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Chlamydomonas reinhardtii displays aversive swimming response to silver nanoparticles

Supplementary Information

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Materials and Methods

Algae growth recipes

Bei (40×): Dissolve 15 g NH_4Cl, 2 g $CaCl_2*2H_2O$ and 4 g $MgSO_4*7H_20$ in 1 L.

TAP (40×): Add 96.8 g of Tris and 40 ml of phosphate buffer to 800 mL of DI water. Adjust the pH to 7 with acetic acid or NaOH. Complete the solution to 1000 mL with water.

Phosphate buffer for TAP: Prepare two solutions, 6.8 g KH_2PO_4 in 50 mL DI water and 8.7 g K_2HPO_4 in 50 mL DI water. Add 30 ml of K_2HPO_4 and adjust the pH to 7 with KH_2PO_4 (approximately 16 mL).

Metal mixture (algae micronutrient additive): See the table below (Table S3) for the list of required chemicals. Dissolve H_3BO_3 and EDTA in ~250 mL boiling DI water. Dissolve $ZnSO_4$ in ~100 mL and all remaining chemicals in ~50 mL. Mix chemicals together in a 1 L flask in the order listed in the table and heat to 70 °C. Adjust pH to 7.0 with 20% KOH (~100 mL). Bring to 1 L with DI water. Stir the solution once per day for 1 to 2 weeks until it becomes purple. Filter the solution (0.45 μm), retain the filtrate in maintain the solution in the refrigerator.

Algae growth protocol

To prepare agar plates, combine 7.50 g agar, 12.5 mL Bei (40x), 12.5 mL TAP (40x) and 0.5 mL of metal mixture and bring to final volume of 500 mL with DI water. Autoclave (L15) and pour into plates aseptically. To prepare liquid growth media, combine 3.125 mL of each of Bei and TAP (×40), as well as 0.125 ml of metal mixture and bring to a final volume of 500 mL with DI water. Aliquot 40 mL into a 125 mL flask and 200 mL into a 500 mL flask and autoclave (L15). Algae were inoculated in a zig-zag pattern onto agar plates every week from a 2 weeks old plate. Cooled liquid growth media was inoculated with 1 loopful of algae from a plate of at least one week old, but less than 2 weeks.

Microfluidics Device Preparation

Fisherbrand™ glass microscope slides (Fisher Scientific) were washed in acid-alcohol overnight and used to base the PDMS devices. Holes for Tygon Microbore Tubing (Cole Palmar, Montreal, QC) were punched using a 20 gauge blunt syringe tip (SAI Infusion Technologies, VWR, Montreal, QC) and connected to 1001 TLL (Hamilton, VWR) using 30 gauge blunt syringe tips (SAI Infusion Technologies, VWR). All other materials, protocols and/or instructions can be found in Englert et al.[28]

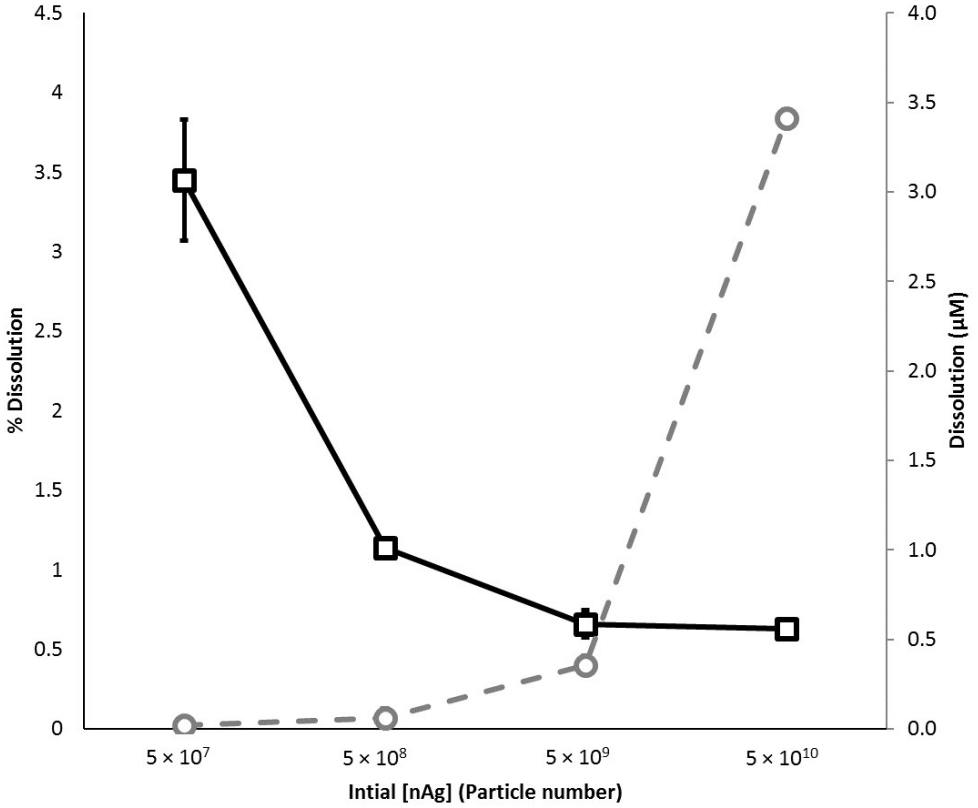

Figure S1. Dissolution of PVP-*n*Ag after 2 h incubation in 1 mM $NaNO_3$ measured by ultracentrifugation and ICP-MS. Black squares denote measured dissolution in µM and grey circles indicate calculated dissolution as a percentage of the total mass of *n*Ag.

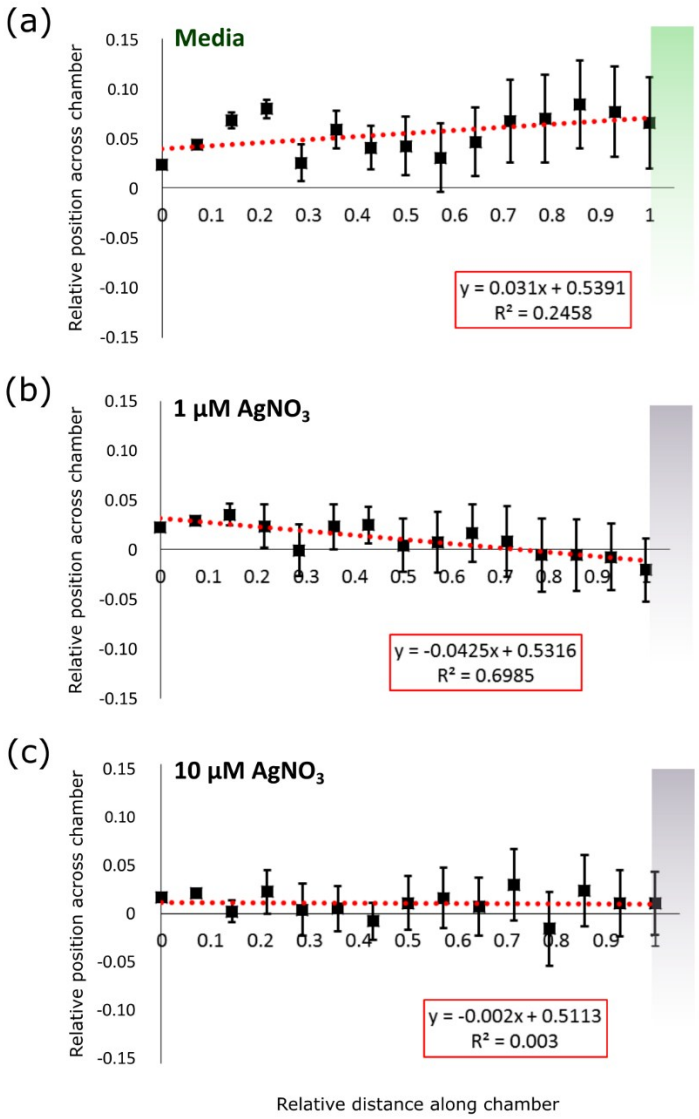

Figure S2. (a) through (c) are the linear regressions corresponding to the frequency plots for each condition presented in Figure 2 (a) through (c), respectively. Error bars indicate 1 standard deviation of the mean of the frequency distribution at each point.

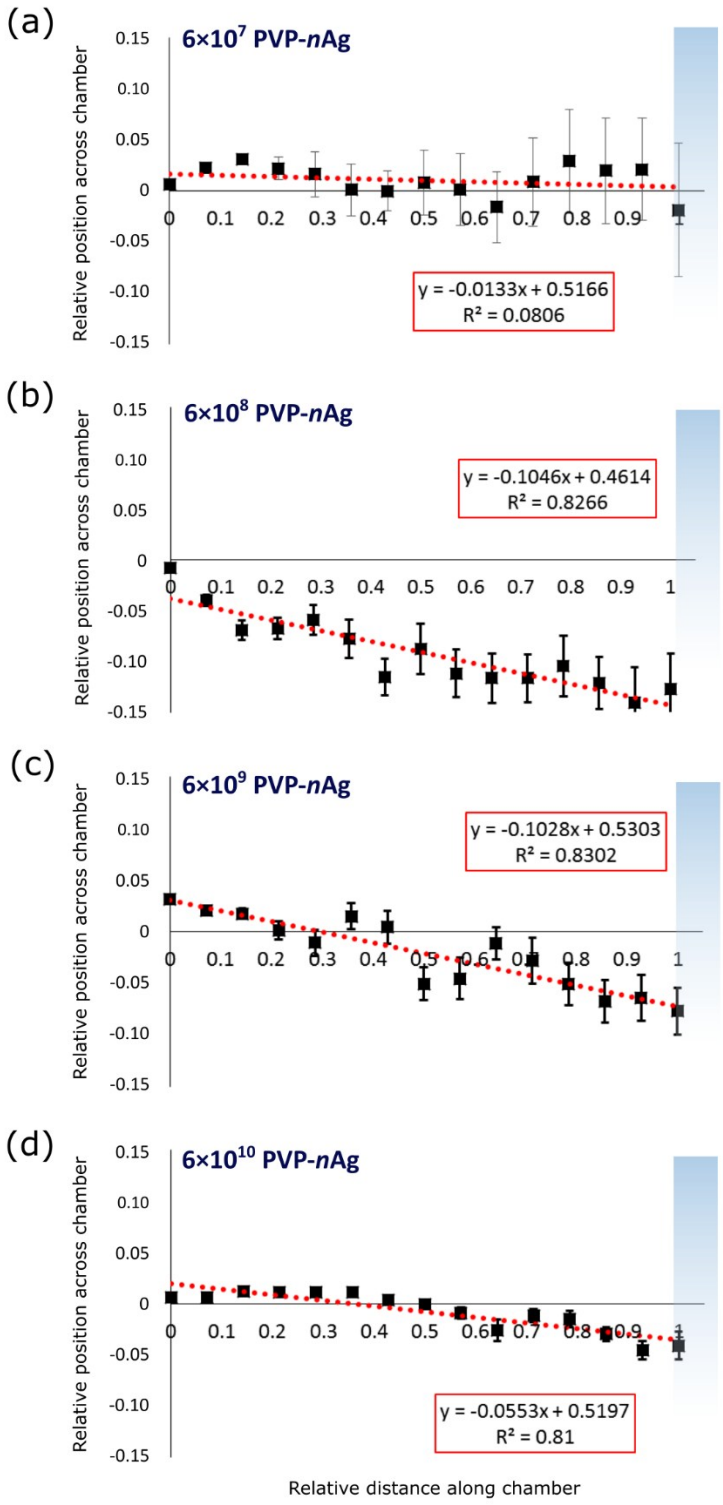

Figure S3. (a) through (d) are the linear regressions corresponding to the frequency plots for each condition presented in Figure 3 (a) through (d), respectively. Error bars indicate 1 standard deviation of the mean of the frequency distribution at each point.

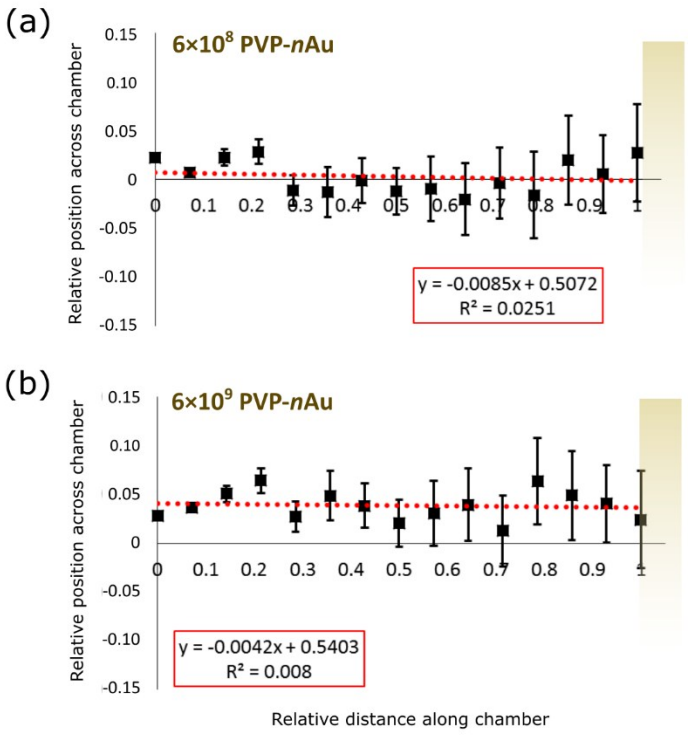

Figure S4. (a) and (b) are the linear regressions corresponding to the frequency plots for each condition presented in Figure 4 (a) and (b), respectively. Error bars indicate 1 standard deviation of the mean of the frequency distribution at each point.

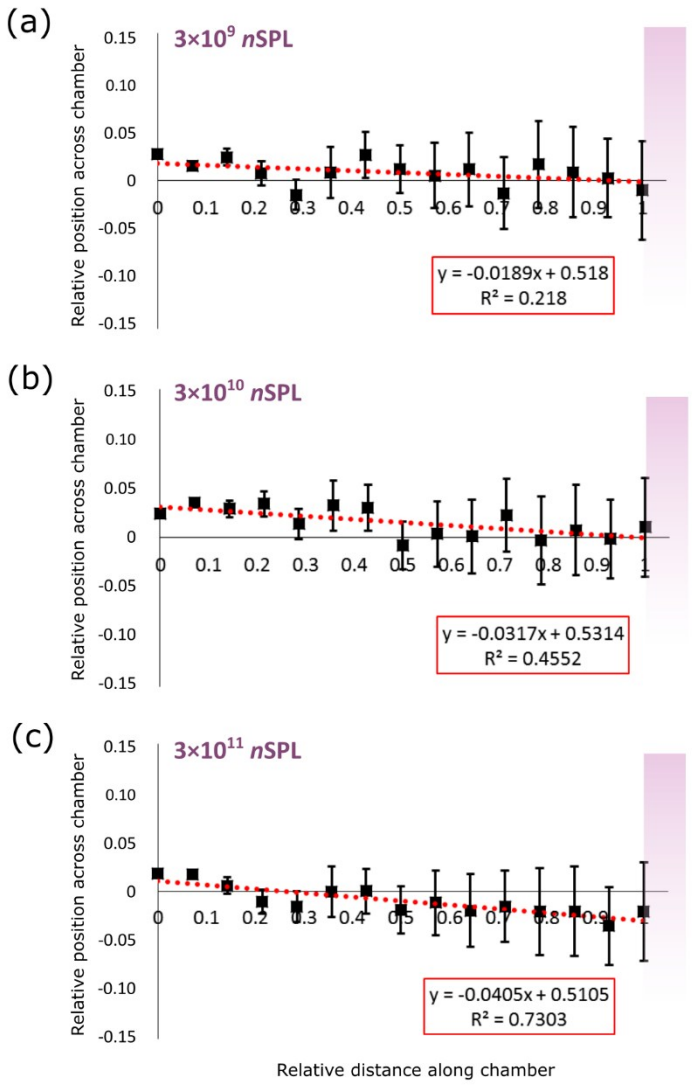

Figure S5. (a) through (c) are the linear regressions corresponding to the frequency plots for each condition presented in Figure 5 (a) through (c), respectively. Error bars indicate 1 standard deviation of the mean of the frequency distribution at each point

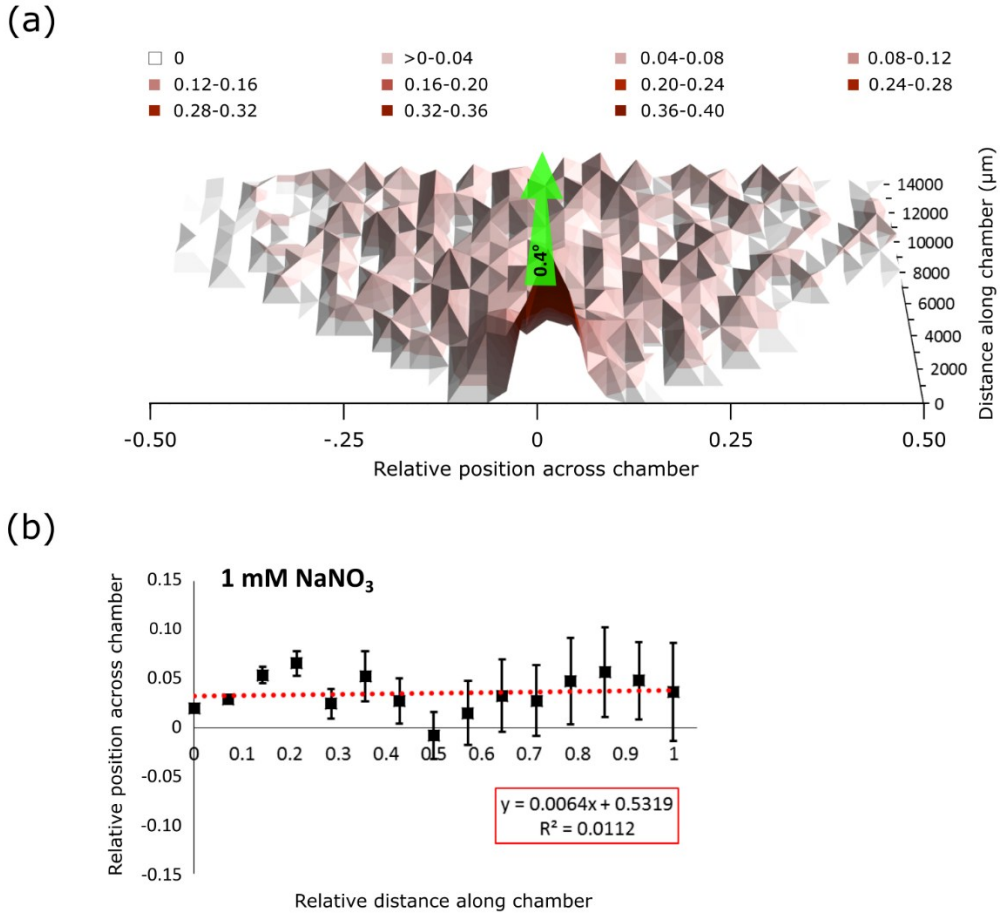

Figure S6. (a) 3-D surface plot showing the relative number of algae observed along the microfluidic chamber at each of the 1000 µm markings. In this condition, both sides of the device were filled with 1 mM $NaNO_3$. Each colour in the legend is associated with a different proportional fraction of algae. (b) The linear regressions corresponding to the frequency plot in (a). Error bars in (b) indicate 1 standard deviation of the mean of the frequency distribution at each point.

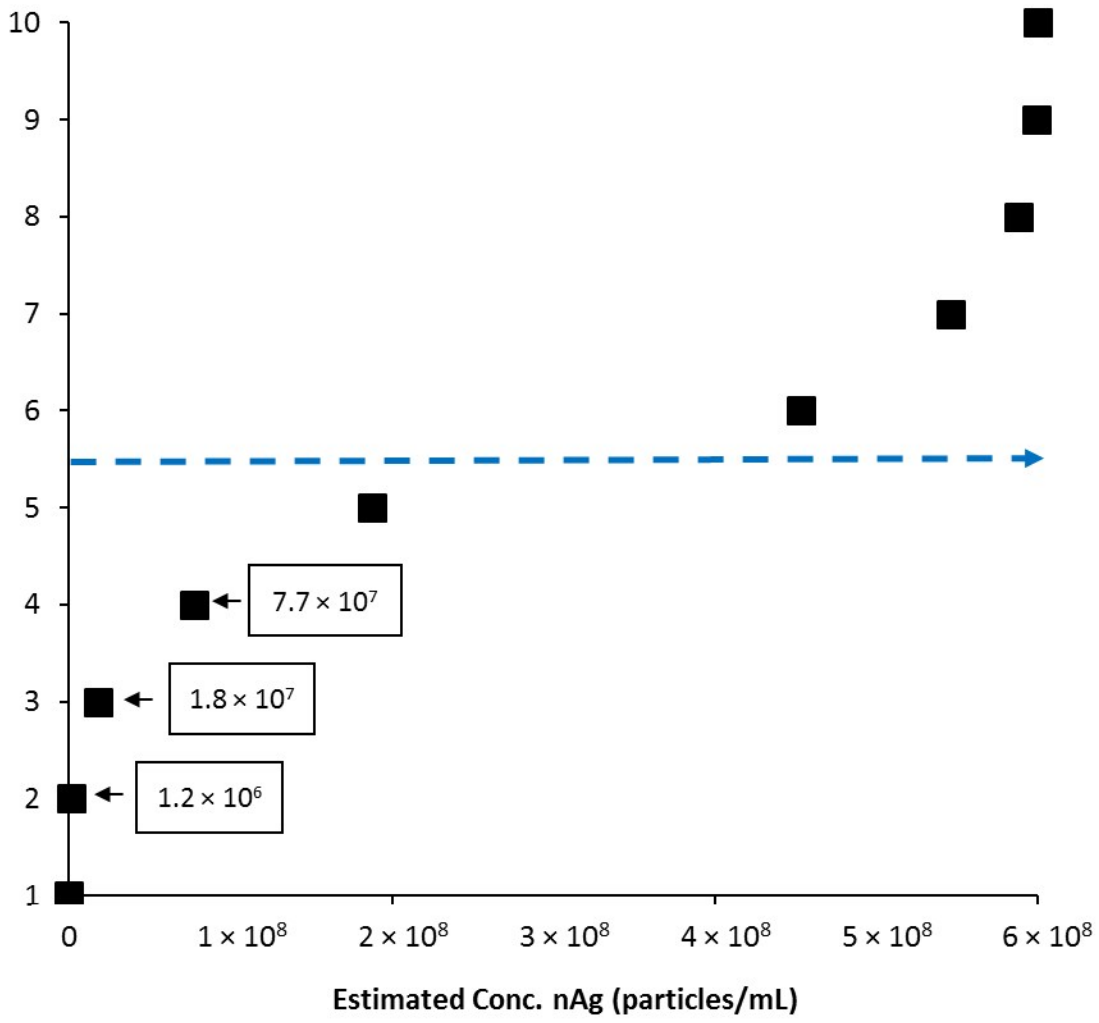

Figure S7. Plot of estimated concentrations of PVP-*n*Ag in the gradient generated by the microfluidic exposure device from inlets of 6×10^8 PVP-*n*Ag (high) and 1 mM $NaNO_3$ (low). These estimated concentrations were calculated assuming perfect 1:1 mixing of fluid streams at each mixing node in the microfluidic gradient generator. Data values are provided for points that are difficult to identify visually. The blue arrow indicates the location of the algae inlet, between the 5th and 6th fluid streams.

Table S1. Average (n=4) hydrodynamic diameters and ζ-potential of PVP-*n*Ag, PVP-*n*Au and *n*SPL.

Particle Type	Ionic Strength (mM)	Polydispersity Index	[a]Mean Size [d_{DLS}] (nm)	ζ-potential (mV)
PVP-*n*Ag	1	0.16	55.1 ± 0.3	49.2 ± 1.3
(6×10^8)	10	0.15	54.4 ± 0.2	48.4 ± 1.6
	100	0.16	54.6 ± 0.4	47. 3 ± 1.9
PVP-*n*Au	1	0.15	54.9 ± 0.2	32.8 ± 1.5
(6×10^8)	10	0.17	55.1 ± 0.3	32.2 ± 1.3
	100	0.14	55.4 ± 0.3	30.6 ± 1.5
***n*SPL**	1	0.13	24.2 ± 0.3	56.2 ± 1.1
(3×10^8)	10	0.16	24.4 ± 0.4	57.1 ± 1.3
	100	0.18	25.3 ± 0.3	48 ± 1.2

[a]The Z-average d_{DLS} is reported.

Table S2. Summary of regression statistics.

Particle Type	[Conc.] (particles/mL)	R^2	Slope of Regression	Angle of Trajectory (°)	*F* score	*p*-value
Media	1 ×	0.25	0.031	+1.77	4.24	0.06
***n*Ag**	6×10^7	0.08	-0.013	-0.76	1.14	0.31
	6×10^8	0.83	-0.105	-5.97	61.97	**~10^{-6}**
	6×10^9	0.83	-0.103	-5.87	63.57	**~10^{-6}**
	6×10^{10}	0.81	-0.055	-3.17	55.42	**~10^{-6}**
***n*Au**	6×10^8	0.03	-0.009	-0.49	0.33	0.57
	6×10^9	0.01	-0.004	-0.24	0.11	0.75
***n*SPL**	3×10^9	0.22	-0.019	-1.09	3.63	0.08
	3×10^{10}	0.46	-0.032	-1.82	10.86	**0.006**
	3×10^{11}	0.73	-0.041	-2.32	35.2	**~10^{-5}**
$AgNO_3$	1 μM	0.70	-0.042	-2.43	30.11	**~10^{-4}**
	10 μM	0.003	-0.002	-0.12	0.04	0.85

Table S3. Chemicals for metal micronutrient solution (for algae growth medium).

Chemical	Molarity (g/mol)	Mass required (g)
H_3BO_3	61.83	11.41
$ZnSO_4 \cdot 7H_2O$	287.55	22.02
$MnCl_2 \cdot 4H_2O$	197.91	5.07
$FeSO_4 \cdot 7H_2O$	278.01	4.99
$COCl_2 \cdot 6H_2O$	237.93	1.61
$CuSO_4 \cdot 5H_2O$	249.68	1.57
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	1235.86	11.08
$NaEDTA \cdot 2H_2O$	372.24	50.06

Representative movie link: https://www.youtube.com/watch?v=_UE59GovcLk

Details: ~20 sec clip of algae swimming in the microfluidic device filled with 1 mM $NaNO_3$ (top) and 6×10^8 particles/mL nAg (bottom).

Representative movie file:

C. reinhardtii slow.gif

Details: Example of algae swimming in the microfluidic device without flow. .gif format, 400× magnification, frame capture rate 30 fps, 0.125× original play speed (double click file to play). The file C. reinhardtii slow.gif is available in the supplementary information zip file.