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Adaptive optics flood-illumination ophthalmoscope with structured illumination capabilities

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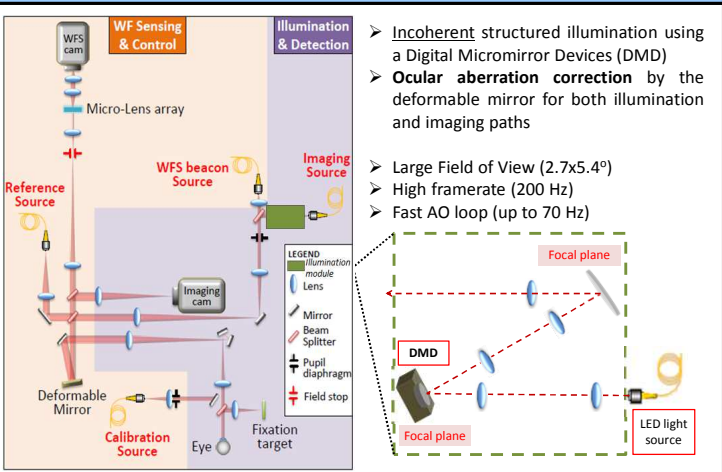
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Purpose: Improve the lateral resolution and achieve optical sectioning with an Adaptive Optics Flood Illumination Ophthalmoscope (AOFIO) by means of tailored Structured Illumination Microscopy (SIM) instrument and algorithms.

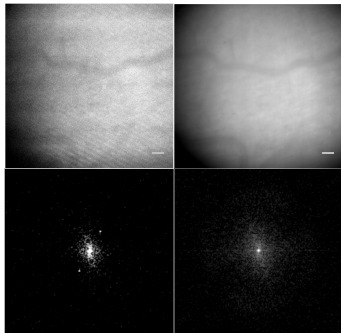
Retinal imaging systems

	⊕	⊖
AOFIO	High framerate and high lateral resolution	Poor axial resolution, low contrast images
AO Scanning Laser Ophthalmoscope (AO-SLO)	Optical sectioning (OS), high contrast images, high lateral resolution	Relatively slow framerate Distortion (scanning)
Optical Coherence Tomography (OCT)	High axial resolution	Relatively more complex (interferometry), Distortion (scanning)

AOFIO using structured illumination



Gofas-Salas et al. [1]



Top : Individual SIM image (left) and average (50 images) conventional image (right). Scale bar 50 μm.
Bottom : Spectrum of the top images. The fringe spacing is about 22 μm in the retina.

- ✓ Demonstration of incoherent fringe pattern projection onto live retina
- ✓ Current work: Improvement of illumination module to avoid illumination fluctuations (DMD).
- ✓ Future work: Reconstruction on retinal images

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[2] S. Chetty and S. Gruppeta, 'Structured illumination microscopy for in-vivo human retinal imaging: a theoretical assessment', *Opt. Express*, vol. 20, no. 23, pp. 25700-25710, Nov. 2012.

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¹ https://github.com/fairSIM/test-datasets/releases/download/OMxv4-Bielefeld/OMx_LSEC_Membrane_680nm.tif

SIM-assisted optically sectioned Bayesian reconstruction for retinal imaging

Traditional approach (static object, sinusoid phase $\phi = 0, 120$ and 240) can't be used: eye motion

Proposed approach: Project a static fringe pattern (one per orientation) and take advantage of the eye motion to obtain sinusoid phase diversity as suggested by [2]. Within the Bayesian framework [3], reconstruct the in-focus layer, the out-of-focus layers' contribution and the retinal shifts, while minimizing noise amplification. This method yields both **optical sectioning and super resolution**.

Multi-layer image formation model [4]:

$$l\text{-th acquired image } i_l = \sum_z h_z * [m_l \cdot (o_z * s_l)] + n_l = \sum_z i_{l,z} + n_l \quad \text{Contribution of each object layer}$$

o_z : 2D layer of the retina at a distance z from the object focal plane

$s_l(x, y) = \delta(x - x_l, y - y_l)$: shifts due to eye motion

$m_l(x, y) = t(x, y) \times [1 + c_l \cdot \cos(2\pi(k_{x,l}x + k_{y,l}y) + \phi)]$: illumination pattern (bell + modulation)

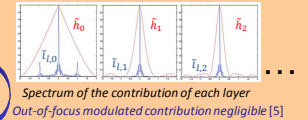
h_z : 2D PSF with a defocus corresponding to a distance z from the object focal plane

n_l : Gaussian noise

*: convolution operator; \sim : Fourier Transform

$$i_l = h_0 * [m_l \cdot (o_0 * s_l)] + \sum_{z \neq 0} h_z * [m_l \cdot (o_z * s_l)] + n_l$$

$$i_l \approx h_0 * [m_l \cdot (o_0 * s_l)] + \sum_{z \neq 0} h_z * [t \cdot (o_z * s_l)] + n_l$$



$$i_l \approx \underbrace{h_0 * [m_l \cdot (o_0 * s_l)]}_{\text{In-focus modulated layer}} + \underbrace{h_1 * [t \cdot (o_{defoc} * s_l)]}_{\text{Out-of-focus non modulated contribution}} + n_l = \mathcal{M}(o_0, o_{defoc}, s_l) + n_l$$

In-focus modulated layer Out-of-focus non modulated contribution

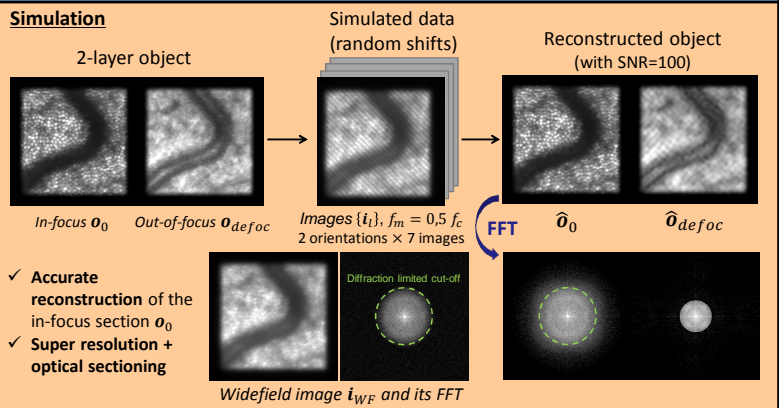
Our reconstruction method:

- Calibrates the instrumental parameters (h_0, h_1)
- Estimates the bell t , the shifts $\{s_l\}$ [6] and the illumination patterns $\{m_l\}$ from the data $\{i_l\}$
- Estimates the Power Spectral Density (PSD) of the object and of the noise for regularization [7]
- Within the Bayesian framework, estimates jointly the in-focus layer o_0 and the out-of-focus contribution o_{defoc} by minimizing numerically the following cost function:

$$F(o_0, o_{defoc}) = \sum_l \left\| \frac{1}{2\sigma^2} [i_l - \mathcal{M}(o_0, o_{defoc}, s_l)] \right\|_2^2 + R(o_0) + R(o_{defoc})$$

where $R(o) = \sum_f \frac{|\hat{o}(f)|^2}{2.PSD_o(f)}$

Reconstructions



Experimental microscope data¹ provided by [8] (3 orientations x 5 phases)

