Gravitaxis in Spherical Janus Swimming Devices: Supplementary Information

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1. Tracking Swimming Janus Spheres in 3D

To track our fluorescent Janus spheres in 3D we used a similar method to that described by.[1] When the spheres are in focus under fluorescence conditions they appear as a solid bright disc. However, when defocussed above the spheres spherical abberations cause a bright ring to appear around the particle, the radius of which is a function of the distance of the sphere from the focal plane, the sphere size and the magnification ([2]) (see Figure 1(a)). Our procedure for extracting 3D particle trajectories from the 2D fluorescence images was as follows.

Generate a calibration chart. We suspended some of our Janus spheres in a 2 wt% solution of gellan gum at 60 °C, which was allowed to cool to room temperature in a glass cell (4.0 x 1.0 x 0.1 cm). Once set, the gellan gum forms a stiff gel immobilizing the spheres. We then focussed on a sphere and took a series of images at increasing z-positions above the sphere. The images were then analyzed using a set of algorithms self-written in Labview to measure the bright ring radius in pixels. This procedure was repeated for each size of sphere and objective used. In Figure 1(b) we have plotted the calibration chart for our $a = 2.40\,\mu$m

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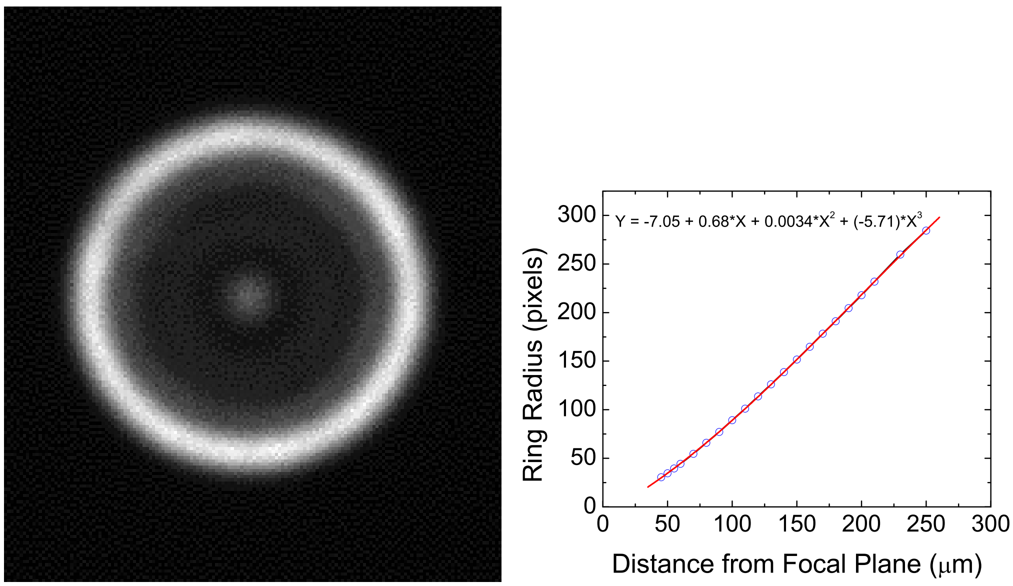
spheres recorded using a Nikon x40 objective.

Particle Image Velocimetry. To track the swimming Janus spheres in 3D, we first focussed about 100 μm above a swimming sphere and then recorded a sequence of 1000 images (11-bit) at 33 Hz using an Andor Neo Camera. The images were then analyzed using a set of self-written algorithms in Labview. Labview has a number of built in functions that enable the quick development of image analysis type functions.



(a) (b)

Figure 1: The radius of the bright ring (1(a)) formed when defocussing above a fluorescent sphere changes with distance. In 1(b) we have plotted the ring radius of our $a = 2.40\,\mu$m platinum coated spheres as a function of distance above the sphere.

1. The images were first smoothed using a Gaussian filter to suppress noise and thresholded using Labview's automatic thresholding algorithm to binarize the image and isolate the object pixels. We then used Labview's built in functions to locate an approximate position of the circular object.

2. A series of lines of pixels running across the circular object, in both the x and y directions were extracted (see Figure 2(a)) and a spline fitted to the intensity values.

The bright ring forms two peaks in the extracted pixels with the point half way between the peaks being either the x or y center of the swimming sphere. By fitting a spline and taking an average over 10 lines we found the (x, y) coordinates of the swimming spheres to sub-pixel accuracy.

3. From the (x, y) center 100 equally spaced lines of pixels were extracted and a spline fitted to the average intensity curve. Labview's spline fitting algorithm takes a value n to determine the number of points in the spline, where the number of points between each pixel value is $(2^n - 1)$, we set $n = 7$ (see Figure 2(b)).

4. The radius in pixels of the bright ring was taken as the peak in the fitted spline and the value compared to the appropriate calibration chart to obtain the distance in micrometers of the sphere from the focal plane of the objective.

In Figure 2(c) we have plotted the calibrated (x, y, z) positions of a swimming $a = 2.40\,\mu$m sphere over a period of about 30 s. The sphere was observed to climb from its starting position towards the objective, with the radius of the bright ring decreasing as it moved. See movie 1.

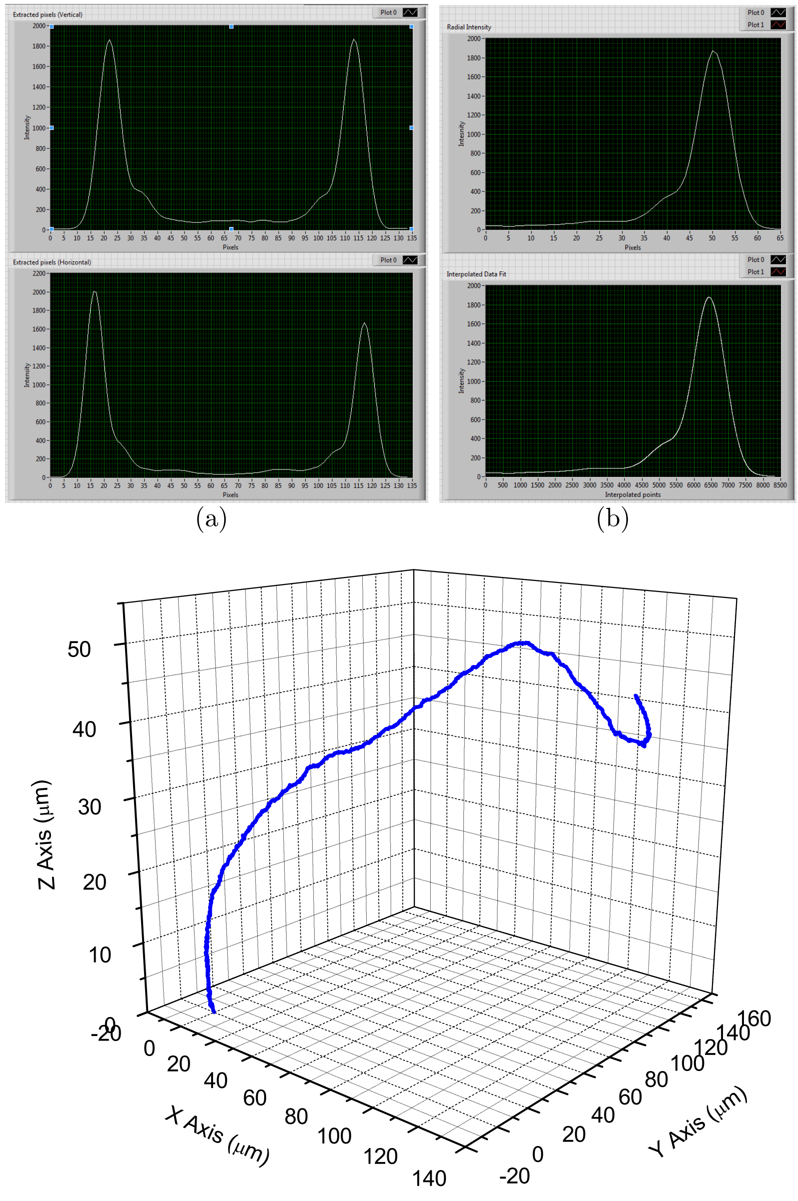


Figure 2: 2(a) The intensity values of a line of pixels extracted across the bright ring used to calculate the (x, y) center. In 2(b) we have plotted the mean intensity values of the pixels radiating from the center of the bright ring and the fitted spline values. The peak toward the end marks the radius of the ring. Plotted in 2(c) is the calibrated (x, y, z) positions of a swimming $a = 2.40\,\mu$m sphere over a period of about 30 s.

2. Center of Mass Calculations

The Boltzmann distribution for the orientation of the Janus spheres is a function of the distance of the center of mass (COM) of the asymmetrically coated spheres from the sphere center. We model the shape of the asymmetric platinum coating on the sphere as an ellipsoidal layer, which is thickest at the sphere pole and tapering to a much thinner layer toward the sphere equator (see Figure 3). This model is consistent with the cap mass calculated from the sedimentation data of our Janus spheres (see main article).

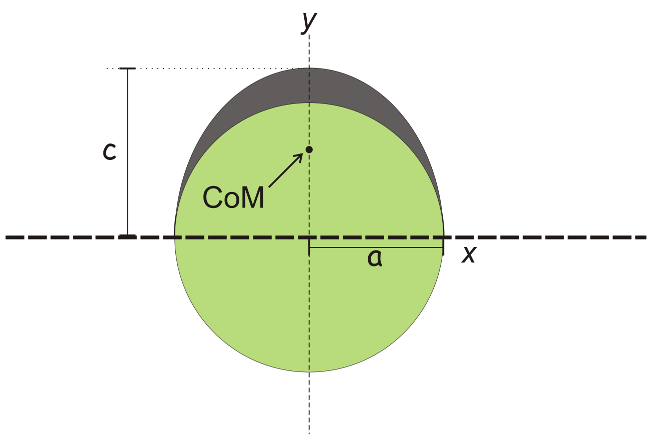


Figure 3: By symmetry the center of mass of the polystyrene spheres with an asymmetric platinum coating (dark grey) lies along the y axis. We model the platinum coating as an ellipsoidal layer with long axis c, on the underlying sphere with radius a.

To find the COM we took the shape of the platinum cap as being a solid half-ellipsoid, with a long axis of length c and short axis of radius a, and then removed a solid half-sphere of radius a. In Figure 3 we can see that by symmetry the COM of the ellipsoidal cap lies along the y axis. By integration it can be shown that the COM of the solid half-sphere is at the point $3/8a$ along the y axis. Similarly, the COM of a solid half-ellipsoid is at the point $3/8c$. Taking the weight of the solid half-sphere as w, then by ratio of their volumes the weight of the solid half-ellipsoid is $\frac{c}{a}w$. The COM along the y axis ($\bar{y}$) can now be calculated using

$$(\frac{c}{a}w - w)\bar{y} = (\frac{c}{a}w)\frac{3}{8}c - (w)\frac{3}{8}a. \quad (1)$$

For our 10 nm thick platinum coating this gives a center of mass as $(\bar{x}, \bar{y}) = (0, 0.75c)$.

3. List of Movies

1. **Movie_1.avi** A 2.40 μm radius Janus sphere with asymmetric platinum coat swimming in a 10 wt% solution of H_2O_2. The sphere is seen to move about 50 μm upwards against gravity - the radius of the bright ring decreases as the sphere moves upwards.

2. **Movie_2.avi** Multiple 0.95 μm radius Janus spheres swimming in a 10 wt% solution of H_2O_2 and viewed in the (x, z) plane. The top of the image is upwards against gravity. The spheres are seen to swim in all directions and frequently change direction.

3. **Movie_3.avi** A single 1.55 μm radius Janus sphere swimming in a 10 wt% solution of H_2O_2 and viewed in the (x, z) plane. The top of the image is upwards against gravity. The sphere demonstrates gravitaxis, traveling against gravity toward the top of the image.

4. **Movie_4.avi** A single 2.40 μm radius Janus sphere swimming in a 10 wt% solution of H_2O_2 and viewed in the (x, z) plane. The top of the image is upwards against gravity. The sphere demonstrates gravitaxis, traveling against gravity toward the top of the image.

References

(1) Peterson, S. D.; Chuang, H. S.; Wereley, S. T. Three-dimensional particle tracking using micro-particle image velocimetry hardware. *Measurement Science and Technology* **2008**, *19*.

(2) Speidel, M.; Jonas, A.; Florin, E. L. Three-dimensional tracking of fluorescent nanoparticles with subnanometer precision by use of off-focus imaging. *Optics Letters* **2003**, *28*, 69–71.