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Focusing light inside scattering media with magnetic-particle-guided wavefront shaping: supplementary material

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This document provides supplementary information to "Focusing light inside scattering media with magnetic-particle-guided wavefront shaping," https://doi.org/10.1364/optica.4.001337. In this document, we first describe the experimental setup in detail. We then provide detailed information about sample preparation, magnetic particle characterization, and cell viability measurement. Finally, we analyze the magnetic guidestar modulation efficiency based on the cell samples with magnetic particles.

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a. Experimental Setup

The experimental setup can be divided into three modules as shown in Figure S1. The first module, named Light Source Regulation, prepares three light beams for the experiment, a sample beam, a reference beam, and a playback beam. These three beams share the same light source, the pulsed laser (532 nm wavelength, 20 ns pulse width, 40 kHz rep rate, QL532-500-RL, CrystaLaser). It should be noted that the principle of this work does not depend on the pulsed nature of the illumination and would also work with a continuous wave (CW) laser source. Both the reference beam and the sample beam are shifted in frequency using two acousto-optic modulators (AOM, AFM-502-A1, IntraAction), respectively. All these three beams are spatially filtered, collimated, and aligned to the same polarization direction as that of the spatial light modulator (SLM, Pluto, Holoeye).

The second module is the DOPC system. This system consists of two key components, a camera (PCO.Edge, PCO.) and an SLM, which are precisely aligned to each other through a plate beam splitter (BSP). A path length compensator is used to match the path length of different k-vectors of the sample beam and playback beam. A pair of lenses (focal length of L4: 200 mm, L5: 75 mm) in a 4-f configuration images the back focal plane of the objective (10x, 0.25 NA, Plan N, Olympus) to the camera. The measured speckle size is on average 9 SLM pixels, resulting in ~2.2×10^5 controlled optical modes with the SLM which contains 2 million pixels. A four-phase stepping approach is used to measure the optical field from the sample. The DOPC system alignment is based on the method described previously in reference [1].

The third module is called Sample Observation as shown in Figure S1. In this module, the sample beam is routed to the sample placed between two electromagnets (cylindrical solenoid, 32mm diameter, 31mm height, 24 V, 6 W, UE 3231, UE-TECH). The measured peak magnetic field and field gradient amplitude at a positon 10 mm away from the magnet surface (sample position) is 17.3 mT and 27.4 mT/mm, respectively. The magnetic field was measured using a Gaussmeter (AlphaLab Inc., GM3). To optimize the magnetic particle displacement, one magnet is placed slightly off axis with reference to the other as shown in Figure S1 to provide a torque for particle rotation. The magnetic particles and the playback light are observed using a microscopic imaging system consisting of an objective (20×, 0.25 NA, SLMPlan N, Olympus), a tube lens (L6, focal length: 200 mm), and a camera (Stingray F145, Allied Vision Technologies).

The measured size of the optical speckle on the target plane was on average 1.5 μm. The number of optical modes being modulated can be estimated based on the mean size of the speckle grain, the size of the target, and its displacement using the following equation,

$$M \approx \frac{2ndl_{\mathrm{tg}}}{l_{\mathrm{sp}}^{\ 2}}, \tag{S1}$$

where n is the number of targets along the direction orthogonal to the direction of target displacement; d is the amplitude of target displacement; l_{tg} is the length of the target; l_{sp} is the mean diameter

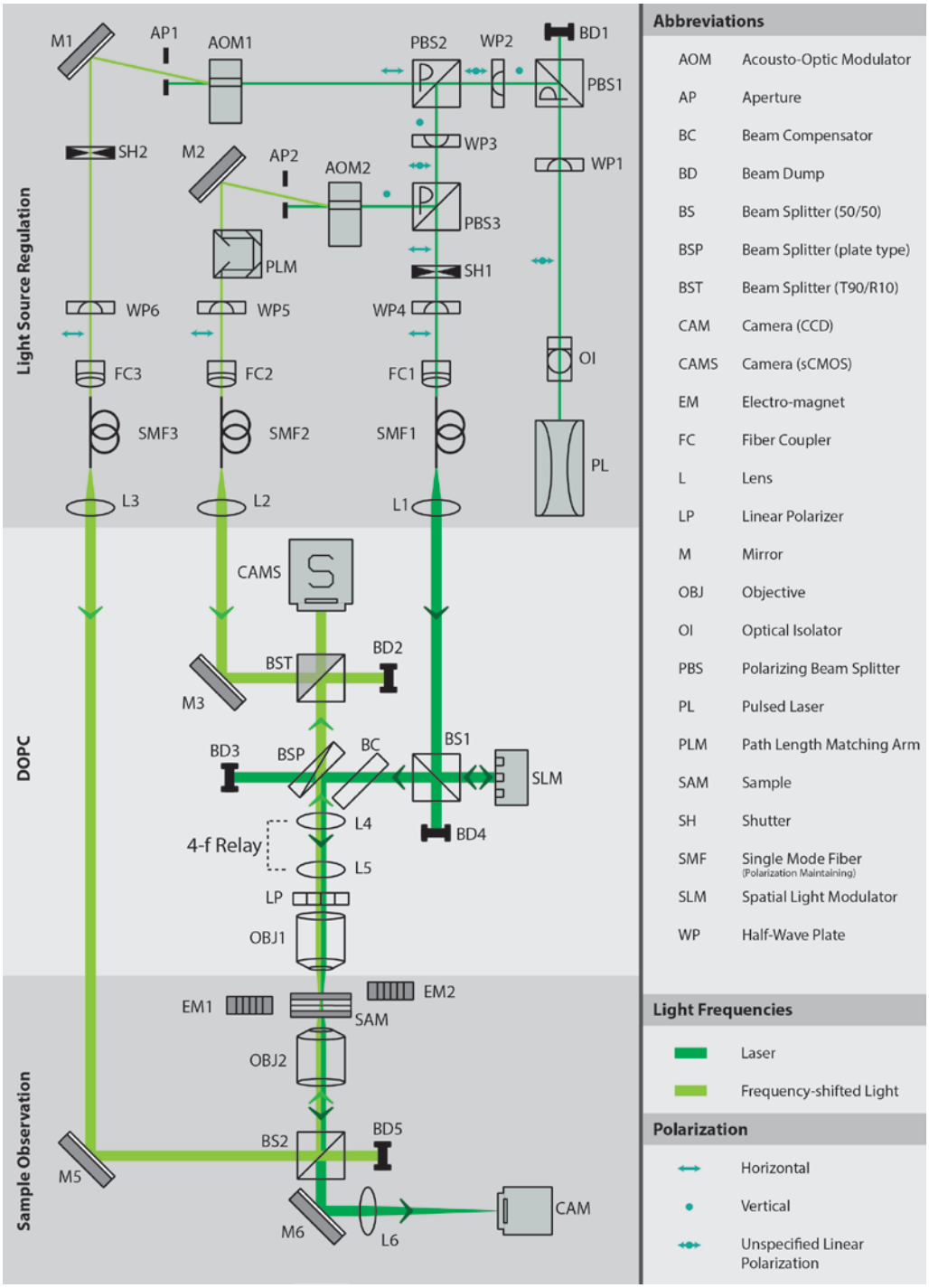


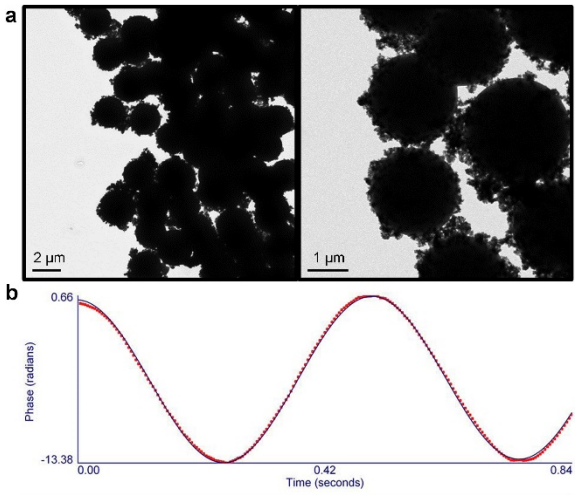
Figure S1. Schematic of the setup.

of the speckle. For the 2.5 μm magnetic particles shown in Fig. 2, we have $n = 2$; $d = 1.7$ μm; $l_{tg} = 2.5$ μm, resulting in $M \approx 8$. For the cell with magnetic particles, we have $n = 1$; $d = 2.2$ μm; $l_{tg} = 11$ μm, resulting in $M \approx 22$.

b. Sample Preparation

For the experiments without living cells, we used polystyrene core paramagnetic particles with a mean diameter of 2.5 μm (PM-20-10, Spherotech). We added 1 μl of the magnetic particle solution (2.5% w/v) into 0.5 ml water, resulting in a concentration of 0.05 mg/ml. This sample was perfused into a rectangular microfluidic channel with a cross section of 50 μm × 500 μm (VitroTubes, VitroCom).

For the experiments involving living cells, we used carboxyl superparamagnetic particles of 453 nm mean diameter (CM-05-10H, Spherotech). We mixed 2 μl of the magnetic particle solution (1% w/v) with 1 ml culture medium (Dulbecco's Modified Eagle's Medium (DMEM) with 10% Fetal Bovine Serum (FBS) and 1% Penicillin and Streptomycin (PS)) and then added the mixed



Run	Mobility	Zeta Potential (mV)	Rel. Residual
1	-2.50	-32.00	0.0235
2	-2.57	-32.86	0.0209
3	-2.64	-33.73	0.0179
4	-2.71	-34.70	0.0209
5	-2.57	-32.91	0.0158
6	-2.71	-34.68	0.0130
7	-2.34	-29.99	0.0170
8	-2.53	-32.37	0.0309
9	-2.45	-31.31	0.0262
10	-2.19	-28.01	0.0140
Mean	-2.52	-32.26	0.0200
Std. Error	0.05	0.66	0.0018
Combined	-2.52	-32.24	0.0069

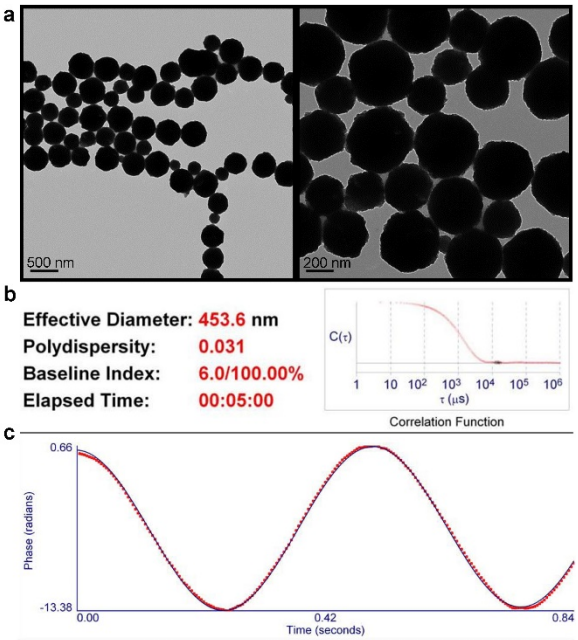
Figure S2. Characteristics of polystyrene core paramagnetic particles. (**a**) TEM images of the polystyrene core paramagnetic particles (Scale bar = 2 µm and 1 µm respectively) and (**b**) Zeta potential of the polystyrene core paramagnetic particles (mV).

solution to the macrophages (RAW 264.7) in a cell culturing dish (35 mm diameter) containing 4 ml culture medium. The initial confluency of the cell sample was ~15%. After culturing for ~36 hours, some of the macrophages engulfed the magnetic particles, and the cell confluency reached ~90%. We then harvested the cells. The media was removed and replaced with 0.5 ml trypsin-EDTA (0.05%, Gibco) and incubated in the incubator for an additional 5 mins. After the cells detached from the plate surface, the sample was transferred into a micro centrifuge tube using a pipette. The sample in the micro centrifuge tube was centrifuged for 3 mins at 4000 rpm. The trypsin-EDTA on top of the cell pellet was replaced with 0.5 ml fresh culture medium. Then the sample was mixed and perfused into a microfluidic channel of the same model as described in the last paragraph. In our experiment, ~10% of the cells engulfed sufficient magnetic particles to generate significant guidestar effect under the external magnetic fields.

The scattering sample was made of 1-mm-thick chicken breast tissue (1mm × 6.3 mm × 6.3 mm). The sliced tissue was sandwiched between a 1-mm-thick glass slide and a 0.17 mm thick coverslip with a 1-mm-thick spacer in between. The samples were then sealed to avoid dehydration during the experiment.

c. Magnetic Particle Characterization

Dynamic light scattering (DLS) and Zeta potential measurements were performed on a Brookhaven 90 Plus/BI-MAS Instrument (Brookhaven Instruments, New York). DLS measurements were obtained by performing 5 runs at 30 s per run and Zeta potential measurements were obtained by performing 10 runs with 30 cycles per run.



Run	Mobility	Zeta Potential (mV)	Rel. Residual
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Combined	-2.52	-32.24	0.0069

Figure S3. Characteristics of carboxyl superparamagnetic nanoparticles. (**a**) TEM images of the carboxyl superparamagnetic nanoparticles (Scale bar = 500 nm and 200 nm respectively). (**b**) Hydrodynamic size (nm) of the carboxyl superparamagnetic nanoparticles and (**c**) Zeta potential of the carboxyl superparamagnetic nanoparticles (mV).

Transmission electron microscopy (TEM) images were obtained with an FEI Tecnai T12 transmission electron microscope at an accelerating voltage of 120keV and images were taken with a Gatan Ultrascan 2K CCD camera. The nanoparticle samples were imaged on 300 mesh carbon/formvar coated grids (Ted-Pella).

d. Cell Viability Measurement

Cell Viability Experiment: RAW 264.7 cells from ATCC (TIB-71) were cultured in complete DMEM (ATCC® 30-2002™) media (10% FBS, 1% PS). For each experiment 4,000 cells were added to each well and after 24 hr, escalating doses of the carboxyl superparamagnetic nanoparticles were added to each well. Final concentration of nanoparticles ranged from 0.625 [µg iron/ml] to 80 [µg iron/ml]. After 3 days of incubation with the nanoparticles, the media was removed and replaced with 100 µL Cell Lysis Buffer (20mM Tris, 2mM EDTA, 150mM NaCl, 0.5% Triton X-100, pH 7.4). Cells were frozen to ensure complete cell lysis. ATP concentration at the time of lysis was measured using the CellTiter-Glo® Assay. ATP concentration is correlated with metabolic activity in cells. In the CellTiter-Glo® Assay, the CellTiter-Glo® substrate is converted into a luminescent substrate which is proportional to the amount of ATP in the cell lysate. In order to normalize to cell number, the amount of double stranded DNA in the cell lysate was measured by

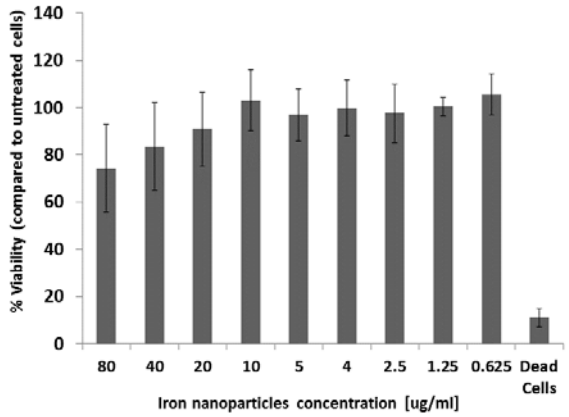

Figure S4. Cell viability after 3 days of incubation with iron nanoparticles. Percentage of viable RAW 264.7 cells 3 days after the addition of different amounts of iron. Error bars show the standard deviations of the results from 3 repeated experiments.

the fluorescence of PicoGreen® reagent. PicoGreen reagent fluoresces upon binding to double stranded DNA. Experimental conditions were normalized to the no treatment control. The viability results are shown in Figure S4.

e. Modulation Efficiency Measurement

The modulation efficiency of the magnetic guidestar was measured based on the cell samples with magnetic particles. The modulation efficiency is defined as the ratio between the modulated light intensity and the light intensity incident on the guidestar, i.e. the percentage of the light being modulated by the guidestar. To directly measure the modulation efficiency, we removed the scattering sample on the DOPC side of the system as shown in Figure S5 and the lens L5 in Figure S1, to directly image the sample to the camera of the DOPC system. In this case, we can image the field on the guidestar plane by implementing the DOPC recording process for both the field-subtraction method and the frequency-modulation method. To calculate the modulation efficiency, we also measured the reference beam light intensity I_r and the sample beam light intensity I_s. For the field-subtraction method, we used the following equation to calculate the modulation efficiency *M* as described in reference [2].

$$\eta = \frac{\left|E_c^{'} - E_c\right|^2}{64 I_s}, \qquad \text{(S2)}$$

where $E_c = \left[(I_0 - I_2) + i(I_1 - I_3)\right] / \sqrt{I_r}$ and $E_c^{'} = \left[(I_0^{'} - I_2^{'}) + i(I_1^{'} - I_3^{'})\right] / \sqrt{I_r}$ are the fields reconstructed from the four intensity images I_k and $I_k^{'}$ ($k = 1, 2, 3, 4$) measured during the 4-phase stepping DOPC recording before and after applying the magnetic field, respectively. Figure S5b shows the image of $\left|E_c^{'} - E_c\right| \sqrt{I_r}$. For the frequency-modulation method, we used the equation

$$\eta = \frac{\left|E_c\right|^2}{16 I_s}, \qquad \text{(S3)}$$

where $E_c = \left[(I_0 - I_2) + i(I_1 - I_3)\right] / \sqrt{I_r}$ is the field reconstructed from the four intensity images I_k (k = 1, 2, 3, 4) measured during the 4-phase stepping DOPC recording when the magnetic field is on. Figure S5 c-e show the $\left|E_c\right| \sqrt{I_r}$ maps, where the AC magnetic field has a fundamental frequency of 25 Hz, while the reference beam frequency is set to 25 Hz (c), 50 Hz (d), and 75 Hz (e).

To compute the modulation efficiency from the captured field images, we applied a 10 μm circular region of interest (ROI) to the images of the cells and averaged the amplitude of the field over the top 10% of the pixels within this ROI. Based on Equation S2 and S3, the modulation efficiency using the field-subtraction method is 29%, while that of the frequency-modulation method is 5% (fundamental frequency), 0.5% (second harmonic), and 0.1% (third harmonic).

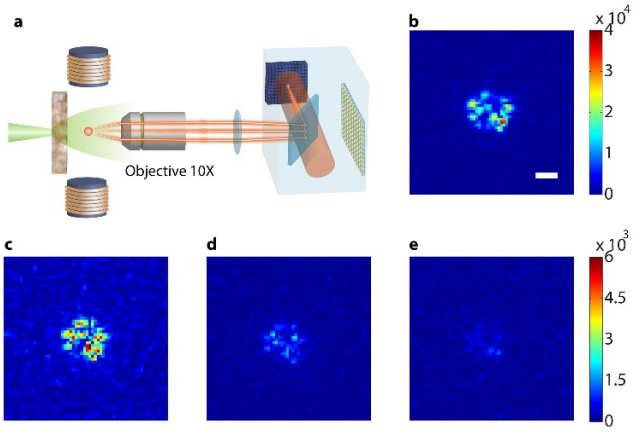

Figure S5 | Measurement of the light-tagging efficiency of the magnetic particle guidestar based on the magnetic particle labelled cell sample. (**a**) Schematic of the setup to measure the light-tagging efficiency of the field-subtraction method and the frequency-modulation method. The light-tagging efficiency was calculated by the ratio between the power of the tagged light and the power of the light passing through the cell with magnetic particles. (**b**) The tagged light field measured by the field-subtraction method, from which we calculated the light-tagging efficiency to be 29%. (**c-e**) The tagged light fields measured by the frequency-modulation method, when the reference beam frequency was shifted by (c) 25 Hz (the fundamental frequency shift of the modulated light), (**d**) 50 Hz (2nd harmonic), and (**e**) 75 Hz (3rd harmonic) relative to the laser frequency. The light-tagging efficiency calculated from the measured field in (**c**) is 5%. Scale bar: 5 μm.

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