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Variable Threshold Method for Determining the Boundaries of Imaged Subvisible Particles

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ABSTRACT

An accurate assessment of particle characteristics and concentrations in pharmaceutical products by flow imaging requires accurate particle sizing and morphological analysis. Analysis of images begins with the definition of particle boundaries. Commonly a single threshold defines the level for a pixel in the image to be included in the detection of particles, but depending on the threshold level, this results in either missing translucent particles or oversizing of less transparent particles due to the halos and gradients in intensity near the particle boundaries. We have developed an imaging analysis algorithm that sets the threshold for a particle based on the maximum gray value of the particle. We show that this results in tighter boundaries for particles with high contrast, while conserving the number of highly translucent particles detected. The method is implemented as a plugin for FIJI, an open-source image analysis software. The method is tested for calibration beads in water and glycerol/water solutions, a suspension of microfabricated rods, and stir-stressed aggregates made from IgG. The result is that appropriate thresholds are automatically set for solutions with a range of particle properties, and that improved boundaries will allow for more accurate sizing results and potentially improved particle classification studies.

Keywords: Flow imaging, Image analysis, Microscopy, Particle Sizing, Physical Characterization, Protein aggregates,

INTRODUCTION

The flow imaging method of particle size analysis has proven increasingly popular for the analysis of biotherapeutics [1]. Compared to other methods, flow imaging is less sensitive to particle refractive index than light obscuration [2 3 4], and image parameters reported by flow imaging enable classification of particles by morphology and brightness. Commercial flow imaging instruments have an optical resolution of approximately 1 µm to 4 µm, yet these instruments are routinely used to size particles down to 2 µm effective diameter. Properly interpreting the particle boundary is key to obtaining accurate particle size and morphology, especially for particles below ≈ 5 µm effective diameter.

The flow imaging method of particle size analysis relies on the definition of the particle boundary by image processing software. In commercial instruments, it is common to