# Overview of the automatic reconstruction method for quantitative phase imaging using a digital holographic microscope operating in non-telecentric regime

**B. Bogue-Jimenez[1], C. Trujillo[2], and A. Doblas[1]**

[1]Department of Electrical & Computer Eng., University of Memphis, USA

[2]School of Applied Sciences and Engineering, Universidad EAFIT, Medellin, Colombia

**SPIE Photonics West BiOS - Quantitative Phase Imaging IX 12389-30**
**January 29, 2023 @ 2.30 pm – 2.50 pm**

Optical Imaging Research Lab

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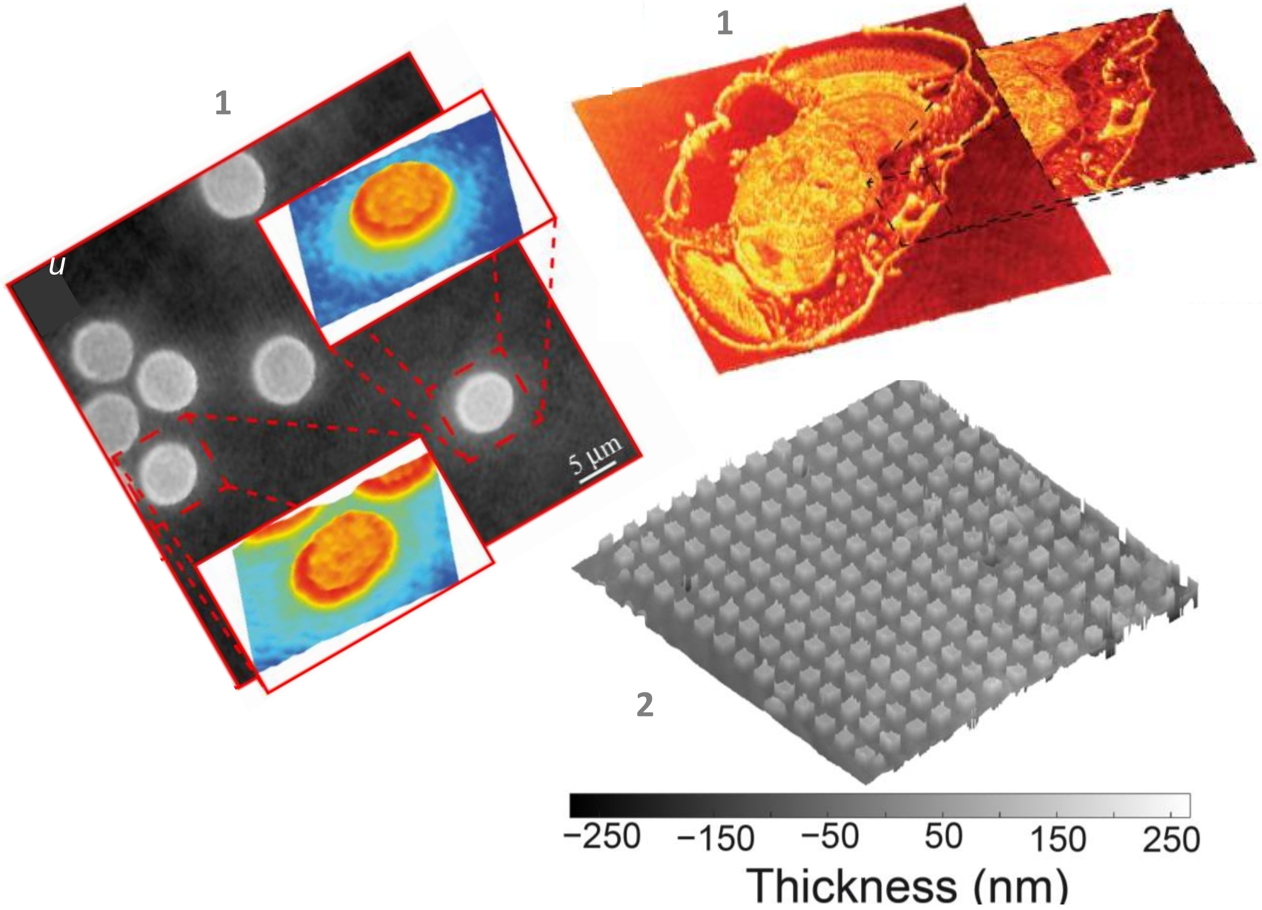
# Digital Holographic Microscopy (DHM)

## Highlights:

- **Quantitative Phase Imaging (QPI)** modality largely used for **material and biological applications**.
- Provides **label-free** (i.e., unstained) **imaging**.
- Reconstructed amplitude and phase information of the sample.
- Off-axis DHM systems allow for single-shot imaging.

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1. A. Doblas, *et. al.*, J. Biomed. Opt. 19, 46022 (2014).
2. A. Doblas *et al.*, Sensors 22, 3793 (2022)

# Optical Recording Stage in DHM

A digital hologram is a *hybrid* imaging method:

- <u>Optical recording stage: </u>interference between two beams

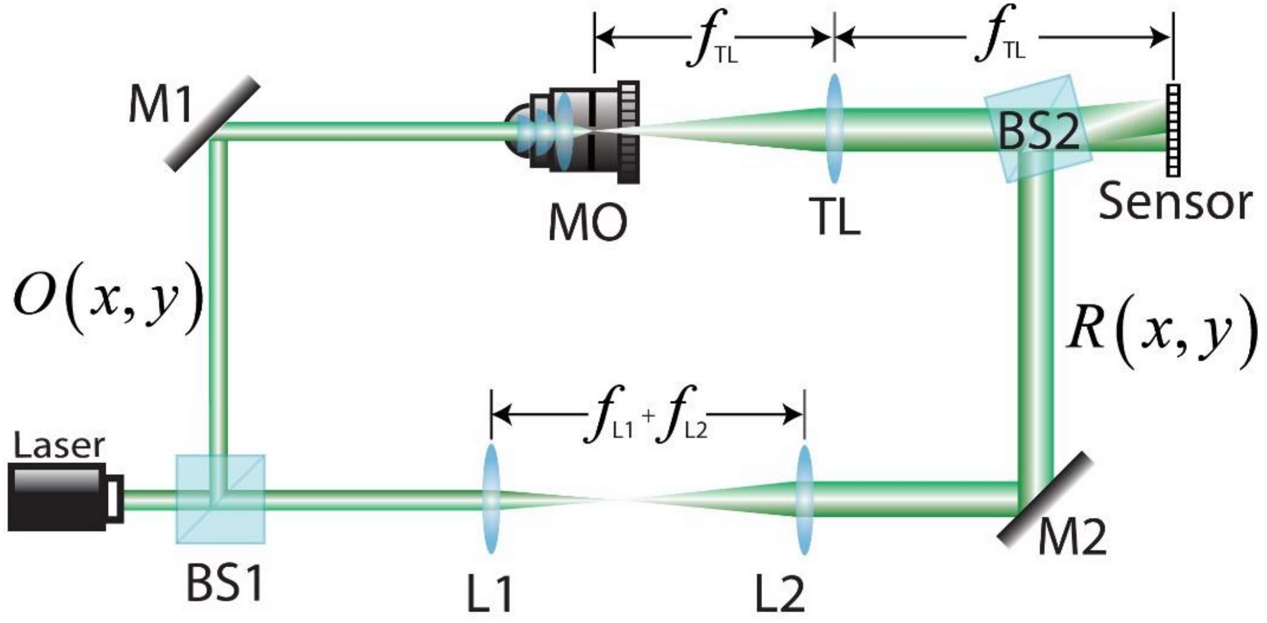
**Reference beam (i.e., plane wave):**

$$r(\boldsymbol{x}) = \exp(\mathrm{i}\,\boldsymbol{k} \bullet \boldsymbol{x})$$

$$\boldsymbol{x} = (x, y); \boldsymbol{k} = (k_x, k_y)$$

Reference angle

**Object beam:** $u_{IP}(\boldsymbol{x}) \propto \frac{1}{M_L^2}\, o\left(\frac{\boldsymbol{x}}{M_L}\right) \otimes_2 P\left(\frac{\boldsymbol{x}}{\lambda f_{TL}}\right)$




$$M_L = -f_{TL} / f_{MO}$$

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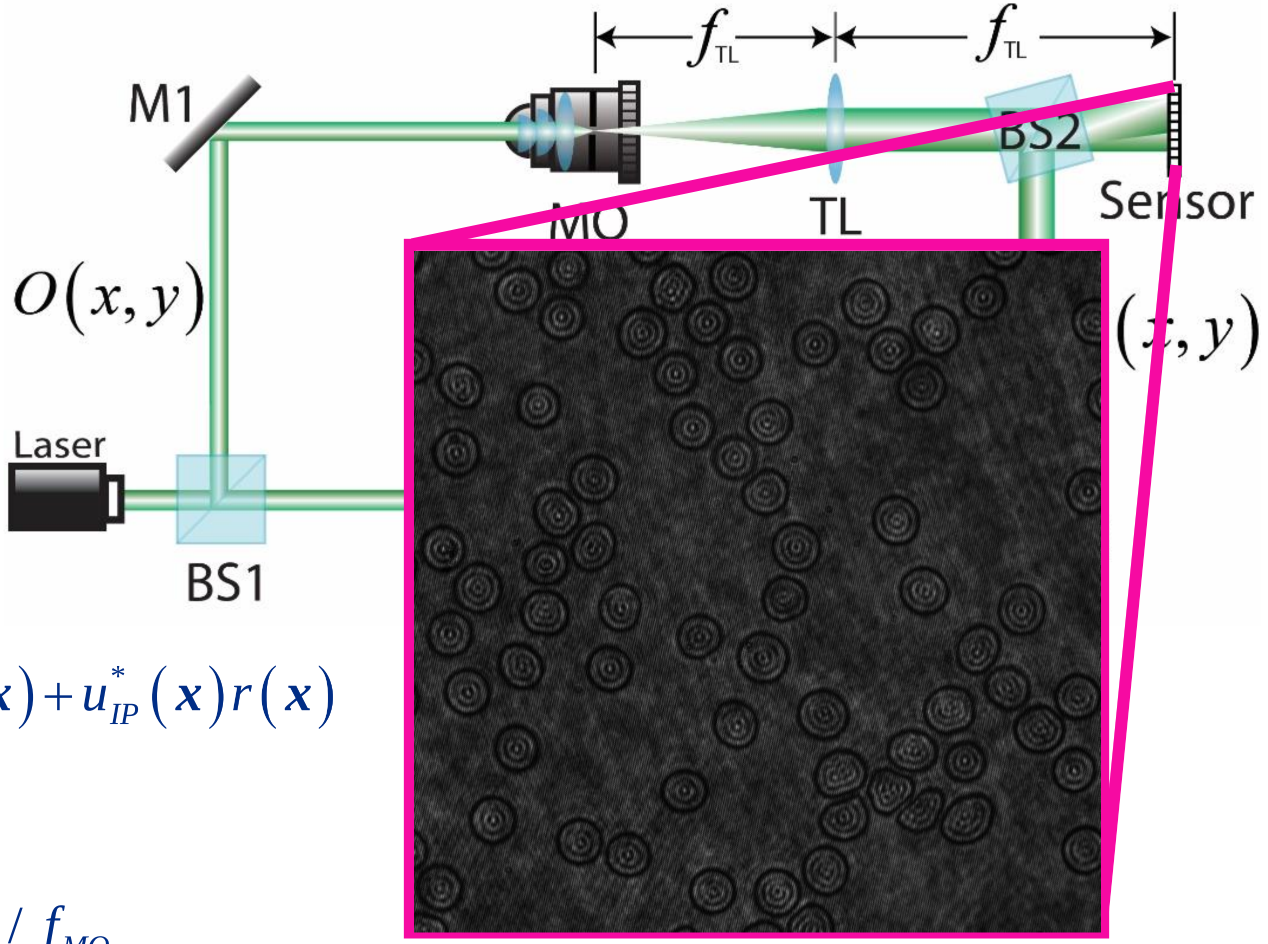
**Hologram:** $h(\boldsymbol{x}) = |u_{IP}(\boldsymbol{x})|^2 + |r(\boldsymbol{x})|^2 + u_{IP}(\boldsymbol{x}) r^*(\boldsymbol{x}) + u_{IP}^*(\boldsymbol{x}) r(\boldsymbol{x})$

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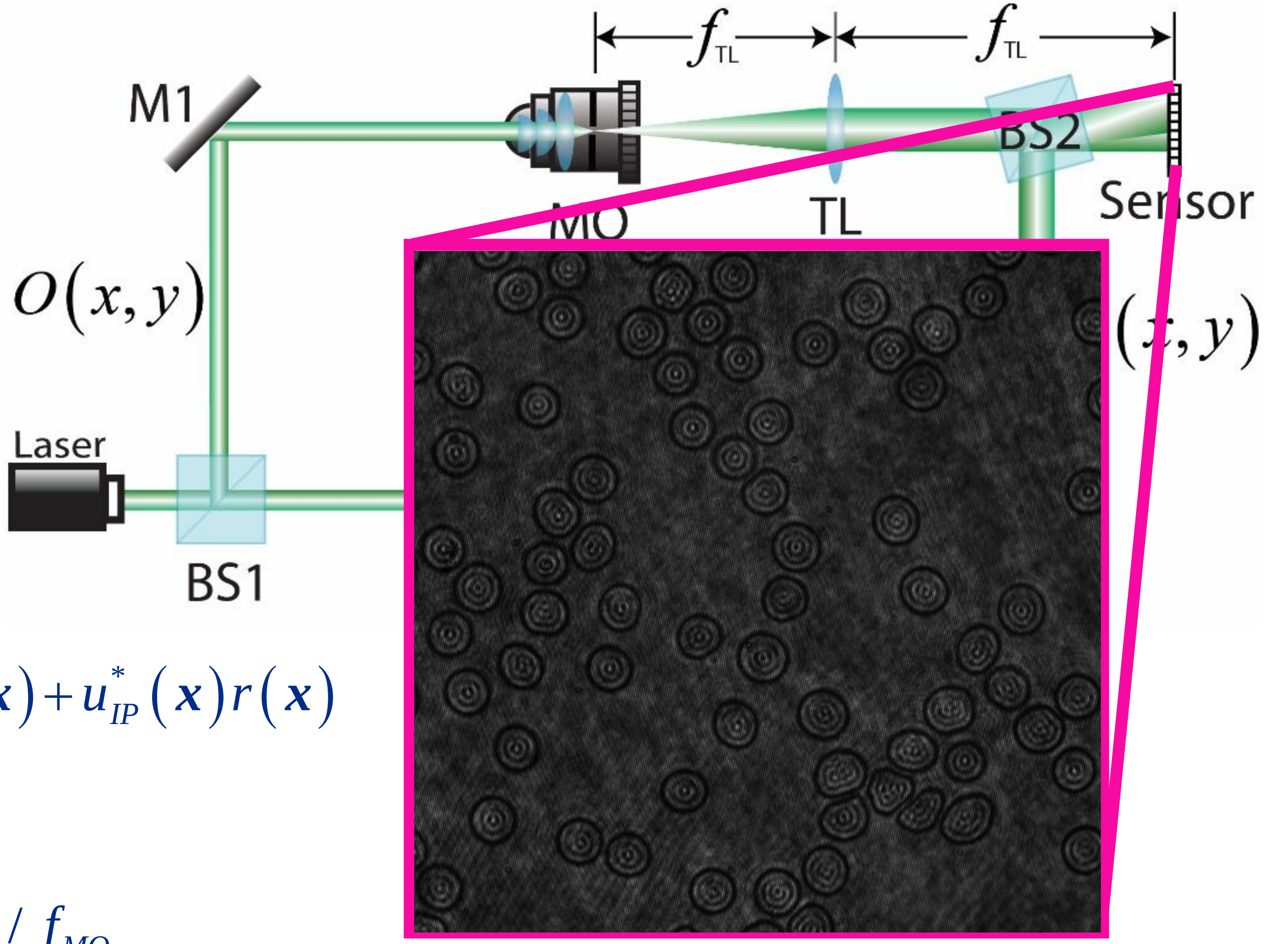
Intensity information

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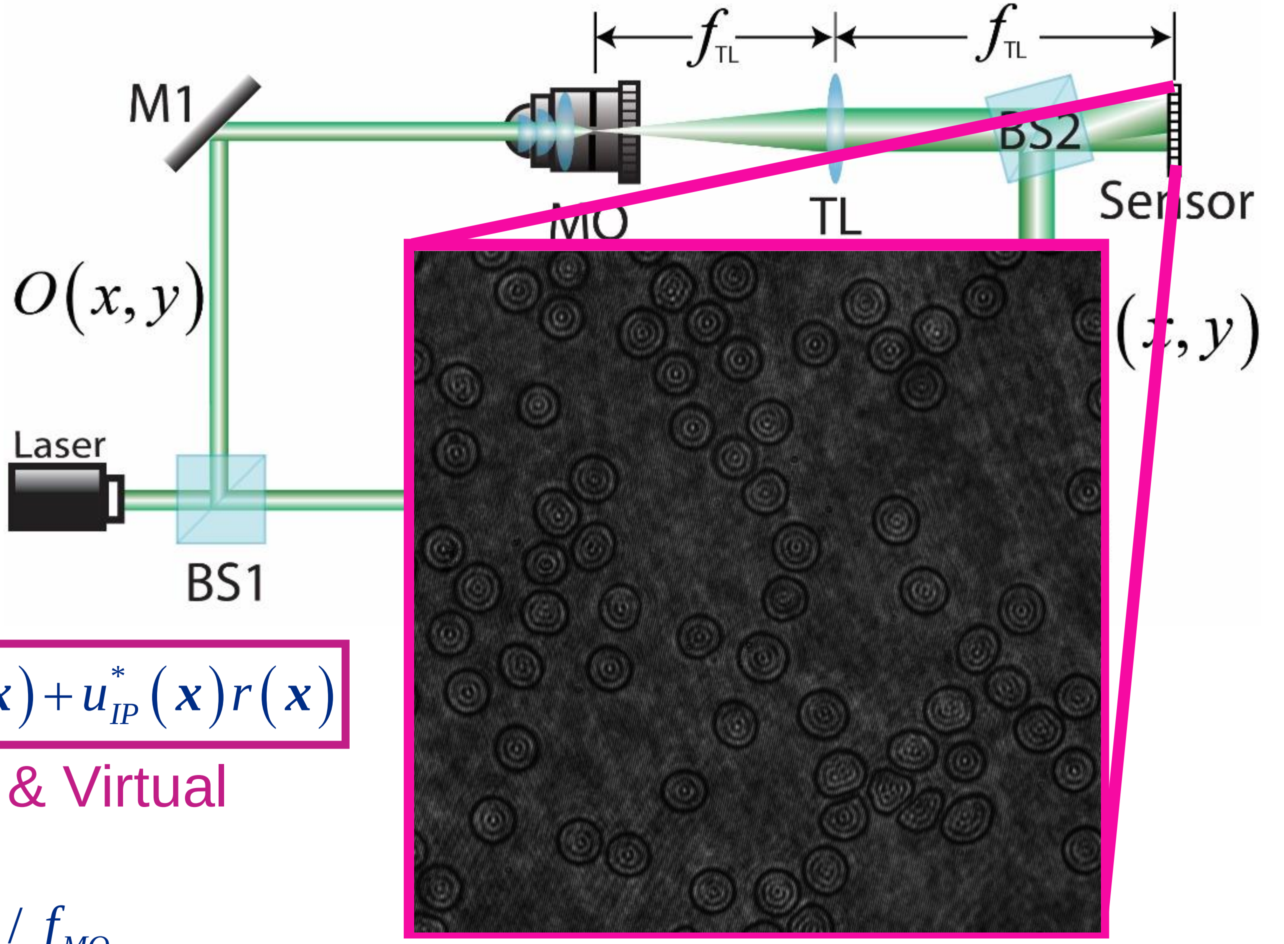
Real & Virtual




$$M_L = -f_{TL} / f_{MO}$$

# Optical configuration of the DHM system: telecentric versus non-telecentric

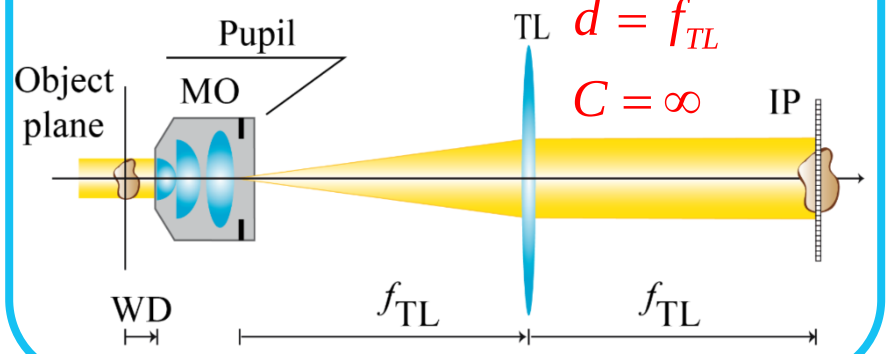
Complex amplitude distribution at the image plane (IP)

$$C = \frac{f_{\mathrm{TL}}^{2}}{f_{\mathrm{TL}} - d}$$

## Telecentric DHM

$d = f_{TL}$

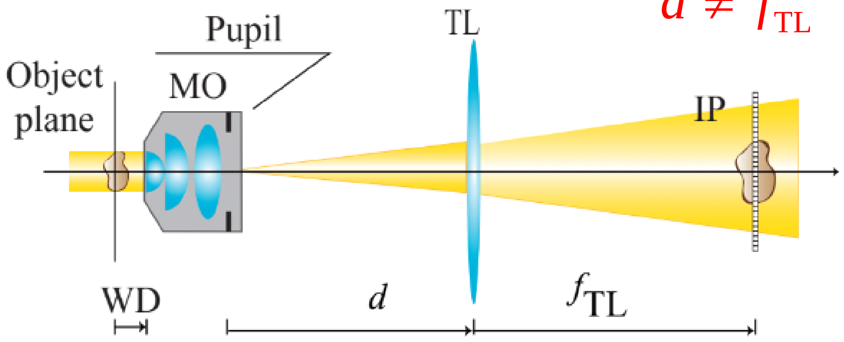
$C = \infty$




$$u_{IP}(\boldsymbol{x}) \propto \frac{1}{M_L^2}\left\{ o\left(\frac{\boldsymbol{x}}{M_L}\right) \otimes_2 P\left(\frac{\boldsymbol{x}}{\lambda f_{TL}}\right)\right\}$$

## Non-Telecentric DHM

$d \neq f_{\mathrm{TL}}$




$$u_{IP}(\boldsymbol{x}) \propto \frac{1}{M_L^2} \exp\left( \mathrm{i}\frac{k}{2C}|\boldsymbol{x}|^2 \right) \times \left\{ o\left(\frac{\boldsymbol{x}}{M_L}\right) \otimes_2 P\left(\frac{\boldsymbol{x}}{\lambda f_{TL}}\right)\right\}$$

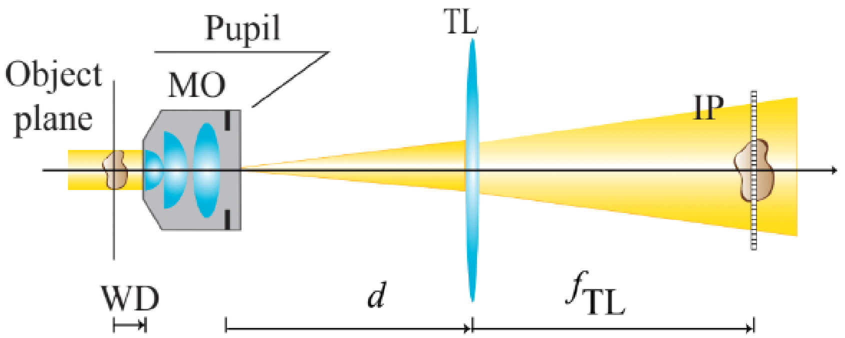
$$M_L = -f_{TL} / f_{MO}$$



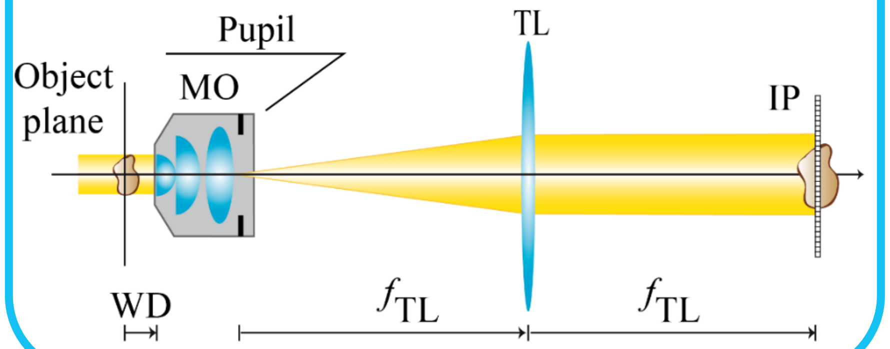

**Doblas** *et al.*, *Opt. Letters* 38, 1352 (2013).

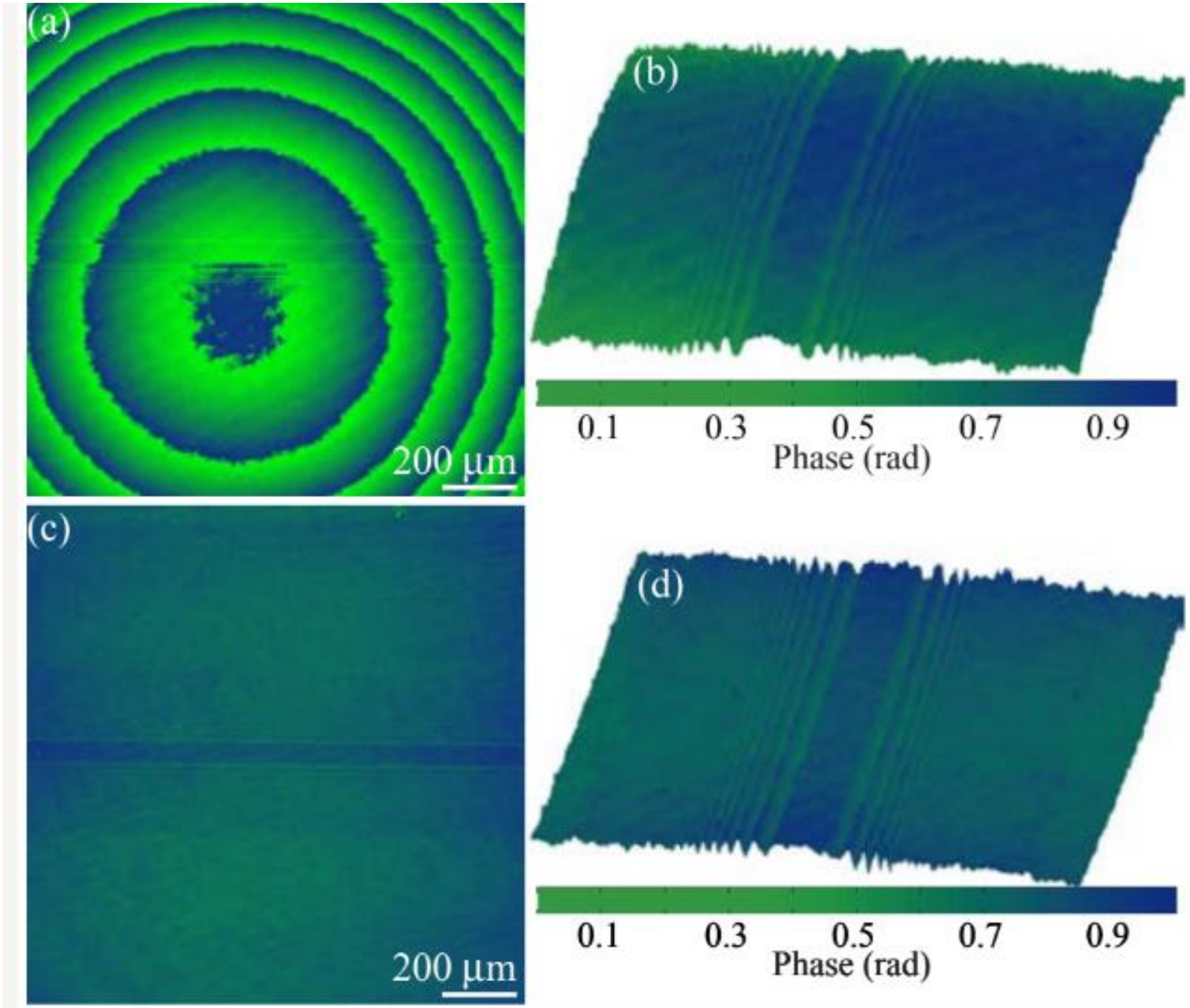
# Optical configuration of the DHM system: telecentric versus non-telecentric

## Non-Telecentric DHM




## Telecentric DHM

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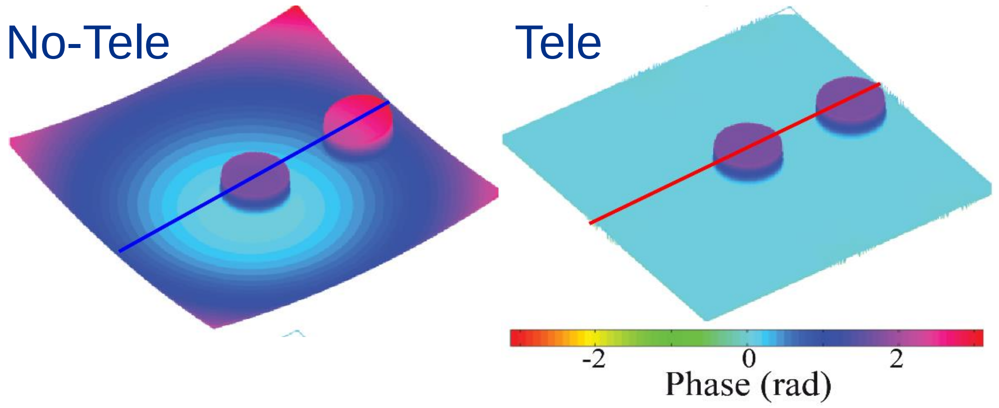
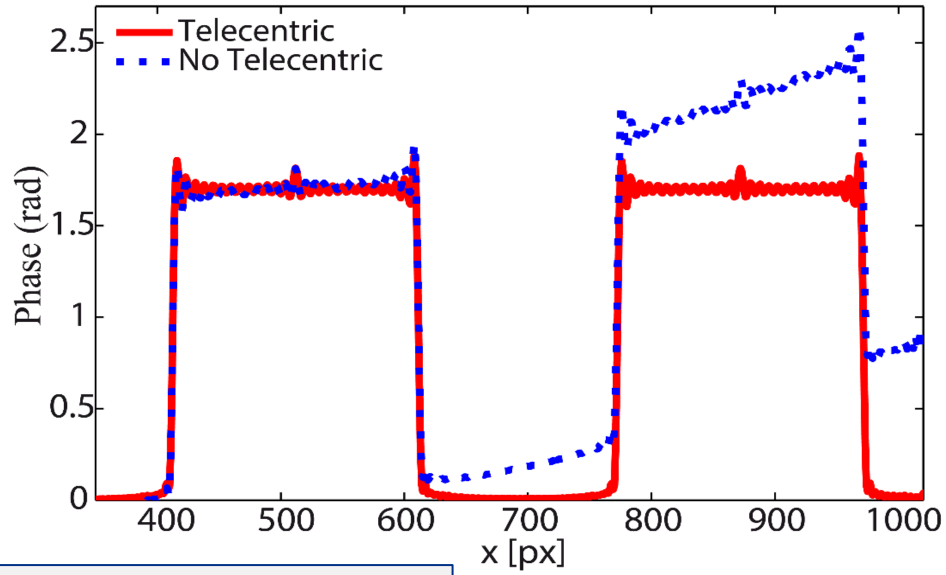
# The performance of DHM technologies relies heavily on computational reconstruction methods to provide accurate phase measurements

- The optical configuration of the imaging systems creates shift-invariant vs shift-variant measurements.
- Telecentric-based DHM systems provide accurate phase values over the whole field of view.
- Non-telecentric DHM systems should compensate for the spherical wavefront associated with a non-telecentric configuration







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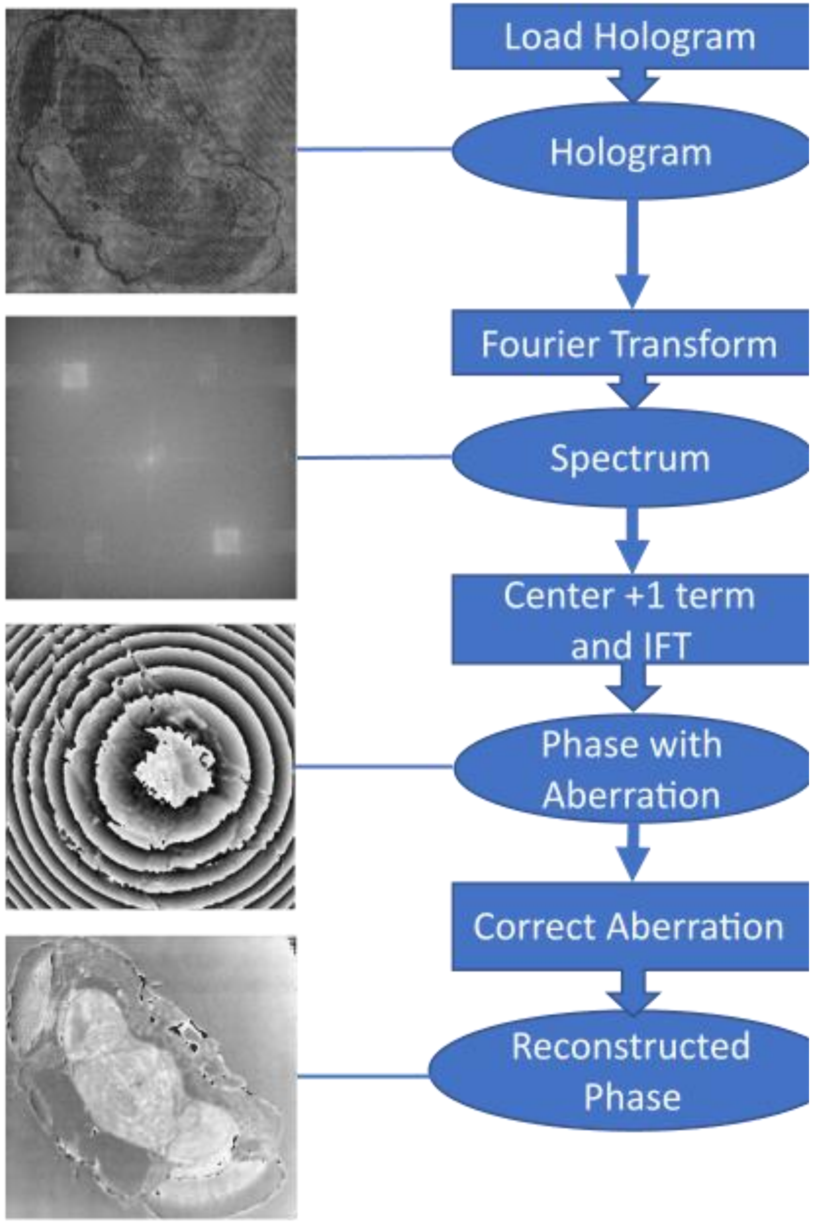
# Requirement of the linear and quadratic compensation for QPI measurements in non-telecentric DHM systems

To reconstruct the phase map from a hologram, one should isolate the +1 term and correct for the distortions:

$$h_F(\boldsymbol{x}) = r^*(\boldsymbol{x})u_{IP}(\boldsymbol{x}) = a_o\left(\frac{\boldsymbol{x}}{M_L}\right)\exp\left[i\varphi_o\left(\frac{\boldsymbol{x}}{M_L}\right)\right]\exp\left[it(\boldsymbol{x})\right]\exp\left[is(\boldsymbol{x})\right]$$



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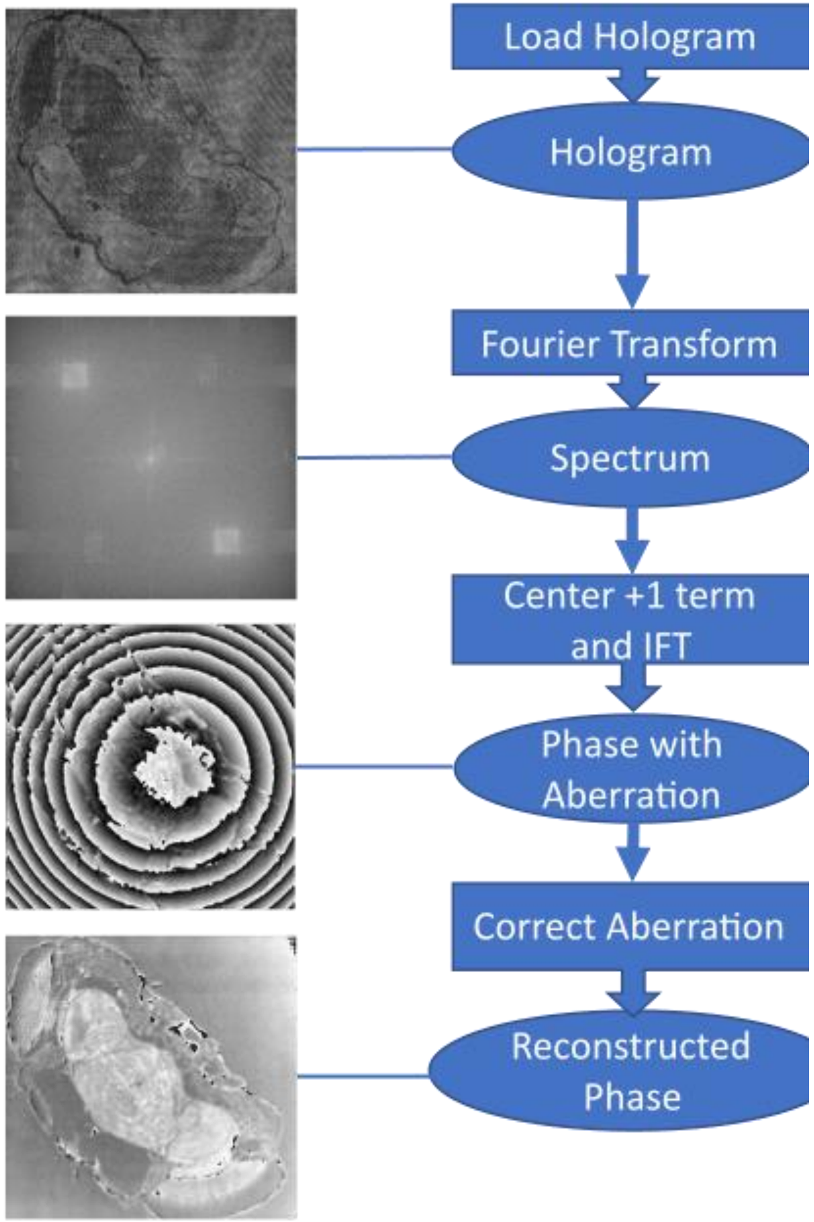
Distortions:

1. Tilt aberrations - $t(\mathbf{x}) = \frac{2\pi}{\lambda}\left(x\sin\theta_x + y\sin\theta_y\right)$



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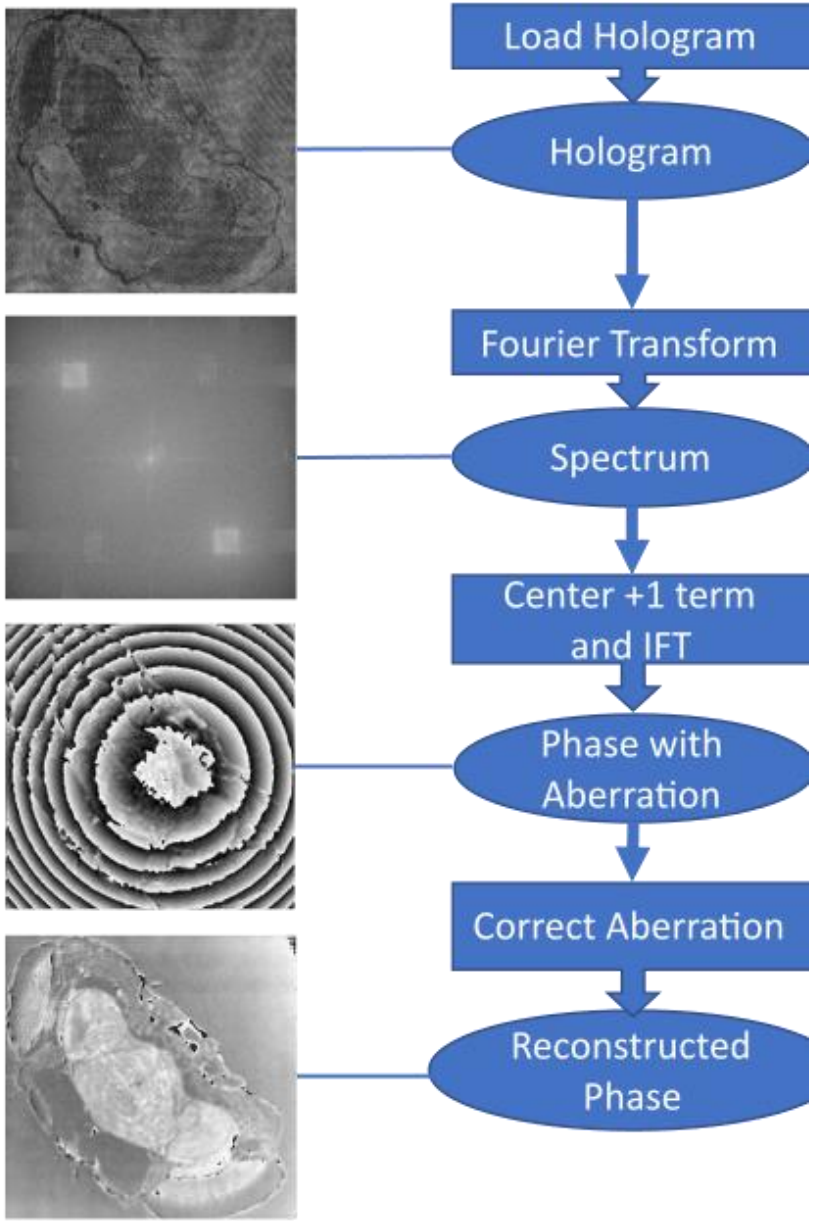
Distortions:

1. Tilt aberrations - $t(\boldsymbol{x}) = \frac{2\pi}{\lambda}\left(x\sin\theta_x + y\sin\theta_y\right)$
2. Spherical aberrations - $s(\boldsymbol{x}) = \frac{k}{C}\left[\left(x - x_C\right)^2 + \left(y - y_C\right)^2\right]$

$$k = \frac{2\pi}{\lambda}$$



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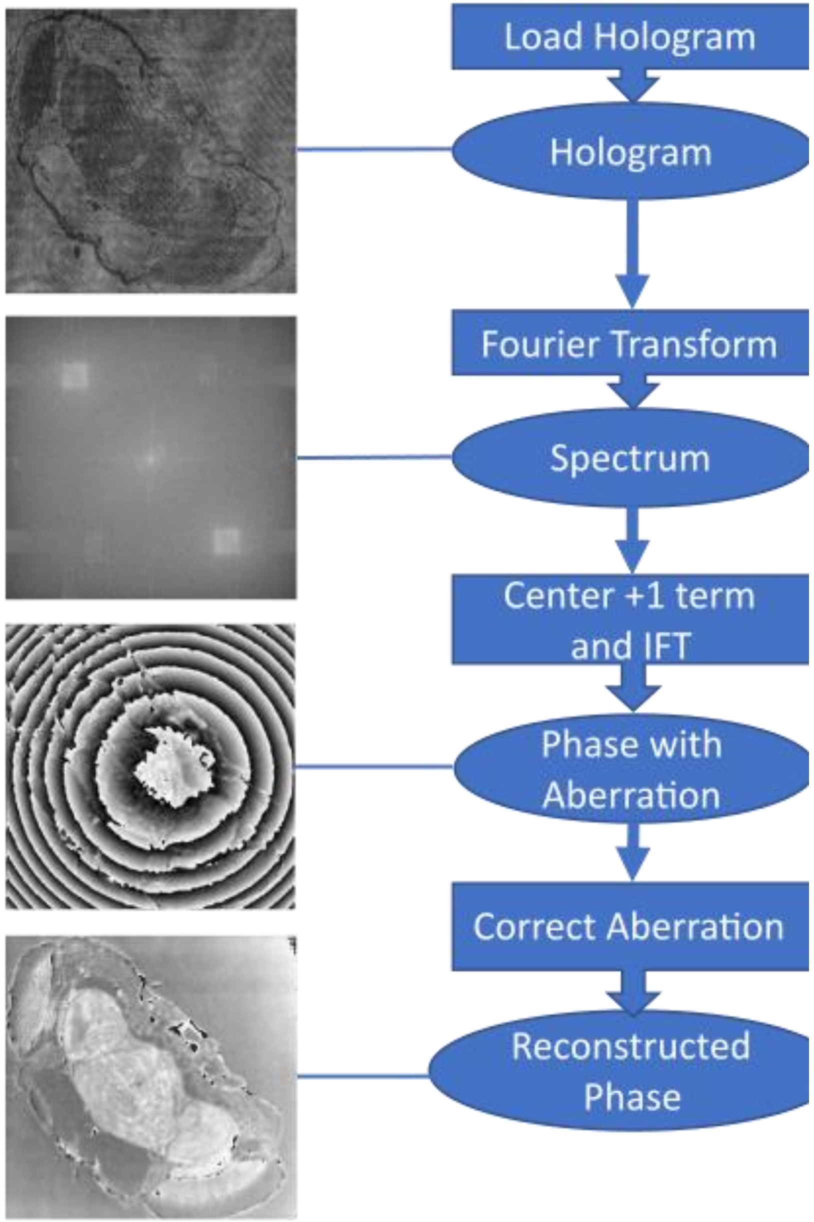
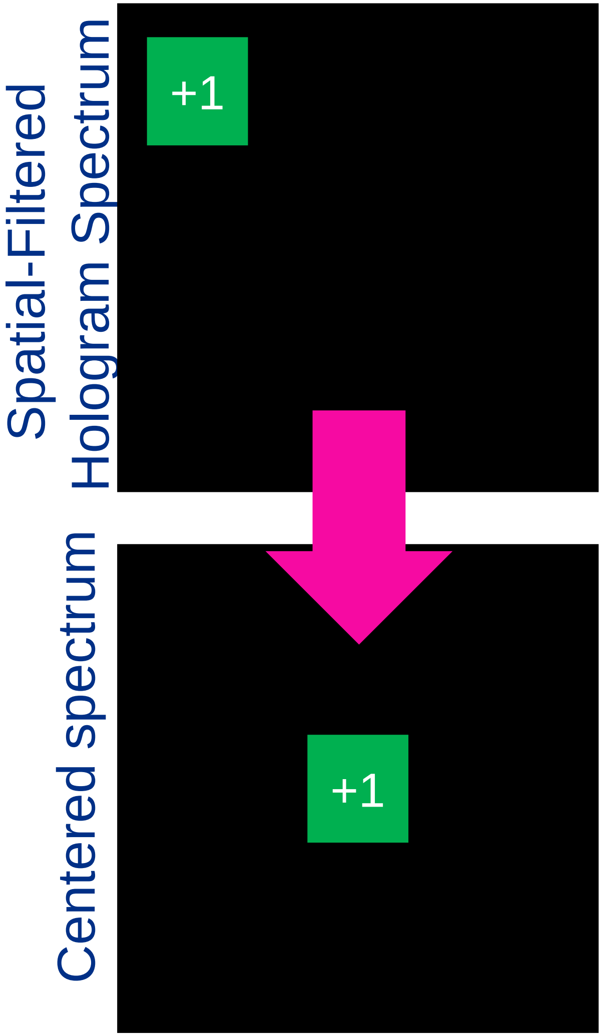
**Corrected** Phase Map: $\boxed{\hat{\varphi}_o(\mathbf{x}) = \mathrm{angle}\left[h_F(\mathbf{x})\right] - t(\mathbf{x}) - s(\mathbf{x})}$




$$k = \frac{2\pi}{\lambda}$$

# Requirement of the generation of a digital reference beam for QPI measurements



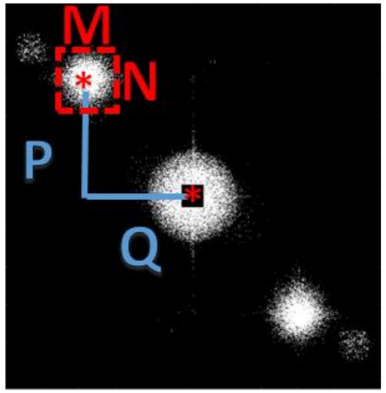

1. Inverse FT of the filtered hologram spectrum $h_F(\boldsymbol{x})$
2. Generation of a digital reference wave

$$r_D(m,n) = \sum_{m,n} \exp\left[ \mathrm{i}\frac{2\pi}{\lambda}\left( m \sin\theta_x X + n \sin\theta_y Y \right)\Delta_{xy} \right]$$

Reference angle, $\boldsymbol{\theta} = (\theta_x, \theta_y)$, is provided by the center pixel position of the order +1, $(P, Q)$

$$\theta_x = \sin^{-1}\left( \frac{|u_0 - P|\lambda}{X\Delta_{xy}} \right)$$

$$\theta_y = \sin^{-1}\left( \frac{|v_0 - Q|\lambda}{Y\Delta_{xy}} \right)$$

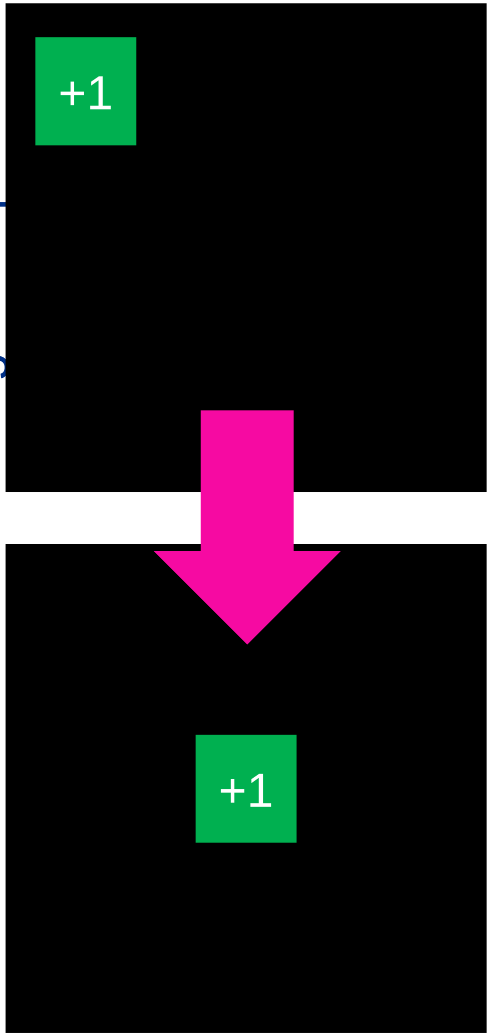



$(X,Y)$ → size of the reconstructed image
$(m,n)$ → index pixel position
$\lambda$ → source's wavelength

$\Delta_{xy}$ → square pixel size
$(u_0,v_0) = (X/2+1,\ Y/2+1)$

# Requirement of the generation of a digital reference beam for QPI measurements

Spatial-Filtered Hologram Spectrum




Centered spectrum

1. Inverse FT of the filtered hologram spectrum $h_F(\boldsymbol{x})$
2. Generation of a digital reference wave

$$r_D(m,n) = \sum_{m,n} \exp\left[ \mathrm{i}\frac{2\pi}{\lambda}\left(m \cdot \sin\theta_x \cdot X + n \cdot \sin\theta_y \cdot Y\right)\Delta_{xy}\right]$$

3. Complex object information without distortion of the reference beam

$$\hat{o}(\boldsymbol{x}) = r_D(\boldsymbol{x}) \cdot h_F(\boldsymbol{x})$$

4. Estimation of the phase map

$$\hat{\varphi}(\boldsymbol{x}) = \tan^{-1}\left(\frac{\mathrm{Im}\left[\hat{o}(\boldsymbol{x})\right]}{\mathrm{Re}\left[\hat{o}(\boldsymbol{x})\right]}\right)$$

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# Need for correct estimation of the center position of the +1 term

The angle of the reference wave is determined by the source's wavelength ($\lambda$), the features of the digital sensor *(X, Y, $\Delta_{xy}$)* and the subtraction between the pixel positions of the DC and +1 terms

$$\theta_x = \sin^{-1}\left(\frac{|u_0 - P|\lambda}{X\Delta_{xy}}\right) \qquad \theta_y = \sin^{-1}\left(\frac{|v_0 - Q|\lambda}{Y\Delta_{xy}}\right)$$

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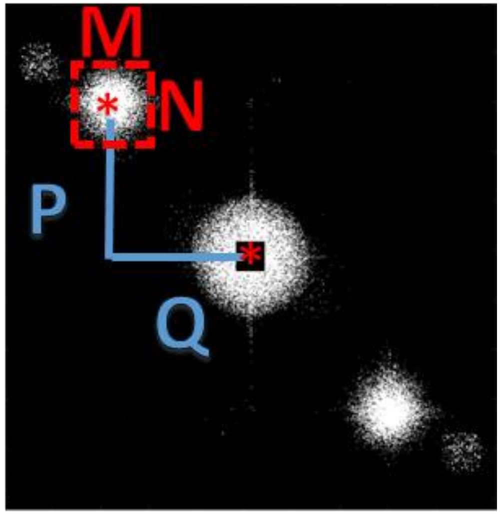


**PROPOSED STEP TO IDENTIFY AND SELECT +1 TERM**

- ❑ Use of Otsu's method for global thresholding to identify the different terms in the hologram's spectrum.
- ❑ The DC term must be blocked before applying the Otsu's method.
- ❑ Apply image segmentation technique to the binarized hologram's spectrum to identify the ±1 terms.

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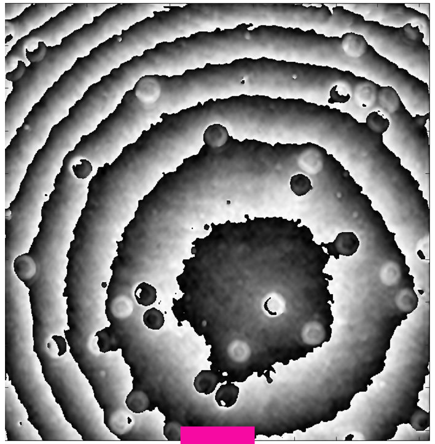
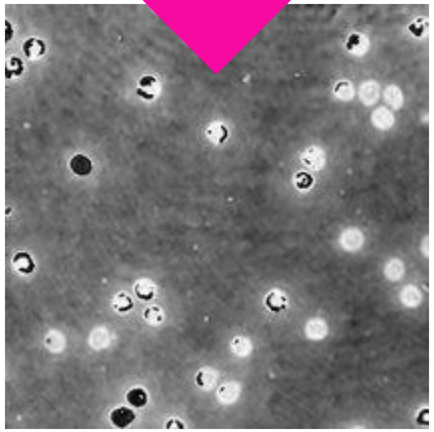
# Requirement of the generation of a digital reference beam for QPI measurements

Phase image after Compensating the linear tilt



Compensated Phase Image



1. Phase Image after compensating the linear tilt

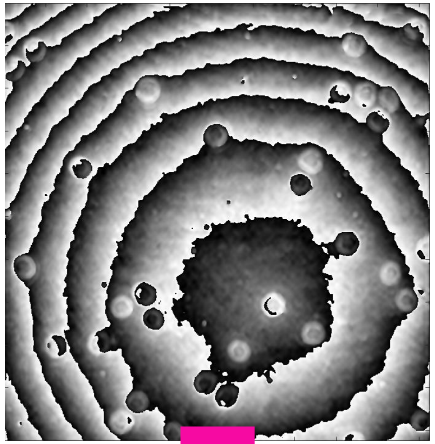
$$\hat{\varphi}'_o(\boldsymbol{x}) = \text{angle}\left[h_F(\boldsymbol{x})\right] - t(\boldsymbol{x})$$

2. Generation of a quadratic phase factor

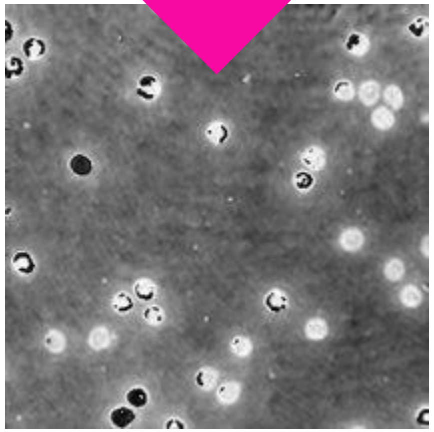
$$s(\boldsymbol{x}) = \frac{k}{C}\left[(x - x_C)^2 + (y - y_C)^2\right]$$

# Requirement of compensating the quadratic phase factor for QPI measurements

Phase image after Compensating the linear tilt



Compensated Phase Image



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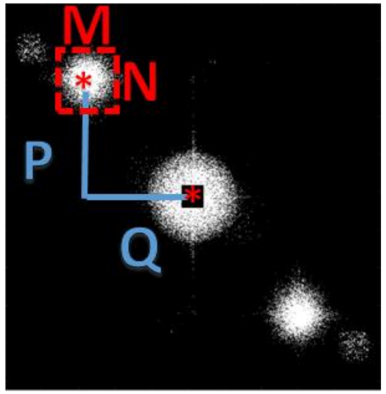
Curvature, ***C*** = ($C_x$, $C_y$), is provided by the size of the order +1, (*M*, *N*)

$$C_x = \frac{(X\Delta_{xy})^2}{\lambda M}$$

$$C_y = \frac{(Y\Delta_{xy})^2}{\lambda N}$$




**Sanchez-Ortiga, Doblas** *et al.*, *Appl. Opt. 53*, 2058 (2014).

(*X*,*Y*) → size of the reconstructed image

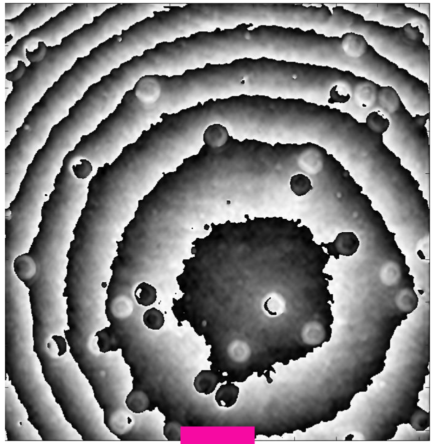
$\Delta_{xy}$ → square pixel size

(*m*,*n*) → index pixel position

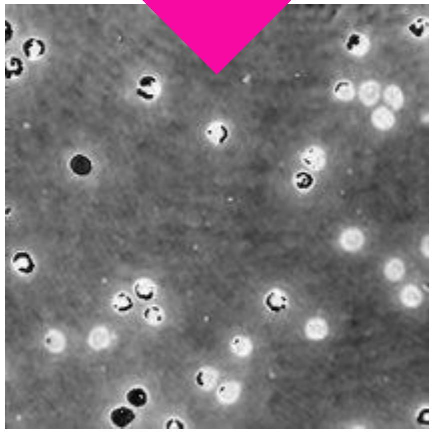
*λ* → source's wavelength

# Requirement of compensating the quadratic phase factor for QPI measurements

Phase image after Compensating the linear tilt



Compensated Phase Image



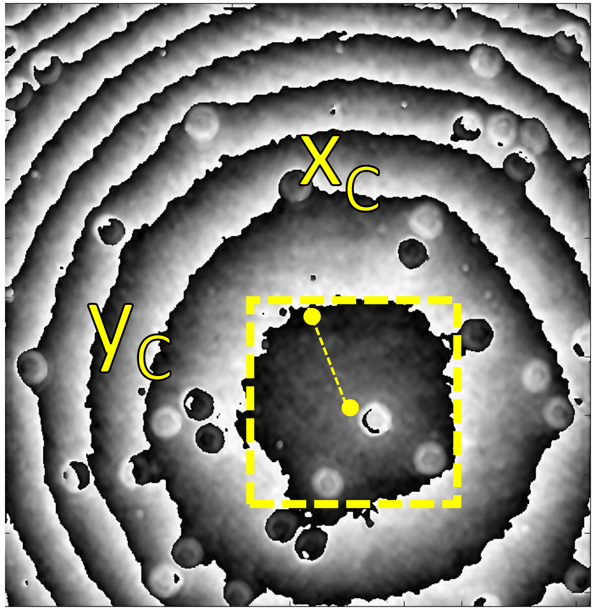
1. Phase Image after compensating the linear tilt

$$\hat{\varphi}'_o(\boldsymbol{x}) = \text{angle}\left[h_F(\boldsymbol{x})\right] - t(\boldsymbol{x})$$

2. Generation of a quadratic phase factor

$$s(\boldsymbol{x}) = \frac{k}{C}\left[(x - x_C)^2 + (y - y_C)^2\right]$$

The center of the quadratic phase factor, ($x_C$, $y_C$), can be measured from the phase image.




($X$,$Y$) → size of the reconstructed image

$\Delta_{xy}$ → square pixel size

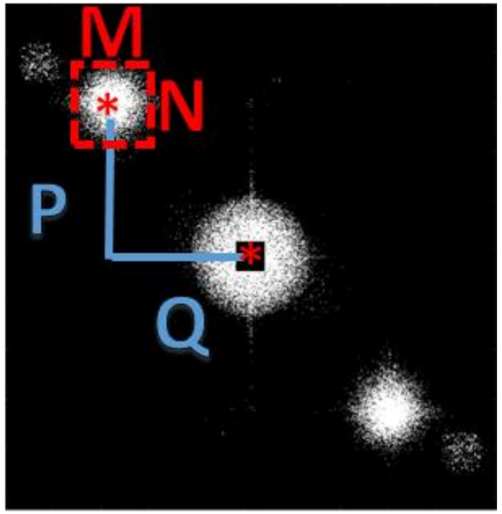
($m$,$n$) → index pixel position

$\lambda$ → source's wavelength

# Need for correct estimation of the quadratic phase factor

The curvature of the quadratic phase factor is determined by the source's wavelength (λ), the features of the digital sensor *(X, Y, $\Delta_{xy}$)* and the size of the +1 term

$$C_x = \frac{\left(X\Delta_{xy}\right)^2}{\lambda M} \qquad C_y = \frac{\left(Y\Delta_{xy}\right)^2}{\lambda N}$$




**PROPOSED STEP TO IDENTIFY AND SELECT +1 TERM**

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- ❑ The DC term must be blocked before applying the Otsu's method.
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*(m,n)* → index pixel position

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# Need for correct estimation of the quadratic phase factor

The center of the quadratic phase factor is determined from the phase image after compensating the linear tilt

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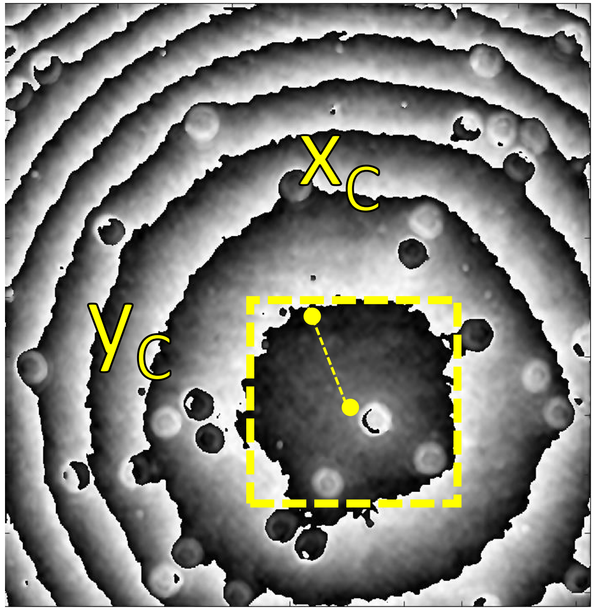
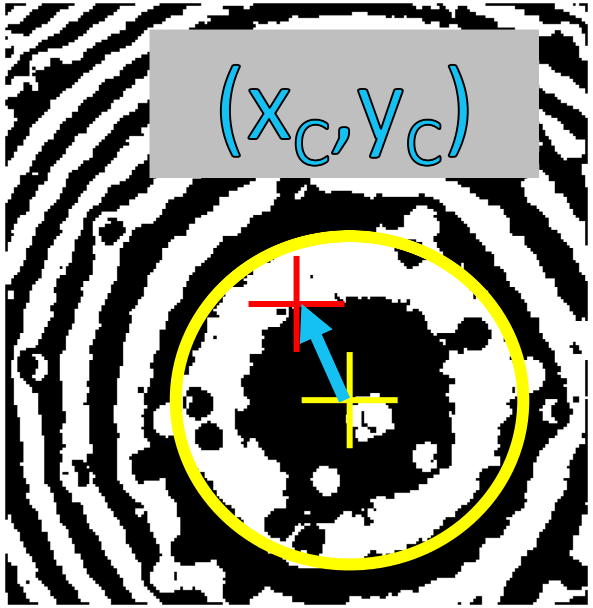


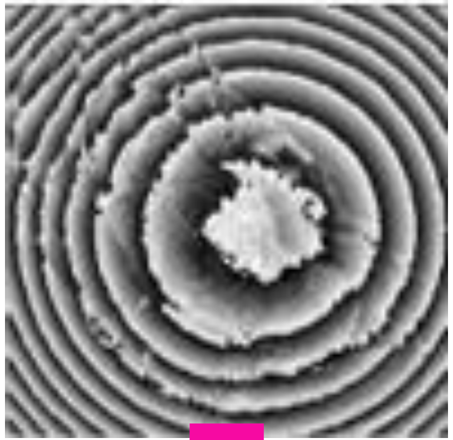

**PROPOSED STEP TO IDENTIFY CENTER OF QUADRATIC TERM**

- Use of Otsu's method for binarizing the phase image.
- Select one of the concentric rings.
- Measure distance of the concentric ring to the center of the image

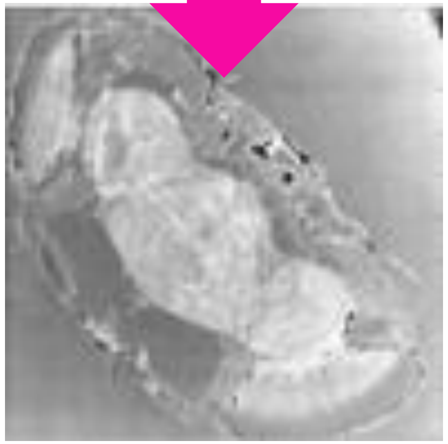


# Need for a fine-tuning compensation through a minimization

Compensated Phase image



Fine-tuning Compensated Phase Image



1. Phase Image after compensating the linear tilt and the quadratic phase factor

$$\hat{\varphi}_o(\boldsymbol{x}) = \text{angle}\left[h_F(\boldsymbol{x})\right] - t(\boldsymbol{x}) - s(\boldsymbol{x})$$

2. Generation of a **residual** quadratic phase factor

$$s_R(\boldsymbol{x}) = \frac{k}{C_R}\left[x^2 + y^2\right]$$

The residual curvature, $C_R$ , estimated by minimizing the standard deviation of the reconstructed phase image (e.g., minimizing the number of phase wraps in the image). We found that the best optimizer was the a hybrid between genetic algorithm and patterns search (ga+ps).

# Summary

- ☑ We outline the implementation steps necessary for automatic reconstruction method for quantitative phase imaging using a digital holographic microscope operating in non-telecentric regime.
- ☑ Our implementation only requires that the user knows the wavelength of the laser used and the camera specifications (i.e., pixel and sensor size).
- ❑ *Comparison of alternative thresholding image segmentation methods and cost functions*
- ❑ *Investigation of alternative approaches to reduce reconstruction times*
- ❑ *Single reconstruction algorithm for telecentric and non-telecentric DHM systems (e.g., generalization)*

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Dr. Jorge Garcia-Sucerquia, School of Physics, Universidad Nacional de Colombia Sede Medellin, Colombia.
Dr. Genaro Saavedra and Manuel Martinez-Corral, Department of Optics, Univ. of Valencia, Spain
Dr. Bahram Javidi, Department of Electrical and Computer Engineering, University of Connecticut, CT.
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**OIRL Research Group**

Assistant Professor: Dr. A. Doblas (adoblas@memphis.edu)
Postdoctoral Researcher: Dr. R. Castenda
Graduate Students in OIRL Group: B. Bogue-Jimenez, S. Patra, K. Dahal.
Undergraduate Students in OIRL Group: K. Balachandran, A. Abraham, J. Brown.

**EAFIT's Applied Optics Research Group**

Associate Professor: Dr. C. Trujillo (catrujilla@eafit.edu.co)
Graduate Students: M. Lopera-Acosta, S. Obando-Vasquez, M. Montoya-Zuluaga.
Undergraduate Students: T. Velez-Acosta, S. Jurado


Optical Imaging Research Lab

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# Q&A