Top FlowCam Studies for Biopharmaceuticals

Machine learning and statistical analyses for extracting and characterizing "fingerprints" of antibody aggregation at container interfaces from flow microscopy images

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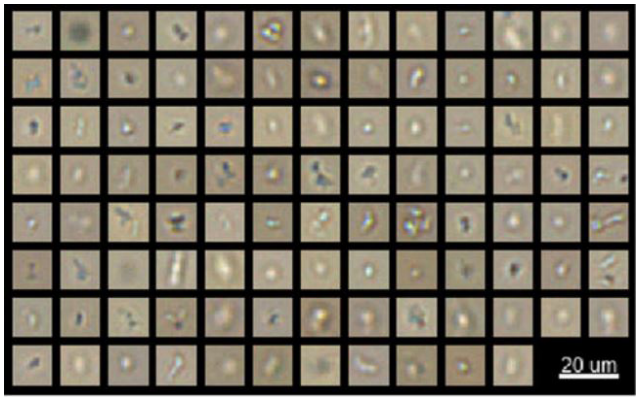
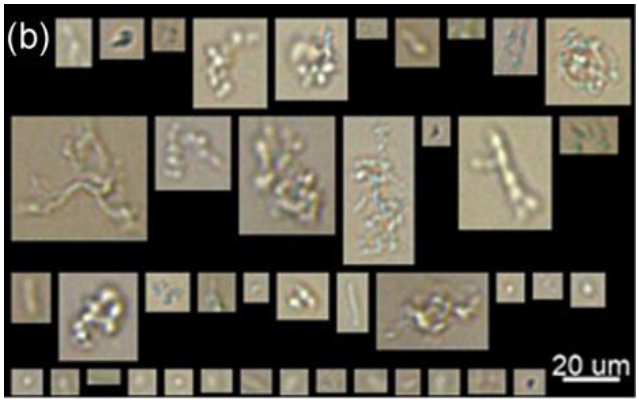
INTRODUCTION "Therapeutic proteins are exposed to numerous stresses during their manufacture, shipping, storage and administration to patients, causing them to aggregate and form particles through a variety of different mechanisms. These varied mechanisms generate particle populations with characteristic morphologies, creating "fingerprints" that are reflected in images recorded using flow imaging microscopy. Particle population fingerprints in test samples can be extracted and compared against those of particles produced under baseline conditions using an algorithm that combines machine learning tools such as convolutional neural networks with statistical tools such as nonparametric density estimation and Rosenblatt transform-based goodness-of-fit hypothesis testing ... As a demonstration, this algorithm was used to compare particles within intravenous immunoglobulin formulations that were exposed to freeze-thawing and shaking stresses within a variety of different containers."

FLOWCAM METHOD "FIM images were recorded with a FlowCam® VS instrument with a ×10 objective, a field-of-view flow cell with a depth of 80 µm and width of 700 µm, and color imaging. The instrument was focused using the built in autofocus protocol for optimal image quality using 20-µm calibration beads. 1% Micro-90 solution followed by filtered ultrapure water were flushed through the instrument before and between measurements. The flash duration of the instrument was adjusted between replicates to achieve a constant background intensity of 150. Three 0.2 ml aliquots were analyzed from each replicate vial. Images were collected at a flow rate of 0.05 ml/min using 15 light and 17 dark pixel thresholds for particle segmentation ... FIM images of particles were imported into Python 2.7. pixels ... Three experimental replicates for each combination of container and stress were used to train the algorithm"

FlowCam images of particles made under the baseline condition:



FlowCam images of particles after freeze-thawing in plastic microcentrifuge tubes:



RESULTS & CONCLUSIONS "the algorithm can easily identify morphology differences between particle populations that had been exposed to [shaking stress and freeze-thaw stress] using only a small number of particle images. Additionally, the algorithm only misidentified unseen test particles made under baseline conditions as being different from the baseline population around 5% of the time—the type I error rate that the test was designed to give ...

The primary advantage of this new approach is its ability to determine, using only a small number of FIM images, if a new sample exhibits significantly different particle populations than those found under baseline conditions. The combination of traditional statistical tools with powerful machine learning algorithms can be used to determine if two samples exhibit a morphology difference that cannot be explained by sample-to-sample variance in particle morphology under a single root cause. This approach is effective at identifying (statistically) significant differences in particle morphology occurring due to different root causes such as manufacturing changes or process upsets that could warrant further investigation.

Forced degradation of cell-based medicinal products guided by flow imaging microscopy: Explorative studies with Jurkat cells

European Journal of Pharmaceutics and Biopharmaceutics (2021)
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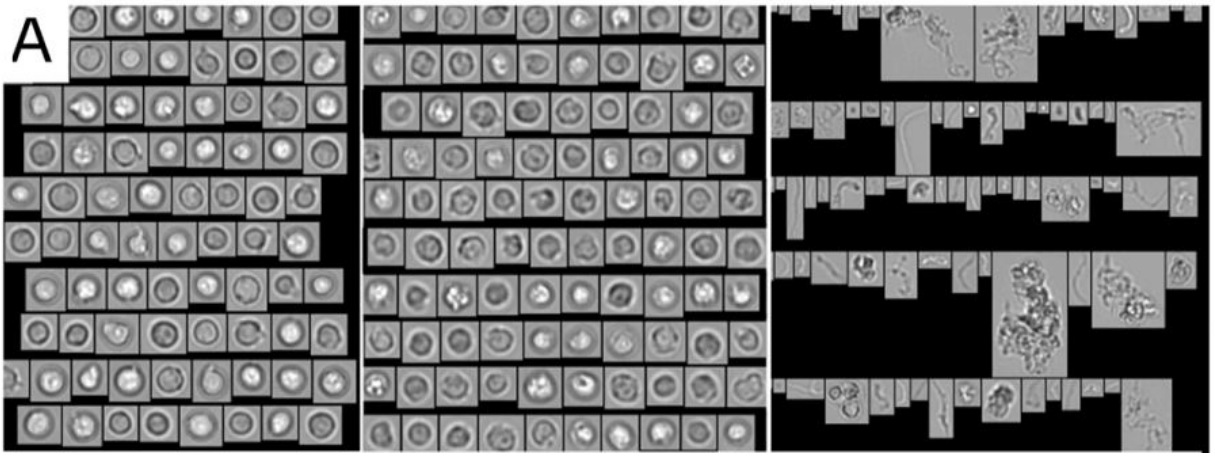
INTRODUCTION Cell-based medicinal products (CBMPs) offer promising opportunities for the treatment of diseases with previously limited or no therapeutic options. However, the complexity and intrinsically fragile nature of these CBMPs create significant challenges in formulation development, analytical characterization, manufacturing, and stability assessment. This recent study used a FlowCam assisted by machine learning software, to measure cell viability and concentration, and to quantify debris particulates. These particulates were the result of forced degradation studies that mimic conditions from cell procurement to administration of product. In particular, the effects of freeze-thawing and shaking on CBMPs were studied.

FLOWCAM METHOD "Cells (viable and dead) and debris particles were quantified by using a FlowCam 8100 equipped with a 50-µm flow cell. The objective used resulted in a 20x magnification and sample imaging was performed by a high-resolution CMOS camera (1920x1200 pixels) at 27 frames per second. In total, a sample volume of 140 µl was measured with an efficiency of approximately 63% (i.e., the imaged sample volume was ca. 87 µl). Particles imaged within the flow cell were detected with intensity thresholds of 12 for light and dark pixels ... Particle images captured with a FlowCam were analyzed by using convolutional neural networks (CNN) ... The CNN was fine-tuned on manually labeled images (4,000–4,500) of each of the three populations: viable cells, dead cells and debris particles."

RESULTS & CONCLUSIONS "The characterization of particulate matter in parenterals is described in pharmacopeial monographs and products should meet the acceptance limits for subvisible particle impurities sized above 10 µm and 25 µm. However, given the particulate nature of cells, it is challenging to fulfil specific particle testing requirements applicable to injectable products, as human cells fall within the subvisible size range (typically 7–30 µm). One of the standard pharmacopeial methods for quantification of subvisible particle impurities, light obscuration, will not discriminate between cells and other particulates. Hence, highthroughput microscopy methods providing morphological data on particles within several hundred µl are promising tools for evaluation of particulates in cell-based products. Previously, the FIM approach was utilized to identify and quantify Dynabeads, which are commonly used during cell activation, in cell suspensions. Moreover, FIM has also found its use in detection of other particulates, such as process impurities in CBMPs. Here, by using FIM-CNN, reliable numbers on the concentration of debris particles sized 1–50 µm could be obtained ...

the FIM-CNN method showed the capability to monitor the levels of an entire debris particle population which may provide relevant information on the potential implications for product quality (consistency and stability) and safety of these drug products."



Examples of FlowCam images selected for training of the CNN model.

Left to right: viable cells, dead cells, debris particles

Extending the Limits: Oil Immersion Flow Microscopy

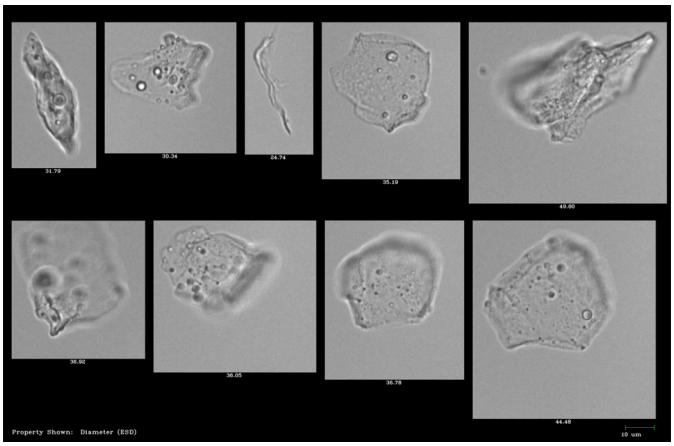
American Laboratory (2019)
C. Sieracki, Fluid Imaging Technologies

INTRODUCTION Particle characterization is one of the most common needs among protein scientists. Imaging data such as flow imaging microscopy (FIM) has emerged as an accepted technique to deliver morphological information of particles ranging in size from 2 µm to 5 mm. Unlike other volumetric methods, FIM can detect translucent, semi-translucent and opaque particles, allowing for the differentiation of protein aggregates and non-proteinaceous particles.

Recently available oil immersion flow imaging microscopy has been shown to increase the optical resolution and thereby increase the range of particle detection down to 300 nm. A typical high-quality oil objective can have a numerical aperture of 1.4, resulting in a resolution of .22µm, approximately 10 times better than a traditional air objective microscope.

The real advantage of this technology is the ability to image and identify particulate content in a fluid where particle morphology is critical to its characterization. Technology for the detection and imaging of nanoparticles has evolved significantly due to the heightened awareness and significance of nanoparticles. The images are invaluable in the identification of the origins of protein aggregation.

METHOD "An oil immersion Nano-Flow Imaging system was configured with a 1.4-NA oil immersion objective. Fisher Scientific size calibration polystyrene beads of 345, 602, and 903 nm were added to 50-µL samples of deionized water and drawn into the instrument using the VisualSpreadsheet software. The instrument was calibrated by sizing these beads and adjusting the calibration constant of the instrument. An edge gradient filter was used to isolate and eliminate beads that were not in focus. Once the instrument was calibrated, the beads were processed again with the instrument. Protein agglomerate samples and NIST protein samples were similarly processed with the oil immersion Nano-Flow Imaging System. For comparison purposes, NIST protein samples were also processed on a 10x air objective flow imaging system."



Protein agglomerates from NIST Protein Reference Standards

RESULTS & CONCLUSIONS "Bead size data show a measurement error of less than 10% ... In the protein agglomerate images [above], the features down to approximately 0.3 µm are clearly visible. In the case of large particles, it can be seen that portions of the particle may be out of focus ...

For the first time in the history of formulation analysis technologies, an imaging flow microscope with properly configured oil immersion optics can be used to analyze samples with particles down to about 300 nm. This opens a new world of feedback to formulation scientists and process technicians. Contaminants can be introduced anywhere in production, and can lead to problems ranging from recalls to serious complications and death. Having an image of a mystery nanoparticle shows scientists where to look for improvements: formulation development, quality assurance, fill finish, or storage and transportation.

Deep Convolutional Neural Network Analysis of Flow Imaging Microscopy Data to Classify Subvisible Particles in Protein Formulations

Journal of Pharmaceutical Sciences (2017)
doi:10.1016/j.xphs.2017.12.008
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INTRODUCTION "Flow-imaging microscopy (FIM) is commonly used to characterize subvisible particles in therapeutic protein formulations. Although pharmaceutical companies often collect large repositories of FIM images of protein therapeutic products, current state-of-the-art methods for analyzing these images rely on low-dimensional lists of 'morphological features' to characterize particles that ignore much of the information encoded in the existing image databases. Deep convolutional neural networks (sometimes referred to as "CNNs or ConvNets") have demonstrated the ability to extract predictive information from raw image data without requiring the selection or specification of "morphological features". However, the inherent heterogeneity of protein therapeutics and optical phenomena associated with subvisible FIM particle measurements introduces new challenges regarding the application of ConvNets to FIM image analysis. In this study, a supervised learning technique leveraging ConvNets is used to extract information from raw images in order to predict the process conditions or stress states (freeze-thawing, mechanical shaking, etc.) that produced a variety of protein particles"

FLOWCAM METHOD "FIM was performed with a FlowCam® VS instrument equipped with a 100-mm flow cell and a 10 objective ... The instrument was focused using the default autofocus procedure on 20-mm calibration beads. Two hundred fifty microliters of sample mixed with 200 mL of ultrapure water were measured for each of the freeze-thaw and shaking þ pH samples.

We trained convolutional neural networks to perform supervised classification tasks such as identifying the aggregation inducing stress (mAb exposed to freeze-thaw, mAb exposed to agitation, IVIG circulated through pump A, or IVIG circulated through pump B) that created a given particle.

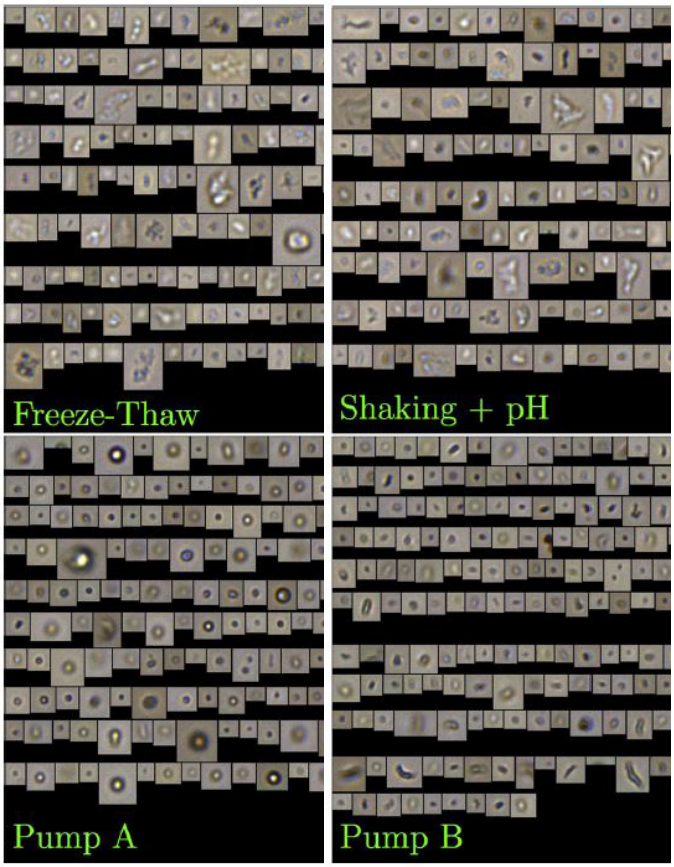

Figure 1. Sample FIM image collages from 4 FIM protein data sets. Clock-wise from top left: freeze-thawed mAb images; mAb experiencing mechanical agitation (shaking) plus pH shock; IVIG processed with a "pump A"; and IVIG processed with "pump B". A ConvNet classifier was used to distinguish these 4 different conditions with high accuracy. Note the heterogeneity and polydispersity of these data sets (expert humans encounter difficulty in classifying the data based on visual inspection of single images).

RESULTS & CONCLUSIONS "A ConvNet was trained to differentiate between FIM images of particles generated via 4 stress conditions ... By using a simple data pooling strategy with a trained ConvNet, we were able to obtain essentially perfect classification of FIM images using a relatively small number of particle images. These results demonstrate that ConvNets can be used to successfully differentiate FIM images of aggregates on the basis of their protein composition (mAb or polyclonal IVIG) and the stress conditions used to generate the particles (freeze-thaw or agitation for mAb,1 of 2 nominally identical pumps for IVIG). Furthermore, these results indicate that both the identity of the protein that may comprise an aggregate and the type of stress used to create the aggregate leave "morphologic fingerprints" to which FIM is sensitive and that can be identified using the ConvNet approach.

Determination of the Porosity of PLGA Microparticles by Tracking Their Sedimentation Velocity Using a Flow Imaging Microscope (FlowCam)

Pharmaceutical Research (2017), doi: 10.1007/s11095-017-2120-8
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Leiden University, Dr. Reddy's Laboratories Ltd.

INTRODUCTION PLGA Microparticles fulfill the needs for controlled release in the area of parenteral pharmaceuticals because of their long clinical experience and favorable performance in terms of biodegradability and biocompatibility. Current approaches to determine porosity of particulate drug delivery systems are based on established methods used in agricultural, petrochemical and constructional engineering. These methods were not developed for use in pharmaceutical laboratories, require large sample volume, and can result in the production of toxic waste, among other drawbacks.

"In this study, FlowCam was used to determine the sedimentation velocity of microparticles in aqueous liquids. The velocity value was then used to derive the density of the microparticles. And from the density and known composition of the microparticles, the porosity was calculated. "

FLOWCAM METHOD "A FlowCAM VS1 system equipped with a 300-µm Field of View (FOV300; 300 µm depth and 1500 µm width) cell and 4× magnification lens was used ... VisualSpreadsheet software version 3 was used to control the system and to process the data."

RESULTS & CONCLUSIONS "Two different methods were explored. In the first method the sedimentation rate of microparticles was tracked in suspending media with different densities. Porosity was calculated from the average apparent density of the particles derived by inter- or extrapolation to the density of a suspending medium in which the sedimentation velocity was zero. In the second method, the microparticle size and sedimentation velocity in one suspending fluid were used to calculate the density and porosity of individual particles by using Stokes law of sedimentation ... Polystyrene beads of different sizes were used for the development, optimization and validation of the methods.

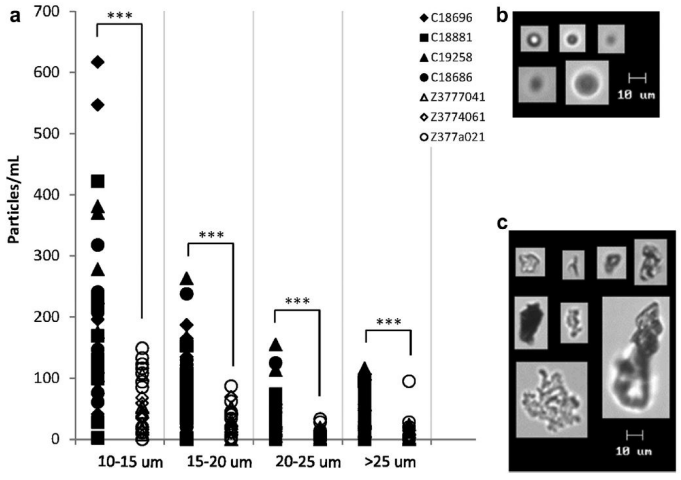
For both methods, porosity values were in excellent agreement with expected values. Both methods were applied to determine the porosity of three PLGA microparticle batches with different porosities (between 4-52%). Both of these methods have proven to be a viable alternative to conventional methods for determining microparticle density and porosity."

Subvisible Particle Content, Formulation, and Dose of an Erythropoietin Peptide Mimetic Product are Associated with Severe Adverse Post-Marketing Events

Journal of Pharmaceutical Sciences (2016)
doi: 10.1016/S0022-3549(15)00180-X
J. Kotarek, C. Stuart et al., US Food & Drug Administration

INTRODUCTION Omontys® – a brand name peginesatide injectable – was voluntarily withdrawn from the market less than a year after the product launch. Clinical trials had demonstrated the drug to be safe and efficacious, but over 40 cases of anaphylaxis, and 7 fatalities were reported soon after the product was introduced to the market. Omontys was manufactured and approved as both a single-use vial (SUV) and a multiuse vial (MUV), which differed in their formulation. Clinical trials primarily used the SUV formulation, but only the MUV formulation was marketed.

FLOWCAM METHOD In this study the FlowCam was used to evaluate the particle profile of SUV and MUV samples. The particle images captured by the FlowCam were then analyzed using VisualSpreadsheet.



Size distribution and concentration of peginesatide particulates 10 mm.

RESULTS & CONCLUSIONS The FlowCam revealed a significantly higher concentration of subvisible particles in the MUV presentation and correlates to the cases of anaphylaxis. Although it is unknown whether the elevated particulate content contributed to these serious adverse events of the drug, the report illustrates the importance of capturing and characterizing subvisible particulates.

The peginesatide samples passed USP <788> limits for particulates, but the methods of particle analysis used by the FDA task force suggest that more sensitive analytical monitoring of SVP (via FlowCam) can differentiate protein aggregates, silicon oil, and air bubbles from other particles that would otherwise go undetected using light obscuration alone.

Protein Aggregation and Particle Formation in Prefilled Glass Syringes

Pharmaceutical Biotechnology (2014)
doi: 10.1002/jps.23973
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University of Colorado, MedImmune

INTRODUCTION "The stability of therapeutic proteins formulated in prefilled syringes (PFS) may be negatively impacted by the exposure of protein molecules to silicone oil–water interfaces and air–water interfaces. In addition, agitation, such as that experienced during transportation, may increase the detrimental effects (i.e., protein aggregation and particle formation) of protein interactions with interfaces. In this study, surfactant-free formulations containing either a monoclonal antibody or lysozyme were incubated in PFS and exposed to silicone oil–water interfaces (siliconized syringe walls), air–water interfaces (air bubbles), and agitation stress (end-over-end rotation)."

FLOWCAM METHOD "For each sample, particles between 2 :m and 2 mm (equivalent spherical diameter) were counted using a Fluid Imaging Technologies Benchtop FlowCam. The FlowCam was fitted with a FC100 flow cell, a 10× objective and collimator, and a 0.5 mL syringe ...

Particle counts were normalized by dividing the number of particles per sample by the total volume imaged per sample to obtain the particle concentration (#/mL). In addition to the samples incubated in syringes, buffer solutions and protein formulations not incubated in syringes were also analyzed by FlowCam."

RESULTS & CONCLUSIONS "Using flow microscopy, particles (≥2 µm diameter) were detected under all conditions ... In addition to the particle images that appeared to reflect primarily spherically shaped silicone oil droplets and non-spherical protein aggregates, there were images of particles that consisted of silicone oil droplets coated with aggregated protein. Furthermore, several images showed large agglomerations of protein-coated silicone oil droplets."

The FlowCam was instrumental in identifying under what circumstance the highest particle concentrations were found; in agitated, siliconized syringes containing an air bubble. The particles formed in this condition consisted of silicone oil droplets and aggregated protein, as well as agglomerates of protein aggregates and silicone oil.