Nonlinear structured-illumination microscopy with a photoswitchable protein reveals cellular structures at 50-nm resolution

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AUTHOR SUMMARY

The light microscope is an almost perfect tool for the life sciences, but its limited resolution means it is often ill suited for investigating life’s smallest and most puzzling mysteries. How is genetic information spatially structured and dynamically orchestrated? How do subcellular structures like organelles and proteins organize and interact with each other? What are the myriad ways in which cells, grow, divide, and communicate on nanometer-length scales?

In the last decade, with the help of technological advances, imaging with a light microscope has improved dramatically, and a number of techniques—collectively termed superresolution light microscopy—have succeeded in the endeavor to improve the resolution, such that it is more relevant on the scale of these biological questions. It is unfortunate then that these superresolution methods typically need thousands of raw images, which may not fully capture biology’s rapid and intricate dynamic processes, or extremely high light intensities are likely to damage biological samples. Here, we present a method that achieves a resolution of approximately 40 nm—or four times the resolution of a conventional light microscope—using 10- to 1,000-fold fewer images and a light intensity six to nine orders of magnitude lower than that of other superresolution methods. Thus, our method brings the field of superresolution microscopy closer to its ultimate goal of realizing these ultrahigh resolutions while retaining the qualities that have made it and will continue to make the light microscope an invaluable resource for biology.

Ernst Abbe formulated the diffraction limit of light more than a century ago, and up until the last few years, physicists and biologists have been operating in its shadow. However, in the last decade, a renaissance in fluorescence light microscopy has taken place, and numerous methods are now able to achieve resolution well beyond this “hard” physical boundary. Resolution of a few tens of nanometers is now possible, whereas previously, still subject to diffraction, the resolution was limited to a few hundreds of nanometers. Each of these subdiffraction or superresolution methods is based on one of two ideas: localization precision or patterned illumination light.

Localization-based technologies exploit the precision to which light from a single molecule can be mathematically determined (1). The goal of these techniques is to localize each individual fluorescent molecule—or fluorophore—in the sample (typically a single cell or region therein) to a precision well below the diffraction limit. Obtaining

Fig. P1. We used fluorescent photoswitching with structured-illumination light to increase the resolution of a wide-field microscope. The off state of the photoswitchable protein Dronpa was driven with a pattern of light (blue), so that only molecules in the minima of the pattern remained fluorescent (green). As the off state was saturated, the region of fluorescent molecules became smaller than diffraction (dashed green). This can be visualized in k-space where high-resolution information exists further away from the origin. Our method (green) resolves information beyond the conventional microscopy (pink) or even structured illumination using linear fluorescence (blue). To demonstrate our technique, we looked at the nuclear pore and the actin cytoskeleton.

Author contributions: M.G.L.G. led the project and conceived of the idea; E.H.R. and M.G.L.G. designed research; E.H.R. performed research; E.H.R., L.S., N.K.H., and M.W.D. contributed reagents/analytic tools; L.S. wrote the reconstruction software and edited the paper; J.J.M. performed a crucial photobleaching experiment; L.W., G.A.J., and E.H.R. built optical hardware; E.H.R. and M.G.L.G. analyzed data; and E.H.R. wrote the paper. The authors declare no conflict of interest.

This Direct Submission article had a prearranged editor.

Freely available online through the PNAS open access option.

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See full research article on page E135 of www.pnas.org.

Cite this Author Summary as: PNAS 10.1073/pnas.1107547108.
We focused the pattern orientations we needed for resolution enhancement. Studies. The illumination pattern was produced by a diffraction (TIRF)-SIM microscope similar to those described in earlier structures with a resolution of 40- to 70-nm resolution.

At a light intensity of switched between two spectrally distinct states using light (photoswitchable fluorescent protein (i.e., a protein that can be six to nine orders of magnitude lower than those needed for stimulated emission, we chose to exploit photoswitching, which resons of approximately 50 nm. Instead of saturation or nonlinear SIM (NL-SIM) technology, such that it could be implemented in three dimensions and better, more light-stable probe were developed, we believe that NL-SIM could be implemented in three dimensions and to living biological samples, as has been done for linear SIM.

In conclusion, we believe NL-SIM to be a powerful approach in the exciting new field of superresolution light microscopy. All superresolution techniques excel in certain aspects and fail in others—the best technique will be determined by the demands of the application. We believe NL-SIM to be the best for those applications that benefit from low light intensity and few exposures, at a resolution of approximately 40 nm in two dimensions with currently available photoswitchable fluorescent probes.