

# *s*<sup>2</sup>*stigmator*: a closed-form solution for single-pass correction of TEM lens astigmatism

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<http://cryoem.bio.purdue.edu>

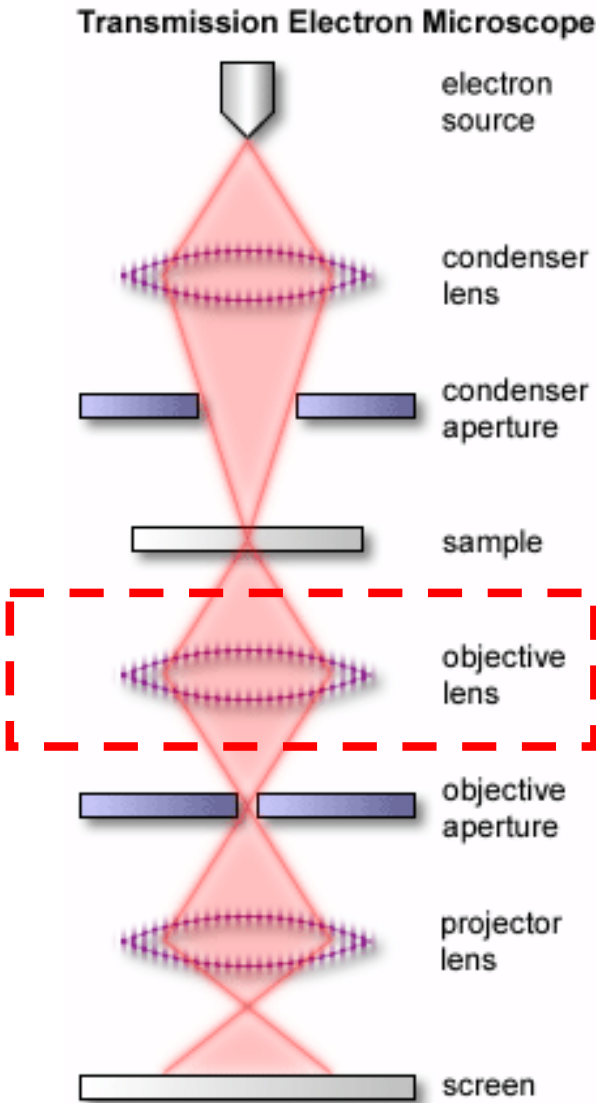
<http://jiang.bio.purdue.edu>



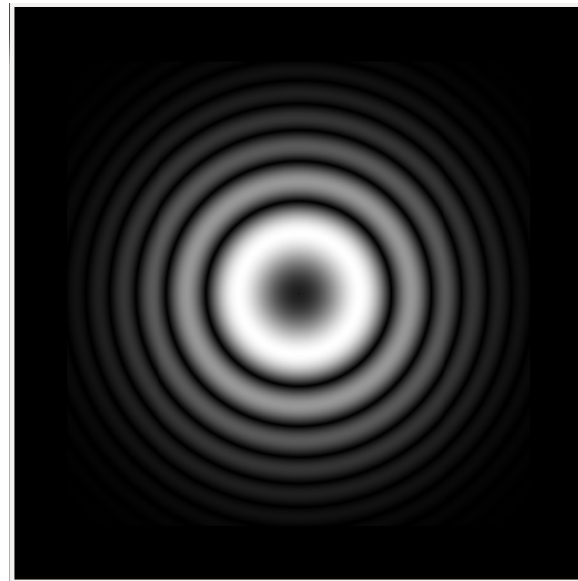
Purdue Cryo-EM Facility  
Markey Center for Structural Biology

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UNIVERSITY

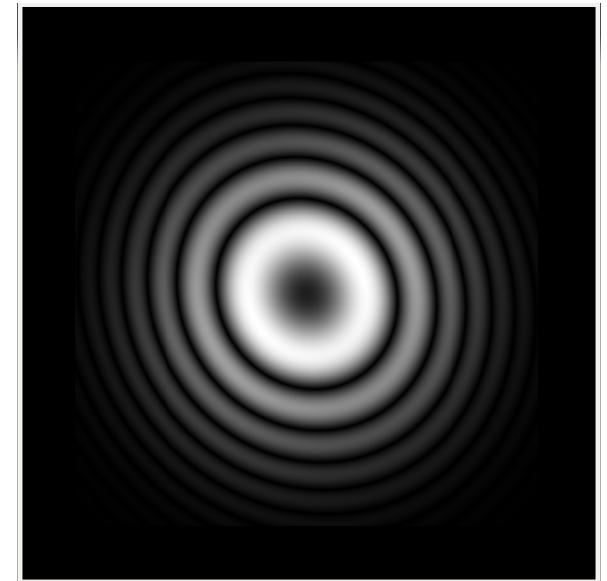
# TEM objective lens astigmatism



The elliptical shape of Thon rings is an indicator of astigmatism.



Astigmatism = 0  
Defocus = 500nm

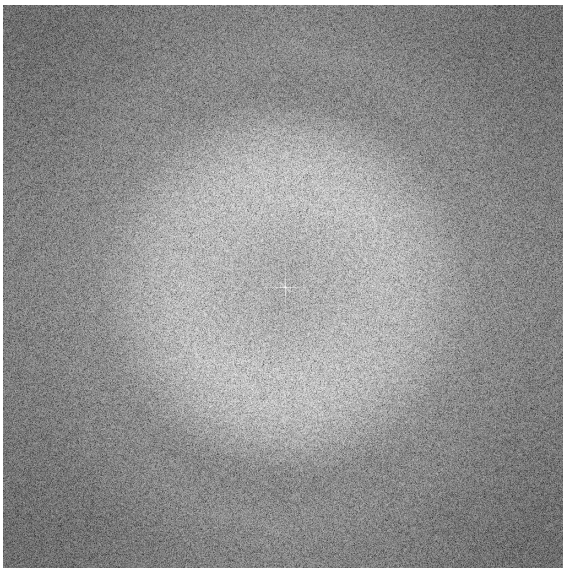


Astigmatism = 100nm  
Angle = 30°  
Defocus = 500nm

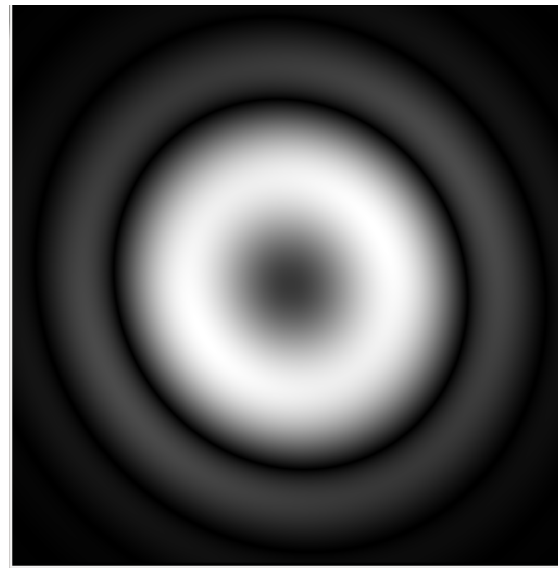
# Visual roundness method

**Very high magnification  
& very small defocus for  
astigmatism correction**

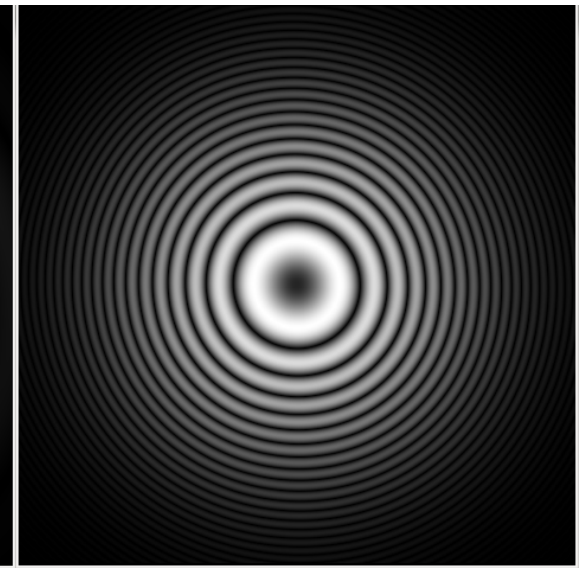
**Lower magnification &  
larger defocus for data  
collection**



Power spectra of an image  
collected from CM200  
(magnification = 200K,  
defocus ~100nm)



Simulated power spectra  
Astigmatism = 50 nm  
Angle = 30 degree  
Defocus = 300 nm



Simulated power spectra  
Astigmatism = 50 nm  
Angle = 30 degree  
Defocus = 1500 nm

# $s^1$ power $\rightarrow$ $s^2$ power

$$CTF(s) = (\sqrt{1 - Q^2} \sin \gamma(s) + Q \cos \gamma(s))$$

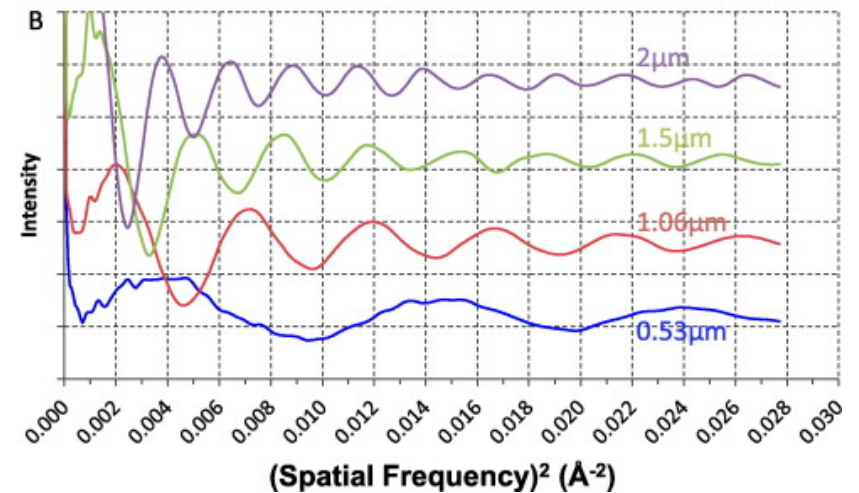
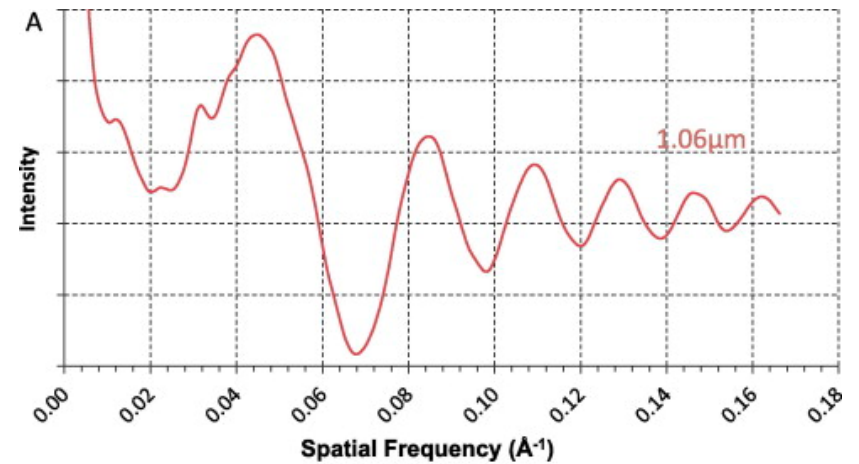
$$= \sin(\gamma(s) + \varphi_0)$$

$$\gamma(s) = 2\pi \left( \frac{f\lambda}{2} s^2 + \frac{C_s \lambda^3}{4} s^4 \right)$$

$$s' = s^2$$

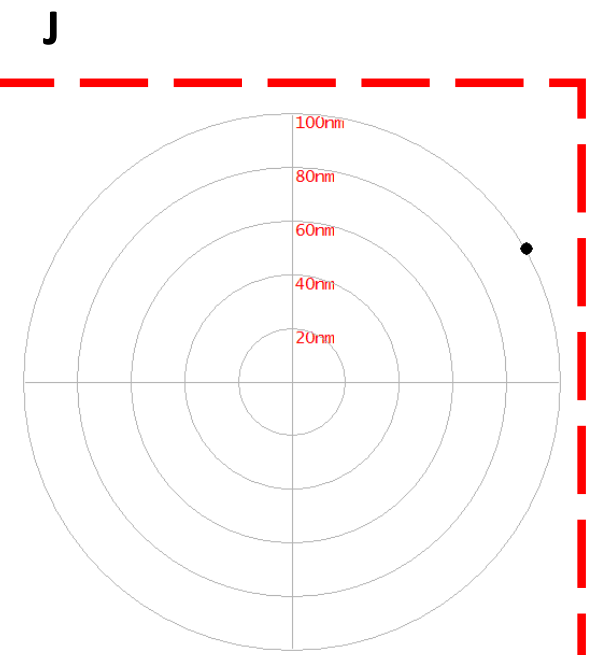
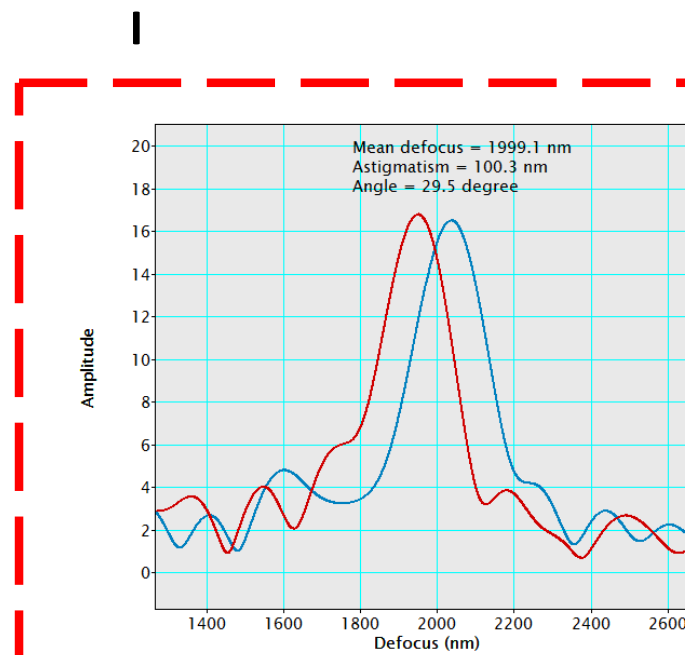
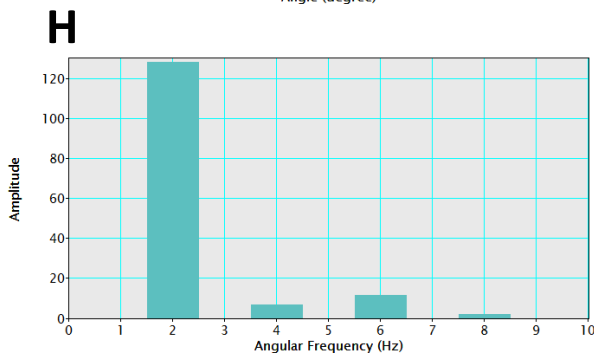
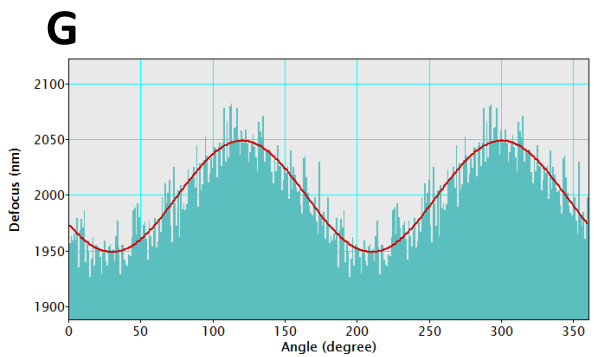
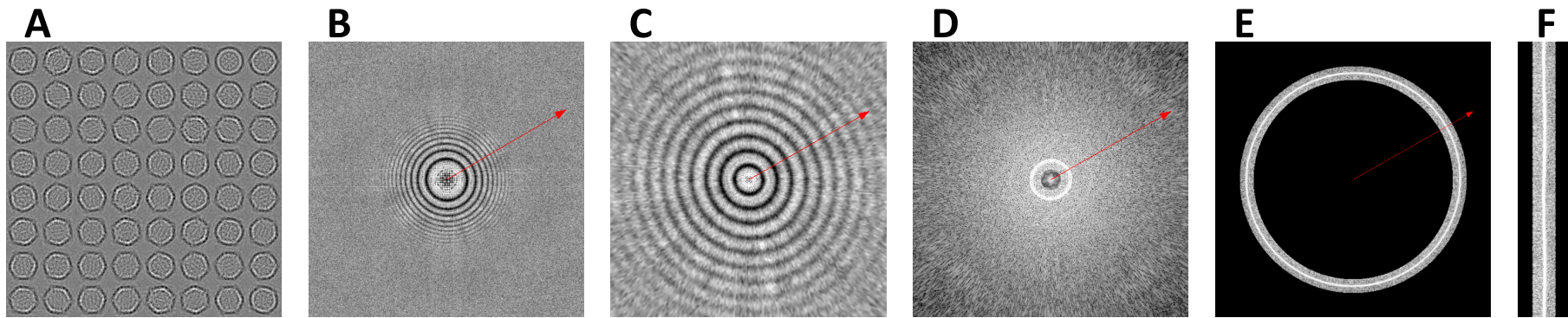
$$\gamma(s') = 2\pi \left( \frac{f\lambda}{2} s' + \frac{C_s \lambda^3}{4} s'^2 \right)$$

$s^1$  power: increasing oscillations

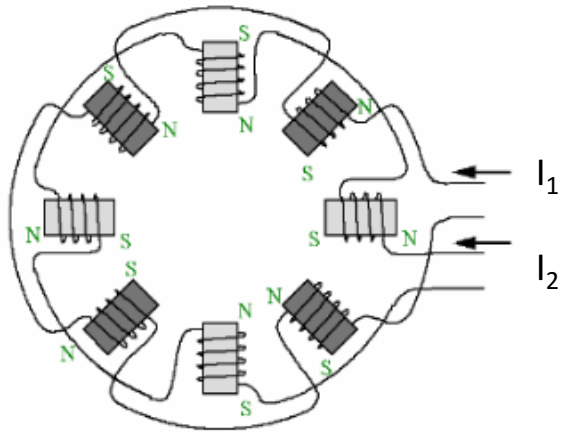


$s^2$  power: uniform oscillations

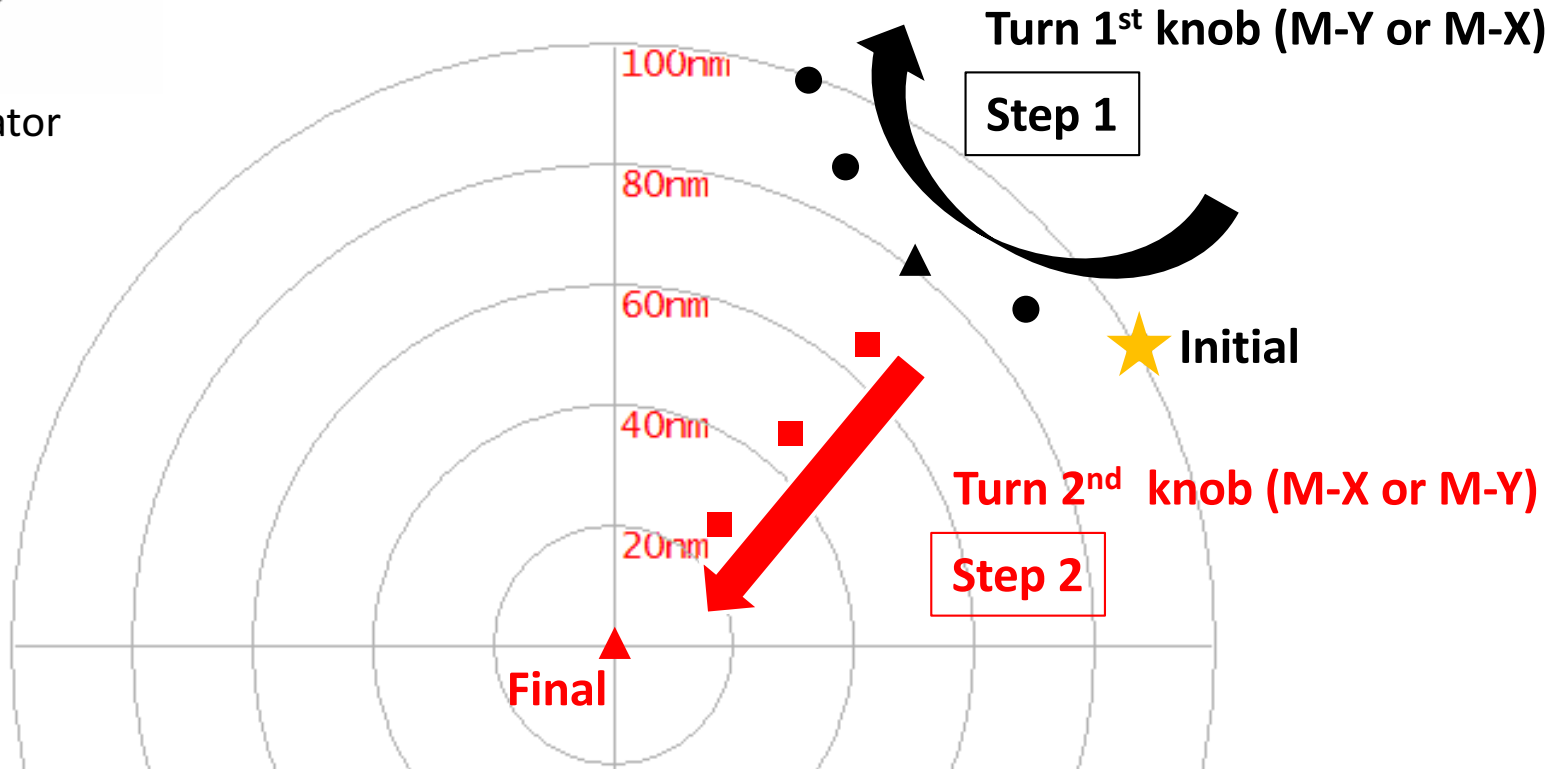
# $s^2$ stigmator: a closed-form algorithm



# Single-pass tuning strategy

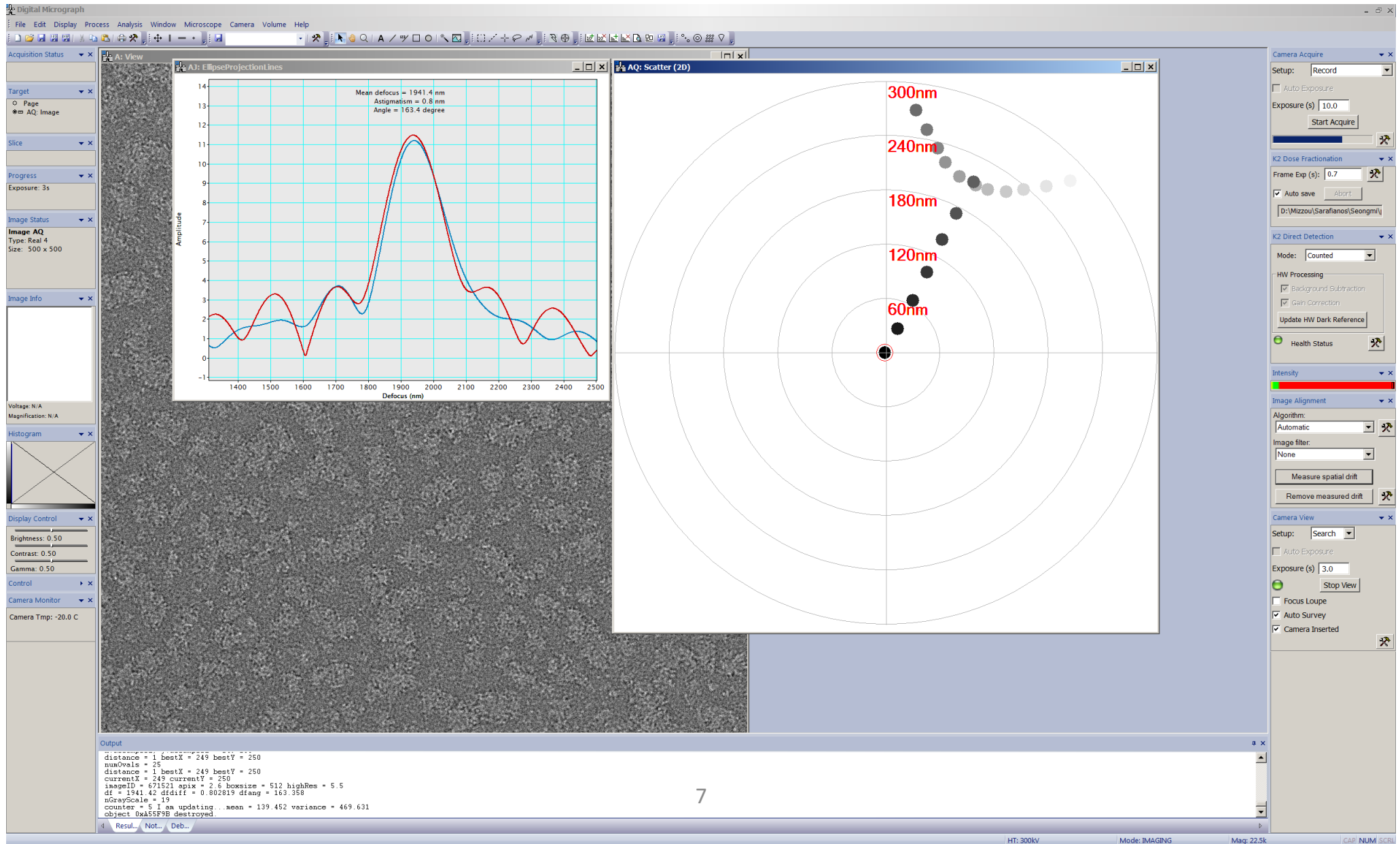


Octupole stigmator



Expected trajectory of the single-pass tuning strategy for astigmatism correction using  $s^2$ stigmator

# Single-pass tuning strategy



# Video: Single-pass tuning strategy

<https://www.youtube.com/watch?v=Fq5zCNq-bBM>

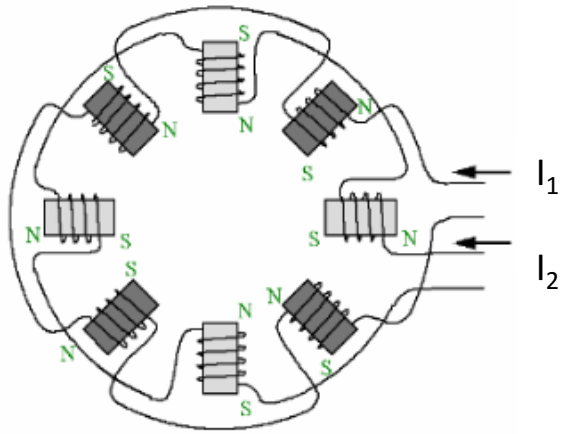
The screenshot displays the Digital Micrograph (DM) software interface. The central window, titled "AI View", shows a grayscale micrograph of a textured surface. The interface is divided into several panels:

- Left Panel:** Contains various control sections including "Adaptation Status", "Target", "Image Status" (showing "Image A", Type: Real 4, Size: 1849 x 1915), "Image Info", "Voltage 100kV", "Magnification: 22.5k", "Histogram" (a blue bell-shaped curve), "Display Control" (Brightness: 0.50, Contrast: 0.50, Camera: 0.50), "Control", "Camera Monitor", and "Camera Temp: -20.0 C".
- Right Panel:** Contains acquisition and processing controls such as "Camera Acquire" (Setup: Record, Exposure: 3.0, Start Acquire), "K2 Data Transmission" (Frame Exp: 0.7, Auto save), "K2 Direct Detection" (Mode: Counted, HV Processing), "Intensity" (a red bar graph), "Image Alignment" (Algorithm: Automatic, Image filter: None), and "Camera View" (Setup: Search, Exposure: 3.0, Stop View).
- Bottom Panel:** An "Output" window displaying text logs, including: "Peak detector = 2.1148e4", "Peak position = 43.5457", "In calculateWignerVectorDirection, apix 2.6 2.6", "Image size 612x112", "Reel shift = 161", and "Initial detection of peak position in polar coordinate 43".

The Windows taskbar at the bottom shows the Start button, system tray, and the date/time: 10:54 PM 8/13/2016.

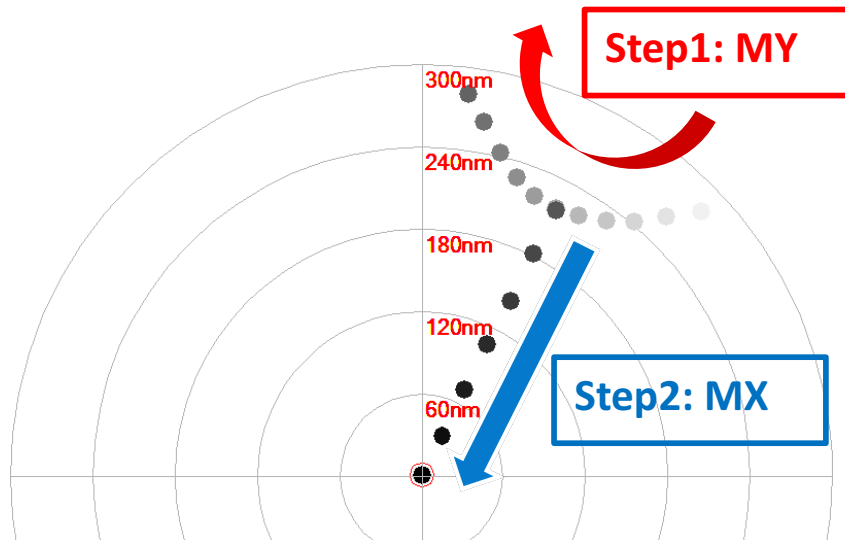


# Arbitrary order of adjusting the two stigmators

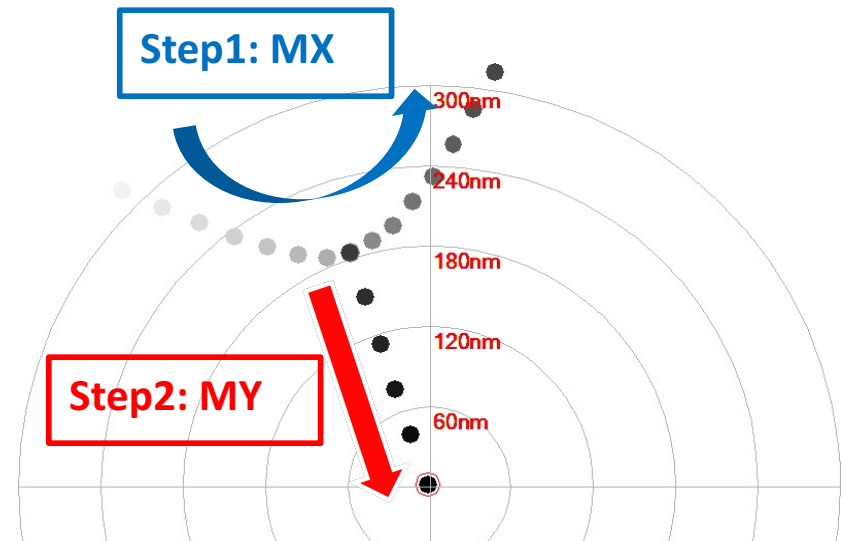


Octupole stigmator

## A : MY-MX trajectory



## B : MX-MY trajectory

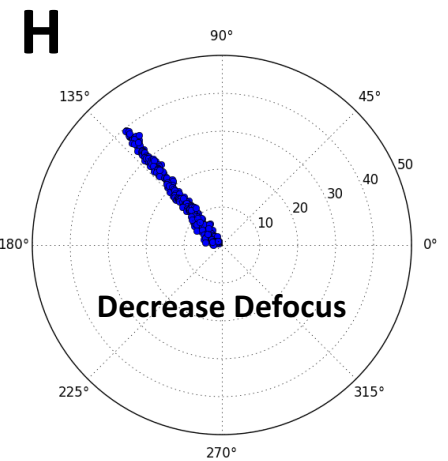
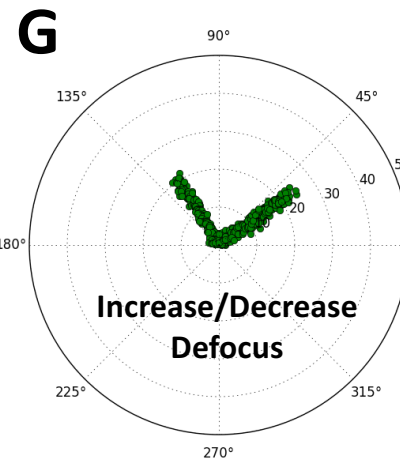
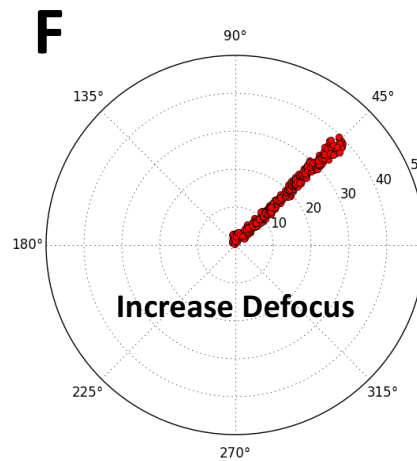
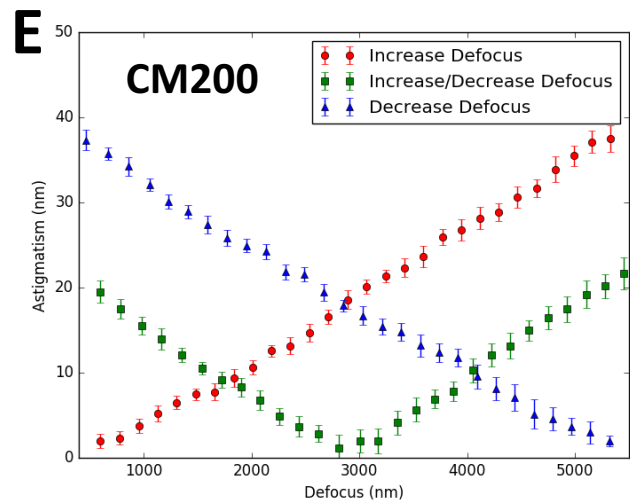
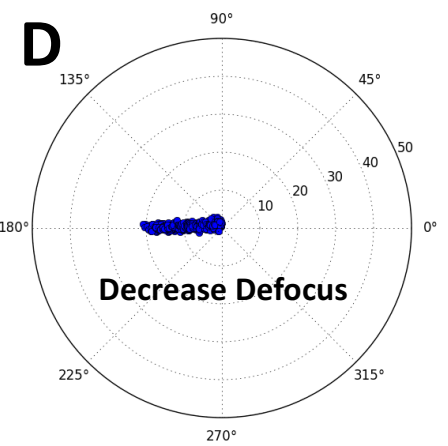
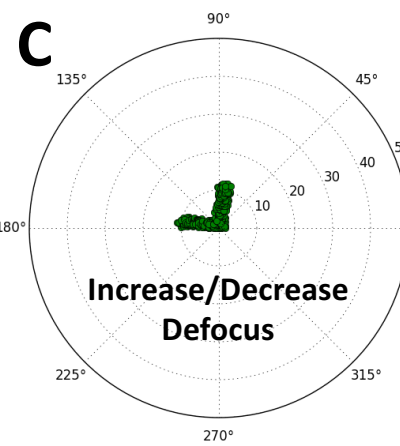
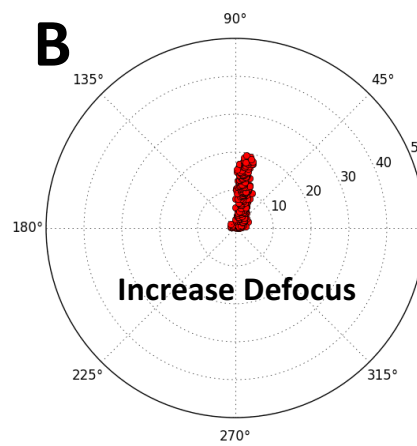
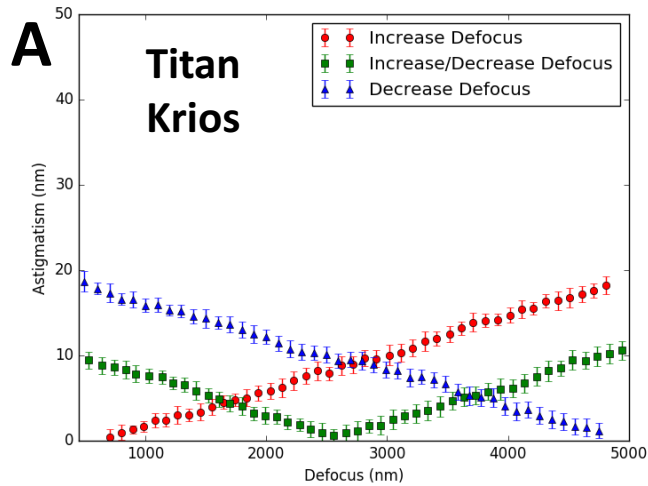


Screenshots of single-pass tuning strategy of astigmatism correction using  $s^2$ stigmator

# *s*<sup>2</sup>*stigmator* method

- Provides real-time feedback for astigmatism correction
- Enhances the sensitivity in ellipticity detection
- Prevents the bias and subjective results from operators
- Establishes an efficient single-pass tuning strategy, instead of blindly playing with the two stigmators
- Guides users to correct astigmatism at any magnification & defocus

# Defocus-dependent astigmatism



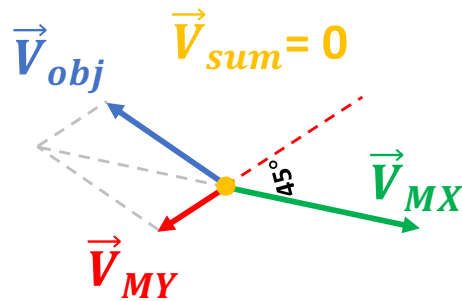
# Defocus-dependent astigmatism

$$\vec{V}_{sum} = \vec{V}_{obj}^0 + \vec{V}_{MX} + \vec{V}_{MY} + \Delta\vec{V}_{obj}^{df}$$

$$= k_{obj} \cdot I_{obj}^0 \cdot \vec{e}_{obj} + k_{MX} \cdot I_{MX} \cdot \vec{e}_{MX} + k_{MY} \cdot I_{MY} \cdot \vec{e}_{MY} + k_{obj} \cdot (I_{obj} - I_{obj}^0) \cdot \vec{e}_{obj}$$

Defocus-dependent astigmatism term  
↑ Scaling factor    ↑ Unit vector

Change of objective lens current when defocus is increased/decreased

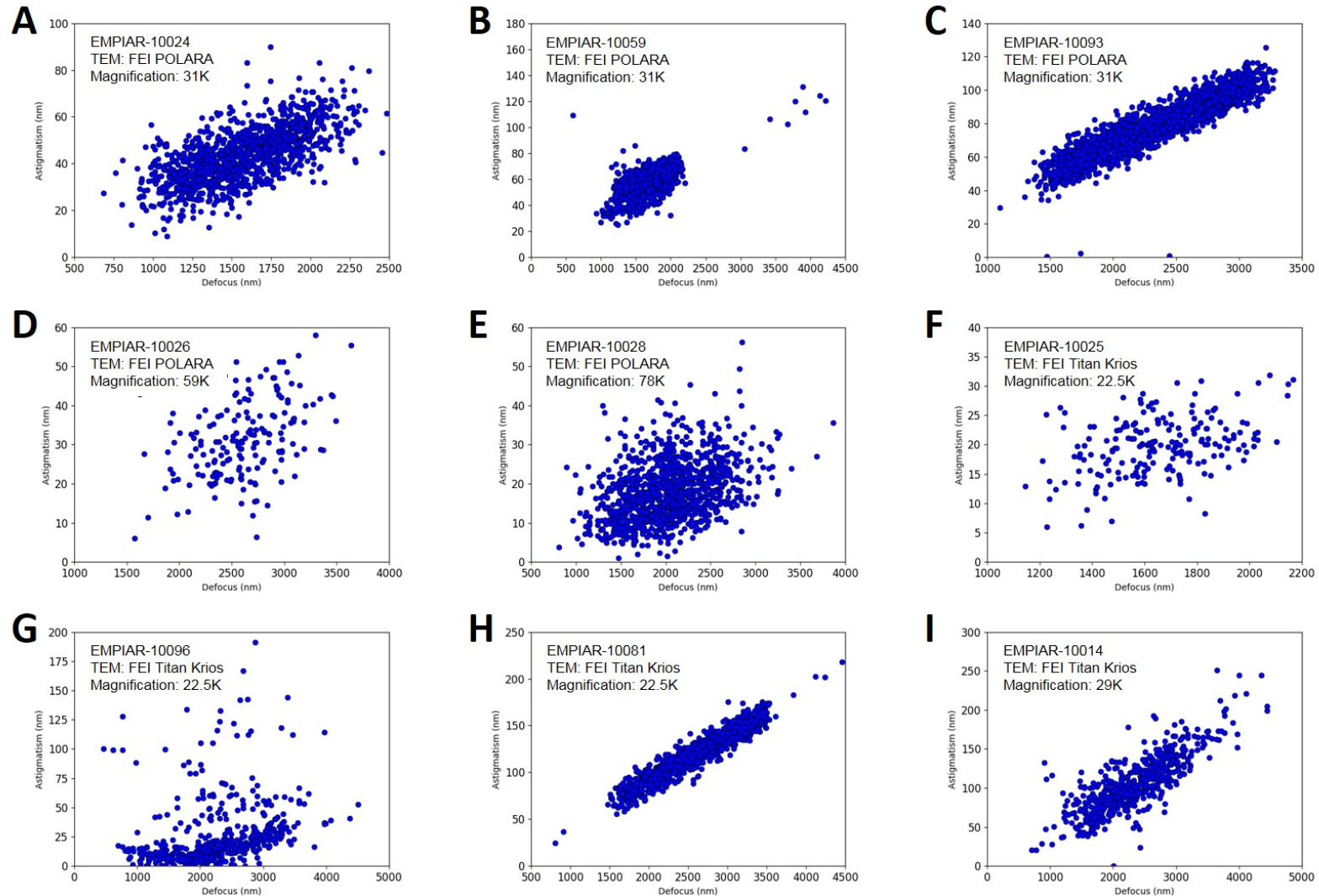


$k$ : a scaling factor representing how strong the dependence is between  $\vec{V}$  and  $I$

$I$ : current

$\vec{e}$ : a unit vector representing the direction of the lens astigmatism ( $\vec{V}$ )

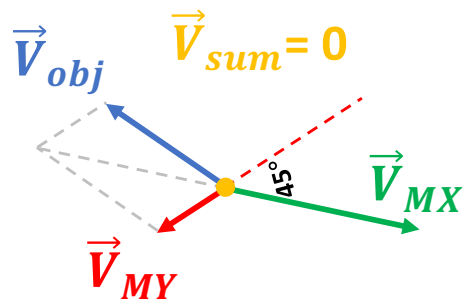
# Observations of defocus-dependent astigmatism in cryo-EM data



# Origin of defocus/magnification dependent astigmatism

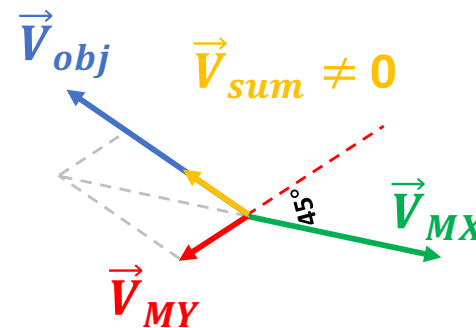
## Microscope alignment

- Before data collection
- High magnification
- Small defocus

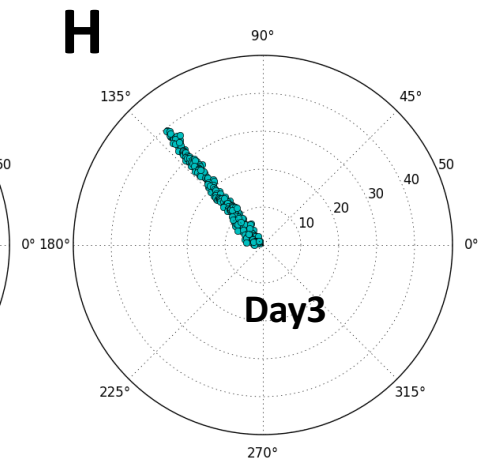
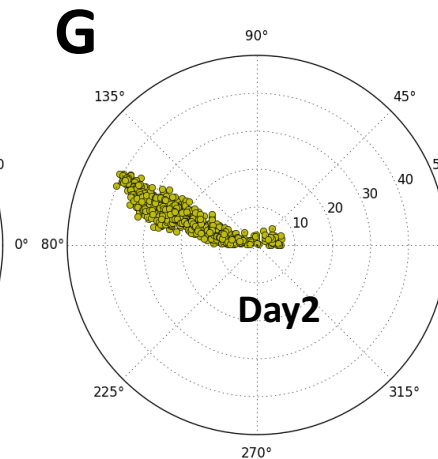
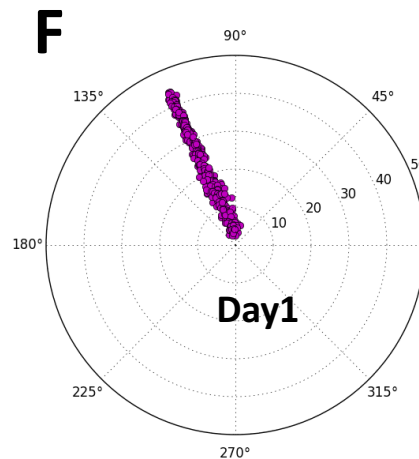
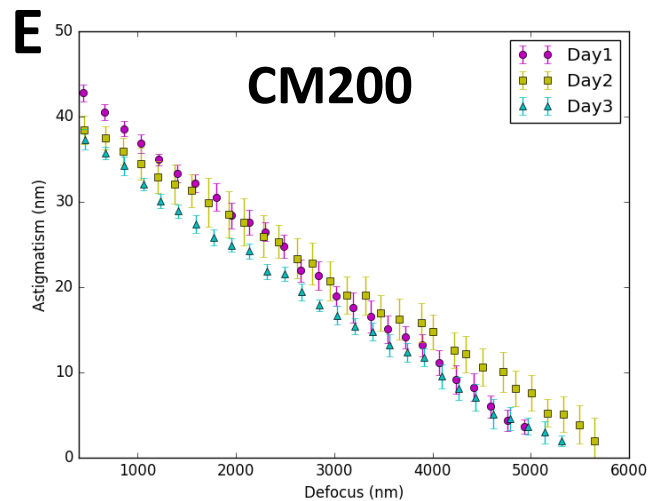
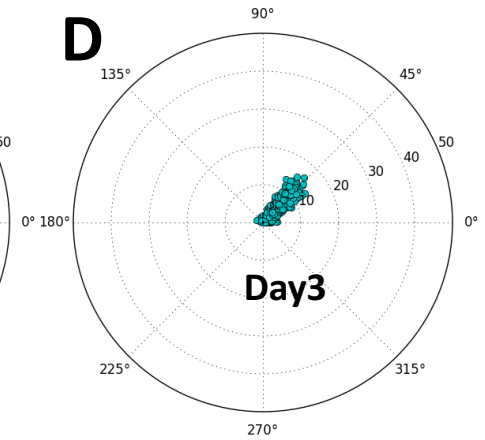
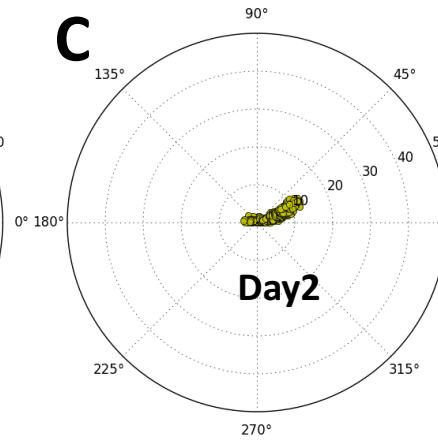
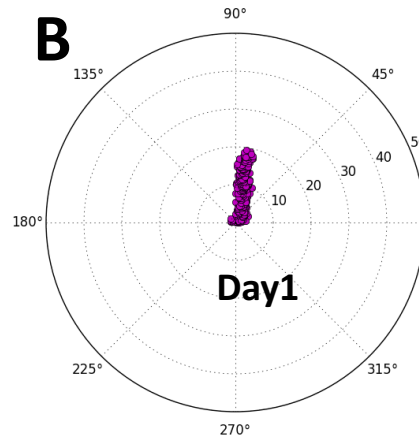
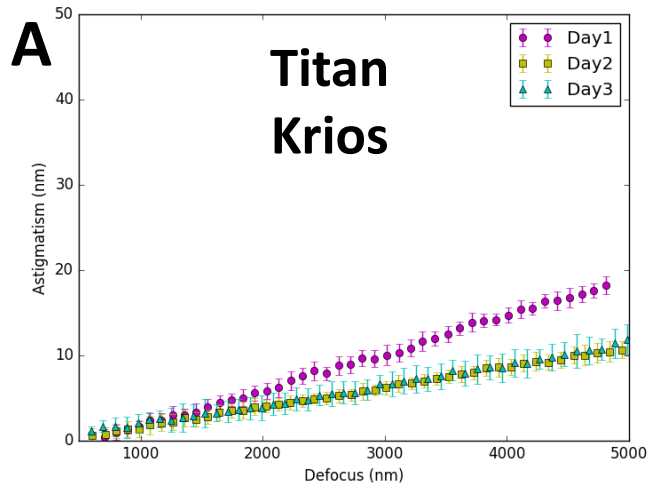


## Data collection

- After astigmatism correction
- Low magnification
- A range of defocus



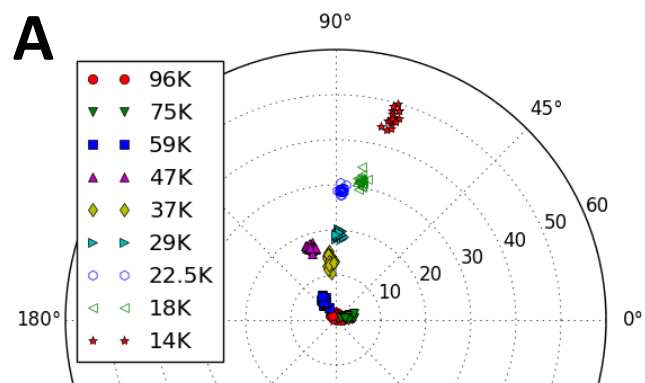
# Variability of defocus-dependent astigmatism



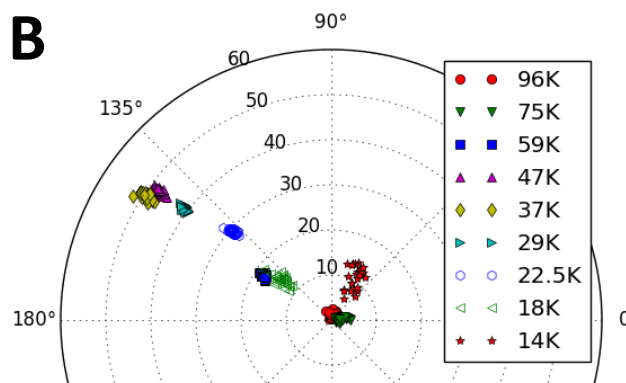
$$\vec{V}_{obj} = k_{obj} \cdot I_{obj} \cdot \vec{e}_{obj}$$

Stochastic variations !

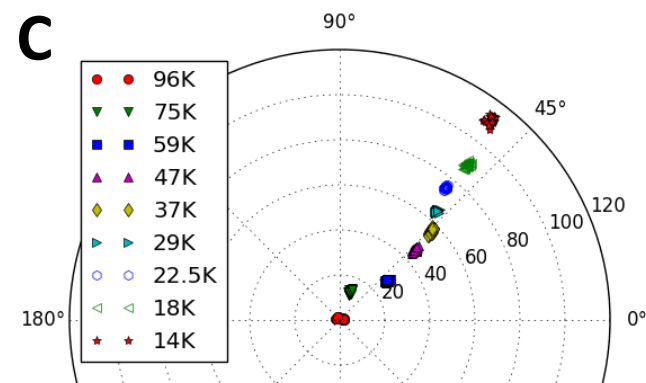
# Magnification-dependent astigmatism (Titan Krios)



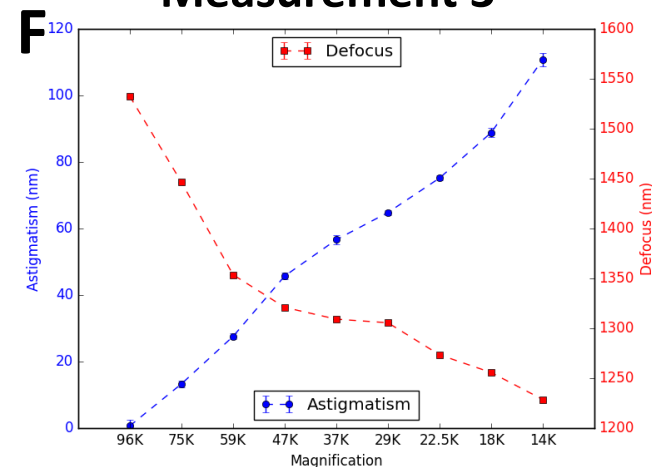
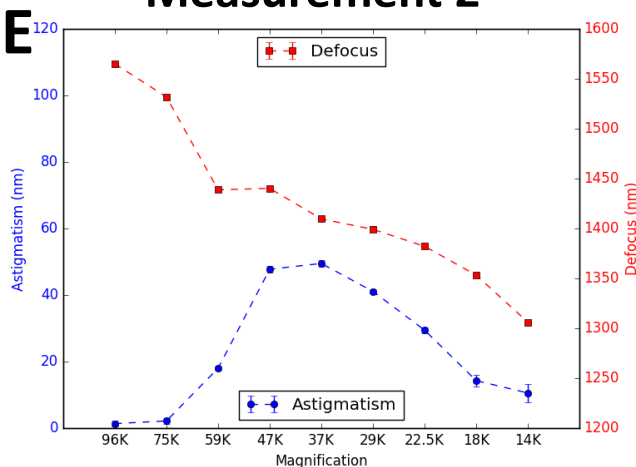
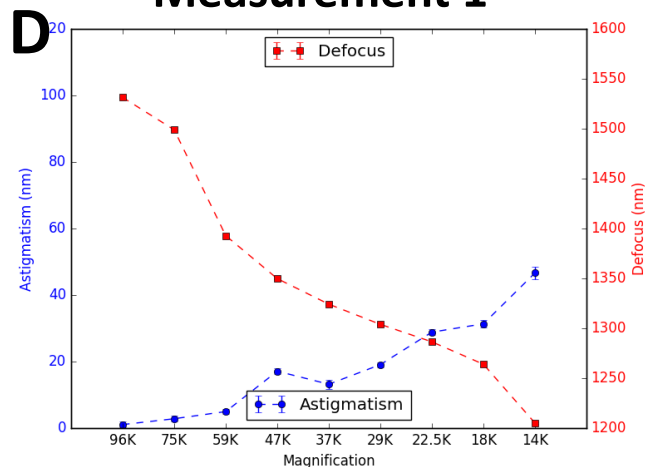
**Measurement 1**



**Measurement 2**



**Measurement 3**





# Recommendations for optimal TEM operations

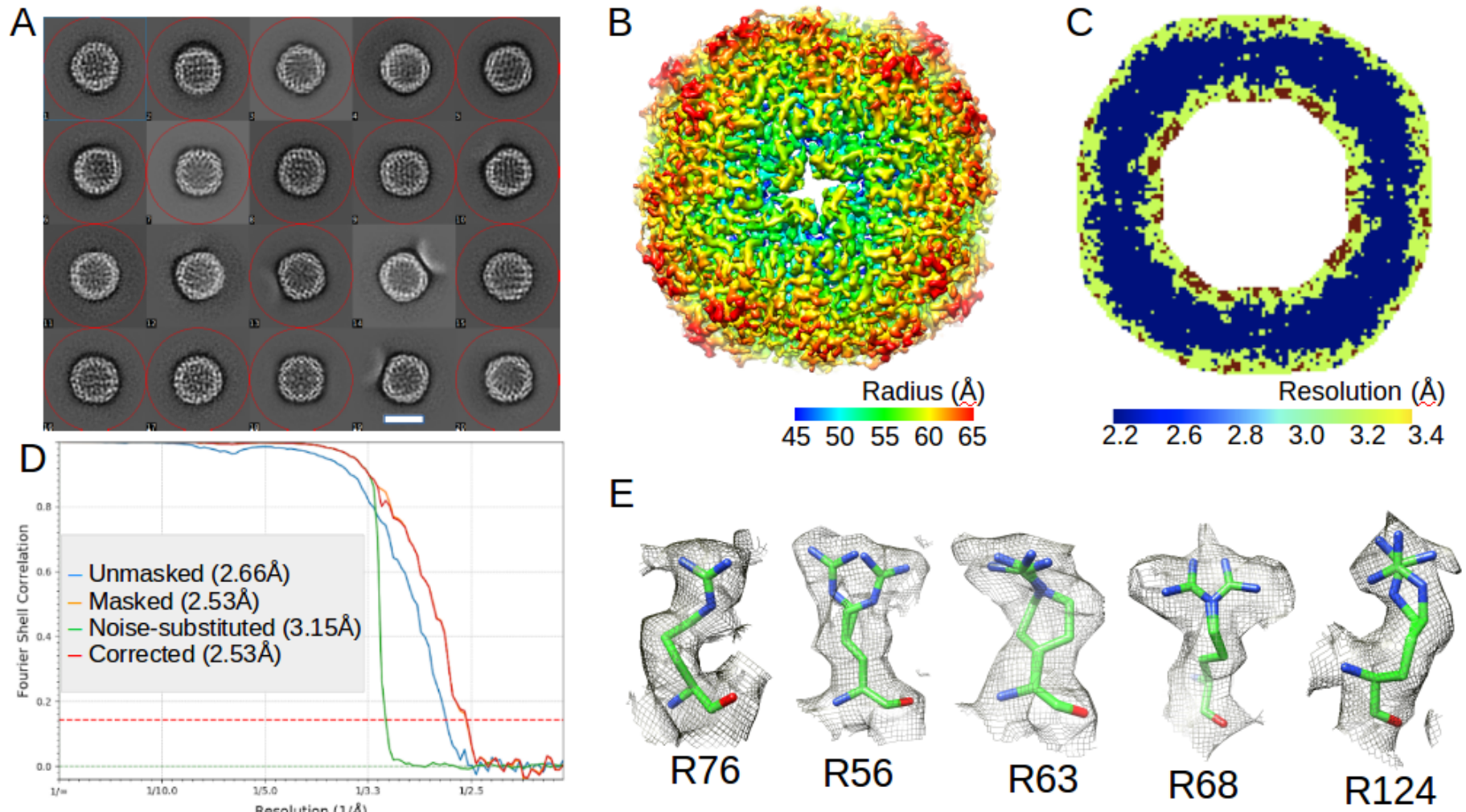
## Instrument Alignment

- mag for instrument alignment = mag for data collection
- defocus for stigmation = median defocus of intended defocus range for data collection

## Data Collection

- mag of focus-mode = mag of exposure mode

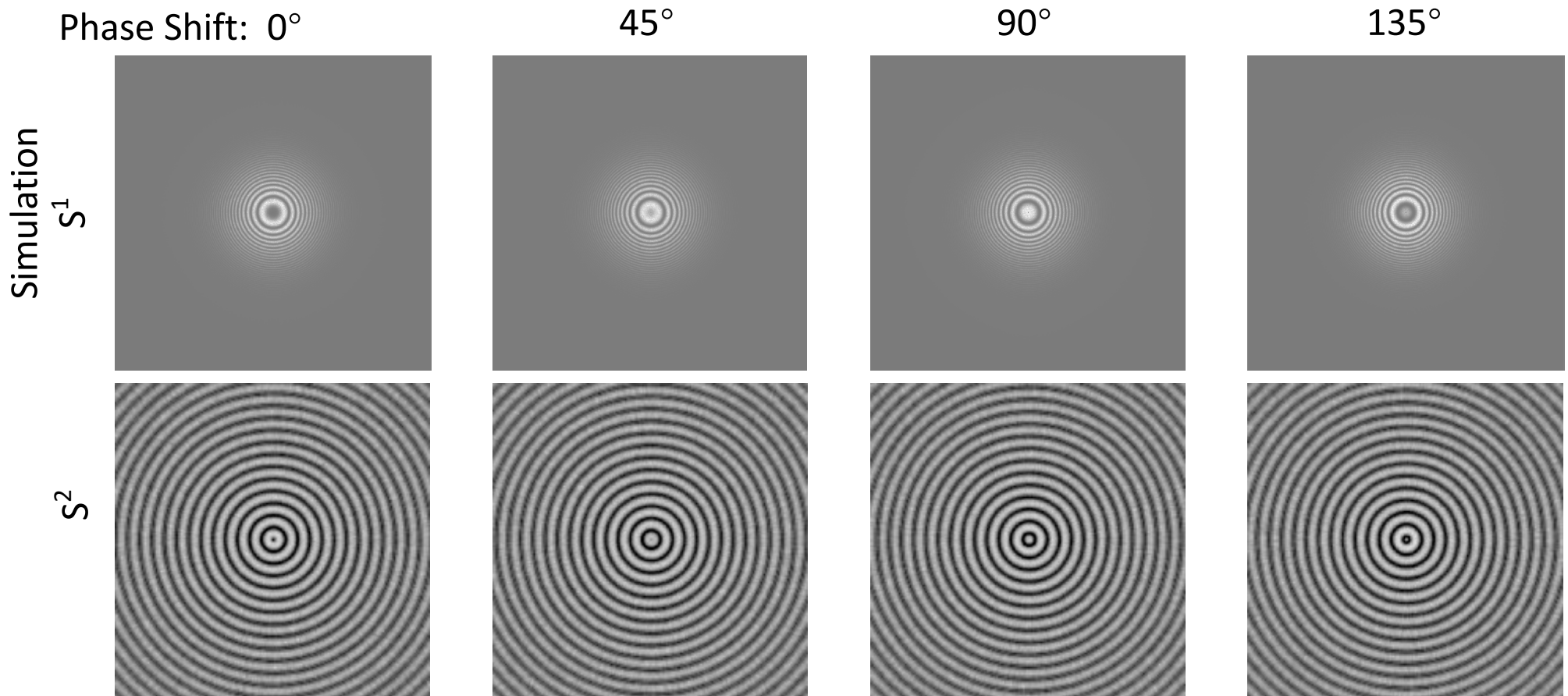
# Volta Phase Plate



*CTF fitting is harder for VPP images*

# $s^2$ ctf: defocus and phase are decoupled

$$CTF(s^2) = \sin\left(2\pi\left(\frac{f\lambda}{2}s^2 + \frac{C_s\lambda^3}{4}s^4\right) + \varphi\right)$$



FFT( $S^2$ )

