

Microarray data analysis of space grown *Arabidopsis* leaves for genes important in vascular patterning. A. J. Weitzel,^{1,2} S. E. Wyatt³, P. Parsons-Wingerter¹.

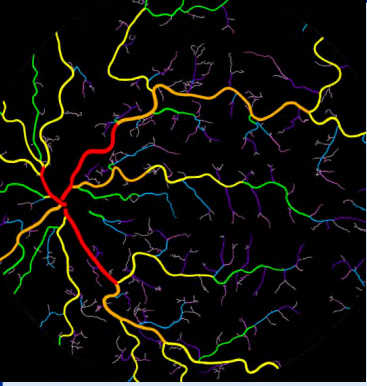
¹Space Biosciences, NASA Ames Research Center, Mountain View, CA

²Biology Undergraduate, Grand Valley State University, Allendale, MI

³Environmental and Plant Biology, Ohio University, Athens, OH

Venation patterning in leaves is a major determinant of photosynthesis efficiency because of its dependency on vascular transport of photoassimilates, water, and minerals. *Arabidopsis thaliana* grown in microgravity show delayed growth and leaf maturation. Gene expression data from the roots, hypocotyl, and leaves of *A. thaliana* grown during spaceflight vs. ground control analyzed by Affymetrix microarray are available through NASA's GeneLab (GLDS-7). We analyzed the data for differential expression of genes in leaves resulting from the effects of spaceflight on vascular patterning. Two genes were found by preliminary analysis to be upregulated during spaceflight that may be related to vascular formation. The genes are responsible for coding an ARGOS like protein (potentially affecting cell elongation in the leaves), and an F-box/kelch-repeat protein (possibly contributing to protoxylem specification). Further analysis that will focus on raw data quality assessment and a moderated t-test may further confirm upregulation of the two genes and/or identify other gene candidates. Plants defective in these genes will then be assessed for phenotype by the mapping and quantification of leaf vascular patterning by NASA's VESsel GENERation (VESGEN) software to model specific vascular differences of plants grown in spaceflight.

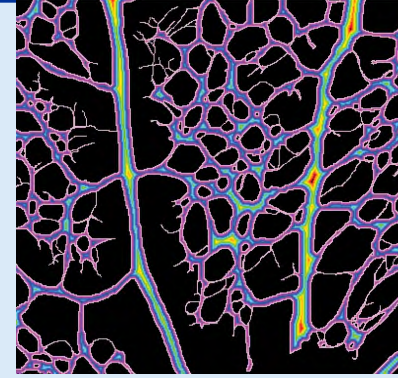
Research supported by a NASA Ames Center Innovation Fund Award.



Human Retina

NASA'S VESGEN Software

Research Discovery Tool
for Fundamental and Translational
Space Biology Research



Mouse Retina

Using NASA's GeneLab for VESGEN Systems Analysis of Vascular Phenotypes from Stress and Other Signaling Pathways

Patricia Parsons-Wingenter
Space Biosciences Research Branch

VESGEN Patents Pending

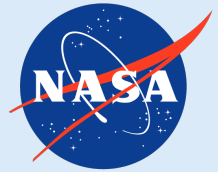


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Collaborators

Alexander Weitzel, USRA Summer 2016 Internship, NASA Ames Research Center & Grand Valley State University,

Ruchi Vyas and Matthew Murray, MORI Associates and Blue Marble Space Institute, NASA Ames Research Center

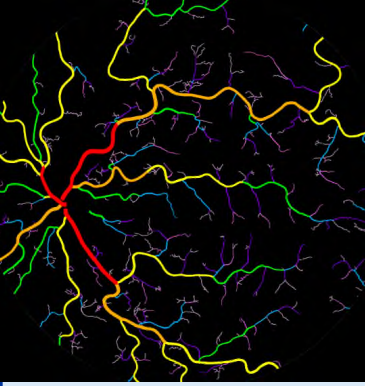
Mary B. Vickerman, Software Systems, NASA Glenn Research Center

Sharmila Bhattacharya Space Biosciences Research Branch, NASA Ames Research Center

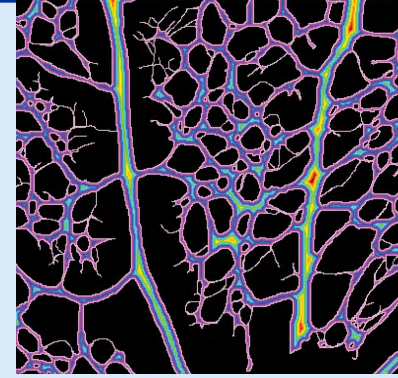
Sarah E. Wyatt, Environmental and Plant Biology, Ohio University, Athens OH

NASA'S VESsel GENeration Analysis [VESGEN] Software

Mapping and Quantification of Branching Vascular Patterns from Physiological Branching Rules

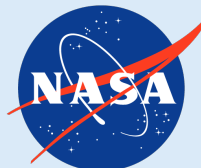


Human Retina



Mouse Retina

- Vascular trees, networks, and tree-network composites from set of weighted parameters for vessel connectivity, tapering and bifurcational branching
- Requirements of fluid dynamics for laminar flow
Aqueous vascular transport by complex distributed system of fractal-based bifurcational branching
- Microvascular rules for fractal-based branching within humans, vertebrates, insects and dicot leaves therefore display many similarities



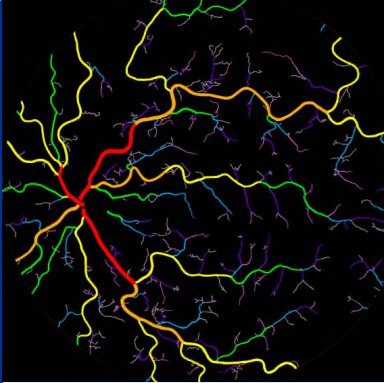


Parsons



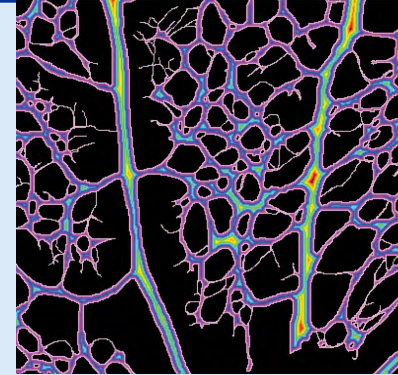
NASA





Human Retina

VESGEN Analysis for Fundamental Space Biology Research with Translational Applications to Astronaut Health and Countermeasures



Mouse Retina

Vascular Trees

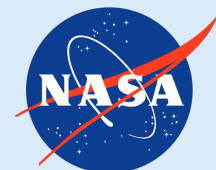
Retinas of Astronauts and Human Bed Rest; Diabetic Retinopathy
Mouse/Avian Coronary Vessels, Chorioallantoic Membrane (CAM), Yolksac

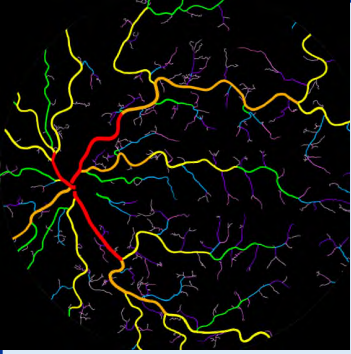
Vascular Networks

Mouse Intestinal Inflammation, CAM Lymphatic Vessels, Abnormal Mouse Corneal
Angiogenesis, *Drosophila* (Fruitfly) Wing

Vascular Tree-Network Composites

Mouse Postnatal Retina
Early Embryonic Coronary Vessels, *Arabidopsis* Leaf Venation

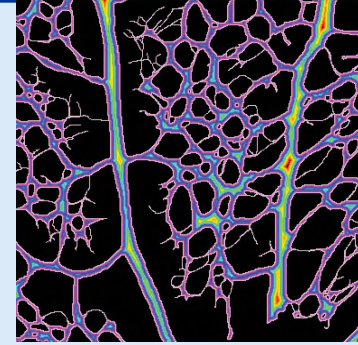




Human Retina

VESGEN

Analysis of Vascular Patterns



Mouse Retina

Research Hypothesis

Vascular patterning offers useful readout of molecular signaling that necessarily integrates crosstalk among complex signaling pathways

Fractal-Based Physiological Branching Rules

from fluid mechanics, anatomy, microscopic observations

Mapping and Quantification by Multiparametric Weighted Analysis

Fractal Dimension, D_f

Vessel Number Density, N_v

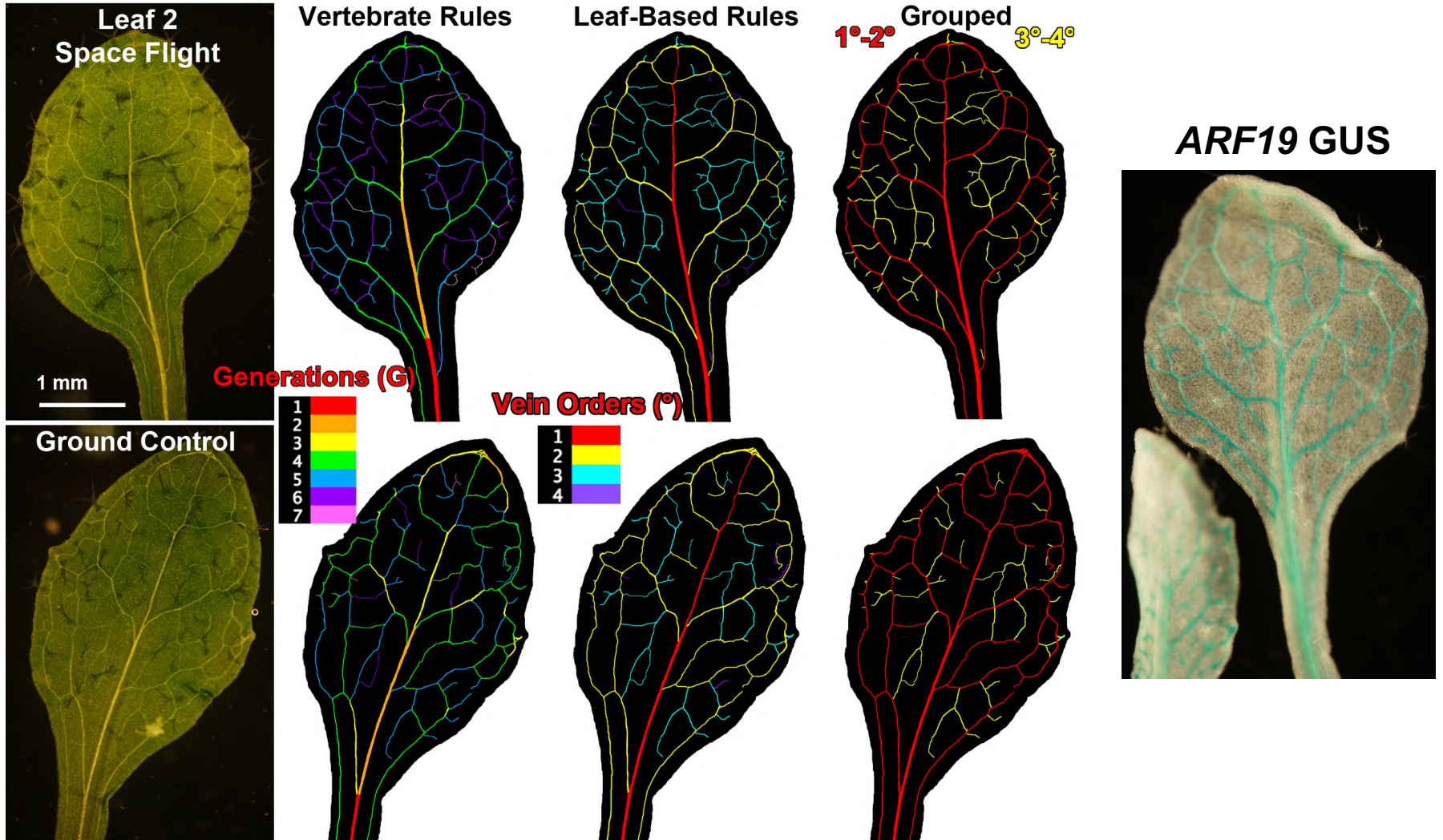
Vessel Length Density, L_v

Vessel Diameter, D_v

Branchpoint + Endpoint Densities, $Br_v + E_v$

Arabidopsis leaves from ISS: STS-130

Juvenile Leaf 2



VESGEN mapping of Arabidopsis leaf venation with bioinformatic analysis

Kang & Dengler, *Int J Plant Sci*

165(2): 231 2004 ©University of Chicago Press

Taxonomy & Phylogeny:

Grouping by Venous
Branching Orders

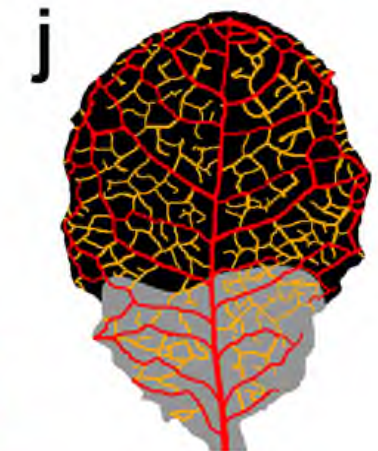
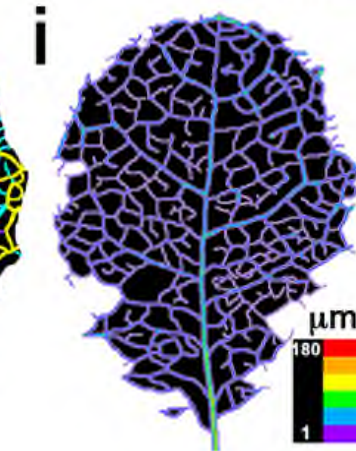
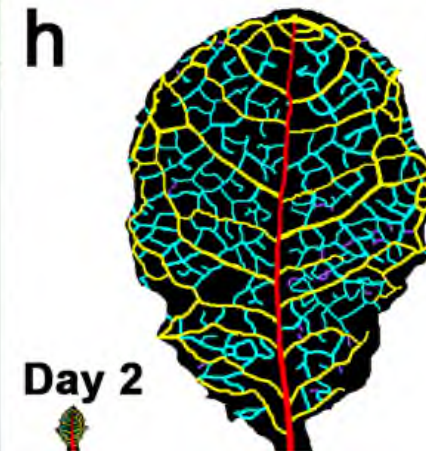
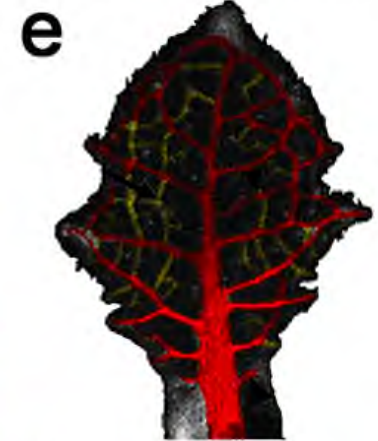
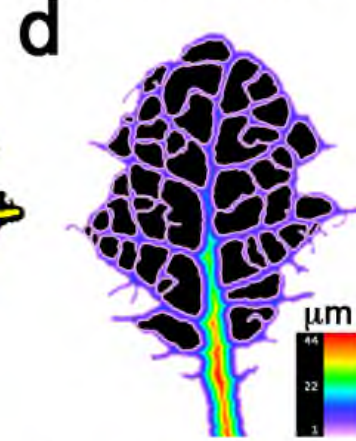
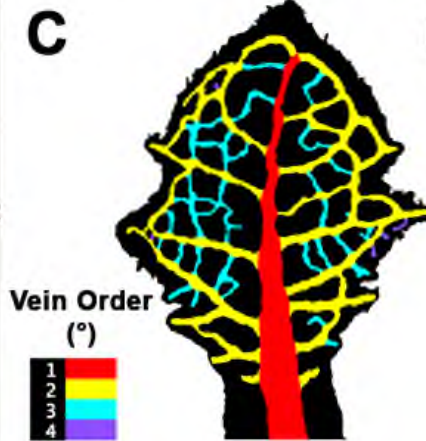
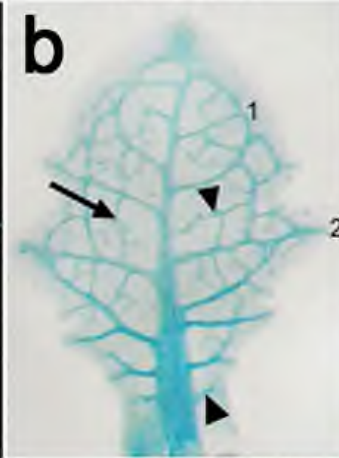
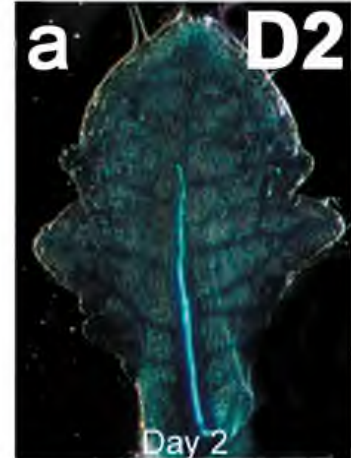
Venous Diameter by
Distance Mapping

Integrative Bioinformatics:

AtHB8::GUS by Structural &
Reticulate Vein Grouping

Differentiated
Xylem

AtHB8::GUS
Expression





GeneLab

Open Science
for Exploration

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The Arabidopsis spaceflight transcriptome: a comparison of whole plants to discrete root, hypocotyl and shoot responses to the orbital environment



2 datasets available for download here:

[ISA-TAB Metadata file](#)
[Raw Data File](#)

| GeneLab Accession Number | GLDS-7 | | | | | | | | | | | | | | | | |
|---------------------------------|--|-----------------------|--|--------------|-------|-------------|--------------|-----------------------|--|----------------|--------------|-----------------------|--|----------------|-----------|-----------------------|--|
| Source Accession Number | E-MTAB-1264 | | | | | | | | | | | | | | | | |
| Contacts | <table border="1"><thead><tr><th>Name</th><th>Role</th><th>Organization</th><th>Email</th></tr></thead><tbody><tr><td>Robert Ferl</td><td>Investigator</td><td>University of Florida</td><td>robferl@ufl.edu</td></tr><tr><td>Anna-Lisa Paul</td><td>Investigator</td><td>University of Florida</td><td>alp@ufl.edu</td></tr><tr><td>Agata Zupanska</td><td>Submitter</td><td>University of Florida</td><td>zupanska@ufl.edu</td></tr></tbody></table> | Name | Role | Organization | Email | Robert Ferl | Investigator | University of Florida | robferl@ufl.edu | Anna-Lisa Paul | Investigator | University of Florida | alp@ufl.edu | Agata Zupanska | Submitter | University of Florida | zupanska@ufl.edu |
| Name | Role | Organization | Email | | | | | | | | | | | | | | |
| Robert Ferl | Investigator | University of Florida | robferl@ufl.edu | | | | | | | | | | | | | | |
| Anna-Lisa Paul | Investigator | University of Florida | alp@ufl.edu | | | | | | | | | | | | | | |
| Agata Zupanska | Submitter | University of Florida | zupanska@ufl.edu | | | | | | | | | | | | | | |

Space Grown *Arabidopsis* with Microarray Data from GeneLab: Identification of Genes Important in Vascular Patterning

(A Weitzel, P Parsons, S Wyatt; ASGSR 2016)

- Analysis of transcriptomic data from space flight and ground control leaves identified differential expression of 22 genes, of which seven may be related to plant vasculature
- Two gene clusters suggest there may be phenotypic changes in leaf venation resulting from development in microgravity
 - **KISS ME DEADLY [KMD]** coding F-box genes
 - **NAM, ATAF1/2, and CUC2 [NAC]** related genes
- Vascular-related changes in leaf gene expression can potentially be phenocopied by mutants in ground-based experiments and corroborated by VESGEN analysis
- Genetic, transcriptional and other molecular changes reported by GeneLab can be mapped to vascular phenotypes by VESGEN by bioinformatic co-localization of single molecular expression

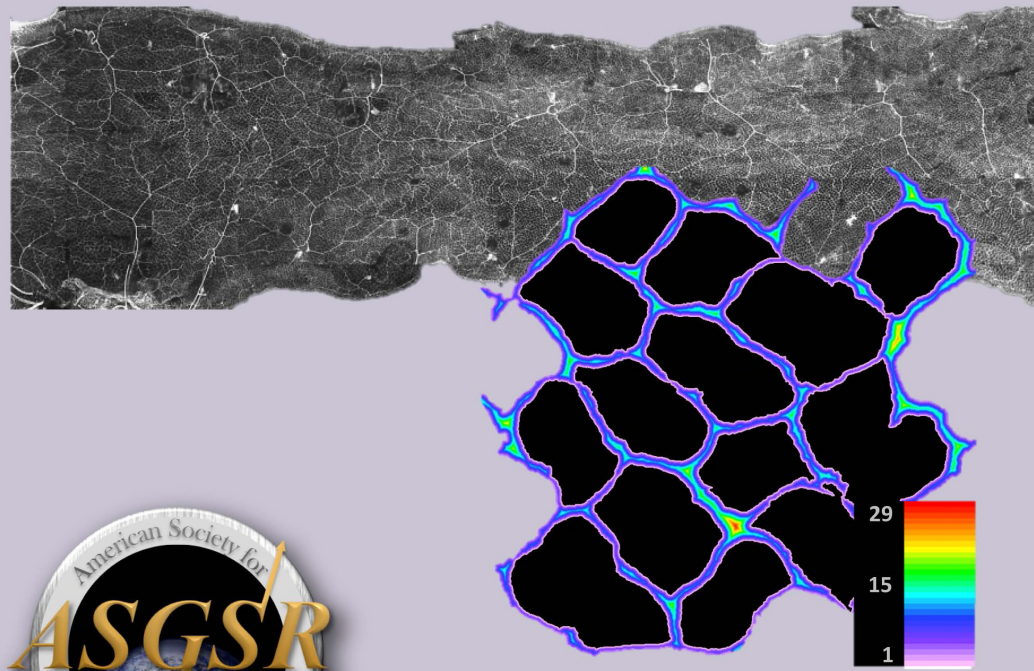


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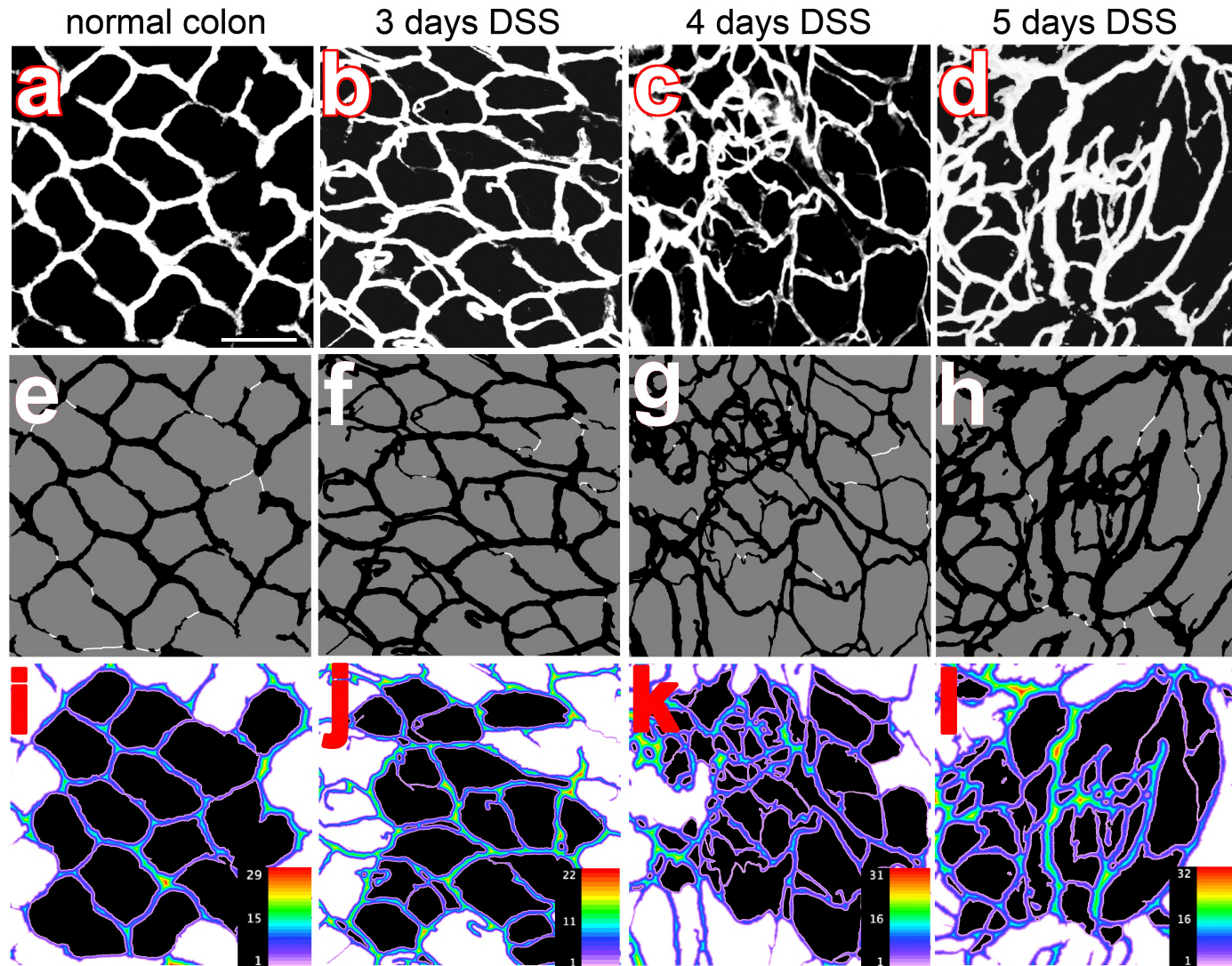


Gravitational and Space Biology

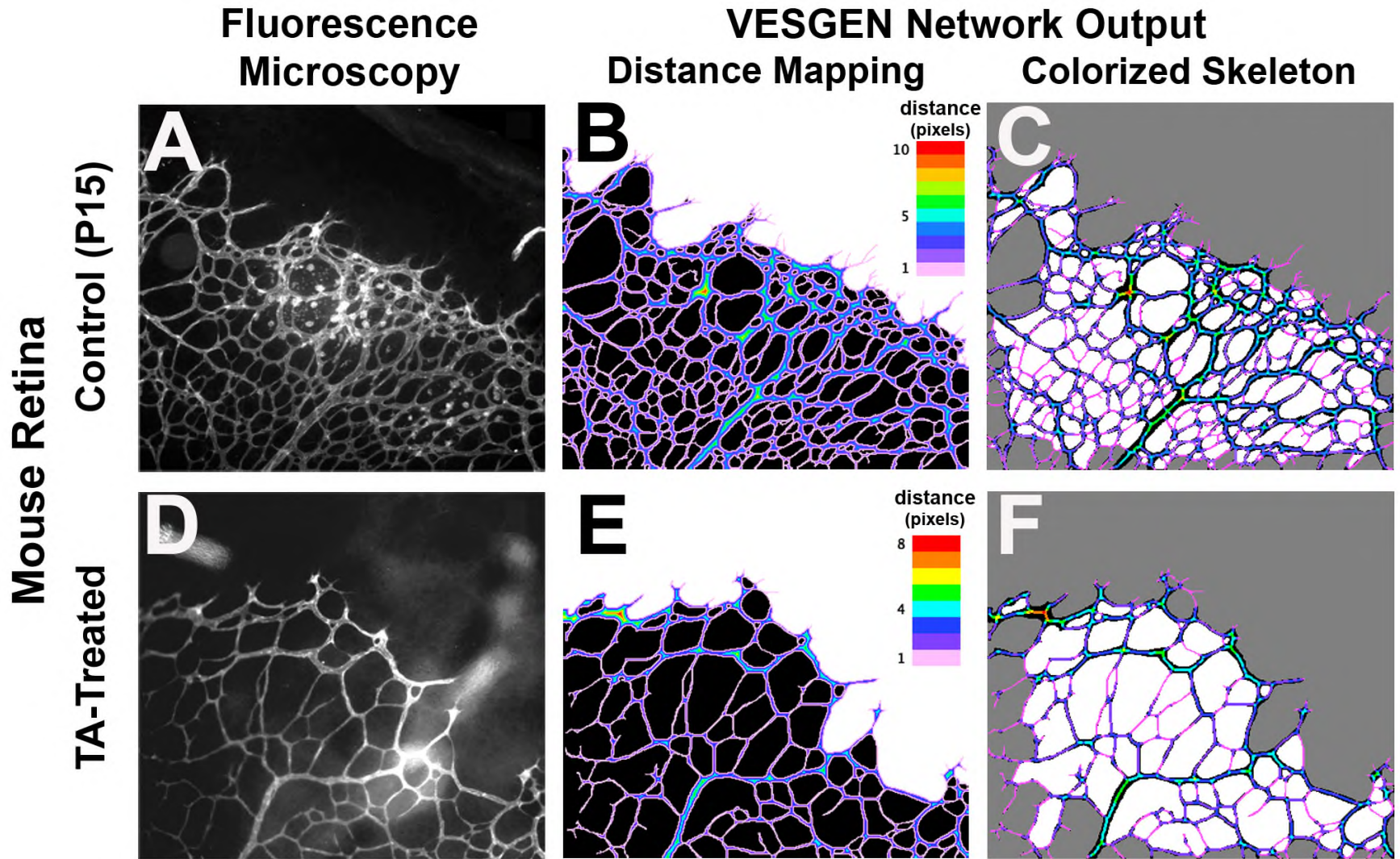
Publication of the American Society for Gravitational and Space Research



VESGEN mapping of vascular networks for progressive GI inflammation progression with mouse model



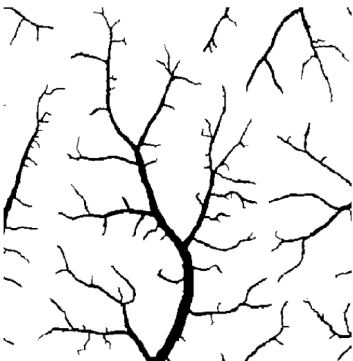
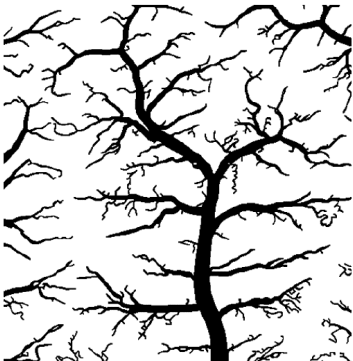
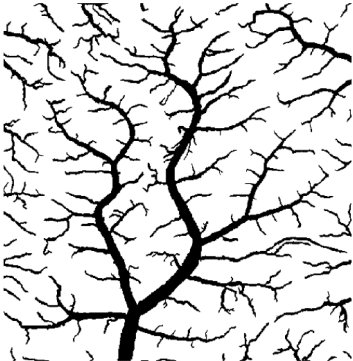
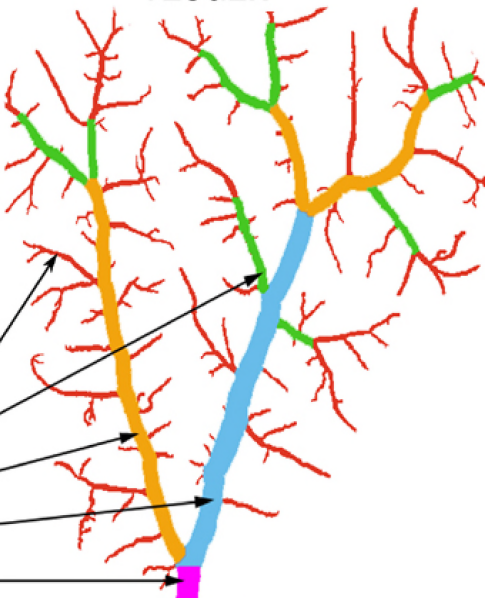
Vascular Networks in Transgenic Mouse Retina



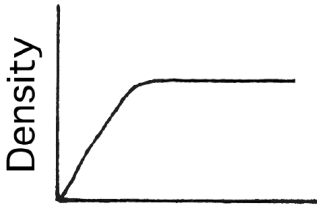
with J Sears & Q Ebrahem (Cole Eye Institute), from Vickerman et al, *Anat Rec* 292(3), 2009

VESGEN Hypothesis 'Fingerprint' or 'Signature' Vascular Pattern as Useful Integrative Readout of Complex Molecular Signaling Pathways

VESGEN



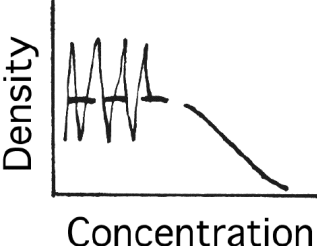
bFGF as Simple Stimulator
Arterio Thromb Vasc Biol 20 (2000)



VEGF as Vascular Complexity Factor: Phenotypic Readout with eNOS Signaling
Microvascular Research 72 (2006)



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

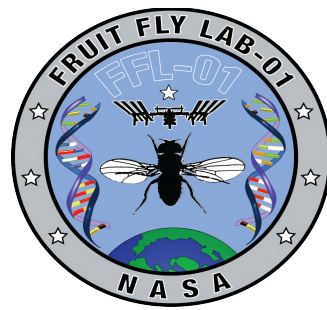


The **form** of an object is a 'diagram of **forces**'

D' Arcy Thompson

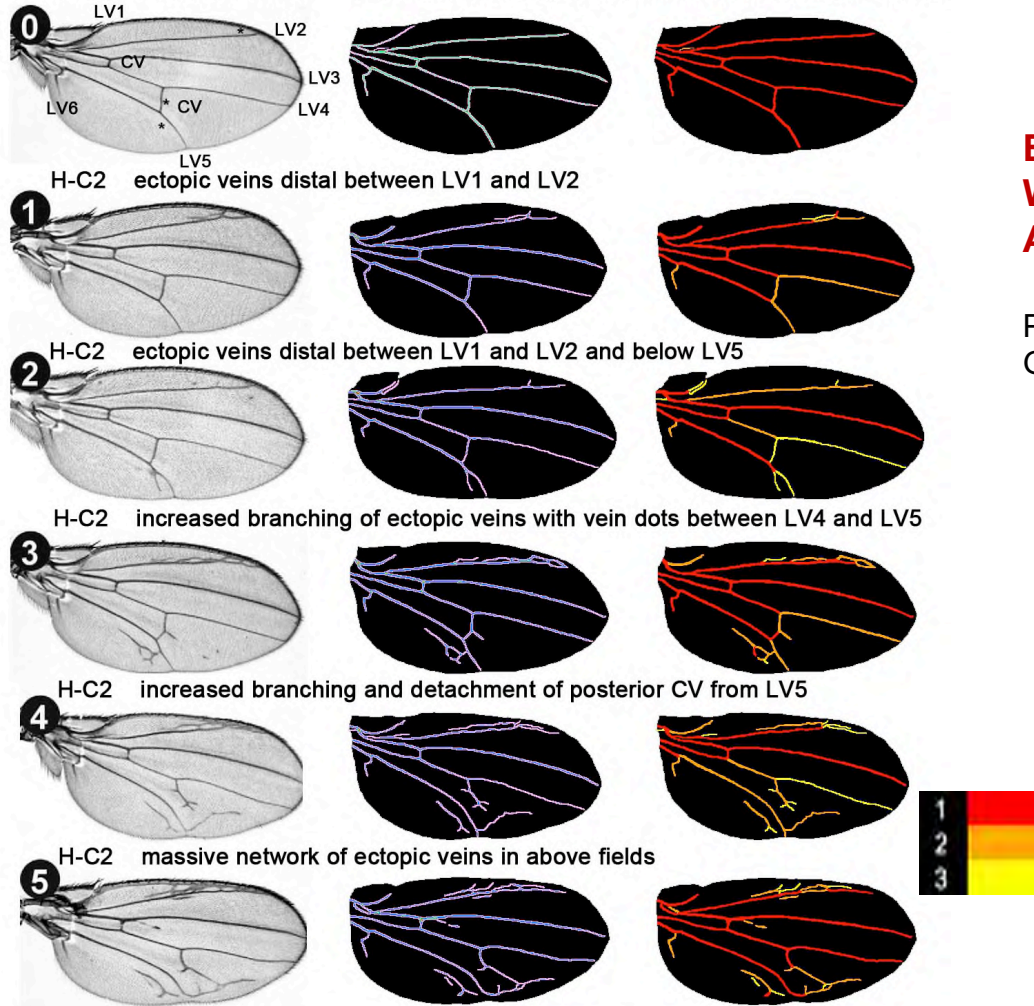
Fruitfly (*Drosophila melanogaster*): Major Genetic Model Organism

Mapping by VESGEN: Hairless (H-C2) overexpression induces phenotypic series of increasing ectopic wing venation



6 Longitudinal Veins (LV) with anterior and posterior Cross Veins (CV)

Wildtype, no heat shock * where veinlets may arise after heat shock in wt or H-C2



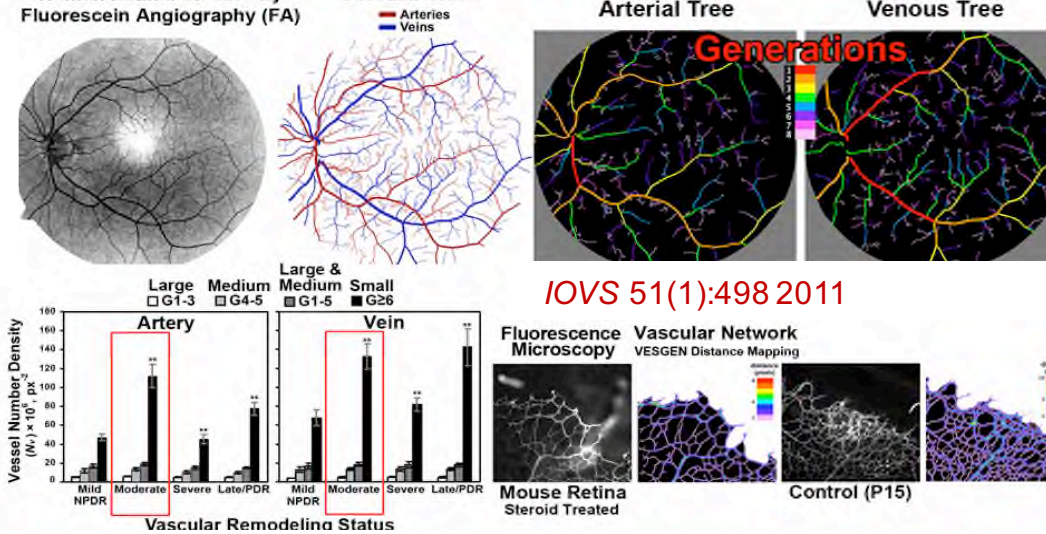
Expression of Genes Involved in *Drosophila* Wing Morphogenesis and Vein Patterning Are Altered by Spaceflight

Parsons, Hosamani, Vickerman, Bhattacharya
Grav Space Res 3(2):54-64 2015, ASGSR 2012

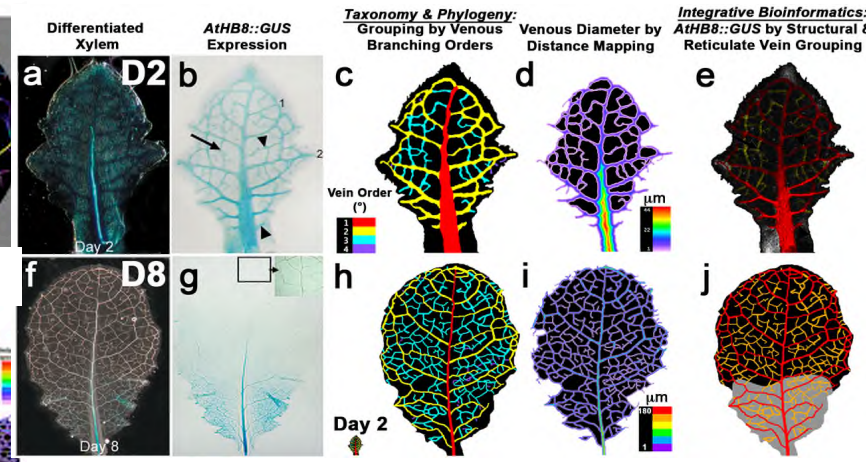
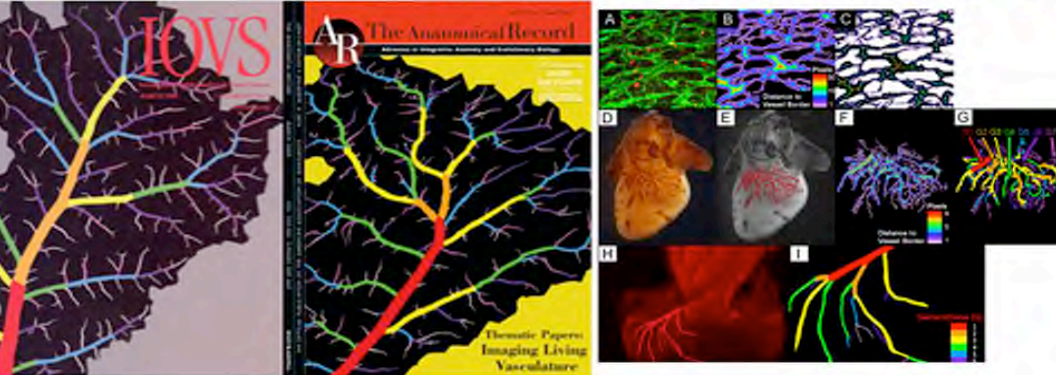
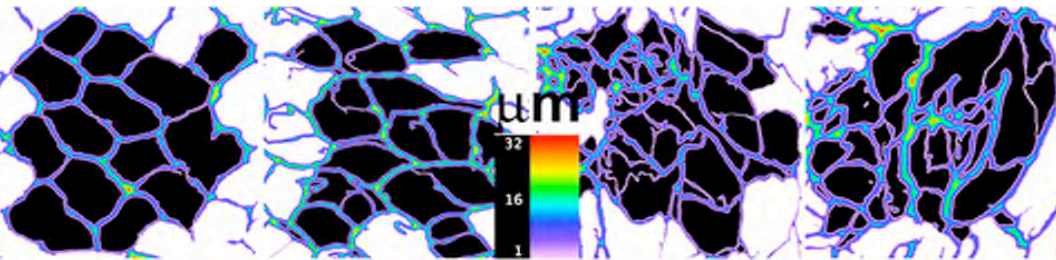
VESGEN Analysis of Vascular Patterning for Fundamental Space Biology with Translational Applications to Astronaut and Terrestrial Health

Insights into progression of visually impairing disease

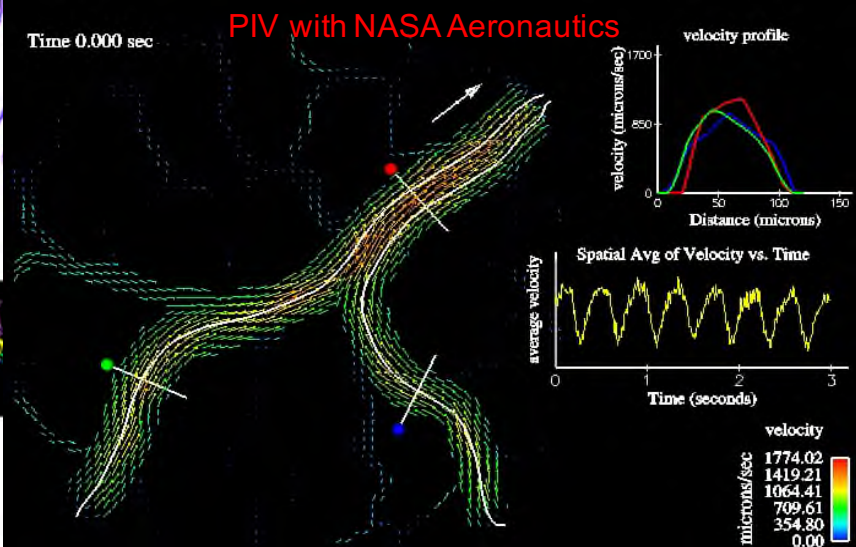
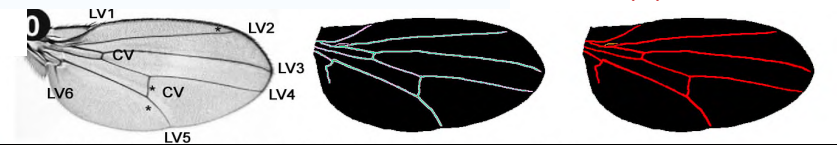
GSR 2(1):68 2014

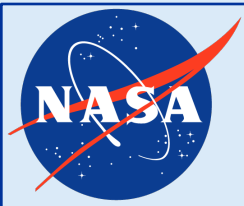


GSB 26(2):2 2012, PloS One 8(5):e64227 2013



Wildtype *where veinlets may arise in H-C2 mutant GSR 3(2):54-64 2015





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Michiko Watanabe, Monica Montano, Karunamuni G

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