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Imaging with illumination and detection arrays

Colin Sheppard

Nano-Physics Department

Italian Institute of Technology (IIT), Genoa, Italy

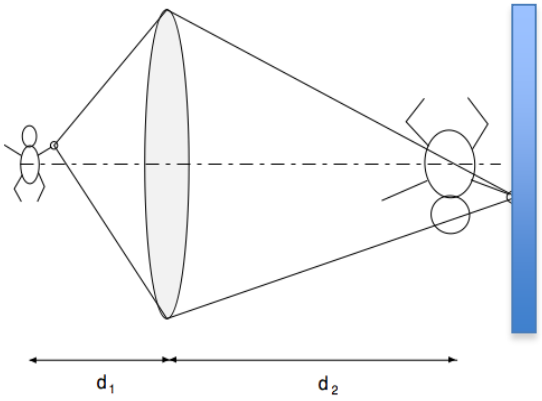
School of Chemistry, University of Wollongong, Australia

colinjrsheppard@gmail.com

Two ways to form an image

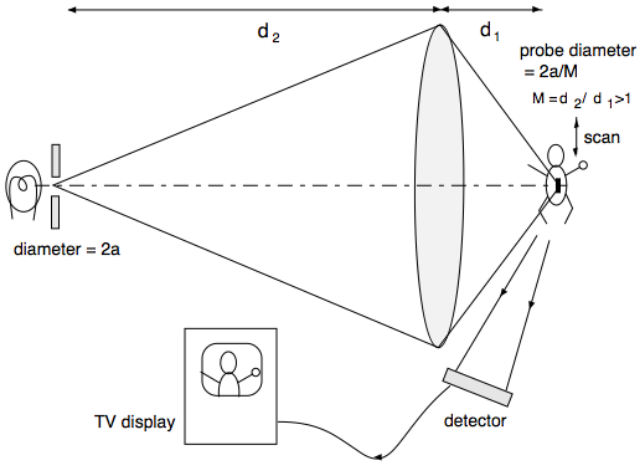
Full-field detection

detector array



Scanning system

single element detector



OPTICA ACTA, 1977, VOL. 24, NO. 10, 1051–1073

Image formation in the scanning microscope

C. J. R. SHEPPARD and A. CHOUDHURY

CHAPTER 1

The Generalized Microscope*

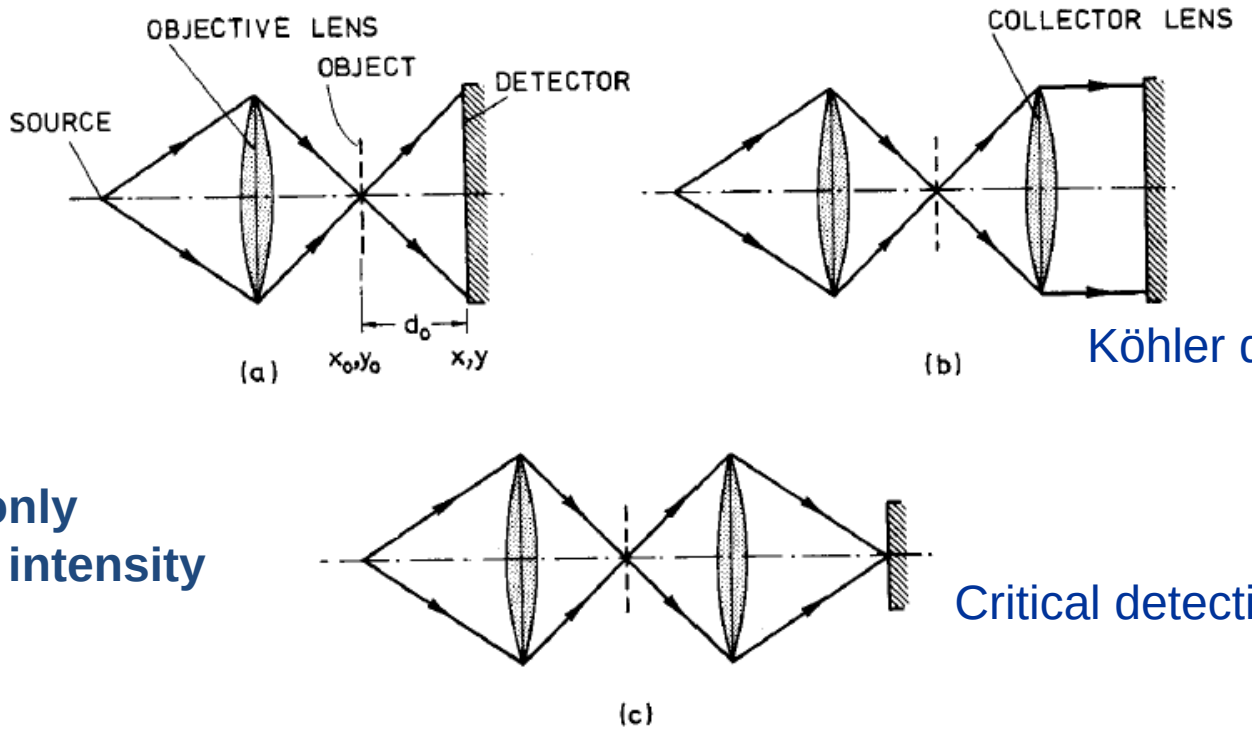
COLIN J. R. SHEPPARD

School of Physics, and Australian Key Centre for Microscopy and Microanalysis, The University of Sydney, NSW 2006, Australia

*This chapter is based upon an invited presentation at the Symposium of the Australian Society for Electron Microscopy, University of Sydney, 1996.

Confocal and Two-Photon Microscopy: Foundations, Applications, and Advances, Edited by Alberto Diaspro.
ISBN 0-471-40920-0

Scanning microscopes of Type 1 (non-confocal)



Köhler detection

Detector only measures intensity

Critical detection

Figure 1. Scanning microscopes of Type 1.

Imaging by **first** lens (objective lens)

C. J. R. SHEPPARD and A. CHOUDHURY
Image formation in the scanning microscope
OPTICA ACTA, 1977, VOL. 24, NO. 10, 1051–1073

Same as ‘single-pixel’ camera!

M. F. Duarte, M. A. Davenport, D. Takhar, J. N. Laska, T. Sun, K. F. Kelly, and R. G. Baraniuk, “Single pixel imaging via compressive sampling,” *IEEE Signal Proc. Mag.*, vol. 25, no. 2, pp. 83–91, March 2008.

But single-pixel camera is not new!

- Logie Baird television (1928)
- Flying spot microscope
  (RC Webb, 1949; Young & Roberts, *Nature*, 1951)
- Scanning electron microscope (Oatley, 1948)
- Non-descanned detector in 2-photon microscope
  (even allows detection through a scattering medium!)

Scanning and conventional microscopes are equivalent

- Based on Principle of Reciprocity
- Holds even with loss or multiple scattering
  (but not inelastic scattering, e.g. fluorescence)
- First shown for electron microscopes

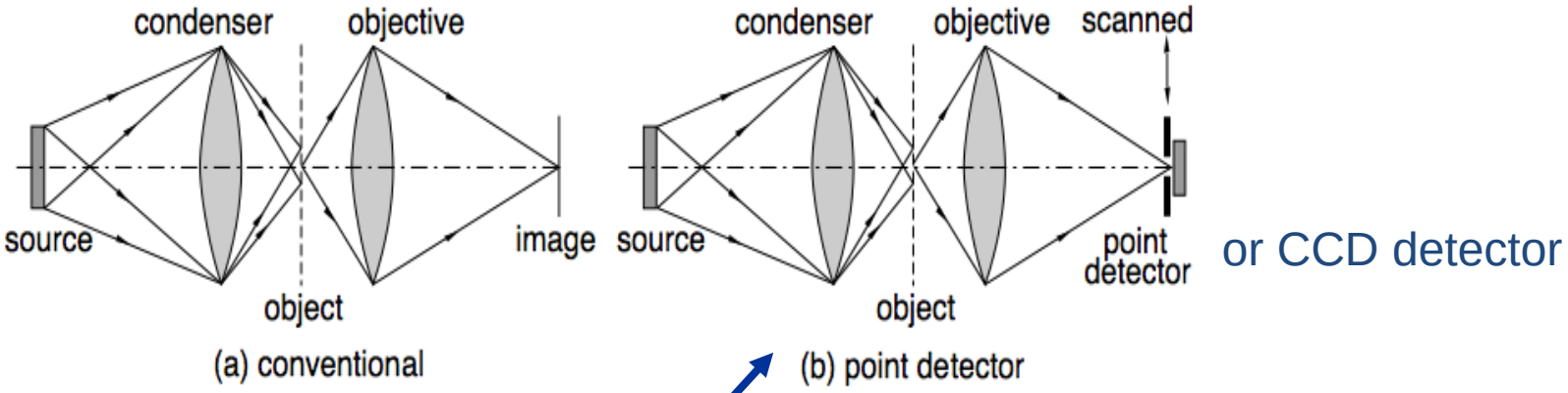
Pogany & Turner, *Acta Cryst.* A**24** 103 (1968)
Cowley, *App. Phys. Lett.* **15** 58 (1969)
Zeitler & Thomson, *Optik* **31** 258 (1970)
Welford, *J. Microscopy* **96** 105 (1972)
Barnett, *Optik* **38** 585 (1973)
Engel, *Optik* **41** 117 (1974)
Kermisch, *J. Opt. Soc. Am.* **67** 1357 (1977)
Sheppard & Wilson, *Optik* **78**, 39-43 (1986);
J. Opt. Soc. Am. A **3**, 755-756 (1986)

WARNING: Some papers say conventional is better, some say scanning is better!
In fact both are the same.
But not for fluoresence: scanning gives better resolution (Stokes shift)

Scanning vs. conventional microscope

Conventional

Conventional with image scanning



Scanning

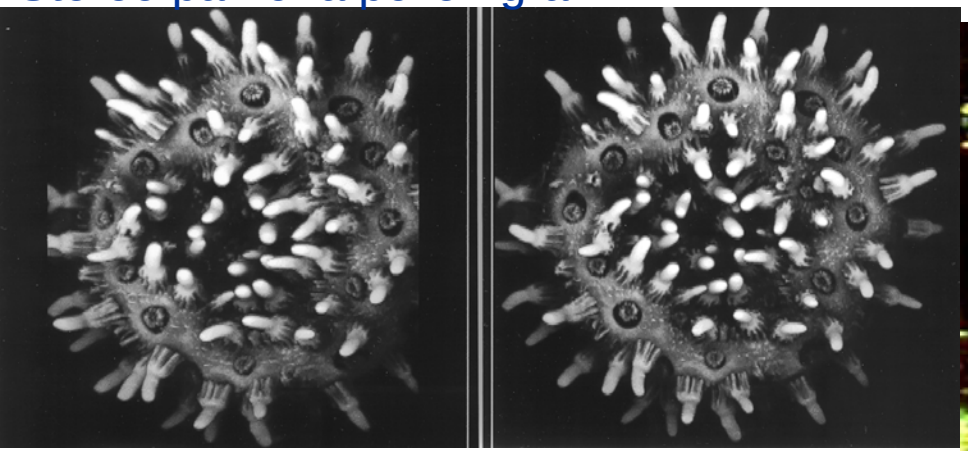
Confocal

Confocal microscopy

- Advantages
  - Optical sectioning
    - 3D imaging
    - Surface profiling
  - Reduced scattered light
    - Imaging through scattering media, e.g. tissue
  - Improved resolution (for small pinhole)
- Reflection
  - Industrial applications, surface profiling
  - Scattering media, tissue (**non-invasive**)
- Fluorescence
  - Autofluorescence or labelled
  - Fixed or living

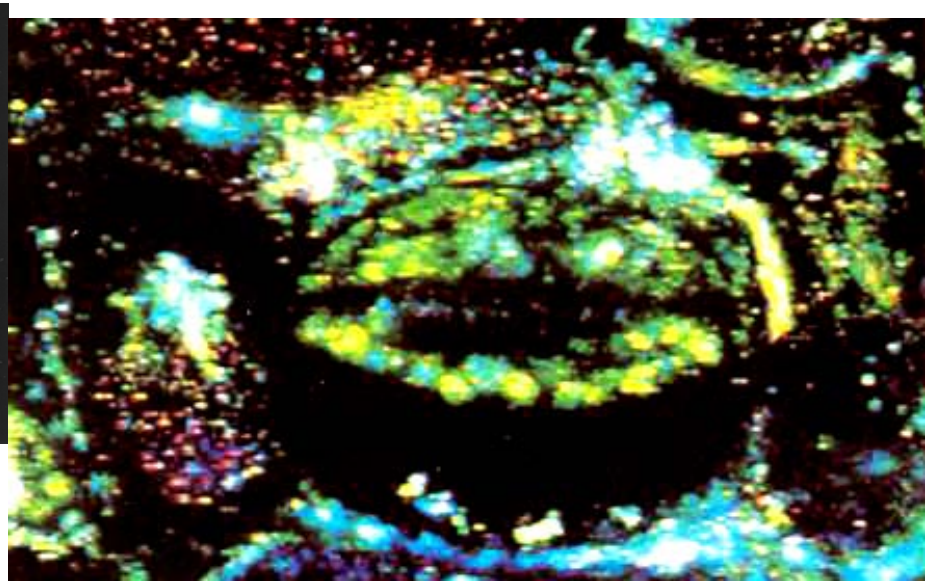
Confocal reflectance (Oxford1974-89)

Stereo pair of a pollen grain



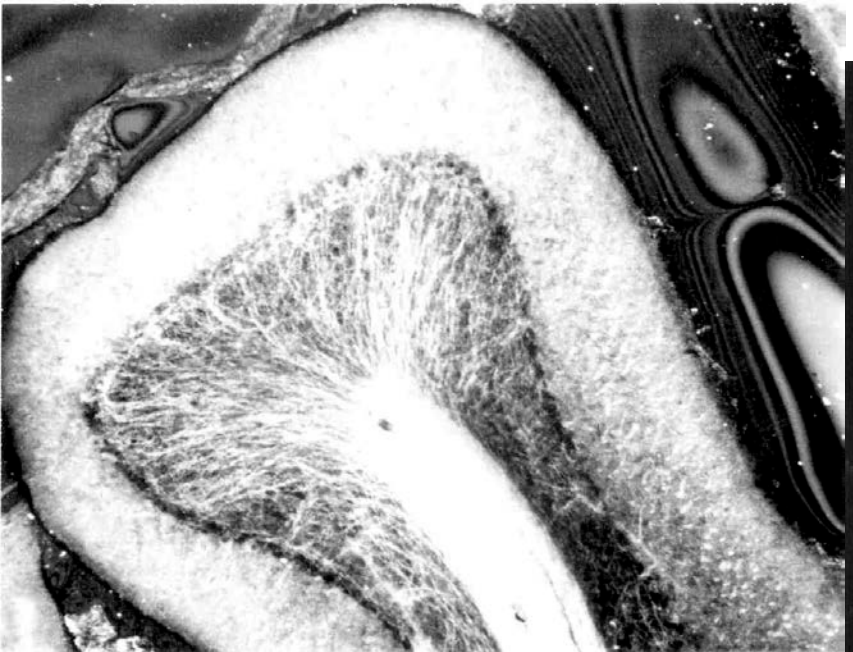
Endeavour, **10**, 17-19 (and cover)(1986)

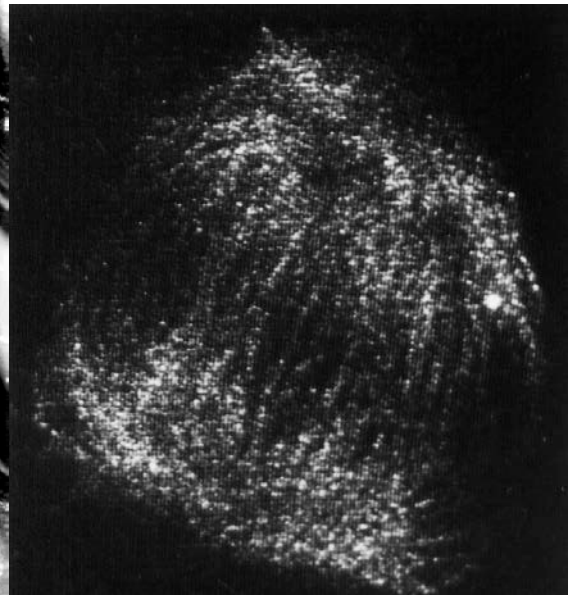
J. Microsc. **165**, 103-117 (1992)



Colour confocal reflection image of a leaf

Rat brain (cerebellum)





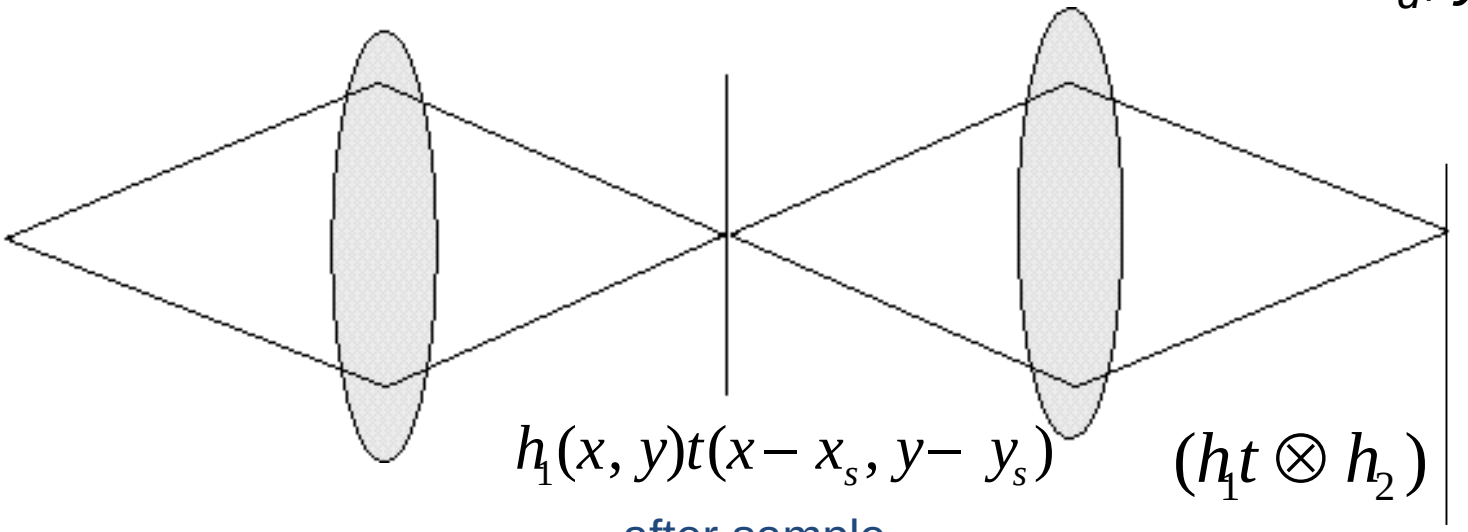
Microtubules labeled with 15nm gold

Inst. Phys. Conf. Ser. No. 98, 1989

Confocal Imaging (non-fluorescence)

h is amplitude PSF

x_d, y_d



after sample

x_s, y_s are scan coordinates

$$I(x_d, y_d) = \left| \iint h_1(x,y)t(x - x_s, y - y_s)h_2(x_d - x, y_d - y)\,dx\,dy \right|^2$$

- Pinhole: x_d, y_d = 0:

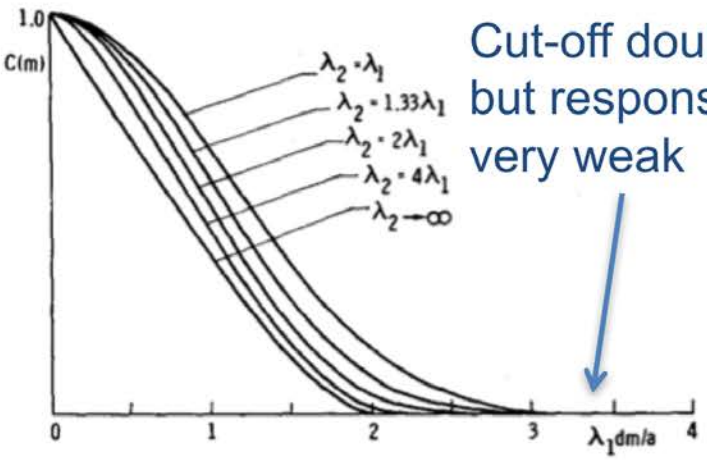
$$I = \left| \left(h_1(x,y)h_2(-x,-y)\right) \otimes t(x,y) \right|^2$$

- h_2 even:

$$I = \left| (h_1 h_2) \otimes t \right|^2$$

  - Same as coherent microscope, with $h_{\text{eff}} = h_1 h_2$
  - Transfer function is convolution of c_1 with c_2

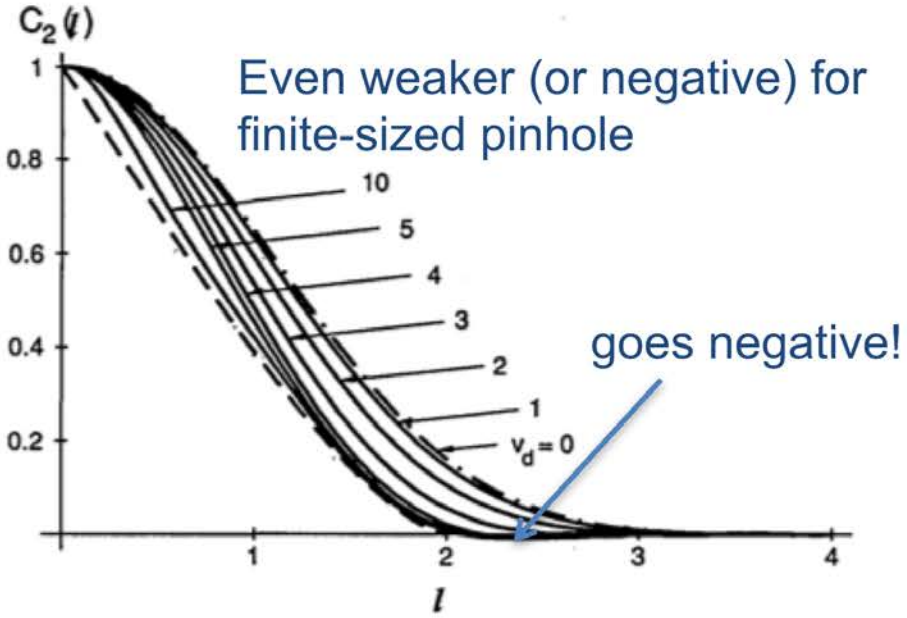
OTF for confocal fluorescence



Cut-off doubled but response is very weak

Fig. 1. Transfer function for the confocal fluorescent microscope for variou rescent wavelengths. The spatial frequency axis is normalised by the in wavelength.

Effect of Stokes shift



Even weaker (or negative) for finite-sized pinhole

goes negative!

Fig. 4. Normalized in-focus (2-D) OTF for different radii of the detector. The dashed curve represents the 2-D OTF when $v_d \to \infty$.

Effect of pinhole size

Plot suggests possibility to use pupil filters to increase the magnitude of the OTF at high frequencies!

Optik 60, No. 4 (1982) 391–396 © Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart

Super-resolution by confocal fluorescent microscopy

I. J. Cox, C. J. R. Sheppard and T. Wilson

JOSA Communications Vol. 9, No. 1/January 1992/J. Opt. Soc. Am. A 151

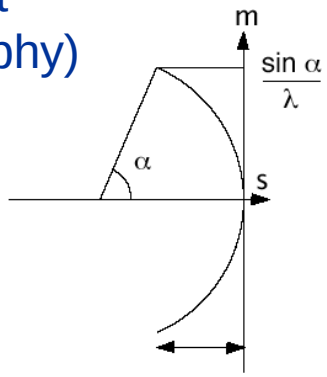
Confocal fluorescent microscopy with a finite-sized circular detector

Min Gu and C. J. R. Sheppard

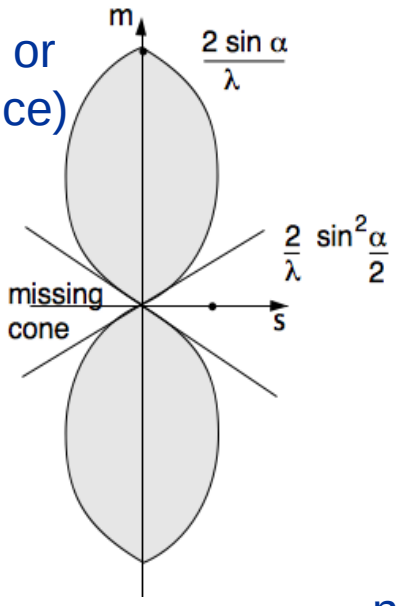
3D Spatial Frequency cut-offs

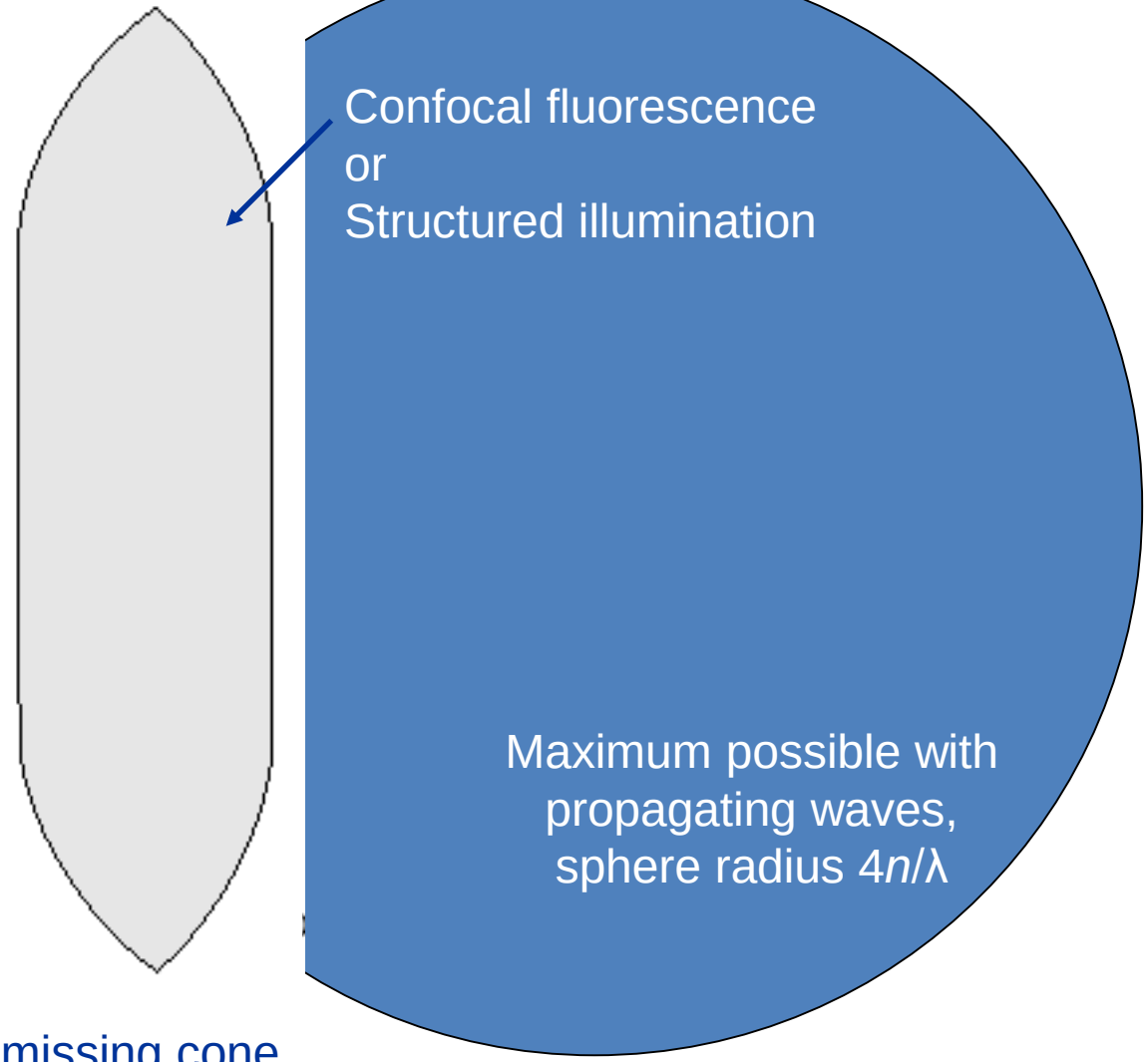
Maximum 4/λ (4*n*/λ in medium, e.g 6/λ)

Coherent (holography)



Abbe limit (large condenser, or Fluorescence)



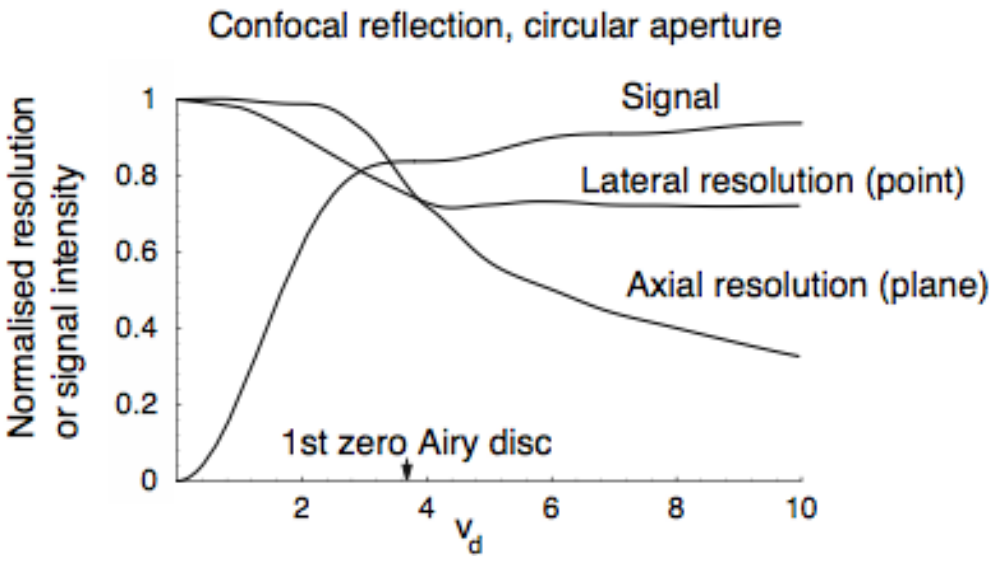


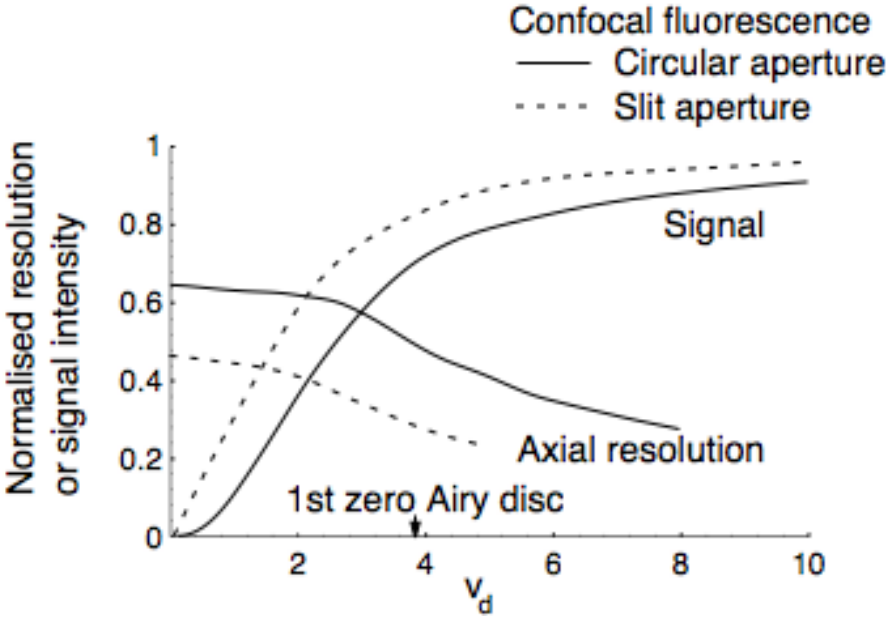
Limitations of confocal microscopy

- Speed
  - Illuminate only one spot at a time
  - In fluorescence, speed limited by saturation of fluorophore
  - Solution: illuminate by more than one spot
    - Spinning disk
    - Line illumination
    - Structured illumination
- Signal level
  - Increasing pinhole size reduces resolution, sectioning
- Resolution
  - 4Pi microscopy
  - STED
  - Localization microscopy (PALM/STORM)
  - Structured illumination/Image scanning microscopy
- Penetration
  - Coherence gating
  - Two/three photon excitation
  - Focal modulation microscopy (FMM)

Main problem: Finite sized pinhole

- Need finite sized pinhole to get adequate signal
- Then resolution improvement is lost





CJR Sheppard and DM Shotton
Confocal Laser Scanning Microscopy,
RMS, Bios, and Springer, 1997

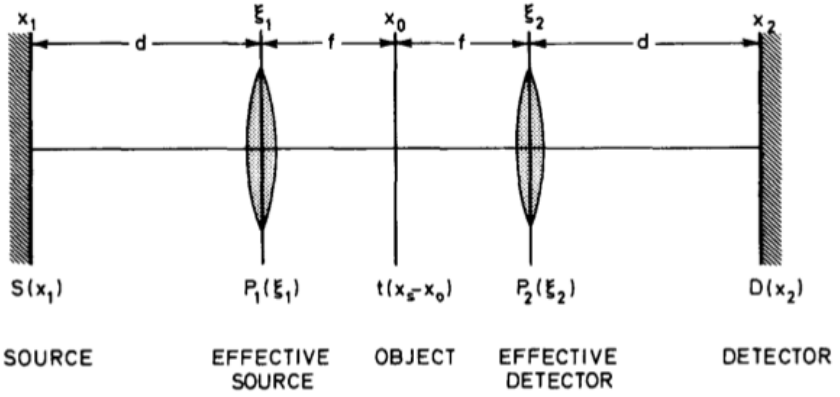
Illumination and detection arrays

- Structured illumination (Lukosz, 1963; Gustafsson, 2000)
- Tandem scanning (spinning disc), Petrán (1968)
- Singular value decomposition (Bertero & Pike, 1982)
- ‘Type 3’: Maximum signal in detector plane (Reinholz, 1987)
- Pixel reassignment (Sheppard, 1988)
- Subtractive imaging (Wilson, 1984; Cogswell & Sheppard, 1990; + many others)
- Source/detector arrays (Benedetti, 1996)
  - Max image
  - Min image gives crosstalk + background
  - Max-Min, Similar to confocal
  - Superconfocal Max+Min-2 Mean

Illumination and detection arrays (II)

- Programmable array microscope (Hanley 1999, Verveer 1998)
  - Scanned array + Detector array. Conjugate image is confocal I_{conf}
  - Non-conjugate image is $I_{conv} - I_{conf}$
  - Random array $I_{conv} + I_{conf}$
- Structured illumination + nonlinear (Heintzmann, 2002; Gustafsson)
- Structured detection, J Lu, Concello, Xie, Lichtmann (2009)
  - SPIN Structured illumination pattern written by modulated beam.
    - Harmonics attenuated by the illumination OTF. Can get modulation pattern without DC offset
  - SPADE “patterned detection” Illumination constant, Detector switched on and off
- Structured detection, RW Lu, Biomed Opt Exp (2013)
  - Digital mask

Scanning microscope with partially coherent source and detector



OPTICA ACTA, 1978, VOL. 25, NO. 4, 315–325

Image formation in scanning microscopes with partially coherent source and detector

C. J. R. SHEPPARD and T. WILSON

Scanning (Type 1)
Confocal with finite pinhole

Image intensity:

$$I(x_s)=\int_{-\infty}^{+\infty}\!\!\iiiint S(x_1)h_1\left(\frac{x_0+x_1/M}{\lambda f}\right)h_1^*\left(\frac{x_0'+x_1/M}{\lambda f}\right)t(x_s-x_0)t^*(x_s-x_0')$$

$$\times h_2\left(\frac{x_0+x_2/M}{\lambda f}\right)h_2^*\left(\frac{x_0'+x_2/M}{\lambda f}\right)D(x_2)\,dx_1\,dx_0\,dx_0'\,dx_2.$$

Transmission cross coefficient (TCC):

$$C(m;p)=\int_{-\infty}^{+\infty}\!\!\iint F_S\left[\frac{\xi_1'-\xi_1}{\lambda Mf}\right]F_D\left[\frac{(p-m)}{M}+\frac{(\xi_1-\xi_1')}{\lambda Mf}\right]P_1(\xi_1)P_1^*(\xi_1')$$

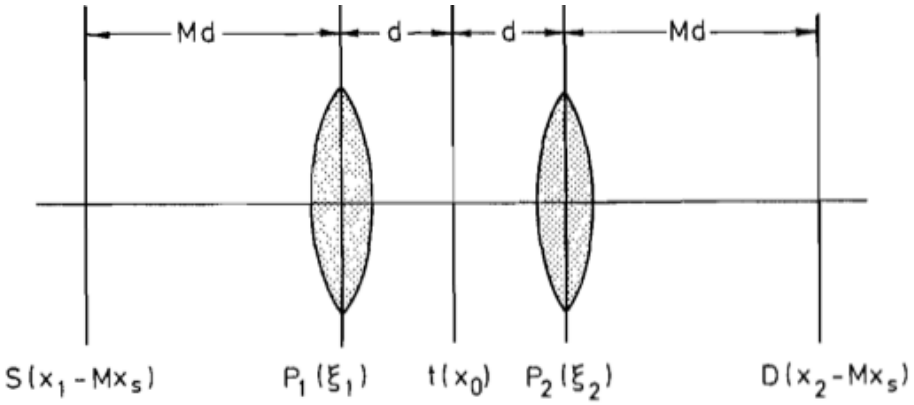
$$\times P_2(\lambda fm-\xi_1)P_2^*(\lambda fp-\xi_1')\,d\xi_1\,d\xi_1'.$$

$F_{S,D}$ are FTs of source, detector:

$$F_S(u)=\int_{-\infty}^{+\infty}S(x_1)\exp\{-2\pi jux_1\}\,dx_1,$$

$$F_D(v)=\int_{-\infty}^{+\infty}D(x_2)\exp\{-2\pi jvx_2\}\,dx_2$$

General microscope with source/detector arrays



Journal of Microscopy, *Vol.* 124, *Pt* 2, *November* 1981, *pp.* 107–117.
Revised paper accepted 10 *March* 1981

The theory of the direct-view confocal microscope

by C. J. R. SHEPPARD *and* T. WILSON, *University of Oxford, Department of Engineering Science, Parks Road, Oxford*

1D theory:

source array

$$I(x_2, x_s) = \int\int\int_{-\infty}^{+\infty} S(x_1 - Mx_s)\, h_1\left(\frac{x_1/M - x_0}{\lambda d}\right) h_1^*\left(\frac{x_1/M - x_0'}{\lambda d}\right) t(x_0)\, t^*(x_0')$$

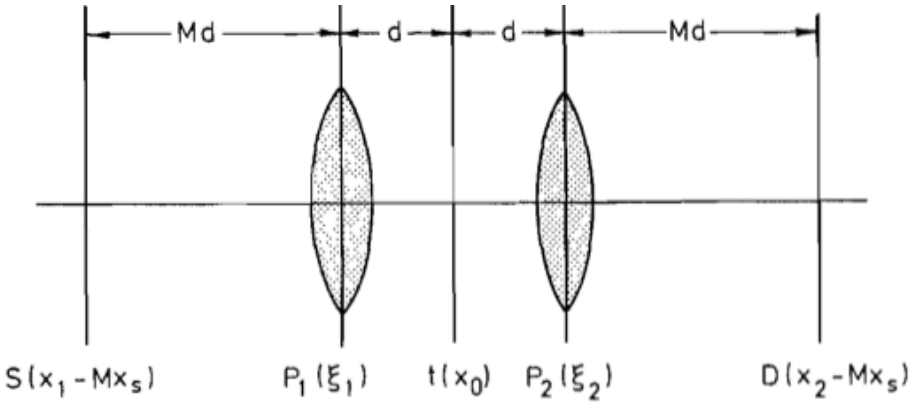
function of 2 variables

$$\times\, h_2\left(\frac{x_2/M - x_0}{\lambda d}\right) h_2^*\left(\frac{x_2/M - x_0'}{\lambda d}\right) D(x_2 - Mx_s)\, dx_1\, dx_0\, dx_0',$$

detector array

- source and detector arrays (scanned)
- reduces to conventional, structured illumination (SIM), scanning, confocal, spinning disk, etc.
- partially coherent system (but can also analyze a fluorescence system)

Fluorescence microscope with source/detector arrays



Corresponding equation for a fluorescence system
Again, applies for conventional, scanning, spinning disk microscopes

H is intensity PSF *T* is intensity object

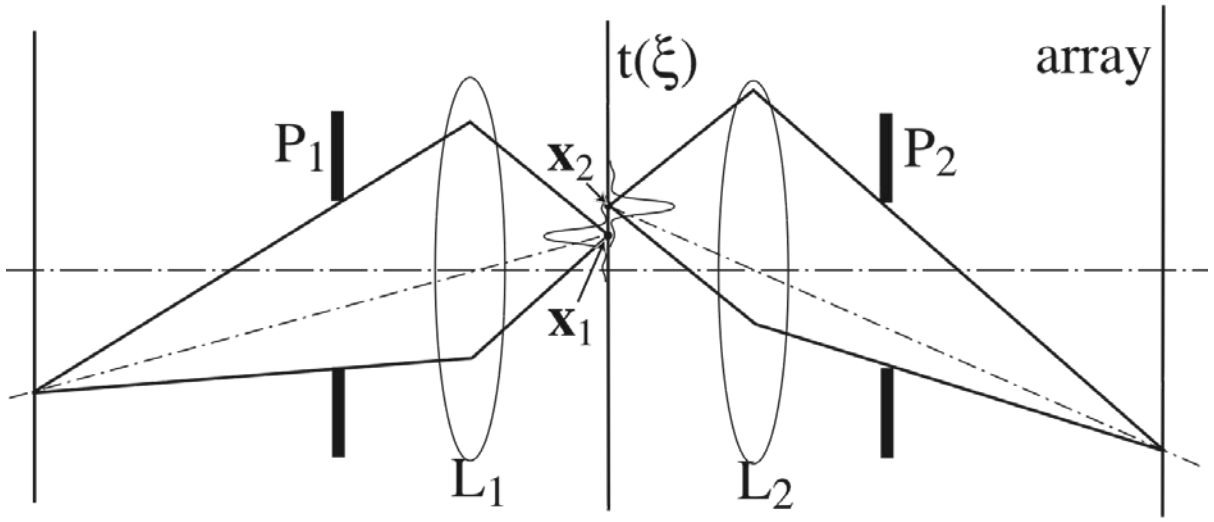
source array

$$I(\mathbf{x}_2,\mathbf{x}_s)=\iint S(\mathbf{x}_1-M\mathbf{x}_s)H_1\left(\frac{\mathbf{x}_1/M-\mathbf{x}_0}{\lambda d}\right)T(\mathbf{x}_0)H_2\left(\frac{\mathbf{x}_2/M-\mathbf{x}_0}{\lambda d}\right)D(\mathbf{x}_2-M\mathbf{x}_s)\,\mathrm{d}\mathbf{x}_1\,\mathrm{d}\mathbf{x}_0.$$

function of 2 (2D) variables

detector array

Fluorescence microscope with source/detector arrays



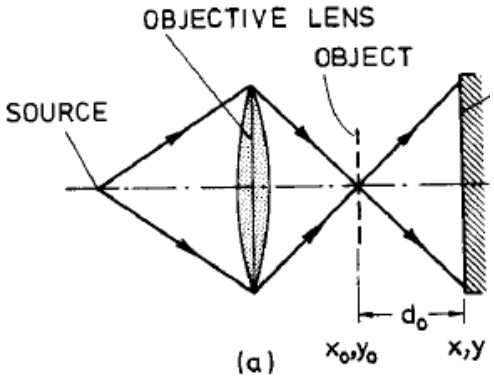
For point source

$$I(\mathbf{x}_1,\mathbf{x}_2)=\int H_1(\mathbf{x}_1-\mathbf{x})H_2(\mathbf{x}_2-\mathbf{x})T(\mathbf{x})\,\mathrm{d}\mathbf{x}$$

Signal at point $\mathbf{x}_2$ when illuminated at point $\mathbf{x}_1$

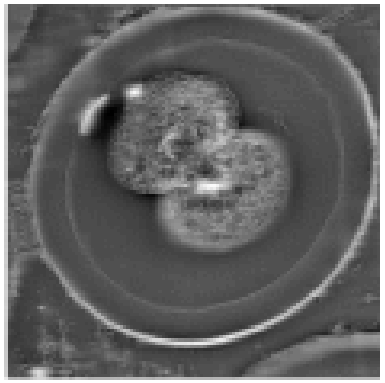
Scanning microscopes with detector array

Detector replaced by detector array



DETECTOR ARRAY

array in Fourier plane, not image plane



phase from AI-DPC (**illumination array**) (Mehta, thesis 2010)

- general case similar to ptychography
- quadrant detector for differential phase contrast (DPC)

Dekkers & de Lang, Differential phase contrast in a STEM, Optik **41**, 452-456 (1974)

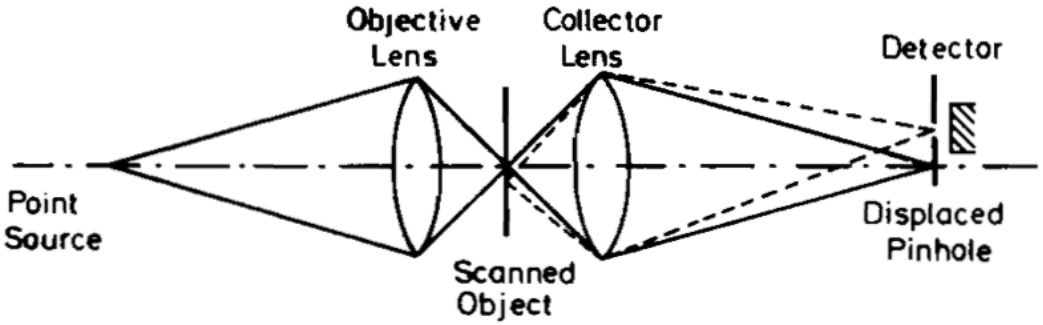
Stewart, On differential phase contrast with an extended illumination source, *JOSA* **66**, 813 (1981)

Ellis, US Patent (1981)

Hamilton & Sheppard, Differential phase contrast in scanning optical microscopy, *J. Microsc.* **133**, 27-39 (1984)

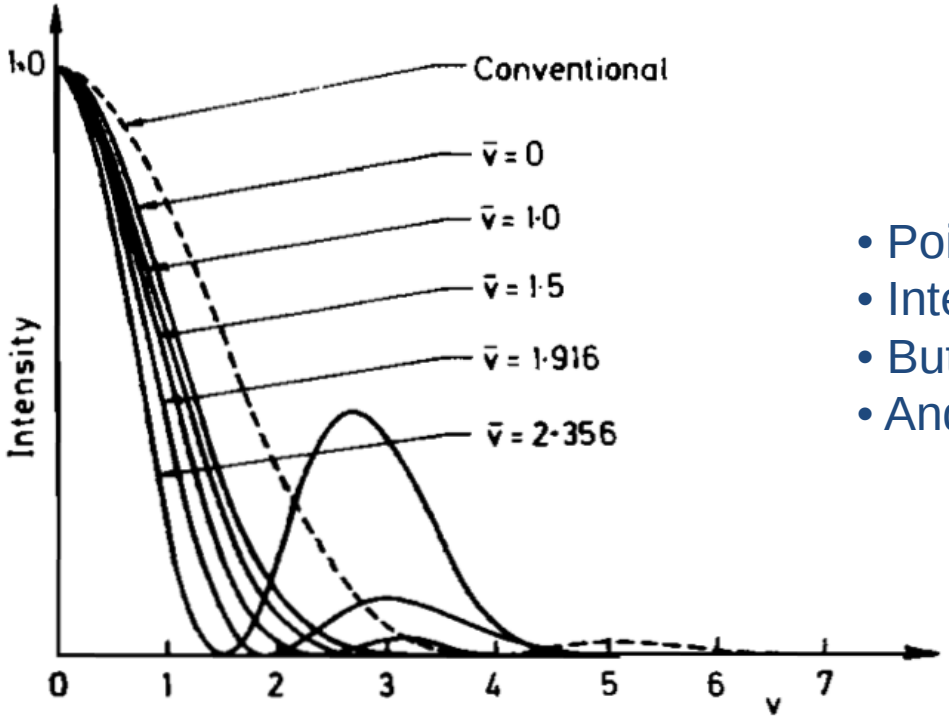
Mehta & Sheppard, Quantitative phase-gradient imaging at high resolution with asymmetric illumination-based differential phase contrast (AI-DPC) *Optics Letts.* **34**, *1924* (2009)

Offset pinhole



PSF:

$$I(v) = \left[\frac{2J_1(v-\bar{v})}{v-\bar{v}}\right]^2 \left[\frac{2J_1(v+\bar{v})}{v+\bar{v}}\right]^2$$



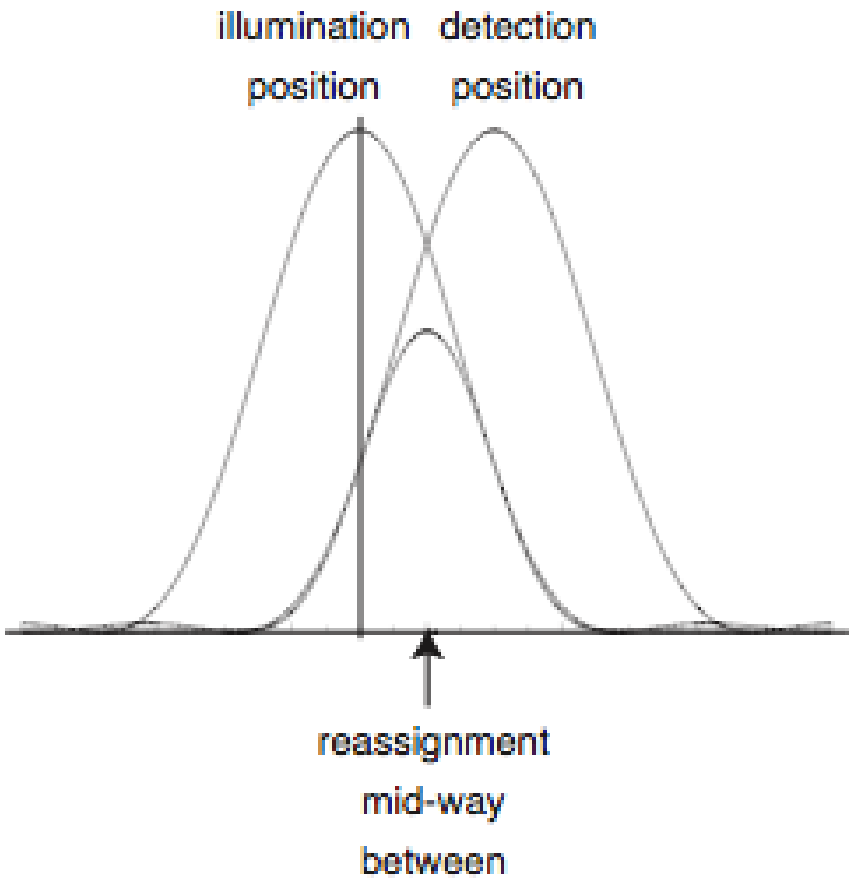
- Point spread function gets narrower
- Intensity decreases
- But increased side lobes
- And effective psf shifts sideways

Improvement in resolution by nearly confocal microscopy

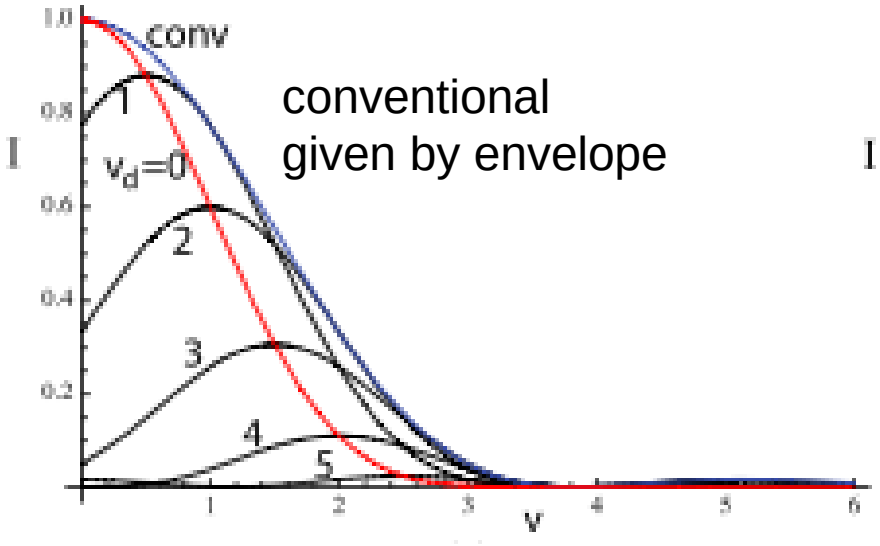
APPLIED OPTICS, Vol. 21, page 778, March 1, 1982

I. J. Cox, C. J. R. Sheppard, and T. Wilson

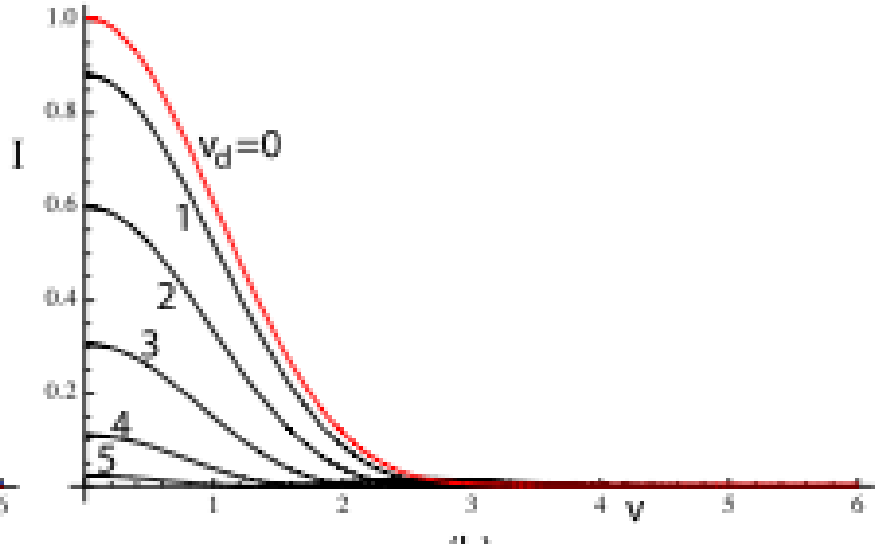
Gives the image of a shifted object point



Offset pinhole & reassignment



conventional
given by envelope



offset pinhole

after reassignment

- Integrate without reassignment: same as conventional
- Integrate with reassignment (to centre of illumination and detection):
  PSF sharpened and signal improved

Pixel reassignment

Abstract

A new explanation for the imaging improvement of confocal microscopy is presented. A method of further increasing the imaging performance is also discussed.

Optical transfer function

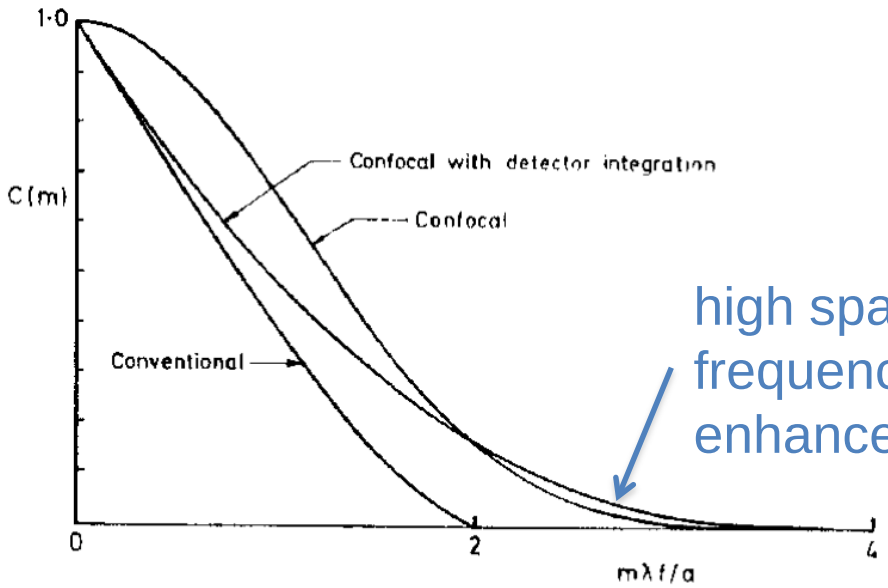


Fig. 2. Incoherent transfer functions for a fluorescence microscope. The radius of the circular pupils is a.

function of $2x_s$

$$I(x_s) = \{|h_1|^2 \otimes |h_2|^2\}(2x_s)$$

$$C(m) = \{(P_1 \otimes P_1^*)(P_2 \otimes P_2^*)\}(m\lambda f/2)$$

$OTF_1 \times OTF_2$

product of rescaled OTFs
(not **convolution** of OTFs
as for confocal)

high spatial frequencies enhanced

Super-resolution in Confocal Imaging

C. J. R. Sheppard,

Optik 80, No. 2 (1988) 53 54

Pixel reassignment

- Considers fluorescence and partially-coherent systems
- Concept that a detector element gives information about points of the object other than the illuminated point
- Introduces pixel reassignment and summation approach
- Explains why a confocal microscope can give superior resolution compared with a conventional one

Super-resolution in Confocal Imaging

C. J. R. Sheppard,

Optik 80, No. 2 (1988) 53 54

Bertero & Pike (from 1982)

Let $f(y)$ be the complex effective transparency (or reflectivity for a reflection microscope) in the object plane; by this we mean that, for a given scanning position, the image $g(x)$ formed by an ideal microscope with a uniformly filled illumination lens is

$$g(x) = \int_{-\infty}^{+\infty} \mathrm{sinc}(x - y)\mathrm{sinc}(y)f(y)dy, \qquad (1)$$

where $\mathrm{sinc}(x) = \sin(\pi x)/(\pi x)$. The basic idea, then, is to record the whole image $g(x)$ at each scanning position and to solve the integral equation (1) for $f(y)$.

i.e. does *not* solve g(x,y) to give f(y)

1748 J. Opt. Soc. Am. A/Vol. 4, No. 9/September 1987 Bertero *et al.*

Analytic inversion formula for confocal scanning microscopy

Bertero & de Mol, *Progress in Optics* (1996)

Eq. (5). A first possibility relies on the exact inversion formula for the imaging equation (28) derived by Bertero *et al.* (1987b):

$$M(x,t) = \frac{4\pi}{\Omega}\cos(\Omega x)\delta(t). \tag{34}$$

In this case, the object at point t is reconstructed only from the data at the same scanning position t and the multiplication by $(4\pi/\Omega)\cos(\Omega x)$ could also be implemented optically by means of a mask (Bertero *et al.* 1992). However, besides Eq. (34) and because of the redundancy of the data in Eq. (29), a whole family of reconstruction kernels can be constructed, all yielding Eq. (33) as overall PSF (Defrise and De Mol 1992), including the following one,

$$M(x,t) = \frac{\pi}{\Omega}\,\delta\left(t - \frac{x}{2}\right) \tag{35}$$

first proposed by Sheppard (1988). In principle, these reconstruction formulas

Super-resolution in confocal scanning microscopy: generalized inversion formulae

M Defrise and C De Mol 1992 *Inverse Problems* **8** 175. doi:10.1088/0266-5611/8/2/001

Image scanning microscopy

PRL 104, 198101 (2010)

Selected for a Viewpoint in *Physics*
PHYSICAL REVIEW LETTERS

Image Scanning Microscopy

Claus B. Müller and Jörg Enderlein*

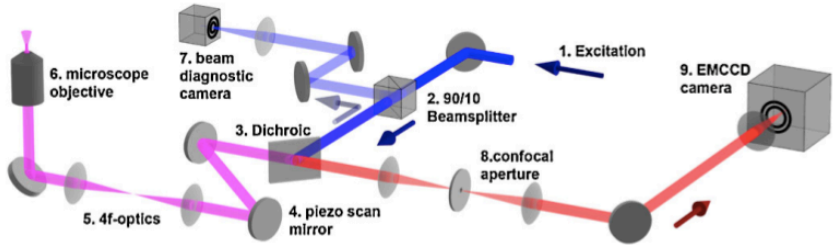


FIG. 1 (color online). ISM Setup, (1) Excitation with supercontinuum white light source and acousto-optic tunable filter, (2) 90/10 nonpolarizing beam splitter cube, (3) major dichroic mirror, (4) piezo scan mirror, (5) 4*f* telescope, (6) UPL APO 60x W microscope objective, (7) beam diagnostic camera, (8) confocal aperture, and (9) EM CCD detection camera system.

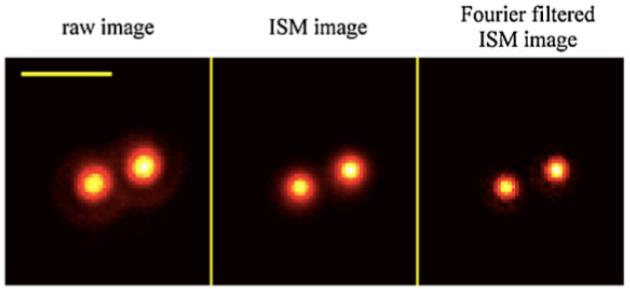
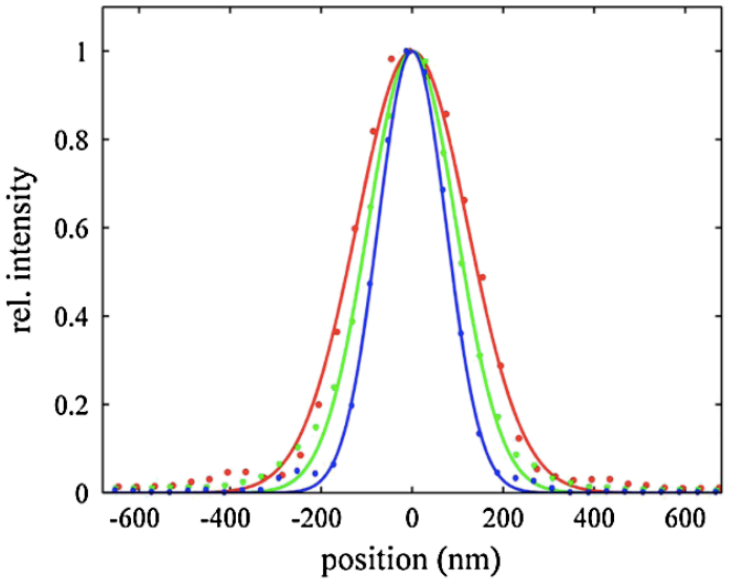
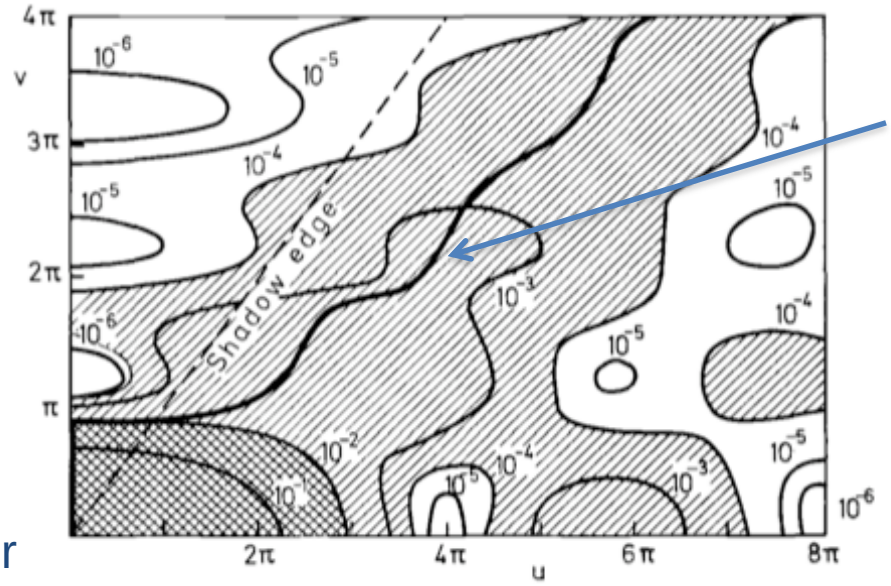


FIG. 2 (color online). Image of a single fluorescent bead of 100 nm diameter. Left panel: CLSM image; middle panel: ISM image; right panel: Fourier-weighted ISM image. The horizontal bar in the left panel has a length of 1 μm.



Optical sectioning

But, for v_{dmax} ➔ ∞, no optical sectioning!
Need to limit size of array



points on detector array > 0.72 AU, image regions away from the focal plane

v = 2.747

(0.72 AU) magic number

Figure 4. The intensity in the confocal image of a single point. The locus of the auto-focus scan of the image is also shown. The cross-hatched region is that in which the intensity is greater than 0·01. The corresponding region for a conventional system is shown shaded.

Locus of u_{lmax} (v)

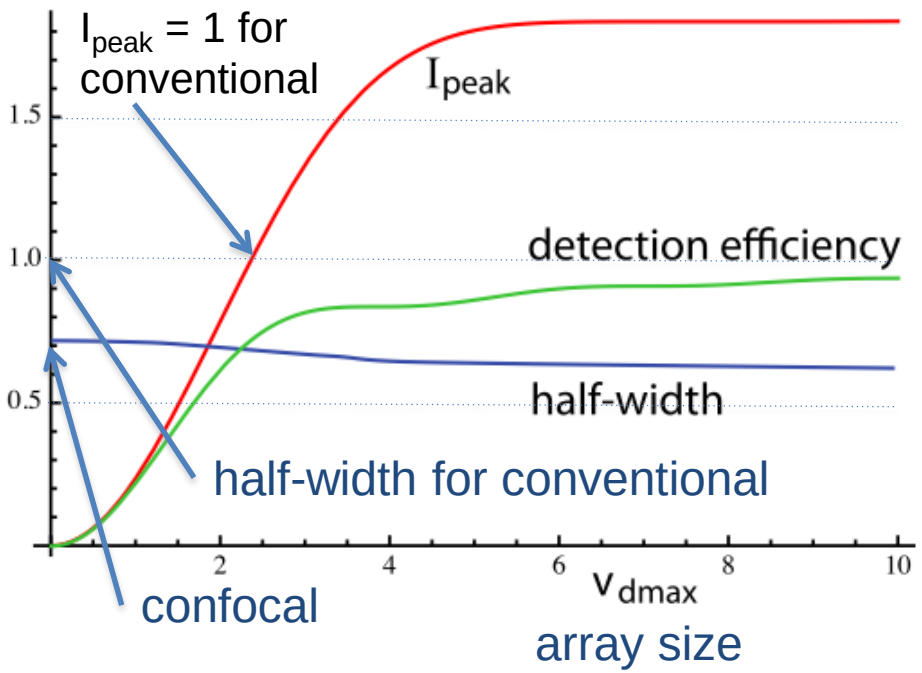
JOURNAL OF MODERN OPTICS, 1988, VOL. 35, NO. 1, 145–154

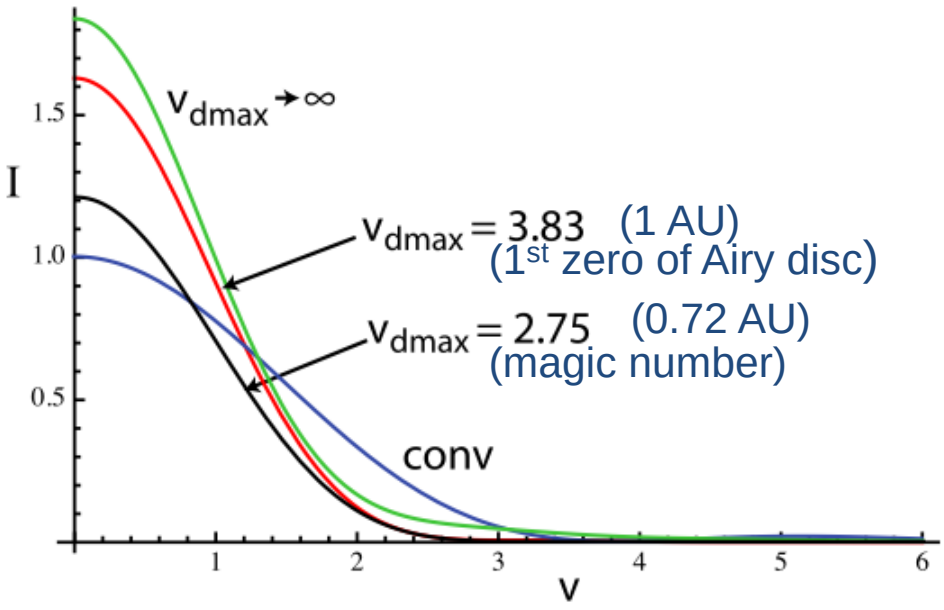
The extended-focus, auto-focus and surface-profiling techniques of confocal microscopy

C. J. R. SHEPPARD and H. J. MATTHEWS

Integration over finite detector array

peak intensity goes above 1!





- Peak is >1!
- Super-concentration
- Beats classical limit of étendue

Resolution and signal strength improve as size of array (v_{dmax}) increases

Peak of point spread function for large array is $4(1-16/3\pi^2) = 1.84$

(4 elements gives ~1.4)

August 1, 2013 / Vol. 38, No. 15 / OPTICS LETTERS 2889

Superresolution by image scanning microscopy using pixel reassignment

Colin J. R. Sheppard,[1,*] Shalin B. Mehta,[2] and Rainer Heintzmann[3,4,5]

Super-resolution in Confocal Imaging

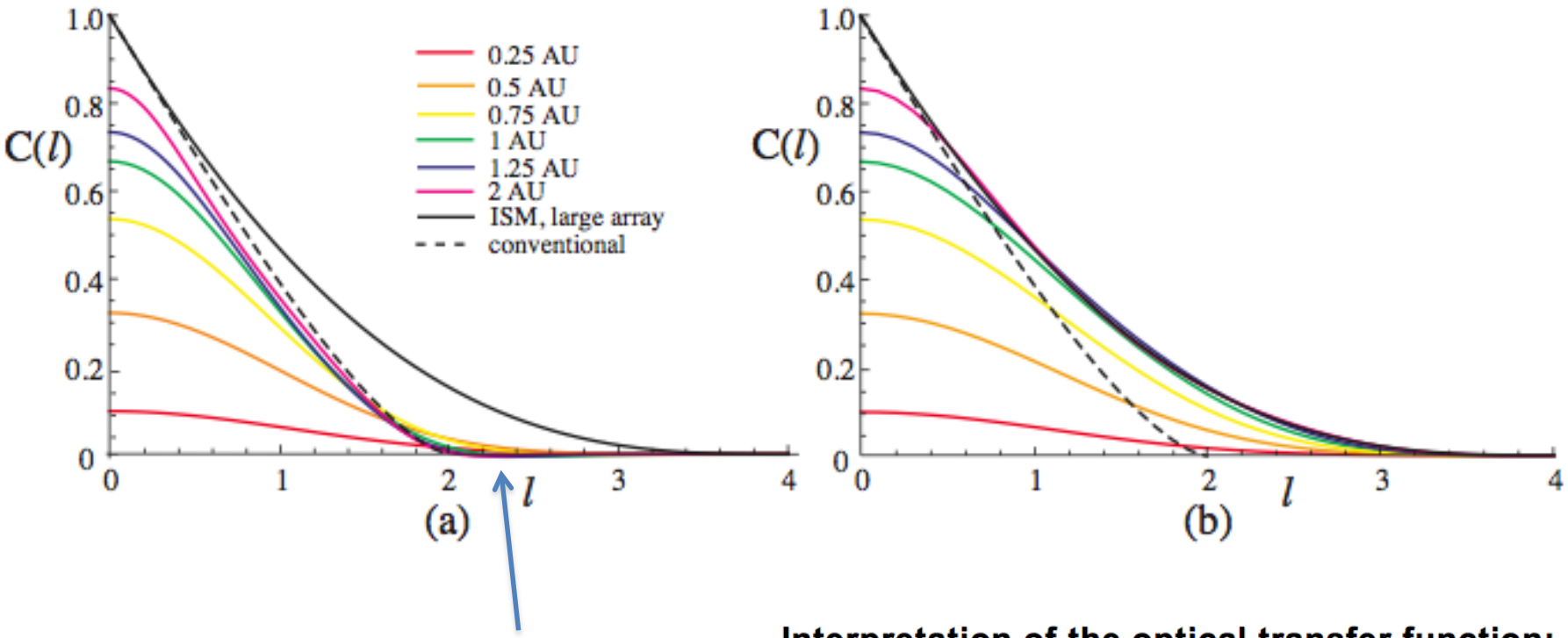
C. J. R. Sheppard,

Optik 80, No. 2 (1988) 53 54

Unnormalized OTF for confocal and ISM

confocal with finite pinhole

ISM with finite detector array



goes negative!

Interpretation of the optical transfer function: Significance for image scanning microscopy

COLIN J. R. SHEPPARD,[1*] STEPHAN ROTH,[2,3] RAINER HEINTZMANN,[2,3] MARCO CASTELLO,[1,4] GIUSEPPE VICIDOMINI,[1] RUI CHEN,[5] XUDONG CHEN,[5] AND ALBERTO DIASPRO[1,4,6]

Opt. Express 24(24), 27280-27287 (2016)

Unnormalized OTF for confocal and ISM

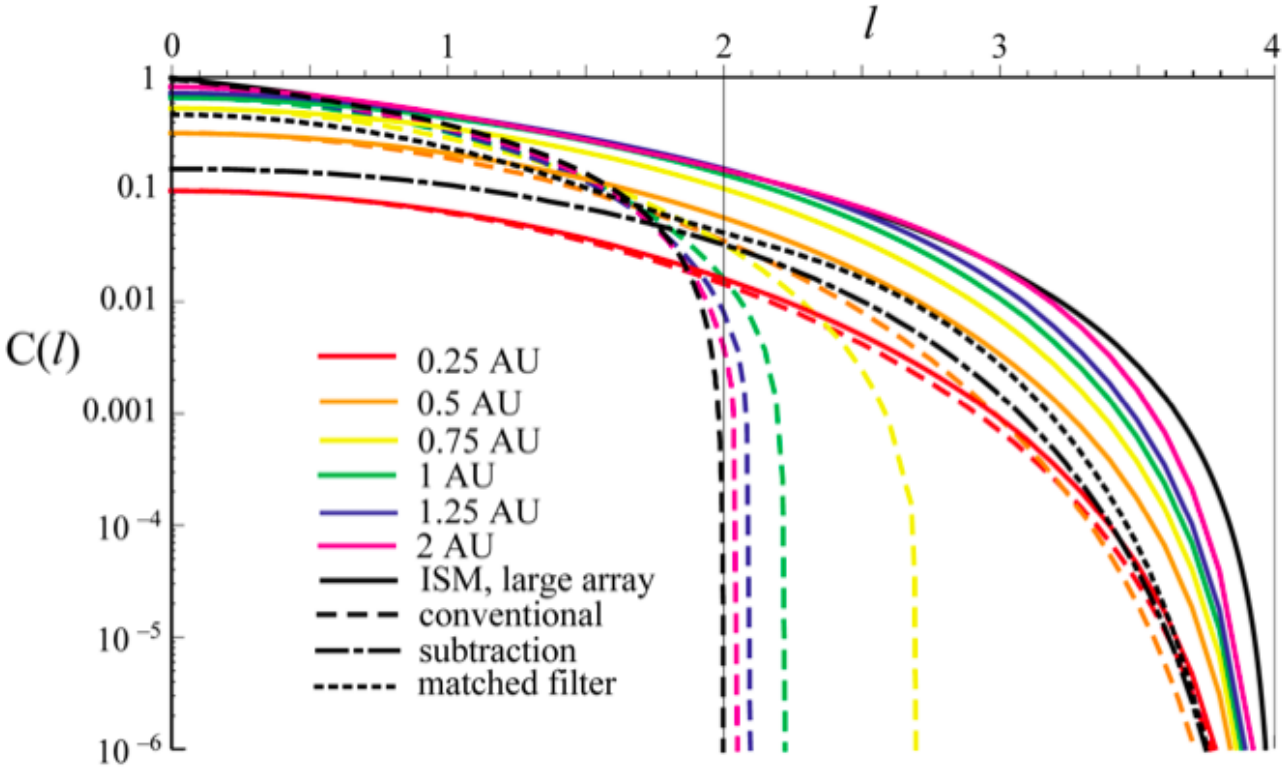


Fig. 5. A logarithmic plot of the unnormalized OTFs for a confocal microscope (dashed lines) and ISM (solid lines) with different pinhole/array sizes. The first positive lobe only of the confocal OTF is shown. The behavior for subtracting images from two pinhole sizes $(I_{0.5AU} - \frac{1}{4} I_{1AU})$, or using a matched filter with two ring detectors is also shown.

Dashed curves:
confocal with finite pinhole

Solid curves:
ISM with finite detector array

Unnormalized takes account of signal level

Unnormalized transfer function proposed in:

Interpretation of the optical transfer function: Significance for image scanning microscopy

COLIN J. R. SHEPPARD,[1,*] STEPHAN ROTH,[2,3] RAINER HEINTZMANN,[2,3] MARCO CASTELLO,[1,4] GIUSEPPE VICIDOMINI,[1] RUI CHEN,[5] XUDONG CHEN,[5] AND ALBERTO DIASPRO[1,4,6]

Vol. 24, No. 24 | 28 Nov 2016 | OPTICS EXPRESS 27280

Proc. R. Soc. Lond. A **387**, 171–186 (1983)
Printed in Great Britain

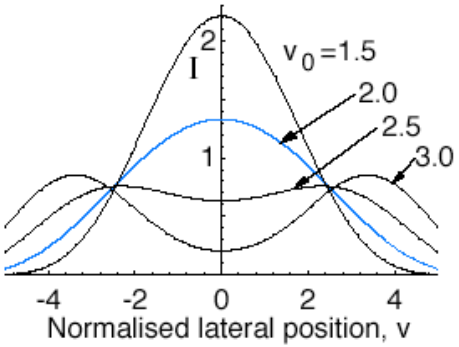
Optical microscopy with extended depth of field

BY C. J. R. SHEPPARD, D. K. HAMILTON AND I. J. COX

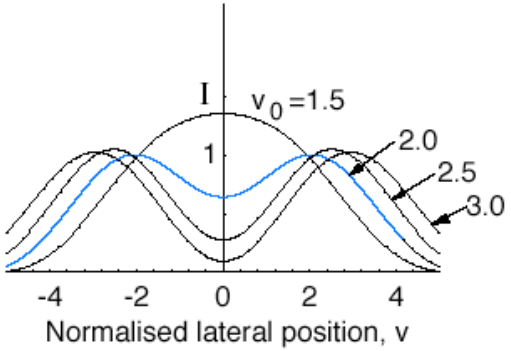
Images of two points

v_0 = 1.92 (~2) corresponds to Rayleigh separation (blue curves)

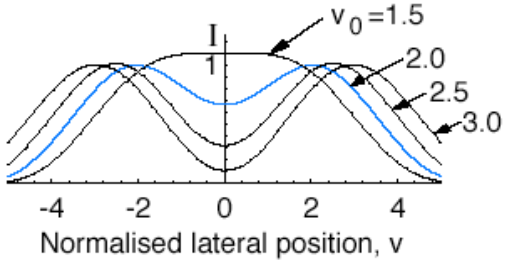
(a) Coherent



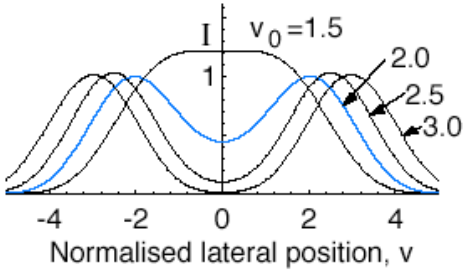
(b) Full illumination



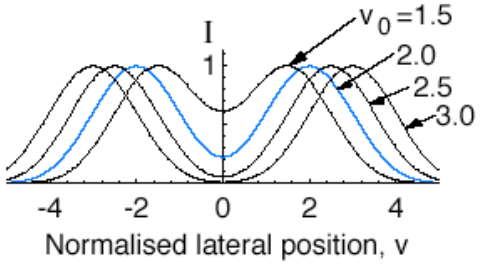
(c) Incoherent



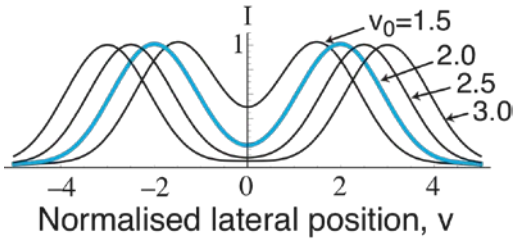
(d) Confocal reflection



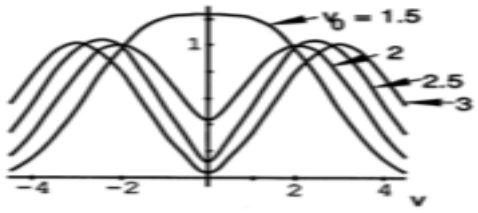
(e) Confocal fluorescence



(f) Fluorescence ISM

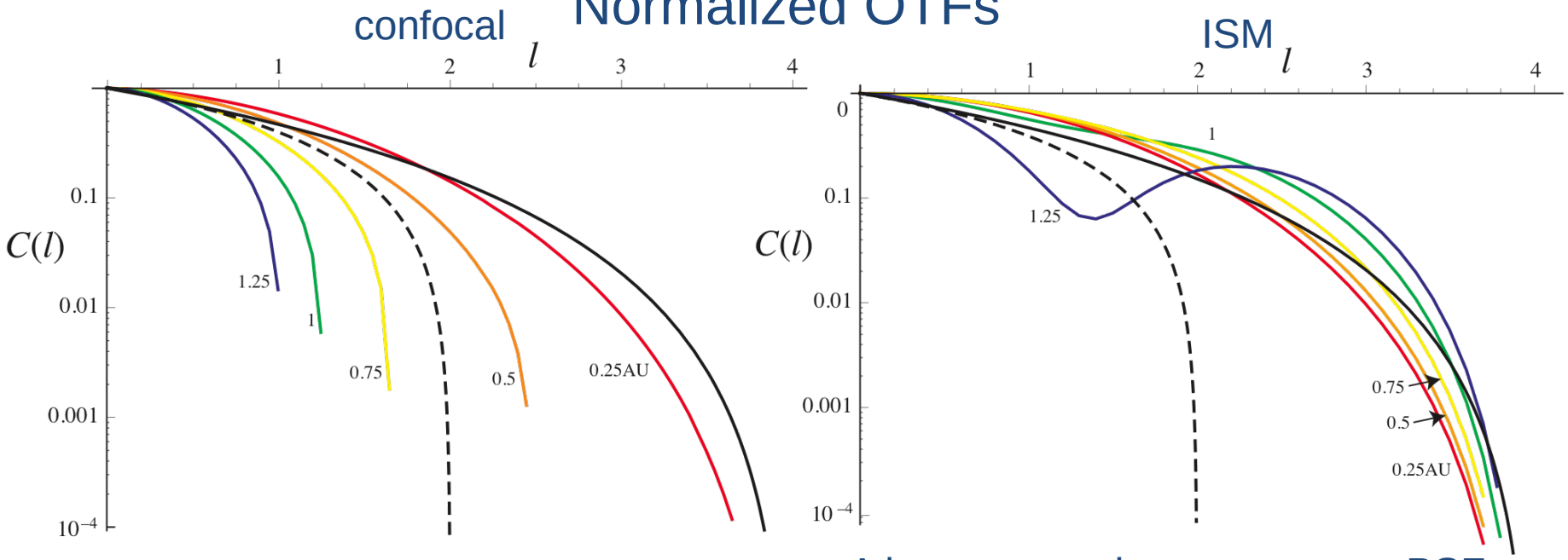


(g) Reflection, maximum detection



Annular ring detector array

Normalized OTFs



For confocal, a ring detector gives a lower cut-off frequency

A large array gives a narrower PSF than a small array, so why not miss out the central part

Dashed black line: conventional
Solid black line: ISM, large array

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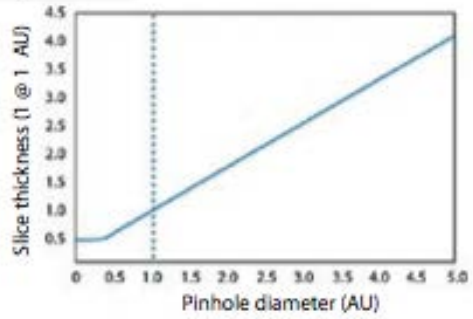
Journal of the Optical Society of America A
OPTICS, IMAGE SCIENCE, AND VISION

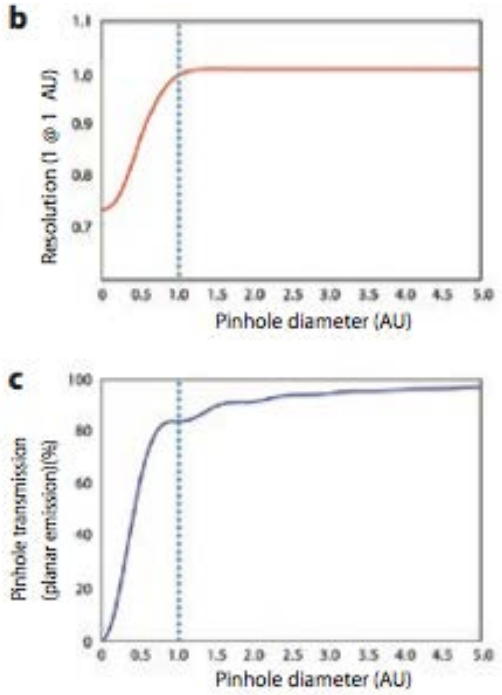
Image formation in image scanning microscopy, including the case of two-photon excitation

Colin J. R. Sheppard,[1,*] Marco Castello,[1,2] Giorgio Tortarolo,[1,2] Giuseppe Vicidomini,[1] and Alberto Diaspro[1,2,3]

Zeiss Airyscan

Over the past 25 years, confocal imaging has become the standard technique for most fluorescence microscopy applications. The increased use of confocal imaging systems in basic biomedical research can be attributed to their ability to produce high-contrast, optically sectioned images while providing enough acquisition versatility to address many sample and application demands





substantial (4-8×) increase in SNR in the final image

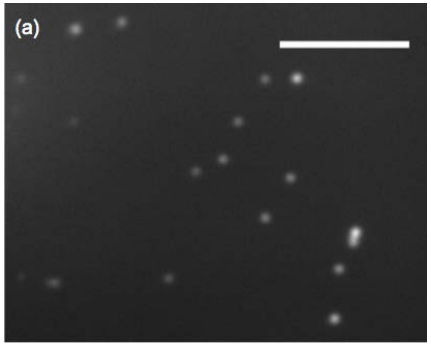
1. Conchello, J.-A. and Lichtman, J.W. *Nat. Methods* **2**, 920–931 (2005).
2. Neu, T.R. and Lawrence, J.R. *Trends Microbiol.* **23**, 233–242 (2015).
3. Sheppard, C.J. *Optik* **80**, 53–54 (1988).
4. Sheppard, C.J., Mehta, S.B. & Heintzmann, R. *Opt. Lett.* **38**, 2889–2892 (2013).

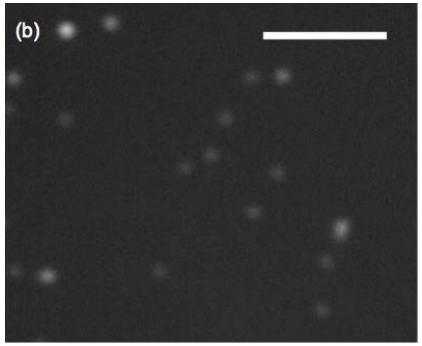
Joseph Huff

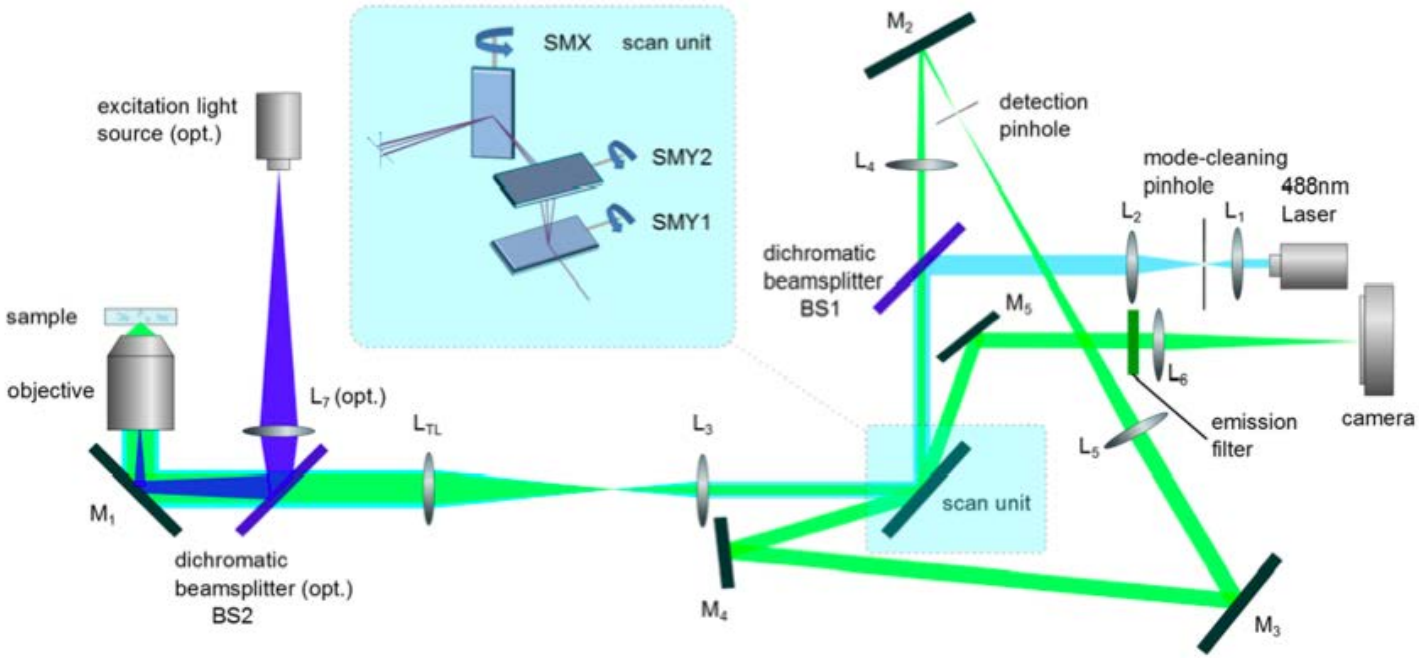
Carl Zeiss Microscopy, LLC, Thornwood, New York, USA. Correspondence should be addressed to J.H. (joseph.huff@zeiss.com).

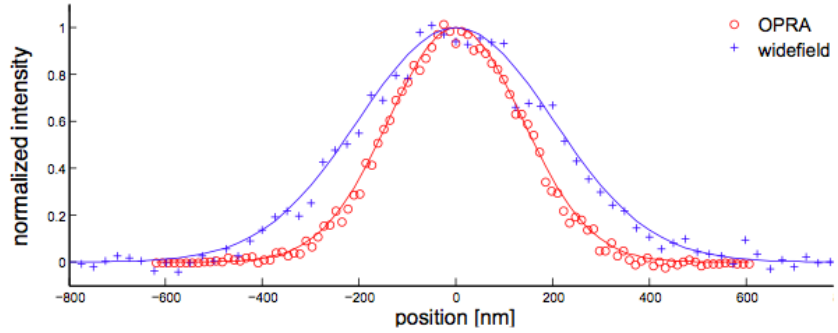
NATURE METHODS | DECEMBER 2015 |

Doing it optically









Roth *et al. Optical Nanoscopy* 2013, **2**:5
http://www.optnano.com/content/2/1/5

Optical Nanoscopy
a SpringerOpen Journal

ORIGINAL ARTICLE **Open Access**

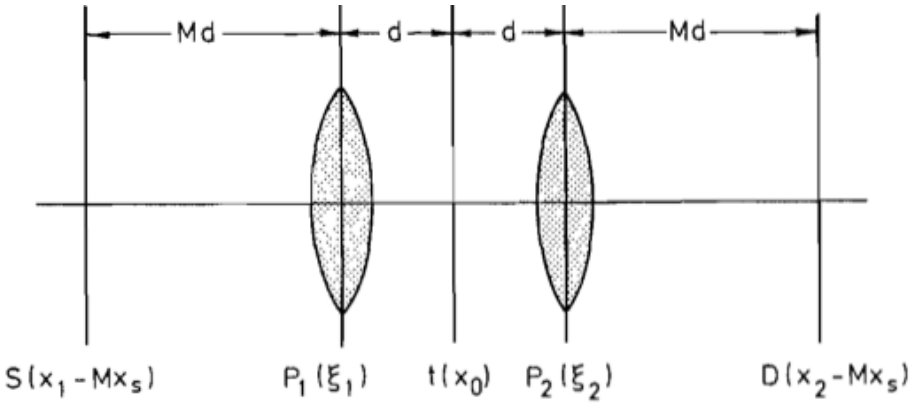
Optical photon reassignment microscopy (OPRA)

Stephan Roth[1,2], Colin JR Sheppard[3], Kai Wicker[1,2] and Rainer Heintzmann[1,2,4*]

Sampling considerations

- Need to sample image at Nyquist rate
- Bandwidth is doubled for pixel reassignment
- If sampling in the detector plane is equal to the sampling of the object illumination, the reconstructed image will exhibit double the sampling rate
- Sampling of the illumination can be at conventional Nyquist rate $v = \pi / 2 = 1.57$, rather than at confocal Nyquist rate
  - Speed advantage over confocal
- This does not contradict information capacity, as multiple images are detected
- Redundancy, so can use compressive sensing

General microscope with source/detector arrays



Journal of Microscopy, *Vol.* 124, *Pt* 2, *November* 1981, *pp.* 107–117.
Revised paper accepted 10 *March* 1981

The theory of the direct-view confocal microscope

by C. J. R. SHEPPARD *and* T. WILSON, *University of Oxford, Department of Engineering Science, Parks Road, Oxford*

1D theory:

source array

$$I(x_2, x_s) = \int\int\int_{-\infty}^{+\infty} S(x_1 - Mx_s)\, h_1\left(\frac{x_1/M - x_0}{\lambda d}\right) h_1^*\left(\frac{x_1/M - x_0'}{\lambda d}\right) t(x_0)\, t^*(x_0')$$

function of 2 variables

$$\times\, h_2\left(\frac{x_2/M - x_0}{\lambda d}\right) h_2^*\left(\frac{x_2/M - x_0'}{\lambda d}\right) D(x_2 - Mx_s)\, dx_1\, dx_0\, dx_0';$$

detector array

- source and detector arrays (scanned)
- partially coherent system (but can also analyze a fluorescence system)
- reduces to conventional, structured illumination (SIM), scanning, confocal, spinning disk, etc.

Fluorescence (incoherent):

$$I(x_1, x_2) = \int H_1(x_1 - x) H_2(x_2 - x) T(x)\, dx$$

General Fluorescence case

2D (or really 4D) image

$$I(x_1, x_2) = \int H_1(x_1 - x) H_2(x_2 - x) T(x)\, dx$$

2D (or 4D) Fourier transform

$$\tilde{I}(m_1, m_2) = \tilde{H}_1(m_1)\tilde{H}_2(m_2)\tilde{T}(m_1 + m_2)$$

Central and difference coordinates

$$m = \frac{m_1 + m_2}{2}, m' = \frac{m_1 - m_2}{2}$$

$$\tilde{I}(m, m') = \tilde{H}_1\left(m + \frac{m'}{2}\right)\tilde{H}_2\left(m - \frac{m'}{2}\right)\tilde{T}(2m)$$

Conventional: $\tilde{H}_1 = \delta\left(m + \frac{m'}{2}\right)$

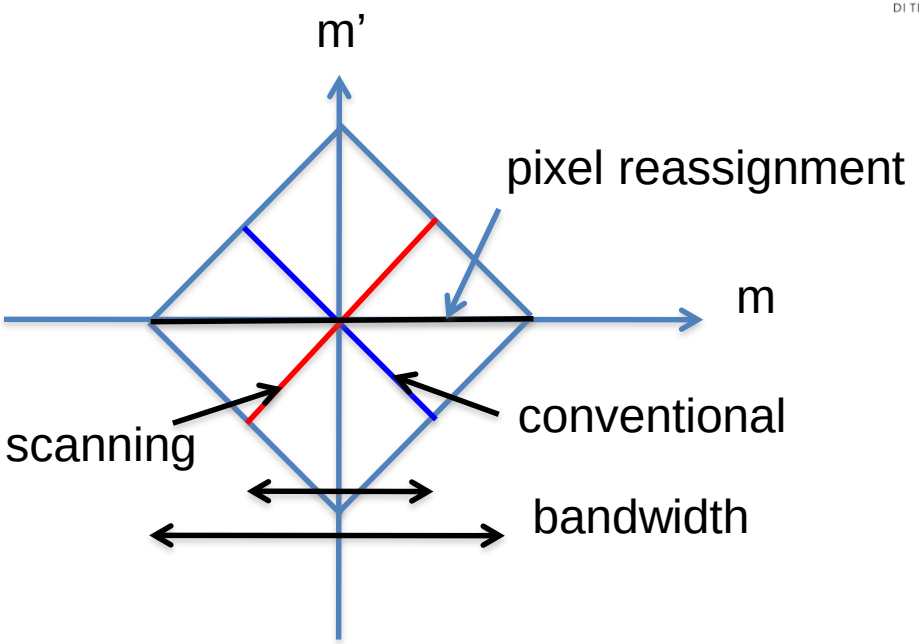
Scanning: $\tilde{H}_2 = \delta\left(m - \frac{m'}{2}\right)$

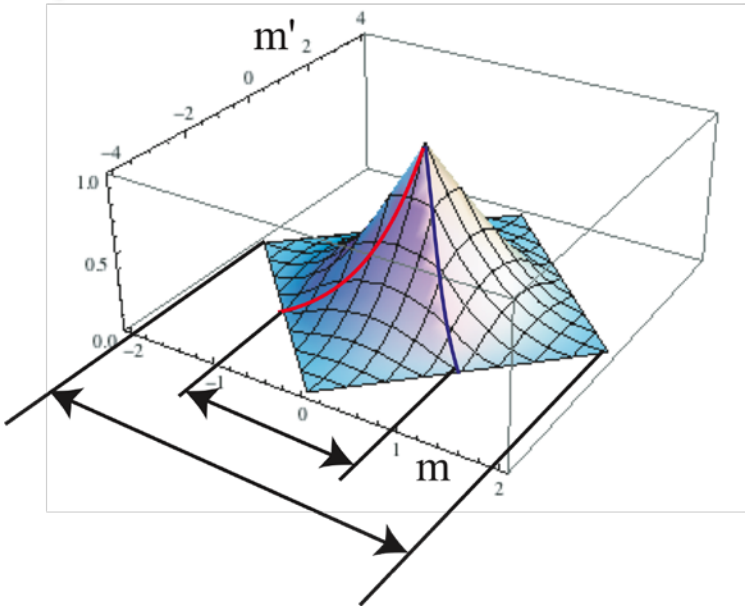
Confocal: $\int dm' \rightarrow \tilde{H}_1 \otimes \tilde{H}_2$

Pixel reassignment:

$m' = 0$

$$\tilde{I}(m, m') = \tilde{H}_1(m)\tilde{H}_2(m)\tilde{T}(2m)$$

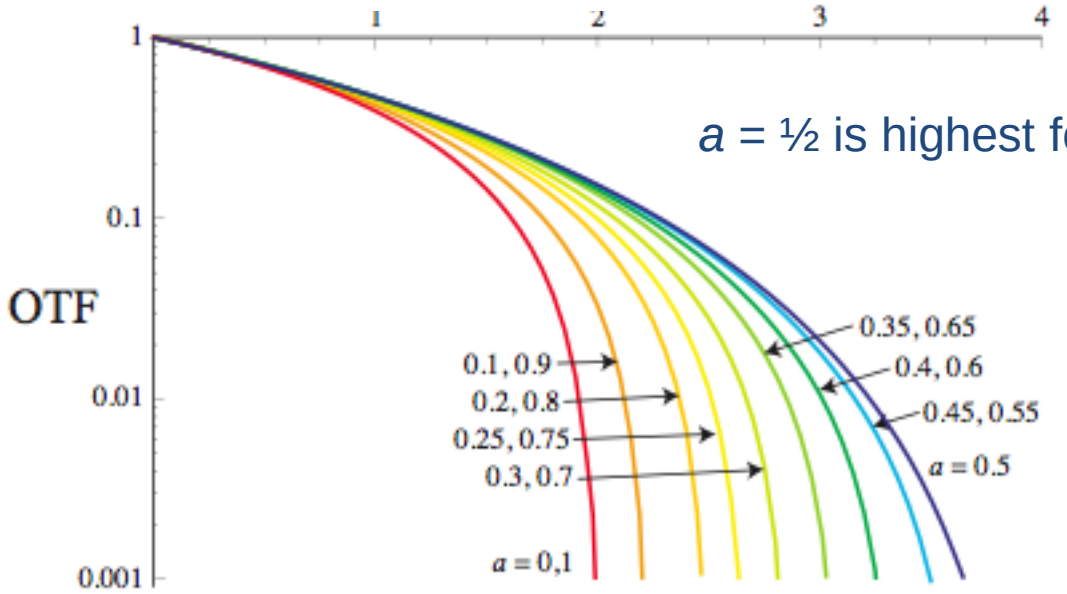




Works for any reassignment factor *a*

- Can use different reassignment factors *a*
- For a large array, OTF is

$$C_{\text{eff}}(l) = C_1[(1-a)l]C_2(al)$$



a = ½ is highest for all spatial frequencies

Fig.4. The OTF for 1PE fluorescence ISM with no Stokes shift, for different values of reassignment factor *a* .

August 1, 2013 / Vol. 38, No. 15 / OPTICS LETTERS 2889

Superresolution by image scanning microscopy using pixel reassignment

Colin J. R. Sheppard,[1,*] Shalin B. Mehta,[2] and Rainer Heintzmann[3,4,5]

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OPTICS, IMAGE SCIENCE, AND VISION

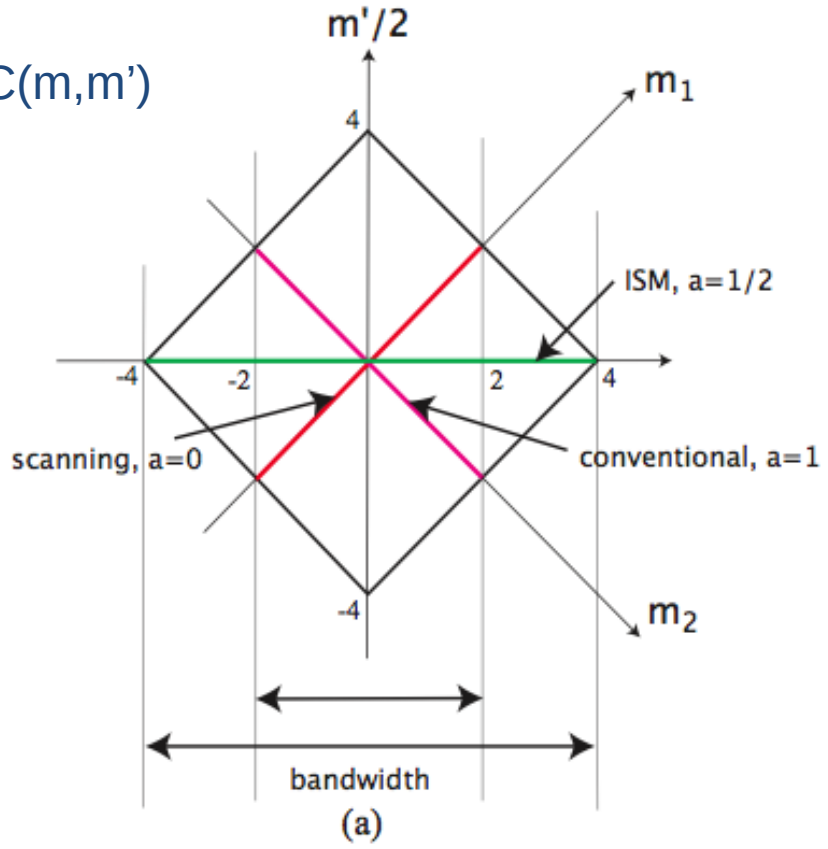
Image formation in image scanning microscopy, including the case of two-photon excitation

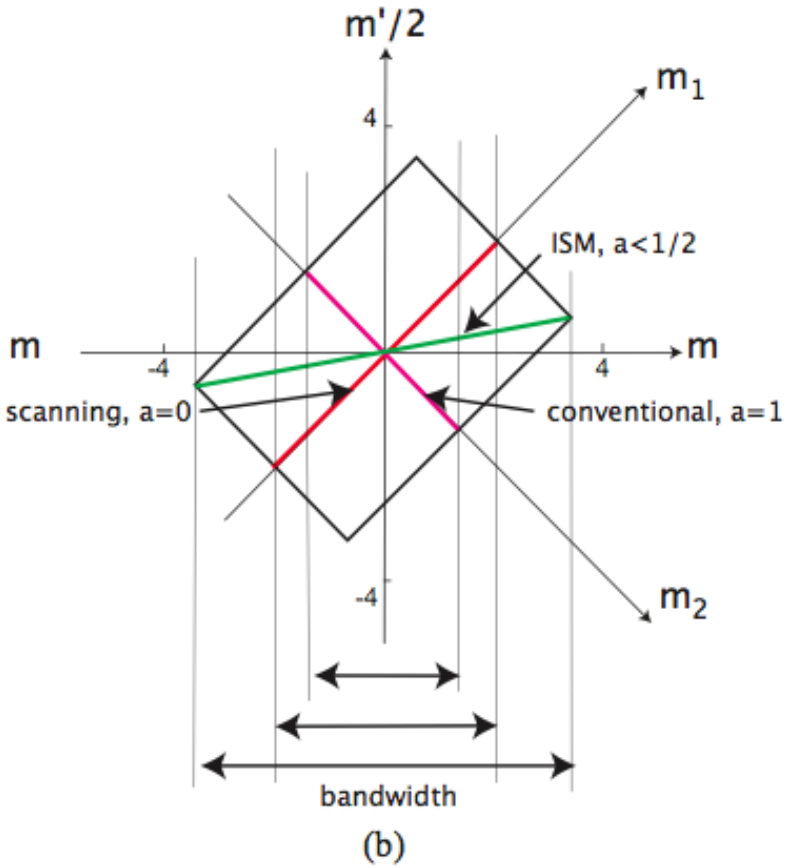
Colin J. R. Sheppard,[1,*] Marco Castello,[1,2] Giorgio Tortarolo,[1,2] Giuseppe Vicidomini,[1] and Alberto Diaspro[1,2,3]

Effect of changing *a*

with Stokes shift

C(m,m')





- Changing *a* changes the slope of a line through the origin
- *a*=0 is scanning, *a*=1 is conventional

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Journal of the Optical Society of America A

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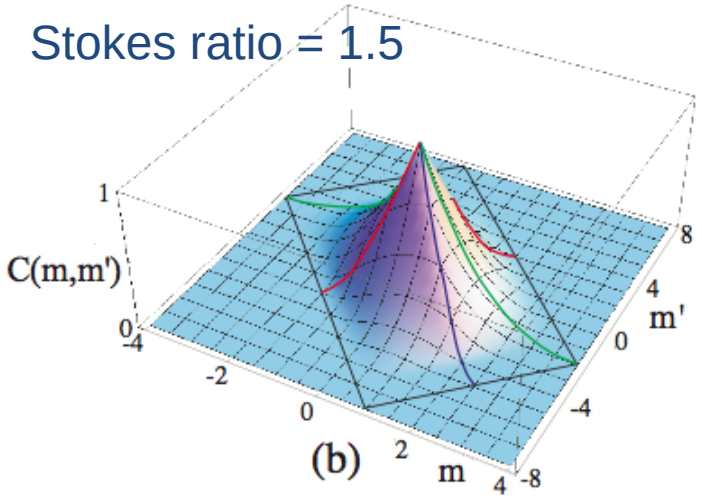
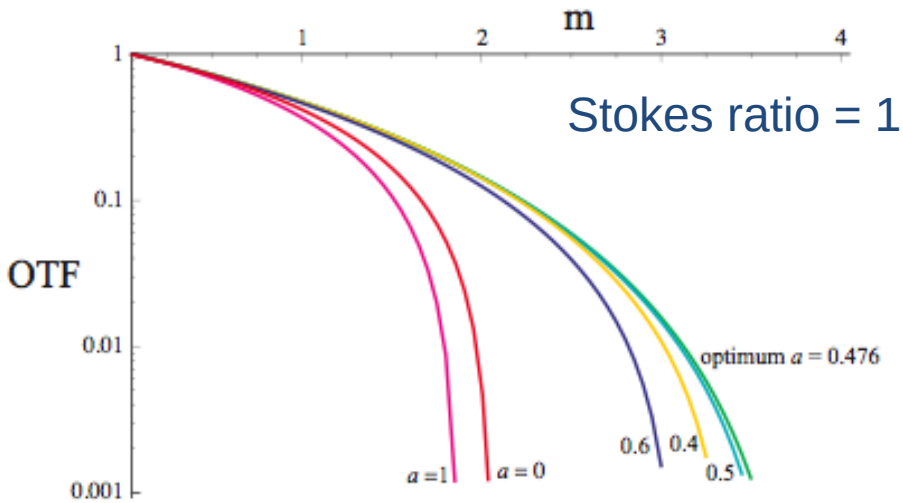
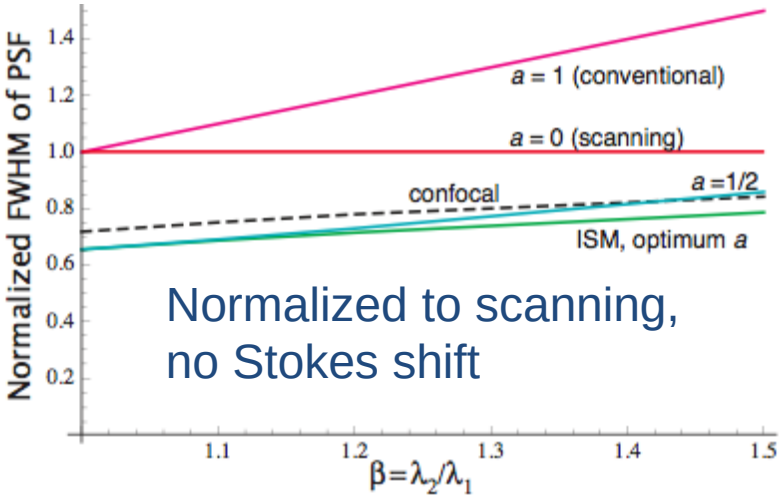
With Stokes shift, large array

Stokes ratio = 1.5







Normalized to scanning, no Stokes shift

Fig.6. The variation in the normalized FWHM of the point spread function with Stokes shift ratio, for different values of the reassignment factor a, for ISM with a large detector array.

- scanning is better than conventional
- true confocal is better
- ISM for optimum *a* is even better
- ISM for *a*=1/2 is better than confocal for Stokes ratio<1.4
- *a*=1/2 is fine for Stokes ratio of 1.1

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Journal of the Optical Society of America A

OPTICS, IMAGE SCIENCE, AND VISION

Image formation in image scanning microscopy, including the case of two-photon excitation

COLIN J. R. SHEPPARD,[1,*] MARCO CASTELLO,[1,2] GIORGIO TORTAROLO,[1,2] GIUSEPPE VICIDOMINI,[1] AND ALBERTO DIASPRO[1,2,3]

Effect of array size and Stokes shift

Curves normalized by FWHM for conventional fluorescence microscope

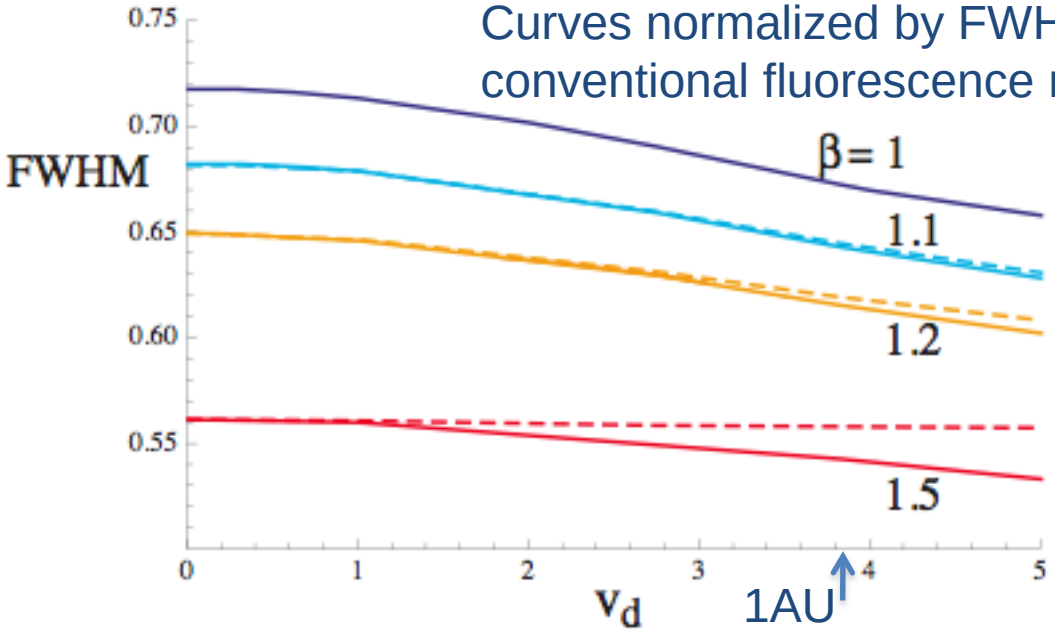

Fig.9. The FWHM of the PSF, normalized by the FWHM for conventional 1PE fluorescence microscopy, for ISM as a function of detector array size, with Stokes ratio β as parameter. The solid curves show the results for reassignment factor $a = 1/(1+\beta)$, and the dashed curves for $a = 1/2$.

- For Stokes ratio of 1.1, *a*=1/2 is OK
- As Stokes ratio increases, the improvement relative to conventional improves
- For small arrays, value of *a* doesn't matter

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Journal of the Optical Society of America A

OPTICS, IMAGE SCIENCE, AND VISION

Image formation in image scanning microscopy, including the case of two-photon excitation

COLIN J. R. SHEPPARD,[1,*] MARCO CASTELLO,[1,2] GIORGIO TORTAROLO,[1,2] GIUSEPPE VICIDOMINI,[1] AND ALBERTO DIASPRO[1,2,3]

Bessel beam, 1977-1980

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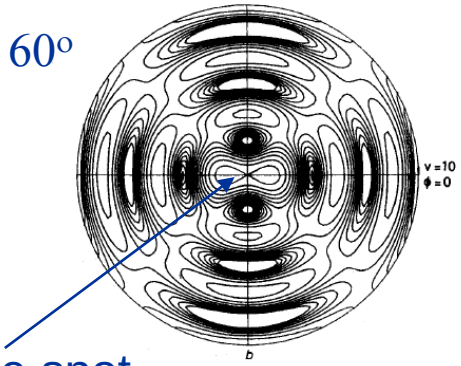
•J_0 beam is propagationally invariant (1978):

The radial distribution of amplitude for a δ ring is given by a zero-order Bessel function in any plane (in the region of validity) perpendicular to the optic axis. That this is so is not surprising because such a wave is the circularly symmetric mode of free space. We are acquainted with modes of this form in circular waveguides, and we can consider free space as the limiting case of a waveguide of very large diameter. Such an overmoded waveguide has an infinity of circularly symmetric modes, that is the scale of the Bessel functions may be chosen at will. A wave with zero-order Bessel-function radial distribution propagates without change.

C. J. R. Sheppard and T. Wilson, "Gaussian-beam theory of lenses with annular aperture," IEE J. Microwaves, Opt. Acoust. **2**, 105–112 (1978).

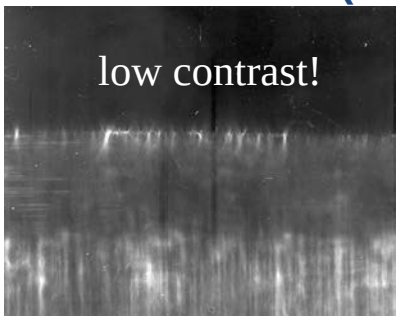
Nonparaxial electromagnetic Bessel beam (1978):

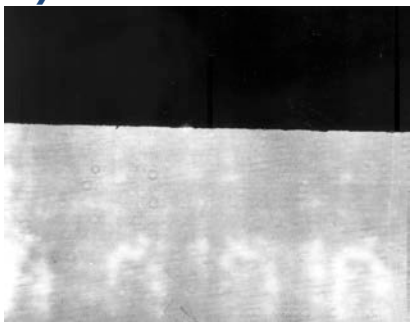


C. J. R. Sheppard, "Electromagnetic field in the focal region of wide-angular annular lens and mirror systems," IEE J. Microwaves, Opt. Acoust. **2**, 163–166 (1978).

Imaging with Bessel beam (1977)

ε = 0.9





C. J. R. Sheppard
The use of lenses with annular aperture in scanning optical microscopy

Optik 48 (1977) No. 3, 329–334

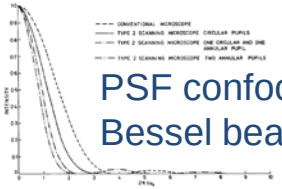
OPTICA ACTA, 1977, VOL. 24, NO. 10, 1051–1073

First paper to use term "confocal microscope" (1977)

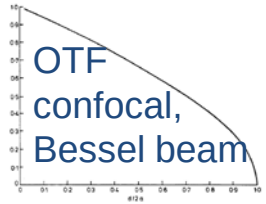
Image formation in the scanning microscope

C. J. R. SHEPPARD and A. CHOUDHURY
Department of Engineering Science, Parks Road, Oxford, England

(*Received* 22 *December* 1976)



Abstract. Fourier imaging in the scanning microscope is considered. It is shown that there are two geometries of the microscope, which have been designated Type 1 and Type 2. Those of Type 1 exhibit identical imaging to the conventional microscope, whereas those of Type 2 (confocal microscopes) display various differences. Imaging of a single point object, two-point resolution and response to a straight edge are also considered. The effect of various arrangements using lenses with annular pupil functions is also discussed. It is found that Type 2 microscopes have improved imaging properties over conventional microscopes and that these may be further improved by use of one or two lenses with annular pupils.



Confocal microscope with Bessel beam (1980):

United States Patent [19] [11] **4,198,571**

Sheppard [45] **Apr. 15, 1980**

[54] **SCANNING MICROSCOPES**

[75] Inventor: **Colin J. R. Sheppard,**

Bessel beam in confocal

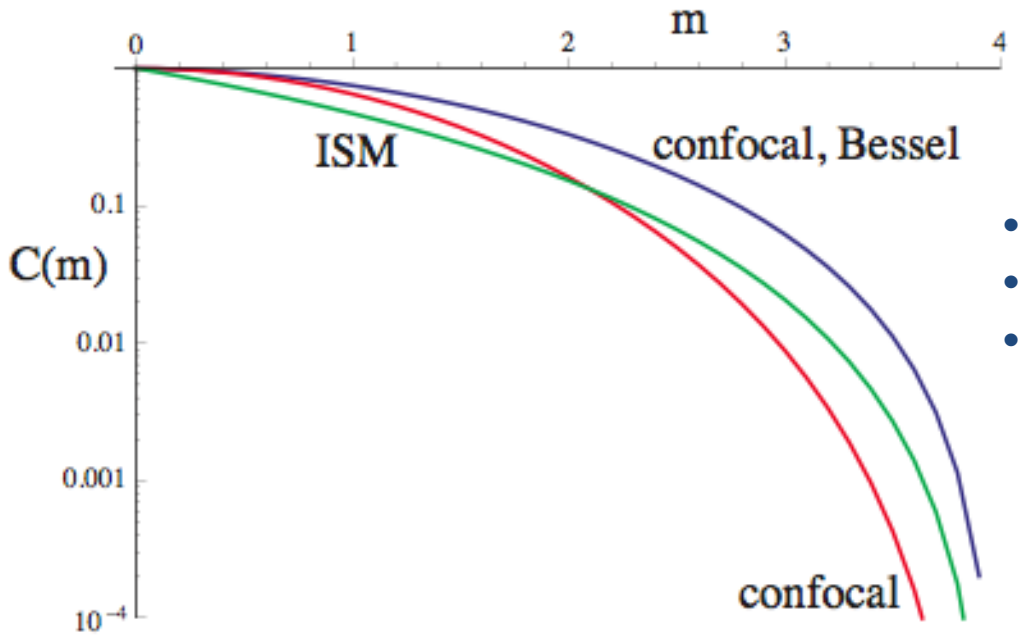


Fig.17. The OTFs for a confocal microscope with illumination by a Bessel beam, a confocal microscope with two circular pupils, and ISM with two circular pupils and a large array. The OTFs are normalized to unity at zero spatial frequency.

- Normalized OTFs
- But confocal has very low signal level
- What about ISM with Bessel beam?

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ISM with pupil filters

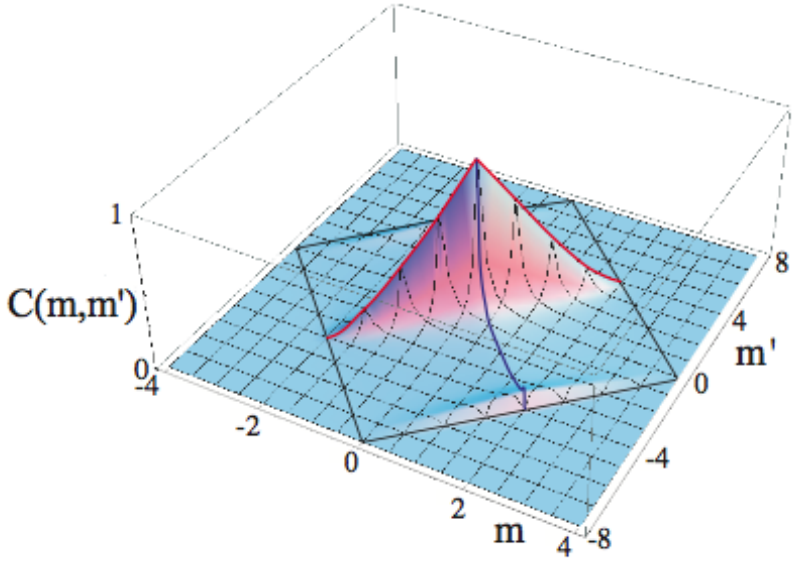


Fig.13. Plot of $C(m,m')$ for 1PE fluorescence with an approximation to Bessel beam illumination (annular lens with $\varepsilon = 0.9$), and no Stokes shift.

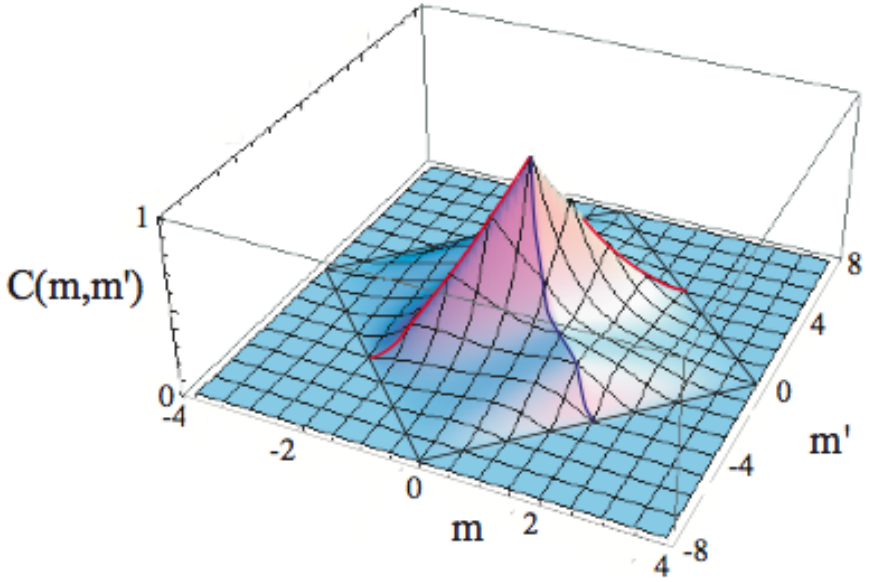


Fig.15. Plot of $C(m,m')$ for 1PE fluorescence with a parabolic amplitude filter $b = 1$ and no Stokes shift.

Bessel beam (annular filter) gives poor response at mid-frequencies because

$$C_{\text{eff}}(l) = C_1[(1-a)l]C_2(al).$$

Parabolic amplitude filter is better

550 OPTICS LETTERS / Vol. 40, No. 4 / February 15, 2015

Optimization of pupil filters for maximal signal concentration factor

C. J. R. Sheppard

Optimum *a* varies with spatial frequency

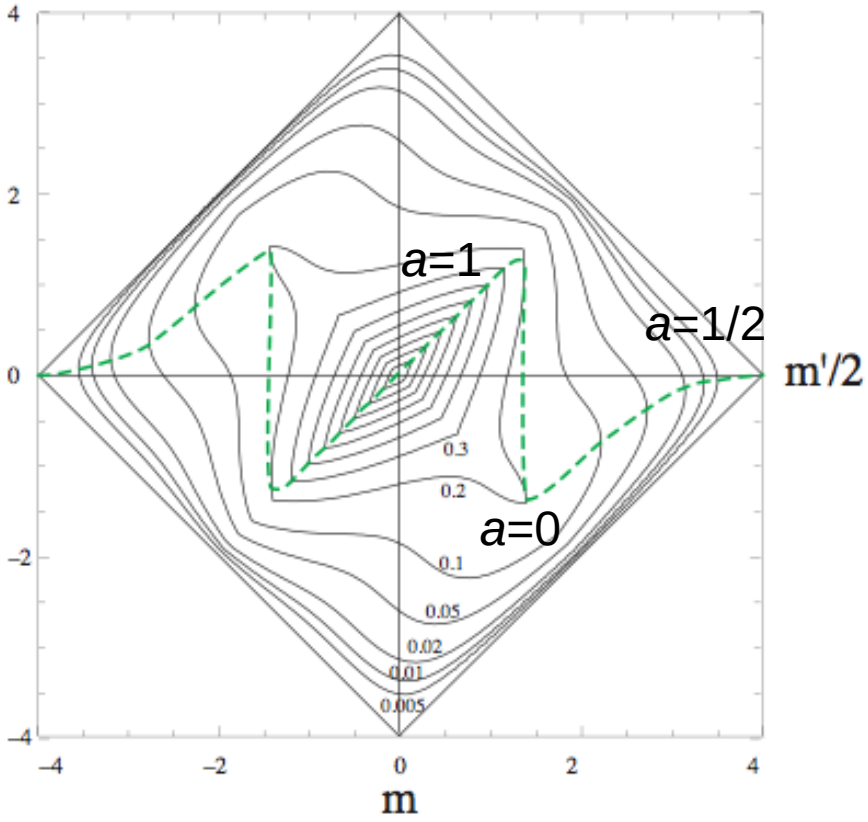
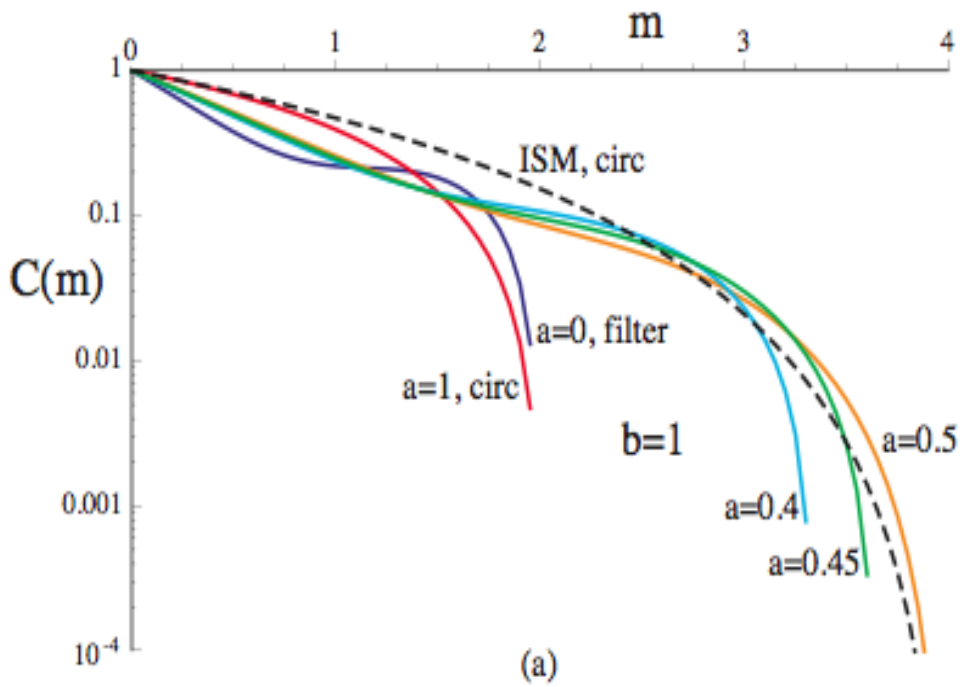


Fig.16. A contour plot of $C(m,m')$ for 1PE fluorescence with a parabolic filter $b=1$ and no Stokes shift. The approximate locus for $a(m)$ to maximize $C(m)$ is shown (dashed line).



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Multiphoton microscopy

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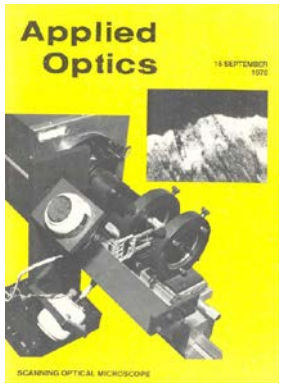
Proposal of different types of scanning nonlinear microscopy based on the high intensity in the focused spot, including two-photon fluorescence and CARS (1978)

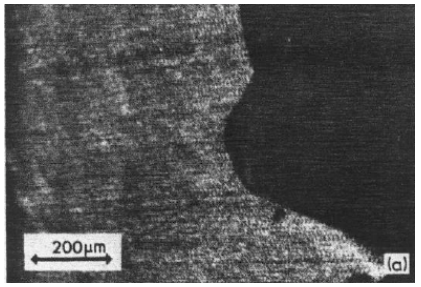
In the scanning optical microscope[1,2] nonlinear interactions are expected to occur between the object and a highly focused beam of light, which we hope will open new ways of studying matter in microscopic detail hitherto not available. Nonlinear interactions[3–5] include the generation of sum frequencies, Raman scattering, two-photon fluorescence, and others. We feel

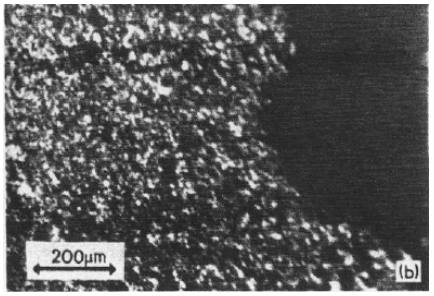
C. J. R. Sheppard and R. Kompfner

Appl. Opt. **17**, 2879-2882 (1978)



First published scanning SHG images (1978)





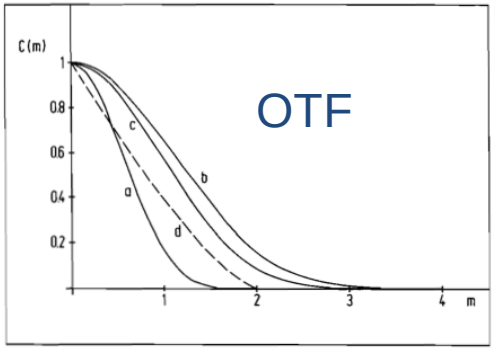
KD*P crystal, SHG images CW NdYAG laser 1064nm **Demonstrates optical sectioning**

Optical and Quantum Electronics 10 (1978) 435–439

Second-harmonic imaging in the scanning optical microscope

J. N. GANNAWAY, C. J. R. SHEPPARD

Image formation (1990)



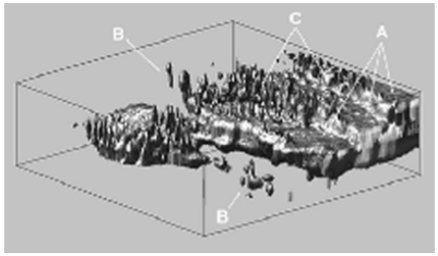
Optik 86, No. 3 (1990) 104–106

Fig. 2. The OTF for various imaging modes: (a) two-photon fluorescence, (b) confocal fluorescence, (c) confocal two-photon fluorescence, (d) conventional fluorescence.

Image formation in two-photon fluorescence microscopy

C. J. R. Sheppard, M. Gu

3D SHG with fs pulses (1998)

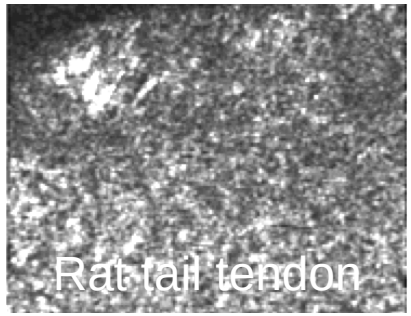


August 1, 1998 / Vol. 23, No. 15 / OPTICS LETTERS 1209

Three-dimensional second-harmonic generation imaging with femtosecond laser pulses

R. Gauderon and P. B. Lukins C. J. R. Sheppard

Radial polarization (2007)



Second harmonic generation polarization microscopy with tightly focused linearly and radially polarized beams

E.Y.S. Yew [a,*], C.J.R. Sheppard [a,b]

Optics Communications 275 (2007) 453–457

Two-photon fluorescence ISM

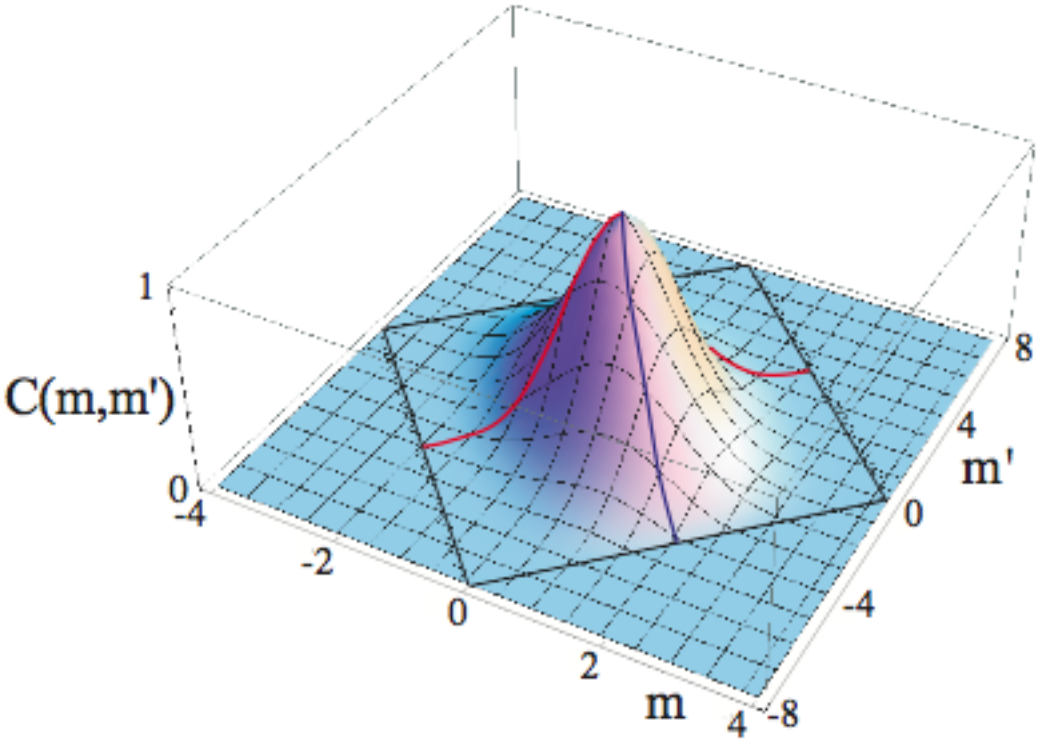


Fig.12. Plot of $C(m,m')$ for 2PE fluorescence, and no Stokes shift.

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Two-photon fluorescence ISM

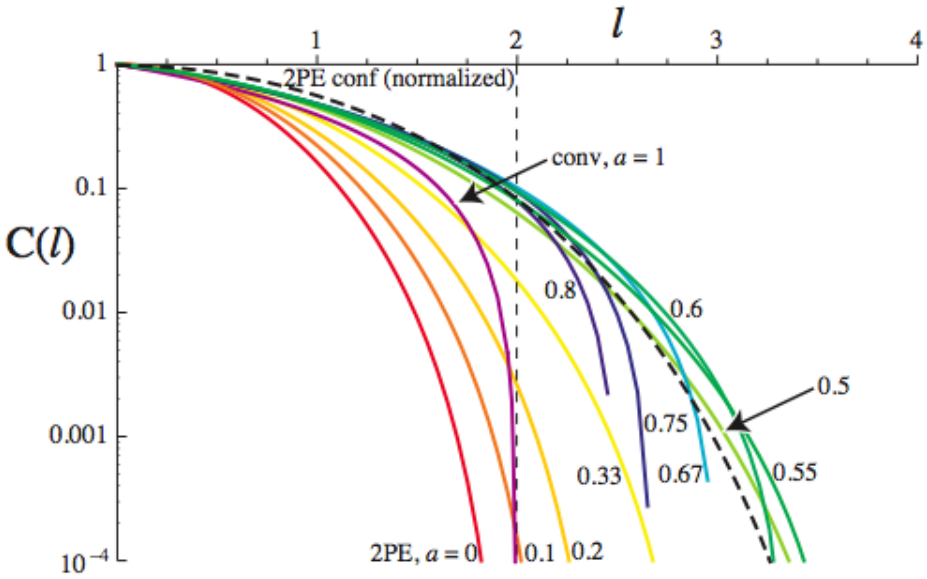

Fig. 1. The OTF for two-photon fluorescence with different reassignment factors, a. A value of zero gives a 2PE fluorescence microscopy with a large detector. A value of unity gives an image identical to that in a conventional 1PE fluorescence microscope. The

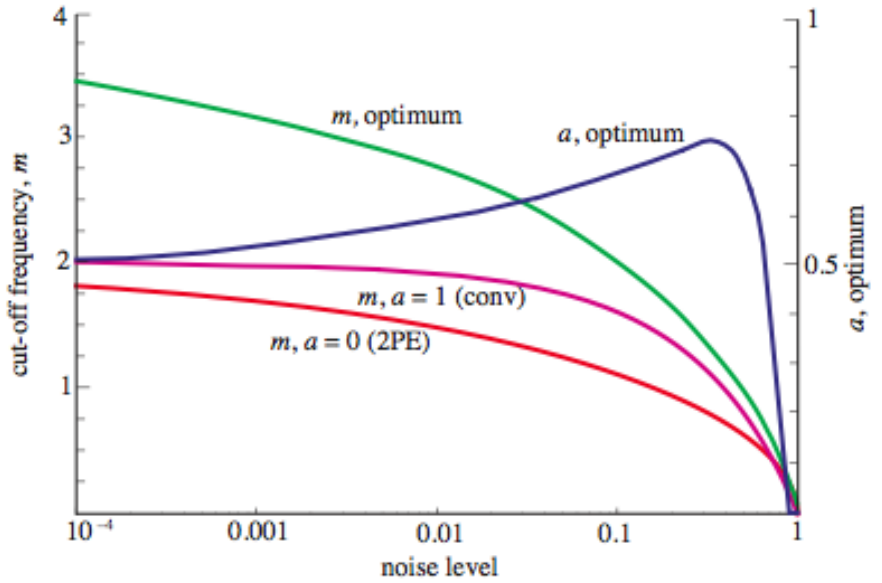

Fig.2. The useful cut-off frequency m, as a function of the noise level, for pixel reassignment with the optimum value of reassignment factor a (green curve). The optimum value of a is also shown (blue curve). The useful cut-off frequencies for conventional 1PE and 2PE are shown for comparison (purple and red curves, respectively).

- Can alter reassignment factor *a*
- OTF is

$$C_{\text{eff}}(l) = C_1[(1-a)l]C_2(al).$$

- Resolution improved compared with two-photon fluorescence with a large single-element detector

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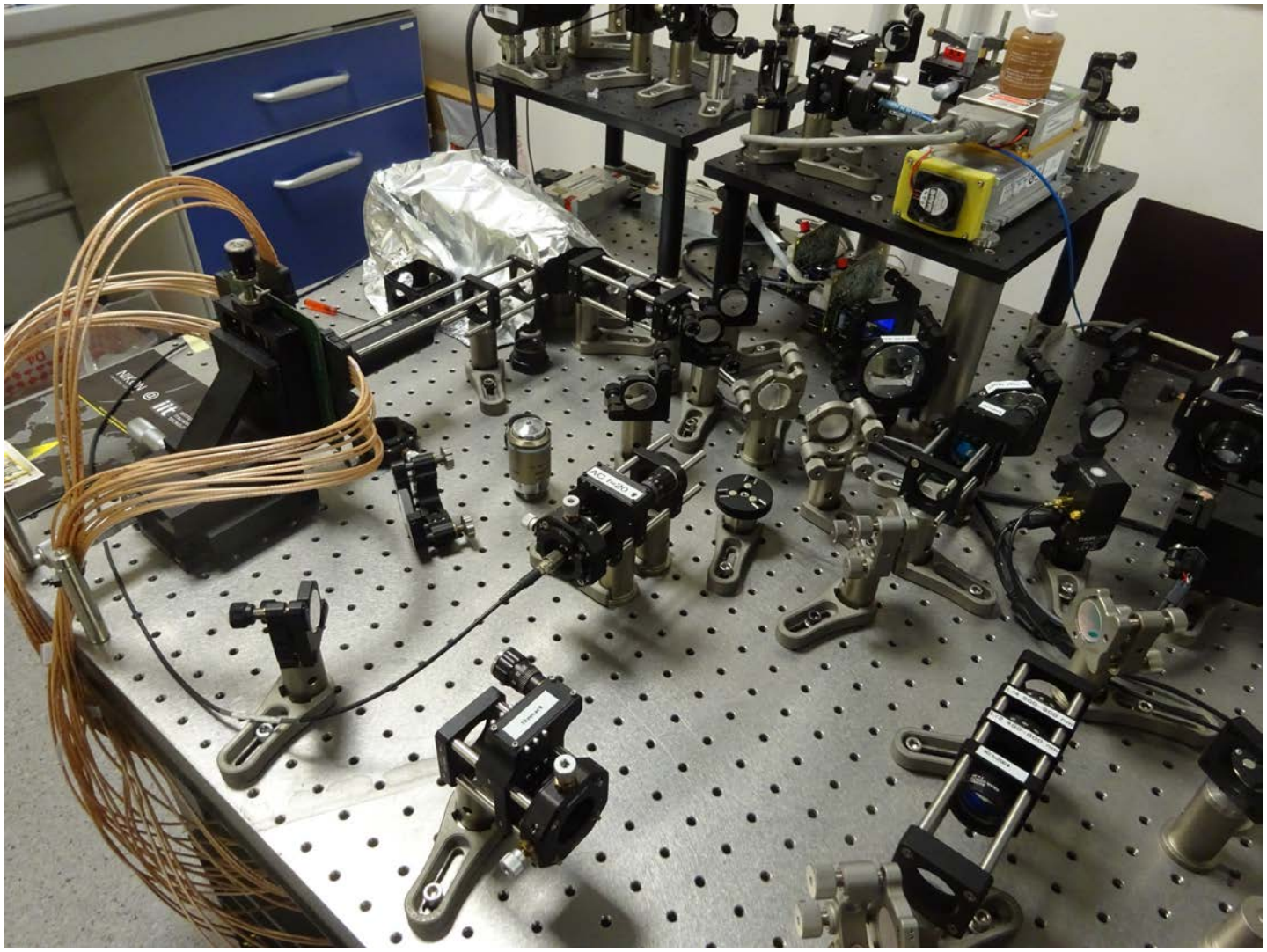
Medusa



Mauro Buttafava & Alberto Tosi
DEIB, Politecnico di Milano,
Via Ponzio 34, Milan, Italy

Marco Castello, Giorgio Tortarolo,
Giuseppe Vicidomini, Alberto Diaspro,
IIT, Genova, Italy

SPAD array

The first APD array designed for microscopy.

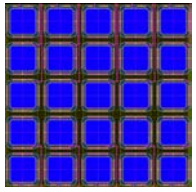
SPADs	Crosstalk probability
First neighbors - orthogonal	< 1%
First neighbors - diagonal	< 0.2%

Hold-OFF Time	Afterpulsing probability
50 ns	6.5%
100 ns	2.4%
200 ns	1.4%

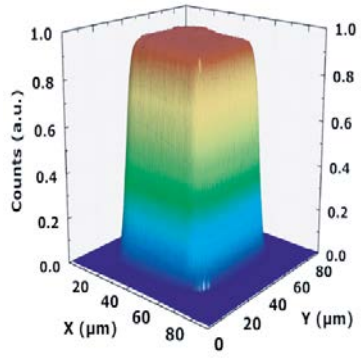
Features:

- 5 x 5 matrix
- 75 µm pitch
- 50 µm x 50 µm active area
- fill factor = $\frac{50^2}{75^2} \approx 44\%$

  (future improvement: microlenses array)
- 25 TTL signals + (3 ch.) communication bus



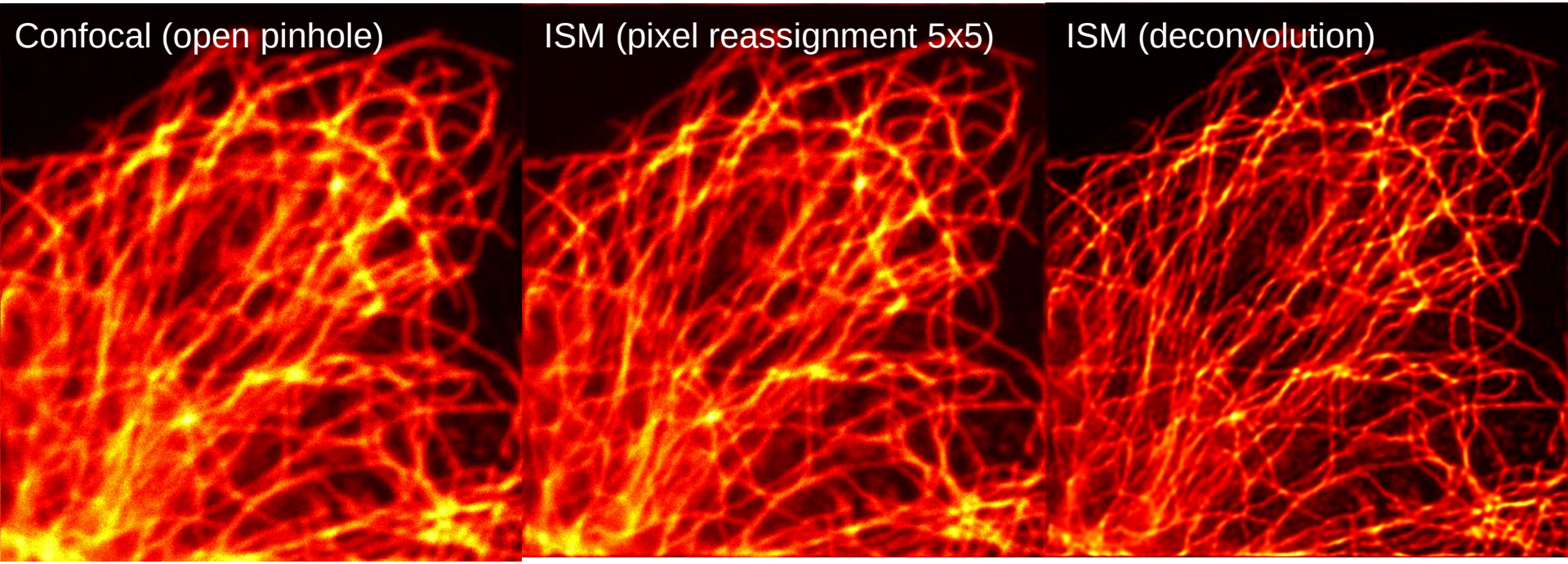
Uniformity:



Mauro Buttafava & Alberto Tosi
DEIB, Politecnico di Milano,
Via Ponzio 34, Milan, Italy

Marco Castello, Giorgio Tortarolo,
Giuseppe Vicidomini, Alberto Diaspro,
IIT, Genova, Italy

Biological sample: Tubuline



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Via Ponzio 34, Milan, Italy

Marco Castello, Giorgio Tortarolo,
Giuseppe Vicidomini, Alberto Diaspro,
IIT, Genova, Italy

Discussion

- Structured illumination can give improved resolution (x2)
- Confocal microscopy gives improved resolution but spatial frequency response at high spatial frequencies is low ($\times\sqrt{2}$ in PSF)
- But signal is also low, so must open pinhole, giving almost no improvement in resolution
- Pixel reassignment increases signal collection efficiency
- Also gives improved resolution, better than confocal
- And speed is increased
- ISM with 2 photon excitation improves resolution
- ISM with pupil filters can improve high frequency response

Single-pixel camera

(a)

(b)

256x256 pixels

1300 random measurements with compressive sensing

32x32=1296 pixels bicubic upsampled

Larkin

http://www.nontrivialzeros.net/Hype_&_Spin/Misleading%20Results%20in%20Single%20Pixel%20Camera-v1.02.pdf

M. F. Duarte, M. A. Davenport, D. Takhar, J. N. Laska, T. Sun, K. F. Kelly, and R. G. Baraniuk, "Single pixel imaging via compressive sampling," *IEEE Signal Proc. Mag.*, vol. 25, no. 2, pp. 83–91, March 2008.

2041 citations