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Biotherapeutics Evaluation Using Artificial Intelligence Assisted Image Analysis

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Particles in biotherapeutic formulations is a potential quality and safety concern. Particle monitoring throughout the life cycle of a therapeutic drug product is critical. Particles may be generated in a drug product during manufacture, shipping, storage and administration. This may reduce product efficacy, but particle formation can also trigger undesirable immune responses [1]. Standard analytical methods utilized to date for product characterization applications use only a small number of morphological features to characterize samples. This may neglect a significant amount of relevant structural information contained in the data [2]. Hence, methods capable of harnessing the large amount of complex digital information encoded within the images have great potential in particle characterization and monitoring.

Artificial intelligence (AI) assisted image analyses is currently being explored and developed within Center for Drug Evaluation and Research (CDER) in collaboration with external collaborators from Ursa Analytics and National Institute of Standards and Technology (NIST), to analyze the images of particles in model drug formulations and compare it to a standard protein surrogate [3]. The goal is to better understand the factors for particle generation during storage, transportation, and other agitation processes that a biotherapeutic product undergoes during its product lifecycle. The talk will focus on application of convolutional neural networks (CNNs or ConvNets) and demonstration of its capability to analyze and classify particles with high efficiency and accuracy (Figure 1 illustrates supervised classification and Figure 2 demonstrates unsupervised image analysis of images collected at CDER). In addition, as a drug product quality control strategy, the added utility of using time stable protein surrogate in combination with CNNs were explored [3] (see Figure 3 from Ref. 3 illustrating the fingerprint approach).

Experiments were conducted using model protein formulations with and without agitation stress. Particle analyses was performed using flow imaging microscopy instrument (FlowCam VS, Yogokawa). To efficiently analyze large set of digital image data collected and further reveal the relevant morphological features, convolutional neural networks (CNNs or ConvNets) were utilized as an emerging tool to address the challenges. The CNN based deep learning image classification and fingerprint approach were performed by Dr. Christopher Calderon from Ursa Analytics [2][3]. In addition, a NIST generated protein surrogate standard (Ethylene tetrafluoroethylene [ETFE]) was utilized for comparison of protein aggregates through AI assisted image analyses classification [3] [4].

Briefly, supervised learning techniques allow ConvNets to extract feature information from raw images and correlate these features to known experimental conditions that generate different particle images exhibiting different morphologies (for example in our case study, we analyzed stressed and unstressed

gamma globulin conditions). Supervised learning relies on the pre-defined labels associated with an image for algorithm training, which in this case are 'unstressed' and 'shaking stress' labels (Figure 1). The trained ConvNets model would then be capable to determine which of the pre-defined labels apply to an image of particulate obtained from an unlabeled condition. Unsupervised classification can extract useful features from images when pre-defined labels are not available and perform an exploratory data analysis without labeling (Figure 2). The fingerprint approach aims to use sample images labeled at macro-level (e.g., NIST ETFE, FDA unstressed, FDA stressed product, Figure 3) in order to reduce the dimension of the spatially correlated image pixel intensities down to two dimensions. For each reference product, a fingerprint can be created; the fingerprint consists of a nonparametric probability density function (PDF) estimate of the two-dimensional representations of images from one known product. Although labels are used in forming reference fingerprints, the approach is capable of detecting process changes or unanticipated new particles [3].

Based on our ongoing research, AI-assisted image analysis has enabled processing large collection of images with high efficiency and accuracy, which would not likely be feasible with existing image processing software. AI assisted image analysis applied early on during product development or at any other stage during life cycle of a drug product could help identify and mitigate potential risks of particulate generation and ensure quality and safety of therapeutic drug products.

Supervised Classification of Particles in Protein Formulation (N_{pool} = 25)

	No-Stress	Shaking
No-Stress	0.99	0.01
Shaking	0.02	0.98

Figure 1. Supervised image classification. Data analysis using supervised image classification for the unstressed and stressed protein formulation. Pooled images classification (N_{pool}=25). 99% accuracy rate with pooled data.

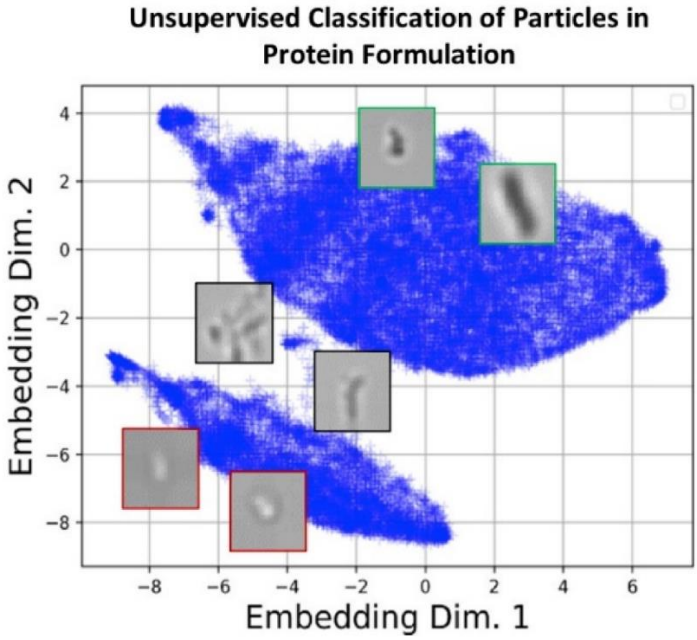

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Figure 2. Unsupervised image classification. Representation of particulates under stressed condition showing clustering of 3 groups. Green, Black and Red framed images represent particles from the three identified clusters.

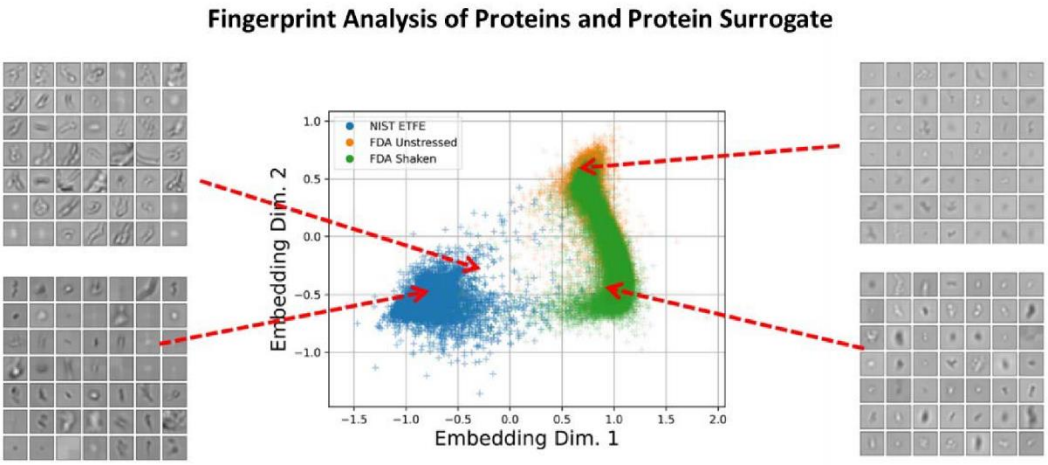

Figure 3. Fingerprint analysis of proteins (stressed and unstressed protein formulation) and protein surrogate (Ethylene tetrafluoroethylene [ETFE standard]) are shown here (Ref.3).

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[5] **Disclaimer:** This article reflects the views of the author and should not be construed to represent FDA's views or policies.

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