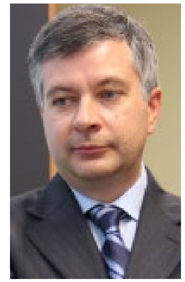
Executive Committee

OSA Therapeutic Laser Applications Technical Group

Chair: Elina A. Vitol

Advisor: David R. Busch, UT Southwestern Medical Center at Dallas

Committee Member: Yannis M. Paulus, Assistant Professor, University of Michigan

Committee Member: Guido Perrone, Associate Professor, Politecnico di Torino

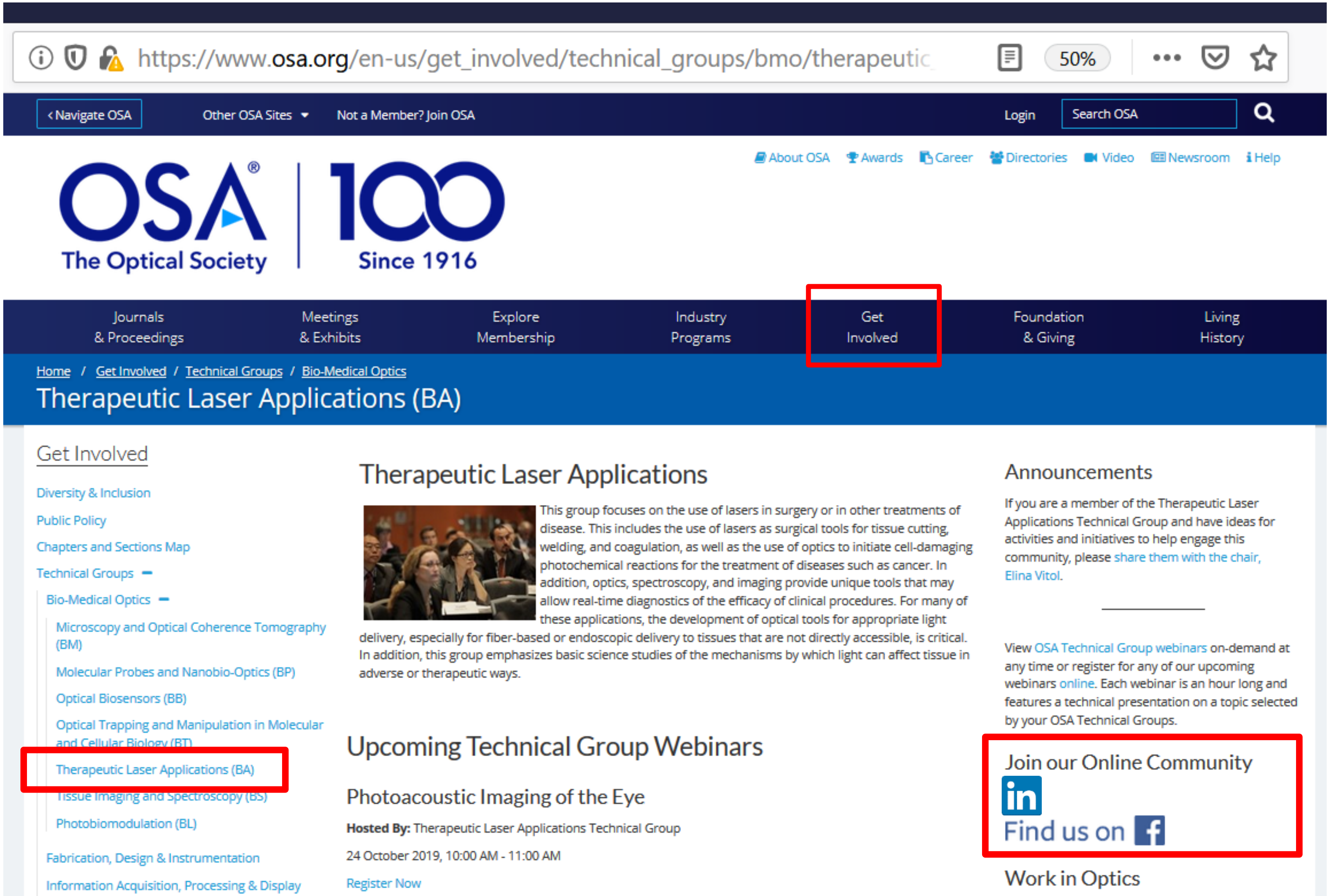
Committee Member: Felix Fanjul-Velez, Associate Professor, University of Cantabria

Industry Liaison: Robbie Thomas, Photon Force, Ltd.

Social Media Liaison: Mariia Shutova, Texas A&M University

Where to find information about the group

Therapeutic Laser Applications Technical Group



We want you to join us!

Therapeutic Laser Applications Technical Group

- Select **Therapeutic Laser Applications** as one of 5 technical groups of interest at your OSA membership account page
- Attend our networking events, webinars and poster sessions
- Join us on LinkedIn and Facebook to keep in touch
- Look out for emails from the committee about group activities
- Interested in presenting your research? Have ideas for technical group events? Want to reach out to your fellow group members?
  - Contact us at elina.vitol@gmail.com or TGactivities@osa.org

Upcoming webinars

Therapeutic Laser Applications Technical Group

1 November 2019, 12 pm EST

Recent advances in tissue biomechanics using Dynamic Optical Coherence Elastography

Kirill Larin, PhD, University of Houston

Registration is now open!

21 January 2020, 11am EST*

Thermomechanical effect of infrared laser for cartilage regeneration

Yulia M. Alexandrovskaya, PhD

Institute of Photon Technologies, Federal Scientific Research Centre “Crystallography and Photonics” of the Russian Academy of Sciences (RAS)

Registration will open in December

Welcome to today's webinar!

OSA Therapeutic Laser Applications Technical Group

PHOTOACOUSTIC IMAGING OF THE EYE

Yannis M. Paulus, M.D., F.A.C.S.

Assistant Professor, University of Michigan
Department of Ophthalmology and Visual Sciences, and
Department of Biomedical Engineering

OCTOBER 24, 2019

Photoacoustic Imaging of the Eye

Yannis M. Paulus, M.D., F.A.C.S.

Assistant Professor

Department of Ophthalmology & Visual Sciences

Department of Biomedical Engineering

University of Michigan Kellogg Eye Center

OSA Therapeutic Laser Applications Technical Group Webinar

October 24, 2019

Disclosures

- Inventor University of Michigan patents
  - Method and Apparatus for Removing Microvessels
  - RetinaScope Apparatus
  - Photomediated Ultrasound Therapy Method and Apparatus
  - Purely Organic Phosphorescent Nanoparticles for In Vivo Oxygen Sensing
  - Laser Ultrasound Body Sculpting
  - Multi-modal imaging for cell tracking
- Co-Founder companies PhotoSonoX LLC, OcuBell.
- CEO of PhotoSonoX LLC
- Consultant for Oraya Therapeutics, Quattro Consulting, Sonify Biosciences, Allergan Regional Advisory Board, Putnam Associated Consulting, Roda Consulting, ENDRA Life Sciences, MediBeacon Inc
- Will discuss several preclinical systems not approved by the FDA

Imaging is critical

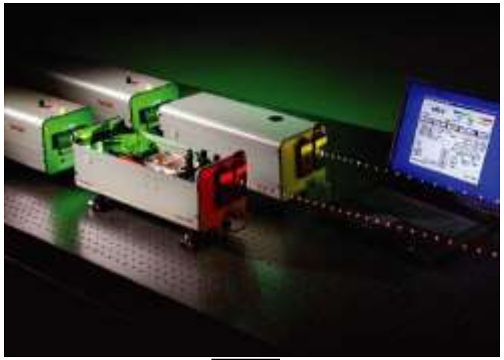
- We can understand and diagnose what we can see
- Early disease detection
- Improved diagnosis
- Improved disease monitoring
- Better patient outcomes
- Precision medicine tailored to each patient's molecular profile
- Improved understanding of pathophysiology
  - Change name: Central Serous Retinopathy to Central Serous Chorioretinopathy
  - Acute Posterior Multifocal Placoid Pigment Epitheliopathy (APMPPE) to Acute Multifocal Placoid Choroidopathy (AMP-C)[1,2]
- **Retina is very unique**. The eye is optically transparent, so we can directly visualize neurons and microvasculature with high resolution optical imaging.

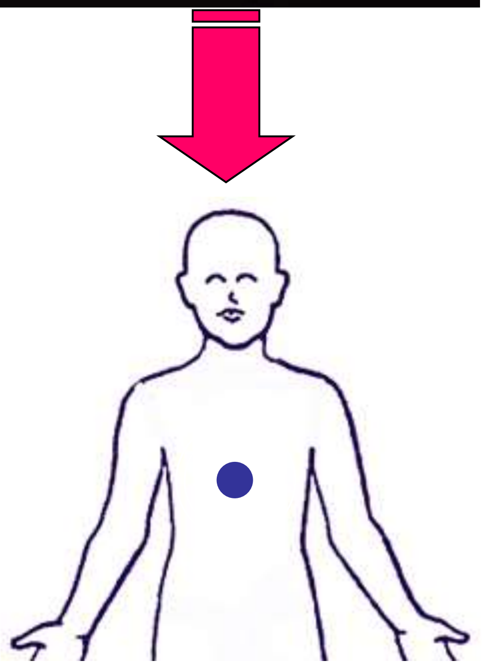
[1]Zhang AY, Han IC, Goldberg MF. Renaming of Acute Posterior Multifocal Placoid Pigment Epitheliopathy (APMPPE) to Acute Multifocal Placoid Choroidopathy (AMP-C). JAMA Ophthalmol. 2017 Mar 1;135(3):185
[2]Jampol LM, Goldstein DA, Fawzi AA. Keeping the Name of Acute Posterior Multifocal Placoid Pigment Epitheliopathy. JAMA Ophthalmol. 2017 Mar 1;135(3):186.

Retinal Imaging today

- Fundus photography 1853
- Ultrasonography (A & B) 1956
- Fluorescein angiography 1961
- Indocyanine green angiography 1972
- Scanning laser ophthalmoscopy 1981
- Optical Coherence Tomography 1991
  - Time domain
  - Spectral domain
  - OCTA
  - Swept Source
  - Intra-operative
  - Hand-held/peds
  - Different wavelength
  - Doppler OCT
- Ultra-wide field imaging
- Fundus autofluorescence (FAF)
- Retinal oximetry
- Fluorescent lifetime imaging ophthalmoscopy (FLIO)
- Real-time image-guidance of laser photocoagulation
- Adaptive Optics
- Handheld/Smartphone-based fundus imaging
- Automated interpretation/ deep learning
- **Photoacoustic Imaging**
- **Multimodal Imaging**
- **Molecular Imaging**

Photoacoustic Imaging





Photoacoustic effect: conversion light to sound.
Optoacoustic = thermoacoustic

Pulsed Laser
▽
Optical Absorption
▽
Heating
▽
Thermal Expansion
▽
Pressure Waves
▽
Acoustic Detection

Optical Path

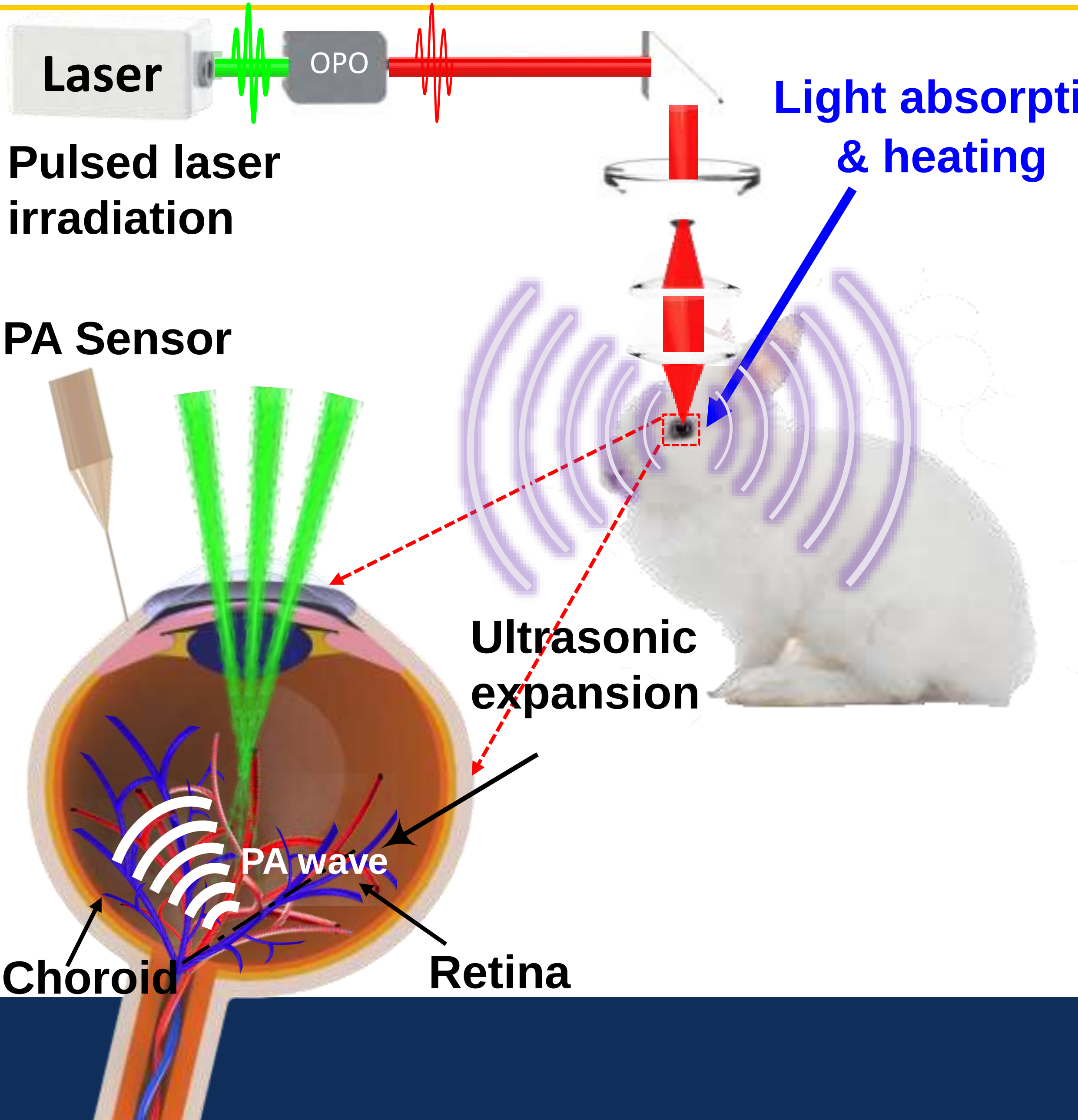
Sound Path

Advantages

1. Deep Penetration
2. High Resolution
3. Speckle-Free

1880: Alexander G. Bell describes photoacoustic princ.
Need t < 20 nsec. Most ~ 5 nsec

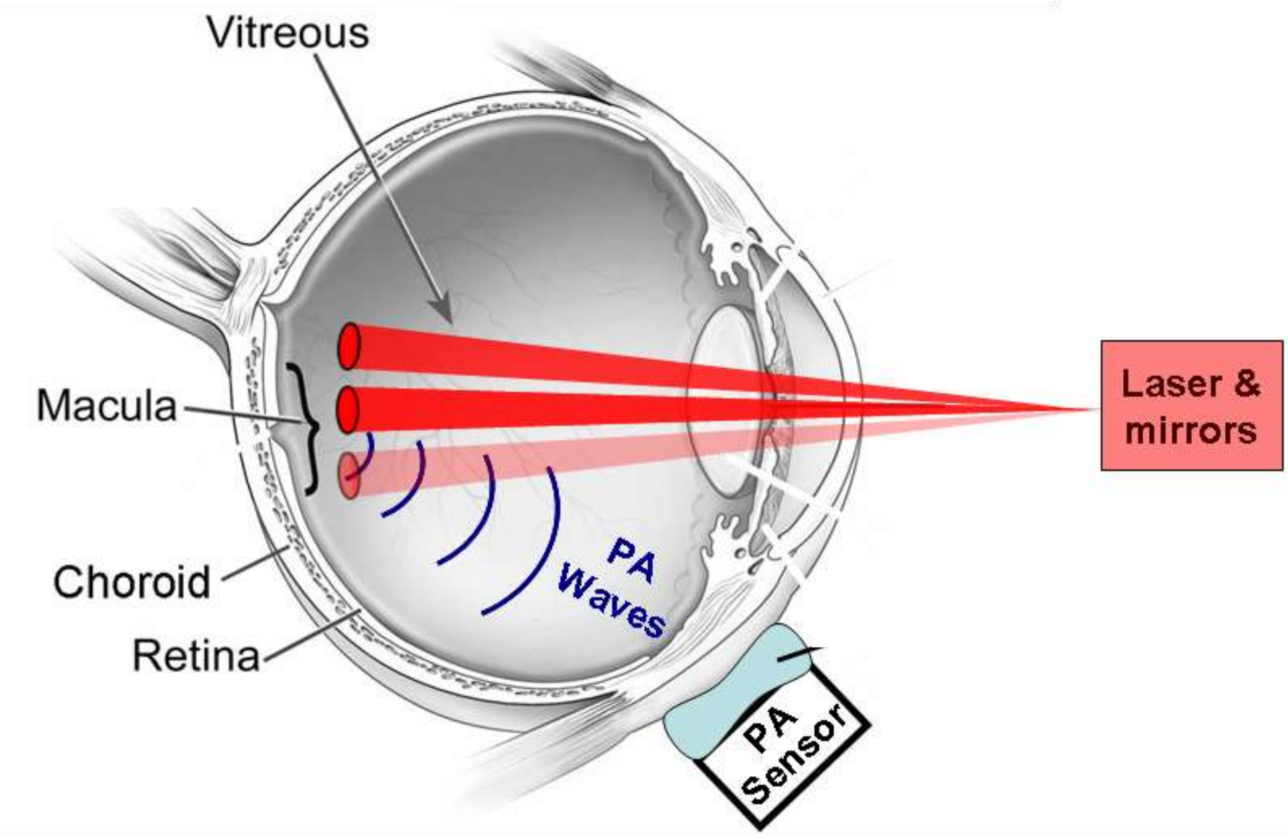
Photoacoustic Ocular Imaging



Advantages:

- Non-invasive, and high contrast
- Structural: 3D vessel structure
- Functional: hemoglobin concentration, oxygen saturation, blood flow
- Molecular information with contrast agents: integrin, growth factor

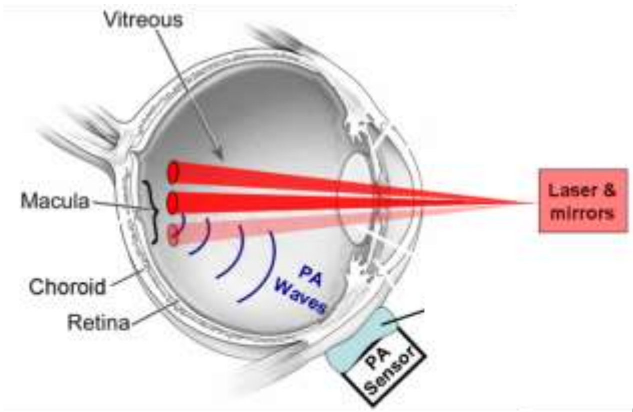
Photoacoustic Ocular Imaging

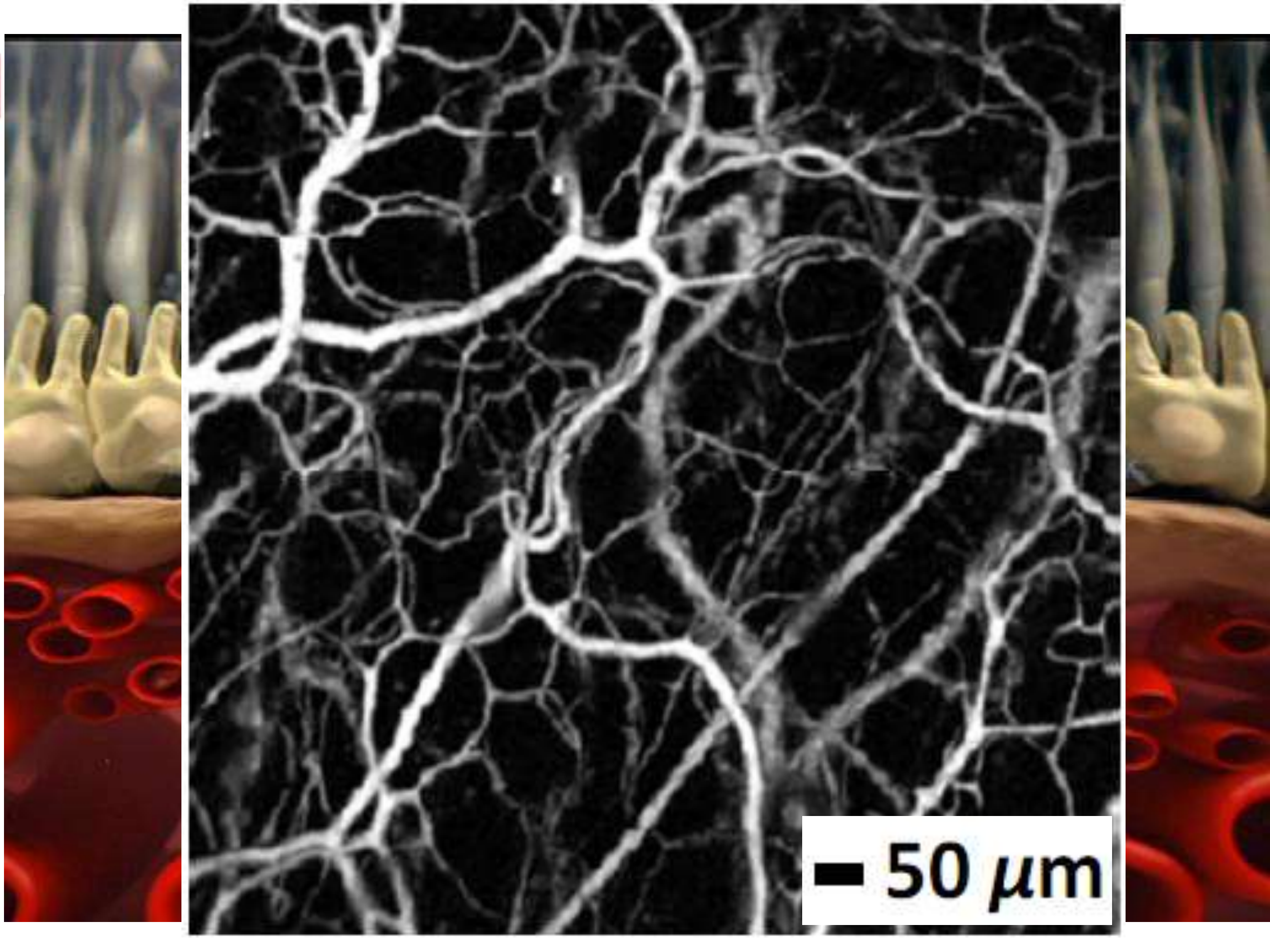


Endogenous absorbers:
Hemoglobin
    Oxy and deoxy
Melanin
DNA/RNA
Lipid
H2O

Exogenous contrast:
Methylene blue
Indocyanine green
Organic nanoparticles
Gold nanorods
Microbubbles

Photoacoustic Ocular Imaging



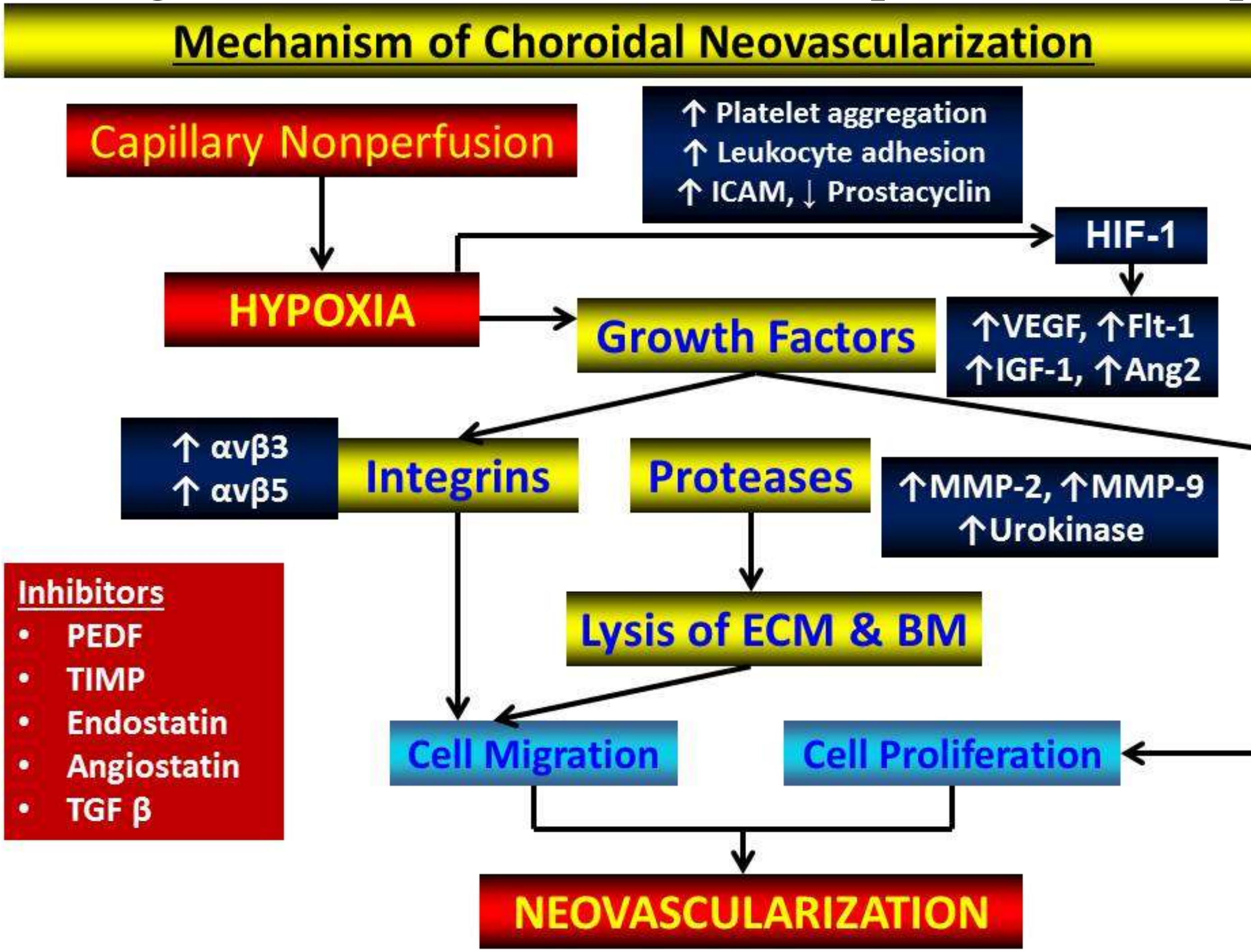


<u>3 μm resolution</u>:

- ✓ OCT image
- ✓ Blood distribution (angiogenesis)
- ✓ Oxygen saturation levels (ischemia)
- ✓ Tissue blood content
- ✓ ICG Photoacoustic Angiography

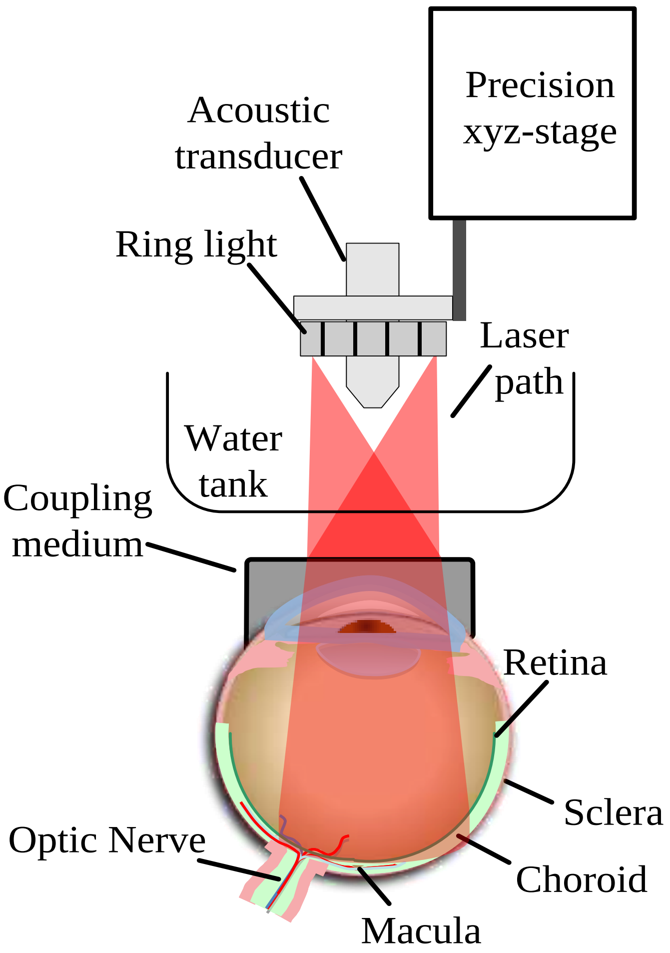
Molecular Imaging

Anatomic changes are the end result from complex molecular pathways

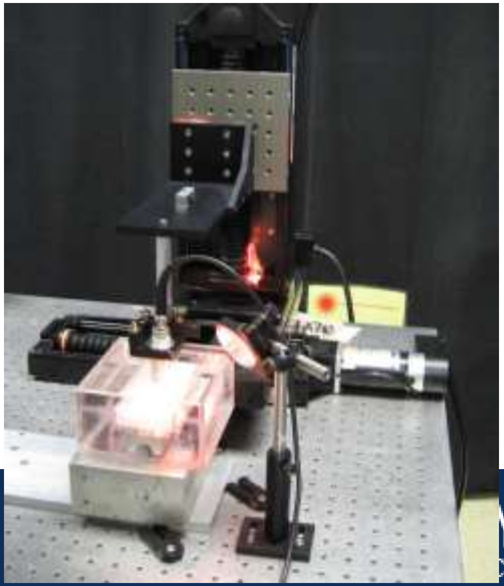


Das A, McGuire PG. Retinal and choroidal angiogenesis: pathophysiology and strategies for inhibition. *Prog Retin Eye Res* 2003;22(6):721-48

Photoacoustic Imaging System

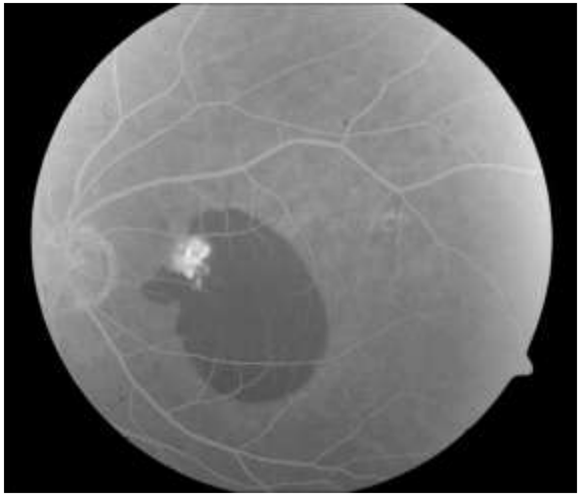


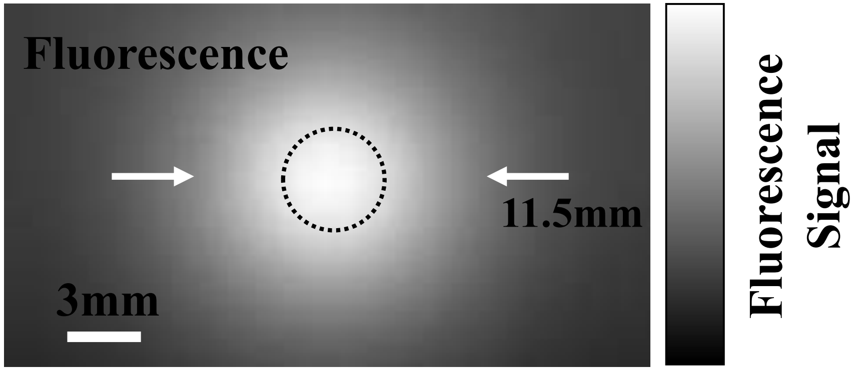
- Tunable, pulsed Nd:YAG laser
- Rep rate 10 Hz, pulse width 5 ns
- Fiber optic ring light
- Retinal laser density 0.5 mJ/cm^2, below ANSI limit
- US transducers 15 & 25 MHz acquire pulse-echo + PA signals
- Axial resolution: 83 & 50 μm
- Lateral resolution: 200 & 240 μm

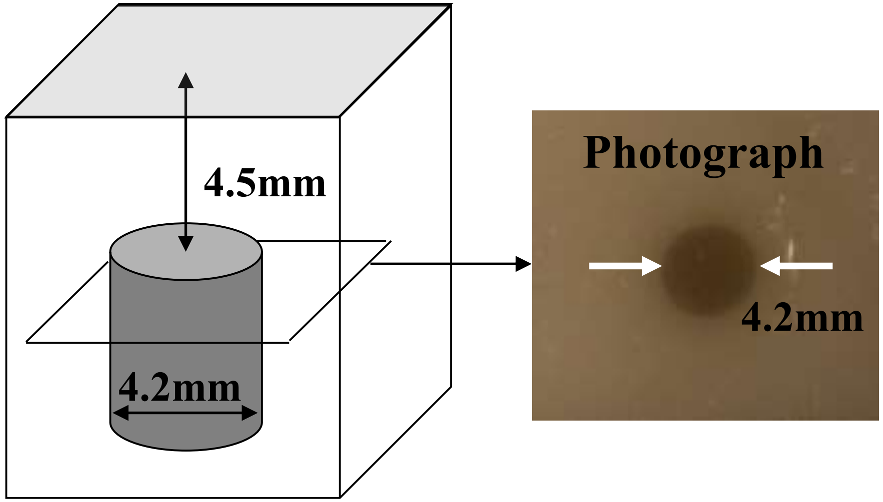


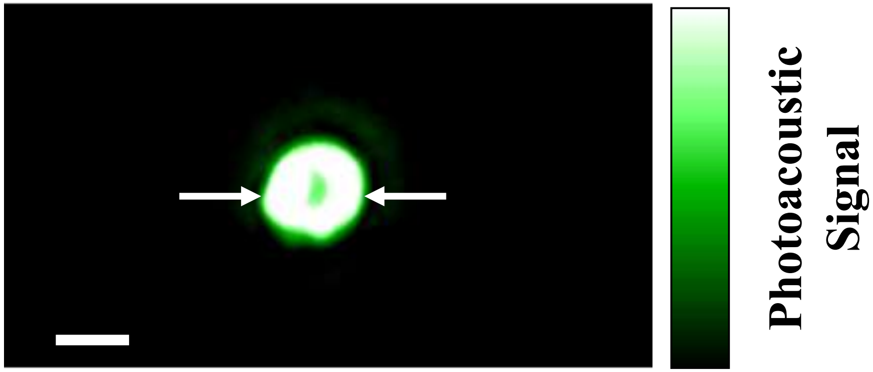
de la Zerda A, Paulus YM, Teed R, Bodapati S, Dollberg Y, Khuri-Yakub, BT, Blumenkranz, MS, Moshfeghi DM, Gambhir SS. Photoacoustic Ocular Imaging. *Optics Letters* 2010; 35(3): 270 – 272.

ICG Photoacoustic Imaging







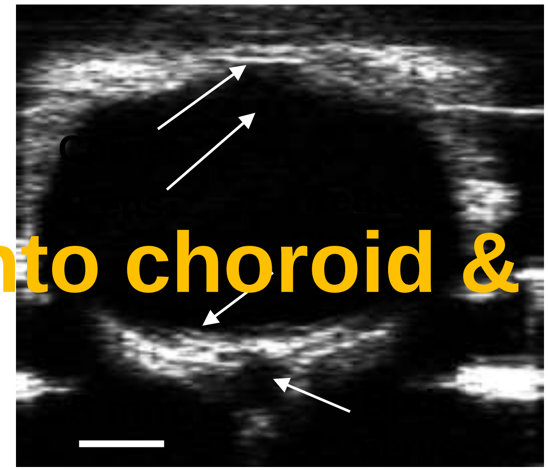


Tissue mimicking phantom filled with ICG

Results: Enucleated Pig Eye

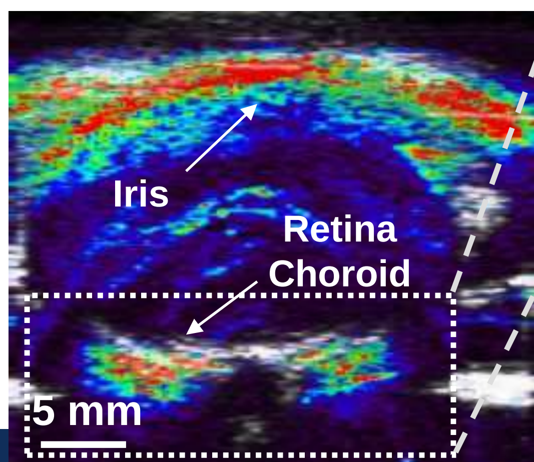
Pig's Eye

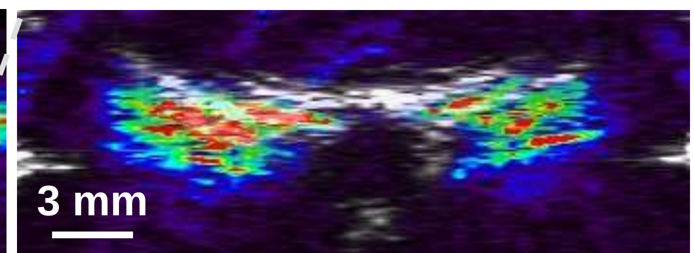
Ultrasound



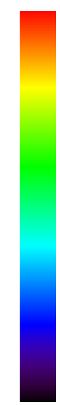
- Deep penetration into choroid & sclera

Photoacoustic





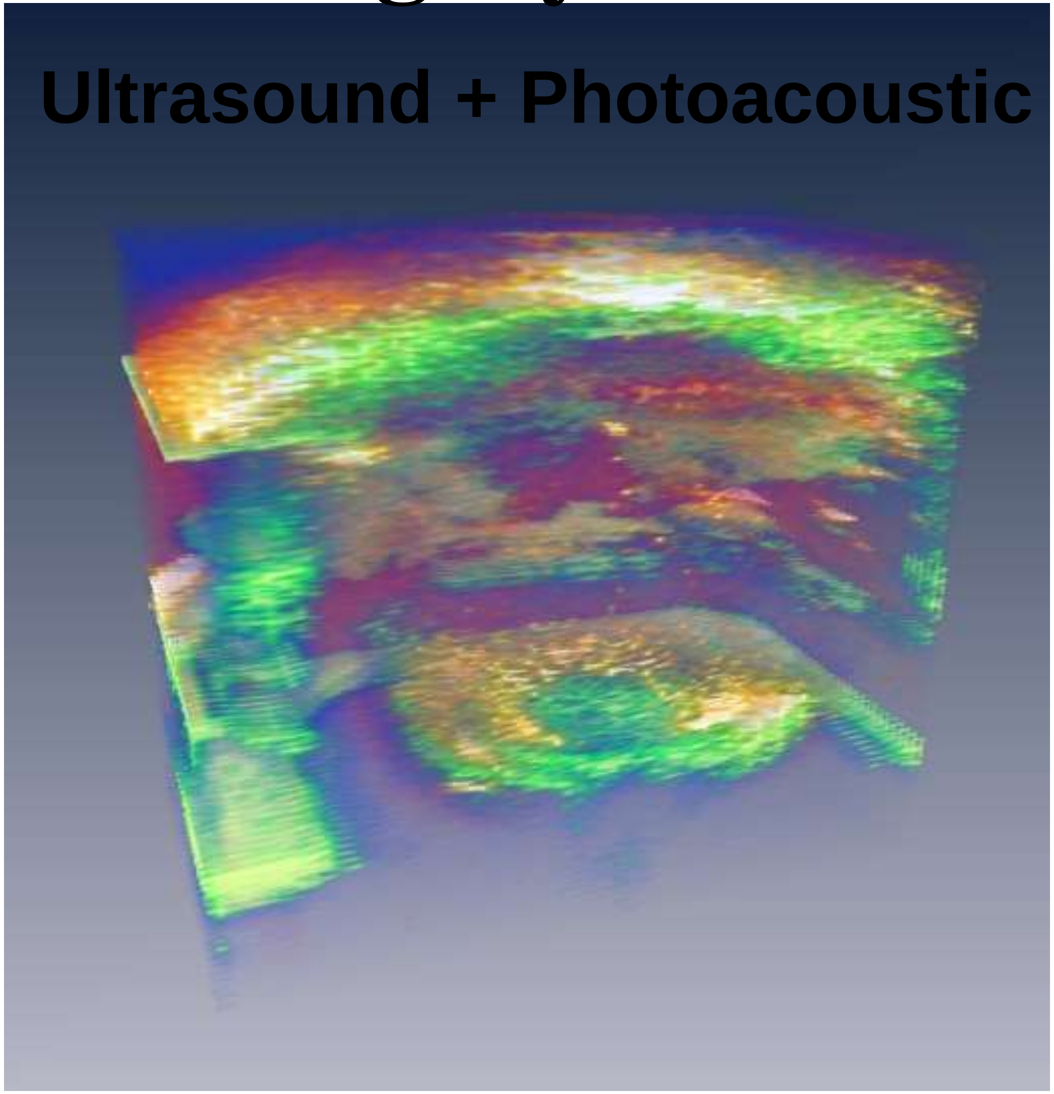
Pig eye: 22 mm
Human eye: 24 mm



700nm(laser depth vs PA (melanin)), 5ns pulses, 0.5mJ/cm^2, 15MHz

KELLOGG EYE CENTER
MICHIGAN MEDICINE

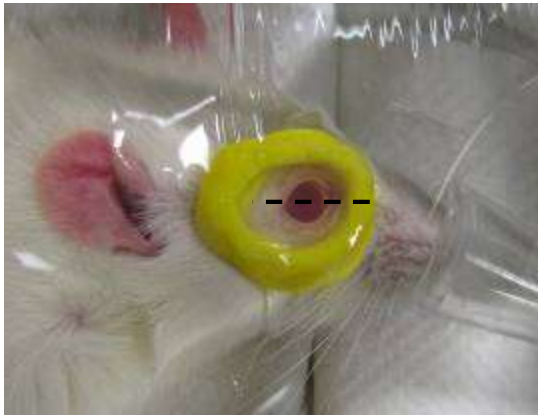
Enucleated Pig Eye



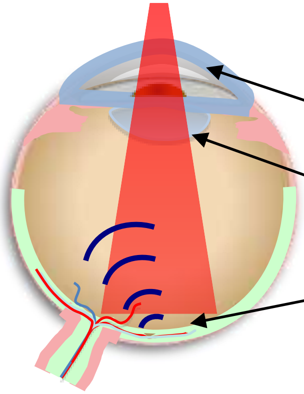
Reconstructed 3D:
Amira, Visage Imaging
63 A-line scans
250 µm apart
8 averages
60 sec acquisition

Live Rat Eye

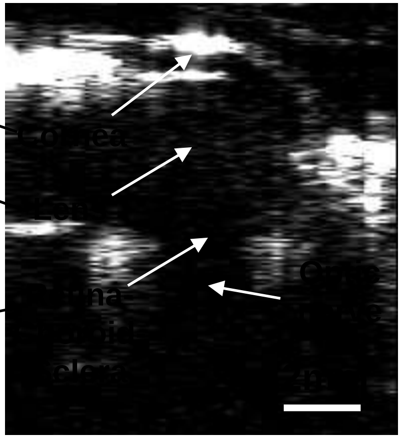
Rat's eye



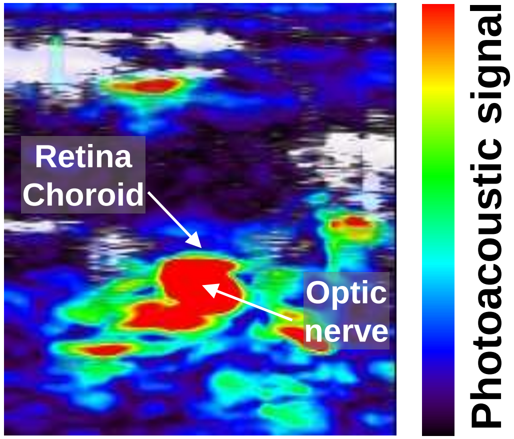
Eye Diagram



Ultrasound



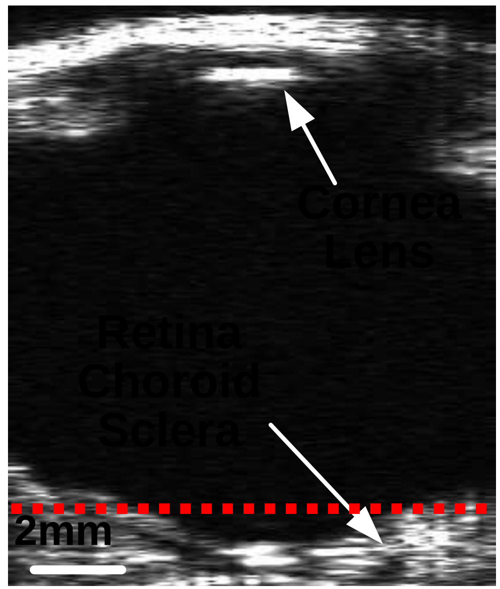
Photoacoustic



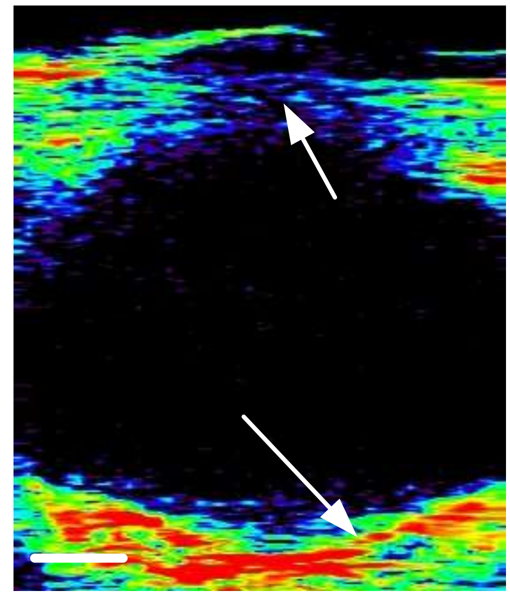
Live Rabbit Eye

Photograph rabbit Vertical Slice

Parasagittal Ultrasound



Parasagittal Photoacoustic



- ➢ **Good signal within safe laser level**

New Zealand rabbits, 6-8 weeks of age

740nm laser, 5ns pulses, 0.5mJ/cm^2, 25MHz focused transducer

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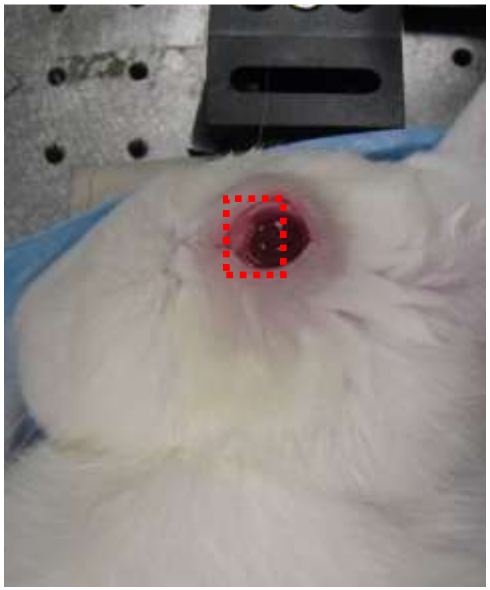
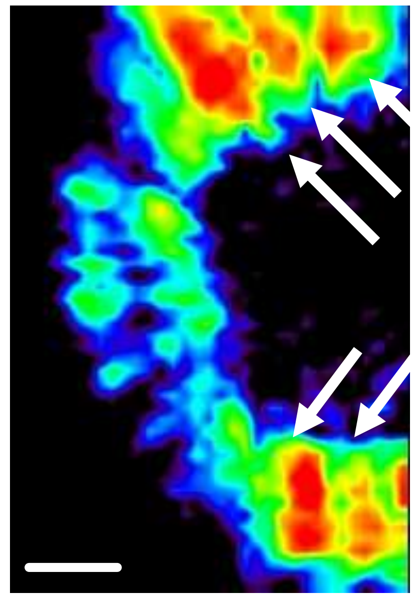
Live Rabbit Eye

Coronal Horizontal Slice



Coronal Photoacoustic



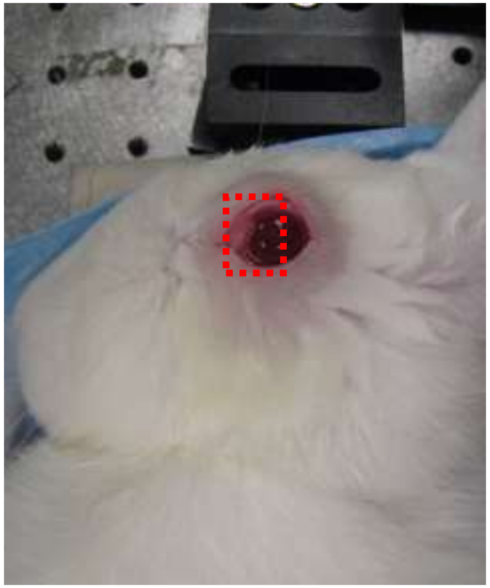
Eye blood vessels

➢ Visualize individual blood vessels

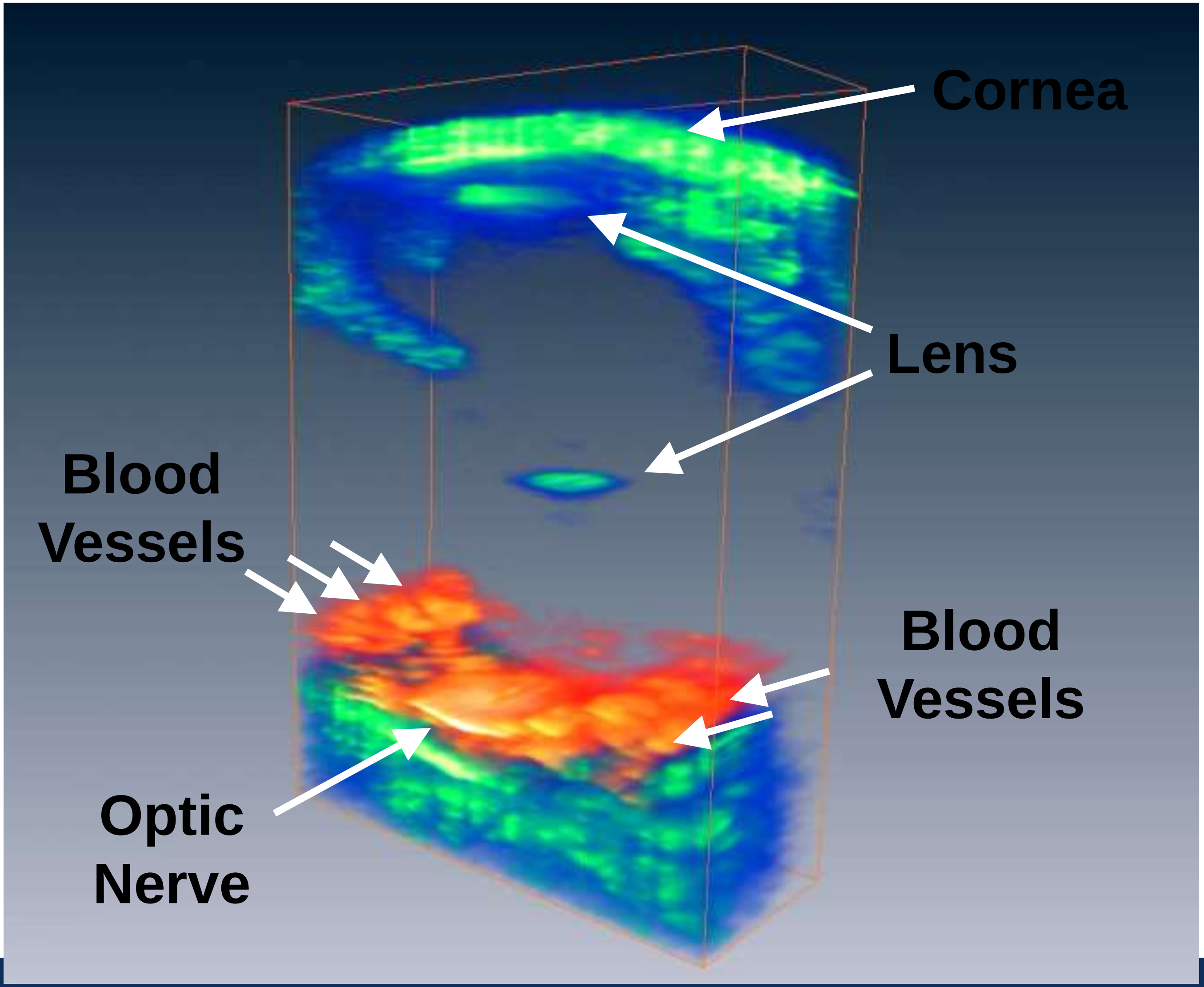
740nm laser, 5ns pulses, 0.5mJ/cm^2, 25MHz focused transducer

Live Rabbit Eye 3D Reconstruction

3D Rendering



32 averages Area: 12 mm x 8 mm 250 µm steps



740nm laser, 5ns pulses, 0.5mJ/cm^2, 25MHz focused transducer

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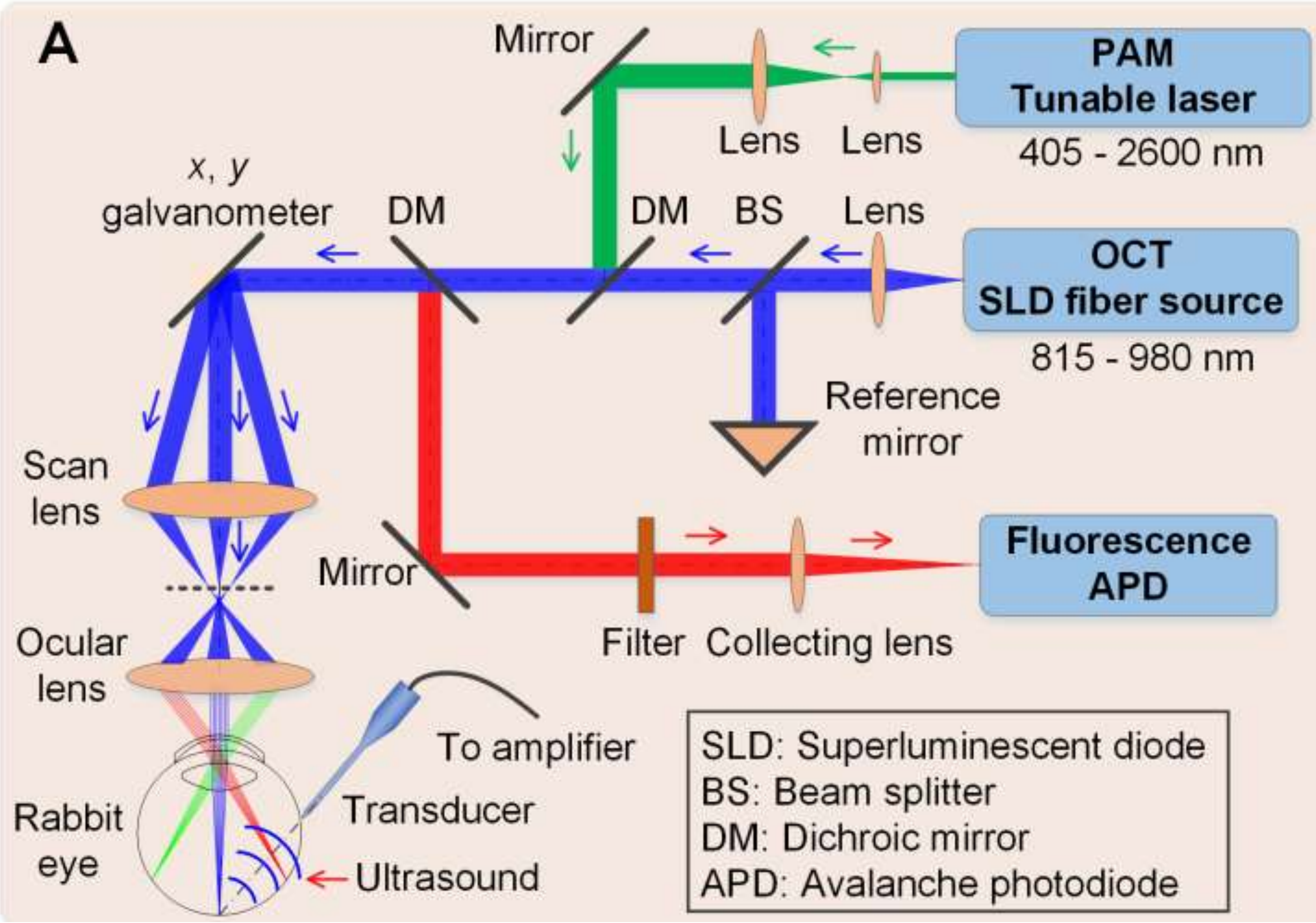
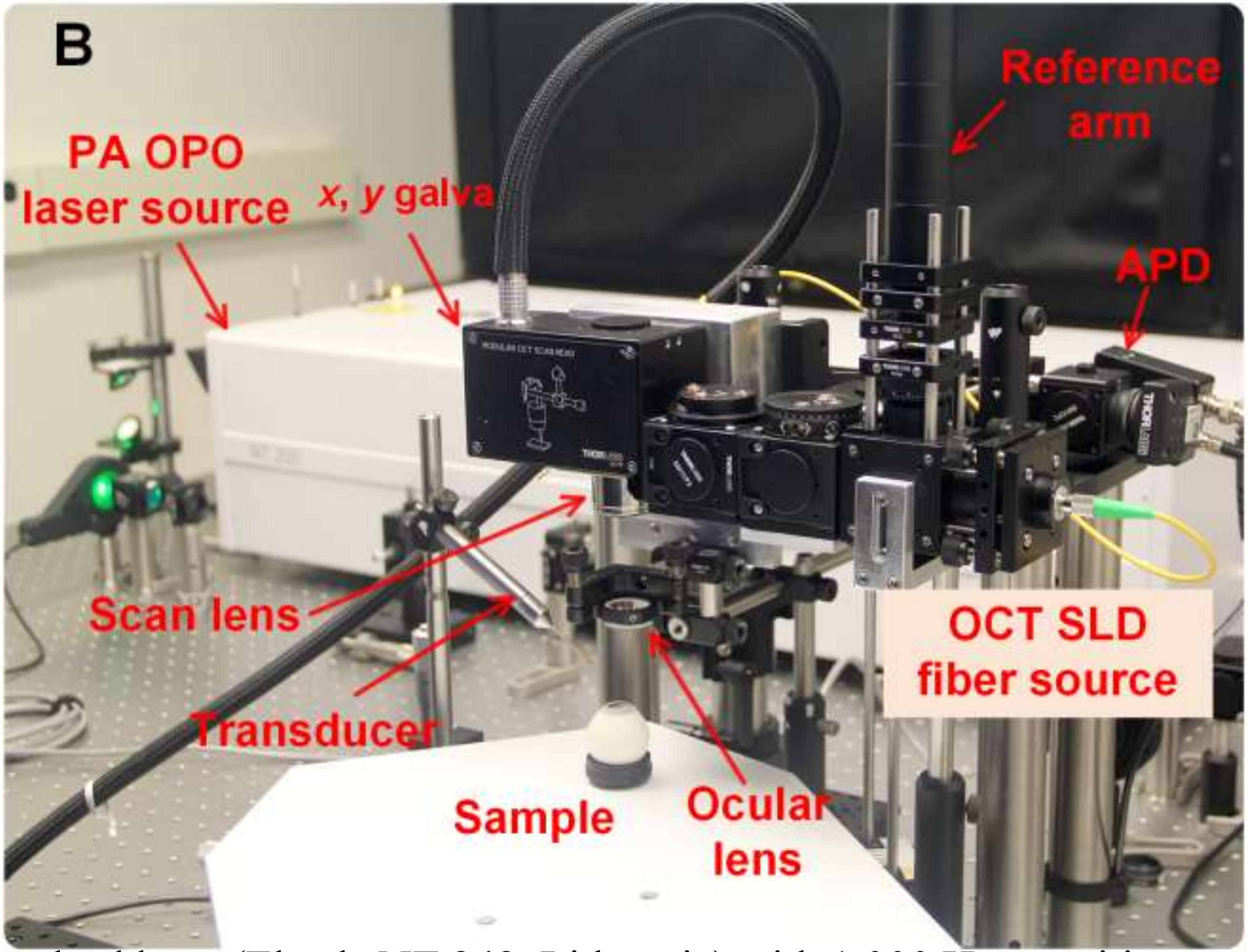
Schematic multimodal photoacoustic microscopy (PAM), OCT, fluorescence microscopy

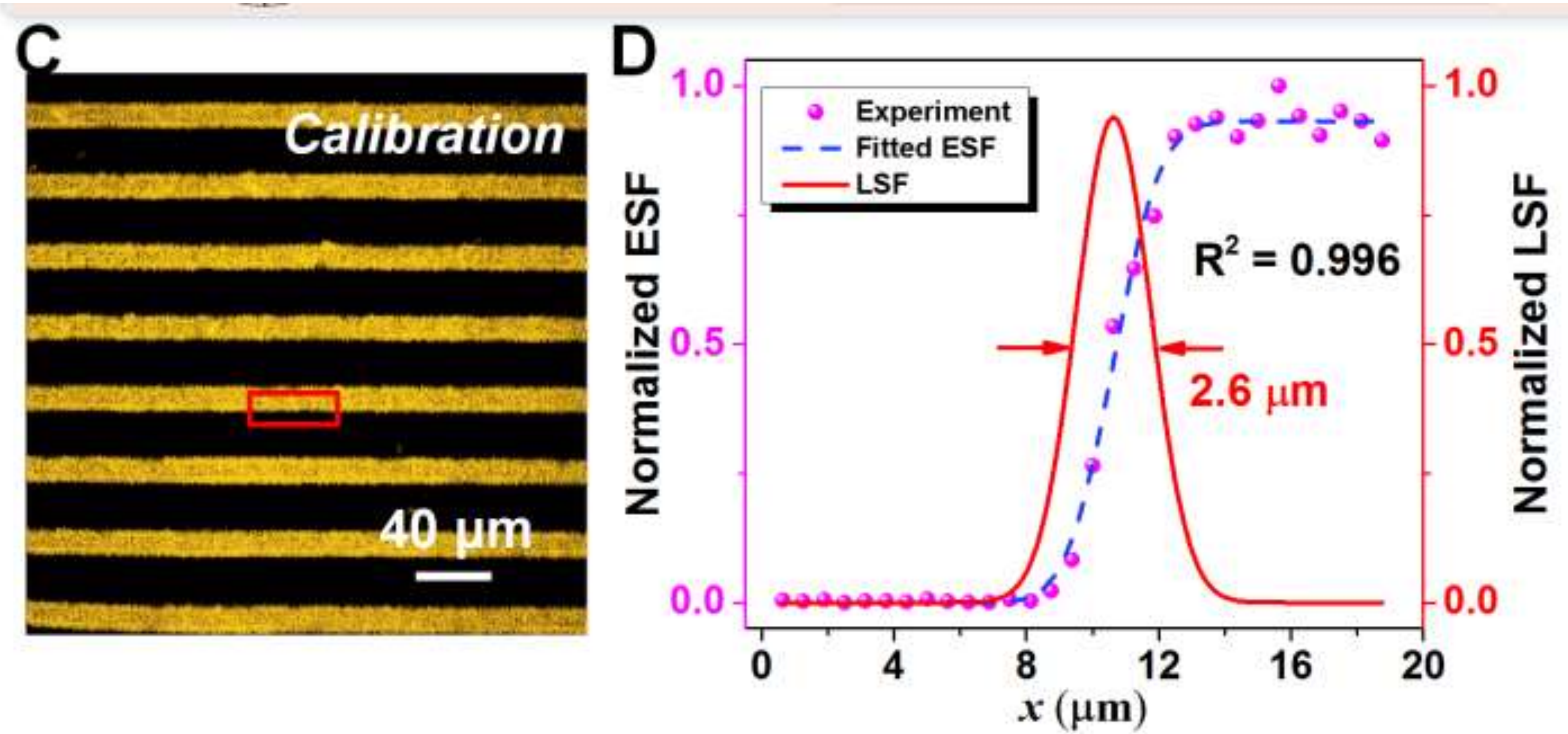




Tunable pulsed laser (Ekspla NT-242, Lithuania) with 1,000 Hz repetition rate
Ultrasound transducer center frequency: 35 MHz
Thorlabs Ganymede-II-High resolution system 36 kHz

Tian C, Zhang W, Phuc VP, Wang X, Paulus YM. Novel Photoacoustic Microscopy and Optical Coherence Tomography Dual-modality Chorioretinal Imaging in Living Rabbit Eyes. *Journal of Visualized Experiments* 2018; 132: e57135. doi:10.3791/57135

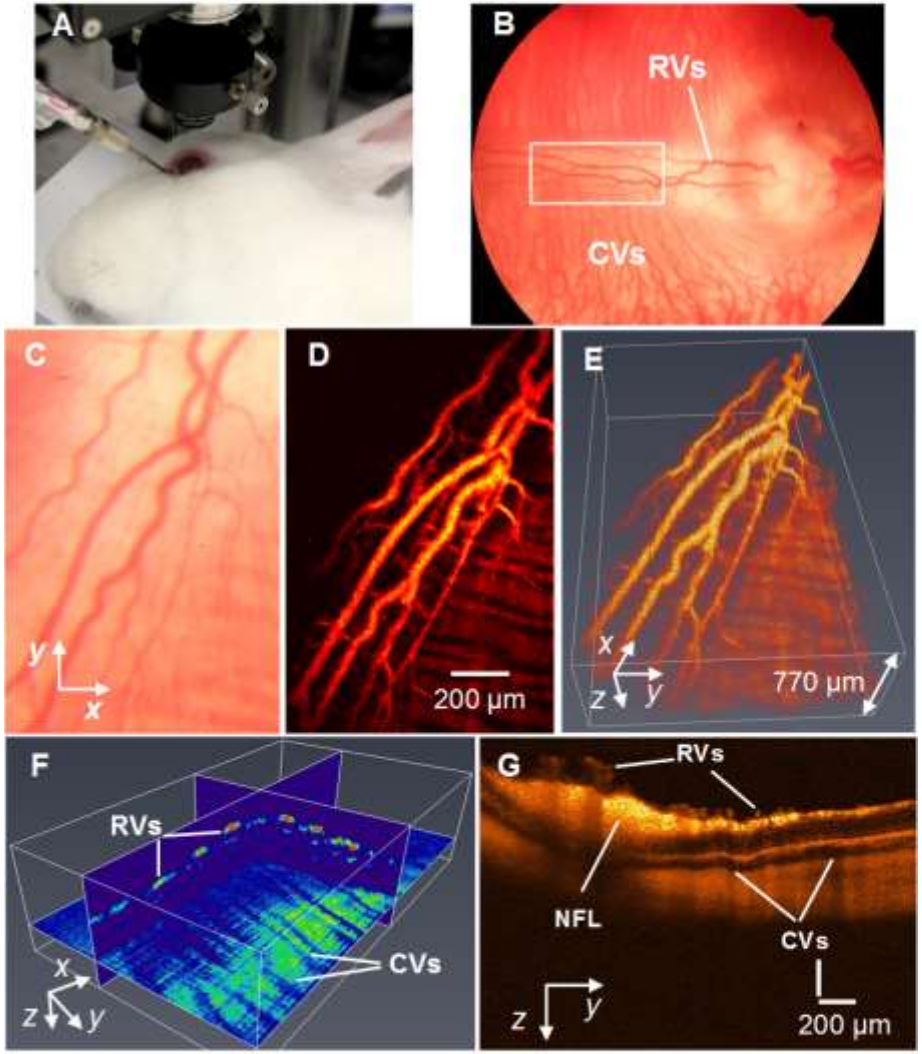
PAM 2.6 µm lateral resolution



A chrome grating was imaged at the focal plane of the scan lens for lateral resolution calibration.

Initial Laser pulse energy: ~30 nJ (half ANSI safety limit)

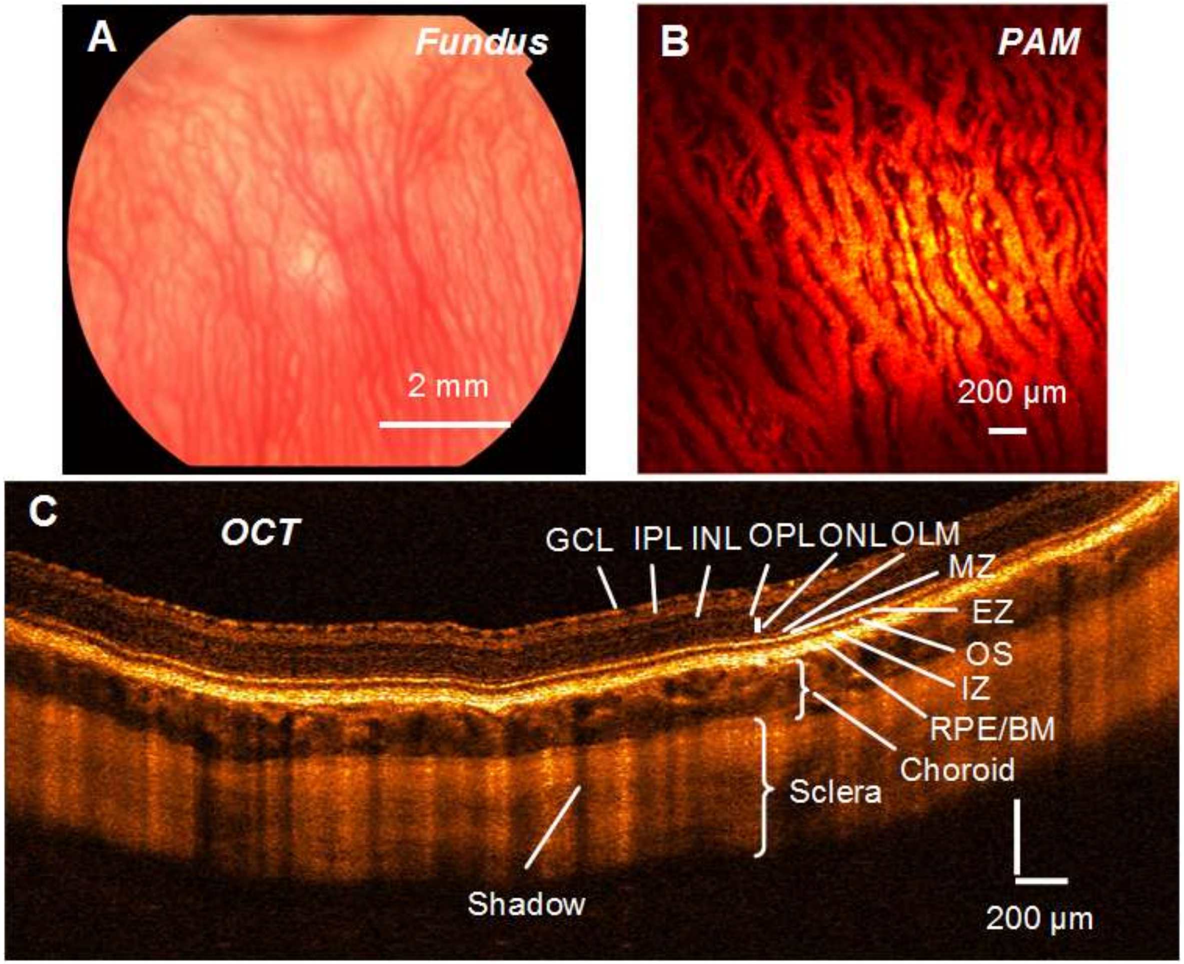
PAM/OCT Retina



A: External photo.
B: Fundus photograph.
C: Photo retinal medullary ray vessels.
D: PAM of retinal (RV) and choroidal vessels (CVs).
E: 3D PAM.
F: 2D orthogonal slices of the PAM image.
G: OCT image showing RVs, CVs, NFL (nerve fiber layer), and retinal layers.

Tian C, Zhang W, Mordovanakis A, Wang X, Paulus YM. Noninvasive chorioretinal imaging in living rabbits using integrated photoacoustic microscopy and optical coherence tomography. *Optics Express* 2017 Jul 10; 25(14): 15947-55.

PAM/OCT Choroid



A: Fundus photograph.
B: PAM signal of the choroid
C: OCT image.

GCL: ganglion cell layer; IPL: inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer; OLM: outer limiting membrane; MZ: myoid zone; EZ: ellipsoid zone; OS: outer segment; IZ: interdigitation zone; BM, Bruch's membrane.

PAM can be performed using a safe laser exposure dose (~80 nJ) below the ANSI safety limit (160 nJ) at 570 nm. Now down to 5% ANSI limit

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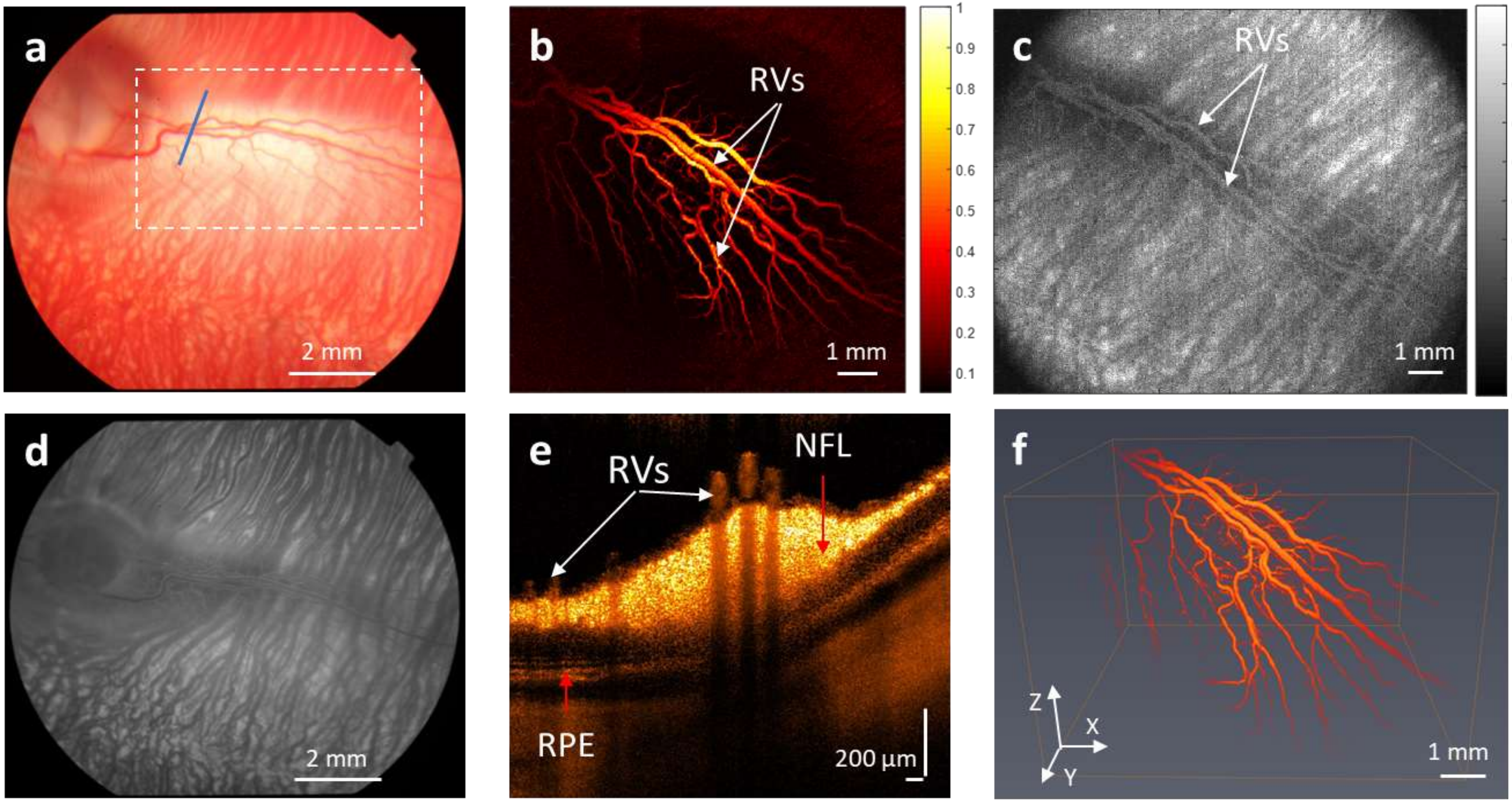
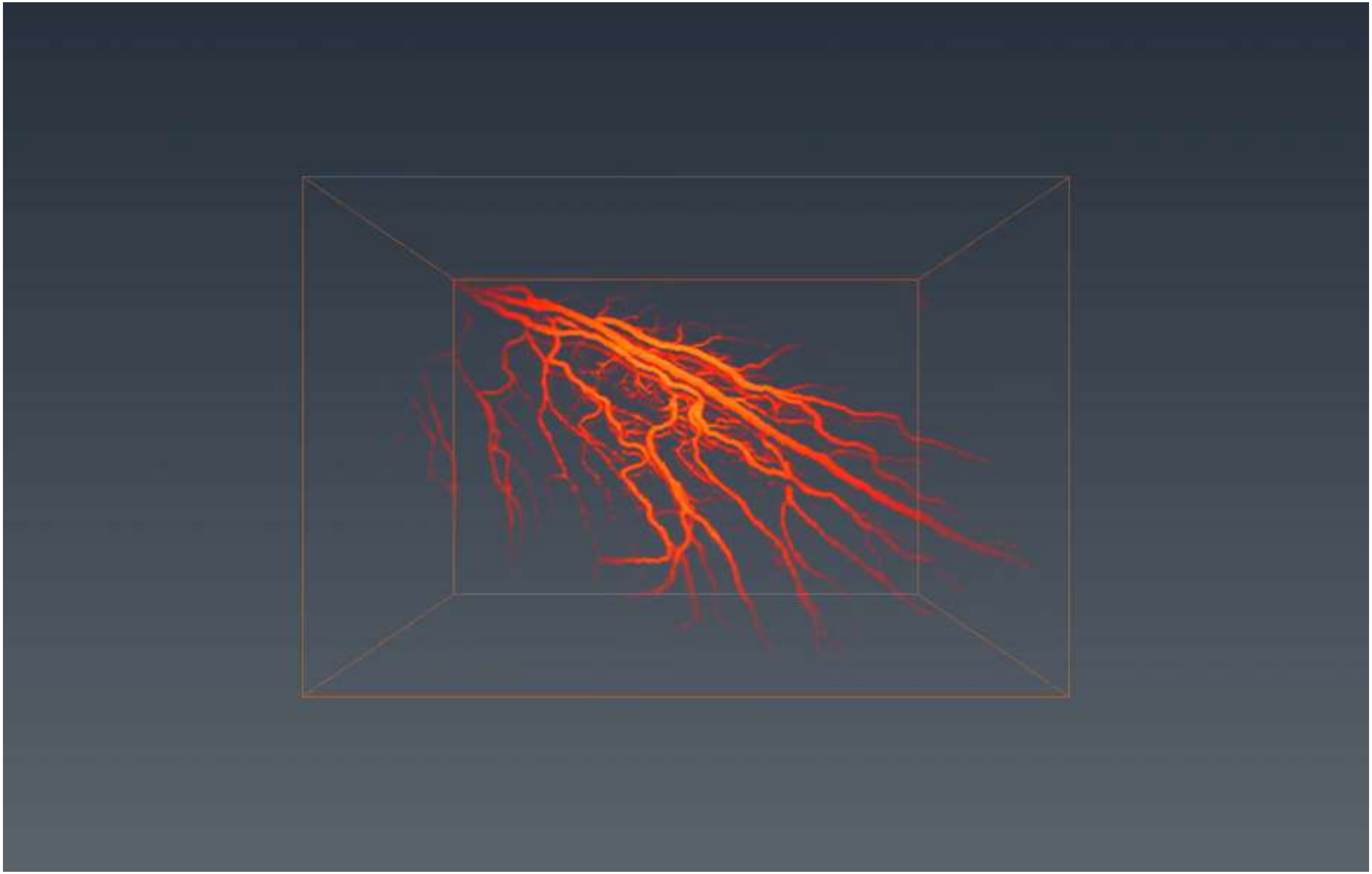
Normal New Zealand white rabbits

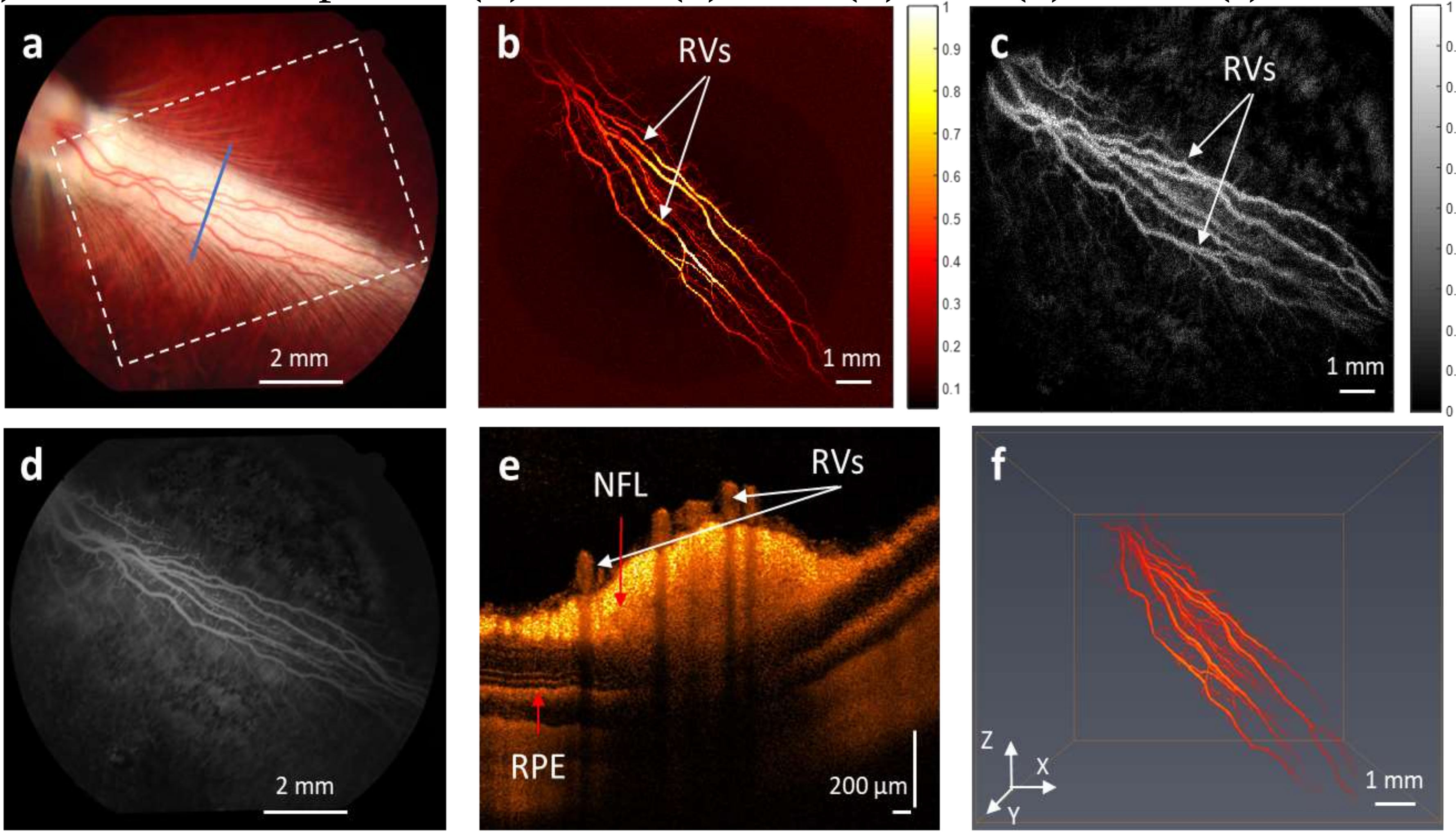
(a) Color fundus photo; (b) PAM (c) FM; (d) FA; (e) OCT; (f) 3D PAM

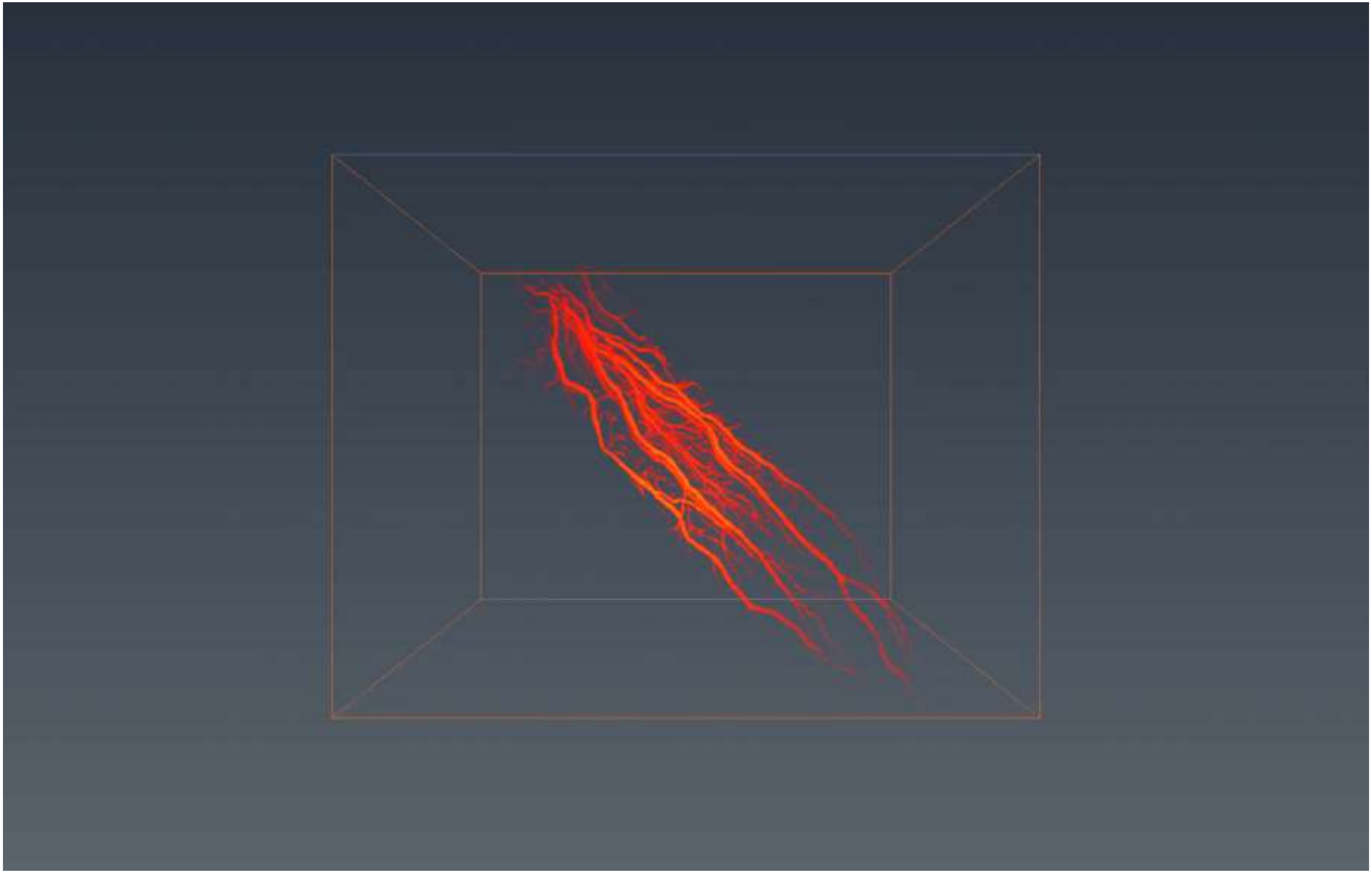




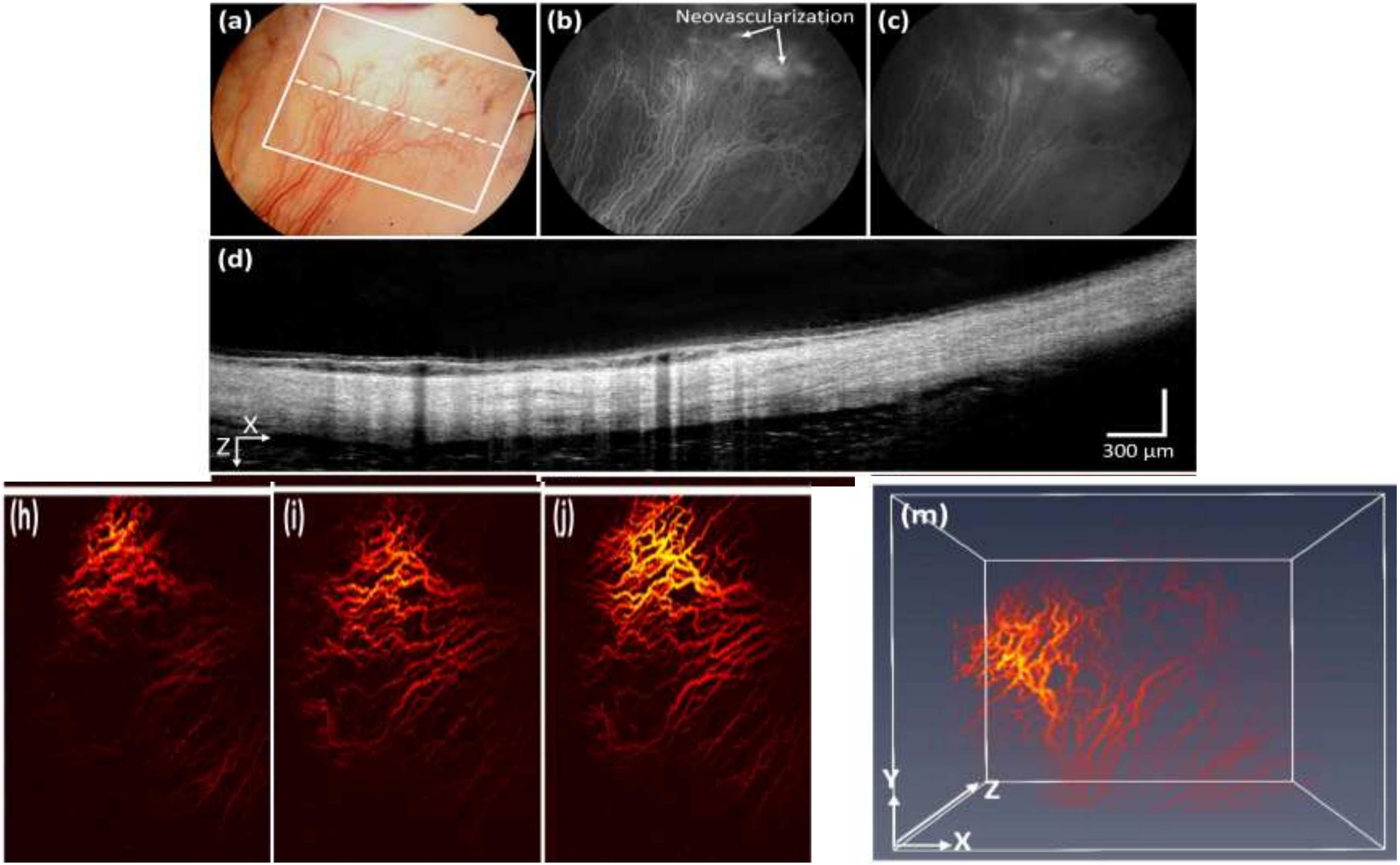
Normal Pigmented rabbits

(a) Color fundus photo; (b) PAM (c) FM; (d) FA; (e) OCT; (f) 3D PAM

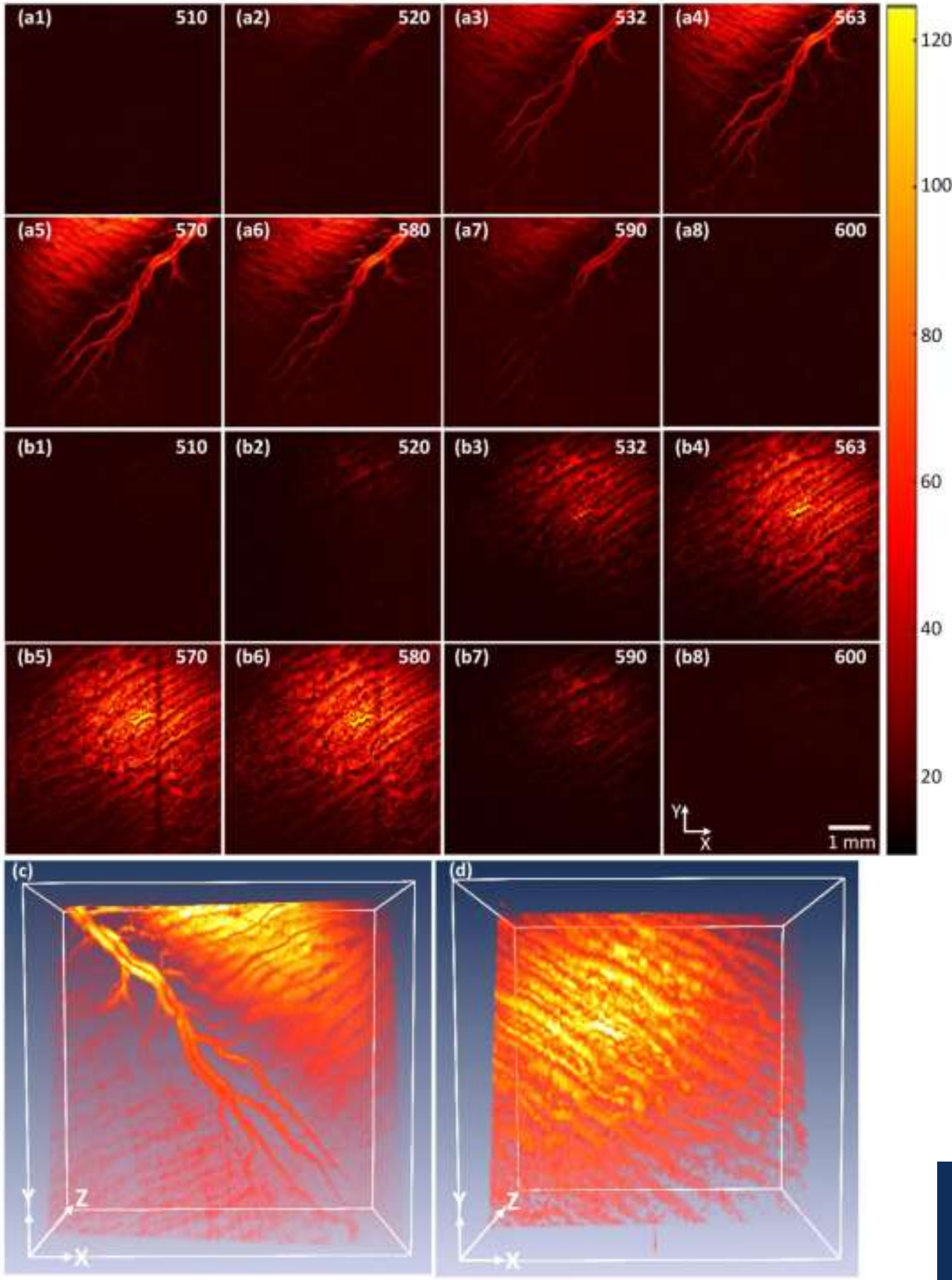




Retinal Vein Occlusion Rabbit Model



Nguyen VP, Li Y, Zhang W, Wang X, Paulus YM. Multi-wavelength, en-face Photoacoustic Microscopy and Optical Coherence Tomography Imaging for Early and Selective Detection of Laser Induced Retinal Vein Occlusion. *Biomedical Optics Express* 2018; 9(12): 5915-5938.



Spectroscopic PAM analysis of the retina and choroid

A. Retinal vessels
B. Choroidal vessels

C and D. 3D reconstructions of A and B

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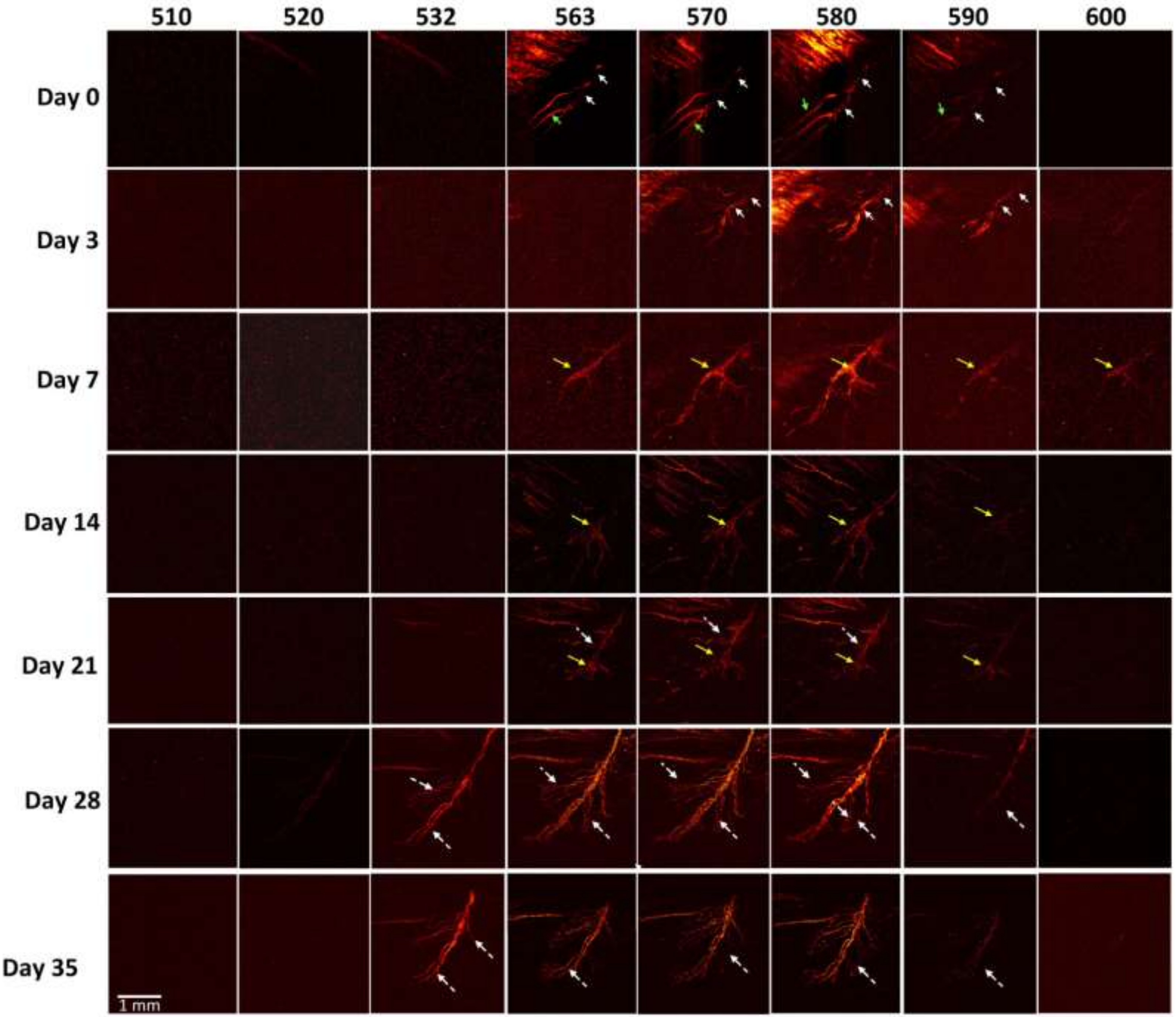
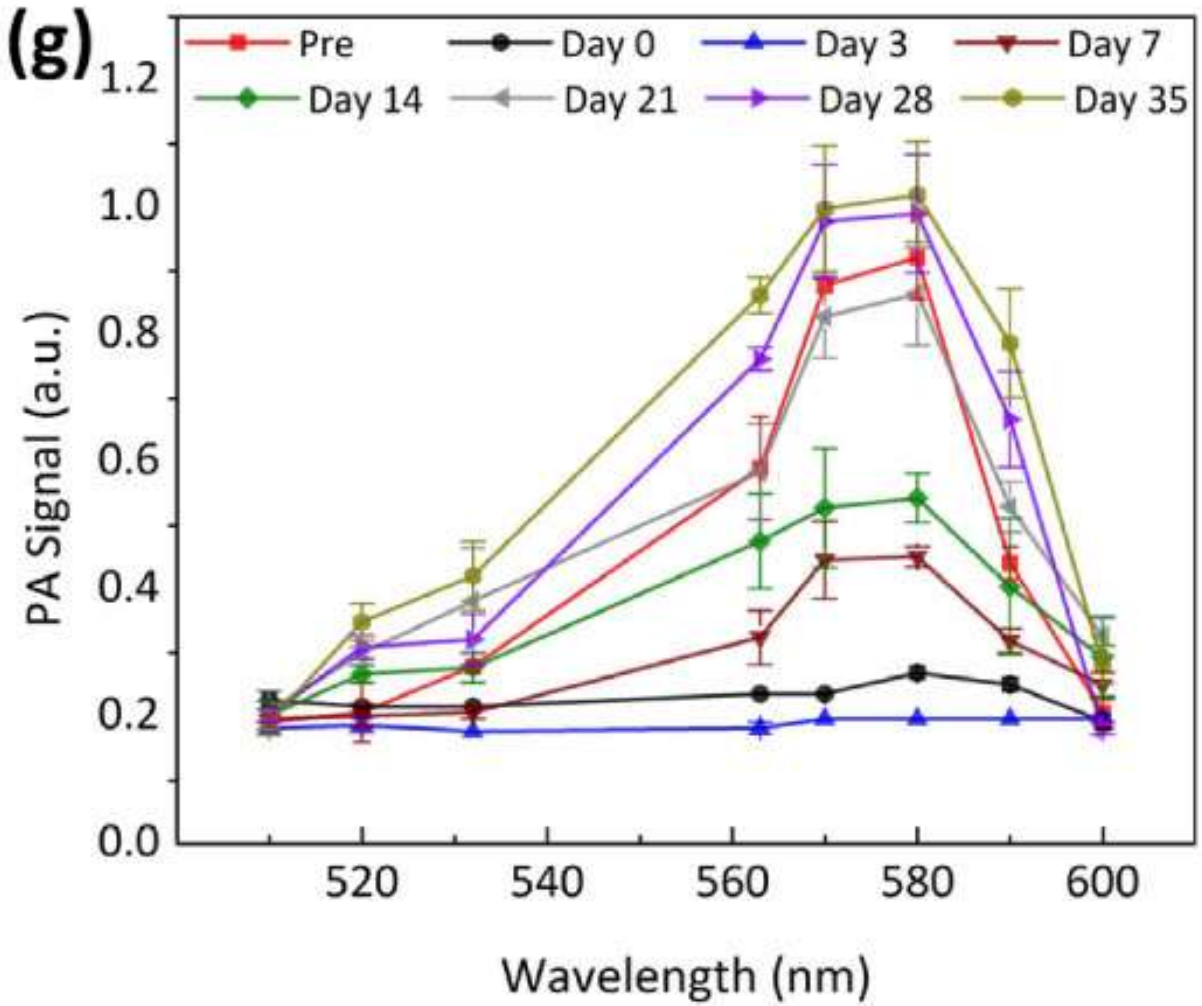
MICHIGAN MEDICINE



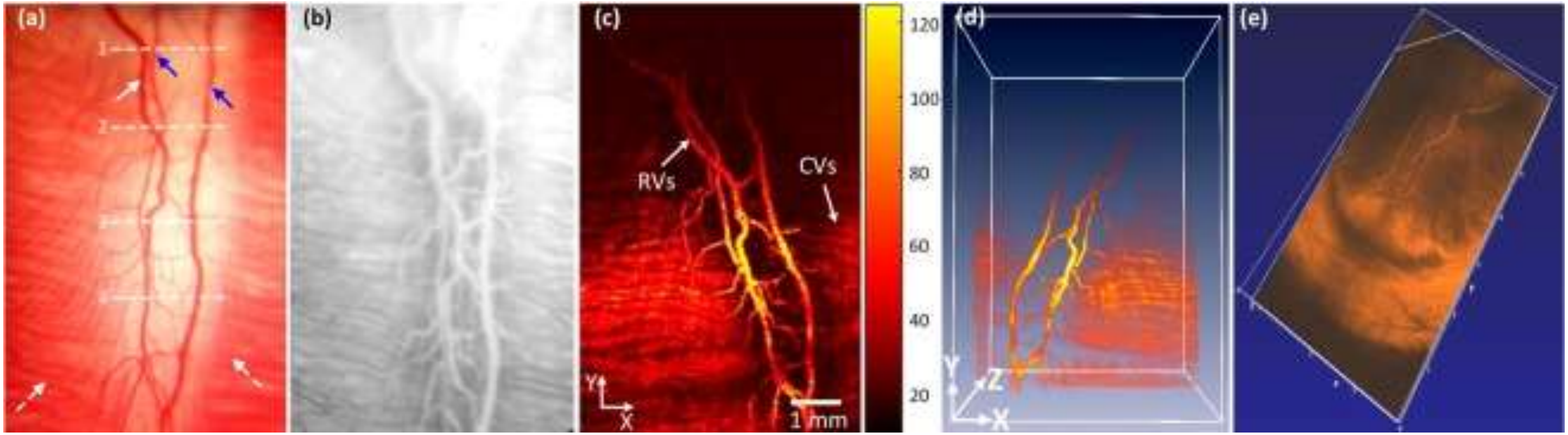
EYE CENTER
ICINE

Quantification of spectroscopic PAM



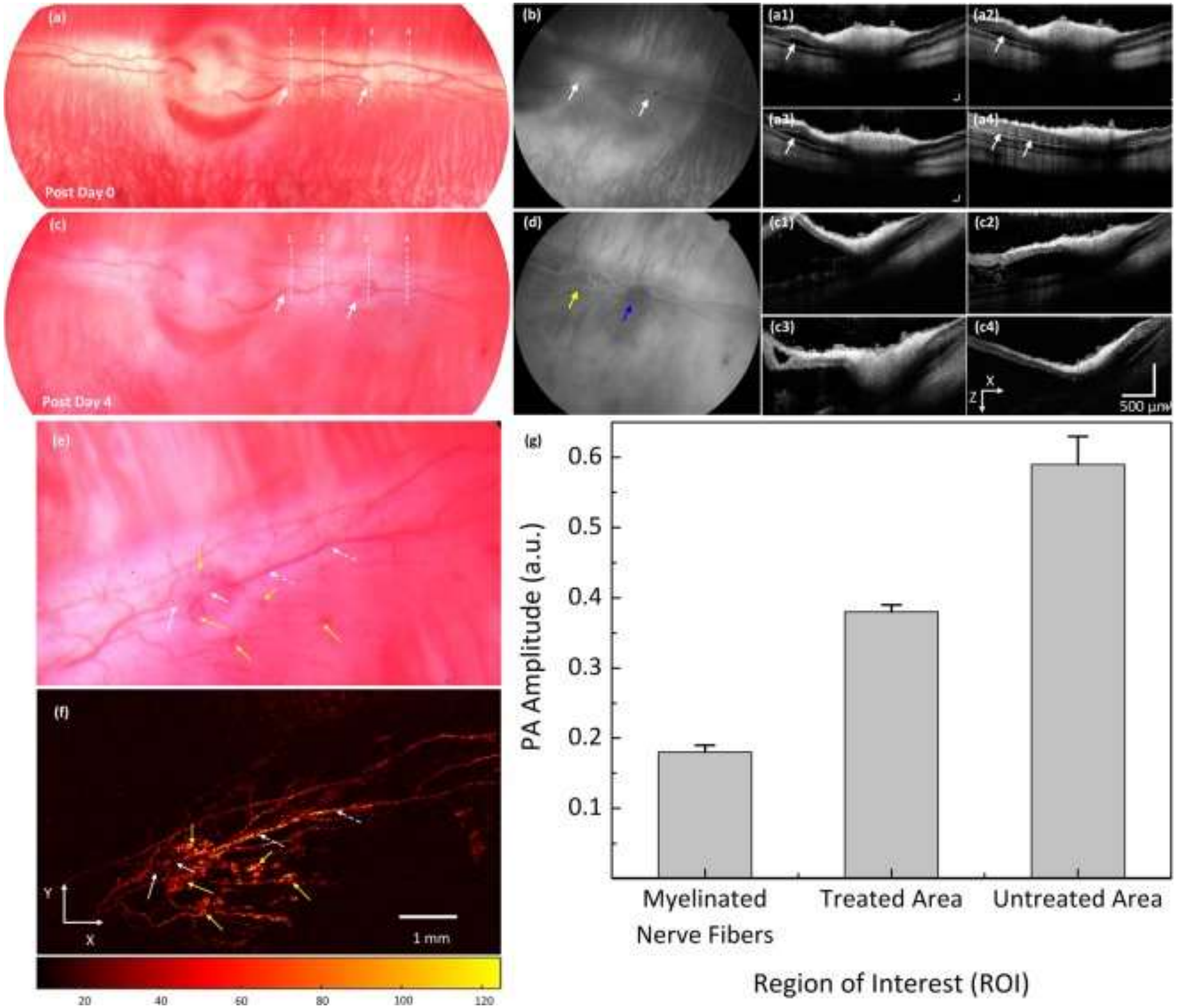
Multimodal imaging retinal blood vessels

(a) Color photo; (b) FA (c) PAM; (d) 3D PAM; (e) 3D OCT



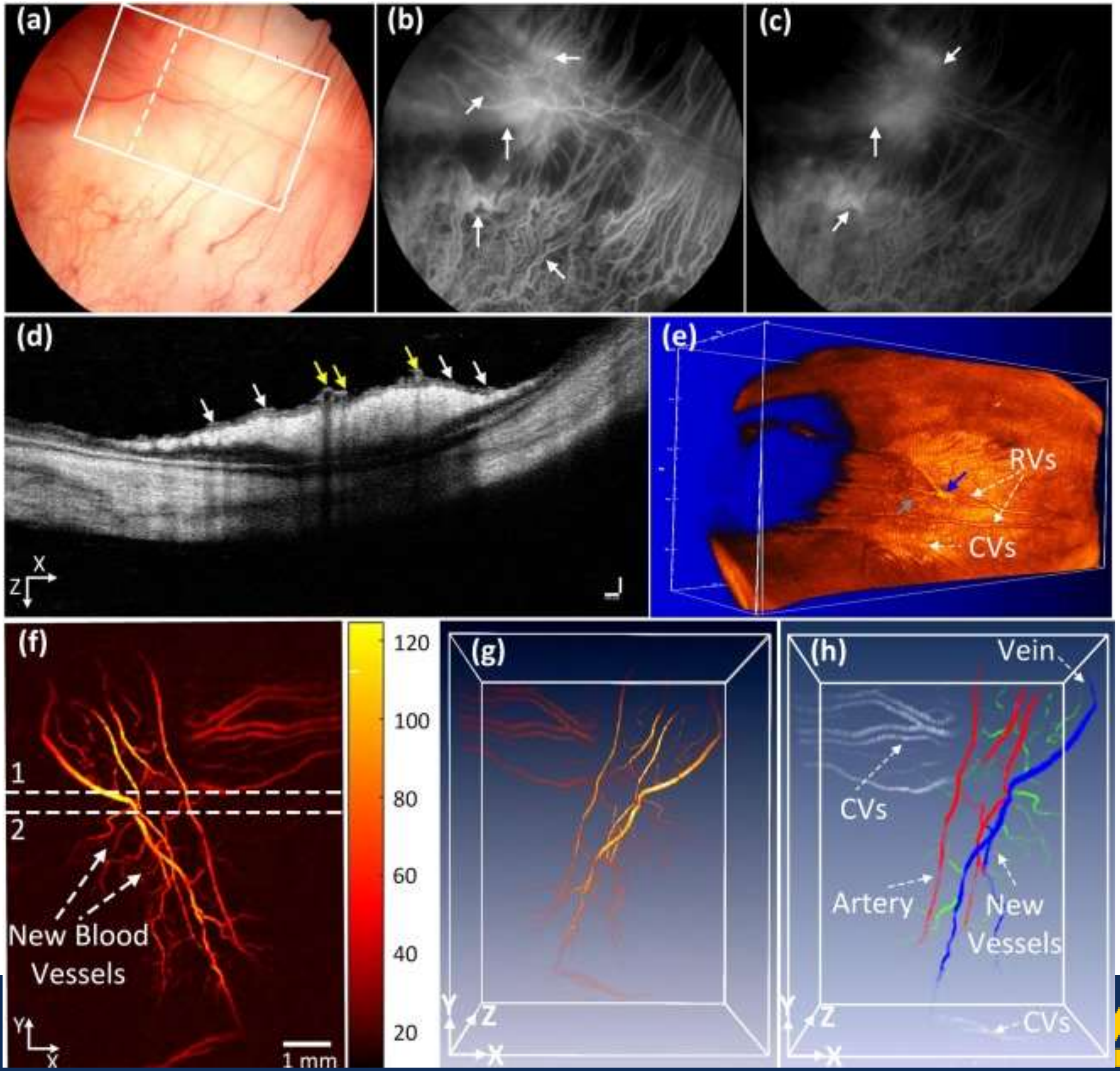
Nguyen VP, Li Y, Zhang W, Wang X, Paulus YM. High-resolution multimodal photoacoustic microscopy and optical coherence tomography image-guided laser induced branch retinal vein occlusion in living rabbits. *Scientific Reports* 2019; 9(1):10560

Retinal vein occlusion



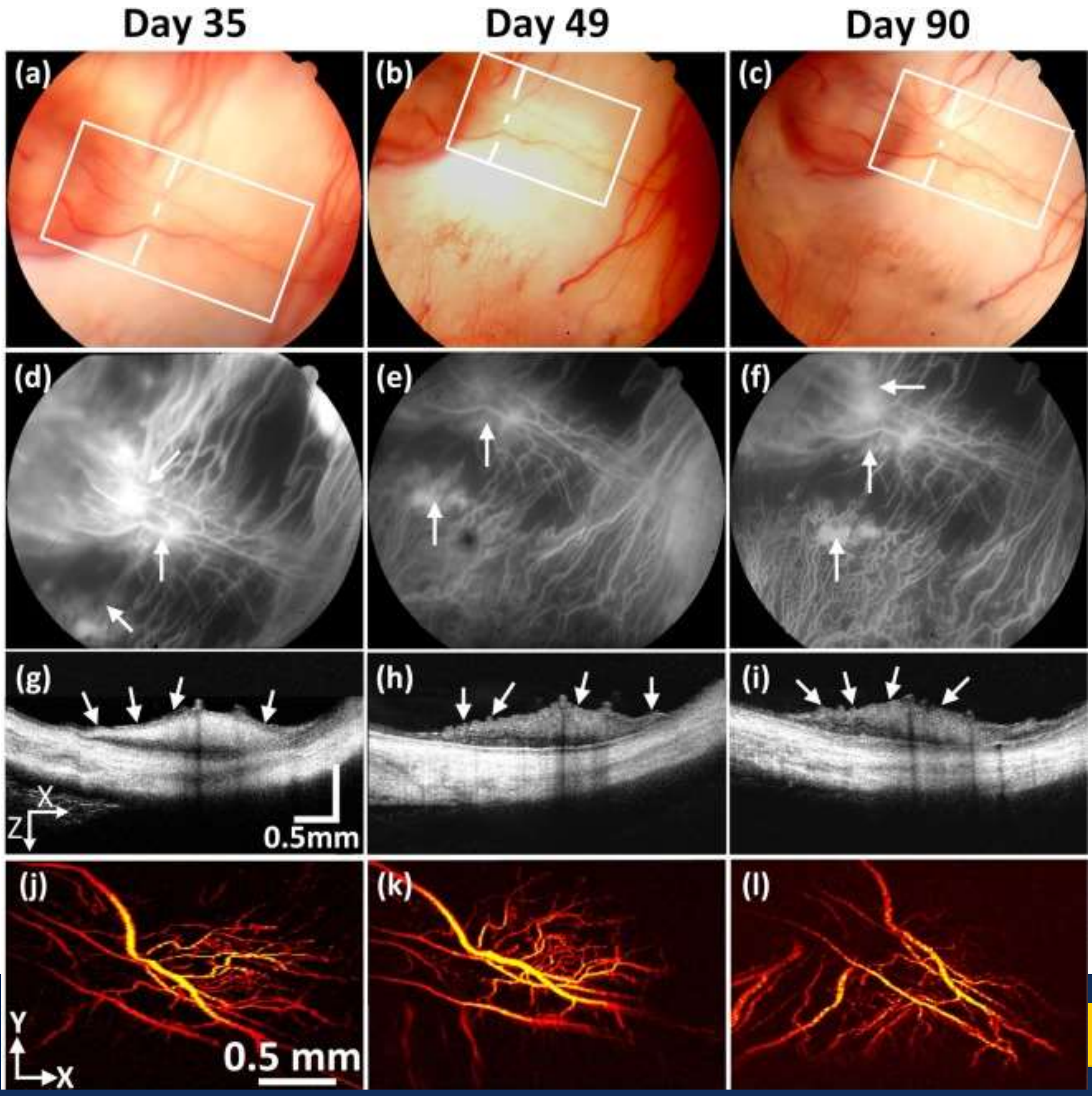
(a,c,e) Color photo;
(b,d) FA
(a1-4) OCT images;
(f) PAM;
(g) PAM amplitude

Retinal neovascularization in RVO



(a) Color photo;
(b,c) FA early and late
(d) OCT; (e) 3D OCT;
(f) PAM;
(g) 3D PAM;
(h) Label type vessels

Retinal neovascularization over time



(a,b,c) Color photo;
(d,e,f) FA
(g,h,i) OCT;
(j,k,l) PAM

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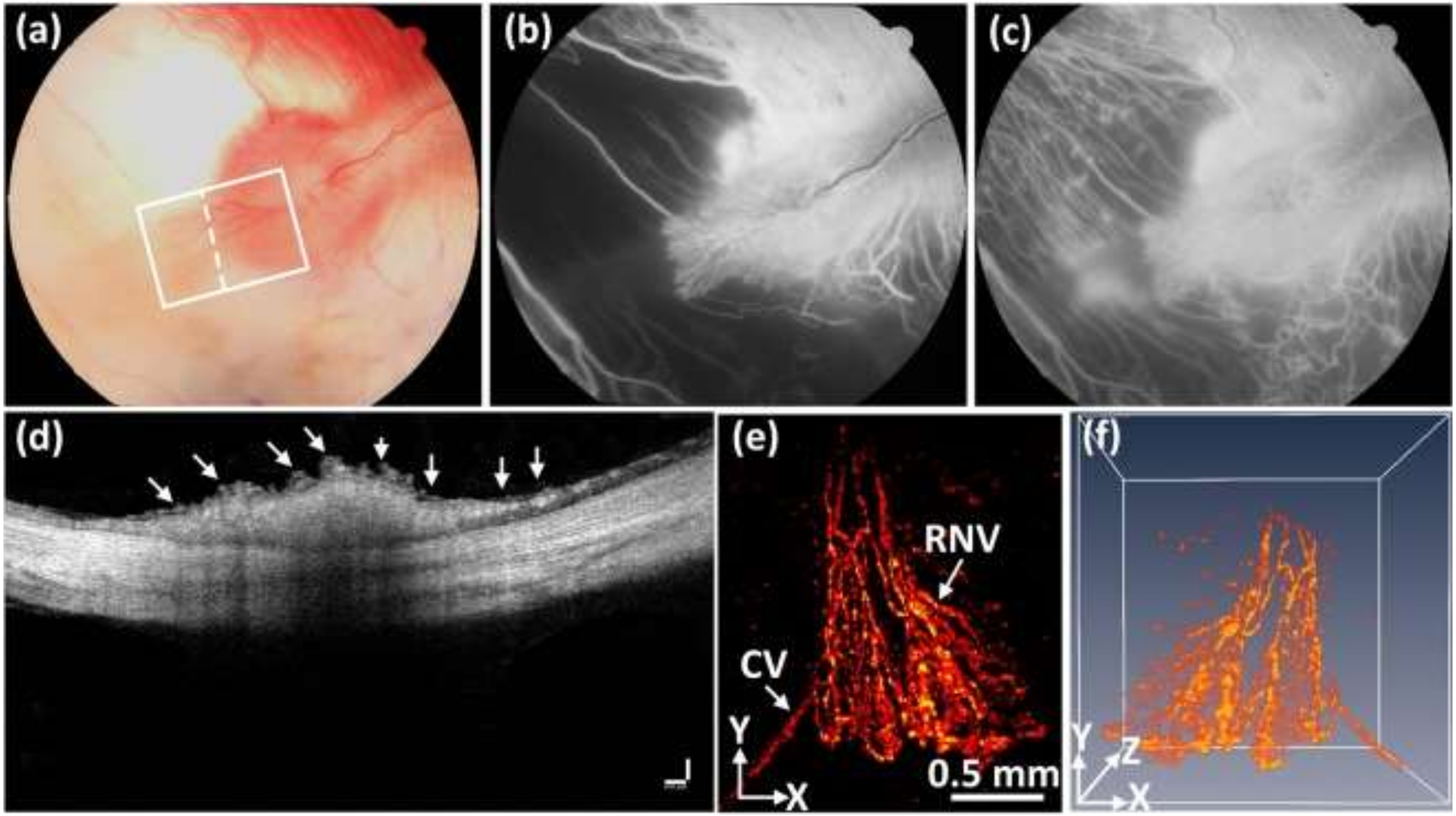
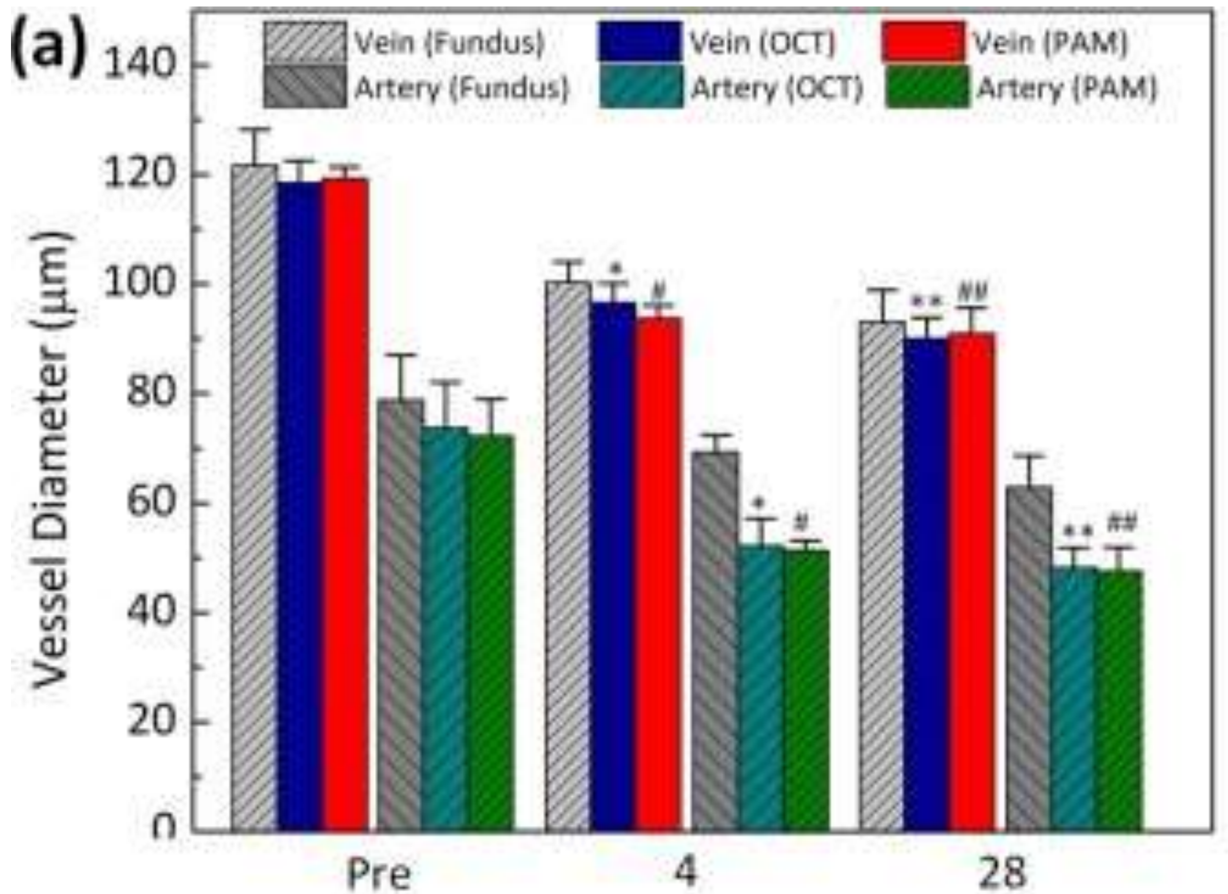
Retinal neovascularization in RVO



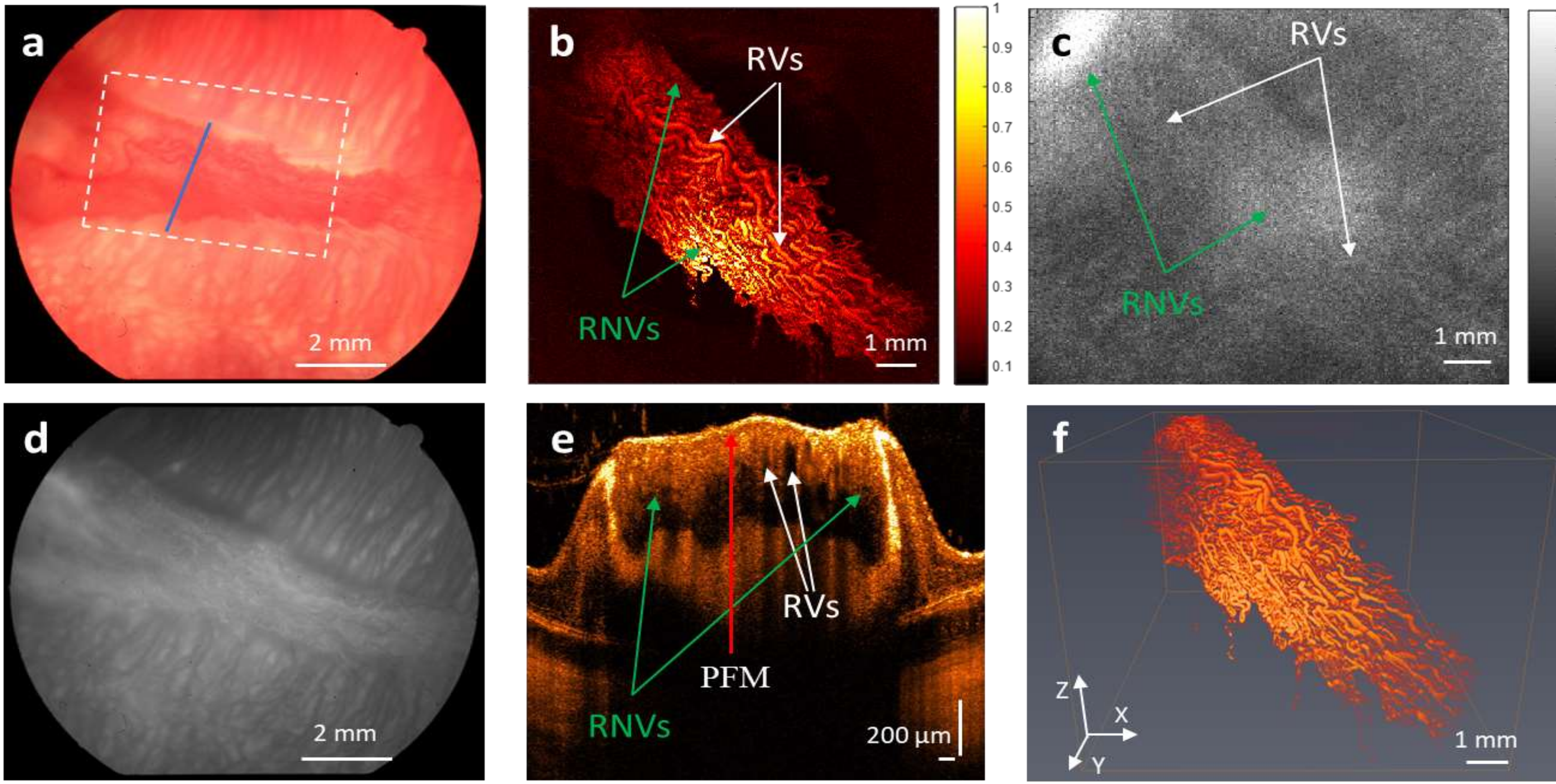
(a) Color photo; (b,c) FA; (d) OCT; (e) PAM; (f) 3D PAM

Quantify vessel diameter photo, OCT, and PAM



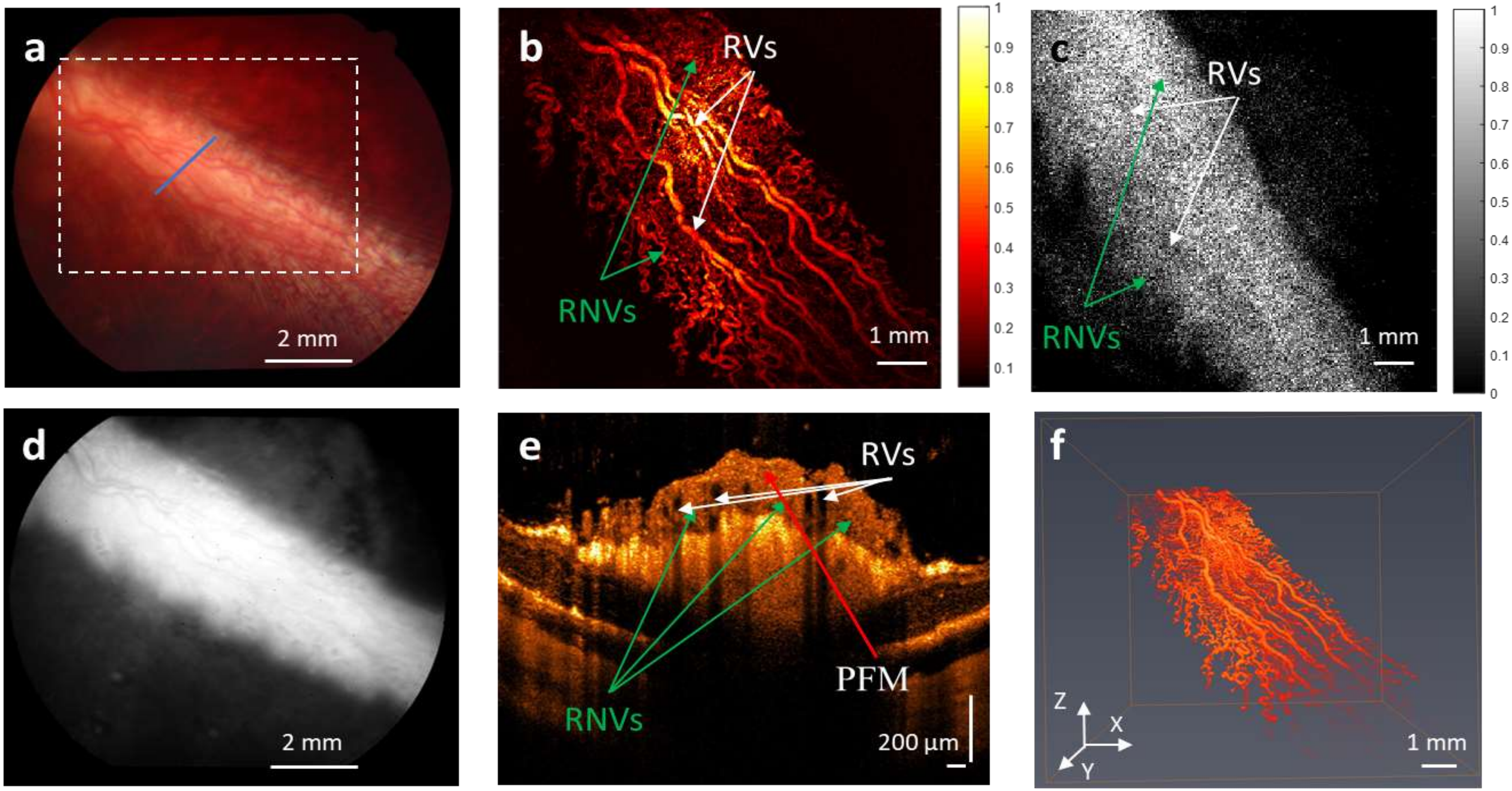
New Zealand white rabbit RNV

(a) Color fundus photo; (b) PAM (c) FM; (d) FA; (e) OCT; (f) 3D PAM



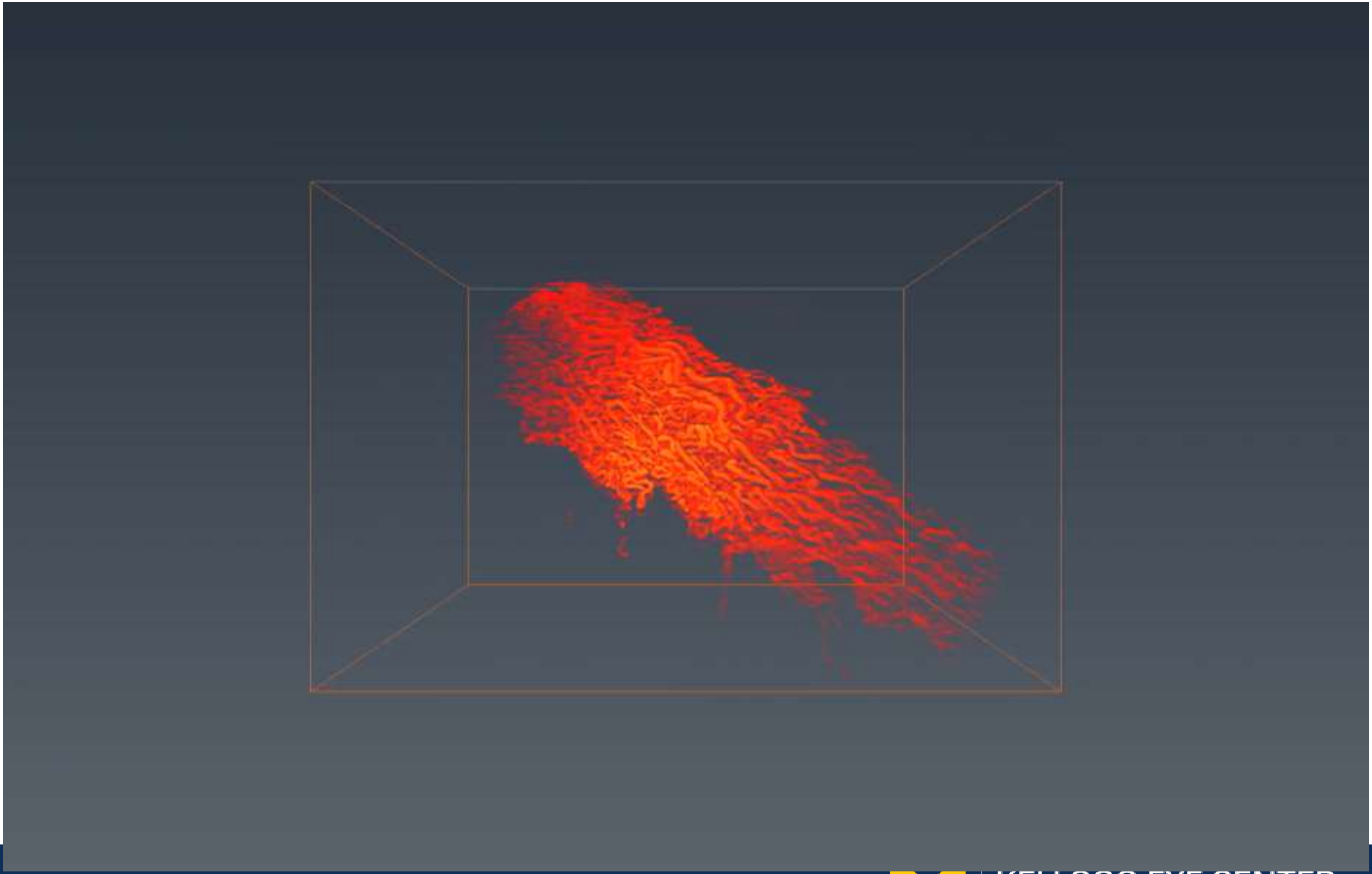
Pigmented rabbits RNV

(a) Color fundus photo; (b) PAM (c) FM; (d) FA; (e) OCT; (f) 3D PAM

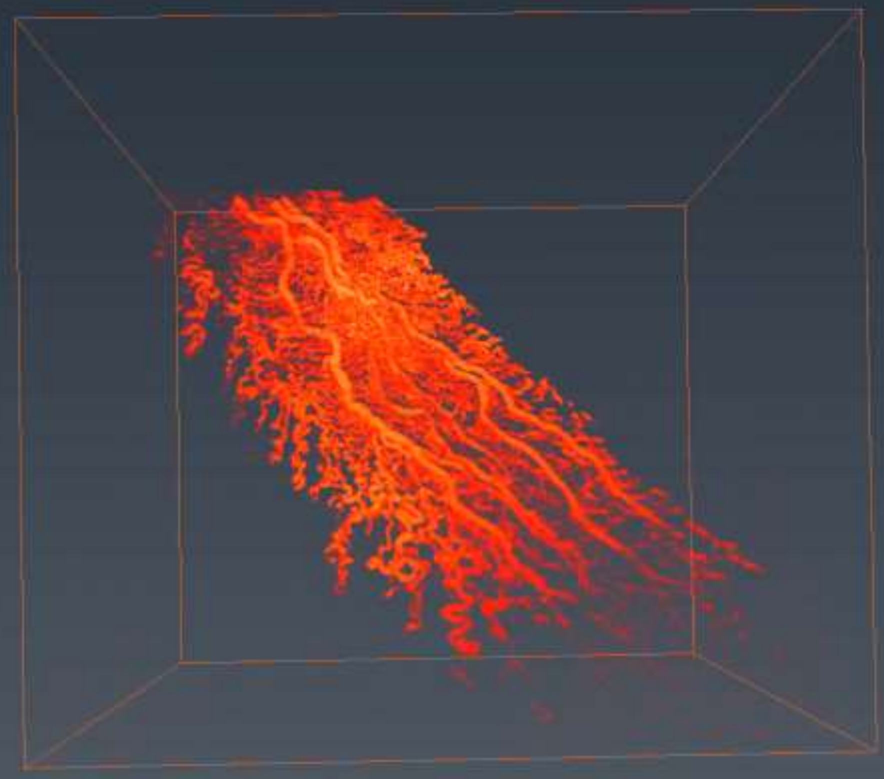


Zhang W, Li Y, Nguyen VP, Huang Z, Liu Z, Wang X, Paulus YM. High resolution, in vivo Multimodal Photoacoustic Microscopy, Optical Coherence Tomography, and Fluorescence Microscopy Imaging of Rabbit Retinal Neovascularization. *Light: Science & Applications* 2018; 7:103.

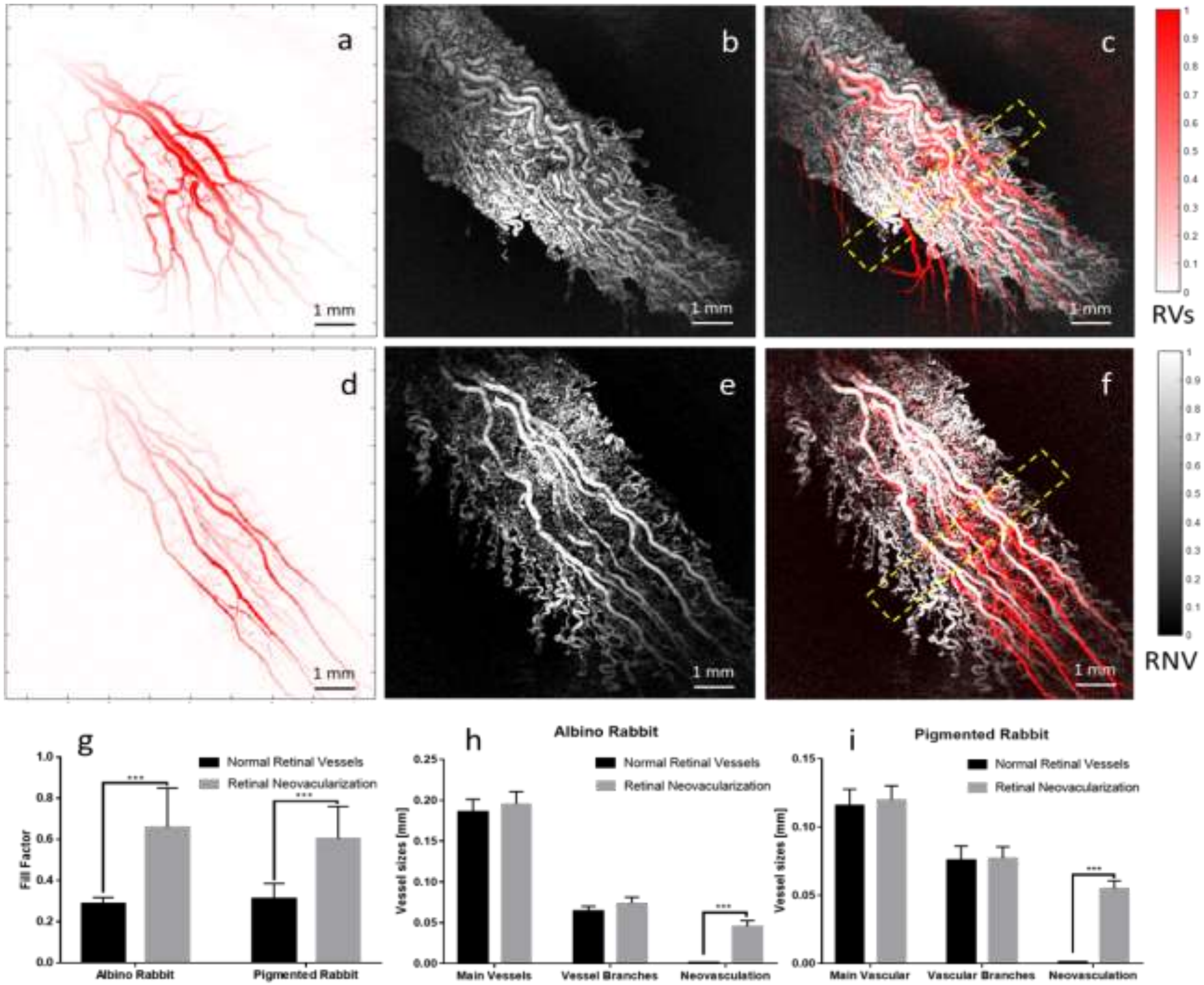
New Zealand white rabbit neovascularization



PAM pigmented rabbit neovascularization



PAM normal vasculature compared to neovascularization



(**a**) Normal retinal vessels in New Zealand white rabbit;

(**b**) RNV induced by VEGF in NZ rabbit;

(**c**) composite pseudo color image of NZ rabbit showing the retinal vessels before and after VEGF injection;

(**d**) normal retinal vessels in pigmented rabbit;

(**e**) RNV induced by VEGF injection in a pigmented rabbit;

(**f**) composite pseudo color image of pigmented rabbit showing the retinal vessels before and after VEGF injection;

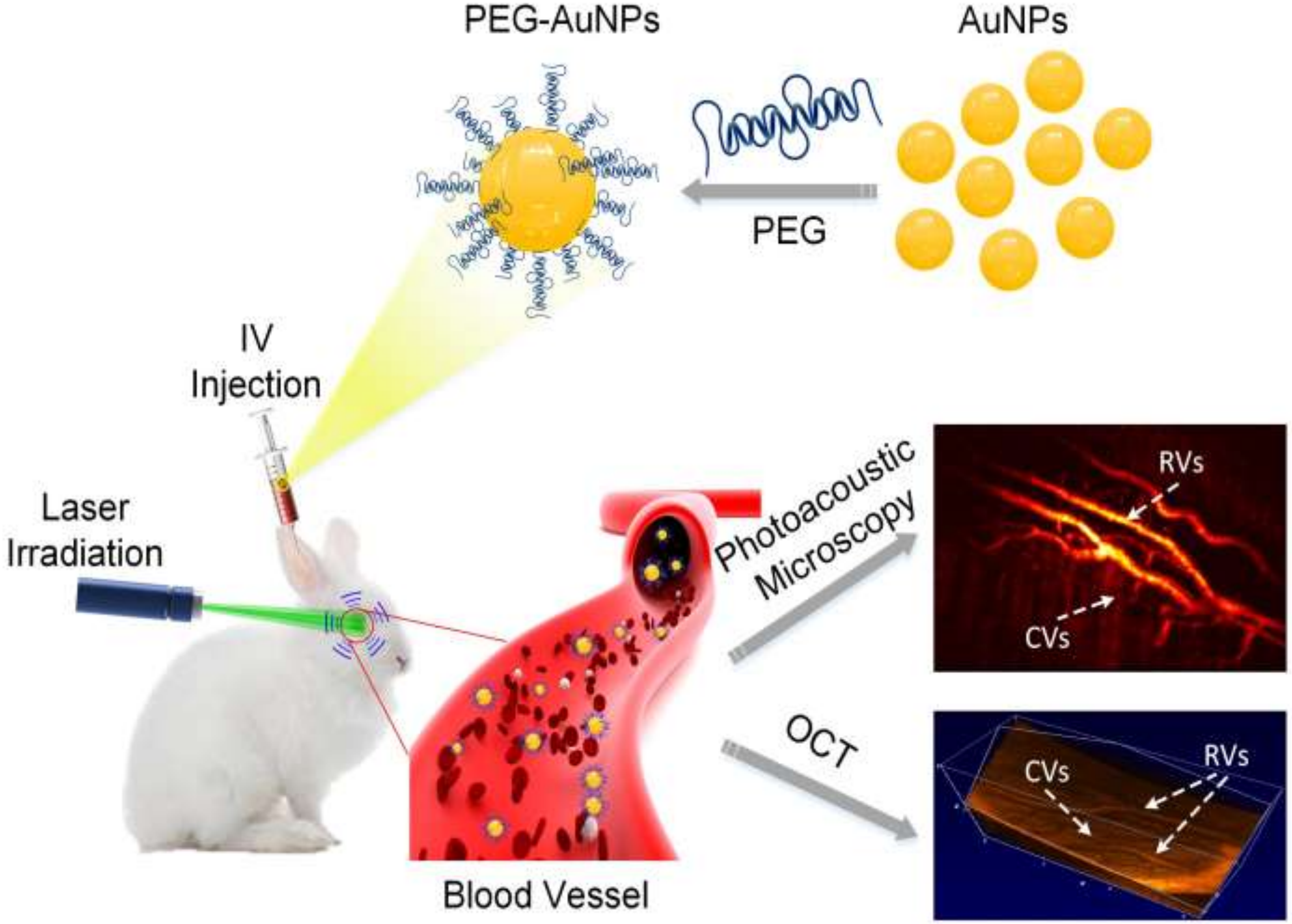
(**g**) quantification of retinal vessels and RNV in NZ and pigmented rabbits before and after VEGF injection;

(**h**) quantification of retinal vessels and RNV in NZ rabbits before and after VEGF injection using the vessel size;

(**i**) quantification of retinal vessels and RNV in pigmented rabbits before and after VEGF injection using the vessel size

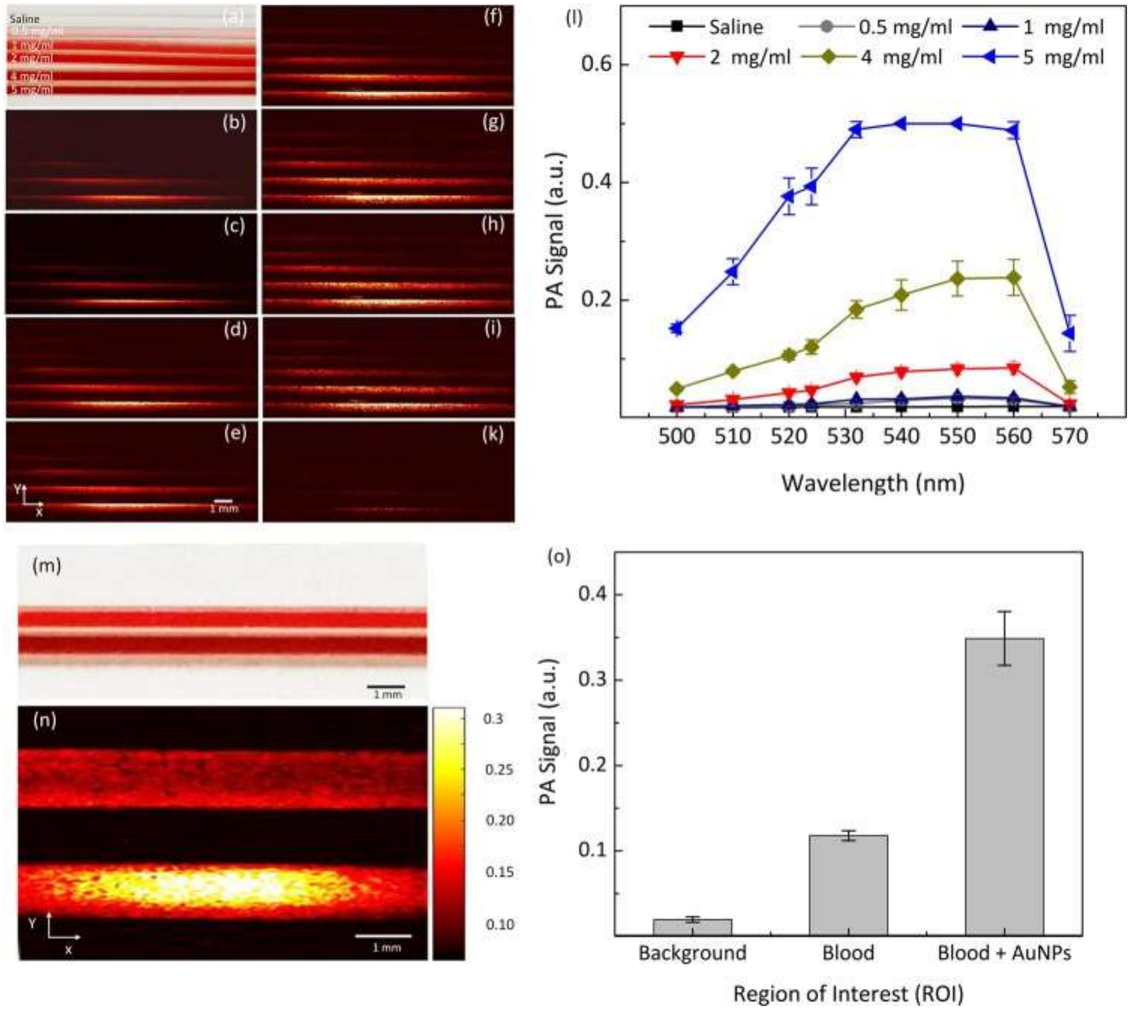
Gold-nanoparticle enhanced PAM imaging

Photoacoustic imaging with gold nanoparticles (AuNP) can significantly enhance signal of PAM and OCT



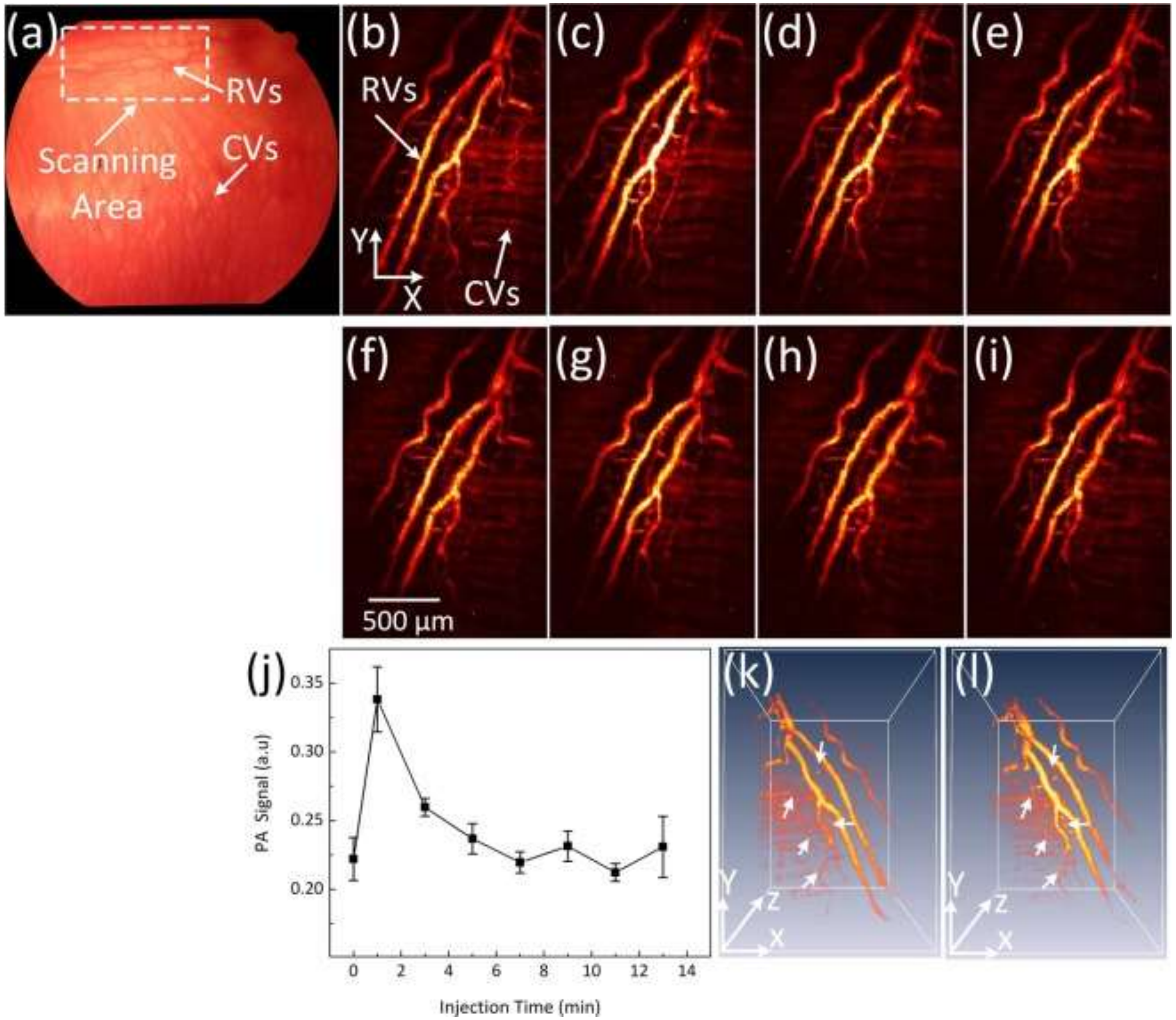
Nguyen VP, Li Y, Qiao W, Liu B, Tian C, Zhang W, Huang Z, Ponduri A, Tarnowski M, Wang X, Paulus YM. Contrast Agent Enhanced Multimodal Photoacoustic Microscopy and Optical Coherence Tomography for Imaging of Rabbit Choroidal and Retinal Vessels *in vivo.* *Scientific Reports* 2019; 9(1):5945.

PA signal with AuNP



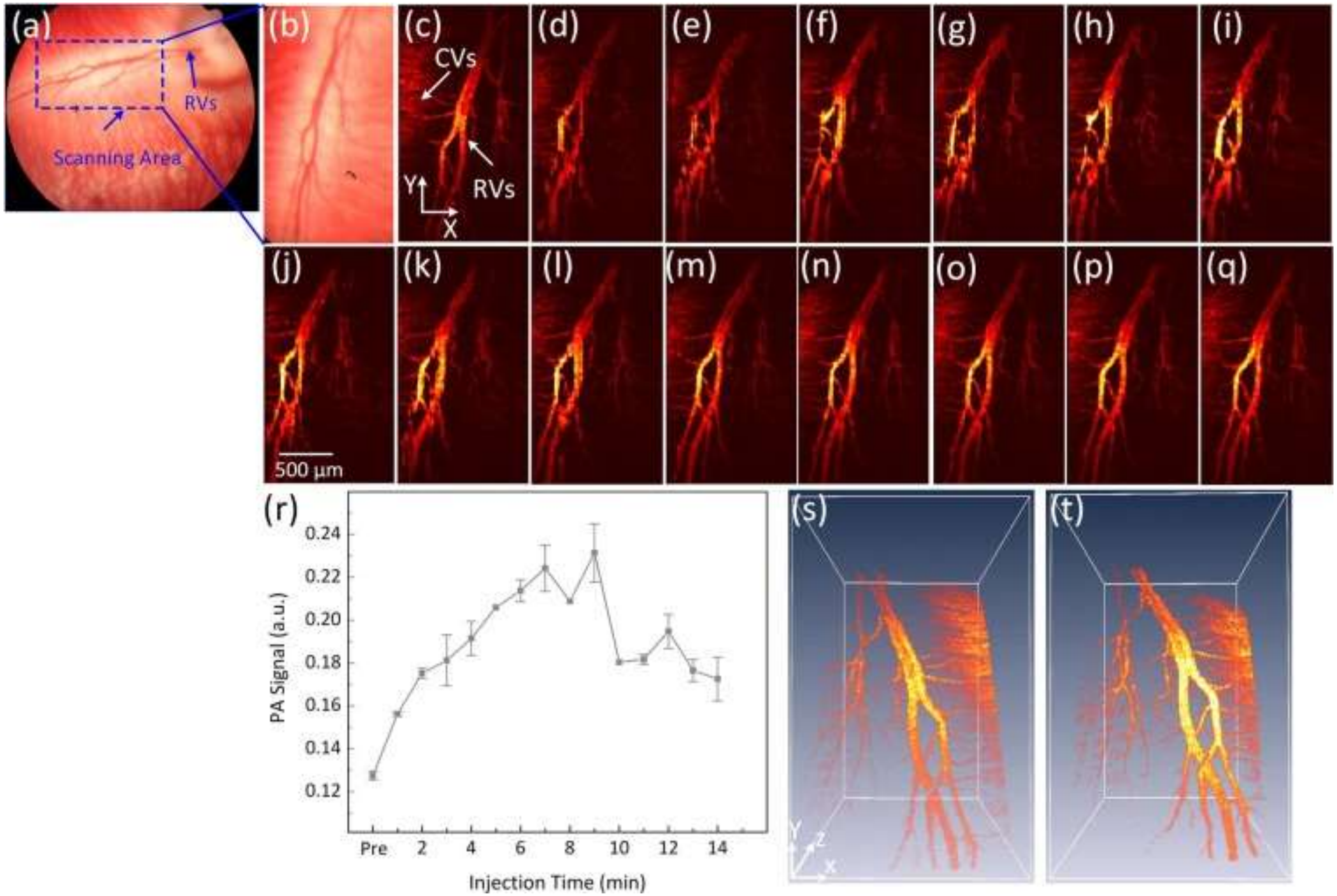
(**a**) Photograph phantoms AuNP various concentrations
(**b-k**) PA images of phantoms wavelength from 500 to 570nm
(**l**) PA signal as function of AuNP concentration and wavelength
(**m**) photographs phantoms blood and blood + AuNP 1:1
(**n**) corresponding PA image
(**o**) PA blood 3-fold higher background; PA blood + AuNP 17.5 fold higher

PA imaging retinal vessels with PEG-AuNP 2 mg/mL



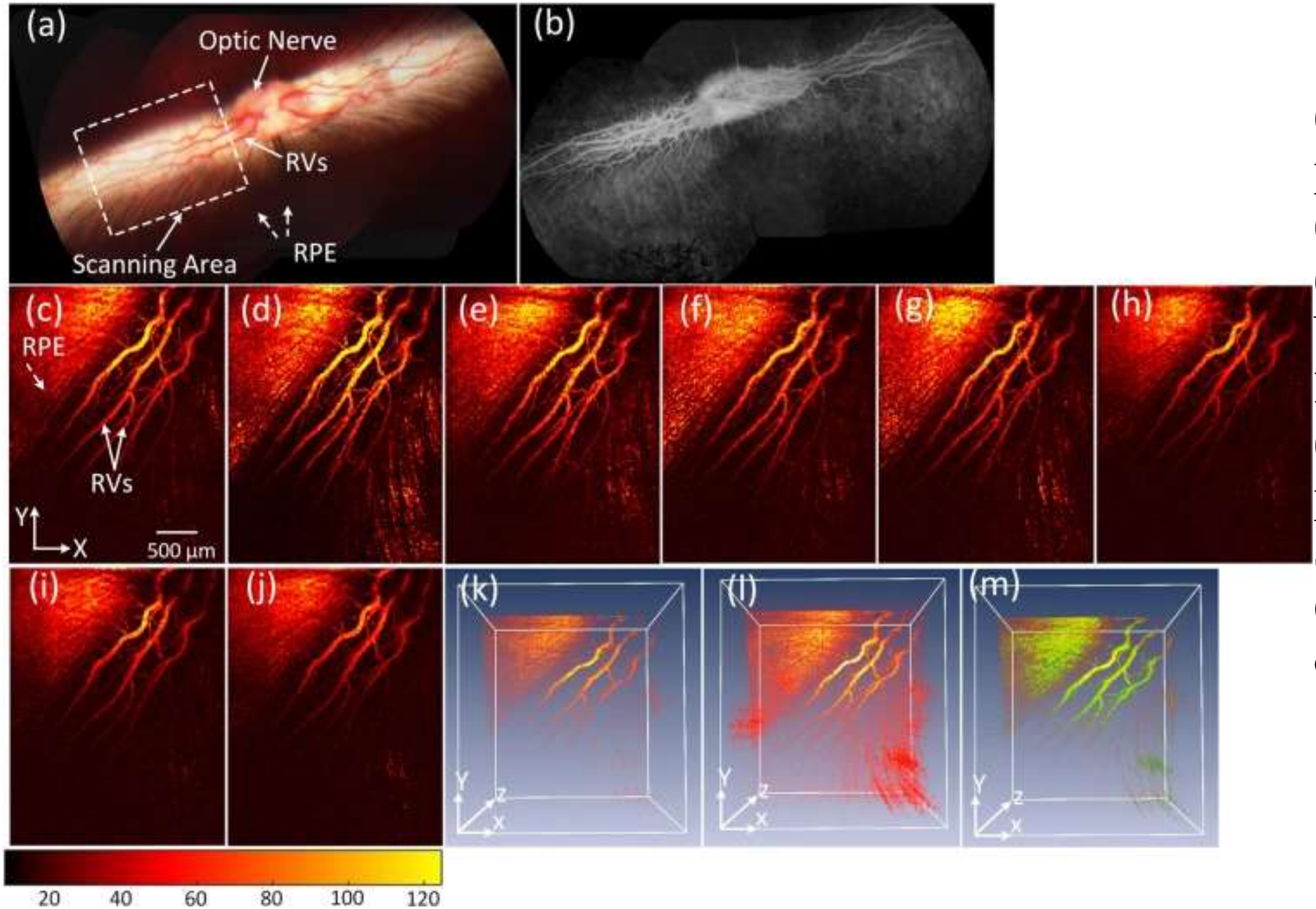
(**a**) Color fundus image of retina.
(**b–i**) PAM images before and after injection of PEG-AuNPs.
(**j**) PA signal amplitude increase 0.22 to 0.34
(**k**) Pre and (**l**) post injection 3D PAM

PA imaging retinal vessels with PEG-AuNP 5 mg/mL



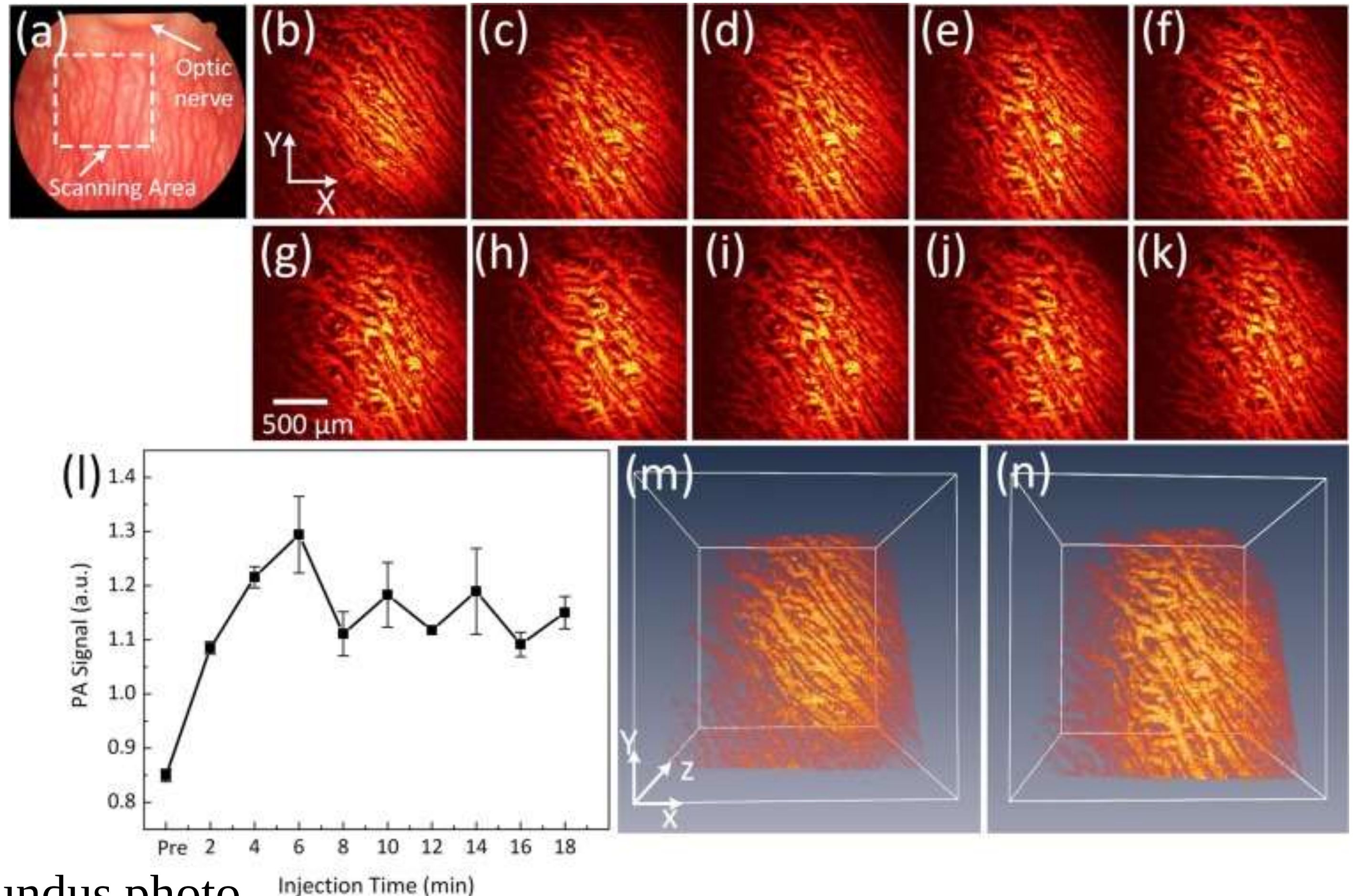
(**a**) color fundus photo (**b**) close-up photo (**c–q**) MIP PA before and after PEG-AuNPs, (**r**) PA signal amplitude over time (**s**) pre and (**t**) post-injection 3D PAM

PA retinal vessels in pigmented rabbits with PEG-AuNP 5 mg/mL



(**a**) color fundus photo
(**b**) FA
(**c–j**) MIP PA before and after PEG-AuNPs,
(**k**) pre and (**l**) post-injection 3D PAM
(**m**) Subtraction of post-pre

PAM imaging choroidal vessels with PEG-AuNP 2 mg/mL



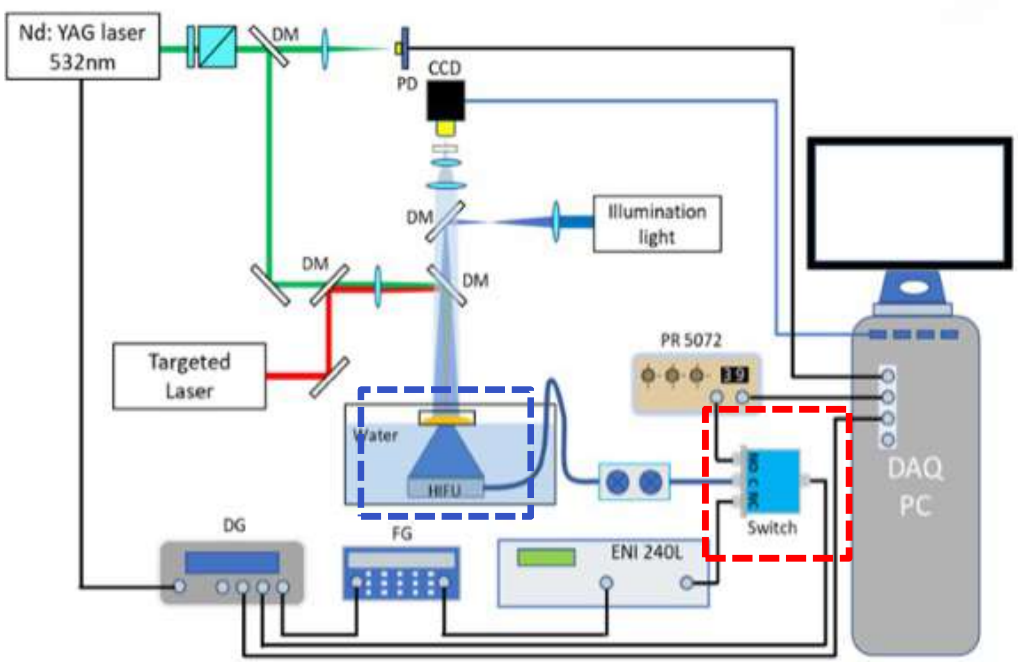
(**a**) Color fundus photo

(**b–k**) PAM images before and after injection of PEG-AuNPs.

(**l**) PA signal amplitude increasing after AuNP

(**m**) Pre and (**n**) post injection 3D PAM

Real-time photoacoustic signal guided therapy

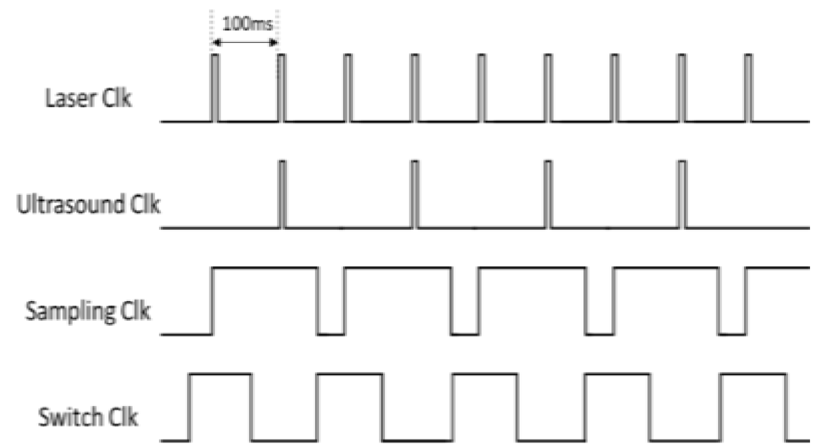


Schematic diagram of real-time PA signal guided PUT system

HIFU: High-intensity focused ultrasound

Switch: Pulse/Receiver Switch

DG: Delay generator FG: Function generator



Time sequencing of the system

Zhang W, Qin Y, Xie X, Hu Z, Paulus YM, Yang X, Wang X. Real-time Photoacoustic Sensing for Photo-mediated Ultrasound Therapy. *Optics Letters* 2019; 44(16): 4063-66.

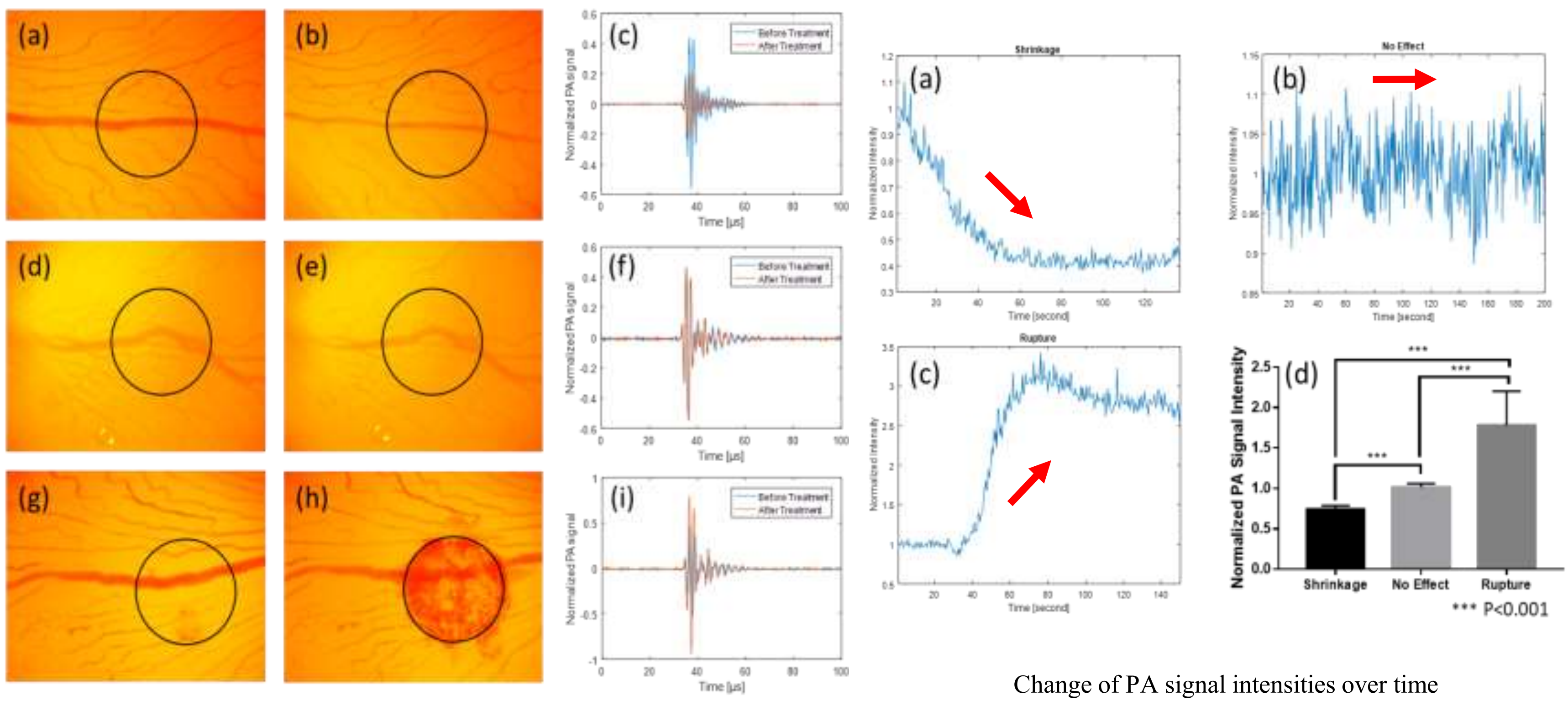
Real-time photoacoustic signal guided therapy

Before | After | Change of Photoacoustic (PA) signal intensity

Shrinkage

No change

Rupture



Change of PA signal intensities over time

Conclusions

- Photoacoustic imaging is a promising modality to noninvasively image blood distribution with a high depth of penetration (retina and choroid)
- Photoacoustic microscopy can be achieved with a high resolution (2.6 μm lateral resolution)
- PAM can be utilized with a safe laser intensity below the ANSI safety limit
- PAM can visualize blood vessels in human-sized eyes (rabbit)
- PAM can visualize retinal vascular pathology such as retinal neovascularization (like in diabetes) and retinal vein occlusion
- Gold nanoparticles can serve as contrast agents to enhance PAM imaging
- Photoacoustic monitoring of retinal vasculature for automated dosimetry and reproducible burns "Smart laser"


Paulus Advanced Retinal
Imaging and Laser Laboratory

Retina Division UM

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- Alliance for Vision Research
- Knights Templar Eye Foundation
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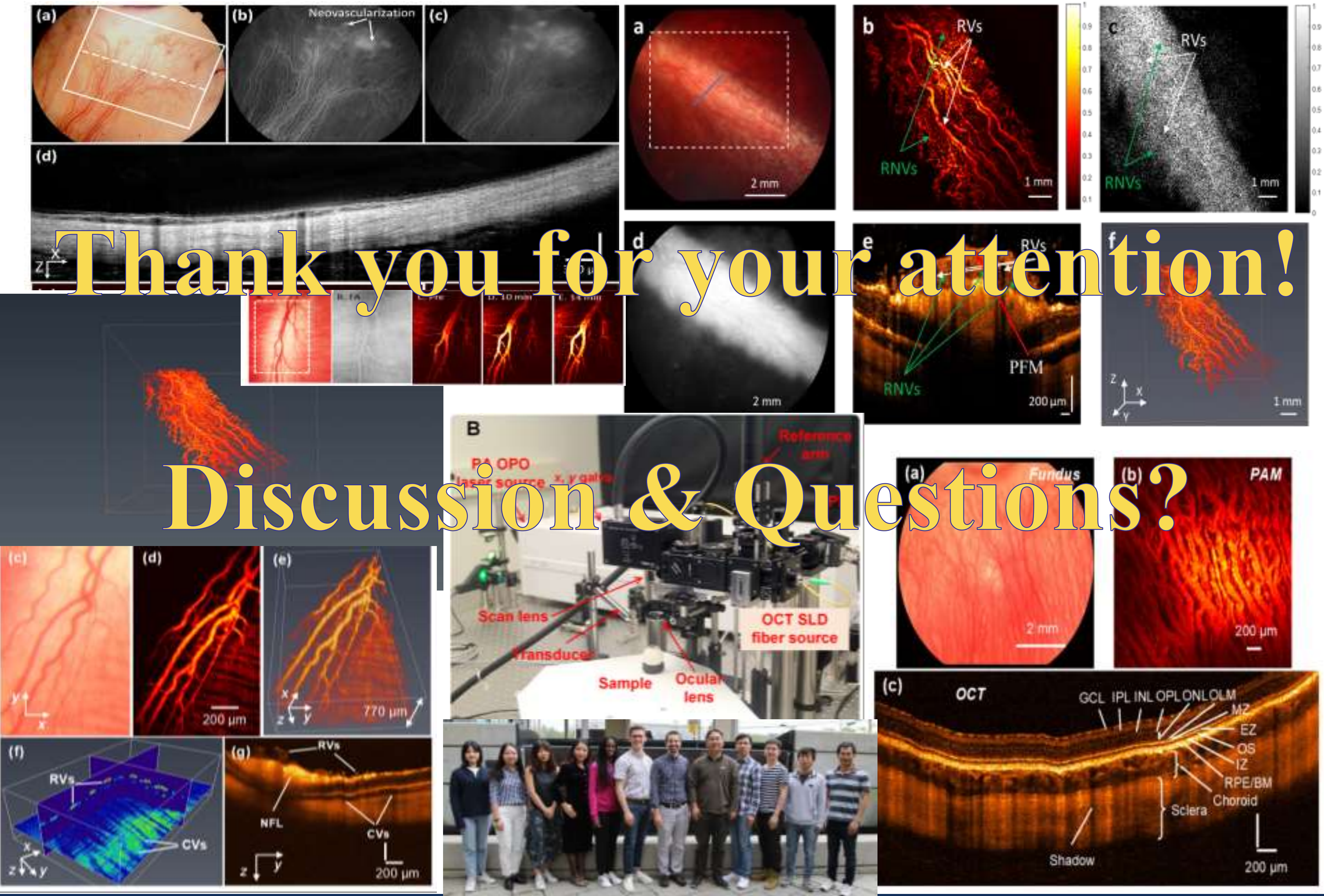
- University of Michigan Translational Research and Commercialization for Life Sciences (MTRAC) # N021025
- Core Center for Vision Research funded by P30 EY007003 from the National Eye Institute
- University of Michigan Department of Ophthalmology and Visual Sciences

Thank you for your attention!

Discussion & Questions?



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