

Circumventing the optical diffraction limit with customized speckles

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Speckle patterns have been widely used in imaging techniques such as ghost imaging, dynamic speckle illumination microscopy, structured illumination microscopy, and photoacoustic fluctuation imaging. Recent advances in the ability to control the statistical properties of speckles has enabled the customization of speckle patterns for specific imaging applications. In this work, we design and create special speckle patterns for parallelized nonlinear pattern-illumination microscopy based on fluorescence photoswitching. We present a proof-of-principle experimental demonstration where we obtain a spatial resolution three times higher than the diffraction limit of

the illumination optics in our setup. Furthermore, we show that tailored speckles vastly outperform standard speckles. Our work establishes that customized speckles are a potent tool in parallelized super-resolution microscopy.

Introduction

When a large number of random partial waves interfere, an irregular pattern of speckled grains, commonly called a speckle pattern or speckles, is created. If the amplitudes and phases of the partial waves independently vary - and the phases are uniformly distributed over a range of 2π - the speckle pattern is fully developed.¹⁻³ Most speckle patterns satisfy Rayleigh statistics, wherein the joint probability distribution of the complex field is circular Gaussian, and the probability density function (PDF) of the intensity is a negative exponential^{1,2} The topology of a Rayleigh speckle pattern can be characterized as an irregular and interconnected low-intensity web with isolated high-intensity islands. While the bright speckle grains are all relatively isometric and isotropic,³ the dark regions surrounding optical vortices are anisotropic.⁴⁻⁷ In various imaging and metrology techniques speckle patterns are used to illuminate samples in order to improve precision, resolution, visibility, and sectioning while simultaneously providing a wide field of view.⁸⁻²¹

In fluorescence microscopy, a sub-diffraction-limited resolution can be obtained

with saturable structured illumination techniques. Examples include stimulated emission depletion (STED) microscopy,^{22,23} ground state depletion (GSD) microscopy,²⁴ and reversible saturable optical fluorescence transitions (RESOLFT).^{25–27} Generally, these techniques rely on spatially modulating the illumination intensity to toggle fluorescence on and off in a spatially selective manner. For example, in RESOLFT, a doughnut-shaped optical beam photoconverts all fluorophores in a region of a sample except those close to the vortex center, and therefore fluorescence is only emitted from a sub-diffraction-sized region. Usually, point scanning of the doughnut beam is required to construct an image which can be very time consuming. Recently, this process has been parallelized using either a one-dimensional (1D) standing wave pattern²⁸ or a two-dimensional (2D) lattice of doughnuts.²⁷ The first method involves rotating or translating the illumination pattern, and as a result, post-processing is required for 2D super-resolution since each individual pattern will only improve the resolution in one direction. In the second method, two incoherently-superimposed orthogonal standing waves create a square lattice of intensity minima, but axial uniformity of the intensity pattern hinders optical sectioning. One way to circumvent this issue is to use the optical vortices in a speckle pattern as the nonlinear structured illumination pattern.²⁹ Because fully-developed speckle patterns rapidly and non-repeatably change along the axial direction, they can be used to obtain a three-dimensional super-resolution image.³⁰ In this context, speckles are advantageous

because they are robust to optical distortions/aberrations while simultaneously enabling three-dimensional super-resolution.^{9–21,30,31} The problem with using Rayleigh speckles, however, is that the anisotropic and strongly fluctuating shapes of their optical vortices leads to non-isotropic and non-uniform improvements in the spatial resolution.

In this work, we introduce and test an ideal family of speckle patterns for nonlinear pattern-illumination microscopy. In order to have isotropic and uniform super-resolution, the speckle patterns need to have the following properties. All vortices in a speckle pattern must have a circular shape and an identical high-intensity halo surrounding each vortex core. Away from the randomly distributed optical vortices, however, the spatial intensity profile of the speckles should be uniform, with minor fluctuations. When this is the case, the speckles' intensity PDF is a narrow peak that can be approximated as a delta function. We thus refer to this family of speckles as “delta” speckles. To enable optical sectioning, the delta speckles' field must be fully developed and as a result the speckles' intensity pattern will rapidly and non-repeatably evolve upon axial propagation. Experimentally, we generate delta speckle patterns by modulating the phase front of a monochromatic laser beam with a spatial light modulator (SLM). Using delta speckle patterns, we photoconvert a planar fluorescent gel sample and measure the fluorescence signal from the unconverted regions in the vicinity of optical vortices. Not only do we demonstrate a $3\times$ enhanced spatial resolution relative to the optical diffraction limit of the illumination optics, but also

show the significant advantages of using customized speckles over Rayleigh speckles in fluorescence microscopy.

Results

Photoconversion and Fluorescence Microscopy. In our experiment, the fluorescent sample is a 25- μm thick layer of the photoconvertible protein mEos3.2 suspended in a gel.³² A continuous-wave (CW) laser beam with a wavelength of $\lambda = 405 \text{ nm}$ is used to photoconvert the protein. The laser's wavefront is modulated by a phase-only spatial light modulator (SLM), which generates a speckle pattern in the far-field (Fourier plane). The polarization of the far-field speckles' field is converted from linear to circular by a quarter-waveplate. The far-field speckle pattern illuminates and photoconverts the sample. The experimental setup is described in the Methods section and a schematic diagram can be found in the Supplementary Materials. Fig. 1a shows part of an example speckle pattern used to photoconvert the sample. The lateral dimension of the entire speckle pattern is 600 μm . The speckle pattern consists of two regions. Within the central region marked by the white dashed square, 300 $\mu\text{m} \times 300 \mu\text{m}$ in size, the speckle pattern obeys delta intensity statistics, i.e. a random array of circular vortices embedded in a nearly uniform intensity background. Outside the square, the speckles adhere to Rayleigh statistics, featuring sparse, nearly circular islands of high intensity, randomly distributed in a dark sea.

The average speckle grain size is 17 μm . See the Methods section for details on how the speckle pattern was created.

After photoconversion, we uniformly illuminate the sample with a $\lambda = 488 \text{ nm}$ CW laser to excite the unconverted proteins in the vicinity of the optical vortices. The unconverted protein fluorescence has a wavelength centered around $\lambda = 532 \text{ nm}$.³² We image the 2D fluorescent pattern with a spatial resolution of about 1.1 μm , which allows us to resolve features in the sample that are smaller than the diffraction limit of our illumination optics. Fig. 1b shows the fluorescence from the uniform protein gel sample after being photoconverted by the speckle pattern presented in Fig. 1a. Outside the central square, the sample is photoconverted by the Rayleigh speckle pattern. The fluorescent pattern consists of a sprawling anisotropic web, which reflects the topology of the low-intensity regions surrounding the optical vortices. In stark contrast, the fluorescence pattern within the central square, which is photoconverted by delta speckles, features isolated fluorescent spots of a much smaller size. They are all created by the optical vortices in the central square of Fig. 1a. The high level of isotropy exhibited by the fluorescent spots originates from the high degree of rotational symmetry that is present in the vortices generating them. Apart from these spots, the fluorescent intensity is uniformly low. This dark background arises from the homogeneity of the delta speckle pattern's intensity away from optical vortices. A qualitative comparison between the distinct fluorescence patterns inside and outside the

central square illustrates how customizing the intensity statistics of speckles can significantly enhance the performance of speckle-based pattern illumination microscopy.

Next, in Fig. 2, we quantitatively assess the performance of delta speckles in our application. We compare a diffraction-limited spot, shown in Fig. 2a, with the fluorescent spots produced in the central region of Fig. 1b to quantitatively determine the resolution enhancement. The diffraction-limited spot is created using the experimentally measured field-transmission matrix of the 405 nm light, and it gives the 2D point spread function (PSF) of our illumination optics. The full width at half maximum (FWHM) of the spot is $17\text{ }\mu\text{m}$, which is in agreement with the theoretically predicted value, $18 \pm 3\text{ }\mu\text{m}$, obtained from the diffraction limit, $\lambda/(2\text{NA})$, where NA is the numerical aperture of the 405 nm illumination optics. Fig. 2b shows an example fluorescent spot created by one of the vortices in the illuminating delta speckle pattern. Its shape is close to circular. We determine the full width at half maximum (FWHM) along both the major axis $a = 6.1\text{ }\mu\text{m}$ and the minor axis $b = 5.0\text{ }\mu\text{m}$ by fitting (see Methods section). The average spot size, $(a + b)/2 \simeq 5.6\text{ }\mu\text{m}$, is three times below the diffraction limit ($17\text{ }\mu\text{m}$).

To determine the isotropy of spatial resolution enhancement produced by the delta speckles, we measure the major axis a and minor axis b of all fluorescent spots in the central region. The aspect ratio b/a is a measure of isotropy. Using measurements from

a total of 89 fluorescent spots stemming from two independent delta speckle patterns, we calculate a histogram of the ratio (b/a) which is shown in Fig. 1c. All of the spots are close to circular. The mean value of b/a is 0.86. The most circular fluorescent spot has $b/a = 0.95$, and the least circular spot $b/a = 0.72$.

The size uniformity of the fluorescent spots is reflected in the box-plot analysis of the major and minor axis widths in Fig. 2d. The mean value of major and minor axis widths, marked by white lines, are $\langle a \rangle = 6.1 \mu\text{m}$ and $\langle b \rangle = 5.2 \mu\text{m}$. The black whiskers show the maximum and minimum of the ensemble. The edges of the blue and red shaded regions denote the upper and lower quartile (25% and 75%) of the data, which are $5.7 \mu\text{m}$ and $6.4 \mu\text{m}$ for the major axis a , $4.9 \mu\text{m}$ and $5.4 \mu\text{m}$ for the minor axis b . The green dashed line indicates the diffraction limit, i.e., the width of the PSF in **a**. Not only do all of the fluorescent spots have a uniform size, but they are also significantly smaller than the diffraction-limited spot size. Therefore, the spatial resolution enhancement provided by delta speckles is homogeneous.

Customized Speckle Statistics. In the previous section, we established the significant advantages of creating isotropic and isomeric vortices in the photoconverting speckle patterns used in fluorescence microscopy. Here we characterize the customized speckles' properties in detail and show how their vortex characteristics are transferred to the fluo-

rescent spots. To this end, we use a simpler experimental setup described in the Methods section.

An example of an experimentally measured delta speckle pattern is shown in Fig. 3a. The corresponding 2D phase profile of the speckle pattern is shown in **b**. While the 2D intensity profile of the speckle pattern is relatively homogeneous apart from the vortices, the corresponding phase profile is random and irregular everywhere. As shown in Supplementary Materials, the complex field of the delta speckles adheres to a circular non-Gaussian PDF. The phase has a uniform probability distribution within the range of $-\pi$ to π and the intensity is uncorrelated with the phase, thus the speckle field is fully developed.

In Fig. 3c, we compare the intensity PDF of an ensemble of 100 delta speckle patterns like the one shown in **a** (purple line) to a Rayleigh PDF (black and gray line). Because the Rayleigh intensity PDF exhibits an exponential decay, the most probable intensity values in a Rayleigh speckle pattern are close to 0. Therefore, the spatial profile of a Rayleigh speckle pattern is dominated by low-intensity regions surrounding the optical vortices. The high-intensity regions, which are the bright speckle grains, are sparse, well separated, and isotropic. In many ways the spatial profile of a delta speckle pattern is the inverse of a Rayleigh speckle pattern. Because the spatially uniform regions of high intensity dominate the spatial profile of a delta speckle pattern, the intensity PDF is narrowly peaked

and centered around the mean value $\langle I \rangle$. The peak's width is a reflection of the intensity fluctuations associated with the speckle grains. The presence of optical vortices, and the surrounding dark regions, in the speckle patterns result in a low-intensity tail in the PDF extending to $I = 0$ (marked by the orange arrow in **c**). Because the probability density in this tail is low, the vortices are sparse and spatially isolated.

Using the spatial field correlation function, $C_E(\Delta r)$, and the spatial intensity correlation function, $C_I(\Delta r)$, (see their definitions in Methods section) we can identify the characteristic length scales in a speckle pattern. For a Rayleigh speckle pattern, $C_I(\Delta r) = |C_E(\Delta r)|^2$, and therefore both the field and the intensity fluctuate on the same length scale. The spatial correlation length, defined as the full width at half maximum (FWHM) of $C_I(\Delta r)$, is determined by the diffraction limit. For a delta speckle pattern, $C_I(\Delta r)$ is narrower than $|C_E(\Delta r)|^2$, as can be seen in Fig. 3**d**. In the delta speckles, $|C_E(\Delta \mathbf{r})|^2$ remains the same as in Rayleigh speckles, because both patterns are generated by a phase-only SLM and their spatial frequency spectra are identical. Because $C_I(\Delta r)$ is narrower than $|C_E(\Delta r)|^2$ in a delta speckle pattern, it indicates that the intensity varies spatially faster than the field. Another difference is that with increasing Δr , the delta speckles' $C_I(\Delta r)$ exhibits a damped oscillation instead of a monotonic decay. These results can be understood as follows: according to the definition of $C_I(\Delta \mathbf{r})$, the features in the speckle patterns that contribute the most to the intensity correlation function are those where the

intensity deviates the most from the mean value. In a Rayleigh speckle pattern, the bright speckle grains have the greatest difference in intensity relative to the mean value. As a result, the speckle grain size determines the FWHM of $C_I(\Delta\mathbf{r})$ in a Rayleigh speckle pattern. Conversely, in a delta speckle pattern, the vortices differ from the mean intensity value more than any other element in the pattern. Therefore, the characteristic feature of the optical vortex dictates $C_I(\Delta\mathbf{r})$. For example, the high-intensity halo surrounding each vortex core is reflected in the negative correlation $C_I(\Delta\mathbf{r}) < 0$ around $\Delta r = 20 \mu\text{m}$, which corresponds to the distance from the vortex center to the high-intensity halo.

Vortex Characteristics. In our photoconversion experiment, the inverse of a speckle pattern is imprinted onto the uniform fluorescent sample. Therefore after photoconversion, the measured fluorescence originates from the vicinity of the optical vortices in the speckle pattern. Here, we investigate the shape and intensity fluctuation of such regions in delta speckles and in Rayleigh speckles. In Fig. 4a,b, we magnify two representative vortex-centered regions in a delta speckle pattern and a Rayleigh speckle pattern each. While the dark region surrounding the vortex cores in the delta speckle pattern is relatively circular, it is elongated and irregular in the Rayleigh speckle pattern. These properties are general and occur within one spatial correlation length from the vortex center. In Fig. 4c, we plot the radial intensity profiles averaged over all vortices in delta (purple line) and Rayleigh (black line) speckle patterns. The intensity rises more rapidly with distance from the vor-

tex center in delta speckles, leading to a smaller region of low intensity than in Rayleigh speckles. Intensity fluctuations from the average profile are described by the shaded area whose edges represent one standard deviation from the mean. The dramatically reduced intensity fluctuations in the neighborhood of vortices, in delta speckles, leads to more consistent vortex profiles than those in Rayleigh speckles.

The core structure of a vortex is characterized by the equal-intensity contour immediately surrounding the phase singularity. As with the fluorescent spots analyzed previously, this structure can be described by an ellipse whose major and minor axis lengths are a and b . The aspect ratio b/a reflects the degree of isotropy of the vortex core structure. In Fig. 4d, we plot the PDF of b/a , $P(b/a)$, obtained from 1,000 independent delta speckle patterns and compare it with that of Rayleigh speckles. For Rayleigh speckles, $P(b/a)$ has a maximum at $b/a = 0$, indicating the most probable shape of the intensity contour around a vortex is a line.⁵⁻⁷ Furthermore, the probability of a circular contour, $b/a = 1$, vanishes, confirming the absence of isotropic vortices in a Rayleigh speckle pattern. Contrarily, $P(b/a)$ for the delta speckles features a narrow peak centered at $b/a = 0.85$, meaning all vortices are nearly circular. For comparison, the probability that a vortex will have $b/a > 0.6$ is 99.7% in a delta speckle pattern, while the probability is only 6% in a Rayleigh speckle pattern.

Protein photoconversion simulation. In this section, we simulate the photoconversion process with a customized speckle pattern. We consider the case of a uniform sample photoconverted by an intensity pattern $I_p(\mathbf{r})$, starting from $t = 0$. The unconverted protein density $\rho(\mathbf{r}, t)$ satisfies the rate equation:

$$\frac{d\rho(\mathbf{r}, t)}{dt} = -qI_p(\mathbf{r})\rho(\mathbf{r}, t) \quad (1)$$

where q is a coefficient describing the photoconversion strength. The solution to this equation is given by

$$\rho(\mathbf{r}, t) = \rho_0 e^{-qI_p(\mathbf{r})t}, \quad (2)$$

where $\rho_0 = \rho(\mathbf{r}, 0)$ is the initial uniform protein density.

The fluorescence intensity from the unconverted proteins $I_e(\mathbf{r}, t)$ is proportional to $\rho(\mathbf{r}, t)$. Thus the negative of the photoconverting intensity $I_p(\mathbf{r}, t)$ is nonlinearly (exponentially) imprinted onto the fluorescence image $I_e(\mathbf{r}, t)$. In Fig. 5, we plot $\rho(\mathbf{r}, t)$ for both delta speckles (Fig. 5a) and Rayleigh speckles (Fig. 5d). With increasing photon-conversion time, the unconverted regions shrink. As can be seen in Fig. 5b & c, for delta speckles the unconverted regions evolve to isolated circular spots of homogeneous size. For Rayleigh speckles, the unconverted regions have irregular shapes and highly inhomogeneous sizes (Fig. 5e & f).

Our model demonstrates that the circularity of the low-intensity region surrounding

each vortex in a delta speckle pattern directly translates to the circularity of the corresponding fluorescent spots in the photoconverted sample. This supports our experimental findings, where the aspect ratio of the fluorescent spots has a mean value of $\langle b/a \rangle = 0.86$, which agrees with the mean aspect ratio of the optical vortices, $\langle b/a \rangle = 0.85$. Similarly, this model explains the homogeneity in size and shape of the fluorescent spots when a delta speckle pattern is used for photoconversion. When a Rayleigh speckle pattern is used for photoconversion, the vanishing likelihood of having circular vortices coupled with the irregular shape of the surrounding low intensity regions results in the scarcity of isotropic fluorescent spots.

Discussion and Conclusion

In summary, we have presented a proof-of-principle demonstration of parallel pattern illumination with a special family of speckle patterns. By customizing the statistical properties of speckle patterns for photonconversion, we obtain a spatial resolution that is three times higher than the diffraction limit of the illumination optics. The isometric, circular vortices in the tailored speckle pattern provide a homogeneous and isotropic spatial resolution enhancement, which cannot be obtained with standard Rayleigh speckles.

Since the photoconversion process is nonlinear, in principle, there is no limit on the

spatial resolution that can be reached. In reality, because the intensity of our photoconverting laser beam is relatively low, the photoconversion process takes a long time (~ 12 hours). During this time, sample drift and protein motion limit the spatial resolution that can be achieved with fluorescence from the unconverted regions. A further increase in spatial resolution is possible with dilute samples of immobile proteins.

In the photoconversion experiment, the illumination optics has a relatively low NA so that the fluorescence spots of size exceeding $4\text{ }\mu\text{m}$ can be well resolved with the detection optics of $1.1\text{ }\mu\text{m}$ resolution. To reach nanoscale resolution, high-NA optics is required to create the photoconverting speckle pattern. In this case, the vector nature of the light field must be considered. It is known that the axial field at a vortex center can be canceled by manipulating the polarization state of light.^{33,34} In a Rayleigh speckle pattern, the intensity contours around a vortex core are elliptical, and the polarization state must be an ellipse with an identical aspect ratio and an identical handedness in order to cancel the axial field.^{29,30} Since the elliptical contours vary in aspect ratio from one vortex to the next in a Rayleigh speckle pattern, it would be challenging, if not impossible, to set the polarization state of a speckle pattern to cancel the axial fields at all of its vortices. In a delta speckle pattern, in contrast, the vortices are almost circular, and half of them have the same handedness. With circularly polarized light, the axial fields will vanish at half of the vortices: specifically those with the same handedness.

In our experiment, a uniform film of purified protein is photoconverted by a speckle pattern. In the Supplementary Materials (Section 1) we show that this technique works with live yeast cells, which are nonuniformly distributed in space.

One additional advantage of delta speckles over Rayleigh speckles is their accelerated decorrelation upon propagation along the axial direction. As shown in the Supplementary Materials, the axial correlation length of a delta speckle pattern is three times shorter than that of a Rayleigh speckle pattern. The faster decorrelation of the delta speckles will enable 3D imaging with finer depth resolution.

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Author contributions N.B. performed the experiments, analyzed the data, and conducted numerical simulations. M.S. fabricated the samples. H.Y. contributed to the design of experimental setup and data acquisition. J.B. and H.C. supervised the research activities. N.B. prepared the manuscript, and the rest edited it and provided feedback.

Competing interests J.B. has financial interests in Bruker Corp. and Hamamatsu Photonics. The rest co-authors declare no competing interests.

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Methods

Photoconversion Experiment Setup. A detailed schematic of our experimental setup is presented in the Supplementary Materials. A CW laser operating at a wavelength of $\lambda = 405$ nm, is used to photoconvert the mEos3.2 protein sample. The laser beam is expanded relative to its source and linearly polarized before it is incident on a phase-only SLM (Meadowlark Optics). The pixels on the SLM can modulate the phase of the incident field between 0 and 2π : in increments of $2\pi/90$. Because a small portion of light reflected from the SLM is unmodulated, we write a binary phase diffraction grating on the SLM and use the light diffracted to the first order for photonconversion. In order to avoid cross-talk between the neighboring SLM pixels, 32×32 pixels are grouped to form one macropixel, and the binary diffraction grating is written within each macropixel with a period of 8 pixels. We use the square array of 32×32 macropixels in the central part of the phase modulating region of the SLM to shape the photoconverting laser light. The light modulated by our SLM is Fourier transformed by a lens with a focal length of

$f_1 = 500$ mm and cropped in the Fourier plane with a slit to keep only the first-order diffraction. The complex field on the Fourier plane of the SLM is imaged onto the surface of the sample by a second lens, with a focal length of $f_2 = 500$ mm. Using a $\lambda/4$ plate, we convert the linearly polarized photoconverting beam into a circularly polarized beam before it is incident upon the sample. In this setup, the full width at half-maximum of a diffraction-limited focal spot is $17\text{ }\mu\text{m}$.

After the photonconversion process, a second laser, operating at a wavelength of $\lambda = 488$ nm, uniformly illuminates the sample and excites the non-photoconverted mEos3.2 proteins. To collect the fluorescence, we use a $10\times$ objective of $\text{NA} = 0.25$ and a tube lens with a focal length of $f_3 = 150$ mm. The 2D fluorescence image is recorded by a CCD camera (Allied Vision Manta G-235B). The spatial resolution of the detection system is estimated to be $1.1\text{ }\mu\text{m}$. The remaining excitation laser light, which is not absorbed by the sample, is subsequently removed by two Chroma ET 535/70 bandpass filters. One filter is placed after the objective lens and reflects the excitation beam off the optical axis of our system. The second one is placed directly in front of the camera.

Purified Protein Sample Preparation. The plasmid for mEos3.2 expression is cloned with PCR and NEBuilder assembly, and transformed into BL21-CondonPlus (DE3) competent cells. The mEos3.2 protein is purified using Ni-NTA His-Bind resin and dialyzed

in dialysis buffer (20 mM Tris, pH 7.5/ 10 mM NaCl/ 1mM EDTA/ 10 mM BME). To immobilize mEos3.2, a mixture containing 42 μ L purified mEos3.2 (0.1 mM), 30 μ L 30.8 % Acrylamide/bis-acrylamide, 0.5 μ L 10 % APS, and 0.5 μ L TEMED is sandwiched between a clean coverslip and a slide to make a 25- μ m-thick gel.

Delta Speckle Generation Method. A delta speckle pattern can be generated following the speckle customization method outlined in our previous paper.³⁵ In this process, first, a Rayleigh speckle pattern is numerically generated by applying a random phase pattern with a uniform probability density in $(-\pi, \pi]$ to the experimentally measured field-transmission matrix of our wavefront shaping system. The recorded Rayleigh speckle pattern's intensity at every spatial location is numerically replaced by a constant value (equal to the mean intensity), while the phase is unchanged. This modified pattern has a delta-function intensity PDF and a circular joint PDF for the complex field. Using this pattern as the starting point in a local nonlinear optimization algorithm, we search for a solution for the SLM phase pattern which generates this pattern.³⁵⁻³⁷ A perfect match is impossible, however, due to the existence of phase singularities. Instead, local minima are found that correspond to speckle patterns with nearly uniform intensity apart from the optical vortices. The resulting intensity PDF has a narrow peak and a tail extending to zero, as denoted by the orange arrow in Fig. 3(c). To generate different speckle patterns with this approach, we simply initialize the SLM with different random phase patterns.

Unconverted Fluorescence Analysis. We use the following procedure to quantitatively analyze the size and shape of the fluorescent spots created by the optical vortices in a photoconverting delta speckle pattern. First, we locate the center of each fluorescent spot: $\mathbf{r}_c = \int \mathbf{r} I(\mathbf{r}) d\mathbf{r} / \int I(\mathbf{r}) d\mathbf{r}$. Using the $38 \mu\text{m} \times 38 \mu\text{m}$ region surrounding each fluorescent spot, which corresponds to 61×61 pixels on the CCD camera, we numerically construct a 2D interpolation of every fluorescent spot. Next, we rotate each interpolated grid around the center \mathbf{r}_c in increments of 1° , over a total of 180° , and record the 1D profile along the horizontal axis for each rotation. Then we fit each of these 1D profiles to a Gaussian function, $I_a \times \exp[-(x - x_0)^2 / (2\sigma^2)] + I_c$, with I_a , x_0 , σ , I_c as the fitting parameters. After determining the maximum and minimum values of σ from all rotation angles for each fluorescent spot, we extract both the major and minor axis widths from the full width at half maximum (FWHM) $2\sigma\sqrt{2\ln(2)}$. For the results shown in Fig. 2, the average R^2 fitting coefficient is 0.996. Generally the rotation angles corresponding to the maximum and the minimum widths are offset by 90° , reflecting the elliptical shape of fluorescent spots.

Speckle Characterization Setup. We use the following setup to characterize the statistical properties of the delta speckles (Figs. 3-5). A linearly-polarized CW laser beam with a wavelength of $\lambda = 642 \text{ nm}$ illuminates a phase-only reflective SLM (Hamamatsu LCoS X10468). The pixels on the SLM modulate the incident light's phase between 0 and 2π :

in increments of $2\pi/170$. We bypass the unmodulated light by writing a binary diffraction grating with a period of 8 pixels on the SLM and work with light in the first diffraction order. Macropixels are formed by groups of 16×16 pixels, each containing the binary diffraction grating. To generate a speckle pattern in the far-field, we modulate the square array of 32×32 macropixels in the central part of the phase modulating region of the SLM. Outside the central square, we display an orthogonal phase grating on the remaining illuminated pixels to diffract the laser beam away from the CCD camera (Allied Vision Prosilica GC660). The SLM and the CCD camera are placed on opposite focal planes of a lens ($f = 500$ mm). In this setup, the full width at half-maximum of a diffraction-limited focal spot is $29 \mu\text{m}$.

Definition of the spatial field/intensity correlation function. The spatial correlation function of the speckles' field is defined as:

$$C_E(\Delta\mathbf{r}) \equiv \frac{\langle E(\mathbf{r})E^*(\mathbf{r} + \Delta\mathbf{r}) \rangle}{\sqrt{\langle |E(\mathbf{r})|^2 \rangle} \sqrt{\langle |E(\mathbf{r} + \Delta\mathbf{r})|^2 \rangle}} \quad (3)$$

where $\langle \dots \rangle$ denotes spatial averaging over \mathbf{r} .

The spatial correlation function of the speckles' intensity is defined as:

$$C_I(\Delta\mathbf{r}) \equiv \frac{\langle \delta I(\mathbf{r})\delta I(\mathbf{r} + \Delta\mathbf{r}) \rangle}{\sqrt{\langle [\delta I(\mathbf{r})]^2 \rangle} \sqrt{\langle [\delta I(\mathbf{r} + \Delta\mathbf{r})]^2 \rangle}} \quad (4)$$

where $\delta I(\mathbf{r}) = I(\mathbf{r}) - \langle I(\mathbf{r}) \rangle$ denotes intensity fluctuations around the mean. Because the spatial correlation functions of the speckle patterns studied in this work are azimuthally

symmetric and depend only on the distance $\Delta r = |\Delta \mathbf{r}|$; $|C_E(\Delta \mathbf{r})|^2$ and $C_I(\Delta \mathbf{r})$ can be represented by $|C_E(\Delta r)|^2$ and $C_I(\Delta r)$.

Techniques For Characterizing Optical Vortices. We use the method outlined in Ref. ³⁸ to locate the optical vortices in our speckle patterns. We use the method outlined in Ref. ⁷ to fit the major and the minor axes of the constant intensity contours and compute the aspect ratios. The intensity profiles in Fig. 4c are obtained by taking the 1D intensity cross section along the horizontal axis of every vortex in 1,000 Rayleigh speckle patterns and 1,000 delta speckle patterns.

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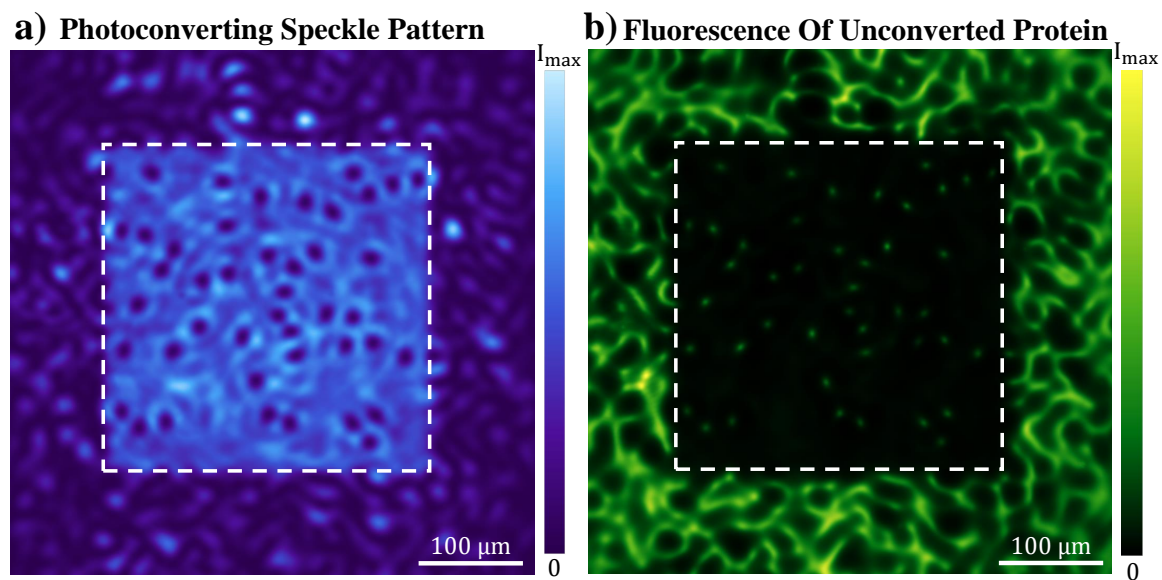


Figure 1: Customizing speckles for nonlinear pattern illumination. An experimentally recorded speckle pattern that illuminates and photoconverts a uniform fluorescent protein sample is shown in **a**. Within the white box the speckles obey delta statistics and outside they obey Rayleigh statistics. In **b**, an experimentally recorded image of the fluorescence from the unconverted regions shows isometric and isotropic spots produced by the vortices in the delta speckles; while the region photoconverted by the Rayleigh speckles features large, irregular, and interconnected fluorescent grains.

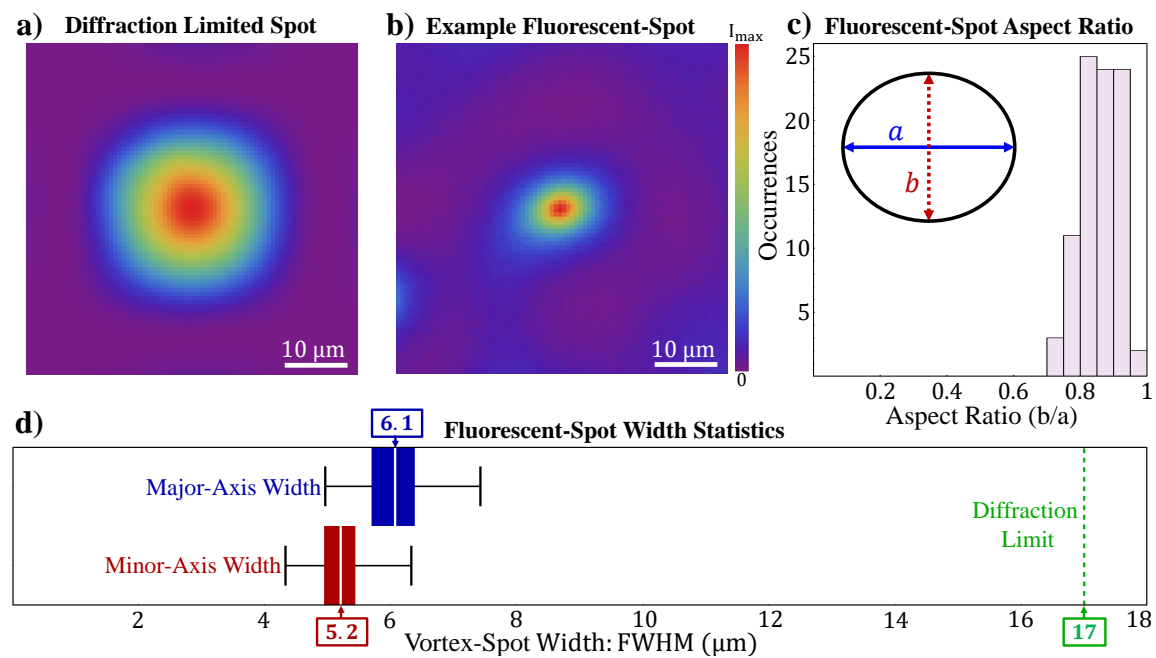


Figure 2: Circumventing the diffraction limit. A diffraction-limited spot of the illumination optics is presented in **a**. In **b**, an example fluorescent spot produced by a vortex in the delta speckle pattern is illustrated. Its size is much smaller than the diffraction-limited spot. Its shape can be fit by an ellipse of major axis width $a = 6.1 \mu\text{m}$ and minor axis width $b = 5 \mu\text{m}$. The aspect-ratio, b/a , histogram of all fluorescent spots produced by the delta speckles is shown in **c**. The inset illustrates an ellipse with the average aspect ratio $\langle b/a \rangle = 0.86$. In **d**, the box-plot analysis of the major and minor axes widths is shown. The white line marks the mean value, and the black whiskers represent the upper and lower bounds of the data. The edges of the blue and red shaded regions mark the upper and lower quartiles (25%, 75%) of the ensemble. The green dashed line indicates the FWHM of the diffraction-limited spot in **a**.

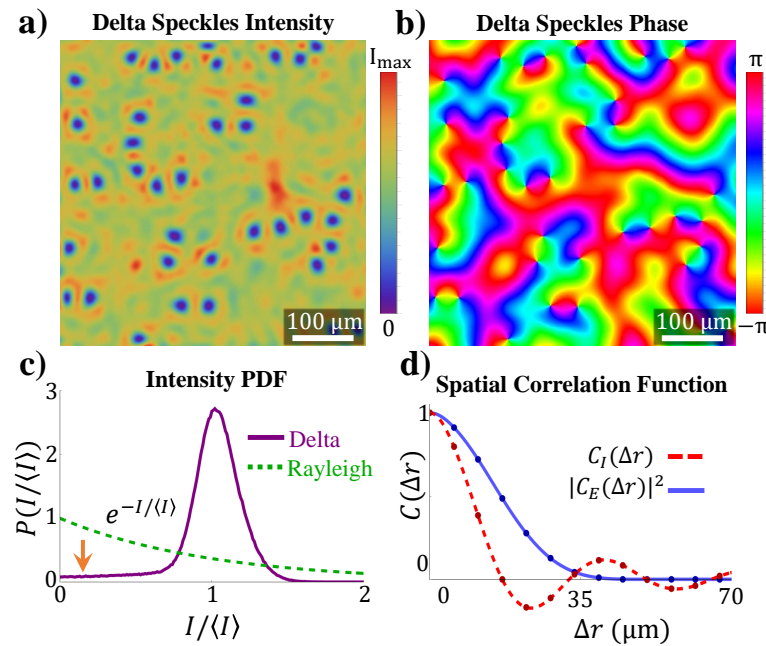


Figure 3: Customized Speckle Statistics. An example delta speckle pattern is presented in **a** and the corresponding phase distribution of its complex field is shown in **b**. The phase is randomly distributed between $-\pi$ and π , indicating that the speckle pattern is fully developed. The intensity PDF (purple solid line) of the delta speckle pattern is shown in **c** and compared with a Rayleigh PDF (green dashed line). The spatial correlation functions of the intensity (red dashed line) and the field (blue solid line) in the delta speckle pattern are shown in **d**. The spatial fluctuations of the intensity are faster than those of the field. The anti-correlation ($C_I < 0$) originates from the bright ring surrounding each vortex core. The plots both in **c** and **d** are obtained from an ensemble of 100 speckle patterns.

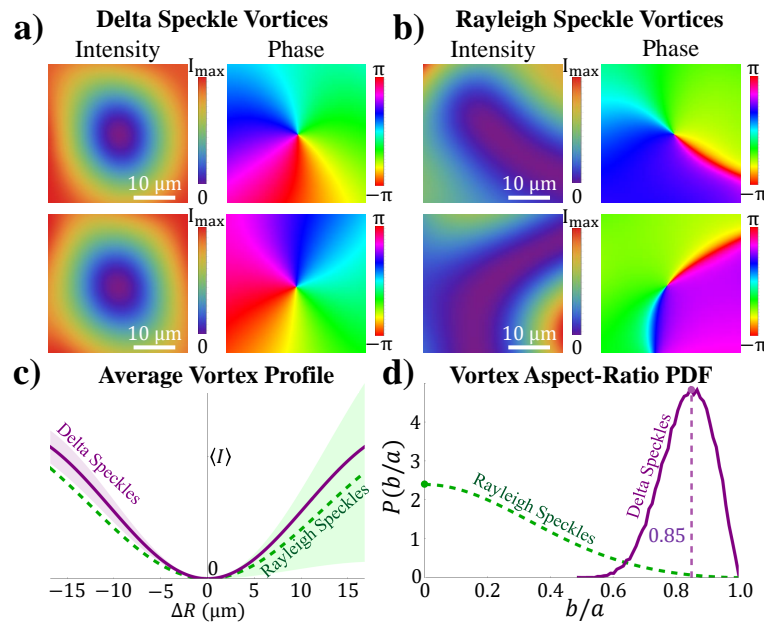


Figure 4: Optical Vortex Characteristics. Two example vortices from a delta speckle pattern, **a**, and from a Rayleigh speckle pattern, **b**, are shown. While the vortices in the delta speckles are nearly circular, the Rayleigh speckles' vortices have highly irregular shapes. This property is reflected in **c** where we plot the average intensity profile of light around vortices in 1,000 delta speckle patterns (purple solid line) and around vortices in 1,000 Rayleigh speckle patterns (green dashed line). The edge of the purple and green shaded regions indicates one standard deviation away from the corresponding mean profile. In **d**, we plot the probability density of the equal-intensity contours' aspect-ratio around the vortices in 1,000 delta speckle patterns (purple) next to the theoretical prediction for Rayleigh speckle patterns (green).

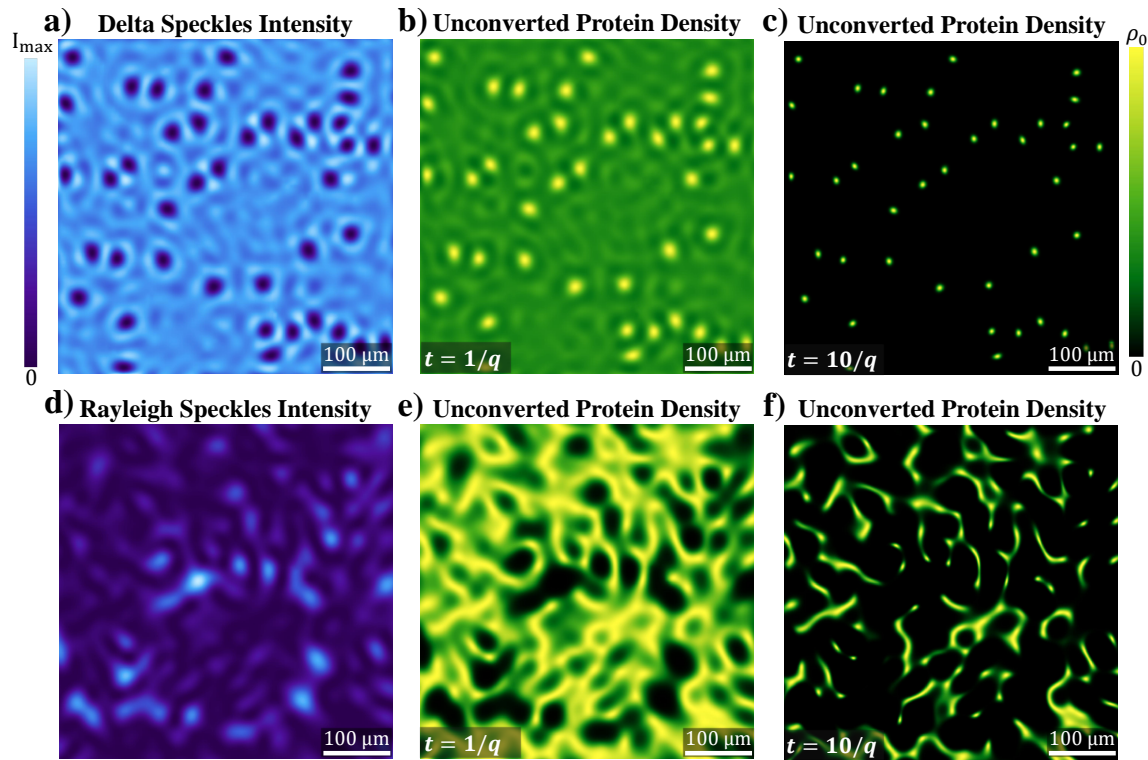


Figure 5: Photoconversion simulation. When a delta speckle pattern is used for photoconversion **a**, the unconverted regions near the vortices quickly evolve into isotropic and isomeric islands as seen in **b** at $t = 1/q$. At long timescales the islands remain, as shown in **c** where $t = 10/q$, yet they have considerably smaller size. When a Rayleigh speckle pattern is used for photoconversion **d**, an interconnected web forms instead of isolated islands at $t = 1/q$ as shown in **e**. Even at long time scales isolated and isotropic islands are rarely seen in **f**.