MicroFD With LyoSim & LyoPAT:
Protocol Development Freeze Dryer

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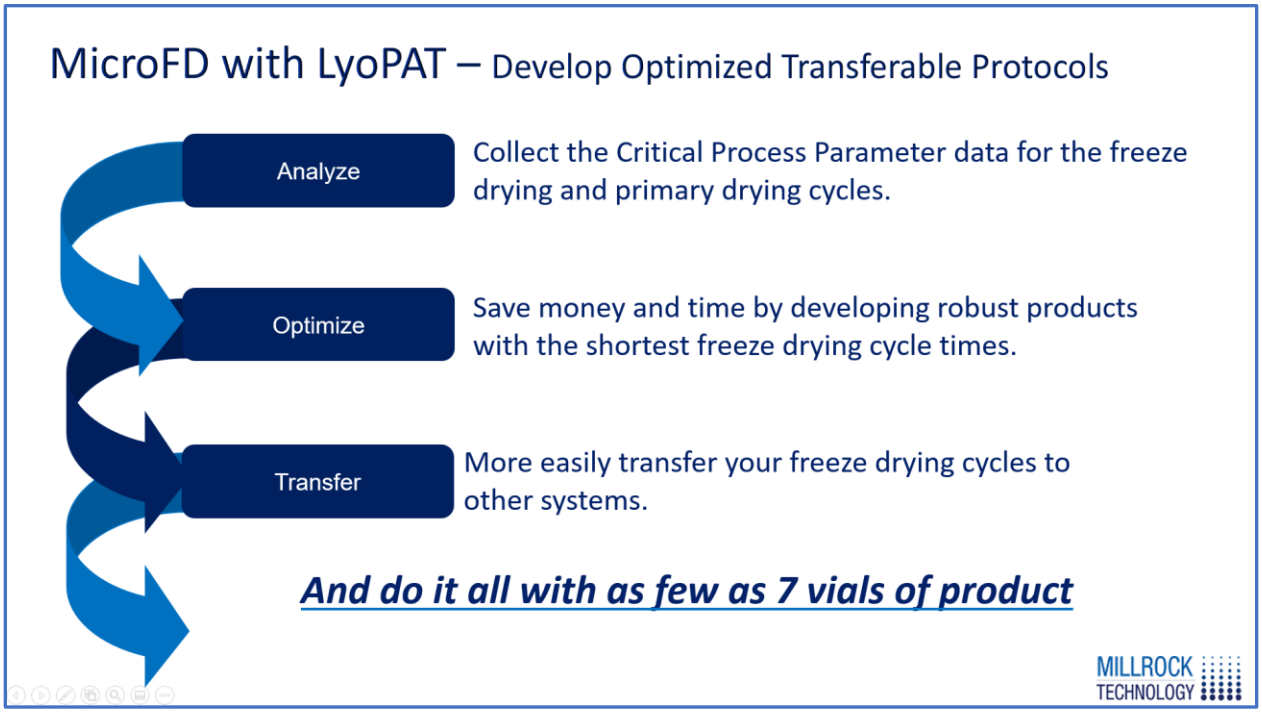
The MicroFD® is unique in that it uses only seven to thirty-seven vials for the development of all critical process parameter data for lyophilization cycle development, optimization, and tech-transfer to larger systems.

The MicroFD can be used to analyze and improve existing freeze-drying protocols or can develop new freeze-drying protocols in a single run.

When using the system for existing protocols, the MicroFD collects the critical process parameter data which can be analyzed and used as a baseline, delivering a full understanding of the existing lyophilization cycle.

The MicroFD® is equipped with tools that further enable the optimization of a lyophilization cycle. The MicroFD® enables both the Freezing and Primary Drying cycles to be optimized to gain the shortest freeze-drying time while developing the critical process parameters needed for tech-transfer to larger, pilot or production systems. Fig. 1

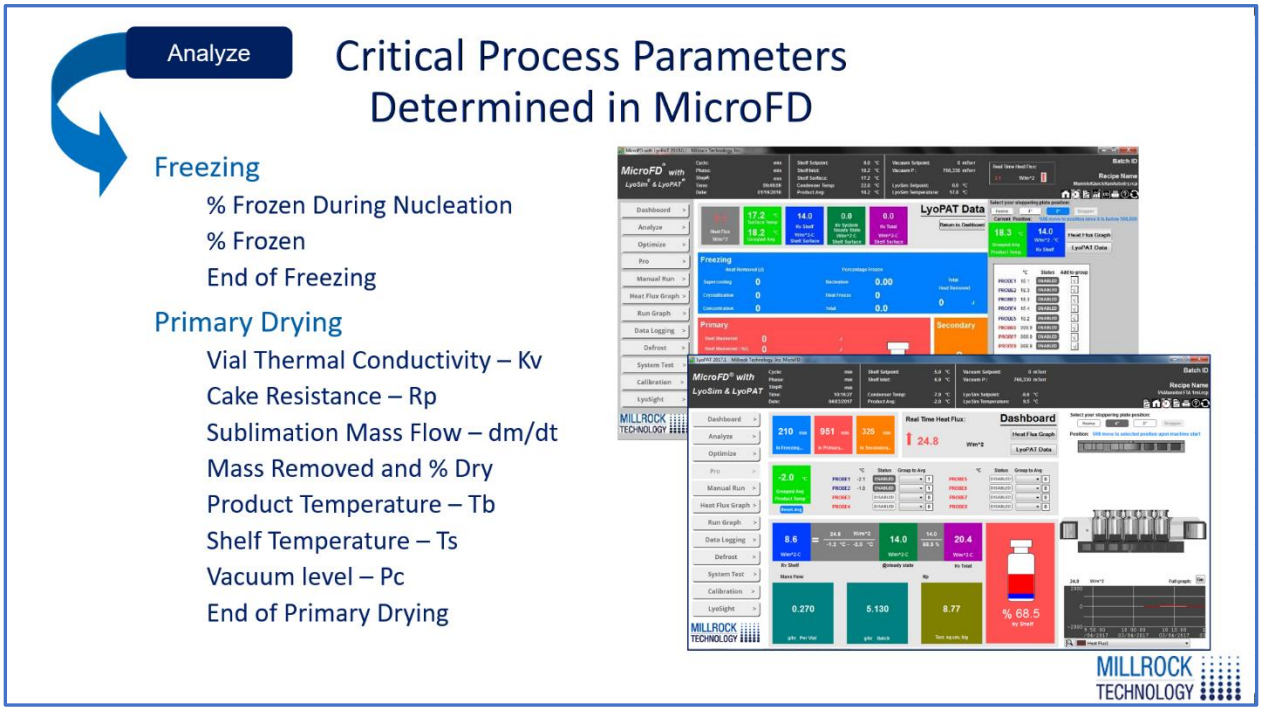
Fig. 1



Unlike other protocol development systems, MicroFD® technology measures the amount of product that is frozen during nucleation, and then indicates the “End of Freezing,” where the product has become stable enough to automatically advance into Primary Drying.

During Primary Drying, the MicroFD® system collects critical process parameters, including Heat Flow, Vial Thermal Conductivity, otherwise known as Kv; Cake Resistance, otherwise known as Rp; Sublimation Mass Flow, % Dry, Product Temperature, Shelf Temperature, Vacuum level, and determines the End of Primary Drying. As this data is collected, it is also reported on-screen, dynamically updated during the process. **Fig. 2**

Fig. 2



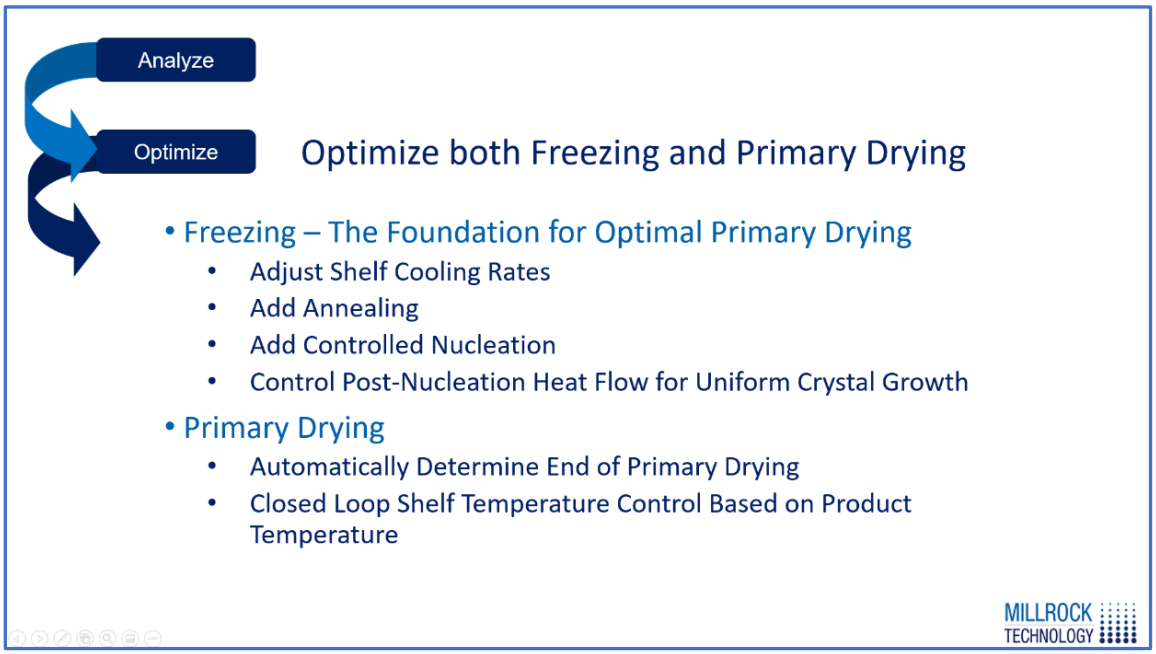
The complete analytics of an existing process provided by the MicroFD® provides the necessary baseline data for optimizing both the Freezing and Primary Drying portions of the lyophilization cycle protocol. This is an advancement in system-based protocol development, entirely unique to the MicroFD® as most systems only focus on the Primary Drying side.

Unlike less robust systems, Millrock Technology’s MicroFD® allows the adjustment of shelf cooling rates during Freezing. It also enables the addition of Annealing and Controlled Nucleation. And unlike any other system, the MicroFD® provides tools for Post-Nucleation Heat Flow for control of ice crystal growth after nucleation. Post-Nucleation Heat Flow and controlling ice crystal growth after nucleation is critical because ninety percent of crystal growth takes place Post-Nucleation.

In Primary Drying, the MicroFD® provides closed loop temperature control (Auto Dry®) where the shelf temperature is controlled based on the product temperature, thereby maintaining the product temperature below its critical point, thus optimizing the Primary Drying cycle. It also

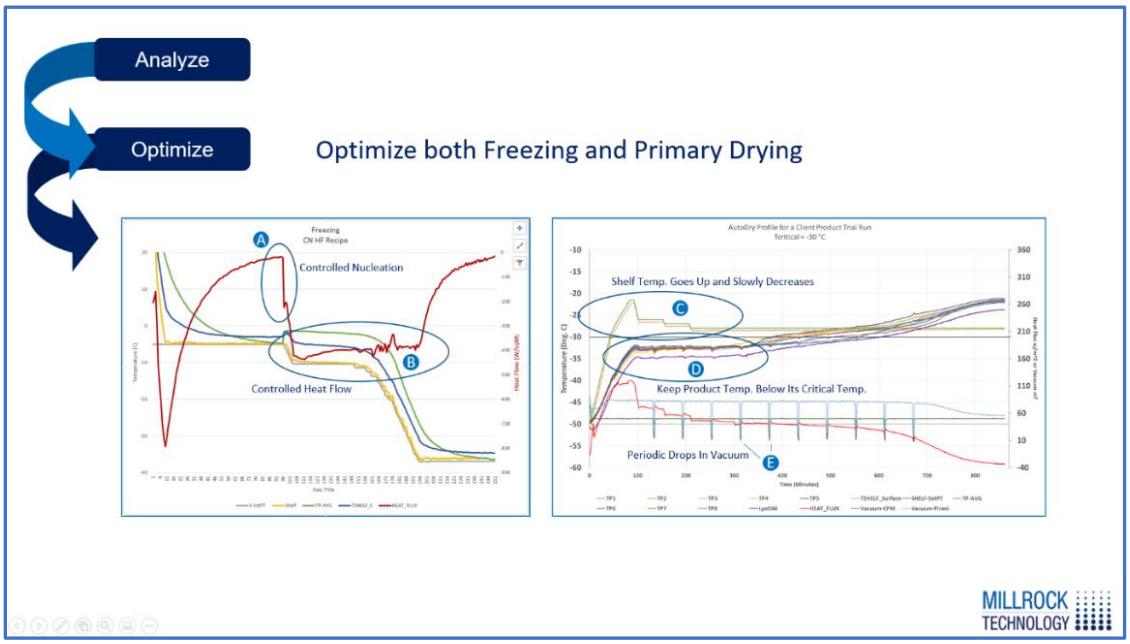
determines the end of Primary Drying automatically using the pressure differential between the capacitance manometer and Pirani. Fig. 3

Fig. 3



The graph on the left side in Fig. 4 is an example of the heat flow during a freezing protocol using controlled nucleation. The product is first supercooled to -5C and allow to stabilize, next FreezeBooster® Controlled Nucleation is performed, and then the heat flow is controlled Post-Nucleation by adjusting the shelf temperature. The red line is the heat flow, similar to what you would see in a DSC where the heat flow increases dramatically. In this case, it is in the negative direction because we are freezing, and then it comes up and stabilizes out. Once the heat flow has stabilized at the end of freezing, it indicates that no more sensible or latent heat is being removed and the frozen solution has reached its maximum freeze concentrate. Fig 4.A

Fig. 4



The result of combining both controlled nucleation and controlled heat flow is that the ice crystal formation both across the batch as well as inside the vial is uniform, which creates the ideal structure for rapid primary drying.

The graph on the right, **Fig 4.B** shows the Primary Drying cycle, where Auto Dry® is used. The shelf temperature is automatically adjusted to optimize the sublimation rate, while keeping the product below is critical temperature.

Fig 4.C Since there is no cake resistance at the beginning of the primary drying cycle, the shelf temperature can be increased aggressively. As the cake resistance builds the shelf temperature is reduced keeping the product temperature below its critical temperature. **Fig 4.D** The result is dramatically reduced processing time.

During this process, periodic pressure drops are performed. **Fig 4.E** The temporary drop in vacuum will result in a reduction in product temperature if the thermocouple sensor is still in ice. If they're in ice, we can use them for temperature control. Once they are out of ice, those sensors are no longer used for process control.

This is a simple and quick example of the type of information MicroFD® delivers during the run.

Fig. 5 is an example, a case study process improvement using Sucrose. The first cycle used a standard protocol where the product was frozen by reducing the shelf temperature to minus forty at half a degree C per minute and then a shelf setpoint of -20C and a vacuum level of 60mT was used in Primary Drying. Then different freezing methods, FreezeBooster Controlled Nucleation and AccuFlux® heat flow control, as well as AutoDry™ primary drying control were introduced to show the resulting improvements of each method. The result was a reduction in freeze drying time of over fifty percent.

Fig. 5

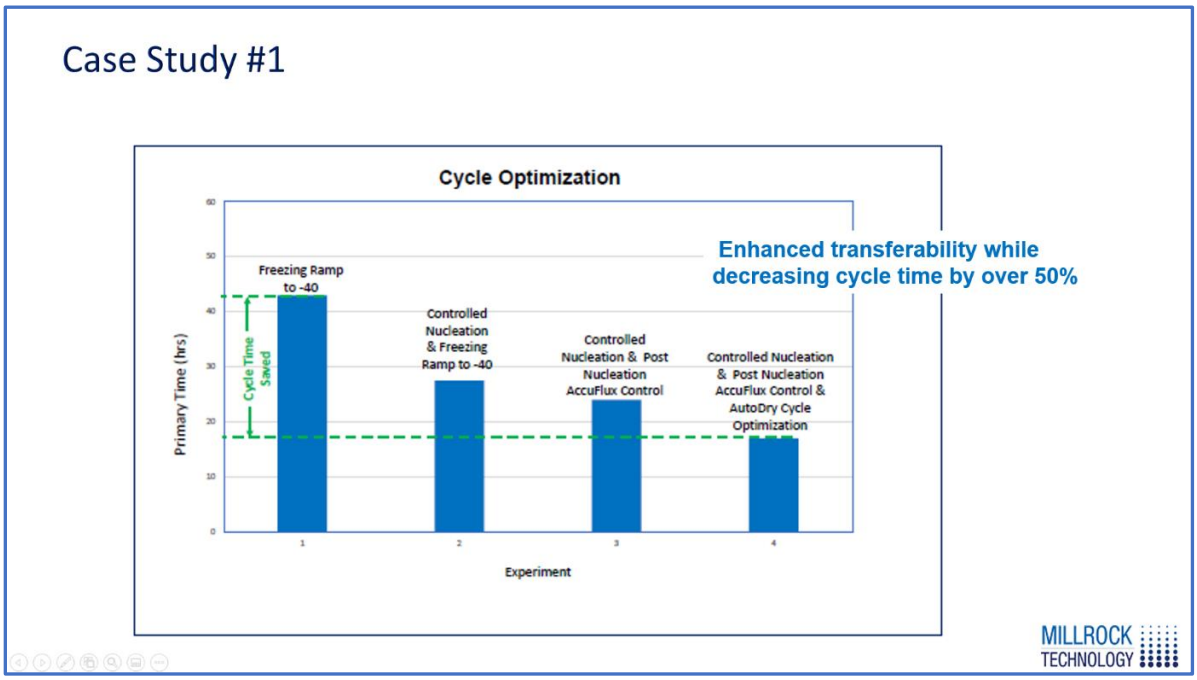
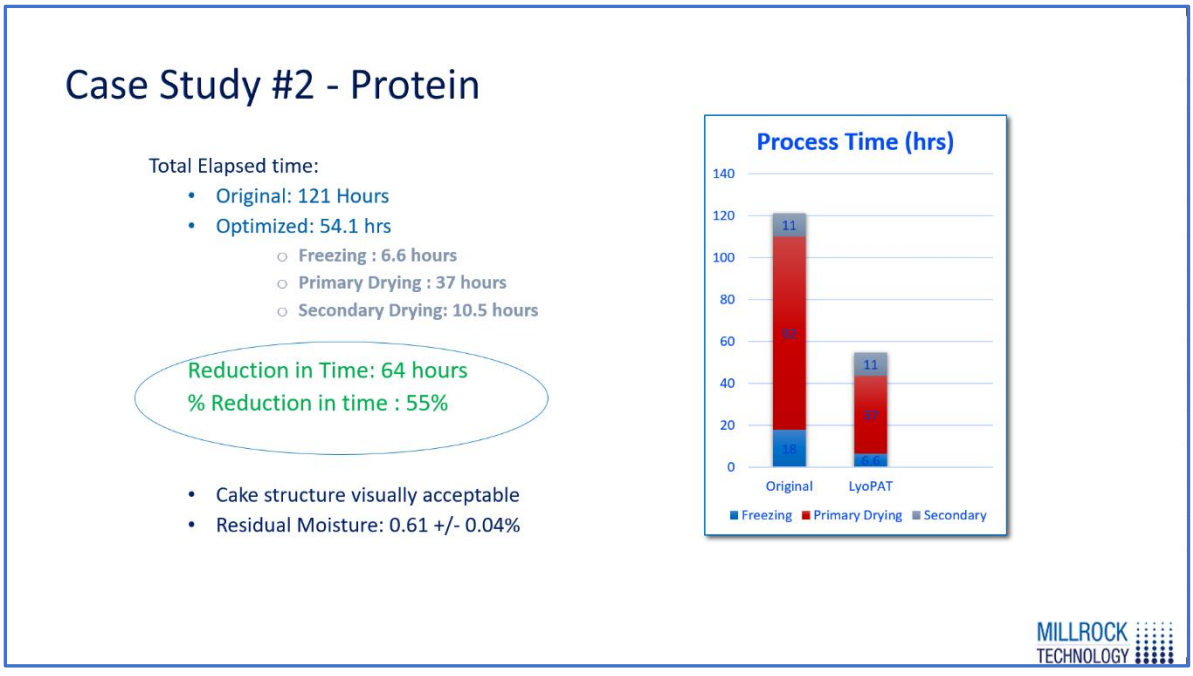


Fig. 6 shows a protein with a 21 mL fill in a 50 mL vial. The original freeze-drying protocol was developed using an older technology that determined the cycle using manometric temperature measurement and estimated calculations. Using this technique, a very conservative run of about 121 hours was produced. After using the tools available in the MicroFD® the processing time was reduced to 54 hours. This is a 55% reduction in primary drying time.

Fig. 6



For scale-up and transfer, the goal is to maintain an equivalent 'thermal history' between the lab unit or source unit and the production or target unit. Fig. 7

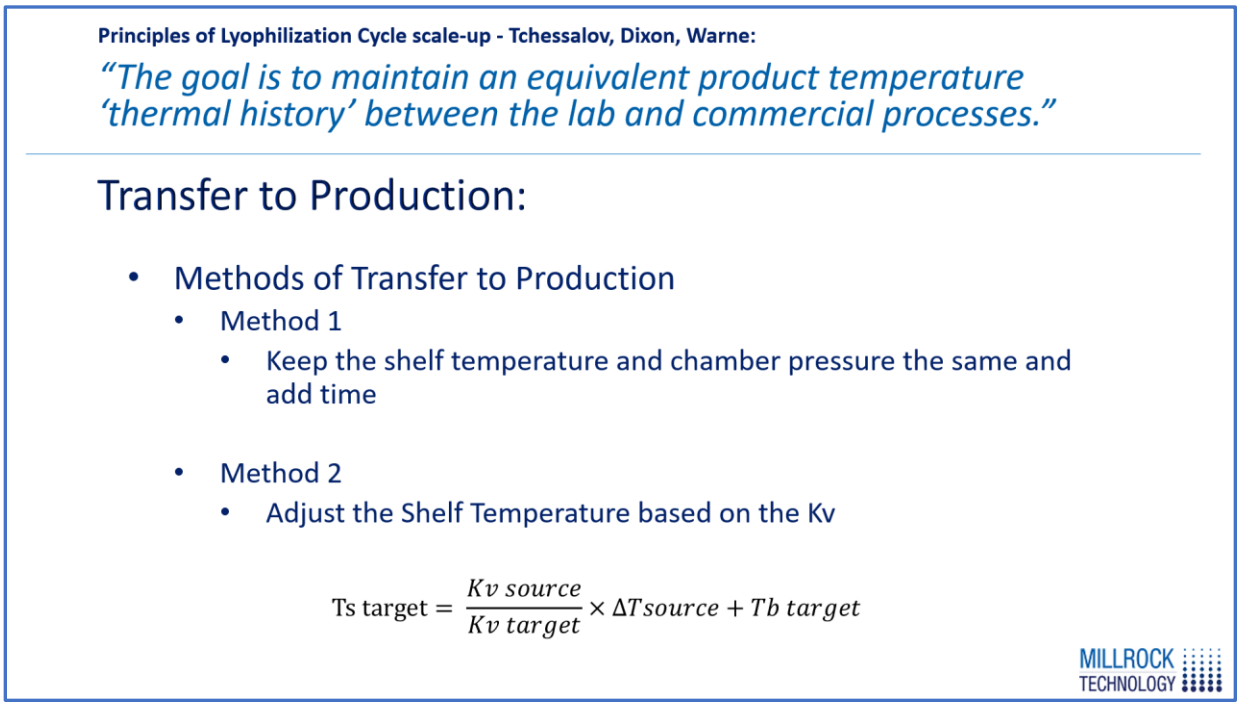
Once an optimized protocol has been developed, two different methods can be used to transfer this protocol between the lab and production unit.

Method #1: Keeping the shelf temperature and chamber pressure the same, while increasing the primary drying cycle time. Larger freeze dryers have lower vial thermal conductivity and therefore the product temperature during primary drying will be lower when using the same shelf temperature and chamber pressure, the result is lower sublimation rates and therefore the cycle time must be increased to enable the product to fully dry. This method usually results in thirty percent longer processing times in the larger system. One advantage of this method is that the result is a conservative cycle in the larger freeze dryer.

Method #2: Utilizing the MicroFD®, the critical process parameter Kv can be directly and continuously measured. Adjust the shelf temperature based on the known Kv values between the source (laboratory) and the target (production) freeze dryer. Due to lower vial thermal conductivity in larger freeze dryers, the shelf temperature in production units would need to be increased somewhere between 1 and 4 degrees C to produce the same thermal history as the lab system.

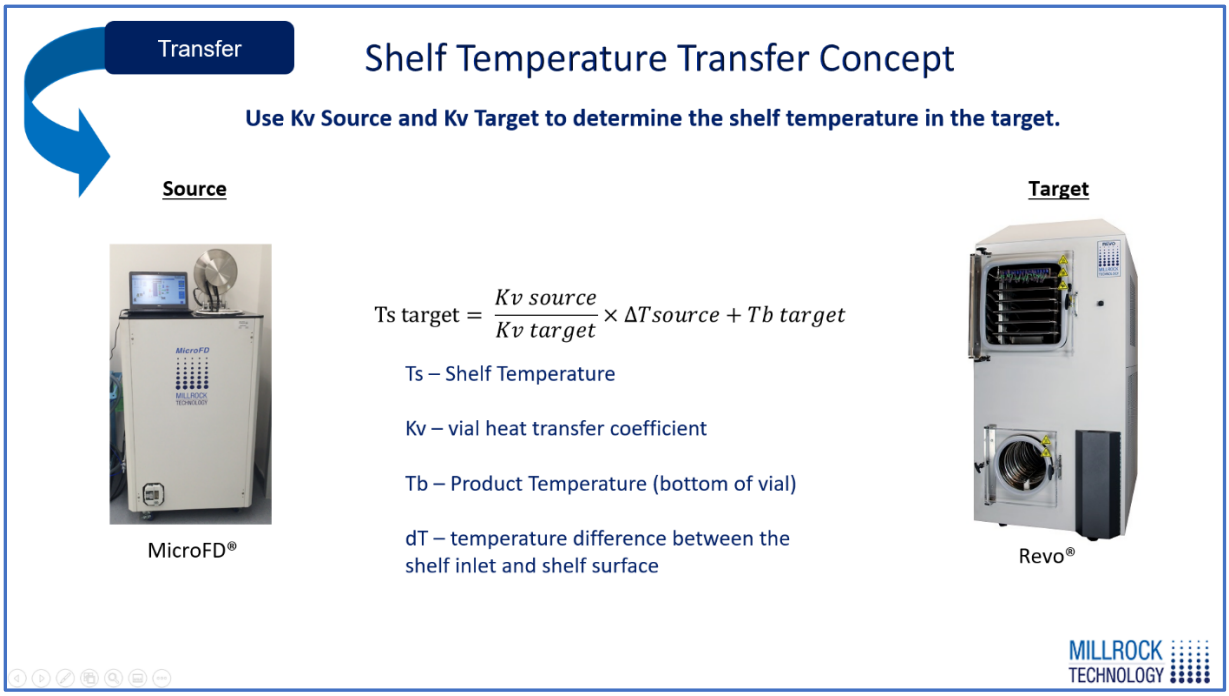
A simple equation is used to determine the target shelf temperature using Kv FIG. 7

Fig. 7



If Kv of the target system is known, the shelf temperature needed to produce the same product temperature can be determined. Fig. 8

Fig. 8



The vial heat transfer coefficient is an efficient way to transfer a cycle and produce the same Primary Drying results. There are two ways determine Kv in a freeze dryer. **Fig. 9**

The first method for Kv determination is the gravimetric method. To determine Kv gravimetrically a batch of product is processed and stopped at after several hours in the primary drying cycle. The amount of material lost is measured and Kv can be calculated for each vial across the batch. This method is very time consuming and subject to error due to the methodology used.

Another, more accurate, method of determining the Kv is called AccuFlux®, which uses the direct heat flow measurement from the heat flux sensor to calculate the Kv. This method allows for a real-time, in-situ measurement of the Kv during the drying process, eliminating the need for multiple time-consuming gravimetric runs.

Equation for measuring Kv when using a heat flux sensor:

Kv = Heat Flux/(Ts-Tb)

At the end of the primary drying process, the MicroFD determines the portion of the total heat flow into the vials that is derived from the shelves, as well as the total heat flow of the system, and can help characterize the sources of heat in a system under different process conditions. These are reported as the Kv Shelf and Kv Total, where the Kv Shelf describes the heat flow into the vial directly from the shelf, and the Kv Total accounts for all the sources of heat seen by the vials.

Fig. 9

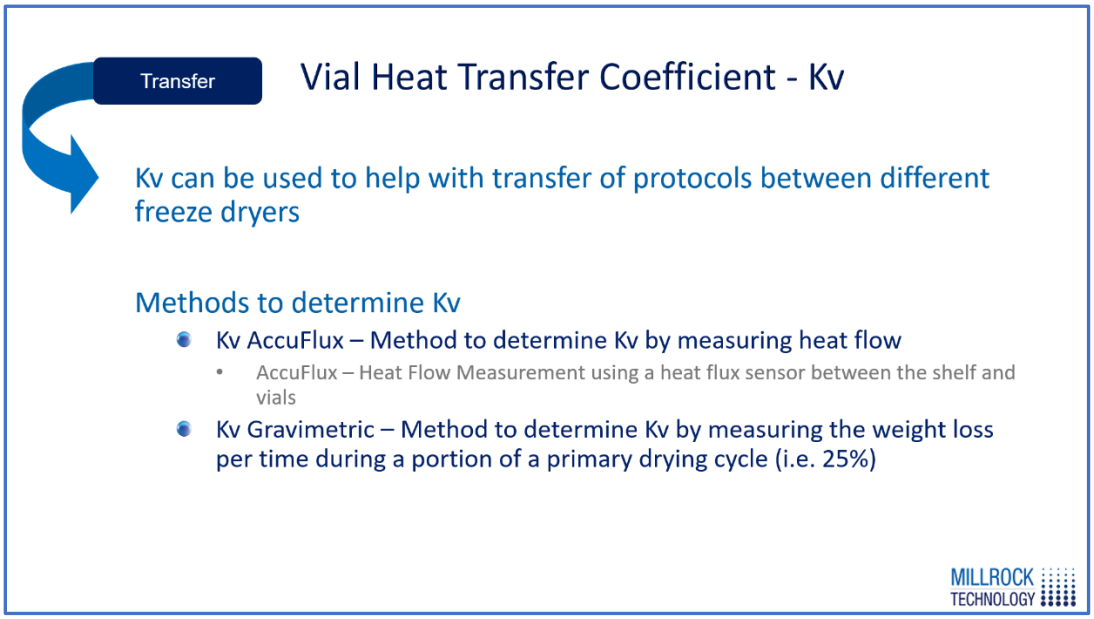
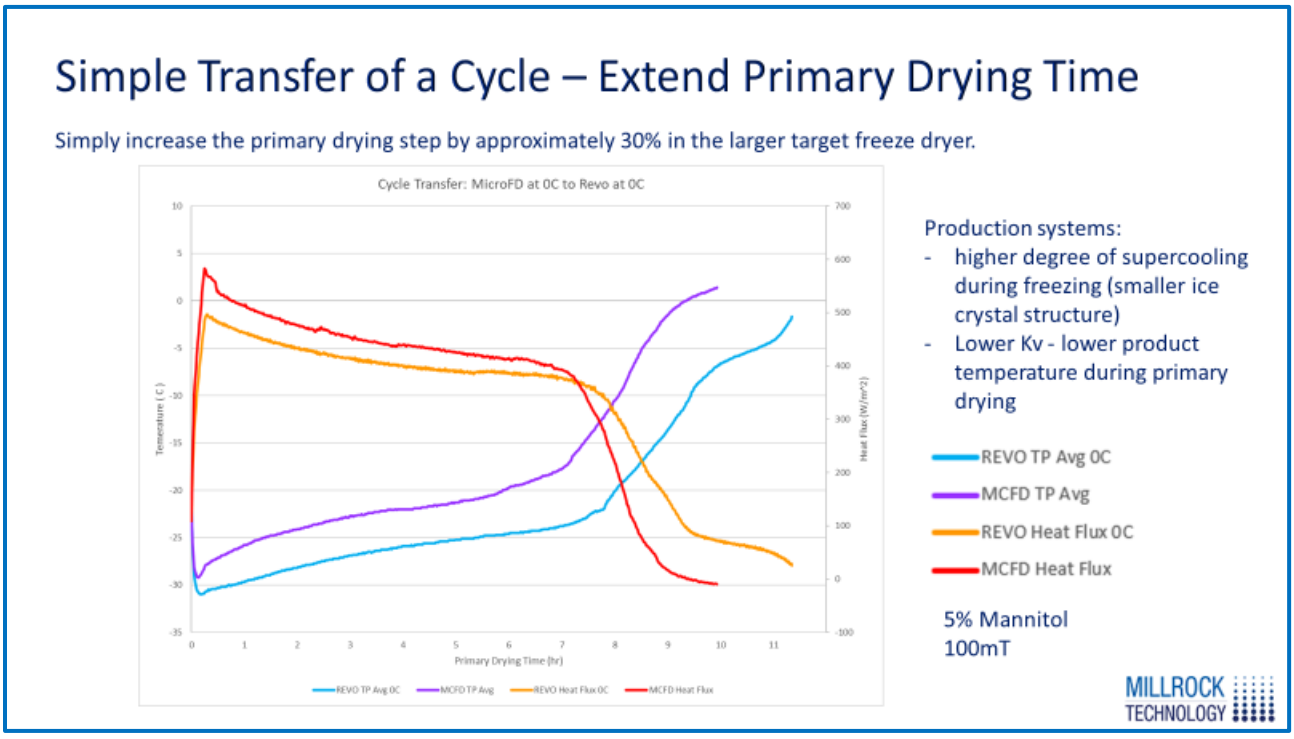


Fig. 10 is an example of the process time or process differences between a Micro Freeze Dryer and a REVO, which was an eight square foot freeze dryer.

Fig. 10



The graph shows both the heat flow and product temperature for Mannitol in a Micro Freeze Dryer (red and purple lines, respectively) and in a REVO™ 8 square foot laboratory freeze dryer (orange and blue lines). **Fig. 10** The shelf was set to 0 degrees C with a chamber pressure of 100 mTorr.

Comparing the same protocol in both systems, you'll notice that the heat flow and product temperature were lower in the larger system. If we just transferred a protocol from the Micro Freeze Dryer to the REVO all we would have to do is extend the Primary Drying time and we have a very conservative cycle transferred between the systems. However, our intent is to produce the same thermal history.

To produce the same thermal history in the larger system the shelf temperature will need to be adjusted. **Fig 11** Using the baseline data developed using the Micro Freeze Dryer, the target shelf temperature can be calculated. In this case the shelf temperature was increased 4 degrees C in the REVO.

Fig. 11

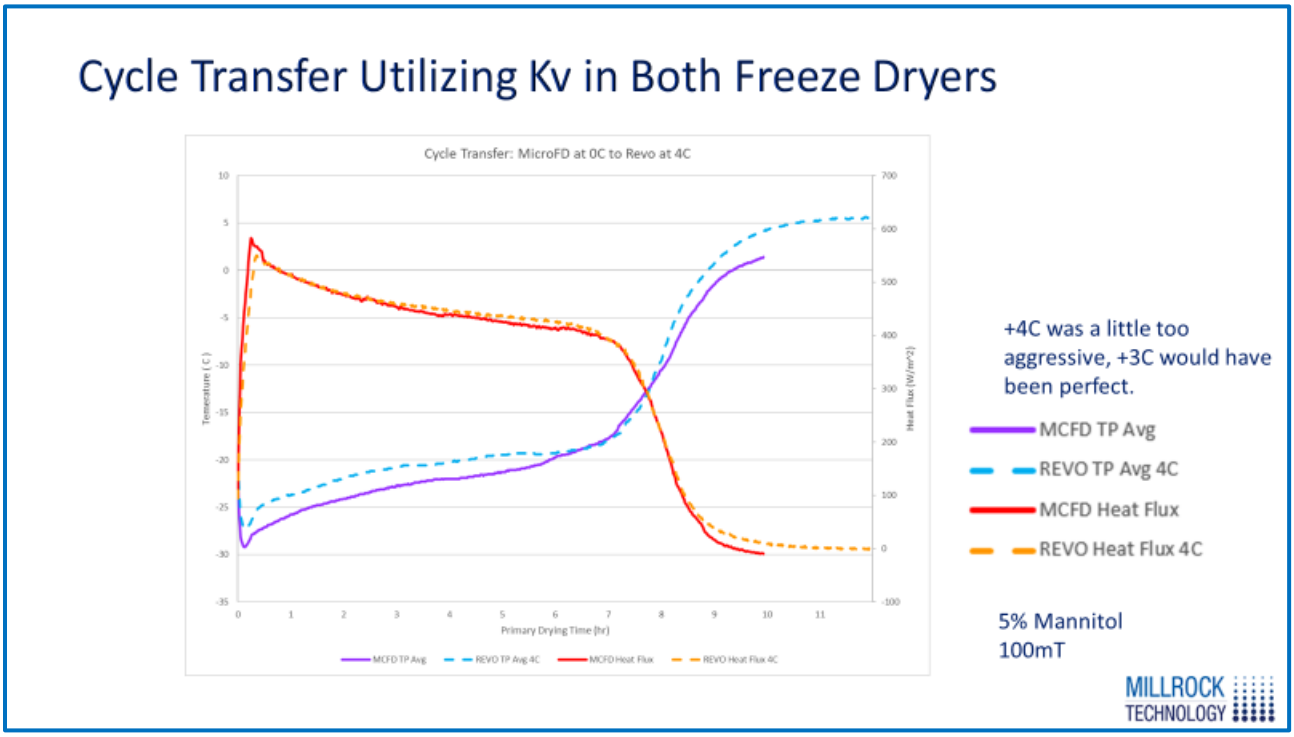
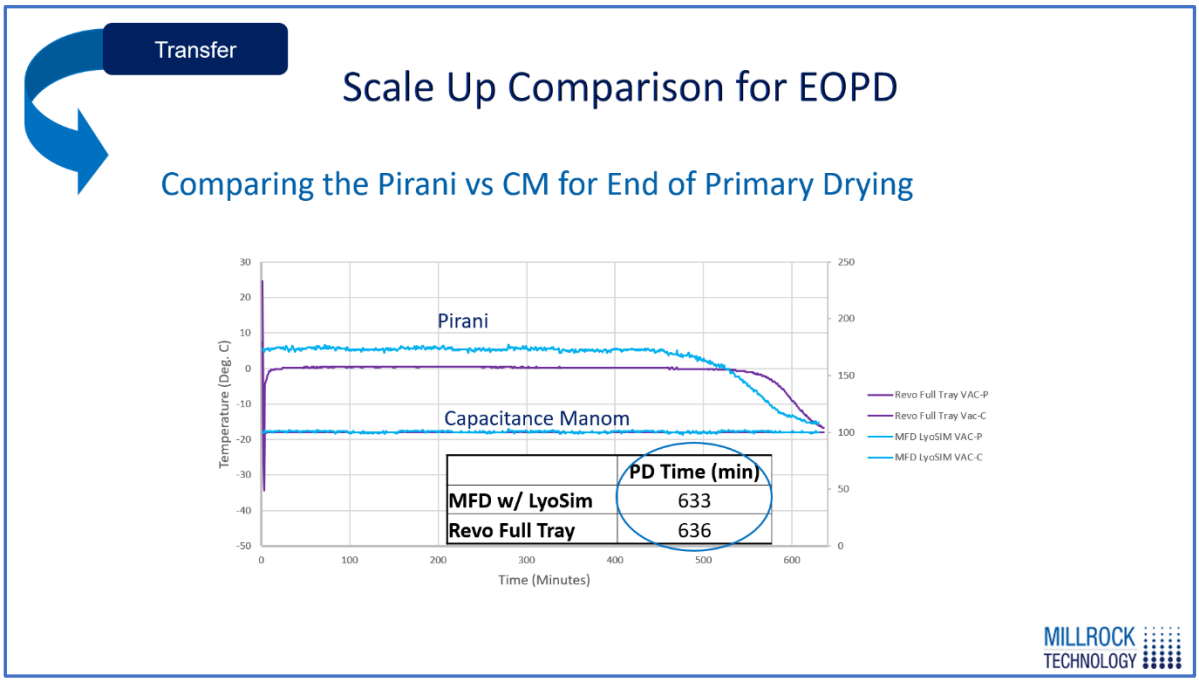


Fig. 11: By increasing the REVO shelf temperature by 4 C, the resulting heat flow and product temperatures was very similar between the MicroFD and the REVO (solid red and dashed orange lines, respectively). In this case, we may have been too aggressive by increasing the shelf 4C. If we had increased the shelf 3 C the thermal history would have been identical between the two systems.

The end of Primary Drying was determined using the capacitance manometer verses the Pirani. Fig 12 The Primary Drying times were within minutes of each other between the two systems. This is an example showing a remarkable advantage in protocol transfer utilizing the MicroFD®.

Fig. 12



The sublimation rates and heat transfer were similar between the two machines when we changed the shelf temperature in the target system. This resulted in a similar processing time. This exercise demonstrates the ability to transfer protocols while producing the same thermal history by simply adjusting the shelf temperature.

A further advantage of testing new products in the MicroFD is that a process can be developed, and protocol tech-transfer can be accomplished using as few as seven to thirty-seven vials. **Fig. 13**

Fig. 13

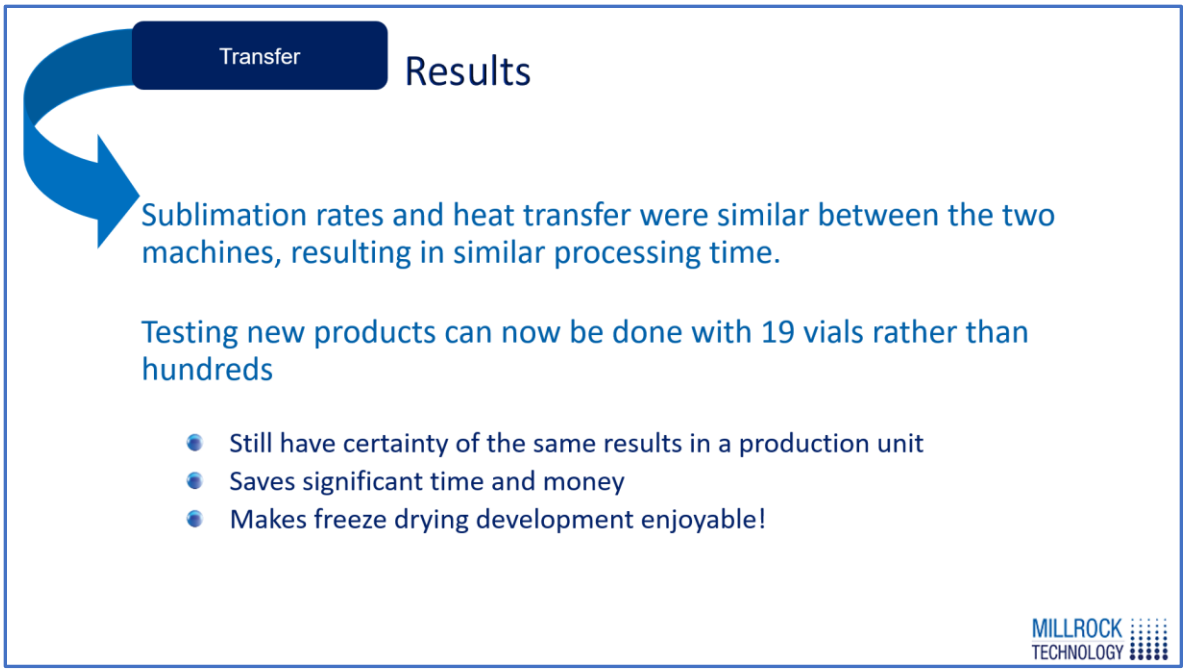
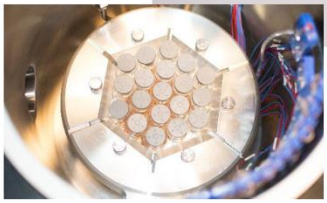


Fig. 14 & Fig. 15: Photo of the Micro Freeze Dryer. It's a very small laboratory instrument. The MicroFD simplifies lyo protocol development by delivering the necessary analytics to determine the critical process parameters with a very small batch of product.

Fig. 14

MicroFD – System Specifications

- System Performance
  - Shelf: -60C to +65C
  - LyoSim: -60C to +60C (independent of shelf)
  - Condenser: -75C
- Shelf and Chamber
  - 6” Diameter Shelf
  - Fluid Filled Shelf and Radiant Plate
  - Stoppering - ElectroMechanical
- Condenser
  - External with Pneumatic Isolation Valve
- Vacuum
  - Pirani and Capacitance Manometer
  - Proportional Vacuum Control



MILLROCK TECHNOLOGY

Fig. 15

LyoSIM® Ring

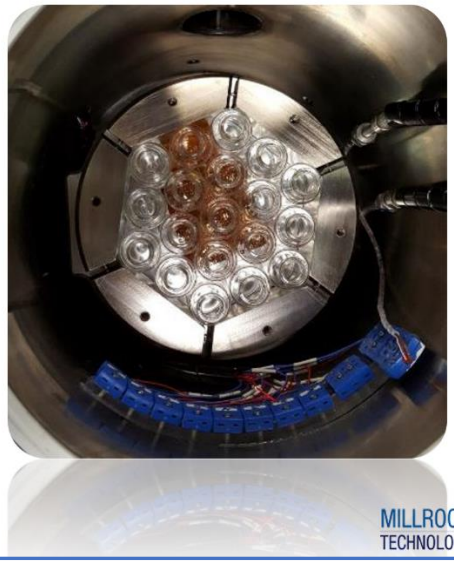
Patent Pending Technology to ensure that the vials are acting like center or edge vials

A Sublimating Vial is a Heat Sink

- The LyoSIM ring is the only technology to simulate a sublimating vial
  - Set the LyoSIM ring to track the temperature of the vial stack

Edge Vials

- The LyoSIM ring can simulate the edge vial effect
  - Set the temperature of the ring to the temperature of the vail corral or wall



MILLROCK TECHNOLOGY

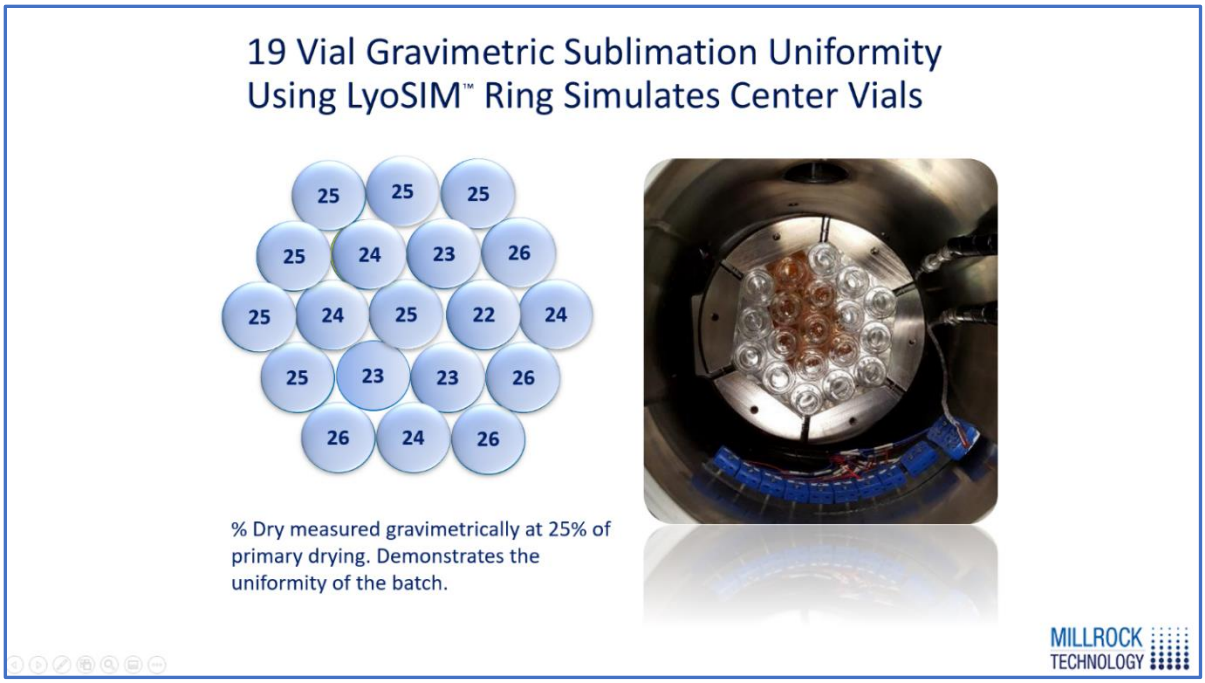
The Micro FD® is set up to produce all the same operating performance as larger systems, including an external condenser with a pneumatic isolation valve, Pirani and capacitance manometer and proportional vacuum control. It is also equipped with Accuflux®, an in-situ real time process monitoring control system that is a Process Analytical Technique (PAT) allowing

monitoring and control of every step in the freezing and primary drying phases of your freeze-drying run.

The LyoSIM® ring [Fig. 15] simulates surrounding vials not physically present in a small batch. The LyoSIM® ring was developed from extensive freezing, primary and secondary drying research. It was determined that cooling the chamber wall does not eliminate the edge vial effect and does not enable a small batch to be used for protocol development. It has been determined that radiation is not the only major effect that creates the edge vial effect. The reason that center vials dry slower is that the vials are surrounded by sublimating vials that are acting like heat sinks and therefore there is less energy available for the sublimation process. Conversely, the edge vial effect is the result of the edge vials not having surrounding vials that reduce the heat available.

The LyoSIM® ring simulates the sublimation effect, or heat sync effect, of vials that would normally be on the outside. The LyoSIM® ring is used for simulating center vials or edge vials. This a very powerful tool, because, by simulating center vials, we get a very uniform distribution across the batch. [Fig 16]

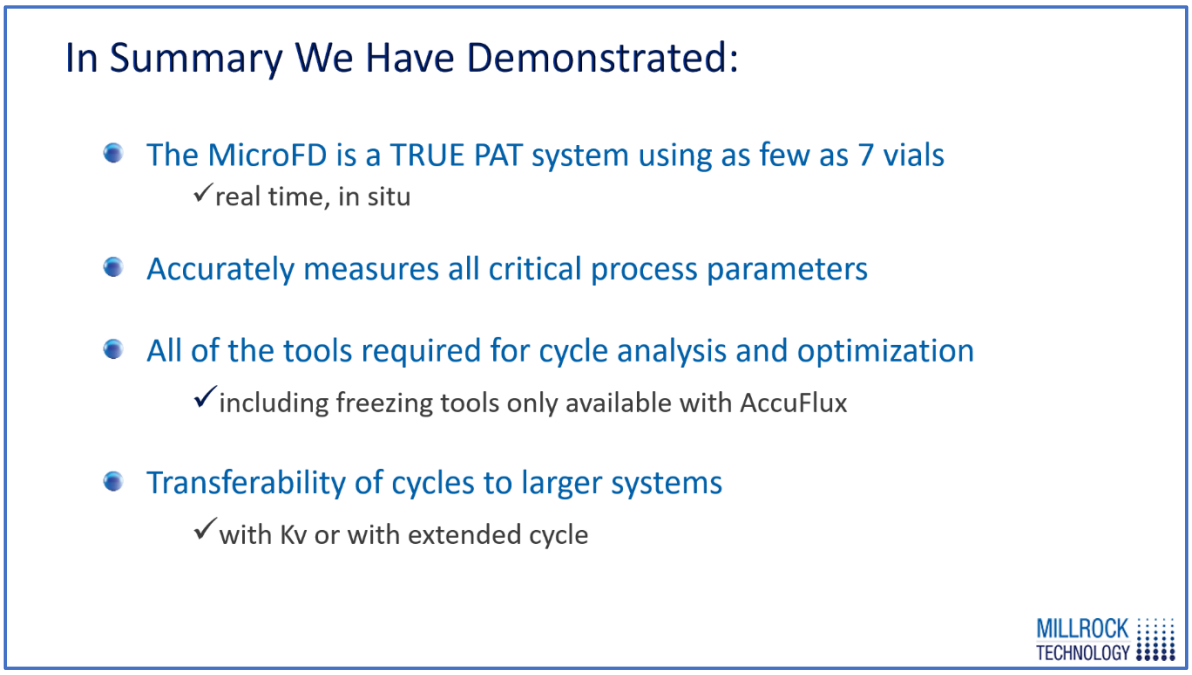
Fig. 16



In summary, we've demonstrated the MicroFD® is a **TRUE** PAT system using as few as seven vials (in most cases 19 vials are used). [Fig. 17] It is real time and in situ, unlike any other system available and it works in both Freezing and Primary Drying. It accurately measures all the critical

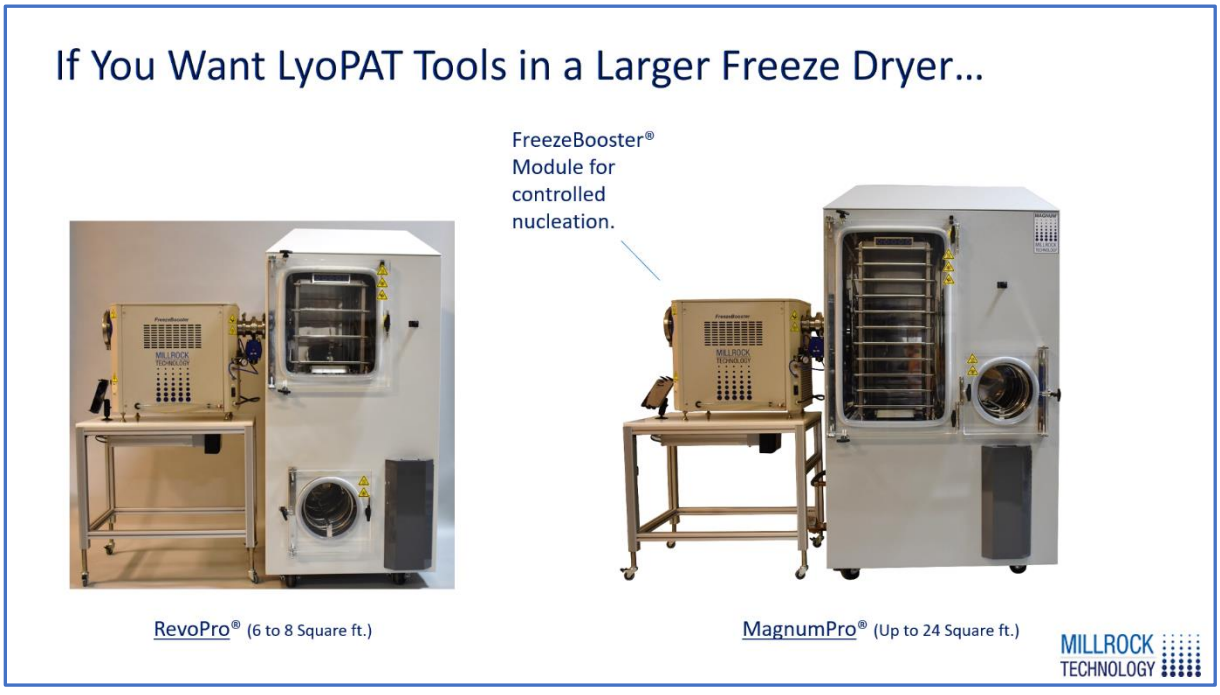
process parameters and provides all the tools necessary for analysis and optimization for protocol tech-transfer.

Fig. 17



We also offer this technology in this case in six to eight square foot systems and up to twenty square foot systems. **Fig 18**

Fig. 18



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