3-1-2013

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Snoeyink, Craig and Wereley, Steven, "Single-image far-field subdiffraction limit imaging with axicon" (2013). *Birck and NCN Publications.* Paper 1359.
http://dx.doi.org/10.1364/OL.38.000625

Single-image far-field subdiffraction limit imaging with axicon

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Received November 5, 2012; revised January 3, 2013; accepted January 11, 2013; posted January 24, 2013 (Doc. ID 179089); published February 21, 2013

This Letter presents a technique for subdiffraction limit imaging termed Bessel beam microscopy (BBM). By placing a lens in series with an axicon in the optical path of a microscope, the diffraction-limited resolution of the base microscope is improved by one third. This improvement is demonstrated experimentally by resolving individual subdiffraction limit fluorescent beads in a close-pack arrangement. The behavior of the BBM system is explored using angular diffraction simulations, demonstrating the possibility of resolving features spaced as little as 110 nm apart when viewed with a 100 × 1.4 NA objective. Unique among super-resolution techniques, BBM acquires subdiffraction limit information in a single image with broadband unstructured illumination using only static geometric optics placed between the microscope and camera.

OCIS codes: 180.2520, 110.0180.

Far-field optical microscopy is a flexible imaging tool that has become a staple of experimental sciences. The diffraction barrier, however, limits the imaging resolution of conventional microscopes to 200–300 nm in the lateral dimension, leaving many subjects unresolvable. New tools intended to lower that barrier have progressed rapidly in recent years. For example, structured-illumination microscopy can double the resolution of a microscope by manipulating the illumination pattern used [1,2]. Alternatively, the diffraction limit can be reduced by shaping the point spread function (PSF) of the microscope. This has been most effectively implemented through scanning confocal microscopy, where a mask can be used to block the intense sidelobes to the PSF that result [3,4]. While the utility of these methods is unquestionable, they do have their drawbacks. Common to all methods is a need to acquire several to many images, as well as specialized illumination, fluorescent tags, or both in order to reconstruct higher resolution information [5].

Here we present a novel (to our knowledge) approach to imaging capable of resolving features below the diffraction limit of a conventional microscope using single image acquisition and without the need for specialized illumination. This system, termed Bessel beam microscopy (BBM), increases spatial resolution by manipulating the light after it has passed through the microscope, as shown in Fig. 1. This approach is related to attempts at increasing the spatial resolution of optical systems through annular apertures in the system pupil [6,7].

The BBM system consists, at a minimum, of two optical elements placed between the microscope and camera. The first is a convex lens placed its focal length away from the imaging plane of the microscope. Immediately following the lens is an axicon, a unique optical element with a conical, as opposed to spherical, surface. This combination transforms the wavefront of a point source into a Bessel beam, which is known for its ability to propagate without diffracting [8,9].

When the Bessel beam is imaged by a camera, the resulting intensity profile has the following form:

$$I(r_c) \propto J_0^2(s), \tag{1}$$

where J_0 is a zero-order Bessel function of the first kind, $s = (2\pi/\lambda)((r_c\alpha(n-1))/D)$, λ is the light's wavelength, r_c is the radial distance from the beam center, α is the surface angle of the axicon, n is the axicon's index of refraction, and D is a component of the system matrix for the optical system in between the axicon and camera [9,10]. The treatment of optical elements after the axicon is included in this derivation, because it allows the experimenter to adjust the width of the Bessel beam's central peak without changing the axicon. In the context of this manuscript, the C and D components of the system matrix can be referred to as the "magnification" and "PSF width" components, respectively. Since Eq. (1) describes the intensity profile of a point source image, it also effectively describes the PSF of the Bessel microscopy system. It should also be noted that this is similar in form to the PSF of an optical system with an annular pupil in the limit of infinitely small pupil width [11].

In this derivation we are using the Rayleigh criterion to describe the resolution of a system [12]. The Rayleigh

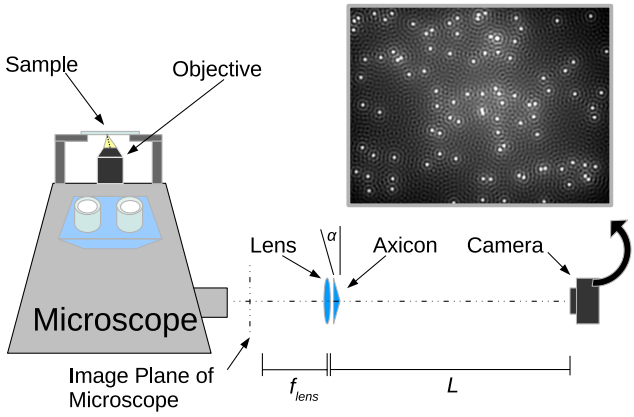

Fig. 1. (Color online) Schematic of basic BBM setup. A convex lens is its focal length away from the image plane. Immediately following is an axicon, then space for the Bessel beam to propagate, and finally a camera.

criterion defines the resolution limit of an optical imaging system as the point source spacing at which the maximum for one point source image lies in the first minimum of the other. Given that the first zero of J_0 is found at $s = 2.401$, the first minimum of Eq. (1) is located at

$$s_0 = 2.401\frac{\lambda D}{2\pi\alpha(n-1)}. \tag{2}$$

Notably, s_0 is a function of only the wavelength, the properties of the axicon, and the optical system after the axicon. The width of the PSF is thus decoupled from the NA of the imaging system.

The nondiffractive property of the Bessel beam plays an important role in the BBM system. Since the intensity profile is constant over extended distances, there is no unique focal plane in which to place the camera. Instead the investigator can move the camera, within limits, to adjust the effective magnification and field of view [9]. These limits are dictated by the distance over which the Bessel beam maintains its integrity, beyond which the system ceases to form coherent images. Using geometry, the largest value of C, the magnification component for the optical system between the axicon and camera for which the interference pattern persists is given by

$$C_{\max} = \frac{r_a D}{\alpha(n-1)}, \tag{3}$$

where r_a is the radius of the beam incident upon the axicon. If nothing is placed between the axicon and camera, $C = L$, the distance between the axicon and camera, and $D = 1$. As a result, this maximum magnification component imposes an upper limit on the axicon–camera distance. If the focal length of the convex lens is chosen to be equal to that of the tube lens for the base microscope, then r_a is equal to the effective aperture of the base microscope and is a function of the objective's NA [13].

As long as the angular magnification of the microscope is preserved, then any distance on the camera sensor is related to the distance in the measurement plane by the following relationship:

$$\frac{d_m}{f_{\text{obj}}} = \frac{d_i}{C}, \tag{4}$$

where d_m is a distance in the measurement plane, d_i is a distance in the imaging sensor, and f_{obj} is the focal length of the microscope objective. To determine the minimum resolvable distance between two point sources in the measurement plane (d_m), the distance in the imaging plane is set to the distance used in the Rayleigh criterion, and the maximum possible value for C, the magnification component, is used from Eq. (3):

$$d_m = 0.38\frac{\lambda}{r_a/f_{\text{obj}}}. \tag{5}$$

Equation (5) can be made comparable to other expressions for diffraction-limited resolution by applying the following definition for numerical aperture: $\text{NA} = r_a/f_{\text{obj}}$. This results in the following expression for diffraction-limited resolution of the BBM system:

$$d_m = 0.38\frac{\lambda}{\text{NA}}. \tag{6}$$

Equation (6) is similar to the familiar diffraction-limited resolution of a conventional microscope ($d_m = 0.61\lambda/\text{NA}$), but with a coefficient of 0.38 as opposed to 0.61 for a conventional microscope. The conclusion is that the addition of the BBM system reduces the minimum resolvable feature size by at least one third.

However, this increase in spatial resolution comes at the cost of light. The Bessel beam used in the BBM system is a result of an overlapping beam of light interfering with itself. At the farthest extent of the beam, only the most distal rays are interfering and contributing to the central peak. This loss of image intensity could limit the application of BBM in cases of especially dim illumination, short camera exposure times, or rapid motion in the field of view.

Experimentally this increase in resolution is demonstrated in Fig. 2. Here, 500 nm fluorescent polystyrene beads emitting at 612 nm are imaged with (a) a $40\times$ 0.6 NA objective and (b) a 40×0.6 NA objective with a BBM attachment. This experiment was chosen because adjacent beads are known to be below the diffraction limit of this microscope and objective and thus indistinguishable [13]. Therefore, the ability to resolve individual particles, as can be clearly seen in Fig. 2(b), is an unambiguous indication that the ultimate resolution of the microscope has been enhanced. As discussed earlier, much of the light from the measurement plane is not used in the formation of the image. Instead it remains as a general haze that surrounds the particles in Fig. 2(b), which lowers the effective signal-to-noise ratio.

The radially averaged power spectrums for the two images shown in Fig. 2 are plotted in Fig. 3. Here, as expected, the spatial frequencies of both the base microscope (dashed) and the BBM-enhanced microscope (solid) are limited to the same degree by the aperture of the microscope objective [10]. However, the power spectrum of the BBM-enhanced microscope shows a distinct increase in the energy present in spatial frequencies adjacent to the cutoff frequency, indicating a possible motivation for the increased resolution.

An angular spectrum diffraction simulation was performed to systematically investigate the difference in resolution offered by the BBM attachment [14]. The microscope has an idealized 100×1.4 NA oil immersion

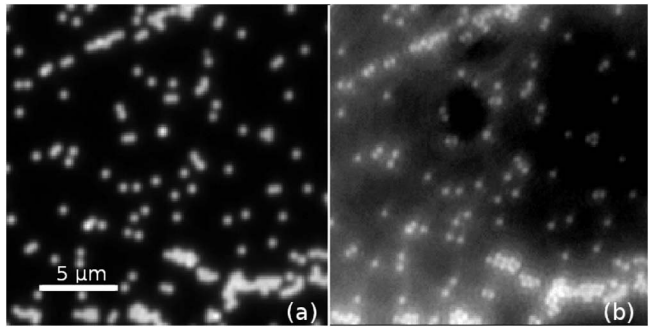

Fig. 2. Images of 500 nm fluorescent beads imaged with a 40×0.6 NA objective microscope both (a) without and (b) with the BBM attachment.

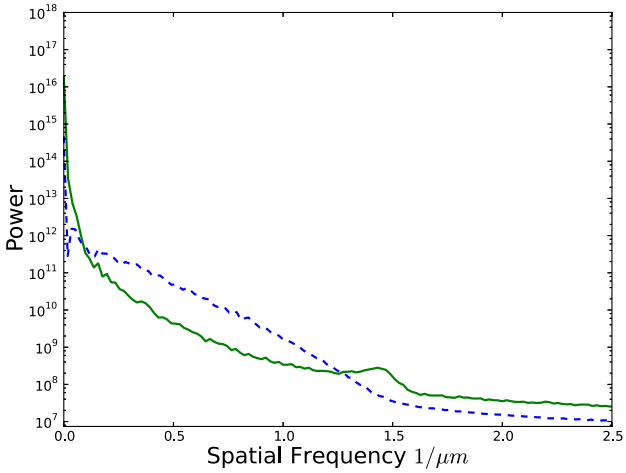

Fig. 3. (Color online) Power spectrum plot of images shown in Figs. 2(a) (dashed) and 2(b) (solid).

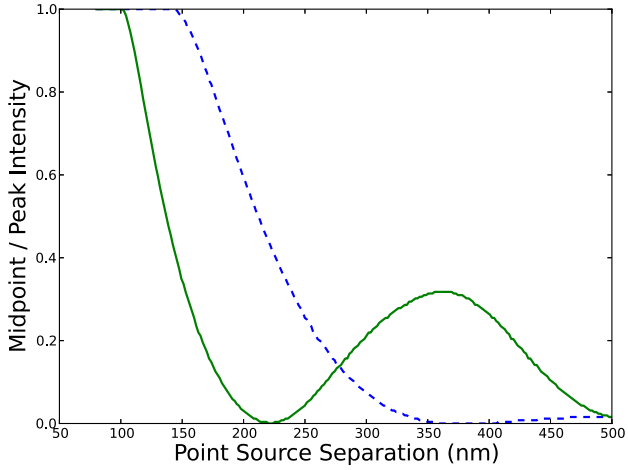

Fig. 4. (Color online) Ratio of peak intensity to midpoint intensity for point sources emitting at 400 nm as a function of separation distance as imaged by a 100×1.4 NA microscope both with (solid) and without (dashed) the BBM attachment.

objective, modeled as a single ideal lens. With this simulation the ratio of peak to midpoint intensity was explored for a range of incoherent point source separations at a wavelength of 400 nm, the results of which are plotted in Fig. 4 for the base microscope both with (solid) and without (dashed) the BBM attachment. For a 26% dip in intensity, a level of contrast consistent with the Rayleigh criterion applied to Airy disk PSF, the BBM system achieves a diffraction-limited resolution that is 33% smaller. This increase in resolution is consistent with the derived maximum resolution in [6]. The reason for the haze in Fig. 2(b) is also evident in Fig. 4. At larger distances the contrast of the BBM system decreases due to the strong sidelobes of the BBM PSF.

The BBM system constitutes a novel approach to super-resolution microscopy. Using simple geometric optics, it is possible to decrease the diffraction-limited resolution by approximately 33%. Further, this resolution improvement is obtainable with broadband illumination and common fluorescent dyes and is acquired in a single image. This imaging method is also customizable. The width of the Bessel PSF and the magnification can be optimized, within limits, for the pixel size of the camera and the feature size of the sample. Finally, it is important to note that the BBM attachment can be added to any traditional imaging system, for example, a telescope, to increase the diffraction-limited resolution.

References

1. M. A. A. Neil, R. Juskaitus, and T. Wilson, Opt. Lett. **22**, 1905 (2003).
2. M. G. L. Gustafsson, J. Microsc. **198**, 82 (2000).
3. M. A. A. Neil, R. Juškaitis, T. Wilson, Z. J. Laczik, and V. Sarafis, Opt. Lett. **25**, 245 (2000).
4. T. R. M. Sales and G. M. Morris, J. Opt. Soc. Am. A **14**, 1637 (1997).
5. S. W. Hell, Science **316**, 1153 (2007).
6. A. Vainrub, O. Pustovyy, and V. Vodyanoy, Opt. Lett. **31**, 2855 (2006).
7. T. R. Sales and G. M. Morris, Opt. Lett. **22**, 582 (1997).
8. J. Durnin and J. J. Miceli, Phys. Rev. Lett. **58**, 1499 (1987).
9. C. Snoeyink and S. Wereley, Opt. Lett. **37**, 2058 (2012).
10. J. Goodman, *Introduction to Fourier Optics* (Roberts, 2004).
11. C. J. R. Sheppard and A. Choudhury, Appl. Opt. **43**, 4322 (2004).
12. M. Born and E. Wolf, *Principles of Optics* (Cambridge University, 1999).
13. C. D. Meinhart and S. T. Wereley, Meas. Sci. Technol. **14**, 1047 (2003).
14. N. Delen and B. Hooker, J. Opt. Soc. Am. A **15**, 857 (1998).