Swimming Microrobot Optical Nanoscopy

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Supporting Information

ABSTRACT: Optical imaging plays a fundamental role in science and technology but is limited by the ability of lenses to resolve small features below the fundamental diffraction limit. A variety of nanophotonic devices, such as metamaterial superlenses and hyperlenses, as well as microsphere lenses, have been proposed recently for subdiffraction imaging. The implementation of these micro/nanostructured lenses as practical and efficient imaging approaches requires locomotive capabilities to probe specific sites and scan large areas. However, directed motion of nanoscale objects in liquids must overcome low Reynolds number viscous flow and Brownian fluctuations, which impede stable and controllable scanning. Here we introduce a new imaging method, named swimming microrobot optical nanoscopy, based on untethered chemically powered microrobots as autonomous probes for subdiffraction optical scanning and imaging. The microrobots are made of high-refractive-index microsphere lenses and powered by local catalytic reactions to swim and scan over the sample surface. Autonomous motion and magnetic guidance of microrobots enable large-area, parallel and nondestructive scanning with subdiffraction resolution, as illustrated using soft biological samples such as neuron axons, protein microtubulin, and DNA nanotubes. Incorporating such imaging capacities in emerging nanorobotics technology represents a major step toward ubiquitous nanoscopy and smart nanorobots for spectroscopy and imaging.

KEYWORDS: Microrobot, scanning, biological imaging, nanoscale propulsion, microlens, super-resolution

The ability to resolve small structures with high resolution has been a topic of enormous interest and importance.1–10 Scanning probe microscopy (SPM) has been widely used to map local nanoscale information with functionalized tips, but such tip scanning processes are hampered by the perturbation induced at the near field and slow imaging speeds within limited scanning areas, especially for operation in liquids and biologically relevant environments.11 The large forces induced by the sharp tip and rigid cantilever usually make it challenging to scan over soft samples.12 Fluorescence nanoscopy approaches have been rapidly advanced recently as powerful super-resolution tools13–16 but might still require sophisticated labeling and image processing algorithms. Recently, micro/nanostructured lenses, such as metamaterials and microsphere lenses,1–9 have shown considerable promise for resolving surface structures below the diffraction limit. While metamaterials have been limited by losses, microsphere lenses are particularly attractive as they are essentially loss-free.17,18 However, the lack of mobility of microsphere lenses impedes their operation for site-specific probing or large-area scanning of real-life samples. Artificial micro/nanomachines, which are tiny devices that overcome low Reynolds number viscous drag and Brownian motion by converting local fuels or external energies into nanoscale locomotion, have recently emerged as powerful nanotechnology tools for biomedical and environmental sciences.19–26 The operation of micro/nanoscale machines and robots have proved useful for cell penetration,27 environmental remediation,28 active materials assembly,29 and nanoscale lithography.30 Engineering small robots with nano-optical elements will provide an alternative paradigm for scanning probe techniques to overcome roadblocks in optical imaging devices.

Here, we introduce a new nanoscopy methodology, denoted swimming microrobot optical nanoscopy (SMON), based on autonomously moving microrobots that rapidly scan over the sample surface. These untethered microrobots, comprising high-refractive-index microsphere lens, utilize chemical locomotion and magnetic guidance for nondestructive “on-the-fly” scanning and imaging over large areas, obviating unwanted tip–sample interactions, macroscopic cantilevers, and complex control systems common to conventional SPM techniques. High-resolution imaging below 50 nm of large areas can be achieved with a conventional white-light or fluorescence microscope. The imaging throughput can be further improved by parallel operation of multiple microrobots. Such self-propelled microrobots with integrated nanophotonic functionality provide a new paradigm for surface imaging toward high-performance, user-friendly, cost-efficient, and readily accessible nanoscopy.

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As illustrated in Figure 1a,b, the microrobot swims autonomously over the sample surface in aqueous solution containing the chemical fuel, focusing light through a high-intensity optical field directly beneath. Subdiffraction surface features scatter this light as near-field evanescent waves, which are magnified and focused by the moving microrobot. The magnified virtual images are captured by the CCD camera behind the objective lens as a video with high-resolution frames, which can be reconstructed as a stitched image with large area of view. 

In Figure 1b, a 15 μm TiO2 microrobot resolves 20 nm nanogaps that are far below the diffraction limit (Supporting Video 2; FWHM analysis in Figure S1 and Figure S2). Figure 1i displays two frames of a 10 μm PS microrobot scanning over fixed neuron axons of a mouse’s brain tissue (Supporting Video 3). Clear magnified 50 nm neuron axons (Figure S3) are resolved by the microrobot without damage to the axon fibers. 

The large-area scanning capacity of SMON has been examined by using a disc with grating nanostructures of 320 nm-wide lines separated 420 nm apart (Figure 2a). The tracking line of Figure 2b displays the motion of a 10 μm PS microrobot scanning the nanograting surface over 3.6 s (taken from Supporting Video 4). Figure 2c displays a reconstructed image of the magnified surface feature, obtained by stitching the magnified scanning area of the microrobot from each frame. By comparing the intensity profiles of the scanned and unscanned areas, we see that SMON significantly enhances the light contrast more than 5-fold, clearly distinguishing the grating structure with 1.8X lateral magnification (Figures 2d,e). The potential scanning area of the microrobots is essentially unlimited within the aqueous environment, allowing the microrobots to approach and scan any location in the whole view of the microscope. Compared to scanning tips, which might induce image artifacts due to the shape inconstancy, the microrobot lenses produce stable and reliable high-resolution images in each frame throughout the scanning process.
The simplicity of SMON enables straightforward parallelization imaging schemes by using multiple imaging microrobots that swarm together or using microrobot ensembles. Figure 2f–h shows the parallel scanning of two magnetically aligned microrobots aligned to greatly accelerate the scan rate of a sample, analogous to parallel AFM cantilevered tips. Colloidal microspheres, capable of forming self-assembled configurations for building complex devices and structures, have been used to explore the parallelized imaging capabilities of microrobot assemblies. Figure 2i–k displays a “side-by-side” paradigmatic dimer assembly of a dual-lens microrobot, enlarging the magnified area in each frame for efficient scanning. This self-assembly is not limited to doublets, as illustrated in Supporting Video 4 for fast imaging using triplet and quadruplet microrobot ensembles. These parallelization and self-assembly strategies could dramatically improve the scanning throughput at a large scale.

The magnification capacity and lateral resolution of SMON are modeled and experimentally verified using PS and TiO$_2$ microspheres with different sizes. Figure 3a shows the FEM simulation of light focused by a 10 μm PS microsphere ($n = 1.59$) in water ($n = 1.33$), which we analyzed with the ray optics method to locate the image plane and calculate the magnification (see Supporting Information). Figure 3b,c shows the focal distances and magnification factors, respectively, for microsphere lens with different refractive indices and sizes. We validated these results experimentally by imaging a 200 nm nanodot array (SEM in Figure S4) with PS microspheres of three sizes (2.16, 4.86, and 10 μm) and 15 μm TiO$_2$ microspheres, as displayed in Figure 3d. The corresponding 3D intensity profiles of the magnified portions over equal-pixel areas are shown in Figure 3e. In agreement with our simulation results, the refractive index plays a more critical role in the lateral magnification, with TiO$_2$ microsphere swimmers offering a magnification factor higher than 4X.

We then estimated the scanning throughput of SMON. As illustrated in Figure 3f, for a microrobot with a diameter of $d$ and a magnification factor of $m$, the lateral scanning length is $d/m$ in each frame. To avoid missing local information between two consecutive frames, the maximum microrobot speed should be $fd/m$, where $f$ is frame rate, providing a maximum scanning rate of $fd^2/m^2$. Experimentally, the microrobot speed is highly dependent on the fuel concentration and ranges from 5 to 35 μm/s (see Figure S5, Supporting Video S5). These microrobot swimming speeds are faster than the regular scanning speeds of AFM, where a typical fast speed of up to 10 μm/s is used for a largest scanning distance of ~100 μm.

The corresponding scanning rates, based on our experimental results for different microrobots, are shown in Figure 3g. Figure 3h displays reconstructed images obtained during a 2 s scanning period using a 10 μm PS nanoscope and fuel concentrations of 2.5%, 5.0%, and 7.5%. The larger stitched images indicate that higher fuel concentrations can dramatically increase the scanning speed without compromising the image quality. Overall, coupling a high-frame-rate microscope with high-speed parallelized microrobots would be optimal for high-resolution high-throughput scanning and imaging. In our current SMON setup, an experimental video rate of 45 frames/s with a 3 μm by 3 μm field of view in each frame, could be achieved using the 15 μm TiO$_2$ microrobot. Such a data acquisition throughput is faster than the recently developed video-rate of far-field optical nanoscopy.
SMON can easily achieve high-resolution imaging of fluorescent and biological samples. Figure 3a displays fluorophore-labeled 50 nm PS nanobeads imaged by a 10 μm PS micro-robot. Beyond resolving the individual nanobeads, the nanoscope also dramatically enhances the fluorescent intensity by 20−25-fold, as highlighted in the fluorescence intensity profile in Figure 4a, while the comparison in Figure 4b highlights the 4X lateral magnification by SMON. The SMON image of a...

Figure 3. Super-resolution focal distance, virtual magnification factor, and imaging speed. (a) FEM simulated electric field distribution of a plane wave passing through a 10 μm PS (n = 1.59) sphere in water (n = 1.33). (b) Virtual magnification factors and (c) focal distances of spherical lenses with different refractive indices and diameters. Blue dotted lines correspond to refractive indices of PS (n = 1.59) and TiO2 (n = 2.1) used in experiments. (d) Magnification of a periodic dot array by spherical lenses made of PS microrobot with diameter of (i) 2.16, (ii) 4.86, and (iii) 10 μm as well as (iv) a 15 μm TiO2 microrobot. Scale: 10 μm. (e) The intensity profiles corresponding to the magnified areas indicated in d. (f) Scheme showing the maximum scanning speed without missing surface information. (g) Dependence of the scan rate upon the microrobot speed and size. Experimental results for scanning rates of different lenses are depicted with circle markers. (h) SMON image of a periodic dot array obtained using a 10 μm PS microrobot and different fuel concentrations: (i) 2.5%; (ii) 5%; and (iii) 7.5% over a 2 s swimming time. Scale bar: 10 μm.

Figure 4. Imaging of fluorescent and biological samples by SMON. (a) Fluorescent intensity profiles of an identical region of fluorescent nanobeads imaged by microscopy (i) and SMON (ii). (b) Fluorescence microscopy (i) and SMON (ii) images showing lateral magnification of fluorescence nanobeads. (c) Fluorescence microscopy (i) and reconstructed SMON (ii) images of a large-area fluorescent nanobead string pattern. (d) Microscopy (i) and SMON (ii) images of microtubules labeled with the rhodamine. (e) Microscopy (i) and SMON (ii) images of ssDNA labeled with YOYO-1. (f) Microscopy (i) and SMON (ii) images of Bacillus globigii spores. Images are representative of >20 data sets of similar quality. Scale bars: 500 nm (a,c,f), 2 μm (b), and 1 μm (d,e).
A typical nanobead shows a FWHM of 81 nm (Figure S6). The strong fluorescence enhancement and photon collection efficiency through the high-refractive index microlens eliminate the long exposure time common to fluorescence microscopy, allowing fast fluorescence scanning with an exposure time of only 20 ms, without compromising the imaging quality. Similar to the fast stochastic optical reconstruction microscopy (STED) microscopy,28 such time resolution is very promising for imaging biological dynamics at the nanoscale. Figure 4c compares an expanded fluorescence microscopy image of a nanobead string to a stitched SMON image of the same sample at the same scale (Supporting Video 6). This degree of magnification and fluorescence enhancement is highly appealing for future single molecule imaging.28 This technique has also been applied to biological samples, using fluorescence SMON to image rhodamine-labeled protein microtubules (Figure 4d). Normalized intensity profiles of the microtubules are displayed in Figure S7, showing a FWHM of ~75 nm, which is comparable to the value of 30–90 nm obtained by advanced super-resolution fluorescence microscopy techniques.29–41 A comparison of microscopy and SMON images of fluorophore-labeled DNA nanotubes illustrates how the microrobot nanoscope can distinguish individual signals from significant background noise (Figure 4e). Note that the strong background noise from the DNA nanotube imaging is from the YOYO-1 dyeing solution, which is directly added to the DNA solution prior to the SMON imaging. Figure 4f illustrates that under white-light illumination, SMON can clearly resolve individual B. globigii spores within large aggregates, indicating potential application within microbiome research. In our imaging experiments, we observed that the biological samples are quite stable without any deformation or cracking after continuous microrobot scanning back and forth for over 50 times. For micromotors swimming in fluid, there is still a small sedimentation height between the micromotor surface and the sample surface.42 Therefore, the spherical microrobot does not come into direct contact with the samples and hence avoiding damage to soft structures. The smooth and stable scanning motion of the microrobot has negligible interactions with the underlying biological samples, while the high resolution and photon-collection efficiency offered by SMON make it attractive for real-time tracking of subdiffraction cellular structures and biological processes.

In summary, we have reported swimming microrobot optical nanoscopy (SMON) as an efficient and high-resolution imaging technique. This microrobot imaging approach relies on controlled and rapid autonomous movement of untethered nano-optical elements over large areas for high-resolution and high-throughput surface imaging. The scalable parallelization schemes and self-assembly of the microrobots can further enhance the imaging throughput. SMON represents a user-friendly, nondestructive and low-cost alternative to complex micromanipulation techniques and can be used for real-time high-resolution imaging of soft biological samples. Furthermore, chemicals such as glucose or urea could potentially be used as biocompatible fuel sources for the microrobots,43 replacing the currently toxic peroxide fuel. Moreover, a fuel-free operation of the microrobot, based on magnetic or acoustic actuation, can replace the catalytic propulsion for digital control and imaging of many live samples. The trajectory of fuel-free microrobots could be readily preprogrammed toward automatic high-throughput surface imaging. Although the current SMON method cannot achieve the atomic-level resolution offered by scanning probe microscopy, replacing scanning tips with micro/nanoscale robots and enabling mobility of nano-optical devices create new opportunities for a variety of applications requiring high-resolution optical imaging and surface topography of large sample areas. We envision that metamaterial superlenses and hyperlenses could also be employed as future microscale imaging robot for site-specific probing and large-area scanning. Beyond improving the resolution of regular white-light and fluorescence microscopy, SMON can also be coupled with other high-resolution techniques, such as stimulated emission depletion (STED) microscopy and structural illumination, to further enhance the resolution synergistically. SMON also offers site-specific optical signal enhancement, thus expanding the parameter space of applicable optical intensities, pulse energies, durations, and wavelengths of spectrometers and optical detectors. The photonic microrobots described in this work should also find applications in reconfigurable nanophotonic systems.

### Associated Content

#### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nanolett.6b03303.

Information about robotic nanoscopes (PDF)

Swimming microrobot optical nanoscopy demonstration, with a 10 μm PS microrobot scanning over a “UC” nanopattern (MPG)

SMON imaging by a 15 μm TiO2 microrobot over a 20 nm nanogap (MPG)

SMON imaging by a 10 μm PS microrobot of mouse neuron axons (MPG)

SMON imaging by 10 μm PS microrobots of 320 nm nanogratings, in singlet, parallel, doublet, triplet, and quadruplet configurations (MPG)

SMON imaging by 10 μm PS microrobots of a 200 nm nanodot at fuel concentrations of 2.5%, 5%, and 7.5% H2O2 (w/v) (MPG)

SMON by a 15 μm TiO2 microrobot of a string of 50 nm fluorescent nanobeads (MPG)

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#### Author Contributions

J.W. and J.L. conceived and designed the experiments. J.L., W.L., T.L., J.Z. and I.R. performed all the experiments. B.B., B.K., and J.L. performed the modeling and simulation. The manuscript was written by J.L., I.R., B.K. and J.W. All authors discussed the results and reviewed the manuscript. J.L., W.L., and T.L. contributed equally to the work.

#### Notes

The authors declare no competing financial interest.

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