Dependence of perceived aggregate size on pixel resolution using an imaging method

Rajat K. Chakraborti and Joseph F. Atkinson

ABSTRACT

In order to assess the experimental artefacts associated with image-based characterization of aggregates, tests were performed to evaluate the effects of aggregate size and shape, relative to the pixel resolution of the imaging device. The basic motivation for the study was to further the understanding of particle separation processes in water treatment and specific goals were to: (1) analyse flocs developed from coagulation and flocculation processes using a non-intrusive measurement technique; and (2) develop relationships between measured aggregate characteristics and accuracy in an imaging method. The main objective of this study was to investigate the dependence of perceived aggregate size on pixel resolution using an imaging method. Analysis of fractal dimensions as a means of quantifying aggregate shape and other geometric properties was used to illustrate the consequences of potential measurement errors that might be generated, based on a given pixel resolution. A variety of pixel resolutions might be realized in an experimental setting by using different camera lenses and magnifications, and this variety is related to the desired degree of measurement accuracy. A relationship between the expected measurement error and the particle properties is established as a function of pixel resolution for both a single spherical particle and a cluster of three spheres. Results of this study provide an estimate to quantify the error in measurements due to pixel resolution obtained from an imaging method depending on the size of the aggregates of interest, and to assess the appropriateness of a particular pixel resolution depending on the desired degree of accuracy for a given analysis.

Key words | fractal dimension, imaging method, particle aggregation, pixel, pixel resolution

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NOTATION

α	pixel resolution
d_o	a characteristic length of the object being measured
λ	pixel size, length scale
A_p	area of pixels
A_o	area of an object
N	number of pixels
λ^2	area of a square pixel
R	dimensionless ratio of areas ($= A_p/A_o$)
E_{area}	error in area measurements
D	fractal dimension
s	second
rpm	revolution per minute
NTU	turbidity unit

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INTRODUCTION

Particle aggregation is a key mechanism in solid–liquid separation processes controlled by various physical, chemical and hydrodynamic conditions. Specifically, coagulation and flocculation are important for enhancing fine particle separation in various processes, such as those in water treatment. The growth of aggregates depends on the relative size of the colliding particles or clusters of particles, their number density, surface charge and roughness, local shear forces and the suspending electrolyte (Amirtharajah & O'Melia 1990; O'Melia 1991; Elimelech *et al.* 1995; Spicer & Pratsinis 1996; Han *et al.* 1997). Either coagulant is intentionally added in engineered processes such as sedimentation, filtration and flotation, or divalent cationic metals are already present in natural systems. In either case, the result is an enhancement of particle growth and subsequent solid–liquid separation (O'Melia 1991, 1998; Elimelech *et al.* 1995).

Mixing in coagulation–flocculation processes assists coagulants to destabilize particle surface charge and therefore helps to adsorb multiple particles in the particle surface, thereby linking the particles together in a complex structure (Amirtharajah & O'Melia 1990; Wiesner 1992; O'Melia & Tiller 1993). The mass density, or structural compactness, is one of the more important properties of aggregates in suspension and needs to be analysed accurately (Tambo & Watanabe 1979; Gregory 1989, 1997, 1998; Odegaard *et al.* 1990; Gregory & Chung 1995). The key element of particle separation processes in engineered systems is the production of aggregates with desired characteristics under controlled conditions (Donaldson *et al.* 1986; O'Melia 1991, 1998). Design of proper coagulant dose to meet a given treatment goal is dependent on precise *in situ* characterization of suspended particles.

Particles play important ecological and water quality roles by presenting reactive surfaces for the transfer of contaminants, influencing metabolic activity, attenuating light, hosting pathogens and bacteria, affecting stoichiometry of particulate constituents, and contributing to net sediment deposition (Effler & Mathews 2004). Monitoring the geometric properties of aquatic particulate aggregates, including accurate determination of *in situ* aggregate size distribution, is therefore important for understanding the transport of particles and particle- associated pollutants.

Mathematical modelling is often used to assess particle-bound contaminant transport and fate in natural and engineered systems. Historically, models have treated suspended sediment as discrete, spherical or nearly spherical particles with some assumed density like that of sand, silt or clay. The settling velocities, the controlling mechanism in solid–liquid separation, often are considered to follow Stokes' law, or some modification thereof, and particle size, shape and other characteristics are often approximated as depth averaged in the water column. In spite of using complex model formulations, results are based on a simplified concept of particles. Within the last 10–15 years, however, as a result of improvements in computer visualization, it is becoming possible to understand the complex structure, transport and deposition characteristics of suspended sediment. In fact, it is observed that suspended particulates in natural and engineered systems typically are flocculated, rather than acting as discrete particles. Due to this complex structure, more detailed descriptions of particles and flocs are needed, and it is also important to evaluate the potential impacts of measurement uncertainty in these analyses.

Precise *in situ* measurement of suspended particles requires tools that are often very expensive and difficult to apply in the field. Many environmental analyses are based on the results of indirect methods of particle analysis and, therefore, the application of those studies must be done with care. Morphological characterization of suspended aggregates usually requires microscopic techniques, which are complementary to macroscopic methods for characterizing flocculated suspensions. For example, with measurements of the active sediment layer, conventional methods of analysing suspension rheometry provide information about the bulk behaviour of the suspension, but it is difficult to extract information concerning the detailed behaviour of individual particles (mostly in the micrometer size range), or their dynamic behaviour and *in situ* properties from such methods.

Several indirect particle measurement methods have been applied. However, most of these methods require sample handling which may alter the shape and size of aggregates (Allen 1997). For example, instruments based on the principle of electrical or optical sensing zone require particles to pass through a small orifice. Miller & Lines (1988) have shown that the results from these types of instrument are valid only for particle diameters up to about 35–40% of the aperture diameter. Another disadvantage of this method is when more than one particle passes at one time through the orifice, distorting the reading. Measurements performed with instruments based on Frauenhofer diffraction are not '*in situ*' and the results are sensitive to the particle concentrations, which may require sample dilution. Moreover, sizes of particles are derived from light scattering theory based on the assumption that particles are spherical.

Photographing particles *in situ* and analysing the images using digital image analysis tools has become one of the most widely used microscopic measurement techniques, providing direct information on particles including floc size distribution and floc structure (Kaye 1984; Donaldson *et al.* 1986; Kramer & Clark 1996; Logan 1999; Chakraborti *et al.* 2000, 2003; Schafer 2002; Ahammer *et al.* 2003; Sterling *et al.* 2004; Atkinson *et al.* 2005). However, the impact of measurement error associated with this technique has not been widely addressed and, more importantly, it has not been quantified, and that is the motivation for the present study. In this work we investigate the implications of pixel size in an imaging method on the accuracy of measurement of natural particles of varying shapes and sizes and quantify the errors associated with the *in situ* image-based measurement technique.

Imaging method as a non-intrusive particle measurement tool

Instrumentation required for image analysis includes a charge coupled device (CCD) digital camera, image acquisition software, a camera control unit, a light source, and image analysis software (Kramer & Clark 1996; Allen 1997; Chakraborti *et al.* 2000, 2003; Bushell *et al.* 2002; Schafer 2002; Ahammer *et al.* 2003; Atkinson *et al.* 2005). Different CCD cameras have different resolutions, meaning there is a range in the number of pixels (e.g. 512 × 512 or 1,024 × 1,024 pixels in a two-dimensional array) available to record a given image.

After acquiring a particle image, the second main step in an image-based measurement approach is analysis of basic geometric properties (Allen 1997), and care must be taken in each of these steps in order to maintain accuracy (Miller & Lines 1988; Kramer & Clark 1996; Schafer 2002). Furthermore, for suspended particles that are light in colour it may be difficult to identify the edges due to contrast problems with the background. This requires appropriate filtering to obtain accurate representations of the particles. Although larger magnification can provide more precise geometric characterization of particles in an image, application of large pixel numbers to avoid experimental artefacts is not always a cost-effective option. Thus, a significant question governing the design of an image analysis-based experiment is how small the pixels must be to provide accurate measurements for a given application.

Given the importance of this question, a quantitative method of evaluating possible errors in estimating geometric properties of floc images using a given pixel resolution (defined as the ratio of the characteristic length of an object and the pixel size) is needed. The present study is designed to formalize a procedure for determining measurement sensitivity based on analysis of several simple shapes meant to represent a single, circular particle and a small cluster of particles, or aggregates. Measures of accuracy are developed in terms of pixel resolution, and experimental errors associated with an image analysis method are determined. In addition, analysis of fractal dimensions is used to illustrate the potential measurement errors that might be generated, based on a given pixel resolution, and also to present the possible implications on particle characterization that may result from uncertainties in the measurements.

MATERIALS AND METHODS

Experimental

Experiments were carried out in a standard 2-litre flocculation jar using alum as coagulant for suspensions taken from the Buffalo River (Buffalo, New York) to observe changes of both surface charge (measured as zeta potential) and residual turbidity. The following stages of aggregation with increasing alum dose were observed: (1) initially, particle surface charge was reduced and the particles became destabilized (the primary aggregation mechanism was the reduction of electrostatic repulsion between particles); (2) charge neutralization was reached when sufficient alum was added so that the originally negatively charged particles were just neutralized, and aggregates were formed when contacts occurred; and (3) further addition of coagulant led to charge reversal and restabilization, where aggregation was not chemically favoured. At higher doses and for an appropriate pH range, 'sweep floc' occurred (with the production of $Al(OH)_3(s)$, which coats particles with a gelatinous and 'sticky' sheath) and caused aggregates to settle more quickly, reducing turbidity. In this study, aggregates that were produced at this 'sweep floc' condition were analysed for floc size distribution and particle characterization using an imaging method.

Images of suspended particles were obtained using a CCD camera (Kodak MegaPlus digital camera, model 1.4) and imaging software (Insight PIV System, TSI Inc., St Paul, Minnesota, USA) (Figure 1). For these experiments, the illumination source was a portable electronic stroboscope (Digistrobe, Cole Parmer Instrument Co. Illinois, USA) to provide a coherent backlighting source. The camera was used with a frame rate of seven frames per second to capture digital images and had a sensor matrix consisting of 1,320 (horizontal) × 1,035 (vertical) pixels. Each pixel was recorded using 8-bit resolution, i.e. there are 256 grey levels for each pixel image. Generally, measurements were conducted at 0.5 pixel = 1 μm. Using this technique, aggregates could be maintained in suspension and images were captured without sample extraction or any other interruption of the experiment. A public domain image analysis software package (NIH *Image*) was used to analyse the digital images and provide geometric information, from which characteristics such as area, perimeter, long and short axes of the aggregates, and fractal dimension could be determined (Chakraborti *et al.* 2000; Atkinson *et al.* 2005).

Materials

For coagulant, a stock solution of alum was prepared by dissolving $Al_2(SO_4)_3 \bullet 18\,H_2O$ (Fisher Scientific, Pittsburgh, Pennsylvania, USA) in deionized water to a concentration of 0.1 M (0.2 M as aluminium). After addition of alum, the suspension was mixed rapidly (~100 rpm) for one minute and then slow-mixed for 20 minutes with a mean velocity gradient, $G = 20\,s^{-1}$. These values are within the normal range used for treatment processes. The mixing was then stopped and images of the resulting aggregated particles in suspension were taken. Experiments were conducted at room temperature (~20–23°C), and the analysed images were obtained with an alum dose of $20\,mg\,l^{-1}$, and with pH maintained at 6.5 by manual addition of acid or base as required. It is to be noted that active pH control in the presence of hydrolysing metal ions in a system open to atmospheric CO_2 exchange may give rise to a dynamic system in which reaction time, i.e. 'floc age', can influence results. This may affect repeatability of the experiments somewhat. However, the main interest of the present study was the accuracy of image analysis, and quantitative

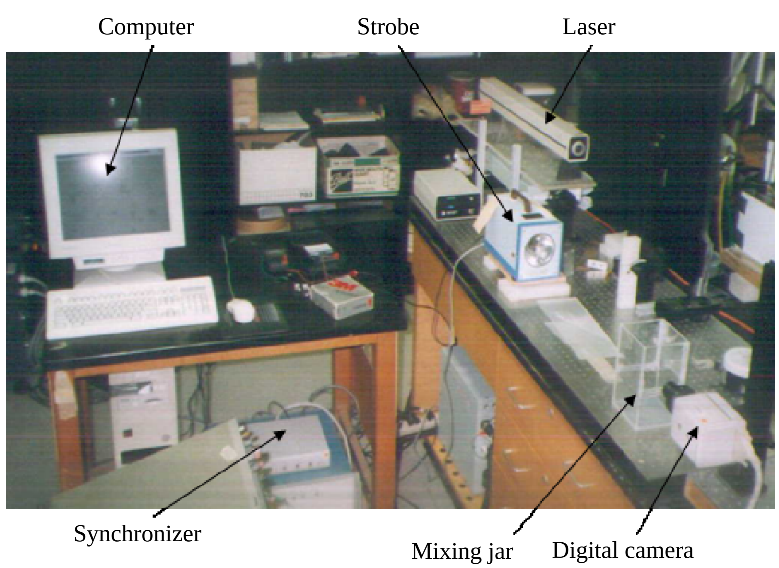

Figure 1 | Various components of an image analysis technique used for particle characterization.

flocculation results were not a priority. The initial turbidity for the Buffalo River suspension was found to be 28 NTU.

RESULTS AND DISCUSSION

Images of suspended aggregates

Figure 2 shows images of natural suspended particles captured and analysed using the imaging method. Clearly, these images show that particles are in fact conglomerates of multiple particles/clusters arranged in various patterns. This is consistent with observations of natural aggregates (Logan 1999; Chakraborti *et al.* 2000, 2003; Atkinson *et al.* 2005). Furthermore, aggregates are elongated and highly porous, with highly convoluted surfaces. Pixel resolution in an imaging method plays a significant role in characterizing such natural aggregates with so much surface irregularity. Qualitatively at least, this observation supports the need to characterize natural aggregates in terms of measures beyond those available in Euclidean geometry as discrete spherical particles. Here we use fractal geometry concepts, primarily the fractal dimension, as an additional descriptor for the particles.

Aggregate size distribution

Analysis of aggregate size distribution is a key design parameter in a particle–liquid separation process. Particle size distributions based on the images are shown in Figure 3, where the initial distribution was obtained just after the rapid mix period was ended and the final distribution corresponds to the 'sweep floc' condition. Also, the frequency of occurrence (number) in each size class is plotted against the aggregate characteristic length. Typically, the average number of aggregates measured from the seven frames obtained (per second) was analysed to evaluate particle size distributions for the 'initial' and 'final' (at the end of the slow mixing) conditions. In this study characteristic length is taken as the major axis of an ellipse fitted to the aggregate (Chakraborti *et al.* 2000). It may be seen that the peak size gradually increases and then it decreases, as length of aggregates increases. In the initial size distribution, particles between 10 and 45 μm with a peak at 18 μm were observed. For the 'final' condition plot, particle size ranged between 16 and 140 μm, with peak size observed at about 22 μm. As expected, compared with the initial size distribution, particle size at the 'final' condition was more spread out and several larger size aggregates developed. It is to be noted that the heterogeneity in aggregate size and shape is expected to lead to variable effects under different physical, chemical and hydrodynamic conditions and, therefore, compared with systems with a uniform distribution of primary particles in the floc structure. This condition highlights the need for proper characterization of suspended particles based on their size and shape in order to evaluate solid–liquid separation processes correctly.

Euclidean vs. fractal approach for particle characterization

As shown in Figure 2, the irregularities in the surface structure of natural particles are varying and mass distribution in the floc structure is highly non-uniform. Historically, efforts to understand individual processes of aggregation have been based on relatively simple systems, assuming impervious spherical particles, with various mechanisms of particle interaction explained using Euclidean geometry. It is clear, however, from images such as those shown in Figure 2 that the Euclidean assumption for aggregate structure does not provide an accurate representation of reality. Based on traditional modelling approaches, these aggregates would be represented as a sphere with diameter related to the area of

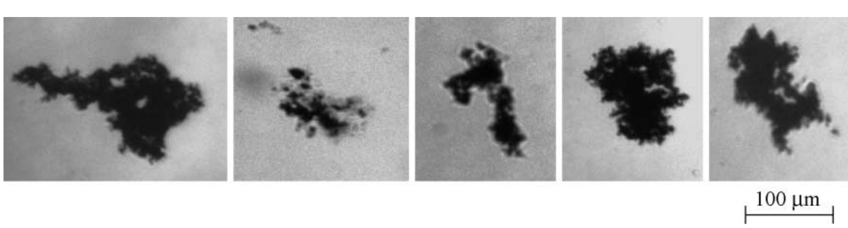

Figure 2 | Images of Buffalo River flocs captured and analysed by an imaging method.

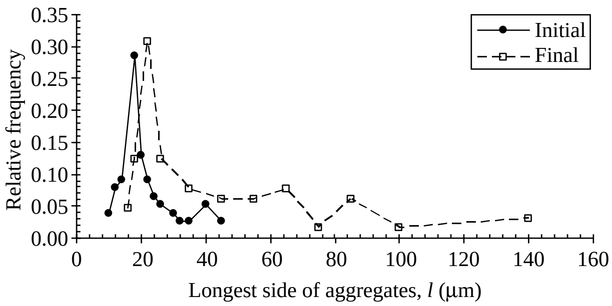

Figure 3 | Particle size distribution of suspended particles measured by an imaging method.

the real aggregate, or equivalent to the characteristic length (long or short side) of the aggregate.

More recently, it has been explicitly recognized that natural aggregates, as shown in Figure 2, are porous with a rugged irregular structure that exhibits statistical self similarity, and that these characteristics suggest different behaviour than that of impervious spheres (Logan 1999; Chakraborti *et al.* 2000, 2003; Atkinson *et al.* 2005). Such aggregates require additional descriptors, and fractal geometry has been found to provide a means of providing additional geometric information. Fractal concepts have been adapted from general theoretical considerations originally discussed by Mandelbrot (1983) and later by Meakin (1988, 1989, 1991, 1999). Fractal theories have been used mainly as a quantifying tool for describing the actual irregular structure of the aggregate, but several studies have also looked at the application of fractal characteristics as a means of analysing the kinetics of aggregation (Meakin 1989; Jiang & Logan 1991; Vicsek 1992; Logan 1999; Chakraborti 2004). Both fractal dimension and aggregate size have an impact on aggregation and vertical settling rate (Sterling *et al.* 2004). Chakraborti *et al.* (2000) demonstrated that a ‘sweep floc’ condition develops flocs which are 0.35 dimensional units smaller than the corresponding Euclidean dimension (a two-dimensional fractal dimension of flocs was found to be 1.65 instead of a corresponding Euclidean dimension of 2). In other words, a difference of 0.35 in fractal dimension units signifies the fluffiness of aggregates, or the degree to which they are not compact spheres. The images of flocs demonstrate that so much difference in fractal dimensional units signifies such a rugged structure of objects and illustrates an important stage of aggregation evolved with a high dose of coagulant.

Pixel resolution and measurement sensitivity

Although shown to be a powerful tool for characterizing particles, the accuracy of image analysis techniques is principally limited by the pixel resolution and size of the image (Ahammer *et al.* 2003). Thus, it is important to quantify the impact of possible inaccuracies involved in an imaging technique and to investigate the implications of such inaccuracies on the estimation of particle properties. In the following discussion, pixel resolution (α) is defined as

$$\alpha = \frac{d_o}{\lambda} \tag{1}$$

where d_o is a characteristic length of the object being measured, and λ is the pixel size. Alternatively, a dimensionless pixel size could be defined as the inverse of α, but it will be more convenient to use α (see below). Obviously, pixel resolution increases as pixel size decreases.

In general, accuracy in an image analysis method can be improved by using lenses to increase magnification, which essentially increases pixel resolution (by decreasing λ) (Kaye 1984, 1989). However, this step is usually costly, and to avoid unnecessary costs it is necessary to determine the required pixel resolution for a given application. As indicated above, the required resolution will be related to the expected size of particles or aggregates to be analysed. A single particle with a well-defined boundary is relatively easy to detect, but for clusters of particles, it is difficult to identify the exact particle surface (Kaye 1984, 1989).

In a two-dimensional imaging technique, a particle is represented by a finite number of square pixel areas that are ‘covered’ by the particle in the camera array of sensors. For example, if (projected) area is to be measured, the total area of pixels A_p covered by an object of true area A_o is calculated as the product of the number of covered pixels (N) and the area of a square pixel (λ^2),

$$A_p = N\lambda^2 \tag{2}$$

Error in the measurement is then given by the difference between A_p and A_o, or, as used below, by the degree to which the ratio $R = A_p/A_o$ deviates from 1.

The pixel size relative to the particle size determines the extent to which the boundary of an object may be accurately represented, considering that the pixel area is square, while the object being measured generally has rounded and irregular surfaces. This issue is illustrated in Figure 4. In Figure 4(a) a circular particle is completely enclosed within a pixel, with sides equal to the diameter of the circle. Using the diameter to represent the characteristic length d_o, the pixel resolution for this situation is $\alpha = 1$. Furthermore, independent of the actual pixel size, $A_o = 0.785A_p$, and $R = 1.27$. The area of the object in this case is thus overestimated by 27%.

Compared with the case in Figure 4(a), a higher pixel resolution ($\alpha = 5$) is obtained in Figure 4(b) using a pixel length one-fifth of the particle diameter. However, the area of the circle is still overestimated by 27%. The equivalent circular area for a 10-μm particle is 78.5 μm^2, whereas the pixel area is 100 μm^2. It is only with higher resolution, as shown in Figure 4(c), that the accuracy can be improved (see discussion below). In Figure 4(d) an image of an irregularly shaped aggregate is shown, along with the pixel representation of the aggregate. Note that the difficulties and possible sources of error in measurement of the aggregate due to pixel resolution are with the pixels extending beyond the boundaries of the object being measured. Again, resolution determines the accuracy with which the pixel array is able to represent the boundaries of the aggregate and, therefore, the accuracy of further calculations of geometrical properties.

Different orientations of a circular particle relative to the pixel array are shown in Figure 5(a). The first panel of Figure 5(a) represents essentially the same situation as in Figure 4(a), where the resolution $\alpha = 1$, and the object is centred inside a single pixel. This orientation of particle location relative to the pixel array is the best-case scenario, in terms of measurement accuracy, since A_p would be even greater if the particle were to register on more than one pixel. This situation is shown in the remaining panels of Figure 5(a), where N is the number of pixels used to register the object. With N varying between 1 and 4, the area ratio R may vary between 1.27 and 5.09, with corresponding over-measurement errors of between 27% and 409%. It is seen that, in addition to resolution, placement of the particle with respect to pixel boundaries is also important in determining accuracy, at least for low pixel resolution.

If the pixel resolution were to be doubled, then a minimum of four pixels would be needed to register the object. Following the same reasoning as above, it can easily be shown that up to nine pixels could be required to register the particle, depending on its placement on the pixel array. In this case R would vary between 1.27 and 2.86. Although this is still a wide range, it is considerably less than the case with $\alpha = 1$ (where $\lambda = d_o$). Additional increases in resolution result in a narrowing of the range of possible values for R. For example, when $\alpha = 5$ as in Figure 4(b), the range is $R = 1.27 - 1.63$.

As the resolution is increased, a point is reached where a square grid of pixels is not needed to cover the circle entirely (Figure 4(c)). This results in an improvement in the accuracy of the pixel representation of the object. With a pixel resolution $\alpha = 8$, 60 pixels instead of 64 are covered by the object. Assuming the circle is centrally placed with reference to the pixel grid, the area ratio is found to be $R = 1.19$. With a further doubling of resolution, $\alpha = 16$ and 216 pixels are needed, compared with a possible $16^2 = 256$ pixels in the square array. This results in $R = 1.07$. Although a distinct improvement, this result still

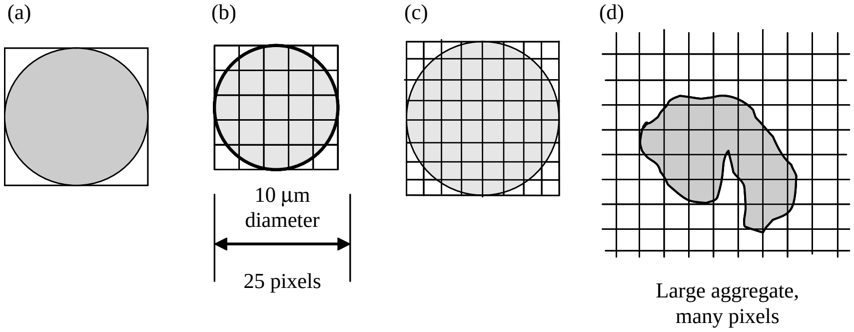

Figure 4 | Particles and various pixel resolutions (shaded areas indicate registered pixels).

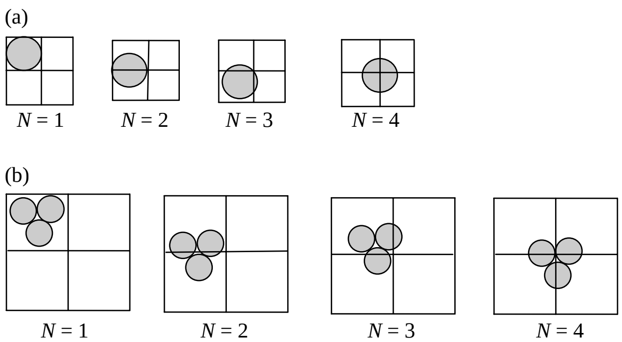

Figure 5 | Pixel size and the number of pixels (*N*) occupied by the particle as a function of particle position. Example: (a) single object, (b) clusters.

gives a possible error of 7% in the measurement of area. As previously discussed, R may also vary for a given resolution, depending on the placement of the object with respect to the pixel array. However, this variability decreases as pixel resolution increases.

To extend the above results, consider a simple aggregate consisting of a triangular cluster of three equally sized circular particles, as shown in Figure 5(b). In this case the diameter of the circle encasing the cluster is used for the characteristic length d_o. In other words, d_o is the diameter of the smallest circle drawn to enclose the cluster. Similar to the case with a single circular particle, different resolutions and placements produce different results for the area ratio R, and as pixel resolution increases, both R and the range of possible values of R decrease.

Results of calculations for a range of resolutions are shown in Figure 6 for both the single particle (Figure 6(a)) and the cluster (Figure 6(b)). In this figure the area ratio is plotted as a function of pixel resolution, and the ranges of area ratios shown for each resolution are based on different placements of the object on the pixel array. These ranges were determined by moving an electronically drawn object (circle or cluster) over a regular grid array and manually counting the number of 'pixels' covered by the object. Different positions were considered for each resolution, giving a range of values of A_p and, therefore, R. At least four to six tests were performed for each resolution, in order to determine the minimum and maximum values for R. The plotted points represent the average value (between minimum and maximum). For both single particles and clusters, R is high when the pixel resolution is low.

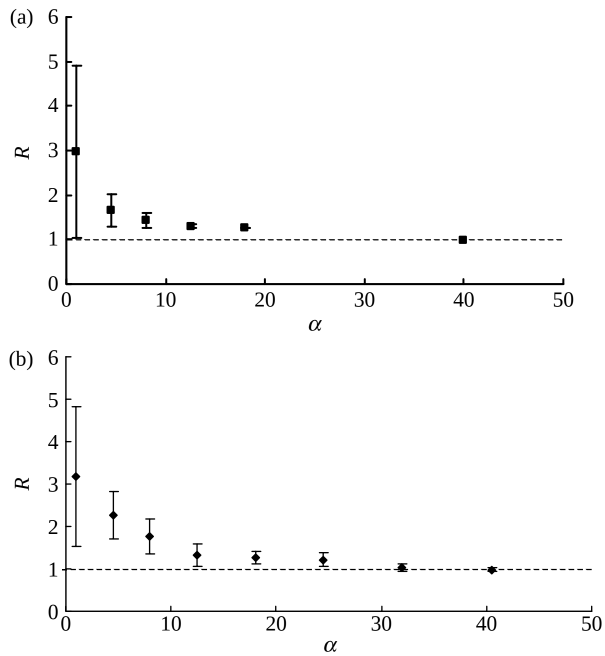

Figure 6 | Effect of pixel size on area measurement for: (a) single object and (b) clusters (triplets). The bars represent (±) one standard deviation about the mean values.

In addition, the range of possible values for R decreases as α increases. It is seen that A_p approaches A_o (or, R approaches 1) for a pixel resolution of 30 or 40 for single particles or clusters, respectively. At this or higher pixel resolution, the area is effectively reproduced by the pixel array. This result is somewhat more conservative than the result reported by Kaye (1984), which suggested that a pixel resolution of about order 10 is required for the delineation of curved and irregular boundaries.

Direct measurements of aggregate properties are used to derive other characteristics such as settling or aggregation behaviour (Logan 1999). For example, the most frequently used shape parameters are calculated using the measured area, length and perimeter (e.g. Schafer 2002). A relationship between the pixel resolution and the associated possible error in measurement (which is generally an overestimation, since $A_p > A_o$) may be developed according to the analysis presented in Figure 4. The error in area measurements (E_{area}) is calculated as:

$$E_{area} = \frac{A_p - A_o}{A_o} = R - 1 \tag{3}$$

The best-fit curve for the mean values presented in Figure 6(a) (single circular particles) is:

$$E_{area} = 2.88\alpha^{-0.97} \quad (R^2 = 0.82) \tag{4}$$

Similarly, the relationship for clusters (Figure 6(b)) is:

$$E_{area} = 3.80\alpha^{-1.02} \quad (R^2 = 0.80) \tag{5}$$

Both of these relations indicate an approximately inverse relationship between error and pixel accuracy, and both equations give similar results for E_{area}. Being based on different object shapes, this result suggests that similar relations might be obtained for other shapes as well, making it more generally useful. In other words, measurement accuracy is expected to be approximately inversely related to pixel resolution for the analysis of generally shaped particles, and equations such as (4) or (5) may be used in determining precision in a digital image analysis technique.

It should be noted that, if aggregates are placed centrally with respect to the pixel array, substituting Equations (1) and (2) in Equation (3), it can be derived that E_{area} and α follow an inverse square relationship. However, due to various possible positioning of objects (as described in Figure 5) within the pixel grids, various possibilities of effective pixel area (A_p) arise which yield uncertainty in the estimates of area ratios (R). As shown in Figure 6, R is not a linear function of α, rather they are related in an asymptotic nature since A_p is not a single value based on any particular pixel resolution. Pixel areas covered by the object are higher with relatively coarser pixels and vice versa, resulting in variability due to different positioning of the object. Standard deviation from the mean is an indicator of the effect of different placement of the single spheres and clusters. As shown in Figure 6, standard deviation in the estimates of R due to variabilities in A_p values is larger with coarse pixels and decreases with finer pixels.

Sensitivity of imaging method: Effect of measurement error on particle characterization

The ability of a system to accurately resolve the smallest particles with irregular surfaces expected in an environmental sample must be determined to attain confidence in the analysis of geometrical properties such as the fractal dimension. Fractal geometry characterizes the distribution of mass within the body of an aggregate, which is often non-homogeneous and difficult to assess, and aggregates with lower fractal dimensions exhibit a more porous and branched structure and have higher aggregation rates (Jiang & Logan 1991; Logan 1999). Moreover, fractal dimension is a sensitive geometric property that describes aggregate structure and various properties of the aggregate (Jiang & Logan 1991; Atkinson *et al.* 2005). Since the (irregular) shape and size of a natural aggregate are best described using fractal geometry, it is required to evaluate the impact of measurement uncertainty on the fractal analysis of particles based on digital images (Ahammer *et al.* 2003). The following discussion is aimed at understanding the possible ranges in estimates of the fractal dimensions that would be associated with measurement errors as described above.

The pixel-based measurement for determining various geometrical characteristics as presented here is also known as a box counting method (Vicsek 1992). The pixels can be considered as small boxes and λ represents a length scale that can characterize any object in a self-similar manner. In a typical box counting method, the fractal dimension (D) is given by (Vicsek 1992):

$$D = \lim_{\lambda \to 0}\left(-\frac{\log(N)}{\log(\lambda)}\right) \tag{6}$$

where N is the number of boxes or pixels covered by the image, and λ is the square box or pixel size, as in Equation 1. Plotting the number of pixels needed to resolve an object resulting from various pixel resolutions as a function of various pixel sizes on a log-log plot returns the fractal dimension of the object from the slope of the line fit through the data. Since the present discussion deals with two-dimensional images, D in Equation 6 is interpreted as the corresponding two-dimensional fractal dimension.

Bushell *et al.* (2002) analysed a fractal aggregate with various pixel resolutions by varying pixel size and reported the associated numbers of pixels needed to describe the floc. They analysed the aggregate using a gradually decreasing pixel size (i.e. using higher resolution), and observed the degree to which surface irregularities or detail of an aggregate could be represented with each level of resolution. This example illustrates the effect of gradually changing pixel resolution on measurement of an object as presented in Figure 4. Using a low pixel resolution with a pixel size of 32 units (i.e. $\alpha = d_o/32$), they found that 131 boxes were required to cover the fractal aggregate. However, this resolution was not sufficient to represent the irregular surfaces of the aggregate very accurately. In order to obtain sharper details for the same aggregate, they used pixels of sizes 32, 16, 8, 4, 2 and 1 (i.e. $\alpha = d_o/32$, $d_o/16$, $d_o/8$, $d_o/4$, $d_o/2$ and $d_o/1$, respectively), and the corresponding number of pixels needed was 131, 386, 1,198, 3,816, 12,578 and 42,397, respectively. In other words, with the smallest pixel size (highest resolution), altogether 42,397 pixels were needed to accurately describe the fractal aggregate geometry. As the pixel resolution increases, more details of the fractal aggregate structure could be represented.

The data from Bushell *et al.* (2002) represent a population of six data points, and the slope of the log-log plot of number of boxes as a function of pixel size returns a fractal dimension, $D = 1.67$ (Figure 7). However, following the above argument, some uncertainty in this result is expected due to different possible resolutions and orientations of the aggregate with respect to the pixel grid. In other words, if the experiment were repeated, different data points could be produced, resulting in a different determination of D. Using Equation 5 to calculate the variation in number of pixels that might be expected to arise from different experimental set-ups (these points are labelled as

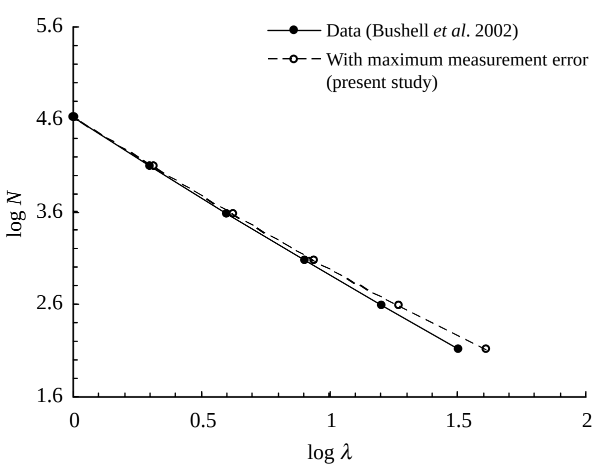

Figure 7 | Log-log plot of number of pixels with the size for describing a computer generated aggregate for Bushell *et al.*'s (2002) data and the corresponding maximum possible error in measurements using an imaging technique.

'with maximum measurement error (present study)' in Figure 7), Bushell *et al.*'s (2002) data were plotted and the fractal dimension was calculated, giving $D = 1.56$. In other words, the uncertainty in calculation of the fractal dimension due to pixel resolution alone changes the value from 1.67 to 1.56, or approximately a 7% decrease. This example demonstrates the uncertainty that might be associated with the fractal dimension estimates when an imaging technique is used for measurements. Depending on the accuracy required in a particular experiment, this much error in the estimate of fractal dimensions and, therefore, impacts on the evaluation of aggregation processes and process designs could be substantial.

It should be noted that the plot with the error in measurements with higher pixel resolution (i.e. with smaller λ) matches well with Bushell *et al.*'s (2002) data, but deviates at decreasing pixel resolutions. The line connecting the points with the error in measurements moves the tail of the plot upwards, resulting in a relatively flatter slope and smaller D. Although this example of error analysis is hypothetical since Bushell *et al.*'s (2002) data were derived by DLCA (diffusion limited cluster aggregation), while most environmental processes are governed by RLCA (reaction limited cluster aggregation), the resulting difference in fractal dimension (i.e. 1.56 instead of 1.67) impacts significantly on the fractal-based analysis of aggregation processes, for example, by altering the calculation of collision frequencies and aggregation kinetics (Jiang & Logan 1991; Logan 1999; Chakraborti 2004; Atkinson *et al.* 2005). For example, a relatively smaller fractal dimension would characterize an aggregate with smaller primary particle concentration, less mass per unit area and larger porosity. This may lead to the design of an environmental system characterized with larger collision frequencies than would occur in reality. Proper measurements are essential since a fractal dimension of 1.56 would be consistent with a relatively loose aggregate, which may actually be relatively more compact (with $D = 1.67$). However, this conclusion is also based on the assumption that the image analysis procedure itself is accurate. Experimental errors may arise at other stages of the experiment that could overwhelm inaccuracies due to pixel resolution, and these various sources of errors need to be evaluated in any given experimental setup.

Interpretation of the impact of physico-chemical processes on aggregate structure also could be significantly different. For example, Chakraborti *et al.* (2000) showed in their coagulation experiments that the (two-dimensional) fractal dimension for lake water flocs changed from 1.96 at an initial condition before addition of alum, to 1.84 at charge neutralization, and to 1.65 at a 'sweep floc' condition (i.e. about 10% change in fractal dimension between charge neutralization and sweep floc conditions). In a further study on coagulation–flocculation of natural suspensions with alum treatment, Chakraborti *et al.* (2003) found that floc fractal dimension for aggregates consisting of latex spheres varied from 1.82 to 1.64 between 10 and 30 minutes of mixing in a standard 2-litre jar test apparatus, with accompanying significant changes in aggregate shape and size. A change of fractal dimension from 1.82 to 1.64 (about 10% change) describes completely different aggregates with different aggregate architecture evolved with time. Therefore, it may be concluded that the above difference between the estimates for D (from 1.56 to 1.67) could be important in terms of analysing aggregation mechanisms in solid–liquid separation processes. For example, the evaluation of detention time in a sedimentation basin or the estimate of particle settling rate will be incorrect if a fractal dimension of 1.56 is assumed for suspended particles instead of their actual fractal dimension of 1.67. Finally, evaluation of fractal dimensions of aggregates and particle characterization based on these measurements (including the particle size distribution data as presented in Figure 3) could cause a greater difference in the estimates of aggregate properties due to possible error in measurements, which in turn would affect modelling of aggregation processes and may lead to an incorrect recommendation (Chakraborti *et al.* 2003; Chakraborti 2004; Atkinson *et al.* 2005).

CONCLUSIONS

A pixel resolution of between 30 and 40 in an imaging method has been found to be sufficient for measurements of detailed geometric information for the irregular surface of an aggregate. That information can be useful for understanding floc dynamics and for treatment and many environmental processes, such as settling, fate and transfer of nutrients and the sorption of toxic chemicals. This resolution can be achieved using suitable camera lenses, or using cameras with greater numbers of pixels available. Finding relationships between the pixel resolution and the expected accuracy of measurements is an important aspect of planning for environmental sampling and analysis, and a prior determination of measurement uncertainty level is also cost effective, in terms of determining required instrumentation. The basic image analysis technique described here can be assembled for several thousands of dollars, although system upgrades, for example using higher resolution or faster cameras, may increase the cost significantly. This study presents an analysis of observations that permit a judgment to be made regarding the trade-off between pixel resolution and area measurement error, on the one hand, and the effect of measurement error on fractal dimension estimates, on the other.

It is seen that the accuracy in measurement using an image analysis system increases with the subdivision of pixels, i.e. by increasing the pixel resolution. After reaching a threshold resolution, however, the deviation in measurement from the actual object diminishes and reaches a condition where measurements are 'correct' (within several per cent). The uncertainty in measurement of an object due to various possible positions of the object with respect to the pixel grids is also reduced with higher resolutions. In the above discussion, however, the uncertainty in particle analysis is demonstrated only for particles with simple shapes, and orientation effects of shapes with non-uniform boundary in the evaluation of populations of particles by image analysis were not investigated. Similar investigations of complex shapes of aggregates could verify the inverse relationship between accuracy and resolution found here, and serve to improve our general understanding of particle structure and aggregation processes. The core component of this study was to quantify the dependency of the pixel resolutions obtained from an imaging method to the size of the aggregates of interest. This study helps to assess the appropriateness of pixel resolution and accuracy in the description of the size characteristics of the particulate matter.

In this study the minimum required pixel resolution is identified for measuring a small particle accurately. It should be kept in mind that the present estimates were based on the assumption of particles with smooth shapes (or more complicated shapes built from smooth and simple shapes), as these facilitated understanding errors associated with an imaging method. The specific relationships for error (Equations 4 or 5) may change slightly when other types of particle are considered, though the evidence thus far suggests the error is approximately inversely proportional to pixel resolution. The example here shows that measurement error could cause significant deviations in fractal dimension estimates of irregular particles at a particular pixel resolution. Such changes in the characterization of aggregates would influence the interpretation of solid–liquid separation processes. Future studies are aimed at understanding the error in measurements using latex particles with known characteristics by measuring actual image pixel numbers surrounding a targeted latex particle with exact area and perimeter. This would provide more opportunities to test aggregates with predetermined complex shapes and sizes to verify the relationships presented in this study and to calibrate the instrumentation. Finally, although our findings are empirically based, the results highlight the fact that the assessment of possible error in measurements would help develop a better understanding of the confidence level and the limitations in a digital particle analysis.

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