

# **Parallel Measurements of Light Scattering and Characterization of Marine Particles in Water: An Evaluation of Methodology**

Dariusz Stramski  
Marine Physical Laboratory  
Scripps Institution of Oceanography  
University of California at San Diego  
La Jolla, CA 92093-0218  
phone: (858) 534-3353 fax: (858) 534-7641 email: [dstramski@ucsd.edu](mailto:dstramski@ucsd.edu)

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<http://www.mpl.ucsd.edu/people/stramski>

## **LONG-TERM GOALS**

The long-term goal of our research is to develop the base of knowledge necessary to:

- (i) understand the magnitudes and variability of the ocean optical properties;
- (ii) predict the inherent and apparent optical properties of the ocean including remote-sensing reflectance, given the types and concentration of suspended particles;
- (iii) retrieve the inherent optical properties and concentration of seawater constituents from remote sensing.

## **OBJECTIVES**

The principal objective of this project is to evaluate various techniques for parallel (or nearly-parallel) determinations of light scattering and particle characteristics using a broad suite of experimental approaches and instruments, including both benchtop and in situ instrumentation. A secondary objective is to characterize variability in the volume scattering function and particle size distribution for various optical water types and samples.

Specific objectives for this reporting period include

- completion of mesocosm experiments to compare different methods of measuring the volume scattering function (VSF) and particle size distribution (PSD)
- collection of field data for assessing and interpreting natural variability in the VSF and PSD for various optical water types and samples.

## **APPROACH**

Our approach consists of experiments designed to directly compare measurements of the volume scattering function and particle size distribution on laboratory and natural particle assemblages of varying composition. For measuring the VSF, we have tested a suite of commercial instruments which

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measure scattering at various angles. These instruments include a Sequoia LISST-100X (32 angles, 0.08-13.5°), a Wyatt Technologies Dawn EOS (18 angles, 22.5-147°), a Wetlabs ECO-VSF (100, 125, and 150°), and a HobiLabs Hydroscat-6 (140°). All instruments utilize a light source of 532 nm, with the exception of the Hydroscat-6 which measures six spectral bands (442, 470, 550, 589, 620, and 671 nm). The combination of these instruments provides a capability for examining the VSF over a broad angular range. With regards to the particle size distribution, we have made direct comparisons of measurements using a Coulter counter, FlowCAM, LISST-100X, and a Spectrex Particle Laser Counter. Each of these instruments employs a different measurement principle of detecting and sizing particles.

A central idea underlying our approach for this project is to conduct mesocosm experiments, in which a large volume tank filled with natural water is subjected to optical and particle measurements and analyses. These experiments are designed to mimic in situ conditions, allowing "non-invasive" measurements on "unperturbed" suspended particles with a suite of instruments that are normally employed at sea. We conduct experiments with differing particle assemblages ranging from standard bead suspensions and specific types of biological particles (e.g. phytoplankton cultures) to heterogeneous assemblages of particles suspended in oceanic water samples. The sampling of natural water used in these experiments covers optically different water types within the coastal zone of San Diego, ranging from the turbid estuary of the Tijuana River to clear oligotrophic waters. To further expand the range of water types, additional field measurements are taken on cruises of opportunity.

In parallel with these tank measurements, sub-volumes of water from the tank are subjected to additional measurements with laboratory bench-top instrumentation and analytical techniques. Some of the lab methods impose no or very little alteration to particles (i.e., immediate non-invasive measurements on sub-volumes taken from the tank without any treatment of the sample) but other lab methods may alter particulate assemblages (i.e., measurements that require some kind of flow of the sample, filtration, dilution, or other treatment).

In addition to parallel mesocosm (large volume tank) and laboratory bench-top (smaller volume sub-samples from the tank) measurements, we also make in situ measurements of light scattering and particle characterization at times when samples of natural ocean water are taken for subsequent tank/lab experiments. Although only a subset of instruments can be deployed in situ, this is an important aspect of our overall approach. These in situ measurements provide a reference suite of characteristics, which allow us to examine the effects associated with water sample storage (e.g., duration, procedure/treatment during storage) between the time of sampling in the field and the time of tank and bench-top measurements in the lab.

In summary, our approach is designed to compare different types of experiments, instruments, and principles involved in the determination of light scatter and particle size distribution in order to develop an understanding of the performance of various methods that are used in oceanography for particle and light scattering characterization.

## **WORK COMPLETED**

During this reporting period, we have completed a series of mesocosm and in situ experiments which permit direct comparison between instruments that measure the VSF, and between instruments that measure the particle size distribution. These experiments were conducted by collecting seawater from

the Scripps pier to fill a large volume tank for subsequent laboratory measurements with the various instrumentation; in situ measurements were also made at the time of collection for comparison with tank measurements. The sampled waters encompass a diverse range of particle characteristics, for example high chlorophyll phytoplankton-dominated communities or assemblages composed primarily of biogenic detritus. Varying contributions of mineral particles were also observed among experiments.

We also participated in two field projects to expand our range of intercomparison measurements in different oceanic environments. During a 2-week study in Monterey Bay in September 2006, we measured particle VSFs and size distributions at 18 stations. We recently completed a 22-day cruise in the Baltic and North Sea (Aug/Sept 2007). Such opportunities afford the opportunity to compare measurements in various optical water types, and cross-compare our measurements with those of other investigators who had identical or similar instrumentation.

## RESULTS

In our previous reports for this project, we described results of experiments comparing measurements of the VSF obtained by different instrumentation for differing particle types. In the present report, we focus instead on comparisons of particle size distribution measurements obtained with three instruments; a Coulter counter Multisizer III (Beckman-Coulter), LISST-100X (Sequoia Sci.), and FlowCAM (Fluid Imaging Tech.). Early results with the Spectrix Particle Laser counter revealed significant issues concerning the accuracy and reliability of measurements, and further work with this instrument was abandoned.

Experiments with monodisperse particle populations (e.g. polystyrene microsphere standards of a known size) suggest that all three instruments yield comparable results in terms of estimating modal particle size. An example illustrating a direct comparison of measurements between the Coulter counter and LISST for several different microsphere standards (1, 2, 5, 10, 20, 50, 100  $\mu\text{m}$ ) is depicted in Figure 1. The Coulter counter provides the higher resolution of the two instruments, and is capable of accurately resolving narrow peaks in a size distribution. The LISST also successfully identifies individual peaks for most of these particles, although the measured distributions are artificially broadened because of the lower resolution and behavior of the inversion algorithm. Difficulties are also observed in accurately resolving the smallest sized particles (e.g. 1 and 2  $\mu\text{m}$ ), suggesting a limitation of the instrument when particles approach the edges of the measured size range.

In natural waters, size distributions are rarely, if ever, monodisperse and instead represent amalgamations of several diverse particle populations of varying sizes, shapes, and chemical composition. Figure 2 compares particle size distributions measured by the three instruments for a representative mesocosm experiment using seawater collected at a depth of 1.5 m from Scripps pier. The PSDs are depicted for both particle number (panel A) and particle volume (panel B) concentrations, and are presented as density functions to compensate for the varying width of size bins within and among instruments. Two measurements using the LISST instrument are shown; an in situ measurement obtained at the time of water collection, and a simulated “in situ” measurement taken within the mesocosm. Subsamples were withdrawn from the tank for measurements with the Coulter counter and FlowCAM.

The measured PSDs all show the similar shape characteristic of natural water samples, with particle number generally decreasing with increasing particle diameter. The presence of individual peaks within the size distribution of this sample are evident, and resolved by all three instruments. A remarkably good agreement in both the shape and magnitude of the measured PSD is observed between Coulter and LISST measurements on the mesocosm (tank) sample. Interestingly, however, the original in situ size distribution as determined by the LISST shows anomalous decreases in the smaller size ranges ( $\sim 2 \mu\text{m}$ ) as compared to the mesocosm measurements; this phenomenon was consistently observed in all experiments. This behavior appears to be associated with enhanced scattering in the largest measured angles for in situ measurements as compared to mesocosm or discrete water samples. At present, we do not have an explanation for this observation.

In contrast, the FlowCAM instrument consistently yields lower particle concentrations than determined with the other two instruments. The FlowCAM was operated with a 20X objective using light scattering/fluorescence triggering for these measurements, and our results suggest a low efficiency at detecting particles when operated in this mode. For particles of  $25 \mu\text{m}$  diameter, particle counts obtained with the FlowCAM are approximately 40% lower than those measured with the Coulter. This underestimation increases with increasingly smaller particles. A major advantage of the FlowCAM is the ability to image and identify particles present in the population; however, it appears that obtaining quantitative information on the overall size distribution with this instrument is difficult.

One metric for examining comparisons between instruments is the median particle diameter calculated from the volume distribution. Figure 3 illustrates a summary of LISST and FlowCAM measurements compared with Coulter-measured size distributions for both laboratory suspensions of monodisperse microspheres and polydisperse natural field samples. The agreement between LISST and Coulter is generally very good for both types of samples. The results comparing FlowCAM with the other two instruments are poorer, and particularly bad for the field samples. The large discrepancy observed in natural water samples results from the low efficiency of the FlowCAM in detecting small particles; the measured particle distributions are thus skewed towards larger particles leading to a significant overestimate of the median particle diameter.

With recently acquired data from field campaigns, we are beginning to examine the interplay between particle size distributions and particle scattering in marine waters. Figure 4 illustrates in situ, depth-resolved measurements of particle scattering and particle size distributions determined by the LISST instrument at a coastal station in Monterey Bay. This shallow station was characterized by an intense phytoplankton bloom restricted to the upper few meters of the water column (chlorophyll *a*  $> 350 \text{ mg m}^{-3}$ ), dominated by the red-tide dinoflagellate *Akashiwo sanguinea*. Beam attenuation at 532 nm varied more than 20-fold over a vertical distance of 12 meters, with values approaching  $15 \text{ m}^{-1}$  near the surface (Fig. 4A). The near-forward VSF(532) of the surface layer increased more than an order of magnitude at most angles, particularly in the smallest angles leading to the presence of a distinct forward scattering lobe (Fig. 4B). The magnitude of the VSF in this layer is considerably higher than reported in the classical measurements of Petzold (1972).

The derived particle size distributions at this station (Fig. 4C) show a dramatic increase in the concentration of particles  $> 2 \mu\text{m}$  in this surface layer as compared to deeper portion of the water column. In particular, a large peak is observed in the distribution centered near a diameter of  $\sim 50 \mu\text{m}$ , which is consistent with microscopic estimations of the general size range of *A. sanguinea* cells. A smaller population of diatoms in this bloom is also revealed by the presence of a lesser peak in the 4–5

$\mu\text{m}$  size range. The presence of these large biological cells vertically within the water column can be estimated to a first approximation by examining the depth-dependence of the median particle diameter (Fig. 4A), which shows strong coherence with the particle scattering and attenuation profile.

## **IMPACT/APPLICATIONS**

The major impact of this project will be to provide an evaluation of the performance of various methods for estimating the volume scattering function and the particle size distribution, characterization of their advantages and disadvantages, and quantification of errors and limitations of individual methods. Based on results of our experiments, we will develop a set of recommendations and improved protocols for the use of various techniques to estimate light scatter and particle characterization. In addition, data generated during this project will contribute to the science of the quantification and understanding of marine particle properties.

## **RELATED PROJECTS**

The coastal zone of San Diego offers an opportunity to examine diverse water types with varying particle assemblages, from the turbid estuary of the Tijuana River to clear oligotrophic ocean water. As part of a NASA funded project, we sampled coastal waters on a regular basis to examine temporal trends in seawater particles and optical properties. The availability of these samples for instrument comparisons, and the extra biological and chemical information they provide about particle assemblages, increases our ability to build a database for comparisons of direct interest to this project.

Other projects and cruises of opportunity in which we have collected additional field data relevant to this project include a north-south transect in the eastern Atlantic (ANT-XXIII/1, NASA), Monterey Bay (COAST experiment, NOAA), and a recent expedition in the Baltic and North Sea.

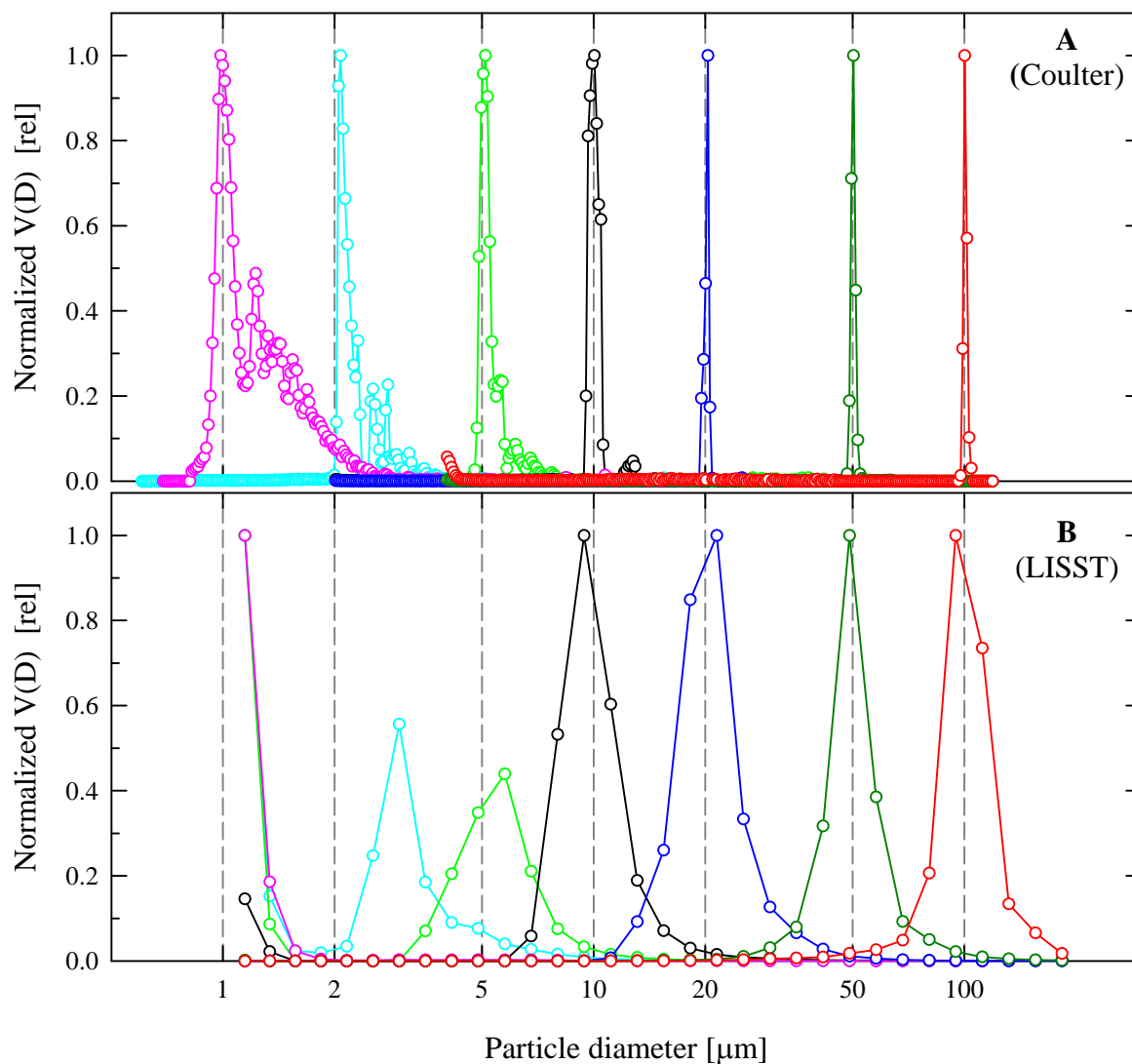
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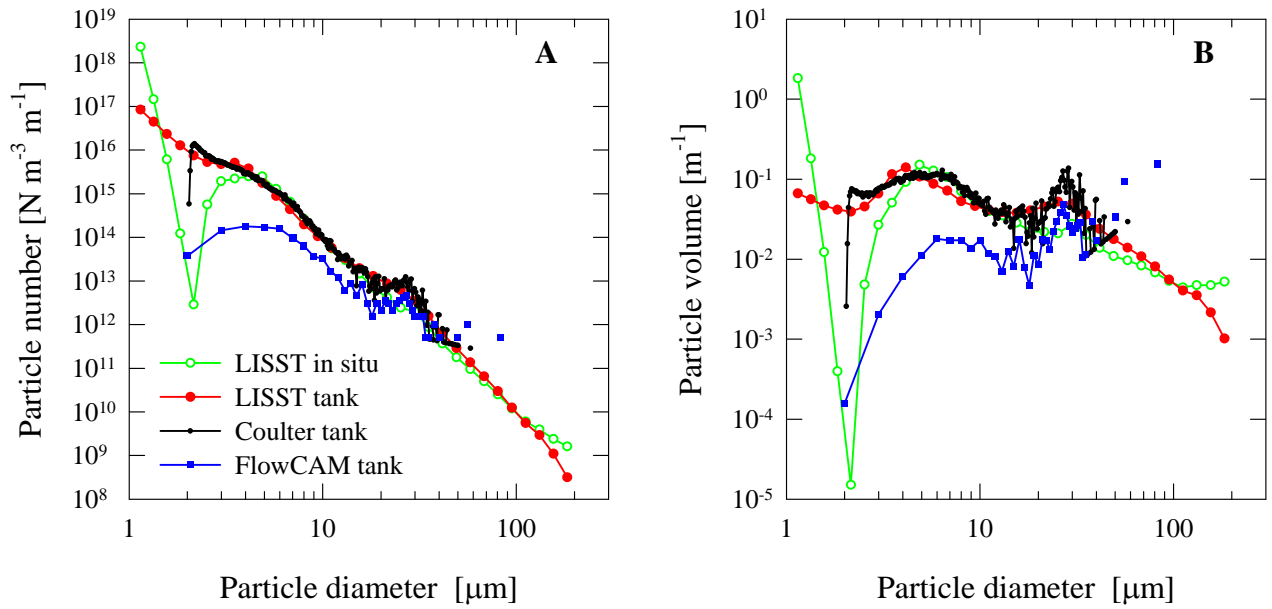
## **PUBLICATIONS**

Reynolds, R.A., D. Stramski, V.M. Wright, and S.B. Wozniak. 2006. Measurement of the volume scattering function using a multi-instrument approach. Ocean Optics OOXII Conference, Montreal, Canada. [published, non-refereed]

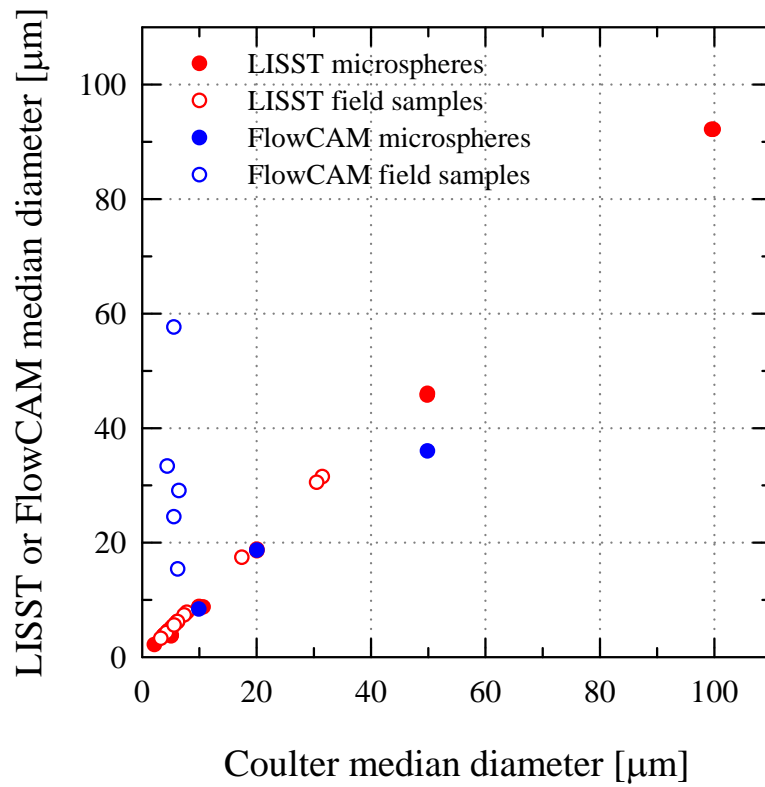
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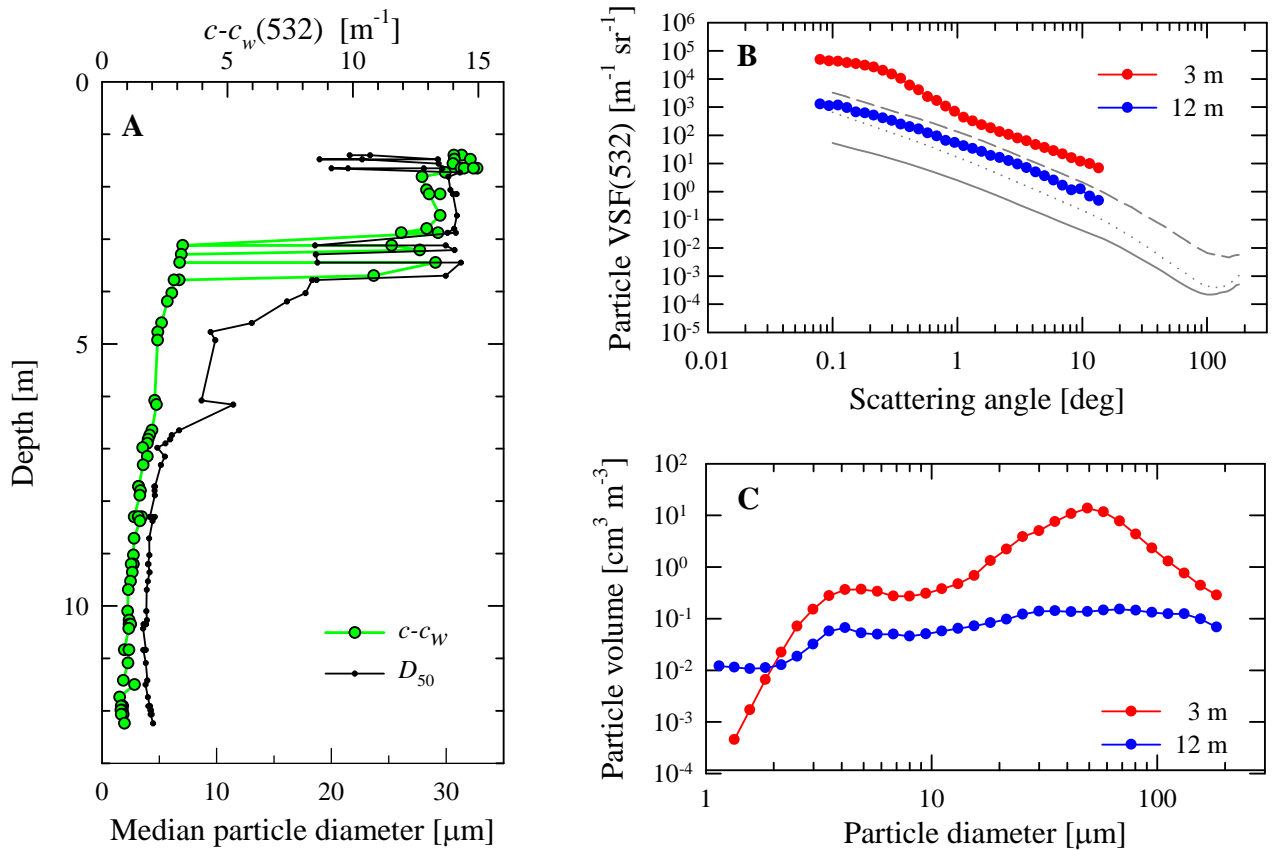
**Figure 1.** Measured size distributions of polystyrene microsphere standards, depicted in terms of particle volume, as determined by a Coulter counter (panel A) and LISST-100X (panel B). Size distributions were measured on individual monodisperse suspensions. For comparative purposes, each size distribution has been normalized to have a value of 1 at the modal peak.



**Figure 2.** Particle size distribution measured with three instruments on a natural seawater sample collected from the SIO pier and placed in a large cylindrical tank. Distributions for both particle number (panel A) and particle volume (panel B) are depicted. For the LISST, measurements done in situ at the time of sampling and within the tank are shown. The Coulter and FlowCAM measurements were made on a subsample of water withdrawn from the tank.



*Figure 3. Scatter plot comparing the median particle diameter derived from particle volume distributions measured with the Coulter counter, LISST-100X, and FlowCAM. Experiments include monodisperse suspensions of polystyrene microspheres of varying size (filled symbols), and natural seawater samples collected from the field (open symbols).*



**Figure 4.** *In situ* measurements of the beam attenuation coefficient at 532 nm and the median particle diameter (A), the particle volume scattering function at 532 nm (B), and the particle size distribution (C) for a station in Monterey Bay (07 Sept. 2006). The contribution of pure seawater has been subtracted from the beam attenuation coefficient. For comparison, the classical measurements of the particle VSF at 514 nm by Petzold (1972) are depicted in panel B for three types of ocean environments (grey lines). This station was characterized by an intense surface phytoplankton bloom dominated by the red-tide dinoflagellate *Akashiwo sanguinea*, as can be seen in the increased particle scattering and distinct peak in the size distribution at 3 m depth.