Wavelength-encoded laser particles for massively multiplexed cell tagging

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Large-scale single-cell analyses have become increasingly important given the role of cellular heterogeneity in complex biological systems. However, no techniques at present enable optical imaging of uniquely tagged individual cells. Fluorescence-based approaches can distinguish only a small number of distinct cells or cell groups at a time because of spectral cross-talk between conventional fluorophores. Here we investigate large-scale cell tracking using intracellular laser particles as imaging probes that emit coherent laser light with a characteristic wavelength. Made of silica-coated semiconductor microcavities, these laser particles have single-mode emission over a broad range from 1,170 nm to 1,580 nm with sub-nanometre linewidths, enabling massive spectral multiplexing. We explore the stability and biocompatibility of these probes in vitro and their utility for wavelength-multiplexed cell tagging and imaging. We demonstrate real-time tracking of thousands of individual cells in a three-dimensional tumour model over several days, showing different behavioural phenotypes.

Although fluorescence microscopy is widely used for cellular imaging, typical fluorophore linewidths, between 30–100 nm, allow no more than a few spectra to fit into the entire visible spectrum without overlap. Consequently, this technique can normally resolve only a handful of labels, preventing concurrent study of many more cell types and subtypes. It is fundamentally challenging to engineer fluorophores or inorganic emitters to have much narrower emission spectra because of the quantum mechanical and thermodynamic broadening of their electronic energy levels. Raman emission from vibrational transitions is narrower (1–2 nm) but over a relatively limited tuning range (20 nm). Multiple fluorescence emitters with dissimilar spectra can be combined, but these approaches relying on intensity-based spectral analysis or nanoscale super-resolution are of limited use for large-scale tracking in optically dense tissues owing to wavelength-dependent absorption and scattering. Unlike molecular engineering, photonic principles harnessing optical resonance and amplification could offer a solution. By placing fluorescent emitters inside an optical cavity with a sufficient quality (Q)-factor, laser emission with extremely narrow, sub-nanometre linewidth can be produced. Previous efforts using dye-doped microspheres have shown the proof of concept of this photonic approach, but the large resonator sizes of around 10 μm have prohibited practical applications.

Here we show microlasers with massive multiplexing capability and optimized properties for cell tagging and tracking applications. We name these imaging probes laser particles (LPs). To miniaturize laser sizes, we used semiconductor materials with high refractive index and gain in a microdisk geometry. Microdisk lasers have been extensively investigated for on-chip applications, but we have developed methods to detach them from the substrate, suspend them in solution and coat them with a biocompatible and protective layer. These LPs occupy only about 0.1% of a typical cell’s volume and generate single narrowband emission peaks (below 0.4 nm), tunable across a wide spectral range of 400 nm. We show their stable performance in cells and present methods of identifying tagged cells using LP-stimulated emission (LASE) microscopy. Finally, we demonstrate massively multiplexed tracking of thousands of individual cancer cells in a three-dimensional tumour spheroid invasion assay. Our work establishes LPs as a new class of luminescent probes that expands optical microscopy for large-scale, comprehensive single-cell analysis.
Single-mode microlasers over a wide spectral range

Of the several semiconductor materials suitable for LPs, we chose the InAlGaAs and InGaAsP quaternary alloys (Fig. 1b), which have small bandgap energy for operation in the second near-infrared window (NIR-II) of 1.0–1.8 μm. This range is attractive owing to its low cell phototoxicity, relatively good penetration into tissues, and no spectral overlap with conventional fluorescent probes. We chose a microdisk design supporting planar whispering gallery mode (WGM) resonances because sufficient passive-cavity Q-factors of >1000 can be obtained with micrometre or submicrometre sizes, and the resonance wavelength is tunable by changing its diameter.

Calculations based on WGM theory (see Supplementary Note 1) predict that, for microdisks with diameters around 2 μm, the sensitivity of the resonance wavelength to small changes in diameter is around 1 nm μm⁻¹. Using electron-beam lithography, we produced batches of microdisks with identical design diameters separated by 10-nm steps. Measurements of their emission wavelengths closely followed theoretical predictions, with a discontinuity when the resonance jumps to a higher order as the diameter increases (Fig. 1c). Measurements of N=100 disks of the same batch show a standard deviation σ=1 nm in resonance wavelength (Fig. 1d). Although this spectral uniformity can be useful and may be further improved, in this work we chose to use ultraviolet lithography instead, so that we could produce microdisks in large quantity (about 3.2 million microdisks per square centimetre of wafer) at much faster speed and lower cost than electron-beam lithography. We allowed microdisk diameters to vary over a range of approximately 200 nm so that the fabricated microdisks have randomly varying wavelengths over the entire gain bandwidth of the semiconductor. We tested different wafer designs including multi-quantum-well structures and bulk semiconductor active layers and obtained comparable performance in terms of laser threshold and emission linewidth (Supplementary Fig. 1). The LPs described below were fabricated from bulk In0.53Al0.13Ga0.34As or In0.4Ga0.6As epitaxial layers.

Microdisks were released from the substrate via wet-etching in hydrochloric acid solution and suspended in water after removing debris using size-selective filters. Scanning electron microscopy (SEM) showed reproducible microdisks with smooth edges (Fig. 1e). For optical characterization, the collected microdisks were embedded in three-dimensional hydrogel matrices (Matrigel). Upon optical pumping with a pulsed ytterbium-doped fibre laser, the output emission from each microdisk was analysed using a near-infrared spectrometer. The lasing threshold was observed at a pump energy (Eₚ) of about 7 pJ for a beam diameter of about 2 μm (Fig. 1f). Above threshold, the output spectrum featured a single peak, with a full-width at half-maximum (FWHM) smaller than 0.4 nm (Fig. 1g, h). The measured intensity varied considerably depending on the microdisk orientation in the hydrogel because WGMs radiate predominantly in the radial direction. The lasing wavelengths of microdisks obtained from a single epitaxial wafer (In0.53Al0.13Ga0.34As on InP) spanned a broad region across 100 nm, supported by the gain bandwidth of the active medium (Fig. 1i).
To extend wavelength coverage, we used five semiconductor wafers with their fluorescence peaks separated by about 80 nm (Fig. 2a). We calculated WGM modes\(^*\) as a function of diameter (Supplementary Fig. 2) to confirm that, at a given diameter, only one or two cavity modes fall inside the gain bandwidth of each wafer (Fig. 2b). Experimentally, microdisks of this diameter range generated single-mode laser emission under normal operation conditions. We were thus able to produce batches of microdisks with single-mode emission at different wavelengths across an ultrawide spectral range from 1,170 nm to 1,580 nm (Fig. 2c).

Silica coating of semiconductor microdisks

Unprotected non-oxide semiconductor materials tend to corrode slowly in water and, under photoexcitation, can generate undesirable electrochemical effects.\(^8,9\) To enable operation in aqueous biological environments, we developed a protocol to passivate the semiconductor surface of the microdisks by coating with silicon dioxide (SiO\(_2\)). Each cycle of a modified Stöber process\(^9\) produced a silica layer about 50 nm thick, and multiple cycles resulted in thicker coating (Fig. 3a, b). Cross-sectional SEM, energy dispersive X-ray spectroscopy and transmission electron microscopy confirmed uniform silica coating (Fig. 3c, d, Supplementary Fig. 3).

Besides material protection, another critical role of the silica coating is decreasing the evanescent field of cavity modes in the surrounding medium and thereby reducing the sensitivity of lasing wavelengths to changes of external refractive index (\(n_{\text{ext}}\)). Finite-difference time-domain calculations predict a wavelength dependence on refractive index (\(\Delta \lambda / \Delta n_{\text{ext}}\)) of 80 nm per refractive index unit (RIU) for uncoated microdisks, which corresponds to a variation of up to 2.4 nm in cell cytoplasm (\(n_{\text{ext}} = 1.36–1.39\))\(^9\); however, this sensitivity decreases with increasing coating thickness (Fig. 3c, f). The wavelength sensitivity of 150-nm-coated microdisks deposited on a glass substrate was measured to be 2.9 ± 0.2 nm per RIU, much lower than the 44 ± 1 nm per RIU for uncoated microdisks (Fig. 3g, h).

Stability of LPs in biological environments

Our standard design coating thickness was 100 nm. Coated microdisks embedded in cell-culture hydrogels had a slightly higher threshold pump energy of about 9 pJ than did uncoated lasers in hydrogels (about 7 pJ), owing to silica having a higher refractive index (1.46) than the hydrogel (1.34) (Fig. 4a). However, silica coating greatly improved the optical stability of microdisks under continuous pulsed excitation (\(E_p = 40\) pJ). After one billion pulses, uncoated microdisks in hydrogels showed output power degradation by about 20%, and a decrease in lasing wavelength of 1.5 nm, both of which were worse than in air (Fig. 4b, c and Supplementary Fig. 4). The degradation of uncoated microdisks is attributed to surface oxidation\(^7\) and photochemical etching of the semiconductor.\(^25\) According to our simulations (see Supplementary Note 1), a surface corrosion of the semiconductor by 2 nm causes a spectral blueshift of about 1.8 nm. Silica-coated microdisks in hydrogels were much more stable, producing constant intensity and a minute wavelength shift of 0.1 nm after emitting a billion laser pulses. Hard silica coating greatly reduced the water-induced degradation of semiconductor materials. Over 30 h in cell culture medium, coated samples showed stable lasing wavelengths within 0.4 nm, whereas uncoated semiconductor microdisks degraded with blueshifts of 11 nm (Fig. 4d).

The silica coating also allows LPs to be functionalized using chemistry already developed for silica-coated nanoparticles. For example, incorporation of fluorescent dye into the silica shell allows fluorescence-based detection of LPs, encapsulation of the silica surface with poly(ethylene glycol) can reduce non-specific cellular interactions\(^7\), and coating with biotin enables conjugation with biomolecules of interest (Supplementary Fig. 5). Thus, it is possible to conjugate LPs with different chemical species that can provide additional functionalities, such as multimodal imaging, biomolecule sensing, or cell-type-specific targeting.\(^7\)

**Biocompatibility of LPs**

The silica coating is also essential to improve biocompatibility of the LPs. Several cell lines efficiently internalized LPs within 24 h of incubation in vitro through the non-specific process of macropinocytosis\(^7\) (Fig. 4c and Supplementary Video 1). A live/dead assay showed that whereas coated LPs had no measurable effect on cell viability compared to controls, significant (\(P < 0.0001\)) toxicity was induced by uncoated microdisks after incubation for 48 and 72 h (Fig. 4f and Supplementary Fig. 6). This result is consistent with previous studies on silica-coated quantum dots, in which the coating layer prevents leakage of toxic ions from the semiconductor surface.\(^8\)
Fig. 3 | Silica coating of III-V semiconductor microdisk lasers. a, SEM images of microdisks before and after 1, 2 or 3 coating cycles. b, Silica shell thickness versus reaction cycles (N ≥ 9 each). Mean ± 95% confidence intervals (CI). c, False-colour cross-sectional SEM image of a coated microdisk cut with focused ion beam. d, Energy dispersive X-ray spectroscopy analysis of different elements along the diameter of a coated microdisk. e, Wavelength shift of a microdisk’s emission versus external refractive index, calculated from finite-difference time-domain simulations for increasing thicknesses of coating. The grey shaded region corresponds to the typical range for refractive index of cytoplasm. f, Sensitivity of the microdisk’s resonance to external refractive index as a function of coating thickness, calculated for small variations around n₁ = 1.37. g, h, Lasing wavelength versus background refractive index for uncoated (g) and 150-nm coated (h) microdisks on glass (N = 7 each). Empty circles are experimental data; dashed lines are linear fits.

Fig. 4 | Stability and biocompatibility of LPs. a, Distribution of lasing thresholds for uncoated microdisks in air (N = 60), uncoated microdisks in Matrigel (N = 54) and coated microdisks in Matrigel (N = 56). b, c, Lasing output intensity (b) and resonance wavelength shift (c) of microdisks under continuous illumination up to 1.8 billion pump pulses (N = 4 each); solid lines are the mean and shaded regions are 95% CI. d, Long-term lasing-wavelength shifts of coated (N = 51) and uncoated microdisks (N = 6) on glass substrate in cell culture medium up to 30 h. Solid lines are the mean and shaded regions are 95% CI. e, Confocal fluorescence images of human cervical cancer (HeLa), mouse breast tumour (4T1) and canine kidney epithelial (MDCK-II) cells with staining for actin (magenta), and nucleus (green), overlaid with brightfield transmission images of LPs (greyscale). f, Cell viability of MDCK-II cells at 24 h, 48 h and 72 h after incubation with LPs. A two-way analysis of variance (ANOVA) was used for statistical analysis [F(2,22) = 16.47] (*P < 0.01, ***P < 0.001; n.s., not significant).
To assess the suitability of our LPs as intracellular probes, we performed time-lapse imaging to observe the migration and proliferation of MDCK-II cells containing LPs for several days (Supplementary Videos 2, 3). As cells divided, LPs were transmitted from a mother cell to daughter cells. Mitotic partitioning of multiple LPs tended to be asymmetric, fitting a skewed binomial probability distribution (Supplementary Fig. 7). Uptake statistics of LPs by MDCK cells after 24h incubation for different initial concentrations are reported in Supplementary Fig. 8. For cells containing up to six particles, their presence did not much affect cell cycle times (Supplementary Fig. 8). A cell proliferation assay also revealed no significant difference in proliferation rate between cells with and without LPs (P = 0.09 at 48h, P = 0.35 at 72h; Supplementary Fig. 8). A few rare cells with an excessive number (>10) of particles were unable to complete mitosis and underwent apoptosis (Supplementary Video 3).

We also performed a wound-healing assay on LP-tagged and untreated MDCK-II cells to confirm that internalized LPs had no appreciable effect on cell motility (Supplementary Fig. 8). Although this data suggests that coated LPs are biocompatible, further studies are needed to exclude any effect on specific cellular functions.

For long-term cell tracking, intracellular LP retention is important to be able to assign a LP unambiguously to a specific cell. Previous works have shown that for sub-micrometre particles the rate of exocytosis decreases with increasing particle size, irrespective of cell type. Particles of diameter 1–3 µm were found to remain internalized in cells for at least six days. We followed hundreds of LP-tagged MDCK-II cells in vitro using time-lapse imaging for over 60h (Supplementary Videos 1–3) and did not observe any clear evidence of exocytosis (Supplementary Video 4). Although we cannot exclude the possibility of LPs being released after cell death, appropriate surface coatings may prevent their re-uptake.

Imaging of intracellular LPs

For high-speed imaging of LPs, we coupled the pump laser and spectrometer to a laser-scanning confocal microscope. The system can acquire the LASE images of LPs (at 100µs per pixel) and the brightfield transmission and confocal fluorescence images of cells and tissues. For example, LASE-fluorescence microscopy showed signatures of individual LPs with varying shapes depending on their orientation inside the cytoplasm of HEK-293 cells expressing membrane-localized green fluorescent protein (GFP) (Fig. 5a).

Using a compact cell-culture incubator on the microscope, we acquired time-lapse LASE images of LPs inside cells. The LPs were identified and tracked over time using an algorithm (see Methods) that exploits both positional and spectral information (Supplementary Note 2 and Supplementary Fig. 9). Tracking LP-tagged MDCK-II cells over 33h in vitro showed that the output wavelengths of LPs in the cytoplasm were stable (Fig. 5b and Supplementary Fig. 10), apart from random fluctuations (0.1 nm) and slower variations. These changes were attributed to the residual sensitivity to the surrounding refractive index that changes slowly by natural cellular processes and rapidly by random-walk movement of LPs inside the cytoplasm. The wavelength variation exhibited a normal distribution with standard deviation σ = 0.18 nm. For LPs with nominal difference in wavelength of Δ = 1 nm, this corresponds to an error probability in identifying LPs of P ≈ 10⁻⁴ (Supplementary Note 3). This error can be reduced by time-lapse spectral measurement averaging random fluctuations or adding redundancy such as positional information.

We then tested the possibility of tagging and imaging cells in tissues. 4T1 cells were stained with a fluorescent dye and loaded with LPs. They were then injected into the tail vein of a membrane-GFP-expressing mouse, in a model mimicking hematogenous micrometastasis. After 15 min, the lungs (where most of the injected cells are trapped) were explanted and imaged with our LASE system. Co-localization of LP emission with the cellular dye demonstrates their reliability as intracellular tags even in scattering tissues (Fig. 5c).

Longitudinal tracking in tumour spheroids

To demonstrate large-scale cell tracking, we used a three-dimensional tumour spheroid model using LP-tagged polyclonal 4T1 breast cancer cells. A single spheroid contained about 70,000 LPs. Using the LASE microscope setup described above, it took 47 min to acquire a z-stack over an imaging volume of 1 × 1 × 0.28 mm³. A computer-automated three-dimensional scan was conducted every hour for 128h, during which the spheroid expanded in size (Fig. 6a and Supplementary Video 5). At each time point, 4,500 to 8,000 LPs were detected, with a good uniformity in wavelength distribution across the 1,180–1,580 nm spectral range (Fig. 6b and Supplementary Fig. 11). The number of LPs detected increased over time, as the tumour grew toward the glass-bottomed plate and more LP-tagged cells entered the imaging volume (although some left the imaging volume). Post hoc analysis of fixed sections of the spheroid revealed that by the end of the experiment the average ratio of LPs per cell was about 0.6 (Supplementary Fig. 11), from which we...
estimated that approximately 20% of the cells are tagged with LPs. Using our tracking algorithm, we were able to track 75–80% of all detected LPs for longer than 24 h, among which 731 were tracked for more than 125 h (Supplementary Fig. 11). To quantify cell motility, we used the position of the imaged LPs as a proxy for the location of the cells containing them. The 3D trajectories reveal a variety of migratory patterns (Fig. 6c). Long trajectories typically show several intermittently slow regions with an interval of about 1 day, which we hypothesize to result from cell division. We found groups of 2–3 LPs travelling in very close proximity, which we interpret as being inside the same cells (Supplementary Fig. 11). Some of the initially co-travelling LPs separated into two distinct paths (Fig. 6d). This phenomenon is probably due to splitting of particles into different descendant cells during cell division.

The longitudinal tracking data permitted various single-cell analyses and grouping in terms of behavioural phenotypes. We classified cells depending on their average motility (that is, the ratio of travel distance to the tracking duration; see Supplementary Fig. 12). While the low-motility group (<25%) cells were found to remain in the core of the spheroid, the trajectories of the high-motility group (>75%) cells feature outward migratory behaviour and invasion into the surrounding gel matrix, particularly along paths near the glass plate (Fig. 6c). These two functionally distinct groups appeared to have originated from statistically distinctive regions in the tumour at early times (Supplementary Fig. 12). From the trajectory of each cell in every 6-h window, the instantaneous velocity was calculated, and velocity maps at each time were obtained (Fig. 6f). The time-lapse video of the velocity maps shows how individual cells move during spheroid growth and invasion (Supplementary Video 6). Interestingly, the high-motility cells had consistently higher speeds than low-motility cells throughout the entire duration and underwent moderate acceleration at 40–60 h at the onset of invasion.
In terms of instantaneous velocity, fast and slow cells (top and bottom quartile) were analysed at each time point. A Geary’s coefficient analysis showed significantly higher spatial correlation of velocities among the fast cells compared to the slow cells and a randomized control, suggesting the presence of non-cell-autonomous behaviours (Fig. 6h). In particular, strong spatial correlation at earlier time points (20–40 h) is attributed to the streams of cells moving in small packs within the spheroid (Supplementary Fig. 12). These results demonstrate the novel capability of gathering large-scale longitudinal single-cell information in situ using LPs.

**Discussion**

We have demonstrated that thickly coated semiconductor microdisks are well suited for cell tagging and tracking applications owing to their low pump energy, excellent stability and biocompatibility. With respect to the spontaneous emission typical of fluorophores, the stimulated emission from single-mode microlasers has several distinct characteristics, such as coherent-state statistics, sharp threshold and picosecond-scale decay times, that are potentially useful for imaging and sensing.

Most remarkably, the spectral width of laser emission is 100 times narrower than that of typical fluorophores. We have harnessed this property for wavelength-encoded tagging and tracking of thousands of densely populated cells in a three-dimensional scattering tissue. The spectral encoding offers a compelling advantage for cell identification and tracking, compared to image-based tracking (such as by light-sheet microscopy) which requires frequent imaging (every few minutes).

Improvements in multiplexing capability are possible. First, the total wavelength span could be expanded to the NIR-I and visible range using appropriate group III–V and group II–VI semiconductor materials. Second, when multiple LPs with different wavelengths are combined, the number of unique identifiers scales as

$$N = \frac{N!}{(N-m)!m!}$$

where \(N\) is the number of colours for singlet LPs (\(m=1\)) and \(m\) is the number of particles in the multiplex. With \(N=1,000\), doublets (\(m=2\)) can have half a million identifiers, and the number increases to 166 million for triplets (\(m=3\)). Preliminary results showed promising feasibility of this massively scalable approach (Supplementary Fig. 13).

Recent work has highlighted the importance of cellular heterogeneity at the single-cell level. Whereas current understanding primarily stems from sequencing of single dissociated cells, emerging spatial transcriptomic techniques promise understanding of cellular identity in the tissue context. Laser-particle-enabled cell tagging could allow sorting and extraction of cells of interest for post hoc analyses such as flow cytometry or single-cell sequencing. Furthermore, it can enable highly multiplexed cell tracking of individual cells, offering rich complementary information on cellular movement, including migration, motility, cell–cell interactions and spatial clustering. The combination of imaging, single-cell tracking, transcriptomic and proteomic assays will enable unprecedented comprehensive evaluation of cell identity and function.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41566-019-0489-0.

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Author contributions
S.-H.Y., N.M. and S.J.J.K. conceived and designed the project. N.M., H.J., H.-M.K., Y.-H.L. and S.-H.Y. designed microdisks. N.M. and A.C.L. fabricated microdisks. N.M. and S.J.J.K. developed the microdisk transfer protocol. S.J.J.K. and S.J.W. developed and performed silica coating of microdisks. N.M. and A.C.L. conducted theoretical simulations. S.J.J.K., S.F. and S.-J.J. performed cell cultures and biocompatibility assays. N.M. and J.W. designed the LASE microscope system. N.M. and S.J.J.K. performed and analysed optical characterization and imaging of LPs. S.J.J.K. and S.-J.J. performed ex vivo lung imaging. S.J.J.K., A.C.L., S.F., S.-J.J. and P.H.D. analysed time-lapse videos of intracellular LPs. N.M., S.J.J.K., A.C.L. and S.-H.Y. prepared figures and wrote the manuscript with input from all authors.

Competing interests
The authors declare the following competing interests: S.-H.Y. holds patents on technologies related to the devices developed in this work. S.-H.Y. and S.J.J.K. have financial interests in LASE Innovation Inc., a company focused on commercializing technologies based on LPs, for a variety of applications in life science and healthcare. S.-H.Y’s interests were reviewed and are managed by Massachusetts General Hospital and Partners HealthCare in accordance with their conflict of interest policies.

Additional information
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silica shell was taken from tabulated values in ref. 44. The simulation region was set with perfectly matched layer boundary conditions on all directions. The distance to the perfectly matched layer boundaries as well as the meshing size were chosen after a series of convergence tests. The optical modes supported by the resonator were excited by a number of randomly placed in-plane dipole emitters, and the resonance wavelength at the bandwidth of interest was recorded as the background refractive index of the silica layer (RIE) process.

**Electron microscopy.** 10 μl of LP suspensions in water were added and air-dried on silicon wafer chips for SEM, and on formvar-carbon-coated nickel mesh grids (Electron Microscopy Sciences) for transmission electron microscopy (TEM). TEM images were obtained using a JEOL JEM 1011 microscope at 80 kV. SEM characterization was performed on a Hitachi S–4800 microscope and a Zeiss Ultra Plus Field-Emission microscope at 2 kV. For cross-sectional viewing, coated microdisks were first milled with Ga+ ions using a dual-beam SEM/focused ion-beam system (Helios Nanolab, FEI Company). Energy-dispersive X-ray spectroscopy and mapping was performed on a Zeiss Supra55VP field emission microscope at 8 kV.

**Optical characterization.** For optical characterizations and imaging of LPs, a commercial laser-scanning confocal microscope (Olympus FV1000) was modified. A pump laser (Spectra Physics GVEN-ISP.POD, 1,060–1,070 nm, pulse duration 3 ns, repetition rate 2 MHz) was coupled to a side port of the laser-scanning unit of the microscope. The emission from LPs was collected from the same port and relayed by a dichroic mirror to a near-infrared spectrometer using an InGaAs linear detector. The spectrometer operated at a spectral range of 1 μm for high-resolution characterization; for LASE imaging, the acquisition of spectrometer data was performed at 100 μs integration and synchronized to the laser-scanning unit. A grating with 600 lines per millimetre was used for high-resolution characterization (0.2 nm resolution, 150 nm span). For LASE imaging, a grating with 200 lines per millimetre (0.6-nm resolution over 1,150–1,600 nm) was used. A near-infrared-optimized, 20X, 0.45-NA (numerical aperture) objective (Olympus IMS LCLPLN20XIR) was used for LASE microscopy. The pump beam under-filled the objective lens (20X) such that the focal beam size was 2 μm, matching the size of LPs. Confocal fluorescence images were acquired with a 40X, 0.7-NA objective (Olympus LUCPLFN40X). A step-stage incubator (Tokai Hit) kept the samples at 37 °C and 5% CO2 during imaging.

Stability of LP emission was performed in air for uncoated microdisks and in hydrogel for both coated and uncoated microdisks. LPs were pumped with $E_t = 40 \text{ pJ}$ pulse at 2 MHz repetition rate. For continuous pumping, spectra from individual LPs were collected for 15 min every 15 s. For long-term stability, emission was collected via LASE imaging every hour for 30 h. The output intensity and wavelength were determined from the area under the spectral curve and the Gaussian-fit centre respectively.

The sensitivity of LP emission to external refractive index was measured in different sucrose solutions prepared with concentrations of 0, 20, 30, and 64% w/w. Their refractive index was measured using a portable refractometer (FISCHER, FR 14, ATAGO). Uncoated microdisks (about 150 μm) were dispensed with different volume ratios of a glass-bottom 96-well plate. For each concentration, 300 μl of sucrose solution was pipetted into each well and a LASE image was acquired. Between successive measurements, the wells were washed twice with ultra-pure water. We note that under this experimental arrangement, only the refractive index of the top half-space is varied, while the bottom half remains constant throughout (glass).

**Cell culture and biocompatibility experiments.** HeLa human cervical cancer cells (ATCC), 4T1 mouse breast tumour cells (ATCC) and MDCK-II canine kidney epithelial cells (ECACC) were cultured and maintained in serum-supplemented cell media following the manufacturer’s guidelines. Membrane GFP-expressing HER-2/3 human embryonic kidney cells were a gift from Dr. E. Canis (Oxford). HeLa cells and 4T1 cells were cultured in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% (v/v) foetal bovine serum (FBS) and 1% (v/v) penicillin-streptomycin. Cells were stained after fixation with AlexaFluor 594 Phalloidin for actin (Thermo Fisher), and DAPI for nuclei (ProLong Gold Antifade Mountant with DAPI, Thermo Fisher) following the manufacturer’s guidelines.

A typical protocol for loading LPS into cells is as follows. Cells were plated in their respective media at a known density in a glass-bottomed, 96-well plate. Laser particles were resuspended in sterile filtered de-ionized water, and counted using a standard hemocytometer. The LP solution was then added to cells, at initial particle-to-cell ratios from 1:1 to 4:1. Immediately afterwards, the requisite amount of 10% PBS was added to maintain isotonicity. The dilution of cell media with addition of LP solution was <10%. The cell media was exchanged to fresh media within 2 h. Cells were then incubated at 37 °C and 5% CO2 for 24–48 h until LP uptake was complete.

Cell viability was assessed via a calcein-AM/ethidium homodimer-1 fluorescent assay (LIVE/DEAD viability/cytotoxicity kit, Thermo Fisher). MDCK-II cells were switched to serum-free MEM-alpha for the duration of the experiment (up to 72 h) to minimize cell proliferation. Uncoated or coated microdisks in water were added to MDCK-II cells such that the particle-to-cell ratio was approximately 1:1. Fluorescent staining was conducted after 24 h, 48 h.
and 72h later. Fluorescence and bright-field microscopy was conducted to quantify particle uptake and cell viability (Keyence BZ-X700 microscope). Cellular proliferation was assessed via a cell counting kit-8 (CCK, Millipore Sigma). After MDCK-II cells were plated, low (4:1 particle-to-cell) and high (32:1) concentrations of microdisks were added as previously described. CCK-8 staining was conducted at 48 and 120h, when cells were approximately 80% confluent. Absorbance measurements were taken using a spectrophotometer (Epoch 2, BioTek Instruments).

Wound healing assay. MDCK-II cells were incubated with and without LPs at an initial LPs-to-cell concentration of 4:1. After 24h, the cells were detached and plated into each chamber of a wound-healing assay insert (20,000 cells per well; culture-insert 2 well, ibidi). After 12h, the inserts were removed. Time-lapse bright-field imaging was performed every 30min on two inserts with LP-tagged cells and two controls. For each insert, we imaged and analysed five different areas of the closing gap. The images were segmented with Fiji and the cell-free area was computed for each time point.

Time-lapse bright-field imaging. To observe the internalization and interaction of cells with the material and automated, time-lapse, bright-field microscope (Keyence BZ-X700). MDCK-II cells were plated with LPs (particle-to-cell ratio of 2:1) in a 96-well glass-bottomed plate as described earlier. Images from different wells were taken every 5min over a 60-h period in a stage-top incubator (Tokai Hit) at 37°C and 5% CO₂. After correcting for drift artefacts, the processed videos were analysed frame-by-frame to record instances of particle uptake, cell division, and suspected exocytosis events. Over 400 individual cell cycles were annotated, from which we calculated the cell cycle time as a function of particle number, and the statistics of particle partitioning due to cell division. Empirical probabilities for LP partitioning were fitted with an asymmetric binomial distribution: \( P(r) = C(n,r) \frac{p^r(1-p)^{n-r}}{\sum_{r=0}^{n} C(n,r) \frac{p^r(1-p)^{n-r}}{\omega W}} \), where \( n \) is the number of particles in the parent cell, \( r \) is the number of particles in the daughter cell, \( C(n,r) = \frac{n!}{r!(n-r)!} \), and \( \omega W \) is a fitting parameter that quantifies the asymmetry of the distribution (\( \omega W = 0.5 \) for a symmetric binomial distribution).

Ex vivo lung imaging. The MGH Institutional Animal Care and Use Committee approved our animal protocol (2017N000021) in accordance with NIH guidelines. Membrane-GFP-expressing ROSA²C mice (https://www.jax.org/strain/007576) were purchased from Jackson Laboratories. 4T1 cells were loaded with LPs in vitro as previously described, and stained with CellTracker Red (Thermo Fisher) following the manufacturer’s guidelines. Approximately 2 × 10⁴ 4T1 cells containing around 1 × 10⁵ LPs in 200 μl of DPBS solution were injected intravenously into the tail vein of a membrane-GFP-expressing adult mouse under anaesthesia. After 30min, the animals was euthanized and the lung was harvested. The entire lung was immersed in DPBS and spread on a glass-bottomed dish (Olympus UPLSAPO 60XW) used for confocal fluorescence and LASE imaging up to 150μm in depth. For large-area imaging, 64 adjacent areas, each spanning 212 × 312 × 3 μm³ were imaged and stitched together in post-processing using Fiji and MATLAB.

Spheroid invasion assay. Approximately 1.5 × 10⁴ LPs were added to 5 × 10⁴ 4T1 cells as previously described and incubated for 48h until confluent in a 96-well cell-culture plate, to ensure complete uptake of LPs. Cells were transferred to an ultralow-attachment-coated round-bottom microplate (CellCarrier) to form spheroids. Each well initially contained approximately 2 × 10⁵ cells and 7 × 10⁵ LPs. After three days of incubation, the spheroids were transferred to a glass-bottomed microplate in 100 μl of serum-supplemented cell media mixed with 100 μl of Matrigel (Corning). After this passage, eventually extracellular LPs are left in the original plate. After 2h, an additional 100 μl of cell media was added. The spheroids were used for LASE imaging after 6h. The spheroid was imaged every hour for a total of 129 measurements \((t=0, 1, \ldots, 128h)\). The imaged region was divided into four adjacent areas acquired sequentially; each area was 480 × 480 × 280 μm³ and was imaged at 320 × 320 × 70 pixels, for 100μs per pixel; total imaging time for each scan was about 48min (about 12min per area). Laser pumping was set at \( E_0 = 80 \) pJ per pulse.

LASE imaging data analysis. A custom Python software was used to analyse the spectra acquired during LASE imaging in real time, recording the data only when they contained peaks greater than a threshold level. Post-processing of the data was performed with a MATLAB custom code. The recorded spectra were further reduced to wavelength peaks by Gaussian fitting, producing a set of wavelength \((λ)\) and position \((r)\) data of each recorded ‘lasing’ event (or pixel). Since many lasing events can be recorded from a single LP, a clustering algorithm was applied to group the data from the same LP. A three-step hierarchical clustering algorithm was used: (1) clustering by position to isolate spatially separate clusters of lasing pixels; (2) clustering by wavelength to distinguish any adjacent particles with different wavelengths; and (3) clustering by position again to separate clusters in the cases where two particles with similar wavelengths were bridged by a third particle with a different emission. Once clusters are identified, their centroid position and mean wavelength were computed to represent corresponding LPs.

For particle in-cell tracking, a metric \((D_j)\) was introduced, which represents a ‘distance’ between two particles identified in two separate measurements in four-dimensional space of wavelength \((λ)\) and position \((r)\); the distance \(D_j\) between the \(i\)th particle in an image and the \(j\)th particle in a preceding image is defined as:

\[
D_j = \left( \frac{(λ_i - λ_j)^2}{2 \Delta λ_i} \right)^\alpha + \left( \frac{(r_i - r_j)^2}{2 \Delta r_j} \right)^\alpha.
\]

where \(\Delta λ\) and \(\Delta r\) are adjustable parameters; we set \(Δλ = 1 nm\), whereas \(\Delta r = \sqrt{2} \Delta λ\), as an estimate of the expected maximum displacement for a LPs in the time interval \(Δt\) between the analysed frames \((D=200μm²/h)\) in the spheroid tracking experiment). For each time point \(t_m\), the distance \(D_j\) of all imaged particles \((i=1 to N_j)\) from those \((j=1 to N_m)\) detected in the previous time \((t_m)\) were calculated. Pairs of particles with a distance smaller than a threshold \(D_j = 2\) were matched, starting from the one with the smallest distance. For the remaining particles, the matching procedure was repeated with the particles detected at earlier times \((t_{m-1}, t_{m-2}, \ldots, t_1)\), and so on.

LPs within the same cell were identified by computing for each couple \(i, j\) of tracked particles their average (geometric) distance \(\overline{D}_{ij} = \frac{1}{N_m-1} \sum_{m=1}^{N_m} (\overline{D}_{ij})_m\) at each time point. Particles with \(\overline{D}_{ij} \leq 2\mu m\) were considered as belonging to the same cell for the entire duration of the tracking and were thus merged in a single trace.

To remove the contribution of intracellular movements of LPs on the overall cellular trajectories, we applied a 6h moving average to the tracked pathways.

Statistics. Data were presented as either box and whisker plots, or as mean and 95% confidence interval. Statistical hypothesis testing for the biocompatibility assays was done using either one-way or two-way analysis of variance (ANOVA), and all F-statistics were reported. Statistical significance was set at \(p = 0.05\). Post hoc comparisons were conducted using Tukey's test. Statistical analyses were performed using GraphPad Prism and MATLAB.

To quantify spatial autocorrelation in the spheroid tracking experiment, we computed Geary’s coefficient as follows: \(C = \frac{(N - 1) \sum_{i,j} (y_i - y_j)^2}{2N \sum_j (y_j - y)^2 - N(y - y)^2}\), where \(N\) is the number of neighbouring cells, and \(o_y\) is a spatial weight matrix that defines the neighbourhood region of interest, or the pack size of spatially correlated cells. A coefficient significantly <1 corresponds to high correlation, \(>1\) corresponds to no correlation, and \(>1\) corresponds to anti-correlation. Before normalization, \(o_y\) contains zeros on the diagonals and ones for neighbouring cells within a given distance. This distance is set to 60μm in Fig. 6b, and is varied from 30μm to 600μm in Supplementary Fig. 12c-e. The matrix is then row-normalized such that each row sums to 1. \(W\) is the sum of all \(o_y\) elements. \(x_i\) and \(x_j\) are either the initial coordinates of cell \(i\) or \(j\) (Supplementary Fig. 12c), the (vector) velocities of cell \(i\) or \(j\) at a given time-point (Fig. 6b) or the overall motility (displacement/time-tracked) of cell \(i\) or \(j\) (Supplementary Fig. 12e). \(x\) is either the mean velocity at a given time point or the mean motility. To generate a control sample, the Geary coefficient was also computed for cells with corresponding cell velocities or motilities randomized. The randomization process was repeated 100 times, and the 95% confidence interval of the random control group was presented.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The data that support the plots within this paper and other findings of this study are available from the corresponding author upon reasonable request.

References
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Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

Confocal images were taken using FluoVIEW software from Olympus; LASE imaging was performed with a custom-made Python software controlling acquisition from the NIR spectrometer

Data analysis

Data were analyzed using custom MATLAB scripts to extract lasing information from spectra (peak wavelength, power, thresholds), to cluster spatial data for localization of laser particles and to simulate WGM resonance

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- **Sample size**: No specific sample size was chosen a priori
- **Data exclusions**: No data was excluded
- **Replication**: All data was reproducible
- **Randomization**: Not applicable
- **Blinding**: Not applicable

Reporting for specific materials, systems and methods

**Materials & experimental systems**

- n/a Involved in the study
- Unique biological materials
- Antibodies
- Eukaryotic cell lines
- Palaeontology
- Animals and other organisms
- Human research participants

**Methods**

- n/a Involved in the study
- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging

Eukaryotic cell lines

Policy information about cell lines

- **Cell line source(s)**: HeLa (ATCC), 4T1 (ATCC), MDCK-II (ECACC), HEK-293 (gift from Prof. Adam E. Cohen, Harvard University)
- **Authentication**: Cell morphology was as expected, no other authentication procedures
- **Mycoplasma contamination**: Cell lines were not tested for mycoplasma
- **Commonly misidentified lines** (See ICLAC register): None

Animals and other organisms

Policy information about studies involving animals. ARRIVE guidelines recommended for reporting animal research

- **Laboratory animals**: Female mouse, mTmG-B6 (Jackson Laboratories), ~7 months
- **Wild animals**: None
- **Field-collected samples**: None