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Rapid particle characterisation using in-flow digital imaging

by L. Brown

Many scientific endeavours involve the use of particulate matter suspended within a liquid. In very few cases are the particles under study large enough to be quantified and analysed by the naked eye. As microscopy has

advanced over the years, with the introduction of instruments such as the Scanning Electron Microscope (SEM), increasingly smaller particles can be studied - even down to the molecular level. This article will look at the issues associated with fluid particle analysis, discuss some of the historical methods used, and introduce a new technology, the continuous imaging fluid particle analyser, which offers an automated method for particle analysis.

Historical methods used in particle analysis

The first use of the microscope to observe and record microscopic life in the 1600s greatly changed the ability of scientists to study objects and phenomena occurring at a level below the limits of the unaided eye. This is certainly true in particle analysis, and the microscope remains even today the most common instrument used for this activity. Microscopy has proven itself to be an invaluable tool for characterising particles. Particle size and shape can greatly affect the performance characteristics of individual components of a mixture, as well as of the final product. The major drawback of standard microscopes for particle analysis is the time required, both for sample preparation, and for counting and measuring properties of the parti-

cles. Additionally, since this time is required for sample preparation and analysis, the microscope can only be used to observe one static sample at a time. While this is fine for basic research in an early discovery phase, it is unacceptable once it is necessary to look at a process in detail. First of all, the second phase of verifying a cause-and-effect relationship found in the discovery phase requires a statistically significant quantity of the particles to be analysed. This process is too time consuming for manual methods, and requires that some level of automation be brought to the measurements. Secondly, since this is an analysis of a process, sampling needs to be accomplished over a time period of the process. Indeed, many of these processes require continuous analysis over a period of time,

an extreme example being the continuous monitoring of particles for purposes of quality control. While microscopy has many benefits for this, it has one important drawback: only a small amount of a given component can be examined at any one time.

As result, several technologies have been developed for automating the rapid measurement of large numbers of particles. One of the earliest technologies developed (and still very commonly used) involves rapidly determining a particle's size by measuring its electrical sensing potential. Most commonly known as the Coulter principle, in this type of system particles suspended in a weak electrolyte are passed through a narrow channel, which has an electric current flowing through it. The particles passing through this channel produce an impedance pulse that is directly proportional to the particle's volume. Assuming a spherical shape, one can quickly derive an Equivalent Spherical Diameter (ESD), which serves as a single number measurement to characterise the particle. These systems can process tens of thousands of particles per minute, which allows rapid characterisation of a sample's properties based upon a distribution of ESDs.

Other technologies that are used in particle analysis systems include light (or laser) obscuration and laser diffraction. All of these methods have the benefit of being able to very rapidly analyse large numbers of particles in a very short period of time. In a conventional microscope, it may take several minutes to count and size less than a hundred particles, while these systems can do the same counting and measuring of tens of thousands of particles in under a minute. From a statistical point of view, having measurements on huge numbers of particles in a sample increases the significance, confidence and repeatability of those measurements dramatically. Large amounts of data are quickly collected in a tabular form, with the results usually presented as a histogram of frequency versus particle diameter (ESD) as shown in Figure 1.

While this information can be extremely helpful, it has one major drawback: all reported measurements assume that each particle is spherical in shape. Additionally, since this assumption is made, the only way of differentiating particle types in a heterogeneous mixture is if their ESD differs significantly enough for them to show as separate peaks on the histogram. Therefore, unless the different particles

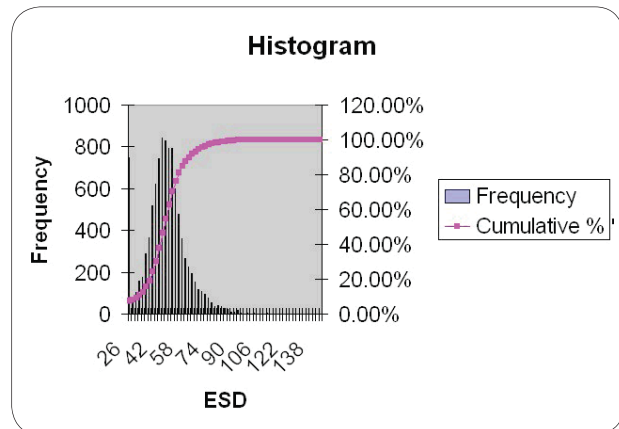


Figure 1. Typical particle size distribution showing frequency and cumulative frequency.

are separated prior to analysis, any histogram represents an aggregate distribution containing many different particle types. It is well documented that particle shape also has an important influence on product performance as well. Particle shape is easily observed using microscopy, but unfortunately, as stated previously, it is extremely time consuming to characterise a statistically significant sample of particles for shape using microscopy.

Continuous imaging particle analysis

A new technology has been developed that combines the benefits of a particle counter and microscopy. This system, called the FlowCAM, consists of a microscope outfitted with a high-resolution digital camera that continuously images particles as they pass through a flow cell in a liquid. A simplified architectural view of the system is shown in Figure 2.

The simplest way to describe how the instrument operates is to show it in operation, as seen in Figure 3. Each particle is isolated from the overall view of the flow cell in real time by the software and stored as a separate particle image. As each particle image is stored, over 23 different particle characteristics can be calculated and saved in a spreadsheet fashion, with each row of the spreadsheet indexed to the individual particle image with which the measurements are associated. This indexing enables the software to present the results of typical spreadsheet operations such as sorting and filtering not only as tabular data, but also simultaneously in visual fashion by displaying the particle images associated with the sorting and filtering results (hence the software's name,

Particle characterisation

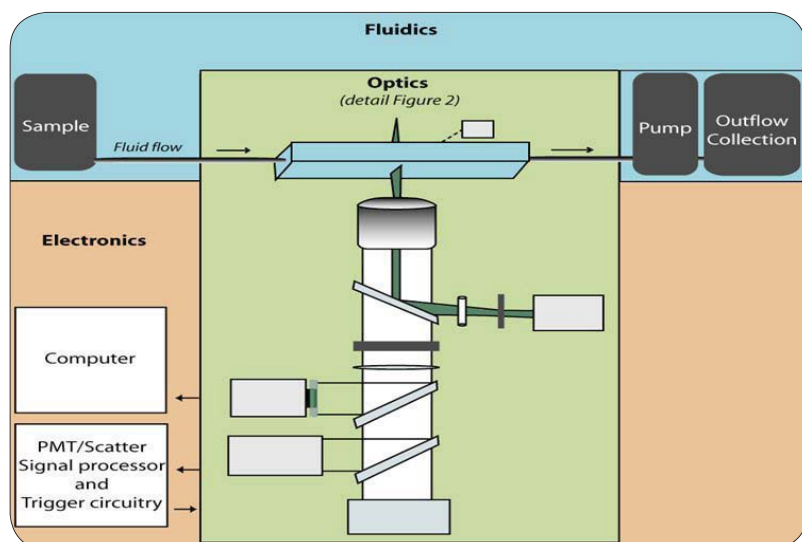


Figure 2. FlowCAM architecture. The sample is pulled through the flow cell by a peristaltic pump, where it is imaged in real time by a high-resolution digital camera.

VisualSpreadsheet).

Since the measurements are made directly from the image, simple measurements such as ESD, length, width, area and volume can be made, along with more complex measurements such as transparency, perimeter, elongation and roughness. These more complex measurements help to describe the particle in higher-level visual terms similar to those that would be used by the eye/brain to distinguish different particle types from each other. The more particle parameters measured, the easier it becomes to algorithmically differentiate particle types using pattern recognition software.

Example of continuous imaging particle analysis

An experiment was performed in order to demonstrate a typical application of this technology. This experiment was simple in nature, the intent being to give the audience a feel for how the technology works, thereby facilitating further discussion on other potential areas for use. Three commercially available chocolate bars were purchased from a grocery store. The first sample is a mass-produced, readily available brand commonly consumed in the US: we will call this sample Common. The second and third samples (both from the same manufacturer, although not the same

as the common sample) are also readily available, but would be considered premium brands. Sample two is very fine milk chocolate, which we will call Premium Milk. Sample three is fine dark chocolate, and will be referred to as Premium Dark.

For each sample processed, four sample runs were made so that repeatability could be shown. All four runs of each sample yielded consistent results, indicating very good repeatability. At the completion of each run, the instrument's software displays summary statistics and distributions (up to 4 distributions can be displayed from a choice of 15 pre-programmed ones). Images can be displayed by selecting them from any of the distributions. A sample

screen shot follows showing a typical result [Figure 4]. Table 1 gives a summary of the overall results found for each sample.

Discussion

Some fairly quick conclusions can be made from the statistical results shown in Table 1.

- 1) The Premium Dark sample contains smaller particles compared to the other two samples.
- 2) The Common sample contains slightly more spherical (higher aspect ratio) and less transparent particles than the Premium Milk sample.

The conclusions that can be made from the particle size measurements (ESD and ESD[4,3]) could be made using any of the other types of particle analysis systems mentioned previously, as all of these systems calculate ESD. Statistically, however, this technique differs from those other techniques in that it can also measure shape-based parameters such as aspect ratio and grey scale-based parameters such as transparency.

Sample	Particles Total	Mean ESD (frequency-weighted)	Mean ESD [4,3] (volume-weighted)	Aspect ratio	Transparency
"Common Milk"	50,283	8.14 μ	31.00 μ	0.71	0.18
"Premium Milk"	40,272	8.74 μ	32.41 μ	0.67	0.23
"Premium Dark"	50,467	7.50 μ	13.22 μ	0.69	0.20

Table 1. Statistical summary results for each sample.

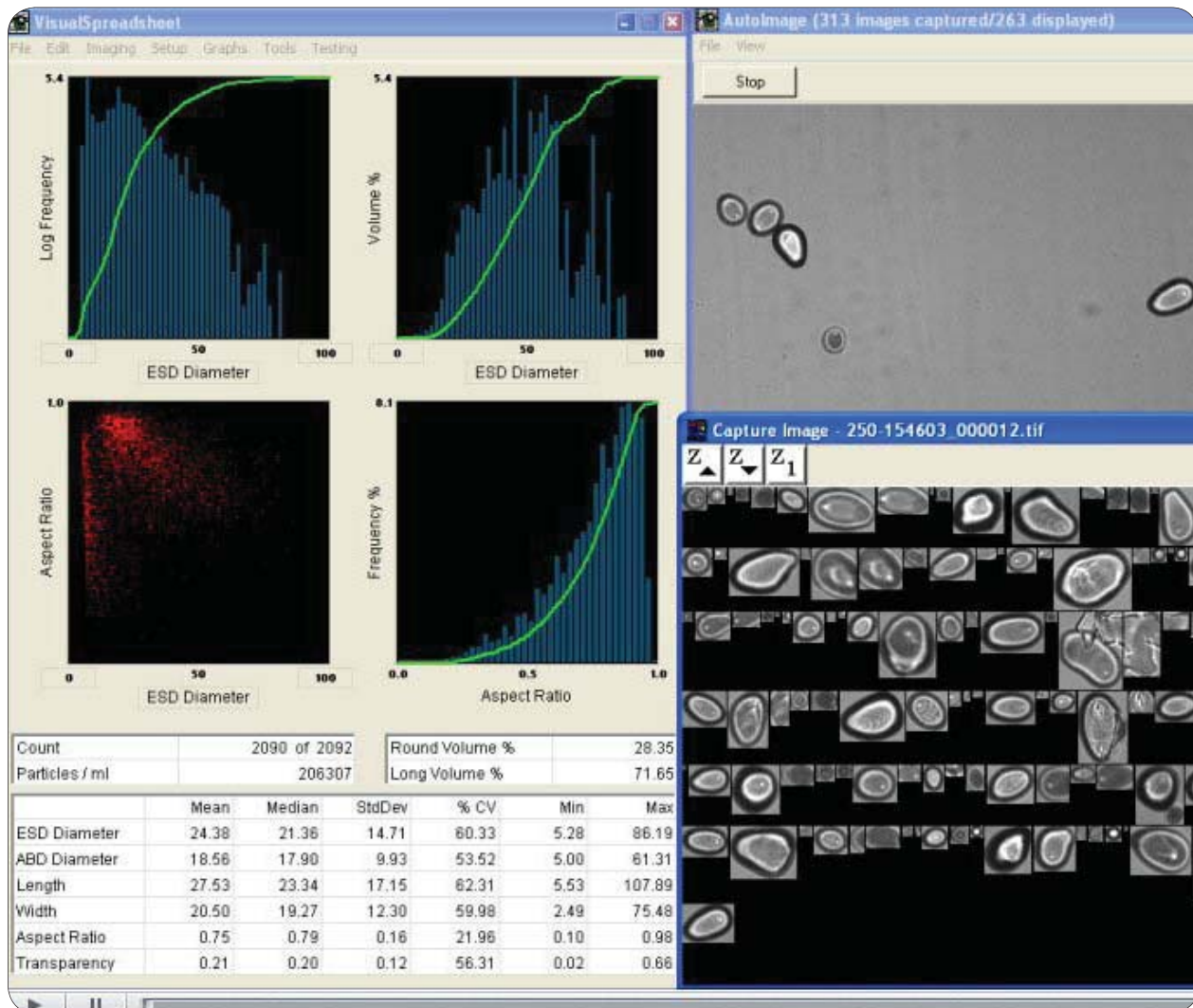


Figure 3. Instrument screen during particle capture. Upper right window shows live camera view of particles passing through flow cell. Lower right window shows particle images as they are captured and stored in real time from the live video frame. Left-hand window shows particle statistics and distributions, which are updated in real time as the particle data are acquired and stored.

While other particle analysis techniques can present data as statistics and distributions, this technique differs substantially in that it also records an image of each particle measured. These images are all stored for later visual and mathematical analysis, giving the scientist the ability to see what was measured. The VisualSpreadsheet software included with this instrument allows the user to interactively display particle images from a region of interest defined from any of the distributions, and to do common spreadsheet operations such as sorting and filtering while presenting the results visually both as graphs and statistics, but also in the form of the particle images themselves.

It was noted while viewing particle images captured for this experiment that many of the particles were crystalline in nature (sugars). One of the most powerful features of an imaging-based particle analysis system is the ability to duplicate and quantify the subjective visual analysis using pattern recognition software. While the individual samples can be reviewed visually by the naked eye to look for similarities/differences in the type of particles found in each sample, this can be time consuming and produces only qualitative results. By using pattern-recognition software, differences in particle composition between samples can be automated and quantified.

Particle characterisation

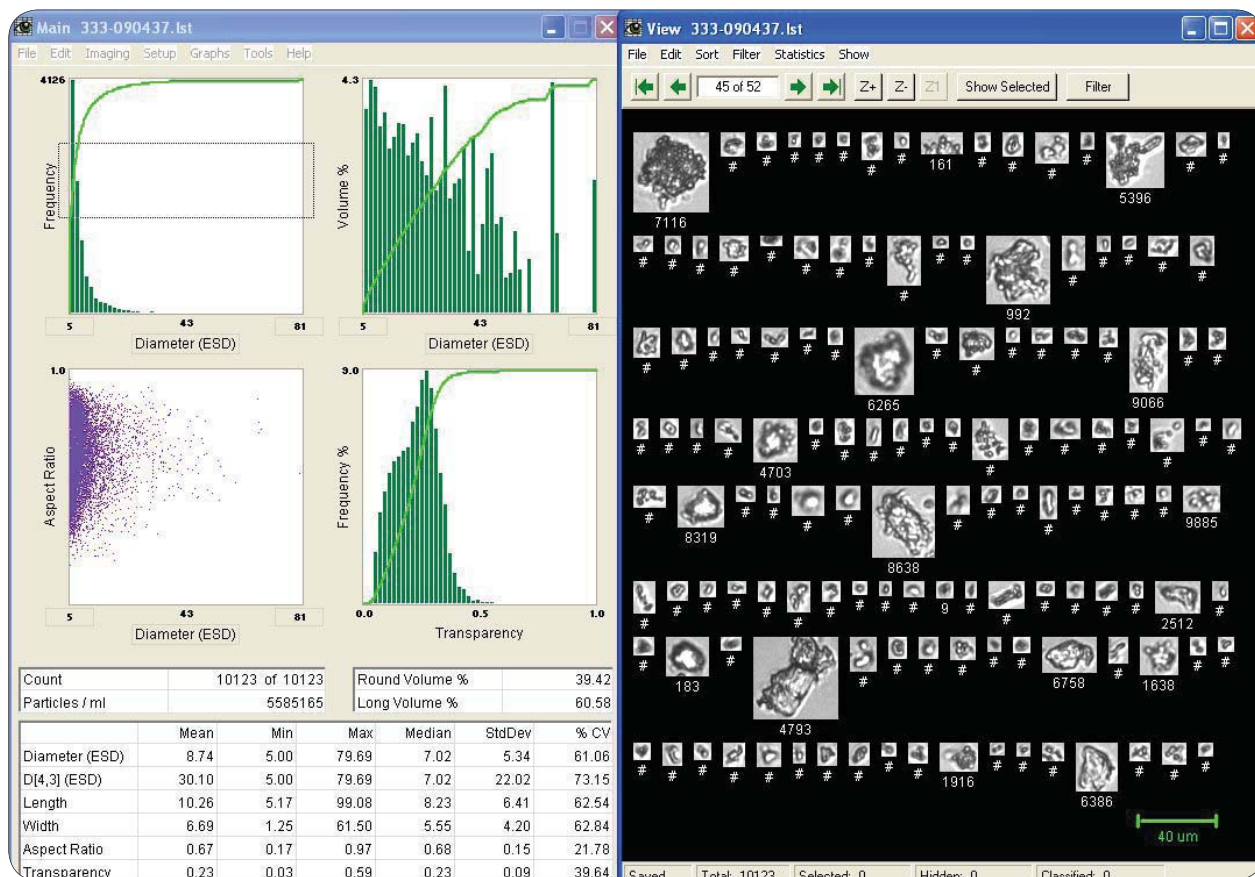


Figure 4. Overall results for run #4 with Premium Milk sample. 10,123 particles were imaged and measured. The particles have a frequency-weighted mean Equivalent Spherical Diameter (ESD) of 8.74µ, a volume-weighted ESD (D[4,3]) of 30.10µ, and a mean Aspect Ratio (width/length) of 0.67. The right-hand window shows some of the particle images.

The first step in the pattern-recognition process is to build a library of particle images that contains typical target particles (the type of particle the software is being asked to find). This is done interactively merely by clicking on particle images that are of the desired type and storing them as a library. To demonstrate how this works with these samples, a library of crystalline particles was built.

Once built, each sample run can be analysed to look for similar particles using the pattern recognition software in VisualSpreadsheet. To look for differences in particle composition within the three

different chocolate samples, each sample was run through the pattern-recognition process using the crystals library. A summary of the results for this analysis is shown in Table 2.

These results agreed exactly with the subjective observation that

had already been made upon visual examination of the particle images: the Premium Dark sample contains very few of these crystalline particles (sugars) compared to the other two samples. This certainly complies with the general qualitative observation that dark chocolates tend to be more

Sample	Particles Total	Total “matched” to library	% Matched
“Common Milk”	50,283	1,041	2.1%
“Premium Milk”	40,272	1,410	3.5%
“Premium Dark”	50,467	372	0.7%

Table 2. Summary results for pattern recognition against “crystals” library.

bitter (less sweet) in taste than milk chocolates. In addition, the Premium Milk sample contained almost two times more crystalline particles than the Common sample. In an unscientific, informal poll conducted among colleagues, all of them described the Premium Milk sample as sweeter than the Common Milk sample. This is, at best, a fairly rudimentary experiment and analysis designed to show how this technique works and how it might be used in the confectionary industry. It is hoped that this brief explanation will stimulate further discussion on how this technique might be further applied.

Uses: R&D, formulation, potential for in-process use

The brief example discussed above shows how powerful pattern-recognition software can be used to quantitatively examine data collected by this instrument in order to determine relative content of specific types of particles in a heterogeneous sample. This example shows a very simple application; the instrument measures over 23 different particle parameters, so filtering and pattern analysis can be applied to isolate many specific different particle types. Multiple libraries can be built and saved so that all incoming data can be compared against those libraries.

This experiment was obviously conducted under laboratory conditions. The majority of the time spent was involved with sample preparation (melting and mixing the chocolate samples). As such, it represents the use of the instrument that would most often be typical of an R&D or formulation environment. In the same configuration, it could also be used for periodic QC during manufacturing; however, this would involve taking a sample and then diluting it prior to analysis.

In order for this technique to be used in process, a system would have to be developed that automatically draws a sample from the process and prepares it for the instrument in a similar fashion as was used here. A prototype system has been built that does exactly this. A computer-controlled system of pumps, valves and mixing components was constructed that draws the sample from the process, mixes it properly for introduction to the instrument, runs it through the instrument, and finally cleans the system out in preparation for the next sample. The entire operation is controlled by software, and can even be set up to continuously monitor the process and cause an alarm to be sounded when certain par-

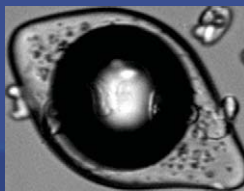
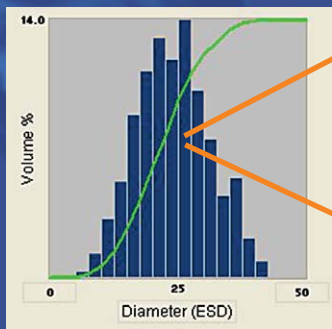
ticle property variables exceed preset limits.

Continuous digital imaging shows great promise as a tool for R&D, formulation and QC. This technique combines, in a single instrument, the speed and statistical benefits of particle counters with the ability to differentiate between different particle types, which is a huge benefit of microscopy.

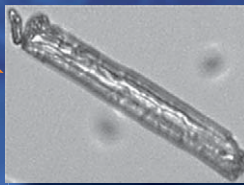
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What's Under the Curve?



or



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