Applications of Adaptive Optics in Fluorescence Microscopy and Ophthalmology

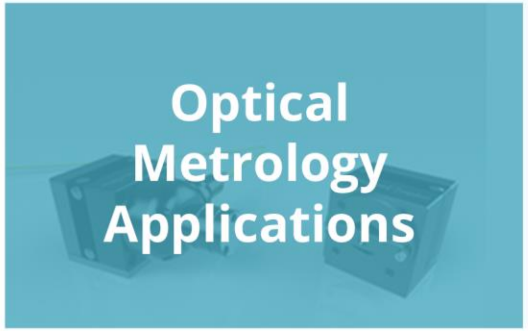
Audrius JASAITIS

Imagine Optic (Orsay, France)

Application Specialist Microscopy

ajasaitis@imagine-optic.com

Imagine Optic - What we do



Leader in Optical Metrology
HASO SH wavefront sensor
WaveView Software

Adaptive Optics for high-power lasers
ILAO Deformable Mirrors
WaveTune Software

Custom-built setups
AOKit Bio

Single Molecule Localization
MicAO 3DSR

Spinning Disk
MicAO SD

Tracking diseases at cellular and microvascular levels

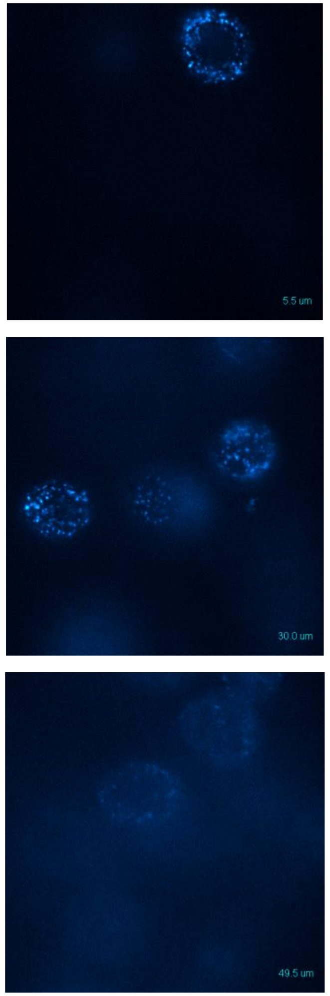
rtx1-e™ adaptive optics retinal camera

The loss of signal in fluorescence microscopy

5µm

30µm

50µm



HeLa cells in agar

- Scattering
- Absorption
- Optical aberrations

  -Loss of the fluorescence signal
  -Loss of the resolution

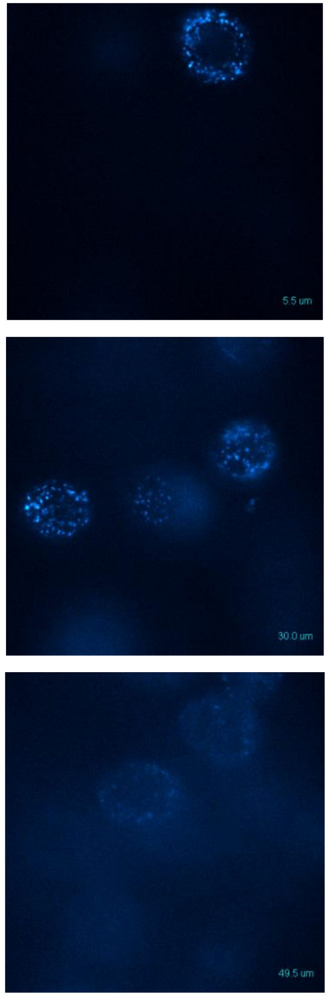
ajasaitis@imagine-optic.com

The loss of signal in fluorescence microscopy

5µm

30µm

50µm



HeLa cells in agar

- Scattering
- Absorption
- Optical aberrations

  -Loss of the fluorescence signal
  -Loss of the resolution

Adaptive optics can correct aberrations and improve the fluorescence signal

Booth (2007) *Phil. Trans. R. Soc. A*, **365**, 2829-2843.

ajasaitis@imagine-optic.com

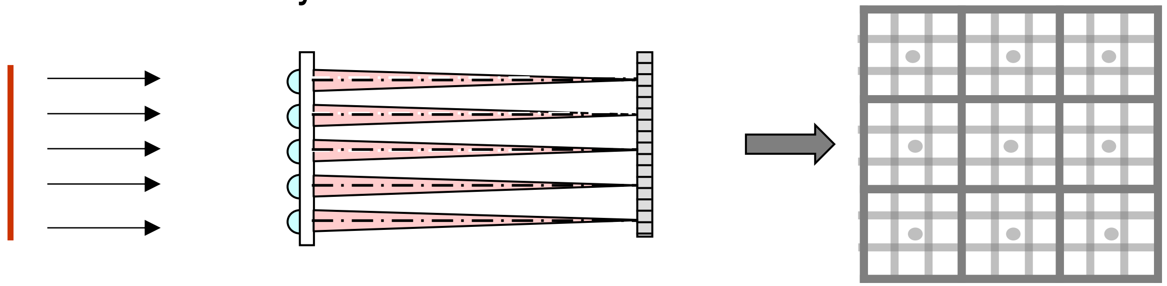
Key Components – Shack Hartman Wavefront sensor

Perfect wavefront

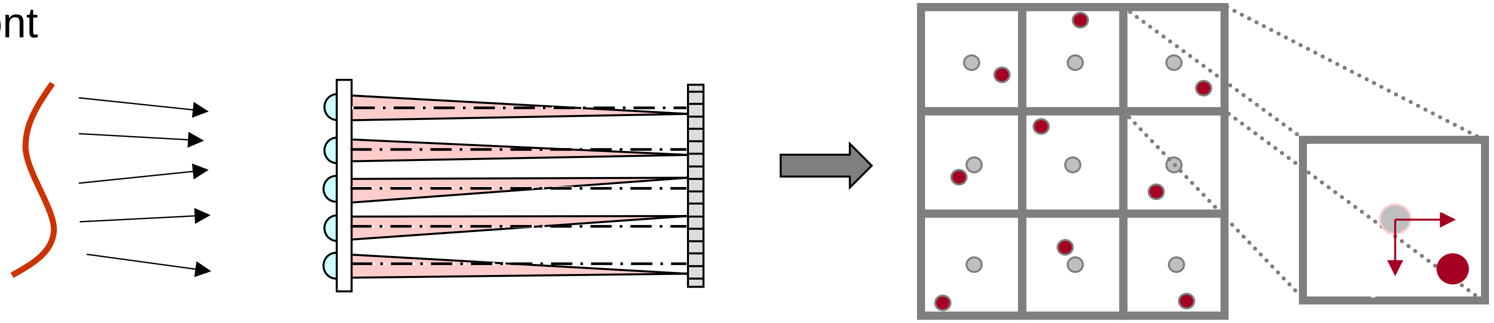
Microlensarra
y

CCD

The view on CCD



Aberrated wavefront

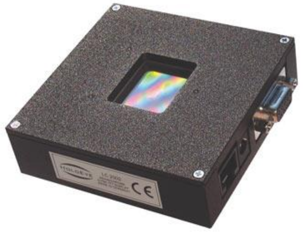


Measure the local slopes
Reconstruct the wavefront

ajasaitis@imagine-optic.com

Key Components – Phase Modulator

Spatial Light Modulator (SLM)

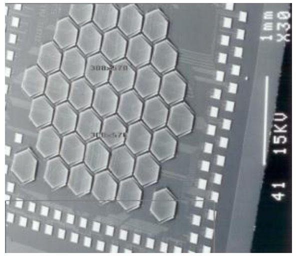


+
Number of elements
Adjustable Phase resolution

−
Speed
Polarized light
(loss of photons)
Wavelength dependence

Beam Shaping (STED, Lattice Light Sheet)

Segmented Deformable Mirror (MEMS)

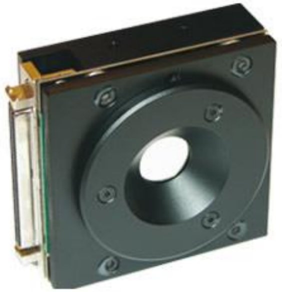


+
Speed
Wide Wavelength range
High frequencies

−
Small Stroke
Segmentation precision

Scattering Compensation

Continuous Membrane Deformable Mirror



+
Wide Wavelength range
High accuracy
High reflectivity
Large dynamic range
Precise Aberration Correction

−
Speed (up to 1kHz)

Aberration Correction

ajasaitis@imagine-optic.com

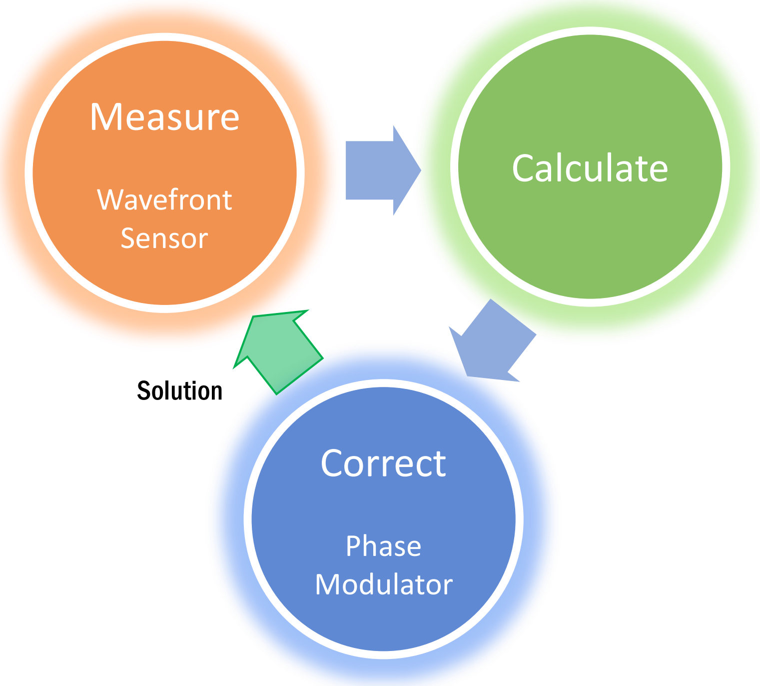
AO correction – Closed-loop optimization

Requirements

Guide star
Wavefront Sensor
Phase modulator

+ "Real" time

− Requires a guide star
Photon Expensive



Booth *et al* (2002) *PNAS*, **99**, 5788-5792.

Rueckel *et al* (2006) *PNAS*, **103**, 17137-17142.

Joel Kuby Lab:
Azucena *et al* (2010) *Opt. Express.*, **18**, 17521-17532.
Azucena *et al* (2011) *Opt. Lett.*, **36**, 825-827.
Tao *et al* (2011) *Opt. Lett.*, **36**, 1062-1064.
Tao *et al* (2011) *Opt. Lett.*, **36**, 3389-3391.
Tao *et al* (2012) *Opt. Express*, **20**, 15969-15982.
Tao *et al* (2013) *Opt. Lett.*, **38**, 5075-5078.

Aviles-Espinoza *et al* (2011) Biomed. *Opt. Express.*, **2**, 3135-3149.

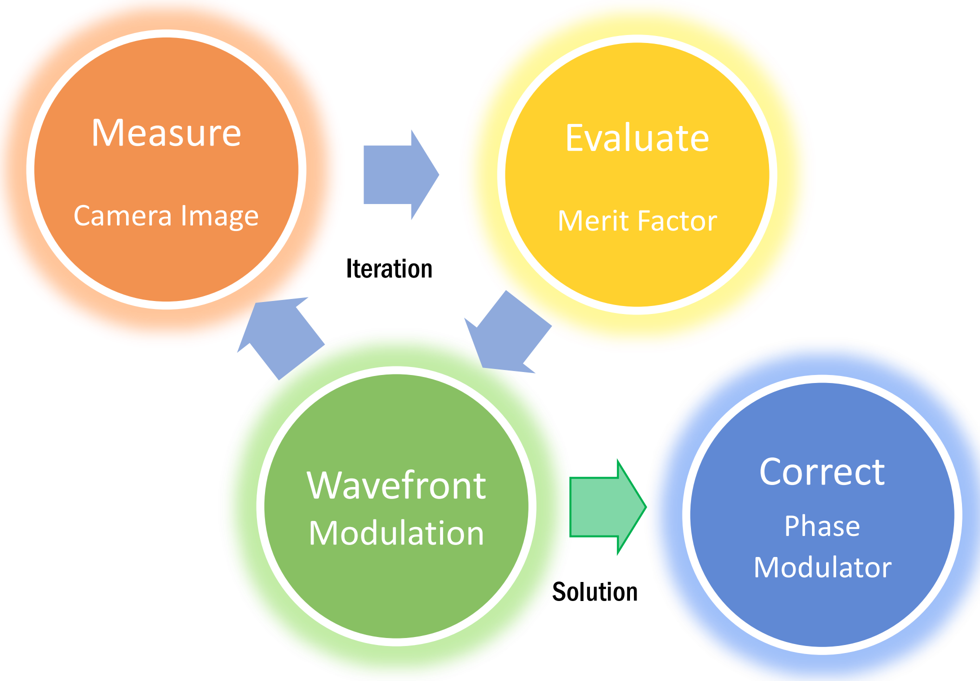
ajasaitis@imagine-optic.com

AO correction – Closed-loop optimization

Requirements
Images from the camera
Iterative Algorithm
Phase modulator

+ Works with all types of samples

− Multiple images
Bleaches the sample



Examples of Iterative algorithms
Phase Retrieval
Pupil segmentation
Genetic
3N

Ji *et al* (2012) *PNAS*, **109**, 22-27.
Kner *et al* (2010) *Proc SPIE*, **7570**, 757006.
Marsh *et al* (2003) *Opt. Express*, **11**, 1123-1130.

Booth, Wilson and Beaurepaire labs:
Débarre *et al* (2009) *Opt. Lett*, **34**, 2495-2497.
Olivier *et al* (2009) *Opt. Lett*, **34**, 3145-3147.
Facomprez *et al* (2012) *Opt. Express*, **20**, 2598-2612.

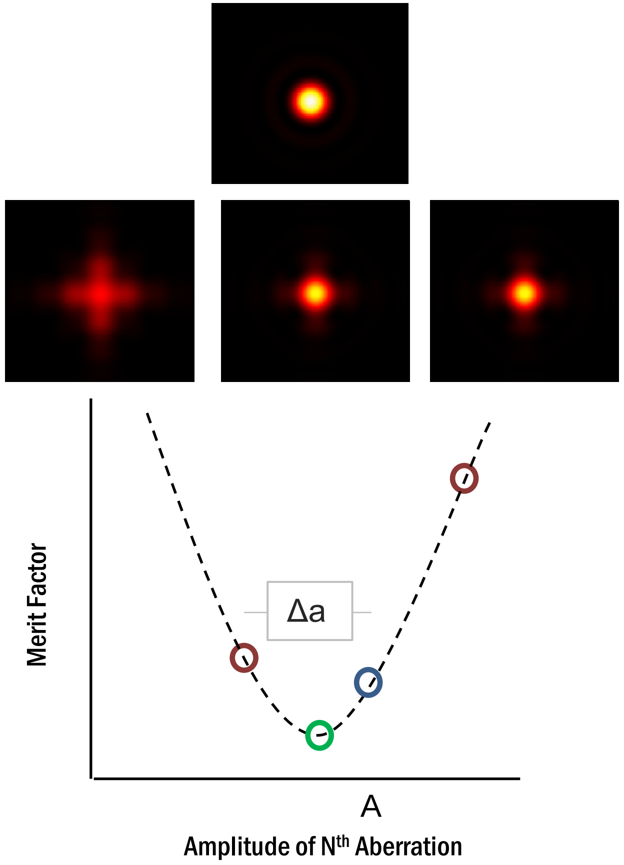
ajasaitis@imagine-optic.com

AO correction – 3N algorithm

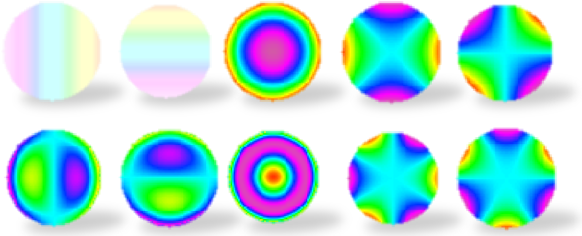
3N algorithm using a point source

Merit Factor = Max Intensity

3 images per mode
(A-Δa, A, A+Δa)



Repeat for N Modes
Typically, 1st order aberrations
(Astigmatism, Spherical, Coma)



$$\phi_{Zernike}(r,\theta) = \Sigma_i a_n^m Z_n^m(r,\theta)$$

Requires roughly **40 images** (2 time optimization)

ajasaitis@imagine-optic.com

AO correction – Aberration Model

Requirements

Model

Phase modulator

\+

Live imaging

No photo-bleaching

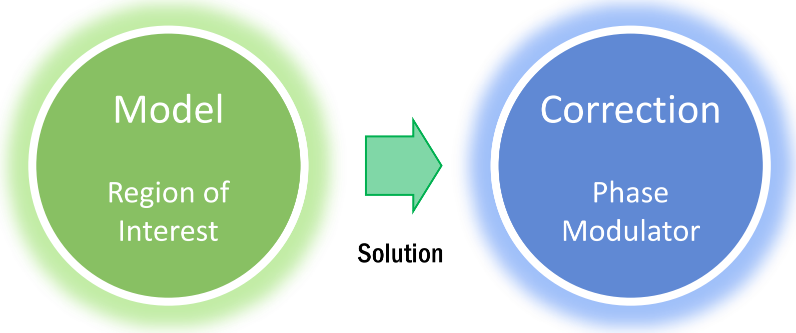
Direct Imaging

–

Works in particular conditions

Homogeneous Samples

Partial phase correction



Booth *et al* (1998) *J. Microscopy*, **192**, 90-98.

Theoretical model

Depth dependence of all Zernike modes

Lenz et al (2014) J. Biophotonics, 7, 29-36.

Booth's model

Spherical aberration correction

Fraisier et al (2015) J. Microscopy.

Experimental model

Spherical aberration correction

ajasaitis@imagine-optic.com

MicAO – the plug & play solution for inverted-frame microscope

The main features :

- Compatible with both 60x and 100x objective lenses
- Compatible with both EMCCD and sCMOS cameras
- Optical bypass option
- Optional wavefront imager
- Can be implemented on both sides of the microscope

Can be used in:

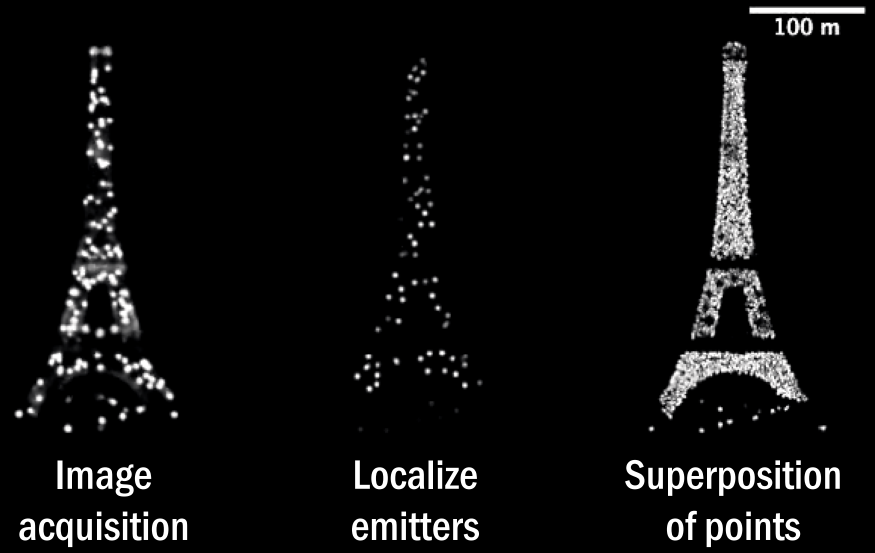
- **PALM/STORM super resolution**
- **Spinning Disk confocal microscopy**

ajasaitis@imagine-optic.com

PALM/STORM basics

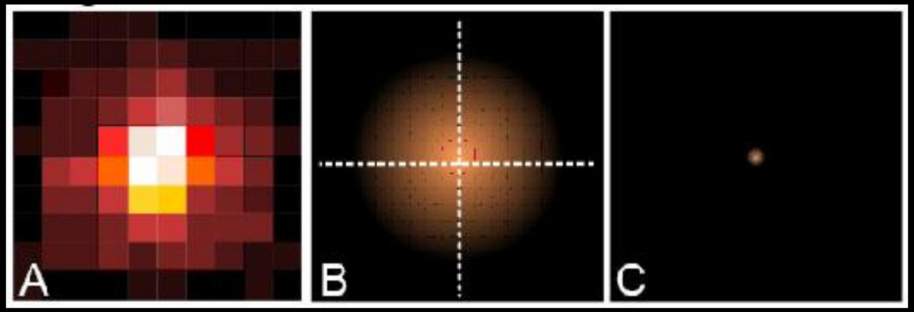
2D

- Widefield excitation in the depth of imaging
- Each fluorophore randomly emits photons
- Fit the fluorophore determine the position
- Record a stack of thousands of images to reconstruct the sample



Localization precision by numerical fitting

200nm in XY &
500nm in Z



5-20nm in XYZ

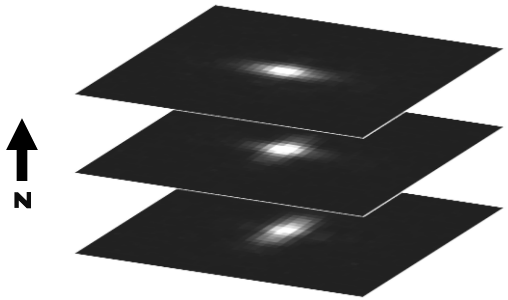
Adaptive optics in SMLM – MicAO 3DSR

MicAO adaptive optics solution

designed for SMLM systems
Placed in detection path
DM & WFS

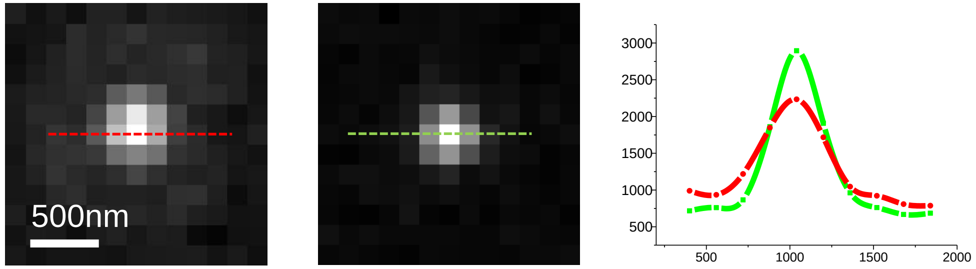
Pure Astigmatic Imaging

Do not lose photons, Deep 3D imaging

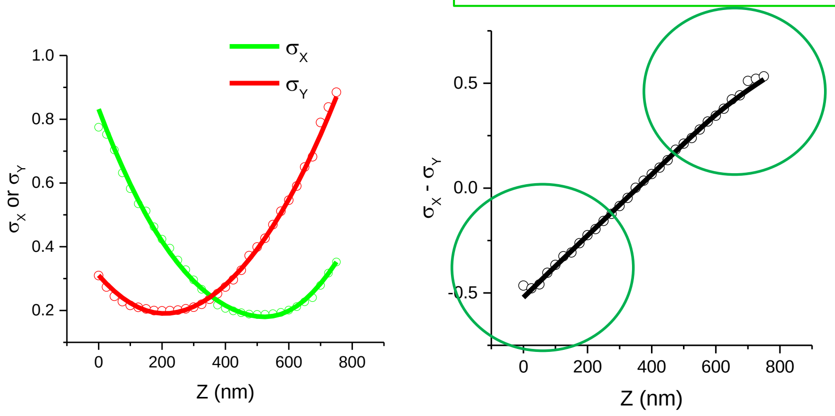


Perfecting the PSF

Correct common aberrations in microscopy



AO Calibration Curve :
high quality Z precision



The goal

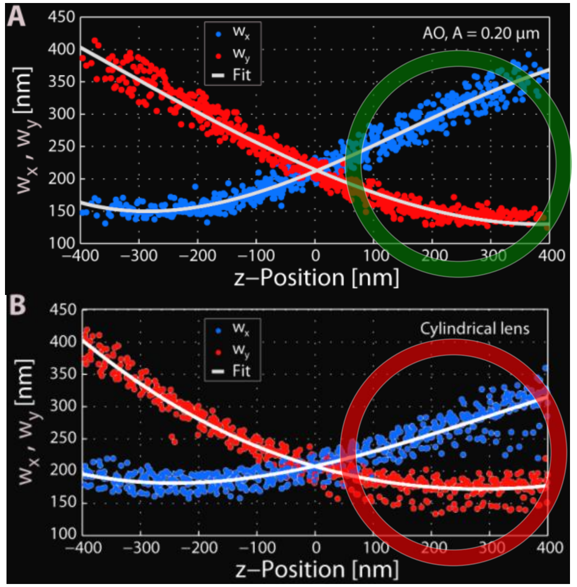
ajasaitis@imagine-optic.com

3D SMLM – Cylindrical lens *vs.* Adaptive Optics

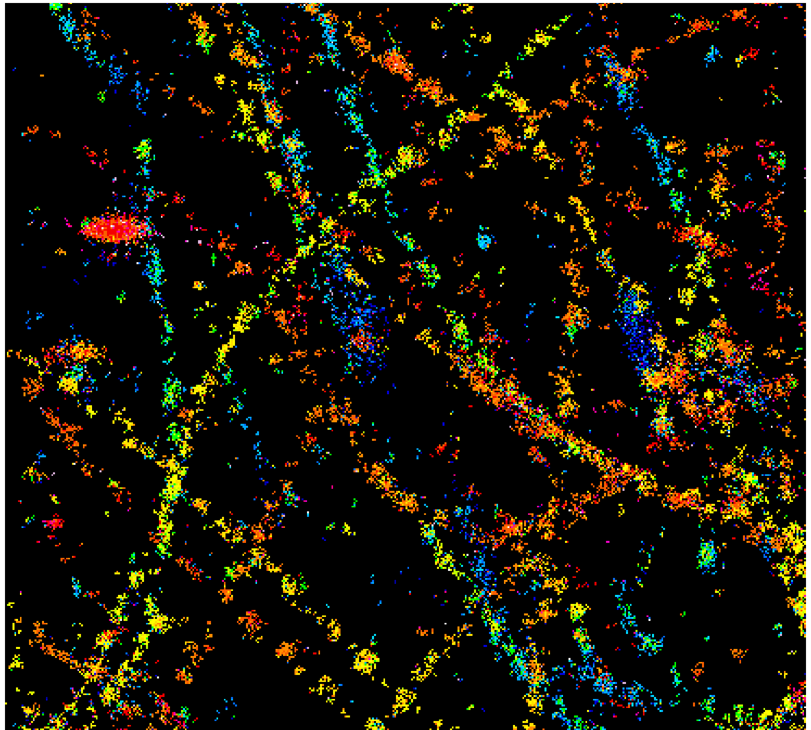
TIRF regime : small residual aberration

Adaptive optics **restores the axial symmetry of calibration curve**

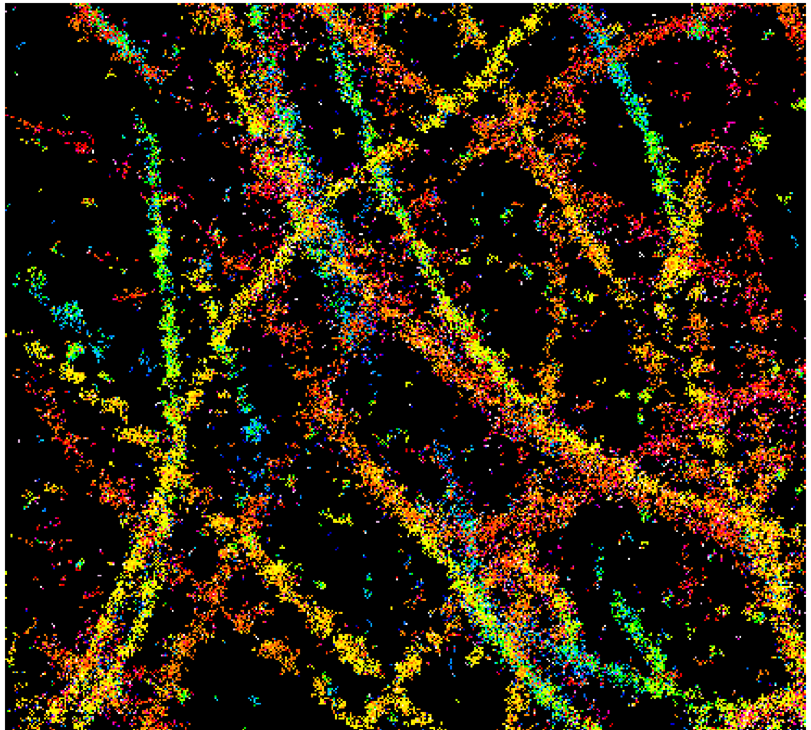
Localization algorithm **rejects aberrated** PSF

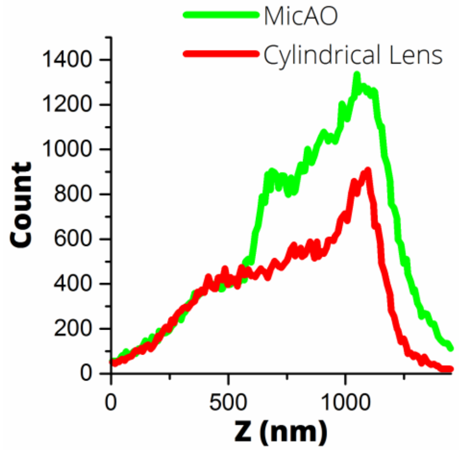


Cylindrical lens



MicAO 3DSR

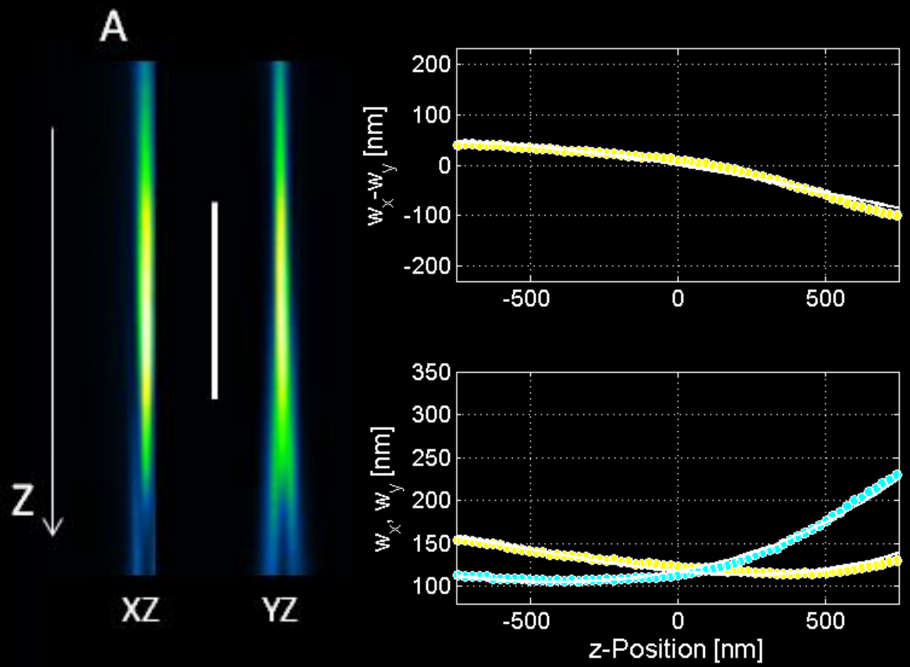




More Counts

MicAO 3DSR – 50µm deep 3D STORM imaging

200nm fluorescent beads at 20µm depth

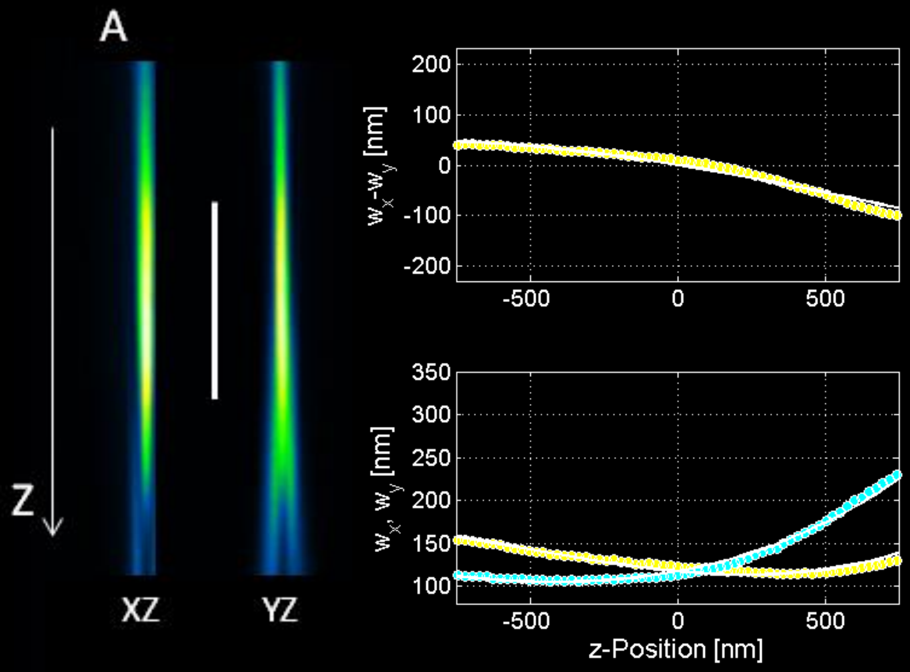


Before correction

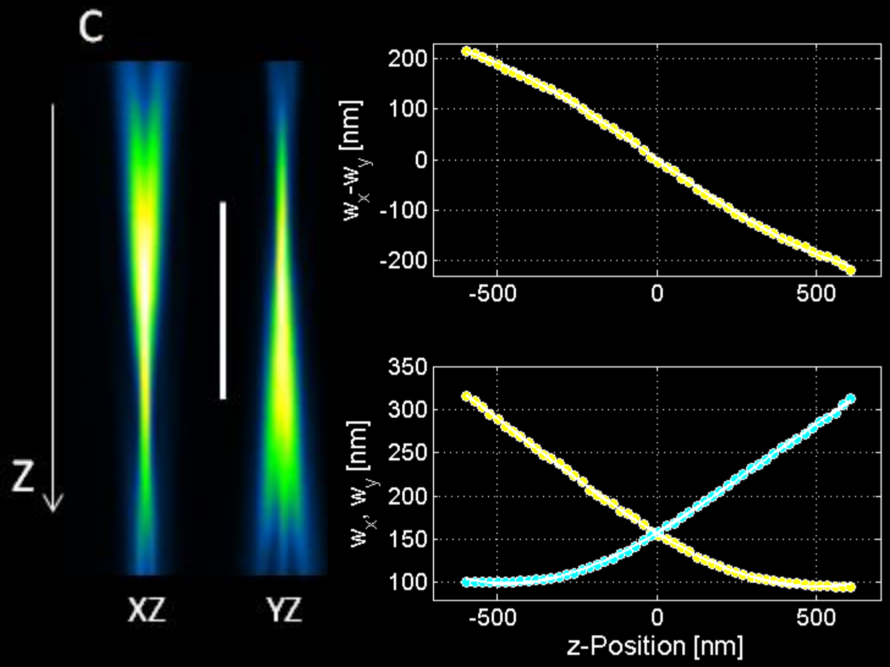
ajasaitis@imagine-optic.com

MicAO 3DSR – 50µm deep 3D STORM imaging

200nm fluorescent beads at 20µm depth



Before correction



Corrected with MicAO 3DSR

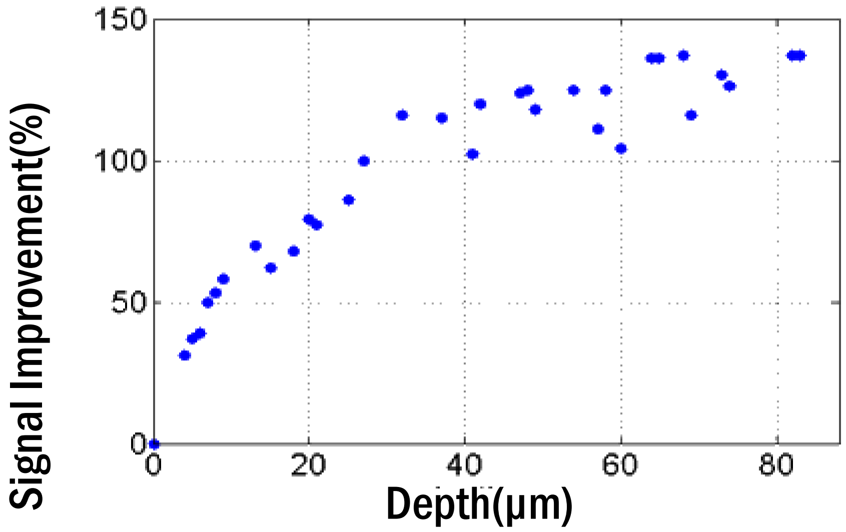
ajasaitis@imagine-optic.com

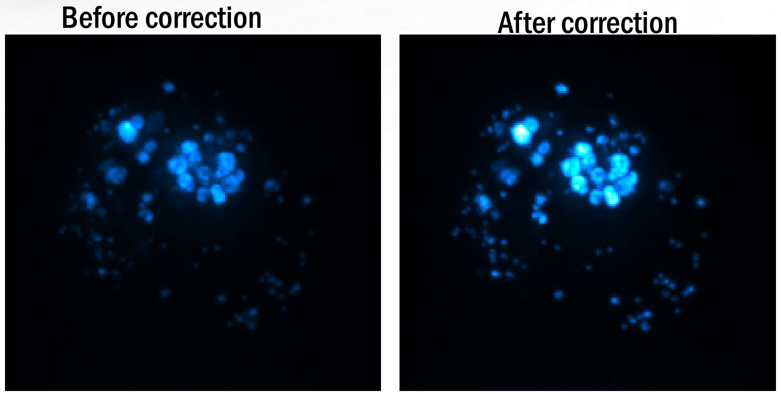
Adaptive Optics – spinning disk microscopy

Designed for Yokogawa spinning disk device (100x, NA>1.33)
Placed in **Excitation** and **Emission** path
Deformable Mirror: Mirao 52e
Mirror calibration with WFS : HASO4 First

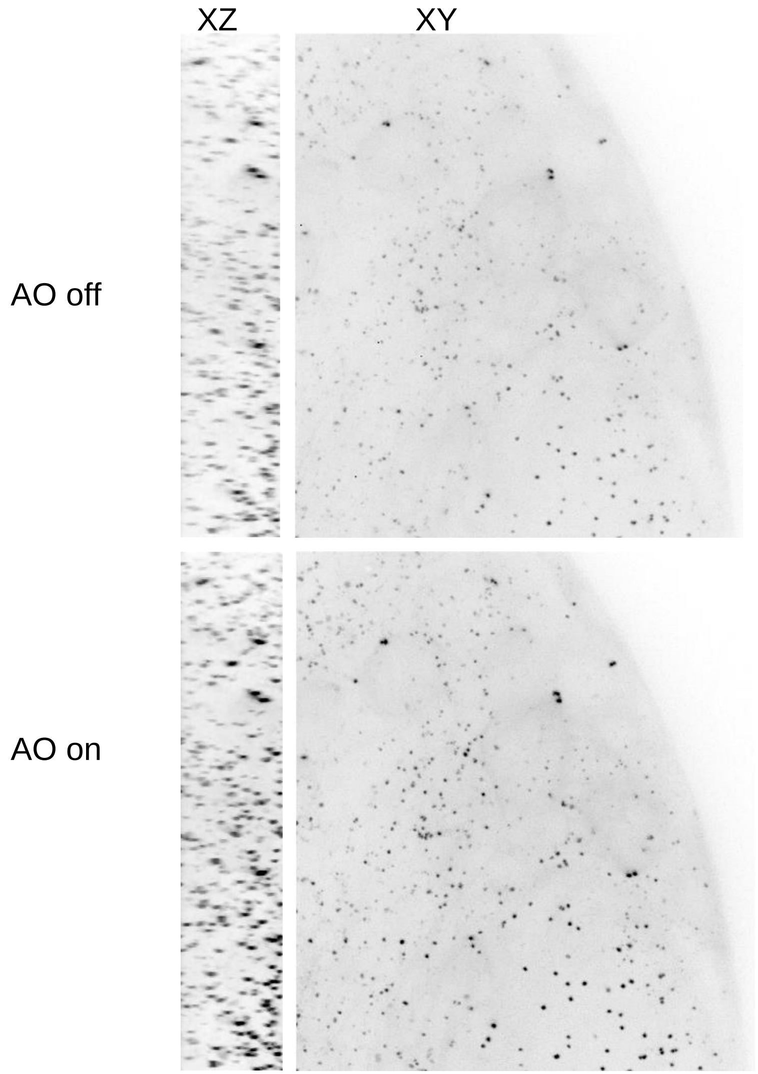
High NA (oil) objectives with Live Samples :
Experimental Spherical Aberration Model of Depth dependence
Fraisier *et al* (2015) *J. Microscopy*.

Direct Imaging using **Aberration Model**
no prior illumination
100% signal gain at 30 um depth





Adaptive Optics – spinning disk microscopy



In vivo centrosomes in Drosophila brain
Fraisier et al (2015) J Microscopy

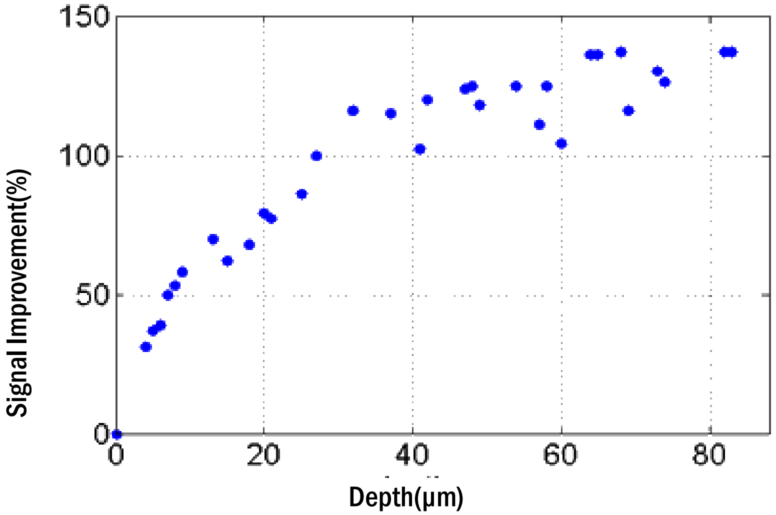
Live Sample Application

Model Correction at 7µm depth

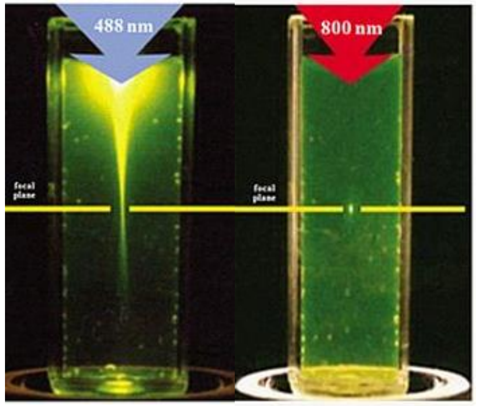
30% fluorescence signal gain

15-25% particle detection increase

Direct Use & **Easy** to Interface



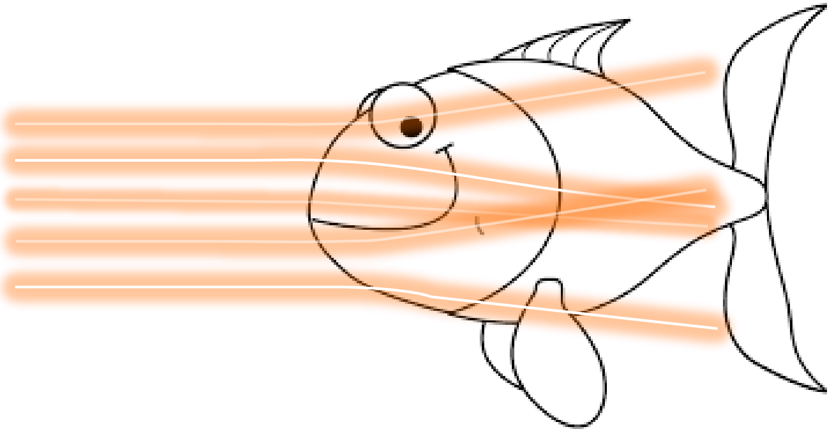
Two-photon excitation microscopy



IR Excitation
Minimized scattering
Higher optical penetration

Minimize Refractive index mismatch
Fixed Samples : Medium associated with Clearing Technique and Objective lens
Live Samples : Water/Glycerol immersion objectives

Sample induced aberrations
AO Last barrier to improve the Signal to Noise ratio



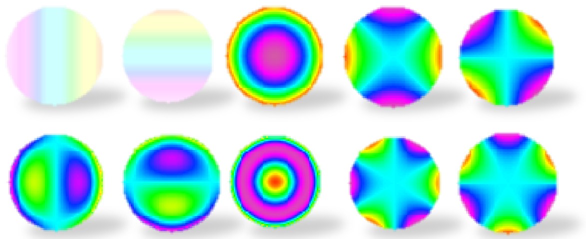
ajasaitis@imagine-optic.com

Iterative algorithms – two-photon excitation microscopy

3N Iterative Algorithm

Lower resolution phase measurement
Easier Implementation - No SH path
No need to modify the sample

3 images per Mode
Repeat for N Modes
Typically, 1^{st} order aberrations
(Astigmatism, Spherical Ab, Coma)

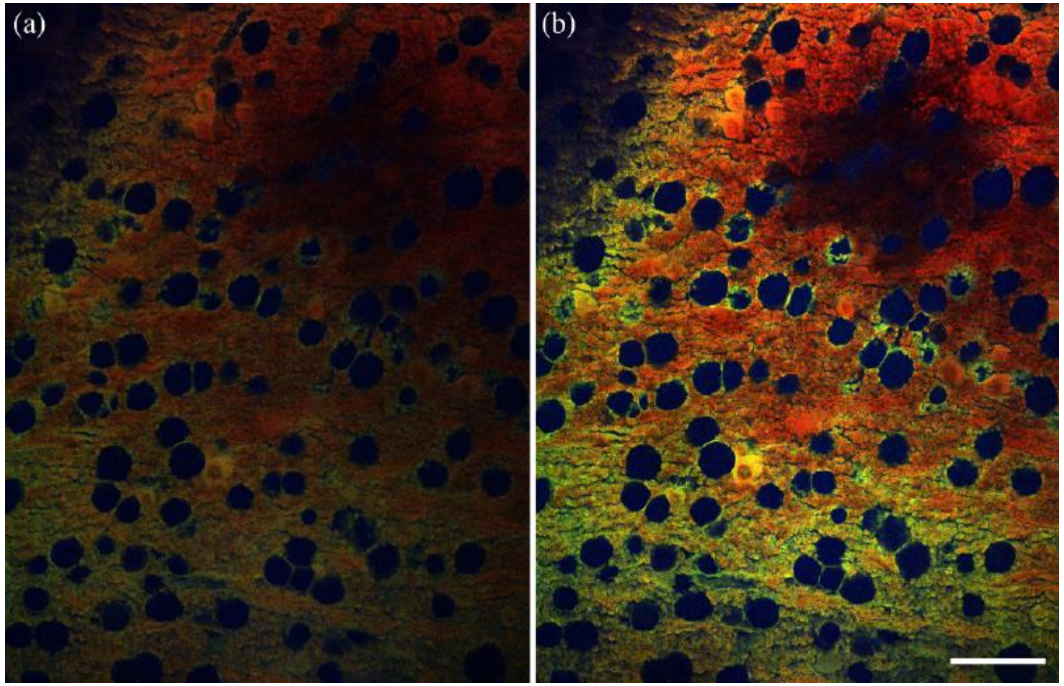


$$\phi_{Zernike}(r,\theta) = \Sigma_i a_n^m Z_n^m(r,\theta)$$

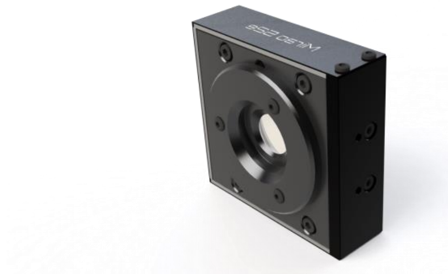
FOV correction
About 20-40 images

3N partial correction
Tradeoff tradeoff

AO off AO on



Zebra fish bone
Facomprez et al, (2012) Proc SPIE

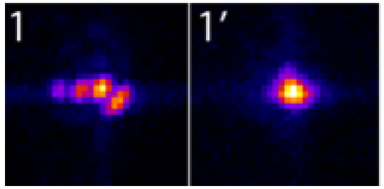


MirAO 52-e DM
15mm diameter
8um PtV Spherical Dyn
10nm surface linearity

ajasaitis@imagine-optic.com

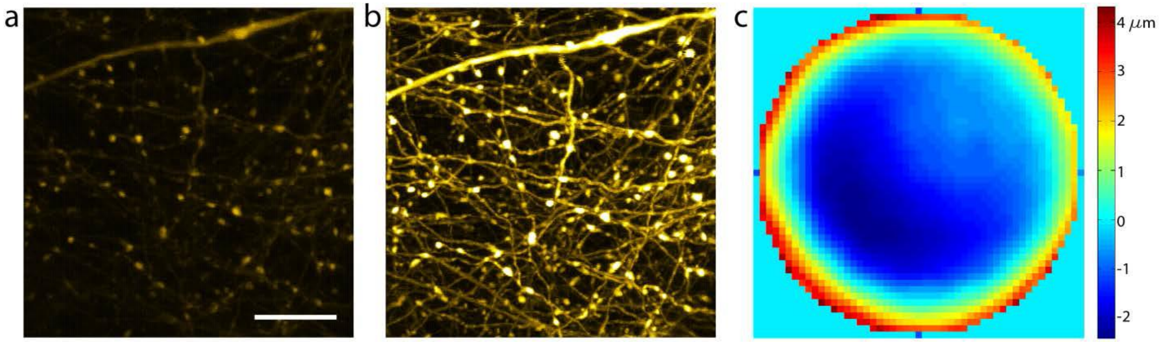
Closed-loop – two-photon excitation microscopy

NIR Guide Star
FRAP excitation laser control
ICG Injection increase GS Signal quality



To scan the Guide Star
Average the WF
Reduce Speckles on the SH

Low Photon Budget SH Sensor
NIR ICG photon Emission
EMCCD based / small number of microlens
0.5Hz Measurement frame rate



Cranial-window Spherical Aberration correction (Surface Aberration)
Wang et al (2015) Nature Communication doi10.1038

Evolve SH Sensor
50 000 photons
3um PtV Spherical Dynamic
Lambda/100 accuracy

ajasaitis@imagine-optic.com

Imaging at the cellular level

Comparison with state-of-the-art scanning laser ophthalmoscope (SLO)

SLO resolution 20 µm

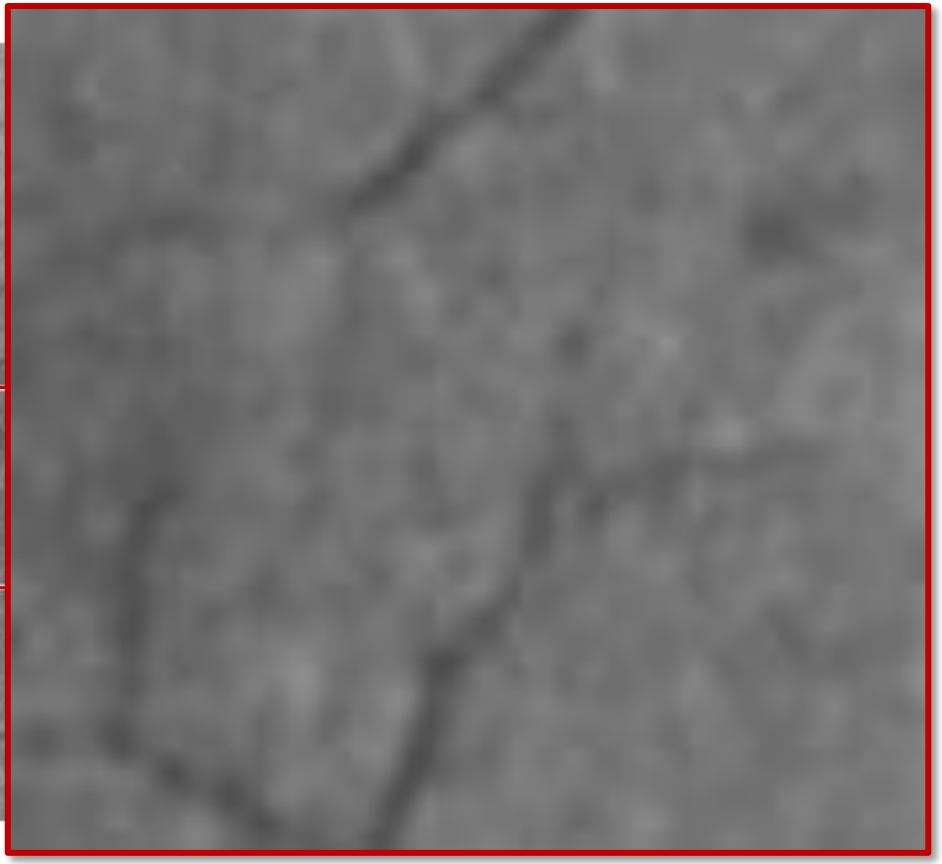
Images: courtesy of Gocho, Kameya et al., Nippon Medical School Hokusoh Hospital, Chiba, Japan

Imaging at the cellular level

Comparison with state-of-the-art scanning laser ophthalmoscope (SLO)

SLO resolution 20 µm

Magnified area : resolution 20 µm



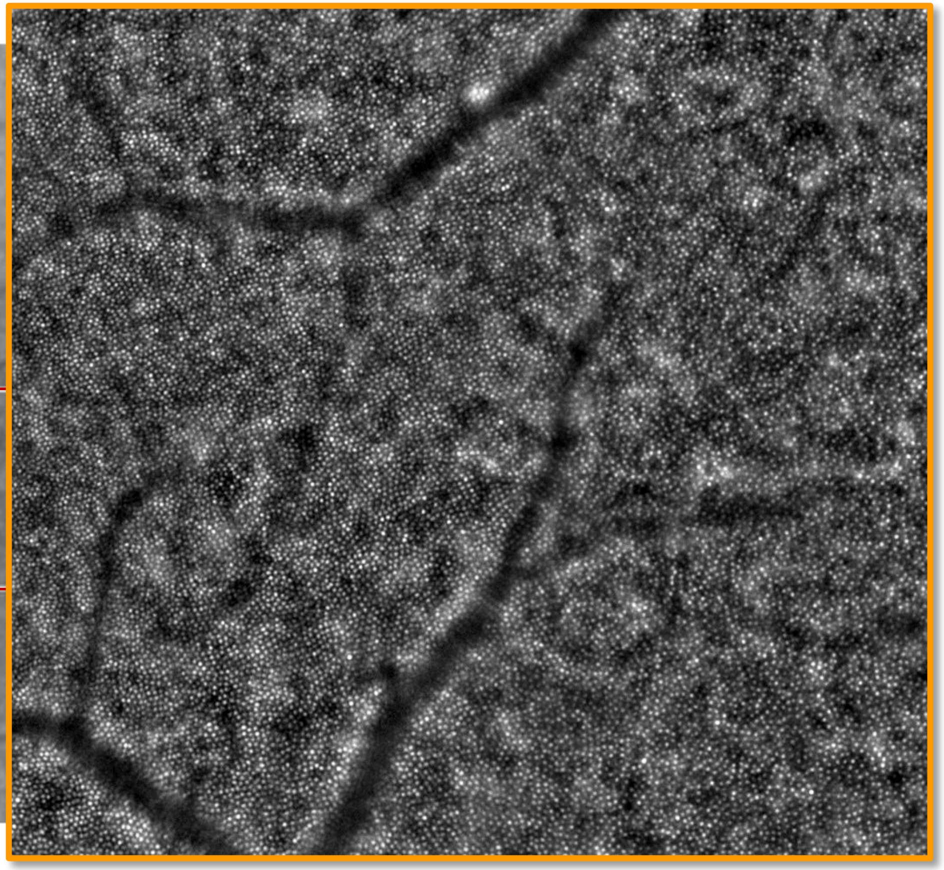
Images: courtesy of Gocho, Kameya et al., Nippon Medical School Hokusoh Hospital, Chiba, Japan

Imaging at the **cellular level**

Comparison with state-of-the-art scanning laser ophthalmoscope (SLO)

SLO resolution 20 µm

rtx1 resolution 2 µm



Images: courtesy of Gocho, Kameya et al., Nippon Medical School Hokusoh Hospital, Chiba, Japan

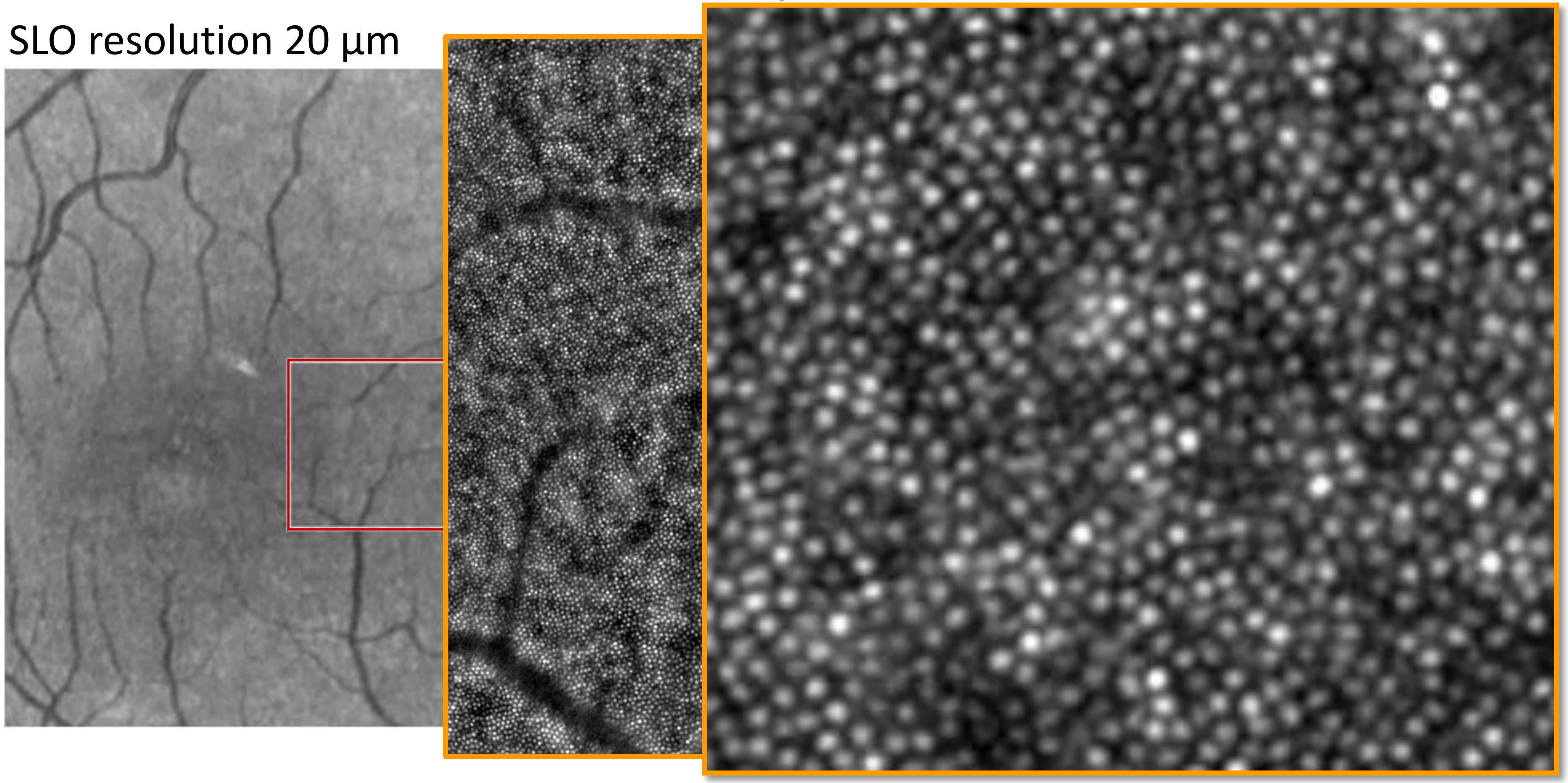
Imaging at the cellular level

Comparison with state-of-the-art scanning laser ophthalmoscope (SLO)

rtx1 resolution 2 µm

Visual cells are visible

SLO resolution 20 µm

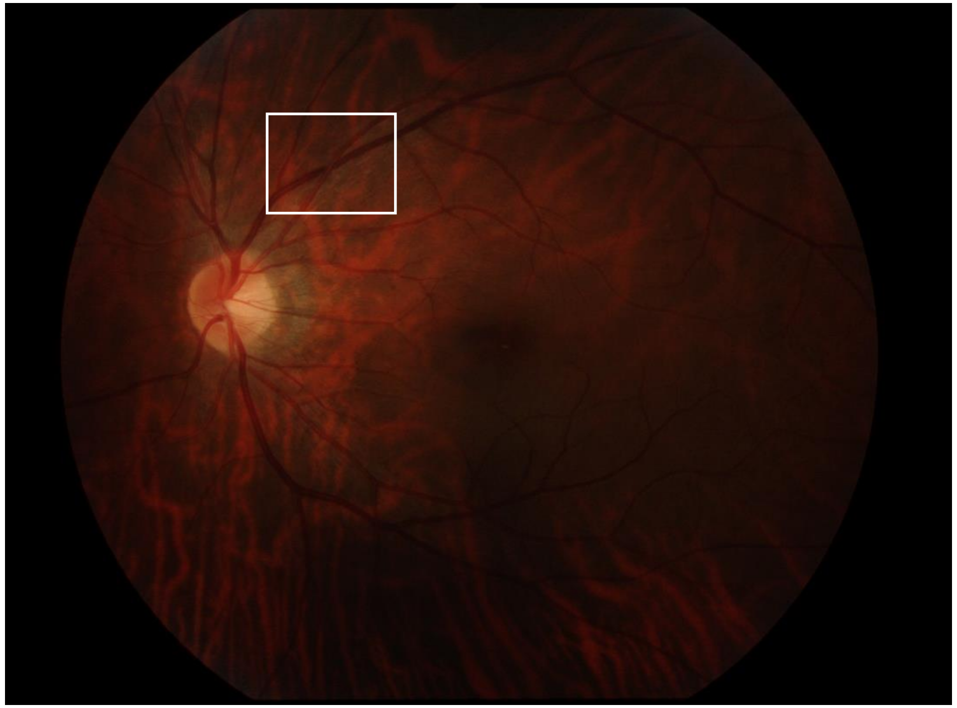


Images: courtesy of Gocho, Kameya et al., Nippon Medical School Hokusoh Hospital, Chiba, Japan

Microvascular imaging

Comparison with a conventional color fundus camera

Resolution 20 μm

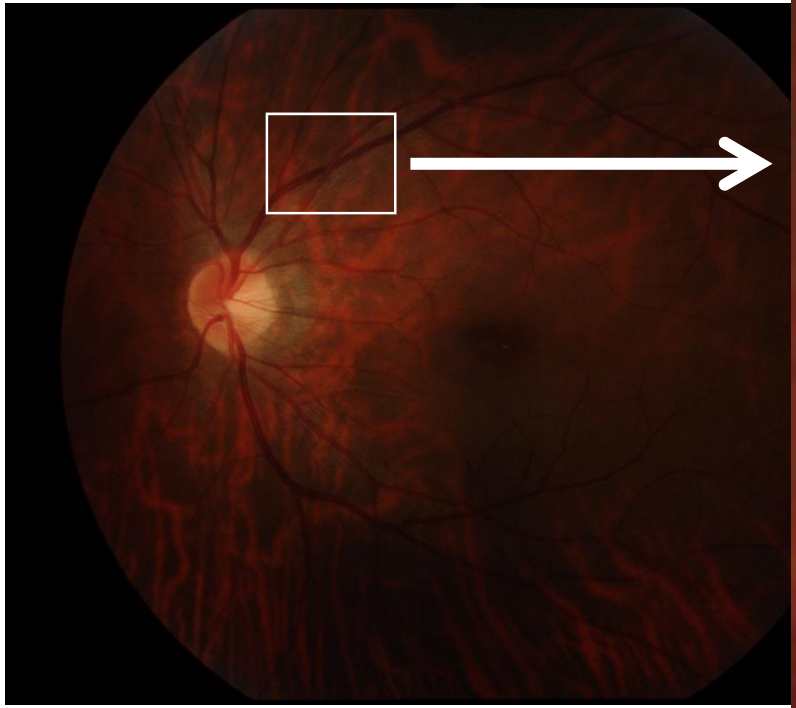


Images: courtesy of Gocho, Kameya et al., Nippon Medical School Hokusoh Hospital, Chiba, Japan

Microvascular imaging

Comparison with a conventional color fundus camera

Resolution 20 µm



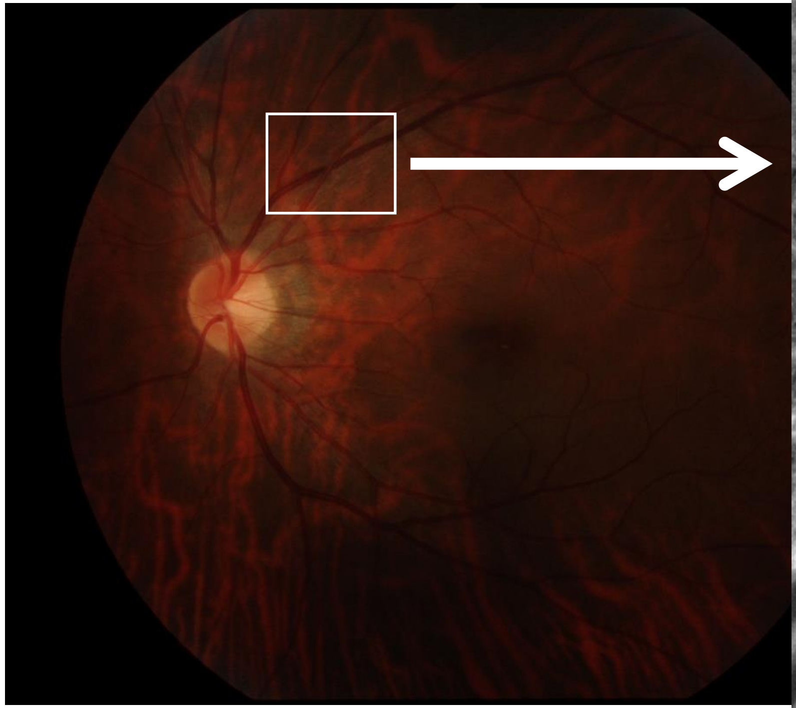
Magnified area : resolution 20 µm

Images: courtesy of Gocho, Kameya et al., Nippon Medical School Hokusoh Hospital, Chiba, Japan

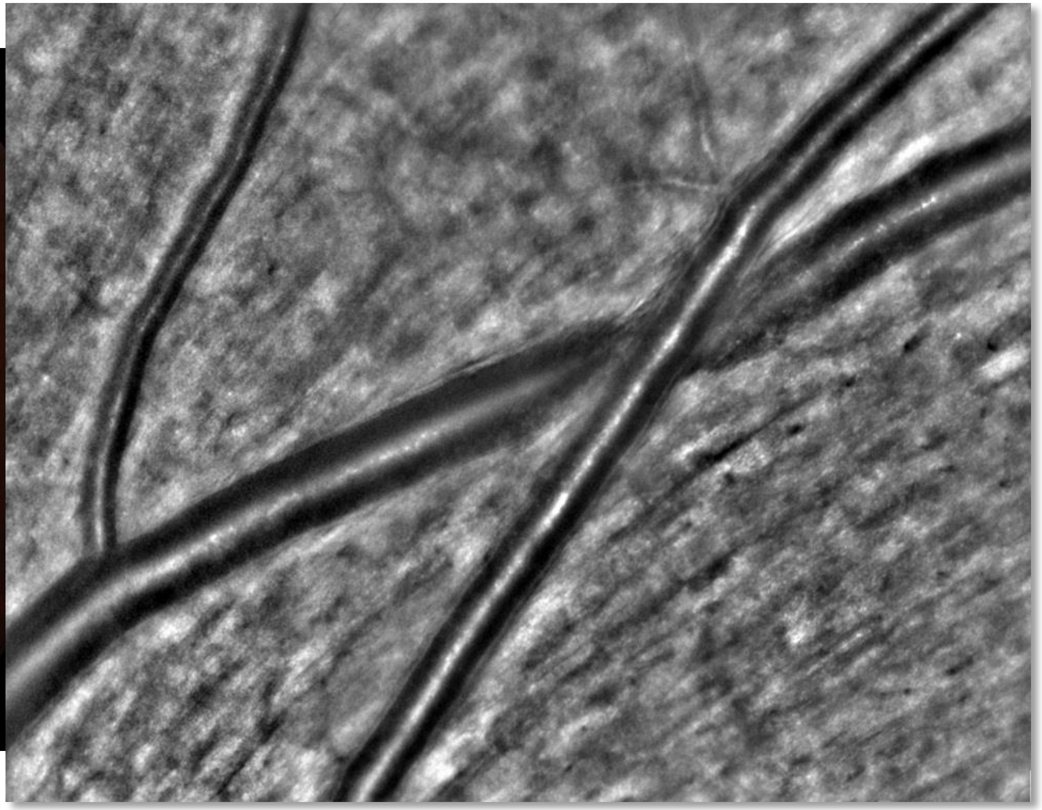
Microvascular imaging

Comparison with a conventional color fundus camera

Resolution 20 µm



rtx1 resolution 2 µm Arteriolar walls are visible

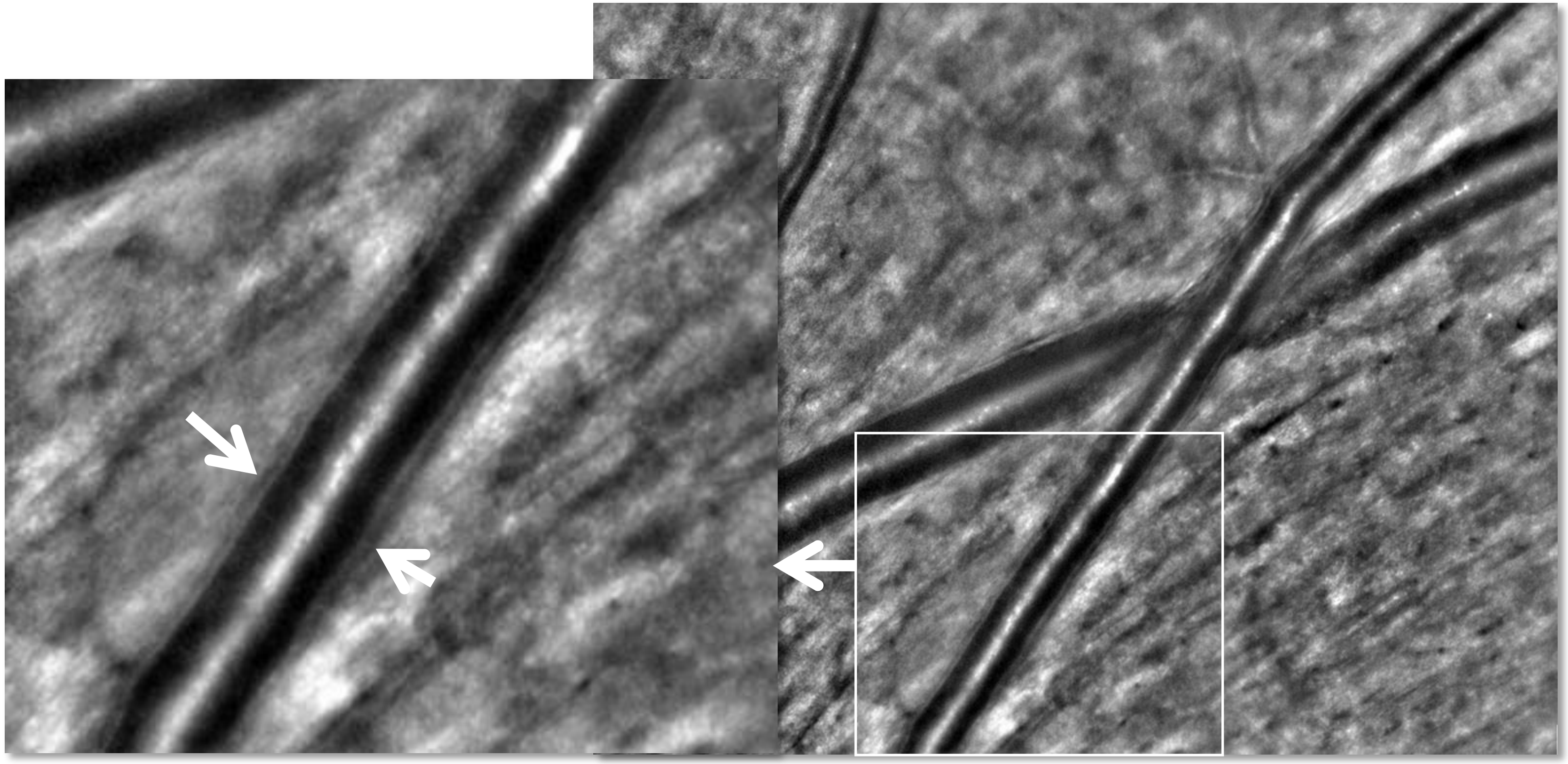


Images: courtesy of Gocho, Kameya et al., Nippon Medical School Hokusoh Hospital, Chiba, Japan

Microvascular imaging

Comparison with a conventional color fundus camera

rtx1 resolution 2 µm Arteriolar walls are visible



Images: courtesy Nippon Medical School Hokusoh Hospital, Chiba, Japan

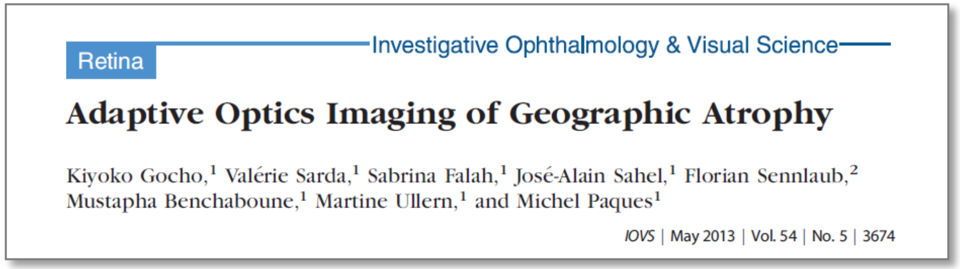
rtx1-e main technical data

Criteria	Specifications
Resolving power	250 line pairs /mm *
Image field	4 deg x 4 deg
Reachable field	29 deg x 20 deg rectangular field
Exposure time for a single image	< 10 ms
Acquisition time for an averaged image	2 s
Illumination wavelength	850 nm

* International standard for retinal cameras: 40 to 80 line pairs /mm

Geographic atrophy in dry AMD

- The rtx1 can detect atrophic progression **in very short times**
- It reveals the **migration** of numerous pigmented cells, previously unseen



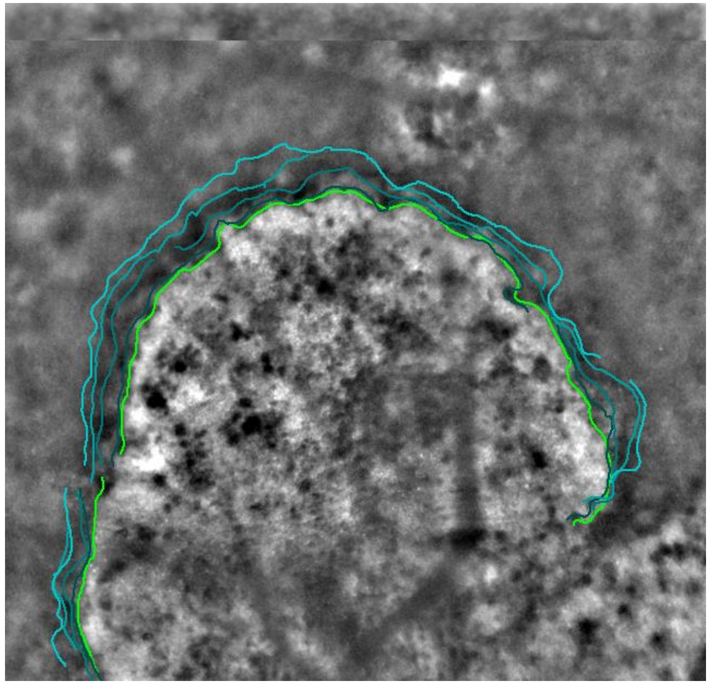
Retina

Investigative Ophthalmology & Visual Science

Adaptive Optics Imaging of Geographic Atrophy

Kiyoko Gocho,[1] Valérie Sarda,[1] Sabrina Falah,[1] José-Alain Sahel,[1] Florian Sennlaub,[2] Mustapha Benchaboune,[1] Martine Ullern,[1] and Michel Paques[1]

IOVS | May 2013 | Vol. 54 | No. 5 | 3674

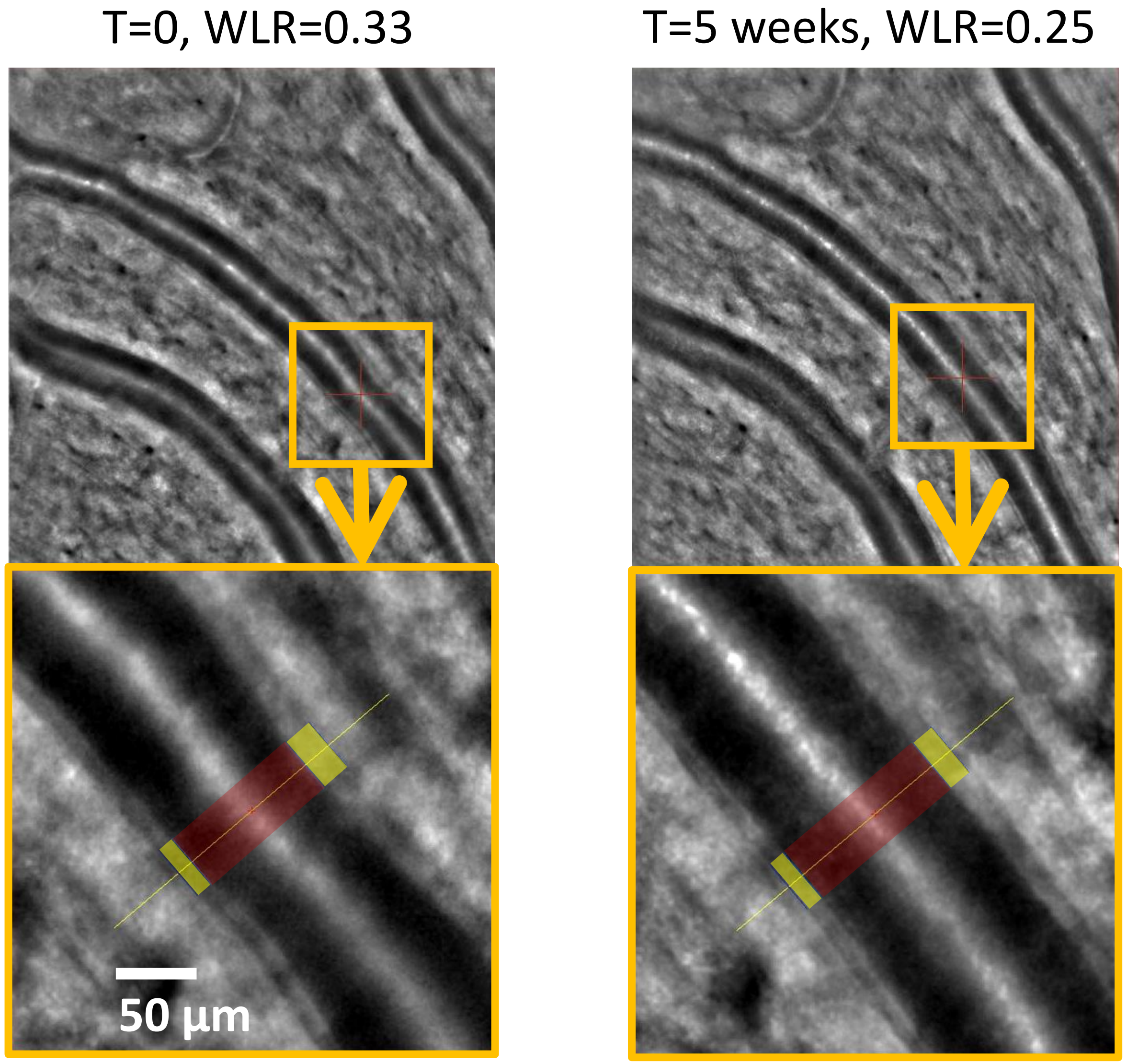


5-month follow-up of an atrophic area

Progression detected in 2 weeks

Images: courtesy of Gocho, Paques et al., Quinze-Vingts National Eye Hospital, Paris, France

Recovery of arteriolar wall structure after anti-hypertensive treatment



Images: courtesy Cardiovascular Prevention Center, Lariboisière Hospital, Paris, France

Thank you and greeting from Imagine Team