FlowCam®

FLUID IMAGING TECHNOLOGIES

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TECHNICAL NOTE

Benefits of FlowCam vs. DLS and NTA for Screening of Biopharmaceuticals

SUMMARY

The problem of protein aggregation and presence of extrinsic particles in biopharmaceutical formulations is not a small one. With the improvement of particle analysis technologies, developers are discovering that drug impurities and protein aggregation occur at multiple phases of formulation development, production, and quality control. The goal is to ensure the production of pure and safe biologics that can be shipped and delivered to patients, ensuring their health and safety.

Many particle analysis instruments commonly used by biopharmaceutical developers have the ability to detect proteins and other particles at the sub-micron level, but do not necessarily detect larger particles that may be present in a sample: e.g. protein aggregates, fibers, glass or silicone particles. The FlowCam 8000 counts, images, and analyzes particles ranging from 2µm to 1mm. Using this instrument as an orthogonal detection method during multiple phases of drug development can assure that a much wider range of particles in a sample are detected, and that drug developers are able to see the full picture of what's in their product.

ABSTRACT

In a study performed by William Bernt of Particle Characterization Laboratories, Inc (Novato, CA), three instruments were used to count and analyze PSD standard samples at a variety of sizes. The following is taken from Bernt's 2017 poster entitled *Screening Biopharmaceuticals with Flow Imaging; Finding the Missing Fraction.*

"Sub-visible proteinaceous aggregates are often a degradation product that can be a major factor in limiting the shelf life and efficacy of biopharmaceuticals. These aggregates may affect product manufacturability, bioactivity and absorption rate. More importantly aggregates may bring about immunogenicity in the patient resulting in a loss of drug efficacy, patient discomfort or even death."

Figure 1. Instruments used in Bernt study. Fluid Imaging Technologies replaced the FlowCAM VS-1 with newer model FlowCam 8000 in 2016.

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ABSTRACT, CONTINUED

"Establishing the presence of such particles is therefore of paramount importance when developing products for infusion or subcutaneous injection. We show how two commonly used particle sizing methods, dynamic light scattering (DLS) and nano-particle tracking (NTA), can easily fail to detect sub-visible micron-sized aggregates even at concentrations ten times greater than those specified by USP 788/787. Furthermore we demonstrate how Flow Imaging Microscopy (FIM) plays a critical role in detecting, characterizing and enumerating these sub-visible particles. Data is also presented on a commercially available 50nm liposomal product."

METHOD

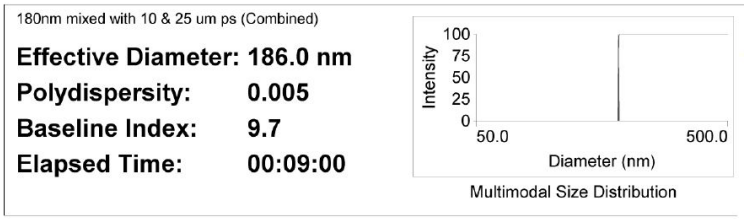
Bernt prepared samples using the following method:

- *"Make up a 10µm PSD standard (Thermo Scientific Corporation) suspension at 120,000 particles per ml (20X UPS-788 limit).*
- *Make up a 25µm PSD standard (Thermo Scientic Corporation) suspension at 12,000 particles per ml (20X USP-788 limit).*
- *Add 4.5ml of each suspension to a fresh Falcon tube. This diluted the concentration of each standard by half (10X the USP-788 limit).*
- *Add 1ml of a 10wt% 186nm NIST traceable PSD standard (final concentration 1wt% 186nm PSD).*
- *Analyze the sample neat through the FlowCam VS-1 10X FC100.*
- *Dilute the sample 1:5,000 for the DLS analysis*
- *Dilute the sample 1:50,000 for the NanoSight analysis."*

RESULTS

Particle size distribution standards of three different sizes (186nm, 10µm, and 25µm) were combined into a single sample and subsequently analyzed by each instrument.

The DLS instrument detected an average particle diameter of 186nm, demonstrating that the instrument detected only the 186nm NIST traceable PSD standard particles, and was not able to detect the 10µm or 25µm particles. (Figure 2)



Run	Eff. Diam. (nm)	Half Width (nm)	Polydispersity	Baseline Index
1	184.3	13.0	0.005	8.0
2	186.4	13.2	0.005	7.5
3	186.9	28.9	0.024	9.0
Mean	185.9	18.4	0.011	8.2
Std. Error	0.8	5.3	0.006	0.4
Combined	186.0	13.2	0.005	9.7

Figure 2. Dynamic Light Scattering Data

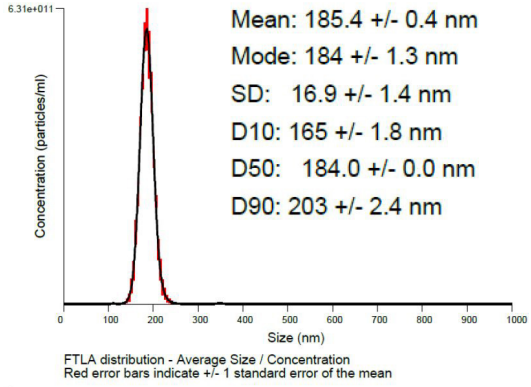


Figure 3. Nano-Particle Tracking Analysis Data

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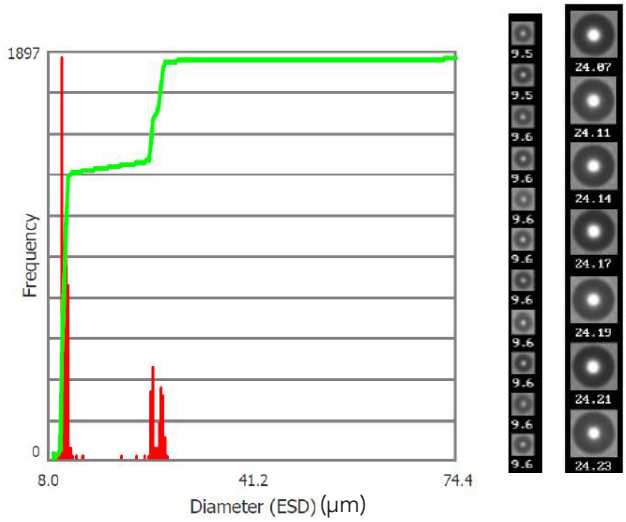
RESULTS, CONTINUED

Particles detected by the NTA instrument had a similar average diameter of 185.4nm showing that the NanoSight was also unable to detect the 10µm and 25µm PSD standard particles. (Figure 3)

FlowCam SEES LARGER PARTICLES

The data from the sample run on the FlowCam shows peaks at 10µm and 25µm (Figure 4), demonstrating that while this FlowCam model does not detect particles in the nanometer range, it works very well to detect, count, and image particles 2µm and larger. This data also shows outliers that could represent extrinsic particles present in the sample, and the imaging capabilities of the FlowCam allow for identification of these individual particles.



Filter	Count	Count %	Volume %	P/ML
10-20um	5249	73.11	41.03	53735
25-300um	1136	15.82	57.08	11630

Figure 4. Flow Imaging Data

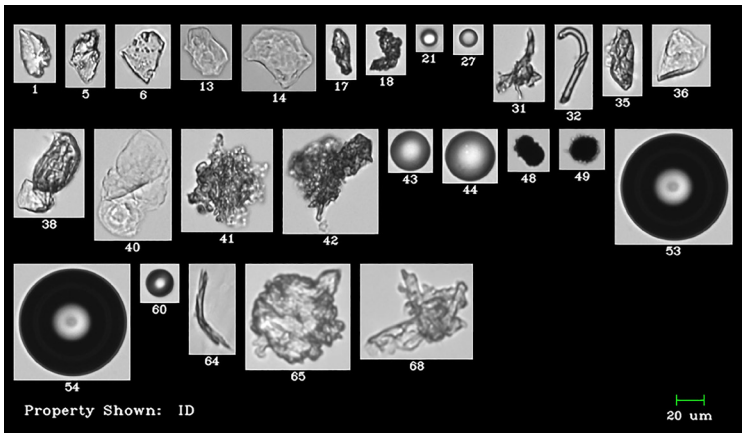


Figure 5. Intrinsic and extrinsic particles imaged by the FlowCam: protein aggregates, glass shards, silicone oil droplets, and other particulate.

CONCLUSIONS

- *"Flow Imaging Microscopy is a critical tool for assessing biopharmaceutical formulations.*
- ***Flow Imaging Microscopy augments other instruments which are designed specifically to characterize the sub-micron size component.***
- *Just because DLS and NTA don't report micron sized particles does not mean that they are not there. Verify!"*

FlowCam 8000

- 2µm-1mm size range
- Advancing thresholding capabilities enable accurate analysis of translucent particles
- Compatible with Automated Liquid Handling system (ALH)

FlowCam Nano

- 300nm-10µm size range
- Uses oil-immersion, flow-imaging technology
- Advancing thresholding capabilities enable accurate analysis of translucent particles

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