Lyophilized Beads: Efficient, Scalable Production

Brian Kirk, VP Engineering, BioDot

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Brian Kirk has been with BioDot since 2001 and has spent that time designing, developing, and marketing high throughput manufacturing systems for many of the world's leading diagnostic and life science companies. As a member of the original team who developed the patented FISHArray™ technology and CellWriter™ workstation for cytogenetics, Brian now leads the Business Development team within BioDot where he leverages BioDot's expertise in high throughput nanoliter and picoliter printing technologies to commercialize important tools for emerging life science markets.

Challenge

Production of lyophilized beads at the microliter scale results can be difficult to achieve with conventional dispensing technologies. Production environments are often forced to manage inconsistent drop volumes, poor and inconsistent bead morphologies, and low throughput. These inconsistencies lead to poor yield and ultimately increase the cost of manufacturing when valuable reagents and personnel time are lost.

Additionally, significant yield loss occurs in common production settings due to a lack of in-process QC control. In most production facilities, waiting to measure bead accuracy until after lyophilization means that entire batches can be lost along with days of production time. This study evaluates the use of a horizontal droplet camera to accurately and effectively measure drop volumes as an in-process, proactive QC tool.

Solution

Using BioDot's patented Sphera dispensing technology, lyophilized beads were created with minimal loss. BioDot's Sphera instrument produced consistent lyobeads at 2µL,10µL, and 30µL, and exhibited a yield of 96%, 96.6%, and 96.6%, respectively.

Faulty beads were minimized with individual partition holes, allowing the beads to form without defects. The dispense process only took 1 hour (20 minutes per dispense volume size) and an additional 75 hours for full lyophilization. By reducing defects and automating the dispense process, BioDot's Sphera saved time and labor in lyobead production.

BioDot's Sphera™ Platform

Method

Three standard volumes (2µL, 10µL, and 30µL) of qPCR mastermix solution were dispensed into isolated wells chilled with liquid nitrogen (LN2). The total number of dispenses were recorded, including a standard set of calibration dispenses (50).

- For 2µL, 845 dispenses (723.75µL total solution) were performed, resulting in 795 lyobeads (50 used in calibration).
- For 10µL, 289 dispenses (1237.50µL total solution) were performed, resulting in 239 lyobeads (50 spent in calibration).
- For 30µL, 139 dispenses (1788.75µL total solution) were performed, resulting in 89 lyobeads (50 used in calibration).

Dispense values including pressures and open times were optimized and recorded. Coefficient of variation and average drop volume were recorded via the on-board horizontal drop camera. Fresh lyobeads were then measured after formation and once more after to lyophilization to understand total end product diameter. Broken, merged (doublet/triplet), and other damaged beads were recorded so that true product yield could be assessed, and shrinkage after drying was also measured.

Results

- The coefficient of variation (CV) for all dispenses was approximately 1%.
- Of the 795 **2µL** droplets, a total of 22 beads were broken (2.8%) and 10 were doublets (1.3%), for a total yield of 96.0%
- Of the 239 **10µL** droplets, a total of 6 beads were broken (2.5%) and 2 were doublets (0.8%), for a total yield of 96.6%
- Of the 89 **30µL** droplets, a total of 2 were broken, (2.2%) and one was a double (1.1%), for a total yield of 96.6%
- Out of 1123 total beads (excluding calibration dispenses), 43 were lost due to breakage or doubling (96.2% yield).

Volume	# of Beads	Yield	Horizontal Drop Camera (CV%)	Frozen Beads (CV%)	Lyophilized Beads (CV%)
2 µl	795	96.0%	0.9%	2.3%	3.9%
10 µl	239	96.6%	1.2%	3.6%	4.2%
30 µl	89	96.6%	0.9%	4.1%	3.6%

As expected, (due to water expansion during the freezing process), we found an increase in volume/diameter when we compared the data from our horizontal drop camera to the measured frozen bead diameters. The average volume increase was 4.5% (2µL), 4.9% (10µL) and 4.4% (30µL). While water expands by an average of 9% when it freezes, it is unclear what the expected increase is for qPCR mastermix.

When comparing volumes from the horizontal camera to the measured volumes after lyophilization, a strong correlation was observed. The horizontal camera had accuracies of 98.8% (2µL), 98.2% (10µL) and 100.1% (30µL) with respect to target volume. Post lyophilization diameters had accuracies of 100.64% (2µL), 100.00% (10µL) and 100.78% (30µL) with respect to target volume.

Comparing the Horizontal Camera Data to Measured Diameters

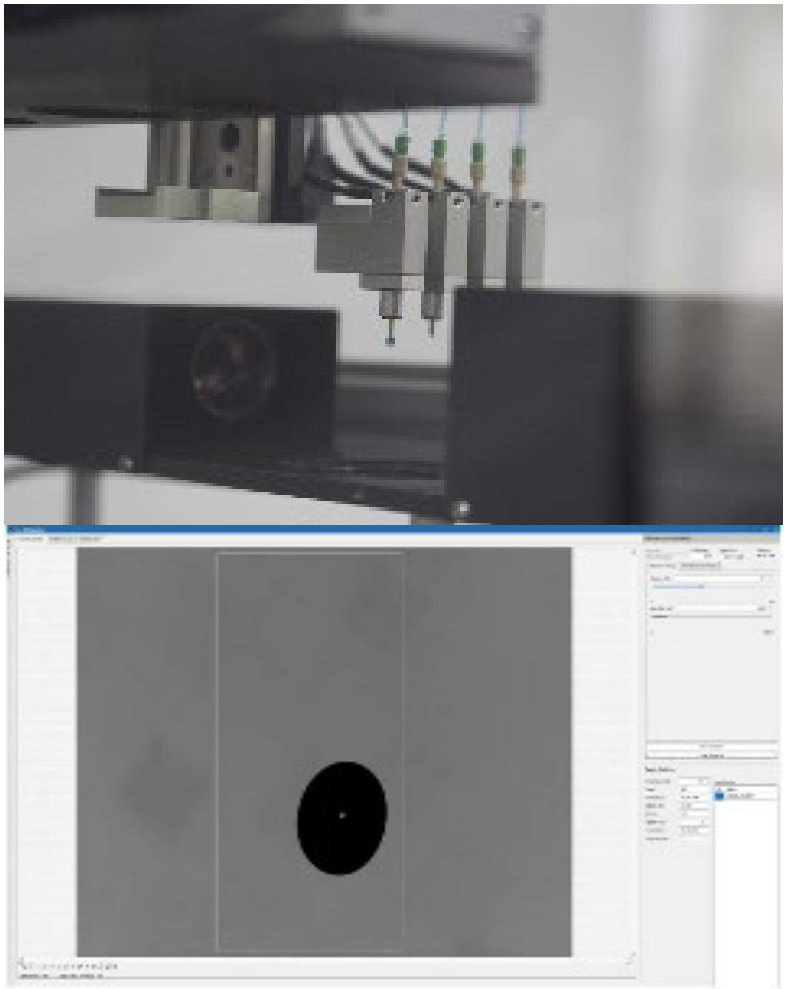
Horizontal Drop Camera			Frozen Beads		Lyophilized Beads	
Volume	Average Drop Camera Volume (uL)	Diameter Accuracy	AVG Frozen Beads Measurement Diameter (mm)	Diameter Accuracy	AVG Lyophilized Beads Measurement Diameter (mm)	Diameter Accuracy
2 µl	1.98	98.8%	1.63	104.5%	1.57	100.6%
10 µl	9.82	98.2%	2.81	104.9%	2.68	100.0%
30 µl	30.04	100.1%	4.03	104.4%	3.89	100.8%

Lyobeads in liquid nitrogen after dispensing

Conclusions

BioDot's Sphera is extremely efficient at dispensing and creating lyobeads. The beads are high quality with minimal loss of beads to breakage, falling apart or adhering to other beads.

Some companies will dedicate in-house engineering resources to develop a custom solution to create lyobeads. This can take months of learning about the nuances of the science. A homemade solution requires continual support and is best assigned to experts in the application. Unleash the power of BioDot's Discrete Droplet Dispensing™, our platform design expertise and global support are available in an established product for lyobead formation.



Images: In-flight capture of 10µL drops (upper left); Drop Camera (lower left); 1000 drops of 4.2µL in volume captured in-flight showing 0.52% CV

About the Sphera™

The BioDot Sphera allows for scalability and versatility. The system can be configured to dispense a range in drop sizes from 2µL-30µL. The system can scale to address changing throughputs by adding dispense channels, in the field. Multiple channels can be installed on a single dispense arm allowing simultaneous on-the-fly dispensing into isolated wells. Multiple passes are repeated with synchronization of freeze cycles and drop times to generate an optimized number of Lyobeads in liquid nitrogen after dispensing of beads per pass.

Lyobead formation relies on uniformity of bead size to the desired drop volume. An integrated camera is used to measure the volume of the bead in-flight to ensure process performance.

Volume, drop rates, speed, placement in and above the isolated wells, and imaging of the dispense are controllable parameters of the lyobead application on a BioDot Sphera™ platform. The Sphera platform can be configured with multiple dispense channels to address various bead throughputs from 10,000 beads per hour per dispense channel.