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Multi Length Scale Imaging of Flocculated Estuarine Sediments; Insights into their Complex 3D Structure

Jonathan Wheatland (1), Andy Bushby (1), Ian Droppo (2), Simon Carr (3), and Kate Spencer (3)

(1) Queen Mary, University of London, School of Engineering & Material Sciences, London, (j.a.t.wheatland@qmul.ac.uk), (2) National Water Research Institute, Environment Canada, Ontario, Canada, (3) School of Geography, Queen Mary, University of London, UK

Suspended estuarine sediments form flocs that are compositionally complex, fragile and irregularly shaped. The fate and transport of suspended particulate matter (SPM) is determined by the size, shape, density, porosity and stability of these flocs and prediction of SPM transport requires accurate measurements of these three-dimensional (3D) physical properties. However, the multi-scaled nature of flocs in addition to their fragility makes their characterisation in 3D problematic.

Correlative microscopy is a strategy involving the spatial registration of information collected at different scales using several imaging modalities. Previously, conventional optical microscopy (COM) and transmission electron microscopy (TEM) have enabled 2-dimensional (2D) floc characterisation at the gross (> 1 μm) and sub-micron scales respectively. Whilst this has proven insightful there remains a critical spatial and dimensional gap preventing the accurate measurement of geometric properties and an understanding of how structures at different scales are related. Within life sciences volumetric imaging techniques such as 3D micro-computed tomography (3D μCT) and focused ion beam scanning electron microscopy [FIB-SEM (or FIB-tomography)] have been combined to characterise materials at the centimetre to micron scale. Combining these techniques with TEM enables an advanced correlative study, allowing material properties across multiple spatial and dimensional scales to be visualised. The aims of this study are; 1) to formulate an advanced correlative imaging strategy combining 3D μCT, FIB-tomography and TEM; 2) to acquire 3D datasets; 3) to produce a model allowing their co-visualisation; 4) to interpret 3D floc structure.

To reduce the chance of structural alterations during analysis samples were first ‘fixed’ in 2.5% glutaraldehyde/2% formaldehyde before being embedding in Durcupan resin. Intermediate steps were implemented to improve contrast and remove pore water, achieved by the addition of heavy metal stains and washing samples in a series of ethanol solutions and acetone. Gross-scale characterisation involved scanning samples using a Nikon Metrology HM X 225 μCT. For micro-scale analysis a working surface was revealed by microtoming the sample. Ultrathin sections were then collected and analysed using a JEOL 1200 Ex II TEM, and FIB-tomography datasets obtained using an FEI Quanta 3D FIB-SEM. Finally, to locate the surface and relate TEM and FIB-tomography datasets to the original floc, samples were rescanned using the μCT. Image processing was initially conducted in ImageJ. Following this datasets were imported into Amira 5.5 where pixel intensity thresholding allowed particle-matrix boundaries to be defined. Using ‘landmarks’ datasets were then registered to enable their co-visualisation in 3D models.

Analysis of registered datasets reveals the complex non-fractal nature of flocs, whose properties span several of orders of magnitude. Primary particles are organised into discrete ‘bundles’, the arrangement of which directly influences their gross morphology. This strategy, which allows the co-visualisation of spatially registered multi-scale 3D datasets, provides unique insights into the true nature floc which would other have been impossible.