Structured Illumination Microscopy method for Adaptive Optics Flood Illumination Ophthalmoscope

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Outline

1. Context of Retinal imaging
2. Presentation of the experimental set-up
3. Structured Illumination Microscopy (SIM) for retinal imaging
4. Simulation results
5. Conclusion and perspectives

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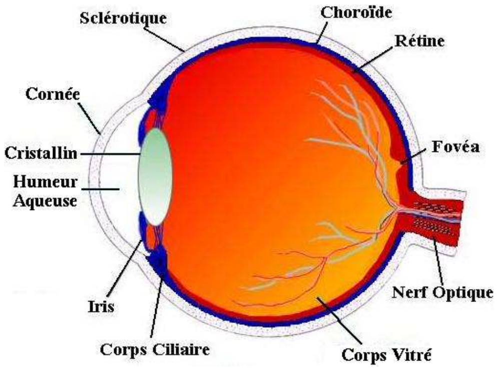
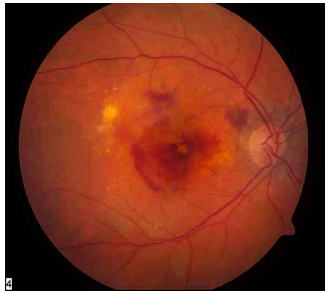
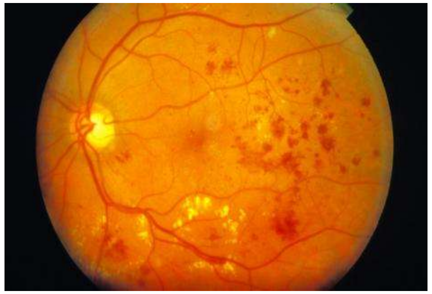
1. Retinal imaging : Context



Glaucoma



AMD



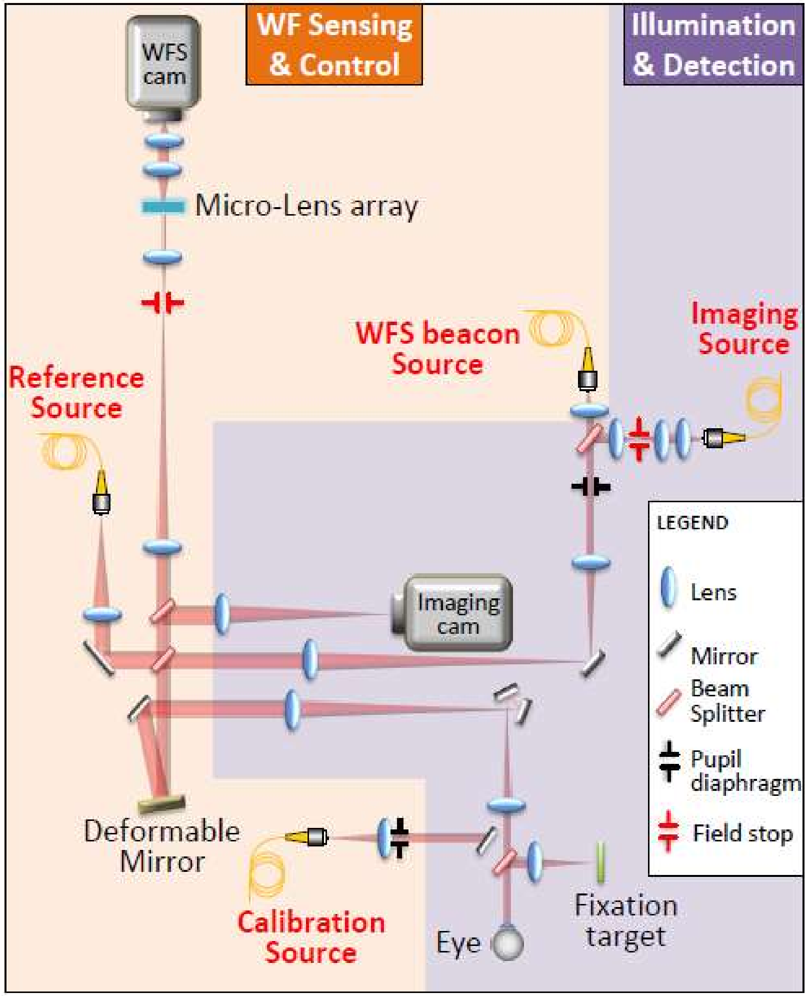
Diabetic retinopathy

→ **High resolution in vivo retina images at the cellular-scale** for early diagnosis

Specificities of retinal imaging :

- Pupil of the eye limits the numerical aperture
- Eye motion
- Dynamic and static optical aberrations
- Low incident illumination flux (Ocular safety standards)

2. Adaptive Optics Flood-Illumination Ophthalmoscope (AO-FIO)

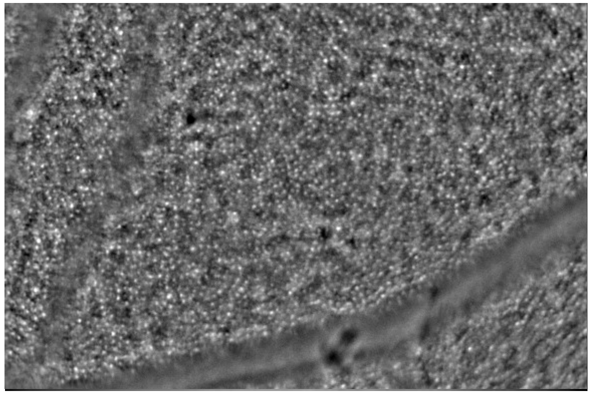


Gofas-Salas et al., 2018

2 subsystems :

- **Wavefront (WF) Sensing & Control :** Measures the WF aberrations and compensates them.
- **Illumination & Detection :** Forms the retina image on a camera.

- **Large Field of View (2.7°x5.4°)**
- **High framerate (200 Hz)**
- **High lateral resolution (2 µm)**

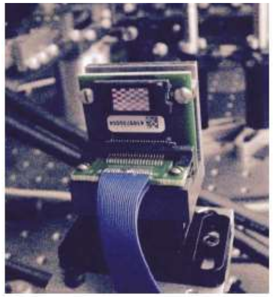


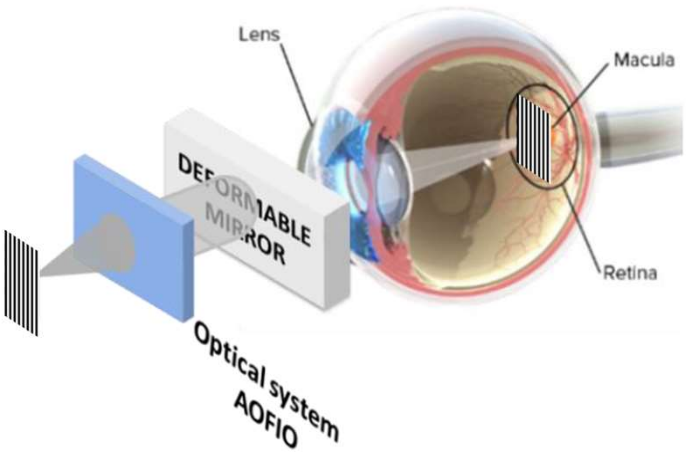
- Enhanced contrast by image processing
- **Poor axial resolution and optical sectioning**

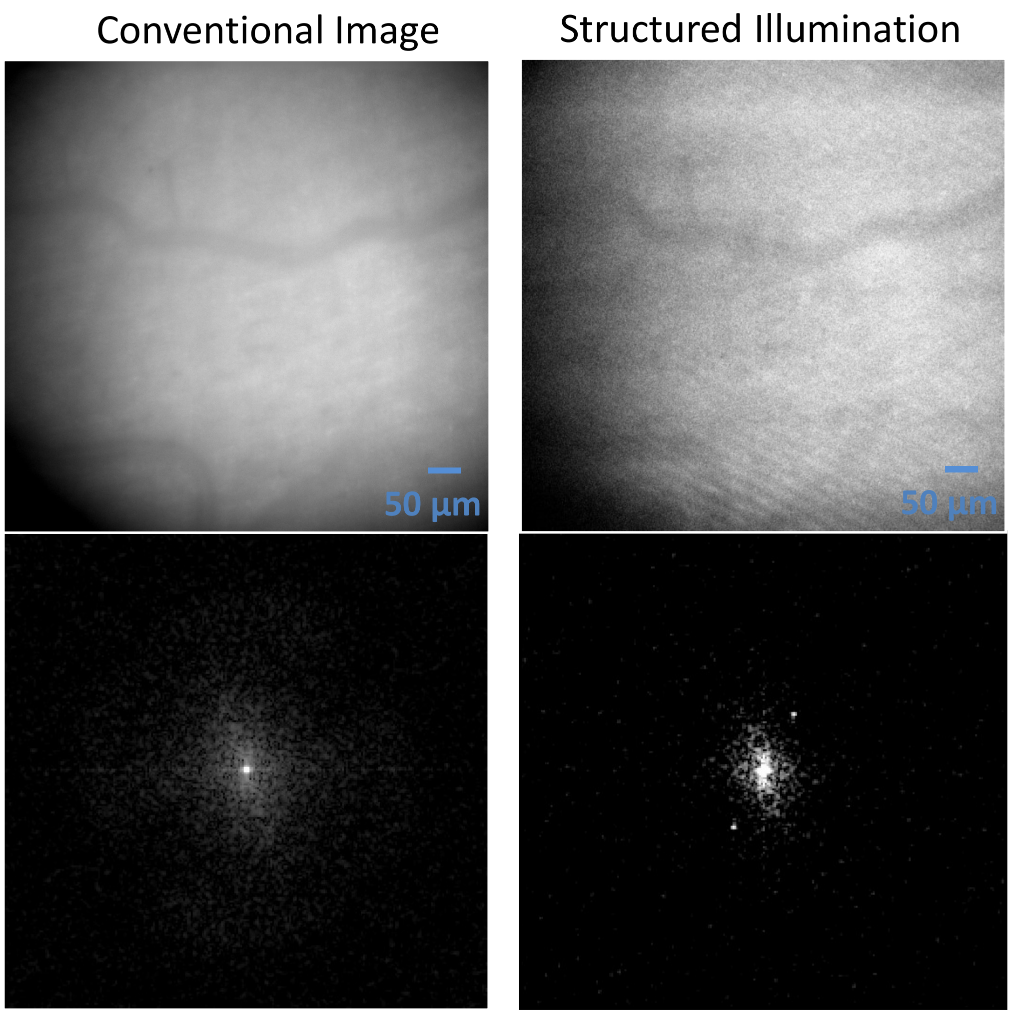
2. AO-FIO + Structured Illumination (SI)

<u>Purpose</u> : Improve the optical sectioning and the lateral resolution using an adapted Structured Illumination Microscopy (SIM) method for retinal imaging

Digital Micromirror Device (DMD)







Fringes are visible !

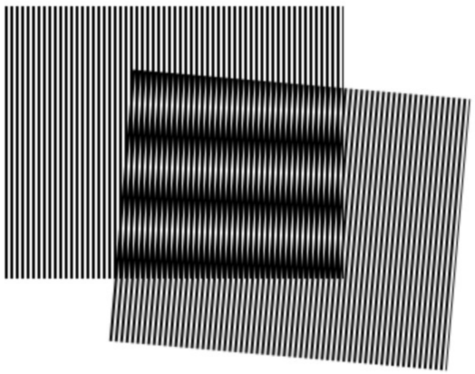
3. SIM for retinal imaging : Principles of SIM

➢ Object illuminated by a sinusoidal light pattern

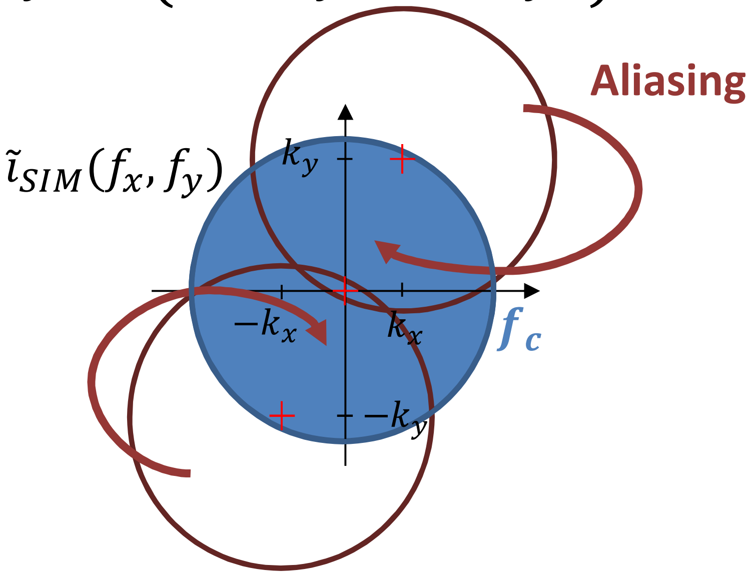
$$m(x,y) = 1 + \text{c}.\cos\big(2\pi(k_x x + k_y y + \phi)\big)$$
$$i_{SIM}(x,y) = PSF(x,y) * \big(m(x,y).o(x,y)\big)$$

$m(x,y)$: Illumination pattern
$i_{SIM}(x,y)$: SIM image
$o(x,y)$: Unknown object

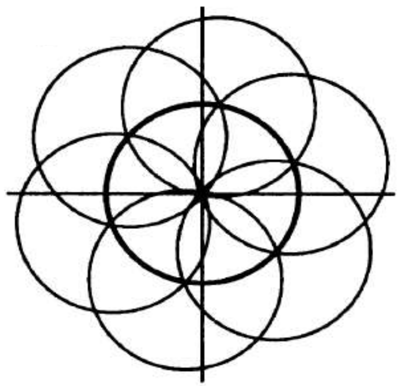
✓ Super Resolution



Moiré effect



Optical spatial frequency cutoff f_c
Replica of the object spectrum

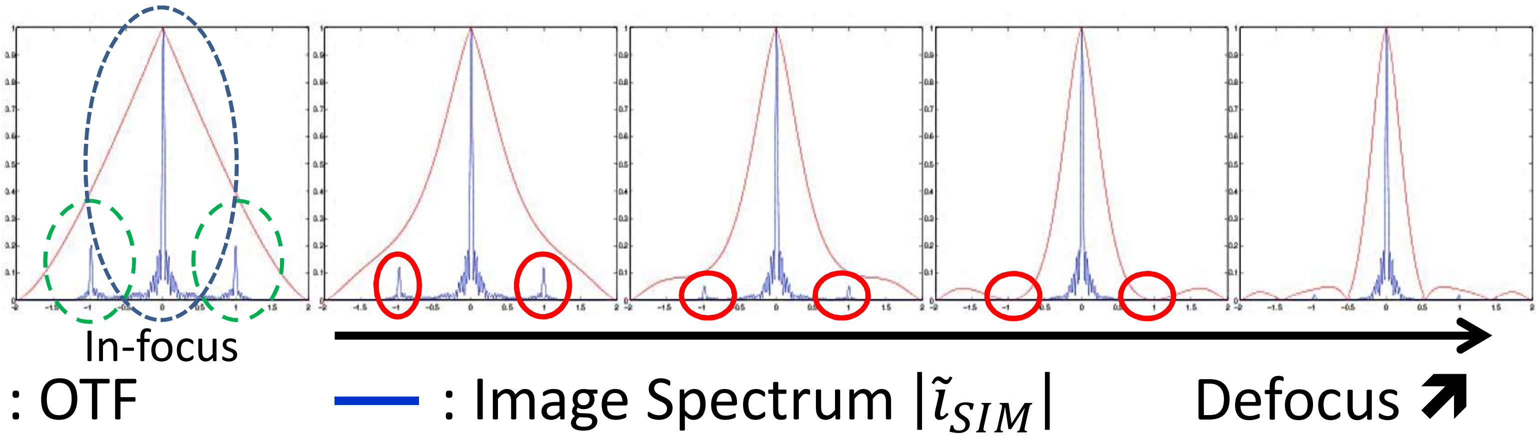


Gustaffson (2000)

3 orientations of the pattern : Isotropic increasing of the accessible spatial frequency domain

✓ Optical sectioning

Effect of defocus on the spectrum of a 2D object and the Optical Transfer Function (OTF) :



Modulation spatial frequency $f_{mod} = \frac{f_c}{2}$, Grupetta, 2013

Image model : $i_{SIM}(x,y) = i_{CV}(x,y) + i_{OS}(x,y)$

- $i_{CV}(x,y)$: Conventional image In and out-of-focus content
- $i_{OS}(x,y)$: **« optically sectioned » (OS) In-focus content**

3. SIM for retinal imaging : Proposed method (1/2)

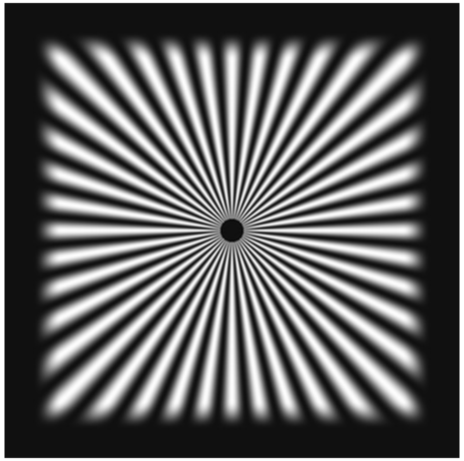
High resolution reconstruction of the object in-focus layer o_0 from the SIM data

- Set of acquired images $\{i_l\}_l$ with structured illumination
- <u>Computation of the OS information</u> : $\{i_{l|OS}\}_l$

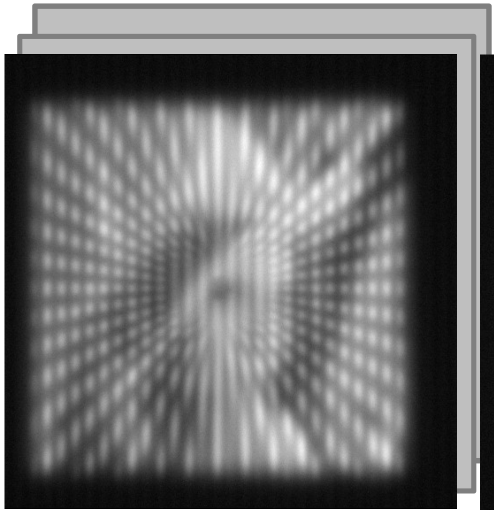
i_{CV}^{exp} : image acquired with the object being homogenously illuminated

MAP estimation of the eye motion shift $s_l(x, y)$

Computation of $i_{l|OS}^{exp} = i_l - i_{CV}^{exp} * \hat{s}_l$



In-focus object o_0



SIM Images $\{\boldsymbol{i}_l\}_l$
with random shifts s_l

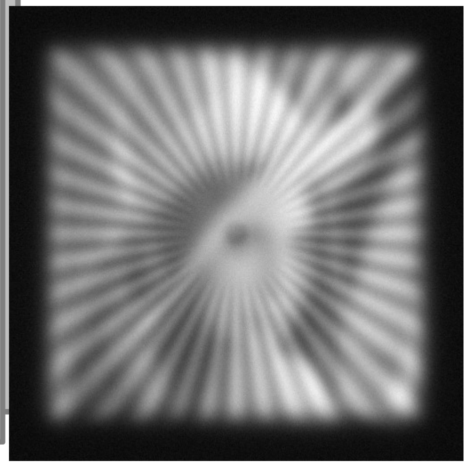
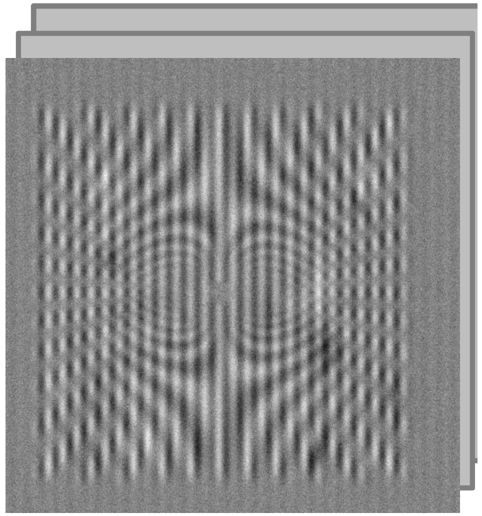
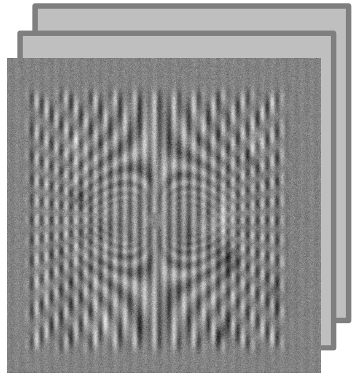


Image i_{CV}^{exp}



Images $\left\{i_{l|OS}^{exp}\right\}_l$

3. SIM for retinal imaging : Proposed method (2/2)



- Inverse problem :

$$i_{l|OS}^{exp} = h_0 * \left(m_l'.(o_0 * s_l)\right) + n_l$$

o_0 : object in-focus layer to reconstruct

h_0 : PSF at the in-focus layer of the object

n_l : Gaussian noise of non-stationary variance $\sigma^2(x,y)$

$m_l'(x,y) = m_l(x,y) - 1$: Cosine part of the illumination pattern

- Regularized cost function :

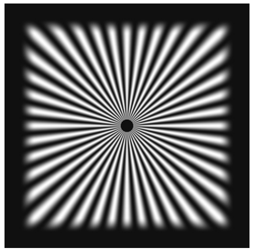
$$F(o_0,\mu) = \sum_l \left\| \frac{1}{2\sigma^2} \left[i_{l|OS}^{exp} - h_0 * \left(m_l'.(o_0 * s_l)\right) \right] \right\|_2^2 + \mu.R(o_0)$$

where $R(o_0) = \sum_f \frac{|\tilde{o}_0(f) - \tilde{o}_{mean}(f)|^2}{PSD_o(f)}$,

PSD_o : Power Spectral Density of the object o estimated from i_{CV}^{exp}

- Numerical minimization

Finally : Reconstruction of the object in-focus layer o_0



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4. Simulation : SIM reconstruction of a simulated 2-layer object

Green circle : optical resolution limit, Yellow circle : SIM resolution limit

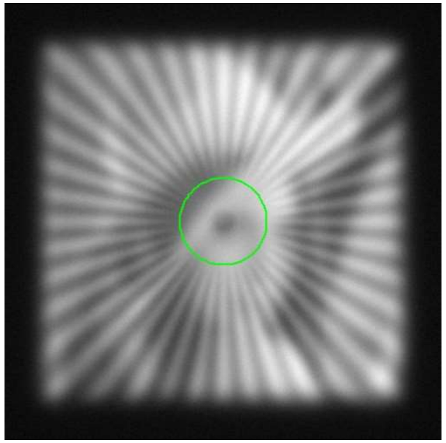
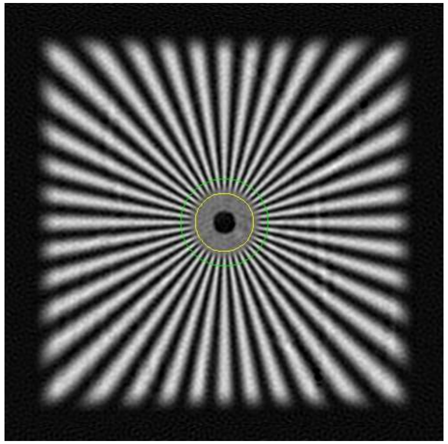
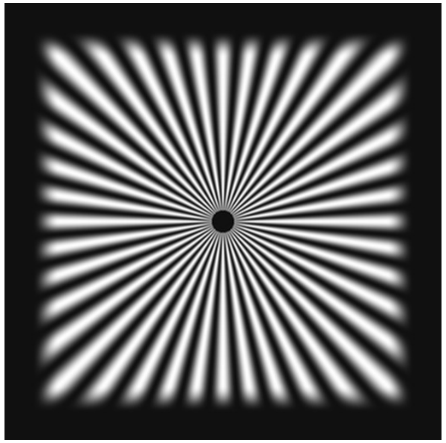


Image i_{CV}^{exp}



Reconstructed layer $\hat{o}_0$



In-focus layer o_0

- SNR : 31.6
- 7 images x 3 orientations = 21 SIM images
- $\mathrm{f}_{\mathrm{mod}} = f_c/2$

→Improvement of lateral resolution + Optical sectioning

Conclusion and perspectives

Conclusion :

- Adapted SIM method for retinal imaging keeping the simplicity of a 2D image model

Specificities :

- Illumination pattern parameters must be precisely known
  → Joint estimation of the illumination patterns ?

Perspectives :

- Elaborate a more accurate retinal image formation model from the experimental data
- Study the influence of the instrumental parameters on the reconstruction quality
- SIM reconstructions on experimental data

References

Thank you for your attention

[1] Gustaffson M.G.L. « Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy ». *Journal of Microscopy*, 198:82-87, May 2000.

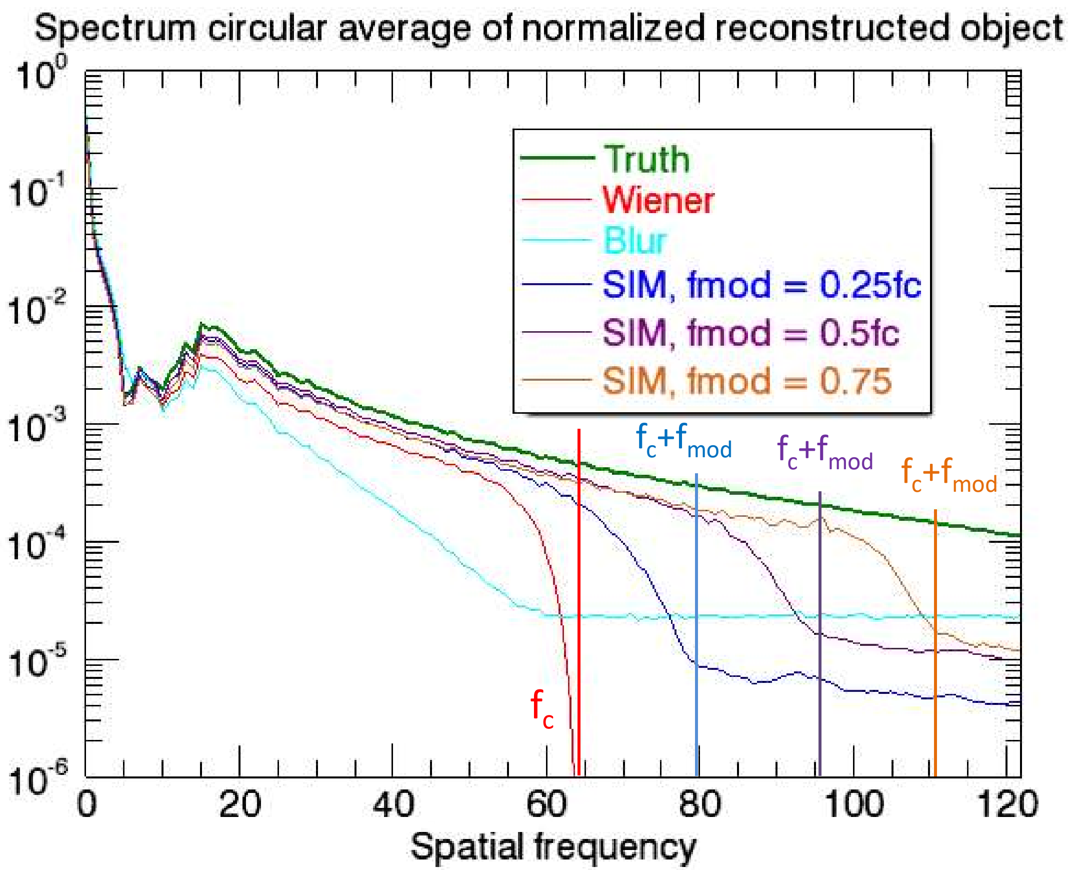
[2] Grupetta S. « Structured illumination for in-vivo retinal imaging ». *Frontiers in Optics* 2013.

[3] R. Baena-Gallé, L. Mugnier, F. Orieux. « Optical sectioning with Structured Illumination Microscopy for retinal imaging : inverse problem approach ». *GRETSI 2017.*

[4] E. Gofas-Salas, P. Mecê et al., "High loop-rate Adaptive-Optics Flood Illumination Ophthalmoscope with structured illumination capability", Appl. Opt. (submitted).

3. Results: SIM reconstruction of a simulated 2-layer object (3/3)

Spectrum of the reconstructed object :



SNR = 31.6, 3 orientations, 7 images/orientation

Adaptive optics for retinal imaging