nanosoft
CRYOGENIC ELECTRON MICROSCOPY INNOVATION

# Blotless Cryo-EM Sample Preparation

Novel blotless technology reproducibly provides unmatched areas of optimal vitrified ice with lower complexity and cost than other blotless systems.

## Objective

NanoSoft is developing a blotless cryo-EM sample preparation technology for plunge freezing-based vitrification. The goal is to offer a sample preparation platform that provides large areas of vitrified ice of appropriate thickness in a repeatable fashion. Furthermore, the platform will prepare samples in ≤ 100 msec to minimize particle interactions with the air/water interface. The goal is to maintain **low technology complexity**, such that these advantages are offered at **much lower prices than other blotless technologies**.

## Technology Proof-of-Concept

Samples were prepared utilizing a Proof-of-Concept prototype of NanoSoft's blotless technology. The chosen sample for Proof-of-Concept testing was Apoferritin in PBS (9 mg/mL). A multitude of replicates were prepared on R 2/2 Quantifoil grids and imaged with a side-entry TEM with CCD camera to partially optimize the Proof-of-Concept prototype.

Following quasi-optimization for this prototype, four samples were prepared and then imaged (without pre-screening) using a Thermo Fisher Scientific Talos Arctica TEM at 200 kV with Gatan K3 direct electron detector. Atlas images (**Fig. 1**) were analyzed to quantify the area of good vitrified ice on each grid, and example high mag images (36,000X) were captured across each grid (**Fig. 2**). For this Proof-of-Concept testing and due to limited imaging time, data was collected on only one grid (of the four) to generate a high-resolution reconstruction from 143 high magnification images (30,283 particles). 25-50 high magnification images were collected on the 3 other grids.

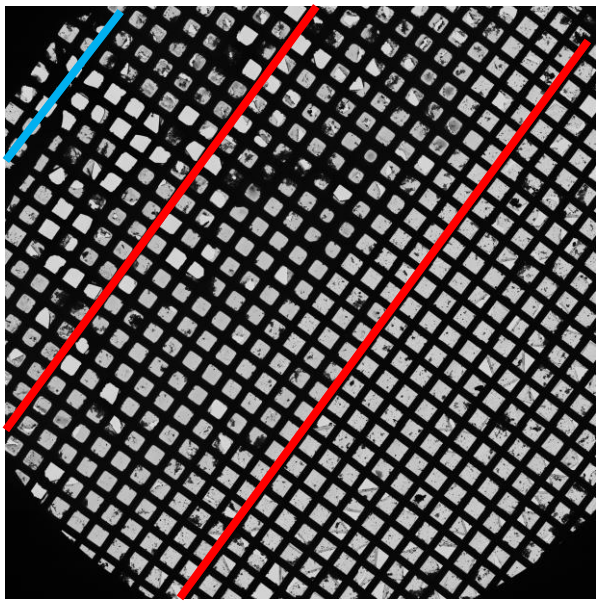

**Fig. 1.** Example of an Atlas from a grid prepared with Proof-of-Concept prototype of blotless preparation technology. **Area between red lines provides large areas of optimal ice as seen in Fig. 2**.

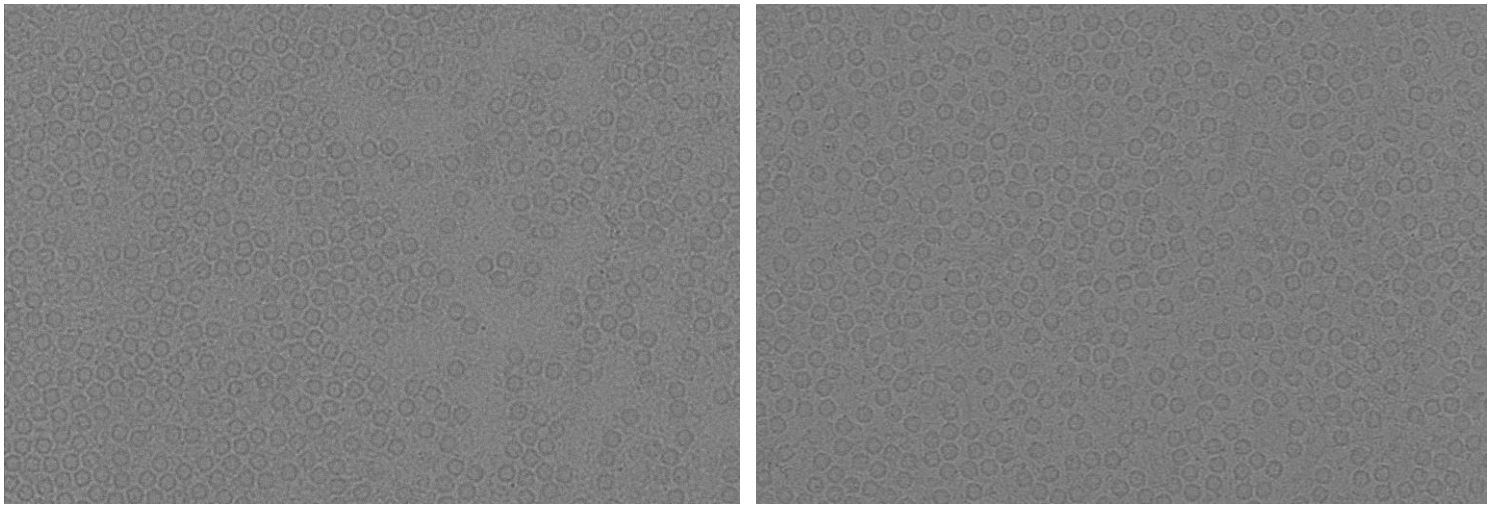

**Fig. 2.** High magnification images of Apoferritin that demonstrate ice quality observed between red lines in **Fig. 1.**

**Key results from the Proof-of-Concept testing include:**

- 75% of grids showed large areas of ice optimal for cryo-EM, with those grids containing an average of 80 such grid squares.
- Our technique reproducibly provided significantly larger areas of optimal ice than other blotless systems (Chameleon, Vitrojet), with a Proof-of-Concept prototype.
- Samples were vitrified within ~225 msec, which leads to fewer particle interactions with the air/water interface and a better distribution of particle orientations (continued development will test proteins with less symmetry than apoferritin and will show more distinct orientations from 2D class averages).
  - **See Noble, Alex J., et al. Nature methods 15.10 (2018): 793-795.**
- Optimal ice quality led to a reconstruction resolution of 2.4 Å (see **Fig. 4,5**), using only 143 images (30,283 particles) and minimal data refinement (Cryolo particle picking followed by typical Relion CTF/ beam tilt refinement and polishing).

**Next Steps of development:**

- Utilize a next generation prototype to optimize the blotless process and validate technology robustness and versatility through testing of various Structural Biology samples.
- Though area of suitable vitrified ice and repeatability are already unmatched, further increase where possible (see **Fig. 3**).

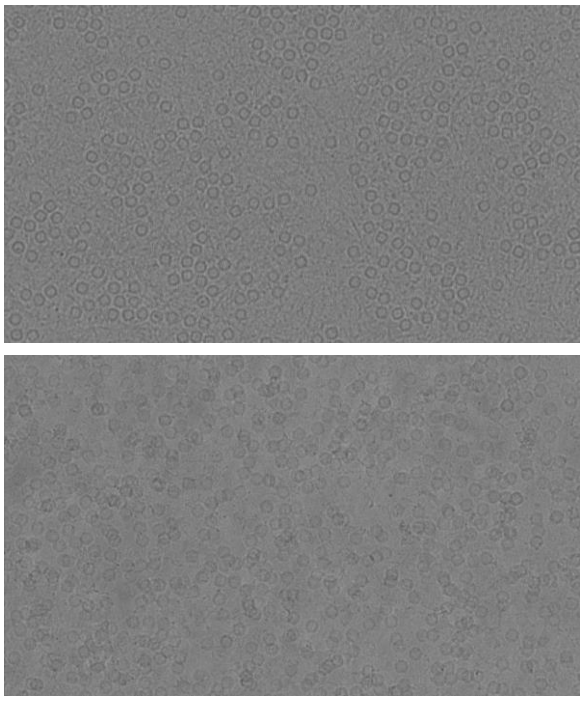

**Fig. 3.** Although large areas of optimal ice are found (top), there are also areas of thick ice (bottom). Bottom is an example of ice seen in between the red and blue line seen in **Fig. 1**.

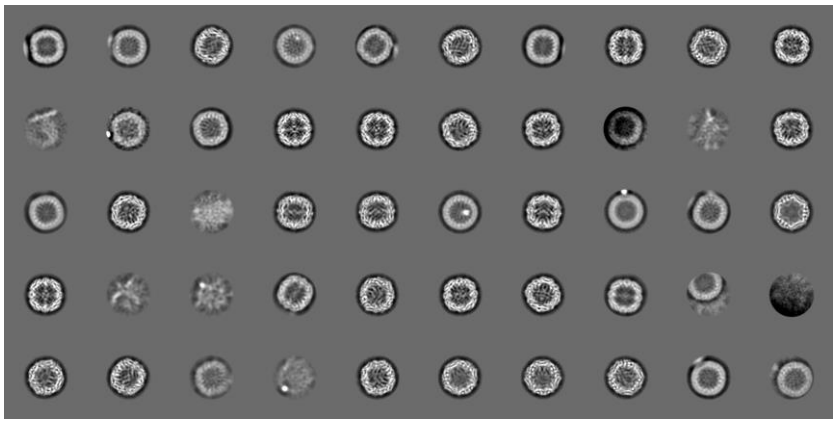

**Fig. 4.** 2D Class Averages from example grid

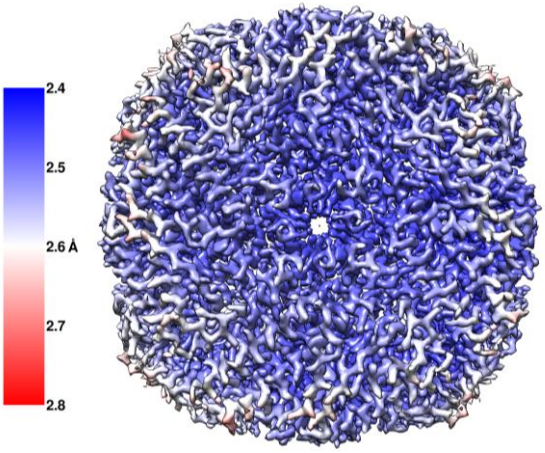


**Fig. 5.** 2.4 Å resolution reconstruction from example grid

## Conclusions

- NanoSoft has developed a blotless technology that reproducibly results in much larger areas of high-quality ice than other blotless technologies (Chameleon or VitroJet), even with a Proof-of-Concept prototype, leading to nearly atomic resolution on protein samples with minimal images and particles.
- The Proof-of-Concept prototype prepared samples fast enough to mitigate air/water interface interactions (225 msec) - **next generation prototype is expected to prepare samples within 100 msec**.
- A next generation prototype is being used to optimize the blotless process towards commercialization of a sample preparation platform. Starting Winter 2020, NanoSoft aims to validate the technology by preparing Structural Biology samples with select researchers - **please contact us if you are interested in preparing your samples either as part of the initial cohort or with future continuing commercialization**.

Find more information at

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