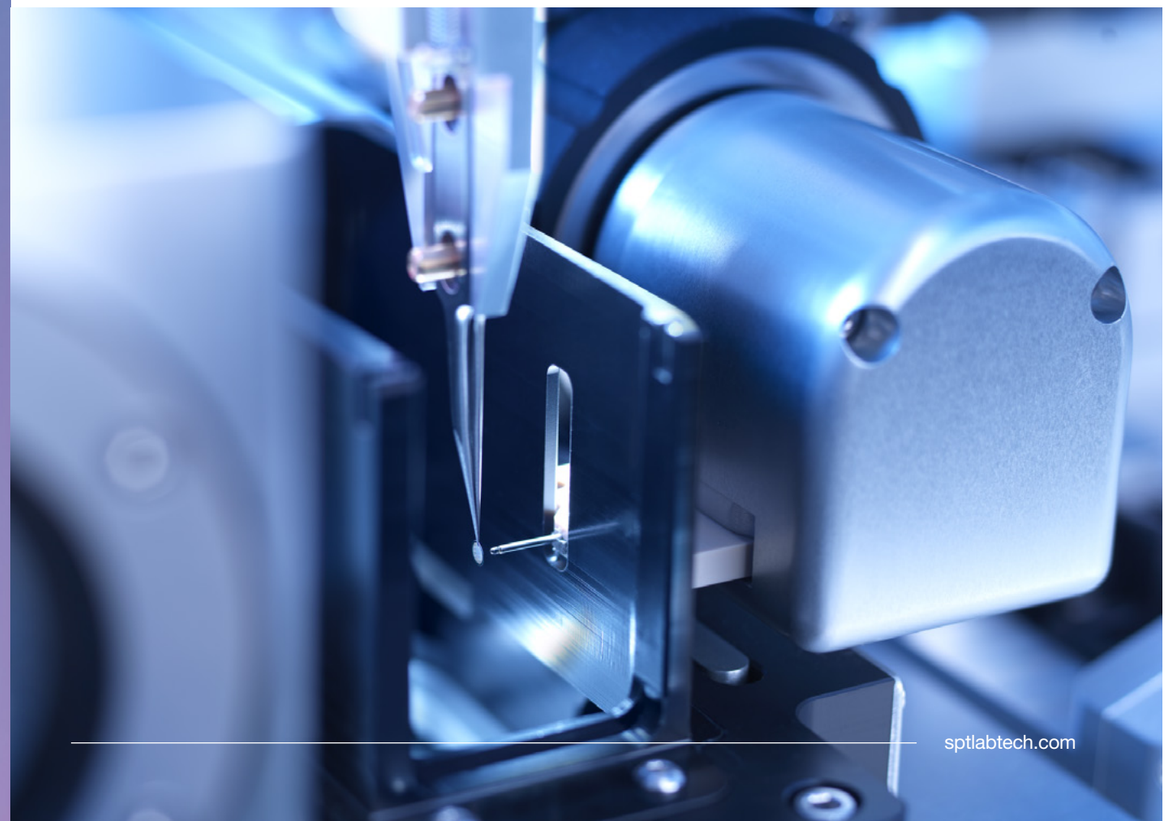
Next-generation sample preparation available on chameleon employs a blotless method, automation, and speed to outrun the effects of particle adsorption to the air water interface while standardizing ice quality and thinness in the hands of novice users. Optimizable vitrification for unique sample behavior can reduce the time and costs associated with trial-and-error experimental optimization necessary using traditional methods.

existing problem	chameleon solution
Grids are easily damaged	Automated grid pickup and delivery
Results dependent on user skill	Guided workflows for instrument set up and use
No traceability	Reporting/tracking of sample preparation
No idea of grid quality ahead of microscope	Indication of appropriate film thickness ahead of imaging
Preferred orientation / aggregation /denaturation	Much shorter dispense to plunge times shows positive impact and improved resolution



chameleon workflow



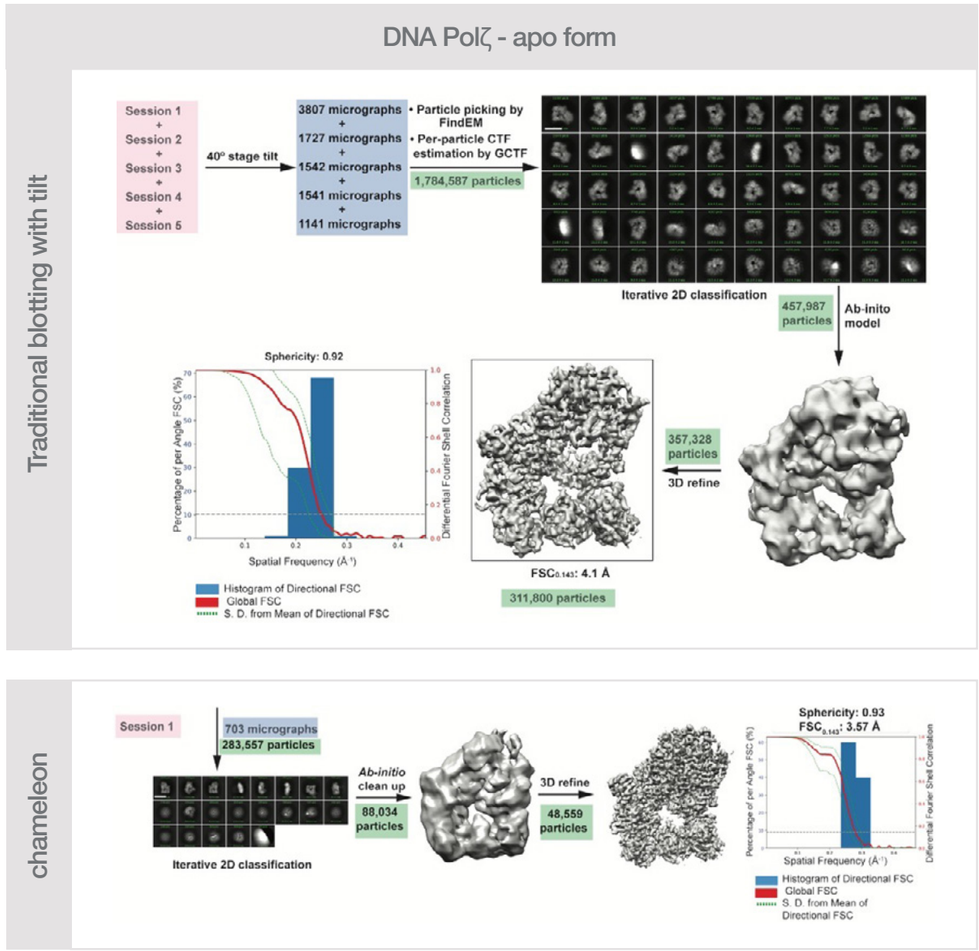


Optimized Vitrification for Your Sample-Specific Behavior

chameleon can be applied to most samples, not just those that are deemed challenging. In cases where traditional methods are struggling however chameleon can be used to address commonly encountered issues such as:

preferred orientation

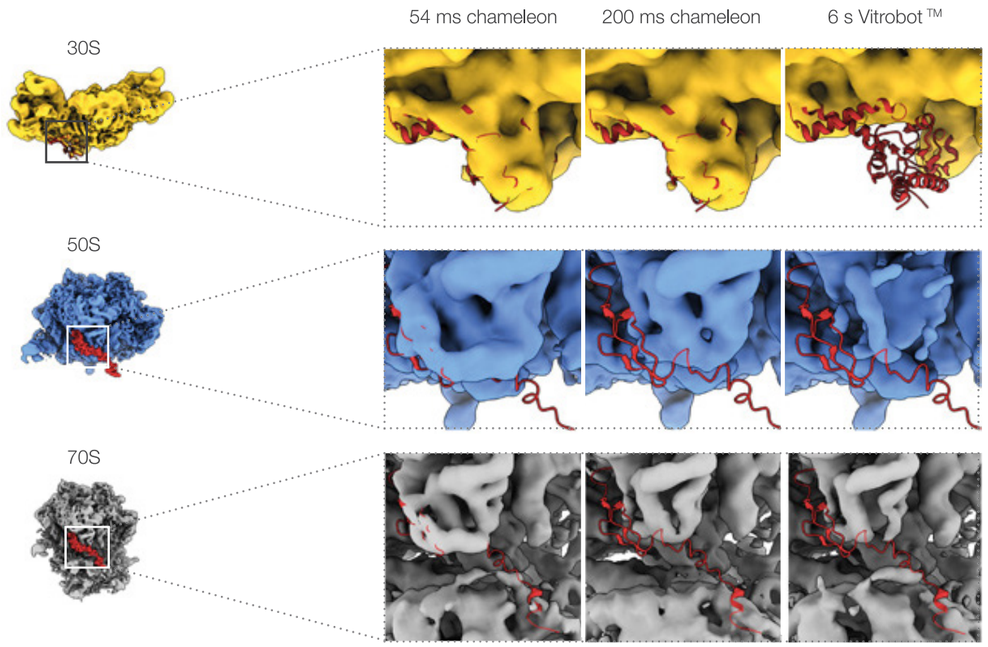
Using equivalent processing methods 134ms chameleon grids produced higher resolution structures with less particles when compared to a 40 deg tilted data set.



Malik, R. et al. Structure and mechanism of B-family DNA polymerase ζ specialized for translesion DNA synthesis. Nat Struct Mol Biol (2020) doi:10.1038/s41594-020-0476-7.

dissociation

Ribosomal subunits demonstrate plunge time dependent dissociation. Subunits missing in the Vitrobot data are present at 200ms (30S) and 54ms (50S and 70S) on chameleon. The 70S is still improving at 54ms.



Klebl et al., 2020, Structure 28, 1–11

denaturation

Ribonucleotide reductase was used to study the effects of sample freezing method and speed on protein concentration and resolution.

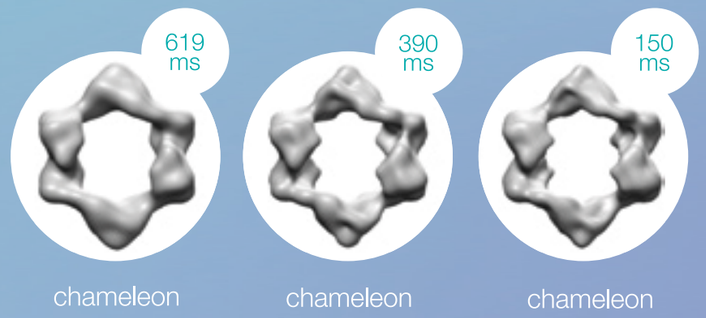
Consistent with literature, blotting concentrates samples on the grid.

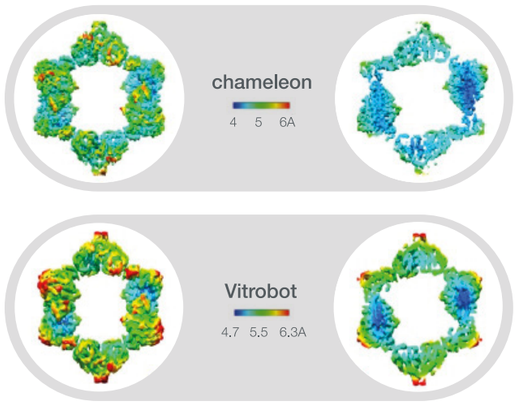
When spraying sample onto self-wicking grids, the number of particles observed is related to the plunge time – **fewer particles are present at faster plunge times**.

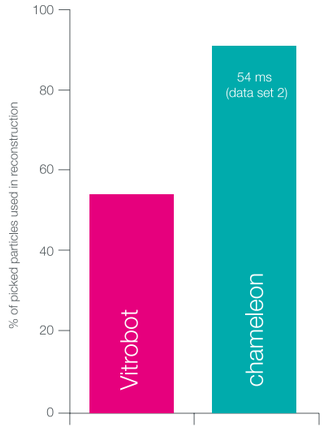
Plunging method	Wicking time (ms)	Protein concentration (mg/mL)	Particles per micrograph (observed, 95% recall)	Particles per micrograph (theoretical)	Ratio (observed particles / theoretical particles)
Vitrobot	n/a	0.5	208	1	208
chameleon	619	1	175	14 - 37	13 - 5
	390	1	269	14 - 37	19 - 7
	150	1	74	14 - 37	5 - 2
	54	4	71	55 - 149	1 - 0.5
	54	6	95	82 - 223	1 - 0.4

Levitz et al, Journal of Structural Biology,Volume 214, Issue 1, 2022.

When particle numbers (~7,500) and processing protocols are held constant, plunge time has an impact on the number of intact particles – **the faster plunge times have more intact particles, which in turn leads to higher resolution reconstructions**.





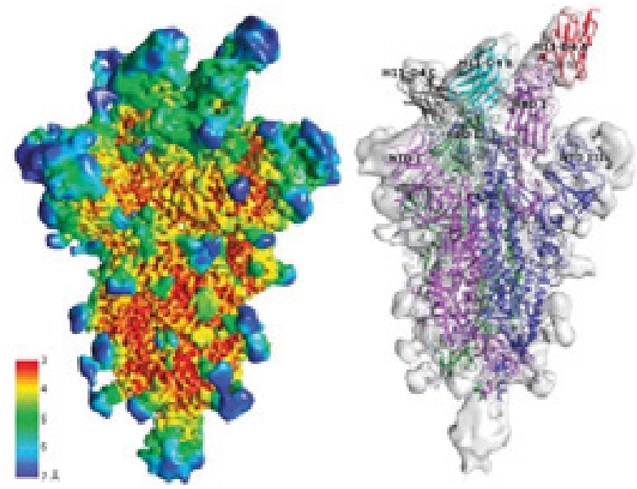


The percentage of picked particles that were used in the final reconstruction is also markedly higher with chameleon. This may allow for shorter data collection times.

reproducibility

A camelid nanobody was identified that binds to the receptor binding domain (RBD) of the Sars-CoV-2 Spike protein, blocking interaction with the ACE2 receptor. The nanobodies bind to all three RBDs in a Spike protein, recognizing the same epitopes and partly overlapping with the ACE2 binding surface.

chameleon was used to overcome sample preparation issues such as uneven ice thickness and to provide reproducible ice quality.



Cryo-EM map of the H11-D4-Spike complex coloured according to local resolution (left). Ribbon diagram of the complex with nanobodies marked in red, teal and dark grey and translucent map indicated in light grey (right).

Ref: Huo, J., Le Bas, A., Ruza, R.R. et al., Nat Struct Mol Biol (2020)

chameleon specifications

minimum sample volume	5 µL
minimum dispense volume	6 nL
sample block temperature control	4ºC to 37ºC
standard and high-speed dispense-to-plunge time	101-2500ms and 54ms
on-line glow discharge	Time and current user selectable
dimensions	(w) 916 mm x (d) 708 mm x (h) 1687 mm 36.1 in x 27.9 in x 66.5 in

how does chameleon benefit me?

blot-free high speed plunging

Self-wicking grids allow rapid on-the-fly dispensing to reduce air water interface effects and potentially address preferred orientation, aggregation or denaturation effects

automated grid handling

Precise and controlled movement of grids eliminates manual damage and loss

on-board optical grid screening

Accept or reject grids ahead of EM screening based on visual images of likely grid quality

intuitive automated workflows

Guided workflows provide easy set-up, use and cleaning of the instrument for any user level

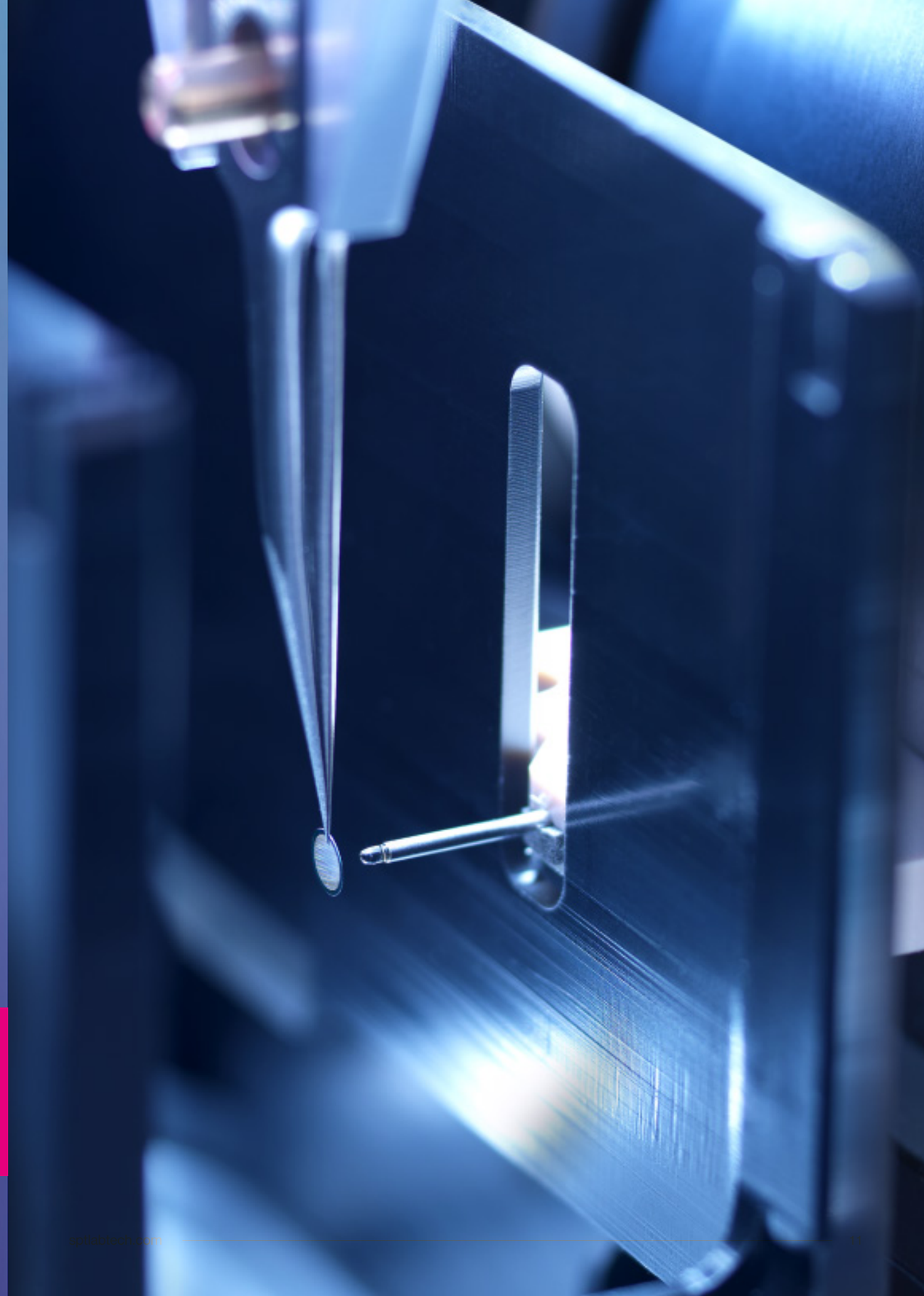
sample tracking and recording

Capture all relevant parameters and grid images for record keeping and future reproducibility

cryogen level sensing/temperature control

Ensure stability and quality of cryogens while safely kept in an automated drawer

Scan me
to find out more



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