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A fully-automated image processing technique to improve measurement of suspended particles and flocs by removing out-of-focus objects

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ABSTRACT

A fully-automated image processing script was developed to analyze large datasets of imaged flocs in dilute turbulent suspensions of mud. In the procedure, out-of-focus flocs are automatically removed from the dataset to attain a more precise floc size distribution. This automated technique was tested against visual inspection of images to ensure that the procedure was only selecting in-focus flocs for inclusion in the size measurements, and the resulting measured sizes were compared to floc measured through manual image processing of the same data. The results show that the automated method is able to accurately measure the floc size distribution by correctly sizing in-focus flocs and removing out-of-focus flocs. The processing procedures were developed with sizing of suspended mud flocs in mind, but the process is general and can be applied for other applications. We show the ability of the method to handle large numbers of images (over 15,000 at a time) by tracking the change in floc size population with time at 1-min intervals over the course of a 160 min floc growth experiment.

1. Introduction

Imaging techniques and analysis methods have been widely used to measure the grain size distribution and settling velocity of fine suspended sediments in both laboratory and field settings (van Leussen and Cornelisse, 1996; Eisma and Kalf, 1996; Syvitski and Hutton, 1998; Nobbs et al., 2002; Govoreanu et al., 2004; Mikkelsen et al., 2004; Kilander et al., 2006; Lintern and Sills, 2006; Mesquita et al., 2009; Lizheng and Huifang, 2010; Xiao et al., 2011). This is especially true when studying flocculated mud particles since no other method can adequately describe their size or morphology without disrupting the flocs and altering their size population when transferring them from suspension to a size measuring device such as a Malvern Mastersizer or Coulter Counter (Chakraborti et al., 2000; Benson and French, 2007; Manning et al., 2007; Kumar et al., 2010).

For typical camera setups, the field of view of the camera is placed within the flow (e.g., Chakraborti et al., 2000; Benson and French, 2007) or within the fluid of a settling column (e.g., Manning et al., 2007; Kumar et al., 2010) and a light source is positioned opposite the camera to provide adequate illumination and contrast between the suspended particle and the fluid (Fig. 1). This lighting technique is known as "bright-field illumination." As a particle or floc passes through the camera's field of view, the backlighting produces a darkened silhouette of the particle against the lighter fluid background that can be captured in the image and measured later in image analysis routines if the pixel to physical length ratio of the sensor and lens combination is known. Sequences of images at known time intervals can then be used to gather information about the particle or floc size distribution with time (Kilander et al., 2006; Benson and French, 2007; Verney et al., 2011), or to measure the settling velocity of particles if the images are collected in a setting column at a high enough sampling or exposure rate (Mikkelsen et al., 2007; Maggi, 2007).

Camera-based data acquisition techniques such as the one described above can generate large volumes of image data that need to be processed before the meaningful data can be obtained. When possible, it is advantageous to automate this process so that higher rates of data an be collected and used (Hatzialekou et al., 1988; Frost and Kuo, 1996; Glaeser, 2004; Smal et al., 2008; Andrews et al., 2010; Irvine-Fynn et al., 2010; Moller et al., 2011). The basic steps involved in extracting particle information from grey scale images often include: reduction in image noise, thresholding the image to distinguish between particles and the background, breaking up particles that are touching, and measurement of the geometric properties of the identified particles left from the thresholding and particle segregation (e.g., Sime and Ferguson, 2003; Graham et al., 2005; Maggi et al., 2006; Benson and French, 2007; Strom et al., 2010). Of these steps, the most critical for image based particle measurement routines is to accurately discriminate between particles and the background using a threshold grey scale value.

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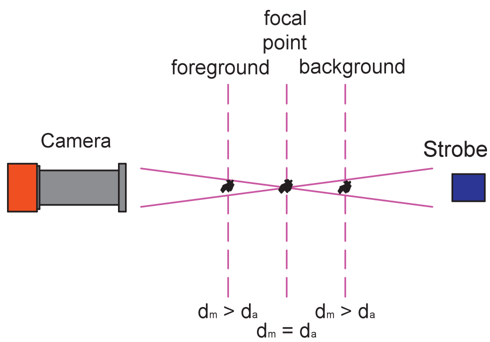

Fig. 1. Schematic of camera and light showing the importance of the point of focus. d_a stands for the actual or true diameter of the floc, and d_m is the measured diameter.

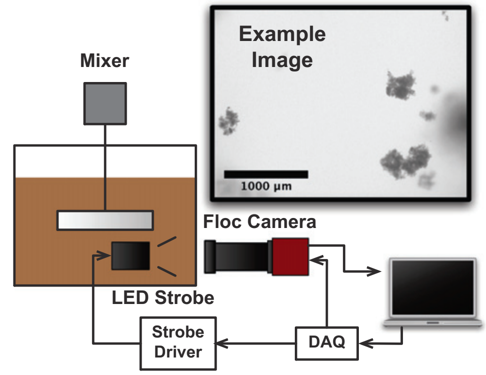

Fig. 2. The system setup with an example image.

If an image is properly thresholded and the physical to pixel length ratio is known through calibration, or by inclusion of a scale within the depth of field, then all particles lying within the plane of focus where the calibration was made can be accurately measured. However, particles located out of the depth of field on either side of the focal plane will appear blurry, which will result in a perceived increase in particles size after image processing (Fig. 1). Sometimes these out of focus particles can be removed during the thresholding step. However, this is not always the case and often out of focus particles are included in the analysis, and these out of focus particles can then introduce inaccuracies in the size measurements (Benson and French, 2007). The objective of this paper is to present an automated image processing method that accurately measures in-focus suspended flocs and removes out-of-focus flocs so that time series of suspended floc populations can be obtained from large sets of images. The image analysis script presented here was developed as part of a larger study on the transformation of mud flocs in fluvial to marine transitions.

2. Floc imaging system

The imaging system used to measure mud floc suspensions consists of a camera and LED strobe controlled and synchronized through a Data Acquisition device (NI-DAQ) and a LabVIEW program written specifically for the setup (Kumar, 2009; Kumar et al., 2010) (Fig. 2). The camera is a 1392 × 1040 pixel AVT Firewire progressive scan, monochrome 8 mm CCD camera fitted with a 2 × primary magnification objective lens (Modular Magnification System kit R-200 plus Objective 9 from Edmund Optics with a 91 mm working distance). Based on the CCD size and 2 × primary magnification, the nominal field of view for the system is 3.2 × 2.4 mm. The camera and optics specifications allow for measurement of flocs from 10 μm to 2.2 mm. The camera can capture single images using an external trigger or can capture continuous images at a rate of up to 17 frames per second. The camera is connected via FireWire which allows for high-speed data transfer, and the camera shutter speed, gain, brightness, gamma, and resolution (camera settings) can be adjusted to achieve the best image possible for the given experimental lighting conditions. The camera is mounted on a heavy steel platform and traversing system that can be moved in the horizontal and vertical directions with fine-scale adjustment knobs for the accurate placement of the camera.

Illumination is provided by an LED spotlight that can be run in continuous, strobe, or trigger mode at varying intensities. The spotlight is controlled by a S4000 Advanced Illumination Strobe source. The continuous lighting mode is used for very slow moving or static fluid conditions. For faster moving flow fields at typical riverine and estuarine turbulence levels, it is not possible to see or freeze the flocs in place in the image using the continuous light source. To remove motion blurring and freeze the flocs in place, the light is strobed by simultaneously triggering the camera and the strobe source.

The pixel to physical length scale ratio for the camera and optics was empirically measured using a Petroff–Hausser counting chamber (Kumar, 2009). The counting chamber is a laser inscribed grid which is typically used to count bacteria. The grid has several graduations of specific lengths. Three length scales were selected for calculating the pixel-to-physical length ratio, those being 1 mm (1000 μm), 0.25 mm (250 μm) and 0.05 mm (50 μm). Several images of the laser counter chamber were taken and then the three length scales were measured at nine locations in the image. By averaging all the pixel to mm values, the calibration for the system was found to be 1 mm = 456 pixels. Based on this conversion, the camera and lens system yield a pixel size of 2.2 μm/pixel.

The depth of field of the lens at the largest aperture was measured by imaging and measuring a marked dot on the surface of the test tank at 0.1 mm increments over a range of distances which spanned before and beyond the focal point (Fig. 3). Based on the calculated size of the marked dot, the measured depth of field is estimated to be 0.4 mm for the system. Within this narrow band, the size of the marked dot was minimized and approximately constant. Outside of this range on either side, the dot size quickly increased due to blurring and its impact on measured size in the image analysis process. The measured values as a function of distance from the focal point also show the importance of only including in-focus particles imaged within the depth of field for accurate floc size measurements.

During experiments on flocculation, using the camera system described above and shows in Fig. 2, images are typically taken at 2 Hz over the course of several hours to characterize the floc size change as a function of time, turbulent shear conditions, and salt levels. Images are taken at this rate to ensure that enough in-focus flocs are imaged to adequately describe the size population at 1-min intervals. Each test produces on the order of 10,000–15,000 images. If only a moderate number of tests are run, say 20, the resulting number of images would be in the range of 200,000–300,000 images (280–420 GB of images). Therefore, to make use of the system and obtain the high temporal resolution data needed to study floc dynamics, an accurate and automated image analysis routine is needed.

3. Image analysis procedure

The fully-automated image processing script developed to process images from the camera system has three major steps that