s²stigmator: a closed-form solution for single-pass correction of TEM lens astigmatism

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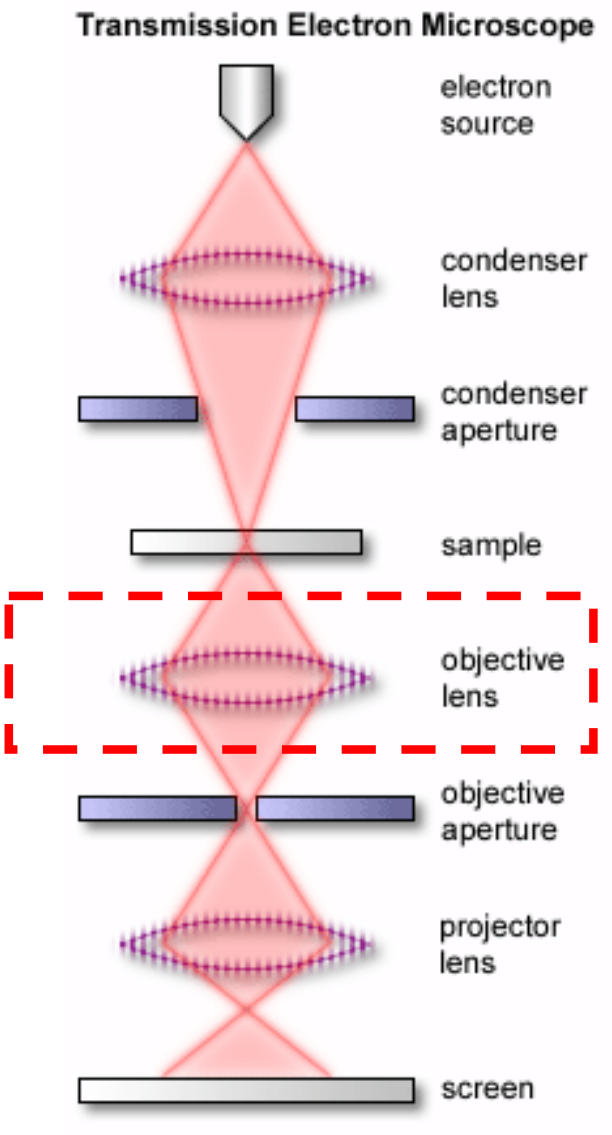
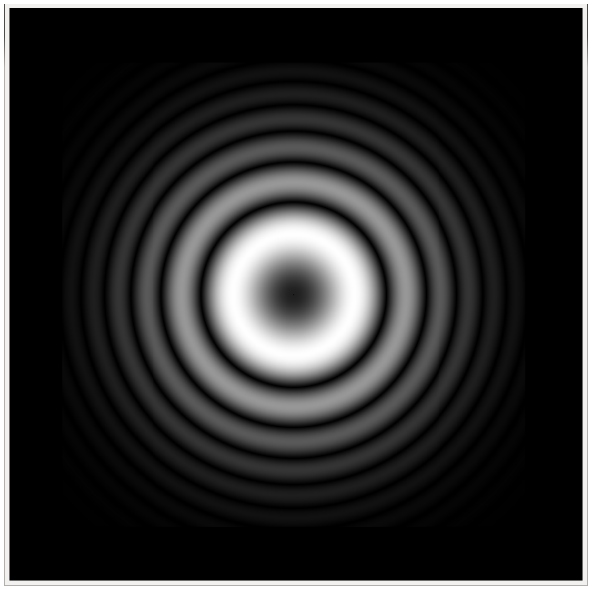
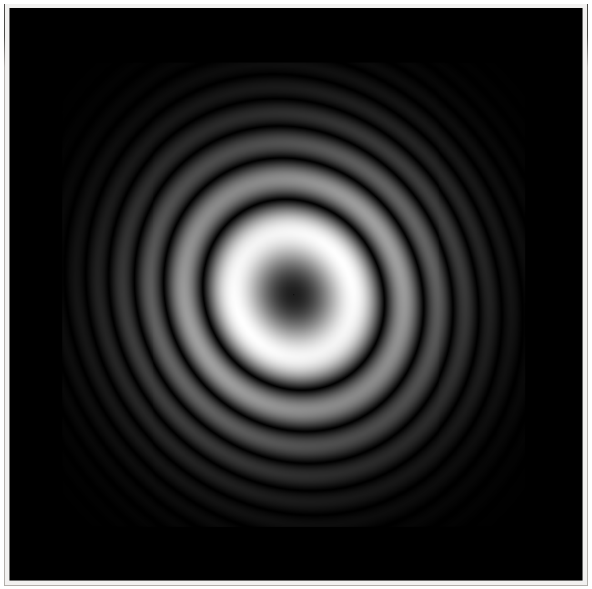
TEM objective lens astigmatism



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



Astigmatism = 0
Defocus = 500nm

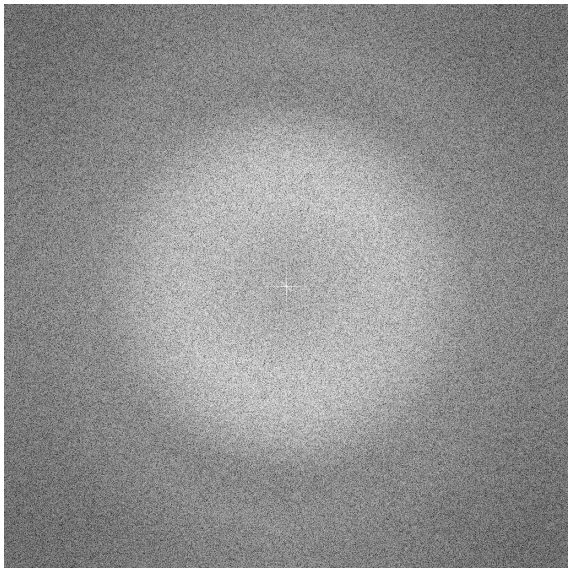


Astigmatism = 100nm
Angle = 30°
Defocus = 500nm

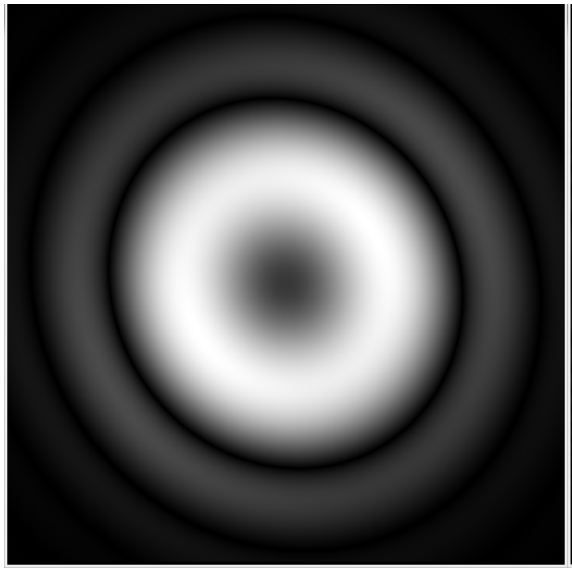
Visual roundness method

Very high magnification & very small defocus for astigmatism correction

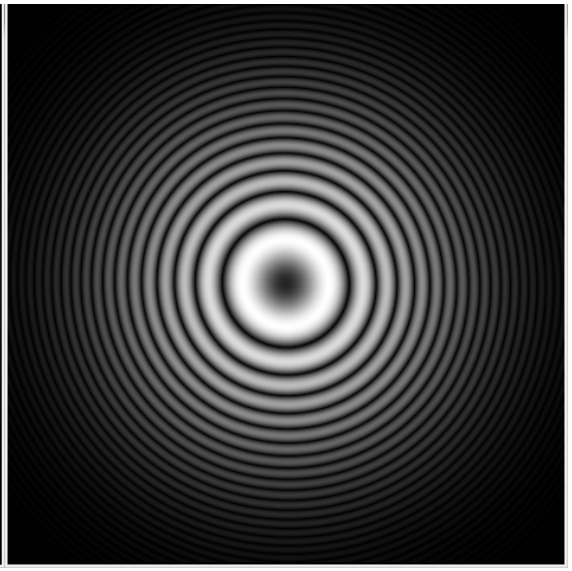
Lower magnification & larger defocus for data collection



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 → s^2 power

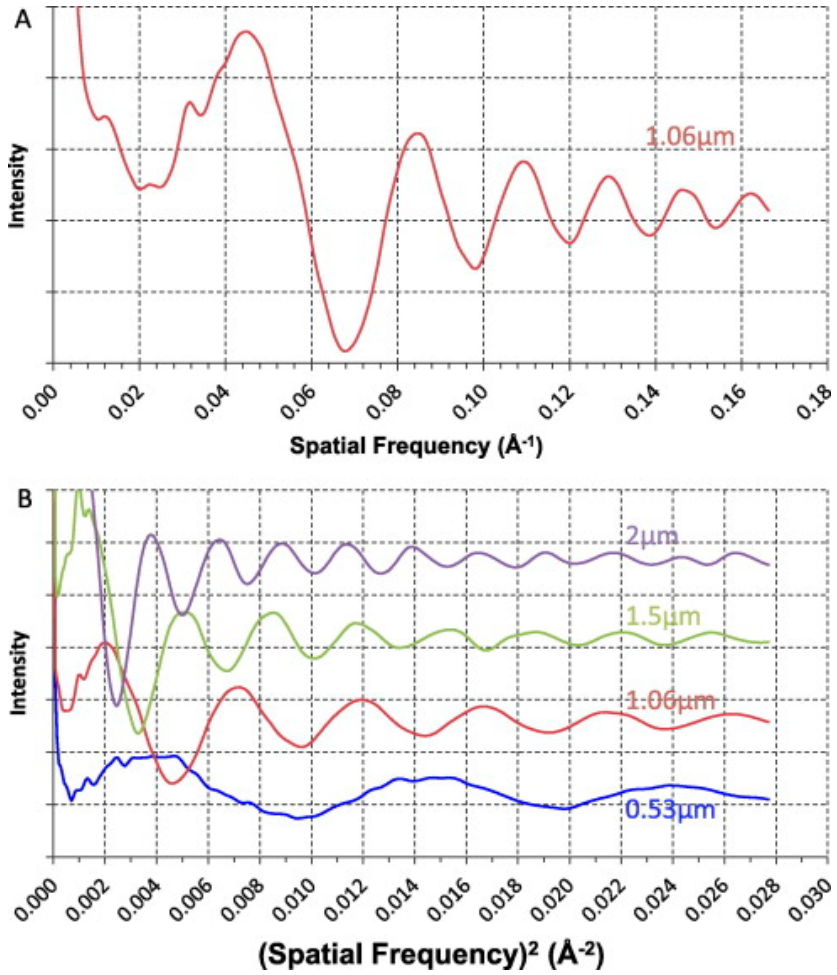
$$CTF(s) = (\sqrt{1-Q^2}\, sin\, \gamma(s) + Q cos\, \gamma(s))$$
$$= sin(\gamma(s) + \varphi_0)$$

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

$$s' = s^2$$

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

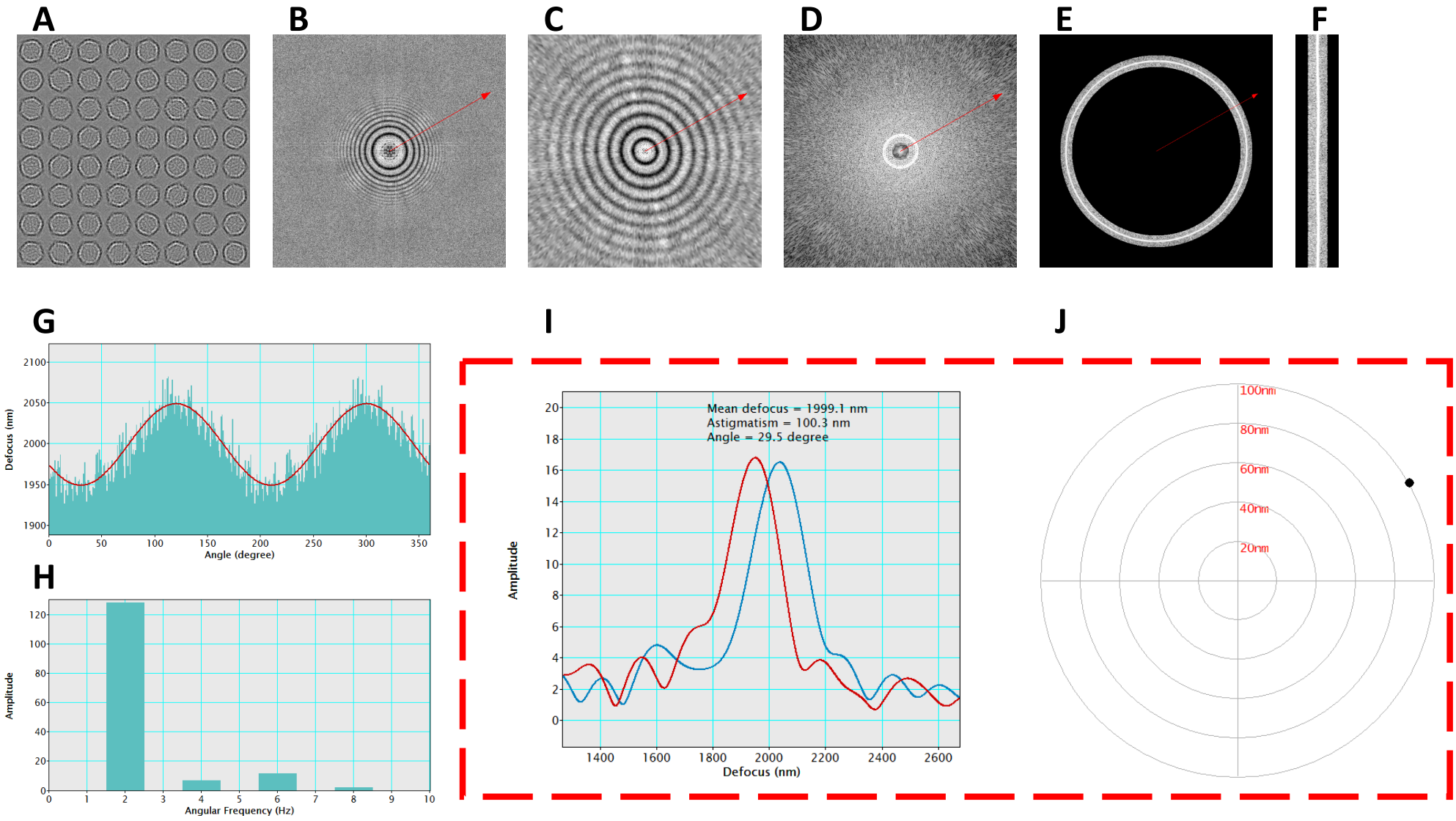
s^1 power: increasing oscillations



s^2 power: uniform oscillations

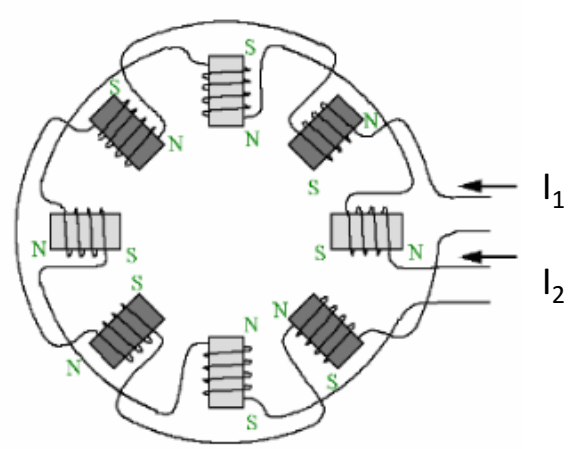
Jiang et al., 2012

s²stigmator: a closed-form algorithm

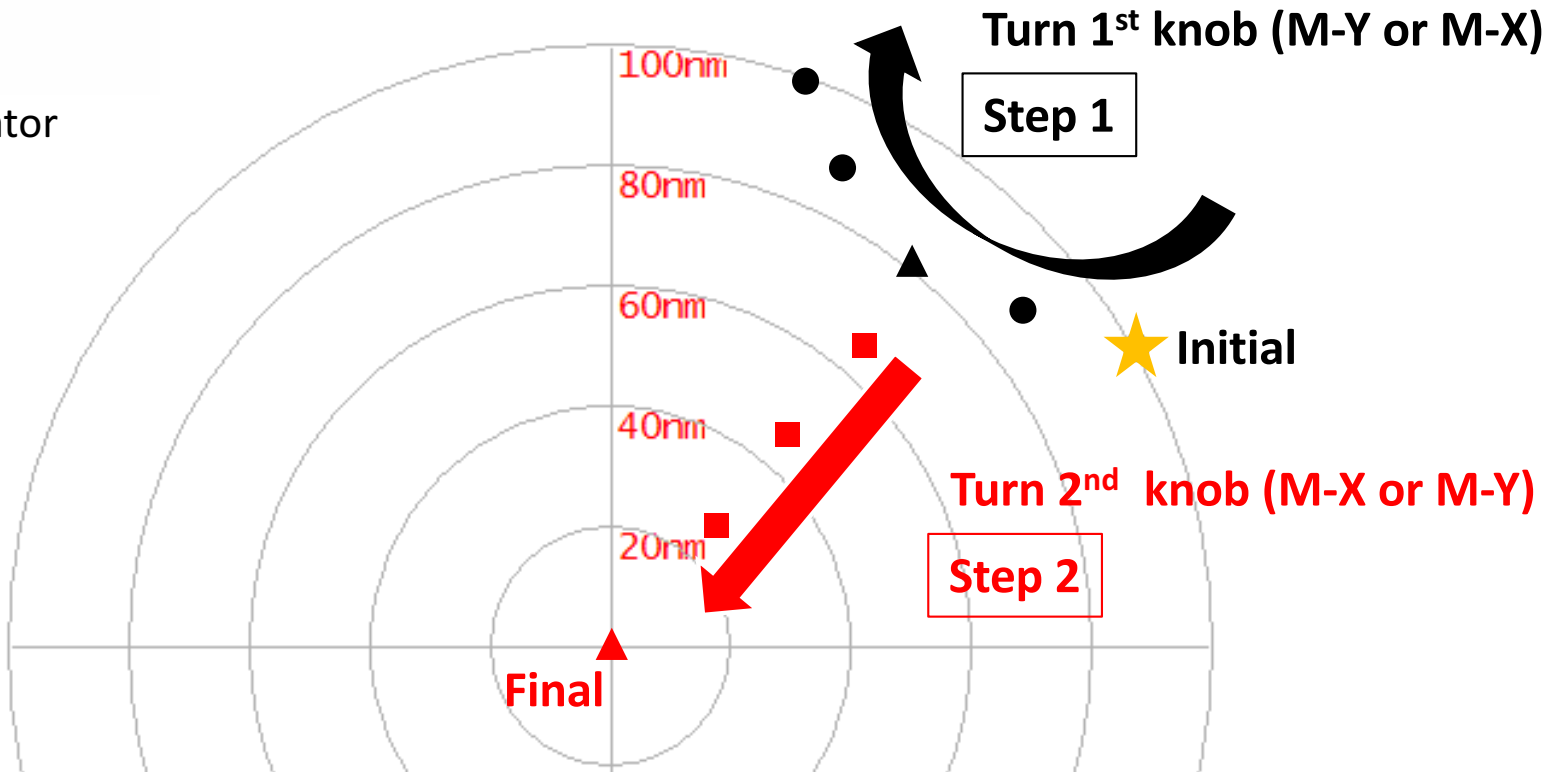


Yan et al., 2017

Single-pass tuning strategy



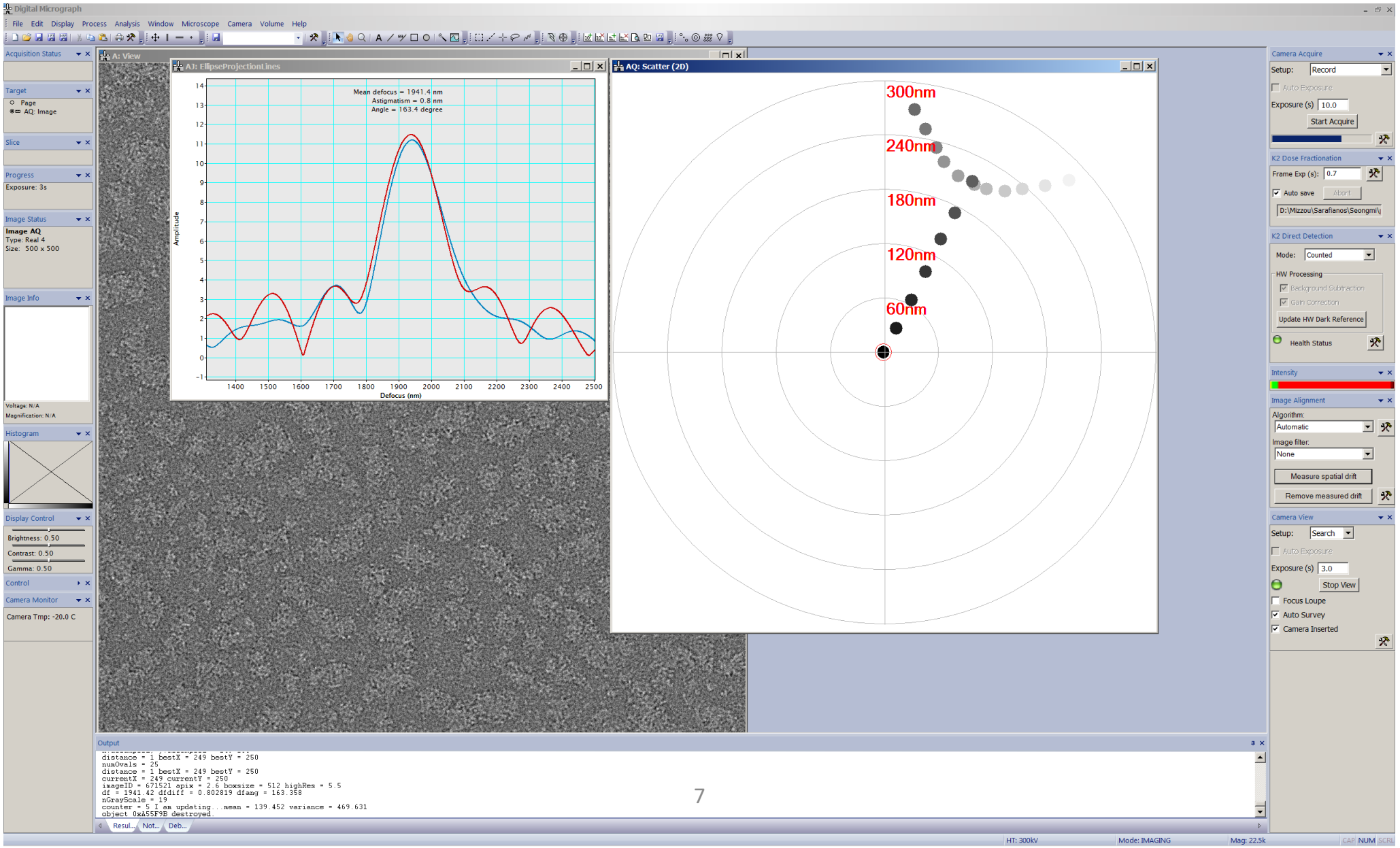
Octupole stigmator



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

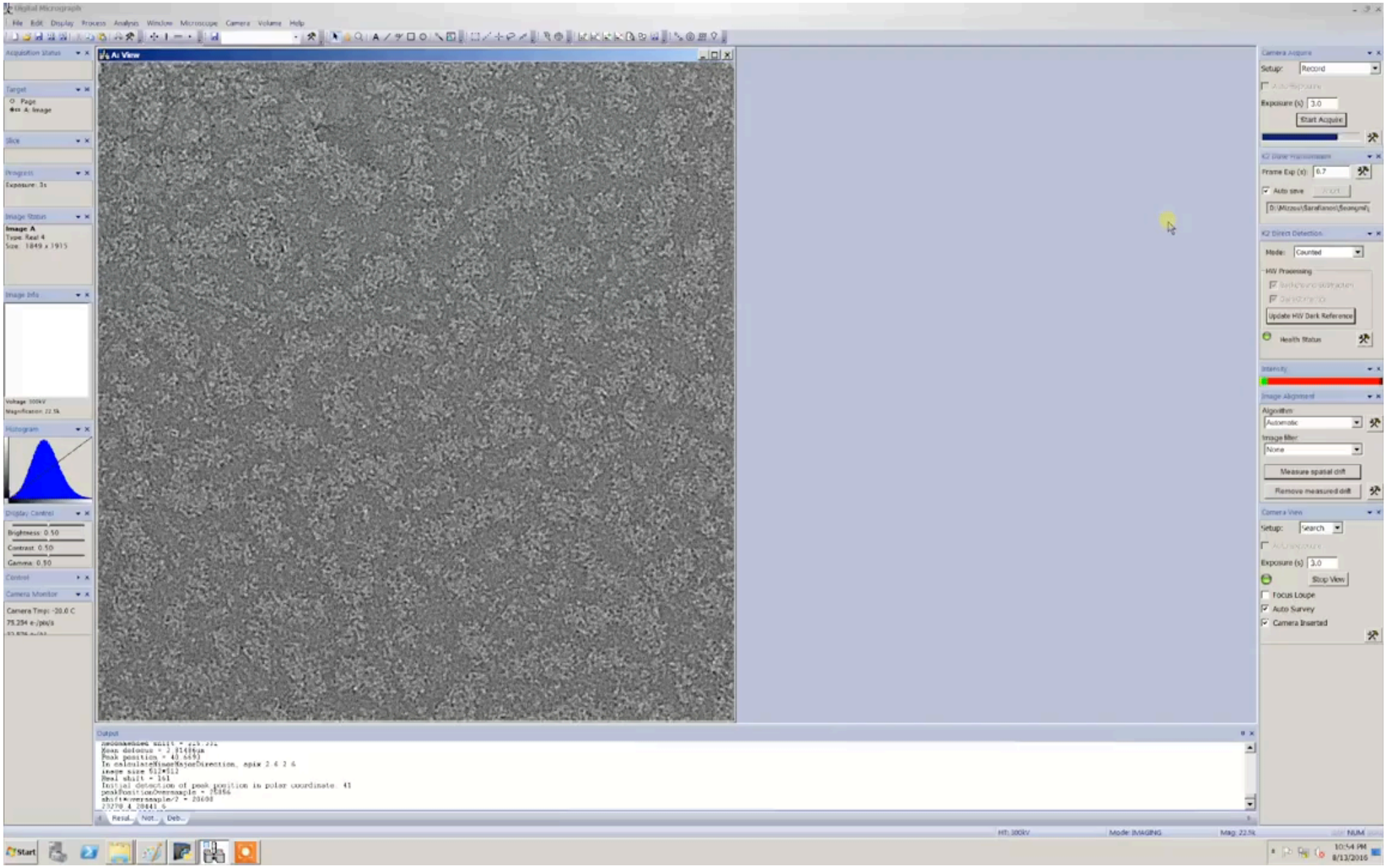
Yan et al., 2017

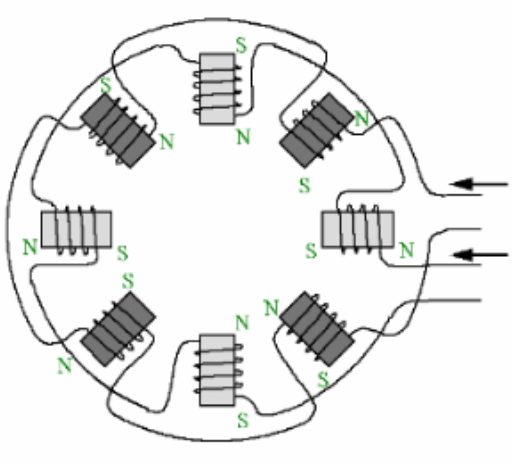
Single-pass tuning strategy



Video: Single-pass tuning strategy

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

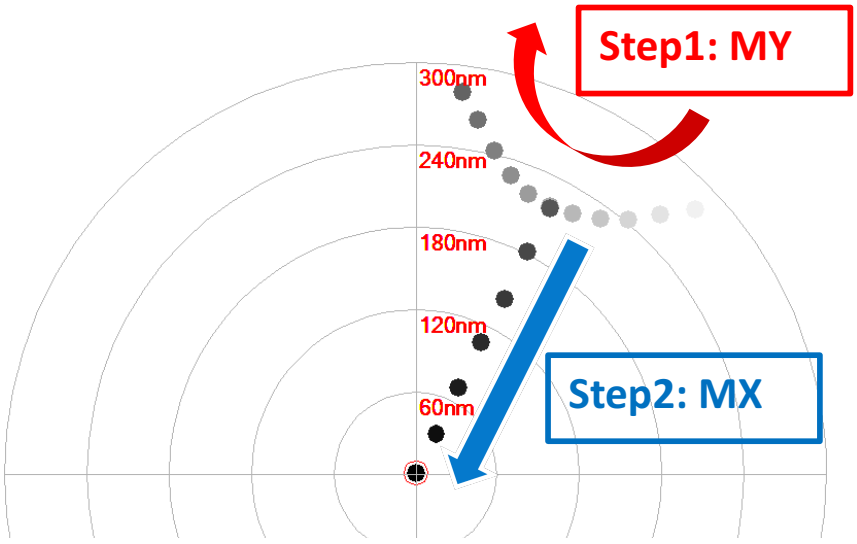




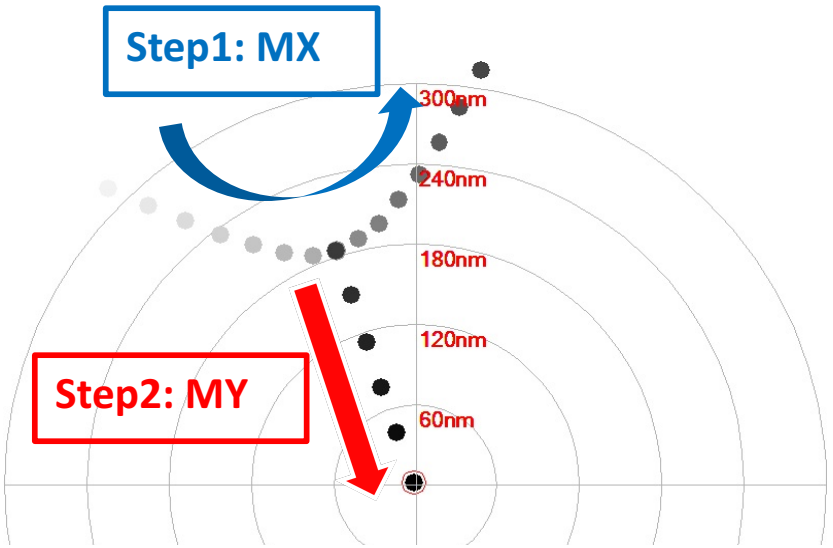
Octupole stigmator

Arbitrary order of adjusting the two stigmators

A：MY-MX trajectory



B：MX-MY trajectory

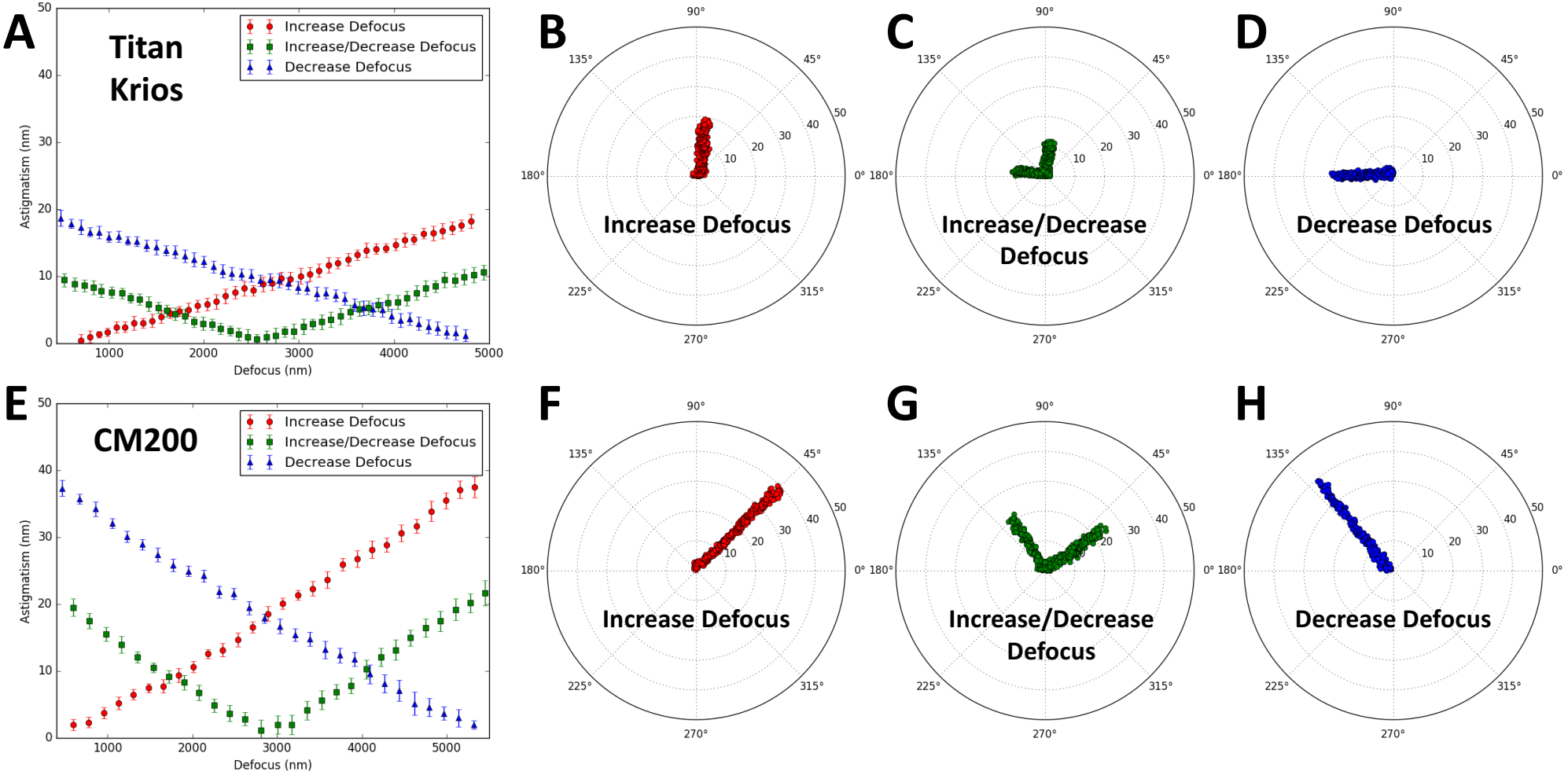


Screenshots of single-pass tuning strategy of astigmatism correction using *s²stigmator*

s²stigmator 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



Yan et al., 2018

Defocus-dependent astigmatism

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

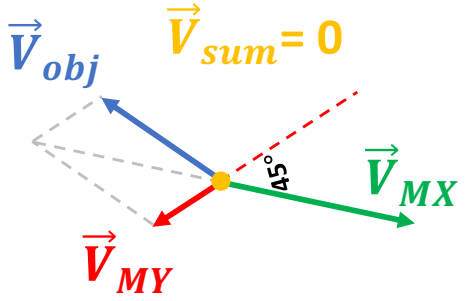
$$= k_{obj} \cdot I^{0}_{obj} \cdot \vec{e}_{obj} + k_{MX} \cdot I_{MX} \cdot \vec{e}_{MX} + k_{MY} \cdot I_{MY} \cdot \vec{e}_{MY} + k_{obj} \cdot (I_{obj} - I^{0}_{obj}) \cdot \vec{e}_{obj}$$

Defocus-dependent astigmatism term

Scaling factor

Unit vector

Change of objective lens current when defocus is increased/decreased

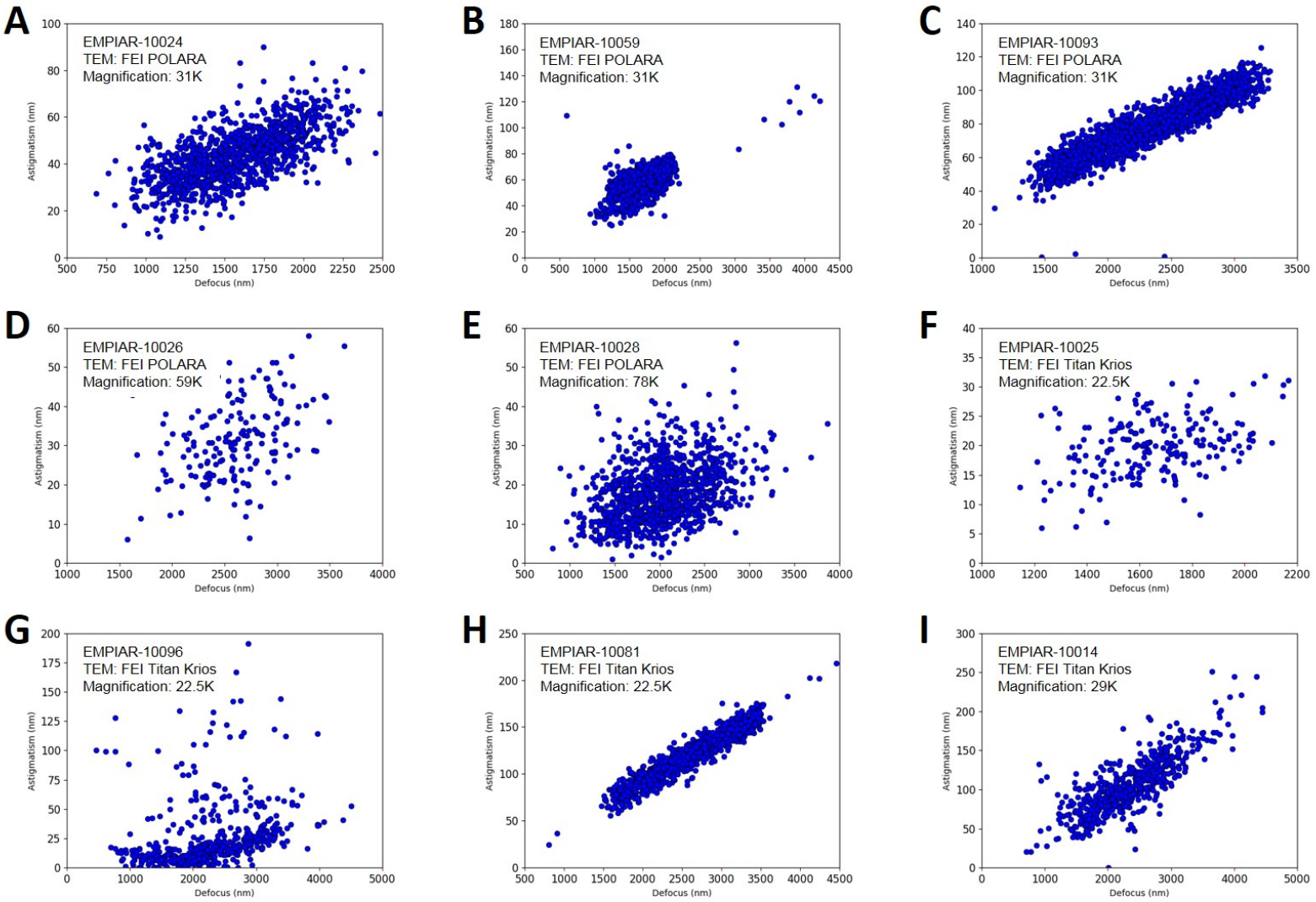


k: a scaling factor representing how strong the dependence is between $\vec{V}$ and I

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

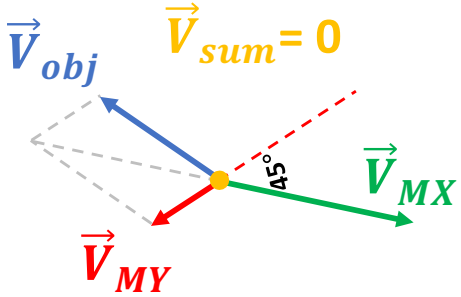


Yan et al., 2018

Origin of defocus/magnification dependent astigmatism

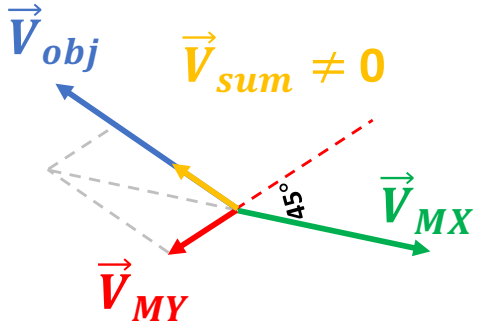
Microscope alignment

- Before data collection
- High magnification
- Small defocus

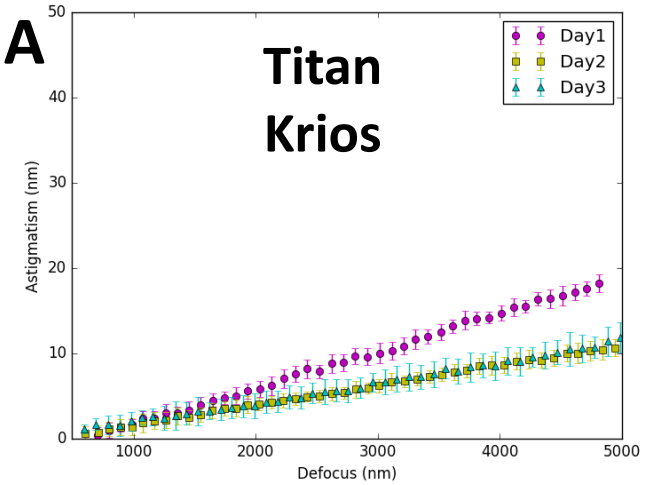


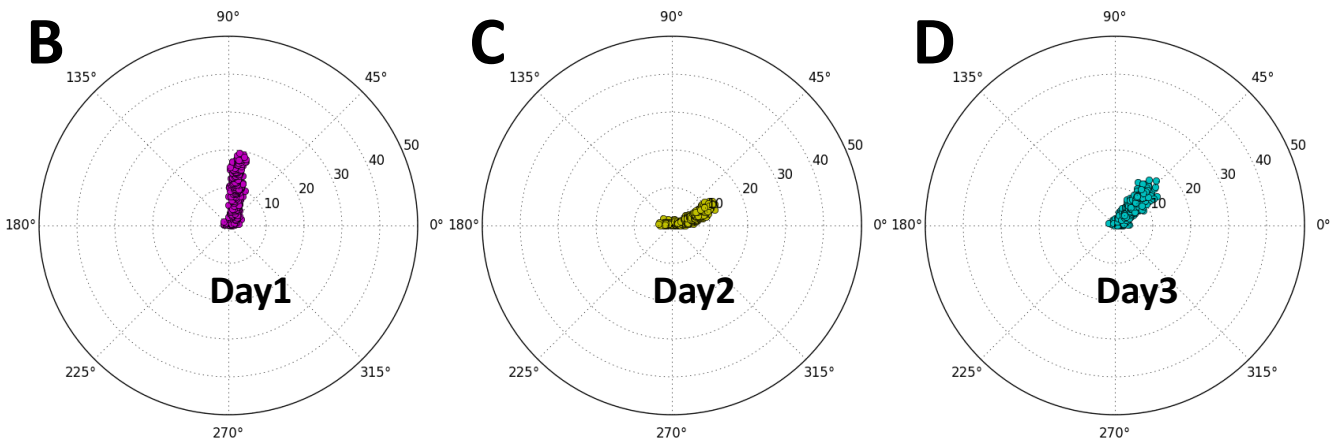
Data collection

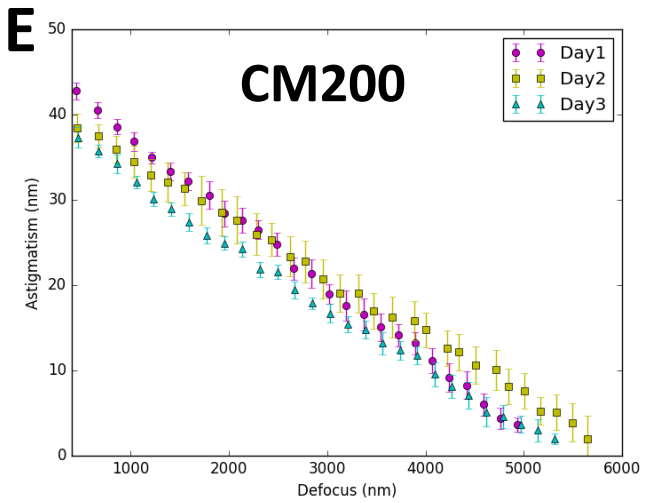
- After astigmatism correction
- Low magnification
- A range of defocus

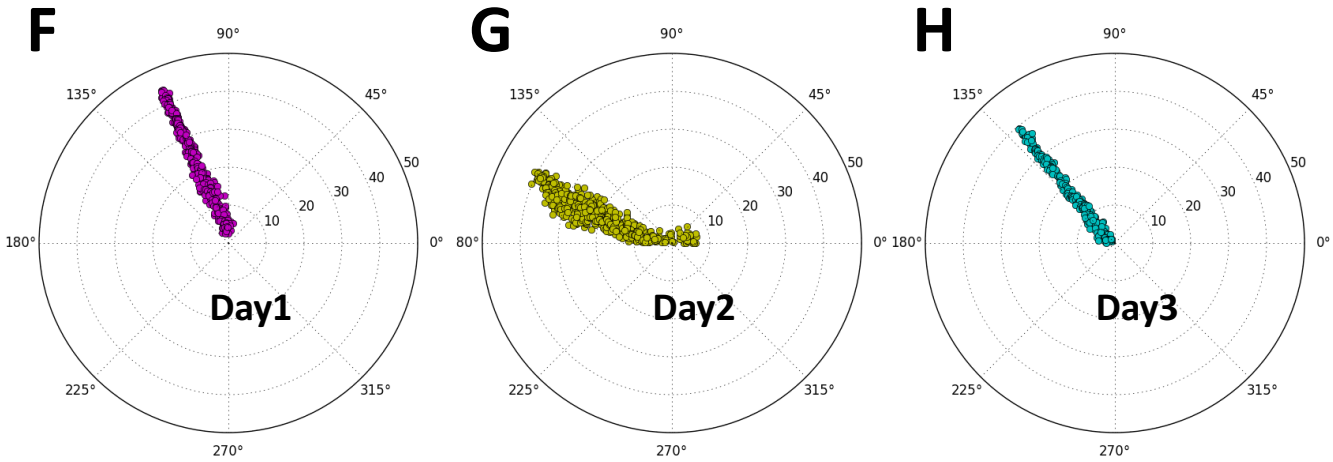


Variability of defocus-dependent astigmatism







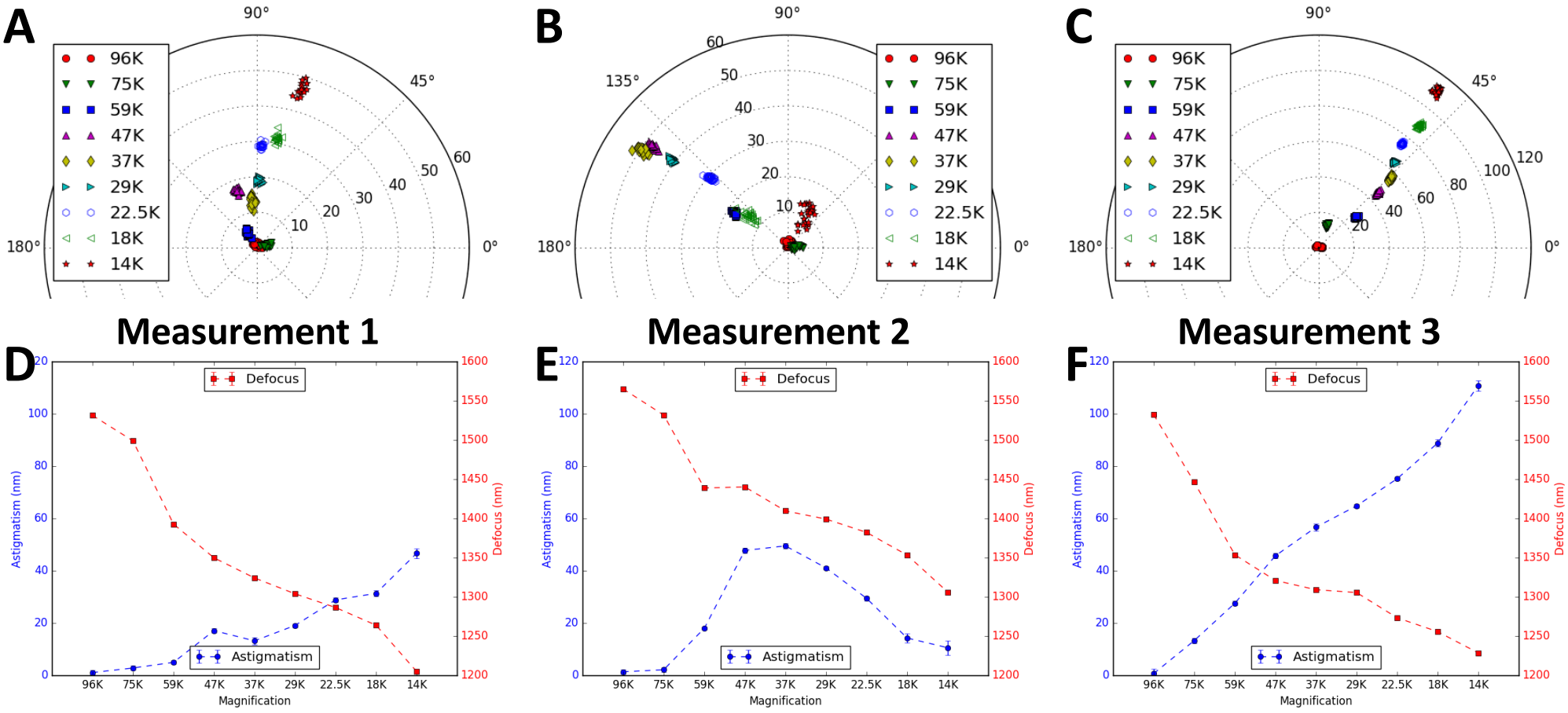


$$\vec{V}_{obj} = k_{obj} \cdot I_{obj} \cdot \vec{e}_{obj}$$

Stochastic variations !

Yan et al., 2018

Magnification-dependent astigmatism (Titan Krios)



Yan et al., 2018

Recommendations for optimal TEM operations

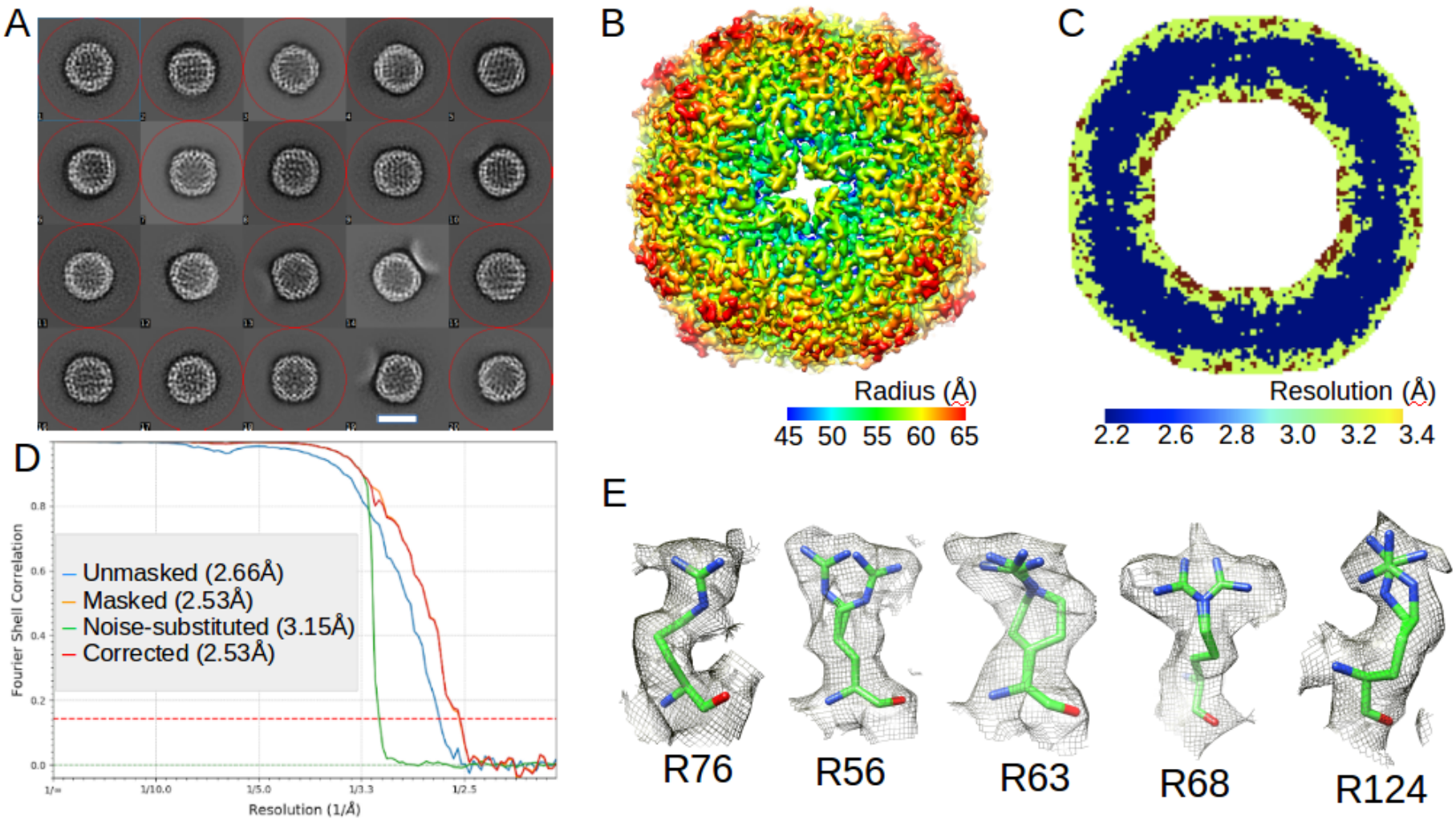
Instrument Alignment

- 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

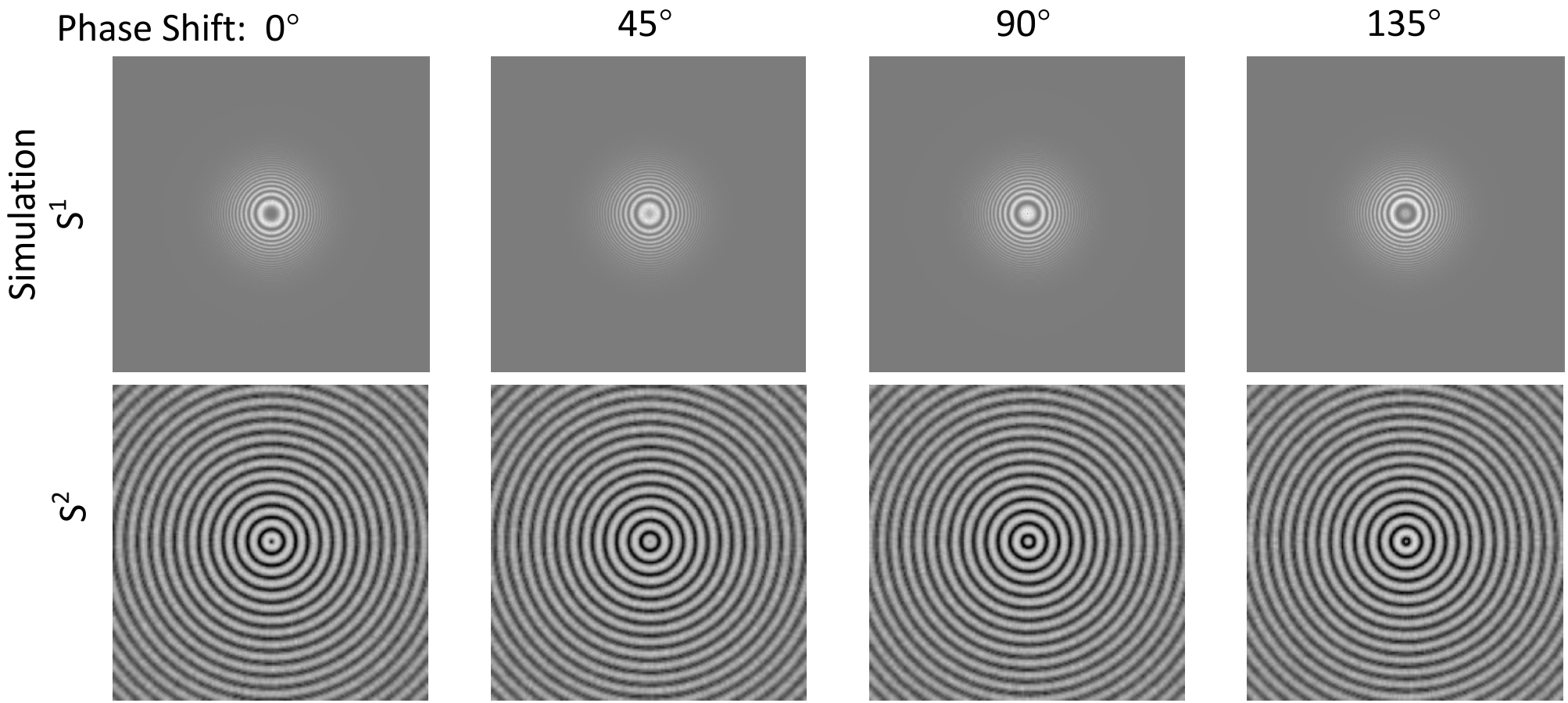
Volta Phase Plate



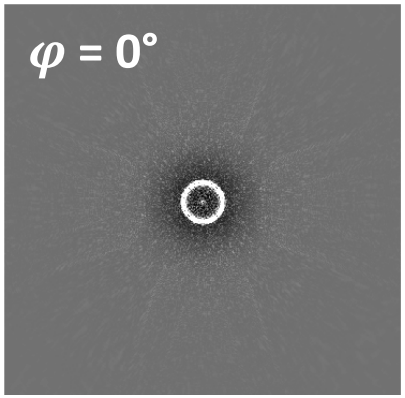
CTF fitting is harder for VPP images

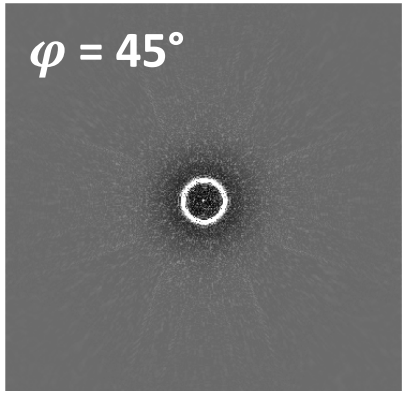
s^2ctf: defocus and phase are decoupled

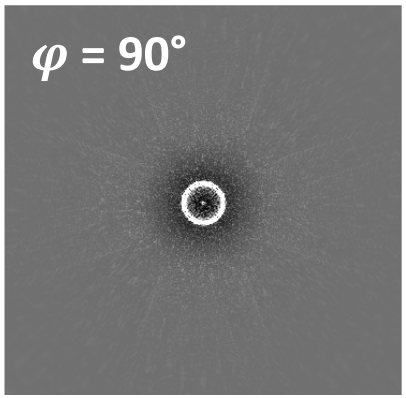
$$CTF(s^2) = \sin(2\pi(\frac{\boldsymbol{f}\lambda}{2}s^2 + \frac{C_s\lambda^3}{4}s^4) + \boldsymbol{\varphi})$$

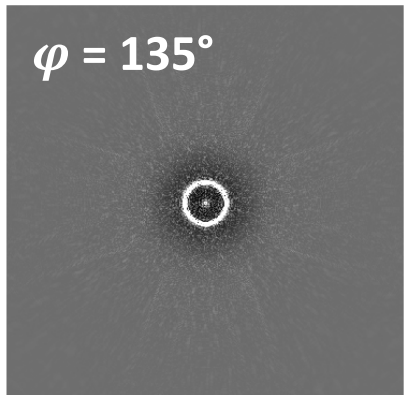


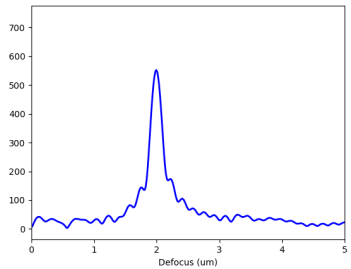
FFT(S^2)

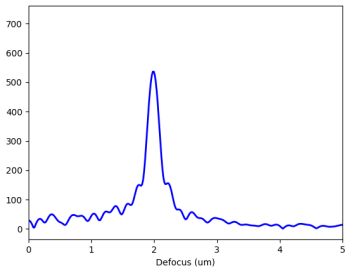

φ = 0°

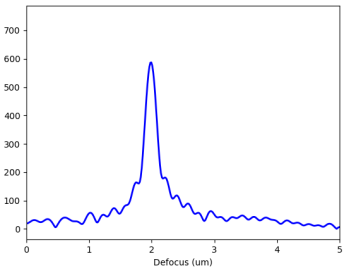

φ = 45°

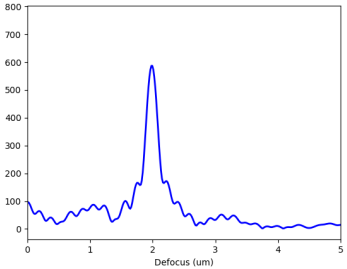

φ = 90°

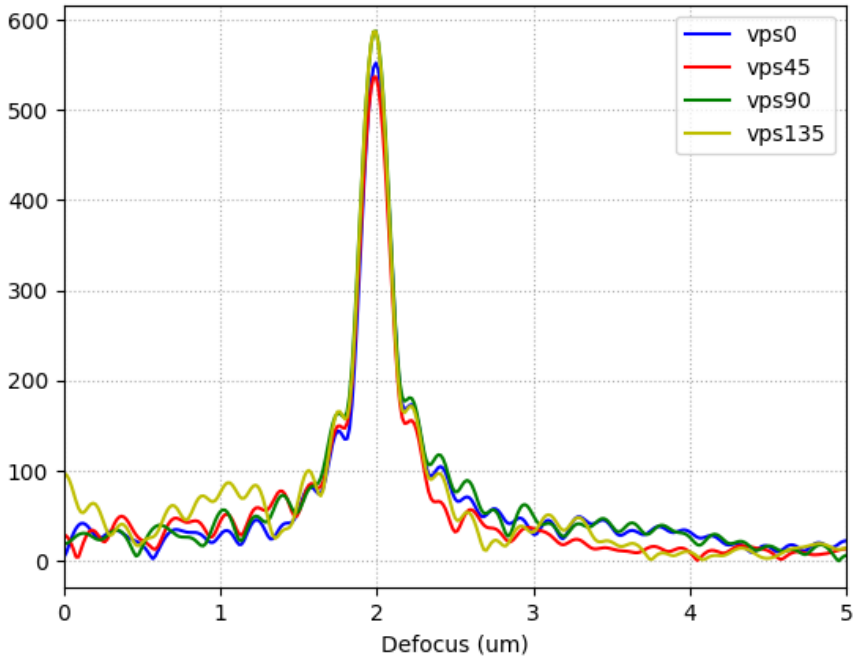

φ = 135°


Defocus (um)


Defocus (um)


Defocus (um)


Defocus (um)


vps0
vps45
vps90
vps135
Defocus (um)