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ISTITUTO ITALIANO DI TECNOLOGIA NANOSCOPY

Partially Coherent Imaging & Phase Contrast Microscopy

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Perfect imaging

Object amplitude transmission

$$t(x,y) = a(x,y)e^{i\phi(x,y)}$$

$a(x,y)$ is modulus (amplitude), real

$\phi(x,y)$ is phase, real

Perfect image

$$I(x,y) = \left| a(x,y)e^{i\phi(x,y)} \right|^2 = a^2(x,y)$$

- No phase information in perfect image!

To see phase, need to have imperfect imaging system:

- Introduce phase (aberration)
- Introduce asymmetry

3 main methods of phase contrast

- Complex Pupil Function
  - Zernike phase contrast
  - Defocus
  - Transport of intensity equation (TIE)
- Phase gradient methods (asymmetry)
  - Schlieren
  - Hoffmann modulation contrast
  - Differential phase contrast (DPC)
  - Wavefront sensing (Shack-Hartmann)
  - Differential Interference Contrast (DIC)
- Interference methods
  - Interference microscopy
  - Digital holographic microscopy (DHM)

Coherent vs. partially coherent

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- 1 Coherent methods (Digital holographic microscopy)
  - Spatial frequencies only on Ewald sphere
  - Limited 3D imaging performance
  - But can get good 3D by holographic tomography
  - Limited spatial resolution
  - Speckle
  - Can reconstruct with Rytov approximation

- 2 Partially coherent methods
  - Improved image bandwidth
  - No speckle
  - More difficult to extract quantitative information

Partially coherent image formation

Propagate mutual intensity through the system:

Image intensity

$$I'(u',v') = \left(\frac{1}{2\pi}\right)^2 \sum_m \sum_n \sum_p \sum_q C(m,n;p,q)\,a(m,n)\,a^*(p,q)\,e^{i[(m-p)u'+(n-q)v']}, \qquad (17)$$

and (15) becomes

$$C(m,n;p,q) = 2\pi \iint_{-\infty}^{+\infty} \gamma(x,y) f(x+m,y+n) f^*(x+p,y+q)\,dx\,dy. \qquad (18)$$

source pupil

$C(m,n;p,q)$ = transmission cross-coefficient (TCC)

m, p are both spatial frequencies in *x* direction
n, q are both spatial frequencies in *y* direction

- System and object separated.
- Although Hopkins propagated mutual intensity, he did not give mutual intensity of the image.

On the diffraction theory of optical images

By H. H. Hopkins

Proc. R. Soc. Lond. A **217**, 408 (1953)

Imaging in a partially-coherent microscope

For non-periodic objects, replace sums by integrals:

C = transmission cross-coefficient (TCC)

object spectrum

$$I(x_s, y_s) = \text{const.} \iiiint_{-\infty}^{+\infty} C(m, n; p, q) T_0(m, n) T_0^*(p, q)$$
$$\exp(2\pi j\{(m-p)x_s + (n-q)y_s\}) dm\, dn\, dp\, dq,$$

Conventional

$$C(m, n; p, q) = \iint_{-\infty}^{+\infty} P_2(x, y) P_1(x + \tilde{m}, y + \tilde{n}) P_1^*(x + \tilde{p}, y + \tilde{q})\, dx\, dy,$$

condenser objective

Confocal microscope:

$$C(m, n; p, q) = \{P_1(\tilde{m}, \tilde{n}) \otimes P_2(\tilde{m}, \tilde{n})\}\{P_1^*(\tilde{p}, \tilde{q}) \otimes P_2^*(\tilde{p}, \tilde{q})\}$$

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

Generalization of coherent imaging

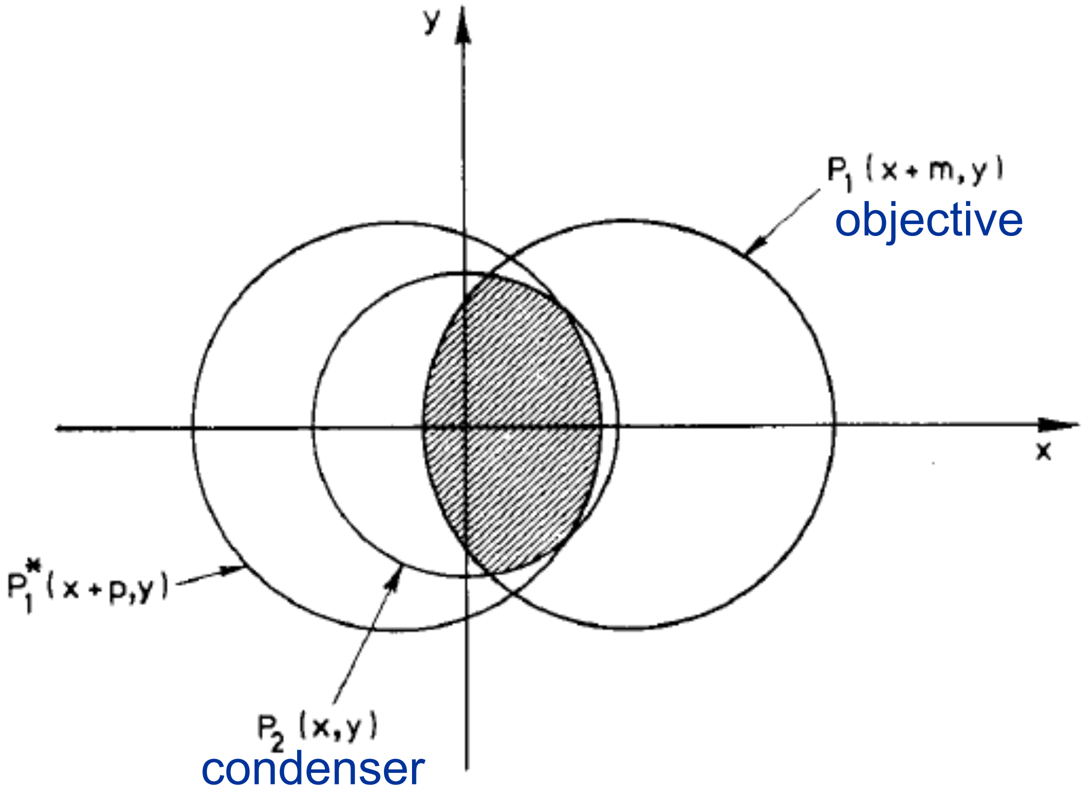
The image cannot be displayed. Your computer may not have enough memory to open the image, or the image may have been corrupted. Restart your computer, and then open the file again. If the red x still appears, you may have to delete the image and then insert it again.

$$I(x) = \left| \int c(m) T(m) \exp[-2\pi i m x]\, dm \right|^2$$

$$= \iint \underbrace{c(m_1) c(m_2)} T(m_1) T^*(m_2) \exp[-2\pi i (m_1 - m_2) x]\, dm_1 dm_2$$

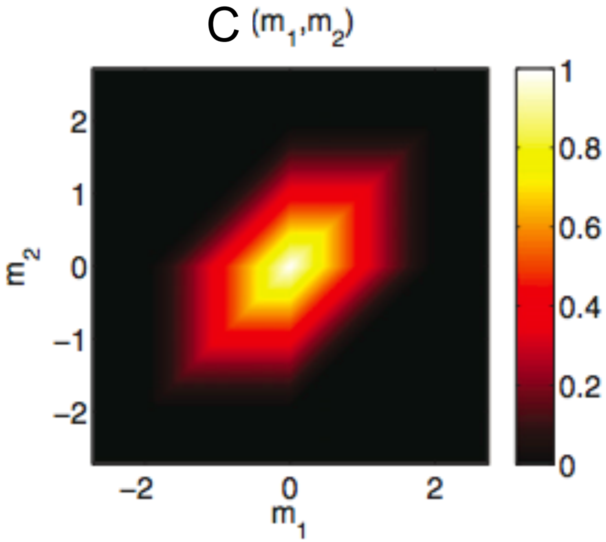
For partially coherent, $C(m_1, n_1; m_2, n_2)$ does not separate

$C(m, 0; p, 0)$ as area of overlap of three circles (conventional system)



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

Transmission cross coefficient (TCC)



$S = 1$

S is coherence ratio (NA_{cond}/NA_{obj})

348 OPTICS LETTERS / Vol. 35, No. 3 / February 1, 2010

Phase-space representation of partially coherent imaging systems using the Cohen class distribution

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

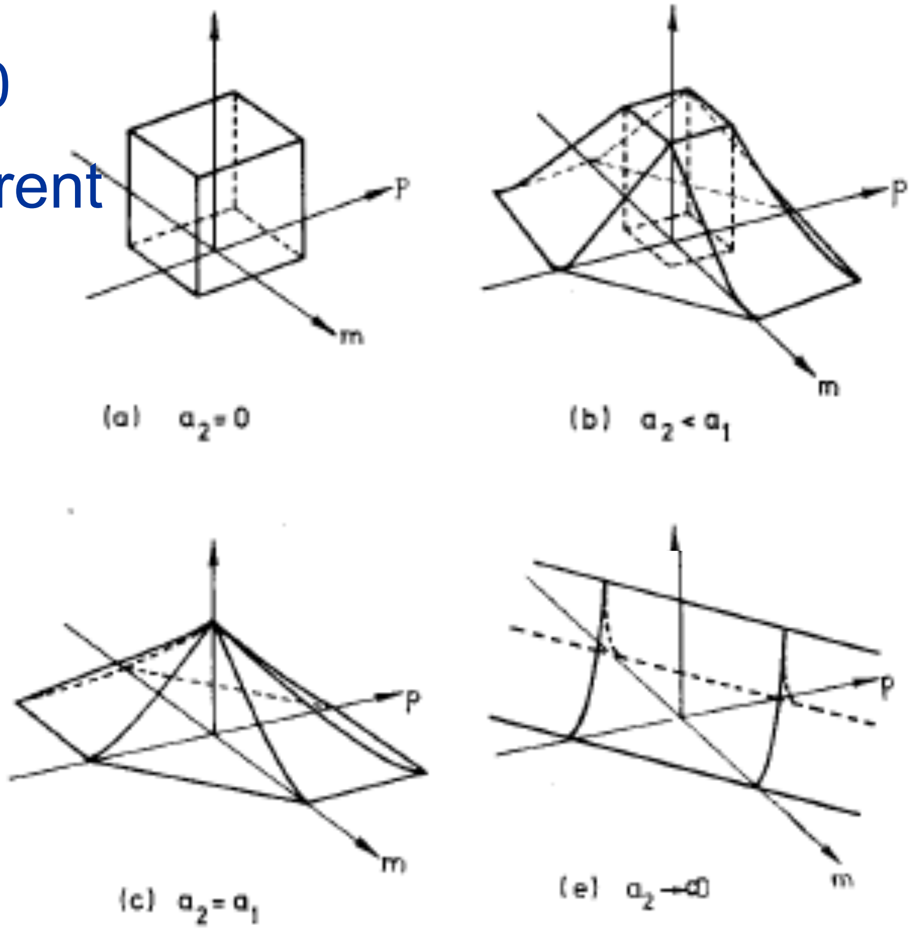
Using the phase-space imager to analyze partially coherent imaging systems: bright-field, phase contrast, differential interference contrast, differential phase contrast, and spiral phase contrast

J. Modern Optics **57**, 718-739 (2010)

Shalin B. Mehta[a,b*] and Colin J.R. Sheppard[a,b,c]

$S = 0$

coherent



$C(m; p)$

(conventional)

$S = 1$

$S \rightarrow \infty$

NB m, p are spatial frequencies both in the x direction

full, complete, or matched illumination

incoherent

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

Introduce central and difference coordinates

The image cannot be displayed. Your computer may not have enough memory to open the image, or the image may have been corrupted. Restart your computer, and then open the file again. If the red x still appears, you may have to delete the image and then insert it again.

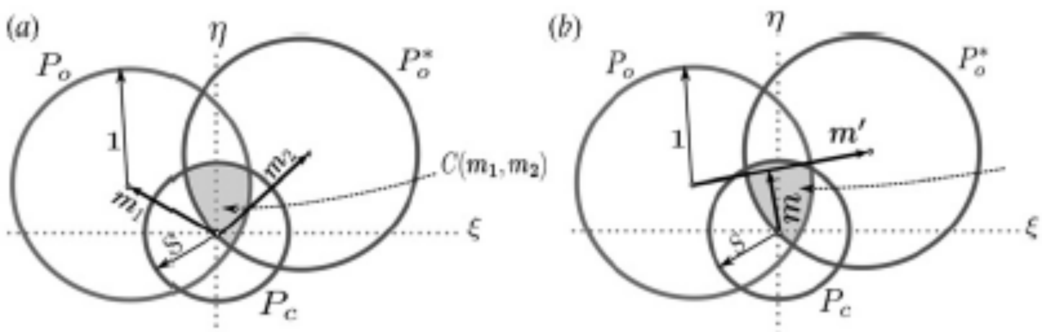
Introduce central and difference coordinates

$$x_1 = x + \Delta x / 2, x_2 = x - \Delta x / 2;$$

$$m_1 = m + \Delta m / 2, m_2 = m - \Delta m / 2$$

$$\Delta m x + m \Delta x = m_1 x_1 - m_2 x_2$$

$$dm_1 dm_2 = dm d\Delta m$$



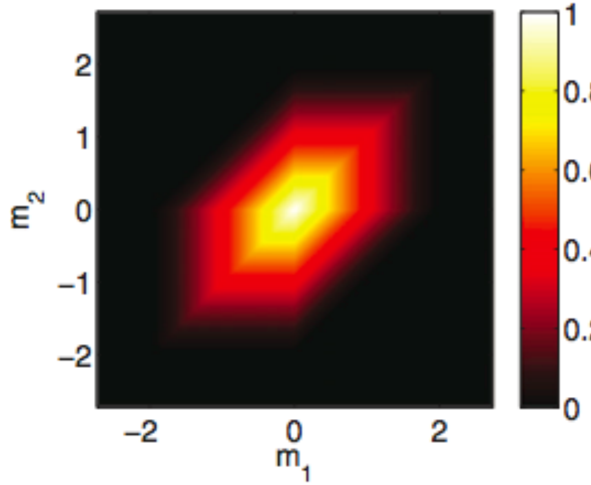
$C(m,\Delta m)$

TCC Transmission cross-coefficient

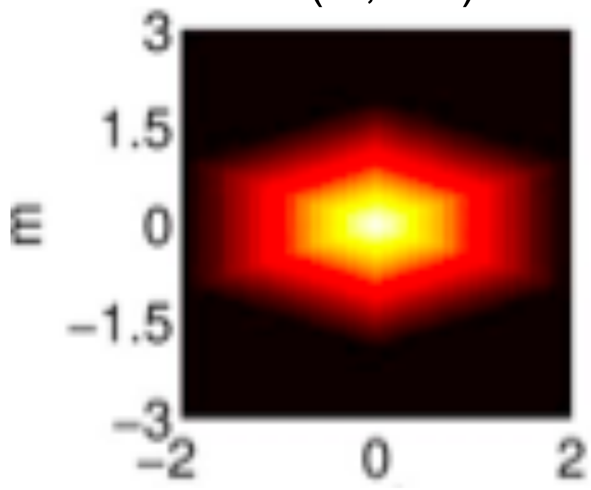
Area of overlap of source and 2 displaced pupils

Transmission cross coefficient (TCC)

C (m_1,m_2)



C(m, Δm)



Δm

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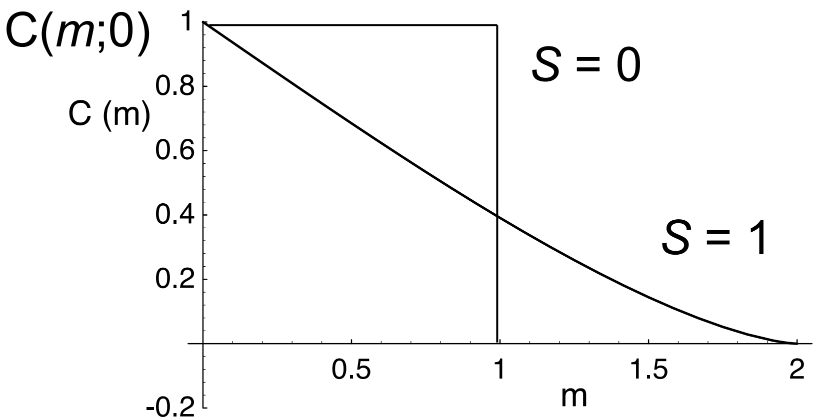
Shalin B. Mehta[a,b*] and Colin J.R. Sheppard[a,b,c]

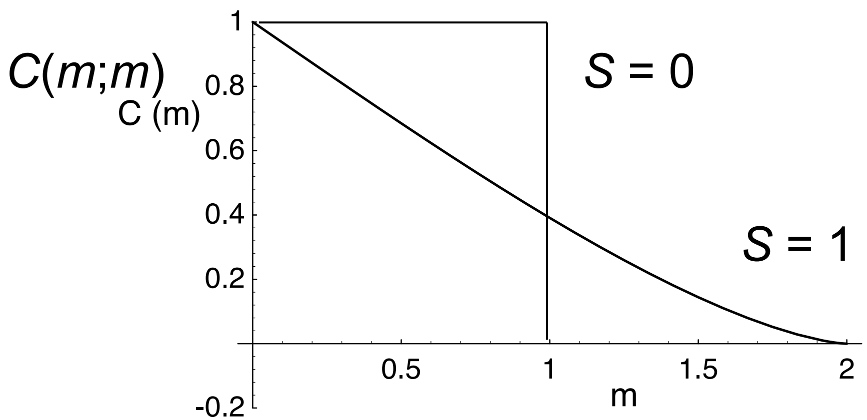
WOTF $C(m;0)$ and PGTF $C(m;m)$ for conventional microscope

Partially coherent imaging is complicated, but it becomes simpler for two special cases:

- Weak object (neglect interference of scattered light with scattered light)
  - Can use if first Born approximation is satisfied.
  - (But not necessarily the inverse)
- Slowly varying phase gradient
  - Can use if Rytov approximation is valid



Weak object transfer function (**WOTF**)



Phase gradient transfer function (**PGTF**)

S is coherence ratio (NA_{cond}/NA_{obj})

Weak object

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$$t(x,y) = e^{b(x,y)} \qquad b(x,y) \text{ complex}$$

- Weak object

$$t(x,y) \approx 1 + b(x,y)$$

Spectrum

$$T(m,n) = \delta(m)\delta(n) + B(m,n)$$

- *B* is skew-Hermitian if *b* is imaginary

Weak object transfer function (WOTF)

- Weak object (*b* is complex)

$$t(x) = 1 + b\cos 2\pi\nu x,$$

- For even *C*:

$$I(x_s) = 1 + 2\,\mathrm{Re}\{bC(\nu;0)\}\cos 2\pi\nu x_s.$$

- Weak object transfer function (WOTF)
- Phase imaged by imaginary part of *C*(*v*;0)

Fourier Imaging of Phase Information in Scanning and Conventional Optical Microscopes

C. J. R. Sheppard; T. Wilson
Philosophical Transactions of the Royal Society of London. Series A, Mathematical and Physical Sciences, Volume 295, Issue 1415 (Feb. 7, 1980), 513-536.

Weak phase object

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- An Hermitian transfer function does not give contrast from a weak phase object
- Make pupil either
    - complex
    - asymmetric

Defocus

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- Earliest method of phase contrast
- Like Zernike, based on changing the phase of the signal
- Only works for a weak object
- Contrast opposite for different defocus directions
- Relative condenser aperture S cannot be too large
- For a coherent system,

  $S = 0$, $\arg[P(\rho)] = u\rho^2/2$, so $\arg[c(m)] = um^2/2$

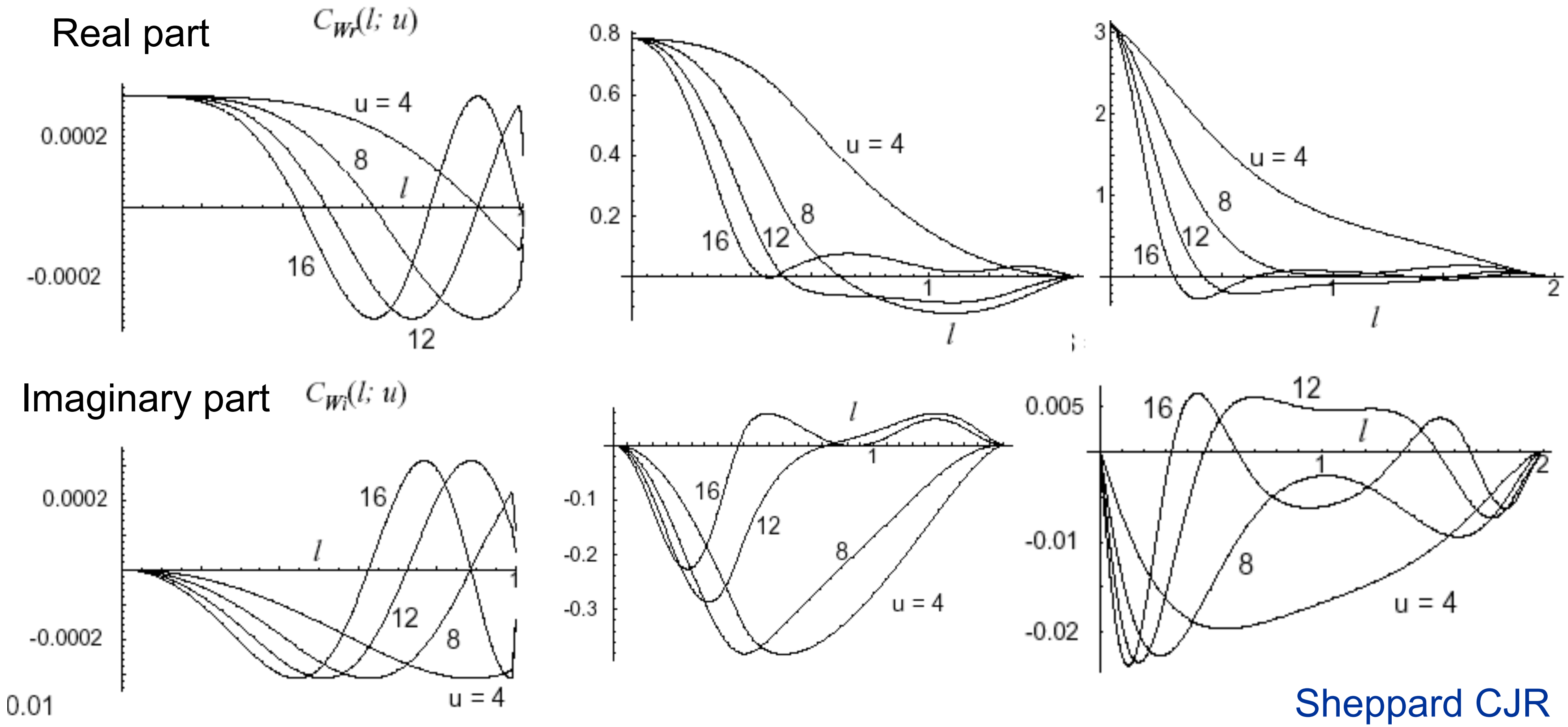
Defocused WOTF

l is radial spatial frequency, $l = (m^2+n^2)^{1/2}$

S=0.01 S=0.5 S=0.99

Real part $C_{Wr}(l;\,u)$

Imaginary part $C_{Wi}(l;\,u)$



Sheppard CJR
Defocused transfer function for a partially coherent microscope, and application to phase retrieval
J. Opt. Soc. Am. A, **21**, 828-831(2004)

Small defocus: analytic expression

$$C_{Wi} = -\frac{1}{2}\pi u l^2 S^2, \; 0 \le l \le 1 - S,$$

$$C_{Wi} = -\frac{u l^2}{2}\left[S^2 \arccos\left(\frac{l^2 + S^2 - 1}{2lS}\right) - \arccos\left(\frac{l^2 - S^2 + 1}{2l}\right)\right]$$

$$-\frac{u}{6l}\left\{\left[S^2 - \left(\frac{l^2 + S^2 - 1}{2l}\right)^2\right]^{1/2}\left((1 - S^2)^2 - \frac{l^2}{2}(1 + l^2 + 7S^2)\right) \qquad 1 - S \le l \le 1 + S.\right.$$

$$\left.-\left[1 - \left(\frac{l^2 - S^2 + 1}{2l}\right)^2\right]^{1/2}\left((1 - S^2)^2 - \frac{l^2}{2}(7 + l^2 + S^2)\right)\right\},$$

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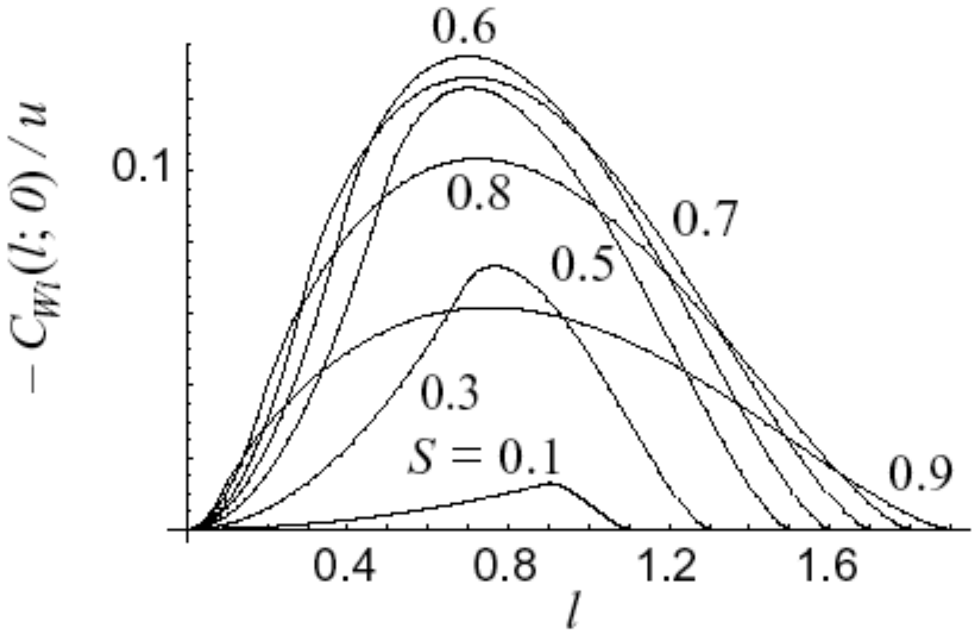
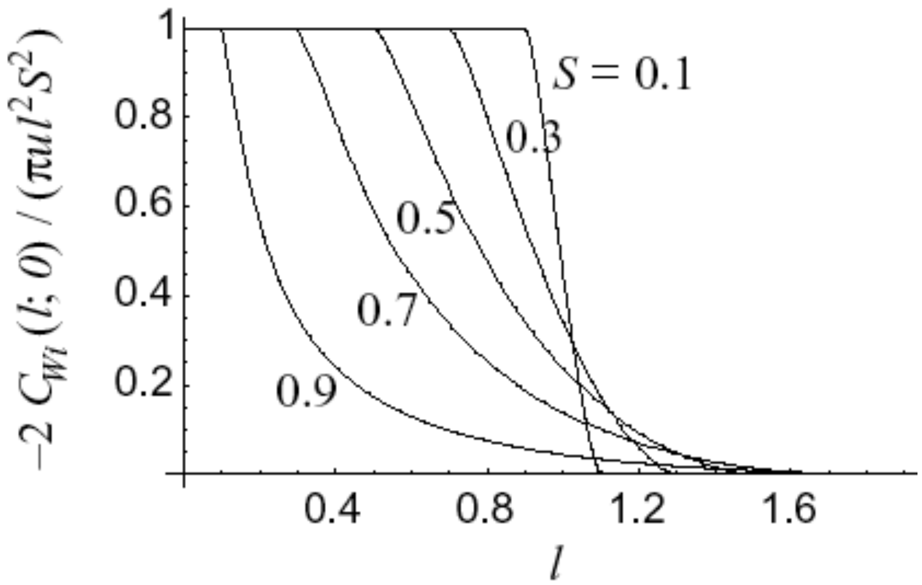
WOTF for phase contrast image

$I(\Delta u) - I(-\Delta u)$



Parabolic for small *l*

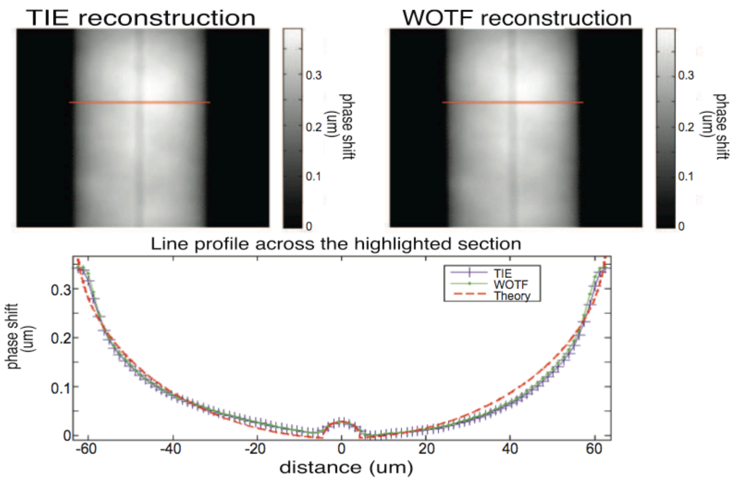
Inverse Laplacian:
Phase restored up to $l = 1 - S$

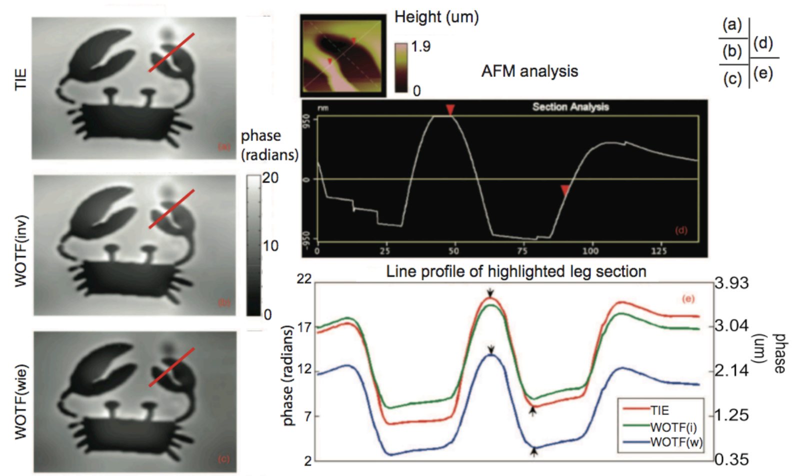


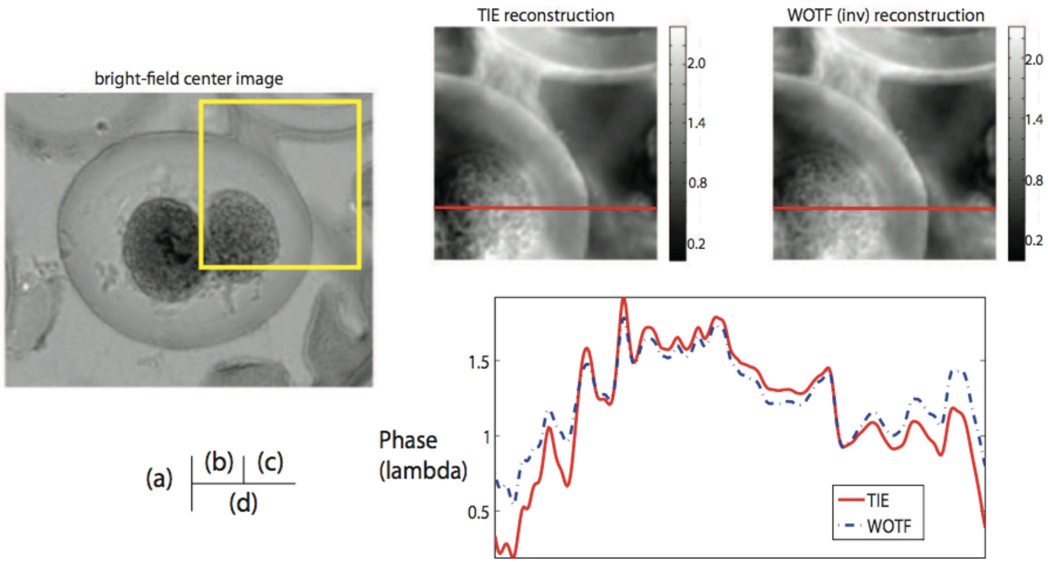
• Or use Wiener filter

Sheppard CJR
Defocused transfer function for a partially coherent microscope, and application to phase retrieval
J. Opt. Soc. Am. A, **21**, 828-831(2004)

Phase measurement using WOTF







Kou SS, Waller L, Barbastathis G, Marquet P, Depeursinge C, Sheppard CJR
Quantitative phase restoration by direct inversion using the optical transfer function,
Opt. Lett. **36**, 2671-2673 (2011).

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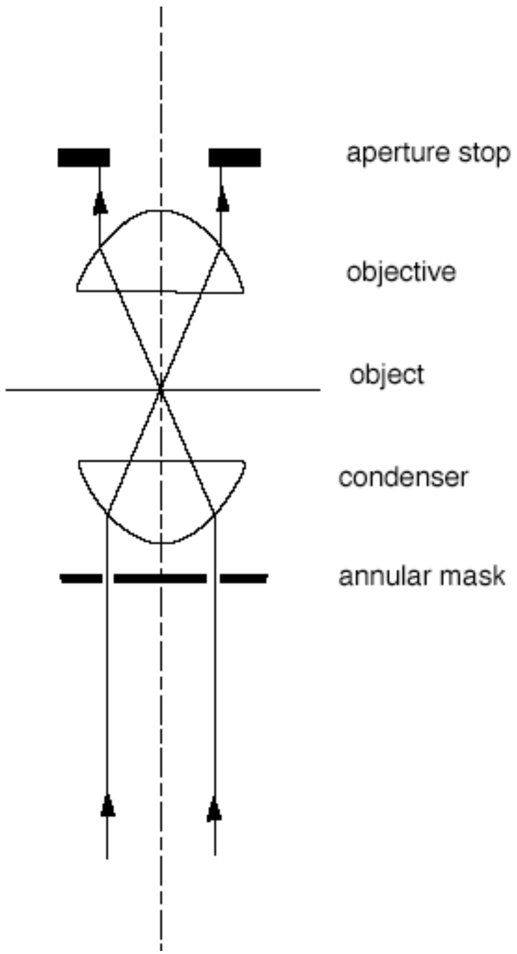
Dark field microscope



- Direct light blocked
- Partially-coherent imaging

Encyclopedia of Modern Optics,
RD Guenther, DG Steel, L Bayvel, eds,
Elsevier, Oxford, **3**, pp. 103-110

Phase Contrast Microscopy

C J R Sheppard, National University of Singapore, Singapore

Dark field

Weak phase object:

$$t(x, y) = 1 + id\cos(2\pi\nu x)$$

d is a real constant

Object spectrum:

$$T(m,n) = \left[\delta(m) + i\frac{d}{2}\delta(m-\nu) + i\frac{d}{2}\delta(m+\nu)\right]\delta(n)$$

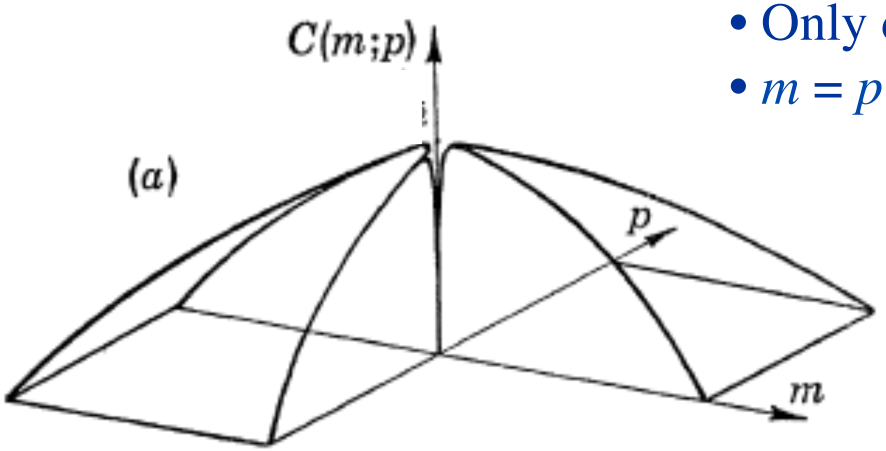
Dark field, no direct light: C(0, 0; 0, 0) = 0, C(ν, 0; 0, 0) = 0

So only terms in C(ν, ν; 0, 0) and C(ν, –ν; 0, 0)

$$I(x,y) = \frac{1}{2}d^2\left[C(\nu,\nu;0,0) + C(\nu,-\nu;0,0)\cos\left(\frac{4\pi\nu x}{M}\right)\right]$$

- Zero for annular dark field system
- Therefore no contrast for a single spatial frequency component

Dark field



- Only difference frequencies imaged
- $m = p$ gives $m - p = 0$

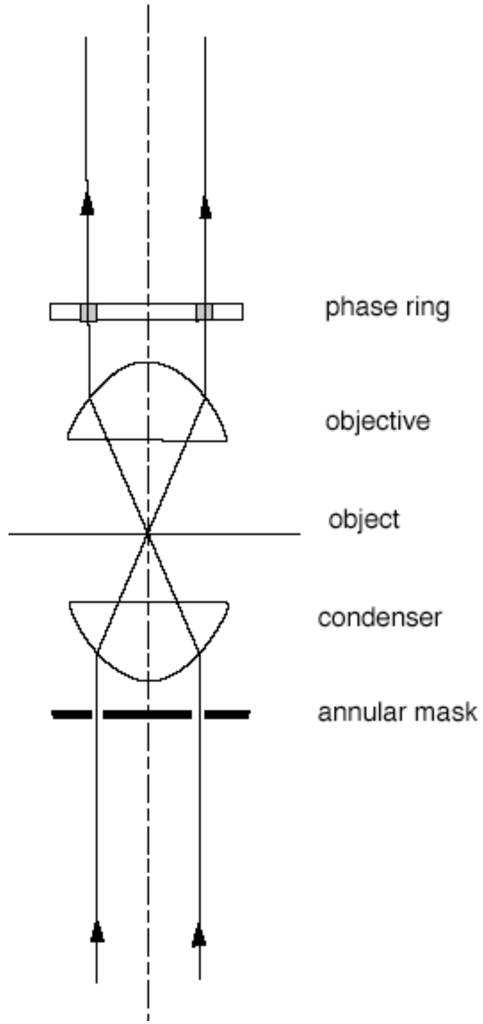
- Sum frequencies (m +ve, p –ve) not imaged

Fourier Imaging of Phase Information in Scanning and Conventional Optical Microscopes

C. J. R. Sheppard; T. Wilson

Philosophical Transactions of the Royal Society of London. Series A, Mathematical and Physical Sciences, Volume 295, Issue 1415 (Feb. 7, 1980), 513-536.

Zernike phase contrast



F. Zernike, "Phase contrast, a new method for the microscopic observation of transparent object," *Physica* **9**, 686-693 (1942).

- Direct light changed in phase
- Partially-coherent imaging
- Direct light on annular cone increases resolution

Encyclopedia of Modern Optics,
RD Guenther, DG Steel, L Bayvel, eds,
Elsevier, Oxford, **3**, pp. 103-110

Phase Contrast Microscopy

C J R Sheppard, National University of Singapore, Singapore

Zernike phase contrast

Weak phase object

$$t(x, y) = 1 + id\cos(2\pi\nu x)$$

Object spectrum

$$T(m,n) = \left[\delta(m) + i\frac{d}{2}\delta(m-\nu) + i\frac{d}{2}\delta(m+\nu)\right]\delta(n)$$

Phase imaging from imaginary part of *C:*

$$I(x,y) = 1 - dC_i(\nu,0;0,0)\cos\left(\frac{2\pi\nu x}{M}\right)$$

If *c* is the amplitude transmittance of the phase ring:

$$I(x,y) = 1 \pm \frac{2d}{c}C(\nu,0;0,0)\cos\left(\frac{2\pi\nu x}{M}\right)$$

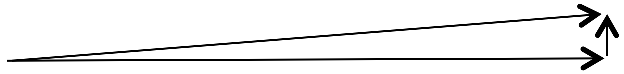
- Phase contrast is amplified

Weak phase object, ϕ small

Brightfield

Total almost unchanged

$$t(x,y) = a(x,y)e^{i\phi(x,y)}$$



Weak phase object $t = a + ia\phi$

Direct light

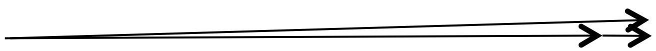
Darkfield

↑ Weak phase object

No direct light to reduce contrast

Zernike

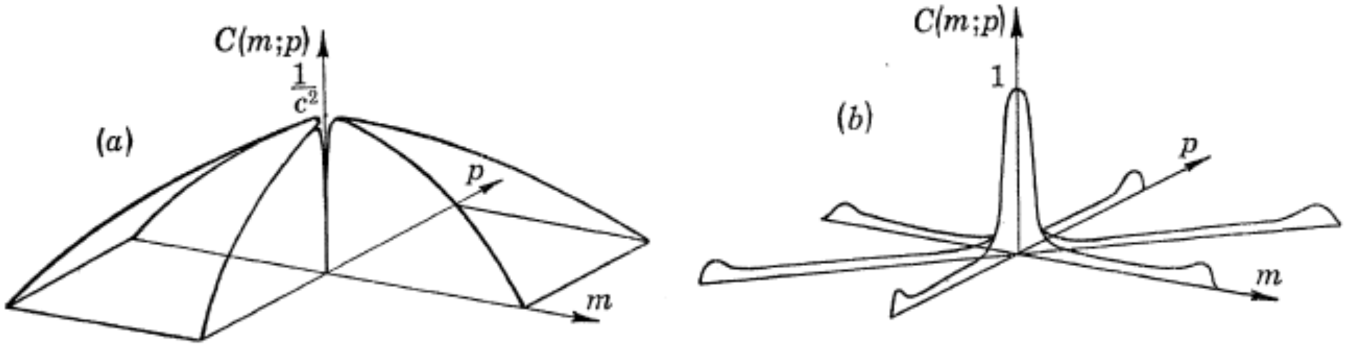
Change in total much larger



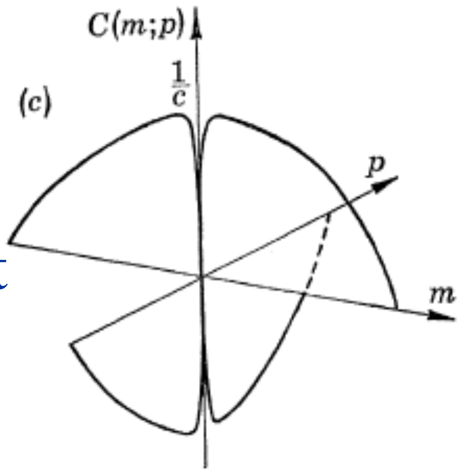
Phase of phase object altered

Direct light

Zernike phase contrast



- Superposed on dark field image



- Imaginary part (phase contrast)

FIGURE 11. Transfer function for conventional Zernike phase contrast.

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Philosophical Transactions of the Royal Society of London. Series A, Mathematical and Physical Sciences, Volume 295, Issue 1415 (Feb. 7, 1980), 513-536.

Zernike phase contrast

- Phase contrast amplified by transmittance of phase ring
- Can make +ve or –ve phase contrast from phase of phase ring
- Difficult to get quantitative information
- Haloes around phase changes

Defocus

- Earliest method of phase contrast
- Like Zernike, based on changing the phase of the signal
- Only works for a weak object
- Contrast opposite for different defocus directions
- Relative condenser aperture S cannot be too large
- For a coherent system,

  $S = 0$, $\arg[P(\rho)] = u\rho^2/2$, so $\arg[c(m)] = um^2/2$

Transport of intensity equation (TIE)

- Teague, *JOSA A* 1434, **73** (1983)
- Streibl, *Opt. Commun.* 6, **49** (1985)
- Barty, Nugent, Paganin, Roberts, *Opt. Lett*. 817, **23** (1998)

Amplitude in image space satisfies paraxial wave equation

$$k\frac{\partial I}{\partial z} = -\nabla_T \cdot \left(I\nabla_T \phi\right)$$

$$k\frac{\partial \ln I}{\partial z} = -\nabla_T^2 \phi - \nabla_T \ln I \cdot \nabla_T \phi$$

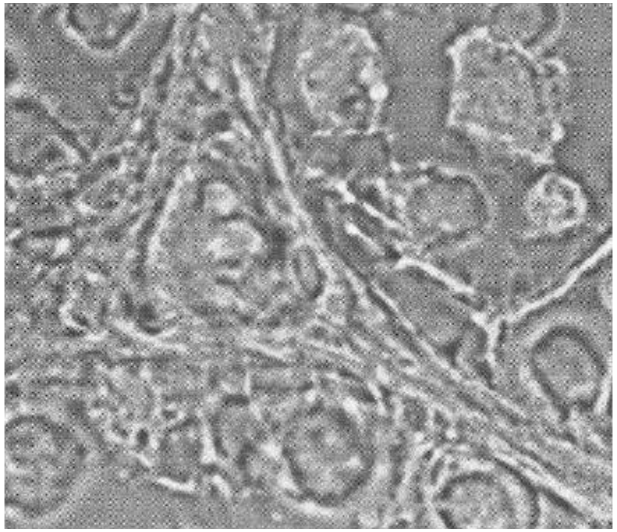
↑ often small

- Similar to eikonal equation
- Wavefront curvature sensing

Phase changes intensity

Photo:
Miguel Porras

Logarithmic derivative image



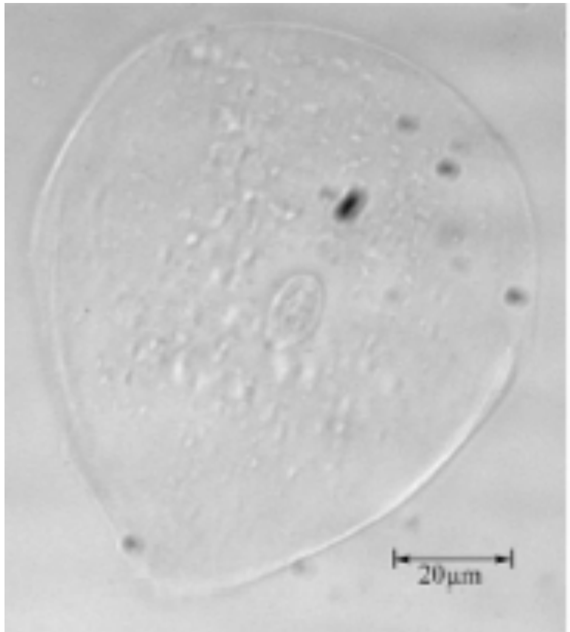
$$\frac{\partial I}{\partial z} = -\nabla_T \cdot \left(I \nabla_T \phi \right)$$

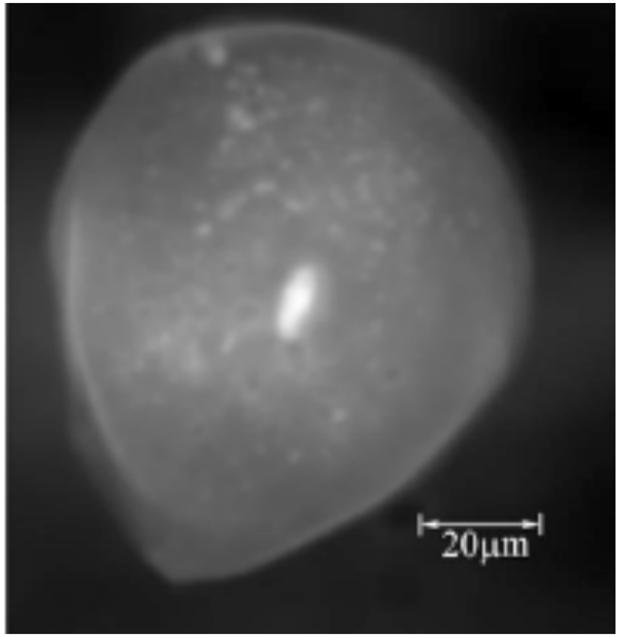
Logarithmic derivative:

$$\frac{\partial \ln I}{\partial z} = -\nabla_T^2 \phi - \nabla_T \ln I \cdot \nabla_T \phi$$

Testicle of rat, Streibl, *Opt. Commun.* 6, **49** (1984)

DIC

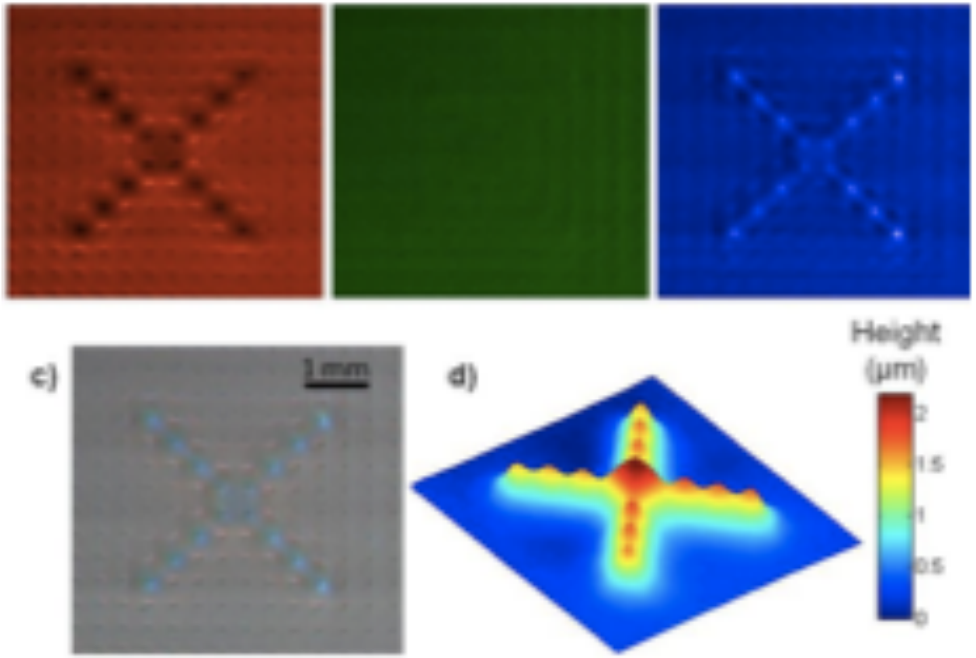


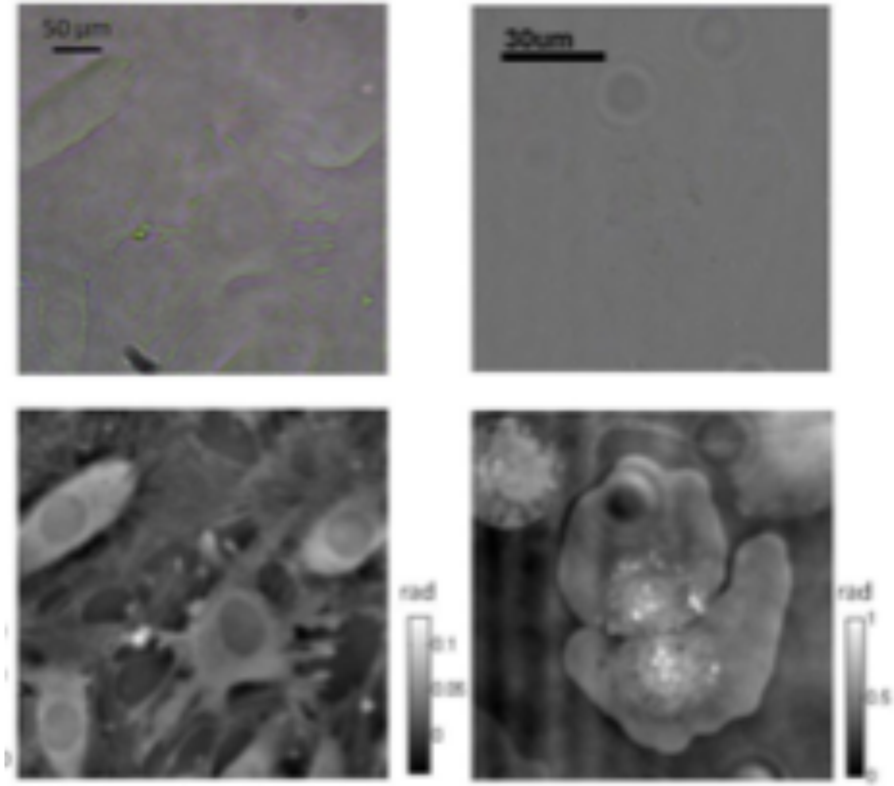


Barty, Nugent, Paganin, Roberts
Opt. Lett, **23**, 817 (1998)

TIE phase image

TIE from colour (single shot)





HMVEC cells

HeLa cells

Phase from chromatic aberrations

Laura Waller,[1,5,*] Shan Shan Kou,[3,6] Colin J. R. Sheppard,[3] and George Barbastathis[2,4]

2010 / Vol. 18, No. 22 / OPTICS EXPRESS 22817

Quantitative phase imaging by TIE

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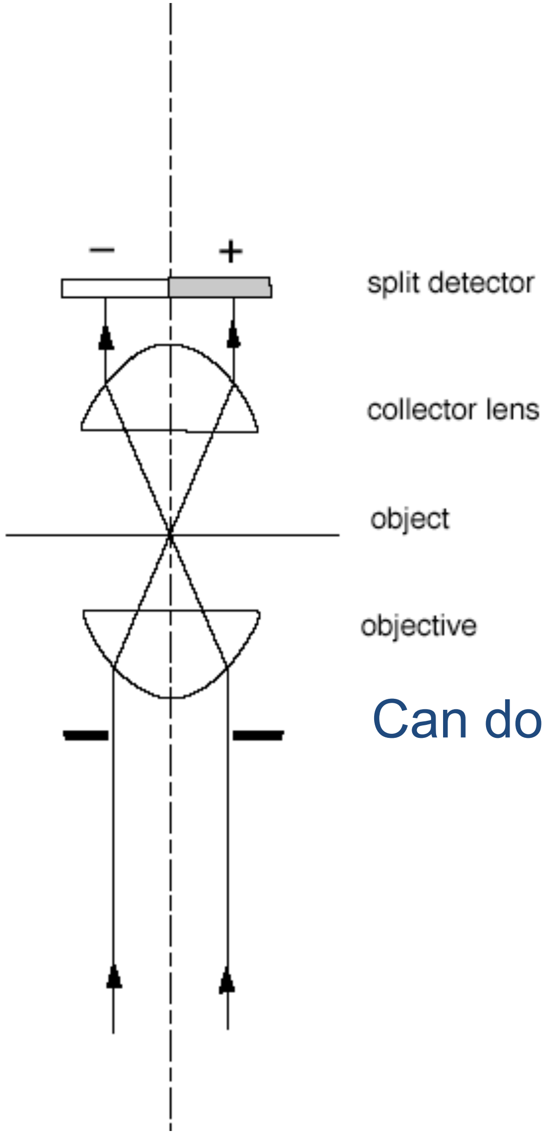
- IATIA system: measure ϕ using TIE equation
- Can then simulate Zernike, DIC, etc. images

Properties of TIE imaging

- Similar to defocus method for weak object, but not limited to weak phase
- Weak signal from low spatial frequencies
- Δz small to approximate $\partial/\partial z$: weak signal
- Measures phase of image not object
- Not enough information to directly recover object phase for strong object
- Problem with 3D imaging:
  Measure $\partial I / \partial z$ so no information on zero axial spatial frequency

Sheppard CJR (2002) Three-dimensional phase imaging with the intensity transport equation, *Appl. Opt.* 41, 5951-5955.

Differential phase contrast (DPC)



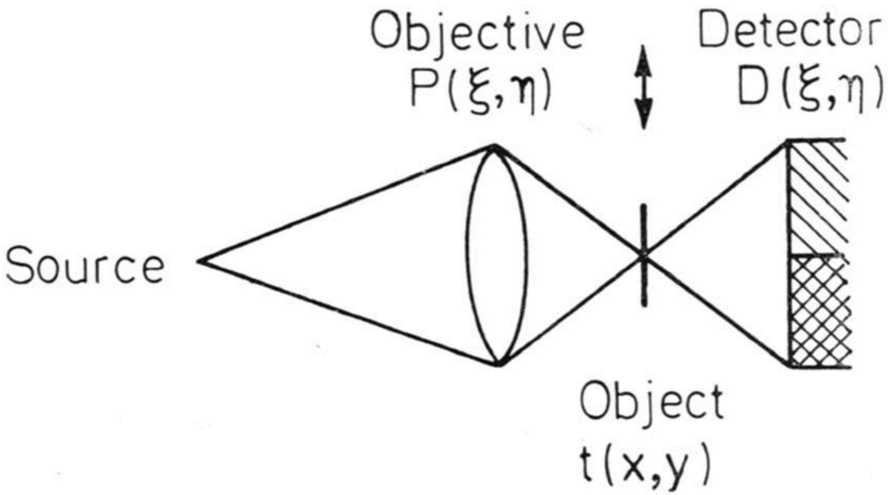
Can do simply in confocal microscope

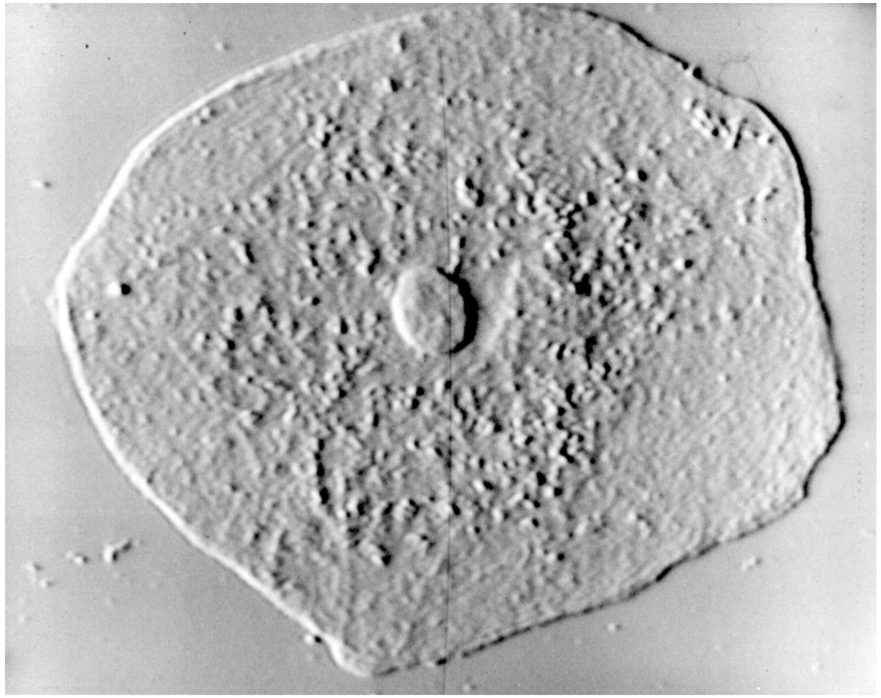
Encyclopedia of Modern Optics,
RD Guenther, DG Steel, L Bayvel, eds,
Elsevier, Oxford, **3**, pp. 103-110

Phase Contrast Microscopy

C J R Sheppard, National University of Singapore, Singapore

Differential phase contrast (DPC)





DPC image of a cheek cell

Journal of Microscopy, Vol. 133, *Pt* 1, *January* 1984, *pp.* 27–39.
Received 1 *November* 1982; *accepted* 29 *March* 1983

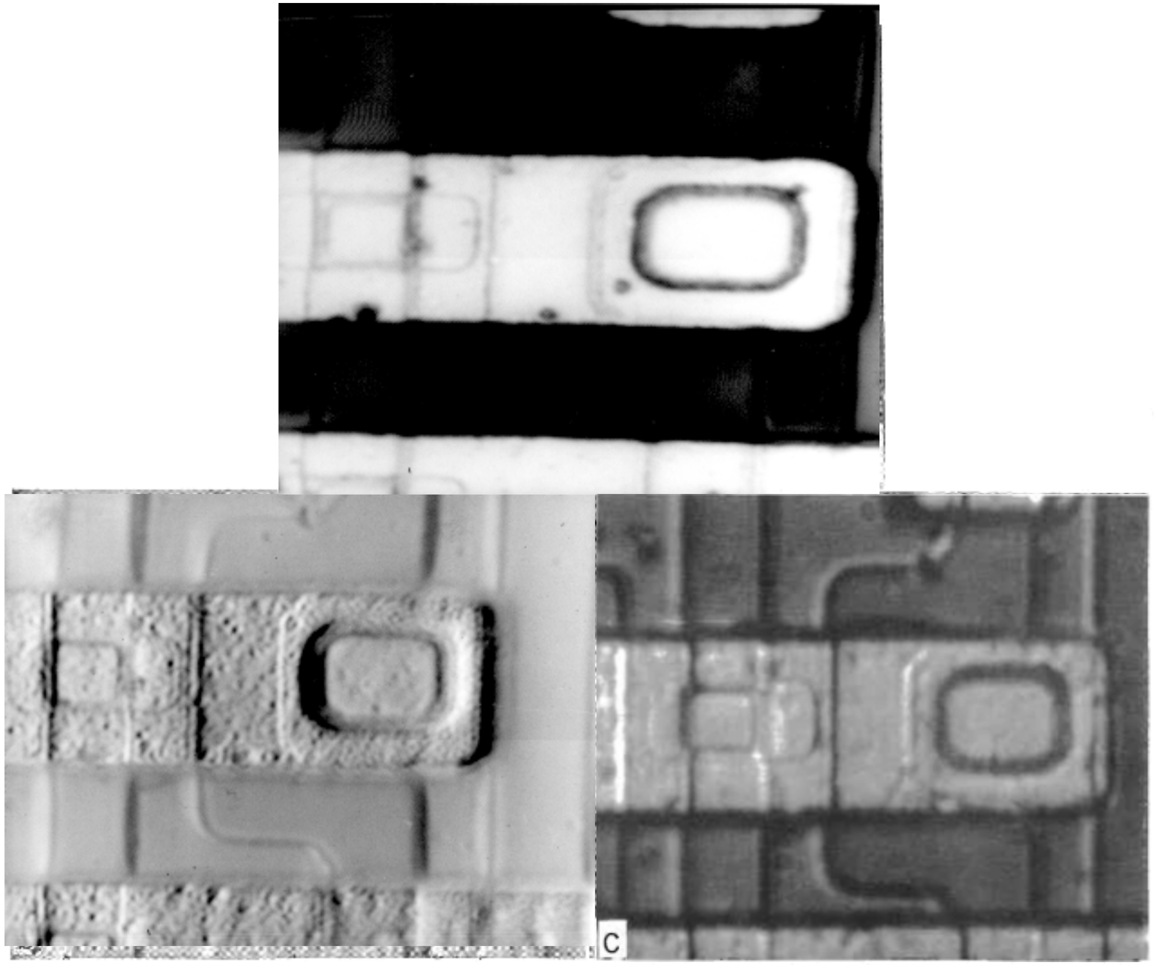
D. K. HAMILTON *and* C. J. R. SHEPPARD,

Differential phase contrast in scanning optical microscopy

Can also do DPC in reflection

Brightfield image of an integrated circuit

DPC



DIC

Fig. 2. An integrated circuit viewed in reflection: (a) amplitude image, (b) differential phase image, (c) a similar region viewed in a Zeiss microscope using Nomarski DIC.

Hamilton DK, Sheppard CJR (1984), *J. Microsc*. **133**, 27-39 (1984)

DPC image of a single monolayer

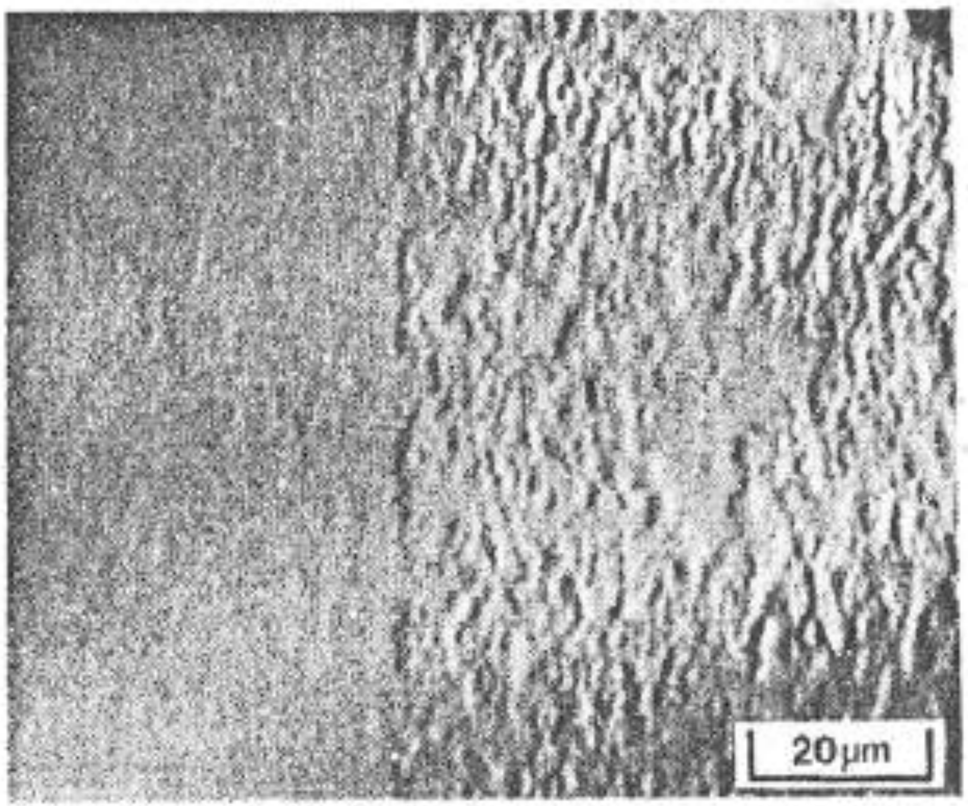


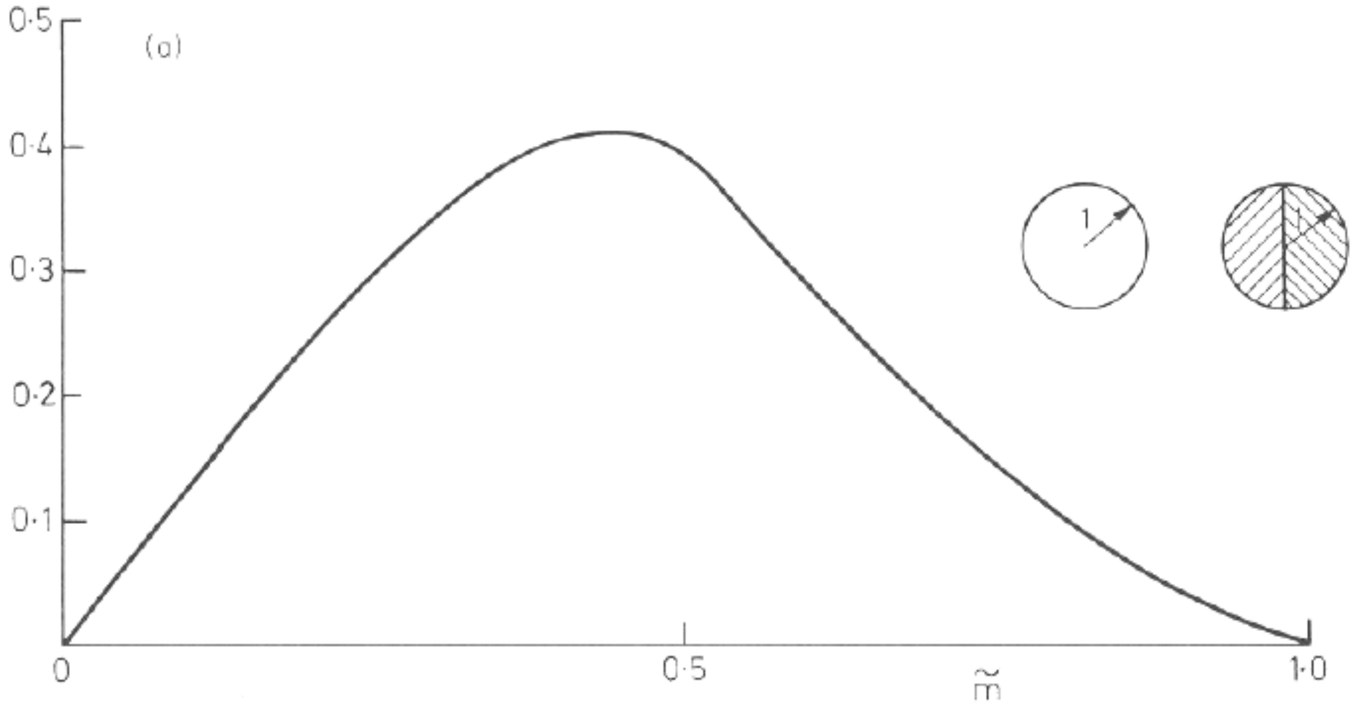
Fig. 2 A differential phase contrast image of a single monolayer.

• Very sensitive to weak phase changes

C.J.R. Sheppard
D.K. Hamilton
H.J. Matthews
Scanning optical microscopy of low-contrast samples
NATURE VOL. 334 18 AUGUST 1988 572

Phase-gradient transfer function $C(m;m)$ (PGTF)

signal

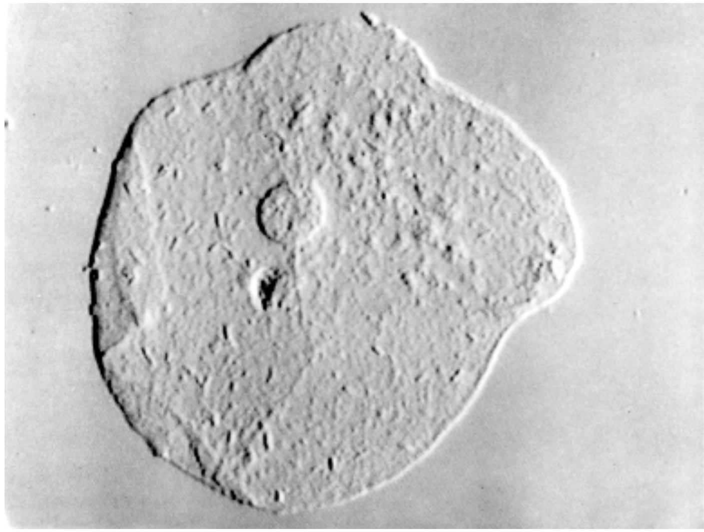


phase gradient (slope)

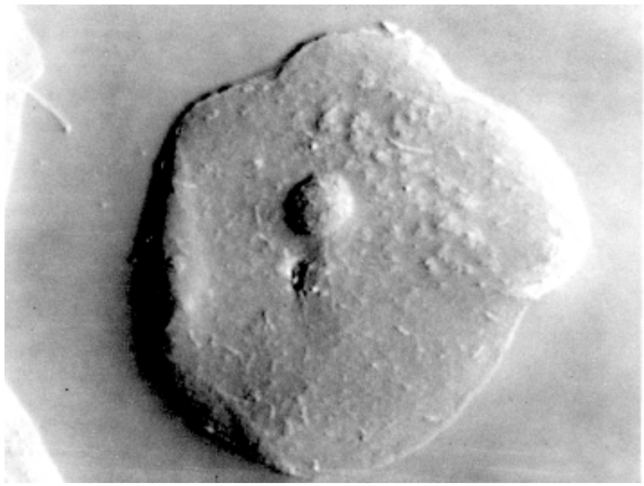
- Anti-symmetrical

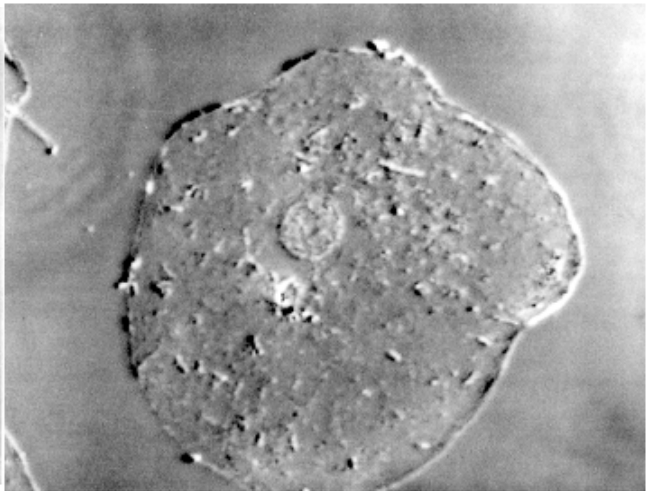
D. K. HAMILTON, C. J. R. SHEPPARD *and* T. WILSON,
Improved imaging of phase gradients in scanning optical microscopy
Journal of Microscopy, Vol. 135, *Pt* 3, *September* 1984, *pp.* 275–286.

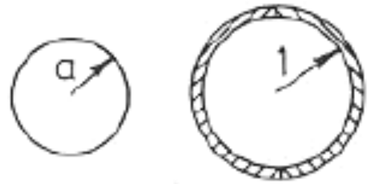
DPC with an annular split detector



Hamilton DK, Sheppard CJR, Wilson T,
J. Microscopy **153**, 275-286 (1984)







- Can adjust contrast/ resolution

$a = 1$
- slow changes in slope

$a = 0.7$
- high spatial frequency response

PGTF for DPC

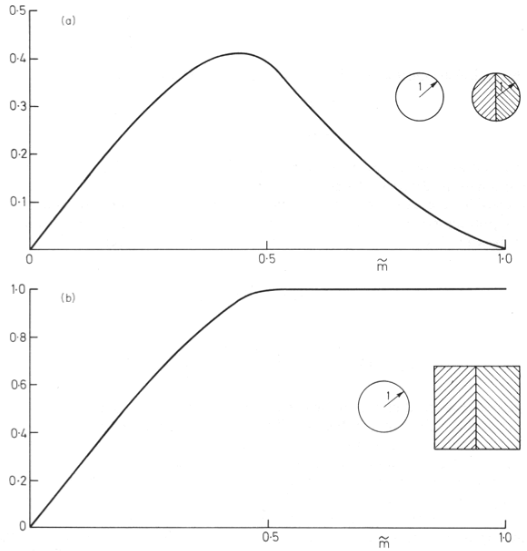


Fig. 2. $C(m; 0)$ and $C(m; m)$ for the transmission scanning optical microscope with an unobscured split detector. (a) Circular detector equal in size to the pupil; (b) square detector larger than the pupil.

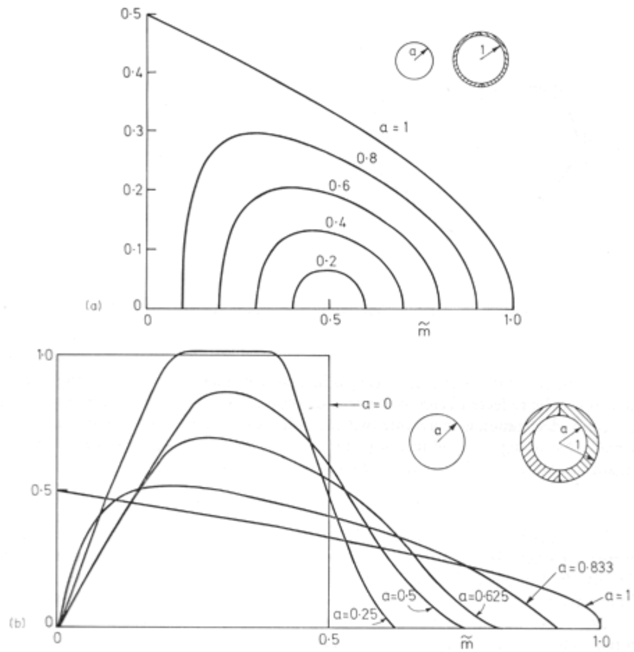


Fig. 4. (a) $C(m; m)$ for the annular detector for various ratios a of pupil radius to annulus radius. When $a=1$ $C(m;0) = C(m;m)$; otherwise $C(m;0)$ is zero. (b) $C(m;m)$ for the circular split detector with a central obscured region equal in radius to the pupil, for various ratios of a pupil racius to detector radius. In this case $C(m;0)$ is always zero.

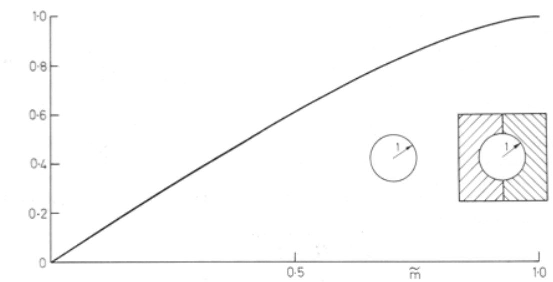


Fig. 5. $C(m; m)$ for a large split detector with a central obscured region equal in radius to the pupil. $C(m; 0) = 0$.

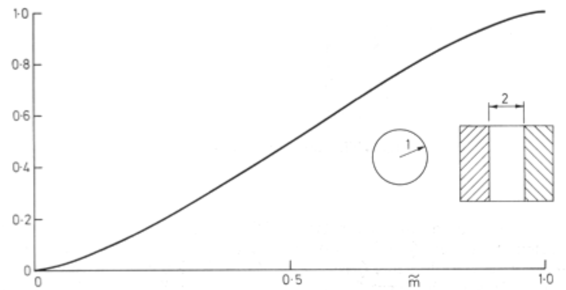
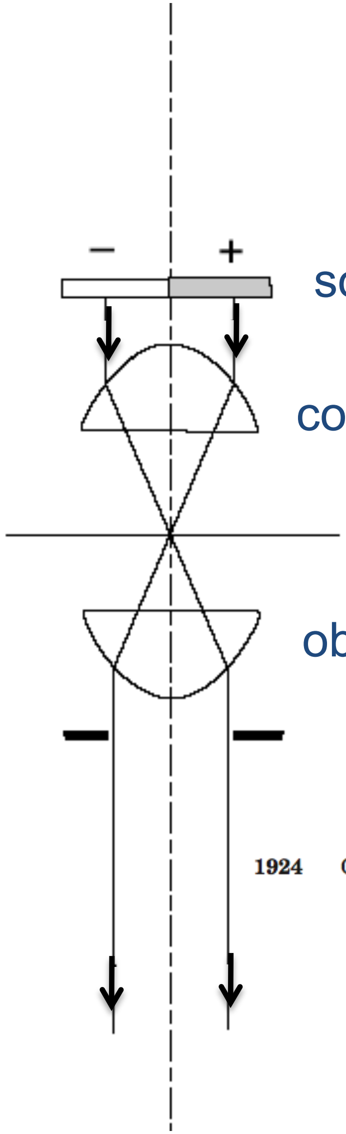


Fig. 7. $C(m; m)$ for a split detector with a central obscured strip.

Often advantageous to have linear behaviour

Hamilton DK, Sheppard CJR, Wilson T, *Journal of Microscopy* **153**, 275-286 (1984)

Asymmetric Illumination DPC (AI-DPC)



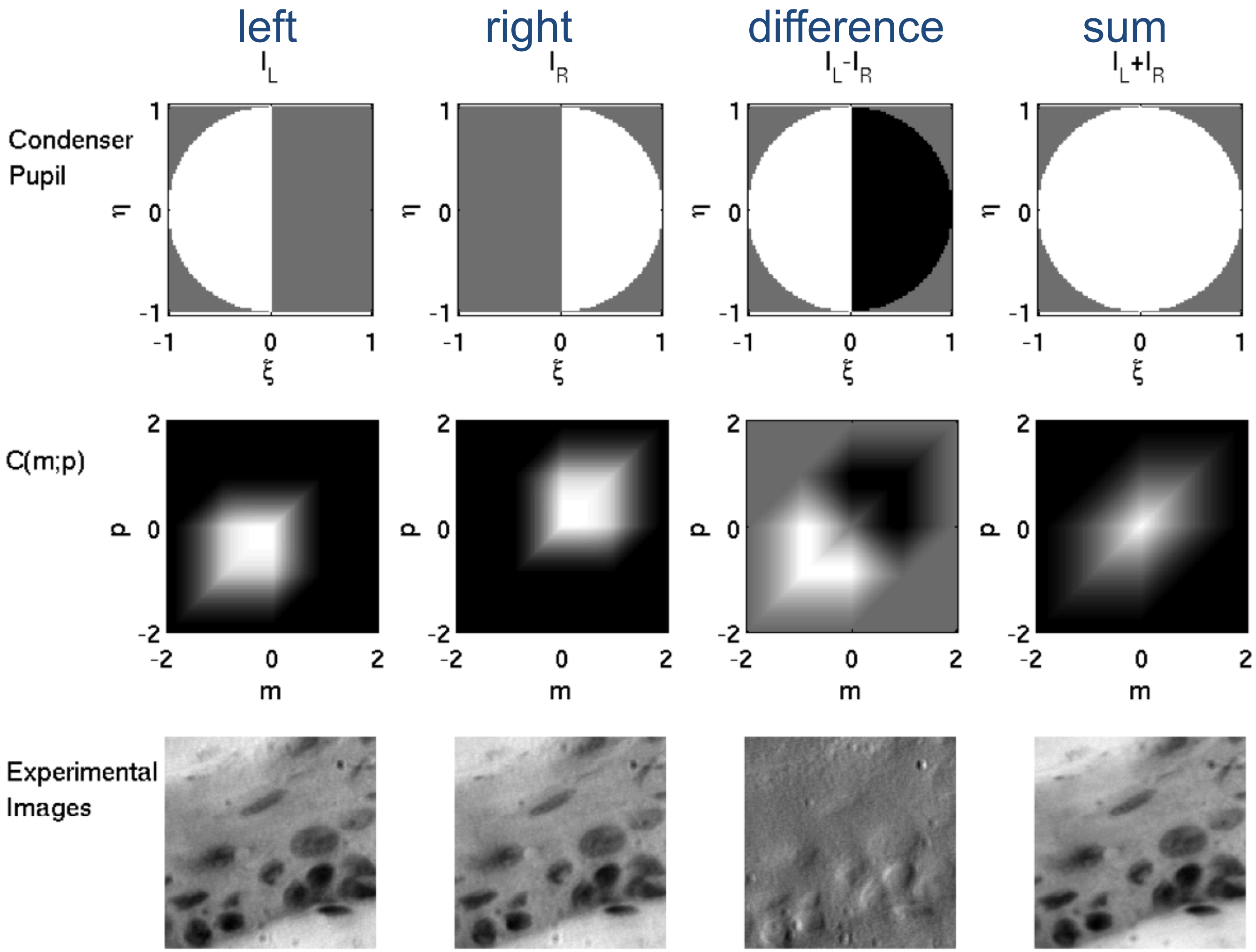
- Can also do in a conventional microscope
- Arrows reversed, source from each semicircle
- Need to take two images
- Or 4 to get $\partial\phi/\partial x$, $\partial\phi/\partial y$

1924 OPTICS LETTERS / Vol. 34, No. 13 / July 1, 2009

Quantitative phase-gradient imaging at high resolution with asymmetric illumination-based differential phase contrast

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

Asymmetric illumination DPC (AI-DPC)



Condenser pupil structures (top row), partially coherent transfer function in direction of differentiation (middle row), and experimental images (bottom row) obtained with AIDPC.
The sample is skin H&E stained section courtesy Graham Wright, TLL and Declan Lunny, IMB.

Phase measurement using DPC

- Integrate phase gradient to get phase (but still constant of integration)

$$\phi = \int \frac{\partial \phi}{\partial x} dx + const.$$

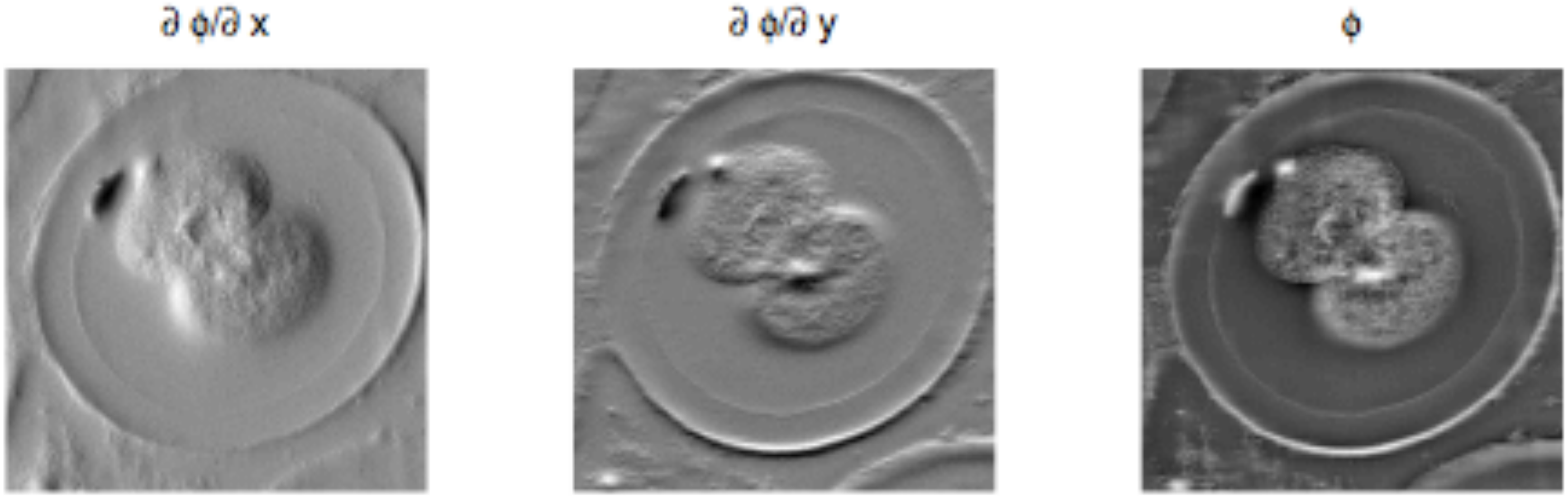
Measure $\partial\phi / \partial x, \partial\phi / \partial y$

$$\phi(x,y) = F^{-1}\left[\frac{F\left[\dfrac{\partial\phi}{\partial x} + i\dfrac{\partial\phi}{\partial y}\right]}{2i\left(\sin 2\pi m\Delta + i\sin 2\pi n\Delta\right)}\right]$$

Arnison, Larkin, Sheppard, Smith, Cogswell, *J. Microsc.* **214**, 7-12 (2004)

Similar to Frankot-Chellappa algorithm
IEEE Trans. Pattern Analysis **10**, 439 (1988)
(Shape from shading)

Phase reconstruction from AI-DPC



S Mehta, Thesis (2010)

History of DPC

First done in electron microscopy

N. H. Dekkers and H. de Lang, "Differential phase contrast in a STEM," Optik **41**, 452-456 (1974).
N. H. Dekkers and H. De Lang, "A detection method for producing phase and amplitude images simultaneously in a STEM," Philips Tech. Review **37**, 1 (1977)

On differential phase contrast with an extended illumination source

W. C. Stewart

813 J. Opt. Soc. Am., Vol. 66, No. 8, August 1976

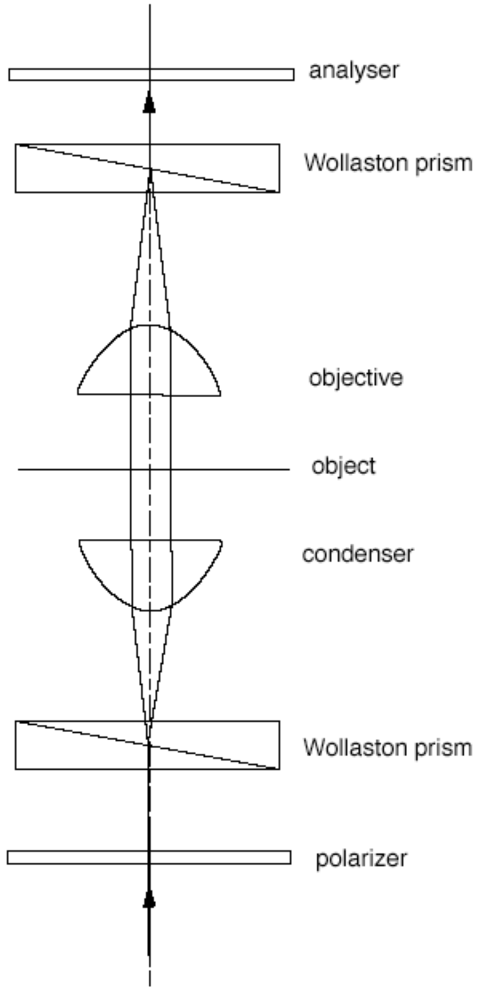
United States Patent [19] [11] **4,255,014**
Ellis [45] **Mar. 10, 1981**

Hamilton DK, Sheppard CJR (1984), *J. Microsc*. **133**, 27-39 (1984)

Nomarski Differential interference contrast (DIC)

ISTITUTO ITALIANO DI TECNOLOGIA

- Phase difference (bias) between two images altered using compensator:
- Translate Wollaston prism
- Rotate polarizing elements



G. Nomarski, "Microinterferometrie differential a ondes polarisés," *J. Phys. Radium* **16**, 9-135 (1955)

Encyclopedia of Modern Optics, RD Guenther, DG Steel, L Bayvel, eds, Elsevier, Oxford, **3**, pp. 103-110

Phase Contrast Microscopy

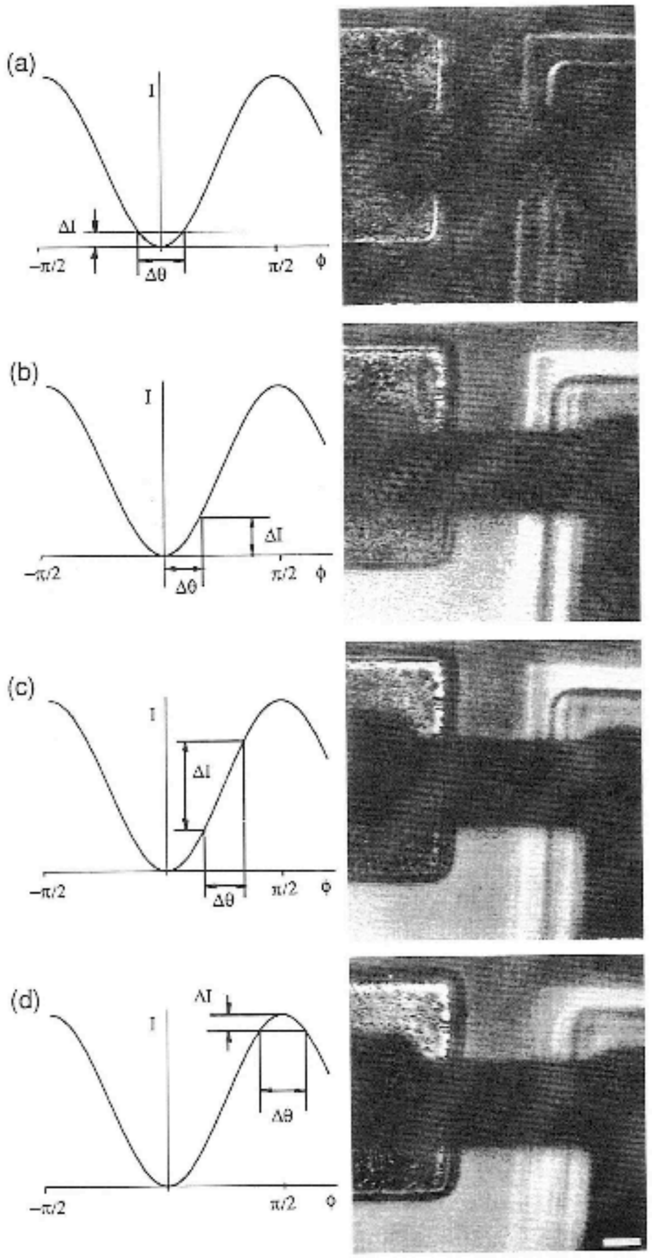
C J R Sheppard, National University of Singapore, Singapore

Nomarski DIC

- Bias changed using shift of Wollaston prism or rotation of polarization

  (Sénarmont compensator)
- Uses polarization, so depends on birefringence of sample
- Can use in conventional or confocal mode

Effect of bias



no bias (dark field)

small bias: used for visual observation

45^o bias: used for CCD detection

90^o bias
(bright field)

Journal of Microscopy, Vol. 165, Pt 1, January 1992, pp. 81–101.
Received 7 December 1990; revised and accepted 6 March 1991

Confocal differential interference contrast (DIC) microscopy: including a theoretical analysis of conventional and confocal DIC imaging

by C. J. Cogswell and C. J. R. Sheppard, Physical Optics Department, School of Physics, University of Sydney, N.S.W. 2006, Australia

Separation of absorption and thickness information

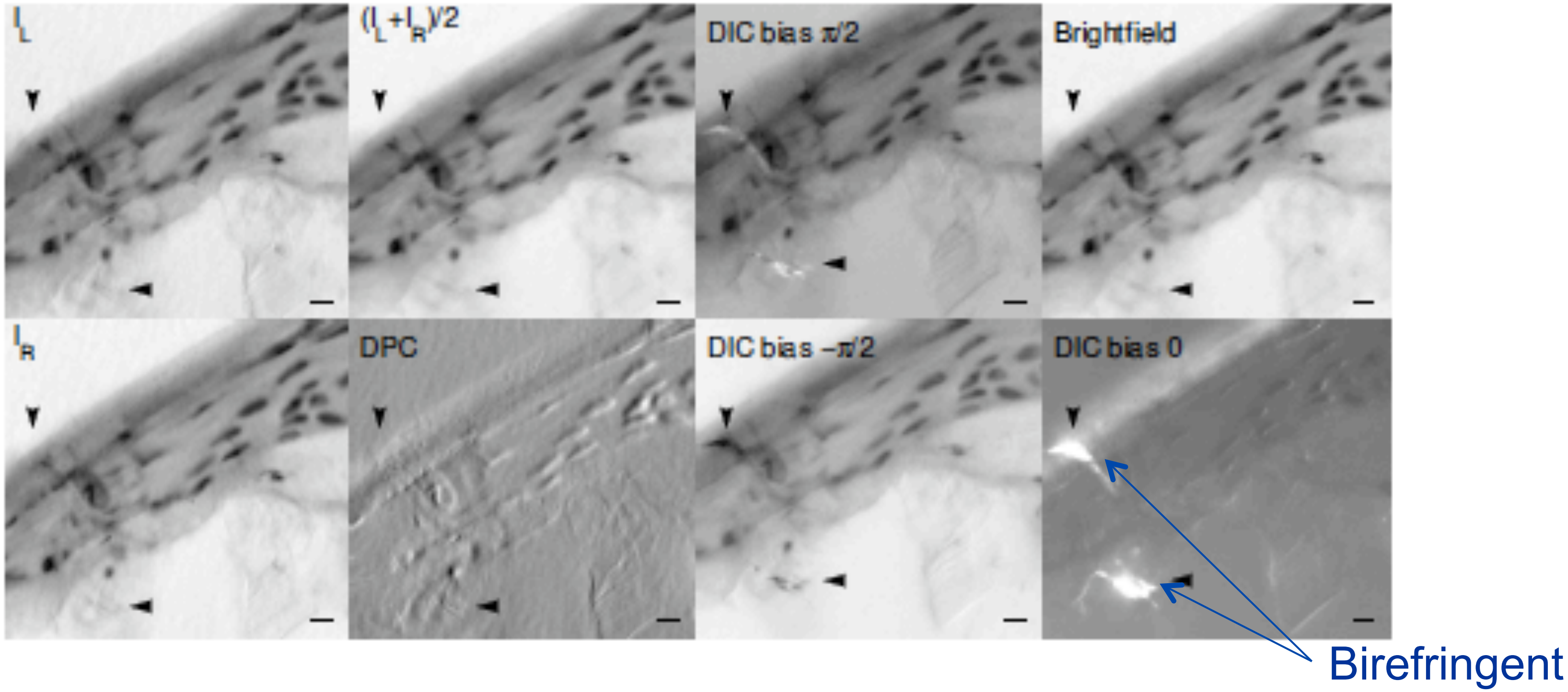


Figure: H&E preparation of $7\mu m$ thick section of human skin. The average and DPC images provide clear separation of absorption and phase information. Birefringent regions may be mistaken as absorbing features in DIC as visible at bias $-\pi/2$. Such regions are indicated by arrow and appear bright under DIC with zero bias. Scale bar is $5\mu m$.
Sample courtesy: Mr. Declan Lunny, Institute of Medical Biology & Dr. Graham Wright, Temasek Life Sciences Laboratory.

Transfer function for DIC

2Δ is the shear

$2\phi_0$ is the bias compensation

$$C_{\text{eff}}(m,n;p,q) = 4C(m,n;p,q)\sin(2\pi m\Delta - \phi)\sin(2\pi p\Delta - \phi).$$

$$C_{\text{eff}}(m,n;p,q) = 2C(m,n;p,q)\{\cos[2\pi(m-p)\Delta] - \cos 2\phi \cos[2\pi(m+p)\Delta] - \sin 2\phi \sin[2\pi(m+p)\Delta]\}.$$

Weak object transfer function $p = 0$ (WOTF)

Odd part: DPC term

$$C_{\text{eff}}(m;0) = 2C(m;0)\,[\cos(2\pi m\Delta)(1-\cos 2\phi_0) - \sin(2\pi m\Delta)\sin 2\phi_0].$$

Strength depends on ϕ_0

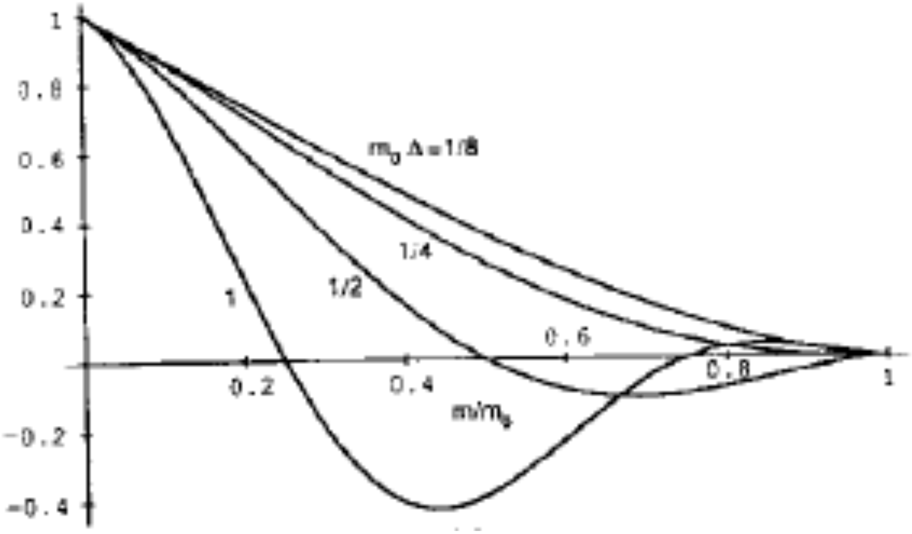
Phase-gradient transfer function $p = m$ (PGTF)

$$C_{\text{eff}}(m;m) = C(m;m)\sin^2(2\pi m\Delta - \phi_0)$$

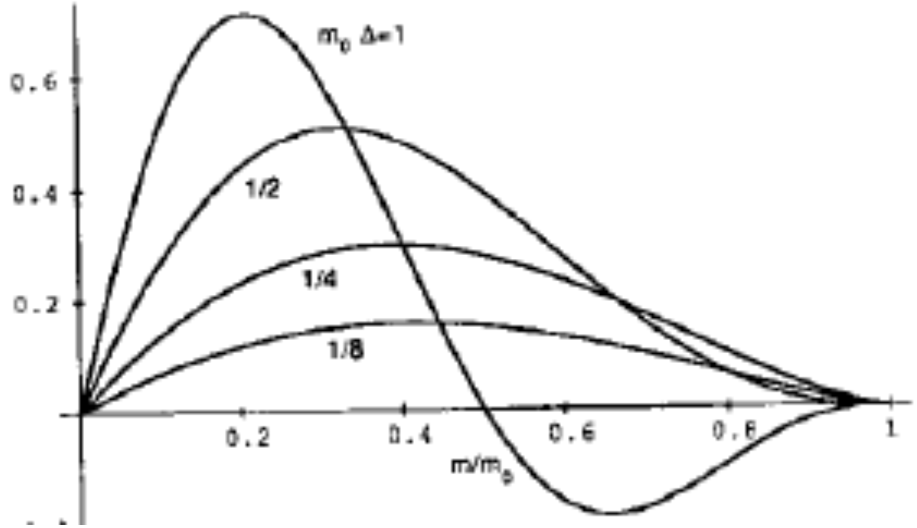
C. J. Cogswell *and* C. J. R. Sheppard, Confocal differential interference contrast (DIC) microscopy: including a theoretical analysis of conventional and confocal DIC imaging

Journal of Microscopy, *Vol.* 165, *Pt* 1, *January* 1992, *pp.* 81–101.

Weak object transfer function for DIC



$m_0\Delta$ too big



Even part (amplitude contrast)

Odd part (DPC)

m_0 is spatial frequency cut-off

C. J. COGSWELL *and* C. J. R. SHEPPARD

Confocal differential interference contrast (DIC) microscopy: including a theoretical analysis of conventional and confocal DIC imaging

Journal of Microscopy, Vol. 165, Pt 1, January 1992, pp. 81–101.

Phase-gradient transfer function for DIC

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- Not anti-symmetrical

•(Small bias)

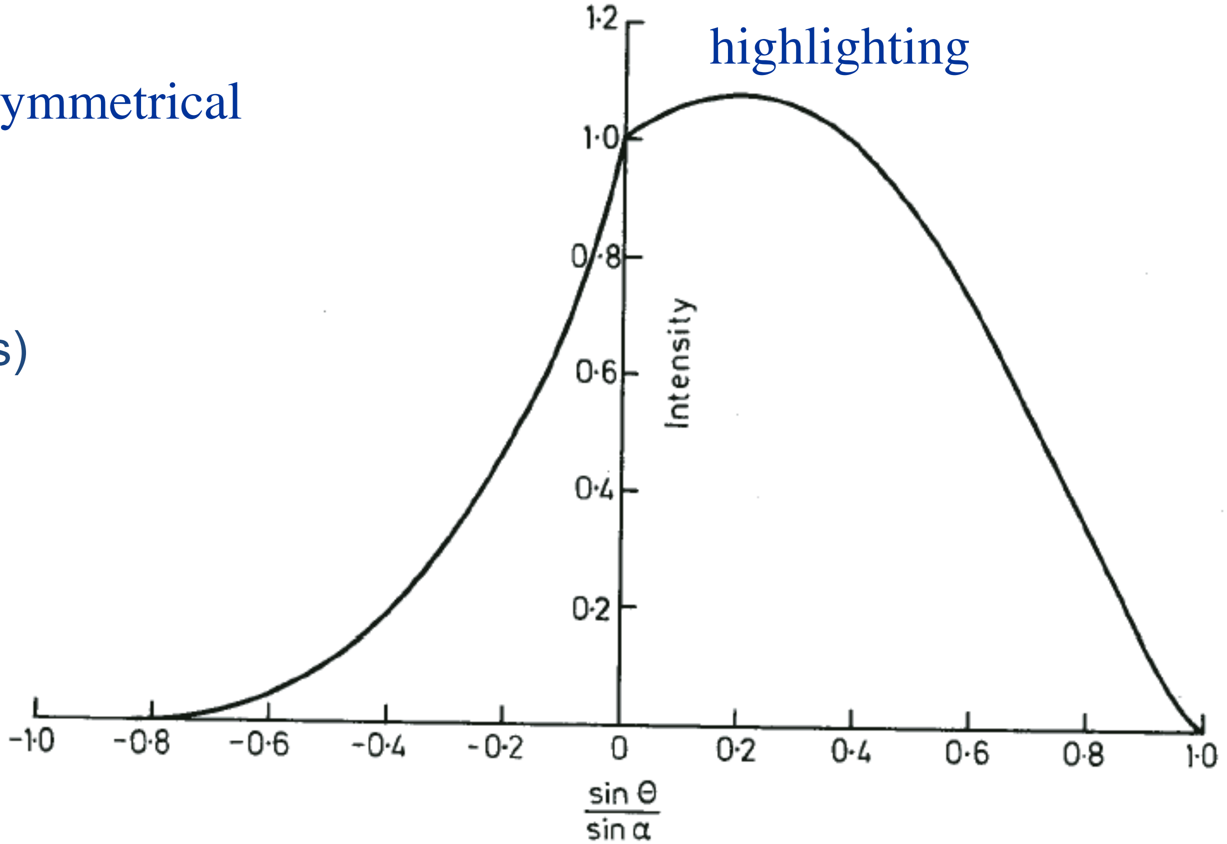


Fig. 12. The signal intensity for a surface at an angle θ, Nomarski DIC with compensator set to just give no zeros in the pass band.

D. K. HAMILTON *and* C. J. R. SHEPPARD
Differential phase contrast in scanning optical microscopy
Journal of Microscopy, Vol. 133, *Pt* 1, *January* 1984, *pp.* 27–39

PGTF for DIC

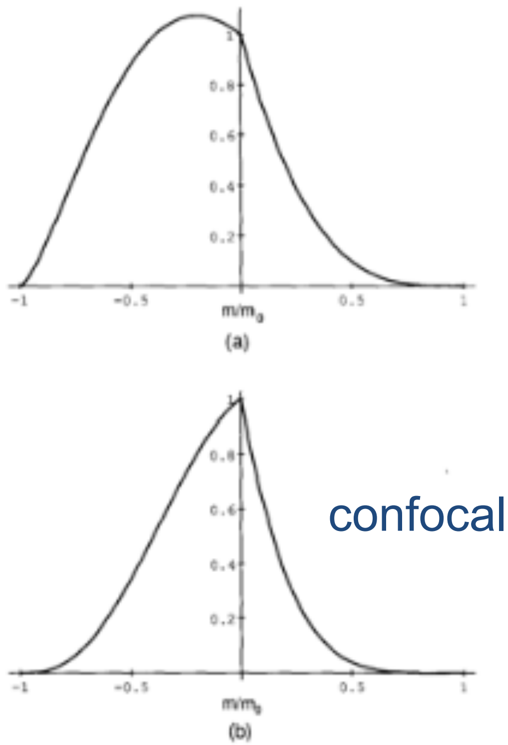


Fig. 5. The phase gradient transfer function for a DIC system with equal condenser and objective apertures, which gives the signal strength as a function of phase gradient $\Psi' = 2\pi m$ for conditions $\phi = 2\pi m_0\Delta$ and ϕ small: (a) conventional and (b) confocal.

small bias

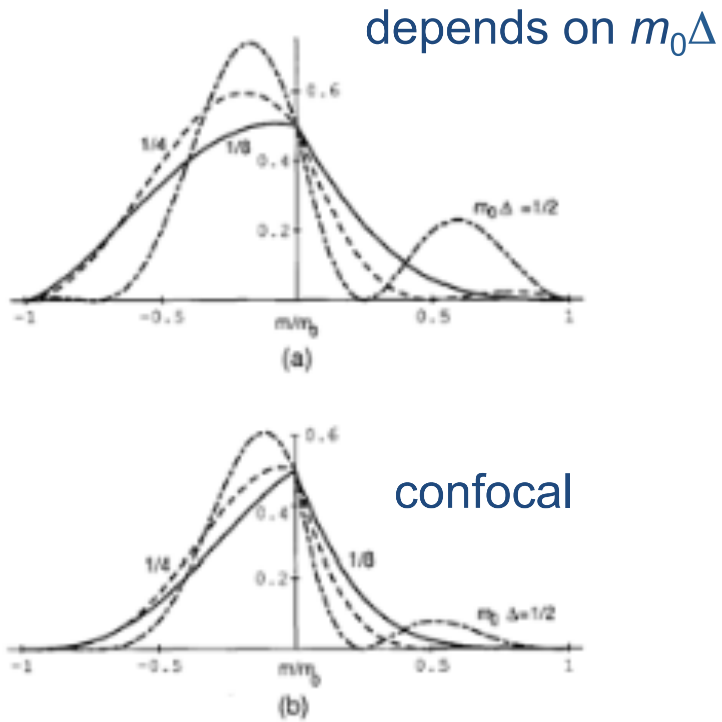


Fig. 6. The phase gradient transfer function for (a) conventional and (b) confocal DIC systems with $\phi = \pi/4$ and values of $m_0\Delta = 1/2$, 1/4 and 1/8.

bias = π/4

- Non-linear behaviour

C. J. Cogswell *and* C. J. R. Sheppard,
Confocal differential interference contrast (DIC) microscopy: including a theoretical analysis of conventional and confocal DIC imaging
Journal of Microscopy, Vol. 165, *Pt* 1, *January* 1992, *pp.* 81–101.

Phase-stepping DIC

- Slowly-varying phase gradient $\partial\phi / \partial x = 2\pi m(x,y)$

- Constant phase gradient deflects light through an angle (prism effect)

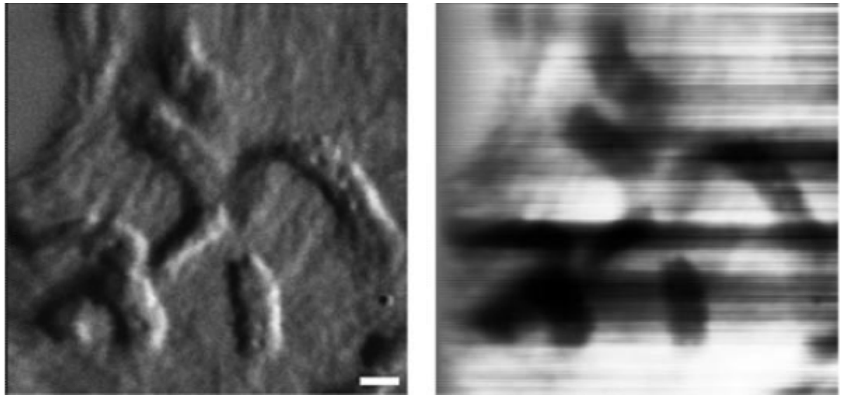
$$I = A + B\cos[2\pi m(x,y)\Delta - \phi_0]$$

ϕ_0 is bias retardation

- Same form as normal interference pattern
  Measure I for different values of bias retardation ϕ_0

- Using phase-stepping algorithm, can recover phase gradient $2\pi m(x,y)$
  Integrate phase gradient to get phase (but still constant of integration)

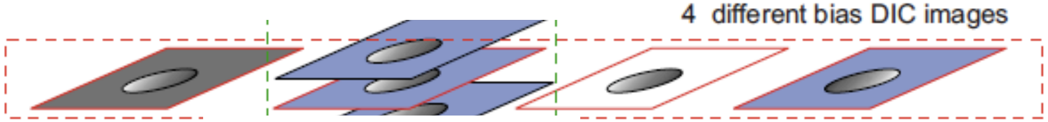
$$\phi = \int \frac{\partial\phi}{\partial x} dx + const.$$

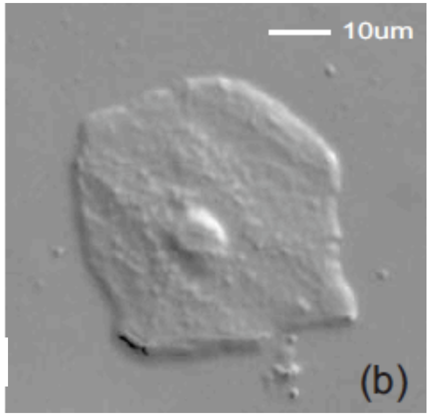


Streaking artifact

M. R. ARNISON*, C. J. COGSWELL*†, N. I. SMITH*, P. W. FEKETE* & K. G. LARKIN*
Journal of Microscopy, Vol. 199, Pt 1, July 2000, pp. 79–84.

Phase-stepping DIC

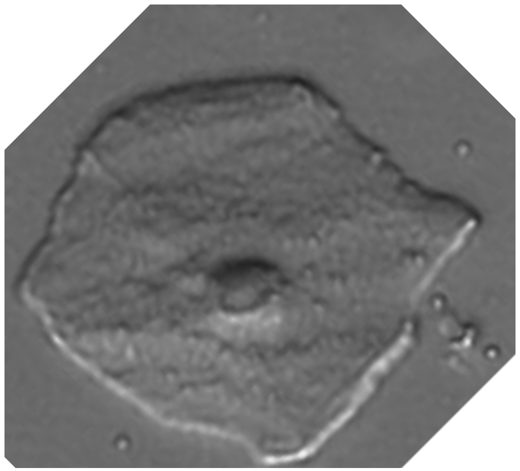


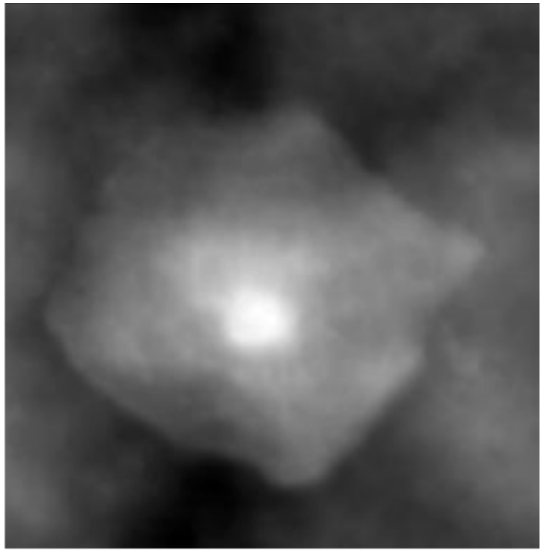


Phase gradient reconstructed from phase-shifting DIC

Shan Shan Kou,[1,2,*] Laura Waller,[3] George Barbastathis,[4,5] and Colin J. R. Sheppard[1,2,6]

February 1, 2010 / Vol. 35, No. 3 / OPTICS LETTERS 447





Quantitative Phase Restoration in Differential Interference Contrast (DIC) Microscopy

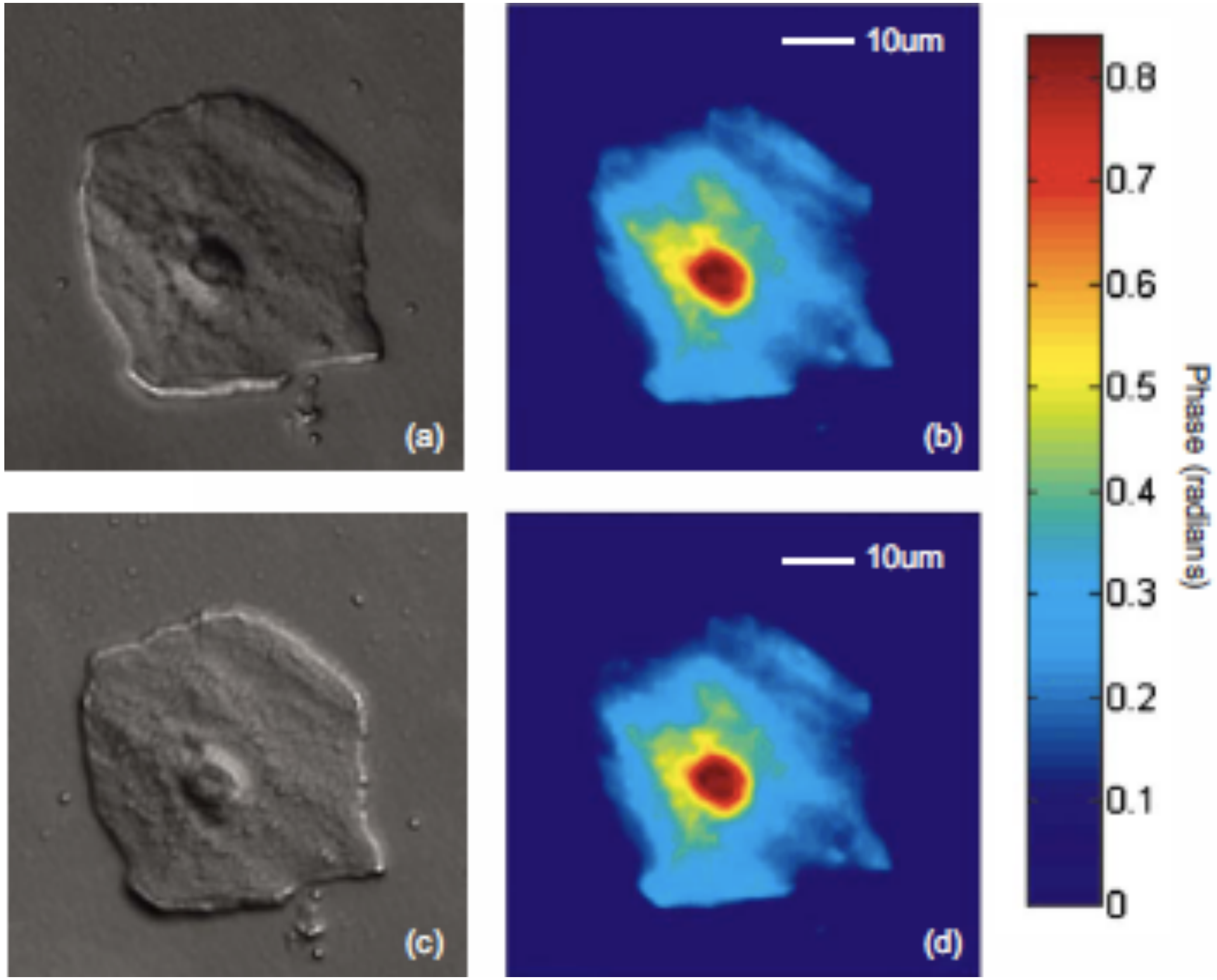
Shan Shan Kou[ab], Colin J. R. Sheppard*[ab]

Optical and Digital Image Processing, edited by Peter Schelkens, Touradj Ebrahimi, Gabriel Cristóbal, Frédéric Truchetet, Proc. of SPIE Vol. 7000, 700005, (2008) · 0277-786X/08/$18 · doi: 10.1117/12.780912

Can use TIE on DIC (TI-DIC)

Bias π/4

Bias 3π/4



DIC

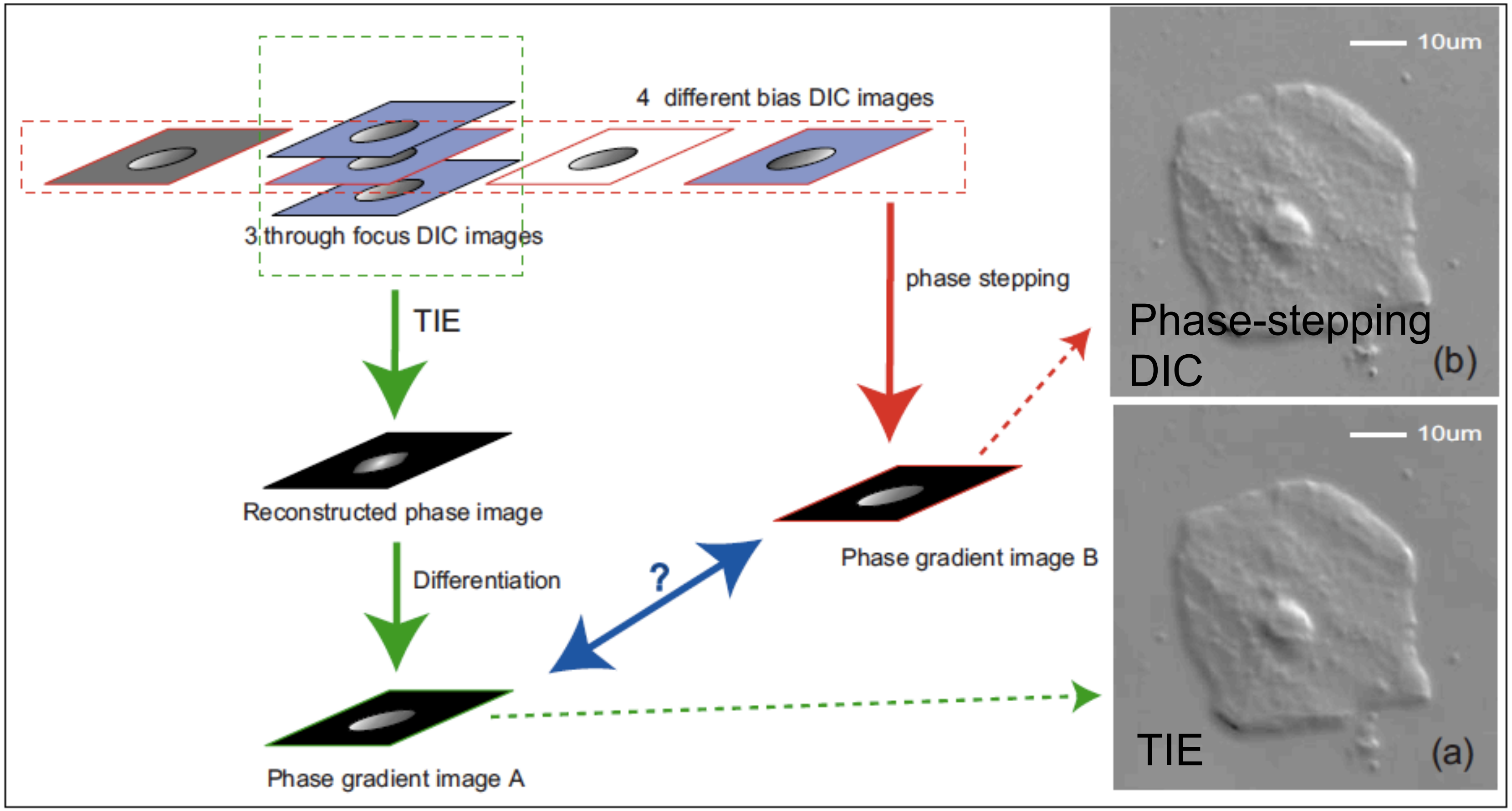
Phase reconstructed from DIC by TIE

Shan Shan Kou,[1,2,*] Laura Waller,[3] George Barbastathis,[4,5] and Colin J. R. Sheppard[1,2,6]

February 1, 2010 / Vol. 35, No. 3 / OPTICS LETTERS 447

PS-DIC and TI-DIC

Phase gradient reconstructed from phase-shifting DIC



Phase gradient reconstructed from DIC by TIE

Shan Shan Kou,[1,2,*] Laura Waller,[3] George Barbastathis,[4,5] and Colin J. R. Sheppard[1,2,6]

February 1, 2010 / Vol. 35, No. 3 / OPTICS LETTERS 447

Quantitative phase from TIE-DIC

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DI TECNOLOGIA

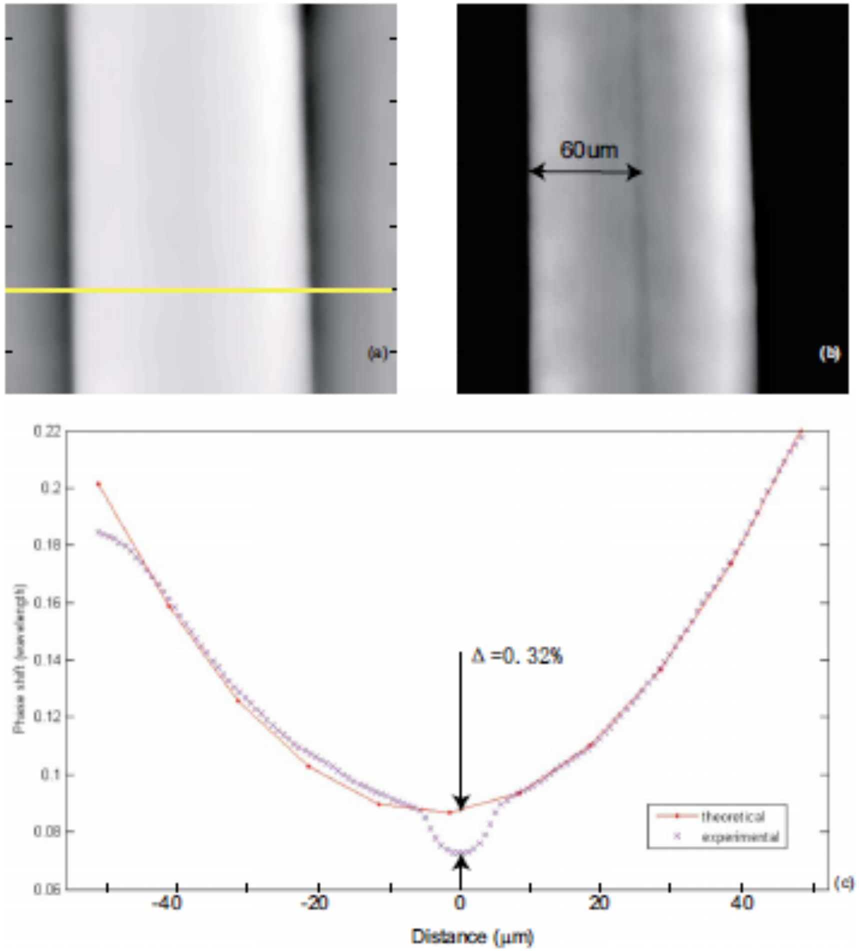

Fig. 3. (Color online) TI-DIC fiber reconstruction. (a) Reconstructed phase profile of the fiber. (b) Same profile after histogram adjustment. (c) Fitting of the fiber profile to theoretical data.

Shan Shan Kou,[1,2,*] Laura Waller,[3] George Barbastathis,[4,5] and Colin J. R. Sheppard[1,2,6]

February 1, 2010 / Vol. 35, No. 3 / OPTICS LETTERS 447

Summary

ISTITUTO ITALIANO DI TECNOLOGIA

- Zernike phase contrast
  - only for weak object
  - not quantitative
  - haloes
- Nomarski DIC
  - good 3D imaging
  - phase stepping for quantitative measurements
  - birefringence a problem (plastic slides)
- DPC
  - not good for 3D imaging
- Defocus
  - only for weak object
- TIE
  - not good for 3D imaging