Scanning superlens microscopy for non-invasive large field-of-view visible light nanoscale imaging

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Nanoscale correlation of structural information acquisition with specific-molecule identification provides new insight for studying rare subcellular events. To achieve this correlation, scanning electron microscopy has been combined with super-resolution fluorescent microscopy, despite its destructivity when acquiring biological structure information. Here we propose time-efficient non-invasive microsphere-based scanning superlens microscopy that enables the large-area observation of live-cell morphology or sub-membrane structures with sub-diffraction-limited resolution and is demonstrated by observing biological and non-biological objects. This microscopy operates in both non-invasive and contact modes with ~200 times the acquisition efficiency of atomic force microscopy, which is achieved by replacing the point of an atomic force microscope tip with an imaging area of microspheres and stitching the areas recorded during scanning, enabling sub-diffraction-limited resolution. Our method marks a possible path to non-invasive cell imaging and simultaneous tracking of specific molecules with nanoscale resolution, facilitating the study of subcellular events over a total cell period.

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To fully explore the basic mechanisms in life and material sciences, and in other areas, research has gradually moved into the nanoscale using various novel microscopy technologies. Traditional electron microscopy, which achieves a molecular- and atomic-level resolution, has played an irreplaceable role in this transition; however, the radiation dose that the specimens are exposed to by the imaging system’s energetic electron beam is comparable to the irradiation from the explosion of a 10-megaton hydrogen bomb 30 m away, which confines the application of electron microscopy to non-destructive cell observation12. Scanning probe microscopes provide another convenient approach to obtain sub-nanometre resolution in both air and liquid, but their invasiveness may induce unrecoverable structural damage or morphology deformation in sensitive specimens. Specifically, the interaction forces they produce may drive adherent cells to detach from a dish surface during scanning with atomic force microscopy (AFM). The adhesion between cells and tips causes cell damage and limits the reusability of the probe and potentially contributes to cell death or necrosis. Recently, advancements in line scanning have accelerated markedly due to the development of super-resolution fluorescence microscopes that circumvent the optical diffraction limit induced by the loss of evanescent waves in far-field imaging, producing nanoscale resolutions3–5. Fluorescent labelling could allow the identification of specific molecules and to explore the functions of these molecules in life4,6; however, this labelling also induces a loss of structural information because a majority of molecules are unlabeled7. The implementation of this labelling also induces a loss of structural information because a majority of molecules are unlabeled7. The implementation of a large fraction of these imaging methods depends on a point-based raster scanning technology, such as the commonly used scanning electron microscopy (SEM)8, scanning probe microscopy (SPM)9,10 and stimulated emission depletion (STED)11 microscopy. The acquisition time increases significantly as the imaging area (for example, > 50 × 50 μm²) is enlarged at a high resolution (for example, <100 nm). In addition, SPM and SEM typically use a slow scanning speed and increase the number of scan lines used to improve imaging quality12, which further leads to time inefficiencies. To increase the imaging speed, parallelized scanning systems have been designed for different types of microscopes (for example, SEM13, SPM14,15 and STED16,17), making the systems more complex and expensive, and requiring sophisticated algorithms to achieve system control and image processing18. Only few of these systems are commercially available currently. Compared with these microscopes, traditional optical microscopes have certain unique advantages, such as the ability for non-invasive, real-time, large-area and fluorescent or white-light imaging, but their resolution is limited by the diffraction limit. Recently, the diffraction barrier has been surpassed by simply introducing dielectrics with a micro-scale spherical configuration when using conventional optical microscopes by transforming evanescent waves into propagating waves18–30. The resolution of this superlens-based microscopy has been decreased to ~50 nm (ref. 26) from an initial resolution of ~200 nm (ref. 21). This method can be further enhanced to ~25 nm when coupled with a scanning laser confocal microscope31. It has achieved fast development in biological applications, as the sub-diffraction-limited resolution of high-index liquid-immersed microscopes has now been demonstrated23,32, enabling its application in the aqueous environment required to maintain biological activity. Experimental results have also shown potential applications in biological observations in both fluorescence29,30 and white-light15 modes. This method inherits the majority of the advantages of traditional optical microscopes; however, the imaging field of view (FOV) is confined by the microsphere superlens’ size and by the aberrations in the boundary area of the FOV. Although the FOV increases linearly with the diameter of the superlens, the resolving ability deteriorates as the diameter increases23. This limited FOV reduces the range of practical applications; therefore, methods, such as probe-based manipulations18,21 and immersing microstructures into elastomers19,20,28, have been reported to locate microsphere superlenses for observations from a specific position. Unfortunately, when using these methods, it is difficult to achieve large-area imaging and to locate specific sub-diffraction-limited targets in a large area. In particular, these targets appear in different areas, such as the distribution of lysosomes in cells. In addition to these methods, microscopes or introduced elastomers still make contact with the samples, and the distance or interaction force between the microspheres and samples cannot be precisely controlled due to the lack of effective feedback mechanisms, which further adds to the invasiveness of this microsphere-based super-resolution imaging method.

In the following, we introduce a non-invasive, environmentally compatible and high-throughput optical microscopy technique called scanning superlens microscopy (SSUM) for large-area, super-resolution imaging and structural information acquisition. The distance or interaction forces between the microsphere superlenses and the specimens are adjusted using AFM principles, allowing the scanning and imaging of large areas. On the basis of the microsphere superlens imaging properties, two different AFM scanning modes that are compatible with microsphere superlens imaging have been demonstrated to achieve microsphere scanning of a large area: the contact mode enables imaging of stiff samples, and the constant-height mode facilitates non-invasive imaging of sensitive specimens. Compared with point-based raster scanning microscopes, this new technique replaces the ‘point’ with an ‘area’ (that is, the middle area of the FOV) of the microsphere superlens has no aberrations; therefore, there is a significant improvement in terms of the time efficiency for large-area imaging.

Results

Description of the microsphere-based SSUM. Figure 1a shows the basic concept of a microsphere-based SSUM. An AFM distance or interaction force control mechanism is introduced to adjust the position of the microscope above the sample. The magnification of the microscope objectives used in the microsphere superlenses-based nanoscale imaging is typically >50 with a limited working distance18,22–26. To be compatible with these objectives, a custom AFM system (Supplementary Fig. 1) illuminates and collects the laser beam of the optical lever using the same objective33,34. The microsphere superlens is attached to an AFM cantilever (Fig. 1c,d) that is fixed during scanning to prevent the distance between microsphere and the objective from changing or to maintain the imaging conditions as much as possible. Raster scanning in the lateral directions and feedback regulation in the vertical direction are achieved using a three-dimensional piezoelectric ceramic (PZT) scanner on which the specimens are placed. The spacing of the raster scanning and the interval of the signal used to trigger camera image recording during lateral scanning are adjusted based on the area of the FOV of microsphere superlenses without apparent aberration (Fig. 1b and Supplementary Fig. 2). Large-area, super-resolution imaging can be achieved by stitching together the recorded images. Here ‘super-resolution’ is defined as resolutions higher than λ/3.8 by considering the solid-immersion effect35,36 and the resolution of SSUM mounted with a 57 μm-diameter BaTiO₃ microsphere is estimated to be λ/6.3 under partial and inclined illumination, where λ is the peak illumination wavelength (Supplementary Note 1 and Supplementary Figs 3 and 4). The resolution could be further improved by the application of smaller microspheres23.
Contact scanning mode. Considering the near-field imaging characteristic of the microsphere superlens, we first demonstrate the capability of large-area imaging by the scanning microsphere using the contact mode. In this study, contact mode indicates that the microsphere instead of the AFM probe tip is in contact with the sample during scanning. To achieve this and to sufficiently scan the image (1) the microsphere diameter should be greater than the tip height if there is a tip on the AFM probe, or an AFM probe without tip should be selected particularly when the microsphere diameter is small. If the microsphere is smaller than the tip (that is, the tip is in contact with the sample and the microsphere is suspended over the sample), it is also possible to achieve super-resolution imaging, but the position of microspheres on the cantilever must be precisely controlled. (2) The microsphere diameter and the cantilever spring constant should be properly selected to prevent vibrations during scanning. For a commercially available cantilever (for example, the TESP probe from Bruker), the microsphere diameter should be $>80 \mu m$, which is a practical conclusion. (3) The selection of the microsphere size should balance the FOV and the resolving ability. (4) The cantilever does not shield the light path for the microsphere imaging (Fig. 1c,d). Figure 2c–e shows the large-area imaging of a Blu-ray disc surface, which contains 200 nm wide stripes spaced by 100 nm groves, that was achieved by scanning with a barium titanate (BaTiO$_3$) microsphere with a diameter of 59 $\mu m$ mounted on a TESP probe cantilever in water. The minimum feature size of the Blu-ray disc is $\approx 100 \text{ nm}$, which cannot be directly observed using an optical microscope. We defined three sample points in 100 nm to satisfy Nyquist criterion and resolve the minimum structure using a commercial AFM (Dimension Icon, Bruker). The total scanning area was $\approx 96 \times 96 \mu m^2$, therefore, 2,880 scan lines were required for AFM scanning, and the real acquisition time was $\approx 4 \text{ h}$ at a scan rate of 0.2 Hz (or 38.4 $\mu m \text{s}^{-1}$) to guarantee imaging quality (Fig. 2a,b). However, only 32 lines of scanning were required when using the microsphere-based SSUM (that is, the imaging efficiency was increased $\times 90$ compared with the traditional AFM method), and the acquisition time was $\approx 3 \text{ min}$. The pixel size for the image obtained by the SSUM was $\approx 14 \text{ nm}$. The AFM method required 6,857 scan lines to acquire the same image, which corresponds to $\approx 9.5 \text{ h}$; therefore, the SSUM is $\approx 214$ more efficient in terms of imaging.

Constant-height scanning mode. For sensitive specimens, such as cells, the interaction forces in contact mode will induce structural deformation or damage; therefore, a non-invasive
method is essential. This non-invasive imaging technique can be achieved using the SSUM within conjunction with a fundamental AFM working principle: the constant-height mode that is commonly used to achieve atomic-resolution images. In this mode, the microsphere is suspended over the samples, and the distance between the microsphere apex and the surface of the PZT scanner is held constant. We first examine the constant-height scanning mode by resolving structures in a central processing unit (CPU) that has specific structures of over a large area by scanning with a 57 μm diameter BaTiO3 microsphere in water at a scan rate of 0.1 Hz. The scan rate is the same in the following experiments unless noted otherwise.

A video recording of the scanning process is provided as Supplementary Movie 1, corresponding to the results shown in Supplementary Fig. 5d. There are certain structures or defects generated in the CPU sample preparation process that influence AFM imaging (Fig. 3c and Supplementary Fig. 5c), but are not apparently observed in the SEM images (Fig. 3b and Supplementary Fig. 5b). Some of these defects are also shown in the optical images (Fig. 3a and Supplementary Fig. 5a); however, they do not prevent the observation of structures in these areas. The influence of these defects on imaging is further weakened, and sub-diffraction-limited structures are resolved when imaged using the microsphere superlenses (Fig. 3d and Supplementary Fig. 5d) compared with the images obtained when they are directly observed using an optical microscope (Fig. 3a and Supplementary Fig. 5a). The virtual image magnification factors of the microsphere superlens in Fig. 3 and Supplementary Fig. 5 are both \( \times 3.4 \). Compared with the features imaged in Supplementary Fig. 5, several structures are located in Fig. 3i–l that can be clearly observed only in the microsphere-enhanced optical images (Fig. 3l). One possible reason for these results is that there is an optically transparent film preventing the energetic electron beam and AFM probes from penetrating and properly imaging the sample, but the film has a negligible influence on visible light transmission or microsphere superlens-based imaging, that is, the microsphere superlens can image sub-diffraction-limited structures below optically transparent films or realize sub-surface imaging.

To further demonstrate the penetration ability of the microsphere superlens, we observe randomly distributed silver nanowires (AgNWs) covered by an 11 nm-thick, optically transparent polystyrene film by scanning with a 57 μm diameter BaTiO3 microsphere in water, as shown in Fig. 4. This scan was conducted using the constant-height scanning mode. With the assistance of the microsphere superlens, sub-diffraction-limited AgNWs covered by the transparent film were clearly resolved (Fig. 4b,i), and a 40 nm-diameter AgNW was located by SSUM in...
The corresponding AFM scanning image is shown in filaments using 55 or 50 (Fig. 5a,c). In other experiments, we also discerned certain with the result directly observed using an optical microscope
detailed surface structural information can be resolved compared
improved surface morphology of cells could be captured. More
diameter of 59 or 63
proteins in one cell25, which form various functional units, it is
algorithm (Fig. 5j). Because there are more than 10,000 types of
image quality can be further improved using a band-pass filter
imaging of a mouse myoblast cell (C2C12) and human breast
cancer cells (MCF-7) using constant-height mode. These scans
were performed using a BaTiO3 microsphere superlens with a
diameter of 59 or 63 μm in water. As shown in Fig. 5b,d, an
improved surface morphology of cells could be captured. More
detailed surface structural information can be resolved compared
with the results shown in Figs 3l and 4b,i; thus, sub-membrane
Fig. 5a–d. Because these filaments are not distributed across the
cell membrane, they should exist inside the cell, which is similar
to the results shown in Figs 3l and 4b;i; thus, sub-membrane
nanostructures that reside inside live cells (Fig. 5f) or fixed cells
(Fig. 5h) beyond the diffraction barrier can be non-invasively
explored over a large area under white-light illumination. The
sub-membrane nanostructures can also be resolved via SEM in
hydrated samples assisted by graphene, but the cell must be fixed,
sub-membrane-extracted and stained, which causes cell death41. The
membrane-extracted and stained, which causes cell death41. The
sample surface is in contact with the microsphere, and a Δz value < 0 indicates deformation towards the objective at the end of the cantilever. These results demonstrate that (1) limited marginal deformation of the cantilever towards the microscope objective (that is, Δz < 0 μm) does not induce apparent changes in imaging when the microsphere superlenses is in contact with the sample; and (2) microsphere superlenses retain their imaging ability even when the microsphere is separated from the samples with sub-diffraction-limited structures (that is, Δz > 0 μm) as long as there is a peak wavelength of incident illumination (~550 nm) for coaxial illumination and five wavelengths for partial and inclined illumination (Fig. 7n) that can be adjusted by the incline angle (Fig. 7o).

Because the image generated by a microscope is the convolution of a point-spread function (PSF) and the object intensity distribution function, the resolution of SSUM can be estimated by a process of convolution with the PSF22,35,36. Using one-dimensional rectangular functions corresponding to two experimental results with minimum feature structures of 80 nm (insets of Fig. 8a), the resolution of SSUM mounted with a 57 μm microsphere is calibrated as the full-width at half-maximum, λ/6.3, of the Gaussian PSF according to Houston’s criterion22 (Fig. 8a, Supplementary Fig. 3 and Supplementary Note 1).

Figure 4 | AgNW imaging in constant-height scanning mode. (a.h) AgNWs are directly observed using an optical microscope. (b.i) AgNWs imaged using a scanning microscope superlens. A × 100 (numerical aperture = 0.8) objective was used in these experiments. (c.j) SEM images. The arrow in f points to a AgNW with a diameter of 40 nm. (g.k) AFM scanning images. (d-f) Local zoomed areas that correspond to the marked areas shown in a-c, respectively. The corresponding AFM scanning image is shown in g. Scale bars, 10 μm (a–c,h–k); 2 μm (d–g).

Imaging characteristics of the microsphere superlens. For traditional AFM constant-height imaging, the tip-sample distance is typically set to be sub-nanometre, and feedback is switched off. Therefore, scanning is confined to a limited area, which is typically measured in nanometres37–39. To elaborate why the microsphere superlenses can achieve a large scanning area in constant-height mode without feedback, we explored the influence of the separation distance (Δz) between the microsphere and samples on microsphere super-resolution imaging (see Methods). In this study, the Blu-ray disc was held by the PZT scanner, vertically scanning away from the microsphere at different illumination conditions (Fig. 7b,c), which were recorded by a camera (inset of Fig. 7a). Figure 7d,e show the cross-sections of the recorded images; a Δz value of zero indicates that the sample surface is in contact with the microsphere, and a Δz value < 0 indicates deformation towards the objective at the end of the cantilever. These results demonstrate that (1) limited marginal deformation of the cantilever towards the microscope objective (that is, Δz < 0 μm) does not induce apparent changes in imaging when the microsphere superlenses is in contact with the sample; and (2) microsphere superlenses retain their imaging ability even when the microsphere is separated from the samples with sub-diffraction-limited structures (that is, Δz > 0 μm) as long as there is a peak wavelength of incident illumination (~550 nm) for coaxial illumination and five wavelengths for partial and inclined illumination (Fig. 7n) that can be adjusted by the incline angle (Fig. 7o).
Figure 5 | Non-invasive observation of cells in white-light mode. A C2C12 cell was imaged using (a) a traditional optical microscope or (b) SSUM. A video recorded while scanning a C2C12 cell is provided as Supplementary Movie 2. MCF-7 cells were observed (c,e,g) without and (d,f,h) with the aid of the microsphere superlens. A × 100 (numerical aperture (NA) = 0.8) objective was used in a,b,g and h, and a × 50 (NA = 0.6) objective was used in c-f. (i) Local zoomed area of the marked area shown in h. (j) After using a band-pass filter algorithm of i. Scale bars, 6 μm (a,b); 10 μm (c-e,g,h); 3 μm (f); 2 μm (i,j).

Figure 6 | Non-invasive white-light and fluorescence microscopy of a C2C12 cell. (a,b) White-light and (c,d) fluorescent imaging of a C2C12 cell (a,c) without and (b,d) with the enhancement of a 56 μm-diameter microsphere superlens. A × 100 (numerical aperture = 0.8) objective was used in these experiments. For fluorescent imaging, the sample was labelled by Alexa Fluor 488-phalloidin to observe actin filaments. Scale bars, 5 μm.
This process is achieved by matching the calculated convolution results to the experimental profiles. A dimensionless parameter, normalized intensity difference (NID = (I_max - I_min)/(I_max + I_min); Fig. 8b), is defined to study the influence of Δz on the resolution of microsphere superlenses under different illumination conditions. The NID calculated by experimental data (scatter data) and by the matched convolution results (solid or dashed curves) with different constants (Con) that are used in resolution estimation (resolution = λ/Con) are shown in Fig. 8c,d. The resolving capability of microspheres improves as Con increases. The resolution is attenuated (or the Con is decreased) with increasing constant (Con) that are used in resolution estimation (resolution = λ/Con). Consequently, the maximum distance that allows for imaging, that is, the structure on sample cannot be observed through the microsphere superlenses as Δz exceeds Δz_max. The incline angle is set to ~64° for partial and inclined illumination. The influence of the inclined angle (θ) on Δz_max. The microsphere diameter is ~60 μm in these measurements. Scale bars, 1 μm (f-m).

Figure 7 | Microsphere imaging properties analyses. (a) Schematic showing the experimental set-up used to study the distance (Δz) between the microsphere apex and the samples (Blu-ray disc) on microsphere imaging properties. The inset shows the image stack recorded during scanning. The left schematics in b and c show two different illumination conditions: coaxial illumination; and partial and inclined illumination. The right images in b and c show the illumination conditions detected during the vertical scanning of an optical beam profiler around the objective focus, where θ is the incline angle of the incident light focused using a × 50 (numerical aperture = 0.6) objective. (d,e) Cross-sections of the images recorded during PZT scanning, as shown in the inset of a, under different illumination conditions. (f-i) Super-resolution images corresponding to the specific positions marked in d and e, respectively. (n) Relationship between Δz_max and the microsphere diameter, where Δz_max is the maximum distance that allows for imaging, that is, the structure on sample cannot be observed through the microsphere superlenses as Δz exceeds Δz_max. The incline angle is set to ~64° for partial and inclined illumination.
As the spherical lens shrinks to the micro-scale, the relationship between the focal length and diameter/Δz (Supplementary Figs 9 and 10) and the dependence of the virtual image magnification on Δz (Supplementary Fig. 11) deviate from the approximation of geometrical optics. To describe the relationship between the near-field magnification and Δz, we present a fitting formula based on the magnification factor calculated by the finite-difference time-domain (FDTD) method simulated focal lengths (f_{FDTD}, M \approx k \times f_{FDTD} / (f_{FDTD} - (D/2 + Δz)), which yields a good match when k \approx 1.2 (Supplementary Fig. 11 and Supplementary Note 2). After demonstrating that the sample reflectivity has a negligible effect on the analyses (Supplementary Fig. 12 and Supplementary Note 3), we determined that the noise signal plays a significant role in the sub-diffraction-limited imaging but has a negligible influence on the imaging of structures larger than the diffraction limit (Supplementary Fig. 13). For microsphere-based super-resolution imaging, the converted propagation signal carrying sub-diffraction-limited structural information decays exponentially with Δz (Supplementary Fig. 14), which follows a similar exponential decay trend of the FDTD-simulated evanescent field generated at the second refraction position on the microsphere surface (Supplementary Fig. 15). The interaction of these evanescent waves and samples is considered to play a role in the origins of the sub-diffraction-limited resolution of the microsphere superlens, which is further supported by the consistency between the theoretically estimated resolution \(2\times(\sqrt{6.5-\Delta z}/9.2)\) by spectral analysis and the experimentally achieved values \(2\times(\sqrt{6.3-\Delta z}/8.4)\) (Supplementary Note 3). The apparent improvement in the partial and inclined illumination on imaging quality (Fig. 7d–m) and the range of Δz that allows for acceptable imaging (Fig. 8c,d and Supplementary Fig. 4a,b) are supported by an analysis of the loss of deterioration from the middle illumination, which mainly introduces propagating light during imaging, and the transfer of the second refraction position by inclining illumination, which shortens the distance for evanescent waves transferring to the sample and induces a spectrum extension of the evanescent waves illuminated on the sample surface (Supplementary Note 3). The evanescent waves with high

Figure 8 | Resolution calibration of SSUM. (a) Insets are two sets of SEM images and SSUM results of the structures in the CPU, in which the dashed line (SSUM-1) and dotted line (SSUM-2) are used to estimate the resolution. The solid curve represents the calibrated convolution result. (b) Definition of NID. The influence of Δz on the resolution under different illumination conditions, that is, (c) coaxial illumination and (d) partial and inclined illumination. Δz is defined in Fig. 7a, and the distance between microsphere and microscope objective does not change. The constants (Con) are the values applied to calculate the resolution (\(\Delta z/\text{Con}\)). The data used to calculate NID (scatter data) are extracted from the experimentally measured cross-sections of the structures on the CPU chip (see the SEM image in the inset of c) using a 60-μm-diameter microsphere similar to that in Fig. 7a–e. In c and d, the solid curves, representing super-resolution, and dashed curves, representing the diffraction confinement, are achieved from the calibrated convolution results, for example, the solid curve in a. The incline angle, as defined in Fig. 7c, was set to \(\sim 70°\) for the experiments under partial and inclined illumination. Scale bars, 1μm (a).
spatial frequencies generated by the microscope are weaker than the propagating waves and the background, a situation that worsens as the frequency increases. This practical restriction prevents the microscope superlens from achieving its theoretical resolution limit. The evanescent field can be enhanced by increasing the illumination intensity; however, this action is accompanied by a promotion of the propagating field. The proposed partial and inclined illumination provides an option to circumvent this problem (Supplementary Note 3).

Discussion
We have shown that non-invasive super-resolution imaging over a large area can be achieved by properly developing the imaging properties of the microscope superlens explored in this study. By replacing the detection point with a microscope superlens, a non-invasive microscope-based SSUM has been demonstrated, and the imaging acquisition efficiency has been improved by \( \sim \times 200 \) compared with a commercial AFM. Because the SSUM imaging time is primarily consumed by the scanning and image stitching processes, the imaging efficiency can be further improved by using more efficient algorithms for image stitching or using a higher-speed camera or parallel scanning purposes or applications. There were 1,056 (Figs 2c, 4b and 5d), 561 (Fig. 6b, d), 272 (Fig. 3d) and 320 (Supplementary Fig. 5d) image tiles used in the image stitching processes. The performances of and time consumed by these two software platforms are compared in Supplementary Fig. 16 and Supplementary Table 1. The quality of the stitched images can be further improved by selective processing using a band-pass filter algorithm or a recursive bilateral filtering algorithm.

Assemblies of the microscope imaging properties. In this study, the imaging conditions were similar to the constant-height scanning mode (that is, the microscope was attached to a TESP cantilever, which was fixed to maintain the distance between the microscope and the objective, as shown in Fig. 7a). Initially, the microscope superlens was in contact with the Blu-ray disc’s surface (that is, \( \Delta z = 0 \) \( \mu m \)) without pre-stress, which can be achieved by monitoring the position-sensitive device signal of the AFM system. Then, the microscope, including the objective driven by a motorized vertical stage, was adjusted to a position where the virtual images generated by the microscope superlens could be clearly observed. Before scanning, a pre-stress was applied by moving the PZT towards the objective; this produced an \( \sim 1 \mu m \) deep pre-deformation at the position where the microscope was in contact. After the PZT was attached, the Blu-ray disc was scanned by the PZT scanner away from the microscope at different illumination conditions (Fig. 7b). The virtual images generated by the microscope superlens in the vertical scanning processes were recorded using a high-speed camera (inset of Fig. 7a).

Data availability. The data sets generated during and/or analysed during the current study are available from the corresponding authors on reasonable request.

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Author contributions
W.L.W. and L.L. conceived of the basic idea of this study and designed its experiments; F.W. and L.L. designed the optical system and performed the experiments; F.W., W.J.L. and L.L. analysed the data and co-wrote this paper; P.Y. programmed the code for the APM scanner; F.W. and Z.L. set up the optical system; W.J.L. and Y.W. supervised the project; H.Y., Y.W. and Z.L. assisted in the experimental processes. All authors contributed to the general discussion and revision of the manuscript.

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