ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/269319468

Conical diffraction as a versatile building block to implement new imaging modalities for superresolution in fluorescence microscopy

Conference Paper *in* Proceedings of SPIE - The International Society for Optical Engineering · August 2014

Impact Factor: 0.2 · DOI: 10.1117/12.2061059

CITATIONS
3

READS
44

8 authors, including:

Clement Fallet
Parrot S.A.

25 PUBLICATIONS **137** CITATIONS

SEE PROFILE

All in-text references underlined in blue are linked to publications on ResearchGate, letting you access and read them immediately.

Available from: Clement Fallet
Retrieved on: 03 June 2016

Conical diffraction as a versatile building block to implement new imaging modalities for superresolution in fluorescence microscopy

Clément Fallet[a], Julien Caron[a], Stephane Oddos[a], Jean-Yves Tinevez[b], Lionel Moisan[c], Gabriel Y. Sirat[a], Philippe O. Braitbart[a], Spencer L. Shorte[b]

[a]Bioaxial SAS, 24 rue du Faubourg St Jacques, Paris, FRANCE;
[b]Institut Pasteur, 25 rue du Dr Roux, Paris, FRANCE;
[c]Universite Paris Descartes, Laboratoire MAP5, 45 rue des Saints-Peres, Paris, FRANCE

ABSTRACT

We present a new technology for super-resolution fluorescence imaging, based on conical diffraction. Conical diffraction is a linear, singular phenomenon taking place when a polarized beam is diffracted through a biaxial crystal. The illumination patterns generated by conical diffraction are more compact than the classical Gaussian beam; we use them to generate a super-resolution imaging modality. Conical Diffraction Microscopy (CODIM) resolution enhancement can be achieved with any type of objective on any kind of sample preparation and standard fluorophores. Conical diffraction can be used in multiple fashion to create new and disruptive technologies for super-resolution microscopy. This paper will focus on the first one that has been implemented and give a glimpse at what the future of microscopy using conical diffraction could be.

Keywords: Superresolution, fluorescence microscopy, conical diffraction

1. INTRODUCTION

Microscopy allows biologists to observe the small details of the living. In order to understand better the complex structures of biological objects, a good resolution is necessary. However, the laws of physics set a limit to the minimal size an imaged spot of light may reach. This boundary was first stated by Ernst Abbe in the 19th century using the wave theory of light. It gives an absolute boundary to the resolution a standard microscope may achieve. Recent techniques have nevertheless succeeded in turning around this problem and achieving higher resolutions. Since the beginning of the 1990's and 2000's, superresolution has gained tremendous attention both in the optical and life science domains after the publications of several seminal papers[1–4] each of them being the foundations of a new technology. We here introduce a new technology based on conical diffraction to create new superresolution functionalities in a standard laser scanning confocal microscope (LSCM). After a short introduction to conical diffraction and its use to create conical diffraction microscopy (CODIM), we will present some typical acquisitions and reconstructions of biological samples. A glimpse at the possibilities of conical diffraction to implement other superresolution modalities will be given in the last section.

2. A BRIEF VIEW OF CONICAL DIFFRACTION AND BIOAXIAL SUPERRESOLUTION

2.1 Conical diffraction

This phenomenon has been known for a long time. Sir Hamilton predicted it in 1832,[5] and Lloyd observed the effect soon after,[6] making it one of the first examples in sciences' history of a phenomenon first described by theory before being observed. This effect was later studied in length by Berry[7–9] and renamed "conical diffraction". This diffraction phenomenon occurs when a beam propagates along an optical axis of a biaxial

Further author information: (Send correspondence to Clement Fallet)
Clement Fallet: E-mail: clement.fallet@bioaxial.com, Telephone: 33 6 7827 2922

Nanoimaging and Nanospectroscopy II, edited by Prabhat Verma, Alexander Egner, Proc. of SPIE
Vol. 9169, 916905 · © 2014 SPIE · CCC code: 0277-786X/14/$18 · doi: 10.1117/12.2061059

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 10/21/2014 Terms of Use: http://spiedl.org/terms

crystal slab (a crystal possessing two optical axes). In internal conical diffraction, the beam propagates as a cone within the crystal, later emerging from it as a cylinder.

Berry described precisely the transformation the beam undergoes.[7] Let (e_x, e_y) be the polarization of the input beam. After passing through the crystal ($n_1 < n_2 < n_3$), the electrical field $\mathbf{E}(\mathbf{R}, Z)$ is described by :

$$\mathbf{E}(\mathbf{R}, Z) = \left[B_0(R, R_0, Z) Id + B_1(R, R_0, Z) \begin{pmatrix} \cos\theta & \sin\theta \\ \sin\theta & -\cos\theta \end{pmatrix} \right] \begin{pmatrix} e_x \\ e_y \end{pmatrix}, \tag{1}$$

where B_0 and B_1 are beams associated with Bessel functions of zero and first order respectively:

$$B_0(R, R_0, Z) = k \int_0^\infty \mathrm{d}P P a(P) e^{-\frac{\mathrm{i}kZP^2}{2}} \cos(kR_0P) J_0(kRP),$$

$$B_1(R, R_0, Z) = k \int_0^\infty \mathrm{d}P P a(P) e^{-\frac{\mathrm{i}kZP^2}{2}} \sin(kR_0P) J_1(kRP),$$

and a(P) is the Fourier transform of the input beam,
$R_0 = \frac{l}{n_2}\sqrt{(n_2 - n_1)(n_3 - n_2)}$ is the radius of the conically diffracted beam, J_0 and J_1 are the Bessel functions of zero and first order.

The incident beam is thus transformed by the crystal into two beams of different types. The B_0 part of the output beam is linked to the incident beam by a Bessel function of zero order and remains of the same topological charge. If the input beam is a Gaussian, the B_0 beam shall remain a Gaussian beam, while if it is a vortex beam, it shall remain a vortex beam. The Bessel function of first order that intervenes on the B_1 beam creates a singularity; if the input beam is a Gaussian beam, the B_1 part of the output beam shall be a vortex beam.[10]

In short, the biaxial crystal can be seen as a way to transform a beam with a constant polarization into a beam with an inhomogeneous distribution of polarization. This inhomogeneous polarization is later converted into an inhomogeneous intensity distribution by passing through a polarizer. By carefully selecting the state of polarization before and after the biaxial crystal, one can generate a whole family of distributions which size is comparable to the Airy disk (see fig.1).

2.2 Bioaxial super resolution: COnical DIffraction Microscopy (CODIM)

The core of the system presented here(CODIM100, BioAxial, Paris, France) is a biaxial crystal. As seen previously, one may generate a wide range of distributions by choosing carefully the polarization states used. This is achieved with a typical polarimeter set up. Before the crystal, a horizontal linear polarizer aligned with a double Pockels cells acts as a polarization state generator (PSG). The Pockels cells are orientated with their fast axis in 45° and 0° respectively. This allows to create any homogeneous state of polarization. In the same way, the light emerging from the crystal encounters first a double Pockels cells orientated at 0° and 45° respectively, before passing through a vertical linear polarizer; this part of the system then acts like a polarization state analyzer (PSA). In this way, all the distributions presented in fig.1 may be generated.

Bioaxial technique enhances the results of fluorescence microscopy by using the special properties of the beams generated by conical diffraction along with a powerful algorithm to retrieve information below the diffraction limit from the sample. The system presents itself as a beam shaping unit (BSU) that is plugged on a standard confocal microscope (Nikon C2, Nikon Corporation, Tokyo, Japan) in lieu of the laser input. The sample is scanned with the distributions generated inside that box. The excitation intensity being low, the fluorescent response of the sample is assumed to depend linearly on the excitation intensity (linear fluorescence regime, no saturation) and is sent on a sCMOS camera (Orca Flash 4.0, Hamamatsu Photonics, Hamamatsu, Japan), plugged at the back port of the microscope. The system generates data-sets made of thousands of images, one for each beam shape and beam position. These images as well as the calibrated distributions used during excitation are fed to a stochastic algorithm. Given the low light doses used in each image and the non-bleaching behavior

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 10/21/2014 Terms of Use: http://spiedl.org/terms

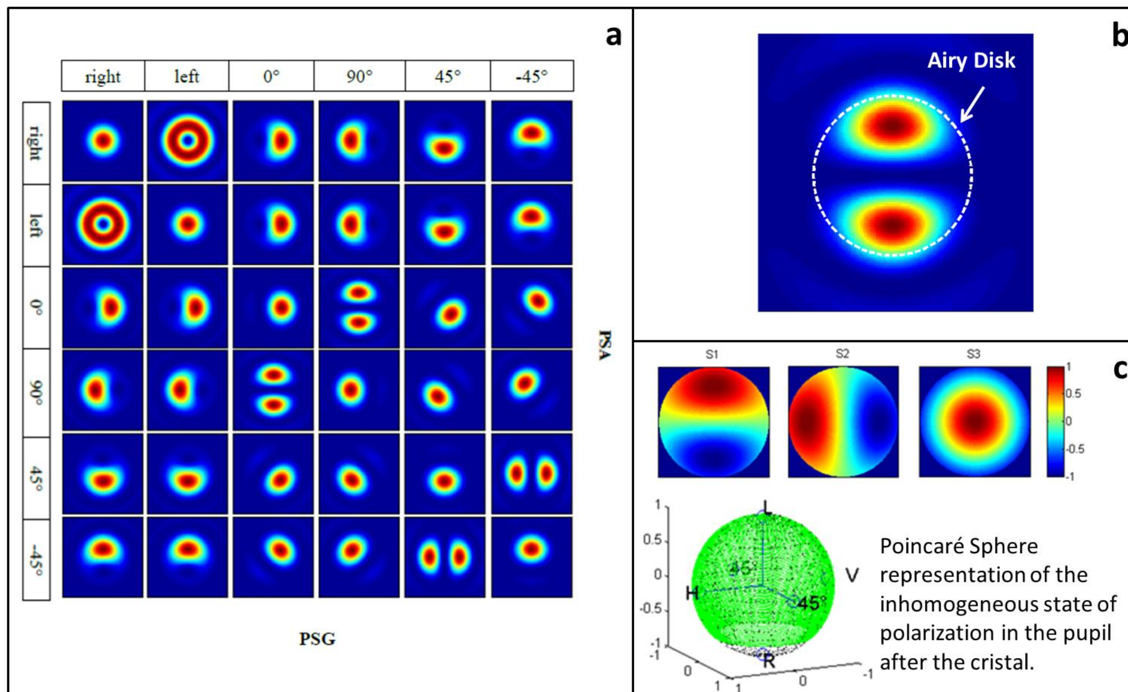

Figure 1. a- Different intensity distributions that may be generated by coupling a biaxial crystal with a Polarization State Generator and a Polarization State Analyzer. b- Generated distributions have a size similar to the Airy disk. c - Stokes vector components in the pupil after the biaxial crystal for **homogeneous left circular input polarization**.

of the sample, the data formation derives from a linear model with limits predicted by Fourier Analysis and recent superresolution theory developments.[11,12] In the framework of Bayesian Inverse Methods, we developed a numerical solver that exploit the data formation model and the noise distribution in modern low-light cameras. It consists in a low-frequency constrained maximum a posteriori (MAP) solved by an accelerated gradient descent scheme,[13] which is easily portable to any a priori formulation provided that a fast proximal operator[14] exists.

3. APPLICATIONS TO BIOLOGICAL SAMPLES

These results were obtained in collaboration with Institut Pasteur, Paris. A description of the system used to acquire the images and of the processing algorithms can be found in.[15] We imaged fixed samples of commercial bovine pulmonary artery endothelial cells (BPAEC) from Life Technologies© (FluoCells® Prepared Slide ♯2) stained with Anti-bovine α-tubulin mouse monoclonal 236-10501 in conjunction with BODIPY FL goat anti-mouse IgG antibody using a 40x NA 0.95 dry objective (CFI Plan Apo Lambda 40X) from Nikon. A Laser Scanning Confocal (Nikon C2, Nikon Corporation, Tokyo, Japan) image of the sample was acquired (left column of fig.2). A $7 \times 7 \mu m^2$ region of interest (ROI) was selected for super-resolution. The ROI was scanned with the distributions generated inside the BSU. The bank of micro-images required for super-resolution was acquired. The super-resolution images are almost devoid of noise thanks to the denoising step in the reconstruction process. They are well contrasted, with a completely black background. Thanks to the flexibility of our technology, even such off-the-shelf samples can be readily imaged using CODIM100. Strikingly the CODIM100 image shows a much higher level of details, resolving tubulin fibers that the conventional image is unable to separate, thus revealing an unexpected complexity in the cytoskeleton network. Both the very high sensitivity and dynamic range of the CODIM100 technology is also clearly demonstrated as very faint structures that usually are below the detection range of super-resolution techniques are fully accounted for.More data confirming these claims can be found in.[15]

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 10/21/2014 Terms of Use: http://spiedl.org/terms

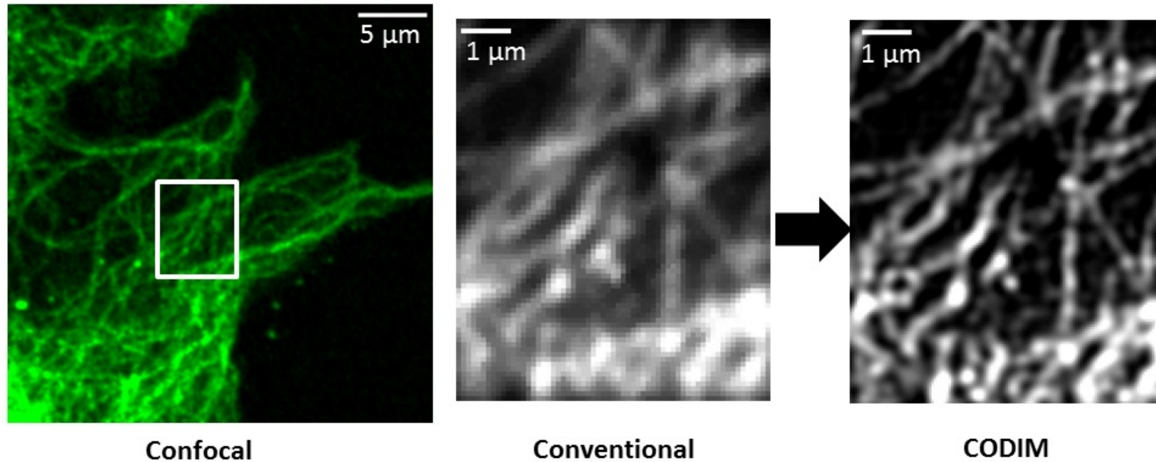

Figure 2. Commercial sample from Life Technologies©. FluoCells prepared slide ♯2 contains bovine pulmonary artery endothelial cells (BPAEC). Anti-bovine α-tubulin mouse monoclonal 236-10501 in conjunction with BODIPY FL goat anti-mouse IgG antibody is labeling microtubules.From left to right: Laser Scanning Confocal image used to select the region of interest for super-resolution, Conventional image of the ROI, CODIM reconstruction of the ROI.

4. CONICAL DIFFRACTION TO CREATE NEW DISRUPTIVE MODALITIES IN MICROSCOPY

Though the work described above focused on super-resolution imaging *via* beam shaping, we are convinced that conical diffraction is an optic tool generic and applicable enough to foster many other approaches. Indeed, biaxial crystals offer many interesting properties on top of their ability to diffract light, and the dependence on these properties on light intensity, wavelength, incidence, *etc.* is well described. Manufacturing processes are mature enough so that one can design a biaxial crystal with all desired properties to support a specific application.

For instance, another family of super-resolution or super-localization techniques, namely single-molecule tracking, could be addressed by conical diffraction. We give here the first description of a technique we named "Dark Tracking". Let's assume the molecule of interest has been tagged by a dye that can be excited at λ_{DT}. We propose here an illumination scheme based on a vortex beam generated by conical diffraction. After an initial detection of the position of the molecule by a classical confocal image at λ_{DT}, the scanner position is adjusted to stimulate the sample so the center of the vortex beam perfectly coincides with the position of the emitter. The fluorescence signal is imaged on a very sensitive camera (eg. EMCCD or sCMOS) enabling a low light detection of the emitter thanks to the high quantum efficiency. The localization scheme is based on the absence of fluorescence signal when the emitter is exactly localized in the center of the vortex beam. The strong intensity gradient close to the center of the vortex enables an accurate localization of the emitter. If the emitter slightly moves, the excitation intensity absorbed by the emitter is not zero anymore, leading to fluorescence which position and intensity are calculated from the image. A feedback loop then re-centers the vortex beam on the emitter and the new position is stored.

The feedback loop can be executed at the camera frame rate (up to 1kHz) leading to real-time single-molecule tracking at high speed over long period of time. By always trying to minimize the fluorescence signal by minimizing the excitation signal on the emitter, we allow the tracking to occur during a longer period of time since bleaching is less likely to happen with such low light doses. The localization accuracy highly depends on the signal-to-noise ratio of the image and thus the background noise (camera noise + autofluorescence signal) needs to be taken into account.

Let's now assume the sample is tagged with two different dyes that can be excited at two different wavelengths λ_C and λ_{DT}. We use two co-propagating beams with different topologies depending of their wavelengths. The first one (λ_C) has a classical gaussian shape and is used to get a confocal image of the context of the sample. The second one (λ_{DT}) is a vortex beam and is used for dark-tracking. By controlling the scanning galvo mirror

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 10/21/2014 Terms of Use: http://spiedl.org/terms

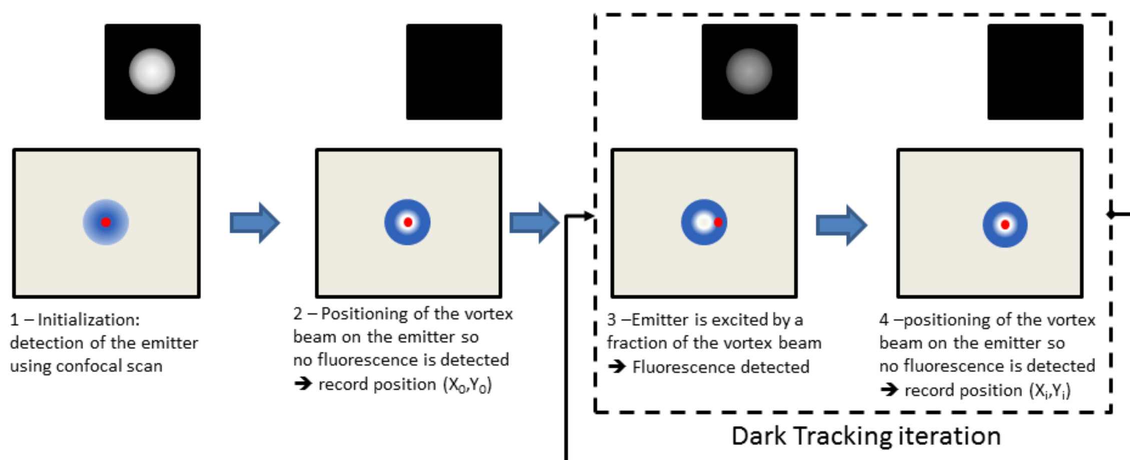

Figure 3. Accurate localization scheme of a single molecule by using a vortex beam as excitation beam in order to lower the light dose received by the molecule during tracking period.

system, the dark-tracking can be executed between every line of the confocal scan of the contextual image, the single-emitter is then tracked at a much higher frequency than the frame rate of the confocal image.

The main advantages of this technique compared to other single-molecule tracking techniques are that it requires only one scanning system and the power sent to the tracked molecule is extremely low. In practice, the use, alignment and shaping of these two beams is not a trivial task if conventional beam-shaping techniques are used such as computer-generated holograms (CGH), spatial-light modulators or spiral phase plates because of the inherent chromaticity and the beam splitting they imply. Conical diffraction can be used to simplify even more single molecule tracking. Through clever optical design and engineering, a biaxial crystal can be tuned to generate the vortex pattern for a wavelength and the fundamental for another one in a common path. This would in turn allow using a single, fiber-coupled- optic path and solve many of the practical challenges that remain.

Dark-tracking technology can be readily used to address a high number of biological questions where conventional single particular tracking over long time periods is hampered by the bleaching of the tracked particles. On-going projects include the tracking of different types of lipids in the plasma membrane over long periods of time to investigate and compare their behavior on different time-scales and different contexts. We are also applying this technique to investigate the mechanisms driving the exchange of signalling molecules between protein micro-domains at the immunological synapses formed by lymphocytes.

5. CONCLUSIONS

In this paper we have presented two different techniques for super-resolution or super-localization based on conical diffraction. In conjunction with scientific cameras enabling detection of low fluorescence signals, these techniques require low light dose at the sample level leading to low photo-toxicity and low photo-bleaching imaging and tracking. The first technique presented (CODIM) has been successfully implemented and used on fixed and live biological samples demonstrating its potential as a new super-resolution modality in life sciences. The second technique (Dark-Tracking) is still theoretical and needs experimental validation. Future developments will focus on the increase of the imaged field of view (FOV) in CODIM and the demonstration of Dark-Tracking in both 2D and 3D modes.

ACKNOWLEDGMENTS

BioAxial greatly benefits from the Imagopole bioimaging core facility of Institut Pasteur and more importantly from their research staff, we hereby thank them for their generous support, time and effort in making this work possible. We extend our thanks to BPI France for supporting the company financially and Paris Biotech Santé for providing us with facilities in an energizing environment.

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 10/21/2014 Terms of Use: http://spiedl.org/terms

REFERENCES

[1] Hell, S. W. and Wichmann, J., "Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy.," *Optics letters* **19**, 780–2 (June 1994).

[2] Gustafsson, M. G., "Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy.," *Journal of microscopy* **198**, 82–7 (May 2000).

[3] Rust, M. J., Bates, M., and Zhuang, X., "Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM)," *Nat Meth* **3**, 793–796 (Oct. 2006).

[4] Betzig, E., Patterson, G. H., Sougrat, R., Lindwasser, O. W., Olenych, S., Bonifacino, J. S., Davidson, M. W., Lippincott-Schwartz, J., and Hess, H. F., "Imaging Intracellular Fluorescent Proteins at Nanometer Resolution," *Science* **313**, 1642–1645 (Sept. 2006).

[5] Hamilton, W., "Third supplement to an essay on the theory of systems of rays," *Trans. R. Irish Acad.* **17**, 1–144 (1837).

[6] Lloyd, H., "On the Phenomena Presented by Light in Its Passage along the Axes of Biaxal Crystals," *Trans. R. Irish Acad.* **17**(1831), 145–157 (1831).

[7] Berry, M. V., "Conical diffraction asymptotics: fine structure of Poggendorff rings and axial spike," *Journal of Optics A: Pure and Applied Optics* **6**, 289–300 (Apr. 2004).

[8] Berry, M., Jeffrey, M., and Lunney, J., "Conical diffraction: observations and theory," *Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences* **462**, 1629–1642 (June 2006).

[9] Berry, M. and Jeffrey, M., "Conical diffraction: Hamilton's diabolical point at the heart of crystal optics," *Progress in Optics* **6638**(07), 13–50 (2007).

[10] O'Dwyer, D. P., Phelan, C. F., Rakovich, Y. P., Eastham, P. R., Lunney, J. G., and Donegan, J. F., "The creation and annihilation of optical vortices using cascade conical diffraction.," *Optics express* **19**, 2580–8 (Jan. 2011).

[11] Candès, E. J. and Fernandez-Granda, C., "Towards a Mathematical Theory of Super-resolution," *Communications on Pure and Applied Mathematics* **67**(6), 906–956 (2014).

[12] Duval, V. and Peyré, G., "Exact Support Recovery for Sparse Spikes Deconvolution," *ArXiv e-prints* (2013).

[13] Nesterov, Y., "Smooth minimization of non-smooth functions," *Mathematical Programming* **103**(1), 127–152 (2005).

[14] Combettes, P. and Wajs, V., "Signal Recovery by Proximal Forward-Backward Splitting," *Multiscale Modeling & Simulation* **4**, 1168–1200 (Jan. 2005).

[15] Caron, J., Fallet, C., Tinevez, J.-Y., Moisan, L., Braitbart, L. P., Sirat, G. Y., and Shorte, S. L., "Conical diffraction illumination opens the way for low phototoxicity super-resolution imaging," *Cell Adhesion* **8**, 0–1 (July 2014).

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 10/21/2014 Terms of Use: http://spiedl.org/terms