Supplementary Note 1: Fourier-optics interpretation of dynamic reflection differential phase contrast

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Introduction

The experiments reported in the main manuscript revealed a form of directional dynamic contrast arising under asymmetric illumination. The observed signal exhibits several characteristic signatures of differential phase contrast (DPC) imaging, including contrast reversal upon illumination inversion, strong dependence on illumination orientation and pupil asymmetry, and recovery of spatial localization through directional Hilbert-transform reconstruction. These observations are characteristic of imaging systems possessing an odd-symmetry transfer function and are widely associated with differential phase contrast and related phase-gradient imaging modalities [1, 2, 3].

Recent developments in reflection-mode phase imaging have shown that asymmetric illumination can generate phase-sensitive contrast in epi-detection geometries through the interaction of scattering and directional optical transfer functions [4]. The mirror-based configuration employed in the present work naturally extends this concept. The reflective substrate not only provides a strong mirror-returned field for interferometric detection but also redirects a substantial fraction of forward-scattered light back toward the objective, enabling the generation of directional contrast in reflection. As a result, the observed image formation appears more closely related to reflection differential phase contrast (RDPC) than to conventional D-FFOCT image formation alone.

The objective of this note is not to derive a rigorous forward model of the system, but rather to provide a phenomenological Fourier-optics framework capable of explaining the principal experimental observations. We show that asymmetric illumination breaks the equivalence between opposite spatial frequencies and generates sensitivity to directional optical quantities closely related to those encountered in DPC imaging. Within this framework, the measured dynamic signal can be interpreted as temporal fluctuations of a reflection differential phase contrast signal rather than as a conventional dynamic interferometric scattering signal.

The principal novelty is therefore not the observation of static RDPC itself, but the demonstration that RDPC signals support temporal fluctuation analysis and can be exploited as a functional imaging modality.

1 Weak phase object approximation

Let $t(\mathbf{r})$ denote the complex object function describing the optical modulation induced by the sample, where $\mathbf{r} = (x, y)$ denotes the transverse coordinates in the sample plane. For a weak phase object,

$$t(\mathbf{r}) = e^{i\phi(\mathbf{r})}, \tag{1}$$

where $\phi(\mathbf{r})$ represents the optical phase delay introduced by the specimen.

Assuming weak phase variations,

$$|\phi(\mathbf{r})| \ll 1, \tag{2}$$

the first-order approximation yields

$$t(\mathbf{r}) \simeq 1 + i\phi(\mathbf{r}). \tag{3}$$

Taking the Fourier transform of Eq. (3) gives

$$T(\mathbf{k}) = \delta(\mathbf{k}) + i\Phi(\mathbf{k}), \tag{4}$$

where $\mathbf{k} = (k_x, k_y)$ denotes the transverse spatial-frequency vector, $\delta(\mathbf{k})$ corresponds to the unscattered or zeroth-order component, and $\Phi(\mathbf{k})$ is the Fourier transform of $\phi(\mathbf{r})$.

Equation (4) provides a convenient framework for interpreting image formation. A weak phase object can be viewed as a strong unscattered field accompanied by weaker scattered components carrying information about the specimen. In the context of differential phase contrast and related imaging techniques, image contrast emerges from the manner in which these scattered components interfere with or are weighted relative to the dominant zeroth-order field.

Although biological cells cannot generally be considered ideal weak phase objects, this approximation provides a useful first-order description that captures the essential Fourier-space mechanisms underlying directional contrast generation. This approximation nevertheless forms the basis of most DPC image-formation models and provides an intuitive description of directional contrast generation [2, 3].

2 Phase gradients and odd spatial frequencies

The key observation is that directional phase-gradient information is associated with odd-symmetry spatial-frequency components. Consider the spatial derivative of the phase distribution along the x direction,

$$\frac{d\phi}{dx}. \tag{5}$$

The connection between phase gradients and spatial frequencies follows directly from the Fourier derivative theorem. Let

$$\Phi(k_x) = \mathcal{F}[\phi(x)] = \int_{-\infty}^{+\infty} \phi(x) e^{-ik_x x}\, dx. \tag{6}$$

The Fourier transform of the spatial derivative is

$$\mathcal{F}\left[\frac{d\phi}{dx}\right] = \int_{-\infty}^{+\infty} \frac{d\phi}{dx} e^{-ik_x x}\, dx. \tag{7}$$

Integrating by parts yields

$$\mathcal{F}\left[\frac{d\phi}{dx}\right] = \Big[\phi(x)e^{-ik_x x}\Big]_{-\infty}^{+\infty} + ik_x \int_{-\infty}^{+\infty} \phi(x) e^{-ik_x x}\, dx. \tag{8}$$

Assuming that the field vanishes at infinity, the boundary term is zero, giving

$$\mathcal{F}\left[\frac{d\phi}{dx}\right] = ik_x \Phi(k_x). \tag{9}$$

Differentiation in real space therefore corresponds to multiplication by the spatial frequency coordinate in Fourier space. Importantly, the factor ik_x changes sign when $k_x \to -k_x$, indicating that phase-gradient information is encoded in odd-symmetry spectral components.

The previous derivation considered a phase gradient along a single coordinate direction. More generally, in two dimensions, the relevant quantity for asymmetric illumination is the phase gradient projected along the illumination direction.

Let $\mathbf{u}$ denote a unit vector describing the illumination direction. The corresponding directional derivative is

$$\mathbf{u} \cdot \nabla\phi(\mathbf{r}) = u_x \frac{\partial\phi}{\partial x} + u_y \frac{\partial\phi}{\partial y}. \tag{10}$$

Using the Fourier derivative theorem along each coordinate direction yields

$$\mathcal{F}\left[\mathbf{u} \cdot \nabla\phi\right] = i(\mathbf{k} \cdot \mathbf{u})\Phi(\mathbf{k}), \tag{11}$$

where $\mathbf{k} = (k_x, k_y)$ denotes the transverse spatial-frequency vector.

The factor $i(\mathbf{k} \cdot \mathbf{u})$ is odd under spatial-frequency inversion,

$$i(\mathbf{k} \cdot \mathbf{u}) \xrightarrow{\mathbf{k} \to -\mathbf{k}} -i(\mathbf{k} \cdot \mathbf{u}). \tag{12}$$

Consequently, directional phase-gradient information resides in odd-symmetry spectral components. Such information is strongly suppressed in imaging systems that weight opposite regions of Fourier space equally. Detection of directional phase gradients therefore requires a mechanism that distinguishes between positive and negative spatial frequencies along a given direction.

This principle forms the basis of differential phase contrast (DPC) imaging, where asymmetric illumination generates an odd transfer function that converts directional phase-gradient information into measurable intensity contrast. More generally, any imaging modality possessing an odd-symmetry transfer function will exhibit sensitivity to directional optical signatures.

In the framework developed in this note, the directional dynamic contrast observed experimentally is interpreted as arising from the interaction between such odd-symmetry optical components and an asymmetric illumination transfer function. The following sections examine how illumination symmetry controls the visibility of these directional signatures.

3 Symmetric illumination

The angular support of the imaging system can be described by a pupil or transfer function $P(\mathbf{k})$. Under symmetric illumination,

$$P(\mathbf{k}) = P(-\mathbf{k}). \tag{13}$$

Positive and negative spatial frequencies are then weighted equivalently. The image formation process is largely invariant under spatial-frequency inversion,

$$\mathbf{k} \to -\mathbf{k}. \tag{14}$$

As a consequence, odd-symmetry components are not efficiently converted into measurable intensity contrast. This is why weak transparent phase objects often exhibit low contrast under symmetric bright-field illumination. In the context of D-FFOCT, symmetric illumination similarly reduces sensitivity to directional optical signatures because contributions from opposite angular components are simultaneously present and weighted in a balanced manner.

4 Asymmetric illumination and symmetry breaking

Asymmetric illumination breaks the symmetry condition in Eq. (13). Instead,

$$P(\mathbf{k}) \neq P(-\mathbf{k}). \tag{15}$$

Positive and negative spatial frequencies are therefore weighted differently. The imaging system becomes sensitive to odd-symmetry components of the optical field, including directional phase-gradient-like terms.

A simple phenomenological representation of the asymmetric scattered field is

$$\Delta E_{\text{asym}} = E_{\text{diff}}^{(+)} - E_{\text{diff}}^{(-)}, \tag{16}$$

where $E_{\text{diff}}^{(+)}$ and $E_{\text{diff}}^{(-)}$ denote diffracted or scattered field contributions originating from opposite regions of Fourier space. Since Eq. (16) is antisymmetric under spatial-frequency inversion, it behaves similarly to a directional derivative operator. To first order, this may be approximated as

$$\Delta E_{\text{asym}} \propto \mathbf{u} \cdot \nabla \phi, \tag{17}$$

where $\mathbf{u}$ denotes the illumination direction.

Equation (17) should be interpreted phenomenologically rather than as a rigorous derivation. It expresses the fact that asymmetric illumination can convert directional phase-gradient-like information into a measurable optical field. This is the central principle underlying differential phase contrast and related oblique-illumination imaging modalities.

5 Connection with differential phase contrast

In differential phase contrast microscopy, asymmetric illumination creates an odd optical transfer function [1, 2, 3]. The resulting image intensity contains a component proportional, to first order, to a directional phase gradient,

$$I_{\text{DPC}}(\mathbf{r}) \propto \mathbf{u} \cdot \nabla \phi(\mathbf{r}). \tag{18}$$

This immediately explains several characteristic features of DPC-like imaging. Reversing the illumination direction changes $\mathbf{u} \to -\mathbf{u}$, which reverses the sign of the contrast. Rotating the illumination direction rotates the measured gradient direction. Increasing the degree of pupil asymmetry increases the strength of the odd transfer-function component, while restricting the pupil support reduces the spatial-frequency bandwidth available for image formation.

The asymmetric-illumination measurements reported in this work exhibit these same signatures: contrast reversal upon illumination reversal, dependence on illumination orientation, enhancement with increasing pupil asymmetry, and partial recovery of spatial localization through directional Hilbert-transform reconstruction. These observations motivate the interpretation of the observed contrast as a dynamic reflection differential phase contrast (D-RDPC) signal acquired on a D-FFOCT platform.

6 Mirror-assisted directional interferometric imaging

The previous section establishes that the observed directional contrast exhibits the characteristic signatures of differential phase contrast imaging. The remaining question is therefore not whether the mechanism is DPC-like, but rather how such a directional signal is generated in the mirror-based geometry used here.

Recent developments in reflection intensity phase microscopy have shown that asymmetric illumination can generate phase-sensitive image contrast in reflection geometries through the interaction of illumination asymmetry, scattering, and the optical transfer function of the imaging system [4]. In contrast to conventional transmission DPC, where directional information is encoded in transmitted light, reflection-mode implementations recover directional information from fields collected through the same objective used for illumination.

The present configuration naturally belongs to this broader family of reflection phase-imaging techniques. Cells are cultured directly on a highly reflective substrate and illuminated asymmetrically through the objective pupil. The mirror provides a strong reflected zeroth-order field while simultaneously redirecting a substantial fraction of the transmitted and forward-scattered light back toward the objective. As a consequence, information that would normally leave the system in transmission can contribute to the detected reflection signal.

A simplified description of the detected field can be written as

$$E_{\mathrm{det}} = E_0^{\mathrm{mirror}} + E_s, \tag{19}$$

where E_0^{mirror} denotes the dominant mirror-returned field and E_s denotes the field carrying information about the specimen.

The detected intensity is therefore

$$I = \left| E_0^{\mathrm{mirror}} + E_s \right|^2, \tag{20}$$

which expands to

$$I = \left| E_0^{\mathrm{mirror}} \right|^2 + |E_s|^2 + 2\,\mathrm{Re}\left[\left(E_0^{\mathrm{mirror}} \right)^* E_s \right]. \tag{21}$$

Because the mirror-returned field is much stronger than the sample-generated field, the useful signal is dominated by the interference term

$$I_{\mathrm{int}} \approx 2\,\mathrm{Re}\left[\left(E_0^{\mathrm{mirror}} \right)^* E_s \right]. \tag{22}$$

The precise composition of E_s may involve multiple scattering pathways, including mirror-returned forward-scattered fields and conventional backscattered contributions. Nevertheless, the experimental observations demonstrate that under asymmetric illumination the detected signal behaves as a directional quantity whose sign and magnitude depend on illumination orientation. Consistent with DPC theory, this directional signal can therefore be phenomenologically represented as

$$I_{\mathrm{dir}}(\mathbf{r}) \propto \mathbf{u} \cdot \nabla \phi(\mathbf{r}), \tag{23}$$

where $\mathbf{u}$ denotes the illumination direction.

Within this interpretation, the mirror should not be viewed merely as a reference surface. Rather, it enables a reflection-mode realization of directional phase-gradient imaging while simultaneously providing the strong reference-like field required for sensitive interferometric detection. The resulting system therefore combines key features of reflection DPC and self-referenced interferometric microscopy.

7 Dynamic contrast

The principal novelty of the present work does not lie in the observation of reflection differential phase contrast itself, but rather in the analysis of its temporal fluctuations. Unlike conventional DPC or reflection phase microscopy, the reported images are not static measurements of a directional optical signal. Instead, dynamic contrast is computed from temporal variations of that signal.

Within the phenomenological framework developed above, the static reflection differential phase contrast signal can be written as

$$I_{\mathrm{RDPC}}(\mathbf{r}, t) \propto \mathbf{u} \cdot \nabla \phi(\mathbf{r}, t), \tag{24}$$

where the explicit time dependence reflects the continuous intracellular activity occurring within living cells.

A dynamic metric may then be constructed from temporal fluctuations of the directional signal. In simplified form,

$$D(\mathbf{r}) \propto \langle |\Delta I_{\mathrm{RDPC}}(\mathbf{r}, t)| \rangle_t \,, \tag{25}$$

where $\langle \cdot \rangle_t$ denotes temporal averaging and

$$\Delta I_{\mathrm{RDPC}}(\mathbf{r}, t) = I_{\mathrm{RDPC}}(\mathbf{r}, t + \tau) - I_{\mathrm{RDPC}}(\mathbf{r}, t), \tag{26}$$

Substituting Eq. (24) into Eq. (25) yields

$$D(\mathbf{r}) \propto \langle |\Delta \left[\mathbf{u} \cdot \nabla \phi(\mathbf{r}, t) \right]| \rangle_t \,. \tag{27}$$

Equation (27) constitutes the central phenomenological result of this note. It suggests that D-RDPC measures temporal fluctuations of a directional phase-gradient-like optical signal rather than the static directional signal itself. In other words, the measured contrast originates not from the directional optical quantity traditionally associated with DPC imaging, but from its temporal variations.

This interpretation differs fundamentally from conventional D-FFOCT, where dynamic contrast is generally viewed as arising from temporal fluctuations of an interferometric scattering signal. In dynamic RDPC, the fluctuating quantity is instead a directional optical quantity generated by the underlying phase-gradient transfer mechanism. Temporal processing therefore transforms a directional structural signal into a functional imaging modality sensitive to fluctuations of that directional contrast.

The precise microscopic origin of the fluctuations remains an open question. Intracellular motion, organelle transport, membrane dynamics, and refractive-index redistribution may all contribute to temporal variations of the directional optical signal. Regardless of their microscopic origin, the present framework provides a simple interpretation of the measured contrast as temporal fluctuations of a directional optical signal.

8 Directional Hilbert reconstruction

A characteristic consequence of differential phase contrast imaging is that the measured signal behaves as a directional derivative of the underlying optical structure. While this derivative-like contrast enhances sensitivity to directional optical gradients, it also reduces spatial localization and can produce the elongated or asymmetric features observed in the raw interferograms.

For a directional signal proportional to

$$I_{\mathrm{RDPC}}(\mathbf{r}) \propto \mathbf{u} \cdot \nabla \phi(\mathbf{r}), \tag{28}$$

the measured image contains predominantly odd-symmetry spatial-frequency components. Information remains present in the image, but is redistributed by the derivative-like transfer function.

The directional Hilbert transform provides a simple means of partially reversing this effect [5, 6]. In Fourier space, the directional Hilbert filter used in this work is

$$H_{\mathbf{u}}(\mathbf{k}) = -i \, \mathrm{sign} \left(\mathbf{k} \cdot \mathbf{u} \right), \tag{29}$$

where $\mathbf{u}$ denotes the illumination direction and $\mathbf{k}$ the spatial-frequency vector.

This operation introduces a phase shift of $\pm\pi/2$ depending on the sign of the spatial frequency along the direction $\mathbf{u}$. For signals dominated by directional derivative-like contrast, the Hilbert

transform therefore acts as a directional integration operator and can partially reconstruct a more localized representation of the underlying structures.

The successful application of directional Hilbert reconstruction in the present experiments provides additional support for the RDPC interpretation. The recovery of localized intracellular structures indicates that the apparent broadening observed under strongly asymmetric illumination does not correspond to a loss of information, but rather to a redistribution of information imposed by the directional transfer function.

The success of the reconstruction provides independent support for the presence of a dominant odd-symmetry directional component in the detected signal. Although the measured contrast cannot be reduced to a pure phase-gradient image, the observed behaviour is consistent with the transfer functions encountered in differential phase contrast imaging. The observation that spatial localization can be substantially recovered after directional reconstruction therefore constitutes independent evidence supporting the D-RDPC interpretation proposed throughout this note.

9 Summary of the phenomenological model

In summary, symmetric illumination suppresses odd-symmetry optical components and produces image contrast that is largely insensitive to directional phase gradients. Asymmetric illumination breaks this symmetry and generates sensitivity to directional optical quantities analogous to those encountered in differential phase contrast microscopy. In the mirror-based geometry used here, forward-scattered light is redirected back toward the objective and interferes with a strong mirror-returned field, producing a reflection-mode directional signal. Temporal fluctuation analysis then transforms this static RDPC signal into a functional imaging modality sensitive to fluctuations of the underlying directional optical quantity. Within this phenomenological framework, the observed dynamic contrast is most consistently interpreted as dynamic reflection differential phase contrast. Equation (27) provides the simplest phenomenological expression of this interpretation.

Conclusion

This supplementary note provides a phenomenological Fourier-optics framework for interpreting the directional dynamic contrast observed under asymmetric illumination. The combined experimental observations, including contrast reversal upon illumination reversal, dependence on illumination orientation, enhancement with increasing pupil asymmetry, and successful directional Hilbert-transform reconstruction, are all naturally explained by an odd-symmetry transfer function closely related to reflection differential phase contrast.

The mirror-based geometry introduces several distinctive features compared with conventional DPC. The reflective substrate provides a strong mirror-returned field, redirects forward-scattered components back toward the objective, and creates a double-pass interaction between the illumination field and the specimen. As a result, the system combines characteristics of reflection phase microscopy, self-referenced interferometric imaging, and directional transfer-function engineering within a single optical configuration.

Most importantly, the measured quantity is not the static directional signal itself, but its temporal fluctuations. Within the proposed phenomenological model, the observed dynamic contrast may therefore be interpreted as fluctuations of a directional phase-gradient-like optical signal. From this perspective, the present method represents a dynamic extension of reflection differential phase contrast imaging.

Although a rigorous forward model remains to be developed, the framework presented here provides a coherent explanation for the principal experimental observations and supports the interpretation that the observed contrast corresponds to dynamic reflection differential phase

contrast. More broadly, the analysis suggests that temporal fluctuation analysis may be applied to directional phase-gradient signals in much the same way that it has previously been applied to interferometric scattering signals. From this perspective, dynamic RDPC establishes a conceptual bridge between temporal fluctuation microscopy and reflection-mode phase-gradient imaging.

References

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