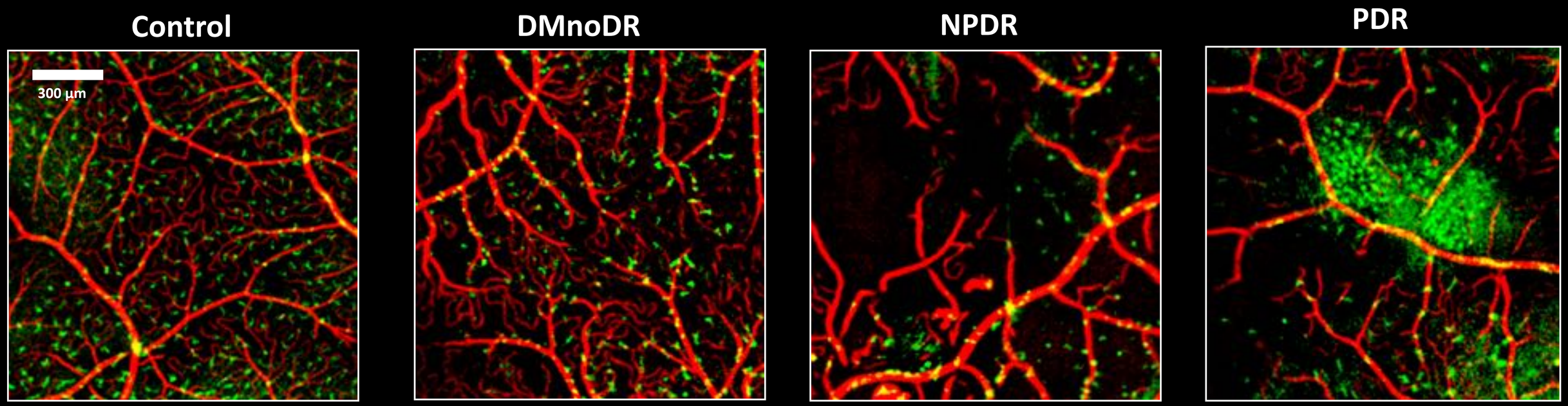
Clinical Imaging of Macrophage-Like Cells in the Living Human Eyes with Diabetic Retinopathy Using En face OCT Reflectance



Richard B. Rosen, Maria V. Castanos, Davis B. Zhou, Tatyana Milman, Reilly Allison, Rishard Weitz, Rachel E. Linderman, Joseph Carroll, Justin Migacz, Toco Y.P. Chui

New York Eye and Ear Infirmary of Mount Sinai

Celebrating 200 YEARS 1820 – 2020

THE NEW YORK EYE AND EAR INFIRMARY

MEDICAL COLLEGE OF WISCONSIN

AOIP ADVANCED OCULAR IMAGING PROGRAM

Disclosures

Richard B. Rosen: OptoVue: Code C; Boehringer-Ingelheim: Code C; Astellas: Code C; Genentech-Roche: Code C; NanoRetina: Code C; OD-OS: Code C; Opticology: Code I: Guardion: Code I; CellView: Code I: Regeneron: Code C; Bayer: Code C; Diopsys: Code C ;Teva: Code C.

Rishard Weitz: CellView: Code I

Toco Y.P. Chui: None

Davis B. Zhou: None

Maria V. Castanos: None

Joseph Carroll: OptoVue: Code F; AGTC: Code F; MeiraGTx: Code C, F; Translational Imaging Innovations: Code I

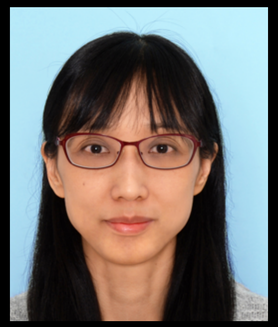
Reilly L. Allison, None

Rachel E. Linderman: OptoVue:Code C

Summary

- Clinical OCT *is* capable of imaging single macrophage-like cells, probably hyalocytes, in eyes of patients with diabetes.
- Changes cell morphology and distribution mirror areas of retinal damage and diabetic progression.
- Clinical utility of this potential biomarker will depend on automation of software and imaging protocols

Acknowledgements


Toco Chui

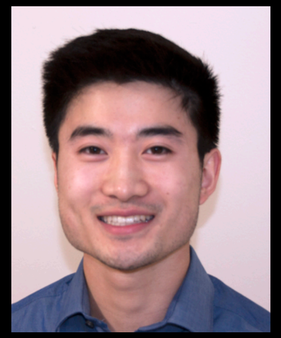

Justin Migacz


Rachel Linderman


Reilly Allison


Joseph Carroll


Maria Castanos


Davis Zhou


Alfredo Dubra


Rishard Weitz


Alexander Pinhas


Jessica Lee


Alex Barash


Patricia Garcia


Jorge Andrade Romo


Shelley Mo

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The Einhorn Family Foundation

The Edith C Blum Foundation

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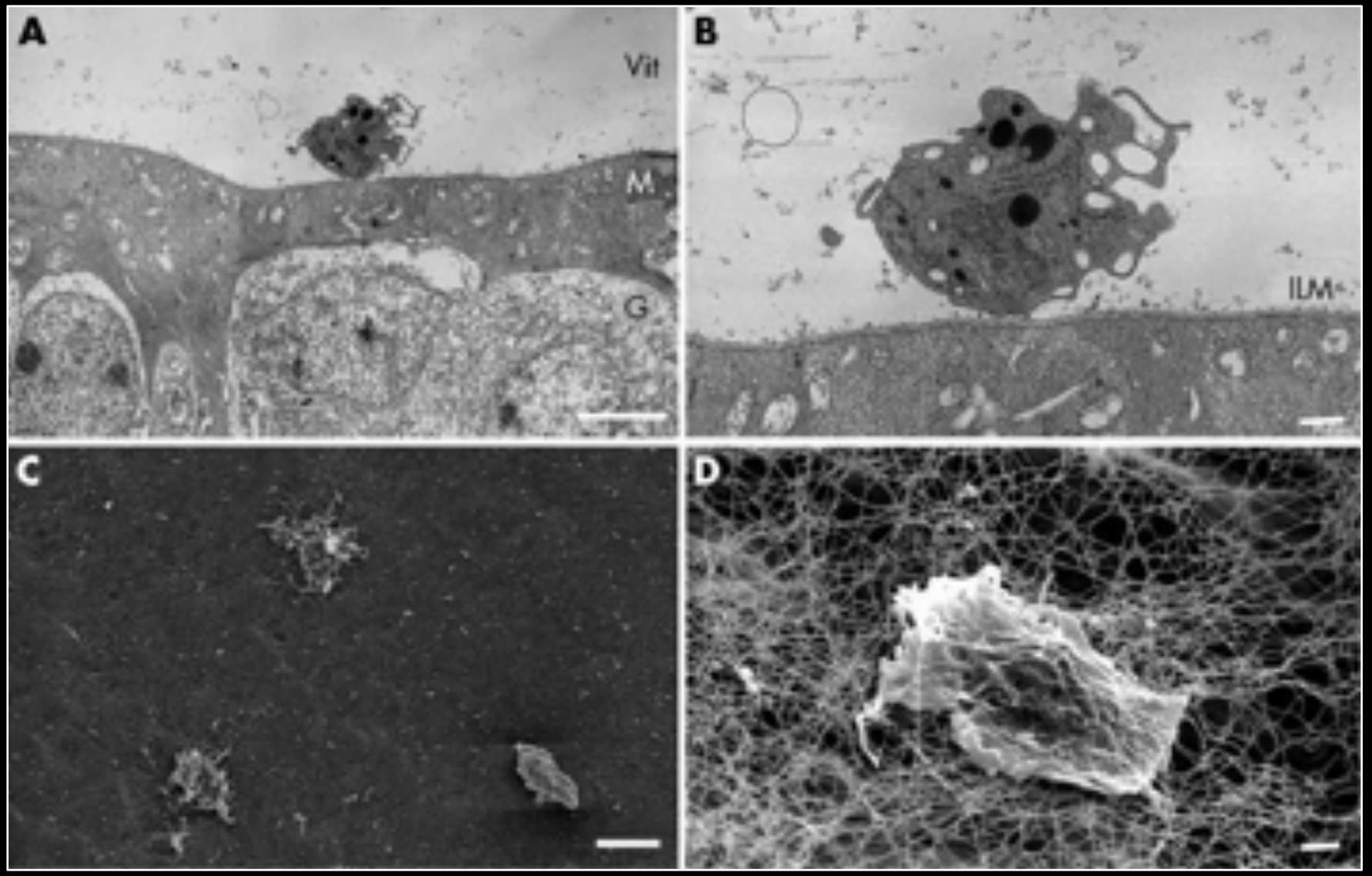
The Jorge N. Buxton, MD Microsurgical Education Foundation.

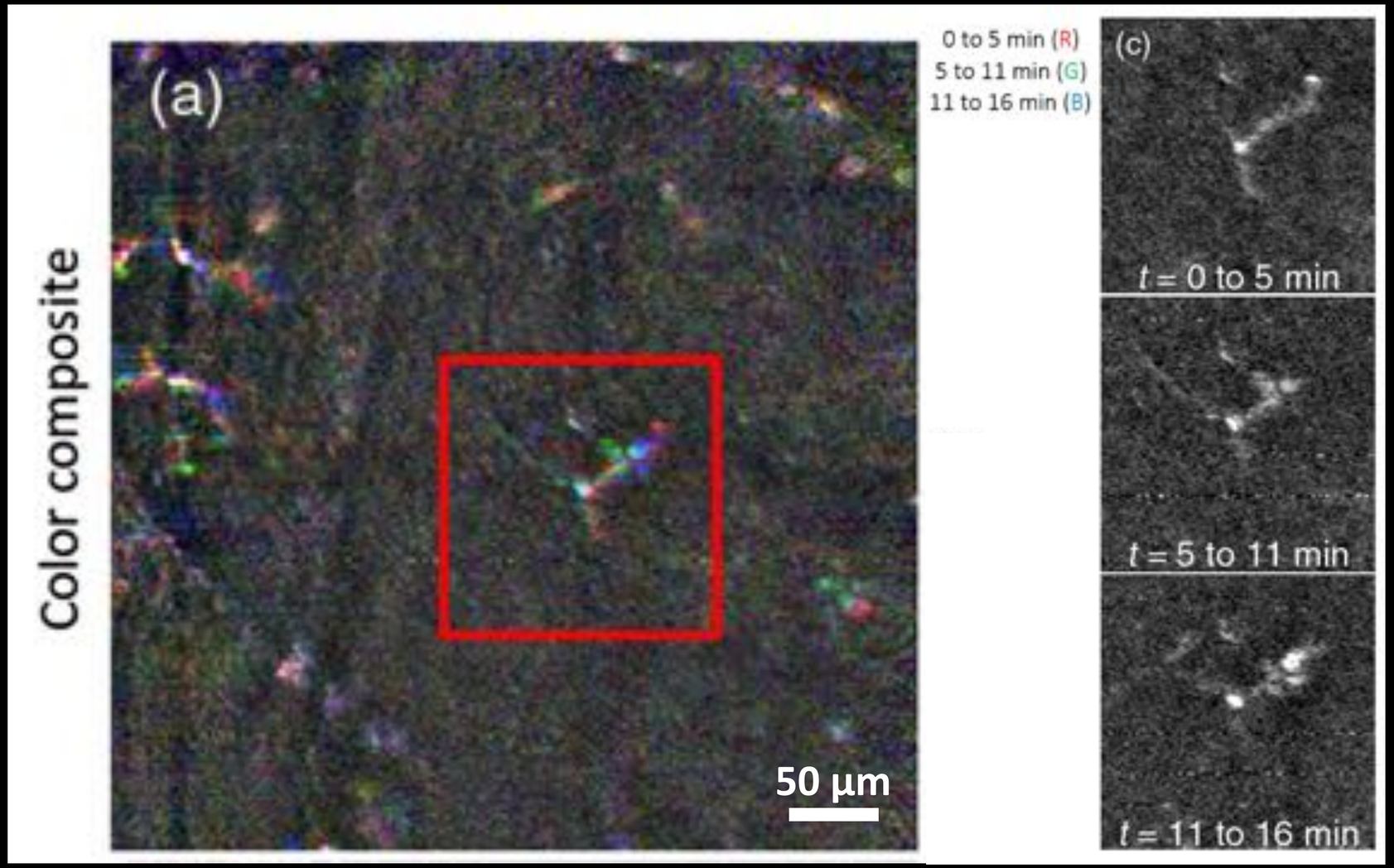
Macrophage-like Cells on the VitreoRetinal Interface



H Qiao et al., The characterisation of hyalocytes: the origin, phenotype, and turnover. Br. J. Ophthalmol, 2005

Adaptive Optics - OCT Imaging of Macrophage-like Cells

Healthy Control, Vitreoretinal Interface, Temporal Retina

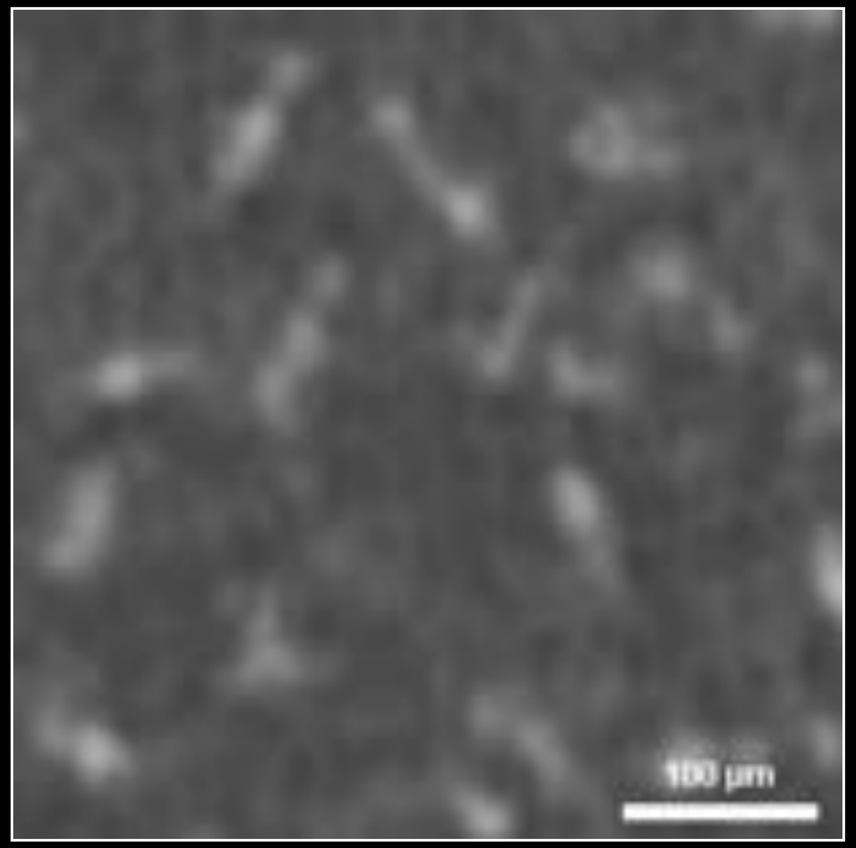


Kurokawa et al., Suite of methods for assessing inner retinal temporal dynamics across spatial and temporal scales in the living human eye, Neurophotonics 2020

Clinical OCT Imaging of Macrophage-like cells

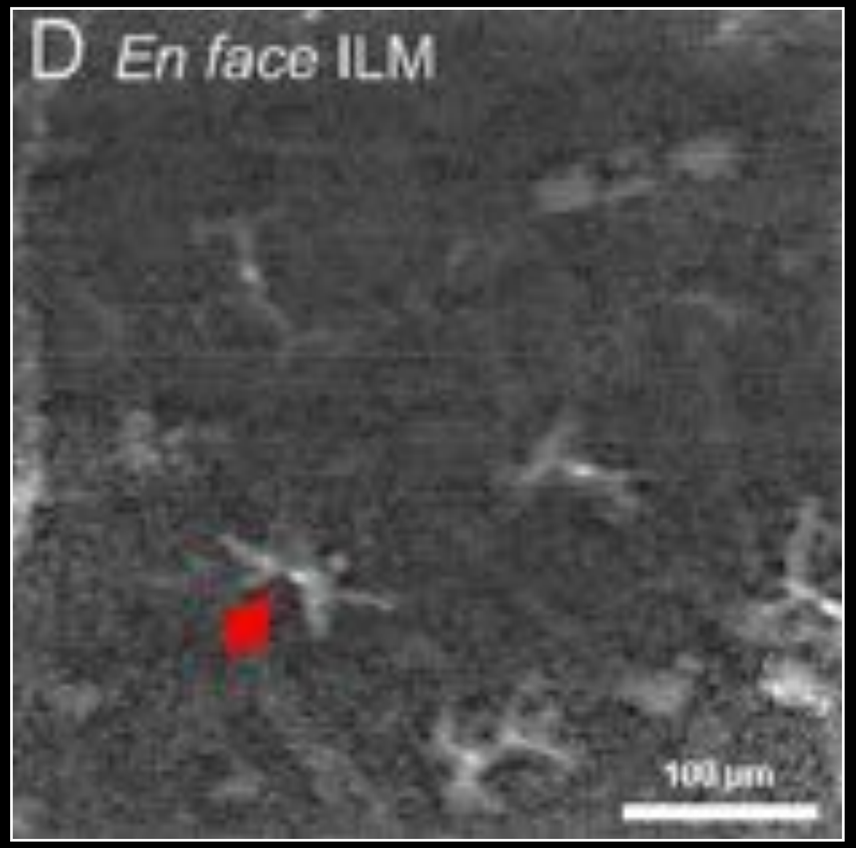
Healthy Controls, Vitreoretinal Interface, Temporal Retina

Clinical OCT



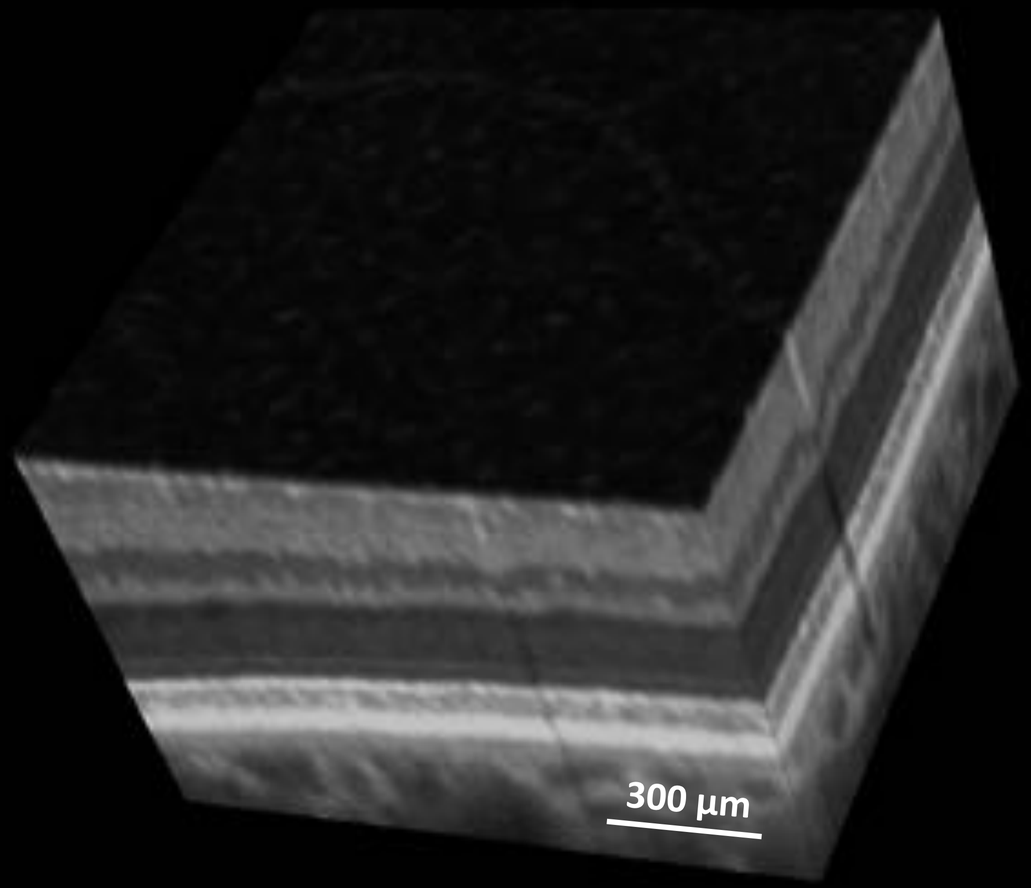
MV Castanos et al., Imaging of Macrophage-Like Cells in Living Human Retina using Clinical OCT, IOVS 2020, in press

Adaptive Optics - OCT

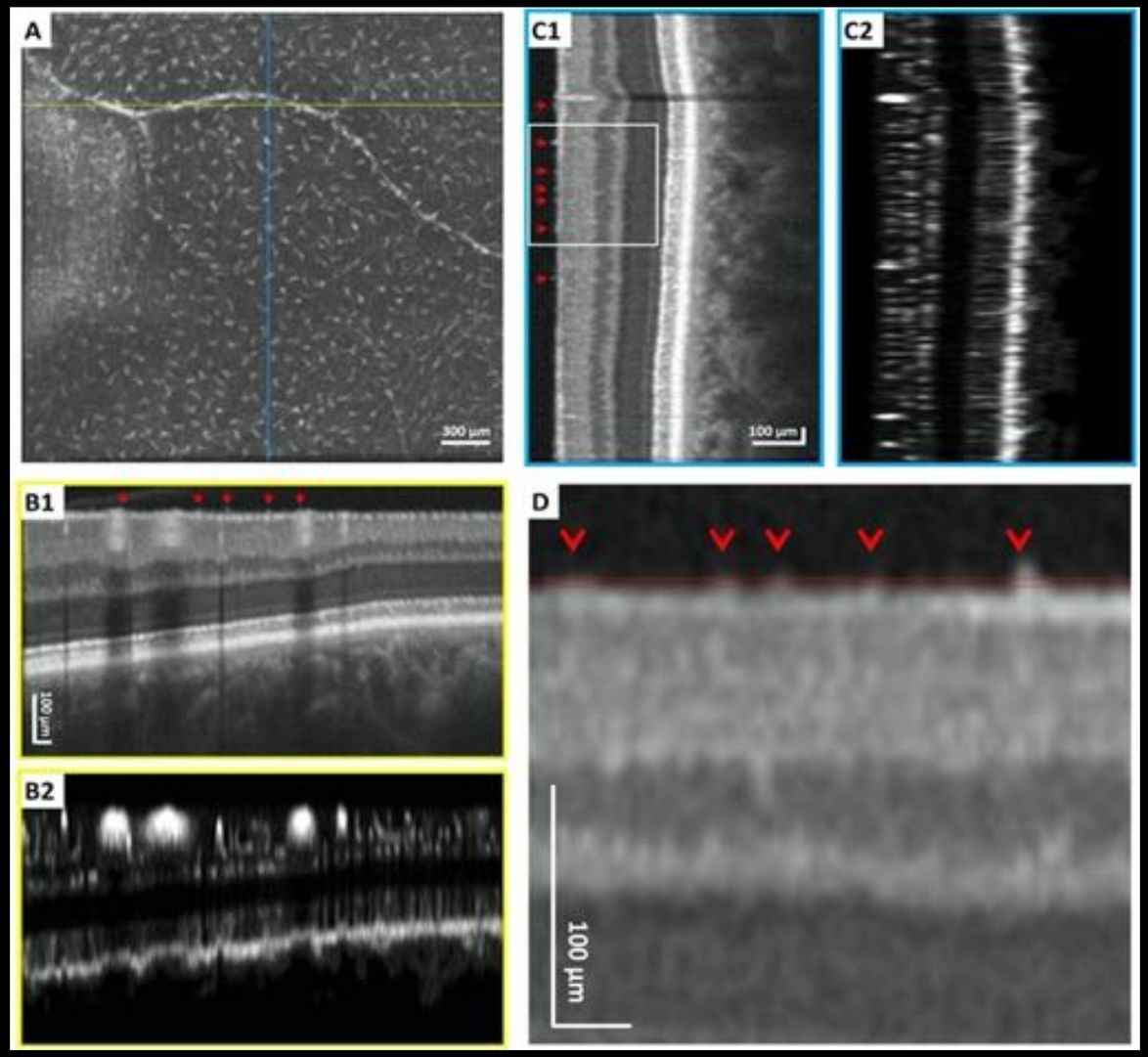


Z Liu et al., Imaging and quantifying ganglion cells and other transparent neurons in the living human retina, PNAS 2017

Clinical OCT Imaging of Macrophage-like cells

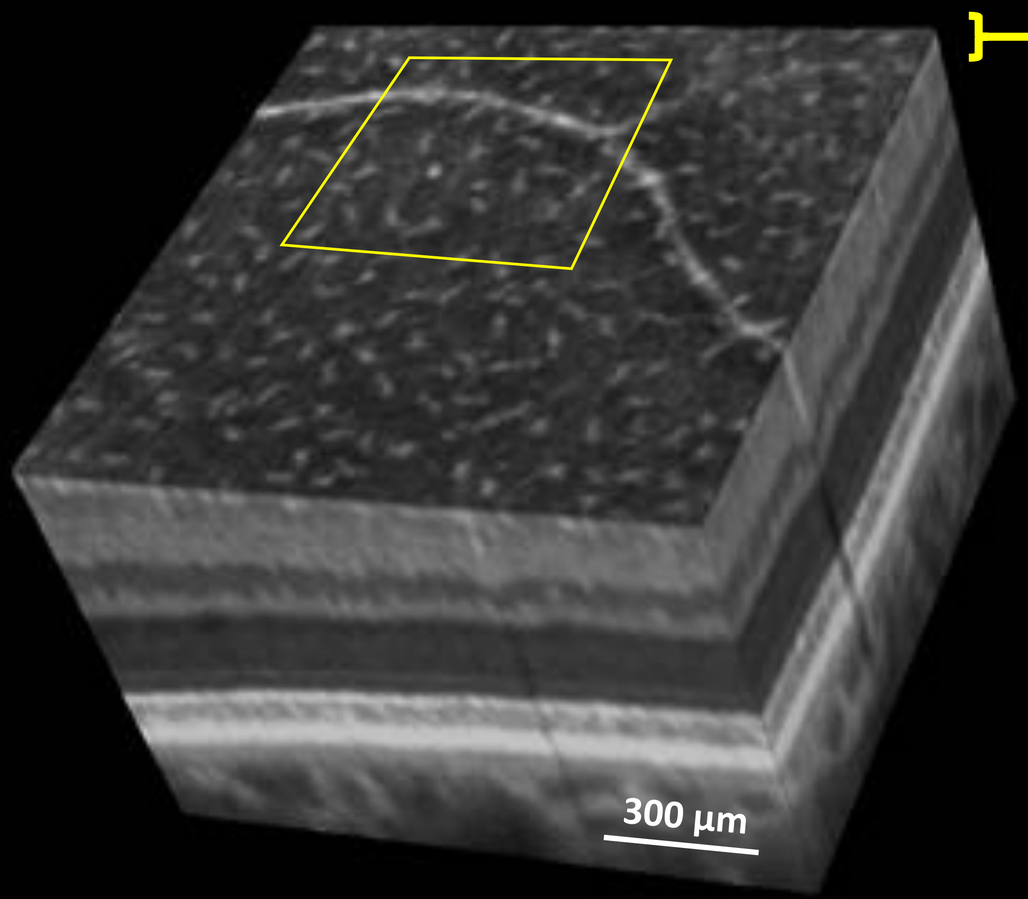


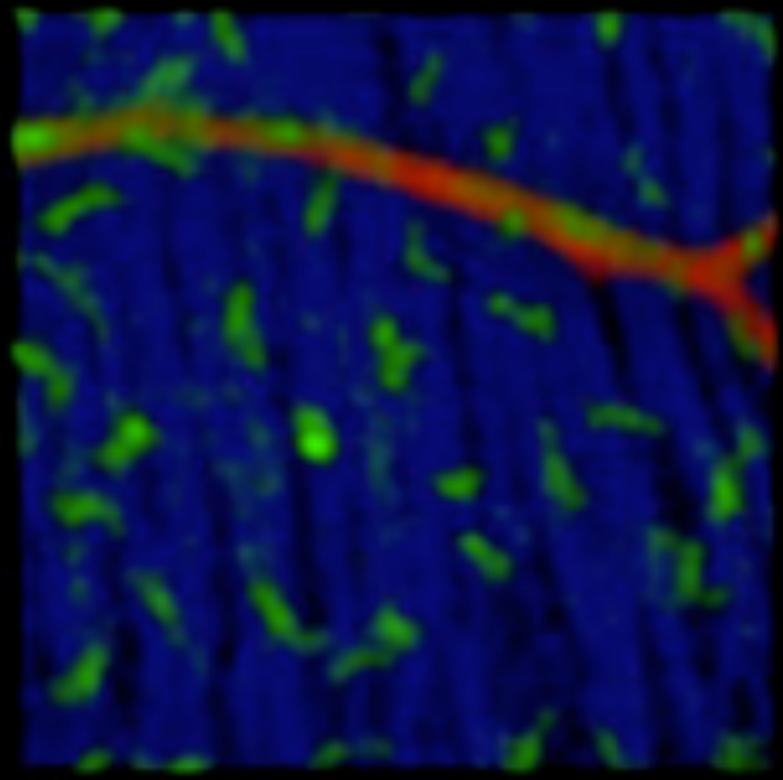
Healthy Control, 34yo, M, OS, Temporal Retina



MV Castanos, DB Zhou, RE Linderman, R Allison, T Milman, J Carroll, J Migacz, RB Rosen, TYP Chui, Imaging of Macrophage-Like Cells in Living Human Retina using Clinical OCT , IOVS 2020;61(6):48.

3-D OCT Shows *Postures* of Macrophage-like Cells on the Vitreoretinal interface

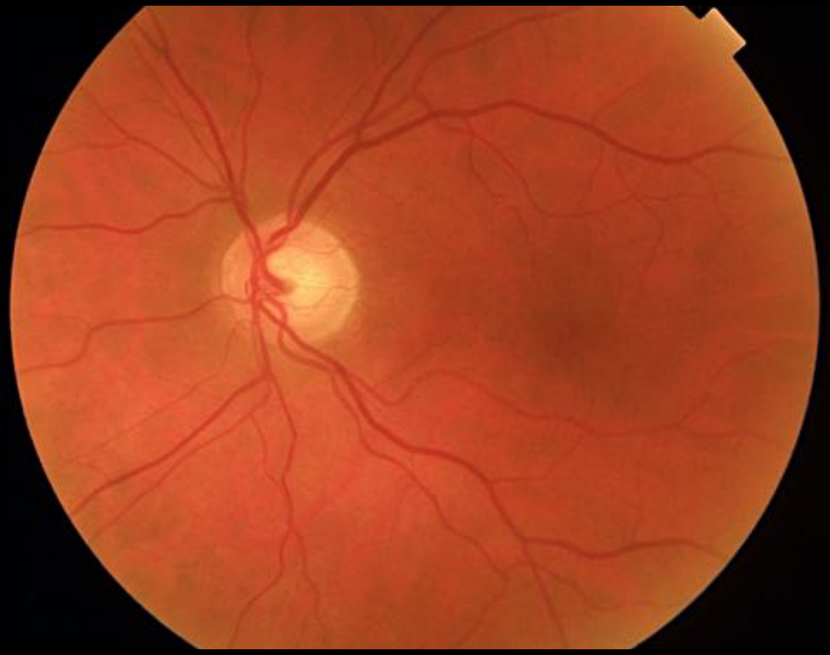




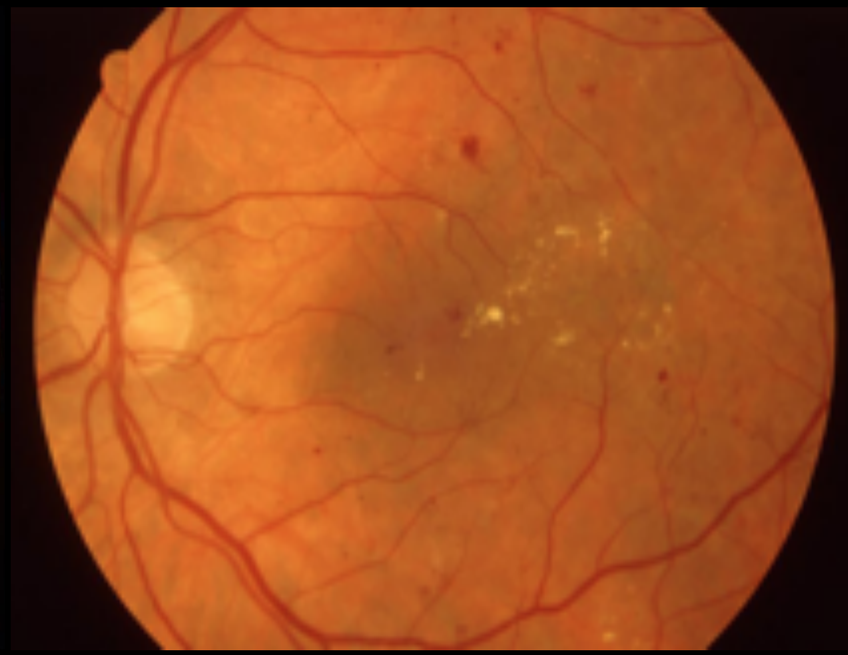
Green : Macrophage-like cells
Blue : RNFBs
Red : Retinal Blood Vessels

Healthy Control, 34yo, M, OS, Temporal Retina

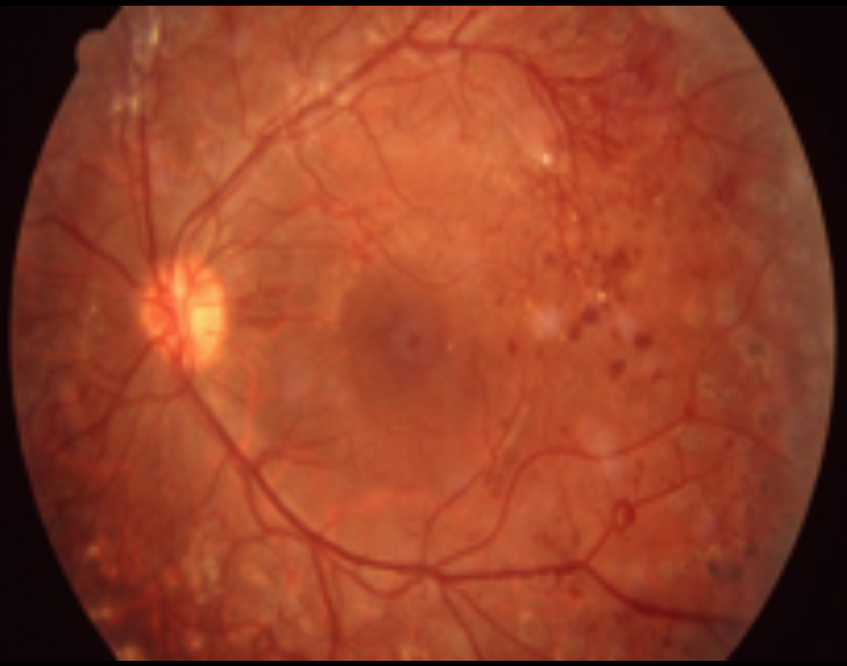
DMnoDR



NPDR



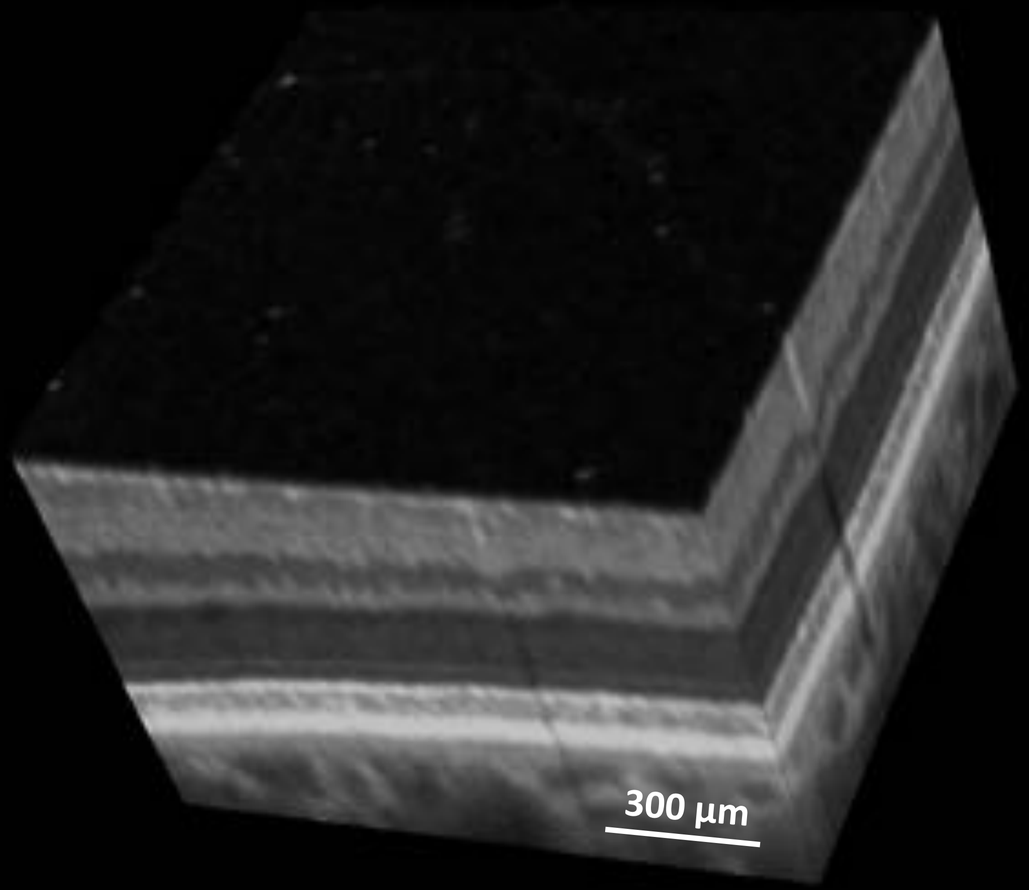
PDR



Can ***Clinical OCT*** be used to visualize macrophage-like cells in diabetic eyes?

Purpose

- To image macrophage-like cells in diabetic eyes using clinical OCT.
- To compare morphology of macrophage-like cells in the healthy and diabetic eyes.
- To quantify distribution of macrophage-like cells in healthy and diabetic eyes using clinical OCT.



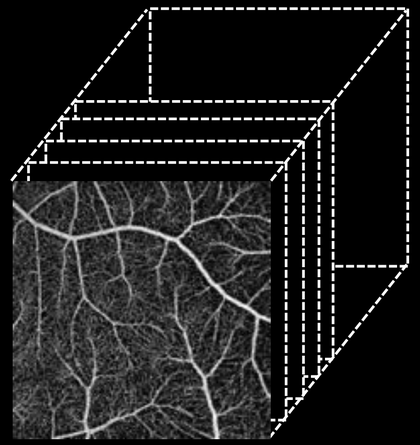
Healthy Control, 34yo, M, OS, Temporal Retina

Methods – Image Acquisition and Processing

13 diabetic retinopathy patients and 17 controls were imaged using a clinical SD-OCT system (Avanti RTVue-XR; Optovue).

1. Optovue Avanti ~10-20 mins

10 Sequential Scans



3x3mm Temporal Scan
OCT-A Full Layer

Healthy Control, 34yo, M

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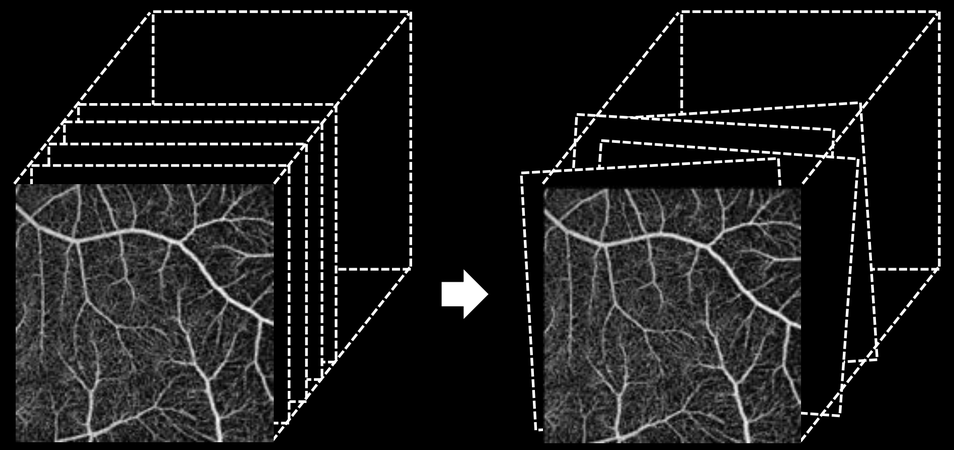
10 Sequential Scans

2. ImageJ ~5 mins

Image Registration & Averaging



3x3mm Temporal Scan
OCT-A Full Layer

Register Virtual Stack Slices
Transformation matrix

Healthy Control, 34yo, M

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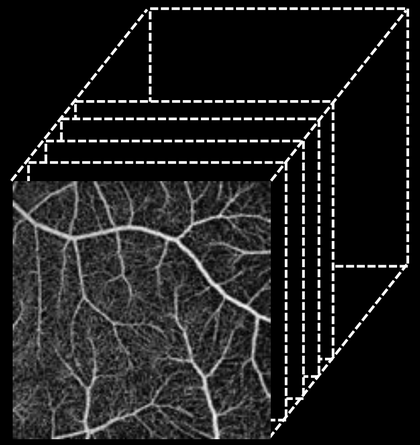

3x3mm Temporal Scan
OCT-A Full Layer

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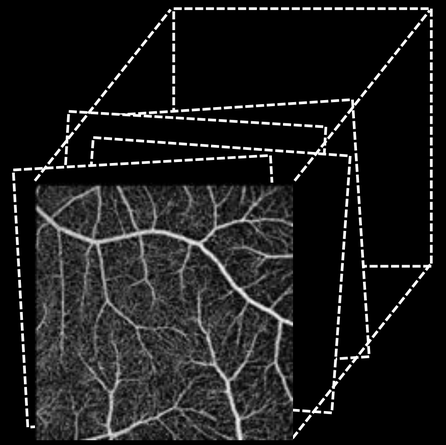


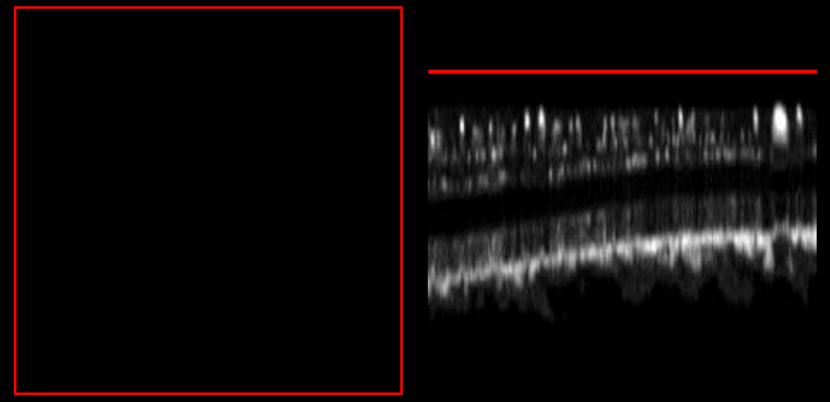
Register Virtual Stack Slices
Transformation matrix

Apply Transformation Matrix

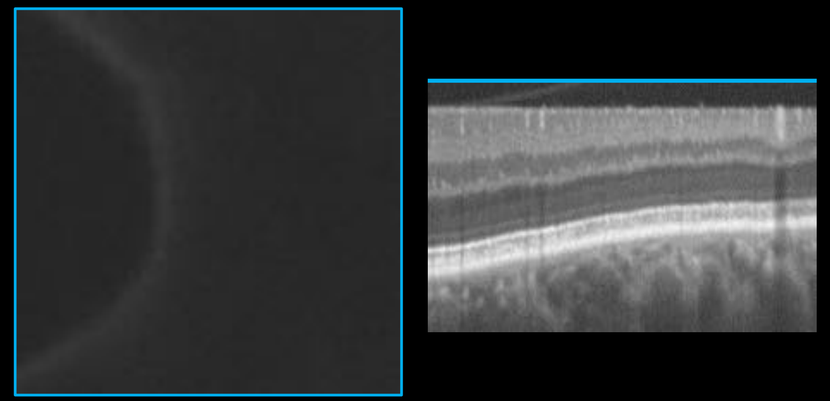
3. Maltab, Optovue, & ImageJ ~3 hrs

3 µm slab OCT-A and OCT-R Stack Registration & Averaging

OCT Angiograph 3 µm slabs



OCT Reflectance 3 µm slabs

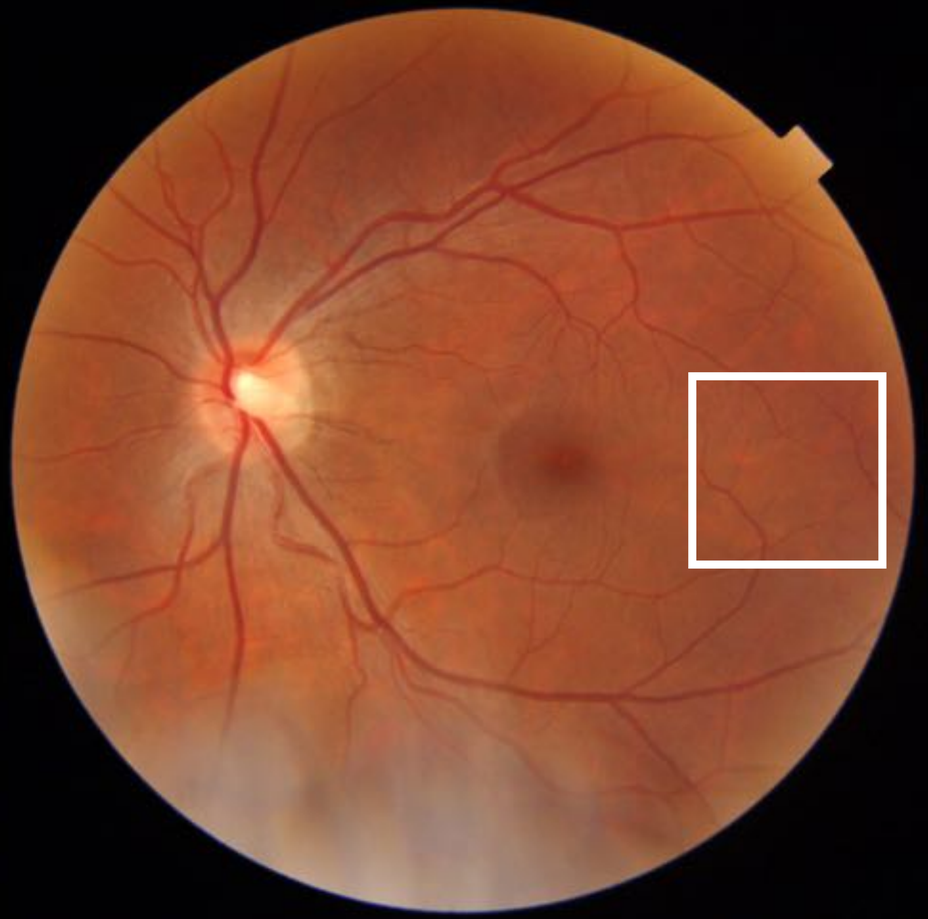


Flattened at Inner Limiting Membrane (ILM)

Healthy Control, 34yo, M

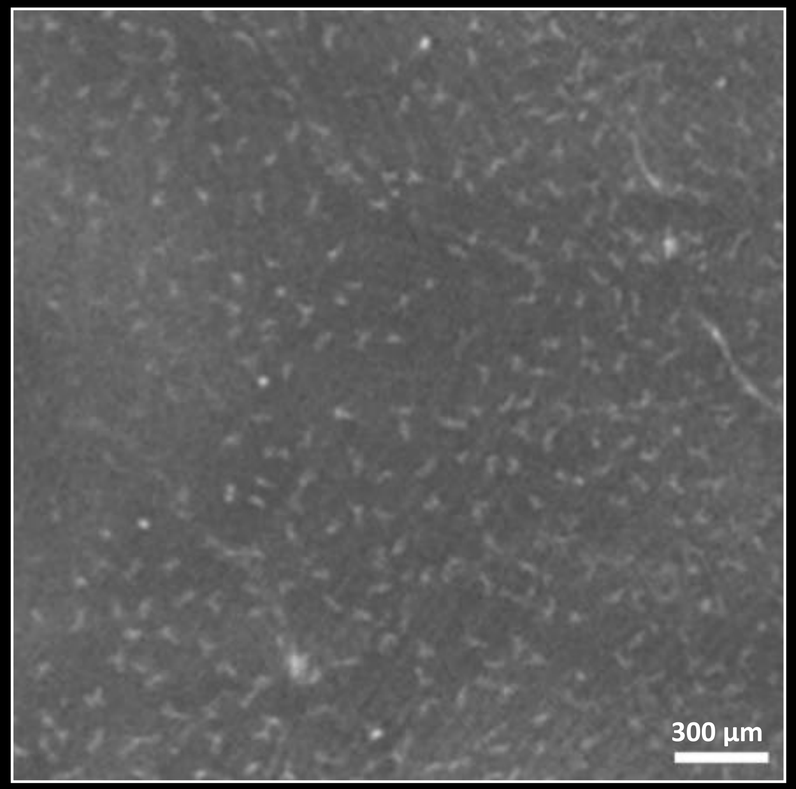
Healthy Control, 25 yo, F, OS

3x3mm Temporal Retina, ~ 9° from the fovea

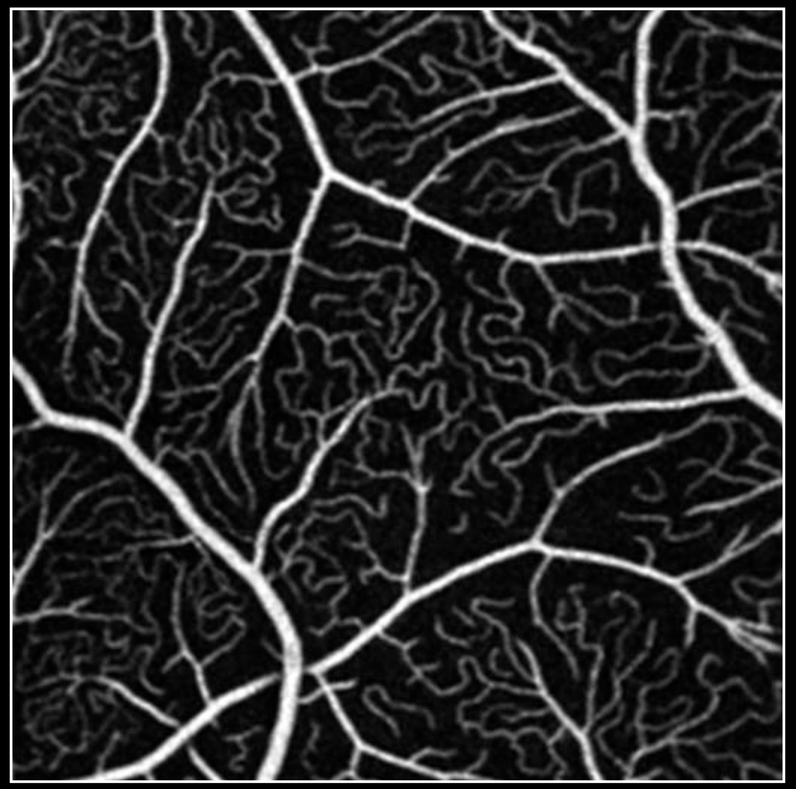


Healthy Control, 25 yo, F, OS

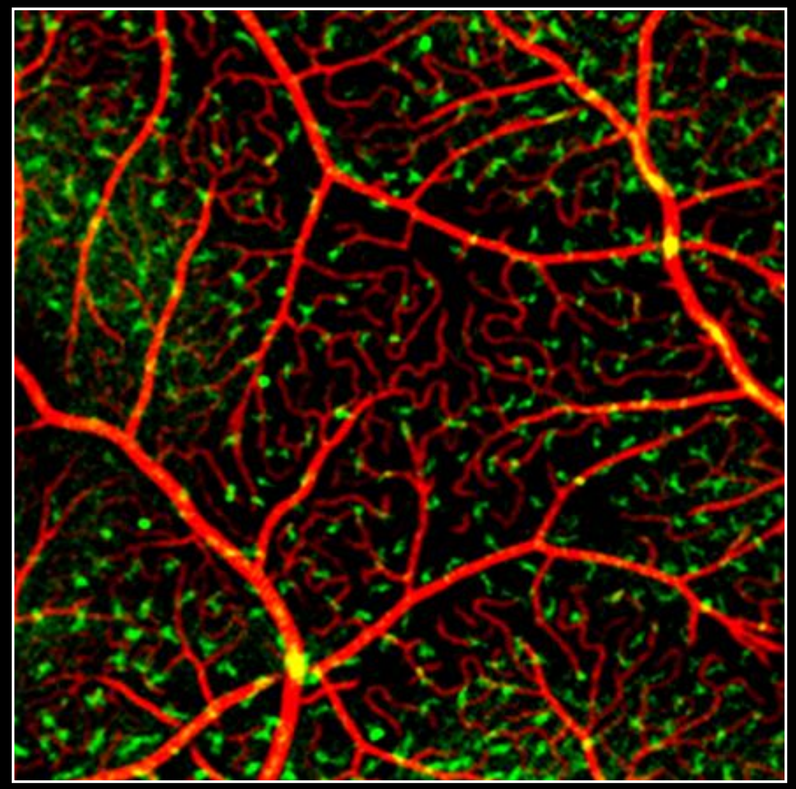
OCT-R Slab
3µm above ILM to ILM surface



OCT-A Slab
ILM surface to 48µm below ILM

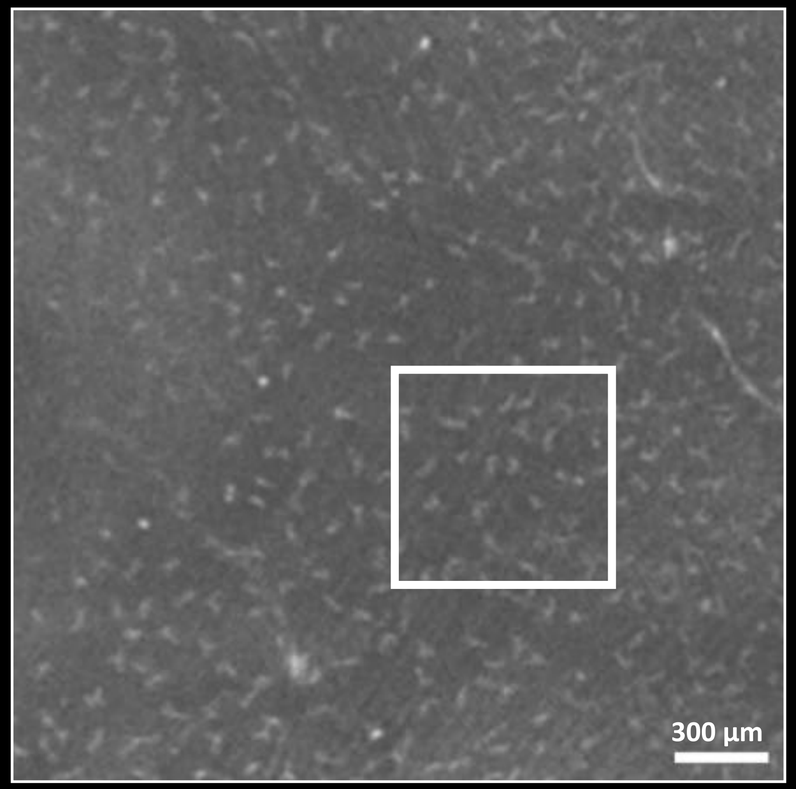


Color Overlay
OCT-R (Green) and OCT-A (Red)



Healthy Control, 25 yo, F, OS

OCT-R Slab
3µm above ILM to ILM surface



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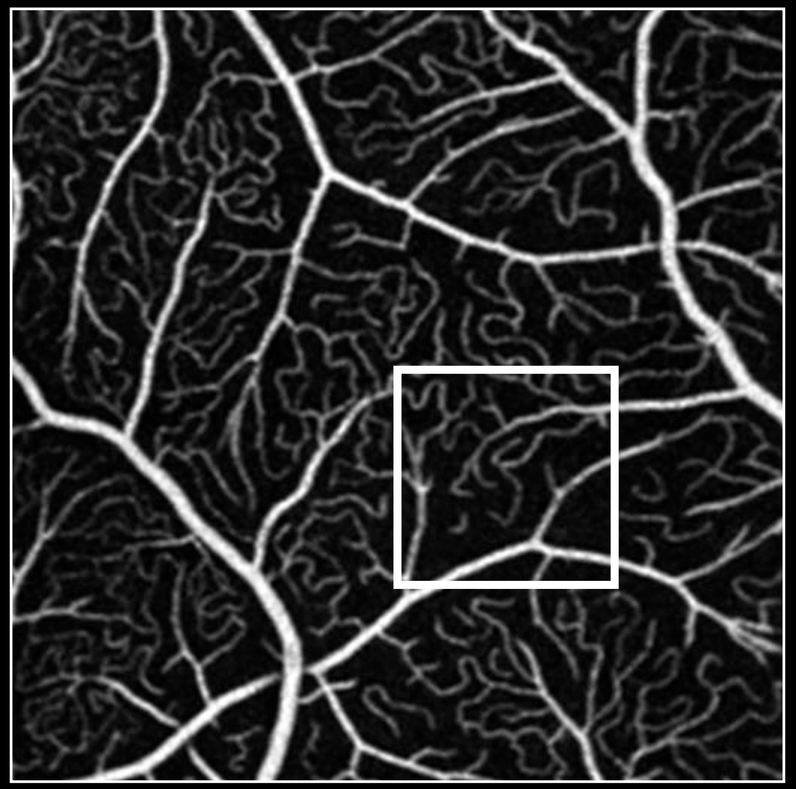
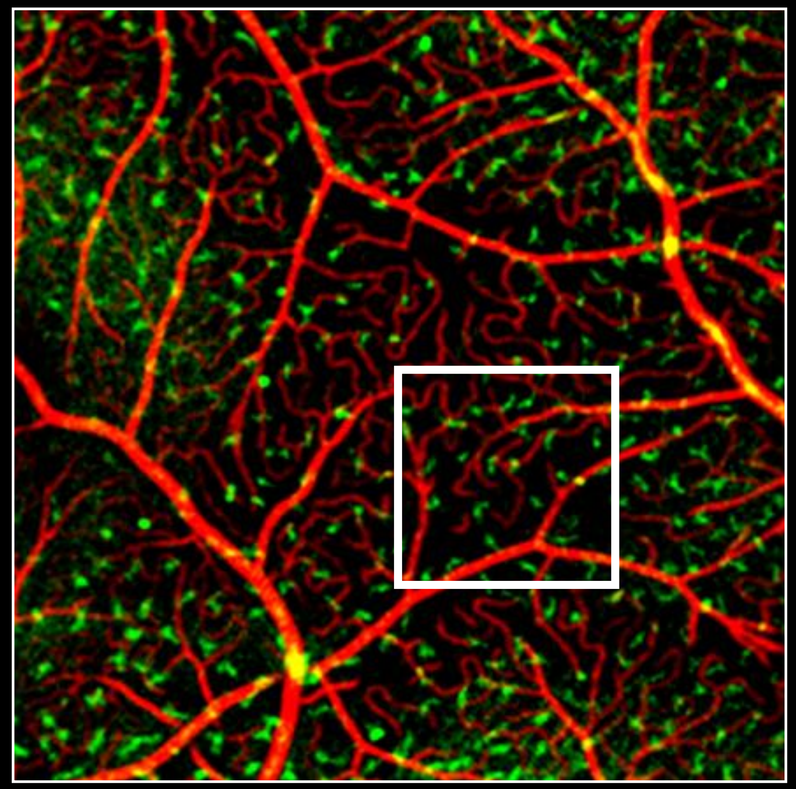
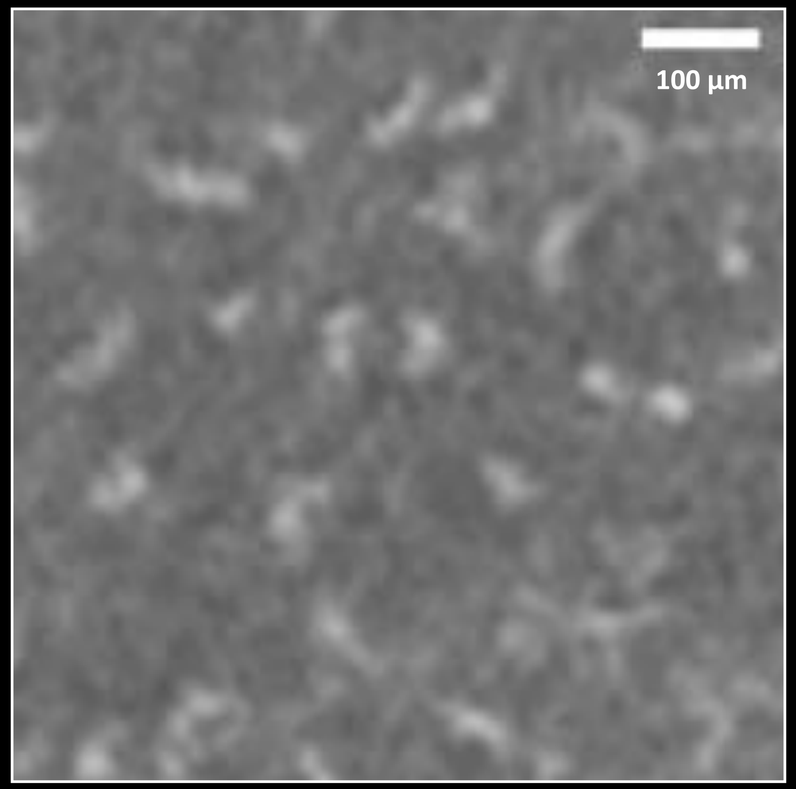


Color Overlay
OCT-R (Green) and OCT-A (Red)



Healthy Control, 25 yo, F, OS

OCT-R Slab
3µm above ILM to ILM surface



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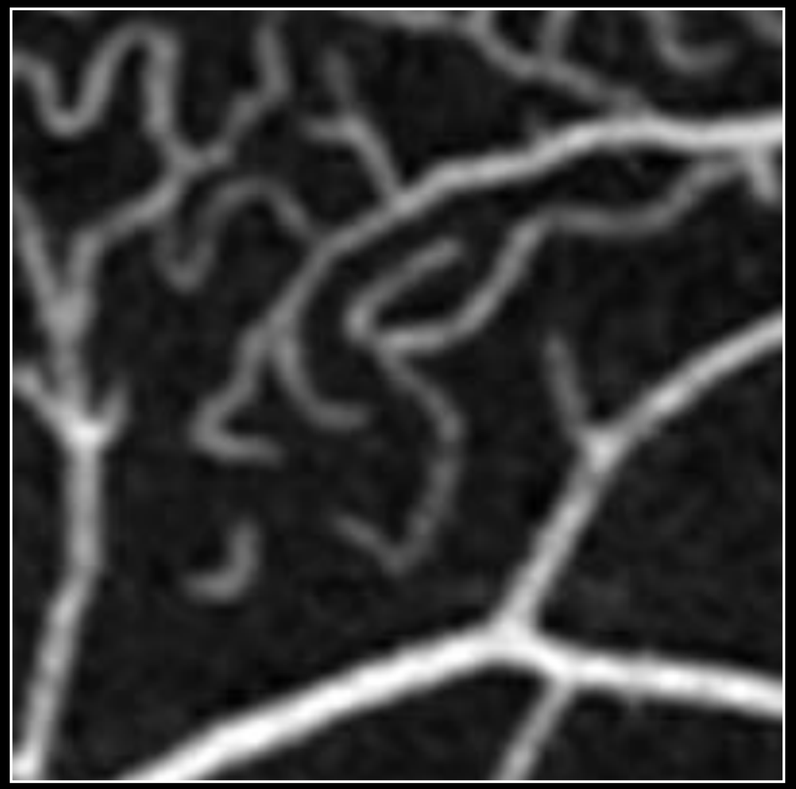

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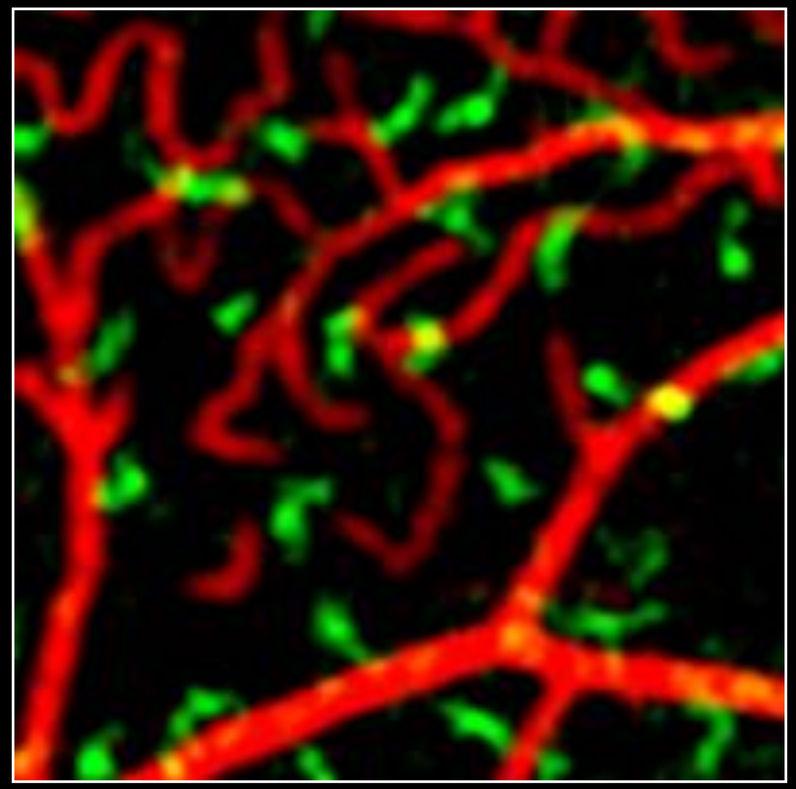
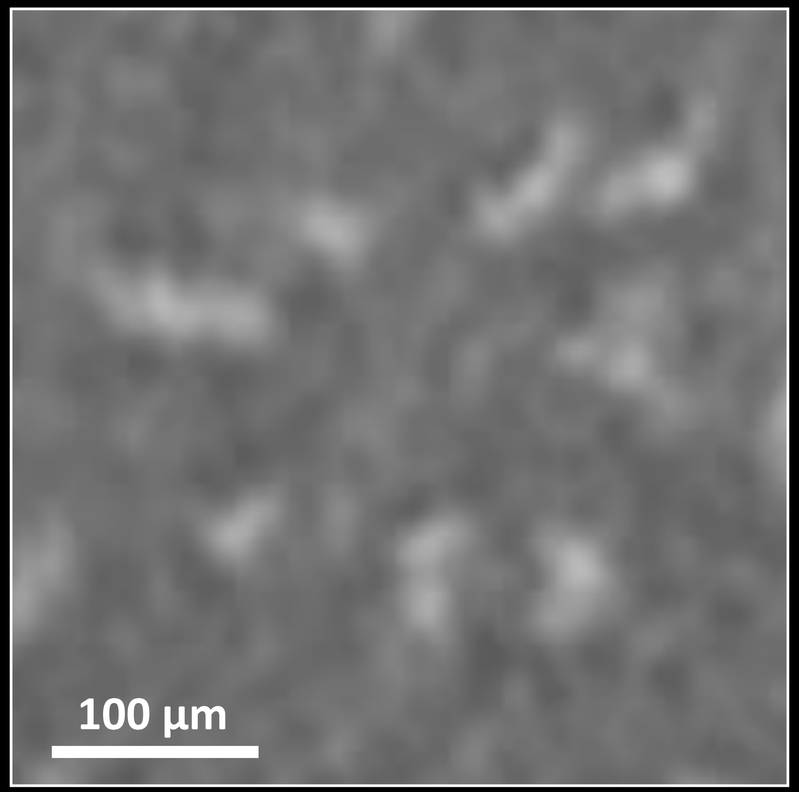


Ramified macrophage-like cells
Slender with spindle-like configuration

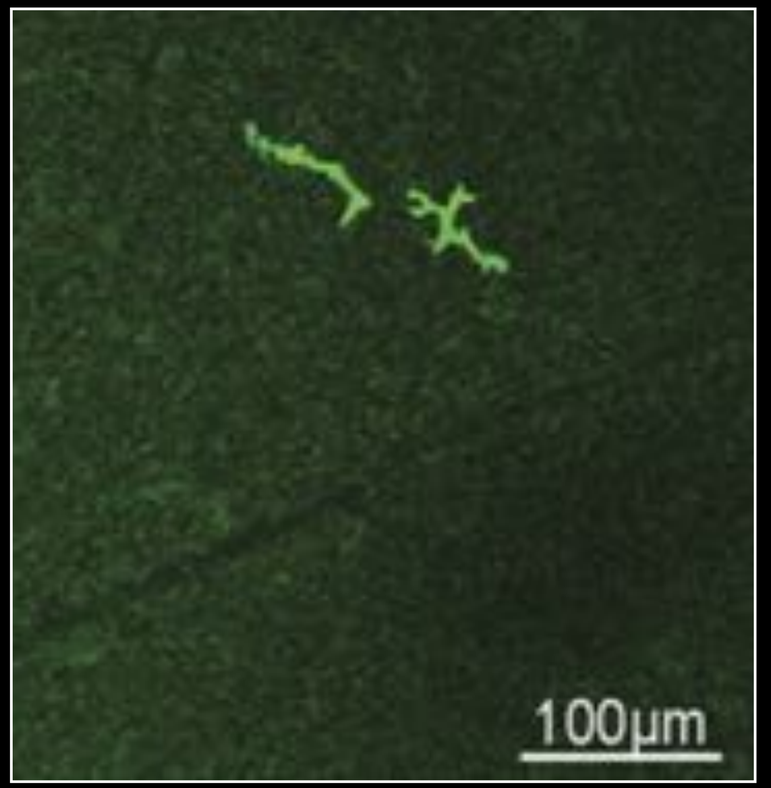
Cells co-localized with blood vessels

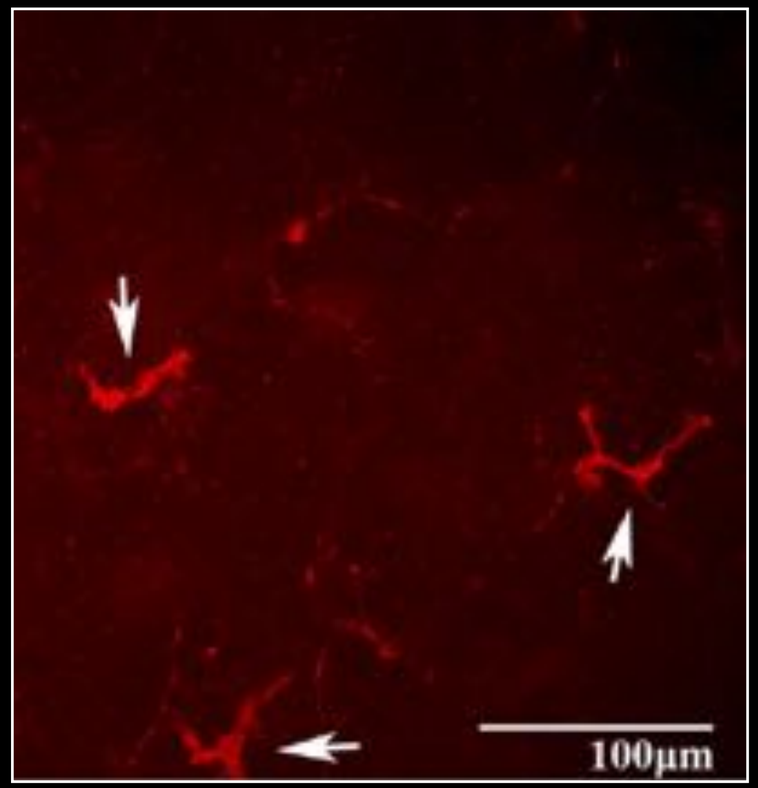
Macrophage-Like Cells at the Vitreoretinal Interface - Hyalocytes (Murine)

Macrophage-like cells
in normal living human retina



Hyalocytes
in normal mouse retina

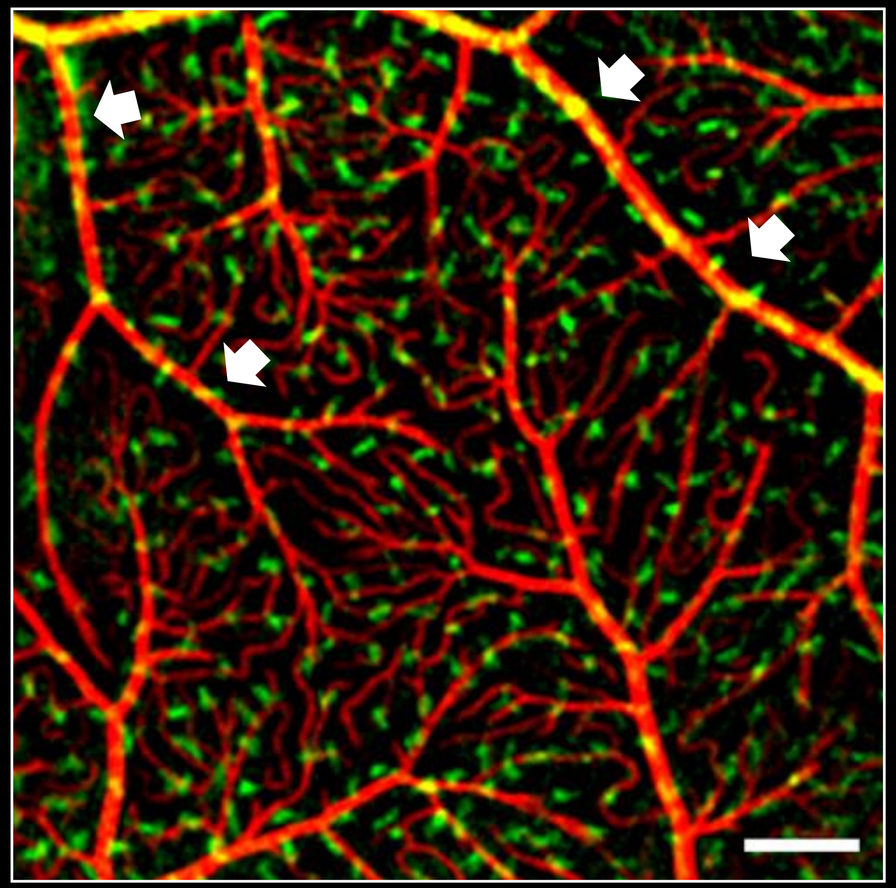




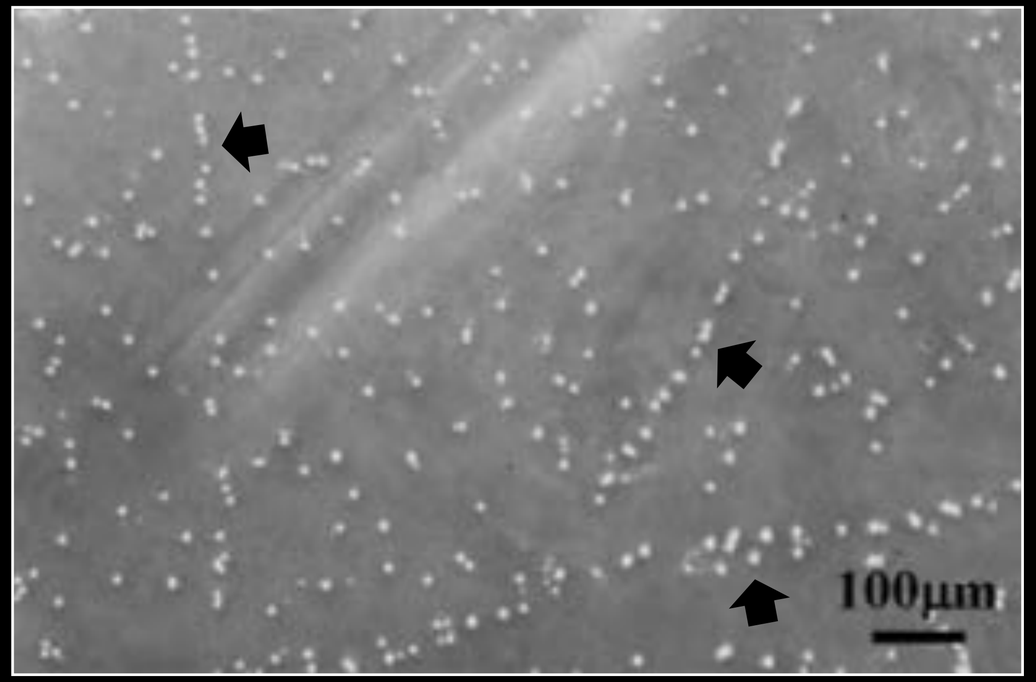
Vagaja. et al., Changes in Murine Hyalocytes Are Valuable Early Indicators of Ocular Disease. Invest Ophthalmol Vis Sci. 2012

Macrophage-Like Cells at the Vitreoretinal Interface - Hyalocytes (Bovine)

Macrophage-like cells
in normal living human retina



Hyalocytes
in normal cow retina

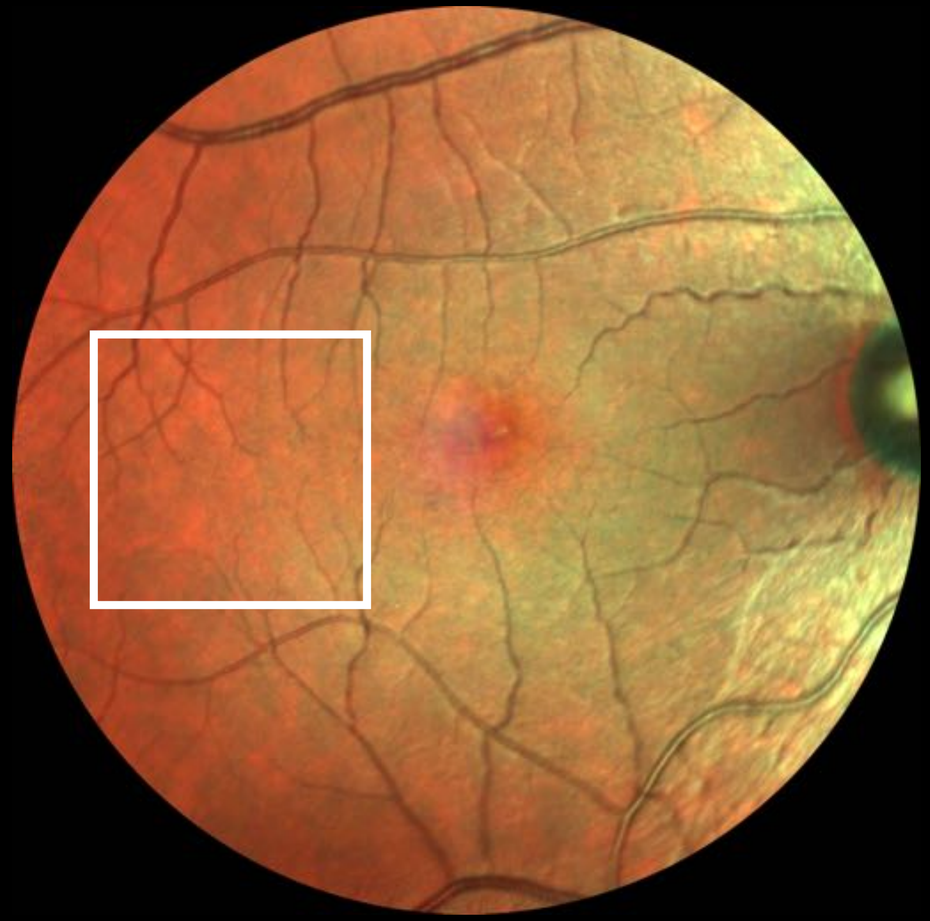


"In the extracted vitreous, the hyalocytes were present mainly on the vitreous gel surface and accumulated like branching blood vessels."

Noda. et al., Functional Properties of Hyalocytes under PDGF-Rich Conditions. Invest Ophthalmol Vis Sci. 2004

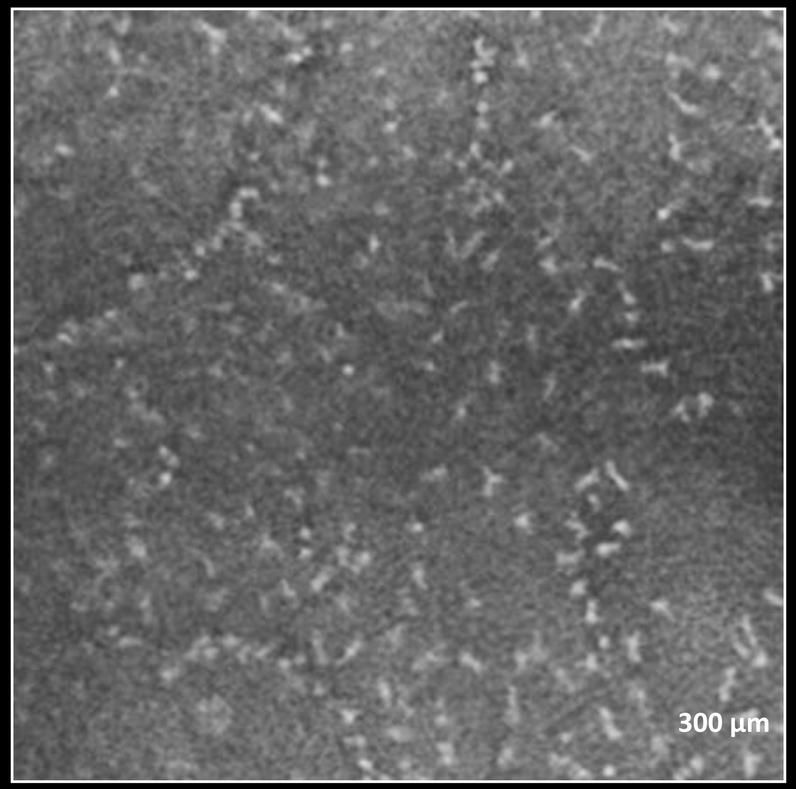
DMnoDR, 53 yo, M, OD

3x3mm Temporal Retina, ~ 9° from the fovea



DMnoDR, 53 yo, M, OD

OCT-R Slab
3µm above ILM to ILM surface



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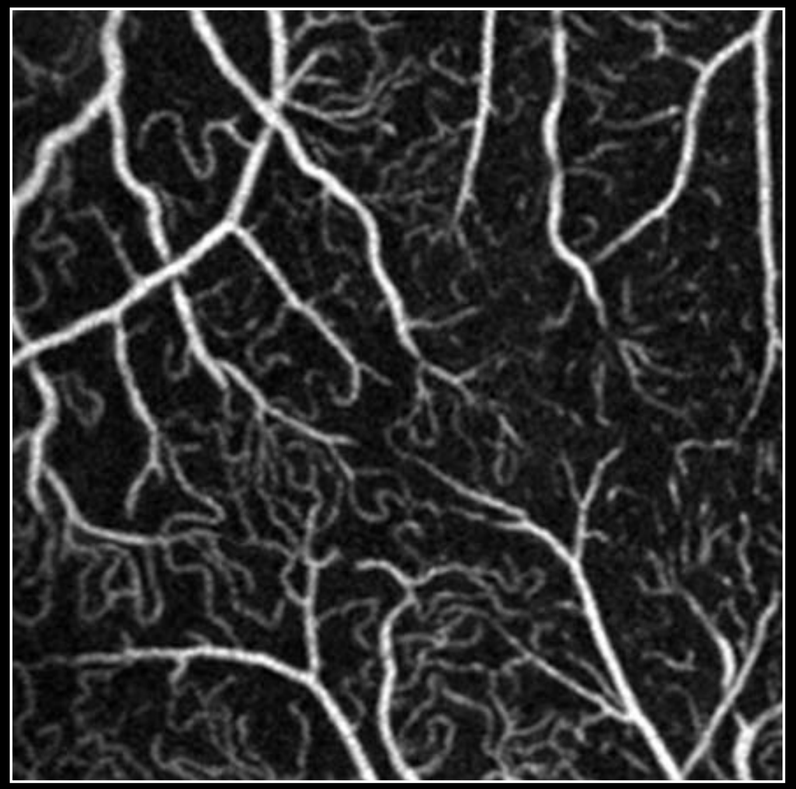
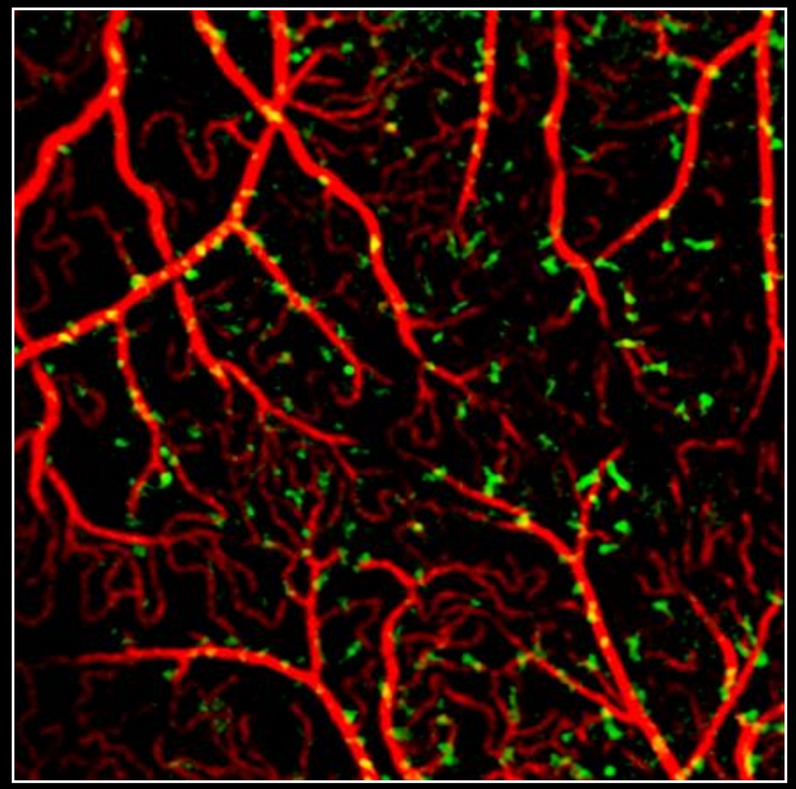


Color Overlay
OCT-R (Green) and OCT-A (Red)



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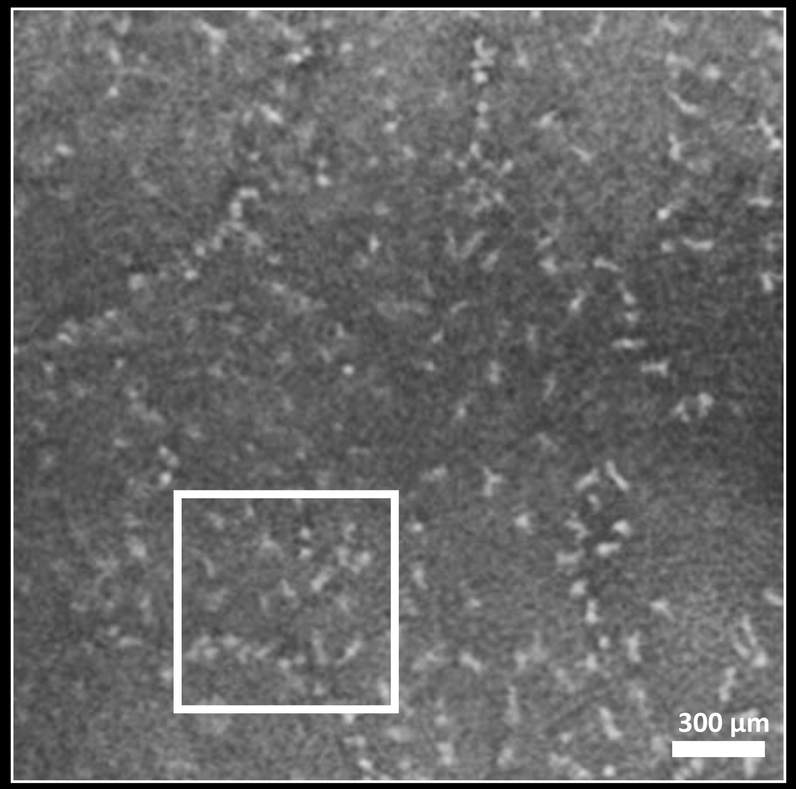
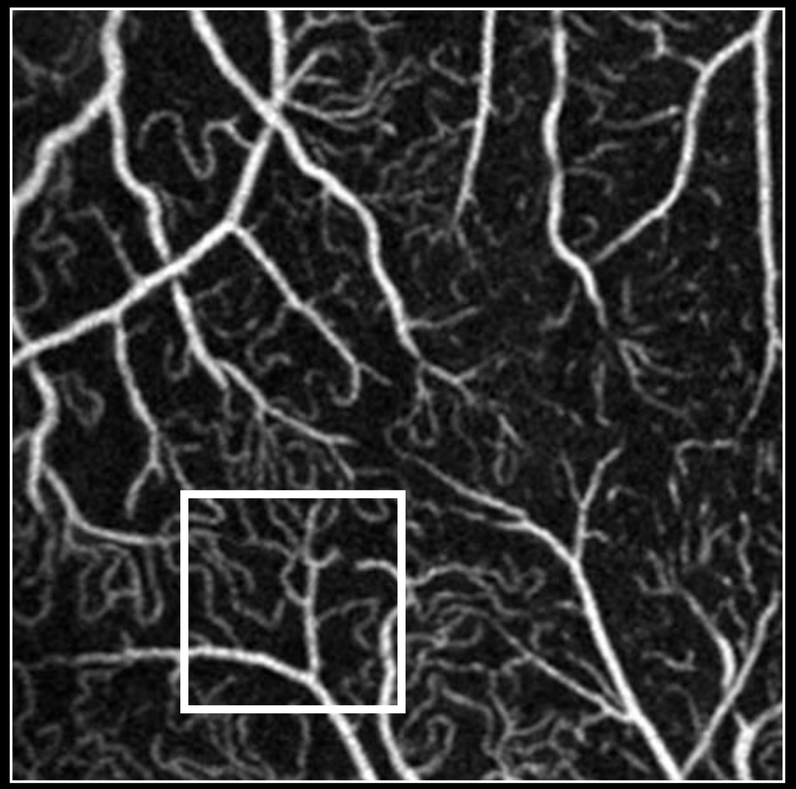
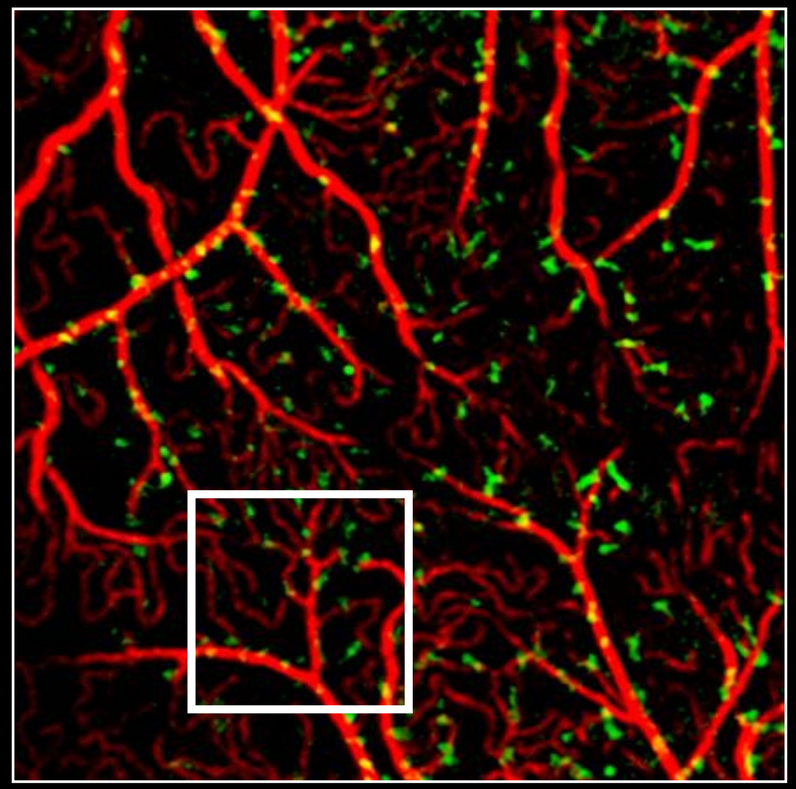


OCT-A Slab
ILM surface to 48µm below ILM

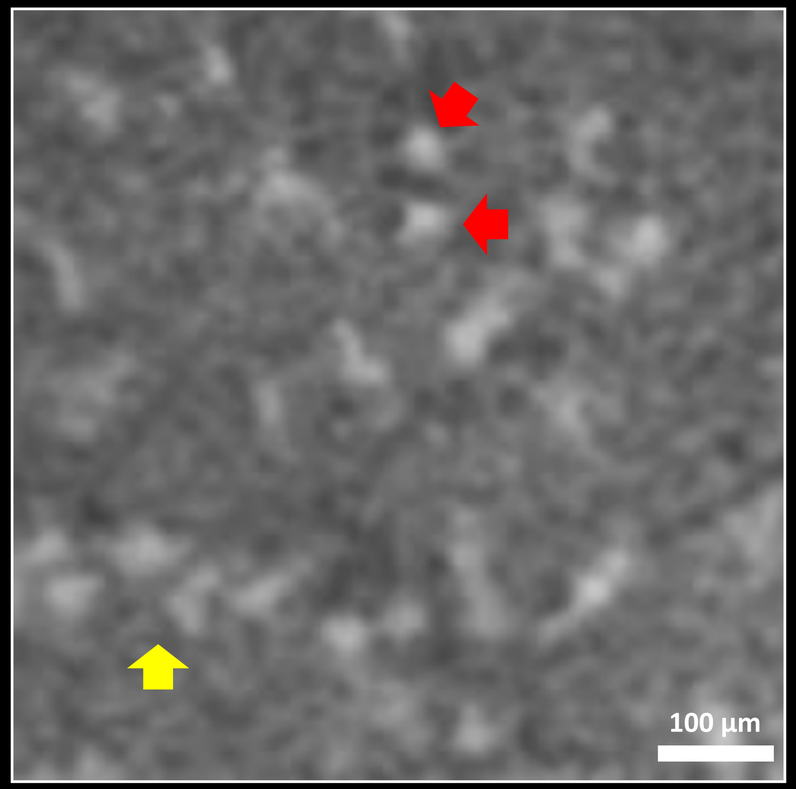


Color Overlay
OCT-R (Green) and OCT-A (Red)

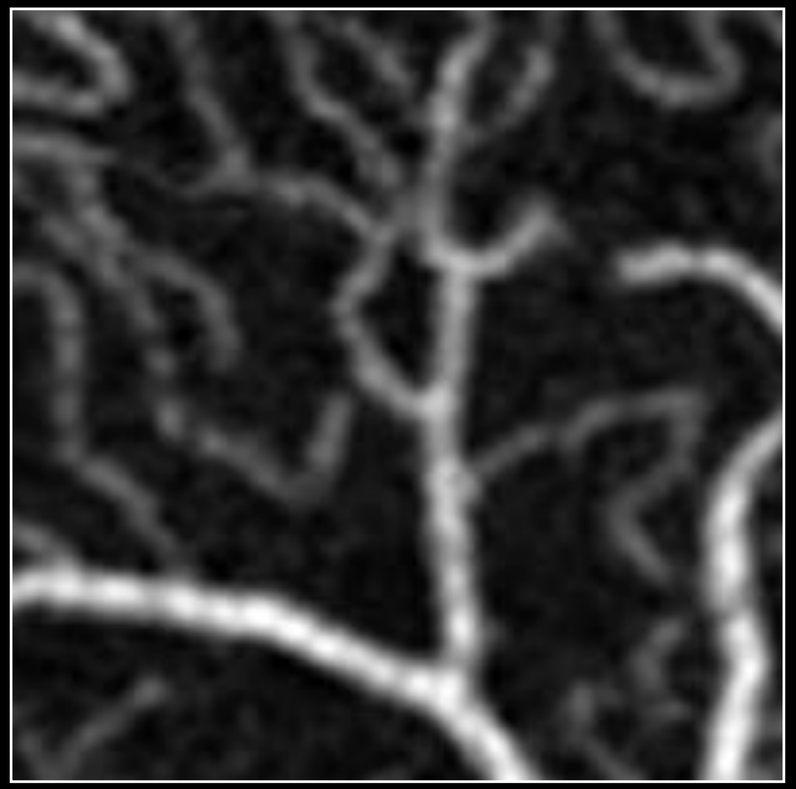


DMnoDR, 53 yo, M, OD

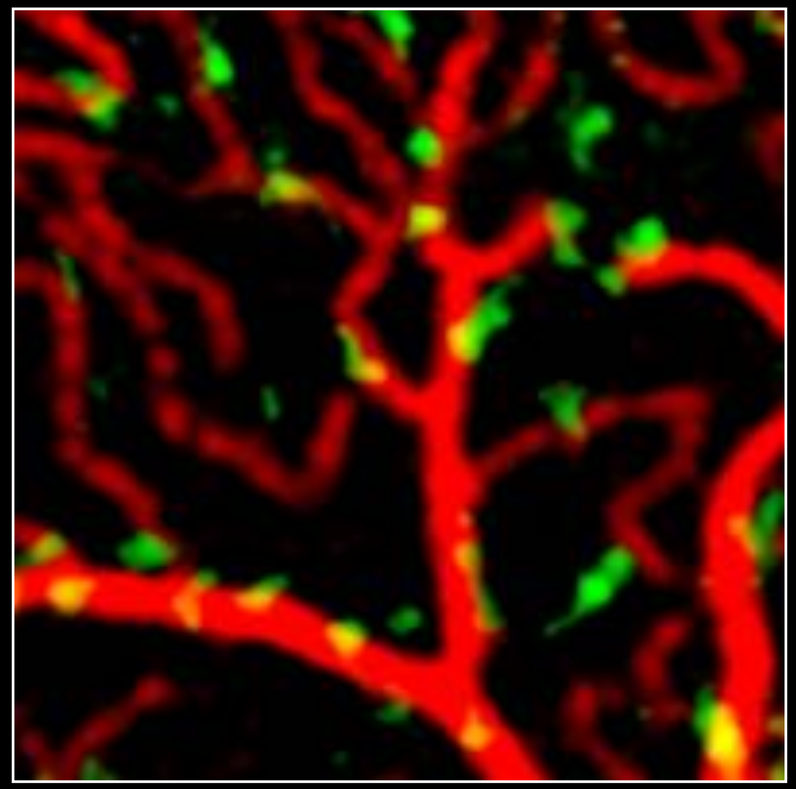
OCT-R Slab
3µm above ILM to ILM surface



OCT-A Slab
ILM surface to 48µm below ILM



Color Overlay
OCT-R (Green) and OCT-A (Red)

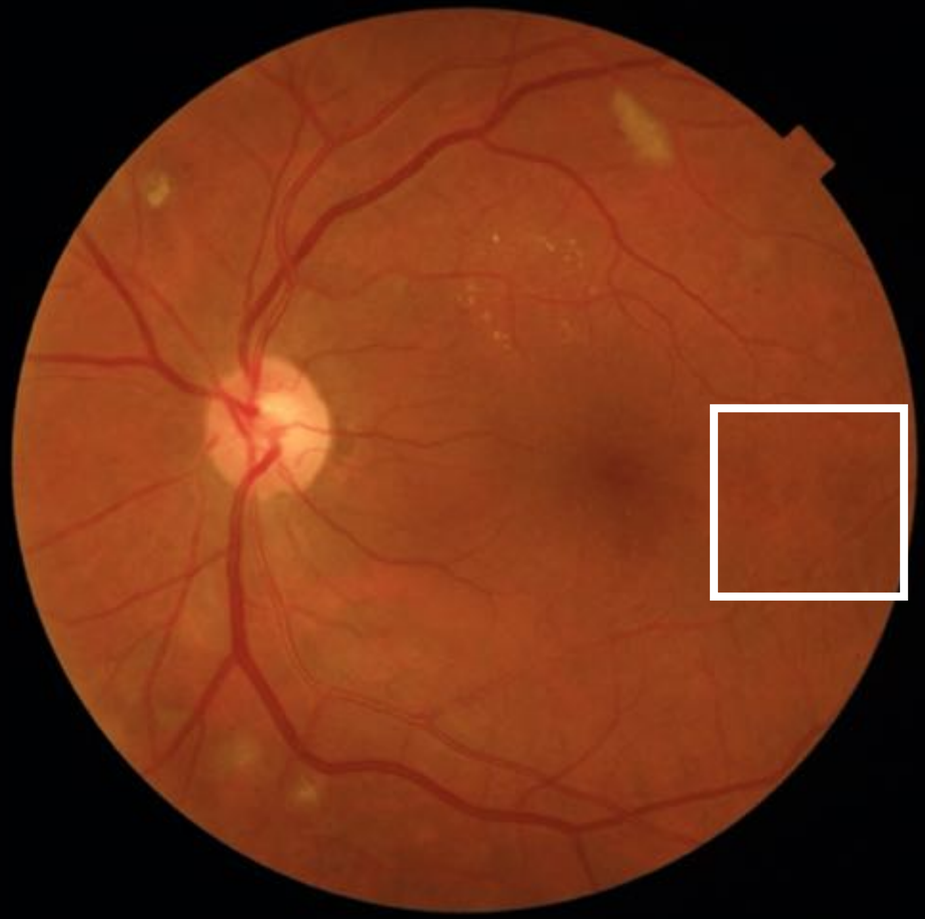


Cells with fewer protrusions (red arrows)
Irregular spatial distribution (yellow arrow)

Cells co-localized with blood vessels

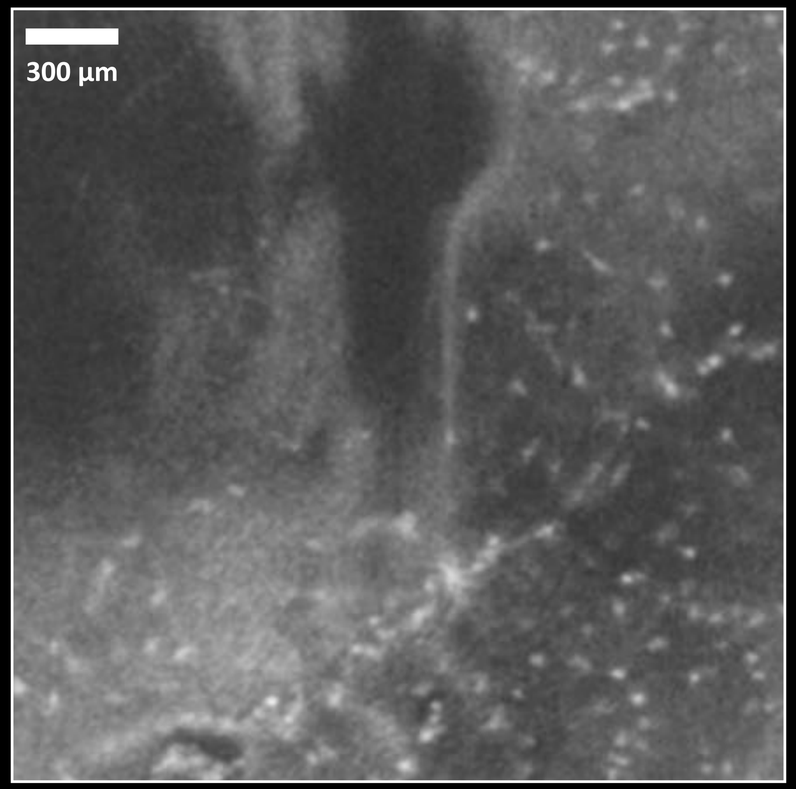
NPDR, 60 yo, F, OS

3x3mm Temporal Retina, ~ 9° from the fovea

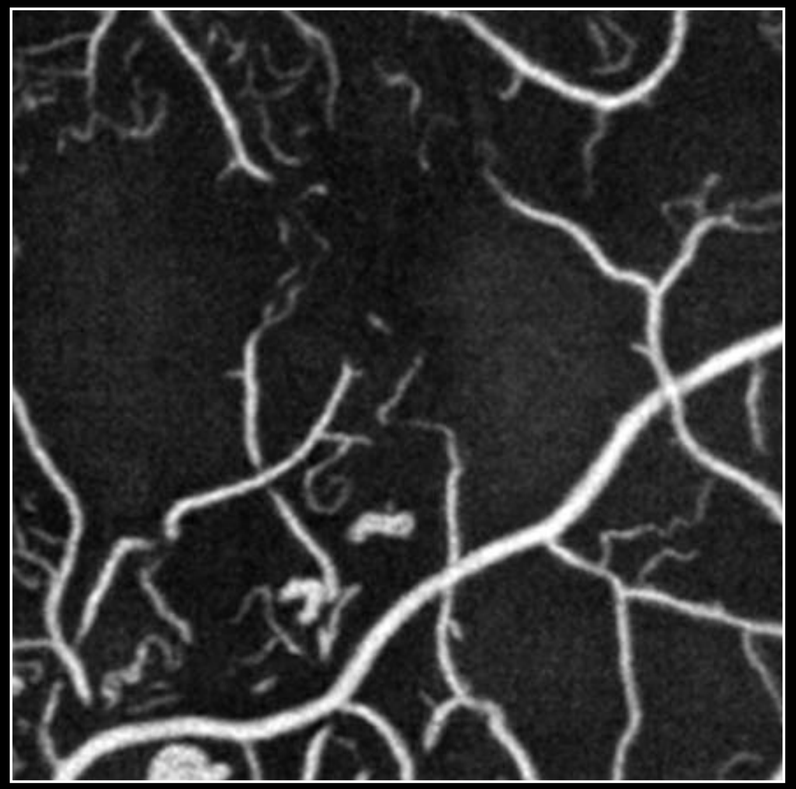


NPDR, 60 yo, F, OS

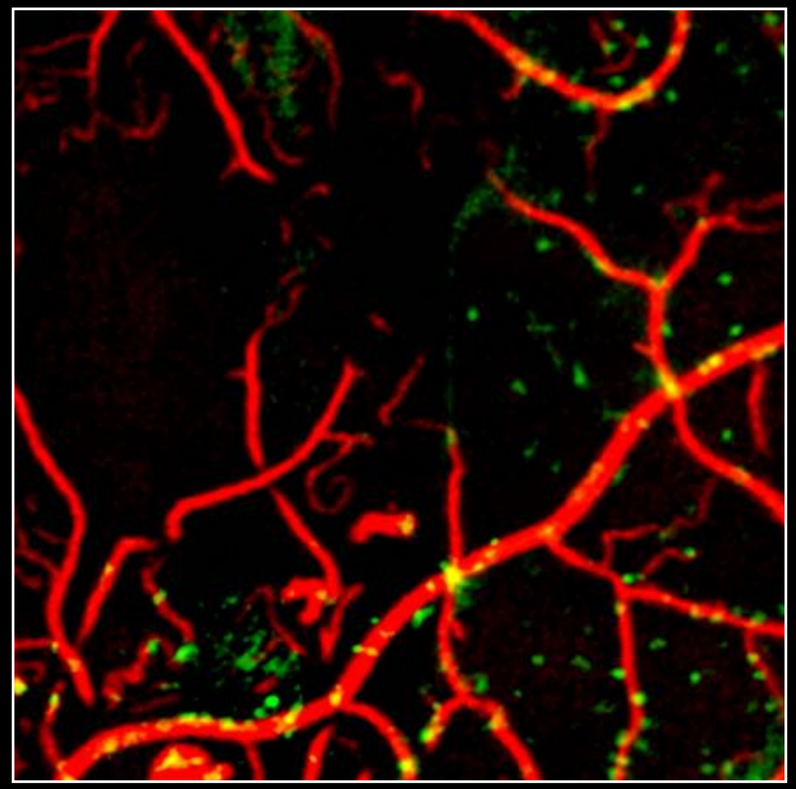
OCT-R Slab
3µm above ILM to ILM surface



OCT-A Slab
ILM surface to 48µm below ILM



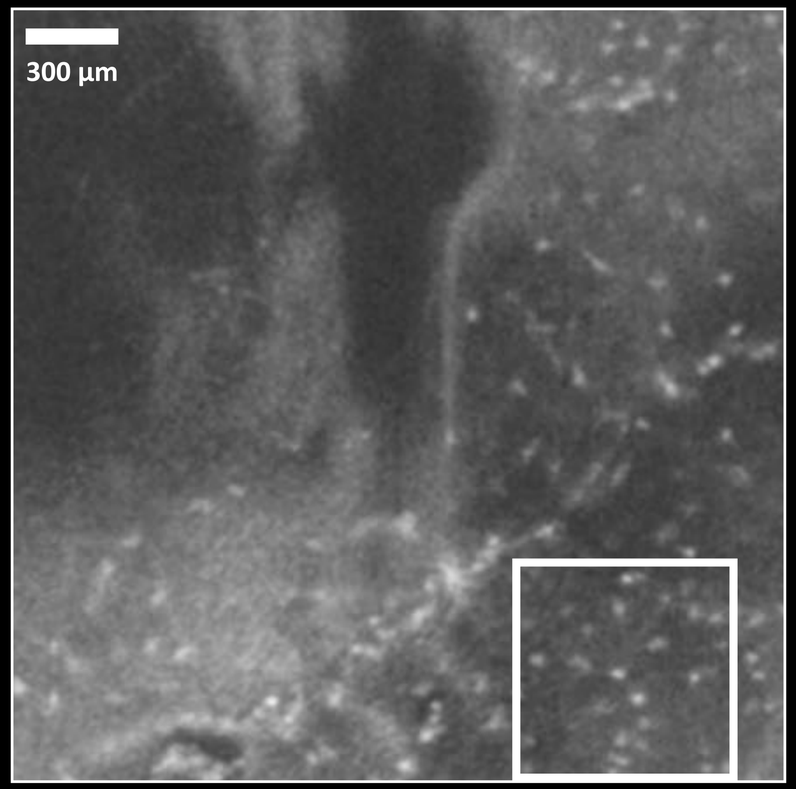
Color Overlay
OCT-R (Green) and OCT-A (Red)



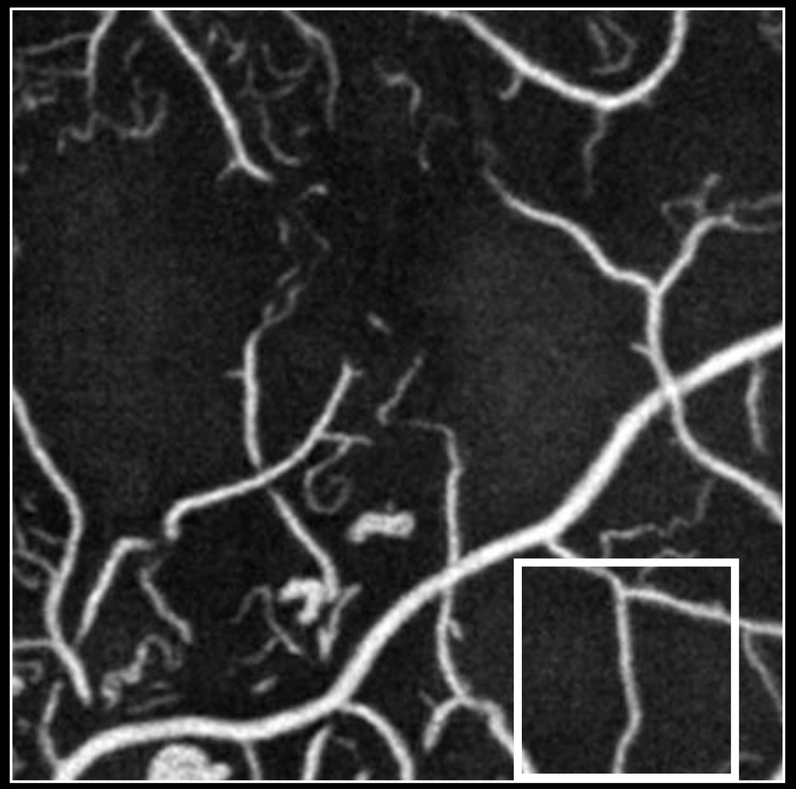
Cells show uneven distribution and clustering along the vessels

NPDR, 60 yo, F, OS

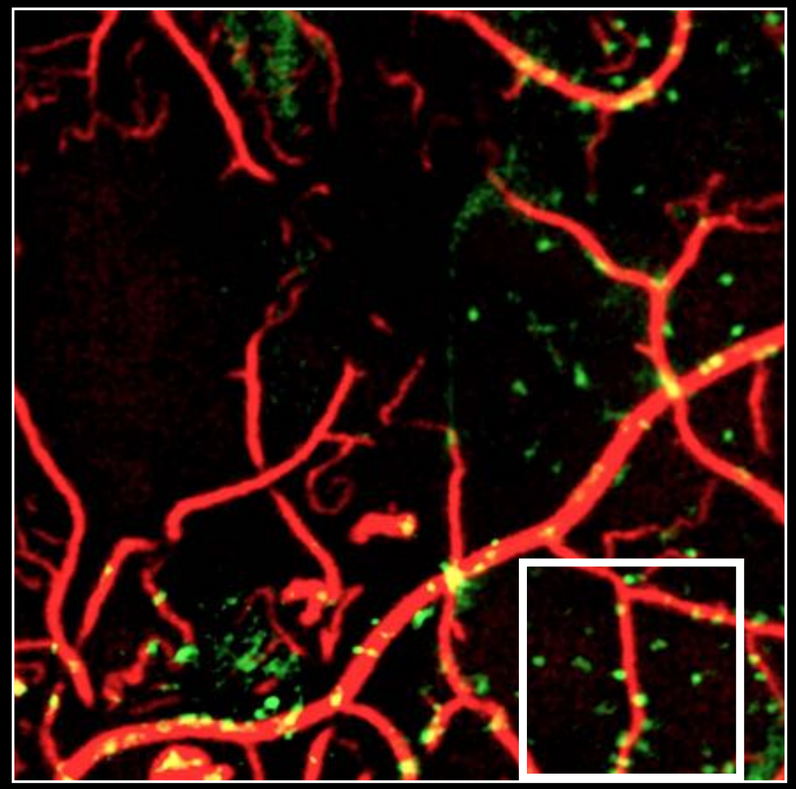
OCT-R Slab
3µm above ILM to ILM surface



OCT-A Slab
ILM surface to 48µm below ILM

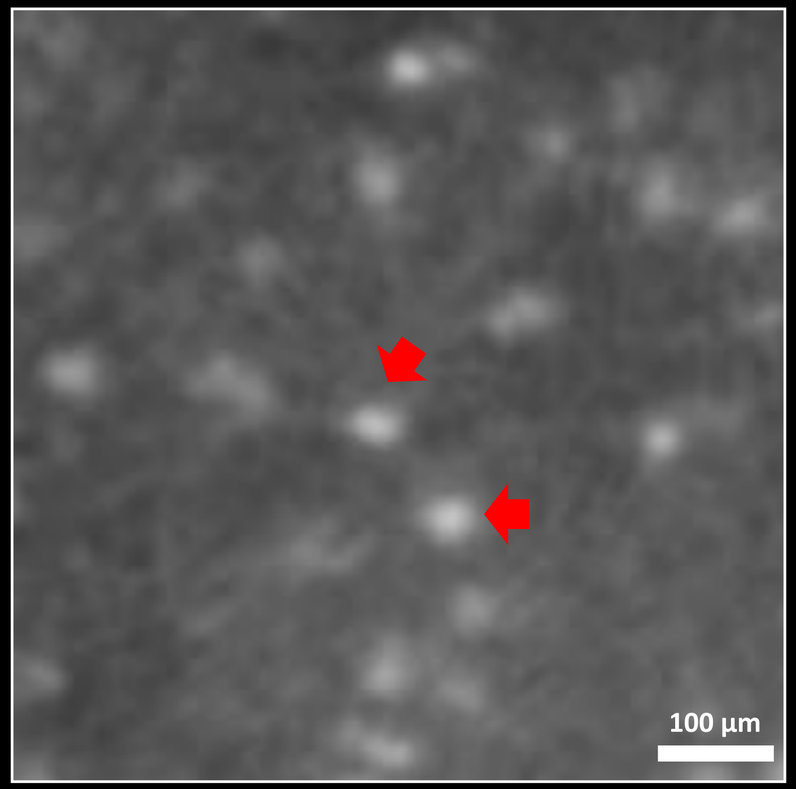


Color Overlay
OCT-R (Green) and OCT-A (Red)

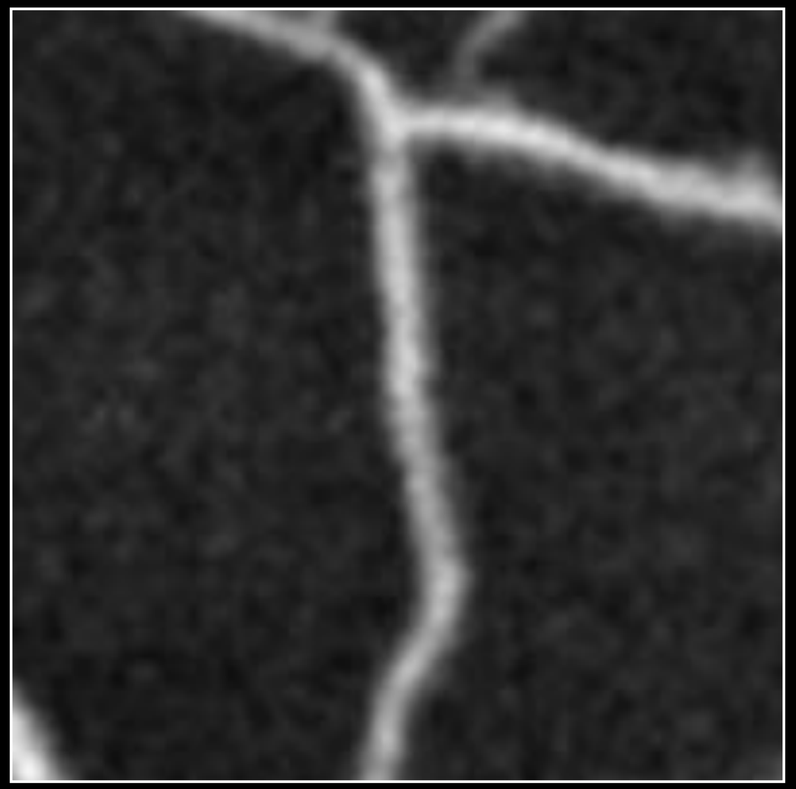


NPDR, 60 yo, F, OS

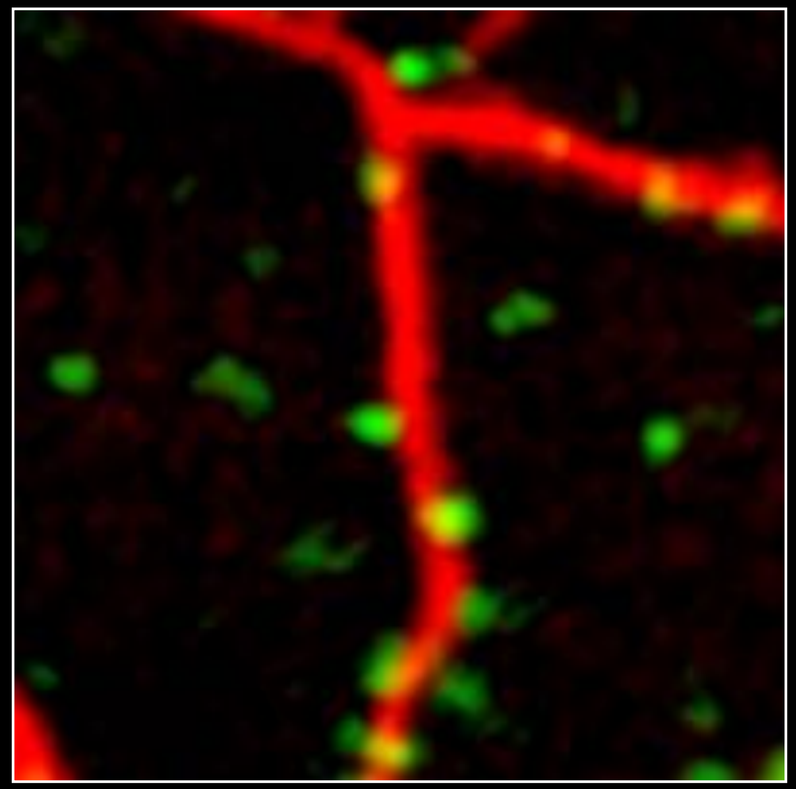
OCT-R Slab
3µm above ILM to ILM surface



OCT-A Slab
ILM surface to 48µm below ILM



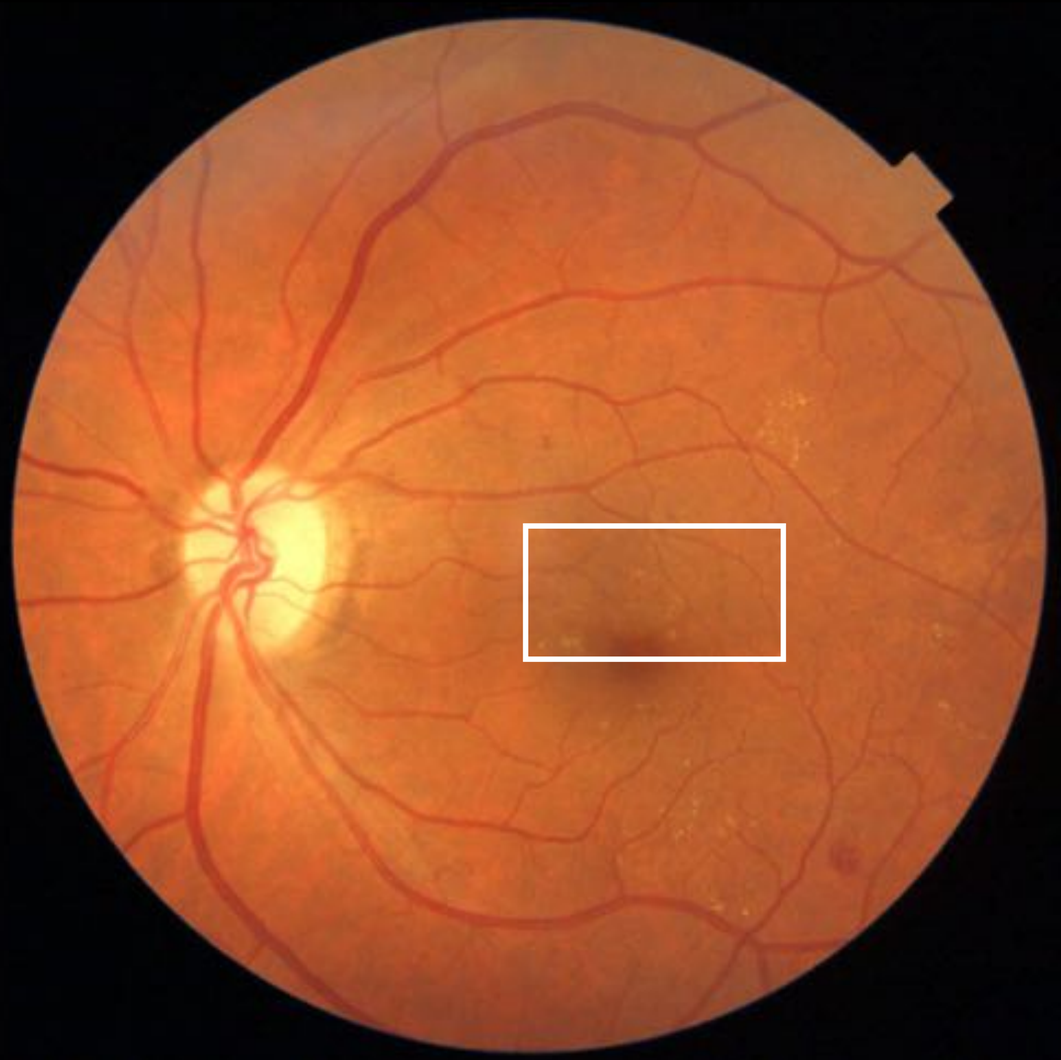
Color Overlay
OCT-R (Green) and OCT-A (Red)

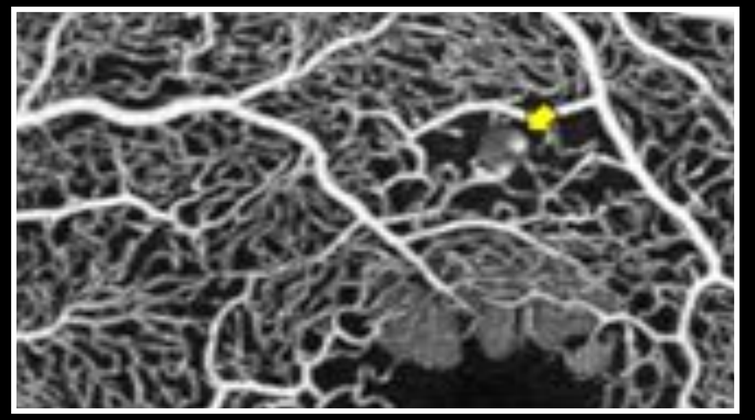


Cells with fewer protrusions (red arrows)

Cells co-localized with blood vessels

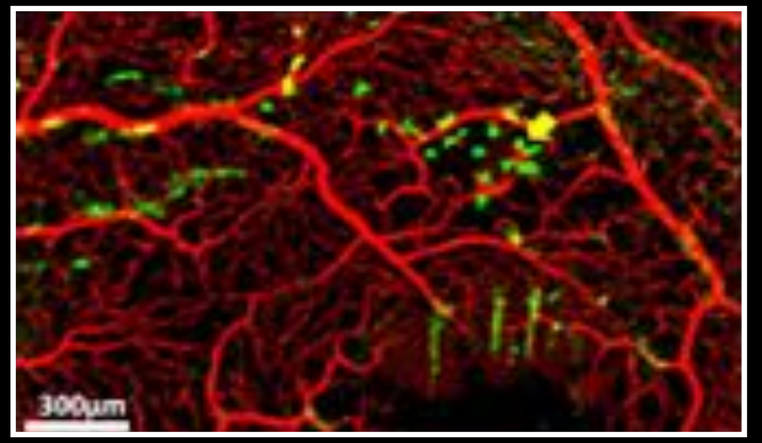
NPDR, 56 yo, F, OS





Full
Vascular Layer
OCTA

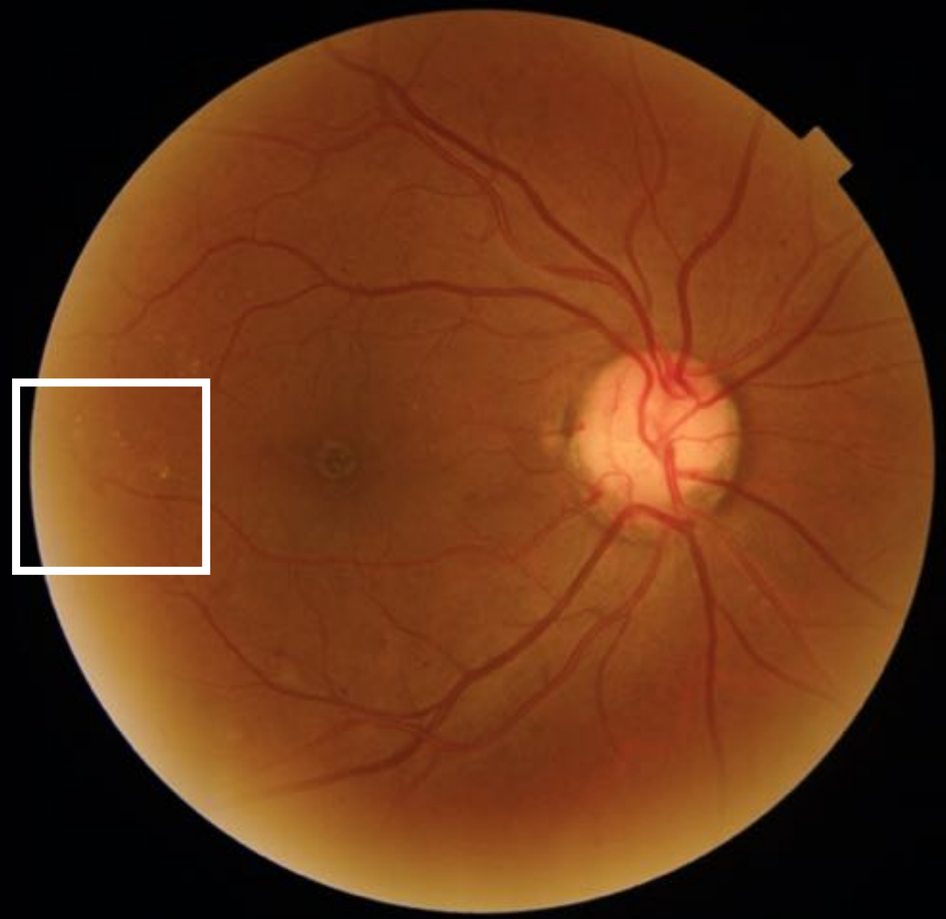
Macrophage -
Like Cell Layer
En Face OCTR
3μ above ILM
to ILM surface



Macrophage -
Like Cell Layer
En Face OCTR
(Green) .
Full
Vascular Layer
OCTA (Red)

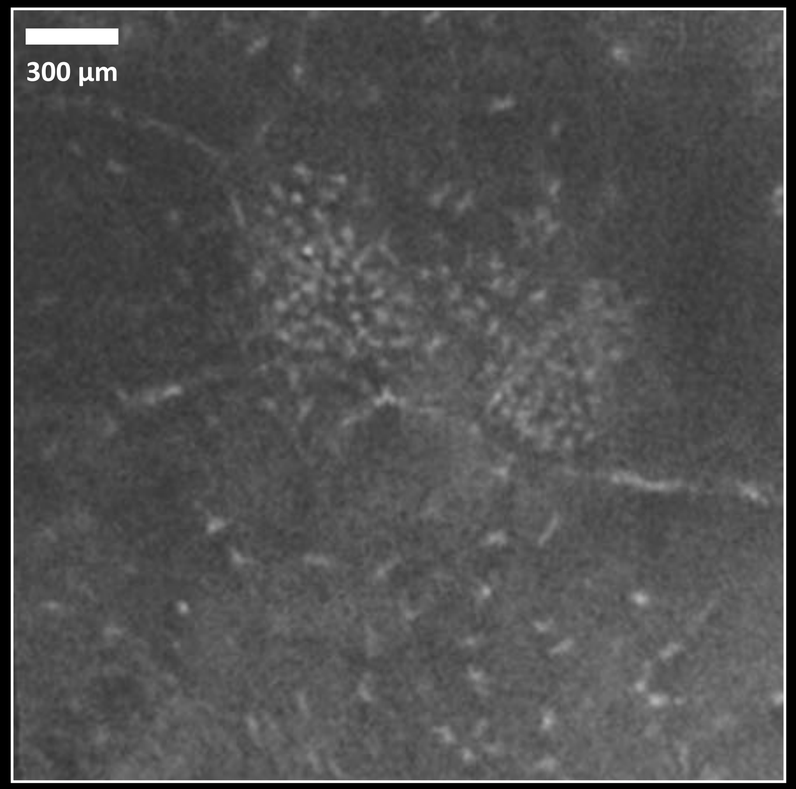
PDR, 53 yo, F, OD

3x3mm Temporal Retina, ~ 9° from the fovea

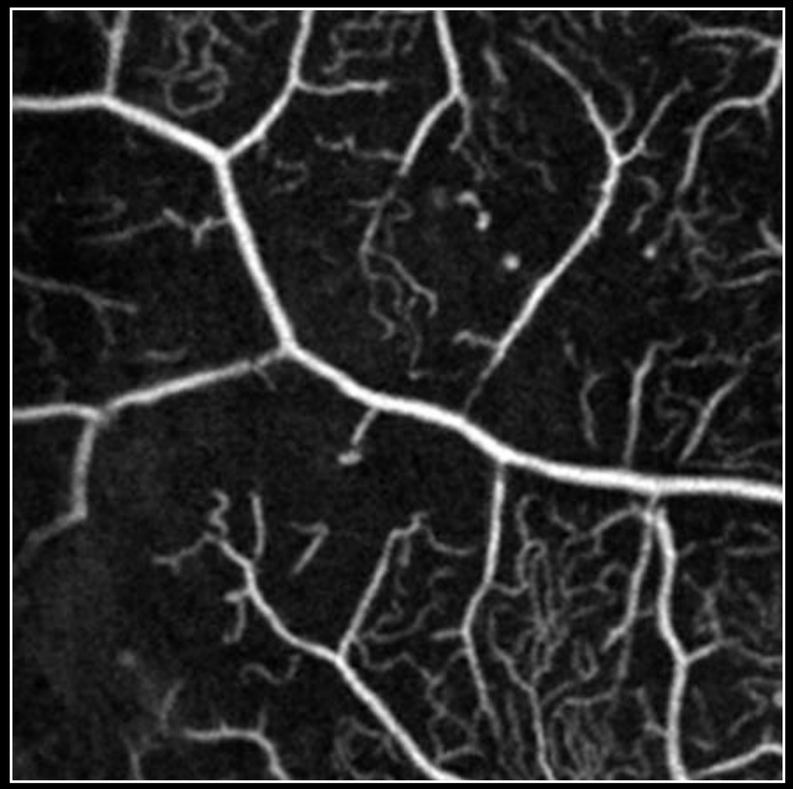


PDR, 53 yo, F, OD

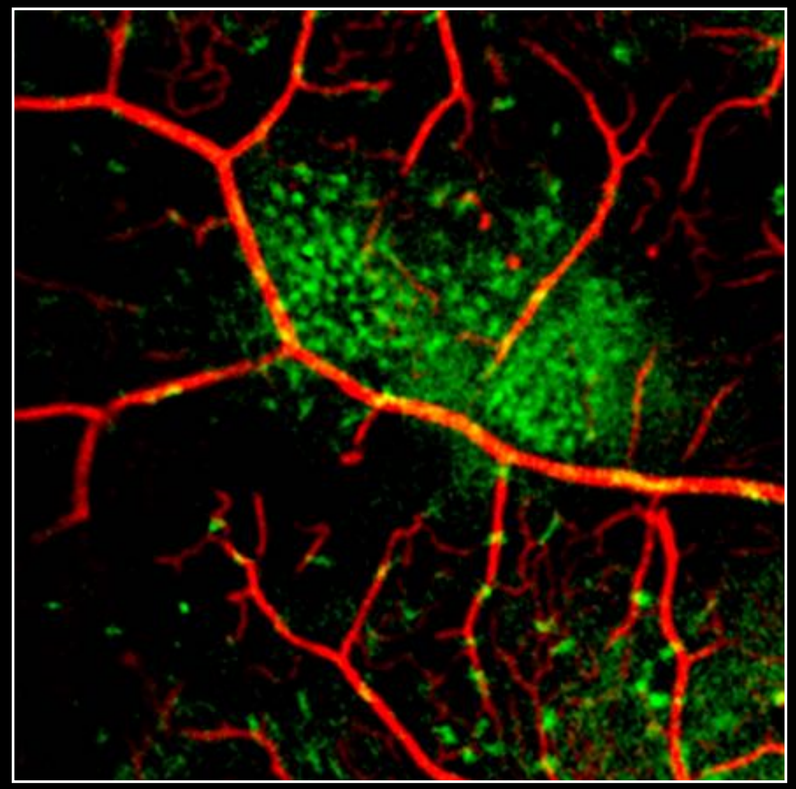
OCT-R Slab
3µm above ILM to ILM surface



OCT-A Slab
ILM surface to 48µm below ILM

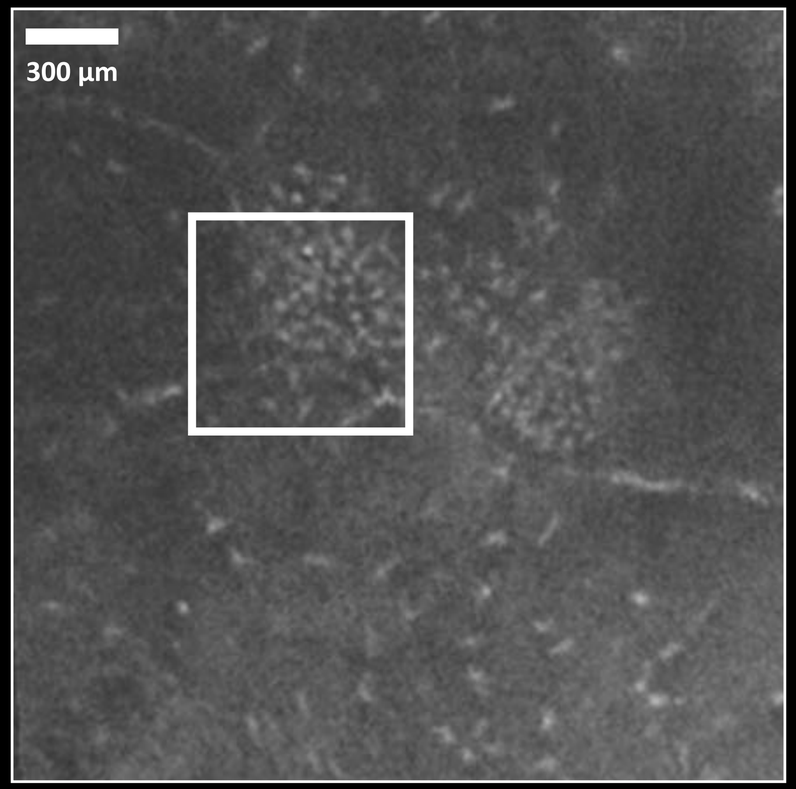


Color Overlay
OCT-R (Green) and OCT-A (Red)

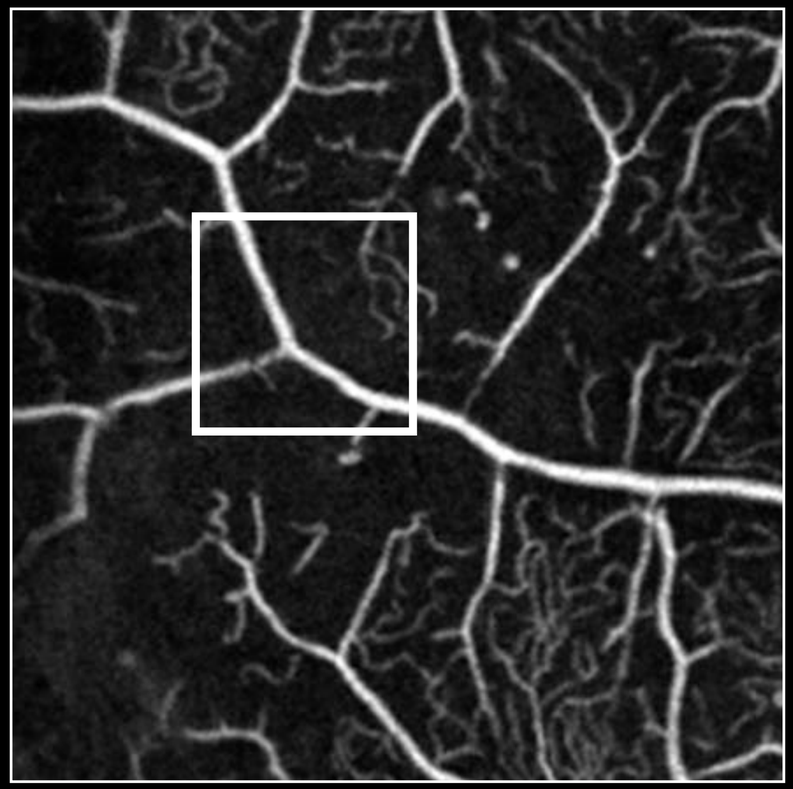


PDR, 53 yo, F, OD

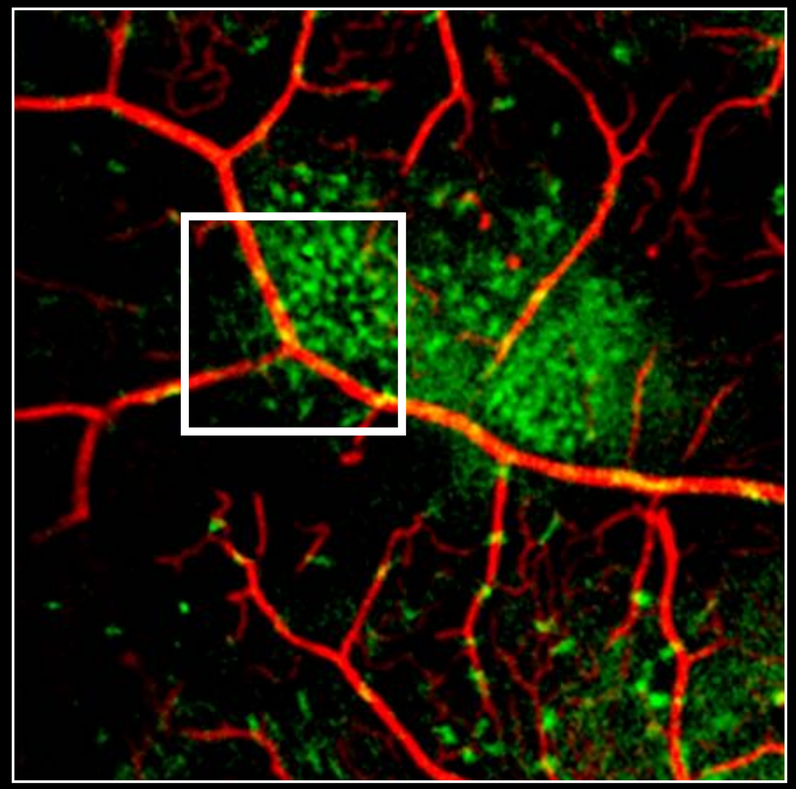
OCT-R Slab
3µm above ILM to ILM surface



OCT-A Slab
ILM surface to 48µm below ILM

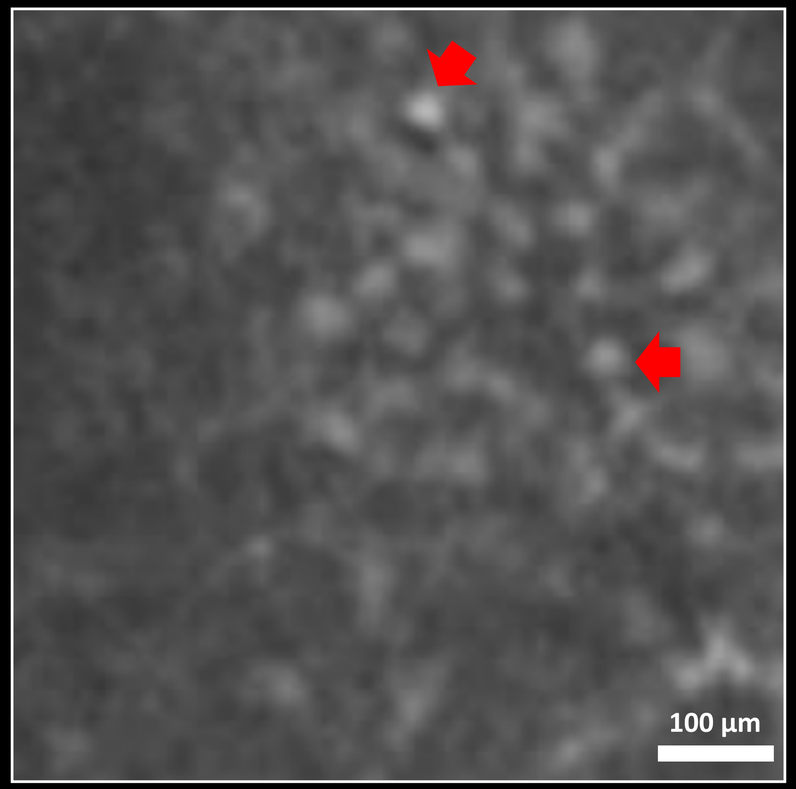


Color Overlay
OCT-R (Green) and OCT-A (Red)

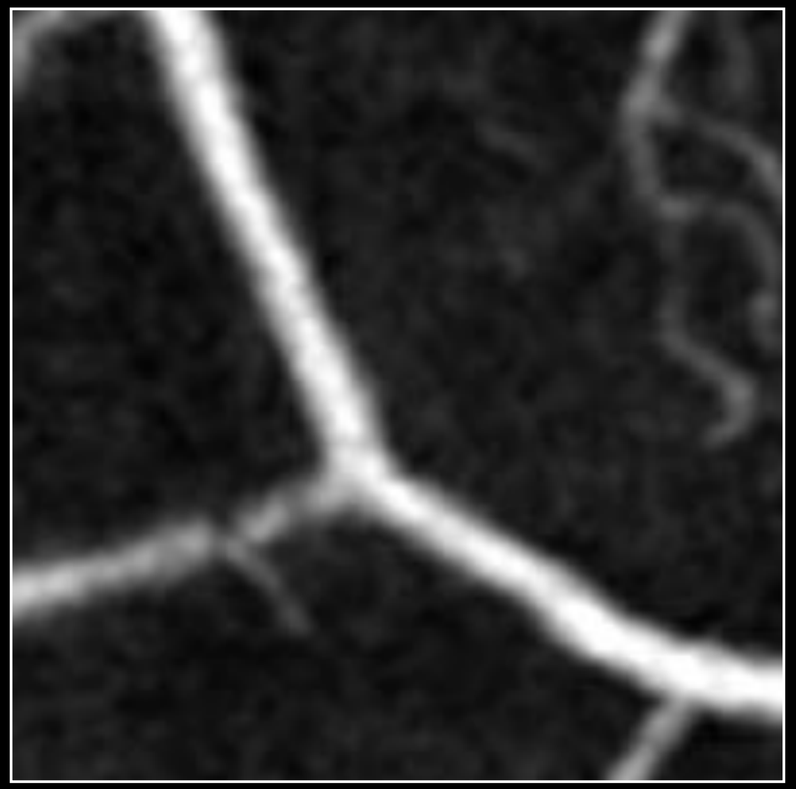


PDR, 53 yo, F, OD

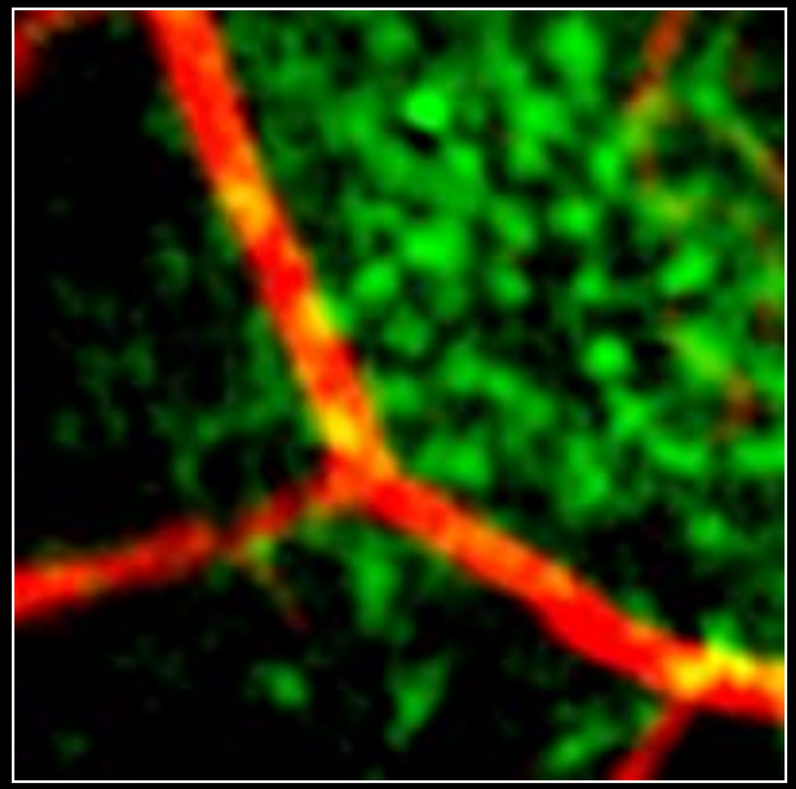
OCT-R Slab
3µm above ILM to ILM surface



OCT-A Slab
ILM surface to 48µm below ILM



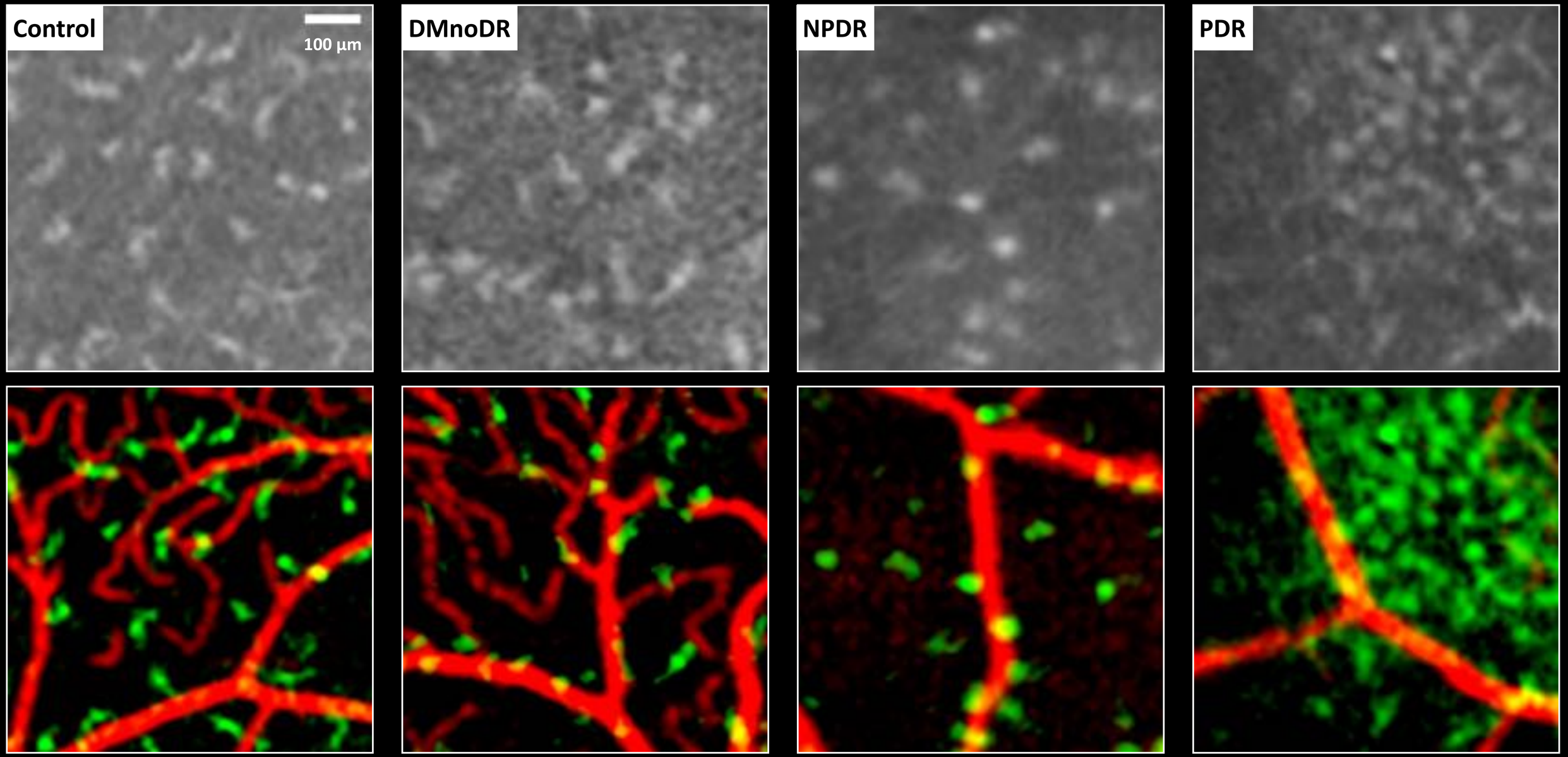
Color Overlay
OCT-R (Green) and OCT-A (Red)



Cells with fewer protrusions (red arrows)
Clustered Cells

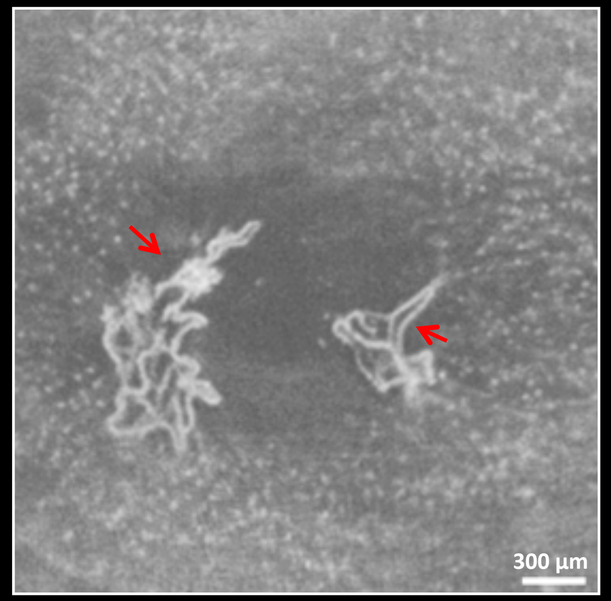
Cells co-localized with blood vessels

Cell Activation and Clustering in DR

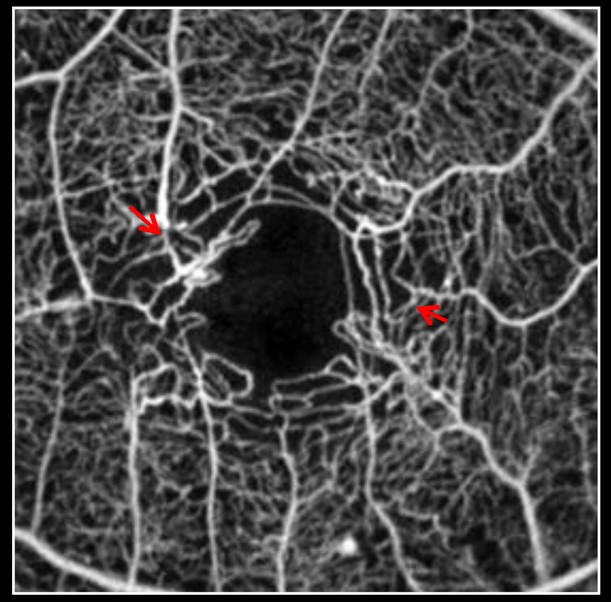


Proliferative Diabetic Retinopathy, 61yo, M, OD

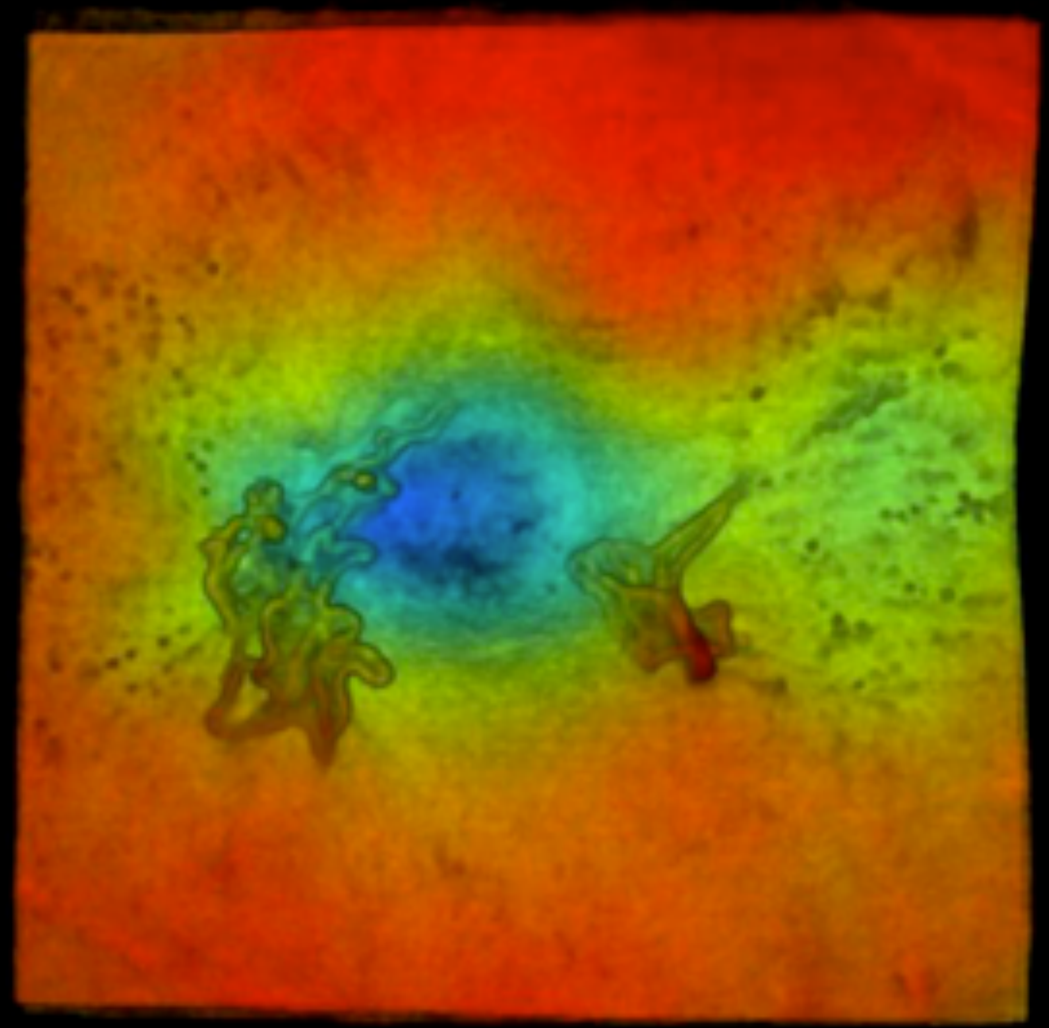
OCT Reflectance
3 µm above ILM

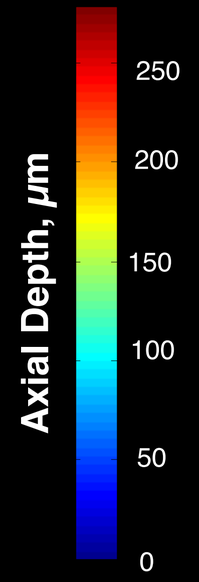


OCT Angiograph
Full Vascular Slab

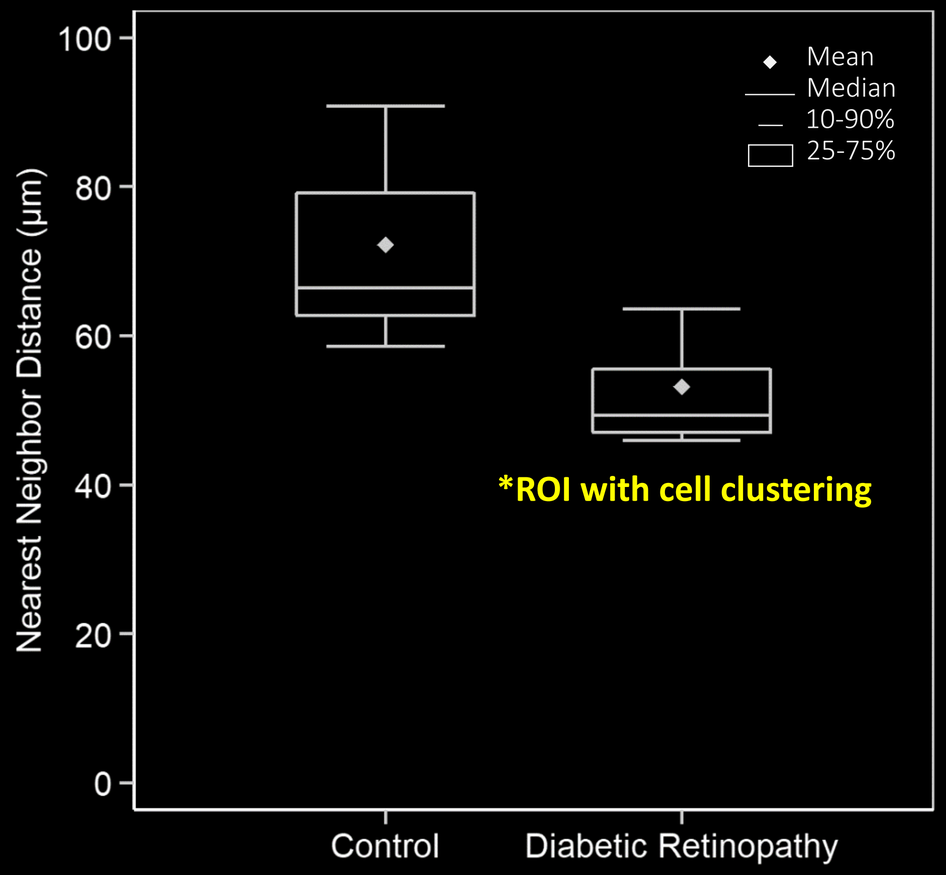


Neovascularization (red arrows) and macrophage-like cell proliferation at the Vitreoretinal Interface

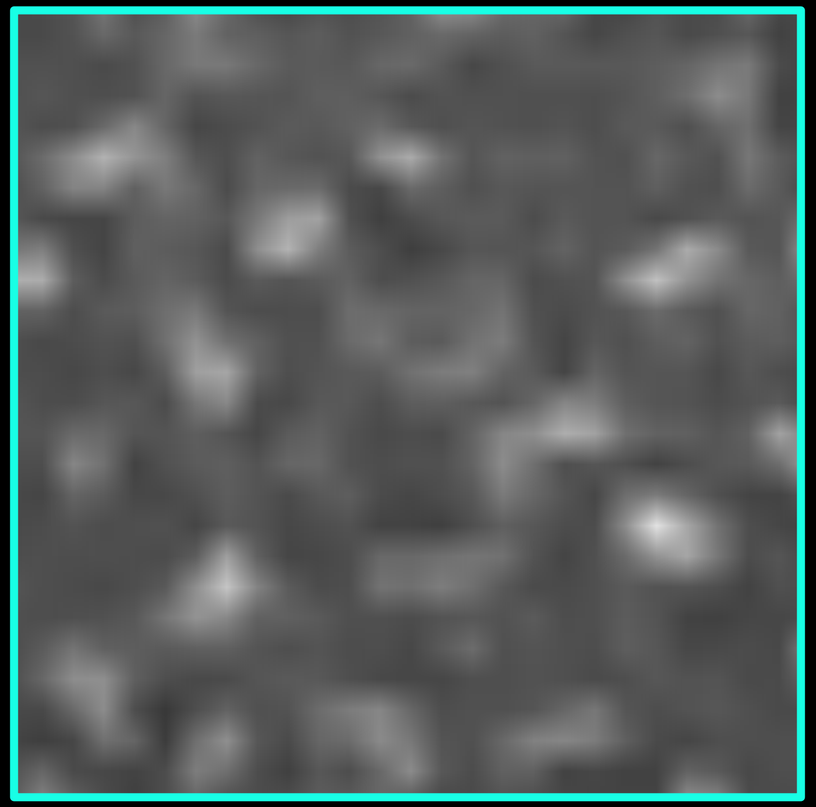




Nearest Neighbor Distance and Cell Density at ROIs



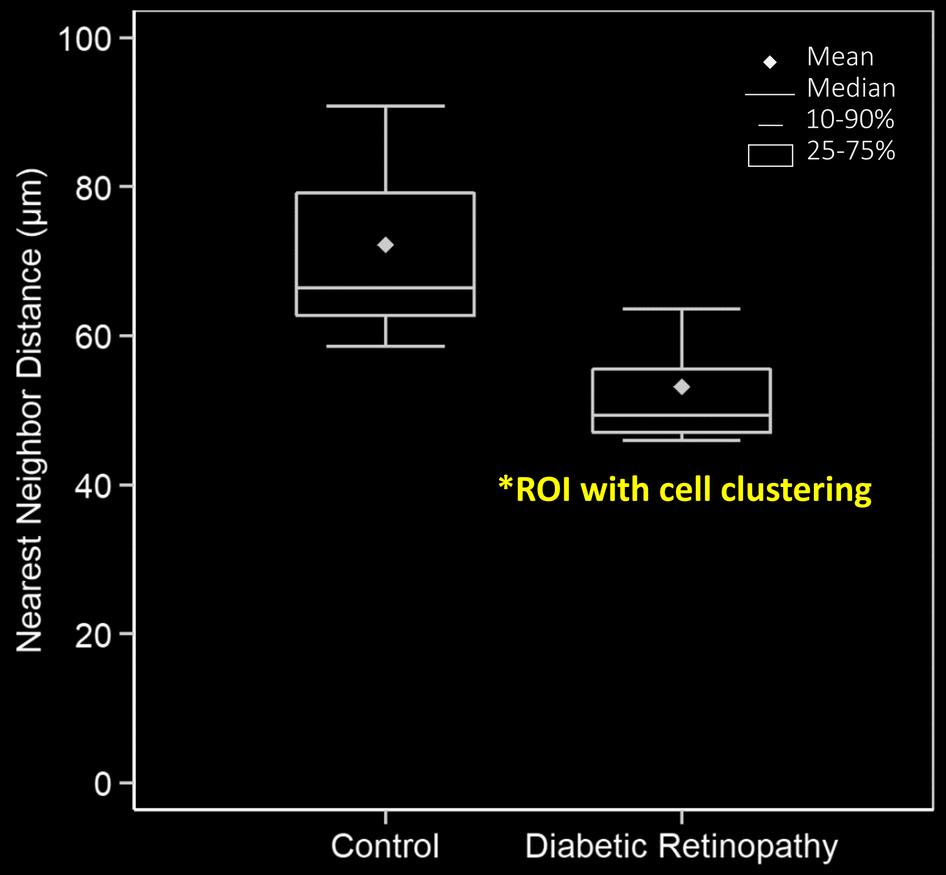
Representative Healthy Control
Temporal OCT-R: 3 µm above ILM



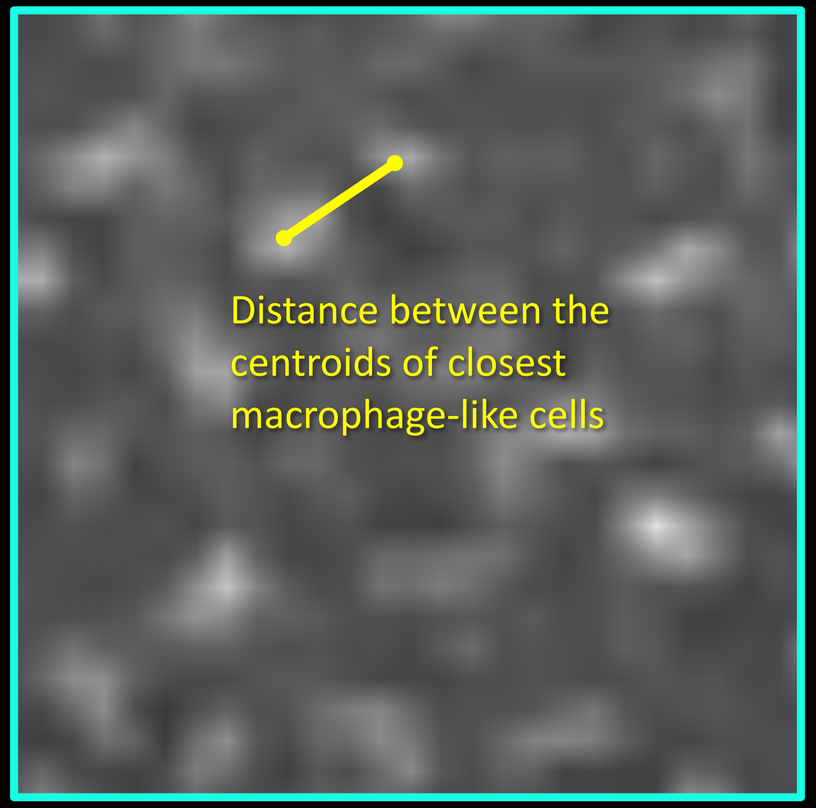
500x500 µm ROI

Cells appear to translocate toward regions of metabolic stress reducing their nearest neighbor distance within the cluster

Nearest Neighbor Distance and Cell Density at ROIs



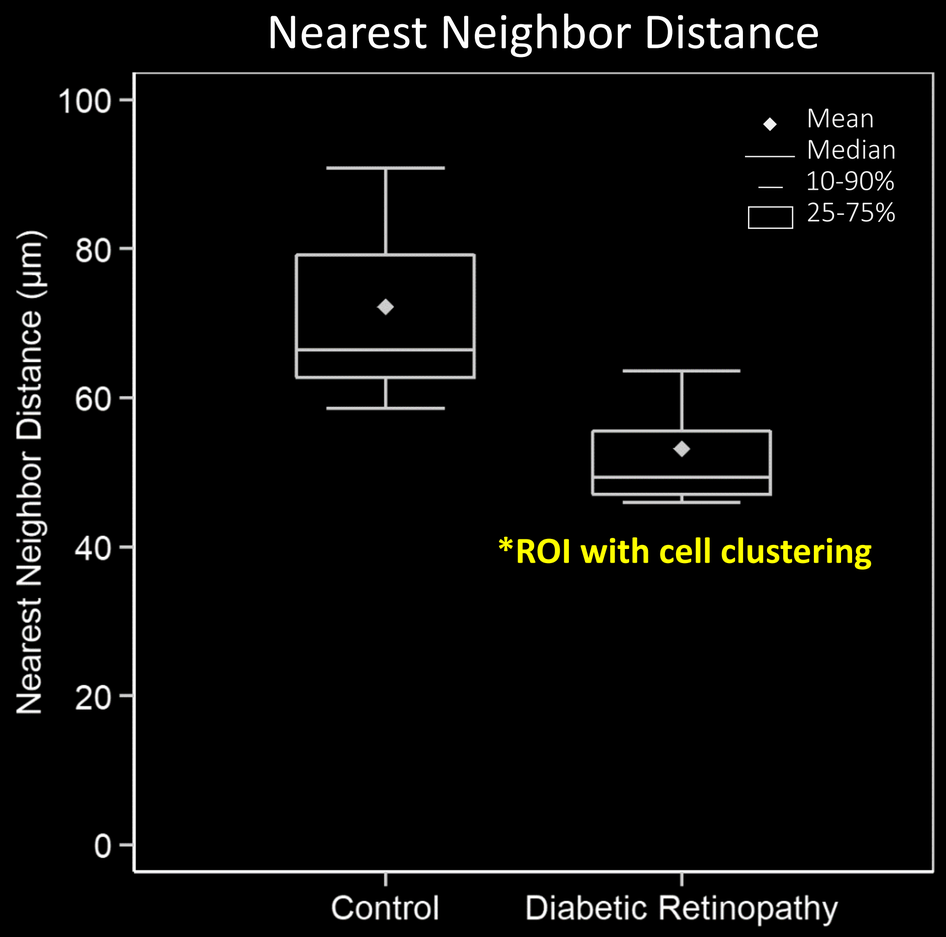
Representative Healthy Control
Temporal OCT-R: 3 µm above ILM

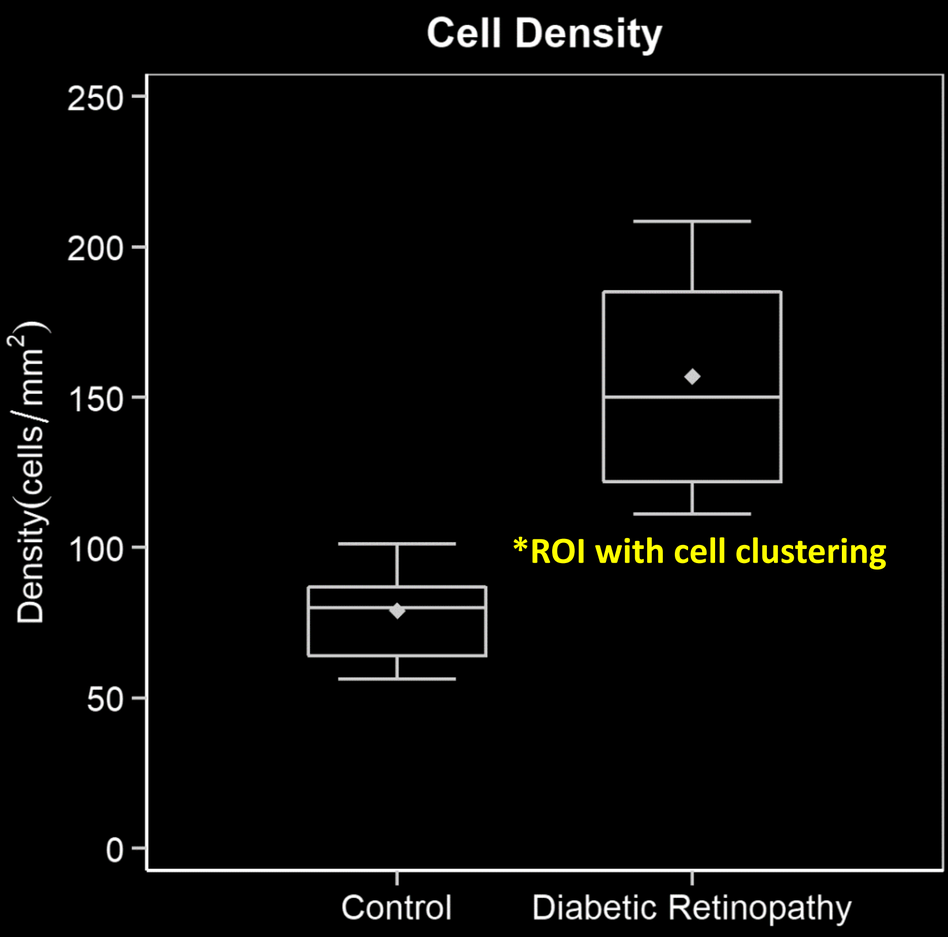


500x500 µm ROI

Cells appear to translocate toward regions of metabolic stress reducing their nearest neighbor distance within the cluster

Nearest Neighbor Distance and Cell Density at ROIs

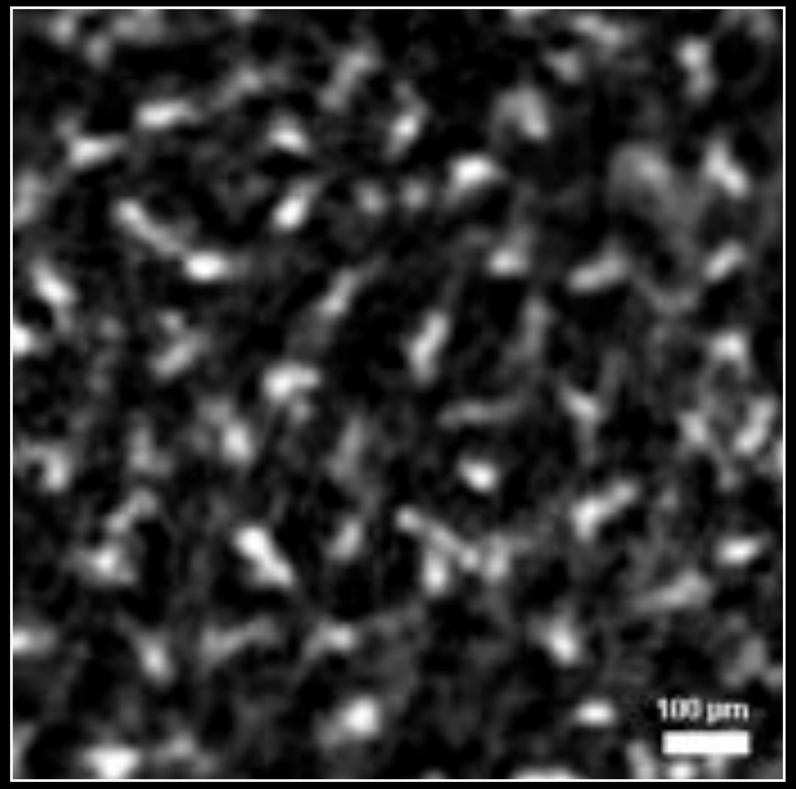




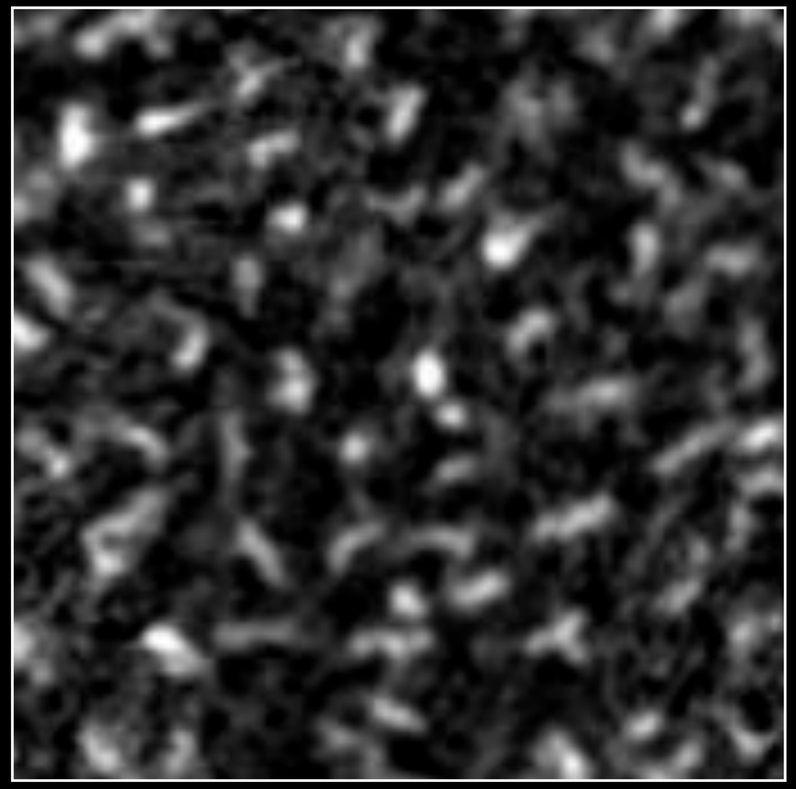
Cells density increases in regions of interest correlate with clinical retina changes

Macrophage-like Cell Translocation Over 6 Hours

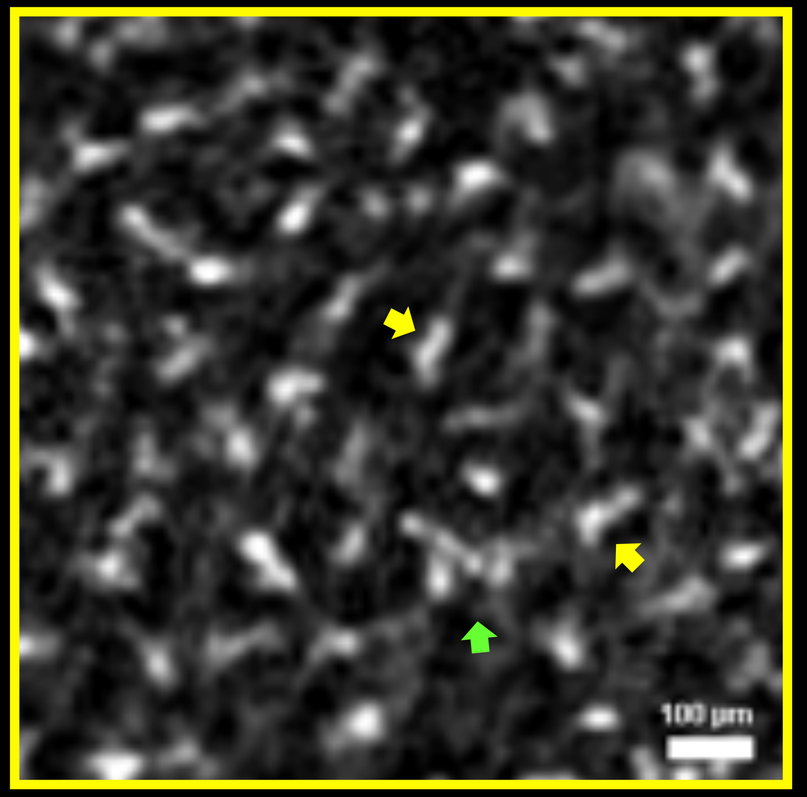
Baseline



Follow up – 6 hours



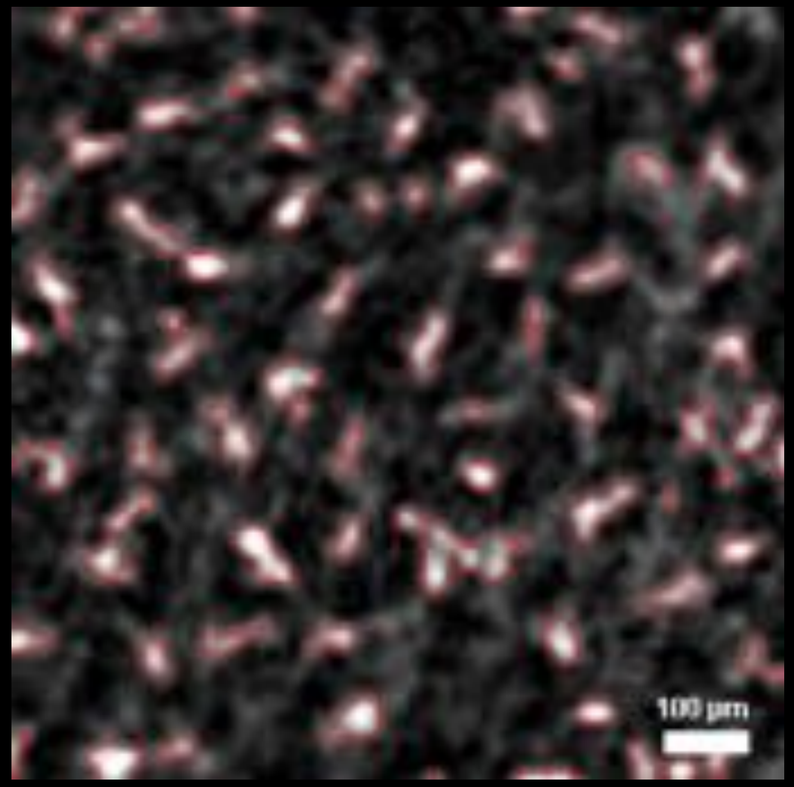
Changes between visits



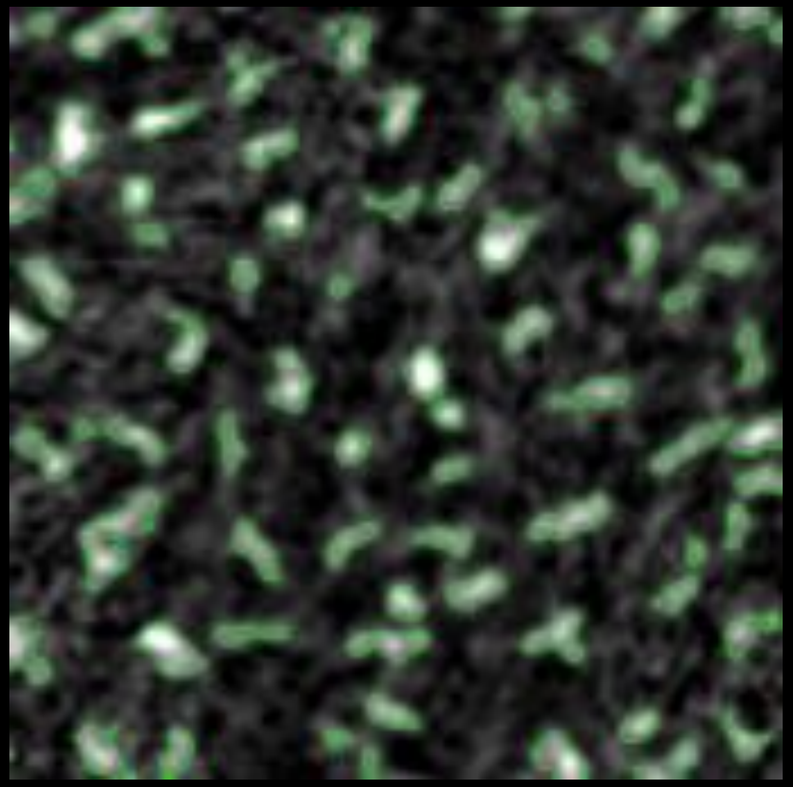
* Simulated cell movement

Changes in Cell Boundaries over 6 Hours

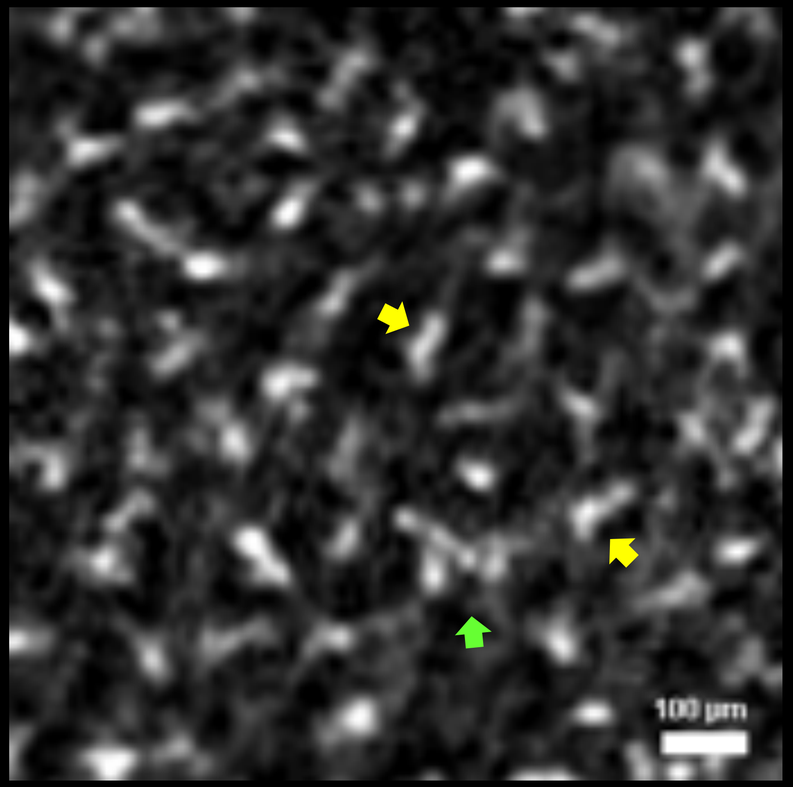
Baseline



Follow up – 6 hours



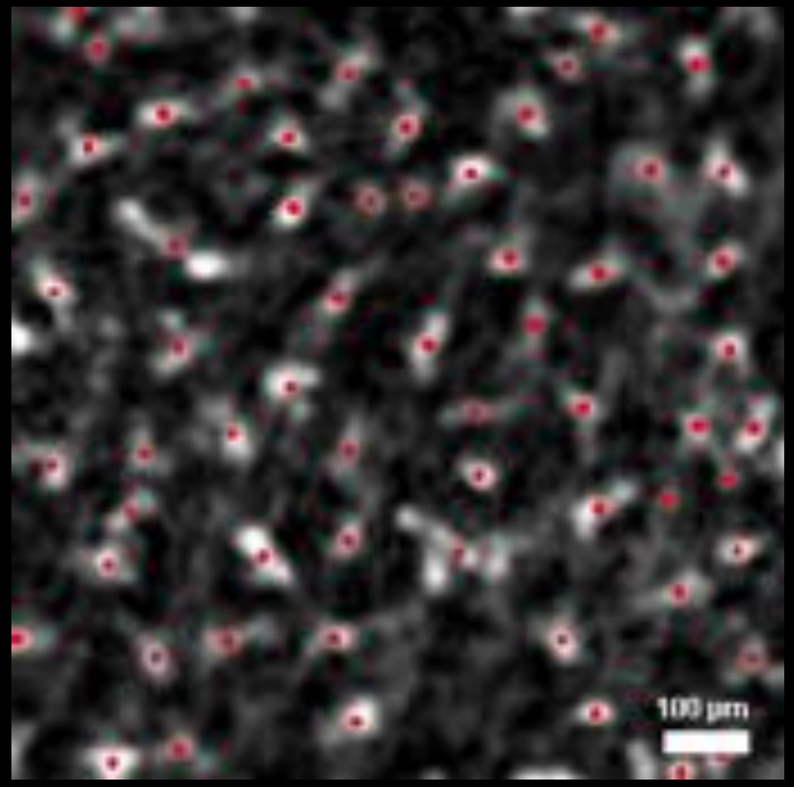
Tweening between visits



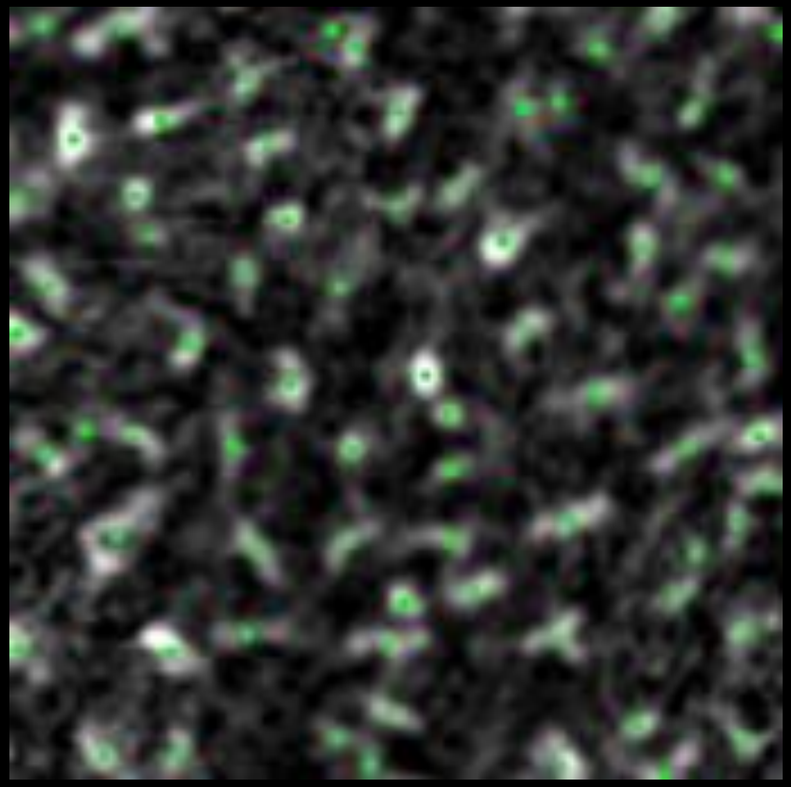
* Simulated cell movement

Changes in Cell Centroids over 6 Hours

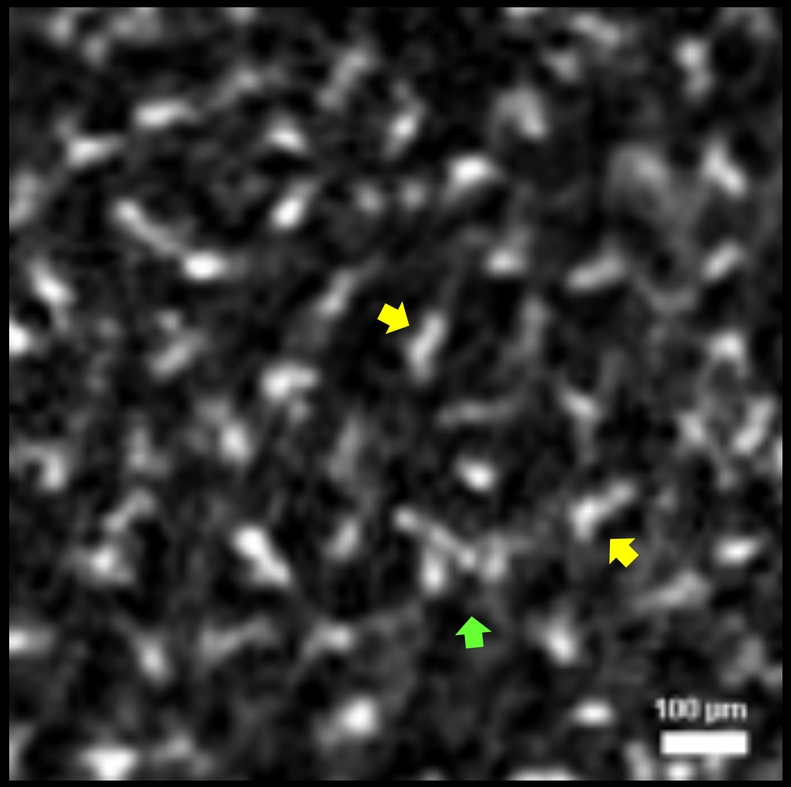
Baseline



Follow up – 6 hours



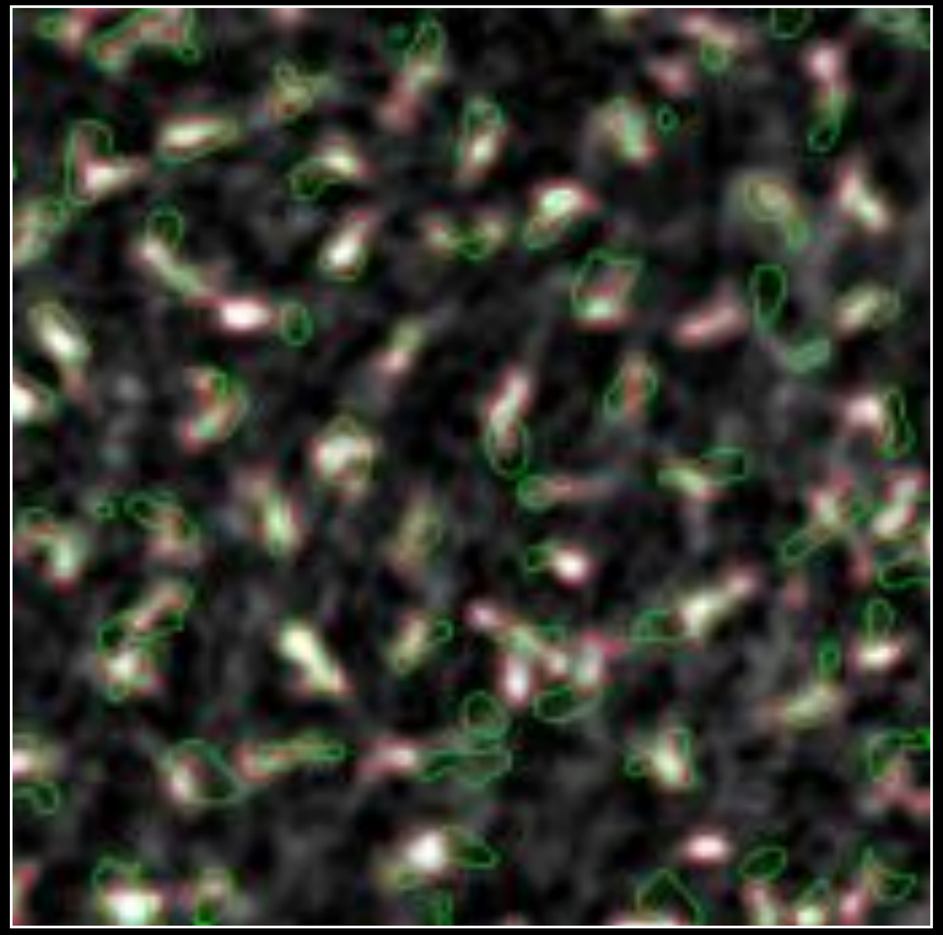
Tweening between visits



* Simulated cell movement

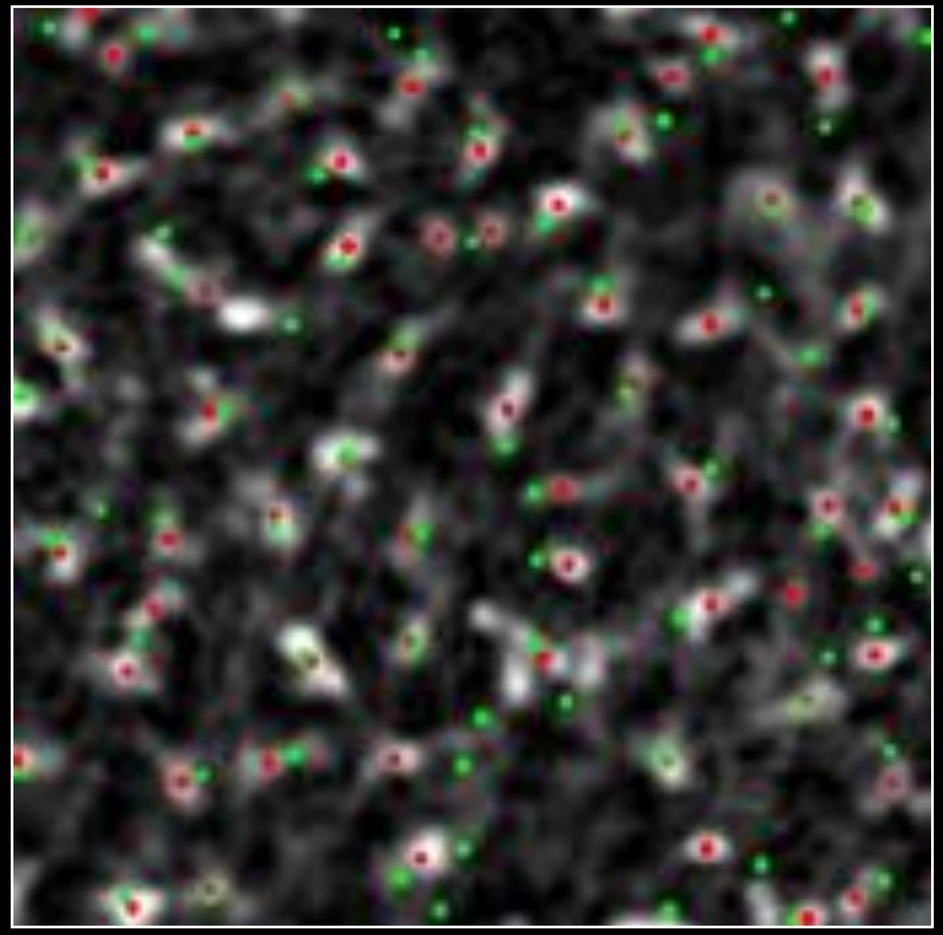
Changes in Cell Boundaries and Centroids over 6 Hours

Baseline vs Follow up – 6 hours



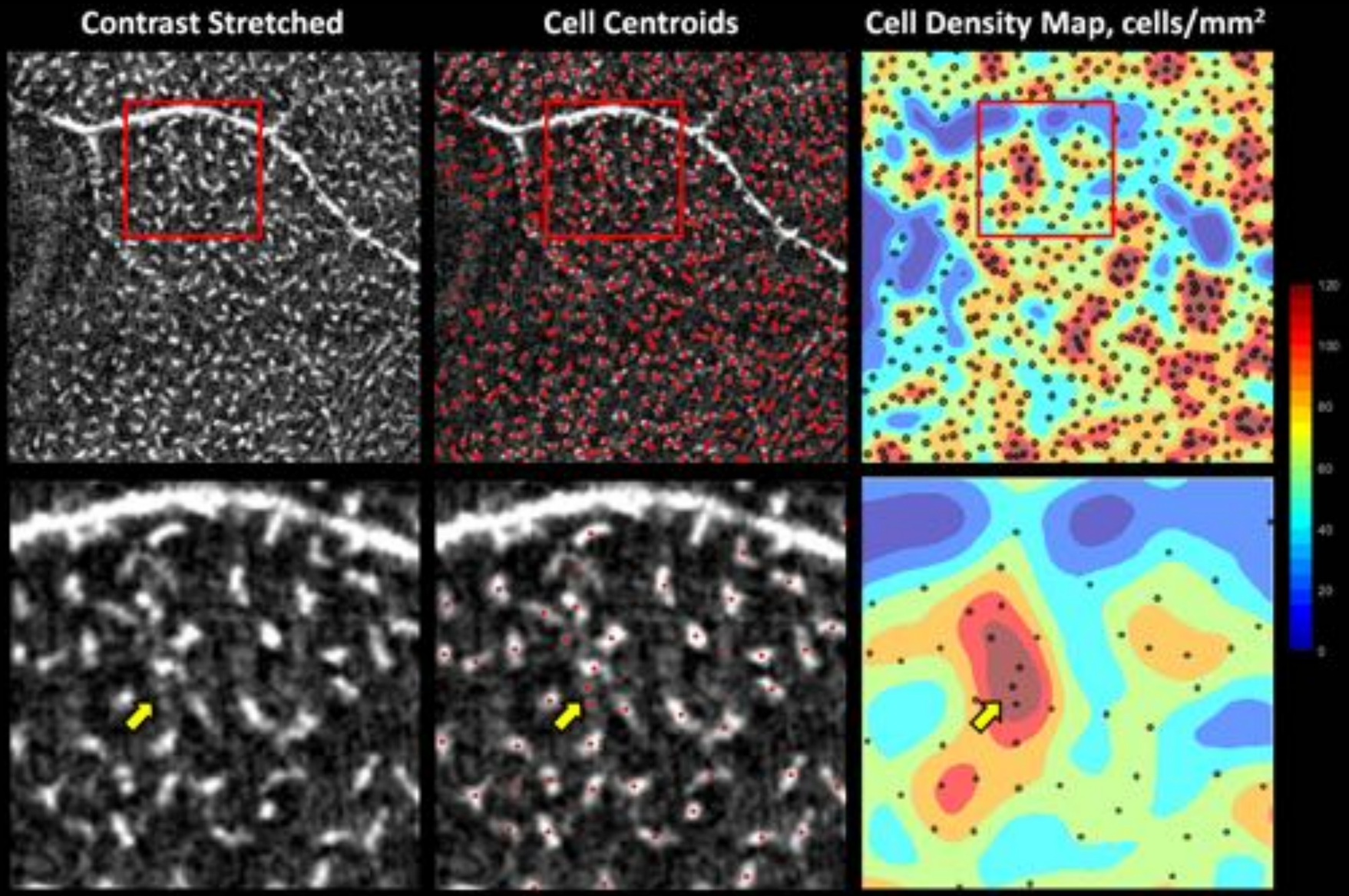
Cell Boundaries

100 µm



Cell Centroids

Automated Cell Identification and Density Mapping

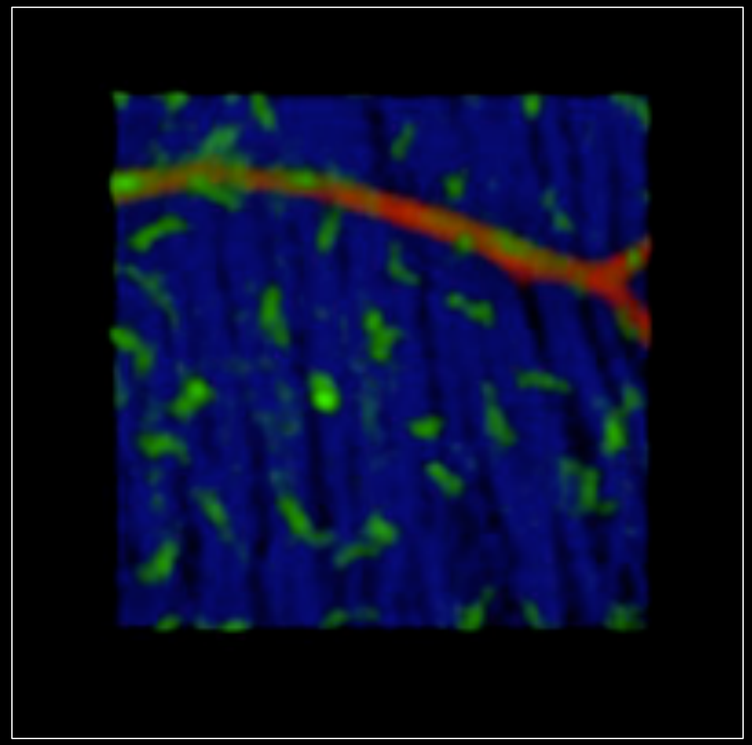


Limitations

- Identity of these cells is still in question, no imaging markers
- Limited sample size and age range of population studied.
- 10 scan acquisition is clinically challenging for some patients.
- Current manual cell identification and counting is labor intensive.

Summary

- Clinical OCT *is* capable of imaging single macrophage-like cells, probably hyalocytes, in eyes of patients with diabetes.
- Changes cell morphology and distribution mirror areas of retinal damage and diabetic progression.
- Clinical utility of this potential biomarker will depend on automation of software and imaging protocols



Thank You for Your Attention