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Confocal Microscopy & Superresolution

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Genoa, Italy
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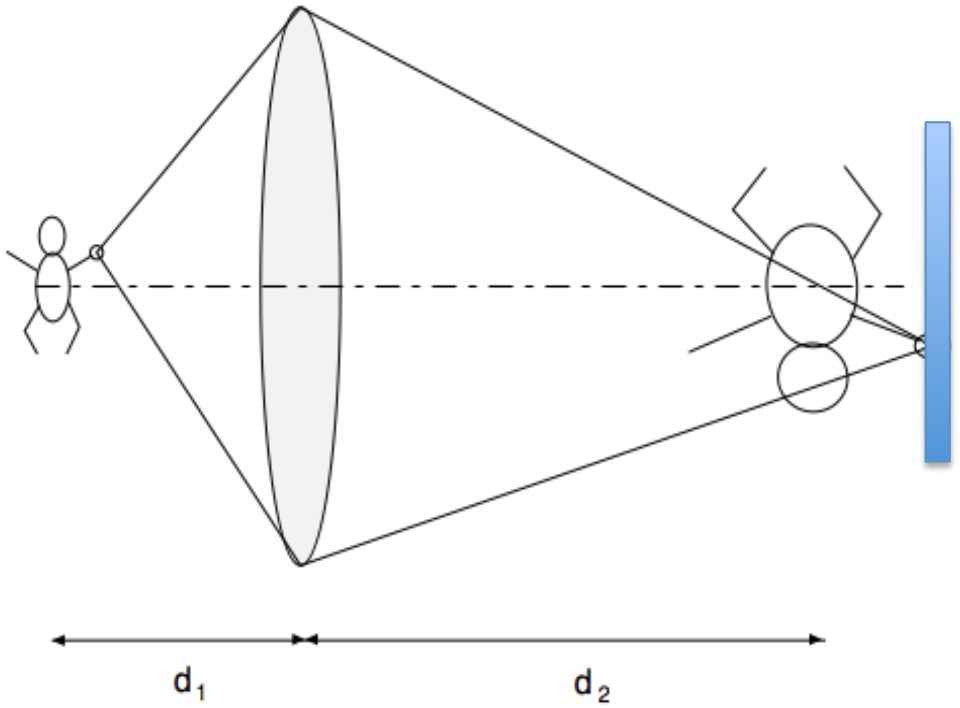
Imaging using a detector array

Can generate an image with a lens and a detector array



wide-field detection

CHAPTER 1

The Generalized Microscope*

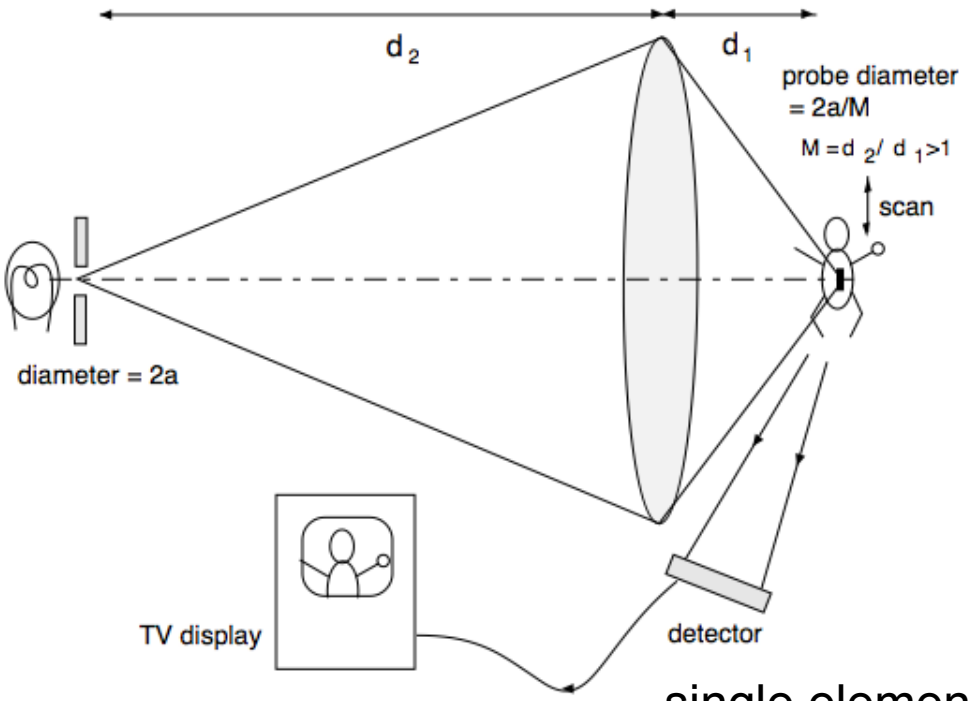
COLIN J. R. SHEPPARD

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

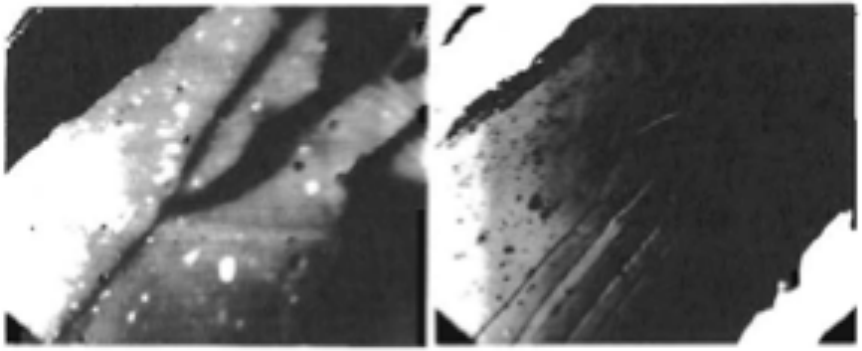
Another way of generating an image: using a scanning system



single element detector

- Detector **does not image**, only collects light.
- Magnification of image is ratio of size of image to amplitude of scan.
- Independent of probe diameter.

Imaging with a focused probe



photovoltage mode reflection mode

A SCANNING OPTICAL MICROSCOPE FOR

THE INSPECTION OF ELECTRONIC DEVICES

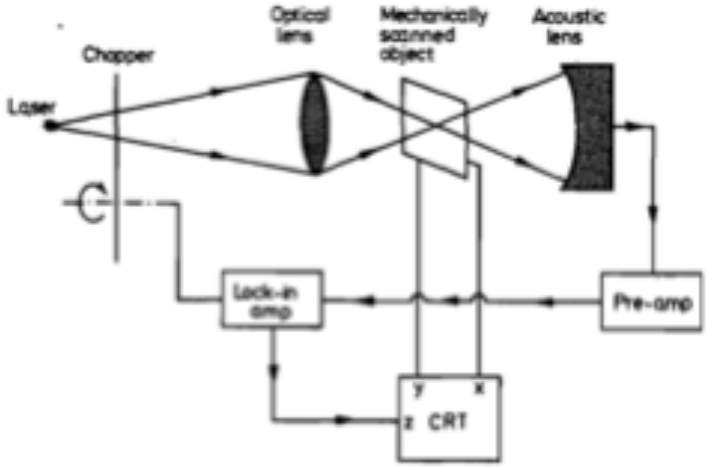
C.J.R. Sheppard, J.N. Gannaway
D. Walsh, T. Wilson

Reprinted from H. Ahmed & W. C. Nixon, *Microcircuit Engineering*.
© Cambridge University Press 1980, Printed in U.S.A.

Imaging in a scanning photoacoustic microscope

I. J. Cox[a] and C. J. R. Sheppard

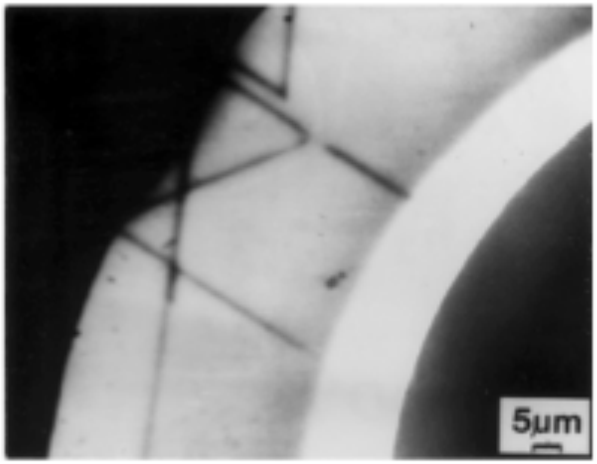
513 J. Acoust. Soc. Am. 76 (2), August 1984



optical psf

$$A(x,x_s) = \iint h_o(x')h_o^*(x')o(x'-x_s) \times h_t(x''-x')t(x''-x_s)h_a(x-x'')dx'\,dx''$$

thermal psf acoustic psf



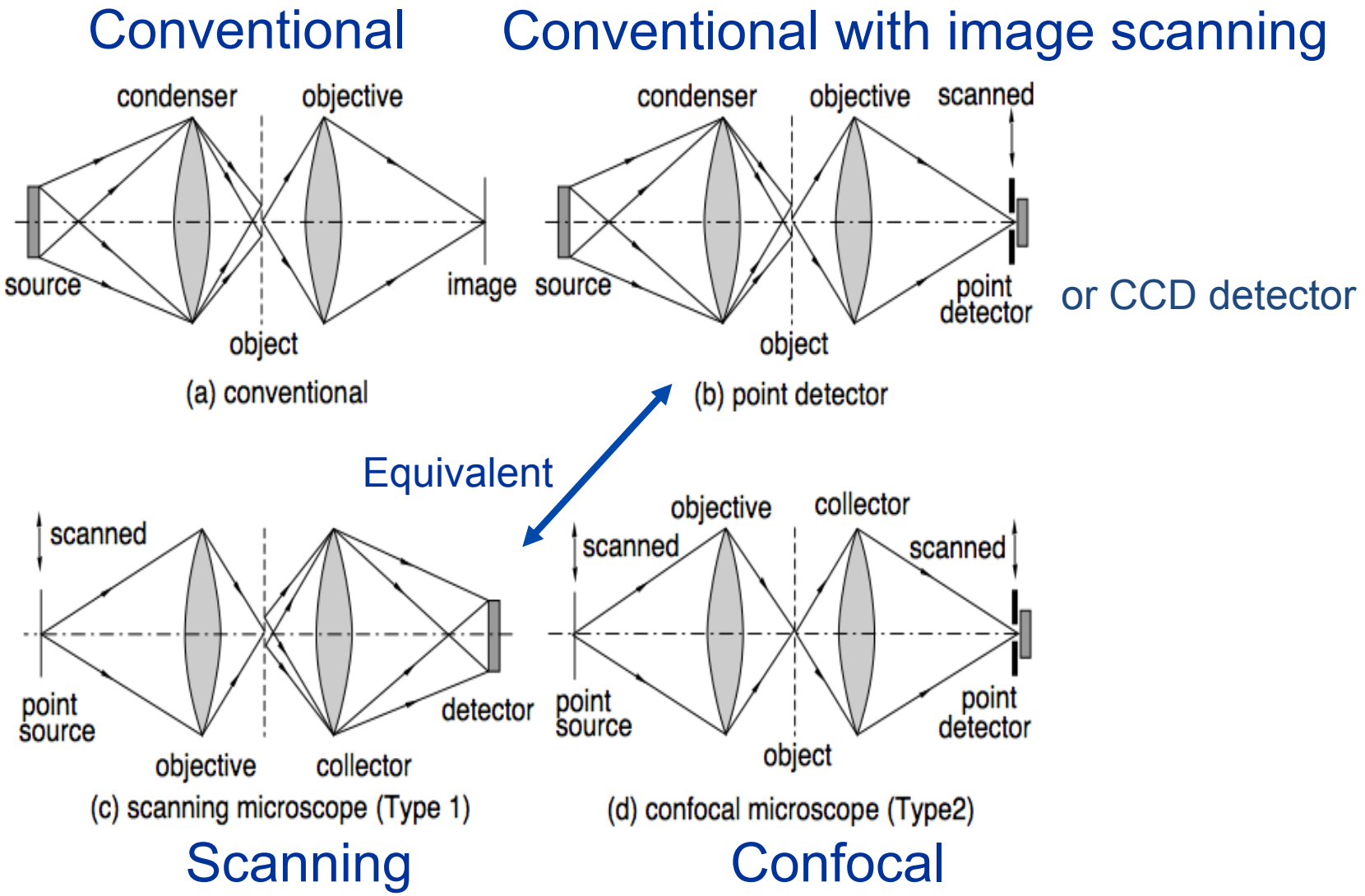
Defects in a silicon transistor,
Optical beam induced current mode,
CJR Sheppard
Applications of scanning optical microscopy,
Proc. SPIE **368**, 88-95 (1982).

Equivalence of scanning and conventional microscopes

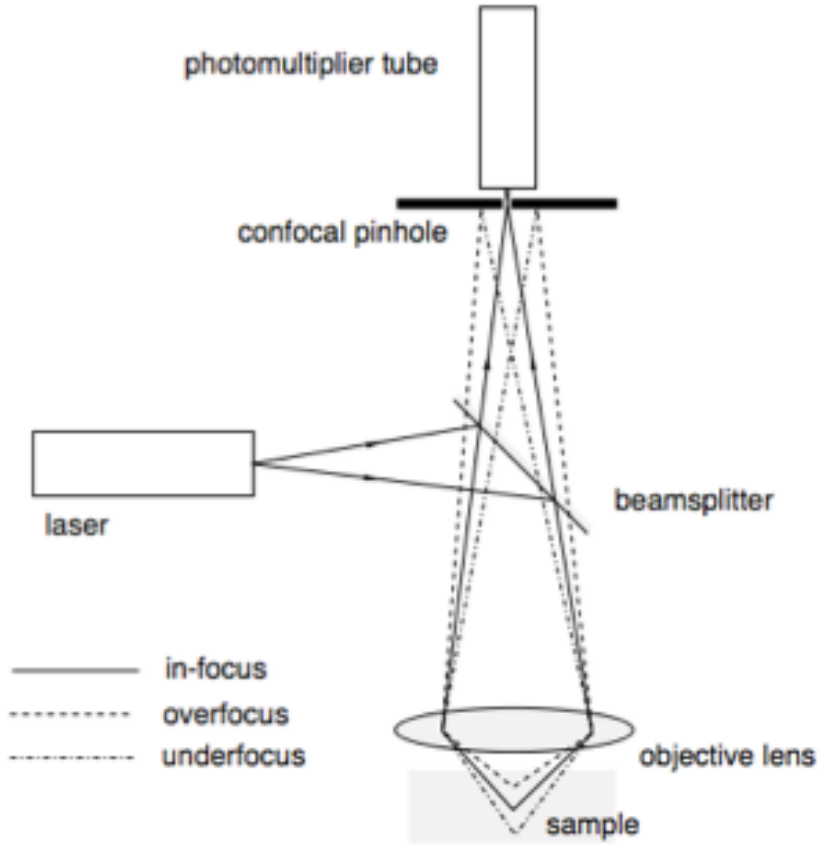
- Based on Principle of Reciprocity
- Holds even with loss or multiple scattering
  (but not inelastic scattering)
- First shown for electron microscopes

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, *Optik* **78**, 39-43 (1986); *J. Opt. Soc. Am. A* **3**, 755-756 (1986)

Scanning vs. conventional microscope



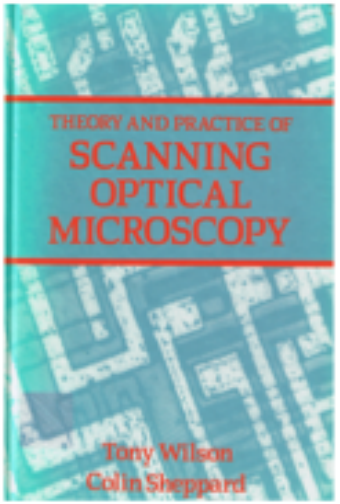
Confocal imaging: schematic diagram



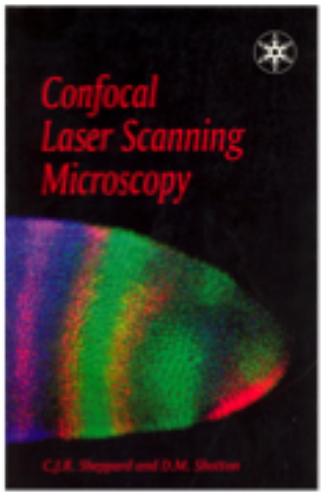
Build up image by scanning:

- Scan sample
- Scan beam
- Scan lens

Reflectance or fluorescence



1984



1997

Optical sectioning

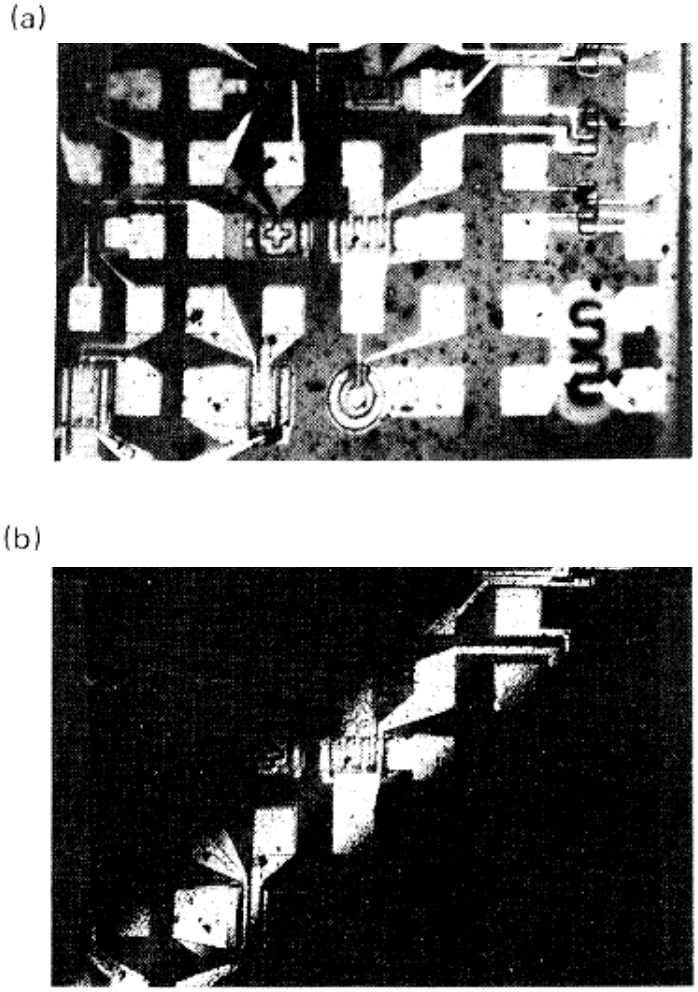


Fig. 3. (a) Conventional scanning microscope image. (b) Confocal-scanning microscope image.

Hamilton DK, Wilson T, Sheppard CJR

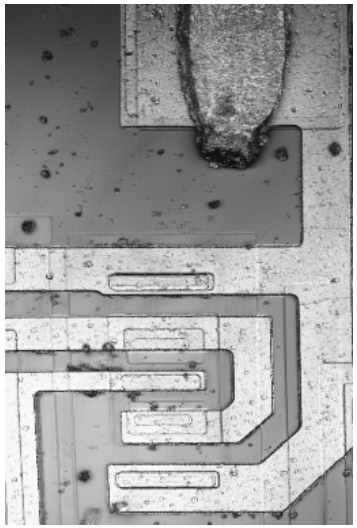
Experimental observations of the depth-discrimination properties of scanning microscopes

*Opt. Letts.***6**, 625-626 (1981)

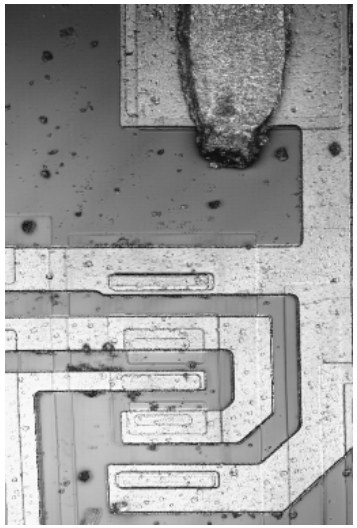
Confocal microscopy

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

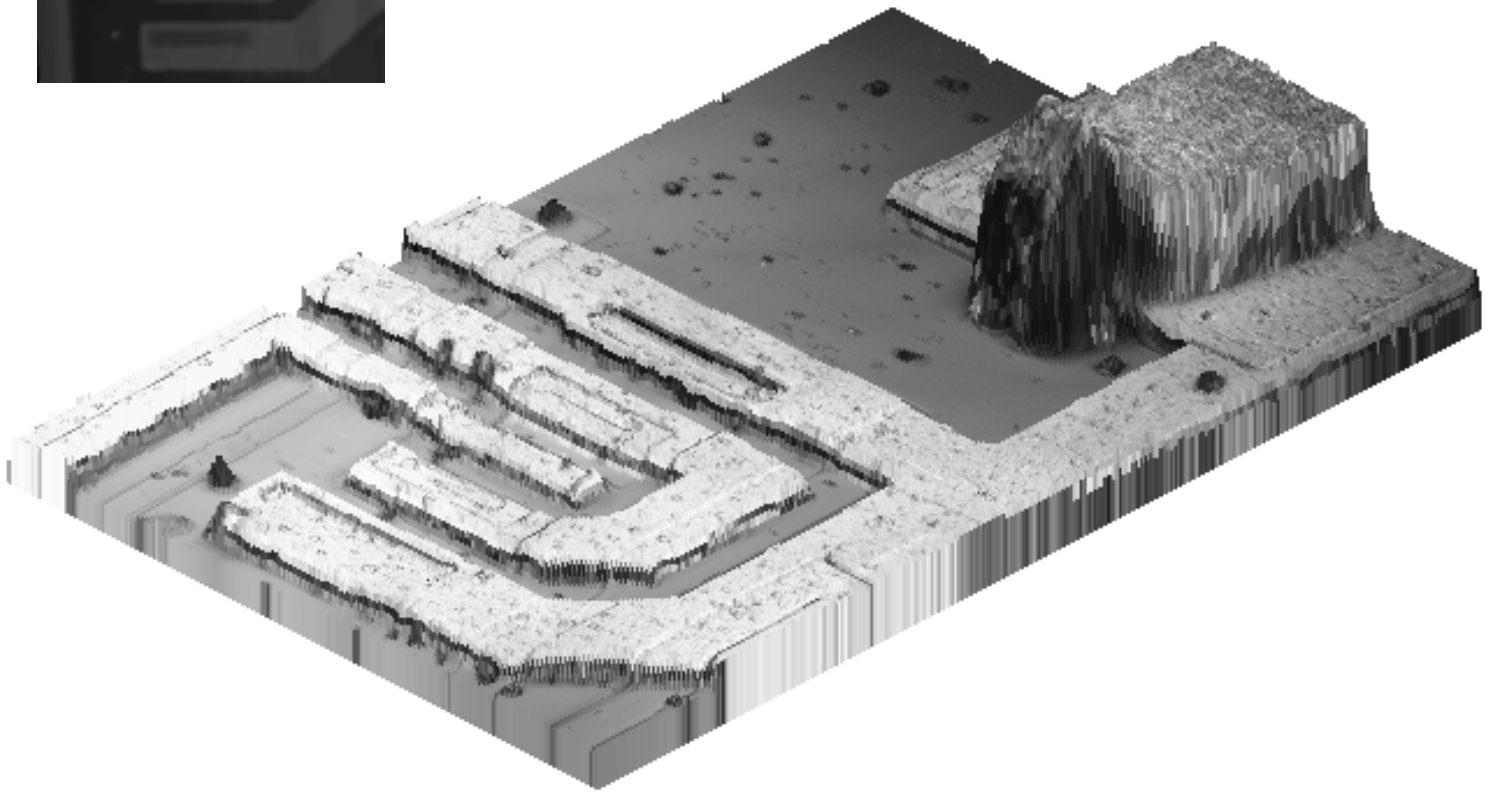
Autofocus and surface profile



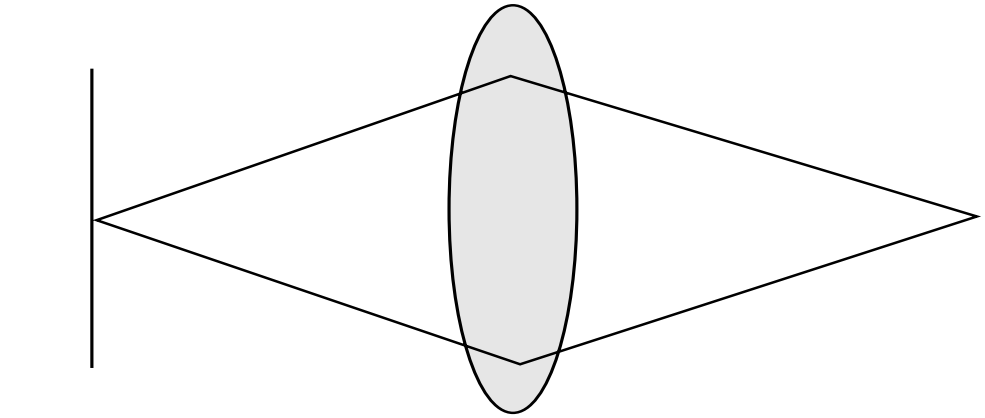
Autofocus and surface profile



Isometric view

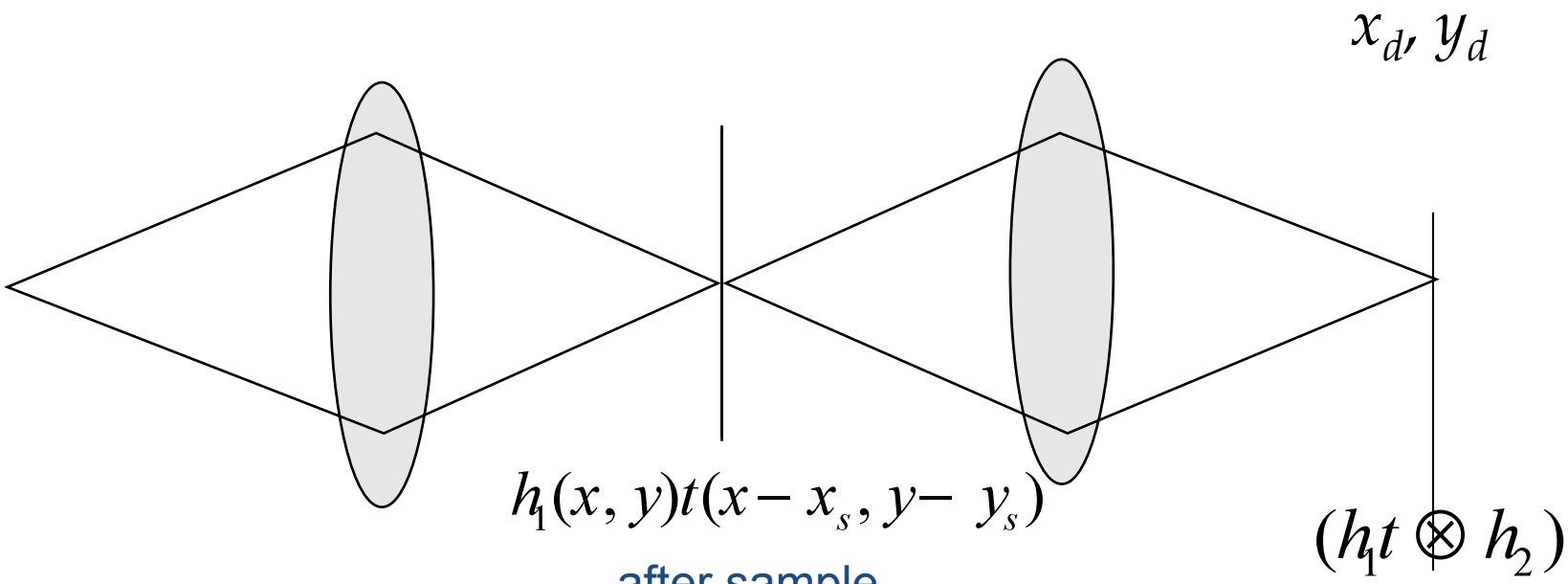


Coherent Imaging



$t(x, y)$ $(t \otimes h)$

Confocal Imaging (not fluorescence)



after sample

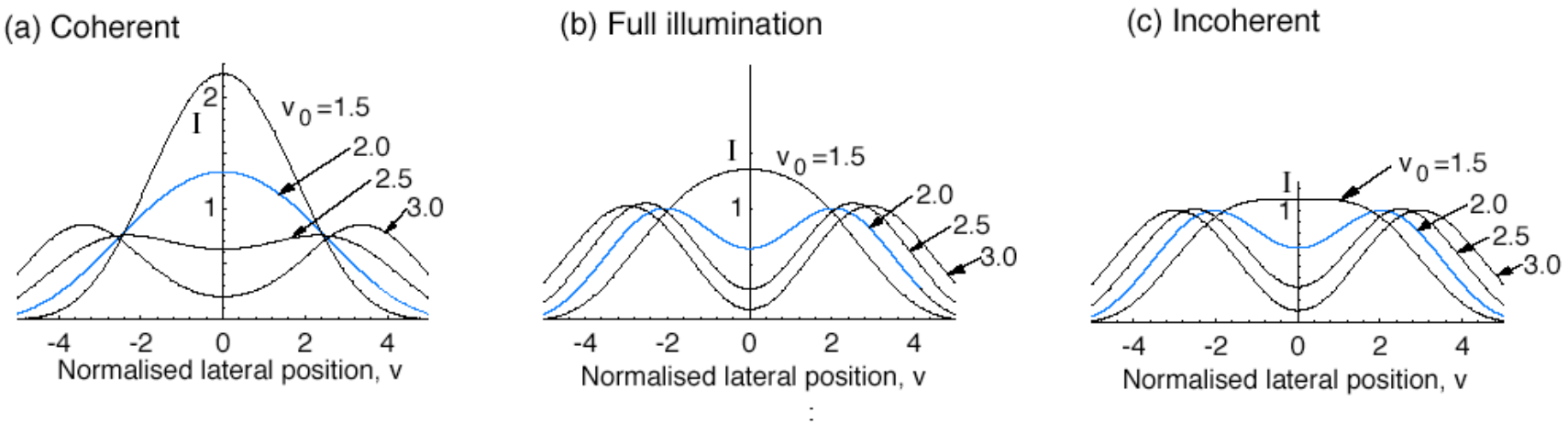
x_s, y_s are scan coordinates

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

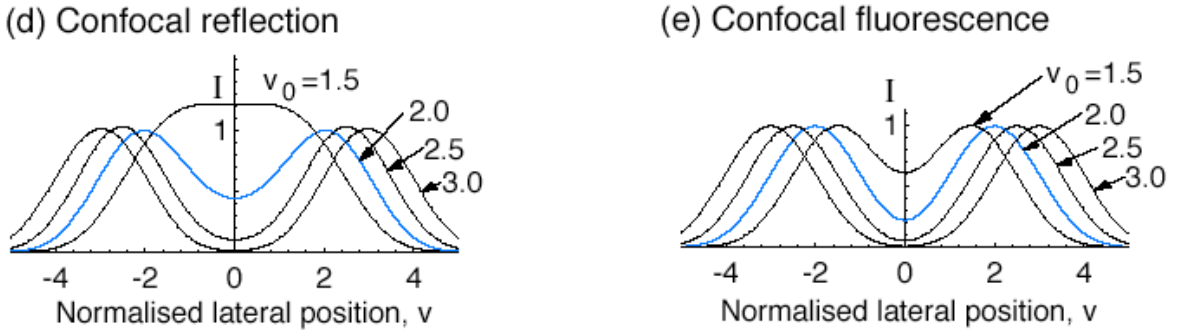
- Pinhole: $x_d, y_d = 0$: $$I = \left| \left(h_1(x,y) h_2(-x,-y) \right) \otimes t(x,y) \right|^2$$
- h_2 even: $$I = \left| (h_1 h_2) \otimes t \right|^2$$

- Coherent microscope, with $h_{\text{eff}} = h_1 h_2$

Images of two points



v_0 = 2.44 corresponds to Rayleigh resolution



Marvin Minsky 1957

Dec. 19, 1961 M. MINSKY 3,013,467

MICROSCOPY APPARATUS

Filed Nov. 7, 1957

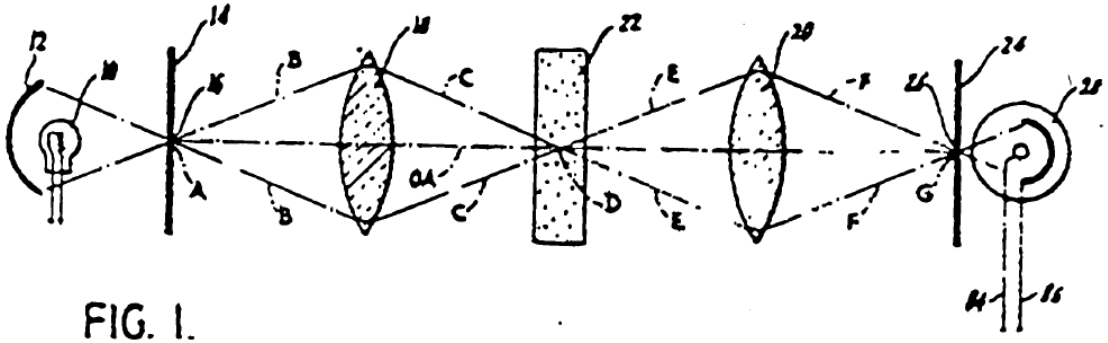


FIG. 1.

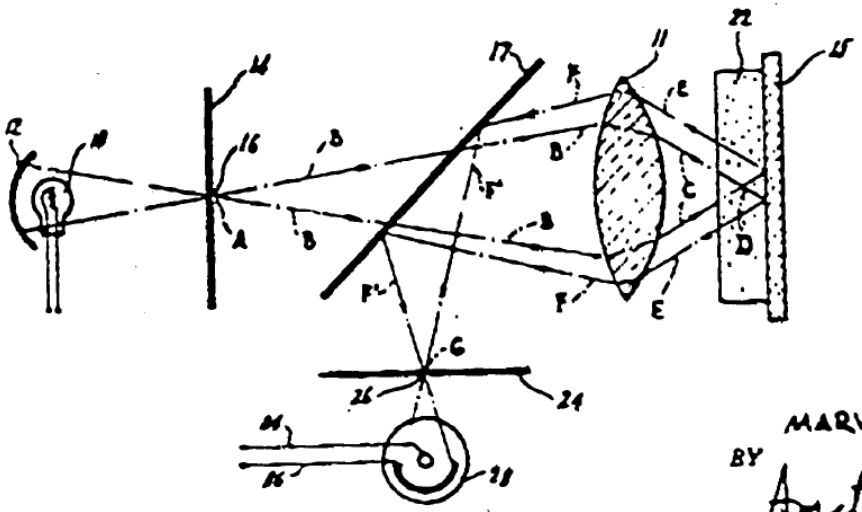
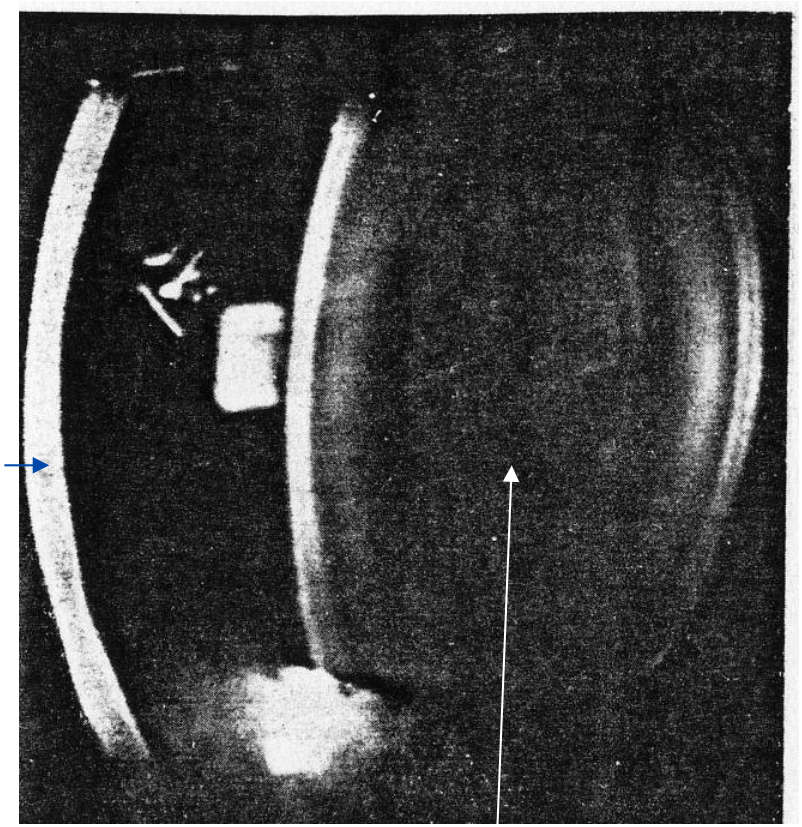


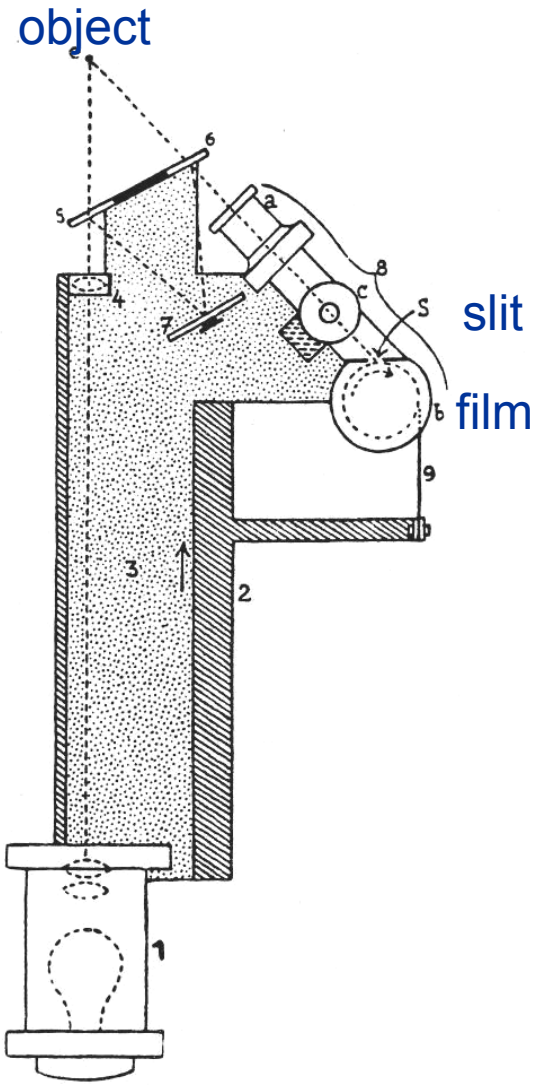
FIG. 3.

INVENTOR.
MARVIN MINSKY
BY Amster & Levy

Goldman, 1940

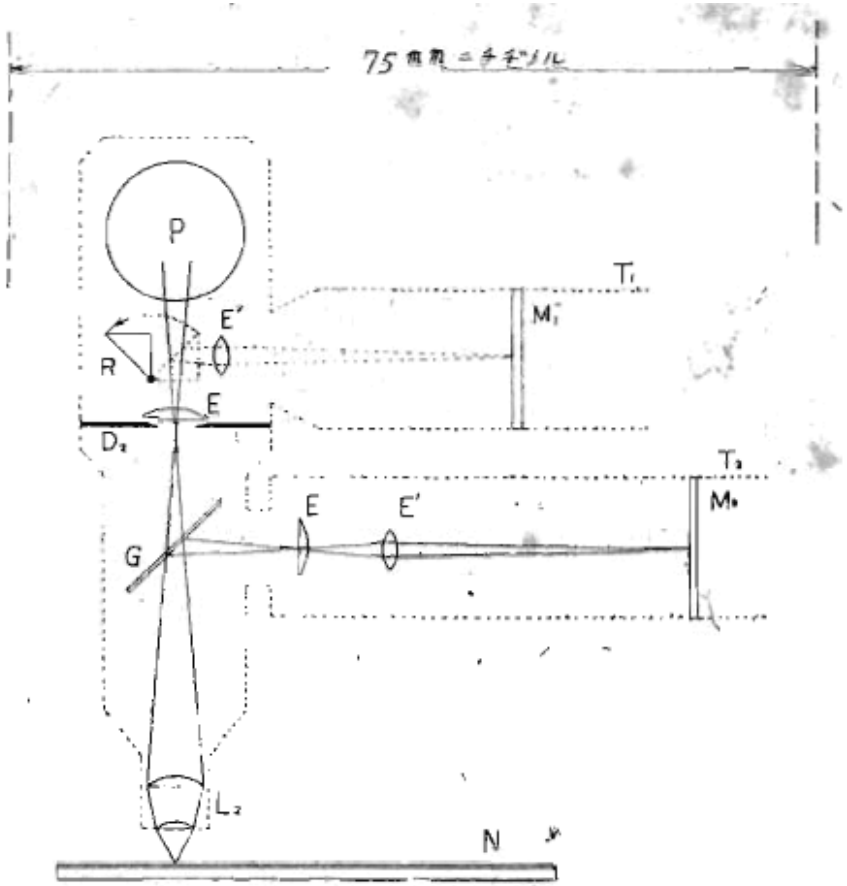
Slit-scanning confocal with angular gating



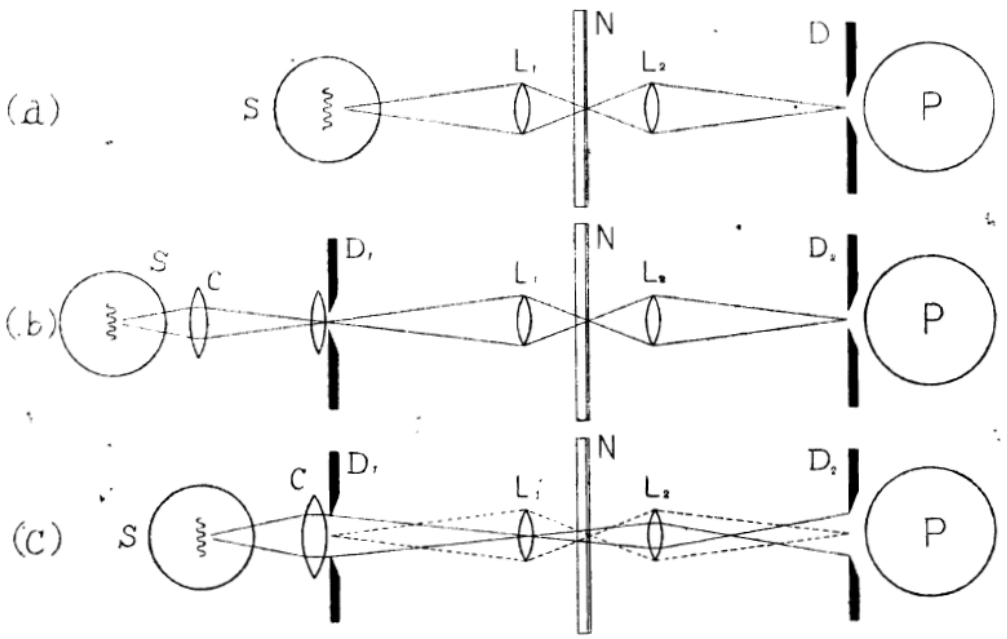


Spaltlampenphotographie und –photometrie, Ophthalmologica **98**, 257-270 (1940).

Z Koana 1942



第 2 圖　測定部分觀察裝置
N；原板．L_2：顯微鏡對物レンズ．G：薄い透明硝子板
D_2：有孔隔壁．E+E′：投影用接眼レンズ．
R：可動式直角プリズム．T_1,T_2：觀察筒．M_1,M_2：すり硝子
P：受光器．

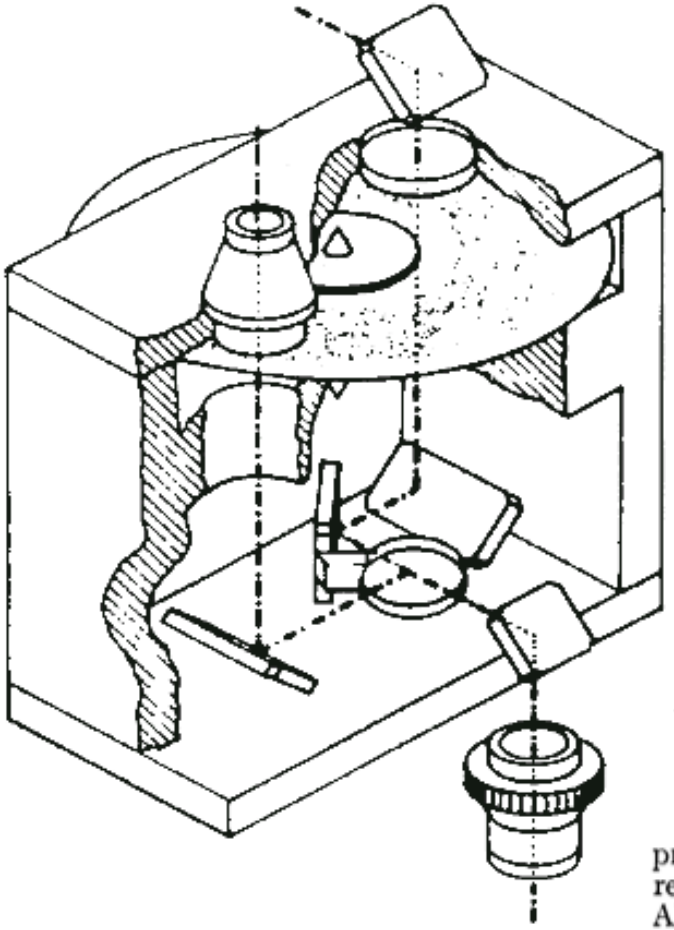


第 1 圖　微小部濃度計に於ける原板照明用光學系
S：光源，C：コンデンサー・レンズ，D：有孔隔壁．
L：顯微鏡對物レンズ．P：受光器

微小部濃度計に關する諸問題

正會員　小　穴　　純

Petráň 1968



Many parallel confocal microscopes

Nipkow disk
top view

[1] An extension of the theory given here indicates that a similar principal could be employed to build an optical microscope with a resolving power greater than that predicted by the Rayleigh and Abbe theories (superresolution in the sense of Herzberger). Such a microscope would be related to the system described theoretically, from another point of view, by W. Lukosz and M. Marchand, Opt. Acta **10**, 241 (1963); see also W. Lukosz, J. Opt. Soc. Am. **56**, 1463 (1966); A. Bachl and W. Lukosz, J. Opt. Soc. Am. **57**, 932 (1967).

FIGURE 1. Tandem scanning reflected light microscope (Petráň *et al.*, 1968).

JOURNAL OF THE OPTICAL SOCIETY OF AMERICA VOLUME 58, NUMBER 5 MAY 1968

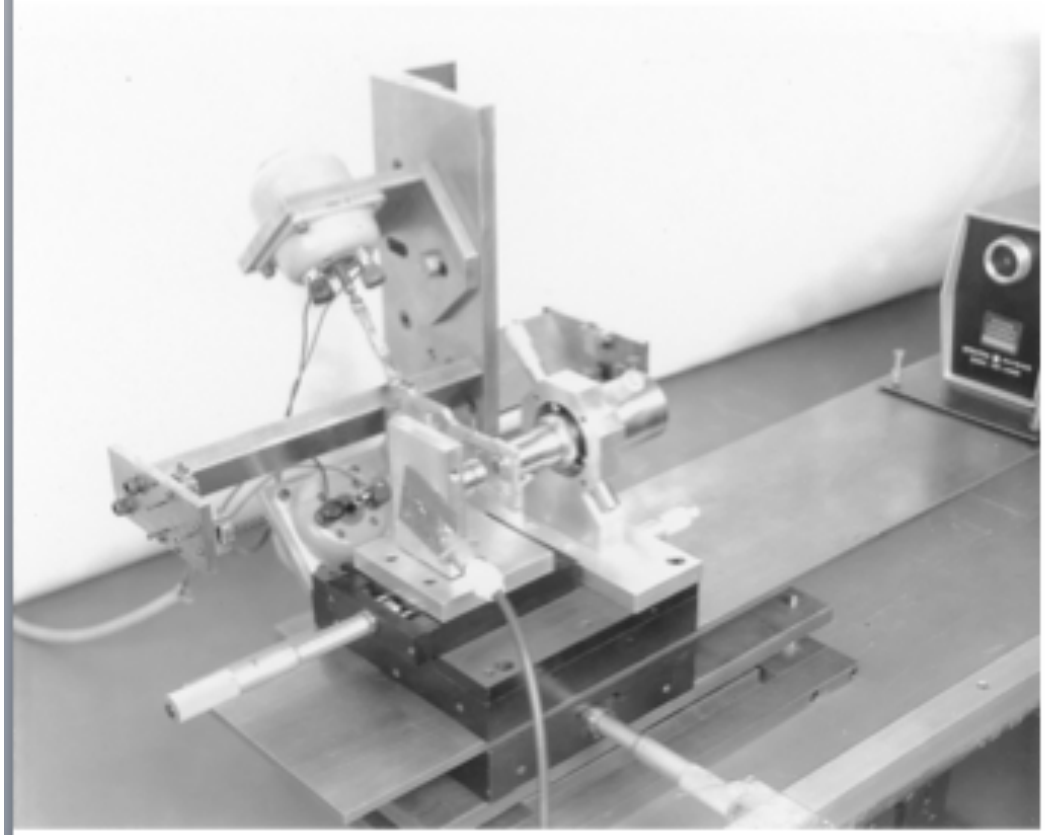
Egger & Petrán, *Science* **157**, 306 (1967)

Tandem-Scanning Reflected-Light Microscope*

MOJMÍR PETRÁŇ AND MILAN HADRAVSKÝ
Institute of Biophysics, Charles University School of Medicine, Plzeň, Czechoslovakia
AND
M. DAVID EGGER AND ROBERT GALAMBOS
Departments of Anatomy and Psychology, Yale University, New Haven, Connecticut

Petran, M. & Hadravsky, M. (1966) *Zpusob a zarizeni pro omezeni rozptylu svetla v mikroskopu pro osvetleni shora*. Czechoslovak Patent No. 128936, application 5-12-66, granted 15-2-68, published 15-9-68.

Oxford microscope, 1975



This short review of the development of Scanning Optical Microscopy at Oxford was originally prepared for MICRO 89, but was lost in the post. It contains some of the early micrographs, none of which has been published before.

15 Years of Scanning Optical Microscopy at Oxford

C. J. R. SHEPPARD

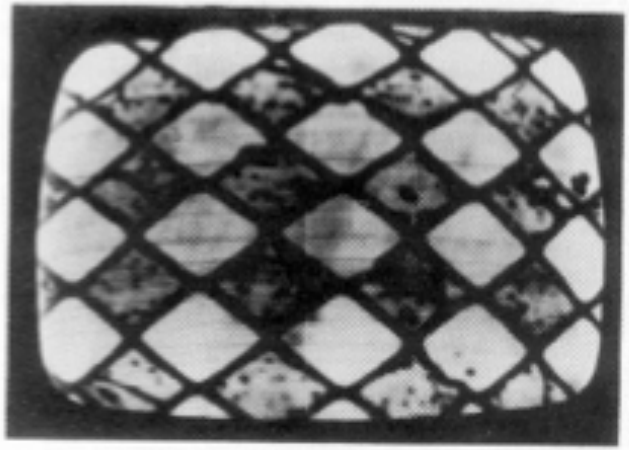


Fig. 1 50 micrometre squares, 20 January 1975.

First image

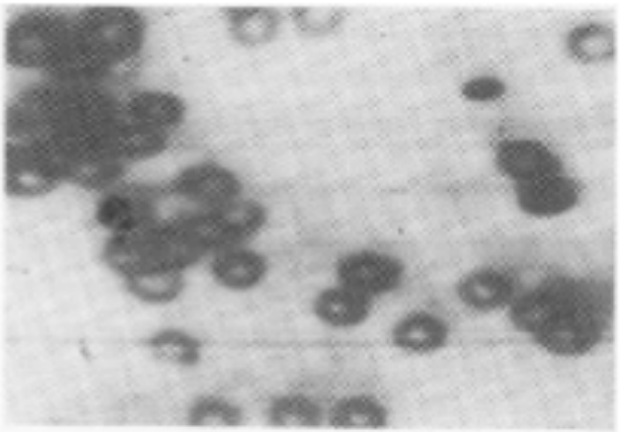


Fig. 7 Blood cells in fast-scan (beam-scanning) microscope, 20 March 1975.

Beam-scanning

Amar Choudhury, Colin Sheppard, Pete Hale & Rudi Kompfner
Oxford, Summer 1976

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

Image formation in the scanning microscope

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

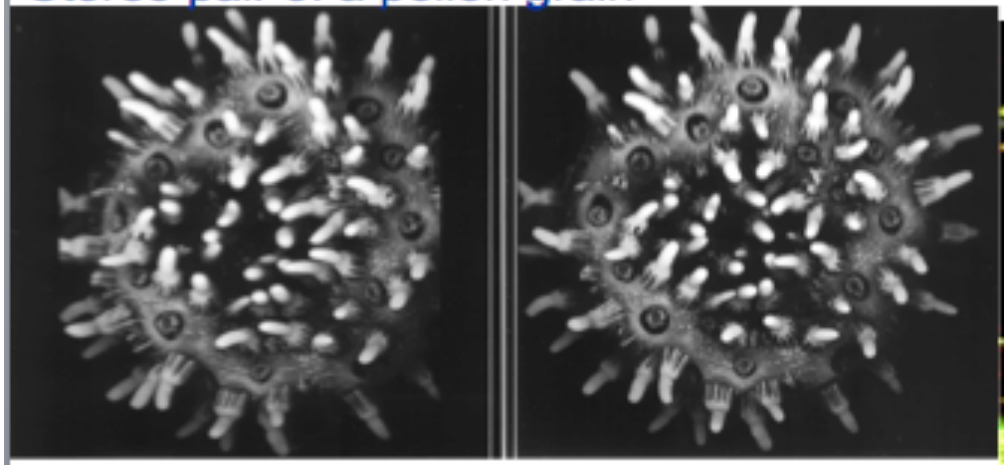
(*Received* 22 *December* 1976)

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.

- First paper to use term "confocal microscope"

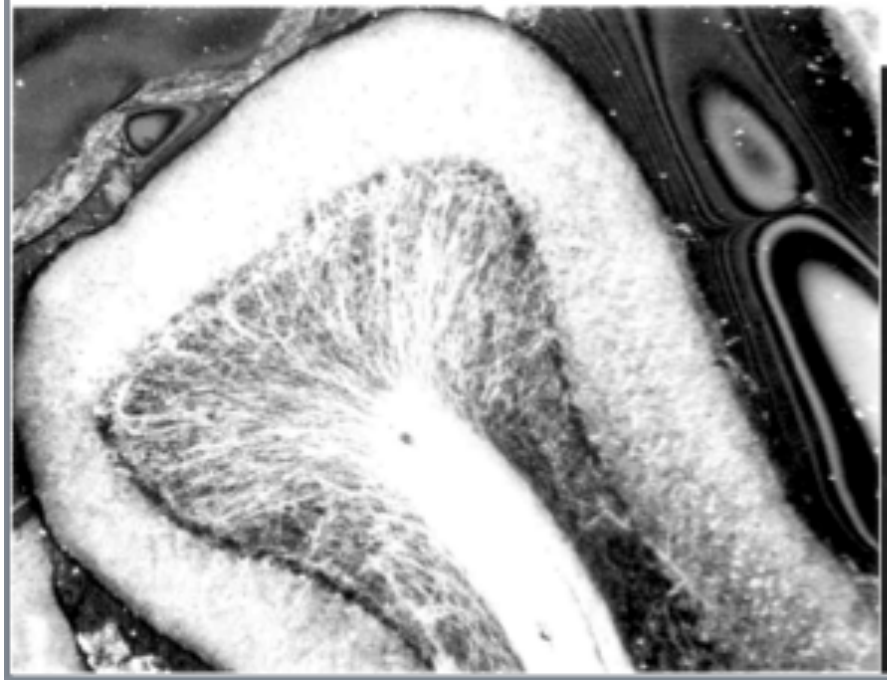
Confocal reflectance

Stereo pair of a pollen grain

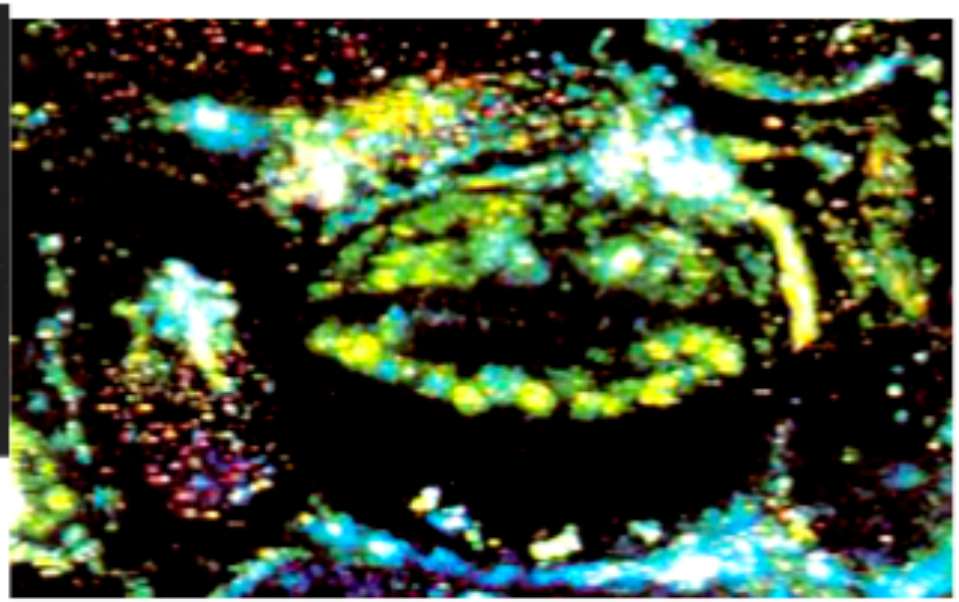


Endeavour, **10**, 17-19 (and cover)(1986)

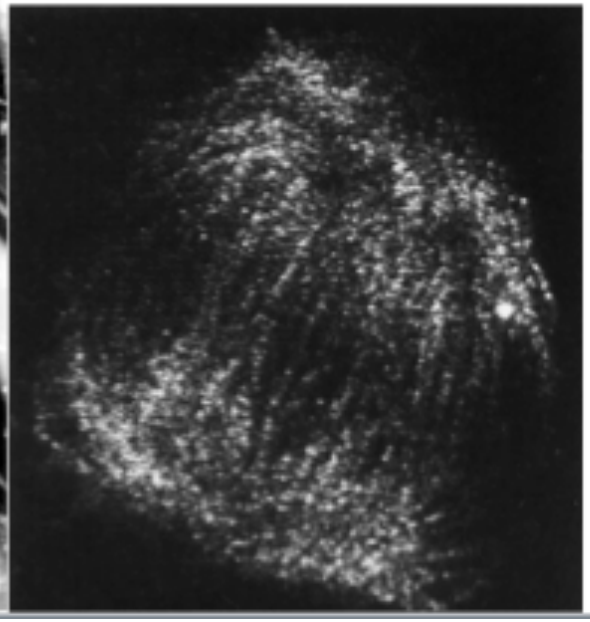
Rat brain (cerebellum)



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



Colour confocal reflection image of a leaf



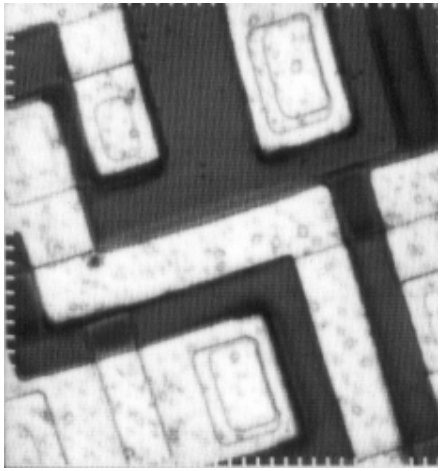
Microtubules labeled with 15nm gold

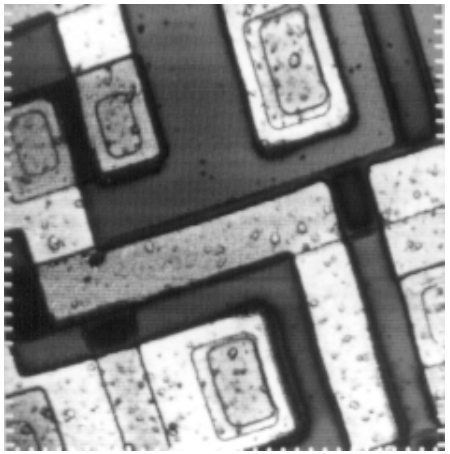
Inst. Phys. Conf. Ser. No. 98, 1989

Confocal microscope with computer

Cox IJ, Sheppard CJR (1983) Digital image processing of confocal images, *Image & Vision Computing* **1**, 52-56 (1983)

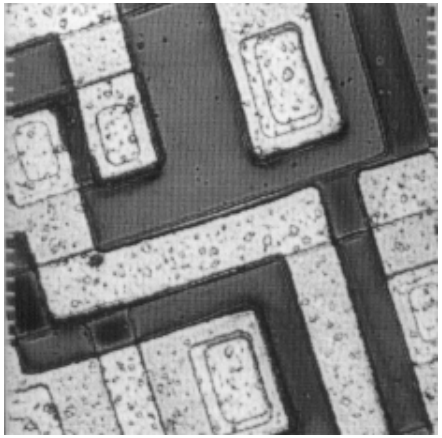
conventional

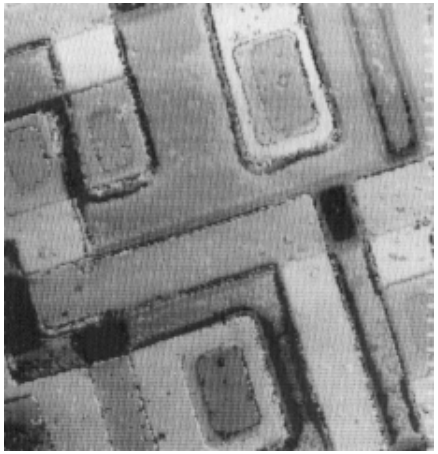




confocal

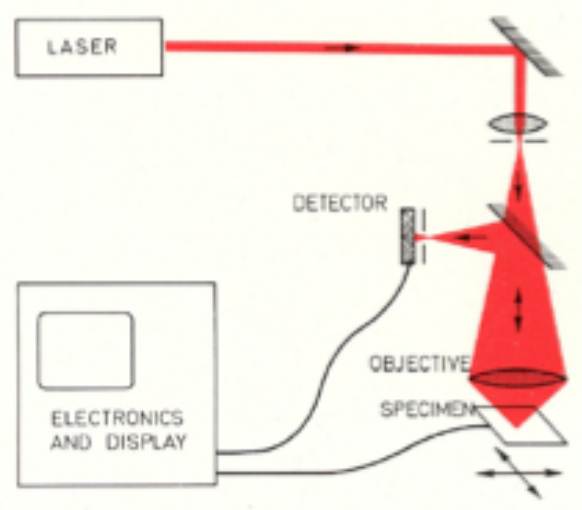
confocal autofocus





surface profile

Commercialization of confocal microscope



Oxford Optoelectronics 1982

LaserSharp SOM100, 1984

BioRad MRC500, 1987

Confocal imaging through scattering medium (confocal gating)

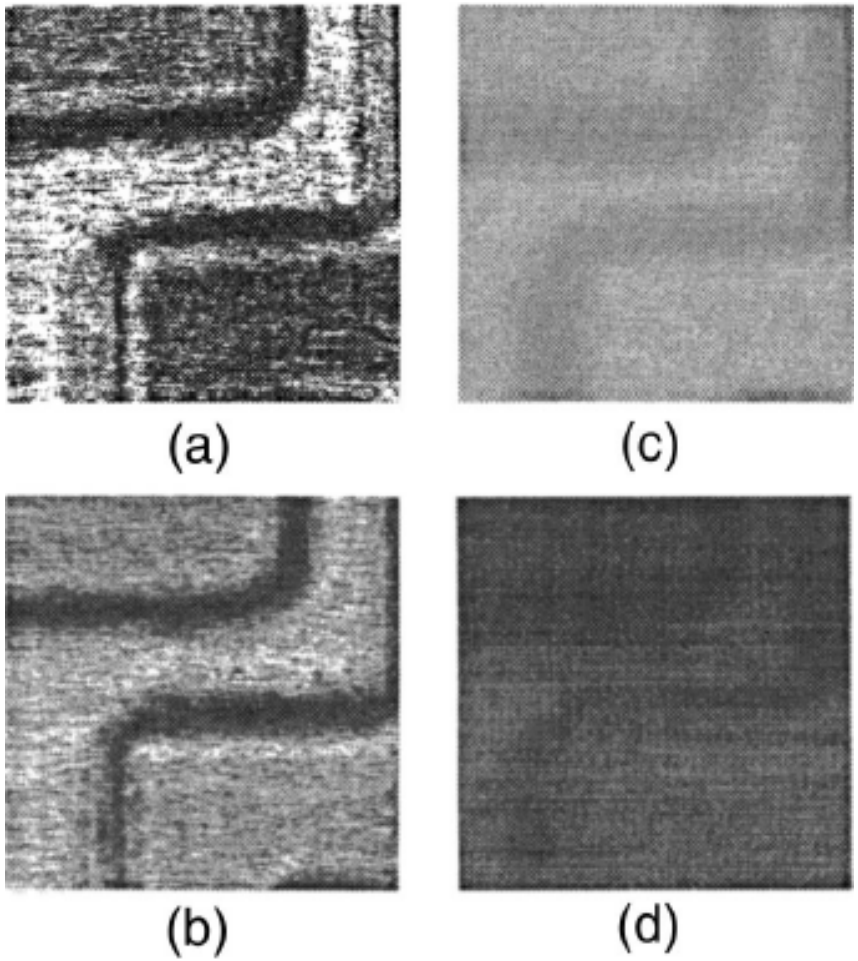


Fig. 3. Images of an IC hidden in a scattering medium (thickness $D \approx 5L_s$; image size 20 μm $\times$ 20 μm) for different values of the pinhole radius r_d: (a) $r_d = 5$ μm, (b) $r_d = 50$ μm, (c) $r_d = 500$ μm, (d) $r_d \to \infty$.

M Gu, T Tannous, CJR Sheppard

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 (fringe projection)
- Size
  - Endoscopic microscopy
- Cost
- Resolution
  - 4Pi microscopy
  - STED
  - Localization microscopy (PALM/STORM)
  - Structured illumination/Image scanning microscopy
- Penetration
  - Coherence gating
  - Two/three photon
  - Focal modulation microscopy (FMM)

3-D imaging methods

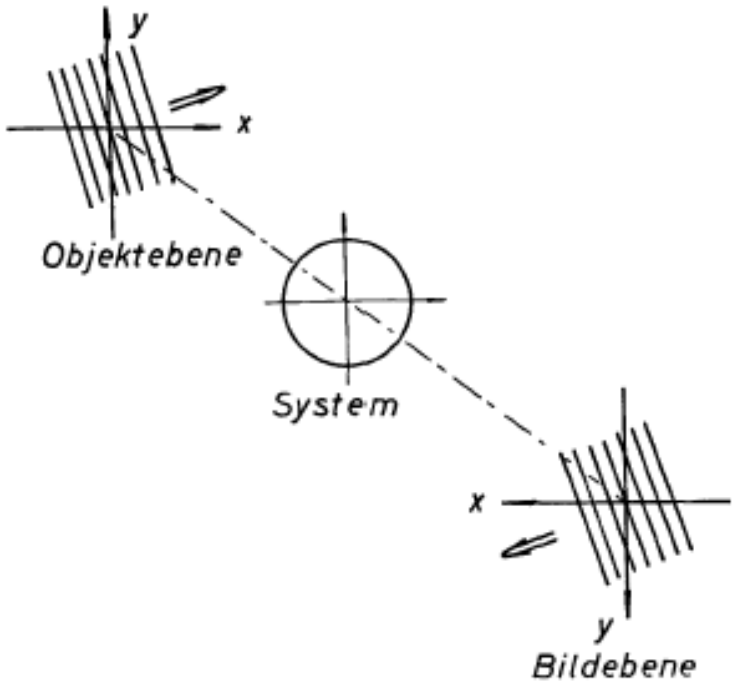
ISTITUTO ITALIANO DI TECNOLOGIA

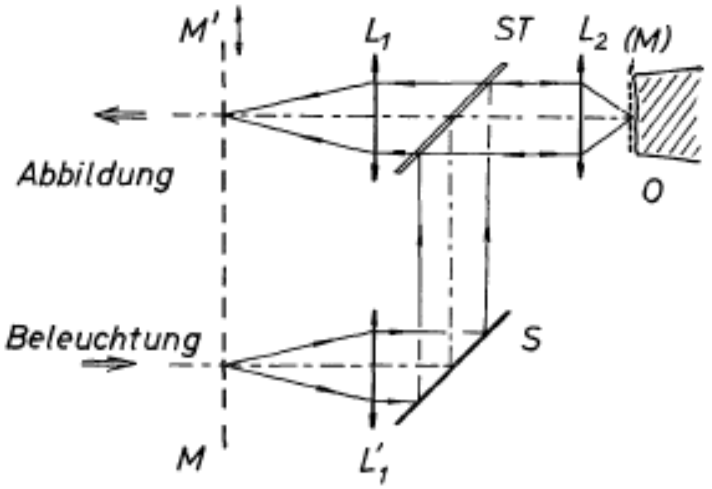
- Confocal
- Digital deconvolution
- Coherence probe/ optical coherence tomography (OCT)
- Multiphoton microscopy:
  - 2-photon fluorescence, SHG
- Structured illumination

Lukosz, 1963

Structured illumination
(or fringe projection)

Optical reconstruction using a second grating





It is known that diffraction sets an upper limit to the resolving power of an optical system or, put more precisely, to the band-width of spatial frequencies that the system can transmit.

A new method is described for obtaining optical images with a resolution exceeding the limits set by diffraction. The optical system itself is not modified, but a mask, or the image of a mask formed by projection is introduced in or near to the object plane. This mask has a variable transmission (for example a grating), and is movable in the object field. A second similar mask is introduced in or near to the image plane, or the plane of an intermediate image, and is moved conjugately with the object plane mask. The image obtained during the scanning by the masks is integrated in time by a receptor of suitable inertia (for example, the eye, or a photographic emulsion).

There results an image of the object with enhanced resolution and contrast (the band-width of the transmitted spatial frequencies is increased, and the frequency response is raised).

The method may be used with coherent, partially coherent or incoherent illumination. Its method of operation and imaging properties are described.

W Lukosz, M Marchand
Optica Acta **10**, 241-255 (1963)

Optical sectioning in line illumination or aperture array microscopes

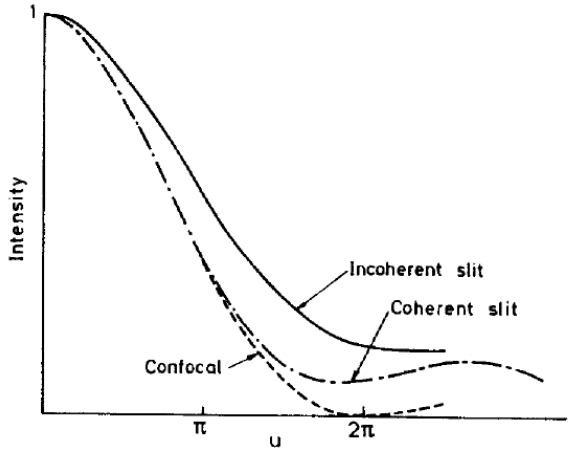


Figure 5. The defocus signal for a scanning microscope with point source and incoherent line detector (or *vice versa*) (solid line), and with point source and point detector (broken line). Also shown is the defocus signal for a system with coherent line source (or detector).

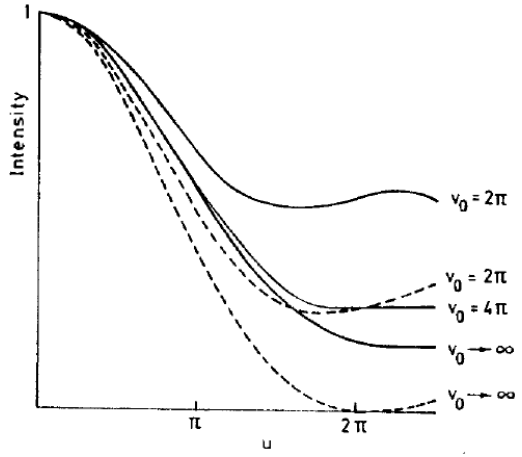


Figure 8. The defocus signal for a direct-view microscope with arrays of either lines (solid lines), or points (dashed lines) (separation v_0).

- Confocal, decays as $1/z^2$
- Line illumination, decays as $1/z$

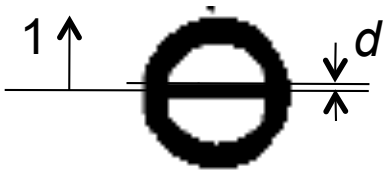
- Aperture array, tends to a constant (cross-talk)

C. J. R. SHEPPARD and X. Q. MAO

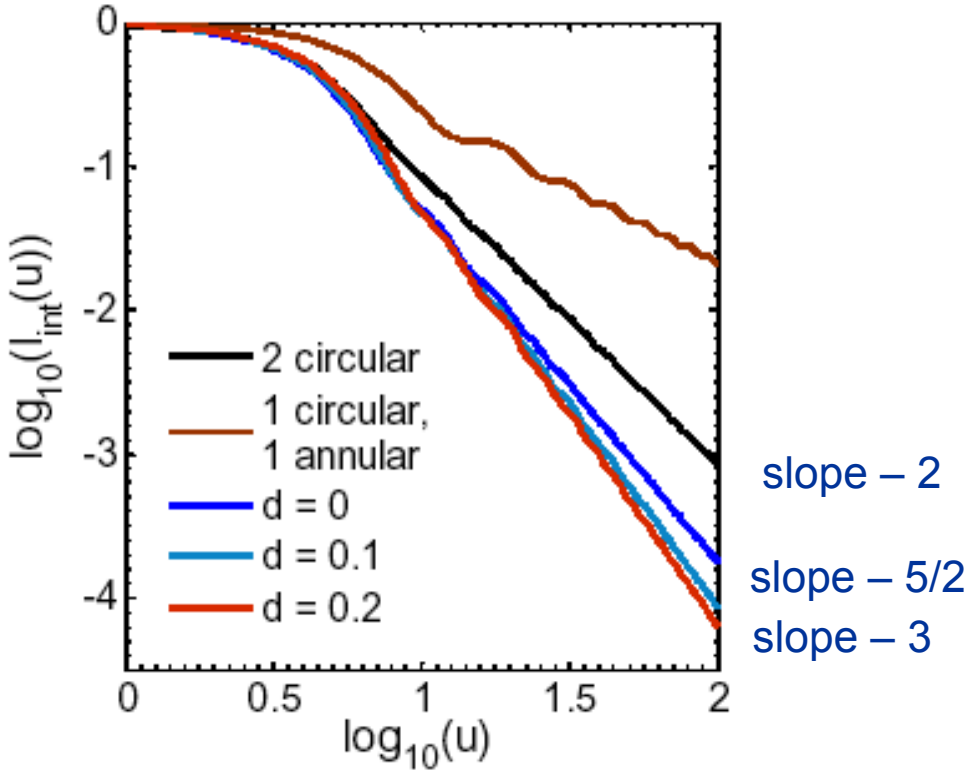
Confocal microscopes with slit apertures

JOURNAL OF MODERN OPTICS, 1988, VOL. 35, NO. 7, 1169-1185

Strength of background



width of divider



slope – 2

slope – 5/2

slope – 3

Fig. 9. The integrated intensity $I_{int}(u)$ for a confocal microscope with two D-shaped pupils and a point detector shown as a log-log plot.

Using D-shaped pupils for illumination and detection, sectioning is improved

The divided aperture technique for microscopy through scattering media

Colin J. R. Sheppard,[1,2,3,*] Wei Gong,[1] and Ke Si[2]

13 October 2008 / Vol. 16, No. 21 / OPTICS EXPRESS 17031

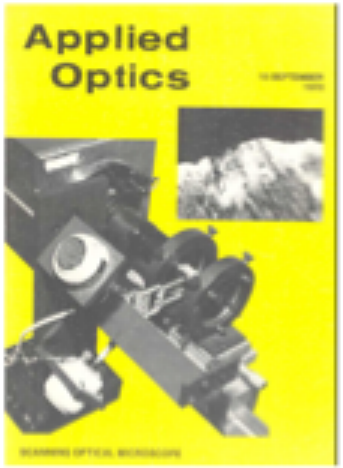
Two-photon microscopy

- Signal proportional to square of illumination intensity
  - Optical sectioning with no pinhole
  - Signal increased using pulsed laser

Multiphoton microscopy

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

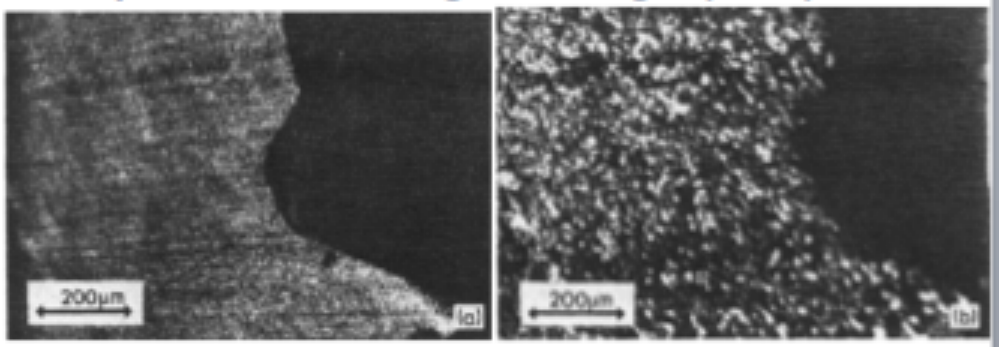
In the scanning optical microscope[1,2] nonlinear interactions are expected to occur between the object and a highly focused beam of light, which we hope will open new ways of studying matter in microscopic detail hitherto not available. Nonlinear interactions[3–5] include the generation of sum frequencies,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



C. J. R. Sheppard and R. Kompfner
Appl. Opt. **17**, 2879-2882 (1978)

Received 1 November 1976; revised manuscript received 22 September 1977.

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

3D SHG with fs pulses

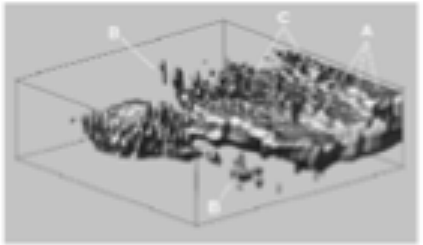
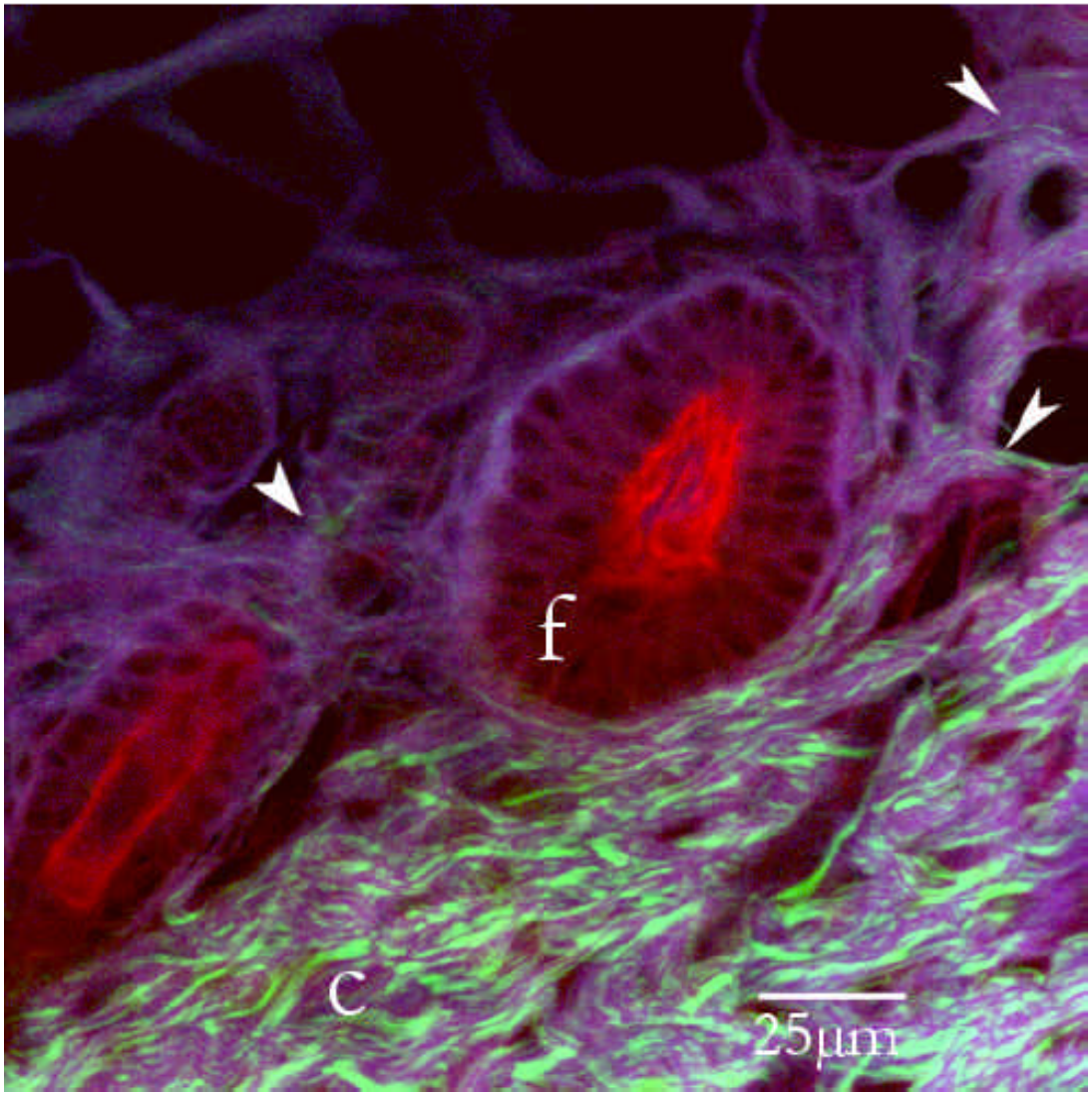


Fig. 4. Side view of a three-dimensional SH generation image (70 μm × 70 μm × 30 μm) of LBO crystal fragments showing A, terraces on the crystal; B, isolated microcrystallites; and C, columnar stacking of microcrystals on the surface of the large LBO crystal.

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

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

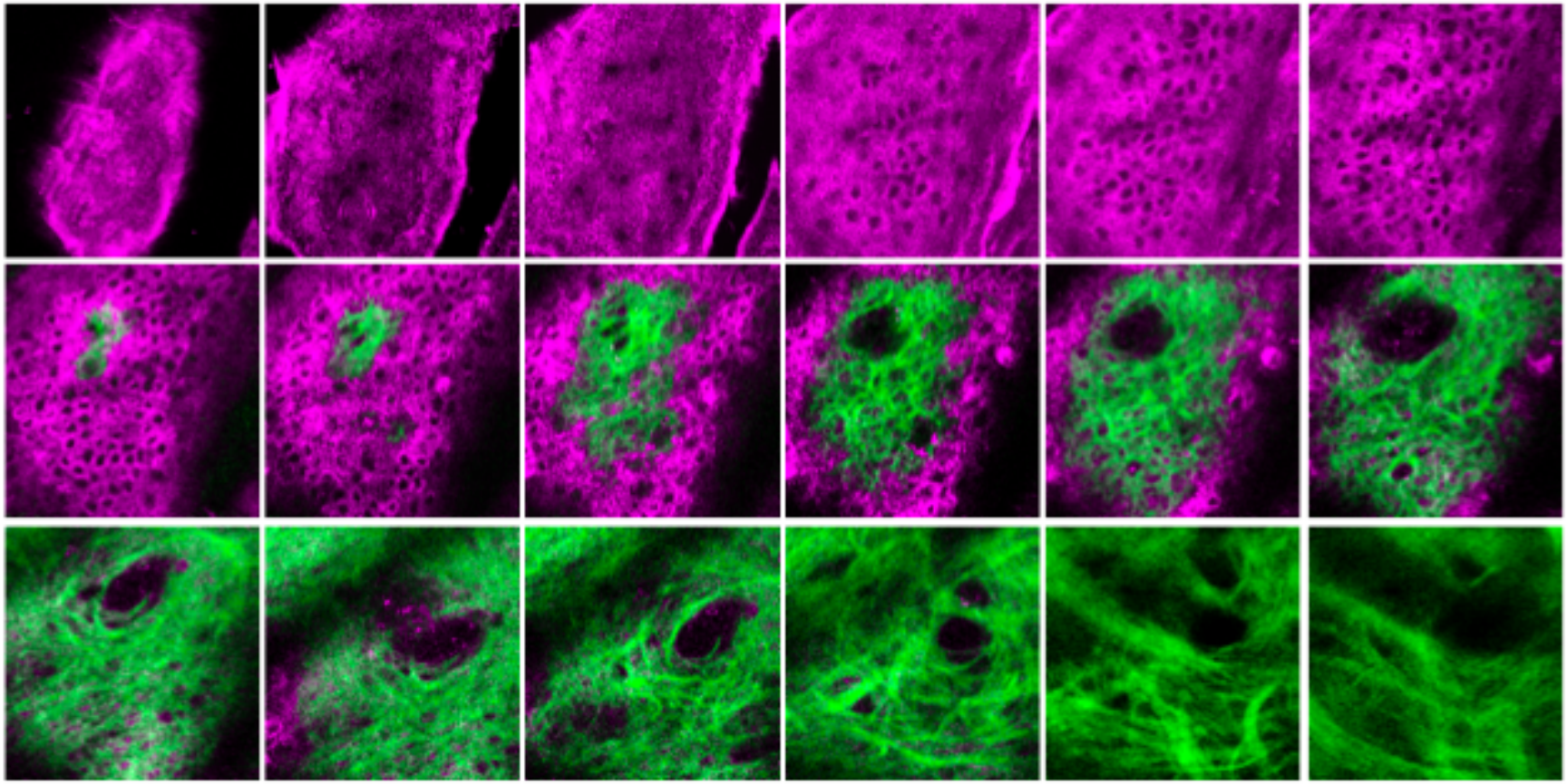
R. Gauderon and P. B. Lukins C. J. R. Sheppard



SHG image (in blue) of collagen in mouse dermis

Cox G, Xu P, Sheppard CJR, Ramshaw J (2003)
Characterization of the Second Harmonic Signal from Collagen,
Proc. SPIE **4963**, 32-40

Harmonic microscopy of my arm

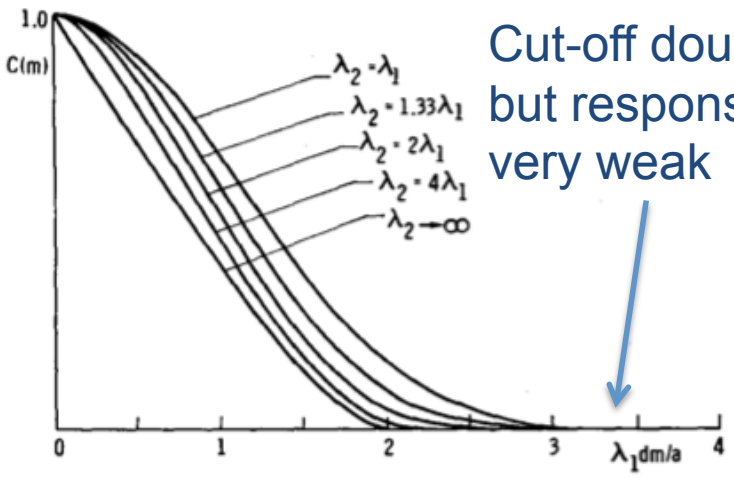


CK Sun, NTU Hospital

SHG THG

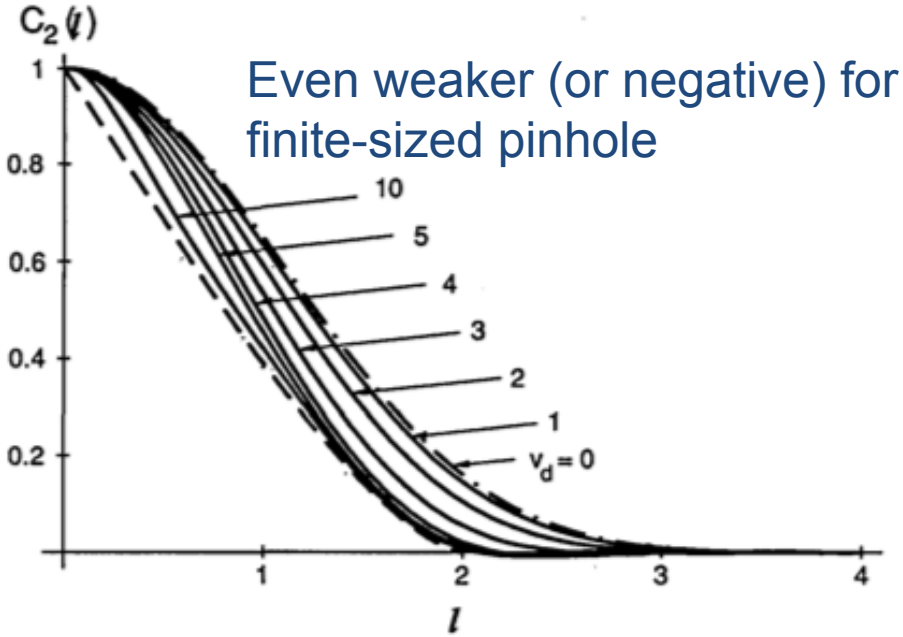
Excitation wavelength: 1230 nm
Bandwidth: 31 nm
Avg. laser power after objective: 90mW
Frame rate: 0.37 Hz
Image size: 120 X 120 µm

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.



Even weaker (or negative) for finite-sized pinhole

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

Suggests possibility to use pupil filters to increase magnitude of OTF!

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

Superresolution

- Classical theory

  Transfer function is band-limited

- Toraldo di Francia (1952):

  Resolution is not a fundamental limit

- Methods of Lukosz, Lohmann (~1960)

  Capacity for information transfer is invariant, not bandwidth
  Increase bandwidth using different polarizations, wavelengths etc.

- Cox and Sheppard (1985)

  Information capacity, but include noise (Shannon)

$$C = \prod \left(1 + B_x L_x\right)\left(1 + B_y L_y\right)\left(1 + B_z L_z\right)\left(1 + B_t L_t\right) \log_2 (1 + SNR)$$

Superresolution methods

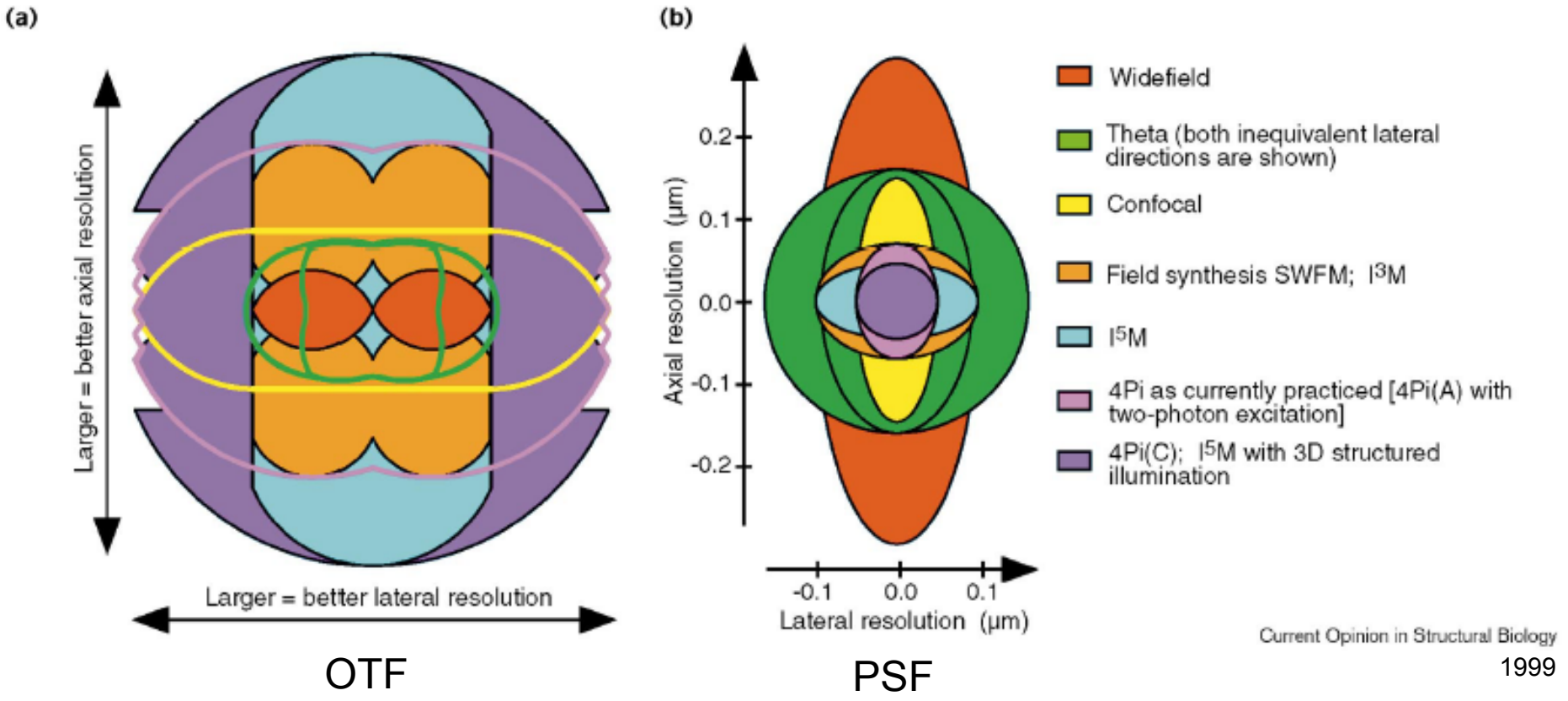
Can trade off another property to improve resolution

- SNR
- Time
- Colour
- Polarization

Distinguish between different classes of 'superresolution'

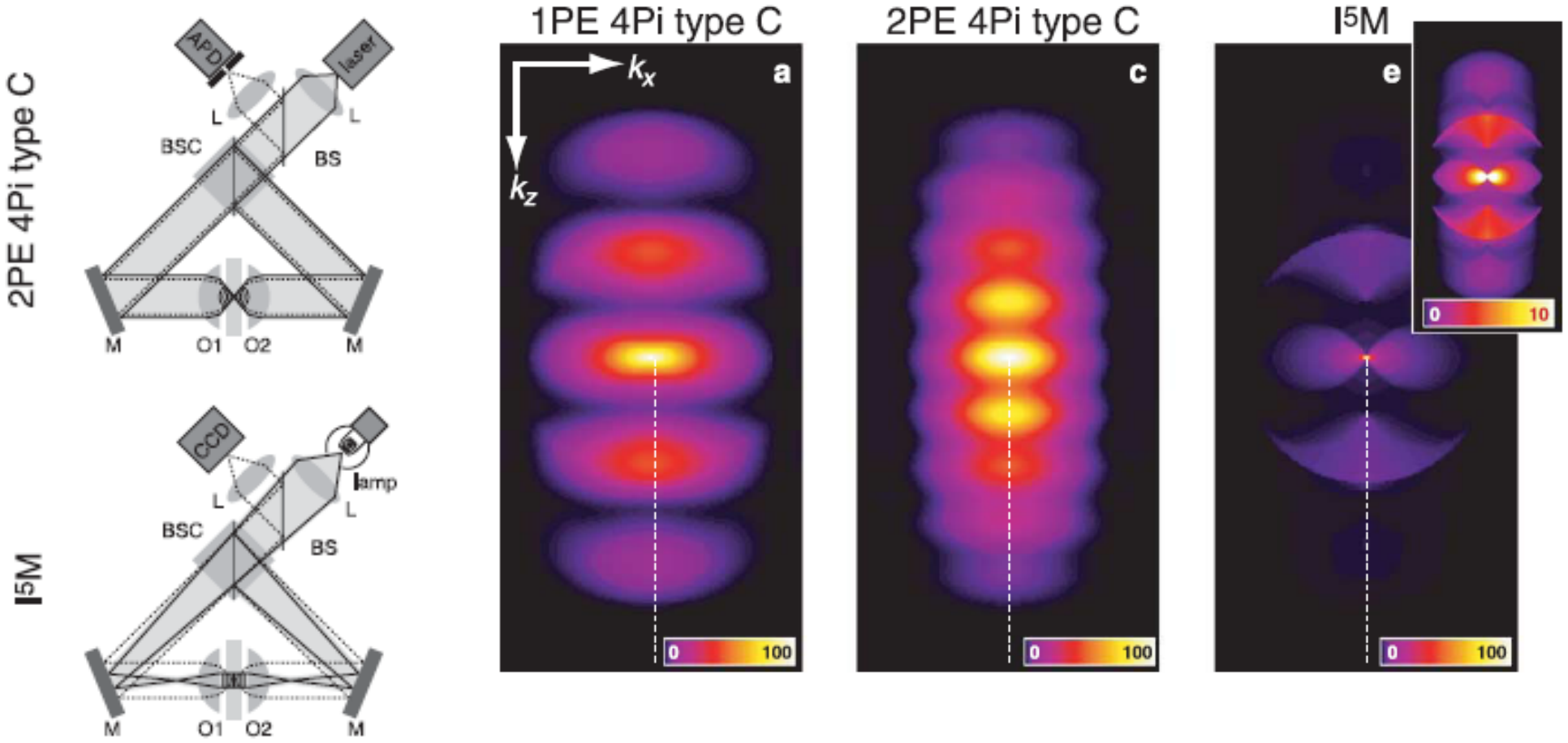
- Class 3: Improve spatial frequency response, but cut-off unchanged
  - 2-point resolution improved
  - Sometimes called ultra-resolution, or hyper-resolution
    - image filtering
    - simple digital deconvolution (Wiener filtering, nearest neighbour)
    - superresolving filters (masks), superoscillations
- Class 2: Cut-off increased, but the effective NA is still < *n*
    - polarization, etc.
    - synthetic aperture
- Class 1b: Cut-off increased, and the effective NA > *n*
    - structured illumination
    - confocal
    - source/detector arrays (ISM)
    - solid immersion lens (SIL)
    - nonlinear imaging
- Class 1a: Cut-off increased, and the effective NA is unlimited
    - STED
    - saturated SIM
    - localization microscopy (PALM/STORM)
    - near-field microscope (SNOM, photon tunneling microscope)
    - deconvolution with constraints

Comparison of different imaging methods



Extended resolution fluorescence microscopy
Mats GL Gustafsson

Comparison of 4Pi and I^{5}M

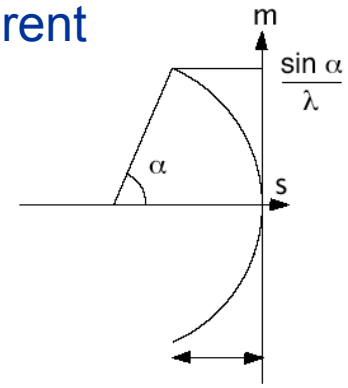


Hell

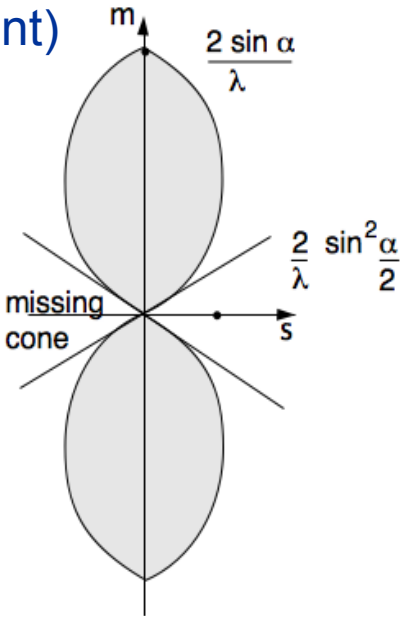
3D Spatial Frequency cut-offs

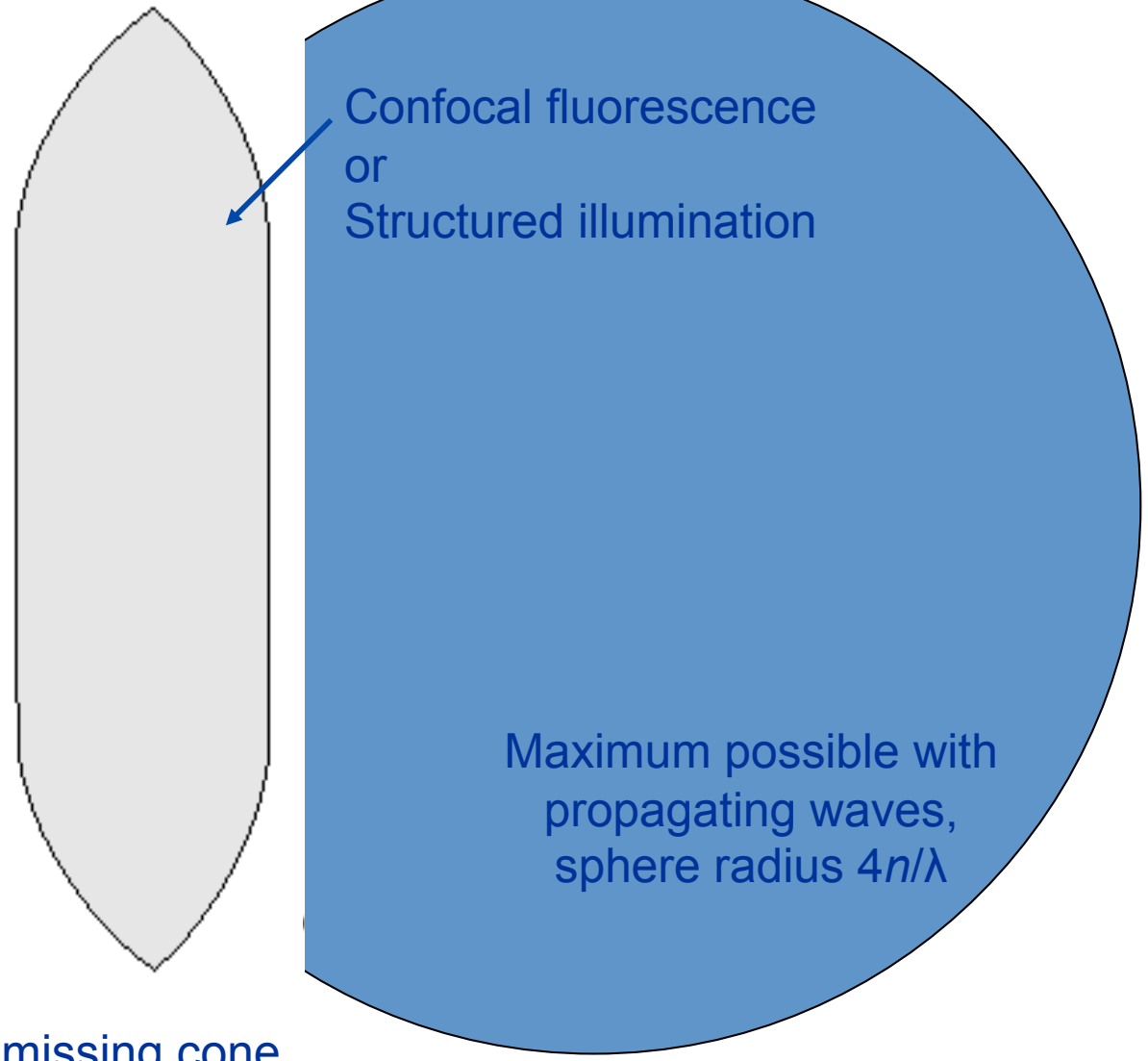
Maximum $4/\lambda$ ($4n/\lambda$ in medium, e.g $6/\lambda$)

Coherent

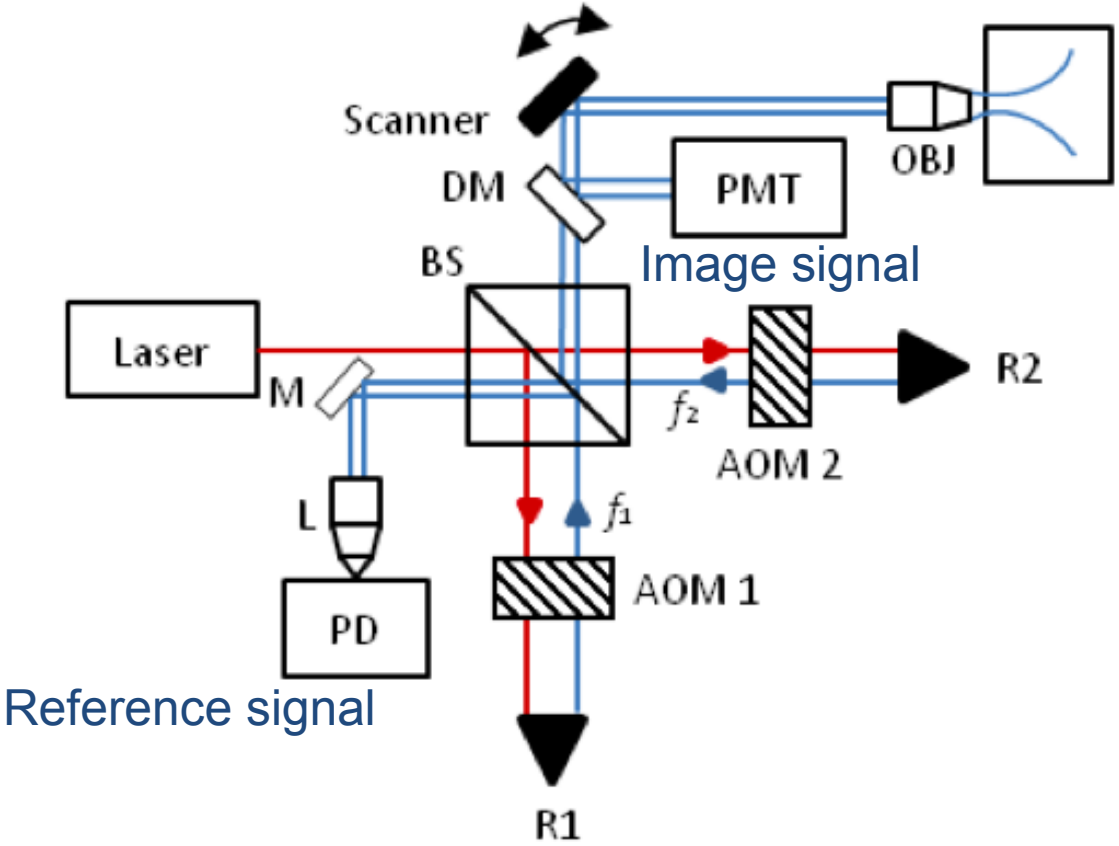


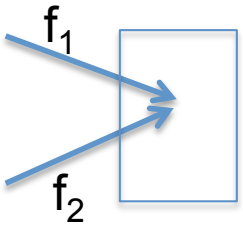
Abbe (incoherent)





Focal modulation microscopy





- Detect beat frequency
- Only get a signal from the focal region, where the 2 beams cross

Focal modulation microscopy

Nanguang Chen[1,2*], Chee-Howe Wong[2], and Colin J. R. Sheppard[1,3]

10 November 2008 / Vol. 16, No. 23 / OPTICS EXPRESS 18764

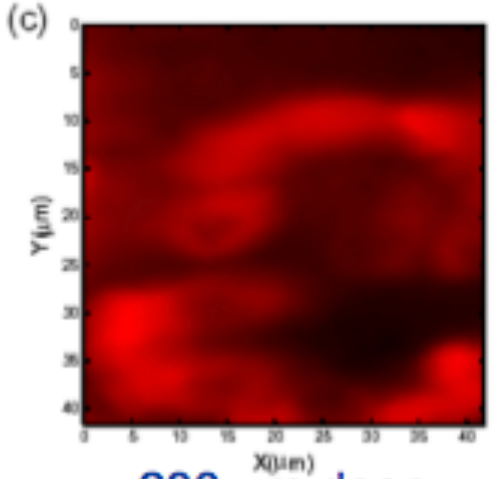
High-speed focal modulation microscopy using acousto-optical modulators

Shau Poh Chong[1], Chee Howe Wong[1], Kit Fei Wong[1], Colin J.R. Sheppard[1,2], and Nanguang Chen[1,*]

Vol. 1, No. 3 / BIOMEDICAL OPTICS EXPRESS 1026

Chondrocytes from chicken cartilage

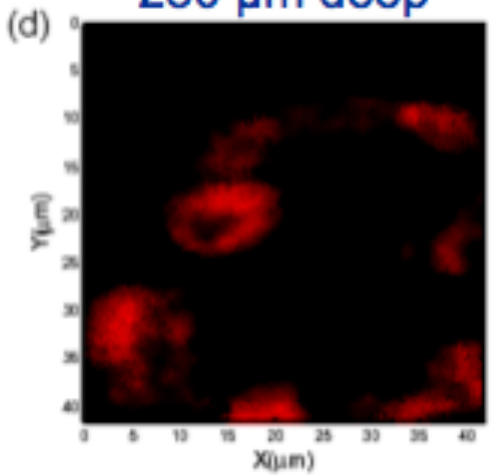
confocal



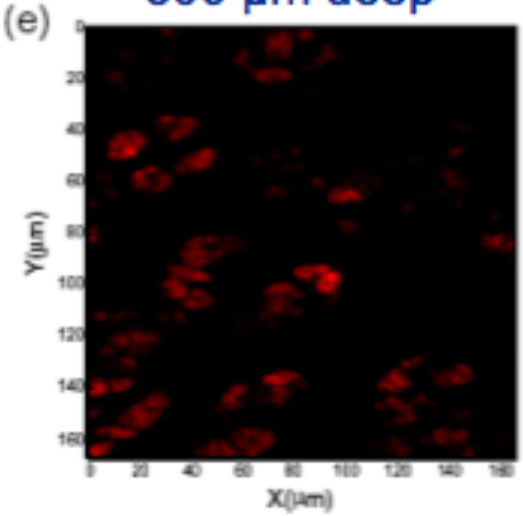
Blur from background

FMM

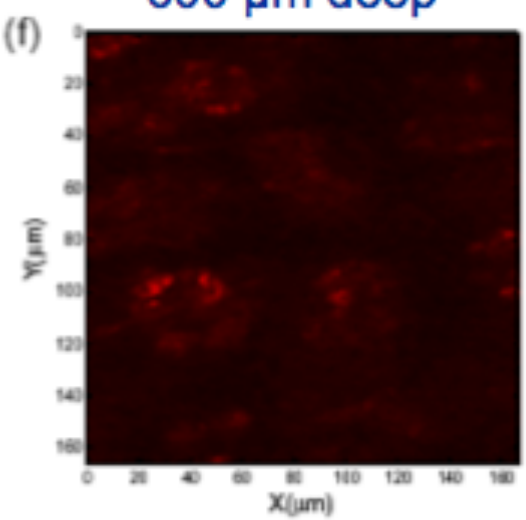
280 μm deep



500 μm deep



600 μm deep

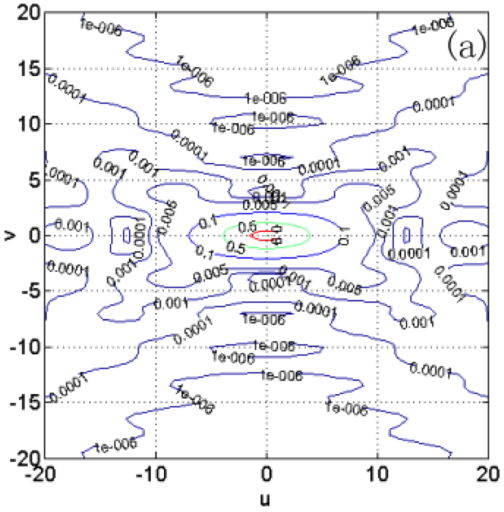


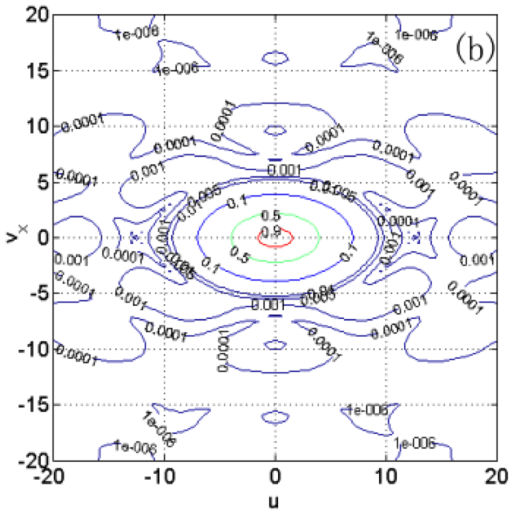
Focal modulation microscopy

Nanguang Chen[1,2*], Chee-Howe Wong[2], and Colin J. R. Sheppard[1,3]

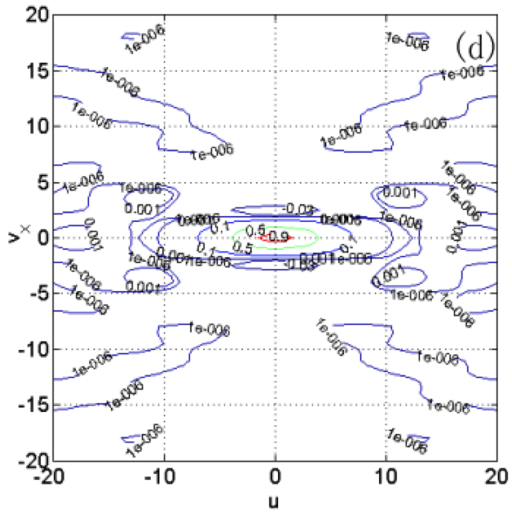
Image of a point object

(a) confocal





(b) D-shaped



(c) FMM

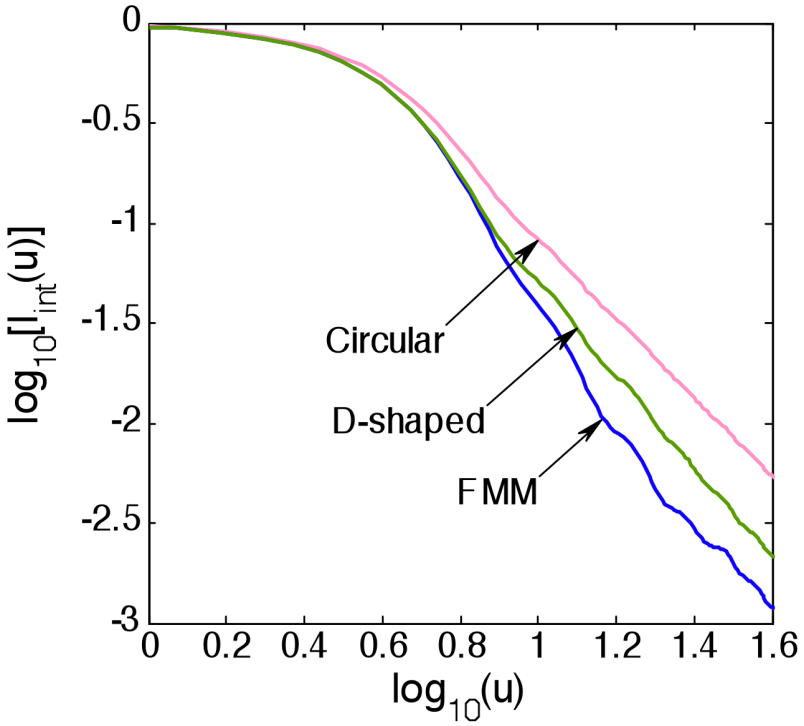
The intensity image of a point object with a point detector, representing the intensity point spread function IPSF.

3508 OPTICS LETTERS / Vol. 34, No. 22 / November 15, 2009

Improved spatial resolution in fluorescence focal modulation microscopy

Wei Gong,[1] Ke Si,[2] Nanguang Chen,[1,3] and Colin J. R. Sheppard[1,2,4,*]

Integrated intensity (background)



Decays as $1/z^3$

The variations of the integrated intensity of FMM, compared with confocal microscope with circular apertures and with D-shaped apertures, for a point detector.

3508 OPTICS LETTERS / Vol. 34, No. 22 / November 15, 2009

Improved spatial resolution in fluorescence focal modulation microscopy

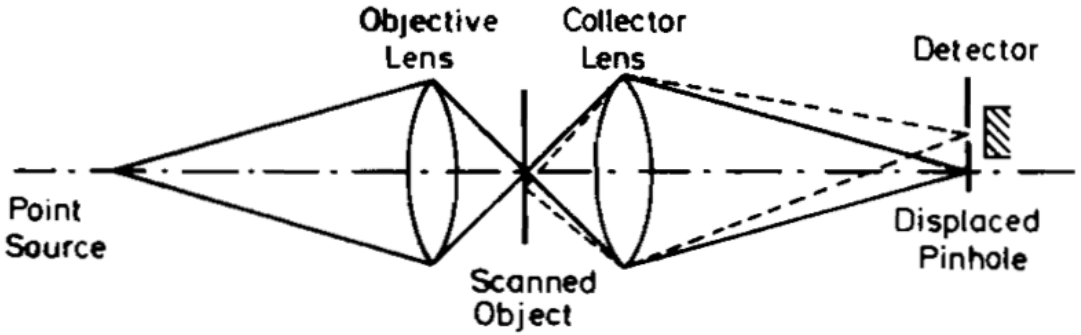
Wei Gong,[1] Ke Si,[2] Nanguang Chen,[1,3] and Colin J. R. Sheppard[1,2,4,*]

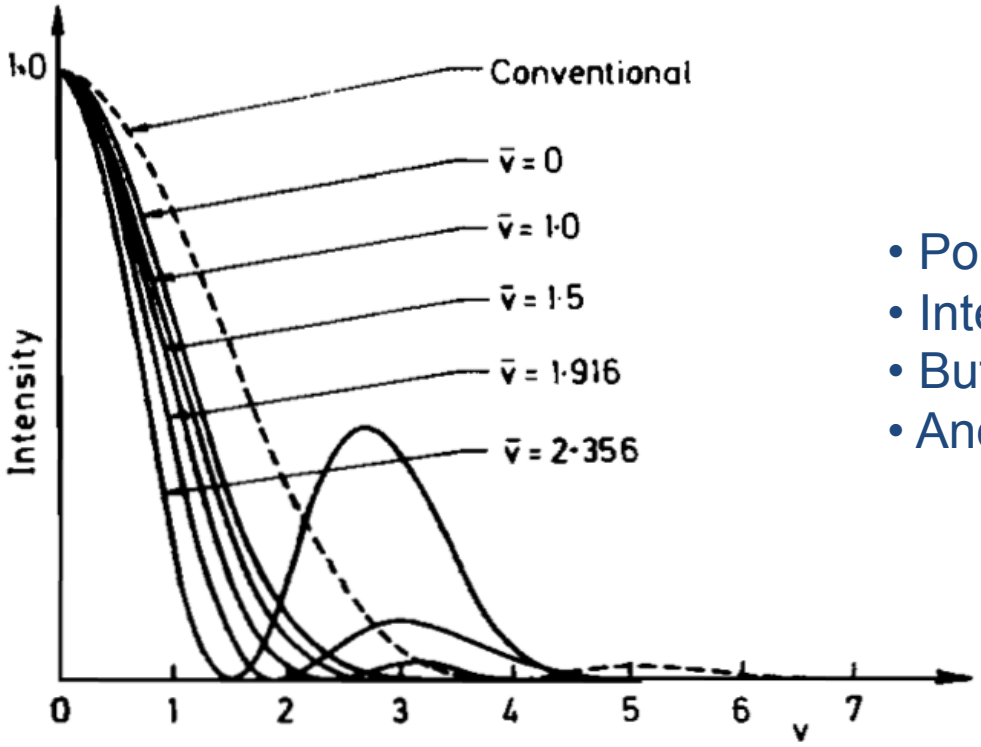
Source/Detector arrays

- Tandem scanning, Petrán (1968)
- Singular value decomposition (Bertero & Pike, 1982)
- ‘Type 3’: Maximum signal in detector plane (Reinholz, 1987)
- **Pixel reassignment (Sheppard, 1988)**
- Subtractive imaging (Cogswell & Sheppard 1990, and others)
- Source/detector arrays (Benedetti 1996)
- Programmable array microscope (PAM) (Hanley, 1998)
- Structured illumination

  (Lukosz, 1963; Gustafsson, 2000)

Offset pinhole





PSF:

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

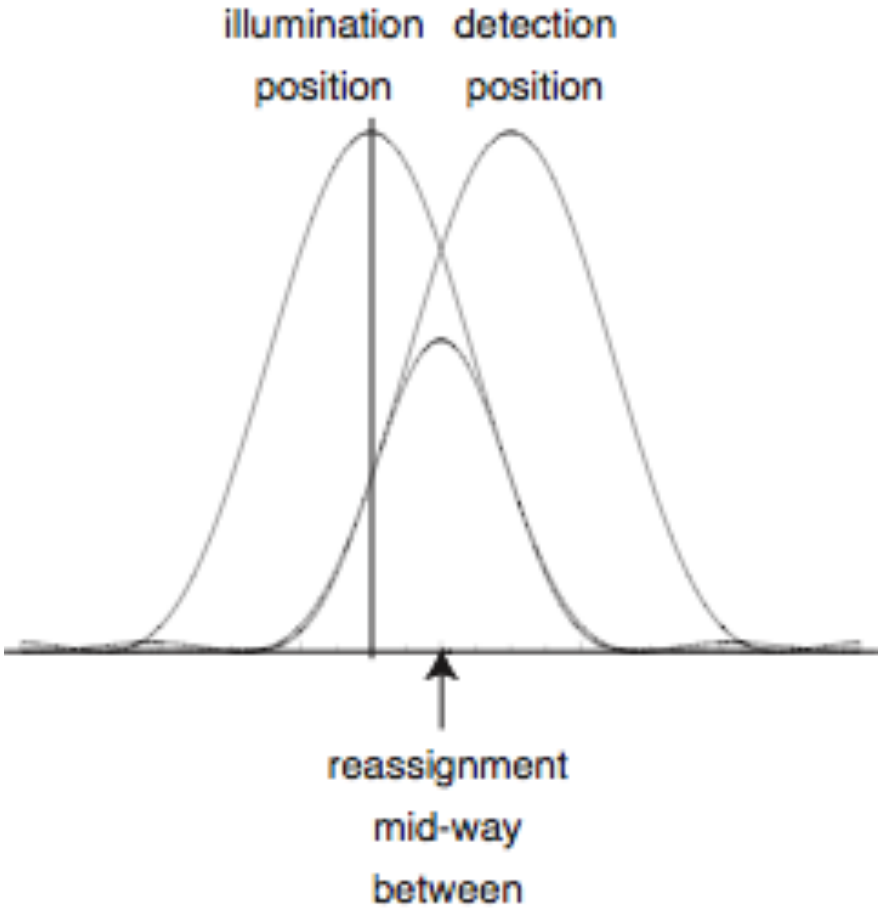
- Point spread function gets narrower
- Intensity decreases
- But increased side lobes
- And effective psf shifts sideways

Improvement in resolution by nearly confocal microscopy

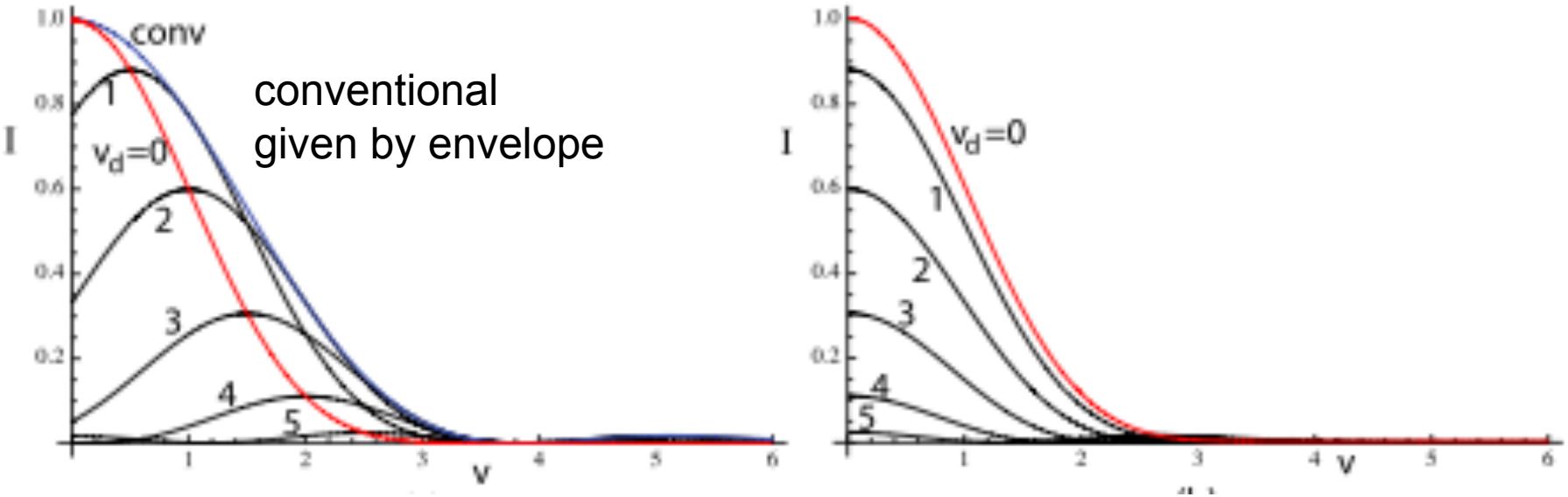
APPLIED OPTICS, Vol. 21, page 778, March 1, 1982

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

Gives the image of a shifted object point



Offset pinhole & reassignment



- 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

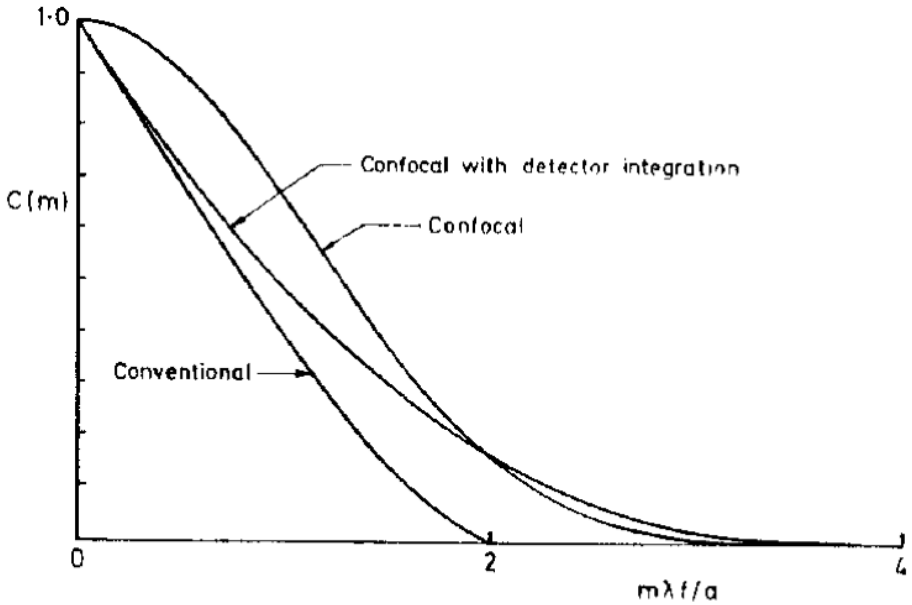


Fig. 2. Incoherent transfer functions for a fluorescence microscope. The radius of the circular pupils is a.

function of $2x_s$

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

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

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

Super-resolution in Confocal Imaging

C. J. R. Sheppard,

Optik 80, No. 2 (1988) 53–54

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*

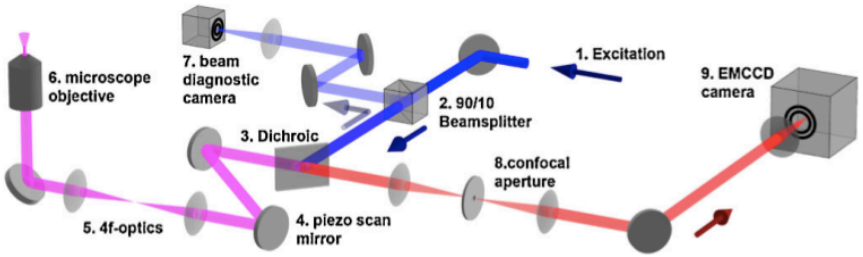


FIG. 1 (color online). ISM Setup, (1) Excitation with supercontinuum white light source and acousto-optic tunable filter, (2) 90/10 nonpolarizing beam splitter cube, (3) major dichroic mirror, (4) piezo scan mirror, (5) 4f telescope, (6) UPL APO 60x W microscope objective, (7) beam diagnostic camera,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

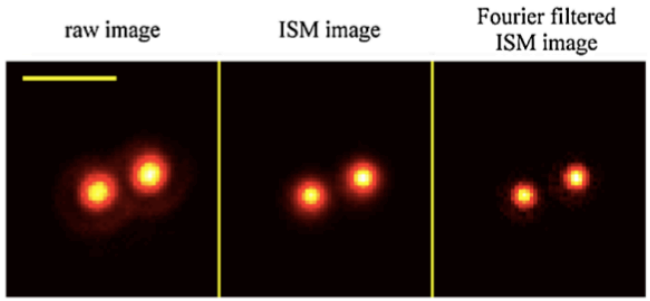
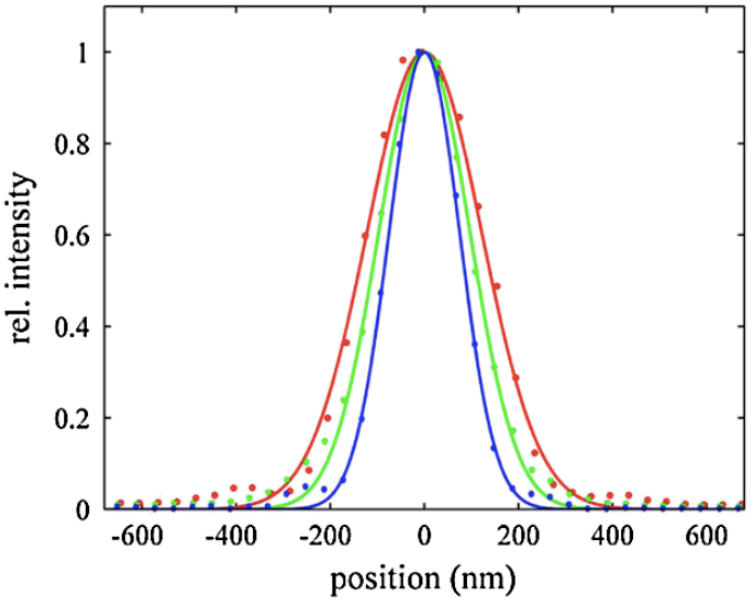
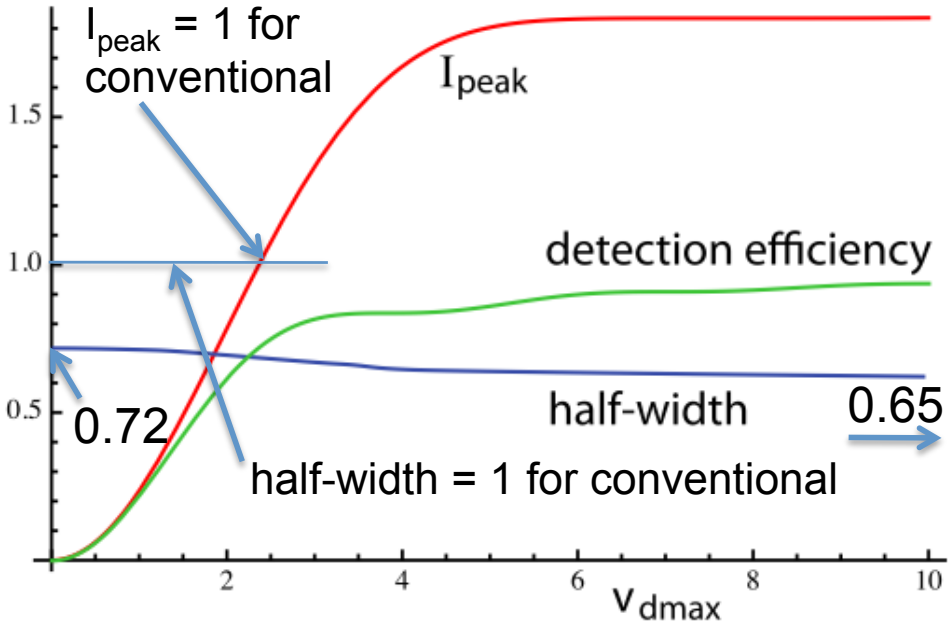


FIG. 2 (color online). Image of a single fluorescent bead of 100 nm diameter. Left panel: CLSM image; middle panel: ISM image; right panel: Fourier-weighted ISM image. The horizontal bar in the left panel has a length of 1 μm.



Integration over finite region

peak intensity goes above 1!

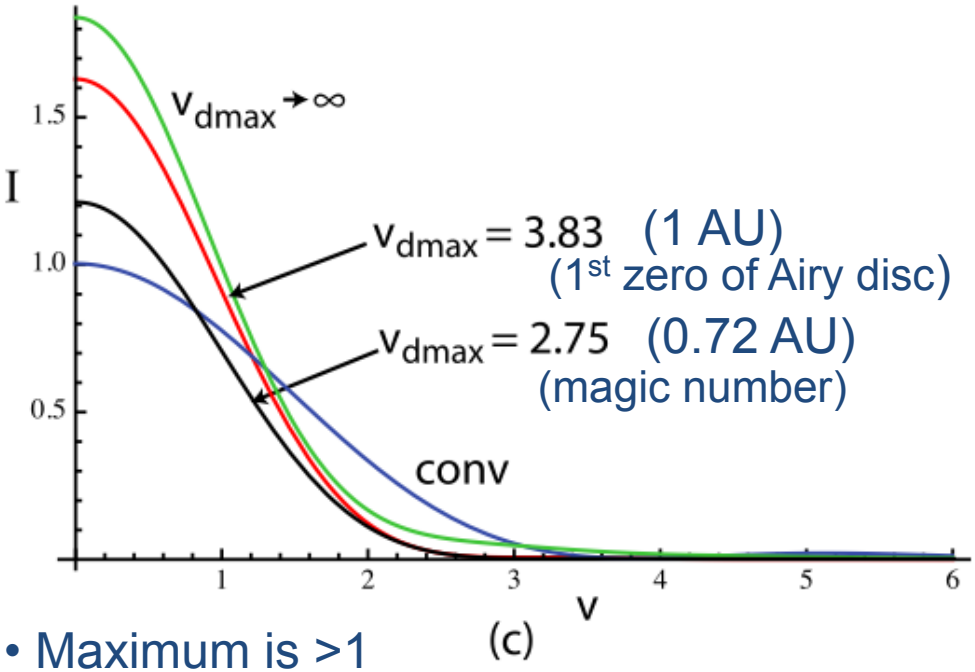


Resolution and signal strength improve as v_{dmax} increases

Maximum of point spread function for large v_{dmax} is

$$4(1-16/3\pi^2)=1.84$$

(4 elements gives ~1.4)



- Maximum is >1
- Super-concentration
- Beats classical limit of étendue

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]

Letter Vol. 41, No. 9 / May 1 2016 / Optics Letters 2109

Optics Letters

Superconcentration of light: circumventing the classical limit to achievable irradiance

Stephan Roth,[1] Colin J. R. Sheppard,[2] and Rainer Heintzmann[1,3,*]

Unnormalized OTF

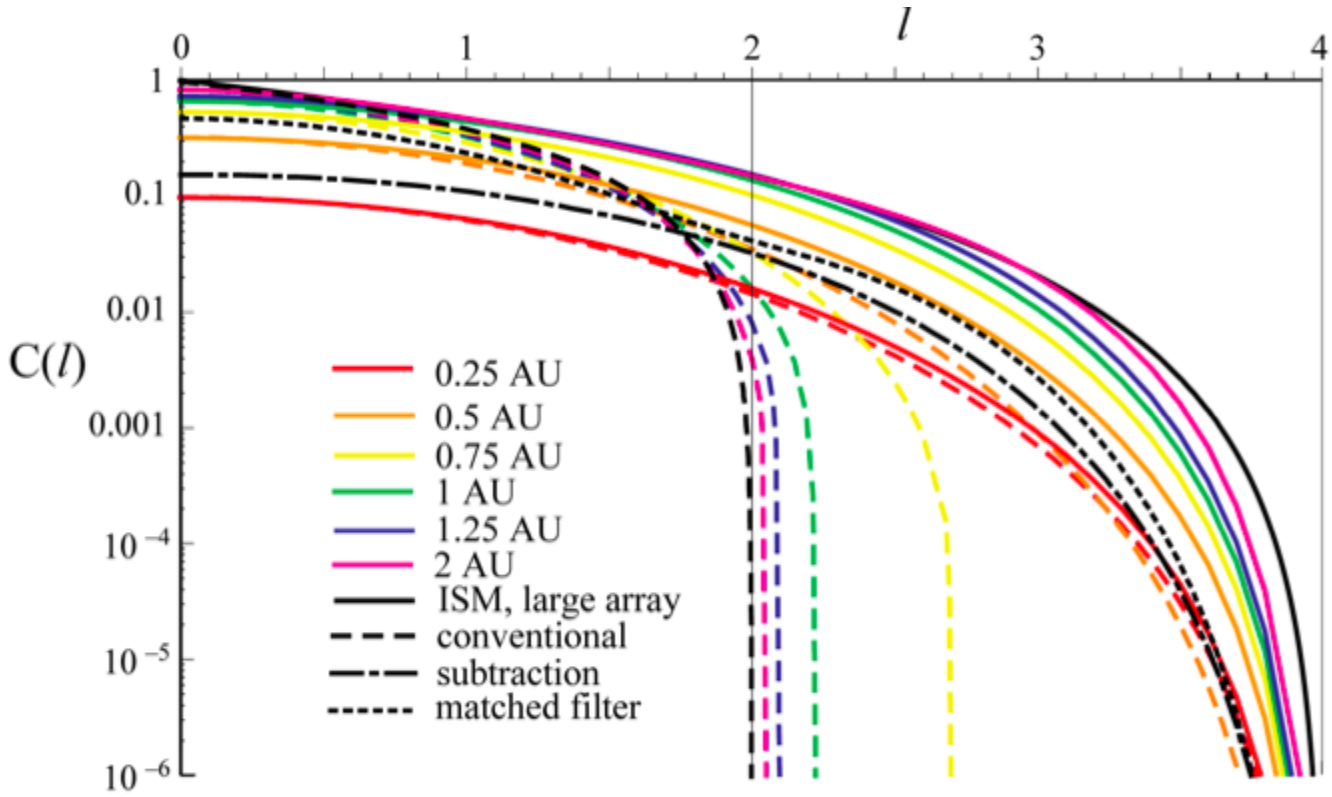


Fig. 5. A logarithmic plot of the unnormalized OTFs for a confocal microscope (dashed lines) and ISM (solid lines) with different pinhole/array sizes. The first positive lobe only of the confocal OTF is shown. The behavior for subtracting images from two pinhole sizes $(I_{0.5AU} - \frac{1}{4} I_{1AU})$,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

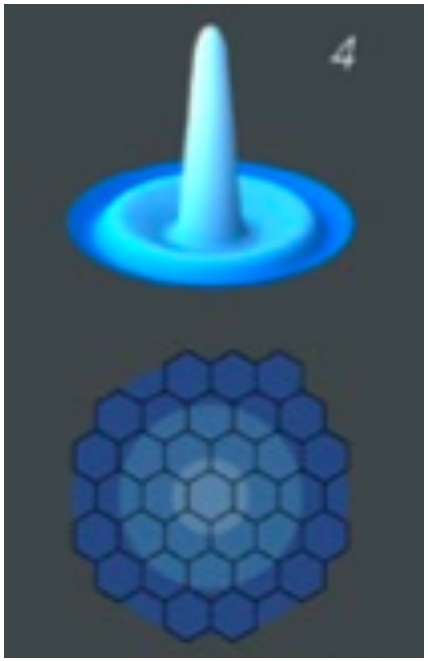
Research Article | Vol. 24, No. 24 | 28 Nov 2016 | OPTICS EXPRESS 27280
Optics EXPRESS

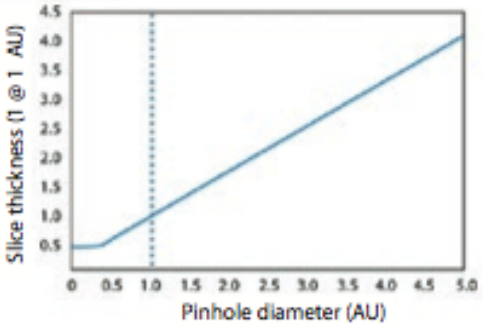
Interpretation of the optical transfer function: Significance for image scanning microscopy

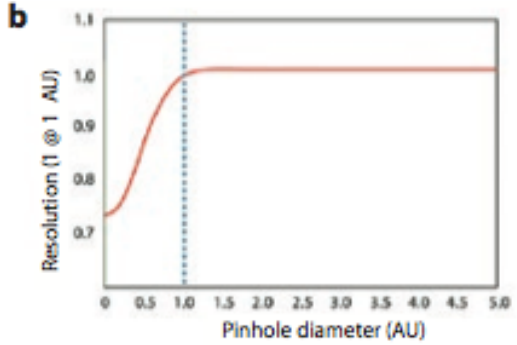
COLIN J. R. SHEPPARD,[1,*] STEPHAN ROTH,[2,3] RAINER HEINTZMANN,[2,3] MARCO CASTELLO,[1,4] GIUSEPPE VICIDOMINI,[1] RUI CHEN,[5] XUDONG CHEN,[5] AND ALBERTO DIASPRO[1,4,6]

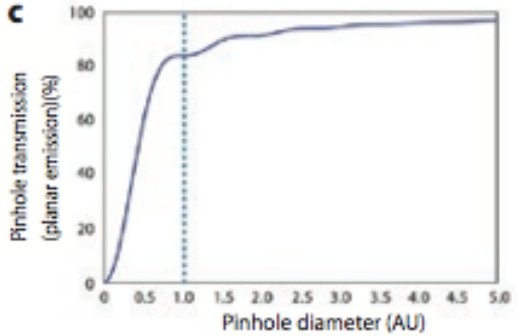
Zeiss Airyscan

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

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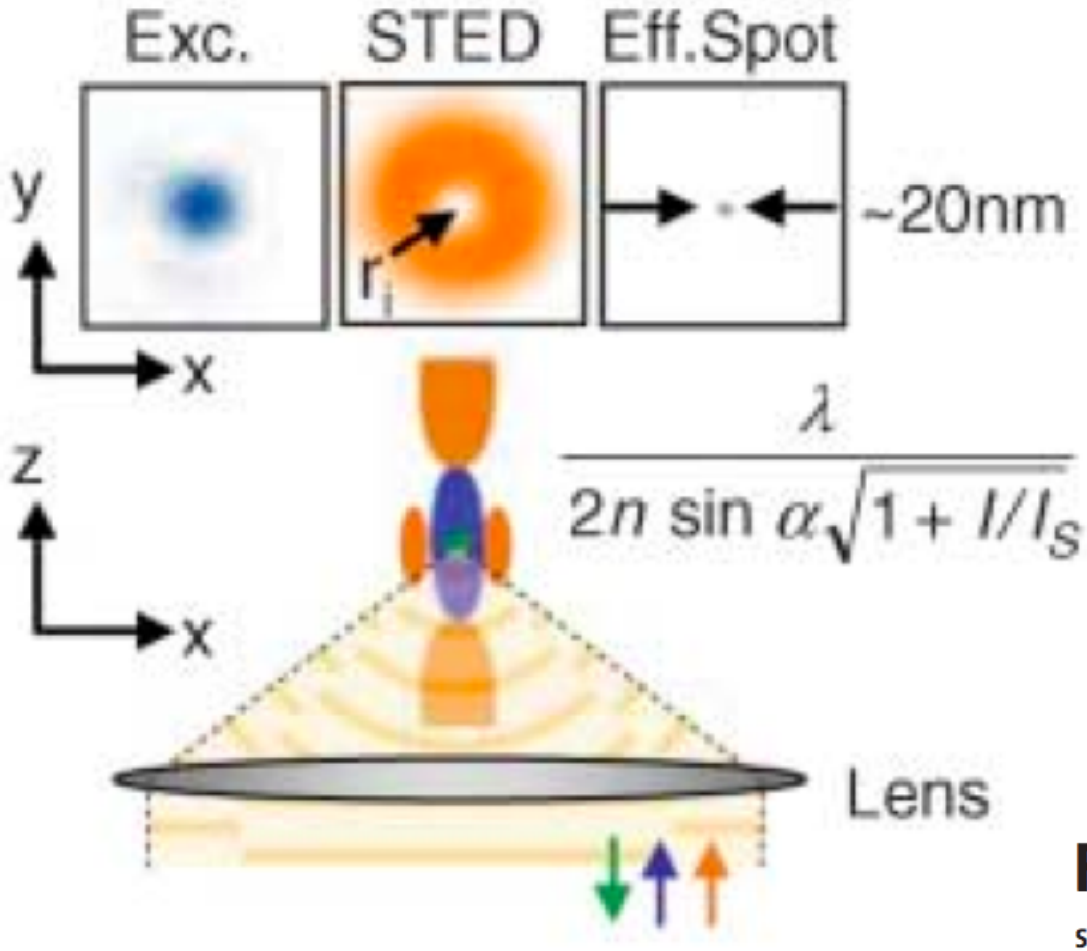
NATURE METHODS | DECEMBER 2015 |

Nonlinear imaging

- Incoherent versus coherent imaging (square law)
- Saturable absorber sharpens point spread function (Choudhury, 1977)
- Nonlinearity of detection in optical storage (Braat, 1980s)
- Nonlinear effects in lithography
- Multiphoton imaging (SHG, 2-photon fluorescence)
- Stimulated emission depletion microscopy (STED) (Hell)
- Saturation in structured illumination (Heinzmann, Gustafsson)
- PALM, STORM

Superresolution microscopy: Stimulated emission depletion microscopy (STED)



STED:
Principle by
Hell, Wichmann (1994)

Far-Field Optical Nanoscopy

Stefan W. Hell

Nonlinear structured illumination microscopy

- Heintzmann, Jovin & Cremer (2002)

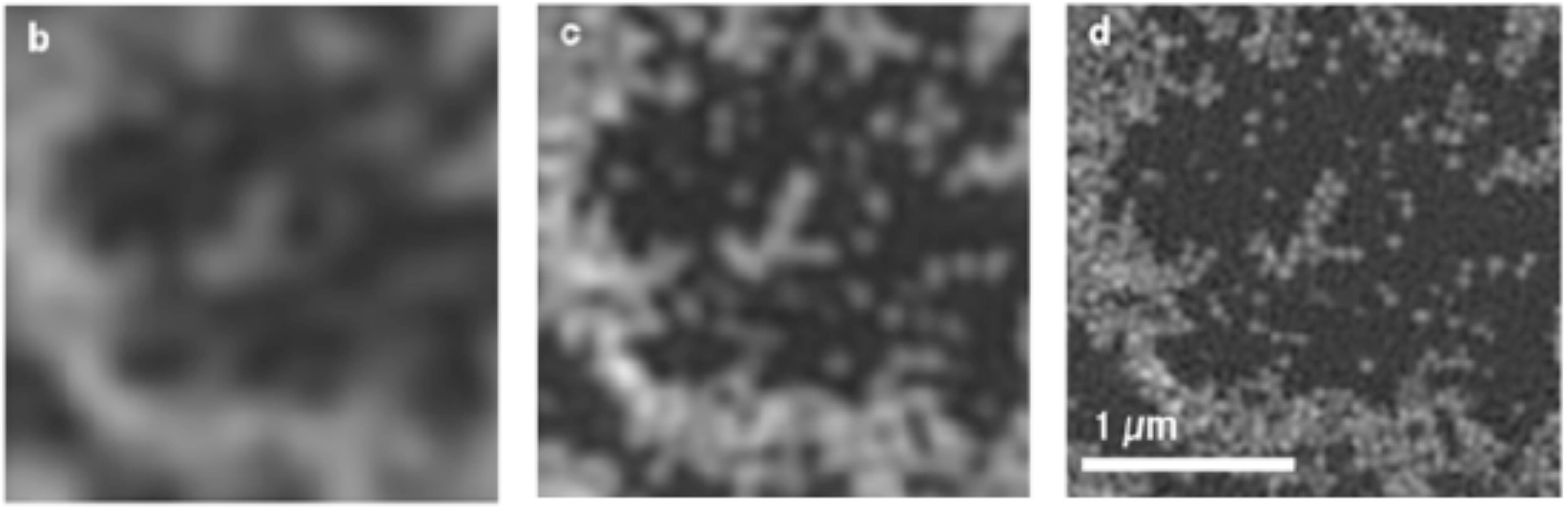
Saturated patterned excitation microscopy—a concept for optical resolution improvement

- Gustafsson (2005)

Nonlinear structured-illumination microscopy: Wide-field fluorescence imaging with theoretically unlimited resolution

Mats G. L. Gustafsson*

Structured illumination microscopy with nonlinearity from saturation



conventional

structured illumination

saturated structured illumination

Fig. 6. A field of 50 nm fluorescent beads, imaged by (a) conventional microscopy, (b) conventional microscopy plus filtering, (c) linear structured illumination, and (d) saturated structured illumination using illumination pulses with 5.3 mJ/cm^2 energy density, taking into account three harmonic orders in the processing. As no scanning is

M Gustafsson, *PNAS* **102**, 13081 (2005)

Localization microscopy

- Lidke *et al. Opt Exp.* 13, 7052 (2005) Blinking of quantum dots

Abstract: In microscopy, single fluorescence point sources can be localized with a precision several times greater than the resolution limit of the microscope. We show that the intermittent fluorescence or 'blinking' of quantum dots can analyzed by an Independent Component Analysis so as to identify the light emitted by each individual nanoparticle, localize it precisely, and thereby resolve groups of closely spaced ($< \lambda/30$) quantum dots. Both simulated and experimental data demonstrate that this technique is superior to localization based on Maximum Likelihood Estimation of the sum image under the assumption of point emitters. This technique has general application to any emitter with non-Gaussian temporal intensity distribution, including triplet state blinking. When applied to the labeling of structures, a high resolution "image" consisting of individually localized points may be reconstructed leading to the term "Pointillism".

Superresolution by localization of quantum dots using blinking statistics

5 September 2005 / Vol. 13, No. 18 / OPTICS EXPRESS 7052

Keith A. Lidke[1], Bernd Rieger[1], Thomas M. Jovin[1] and Rainer Heintzmann[1,2]

- Betzig *et al. Science* 313, 1642 (2006) PALM
- Hess *et al. Biophys. J.* 91, 4258 (2006) FPALM
- Rust *et al. Nature Methods* 3, 793 (2006) STORM

Conclusions

- Distinguish between true superresolution and others
- Some methods can give improvement in resolution with an unchanged spatial frequency cut-off
    - Pupil filters
- Some methods can increase spatial frequency cut-off by factor of two
    - Confocal
    - Structured illumination
    - Source/detector arrays
- Some methods can give further increased bandwidth
    - Nonlinear or switching
    - Near field