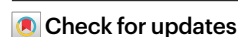


Rationalized deep learning super-resolution microscopy for sustained live imaging of rapid subcellular processes

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The goal when imaging bioprocesses with optical microscopy is to acquire the most spatiotemporal information with the least invasiveness. Deep neural networks have substantially improved optical microscopy, including image super-resolution and restoration, but still have substantial potential for artifacts. In this study, we developed rationalized deep learning (rDL) for structured illumination microscopy and lattice light sheet microscopy (LLSM) by incorporating prior knowledge of illumination patterns and, thereby, rationally guiding the network to denoise raw images. Here we demonstrate that rDL structured illumination microscopy eliminates spectral bias-induced resolution degradation and reduces model uncertainty by five-fold, improving the super-resolution information by more than ten-fold over other computational approaches. Moreover, rDL applied to LLSM enables self-supervised training by using the spatial or temporal continuity of noisy data itself, yielding results similar to those of supervised methods. We demonstrate the utility of rDL by imaging the rapid kinetics of motile cilia, nucleolar protein condensation during light-sensitive mitosis and long-term interactions between membranous and membrane-less organelles.

All living organisms execute elaborate bioprocesses. Fluorescence microscopy is essential for elucidating the dynamics and functions of various bioprocesses in live cells^{1–4}. Its power has strengthened with recently developed super-resolution (SR) techniques. However,

the increase in spatial resolution with any SR method is accompanied by tradeoffs with other imaging metrics that are equally important for understanding bioprocesses^{5,6}. Structured illumination microscopy (SIM) is usually recognized as the optimal SR method for live

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cell imaging because it requires fewer raw images and lower illumination intensity than other SR techniques^{3,6}. Nonetheless, SIM-related techniques generally require a decent signal-to-noise ratio (SNR) for each raw image to prevent reconstruction artifacts^{6,7}. Consequently, photobleaching and phototoxicity restrict the imaging performance of existing SR-SIM techniques.

In addition to advances in microscope hardware, computational approaches have become increasingly important for image restoration and enhancement in optical microscopy. Traditional algorithms employ analytical models with certain assumptions to iteratively restore/denoise SR images^{7,8}. However, microscope imaging is a statistically complex process; thus, handcrafted analytical models are limited by the accuracy of their assumptions, often resulting in losses in spatial and temporal resolution (Supplementary Fig. 1 and Supplementary Video 1). Deep neural networks (DNNs) are capable of learning not only the pseudo-inverse functions of image transformation processes but also the stochastic characteristics of good solutions by leveraging paired end-to-end transformation images^{9,10}. Deep learning algorithms have demonstrated outstanding performance in various image transformation tasks, including denoising and image SR. However, these tasks are essentially ill-posed problems¹⁰, indicating that several solutions exist for a given input in the high-dimensional manifold of all possible inferences. Moreover, DNNs learn low-frequency modes faster and more robustly than high-frequency modes, a phenomenon called spectral bias¹¹ (Supplementary Note 1a and Extended Data Fig. 1), suggesting that reconstruction of high-frequency rich SR images from diffraction-limited counterparts is more challenging than denoising diffraction-limited images. The effects of ill-posed problems and spectral bias are exacerbated as the input images become noisier, contain more complex structures or extend from two dimensions (2D) to three dimensions (3D). Finally, the acquisition of high-quality ground truth (GT) for training DNNs is not trivial in most bioimaging applications.

In this study, we developed rDL methods for SIM and LLSM. The rDL network architecture, which is distinct from existing DNNs for image transformation, incorporates a pre-characterized physical model of each microscopy technique (for example, the prior knowledge of illumination patterns used in SIM) into the network to guide network training and regulate the network output to a lower-dimensional manifold that is closer to the GT than other methods (Supplementary Notes 1 and 2). Moreover, instead of mitigating the artifacts embedded in the reconstructed SR image^{7,8}, we apply the rDL SIM model to denoise the diffraction-limited raw SIM images, ensuring high-quality reconstruction via the conventional algorithm while eliminating the spectral-bias-induced resolution degradation in the final SR image. Furthermore, we use bioimaging sampling continuity in the temporal or spatial domain to develop a temporally/spatially interleaved self-supervision mechanism for implementing rDL LLSM denoising without the need for GT images. We demonstrate that rDL SIM and rDL LLSM enable high spatiotemporal resolution and long-term observation of more than ten types of bioprocesses that are too light-sensitive, too rapid or too long to be recorded in live via state-of-the-art SR microscopy.

Results

Development and characterization of rDL SIM

The multimodal SIM (Multi-SIM) system was developed from our previous grazing incidence SIM (GI-SIM) system¹², which integrated total internal reflection fluorescence (TIRF)-SIM, GI-SIM and 3D-SIM. We characterized the illumination patterns and point spread function (PSF) of different SIM modalities under high SNR conditions. Because illumination patterns and PSF are natural attributes of the SIM system that are independent of observed specimens or available SNR levels of different experiments, these deterministic properties can be used to regulate the denoised raw images in compliance with the physical model of SIM (Supplementary Note 1).

Next, we devised the rDL network consisting of three deep residual convolutional neural network branches trained in a supervised manner (Fig. 1a and Supplementary Fig. 2). Branch 1, named the primary feature extracting (PFE) branch, was trained to extract the features of observed specific biological structures (Fig. 1b) and separate these features from the noise features (Supplementary Fig. 3a,b). However, if these features were directly decoded as the final output¹³, the denoised raw image barely exhibits Moiré fringes (arrows in Fig. 1c,e). This drawback was generally observed with either deep learning or analytical algorithms (Supplementary Fig. 4b,c)^{14,15}. Thus, SIM images reconstructed via conventional deep learning denoising models (Fig. 1c) often cannot reconstruct the SR information and fail to resolve the fine structures compared to GT-SIM images (Fig. 1e,f). Branch 2, named the Moiré pattern extracting (MPE) branch, integrated the physical model of SIM into the rDL framework as follows: (1) infer the SR image from the corresponding raw SIM images using a pre-trained deep Fourier channel attention network (DFCAN)¹⁶ for a specific specimen; (2) multiply the SR image by pre-characterized structured illumination (SI) patterns and then convolve with the PSF to generate pattern-modulated raw images with high-contrast Moiré fringes; and (3) extract features from the pattern-modulated images (Supplementary Figs. 3c and 5). Branch 3, named the feature coalescing and denoising (FCD) branch, was designed to adaptively integrate features learned from the raw and pattern-modulated images. These features were decoded as the final output of the denoised images, possessing the same biological structures and Moiré fringes as the raw GT images (Fig. 1d,e and Supplementary Fig. 3d), which ensured high-quality SIM reconstruction. Of note, although the rDL denoising network should preferably be trained for each type of biological structure, we found that the strategy of physical model rationalization allowed the rDL model trained with specific structures (for example, F-actin), to be well-generalized onto other structures (for example, clathrin-coated pits (CCPs) or microtubules (MTs)) (Extended Data Fig. 2a,b).

In addition, although the illumination patterns and PSF are deterministic characteristics of a SIM system, they suffer from certain variations relative to the pre-characterized parameters in daily experiments, presumably because of either the small refractive index change of the immersion oil due to the heating effect by excitation light illumination or the poor SNR imaging condition. We compared the stability of pattern parameters estimated from 45,000 timepoints of endoplasmic reticulum (ER) data between CARE-SIM¹⁵ and rDL SIM. After CARE denoising, the estimated pattern period, orientation and initial phase still exhibited large variations (blue curve in Fig. 1g and Extended Data Fig. 2c); in contrast, rDL denoised raw images permitted a stable estimation of pattern period and orientation during SIM reconstruction. Nonetheless, a certain variation of less than $\pm 0.192\pi$ remained in the initial phase estimation of rDL SIM (orange curve in Fig. 1g). To test the sensitivity of rDL SIM to the initial phase error, we employed the incorrect patterns, whose initial phases were deviated by $< \pm 0.2\pi$, to generate pattern-modulated raw images in the MPE branch for the rDL denoising model. The peak signal-to-noise ratio (PSNR) of the rDL SIM images with $\pm 0.2\pi$ phase deviation was decreased by only 1% relative to the one without phase error (Fig. 1h), and there were no obvious artifacts in the rDL SIM images (Extended Data Fig. 2d,e). Furthermore, we examined the robustness of rDL SIM to the aberrant PSF in the MPE branch distorted by spherical or coma aberration, which correspond to the common misalignments of refractive index mismatch and placing the specimen oblique to the optical axis of the objective lens, respectively. We found that the PSNR of rDL SIM images decreased by less than 2.3% until the two aberrations were as high as $\pm 0.4\lambda$, which was sufficient to induce substantial distortion into the PSF (Fig. 1i and Extended Data Fig. 2f). These results illustrate the strong robustness of rDL SIM to the unexpected variations in practical experiments (Supplementary Note 2b).

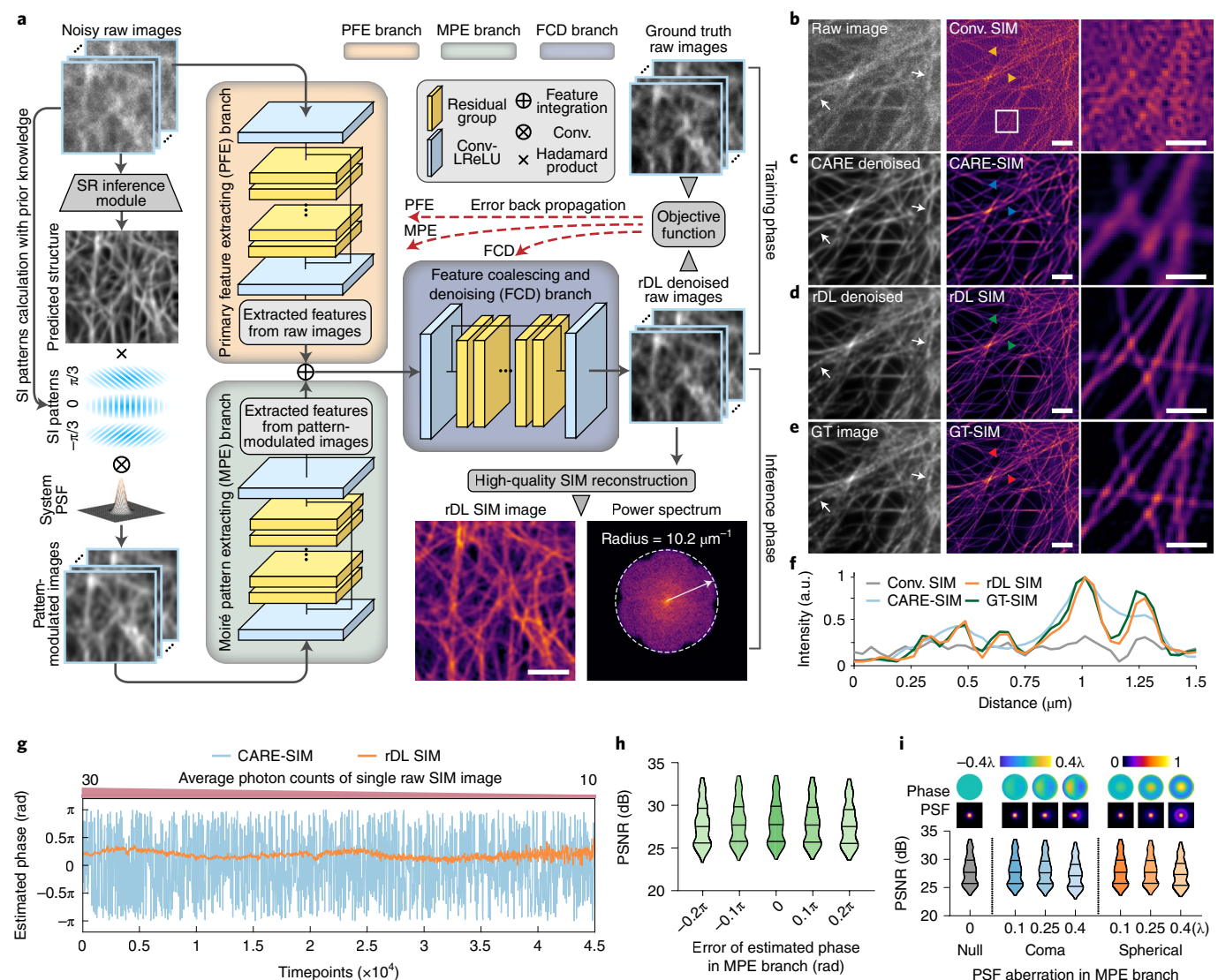


Fig. 1 | rDL SIM method. **a**, The architecture of the rDL network comprised of three branches. **b**, Noisy raw SIM image of MTs acquired under low SNR conditions (left) and the corresponding conventional (Conv.) SIM image without denoising (right). **c**, Denoised raw SIM image of **b** by the deep learning (DL) denoising method of CARE¹⁵ without physical model regularization (left) and the corresponding CARE-SIM image (right). **d**, Denoised raw SIM image of **b** by the rDL method with physical model regularization (left) and the corresponding rDL SIM image (right). **e**, GT raw image acquired under high SNR conditions (left) and the corresponding GT-SIM image (right). The arrows in **b–e** indicate the examples of Morie fringes that are almost invisible in raw image of low SNR (**b**) or denoised image by CARE (**c**) but faithfully restored by rDL (**d**) compared to the GT raw image (**e**) (Supplementary Video 2). **f**, Comparison of the intensity

profile plots of Conv. SIM (gray), CARE-SIM (blue) and rDL SIM (orange) with GT-SIM (green) along the lines indicated by the two arrowheads in **b–e**. **g**, Plot of the illumination pattern's initial phase for the 45,000 timepoints of ER data in Supplementary Video 8 estimated by CARE-SIM and rDL SIM. **h**, The robustness of rDL SIM to the initial phase error of the illumination pattern used in the MPE branch, showing that the PSNRs of the rDL SIM images with $\pm 0.1\pi$ and $\pm 0.2\pi$ phase error in MPE branch are similar to the images without phase error, $n = 144$. **i**, The robustness of rDL SIM to the aberrant PSF used in the MPE branch, showing that the PSNRs of the rDL SIM images with spherical or coma aberration distorted PSF in the MPE branch are similar to the images without aberration distortion, $n = 144$. Scale bars, 1 μm (**a**), 1.5 μm (**b–e**) and 0.5 μm (zoom-in regions of **b–e**). a.u., arbitrary units.

Comparison of rDL SIM and state-of-the-art SIM methods

Next, we performed a systematic evaluation of rDL SIM and other state-of-the-art SIM methods, including DFCAN/DFGAN-SIM¹⁶ and Hessian-SIM⁷, by using of our published BioSR dataset (Methods). Figure 2a shows that, under low SNR conditions, the deep learning super-resolution (DLSR) methods generally outperform Hessian-SIM, which employs an analytical model with certain assumptions⁷; although both DFCAN-SIM and DFGAN-SIM could infer perceptually good SR images, rDL SIM more precisely reconstructed the fine structures of MT and F-actin. Moreover, we quantified the PSNR and resolution of F-actin SIM images as a function of fluorescence intensity (Fig. 2b), which revealed that rDL SIM offered the greatest accuracy and resolution

close to the GT-SIM among the four SIM methods at all the fluorescence levels. Compared to the DLSR models without using the generative adversarial network (GAN) strategy (for example, DFCAN), the rDL scheme eliminated the spectral bias effect that otherwise remarkably degraded the resolution of reconstructed SR images (Extended Data Fig. 1 and Supplementary Note 1a). Compared to the GAN-based DLSR models (for example, DFGAN), the rDL scheme suppressed the SR image reconstruction uncertainty—that is, model uncertainty due to inadequate representativeness of the training data (Fig. 2c, Extended Data Fig. 3 and Supplementary Note 2d). Compared to CARE-SIM and Hessian-SIM relying on the conventional SIM reconstruction algorithm, rDL SIM improved the strength of SR components by approximately

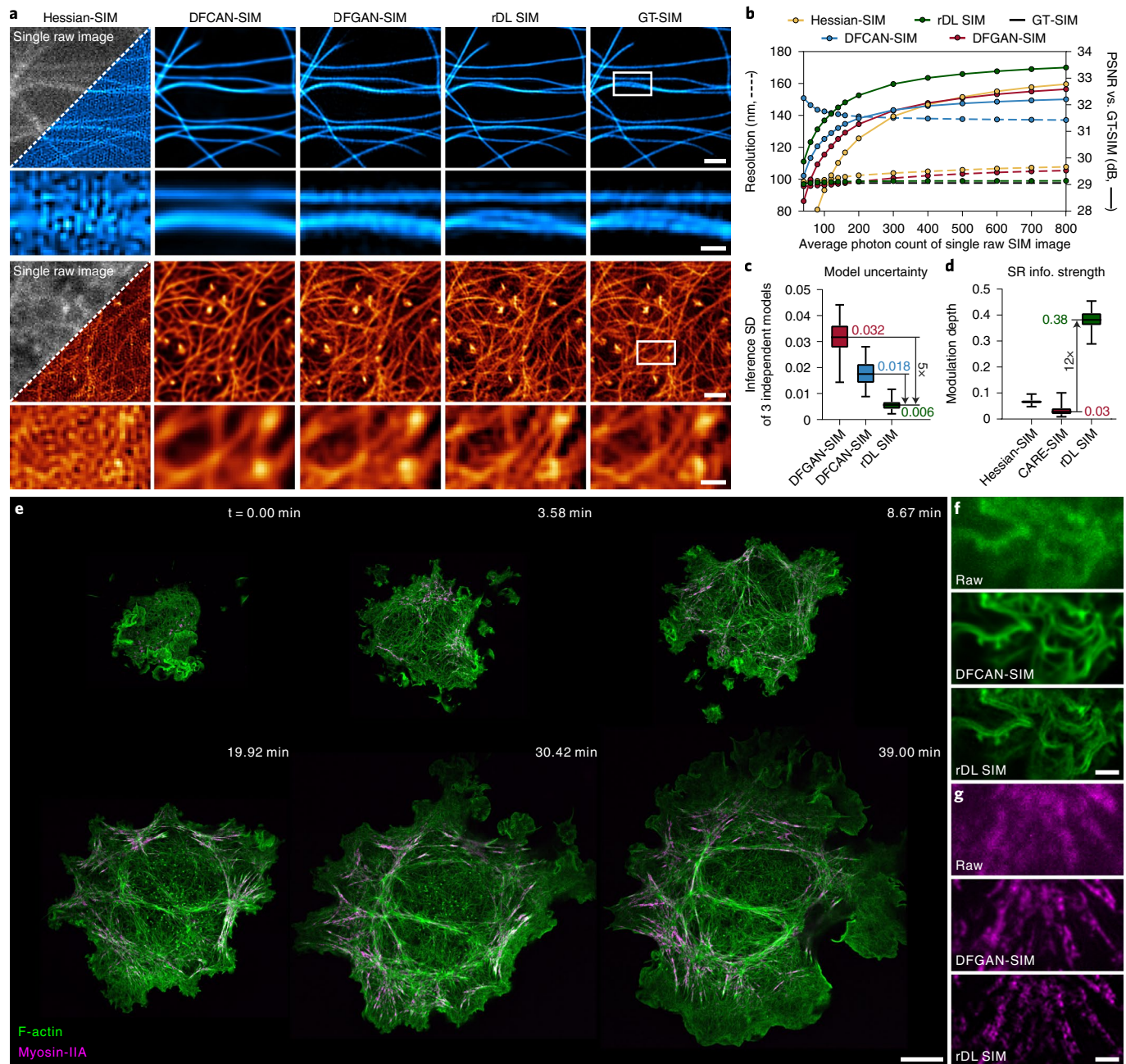


Fig. 2 | Comparison of rDL SIM with state-of-the-art SIM methods. a, Representative SR images of MTs (upper panel) and F-actin (lower panel) reconstructed by Hessian-SIM (first column, $\mu = 100$, $\sigma = 1$), DFCAN-SIM (second column), DFGAN-SIM (third column) and rDL SIM (fourth column). GT-SIM images acquired at high SNR are shown for reference. **b**, PSNR (solid line) and resolution (dashed line) comparisons of the four SIM methods with the F-actin data at different imaging conditions, $n = 150$. **c**, Model uncertainty comparison of rDL SIM with DFCAN-SIM and DFGAN-SIM, quantified by the SR images variation reconstructed by three independently trained models, $n = 150$. **d**, Comparison of SR information strength of F-actin data

reconstructed by rDL SIM, Hessian-SIM and CARE-SIM. The SR information was represented by the modulation depth estimated via the conventional SIM reconstruction algorithm, $n = 150$. **e**, Time-lapse rDL TIRF-SIM images showing the coordinated remodeling dynamics of F-actin (green) and myosin-IIA (magenta) over the hour-long spreading process after placing a U2OS cell onto a coverslip (Supplementary Video 3). **f**, Comparison of the SR image of F-actin reconstructed by rDL SIM and DFCAN-SIM. **g**, Comparison of the SR image of myosin-IIA reconstructed by rDL SIM and DFGAN-SIM. Center line, medians; limits, 75% and 25%; whiskers, maximum and minimum. Scale bars, 1 μm (**a**), 0.3 μm (zoom-in images in **a**), 8 μm (**e**) and 1 μm (**f, g**).

ten-fold, represented by the enhanced modulation depth of the illumination patterns (Fig. 2d).

We further assessed the SR imaging capability of these SIM methods with live cell specimens. Cell adhesion and migration are often disrupted by the high light illumination of conventional SR microscopy¹⁷. rDL TIRF-SIM achieved hour-long time-lapse recording of cell spreading after cells co-expressing mEmerald-Lifeact and mCherry-myosin-IIA were placed on a coverslip (Fig. 2e), which clearly

resolved the coordinated dynamics between F-actin and myosin-IIA, generating the protrusion and retraction oscillation of lamellipodia (Supplementary Video 3). In contrast, we found that, at this low fluorescence level, DFCAN-SIM and DFGAN-SIM rarely resolved the fine structures of parallelly aligned actin filaments and opposed heads of bipolar myosin-IIA groups (Fig. 2f,g). Additionally, rDL TIRF-SIM was able to visualize clathrin-mediated endocytosis in gene-edited SUM159 cells endogenously expressing clathrin-EGFP for more than 5,000

frames lasting more than 45 minutes (Supplementary Video 4), which corresponds to prolonging the imaging duration by more than 30-fold compared to conventional TIRF-SIM¹⁸. Moreover, the SR information of hollow, ring-like structure of CCPs was continuously reconstructed in high fidelity by rDL SIM, which was unrestored by CARE-SIM (Extended Data Fig. 4a). Taken together, these comparisons illustrate the superior SR imaging capability of rDL SIM.

Characterizing the ultradynamics of motile cilia

Motile cilia are extremely dynamic organelles that continuously beat in unison. Characterizing the ciliary beat frequency (CBF) and the trafficking of intraflagellar transport (IFT) inside cilia are challenging for current fluorescence microscopy because it necessitates the imaging of high resolution, fast speed and long duration simultaneously. Here, we applied rDL GI-SIM to visualize the free cilia against the bottom of a coverslip (Methods). To achieve ultra-fast SR imaging, we shortened the exposure time of each raw image to 0.1 ms and employed the interleaved reconstruction strategy¹⁹. This enabled GI-SIM imaging at a speed of 684 frames per second (fps). At this high speed, the conventional GI-SIM image was overwhelmed by reconstruction artifacts (Fig. 3a, right). To eliminate these artifacts, we trained the rDL denoising model to learn the precise mapping relationship between low SNR and high SNR datasets of cilia immobilized in agarose²⁰. With 97 nm spatial and 684 fps temporal resolution, we clearly resolved the hollow tubular structure of the ciliary axoneme (Fig. 3a, left) and the ciliary beating dynamics, which were characterized by sideways sweeping and back-and-forth motions (Fig. 3b). This high spatiotemporal resolution imaging could record 60,000 frames without causing noticeable phototoxicity and photobleaching effects (Supplementary Video 5). We traced the distal ends of the cilia ($n = 107$) to quantify the CBF of mouse ependymal cells (mEPCs), which ranged from 17 Hz to 49 Hz with an average of 32 Hz (Fig. 3c). The CBF of brain ependymal cells was higher than that of olfactory or tracheal cells^{20,21}, which might reflect the difference in fluid clearance efficiency in the brain versus other organs.

We next applied two-color rDL GI-SIM imaging to visualize the spatiotemporal remodeling of IFT trains (EGFP-IFT81), extending the observation window from -10 seconds^{22,23} to -3 minutes (Supplementary Video 6). With rDL GI-SIM, the IFT trains were resolved as three groups moving along MT tracks on the left, middle and right sides of the projected footprint of the ciliary axoneme (Fig. 3d). This allowed us to analyze the behaviors of IFT trains, although multiple trains often coexist within each axoneme (Supplementary Video 7). We found that the population of retrograde trains was approximately four-fold more abundant than that of anterograde trains ($n = 738$ versus $n = 186$ from 29 cilia) (Methods). Second, 41% of the 738 retrograde trains stopped and paused for some time before reaching the ciliary base (Fig. 3e and Extended Data Fig. 4b). Third, 35% of the paused retrograde trains unexpectedly reversed their direction of motion in the middle of MT tracks (Fig. 3f and Extended Data Fig. 4c). Fourth, faster IFT trains could collide with slower trains; after a short period of co-movement, the IFT trains might reorganize the IFT complexes between different trains (Fig. 3g) or split the original IFT trains (Extended Data Fig. 4d). In brief, rDL GI-SIM enables the study of the intricacy of IFT complex behaviors.

Contact between membranous and membrane-less organelles

Recent studies have revealed that different organelles interact with one another to synergize in a wide variety of bioprocesses⁴. Functional interorganelle contact sites (CSs) include protein complexes that tether opposing membranes together⁴, allowing for sustained correlated motion between contacting organelles. The high-speed and long-time SR imaging capabilities offered by rDL SIM are very helpful to distinguish the correlated motions from random interactions. With rDL GI-SIM, we were able to record the interactions between ER and peroxisomes (POs) in live COS-7 cells (Methods) at a high speed of 182 fps for more than 45,000 frames (Fig. 3h and Supplementary Video 8).

We tracked the displacements of individual POs and then computed the Mander's coefficient of each PO with its adjacent ER tubules for each frame to identify the functional ER-PO contacts (Methods and Extended Data Fig. 5a,b). We found that 82% of the POs (112 of 137 from 14 cells) were closely associated with ER, which usually restricted PO mobility (Fig. 3i and Extended Data Fig. 5c). We observed a moving PO that pulled out and elongated the ER tubule along its trajectory, reshaping the local ER network (Extended Data Fig. 5d).

In addition to membranous organelles, various membrane-less organelles have been identified—for instance, DNA binds to cyclic GMP-AMP synthase (cGAS) to form cGAS-DNA condensates²⁴. To examine whether cGAS-DNA condensates interact with other organelles, we applied rDL GI-SIM to image a HEK293T cell line stably expressing cGAS-Halo and mEmerald-KDEL (Methods). We found that most cGAS-DNA condensates were closely associated with adjacent ER tubules or corralled by ER polygons, stabilizing cGAS-DNA condensate positions over time (Fig. 3j,k and Supplementary Videos 9 and 10). As cGAS-DNA condensates grew larger, the surrounding ER polygons adaptively enlarged to accommodate the larger condensates (Fig. 3l). Moreover, some cGAS-DNA condensates moved directionally over long ranges (Supplementary Video 11), during which the ER tubules were reshaped to retain the contacts with moving cGAS-DNA condensates (Fig. 3m). These observations demonstrate that the ER membrane contacts cGAS-DNA condensates, which might regulate the biogenesis, position and relocation of cGAS-DNA condensates.

SR live cell 4D imaging with rDL 3D-SIM and rDL LLS-SIM

Volumetric SR imaging requires more high SNR raw images than planar imaging. Volumetric SR techniques have rarely been used to study bioprocesses that span the whole cell, even under overexpression conditions. To extend the capability of long-time SR imaging from 2D to 3D, we integrated the rDL denoising strategy into 3D-SIM and LLS-SIM. Although the wide-field epi-illumination configuration used in 3D-SIM results in a high fluorescence background and rapid photobleaching, rDL 3D-SIM can consistently produce high-quality SR images of mitochondrial cristae for more than 400 volumes (Fig. 4a and Supplementary Video 12), capturing cristae dynamics during mitochondrial fission (Fig. 4b). In contrast, conventional 3D-SIM produced uninterpretable SR images under the same conditions (Fig. 4a).

To address the limitations of epi-illumination while retaining the SR capability, we implemented rDL SIM into a home-built LLSM platform to develop the rDL LLS-SIM system. Although nucleoli are known to undergo disassembly and reassembly during mitosis, the volumetric dynamics of nucleolar condensation are difficult to observe at high spatiotemporal resolution with existing SR techniques. We used rDL LLS-SIM to image HeLa cells with mEmerald-NPM1 knocked in and stably expressing mCherry-H2B and Halo-RPA49, for more than 2.5 hours at a speed of 12 seconds per three-color volume covering the entire mitosis (Fig. 4c, Extended Data Fig. 6a and Supplementary Video 13). Compared to conventional LLS-SIM, the rDL LLS-SIM revealed the nested structure of the nucleolus, and the innermost fibrillar center (FC) marked by RPA49 was surrounded by the outermost granular component (GC) marked by NPM1 (Fig. 4d). During prometaphase, we observed that the dissociation of RPA49 from the FC preceded the disassembly of the GC (Extended Data Fig. 6b); however, several RPA49-foci maintained their association with chromatin. During anaphase, before the nuclear envelope formed, small NPM1-foci assembled preferentially around chromosomes. Independent of NPM1-focus condensation, the retained RPA49-foci gradually enlarged, and new RPA49-foci started to condense into prenucleolar bodies (PNBs) (Fig. 4e). During telophase, the diffusing NPM1 protein began to condense around these PNBs (Fig. 4f), and the previously formed NPM1-foci also fused into nearby PNBs (Extended Data Fig. 6c), which caused PNBs to rapidly expand and coalesce one another, forming large multi-layered condensates, eventually maturing into nucleoli (Supplementary Video

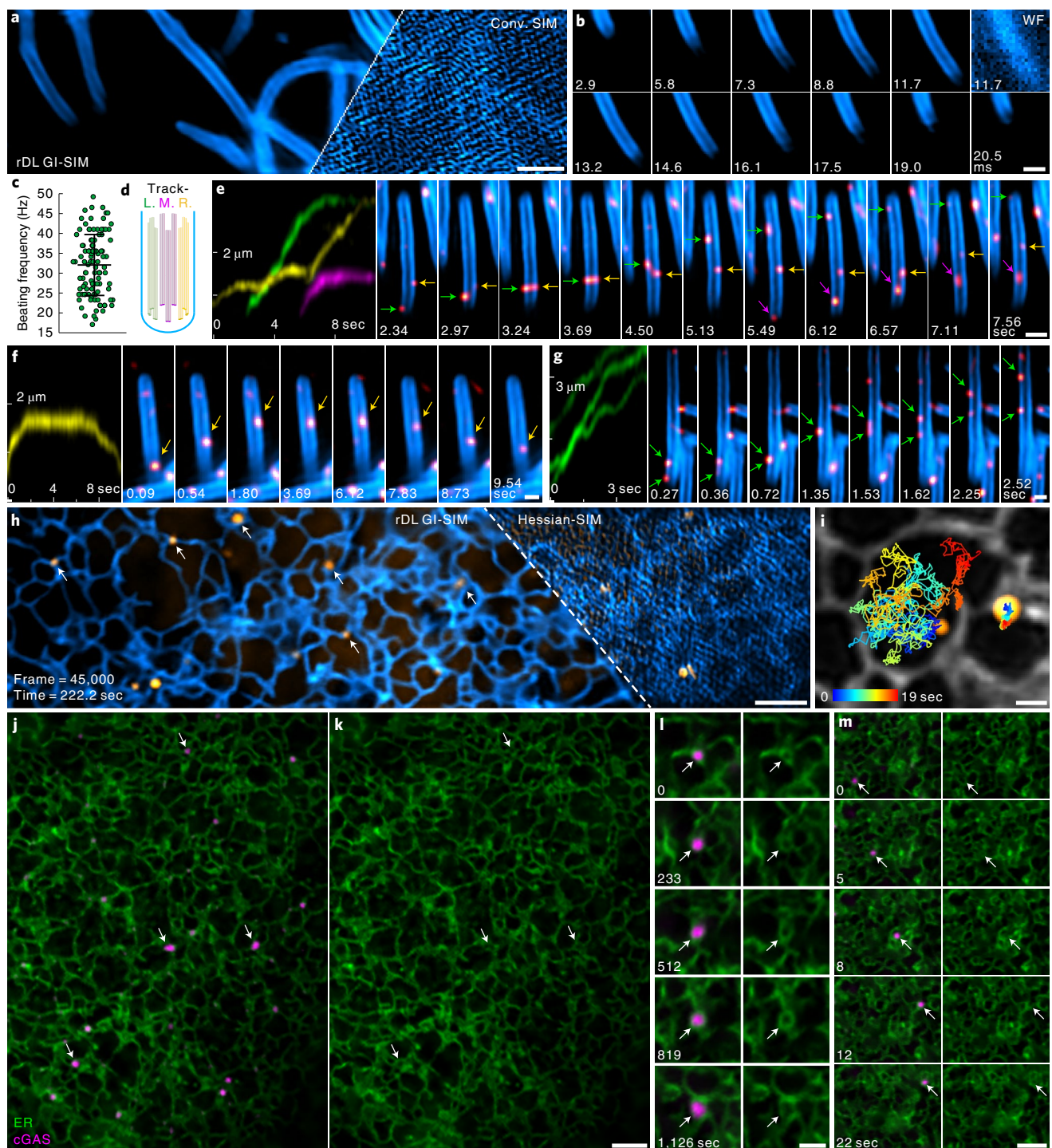


Fig. 3 | rDL SIM imaging of motile cilia and organelle interactions. **a**, Single timepoint from a movie of beating motile cilia in mEPCs via rDL GI-SIM imaging at 684 fps (left), at which conventional GI-SIM image is overwhelmed by reconstruction artifacts (right) (Supplementary Video 5). **b**, Time-lapse images showing that a cilium undergoes stereotypical back-and-forth motion over a period of ~24 ms. At 11.7 ms, the hollow, tube-like structure is well-resolved in the rDL GI-SIM image (left) but cannot be observed in the diffraction-limited wide-field (WF) image (right). **c**, Quantification of ciliary beating frequency. Mean $\pm$ s.d. derived from $n = 107$ cilia is shown. **d**, Schematic of the MT tracks in the ciliary axoneme. **e–g**, Kymographs and time-lapse images of the processive dynamics of IFT trains moving along the left (green), middle (magenta) and right (yellow) tracks in the projected footprint of the ciliary axoneme. **h**, Two-color rDL GI-SIM image of ER (blue) and PO (yellow) at the 45,000th timepoint from a long-term movie recording the interaction dynamics of ER–PO (indicated by arrows) in a live COS-7 cell, wherein Hessian-SIM cannot effectively suppress

the reconstruction artifacts at the low SNR condition (right) (Supplementary Video 8). **i**, The PO in association with ER exhibits a confined motion trajectory (right), whereas the PO that has no stable association with ER exhibits a diffusive motion trajectory (left). **j**, Two-color rDL GI-SIM image reveals that most of the cGAS–DNA condensates (magenta) are tightly corralled by the ER tubules (green) (Supplementary Videos 9 and 10) after transfection of 45-bp double-stranded DNA into HEK293T cells stably expressing mEmerald–KDEL and cGAS–Halo. **k**, The ER channel shows only the ER polygons surrounding the corresponding cGAS–DNA condensates, as indicated by the arrows. **l**, Time-lapse images illustrating that, as a cGAS–DNA condensate grew, its surrounding ER polygon became larger accordingly. **m**, Time-lapse images illustrating that, when a cGAS–DNA condensate undergoes directional movement, the surrounding ER tubules are deformed correspondingly, implying tight contact between the ER and cGAS–DNA condensates (Supplementary Video 11). Scale bars, 1 μ m (**a**), 0.5 μ m (**b,e**), 0.3 μ m (**f,g**), 2 μ m (**h,k,m**), 0.5 μ m (**i**) and 1 μ m (**l**).

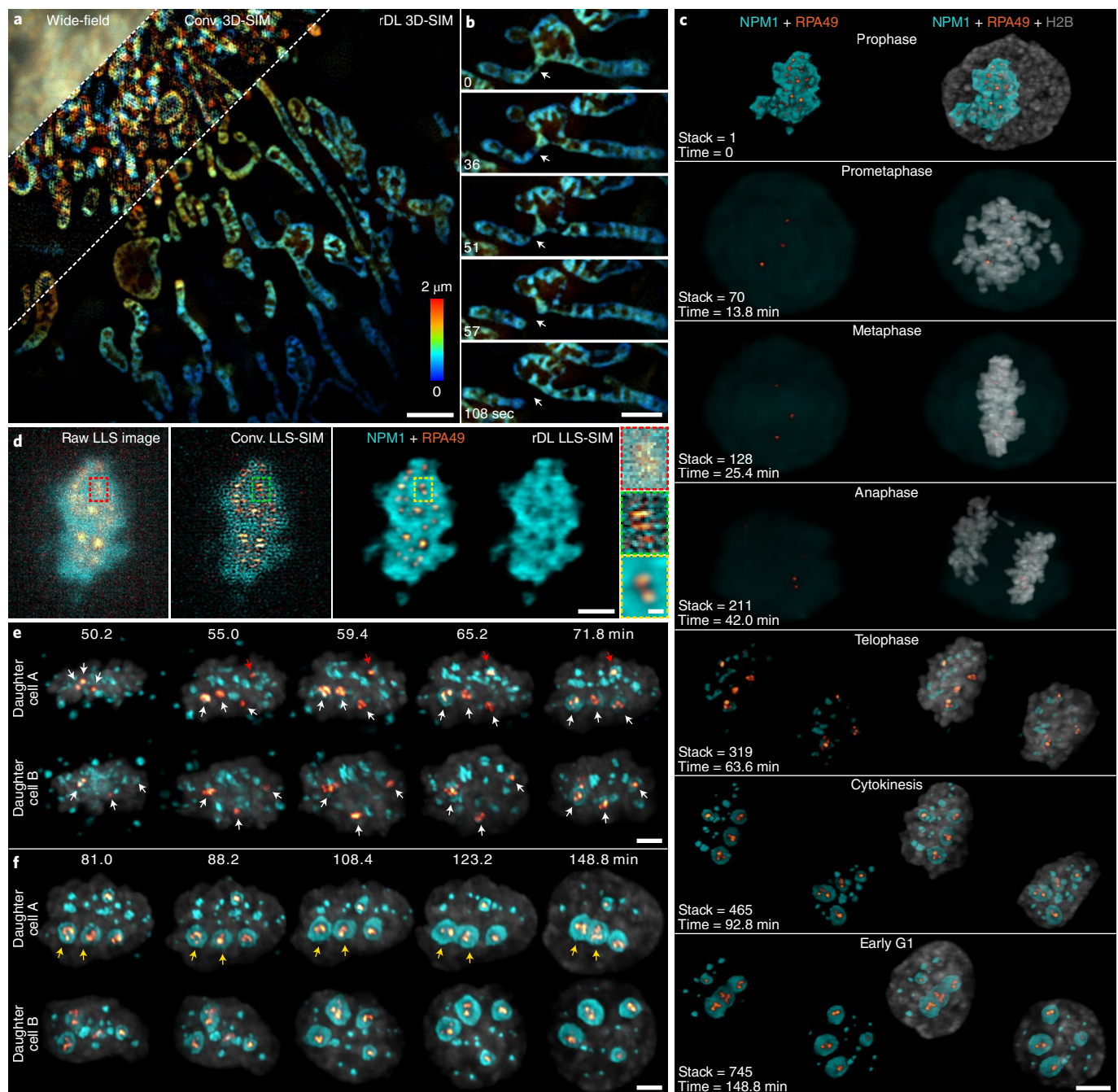


Fig. 4 | Volumetric long-term SR imaging via rDL 3D-SIM and rDL LLS-SIM. a, Representative rDL 3D-SIM image of a live U2OS cell expressing mEmerald-PHB2, color-coded for distance from the substrate. **b**, Time-lapse rDL 3D-SIM images of cristae remodeling during mitochondrial fission (Supplementary Video 12). **c**, Time-lapse rDL LLS-SIM three-color images of mEmerald-NPM1, mCherry-H2B and RPA49-Halo, showing the disassemble and reassemble process of nucleoli during mitosis (Supplementary Video 13). **d**, rDL LLS-SIM image (right) resolves the nested structure of the nucleolus, whereas the conventional LLS-SIM image

(left) is riddled with artifacts at the low SNR condition. The boxed regions in the three images are magnified on the right. **e**, Time-lapse images showing that, in telophase, new RPA49-foci start to form (red arrow), and the existing RPA49-foci gradually enlarge (white arrows) as NPM1 condenses around them. **f**, Time-lapse images showing that two multi-layered condensates of NPM1 and RPA49 coalesced into large nucleoli (indicated by yellow arrows). Scale bar, 3 μm (**a,d,e,f**), 2 μm (**b**), 5 μm (**c**) and 0.5 μm (zoom-in region of **d**).

13). Additionally, we observed that the large RPA49-foci in the FC layer were occasionally partitioned into several independent foci during telophase (Supplementary Fig. 6), which is an unusual behavior for liquid condensates. Moreover, the NPM1-foci outside the nuclear envelope were disassembled in the cytoplasm rather than assembled into larger condensations, presumably due to the biochemical differences between the nucleoplasm and cytosol. These results reveal the spatiotemporally coordinated multi-layer condensation dynamics

of nucleoli during mitosis, demonstrating that rDL LLS-SIM can provide volumetric imaging of light-sensitive bioprocesses with minimal invasiveness.

Temporally interleaved self-supervised rDL LLSM for high-speed volumetric imaging

Although pairs of noisy and GT images can effectively train a denoising model, acquiring high-quality GT data is difficult and sometimes

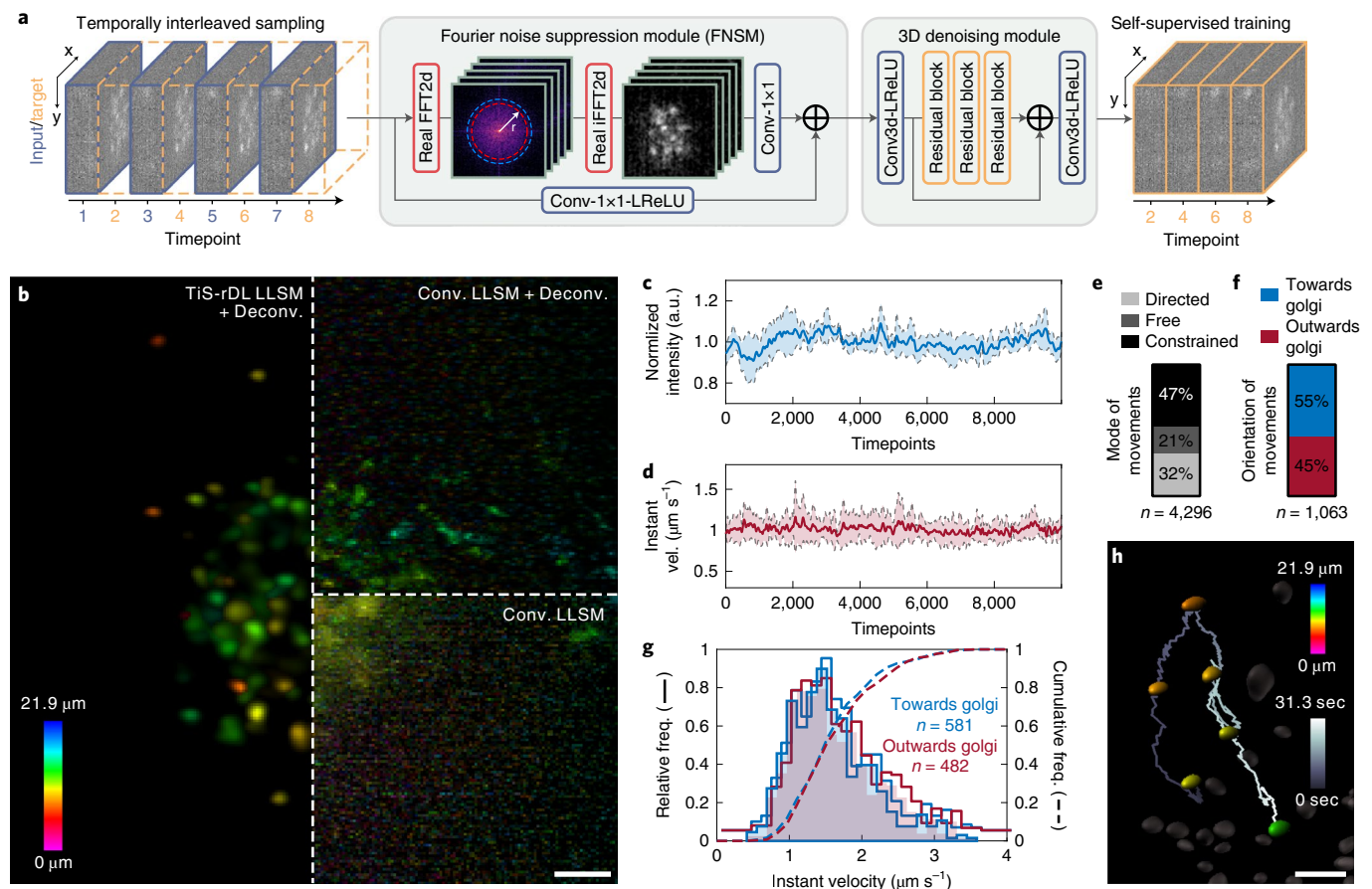


Fig. 5 | Volumetric, high-speed TiS-rDL LLSM imaging of SiT-containing transport intermediates. a, Schematic of the TiS-rDL denoising network. **b**, Representative TiS-rDL LLSM image of a live R1 cell expressing mEmerald-SiT, a Golgi enzyme, color-coded for distance from the substrate, at a speed of 10 volumes per second for 10,000 whole-cell images. However, at such low SNR, a conventional LLSM image (bottom right) even after deconvolution (top right) hardly conveys usable information. **c,d**, Time courses of the normalized total fluorescence (**c**) and the instantaneous velocity of SiT transport intermediates (**d**) over the imaging duration of 10,000 timepoints. Both plots are relatively stable for the entire imaging duration, suggesting that the photobleaching

and phototoxicity are negligible in rDL LLSM. The shaded area refers to the standard deviation ($n = 4$ cells). **e**, The proportion of SiT transport intermediates undergoing directed movement, free diffusion and constrained diffusion. **f**, The proportion of SiT transport intermediates having directed movement trafficking towards and outwards from the Golgi complex. **g**, Quantification of the average velocity of SiT transport intermediates moving towards and outwards from the Golgi complex. **h**, Trajectory of SiT transport intermediates undergoing processive bidirectional motion. The same structure at six timepoints is temporally color-coded and projected along the trajectory that is coded in grayscale. Scale bar, 2 μm (**b**) and 1.5 μm (**h**). a.u., arbitrary units.

impractical for dynamic biological specimens. To extend the applicability of the rDL scheme, we improved the sampling rate of the LLSM to 1,000 z-slices per second (Methods), ensuring the temporal continuity of noisy raw image series. Inspired by the Noise2Noise configuration²⁵, we exploited the duplicity of information in consecutive noisy raw images and the optical transfer function (OTF) model of LLSM to construct the temporally interleaved self-supervised rDL (TiS-rDL) denoising network (Fig. 5a and Supplementary Notes 3b–d). TiS-rDL produced denoised results similar to those obtained with GT supervision (Fig. 5b and Extended Data Fig. 7a–c). We first used TiS-rDL LLSM to investigate the ultradynamics of Golgi transport intermediates containing sialyl-transferase (SiT). At the high volumetric imaging speed of 10 whole-cell volumes per second, TiS-rDL LLSM produced high-quality images of the collective behaviors of transport SiT vesicles for 10,000 volumes (Supplementary Video 14). During the long observation period, the normalized total fluorescence and averaged instantaneous velocity of the SiT vesicles remained stable (Fig. 5c,d; $n = 4$ cells), suggesting that TiS-rDL LLSM caused negligible photobleaching and phototoxicity. The high-quality TiS-rDL LLSM images enabled us to track and analyze the movement of SiT vesicles (Methods). We found that ~47%, ~32% and ~21% of the SiT vesicles underwent constrained diffusion, directed

movement and free diffusion, respectively (Fig. 5e). Half of the SiT vesicles that exhibited directed movement moved towards Golgi cisternae, whereas the other half moved away (Fig. 5f) at similar speeds (Fig. 5g). In addition, a small fraction of the SiT vesicles underwent bidirectional processive motion (Fig. 5h). These results reveal the heterogeneous yet dynamic behaviors of Golgi-derived vesicles.

Visualizing organelle inheritance in mitosis via SiS-rDL LLSM

The TiS-rDL scheme is suitable for denoising the time-lapse data of temporal continuity, but high-speed live cell imaging is not applicable to light-sensitive bioprocesses. For instance, because of the vulnerability of mitotic cells, two-color confocal imaging is usually deployed in a single x–y slice with an interval of several minutes²⁶; conventional LLSM imaging waits for 5–10 minutes for mitotic cells to recover from light illumination every 100 two-color volumes²⁷. To further extend the applicability of the rDL scheme, we devised the spatially interleaved self-supervised rDL (SiS-rDL) scheme that used the spatial continuity of Nyquist sampling in the z-axis of each volumetric dataset and gap-amending regularization to construct a self-supervised denoising network (Fig. 6a,b, Extended Data Fig. 7d–g and Supplementary Note 3e). Despite extensive research on the mechanisms regulating genome

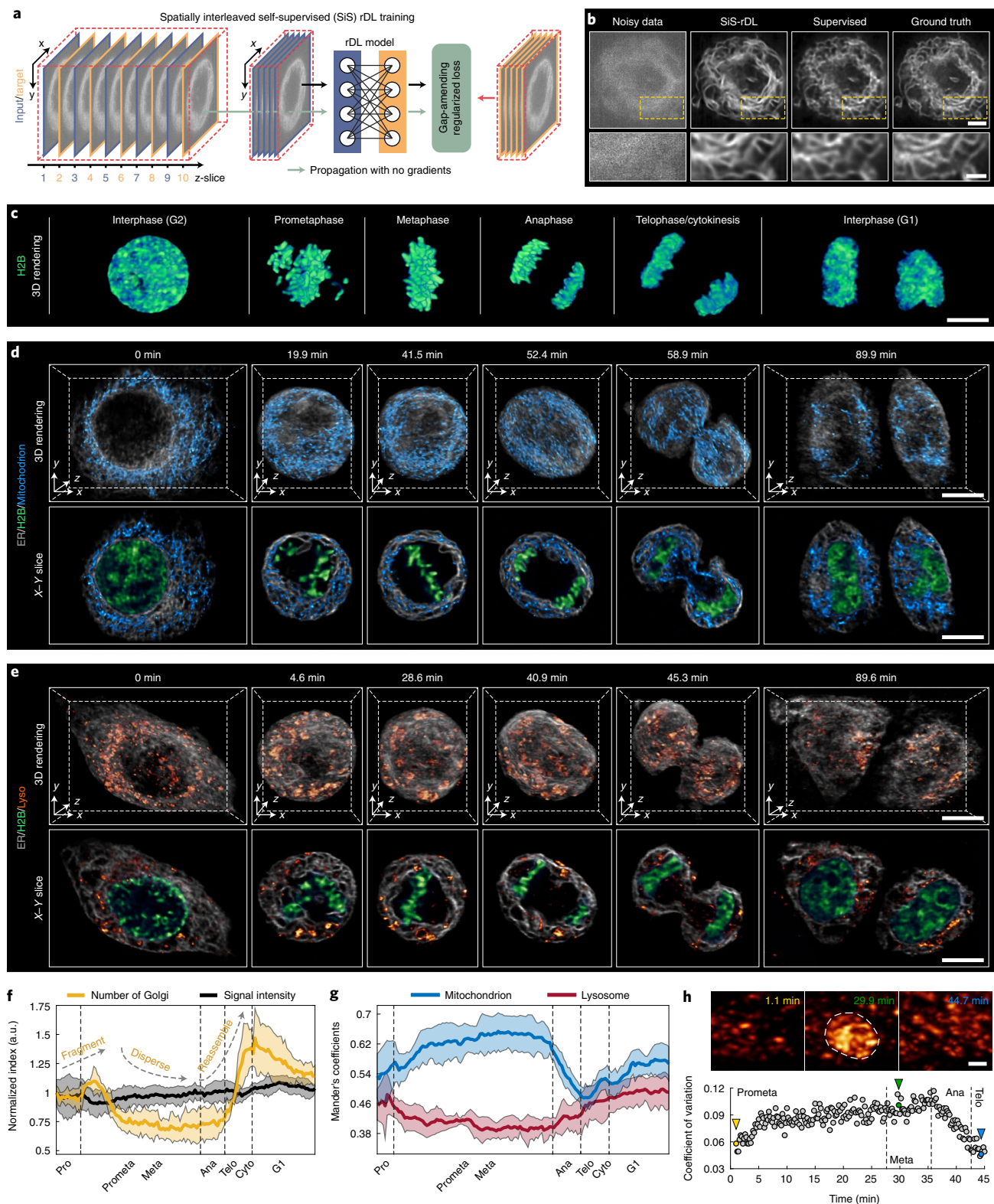


Fig. 6 | Inheritance and interactions of membrane organelles in mitosis via SiS-rDL LLSM. a, Schematic of the SiS-rDL denoising network. **b**, Representative LLSM images of ER denoised by SiS-rDL and traditional supervised learning models. **c**, 3D rendering images of H2B-labeled chromosomes mark the different stages of mitosis. **d, e**, Three-color rDL LLSM images of ER, H2B and mitochondria (**d**) or Lyso (**e**) showing the remodeling processes of three organelles as well as their interaction dynamics with ER during mitosis (Supplementary Videos 16 and 17). The upper row in **d, e** presents the 3D rendering images of the whole-cell volume; the bottom row presents the corresponding lateral slice images

from the middle of the whole-cell volume. **f**, Quantification of the population of SiT-containing puncta (yellow) and the normalized total fluorescence (black) over the time course of mitosis. **g**, Plots of Mander's coefficients reflecting the interactions between ER and mitochondria (blue) or Lyso (red) over the time course of mitosis. **h**, Plot of the coefficient of variation reflecting the kinetics of Lyso aggregation over the time course of mitosis. The upper row shows representative morphological changes in regional Lyso from prometaphase to telophase. Scale bar, 5 μ m (**b**), 10 μ m (**c–e**) and 2 μ m (**h**). a.u., arbitrary units.

partitioning, few studies have investigated how various organelles properly allocate into each daughter cell. The SiS-rDL LLSM allowed us to record the spatiotemporal relationship of chromosomes, ER and other organelles in HeLa stable cell lines (Methods) for ~1,000 three-color whole-cell volumes at 6-second intervals. Consistent with previous studies²⁸, we observed that prometaphase ER transitioned from a reticular network to an extended array of sheets, and the sheets remained until the end of telophase (Fig. 6c–e, gray), and the Golgi apparatus disassembled and reassembled during mitosis without photobleaching (Fig. 6f, Extended Data Fig. 8 and Supplementary Video 15).

Moreover, the increased spatiotemporal resolution of SiS-rDL LLSM enabled us to examine the dynamic interactions between ER sheets and other organelles (for example, mitochondria (Mito; Fig. 6d) and lysosomes (Lyso; Fig. 6e)) during mitosis. We found that Mito-ER contacts were enhanced at the beginning of prometaphase, coinciding with ER sheets formation. The enhanced Mito-ER contacts were maintained until metaphase, and then, as the chromosomes partitioned, the Mito-ER contacts decreased to a relatively low level. When the ER sheets were transformed back into the reticular network during cytokinesis, Mito-ER contacts were progressively strengthened, eventually reaching the level of G1 phase (Fig. 6g, blue curve, and Supplementary Video 16). Additionally, we observed that both mitochondrial fission and fusion were retained during metaphase even at low activity levels (Supplementary Fig. 7).

Different from the mitochondria and Golgi apparatus, the cohorts of neighboring Lyso started to aggregate into large entities dispersed throughout the cytoplasm in prometaphase. Although the aggregation of Lyso at the onset of mitosis resulted in fewer Lyso-ER contacts (Fig. 6g, red curve), almost all Lyso aggregates were still closely associated with ER sheets (Fig. 6e and Supplementary Fig. 8). This might help stabilize the Lyso in each half of the dividing cell as the chromosomes align in the equatorial plate (Supplementary Video 17). During anaphase, the aggregated Lyso gradually disassembled into individual vesicles as the chromosomes moved to opposite sides of the cell (Fig. 6e,h). The Lyso that disengaged from the aggregates rapidly resumed their interactions with the ER, and the Lyso-ER contact returned to the interphase level (Fig. 6g, red curve). These observations with SiS-rDL LLSM reveal the diverse strategies that cells use to segregate organelles during mitosis, with the ER likely playing a unifying role.

Discussion

By synergizing the advantages of both optical front-end and algorithmic back-end methodologies, rDL SIM and rDL LLSM substantially advance SR live cell 2D/3D imaging. We emphasize the following advantages of rDL methods. (1) Compared to the conventional DLSR algorithms that simply introduce the physical constraints into the loss function, rDL methods first incorporate the deterministic physical model of a specific microscopy into network training and inference, largely reducing the ill-posedness of the final SR image. (2) rDL methods are applied to denoise the raw SIM images, in which high-frequency information beyond the diffraction limit is downmodulated as low-frequency Moiré fringes. This strategy eliminates the spectral bias effect of directly inferring the SR images but also ensures high-quality SR image reconstruction. (3) Existing DLSR methods suffer from strong model uncertainty, which depends on the representativity of the training dataset and the network architecture²⁹, reducing the quantifiability and fidelity of SR images crucial for scientific research. We found that the physical-model-rationalized three-branch architecture reduced the model uncertainty, providing physically feasible inferences that can be more readily generalized into the regimes uncovered by training data. (4) The rDL concept is widely compatible and has broad functionality. In particular, SiS-rDL, which uses the inherent spatial continuity necessitated by Nyquist sampling, is widely compatible with other 3D imaging techniques. The denoised results of SiS-rDL are as good as, if not better than, those of GT-supervised methods. Moreover, other

prior knowledge, such as the OTF's shape and coverage of specific microscopy, and the sparsity or continuity of biological structures, can be integrated into the rDL scheme (Supplementary Note 3). These characteristics underlie the superior performance of the rDL methods, greatly expanding the applicable range of SR live imaging.

Further improvements in rDL methods are expected. The combination of adaptive optics^{30,31} with the rDL LLS-SIM system should enable minimally invasive high-speed five-dimensional (5D) (x–y–z–time–color) SR imaging in optically challenging specimens. Moreover, because rDL methods have lower fluorescence demands for SR images, they provide additional bandwidth to expand the color dimension, allowing more proteins to be imaged simultaneously through spectral unmixing³² or combinatorial labeling approaches³³.

In conclusion, the rDL methodologies described and demonstrated here satisfy an unmet need for minimally invasive 2D/3D imaging of intracellular dynamics at ultra-high spatial and temporal resolution, high fidelity and quantifiability for long durations. The implementation and improvement of the rDL methods show great promise for shedding light on diverse biological phenomena.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41587-022-01471-3>.

References

- Moore, A. S. et al. Actin cables and comet tails organize mitochondrial networks in mitosis. *Nature* **591**, 659–664 (2021).
- Liao, Y.-C. et al. RNA granules hitchhike on lysosomes for long-distance transport, using annexin A11 as a molecular tether. *Cell* **179**, 147–164 (2019).
- Phillips, M. J. & Voeltz, G. K. Structure and function of ER membrane contact sites with other organelles. *Nat. Rev. Mol. Cell Biol.* **17**, 69–82 (2016).
- Prinz, W. A., Toulmay, A. & Balla, T. The functional universe of membrane contact sites. *Nat. Rev. Mol. Cell Biol.* **21**, 7–24 (2020).
- Schermelleh, L. et al. Super-resolution microscopy demystified. *Nat. Cell Biol.* **21**, 72–84 (2019).
- Wu, Y. & Shroff, H. Faster, sharper, and deeper: structured illumination microscopy for biological imaging. *Nat. Methods* **15**, 1011–1019 (2018).
- Huang, X. et al. Fast, long-term, super-resolution imaging with Hessian structured illumination microscopy. *Nat. Biotechnol.* **36**, 451–459 (2018).
- Zhao, W. et al. Sparse deconvolution improves the resolution of live-cell super-resolution fluorescence microscopy. *Nat. Biotechnol.* **40**, 606–617 (2022).
- de Haan, K., Rivenson, Y., Wu, Y. & Ozcan, A. Deep-learning-based image reconstruction and enhancement in optical microscopy. *Proc. IEEE* **108**, 30–50 (2019).
- Belthangady, C. & Royer, L. A. Applications, promises, and pitfalls of deep learning for fluorescence image reconstruction. *Nat. Methods* **16**, 1215–1225 (2019).
- Rahaman, N. et al. On the spectral bias of neural networks. *Proc. of the 36th International Conference on Machine Learning*. 5301–5310 (PMLR, 2019).
- Guo, Y. et al. Visualizing intracellular organelle and cytoskeletal interactions at nanoscale resolution on millisecond timescales. *Cell* **175**, 1430–1442 (2018).
- Shah, Z. H. et al. Deep-learning based denoising and reconstruction of super-resolution structured illumination microscopy images. *Photonics Res.* **9**, B168–B181 (2021).

14. Dabov, K., Foi, A., Katkovnik, V. & Egiazarian, K. Image denoising by sparse 3-D transform-domain collaborative filtering. *IEEE Trans. Image Process.* **16**, 2080–2095 (2007).
 15. Weigert, M. et al. Content-aware image restoration: pushing the limits of fluorescence microscopy. *Nat. Methods* **15**, 1090–1097 (2018).
 16. Qiao, C. et al. Evaluation and development of deep neural networks for image super-resolution in optical microscopy. *Nat. Methods* **18**, 194–202 (2021).
 17. Kiepas, A., Voorand, E., Mubaid, F., Siegel, P. M. & Brown, C. M. Optimizing live-cell fluorescence imaging conditions to minimize phototoxicity. *J. Cell Sci.* **133**, jcs242834 (2020).
 18. Li, D. et al. Extended-resolution structured illumination imaging of endocytic and cytoskeletal dynamics. *Science* **349**, aab3500 (2015).
 19. Ma, Y., Li, D., Smith, Z. J., Li, D. & Chu, K. Structured illumination microscopy with interleaved reconstruction (SIMILR). *J. Biophotonics* **11**, e201700090 (2018).
 20. Oltean, A., Schaffer, A. J., Bayly, P. V. & Brody, S. L. Quantifying ciliary dynamics during assembly reveals stepwise waveform maturation in airway cells. *Am. J. Respir. Cell Mol. Biol.* **59**, 511–522 (2018).
 21. Jing, J. C., Chen, J. J., Chou, L., Wong, B. J. F. & Chen, Z. Visualization and detection of ciliary beating pattern and frequency in the upper airway using phase resolved doppler optical coherence tomography. *Sci Rep.* **7**, 8522 (2017).
 22. Chien, A. et al. Dynamics of the IFT machinery at the ciliary tip. *eLife* **6**, e28606 (2017).
 23. Ott, C. & Lippincott-Schwartz, J. Visualization of live primary cilia dynamics using fluorescence microscopy. *Curr. Protoc. Cell Biol.* **Chapter 4**, Unit 4.26 (2012).
 24. Du, M. & Chen, Z. J. DNA-induced liquid phase condensation of cGAS activates innate immune signaling. *Science* **361**, 704–709 (2018).
 25. Lehtinen, J. et al. Noise2noise: learning image restoration without clean data. Preprint at <https://arxiv.org/abs/1803.04189> (2018).
 26. Sengupta, P. et al. ER trapping reveals Golgi enzymes continually revisit the ER through a recycling pathway that controls Golgi organization. *Proc. Natl Acad. Sci. USA* **112**, E6752–E6761 (2015).
 27. Chen, B. C. et al. Lattice light-sheet microscopy: imaging molecules to embryos at high spatiotemporal resolution. *Science* **346**, 1257998 (2014).
 28. Lu, L., Ladinsky, M. S. & Kirchhausen, T. Cisternal organization of the endoplasmic reticulum during mitosis. *Mol. Biol. Cell* **20**, 3471–3480 (2009).
 29. Kendall, A. & Gal, Y. What uncertainties do we need in Bayesian deep learning for computer vision? in *Advances in Neural Information Processing Systems* 30. <https://papers.nips.cc/paper/2017/hash/2650d6089a6d640c5e85b2b88265dc2b-Abstract.html> (NIPS, 2017).
 30. Liu, T.-L. et al. Observing the cell in its native state: imaging subcellular dynamics in multicellular organisms. *Science* **360**, eaaq1392 (2018).
 31. Ji, N. Adaptive optical fluorescence microscopy. *Nat. Methods* **14**, 374–380 (2017).
 32. Valm, A. M. et al. Applying systems-level spectral imaging and analysis to reveal the organelle interactome. *Nature* **546**, 162–167 (2017).
 33. Lubeck, E. & Cai, L. Single-cell systems biology by super-resolution imaging and combinatorial labeling. *Nat. Methods* **9**, 743–748 (2012).
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Methods

Multi-SIM system

The Multi-SIM system was developed from our previous GI-SIM setup¹². In brief, the Multi-SIM system was built based on an invented fluorescence microscope (Ti2E, Nikon). Three laser beams of 488 nm (Genesis-MX-SLM, Coherent), 560 nm (2RU-VFL-P-500-560, MPB Communications) and 640 nm (LBX-640-500, Oxxius) were collinearly combined and then passed through an acousto-optic tunable filter (AOTF, AOTFnc-400.650, Quanta Tech), which selects a desired laser wavelength coupled onto an afterwards light path and flexibly controls its power and exposure time. The selected laser light was expanded and then sent into an illumination pattern generator, which is composed of a ferroelectric spatial light modulator (SLM, QXGA-3DM, Forth Dimension Display), a polarization beam splitter and an achromatic half-wave plate. The grating patterns of specific imaging mode—that is, 3-phase × 3-orientation at 1.43 NA for TIRF-SIM; 3-phase × 3-orientation at 1.38 NA for GI-SIM; and 5-phase × 3-orientation at 1.2 NA for 3D-SIM—was displayed sequentially onto the SLM and synchronized with the programmed ‘ON’ time of AOTF. Light diffracted by the grating pattern was passed through a polarization rotator consisting of a liquid crystal cell (Meadowlark, LRC-200) and quarter-wave plate, which rotated the linear polarization of diffracted light according to the orientation of ±1 diffraction orders, so as to maintain the s-polarization necessary to maximize the pattern contrast for all pattern orientations. The diffraction orders, except for ±1 orders for TIRF-SIM and GI-SIM and 0 and ±1 orders for 3D-SIM, were filtered out by a spatial mask and then relayed onto the back focal plane of the objectives (1.49 NA, Nikon). The raw SIM images excited by different illumination patterns were sequentially collected by the same objective and then separated by a dichroic beam splitter (Chroma, ZT405/488/560/647tpc), finally relayed onto an sCMOS camera (Hamamatsu, Orca Flash 4.0 version 3). The nine raw images of TIRF-SIM or GI-SIM and 15 × N_{z-slice} raw images of 3D-SIM were denoised by the rDL network first and then reconstructed using the previous algorithm^{18,34,35}.

LLS-SIM system

The home-built LLS-SIM system was developed from the original design²⁷. Similarly to the laser combinator and pattern generator used in the Multi-SIM system, three lasers of 488 nm, 560 nm and 640 nm (MPB Communications) were controlled by an AOTF and then illuminate the LLS pattern displayed on the SLM. The LLS-SIM patterns of 3 phases were displayed sequentially and synchronized with the exposure time controlled by AOTF. The diffracted light was filtered by an annular mask equivalent to 0.5 outer NA and 0.375 inner NA for the excitation objective (Special Optics). Subsequently, the filtered excitation light passed through a pair of galvo mirrors (x-galvo and z-galvo) (Cambridge Technology, 6210H). In LLS-SIM mode, the LLS patterns of 3-phase were sequentially displayed onto the SLM and synchronized with the programmed ‘ON’ time of AOTF and then scanned by the z-galvo objective in a step size of 0.2 μm or by sample piezo in a step size of 0.39 μm to acquire the volumetric raw images. To achieve high acquisition rate in LLSM mode, a fixed illumination pattern was quickly dithered by x-galvo and then scanned by using sample piezo in a step size of 0.39 μm rather than using z-galvo objective scan mode, because the sample holder has lighter weight than the objective, which allows the sample piezo to run in high speed with small hysteresis. Moreover, instead of driving the sample piezo with the ramp wave, we used the triangle wave to minimize the flyback time when reversing the scanning direction of the piezo stage. All of these measures allow LLSM to operate at 1,000 z-slices per second acquisition rate and implement the TiS-rDL method in 3D whole-cell imaging regime. Live cell specimens were maintained in a custom-designed microscope incubator (Okolab, H301-LLSM-SS316) to maintain the physiology condition of 37 °C and 5% CO₂ during imaging. Fluorescence emission was collected by the detection objective (Nikon, CFI Apo LWD 25XW, 1.1 NA) and captured by an sCMOS camera (Hamamatsu, Orca Fusion). The raw images of

LLS-SIM or LLSM were denoised by the rDL network first and then deconvolved using an iterative Richardson–Lucy algorithm²⁷. The PSFs for deconvolution were measured using 100-nm-diameter beads for each excitation wavelength.

Network architecture of the rDL denoising module

Fed with the noisy raw SIM images and the pattern-modulated images, the rDL denoising module is aimed to extract hierarchical features from these input data and rationally reassemble them into clear raw SIM images. The network architecture of the rDL denoising module consists of three branches—the PFE branch, the MPE branch and the FCD branch—each constructed by similar fundamental modules based on residual learning^{36,37} and the squeeze-and-extract (SE) mechanism³⁸ (or called the channel attention (CA) mechanism)³⁹.

Specifically, the PFE branch begins with a convolutional layer activated by a leaky ReLU (LReLU) activation function, which is formulated as

$$LReLU(x, \alpha) = \max(0, x) - \alpha \max(0, -x) \quad (1)$$

where α is the leaky factor set as 0.1 in our experiments. Then, the output feature maps are fed into m_{PFE} sequential residual groups (RGs), and a long skip connection is introduced to stabilize the training. Each RG is comprised of n channel attention block (CAB) with a skip connection that directly delivers its input to the end:

$$RB(x) = x + \underbrace{CAB(CAB(\dots CAB(x)))}_{n \times CAB} \quad (2)$$

Each CAB consists of two Conv-LReLU modules, a CA module that implements the CA mechanism and a short skip connection:

$$SCAB(x) = x + y \times LReLU(C_{up}(\delta(C_{down}(Pooling(y))))), \quad (3)$$

$$y = LReLU(Conv(LReLU(Conv(x))), \quad (4)$$

where $Pooling(\cdot)$ is the global pooling layer to calculate one descriptor for each feature map; C_{up} and C_{down} are the weight sets of two convolutional layers used to adaptively recalculate the global descriptors; and $\delta(\cdot)$ denotes the sigmoid activation function. Finally, the hierarchical extracted features from noisy raw SIM images are output by another Conv-LReLU module.

The architectures of the MPE branch and the FCD branch are similar to the PFE branch with the differences mainly lying in the network depth. For the MPE branch, we adopt a smaller number of RBs on account that the major purpose of the MPE branch is to extract the pattern information from input pattern-modulated images, which is relatively a straightforward task compared to image restoration. For the FCD branch, we employ an additional Conv-LReLU module with an expanded channel number, and another CA module before the final Conv-LReLU, to further enrich the abundant features and make the utmost use of them. Typically, we set $m_{PFE} = 5$, $m_{MPE} = 2$, $m_{FCD} = 5$ for rDL TIRF-SIM and GI-SIM and $m_{PFE} = 2$, $m_{MPE} = 1$, $m_{FCD} = 2$ for rDL LLS-SIM and rDL 3D-SIM (Supplementary Table 1). The overall architecture of the rDL denoising module is illustrated in Supplementary Fig. 2.

The three branches of the rDL denoising module are trained synchronously as an integral with the objective function defined as:

$$L(\hat{Y}, Y) = MSE(\hat{Y}, Y) + \lambda(1 - SSIM(\hat{Y}, Y)), \quad (5)$$

where $\hat{Y}$ and Y denote the predicted raw images and the clear raw GT images, respectively, and λ is the weighting scalar to balance the contribution of mean squared error (MSE) loss and structural similarity index measure (SSIM) loss, which is set to 0.1 empirically in our experiments.

Pattern-modulated image generation

To generate the pattern-modulated images for the MPE branch, the amplitude of the wave vector $\hat{\mathbf{p}}$ and the initial phase describing the illumination pattern should be determined for each pattern orientation from the raw SIM images. The parameter fitting was performed using the same algorithm as the original version described in ref. ³⁴. In brief, it is conducted as follows. (1) Average the raw SIM images acquired with the same illumination pattern orientation and phase from 3 to 20 (according to imaging conditions and the type of specimens) successive timepoints to improve the signal-to-noise and then separate different harmonic components by a linear matrix. (2) Estimate the preliminary wave vector $\hat{\mathbf{p}}$ by localizing the maximum of the cross-correlation function between the overlapping region of adjacent harmonic components. (3) Fine-tune the wave vector to maximize the modulation amplitude of the harmonic component via linear search optimization. (4) Calculate the initial phase φ_0 of the wave vector $\hat{\mathbf{p}}$ through a complex linear regression.

The pattern-modulated images were then generated following the physical model of the raw image acquisition process, which can be formulated as:

$$D(\mathbf{r}) = P(\mathbf{r}) \otimes [I(\mathbf{r}) \cdot S(\mathbf{r})] \quad (6)$$

where $S(\mathbf{r})$ denotes the fluorescence distribution of the sample, which is substituted by the deep learning SR image output from the pre-trained SR image inference network, DFCAN; $I(\mathbf{r})$ denotes the excitation pattern; and $P(\mathbf{r})$ is the system PSF.

In 2D-SIM modes (that is, TIRF-SIM and GI-SIM), the illumination pattern $I(\mathbf{r})$ is simulated by superposing two symmetrical plane waves described by $\hat{\mathbf{p}}$ and $-\hat{\mathbf{p}}$ and calculating the intensity of the resulted electronic field as follows:

$$E_1(\mathbf{r}) = e^{i(\pi\hat{\mathbf{p}} \cdot \mathbf{r} + \varphi_0 + \delta\varphi)}, \quad (7)$$

$$E_{-1}(\mathbf{r}) = e^{-i\pi\hat{\mathbf{p}} \cdot \mathbf{r}}, \quad (8)$$

$$I(\mathbf{r}) = |E_{-1}(\mathbf{r}) + E_1(\mathbf{r})|^2. \quad (9)$$

where $\delta\varphi$ is the equally spaced phase shift. Then, the pattern-modulated 2D-SIM raw images can be generated in Fourier space by calculating the dot product of the experimentally measured OTF (that is, the Fourier transform of PSF) with the Fourier transform of the pattern-modulated fluorescence signals as:

$$D_{2D-SIM}(\mathbf{r}) = \mathcal{F}^{-1}\{O(\mathbf{k}) \cdot \mathcal{F}\{I(\mathbf{r}) \cdot S(\mathbf{r})\}\} \quad (10)$$

where $\mathcal{F}$ and $\mathcal{F}^{-1}$ denote fast Fourier transform and inverse fast Fourier transform, respectively.

In 3D-SIM modes, $I(\mathbf{r})$ is simulated by calculating the interference intensity of three plan waves described by $-\hat{\mathbf{p}}$, 0 and $\hat{\mathbf{p}}$, which can be represented as:

$$E_0(\mathbf{r}) = e^{i\frac{2\pi n}{\lambda_{exc}} r_z}, \quad (11)$$

$$E_1(\mathbf{r}) = e^{i\left(2\pi\hat{\mathbf{p}} \cdot \mathbf{r}_{xy} + 2\pi\sqrt{\left(\frac{n}{\lambda_{exc}}\right)^2 - |\hat{\mathbf{p}}|^2} r_z + \frac{1}{2}\varphi_0 + \frac{1}{2}\delta\varphi\right)}, \quad (12)$$

$$E_{-1}(\mathbf{r}) = e^{i\left(-2\pi\hat{\mathbf{p}} \cdot \mathbf{r}_{xy} + 2\pi\sqrt{\left(\frac{n}{\lambda_{exc}}\right)^2 - |\hat{\mathbf{p}}|^2} r_z - \frac{1}{2}\varphi_0 - \frac{1}{2}\delta\varphi\right)}, \quad (13)$$

$$I(\mathbf{r}) = |E_{-1}(\mathbf{r}) + E_0(\mathbf{r}) + E_1(\mathbf{r})|^2. \quad (14)$$

where n denotes the refractive index of the immersion medium, and λ_{exc} denotes the excitation wave length. In the acquisition procedure

of 3D-SIM, the sample stage moves axially, whereas the objective and excitation pattern are spatially fixed. Following this scheme, the pattern-modulated raw 3D-SIM image volumes are generated in a slice-by-slice manner with the i^{th} z-slice calculated by:

$$D_{3D-SIM}(\mathbf{r}_{xy}, z_i) = \left[\mathcal{F}^{-1}\left\{O(\mathbf{k}) \cdot \mathcal{F}\left\{I(\mathbf{r}) \cdot S\left(\mathbf{r}_{xy}, z + \frac{N_z}{2} - z_i\right)\right\}\right\}\right]_{mid}, \quad (15)$$

where $[\cdot]_{mid}$ represents the median z-slice of the image volume, and N_z is the total number of z-slices.

In LLS-SIM modes, each slice of the acquired image volume shares the same excitation pattern, which is approximatively sinusoidal and can be reproduced following Eq. (9). The i^{th} z-slice of the pattern modulated image volume can be generated by:

$$D_{LLS-SIM}(\mathbf{r}_{xy}, z_i) = \mathcal{F}^{-1}\{O(\mathbf{k}) \cdot \mathcal{F}\{I(\mathbf{r}) \cdot S(\mathbf{r}_{xy}, z_i)\}\}. \quad (16)$$

Dataset acquisition, pre-processing and rDL network training

The training datasets of different biological specimens were acquired via the home-built Multi-SIM and LLS-SIM system. For each type of specimens, we typically acquired raw SIM images from about 35 distinct regions of interest (ROIs) of 512×512 pixels. For each ROI, a group of raw images excited at 3–6 different levels of light intensity was sequentially acquired to cover the fluorescence level of very low (<40 average photon counts) to medium (150–300 average photon counts), and then the excitation intensity was elevated to achieve high fluorescence of more than 1,000 average photon counts to acquire the corresponding GT raw images, which can be reconstructed into high-quality GT-SIM images via the conventional SIM reconstruction algorithm. To generate the training dataset for SR inference module and rDL denoising module, respectively, the dataset was augmented into ~30,000 image patch pairs of raw SIM patches (128×128 pixels for TIRF-SIM, $64 \times 64 \times 11$ voxels for 3D-SIM and $64 \times 64 \times 16$ voxels for LLS-SIM or LLSM) and their corresponding GT-SIM patches. One-third image pairs of the augmented dataset were employed to train the SR module, and the other two-thirds were used to train the rDL denoising module. Either the SR inference module or the rDL denoising module was trained with the data of all fluorescence levels unless otherwise noted.

In this work, the rDL networks were trained on a computer workstation equipped with a Xeon(R) Gold 6134 CPU at 3.20 GHz (Intel) and two RTX 3090Ti graphic processing cards (NVIDIA) with Python version 3.6 and TensorFlow version 2.4.0. The training process of an rDL SIM model consisted of two stages. First, train the SR inference module; and second, train the rDL denoising module by using the pre-trained SR inference module. Specifically, the 2D and 3D SR inference modules were trained with a learning rate of 1×10^{-4} and a batch size of 4 and 2, respectively. The training process typically lasted for ~24 hours with ~200,000 mini-batch iterations. Then, the 2D and 3D rDL denoising modules were trained with the learning rate of 1×10^{-4} and 5×10^{-5} , respectively. The training time of an rDL denoising module was typically 3–5 times longer than that of an SR inference module, with a total mini-batch iteration of ~300,000 and ~100,000, lasting ~90 hours and ~120 hours for 2D and 3D models, respectively. In particular, for the 2D rDL denoising module, images of the same orientations were recognized as a three-channel 2D tensor, and the raw images of 3-phase $\times$ 3-orientation were together fed into the network with a batch size of 3 in each mini-batch iteration. For the 3D rDL denoise model, each raw image stack of a single pattern phase was recognized as an independent 3D tensor, and, in each training iteration, we randomly select three raw image stacks of the same ROI to constitute a training batch. The reason is that the 3D models were subject to relatively weaker capability of feature extraction than the 2D models, presumably due to the shallower and smaller architecture of 3D models. We empirically found that implementing the 3D model in such a single-channel manner could enhance the high-frequency details in the resulted rDL 3D-SIM images.

The training procedures and configurations of TiS-rDL and SiS-rDL denoising networks were similar to those of the rDL denoising module described above, and the detailed training information for each type of biology structure used in this work is listed in Supplementary Table 2.

Comparison of rDL SIM and other SIM methods

To validate the superiority of the rDL SIM, we used our published BioSR dataset to quantitatively evaluate the performance of rDL SIM and other state-of-the-art SIM methods, including DFCAN-SIM, DFGAN-SIM and Hessian-SIM. Specifically, the raw SIM data of the highest signal level for MT and F-actin in BioSR were degraded to low SNR by adding Poisson and Gaussian noise following the fluorescent image acquisition model⁴⁰, producing well-registered testing data at 14 precisely escalating signal levels from 40 to 800 average photon counts¹⁶. For each type of biological specimen and DNN, only one model was trained to process the data of all signal levels. It is worth noting that, although the DFCAN-SIM did not precisely infer the fine structures of MT or F-actin (second column in Fig. 2a), which were used to generate the auxiliary pattern-modulated raw images in the MPE branch for rDL denoising, the final rDL SIM images successfully corrected these inaccurate inferences in DFCAN-SIM images. In Fig. 2b, the PSNR was calculated after a commonly used image normalization procedure¹⁶, and the resolution was measured by the decorrelation analysis⁴¹ from 150 testing F-actin images for each signal level.

Data post-processing for rDL LLSM

It is known that the non-uniformity among the millions of pixels of sCMOS camera usually induces a patterned background⁴⁰. Although the self-supervised denoising method effectively removes random noise without fixed patterns²⁵, it is not suitable to deal with the camera background. After TiS-rDL or SiS-rDL denoising, we suppressed the camera background by the following steps: (1) average each image stack axially into a single image, which contains both sample structures and the enhanced camera background; (2) perform the fast Fourier transform to this averaged image and filter out the information within the OTF of the LLSM system and then obtain the camera background-only image via inverse fast Fourier transform; and (3) subtract the noise image from the denoised stack in a slice-by-slice manner and apply a 2D Gaussian filter with a standard deviation of 0.6 pixels to obtain the camera background-free rDL LLSM image stack. To further improve the contrast and resolution of rDL LLSM images, we applied the commonly used Richardson–Lucy deconvolution with 5–8 iterations for the time-lapse data in Figs. 5 and 6.

Organelle contact quantification

The contact level between POs and ER tubules was evaluated by Mander's overlap coefficient (MOC) (Extended Data Fig. 5b). Considering that ER–PO contact sites locate at the periphery of POs, we first identified the boundary for each PO by the following procedure: (1) estimate the background of the ROI of PO by applying a Gaussian filter of $\sigma = 10$ pixels; (2) smooth the original rDL GI-SIM image by another Gaussian filter of $\sigma = 1$ pixel and then subtract the estimated background; (3) calculate a binary mask by applying a threshold to the background-subtracted image; (4) extract the boundary of each PO using the Sobel operator; and (5) convolve the PO boundary image with the equivalent PSF of rDL GI-SIM to obtain a potential contact region of POs. Subsequently, a binary ER mask $Mask_{ER}$ was calculated following steps 1–3 described above. Then, the MOC of the PO periphery relative to the ER tubules could be calculated by:

$$MOC = \frac{\sum_i D_i \cdot Mask_{ER,i}}{\sum_i D_i}, \quad (17)$$

where D_i and $Mask_{ER,i}$ denote the value of the i^{th} pixel in the ROI of the PO image and ER mask, respectively. We calculated the MOC of each

ER–PO contact event for every timepoint of its lifetime. Then, the ratio of mean value and standard variation of the MOC curve was calculated to identify if the specific ER–PO formed bona fide functional contact sites or not with an empirical threshold of 0.6 (Extended Data Fig. 5c).

Similarly to the 2D scenario, the 3D contacts between ER and mitochondria or lysosomes during mitosis (Fig. 6g and Supplementary Video 16) were also evaluated via volumetric MOC, which was computed following the procedure described above. Specifically, the Gaussian filter, the Sobel operator and the PSF of the LLSM system were upgraded to 3D version for volumetric boundary extraction. Then, the MOC of mitochondrion or lysosome boundary relative to the ER was calculated by Eq. (17) for each timepoint of the rDL LLSM data.

Tracking of SiT-Golgi vesicles

Tracking of SiT-Golgi vesicles was performed in Imaris 9.0.1 (Oxford Instruments) (Fig. 5d–h). To determine the orientation of each trajectory relative to the stacked Golgi complex, we first estimated the centroid of the Golgi complex by averaging the center coordinates of all Golgi vesicles that aggregated close to the nucleus. Then, a locally weighted scatterplot smoothing (LOWESS) algorithm⁴² was applied to temporally stabilize the vibration of the estimated centroid. The moving direction of each SiT-Golgi vesicle was represented by the vector $\mathbf{v}_{\text{Golgi-vesicle}}$ pointing from its initial to end spots. The orientation of the trajectory of SiT-Golgi vesicle relative to the Golgi complex was represented by the vector $\mathbf{v}_{\text{Golgi-centroid}}$ pointing from the initial spot of the trajectory to the centroid of the stacked Golgi complex. Then, the intersection angle between the two vectors can be calculated as:

$$\theta = \arccos \frac{\mathbf{v}_{\text{Golgi-vesicle}} \cdot \mathbf{v}_{\text{Golgi-centroid}}}{|\mathbf{v}_{\text{Golgi-vesicle}}| |\mathbf{v}_{\text{Golgi-centroid}}|}, \quad (18)$$

where $\cdot$ denotes the dot product, and $|\cdot|$ denotes the modulus operator. In this work, we categorized the vesicles of $\theta < 60^\circ$ as moving towards the Golgi complex, whereas the vesicles of $\theta > 120^\circ$ were categorized as moving outwards from the Golgi complex.

To characterize the dynamic behavior of SiT-Golgi vesicle movement, we calculated the mean square displacement (MSD) for the trajectories that lasted longer than ten timepoints and then fit the MSD using the following model via the MATLAB (MathWorks, 2017b) function `lsqcurvefit`:

$$MSD(\tau) = A\tau^\alpha, \quad (19)$$

where α is the anomalous exponent used to categorize the movements⁴³. In this work, the SiT-Golgi vesicle movement was categorized as constrained diffusion, free diffusion or directed movement, if the α of its MSD curve satisfies $\alpha < 0.9$, $0.9 < \alpha < 1.2$ or $\alpha > 1.2$, respectively⁴⁴.

3D distribution characterization of Lyso during mitosis

The Lyso distribution over the whole-cell volume was characterized by the following procedure:

- Detect and localize the centroid of the Lyso using Imaris 9.0.1 (Oxford Instruments) for each timepoint of rDL LLSM data.
- Calculate the average nearest neighbor distances (NNDs) between each Lyso and its n nearest neighbors in a single cell:

$$NND_i = \frac{1}{n} \sum_{j=1}^n \|P_i - P_j\|_2, \quad (20)$$

where NND_i denotes the NND of the i^{th} Lyso; P_i and P_j denote the coordinates of the i^{th} and the j^{th} Lyso, respectively; and $\|\cdot\|_2$ represents the Euclidean distance.

- Calculate the coefficient of variation by:

$$\text{Coefficient of variation} = \frac{\text{var}(NND)}{\text{mean}(NND)}, \quad (21)$$

where $var(\cdot)$ and $mean(\cdot)$ represent the variation and average value of NND, respectively.

The escalating tendency of the coefficient of variation represented the distribution of Lyso getting inhomogeneous. In other words, the Lyso tended to aggregate into large entities, as we observed in prometaphase and metaphase of mitosis (Fig. 6h).

Cell culture, transfection and staining

COS-7, HeLa and HEK293T cells were grown in DMEM (Gibco) supplemented with 10% FBS (Gibco) and 1% penicillin–streptomycin, and U2OS cells were grown in MCMM (Gibco) supplemented with 10% FBS (Gibco) and 1% penicillin–streptomycin. All cell lines were cultured at 37 °C and 5% CO₂ until 60–80% confluency was reached. For live cell imaging experiments, the coverslips were pre-coated with 50 mg ml⁻¹ of collagen for 1 hour, and cells were then seeded onto the coverslips to achieve ~70% confluence before transfection. Transfections were executed using Lipofectamine 3000 (Invitrogen) according to the manufacturer's protocol. Cells were imaged 24–36 hours after transfection in a stagetop micro-incubator (Okolab) maintained at 37 °C and 5% CO₂. Where indicated, the cells transfected with HaloTag plasmids were labeled with JF646 or JF549 ligand following the published protocol⁴⁵, and the cells were imaged immediately afterwards.

R1 mES cells were grown in Knockout DMEM (Thermo Fisher Scientific) supplemented with 100 U ml⁻¹ of penicillin–streptomycin, 1× MEM NEAA, 1 mM sodium pyruvate, 1 mM L-glutamine (all from Thermo Fisher Scientific), 0.1 μM 2-mercaptoethanol (Sigma-Aldrich), 1 mM MEK inhibitor (PD0325910, MCE), 33 mM GSK3β inhibitor (CHIR99021, MCE) and 100 U ml⁻¹ of leukemia inhibitory factor (LIF, EMD Millipore) without feeder cells at 37 °C with 5% CO₂ in a humidified incubator.

Multiciliated mEPCs were cultured as described previously with minor modifications^{46,47}. In brief, newborn mice were anesthetized on ice for 5–8 minutes before telencephala were dissected out from brain in cold dissection solution (161 mM NaCl, 5 mM KCl, 1 mM MgSO₄, 3.7 mM CaCl₂, 5 mM HEPES and 5.5 mM glucose, pH 7.4). The telencephala were digested in dissection buffer (10 U ml⁻¹ of papain, 0.2 mg ml⁻¹ of L-cysteine, 0.5 mM EDTA, 1 mM CaCl₂, 1.5 mM NaOH and 0.15% DNase I) for 30 minutes at 37 °C after removing the olfactory bulb, meninges and hippocampus. Then, fresh DMEM supplemented with 10% FBS and 1% penicillin–streptomycin was added to resuspend cells with pipetting up and down gently ten times. The released cells were collected at 400g for 5 minutes at room temperature and plated into a flask pre-coated with laminin (Sigma-Aldrich, L2020). Neurons were mechanically shaken off by striking the flask roughly 2 days after incubation. The remaining cells were digested with trypsin and transferred into laminin-coated 12-mm coverslips. After cells reached full confluence, serum was removed from medium to induce multiciliate mEPCs. Adenovirus packaging was produced and used to infect mEPCs as described previously⁴⁶. Serum starvation day 5 or later mEPCs were, thus, incubated with 200 nM LysoTracker (Thermo Fisher Scientific, L7528) for 30 minutes to fluorescently label their multicilia. Then, the mEPCs were laid flat against the bottom coverslip and imaged. In this manner, the ciliary tips tended to beat in the form of back-and-forth or sideways sweeping, whereas the ciliary bases stayed relatively stationary, which were the characteristics used to distinguish the orientation of cilia in this study.

Experiments involving mouse tissue were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of the Chinese Academy of Sciences Center for Excellence in Molecular Cell Science, Institute of Biochemistry and Cell Biology.

Lentivirus packaging and stable cell line

Lentivirus was produced by co-transfecting the plasmids of psPAX2 and pMG2.G into HEK293T cells grown to approximately 70% confluency in six-well plates using Lipofectamine 3000 (Invitrogen) following the manufacturer's protocol. After 2 days, supernatant containing viral

particles was harvested and filtered with a 0.45-μm filter (Millipore). Supernatant was immediately used for transduction or stored at –80 °C in aliquots. HeLa cells were grown to 10–20% confluency in six-well plates, and 100–300 μl of filtered viral supernatant was added to the cells. Media containing virus were replaced with fresh growth medium 24 hours after infection.

To establish cell lines expressing multiple constructs, HeLa or HEK293T cells were infected with lentiviruses carrying corresponding fluorescent protein-tagged organelle markers. Specifically, cGAS-Halo and mEmerald-KDEL for HEK293 cells used in Fig. 3j–m; mCherry-H2B and Halo-RPA49 for HeLa cells used in Fig. 4c–f; SiT-Halo for R1 cells used in Fig. 5; calnexin-mEmerald, mCherry-H2B and SiT-Halo for HeLa cells used in Extended Data Fig. 8; calnexin-mEmerald, Mito-dsRed and Halo-H2B for HeLa cells used in Fig. 6d; and calnexin-mEmerald, mCherry-H2B and Halo-LAMP1 for HeLa cells used in Fig. 6e. Forty-eight hours after transduction, the positive cells were enriched by fluorescence-activated cell sorting (FACS) (FACS Aria II, BD Biosciences) and then plated one per well into 96-well plates. Monoclonal cells were used for our experiments.

Genome-edited cell lines

HeLa cells were genome edited to incorporate mEmerald into the xx-terminus of NPM1 using the CRISPR–Cas9 approach. The single-guide RNA targeting sequences is 5'-TCGCTCTCGCCGCGCAAT-3' for NPM1. Target-specific oligonucleotides were cloned into pX330-1×2 (Addgene, 58766) plasmid. HeLa cells were transfected with 2.5 mg of Cas9 plasmid containing the guide sequence and 1.25 mg of non-linearized repair plasmid. The cells expressing GFP were sorted and enriched after 48 hours. The cells were grown for approximately 1 week, and then individual colonies were picked into a 96-well plate. Cells were expanded and genotyped by PCR and western blot. Clones with a homozygous knock-in tag were further expanded and used for experiments.

SUM159 cells were genome edited to incorporate EGFP to the C-terminus of clathrin light chain A (clathrin-EGFP) using the TALEN-based approach as described⁴⁸. The clathrin-EGFP-expressing cells were enriched by two sequential bulk sorting.

Reporting summary

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

Data availability

The published BioSR dataset was used to train the rDL models for the biological structures of ER, MT, actin and CCPs. The training data for the rDL models of other biological structures were also uploaded into the figshare repository of the BioSR dataset, which is publicly accessible via <https://doi.org/10.6084/m9.figshare.13264793>. Source data are provided with this paper.

Code availability

The source codes of rDL SIM, several representative pre-trained models as well as some example images for testing are publicly accessible via <https://github.com/qc17-THU/rDL-SIM>.

References

- Gustafsson, M. G. et al. Three-dimensional resolution doubling in wide-field fluorescence microscopy by structured illumination. *Biophys. J.* **94**, 4957–4970 (2008).
- Muller, M., Monkemoller, V., Hennig, S., Hubner, W. & Huser, T. Open-source image reconstruction of super-resolution structured illumination microscopy data in ImageJ. *Nat. Commun.* **7**, 10980 (2016).
- He, K., Zhang, X., Ren, S. & Sun, J. Deep residual learning for image recognition. *Proc. of the IEEE Conference on Computer Vision and Pattern Recognition*. 770–778 (2016).

37. Szegedy, C., Ioffe, S., Vanhoucke, V. & Alemi, A. A. Inception-v4, Inception-ResNet and the impact of residual connections on learning. <https://ojs.aaai.org/index.php/AAAI/article/view/11231>. Thirty-First AAAI Conference on Artificial Intelligence (2017).
38. Hu, J., Shen, L. & Sun, G. Squeeze-and-excitation networks. *Proc. of the IEEE Conference on Computer Vision and Pattern Recognition*. 7132–7141 (2018).
39. Zhang, Y. et al. Image super-resolution using very deep residual channel attention networks. *Proc. of the European Conference on Computer Vision (ECCV)* 286–301 (2018).
40. Liu, S. et al. sCMOS noise-correction algorithm for microscopy images. *Nat. Methods* **14**, 760–761 (2017).
41. Descloux, A., Grussmayer, K. S. & Radenovic, A. Parameter-free image resolution estimation based on decorrelation analysis. *Nat. Methods* **16**, 918–924 (2019).
42. Cleveland, W. S. LOWESS: a program for smoothing scatterplots by robust locally weighted regression. *Am. Stat.* **35**, 54 (1981).
43. Qian, H., Sheetz, M. P. & Elson, E. L. Single particle tracking. Analysis of diffusion and flow in two-dimensional systems. *Biophys. J.* **60**, 910–921 (1991).
44. Ba, Q., Raghavan, G., Kiselyov, K. & Yang, G. Whole-cell scale dynamic organization of lysosomes revealed by spatial statistical analysis. *Cell Rep.* **23**, 3591–3606 (2018).
45. Grimm, J. B. et al. A general method to improve fluorophores for live-cell and single-molecule microscopy. *Nat. Methods* **12**, 244–250 (2015).
46. Zhao, H. et al. Fibrogranular materials function as organizers to ensure the fidelity of multiciliary assembly. *Nat. Commun.* **12**, 1273 (2021).
47. Delgehyr, N. et al. Ependymal cell differentiation, from monociliated to multiciliated cells. *Methods Cell. Biol.* **127**, 19–35 (2015).
48. Sanjana, N. E. et al. A transcription activator-like effector toolbox for genome engineering. *Nat. Protoc.* **7**, 171–192 (2012).

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Author contributions

Dong Li and C.Q. conceived the idea. Dong Li and Q.D. supervised the research. Dong Li, C.Q. and Di Li designed the experiments. Di Li, Y.L. and S.Z. built and improved the microscope systems at the Institute of Biophysics, under the supervision of Dong Li. Di Li, Y.L., C.L., Y.G., T.J., C.F. and N.L. prepared samples and performed experiments. C.Q., Di Li, Y.L., K.L. and Y.Z. analyzed the data, with conceptual advice from Dong Li, K.H., X.Z., and J.L.-S. C.Q., Di Li and Y.L. composed the figures and videos, under the supervision of Dong Li. Dong Li, C.Q., Q.D. and J.L.-S. wrote the manuscript, with input from all authors. All authors discussed the results and commented on the manuscript.

Competing interests

Dong Li and C.Q. have pending patent applications on the presented frameworks. The other authors declare no competing interests.

Additional information

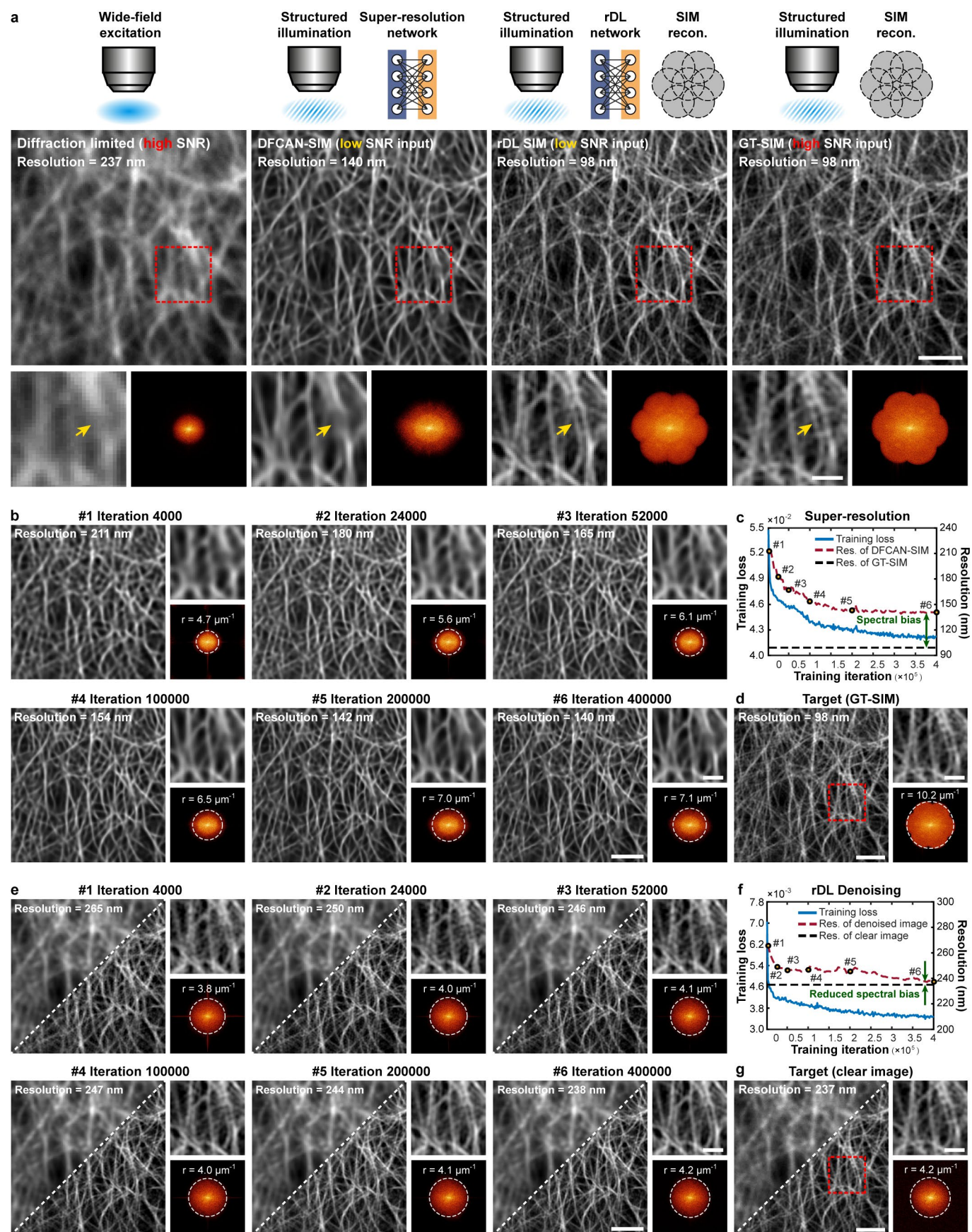
Extended data is available for this paper at <https://doi.org/10.1038/s41587-022-01471-3>.

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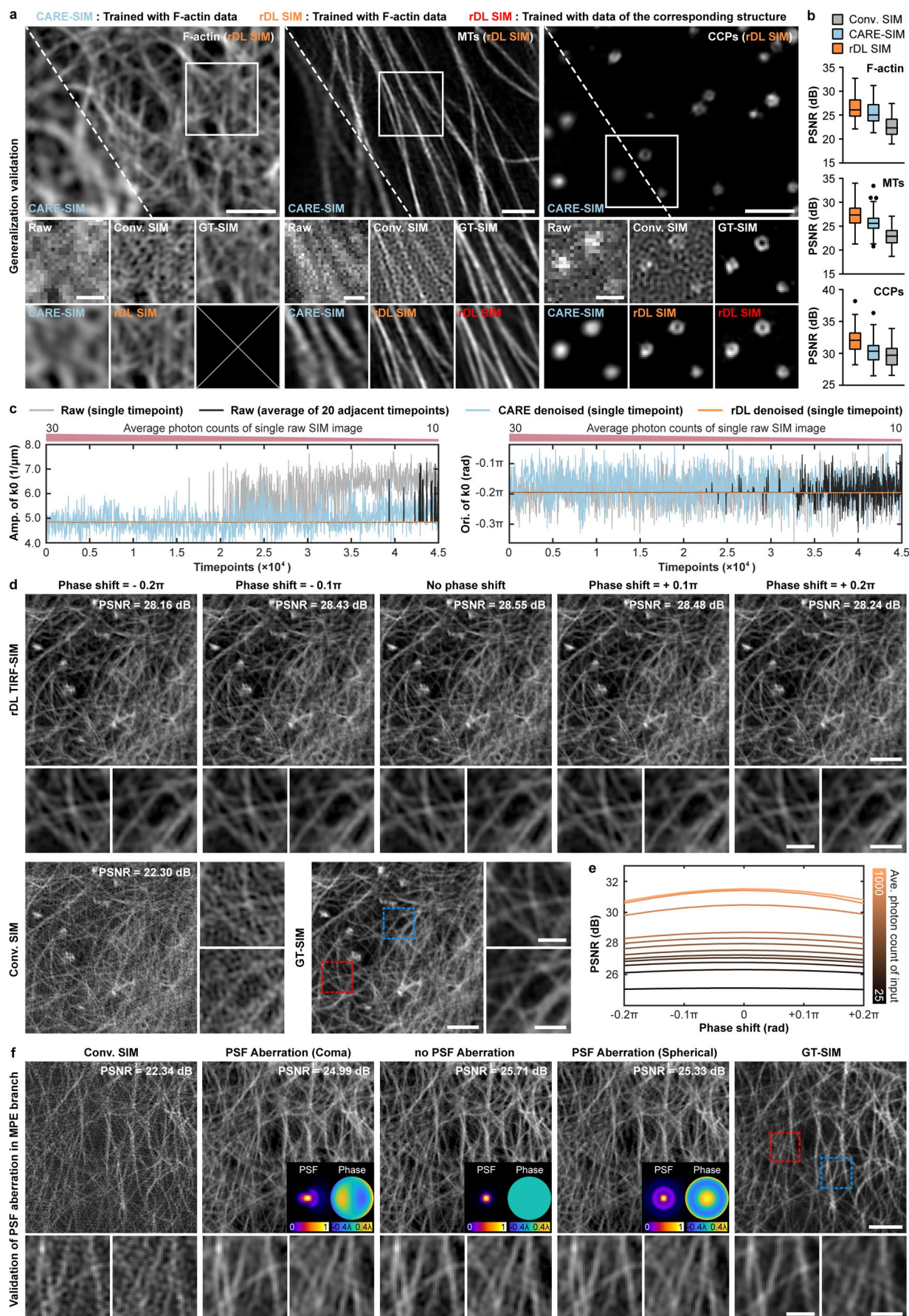
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Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | Illustration of the spectral bias effect in deep learning super-resolution (DLSR) task. a, Concepts (upper row) and typical results (lower row) of wide-field, DFCAN-SIM, rDL SIM and conventional SIM imaging. **b,** Typical DFCAN-SIM images of F-actin output at 4000, 24000, 52000, 100000, 200000, and 400,000 training iterations, respectively. **c,** The evolutions of the training loss and the resolution of the intermediate outputs during the training process of the DFCAN-SIM model of F-actin dataset (averaged from $n = 20$ test images). The curves shows that there exists a significant resolution gap between DFCAN-SIM (red dashed line) and GT-SIM (black dashed line) images, which implies DLSR models suffer from severe spectral bias. **d,** The corresponding GT-SIM image of the same ROI with (b) for reference. **e,** Typical rDL denoised

images (top left) and rDL SIM images (bottom right) of F-actin output at 4000, 24000, 52000, 100000, 200000, and 400,000 training iterations, respectively. **f,** The evolutions of the training loss and the resolution of the intermediate outputs during the training process of the rDL denoising model of F-actin dataset (averaged from $n = 180$ test images). Compared with DLSR task in b and c, the rDL denoising of raw SIM images is hardly impaired by the spectral bias problem, preventing the resolution degradation of rDL SIM image relative to the GT-SIM image, as illustrated in (a). **g,** The corresponding ground truth raw SIM image of the same ROI with (e) for reference. Scale bar, $3\ \mu\text{m}$ (a, b, d, e, g), $0.5\ \mu\text{m}$ (zoom-in regions of a, b, d, e, g). Gamma value, 0.7 for DFCAN-SIM images, rDL SIM images and GT-SIM images.

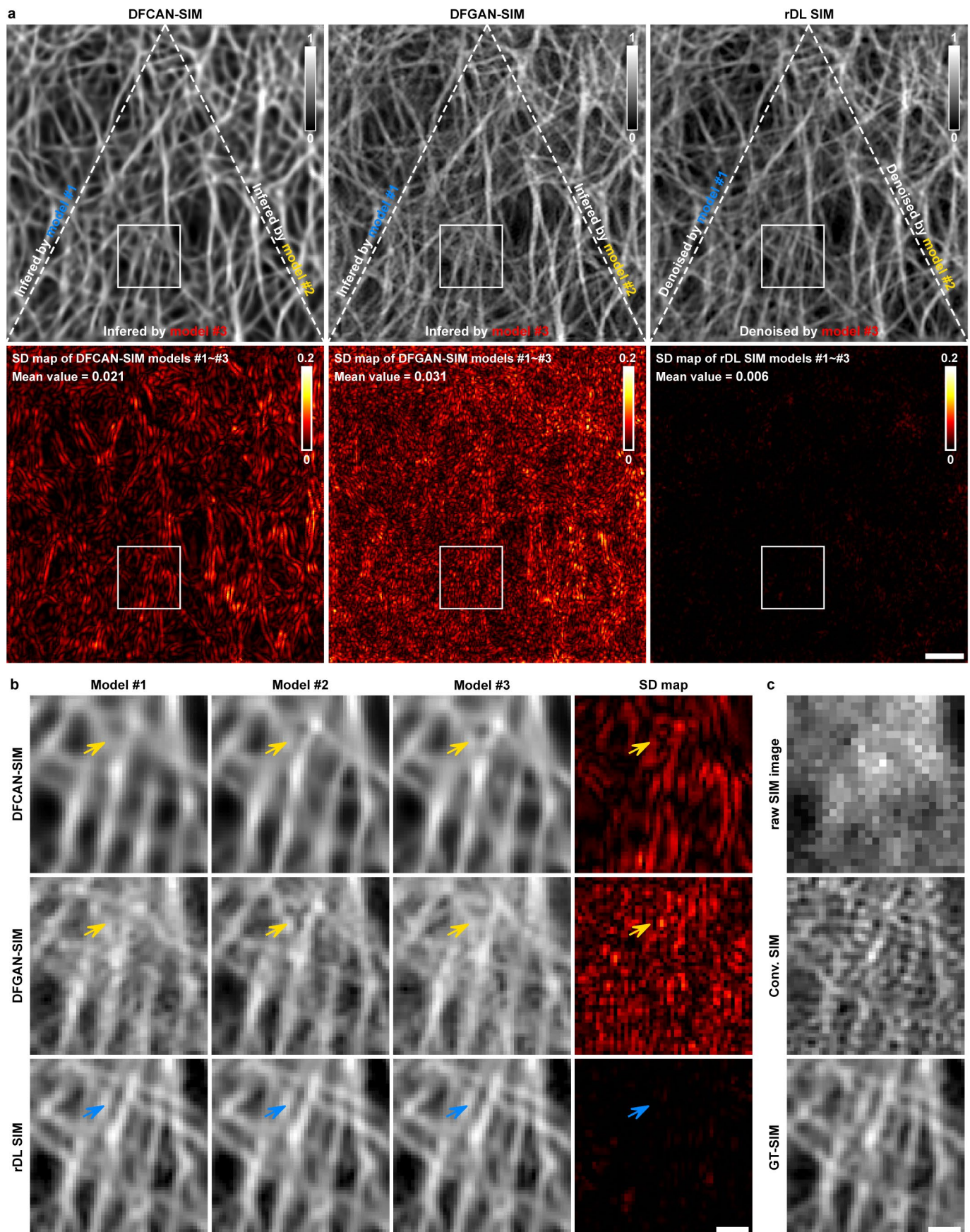


Extended Data Fig. 2 | See next page for caption.

Extended Data Fig. 2 | Generalization and Robustness validation of rDL

SIM. **a**, Typical SR images of F-actin, MTs and CCPs reconstructed by rDL SIM (top right corner) and CARE-SIM (bottom left corner) trained with only F-actin data. Zoom-in regions show the comparisons of raw SIM images, GT-SIM images and SR images reconstructed by conventional SIM (Conv. SIM), CARE-SIM, rDL SIM trained with F-actin data (orange) and rDL SIM trained with corresponding specimens (red). These results illustrate the strong generalization capability of rDL SIM. **b**, PSNR quantifications of CARE-SIM and rDL-SIM in terms of F-actin, MTs and CCPs, where both models are trained with only F-actin data. The PSNRs of Conv. SIM are shown for reference, $n = 145$. Center line, medians; limits, 75% and 25%; whiskers, the larger value between the largest data point and the 75th percentiles plus $1.5 \times$ the interquartile range (IQR), and the smaller value between the smallest data point and the 25th percentiles minus $1.5 \times$ the IQR; outliers, data points larger than the upper whisker or smaller than the lower whisker. **c**, Plots of amplitudes (left) and orientations (right) of the pattern wave vector for

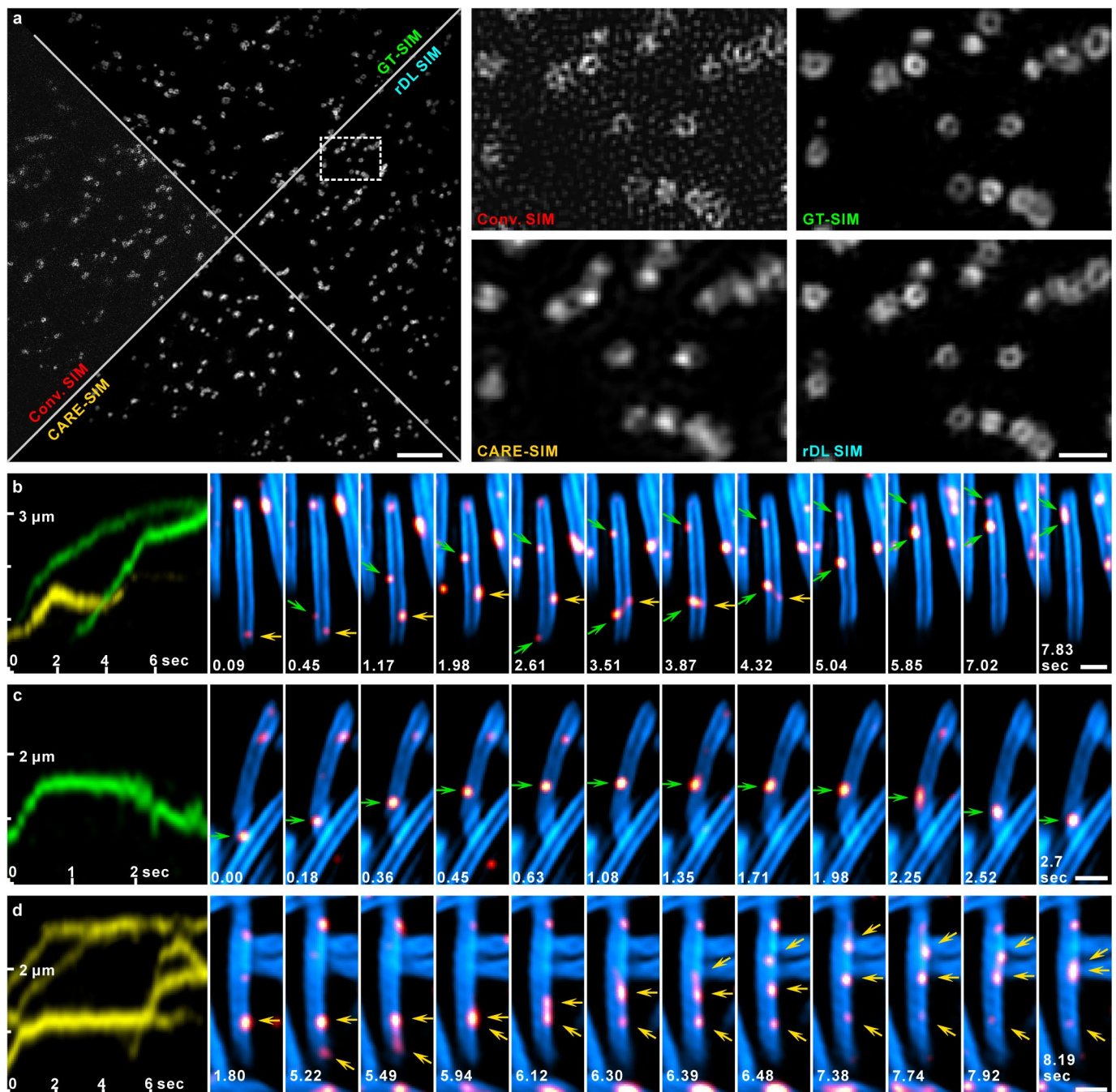
the 45,000-time-point ER data shown in Fig. 3h and Supplementary Video 8. The pattern parameters are estimated from the single time point of raw SIM images (gray lines), averaged 20 consecutive time points of raw SIM images (black lines), single time point of CARE denoised images (blue lines) and rDL denoised images (orange lines). **d**, Representative rDL SIM images of F-actin with the pattern initial phase shifted by -0.2π , -0.1π , 0, 0.1π and 0.2π , respectively, relative to that of GT-SIM. Conv. SIM and GT-SIM images are shown for reference. The PSNRs are labelled at the top right of all Conv. SIM images and rDL SIM images. **e**, PSNR quantifications of the rDL SIM images at different phase errors under different fluorescence intensity levels in the cases of F-actin, $n = 50$. **f**, Representative rDL SIM images of F-actin with spherical or coma aberration distorted PSF in MPE branch. Conv. SIM and GT-SIM images are shown for reference. Scale bar, $1\mu\text{m}$ (a), $0.5\mu\text{m}$ (zoom-in regions in a), $1.5\mu\text{m}$ (d and f), and $0.5\mu\text{m}$ (zoom-in regions in d and f). Gamma value: 0.7 for all SIM images of F-actin.



Extended Data Fig. 3 | See next page for caption.

Extended Data Fig. 3 | Uncertainty comparison of DFCAN-SIM, DFGAN-SIM and rDL SIM models. **a**, Representative SR images of F-actin produced by the three models of DFCAN-SIM, DFGAN-SIM and rDL SIM that are independently trained with the same dataset (upper), and the corresponding standard deviation (SD) maps (lower). **b**, The magnified regions of the white box in (a). **c**, The raw SIM

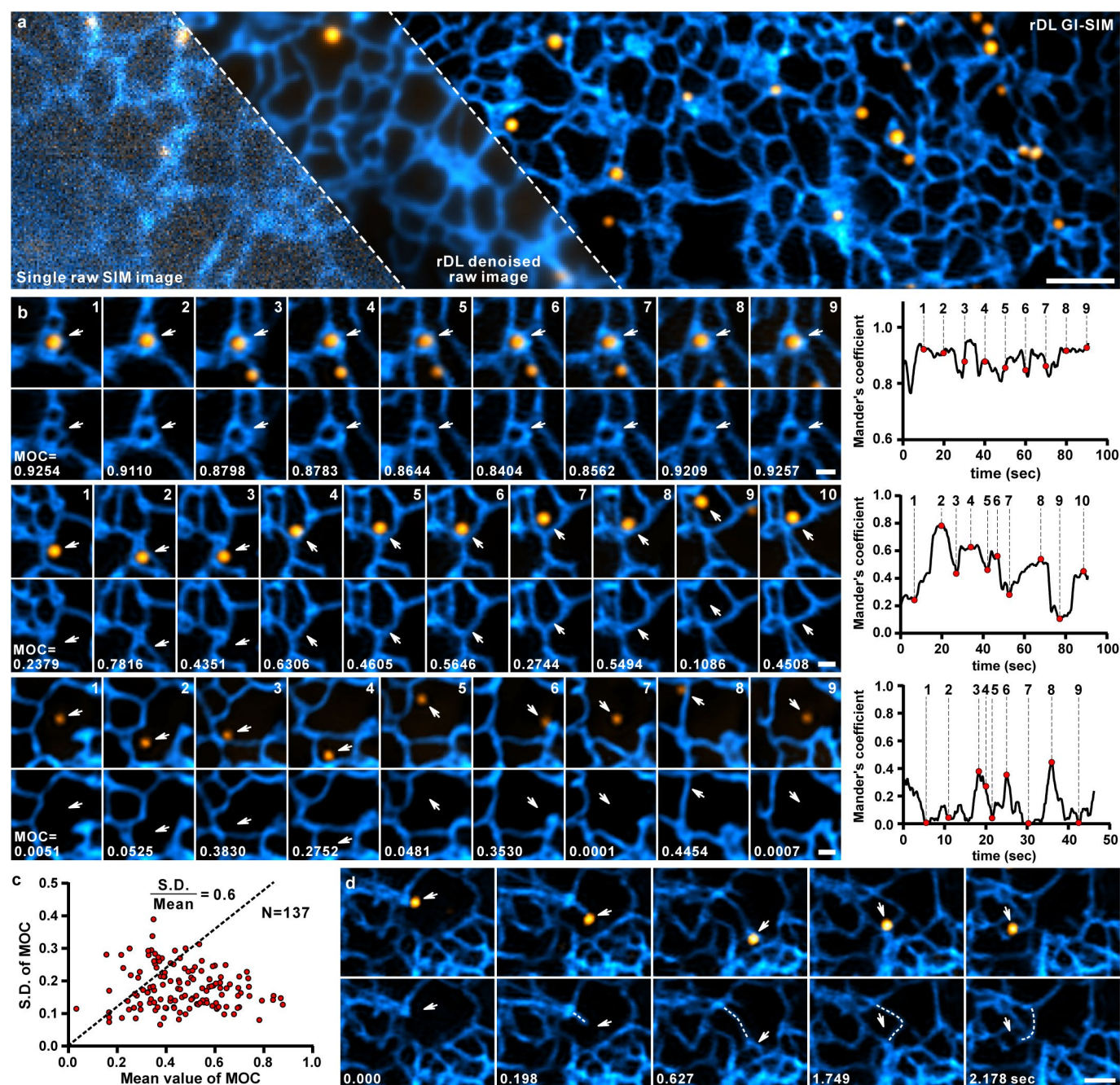
image, Conv. SIM and GT-SIM images shown for reference. The arrows indicate the fine structures of F-actin that exhibit large reconstruction variation by both DFCAN- and DFGAN-SIM models, whereas that are stably reconstructed by rDL SIM with high consistency to the GT-SIM image. Scale bar, 1 μm (a), 0.3 μm (b, c). Gamma value, 0.7 for all SIM images of F-actin.



Extended Data Fig. 4 | rDL SIM resolves the ring structure of CCPs and visualizes multiple IFT trains' unusual behaviors in ependymal motile cilia.

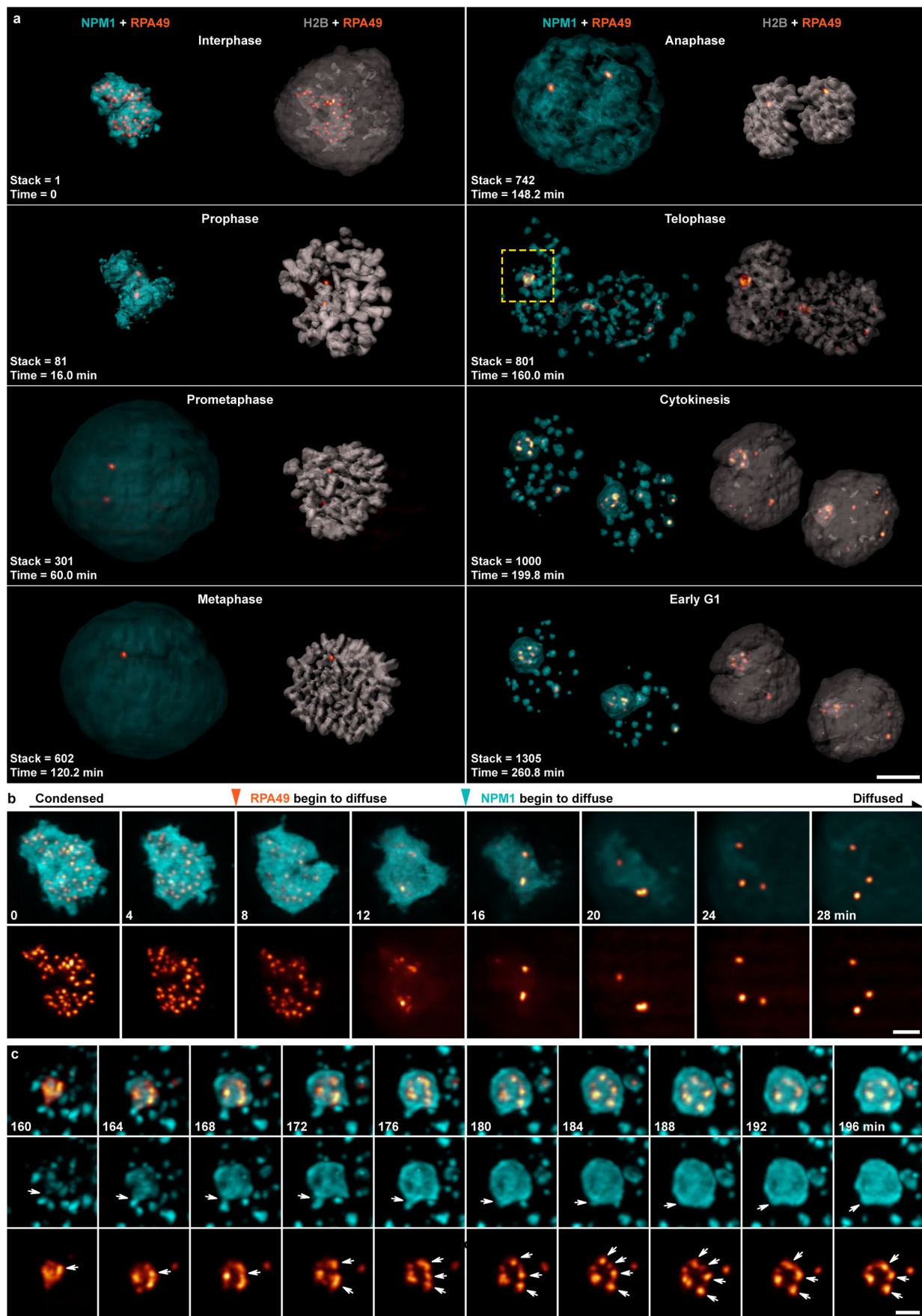
a, Representative SR images of CCPs reconstructed by conventional (Conv.) SIM, CARE-SIM and rDL SIM, whose raw images were acquired at 18-fold lower fluorescence than those of GT-SIM. It shows that rDL-SIM provides high-fidelity ring-like structure and prevents the reconstruction artifacts. (Supplementary Video 4). **b-d**, Kymographs (left) and time-lapse images (right) show that, (b) IFT trains stopped halfway or paused for a while before reaching the ciliary base; (c)

IFT trains reversed their direction of motion in the midway of the microtubule (MT) tracks; (d) Two IFT trains moving along the same MT track collided with each other, which remodeled them into three independent IFT trains. As described in Fig. 3d, the IFT trains are resolved into three groups that transported along the microtubule tracks residing at the left (green), middle (magenta), and right (yellow) sides of the ciliary axoneme. Scale bar, 5 μ m (a) and 0.5 μ m (zoom-in regions of a, b-d).



Extended Data Fig. 5 | Interactions between endoplasmic reticulum (ER) and peroxisomes (POs) as seen by rDL GI-SIM. a, Comparisons of single raw SIM image (left), rDL denoised raw image (middle) and rDL GI-SIM image (right) in the case of two-color live-cell imaging of ER and POs. Scale bar, 2 μm . **b**, Time-lapse two-color images of ER and POs, and the corresponding dynamic characteristics of Mander's overlap coefficient (MOC) for POs that are stably contacted with

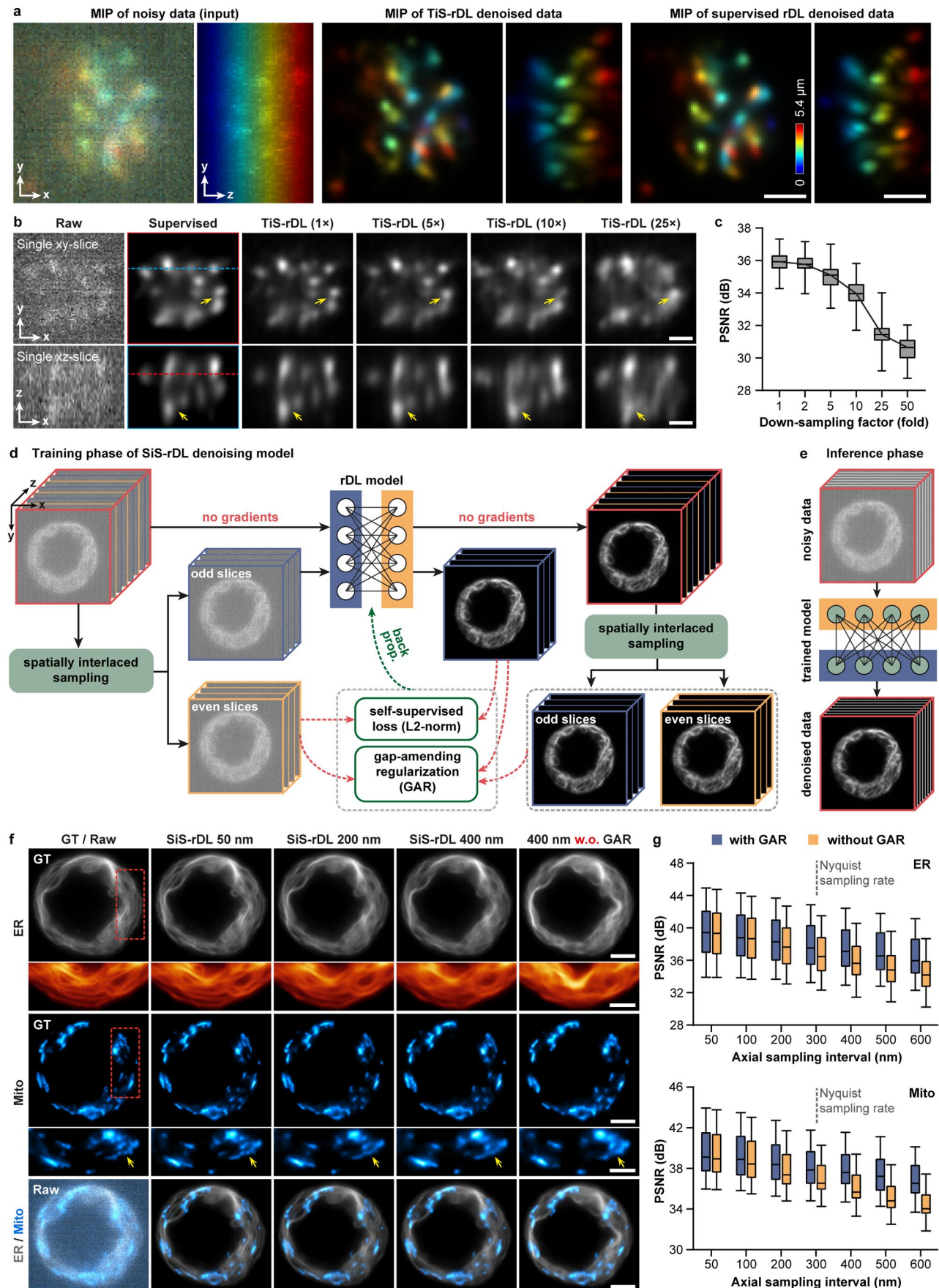
ER over time (top and middle rows) or that is freely moving without stable association with ER (bottom row). Scale bar, 0.5 μm . **c**, Distribution of the ER-PO MOCs' standard deviation (S.D.) versus their mean values, $n = 137$. **d**, Time-lapse images of a tubular ER generation event by hitchhiking on a moving PO. Scale bar, 1 μm . Gamma value, 0.7 for rDL GI-SIM images of ER, and 0.8 for rDL GI-SIM images of POs.



Extended Data Fig. 6 | See next page for caption.

Extended Data Fig. 6 | Visualizing the behaviors and interactions of NPM1 and RPA49 during cell mitosis via rDL LLS-SIM. a, 3-D rendering of three-color time-lapse images of fibrillar centre (FC, labeled by Halo-RPA49) and granular component (GC, labeled by mEmerald-NPM1), and chromosome (labeled by mCherry-H2B) at different stages of mitosis in a HeLa cell, knocking-in mEmerald-NPM1 and stably expressing mCherry-H2B and Halo-RPA49. **b**, Time-lapse max intensity projections of NPM1 and RPA49 showing that the dissociation

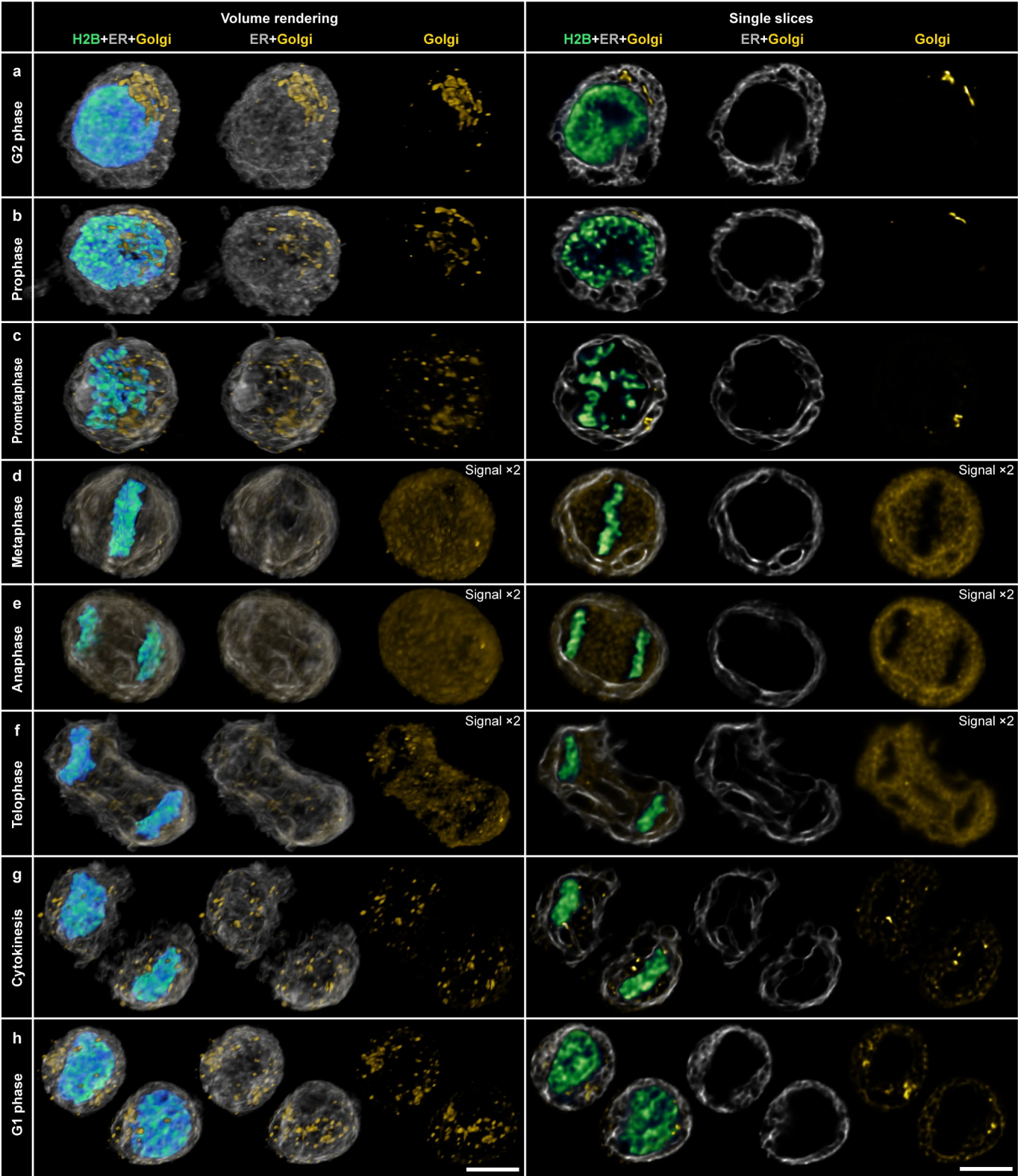
of RNA polymerase I subunits (*e.g.*, RPA49) from the innermost FC preceded the disassembly of the outermost GC during prophase to prometaphase. **c**, Time-lapse max intensity projections of NPM1 and RPA49 showing that small nucleolar-derived foci (NDFs) of NPM1 coalesced into giant ones (indicated by arrows in the second row), while the innermost RPA49 marked FC foci of large size could be partitioned into several independent small foci during telophase (arrows in the third row). Scale bar, 6 μm (a), 3 μm (b), 2 μm (c).



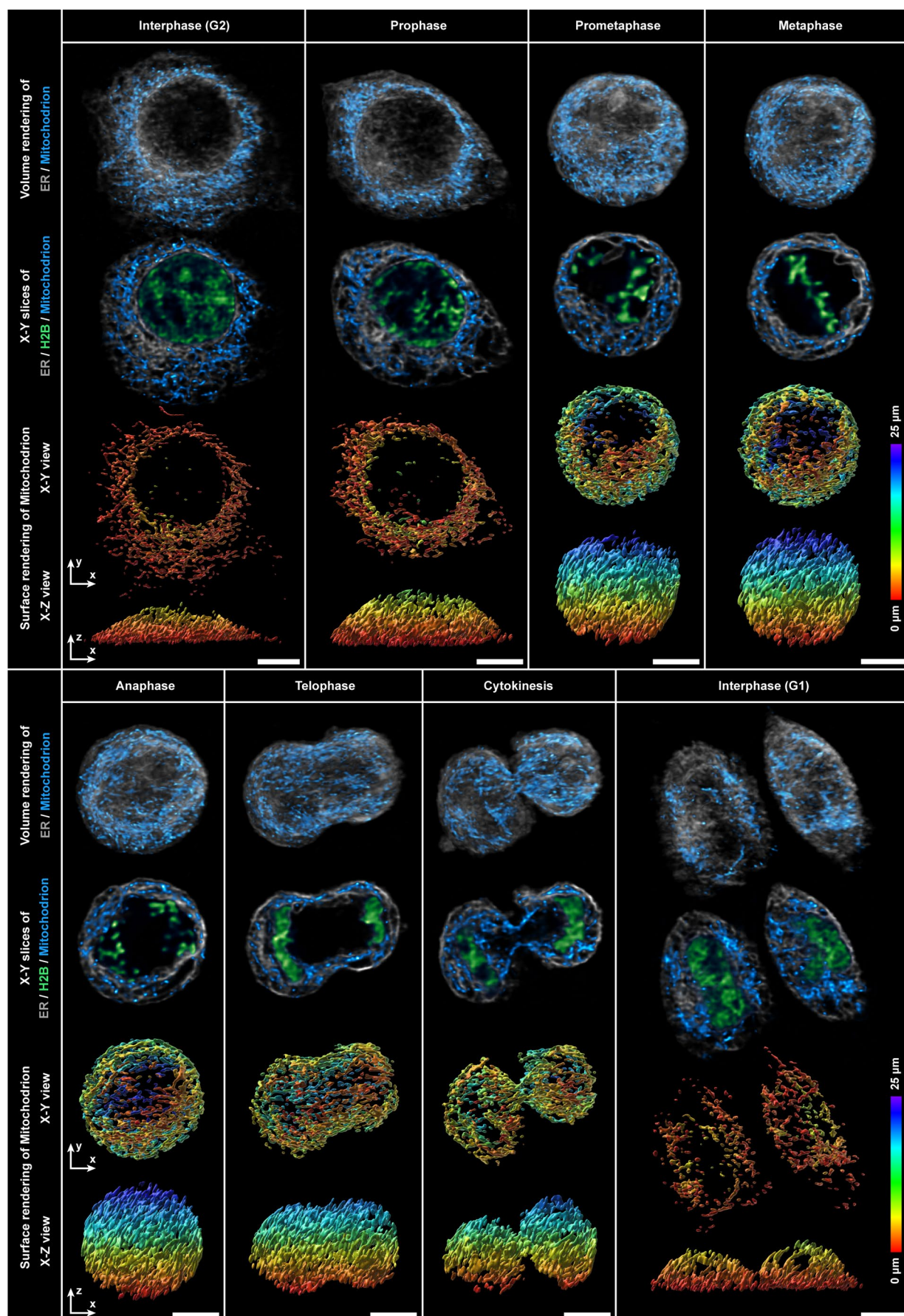
Extended Data Fig. 7 | See next page for caption.

Extended Data Fig. 7 | Temporally and Spatially interleaved self-supervised learning of rDL denoising networks. **a**, Representative LLSM images of Golgi SiT-vesicles denoised by TiS-rDL self-supervised and rDL supervised learning models. Depth color-coded maximum intensity projections (MIPs) of input noisy raw data (left), the images stack denoised by the TiS-rDL model (middle) and by the supervised learning model (right). These results illustrate the performance of TiS-rDL denoising model is as good as the rDL model trained with the supervision of high-SNR images. **b, c**, Representative TiS-rDL denoised images of Golgi SiT-vesicles (b) and statistical analysis in terms of PSNR under the conditions of temporally down-sampled at different factors (c), $n = 50$. Center line, medians; limits, 75% and 25%; whiskers, maximum and minimum. Noisy raw images and images denoised by the supervised model are shown for reference. These

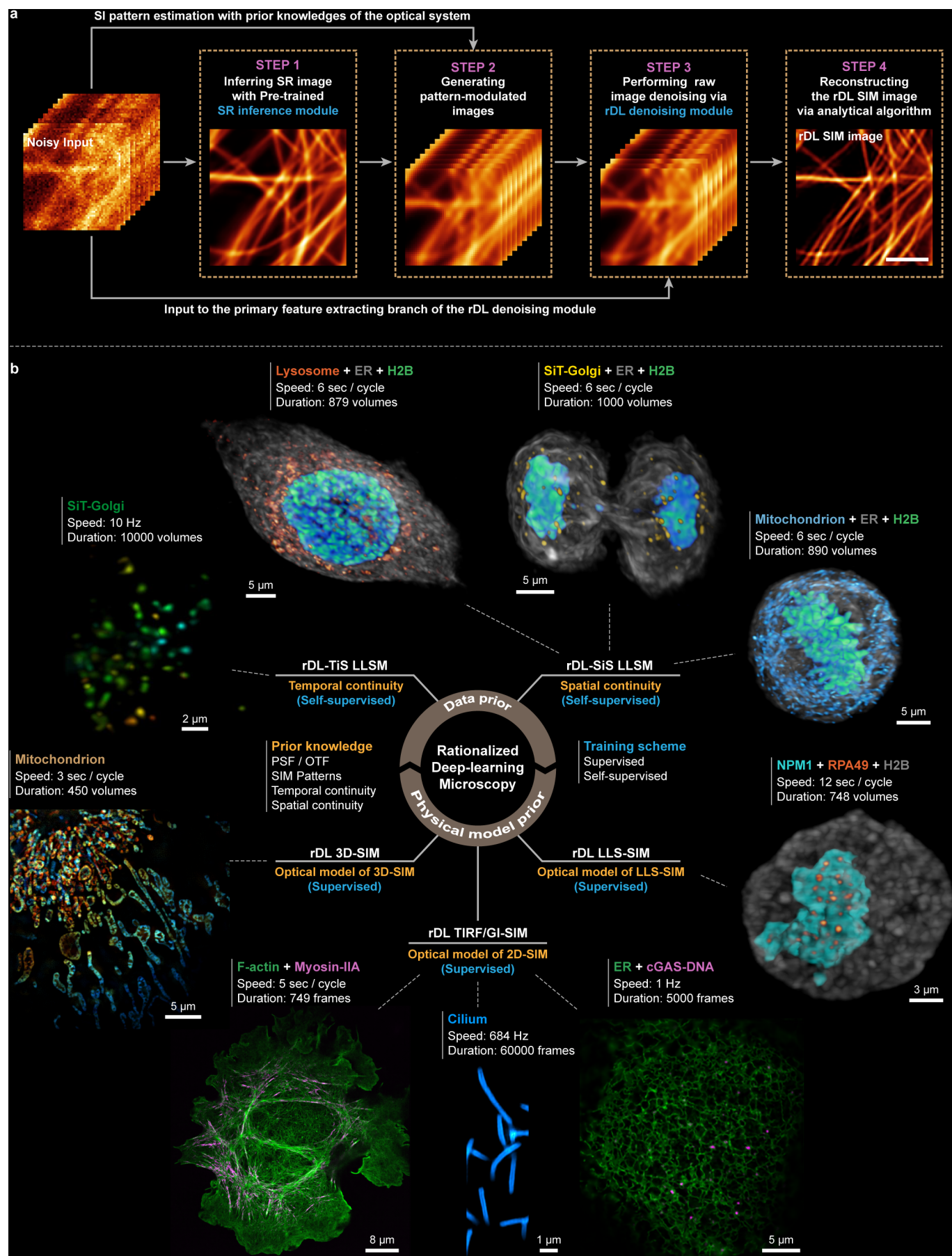
results indicate that the decrease of temporal sampling rate gradually affects the output fidelity of the TiS-rDL method. **d, e**, Schematic of SiS-rDL denoising network training (d) and inference (e). **f, g**, Representative outputs (f) and PSNR comparisons (g) of SiS-rDL denoising models with and without gap-amending regularization (GAR) in terms of ER and Mito in mitotic cells at different axial sampling intervals of 50, 100, 200, 300, 400, 500, and 600 nm, $n = 31$ for ER, 42 for Mito. Center line, medians; limits, 75% and 25%; whiskers, 95% and 5%. These results show that GAR effectively improves the performance of SiS-rDL models; The PSNR of SiS-rDL denoised images decreases by less than 4% before the axial sampling rate is lower than Nyquist criterium, that is, 300 nm for LLSM, which suggests the general applicability of the SiS-rDL method for 3D imaging. Scale bar, 2 μm (a, b), 5 μm (f), 3 μm (zoom-in regions of f).



Extended Data Fig. 8 | Three-color rDL LLSM images of ER, H2B, and Golgi apparatus at different stages of mitosis. Three-color 3-D rendering (left panel) and single slices of X-Y view (right panel) showing the remodeling process of Golgi apparatus, as well as their interactions with ER at different stages of mitosis (A-H). Scale bar, 10 μ m.



Extended Data Fig. 9 | 3-D rendering of three-color rDL LLSM images of ER, H2B, and mitochondria (Mito) at different stages of mitosis. For each stage of mitosis, images of volume rendering (first row), single slices of X-Y view (second row), color-coded in z axis surface rendering of Mito in X-Y view (third row) and X-Z view (fourth row), respectively, are shown to visualize the segregation process of Mito and their interaction dynamics with ER during mitosis. Scale bar, 8 μm.



Extended Data Fig. 10 | See next page for caption.

Extended Data Fig. 10 | Sustained live imaging enabled by rationalized deep learning microscopy. a, Flowchart of the rDL SIM denoising and reconstruction algorithm. Scale bar, 1 μm . **b,** Synopsis of rDL imaging modalities, including (i) rDL TIRF-SIM, rDL GI-SIM, rDL 3D-SIM, and rDL LLS-SIM that utilize the physical model of SIM to guide the network training and inference processes; (ii) rDL-TiS LLSM and rDL-SiS LLSM, which utilize the spatial/temporal continuity of acquired

biological data to implement self-supervised denoising, yielding comparable results to the supervised methods. The rDL methods enable investigations into the fine spatial details, rapid kinetics and long-time dynamics of a variety of bioprocesses, showing great promise for shedding light on diverse biological phenomena.

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- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
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- ☒ ☐ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

No collected data. All data were acquired using our home-built Multi-SIM system, LLSM-SIM system and home-written control softwares in which the details can be found in the Methods section. The methods and conclusion reported in this work is not specific to the control program, but can be generalized to other optical imaging systems.

Data analysis

The statistical evaluation of proposed methods and analysis of biological data were performed with Matlab 2017b, Fiji and Imaris 9.0.1. The rDL denoised LLSM images were deconvolved using an iterative Richardson-Lucy algorithm performed by the Matlab function of deconvlucy. The deep learning models were trained, validated and tested using python3.6.10 and Tensorflow2.4.0. The source code of rDL deep learning model was uploaded into GitHub (<https://github.com/qc17-THU/rDL-SIM>), and the download information was added into the code availability section.

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Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Source data are provided with this paper. The published BioSR dataset was used to train the rDL models for the biological structures of ER, microtubule, actin, and clathrin coated pits in Figs. 1b, 2a-d, 3h-m, Extended Data Figs. 1-3, 4a, 5. The training data for the rDL models of other biological structures was also uploaded into

the figshare repository of BioSR dataset, which is publicly accessible via <https://doi.org/10.6084/m9.figshare.13264793>. All other data that support the findings of this study are available from the corresponding author on request.

Field-specific reporting

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For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was not predetermined based on statistical calculations. But for every experiment in this study, we performed 3~150 replications to ensure reproducibility. The sample size (n) of each experiment is provided in the figure legends in the main manuscript and supplementary information files.
Data exclusions	No data was excluded from the analysis.
Replication	The number of repetitions for each experiment is provided in the figure legends in the main manuscript and supplementary information files and all repetitions showed similar characteristics and performance.
Randomization	Samples were randomly assigned by independent persons.
Blinding	Data acquisition and analysis were being blinded to the experimental groups.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

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)	COS-7, U2OS, HeLa, HEK293T and R1 cell lines were originally from ATCC. COS-7, U2OS, HeLa, and HEK293T cell lines are gifts from Dr. Junjie Hu's lab in institute of Biophysics. R1 cell line is gift from Dr. Guohong Li's lab in Institute of Biophysics. SUM159 cell line is gift from Dr. Tom Kirchhausen's lab in Harvard Medical School, Boston Children's Hospital.
Authentication	These cell lines have been authenticated by the vendor.
Mycoplasma contamination	The cell lines used in this study are not contaminated by mycoplasma.
Commonly misidentified lines (See ICLAC register)	There is no misidentified cell line used in this study.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Wild-type P0 and 4-week male or female C57BL/6J mice were used for primary cell culture. The mice were housed under specific-
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Laboratory animals	pathogen-free (SPF) conditions in cages and a 12/12-hr light/dark photoperiod at 20-26 degree. The humidity of the housing room was maintained at 40-70% humidity.
Wild animals	We did not use any wild animals.
Field-collected samples	We did not use any field-collected samples.
Ethics oversight	Experiments involving mouse tissue were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of CAS center for excellence in molecular cell science, Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences.

Note that full information on the approval of the study protocol must also be provided in the manuscript.