

Objective

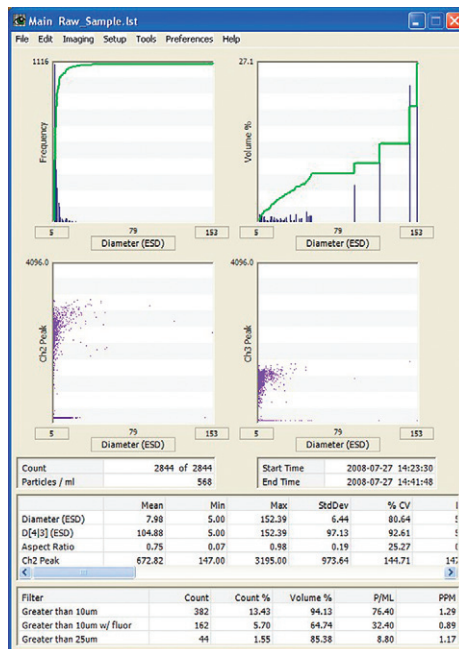
Aggregation of proteins in parenteral drugs is a serious issue, and must be closely monitored to meet FDA regulations. Of particular importance is consistent reporting of particles larger than 10µm and particles larger than 25µm found in the formulation. These particles can be both protein aggregates and “other” particles, such as silicone oil droplets or air bubbles.

While microscopy can be used for this type of analysis, the concentration of these particles is typically so low that it is time prohibitive to analyze using a microscope. As a result, formulators and QC personnel have typically turned to automated particle sizing methods such as light obscuration, electrozone sensing and laser diffraction. The problem with these techniques is that they use measurement techniques that assume all particles are spherical in shape, recording only an Equivalent Spherical Diameter (ESD) for each particle. This can lead to costly miscounts when “other, allowable” particles such as silicone droplets get counted. Inevitably, this could lead to the premature disposal of a batch due to it being “out of spec”. Light obscuration has the further issue of not being able to detect transparent particles.

Method

The FlowCAM® is ideally suited to this application, because it stores a digital image of each particle along with up to 26 different measurements. This means that particles can be characterized based upon size *and* shape, thus enabling automatic differentiation between protein aggregates and silicone droplets, for instance. Additionally, since all particles are captured as images and saved, the results can be examined visually to insure accuracy.

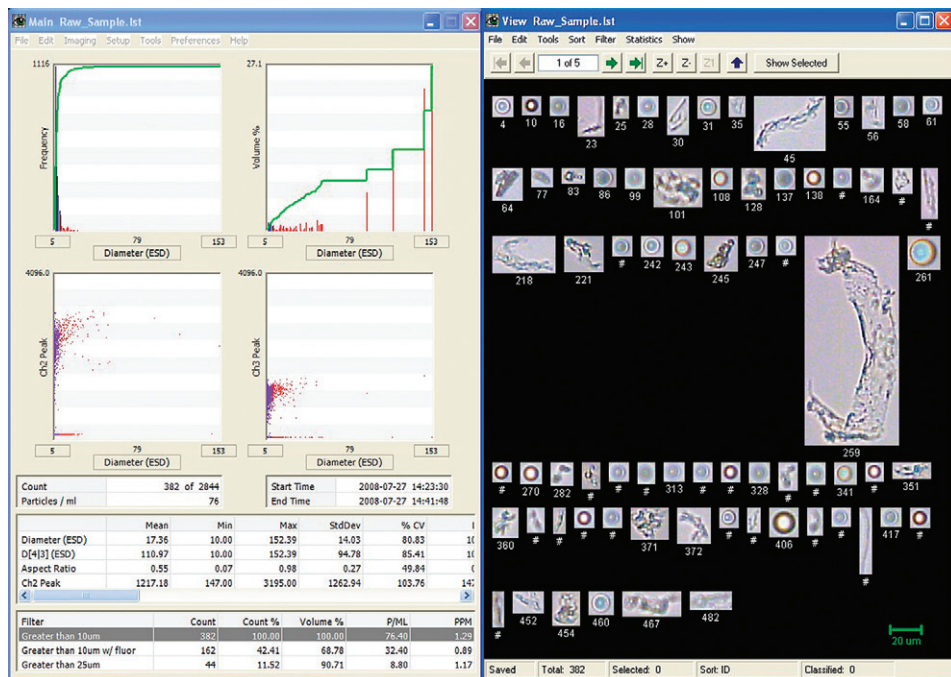
Detection of Protein Aggregates in Parenteral Drug Formulations



The screenshot above shows the results of a FlowCAM run (AutoImaging mode) with a parenteral formulation sample. Summary statistics show that 2,844 particles total were found in the sample. Additional statistics such as mean ESD are also calculated.

In the lower portion of the window, one can see results of some basic particle filters defined in the VisualSpreadsheet® software, specifically for particles greater than 10µm and particles greater than 25µm in ESD. The actual particles associated with each of these filter results can be displayed simply by clicking on the results row as shown below. In this case we are displaying all particles greater than 10µm in ESD. The particle images are displayed in multiple “pages” (collage image files), with the image below showing “page 1 of 5”. The user can navigate through these pages by using the arrow buttons.

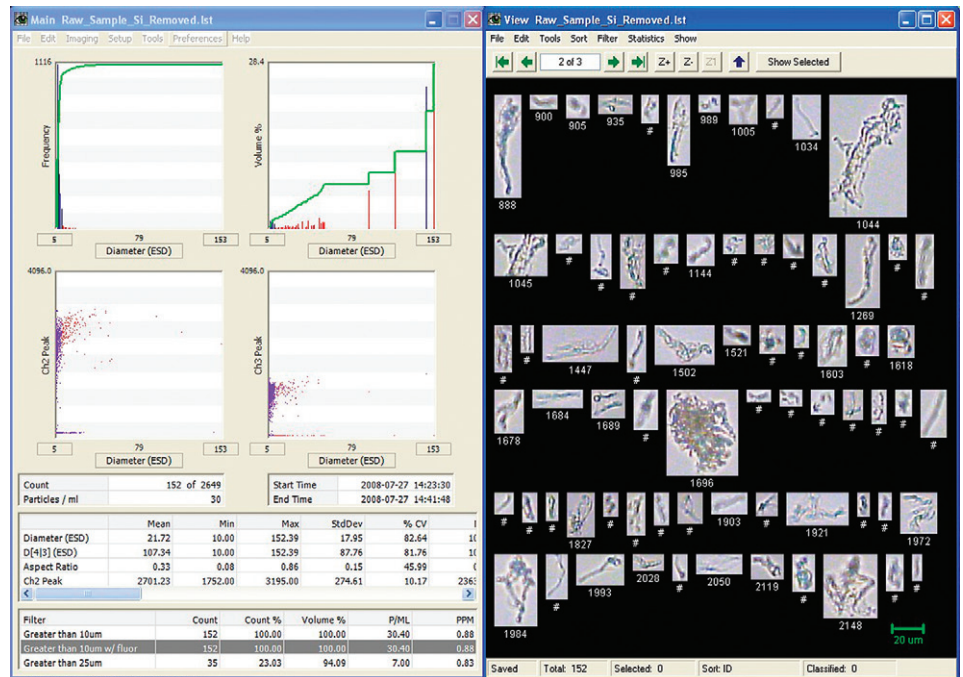
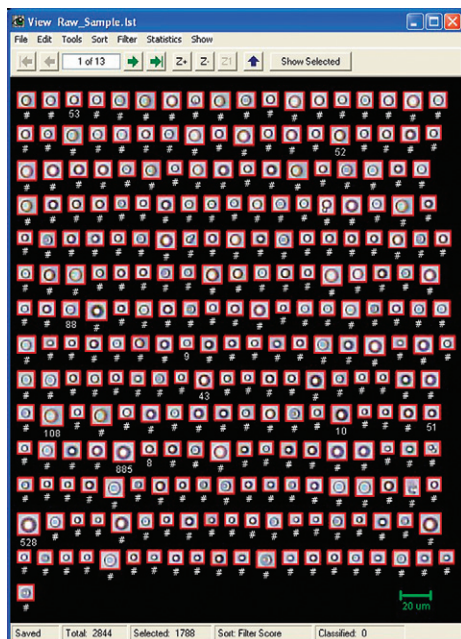
In this particular sample, we can see that many of the particles >10µm in ESD are in fact silicone droplets which actually are “allowable” (they are introduced during the filling process, but are not harmful). So, it would be useful to eliminate the silicone droplets to get a realistic accounting of the number of particles >10µm.



Fortunately, the VisualSpreadsheet software makes the process of removing the silicone droplets very straightforward. By selecting some examples of the particles one wishes to find, statistical pattern recognition can be used to find all of those particles (in this case, the silicone droplets) as shown below.



As can be seen, five silicone droplets were selected. Once the statistical filter is invoked, the resulting matching images are displayed as shown below.



Out of the 2,844 particles originally found, 1,788 are found to be silicone droplets. Those particles can then be deleted from the run and the data saved as a new file. When this file is opened, we can see that the statistics for each of the original filters change based upon the removal of the silicone droplets as seen in the screen shot above.

The summary statistics indicate that of the original 382 particles detected which were >10µm in ESD, 195 of these were silicone droplets, leaving 187 that are actual protein aggregates. This represents a significant change in the number of particles found that are greater than 10µm, and could be the difference between whether a batch is accepted or rejected in QC.

The VisualSpreadsheet software can combine the two operations performed in this example (filtering out the silicone droplets and reporting the filter results for particles >10µm and >25µm) into a single step, thus allowing the operator merely to introduce the sample to the FlowCAM and invoking the run.

Summary Results:

Data Set	# >10µm	# >25µm
“Raw”	382	44
Si Removed	187	41

In addition to the technique described above, the FlowCAM and VisualSpreadsheet also have two unique capabilities which can be used in this application. If desired, proteins can also be stained with a fluorescence dye, and the FlowCAM can be set to “trigger” (take images) only when a fluorescing particle passes through the flow cell (the fluorescence signal can also be recorded for each particle).

Finally, the FlowCAM can also be used in “scatter trigger” mode, whereby the camera is only triggered when a particle larger than a certain size (in this case, 10µm) passes through the flow cell.

FlowCAM represents a powerful new tool for the detection and counting of protein aggregates in parenteral drug formulations.

