FREEZING AND SCALE-UP

Turning Ice Nucleation From a Guess Into a Measurement

Using LyoVision™ infrared imaging during the freezing step, we recorded nucleation in each vial in view: the first vial nucleated about 1.4 hours into freezing, the last 32 minutes later, and nucleation temperatures varied by nearly 5°C across vials.

LyoWave, Inc. | Lab-scale freezing study | November 2026

~5°C
Spread in nucleation temperature across vials in a single freezing step

32 min
Between the first and last tracked vial to nucleate

The challenge

Ice nucleation temperature is a key driver of primary drying rate (Searles et al., 2001). The deeper a formulation supercools before it nucleates, the smaller its ice crystals. Smaller crystals leave smaller pores in the dried cake, which resist vapor flow and slow drying.

Nucleation is random. Vials on the same shelf nucleate at different times and temperatures. Conditions also differ between sites: laboratory samples carry more particulate matter and nucleate at higher temperatures than product filled in a particulate-controlled manufacturing environment (Patel et al., 2009; Patel and Pikal, 2011).

A cycle developed on lab nucleation behavior can therefore dry differently in manufacturing. Without measuring nucleation vial by vial, teams have to guess how much variation they are carrying into scale-up.

The approach

Equipment. A LyoVision™ long-wave infrared camera (8 to 14 µm) mounted to a standard 3- or 4-inch sanitary port on a Millrock Stellar® laboratory freeze-dryer with a single 10 x 18 inch shelf. A gold-plated mirror suspended above the unstoppered vials gave the camera a direct, top-down view of the formulation. The gold coating reflects about 99% of infrared energy in the camera's band. This configuration is intended for laboratory development studies.

Product and container. 5% w/v sucrose in 10 mL vials with a 3 mL fill. Six vials were tracked.

Freezing protocol. Shelf cooled from 20°C to -40°C at 0.5°C/min, then held for 3 hours.

Method.

1. Track the temperature of each vial in view through the freezing step.
2. Identify nucleation as a sharp temperature rise to the equilibrium freezing temperature, followed by a return toward shelf temperature as the vial solidifies.
3. Record the time and temperature at which each vial nucleates.
4. Estimate the ice formed at nucleation from each vial's supercooling, using an energy balance.

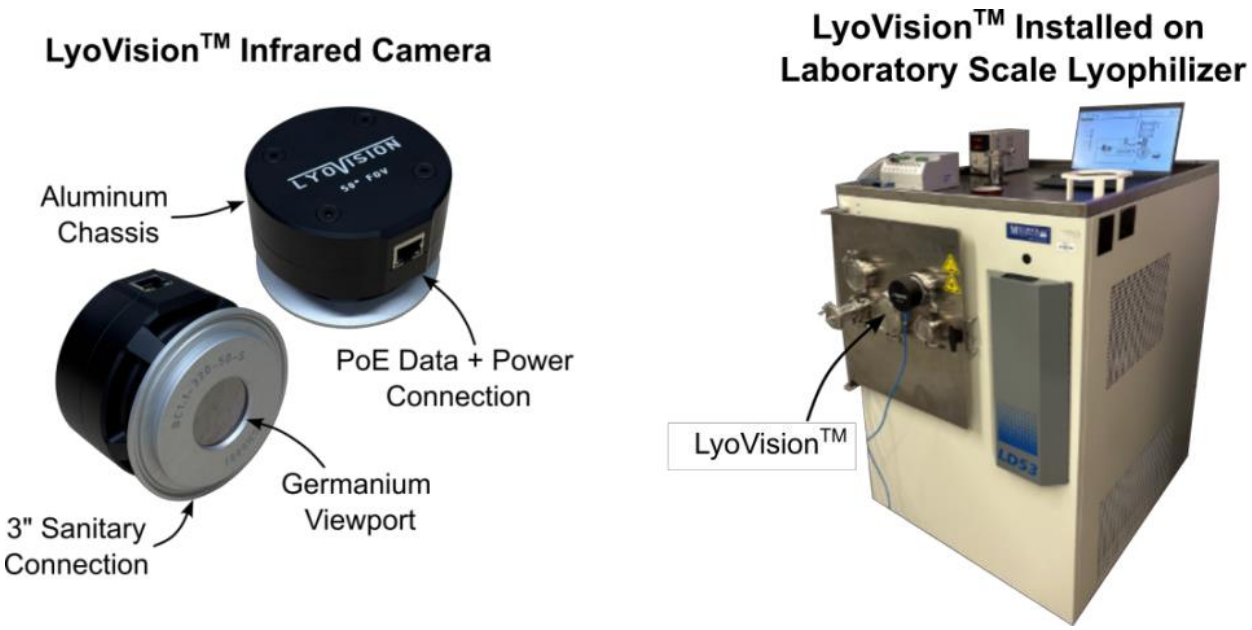


Figure 1. LyoVision™ camera (left) and a representative installation on a laboratory-scale freeze-dryer (right).

Results

Nucleation spread across 32 minutes and nearly 5°C within a single freezing step.

- **Timing.** The first vial nucleated about 1.4 hours into the freezing step, and the last nucleated 32 minutes later.
- **Temperature.** The temperature at which vials nucleated varied by nearly 5°C.
- **Effect on ice.** The deeper a vial supercools, the more of its water freezes at the moment of nucleation, before slower solidification freezes the rest. A 5°C spread in nucleation temperature corresponds to about a 2x variation in the fraction of water frozen at nucleation.

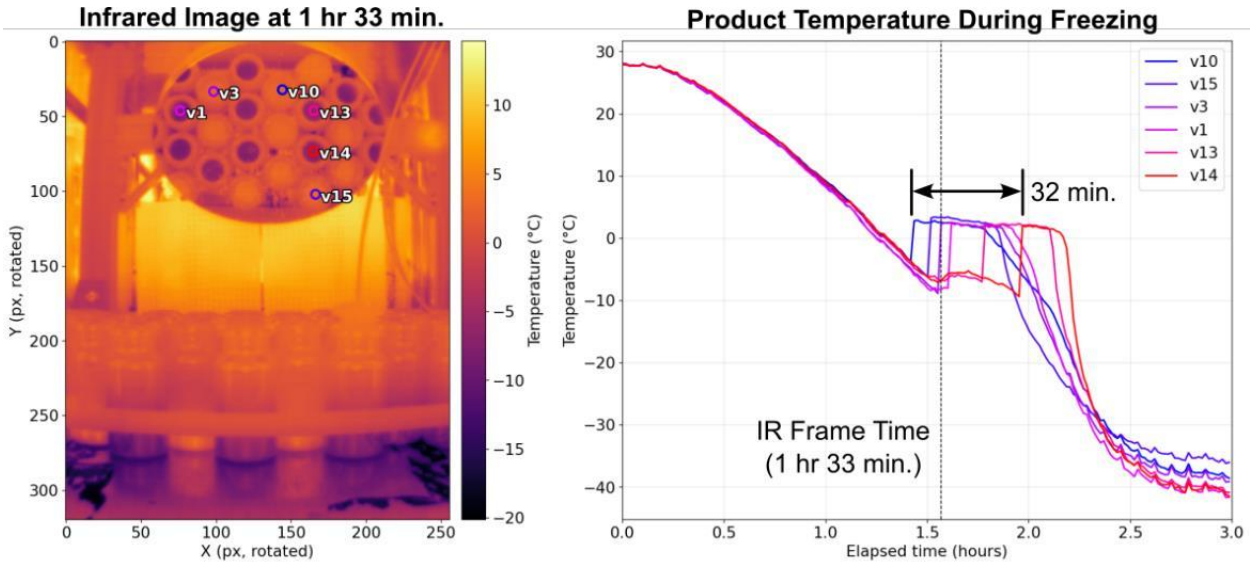


Figure 2. Left: infrared image at 1 hour 33 minutes, with the six tracked vials labeled in the mirror view. Right: product temperature during freezing for each tracked vial, with the 32-minute span between first and last nucleation marked.

What it means for process development and manufacturing teams

Infrared imaging turns nucleation from an assumption into a measurement.

- **Quantified variability.** Each vial in view yields its own nucleation time and temperature, so teams can see the spread instead of inferring it after drying.
- **Scale-up uncertainty.** Knowing how widely nucleation varies in the lab supports data-driven estimates of how drying could change at a different nucleation temperature in manufacturing.
- **Freezing protocol decisions.** The same measurement provides quantitative benchmarking when comparing freezing protocols, including methods intended to control nucleation.
- **No product contact.** The camera reads temperature without thermocouples in the vials.

Limits

- **Lab configuration.** The mirror view requires unstoppered vials and a mirror above the shelf. It is designed for laboratory development, not for production runs.
- **One example.** This note reports a single 5% sucrose solution in 10 mL vials with a 3 mL fill.
- **Vials in view only.** We tracked six vials visible to the camera, not the whole batch. They may not represent every position on the shelf.
- **Single run.** We report one freezing run, so these results do not show run-to-run variability.
- **Calculated, not measured.** The 2x variation in initial ice is calculated from the nucleation temperature spread, not measured directly.
- **Absolute temperatures.** LyoVision™ is rated to ±2°C accuracy with LyoWave calibration. Readings at the freezing plateau ran about 2 to 3°C above the solution's expected equilibrium freezing point of about -0.3°C. Every vial was read by the same camera in the same frames, so this offset does not change the spread between vials.

- **Scale.** The example ran on a laboratory freeze-dryer with a single shelf. This note makes no claim about nucleation behavior in a specific production system.

NEXT STEP

Talk with us about your freezing step

If freezing variability is a question in your scale-up, tell us about your product, containers, and equipment, and we will follow up. LyoVision™ mounts to a standard 3- or 4-inch sanitary port on existing freeze-dryers.

Contact us at lyowave.com/contact

Source and references

Source. LyoWave, Inc. laboratory data, October 19, 2026.

References. Searles, J. A., Carpenter, J. F., and Randolph, T. W. "The ice nucleation temperature determines the primary drying rate of lyophilization for samples frozen on a temperature-controlled shelf." *Journal of Pharmaceutical Sciences* 90(7), 860-871 (2001).

Patel, S. M., Bhugra, C., and Pikal, M. J. "Reduced pressure ice fog technique for controlled ice nucleation during freeze-drying." *AAPS PharmSciTech* 10(4), 1406-1411 (2009).

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