Patterned Light Boosts the Resolution of a Lens

Abstract

A method to boost the resolution of a low numerical aperture (NA) lens by patterned light illumination is outlined and a proof-of-concept experimental demonstration is presented. In this method, a sample is illuminated by a series of finely detailed light patterns made by interference of coherent beams and a low NA lens and a CCD camera record a series of low resolution images. A high resolution image of the sample is then generated from a series of low resolution images guided by the knowledge of the illuminating patterns. A resolution greater than the best possible performance of a 0.3 NA lens whose working distance is less than 2 mm was experimentally demonstrated using a 0.1 NA lens at a working distance of 18 mm, breaking the link between the NA and the working distance for the first time. The result led to a foundation of important new possibilities in optical microscopy.

Optical microscopy has been playing an instrumental role in advancement of science and technology. Although vest amount of efforts have been made to improve the performance of the optical microscopy, the lens is still the principal determinant of its performance. However, physics of the lens imposes important limitations on optical microscopy. For example, the four key performance parameters of microscopy imaging, resolution, working distance, field of view, and depth of focus are unfavorably linked to each other by the lens. That is, high resolution, which is desirable, requires short working distance and small field of view, both of which are undesirable. Furthermore, as the wavelength of the light becomes shorter, it becomes increasingly difficult to achieve and maintain desired precision through the lens. This explains why it is such a challenging task to build a precision optical system for a very short wavelength of light, such as X-ray. A direct consequence of these limitations is that the kinds of problems that can be addressed using optical microscopy are still largely limited and the framework offered by the conventional optical system is not adequate in addressing the growing needs to overcome these limitations.

In this paper, we propose an alternative framework for an optical system that is promising in overcoming the above limitations and present a proof-of-concept experimental demonstration. The basic idea behind this proposal is to replace the physical perfection, which is hard to achieve and fixed once it is physically defined, with controllability and computational complexity.

Fig. 1 shows a schematic of the proposed system [1, 2]. Here, a low numerical aperture (NA) lens is employed at the detector side and resulting low resolution of the imager is boosted using a special illuminator made of N intersecting coherent beams. The sample is placed where the beams overlap. Interference between beams fills the region occupied by the sample with finely structured light pattern, which illuminate the sample. This is in contrast to the conventional microscopy where the sample is illuminated by a uniform light.

If the fine structures in the sample are too small to be resolved by the lens, the camera will only see a blurred image of the sample. Now suppose we illuminate the sample with a series of light patterns each with different fine structure in it. This can be done by changing the amplitudes and the phases of the interfering beams that produce the pattern. The camera will still see a series of blurred images, but the brightness of these images will change over time, depending on how well at each time the fine structures in the pattern matches the fine structures in the sample. This change in brightness conveys information on the unresolvable
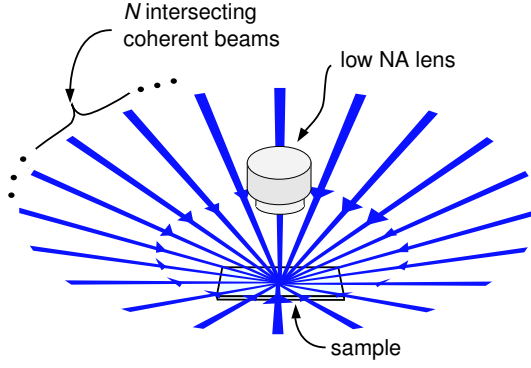
FIG. 1: A schematic showing the proposed setup to boost the resolution of a low NA lens. 15 intersecting coherent beams are arranged in a cone and a sample is placed where they overlap. Interference between beams creates finely detailed light pattern that illuminate the sample. See text for details.

fine structures in the sample. A high resolution image of the sample then can be generated from a series of low resolution images guided by the knowledge of the illuminating patterns.

Fig. 2 shows computer-simulated images of a point source acquired with 0.1 NA lens for two different illumination conditions. Fig. 2a corresponds to the conventional, uniform illumination case. The spread of intensity, or the blurriness of the image, represents the ultimate resolution limit of the 0.1 NA lens for a given wavelength of light. Fig. 2b shows the counterpart image for the proposed patterned light illumination made by 31 beams. Comparison of the full-width-half-maximum of the spread of intensity between the two cases shows an order of magnitude increase in resolution by the use of the patterned light illumination[6].

Key requirement in realizing this concept is the ability to project high resolution light patterns from the interference of sufficiently many beams. Furthermore, the identity of the projected pattern needs to be known within desired precision, which requires nano-meter precision. Resolution improvement by use of patterned light has been reported in microscopy [3–5]. However, the pattern set that can be generated by the reported works is very limited due to the fact that there are at best a few beams to generate the patterns. As a consequence, high NA lens is still required at the detector side. We have enabled the use of a very low NA lens, whose NA is as small as 0.1, at the detector side by developing a novel light pattern projector that utilizes several dozens of beams.

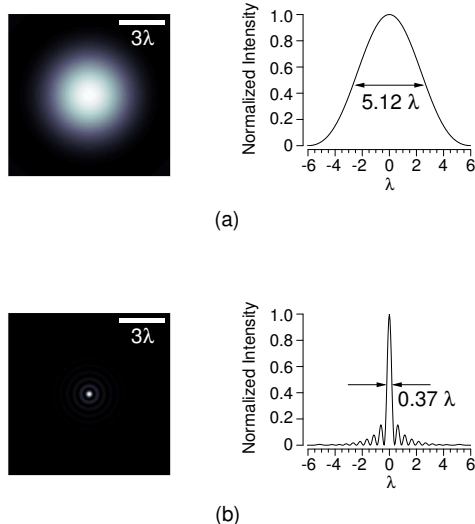In the developed illuminator, a single coherent beam from argon ion laser (model I304C,

FIG. 2: Computer-simulated images of a point source acquired with 0.1 NA lens under two different illumination conditions; the conventional uniform light (a) and the proposed patterned light (b). λ is the wavelength of the light. Next to the each image is the plot of the intensity along a line that passes the center of the image as a function of λ. The marked width in each plot corresponds to the full-width-half-maximum. For the case of the patterned light illumination in (b), 31 equally spaced beams arranged in a cone were used. The angle between the optical axis and the direction of propagation of each beam, or the *synthetic* cone angle of the illuminator, was 78 degrees.

Coherent, Inc., Santa Clara, California) was split into 31 diffracted beams by an acousto-optic deflector (AOD, model LS55-V, Isomet Corporation, Springfield, Virginia). A home made assembly of mirrors then converted an array of diffracted beams into a converging cone of beams, making them overlap and produce interference patterns. The amplitude and the phase of the individual diffracted beam were electronically controlled by the computer-generated drive signal for the AOD. This architecture for generating and controlling multiple beams enabled fast switching between patterns out of pre-selected pattern set without any moving part involved [1]. However, the desired precision cannot be achieved and maintained with the physical system only. Rather than trying to build a perfect physical system, we have developed a pattern calibration method that dynamically assesses mechanical imperfections. A more detailed description on the illuminator and the calibration method for it will be presented in a separate paper.

Here, we report a proof-of-concept experimental demonstration of the proposed method.
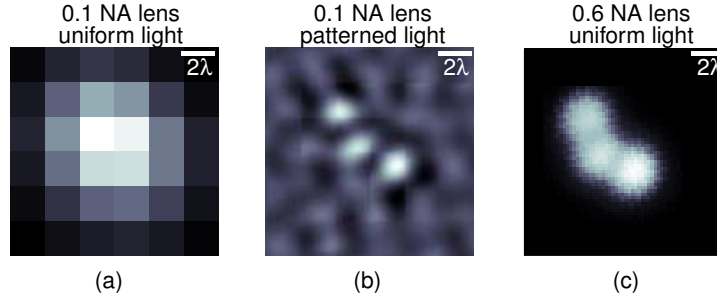
FIG. 3: The same region of a sample made of fluorescent polystyrene beads with diameter 1 μm imaged using three different techniques. (a) 0.1 NA lens with the conventional uniform light illumination. (b) 0.1 NA lens with the proposed patterned light illumination. (c) 0.6 NA lens with the conventional uniform illumination. $\lambda = 488 \ nm$. For (a) and (b), the same lens and camera were used and a region covered by 6×6 physical pixels are shown. For each physical pixel in (a), 20×20 sub-pixels were computationally inserted in (b) based on the proposed method. Notice the image in (c) still shows the presence of noticible blurring due to the finite aperture size. Comparing (b) and (c) suggests the proposed method whose an image formation operation is computational, not physical, can provide *sharper* image of the object.

A sample made of 1 μm diameter fluorescent polystyrene beads (Molecular Probes, Eugene, Oregon) was prepared and then imaged using a lens with the NA of 0.1. A series of low resolution images were acquired while the sample was illuminated by a series of pre-selected light patterns. Each time a pair of beams were interfered to illuminate the sample with the two-dimensional sinusoidal function of space (fringe pattern). The spatial frequency and spatial phase of the fringe pattern were changed so that a total of 656 patterns were projected. The exposure time at each frame was 0.6 sec.

The raw data was analyzed on a pixel by pixel basis. Time series of brightness for each pixel was processed to yield 165 distinct Fourier coefficients of an unknown feature within that pixel. These Fourier coefficients were combined to generate an image of the unknown feature. [7] This procedure was repeated for all pixels and images independently reconstructed for different pixels were then combined.

Figure 3a and figure 3b compares the two images of the same region of the sample acquired with the same lens (with 0.1 NA) and the camera under two different illumination conditions; the conventional uniform illumination and the proposed patterned illumination, respectively.

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In both cases, a region covered by 6×6 physical pixels are shown. The sample, verified by an image in Fig. 3c that was acquired with a lens with 0.6 NA using the uniform illumination, contains a constellation of three beads that are close to each other. Fig 3a clearly shows that the NA of the lens is too low to resolve fine features in the sample (the constellation of the beads). Computer simulation shows that, even under perfect imaging condition without any noise and with infinitesimally small pixel size, this particular constellation cannot be resolved due to the fundamental resolution limit. Beating of this fundamental limit is demonstrated in Fig. 3b that shows the counterpart image of Fig. 3a, acquired using the patterned illumination. A constellation of three adjacent beads is clearly seen in the image. This image was generated by computationally inserting 20×20 virtual sub-pixels into each physical pixels.

According to computer simulation, NA of 0.32 or higher is required to resolve a pair of beads each with 1 μm diameter that are adjacent to each other, under ideal imaging condition. The best lens from a leading manufacturer with the NA of this value or higher requires working distance shorter than 2 mm. We have experimentally demonstrated the same resolution at a working distance of 18 mm. In principle, current prototype system can achieve a resolution better than the theoretical limit of 0.97 NA lens at the same working distance. The working distance of the 0.97 NA lens is on the order of several hundred microns.

In this paper, we outlined an optical microscopy method where the resolution of a low NA lens can be boosted by a series of patterned light illumination. We also presented a proof-of-concept experimental demonstration of the proposed method. The result clearly led to a foundation for new avenues in optical microscopy. First, achieving high resolution without sacrificing other important performance measures will open up the possibilities of previously impossible investigations in biomedical sciences, precision metrology, projection lithography, etc. Secondly, the underlying philosophy of this method, which is to replace the physical perfection with electronic control and computation offers a promising new framework where an imaging system based on UV or X ray can be built.

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[3] B. Bailey, V. Krishnamurthi, D. L. Farkas, D. L. Taylor, and E. Lanni, Proc. SPIE **2184**, 208 (1994).

[4] M. G. L. Gustafsson, J. Microsc. **198**, 82 (1999).

[5] J. T. Frohn, H. F. Knapp, and A. Stemmer, Proc Natl Acad Sci USA **97**, 7232 (2000).

[6] It is worth noticing what determines the ultimate resolution limit for the two cases for a given wavelength of light. In uniform illumination case, the cone angle of the lens, expressed as the numerical aperture, sets the resolution limit. In patterned light illumination case, the highest spatial frequency in the illuminating pattern sets the resolution limit, which is determined by the angle between the optical axis and the beam.

[7] The result shown in Fig. 3 is the reconstruction made from the phases of the Fourier coefficients only, without the amplitudes. For each physical pixel, the amplitudes of all Fourier coefficients were made equal to the average brightness of the pixel. This is due to the fact that for the current prototype projector, the difference in amplitudes between beams is significant.