NANUQ™

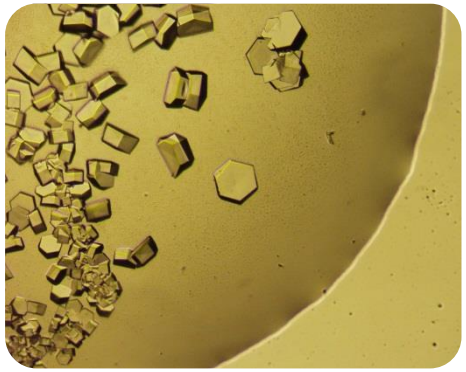
Advanced Cryocooling Technology for Biomolecular Cryocrystallography

MiTeGen, LLC
Ithaca, NY

Crystallography requires good crystals - *and that they stay good* to the end of data collection

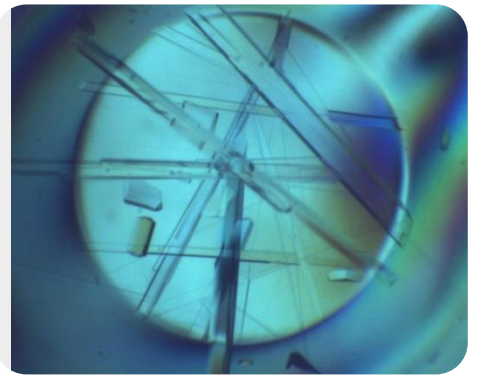
- Getting good crystals requires

  Time + Effort + Resources ($)



- Once you have good crystals, don't **damage** or **waste** them.

- Use protocols that **maximize data quality**, **maximize throughput**, **minimize crystal-to-crystal variability**, and **minimize** risks of crystal **contamination**, **damage** and **loss**.



Use MiTeGen's ***NANUQ™*** automated cryocooler!

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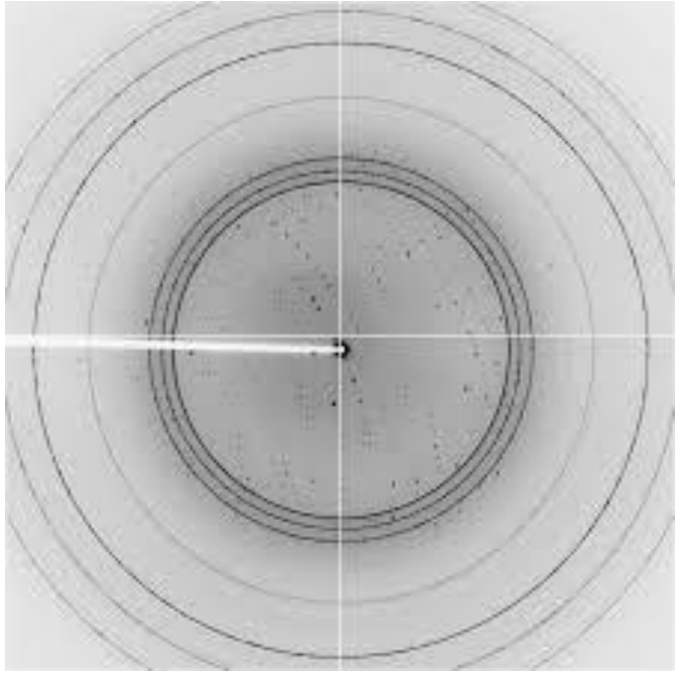
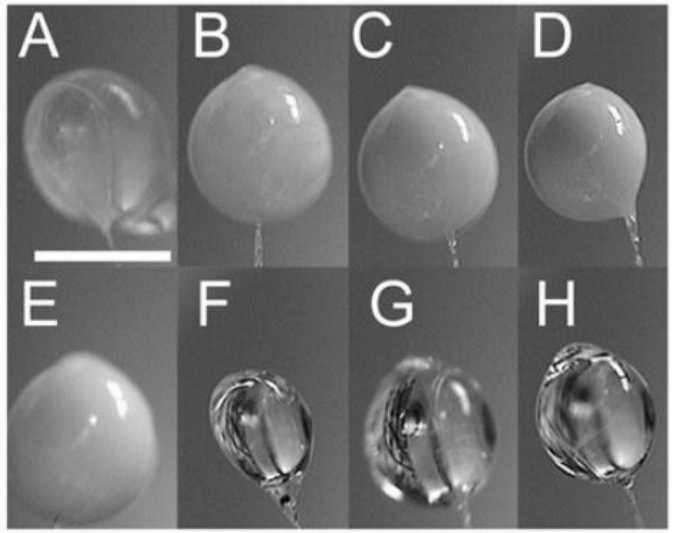
Challenges in Biomolecular CryoCrystallography



- Cooling **damages crystals**, especially when ice forms.
- **Ice forms** in **inside** and **outside crystals**.
- **Ice** accumulates in LN_2 and **contaminates crystals**.
- **20% of PDB deposited data sets** - and a much larger fraction of all data sets - **show structure factor errors due to ice.**
- High-value targets **with large solvent contents** (e.g., membrane proteins) and **large solvent cavities** (e.g., large complexes) are the most challenging to cool successfully.

You have Ice? Why not just use more cryoprotectant?

- **Cryoprotectant soaks** reduce ice, but can **damage** or **dissolve** crystals.
- Cryoprotectants can **modify protein conformation.**
- **Cryoprotectants reduce electron density contrast** and **resolution.**
- Cryoprotectants complicate electron density in solvent cavities.
- **Cryoprotectants** can **displace** or be mistaken for **ligands** (e.g., in HT fragment screening)



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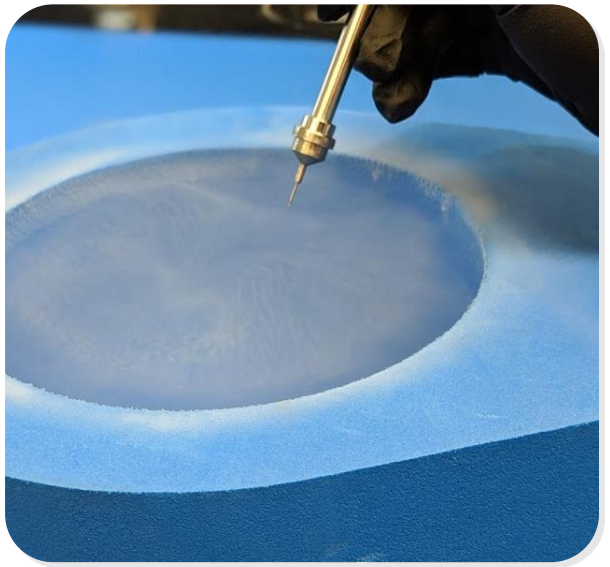
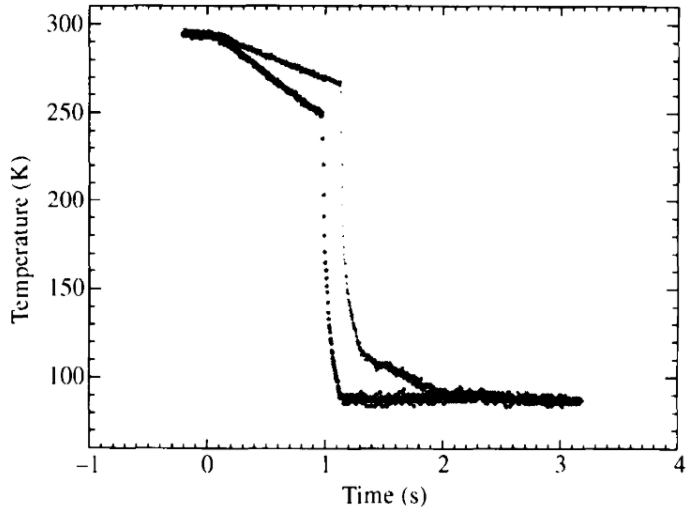
Challenges in cooling by hand plunging in LN_2

- Cooling **damage** depends on **cooling rate** and **cryoprotectant concentration**.
- **Cooling rates** in hand plunging are **modest** (100-1000 K/s) , so **cryoprotectants** are often needed **to eliminate ice.**
- **Cooling times** in hand plunging are **0.1 to 1 s**, **too long** to **kinetically capture** the biological temperature **structural ensemble**.





T.-Y. Teng & K. Moffat, 1998

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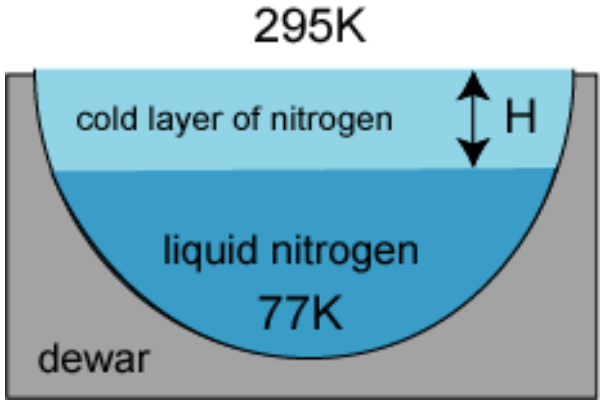
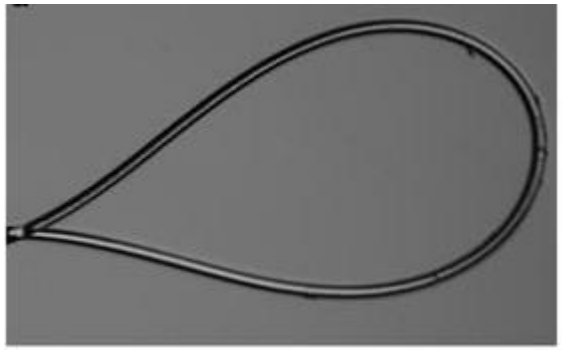
Challenges in cooling by hand plunging in LN_2

- **Cooling rates** are **highly irreproducible** (by a factor up to **100** ×) due to cooling in **cold gas** above LN_2 and to variable plunge speeds.
- Cooling rate variability is a **major cause of crystal nonisomorphism**.
- Why does one crystal diffract better than the next? Was it how it was grown, how it was cooled, or ... ?
- **Crystals** are **lost** during hand plunging and during manual loading into pucks. **Loops** sent to synchrotrons often arrive with **no crystals**.





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What Would an Optimal Cryocrystallography Workflow Look Like?

1. Mount as-grown crystals and check diffraction at **room T** using MiTeGen's MicroRT™ system. Triage bad crystals.

2. Harvest crystals **in a humidity-controlled environment**.

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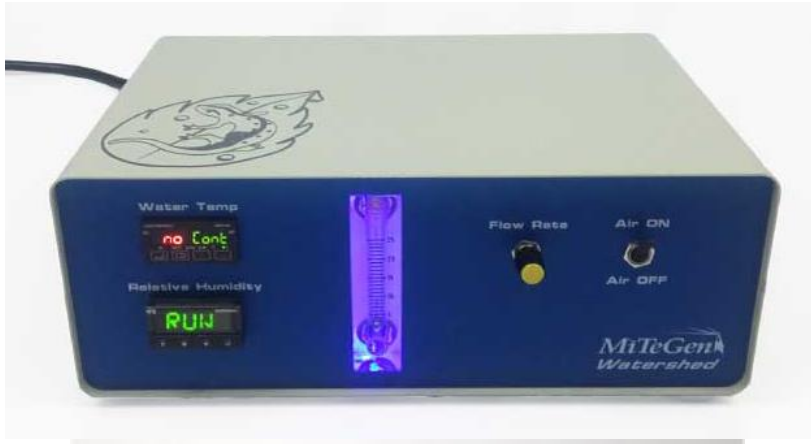
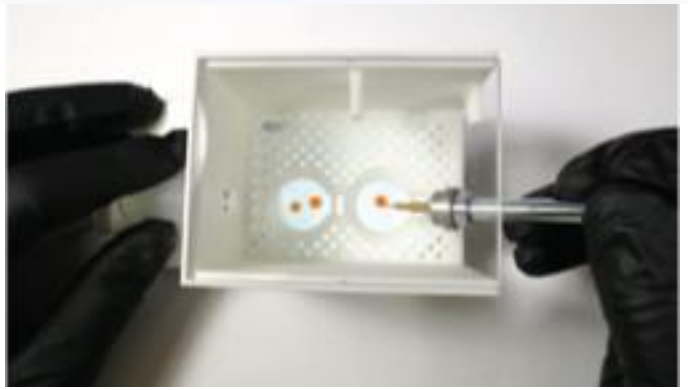
Watershed™ Humidity Control System





- *Prevent drops from drying out while harvesting*
- *Perform controlled crystal dehydration to improve diffraction*

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What Would an Optimal Cryocrystallography Workflow Look Like?

1. Mount as-grown crystals and check diffraction at **room T** using MiTeGen's MicroRT™ system. Triage bad crystals.
2. Harvest crystals **in a humidity-controlled environment**.
3. Do a quick swipe through oil or a 10% cryo solution. **Do not soak in penetrating cryoprotectants.**
4. **Remove excess liquid** from around the crystal (and use MiTeGen's MicroLoops or MicroMounts).
5. **Cool as fast as possible** in **ice-free LN2**.
6. Store in an **ice-free** environment until data collection.

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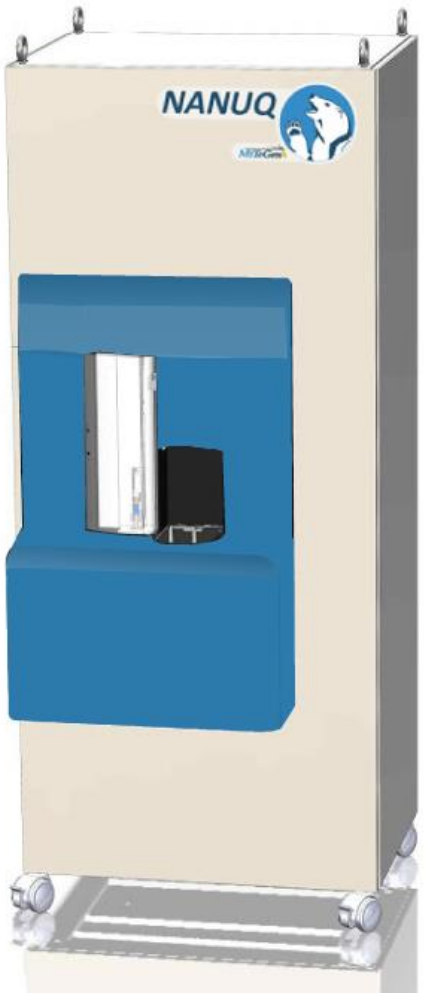
NANUQ™:

- Eliminates ice formation and ice contamination
- Minimizes sample-to-sample variability
- Provides complete control of every plunge – ultrafast cooling, slow cooling, or anything in between
- Standardizes cooling for every crystal and every user
- Automates critical steps between crystal harvesting and X-ray data collection



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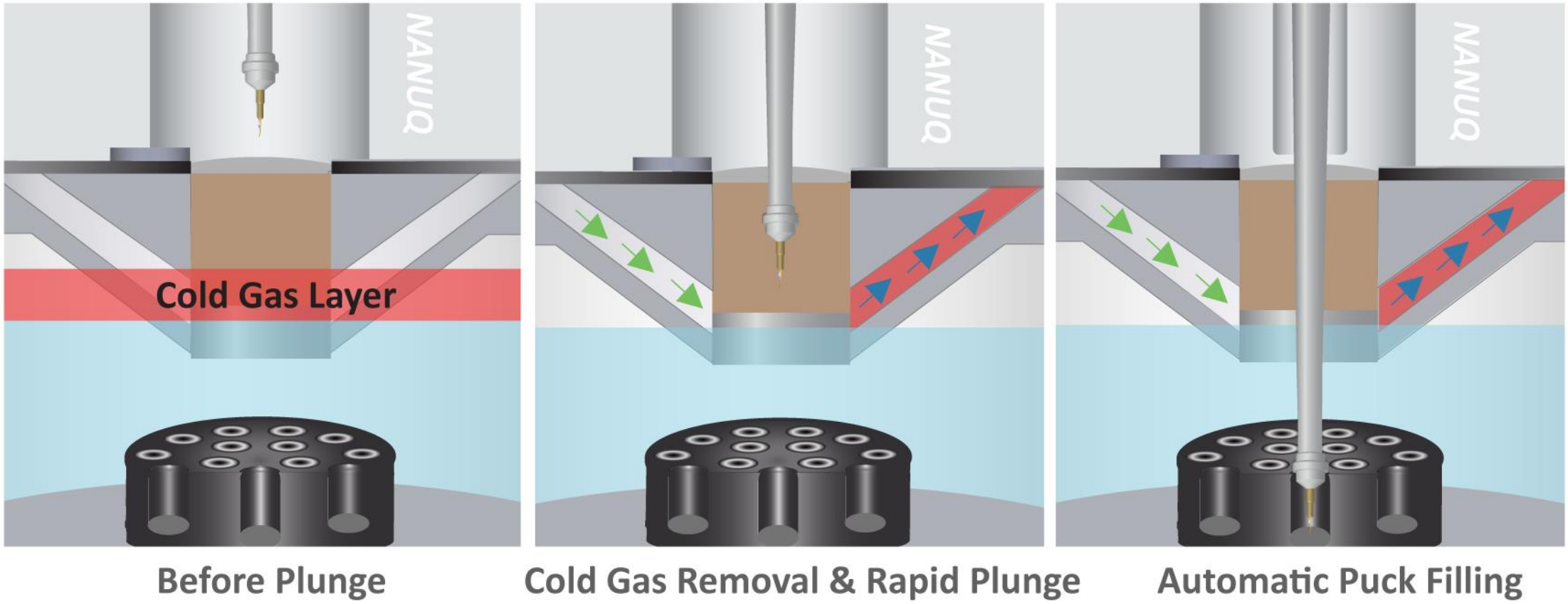
NANUQ™: Key Design Features and Patented Technology



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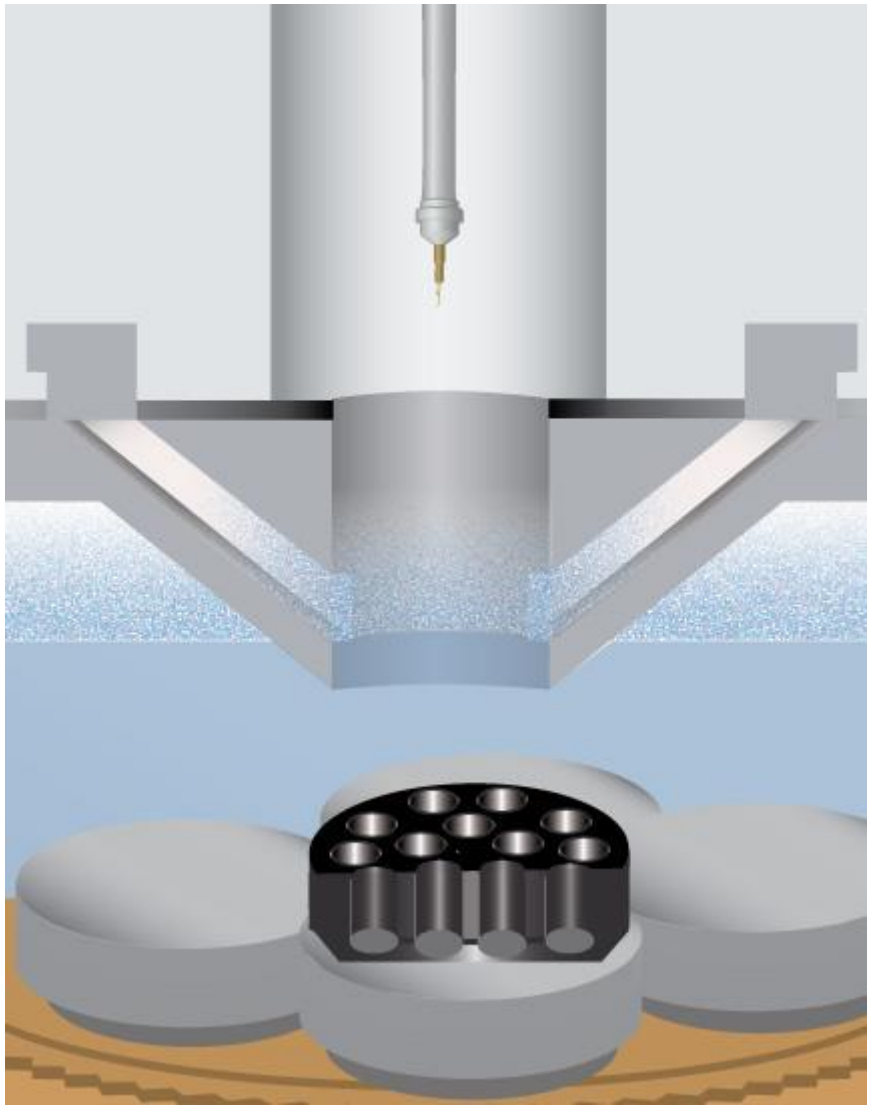
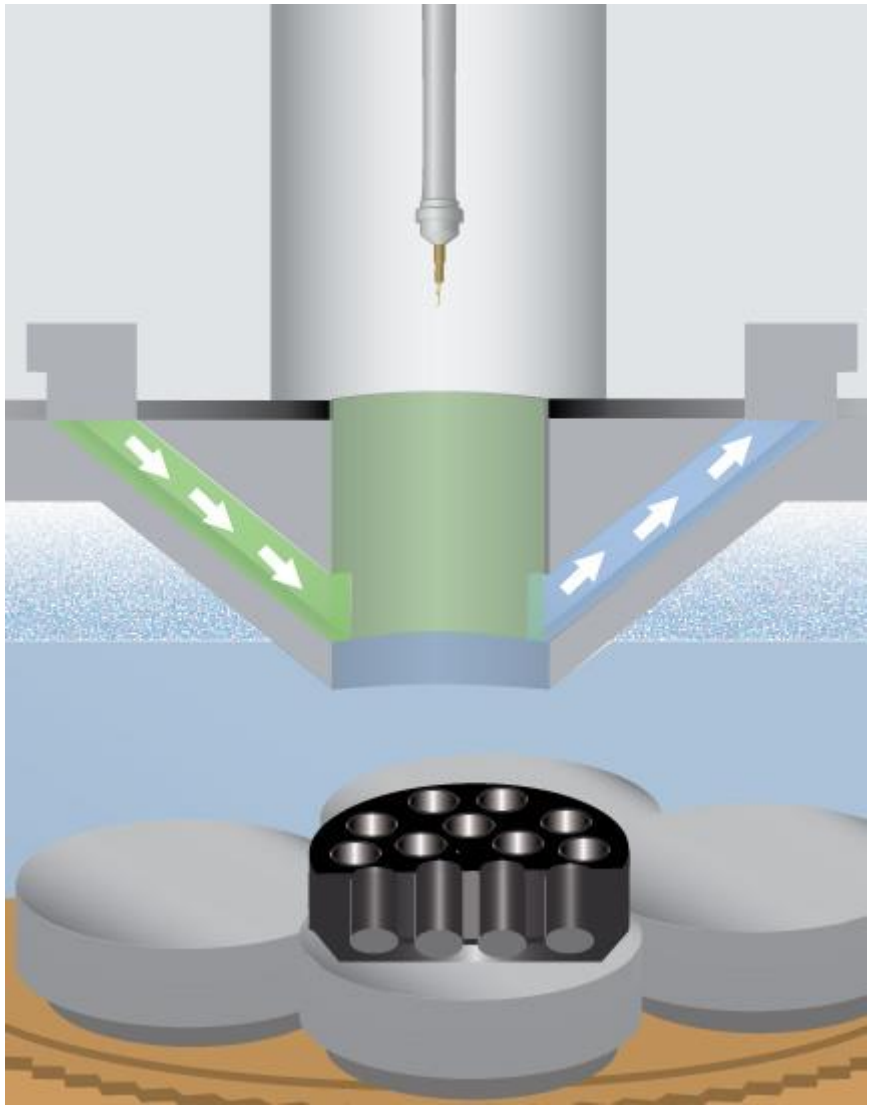
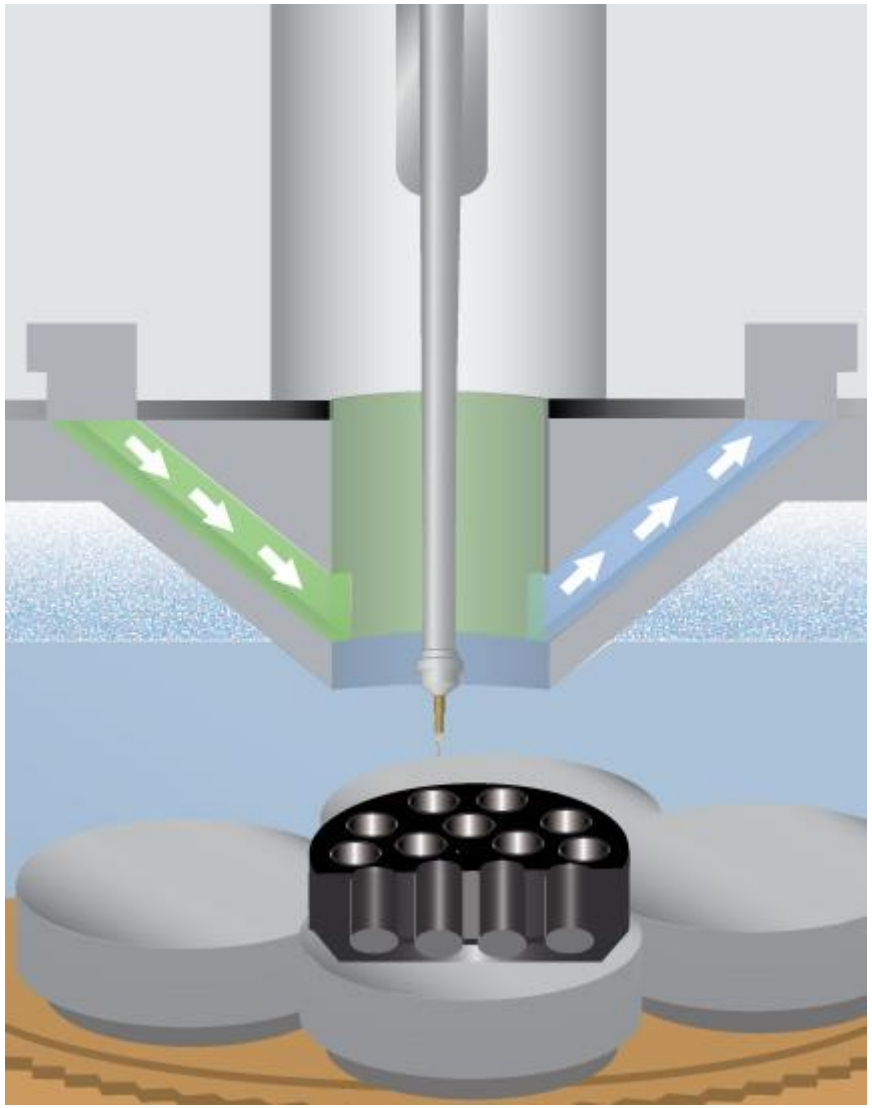
NANUQ™: Hyperquenching (Fast Cooling) Plunge Sequence



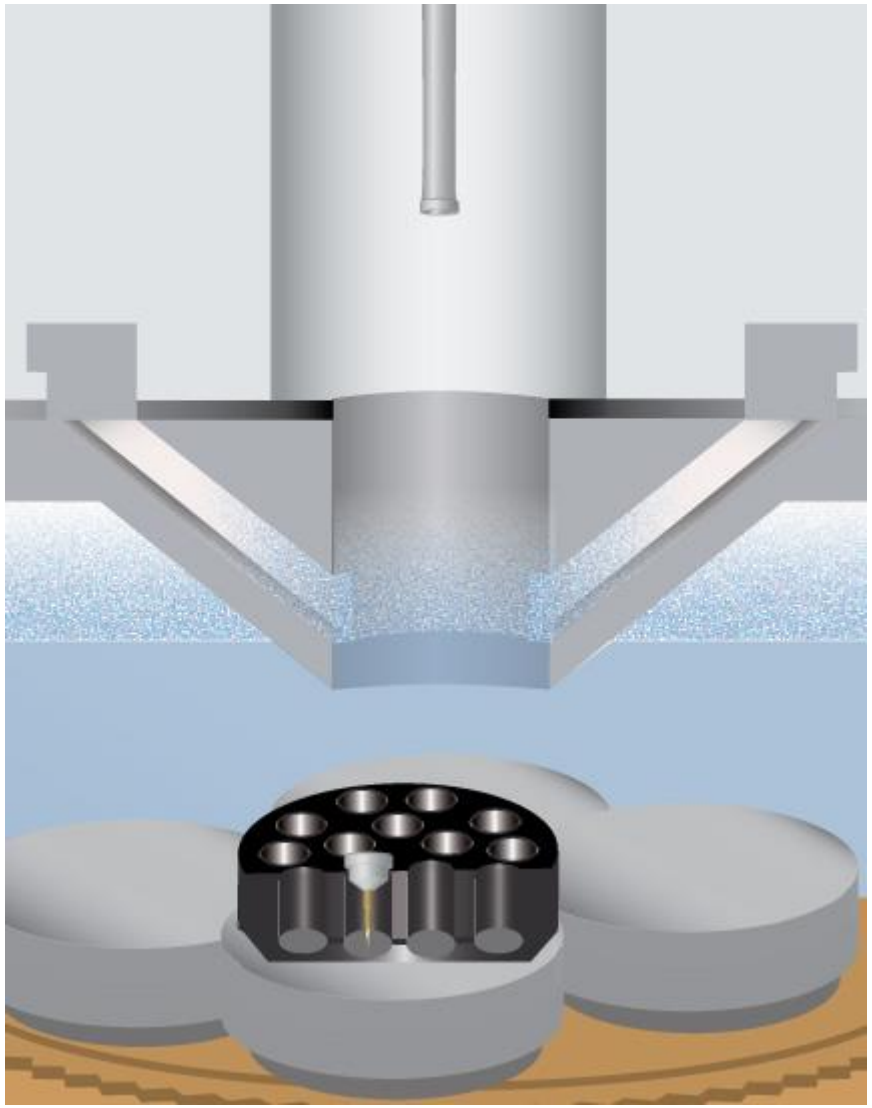
NANUQ™: Hyperquenching Plunge Sequence



NANUQ™: Hyperquenching Plunge Sequence



NANUQ™: Hyperquenching Plunge Sequence

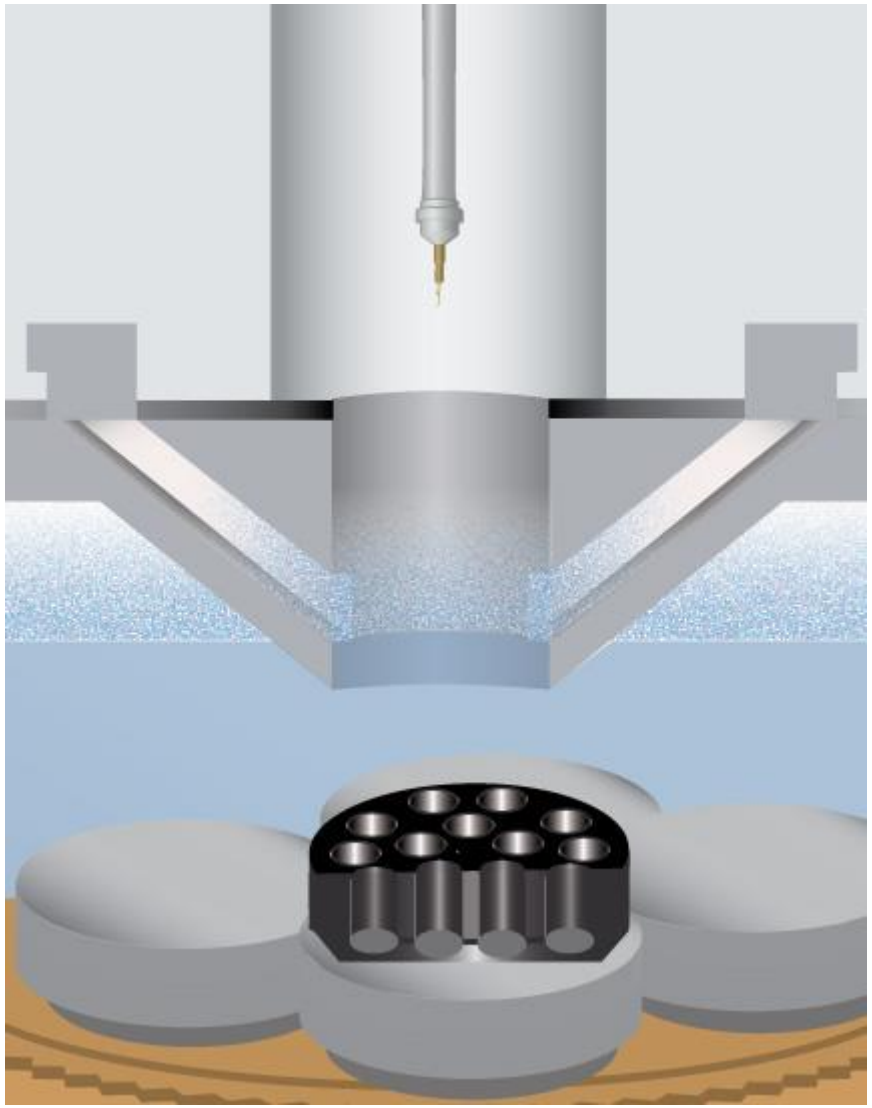


NANUQ™: Slow- and Variable-Rate Cooling

The fastest cooling may not always give the best results.

- **Large crystals** can develop internal thermal gradients during fast cooling that can lead to crystal damage.
- Flows of internal solvent during cooling can increase disorder. Allowing more time for this flow can reduce disorder.
- The protein and lattice may undergo favorable relaxations during slow cooling that improve overall crystal order.

NANUQ™: Slow / Variable-Rate Cooling Plunge Sequence



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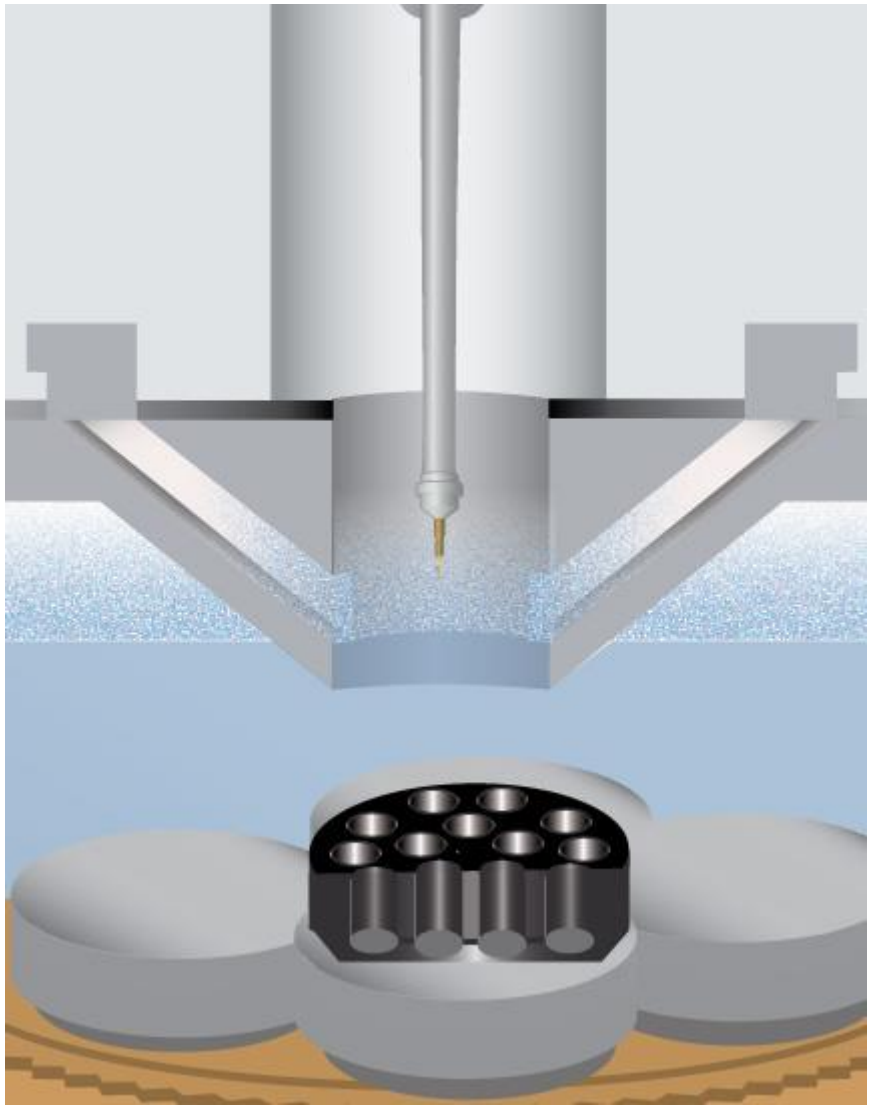
NANUQ™: Slow / Variable-Rate Cooling Plunge Sequence



Cooling rate determined by sample transit time through cold gas, i.e., by plunge speed

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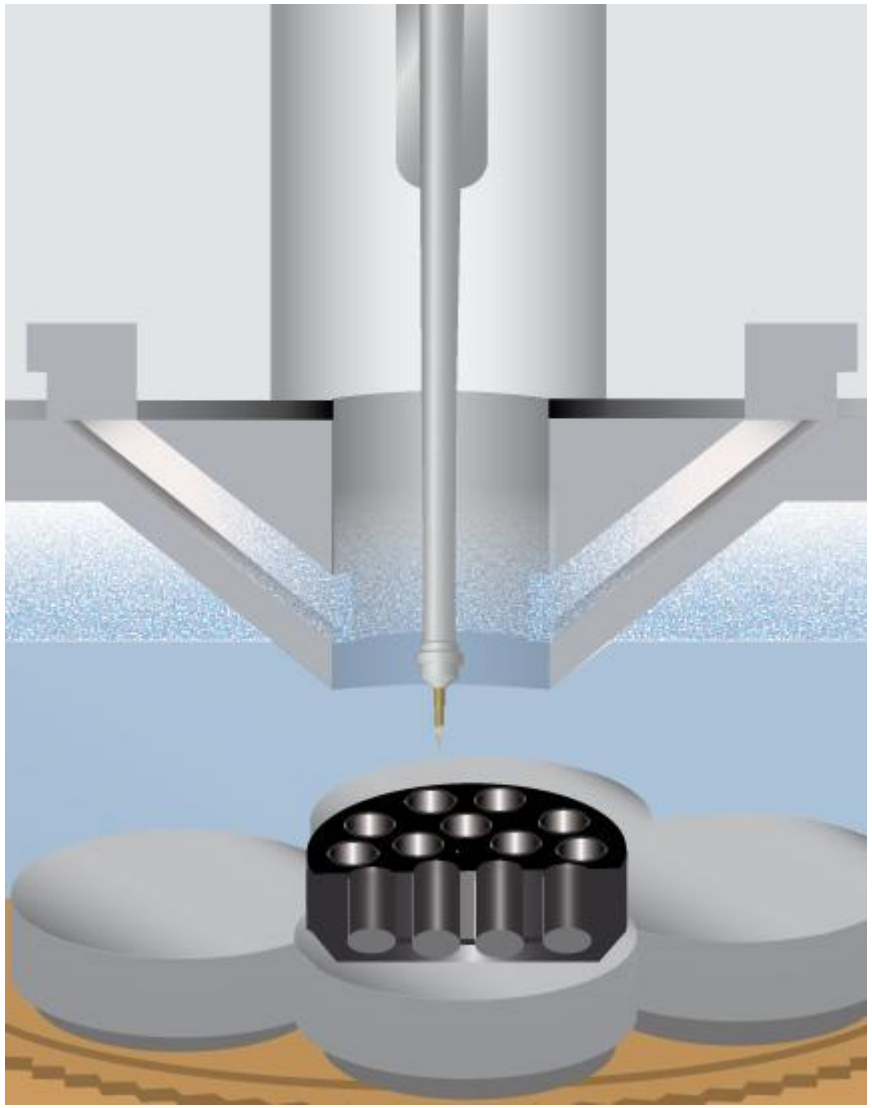
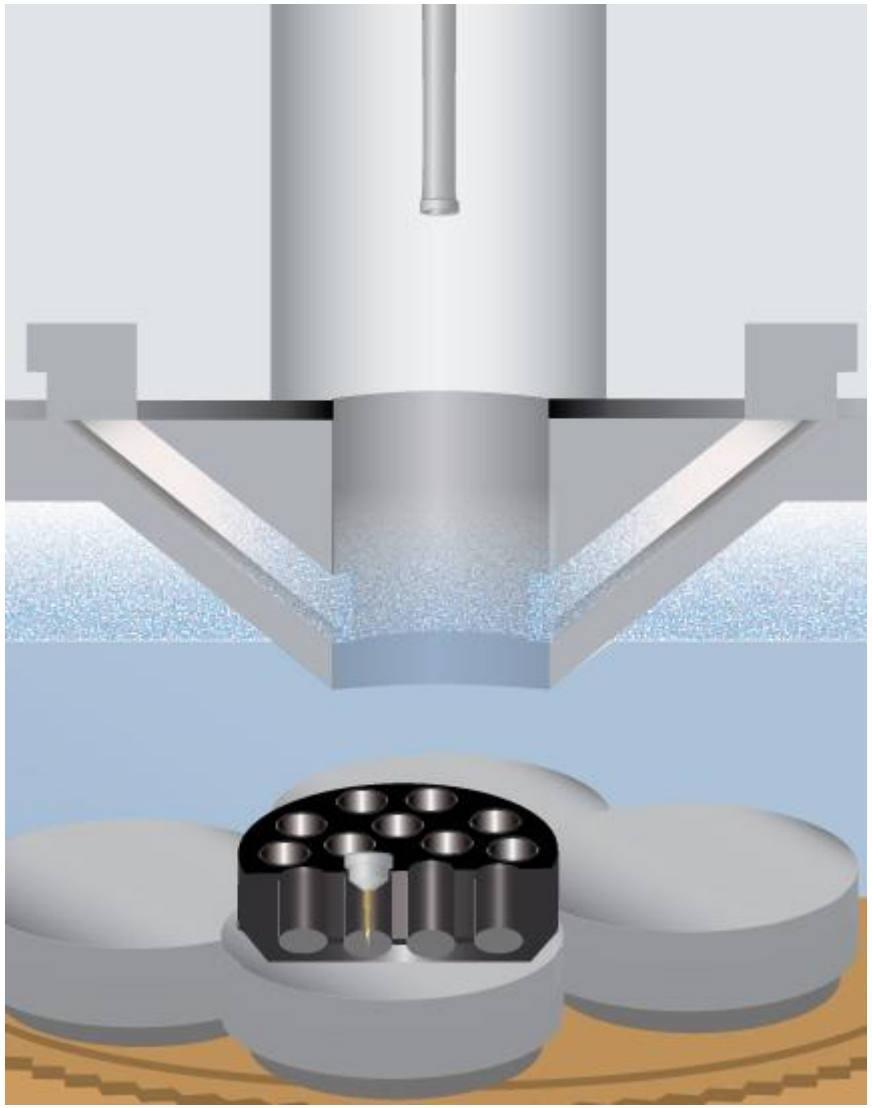
NANUQ™: Slow / Variable-Rate Cooling Plunge Sequence

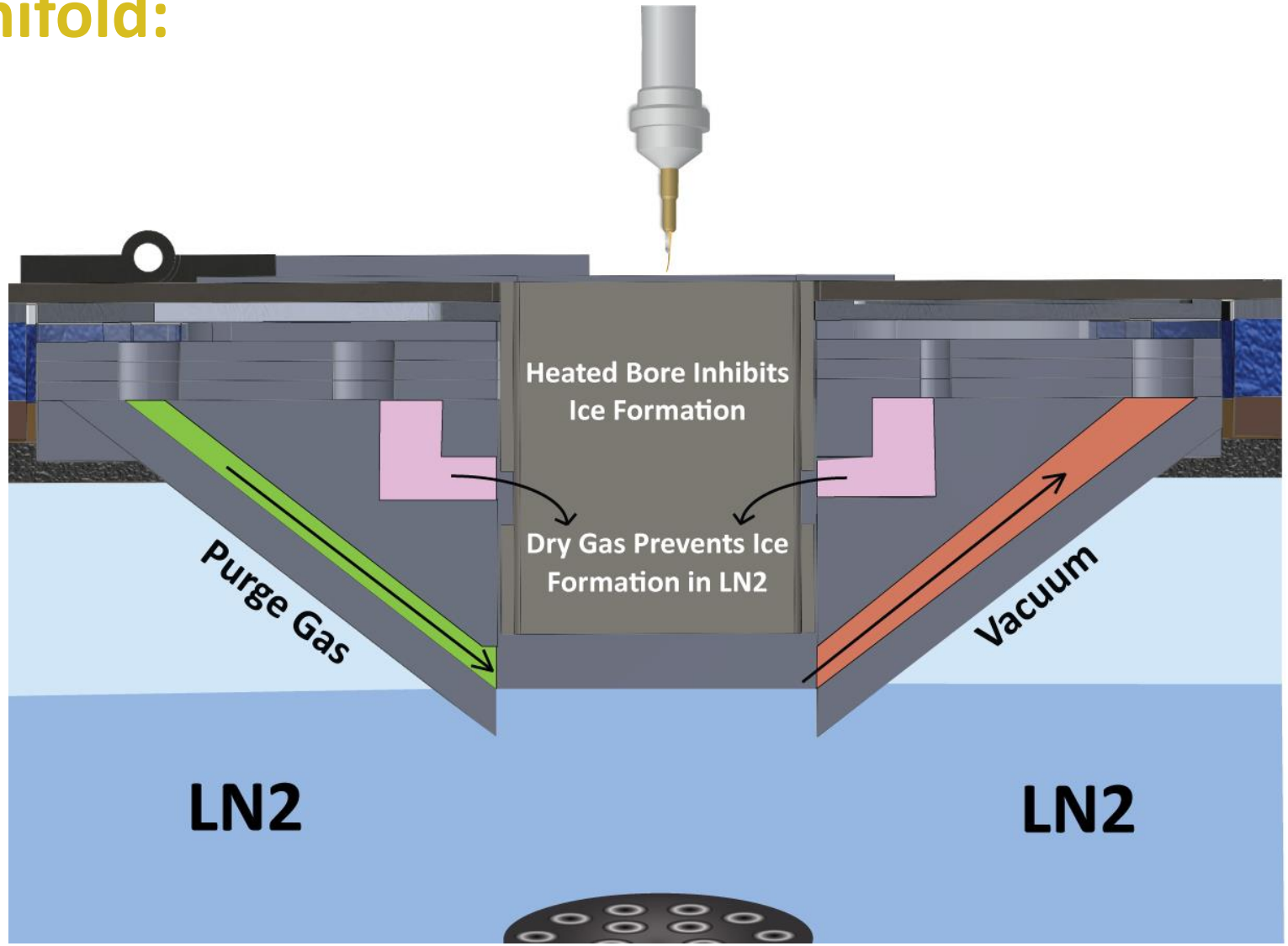


NANUQ™: Slow / Variable-Rate Cooling Plunge Sequence



NANUQ™: Key Design Features and Patented Technology

Plunge manifold:



NANUQ™: Key Design Features and Patented Technology

Plunge manifold:

- **Controls plunge environment** to remove or define cold gas layers above the LN_2 and provide a controlled temperature plunge path.
- **Eliminate cold gas** for fastest cooling.
- **Trap cold gas** for slow / variable rate cooling.
- Eliminates waves on the LN_2 surface for fastest cooling.
- Isolates the LN_2 and cold surfaces from ambient air, eliminating frosting.
- Computer-controlled dry gas flows, vacuum, and heaters **minimize LN_2 consumption**.

NANUQ™: Key Design Features and Patented Technology

High speed sample translation stage:

- **Programmable sample plunge speeds** from **3 m/s** down to **< 0.01 m/s**.
- **Remove cold gas** and **plunge fast** for **fastest cooling**.
- **Trap cold gas** and **plunge slow** for **slower** cooling.

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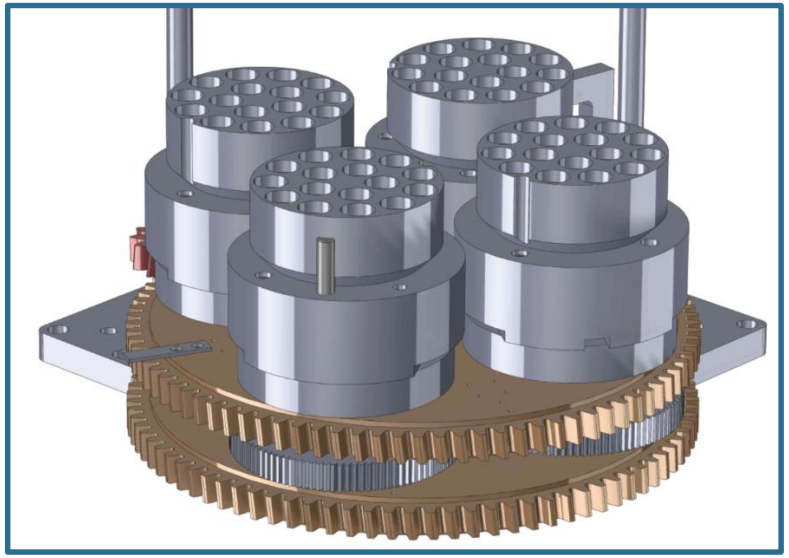
NANUQ™: Key Design Features and Patented Technology

Automated sample puck loading:

- Carousel holds **4 Uni-Pucks** and 64 samples in LN_2.
- Pucks are positioned in a programmable sequence to capture cold samples and organize them for optimal synchrotron data collection.
- Pucks are easily loaded into *NANUQ* and then transferred to a dry shipper or storage Dewar.
- All **manual handling** of cold samples is **eliminated**.



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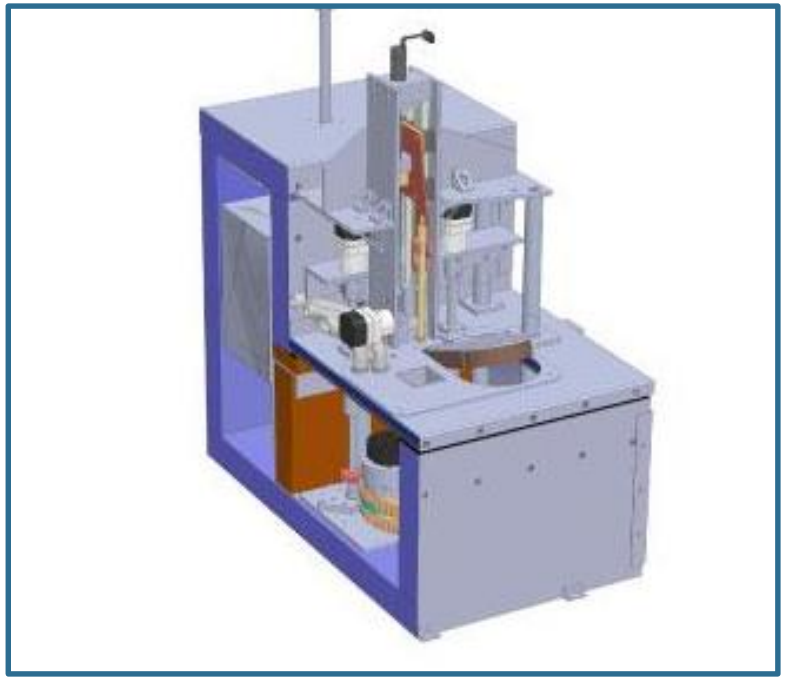
NANUQ™: Key Design Features and Patented Technology

High capacity LN_2 storage:

- Large LN_2 capacity and efficient insulation allow continuous operation for **>12 hours** in high-throughput applications **without frosting**.



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NANUQ™: Key Design Features and Patented Technology

Automated, computer-controlled operation:

- Automatically or manually trigger sample plunge
- Time from crystal harvest to fully cryocooled is < 4 seconds
- Sample cycle time < 15 secs; cool 4 samples/minute
- Minimizes time for crystal dehydration, maximizes throughput

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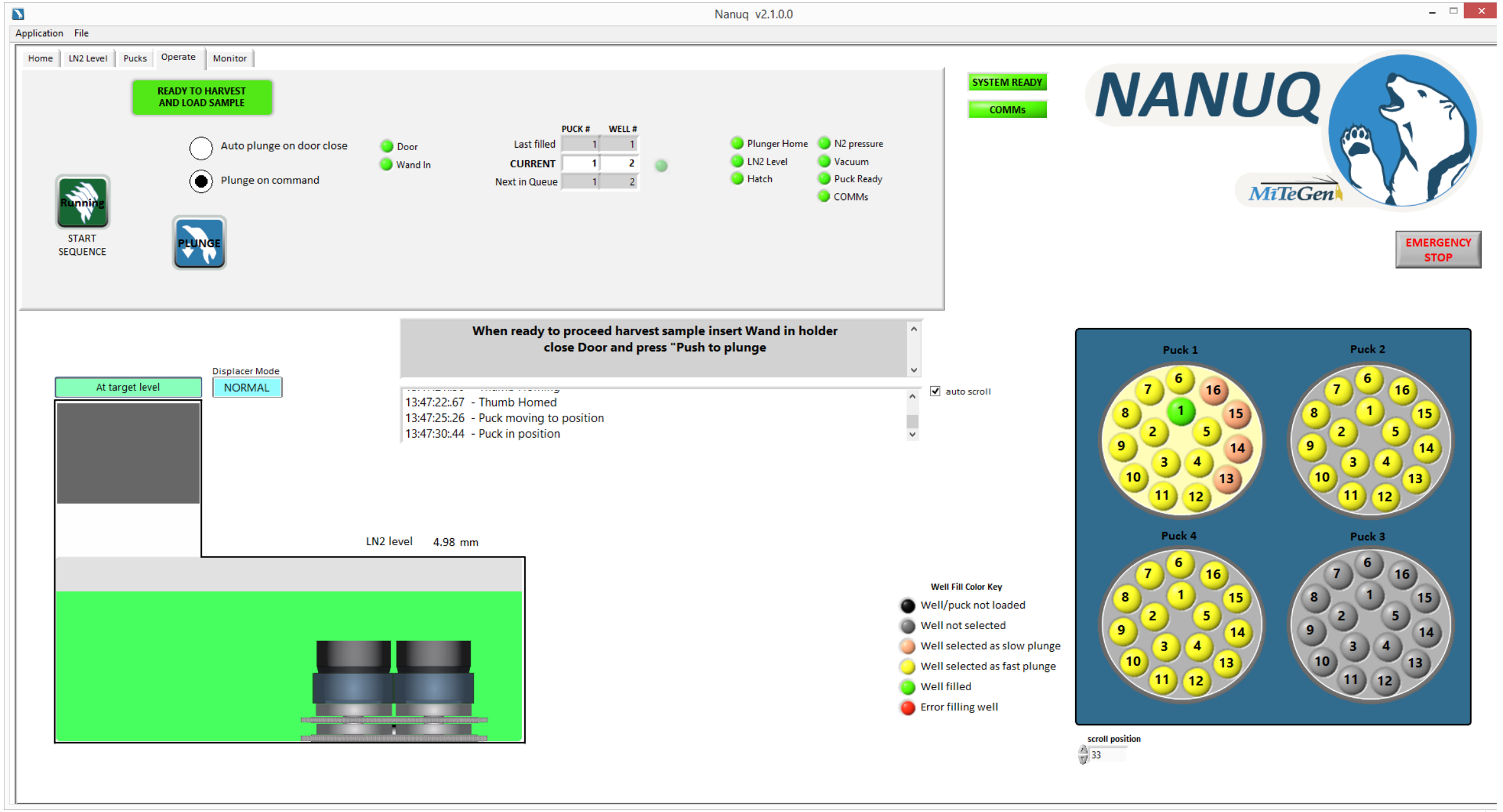
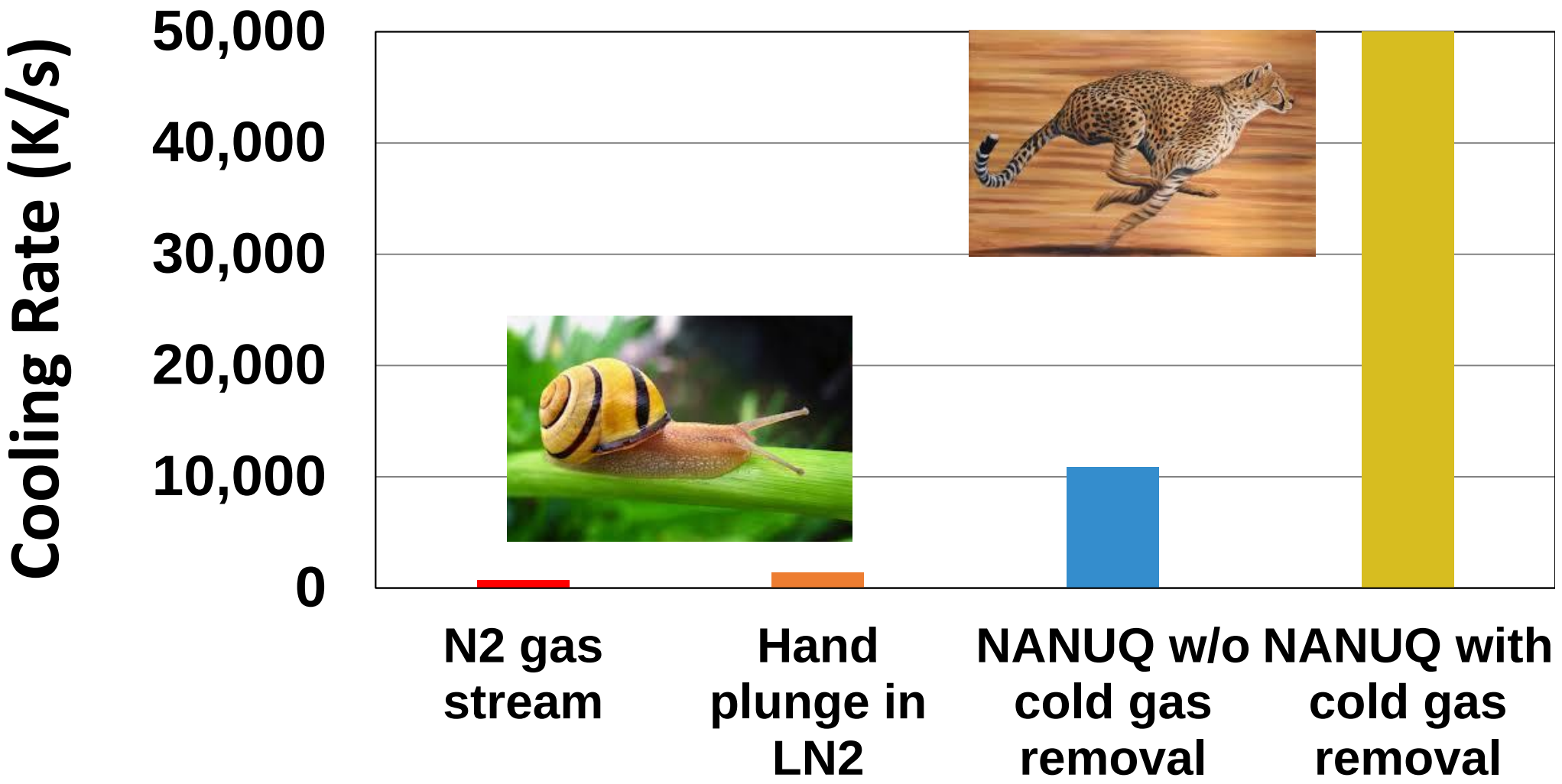

Nanuq v2.1.0.0
Application File
Home LN2 Level Pucks Operate Monitor
READY TO HARVEST AND LOAD SAMPLE
Auto plunge on door close
Plunge on command
Door
Wand In
PUCK # WELL #
Last filled 1 1
CURRENT 1 2
Next in Queue 1 2
Plunger Home
LN2 Level
Hatch
N2 pressure
Vacuum
Puck Ready
COMMs
SYSTEM READY
COMMs
NANUQ
MiTeGen
EMERGENCY STOP
Running
START SEQUENCE
PLUNGE
When ready to proceed harvest sample insert Wand in holder close Door and press "Push to plunge
13:47:22:.67 - Thumb Homed
13:47:25:.26 - Puck moving to position
13:47:30:.44 - Puck in position
auto scroll
At target level
Displacer Mode
NORMAL
LN2 level 4.98 mm
Puck 1
Puck 2
Puck 4
Puck 3
Well Fill Color Key
Well/puck not loaded
Well not selected
Well selected as slow plunge
Well selected as fast plunge
Well filled
Error filling well
scroll position
33

NANUQ™ : Key Advantages

Maximize cooling rates using LN_2:



NANUQ™ : Key Advantages

Maximize cooling rates using LN_2:

- Cooling rates to **>50,000 K/s**.
- **Cooling times < 2 ms** ⇒ **better kinetic capture** of room temperature structure & ligand binding configuration.
- Can also cool at **intermediate (100 K/s)** and **slow (down to 1 K/s) rates** to **simulate gas stream cooling** and for quasi-equilibrium cooling, which may give better diffraction outcomes (e.g., for large crystals).
- **Highly reproducible cooling** ⇒ science-based **optimization of cooling and diffraction**.

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NANUQ™: Key Advantages

Minimize cryoprotectant concentrations:

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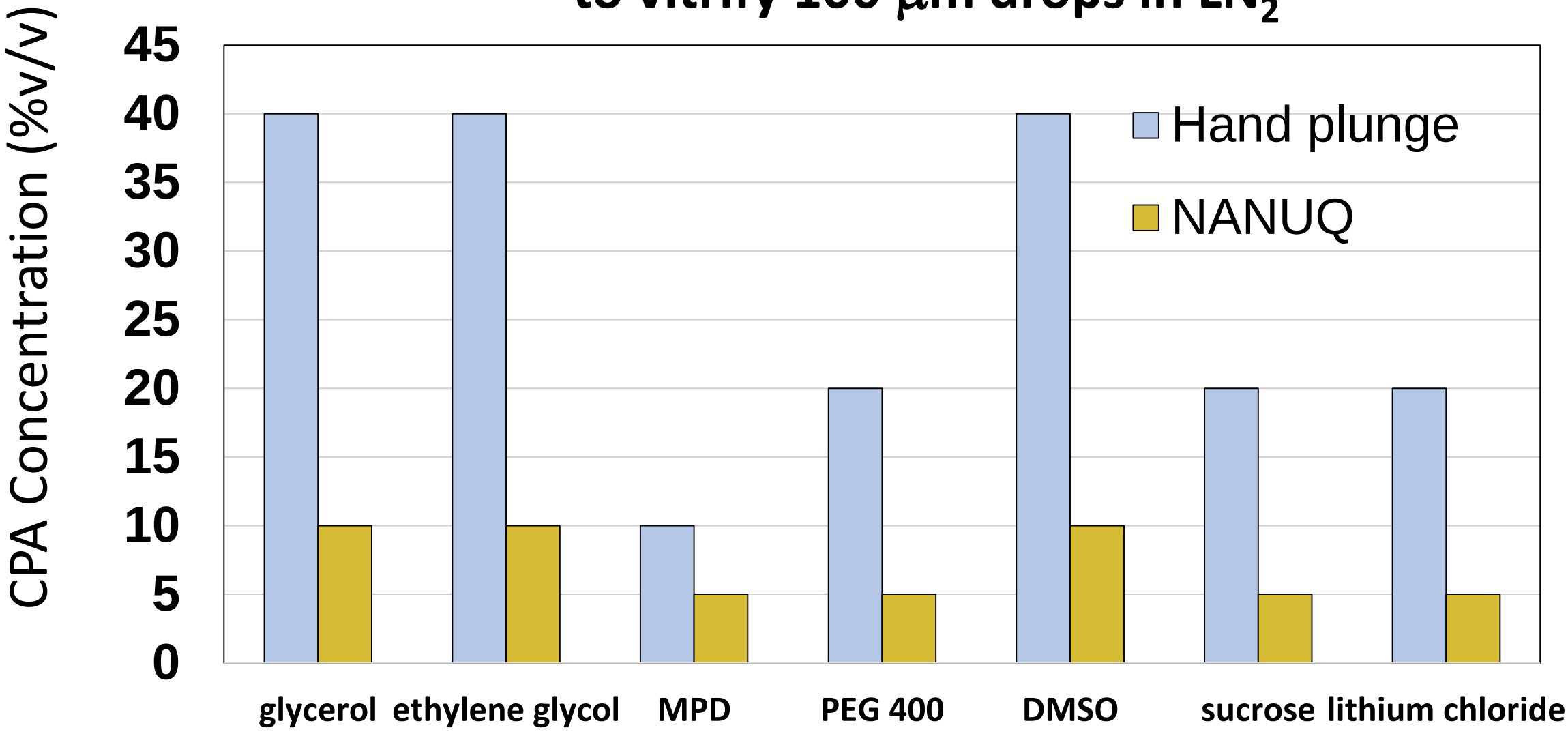
Minimum CPA concentration (%v/v) to vitrify 100 μm drops in LN_2



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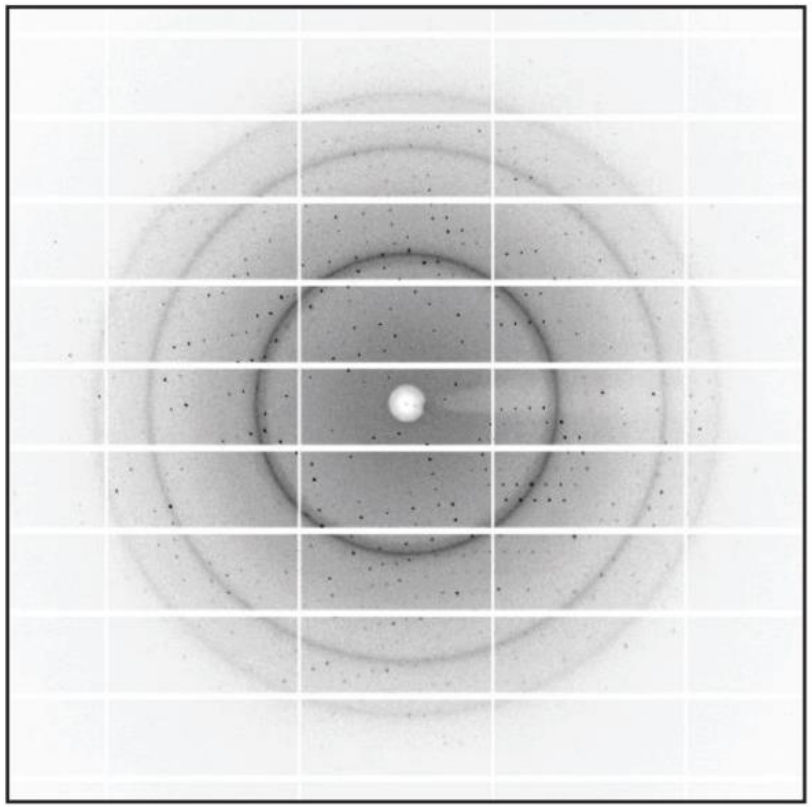
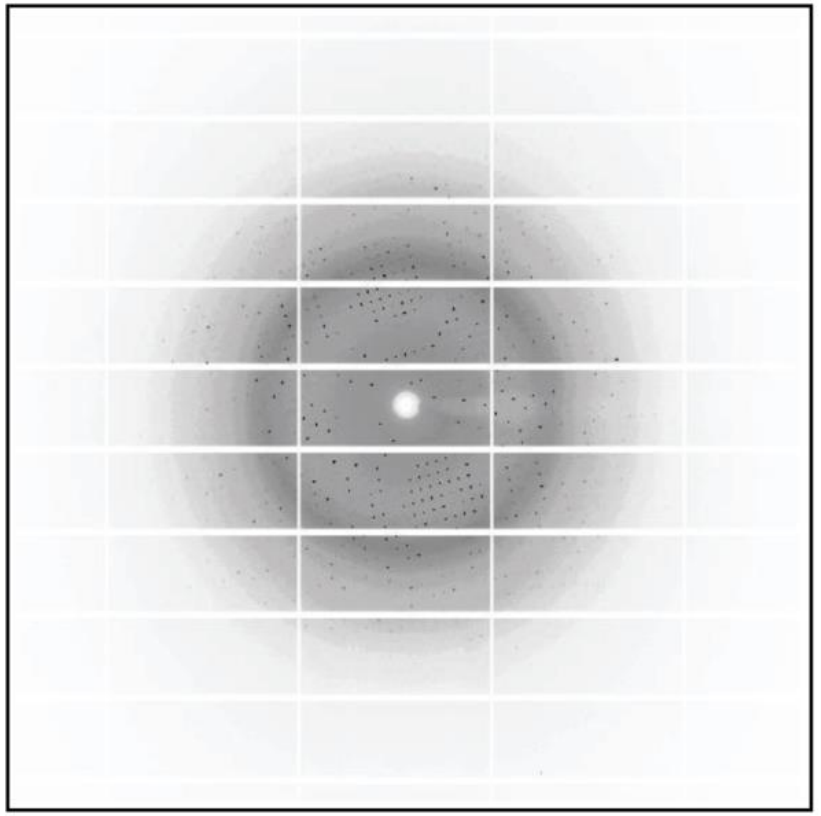


Hand plunged (LN2)
Sample: CA IX-mimic Crystal
Cryprotectant: 20% sucrose
Result: Significant ice rings



Cryocooled on NANUQ™ (LN2)
Sample: CA IX-mimic Crystal
Cryprotectant: none
Result: No ice rings

U. of Florida

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NANUQ™: Key Advantages

Use less (no) cryoprotectant, simplify cryoprotection:

- **No penetrating cryoprotectants required** to prevent internal ice. Only add for contraction matching.
- **No cryo soaks necessary** - just quick swipes through a dilute cryo solution / oil to protect / remove external solvent.
- No crystal damage or dissolving by cryoprotectants.
- **Ideal for ligand/fragment screening:** no ligand displacement by or confusion with cryoprotectants.

NANUQ™: Key Advantages

- Cool **immediately** after harvesting, with **complete confidence**.
- **No ice** from **cooling**, **LN_2** or **frosting** during puck loading.
- **No lost crystals** due to manual plunging / loading accidents.
- Easily **sequence and track** samples in pucks for optimized data collection.
- Get **everyone** in your lab using the **same reliable protocols**.
- Provides the **reproducibility** and **flexibility** to rationally optimize your cooling protocols.

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How Can You Get the Technology?

NANUQ™ shipped and installed at a customer location.

We can help with instrumentation proposal writing and justification statements.

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Access the Technology:
NANUQ™ Facility in Ithaca, NY Open to Users

NANUQ™ Access Facility
Cool your crystals with NANUQ™

Getting Your Crystals to MiTeGen:

- Bring your crystals via car, or ship them to us.
- Come in advance and set up plates. We can monitor them.
- Send your solutions + plates. We can set them up manually & monitor them.

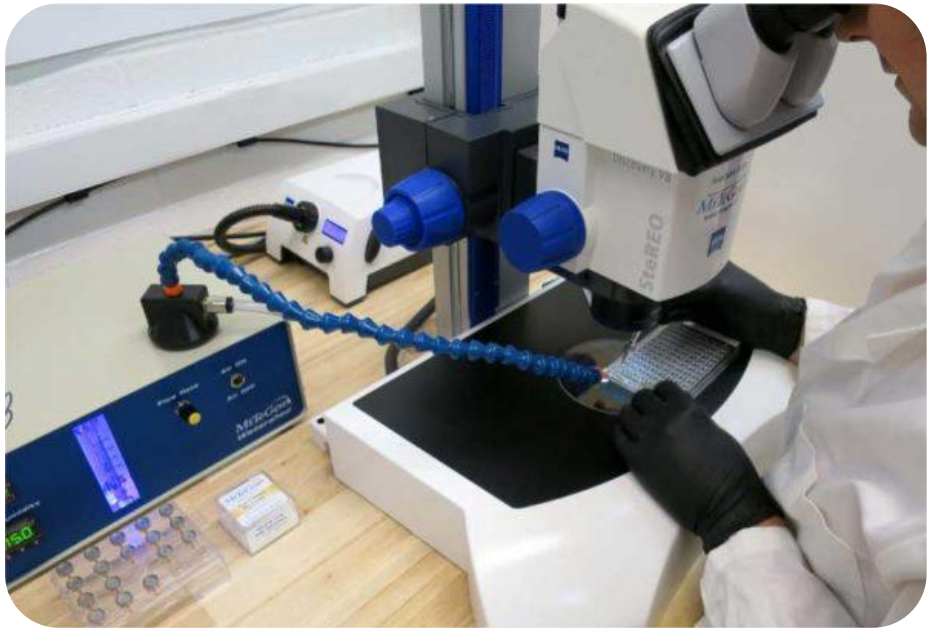
Cooling Your Crystals:

- Do it yourself with our support.
- Have our expert staff harvest, cool, & ship them anywhere.

NANUQ™ Access Facility
Cool your crystals with NANUQ™

Available equipment:

- Vibration-free Crystallization Incubator
- Zeiss V8 & V20 Microscopes with imaging
- Watershed™ Humidity Controlled Harvesting
- Worthington HC34 & HC35 Refrigerators
- Worthington CX100 Dry shippers
- Harvesting loops, tools, materials....



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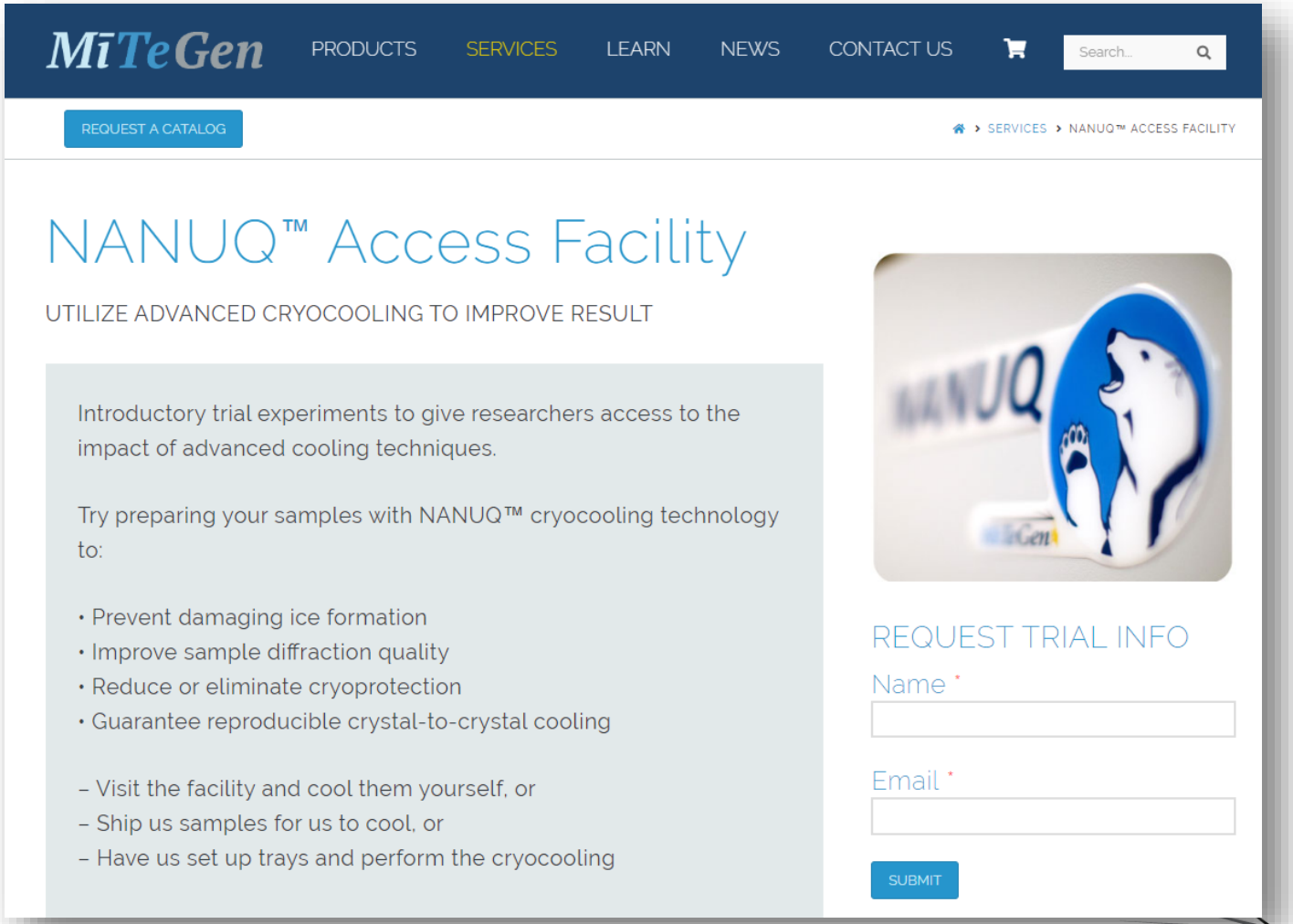
NANUQ™ Access Facility
How to Apply for Access Time

1. Visit our NAF Website:
   - www.mitegen.com/NAF

2. Fill out the Request Trial form

3. Our team will be in touch to get the process started!

We look forward to having you see the difference NANUQ™ can make!



Thank you!

- From Everyone at MiTeGen!

Contact:
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+1-607-266-8877