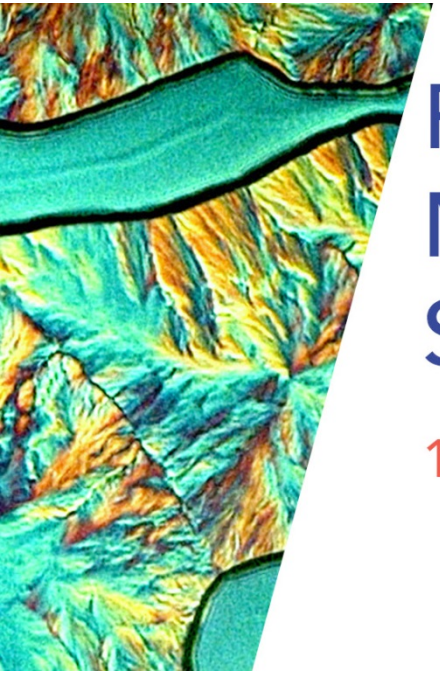
Functional Imaging: Eliciting, Measuring and Interpreting Intrinsic Signals in the Retina

Presented by:

OSA Clinical Vision Sciences Technical Group

The OSA Clinical Vision Sciences Technical Group Welcomes You!



Functional Imaging: Eliciting, Measuring and Interpreting Intrinsic Signals in the Retina

12 December 2018 • 12:30 EST

OSA Clinical Vision Sciences Technical Group

OSA Clinical Vision Sciences Technical Group

Technical Group Leadership 2018-2019

Chair
Krystel Huxlin, Ph.D.
University of Rochester, USA

Vice-chair
Jessica I. W. Morgan, Ph.D.
University of Pennsylvania, USA

OSA Clinical Vision Sciences Technical Group

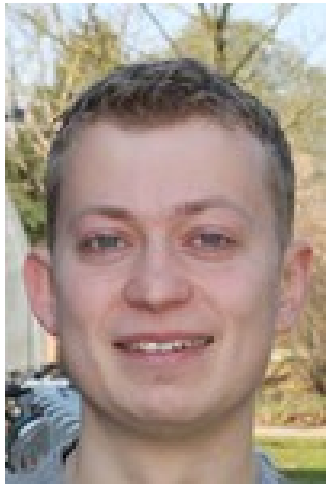
Technical Group at a Glance

- Focus
  - Investigate visual function in disease, development, and aging
  - Study mechanisms, new assessment techniques, efficacy of treatment, and prevention of visual function deficits
- Mission
  - To benefit *YOU*
  - Webinars, publications, technical events, networking events
  - Interested in presenting your research? Have ideas for TG events? Contact us!
- Find us here
  - Krystel Huxlin: khuxlin@ur.rochester.edu
  - Jessica I. W. Morgan: jwmorgan@pennmedicine.upenn.edu
  - Website: www.osa.org/vs

Today's Webinar

Functional Imaging: Eliciting, Measuring and Interpreting Intrinsic Signals in the Retina

Robert F. Cooper, Ph.D.
University of Pennsylvania



Dierck Hillmann, Ph.D.
Thorlabs and University of Lübeck

Mehdi Azimipour, Ph.D.
University of California, Davis

OSA Clinical Vision Sciences Technical Group

Examining the health of individual photoreceptors in the living eye

Robert F Cooper, PhD

University of Pennsylvania

Acknowledgements


Jessica Morgan


David Brainard


Will Tuten


Min Chen


Grace Han

National Eye Institute (NEI)
Research Today...Vision Tomorrow

Research to Prevent Blindness
The Catalyst for Eye Research

Paul and Evanina Mackall Foundation Trust

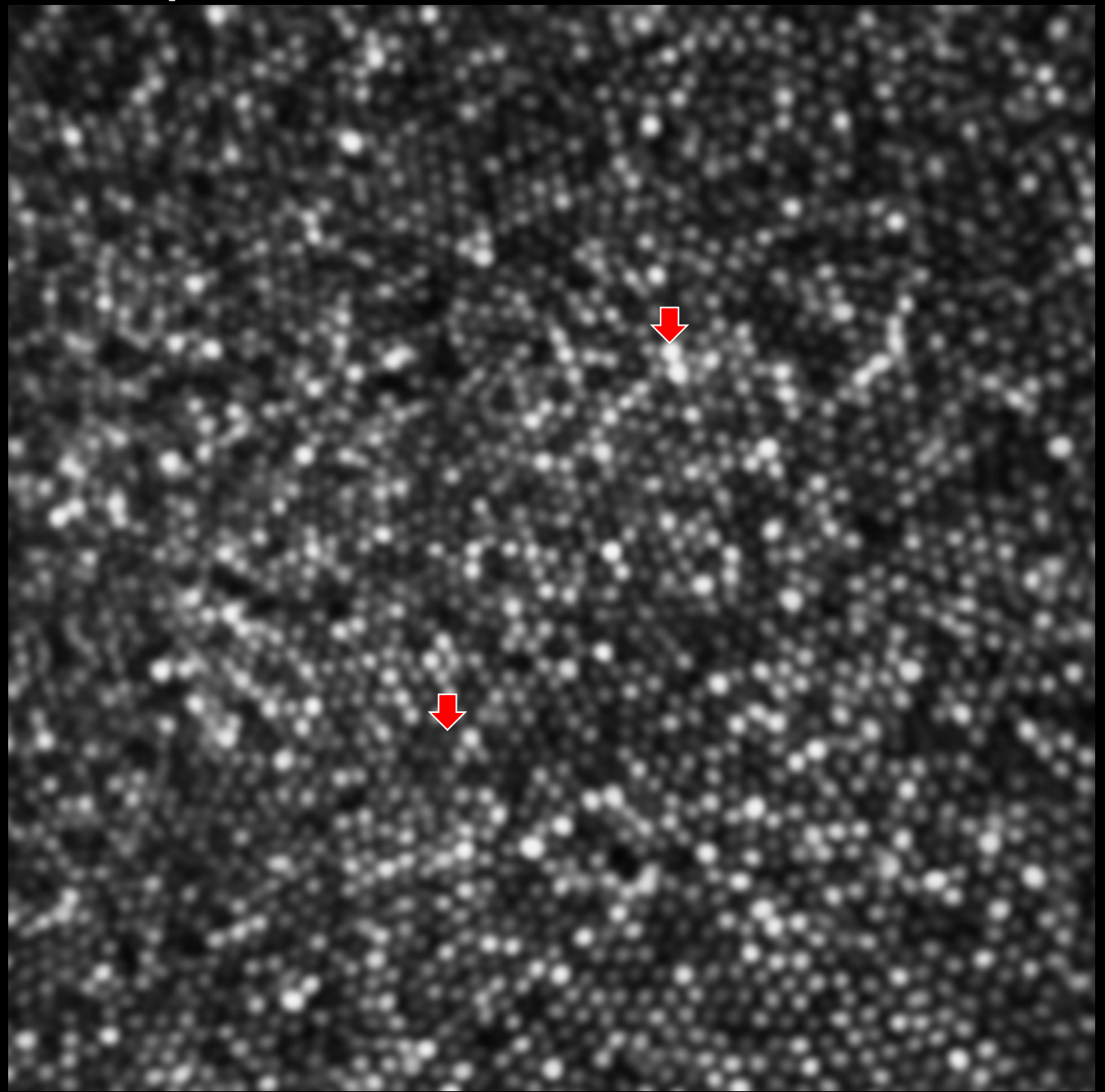
FOUNDATION FIGHTING BLINDNESS

F. M. Kirby Foundation

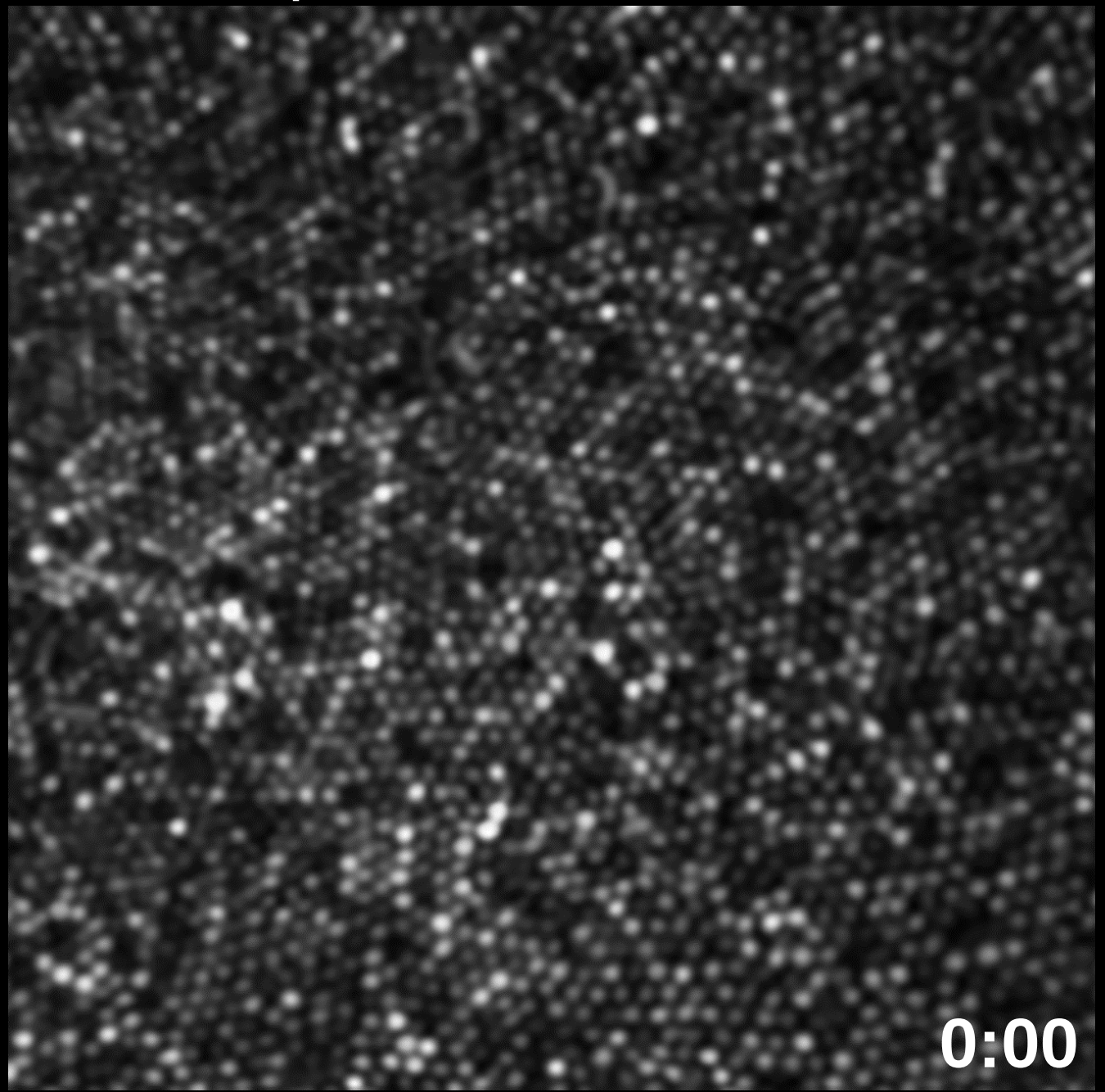
BRAINARD LAB

Disclosure: I am a co-inventor on a provisional patent for work related to this talk.

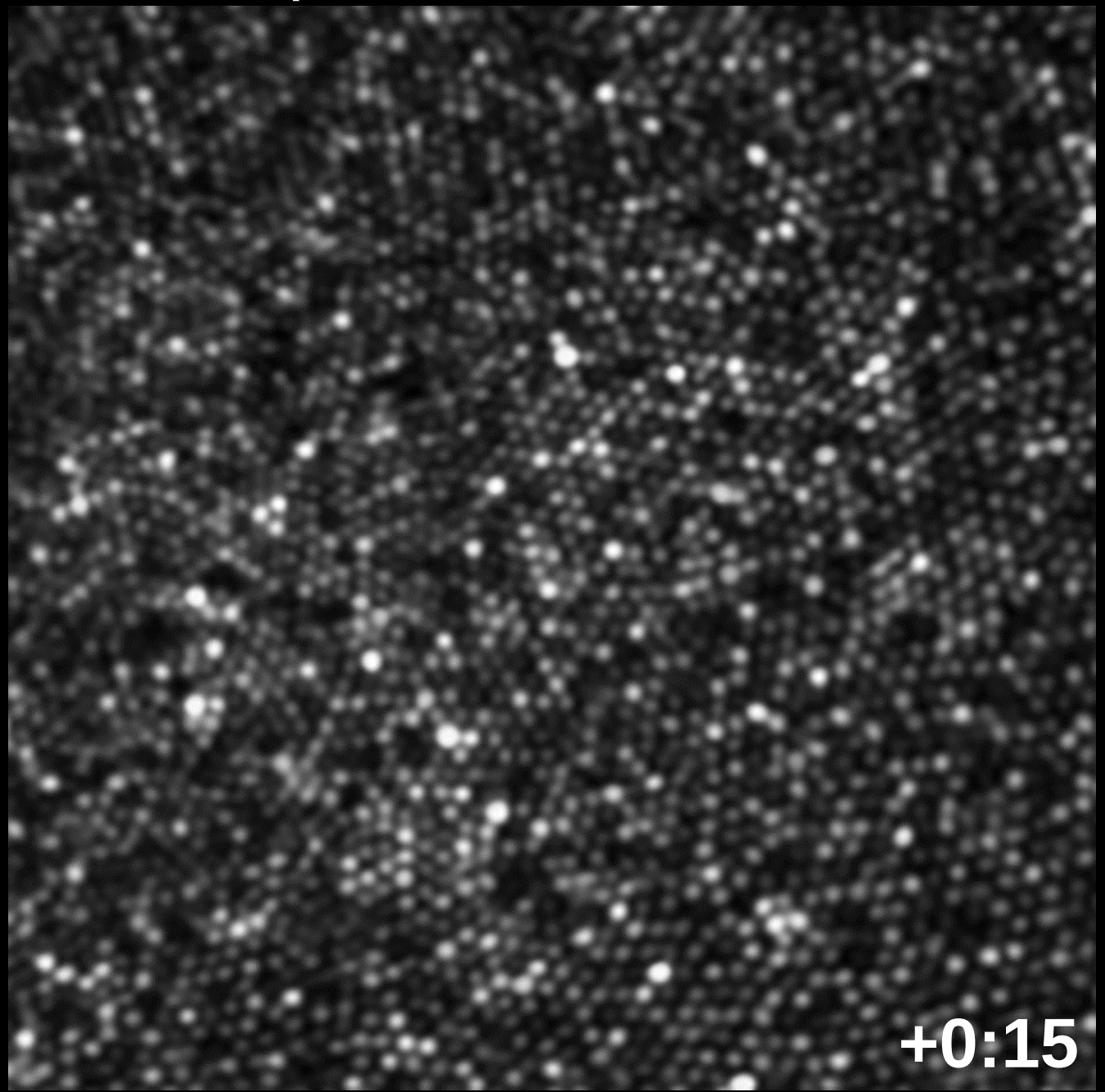
NIR photoreceptor reflectance varies **across the retina**



NIR photoreceptor reflectance varies **over time**



NIR photoreceptor reflectance varies **over time**



The road to a biomarker of cone function

1. Quantifiable / Repeatable
2. Dose sensitive
3. Functionally significant
4. Clinically relevant
5. Optimized

The road to a biomarker of cone function

1. Quantifiable / Repeatable
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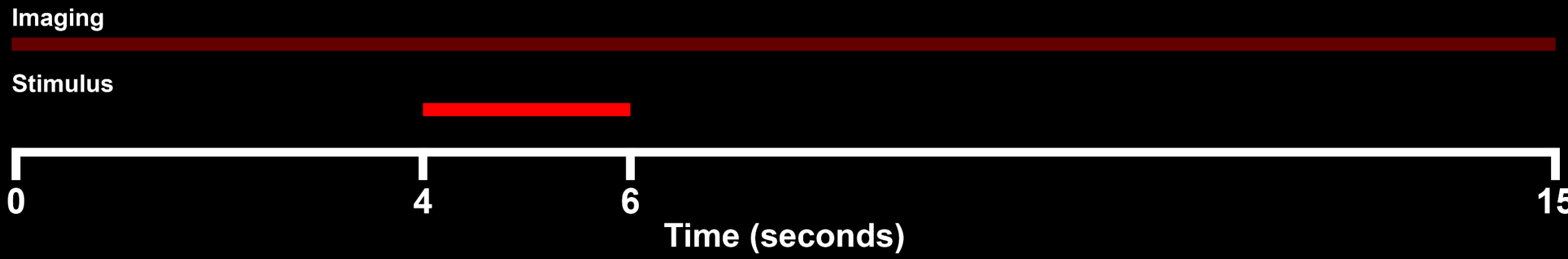
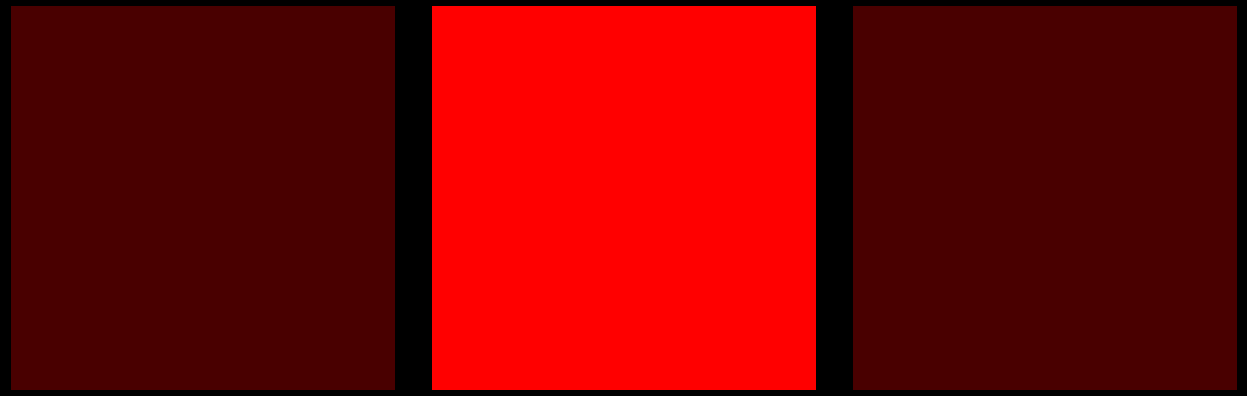
Designing an intrinsic experiment



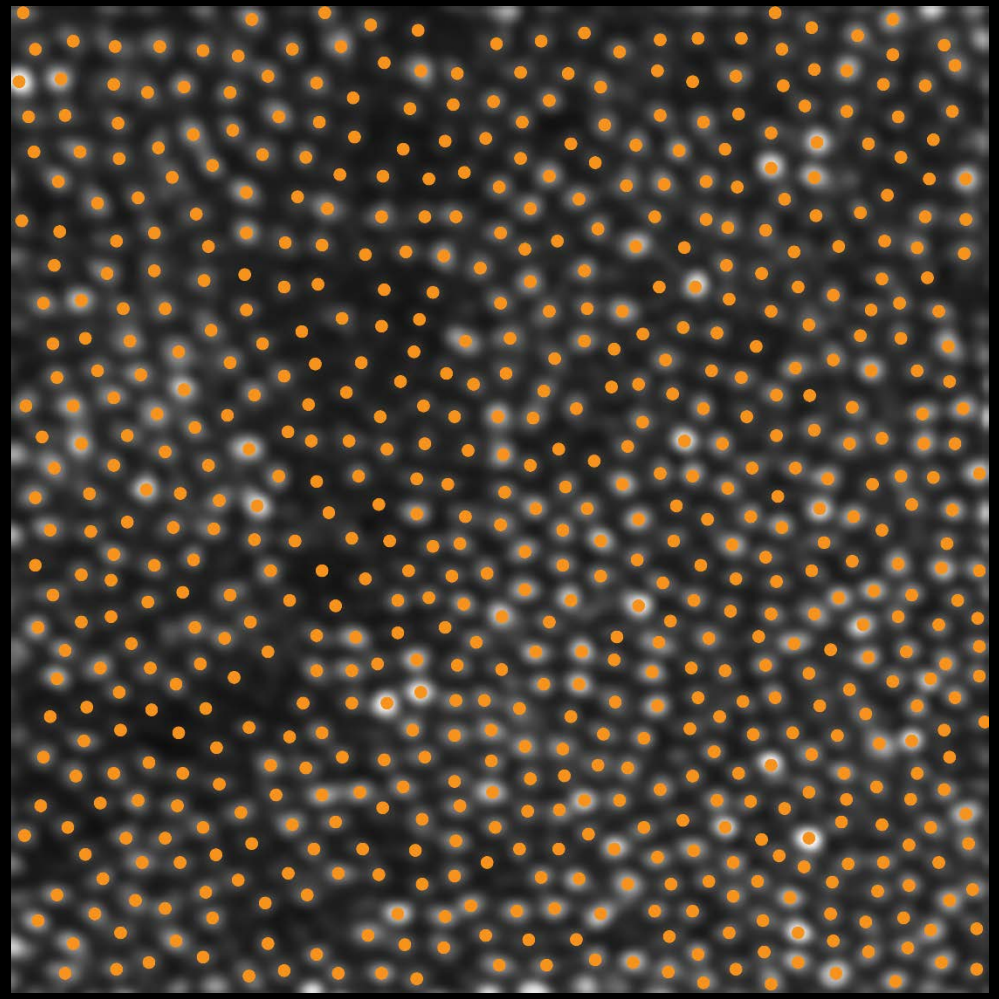
Subject View:



1. While imaging the cones with near infrared light, apply a visible light stimulus to the retina at the same location as the imaging field.
2. While imaging the cones with near infrared light, do nothing.

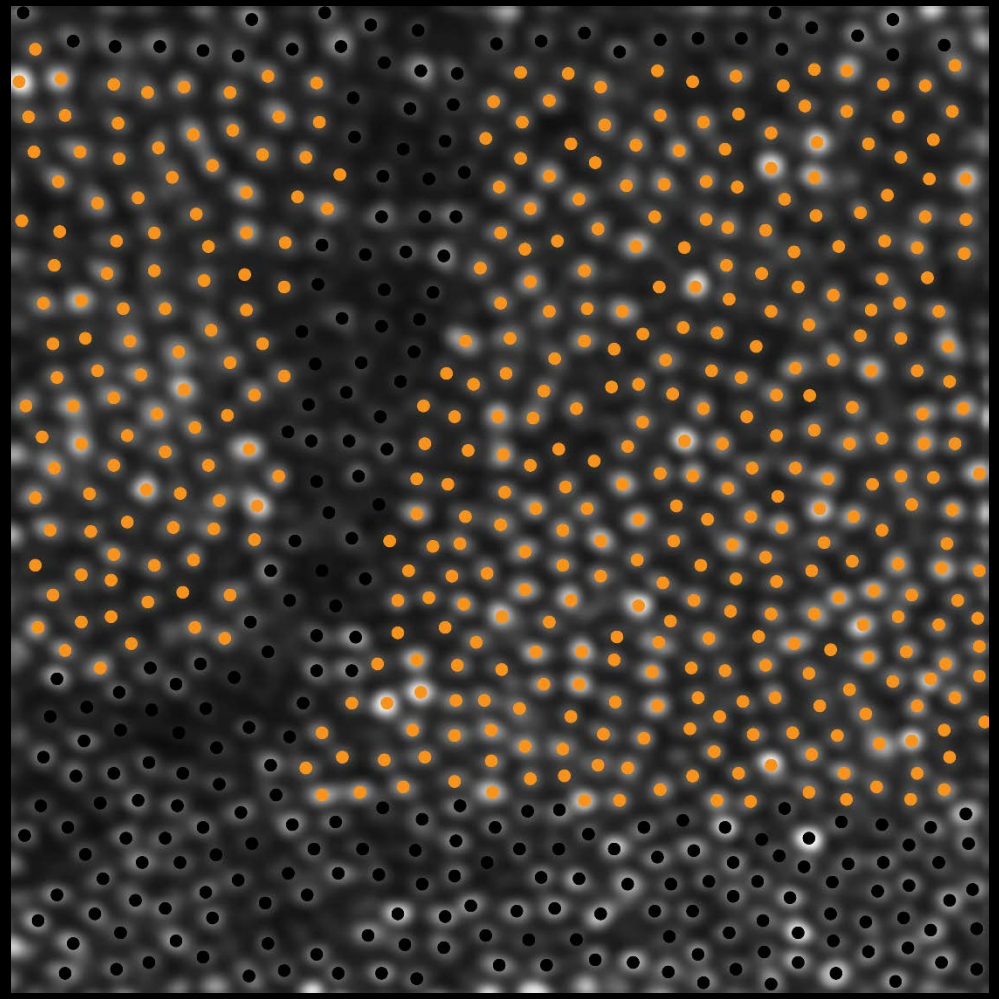
Extracting the cone reflectance

- Co-register all frames from each image sequence (Dubra & Harvey 2010, Thévenaz et. al. 1998)
- Crop to common area
- Identify cone locations



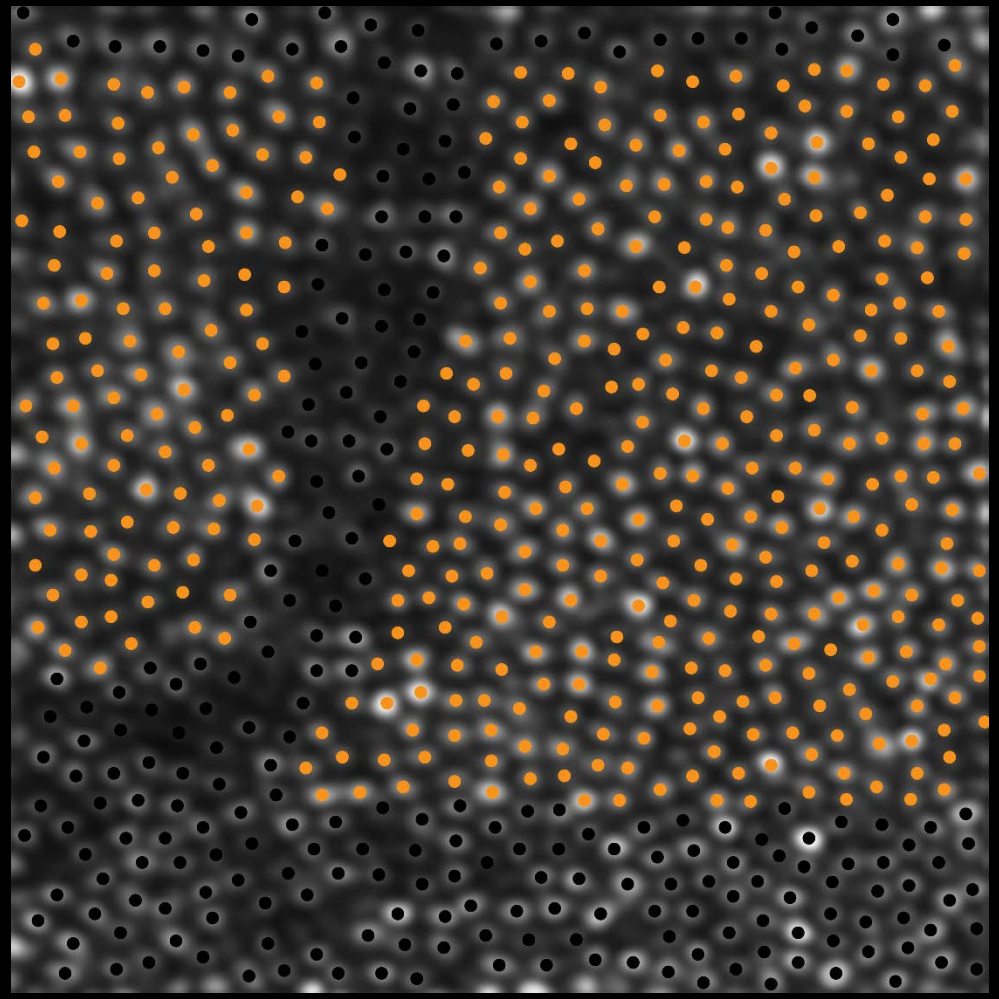
Extracting the cone reflectance

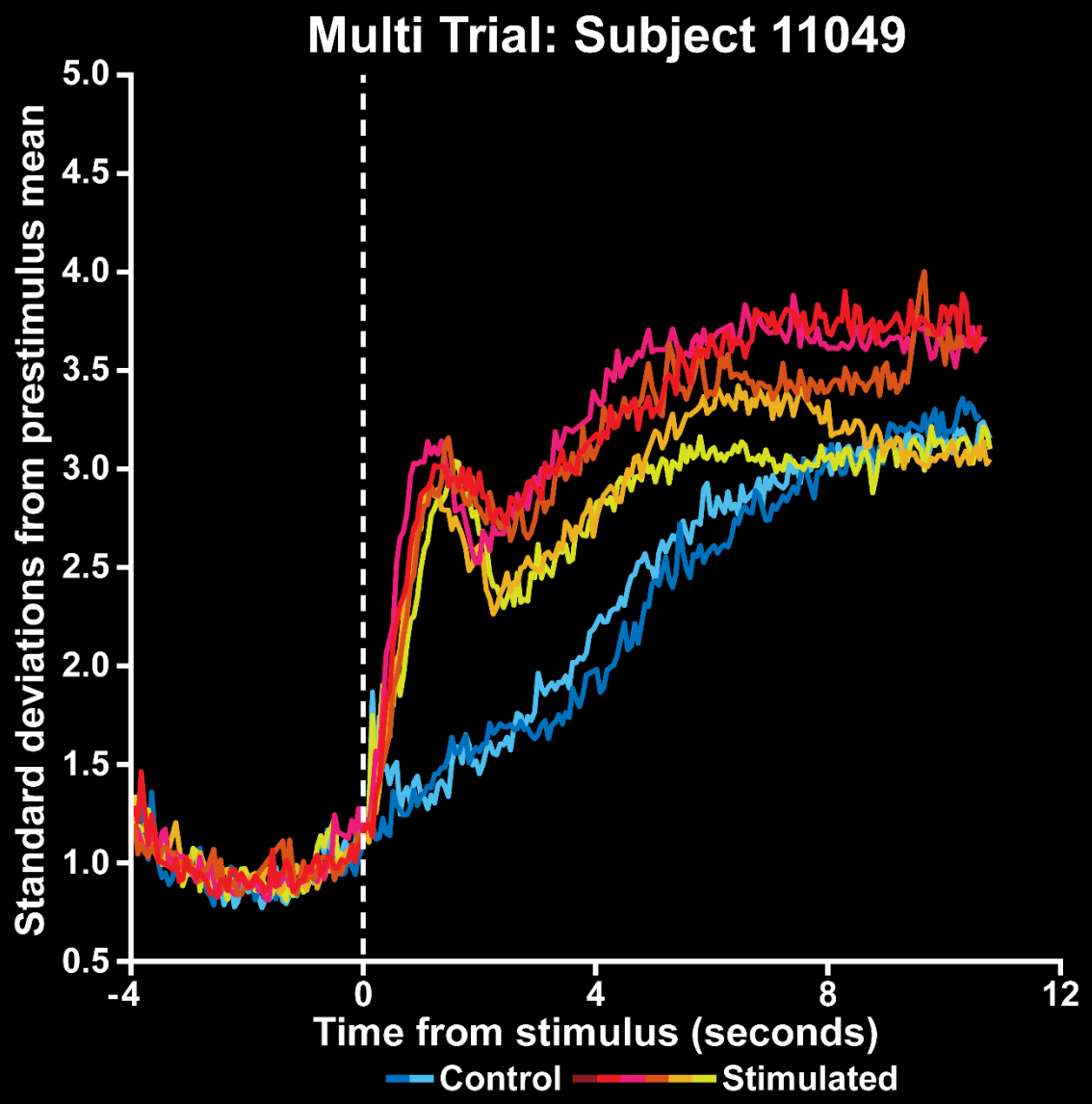
- Co-register all frames from each image sequence (Dubra & Harvey 2010, Thévenaz et. al. 1998)
- Crop to common area
- Identify cone locations
- Mask out cones under vasculature (Tam et al. 2010)

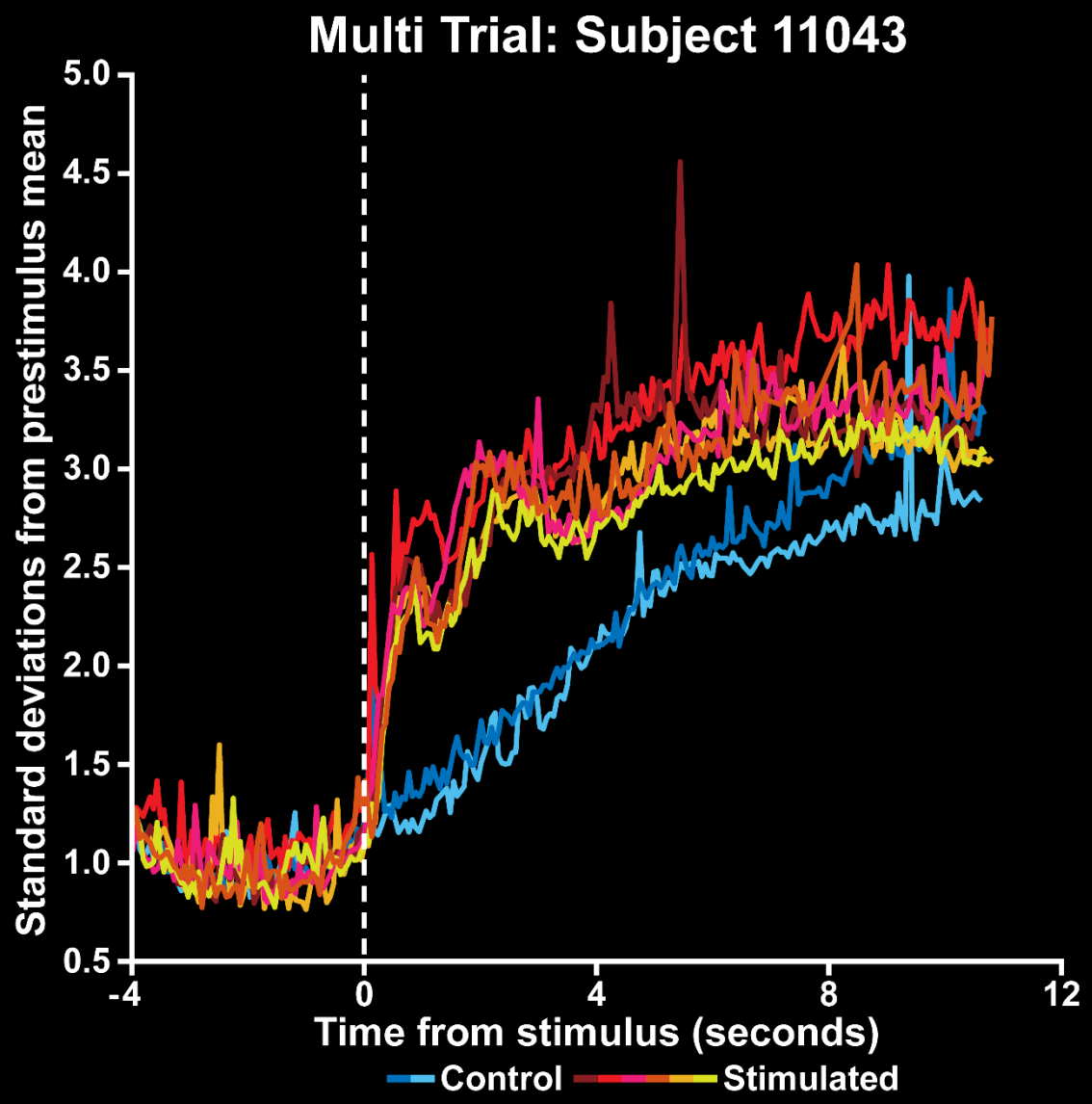


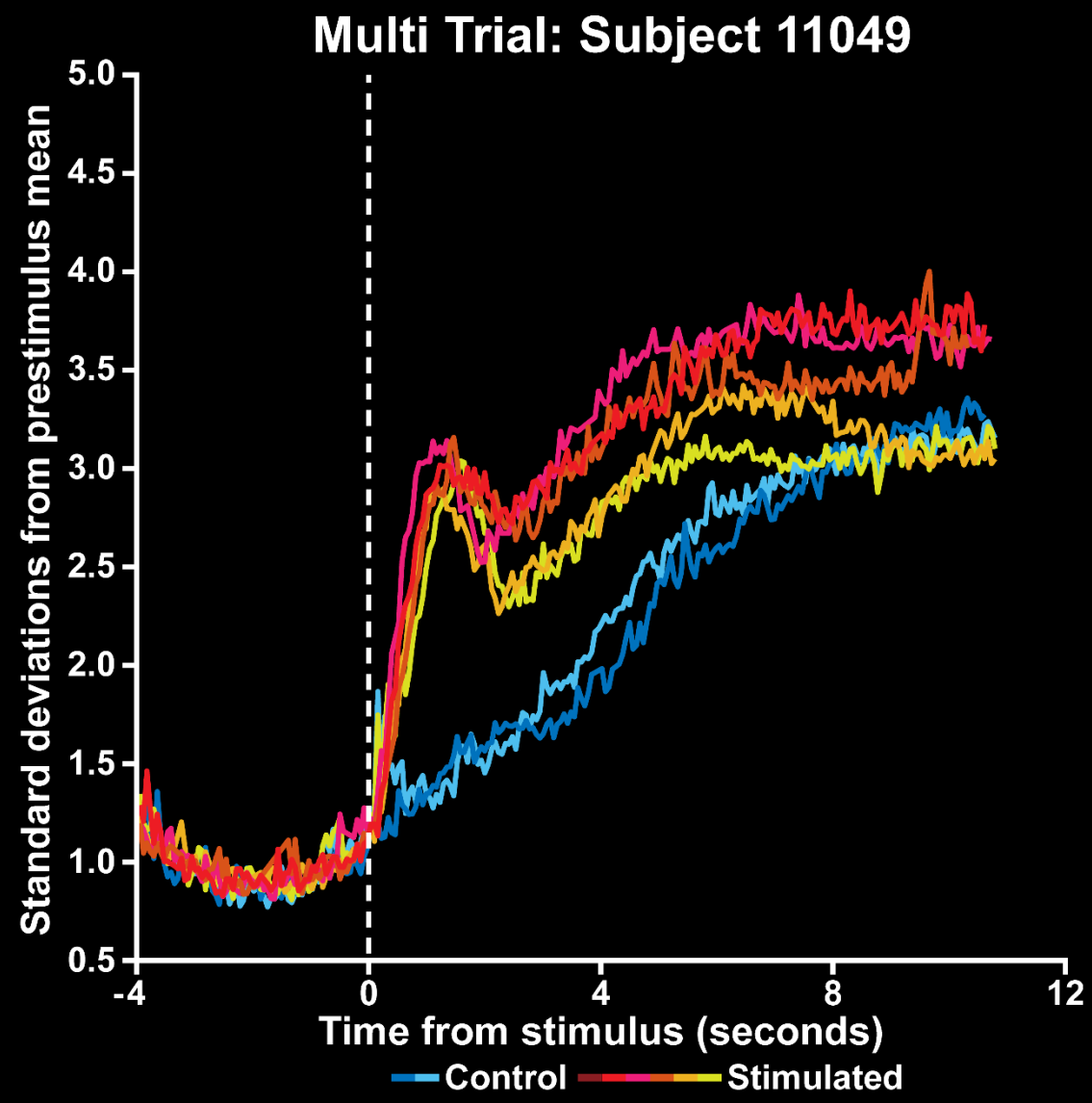
Extracting the cone reflectance

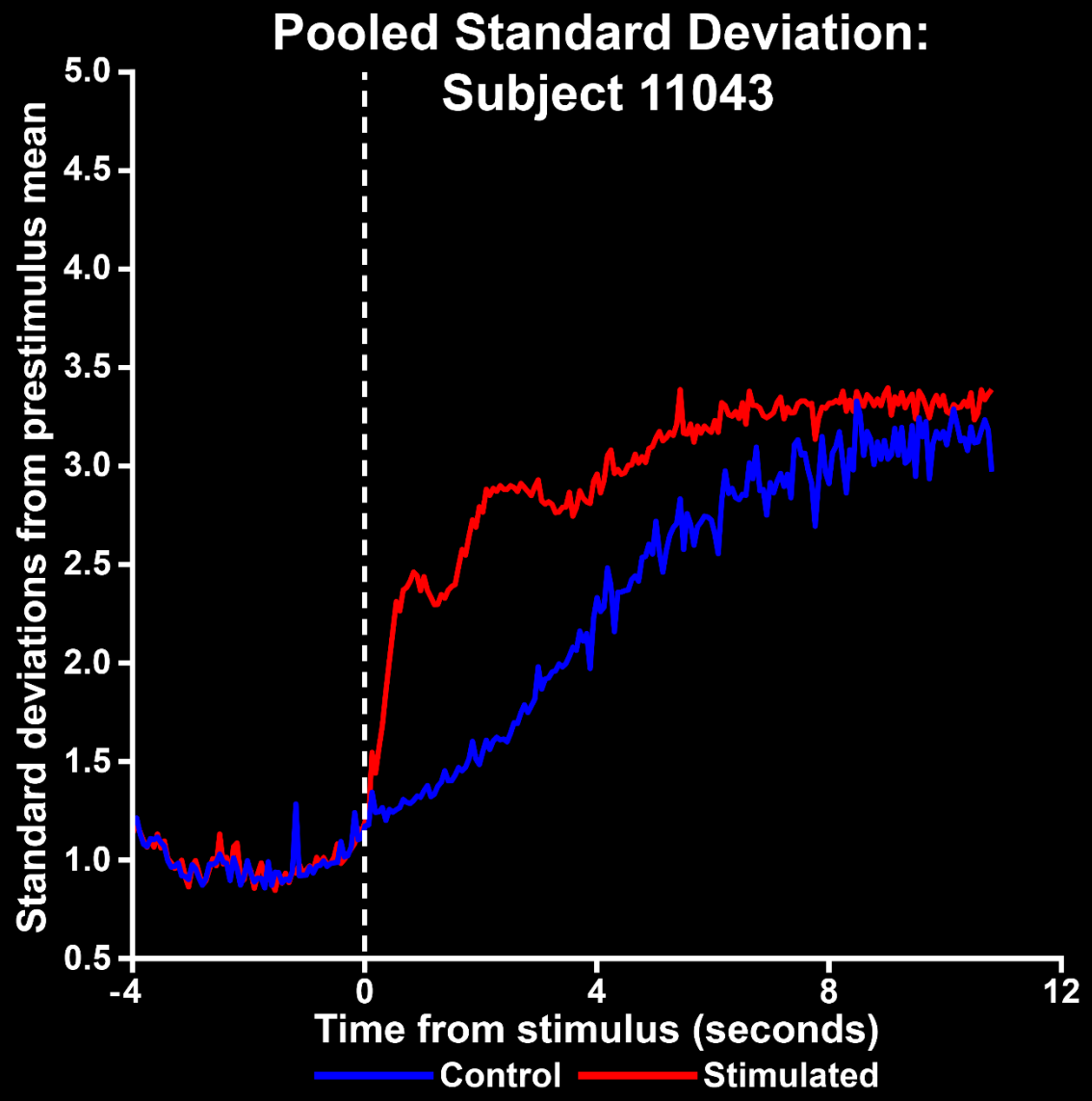
- Image mean affected by eye dryness, AO correction
  - ✓ **Normalized** cone reflectance to image mean
- Characterizing the *response* to the stimulus
  - ✓ **Standardized** each signal to pre-stimulus mean and standard deviation

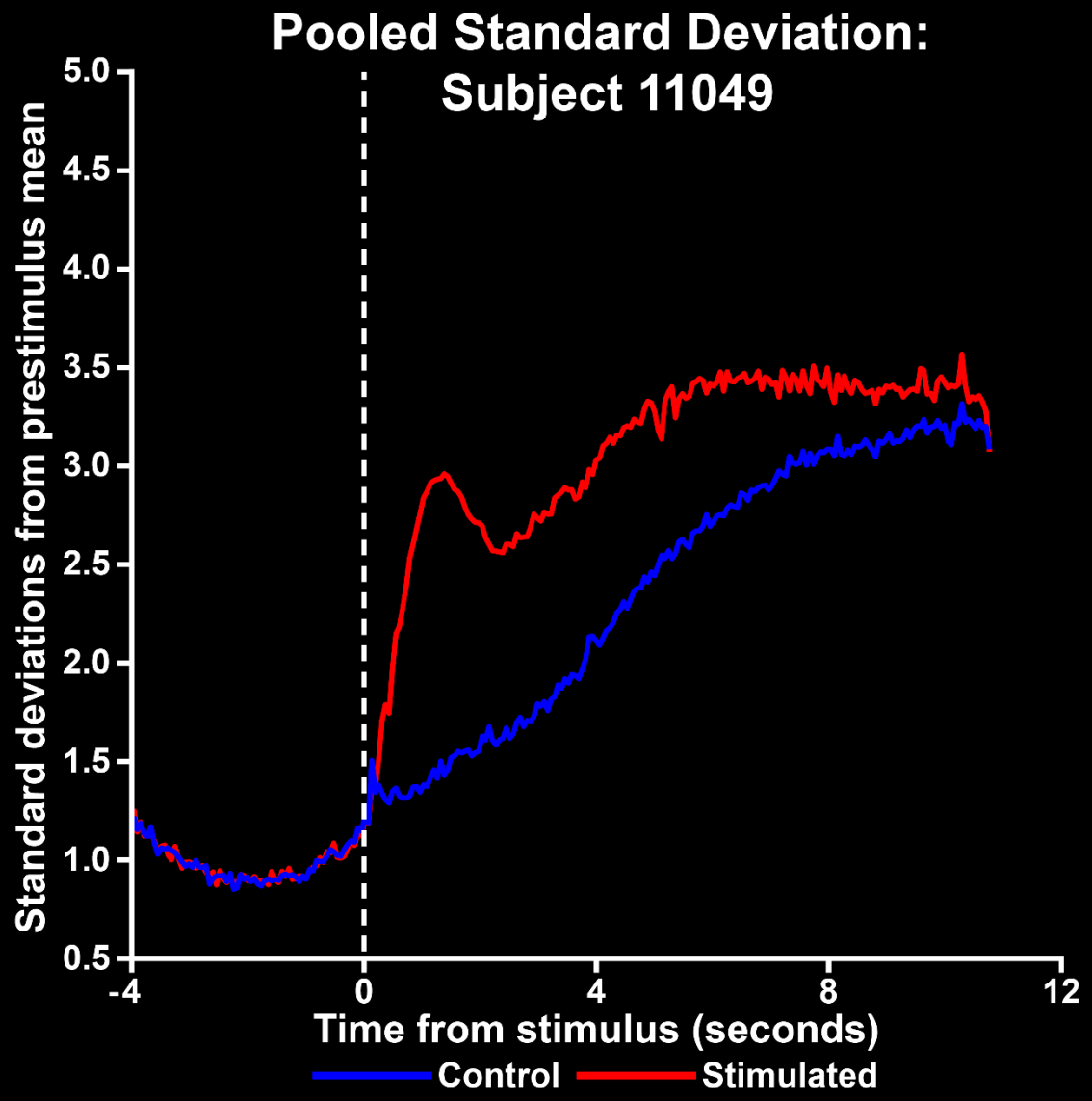



Multi Trial: Subject 11049
Standard deviations from prestimulus mean
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
-4
0
4
8
12
Time from stimulus (seconds)
Control
Stimulated


Multi Trial: Subject 11043
Standard deviations from prestimulus mean
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
-4
0
4
8
12
Time from stimulus (seconds)
Control
Stimulated


Multi Trial: Subject 11049
Standard deviations from prestimulus mean
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
-4
0
4
8
12
Time from stimulus (seconds)
Control
Stimulated


Pooled Standard Deviation:
Subject 11043
Standard deviations from prestimulus mean
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
-4
0
4
8
12
Time from stimulus (seconds)
Control
Stimulated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Standard deviations from prestimulus mean
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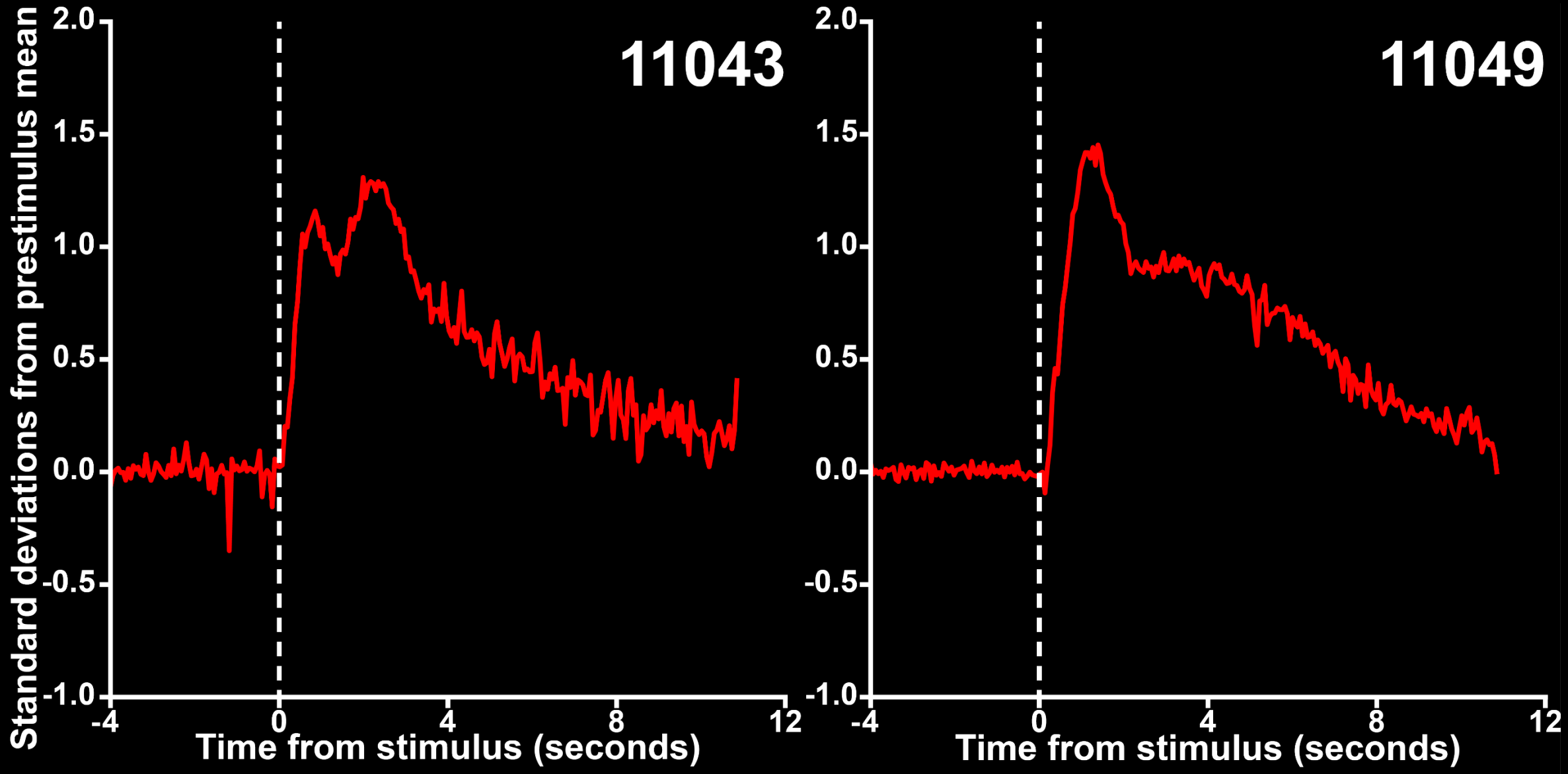
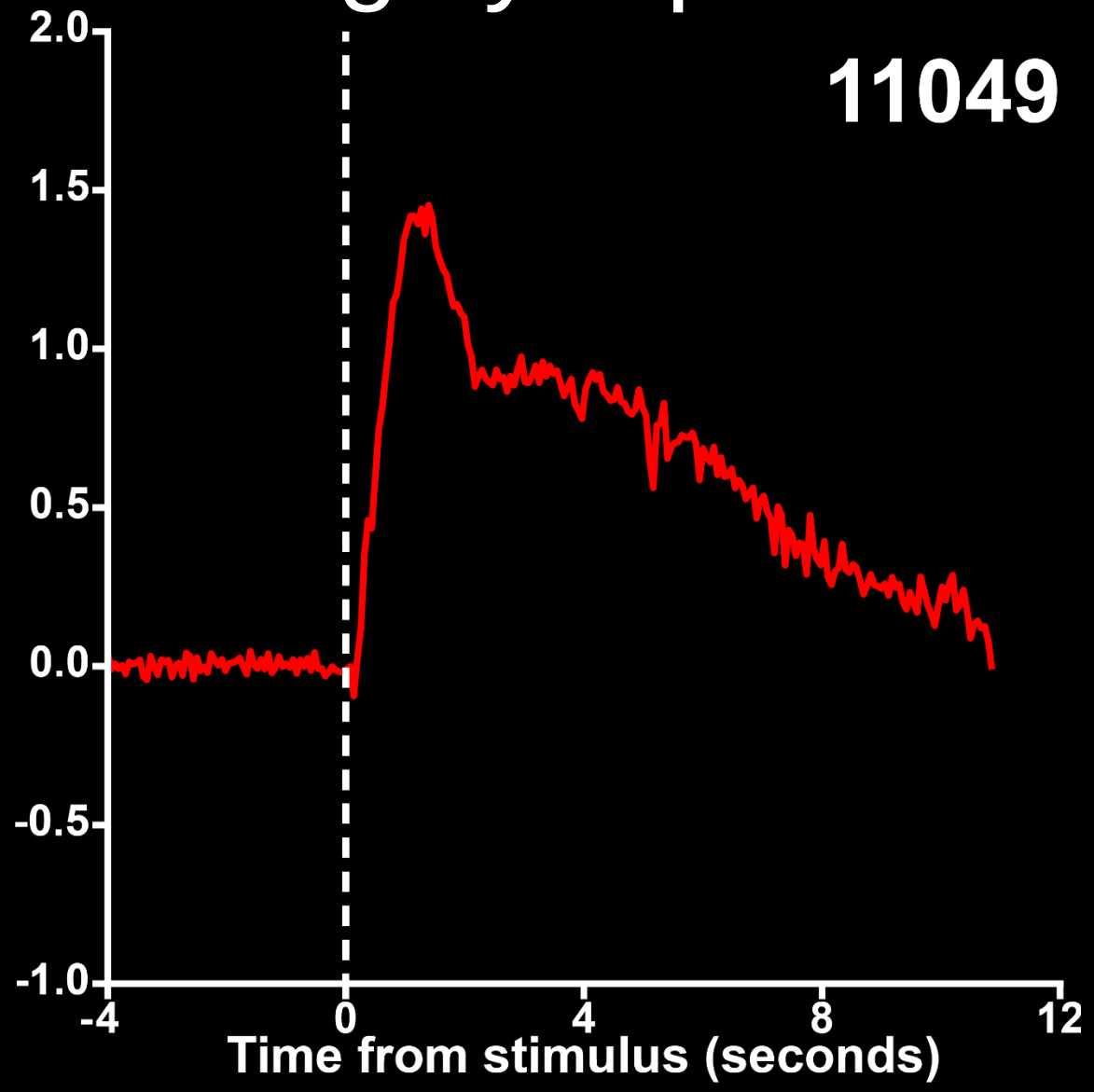
3.0
2.5
2.0
1.5
1.0
0.5
-4
0
4
8
12
Time from stimulus (seconds)
Control
Stimulated

The intrinsic reflectance response

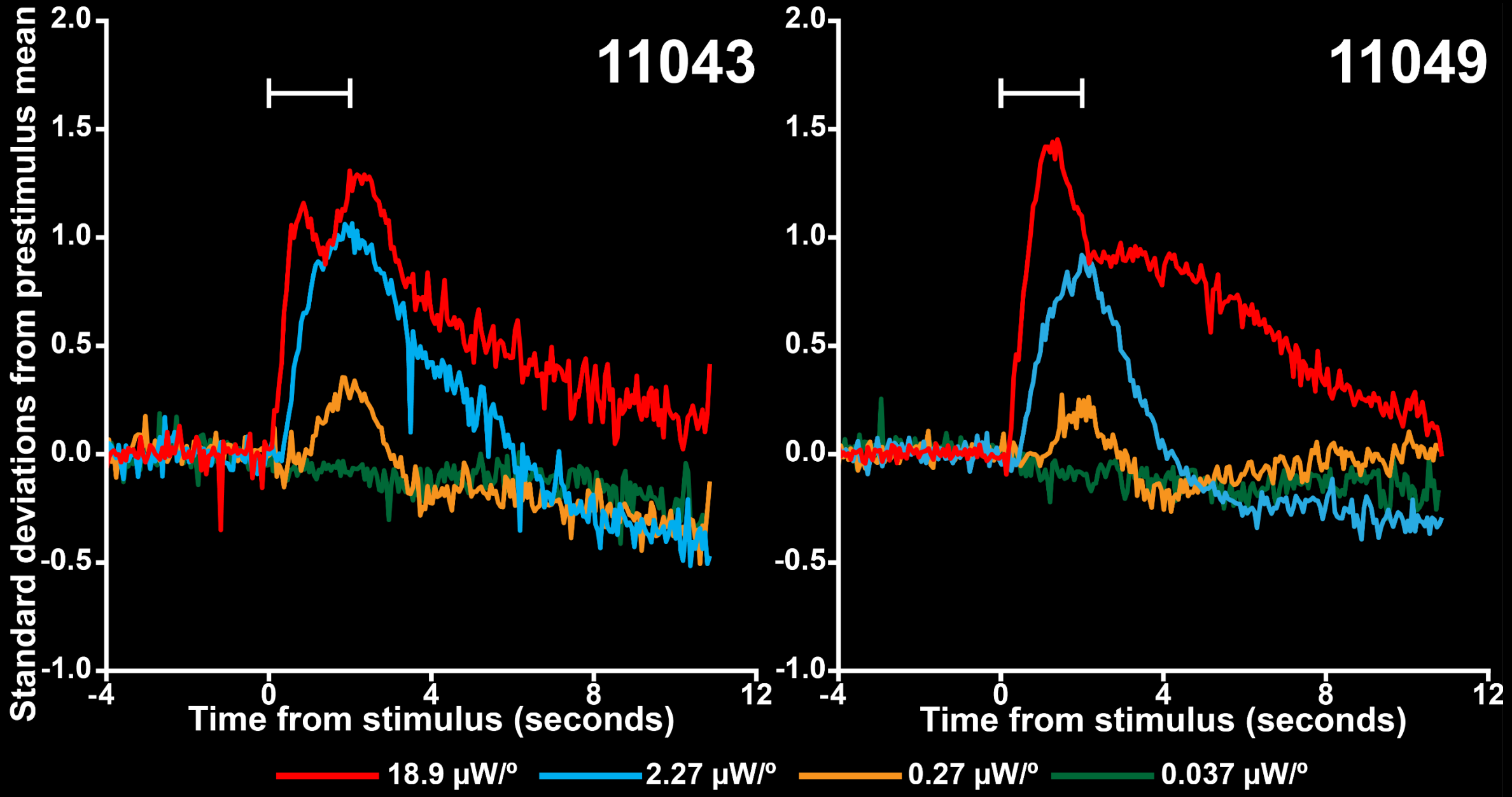


The reflectance response is highly repeatable

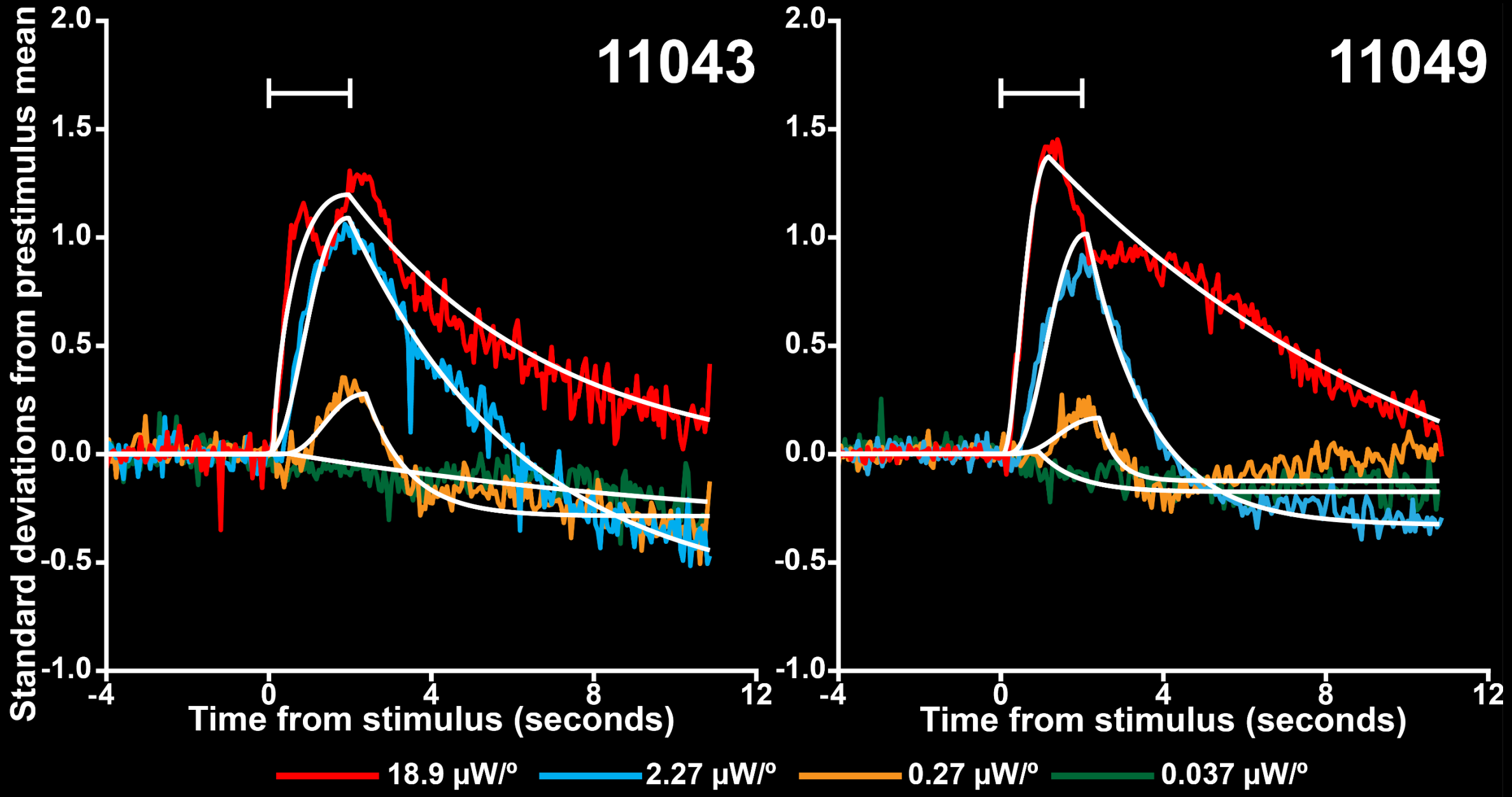
- ✓ Quantifiable / Repeatable
2. Dose sensitive
3. Functionally significant
4. Clinically relevant
5. Optimized



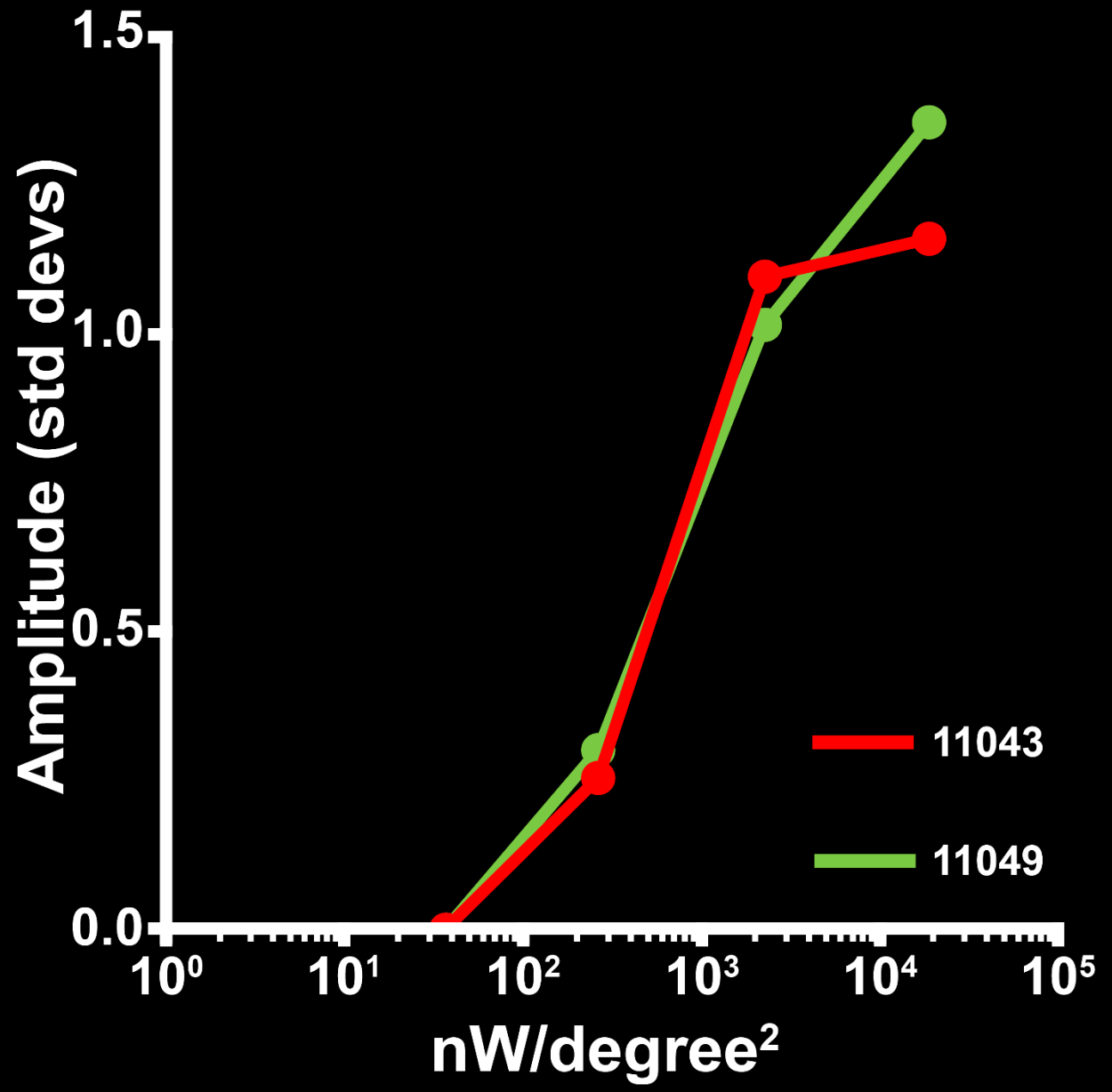
The reflectance response *increases* with brighter stimuli



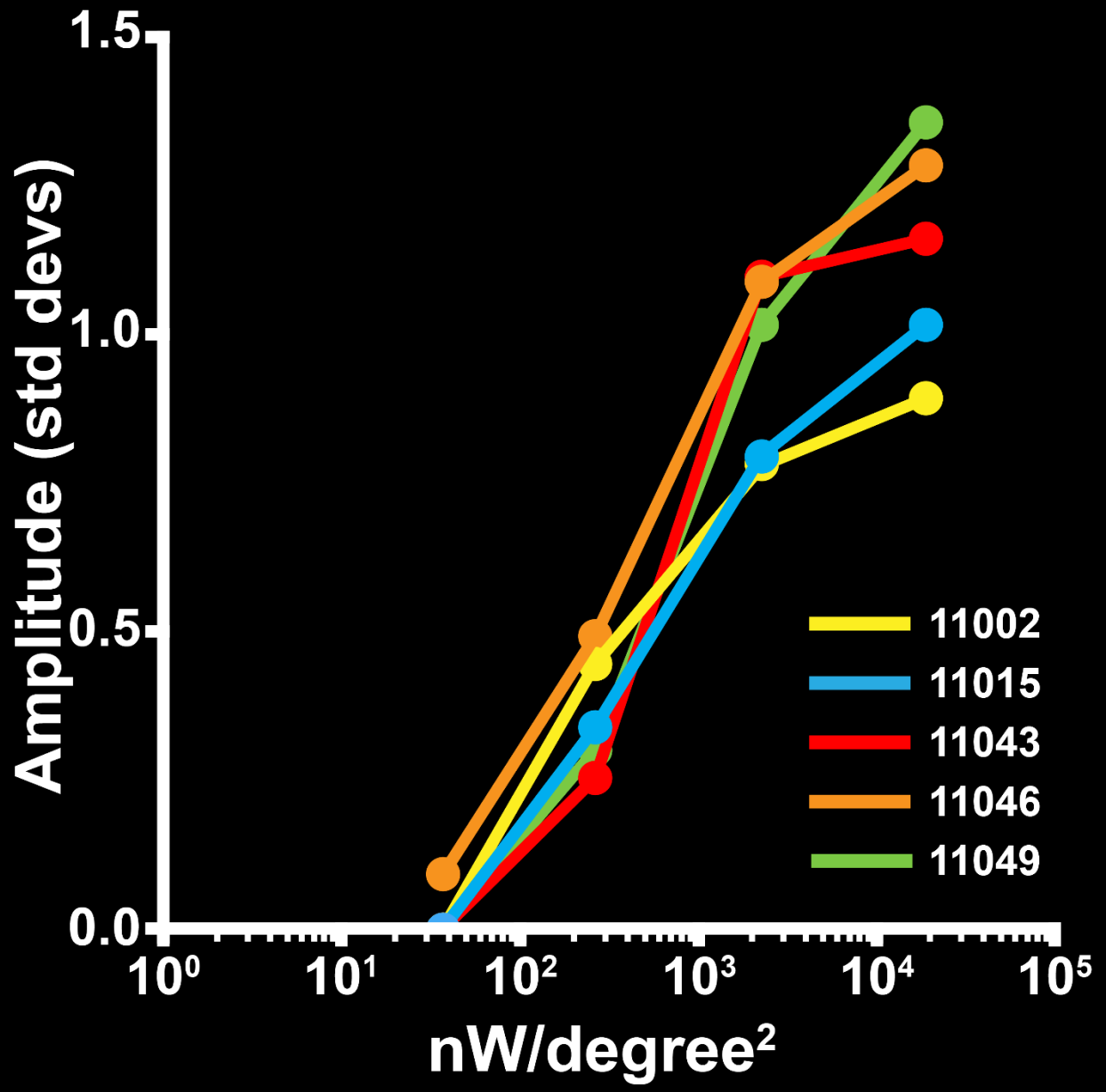
Quantifying the reflectance response



The reflectance response is dose sensitive



The reflectance response is dose sensitive



The reflectance response is dose sensitive

- ✓ Quantifiable / Repeatable
- ✓ Dose sensitive
3. Functionally significant
4. Clinically relevant
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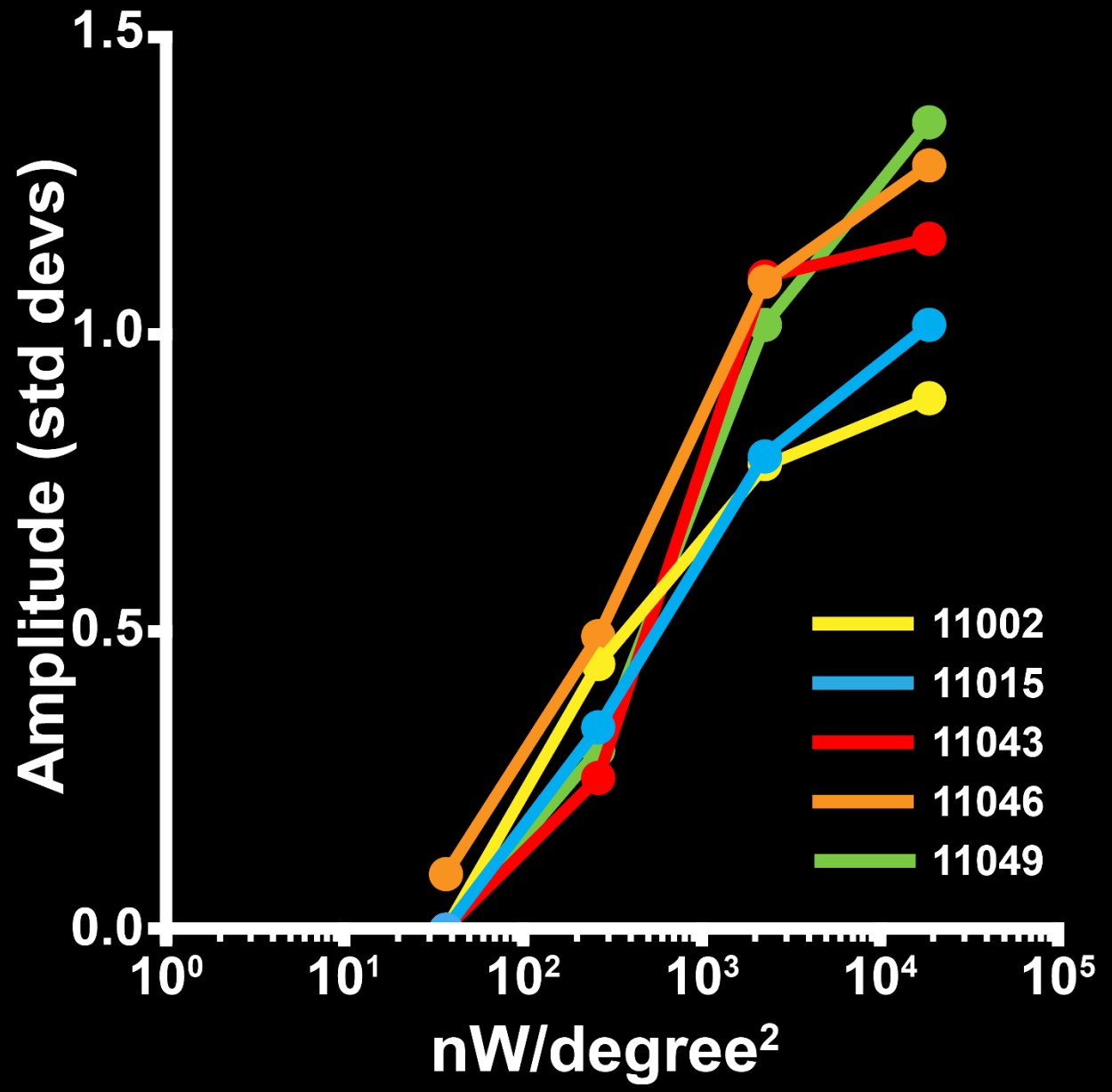

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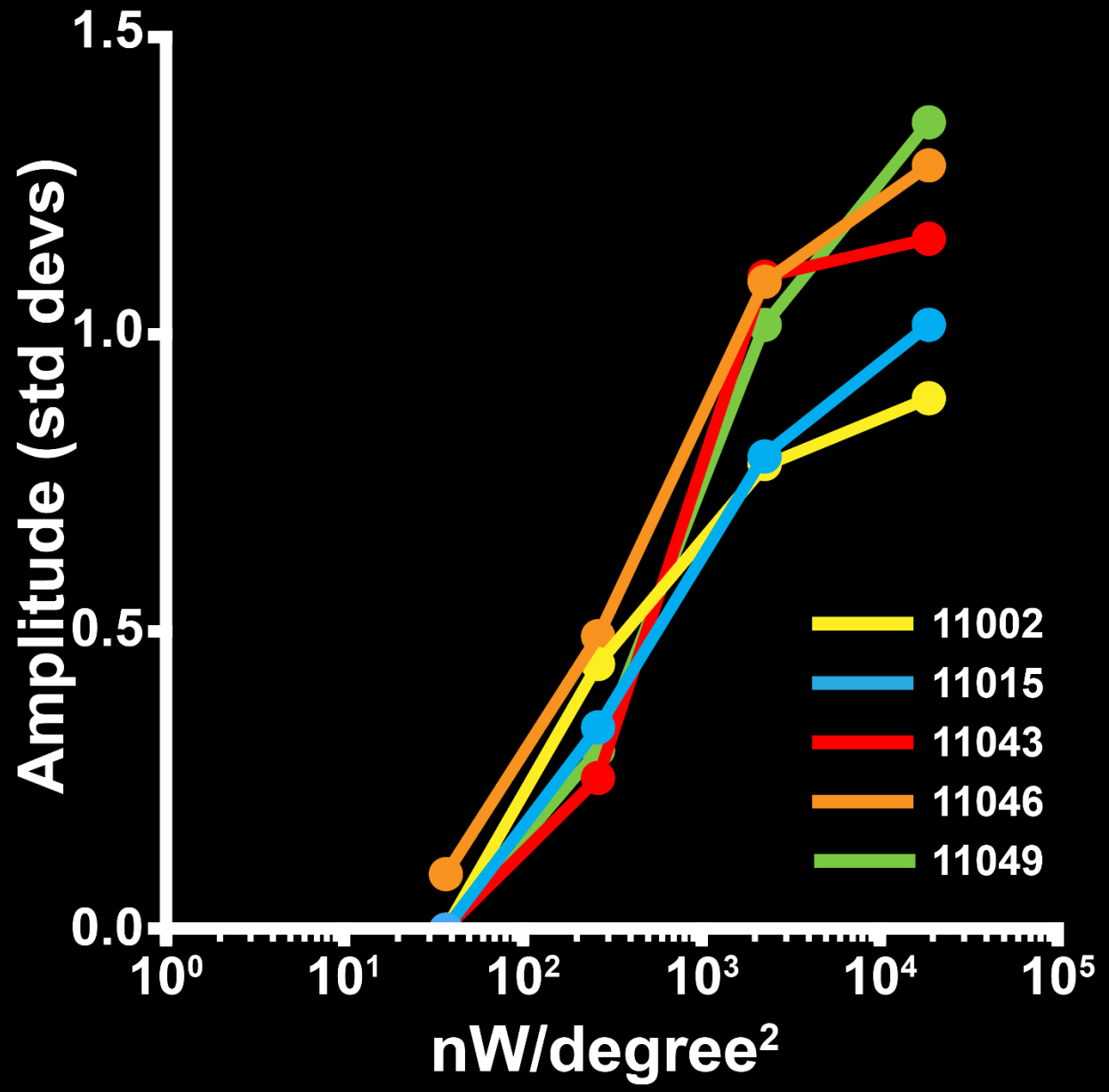
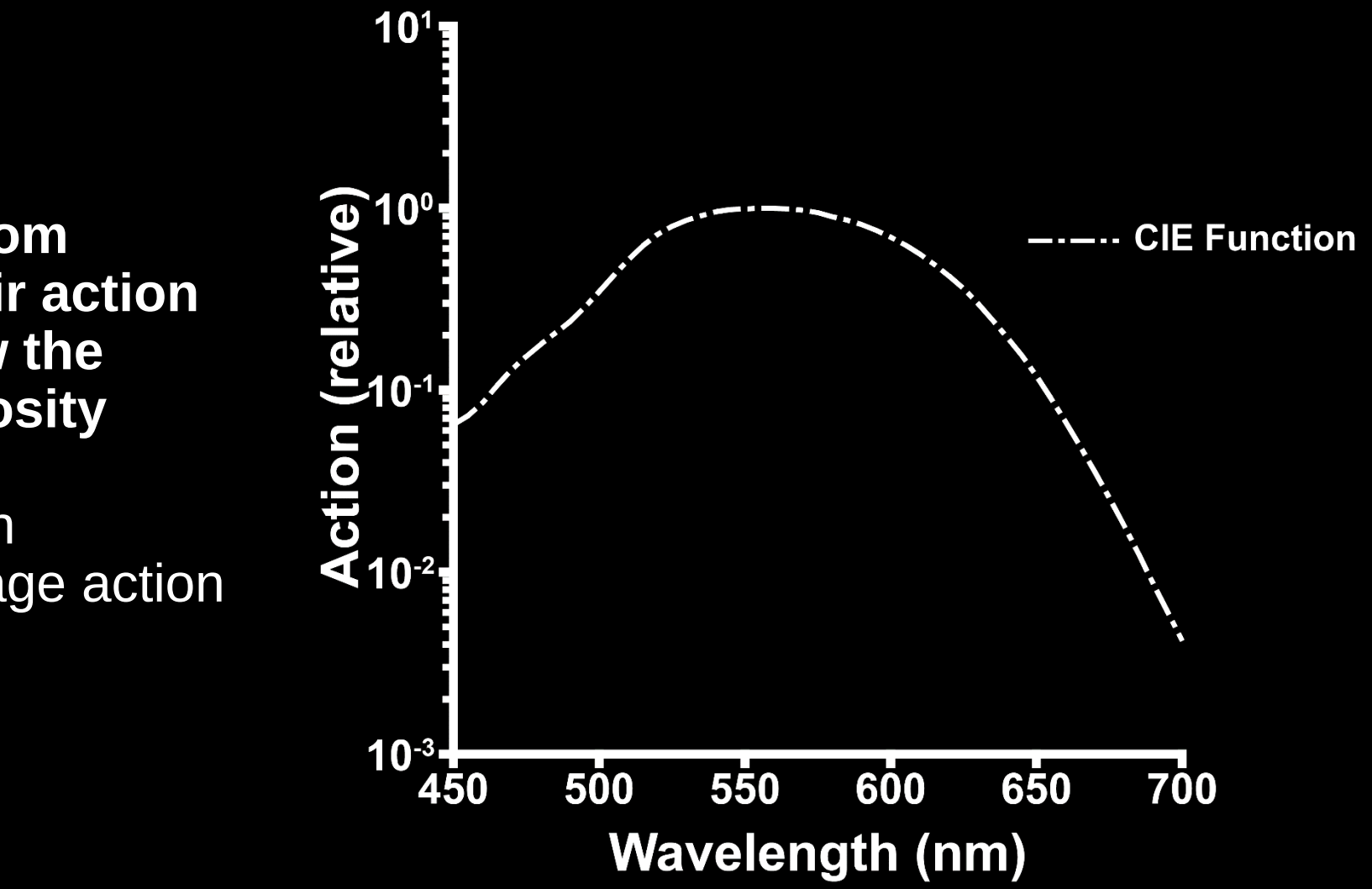


The action spectrum of the response should be consistent with phototransduction

- **If these signals arise from phototransduction, their action spectrum should follow the human photopic luminosity function.***

  *The luminosity function approximates the average action spectrum of the cones.



Sharpe et al., 2005

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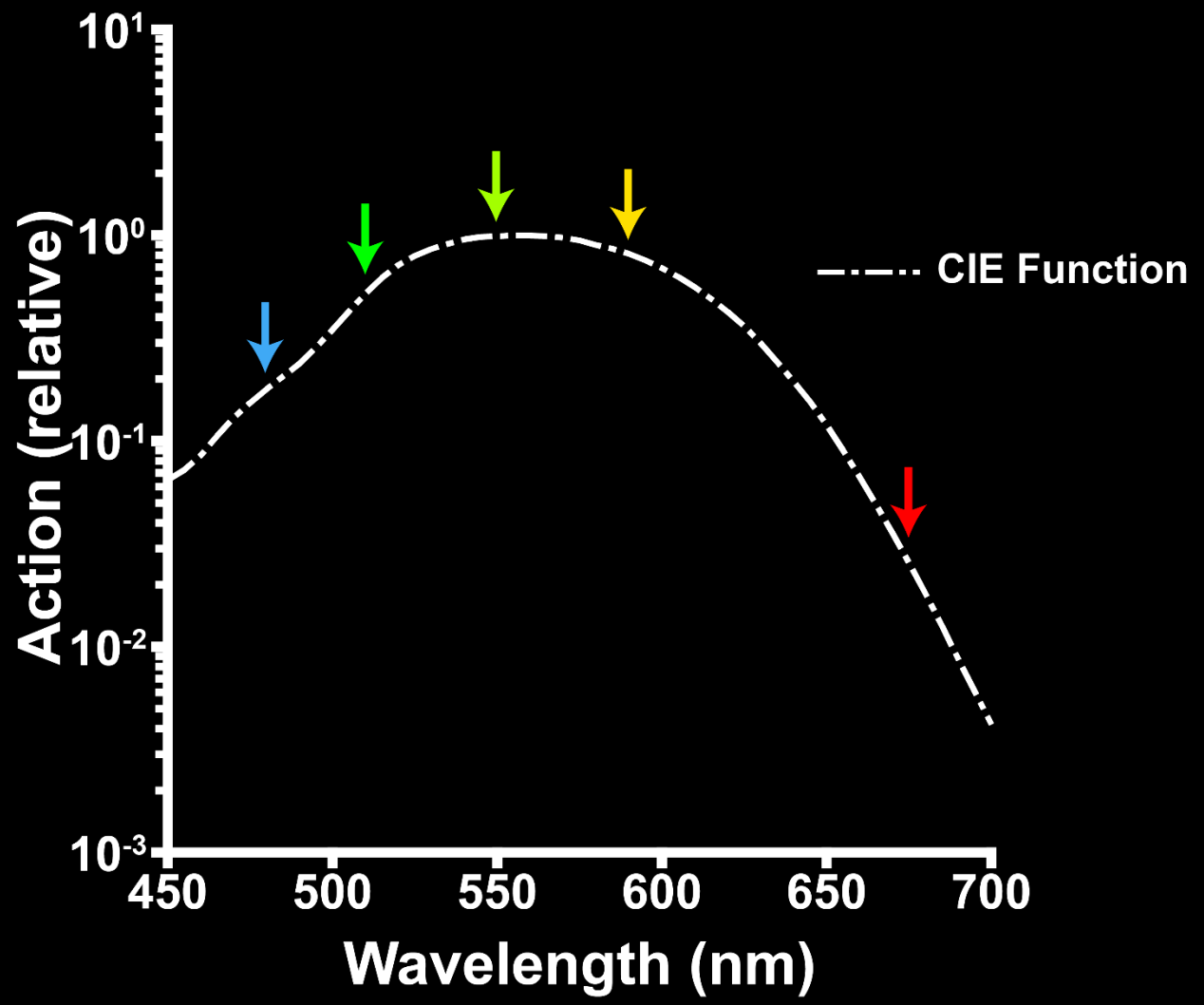
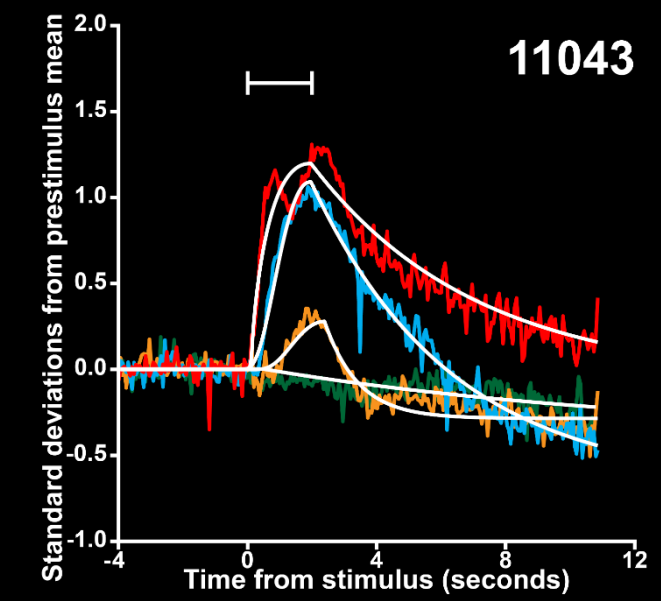
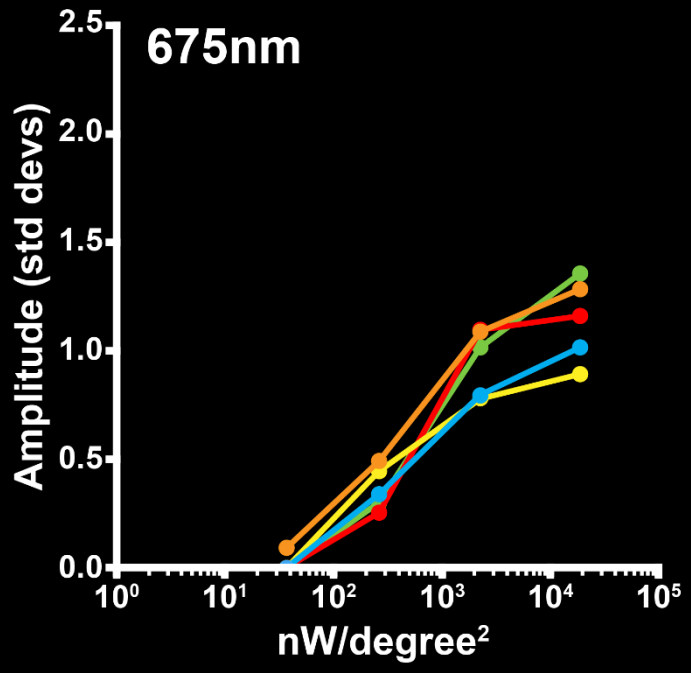


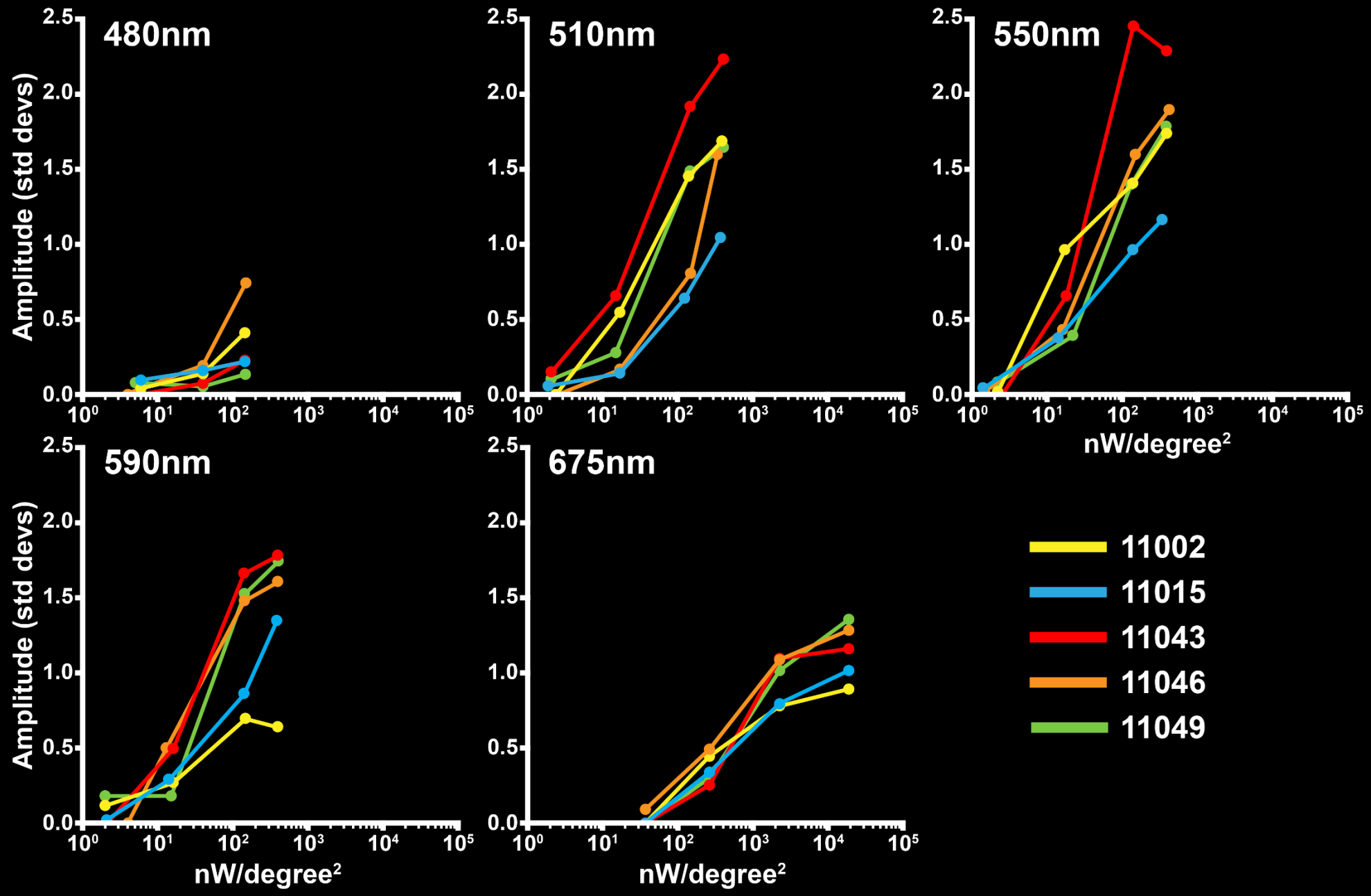
Sharpe et al., 2005



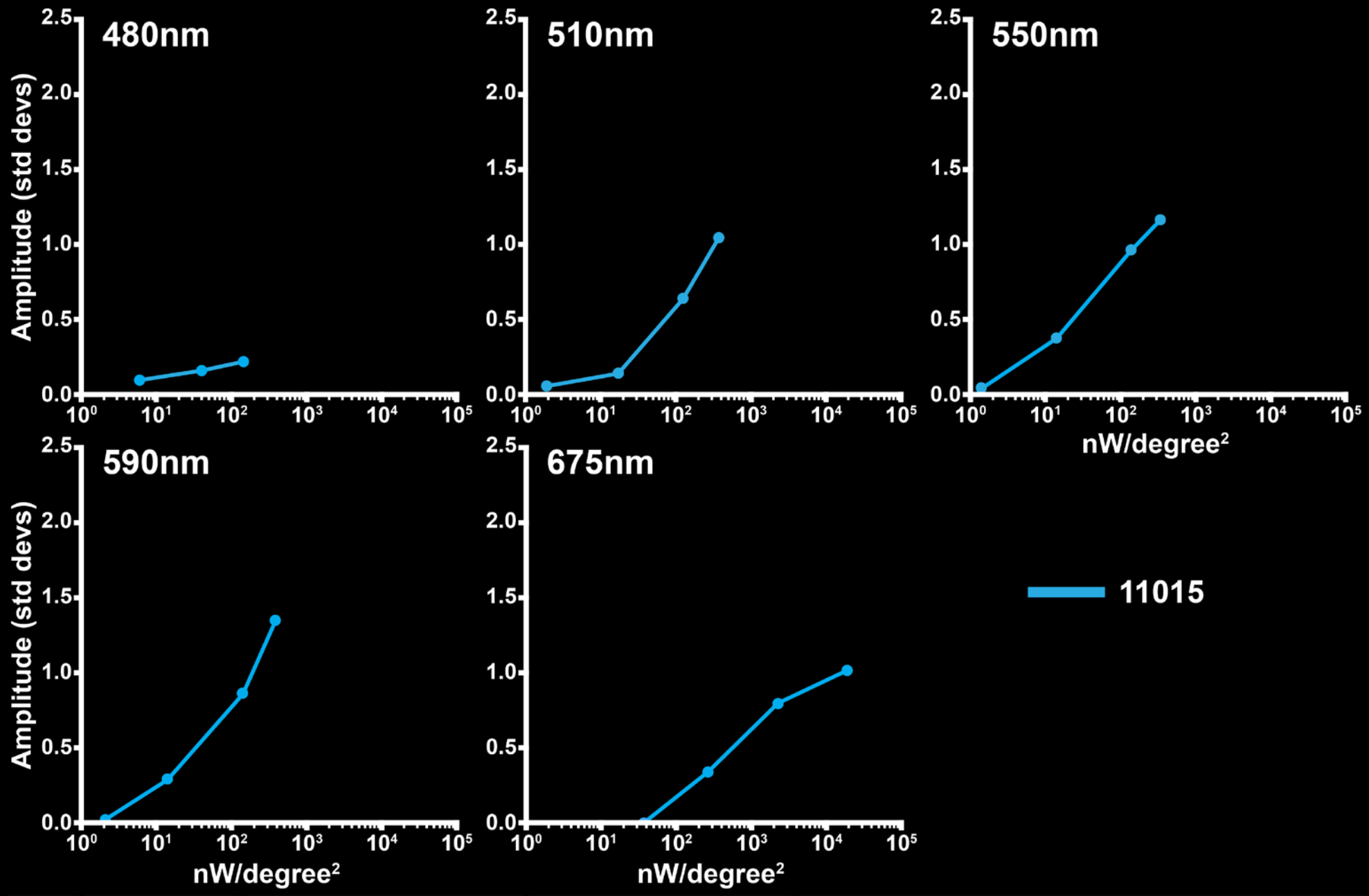


11002
11015
11043
11046
11049

The reflectance response *increases* for all wavelengths



Determining the action spectrum



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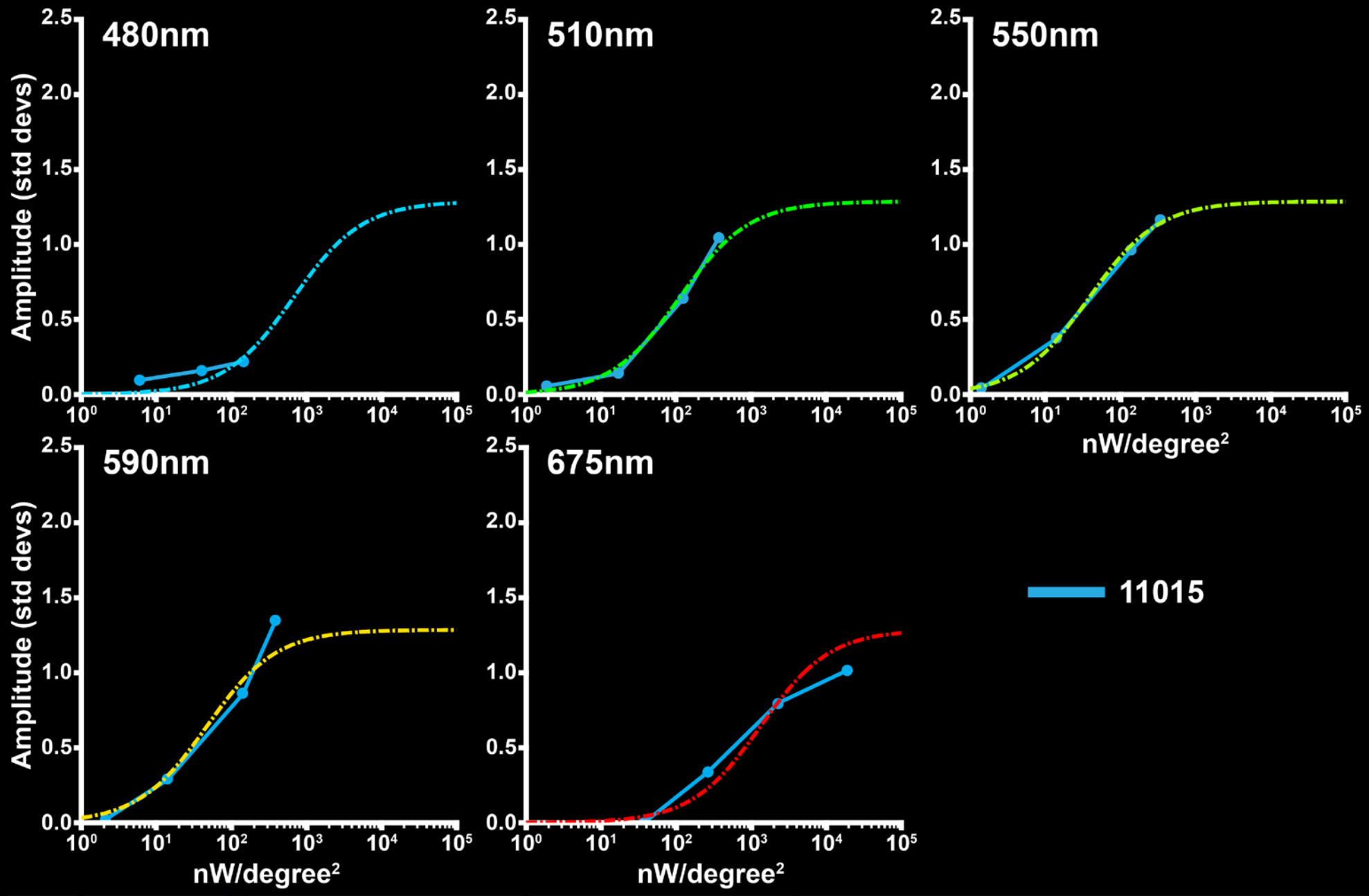

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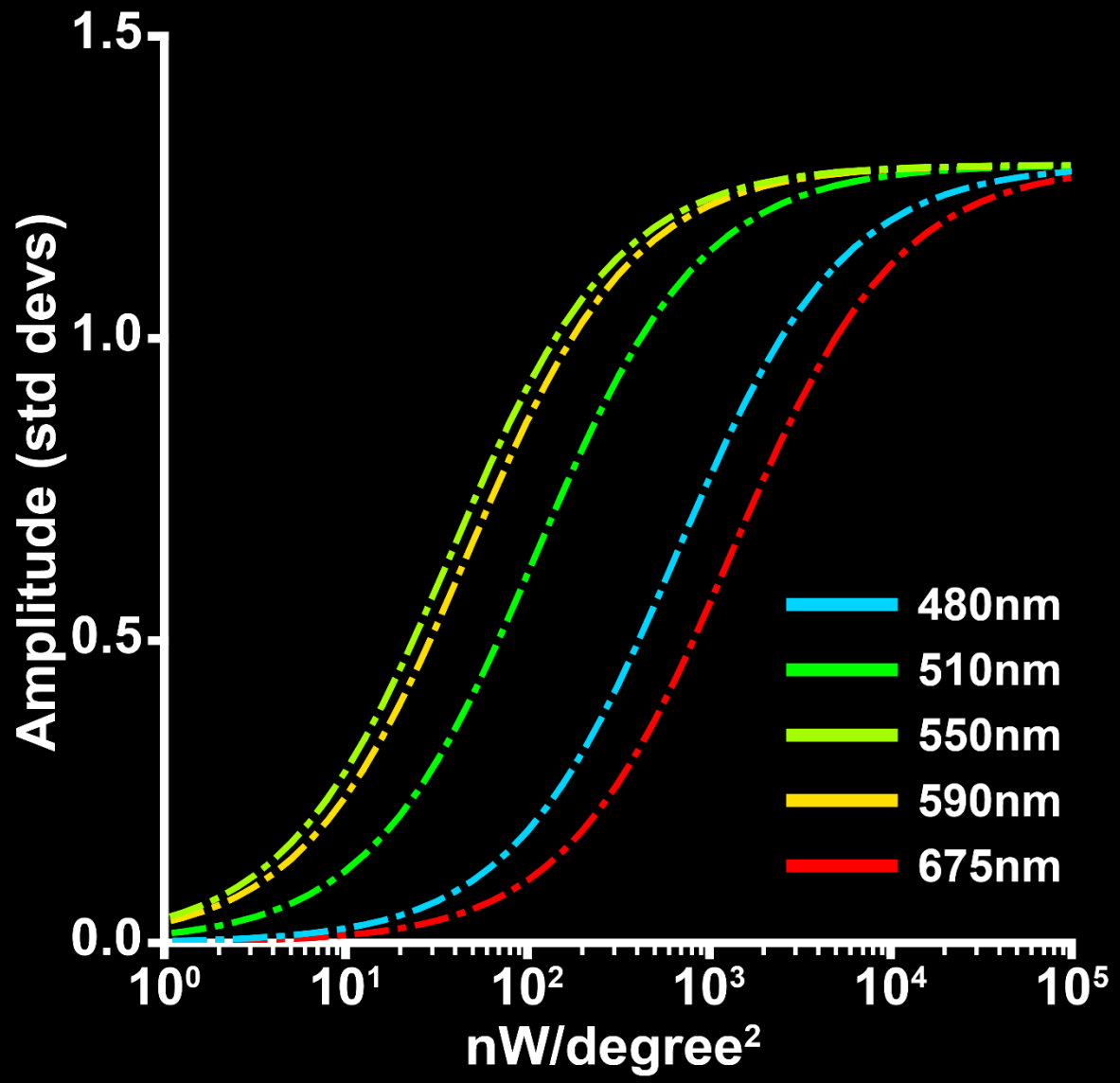

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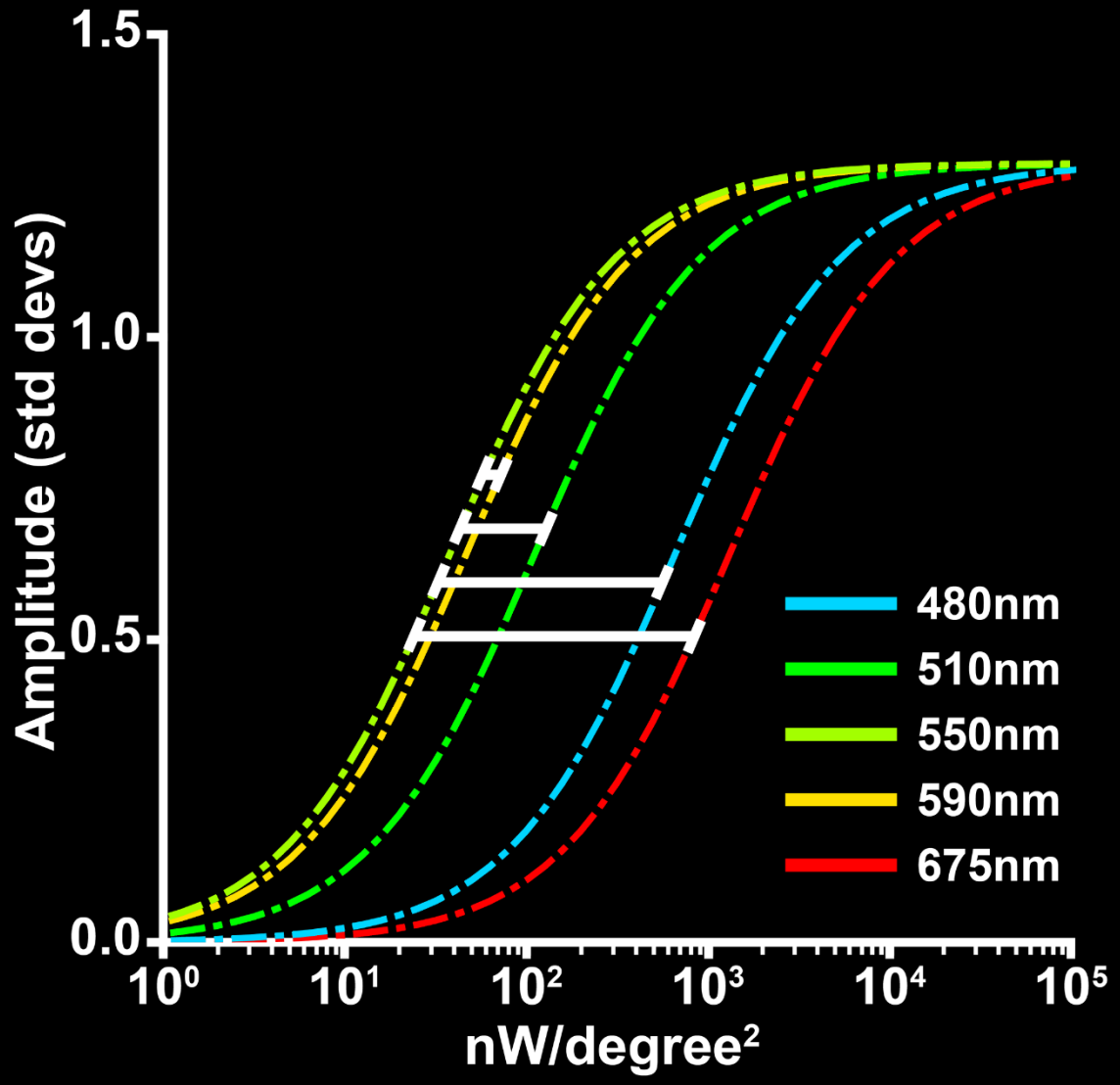

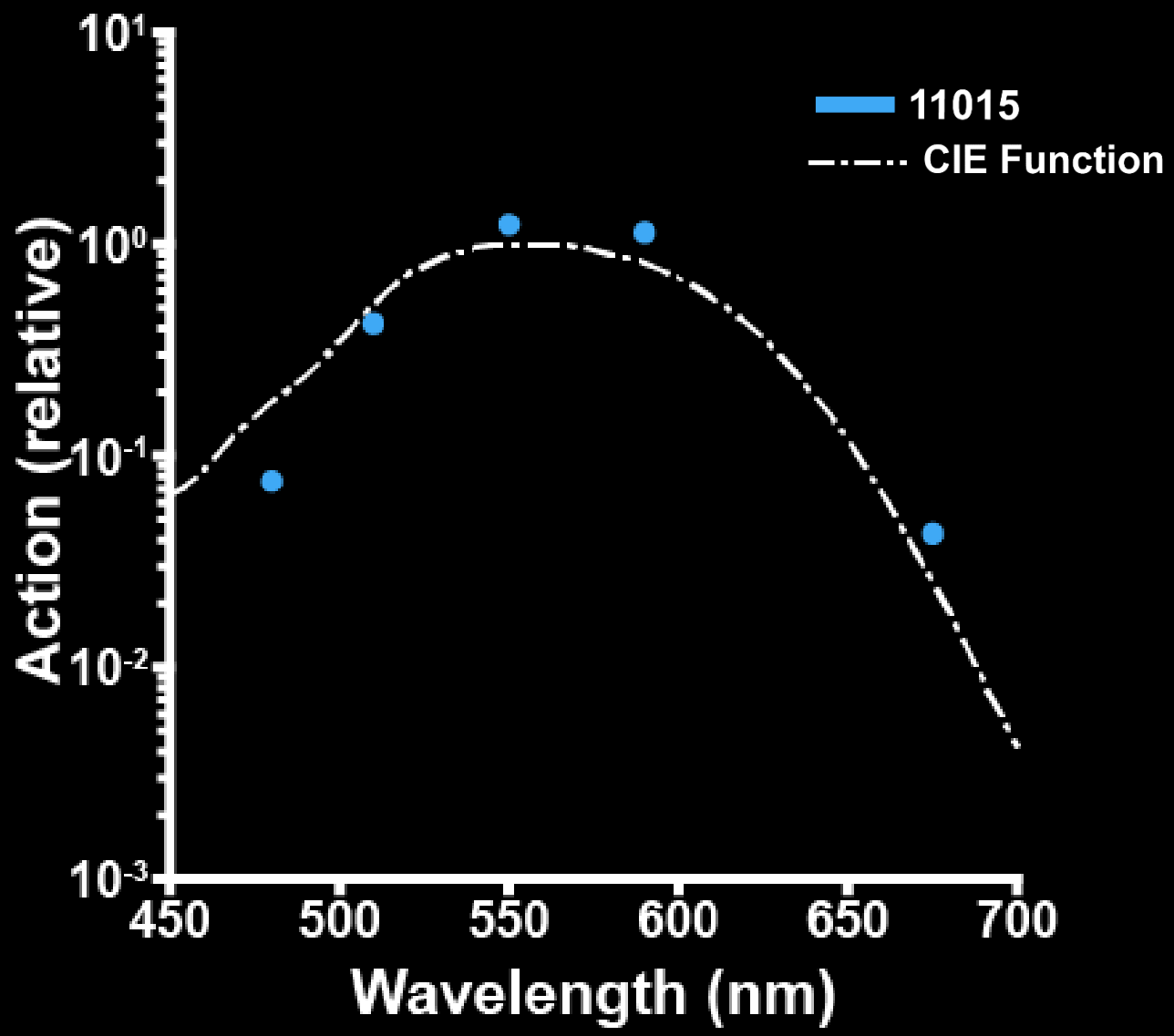

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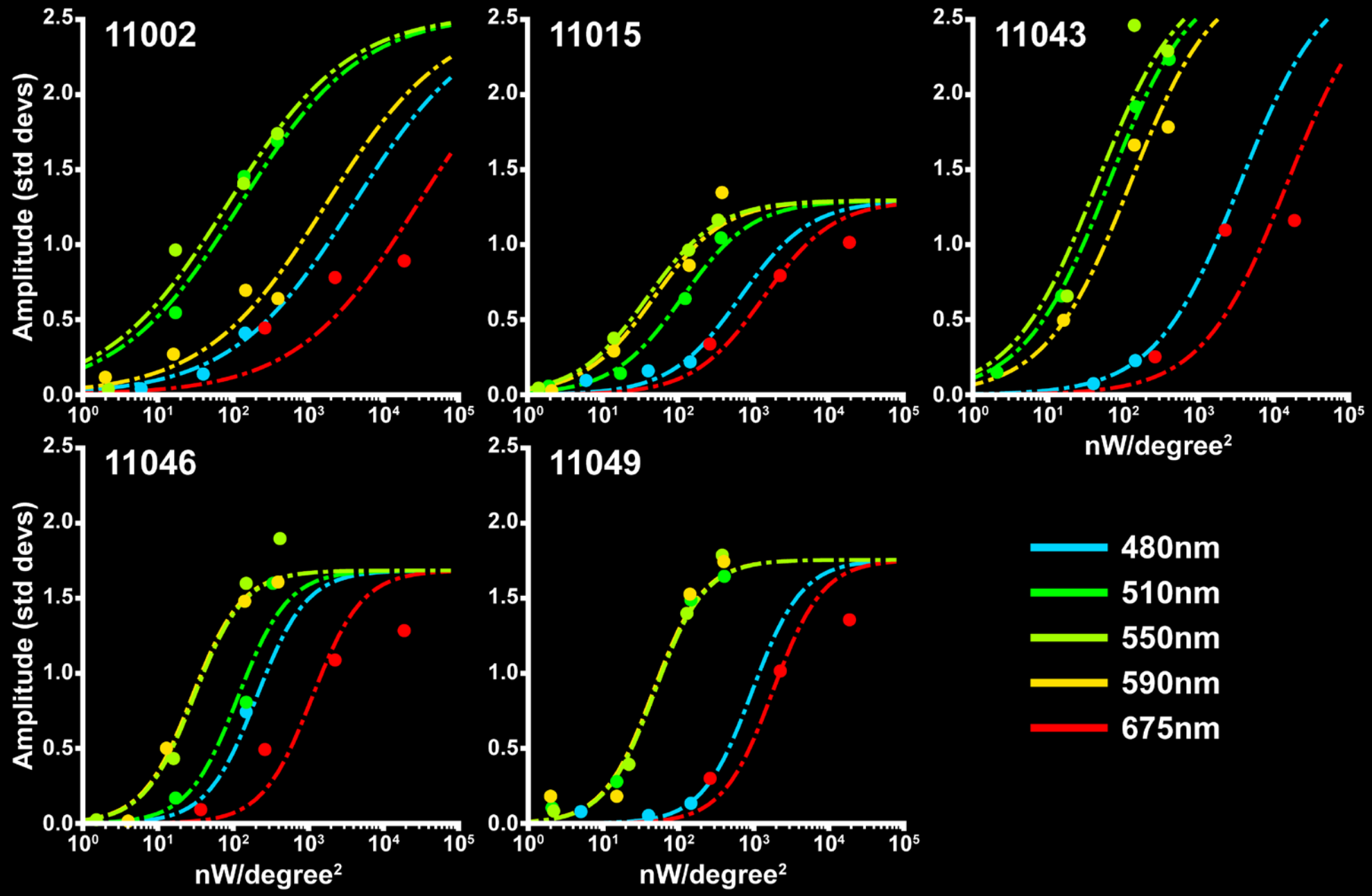
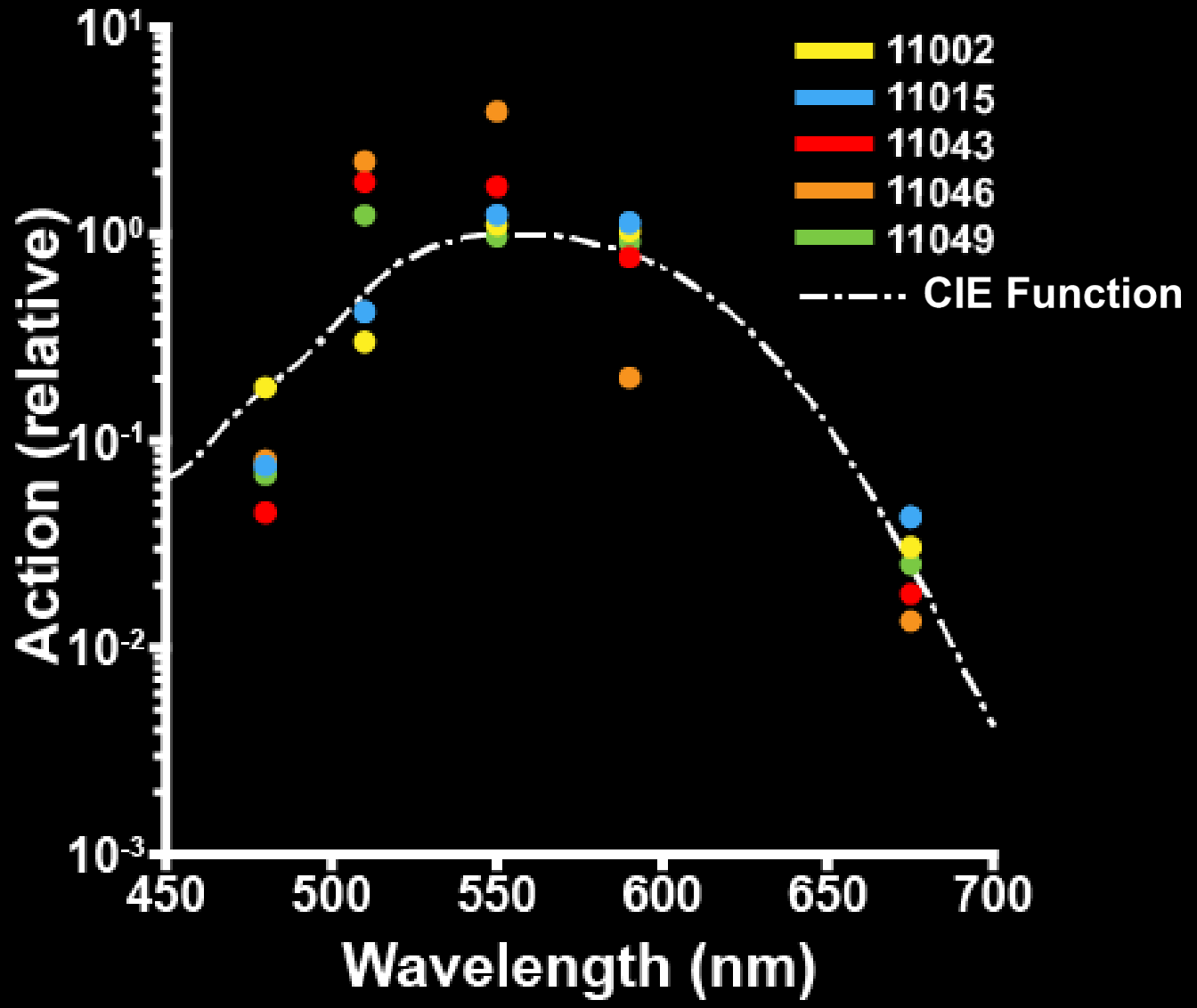


The action spectrum of the intrinsic reflectance response approximates the photopic human luminosity function



Cooper et al., 2017

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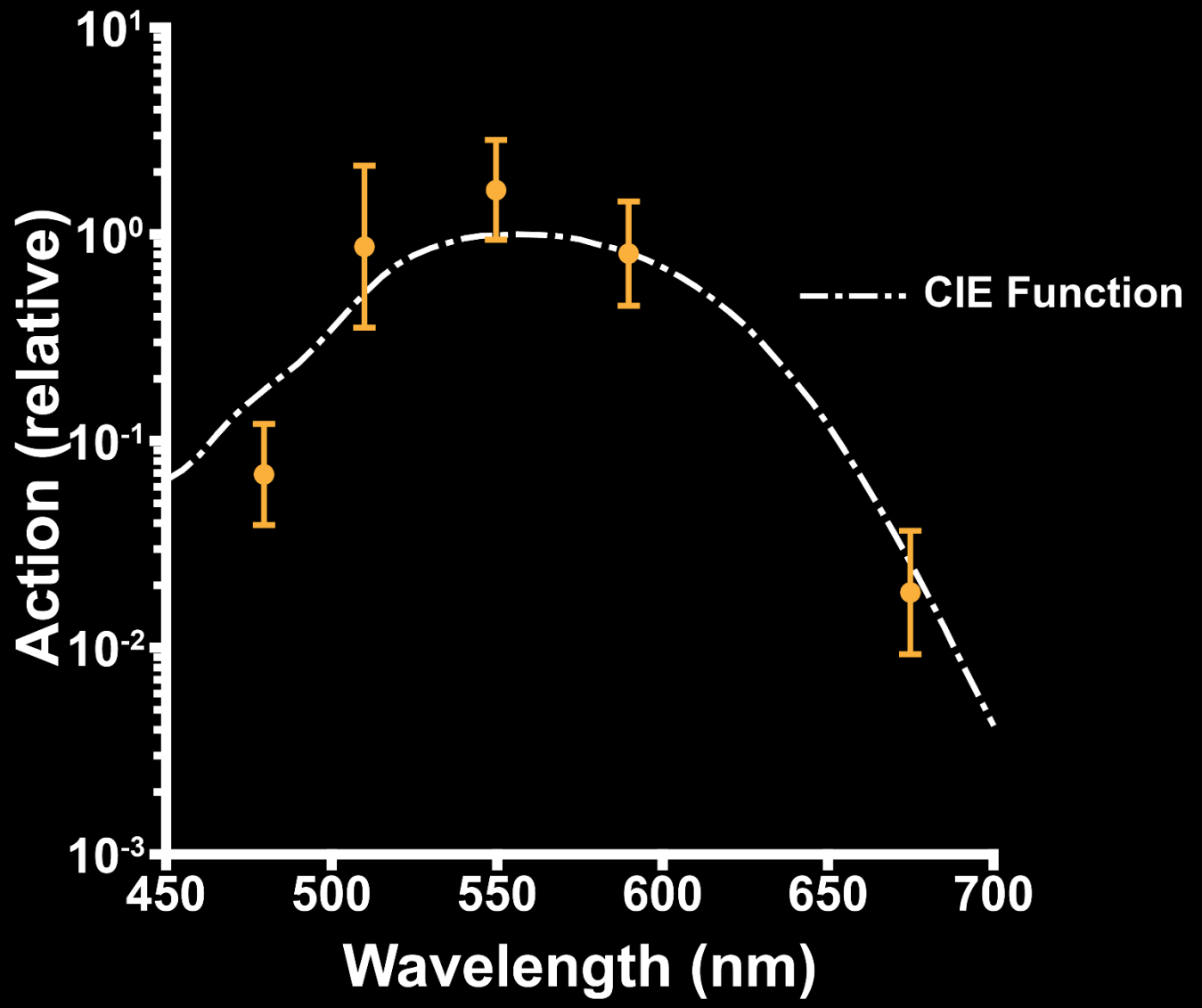
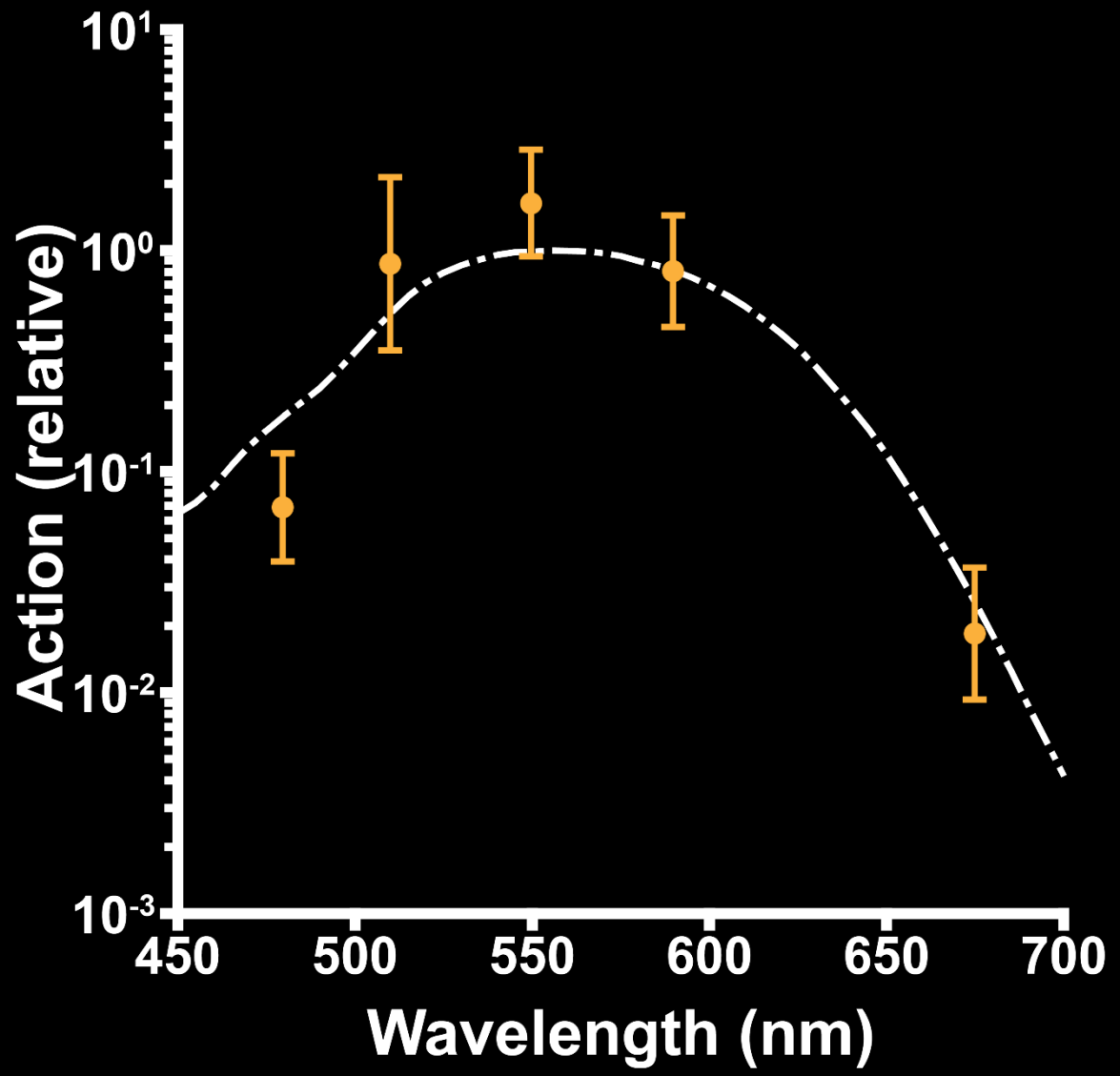


Cooper et al., 2017

The cone intrinsic reflectance response is mediated by phototransduction.

- ✓ Quantifiable / Repeatable
- ✓ Dose sensitive
- ✓ Functionally significant
4. Clinically relevant
5. Optimized



Cooper et al., 2017

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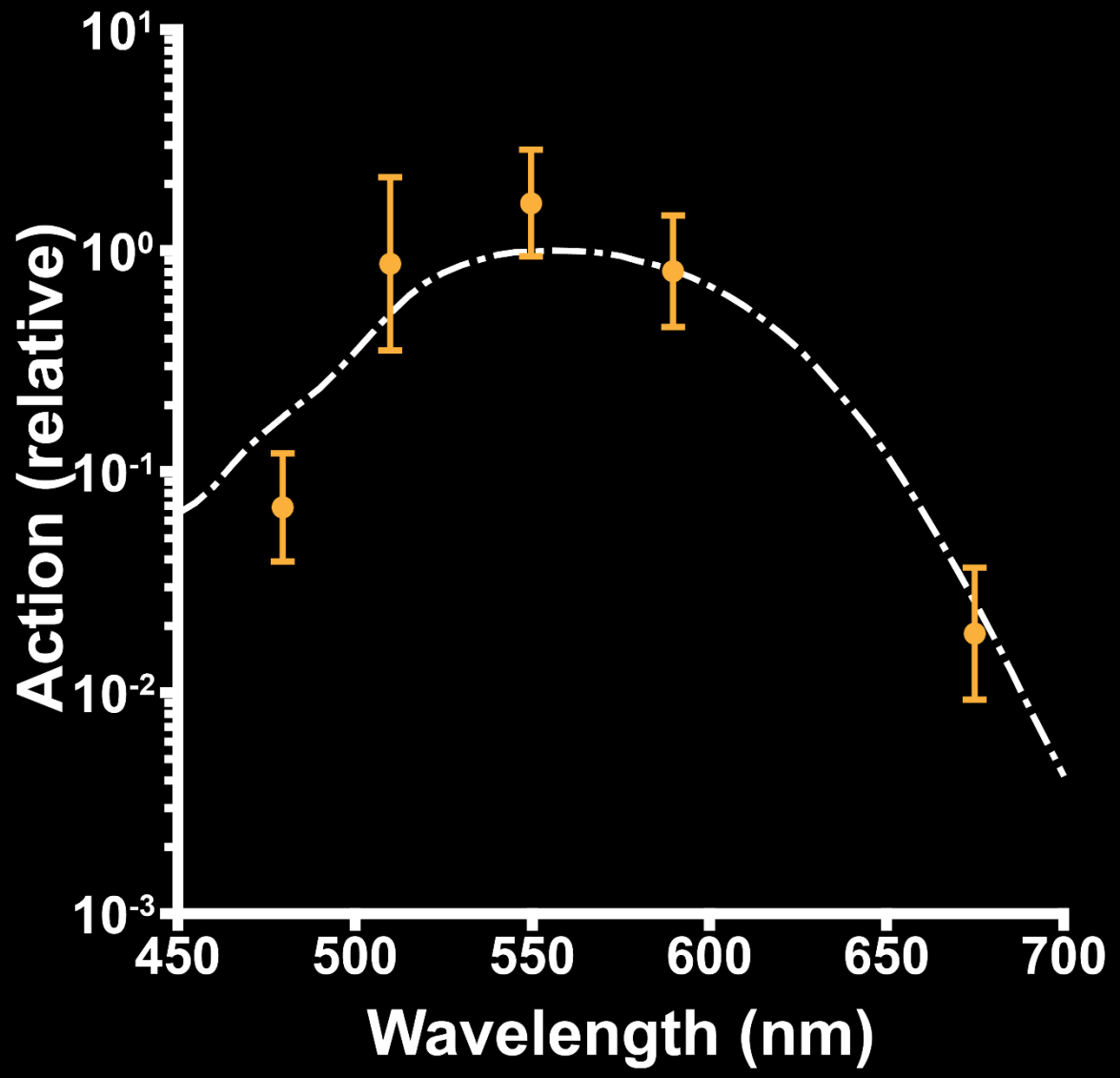


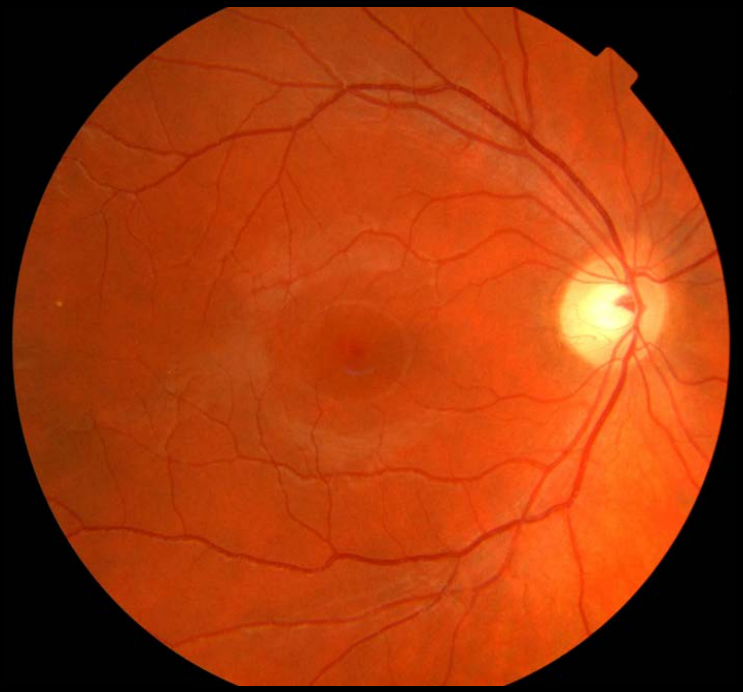
Cooper et al., 2017

Choroideremia (CHM)

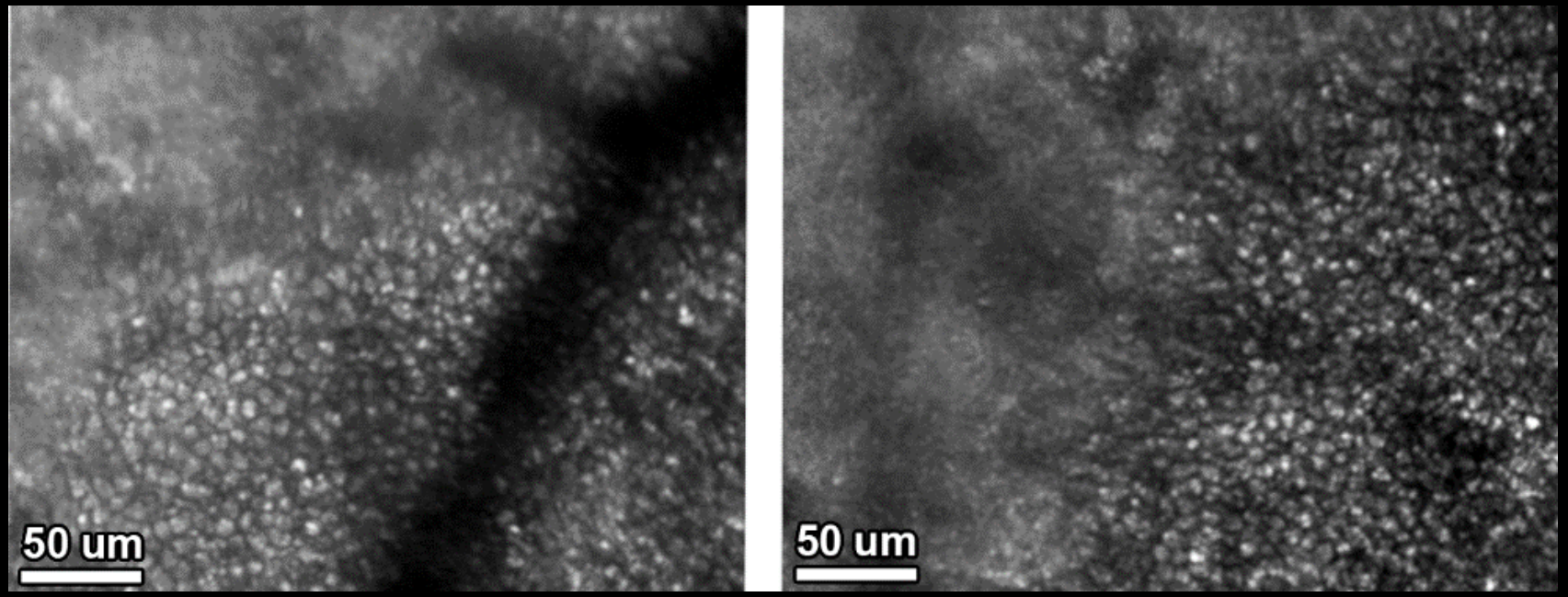
- X-linked inherited retinal degeneration
- Symptomatic in childhood
  - Night blindness
  - Visual Field loss
    - Initially in the periphery leading to tunnel vision
- Blindness in 30’s-40’s, although many patients maintain central vision until age 40-50

CHM

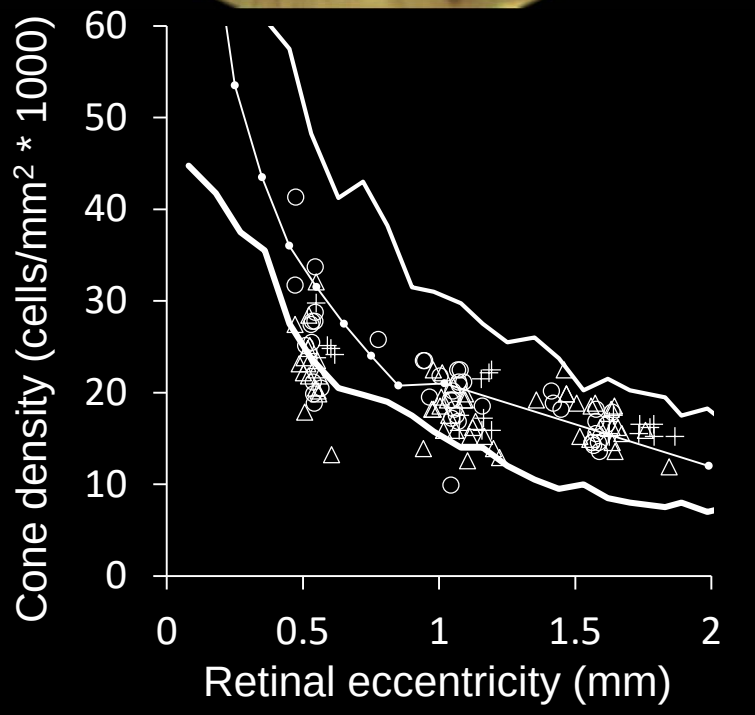
Normal



Choroideremia (CHM)

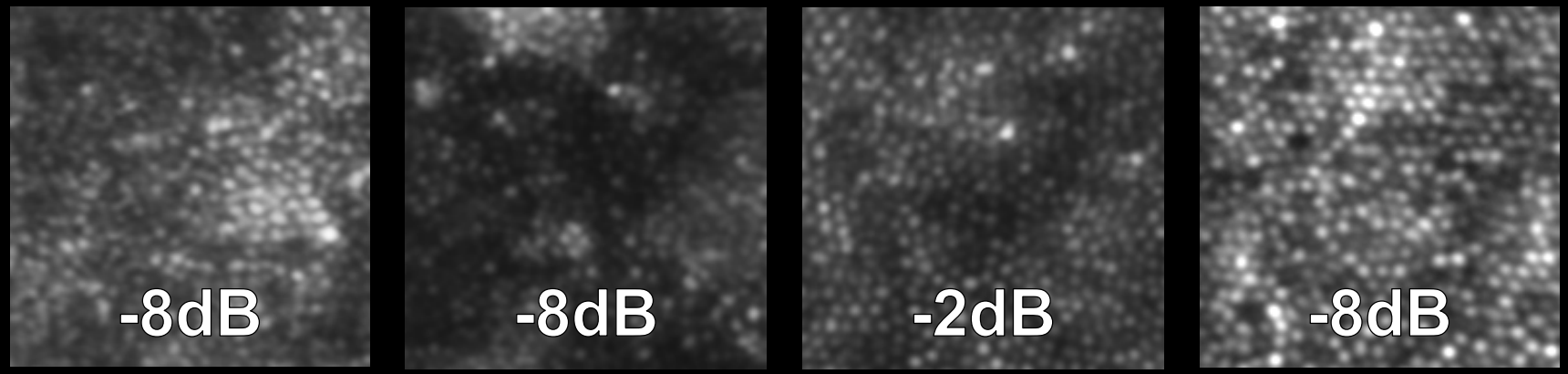


Morgan *et al.,* 2014



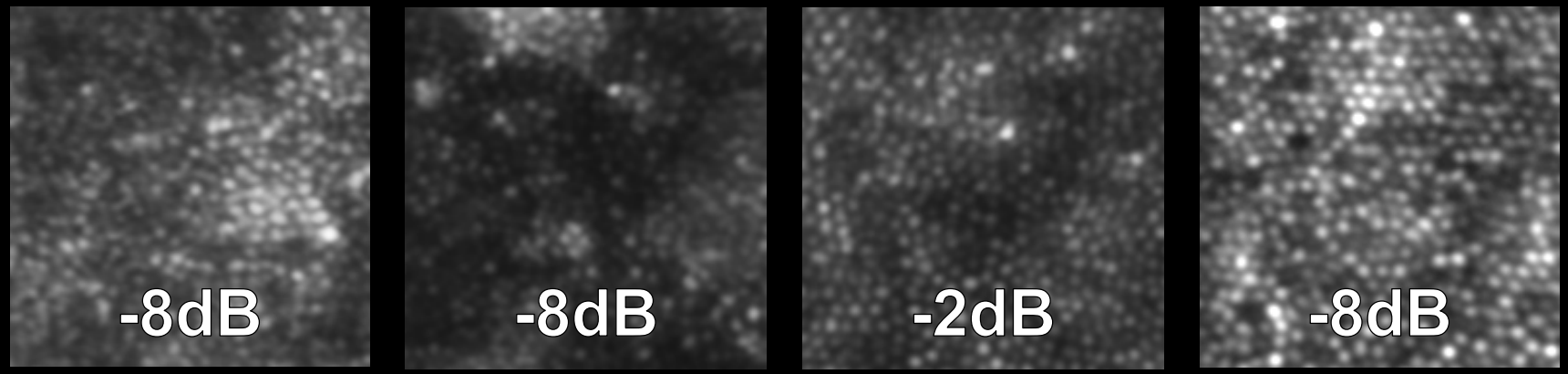
Assessing function with a clinical gold standard

Choroideremia

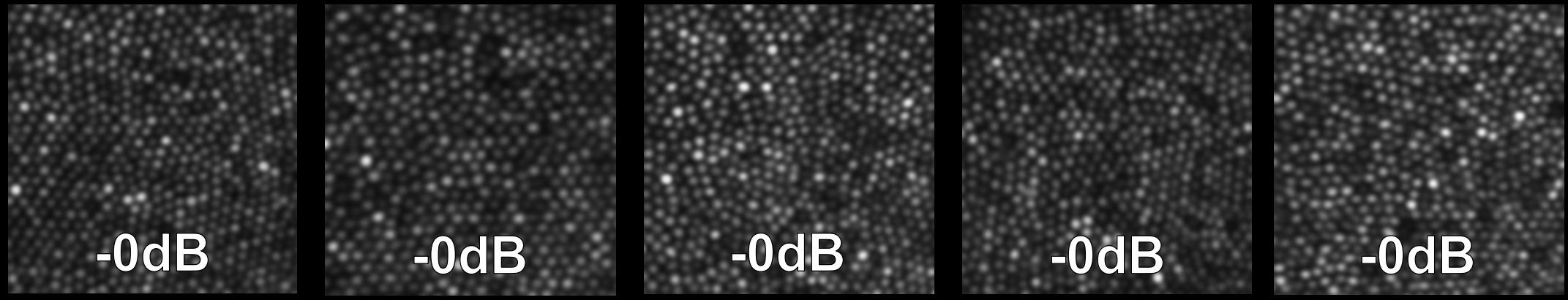


Assessing function with a clinical gold standard

Choroideremia

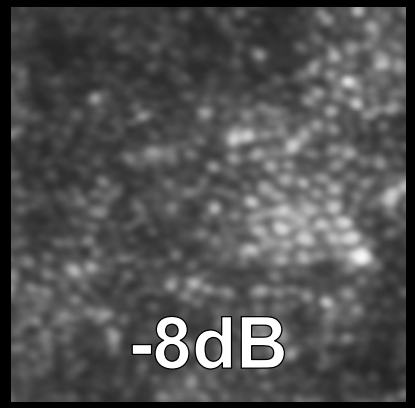


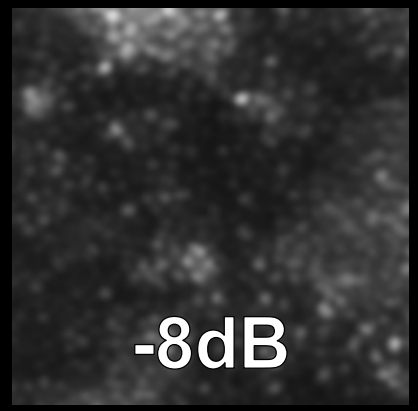
Controls

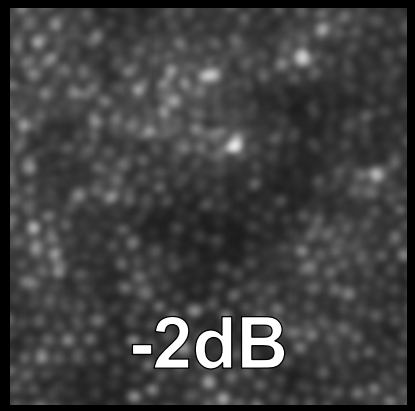


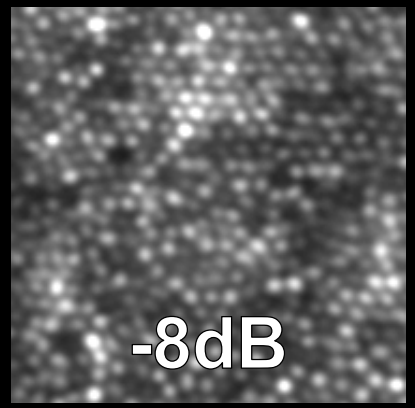
The reflectance response in controls

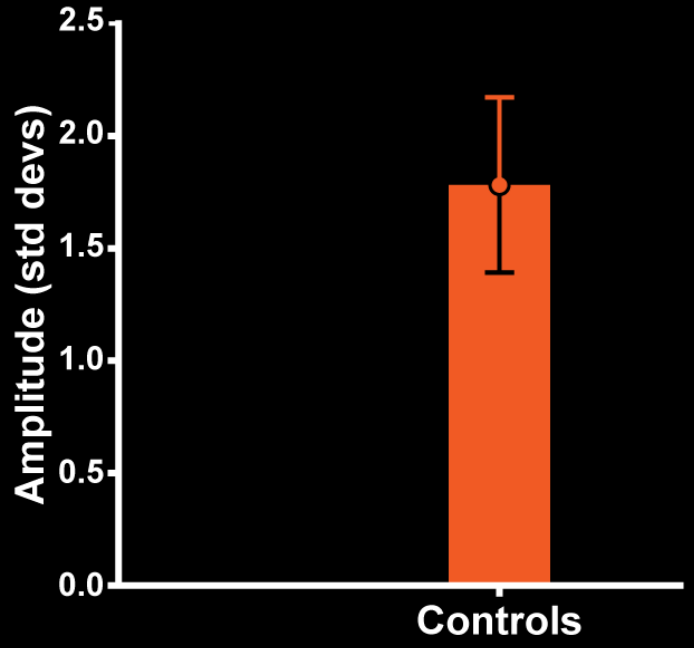
Choroideremia











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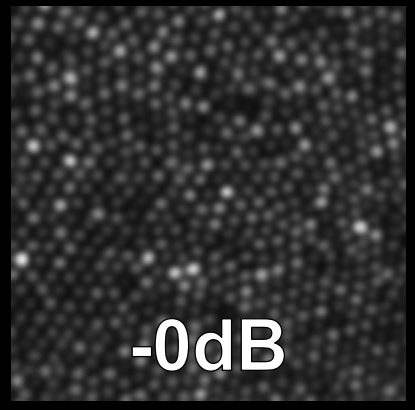
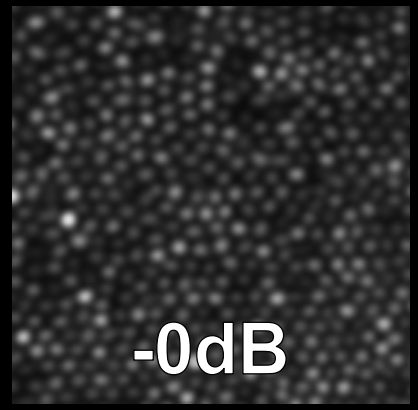
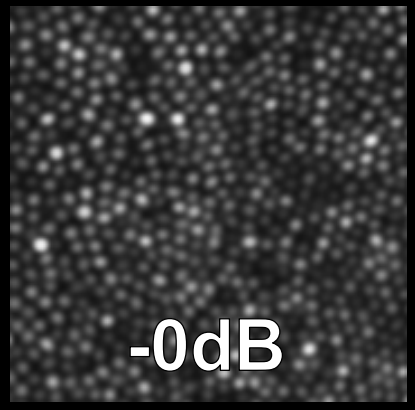
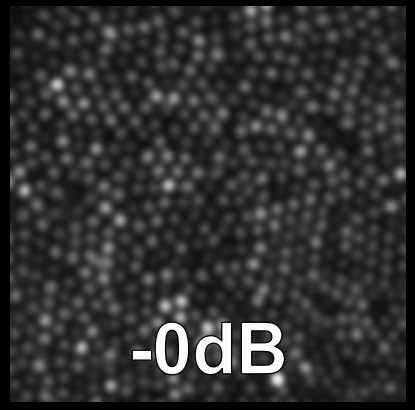
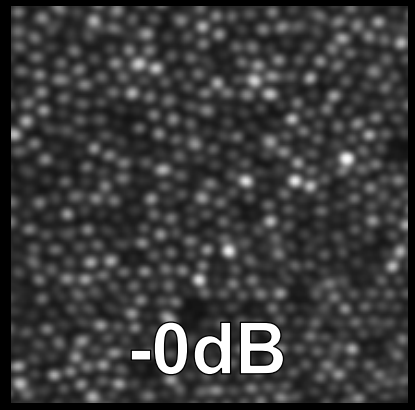




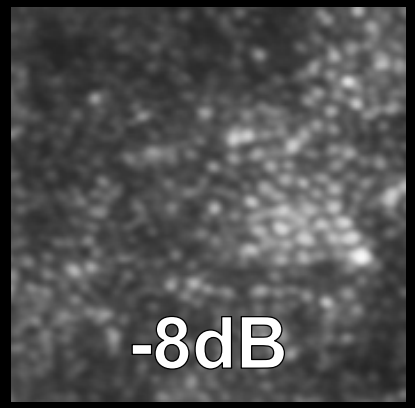


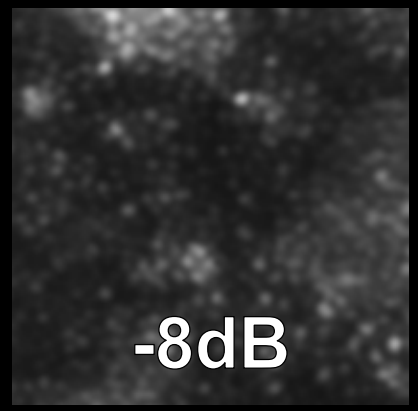


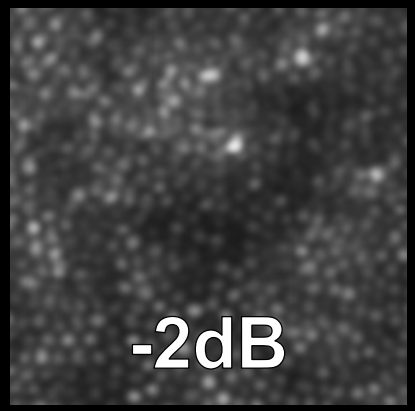


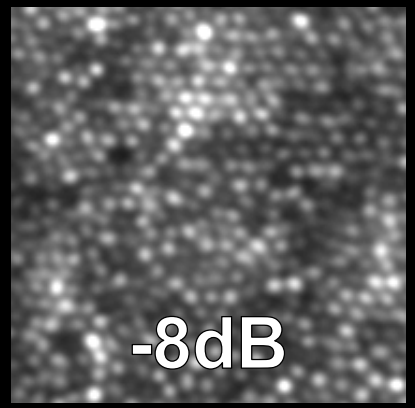
The reflectance response in choroideremia

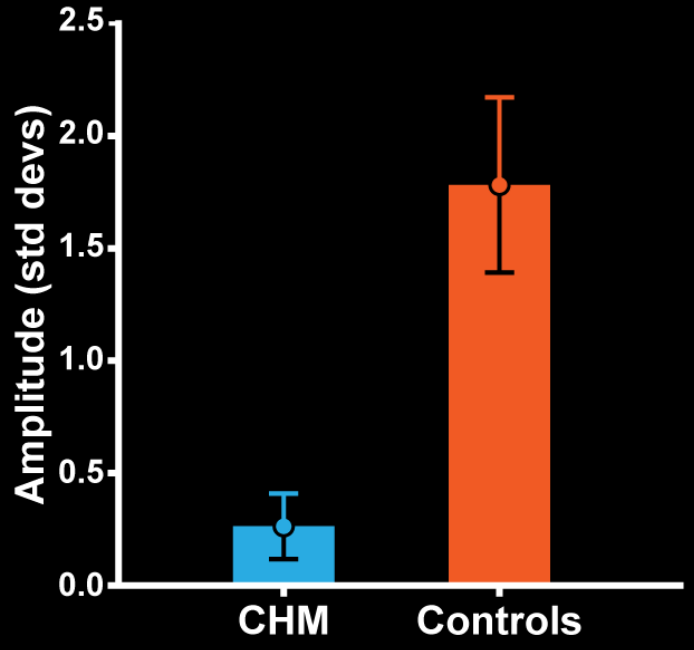
Choroideremia











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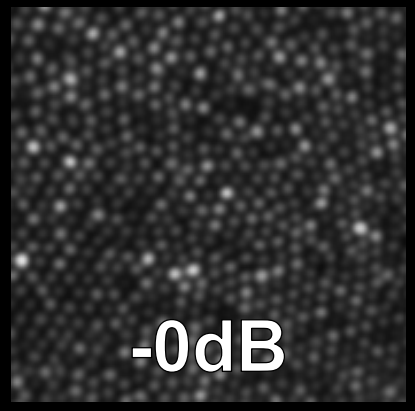
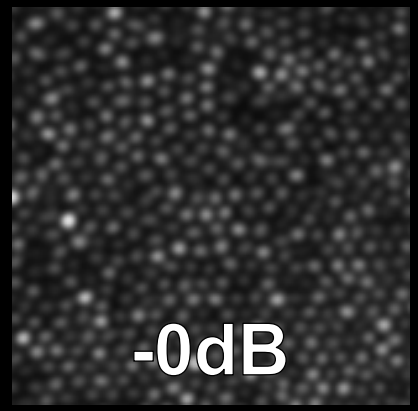
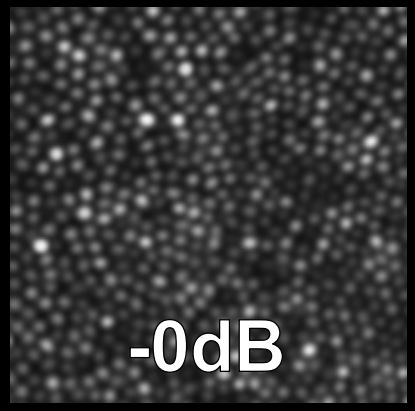
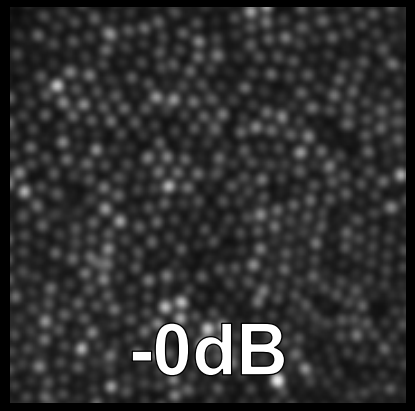
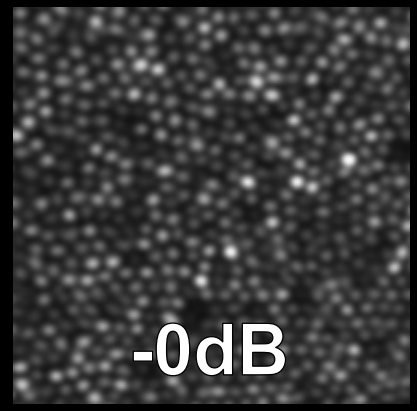




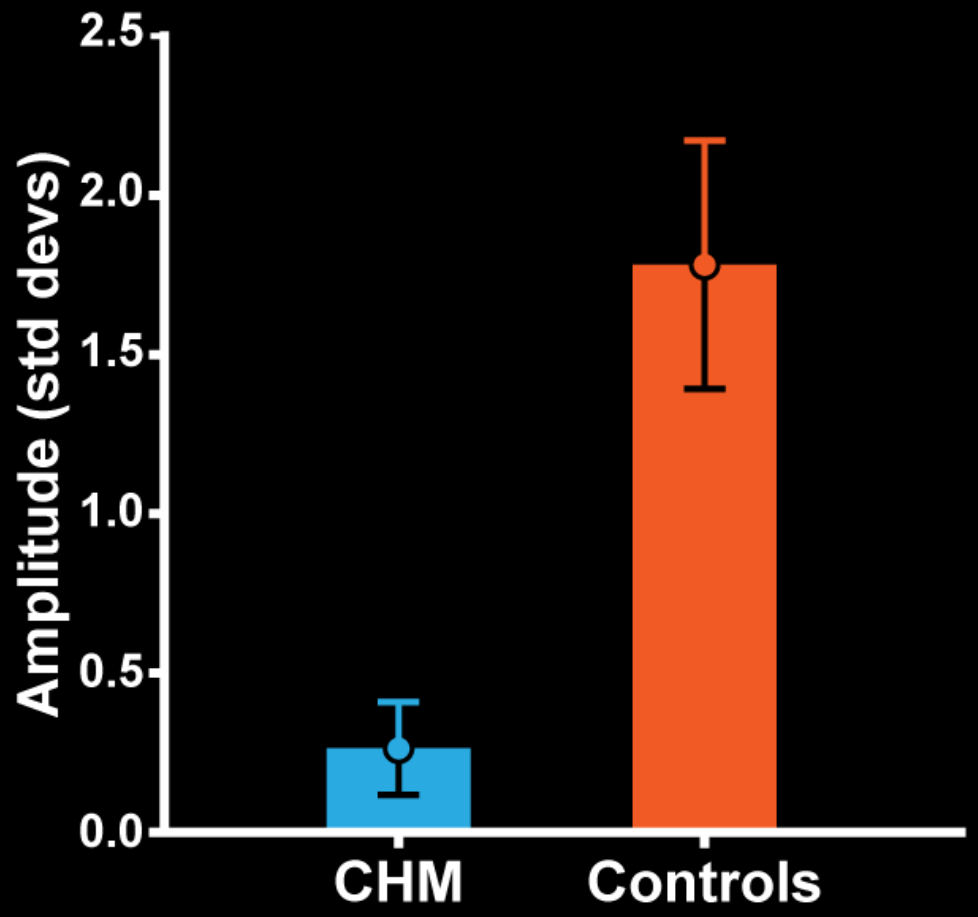






The cone intrinsic reflectance response has translational applications

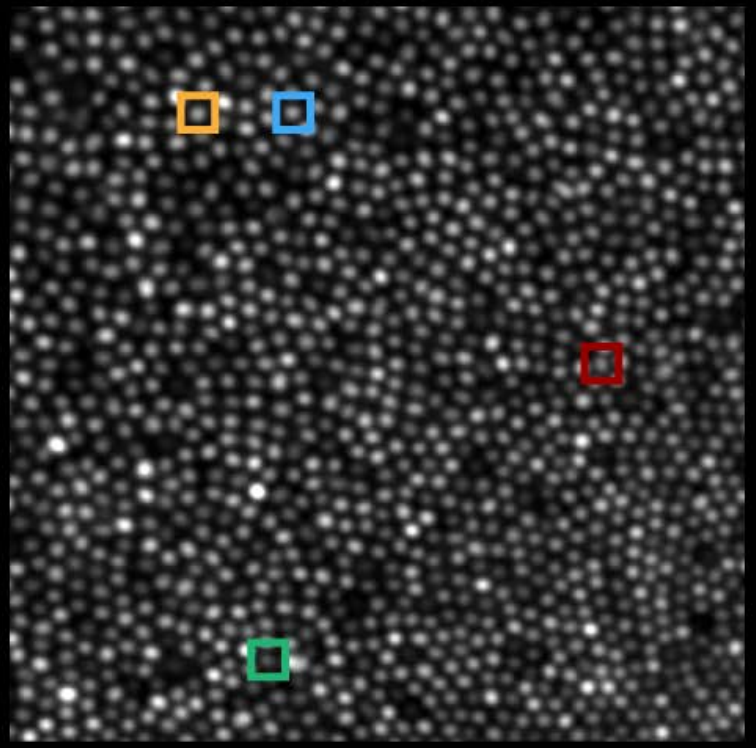
- ✓ Quantifiable / Repeatable
- ✓ Dose sensitive
- ✓ Functionally significant
- ✓ Clinically relevant
5. Optimized

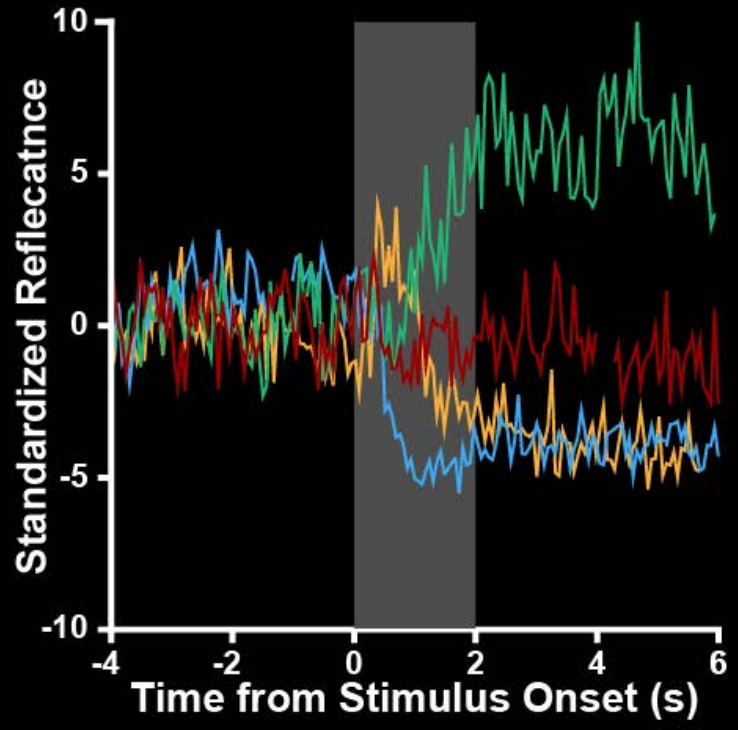


Pushing the limits

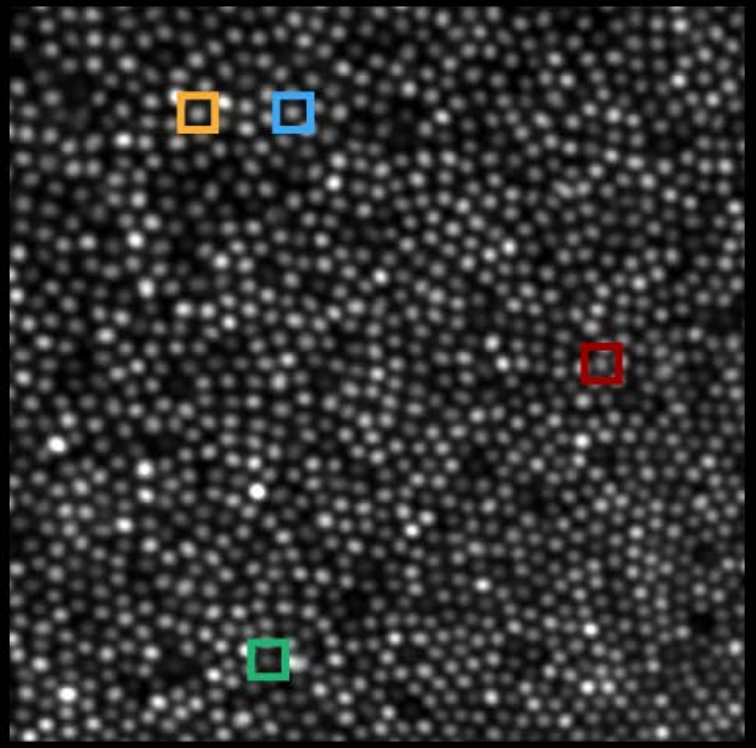
- ✓ Quantifiable / Repeatable
- ✓ Dose sensitive
- ✓ Functionally significant
- ✓ Clinically relevant
5. Optimized

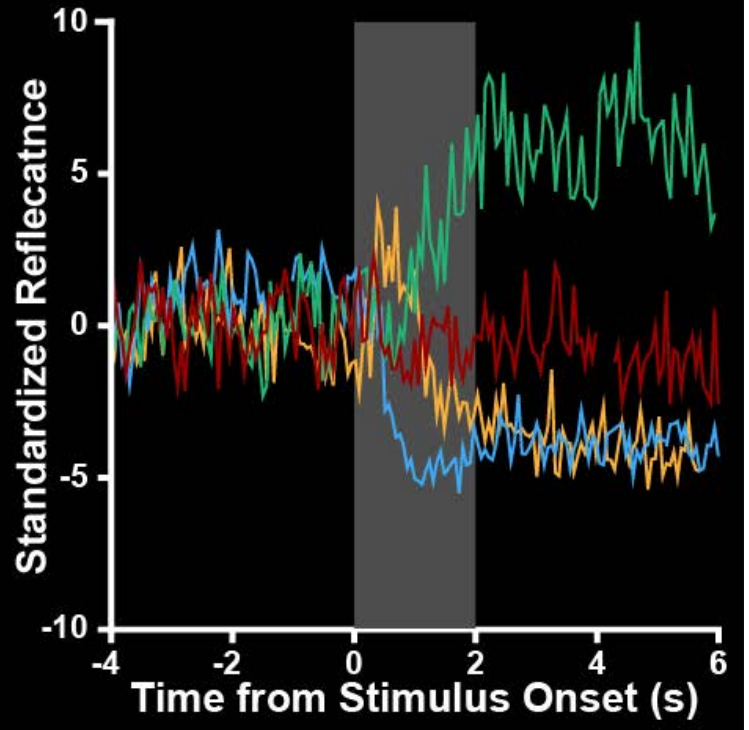
The reflectance response of a cone population is heterogeneous across cones





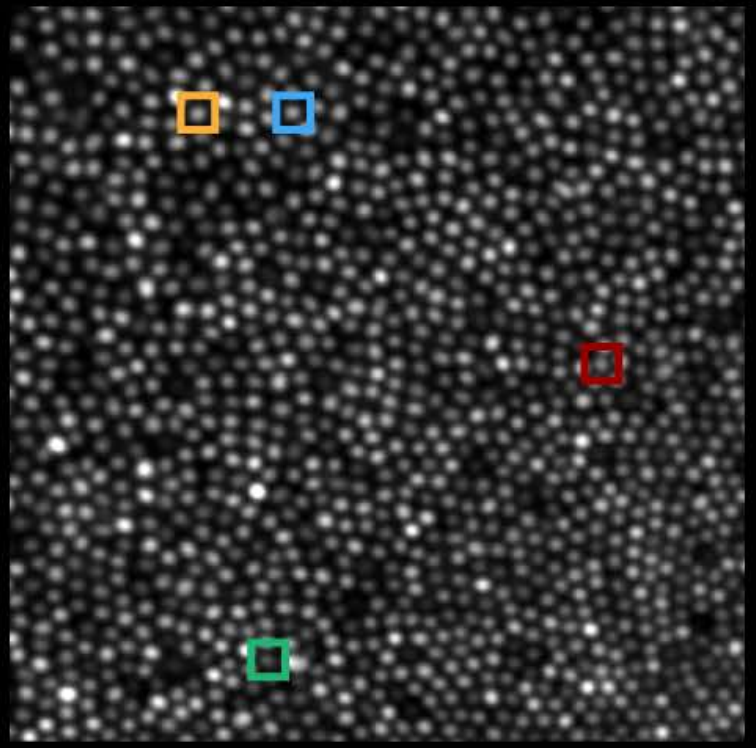
The reflectance response of a cone population is heterogeneous across cones

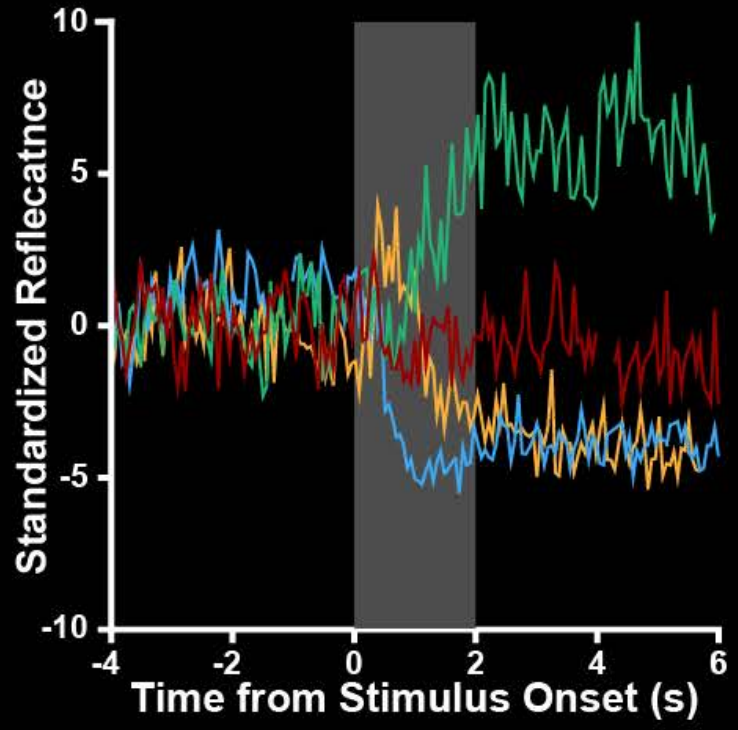


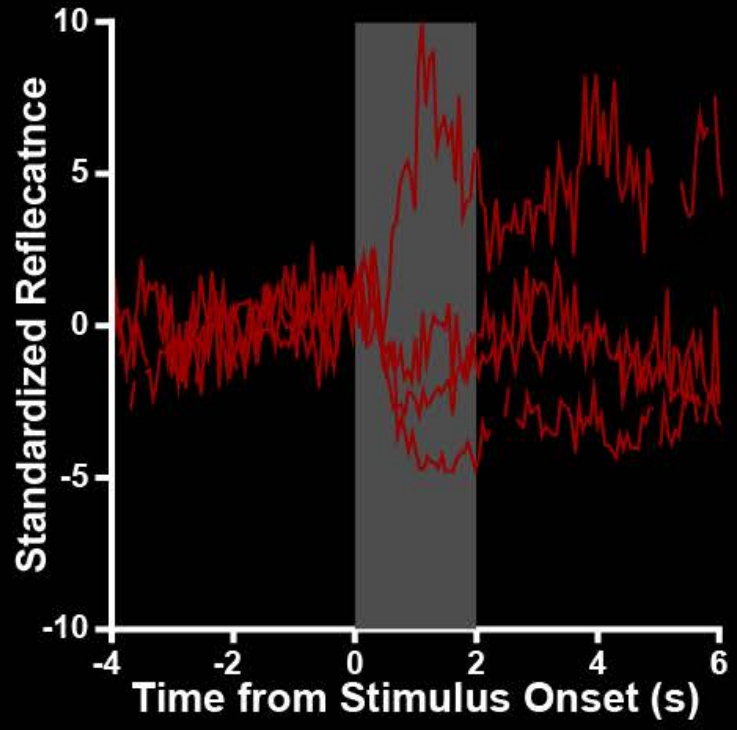


?

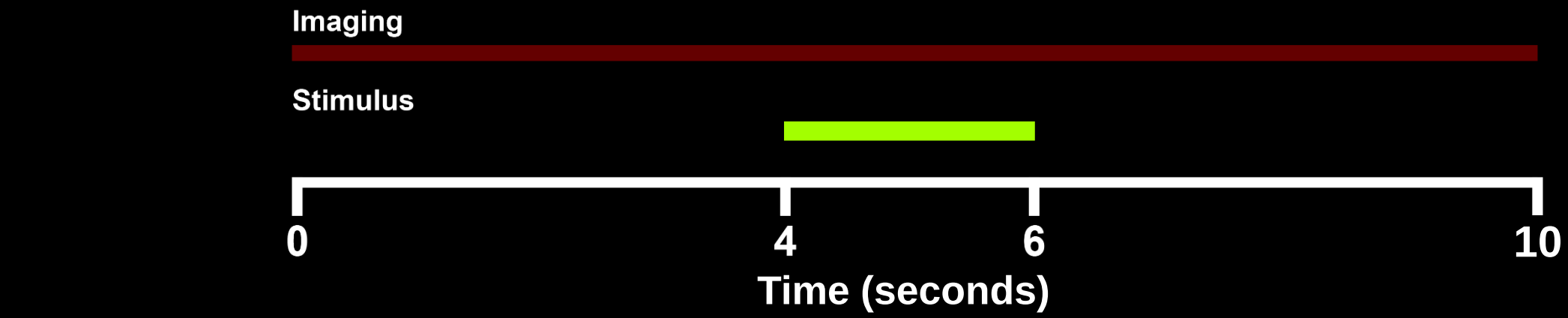
The reflectance response of a single cone is heterogeneous across trials

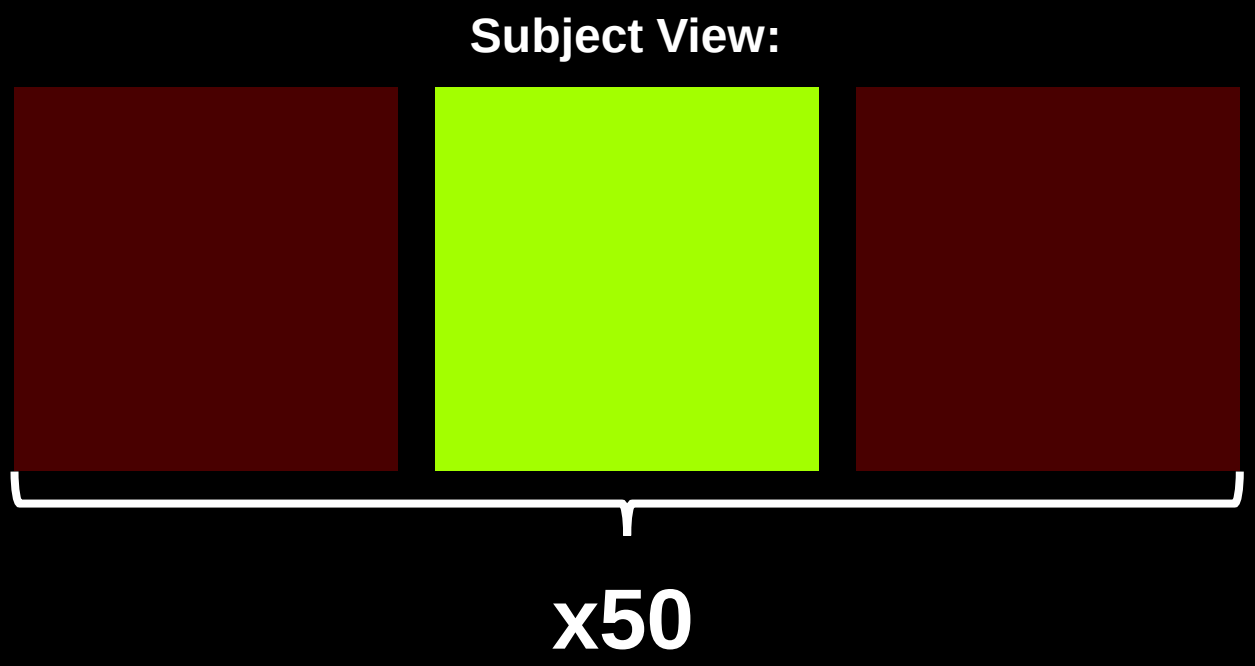






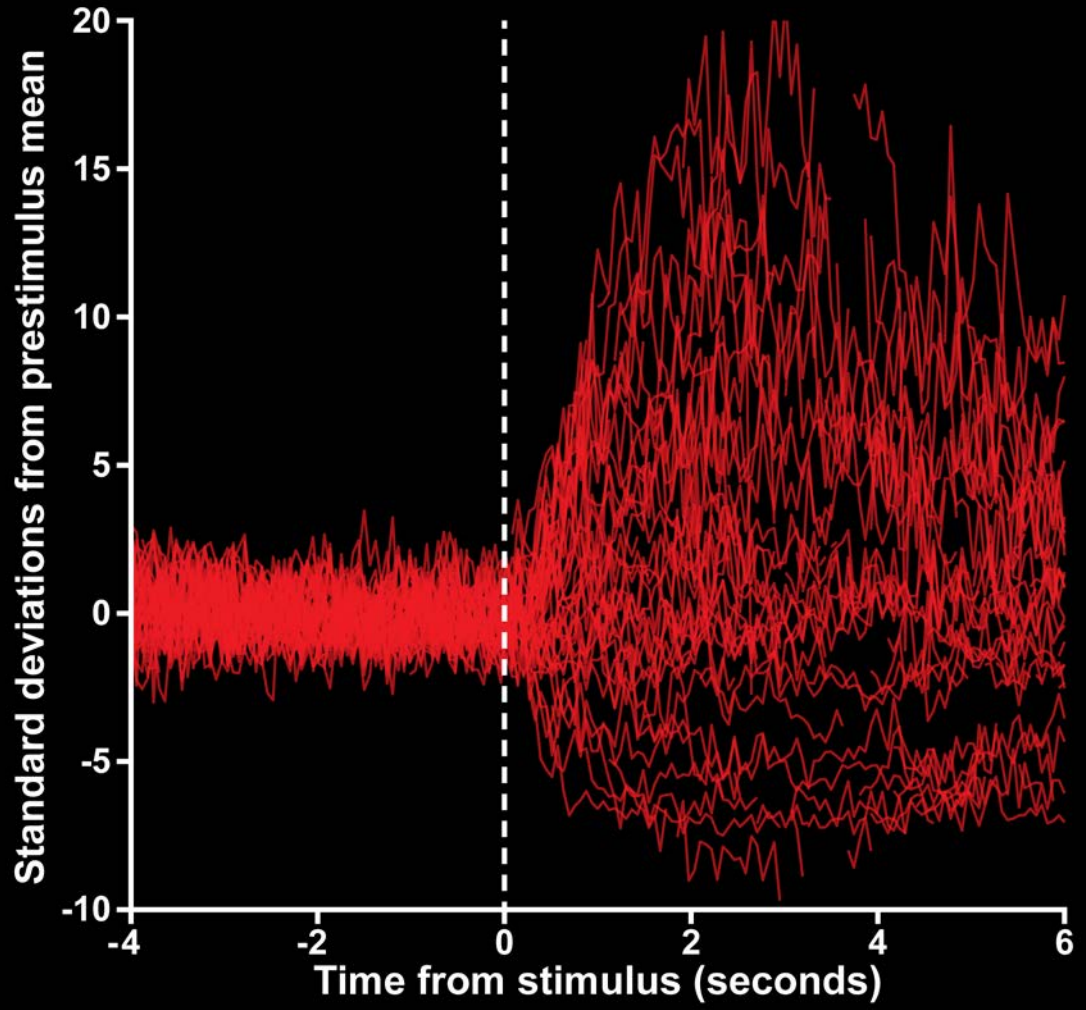
Detecting the intrinsic response of a single cone

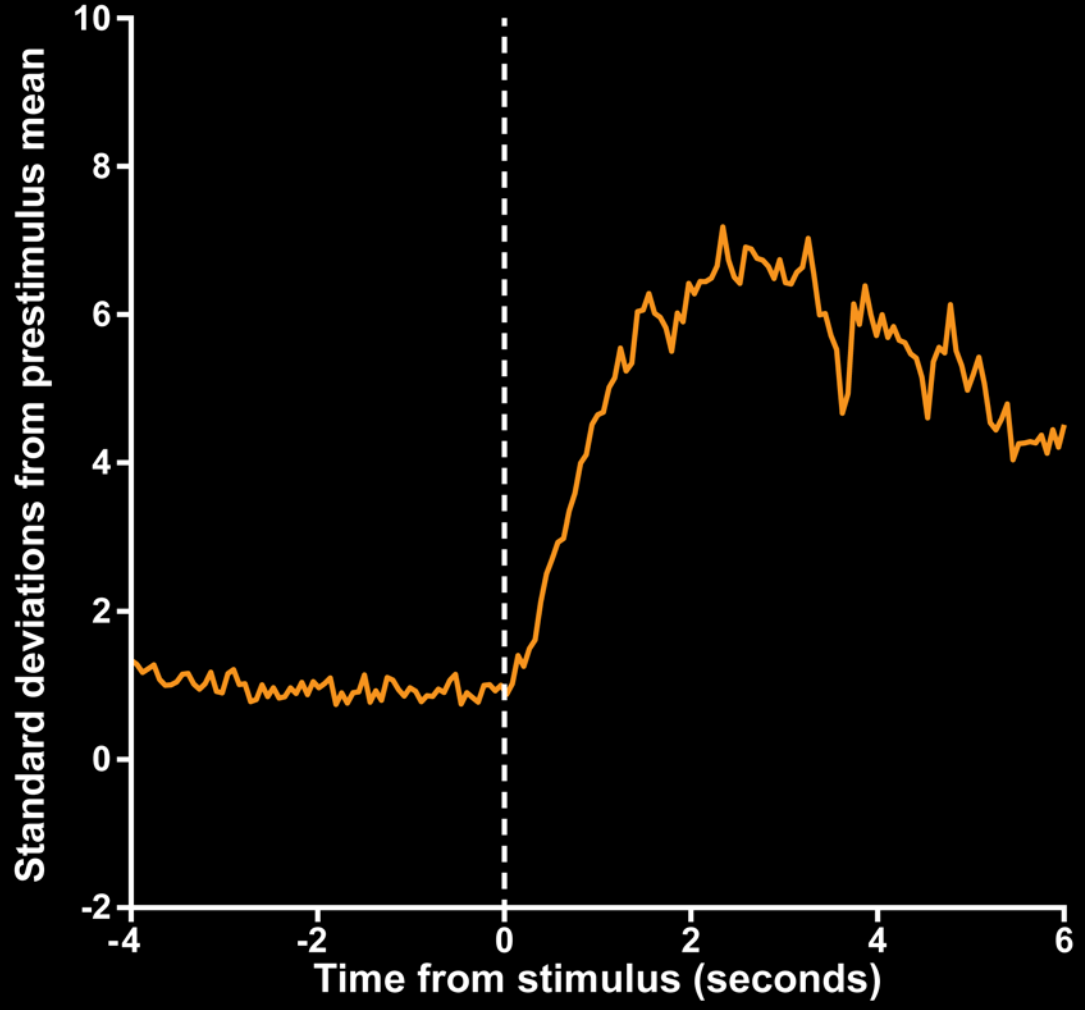




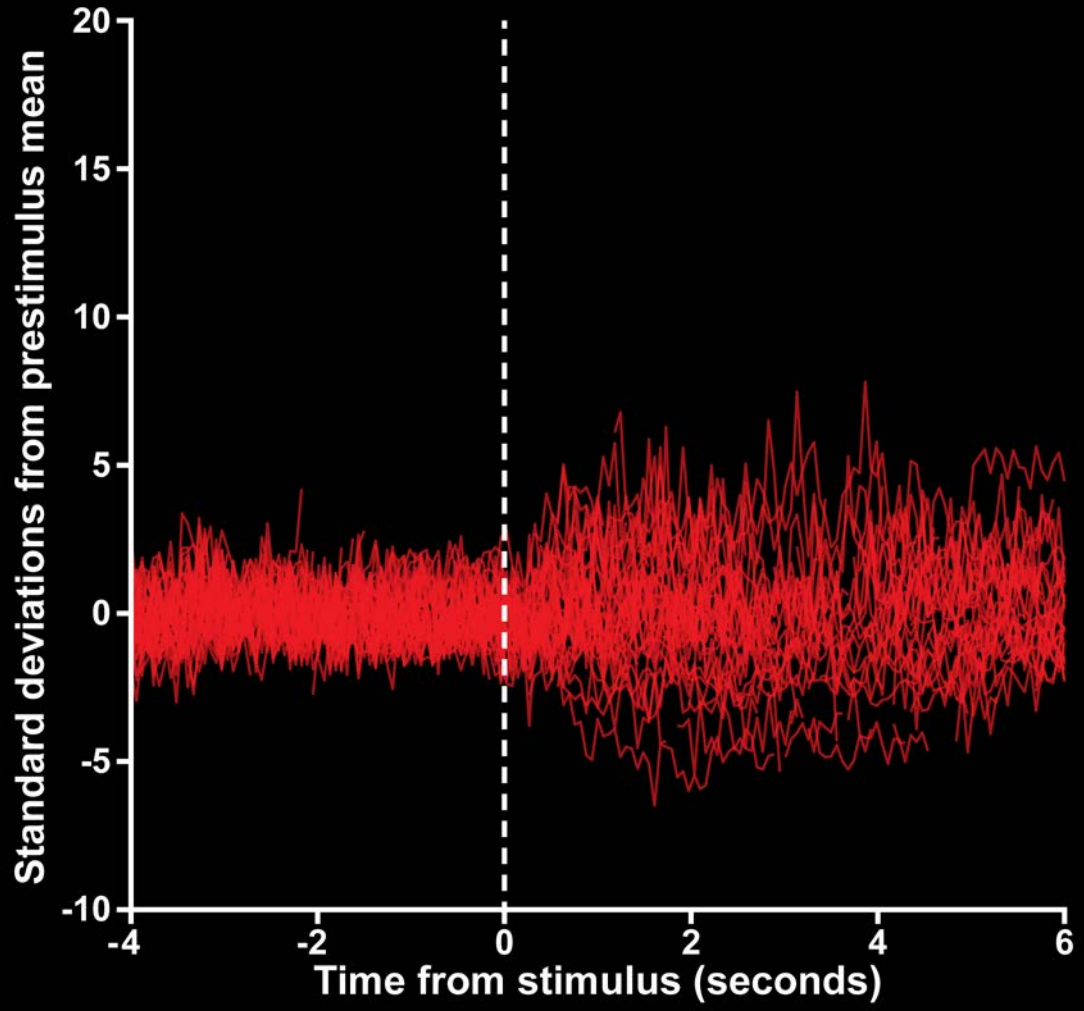
1. While imaging the cones with near infrared light, apply a visible light stimulus to the retina at the same location as the imaging field.
2. While imaging the cones with near infrared light, do nothing.

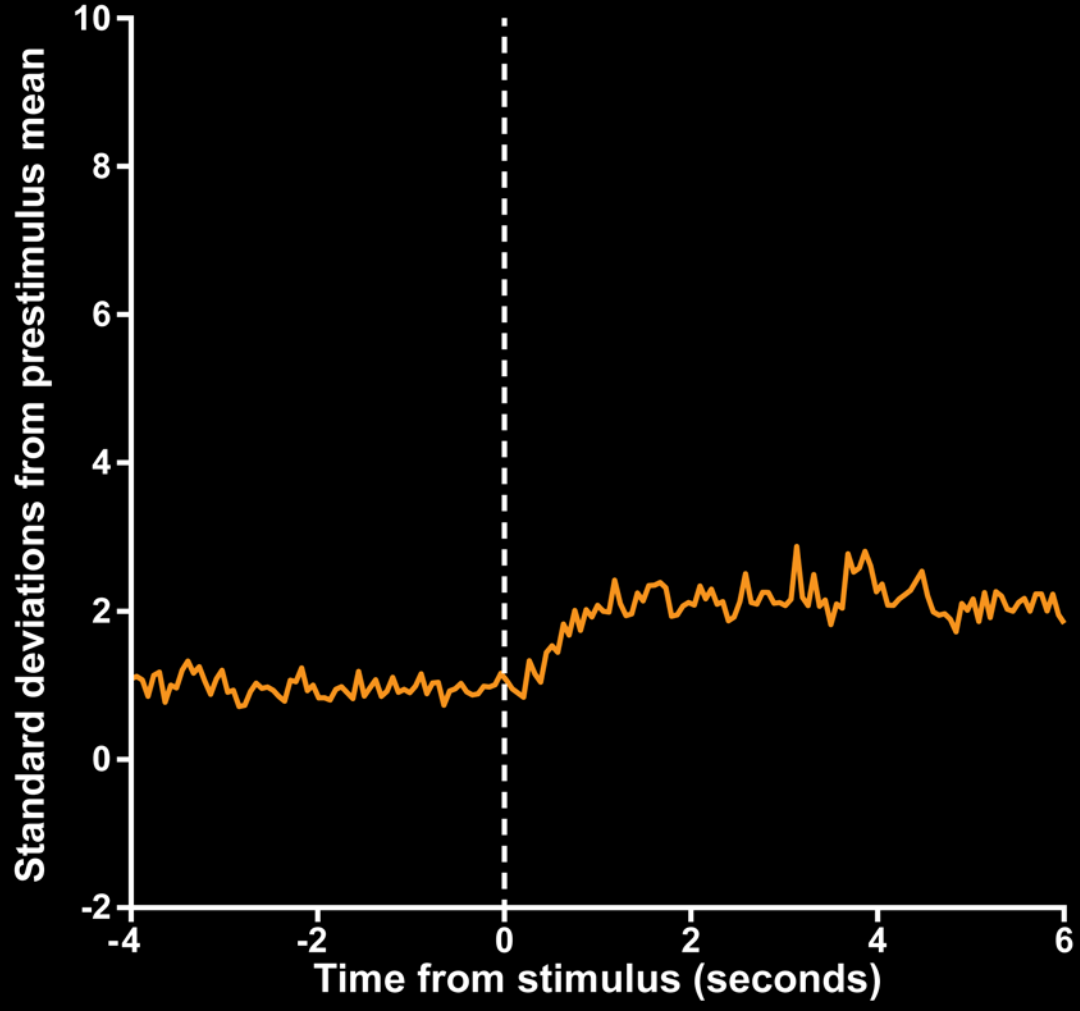
Individual cones exhibit an intrinsic reflectance response



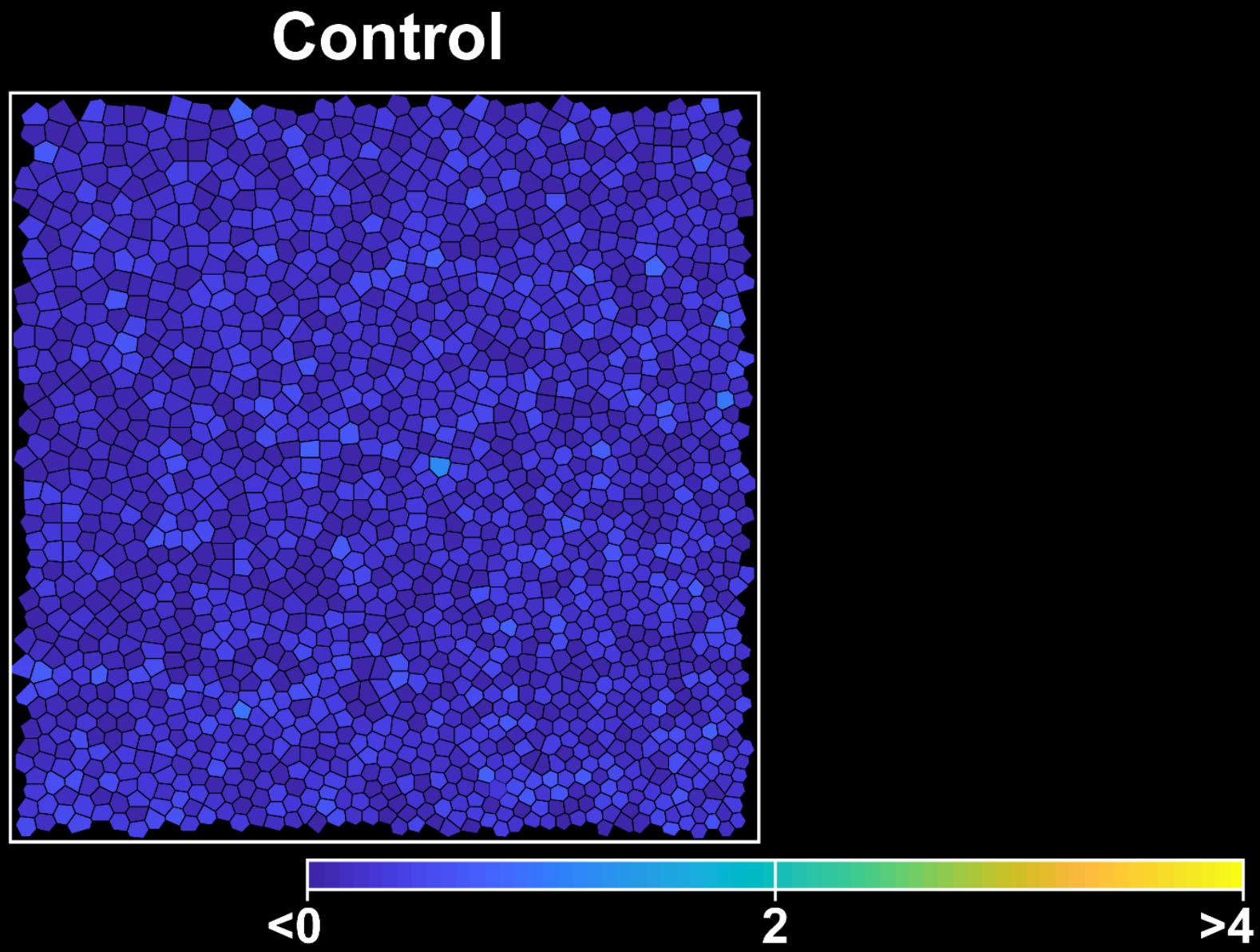


Individual cones exhibit an intrinsic reflectance response

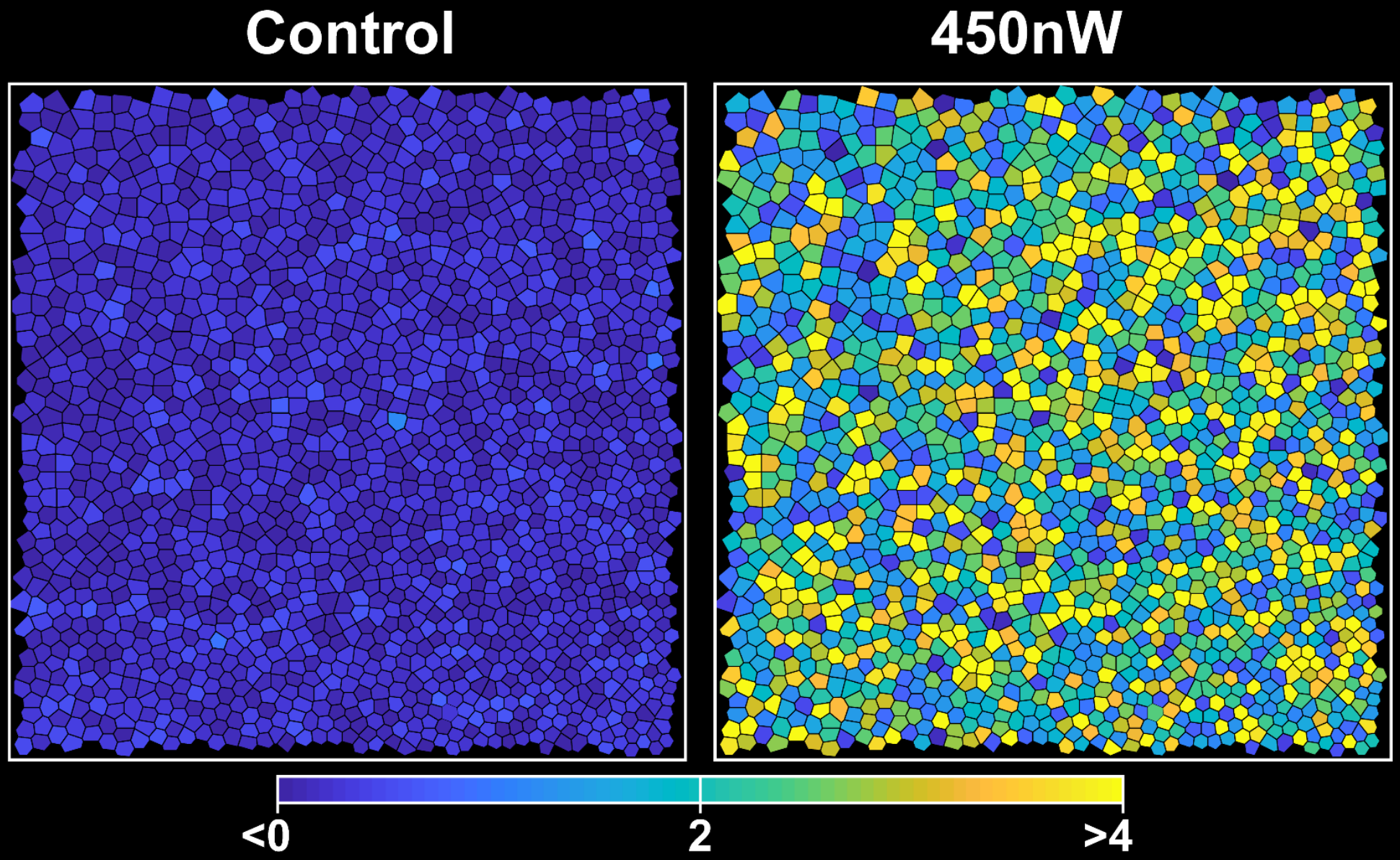


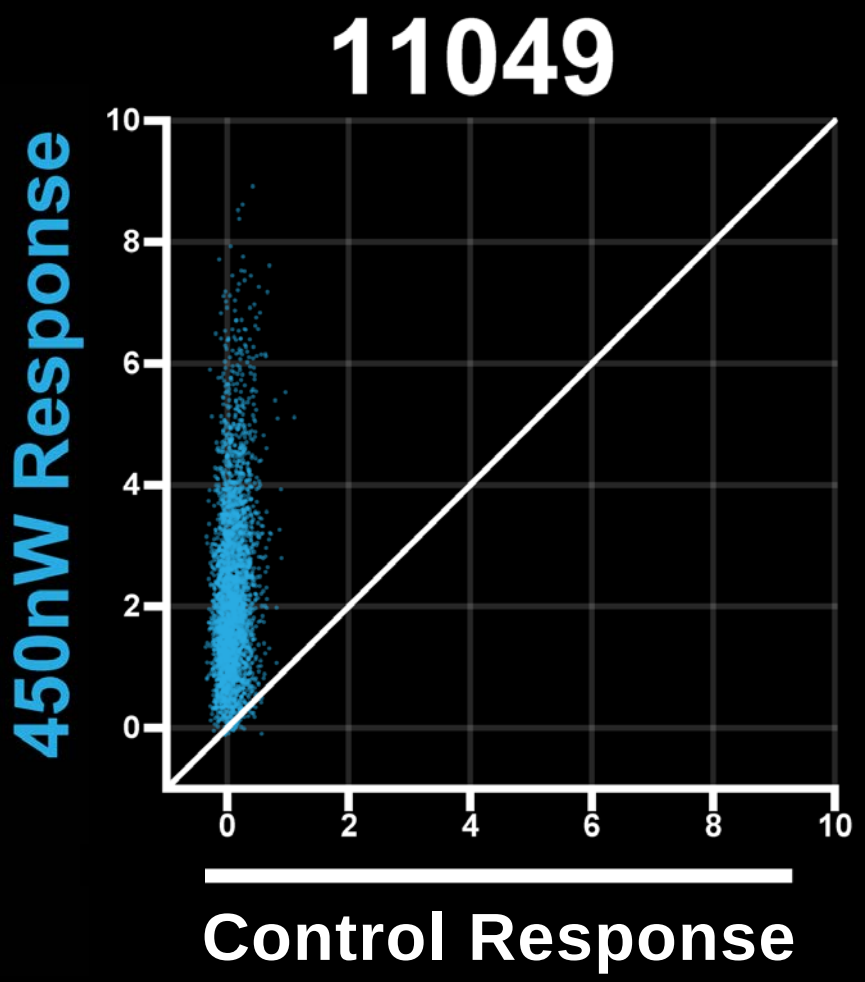


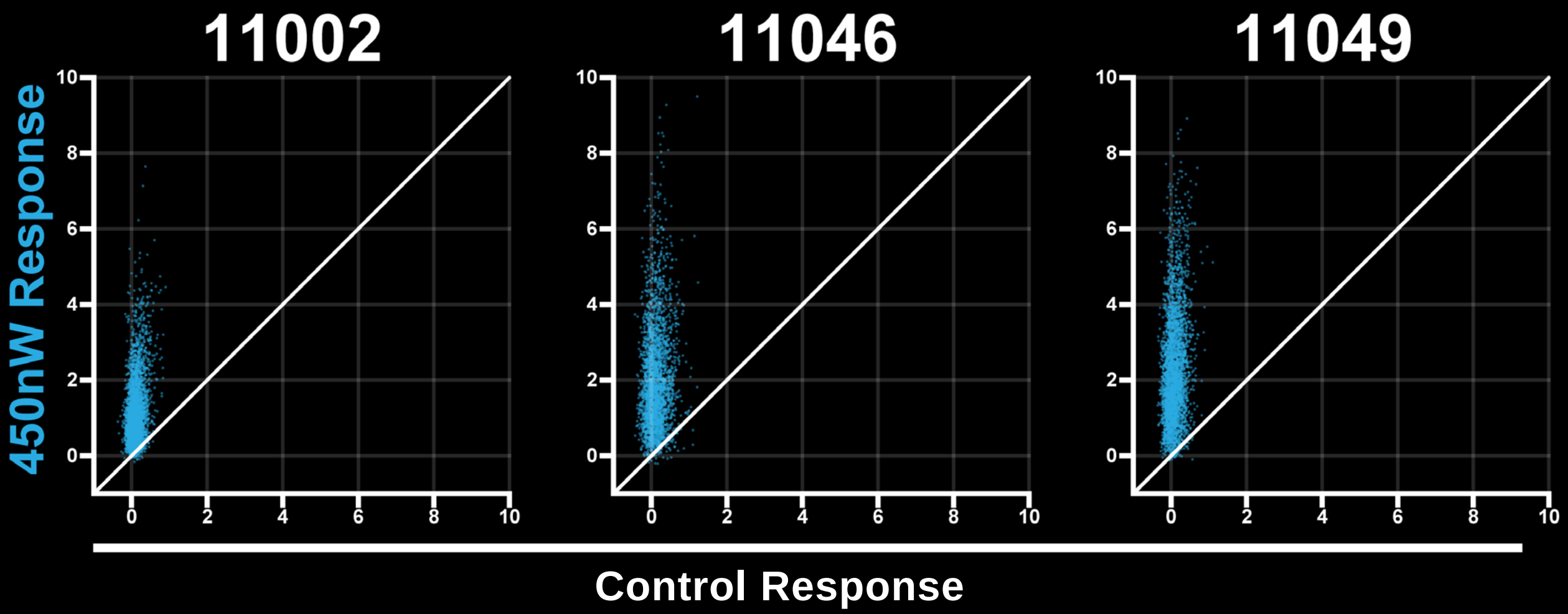
The single cone intrinsic reflectance response



The single cone intrinsic reflectance response




11049
450nW Response
10
8
6
4
2
0
0
2
4
6
8
10
Control Response


11002
11046
11049
450nW Response
Control Response
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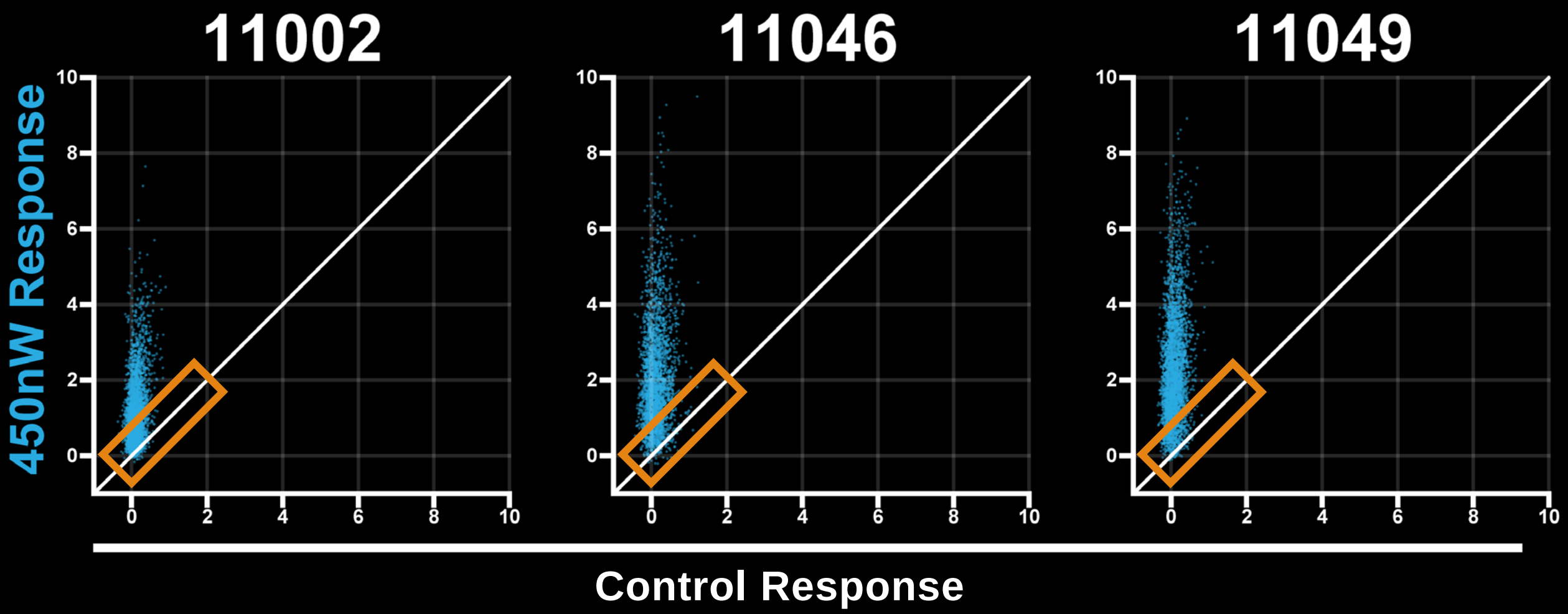
11002
11046
11049
450nW Response
Control Response
0
2
4
6
8
10

Individual cones exhibit an intrinsic reflectance response

- ✓ Quantifiable / Repeatable
- ✓ Dose sensitive
- ✓ Functionally significant
- ✓ Clinically relevant
- ✓ Optimized

The intrinsic reflectance response is a biomarker of cone function

- ✓ Quantifiable / Repeatable
- ✓ Dose sensitive
- ✓ Functionally significant
- ✓ Clinically relevant
- ✓ Optimized

Using intrinsic optical signals to visualize photoreceptor activity in human retina

Dierck Hillmann,[1,2] Clara Pfäffle,[2,3] Hendrik Spahr,[2,3] Sazan Burhan,[2] Lisa Kutzner,[2] Felix Hilge,[2] and Gereon Hüttmann[2,3,4]

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[4]Airway Research Center North (ARCN), German Centre for Lung Research (DZL)

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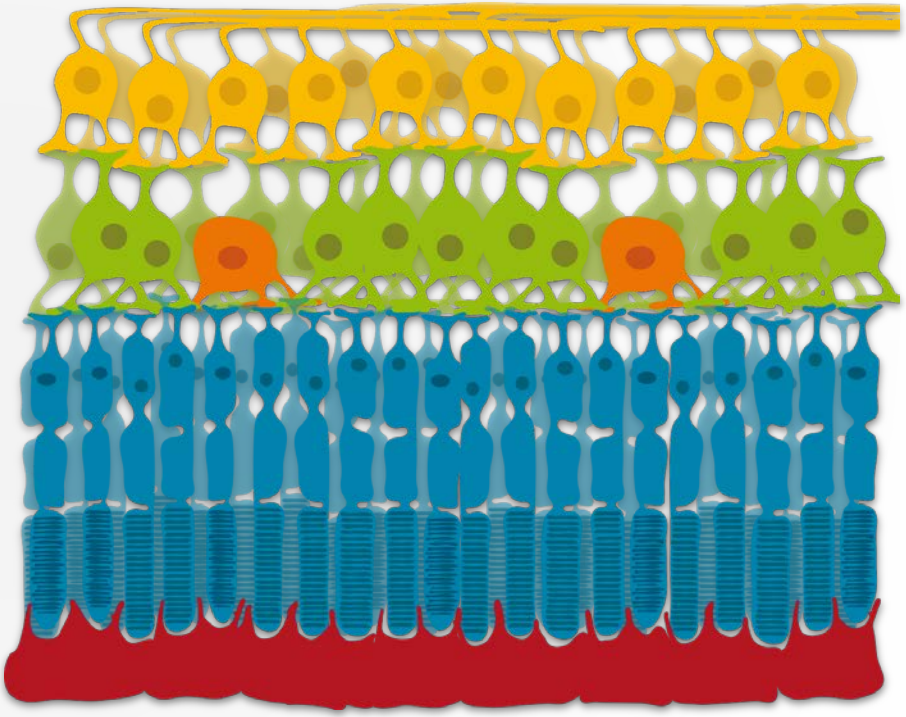
UNIVERSITÄT ZU LÜBECK
INSTITUT FÜR BIOMEDIZINISCHE OPTIK

The human retina

- Layered structure of the retina
  - RPE
  - photoreceptors
  - bipolar cells
  - ganglion cells
  - nerve fibres
- Seeing begins in the photoreceptors
  - light sensitive receptors
  - photoabsorption initializes a signaling cascade
- Neurons and nerves
  - initial post processing
  - transmit the signal to the brain



Reactions of photoreceptor to stimulation

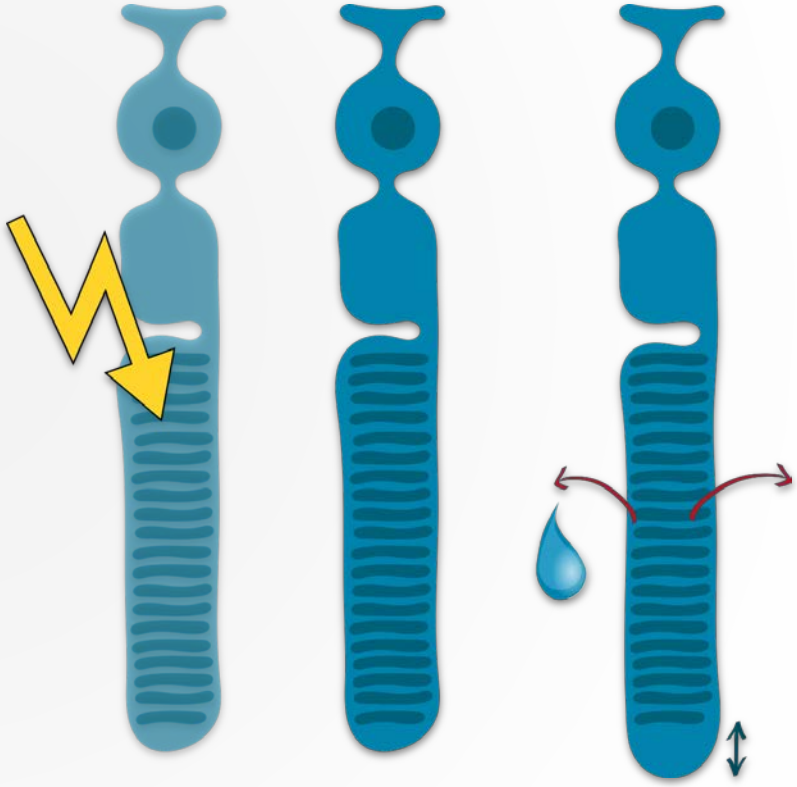
Motivation to visualize photoreceptor activity

- Research on phototransduction or perception
- Clinical diagnosis
- Therapy monitoring

Optical detection of activity with intrinsic signals

- Changes in backscattering
- Changes in optical path length
  - refractive index
  - length

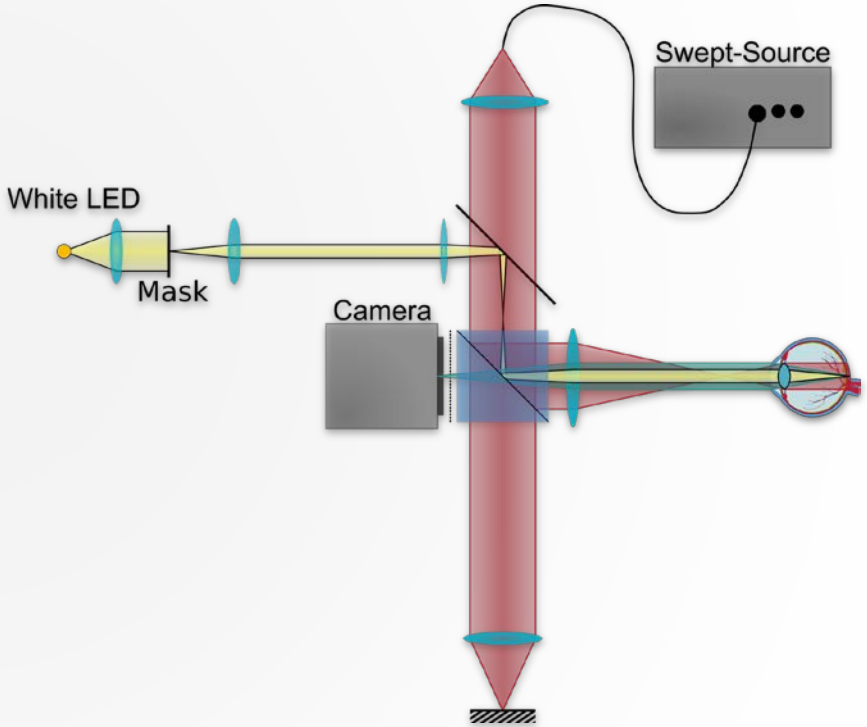
Any possible change is expected to be very small



A special OCT system...

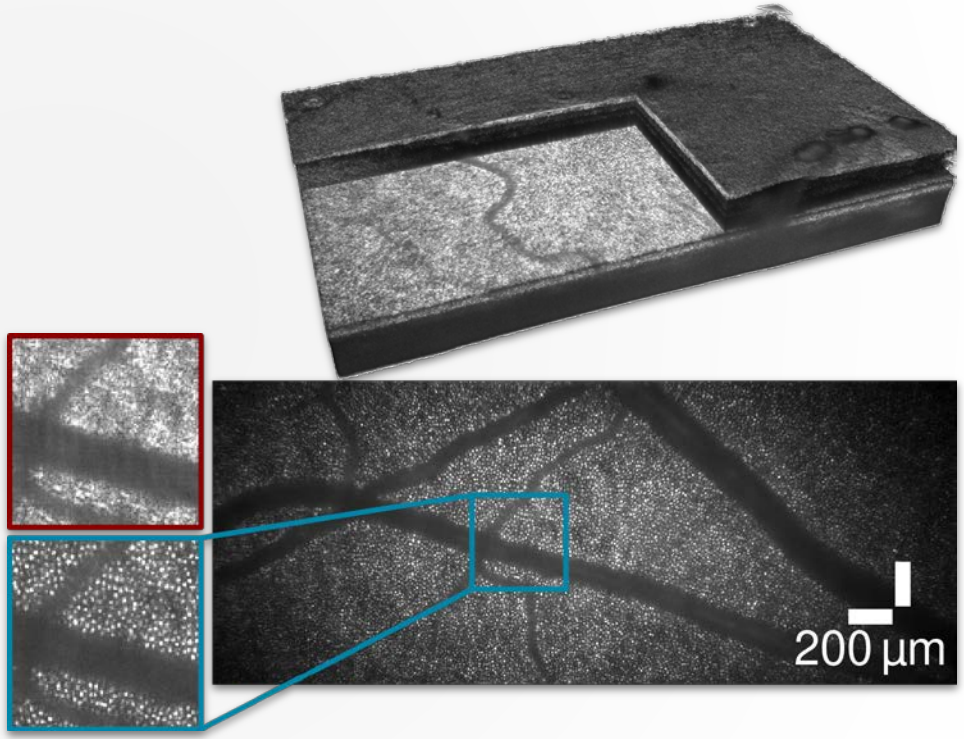
Full-Field Swept-Source Optical Coherence Tomography

- High-Speed Camera (Photron SA-Z, 70,000 frames/s, with 640 x 368 pixels)
- Superlum Broadsweeper ($\Delta\lambda$ = 50 nm, λ_0 = 840 nm)
- During the laser sweep, each pixel of the camera acquires one A-scan
- Volumetric imaging
- 6 ms for one volume, up to 166 volumes/s
- More than 40 MHz A-scan rate
- Laterally phase stable
- White light stimulus with about 10 µW



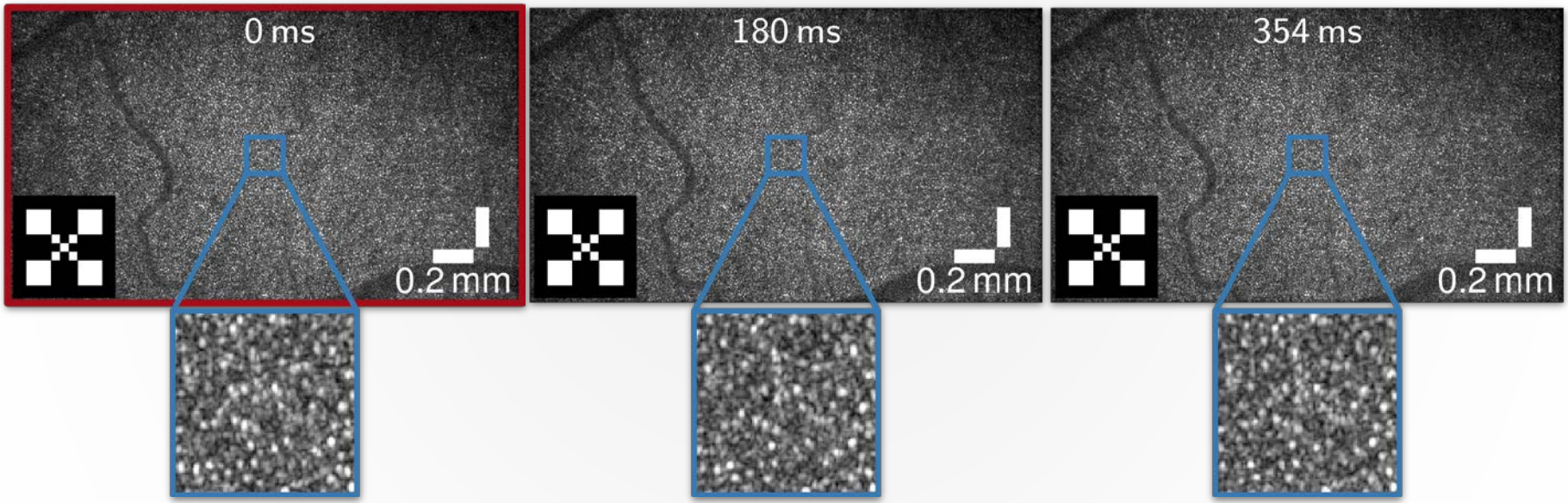
Possibilities of phase sensitive OCT

- Post-processing
  - intra-volume motion and dispersion correction
  - inter-volume motion correction
  - computational aberration correction
  - co-registration
  - segmentation
- Tracking single photoreceptors over a few seconds with high temporal resolution



Does reflected intensity change?

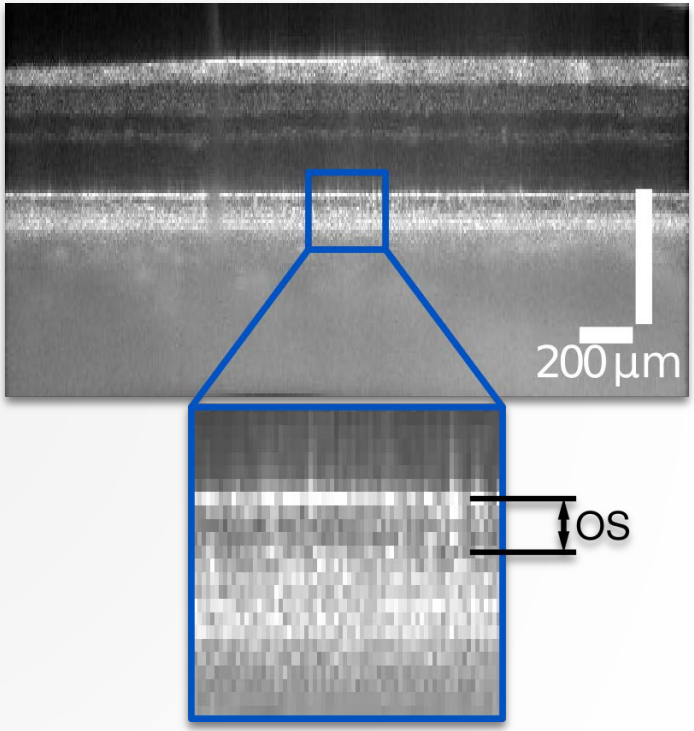
- 50 ms stimulus
- 6 ms between volumes



- No reproducible, clear changes observed

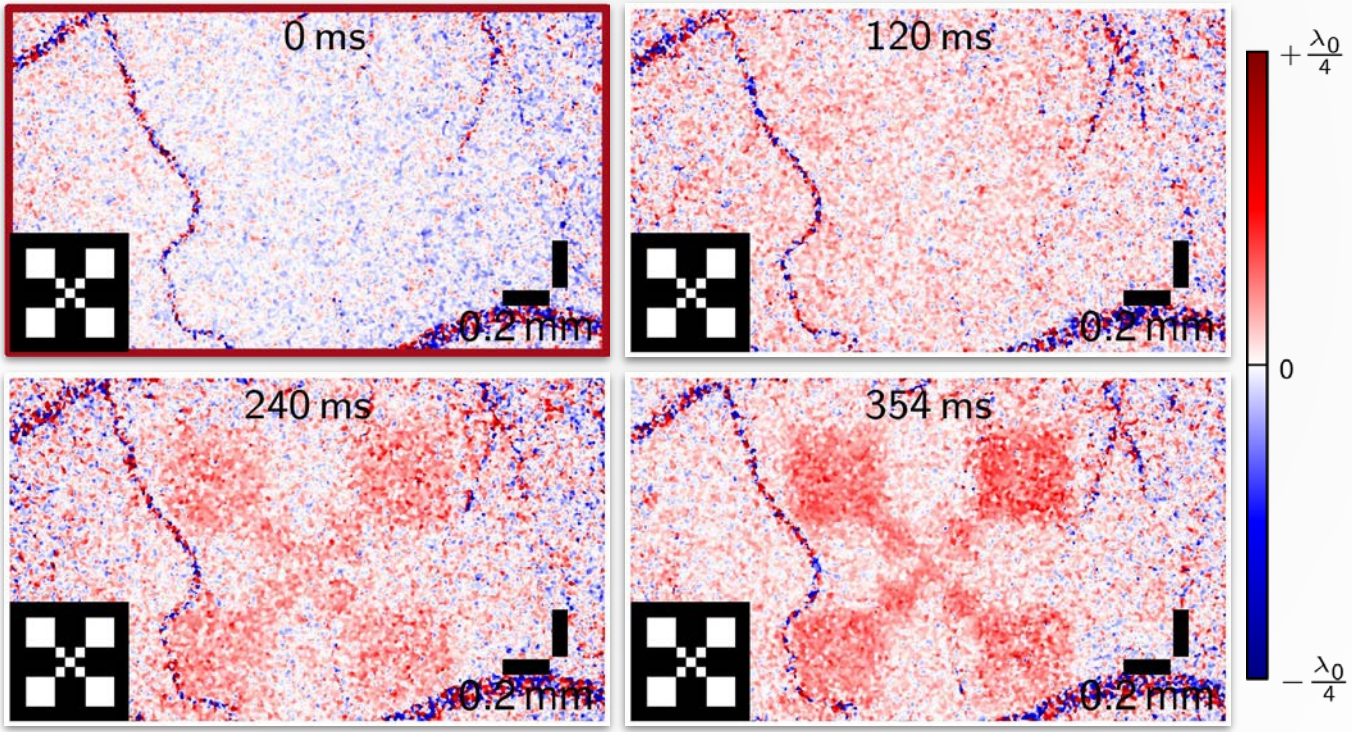
Principle of phase evaluation

- Only phase changes give information
- **Axial phase difference** between the
  - Inner segment/Outer segment
  - Outer segment tips
- **Temporal phase difference** to a pre-stimulus volume



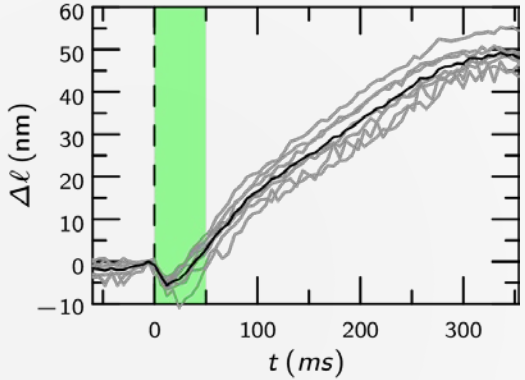
Observed changes in phase

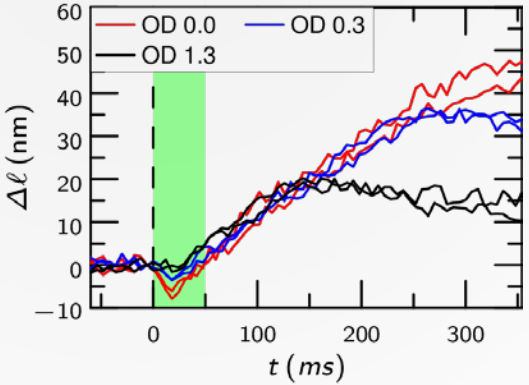
- Same measurement (50 ms stimulus, 6 ms/volume); with phase evaluation

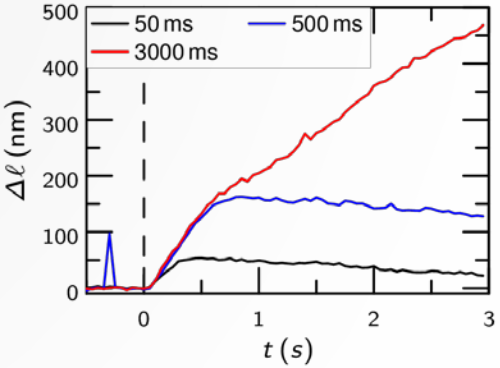


Studying the time course and signal dependencies

- Phase changes are repeatable
- Signal slope seems to be independent of stimulus strength
- Initial “dip” dependent on stimulus strength
- Signal increases with longer stimulation

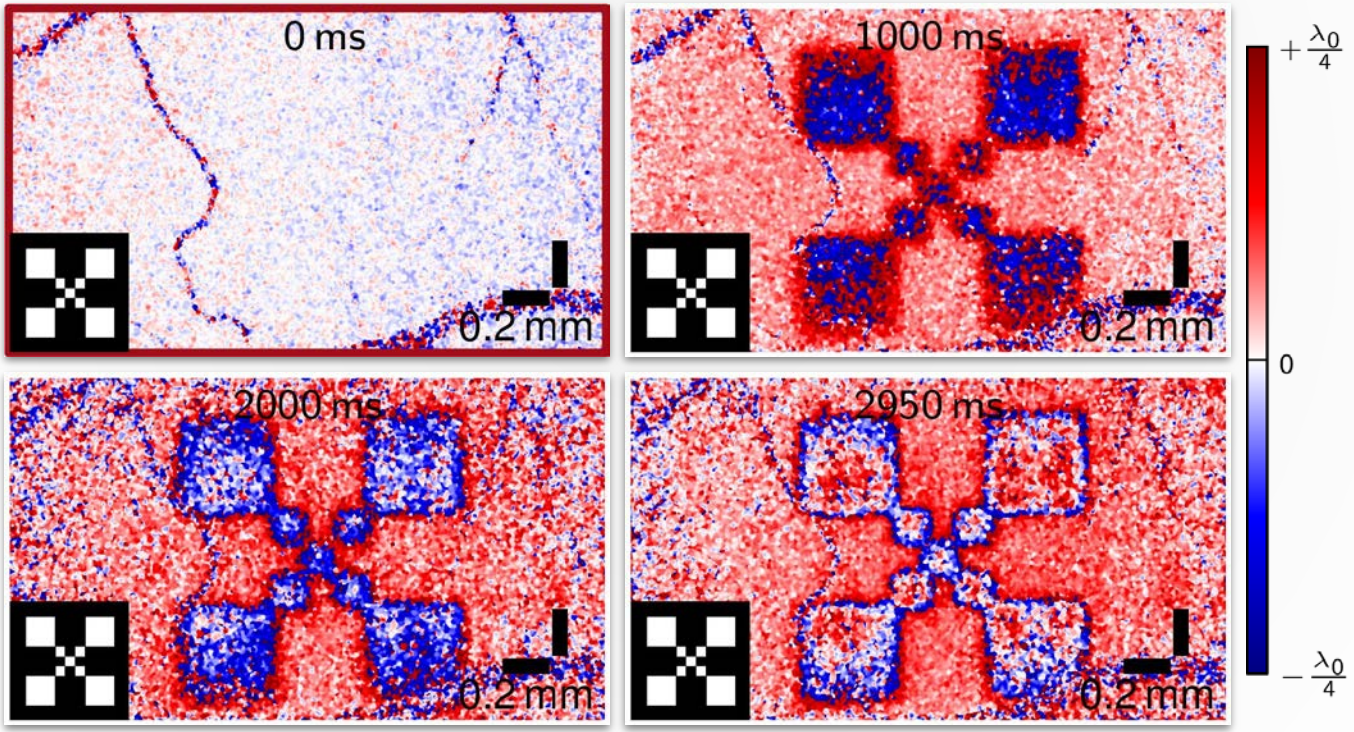






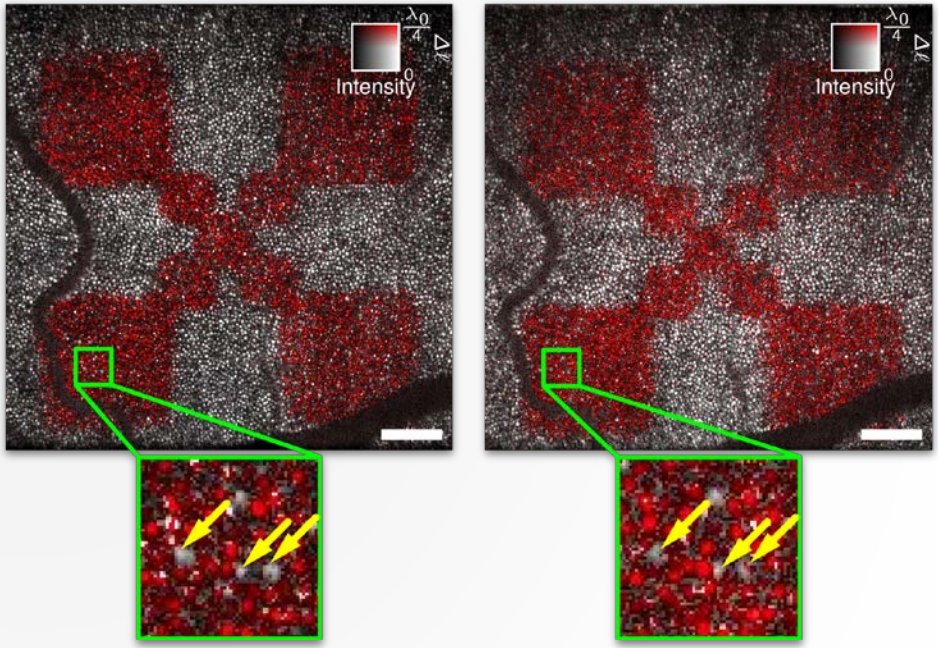
Enhancing the signals...

- Continuous stimulation and longer measurement time enhances signals



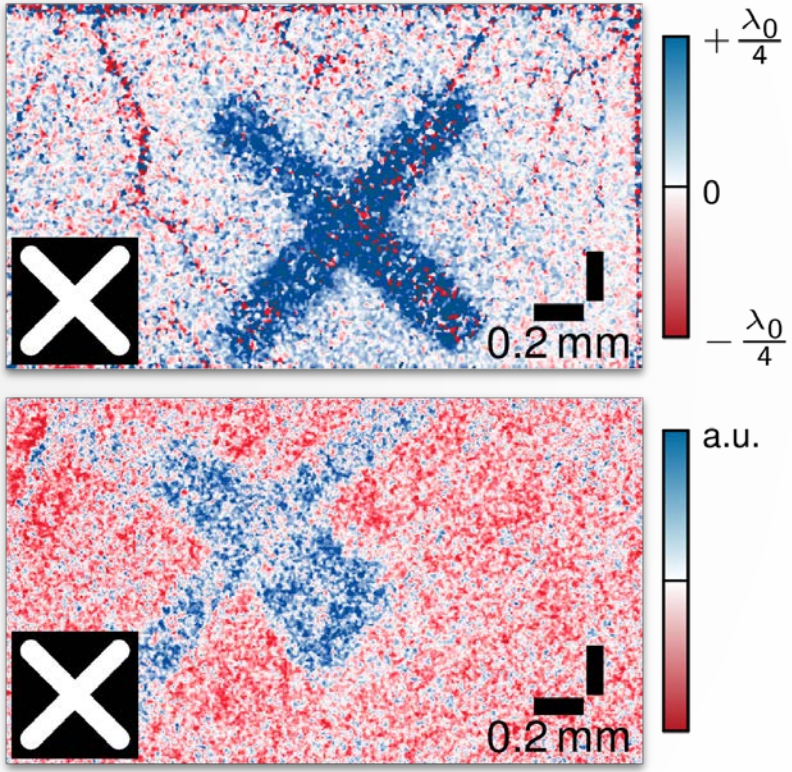
Activity of single cone photoreceptors

- Attempt to assign the activation signal to single cones
  - Filtering the signals in time
  - Setting a certain threshold
- Same cones appear to not react
  - Reproducible in a 10 minute time frame



Visualizing ganglion cell activity

- Method can be applied to **ganglion cell** layer
- Signal is significantly **weaker** and requires additional post processing
- When compared to the photoreceptor response, signal is **laterally shifted** and **deformed**



Conclusion and Outlook

- We observed clear intrinsic optical signals upon light stimulation with high-speed full-field swept-source OCT in living humans
- We visualized
  - photoreceptor activity
  - ganglion cell activity
- We measured time course and dependencies on the stimulation
- Results suggest osmotically driven process linked to ion influx/efflux and photo current

References

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7. Hillmann, D. *et al.* In vivo optical imaging of physiological responses to photostimulation in human photoreceptors. *Proc. Natl. Acad. Sci.* **113,** 13138–13143 (2016).
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9. Hillmann, D. *et al.* Aberration-free volumetric high-speed imaging of in vivo retina. *Sci. Rep.* **6,** 35209 (2016).

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- Gesa Franke
- Peter Koch

VSRI VISION SCIENCE AND ADVANCED RETINAL IMAGING LABORATORY
UNIVERSITY OF CALIFORNIA, DAVIS

Imaging structure and function in the living human retina with adaptive optics optical coherence tomography

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Postdoctoral Research Fellow

Vision Science and Advanced Retinal Imaging Laboratory,

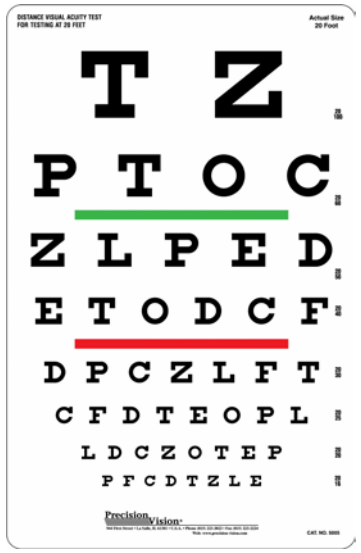
UC-Davis Eye Center

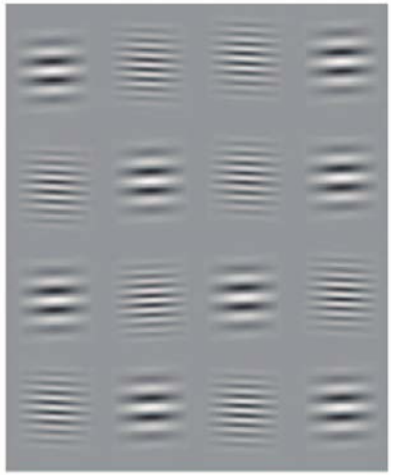
Existing Methods for Testing Retinal Function

Subjective testing

- Visual acuity / contrast sensitivity tests
- Psychophysics

Poor spatial resolution; subjective effects often manifest late in disease progression

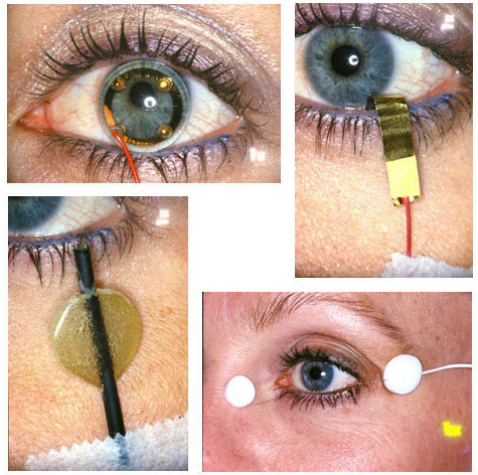




Electrophysiology

- ERG
- mfERG

Poor spatial resolution; slightly invasive; long duration of tests; crosstalk between photoreceptors and postreceptoral neurons



Significance of Functional Photoreceptor Imaging

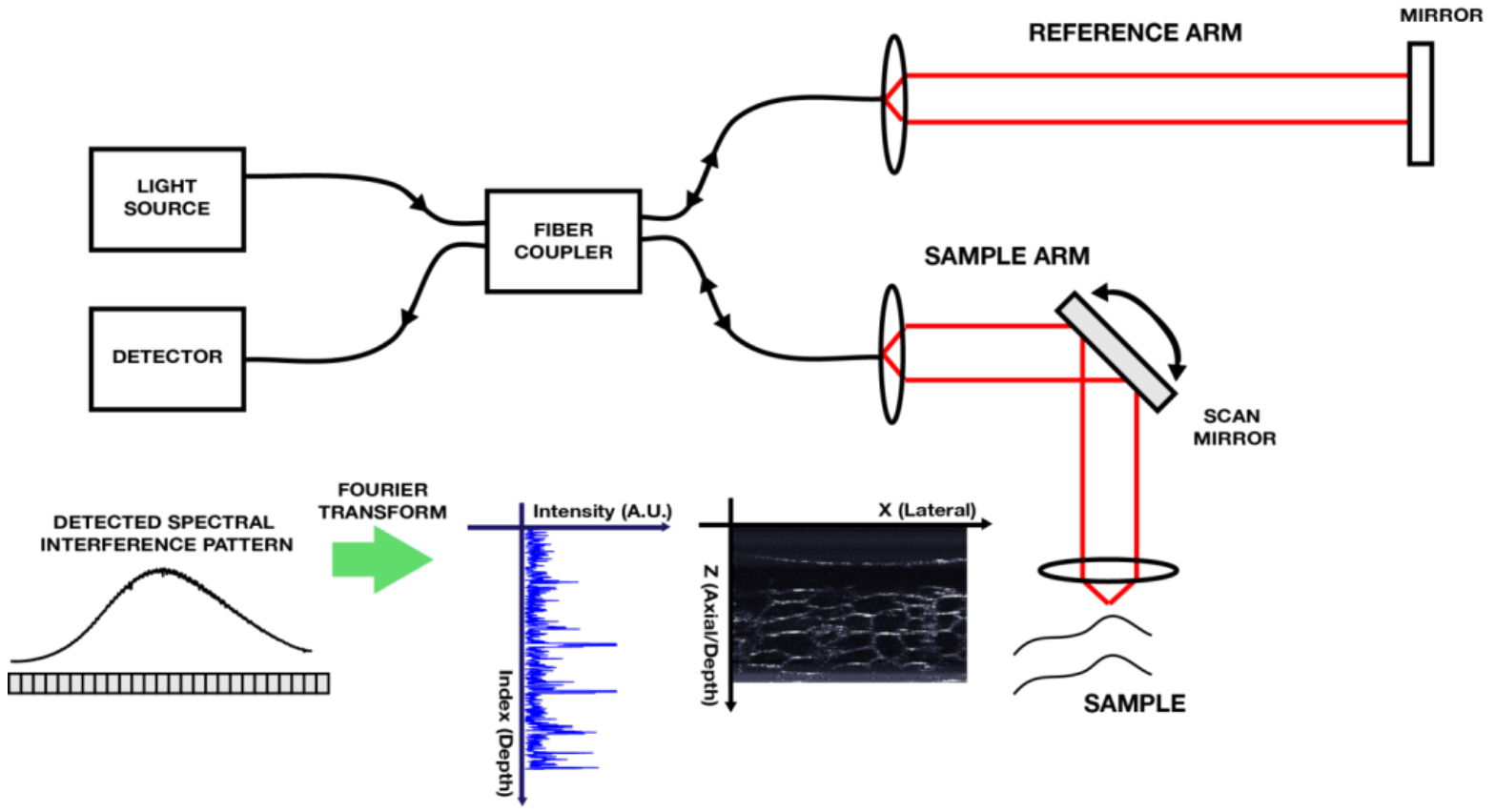
Clinical significance:

- Earlier detection of retinal degenerations
- Improved assessment of therapeutic efficacy (stem cells, gene therapy, drugs)
- Disease monitoring with better sensitivity and spatial resolution

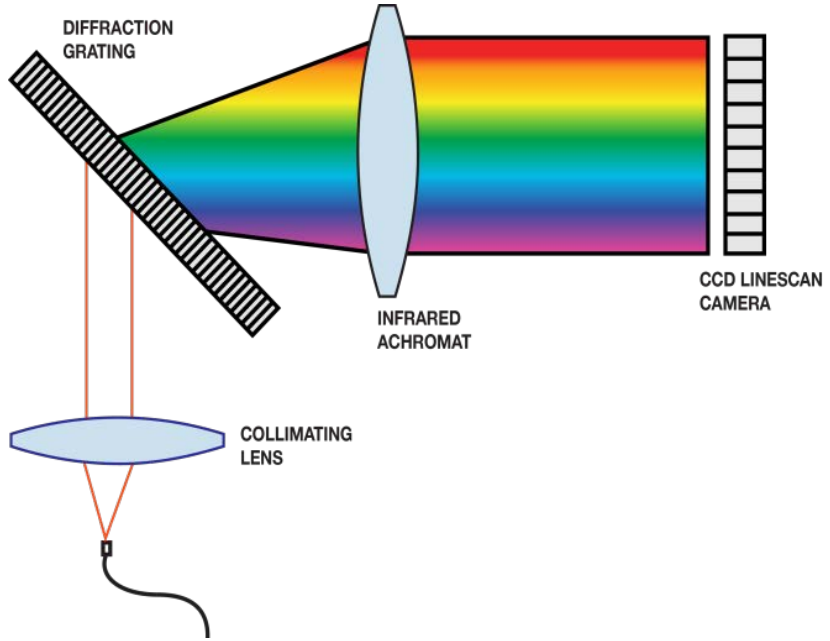
Research significance:

- Improving our understanding of disease mechanisms (e.g., combined with structural RPE imaging and choriocapillaris angiography in AMD)
- Phototransduction is a well-understood process, but has not been studied extensively *in vivo*

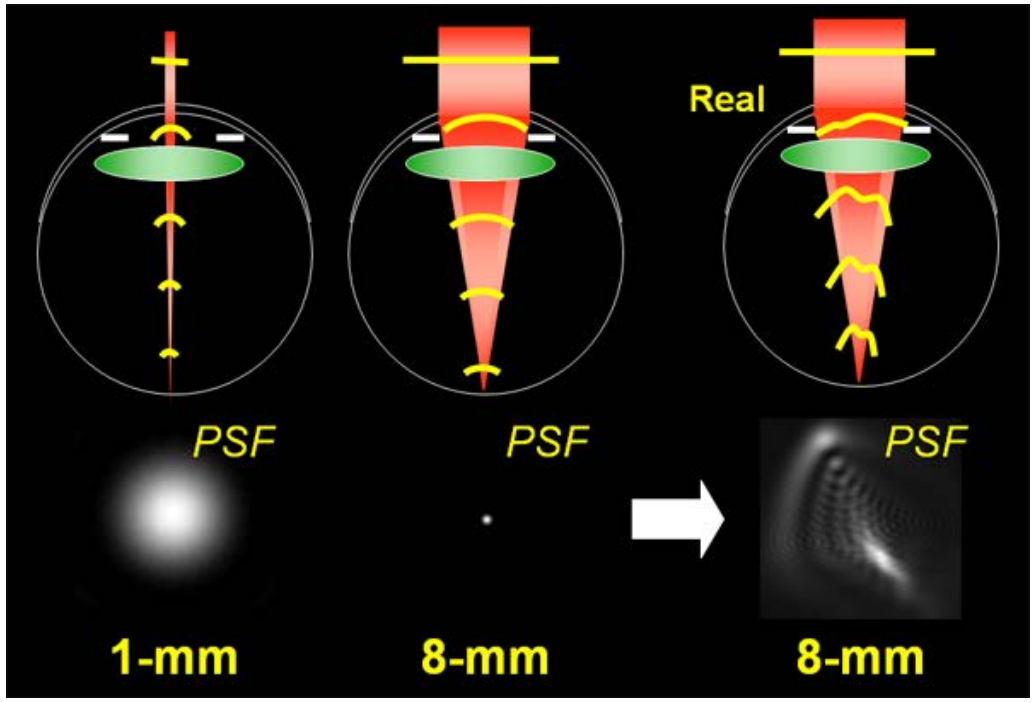
OCT Principle



Detector



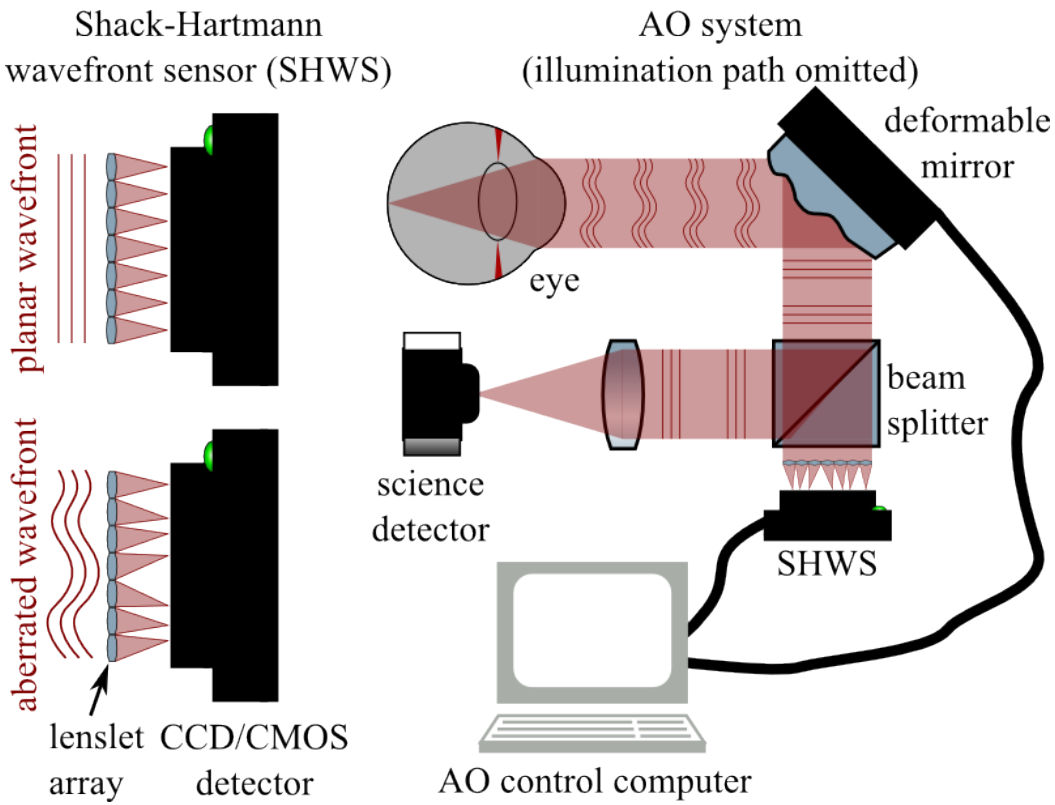
Aberrations in the Eye



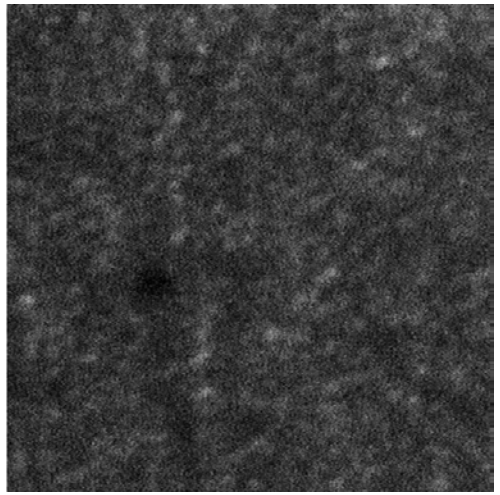
http://www.zmpbmt.meduniwien.ac.at

http://roorda.vision.berkeley.edu/

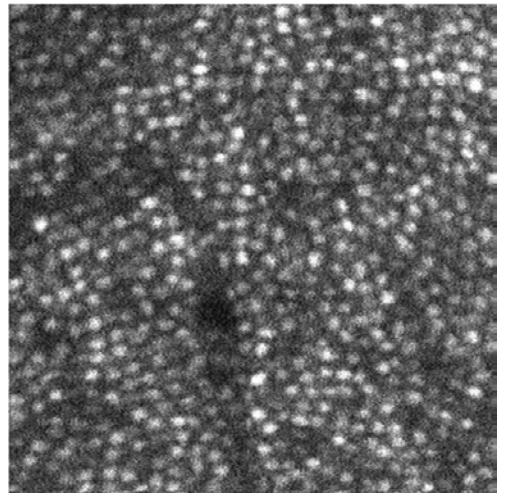
Principle of Adaptive Optics



(A)

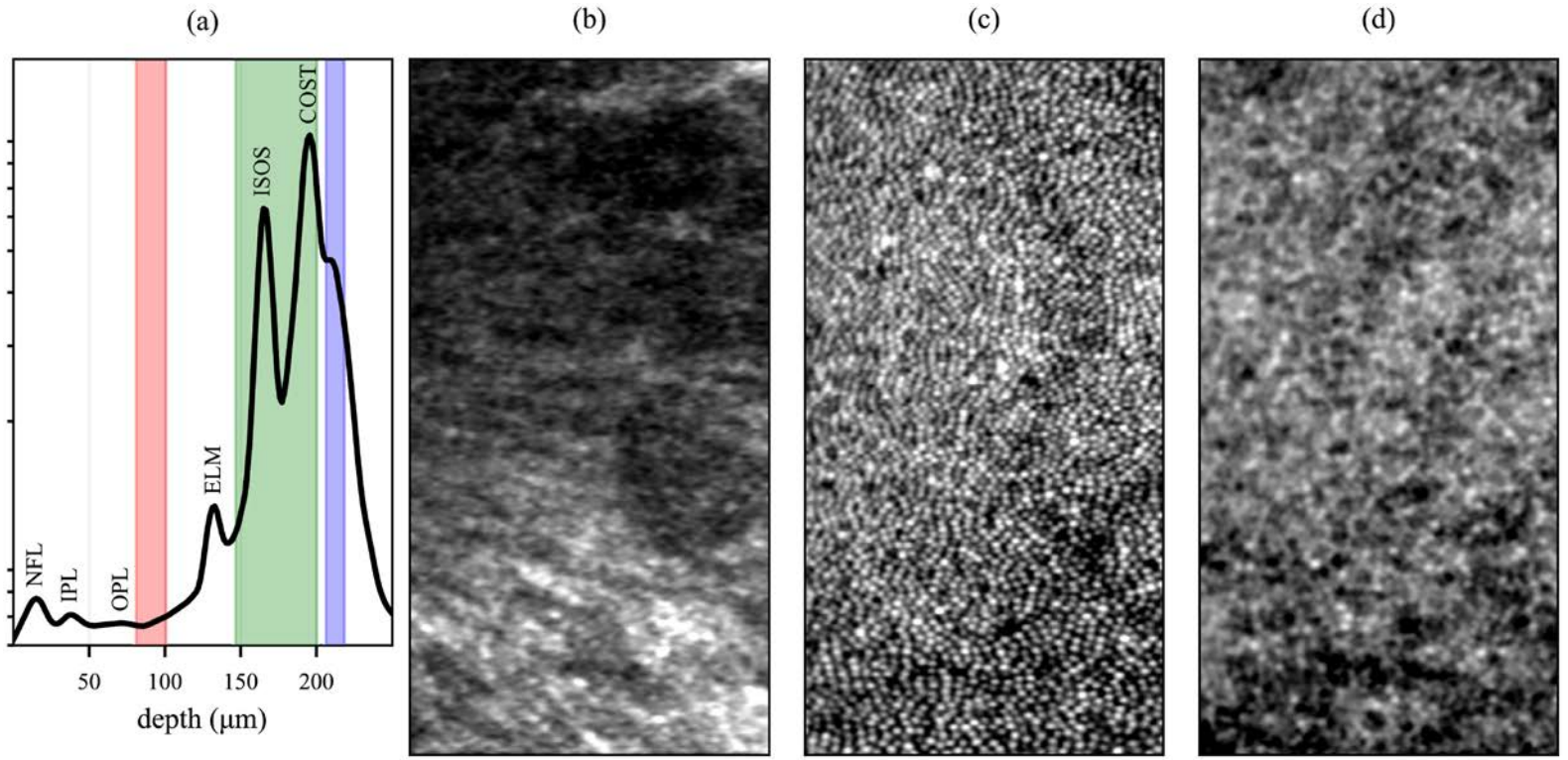


(B)



Mosaic of photoreceptors, (A) before and (B) after AO correction.

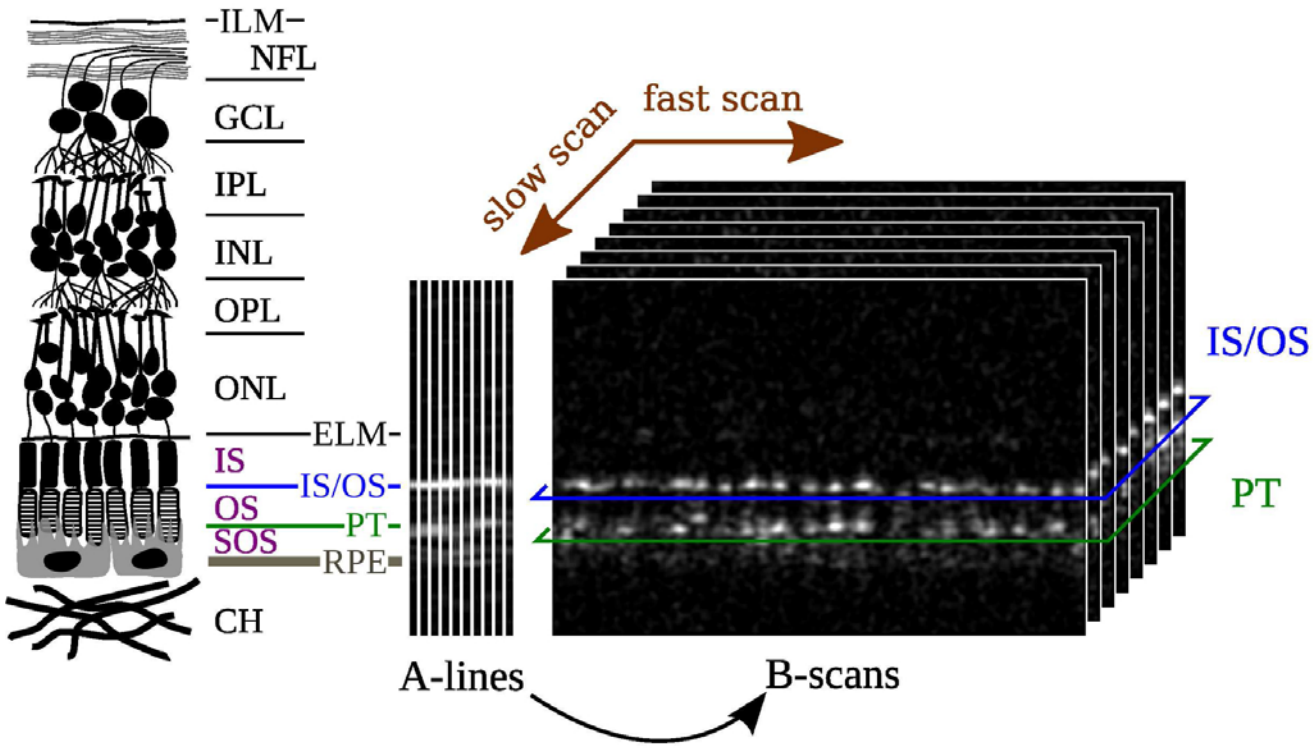
AO-OCT Provides Three-dimensional Cellular Resolution



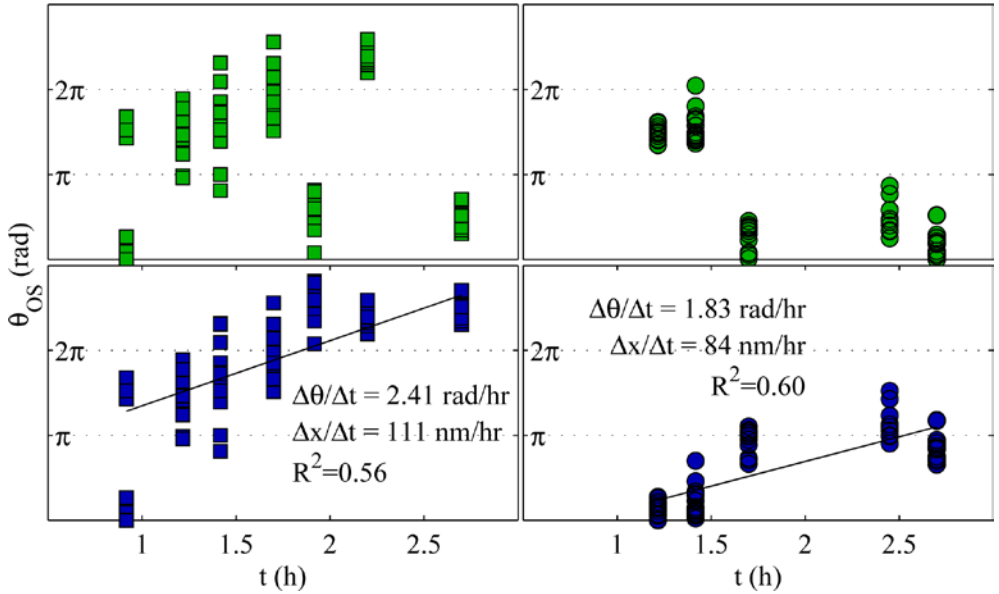
Averaging the AO-OCT volume in the two lateral dimensions produces a longitudinal reflectance profile, shown (a) in log scale. By extracting and averaging together corresponding depths of interest from the motion-corrected volumetric image, projections of (A) Henle fiber layer (HFL), (B) cone outer segments (COS), and (C) retinal pigment epithelium (RPE) layers can be produced.

Azimipour M, Zawadzki RJ, Gorczynska I, Migacz J, Werner JS, Jonnal RS (2018) Intraframe motion correction for raster-scanned adaptive optics images using strip-based cross-correlation lag biases. **PLoS ONE** 13(10): e0206052.

Referenced Phase Measurement in OCT



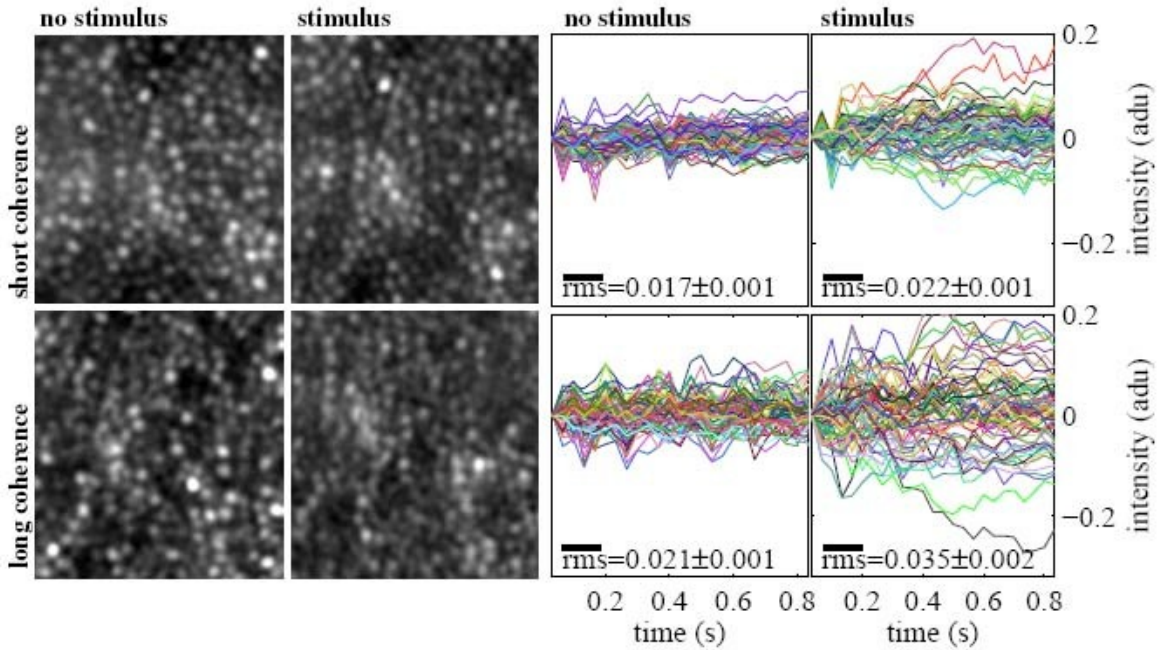
Conceptual diagram of OCT imaging. OCT is principally a depth imaging modality.

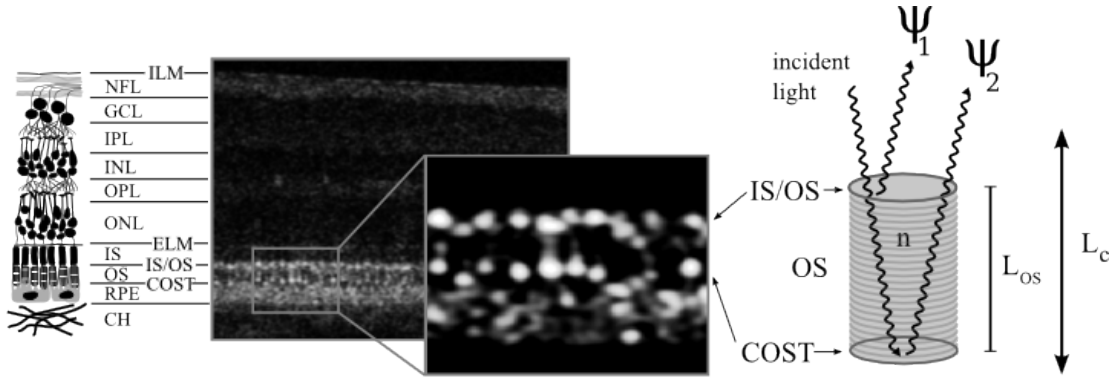


Representative phase changes in two single cones. For each cone, the temporally wrapped data are shown in the top plot (green markers) and temporally unwrapped data shown in the bottom plot (blue markers).

Ravi S. Jonnal, Omer P. Kocaoglu, Qiang Wang, Sangyeol Lee, and Donald T. Miller, "Phase-sensitive imaging of the outer retina using optical coherence tomography and adaptive optics," Biomed. Opt. Express 3, 104-124 (2012)

Early Evidence of Light-Induced OS Swelling

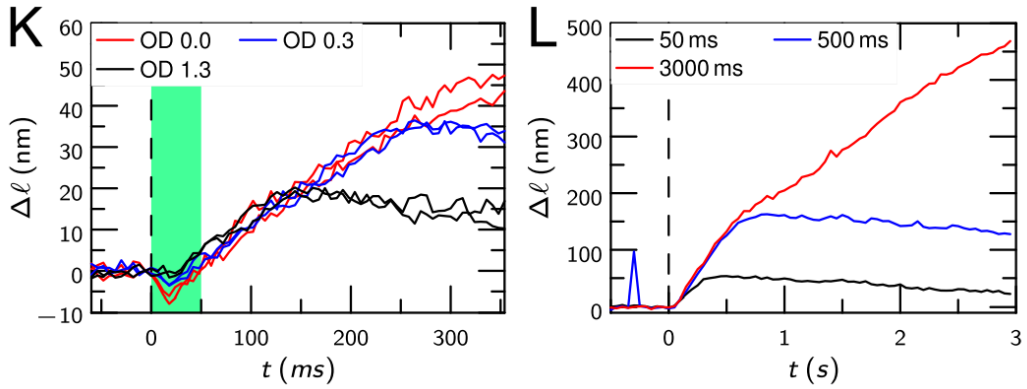




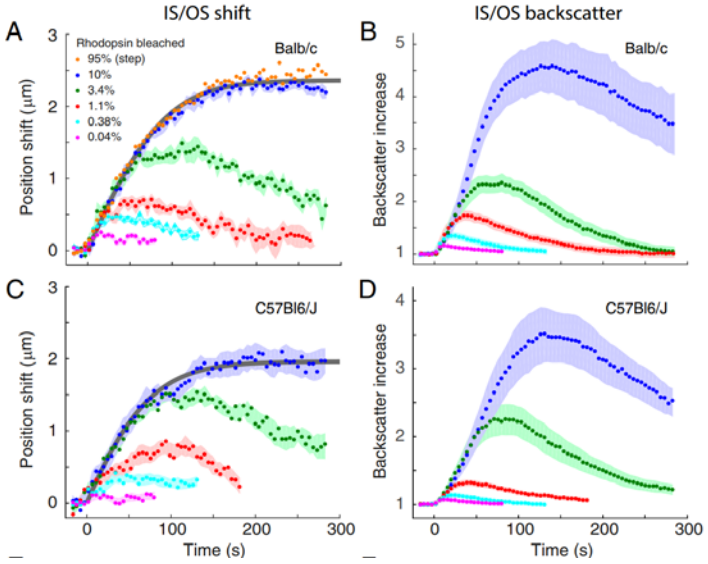
Cone mosaic of the same retinal patch under four different imaging conditions: short coherence and no stimulus (upper left), short coherence and stimulus (upper right), long coherence and no stimulus (lower left), and long coherence and stimulus (lower right). The long coherence/stimulus video shows the most scintillation-nearly every cone scintillates. A few cones appear to scintillate in the short coherence/stimulus case.

Ravi S. Jonnal, Jungtae Rha, Yan Zhang, Barry Cense, Weihua Gao, and Donald T. Miller, "In vivo functional imaging of human cone photoreceptors," Opt. Express 15, 16141-16160 (2007)

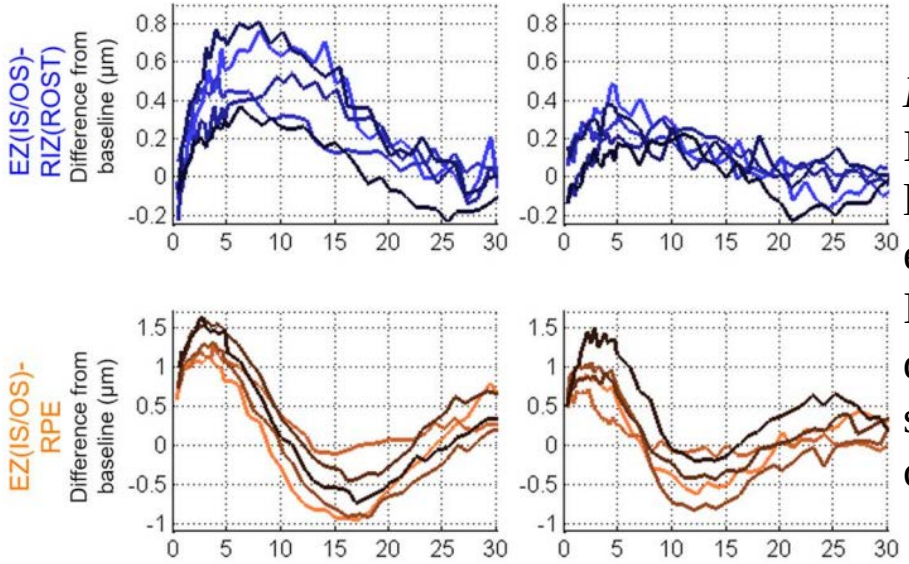
Recent Measurements of Light-Induced OS Swelling



Hillmann et al., 2016
In response to light, peripheral human cones elongate, as shown by phase-sensitive swept-source OCT with digital aberration correction.



Zhang et al., 2017
In response to light, mouse rods elongate (and scatter more), shown with conventional OCT.



Lu et al., 2017
In response to light, human rods elongate, and the IS/OS-RPE distance changes., shown with conventional OCT.

Adaptive Optics Swept-source OCT at 1.6MHz

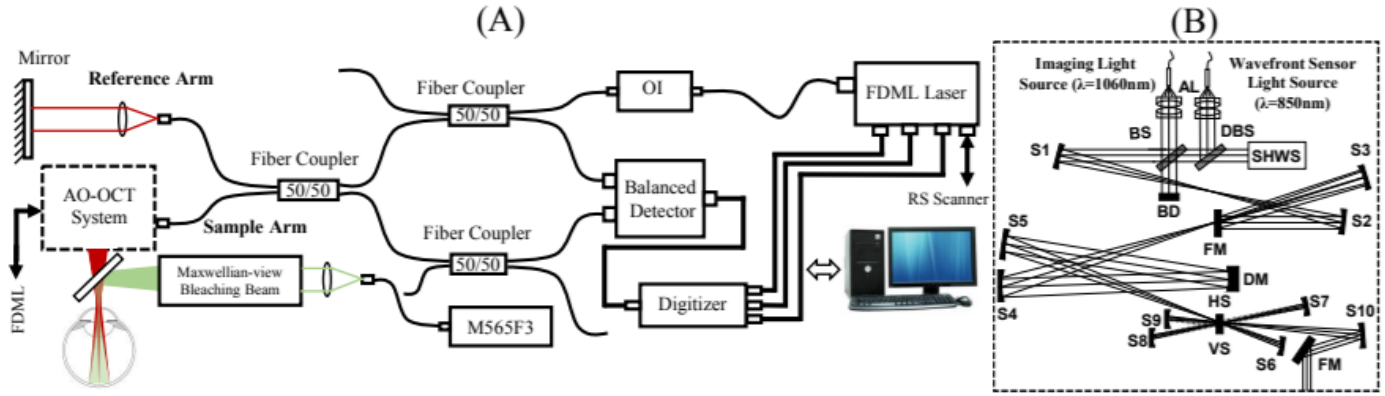


Fig. 1. **(A)** Schematic of the AO-FDML OCT imaging system integrated with Maxwellian-view optical system for bleaching photoreceptors. **(B)** An expanded view of the AO scanning system: DM, deformable mirror; SHWS, Shack-Hartmann wavefront sensor; AL, achromatic lens; S, spherical mirror; FM, flat mirror; BS, beam splitter; DBS, dichroic beam splitter; HS, horizontal scanner; VS, vertical scanner; BD, beam dump; OI, optical isolator.

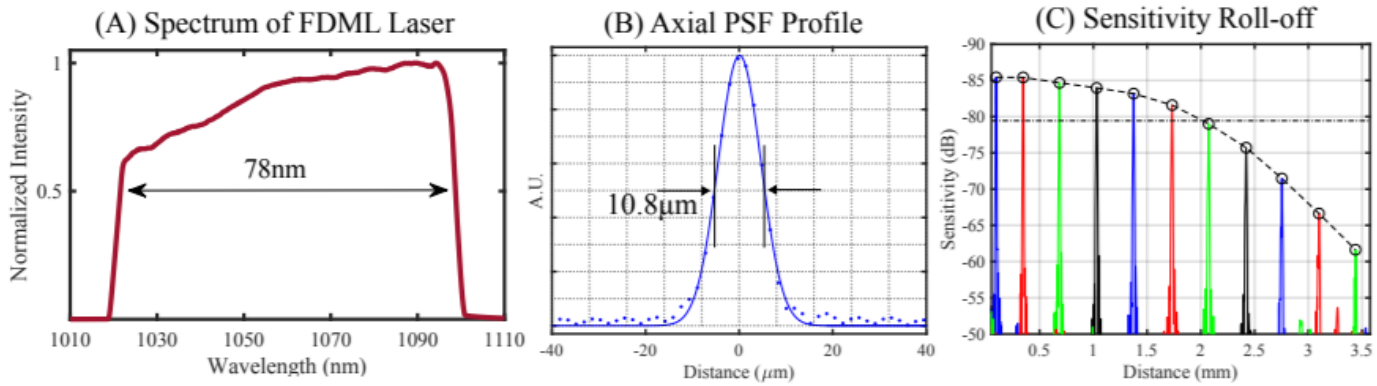


Fig. 2. **(A)** Spectrum of FDML laser. **(B)** Axial point spread function and **(C)** sensitivity roll-off of the imaging system.

Mehdi Azimipour, Justin V. Migacz, Robert J. Zawadzki, John S. Werner, Ravi S. Jonnal, "Functional retinal imaging using adaptive optics swept-source OCT at 1.6MHz", **bioRxiv** 420240 (under review)

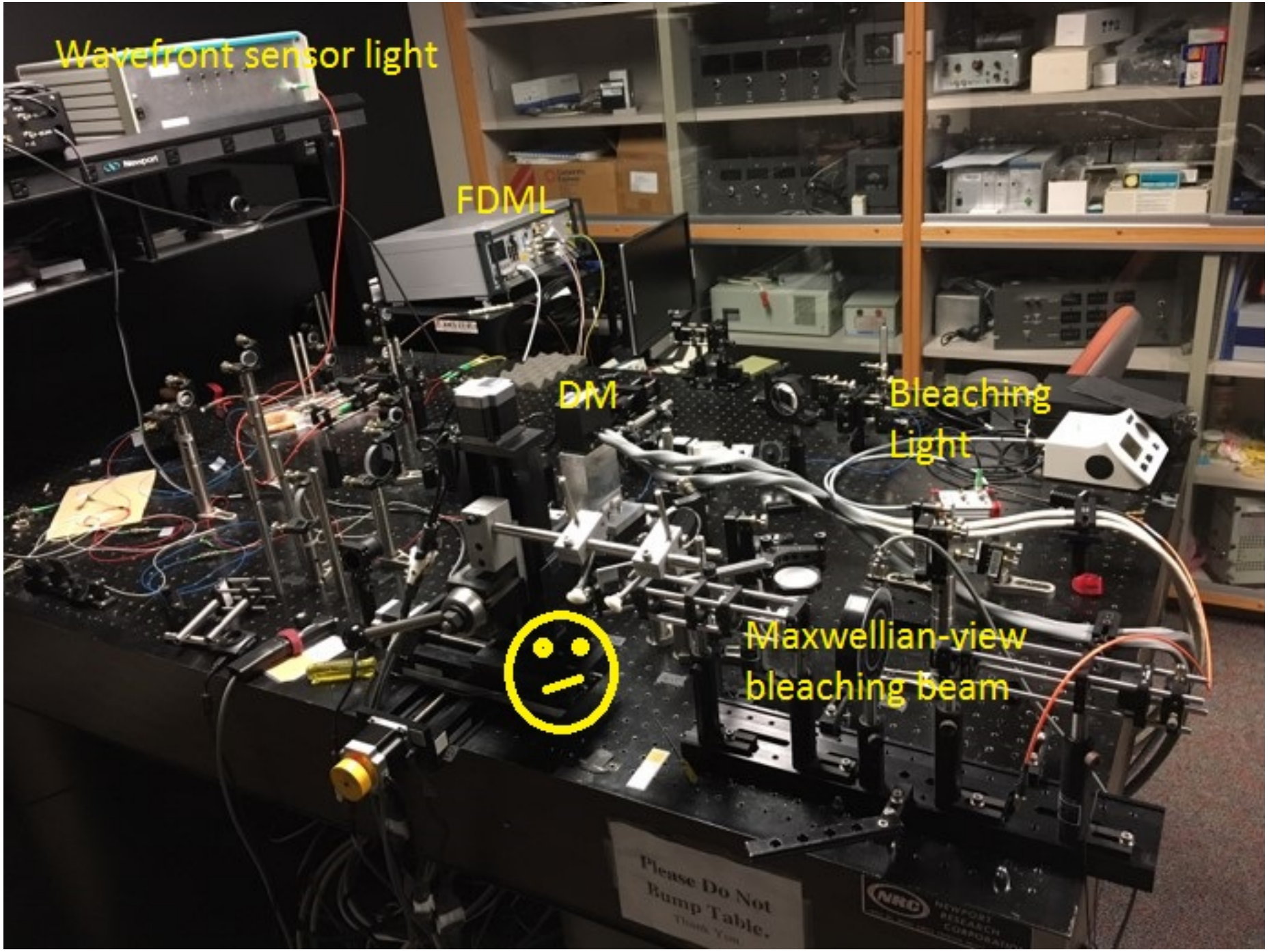
Adaptive Optics Swept-source OCT at 1.6MHz

- ✓A critical feature of this system's design is its speed. For the most intense stimuli, we observed initial phase changes of up to 50 rad/s. In order to correctly unwrap phase, the phase change between consecutive samples should be less than π radians.

Table 1. Specifications of the AO-FDML system and scanning parameters during imaging.

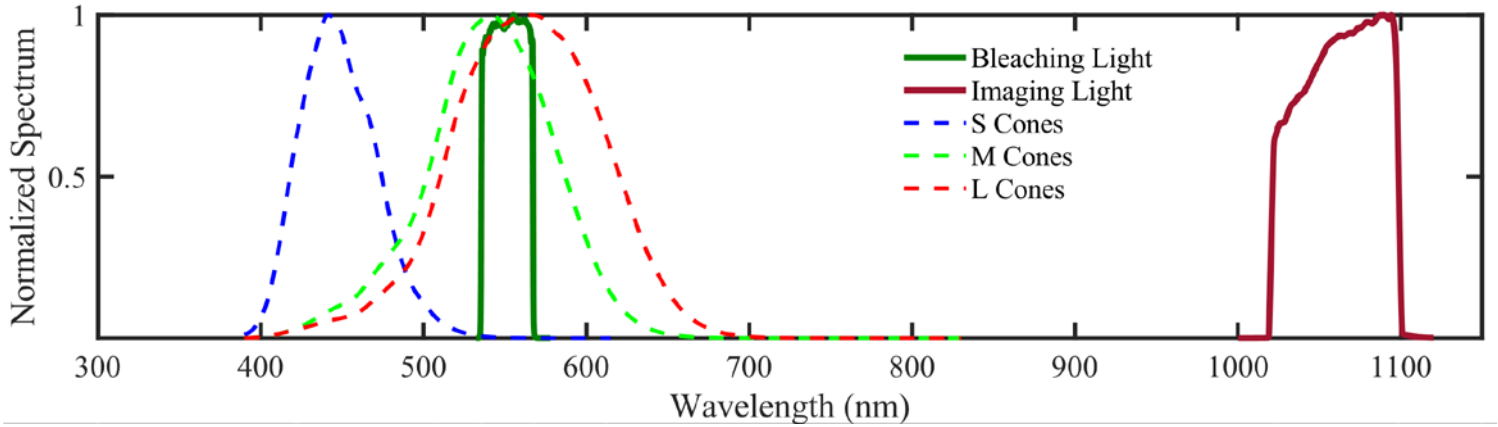
Laser center wavelength	$1063nm$
Spectral bandwidth (FWHM)	$78nm$
Laser A-scan rate	$1.64MHz$
B-scan rate	$5kHz$
Volume rate	$32Hz$
Optical power at cornea	$1.8mW$
Axial resolution in air	$10.8\mu m$
Measured sensitivity	$-85.4dB$
Laser phase noise (rms)	$2.6mrad$

Mehdi Azimipour, Justin V. Migacz, Robert J. Zawadzki, John S. Werner, Ravi S. Jonnal, "Functional retinal imaging using adaptive optics swept-source OCT at 1.6MHz", **bioRxiv** 420240 (under review)

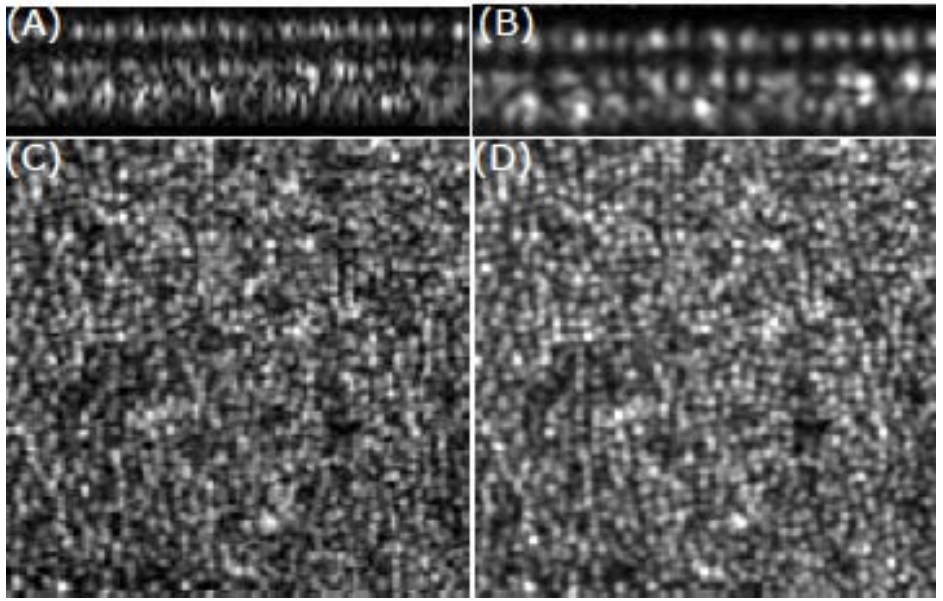

Wavefront sensor light
FDML
DM
Bleaching
Light
Maxwellian-view
bleaching beam
Please Do Not
Bump Table.

Functional Retinal Imaging in Human

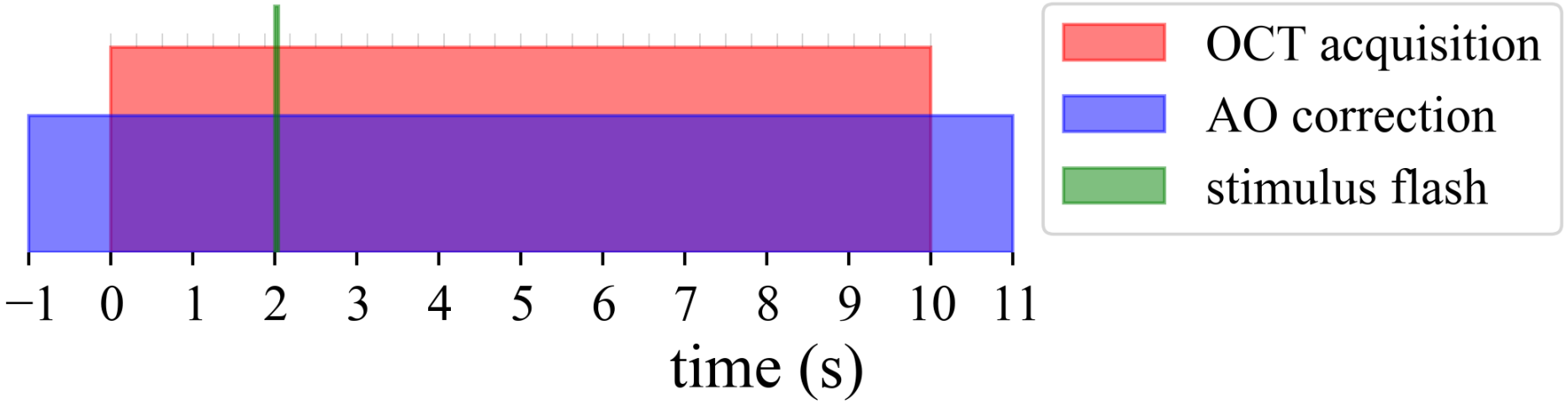
Normalized spectrum of imaging light source, bleaching light, and also normalized relative response of ‘S’, ‘M’, and ‘L’ cones.



Strip-based registration permits averaging of AO-OCT volumes. Top panel shows (A) single B-scan and (B) average of 30 B-scans. En-face projection of cone mosaic from a (C) single and (D) average of 30 motion-corrected volumes of a 1×1 degree patch acquired at 2.5º temporal from the foveal center.



Imaging Protocol



Four normal subjects, dilated and dark adapted for 15 min

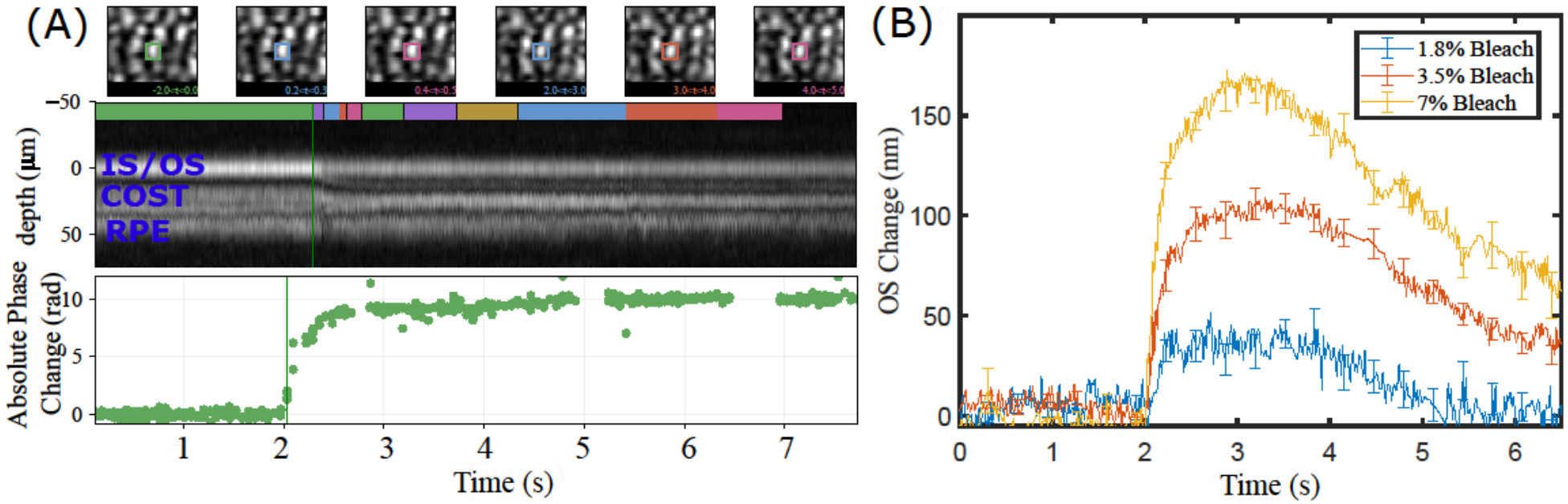
Closed-loop AO correction of 6.75 mm pupil, with 50-100 nm RMS residual error

OCT volumes acquired at 32Hz for 10 seconds

10 ms stimulus flash delivered at 2-second mark

Mehdi Azimipour, Justin V. Migacz, Robert J. Zawadzki, John S. Werner, Ravi S. Jonnal, "Functional retinal imaging using adaptive optics swept-source OCT at 1.6MHz", **bioRxiv** 420240 (under review)

Results



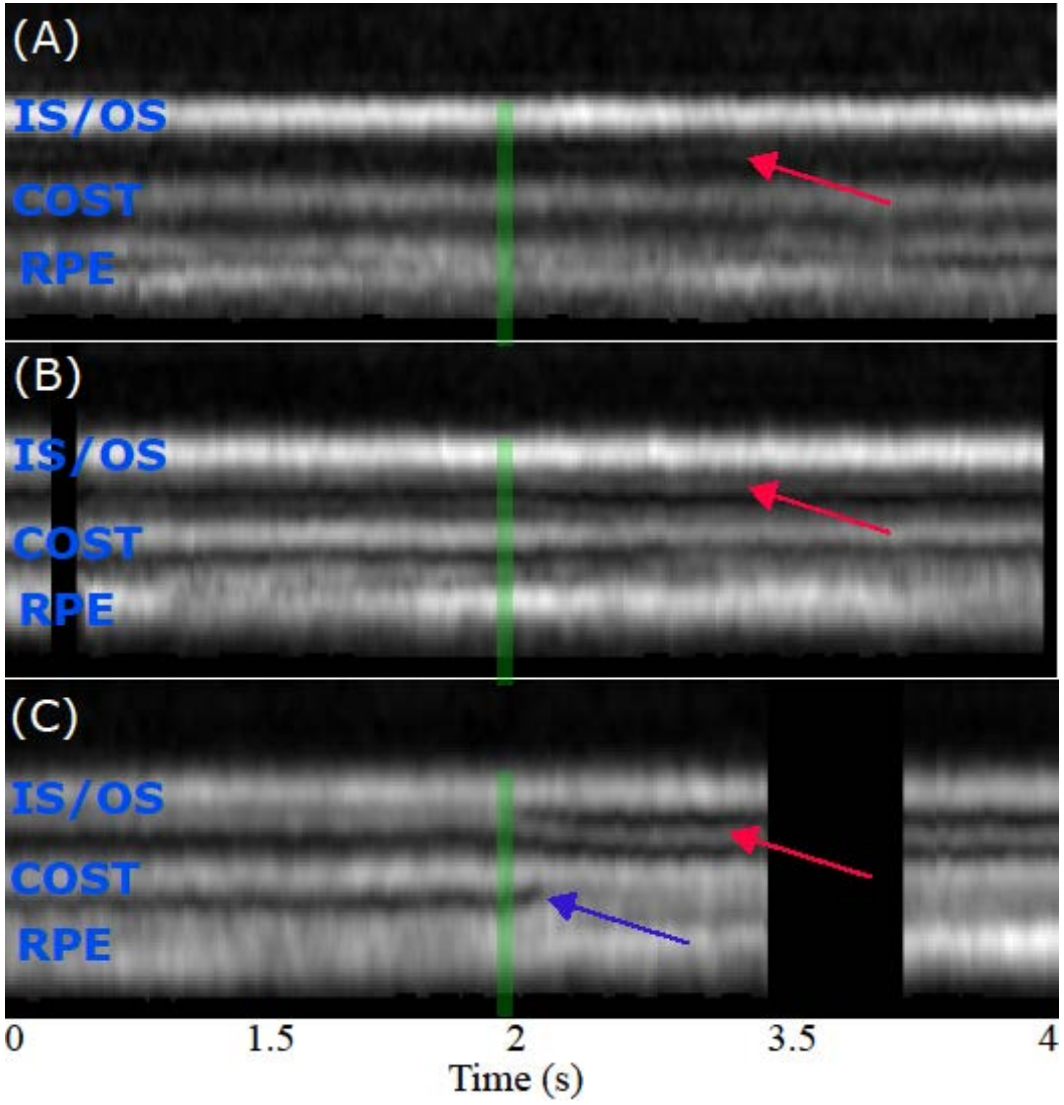
(A) Response of a single cone to 70% photopigment bleaching stimuli. The top row shows examples of motion-corrected projections of the cone's neighborhood. A time-series of the cone's axial profile (M-scan) is shown below the projections, with a green line indicating the stimulus flash. The phase difference between the IS/OS and COST was monitored as a function of time and can be seen in the bottom plot. (B) OS length change as a function of time for lower L/M photopigment bleaching percentages of 1.8, 3.5 and 7. Each curve was produced by averaging responses of 10-30 cones.

M. Azimipour, J.V. Migacz, R. J. Zawadzki, J .S. Werner, R. S. Jonnal, “Functional retinal imaging using adaptive optics swept-source OCT at 1.6MHz”, **bioRxiv** 420240 (under review)

Results

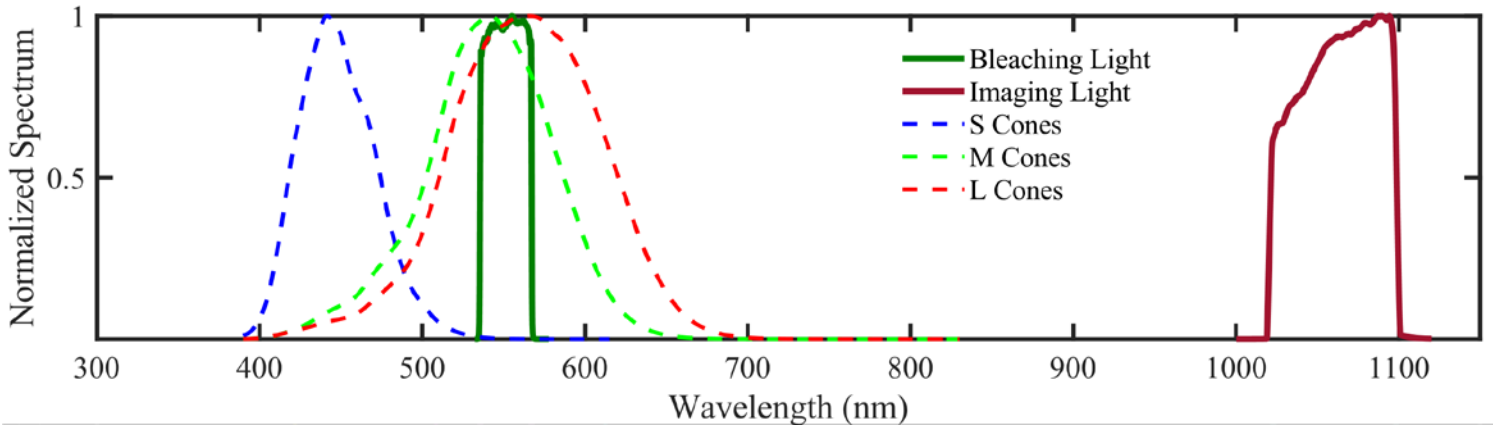
Changes in the axial morphology of cones for photopigment bleaching percentages of (A)1.8%, (B) 7% and (C) 70%. Red arrows shows appearance of an extra band between IS/OS and COST. The blue arrow indicates changes observed in the RPE and subretinal space.

- ✓ The extra band in OS could be generated, for instance, by an abrupt change in disc spacing or concentration of a visual cycle intermediate.
- ✓ The contrast reduction between COST and RPE could be an indication of melanosome movement into the apical part of the RPE cell.

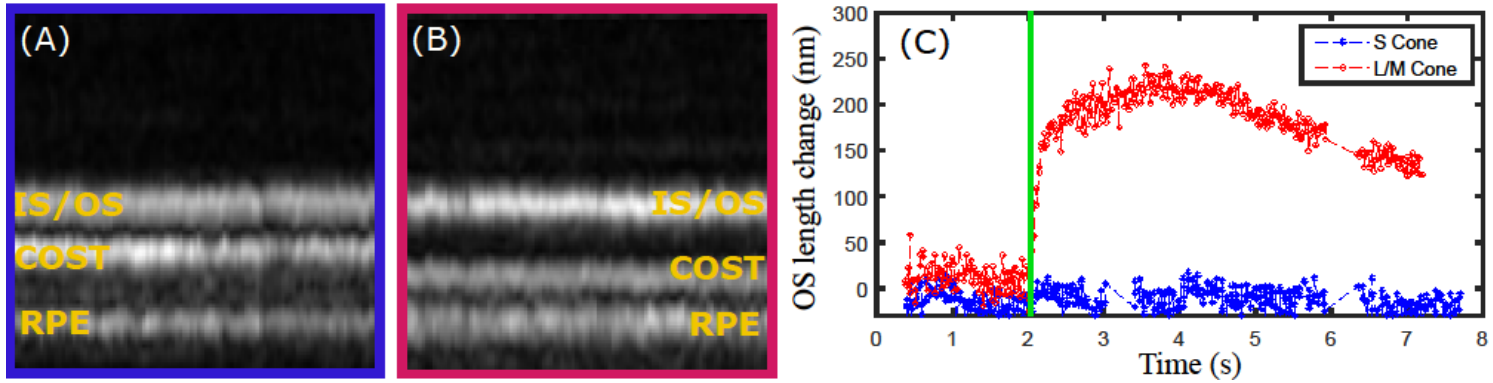


Possible S-cone mosaic investigated with AO-OCT

Normalized spectrum of imaging light source, bleaching light, and also normalized relative response of ‘S’, ‘M’, and ‘L’ cones.



Time-series of the cone’s axial profile (M-scan): (A) possible "S" cone, (B) "L" or "M" cone. (C) Response of the cones shown in panels "A" and "B" to a 10ms bleaching flash delivered at 2s. The cone with shorter OS length did not show any response to the stimuli.



Possible S-cone mosaic investigated with adaptive optics optical coherence tomography (under preparation)

Conclusions

1. Functional AO-OCT permits the measurement of light-induced changes in the cones (and potentially other retinal layers) with cellular resolution.
   These changes include:
   - OS swelling, encoded in the phase of the outer retinal bands
   - Possible changes in the intensity of the bands
   - Movement of bands (or sub-bands) corresponding to subcellular shifts of scatterers or translocation of biomolecules
2. High-speed (30 Hz+) volume rates are critical for measuring the rapid phase changes in cones.
3. Due to variations in the band movements among cells, AO is likely necessary to visualize and study these changes.
4. AO-OCT offers a unique set of biomarkers of photoreceptor function.

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