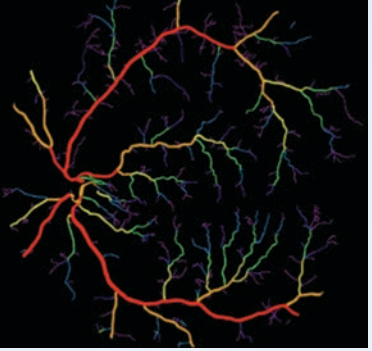
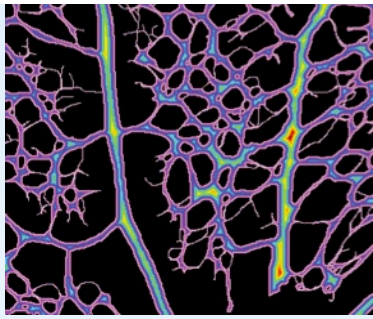




New Therapeutic Window of Regenerative Opportunity in Diabetic Retinopathy by VESGEN Analysis

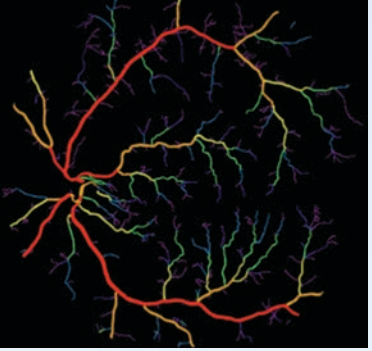
Patricia Parsons-Wingerter, PhD
Biomedical Research Engineer, Bioscience and Engineering Branch
Research & Technology Directorate

Of Blood Vessels

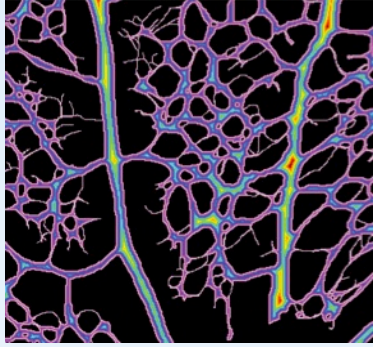
Glenn Research Center

VESGEN Patent Pending

at Lewis Field



with VESGEN Software as Research Discovery Tool



Multi-Scale mapping of vascular pattern for development of regenerative and preventive therapies targeting diseases dependent on microvascular remodeling

C. Blood Vessels

Glenn Research Center

VESGEN Patent Pending

at Lewis Field

Vascular Alterations, Visual Impairments (VIIP) & Increased Intracranial Pressure (ICP), Immunosuppression & Bone Loss:
NASA-defined risk categories for human space exploration and ISS Utilization

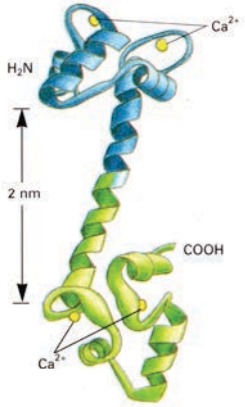
Abstract

Vascular pattern may serve as a useful new biomarker principle of complex, multi-scale signaling in pathological, physiological angiogenesis and microvascular remodeling. Each angiogenesis stimulator or inhibitor we have analyzed, including VEGF, bFGF, TGF-beta1, angiostatin and triamcinolone acetonide, has induced a novel ‘fingerprint’ or ‘signature’ biomarker vascular pattern that is spatio-temporally unique. Remodeling vasculature thereby provides an informative read-out of dominant molecular signaling, when analyzed by innovative, fractal-based VESsel GENeration (VESGEN) Analysis software. Using VESGEN to analyze ophthalmic clinical vascular images, we recently introduced a potential paradigm shift to the understanding of early-stage progression that suggests new regenerative opportunities for human diabetic retinopathy (DR), the major blinding disease for working-aged adults. In a pilot study, we discovered that angiogenesis oscillates as a surprising, homeostatic-like regeneration of retinal vessels during early progression of DR (*IOVS* 51(1):498). Results suggest that the term ‘non-proliferative DR’ may be a misnomer. In new studies, normalization of the vasculature will be determined from the response of vascular pattern to therapeutic monitoring and treatment. We have mapped and quantified *in vivo* experimental models of angiogenesis, lymphangiogenesis and intravital blood flow from cellular/molecular to higher systems levels that include a murine model of infant retinopathy of prematurity (ROP); developing and pathological coronary and placental-like vessel models; progressive intestinal inflammation, growing murine tumors, and other pathological, physiological and therapeutically treated tissues of transgenic mice and avian embryos.

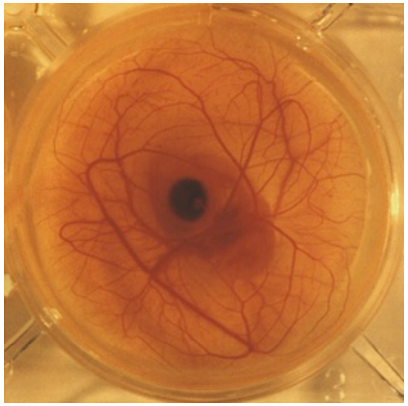
Motivation for Microvascular Quantification and Mapping by VESGEN

NASA IR&D to NIH

1. Molecules *in Vitro*

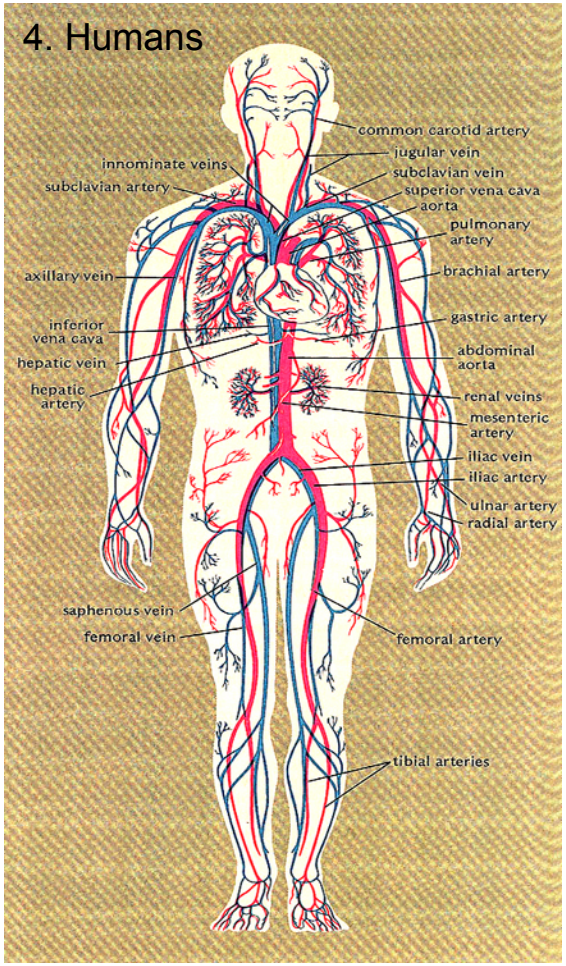


2. Avian Eggs

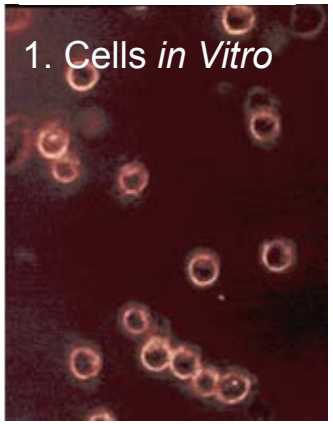


3. Mouse

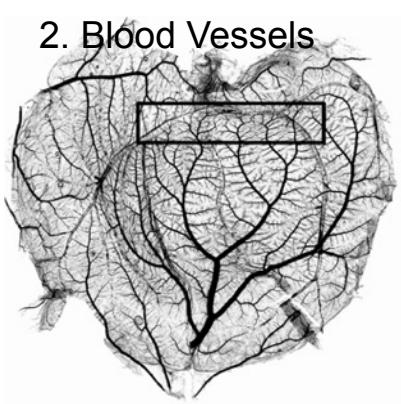
4. Humans



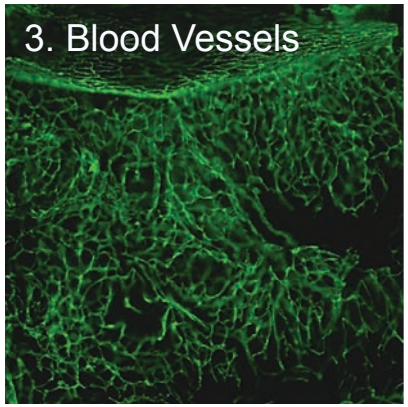
1. Cells *in Vitro*

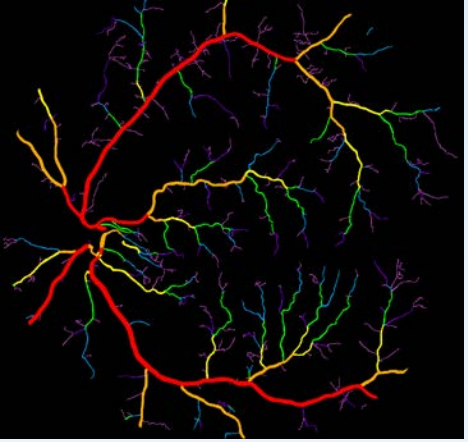


2. Blood Vessels



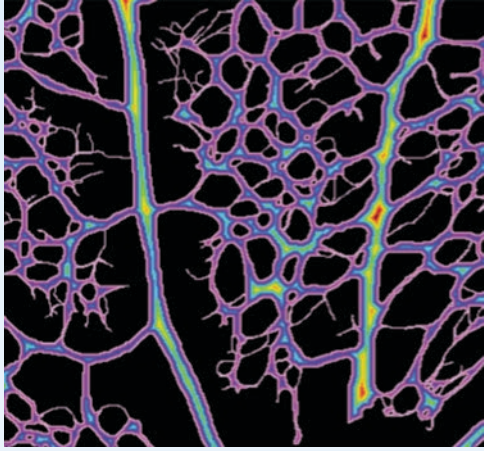
3. Blood Vessels





VESGEN

Mapping and Quantification of Branching Vascular Pattern



Human Retina

Mouse Retina

Vascular Trees

Diabetic Human Retina

Avian CAM, Yolksac and Mouse/Avian Coronary Vessels

Vascular Networks

Mouse Intestinal Inflammation, CAM Lymphatic Vessels, Abnormal Mouse Corneal Angiogenesis

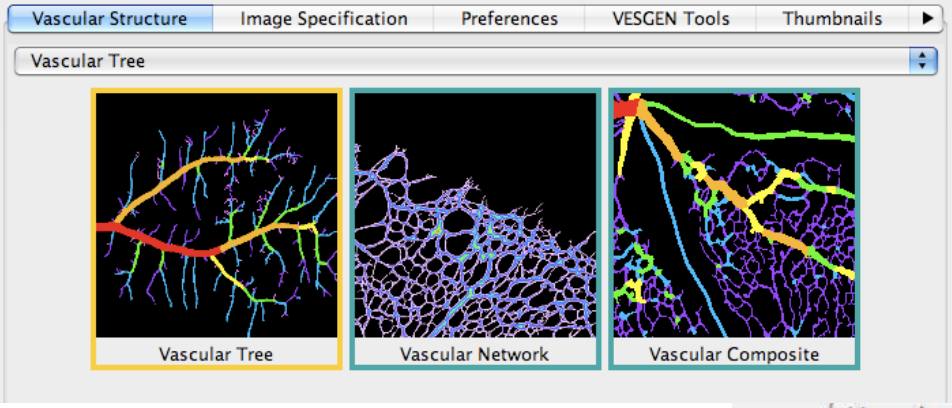
Vascular Tree-Network Composites

Mouse Postnatal Retina

Early Embryonic Coronary Vessels, Juvenile and Adult Leaf Venation

Glenn Research Center at Lewis Field

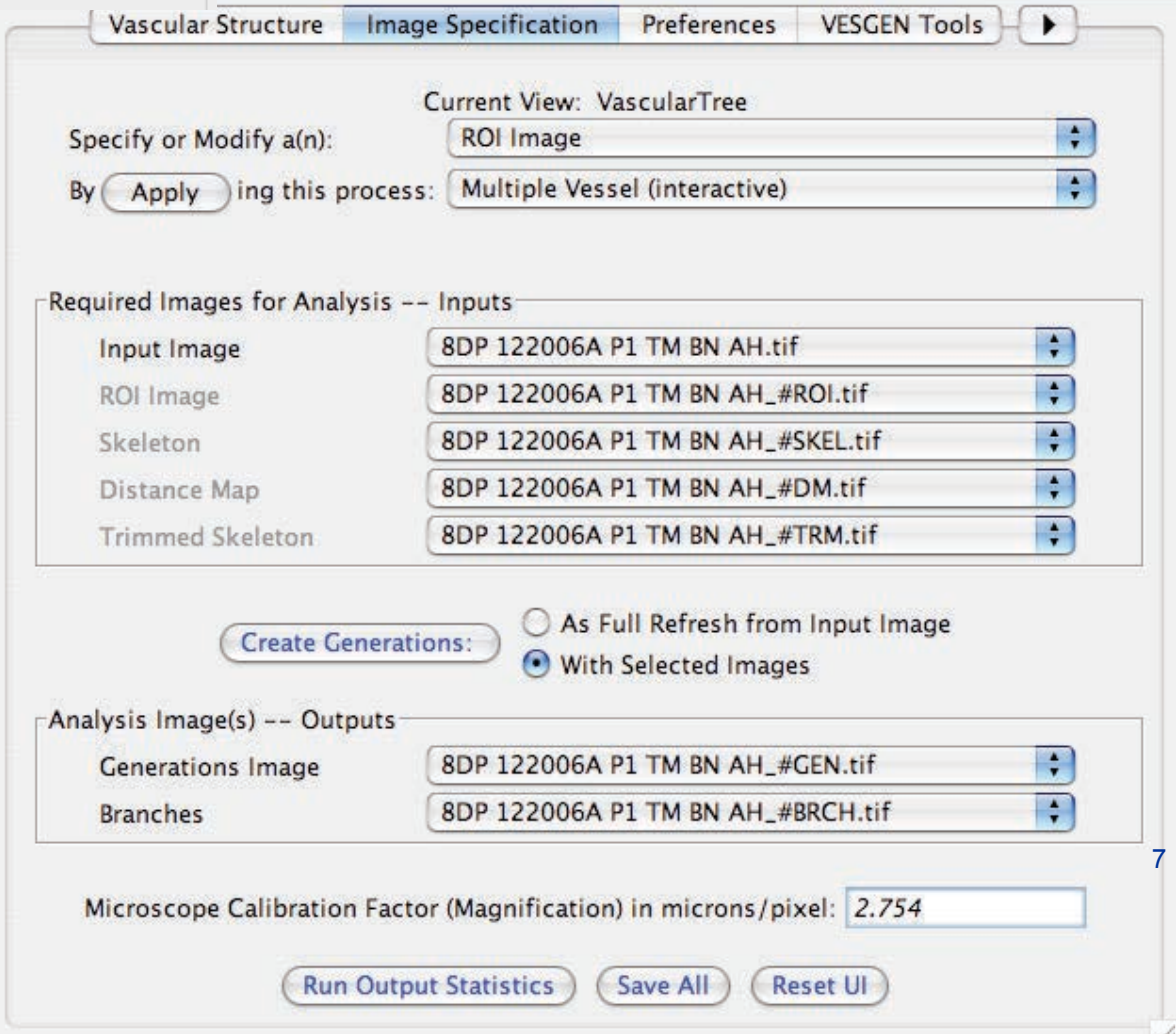
VESGEN Patent Pending



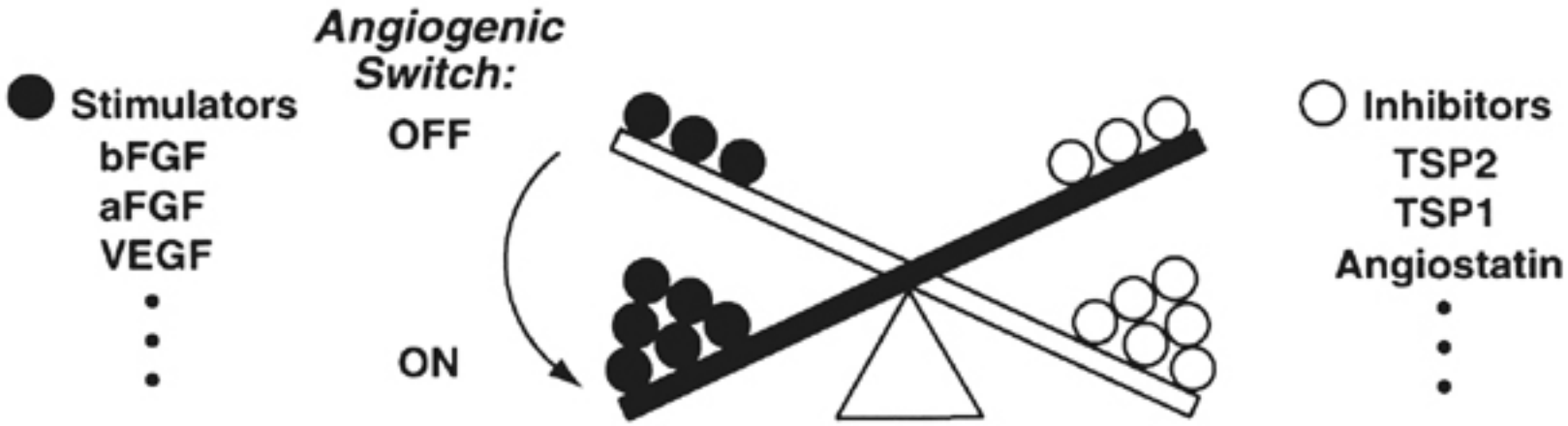
Panel to specify vessel type

Main panel

- Image specification
- Algorithm selection
- Process initiation



Dynamic Balance Hypothesis



adapted from Hanahan and Folkman, *Cell* 86(3):353-64 (1996)

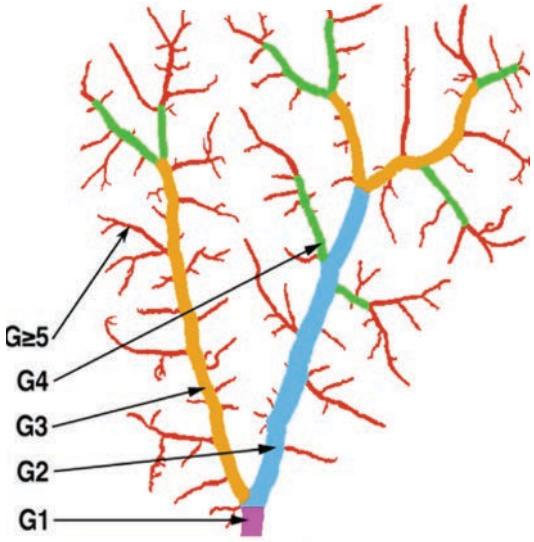
Long-Term Translational and Basic Research Hypothesis

Vascular patterning provides integrative, insightful read-out of dominant molecular regulators in complex signaling pathways of angiogenesis and microvascular remodeling

Fractal-Based VESsel GENeration Analysis (VESGEN) Software

Fractal Dimension, D_f
Vessel Number Density, N_v
Vessel Length Density, L_v
Vessel Diameter, D_v
Branchpoint + Endpoint Densities, Br_v+E_v

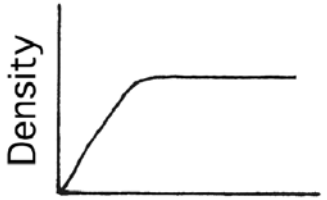
VESGEN Hypothesis: *'Fingerprint'* or *'Signature'* Vascular Pattern As Integrative Readout of Complex Signaling



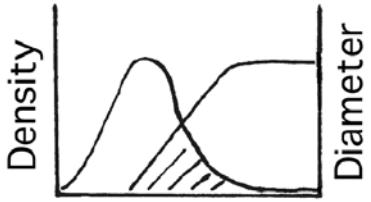
The form of an object is a 'diagram of forces'

- D' Arcy Thompson

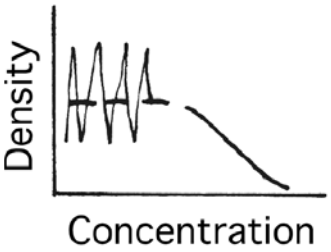
bFGF as Simple Stimulator
Arterio Thromb Vasc Biol 20 (2000)



VEGF as Complexity Factor
Microvascular Research 72 (2006)

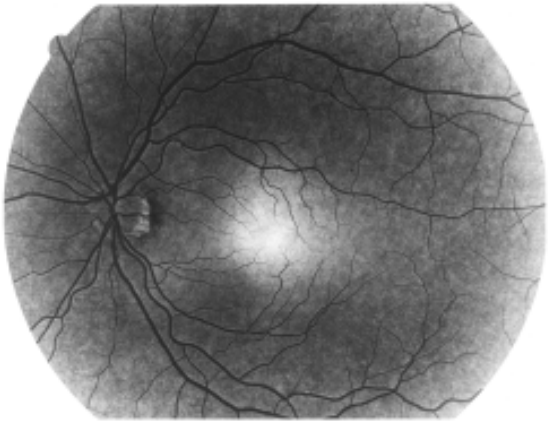


TGF-β1 as Simple Inhibitor but Complex Potentiator
Microvascular Research 59 (2000)

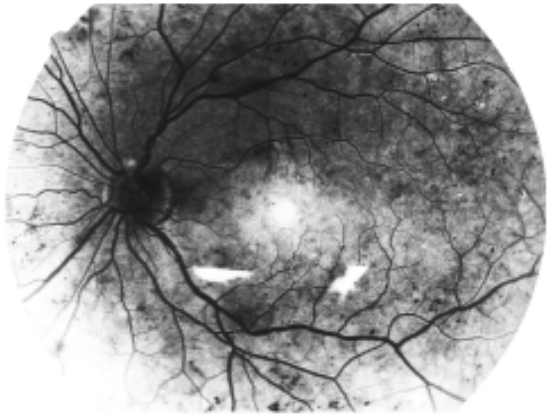


Progression of Diabetic Retinopathy by Clinical Fluorescein Angiography

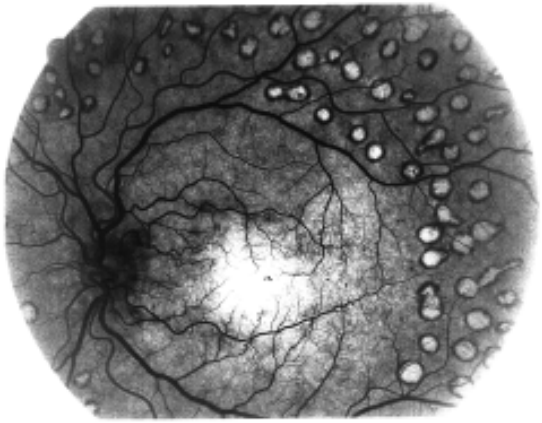
Normal



NPDR

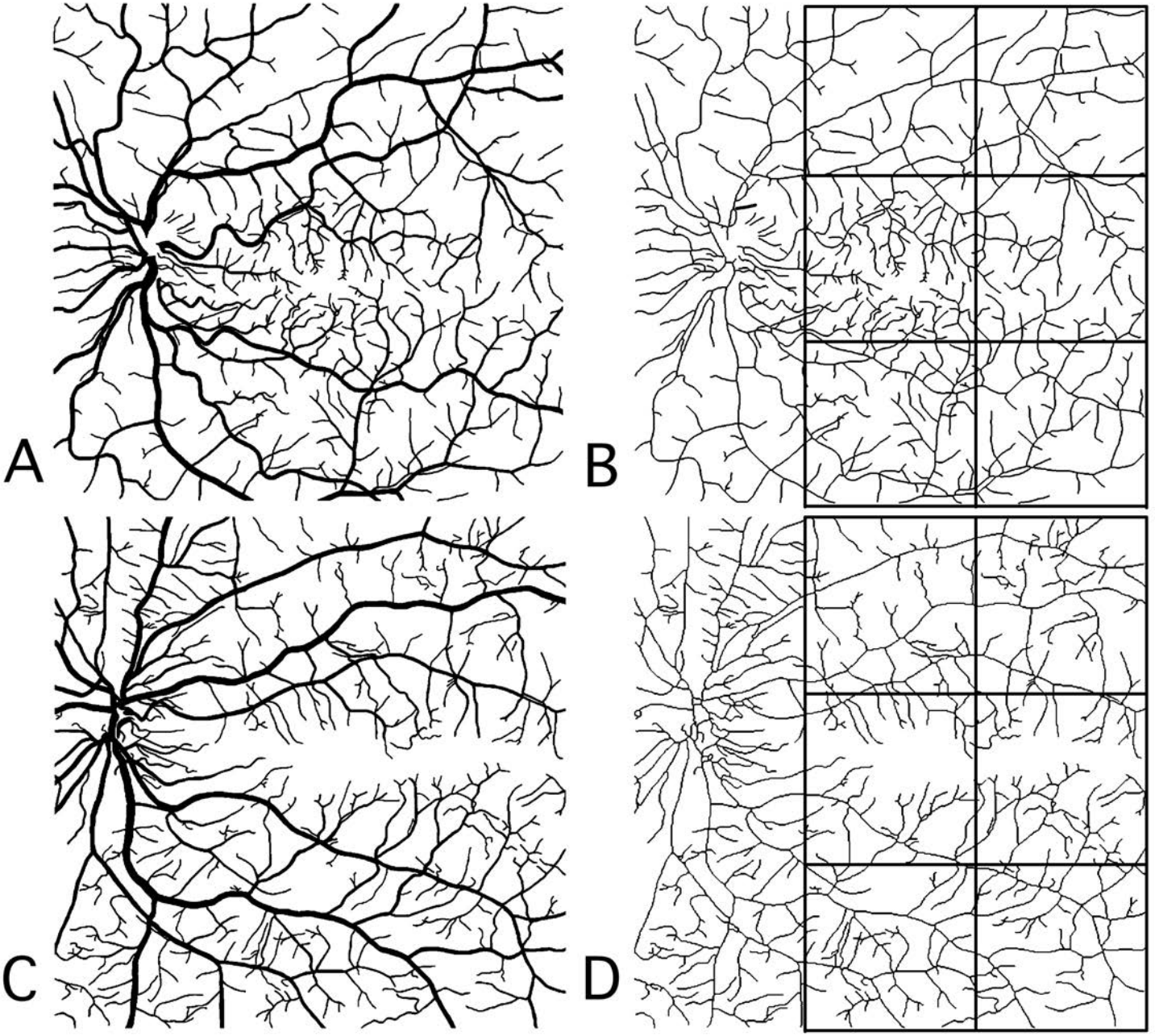


PDR
after Laser Ablation



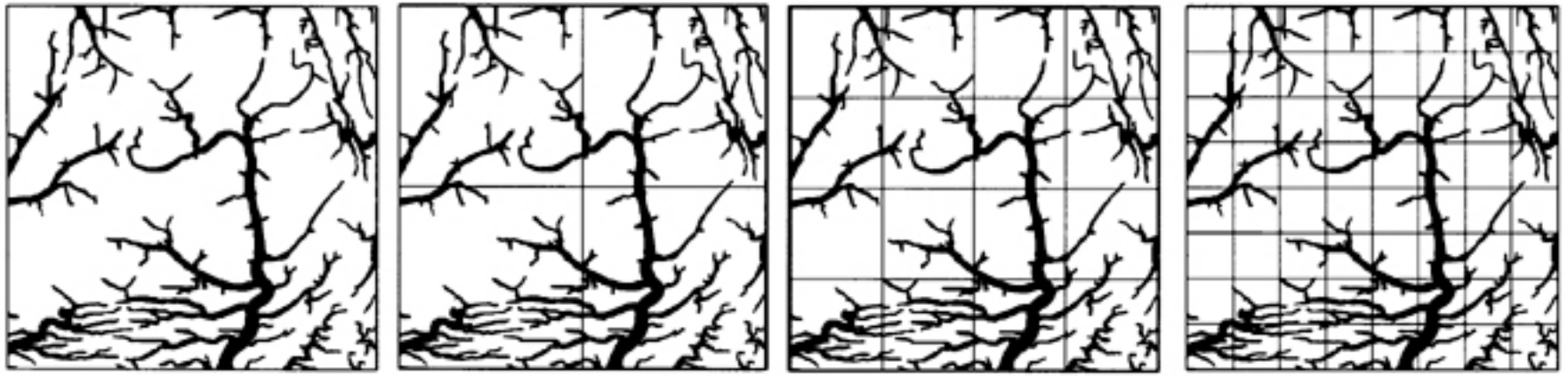
EARLY ***Vascular*** Nonproliferative DR (NPDR)

LATE ***Vascular*** Proliferative DR (PDR)

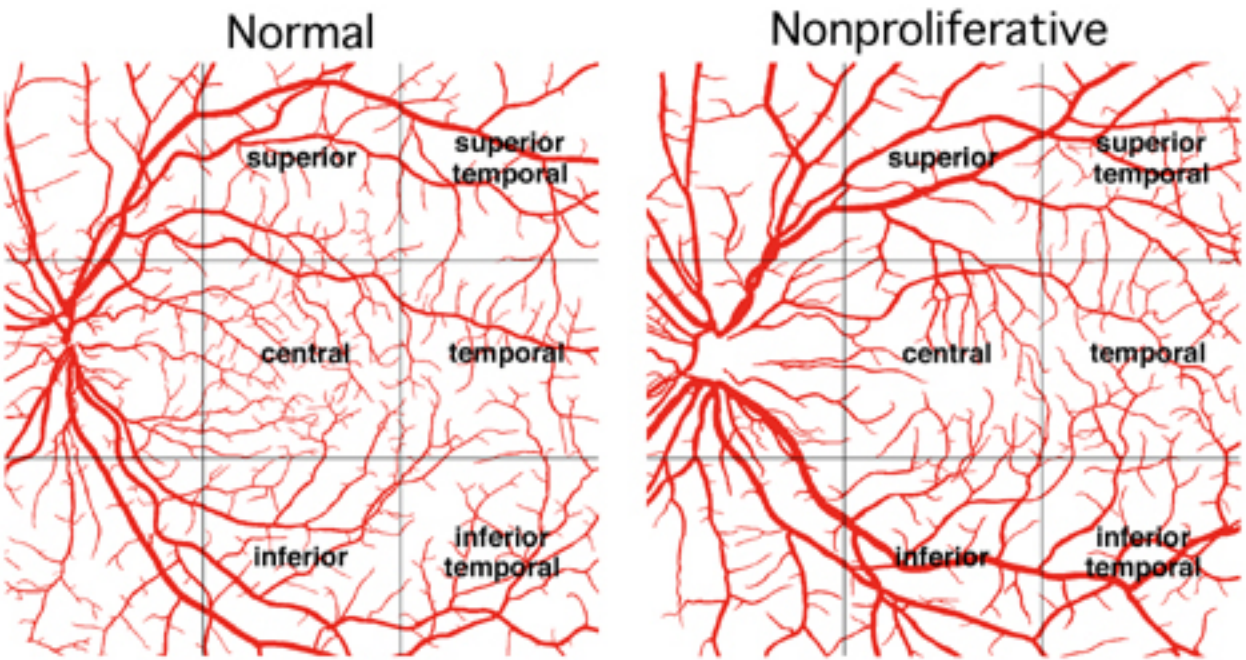


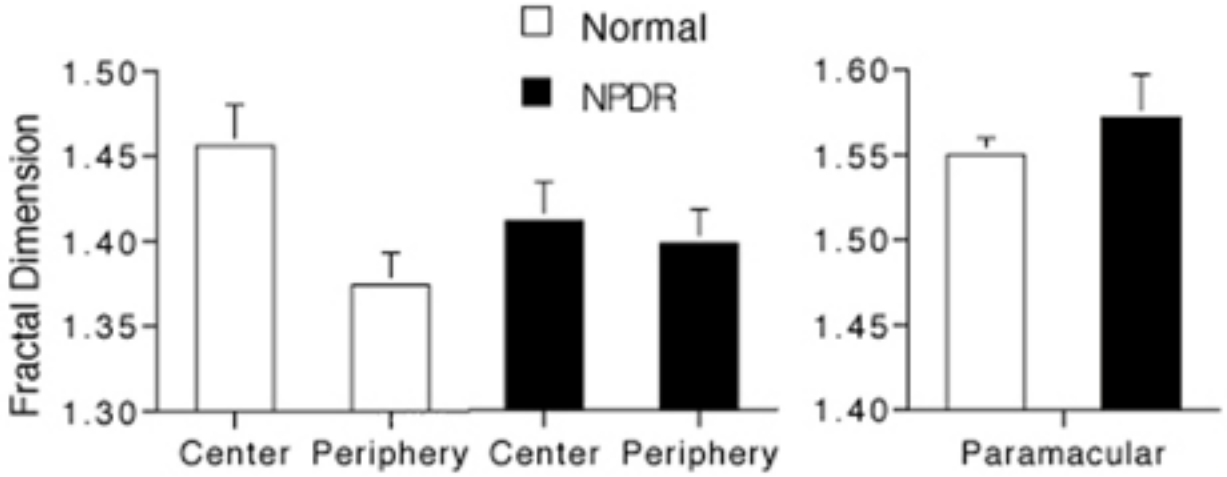
Current Eye Research 24(4):274-280(2002)

Fractal Dimension (D_f) by Box-Counting (▬ , ■)

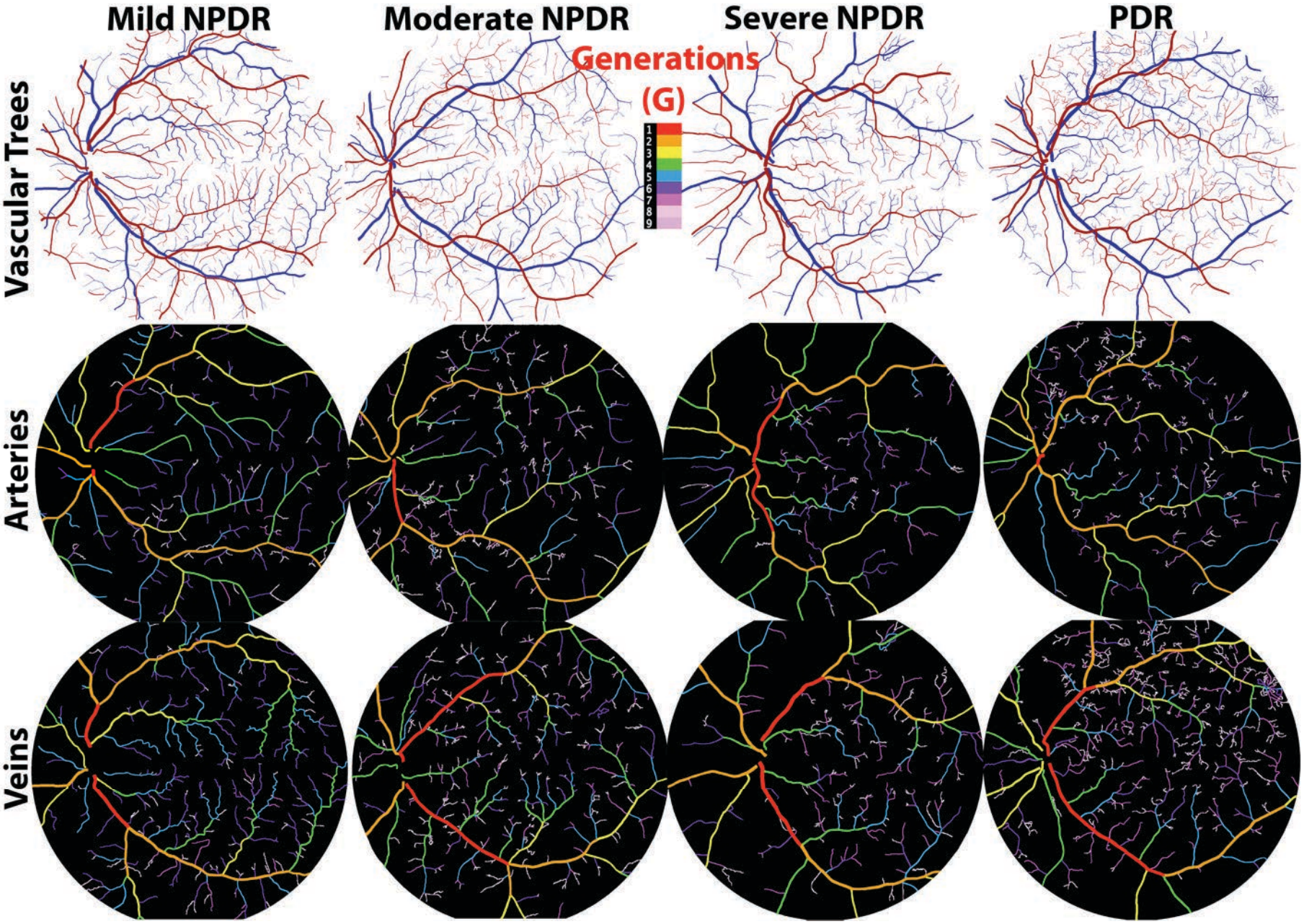


Vascular Pattern in the Human Retina Is Altered in Early-Stage Diabetes





Mapping of Progressive Diabetic Retinopathy by VESGEN

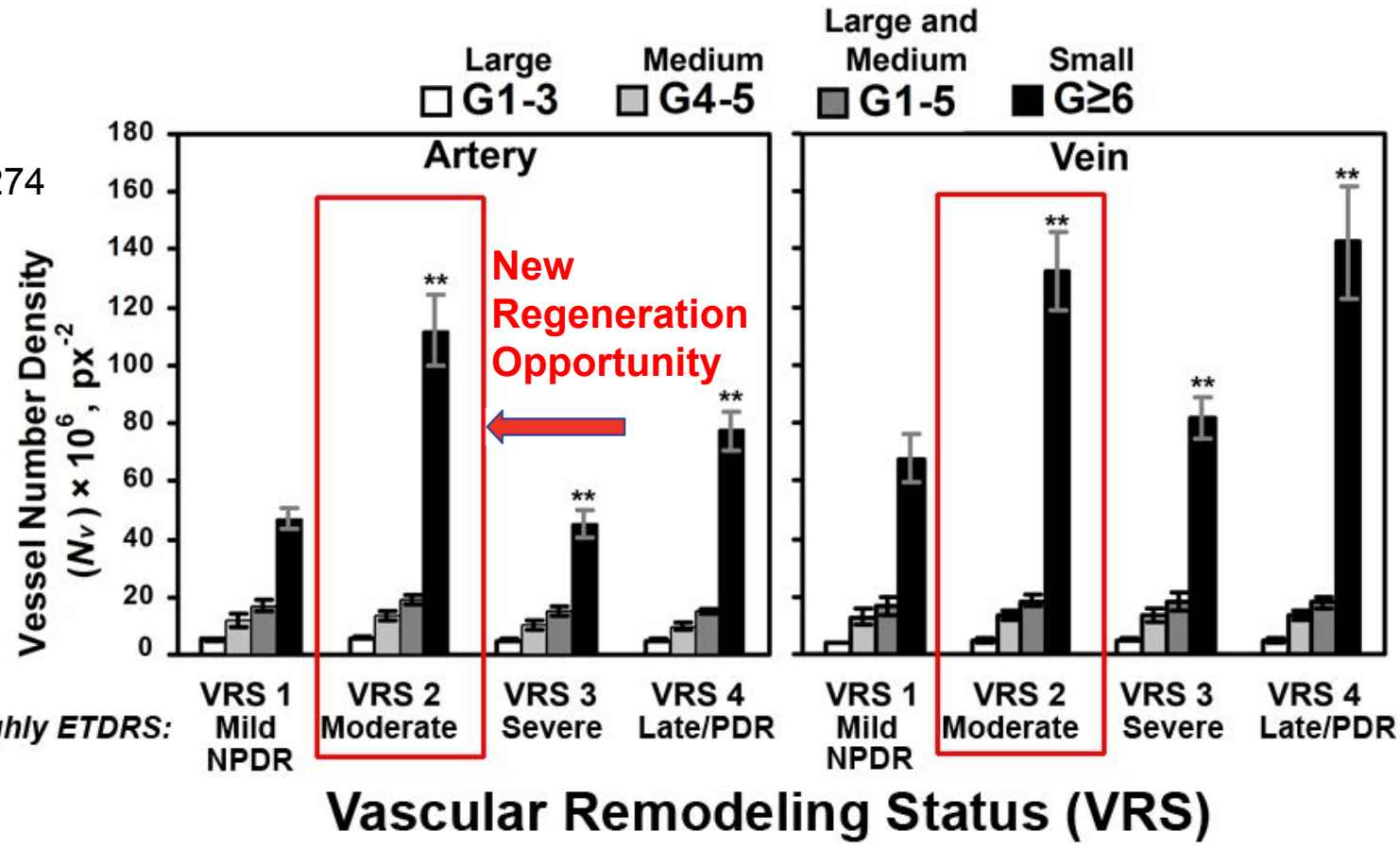


P Parsons-Wingerter, K Radhakrishnan, M B Vickerman, P K Kaiser, *IOVS* 51(1):498-507 (2010) and in progress

Angiogenesis Oscillates with Vascular Dropout during Progression of Diabetic Retinopathy

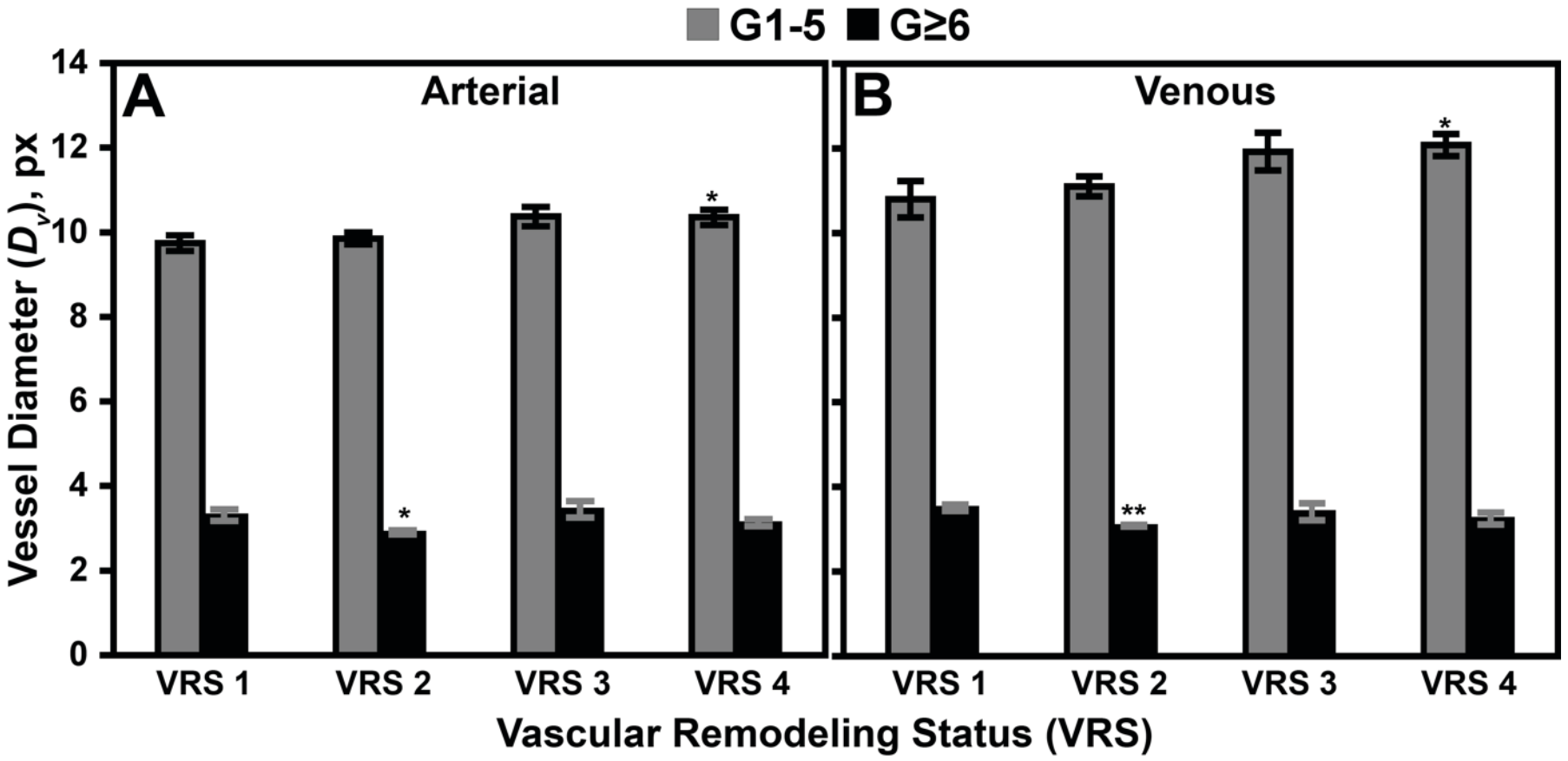
Normal Retina

Curr Eye Res 24(4):274



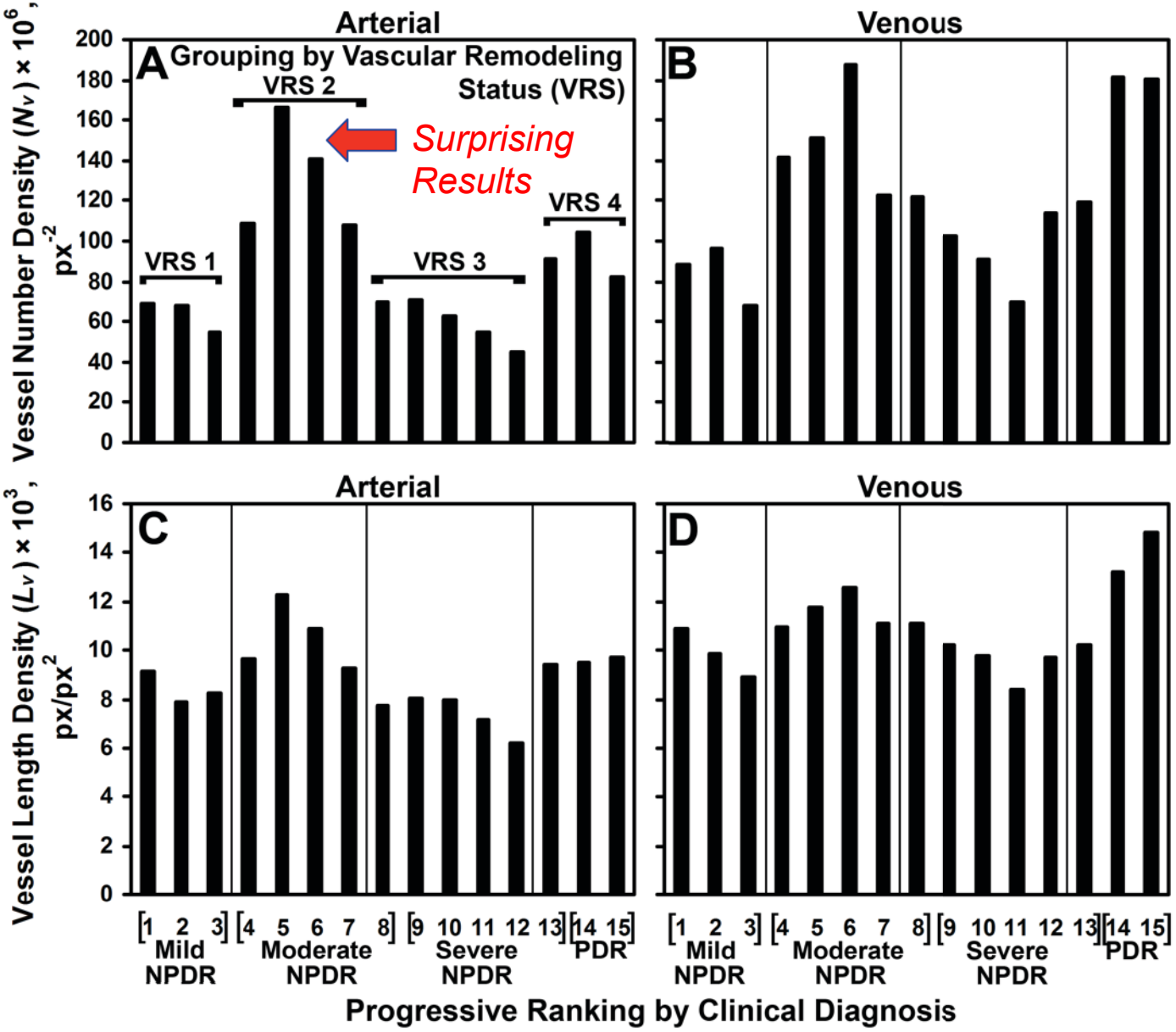
P Parsons-Wingerter, K Radhakrishnan, M B Vickerman, P K Kaiser, *IOVS* 51(1):498-507 (2010) and in progress

Slight Trend toward Increasing Diameter of Larger Vessels during Progression of Diabetic Retinopathy



Parsons, Radhakrishnan, Vickerman & Kaiser, *Investigative Ophthalmology & Visual Science* 51(1):498-507 (2010)

Grouping by Vascular Remodeling Status (VRS)



P Parsons-Wingerter, K Radhakrishnan, M B Vickerman, P K Kaiser, *IOVS* 51(1):498-507 (2010) and in progress

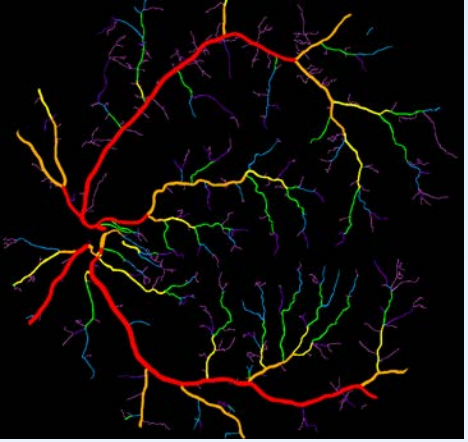
Conclusions on Novel Vascular Disease Biomarkers during Progression of Diabetic Retinopathy

New, surprising discovery on early-stage angiogenesis during moderate NPDR: ***Does the retina retain the capacity to regenerate itself?***

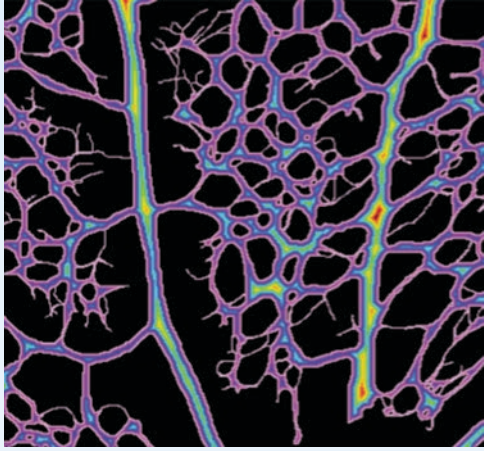
VESGEN as Research Discovery Tool

Are results important for early-stage regeneration in other inflammatory diseases such as diabetic nephropathy and tumors?

C. Blood Vessels



VESGEN



Human Retina

Mouse Retina

Vascular Pattern as Informative Biomarker and Integrative Readout of Complex Signaling Pathways for Angiogenesis, Lymphangiogenesis and Other Microvascular Remodeling

C. Blood Vessels

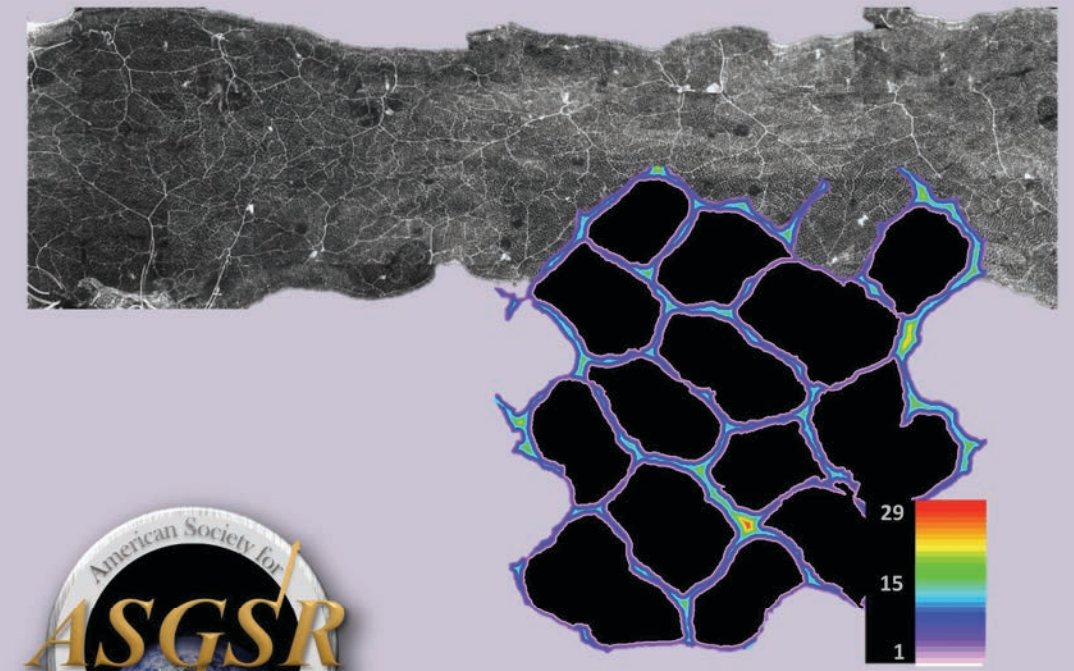
Glenn Research Center at Lewis Field

VESGEN Patent Pending

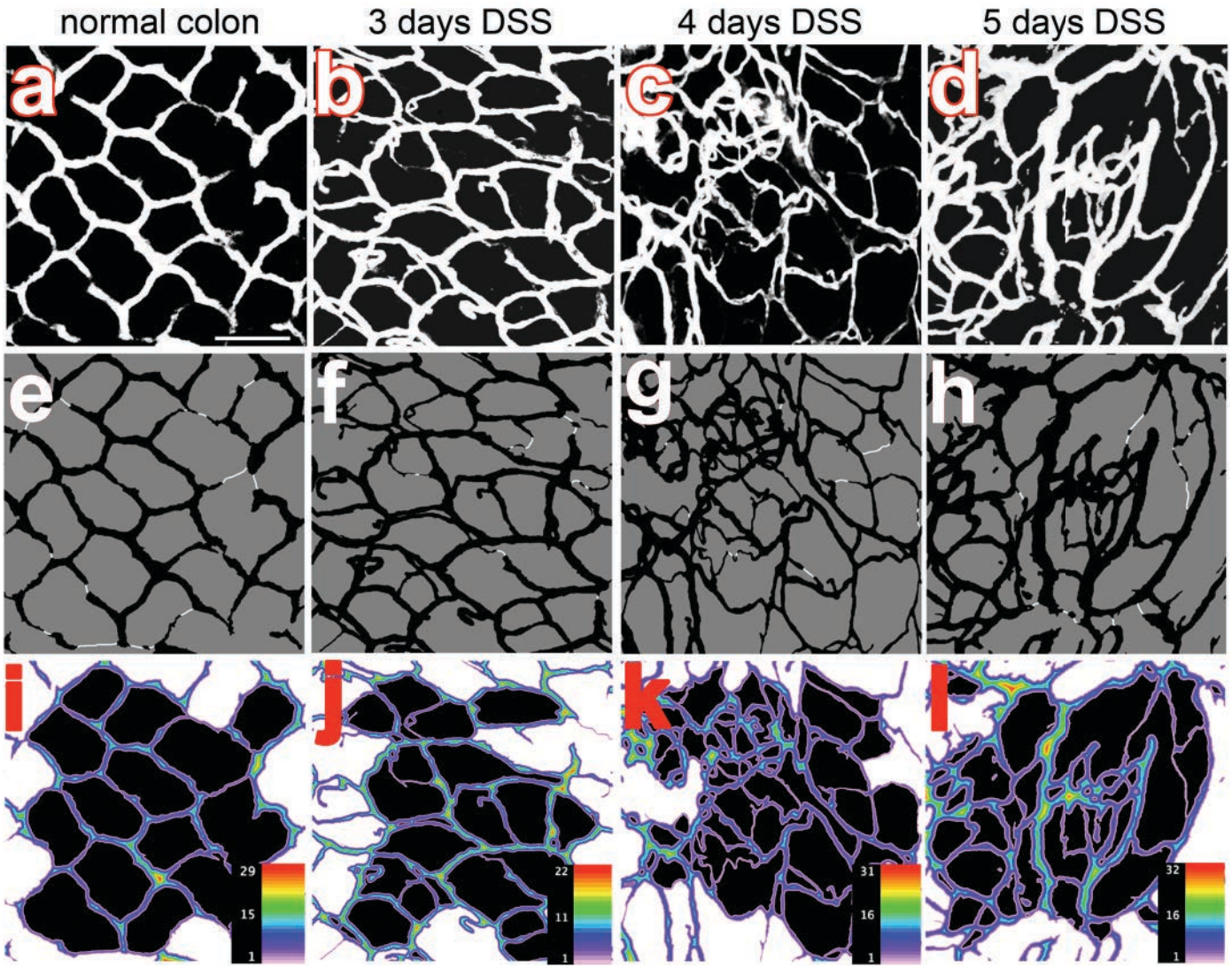
Volume 26, Number 2 October 2012

Gravitational and Space Biology

Publication of the American Society for Gravitational and Space Research



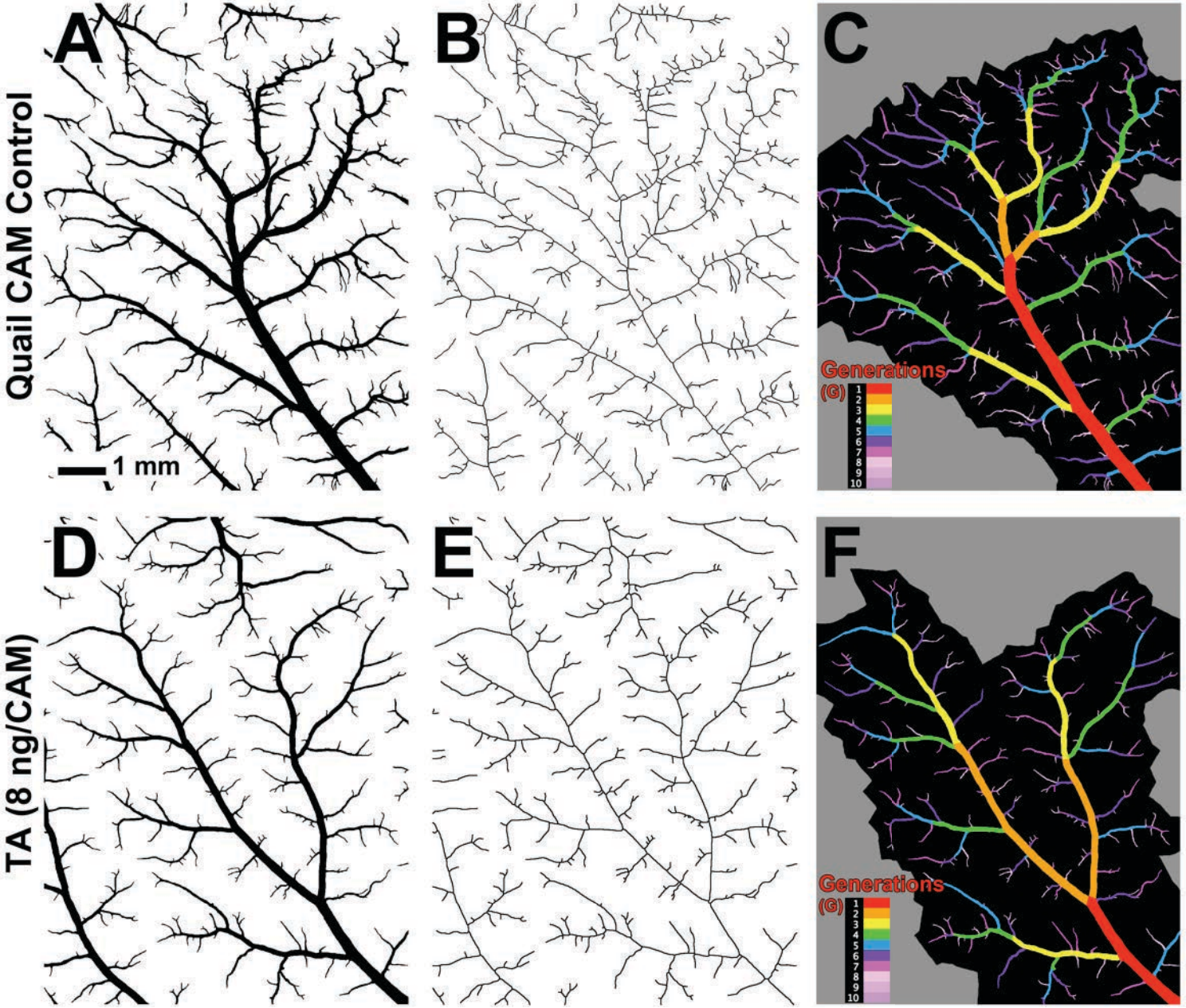
VESGEN mapping of vascular networks with GI inflammatory progression in experimental mouse DSS model



P Parsons and H-C Reinecker, accepted to *Grav Space Biology*

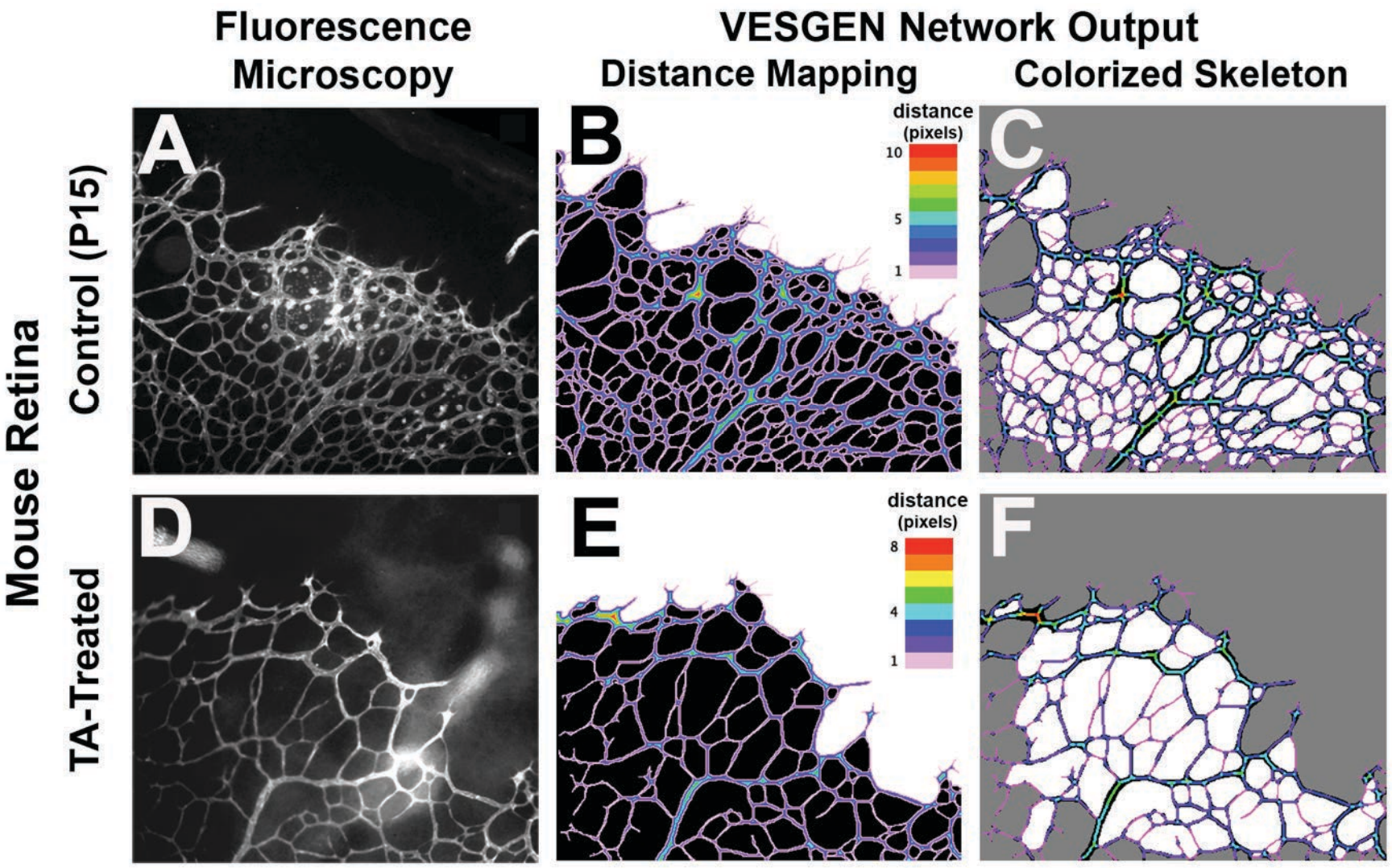
VESGEN Patent Pending

Triamcinolone Acetonide (TA) Steroid Treatment in CAM Vascular Tree



Reviewed in *Anatomical Record* 2009; *Investigative Ophthalmology & Visual Science* 2008

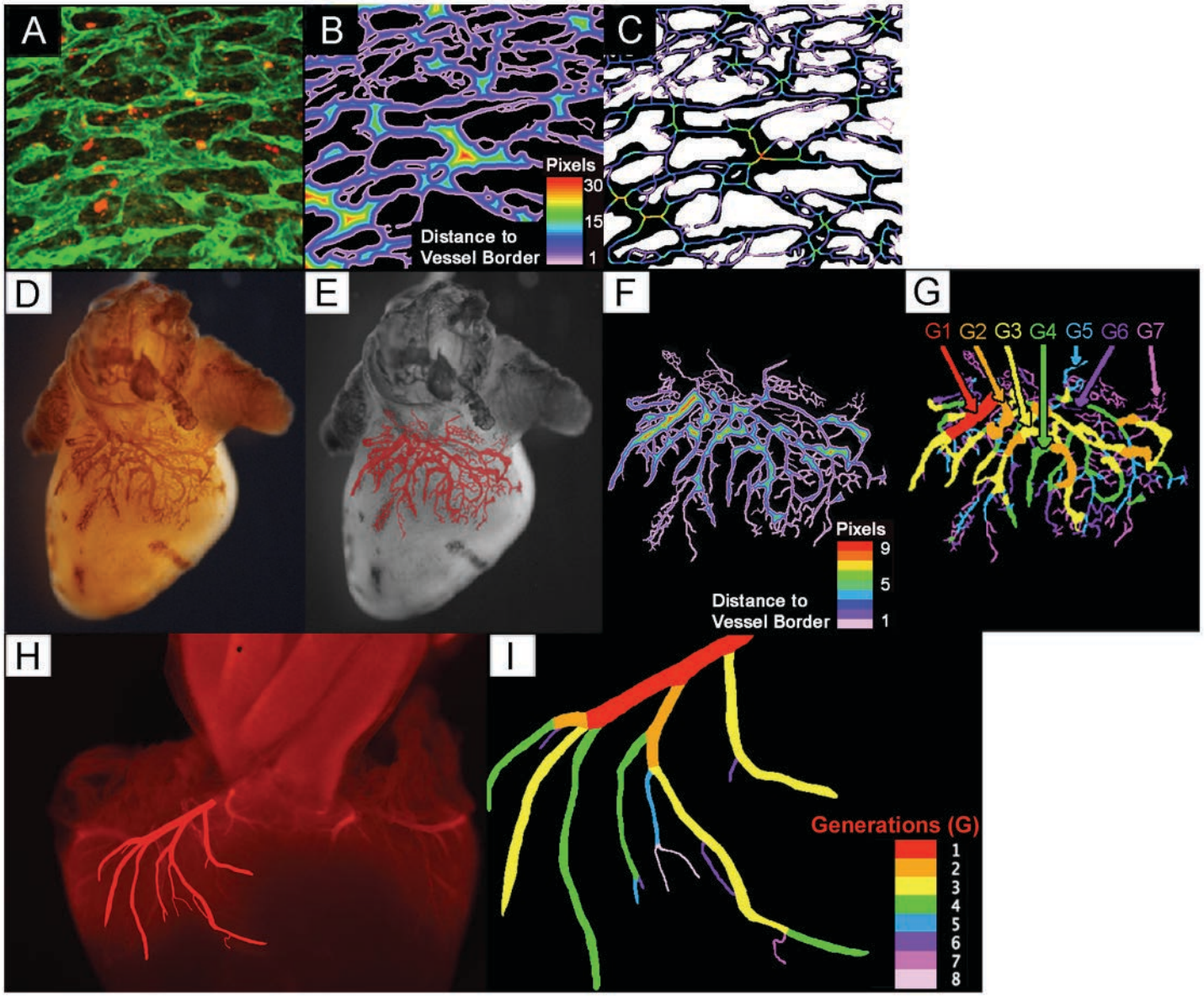
Vascular Networks in Transgenic Mouse Retina



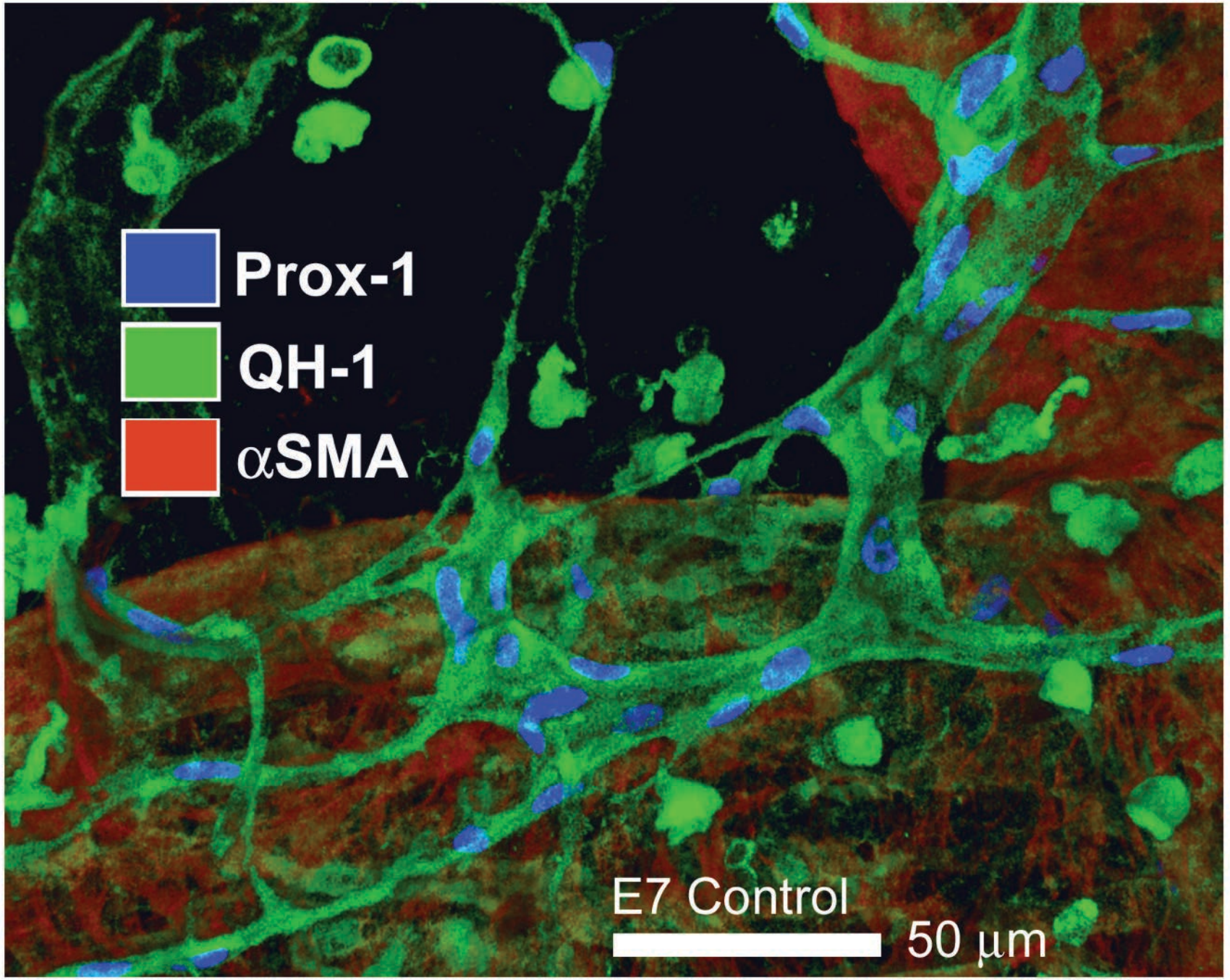
with J Sears & Q Ebrahem (Cole Eye Institute), from Vickerman et al, *Anatomical Record A* 292(3), 2009

VESGEN Patent Pending

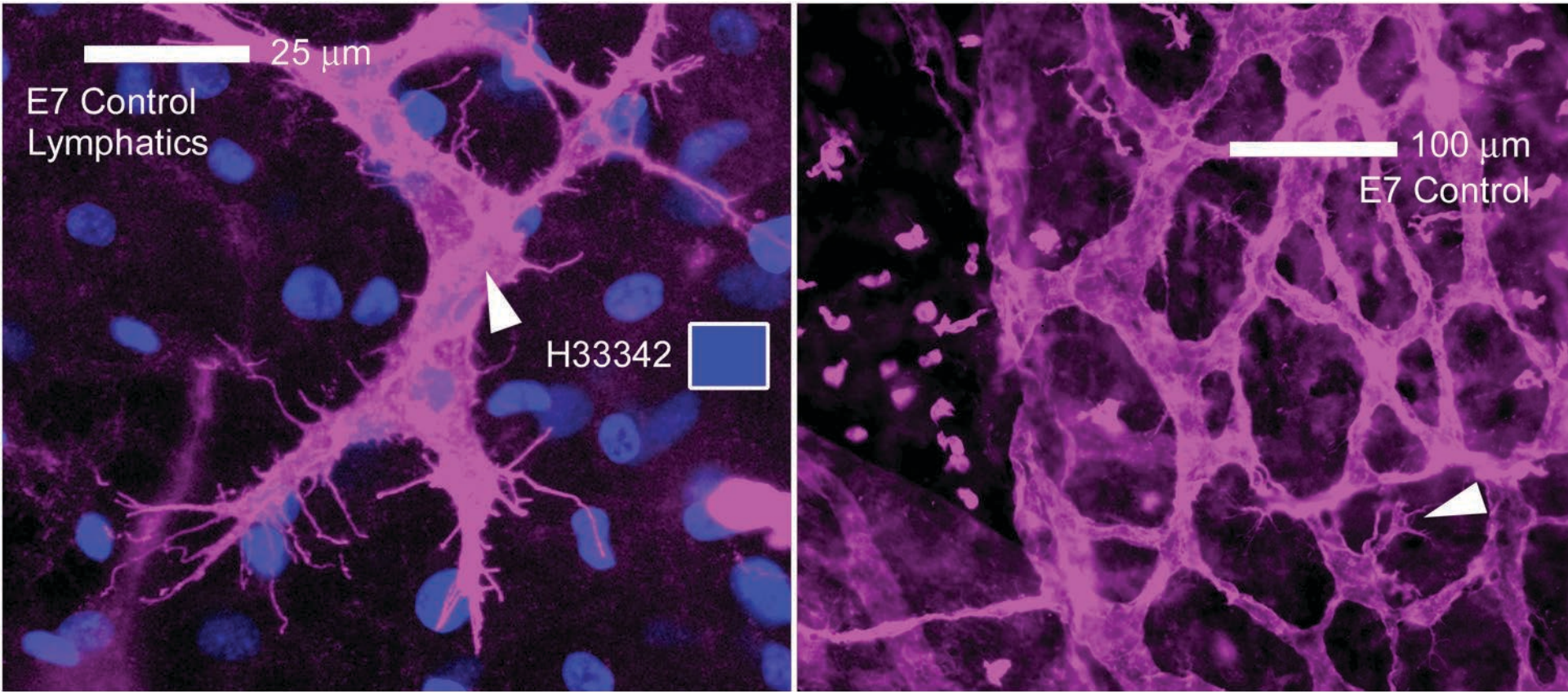
Coronary Vessel Network-to-Tree Transitions

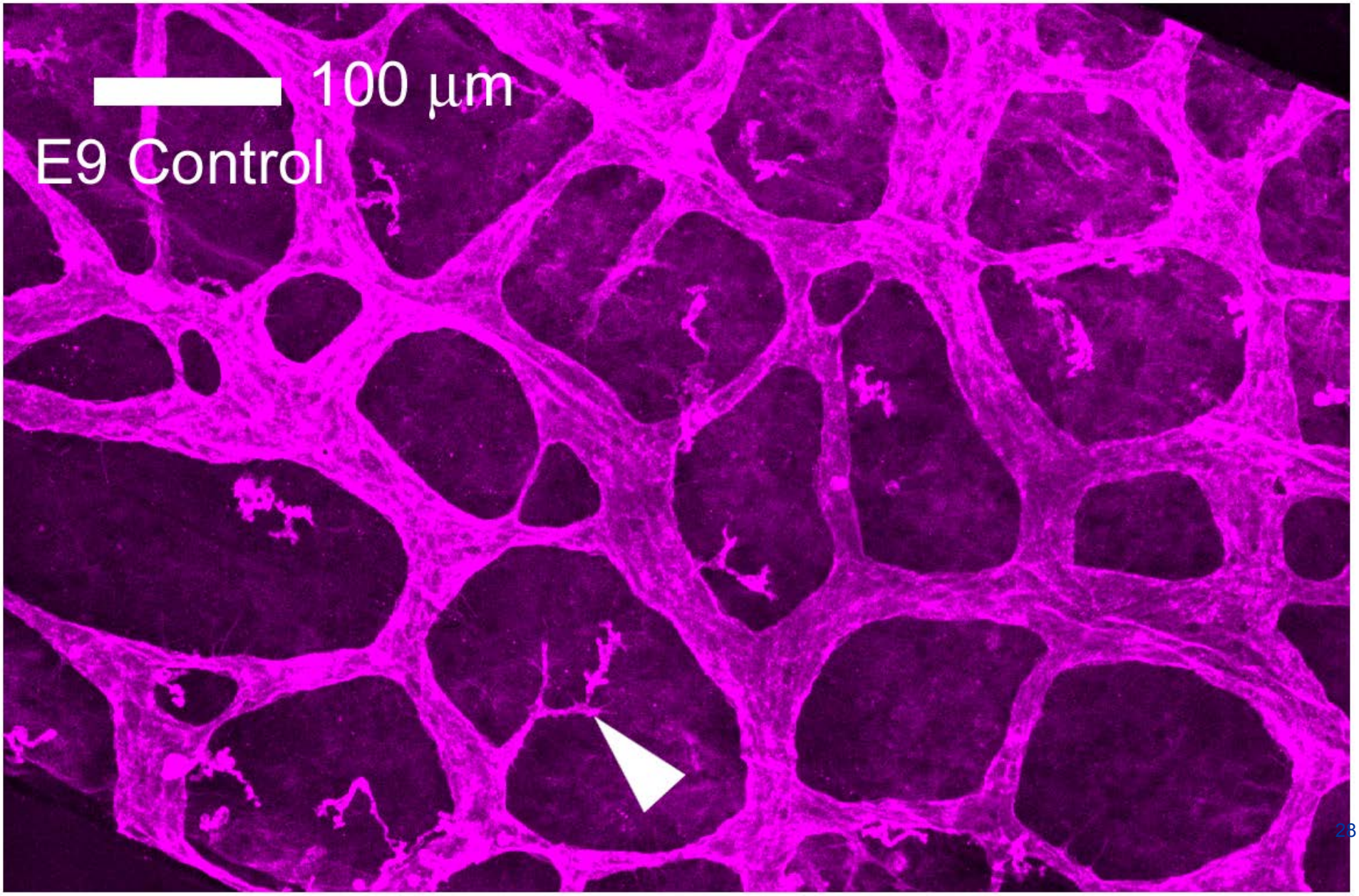


Vickerman et al, VESGEN Review, *Anatomical Record A* 292(3), 2009

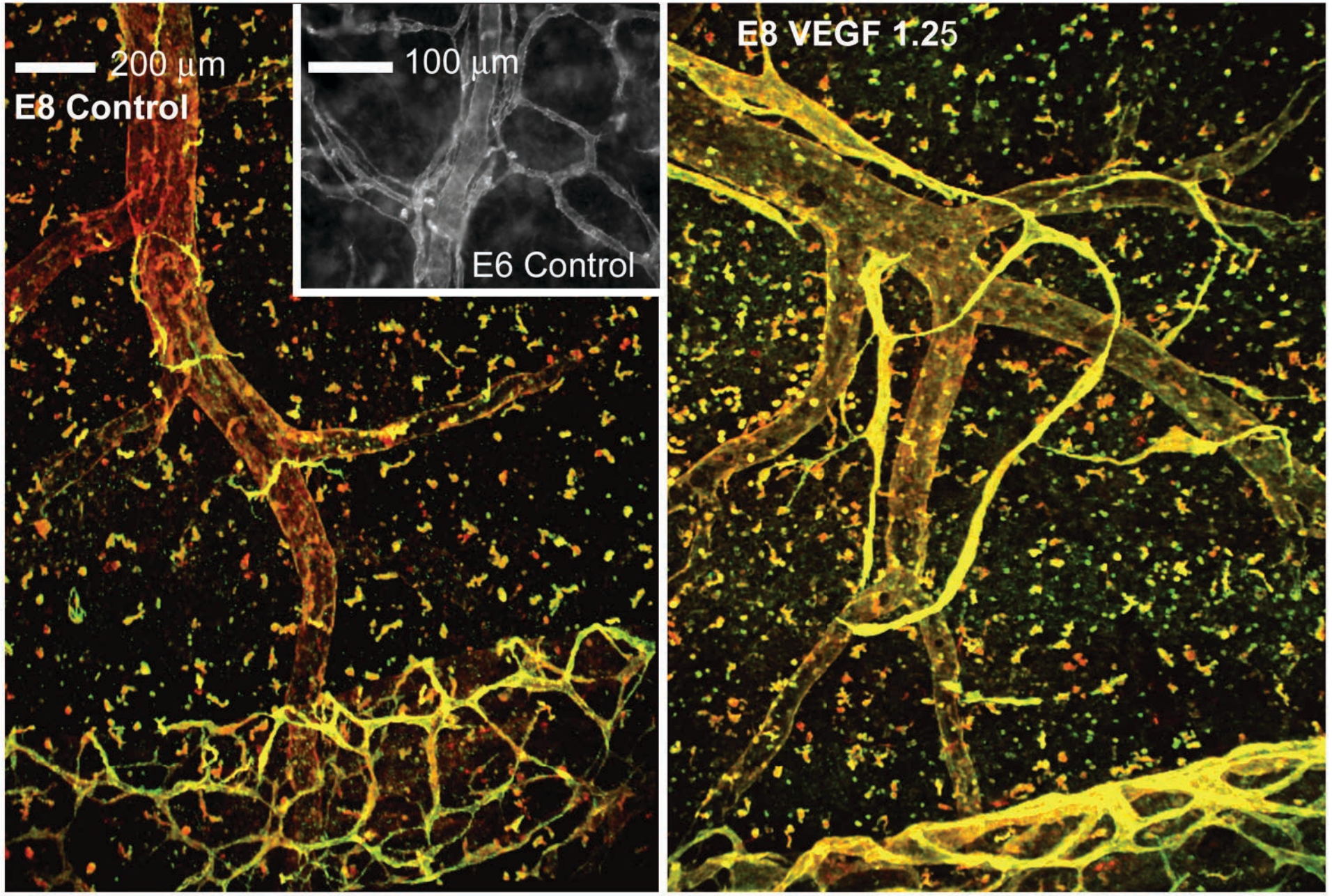

Prox-1
QH-1
αSMA
E7 Control
50 μm

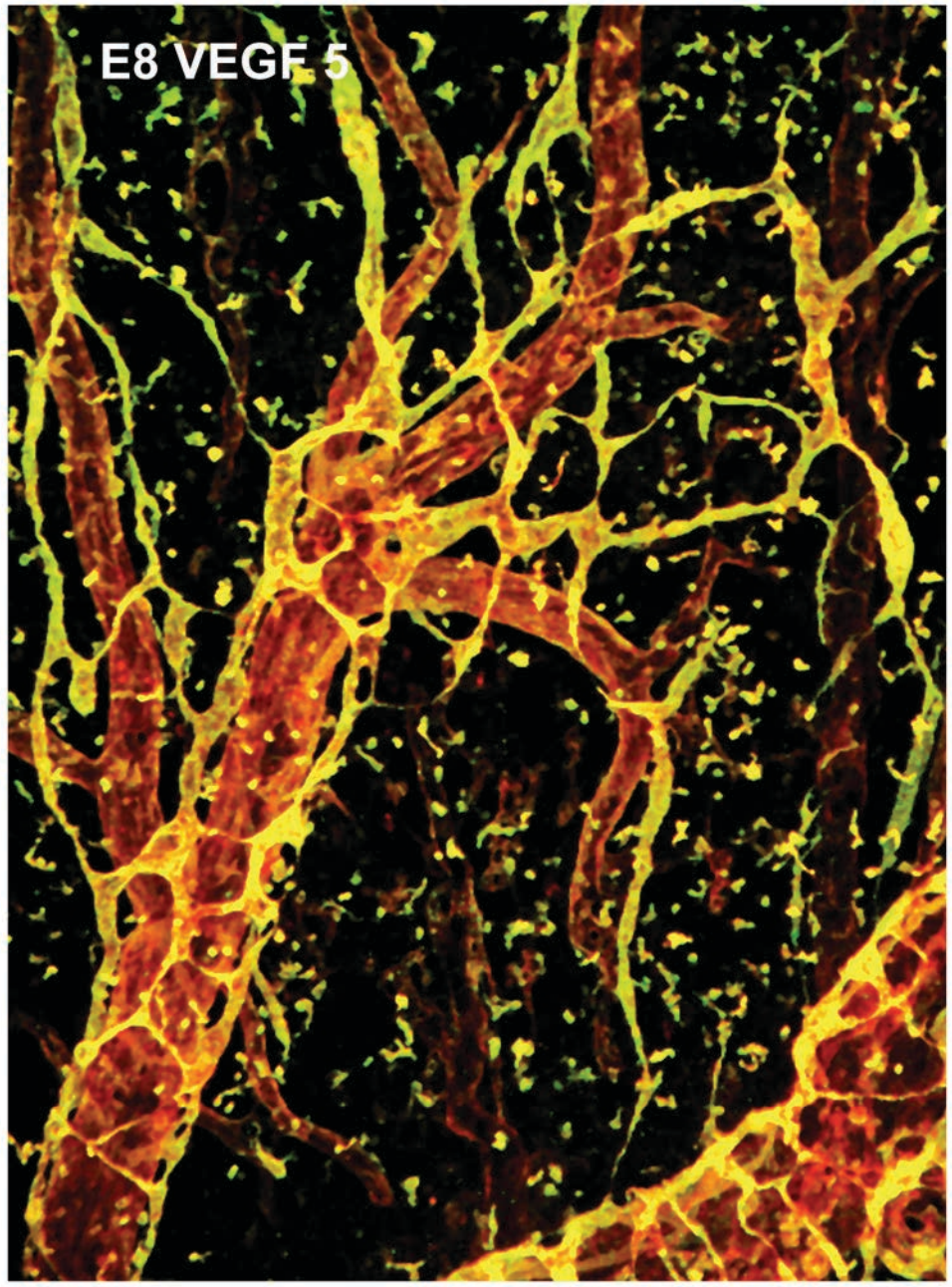
Lymphangiogenic Sprouting: By Filopodial Guidance?

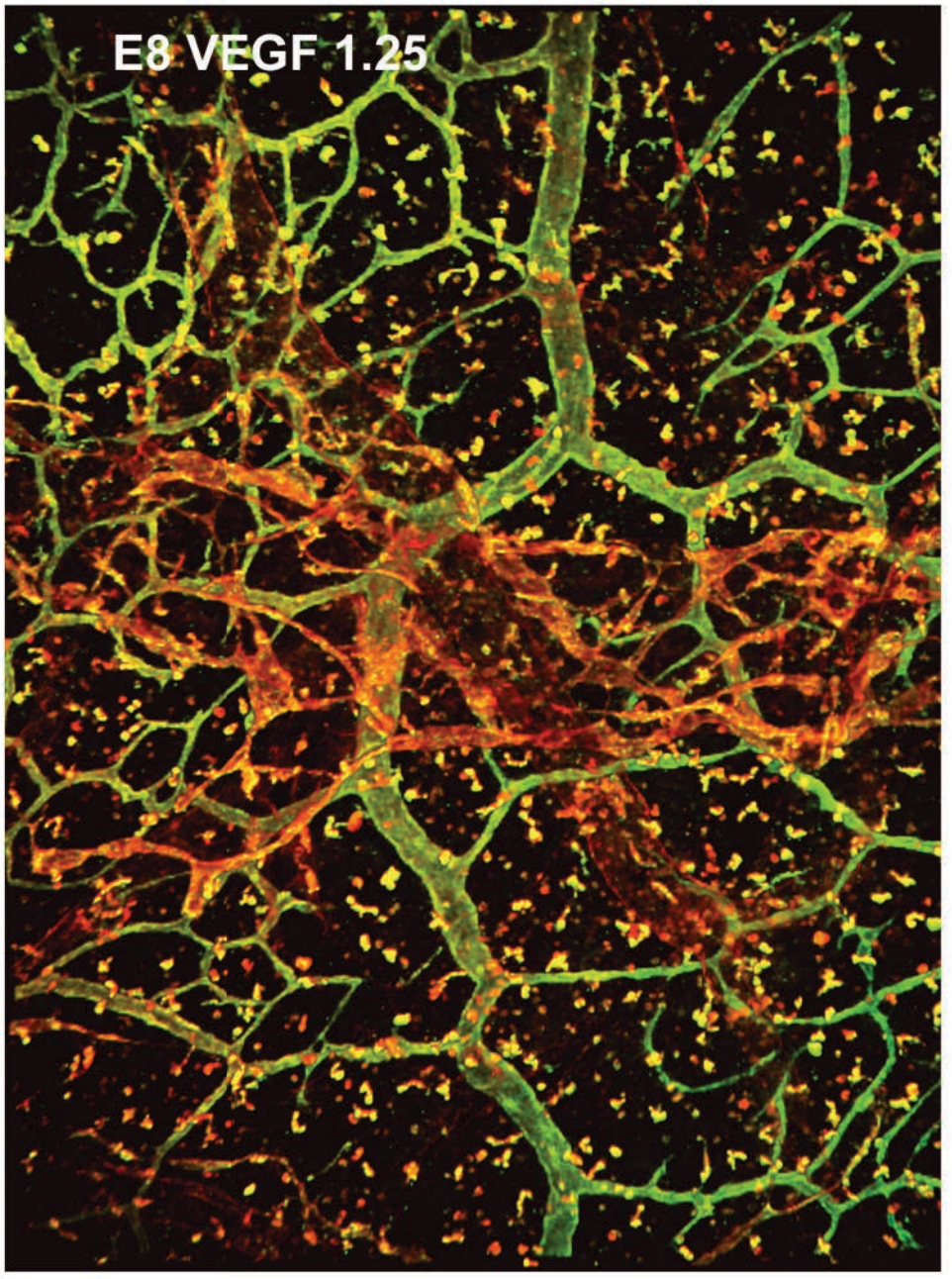


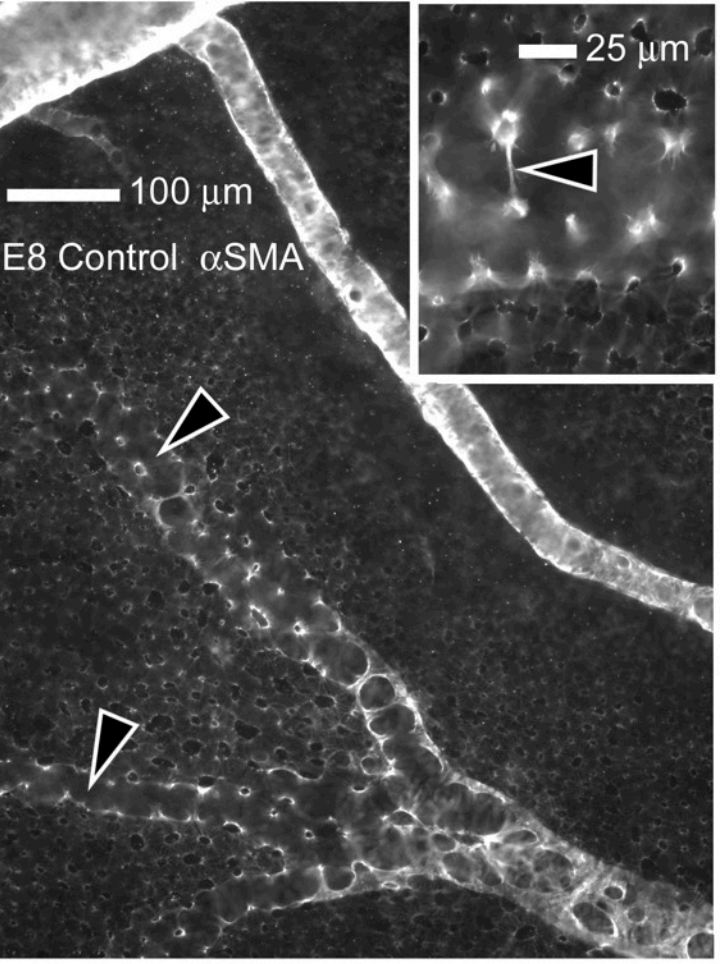


Parsons et al, *Microvascular Research* 167(1):193-211(2005)

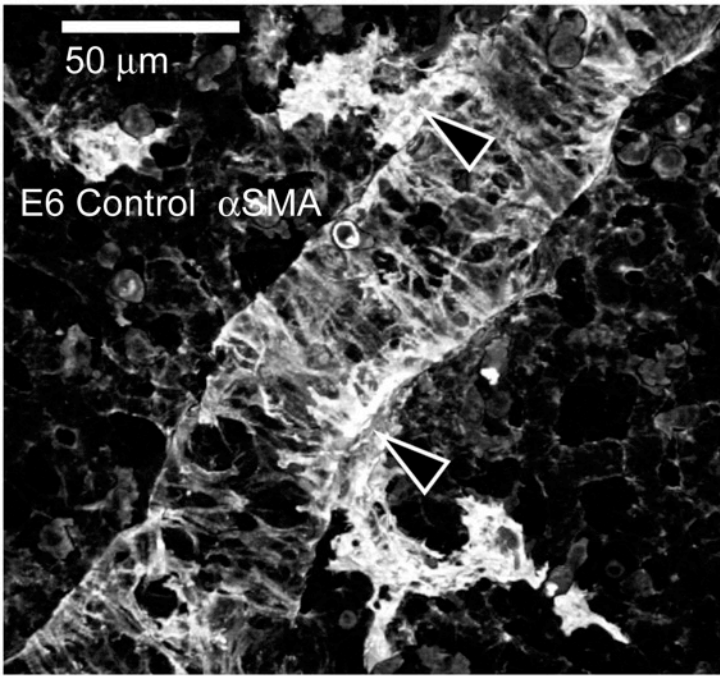

200 µm
E8 Control
100 µm
E6 Control
E8 VEGF 1.25

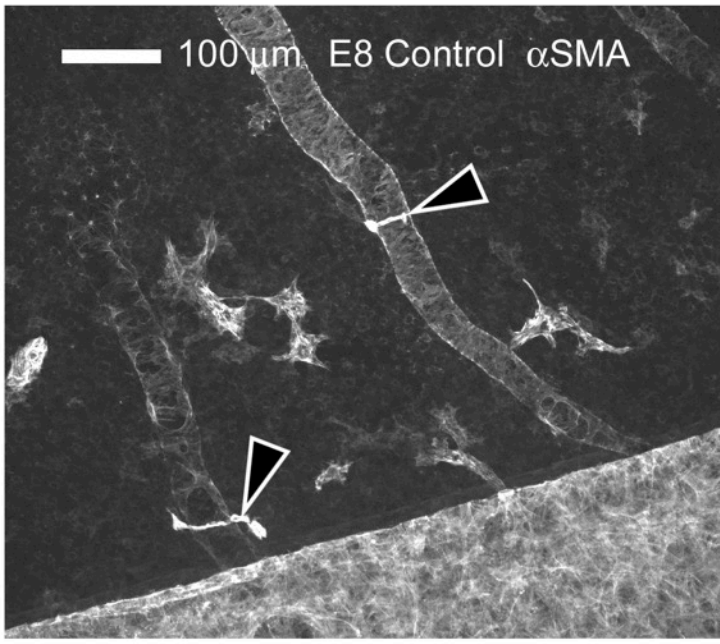

E8 VEGF 5

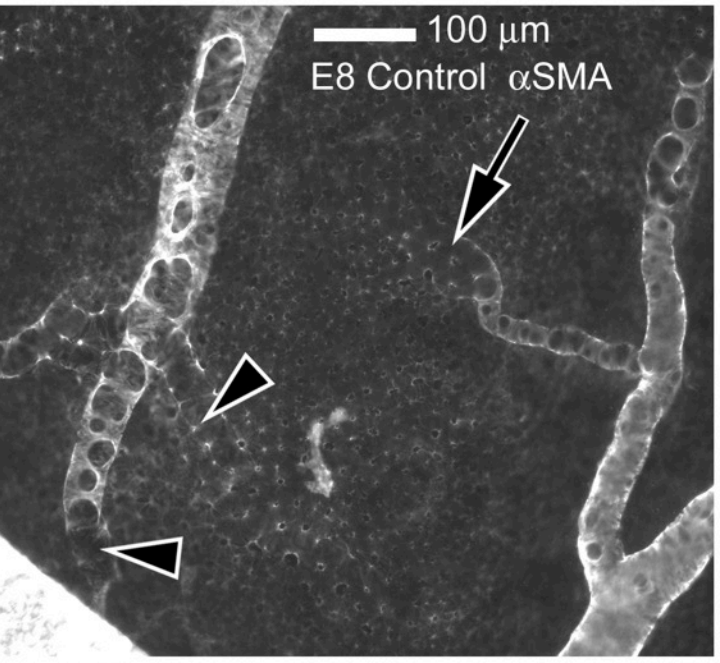

E8 VEGF 1.25

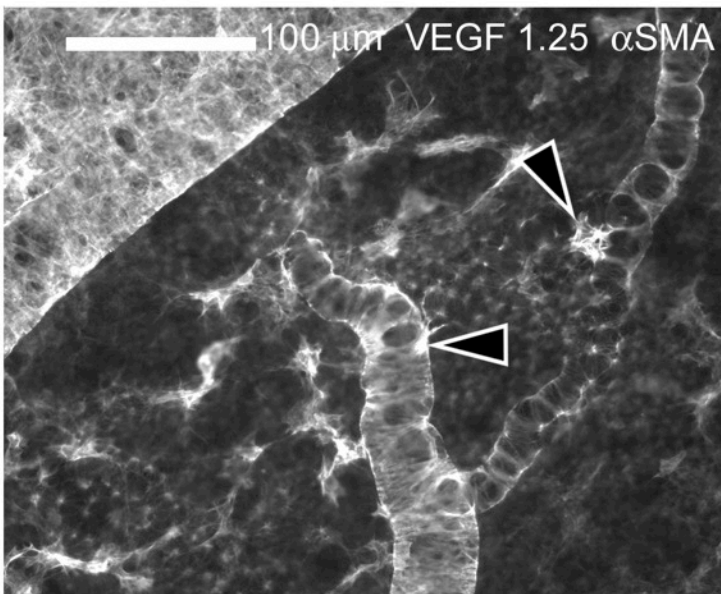

100 µm
E8 Control αSMA

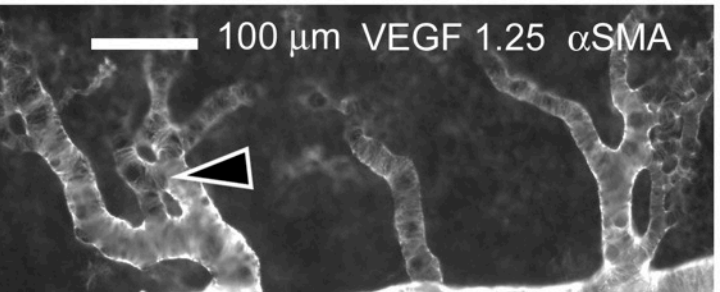
25 µm


50 µm
E6 Control αSMA


100 µm E8 Control αSMA


100 µm
E8 Control αSMA


100 µm VEGF 1.25 αSMA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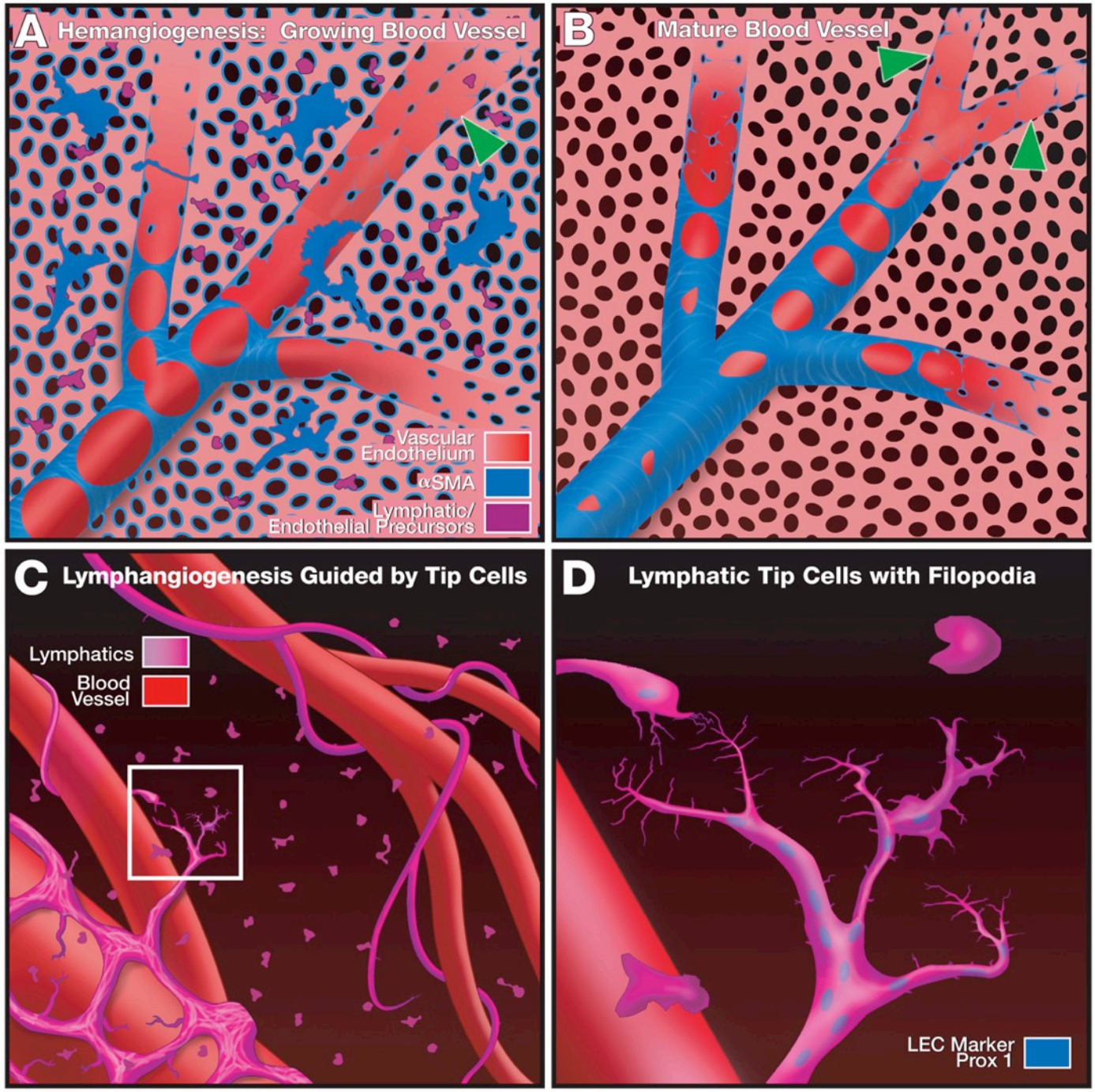
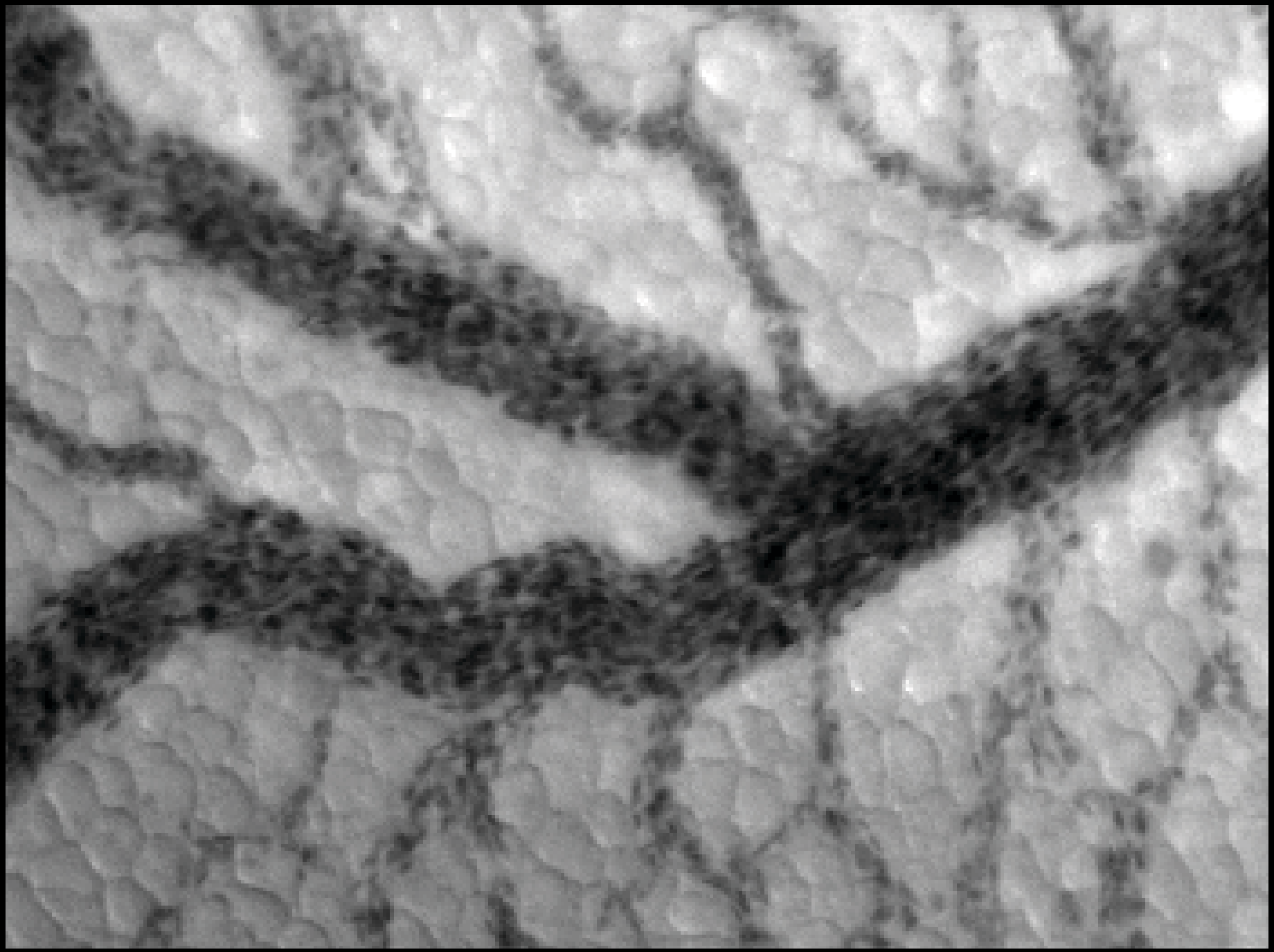


Fig. 7 Parsons-Wingerter *et al.*



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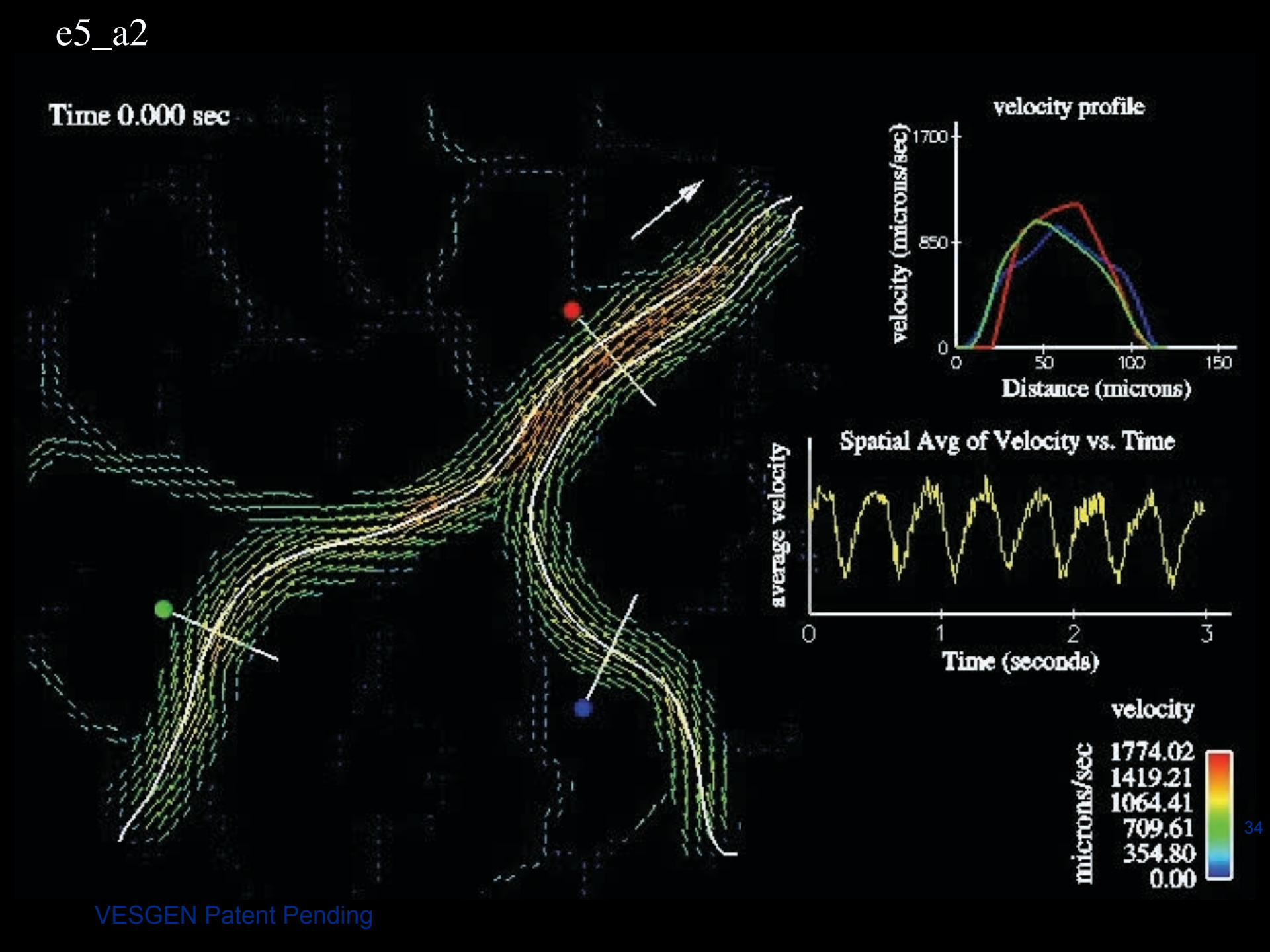

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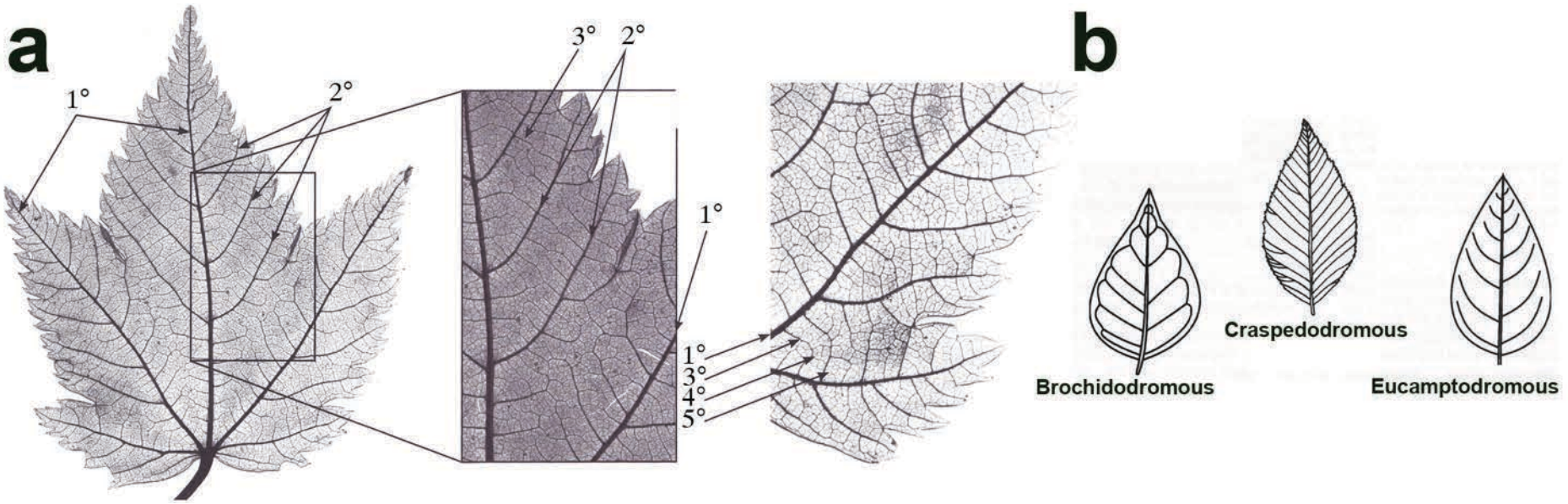
Taxonomic/Phylogenetic Identifiers

Botanical rules for leaf vascular patterning by branching order

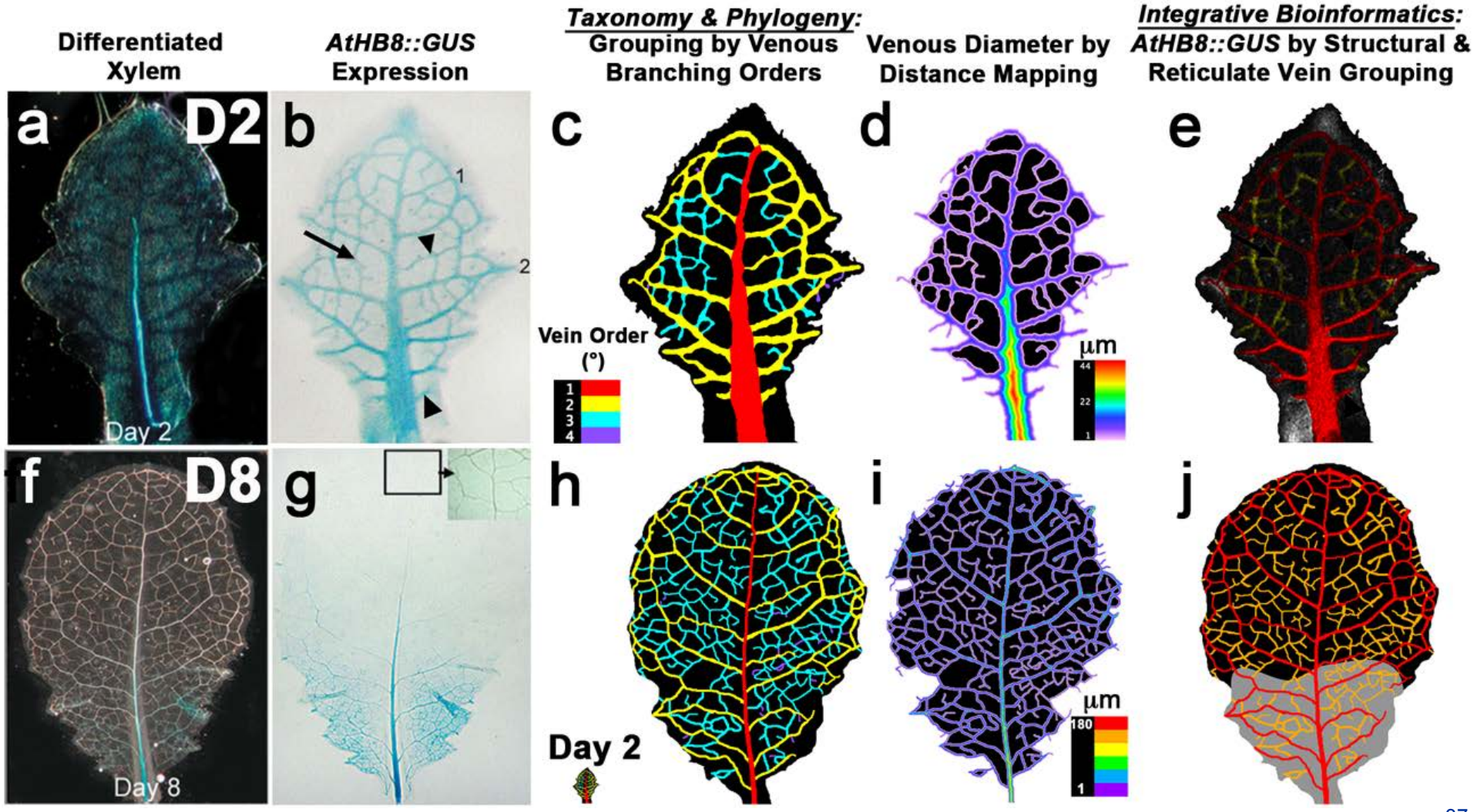


Acer argutum

[a]Ellis, Daly, Hickey et al, Manual of Leaf Architecture, 2009

[b]Roth-Nebelsick, Uhl, Mosbrugger, Kerp, Annals of Botany 887:553-566, 2001

New VESGEN analysis of leaf venation for *Arabidopsis* with first bioinformatic dimensional analysis



P Parsons, M Vickerman,A-L Paul, R Ferl submitted to ASGSR 2012 New Orleans

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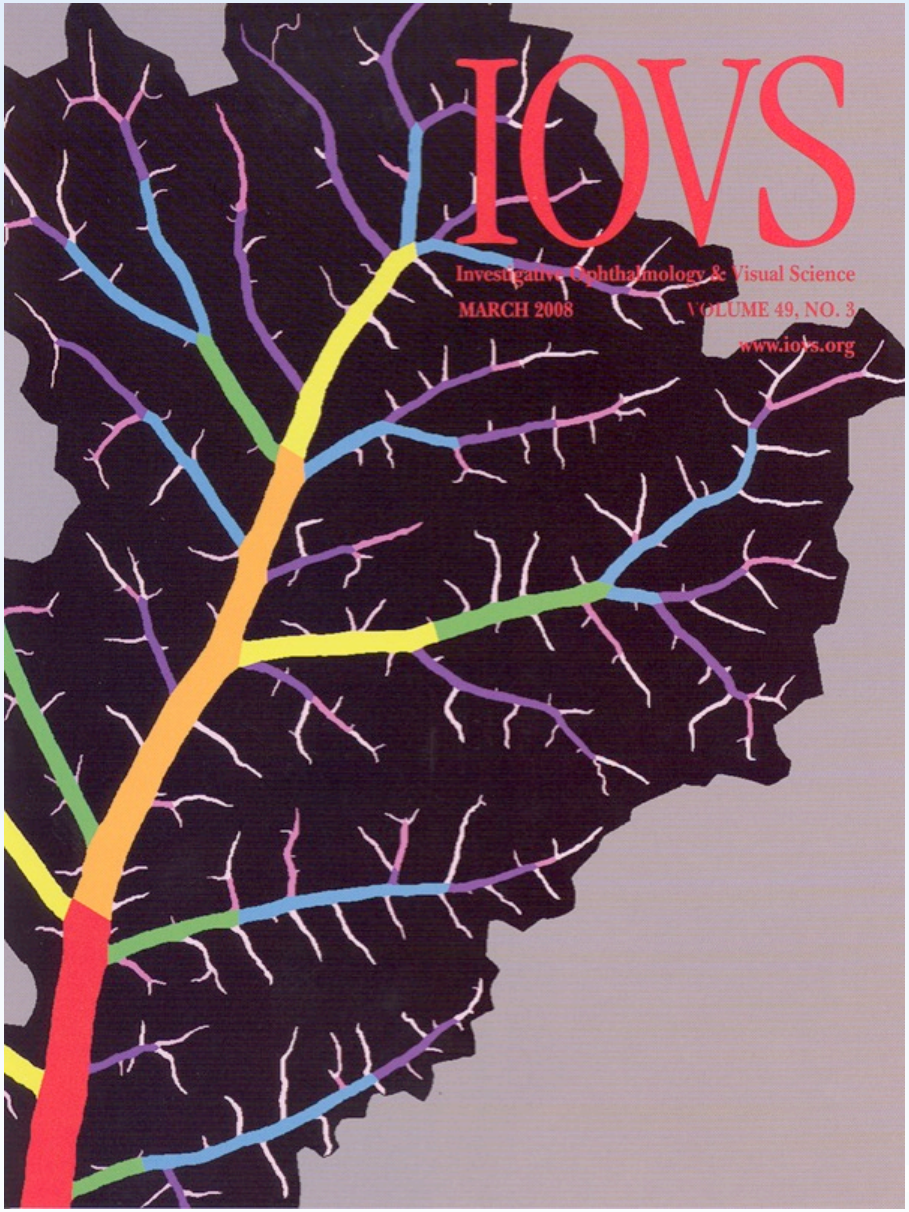

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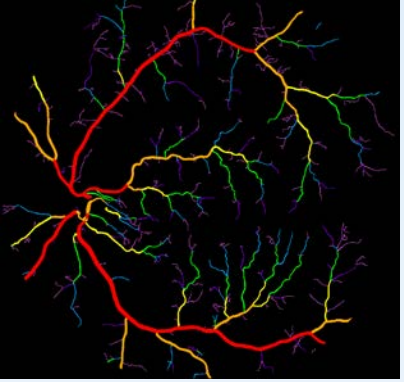
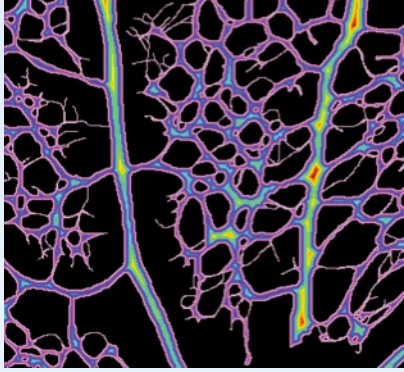
at Lewis Field



Novel Angiogenesis and Vascular Dropout Biomarkers by VESGEN



Potential New Window of Therapeutic Opportunity for Early-Stage Regenerative Treatment

Surprising Oscillation of Angiogenesis with Vascular Dropout during DR Progression

- **First demonstration of angiogenesis during Moderate NPDR**
- **New longitudinal studies with Maria Grant**

C. Blood Vessels

VESGEN Patent Pending

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C. Blood Vessels