OMCEC: A Novel Method for Simultaneous Detection of Composition, Geometry and Motion of Suspended Particles

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Abstract
Here, we introduce OMCEC, a laboratory-based system that simultaneously measures the material composition, geometric properties, and motion of individual suspended aggregates in a non-invasive and non-destructive way. OMCEC consists of a full-color high-resolution optical system and real-time algorithms for material segmentation based on light spectra emission analysis, quantification of various geometrical properties, and motion detection with particle tracking velocimetry (PTV). We show applications of OMCEC for the composition analysis of aggregates made of biological matter and (i) mineral and (ii) microplastics.
Keywords: biomass fraction, cell colonization, microplastics, settling velocity, optical measurement.

1. Introduction

Natural waters contain a wide variety of suspended particulate matter (SPM) such as sediment, organic debris, solid contaminants like heavy metals and microplastics, and living microorganisms. SPM can exist as single particles, aggregates of one material, or aggregates of polydisperse materials. Each one has different characteristics and dynamics from each other, and these make it challenging to predict SPM transport and distribution in the water column. While extensive literature can be found on the dynamics of one-material SPM, little is known about mixed SPM.

Unlike non-living materials that bond to each other and break up by physical/chemical forces, living microbial cells can grow biofilms on SPM and alter their properties by colonization and metabolism. These complex processes cause biomass-affected SPM to have distinct size, shape, internal structure, and dynamics as compared to biomass-free SPM (Tang and Maggi, 2016). However, the extent to which biomass affects the SPM dynamics is still not well characterized. One of the reasons is limitations in experimental measurements of biomass content on individual SPM that require destructive procedures altering the SPM properties.

This paper introduces the OMCEC (Optical Measurement of CEll Colonization) system, a novel tool for studying the biomass effects on SPM properties and dynamics in a non-invasive and non-destructive way. OMCEC was first released in Nguyen et al. (2017) for composition analysis of montmorillonite and *Saccharomyces cerevisiae* cells, and has been generalized in this work for uses with different materials and the coupling with the particle tracking velocimetry (PTV) method for motion detection. OMCEC applications to composition analysis of biomass-affected minerals and microplastics SPM are shown and discussed.

2. OMCEC System

OMCEC is a laboratory-based system that includes a high-resolution full-color optical setup to acquire images of settling SPM and image analysis algorithms to systematically separate materials, characterize SPM geometric properties, and detect SPM motion.

2.1. Material preparation

Material separation is based on the difference in the emission spectra of materials. Staining techniques were applied to non-living materials when the test materials have similar emission spectra. In one application of OMCEC (Nguyen et al., 2017), clay minerals were stained with fluorescent dye Rhodamine B so as to be distinguished from *Saccharomyces cerevisiae* cells (emission peaks higher and lower than 550nm, respectively). Other fluorescent dyes that can be used to gain wider spectral emissions are Fluorescein staining on minerals (emission peak less than 550 nm; Diaz et al., 2013) and Nile Red staining on nylon, PET, and PP microplastics (Cole, 2016). Mixed SPM with different materials were then prepared and incubated at specific environmental conditions like nutrient concentrations, turbulence shear, light intensity, and salinity.

2.2. Image acquisition

Ideally, when SPM was incubated in the upper part of our settling column as in Tang and Maggi (2016), SPM freely settled directly into the OMCEC optical system in the lower column through a 5mm hole. In other instances, SPM was gently transferred to the OMCEC optical system by pipetting as in Nguyen et al. (2017) without incubation in the stirred settling column. The OMCEC optical system consists of a Prosilica GT3400C color CCD equipped with a high magnification Navitar 12X Body Tube lens and a light sheet generated by optic fibers connected to a cool white Cree LED. The resolution varied from 1.97 to 2.75 μm depending on the test materials. Sequential frames were acquired and used for the PTV motion detection.

2.3. Image analysis algorithms

The image processing algorithm (Nguyen et al., 2017) includes noise removal and isolation of individual in-focus SPM to retrieve SPM geometric properties and velocities. The SPM size is estimated as the least square covering the SPM image, and the 3D fractal dimension of fractal SPM was reconstructed from the perimeter-based fractal dimension (Maggi and Winterwerp, 2004) or using the light intensity spectrum method (Tang and Maggi, 2016). SPM aggregates are tracked across two sequential frames by matching their vertical position, size, and projected area using the probabilistic method developed in Tang and Maggi (2016). SPM vertical and horizontal velocities were calculated as the distances traveled by the tracked SPM over the time difference between the two frames.

The material separation algorithm classifies the materials in each SPM by means of the red, green, and blue spectral intensities of each pixel in the threshold graph. This graph is a calibration nomogram of the RGB color channels of virgin material image pixels over the color reference space and uses the bisector of the two 99% confidence intervals of each material as the threshold function to detect any separable materials. The primary staining colors should be chosen to give the best separation with the least error (less than 5%). To separate reddish stained minerals and yellowish microbial cells (Fig. 1A), R and G were used as the reference axes (see the threshold graph in Nguyen et al., 2017). In another application to mixed SPM of blueish microplastics and yellowish microbial cells (Fig. 1B), (R+G) and (G+B) were used as the references. Errors stemmed from image resolution and lighting were corrected as in Nguyen et al. (2017). Material volume fractions were calculated as the total pixels of one material over the total SPM pixel count.

3. OMCEC Applications

The OMCEC system was applied to different material combinations at different size scales. We were able to simultaneously obtain size (L), settling velocity (v), and biomass volume fraction (f_b) of individual mixed SPM. Fig. 1-A1 shows an example of SPM made of rhodamine-B-stained montmorillonite and biological matter sampled from Black Wattle Bay, NSW, Australia. Fig. 1-B1 shows an example of SPM made of microplastics and biological matter sampled from Hawkesbury River, NSW, Australia. Microplastics were the sawdust of blue polyurethane pipe. Fig. 1-A2 and B2 report the segmented materials from Fig. 1-A1 and B1, respectively. OMCEC can be applied broadly to study microbial effects on the transport of different materials, including sediment, microplastics, and other solid contaminants.

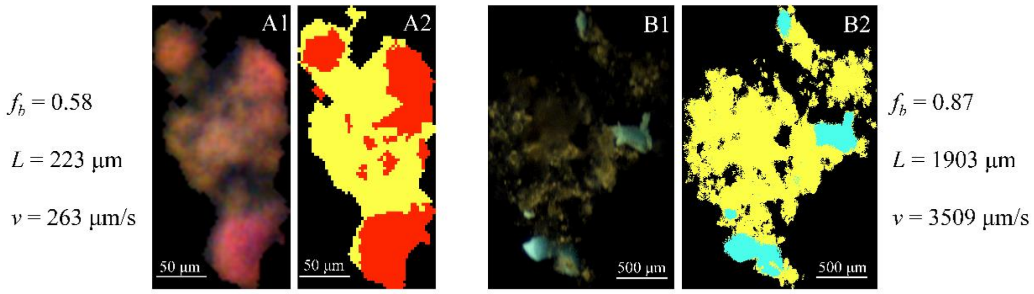

Figure 1. Applications of OMCEC to mixed SPM made of biological matter and (A) minerals, (B) microplastics

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