FLUID IMAGING TECHNOLOGIES

Particle Analysis with Vision®

FlowCAM® Tech Brief #1

Count Versus Concentration

Summary: The term "particle count" is often misused in the industry when the proper term is really concentration. This Tech Brief will explain what the terms "count" and "concentration" actually mean in a dynamic imaging particle analysis system such as FlowCAM®.

Definitions:

Count: Enumeration of the exact number of particles or cells contained in a given volume analyzed.

Concentration: A *calculation* of count per unit volume based on a *sample* of the given volume analyzed.

Volume Efficiency: The percent of the entire volume actually sampled to calculate the concentration.

Based on these definitions, we can conclude that:

if Volume Efficiency = 100%
then Concentration = Count !

Particle Counters: True particle counters, based on techniques such as the Coulter principle, light obscuration, etc., count particles one at a time by passing them through an orifice or by using other techniques such as sheath fluid to ensure that only one particle at a time passes by the detector. Some other particle analysis systems actually recirculate the sample through the detector, and therefore can only be used to determine particle size distribution, *not* count.

Imaging Particle Analysis: In a dynamic imaging particle analysis system such as FlowCAM, particles are imaged in a 2-dimensional space as they move past the camera's field of view (FOV) as shown in Figure 1.

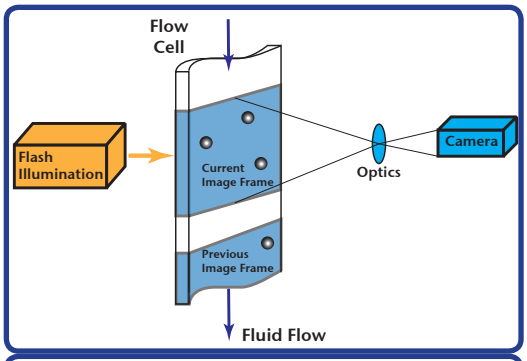

Figure 1: Particles captured in FlowCAM.

Issues Common to Imaging Particle Analyzers: Figure 1 shows how the FlowCAM captures particle images as they pass through the flow cell. However, in order to do true counting of particles, the flow rate of the liquid through the flow cell and the frame rate at which the camera captures those particles would both need to be perfectly optimized for 100% Volume Efficiency. If the flow rate and frame rate are not perfectly matched, then the possibility exists for overcounting due to multi-imaging of individual particles or undercounting due to missing particles in-between frames, as shown in Figure 2.

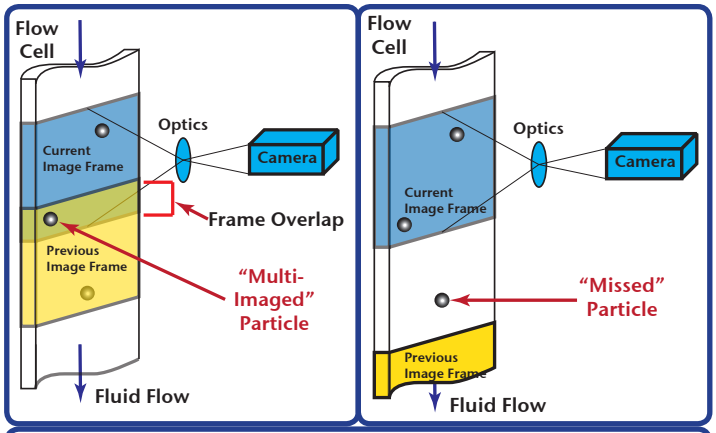

Figure 2: The effect of non-matched flow rate and frame rate: On the left, the system is oversampling, and can count a single particle multiple times. On the right, the system is undersampling, and can miss particles. Either issue produces an inaccurate count.

In either of these two cases (oversampling or undersampling), the count would be inaccurate. Additionally, in a very sparse sample, the calculated concentration might be slightly off as well, because over or undercounting of even one particle may be enough to skew the concentration calculated. As mentioned before, in any system that recirculates the sample, neither count nor concentration can be reported due to the higher probability of multi-imaging particles over time.

Fortunately, the FlowCAM offers powerful and easy-to-use tools for controlling volume efficiency. By using a computer-controlled, high precision syringe pump, FlowCAM's VisualSpreadsheet® software can precisely control both the flow rate through the flow cell *and* the frame rate of the camera.

Another issue that needs to be kept in mind when discussing volume efficiency is the camera's Field of View (FOV) as it covers the flow cell width. Most imaging particle analyzers use a flow cell that is *wider* than the camera FOV. This means that particles passing outside the FOV will not be captured or counted, also leading to incorrect count and/or possibly concentration reported. To overcome this, in applications where an *absolute count* or concentration is important, the FlowCAM® can be optionally equipped with a specially made Field of View flow cell. Figure 3 below shows the difference.

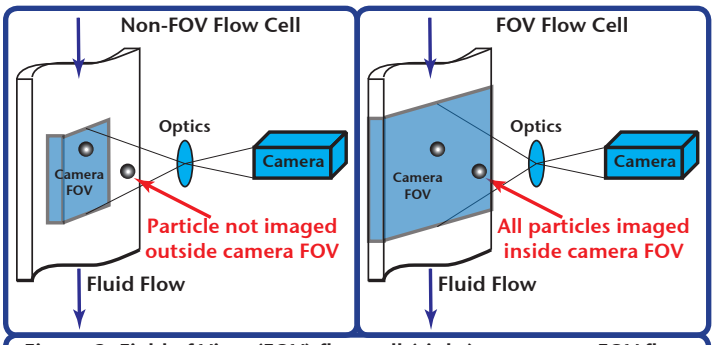

Figure 3: Field of View (FOV) flow cell (right) versus non-FOV flow cell (left). The non-FOV flow cell will miss particles *even if* the flow rate and camera rate have been optimized.

<u>How FlowCAM can be Optimized for Proper Count & Concentration Calculations:</u> The FlowCAM FOV flow cell and VisualSpreadsheet® software combine to enable accurate, repeatable count and concentration results, using an easy-to-use interface. Figures 4A and 4B show screen shots of the software interface for inputting the sample volume, and then controlling the flow rate and frame rate. Once those three values are entered into the "Settings" portion of the interface, VisualSpreadsheet *automatically* calculates the volume efficiency and run time for the given volume. All functions of the high precision syringe pump are controlled by the computer.

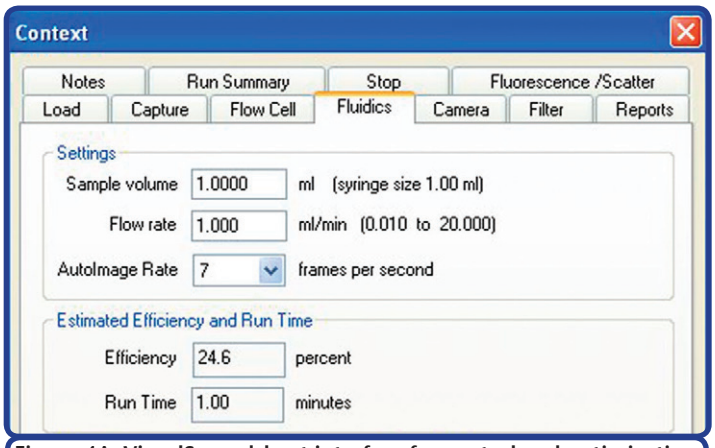

Figure 4A: VisualSpreadsheet interface for control and optimization of flow rate and camera rate. In this instance, a 1ml sample would be analyzed using a 1 ml/min flow rate and 7 fps frame rate, yielding an Efficiency of 24.6% and a run time of 1 minute. The Volume Efficiency and Run Time are automatically calculated for the chosen settings by the software.

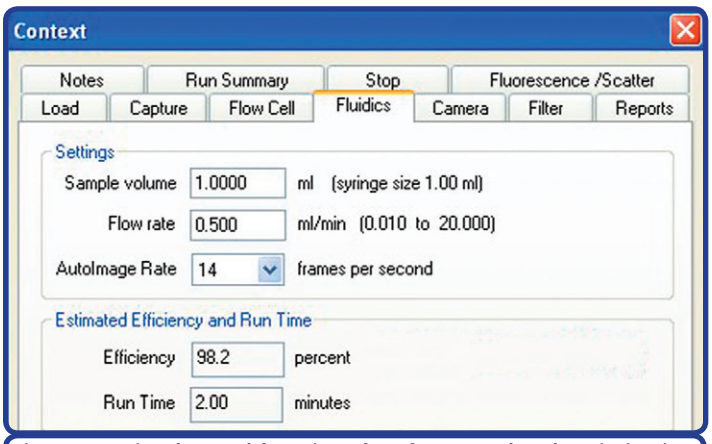

Figure 4B: VisualSpreadsheet interface for control and optimization of flow rate and camera rate. In this instance, a 1ml sample would be analyzed using a 0.5 ml/min flow rate and 14 fps frame rate, yielding an Efficiency of 98.2% and a run time of 2 minutes. The Volume Efficiency and Run Time are automatically calculated for the chosen settings by the software.

In Figure 4A, you can see a flow rate of 1.000 ml/min was chosen with a frame rate of 7 fps, yielding an efficiency of 24.6% and a run time of 1 minute. In Figure 4B, the flow rate has been slowed down to 0.500 ml/min and the frame rate increased to 14 fps, yielding an efficiency of 98.2% and a run time of 2.00 minutes for the same sample volume.

If simply calculating concentration on a fairly dense sample, then one does not need to use an FOV flow cell, and volume efficiency is not important. In this case, concentration is calculated as:

$$P/mL = \frac{\text{Particle Count}}{\text{Total Volume Imaged}}$$

However, when the sample is sufficiently sparse (<1,000 particles/ml) or an exact *count* is required, then the FOV flow cell should be used, and volume efficiency should be optimized to near 100%. The beauty of the FlowCAM hardware and VisualSpreadsheet combination is that all the calculations necessary to achieve this high volume efficiency are automatically done via the simple interface shown in Figures 4A and 4B.

By understanding these issues, and making it easy to optimize the system to take them into account, the FlowCAM ensures that the results you obtain are exactly what you expected them to be, whether it be true count or concentration.

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