An ultraviolet dyegraph for measuring the chemical disturbances of sinking particles and swimming plankton

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Abstract

Sinking particles and swimming plankton may deform ambient chemical gradients, leaving long trails of chemical disturbance that dissipate on a diffusive timescale. Imaging this phenomenon in a laboratory setting requires a large field of view, a broad depth of field, and a passive chemical substance that does not influence the hydrodynamics. These requirements obviate the use of traditional schlieren optical systems, which rely on density stratification and their consequent buoyancy forces, and modern planar laser-induced fluorescence, which has a narrow depth of field. Further, the light used to induce plankton movement must be kept separate from the recorded signal. Here, we describe a new ultraviolet “dyegraph” technique that quantifies dye disturbance caused by sinking spheres and swimming zooplankton. An experimental tank is filled with seawater and a gradient of an ultraviolet-absorbing pigment. Using modified shadowgraph optics, ultraviolet light spreads from a pinhole to a collimating lens, passes through the tank to a telecentric lens, and is recorded by a camera fitted with an ultraviolet bandpass filter. The dye is transparent in visible light but appears dark in the imaging system. The 3D chemical disturbance is reconstructed from images using tomographic inversion. After outlining the method, we present examples of chemical deformation trails generated by sinking spheres and live, swimming copepods.

Microscale gradients of chemicals often constrain the rates of growth, grazing, predation, and mating in planktonic communities (Pasciak and Gavis 1974; Dodson 1988; Ploug et al. 1999; Long et al. 2007; Yen and Lasley 2010; Seymour et al. 2017). The primary sources of chemical microstructure are thought to be excretions from plankton and turbulent stirring of large-scale gradients (Azam 1998; Stocker 2012). While turbulence is pronounced near boundaries, the pelagic habitat of plankton can be relatively quiescent in certain regions and seasons (Peters and Marrase 2000; Waterhouse et al. 2014; Fuchs and Gerbi 2016). In addition to stirring by ambient turbulence, there is mounting evidence that swimming plankton and sinking or floating particles can also stir ambient chemical gradients. Katija and Dabiri (2009) observed jellyfish dragging dye injected ahead of their swimming path in a highly stratified lake. Noss and Lorke (2014) measured increased scalar dissipation rates of a dye gradient in the vicinity of swimming daphnia in an experimental tank. Recent simulations of a sphere sinking through a linear chemical gradient indicate that the gradient is deformed and enhanced in a long trail that persists on a diffusive time scale, much longer than the velocity disturbance that created it (Inman et al. 2020). Further experiments in a controlled laboratory setting are needed to quantify the structure and extent of gradient enhancement by sinking particles and swimming plankton.

Imaging trails of deformed chemicals – “gradient deformation trails” – presents a number of challenges that are not met by current methods. Traditional shadowgraph or schlieren techniques rely on the refraction of light by disturbances in density stratification (Yick et al. 2007). Biologically relevant chemicals in the ocean such as nitrate or dissolved organic matter generally do not influence the density of the fluid, making these methods unsuitable for characterizing the stirring of gradients of these chemicals. Planar laser induced fluorescence (PLIF) has been used to measure scalar dissipation by swimming zooplankton (Noss and Lorke 2014), but the narrow imaging plane does not capture the full trail of chemical disturbance and the laser may affect swimming behavior. Both a large observation window and large depth of field are required to capture the trail. Here, we describe a new imaging system to quantify a vertical dye gradient stirred by sinking particles and swimming zooplankton in controlled laboratory conditions.
Calanoid copepods are ubiquitous in the world’s oceans; many species perform diel vertical migrations, making them an ideal choice for zooplankton-swimming experiments (Mauchline 1998). Such phototactic zooplankton can be induced to swim in a desired direction using a light stimulus. To prevent the stimulating light from interfering with the imaging, the wavelength of light used to guide plankton swimming must be separated from the imaging wavelength. Wilhelmus and Dabiri (2014) used a blue-green laser to initiate vertical migrations of *Artemiasalina* and tracked microparticles using a red planar laser. We have determined that *Calanus pacificus* copepods swim in response to white light but do not react to ultraviolet wavelengths. To conduct imaging of the trails created by swimming zooplankton, we therefore utilize a pigment that absorbs ultraviolet light but is transparent in the visible spectrum.

The modified shadowgraph optical system described here measures the concentration field of an ultraviolet-absorbing dye stirred by spheres and copepods in a tank. The dye represents an ambient nutrient such as nitrate that typically occurs as a vertical gradient through the base of the euphotic zone. Parallel-light shadowgraphs afford a broad field of view, large depth of field, and negligible magnification through the focal plane. This means that an object in the imaging volume of the tank is imaged at essentially the same size, and is in focus regardless of its position. While shadowgraphs are much simpler than Schlieren optical systems, they are not often used in modern flow visualization because the shadow cast by a density anomaly is not a 1:1 representation of the flow that caused it (Settles 2001). We overcome this limitation in our apparatus by modifying the shadowgraph to image the specific wavelengths absorbed by the dye, rather than the density gradients alone. Because the light rays are collimated, the light intensity recorded by this “dyegraph” is the integrated dye concentration along a ray path, projected onto the imaging plane of the camera. A 3D disturbance of the dye field can then be reconstructed using tomographic inversion, assuming axisymmetry. Images acquired by the dyegraph include light that is refracted by density gradients; however, this interference is minimal. To our knowledge, this is the first system designed to record 3D disturbances by moving objects of a 1D ambient chemical field (an assertion supported by G. S. Settles, pers. comm.). The dyegraph allows quantification of variables relevant to gradient deformation, such as the maximum distance an isosurface of chemical is displaced.

**Materials and procedures**

**Optics**

Illumination for a shadowgraph consists of a pinhole light source that projects onto a collimating lens. Collimated light then passes through the imaging volume to a second lens which focuses the light onto a camera sensor. Objects or dye in the imaging volume obstruct the light and appear dark in shadowgraph images. Because the light is collimated, the field of view is determined by the size of the lenses, and there is no magnification with distance through the imaging volume.

In our system, the light source is an ultraviolet 3 W LED (LED Engin LZ1-10U600, 0.6 A/4.2 V) with peak emission at 365 nm that passes through a 1 mm pinhole (Fig. 1). A diffuser placed between the LED and the pinhole blurs the diode pattern, which would otherwise be visible in the image. The UV LED is attached to a heatsink mounted on a translation stage to allow fine adjustments. Ultraviolet light is collimated by a 20 cm diameter, 40 cm focal length plano-convex lens (Edmund Optics) positioned in line with the light source using a custom-designed adjustable lens mount. After passing through the imaging volume, light rays are received by a bi-telecentric lens (Opto Engineering TC23192) that reduces distortion from oblique light rays. A 15 cm diameter circle of
illumination with a resolution of 73 μm is recorded at 5 frames s⁻¹ by a 5 megapixel CMOS camera with a 1.69 cm detector (Point Grey GS3-U3-51S5M-C, 1.3 ms shutter/1.25 gamma). Because white light is used to induce zooplankton phototaxis, a 275–375 nm ultraviolet bandpass filter is fixed to the camera to prevent visible wavelengths from interfering with the image.

The shadowgraph optics are calibrated with images of a checkerboard pattern, and processed using the Matlab Computer Vision System Toolbox (The MathWorks 2016). Within the field of view, 75% of the area displays less than 1 pixel of radial distortion, with a maximum of 1.8 pixels of radial distortion near the edges of the lens.

**Experimental tank**

The inner dimensions of the experimental tank are 30 cm tall, 12 cm wide, and 5 cm long in the direction of light rays, containing a 1.8-liter volume (Fig. 2). The image produced by the dyegraph is then a circular section 12 cm wide and 15 cm tall, integrating 5 cm through the tank interior. The tank is constructed out of 9.5-mm thick UV-transmitting acrylic, typically used in tanning beds (Plexiglas G-UVT). The tank rests on 8 cm tall supports that provide access to a single port for filling from the bottom, as well as space to place a visible-spectrum LED for driving plankton phototaxis. The base of each tank is fastened to an optical breadboard on a vibration-damped table. Four regularly spaced ports in each side wall of the tank permit water samples to be extracted with a syringe for density or chemical measurements. The side ports consist of a nylon bulkhead fitting capped with 3-mm thick silicone septa, allowing a needle to be inserted into the center of the tank.

**Dye**

The dye is a commercially available ultraviolet-absorbing pigment produced by Lycus for use in cosmetics; benzophenone-9 (Maxgard 1800) is water soluble, nontoxic, and absorbs 365 nm wavelengths. Dissolved in seawater, the dye is colorless in the visible spectrum but appears dark when imaged using the ultraviolet shadowgraph.

The attenuation of light by dye pigments is described by

\[ I(x) = I_0 e^{-a\int C(x) dx} \]

where \( I \) is the measured pixel intensity, \( I_0 \) is the pixel intensity with no dye present, \( a \) is the attenuation coefficient, \( C \) is the concentration of dye, and \( x \) is in the direction of the light rays. A calibration curve for dye concentration vs. average pixel intensity was created by filling the experimental tanks with known concentrations of dye dissolved in seawater (Fig. 3). The equation for dye concentration as a function of pixel intensity in a tank of thickness \( X \) is

\[ C = -0.002 \ln \left( \frac{I}{I_0} \right) \frac{X}{X} - 0.002 \]

where the first coefficient is the attenuation \( a^{-1} = 0.002 \text{ kg m}^{-2} \) and the standard deviation is ± 0.002 kg m⁻³.

The value of the molecular diffusivity of benzophenone-9 is required for determining nondimensional fluid parameters, but has not previously been determined. We estimate the temperature-dependent molecular diffusivity of benzophenone-9 using the Stokes–Einstein relationship and verify it by observing diffusion of a layer of dye in the experimental tank. The Stokes–Einstein equation defines the diffusivity of a solute in terms of the viscous drag on a single spherical molecule. Edward (1970) adapted the relation for nonspherical molecules:

\[ D = \frac{kT}{n
fr(r/f_0)} \]

where \( k \) is the Boltzmann constant, \( T \) is the absolute temperature, \( n \) is an empirical correction factor, \( \nu \) is the dynamic viscosity, \( r \) is the van der Waals radius of the molecule, and \( f/\nu_0 \) is the frictional coefficient for ellipsoids. The radius of the benzophenone-9

![Fig 2.](image-url)
Inman et al.

An ultraviolet dyegraph

**Fig 3.** Calibration curve for light intensity vs. dye concentration. $I$ is the mean pixel intensity, $I_0$ is the mean pixel intensity of seawater without dye, and $X = 5$ cm is the inner thickness of the tank. The standard deviation of the error from the line of best fit is 0.002 kg m$^{-3}$.

molecule is estimated as $r = (3V/4\pi)^{1/3} = 4.9227$ Å, where $V$ is the sum of the incremental atomic volumes (Bondi 1964). For $T = 291.65$ K (18.5°C), $n = 5.97$, and $f/f_0 = 1.05$, the diffusivity of benzophenone-9 is $D = 3.7141 \times 10^{-10}$ m$^2$s$^{-1}$. Recording the diffusion of a dye layer in the experimental tank at 18.5°C, the analytical solution to the diffusion of a delta function is fit to the vertical gradient of dye at two time points resulting in a mean diffusivity of $D = 3.2914 \times 10^{-10}$ m$^2$s$^{-1}$ with a standard deviation of $\pm 1.775 \times 10^{-10}$ m$^2$s$^{-1}$ (see Inman 2018). The two estimates agree and the Stokes–Einstein relation is used hereafter to accommodate varying temperatures in the experiments.

**Stratification**

The tank is filled with salinity-stratified seawater using the double-bucket method (Oster 1965). The first beaker (A) of saltier seawater is pumped into a second beaker (B) of fresher seawater that is mixed on a stir plate (Fig. 4). A second pump operating at twice the rate of the first pump transports the mixed seawater into the bottom of the tank, creating a linear salinity gradient. A negative vertical dye gradient is superimposed on the salinity stratification by adding dye to beaker A. The magnitude of the dye gradient is increased by adding the dye to the first beaker after filling begins. To create an isolated dye layer, dye is injected inline through a threeway stopcock midway during filling. The salinity stratification causes the dye to flatten into a layer that is pushed up to the middle of the tank from below by the pump.

We tested several configurations of peristaltic pumps and gear pumps during preliminary tank fillings. Peristaltic pumps produce a pulsating flow that can cause mixing in the bottom of the tank, but the average flow rate is fairly steady with increasing back pressure from the water level in the tank or beaker. On the other hand, gear pumps generate smoother flow, but the average flow rate decreases with back pressure. As such, a Labconco peristaltic pump is used to transfer water between the first and second beakers, while a Cole-Parmer gear pump with a Micropump head moves fluid between the second beaker and the experimental tank. The gear pump speed is adjusted while filling the tank to compensate for back pressure. Heating of the fluid by the stir plates and pumps can cause convection in the tank. We regulate the temperature using a Haake K20 water bath connected to the two water-jacketed beakers and a segment of water-jacketed 316 stainless steel tubing. After filling, the tank is covered and allowed to stand for 8 h to relax any residual flows.

We confirm the linearity of the density gradient by using a syringe to take 10 mL samples from the center of the tank through each of the four side ports after the conclusion of an experiment. The exact sampling locations are determined from images by measuring the distance from the tip of the needle to a reference pixel in a mark on the tank wall. The densities of the samples are measured with a Rudolph Research Analytical densitometer thermostated at 20°C and precise to 0.01 kg m$^{-3}$. Images of the linear dye gradients are also used to verify the linearity of the density gradient. The Brunt–Väisälä frequency is calculated using

$$N = \sqrt{\frac{g \partial \bar{\rho}}{\rho_0 \partial z}}$$

where $g$ is the gravitational acceleration, $\bar{\rho}$ is the measured density, and $\rho_0$ is the mean density in the tank. Salinity and kinematic viscosity are calculated from the average density and temperature for each experiment using the Gibbs-SeaWater Oceanographic Toolbox and the Seawater Thermophysical Properties Library (McDougall and Barker 2011; Nayar et al. 2016).

**Spheres**

To study the deformation of dye gradients by moving objects, we performed experiments with either sinking spheres or swimming copepods. For the first set of experiments, we used polystyrene spheres produced by Cospheric with a diameter of 4.8 mm and densities ranging from 1023 to 1030 kg m$^{-3}$. Spheres were wetted with seawater and placed in the top of the tank with tweezers. We quantified the sinking speed by tracking the centroid of the sphere from one image to the next at 5–12 positions within the 15 cm vertical field of view. Because the tanks were density stratified, the spheres slowed as they descended. The mean sinking rates $W$ of 23 separate sphere drops varied from 5.1 ± 0.4 to 20.8 ± 0.5 mm s$^{-1}$. The Reynolds ($Re$), Froude ($Fr$), and Péclet ($Pe$) numbers were calculated using the average velocity of the sphere, $W$. These nondimensional numbers are $Re = 2aW/\nu$, $Fr = W/\alpha N$, and $Pe = 2aW/D$, where 2$a$ is the spherical diameter, $\nu$ is the
and placed them in a 16\microscope. We separated adult submarine canyon. The contents of the cod ends were brought in experiments based on their phototactic behavior (see Inman 2018). The code does not allow for a solid object to block the axis of symmetry, and so pixels occupied by the sphere are replaced with an average of five horizontal pixels adjacent to the sphere. Adding the background dye rarely in a straight line – and passively sank downward. The copepods did not appear to react to the presence of the dye or the UV light source.

**Image analysis**

The dyegraph imaging system records the 2D projection of the 3D dye field. Assuming that the disturbance of the dye field by a moving object is axisymmetric, we can reconstruct the 3D dye field using an inverse Abel transform (Pretzler 1991). First, image pixel intensity is converted to dye concentration using the calibration above. Then, an average of up to 60 frames of undisturbed fluid is subtracted from images of the moving object to give the dye anomaly. Five consecutive images (one second’s worth) tracking the object are averaged and processed with a Wiener filter to reduce high-frequency noise caused by air moving between the tank and the lenses.

From the dye anomaly image $F(y, z)$, the radially symmetric original distribution of dye anomaly $f(r, z)$ can be calculated using the Abel inversion,

$$f(r, z) = -\frac{1}{\pi} \int_r^\infty \frac{dF(y, z)}{dy} \frac{1}{\sqrt{y^2 - r^2}} dy$$  \hspace{1cm} (5)$$

where $r = 0$ is the axis of symmetry, $R$ is the edge of the domain, and $F(R, z) \approx 0$. Note that this transformation changes the coordinate system from Cartesian $(x, y, z)$ to cylindrical $(r, \varphi, z)$. The integral is evaluated numerically using the series expansion method described in Pretzler (1991) and coded by Killer (2016). The code does not allow for a solid object to block the axis of symmetry, and so pixels occupied by the sphere are replaced with an average of five horizontal pixels adjacent to the sphere. Adding the background dye
concentration to the dye anomaly from the Abel inversion yields a 2D vertical slice through the axis of the disturbed dye field.

We process images of passively sinking copepods in a similar manner. Trails left by swimming copepods, however, are more difficult to analyze because small variations in swimming direction prevent image averaging following the copepod: bends in the trail would not align. Instead we averaged images in the reference frame of the tank to preserve the trail. Unfortunately, detail around the copepod is lost in this averaging. Copepod trails are not fully axisymmetric because the swimming appendages are located in two longitudinal rows on the ventral side of the body. However, particle image velocimetry (PIV) experiments of free swimming calanoid copepods indicate that the downstream velocity field for each row of appendages is roughly axisymmetric during cruising, if the organism is not rotating (Catton et al. 2007, 2012). We can then assume that the downstream dye field disturbance is axisymmetric aligned with a row of appendages, and tomographically invert these image segments separately. Based on the orientation of the copepod with respect to the camera, the segment is chosen so that the disturbance caused by one row of appendages does not overlap with the other.

**Numerical simulations**

To assess the accuracy of the dyegraph method, numerical simulations of a sphere sinking through a chemical gradient are run for comparison. A full description of the model is available in Inman et al. (2020). In brief, the non-dimensionalized Navier–Stokes equations are solved on a curvilinear grid using finite differences and the successive over-relaxation method. Simulations are conducted in the reference frame of the sphere with the outer boundary located 1200 sphere diameters away from the origin in all directions. Using the same non-dimensional parameters as a given experiment, the model is run until the sphere has moved an equivalent distance from an impulsive start.

**Assessment**

To demonstrate the method, we present images of a 4.8 mm sphere sinking through linear dye and salinity gradients (Fig. 5). Both dye concentration and salinity increase toward the bottom of the tank. The velocity of the sphere decreased from 8.2 mm s\(^{-1}\) at the top to 7.6 mm s\(^{-1}\) at the bottom of the field of view. Average fluid parameters calculated for the sinking sphere were \(Re = 38.4\), \(Fr = 27.9\), and \(Pe\) numbers.
Lower concentrations of dye dragged down by the sphere are visible as a trail that spans the 15 cm vertical field of view (Fig. 5a). The dye anomaly in the trail and near the sphere is more apparent after subtracting the (stationary) background dye concentration (Fig. 5b). This view represents the integrated dye anomaly projected onto the camera, and accordingly the values go to zero on either side of the trail. The dim feathery pattern surrounding the trail is caused by irregularities in the underlying dye gradient. The Abel inversion of the dye anomaly is shown in Fig. 5c. Adding the background dye concentration to the Abel inversion represents a 2D slice through the axis of the trail, as shown by the contours in Fig. 5d. The 3D dye concentration field is recreated by rotating the 2D slice about the axis of symmetry. The dye concentration throughout the trail is less than at the top of the background gradient, indicating that dye is dragged from far above the field of view.

Smoothed contours of dye concentration separated by $5.5 \times 10^{-3}$ kg m$^{-3}$ are in excellent agreement with contours produced by a numerical simulation with the same $Re$, $Fr$, and $Pe$ numbers (Fig. 5d). As with the model, the experimental contours are contiguous between their background location and their attachment point on the sphere. Close to the centerline, noise amplified by the Abel inversion makes the structure of dragged dye contours more difficult to determine. Nonetheless, the dye anomaly near the sphere on the axis of the trail corresponds to the farthest a dye contour was dragged by the sphere. Dividing the maximum dye anomaly by the background gradient gives the maximum distance a contour is dragged, in this case 47.3 sphere diameters (22.7 cm). The farthest a contour is dragged in the equivalent simulation is 54.3 sphere diameters. Given that the experimental and simulated contours have the same shape, it is likely that the 14% lower value derived from the experiment is caused by smoothing of noise along the centerline.

Because the refractive index of a fluid depends on its density, disturbing the density field causes fluctuations in the illumination recorded by the shadowgraph. The differences in light intensity are proportional to the Laplacian of the density anomaly (Settles 2001). The trail seen in Fig. 5b is thus a combination of the dye anomaly and the second spatial derivative of the density anomaly. Because one is the result of absorbing light and the other of bending light, it is difficult to predict how the two interact. To quantify the relative contributions of the dye and density disturbances to the imaged signal in the trail, spheres were dropped through tanks filled to the top with a salinity gradient but only midway with a dye gradient (i.e., the dye was added to the first bucket after filling began).

![Fig 6](image-url) **Fig 6.** (a) A raw image with the background subtracted. Dye extends from the dashed line to the bottom. The Laplacian of the density anomaly is visible above the dashed line. (b, c) Horizontal cross sections through the trail of two experiments with similar $Re$ and $Pe$ but different stratifications. The solid line is the lower density stratification case with $Re = 31.1$, $Fr = 20.4$, $Pe = 4.63 \times 10^4$, and $N = 0.129$. The dashed line is the higher stratification case with $Re = 28.4$, $Fr = 12.5$, $Pe = 4.24 \times 10^4$, and $N = 0.193$ s$^{-1}$. (b) In a section without dye (e.g., above the dashed line in (a)), the Laplacian of the density anomaly is recorded and the higher stratification signal maximum is more than double that of the lower stratification case. (c) With dye present (e.g., below the dashed line in (a)), the magnitude of the dye anomaly is slightly lower for the higher stratification case, showing that the Laplacian of the density anomaly does not amplify the dye anomaly signal.
The density anomaly alone is visible at the top of the image, while the bottom displays the combined dye and density anomalies (Fig. 6a).

Figure 6b,c shows horizontal cross sections through the Abel-inverted trails of two sphere descents through similar dye gradients but different salinity gradients. The Re and Pe numbers are roughly the same and the Fr numbers differ, but not enough to change the flow dramatically (Inman et al. 2020). The experiment with the greater background density gradient has a stronger signal in the absence of dye (Fig. 6b). If the refraction and absorption effects are additive or multiplicative, the experiment with the stronger density gradient would also display a proportionately larger signal in the presence of dye. However, this is not the case (Fig. 6c): the higher density stratification case shows a slightly lower combined dye and density anomaly signal than the weaker stratification case. Therefore, as long as the dye gradient is sufficiently large, refraction caused by the density anomaly does not greatly interfere with the magnitude of the dye anomaly signal.

Trails of dye disturbance left by swimming copepods also spanned the vertical field of view, although rarely in a straight line. Subtracting the background dye gradient, the trail is visible behind an adult female copepod swimming upward through a tank configured with higher dye concentration at the bottom of the tank (Fig. 7a). The body is in three-quarter view with the ventral side facing left, leaving the trail downstream of the left row of appendages roughly unobstructed by the right. The trail appears broader and more diffuse in relation to the body of the copepod when compared to the trail left by a sphere. Choosing the left row appendages as the axis of symmetry, the reconstructed dye concentration field reveals that dye is pushed downward in reverse of the swimming direction (Fig. 7b). This is consistent with the “breaststroke” motion of the cephalic appendages observed in PIV studies (van Duren and Videler 2003; Jiang and Osborn 2004; Kiørboe et al. 2014).

**Discussion**

In both sphere and copepod experiments, the dyegraph optical system captures trails of dye anomaly anticipated by numerical simulations. Despite the simplicity of the optics, this modified shadowgraph provides a 15-cm diameter field of view with little radial distortion, ample depth of field with no change in magnification, and resolution primarily limited by the sensor of the camera. Similar characteristics would be difficult to achieve with schlieren or PLIF techniques. Comparison with numerical simulations indicate that the 3D dye field could be accurately reconstructed using an Abel inversion. Due to the large field of view and resolution of the camera, the dye field near the sphere or copepod was not as well resolved as has been demonstrated with schlieren or PLIF systems (Yick et al. 2009; Noss and Lorke 2014). In our dyegraph system, the Laplacian of the density anomaly—the intended subject of traditional shadowgraphs—could interfere with dye concentration measurements; however, weak stratification and strong dye gradients mitigate this potential problem. An additional benefit of imaging dye is that integration is not required after tomographic inversion, as is the case with the schlieren technique. As opposed to density stratification, the dye can be configured as gradients or thin layers that do not influence the hydrodynamics of the experiment. The dyegraph technique is well suited for recording chemical disturbances by swimming organisms that occur over much larger spatial scales than the organism.

The method can be modified to record dye disturbances by < 1 mm particles and plankton by simply increasing the resolution of the camera. A smaller dye disturbance signal can be enhanced by reducing the length of the tank in the direction of the light rays, so that there is less fluid to image through. Another potential extension of the dyegraph method is measuring dye as a proxy for chemicals released by sinking organic matter or zooplankton. In such an experiment, copepods and their fecal pellets would be cultured in a known concentration of dye, rinsed, and placed in a tank of pure seawater. If the dye is not metabolized, copepod excretions or plumes released by sinking fecal pellets could be recorded. The 3D dye field can be reconstructed by the dyegraph if the signal is axisymmetric, but not if it is asymmetric. In the case of asymmetry,
the integrated 2D dye concentration recorded by the camera could be useful for determining the rate of release and subsequent diffusion of the chemical. Further, an array of 5–15 smaller dyegraphs arranged in a semicircle around a cylindrical tank may provide enough overlapping views for stochastic tomographic reconstruction of an arbitrary 3D dye field (Gregson et al. 2012).

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Conflict of Interest
None declared.