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Three dimensional imaging by partially coherent light under non-paraxial condition

Yongjin Sung¹ and Colin J. R. Sheppard^{2,*}

¹G. R. Harrison Spectroscopy Laboratory, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA

²Division of Bioengineering and Department of Biological Sciences, National University of Singapore, Singapore 117576

Abstract

In this paper, we present the theory of 3-D imaging using partially coherent light under the non-paraxial condition. Using the linear system approach, we derive the image intensity in terms of the 3-D non-paraxial transmission cross-coefficient (TCC) and the transmission function defined in this paper. We present that the 3-D TCC can be calculated by multiple applications of the 3-D FFT instead of the 6-D integral in the original formula. Using the simplified formula, we simulate phase contrast and Nomarski-DIC (differential interference contrast) imaging of a transparent 3-D object. Within our knowledge, the 3-D model for the DIC based on the 3-D non-paraxial TCC is the most rigorous approach that has been suggested. It demonstrates clearly the optical sectioning effect of DIC.

1. INTRODUCTION

Three-dimensional (3-D) imaging techniques are developing fast in microscopy because they can provide an incomparable amount of information compared to that in the conventional two-dimensional modality. For example, tomographic phase microscopy provides the 3-D refractive index map of a live biological sample with resolution twice that of the coherent diffraction limit [1,2]. However, it requires a complicated set-up and reconstruction process, and suffers from speckle noise. Partially coherent light can be useful in the latter respect, but the reconstruction process is much more complicated: better understanding of the 3-D imaging theory will be necessary.

The 3-D transfer function which characterizes an imaging system has been developed within the first Born approximation by several researchers. McCutchen [3] showed that the coherent transfer function for a 3-D object is a projection of an objective pupil onto the Ewald sphere. For incoherent imaging, Frieden [4] derived the 3-D optical transfer function under the paraxial approximation, and Sheppard [5] derived an analytic formula for the non-paraxial case. For partially coherent imaging, Streibl [6–8] described 3-D image formation in terms of the mutual intensity transfer, and Sheppard [9] derived a more general formula in terms of the transmission cross-coefficient (TCC) or the partially coherent transfer function. However, both approaches used the paraxial approximation, which is more suitable for a low numerical aperture system. In this paper, we present the theory of 3-D imaging using partially coherent light under non-paraxial, but scalar and Debye, approximation.

2. NON-PARAXIAL MODEL OF 3-D PARTIALLY COHERENT IMAGING

We start from the optical transfer of the Green's function for the Helmholtz operator. Consider a point source in the 4-f imaging system as shown in Fig. 1.

Spherical waves emitted from the point source are described by the Green's function, $G_0(\vec{x})$ for the Helmholtz operator [10].

$$(\nabla^2 + k_0^2)G_0(\vec{x}) = -\delta(\vec{x}), \quad (1)$$

in which $k_0 = 2\pi/\lambda$, λ is the wavelength of the incident beam in the medium, and $\vec{x} = (X, Y, Z)$ is the spatial coordinate.

From the symmetry of the imaging system, the field right after the second objective lens is the converging wavefront cut off by the finite numerical aperture. Under the Debye approximation, the field distribution in the image space formed by the finite converging wavefront can be expressed as [3]:

$$U(\vec{r}) = \frac{1}{i4\pi\lambda} \iint_{\Omega} A(\vec{q}) e^{-i2\pi\vec{q}\cdot\vec{r}} d\Omega, \quad (2)$$

in which $\vec{r}$ is a spatial coordinate vector normalized by λ , dS is the incremental area on the converging wavefront, $d\Omega$ is the incremental solid angle which dS subtends at the origin of the image space, $\vec{q}$ is a unit vector in the direction from the origin to dS . Note that the Debye approximation is not really an approximation if the lens pupils are placed in the back focal planes of the lenses, as is usual in practice. McCutchen [3] found that the Fourier transform of Eq. (2) can be connected to the pupil function of the objective lens, which can be written in the current notation as

$$\tilde{U}(\vec{p}) = \frac{1}{i4\pi\lambda} P_{obj}(u, v) \delta(|\vec{p}| - 1), \quad (3)$$

where $\vec{p} = (u, v, w)$ is a frequency vector corresponding to the normalized spatial coordinate $\vec{r} = (x, y, z)$, $P_{obj}(u, v)$ is the 2-D objective pupil function, $\delta(|\vec{p}| - 1)$ represents the projection of the pupil function onto the Ewald sphere.

Now we consider the scattering of transmitted light by a 3-D sample, and its transfer through the imaging system. Under the first Born approximation, the scattered field in the image space can be written

$$u_{scat}(\vec{x}) = - \int u_{in}(\vec{x}') f(\vec{x}') U(\vec{x} - \vec{x}') d^3x', \quad (4)$$

in which $u_{in}(\vec{x})$ is the incident field, $f(\vec{x})$ is the scattering potential defined as [11]

$$f(\vec{x}) = k_0^2 (n_0^2 - n(\vec{x})^2), \quad (5)$$

where $n(\vec{x})$ is the complex refractive index, and n_0 is the refractive index of the medium. (I.e. not of free space. The refractive index changes are smaller with respect to the medium.)

Normalizing the spatial coordinates by $\vec{r} = \vec{x}/\lambda$ in Eq. (4), and including the transfer of the incident field, we can write the output field in the image space as follows:

$$u_{out}(\vec{r}) = \int u_{in}(\vec{r}') T(\vec{r}') H(\vec{r} - \vec{r}') d^3 r', \quad (6)$$

in which $T(\vec{r}) = 1 + i\pi(n_0^2 - n(\vec{r})^2)$, $H(\vec{r}) = i4\pi\lambda U(\vec{r})$, $\tilde{H}(\vec{p}) \equiv F\{H(\vec{r})\} = P_{obj}(u, v)\delta(|\vec{p}| - 1)$.

Note that Eq. (6) assumes that 3-D imaging is space invariant, as also assumed by Frieden [4]. But it is known that 3-D imaging cannot be space invariant, as a defocus of the image plane will introduce aberration if the sine condition is satisfied. This problem can be overcome by interpreting Eq. (6) as referring to direct imaging in the transverse coordinates, but to scanning the object axially to bring the relevant plane of the object to the conjugate of the image plane. This is exactly what is done in practice in generating a 3-D image data set.

The partially coherent imaging can be described more easily by the mutual intensity defined as follows:

$$J_{out}(\vec{r}_1; \vec{r}_2) = \langle u_{out}(\vec{r}_1, t) u_{out}^*(\vec{r}_2, t) \rangle. \quad (7)$$

Substituting Eq. (6),

$$J_{out}(\vec{r}_1; \vec{r}_2) = \iint T(\vec{r}'_1) T^*(\vec{r}'_2) J_{in}(\vec{r}'_1; \vec{r}'_2) H(\vec{r}_1 - \vec{r}'_1) H^*(\vec{r}_2 - \vec{r}'_2) d^3 r'_1 d^3 r'_2, \quad (8)$$

where $J_{in}(\vec{r}_1; \vec{r}_2)$ is the mutual intensity of the incident light.

Under the Debye approximation, $J_{in}(\vec{r}_1; \vec{r}_2)$ can be simplified to a function only of $\Delta\vec{r} = \vec{r}_1 - \vec{r}_2$ [6,12].

$$J_{in}(\vec{r}_1; \vec{r}_2) \approx J_{in}(\Delta\vec{r}) = \int \tilde{J}_{in}(\vec{p}) \exp[i2\pi(\vec{p} \cdot \Delta\vec{r})] d^3 p, \quad (9)$$

where $\tilde{J}_{in}(\vec{p}) = |P_{cond}(u, v)|^2 \delta(|\vec{p}| - 1)$, $|\vec{p}| = (u, v, w)$. This is equivalent to assuming a Schell model illumination beam in which the illumination intensity is uniform, which is a good approximation for Köhler microscope illumination.

For convenience, we use the linear system approach that Goodman [13,14] adopted for the partially coherent imaging of a 2-D object. For the 3-D imaging, here we define a 6-D Fourier transform as follows:

$$\tilde{J}_{out}(\vec{p}_1; \vec{p}_2) \equiv \iint J_{out}(\vec{r}_1; \vec{r}_2) \exp[-i2\pi(\vec{p}_1 \cdot \vec{r}_1 + \vec{p}_2 \cdot \vec{r}_2)] d^3 r_1 d^3 r_2. \quad (10)$$

The 6-D Fourier transform of the mutual intensity in the image space can be written as

$$\tilde{J}_{out}(\vec{p}_1; \vec{p}_2) = \tilde{H}(\vec{p}_1) \tilde{H}^*(-\vec{p}_2) \int \tilde{J}_{in}(\vec{p}) \tilde{T}(\vec{p}_1 - \vec{p}) \tilde{T}^*(-\vec{p}_2 - \vec{p}) d^3 p, \quad (11)$$

in which $\tilde{T}(\vec{p}) \equiv \int T(\vec{r}) \exp[-i2\pi(\vec{p} \cdot \vec{r})] d^3 r$, and $\tilde{H}(\vec{p})$, $\tilde{J}_{in}(\vec{p})$ are defined in Eq. (6) and (9), respectively.

The corresponding intensity can be calculated by

$$I_{img}(\vec{r}) = J_{out}(\vec{r}; \vec{r}) = \iint \tilde{J}_{out}(\vec{p}_1; \vec{p}_2) \exp[i2\pi(\vec{p}_1 + \vec{p}_2) \cdot \vec{r}] d^3 p_1 d^3 p_2. \quad (12)$$

To summarize, under the non-paraxial condition the image intensity of a 3-D object by partially coherent light can be written as follows:

$$I_{img}(\vec{r}) = \iint C(\vec{p}_1; \vec{p}_2) \tilde{T}(\vec{p}_1) \tilde{T}^*(\vec{p}_2) \exp[i2\pi(\vec{p}_1 - \vec{p}_2) \cdot \vec{r}] d^3 p_1 d^3 p_2, \quad (13)$$

in which $C(\vec{p}_1; \vec{p}_2) = \iint \tilde{J}_{in}(\vec{p}) \tilde{H}(\vec{p} + \vec{p}_1) \tilde{H}^*(\vec{p} + \vec{p}_2) d^3 p$ is the six-dimensional TCC.

Streibl [6] derived expressions for the optical transfer, in a paraxial system, of a weak object (i.e. neglecting the bilinear terms) in the Born approximation. In Eqs. (26), (27) he described what he called the absorption and phase optical transfer functions. However, in our view this terminology is misleading, as these equations describe the spatial frequency content of the image, rather than the spatial frequencies of the object that are imaged, and therefore should not be considered as transfer functions. Thus his Figs. 3(a) and (b), for the coherent case, each show two Ewald spheres, implying that positive and negative axial spatial frequencies are imaged, whereas we know from the usual Ewald sphere construction that negative axial spatial frequencies only are imaged.

3. SIMPLE FORMULA FOR 3-D PARTIALLY COHERENT IMAGING

In the previous section, we derived a non-paraxial model of 3-D partially coherent imaging, which can be summarized to Eq. (13). However, its calculation requires prohibitively large computation time for the 6-D integral of the TCC. In this section, we present a simple formula that can be calculated only by multiple applications of the 3-D fast Fourier transform (FFT).

As qualitatively described by Sheppard et al. [9], the 3-D non-paraxial TCC, $C(\vec{p}_1; \vec{p}_2)$ is the overlap integral of three hemi-spherical shells. Three shells can intersect in a surface, in a line, or at a point depending on the relative amount of shift, $(\vec{p}_1, \vec{p}_2)$. For example, for $\vec{p}_1 = \vec{p}_2 = (0,0,0)$, they overlap and collapse into one hemi-spherical shell centered at the origin. Then, the overlap integral is the area of the surface weighted by the modulated pupil functions. In another case, three shells can make a line intersection when two of them overlap while the third shifts with respect to the other two. In terms of the relative shift, the following combinations are possible: (i) $\vec{p}_1 = (0,0,0)$, $\vec{p}_2 = (0,0,0)$ (ii) $\vec{p}_1 = (0,0,0)$, $\vec{p}_2 = (0,0,0)$ (iii) $\vec{p}_1 = \vec{p}_2 = (0,0,0)$. The overlap integral in this case is the length of the intersecting line. Finally, three shells can intersect at a point or points. The combination of the relative shift to make the point intersection is more tricky, but, the overlap integral, which is the incremental volume of an intersecting point, is much smaller than the first two cases, line or surface contact. We discuss more about this at the end of this section. It is worthwhile to note that when $\vec{p}_1 = \vec{p}_2$, the integrand of Eq. (13) does not depend on the spatial coordinate, and its contribution is limited to the enhancement or degradation of the contrast. Hence, we merge these contributions into the background intensity.

To summarize, the 3-D non-paraxial TCC makes significant contribution to the image when $\vec{p}_1 = (0,0,0)$, $\vec{p}_2 = (0,0,0)$ or $\vec{p}_1 = (0,0,0)$, $\vec{p}_2 = (0,0,0)$. For these conditions, $C(\vec{p}_1; \vec{p}_2)$ can be written as follows:

$$C(\vec{p}_1; \vec{0}) = (P_{cond}^{3D} P_{obj}^{3D} * P_{obj}^{3D})(\vec{p}_1) \equiv C_1(\vec{p}_1), \quad (14)$$

$$C(\vec{0}; \vec{p}_2) = C(\vec{p}_2; \vec{0})^* = C_1(\vec{p}_2)^*, \quad (15)$$

where $P_{cond}^{3D}(\vec{p}) = |P_{cond}(u, v)|^2 \delta(|\vec{p}| - 1)$, $P_{obj}^{3D}(\vec{p}) = P_{obj}(u, v) \delta(|\vec{p}| - 1)$, $*$ is the 3-D autocorrelation defined as follows:

$$(f * g)(\vec{p}) \equiv \int f^*(\vec{q}) g(\vec{p} + \vec{q}) d^3 q. \quad (16)$$

In case of bright field imaging using the same numerical aperture for both objective and condenser lenses, Eq. (13) can be reduced to the autocorrelation of two uniform hemispherical shells (neglecting the aplanatic factor). It is same as the optical transfer function for high numerical aperture, which Sheppard et al. [5] has derived. In the current notation,

$$C_1(\vec{p}) = \frac{4}{\pi K} \arccos \left[\frac{1}{M} \left(\frac{2 \cos \alpha}{|w|} + 1 \right) \right], \quad (17)$$

$$\text{in which } \vec{p} = (u, v, w), M = \frac{2(u^2 + v^2)^{1/2}}{K|w|} \left(1 - \frac{K^2}{4} \right)^{1/2}, K = (u^2 + v^2 + w^2)^{1/2}.$$

However, most of the imaging modalities adopt various modulations for either condenser or objective pupil functions, or for both, so numerical computation of Eq. (14) is required. Substituting Eqs. (14–15) into Eq. (13), the overall image intensity can be simplified to

$$I_{img}(\vec{r}) \cong I_0 + 2\text{Re}[\alpha \int C_1(\vec{p}) \tilde{T}(\vec{p}) \exp[i2\pi(\vec{p} \cdot \vec{r})] d^3 p], \quad (18)$$

in which I_0 is the background intensity, $\alpha = \tilde{T}^*(0 \rightarrow) k_{res}^3$, k_{res} is the thickness of the Ewald sphere or the bandwidth of the incident light.

The integrals in the parenthesis are nothing more than inverse Fourier transforms, which can be efficiently calculated using the FFT algorithm [15].

$$I_{img}(\vec{r}) = I_0 + 2\text{Re} \left[\alpha F^{-1} \left\{ C_1(\vec{p}) \tilde{T}(\vec{p}) \right\} \right], \quad (19)$$

$$\text{in which } \tilde{T}(\vec{p}) = F\{1 + i\pi(n_0^2 - n(\vec{r})^2)\}, C_1(\vec{p}) = (P_{cond}^{3D} P_{obj}^{3D} * P_{obj}^{3D})(\vec{p}).$$

It is interesting to notice that the Eq. (19) has a similar form as the weak transfer function Sheppard et al. derived in the paraxial case. However, as shown in the form of the transmission function, Eq. (19) can be applied for a more general object within the first Born approximation.

Going back to the issue about relative strength of the point and the line intersection case, we compare the image intensity calculated by each of the two contributions. We perform a numerical simulation using a modified 3-D Shepp-Logan phantom, whose magnitude is adjusted to the refractive index of usual biological samples (1.35–1.37) [16]. Fig. 2(b) and

(c) show the horizontal and the vertical cross-section of the phantom, whose color represents the refractive index. Remember that we used the first Born approximation, which is valid when the overall phase delay across a sample is less than π . The phantom has a maximum phase delay around 2.5, so the first Born approximation is still applicable, although the Rytov approximation is usually preferred in biological samples [2].

For the calculation of the overlap integral, we consider the intersection of spheres instead of hemi-spheres of particular size, which simplifies the estimation. From geometry, three spheres can intersect in at least one point when the following condition is satisfied:

$$|\vec{p}_1| |\vec{p}_2| |\vec{p}_1 - \vec{p}_2| \leq 2r |\vec{p}_1 \times \vec{p}_2|, \quad (20)$$

in which $\vec{p}_1, \vec{p}_2$ are vectors from the center of the first sphere to the centers of the other two, r is the radius of three spheres. The equality sign in Eq. (20) corresponds to intersection of the three spheres in a single point, whereas the $<$ sign corresponds to intersection in two points. In the case when the spatial coordinates are normalized by λ , the radius r becomes 1.

Neglecting the varying shapes of the intersecting volumes, Eq. (13) for the point intersection case may be approximated by

$$I_{img}(\vec{r}) \approx k_{res}^3 \sum_{\vec{p}_1} \tilde{T}(\vec{p}_1) \left\{ \int C_{point}(\vec{p}_1; \vec{p}_1 + \vec{p}) \tilde{T}^*(\vec{p}_1 + \vec{p}) \exp[i2\pi(\vec{p} \cdot \vec{r})] d^3 p \right\} \quad (21)$$

in which $C_{point}(\vec{p}_1; \vec{p}_2) = 1$ for $\vec{p}_1, \vec{p}_2$ ($\vec{p}_1$ or $0 \rightarrow$) satisfying Eq. (20), and 0 otherwise.

$C_{point}(\vec{p}_1; \vec{p}_2)$ is a function defined in a six-dimensional space, which is hard to visualize. Instead, Fig. 3 shows $C_{point}(\vec{p}_1; \vec{p}_2)$ in a three-dimensional $\vec{p}_2$ space for various values of $\vec{p}_1$. $C_{point}(\vec{p}_1; \vec{p}_2)$ has 1 for $\vec{p}_2$ in the donut-shaped region that is shifted and deformed depending on the choice of $\vec{p}_1$.

Fig. 4 compares horizontal cross-sections of the bright field image reconstructed using the line and the point intersection terms, Eq. (19) and Eq. (21) respectively. The intensity in Fig. 4(b) changes by less than 1% of Fig. 4(a), which justifies our neglect of the point intersection cases above. Although the point intersection terms provide sharper boundaries, they are overwhelmed by the stronger, smeared out image by the line intersection terms in the bright field image.

4. APPLICATIONS

In this section, we test Eq. (19) to simulate various 3-D imaging techniques using partially coherent light. Changing the condenser and the objective pupils, Eq. (19) can be used to simulate various imaging modalities. More specifically, in this section we simulate phase contrast and Nomarski differential interference contrast (DIC), and compare them with bright field imaging without any modulation. Fig. 5 shows the 2-D pupil functions for the condenser and the objective lens used for phase contrast. The 3-D pupil functions used in Eq. (19) are calculated by projecting the 2-D functions onto the Ewald sphere. The condenser pupil is transparent over a limited portion with a ring shape, while the objective lens provides $\pi/2$ phase retardation and 80% absorption of the field through the ring colored as a light blue region in Fig. 5(b).

In case of the Nomarski DIC, there is no modulation in the pupil functions. Instead, two beams shifted by a Wollaston prism pass through a sample and then interfere after being merged by another Wollaston prism [17]. The self-interference without any blocking pupils

enables the imaging of a transparent sample with higher resolution and better sectioning capability. In terms of the image formation model, most of the works are for 2-D [18–20]. Preza et al. [20] suggested a 3-D model, which is an extension of their 2-D model based on the defocused point spread function. However, as Mehta et al. [19] pointed out, their model is closer to what he calls Kohler-DIC (a system with no Wollaston prism in the input arm), which may be used for Nomarski-DIC for condenser apertures smaller than around 0.4 times the objective aperture. We show that the 3-D non-paraxial TCC derived in the previous section can be used to analyze Nomarski-DIC in a more efficient and rigorous way.

As briefly mentioned, Nomarski-DIC uses the self-interference of two beams passing through a slightly different regions of a sample. In the TCC approach, it can be described by rewriting the transmission function as follows:

$$T_N(\vec{r}) = T(\vec{r} + \Delta \vec{e}_\Delta) \exp(-i\varphi) + T(\vec{r} - \Delta \vec{e}_\Delta) \exp(i\varphi), \quad (22)$$

where $\vec{e}_\Delta$ is a unit vector in the shearing direction, 2Δ is the amount of shift, 2φ is the bias.

For convenience, let us define the X axis as the shearing direction without loss of generality. The Fourier transform of the transmission function can be written as

$$\tilde{T}_N(\vec{p}) = 2\tilde{T}(\vec{p}) \cos(2\pi m\Delta - \varphi). \quad (23)$$

Substituting into Eq. (18),

$$I_N(\vec{r}) = I_0 + 2\text{Re} \left[\alpha F^{-1} \left\{ C_N(\vec{p}) \tilde{T}(\vec{p}) \right\} \right], \quad (24)$$

in which $C_N(\vec{p}) \equiv 2 \cos(2\pi m\Delta - \varphi) C_1(\vec{p})$ is the effective transfer function for the Nomarski-DIC.

Fig. 6 shows images calculated by simulating bright field, phase contrast, and Nomarski-DIC using the method described above. The sample in the simulation has a real refractive index, so it is transparent under bright field. Vague boundaries of the phantom in Fig. 6(a) and (d) are due to the second order interaction of scattered light from the sample. Fig. 6(b) and (e) present the horizontal and the vertical cross-section of the phase contrast image using the condenser and the objective pupil in Fig. 5. External and internal boundaries of the phantom are clearly visible now. The halo around the phantom is one of the main features of the phase contrast. Fig. 6(c) and (f) present the cross-sections of the Nomarski-DIC image. Using wider numerical apertures, it provides better resolution and optical sectioning. It does not contain the halo artifact either.

The benefit of the TCC approach is to get insight about the frequency response of an imaging system. Fig. 7 shows cross-sections of the effective transfer function for the Nomarski DIC, which was defined in Eq. (24). Since it uses the full aperture, the frequency support is the same as for bright field imaging. However, for the visualization of a transparent sample, it modulates the frequency spectrum asymmetrically in the shearing direction. Hence, some features whose frequency spectrum falls in the low transmission region may not be imaged for this setting of parameters. Finally, Kohler-DIC can be simulated using the effective transfer function that can be written as

$$C_K(\vec{p}) = (P_{cond}^{3D} P_K^{3D} * P_K^{3D})(\vec{p}), \quad (25)$$

in which $P_K^{3D}(\vec{p}) = 2\cos(2\pi m\Delta - \varphi)P_{obj}^{3D}(\vec{p})$.

5. CONCLUDING REMARKS

In this paper, we have presented the theory of 3-D imaging using partially coherent light under the non-paraxial, but scalar and Debye, approximation. Starting from the optical transfer of the Green's function for the Helmholtz operator, we derived the image intensity in terms of the 3-D non-paraxial TCC. By an inspection over the overlap integral, we derived a simple formula that can be efficiently calculated. Using the derived formula, we simulated phase contrast and Nomarski-DIC to image a modified 3-D Shepp-Logan phantom. The calculated images revealed major features of each technique, and the frequency response of the Nomarski-DIC was presented using the effective transfer function.

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Fig. 1.
Optical transfer of a point source.

Fig. 2. Modified 3-D Shepp-Logan phantom, the magnitude of which is adjusted to the refractive index of usual biological samples. (b) and (c) are the horizontal and the vertical cross-section, respectively. The scale bar is $5\mu\text{m}$.

Fig. 3. $C_{po\ int}(\vec{p}_1; \vec{p}_2)$ in $\vec{p}_2$ space for (a) $(p_{Ix}, p_{Iy}, p_{Iz}) = (0.46, 0, 0)$, (b) $(p_{Ix}, p_{Iy}, p_{Iz}) = (3.68, 0, 0)$, (c) $(p_{Ix}, p_{Iy}, p_{Iz}) = (1.84, 1.84, 1.84)$. Unit of frequency is μm^{-1} .

Fig. 4. Horizontal cross-sections of bright field image reconstructed using (a) line, and (b) point intersection terms (after subtracting background). x is horizontal and y is vertical.

Fig. 5. 2-D pupil functions used for the calculation of the phase contrast in Fig. 6(b), (e): (a) condenser (b) objective pupil function. The 3-D pupil function is calculated by projecting the 2-D pupil function onto the Ewald sphere. Dark grey region in (a) is opaque, and light blue region in (b) induces phase retardation with some absorption of transmitted light.

Fig. 6.

Comparison of cross-sections imaged by (a),(d) bright field, (b),(e) phase contrast and (c),(f) Nomarski DIC. (a),(b),(c) are horizontal cross-sections, (d),(e),(f) are vertical cross-sections. For phase contrast, the condenser and the objective pupil functions in Fig. 2 are used. For Nomarski-DIC, shearing is given in X direction with $2\Delta = \lambda/4NA$, $2\phi = \pi/2$. The optical axis is in Z direction and the scale bar is $5\mu\text{m}$.

Fig. 7. Cross-sections of the effective transfer function for Nomarski DIC ($2\Delta = \lambda/4NA$, $2\phi = \pi/2$): (a) U - V cross-section, (b) U - W cross-section. (U, V, W) is the spatial frequency corresponding to the spatial coordinate (X, Y, Z) , in which X is the shearing direction and Z is the optical axis.