Using the Hilbert transform for 3D visualization of differential interference contrast microscope images

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Summary

Differential interference contrast (DIC) is frequently used in conventional 2D biological microscopy. Our recent investigations into producing a 3D DIC microscope (in both conventional and confocal modes) have uncovered a fundamental difficulty: namely that the phase gradient images of DIC microscopy cannot be visualized using standard digital image processing and reconstruction techniques, as commonly used elsewhere in microscopy. We discuss two approaches to the problem of preparing gradient images for 3D visualization: integration and the Hilbert transform. After applying the Hilbert transform, the dataset can then be visualized in 3D using standard techniques. We find that the Hilbert transform provides a rapid qualitative pre-processing technique for 3D visualization for a wide range of biological specimens in DIC microscopy, including chromosomes, which we use in this study.

Introduction

Biomedical researchers are increasing their use of 3D visualization in examining the behaviour of small structures such as the cytoskeleton and chromosomes during mitosis. Much of this work is carried out using fluorescence, especially confocal fluorescence, which is ideally suited to 3D analysis (Terasaki & Dailey, 1995; Hepler & Gunning, 1998). However, there is a continuing need to observe unstained transparent features within biological specimens, accounting for the continuing popularity of differential interference contrast (DIC) in conventional microscopes (Cole et al., 1995). The use of DIC is currently limited to 2D, as attention has only recently been paid to 3D visualization for transmission DIC. This contrasts with the advanced development of 3D visualization techniques for fluorescence.

We are investigating 3D DIC microscopy, in both conventional and confocal modes. We have found that standard 3D image processing and visualization techniques, such as those used for confocal fluorescence, are inappropriate for DIC.

DIC produces an image combining the phase gradient and amplitude of the specimen. For weakly absorbing objects, the image will be mostly phase information. This provides structural information about otherwise transparent details of the specimen, where there are changes in refractive index in features such as cell components. The response to the phase-gradient is non-linear, so without correction to a linear response, any resulting 3D visualization will be qualitative only, just as for the original 2D DIC images. In this paper we concentrate on such qualitative imaging.

A simulated 1D cross-section of a phase object and its DIC phase gradient is shown in Fig. 1. This produces a 2D image which has a bas relief appearance, as shown in Fig. 2(a). Specimen features (in this case, a few metaphase chromosomes from an orchid root tip preparation) appear with highlights and shadows at the edges, against a grey background. This provides a boost in high spatial frequency response (Cogswell & Sheppard, 1992), together with increased contrast for human viewing of 2D images. Meanwhile, axial resolution is increased, with optical sectioning occurring for high spatial frequency features in the specimen. However, unlike confocal fluorescence, this optical sectioning does not apply to low spatial frequencies in DIC.

A common approach to computer display of 3D microscopy datasets of biological specimens is 3D volume visualization (Kriete, 1992; Chen et al., 1995). For
fluorescence imaging, this relies on making the background transparent. This is easily done if the background is approximately black, indicating low fluorescence signal (see Fig. 1a). In the DIC case, the background is grey with small deviations in intensity. Making grey transparent in a 3D volume will reveal only the lateral shells of specimen features (Fig. 1b). Image processing techniques appropriate to gradient images need to be found and applied to DIC datasets before they can be displayed meaningfully in 3D.

Several authors have recently outlined methods that could be used to prepare DIC images for 3D visualization. Line integration and deconvolution (Kam, 1998) is an iterative method requiring calibration to the particular microscope in use. Variance filtering and directional integration using iterative energy minimization have also been proposed (Feineigle et al., 1996). The rotational diversity technique (Preza et al., 1998) combines numerous images taken of the specimen at different rotations. Direct deconvolution may be used, calibrated and optimized to reduce noise with a Weiner filter (van Munster et al., 1997).

In this study we explore the suitability of the Hilbert transform for DIC image visualization. As a starting point we take the simple case of directional integration in 2D, as a method of retrieving the phase, given the phase gradient supplied by DIC. We then propose the 2D Hilbert transform as a qualitative alternative.

**Image processing methods**

**Test specimen**

In choosing a test specimen for studying DIC image processing methods, there is a balance between quantitative yet trivial specimens such as latex spheres, to complicated biological specimens which can best be appraised qualitatively, based on a knowledge of expected biological features. Particularly for segmentation of specimen features, the statistics and parameters used must be customised to the morphological characteristics of the class of specimens being examined. For this study, we chose to use plant chromosomes, as our emphasis is on creating qualitative techniques for visualization of cell morphology.

**Integration**

The obvious mathematical approach to recover a function from its gradient is integration. DIC effectively provides an optical differentiation. The DIC image is related to the phase gradient of the specimen, and the ability of the DIC system to resolve small differences in specimen phase is related to the amount of optical shear introduced by the DIC prism geometry (Pluta, 1989). In the imaging plane, the direction of differentiation is determined by the orientation of the Wollaston prism, and for DIC, this is often set at 45° to the lateral axes of the image. The first step in obtaining an accurate integration is to align the direction of shear or differentiation with one of the Cartesian axes of the digitized image. In our case, we have chosen to align the shear angle, and thus the direction of differentiation, to be parallel with the x axis of our image.
The integration may then be applied horizontally, line by line, to the digital image. Each line integrated will have an uncertainty from small errors in pixel values and an unknown constant of integration, which is lost by the differential imaging method. This constant will probably be different for each line, producing horizontal streaking in the image. In addition, integration acts as a low pass filter, suppressing high spatial frequency information in the image.

**Hilbert transform**

Turning to a spatial frequency analysis, a (real space) differentiation corresponds to a multiplication by the value of the spatial frequency parallel to the shear for each point in frequency space. Integration is then just the inverse, namely division by the spatial frequency. Both of these processes are antisymmetric (or odd) but the division selectively attenuates higher frequency components. What is needed is an antisymmetric transform or process that does not change the relative balance of the various frequency components. Such a transform exists and is known as the Hilbert transform (Bracewell, 1978; Oppenheim & Schafer, 1989). In 1D real space, the Hilbert transform \( H \{f(x)\} \) is defined as

\[
H\{f(x)\} = \tilde{f}(x) = \frac{1}{\pi} \int_{-\infty}^{\infty} \frac{f(x')}{(x' - x)} \, dx'.
\]

(1)

In Fourier space the apparent problems with the above singular integral disappear and the Hilbert transform results from a simple multiplication by a signum function:

\[
\hat{F}(s) = \int_{-\infty}^{\infty} \hat{f}(s) \exp(-2\pi isx)dx,
\]

(2)

\[
\hat{F}(s) = isgn(s)\hat{F}(s),
\]

(3)

where \( s \) is the spatial frequency, and \( sgn(s) \) is the signum function, which is equal to +1 for positive \( s \) and −1 for negative \( s \).

In essence, the Hilbert transform keeps all the positive frequency components the same but reverses the sign of all the negative frequency components. Effectively, this makes the image features symmetric, removing the asymmetrical highlight and shading of objects. A simulation in one dimension is shown in Fig. 1(c). Note there is some ringing on either side of the peaks in the phase gradient. In fact the ringing is mainly due to the initial edge enhancement afforded by the derivative operation of the DIC optical system.

In two dimensions the Hilbert transform is not uniquely defined because the line between the positive and negative regions of the associated 2D signum function may have any orientation. In practice we choose the line orientation to be perpendicular to the DIC shear direction because it corresponds to the direction used by the integration method, and gives optimal results. The extension of the Hilbert transform into two dimensions is not well known in the area of digital image processing but it does occur frequently in the Fourier based analysis of 2D fringe patterns. An early use of what is essentially a 2D Hilbert transform occurs in Bone et al. (1986). In that instance the transform method was not actually called the Hilbert transform, but it can be shown to be essentially the same.
FFT and imaginary parts by the Hilbert mask (a 2D step function). The middle step shows a multiplication of the real part of the complex Fourier image, and 'Im' indicates the imaginary part. The Hilbert transform more useful for 3D visualization than method. Firstly, the image shows unacceptable horizontal streaking. This may be attributed to the unknown constant of integration, which varies line by line. In addition, the image after integration is considerably blurred in contrast with the original, as is to be expected due to the low-pass filtering effect of integration.

The horizontal streaking problem may be dealt with by further developing directional integration in conjunction with an iterative energy minimization technique (Feineigle et al., 1996). Alternatively, directional integration may be combined with iterative deconvolution (Kam, 1998). This last method requires calibration using a measurement of the shear produced by the DIC prism, and does not completely eliminate the streaks. Both methods produce the smoothing effect inherent to integration.

Applying a Hilbert transform gives a markedly improved result (Fig. 2c) compared with straightforward directional integration. The Hilbert transform produces a clear contrast between the features and the background. There is perhaps a slight ringing around the boundaries of the chromosomes (which is in agreement with the 1D simulation in Fig. 1). However, this is removed outside the boundaries for visualization using a transparency function modulated by intensity; as it is of lower intensity than the features. In our experience, the Hilbert transform has produced similar quality results across numerous DIC datasets.

The Hilbert transform may be compared with the variance (Feineigle et al., 1996) in that it is a simple single pass computation that transforms the bas relief appearance into an intensity. However, the variance only highlights the edges of specimen features, whereas, within regions of higher phase, the Hilbert transform always has intensity levels higher than the background (see Fig. 1c). This makes the Hilbert transform more useful for 3D visualization than the variance.

Other more advanced techniques for reconstructing the phase from DIC images include Wiener filtered deconvolution (van Munster et al., 1997) and rotational diversity (Preza et al., 1998). Both these methods use quantitative models of the DIC imaging system in an effort to accurately restore the phase. Wiener filtered deconvolution requires a measurement of the DIC prism shear, and involves a trade-off between noise and high and low frequency components in the image. It also assumes there is no absorption in the specimen and that DIC has a linear response to phase in the specimen, which are generally not valid assumptions for complex biological objects at high numerical aperture. The rotational diversity technique involves taking multiple images of the specimen at differing rotations with respect to the DIC shear angle and then combining them using iterative optimization against the imaging model. This overcomes the loss of information for features perpendicular to the shear, a drawback to DIC imaging not addressed by other methods.

By contrast, the Hilbert transform is not necessarily a
quantitatively accurate technique, such as integration or deconvolution might be. However, the images recorded using DIC are initially a mixture of non-linear phase and amplitude information. In these qualitative conditions it seems appropriate to use an image processing method that does not attenuate features of interest, namely high spatial frequency information and contrast of specimen objects from the background.

In terms of the technique itself, the Hilbert transform has the advantages that it requires no optical modification to the microscope, it is a simple single pass computation, and it does not require calibration for the shear of the DIC prism.

**3D visualization**

The Hilbert transform may be applied in turn to each 2D slice of a 3D dataset. A visualization of one such dataset is shown in Fig. 4, produced using the Hilbert transform and selective opacity based on intensity. The 3D images are from a focus series of orchid root tip metaphase chromosomes (of which Fig. 2a is one optical section). Comparison between 2D slices and the 3D visualization indicates that subtle structure available in the dataset is being borne out by the visualization. In particular, changes in phase along the z axis are clearly resolved. This fine detail was validated as being accurate by comparison with the features appearing in different z planes within the original 2D image slices (Fig. 5). This is especially evident when the dataset is rotated in an animation.

**Conclusion**

We have demonstrated that the Hilbert transform is a useful qualitative technique for effective 3D visualization of transmission DIC datasets. It overcomes the problems of streaking and high spatial frequency loss that occur in line-by-line integration techniques and it can be used for images of complex biological specimens that contain both phase and amplitude information. Because of its simplicity, the Hilbert transform technique can rapidly be applied to DIC datasets comprised of multiple focus planes using batched post-processing. This holds promise for future applications for 3D visualization of live-cell processes over time.

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![Fig. 4. A 3D visualization of a DIC through-focus series dataset (of which Fig. 2a is one optical section). A Hilbert transform was performed on each 2D slice in the stack. During visualization selective opacity was applied to voxels (3D pixels) according to their intensity. Horizontal scale is the same as Fig. 2, the volume height is 3.6 μm.](image)

![Fig. 5. Selected unprocessed 2D slices from the 3D dataset used in Fig. 4, cropped to highlight features that change through focus that are also clearly visible in the 3D visualization. This demonstrates that these fine details are preserved in the visualization. The three slices shown are at focal depths of 0.6 μm, 1.2 μm and 1.8 μm. The corresponding chromosome in Fig. 4 is in the middle right of the image.](image)
References


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