Dynamic Digital Image Analysis; Emerging Technology for Particle Characterization

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Abstract

The feasibility of applying dynamic imaging analysis technology to particle characterization has been evaluated for application in the water sector. A system has been developed which captures in-situ images of suspended particles in a flowing sample stream and analyzes these images in real time to determine particle size and concentration. The technology can measure samples having a wide range of particle sizes (~1.5 to 1000µm equivalent circular diameter) and concentrations (zero to >1million/ml). The system also provides magnified images of particles for visual analysis of properties such as size, shape and grayscale level. There are no sample preparation requirements and statistically accurate results are produced in less than three minutes per sample.

The major design challenges in developing a practical system include obtaining adequate contrast for the range of particle materials found in typical water samples and achieving this under operating conditions permitting an adequate sample processing rate for real time feedback of results. The overall system architecture is described. Performance of the instrument is reported in reference to industry accepted particle standards and applications as an analytical tool for the water and wastewater industries are considered.

Keywords

Characterization, dynamic, imaging, optical, particles, water quality

INTRODUCTION

Particle analysis is a primary means used to characterize water supplies, optimize treatment processes, and to contribute to the overall scientific understanding of water quality. Despite the importance of solids characterization in water purification, the range of instrument technologies available to assist in this task has been limited.

For precise particle characterization, microscopic analysis is an accurate, direct technique and is insensitive to particle optical properties. With the introduction of digital cameras and sophisticated image processing tools, microscopy also offers image analysis capabilities. For the water industry the drawbacks to this technique include the time required for sample preparation and analysis as well as the requirement for a high degree of operator expertise. Particle analysis technologies in use in other industries (e.g. healthcare, pharmaceuticals, chemical processing, etc.) have potential value for water quality researchers. However the high cost and application-specific designs of these instruments have prevented widespread adoption in the water quality field.

This paper will discuss the development of a first generation instrument applying dynamic particle analysis (DPA) technology to particle characterization for water quality applications. The technique combines the advantages of direct measurement (as in traditional microscopy) with the rapid analysis typical of other optical techniques such as light scattering and obscuration. Specifically, the objective of the development is to produce a technology to achieve the following:
Simultaneous counting and sizing of particles broadly distributed in size (from 1.5 to 1000µm) and concentration (from 1 to 1x10^6 particles/ml)
Size measurement accuracy (±5%) independent of particle optical properties
Rapid, real-time, statistically accurate analysis (less than 3min/ml)
Selective image acquisition capabilities (based on particle size)
Acceptable cost/performance combination to allow general application in the sector

METHODS
Experimental Setup
The principle of operation of the Dynamic Particle Analysis (DPA) system is shown below in Figure 1. A sample fluid containing suspended particles is drawn via a gentle vacuum through a flow cell capillary. An optical light source incorporating a high radiance blue LED illuminates the flowing sample. The resultant signal is projected by the optical magnification system onto the photo-detector array. The signal from each pixel is read and processed by the system PC to produce and analyze the final image.

All particulate matter within the flowing sample blocks or scatters the light emitted from the LED relative to the surrounding fluid, creating an intensity variation on the corresponding pixel array. An ‘intensity threshold’ is used to determine whether or not the pixel lies wholly or partially within a particle image. From the number of pixels within each image and the known magnification, the projected 2-D area is calculated and converted to an equivalent circular diameter.

The optical system’s field of view (FOV) and the physical geometry of the capillary define the optical sampling volume and permit the particle concentration to be determined.

A software interface allows the user to count particles with an equivalent circular diameter ranging from 1.5 to 1000µm at customized (and variable) step sizes or ‘buckets’. The user interface also allows the instrument to be configured to capture selected images (black and white or 10-bit grayscale).

![Figure 1 – Dynamic Particle Analysis System Overview](image-url)
Technical Challenges

There are two primary design considerations to apply dynamic imaging techniques to water quality analysis. The first is the ability to form images with sufficient contrast for the broad range of particle material types found in water. Second is the ability to form these images for an adequate volume of water (and therefore number of particles) to achieve statistically accurate results in an acceptable period of time. These issues are discussed in more detail below.

**Material Resolution.** The ability of the system to detect and measure any particle depends on its ability to form an image with adequate contrast. Adequate contrast allows pixels to be identified when their signal is reduced because they lie, wholly or partially, within the particle image. This in turn depends on the image contrast provided by the illumination and optical magnification system, on the sensitivity and noise performance of the detector array and on the threshold used to differentiate pixels. The results described in this paper were obtained using direct bright-field illumination without using additional optical contrast enhancement techniques. The detector was a standard 9.66 x 7.74 mm array of 1032 x 1288 pixels. Using 10 bit A/D conversion, threshold levels for image detection could normally be set at 96.4% relative to the background illumination. In order to minimize the effects of static intensity irregularities resulting from the light source, capillary or magnification system, the system software recorded the bright-field intensity of each pixel with particle-free water flowing in the capillary. These static values were compared to those obtained with the particles present.

**Processing Volume:** The volume of water processed in each frame depends on the field-of-view (FOV) determined by the magnification system and sample depth defined by the capillary geometry. While lower magnification will increase the FOV, a limit is set by the requirement that the image of the smallest particle must occupy a minimum number of pixels in order to accurately determine its area. The maximum depth of sample flow is determined by a number of attributes of the magnification and subsequent image processing systems.

**Depth-of-Field (DOF) Effect:** In conventional microscopy, the particles are placed onto filter paper and manually positioned at the focal plane of the microscope. Since the particles are in-focus, each particle image corresponds exactly, other than magnification, with its original particle. In a typical microscope used at high magnification, the depth over which particles remain in focus is small (typically 6µm for 20x magnification). In the DPA system, particles are not located in a plane but are distributed within a finite depth defined by the sample cell design. Images of particles located above or below the position of best focus experience out-of-focus effects such as enlargement and loss of contrast.

The depth-of-focus of a magnification system can be increased and the DOF enlargement reduced by reducing its aperture using diaphragms. An undesirable side effect of aperture reduction is the introduction of other forms of distortion resulting in enlargement and loss of contrast of a particle image even when the particle is located at the position of best focus. In turn, these effects may be minimized by operating the magnification system with a larger aperture and by restricting the particles to a very small depth close to the position of best focus. However, such a restriction will result in a reduction in optical sampling volume and thereby increase the required measurement time for statistically accurate analysis.

Because the system processes the signal from each pixel in order to derive information from the particle image, it is possible to apply compensation factors as each frame is captured. To the extent that these image distortion effects are consistent and independent of particle properties such as material and structure, this approach allows the system to be operated with a substantial increase in optical sampling volume.
The DOF enlargement effect is illustrated in Figure 2 below. In order to statistically compensate for this enlargement, the maximum enlargement as a function of location within the DOF (defined physically by the sample cell) was measured. Considering the particles are randomly distributed with respect to their displacement from the focal plane, a statistical model was then developed which compensates for the DOF effect. Using the model it was determined that accurate and consistent results could be obtained even when the DOF enlargement was relatively large (e.g. 42% for a 10µm particle). Application of this compensation algorithm based on this model allowed the sample depth to be increased, and the sampling time to be reduced by almost two orders of magnitude.

![Schematic of Optical Sampling Volume](image)

**Figure 2 – DOF Effect**

**Low Pixel Effects:** As noted earlier, the lowest system magnification is determined by the minimum number of pixels required to measure the smallest targeted particles. It was calculated that with this instrument configuration, images of approximately 20 pixels, equating to approximately 10µm, would be necessary to remain within the target sizing error range.

The instrument’s range may be extended to smaller particles by increasing the magnification resulting in a larger image projection and therefore a larger number of triggered pixels. However, higher magnification decreases the optical sampling volume and increases measurement time.

An alternative solution involves developing a statistical model capable of correlating the probability of a resulting image (pixel count) to particle diameter at the instrument’s specified intensity threshold. A series of look-up tables (corresponding to different particle sizes) was produced and incorporated into an algorithm to assign the most probable particle size given the number of pixels triggered. This technique effectively extends the instrument’s measurement range to 1.5µm without increasing magnification.
RESULTS AND DISCUSSION

The performance of the instrument has been characterized with respect to Size Determination and Consistency, Concentration Measurement Repeatability and Limits, and Material Dependence. The measurement data presented for particle size and concentration were obtained using a magnification and sample depth which allowed 1ml of sample to be measured in three minutes. The minimum and maximum particle diameter for this magnification and capillary were 1.5 and 300µm respectively. The data shown is largely concentrated at the bottom end of this size range, from 1.5 to 10µm, where conditions for the instrument are more challenging. This also represents the size range of greatest interest in measurements on finished water.

Size Determination and Consistency

In order to assess the sizing accuracy of the DPA, industry accepted polystyrene particle size standards were analyzed. The instrument was configured to identify and count particles with equivalent circular diameters ranging from 1.5µm to 20µm in 0.5µm steps. Eight separate runs of 1ml each were analyzed for each sample size. As illustrated in Table 1, accuracy is expressed as the number of particles falling outside of the expected steps or ‘buckets’ relative to the known distribution of standard sample.

<table>
<thead>
<tr>
<th>Particle Size Standard Attributes</th>
<th>Measurement Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (µm)</td>
<td>1.588</td>
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<tr>
<td>Tolerance (+/- µm)</td>
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<td>Range (µm)</td>
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<td>Average</td>
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<td>Std. Dev.</td>
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<tr>
<td>Particles Smaller Than</td>
<td>NA **</td>
</tr>
<tr>
<td>Target Bucket (%)</td>
<td>NA **</td>
</tr>
</tbody>
</table>

Table 1: Instrument Sizing Accuracy

Figure 3 depicts the analysis results of 2.5µm, 5µm, and 10µm particle standards in terms of sizing repeatability. Concentrations were approximately 600,000 particles/ml, 50,000 particles/ml, and 12,000 particles/ml respectively. For display purposes the curves have been normalized for concentration.

The instrument’s capability to resolve distributions of similar sizes is shown in Figure 4. A composite mixture of 2µm, 2.5µm and 5µm particle size standards was produced with particle concentrations approximately 50,000 counts/ml for each. Three runs of 1ml were analyzed and the results indicate a resolvability of better than 0.5µm for small particles.
Concentration Measurement Repeatability and Limits
In order to assess the concentration measurement repeatability, samples with concentrations from 12,000/ml to 800,000/ml were evaluated. As shown in Table 2, the concentration measurements were repeatable across the range of concentrations, with a worst case of ± 3.8% for a 2µm sample with a concentration of 793,000 particles/ml.

The ability to accurately size samples with very high concentrations was explored using 2.5µm particle size standards prepared at concentrations of approximately 0.5x10^6/ml, 1x10^6/ml, and 2x10^6/ml. Figure 5 shows the enlargement effect of coincident particles while figures 6, 7, and 8 are system images which illustrate the particle coincidence. Note that the effective concentration limit can be substantially increased using a flow cell with a smaller sample depth.

Prior to each set of runs, 1ml of 0.2µm filtered DI water was analyzed in order to assure the instrument did not produce false positives. In each case no particles were detected, suggesting the instrument is suitable for applications with very low particle concentrations.

<table>
<thead>
<tr>
<th>Particle Size Standard Attributes</th>
<th>Measurement Results</th>
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<tr>
<td>Mean (um)</td>
<td>5.86</td>
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<td>Tolerance (+/- um)</td>
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<tr>
<td>Range (um)</td>
<td>1.40 to 2.77</td>
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<td>Average Concentration (count/cc)</td>
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<tr>
<td>Standard Deviation (count/cc)</td>
<td>163</td>
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<tr>
<td>Repeatability (+/- %)</td>
<td>2.1%</td>
</tr>
</tbody>
</table>

Table 2: Instrument Counting Repeatability
Material Dependence

Smaller particles which are more transparent, smoother, and have a refractive index closer to water will provide images with the lowest contrast and therefore be the most difficult for the DPA instrument to resolve. In order to assess the instrument’s ability to detect, size, and count particles of different material composition, particle populations of various substances including polystyrene, borosilicate glass, silica, and graphite were analyzed.

Figure 9 shows the size distributions resulting from analysis of 5µm polystyrene, and 10µm borosilicate glass particle size standards. The refractive index of the borosilicate glass supplied was 1.59 (at 589nm) with a density of 2.5g/cm³. In comparison polystyrene has a refractive index of 1.56 (at 589nm) and a density of 1.05g/cm³.

Using the manufacturer’s size distribution parameters for the borosilicate sample, the 10µm particles were analyzed and sized with accuracy of +2.53%/-0.48% (standard deviations of 2.16% and 0.12% respectively) for three 1ml runs. This result was well within the ranges determined for both the 5µm and 10µm polystyrene samples.

While all material substances analyzed were found to be easily detected, limitations in the availability of precise particle size standards emphasizes this as an area for future investigation.
Water Analysis

In order to evaluate the ability of instrument to work with a water sample containing unknown concentrations of unknown materials, the instrument was used to analyze tap water extracted from the Brightwell Technologies facility (City of Ottawa).

Figure 11 shows the analysis of the tap water. Three separate runs of 1ml each were analyzed. The results indicate that approximately 120 particles/ml ranging from 1.5µm to 4µm were detected in the water samples. Additionally, as many as 35 particles were detected in the 4µm to 8µm.

In order to explore the ability of the instrument to capture selective high quality gray scale images, surface water extracted from a nearby river was analyzed. The instrument was configured to determine the presence of particles larger than 25µm in diameter and store the frame captured by the digital camera. Processing of stored frames resulted in many interesting images including the organism shown in Figure 12.
CONCLUSIONS
Experiments carried out with a first generation instrument have demonstrated the feasibility of applying dynamic digital imaging to characterize particle populations in water. The technology has been shown to be capable of measuring particles in sizes from 1.5 to 1000 µm in concentrations ranging from zero to one million (smaller) particles per ml with a high degree of accuracy and consistency. Operation of the instrument has been found to be independent of particle material for a range of materials having different optical properties. The measurement time required for the instrument to obtain statistically accurate results has been significantly reduced by operating with a lower magnification and increased sample depth and by employing statistical image processing to compensate for the resulting optical distortions. The feasibility of capturing high quality images selected by the system software according to user criteria has been demonstrated.

Based on the results obtained to date, the dynamic digital image technique appears to have considerable potential for a wide range of applications throughout the water quality sector.

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