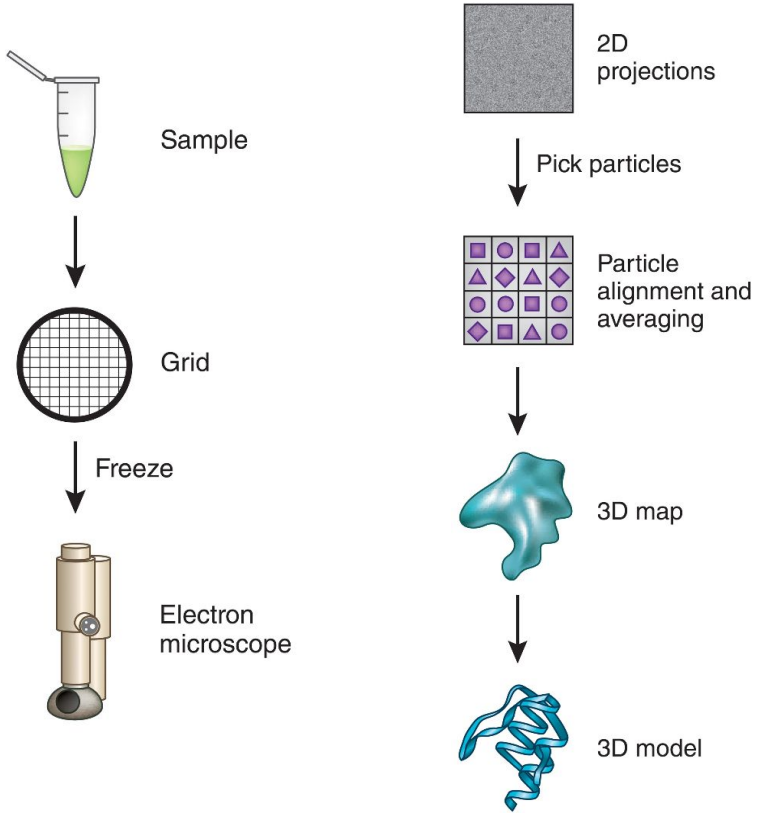
Optimization of the Chameleon System

Strategy design and implementation to minimize preferential orientation and other Air-Water interface problems in cryoEM grid preparation

A Quick Refresher on CryoEM:

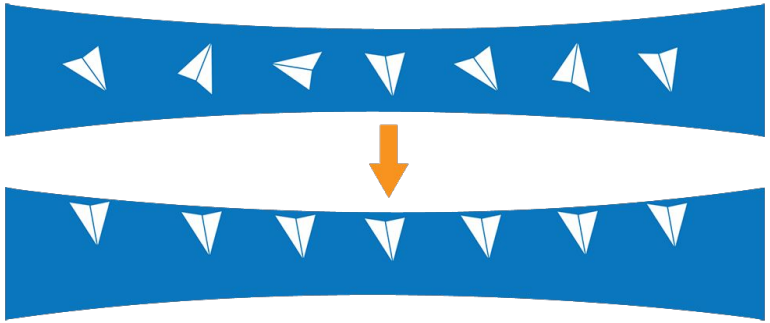
- Resolution used to be too low to resolve proteins
- Better tech (ie: direct electron detection) allows for drastically better resolution
- Human ingenuity (literally) filled the remaining gaps

- Liquid ethane is used freeze in solution samples in vitreous ice
- Thousands of 2D projections are aligned and averaged to produce a 3D structure
- Multiple advantages compared to other imaging techniques



Doerr, A. Single-particle cryo-electron microscopy. https://doi.org/10.1038/nmeth.3700

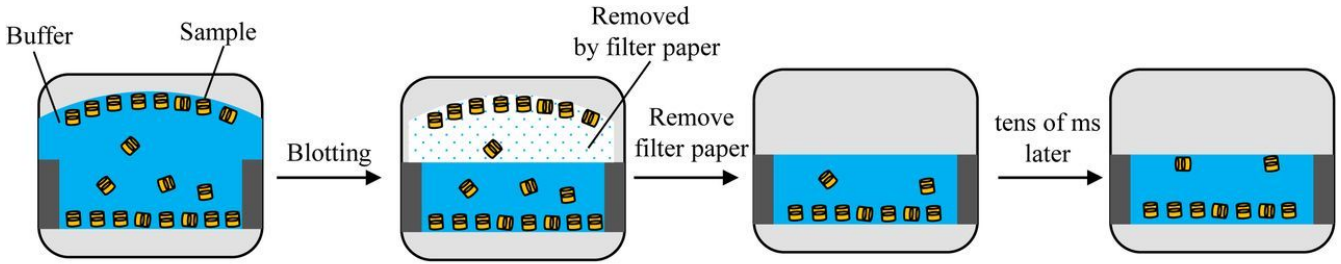
The Air-Water Interface: The Main Culprit



nanoScience Instruments Blog. The Air Water Interface and Sample Preparation for Cryo-EM

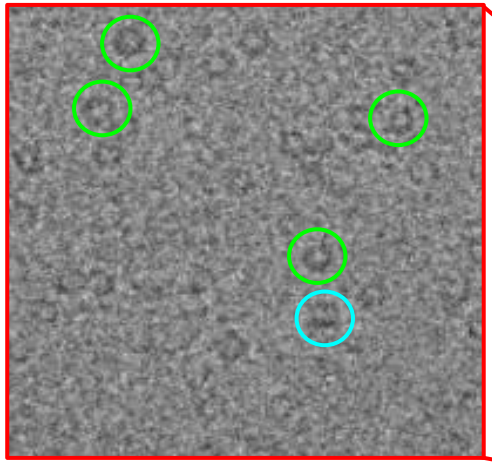
Interactions with the Air-Water Interface (AWI) can lead to:

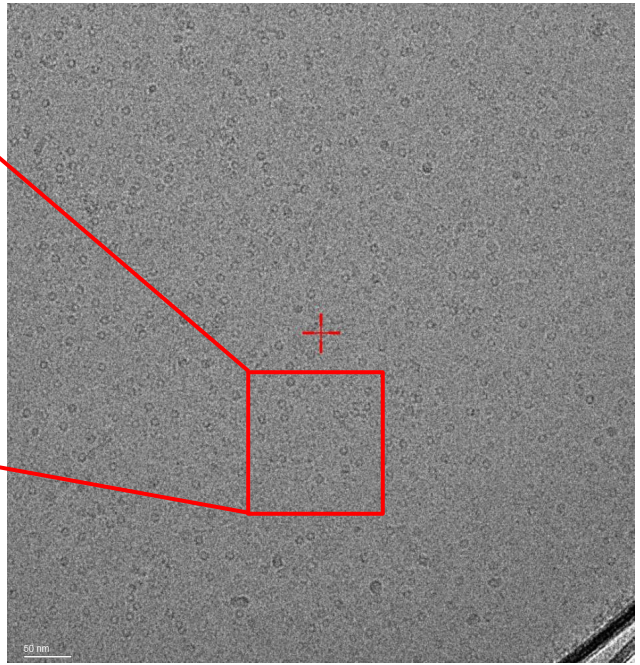
- **Protein deformation**
- **Preferential Particle Orientation (PPO)**
- **Unwanted hydrophobic interactions**

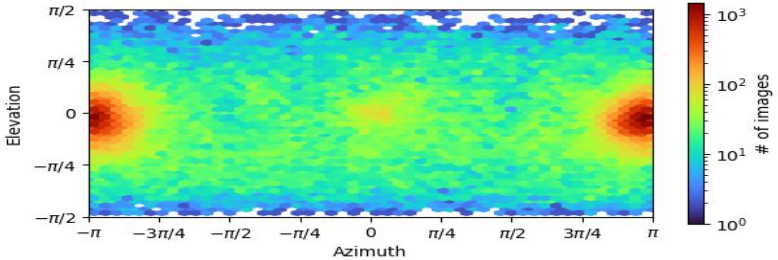


Kang, J. Theoretical framework and experimental solution for the air-water interface adsorption problem in cryoEM. https://doi.org/10.1101/2023.05.23.541984

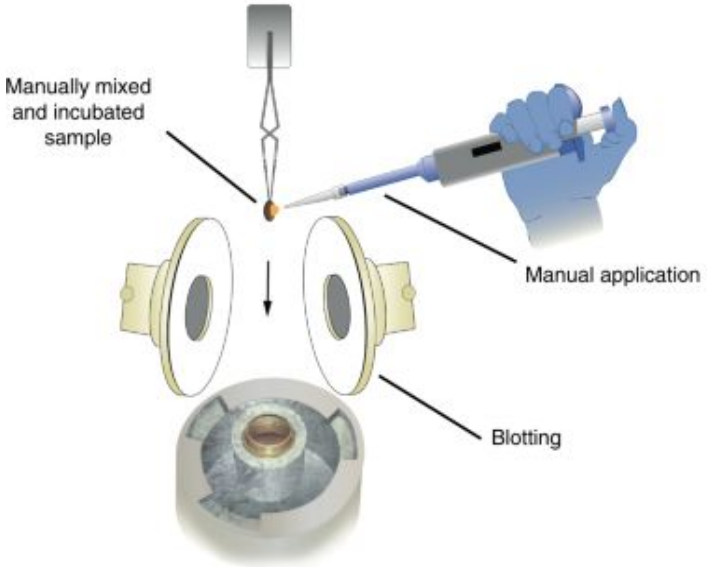
Preferential Orientation And Why It's a Problem







Why is it so hard to address with traditional methods?



https://www.bioz.com/result/standard%20vitrobot%20ethane%20nitrogen%20container/product/Thermo%20Fisher

https://www.cryoemcenters.org/merit-badge/tfs-vitrobot-mark-iv/

Steps Using Vitrobot:

1. Glow discharge grid
2. Pipette onto grid
3. Blot
4. Plunge
5. Analyze

Automating grid preparation using Chameleon

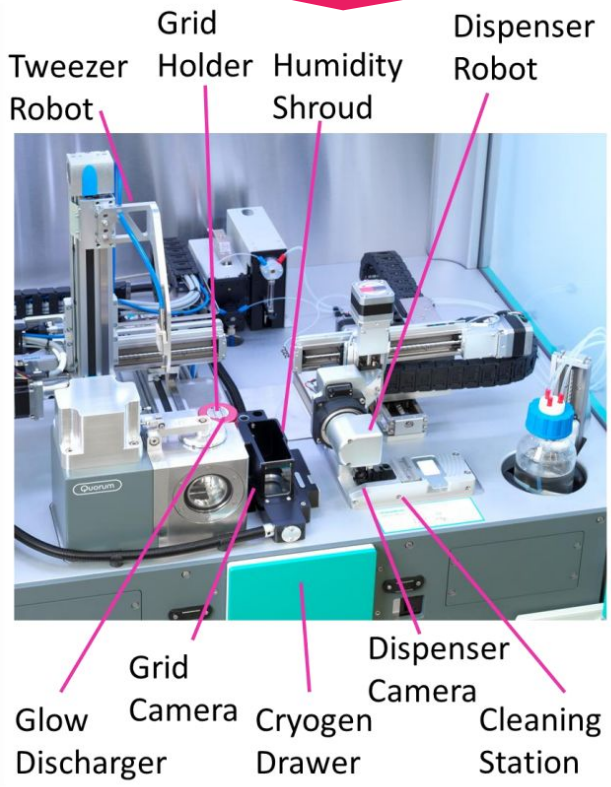
A new system with many advantages:

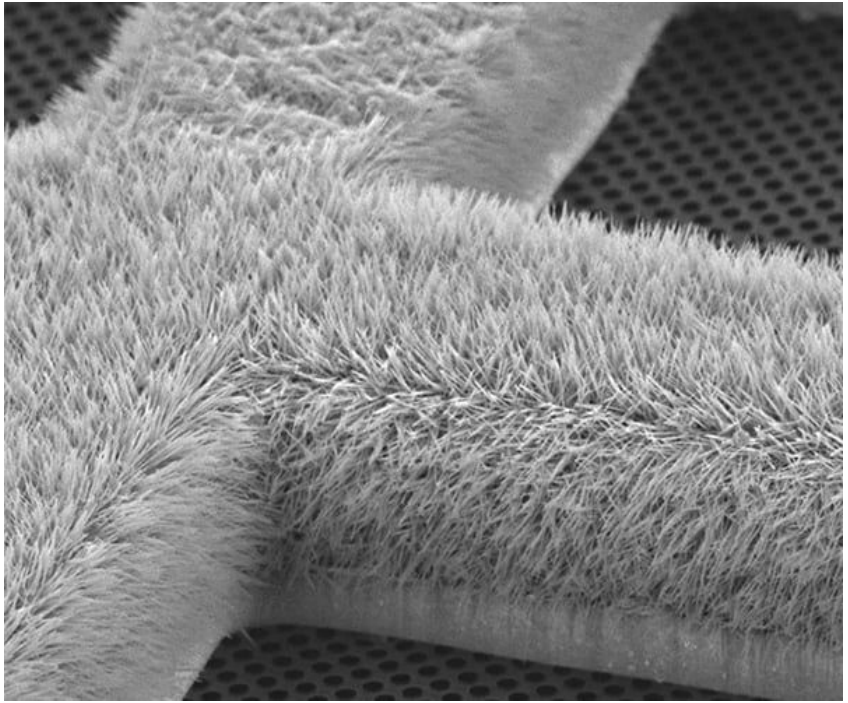
- **No blotting step**
- **Minimal sample volumes for each grid**
- **Fully automated**

But most importantly, decreases time from sample application to freezing by orders of magnitude!

Image courtesy of SPTlabtech Chameleon training material

A closer look at what's under the hood

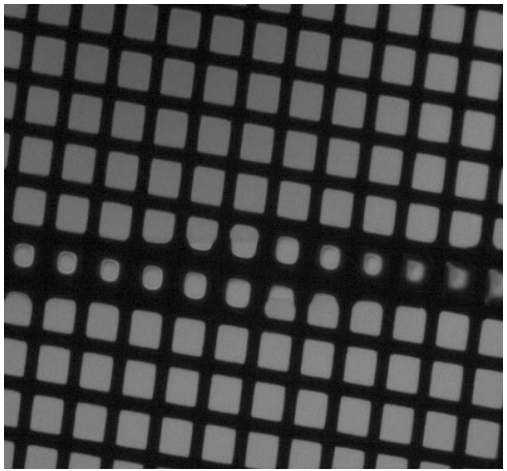


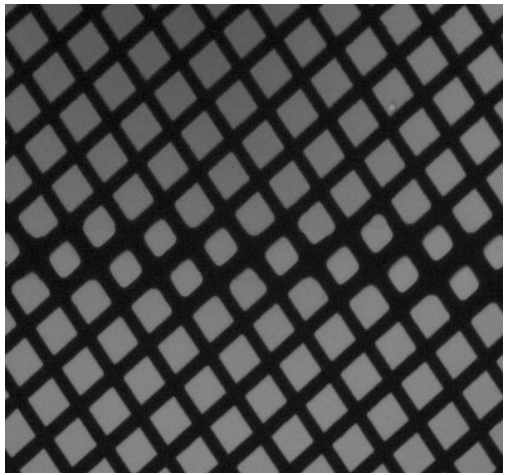


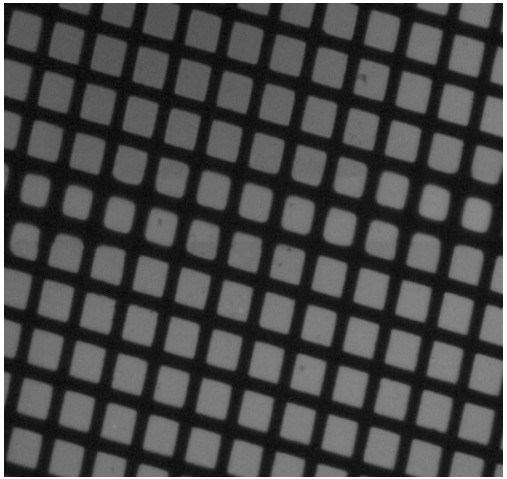
Images courtesy of SPTlabtech Chameleon training material

So lets see these grids in action

Fully wick any protein sample....







...all in as little as 54 ms!

Individual grids can then be characterized based on wicking

rid characterization step in Chameleon software

Project goal and workflow

Weekly Workflow:

- Prepare grids with different variables
- Compare two samples with different conditions
- Screen resulting grids and determine which had less PPO

Goals

- Test a variety of samples in a variety of conditions
- Determine how these conditions affect orientation in PPO proteins
- Identify any patterns that emerge
- Develop an SOP for future in-house and visiting researchers

Proteins and conditions tested

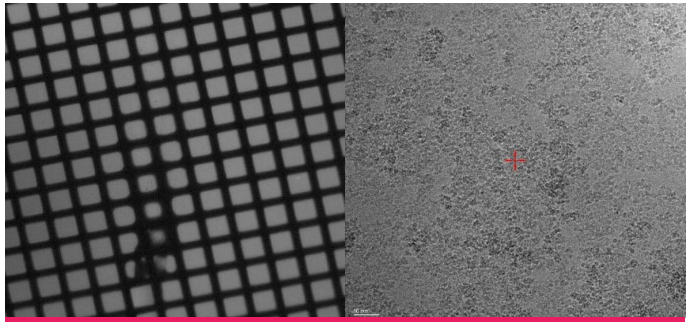
Proteins:

- Acetylcholine Receptor (AChR)
- ClpE
- ClpE/P/FITCcas complex
- ClpX/P complex
- Polymerase Epsilon Complex

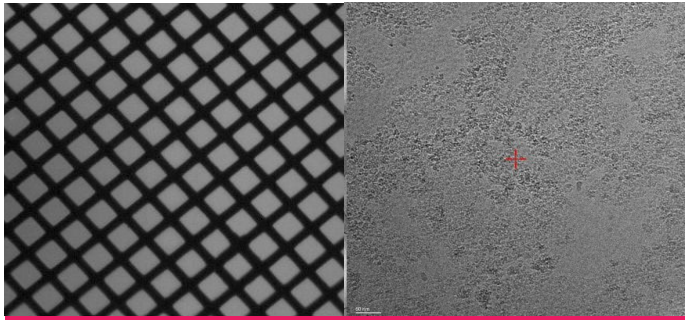
Conditions Tested:

- Glow discharge amperage all done at 12 mA
- 20 to 140 sec glow discharge time
- 54 to 2500 ms wicking time
- Buffer with and without glycerol
- Internal vs external incubation

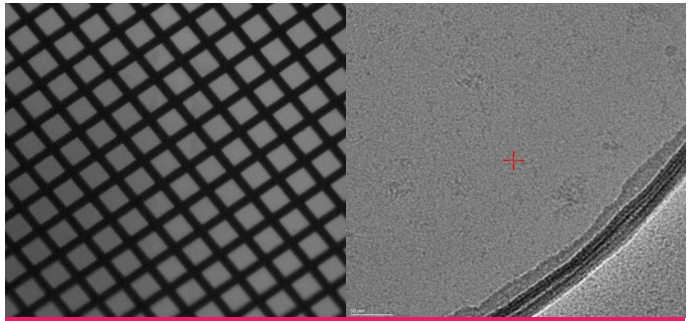
Example micrographs of screened grids



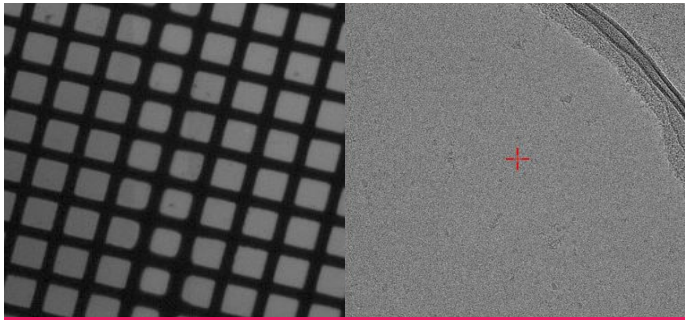
AChR - 40 sec GD - 757 ms Plunge



AChR - 60 sec GD - 1232 ms Plunge

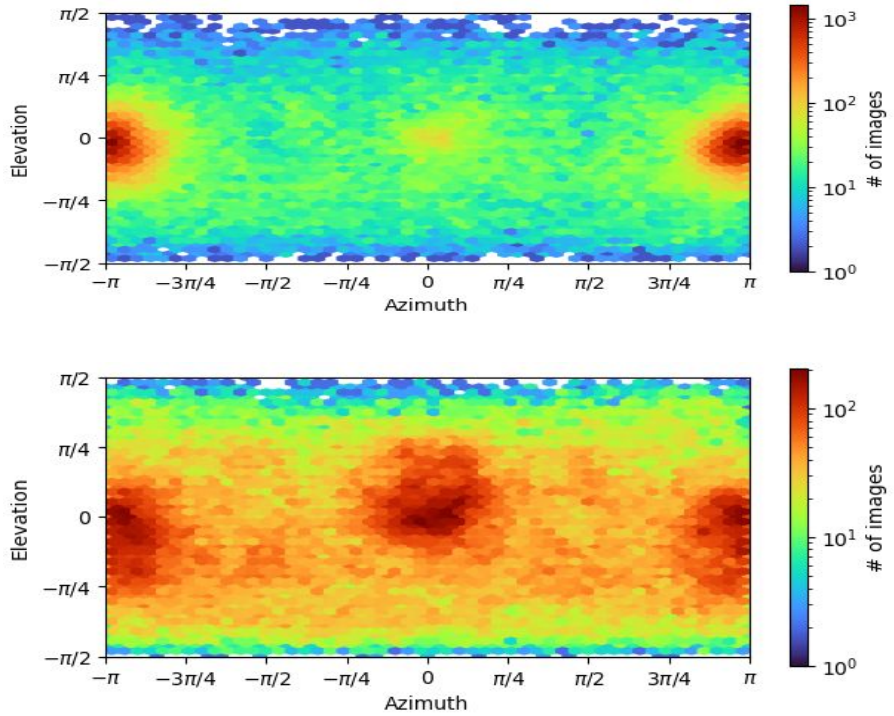


PolE+Glyc-60 sec GD-350 ms Plunge



ClpG - 60 sec GD - 125 ms Plunge

Some promising results



Angular Distribution of Vitrobot (top) vs Chameleon (bottom) prepared ClpXP grids

Bad results do not a failed project make

Despite not preparing grids with minimal PPO, there is still plenty of good data and a overall results…

Practical Benefits:

- One person to learn the complex machine is more efficient
- Easy one-on-one training of in-house researchers
- One person to understand all the issues and how to troubleshoot
- Plenty of data to develop a good strategy and SOP

Lessons learned

- Playing with plunge time to get perfect wicking is a dead end
- Glow discharge time and variables are the only machine variables that can be adjusted
- It’s better to have overwicked grids than perfect ice thickness
- 60 sec GD time for unknown samples
- The 2-stripe mode is a waste of grids

Final strategy and conclusion

For samples with AWI problems:

- All plunge times should be set to 54 ms
- Only use the 1 stripe mode
- Aim to prepare 4 grids/sample
- First grid should be activated at 60 sec/12 mA
- Second grid should change amperage to 15-20 mA
- Second and third grids should use same amperage, but change time by +/- 5 sec depending on wicking of first 2 grids

Thank you for you attention

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