Omontys® – a brand name peginesatide injectable – was voluntarily withdrawn from the market less than a year after the product launch.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.

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®.

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.

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).

Using FlowCam 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.
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 0.22µm, 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.

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.

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 interpolation 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.

NIST Protein Samples as imaged by the FlowCam Nano at 40x.
Flow-imaging microscopy (FIM) FlowCam, 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 particle morphologies.

For example, one ConvNet was trained to differentiate between 2 different mixtures of protein aggregates and silicone oil microdroplets. Error-free classification of these 2 formulations of silicone oil and protein mixtures was obtained.

We demonstrate that the new classifier, in combination with a “data pooling” strategy, can nearly perfectly differentiate between protein formulations in a variety of scenarios of relevance to protein therapeutics quality control and process monitoring using as few as 20 particles imaged via FIM.