

## APPLICATION NOTE

# FlowCam Nano provides counts, sizes and images of nano- and microparticles: Application to a therapeutic protein pumping study

Cheng Her<sup>1</sup>, Chris Sieracki<sup>2</sup>, Kent Peterson<sup>2</sup>, Christian Mills<sup>2</sup>, John Carpenter<sup>1</sup>

## ABSTRACT

Sub-visible particle characterization is a critical method for assessing drug substance and drug product quality and stability. In this study, we evaluated the capabilities of FlowCam Nano from Yokogawa Fluid Imaging Technologies (which utilizes patented oil immersion flow microscopy), in conjunction with a peristaltic pumping study that focused on protein particle formation generated during filling pump operations.

Initial experiments showed that FlowCam Nano can generate highly resolved images of both nano and microparticles, allowing assessment of morphology for micron-sized and submicron particles. In addition, particle sizes and counts are obtained for particles as small as 0.3 microns. This capability is extremely useful. For example, the peristaltic pumping study showed that there were relatively high levels of both nano- and microparticles formed when intravenous immunoglobulin (IVIG) formulations were pumped. Also, it was observed that during post-pumping agitation there was a substantial increase in microparticle concentrations coinciding with a large depletion of nanoparticles. *This result suggests that agglomeration was the likely mechanism for agitation-induced increases in microparticle concentrations.*

In addition, results obtained with FlowCam Nano were sensitive enough to observe manifestations in the differences of colloidal stabilities in protein formulations in different buffers (Figures 6A and 6B). During pumping operations, and post-pumping agitation studies, FlowCam Nano was able to show the differences in particle size distributions for samples in phosphate buffered saline (PBS) version 0.25 M glycine buffer. IVIG is much less colloidal stable in PBS than in glycine buffer, and presumably, particles of the protein are also. During agitation of pumped IVIG in PBS, the diameter of particles increased much more rapidly than was observed during the same treatment in glycine buffer.

Figure 1. (right) FlowCam Nano images of polystyrene bead standards. The certified ranges of those beads were 0.345 +/- 0.007  $\mu$ m (A) and 0.600 +/- 0.009  $\mu$ m (B)

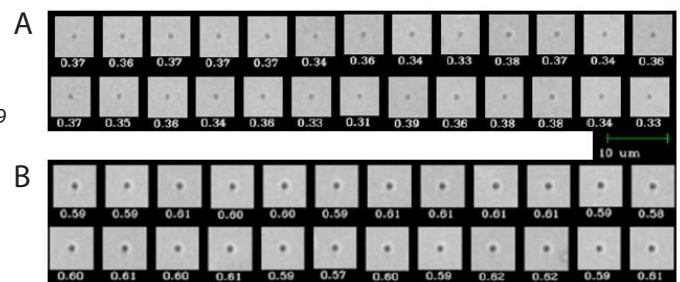
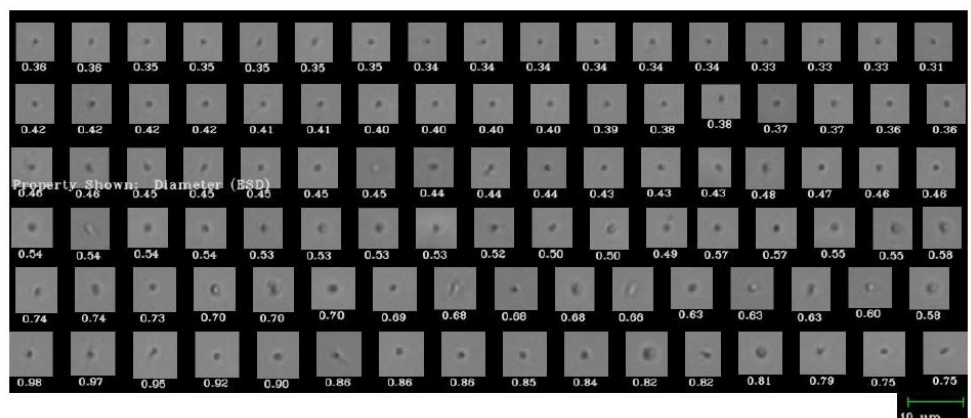


Figure 2. (below) FlowCam Nano images of a population of submicron particles from pumped IVIG in PBS



## FlowCam Nano provides counts, sizes and images of nano- and microparticles

Overall, this study demonstrated that FlowCam Nano has unique capabilities that are very useful for characterizing and quantifying nano- and microparticles in therapeutic protein products and for gaining insights into effects of processing steps and formulations. The ability to characterize and quantify nano- and microparticles simultaneously will allow us to better assess the causes of particle formation and develop more effective control strategies.

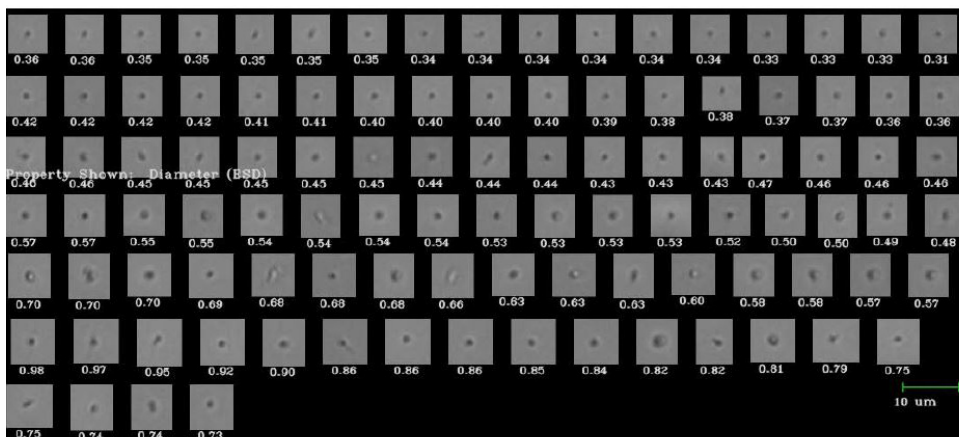


Figure 3. FlowCam Nano images of a population of submicron particles of pumped IVIG in glycine buffer

### METHODS AND MATERIALS

Using a Flexicon PF6 aseptic peristaltic filler pump, we emulated filler processes of those seen in pharmaceutical filling lines using 1 mg/mL of IVIG in PBS pH 7.4 and 0.25 M glycine pH 4.2. For these experiments evaluating oil immersion flow microscopy, a 500 mL fill volume was used, along with Pharmed BPT (Saint-Gobain), due to this particular tubing generating larger amounts of nanoparticles than other tubing tested previously. Post-pumping agitation was done using an ATR Rotamix at 15 RPM. Particle analysis was done using oil immersion flow microscopy (FlowCam Nano).

### FLOWCAM NANO SAMPLING PROCEDURE

For maximum accuracy in sizing small particles (less than 1 micron), a segmentation threshold setting of 5.00 was used, segmenting on dark pixels only. An Edge Gradient filter was applied to display only particles with an edge gradient above 30, to ensure that only accurately measurable particles were visible. A dilution ratio of 0.10 was applied to reflect the portion of imageable depth that the FlowCam Nano was able to capture within the edge gradient filter's restrictions. A flow rate of 0.02 mL/min was used to maximize image clarity. Protein samples were processed using the following method:

After the prior run, clean flow cell with 0.2 mL of roughly 2% Contrad 70 solution. The cleaning solution ensures that no aggregates adhered to the flow cell between samples. Next, rinse flow cell with 0.2 mL DI water. Visually verify in Setup/Focus window that no cleaning solution or contaminants remain in flow stream. Vortex the sample using a Vornado Miniature Vortex Mixer. Pipette 0.12 mL of sample into sample introduction pipette tip (this varied by sample density, as high image counts made massive files). Using Camera View, advance lower sample meniscus past the viewable area.

Size calibration standards were run using the same procedure, with the modification that only a rinse (not a cleaning) step was used. The size standard solutions were a 20:1 dilution of Duke Standards 0.350  $\mu\text{m}$   $\pm$  0.007  $\mu\text{m}$  Polystyrene Particle Counter Size Standards and a 20:1 dilution of Duke Standards 0.600  $\mu\text{m}$   $\pm$  0.009  $\mu\text{m}$  Polystyrene Particle Counter Size Standards.

## FlowCam Nano provides counts, sizes and images of nano- and microparticles

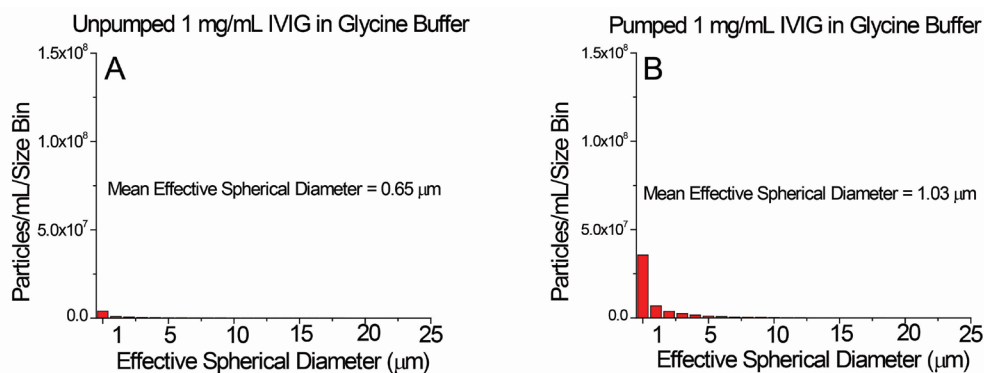


Figure 4. Particle size distribution data of unpumped (A) and pumped (B) 1 mg/mL IVIG in 0.25 M glycine buffer pH 4.2 via FlowCam Nano<sup>3</sup>

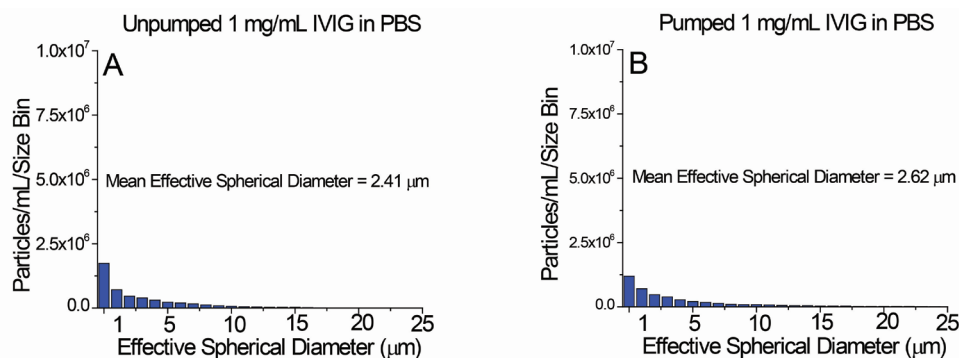


Figure 5. Particle size distribution data of unpumped (A) and pumped (B) 1 mg/mL IVIG in PBS pH 7.4 via FlowCam Nano<sup>3</sup>

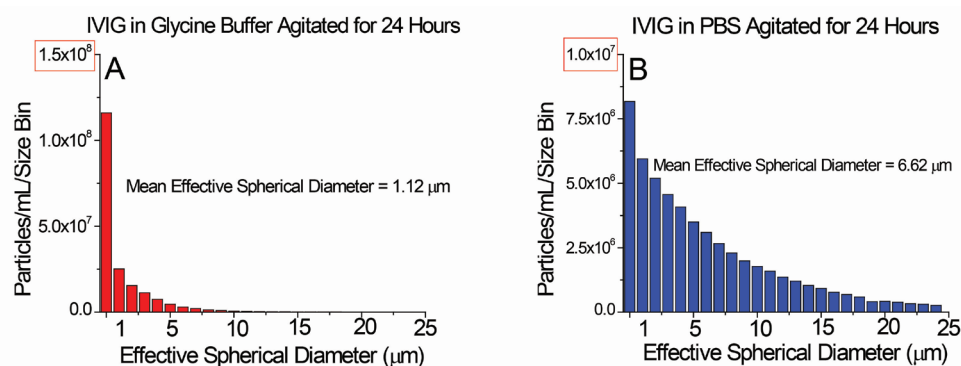


Figure 6. Comparison of pumped IVIG agitated for 24 hours in glycine (A) and PBS (B)<sup>3</sup>  
 \*\*Y-axes are not normalized for these two graphs

## DISCUSSION

As seen in Figures 4 and 5, FlowCam Nano showed that as IVIG is pumped, the size distribution shifts toward larger microparticles in both buffers. This is substantiated by an increase in the mean spherical diameter for the entire particle population. In Figures 6A and 6B, IVIG is shown to be much more colloiddally stable in glycine than PBS. The size distribution of particles for IVIG in glycine is more concentrated in the submicron population and the size distribution of particles for IVIG in PBS is more evenly distributed up to 25 μm.

## CONCLUSIONS

Overall, this study demonstrates that FlowCam Nano has unique capabilities that are very useful for characterizing and quantifying nano- and microparticles in therapeutic protein products and for gaining insights into effects of processing steps and formulations. The ability to characterize and quantify nano- and microparticles simultaneously will allow us to better assess the causes of particle formation and develop more effective control strategies.

<sup>1</sup>University of Colorado-Denver, Skaggs School of Pharmacy and Pharmaceutical Sciences

<sup>2</sup>Yokogawa Fluid Imaging Technologies, Inc.

<sup>3</sup>Particles above 2 μm are outside the size range of FlowCam Nano and may be subject to sizing errors



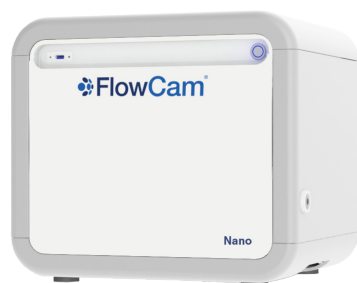
## FlowCam Nano provides counts, sizes and images of nano- and microparticles

Sample (Fraction)	Particles $\geq 0.3$ (particles/mL)	Particles $\geq 1$ (particles/mL)	Particles $\geq 5$ (particles/mL)	Particles $\geq 10$ (particles/mL)
Control	$4.0 \times 10^6$	$9.1 \times 10^5$	$2.8 \times 10^5$	$1.2 \times 10^5$
1	$3.6 \times 10^7$	$6.7 \times 10^6$	$9.2 \times 10^5$	$2.3 \times 10^5$
16	$1.1 \times 10^8$	$1.4 \times 10^7$	$1.2 \times 10^6$	$2.4 \times 10^5$
32	$4.8 \times 10^5$	$2.3 \times 10^5$	$4.7 \times 10^4$	$2.0 \times 10^4$
48	$1.2 \times 10^8$	$2.5 \times 10^7$	$4.6 \times 10^6$	$6.4 \times 10^5$
Sample Agitated	Particles $\geq 0.3$ (particles/mL)	Particles $\geq 1$ (particles/mL)	Particles $\geq 5$ (particles/mL)	Particles $\geq 10$ (particles/mL)
Control	$4.0 \times 10^6$	$9.1 \times 10^5$	$2.8 \times 10^5$	$1.2 \times 10^5$
1 Hour	$1.9 \times 10^8$	$4.7 \times 10^7$	$1.7 \times 10^6$	$9.4 \times 10^4$
4 Hours	$1.8 \times 10^7$	$3.2 \times 10^6$	$3.6 \times 10^5$	$2.0 \times 10^4$
8 Hours	$4.5 \times 10^5$	$2.3 \times 10^5$	$3.4 \times 10^4$	0
24 Hours	$1.2 \times 10^8$	$2.5 \times 10^7$	$4.6 \times 10^6$	$6.6 \times 10^5$

Figure 7. Size distribution by particle concentration determined by FlowCam Nano of 1 mg/mL IVIG in 0.25 M glycine buffer during the pumping run (top) and post-pumping agitation (bottom)<sup>3</sup>

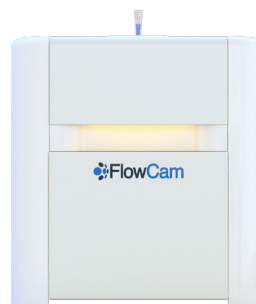
Sample (Fraction)	Particles $\geq 0.3$ (particles/mL)	Particles $\geq 1$ (particles/mL)	Particles $\geq 5$ (particles/mL)	Particles $\geq 10$ (particles/mL)
Control	$1.2 \times 10^6$	$7.2 \times 10^5$	$2.3 \times 10^5$	$1.1 \times 10^5$
1	$1.8 \times 10^6$	$7.2 \times 10^5$	$2.4 \times 10^5$	$8.0 \times 10^4$
16	$1.1 \times 10^6$	$4.7 \times 10^5$	$1.4 \times 10^5$	$5.4 \times 10^4$
32	$1.7 \times 10^6$	$7.7 \times 10^5$	$3.2 \times 10^5$	$1.4 \times 10^5$
48	$2.1 \times 10^6$	$9.6 \times 10^5$	$4.6 \times 10^6$	$2.6 \times 10^5$
Sample Agitated	Particles $\geq 0.3$ (particles/mL)	Particles $\geq 1$ (particles/mL)	Particles $\geq 5$ (particles/mL)	Particles $\geq 10$ (particles/mL/size)
Control	$1.2 \times 10^6$	$7.2 \times 10^5$	$2.3 \times 10^5$	$1.1 \times 10^5$
1 Hour	$1.9 \times 10^6$	$9.5 \times 10^5$	$3.4 \times 10^5$	$1.3 \times 10^5$
4 Hours	$1.4 \times 10^6$	$9.0 \times 10^5$	$4.8 \times 10^5$	$3.2 \times 10^5$
8 Hours	$3.2 \times 10^6$	$2.2 \times 10^6$	$1.3 \times 10^6$	$8.4 \times 10^5$
24 Hours	$8.2 \times 10^6$	$5.9 \times 10^6$	$3.5 \times 10^6$	$1.8 \times 10^6$

Figure 8. Size distribution by particle concentration determined by FlowCam Nano of 1 mg/mL IVIG in PBS during the pumping run (top) and post-pumping agitation (bottom)<sup>3</sup>



## FlowCam Nano

- 300 nm-2  $\mu$ m size range
- Uses oil immersion flow-imaging technology
- Offers the highest resolution images of nanoparticles available on the market today



## FlowCam 8100

- 2  $\mu$ m-1 mm size range
- Advanced thresholding capabilities enable accurate analysis of translucent particles
- Compatible with Automated Liquid Handling system (ALH)