Oscillation of Angiogenesis and Vascular Dropout in Progressive Human Vascular Disease

Vascular Pattern as Useful Read-Out of Complex Molecular Signaling

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Oscillation of Angiogenesis and Vascular Dropout in Progressive Human Vascular Disease

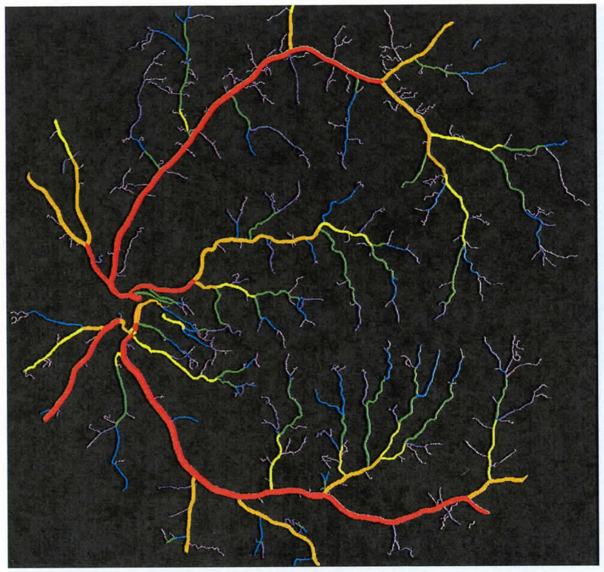
When analyzed by VESsel GENeration Analysis (VESGEN) software, vascular patterns provide useful integrative read-outs of complex, interacting molecular signaling pathways. Using VESGEN, we recently discovered and published our innovative, surprising findings that angiogenesis oscillated with vascular dropout throughout progression of diabetic retinopathy, a blinding vascular disease. Our findings provide a potential paradigm shift in the current prevailing view on progression and treatment of this disease, and a new early-stage window of regenerative therapeutic opportunities. The findings also suggest that angiogenesis may oscillate with vascular disease in a homeostatic-like manner during early stages of other inflammatory progressive diseases such as cancer and coronary vascular disease.


Cardiovascular Alterations, Immunosuppression & Bone Loss:
NASA-defined risk categories for human space exploration
2

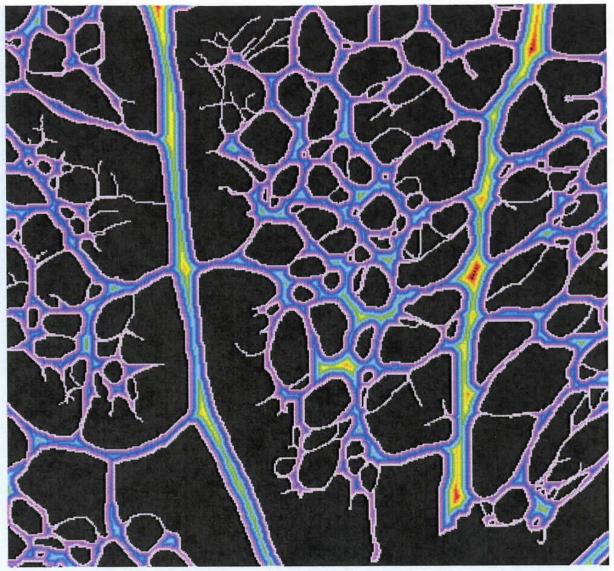
Recent results with Peter Kaiser MD, Cole Eye Institute
Oscillation of vessel density with progression of diabetic retinopathy

"Eye as a Window to the Body"
True of other vascular-dependent progressive diseases such as solid tumors?

Vascular pattern as integrative read-out of complex signaling
VESGEN software for mapping and quantification of progressive angiogenesis and microvascular remodeling



VESGEN
APPLICATIONS



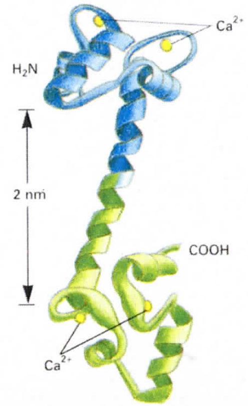
Vascular Trees
Human Retina
Avian CAM, Yolksac and Murine/Avian Coronary Vessels
(Solid Tumors?)

Vascular Networks
Mouse Postnatal Retina
CAM Lymphatic Vessels

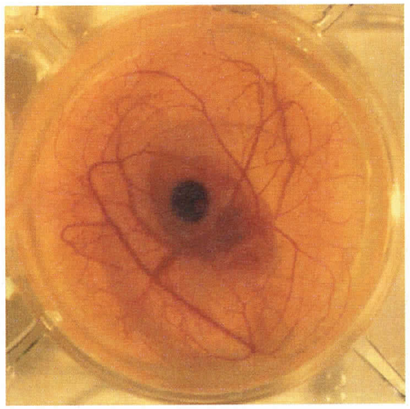
Vascular Tree-Network Composites
Normal and Abnormal Embryonic Coronary Vessels

Mapping and Quantification of Microvascular Remodeling and Angiogenesis by VESGEN

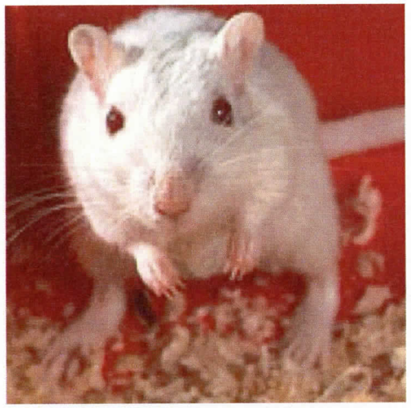
Molecular Regulation

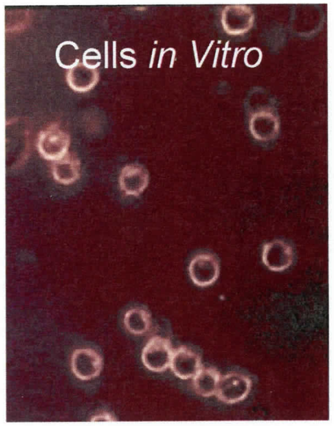


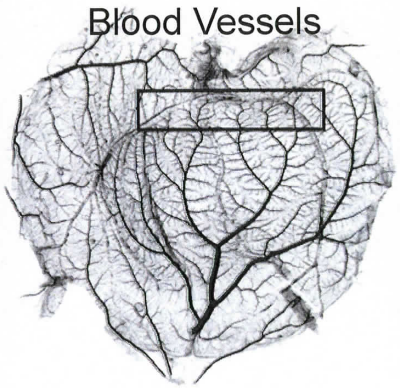
Avian CAM

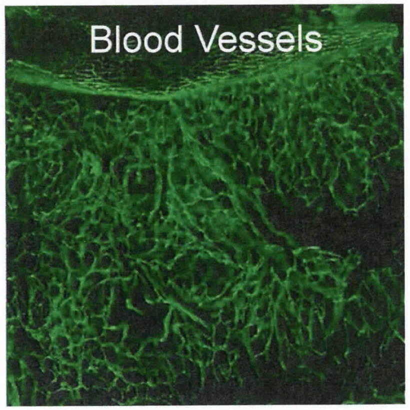


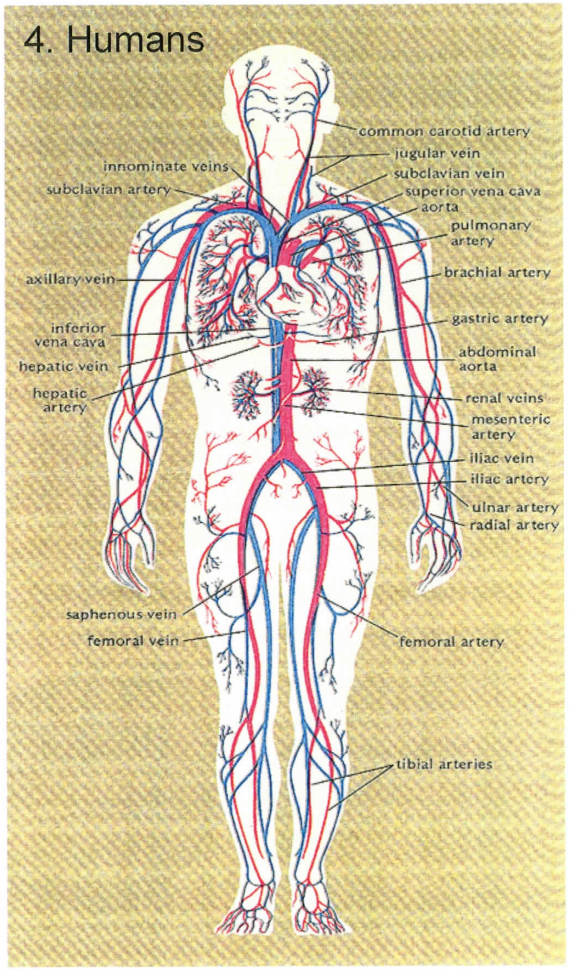
Transgenic Mouse

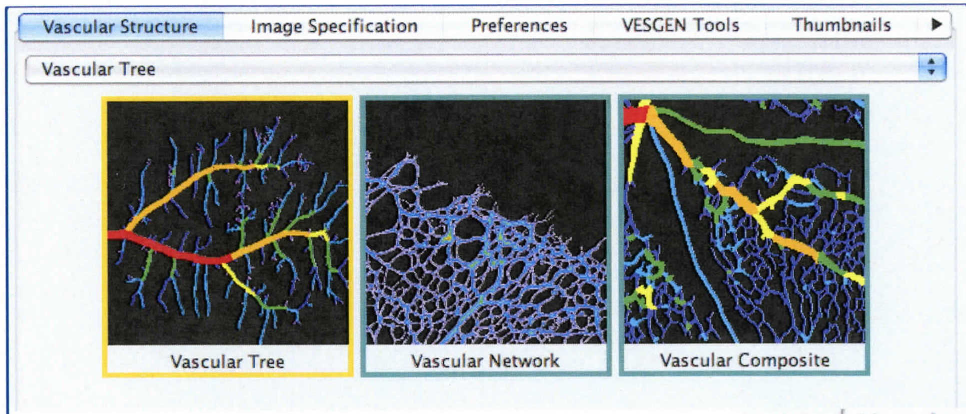








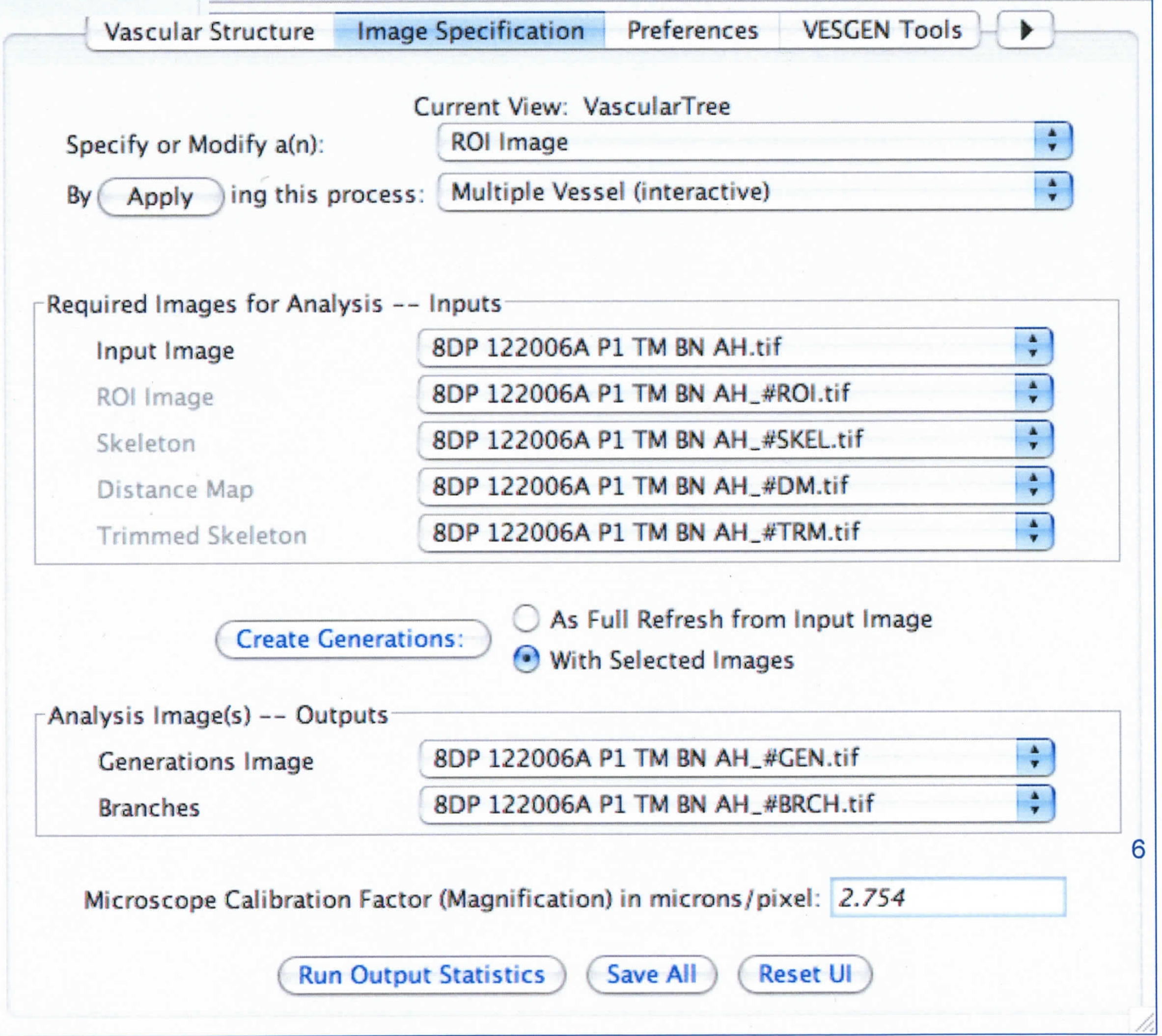


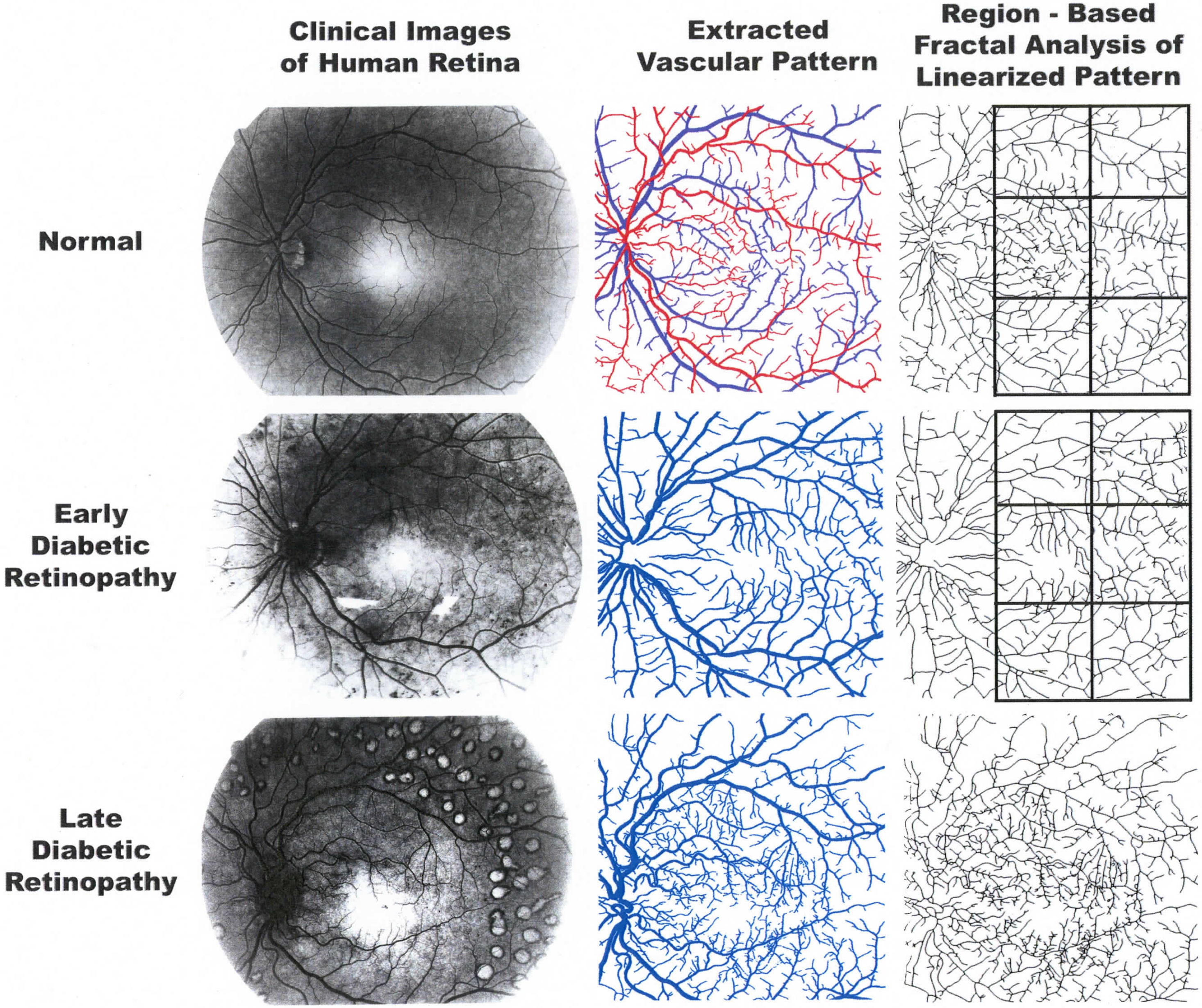


Panel to specify vessel type

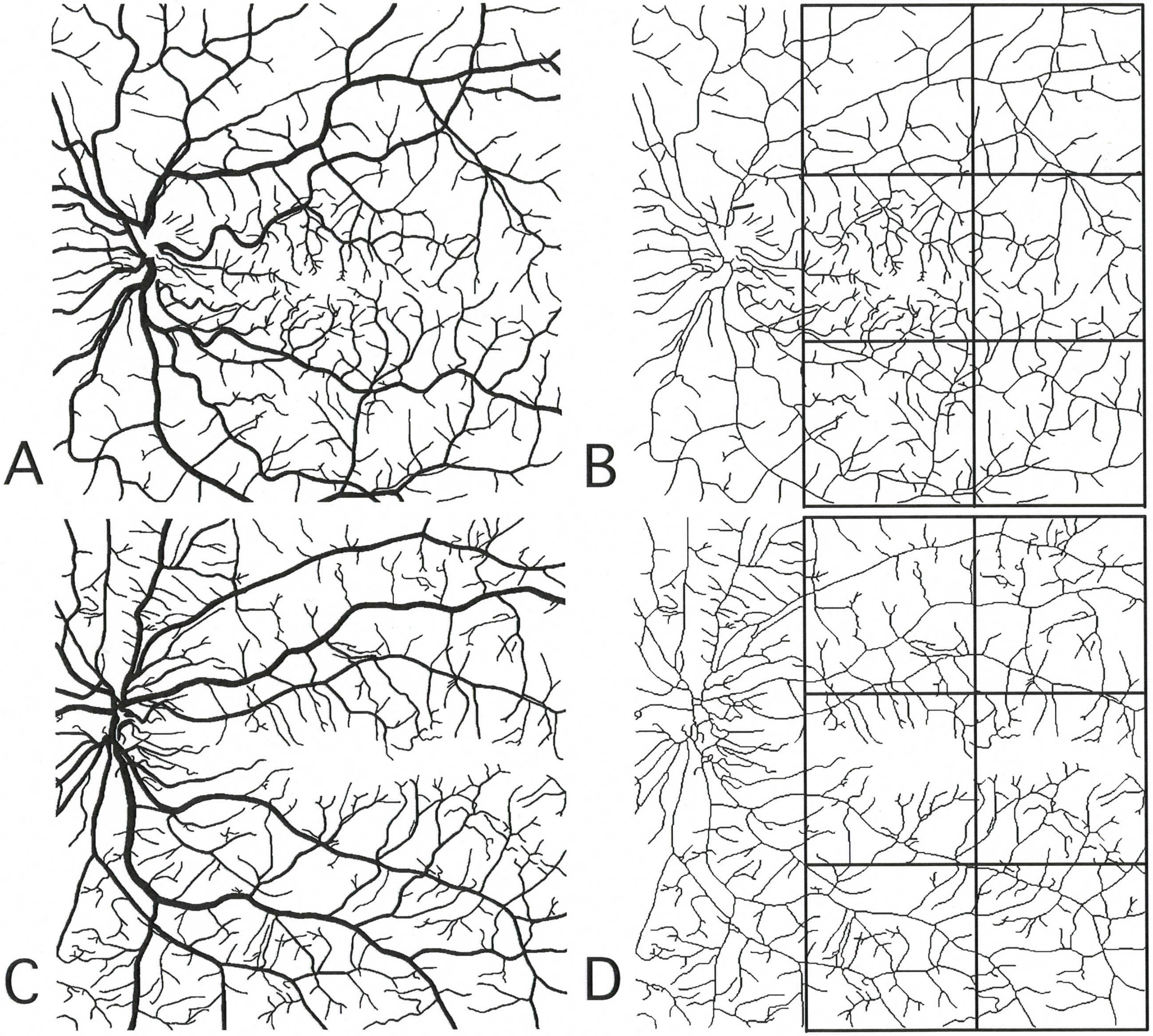
Main panel

- Image specification
- Algorithm selection
- Process initiation



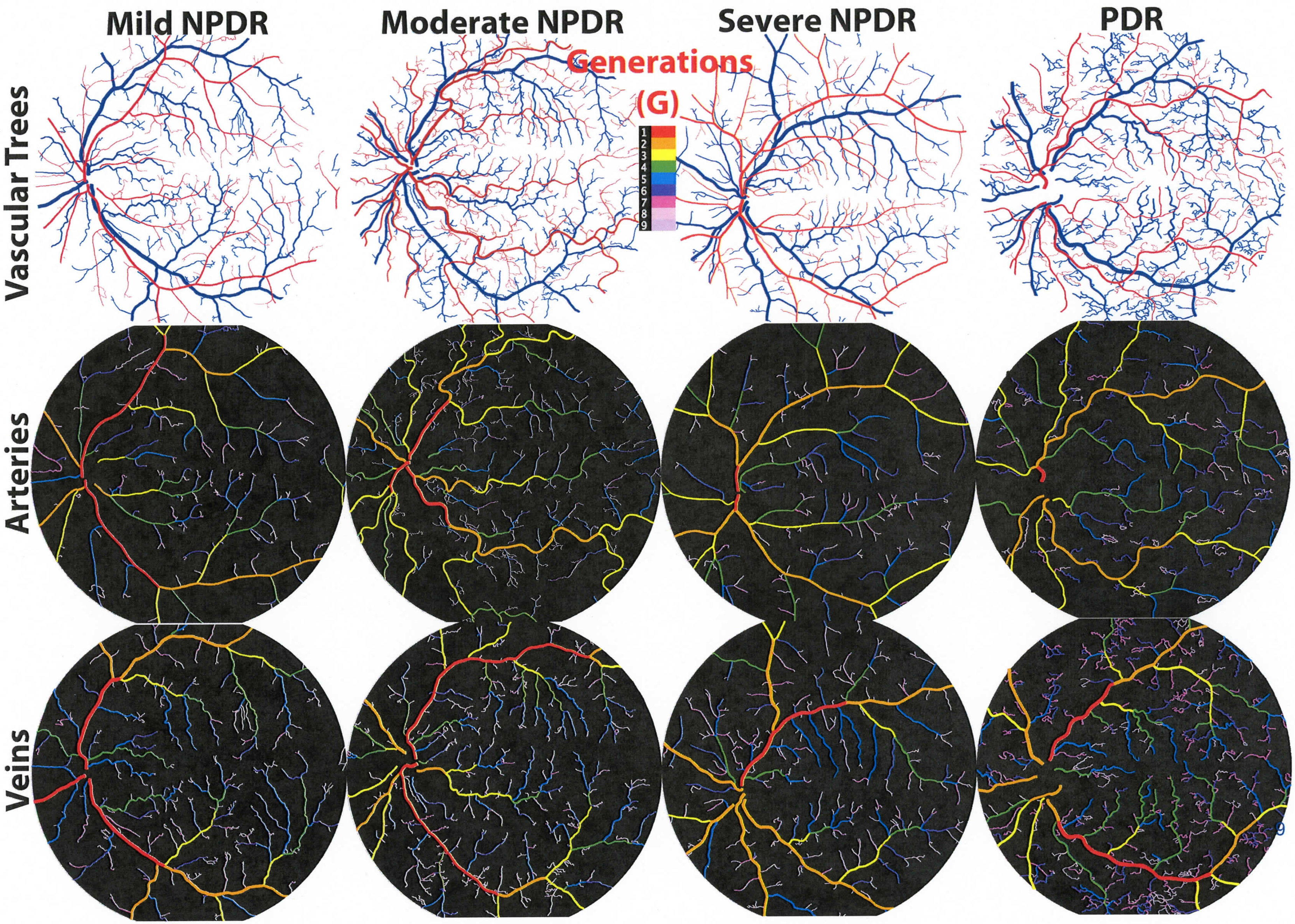


for *Current Eye Research* 24(4):274-280(2002)



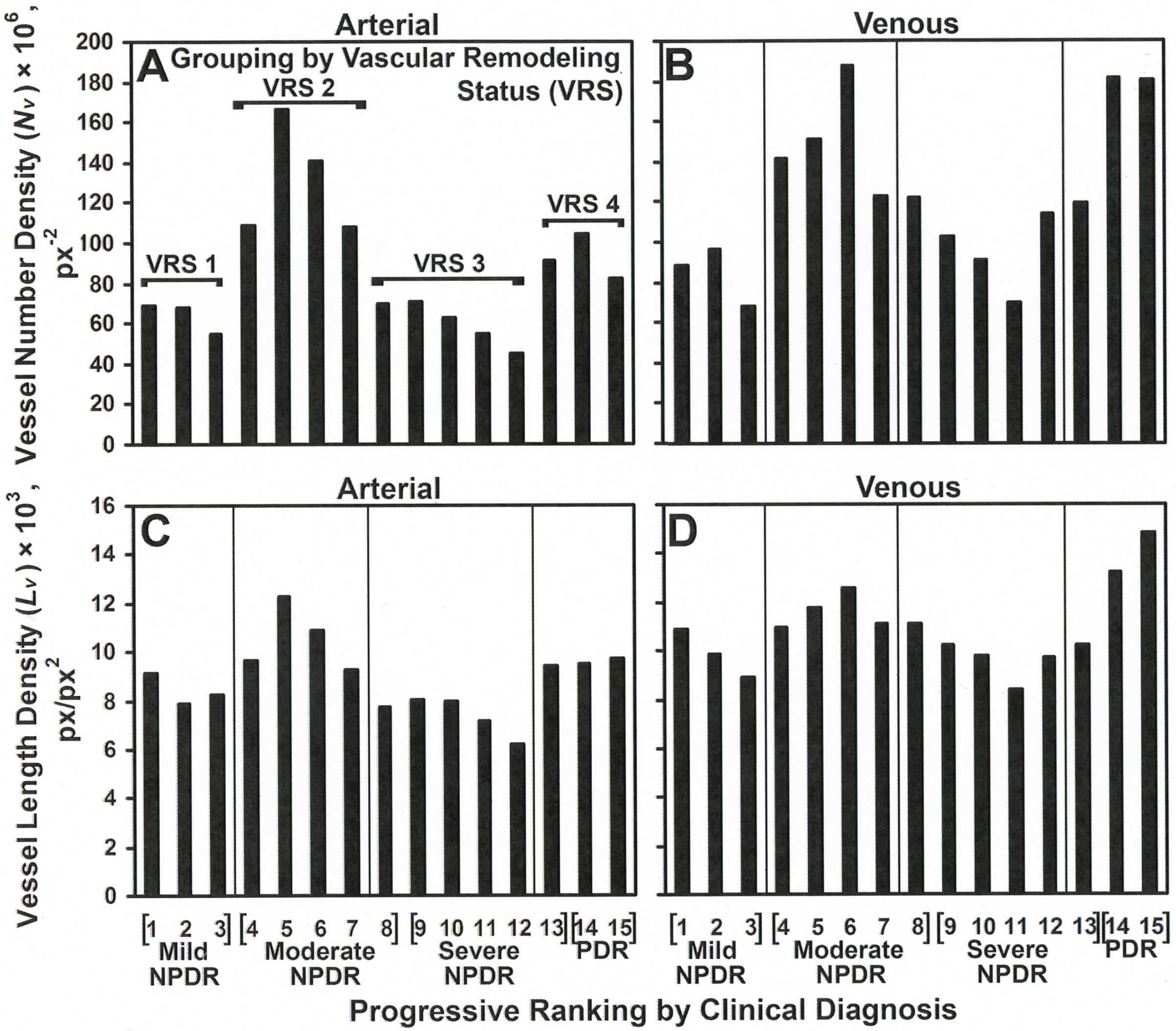
Current Eye Research 24(4):274-280(2002)

Mapping of Progressive Diabetic Retinopathy by VESGEN



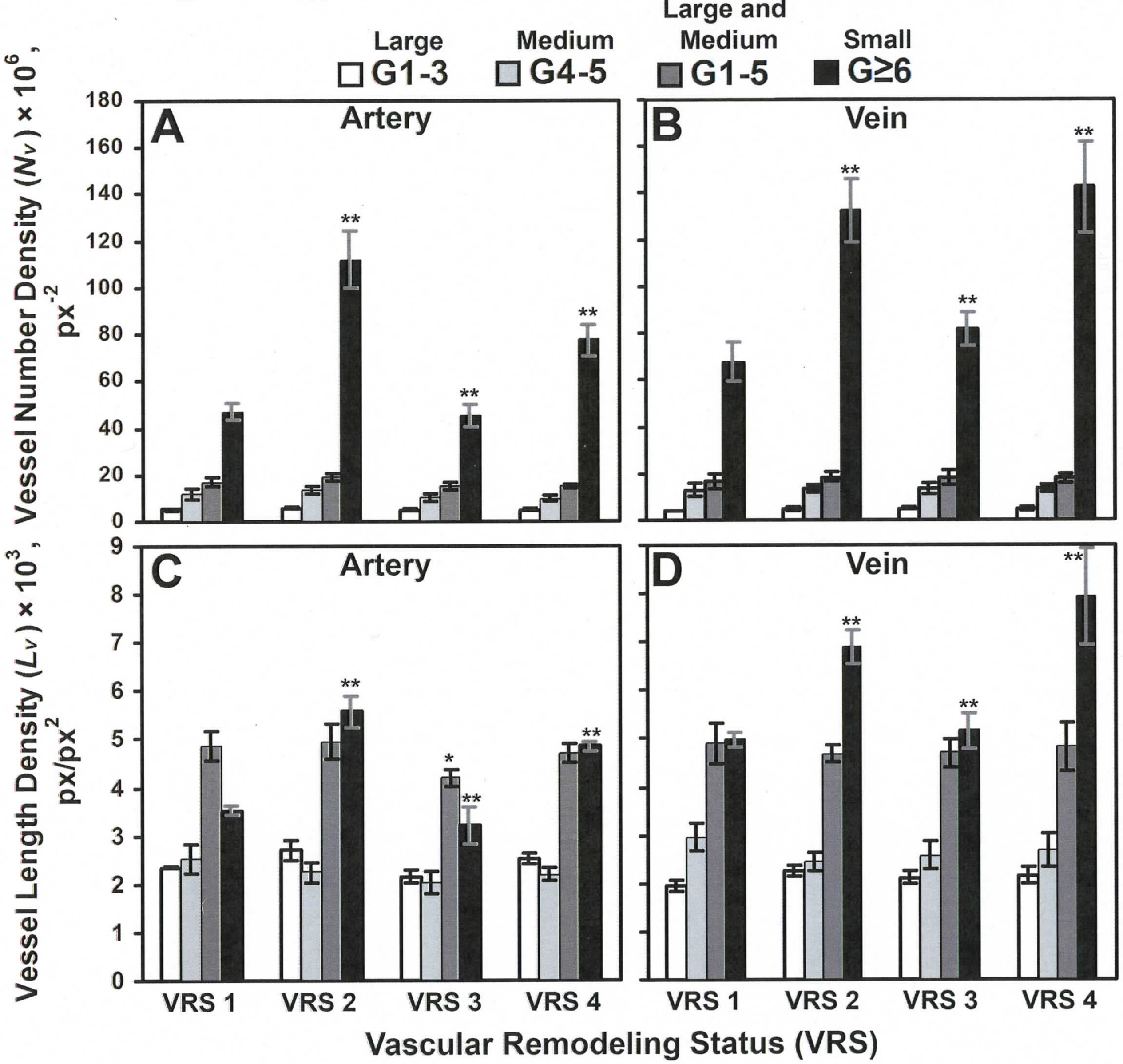
K Radhakrishnan, P Parsons-Wingerter, M B Vickerman, P K Kaiser, *IOVS* 51(1):498-507 (2010) and in progress

Grouping by Vascular Remodeling Status (VRS)



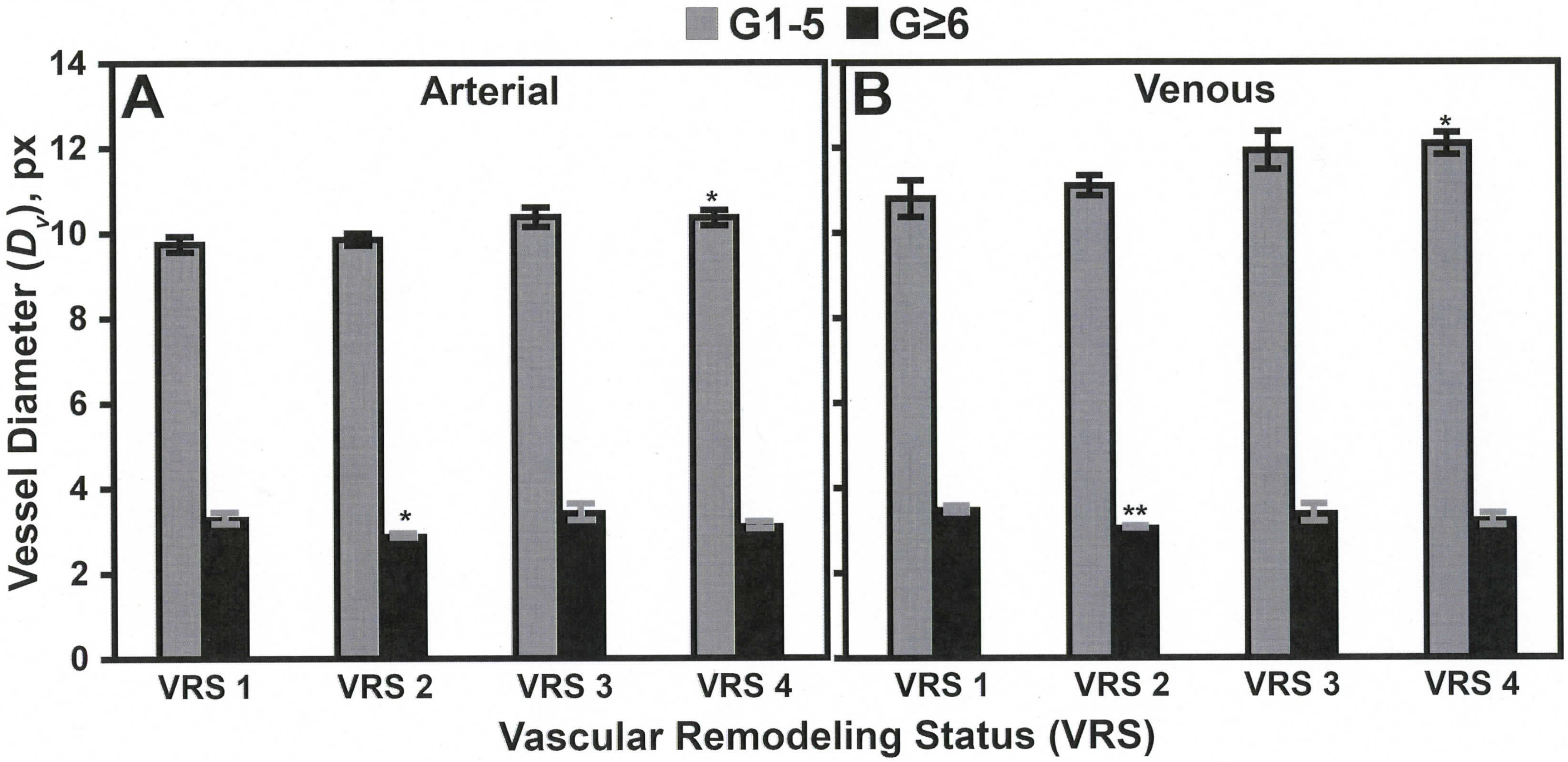
Parsons, Radhakrishnan, Vickerman & Kaiser, *Investigative Ophthalmology & Visual Science* 51(1):498-507 (2010)

Angiogenesis Oscillates with Vascular Dropout during Progression of Diabetic Retinopathy



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

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)

Oscillation of Angiogenesis with Vascular Dropout: *Systems Biology Analysis*

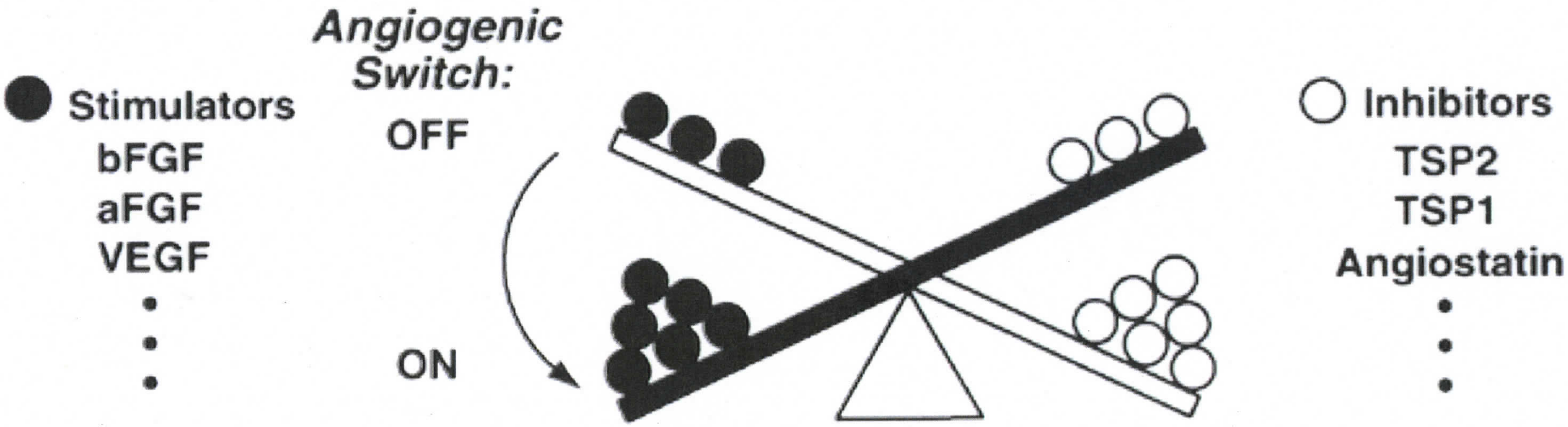
Space-Filling Capacity of Arterial and Venous Trees by VESGEN Analysis of Branching Generations ($G_1 \ldots, G_8$ *or* G_9) as f (D_f, N_v, L_v, $Br_v + E_v$, D_v, T_v, $\theta \ldots$)

Vessel Number Density, N_v
Vessel Length Density, L_v
Vessel Diameter, D_v

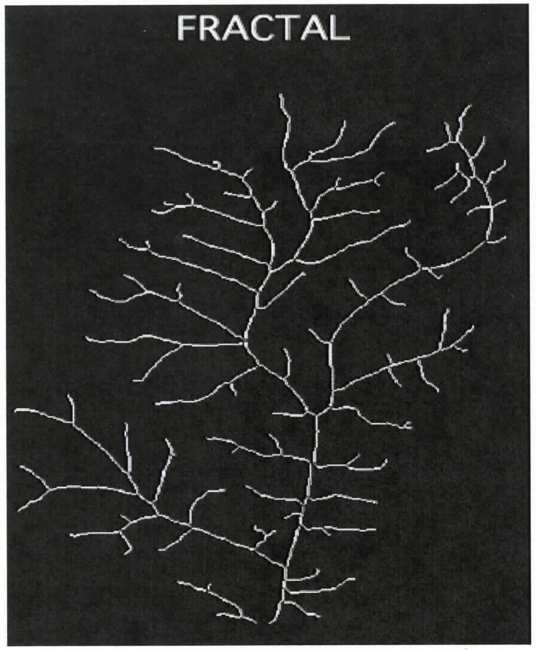
Fractal Dimension, D_f
$Br_v + E_v$ from Branch Point Density, Br_v and Endpoint Density, E_v

Basic Research to Innovative Translational Medicine

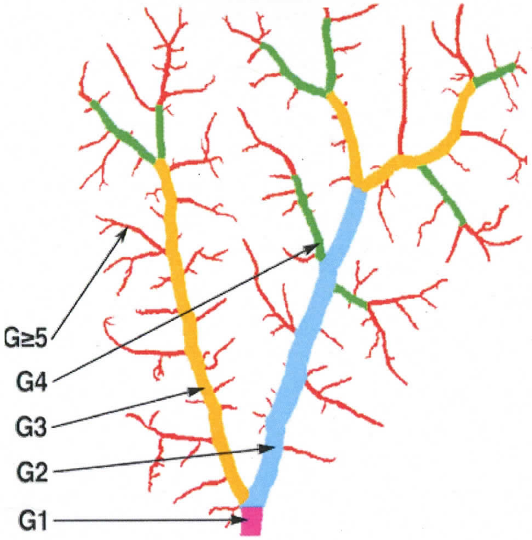
Dynamic Balance Hypothesis



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



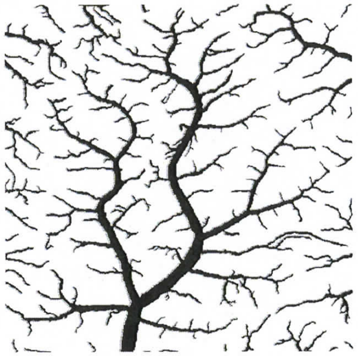
VESGEN



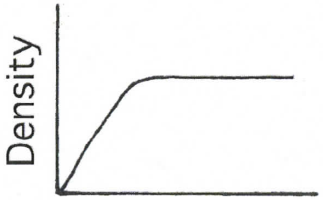
The form of an object is a 'diagram of forces'

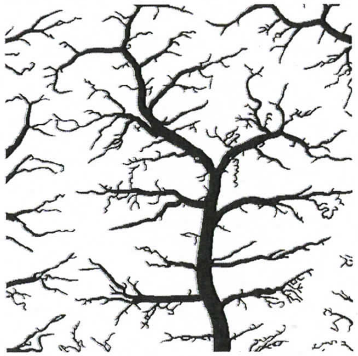
- D'Arcy Thompson

VESGEN Hypothesis: *'Signature'* Vascular Patterns

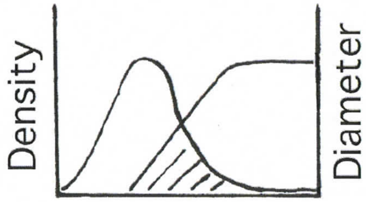


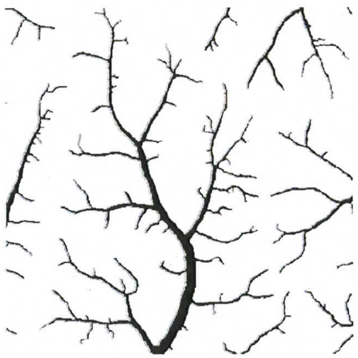
FGF-2 as a Simple Stimulator
(Fibroblast Growth Factor-2)



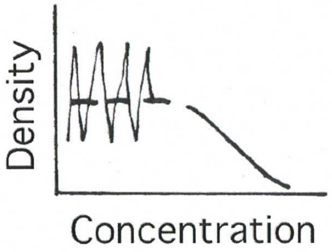


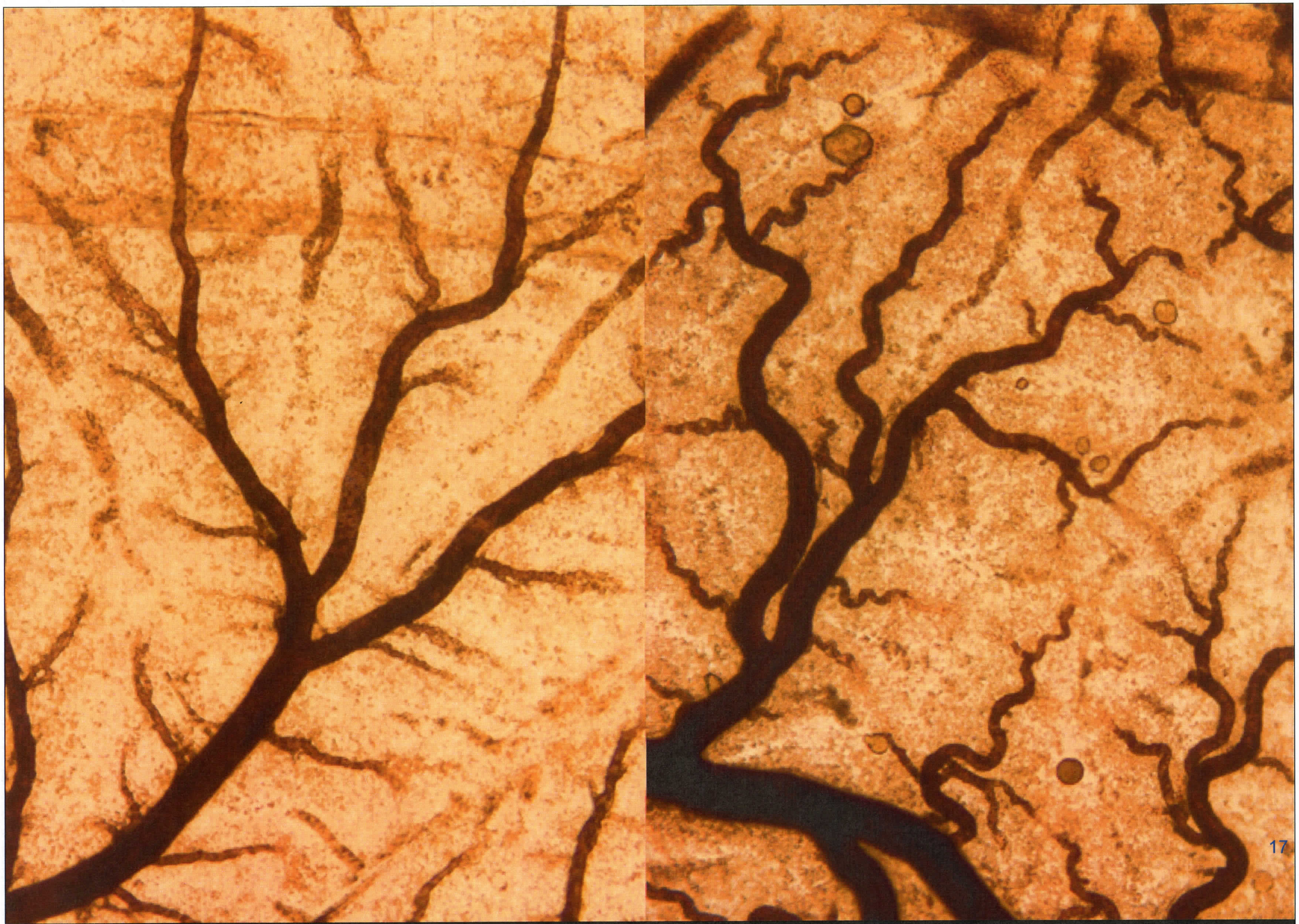
VEGF as a Complexity Factor
(Vascular Endothelial Growth Factor-2)

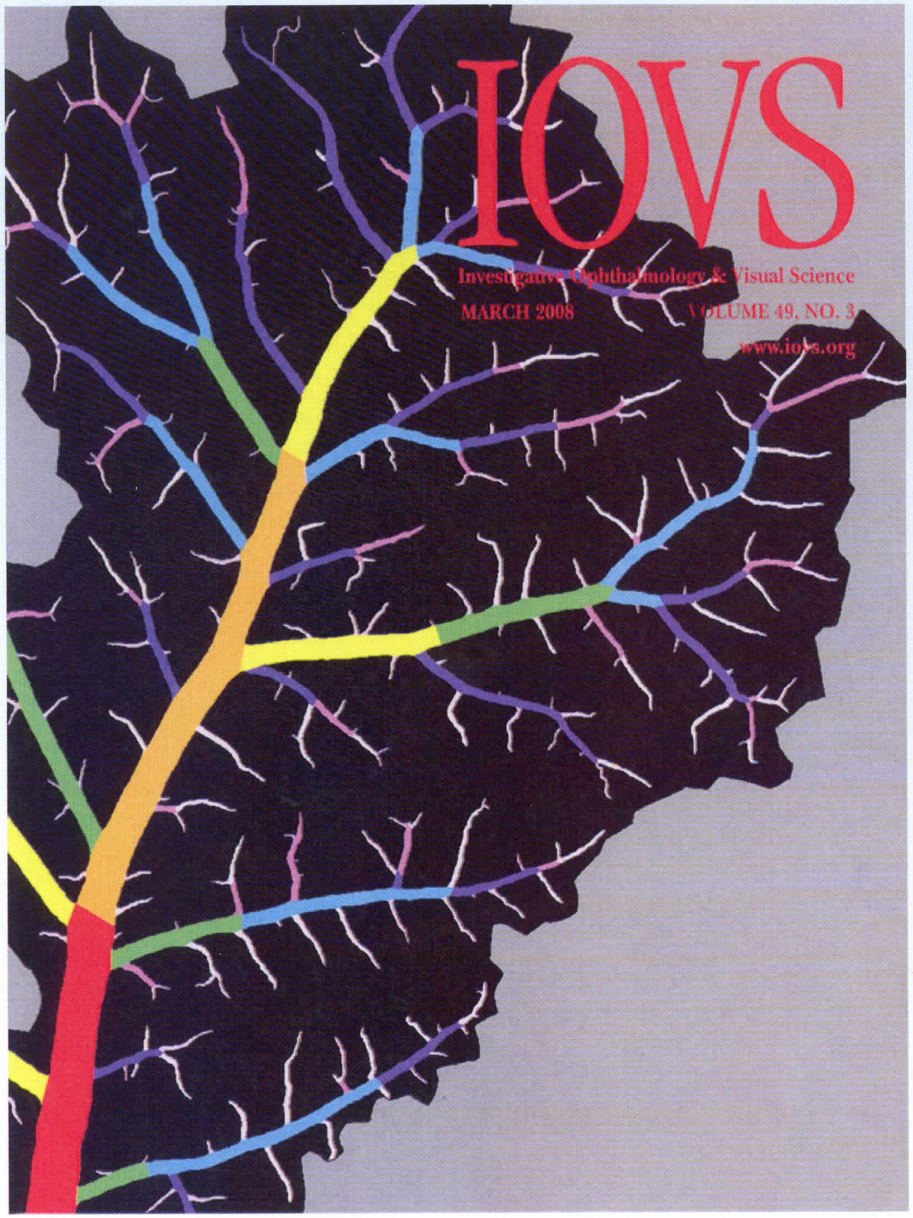




TGF-β1 as a Simple Inhibitor
But Complex Potentiator
(Transforming Growth Factor-β1)



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VOL. 292 NO. 3 MARCH 2009
AR
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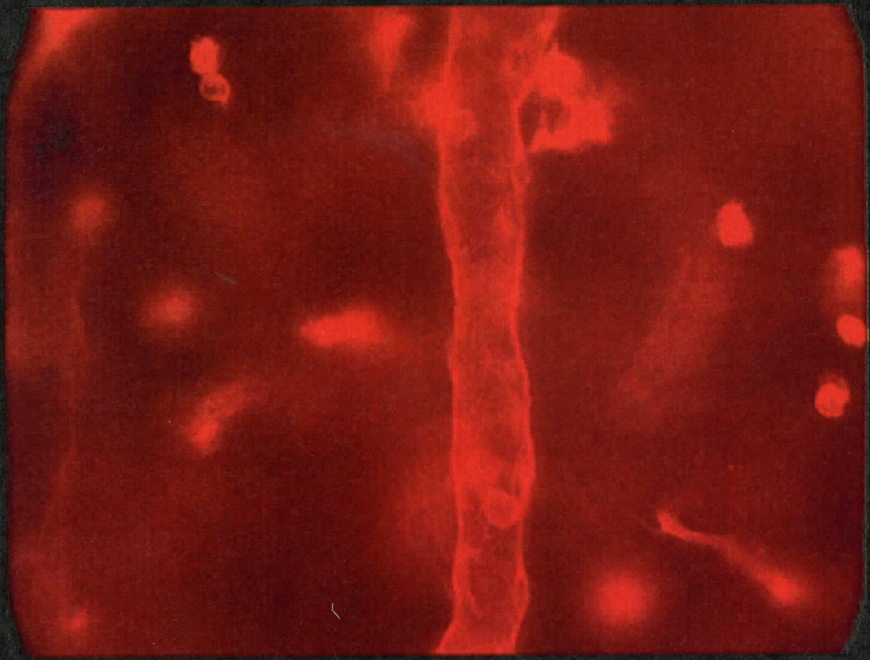
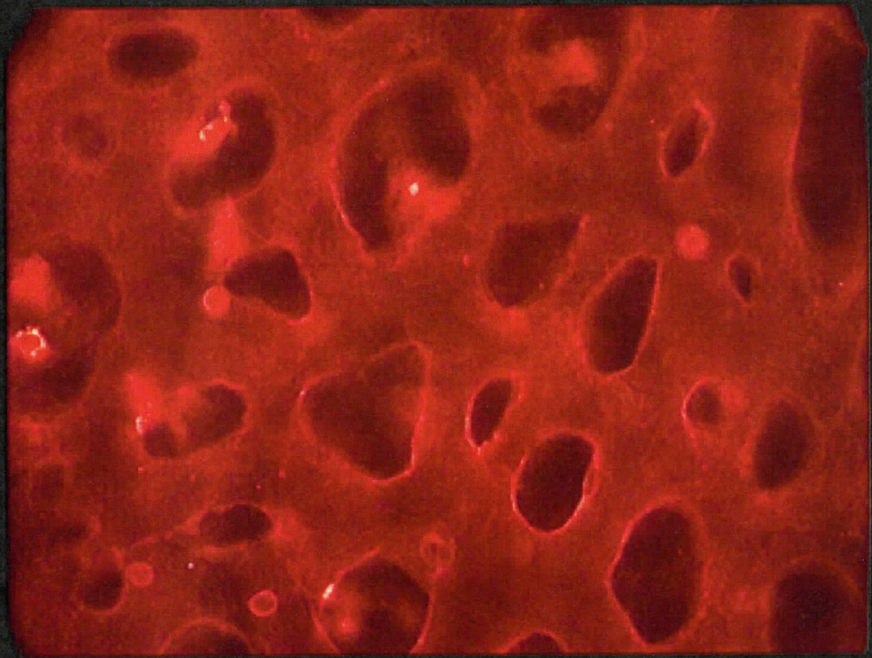
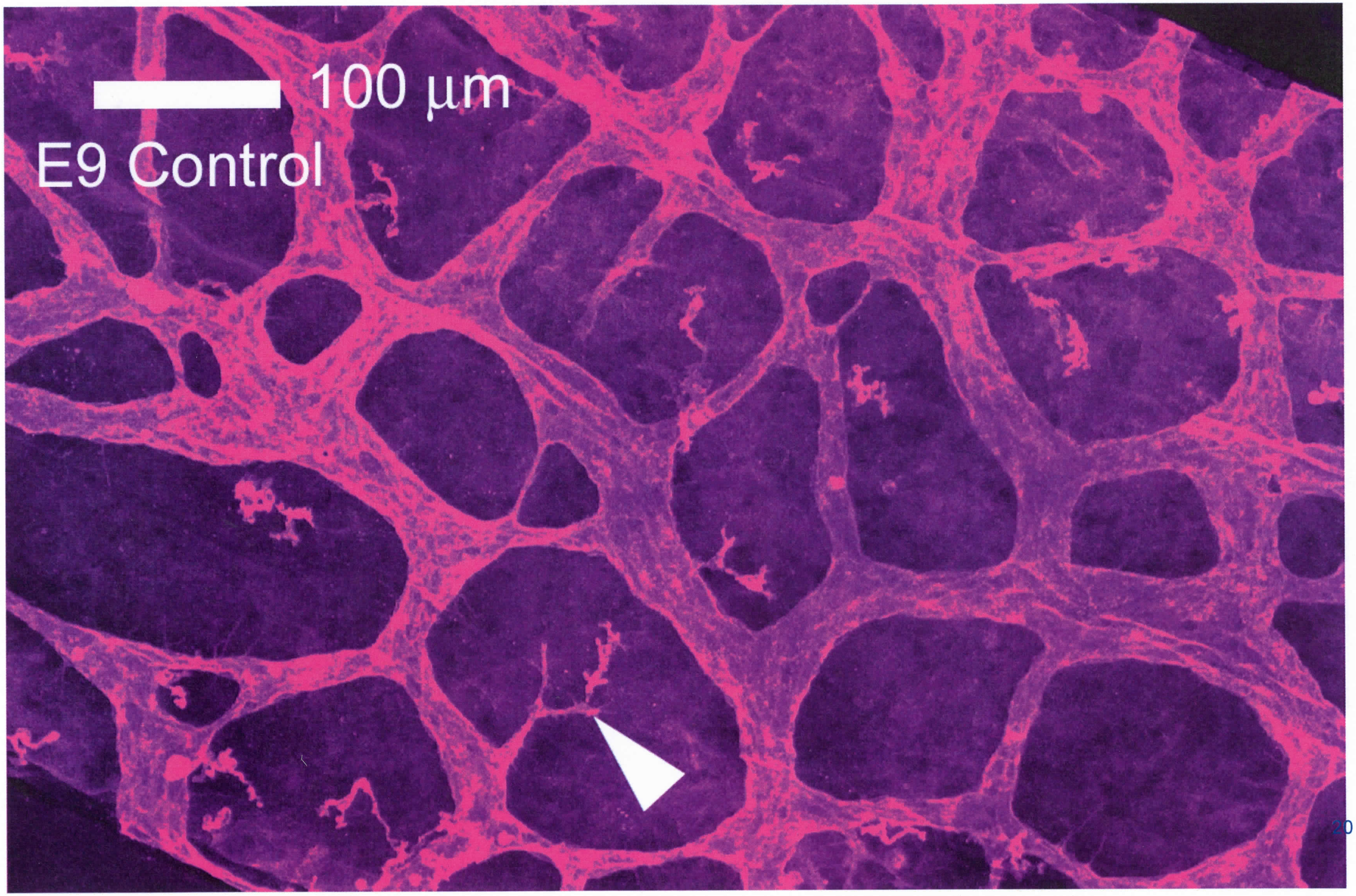
of Excellence in
ANATOMICAL
PUBLICATION
Thematic Papers:
Imaging Living
Vasculature

Vessel



Capillaries





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

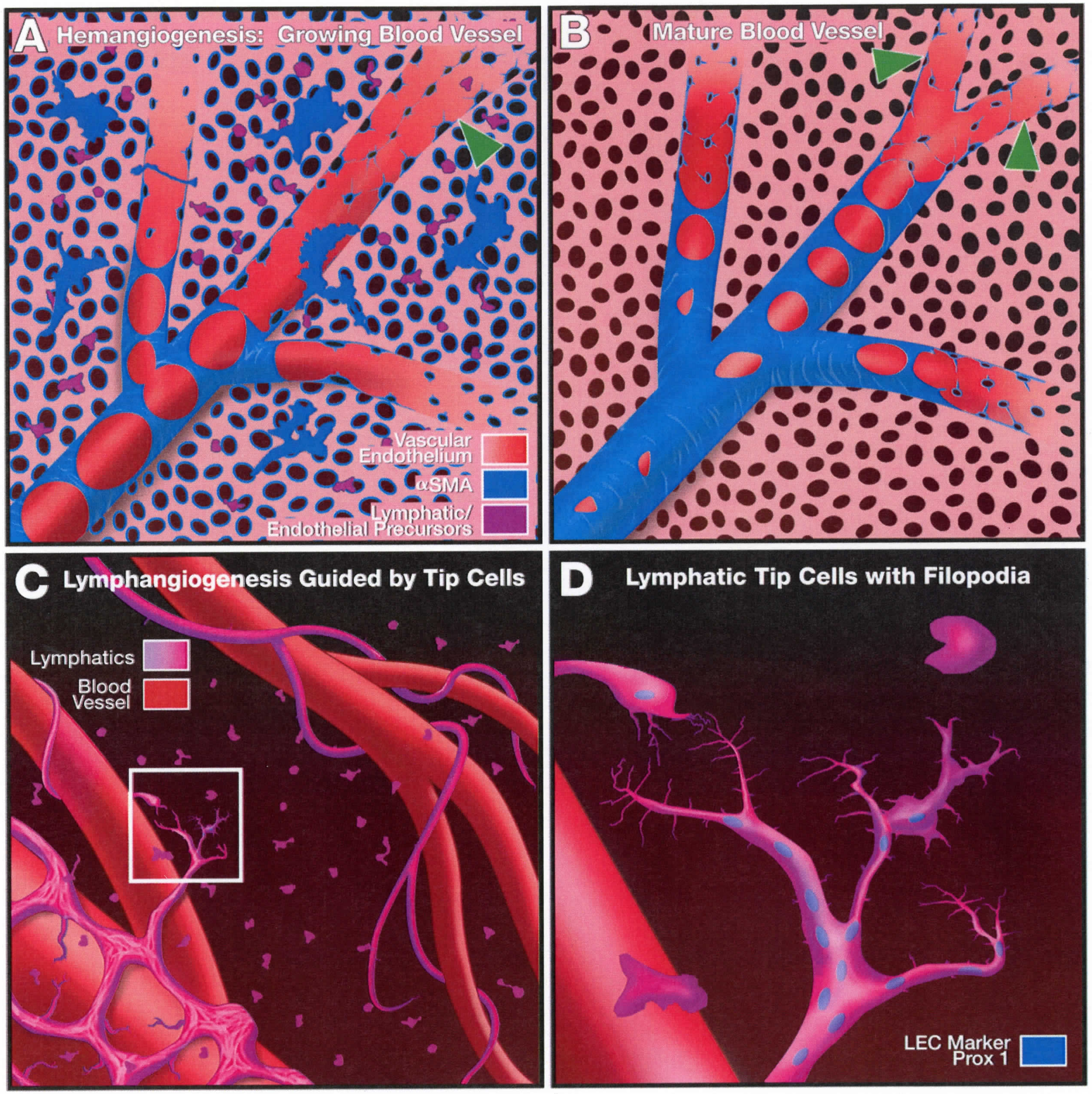
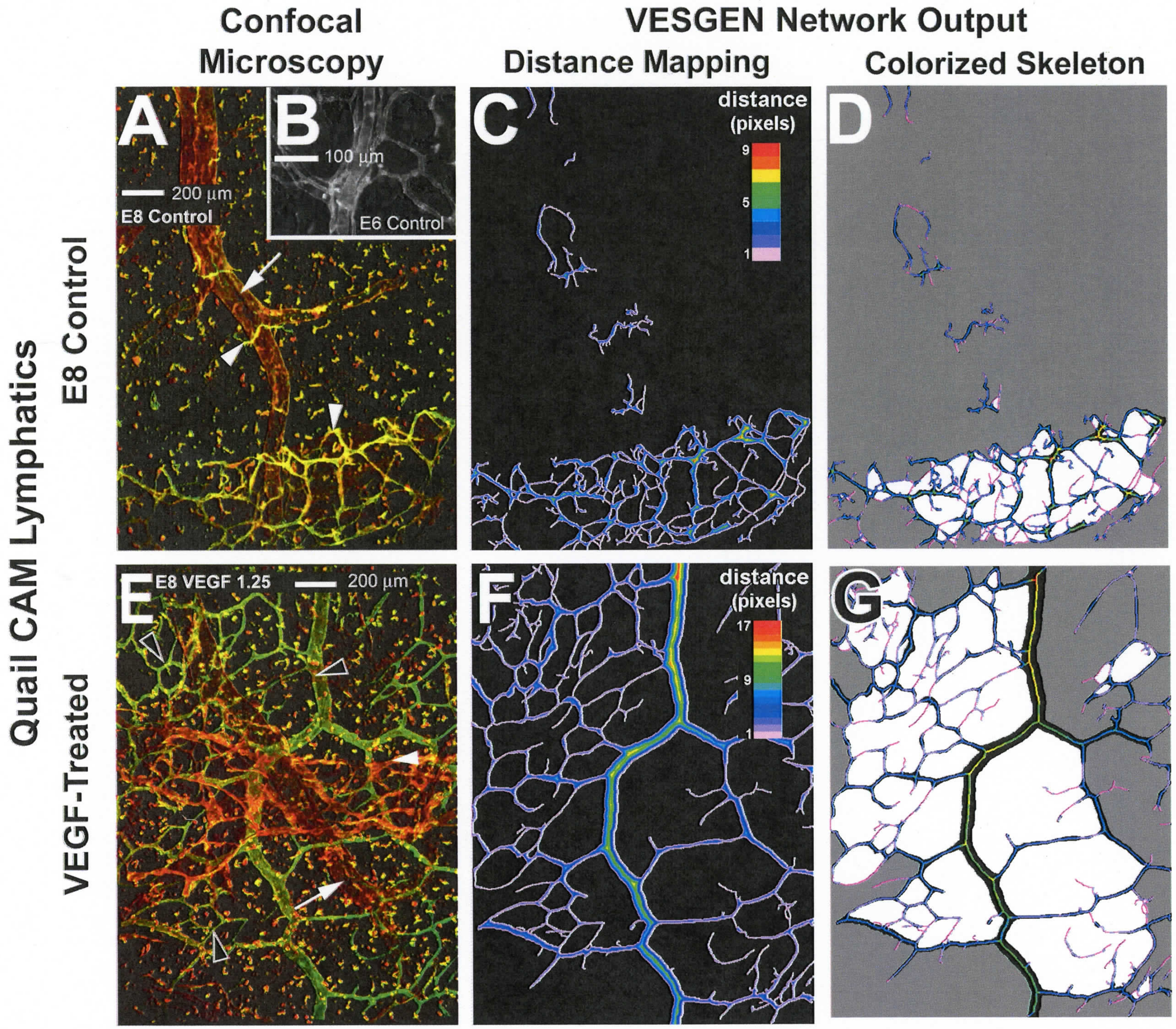


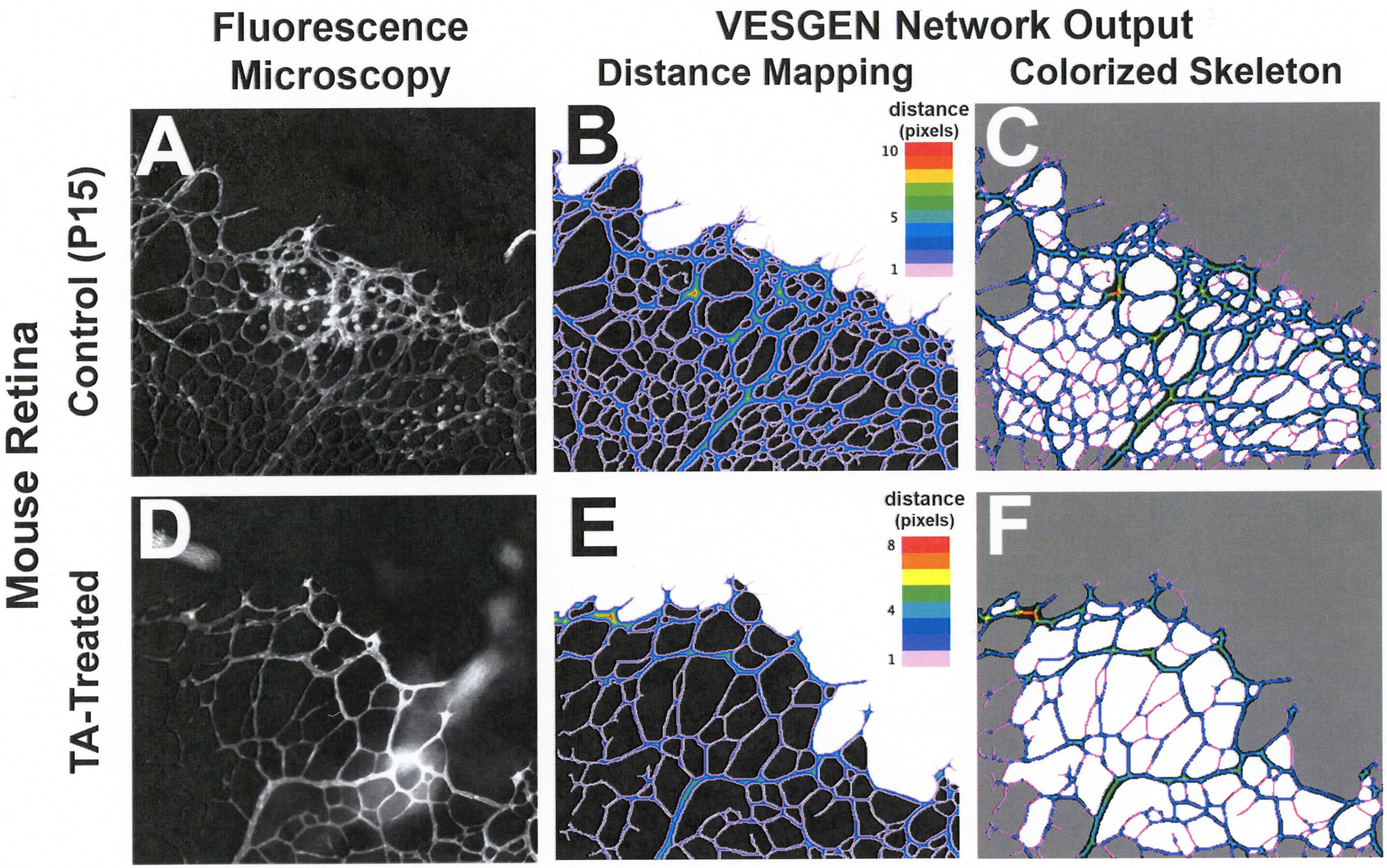
Fig. 7 Parsons-Wingerter *et al.*

CAM LYMPHATIC NETWORK



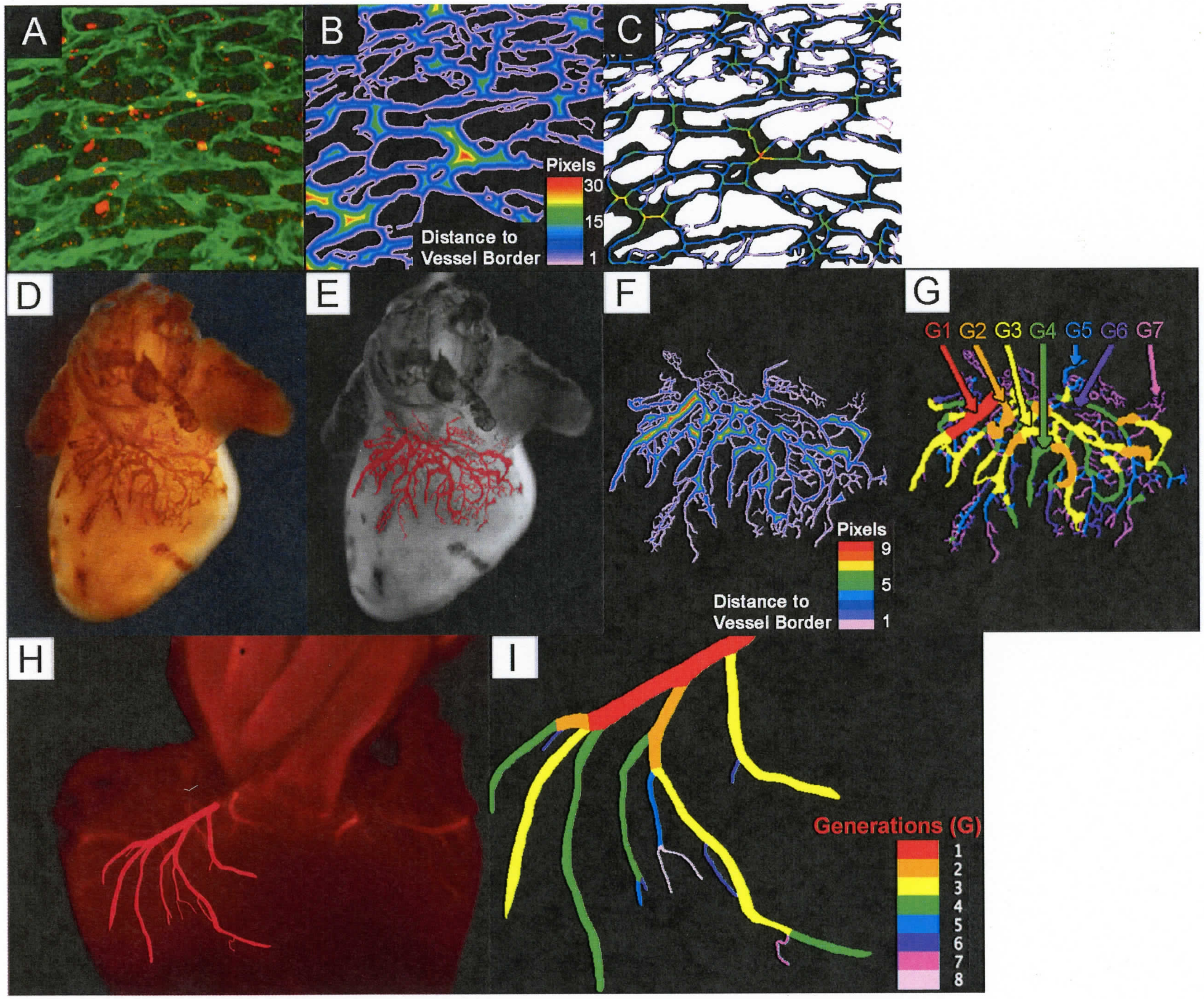
Reviewed in *Anatomical Record A* 2009; *Anatomical Record A* 288A:233 (2006)

VASCULAR NETWORKS IN TRANSGENIC MOUSE RETINA



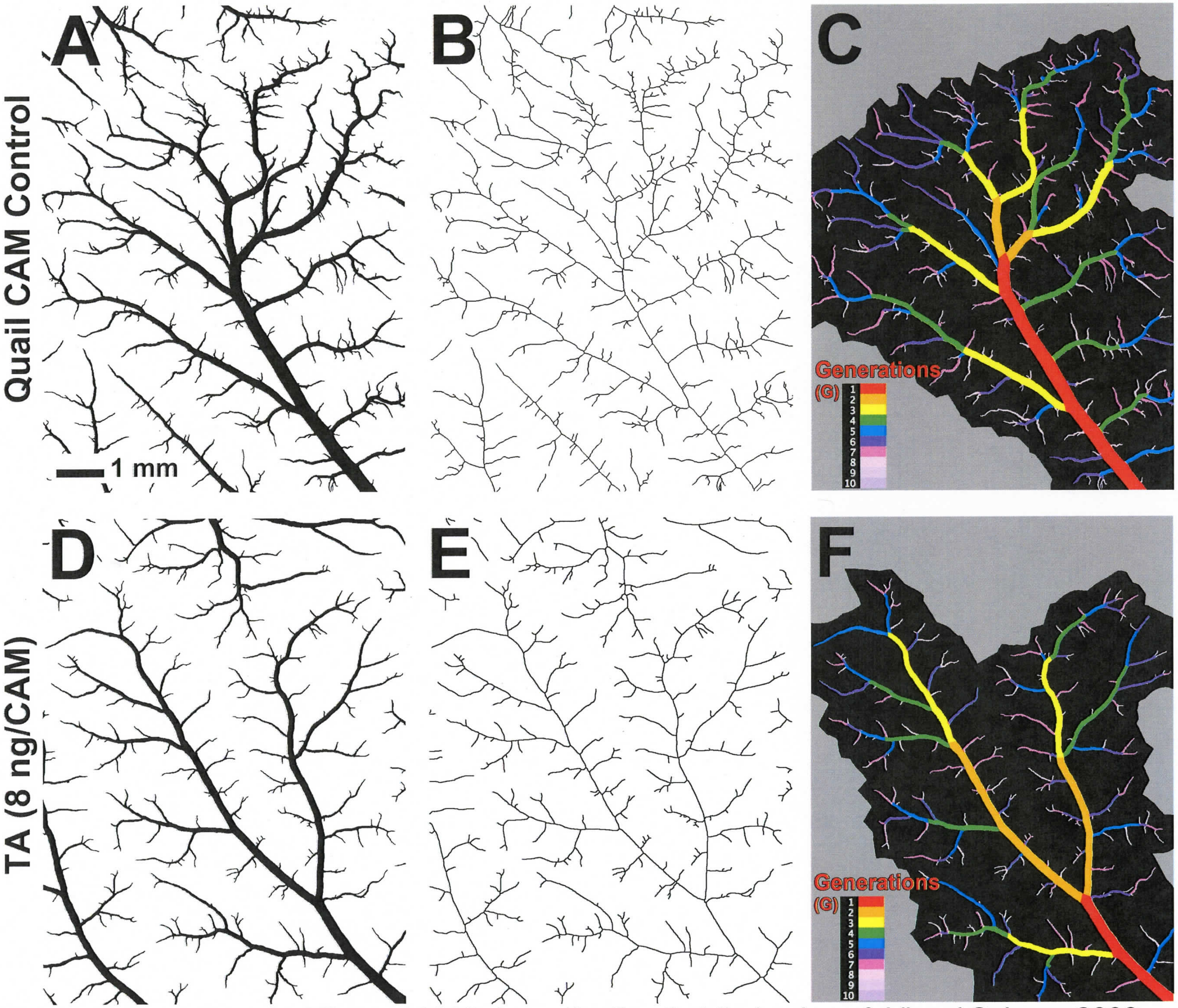
with J Sears & Q Ebrahem (Cole Eye Institute), from Vickerman et al, *Anatomical Record A* 292(3), 2009

CORONARY VESSEL NETWORK-TO-TREE TRANSITIONS

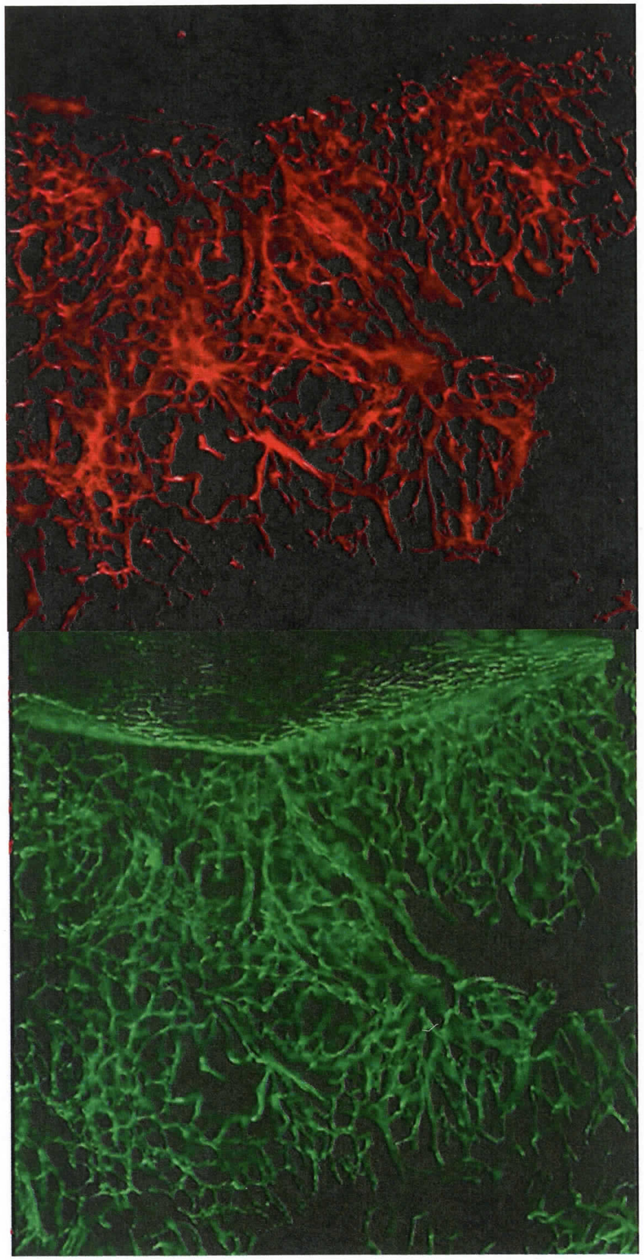


Vickerman et al, VESGEN Review, *Anatomical Record A* 292(3), 2009

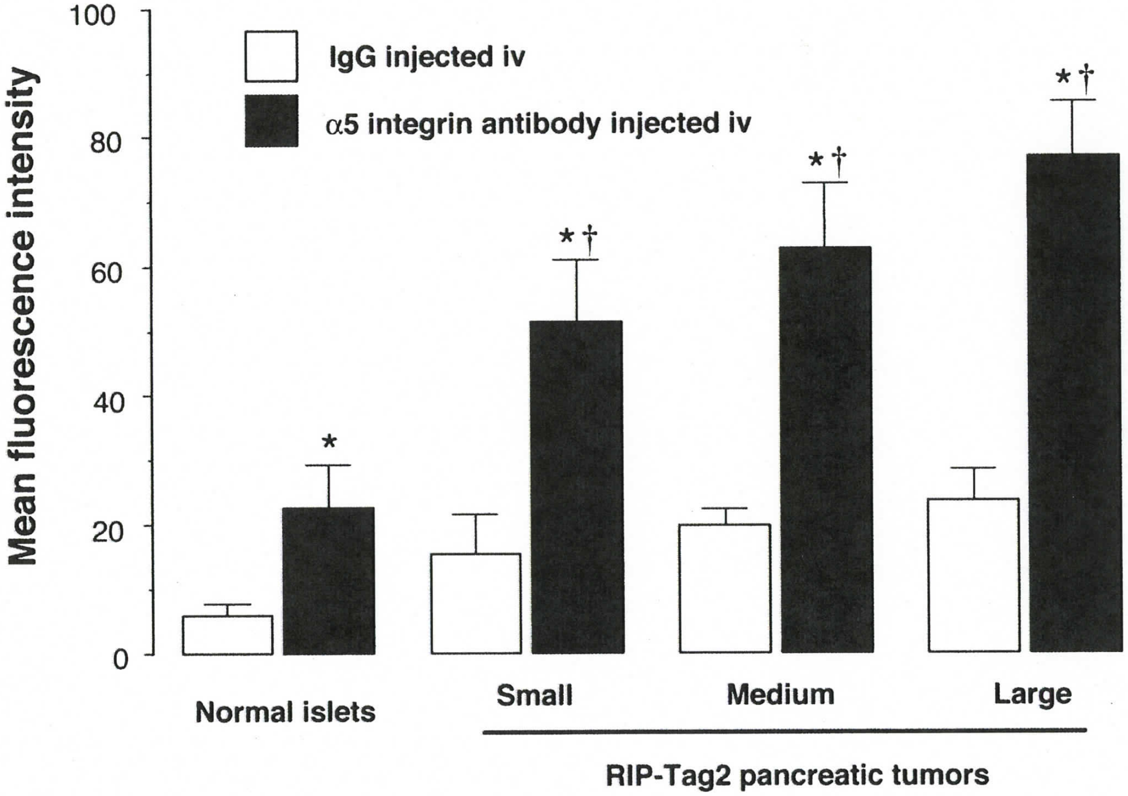
TA Treatment in CAM Vascular Tree



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

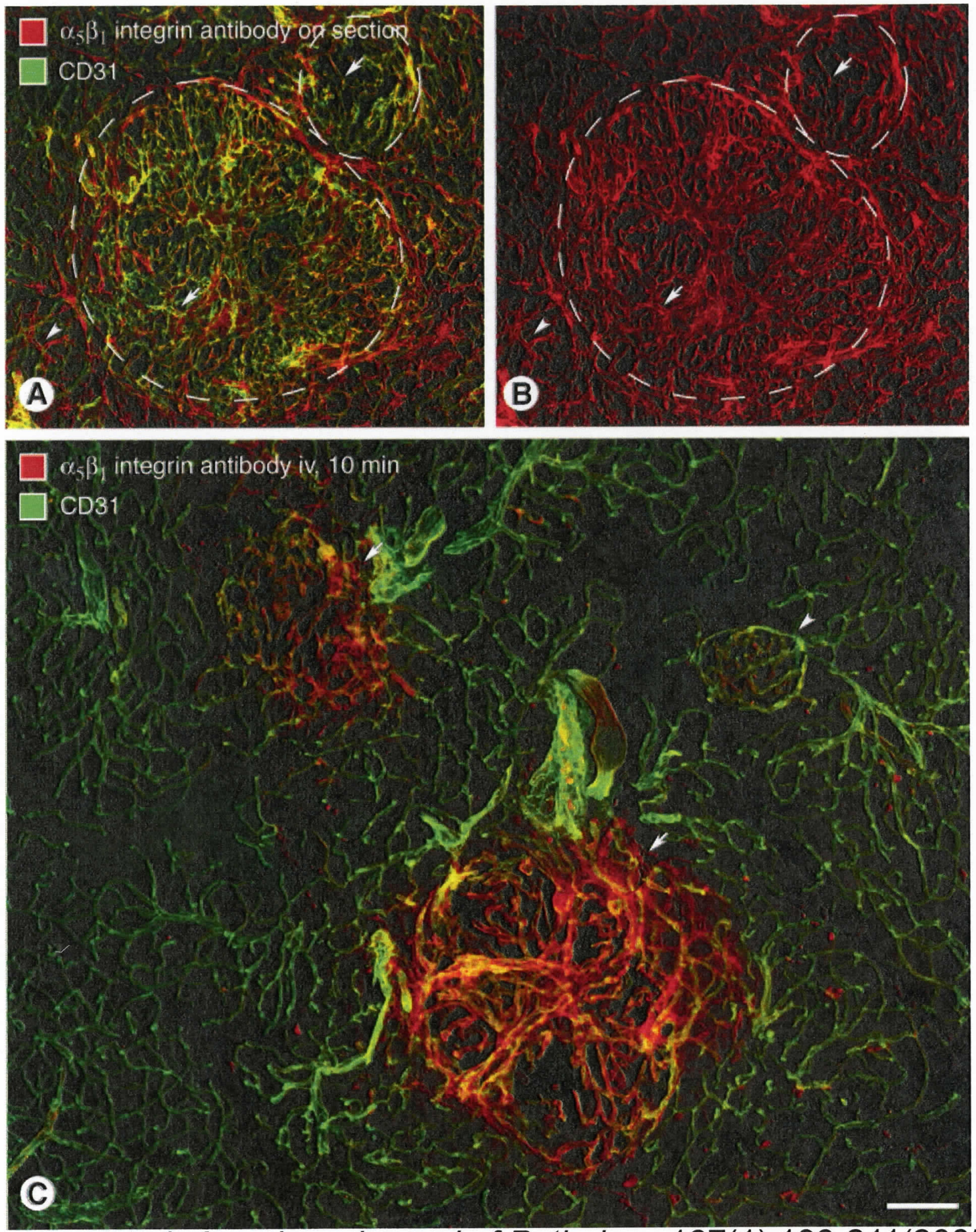


Vascular Targeting of Tumor Vessels In RIP-Tag Transgenic Mice by iv Injection

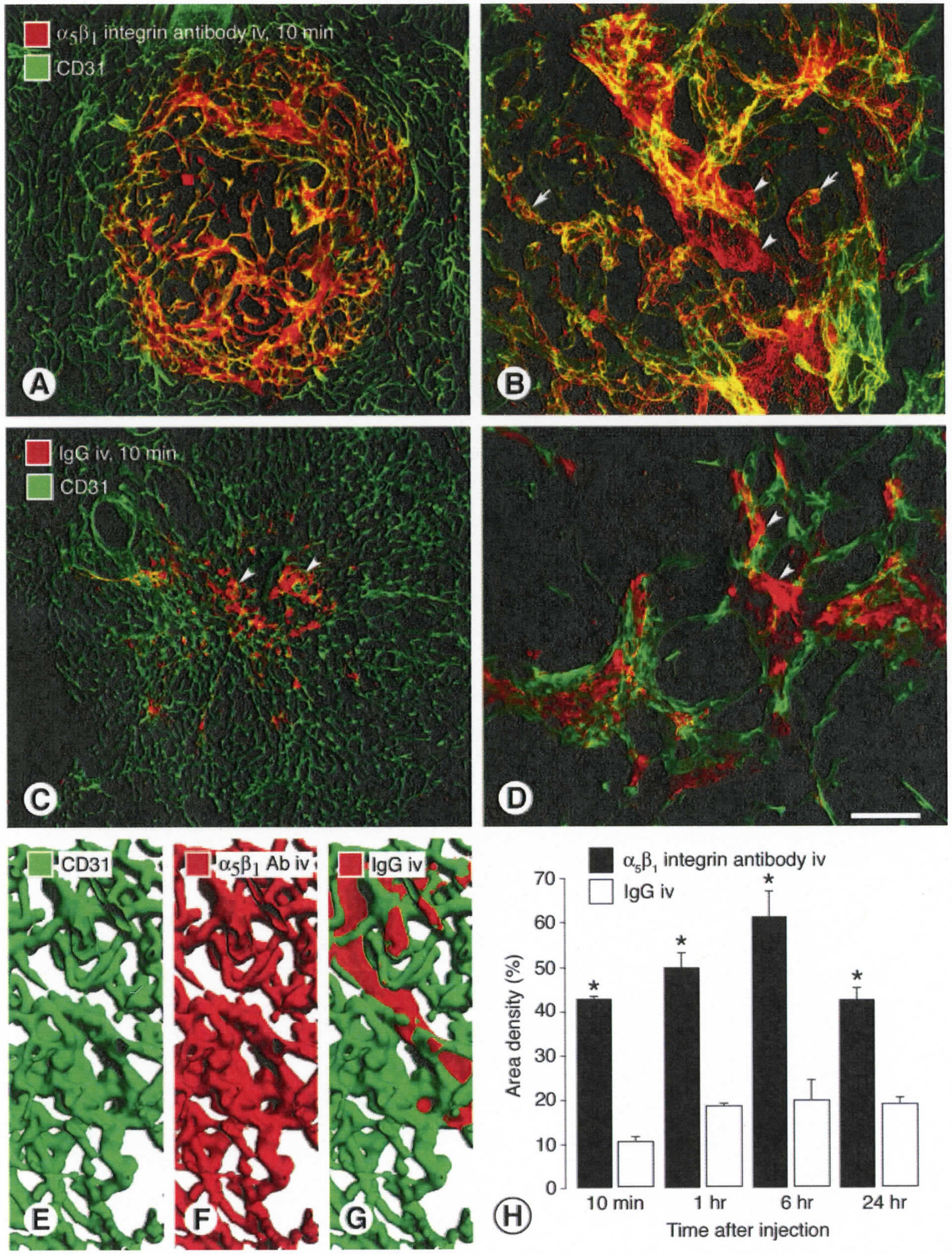


Parsons et al, *American Journal of Pathology* 167(1):193-211(2005)

Vascular Targeting: $\alpha5\beta1$ Integrin Antibody *iv* in Transgenic RIP-Tag2 Pancreatic Tumors

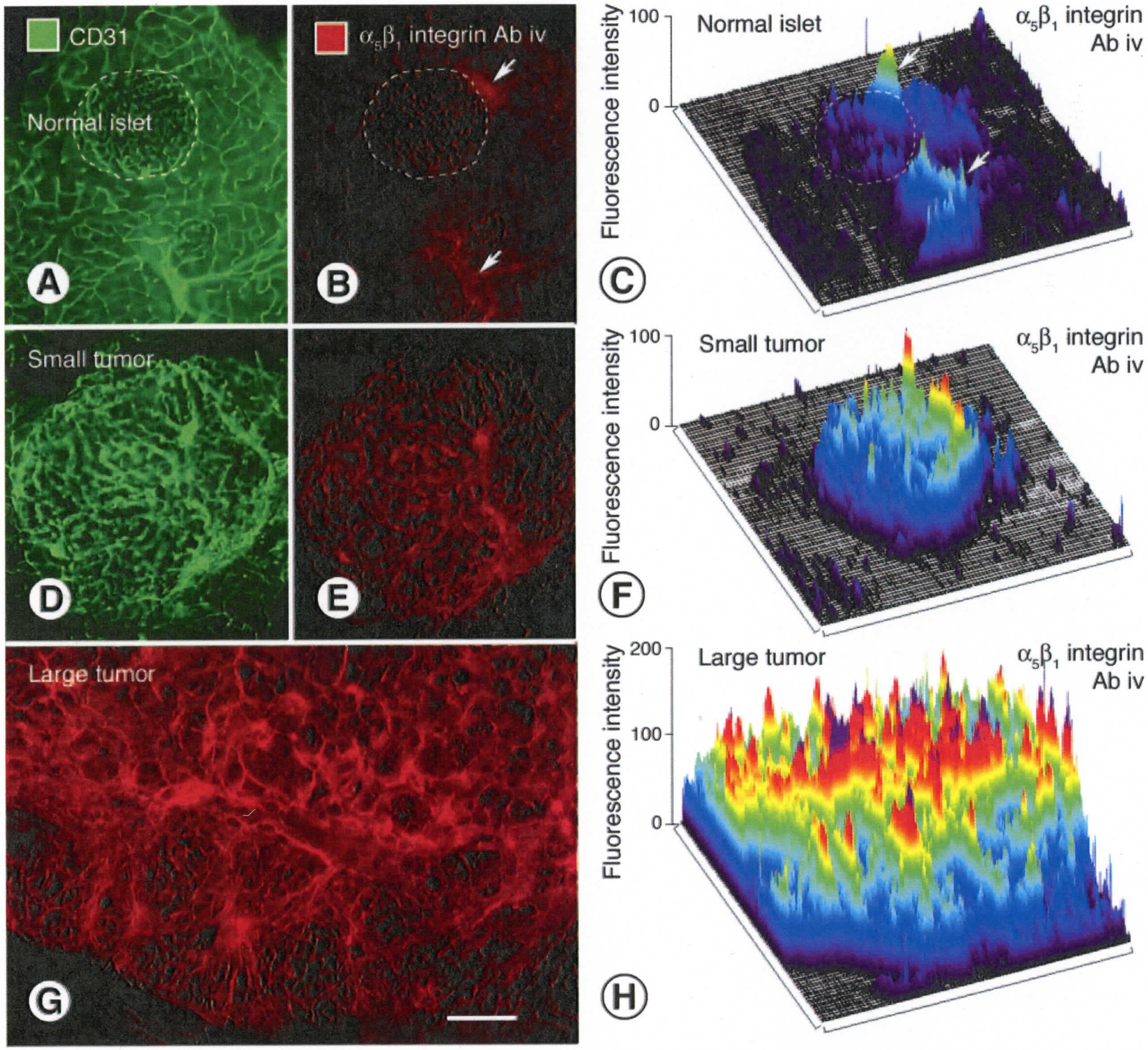


Parsons et al, *American Journal of Pathology* 167(1):193-211(2005)



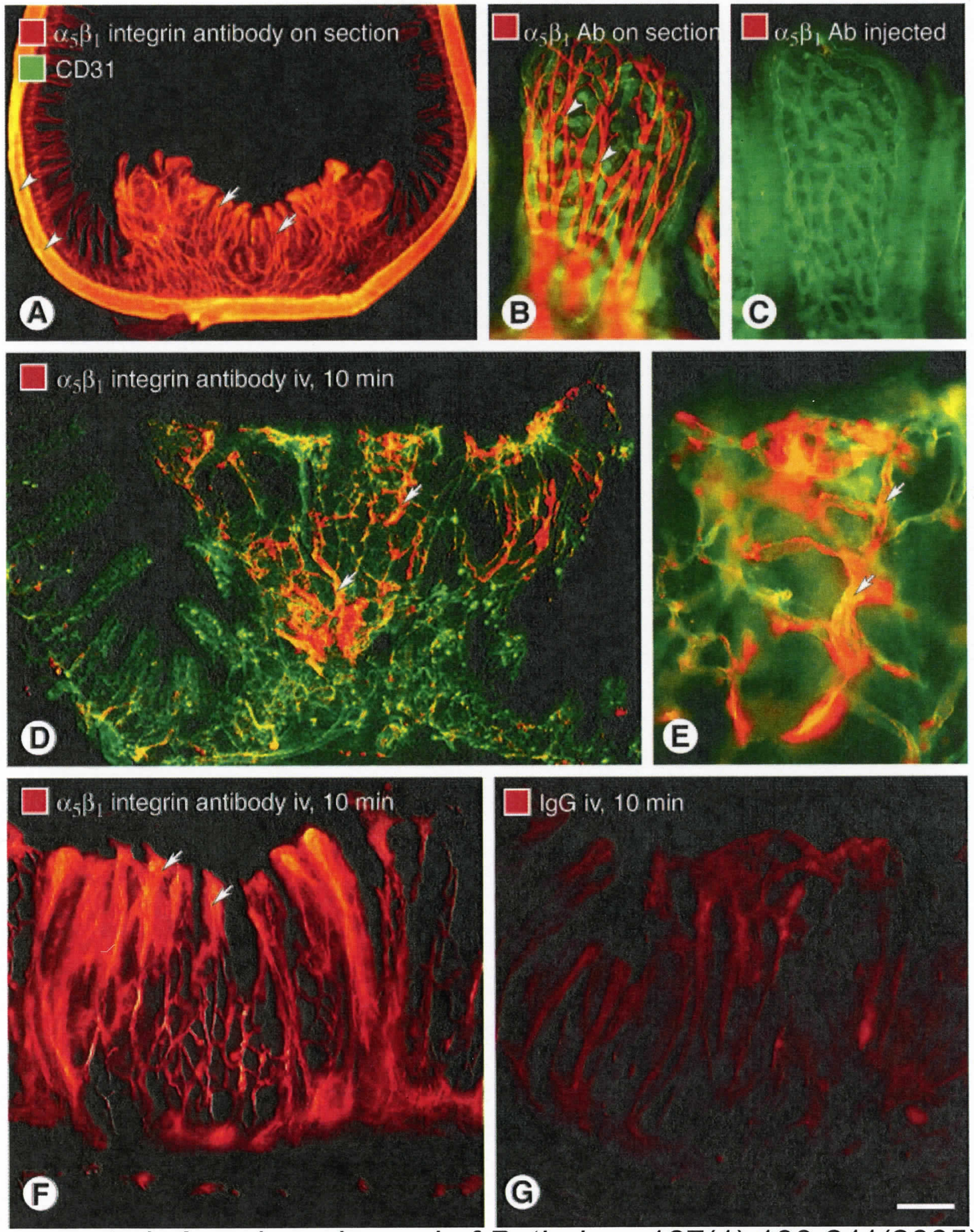
Parsons et al, *American Journal of Pathology* 167(1):193-211(2005)

Vascular Targeting: Increasing Expression of $\alpha5\beta1$ Integrin in Blood Vessels with RIP-Tag2Tumor Progression



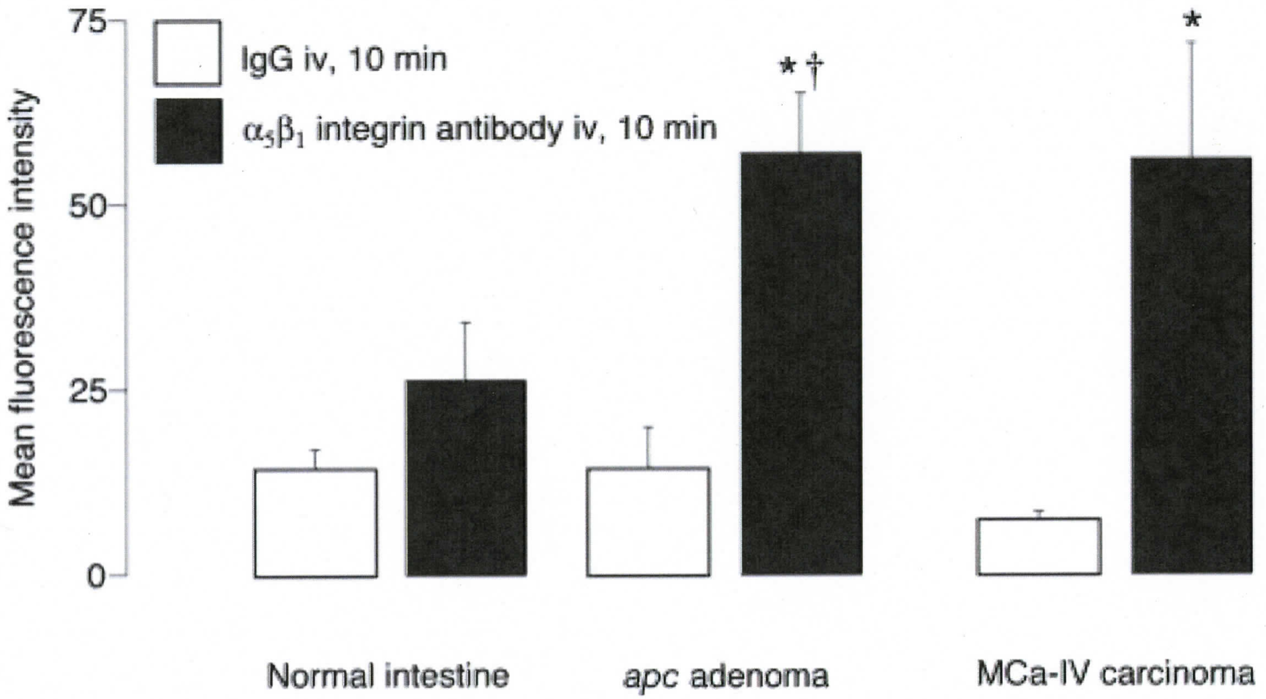
Parsons et al, *American Journal of Pathology* 167(1):193-211(2005)

Vascular Targeting: $\alpha5\beta1$ Integrin Antibody *iv* in Transgenic *apc* Intestinal Tumors



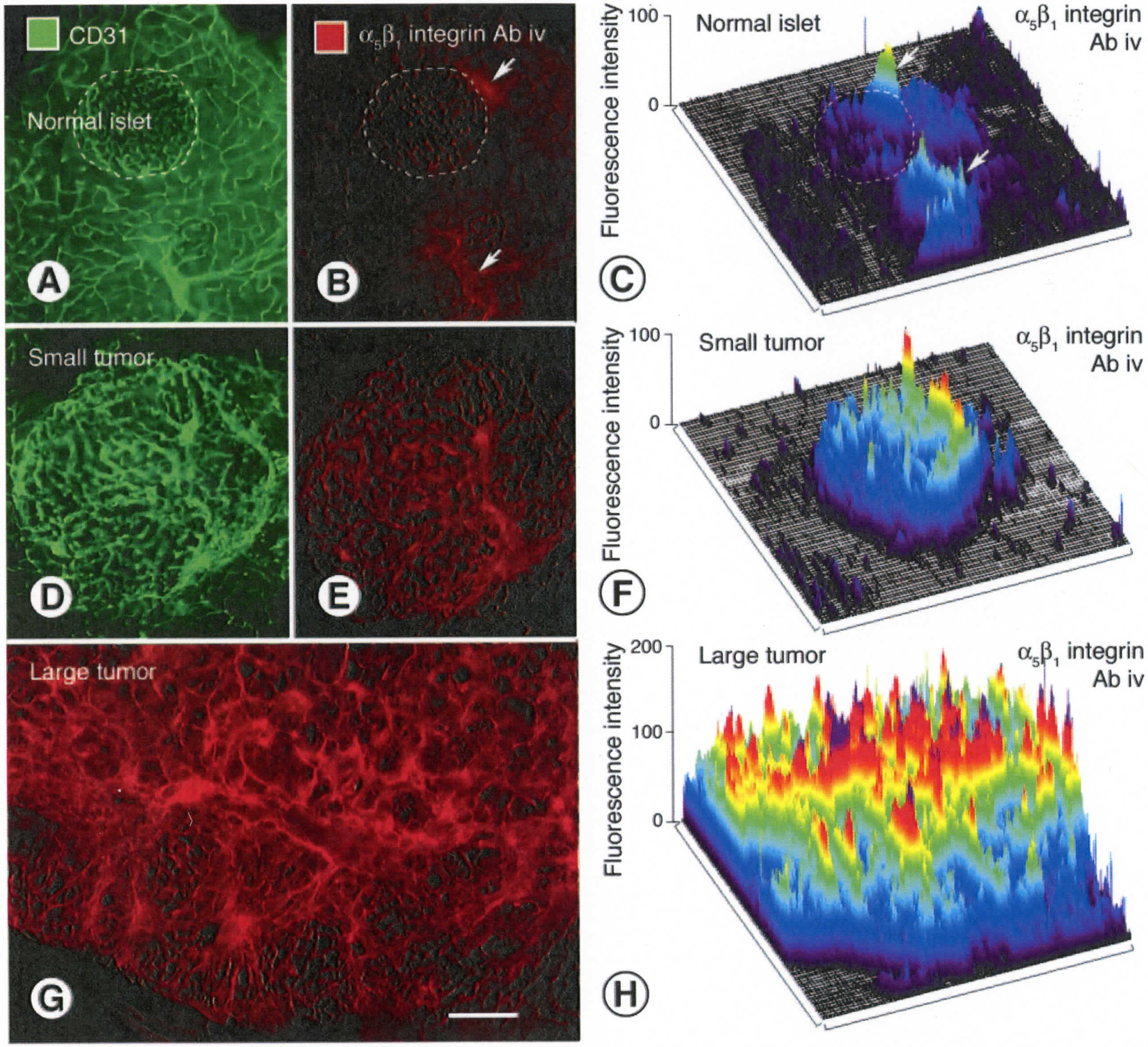
Parsons et al, *American Journal of Pathology* 167(1):193-211(2005)

Increased Expression of $\alpha 5\beta 1$ Integrin Antibody *iv* in Transgenic *apc* Intestinal and MCa-IV Mammary Tumors

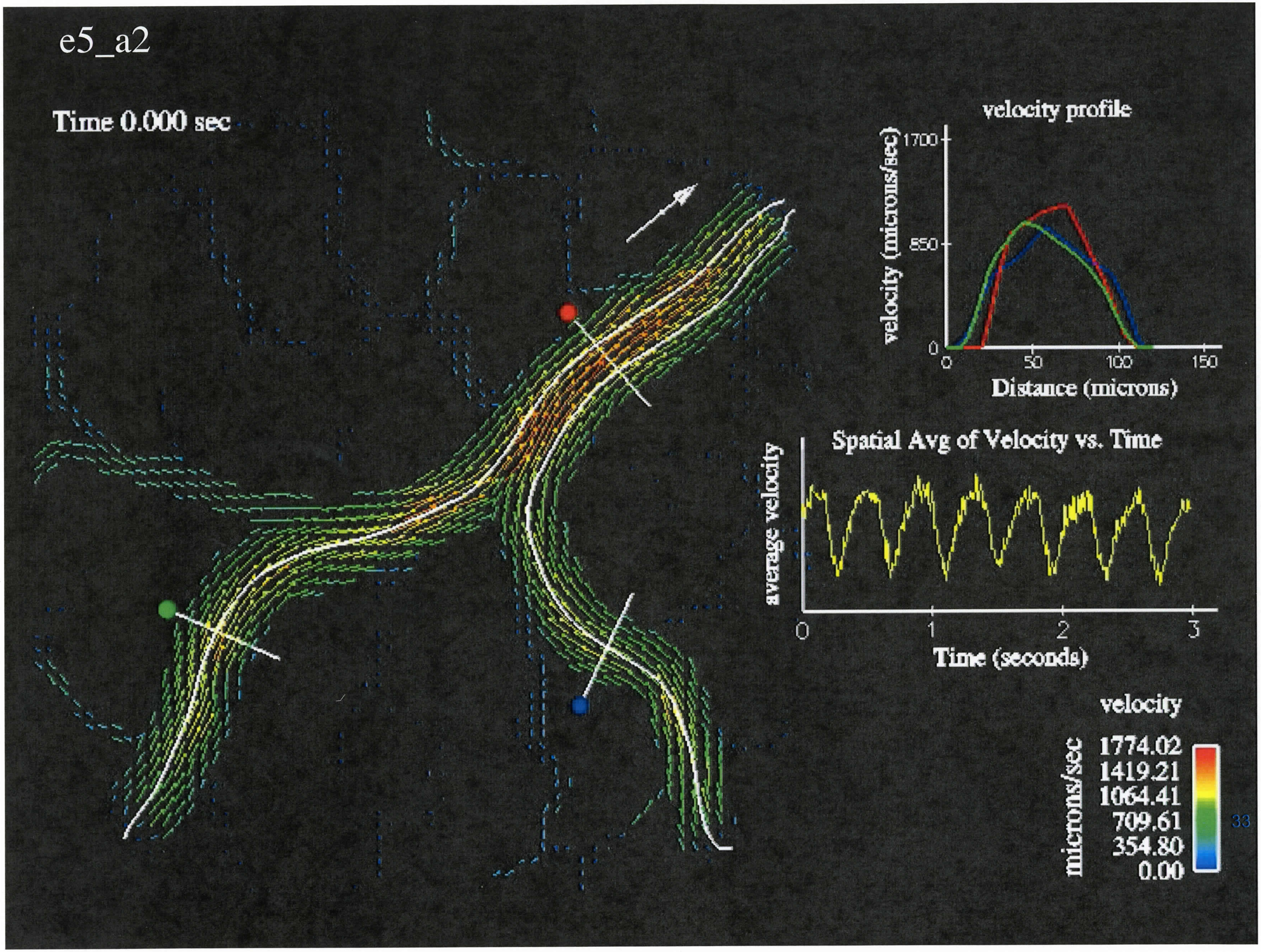


Parsons et al, *American Journal of Pathology* 167(1):193-211(2005)

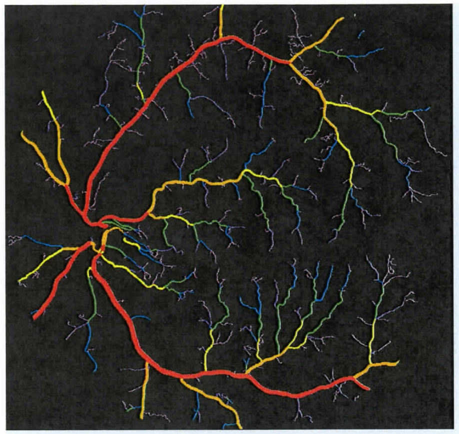
FRACTAL SCALING of Vascular Network and Integrin Expression with Tumor Progression



Parsons et al, *American Journal of Pathology* 167(1):193-211(2005)

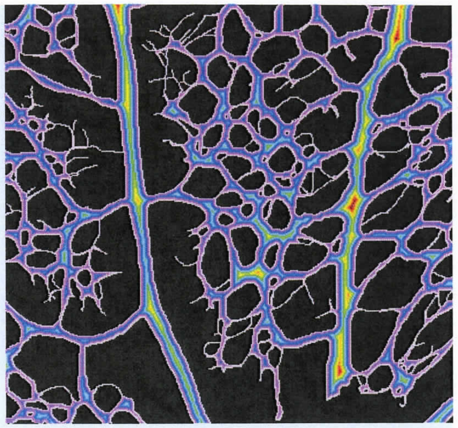


e5_a2
Time 0.000 sec
velocity profile
velocity (microns/sec)
1700
550
0
0
50
100
150
Distance (microns)
Spatial Avg of Velocity vs. Time
average velocity
0
1
2
3
Time (seconds)
velocity
microns/sec
1774.02
1419.21
1064.41
709.61
354.80
0.00



VESGEN

for Vascular Mapping & Quantification



Integrative Systems Biology:
'Fingerprint' or 'Signature' Mapping of Dominant Vascular Patterns Induced by VEGF and Other Angiogenesis Regulators

Fractal-Based VESGEN for Multi-Parametric Pattern Analysis according to Branching Generation

Clinical Research Application: **Oscillation of Angiogenesis with Vascular Dropout in Diabetic Retinopathy**

General paradigm for progression of other diseases such as cancer?

Integrated Scaling of Vascular Anatomy with Signaling Regulators such as $\alpha 5\beta 1$ Integrin during Tumor Progression

Acknowledgements

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Lerner Research Institute
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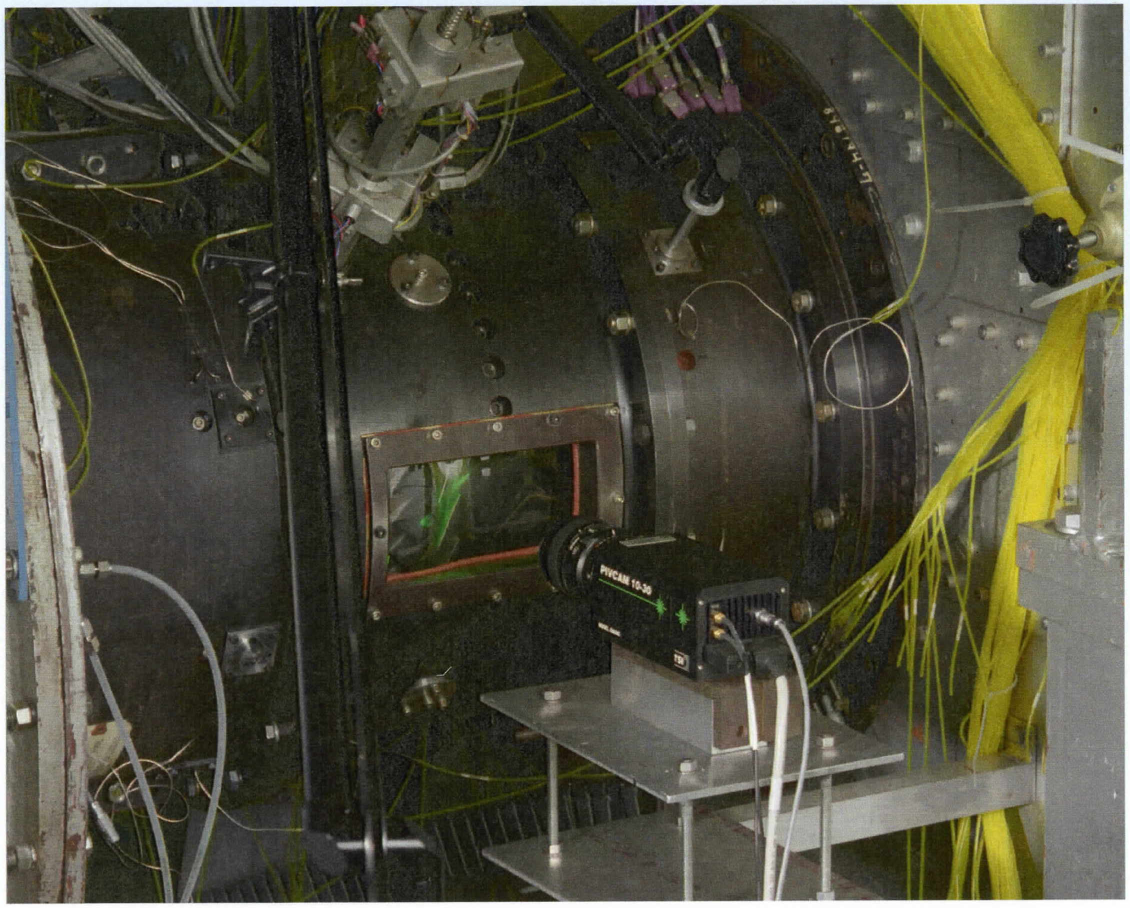
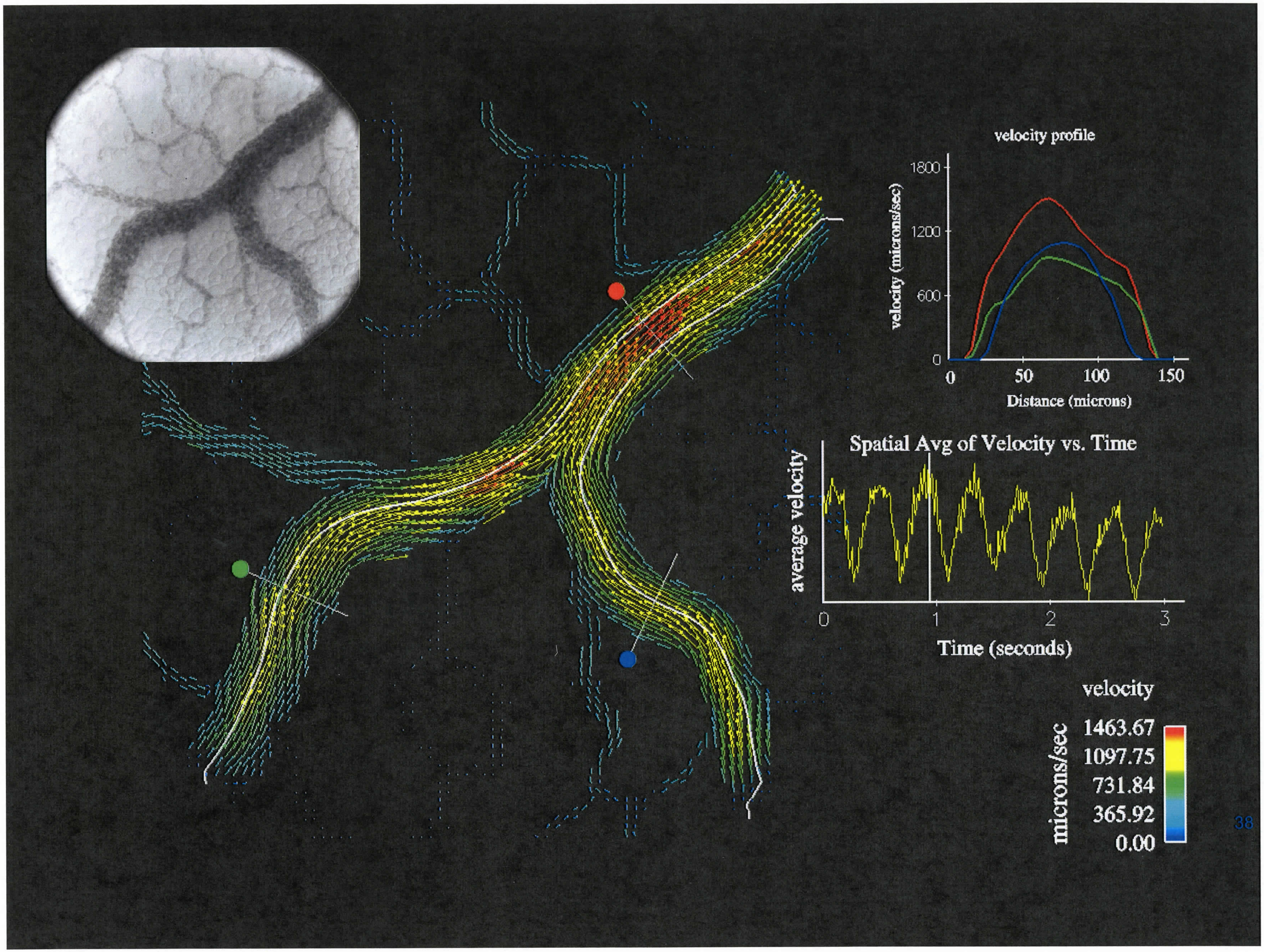
University of New Mexico School of Medicine/Ohio Aerospace Institute
Krishnan Radhakrishnan MD PhD

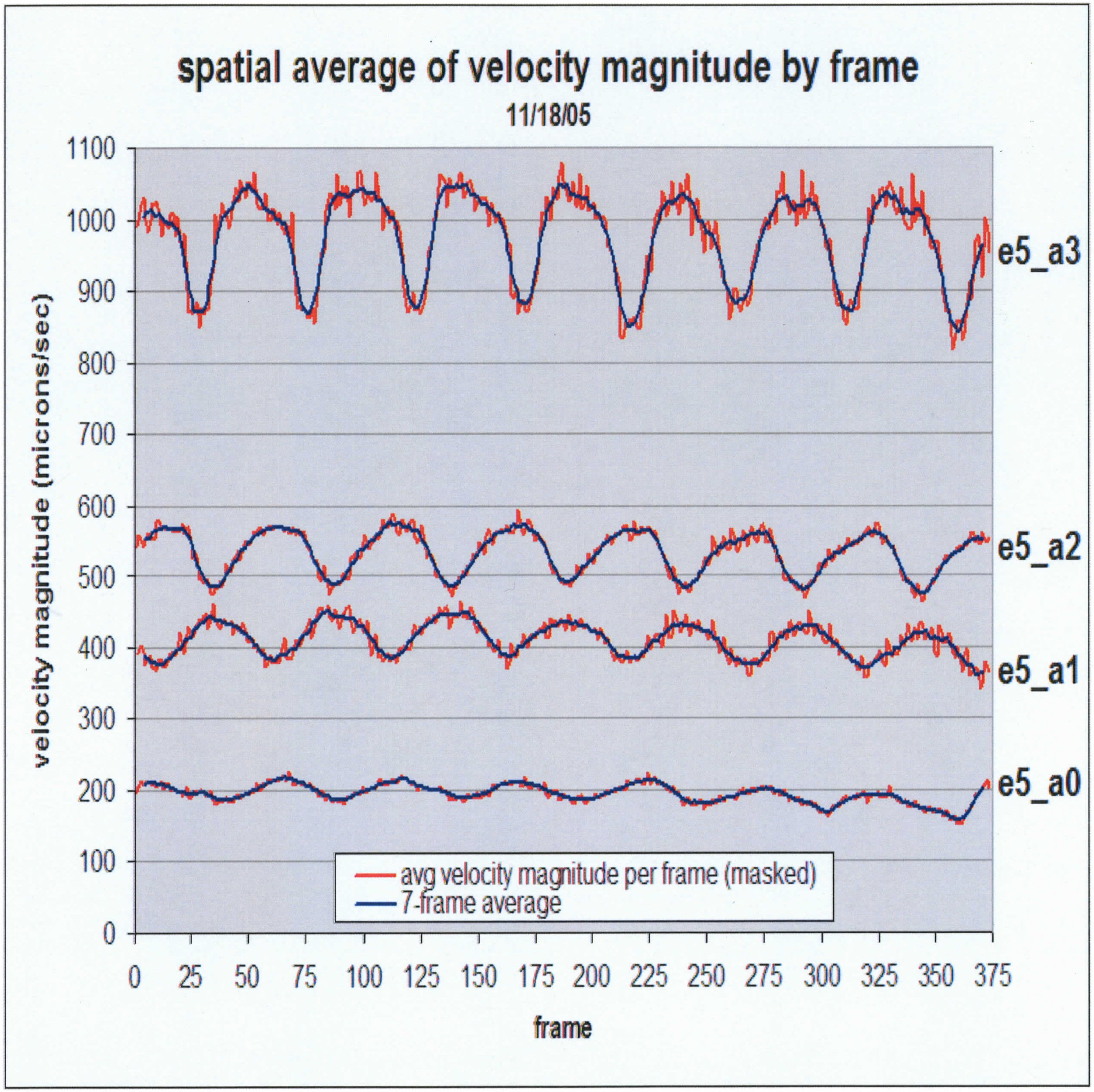
University Hospitals, Case Western Reserve University
Steven Fisher MD, Hong-Bin Liu PhD
Michiko Watanabe PhD, Ganga Karunamuni BS, Monica Montano PhD

NASA, NIH, NSF (University of Washington)

Particle Imaging Velocimetry (PIV) for Analysis of Blood Flow *in Vivo*

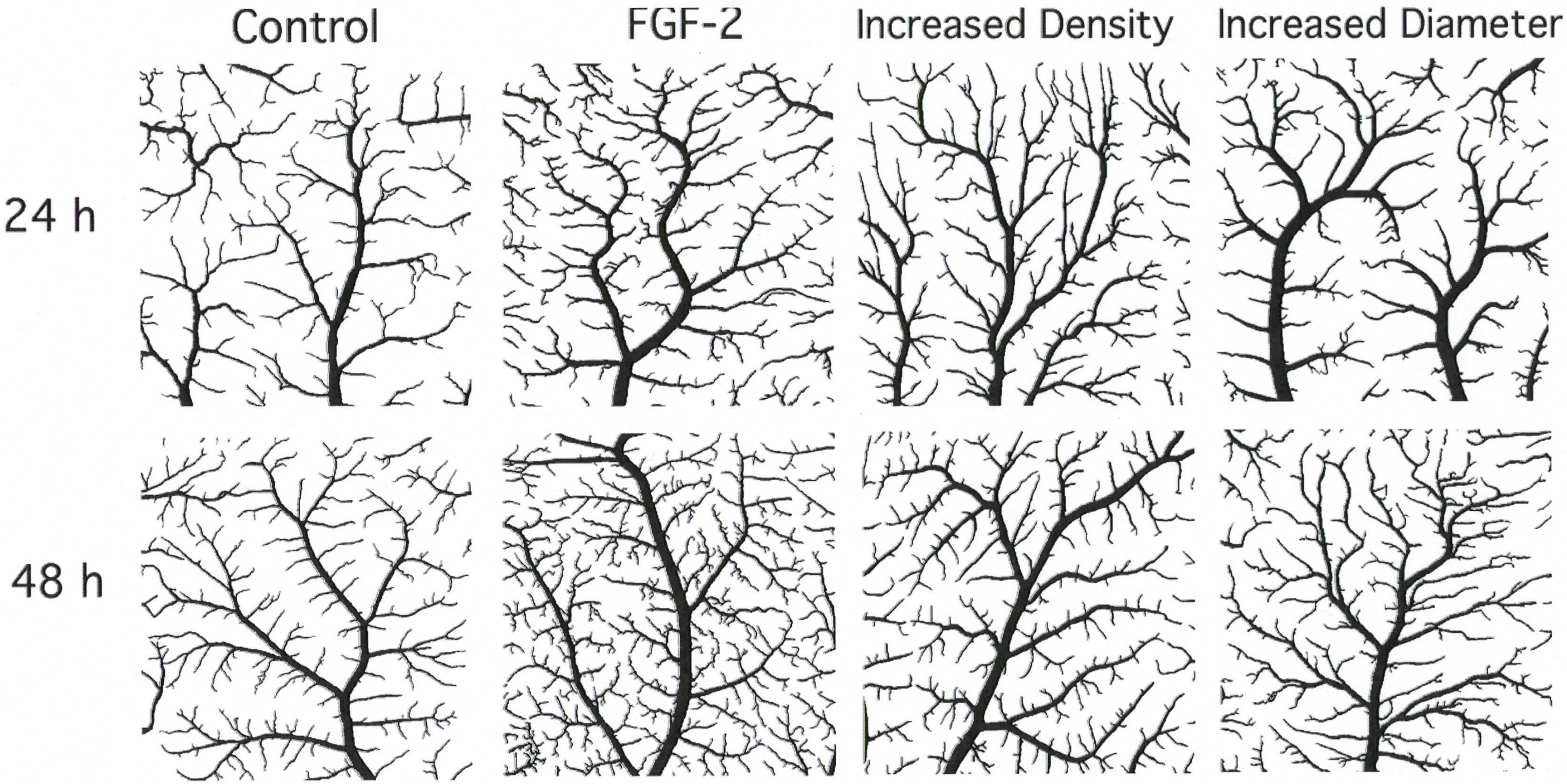



velocity profile
1800
1200
600
0
velocity (microns/sec)
0
50
100
150
Distance (microns)
Spatial Avg of Velocity vs. Time
average velocity
0
1
2
3
Time (seconds)
velocity
microns/sec
1463.67
1097.75
731.84
365.92
0.00



spatial average of velocity magnitude by frame
11/18/05
velocity magnitude (microns/sec)
1100
1000
900
800
700
600
500
400
300
200
100
0
0
25
50
75
100
125
150
175
200
225
250
275
300
325
350
375
frame
e5_a3
e5_a2
e5_a1
e5_a0
avg velocity magnitude per frame (masked)
7-frame average

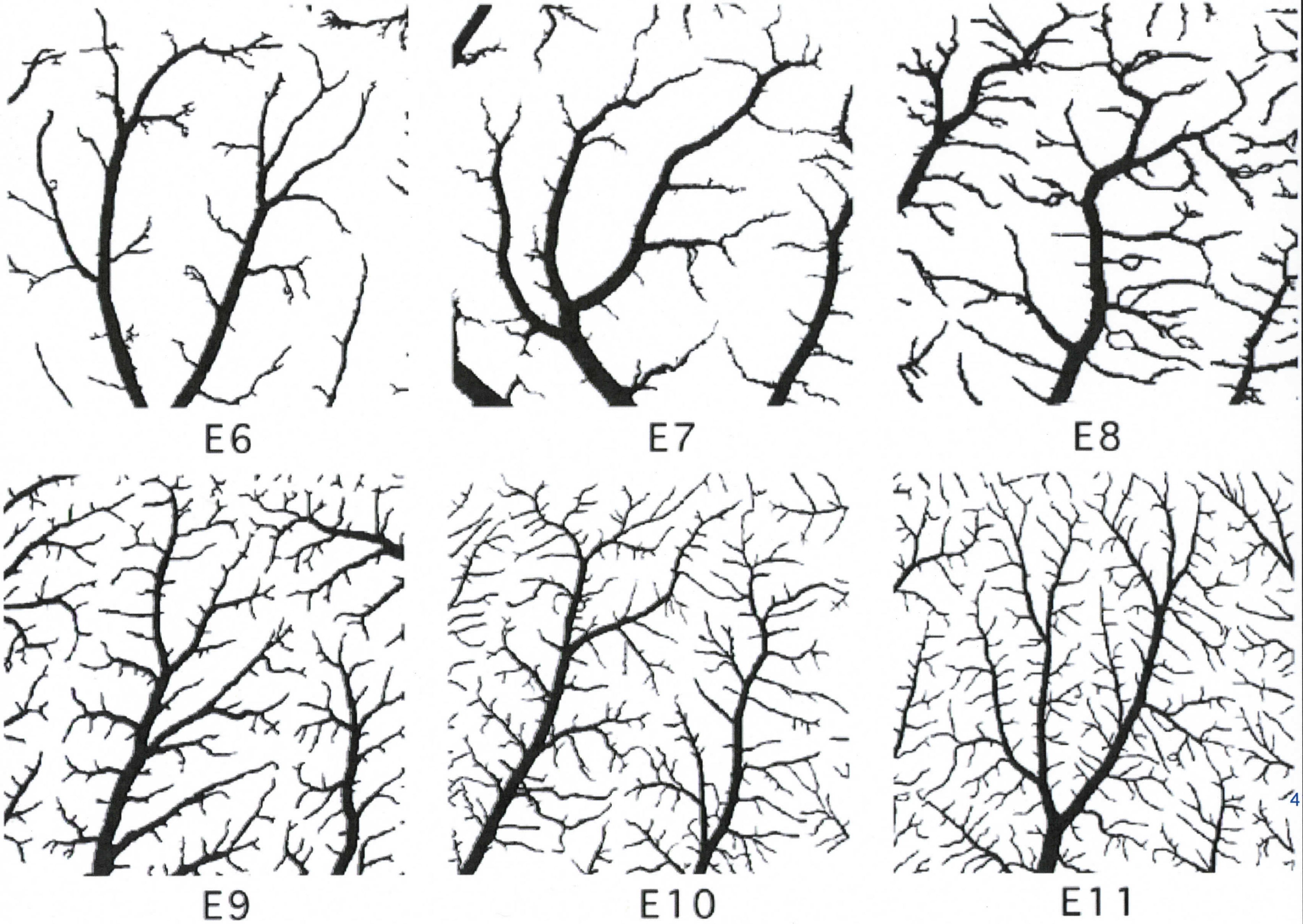
Stimulation of Angiogenesis



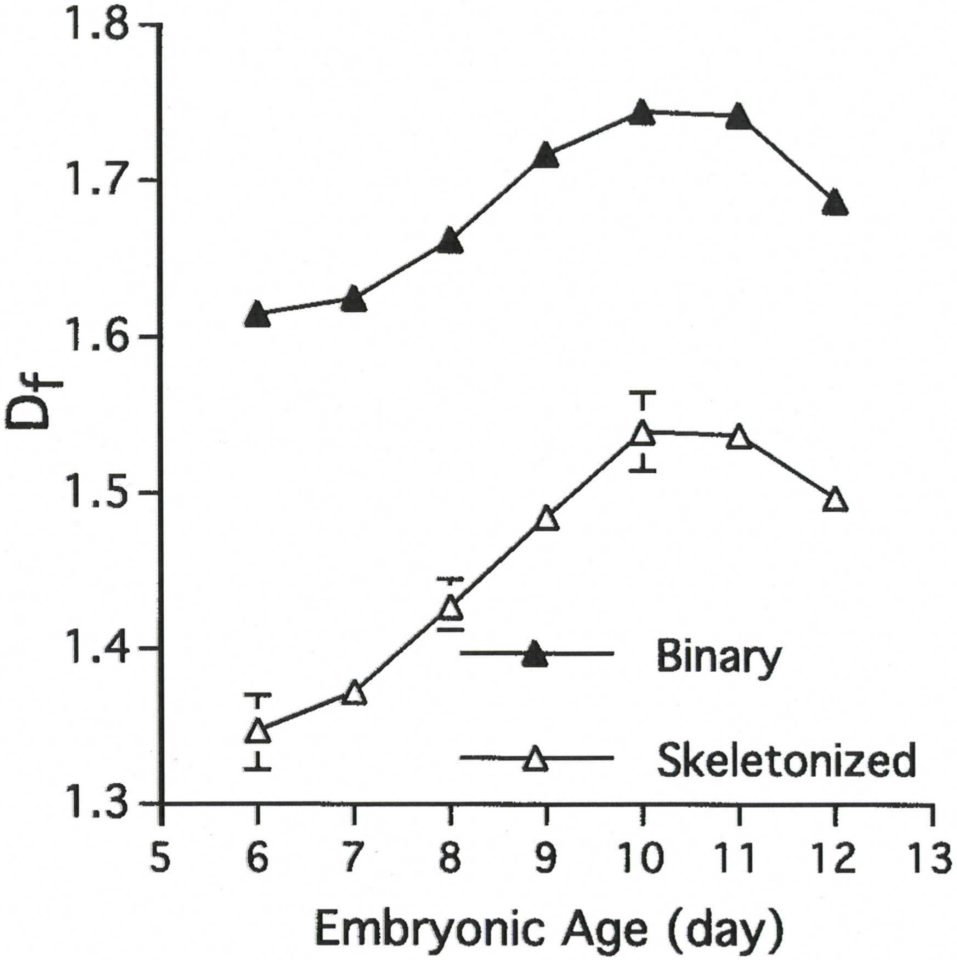
Microvascular Research 72(3):91-100 167(1):193-211(2006)

Arteriosclerosis Thrombosis Vascular Biology 20:1250-1256 (2000)

Angiogenesis in the Developing Quail CAM

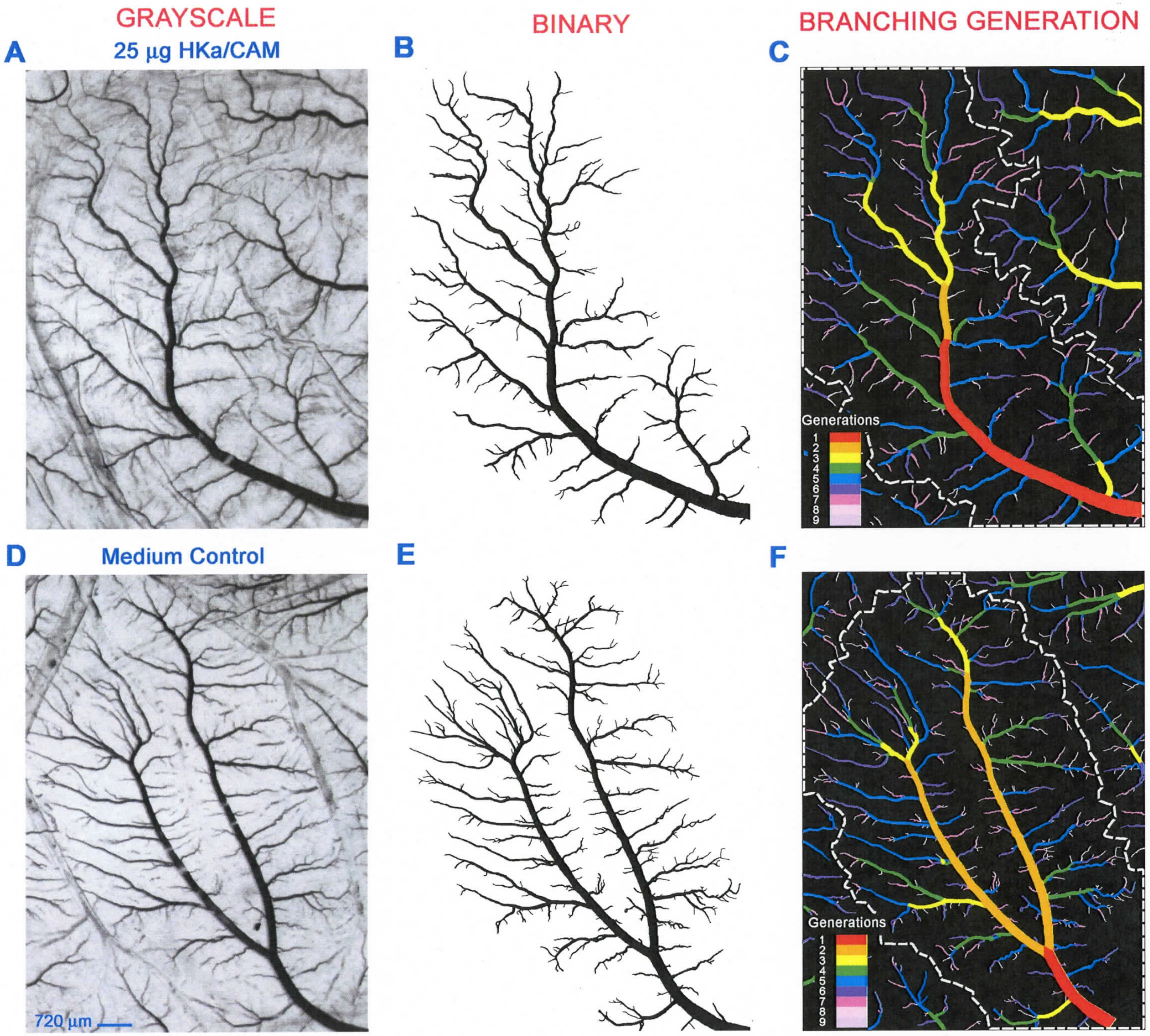


The Rate of Angiogenesis in the Quail CAM is a Linear Process



Parsons et al, Microvascular Research 55(3):201(1998)

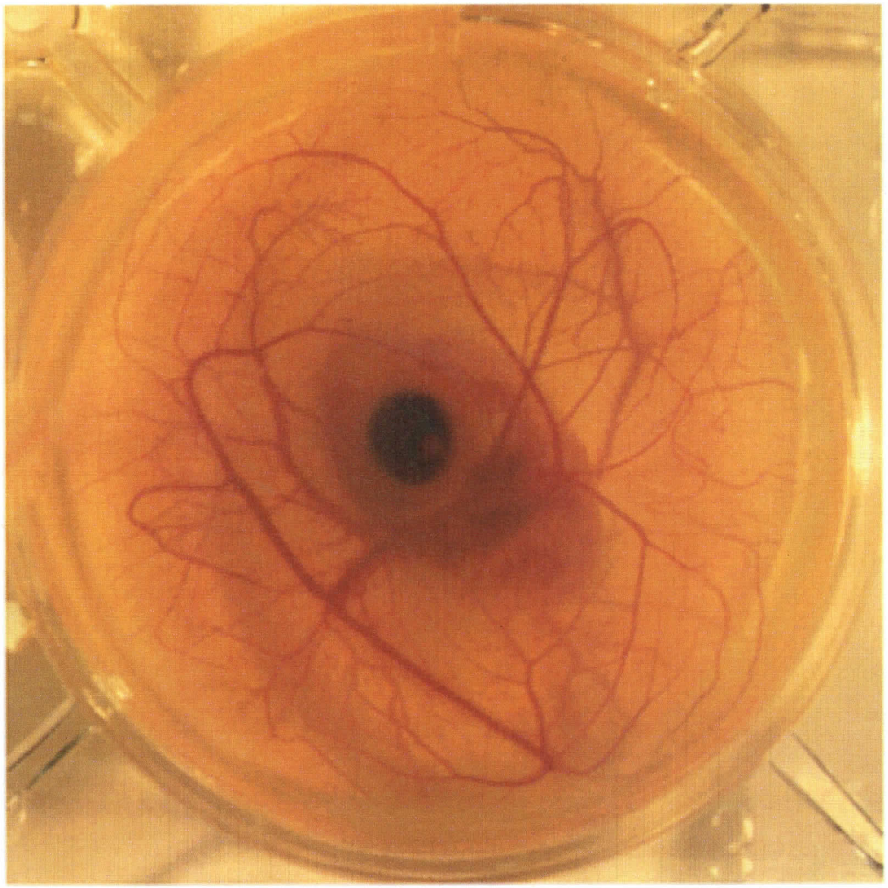
HMW Kininogen Treatment in CAM Vascular Tree



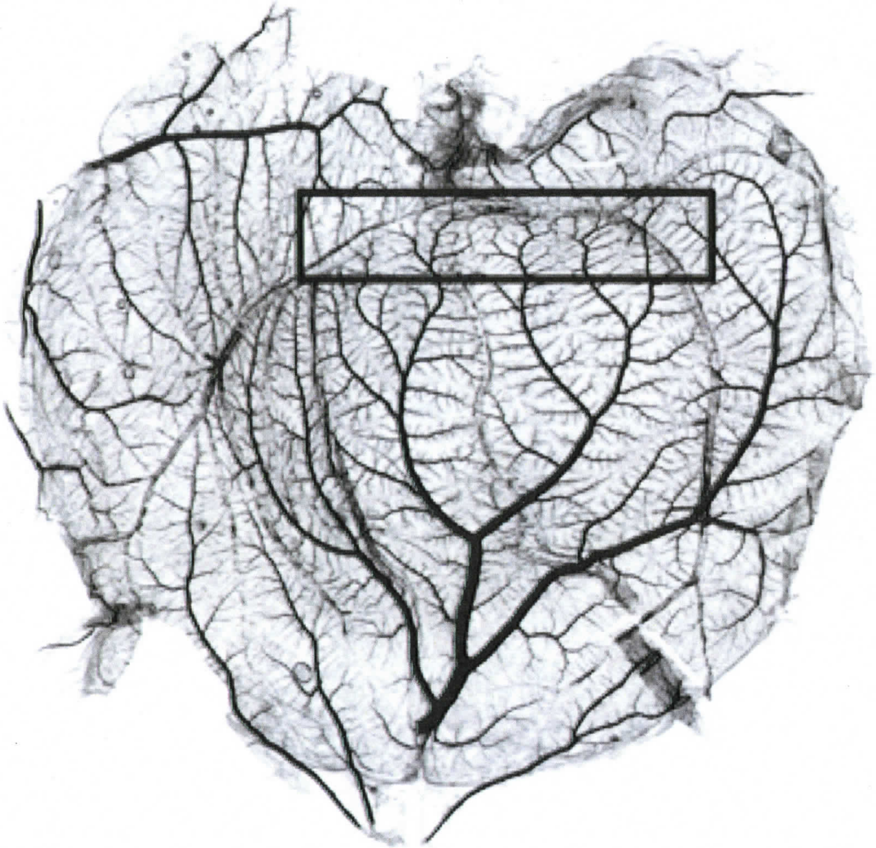
Keith McCrae, Hematology & Oncology

*Engineering Testbed for **VESGEN** Innovations:*

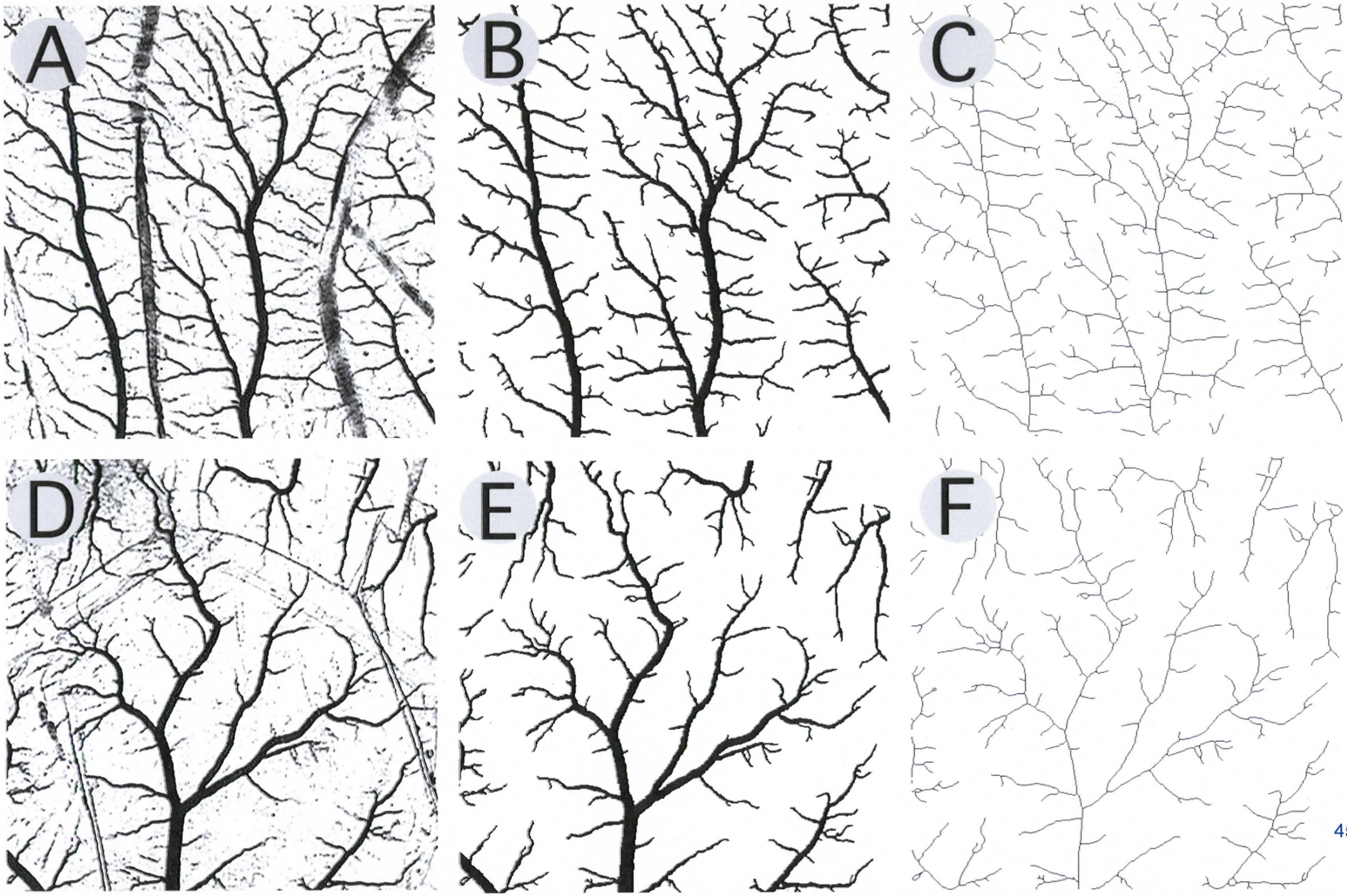
Quail Chorioallantoic Membrane (CAM)



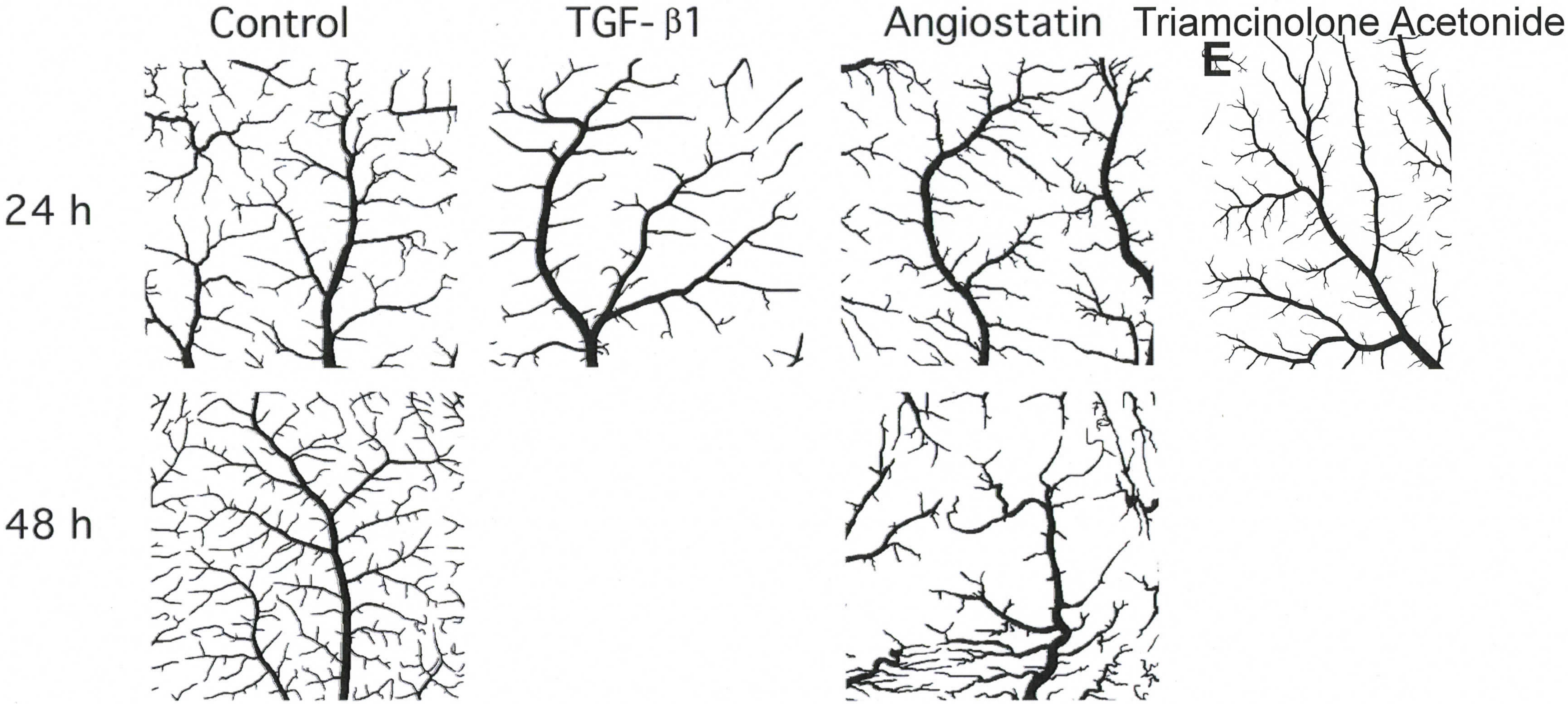
Embryo



CAM Specimen


A
B
C
D
E
F

Inhibition of Angiogenesis



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

Microvascular Research 59:221-232(2000)
Microvascular Research 55:201-214(1998)

Unique *'Signature'* Patterns:

Vasculature as Integrative Read-Out System of Complex Molecular Signaling

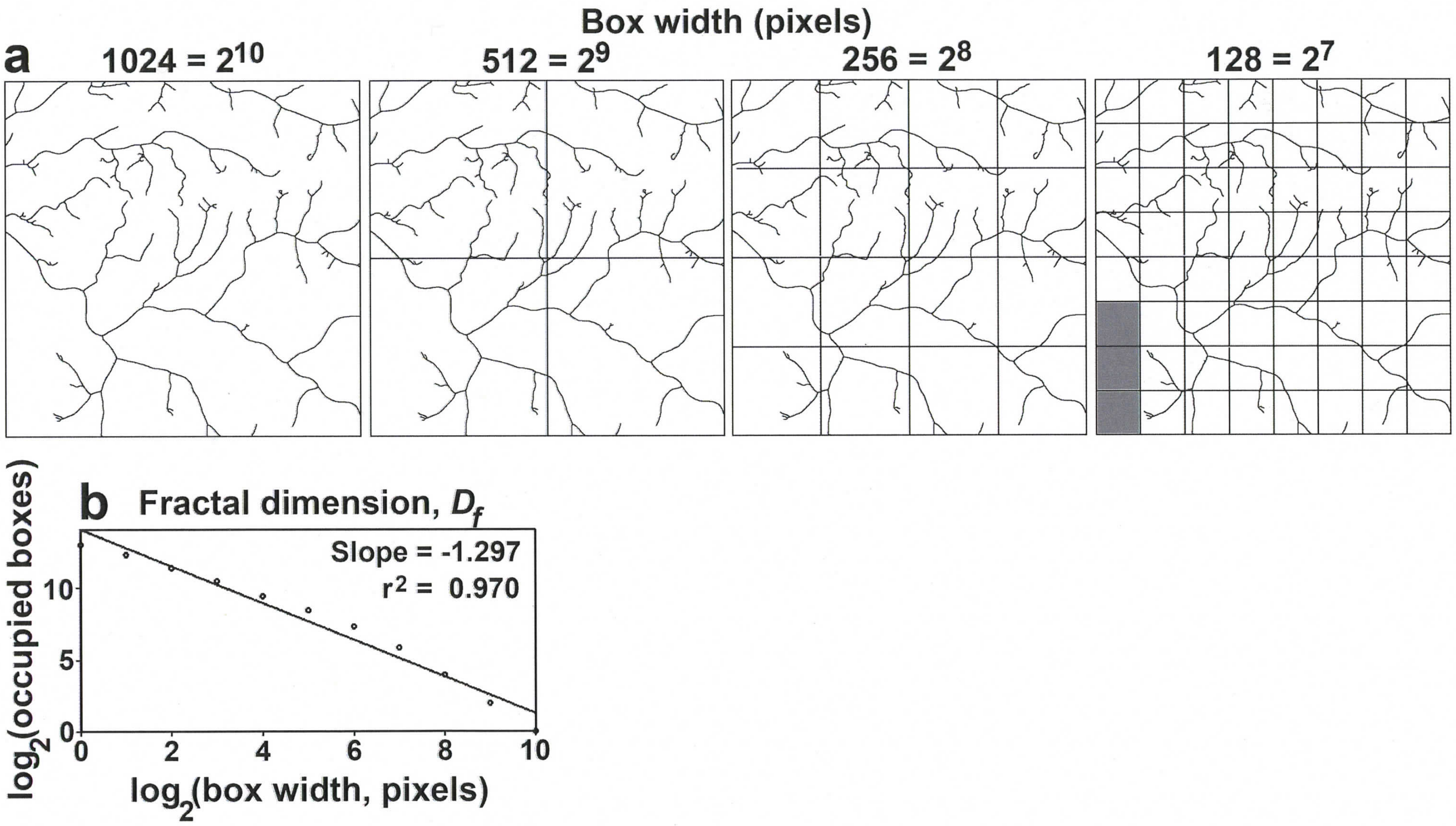
Observation

Dominant molecular regulators of vascular remodeling and angiogenesis induce vascular patterns that are spatio-temporally unique

Hypothesis as Consequence

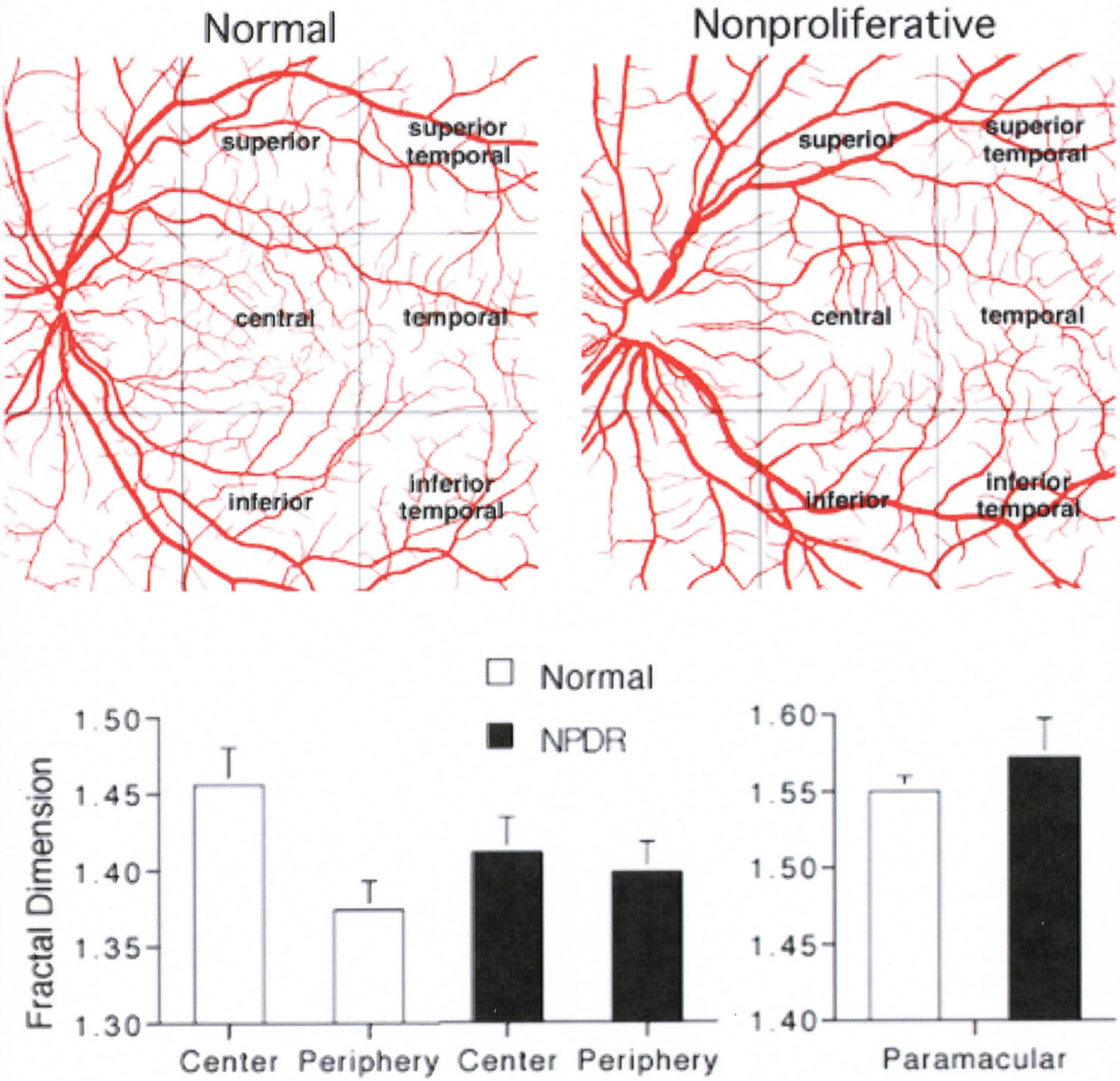
Dominant regulators can be deduced from alterations in vascular pattern as integrative read-out of complex molecular and systems signaling

Box-Counting Algorithm for Fractal Dimension



K Radhakrishnan, P Parsons-Wingerter, M B Vickerman, P K Kaiser

Vascular Pattern is Altered in Early-Stage Diabetic Retinopathy



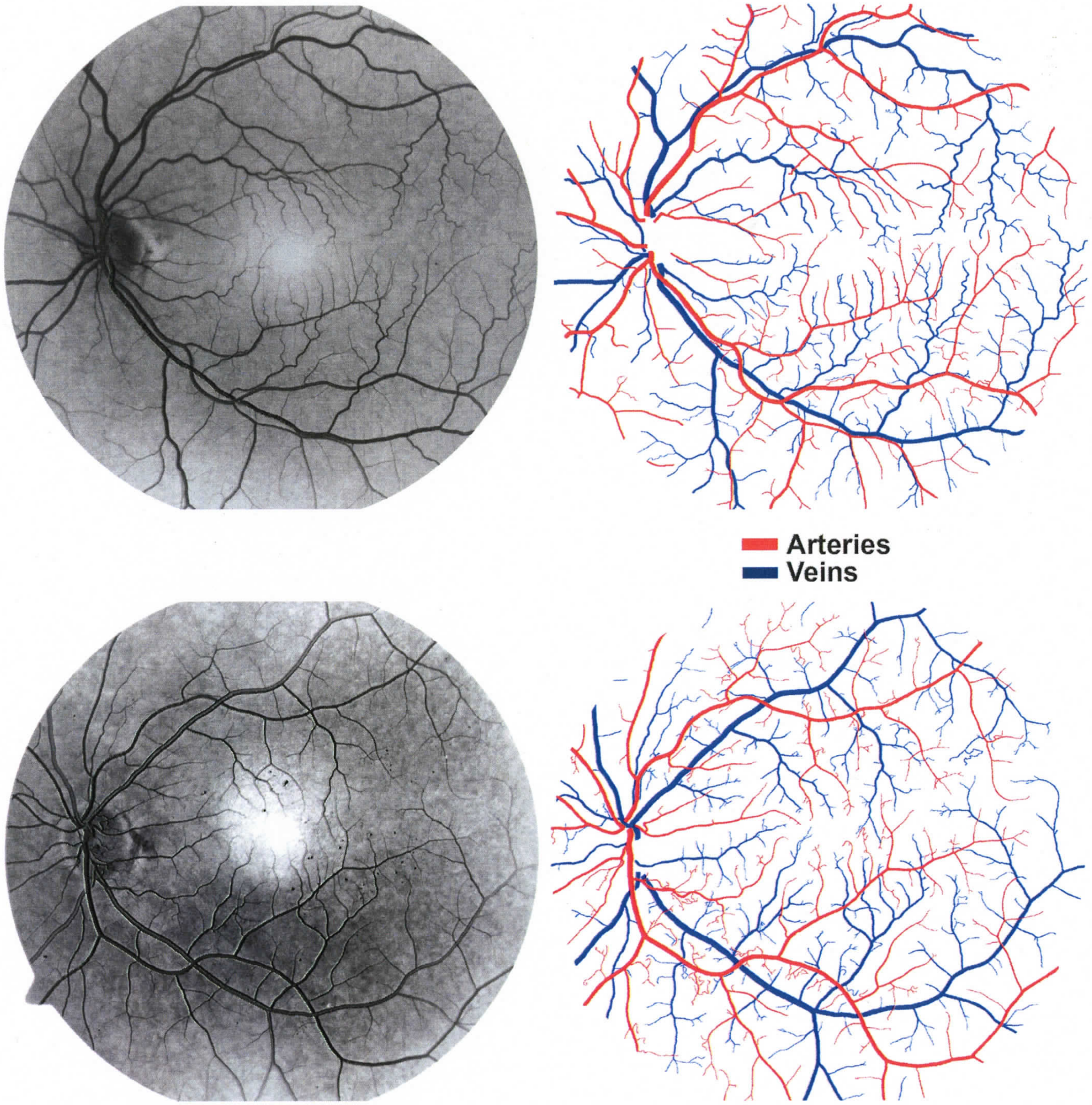
Clinical Fluorescein Angiography of Human Retina

50° FA

Vascular Trees

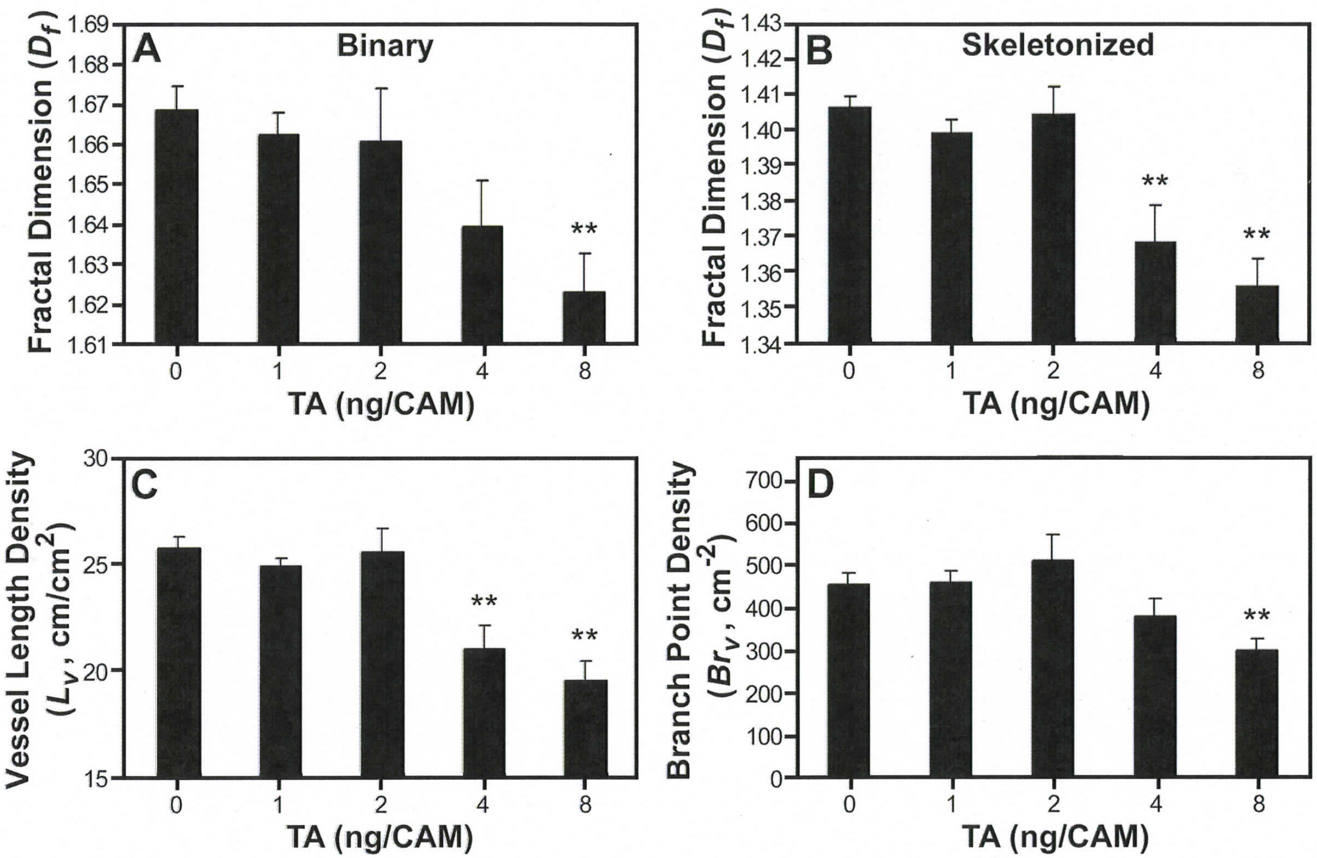
Mild NPDR

Mild/Moderate NPDR



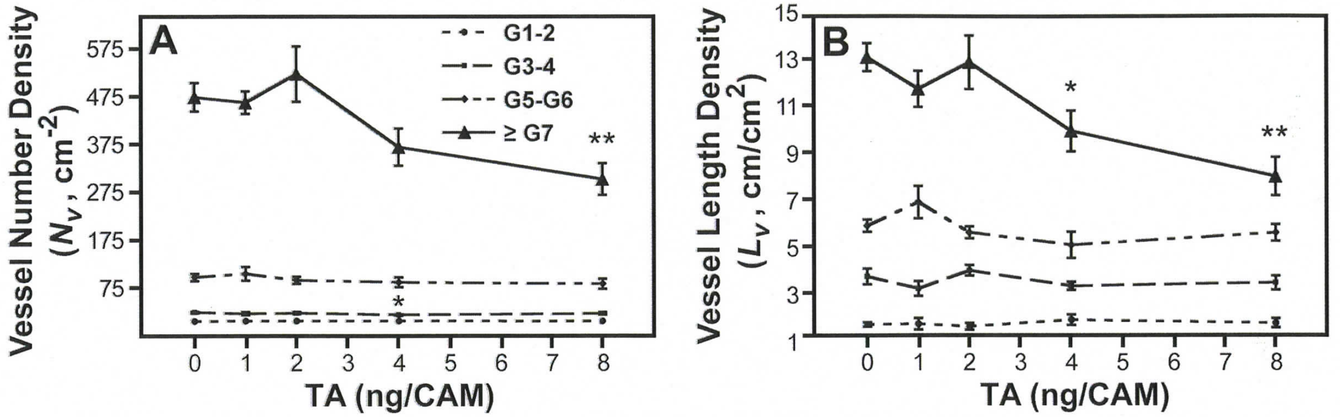
Krishnan Radhakrishnan, Peter Kaiser (Cole Eye Institute)

Steroid Triamcinolone Acetonide(TA): Inhibition of Angiogenesis in the CAM

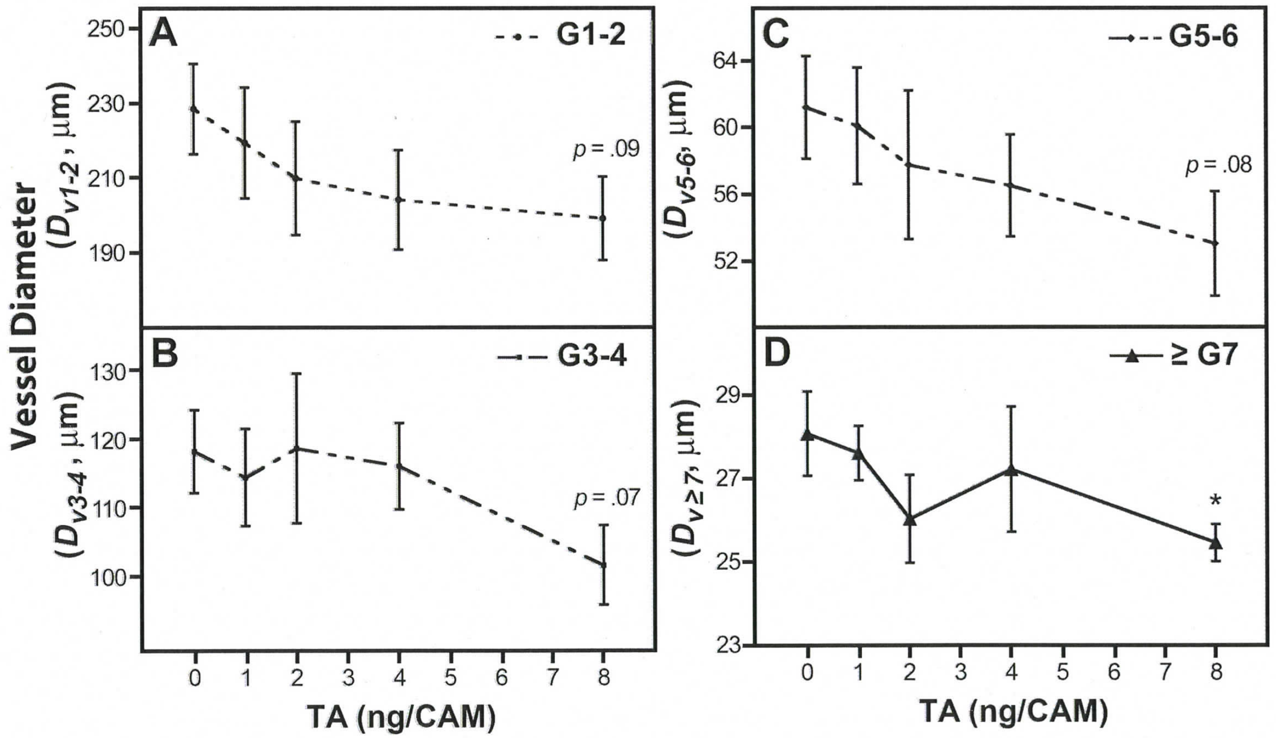


McKay et al, *Investigative Ophthalmology & Visual Science* 49:1184 2008

TA Inhibition Selectively Targets Small Vessels

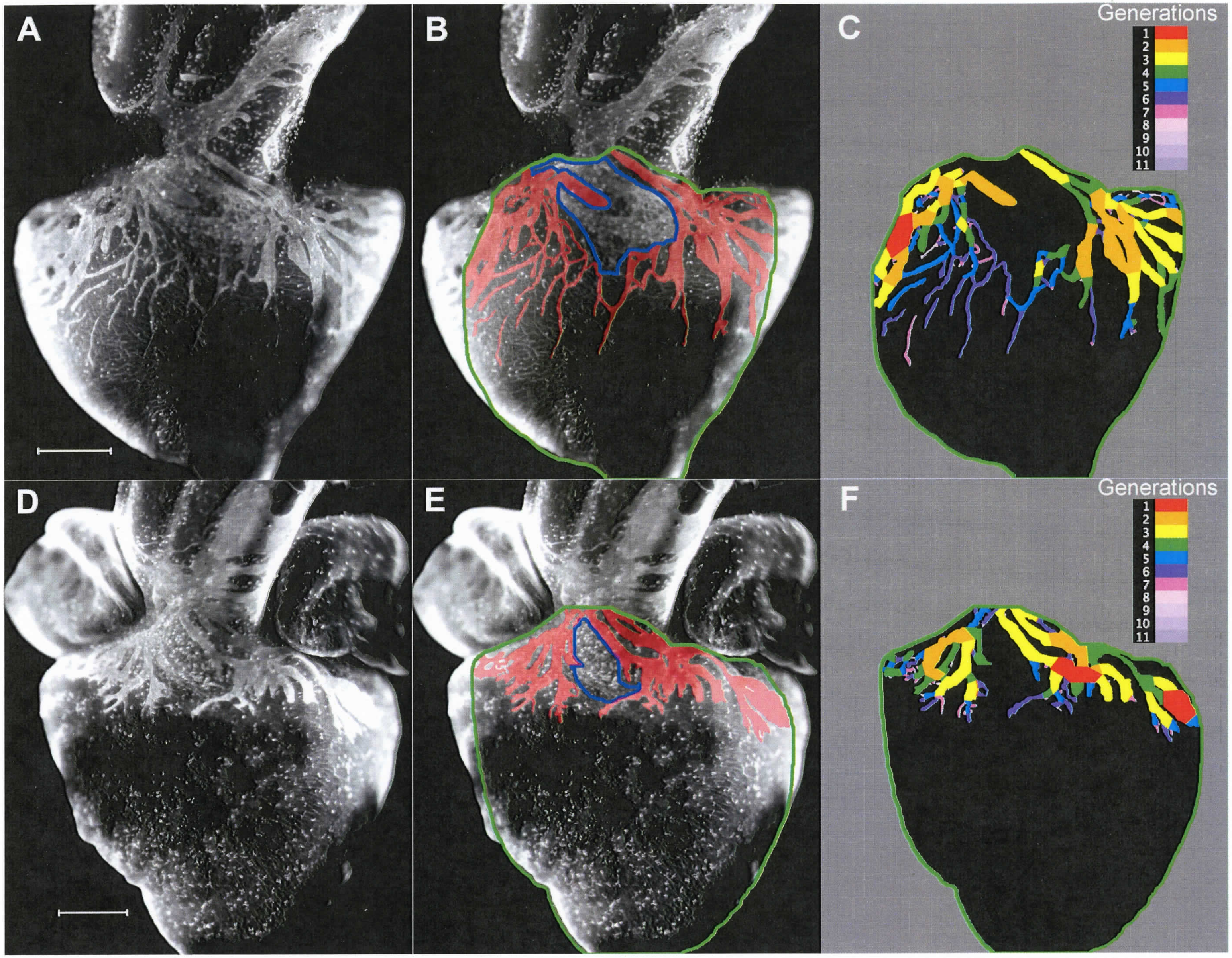


and Thins Vessels throughout the Vascular Tree



McKay et al, *Investigative Ophthalmology & Visual Science* 49:1184 2008

VEGF TRAP Expression in Developing Coronary Tree



Steven Fisher, Hong-Bin Liu (Cardiology), Krishnan Radhakrishnan