Phase estimation in

Differential Inteference Contrast (DIC) Microscopy

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Motivation

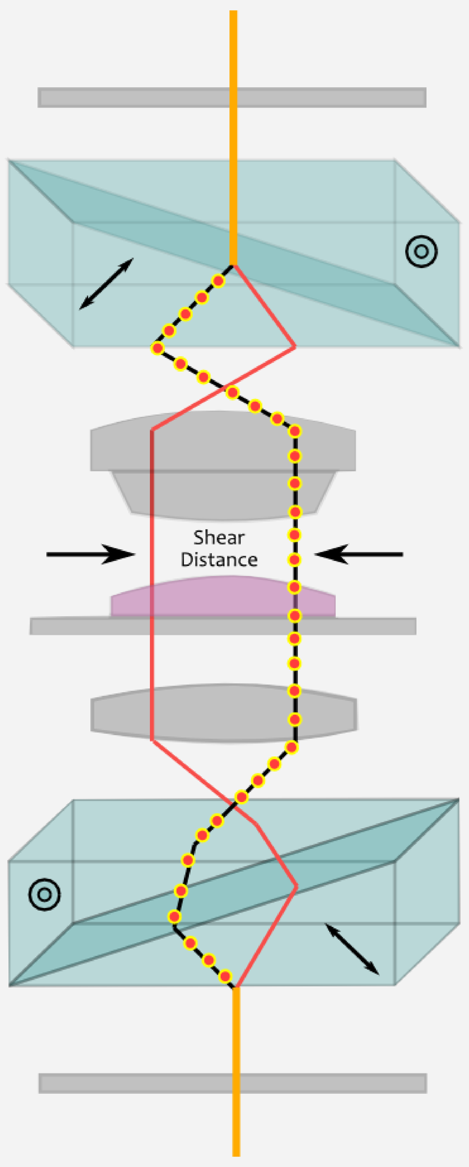

Fig 1. DIC system

DIC microscopy allows to image unstained biological specimens, it achieves **high lateral resolution** and produces **high contrast** images of phase objects.

It is accomplished from the interference of two waves that have a lateral differential displacement (shear) and are phase shifted relative one to each other.

The resulting intensity image is given by a **nonlinear function** of the hidden phase gradients of the object.

Previous works for phase estimation in DIC microscopy have considered methods such as Phase shifting [1], Transport of Intensity [2], and Rotational-Diversity [3].

In order to retrieve information hidden in the phase gradients, it is necessary to solve a nonlinear, ill-posed inverse problem.

Proposed approach: **We present a gradient-based optimization method which minimizes the sum of a nonlinear least-squares discrepancy measure and a smooth approximation of the total variation.**

Polychromatic rotational-diversity model

The intensity image is described by the non-linear function of the phase gradient in the shear direction given by

$$i_{k,\lambda}(x) = a_1 \left| \left(e^{-j\frac{\phi}{\lambda}} * h_{k,\lambda} \right)(x) \right|^2 \quad (1)$$

where a_1 is a constant; $k \in \{0,1,\dots,K-1\}$; $x \in \chi = \{0,1,\dots,N-1\}^2$, where N is the number of elements in each dimension of the object and image space.

$e^{-j\frac{\phi(x)}{\lambda}}$ is the specimen's transmission function, being $\phi(x)$ **the specimen's phase function we want to retrieve.**

$h_{k,\lambda}(x)$ is the k-th rotation of the polychromatic DIC amplitude point spread function.

$$h_{k,\lambda}(x) = \frac{1}{2}\left[e^{-j\Delta\theta} p_{k,\lambda}(x - \Delta x, y) - e^{j\Delta\theta} p_{k,\lambda}(x + \Delta x, y) \right] \quad (2)$$

expressed in terms of the coherent PSF of the microscope's objective lens $p_{k,\lambda}(x)$ at angle τ_k and for the wavelength λ, where $2\Delta\theta$ is the DIC bias retardation and $2\Delta x$ is the shear.

Gradient-based Phase Estimation

$$\min_{\phi \in \mathbb{R}^{N^2}} J(\phi) \equiv \sum_{\lambda=\lambda_1}^{\lambda_3} \sum_{k=0}^{K-1} \sum_{x \in \chi} \left[o_{k,\lambda}(x) - i_{k,\lambda}(x) \right]^2 + \mu J_{TV}(\phi) \quad (3)$$

where $o_{k,\lambda}$ is the k-th observed polychromatic image, $i_{k,\lambda}$ is the k-th theoretical polychromatic image as shown in equation (1), J_{TV} is a smooth approximation of the total variation and μ is the regularization parameter.

Because of model (1):

- The true phase might be recovered only up to a real constant: $J(\phi + c\,\mathbb{1}) = J(\phi)$, where $\mathbb{1}$ is the vector of all ones.

Problem (3) has been approached by using a gradient-descent method defined as

$$\phi^{(m+1)} = \phi^{m} - \alpha_m \nabla J\left(\phi^{(m)}\right) \quad (4)$$

where α_m is the step size.

The components of gradient are given by

$$\frac{\partial J(\phi)}{\partial \phi(x_0)} = \sum_{\lambda=\lambda_1}^{\lambda_3} \sum_{k=0}^{K-1} -\frac{4}{\lambda} \xi(\phi, x_0, h_{k,\lambda}) + \mu \frac{\partial J_{TV}(\phi)}{\partial \phi(x_0)} \quad (5)$$

here

$$\xi(\phi, x_0, h_{k,\lambda}) = \Im\left\{ \left[\left(r_{k,\lambda} * \left(h_{k,\lambda} \circledast e^{-j\frac{\phi}{\lambda}} \right) \right) \circledast h^*_{k,\lambda} \right](x_0) . e^{j\frac{\phi(x_0)}{\lambda}} \right\} \quad (6)$$

Convolution-based: Compact and fast computations

$\Im(\cdot)$ denotes the operator that takes the imaginary part of its argument, $r_{k,\lambda} = i_{k,\lambda} - o_{k,\lambda}$, $h^*_{k,\lambda}(x_0) = \overline{h_{k,\lambda}(-x_0)}$,
$*$ is the componentwise product and $\circledast$ is the convolution operator.

Choice of the step size parameter

The stationarity of the limit points of the sequence generated by (4) is guaranteed by choosing the step size $\alpha_m = \alpha_m^{(0)} \gamma^{l_m}$ where $\gamma \in (0,1)$ and l_m is the smallest natural number satisfying the Armijo condition, being $\beta \in (0,1)$

$$J\left(\phi^{(m)} - \alpha_m \nabla J(\phi^{(m)})\right) \le J(\phi^{(m)}) - \beta \alpha_m \left\| \nabla J(\phi^{(m)}) \right\|^2 \quad (7)$$

The initial guess $\alpha_m^{(0)}$ is chosen using a rule recently proposed by Fletcher [4]:

- It is based on the storage of q consecutive gradients and steplengths.
- It computes q Ritz values approximating some eigenvalues of the Hessian matrix of J.
- It improves speed of convergence.

Numerical Tests

Physical parameters			
Image size	64 x 64 pixels	Objective	10x /0.3 N.A.
Pixel size	$0.30\mu m$ x $0.30\mu m$	Bias	$2\Delta\theta = 1.57 rad$
Physical size	$19.2\ \mu m$ x $19.2\ \mu m$	Shear	$\Delta x = 0.68 \mu m$

Algorithm set up parameters	
Initialization	$\phi^{(0)} = 0$
Step size	Every $q = 4$ iterations
Line search	$\gamma = 0.5$ and $\beta = 10^{-4}$
Regularization	$\mu_{cone} = 10^{-2}$; $\mu_{cross} = 4 * 10^{-2}$
TV smoothing	$\delta_{cone} = 10^{-2}$; $\delta_{cross} = 10^{-3}$
Stopping rule	$\left\| \phi^{(m)} - \phi^{(m-1)} \right\| < 10^{-5}$

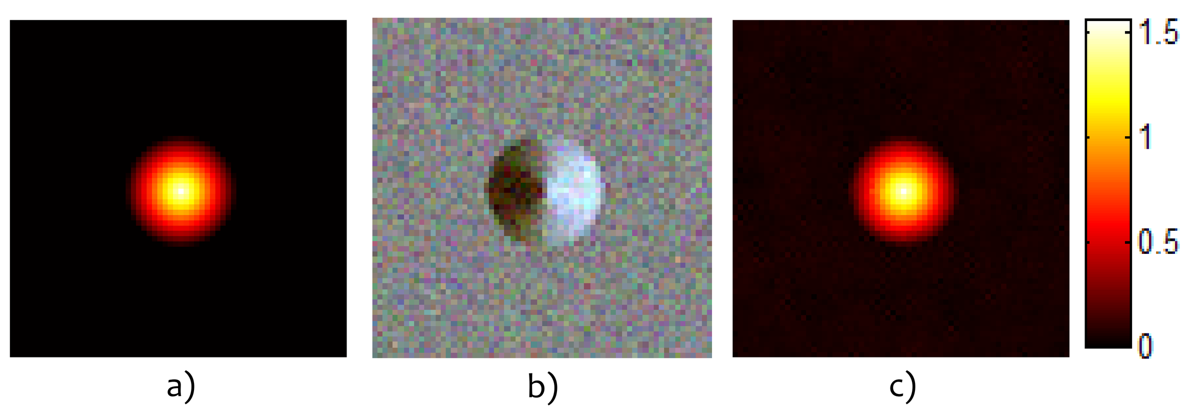

Fig 2. Cone test problem. a) True object, b) Noisy DIC color image taken at angle $\tau_0 = 0^{\circ}$ and SNR = 4.5, c) Reconstructed phase.

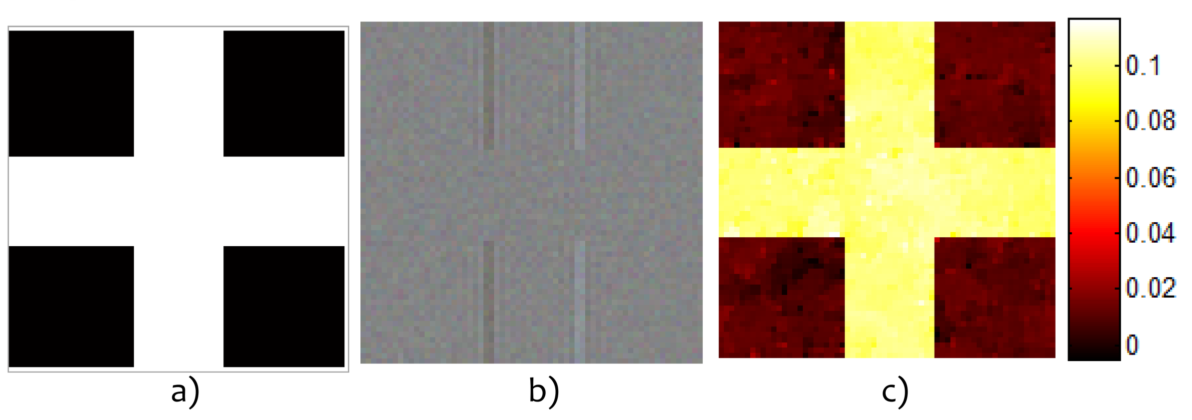

Fig 3. Cross test problem. a) True object, b) Noisy DIC color image taken at angle $\tau_0 = 0^{\circ}$ and SNR = 4.5, c) Reconstructed phase.

- For the cone, the relative error is below 6% in the most noisy case.
- For the cross, the shape is well-recovered, as well as the phase difference.
- In both cases, the proposed approach seems to be quite robust to noise.

Discussion

Comparison with the Barzilai-Borwein rule:

$$\alpha_m^{BB1} = \frac{\left(\phi^{(m)} - \phi^{(m-1)}\right)^T \left(\phi^{(m)} - \phi^{(m-1)}\right)}{\left(\phi^{(m)} - \phi^{(m-1)}\right)^T \left(\nabla J(\phi^{(m)}) - \nabla J(\phi^{(m-1)})\right)} \quad (8)$$

Error: $\|\phi^{(m)} - \phi^* - \bar{c}\mathbb{1}\| / \|\phi^*\|$ where ϕ^* is the true object, $\mathbb{1}$ is the vector of all ones and $\bar{c} = \sum_{x \in \chi} [\phi^{(m)}(x) - \phi^*(x)]/N$.

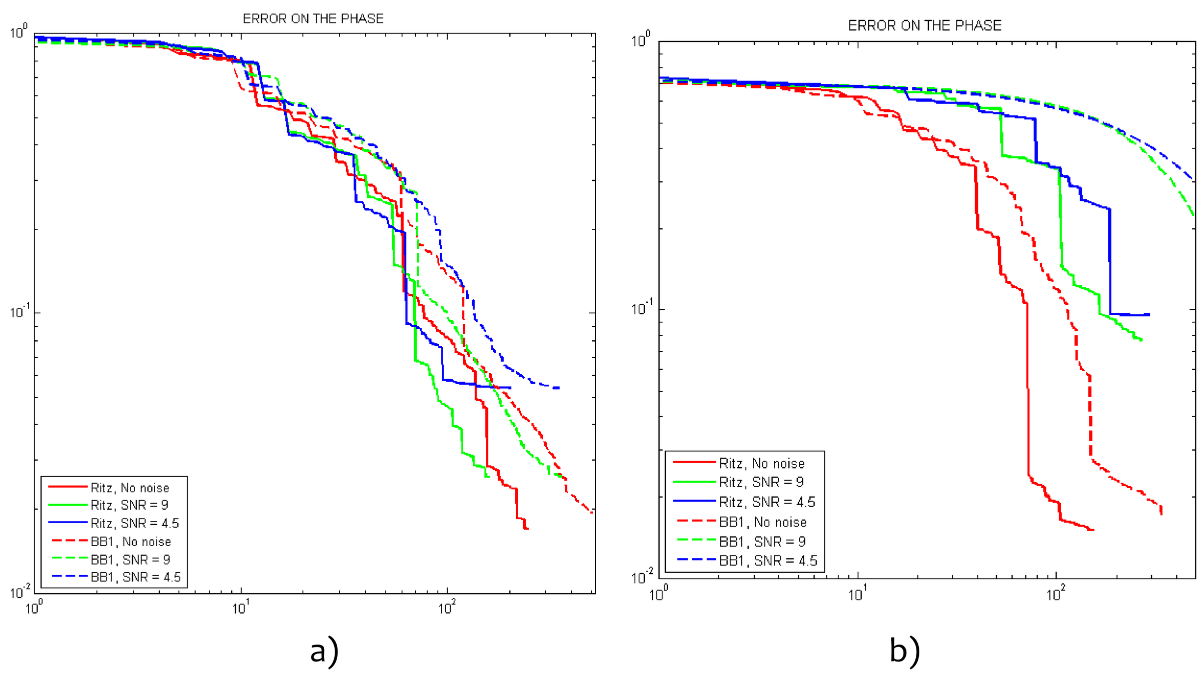

Fig 4. Relative error versus the number of iterations; a) Cone; b) Cross

- The proposed choice for the step size outperforms significantly the standard BB1 approach, in terms of **efficiency, accuracy and robustness to noise.**
- BB1 recovers a coarse estimate in 500 iterations, while our approach **provides an error below 10% in roughly 300 iterations.**
- **The number of iterations is reduced of nearly 50%.**

Conclusions

Numerical experience showed that:

- The method is robust with respect to the presence of high rates of noise.
- It overcomes the assumption of no previous knowledge of the initial guess.
- It provides very good estimations in less iterations than a standard gradient descent method.
- The computational time is significantly reduced thanks to our convolution-based formulation.

Future Work

- Verify the proposed method on real experimental images.
- Consider different formulations of problem (3), e.g. by doing change of variables $u = e^{-j\frac{\phi}{\lambda}}$ subject to $\|u\|=1$

References

[1] Lin W., Yu S., and Lin S., "Accelerating phase shifting technique in quantitative differential interference contrast system for critical dimension measurement of TFT substrate," SID Int. Symp. Dig. Tec., vol. 43, no. 1, 2012.

[2] Bostan E., Froustey E., Rappaz B., Shaffer E., Sage D., and Unser M., "Phase retrieval by using transport-ofintensity equation and differential interference contrast microscopy," in Proc. 21th IEEE International Conference on Image Processing, 2014, pp. 3939–3943.

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[4] R. Fletcher, "A limited memory steepest descent method," Math. Program., vol. 135, no. 1–2, pp. 413–436, 2012.

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