WE ARE ON

OSA Applications of Visual Science Technical Group

Unveiling the Living Eye with Multiphoton Techniques

Christina Schwarz, University of Tübingen
Juan M. Bueno, Universidad de Murcia

Applications of Visual Science Technical Group

Technical Group executive committee (until December 2020!):

E. Josua Fernandez, University of Murcia, Spain (Chair), enriquej@um.es

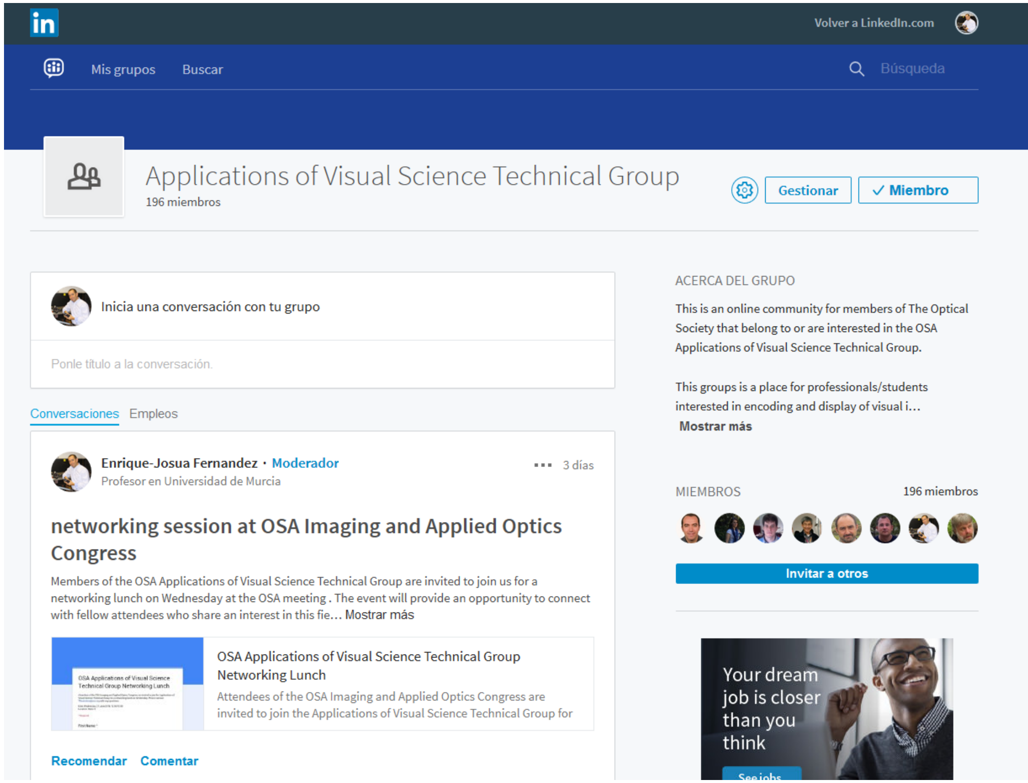
Karen Hampson, University of Oxford, UK (Vice Chair)

Juan Tabernero, Anglia Ruskin University, UK/ University of Murcia, Spain (Vice Chair)

Applications of Visual Science Technical Group

Contact your Technical Group and Get Involved!

- Linked-In site (global reach)
- Announce new activities
- Promote interactions
- Complement the OSA Technical Group Member List



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OSA Applications of Visual Science Technical Group

MEET THE NEW COMMITTEE (starting Janurary 2021):

Chair

Karen Hampson

University of Oxford

Vice-Chair

Laura Young

University of Newcastle

Officer for Media and Webinars

Maria Viñas

Harvard Medical School

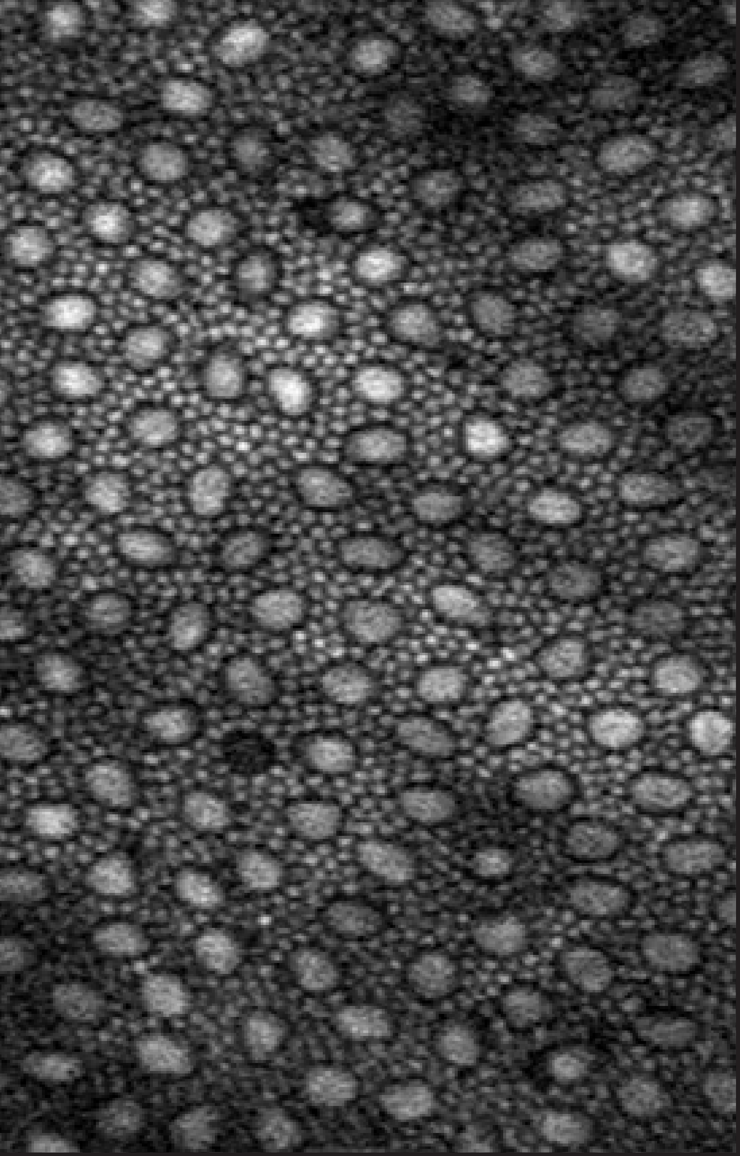
karen.hampson@eng.ox.ac.uk

Applications of Visual Science Technical Group

Unveiling the Living Eye with Multiphoton Techniques

Second Harmonic Generation Microscopy of the Cornea,
Prof. Juan M. Bueno

Multiphoton Imaging of the Living Retina,
Dr. Christina Schwarz



Multiphoton Imaging of the Living Retina

Christina Schwarz

Institute for Ophthalmic Research
University of Tübingen, Germany

Center for Visual Science
University of Rochester, NY, USA

OSA Webinar: Unveiling the living eye with multiphoton techniques

November 4, 2020

EBERHARD KARLS UNIVERSITÄT TÜBINGEN

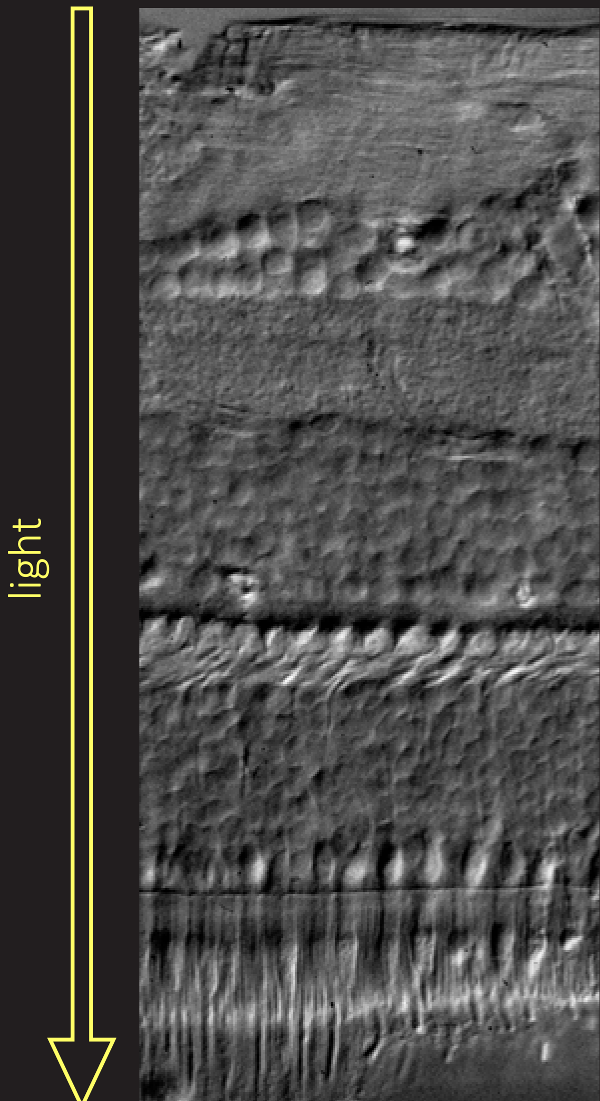
Universitätsklinikum Tübingen

aria
ADVANCED RETINAL IMAGING ALLIANCE

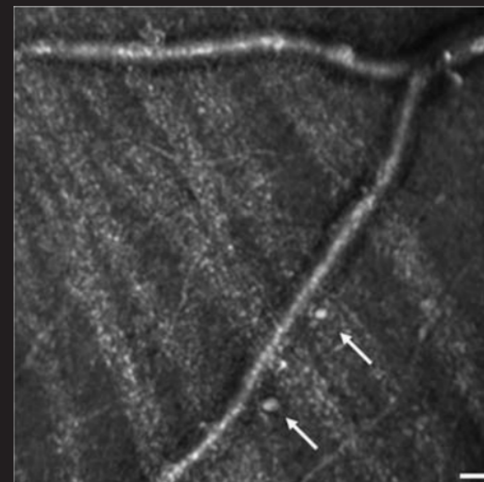
1850 MELIORA

The retina – a multi-layered, translucent tissue

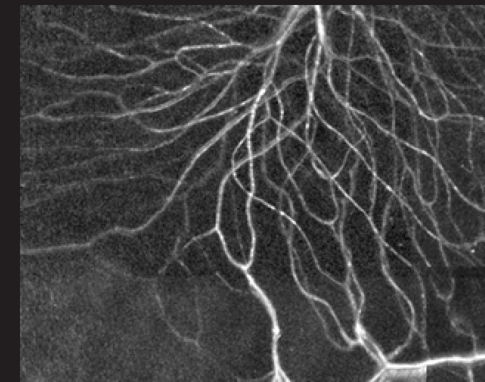
Imaging retinal structure: confocal reflectance mode



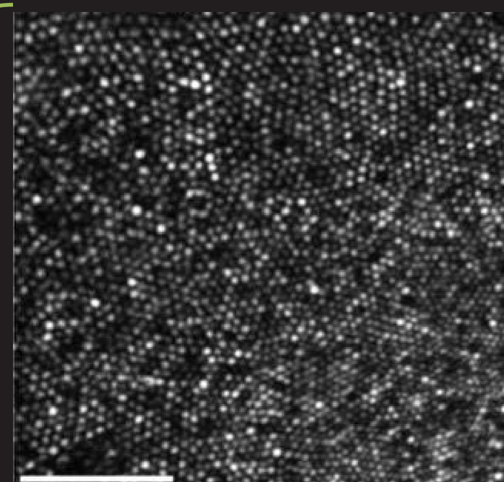
primate retina (*ex vivo*)
Courtesy Steve Massey



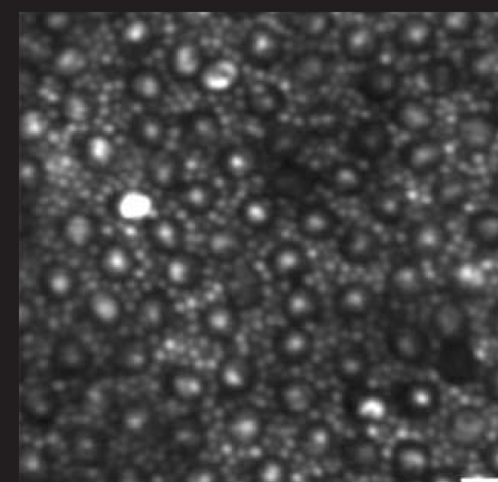
Nerve fibers
Rossi et al, Eye 2011



Retinal vasculature
Courtesy Jesse Schallek

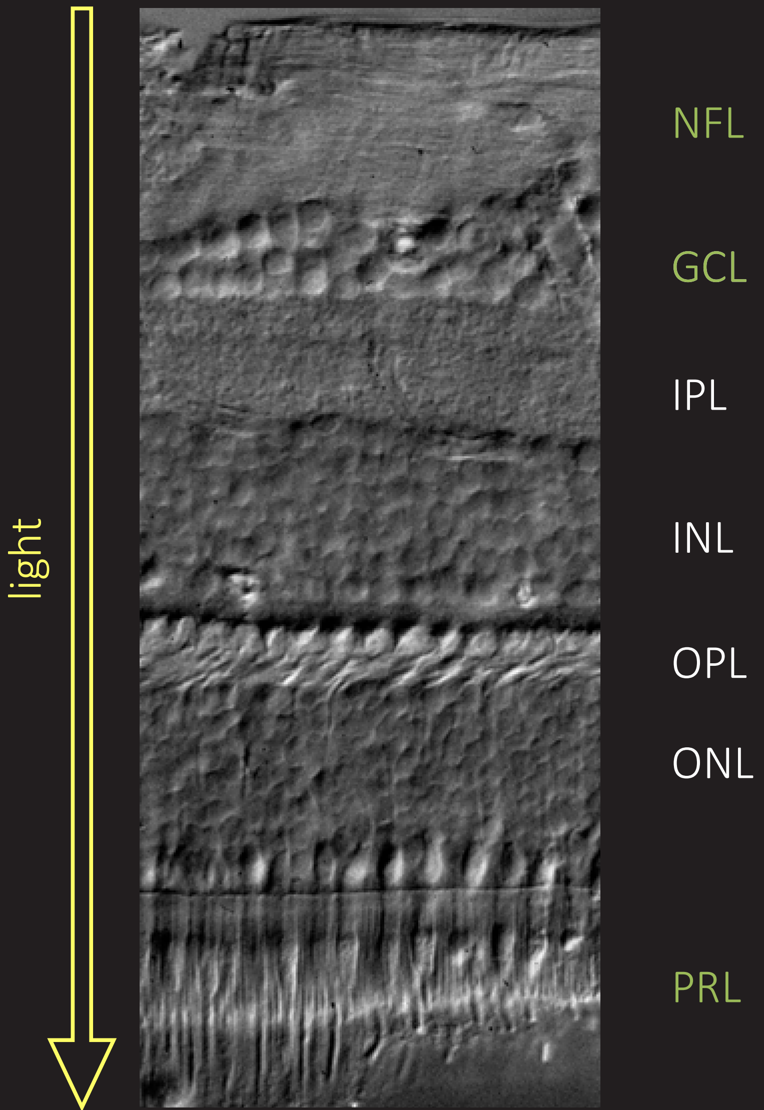


Foveal cones
Dubra and Sulai, BOEx 2011

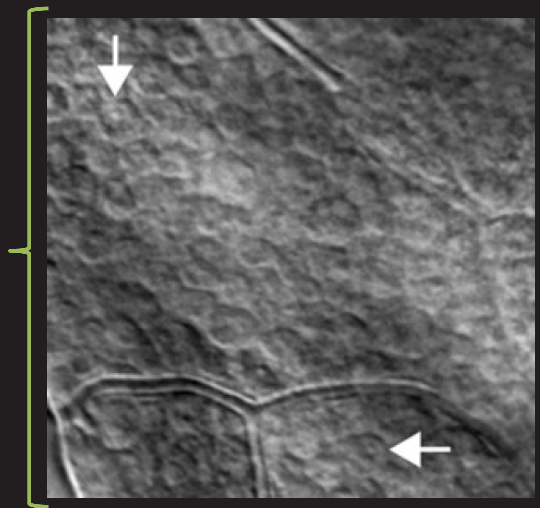


Peripheral rods and cones
Dubra et al, BOEx 2011

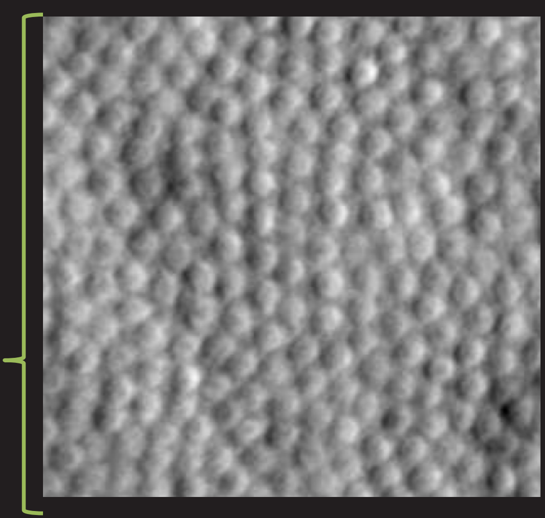
Imaging retinal structure with offset-aperture detection



primate retina (*ex vivo*)
Courtesy Steve Massey



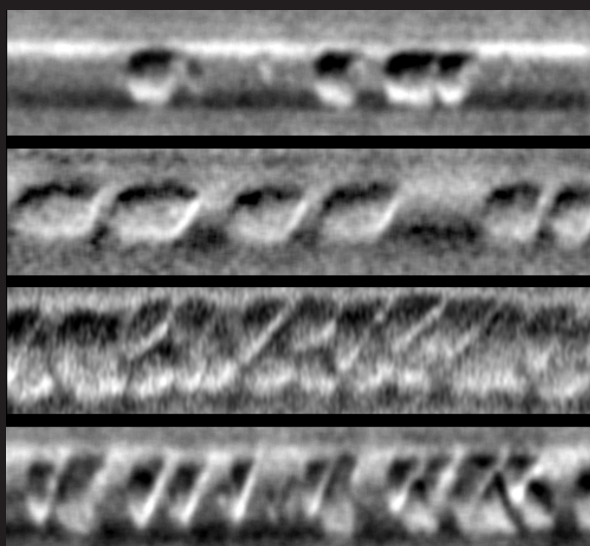
Unlabeled ganglion cells
Rossi et al, PNAS 2017



Cone inner segments
Scoles et al, IOVS 2014

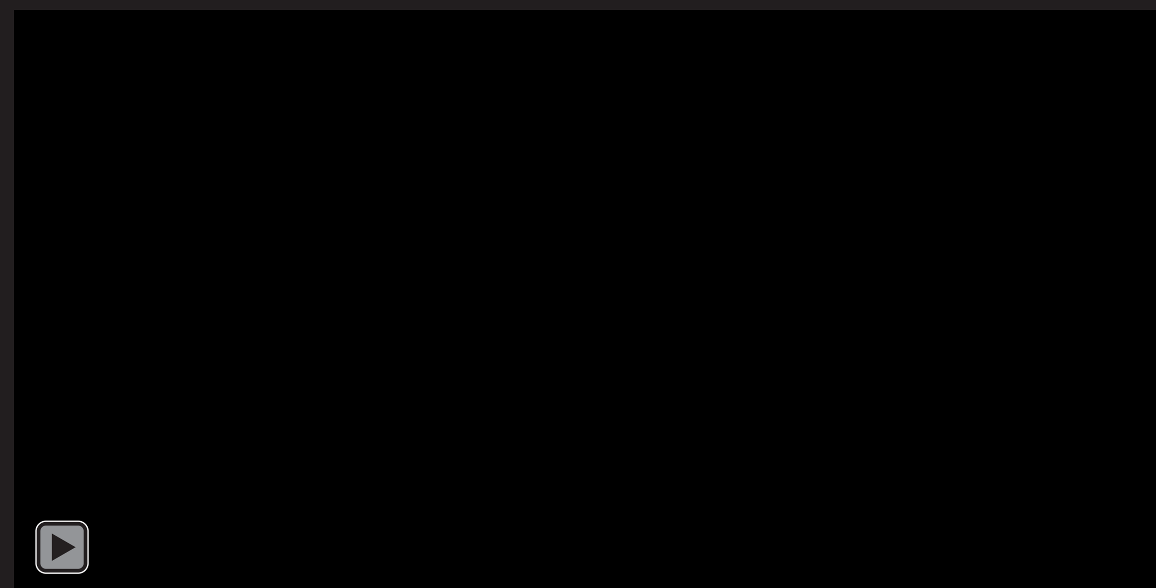
Imaging dynamic features with time-lapse offset-aperture imaging

Blood cells within capillaries



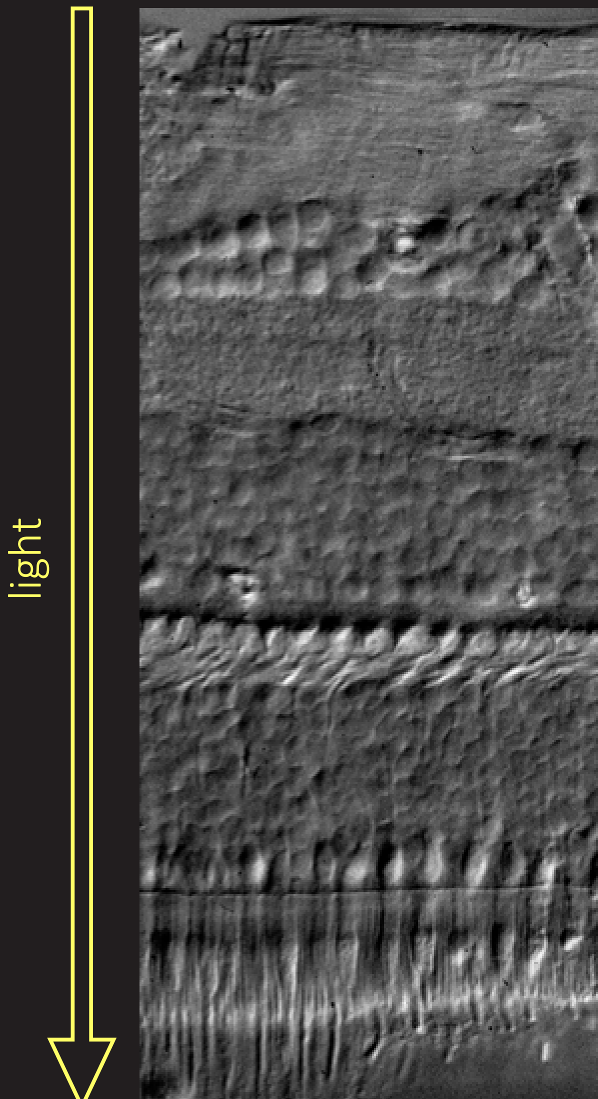
Guevara-Torres et al, BOEx 2016

Immune response after ocular injection of lipopolysaccharide



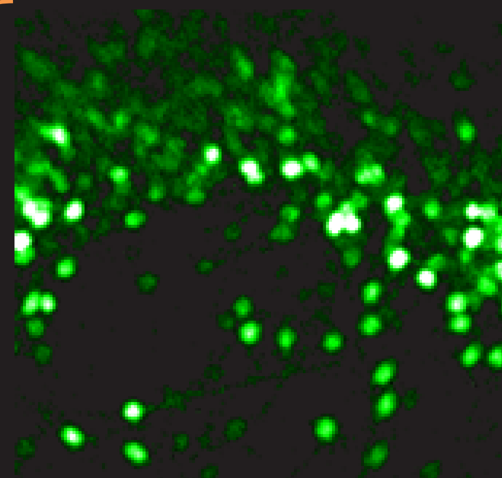
Joseph, Chu et al, eLife 2020

Possibilities for functional imaging of the retina



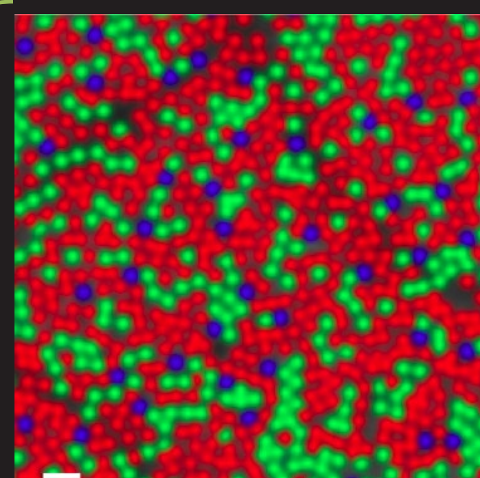
primate retina (*ex vivo*)
Courtesy Steve Massey

NFL
GCL
IPL
INL

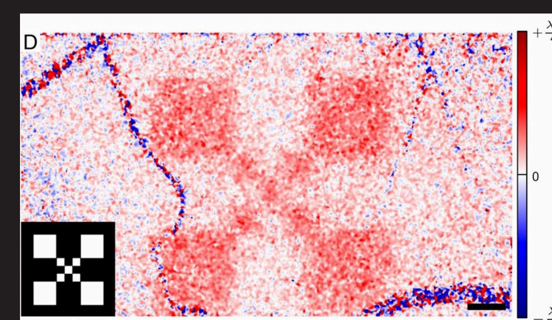


GCaMP labeled ganglion cells
Courtesy Juliette McGregor

OPL
ONL
PRL

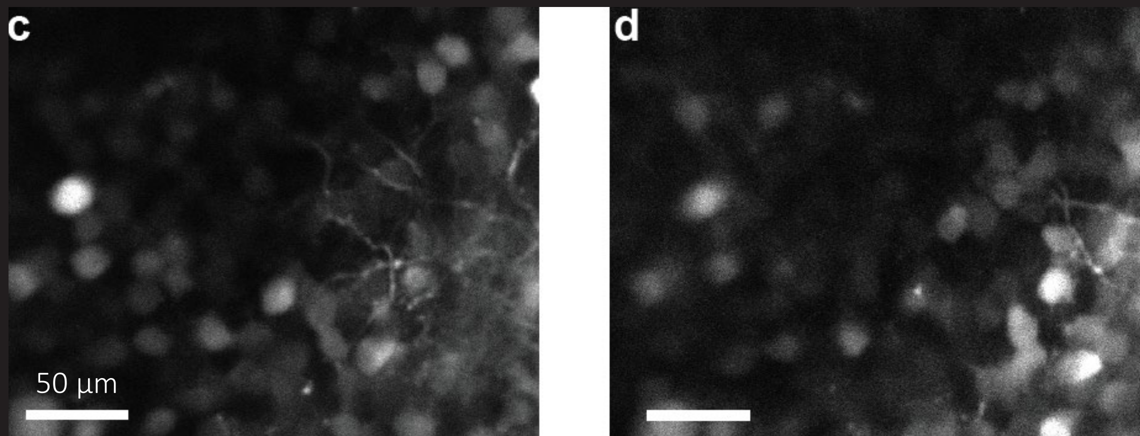


Photopigment densitometry
Sabesan et al, PlosOne 2015



Optical coherence tomography
Hillmann et al, PNAS 2016

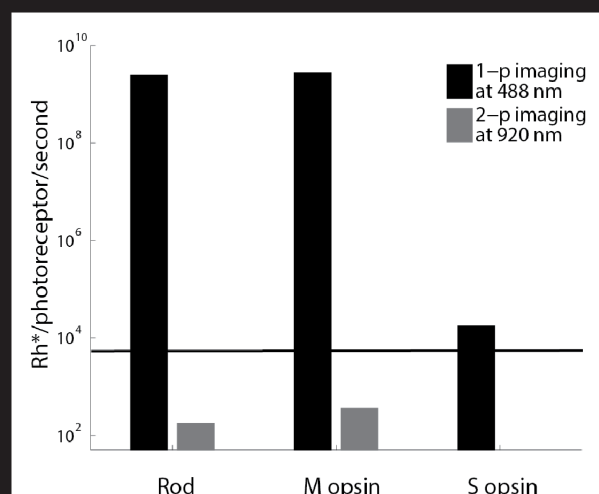
Two-photon imaging provides better resolution and weaker stimulation of photoreceptors



0 µm

-5 µm

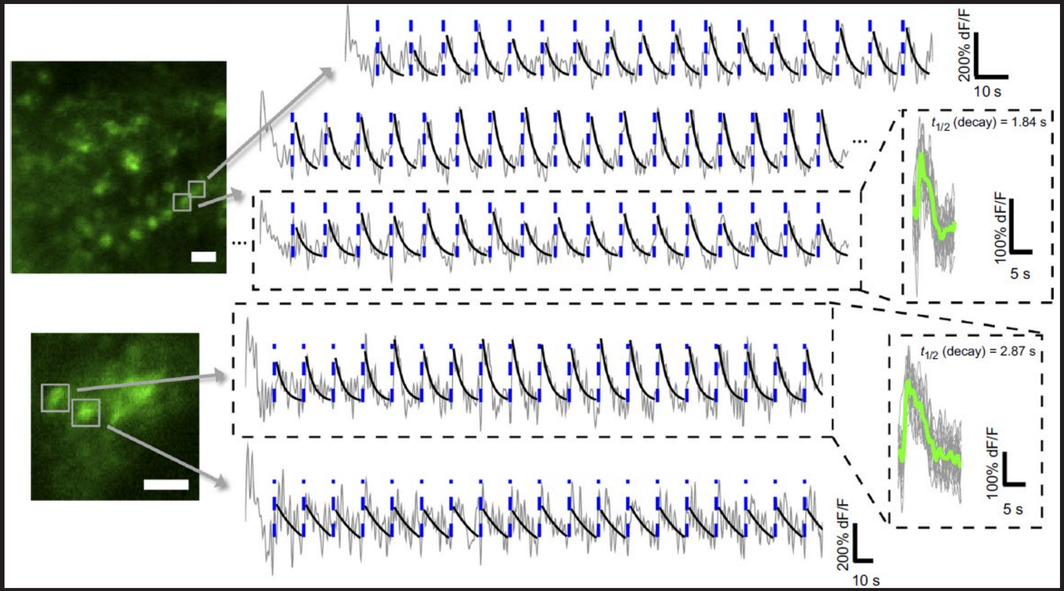
GFP-labeled RGCS, dendrites visible



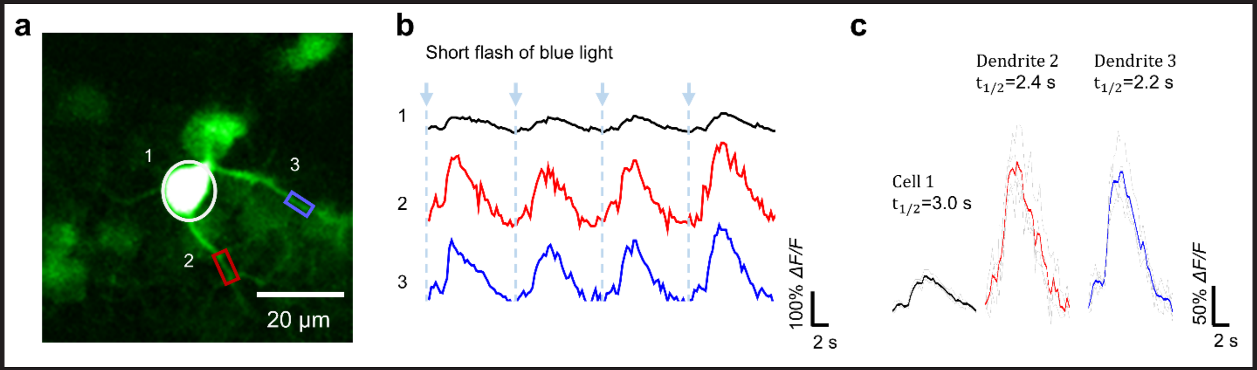
Sharma et al, BOEx 2013

Functional calcium imaging allows tracking of RGC responses to light stimulation

GCaMP6s-labeled RGCs

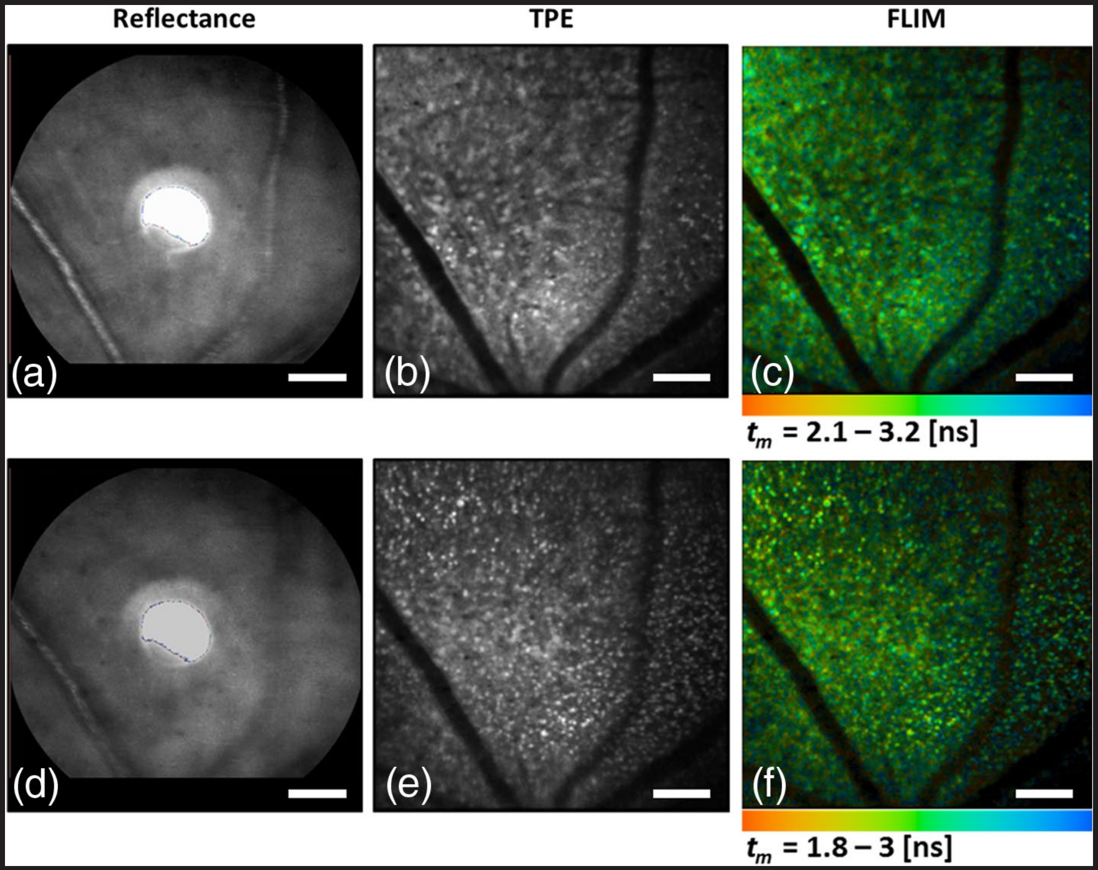


Bar-Noam et al, LSA 2016



Qin et al, LSA 2020

Simultaneous functional calcium imaging of many RGCs at a time

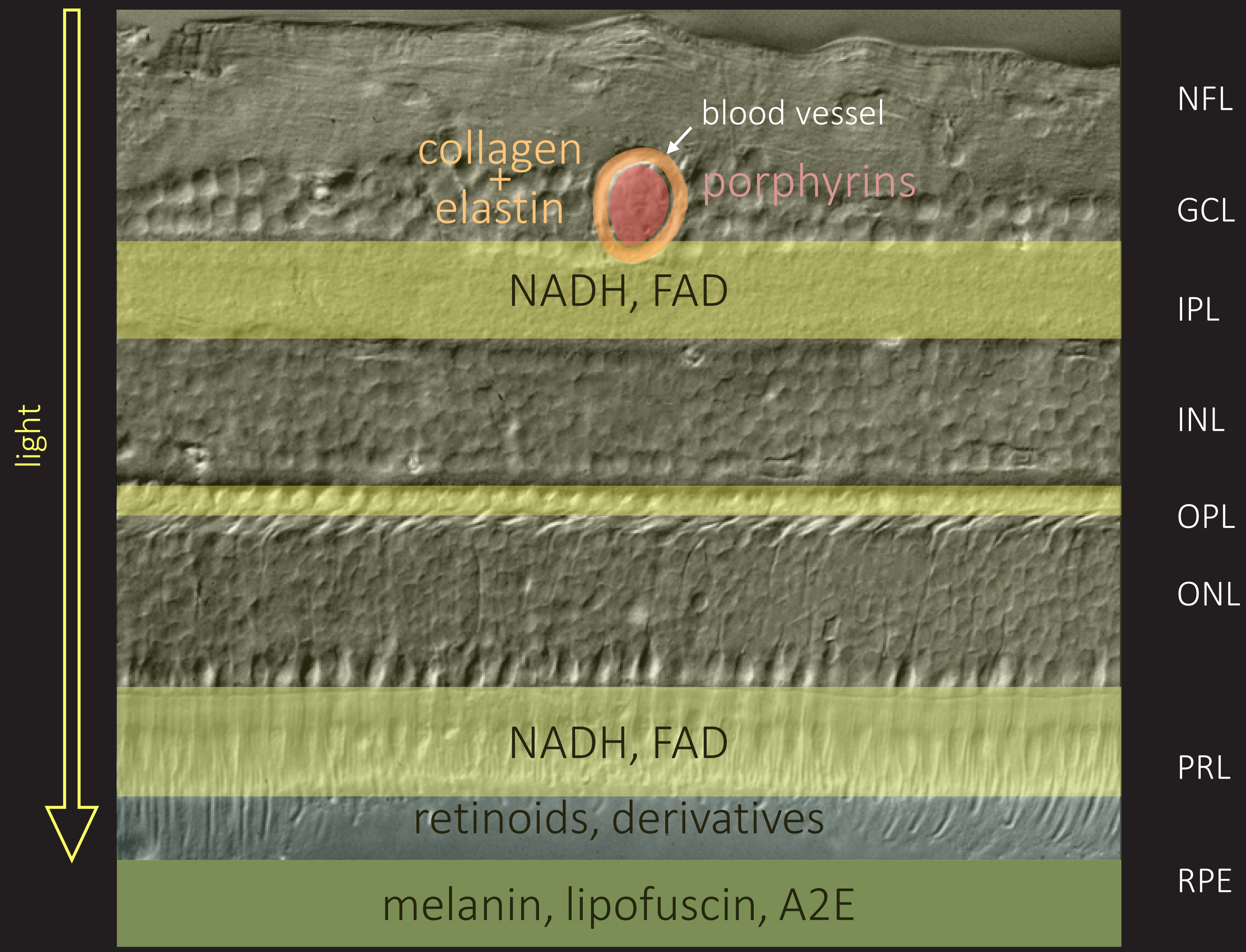


Thy1-GCaMP3

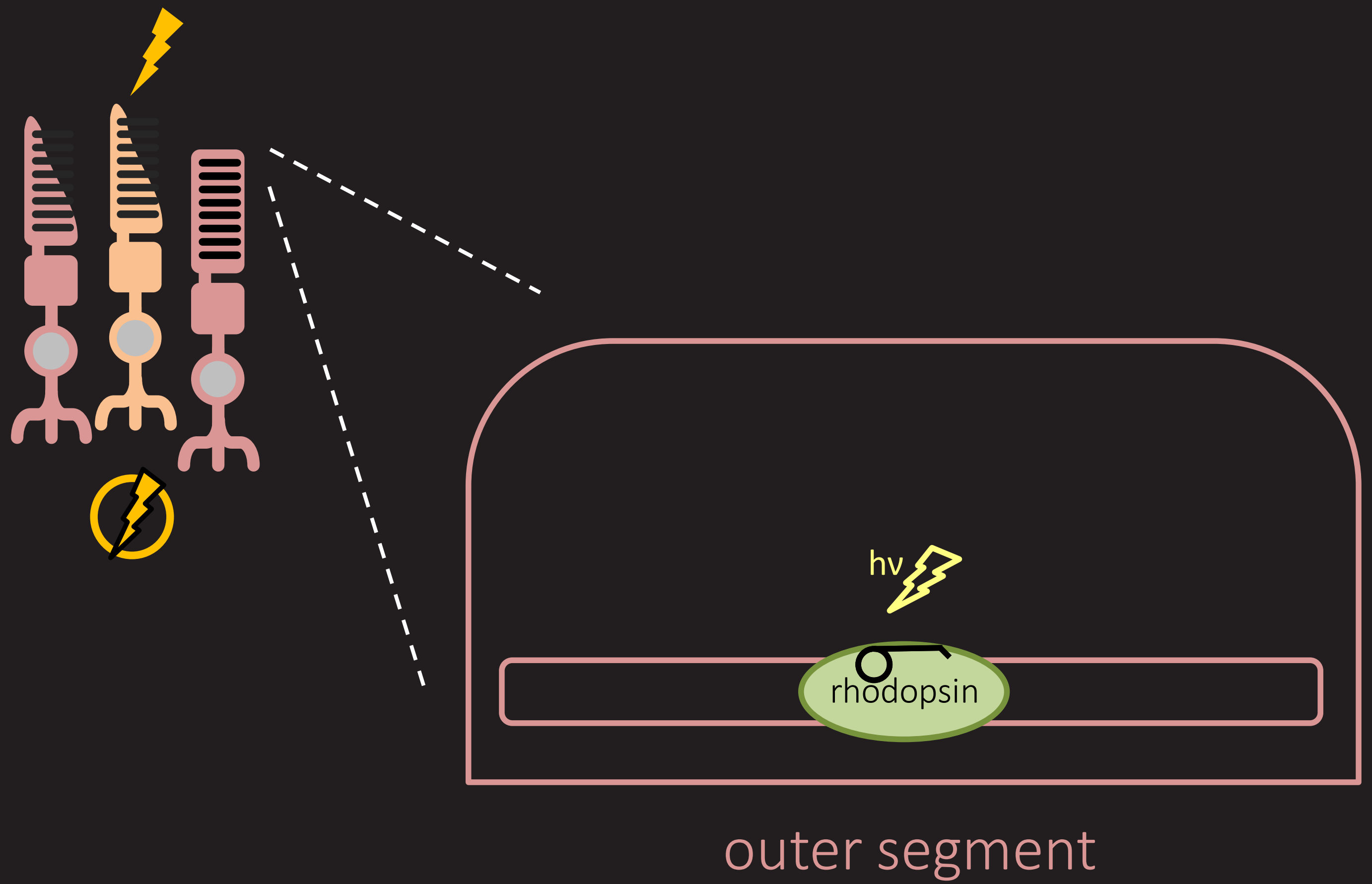
Kamali et al, JBO 2018

Imaging endogenous fluorophores in the living eye indicative of cellular function

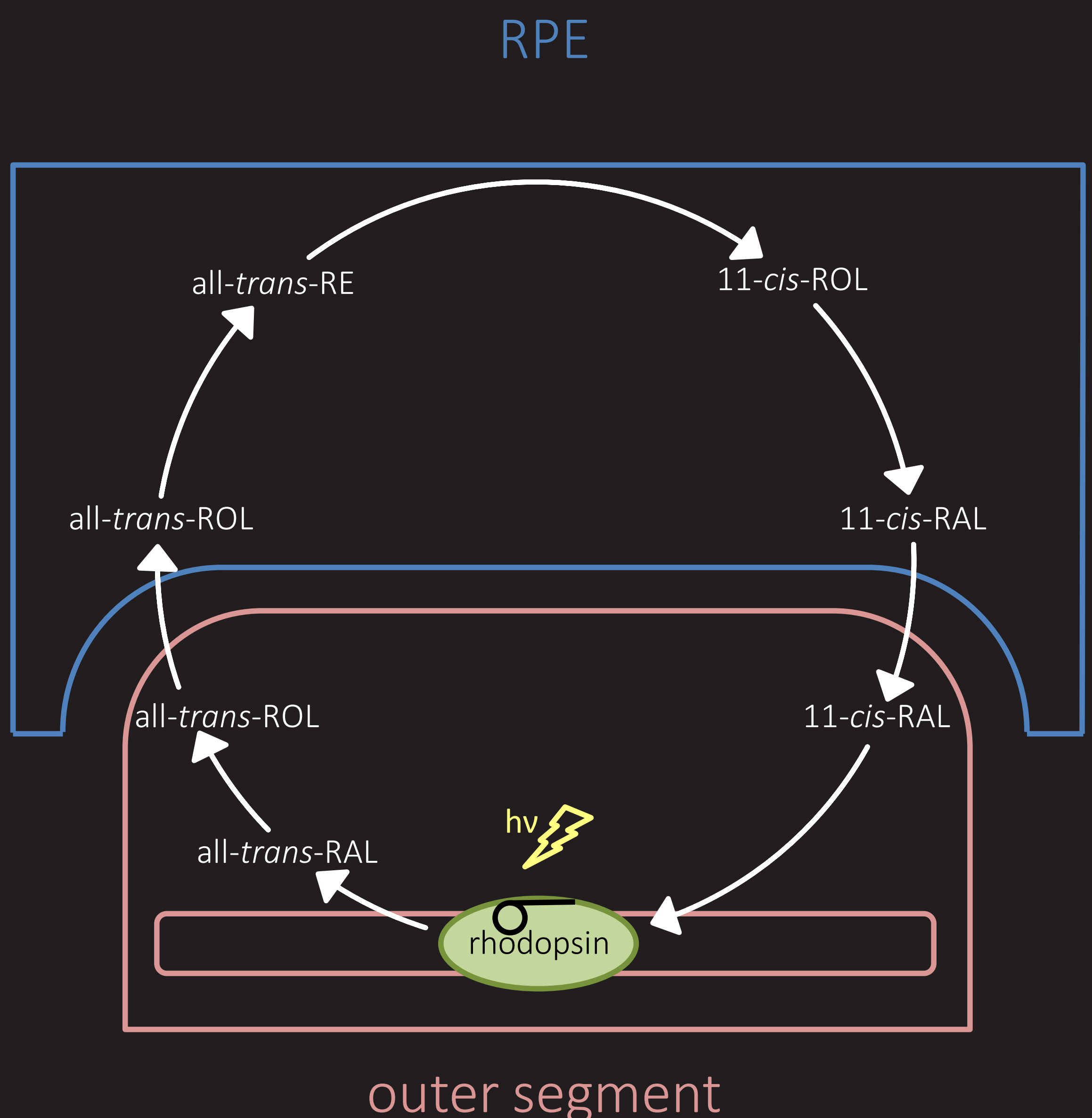
Endogenous fluorophores in the retina



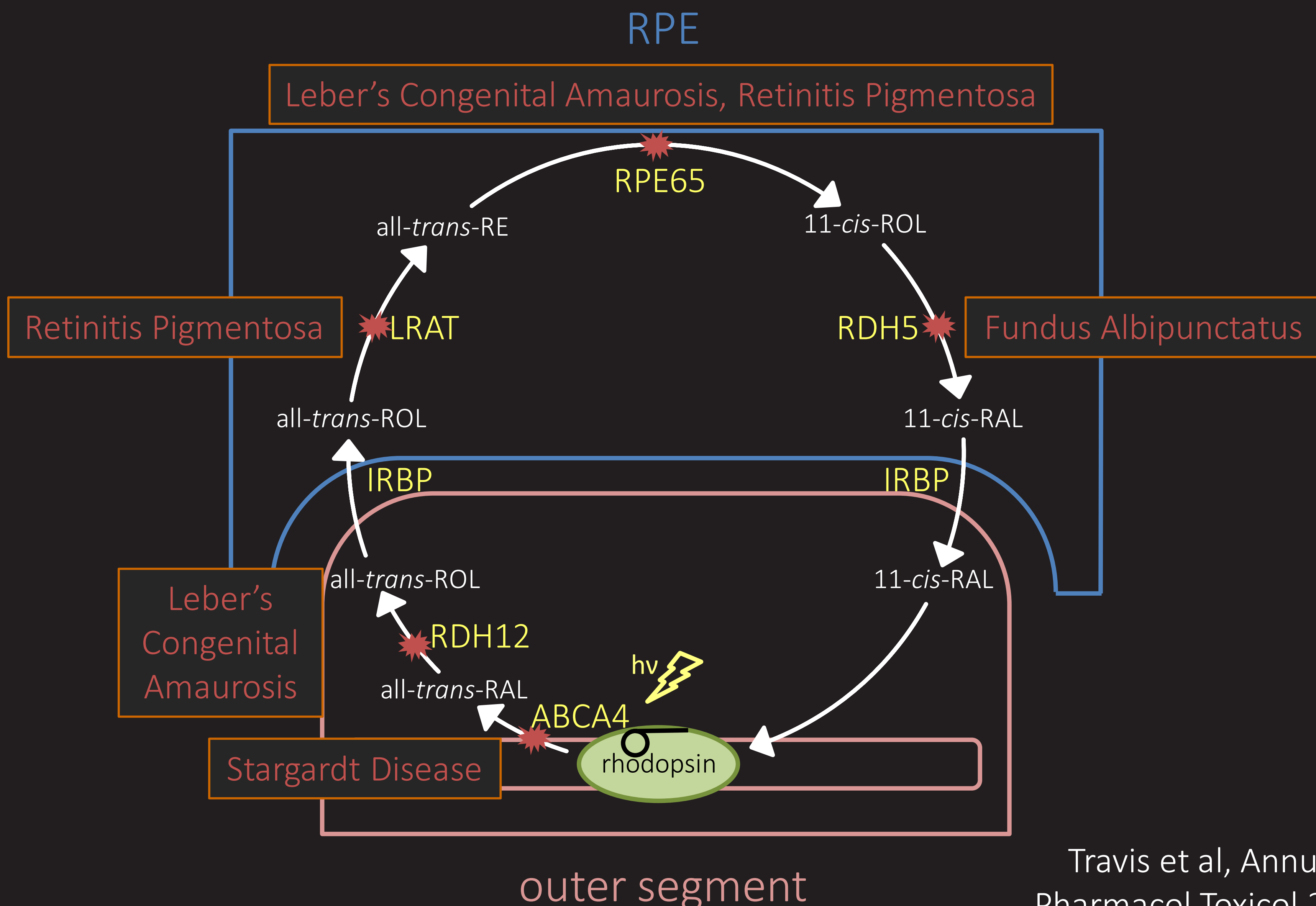
Retinol and the visual cycle



Retinol and the visual cycle



Retinol and the visual cycle



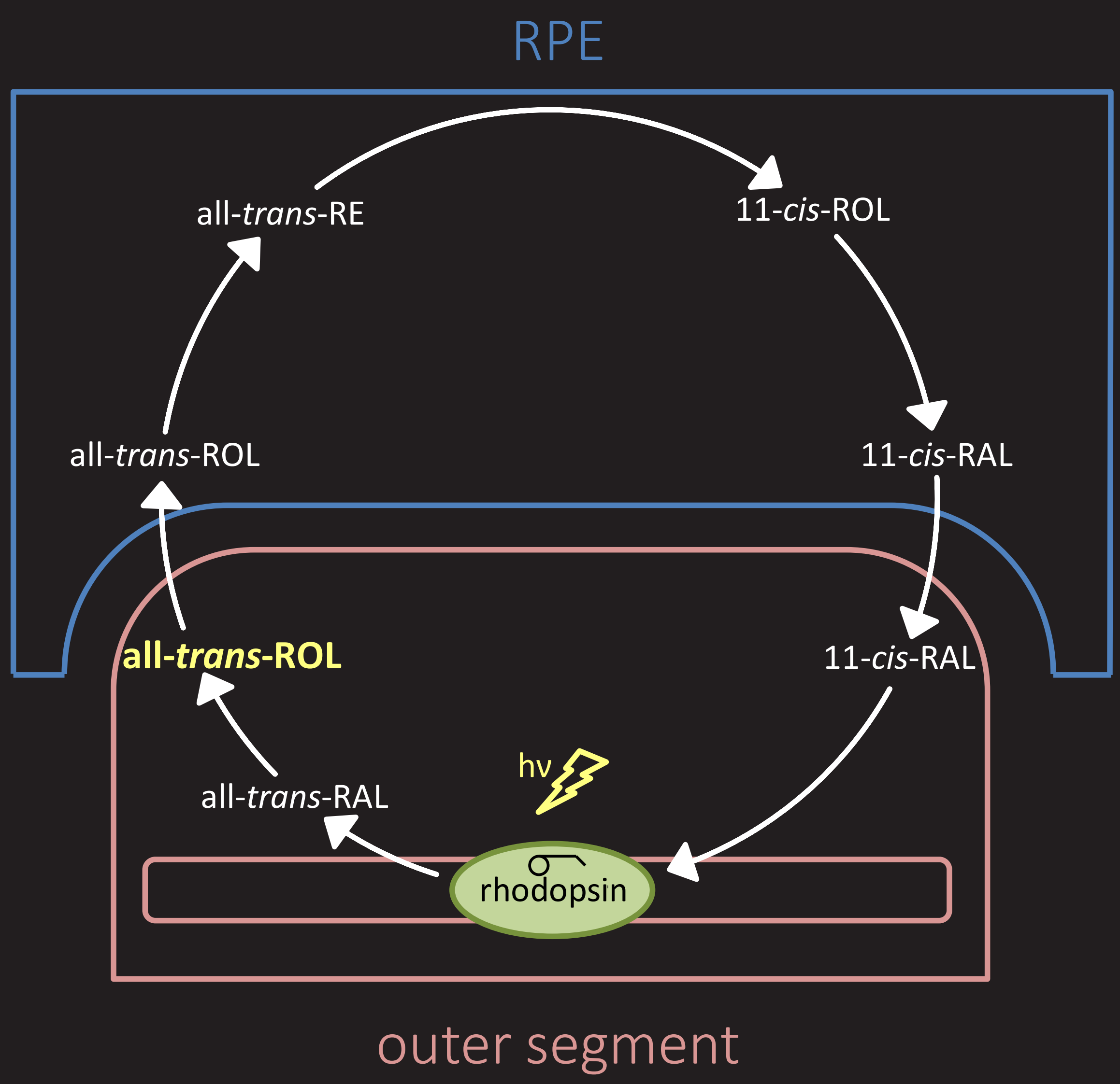
Travis et al, Annu Rev Pharmacol Toxicol 2007

Available methods to track the visual cycle

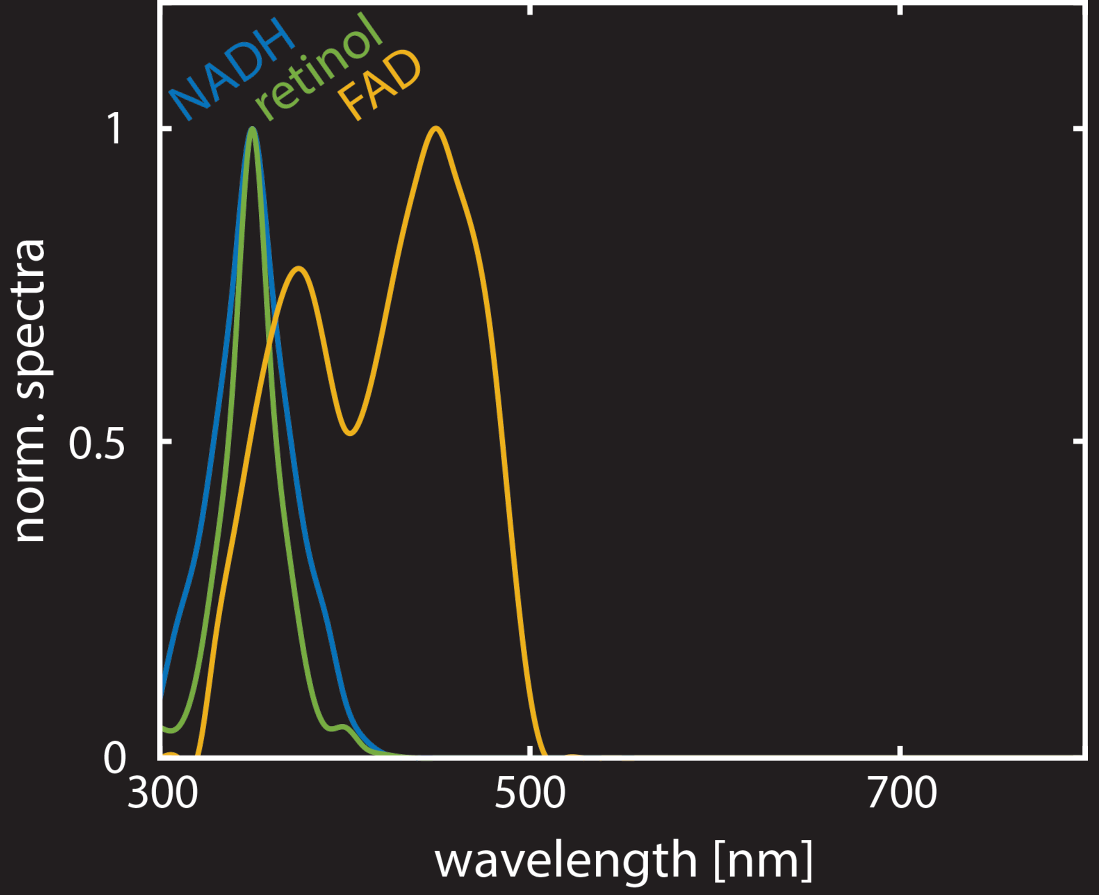
- Electroretinography (ERG)
  - Voltage change
  - Signal from all retinal layers and cell classes is intertwined
- Pigment densitometry
  - Change in pigment density
  - Afflicted with artifacts due to neural responses and vascular changes
- Psychophysics
  - requires patient feedback

To accelerate diagnosis of disease and treatment development, there is a need for objective methods to quantify visual cycle kinetics on the single-cell level!

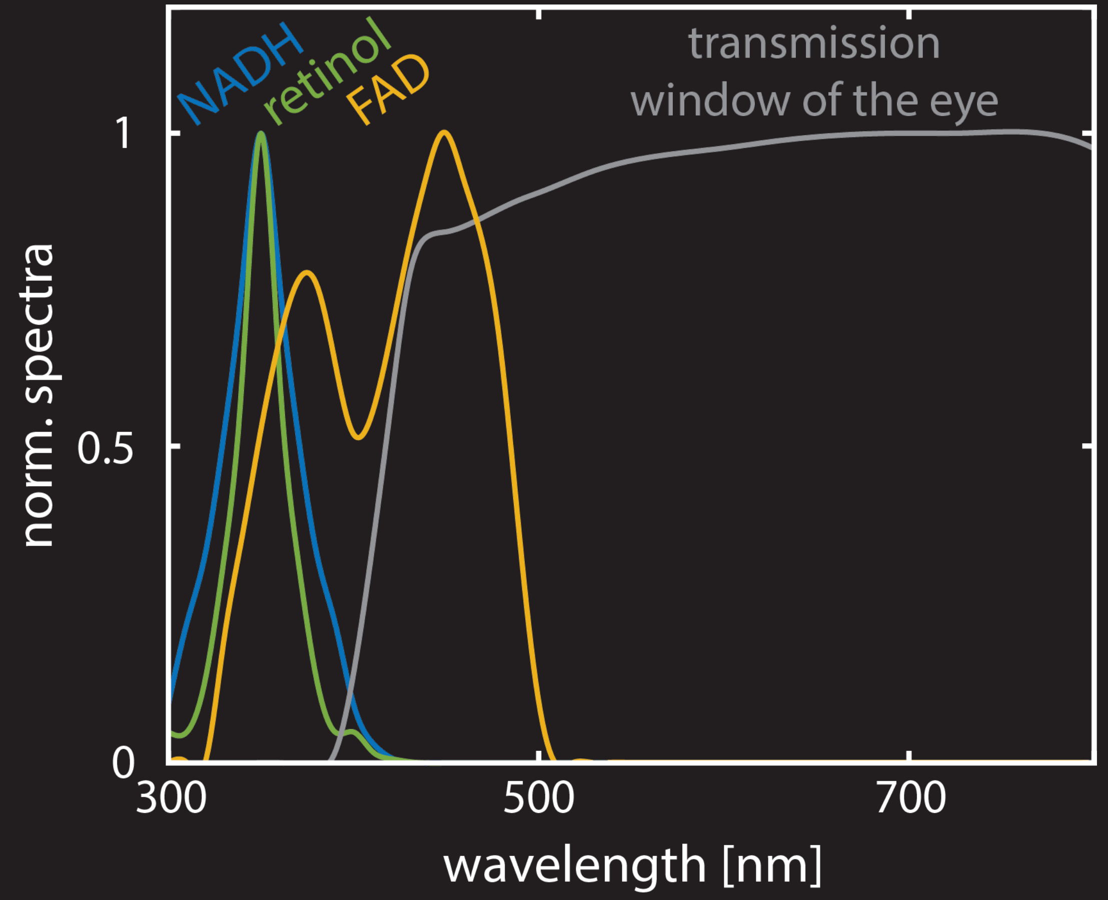
In vivo two-photon ophthalmoscopy can assess visual cycle function



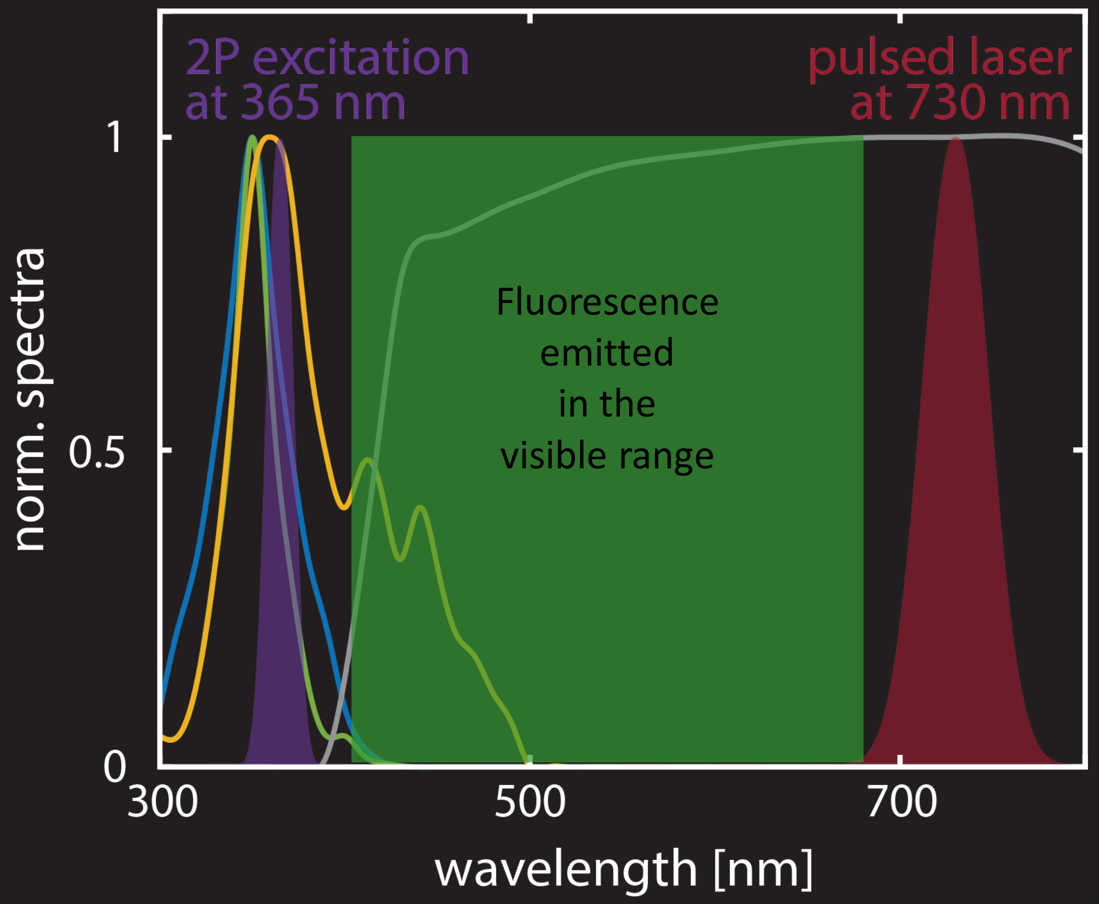
Endogenous retinal fluorophores are excitable in the UV band...



…but the water window blocks this range



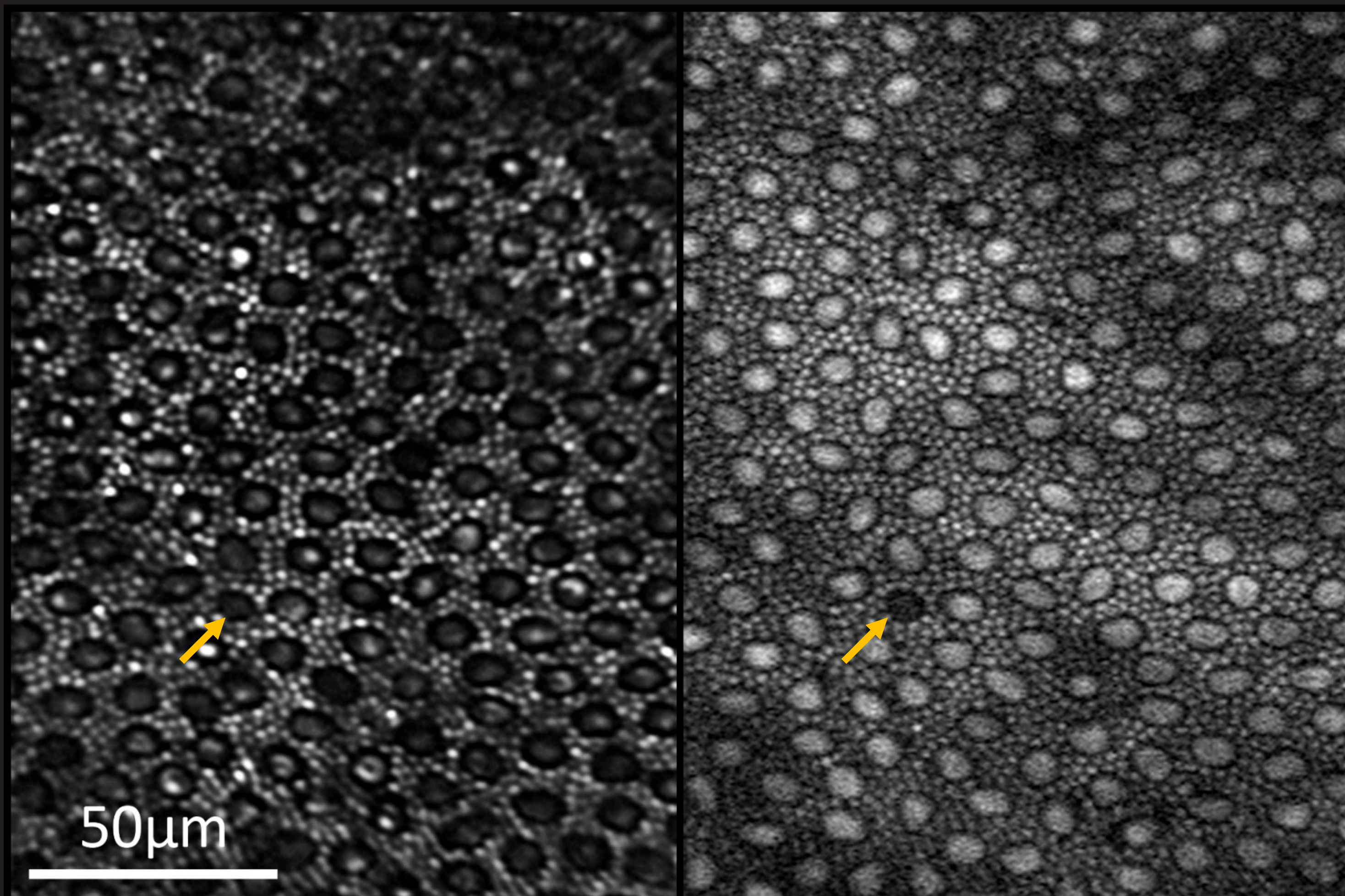
Two-photon ophthalmoscopy can excite these fluorophores in the living eye



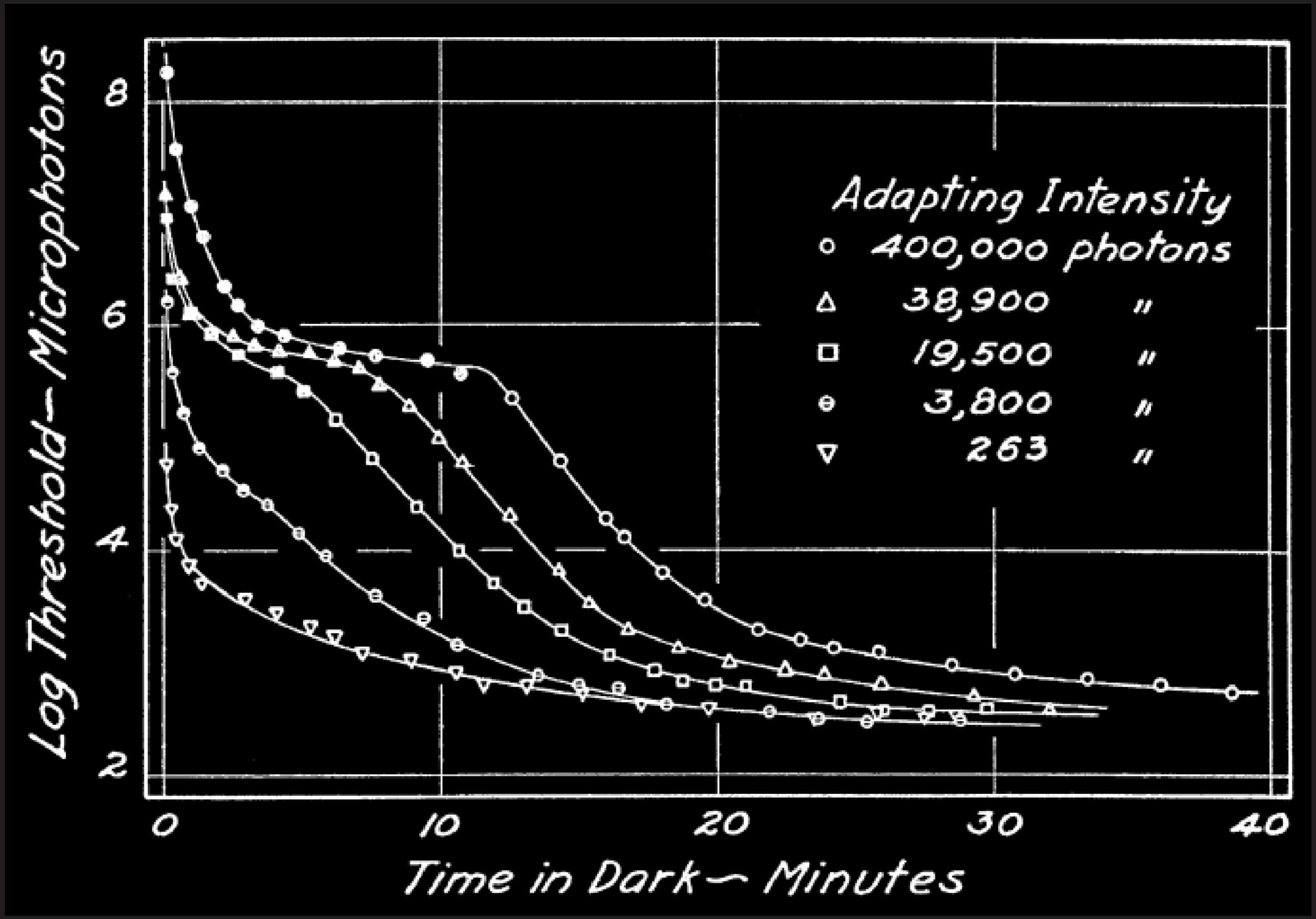
Reflectance and fluorescence images of photoreceptors provide complementary information

reflectance

two-photon excited fluorescence (TPEF)



Does the fluorophore at the photoreceptor layer show a dark adaptation-like behavior?

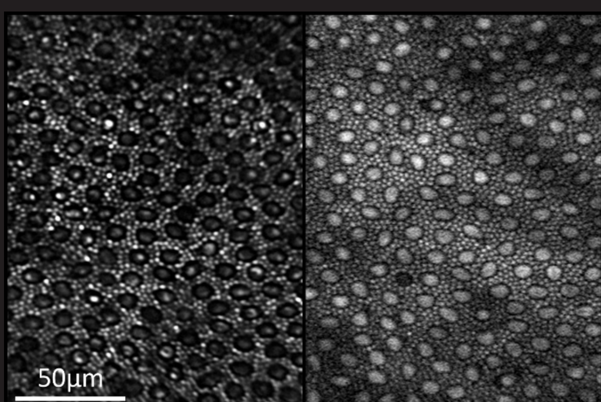


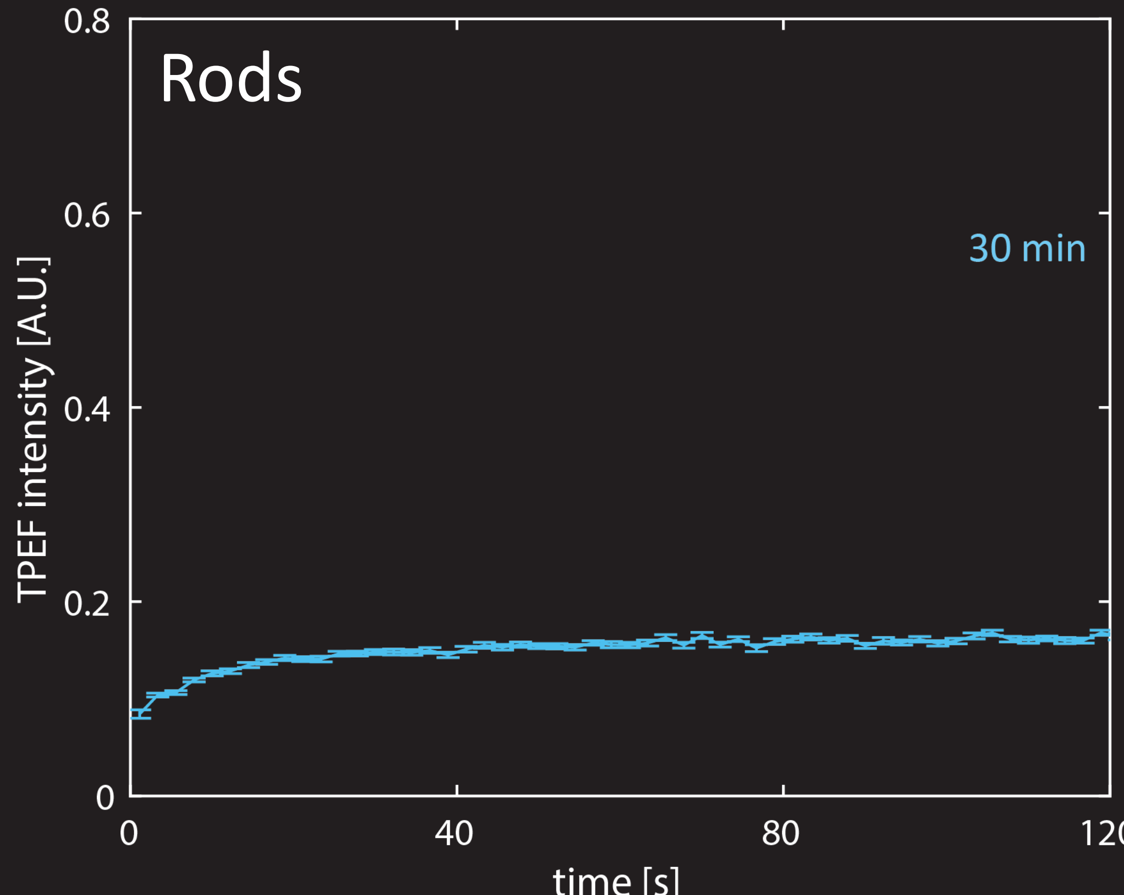
Hecht et al., J Gen Physiol 1937

Autofluorescence during light and dark adaptation

wait for 30 min

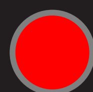
REC



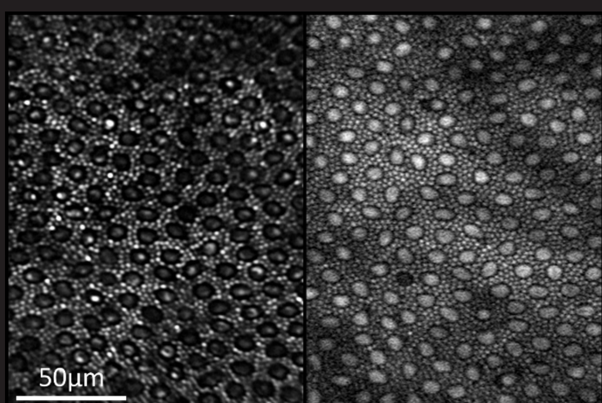


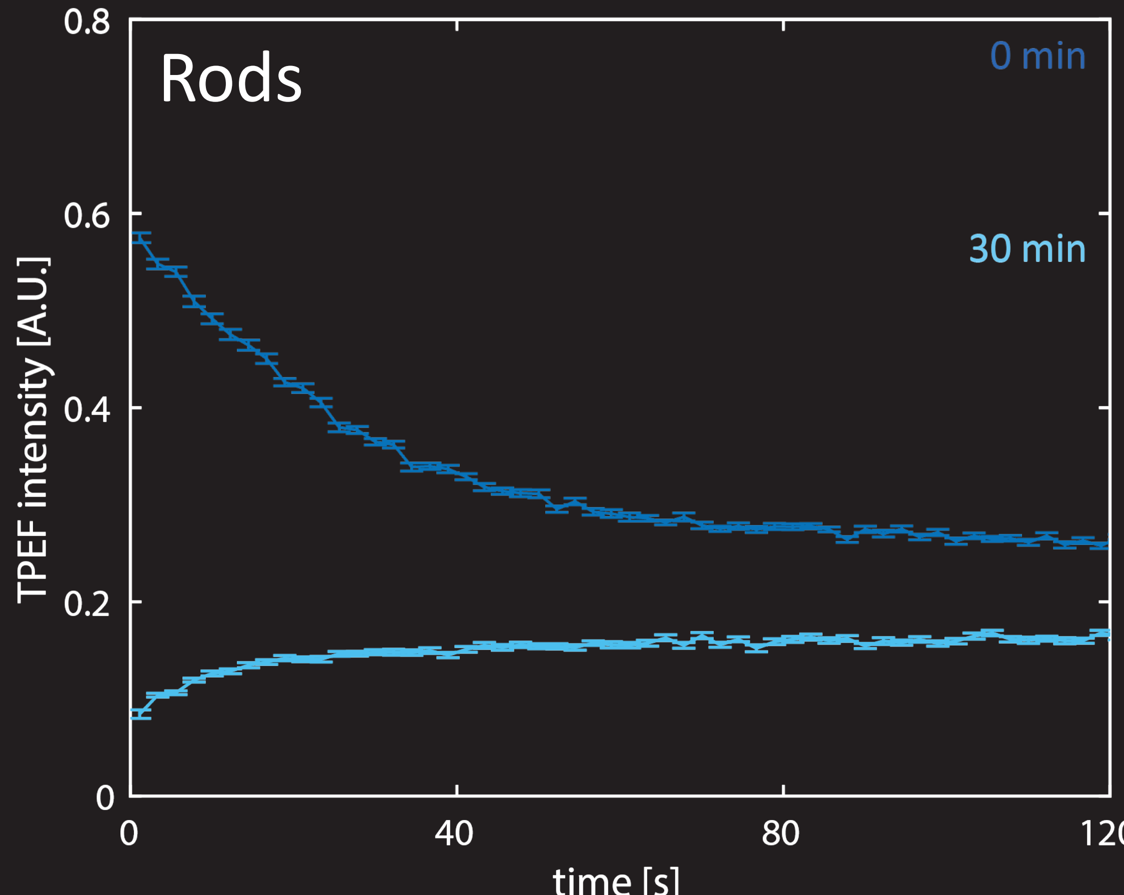
Sharma et al, IOVS 2016

Autofluorescence during light and dark adaptation



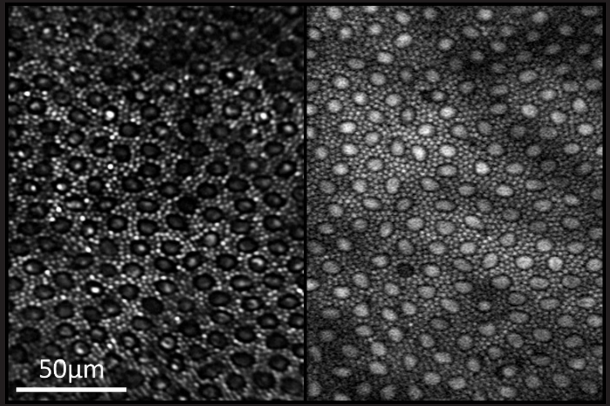
REC

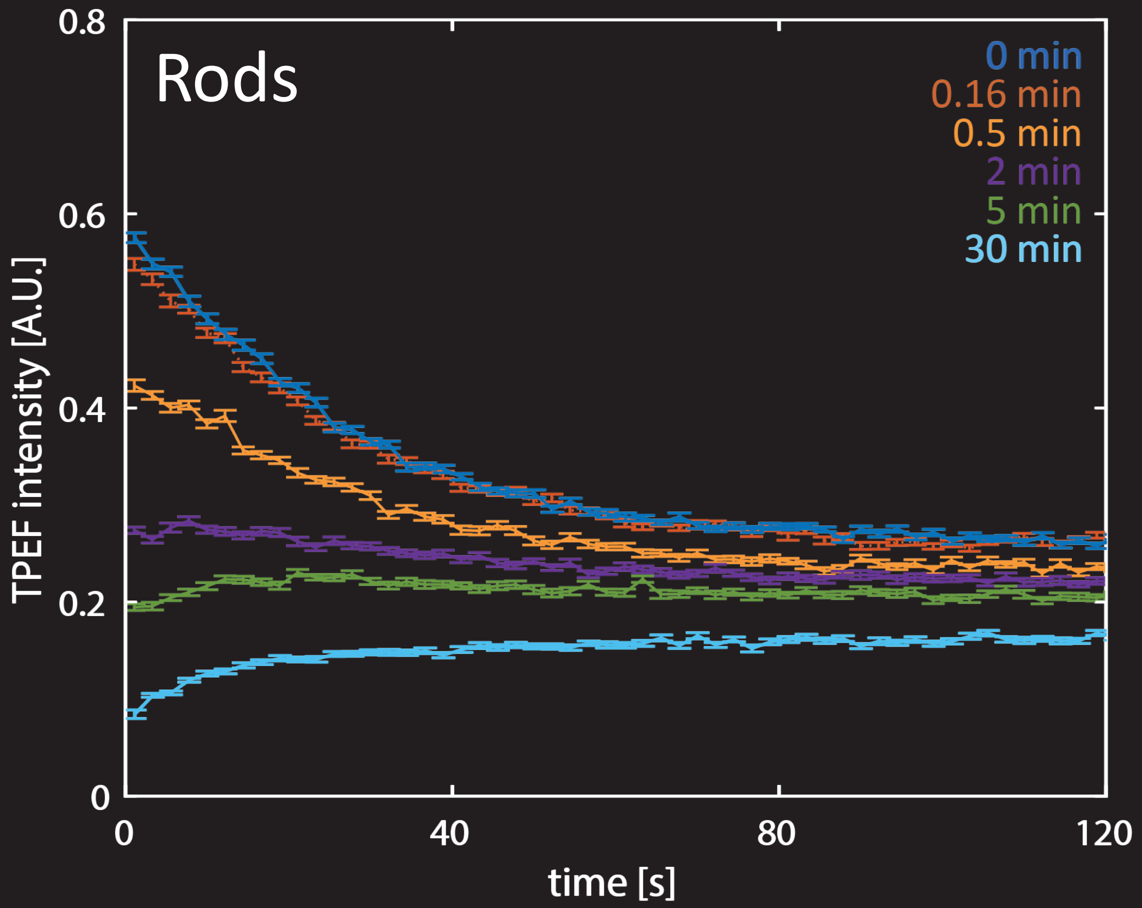




Sharma et al, IOVS 2016

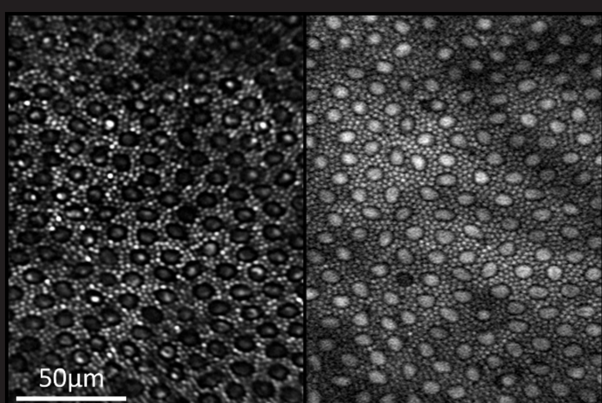
Autofluorescence during light and dark adaptation

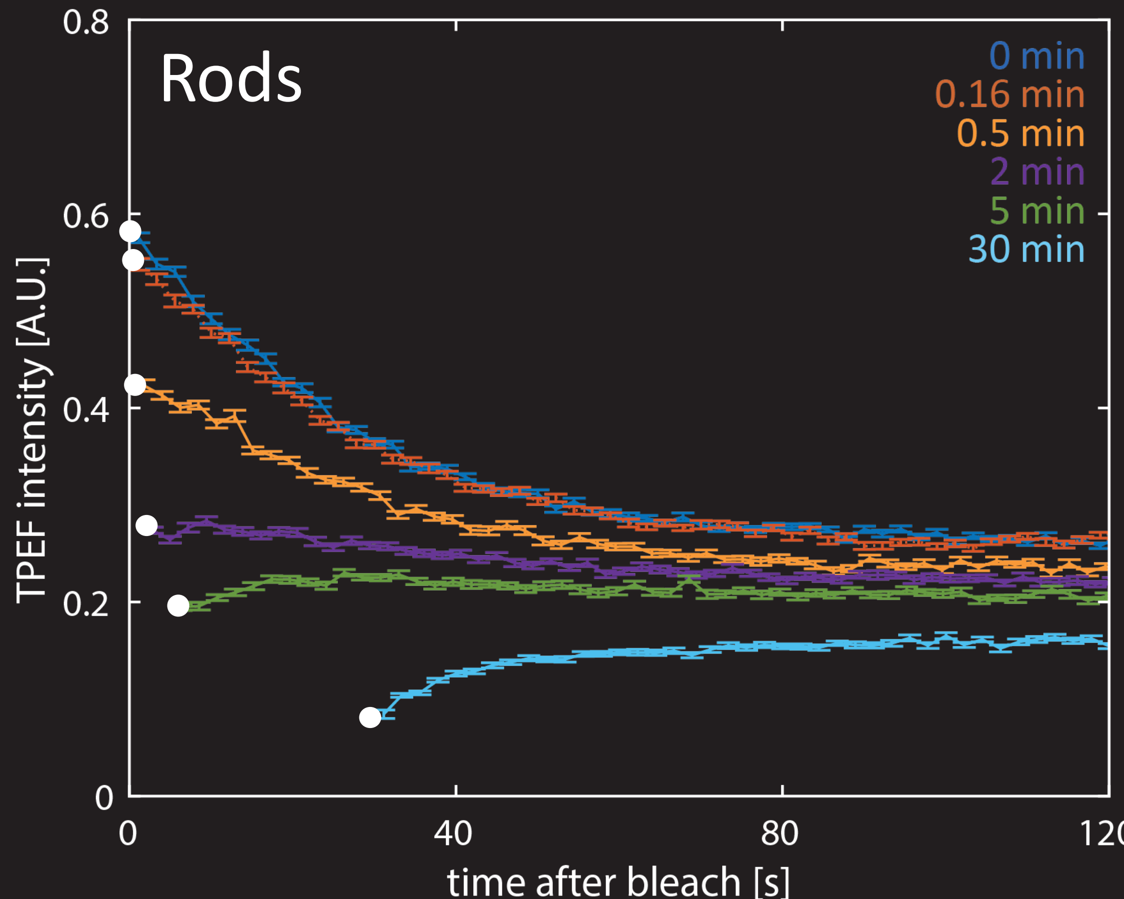




Sharma et al, IOVS 2016

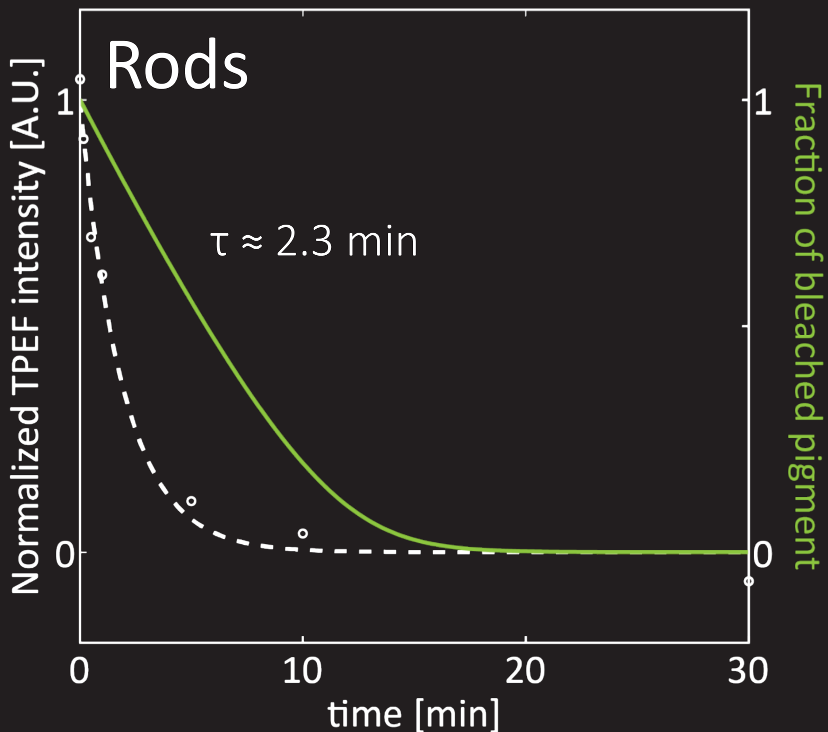
Autofluorescence during light and dark adaptation

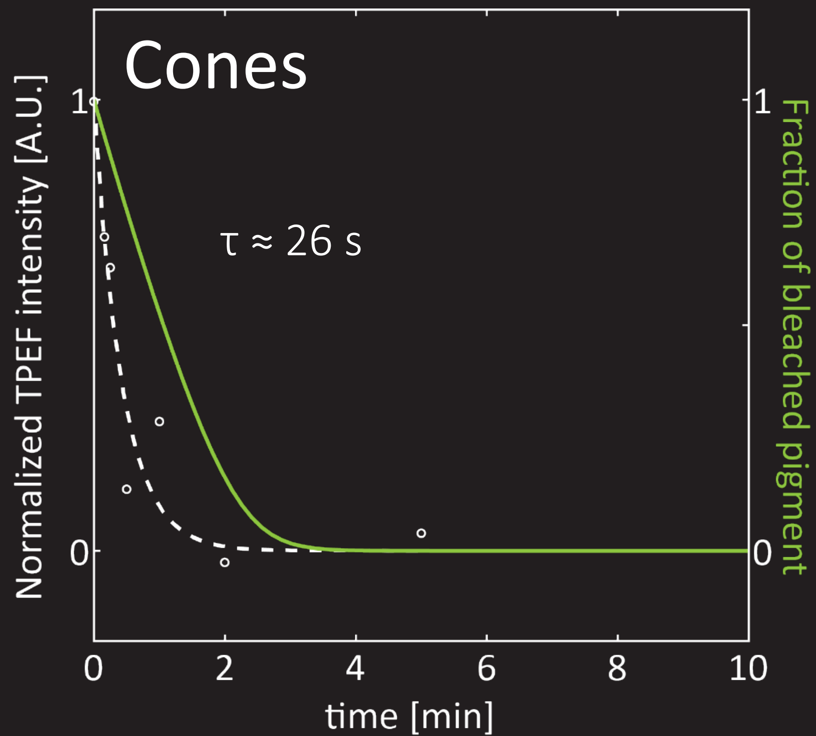




Sharma et al, IOVS 2016

Fluorescence decrease is different from photopigment regeneration





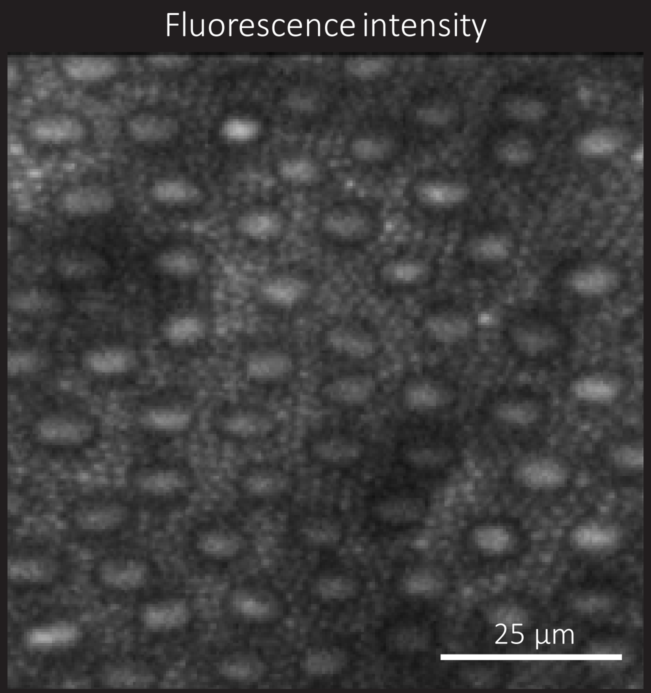
Fluorescence decrease ~4x faster than photopigment regeneration

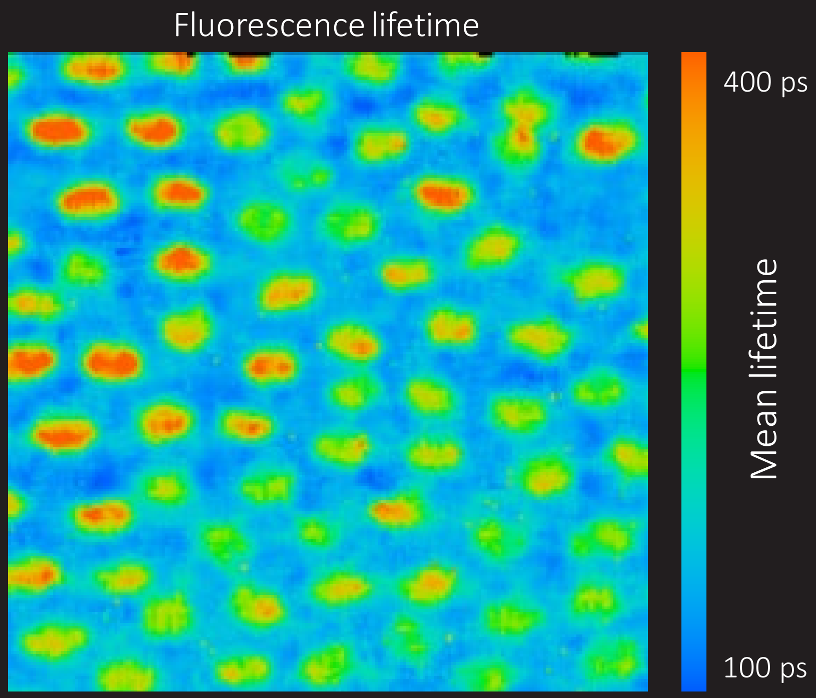
→ Fluorophore is intermediate product of the visual cycle

Fluorescence decrease 4-5x faster in cones than in rods

Sharma et al, IOVS 2016

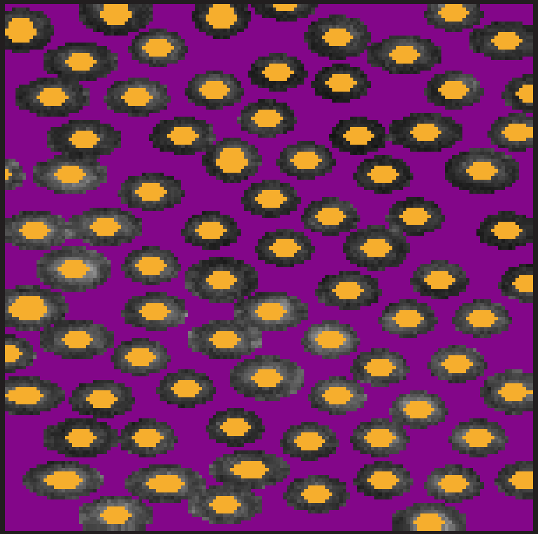
AOFLIO reveals longer fluorescence lifetime in cones than in rods

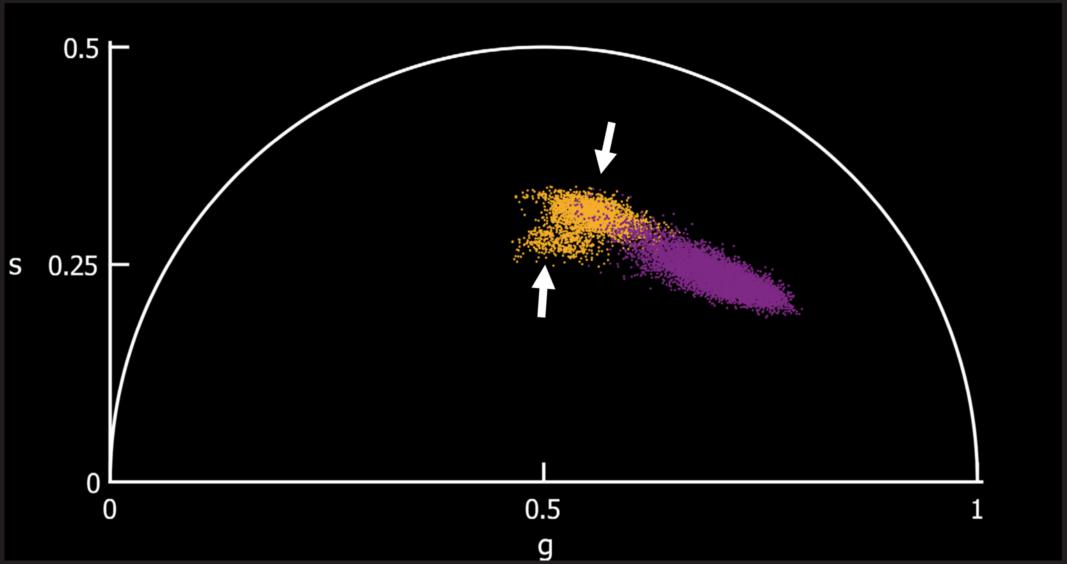




Walters, Feeks et al, in preparation

Subsets of photoreceptors are distinguishable



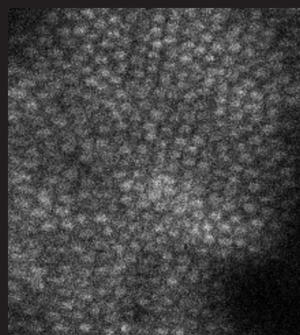
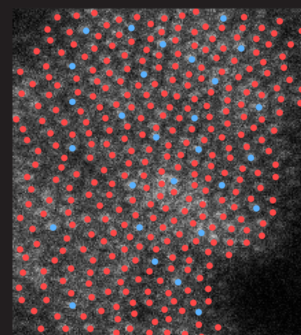
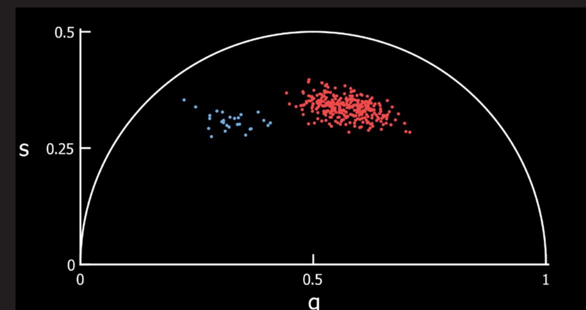


Phasor analysis

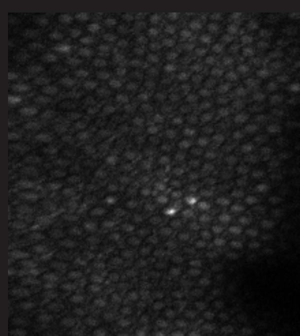
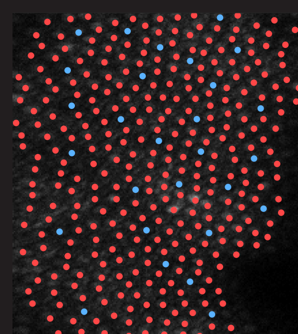
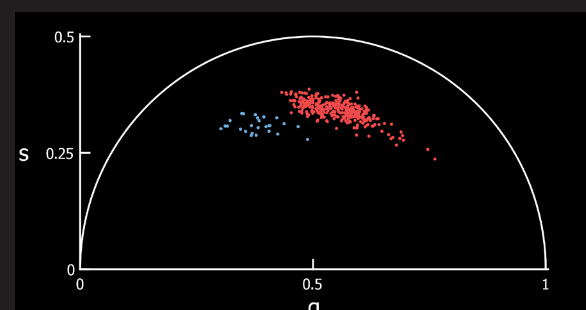
Slide courtesy Khang Huynh

Clustering of cones is repeatable

Day 1,
3 mW

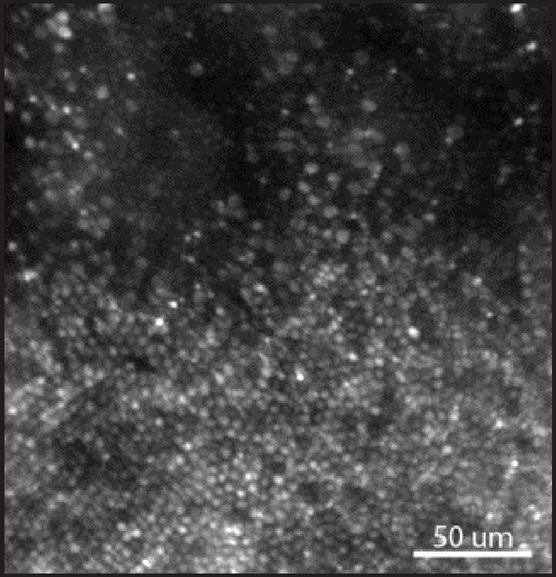
Day 2,
3 mW

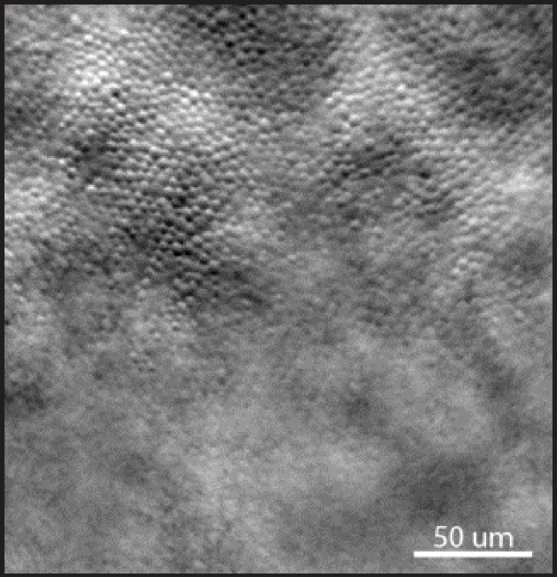
Slide courtesy Khang Huynh

Model 1 – AAV induced outer segment degeneration

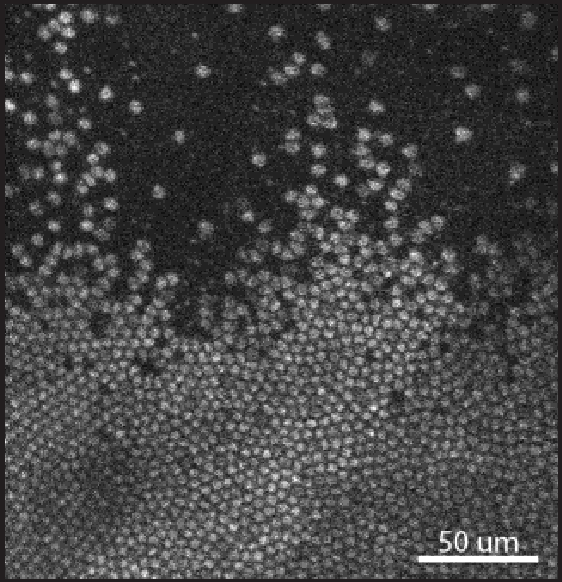
Reflectance



Offset Aperture

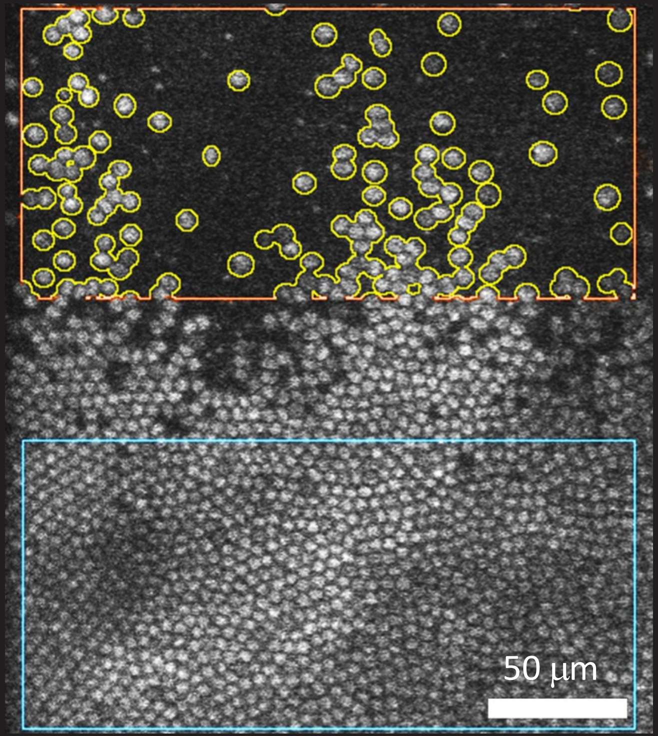


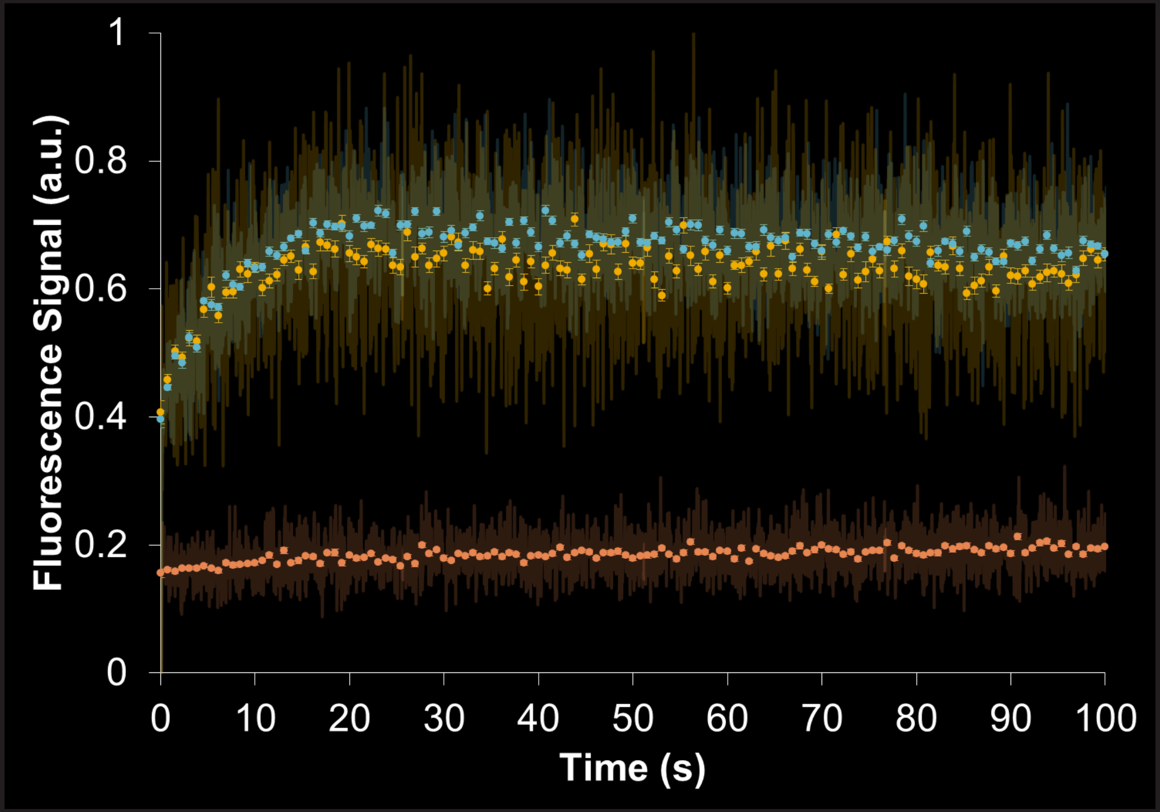
TPEF



Walters et al, BOEx 2018

Impairment of the retinoid cycle

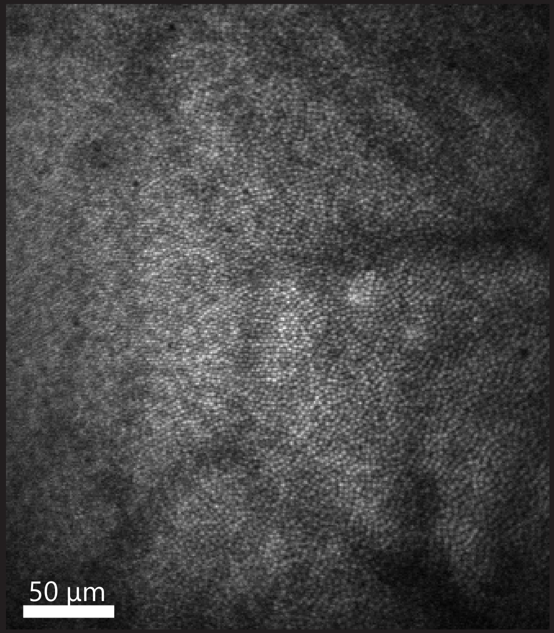


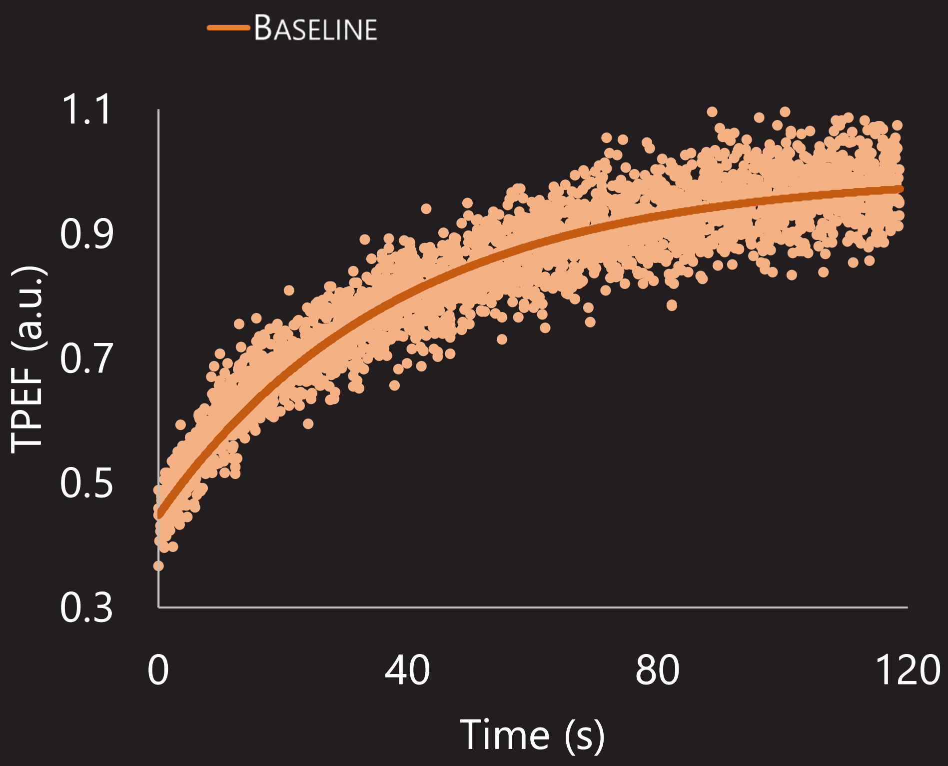


Walters et al, BOEx 2018

Model 2 – Short periods of systemic hypoxia

TPEF

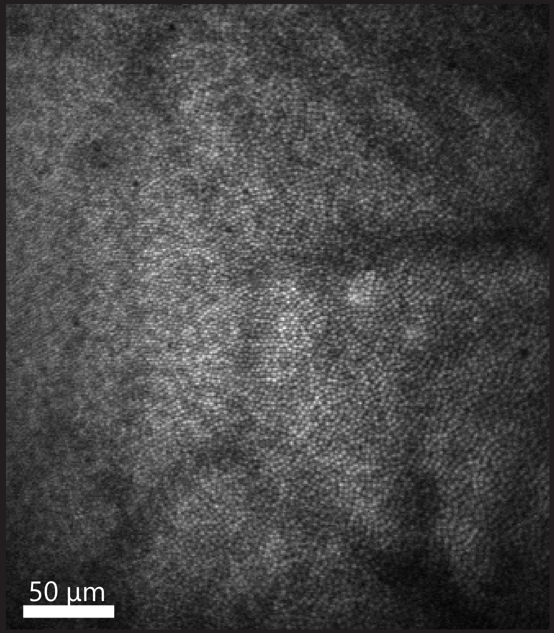


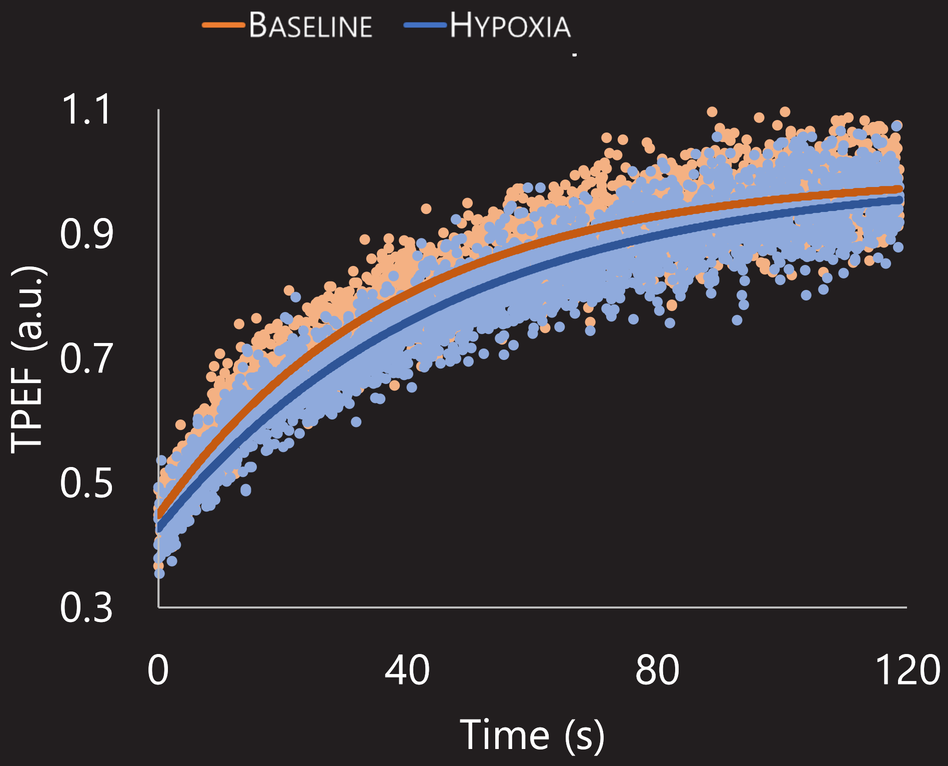


Walters et al, in preparation

Systemic hypoxia alters the time course of TPEF

TPEF

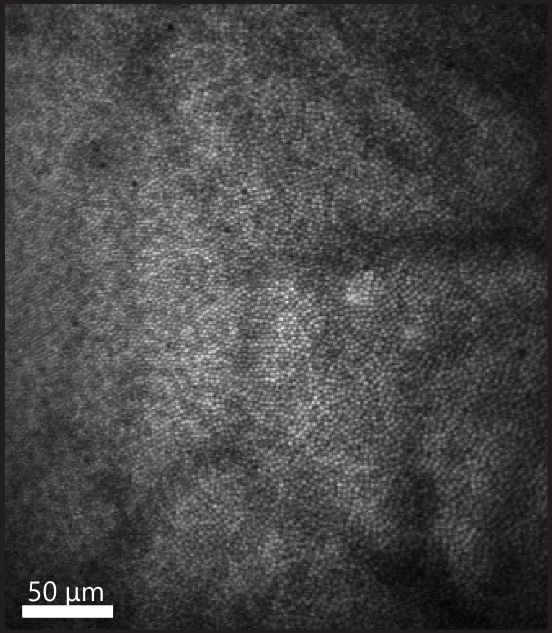


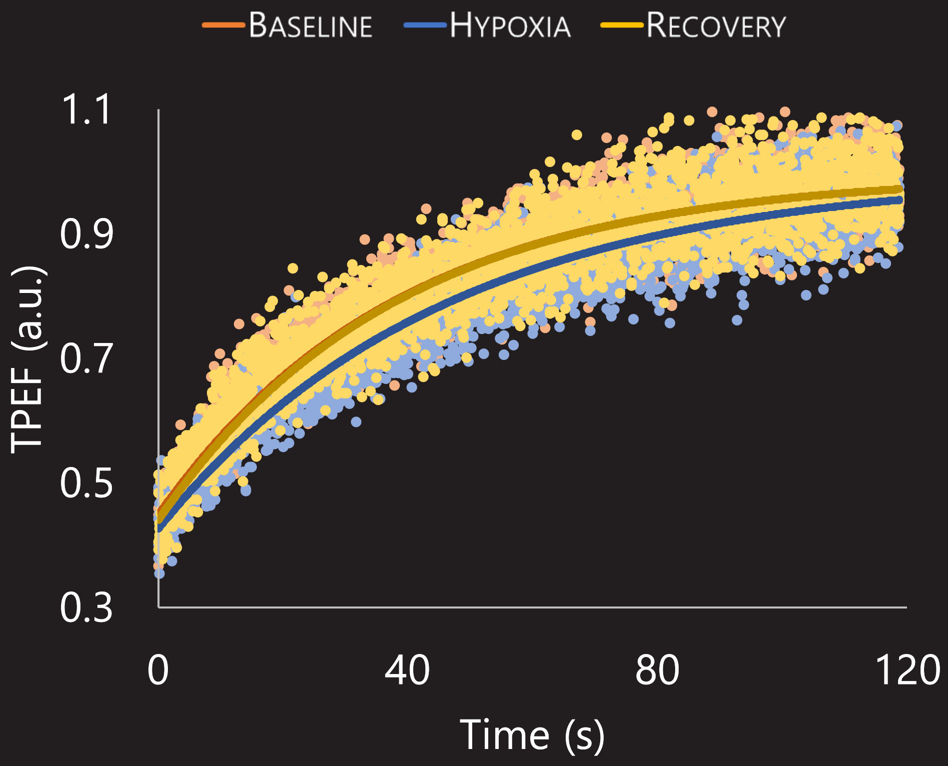


Walters et al, in preparation

Systemic hypoxia alters the time course of TPEF

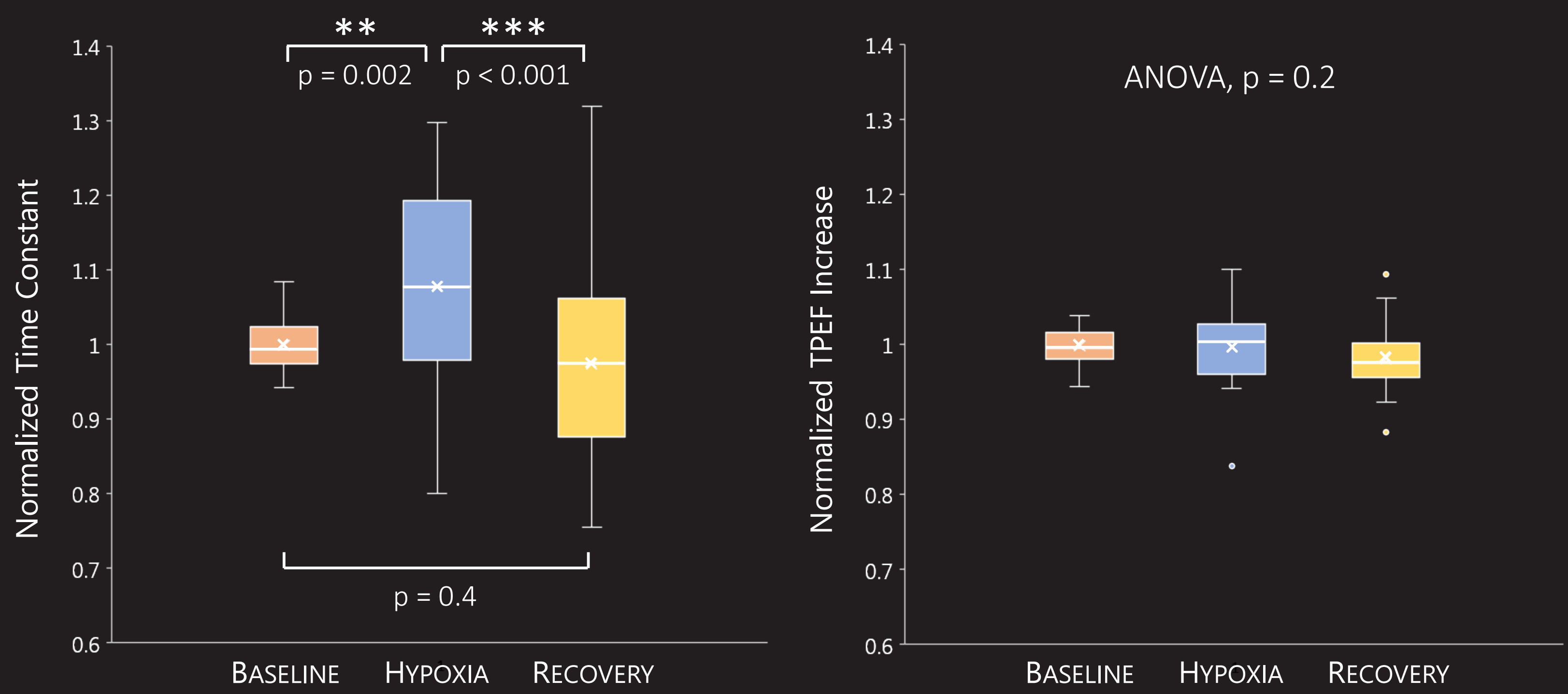
TPEF





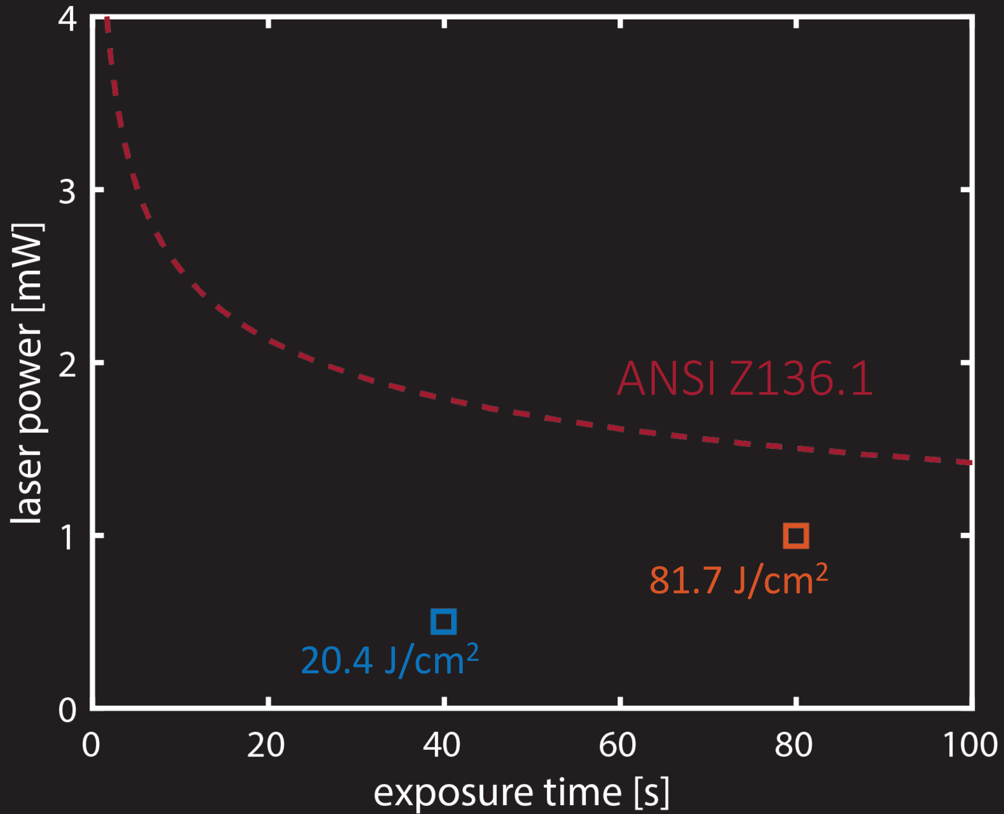
Walters et al, in preparation

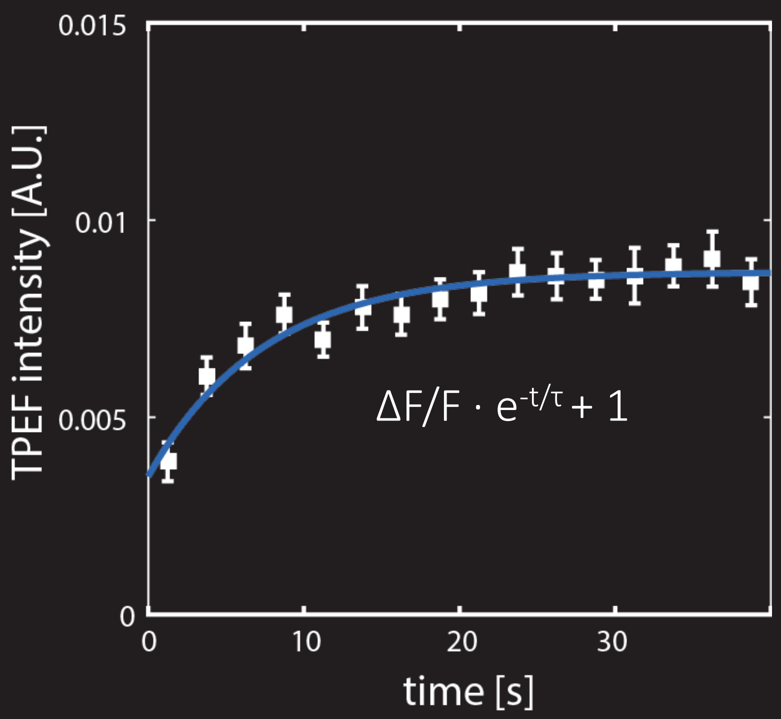
Time constant of TPEF increases during hypoxia
Fractional TPEF increase is unaffected



Walters et al, in preparation

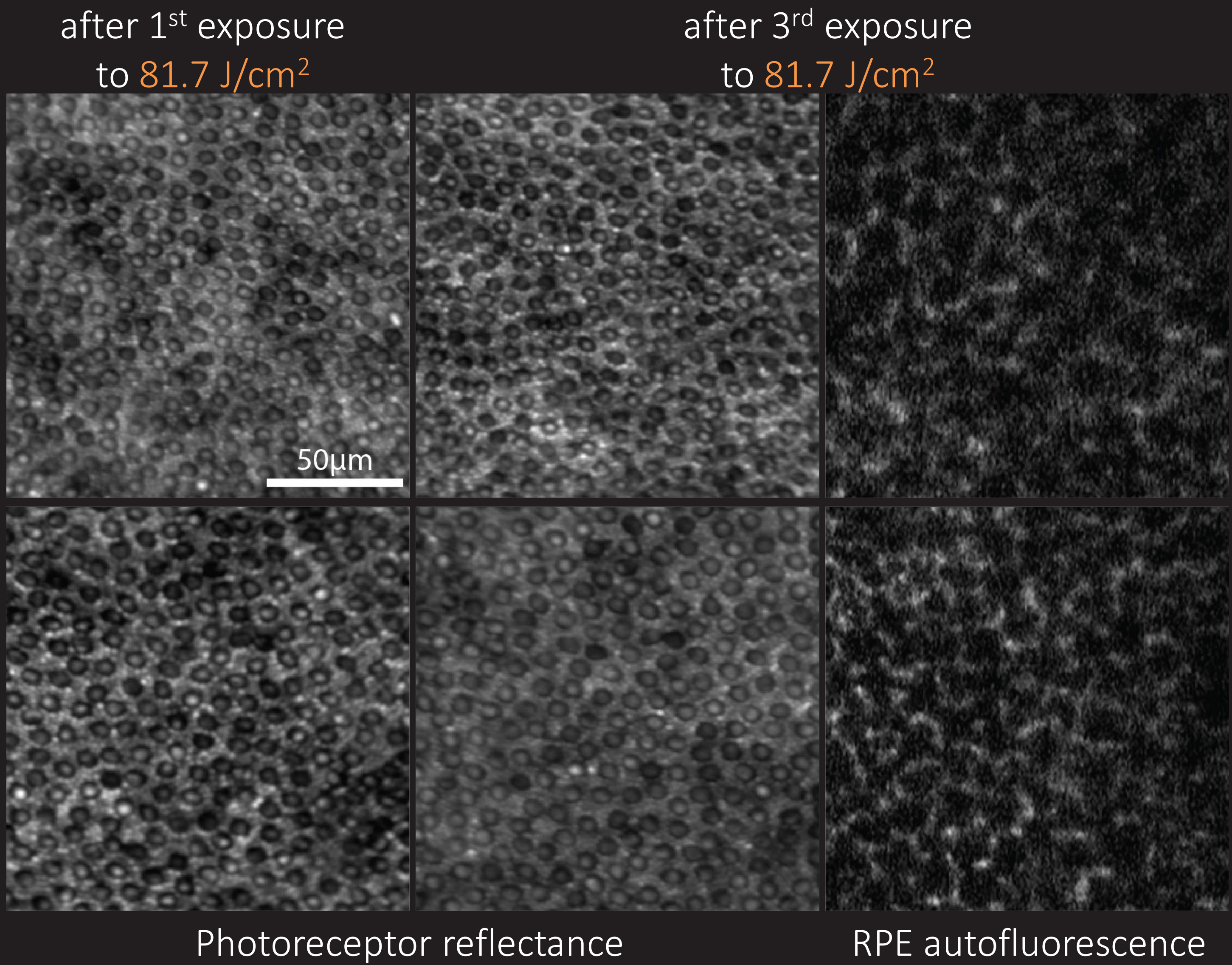
Functional two-photon imaging is possible within current safety standards





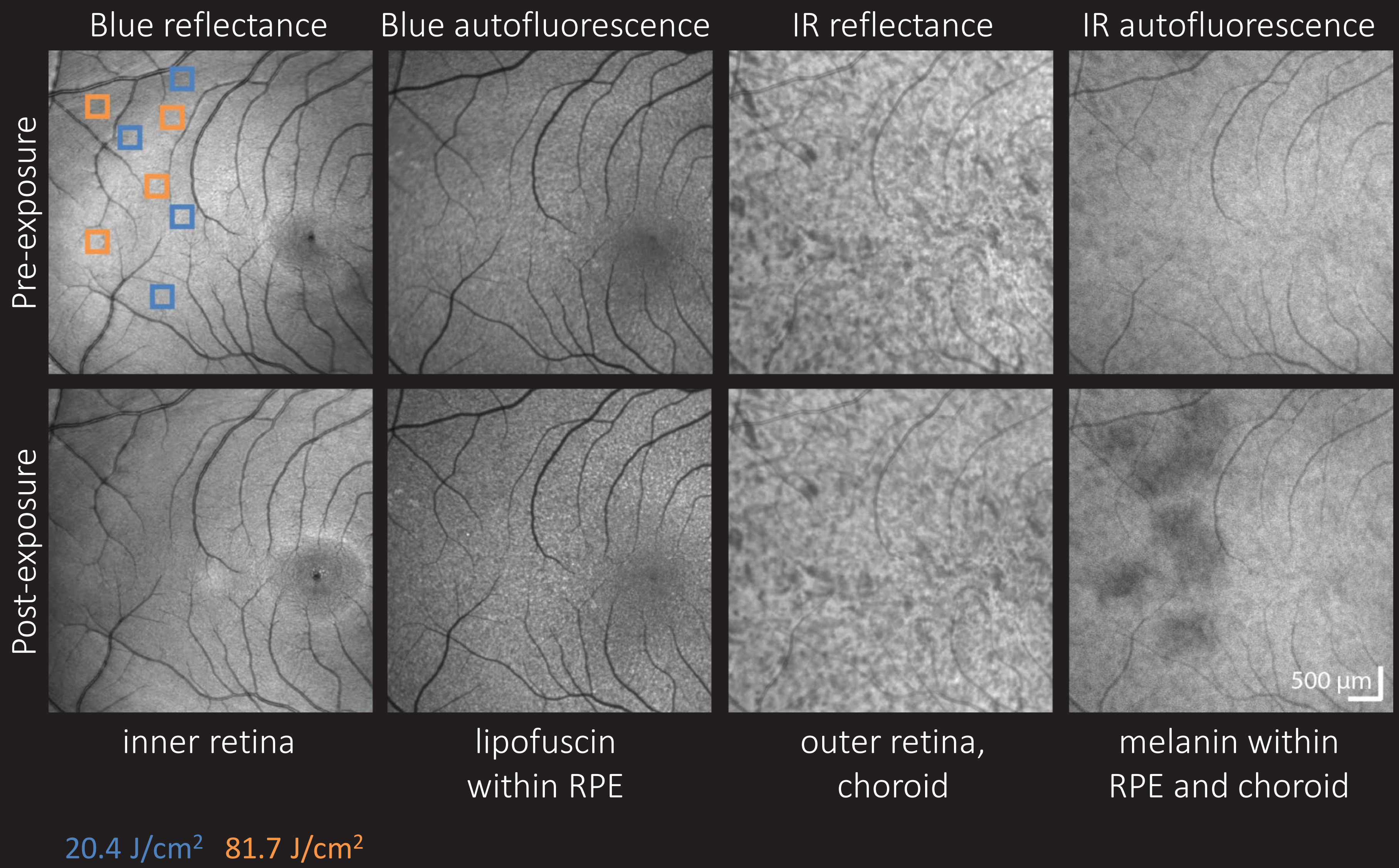
Schwarz et al, BOEx 2016

Photoreceptors and RPE appeared normal



Schwarz et al, BOEx 2016

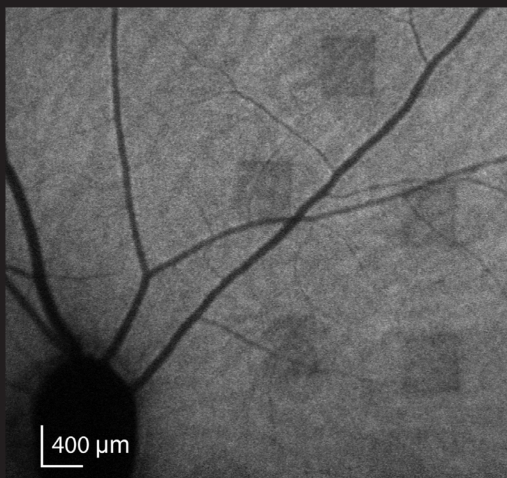
Only IR autofluorescence is affected



Schwarz et al, BOEx 2016

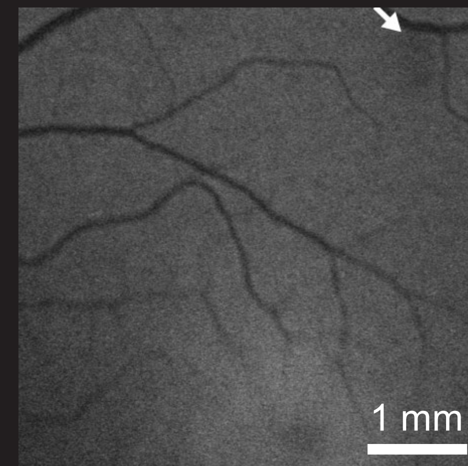
IRAF reduction with CW exposures (790 nm)

Macaque



$110\ J/cm^2$

Human

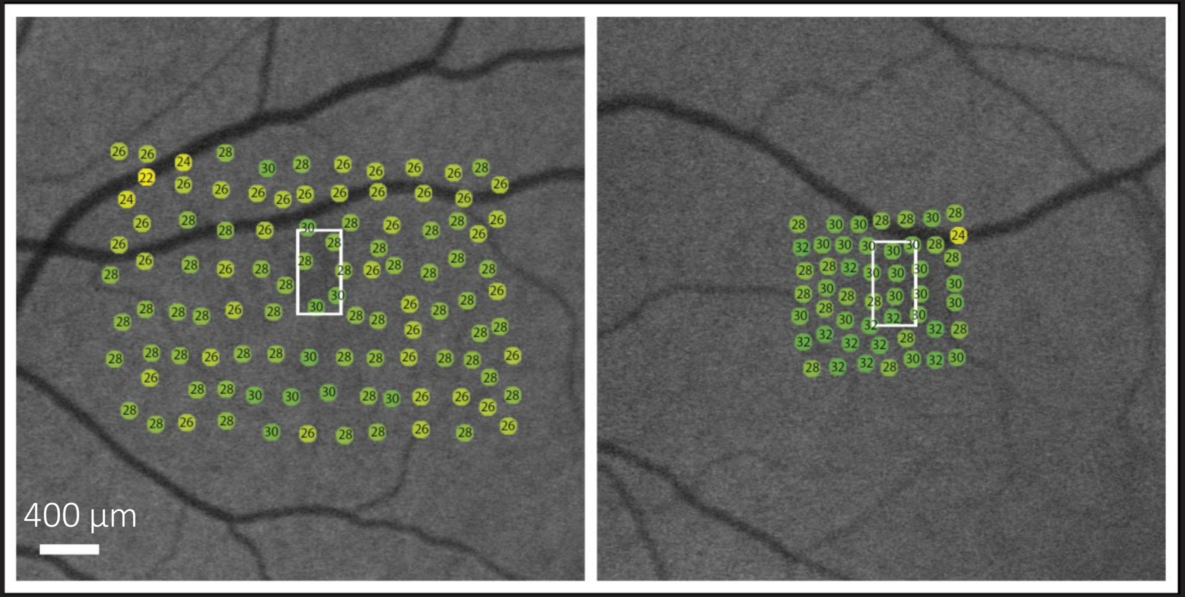


~$190\ J/cm^2$

- Occurs for exposures below ANSI MPE
- Photochemical effect

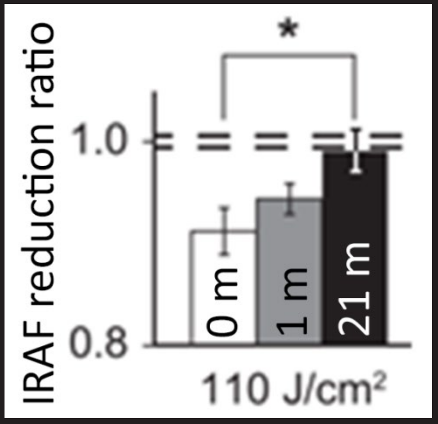
Masella et al, IOVS 2014

IRAF reduction had no measurable functional consequences



Direct ophthalmoscopy, Goldmann visual fields, multifocal ERG, photopic microperimetry (MAIA) within normal range

IRAF showed slow but full recovery



Masella et al, IOVS 2014

Summary

- Two-photon imaging of exogenous fluorophores (e.g. GCaMP) allows to study cell function in response to visual stimulation as realistically as possible in the living eye .
- Two-photon ophthalmoscopy of endogenous fluorophors can assess photoreceptor function. The technique is sensitive to differences in cell physiology and to interventions expected to alter visual cycle kinetics.
- Functional two-photon ophthalmoscopy is possible at safe light levels. Still, the cause and consequence of IRAF reduction requires further research.

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