

Optical scattering properties of phytoplankton: Measurements and comparison of various species at scattering angles between 1° and 170°

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Abstract

We describe the results of a laboratory scattering experiment performed to investigate differences in the optical scattering properties between five common phytoplankton species (*Dunaliella tertiolecta*, *Isochrysis galbana*, *Nanochloropsis*, *Skeletonema costatum*, and *Thalassiosira weissflogii*). Data sets were taken at scattering angles 1° to 170° at incident polarizations parallel and perpendicular to the scattering plane. Differences between the species were especially apparent in the slopes at near-forward (1°–10°) angles and the enhanced scattering at near-backward (150°–165°) angles. There were also notable differences observed between the depolarization ratios, especially at scattering angles between 70° and 110°. The data are shown to be not only helpful in differentiating several species from each other, but also in separating the phytoplankton from bubble/hydrosol contributions to the ocean's inherent optical properties. The results presented here motivate the development of in situ large-angle polarimetric scatterometers and associated retrieval algorithms.

Phytoplankton has a dominant influence on the optical properties of the open ocean (Bricaud and Morel 1986). Remote sensing techniques such as those used in Sea-viewing Wide Field-of-view Sensor (SeaWiFS) and Moderate Resolution Imaging Spectroradiometer (MODIS) take advantage of the effects of cells on ocean color when interpreting reflectance measurements. These properties are also interesting from a biological perspective because they affect the light harvesting abilities of the cells. Phytoplankton is a vital component of both the global and local ecology; its effects on the environment include nutrient depletion, production of photosynthetic byproducts, and changes in the optical properties of the surrounding waters (Garver and Siegel 1997, Moisan and Mitchell 2001). The ability of the ocean to absorb greenhouse gases is also affected by the type of phytoplankton dominating an area, and a better understanding

of how this process works is required (Bisett 2001). In spite of all this, little data are available on the scattering behavior of phytoplankton cells themselves, although it seems likely that scattering will differ between species depending on size, composition, shape, pigmentation, etc. (Morel 1987). Studies do exist addressing the volume scattering function (VSF), or scattered radiance given input irradiance, but knowledge of the optical scattering matrix (which includes polarization effects) is crucial.

Generally, past phytoplankton studies have not fully characterized individual species for a range of angles from forward (approaching 0°) to backward (near 180°) angles for differing polarizations. For example, Slade and Boss (2006) and Agrawal (2005) consider forward angles <20°, whereas Vaillancourt et al. (2004) and Ulloa et al. (1994) study primarily backscattering. Large-angle experiments have been performed, of course: Volten et al. (1998) covers intermediate angles between 20° and 160°, Witkowski et al. (1998) looked at the large-angle scattering matrix for a single species, and Shao et al. (2006) recently presented compelling theoretical as well as experimental results in the 40° to 140° range (albeit without considering polarization effects). Chami et al. (2006) discuss spectral aspects of scattering at large angles. A review of phytoplankton scattering basics is included in Kokhanovsky (2006).

Our study measures the normalized scattering functions of five common species of phytoplankton (*Dunaliella tertiolecta*, *Isochrysis galbana*, *Nanochloropsis*, *Skeletonema*

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Acknowledgments

We thank the University of Maryland's Chesapeake Biological Lab (CBL) for growing the phytoplankton cultures used in this study. We also thank Kyle Ball and David Hadka of Penn State ARL for helping to prepare the manuscript.

This work was supported by NAVAIR under Contract N00421-01-C-0223 PO 0002 at Patuxent River, Maryland. This project was administered through the Pennsylvania State University's Applied Research Lab (Penn State ARL).

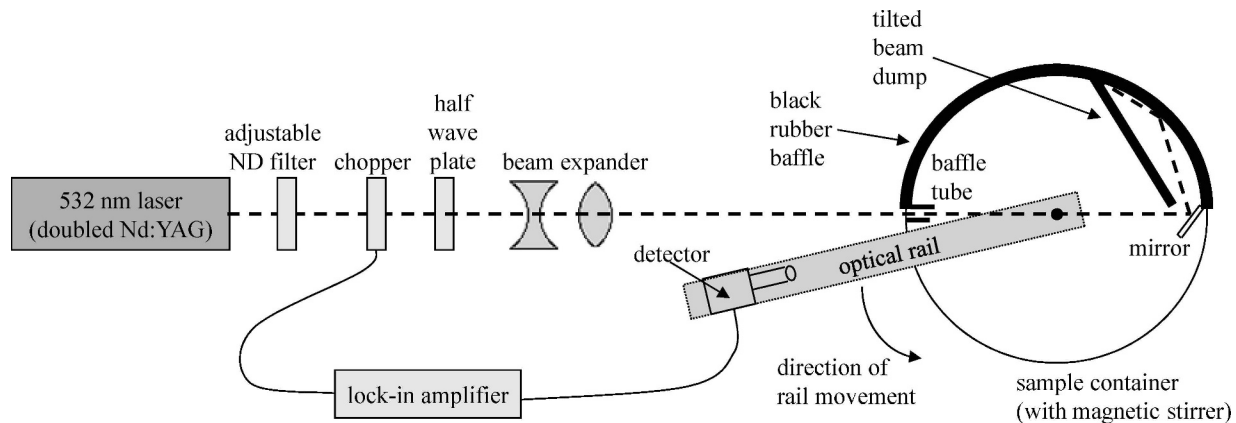


Fig. 1. Diagram of the experimental setup.

costatum, and *Thalassiosira weissflogii*) at orthogonal polarizations and at angles between 1° and 170° . Because the selected species differ not only in size distribution, but shape and internal organelle composition, it was hypothesized that scattering, especially polarization effects, would differ. Thus, species could be differentiated from each other as well as other hydrosols. Similar to the discussion presented by Lee and Lewis (2003), the ultimate point of such data would be to motivate the development of dedicated instruments for in situ measurements of various components of the inherent optical properties of the oceans. This capability will have impact on ocean color interpretation (Chami et al., 2006), ocean ecologists, and other researchers interested in the radiative transfer of light in natural water.

Experimental setup

The experimental setup, shown diagrammatically in Fig. 1, is a polar nephelometer adapted from that used by Laux et al. (2002) and Coakley et al. (unpubl.). A doubled Nd:YAG (neodymium-doped yttrium aluminum garnet) laser at 532 nm laser was chosen as the source because of the applications of that particular wavelength in remote sensing of the ocean. The beam first passed through a neutral density filter wheel (this enabled us to keep the detector within its linear range) and a chopper to enable detection by a lock-in amplifier. Next, the beam was transmitted through a half-wave plate to set its polarization as parallel or perpendicular to the scattering plane, which is defined as the plane that includes the incident beam and the detector optical rail. After the half-wave plate, the laser beam was passed through a beam expander to increase the beam diameter within the sample. A photomultiplier tube was used as the detector; the response of the detector was polarization-independent. The detector field-of-view was $<0.5^\circ$ FWHM (Full-Width at Half Maximum). Each data set was measured by moving the rotation stage in one degree increments and recording the scattered signal.

The sample cell used was a 255-mm-diameter Pyrex® glass cylinder with a magnetic stirrer. For a circular interface, the field of view of the detector is always perpendicular to the tangent of the sample cell wall; this

retains the polarization ratio of the incident beam. Two setups were used within the cell, depending upon whether forward or backward angle scattering intensities were being measured. For the forward angles (angles $<90^\circ$) and some of the middle angles (90° – 110°), reflections were controlled by using both a lens tube surrounding the input beam and a black rubber baffle placed at the back of the cell. For the backward angles (angles $>110^\circ$), a beam dump was used in addition to the lens tube and baffle previously mentioned. The beam dump was used to prevent reflections from the front of the cell from interfering with the backward scattered light signal. The presence of the baffling did not interfere with the scattering volume for angles between 1° and 170° . As the detector was moved in an arc around the sample cell, the detector view angle changed the apparent beam path length within the sample. As pointed out by Lee and Lewis (2003), the greatest uncertainty in the data handling occurs in determination of this scattering volume. To account for these differences, the data were weighted by the sampling volume at each respective angle.

A correction was needed to separate scattering caused by the background medium from that caused by the target scatterers. Background measurements were performed before adding any scatterers to the medium. Distilled water was the medium used for the polystyrene calibration beads (index of refraction, $n = 1.59$); filtered seawater was used for the phytoplankton measurements (this was done expeditiously and with a magnetic stirrer, acknowledging the fact that organic material in filtered seawater can reassemble into gel particle and impact the measurements). Multiple measurements of both backgrounds were performed to check stability. These background measurements were subtracted from the experimental results to account for scattering caused by the background medium and sample cell.

In order to have confidence in the experimental data, it was necessary to compare the corrected measurements to scattering theory. In this case, polystyrene beads of various sizes were used. As the beads were almost perfectly spherical, the results could be compared to Mie theory calculations. Figure 2 shows the results for $5\text{-}\mu\text{m}$ (comparable in size to many phytoplankton species) beads for parallel polarization; the data for perpendicular polarization are similar, and both follow the structure of the Mie

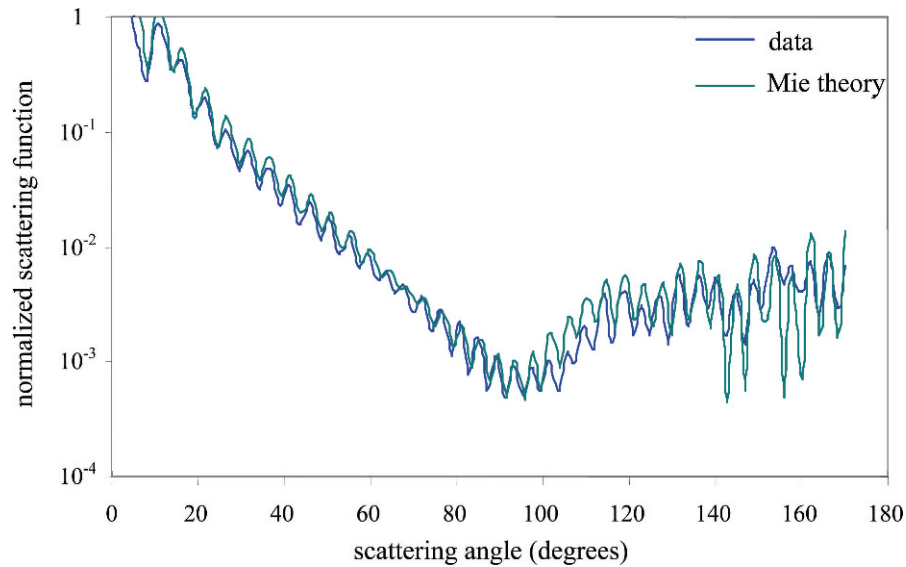


Fig. 2. Normalized scattering function versus scattering angle for 5- μm spherical beads ($n = 1.59$) in distilled water at incident parallel polarization. The data were taken at one degree intervals between 5° and 170° and plotted along with Mie scattering theory for the same size sphere for comparison. The data were normalized by dividing all data points by the reading for 5° . Similar results occurred for incident perpendicular polarization.

theory calculations. Some relative changes between maxima and minima are not as marked in the data as in the theory, likely the result of spatial averaging due to the finite receiver field of view (especially at very small angles) or a slight mismatch in size distribution between beads and Mie calculations. Despite this discrepancy, it is clear from these results that the setup and data handling result in a scattering structure that accurately reflects that found in theory, and this setup can be expected to provide equally accurate results in experimental scattering targets. Of course, natural suspensions will likely have less structure in their scattering functions because they are more polydisperse.

Selected phytoplankton species

The species of phytoplankton used in this experiment were grown at the University of Maryland's Chesapeake Biological Lab (CBL). Five species from three different classes were chosen from CBL's stocks:

D. tertiolecta belongs to the class *Chlorophyceae* and is a nearly spherically shaped cell about 5–10 μm in diameter. Its genus is characterized by the presence of two flagella and an elastic cell envelope and the lack of visible cell walls. This species is found in coastal areas and the Atlantic Ocean and is brownish-green in color. Belonging to this same class is *Nanochloropsis*, also known as *Nanochloris*. It is a small species and is approximately 2–4 μm in diameter.

I. galbana belongs to the class *Pyrrhomonophyceae* and is a dinoflagellate. It is found in the same areas as *D. tertiolecta* and is approximately 5 μm in diameter.

S. costatum (2–21- μm diameter) and *T. weissflogii* (6–20 $\mu\text{m} \times 8$ –15 μm) are both diatoms (class Bacillariophyceae). *S. costatum* is considered the dominant species in temperate coastal waters by Sieburth (1979). Its cells form

in chains by use of external tubes, and its environment is considered “cosmopolitan,” meaning that it can be present anywhere in the world's oceans excepting the high Arctic and Antarctic. *T. weissflogii* cells form a modified ring connected by organic threads. The species is often found in fresh to brackish waters and lacks the chain formation when found in bloom concentrations.

Concentrations of each culture sample used for the experiment were chosen well below the level where multiple scattering became significant. This threshold was determined experimentally as the point when backscatter no longer increased linearly with scatterer concentration.

Experimental results

Figure 3 shows the experimental scattering results for each species, measured at incident perpendicular and parallel polarizations. The near-forward angles are similar for all species, although *Nanochloropsis* has a shallower slope in this regime. *I. galbana* and *D. tertiolecta* plots have similar shape in the backscattering region. In fact, all species show increased scattering in the backscattering region between 150° and 160° , although the position and shape of the increase varies somewhat. The major difference between polarizations in all species occurs between 60° and 120° . Three species, namely *Nanochloropsis*, *S. costatum*, and *T. weissflogii*, show a pronounced minimum between 90° and 110° for parallel polarization but not for perpendicular polarization. *I. galbana* has a less pronounced minimum that falls near 120° ; *D. tertiolecta* has a smoother curve and displays more scattering in the middle angles than the other four species.

The fact that *Nanochloropsis* was the smallest species investigated explains the difference in slope in the forward angles because Mie theory predicts a relationship between

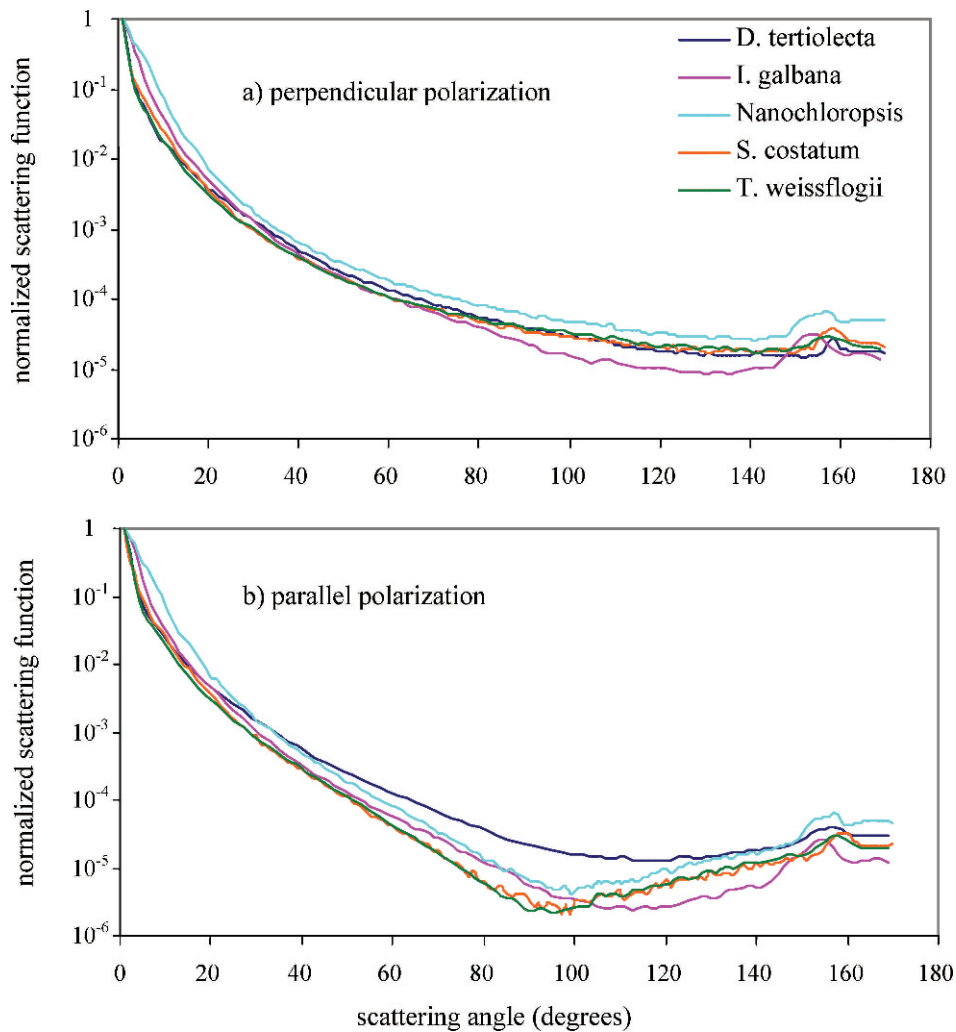


Fig. 3. Normalized scattering function versus scattering angle comparison for the different phytoplankton species in filtered seawater for incident (a) perpendicular and (b) parallel polarization. The data were taken for 1° – 170° in one degree increments. The data were normalized by dividing all of the data points of a species by that species' reading at 1° .

the slope of the forward angles and the size distribution of the scatterer (specifically, larger scatterers have stronger forward scatter, consequently steeper slopes in the scattering functions; Bohren and Huffman 1984). Differences in the scattering properties of the other species may be explained by the work of Volten et al. (1998) and Witkowski et al. (1998), who demonstrated that vacuoles and other internal structures can significantly affect scattering. For example, the lower scattering of perpendicular polarization by *I. galbana* in the middle angles is likely due to its relative lack of internal structures. *D. tertiolecta* and *I. galbana*'s flagella account for their lack of a pronounced minimum around 90° compared to the other three species. The differences in the backward angle scattering can be explained by the number of chloroplasts within a cell. *D. tertiolecta* and *S. costatum* have a similar number per cell (one to two), hence they have similar scattering effects in this region. In contrast, *T. weissflogii* has

numerous chloroplasts arranged around the periphery of the cell, yielding a distinct backscattering characteristic.

Petzold (1972), Lee and Lewis (2003), and Agrawal (2005), among others, have performed similar experiments to ours on actual ocean waters containing multiple species, sediment, bubbles, etc. It should be noted that none of these studies explicitly discuss growth conditions, which can have an impact on the data (e.g., Voss et al. 1998). Comparing the current data to Petzold (1972) as redrawn in Mobley (1994), the log/linear plots of Petzold are similar in shape to the current data, which should be no surprise, as virtually all natural hydrosols will exhibit this similar structure. Petzold's "coastal ocean" data also include the rise at the near-backward ($>170^{\circ}$) angles, although in our present data an additional increase in scattering at 150° to 160° is superimposed. Additionally, several of the plankton scattering curves in our results have a pronounced dip near 90° , which is absent in the Petzold coastal water plots

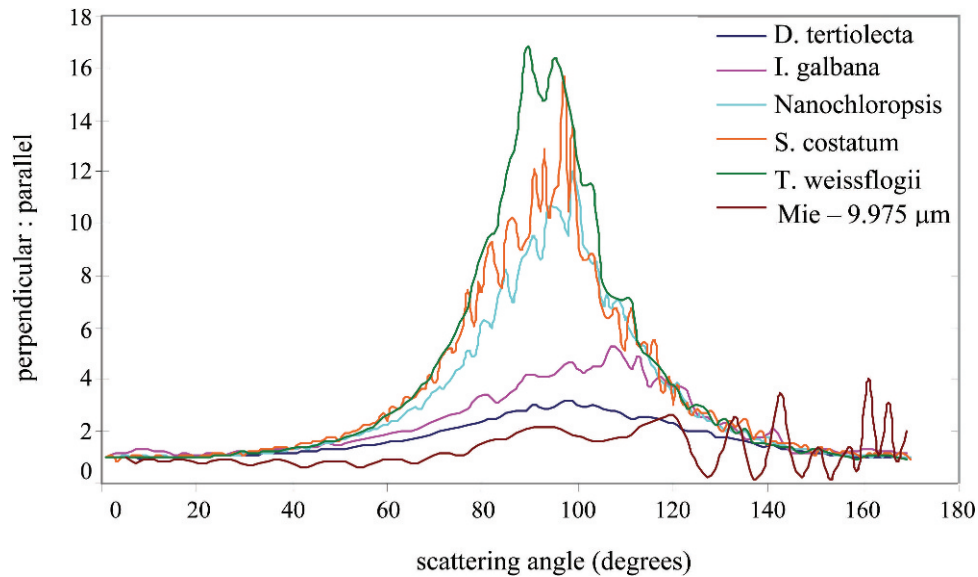


Fig. 4. Ratio of polarized scatter (perpendicular : parallel) plotted versus scattering angle for the different phytoplankton species and Mie theory calculations for $\sim 10\text{-}\mu\text{m}$ -diameter spheres ($n = 1.59$).

(easily explained by sediment and other hydrosol contributions to the total VSF). One has to tread carefully with such comparisons, however; Agrawal (2005) notes that his data quantitatively “only occasionally agree with” the widely referenced Petzold (1972) data.

To further explore the polarization results from our experiment, the ratios of normalized perpendicularly-polarized scattering to normalized parallel-polarized scattering data for each species are plotted together in Fig. 4. Also shown for comparison are the results for $10\text{-}\mu\text{m}$ spherical beads, calculated from Mie theory. All of the phytoplankton exhibit near unity ratio for both forward and backscatter. In the middle angles, the ratios increase for all phytoplankton (differentiating them from the Mie results), peaking around 90° at between 3 for *D. tertiolecta* and 17 for *T. weissflogii*. The strongest ratios, as perhaps expected, are for *S. costatum* and *T. weissflogii*, which are both diatoms and decidedly non-spherical. The Mie plot develops a distinct oscillatory pattern starting around 90° (Bohren and Huffman 1984). It can be concluded that spherical (bubbles and other small hydrosols) and non-spherical particles (many plankton) cannot be easily differentiated by their forward angle or backscatter polarization ratios, but can be differentiated in the middle angles between 70° and 120° . Extending this, some of the phytoplankton species themselves can be differentiated in this regime.

The five species of phytoplankton studied here (*D. tertiolecta*, *I. galbana*, *Nanochloropsis*, *S. costatum*, and *T. weissflogii*) exhibit discernable differences in their relative scattering properties, including polarization effects. The Agrawal (2005) and Petzold (1972) data, both taken in situ in coastal waters, are generally similar in shape to the present data, although several of the plankton scattering curves in our results have a pronounced dip near 90° , which is absent in the coastal water plots. The differences are due

to a number of factors, possibly including the lack of bubbles or the filtering out of organic matter in our laboratory experiment. Shao et al. (2006) examined some other organisms, primarily picoplankton (e.g., *Ostreococcus sp.* Strain CCE9901) and found scattering functions similar in shape to ours as well. Of particular interest are their polarization results, which like ours exhibit a strong signature in the 60° to 120° range. Volten et al. (1998) had an experimental setup perhaps closest to ours, including defining an equivalent depolarization ratio. They examined different species than we did, however, and their ratios (as compared to our results in Fig. 4) did not show nearly as much diversity in values between species.

The effects of internal and external cell structures and absorption by pigments on scattering need to be investigated. This will enable a better understanding of the differences between the species that have been observed in this experiment. The effects of pigments also help explain the discrepancies seen in scattering behavior by two species when their pigment content varied between samples. Such conditions necessitate multi-spectral studies of the scattering properties (e.g., Chami et al. 2006).

Complete characterization of optical scattering in photic zone ocean waters must include both plankton and bubble/hydrosol effects. Coakley et al. (unpubl.) found significant differences between the scattering properties of bubbles in pure water, bubbles in seawater, and phytoplankton. In particular, the initial rise in the bubble depolarization ratio curve occurs near 80° , rather than near 60° as in the phytoplankton curve (Fig. 4). Both Coakley et al. (unpubl.) and Zhang et al. (2002) found that the bubble scattering curve differs from the phytoplankton scattering functions (Fig. 3) in that bubbles produce a critical angle scattering “bump” between 60° and 80° . These differences will enable retrieval of algorithm separation between plankton, bubbles, and other hydrosols.

The goal of this work was to verify the hypothesis that strong measurable differences in optical scattering exist between various common phytoplankton species as well as the natural background. Our results, in league with others, are motivation for further experiments, addressing not only other species and hydrosols, but different wavelengths as well. Such data will lead to inversion algorithm and instrument development (when we acquire additional data, an information content analysis will quantitatively determine the principle components of interest). The dedicated instrument will ultimately be designed around optimal fixed angles and wavelengths, enabling robust deployment.

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Received: 17 October 2006

Accepted: 13 July 2007

Amended: 7 September 2007