Omontys® – a brand name peginesatide injectable – was voluntarily withdrawn from the market less than a year after the product launch.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

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.
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.