1   **Millimeter scale profiles of chlorophyll fluorescence: deciphering the microscale spatial structure of**
2   **phytoplankton.**

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4   Mark J. Doubell¹, Jennifer C. Prairie², Hidekatsu Yamazaki³

5   ¹South Australian Research and Development Institute, 2 Hamra Avenue, West Beach, South Australia
6   5024, Australia.

7   ²Department of Mathematics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina.

8   ³Faculty of Marine Science, Tokyo University of Marine Science and Technology, 5-7, Konan 4, Minato-ku,
9   Tokyo 108-8477, Japan.

10   *Corresponding author address: Hidekatsu Yamazaki, E-mail: hide@kaiyodai.ac.jp

11

12   **Abstract**

13   Marine food webs and biogeochemical cycles are driven by interactions between individual phytoplankton
14   and other micro-organisms embedded within turbulent flows. Understanding the causes and ecological
15   consequences of these interactions requires measurement of the spatial distribution of organisms across
16   sub-meter scales relevant to their activities. However, estimates of many microscale processes (e.g.,
17   encounter rates, competition) are implicitly based on a random distribution of plankton despite increasing
18   evidence of patchy distributions of turbulence and phytoplankton at the oceans microscale. Further
19   complicating our understanding of microscale phytoplankton ecology, recent studies have suggested that
20   the high levels of fluorescence variability measured at sub-centimeter scales may be due to the detection of
21   separate, large phytoplankton particles (i.e. large cells, chains and aggregates) rather than ‘patches’ of
22   increased cell abundances. By comparing coincident fluorescence estimates measured with millimeter (μL)
23   and centimeter (mL) scale resolution, we show that estimates of phytoplankton biomass made at centimeter
24   scales are consistent with averaging discrete variations in fluorescence measured at millimeter scales and
25   that a critical scale exists where measures of fluorescence variability transitions from representing an
26   individual to a patch. Application of nearest neighbor analysis to the discrete fluorescence patterns showed
27   deviations from complete spatial randomness towards clustering across scales of millimeters to tens of
28   centimeters. The strength of the deviation from random increased significantly in regions of elevated
29   phytoplankton concentrations. No relationship was observed between fluorescent particle concentrations or
30   nearest neighbor distances with the rate of dissipation of turbulent kinetic energy. Our results provide
31   empirical evidence that the scale at which phytoplankton distributions are estimated by chlorophyll

32 fluorescence may be critical to the interpretation of microscale phytoplankton spatial patterns and that
33 biological processes may strongly influence the microscale structure and ecology of marine systems.

34

35 **Keywords**

36 phytoplankton, turbulence, microstructure, marine ecology

37 1.1 Introduction

38 A microscopic inspection of seawater reveals that plankton and many of the resources for which they
39 compete are distributed discretely in a dynamic seascape of organic matter (Azam and Malfatti 2007). For
40 example, phytoplankton occur as individual cells, chains or aggregates, all of which are competing for
41 nutrient molecules as well as providing food for grazers and loci for bacteria and viruses. Embedded within
42 turbulent flows, interactions among phytoplankton, other organisms and biogenic particles drive plankton
43 trophodynamics and biogeochemical cycling with cumulative influences for ocean systems predicted
44 across multiple scales (Azam and Worden 2004). Advancing our understanding of the influence of
45 individual interactions between organisms on marine systems therefore requires the measurement of
46 phytoplankton distributions (as well as other microscopic organisms) and turbulence across the nanometer
47 to centimeter scales (microscale) relevant to their activities (Azam and Malfatti 2007).

48
49 Phytoplankton play a central role in plankton ecology and are strongly influenced by turbulence across its
50 entire size spectra (Kiørboe 1993, Martin 2003, Jumars et al. 2009). At the smallest dissipative scales of
51 turbulence, eddies of the order of millimeters interact intimately with phytoplankton influencing many
52 fundamental ecological processes such as behavior (Dower et al. 1997), nutrient uptake (Karp-Boss et al,
53 1996), plankton trophodynamics (Rothschild and Osborn 1988) and organism growth (Peters et al. 2006).
54 However, since microscale turbulent motions have typically been regarded to be a homogenizing factor, the
55 modeling of microscale planktonic processes such as encounter rates (Rothschild & Osborn 1988),
56 coagulation (Jackson 1990), nutrient competition (Siegel 1998) and ecosystem metabolism (Rothschild

1992) have implicitly assumed a random (Poisson) spatial distribution of particles. Progress in our understanding of the microscale structure of turbulence at the scale of phytoplankters (Jumars et al. 2009) now reveals that vortical motions may lead to aggregation (Crimaldi et al. 2006). Similarly, biological processes such as reproduction have the potential for causing non-random, aggregative distribution patterns (Young et al. 2001). As technological advances allow for the measurement of phytoplankton distributions with greater resolution, deciphering the *in situ* spatial patterns observed over a range of natural systems is fundamental to developing our knowledge of the mechanisms underpinning the microscale spatial structure and ecology of marine ecosystems (Levin 1992).

The microscale distribution of phytoplankton has recently been studied with a variety of high-resolution instruments including water sampling devices (Waters et al. 2003), microstructure profiling fluorometers (Desiderio et al. 1993, Wolk et al. 2001, Doubell et al. 2006, 2009) as well as fluorescence imaging systems (Franks et al. 2001, Prairie et al. 2010) and holography (Katz et al. 1999, Davis et al. 2005, Malkiel et al. 2006). Various fluorescence based measurement systems have all shown increasing levels of fluorescent intermittency with a reduction in sample volume size (Desiderio et al. 1993, Franks et al. 2001, Doubell et al. 2006, 2009) attributable to the preferential detection of larger individual cells, chains and aggregates (Franks 2005). Coincident measurements of the spatial distribution of phytoplankton and turbulence has remained a technically challenging endeavor limited to microstructure profilers that contain adjacent fluorescence and shear sensors (Wolk et al. 2001, 2002, Mitchell et al. 2008, Doubell et al. 2009). Moreover, efforts to understand the mechanisms driving the high levels of observed spatial variability using

77 conventional (Gaussian) models such as spectral analysis (Denman et al. 1995) appear to be limited by the
78 discrete nature of phytoplankton distributions when resolved at these small scales (Franks 2005, Doubell et
79 al. 2009). These findings suggest that for any given system, a critical scale exists such that measures of
80 chlorophyll fluorescence (used as an indicator of phytoplankton biomass) transition from representing a
81 continuum (i.e. Gaussian process) to representing a discrete variable (i.e. point process). Identifying this
82 scale is central to the interpretation of microscale phytoplankton patchiness.

83

84 In this context, the first part of this paper identifies the length scale at which the measurement of
85 phytoplankton fluorescence transitions from a bulk property to discrete phytoplankton particles by
86 investigating the probability density function (PDF) of the fluorescence signal measured over different
87 sample volume sizes. Second, we evaluate the spatial structure of separate fluorescent particles through the
88 application of point-process statistics (Diggle 1983, Rothschild 1992). Specifically, the statistical
89 distribution of nearest neighbor distances (NND) is compared to those drawn from a random (Poisson)
90 distribution in order to identify the microscale spatial structure of phytoplankton. Finally, spatial and
91 temporal shifts in particle concentrations and NND are compared with changes in the background particle
92 concentrations and turbulence conditions to understand how the spatial distribution of fluorescent particles
93 fit with the patterns and processes observed at larger scales.

94

95   **2.1 Methods**

96   **2.1.1 Field site and sampling**

97   Tokyo Bay is a semi-enclosed bay with an estuarine circulation which is intensified during summer due to
98   increased freshwater input into the bay and increased thermal stratification (Nakayama et al. 2005). At the
99   narrow mouth (6 km wide, 50 m depth) out-flowing freshwater overlies denser in-flowing seawater creating
100   strong stratification. The vertical structure of the water column is periodically modulated by tidal mixing
101   which restricts seawater exchange in the bay (Yanagi et al. 2003). As a consequence of the limited exchange
102   and increased eutrophication of the inner bay region, frequent bloom events including harmful algal blooms
103   typically occur throughout the spring, summer and autumn periods (Han and Furuya 2000).

104

105   We sampled at the entrance of Tokyo Bay (35°16'26" N, 139°43'14" E) using the Tokyo University of
106   Marine Science and Technology *RTV Seiyō Maru* on May 18 and 19, 2009. Profiling was conducted every
107   15 minutes over a 16 hour period using a TurboMAP-L microstructure profiler (Doubell et al. 2009).
108   TurboMAP-L provided undisturbed measures of turbulent shear ($\partial u / \partial z$, $\partial v / \partial z$), *in vivo* chlorophyll-a
109   fluorescence, turbidity as well as standard hydrographic parameters (conductivity, temperature and depth).
110   TurboMAP-L sensors are sampled at a rate between 64 and 512 Hz for different channels. Typical free-fall
111   descent rates were about 0.65 m s⁻¹.

112

113   TurboMAP-L contains two fluorescence sensors for the measurement of microscale phytoplankton
114   distributions. The sensors are separated by a distance of 12 cm and both sensors sample at 256 Hz.

115 Fluorescence concentrations measured with the light-emitting-diode (LED) fluorescence/turbidity sensor
116 are averaged over a sample volume of approximately 4 mL and have a spatial resolution of approximately 2
117 cm (Wolk et al. 2001, 2006). The LASER sensor, with a sample volume of 32 μL can resolve spatial
118 features as small as 1 mm and at typical descent rates gives independent measures of the fluorescence field
119 approximately every 2-3 mm (Doubell et al. 2009). Both fluorescence sensors are calibrated so that the
120 normally arbitrary units used to measure chlorophyll *a* fluorescence are approximately equivalent to $\mu\text{g L}^{-1}$
121 (Wolk et al. 2001, 2002, Doubell et al. 2009).

122
123 Intense spikes in fluorescence measured by the TurboMAP-L LASER fluorescence sensor have been
124 shown to be consistent with the measurement of separate particles with a mean size of 1 mm in Tokyo Bay
125 (Doubell et al. 2009). Additional comparisons of the TurboMAP-L LASER profiles with fluorescence
126 images obtained using a planer laser imaging system (Prairie et al. 2011) have shown that the high
127 fluorescence concentration of spikes measured by TurboMAP-L are driven by the preferential detection of
128 large individual ($>20\ \mu\text{m}$) fluorescent particles (Franks 2005). The composition of these particles is varied
129 and includes individual large cells, chains and aggregates (Franks et al. 2008, Prairie et al. 2010, 2011).
130 Since identification of the composition of fluorescence peaks was not possible in this study we simply refer
131 to these peaks as fluorescent particles.

132
133 Supplementary measures of phytoplankton community composition, species abundance and biomass were
134 made by water sampling and standard CTD and fluorescence profiling using a Seapoint system. Near

135 surface and bottom water samples were taken at 10 and 40 m depths and 1 L samples for phytoplankton
136 identification and enumeration were fixed with buffered formalin (1% final concentration). Water samples
137 were settled using the Utermöhl technique and counted under an inverted microscope.

138

139 **2.1.2 Fluorescence PDF fitting**

140 Fluorescence measures from the LED sensor were first low-pass filtered at 50 Hz to suppress sensor noise
141 whilst retaining the spatial scales resolved by the sensor (Wolk et al. 2006, Sato & Yamazaki 2008). No
142 correction was required for fluorescence measured by the LASER sensor (Doubell et al. 2009). The
143 fluorescence signal for each sensor was then divided into 4 m sub-regions (number of samples~1600) and
144 log-transformed. The probability density function (PDF) for each sub-region was then determined using the
145 histogram estimated from the empirical cumulative distribution function (CDF).

146

147 **2.1.3 Point process analysis**

148 For each sub-region of fluorescence measured by the LASER sensor, the locations of fluorescent peaks,
149 assumed to be discrete fluorescent particles ($s_1, \dots, s_n$), was used to form a point pattern of events on a line
150 segment (R) of length 4 m. R was chosen as 4 m as this provided sufficient data for the accurate
151 determination of the dissipation rate of turbulent kinetic energy and particle number counts (>350) for the
152 nearest neighbor analysis. Each particle location, s_i , corresponded to the depth of the local fluorescence
153 maximum for each peak above a threshold fluorescence value. Threshold values were conservatively set to
154 the mean fluorescence value for each R . Using this method, particles could not occur at a separation

155 distance smaller than the sample interval determined by descent speed of the profiler (~2.5 mm).

156

157 The particle concentration (λ) is simply the number of particles counted in each R , standardized by the total
158 volume of water sampled in each R to give the number of particles per liter. Particle concentrations were
159 then compared to the mean chlorophyll concentration (CHL) and the dissipation rate of turbulent kinetic
160 energy (ε) for each R

161

162 Based on the TurboMAP-L configuration and assuming the turbulence is locally isotropic, the dissipation
163 rate of the turbulent kinetic energy, ε (W kg^{-1}), can be estimated from the shear spectrum,

164
$$\varepsilon = \frac{15}{4} \nu \left(\left\langle \left(\frac{\partial u'}{\partial z} \right)^2 \right\rangle + \left\langle \left(\frac{\partial v'}{\partial z} \right)^2 \right\rangle \right) \quad (1)$$

165 where $\langle \cdot \rangle$ is the ensemble average, u' and v' are the fluctuations of horizontal velocity components and
166 ν is the kinematic viscosity (Tennekes and Lumley 1972). The shear variance was estimated by integrating
167 the power spectra of the velocity shears, $\frac{\partial u'}{\partial z}$ and $\frac{\partial v'}{\partial z}$, between 1 cycle per meter (cpm) and half the
168 Kolmogorov scale. A correction is made to recover the unresolved variance using the Nasmyth empirical
169 spectrum (Oakey 1982).

170

171 To further explore the spatial structure of fluorescence particles, the nearest neighbor particle-particle
172 distance (NND) was calculated. This is defined as the distance between a randomly chosen particle and the
173 nearest neighboring particle in R . The empirical cumulative distribution function (CDF) of NND is given
174 by:

$$\hat{G}(NND) = \frac{\#(NND_i \leq NND)}{N} \quad (2)$$

where N is the number of particles randomly sampled from R .

$\hat{G}(NND)$ was then compared to the distribution expected for complete spatial randomness (CSR) by generating a random (Poisson) distribution of N particles in a line segment of length R using the Monte Carlo technique (Diggle 1983). Monte Carlo simulations assumed a minimum separation distance equal to the mean sample interval for each R , thus mimicking the data collected from the TurboMAP-L system.

To quantify the extent of deviation from CSR the ‘z score’ (Freedman et al. 1980) was calculated as:

$$z = \frac{\overline{X}_S - \overline{X}_r}{\sigma_r / \sqrt{N}} \quad (3)$$

where $\overline{X}_S$ is the mean NND of a sample, N is the number of particles in the sample, while $\overline{X}_r$ and σ_r are the mean and the standard deviation of the NND obtained from the random simulation. For a result to be statistically significant ($p < 0.05$) the z score should have a magnitude >1.96 . A $z > 1.96$ indicates a spatially uniform mean distance between particles, whereas a $z < -1.96$ indicates clustering. Additionally, we show how spatio-temporal changes in the NND , summarized by the mean nearest neighbor distance (m_{NND}) for each R , are related to changes in particle concentration (λ) and the dissipation rate of turbulent kinetic energy (ϵ).

194   **3.1 Results**

195   **3.1.1 Oceanographic conditions**

196   The overlay of warm, fresh surface waters above cool, salty bottom waters was a consistent feature during
197   the experiment (Fig. 1). Although wind speeds remained light ($< 5 \text{ m s}^{-1}$), tidal mixing caused variations in
198   the vertical structure of the water column. Temporal shifts in the extent of stratification are clearly seen in
199   the vertical density gradient. The density gradient can be expressed in terms of the buoyancy frequency, N ,
200   defined as:

$$201 \quad N^2 = -\frac{g}{\rho_o} \frac{\partial \rho}{\partial z} \quad (4)$$

202   where where g is the gravitational acceleration, z the vertical coordinate taking positive upward, ρ_o the
203   mean density, and ρ the local density. Estimates of N^2 ranged between 10^{-3} to 10^{-5} s^{-2} during periods of
204   strong and weak stratification, respectively (Fig 1C, E). In contrast to the passive traces of temperature and
205   salinity, turbulence intensity showed high levels of spatial and temporal variability (Fig 1D) with estimates
206   of the turbulent kinetic energy dissipation rate ranging between orders of 10^{-5} to $10^{-9} \text{ W kg}^{-1}$.

207

208   **3.1.2 Bulk patterns of phytoplankton**

209   The Seapoint (data not shown) and TurboMAP-L LED fluorescence sensors (Fig. 1F) were consistent and
210   showed that bulk chlorophyll levels always exceeded $1 \mu\text{g L}^{-1}$ throughout the water column. Chlorophyll
211   concentrations in surface waters increased during the second half of experiment, reaching concentrations up
212   to $11.1 \mu\text{g L}^{-1}$. The spatial and temporal changes observed in phytoplankton biomass inferred by
213   fluorescence profiling were also consistent with estimates of phytoplankton abundance taken from water

214 samples (Fig.2).

215

216 Phytoplankton abundances determined from microscopy ranged from approximately 0.17×10^6 cells L^{-1} in

217 the surface and bottom waters at the beginning of the observational period to reach concentrations as high

218 and low as 1.10×10^6 and 0.08×10^6 cells L^{-1} in the surface and bottom waters, respectively. Diatoms

219 consisting mainly of *Skeletonema costatum*, *Rhizosolenia setigera*, *Chaetoceros lorenzianum*,

220 *Thalassionema nitzschioides* and *Nitzschia spp.* dominated the community composition throughout the

221 sampling period and contributed between 76 to 92% of the total cell abundances. Dinoflagellates consisting

222 of mainly *Peridinales* and *Gymnodinales* represented the next most dominant group and contributed to

223 between 11 and 23% of the total cell abundances. Elevated cell abundances measured in the surface waters

224 towards the latter half of the study period were associated with a shift in the dominant diatom species to

225 *Rhizosolenia fragilissima* and *Cylindrotheca closterium* as well as a significant increase in the abundance

226 of *Peridinales*.

227

228 **3.1.3 Microscale fluorescence distributions**

229 Comparison of fluorescence profiles from each of TurboMAP-L fluorescence sensors clearly shows that the

230 extent of phytoplankton spatial variability becomes increasingly intermittent when measured with

231 increased resolution (Fig. 3). Microscale variability measured by the LED sensor varied by less than 2-fold

232 across centimeter to meter scales. In contrast, fluorescence levels measured by the LASER sensor varied by

233 between 2 to 10-fold across millimeter size peaks, and were typically greater than changes measured by the

LED sensor across the entire water column sensor.

Histograms of log-normalized fluorescence values measured by the LED and LASER sensors for each 4 m sub-region (Fig. 4A, C) were best fit by normal and Fréchet (also known as a type II extreme value distribution) distributions respectively (Kolmogorov-Smirnov test, $P < 0.05$). Low-pass filtering of the LASER fluorescence signal at 5 Hz (equivalent to the sample volume of the LED sensor) gave a log-normal PDF statistically equivalent (Kolmogorov-Smirnov test, $P < 0.001$) to the PDF obtained from the LED sensor (Fig. 4E). Since a log-transformation forces a skewed random variable to approach a normal distribution, the clear shift from a log-normal to a strongly skewed Fréchet distribution with a reduction in sample volume demonstrates the existence of a critical scale at which the underlying nature of the fluorescence field diverges.

For phytoplankton to be viewed as a continuous variable, biomass estimates using fluorescence must be averaged over a volume large enough that the contribution of variations in the fluorescence of individual particles to the local variance is negligible. To quantify the transition scale, the energy density per unit volume (and equivalent length scale) (E) was calculated as the ratio of the variance to the sample volume size for LASER fluorescence measures averaged over increasing volume sizes. Figure 5 shows that the mean fractional change in E decreases rapidly from a maximum at the smallest resolved volume/ length scales ($\sim 32 \mu\text{L}$, 1 mm) becoming negligible at larger samples volumes/length scales. The influence of variations in the fluorescence of individual of particles is reduced by 95% when estimates of E are averaged

254 over a sample volume of 0.16 mL (equivalent to a spatial scale of ~0.5 cm) and become negligible (99%
255 reduction) when averaged over sample volumes of 0.42 mL (~1.3 cm). Hence, for the given system,
256 estimates of fluorescence made at or above this transitional scale, such as done by the LED sensor (Fig. 3),
257 the distribution of phytoplankton is smooth and is based on averaged values in a continuum. For measures
258 made below this transitional scale, individual variations in fluorescence become better resolved with a
259 decrease in sample volume size, providing insight into the structure and behavior of individual
260 phytoplankton particles.

261

262 **3.1.4 Spatial Point Patterns**

263 Particle concentrations (λ) for each R ranged from 3515 to 6113 particles L^{-1} (Fig. 6A) and were
264 significantly correlated with increases in the bulk chlorophyll concentration ($r^2 = 0.36$, $P < 0.01$). No
265 significant relationship was observed between particle concentrations and the rate of turbulent kinetic
266 energy dissipation (ε) (Fig. 6B). Fluorescence peaks identified as particles constituted approximately 5% of
267 the cell counts estimated by microscopy (Fig. 2) indicating only large, dominant fluorescent particles were
268 detected by the LASER sensor and are included in this analysis. Whilst smaller cells of low fluorescence
269 concentration are not individually identifiable, they do contribute to small shifts in the baseline
270 fluorescence level (i.e. non zero values) between regions of high and low cell abundances (see Fig. 3B) as
271 well as to estimates of the bulk chlorophyll concentration.

272

273 Nearest-neighbor distances (NND) during the experiment ranged from 2 to 56 mm. For the majority of

sub-regions analyzed the empirical CDF of *NND* showed similar probability distributions and were characterized by distinct regions (Fig. 7A). At length scales between approximately 2 and 8 mm the CDF curve climbed steeply, but remained within the confidence intervals for CSR suggesting the distribution of particles was not significantly different from random at these scales. For *NND* typically between about 8 to 20 mm the CDF consistently lay above the upper 95% confidence interval for CSR indicating significant clustering at these scales. At larger nearest neighbor distances ($NND > \sim 20$ mm) the CDF typically flattened in agreement with CSR. Several sub-regions of *NND* showed no significant departure from CSR across all *NND* (Fig. 7B). The distribution of z scores (Fig. 8) showed that 98% of the sub-regions analyzed showed significant clustering of particles ($P < 0.05$), at some length scale with deviations from random increasing significantly with particle concentrations ($r^2 = 0.34$, $P < 0.01$). Consistent with the observed increase in the clustering of particles, mean nearest neighbor distances estimated from the *NND* CDF for each *R* (Fig. 9A) decreased from approximately 13 to 7 mm and were strongly negatively correlated with increases in the particle concentrations ($r^2 = 0.58$, $P < 0.01$). No relationship was observed between the mean nearest neighbor distance and the rate of turbulent kinetic energy dissipation (Fig. 9B).

289   **4.1 Discussion**

290   Profiles of *in vivo* chlorophyll fluorescence are commonly used to infer the distribution of phytoplankton
291   biomass. The application of high-resolution profiling fluorometers to studies of microscale phytoplankton
292   distributions have revealed the common presence of intermittent millimeter to centimeter sized
293   fluorescence peaks across a range of marine systems. Whether these peaks represent increases in biomass
294   (Cowles et al. 1993, Doubell et al. 2006, Mitchell et al. 2008) or are due to the detection of discrete
295   fluorescent particles (Franks 2005, Doubell et al. 2009) has important implications for understanding the
296   causes and consequences of the microscale structure of phytoplankton. In this study, we show that the scale
297   at which microscale patterns of phytoplankton fluorescence are estimated may be critical to the
298   interpretation of the observed spatial variability. In addition, nearest neighbor analysis of discrete
299   fluorescence particles demonstrates that biological processes may be an important factor in structuring the
300   spatial distribution of phytoplankton across scales of millimeters to tens of centimeters.

301

302   **4.1.1 Spatial scales for the measurement of phytoplankton distributions**

303   During the observation period, bulk chlorophyll concentrations measured by the TurboMAP-L LED sensor
304   and cell abundances estimated from discrete water samples remained high and always exceeded $1 \mu\text{g L}^{-1}$
305   and $10^5 \text{ cells L}^{-1}$, respectively. Changes in phytoplankton biomass and cell abundances in space and time
306   were consistent with each other and varied by up to 11-fold across the water column. The consistency of
307   these measures indicates the observed finescale ($>1 \text{ m}$) changes in biomass were driven predominantly by
308   shifts in the abundance of the dominant large phytoplankton cells (i.e. diatoms).

309

310 Previous comparisons of the 1-meter averaged fluorescence levels between the two TurboMAP-L
311 fluorescence sensors have shown a strong correlation with chlorophyll-*a* measures made using
312 conventional fluorometers attached to CTD assemblies (Doubell et al. 2009). At smaller scales the
313 measured distribution of phytoplankton fluorescence becomes increasingly intermittent with a decrease in
314 sample volume size (spatial resolution) from mL (cm) to μ L (mm). The measurement of discrete particles
315 of varying fluorescent concentrations by the small (32 μ L) sample volume of the LASER sensor gave a
316 highly skewed Fréchet PDF significantly different to the log-normal PDF of fluorescence given from the
317 LED sensor for which estimates of *in vivo* phytoplankton fluorescence are averaged over a much larger
318 sample volume (4 mL). Moreover, spatial averaging of the LASER fluorescence profiles resulted in a shift
319 in the PDF from a Fréchet to a log-normal distribution, consistent with that measured by the LED sensor at
320 larger scales. This finding demonstrates that, (i) estimates of chlorophyll biomass made at centimeter scales
321 are consistent with the volume/spatial averaging of individual variations in particle fluorescence made at
322 smaller scales and, (ii) a critical transitional scale exists for the measurement and interpretation of
323 phytoplankton distributions.

324

325 Since phytoplankton occur as discrete particles (i.e. cells, chains, aggregates), for estimates of
326 phytoplankton biomass to be considered as a continuum, measures of *in vivo* cell fluorescence must be
327 averaged over a volume large enough such that individual particles do not contribute to the total variance in
328 fluorescence (Franks 2005). For the region and period of this study, we show that the scale at which the

329 distribution of phytoplankton fluorescence can be considered as discrete or as a continuum transitions
330 around a sample volume/spatial scale of approximately 0.42 mL and 1.3 cm, respectively. This result is
331 generally consistent with scaling arguments that suggest volume averaged measures of randomly
332 distributed phytoplankton transition from being discrete to a continuum at a length scale (L_d) given by
333 $L_d = 7P^{-1/3}$, where P is the abundance of particles in m^{-3} (Siegel 1998). For the fluorescent particle
334 concentrations of approximately 5000 L^{-1} observed in this study L_d is estimated to be ~ 4 cm. While the
335 predicted theoretical length scale is slightly larger than estimated in this study the scales for measuring the
336 spatial arrangement of individual phytoplankton by fluorescence do not only depend on the abundance of
337 organisms present, but will also be influenced by the size and type of particles present (Siegel 1998) as well
338 as the underlying structure of their distribution (Rothschild et al. 1992) for which we observed significant
339 evidence of clustering.

340

341 The existence of a critical transitional scale in the measurement of phytoplankton distributions by
342 fluorescence has important implications for the interpretation and choice of methods used to investigate the
343 microscale structure of phytoplankton. Depending on which side of this transitional scale measures of
344 phytoplankton are made, the interpretation of local increases in fluorescence will differ. At larger scales,
345 phytoplankton distributions can be viewed as a continuous, permitting us to study the macroscopic behavior
346 of phytoplankton ‘patches’ in fluids. This explains why previous observations of phytoplankton estimates
347 made above these scales have shown matching turbulent and phytoplankton spectra following the $k^{5/3}$
348 turbulence model (Denman et al. 1995, Yamazaki et al. 2006). For smaller scales, as suggested by Franks

(2005), analysis methods such as spectral analysis may in many instances be inappropriate and investigations into the spatial structure of phytoplankton particles will require the use of discrete analysis techniques such as point process models (Diggle 1983, Rothschild 1992, Malkiel et al. 2006).

4.1.2 Spatial structure of discrete phytoplankton particle distributions

The ability to measure the distribution of discrete fluorescent particles, albeit limited to the larger size fraction of phytoplankton particles and with a minimum sample interval of ~2.5 mm, provided a comprehensive dataset to apply the theoretical framework of point processes to investigate the spatial structure of discrete phytoplankton particles. Particle concentrations ranged between ~3500 to 6000 L⁻¹ and showed a significant positive correlation with changes in the bulk chlorophyll across sub-regions. Not surprisingly, *MND* summarized by the mean nearest neighbor distance, decreased with an increase in particle concentration. Under these conditions the spatial distribution of particles showed significant evidence for clustering in 98 % of the analyzed sub-regions, with the strongest deviations from random observed at spatial scales of ~8-20 mm and in regions of increased particle concentrations. With no relationship observed between particle concentrations or mean *MND* and turbulent energy dissipation rates, our findings suggest that biological processes may strongly influence the distribution of phytoplankton particles over millimeters to centimeter scales.

Although models have been presented for the microscale clustering of phytoplankton (Rothschild 1992), previous observations of the spatial structure of discrete phytoplankton cells and aggregates have generally

369 shown a random distribution of particles across millimeter to centimeter scales (Franks 2005, Malkiel et al.
370 2006, Franks and Jaffe 2008). We propose that the dominance and high abundance of diatoms measured in
371 this study may be a significant factor influencing the formation of the observed clustered distribution
372 patterns. Diatoms, including *Chaetocerus spp.* observed throughout this study, have several
373 morphological and physiological features including increased coagulation efficiency (Hansen and Kiørboe
374 1995) and the generation of transparent exopolymeric particles (Passow et al. 1994) which provide multiple
375 mechanisms for increased aggregation between cells, chains and other particles (Crocker and Passow 1995).
376 Increases in seawater viscosity associated with the release of phytoplankton-produced organic matter may
377 also aid the aggregation between cells by dampening the effect of turbulence at the smallest scales
378 (Jenkinson 1993). For this study, the close association of phytoplankton cell abundances with biomass and
379 particle concentration estimates, coupled with the increased levels of turbulence ($\epsilon > 10^{-7} \text{ W kg}^{-1}$) observed
380 in surface waters, would lead to increased coagulation rates (Jackson 1990) and aggregate formation
381 (Kiørboe 2001) and offers a plausible explanation for the finding of strong clustering patterns.

382

383 **4.1.3 Ecological consequences**

384 The ability to predict the contribution of individuals to measures of chlorophyll spatial variability has
385 important implications in regard to the application of microscale phytoplankton distribution patterns to
386 investigate oceanic processes across a range of scales. As plankton ecology depends on discrete
387 inter-organism interactions, our finding of clustered fluorescent particle distributions is important and
388 suggests that, for particular systems and times, rates of many microscale processes, including infection

389 (Riemann and Middleboe 2002), bacterial colonization (Alldredge et al. 1986, Mitchell et al. 1996),
390 trophodynamics (Rothschild & Osborn 1988), resource competition (Siegel 1998), aggregate formation
391 (Jackson 1990) and community metabolism (Rothschild 1992) will be higher than those modeled for a
392 random distribution of planktonic particles. Moreover, inferences based on estimates of phytoplankton
393 biomass through the measurement of phytoplankton chlorophyll fluorescence such as primary production
394 and oceanic carbon flux (Longhurst 1995) will be strongly affected by differences between the chlorophyll
395 concentration estimated at the patch scale and at the scale of discrete phytoplankton particles.

396

397 This paper is a first step in presenting empirical results and analysis methods relevant to the application of
398 high-resolution profiling fluorometers to understanding the scale-dependent spatial structure of
399 phytoplankton. Only through the development of new technologies capable of providing better resolved
400 datasets across a range of ecosystems and the application of appropriate analysis methods will we develop
401 our understanding of the mechanisms underlying the structural organization of planktonic organisms and
402 ecology of marine ecosystems.

403

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575 **Figure Captions**

576 Figure 1. Time series of the vertical distribution of physical and biological properties measured using the
577 TurboMAP-L microstructure profiler at the mouth of Tokyo Bay on May 18 and 19, 2009; (A) temperature
578 ($^{\circ}\text{C}$), (B) salinity (psu), (C) density (kg m^{-3}), (D) rate of turbulent kinetic energy dissipation (W kg^{-1}), (E)
579 buoyancy frequency (s^{-2}) and (F) chlorophyll ($\mu\text{g L}^{-1}$). High and low tides occurred at 00:50 and 07:22
580 hours on the 19th of May, respectively.

581
582 Figure 2. Phytoplankton abundance and community composition estimated from surface (s) and bottom (b)
583 water samples during the course of the experimental period, (A) May 18, 18:00 hr, (B) May 18, 21:00 hr,
584 (C) May 19 04:00 hr and (D) May 19, 14:00 hr.

585
586 Figure 3. Examples of the fluorescence microstructure measured simultaneously by the TurboMAP-L LED
587 (light grey) and LASER (dark grey) sensors. Profiles were taken at (A) 18:50 hr, May 18 and (B) 13:00 hr,
588 May 19. The LED and LASER sensors are separated by a distance of 12 cm. The normally arbitrary units of
589 fluorescence are calibrated to give estimates of biomass in $\mu\text{g chlorophyll L}^{-1}$.

590
591 Figure 4. Representative density histogram (grey bars) of log normalized fluorescence values ($n \sim 1600$)
592 measured by the TurboMAP-L (A) LED and (C) LASER sensors. The histogram for the LED sensor was
593 fitted ($P < 0.05$, Kolmogorov-Smirnov test) with a log-normal probability distribution function (PDF). In
594 comparison, the distribution of fluorescence values from the LASER sensor was highly skewed and best fit

595 with a Fréchet extreme value PDF ($P < 0.05$, KS test). Corresponding Q-Q plots show the comparison of the
596 distribution of the LED (B) and LASER fluorescence values (D) to theoretical log-normal and Fréchet
597 extreme value distributions, respectively. (E) Comparison of the LED log-normal PDF (black line, shown
598 in A) to the PDF given by low-pass filtering the LASER fluorescence values (shown in C) over spatial
599 scales equivalent to the sample volume of the LED sensor (grey line). The PDF's are statistically equivalent
600 ($P < 0.001$, KS test) and show the statistical convergence of measures made with the LASER sensor at
601 millimeter scales to the distribution of fluorescence measured with the LED sensor at centimeter scales.

602

603 Figure 5. Mean fractional change in the energy density (E) with sample scale (s) estimated for each
604 sub-region ($n \sim 1600$) of fluorescence. s_{vol} and s_{len} are the sample volume size and equivalent averaging
605 length scale, respectively. Dashed and dash-dot lines show the sample scale at which the original estimate
606 of E are reduced by 95 % and 99%, respectively.

607

608 Figure 6. Changes in particle concentrations (λ , particles L^{-1}) determined from the TurboMAP-L LASER
609 sensor over 4 m sections plotted vs. (A) bulk chlorophyll-*a* concentration (chl , $\mu g L^{-1}$) and (B) the rate of
610 turbulent kinetic dissipation (ε , $W kg^{-1}$). Significant linear regressions ($P < 0.01$) are shown (grey dashed
611 line).

612

613

614

Figure 7. Representative cumulative distribution function of nearest neighbor distances ($\hat{G}(NND)$) measured over a 4 m sub-section on May 19 at 19:00 hours in, (A) surface (9-13m) and (B) bottom (35-39 m) waters. Grey dashed lines show the upper and lower 95 % confidence envelopes estimated for a Poisson random process over 100 Monte-Carlo simulations. For surface waters the departure from the upper simulation envelope provides strong evidence for clustering at length scales of approximately 5 to 25 mm. In contrast, for bottom waters no significant departure random was observed.

Figure 8. Distribution of the nearest neighbor distances z-score with particle concentrations (λ , particles L^{-1}). Z-score values greater or less than 1.96 or -1.96 (dark grey dashed lines) indicate statistically significant ($P < 0.05$) regularity or clustering, respectively. Significant linear regression ($P < 0.01$) is shown (light grey dashed line).

Figure 9. Relationship between changes in the mean nearest neighbor distance (m_{NND} , mm) for each 4 m section with, (A) particle concentrations (λ , particles L^{-1}) and, (B) the rate of turbulent kinetic dissipation (ϵ , $W\ kg^{-1}$) and the Kolmogorov length scale (grey solid line). The significant linear regression ($P < 0.01$) in (A) is shown (grey dashed line).

Figure 1

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