Theoretical development and experimental evaluation of imaging models for differential-interference-contrast microscopy

Chrysanthe Preza and Donald L. Snyder
Institute for Biomedical Computing, Washington University, 700 South Euclid Avenue, St. Louis, Missouri 63110, and Department of Electrical Engineering, Washington University, 1 Brookings Drive, St. Louis, Missouri 63130

José-Angel Conchello
Institute for Biomedical Computing, Washington University, 700 South Euclid Avenue, St. Louis, Missouri 63110

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Imaging models for differential-interference-contrast (DIC) microscopy are presented. Two- and three-dimensional models for DIC imaging under partially coherent illumination were derived and tested by using phantom specimens viewed with several conventional DIC microscopes and quasi-monochromatic light. DIC images recorded with a CCD camera were compared with model predictions that were generated by using theoretical point-spread functions, computer-generated phantoms, and estimated imaging parameters such as bias and shear. Results show quantitative and qualitative agreement between model and data for several imaging conditions. © 1999 Optical Society of America [S0740-3232(99)01509-4]

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1. INTRODUCTION

Transmitted-light Nomarski differential-interference-contrast (DIC) microscopy is a widely used microscopy modality that was developed over 40 years ago for the study of unstained transparent biological specimens. Such specimens, known as phase objects, cannot be seen when in focus under an ordinary transmitted-light microscope. Phase objects retard or advance light that passes through them because of spatial variations in their refractive index and/or thickness, and thus they can be examined with special microscopes that allow the visualization of phase variations. Alternatively, phase variations can be converted to intensity variations by the use of interference methods.

A widely used interference method is DIC microscopy in which a two-dimensional (2-D) image is formed from the interference of two mutually coherent waves that have a lateral differential displacement of a few tenths of a micrometer (called the shear) and are phase shifted relative to each other. This is accomplished by illuminating a specimen with a plane-polarized beam that has been split into two orthogonally polarized, mutually coherent components by a Wollaston prism and afterward recombined into a single beam by another prism and analyzer before being detected (see Fig. 1). The amplitude and thus the intensity of the resulting beam are a function of the phase difference between the two waves. Therefore the intensity distribution in measured DIC images is given by a nonlinear function of approximately the spatial gradient of a specimen’s optical-path-length distribution (integral of refractive index over length) along the direction of shear (perpendicular to the optical axis).

Conventional DIC microscopy has been used to study both thin and thick living specimens. A three-dimensional (3-D) image of a thick specimen is obtained through the method of optical sectioning by collecting a set of 2-D images while the specimen is moved through focus. DIC microscopy has been known to have good optical-sectioning capability (i.e., it has a shallow depth of field), which is a consequence of the combination of a high numerical aperture (NA) used for the illumination and the shearing of the wave fronts. However, comparisons of confocal and nonconfocal 3-D DIC images have shown that a confocal DIC microscope rejects more out-of-focus information than a conventional DIC microscope. As in other microscopy modalities, improvements in DIC images can be achieved by computational methods designed to undo degradations introduced by the optical system. To be able to undo these degradations, one must have a mathematical description of the image-formation process. Therefore the development of model-based computational methods for DIC microscopy is essential for quantitative interpretation of DIC images. A first step toward improving DIC images is the derivation of a reasonable imaging model that can predict these degradations.

In this paper a general model for DIC image formation is derived, and results from testing different cases of the model are presented. The paper is organized as follows. Section 2 summarizes related work. In Section 3 the model is derived for 2-D imaging and then extended to three dimensions. Section 4 describes the data collection and the calculation of the model predictions. Section 5 is a comparison study between measured DIC images and the model predictions. The last section summarizes our results. For completeness, we include in this paper portions of our work that were presented elsewhere.
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We have derived and evaluated a general image-formation model for DIC microscopy under partially coherent illumination. This was accomplished by first deriving a 2-D model and then extending it to three dimensions, assuming weak optical interactions within the specimen. The derivation of the 2-D model was based on two different approaches, each of which led to the same result. First, we extended the frequency theory of Cogswell and Sheppard from coherent to partially coherent illumination by propagating the mutual intensity of the light through a thin specimen and the optical system based on the theory of image formation in partially coherent light for transmitted-light optics described by Born and Wolf (pp. 526–532). We arrived at the same model by propagating the complex amplitude of the illuminating wave field under Köhler illumination through a thin specimen and the optical system.

In what follows, we first present the DIC PSF and then derive the general imaging model. Special cases of this model are shown to be equivalent to models developed by others. Evaluation of the model under certain imaging conditions is presented in Section 5.

A. Differential-Interference-Contrast Point-Spread Function

In a DIC microscope, the image is formed from the difference between the amplitudes of two waves that are phase shifted relative to each other and are separated by a lateral shear $2\Delta x$ (expressed in length units), introduced by the Wollaston prism (see Fig. 1). Without loss of generality, we assume that the shear is along the $x$ direction. The phase difference between the two waves is due to the fact that the waves travel through different regions of the specimen. Furthermore, a uniform phase difference between the two waves, the bias retardation $2\Delta \phi$ (expressed in radians), can be introduced by translating the sliding prism along the shear direction. Assuming coherent illumination, perfect polarizing components, and the absence of instrumental stray light, then, when the phase difference that is due to the specimen is zero, we can write the amplitude difference between the two waves as

$$h(x, y) = (1 - R)\exp(-j\Delta \phi)k(x - \Delta x, y) - R\exp(j\Delta \phi)k(x + \Delta x, y),$$

where $R$ is the amplitude ratio (the amplitude of one wave field divided by the sum of amplitudes of the two wave fields) and $k(x, y)$ is the amplitude PSF for transmitted-light optics under coherent illumination. The complex-valued function $h(x, y)$ is the amplitude PSF for DIC optics; a calculated $h(x, y)$ is shown in Fig. 2. It should be noted that the squared magnitude of the DIC PSF, $|h(x, y)|^2$, predicts the DIC image of a pinhole, i.e., an intensity point, rather than the DIC image of a delta phase function (see the derivation in Appendix A).

As is evident from Eq. (1), in addition to the NA of the objective lens, several other physical parameters determine the DIC PSF. First, the shear, which is fixed in conventional DIC microscopes, is determined by the deviation angle of the Nomarski prism and the focal length of the objective lens, and it is usually on the order of the resolving power of the microscope objective (see Ref. 4, p.
Fig. 3. The effect of the bias on the DIC PSF is shown in Fig. 3 in Ref. 6) in the DIC imaging purely phase objects. Furthermore, the bias retardation is adjusted by sliding the second Wollaston prism to optimize contrast. The effect of the bias on the DIC PSF is shown in Fig. 3.

The 3-D PSF of a DIC system, \( h(x, y, z) \), can be obtained from Eq. (1) and the defocused PSF for transmitted-light optics, \( k(x, y, \Delta z) \), which can be modeled as the 2-D Fourier transform of the generalized pupil function

\[
p(x, y, \Delta z) = |p(x, y, \Delta z)| \exp\left(2\pi i w(x, y, \Delta z)/\lambda\right),
\]

where \( w(x, y, \Delta z) \) is an effective path-length error (see Ref. 17, p. 121) and \( \Delta z \) is the amount of defect in the focus that is due to a displacement of the object plane along the \( z \) (optical) axis. Detailed derivations of \( k(x, y, \Delta z) \) can be found in Refs. 15 and 18–20. A review and a comparison of various models for the defocused PSF and the order of approximations made in each model can be found in Ref. 18. In our work, computation of \( k(x, y, \Delta z) \) is based on the PSF model by Gibson and Lanni,20 which was shown to be accurate enough for 3-D serial-sectioning microscopy. Section images through the volume of a calculated \( h(x, y, z) \) are shown in Fig. 4.

B. Frequency Support of the Point-Spread Function

The Fourier transform of the 2-D DIC PSF \( h(x, y) \), assuming \( R = 0.5 \) in Eq. (1), can be written as

\[
H(f, g) = -j \sin(2\pi f \Delta x + \Delta \theta) K(f, g),
\]

where \( K(f, g) \) is the coherent transfer function, defined as the Fourier transform of the coherent PSF \( k(x, y) \). For a circular aperture, \( K(f, g) \) is circularly symmetric, and it is equal to 1.0 inside a circle with radius equal to \( f_c = NA/\lambda \) and zero outside the circle, where NA is the numerical aperture of the objective lens, and \( \lambda \) is the wavelength of the illumination light. Thus the cutoff frequencies of \( H(f, g) \) are \( f_c = g_c = NA/\lambda \). Because \( K(f, g) \) is a real function, the real part of \( H(f, g) \) is zero.

Profiles of calculated \( H(f, g) \) functions for different bias values are shown in Fig. 5(a), and horizontal profiles of \( H(f, 0) \) are plotted in Fig. 5(b).

As evident from Fig. 5(b) and Eq. (2), \( H(0, 0) \) equals zero for bias \( 2\Delta \theta \) equal to zero, while, for a nonzero bias, \( H(0, 0) = -j \sin \Delta \theta \). Furthermore, for a nonzero bias, \( H(0, g) \) is not zero along the \( g \) axis (i.e., perpendicular to the direction of shear) as it is for a zero bias, as evident from the vertical profiles in Fig. 5(a). This is important because it shows that some frequencies of an object with a phase function that does not change along the shear direction are passed through the objective lens. Such an object has frequency components only in the direction perpendicular to the shear direction, and thus, with a nonzero bias, some of these frequency components pass through the DIC microscope. In fact, some information about such a specimen is actually imaged by the microscope (see Ref. 14, p. 173).

Fig. 4. xy- and xz-section images from a 3-D calculated DIC PSF of a 10×/0.3-NA lens with bias equal to 0.0 rad: (a) real part of the complex amplitude, (b) imaginary part of the complex amplitude, (c) squared magnitude. In the images black represents the minimum negative value in (a) and (b) and a zero value in (c), while white represents the maximum positive value of each image. For the xy images, \( z = 1.7 \mu m \) away from focus. The shear distance is equal to 0.68 \( \mu m \), and the direction of shear is along the x axis. The scale bar is approximately 3 \( \mu m \).

Fig. 5. Effect of the bias on the Fourier transform of the PSF of a 10×/0.3-NA dry lens. Vertical (top) and horizontal (bottom) profiles through the center of the imaginary part of \( H(f, g) \) are shown. The bias values are shown by the curves, and they are in radians. We note that the edges of the curves are sloped because the sampling is coarse.

The Fourier transform \( H(f, g, j) \) of the 3-D DIC PSF \( h(x, y, z) \) is shown in Fig. 6. \( H(f, g, j) \) is the DIC 3-D amplitude transfer function for a closed condenser aperture. The spatial-frequency cutoffs of \( H(f, g, j) \) are \( f_c \).
= g_c = NA/λ in the fg plane and _j_c_ = (NA)_2/(nλ) along the j axis, where n is the refractive index of the objective
lens's immersion medium. Thus, based on the Nyquist frequency required by the sampling theorem, an adequate
discrete representation of the PSF can be obtained with samples dx = dy = λ/(2NA) in the xy plane and
dz = nλ/[2(NA)_2] along the z axis.

C. Derivation of the Two-Dimensional Imaging Model

The derivation given in this section is based on propagating the complex amplitude of the illuminating wave field
under Köhler illumination through a thin specimen and the optical system. In Köhler illumination an extended
light source emitting incoherent light is focused by a col-

lector lens in the front-focal plane of the condenser lens as shown in Fig. 7. The condenser then illuminates the
specimen, which is specified by a complex amplitude

\( U_o(x, y, z) \) along the z axis.

Let the complex amplitude of the wave field in the
front-focal plane of the condenser lens, \( U_{ij}(ξ) \) be a white
random process with zero mean and covariance

\[ \text{E}\{U_{ij}(ξ)U_{ij}^*(ξ')\} = a(ξ)δ(ξ - ξ') \]

where \( a(ξ) \) is the intensity of the light source and equals zero at points that
lie outside the circular aperture \( D_c \) of the condenser lens, \( δ(ξ) \) is a 2-D Dirac delta impulse function, and \( * \) denotes
the complex conjugate. Then the complex amplitude
\( U_{ij}(ξ) \) of the wave field in the back-focal plane of the con-
denser lens and right before the specimen can be obtained by the Fresnel superposition integral

\[ U_c(x, y) = \int_{-∞}^{∞} U_{ij}(ξ)h_c(ξ; x, y)\,dξ, \]

where \( h_c(ξ; x, y) = 1/\text{πfc} \exp(j2πξ(x, y)/\text{fc}) \) specifies the
complex amplitude of the illuminating plane waves (see Fig. 7) and \( f_{\text{con}} \) is the focal length of the condenser lens.

Under the paraxial approximation, the complex amplitude
of the wave transmitted by the specimen is simply

\[ U_o(x) = f(x)U_c(x) \]

Assuming that the DIC microscope (excluding the condenser lens) is a linear shift-
invariant system characterized by the PSF \( h(x) \) [Eq. (1)],

we can express the complex amplitude of the wave field in the image (detector) plane as

\[ U_i(x, y) = \int_{-∞}^{∞} U_o(x)h(x - x_0)\,dx_0. \]

Fig. 6. Fourier transform H(f, g, j) of the 3-D DIC PSF of a
10×/0.3-NA lens with bias = 0.0 rad, shear = 1.0 μm, and
550-nm illumination light wavelength: (a) real part of the com-
plex amplitude, (b) imaginary part of the complex amplitude, (c)
squared magnitude. The fg-section images (top row) are cuts
through the 3-D image at the lines shown in the f_j-section im-
ages, while the fj-section images (bottom row) are through the
center of the volume as shown by the lines in the fg-section im-
ages. The direction of shear is along the f axis. The spatial-
frequency cutoffs are \( f_c = g_c = 0.545 \mu m^{-1} \) and \( j_c =
0.083 \mu m^{-1} \). Black regions in (a) and (b) represent negative
values, while in (c) they represent values equal to zero.

Fig. 7. Köhler illumination. An incoherent light source is focused in the front-focal plane of the condenser lens, and thus the specimen
is illuminated by plane waves with normal vectors \((k_x, k_y, k_z)\), where \( k_x = 2π/λ f_{\text{con}}, k_y = 2π/λ f_{\text{con}}, k_z = (2π/λ)^2 - k_x^2 - k_y^2\),
which make angles \( θ_x = \sin^{-1}(f/λ f_{\text{con}}) \) and \( θ_y = \sin^{-1}(g/λ f_{\text{con}}) \) with the yz and xz planes, respectively (see Ref. 17, p. 49). The direction of
each plane wave depends on the illuminating point \((ξ, η)\) in the front-focal plane of the condenser lens. The DIC components are not
shown here for simplicity.
The intensity in the image is then obtained from \( i(x) = E[U_i(x)U_i^*(x)] \), which yields the expression

\[
i(x) = \int_{-\infty}^{+\infty} a(\xi) \left| \int_{-\infty}^{+\infty} f(x_0) h(x - x_0) h_2(\xi; x_0) d\xi \right|^2 dx_0.
\]

(5)

Equation (5) is a general formulation of the intensity in the image plane of a DIC microscope and describes partially coherent imaging. Limiting cases of the model can be obtained by specifying the intensity \( a(\xi) \) of the source. The coherent-illumination limit of the model, obtained by restricting \( a(\xi) \) to be zero except at a single point, predicts Holmes and Levy's intensity expression and yields the same frequency transfer function reported by Cogswell and Sheppard (see Subsections 3.C.1 and 3.C.2 below).

1. 
Coherent Illumination

To describe coherent illumination, we let \( a(\xi) = a(\delta(\xi)) \), which corresponds to closing the condenser aperture down to a single point. In this case Eq. (5) becomes

\[
i(x) = \frac{1}{\pi} \left| \int_{-\infty}^{+\infty} f(x_0) h(x - x_0) d\xi \right|^2,
\]

(6)

where \( a_1 = a/(\lambda f_{\text{con}})^2 \). We refer to Eq. (6) as the point-aperture model. It is easy to show that Eq. (6) predicts the expression derived by Holmes and Levy. First, we substitute Eq. (1) (with \( R = 0.5 \)) in Eq. (6) and let

\[
c(x) = 0.5 |a_1| \int_{-\infty}^{+\infty} f(x_0) k(x - x_0) dx_0
\]

\[
= |c(x)| \exp(-j_\phi_c(x)),
\]

(7)

Then Eq. (6) may be rewritten as

\[
i(x, y) = |\exp(-j\Delta \theta) c(x - \Delta x, y) - \exp(j\Delta \theta) c(x + \Delta x, y)|^2
\]

\[
= |c(x_1, y)|^2 + |c(x_1 + 2\Delta x, y)|^2
\]

\[
- 2|c(x_1, y)||c(x_1 + 2\Delta x, y)| \cos[\phi_c(x_1, y) - \phi_c(x_1 + 2\Delta x, y) + 2\Delta \theta],
\]

(8)

where \( x_1 = x - \Delta x \). Equation (8) is the same as Eq. (28) in Ref. 12 with the specified shear and bias parameters.

It can also be shown that Eq. (6) predicts the expression given by Pluta, which assumes geometric optics. Assuming an ideal PSF (i.e., ignoring the blurring effects of the PSF), i.e.,

\[
h(x, y) = 0.5 \exp(-j\Delta \theta) \delta(x - \Delta x, y) - 0.5 \exp(j\Delta \theta) \delta(x + \Delta x, y),
\]

(9)

and that the specimen's transmission function is \( f(x) = \exp(-j\phi(x)) \), Eq. (6) reduces to

\[
i(x, y) = a_1 \sin^2 \left( \frac{1}{2} \phi(x - \Delta x, y) + \Delta \theta \right).
\]

(10)

Equation (10) is a geometric-optics model for DIC imaging, which is usually reduced by assuming that the wavefront shear \( 2\Delta x \) is very small (differential) relative to the size of the specimen, and thus the phase difference in Eq. (10) can be written approximately as a phase gradient:

\[
i(x, y) = a_1 \sin^2 \left( \frac{1}{2} \frac{\partial \phi(x, y)}{\partial x} + \Delta \theta \right).
\]

(11)

Equation (11) agrees with Eq. (7.27) in Ref. 4. Thus, for ideal imaging under coherent illumination, the intensity in the DIC image is related to the gradient of the specimen's phase function along the direction of shear, and it is independent of the specimen if the specimen's phase function is constant in that direction. We emphasize that the latter is true only for ideal imaging, and it is due to the geometric-optics approximation for the PSF [Eq. (9)]. In general, some information about a specimen's phase function that is constant along the direction of shear is expected to be imaged by the DIC microscope (see Ref. 14, p. 173).

2. Frequency Transfer Theory

The purpose of this subsection is to compare our image-formation model with the frequency transfer theory derived by Cogswell and Sheppard and not to present a detailed analysis of frequency transfer through the DIC microscope as in Ref. 6. To do that, we first rewrite the detected intensity [Eq. (5)] as follows:

\[
i(x) = \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} f(x_0) f^*(x_0') j_s(x_0 - x_0') h(x - x_0)
\]

\[
\times h^*(x - x_0') dx_0 dx_0',
\]

(12)

where

\[
j_s(x_0 - x_0') = E[U_c(x_0)U_c^*(x_0')]
\]

\[
= 1/(\lambda f_{\text{con}})^2 \int_{-\infty}^{+\infty} a(\xi) \exp \left[ j 2\pi[(x_0 - x_0')^2/\lambda f_{\text{con}}] \right] d\xi,
\]

is the mutual intensity of the wave field illuminating the specimen. [Note that for Köhler illumination the mutual intensity depends on the difference of the coordinates, i.e., \( j_s(x_0; x_0') = j_s(x_0 - x_0') \).] Equation (12) can be derived directly by propagating the mutual intensity \( j_s(x_0 - x_0') \) through the system in the same way that it was derived in Ref. 15 for transmitted-light optics. Equation (12) can then be rewritten (see Ref. 15, p. 529) as

\[
i(x) = \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} T_{\text{DIC}}(f; f') F(f) F^*(f')
\]

\[
\times \exp(j 2\pi(f - f') \cdot x') df df',
\]

(13)

where \( F(f) \) is the 2-D Fourier transform of \( f(x_0) \),

\[
T_{\text{DIC}}(f'; f') = \int_{-\infty}^{+\infty} H(f + f')H^*(f + f') j_s(f) df
\]

(14)

is the transmission cross-coefficient of the system, \( H(f) \) and \( j_s(f) \) are the Fourier transforms of \( h(x) \) and \( j_s(x) \), respectively, and all boldface letters denote vectors of spatial frequencies (i.e., \( f = (f'_x, f'_y) \)). \( T_{\text{DIC}}(f; f') \) expresses the combined effect of the illumination and the optics on the object and characterizes the frequency transfer properties of an optical system under partially
coherent illumination, as can be shown by the Fourier transform of the image intensity [Eq. (13)],

\[
I(f) = \int_{-\infty}^{\infty} T_{DIC}(f' + f, f') F(f' + f) F^*(f') df'.
\] (15)

Another function that characterizes frequency transfer in an optical system under partially coherent illumination is the frequency response function of the system (see Ref. 15, p. 527), defined as

\[
M_{DIC}(f) = H(f)H^*(-f'),
\] (16)

which relates the Fourier transforms \( J_s(f, f') \) and \( J_j(f, f') \) of the mutual intensities at the object and image planes, respectively, as follows: \( J_j(f, f') = M_{DIC}(f, f') \times J_s(f, f') \). The DIC effective frequency response function is obtained by inserting Eq. (2) in Eq. (16), yielding

\[
M_{DIC}(f'; f^*) = \sin(2\pi f'\Delta x + \Delta \theta) \times \sin[2\pi(-f^*)\Delta x + \Delta \theta] M(f'; f^*),
\] (17)

where \( M(f'; f^*) = K(f')K^*(-f^*) \) is the frequency response function for transmitted optics.

We note that under coherent illumination \( J_s(f) = \delta(f) \) (see Ref. 14, p. 34), and thus Eq. (14) becomes equivalent to \( M_{DIC}(f'; -f^*) \), which is the same as the expression reported by Cogswell and Sheppard as Eq. (13) in Ref. 6. Thus their expression is the coherent limit of Eq. (14). Plots of \( M_{DIC}(f'; -f^*) \) for various cases and a detailed analysis of frequency transfer through a DIC microscope under coherent illumination can be found in Ref. 6.

D. Extension of the Imaging Model to Three Dimensions

Assuming an optically weak specimen, distortion of the wave field that is due to multiple refraction through the specimen can be regarded as a secondary effect. With this assumption the first-order Born approximation (see Ref. 15, p. 453), which permits the use of linear superposition, is valid. To our knowledge, all existing 3-D models for transmitted-light optics are based on the first-order Born approximation\(^{21-23} \) because it simplifies the models.

We have extended the 2-D model [Eq. (5)] to three dimensions by assuming that an optically weak 3-D specimen with thickness \( t \) along the \( z \) axis is a set of \( N \) planar specimens of thickness \( \Delta z = t/N \) and that a wave field that interacts with one plane in the specimen does not interact with the other planes. This allows the determination of the specimen's 3-D image from the linear superposition of 2-D images. A reasonable question here is what to superimpose: amplitudes or intensities? To answer this question, we must examine the issue of temporal coherence between two wave fields that interact with two different specimen planes that are a distance \( D_z \) apart.

One way to do that is to compare the coherence length of the illuminating source, \( \lambda_z \), with the smallest and largest possible optical-path-length difference. The smallest and largest \( D_z \) of interest are the \( z \)-axis sampling distance (based on the Nyquist frequency required by the sampling theorem) and the thickness of the specimen, \( t \), respectively. If \( \lambda_z \gg \lambda/2t \), where \( \lambda/2t \) is the average refractive index of the specimen, then temporal coherence holds among all waves interacting with the specimen and thus wave fields can interfere at the detector; in this case the amplitudes of the different wave fields must be superimposed. On the other hand, if \( \lambda_z \ll \lambda/2t \), then temporal incoherence holds between any two waves interacting with different specimen planes and thus the intensities of the wave fields must be superimposed. For all other values of \( \lambda_z \), temporal partial coherence holds. Because of the complexity of the partial-coherence case, in practice, assumptions are made to justify the use of temporal coherence or incoherence in the derivation of a model for a particular application. The choice of the best approximation depends on the various parameters used in an application. Instead of making any assumptions, we derived and tested (see Section 5) two models—one based on superposition of amplitudes and the other based on superposition of intensities—to determine which is a better approximation.

When the image of a 3-D object can be obtained by the superposition of the intensity images of the planes that make up the object, then the 3-D image can be obtained by first introducing the specimen plane \( z_0 \) in Eq. (5):

\[
i(x, z) = \int_{-\infty}^{+\infty} i_o(x, z - z_0) d z_0.
\] (19)

\( h(x, z_1 - z_0) \) is the 2-D PSF defocused by a distance \( \Delta z = z_1 - z_0 \). Alternatively, Eq. (5) can be extended to three dimensions by integrating amplitudes over \( z_0 \), yielding a 3-D intensity:

\[
i(x, z) = \int_{-\infty}^{+\infty} a(\xi) \int_{-\infty}^{+\infty} f(x_0, z_0) h(x - x_0, z - z_0) \times h(\xi; x_0) dx_0 dz_0 d \xi.
\] (20)

The coherent-illumination limit of this general 3-D model can be obtained by letting \( a(\xi) = a(\delta) \). The resulting simplified model is the extension of Eq. (6) to three dimensions, and as in the case of the 2-D model, we refer to it as the 3-D point-aperture model.

4. METHODS

To test the DIC imaging model, we constructed several simple phantom specimens whose geometries and refractive-index distributions could be determined. Because our phantom fabrication facilities are limited to ge-
omeries of several micrometers, the models were primarily tested at low magnifications. These phantoms were imaged with conventional DIC microscopes with the use of quasi-monochromatic light (this is accomplished by placing a bandpass filter with a relatively narrow bandwidth in front of the light source) and different imaging conditions. The images were recorded by using a cooled CCD camera. First, a 2-D phantom with shallow grooves was used for quantitative tests of the 2-D model. Second, a cross-shaped phantom and a bead phantom were used to test the 3-D models.

A. Phantom Specimens
Two of the phantom specimens were constructed at the Photonics Research Laboratory (Electrical Engineering Department, Washington University, St. Louis, Mo.). The simple 2-D groove phantom was fabricated by etching two parallel grooves in a glass slide, each groove approximately 7 μm (±60 nm) wide and 60 nm (±5 nm) deep. The grooves were measured with a stylus profilometer (Alpha Step, Tencor Instruments) to be approximately 55–65 nm deep and 40 μm apart. Based on the etching process, the grooves are expected to have sharp edges.

The 3-D cross phantom was constructed from resins with well-known refractive indices24 with the use of a computer-driven laser beam, and it consists of two perpendicular bars placed one on top of the other (see Fig. 8). The two bars have width and height of approximately 9 μm and refractive index slightly higher than the refractive index of the medium that surrounds them. This phantom was chosen because it has the ability to test the validity of intensity or amplitude superposition for weak specimens, an assumption that was used in extending the 2-D model to three dimensions (see Subsection 3.D).

The bead phantom consists of a 4-μm-diameter polystyrene bead of refractive index n2 = 1.59 embedded in an optical cement of refractive index n1 = 1.524 ± 0.0001 (Optical Adhesive No. 65, Norland Products, Inc., New Brunswick, N.J.). Optical cement was dropped on top of the beads, dried on a coverslip, and cured by a 10-s exposure to ultraviolet light from a 100-W mercury arc lamp.

B. Data Acquisition
A DIC image of the groove phantom was acquired at the Laboratory for Radiobiology, Academic Medical Center of the University of Amsterdam, The Netherlands, with the use of an Ortholux II (Leitz, Germany) DIC microscope with a Leitz DIC 25×/0.5-NA dry objective lens and a 0.9-NA dry condenser lens with the condenser aperture open. This microscope has a calibrated DIC bias setting that is adjusted by rotating a polarizer (de Sénarmont method25). The DIC bias was set to be approximately π/2 rad. A bandpass filter (MAD8-1, Schott Glaswerke, Germany) with peak at 550 nm and bandwidth of 8 nm around the peak was fitted in front of the light source for quasi-monochromatic illumination. The images were acquired with a cooled CCD camera (Lambert Instruments, The Netherlands) equipped with a Kodak KAF0400 CCD chip (9-μm well size). The effective pixel size in the recorded images is 0.36 μm. A DIC image of small latex beads (460-nm diameter; DOWlatex, 41984, Serva, Germany) was also measured at a bias setting equal to π/2 rad for the determination of the shear parameter. The shear for the 25×/0.5-NA lens was determined to be approximately equal to 1.0 μm along the 45° axis by computing the coordinates of the center of the bright spot and the center of the dark spot in the image of the bead using a method similar to the one described by van Munster et al.26 Calibration images, dmin and dmax, were also acquired at biases of 0 and π rad, respectively, of an empty region on the slide by using the same exposure time as that for the other images. These images were used to correct the measured DIC images for CCD camera dark current and nonuniform flat-field response and to normalize the DIC image values. For the correction, dmin was subtracted from each DIC image; then the result was divided by dmax − dmin (see Ref. 14, pp. 48–50).

DIC images of the cross phantom and the bead phantom were acquired by using an Olympus IMT2 inverted microscope (Olympus Corporation, Lake Success, N.Y.) with the IMT2-NIC attachment for DIC, which has a 0.55-NA dry condenser lens. The images were recorded by using a cooled (−45°C) CCD camera (Photometrics Ltd., Tucson, Ariz.) equipped with a Kodak KAF1400 CCD chip (6.8-μm well width). A bandpass filter with peak at 535 nm and full width at half-maximum equal to 64.5 nm (IF550, Olympus) was placed in front of the light source (a tungsten halogen lamp; BRL, Ushio, Cypress, Calif.) for quasi-monochromatic illumination. The bias setting, controlled by sliding the Wollaston prism, is not calibrated on this microscope.

The cross was imaged with an Olympus S Plan Achromat 10×/0.3-NA dry objective lens and the condenser aperture closed (for a closed aperture, the effective condenser NA is 0.082) at four different orientations. The effective pixel size in each 2-D recorded image is 0.68 μm. The spacing between focal planes along the z axis (controlled by a stepping motor) was set to 3 μm. First, the two bars of the cross were aligned with the vertical and horizontal axes. The cross was then rotated manually (by rotating the specimen slide) by approximately 13°, 32°, and 47° clockwise.

The bead phantom was imaged with an Olympus LWD CD Plan 40×/0.55-NA dry objective lens and the con-
denser aperture either closed or partially open. The pixel size in each 2-D image is 0.17 μm, and the spacing between focal planes was set to 1.8 μm.

A DIC image of small latex beads (210-nm diameter; Polysciences, Warrington, Pa.) with refractive index equal to 1.59 and air dried on a slide was also acquired with the 10×/0.3-NA objective lens for the determination of the shear parameter and for model testing. The shear determined from the bead image (using the method mentioned above) is approximately equal to 1.0 μm along the 135° axis (see Fig. 13 below).

C. Model Predictions

Model predictions were obtained through simulations using theoretically determined PSF's and computer-generated phantoms that approximate the phantom specimens described above. Synthetic 2-D and 3-D DIC images were generated by using both the general models [Eqs. (5), (19), and (20)] and the point-aperture models [Eq. (6) and its corresponding extensions to three dimensions].

Theoretical PSF's were calculated by using Eq. (1) with R = 0.5, λ = 550 nm, various bias values, and specified shear values for each of the objective lenses used: 10×/0.3-NA, 25×/0.5-NA, and 40×/0.55-NA. The sampling rates used for the PSF calculations were based on the Nyquist condition (see Subsection 3.B).

The shear value 2Δx was determined to be equal to 1 μm for the three lenses. It is noted that the exact shear value used in the computation of the model predictions is affected by the pixel size used, and thus some deviation from the above value was necessary in some cases.

The bias 2Δθ was estimated from the average value I_b of a region in the correct DIC image with no specimen by using the expression Δθ = sin⁻¹(I_b); it is easy to show that Eq. (6) reduces to I_b(x) = sin²(Δθ) = I_b for f(x) = 1 (see Ref. 14, pp. 176–178). Other methods for determining the bias can be found in Refs. 11 and 27.

The computer-generated phantoms represent the phase function of each phantom specimen, which was calculated by using φ = 2π(n_2 - n_1)Δτ/λ, where n_2 is the uniform refractive index of a structure in the phantom (i.e., groove, bars, or bead), n_1 is the uniform refractive index of the surrounding medium, Δτ is the thickness of a thin section of a 3-D phantom (or the thickness of a 2-D phantom), and λ is the wavelength of light. This is a relative phase with respect to the background, which is assumed to have zero phase.

An implementation of Eqs. (5) and (20) was developed based on the following approach. First, the illumination aperture is approximated by a lattice of points. Then, for each point in the aperture, a subimage is computed by performing a convolution operation between the PSF h(x) and the product term f(x)h_t(x). The linear convolution operation is approximated with a circular convolution by using fast Fourier transforms. To minimize aliasing errors, instead of padding with zeros, we separate the phantom images into two parts, background and structure, and superposition is used to determine the convolution result. This is done because our phantom's transmission functions have a constant nonzero background and thus the values in the phantom images do not fall near zero toward the edges of the images.

The final image is obtained by integrating over the aperture. The integration over the condenser aperture D_c is approximated with a summation.

Implementation of the function h_t(ξ, x) requires evaluation of complex exponentials with frequencies u = ξ/(λf_con) and v = η/(λf_con). For a given radius r_c = \sqrt{x^2 + y^2}, the highest frequency, u_r = \sqrt{u^2 + v^2}, is given by

\[ u_r = \frac{r_c}{\lambda f_{\text{con}}} = \frac{r \tan \alpha}{\lambda}, \]  

where r = r_c/r_{\text{max}}, r_{\text{max}} = f_{\text{con}} tan \alpha is the maximum radius of D_c, \alpha = sin⁻¹\left(\frac{\text{NA}_{\text{con}}}{n}\right) is the acceptance angle of the condenser lens, NA_{\text{con}} is the NA of the condenser lens, and n is the refractive index of the immersion medium used with the condenser lens. Thus, as the acceptance angle \alpha (or equivalently NA_{\text{con}}) or the normalized condenser aperture r increases, complex exponentials with higher frequencies need to be computed. At some point it becomes impossible to sample these functions adequately without decreasing the pixel size and at the same time increasing the number of points in the aperture lattice and thereby the computation time. This sampling problem gives rise to artifacts in the model predictions as the condenser aperture increases. In our computations artifacts were sometimes observed for condenser apertures equal to 35% of the maximum radius, although the exact size varies with the acceptance angle of the lens, the image size, and the pixel size.

The point-aperture assumption simplifies the model equation, and thus this model is easier to implement, and less time is required for its computation, than the general model. For the point-aperture model, only one convolution operation is performed, and there is no need to compute complex exponentials as in the case of the general model.

The program, written in the C programming language, was executed on an SGI R10000 180 MHz processor (Silicon Graphics, Inc., Mountain View, Calif.). Results from a performance analysis of the implementation using a commercially available program called “quantify” (Rational Software Corporation, Santa Clara, Calif.) showed that the implementation spends approximately 73% of the time executing fast Fourier transforms. For the 2-D point-aperture model, the program takes approximately 6 s for a 512 × 512 image, while for the 3-D point-aperture model, it takes approximately 55 s for an image with 128³ pixels. The time needed to compute the general model (2-D or 3-D) is equal to the time that it takes to compute the point-aperture model scaled by the number of points in the aperture lattice.

5. RESULTS

In this section we present predictions obtained with our imaging models. Some of the model predictions are compared with measured images. In some cases exact values for imaging parameters such as bias, shear, and condenser aperture size were not available because of lack of
system calibration. Furthermore, large aperture sizes could not be incorporated in the computation of the model predictions because of the required computational complexity (see also the discussion in Subsection 4.C).

A. Two-Dimensional Model

2-D model predictions were generated by using a computer-generated groove phantom (Fig. 9). As evident in Fig. 9(a), the model prediction obtained with the point-aperture model \( r \rightarrow 0.0 \), which corresponds to coherent imaging, shows oscillations that are characteristic of coherent imaging of a sharp step (Ref. 17, pp. 131–132). These oscillations are not visible in partially coherent imaging, i.e., when the condenser aperture is partially open [Fig. 9(b), \( r = 0.3 \)].

Comparison of a model prediction (synthetic image) of the groove phantom with a measured DIC image of the phantom (Fig. 10) shows good qualitative agreement. The two images appear to be similar and show a distance of approximately 7 \( \mu m \) between the groove edges, which is consistent with the groove's width. Quantitative differences between model and data are evident from the profiles shown in Fig. 10(c). The measured DIC image has lower and wider peaks than the synthetic DIC image.

This mismatch could be attributed to several differences between the actual imaging conditions and the ones assumed in the generation of the synthetic image: (1) condenser aperture size, (2) errors in the bias value and the shear value and direction, (3) PSF errors, and (4) deficiencies in the imaging model. It is noted that the condenser aperture used for the model prediction is smaller than the actual condenser aperture because of computation problems for larger apertures.

B. Three-Dimensional Model

Because of the computational complexity of the 3-D general model (see the discussion in Subsection 4.C), most of the model predictions presented in this subsection were computed by using the point-aperture model. Although the point-aperture model is an approximation of the general model, evaluation of the point-aperture model provides a good starting point toward the evaluation of the general model. 3-D point-aperture model predictions were generated for the cross phantom and a small bead.
(210-nm diameter), while model predictions for various aperture sizes were generated for the bead (400-nm-diameter) phantom. For simplicity, 2-D cross-section images from the 3-D images are shown in the figures.

Model predictions of the cross-shaped phantom computed with the point-aperture model were compared with measured images acquired with a closed condenser aperture. Two specific issues were tested in these comparisons: (1) the superposition assumption used to extend the 2-D model to three dimensions based on the temporal coherence of the light and (2) the ability of the model to predict the direction sensitivity of DIC imaging. In what follows we describe results from these comparisons.

To test the superposition assumption used to extend the 2-D model to three dimensions (see Subsection 3.D), we generated model predictions with both Eqs. (19) and (20) (assuming a point condenser aperture for simplicity) and a computer-generated crossed-bars phantom [Figs. 11(b) and 11(c), respectively]. Measured images of the crossed-bars phantom specimen (Fig. 8) acquired with a closed condenser aperture were compared with these model predictions. One such comparison is shown in Fig. 11. As evident from the figure, the model prediction obtained by superimposing amplitudes [Fig. 11(b)] shows good qualitative agreement with the measured image [Fig. 11(a)], while the model prediction obtained by superimposing intensities [Fig. 11(c)] fails to capture the distinct features of the measured DIC image. This result suggests that assuming temporal coherence between the interfering wave fields is a better approximation than temporal incoherence. We note that, for our experimental parameters, the condition \( l_c \gg \Delta r \) that ensures temporal coherence (see the discussion in Subsection 3.D) does not hold because the coherence length of the illuminating light, \( l_c \), is small; \( l_c = 0.664c/\Delta \nu = 2.9 \mu m \) (see Ref. 16, p. 168), where \( c \) is the speed of light in air and \( \Delta \nu = 6.8 \)
107 MHz is the half-power bandwidth of the excitation filter (Olympus IF550), which was placed in front of the light source. We note that using a filter with a narrower bandwidth would yield a larger $l_c$, which could ensure temporal coherence between the interfering wave fields. In what follows we show model predictions obtained based on the temporal coherence assumption [i.e., with Eq. (20)] only.

Another comparison of a measured image of the cross-shaped phantom with a model prediction is shown in Fig. 12. Because the model prediction was obtained with the point-aperture model, it shows oscillations characteristic of coherent imaging of a sharp step. As evident from Fig. 12, the spread along the $z$ axis in the model prediction is consistent with that observed in the measured image. An apparent tilt along the $z$ axis observed in the measured image [Fig. 12(a)] is probably due to a misalignment in the microscope that is not accounted for by the model.

A similar tilt along the $z$ axis (off-axis aberration) is observed in the measured image of a small bead [Fig. 13(a)] but not in the synthetic DIC image of the bead [Fig. 13(b)]. Another discrepancy between model and data evident in Fig. 13 is due to the diffraction rings observed in the synthetic image, which are not visible in the measured image because of noise.

To test how well the model accounts for the direction sensitivity of DIC imaging, we compared measured images of the cross phantom acquired at different orientations of the cross with model predictions. For simplicity, we show only $xy$-section images that correspond to a cut through the middle of a 3-D volume (Fig. 14). Several similarities between the images are noted with respect to object features that are present in the images. At the first orientation of the phantom, the shear direction makes a 45° angle with both bars, and thus the edges of both bars are visible [Fig. 14(a)]. As the phantom is rotated clockwise, the phase-gradient information of one of the bars dominates, making that bar more visible in the image. Finally, only the bar that is oriented perpendicular to the direction of shear is visible, while the other one, along the direction of shear, disappears [Fig. 14(d)]. The asymmetry evident in the measured images [i.e., the difference in the width of the bar edges in the top row of Figs. 14(b)–14(d)] suggests that the bars do not have a square cross section, which was assumed for the computer-generated phantom used in the model predictions.

3-D model predictions of a computer-generated bead phantom were generated with the general model for different aperture sizes (Fig. 15). Artifacts were observed in the model predictions for aperture sizes larger than 35% of the maximum aperture. One such artifact is the bright intensity in the center of the bead image in Fig. 15(d). Measured images of the bead phantom acquired with different aperture openings of the condenser lens do not show this artifact, but instead they show an asymmetry along the $z$ axis that seems to be due to spherical aberration (Fig. 16). To confirm this, we generated a model prediction by using a PSF with spherical aberrations (Fig. 16(a)).
Comparison between data (Fig. 16) and model (Fig. 17) shows a qualitative agreement for the observed asymmetry along the z axis. The aberrant PSF was computed by using the Gibson and Lanni model, 20 which incorporates spherical aberrations introduced by deviations of the thickness and the refractive index of layers separating the object plane from the objective lens from the design conditions. We note that the amount of spherical aberration introduced to account for the observed asymmetry in the measured images is much larger than what would have been expected for the relatively thin bead phantom. Part of the observed spherical aberration may be due to residual aberrations reported for this objective lens by other users. Unfortunately, this was the only DIC lens available to us with a NA at least as large as the NA of the condenser lens.

6. SUMMARY AND CONCLUSIONS
The derivation of models for 2-D and 3-D DIC imaging based on first principles has been presented. Evaluation of the models with three physical phantom specimens (a 2-D groove, a 3-D cross-shaped phantom, and a bead) has shown a number of similarities between model and data suggesting that although our models are not perfect, they do capture major features in the measured image. Currently, model predictions are limited to small condenser apertures. Computation of model predictions for...
large aperture sizes yield artifacts due to inadequate sampling (see discussion in Section 4.3). We expect that these sampling problems can be overcome with the increase of computing power (faster processors and more memory) available to us. By overcoming these current computational problems and by generating synthetic DIC images with larger condenser apertures, we expect that the match between model and data will improve.

Several system parameters were estimated in order to best approximate DIC imaging formation with our model. Furthermore, in the case of the cross-shaped phantom, the exact phantom geometry was not known exactly and thus, estimated dimensions were used. In addition to errors in these estimated system and phantom parameters, aberrations in the optical system could be partly responsible for the observed differences between model and data. Optical system aberrations due to incompletely corrected optical components or due to non-ideal set-up of the system can give rise to artifacts in the measured images (see detailed discussion in Ref. 29, pp. 405–412 and Ref. 20) that are not accounted for by the model. Thus, for further model testing a well-calibrated DIC microscope equipped with high-NA optics and high-resolution phantom specimens with known geometries will be used.

The development and evaluation of the DIC imaging formation models presented in this paper is a necessary first step for the development of model-based image-processing methods for DIC microscopy and thus should facilitate future development of such methods. Although further evaluation of our DIC models is needed, our results have shown that the model has the potential to capture the main features of measured DIC images.

APPENDIX A:
DIFFERENTIAL-INTERFERENCE-CONTRAST IMAGE OF A POINT PHASE OBJECT

A point phase object has a phase function \( \phi(x) = \phi_1 \delta(x) \), where \( \phi_1 \) is a constant and \( \delta(x) \) is a 2-D Dirac delta function and a complex amplitude transmission function

\[
   f(x) = \exp[-j \phi(x)] = \begin{cases} 
   \cos \phi_1 - j \sin \phi_1, & x = (0,0) \\
   1 & \text{otherwise} 
   \end{cases} 
\]  

From Eq. (6) the intensity in the DIC image of this object can be written as

\[
   i(x) = a_1 \left[ (b_1 + j b_2) \int_{-\infty}^{\infty} \delta(x_0) h(x-x_0) dx_0 \right. \\
   \left. + \int_{-\infty}^{\infty} h(x-x_0) dx_0 \right] \\
   = a_1 \left[ (b_1 + j b_2) h(0,0) + H(0,0) \right] \\
   = a_1 \left[ (b_1 + b_2) h(0,0) + 2 \Re \{ (b_1 + j b_2) h(x) \} \right] \\
   = 2 \Re \{ (b_1 + j b_2) h(x) \} \\
   = 2 \Re \{ b_1 + j b_2 \} \text{Re} \{ h(x) \} \\
\]

where \( b_1 = \cos \phi_1 - 1 \) and \( b_2 = \sin \phi_1 \), \( H(f) \) is the Fourier transform of \( h(x) \), and \( \Re \{ \cdot \} \) denotes the real part of a complex-valued function. The first term on the right-hand side of Eq. (A2) is the image of an intensity point object (i.e., a pinhole). The second term \( |H(0,0)|^2 = \sin^2 \Delta \phi \). However, the last term is not a constant, and thus we conclude that the image of a phase point object is different from the image of an intensity point object.

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The corresponding author, C. Preza, can be reached by e-mail, preza@bc.wustl.edu; phone, 314-362-2938; or fax, 314-362-0234.

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