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Testing Precision Limits of Neural Network-Based Quality Control Metrics in High-Throughput Digital Microscopy

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Flow imaging microscopy and microfluidic imaging (together referred to as FIM) have been used for many years as orthogonal methods to the USP <787> and <788> light obscuration (LO) particle count test for injectable drug products. In addition to providing particulate size and count information, FIM delivers images of the particles encountered during the test. Historically, these images have had limited use because they have poor image contrast, and it is difficult for humans to extract quantifiable characteristics from the images. However, convolutional neural networks (CNN) have demonstrated utility in image analysis and extracting textural and morphological information that the human eye cannot interpret. This paper discusses a revolutionary use of a CNN-based approach (Fingerprints) for identifying particles from both known and unknown causes. This novel approach can also verify instrument functionality using a surrogate protein particle standard made from ethylene-tetrafluoroethylene (ETFE) particles produced by NIST. The CNN and standard together demonstrate the utility of the textural and morphological features contained within the images and their statistical relevance in pharmaceutical manufacturing.

Light obscuration (LO) has been the compendial method for lot release of injected or implanted drug products since 1985. The output of data is particle size and count, and the method has known technical shortcomings (e.g., reports particle size as the diameter of a circle having an equivalent cross-section, may underestimate the size and count in low index contrast materials, counts air or silicone bubbles as particles, etc.) [1]. While FIM methods provide orthogonal size/count data, research has found that complex correction factors are required to correlate FIM size/count information with data provided by LO[2]. In addition, protein therapeutics pose unique challenges to LO. Consequently, the size and count results delivered from FIM fall short of utilizing the available information contained in the images (i.e., morphological characteristics, texture, density, etc.) to provide accurate information about particle populations to ensure patient safety and drug efficacy.
The authors conducted a series of tests using FlowCam® VS, γ-globulin and ETFE particles for comparative and contextual reference. This CNN-based approach recognizes the textural and morphological features and delivers statistical precision and actionable data by leveraging particle images. Once the fingerprint is created this unique approach provides actionable data

- Comparative process results identify when processes produce different particles
- Recognize new particle populations and, once failure conditions are known, the fingerprint can be retrained to include the recent aggregation source, making future analysis of process variation quick and straightforward
- Statistical precision, even with low particle counts – like those in fill-finish operations
- A mechanism to confirm instrument performance with a known standard
- Ability to overcome sub-optimal optical settings – consistency is the key

Why does this matter? Protein drugs are inherently prone to aggregation throughout manufacturing, shipping, storage, and administration. Furthermore, the risk of aggregation increases with higher protein concentration. Protein aggregate particles are proven to be highly variable and impact drug efficacy, immunogenicity, and patient safety[3–10].

ParticleSentry[AI] accurately and reliably characterizes protein aggregates AND identifies new aggregates and variation in drug product, enabling better control of drug quality.

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Endnotes:

[1] *United States Pharmacopeia and National Formulary.* USP 1-May-2021 <1787>. Measurement of Subvisible Particulate Matter in Therapeutic Protein Injections. Accessed 14 May, 2021 https://online.uspnf.com/uspnf/document/1-GUID-5BAAFB9B-8849-497D-BAEF-A0BCC19FBDB1_3_en-US.

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