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Increased Localization Precision by Interference Fringe Analysis

Carl G. Ebeling^{1,‡}, Amihai Meiri^{2,*}, Jason Martineau¹, Zeev Zalevsky³, Jordan M. Gerton¹, and Rajesh Menon²

¹Department of Physics and Astronomy, University of Utah, Salt Lake City, UT 84112, USA

²Department of Electrical and Computer Engineering, University of Utah, Salt Lake City, UT 84112, USA

³Faculty of Engineering, Bar-Ilan University, Ramat-Gan, Israel

Abstract

We report a novel optical single-emitter-localization methodology that uses the phase induced by path length differences in a Mach-Zehnder interferometer to improve localization precision. Using information theory, we demonstrate that the localization capability of a modified Fourier domain signal generated by photon interference enables a more precise localization compared to a standard Gaussian intensity distribution of the corresponding point spread function. The calculations were verified by numerical simulations and an exemplary experiment, where the centers of metal nanoparticles were localized to a precision of 3 nm.

Introduction

Single particle localization¹ uses the Airy disk profile of imaged sparse single emitters to find the exact position of optical markers. These markers, mainly fluorophores^{2,3}, quantum dots^{4–6} and metal nanoparticles^{7–11} are often used to tag a biological sample (cell, proteins, organelles *etc.*) and to characterize intracellular structures and dynamics. The localization precision of these markers is better than the diffraction limit, down to tens of nanometers, and even a few nanometers in the case of bright samples^{2,3,12,13}. The localized position can be used to track the motion of the nanoparticle, and to characterize the dynamic and elastic properties of the investigated sample^{2,14}, or to image the sample with a sub-diffraction resolution of 20 nm to 50 nm^{15–17}.

Localization precision is a limiting factor in these particle tracking and super resolution techniques, and is limited by the signal-to-noise ratio of the collected photons. Specifically, the uncertainty in the location of a single emitter is $s/\sqrt{N}$ in the background negligible regime, where N is the number of detected photons, and s is the effective standard deviation of the point spread function (PSF), taking into account the detector-pixelation effect^{12,18,19}. This dependence is precision-limiting either when fast imaging is required or when the probes are weakly emitting.

*meirial@gmail.com.

‡These authors contributed equally.

In this paper, we propose an alternative to conventional localization approaches, one that involves measuring a relative phase acquired by the emitted photons through the detection path, in addition to the count rate. In the past, instruments have been designed to utilize interference^{20,21} that increases localization precision in the axial direction. Those methods rely on intensity differences between optical channels to record interference effects, rather than analysis of the fringe patterns directly. Several techniques modified the shape of the emitted signal to obtain precise axial position of an emitter; however, all these techniques were limited by the same theoretical limit of $s/\sqrt{N}$ for the in-plane localization precision. In the case of the double helix point spread function^{22–24}, it was shown that the DH-PSF has a lower limit (i.e. higher theoretical precision) for in-plane localization, however the improvement was limited and an estimator which can utilize this improvement was not introduced. We show that by using the acquired phase, the theoretical limit for localization precision can be fundamentally smaller than $s/\sqrt{N}$, thereby allowing for much faster imaging or being able to image dimmer sources with the same precision. We performed theoretical analysis and built a photon-interference system based upon a confocal design to measure this effect. In these proof-of-concept experiments, we localized the center of metal nanoparticles with a precision (standard deviation) as small as 3 nm. This Fourier-Interference-Localization Microscopy (FILM) has the potential to improve localization precision of scattering objects.

Theory

Consider the schematic shown in Fig. 1. Photons that are emitted from a point source exactly on the optical axis will emerge propagating parallel to the optical axis after an infinity-corrected microscope objective. On the other hand, if the point source was positioned off-axis, the photons will propagate at an angle to the optical axis due to the fact that the spatial location and the angular propagation of the emission beam in the Fourier plane are conjugate pairs. The propagation angle is proportional to the off-axis displacement of the point source. Here, we introduce a phase grating based Mach-Zehnder interferometer into the detection path which allows the signal to self-interfere, leading to an interference pattern on a detector placed at the interferometer output. The phase difference between the two arms of the interferometer depends very sensitively on the input angle, which directly maps to a lateral position in the sample plane. Denoting the path length in each of the arms as $P(\alpha \pm \beta)$, the phase difference between the arms is

$$\phi = \frac{2\pi (P(\alpha + \beta) - P(\alpha - \beta))}{\lambda}, \quad (1)$$

where

$$P(\alpha \pm \beta) = \frac{L}{\cos(\alpha \pm \beta)} \quad (2)$$

Here α is the grating diffraction angle, and β is the position dependent propagation angle, which can be approximated by $\beta = x_0 / f$, where x_0 and f are the nanoparticle's position and

the focal length of the objective lens, respectively. The phase difference can then be written as

$$\phi = \frac{2\pi L}{\lambda} \left[\frac{1}{\cos(\alpha + x_0/f)} - \frac{1}{\cos(\alpha - x_0/f)} \right] \quad (3)$$

The transmission grating interferometer produces a sinusoidal variation along the x -axis due to the wavefront of the two recombined beams not being constant across the detection plane of the interferometer; it is the interference of these two wavefronts that give rise to the interference or fringe pattern. The resultant is then the interference pattern of two (partially) coherent beams, which is given by [2]:

$$q(x) = f(x) [1 + \gamma \cos(\omega x + \phi)], \quad (4)$$

where γ is the interference fidelity (between 0 and 1), ω is the spatial frequency of the interference pattern, and ϕ is the phase of the fringe pattern, which corresponds to the path length difference between the interferometer arms. Due to the Fourier domain signal, $f(x)$ has the shape of the Optical Transfer Function (OTF) of the system.

The fringe frequency ω is related to the angle by

$$\omega = \frac{2\pi}{\lambda} [\sin(\alpha + \beta) + \sin(\alpha - \beta)] \quad (5)$$

The phase of the interference (or fringe) pattern on the detector for on-axis photons is thus distinct compared to that for slightly off-axis photons. By utilizing this phase difference, a point source can be localized to a higher precision than is otherwise possible for the same imaging conditions.

For single particle localization, we are interested in the localization precision of the source. According to the Cramer-Rao Lower Bound (CRLB), the uncertainty in the location of the single emitter is given by I^{-1} where I is the Fisher information matrix, which in turn, may be calculated from the FILM signal in eq. (4), $q(x, y)$ as

$$I(\theta) = N \int_{\mathbb{R}^2} \frac{1}{q(x, y)} \left[\frac{\partial q(x, y)}{\partial x}, \frac{\partial q(x, y)}{\partial y} \right]^T \left[\frac{\partial q(x, y)}{\partial x}, \frac{\partial q(x, y)}{\partial y} \right] dx dy, \quad (6)$$

where N is the number of detected photons^{18,25}. The number of detected photons is the key variance in all single emitter localization schemes. When only the intensity is measured, the localization precision is inversely proportional to the square root of the number of detected photons^{12,18}. We refer to this as the Gaussian PSF case. In our approach, the extra phase information allows for an enhanced precision in the localization information, due to the fact that $q(x, y)$ is a highly varying function. To elucidate this impact, we calculated the localization precision of the center of a point emitter by numerically evaluating eq. (6). To do that we first numerically normalized $q(x)$ such that its integral over the entire space will equal 1. The results are summarized in Fig. 2(a). The conventional Gaussian PSF fitting

scenario (blue dashed line) shows the familiar $1/\sqrt{N}$ dependence. The FILM case with ideal fringe visibility ($\gamma = 1$) shows the same $1/\sqrt{N}$ dependence, but with a scaling factor that yields an increase in the localization precision, as shown by the red dashed line. For FILM, an interferometer length of 1 m and grating diffraction angle of 30° were assumed. The localization precision is increased by a factor of 2 compared to the conventional PSF Gaussian fitting.

We further ran one-dimensional Monte-Carlo simulations to test the ability of FILM to improve localization precision. In these simulations, we used the same configuration for which we calculated the CRLB curve. We first generated a calibration curve using a noise-free scenario in a range of positions from -50 nm to 50 nm. We then simulated one single emitter at a time, calculated $f(x) = OTH(x)$ and imposed the interference fringes using eqs. (1)–(5) for the phase and fringe period. Since the Fourier domain signal may be distributed over many pixels, thus decreasing the signal-to-background ratio, we de-magnified the image by a factor of 6.8. This kind of de-magnification can be implemented using a simple 4F system. The nanoparticle position was randomly selected between -30 nm and 30 nm for each iteration, and shot noise was added by sampling a Poisson random process with a mean value that corresponds to each individual pixel values. To obtain accurate fitting we started by fitting the one dimensional fringe signal to a Gaussian shape to extract initial values for the center of the signal, envelope width (standard deviation), amplitude and background. We then divided the signal by the fitted Gaussian and used least squares fitting to a harmonic function to find initial values for the fringe frequency, visibility and the phase. We then used these two sets of initial values to obtain the nanoparticle position by fitting to eq. (4). The

localization error was calculated as $\Delta x_{rms} = \sqrt{E\{(\hat{x} - x_0)^2\}}$, where x_0 is the real position of the nanoparticle, and $\hat{x}$ is the calculated position. The expectation, $E\{\}$, was taken over all the iterations for a single photon count value. The results can be seen in Fig. 2(a), solid red line. We see that the localization error in FILM is smaller than the theoretical limit in the case of Gaussian PSF.

While shot-noise limited simulations and CRLB calculations show the potential of localization precision in a given method, background photons as source of noise, which are known contributors to localization error¹², should be taken into account. To test its effect on localization precision using FILM we added background photons to each pixel and introduced shot noise by sampling a Poisson random variable with mean that corresponds to the sum signal and background values. We assumed a photon count of $N=1000$. We then localized the single emitter using the fringe-fitting method. We compared this with Gaussian fitting of a single emitter in the conventional case. For this case we used pixel size of 50 nm. We can see in Fig. 2(b) that Fourier plane localization is less sensitive to background noise by inspecting the slope of the curves. Further, at $N=1000$, even when the background is 10 photons/pixel, the localization error is lower in the case of FILM than the CRLB limit for Gaussian PSF.

We also tested the influence of fringe visibility (γ) on the localization error. In these simulations the number of photon remained constant ($N=1000$) and the fringe visibility was

changed between 0.1 and 1. The results can be seen in Fig. 2(c), where a fringe visibility of 0.6 already showed improvement over Gaussian PSF.

Experimental Results and Discussion

We set up a confocal scanning imaging system in a scan/descan configuration, as shown in Fig. 3. A $\lambda = 561$ nm laser (Coherent, Sapphire 561 LP) was collimated and directed to the sample using a biaxial scanning mirror (Optics in Motion, OIM101). The scanning mirror, along with a scan and tube lens, are arranged in a $4f$ configuration such that the back pupil plane of the infinity corrected objective lens (Zeiss, Plan-Apochromat 63/1.40 Oil Iris) is conjugate to the scanning mirror plane. Using a flip mirror, light coming from the sample was then either directed to an APD (Micro Photon Devices, PDM APD) to obtain a conventional confocal image, or to an interferometer whose output is recorded by an EMCCD camera (Andor iXon Model DV885) to record the output of the interferometer. A second $4f$ system is used to project a Fourier plane image onto the entrance grating of the interferometer. The objective lens position is controlled using a piezo-driven focus stage (Physik Instrumente P-725 PIFO Objective Piezo), and the sample position is controlled with an x - y flat scanner (Prior, H117IX3). For the grating system, we used custom-made matched gratings optimized for the ± 1 diffraction orders. The aperture of the objective was stepped down to an effective NA of 0.7 to prevent clipping of the optical signal by the grating area. One should note that this is not a general restriction of the system, and NA of 1.4 can be used with larger gratings, effectively doubling the obtained localization precision. With knowledge of the objective properties, we can numerically convert the input angle into the interferometer, β , to a displacement of the focused spot in the sample plane.

For simplicity, in these preliminary experiments we utilize a modulation that is one-dimensional only. With the appropriate optical design and grating construction, this principle can be extended to a two-dimensional system.

As a point source in our experiment, we used a 100 nm Au nanoparticle on a silicon wafer. The 100 nm nanoparticles are not the limit for the system and were chosen for demonstration purposes only. Every nanoparticle, or other scattering beads, that can be imaged under these conditions, and which has a size smaller than the diffraction limit can be used for this method. Such photo-stable labels were previously used for tracking purposes^{7,14,26}.

We measured the light scattered from the silicon wafer, where the nanoparticle appears as a dark diffraction-limited spot (dark emitter, see Fig. 4(a)), due to their large extinction cross-section²⁷. A measured interference pattern (CCD image of the collected light through the interferometer) is shown in Fig. 4(b). After using the APD to isolate a single nanoparticle, the sample stage was used to position the nanoparticle relative to the center of the focus of the excitation beam. The stage was then moved in x through four 100nm steps, thus moving the nanoparticle with respect to the center of the excitation spot. Each position of the nanoparticle will give a slightly different spatial separation between the center of the excitation PSF and the location of the nanoparticle, and hence, a unique phase value φ is measured on the camera. For each position of the stage, 100 camera frames of the

interference pattern were recorded. Each recorded frame was then projected on to the x-axis by summing the columns and fit using eq. (4), where for $f(x)$ we used a Gaussian distribution. We used the same localization procedure as in the simulations with a slight change. The parameter of ω was used to discriminate poor localizations within the data sets. In some instances, the localization did not result in small residuals. In both of these cases, a small constrained random perturbation of the initial values was applied several times, and the result with lowest residuals was selected. Averaging over each set of 100 frames for each position of the nanoparticle yields an average value for the fringe spatial frequency of $\omega = 0.18 \text{ pixel}^{-1}$ with fringe visibility $\gamma = 0.91$. A plot of the extracted phase, ϕ , for four different stage positions, each separated by 100 nm steps, is shown in Fig. 4(c). The standard deviation of measured values of ϕ at each position is $\sim 70\text{--}90 \text{ mrad}$. Looking at eq. (3), we can use a Taylor series expansion to the second order around α . In this expansion we have to note that x_0 is on the order of magnitude of hundreds of nanometers at most, in the case of a scanning system, and the objective focal length f is in millimeters, thus $x_0 / f \ll 1$, therefore any high order term of x_0 / f will disappear. The second order term in the expansion disappears due to symmetry, and we are left only with

$$\Delta\phi = \frac{2\pi L}{\lambda} \left[\frac{1}{\cos(\alpha)} + \frac{\sin(\alpha)}{\cos^2(\alpha)} \left(\frac{x_0}{f} - \alpha \right) \right] \quad (7)$$

According to eq. (7), the relation between the phase and the spatial shift is linear, therefore we can find the slope of the ϕ versus x curve, by linear fitting of the experimental data. We calculated the corresponding localization standard deviation to be between 3nm and 5nm. Smaller steps during this calibration experiment essentially gave the same experimental results, thereby showing that calibration at 100nm steps is sufficient.

To compare the localization precision of FILM to that of localization using a traditional Gaussian PSF in a confocal setup, we acquired 100 frames of a single nanoparticle using the APD. We then performed a standard localization procedure by fitting these images to a Gaussian; the standard deviation of fitted Gaussian centers for these 100 measurements was $\sim 10 \text{ nm}$. By measuring the emitted power from the sample we can compare the total photon flux incident on the APD with that incident on the entrance grating of the interferometer. The photon count incident on the grating was 12% higher than that on the APD. Taking into account the higher quantum efficiency of the camera (0.6 vs 0.5 of the APD), this accounts for only $\sim 14\%$ of the more than 50% improvement in localization precision.

As can be seen in the phase inset of Fig. 4(c), the value of ϕ can wrap around 2π ; careful design of the system can result in phase values between 0 and 2π to eliminate ambiguity. Since the angle that light is incident on the gratings depends on the nanoparticle position, and the fringe period has a weak but detectable dependence on the entrance angle, β , the coarse position of the nanoparticle can also be extracted using the fringe frequency, see Fig. 4(d). We calculated the standard deviation of the extracted fringe period values for each position, and the largest standard deviation was $4.5 \cdot 10^{-6} \text{ pixel}^{-1}$, which corresponds to 13.1 nm in terms of localization precision. Therefore, a precise localization cannot be obtained

using this method, however, a coarse localization can be obtained, thus eliminating phase ambiguity.

When using the fringe frequency to obtain localization, one should consider the Nyquist criterion. The highest fringe frequency that can be used is determined by the pixel size P , i.e.

$2\pi / \omega > 2P$. Equation (5) can be also written as $\omega = \frac{4\pi}{\lambda} [\sin(\alpha)\cos(\beta)]$. Looking at the derivative, ω / x is 0 when $x=0$, i.e. the center of the imaged emitter overlaps the center of the illumination beam. The positions in Fig. 4(d) corresponds to the stage position, where there is a slight deviation between stage position 0 and $x=0$, thus the non-zero slope.

Combining the less accurate fringe frequency dependence with the fine localization obtained using the phase values, allows for coarse-grained scanning without loss of localization precision. Such a calibration curve can be used to localize single nanoparticles given an extracted phase value from a Fourier domain interference pattern.

One has to consider the effect of a slight defocus on the obtained results. If the calibration curve and the investigated nanoparticle are obtained on the same plane, a slight defocus will have no effect. If, on the other hand, the calibration curve is obtained using an emitter, which is in a different plane than the sample, the slight defocus will cause a constant phase shift of the fringes, thus resulting in a constant shift in the acquired position. If all the emitters have a constant shift in the estimated position, the constant shift can be eliminated by choosing an arbitrary particle as the origin and shifting all the localized locations accordingly.

To show that we localize actual single emitters using the proposed technique, we imaged three nanoparticles separated approximately $2\text{ }\mu\text{m}$ from each other, (Fig. 4(e), top). Using a calibration curve, the position of each nanoparticle relative to the illumination center could be determined from the measured phase values as the sample was scanned along the axis connecting the three nanoparticles. The actual nanoparticle position was then determined by $x = \hat{x}_0 + mL$, where $\hat{x}_0$ is the estimated position relative to the illumination beam (i.e. position within the scanning pixel), m is the scanning pixel index and L is the scanning step. The scanning step (pixel) for this measurement was $1\text{ }\mu\text{m}$, and 25 frames were averaged at each position. To calculate the positions of the three nanoparticles, the left nanoparticle was used as the reference point ($x = 0$). A calibration curve was then used to find the position of the middle and right nanoparticles relative to the left nanoparticle. For comparison, the three nanoparticles were scanned conventionally in confocal mode (with 40 nm step size) and imaged onto the APD. A conventional Gaussian localization algorithm was then applied to the image data, again using the position of the left nanoparticle as a reference. The results of the two fitting techniques are shown in Fig. 4(e) and demonstrate good agreement with each other, indicating that the FILM scheme can be used to localize single emitters. Slight discrepancies in values between the two methods is dominated by the position accuracy of the scanning stage, which is 50 nm . Due to the positioning accuracy of the stage, this last experiment cannot be used to characterize the localization precision of the technique, but it clearly shows that the nanoparticles, rather than direct reflections from the samples, are localized.

Importantly, the phase-sensitive approach yields more precise localization even though the sampling density for the scan was very low (1 μm step size), compared to the conventional confocal localization with 40-nm step size. In the case of the APD, the scan covered an area of $7\mu\text{m} \times 2\mu\text{m}$, with a 40nm scanning pixel size and 1.5ms pixel dwell time, for a total image acquisition time of $\sim 13\text{s}$. Measuring the position via the phase value of the fringes in the FILM signal, only 7 frames of the EMCCD were acquired over the 7 micron scan along the x direction, as only one data point per single nanoparticle is needed to estimate the position. At a 50ms frame rate, the acquisition time was 350ms, over an order of magnitude faster than that with the APD. Therefore this technique also offers speed advantage over conventional scanning methods. The acquisition time depends on the scanning step size and imaging instrument speed. FILM scanning speed was limited by the camera, therefore higher acquisition speeds may be obtained. It was shown in the past that nanoparticles smaller than 60 nm can be imaged with camera integration times shorter than 50 ms^{28,29}. Even if the APD scanning step size is 100 nm, this will decrease the acquisition speed by 6.25 times, still slower than the FILM scanning speed. To image a diffraction limited spot using a scanning system requires at least 4–5 pixels, where in the case of FILM 1 pixel is required in a diffraction limited spot. Using our current set-up with 1.5 ms dwell time for the APD and 50 ms for the camera, results in scanning time of 24 ms to 38 ms. However, one can choose to increase the spot size in FILM, thus increasing the FILM step size, as discussed later, and obtain faster imaging.

In the above measurements, backscattering of the laser from the sample leads to a specific fringe pattern, which is altered by the presence of a nanoparticle in the laser focus. To determine which scan positions have nanoparticles, the flip mirror was used to deflect the collected signal onto an APD to acquire a conventional confocal image of the scan area. At scan positions corresponding to nanoparticle locations, the fringe pattern image of the scan area exhibits a clear shift in phase relative to the Gaussian envelope function that defines the interference zone. For darkfield or fluorescence imaging, where the signals are expected to be “positive” (larger than background) the use of an APD would be completely redundant since the fringe pattern would appear on the camera only when the scattering nanoparticle is within the laser focus.

The detection efficiency in localization microscopy is an important factor in the localization precision. Considering the efficiency of the grating system, for a conventional binary phase grating, the ± 1 orders contain $\sim 41\%$ of the incident power each, thus providing an overall efficiency of 82%. This theoretical value can be obtained experimentally using gratings fabricated via e-beam lithography, photolithography, or other precise fabrication techniques. For the second grating, only one order is selected for each of the two interferometer arms, thus combined, the efficiency of the grating system is $\sim 33\%$. Due to the $\sqrt{N}$ dependence of the precision, this loss of photons results in loss of precision of $\sim 42\%$. Since our method is capable of increasing the localization precision by a factor of ~ 2 , the localization precision is still higher than conventional Gaussian fitting. More importantly, the experimental comparison between the Gaussian fitting and FILM took that into account by comparing the number of photons incident on the grating system with that incident on the APD.

The precision reduction due to loss of photons can be further minimized by using a single grating. To obtain the interference pattern one has to combine the two beams from the two interferometer arms at an angle. The angle determines the fringe period. To get a high fringe period, this angle between the two signals has to be small, thus increasing the interferometer length. Due to space constraints in our system we added another grating. By adding a second grating, the output angle of each arm will be $\theta_{out} = \theta_{\pm 1} + \theta_2$, where $\theta_{\pm 1}$ is the propagation angle at each of the arms, and θ_2 is the diffraction angle of the second grating. This allows us to have a small output angle (by setting the mirror angles), with a shorter interferometer. By removing the second grating and having a longer interferometer, the efficiency of the grating system is ~80%. Furthermore, in this setup, the grating is not necessary, and a beam splitter can be used instead. The efficiency here will depend on the incident angle, and the alignment may be more complex.

The particle size is not limited by the FILM method but by the chosen imaging modality. Nanoparticles a few nanometers in size were imaged using dark-field²⁹ microscopy or iSCAT³⁰ for localization purposes. The FILM method can use the dark field higher detection efficiency with the grating system (as the losses due to the gratings can be reduced to ~20%) to obtain higher localization precision than what is obtained using simple Gaussian fitting. A 2.2 nm Au nanoparticle has a scattering cross section 3.3 times higher than a 1.8 nm nanoparticle at a wavelength of 532 nm. Therefore, even when choosing a configuration with higher losses for convenience, as discussed in the previous paragraphs, where only 33% of the photons are collected, by replacing 1.8 nm nanoparticles with 2.2 nm, the same number of photons will be collected, maintaining the improvement factor of FILM, and the ability to image the nanoparticles will not be compromised. Moreover, larger nanoparticle/scattering beads were proven useful in the past, even with sizes of hundreds of nanometers^{14,31}.

Conclusion

In this paper, we showed that localization precision for isolated scatterers/emitters could be enhanced by extracting phase information from the scattered/emitted photons. The phase can be measured by allowing photons to interfere in a Mach-Zehnder interferometer positioned between the microscope objective and detector. By combining such a phase measurement technique with conventional localization schemes, the achievable precision of localization-based techniques can be increased significantly. Furthermore, this technique allows scanning of the sample with larger scanning steps than conventional scanning systems, allowing for faster scanning. For example, the total scanning time that was required to generate the APD image in Fig. 4 was 15 seconds, compared to 350ms required to scan the same 7 μ m range. This scanning speed corresponds to 300 μ m for 15 seconds. Here we showed that a scanning step of 1 μ m allows for the localization of a point emitter with high precision.

FILM can be applied for particle tracking of single metal nanoparticles (or any monochromatic scattering/absorbing point source). A diffraction limited excitation spot can be regarded as wide compared to the size of a point emitter. The only limitation it imposes is the travel distance of the tracked object. This can also be overcome with coarse correction of the position of the scanning spot using piezo-mirrors to ensure that the tracked object is close to the center of the beam. In nanometer scale tracking, the total distance travelled by

the object may not be more than a few PSF widths, or even less than a PSF width. For example, Yildiz et. Al. tracked Myosin motion over $1.5\mu\text{m}^2$, i.e. ~ 2 PSFs, and kinesin³² over 300nm, less than the PSF width. But more importantly, this method is not strictly limited to diffraction limited excitation spot. An arbitrary sized spot can be chosen, as long as there is a single emitter within this spot. A proper modification of the model, to allow better localization will be required in this case.

In particle tracking techniques, one is usually interested in tracking a single particle, or at least a single particle within a diffraction limited spot. To track multiple nanoparticles one has to make sure they are separated, or use different color markers. Metal nanoparticles have different scattering spectra, depending on their size, shape, material etc. To track multiple nanoparticles within a diffraction limited spot, there is a need for different nanoparticle species, with different scattering resonance peaks, and different lasers. Then a dichroic mirror can be used to split the two color signals into different interferometers.

Extending the technique to super-resolution microscopy requires adapting the proposed method to previously suggested nanoscopy methods using metal nanoparticles^{8–10}. In addition, applying the same principles to non-coherent fluorophore emission by allowing the broad-band beams to interfere in the same place by applying optical-path-length correction, which may be a subject of future work, will result in Fourier domain super-resolution imaging technique with enhanced resolution.

To extend this principle to two-dimensional localization, two-dimensional gratings are required, with two additional mirrors that will reflect the light back to interfere on the camera, in the same manner as in one-dimension. One can then find the phase shift in x and y independently to obtain the x and y position of the single emitter. Since the localization in x and y is done independently (separability of the Fourier transform), the theoretical analysis is valid for two-dimensional localization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Schematic of the interferometric-based FILM. (a) The signal from an on-axis emitter will propagate parallel to the optical axis after a Fourier transform generated by the lens. If the emitter were off-axis, the signal will propagate at an angle entering the interferometer after Fourier transform. (b) An on-axis source emits photons that acquire a different phase on their paths to the detector compared to those from (c) an off-axis source. By allowing each photon to interfere in the grating-mirror interferometer and capturing the interference

patterns on a CCD camera, it is possible to extract their phase and increase the localization precision of each emitter.

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Fig. 2.

Theoretical analysis and simulation results. (a) Theoretical limit of localization error of a single emitter as a function of the number of photons for the conventional case (blue dashed), FILM with ideal fringe visibility (red dashed) and fringe visibility obtained in our experiments (black dashed lines). The localization error was calculated using the Cramer-Rao Lower Bound (CRLB). Solid red line indicates the localization error obtained in our numerical simulations. (b) Localization error obtained by numerical simulations of a single emitter as a function of background photons, with constant signal photons $N=1000$, for Gaussian PSF, FILM. For comparison, CRLB curve when $N=1000$ is also shown.

Fig. 3.

Experimental setup. A confocal scanning imaging system was used for the experiments, where 5 lenses were used to obtain the Fourier transform of the optical signal. The input to the grating system lies in the Fourier plane, thus converting spatial shift in the sample plane, to propagation angle in Fourier plane. This is converted to path length difference using the grating interferometer, where the fringe pattern is recorded onto the EMCCD camera.

Fig. 4.

Experimental results (a) Single nanoparticle appearing as dark spot when imaged using the APD. Scale bar is 200 nm. (b) The measured interference pattern of photons from an on-axis beam representing a nanoparticle on the optical axis. Scale bar is 1 mm. The scale for the APD image indicates photon counts. For the EMCCD image, since this is a Fourier plane image, the background photons cannot be counted separately from the signal photons. (c) Phase extracted from the interference patterns as a function of emitter position relative to the stage position (red) and calibration curve obtained by a linear fit (black). The calculated standard deviation indicates that displacements as small as 5 nm can be clearly discerned. Inset: phase values (green) and unwrapped phase (blue) for the 400 frames captured (4

positions, 100 frames per position). The steps correspond to nanoparticle positions. (d) Extracted fringe frequency values for different emitter positions. (e) Localization of three nanoparticles (not in scale), at positions $x_0, 2L + x_1, 2L + x_2$, where L is the scanning pixel (step) size, using conventional Gaussian method (gray) of the nanoparticles from the APD image, and FILM (red). The values obtained by conventional fitting of Gaussian PSF from the APD image are 0, 2067 nm, 4160 nm and for the phase fitting using the FILM technique, the values are 0, 2099 nm, 4150 nm. The widths of the Gaussian profiles correspond to the expected standard deviations of localization using both methods. TOP: APD image. Scale bar is $1\mu\text{m}$.

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