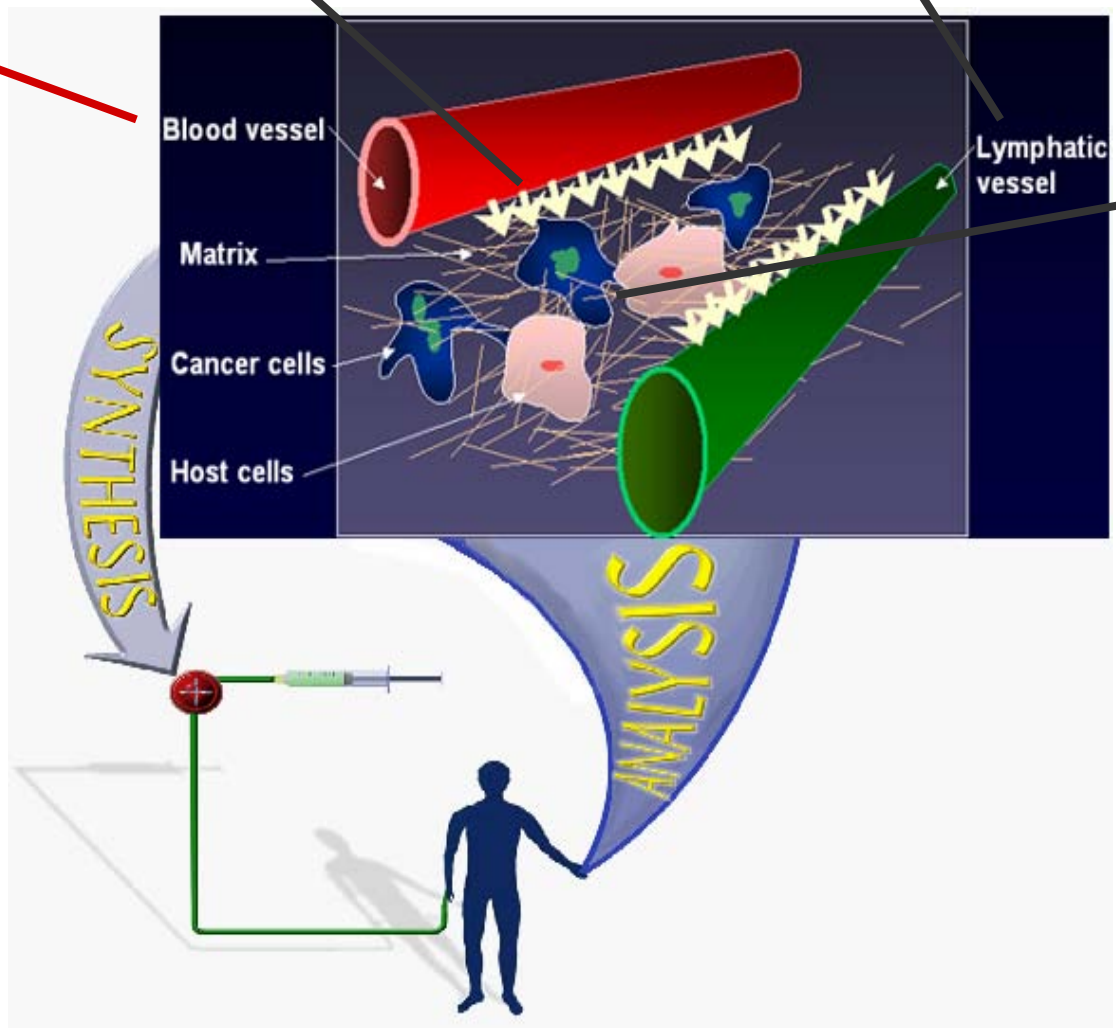
# Delivery of Molecular and Cellular Medicine to Tumors

**Vascular Transport (Lecture I)**

**Interstitial and Lymphatic Transport (Lecture II)**

**Angiogenesis & Microcirculation (Lecture I)**

**Vascular Normalization (Lecture II)**



# DELIVERY OF MOLECULAR MEDICINE TO TUMORS

## Lecture I: Tumor Angiogenesis and Microcirculation

### OVERVIEW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## TUMOR MODELS

### Isolated Tumor Preparation

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# Outline

- How do we study drug delivery and tumor physiology?
- How does host-tumor interaction affect tumor physiology and therapeutic response?
- How do we measure tumor blood flow?
  - Directly or indirectly
  - Microscopically or macroscopically
- How does tumor blood flow compare with normal blood flow?
  - Temporally
  - Spatially
- What parameters govern tumor blood flow and how do we measure them?

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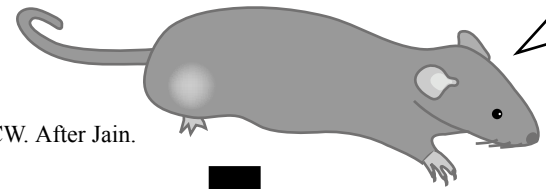
# Understanding Physiological Barriers

**Novel Drug**



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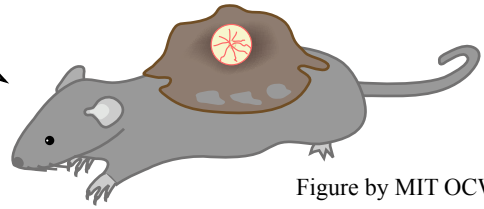

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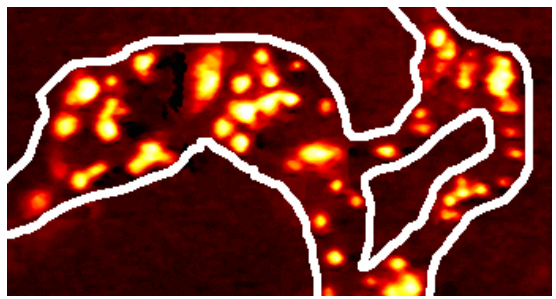


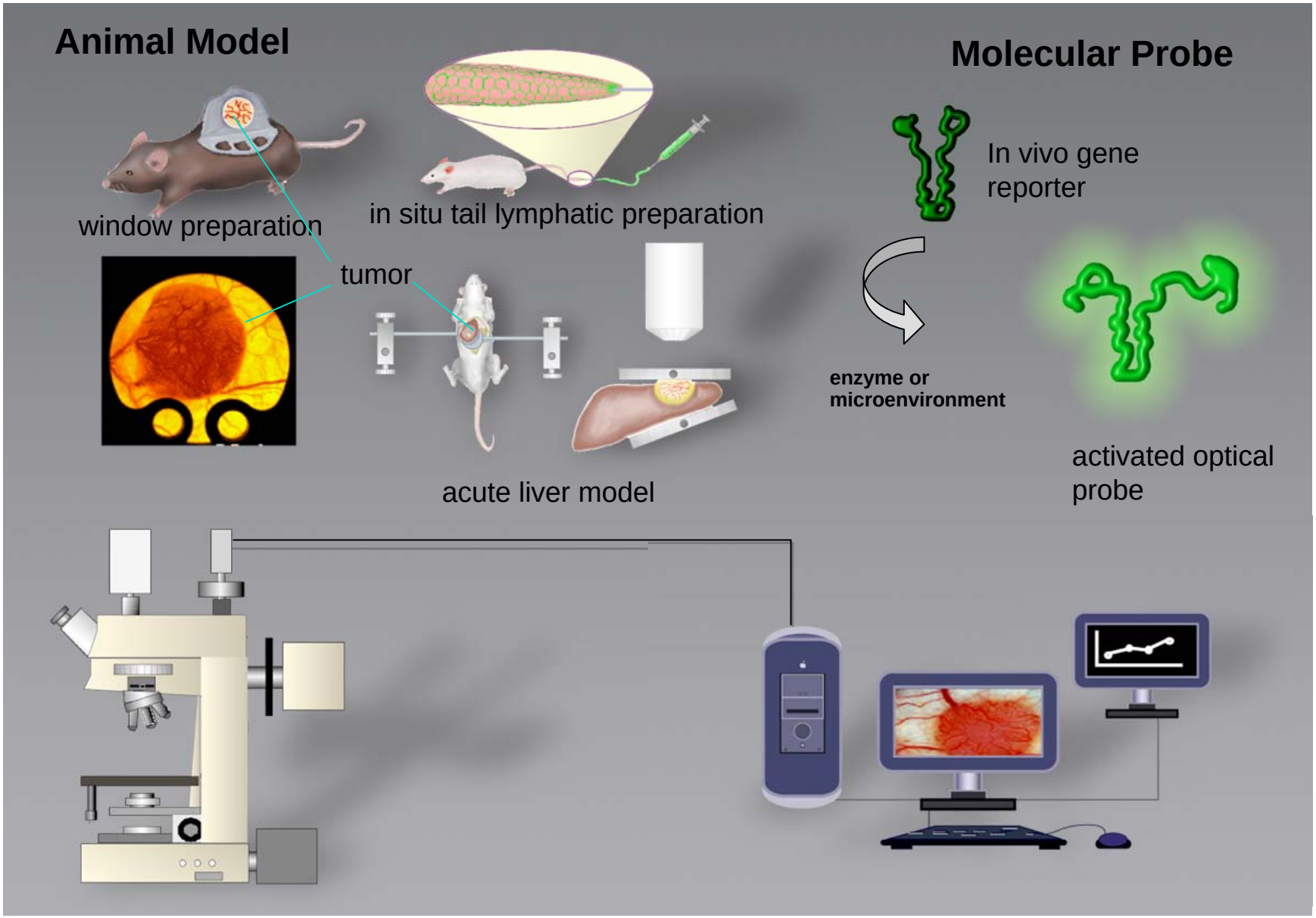
Drug Concentration

Visualization & Quantification

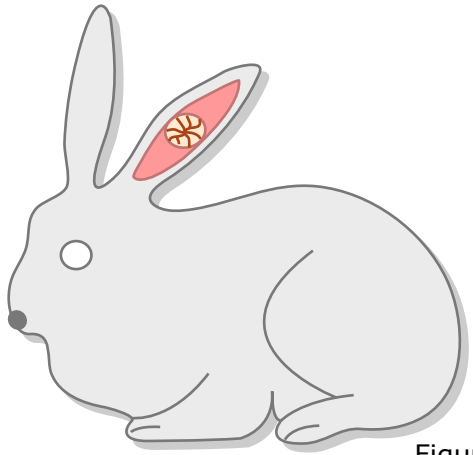
**Tumor Regression, Survival**

**Insight into Physiological Barriers**

**2005**



# Chronic Windows Preparations for Intravital Microscopy



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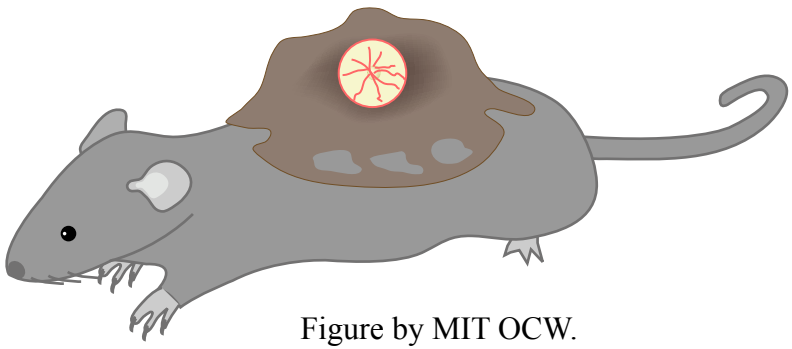
**Rabbit Ear Chamber**



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**Dorsal Skinfold Chamber**

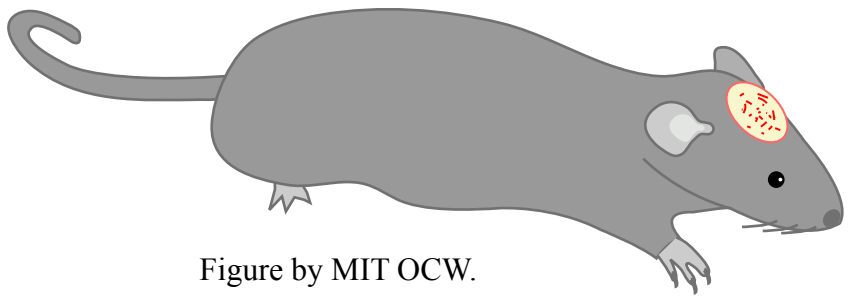

Figure by MIT OCW.

**Cranial Window**

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# Acute Preparations for Intravital Microscopy

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Source: Jain, R.K., L.L. Munn, and D. Fukumura. "Dissecting Tumor Pathophysiology using Intravital Microscopy." *Nature Reviews Cancer* 2 (2002): 266-276.

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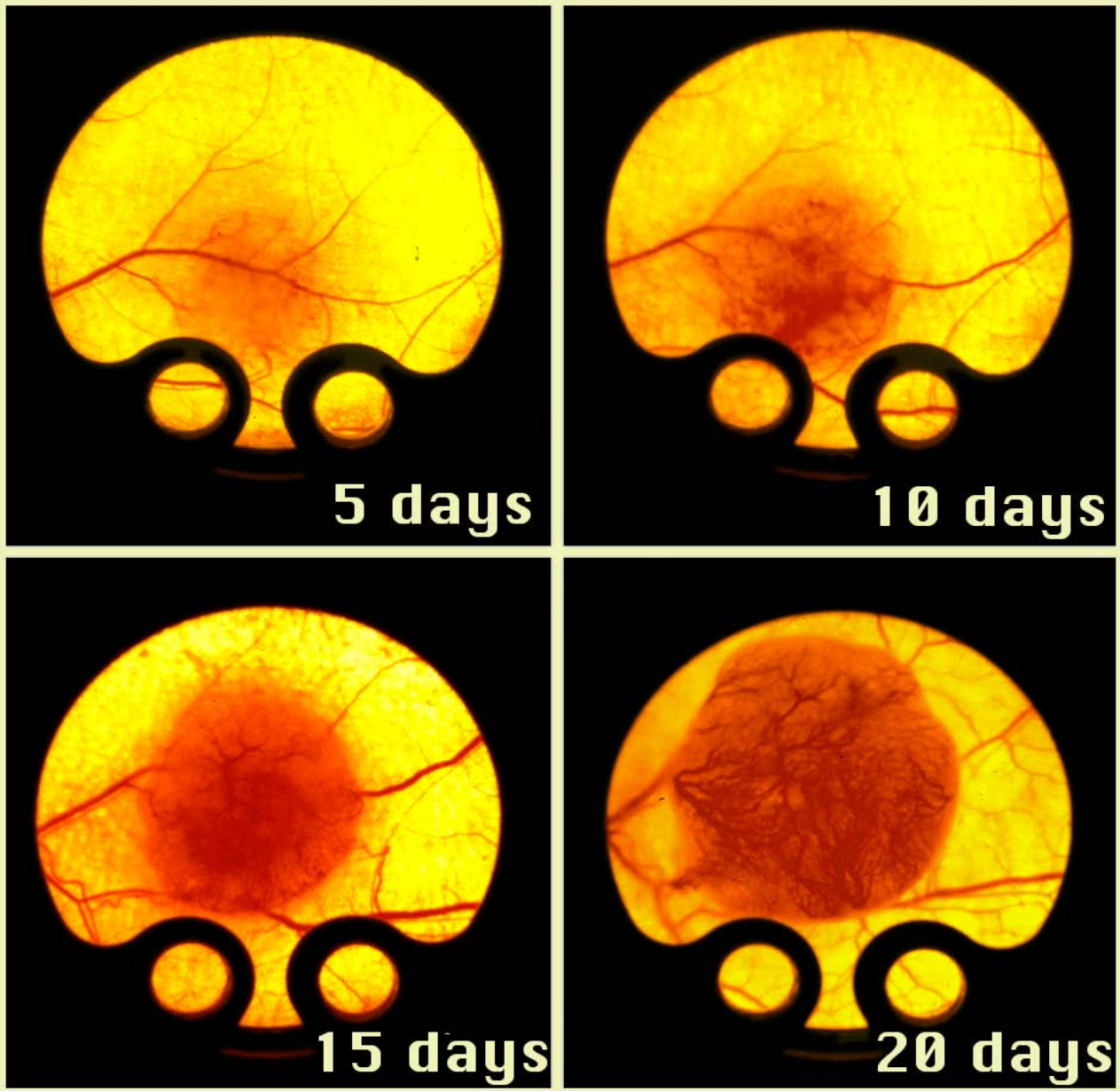
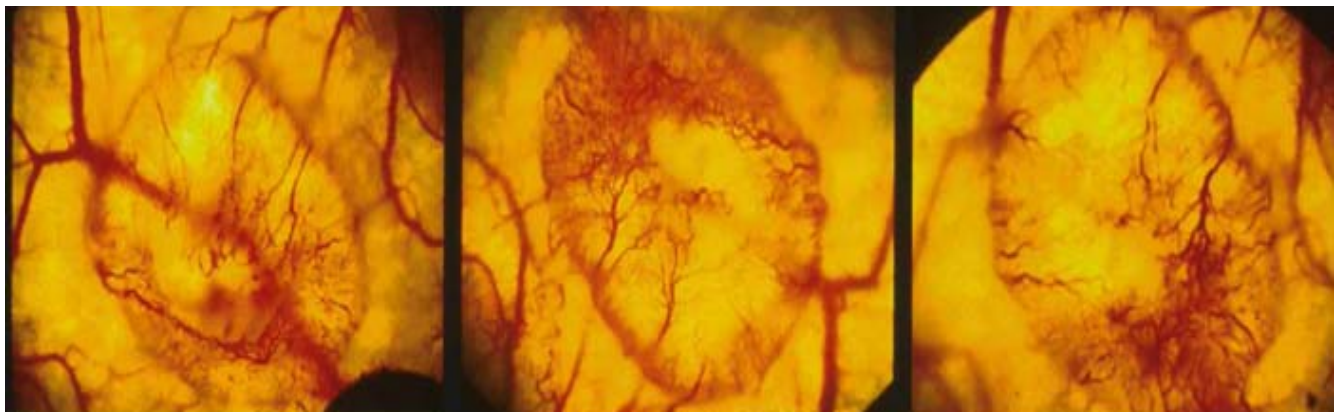
# Growth of LS174T in Dorsal Window




Courtesy of the American Association of Cancer Research. Used with permission. Leunig, M., F. Yuan, M. D. Menger, Y. Boucher, A. E. Goetz, K. Messmer, and R. K. Jain. "Angiogenesis, Microvascular Architecture, Microhemodynamics, and Interstitial Fluid Pressure During Early Growth of Human Adenocarcinoma LS174T." *Cancer Research* 52 (1992): 6553-6550

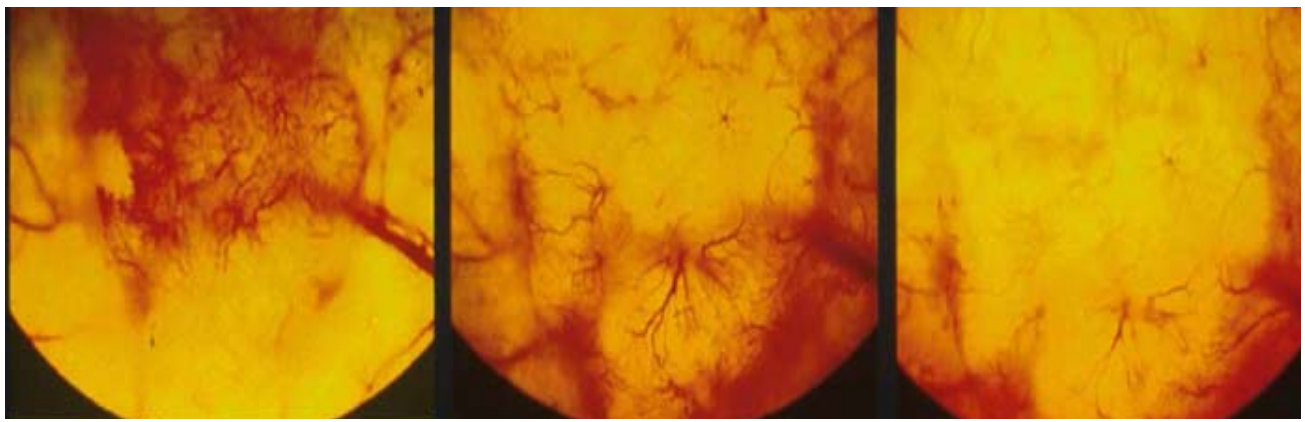
# Regression of LS174T by anti-VEGF antibody

Control



Before treatment | 3 days | 7 days

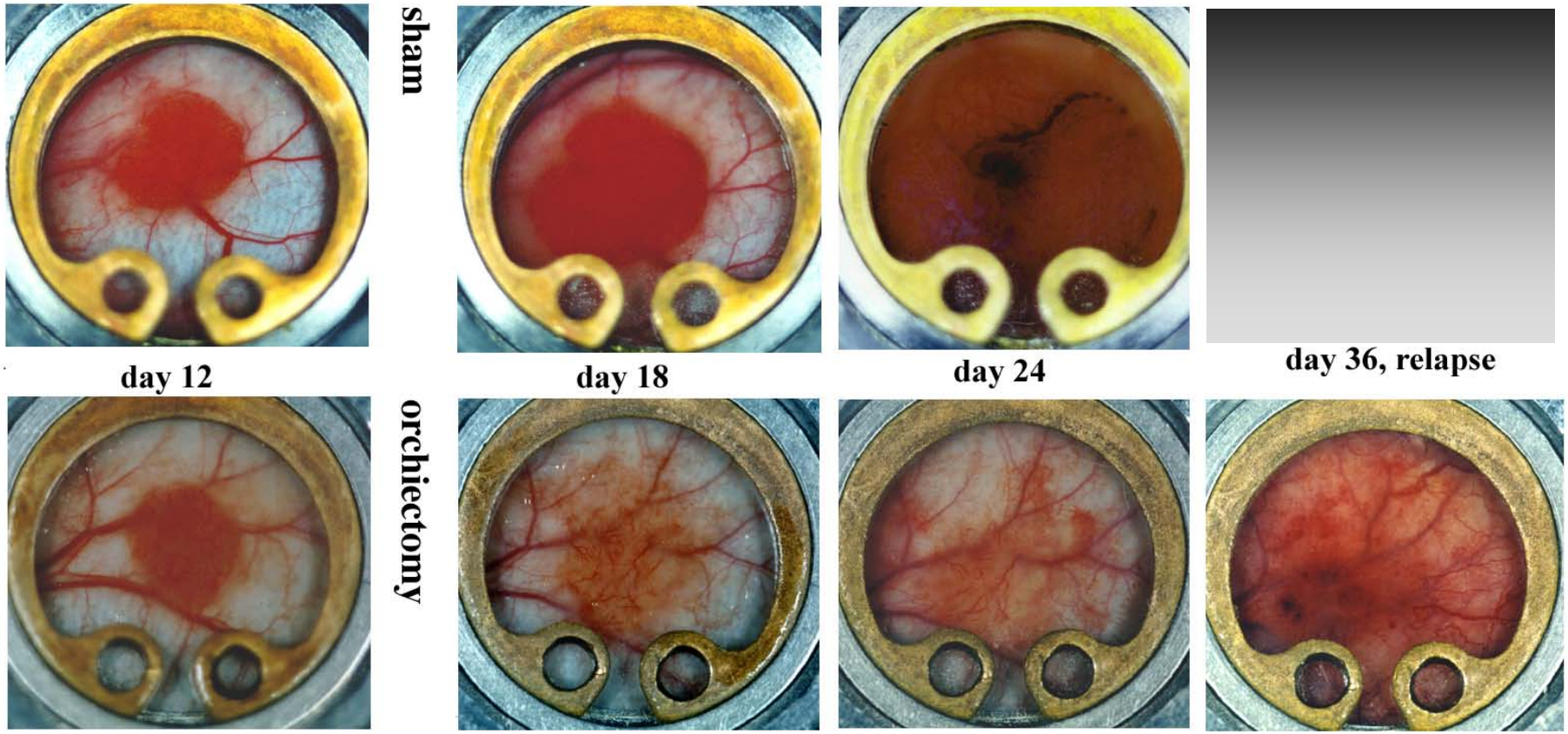
Treatment





Reference: *Yuan et al. PNAS (1996)*

# Tumor relapse after regression



# Vessels Induced by Defined-Growth Factors: Effect of Host Microenvironment

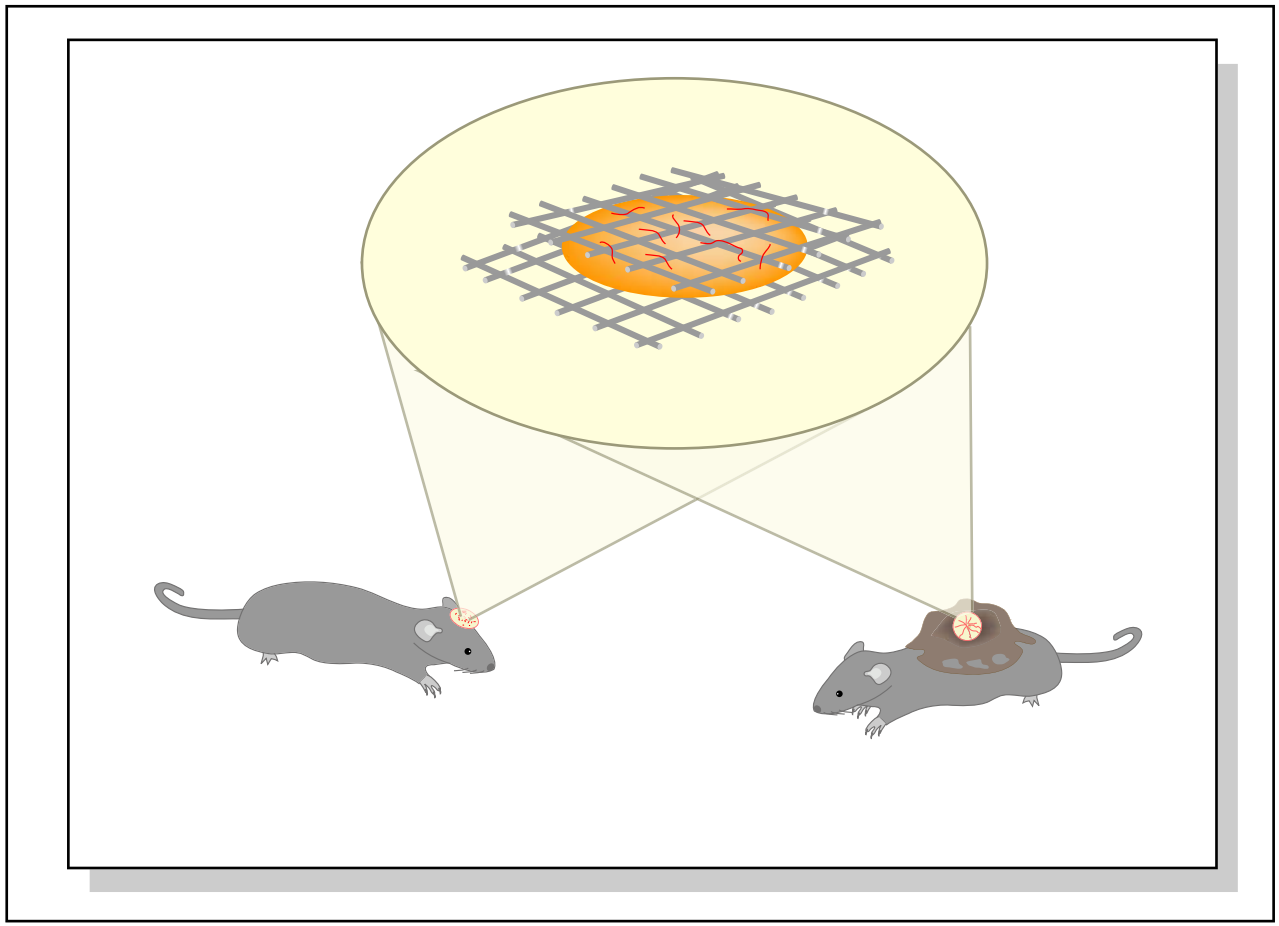


Figure by MIT OCW. After Dellian et al., 1996.

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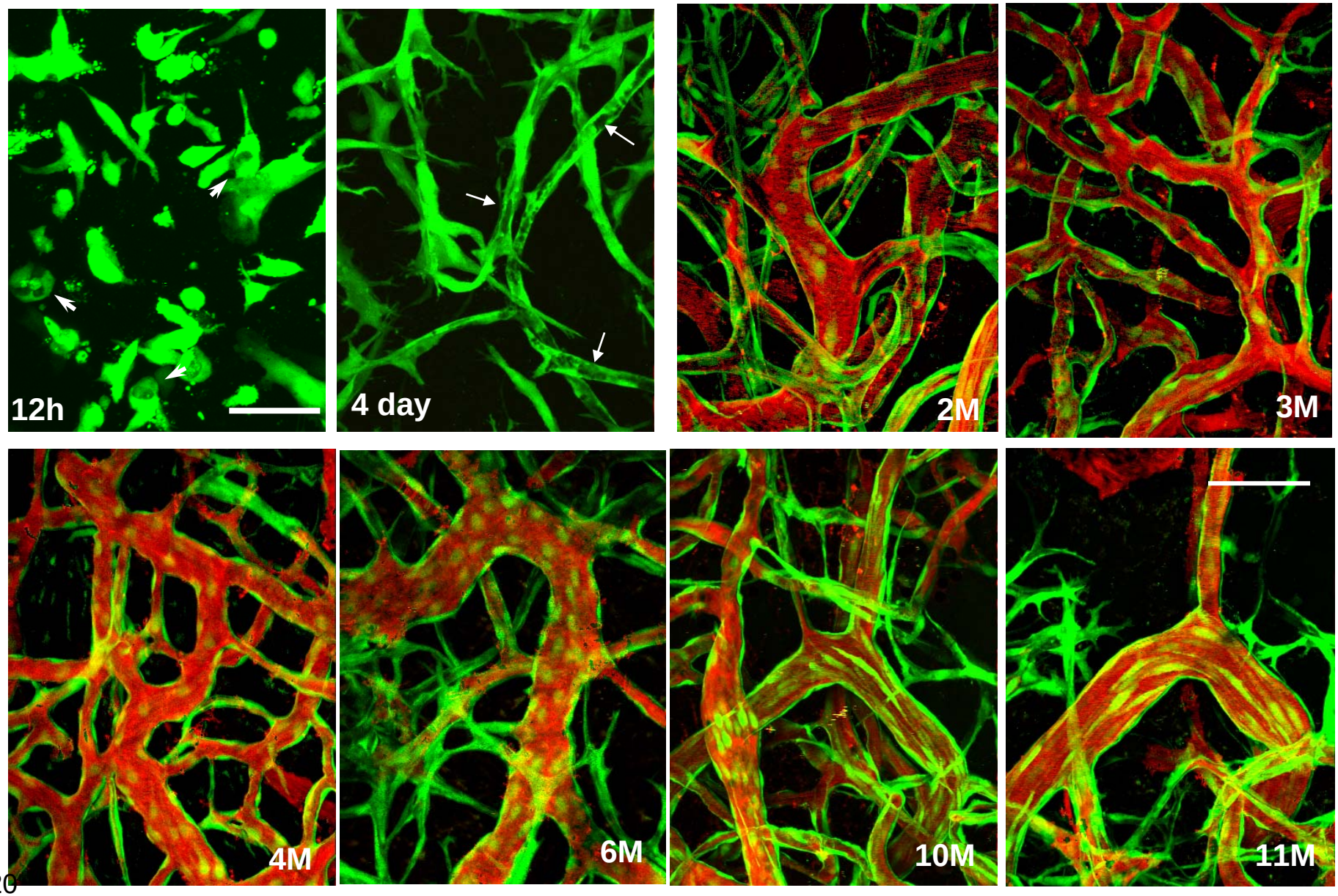
# Creation of long lasting blood vessels






Reference: *Koike et al., Nature, (2004)*

# Monitoring Gene Expression *In Vivo*

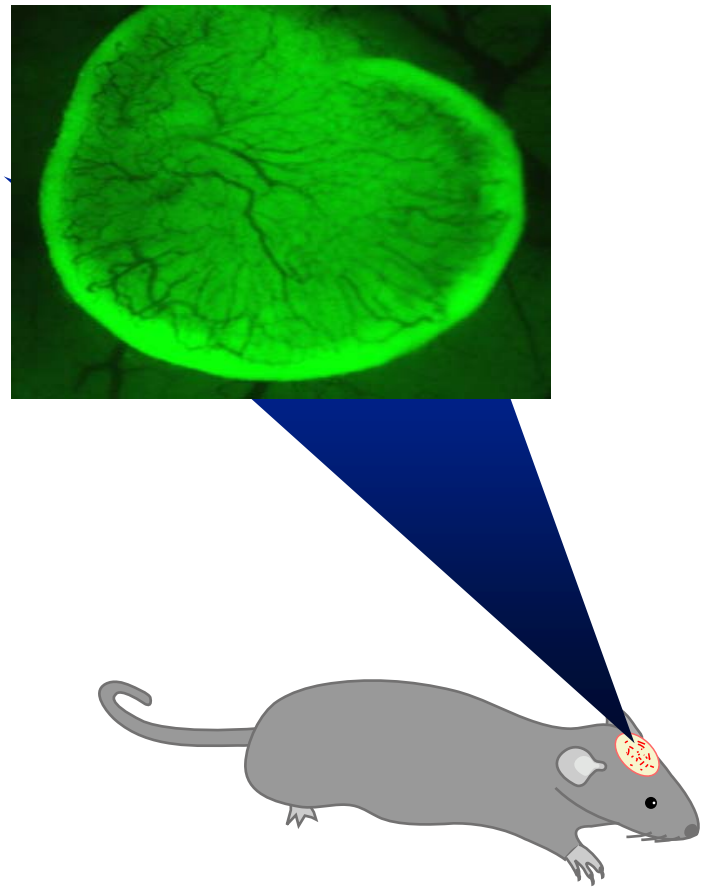
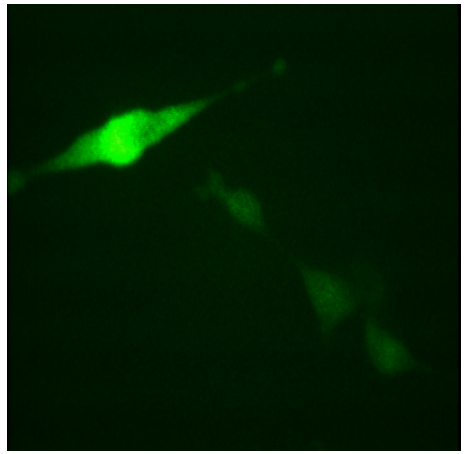
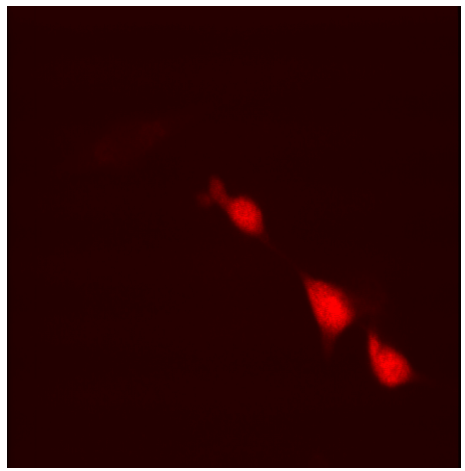


Figure by MIT OCW. After Jain.





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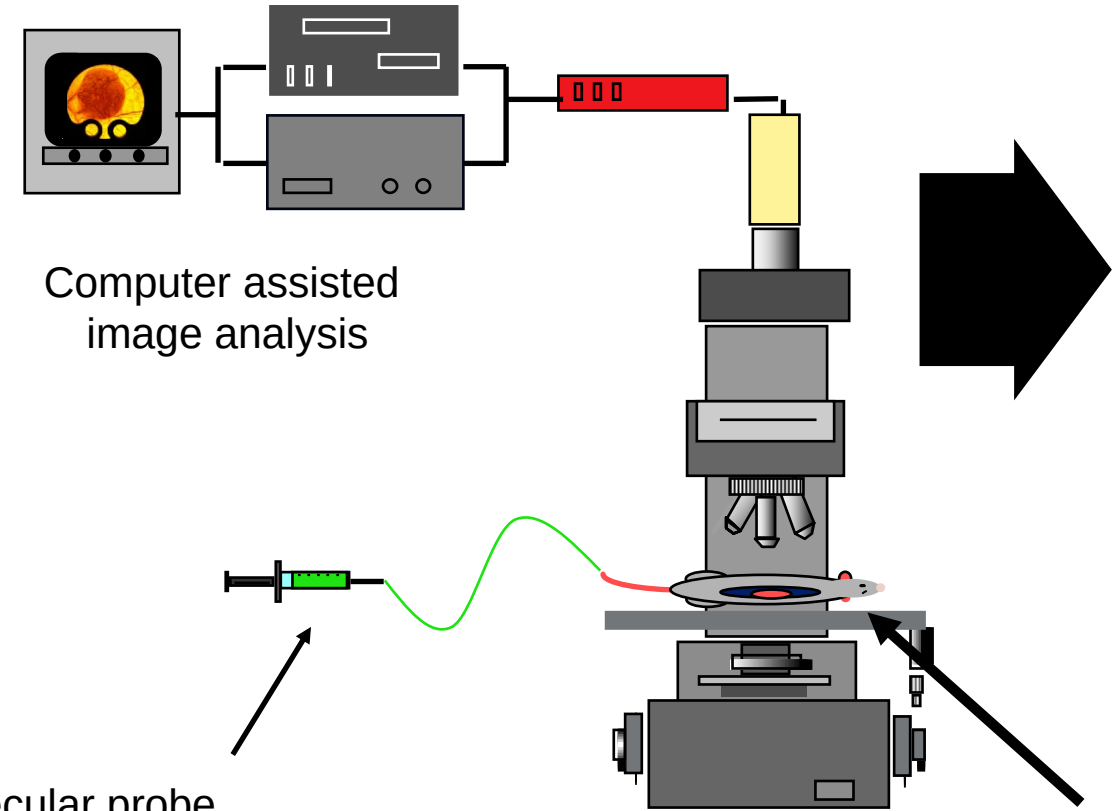
# Dissecting Tumors using Intravital Microscopy



Computer assisted
image analysis

- **Molecular imaging**
- **Cellular imaging**
- **Anatomical imaging**
- **Functional imaging**
- **Therapeutic response**

Molecular probe
or engineered cells

Genetically engineered mice &
Tumor models

# Host-Tumor Interactions

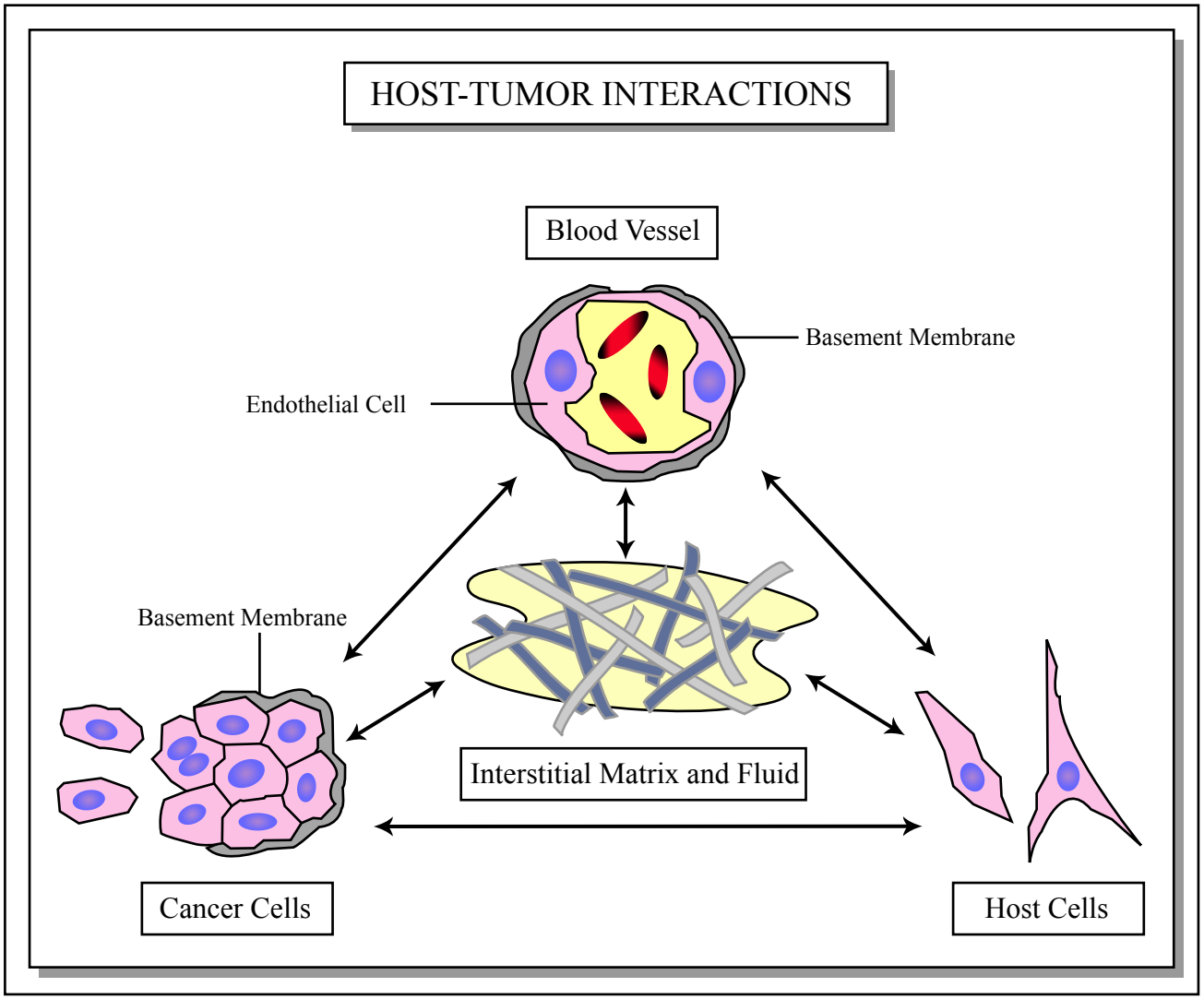



Figure by MIT OCW. After Jain, 2003.

# VEGF - Promoter is Activated During Wound Healing

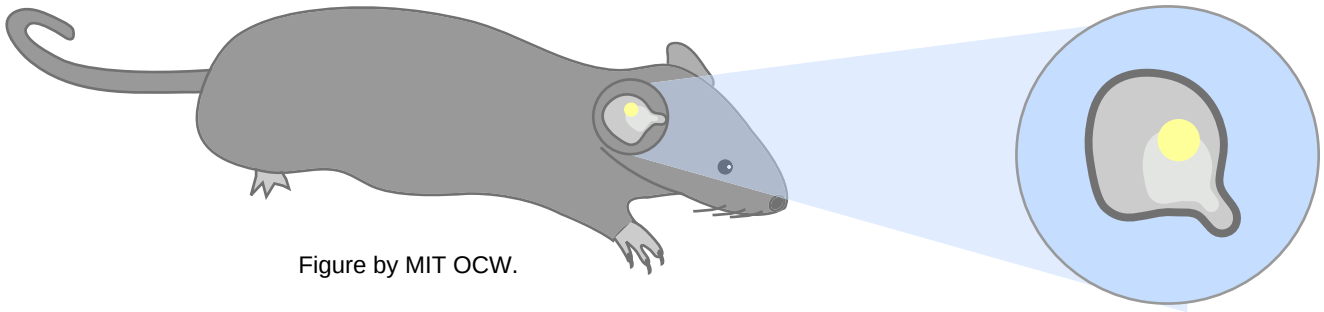
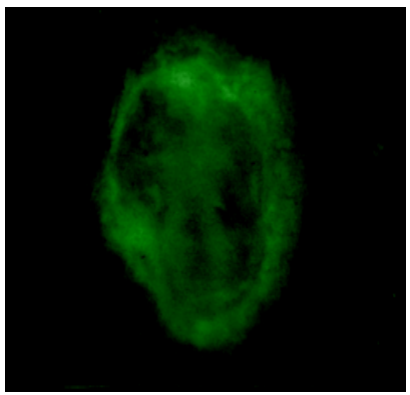
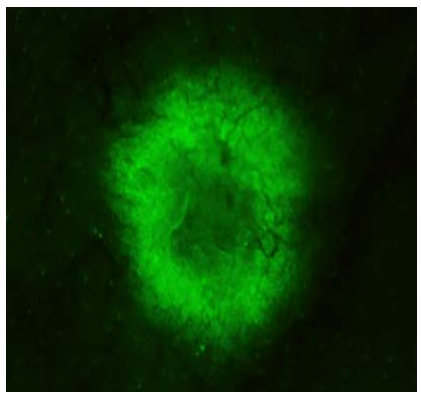


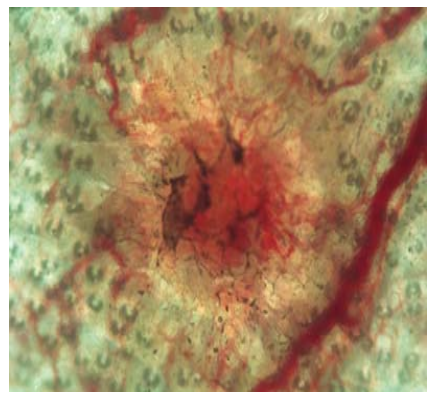
Figure by MIT OCW.



1 week



2 weeks



Bar=500μm

Trans-illumination

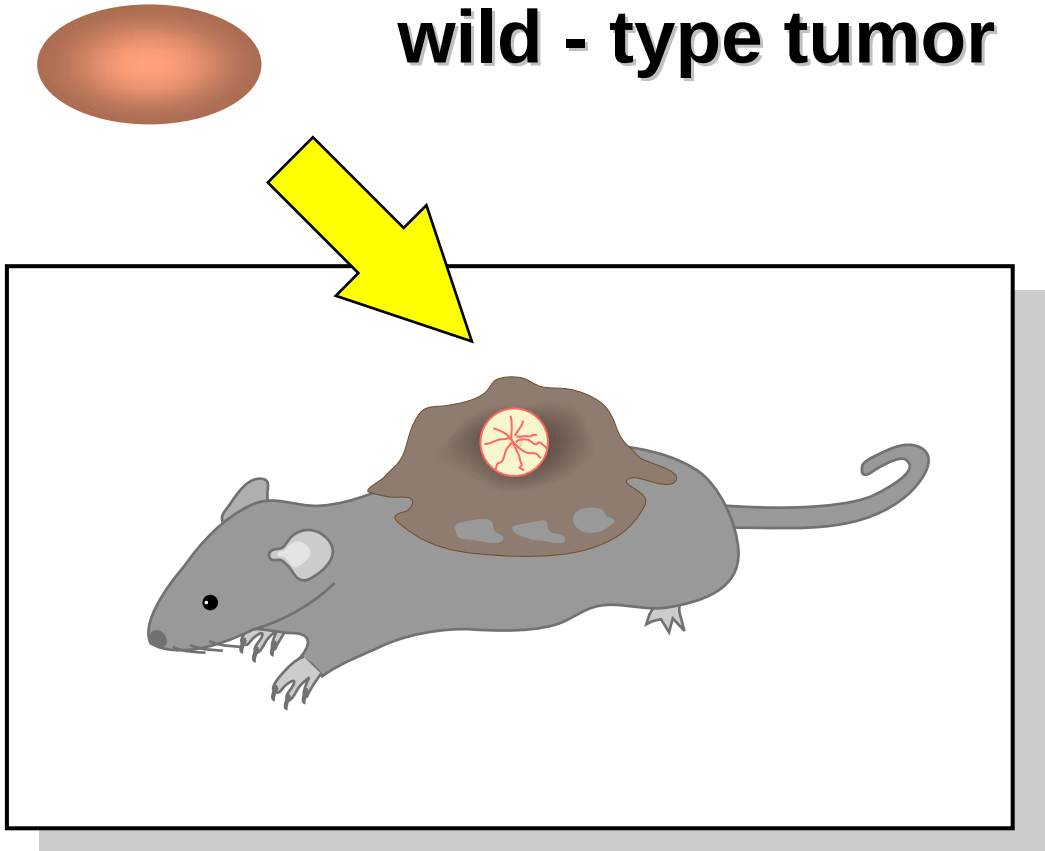
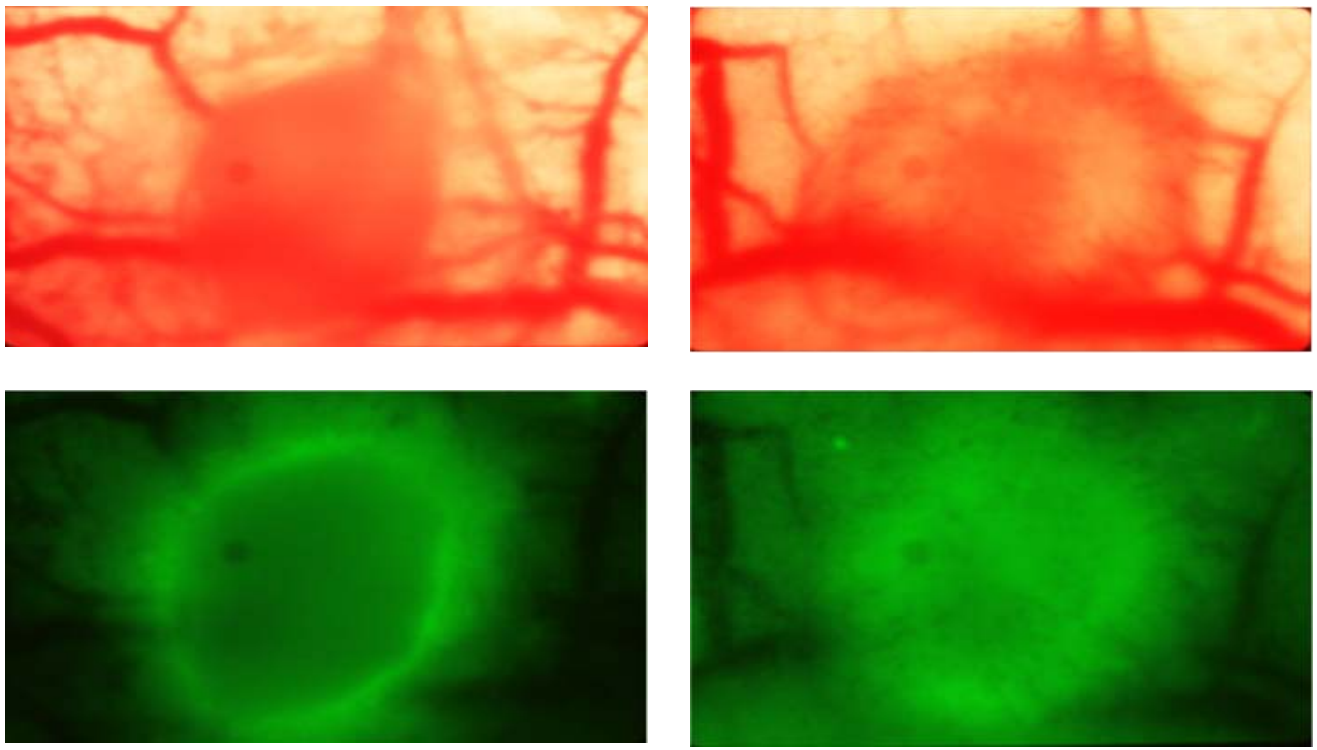



Figure by MIT OCW. After Jain.

**VEGF - GFP transgenic mouse**

2005

# VEGF Promoter is Activated in Stromal Cells in Tumors



1 week

2 weeks



Reference: *Fukumura et al., Cell, (1998)*

**2005**

# Location of activated stromal cells

Images removed for copyright reasons. See: Fig. 2 in Brown E. B., R. B. Campbell, Y. T. Suzuki, L. Xu, P. Carmeliet, D. Fukumura, and R. K. Jain. "In vivo measurement of gene expression, angiogenesis and physiological function in tumors using multiphoton laser scanning microscopy." *Nature Medicine* 7 (2001): 864-868.

2005

# Host cells expressing VEGF migrating in a tumor

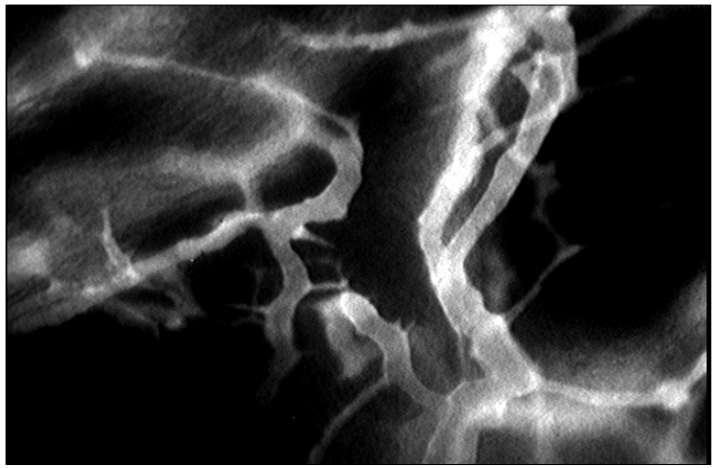


**1 Frame per hour**

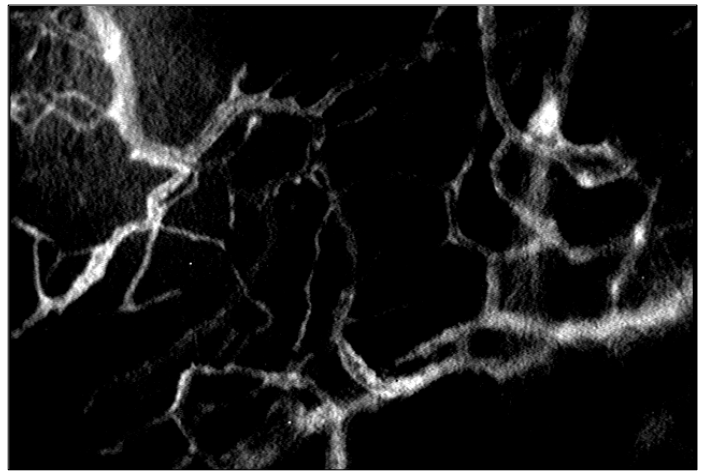
2005

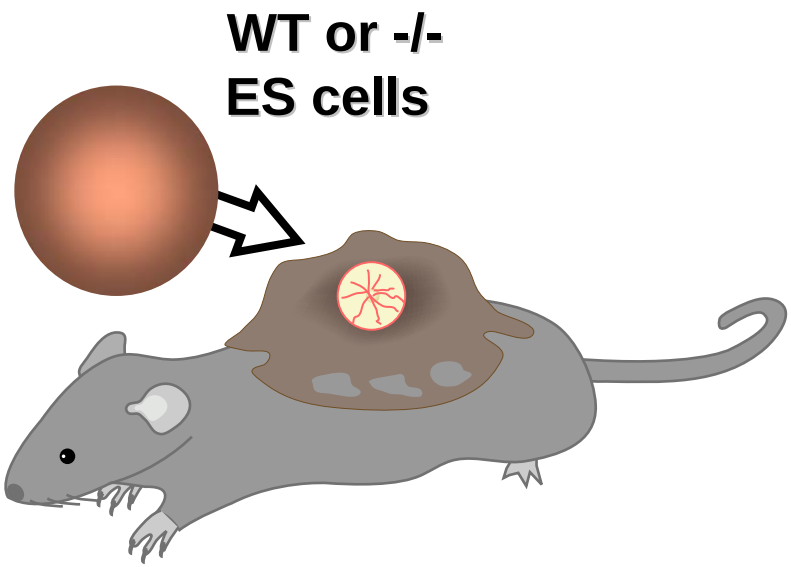
# Stromal cells produce ~50% of VEGF in tumors

WT



VEGF $^{-/-}$





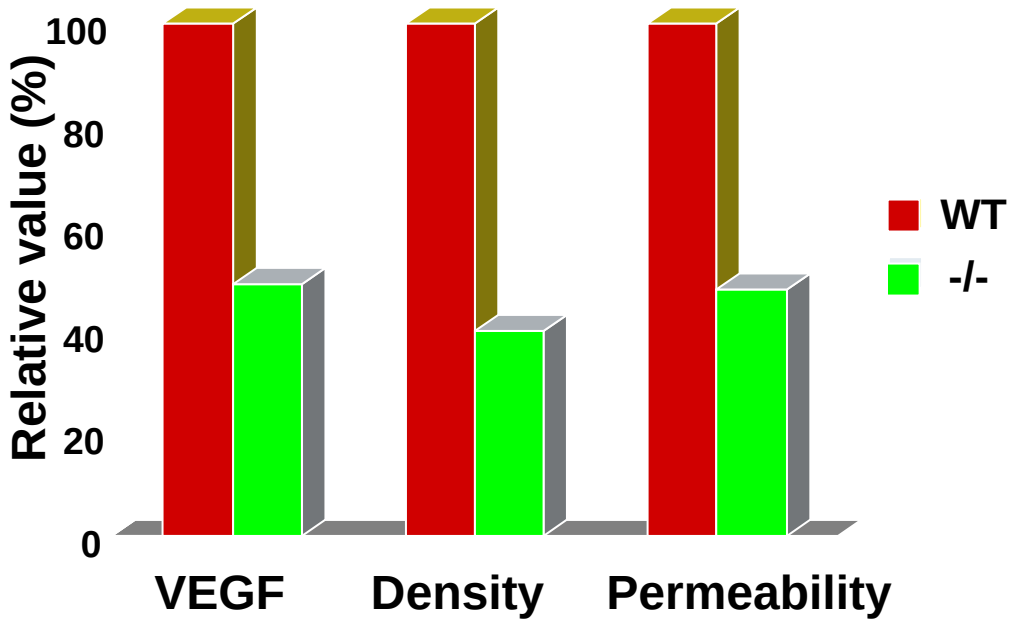






Figure by MIT OCW. After Jain.

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# Orthotopic primary tumor suppresses secondary tumor growth

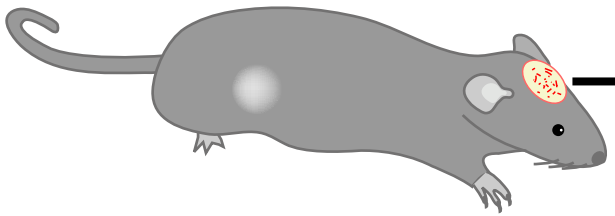


Figure by MIT OCW. After Jain.

**Ectopic (subcutaneous) 1° tumor**

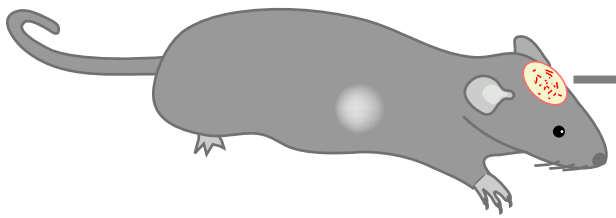
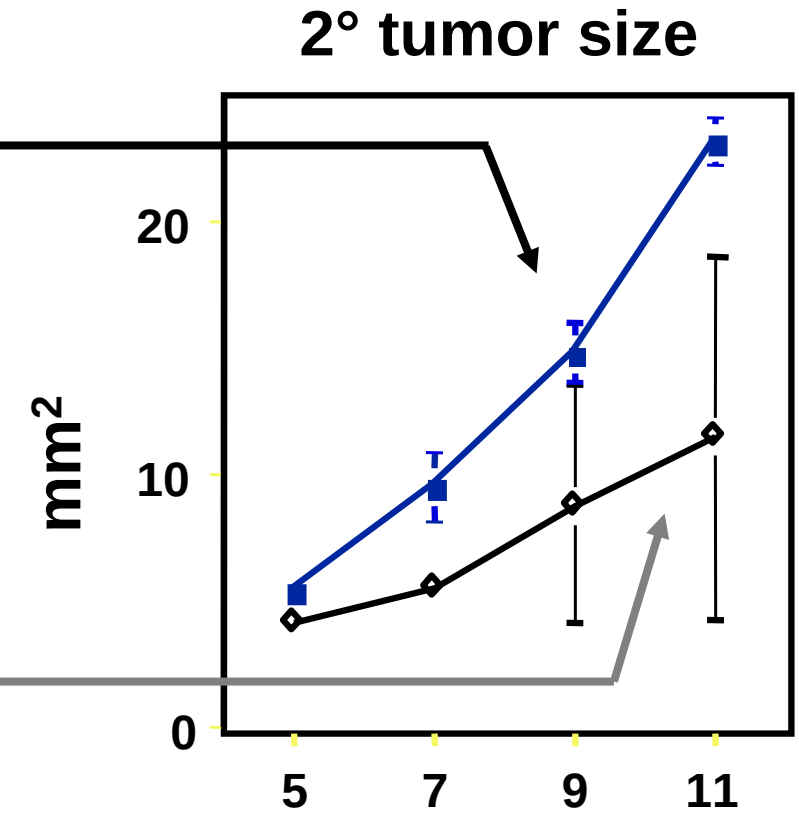
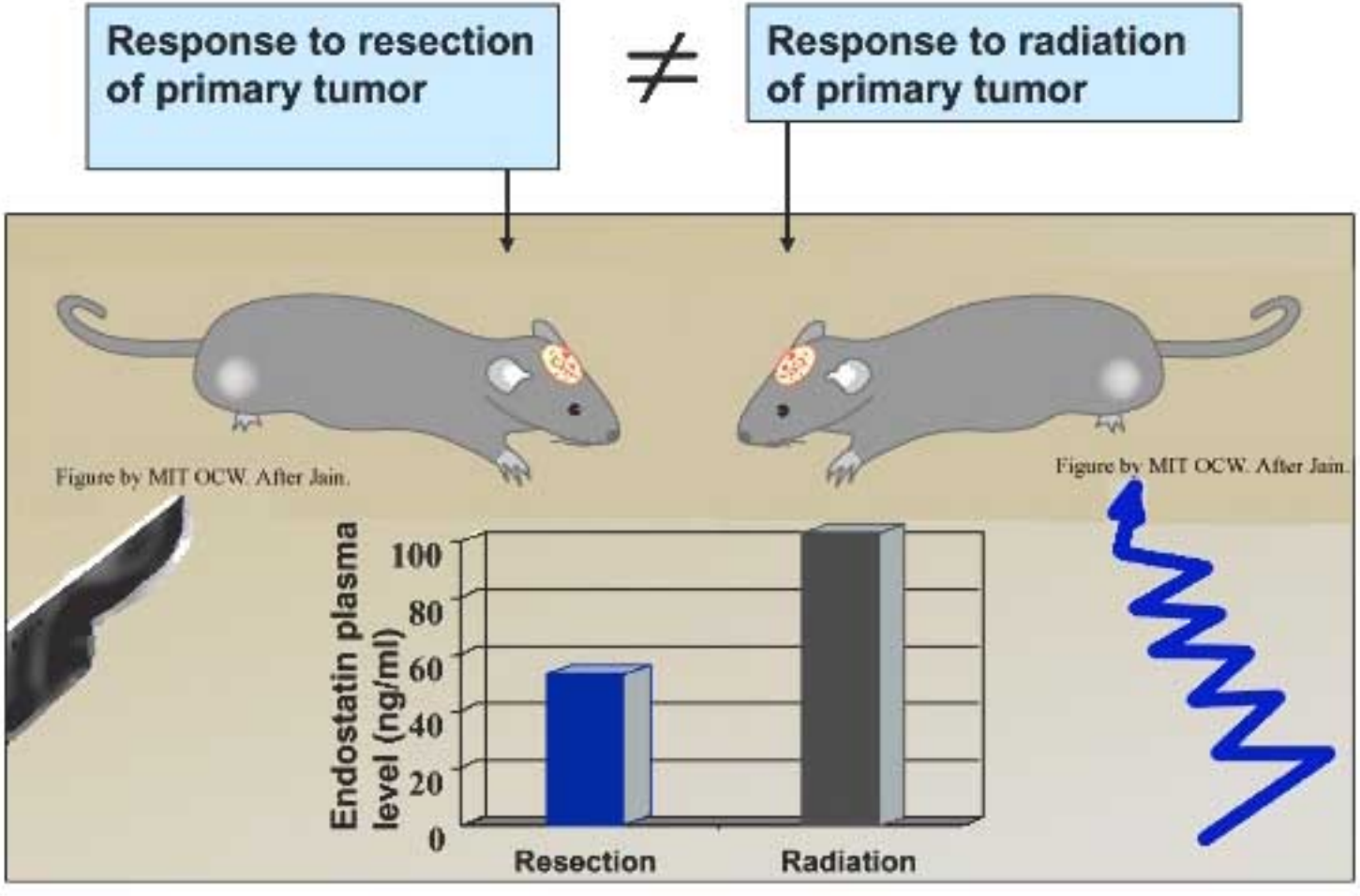


Figure by MIT OCW. After Jain.

**Orthotopic (gallbladder) 1° tumor**



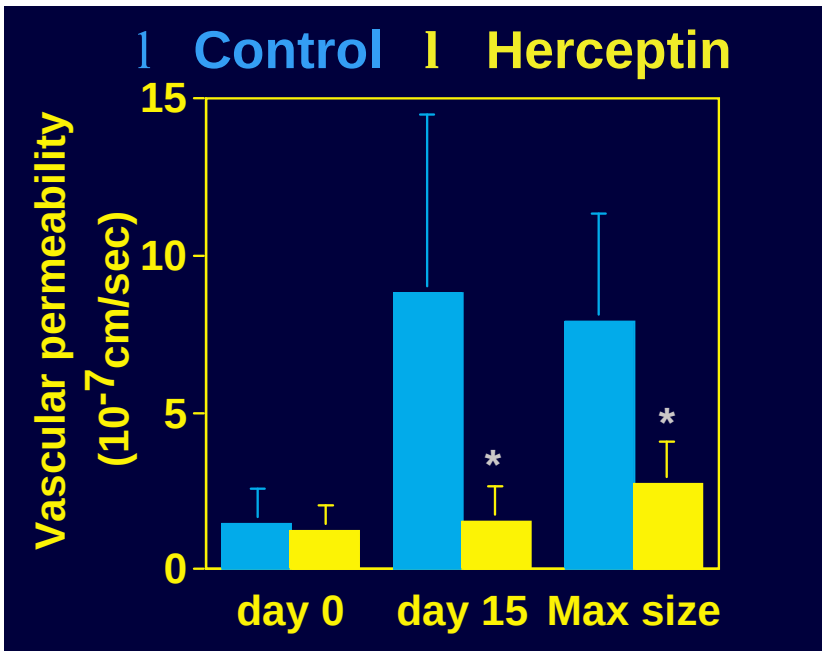
# Resection and radiation differentially affect distal angiogenesis

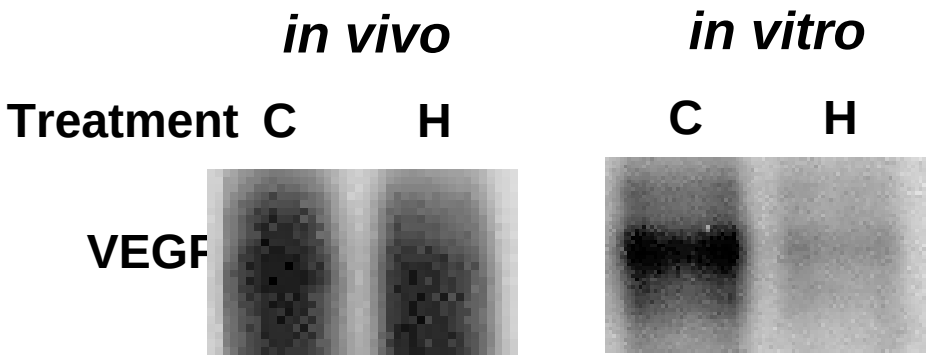


2005

# Role of host cells in therapeutic response

Images removed for copyright reasons. See: Fig. 1a in Izumi, Y., L. Xu, E. di Tomaso, D. Fukumura, and R. K. Jain. "Tumour biology: herceptin acts as an anti-angiogenic cocktail." *Nature* 416 (2002): 279-280.



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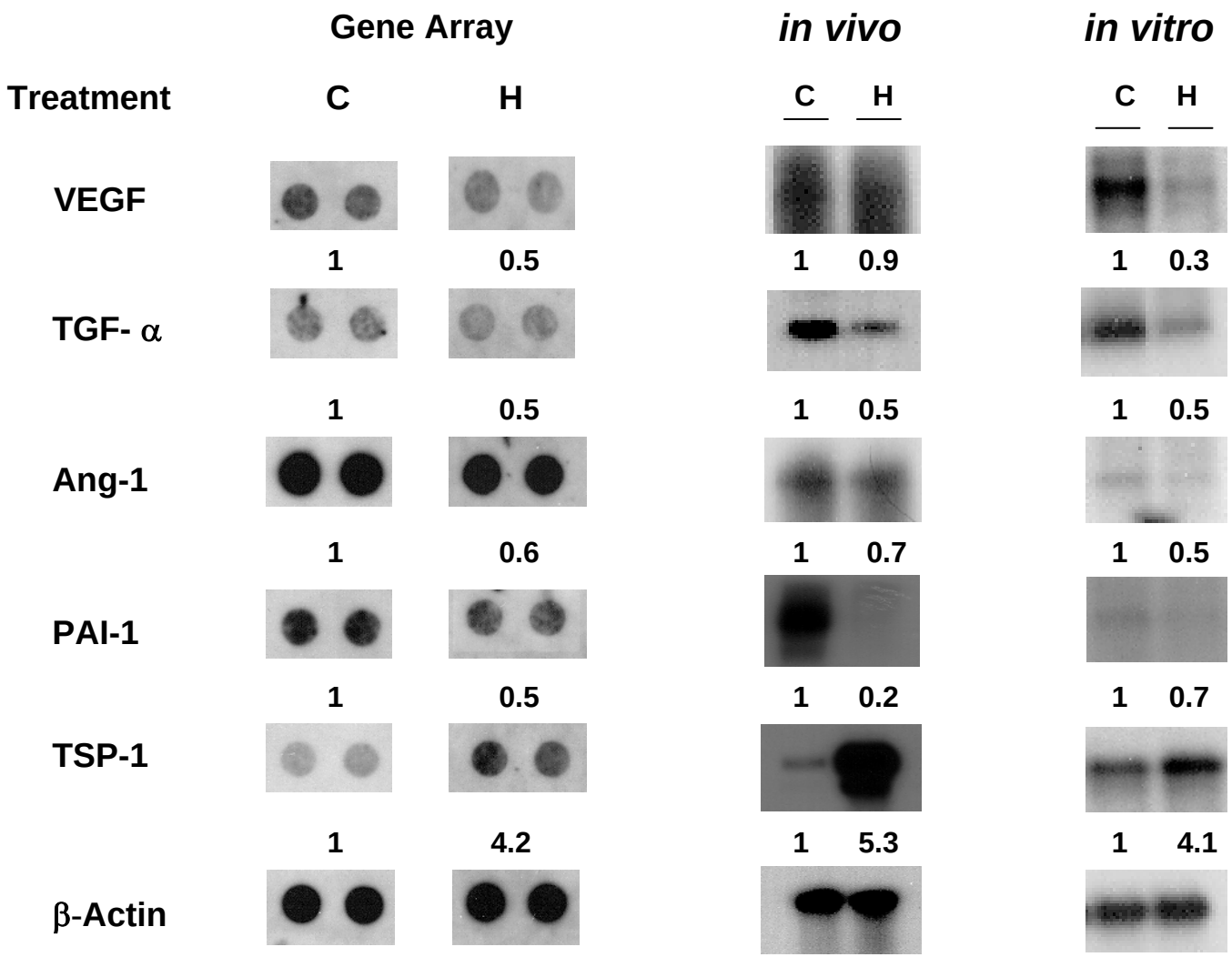
# Herceptin mimics an anti-angiogenic cocktail






Reference: *Izumi et al. Nature (2002)*

# Outline

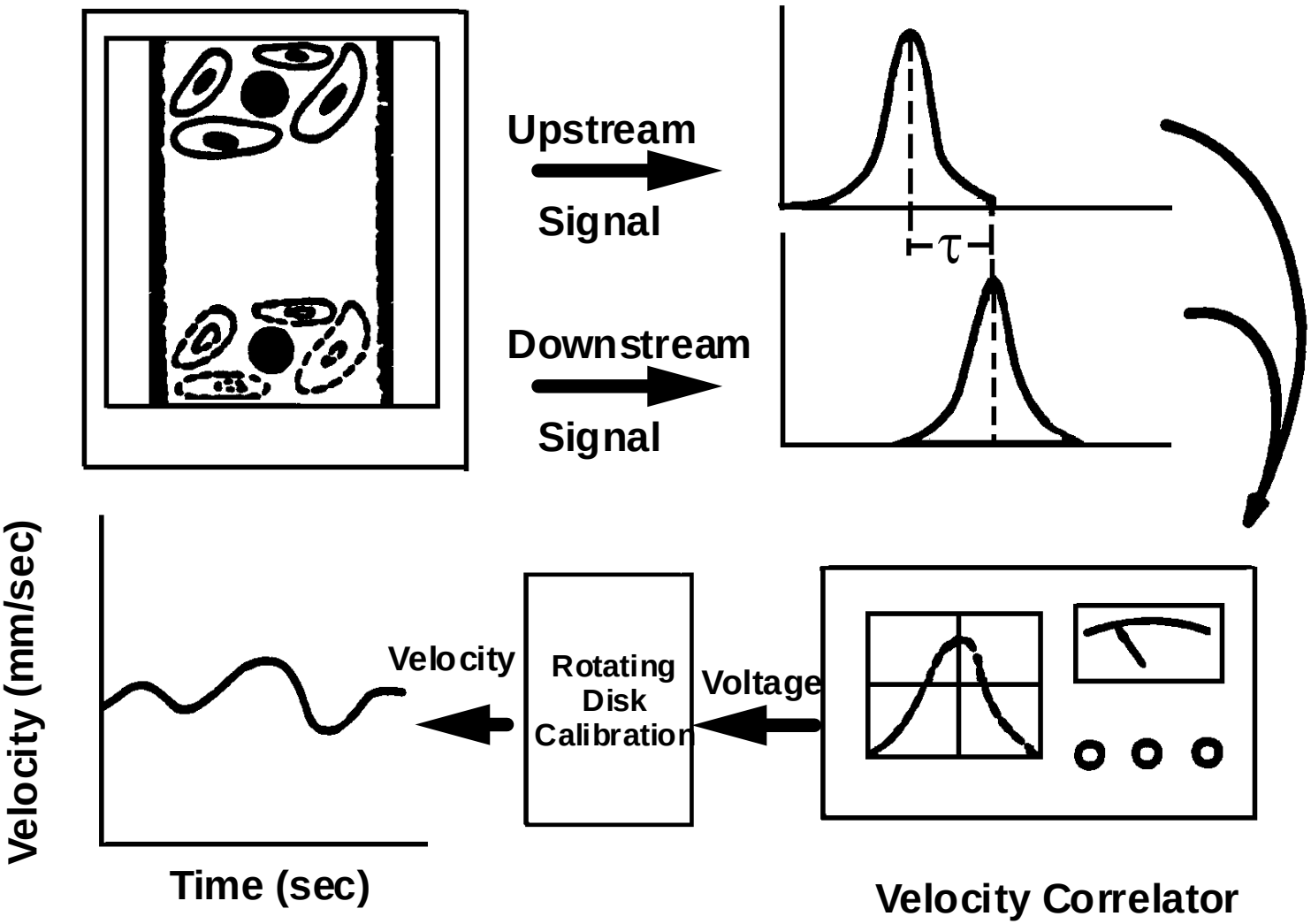
- How do we study drug delivery and tumor physiology?
- How does host-tumor interaction affect tumor physiology and therapeutic response?
- How do we measure tumor blood flow?
  - Directly or indirectly
  - Microscopically or macroscopically
- How does tumor blood flow compare with normal blood flow?
  - Temporally
  - Spatially
- What parameters govern tumor blood flow and how do we measure them?

# How Do We Measure Blood Flow?

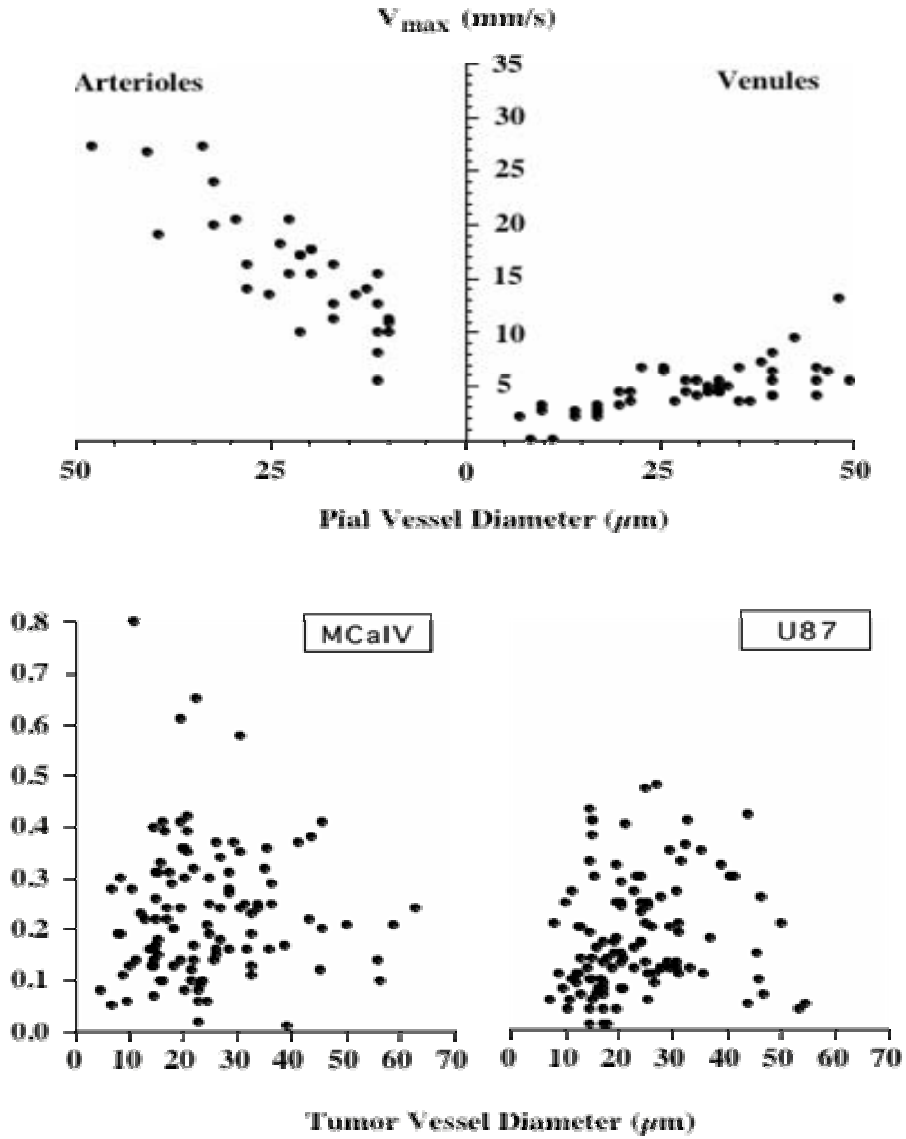
# Microscopic Methods

- **Flow rate of blood in individual vessels can be measured by measuring RBC velocity and cross-sectional area (A):**
  - **$Q = \alpha V_{RBC} A$**
  - **$\alpha$: depends on velocity profile and relative speed of RBC / plasma.**
  - **= 0.500 80 < D < 140 mm**
  - **= 0.625 17 < D < 80 mm**
  - **= 0.790 ? < D < 10 mm**
- **$V_{RBC}$ can be measured**
  - **Visually : Anton Van Leeuwenhoek (~1675)**
  - **Photographically**
  - **Opto–Electronically (most common)**
  - **Two-slit method**

# Schematic of Velocity Measurement

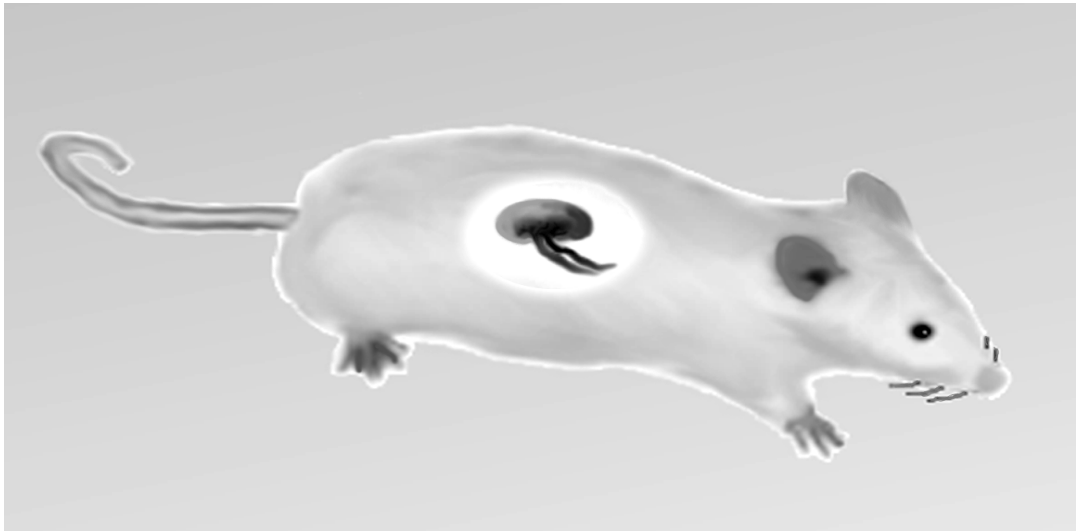


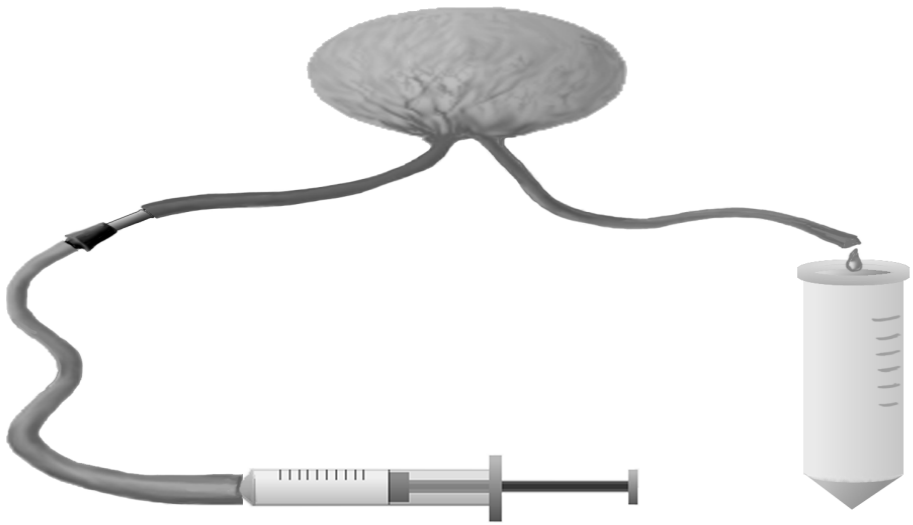
**2005**




Courtesy of the American Association for Cancer Research. Used with permission. Yuan, F., H. A. Salehi, Y. Boucher, U. S. Vasthare, R. F. Tuma, and R. K. Jain. "Vascular permeability and microcirculation of gliomas and mammary carcinomas transplanted in rat and mouse cranial windows." *Cancer Research* 54 (1994): 4564-4568.

**2005**







**Reference:** ***Kristiansen et al., Microvascular Research, (1994)***
***Less et al., Microcirculation, (1997)***

# Macroscopic Methods – Indirect

- Microsphere technique
- Uptake of radioactive tracers (e.g. $^{86}Rb$; $^{14}C$/$^{131}I$-AntiPyrine)
- Clearance of radio-tracers (e.g. $^{133}Xe$; $^{85}Kr$)
- PET
- NMR
- Ultrasound
- Thermal clearance
- Thermal probe
- Laser doppler
- Plethysmography
- Electromagnetic flowmeter



Reference: *Jain & Ward-Hartley, IEEE Trans., S-US, (1984); Vaupel and Jain, (1991); Eskey et al., JNCI, (1994)*

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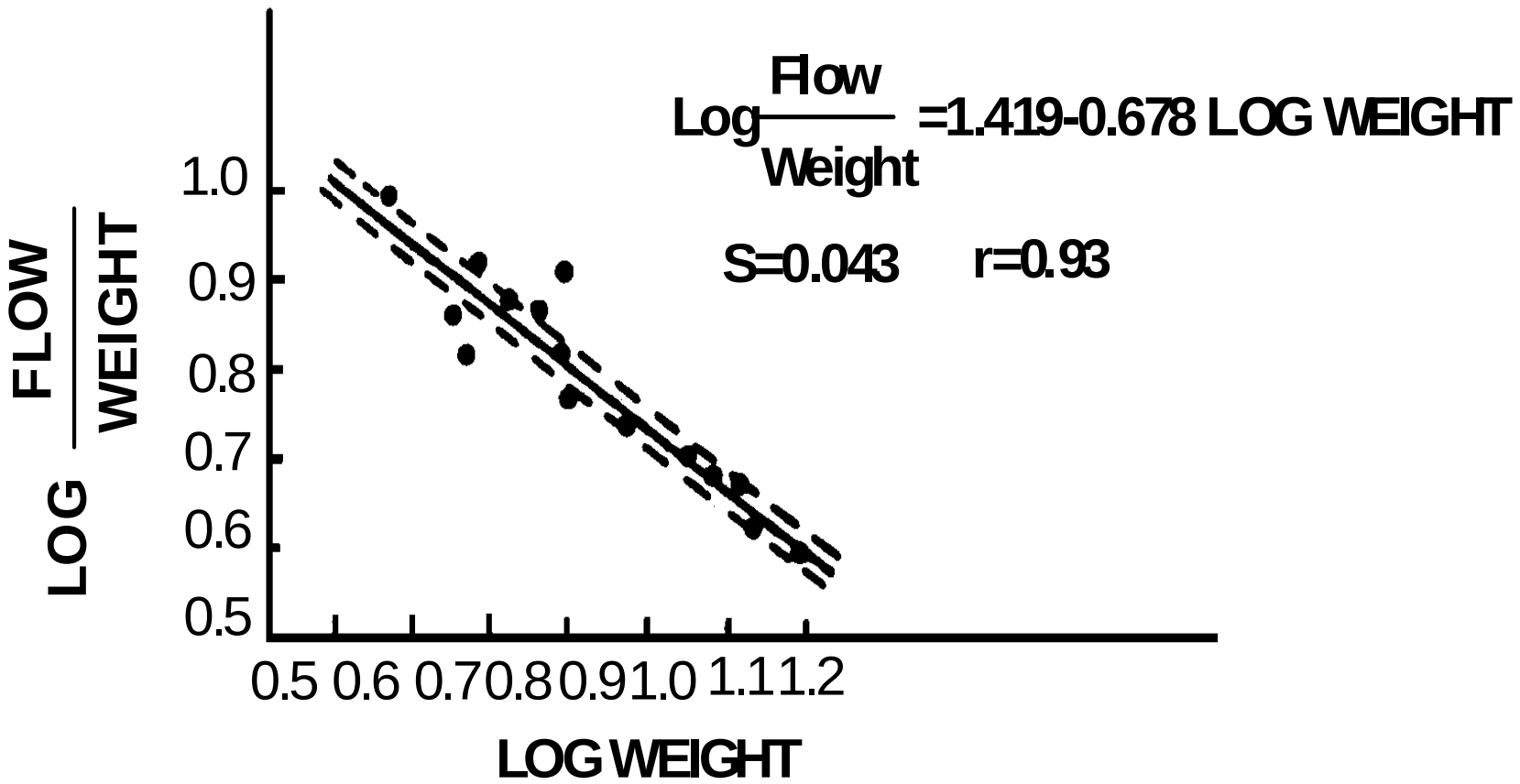
- **As tumors grow larger, their average perfusion rate decreases, in general, due to development of necrotic foci.**

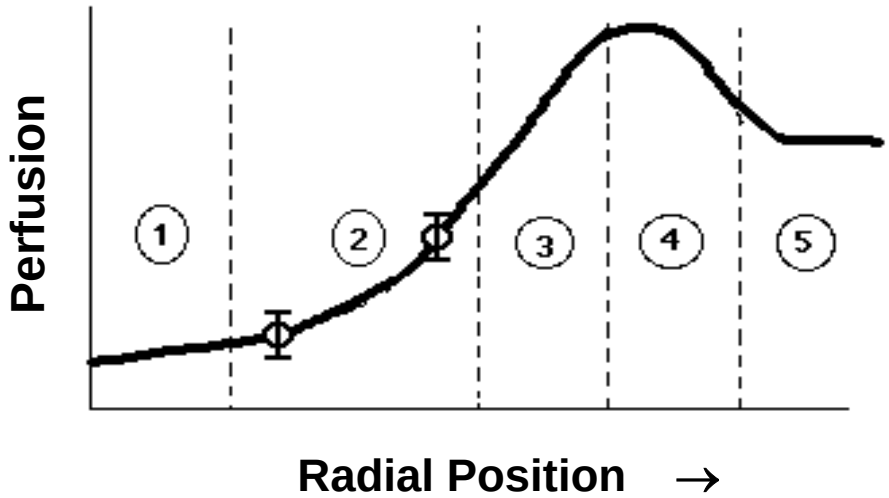





Reference: *Gullino, (1968)*

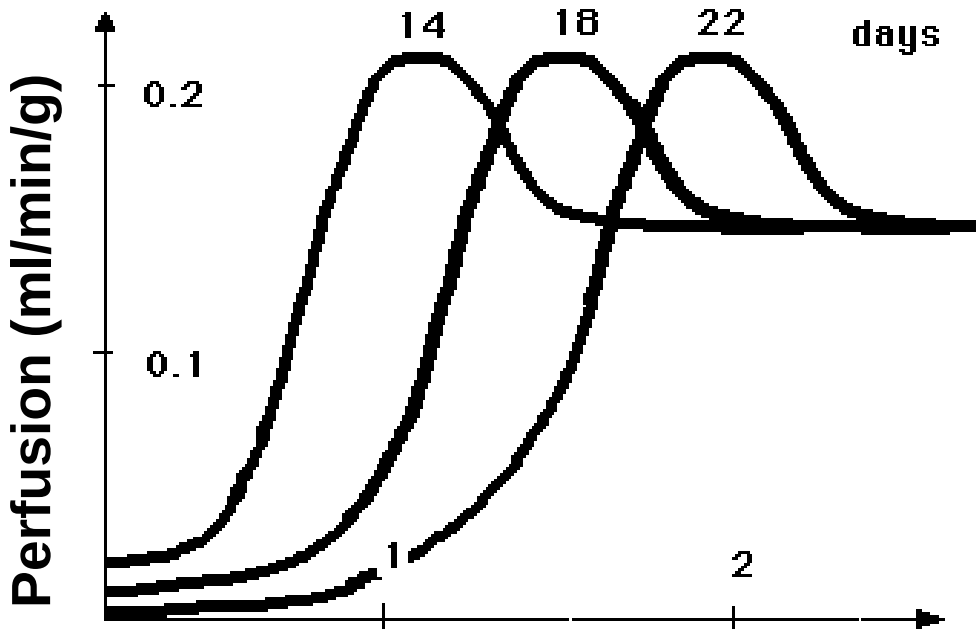
# Quantitative Data

- **2-D Tumors (Endrich et al, JNCI, 1979)**
- **At a fixed time**




**1) Necrotic zone**
**2) Semi-necrotic zone**
**3) Stabilized tumor circulation (A + V's)**
**4) Advancing front (percolation)**
**5) Normal tissue**

- **As a function of time**




- **Since the fraction of necrotic and semi-necrotic tissue increases with growth=> average perfusion rate decreases.**

# What Parameters Govern Tumor Blood Flow?

$$Q = \frac{\Delta P}{FR}$$

| | | |
|---|---|---|
| Q | = | Flow rate (Vessel/Tissue) |
| $\Delta P$ | = | Pressure difference between arterial and venous ends |
| FR | = | Flow resistance |
| | = | $\eta \cdot Z$ |
| $\eta$ | = | Apparent viscosity |
| | | (Viscous resistance) |
| Z | = | Geometrical resistance |

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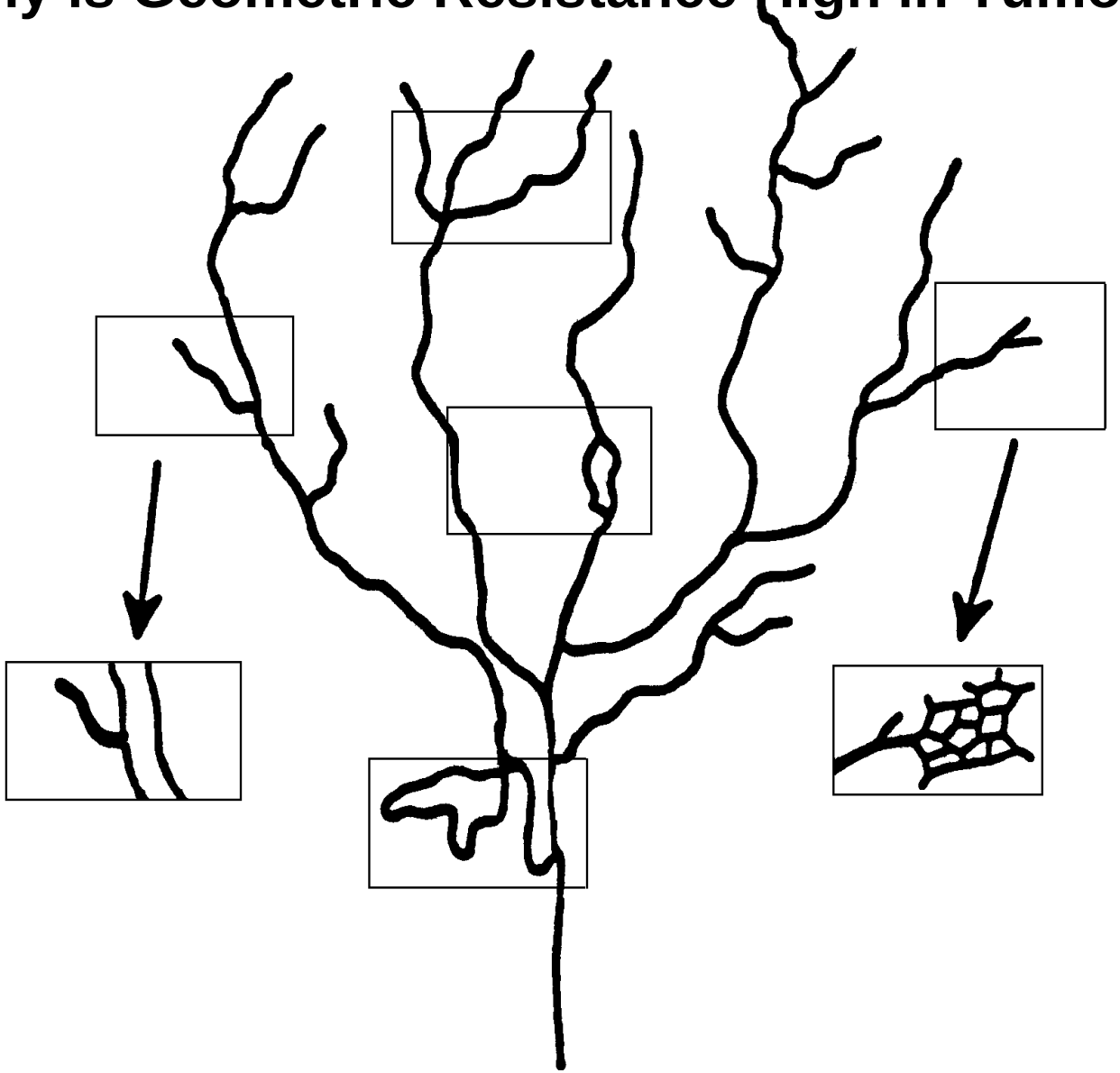
# Why is Geometric Resistance High in Tumors?

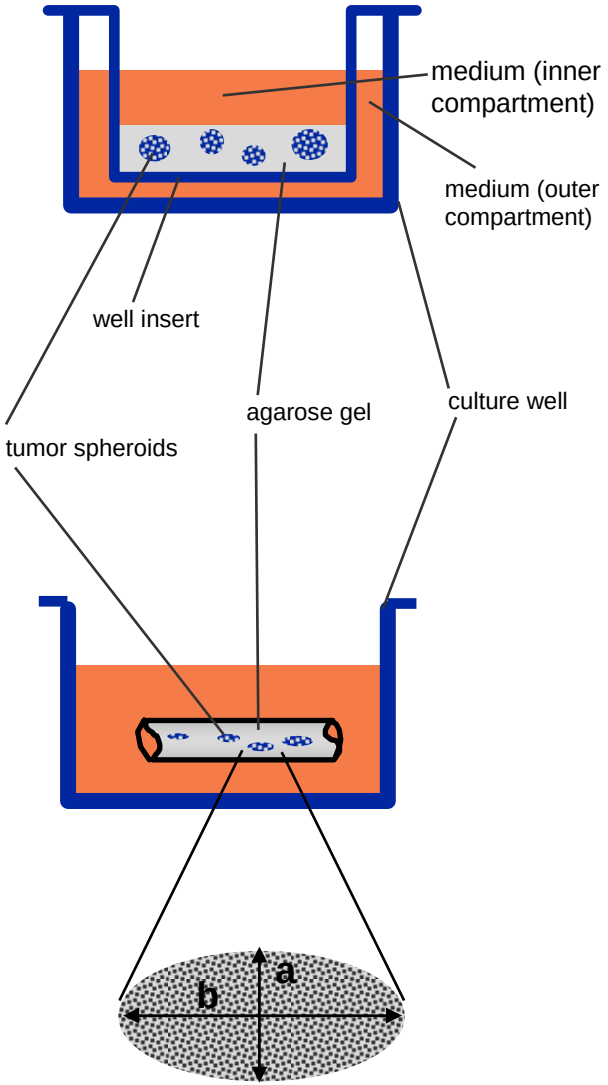


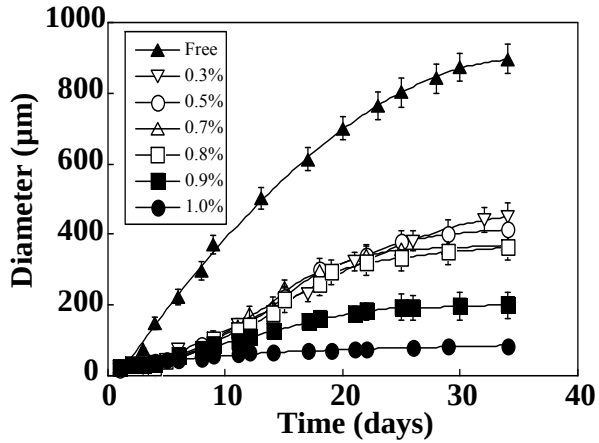
***Peculiar Branching Patterns of Tumor Vessels***

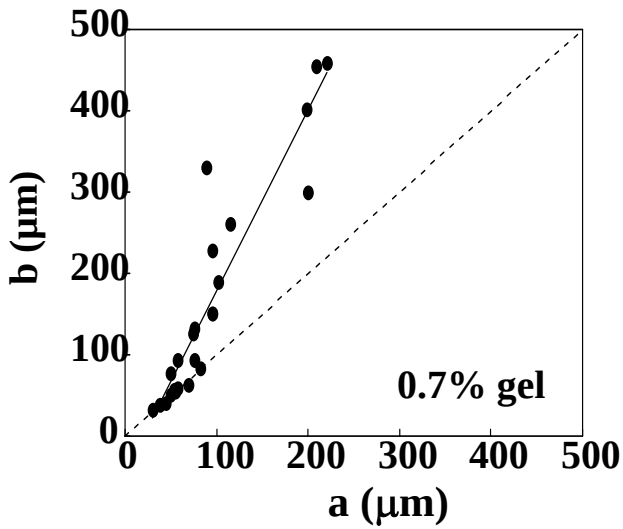
**Reference:** ***Less, Skalak, Sevick and Jain, Cancer Research, (1991)***
***Baish and Jain, Cancer Research, (2000)***

# Why is Geometric Resistance High in Tumors?

*Tumor vessels can be compressed/collapsed by growing cells*



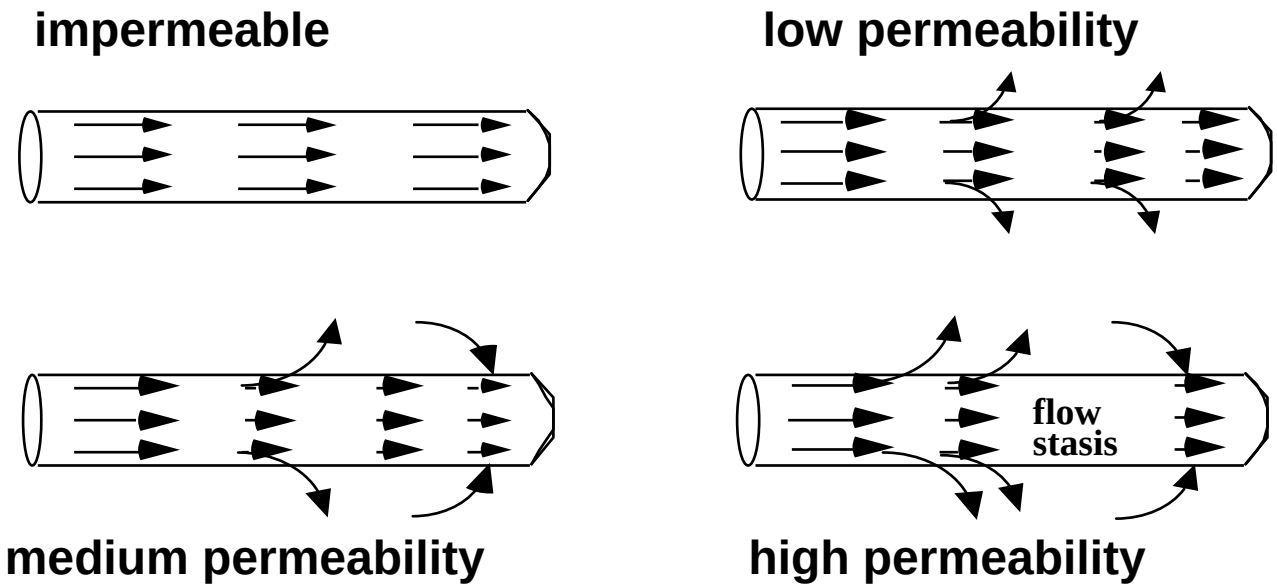






Reference: ***Helmlinger et al., Nature Biotechnology, (1997)***

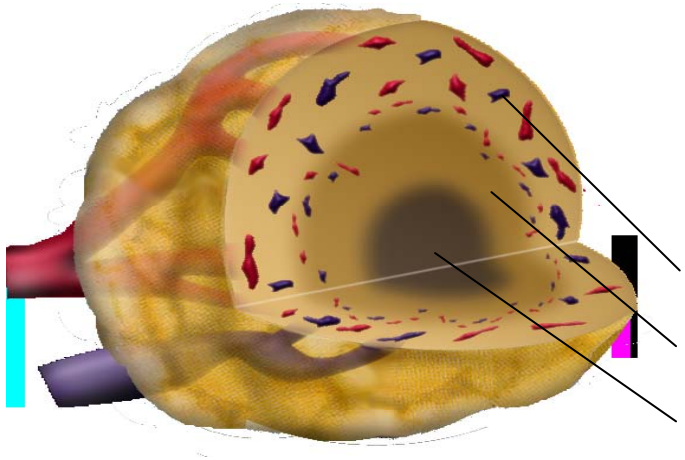
# Can Elevated IFP and Hyperpermeability Decrease Tumor Blood Flow?



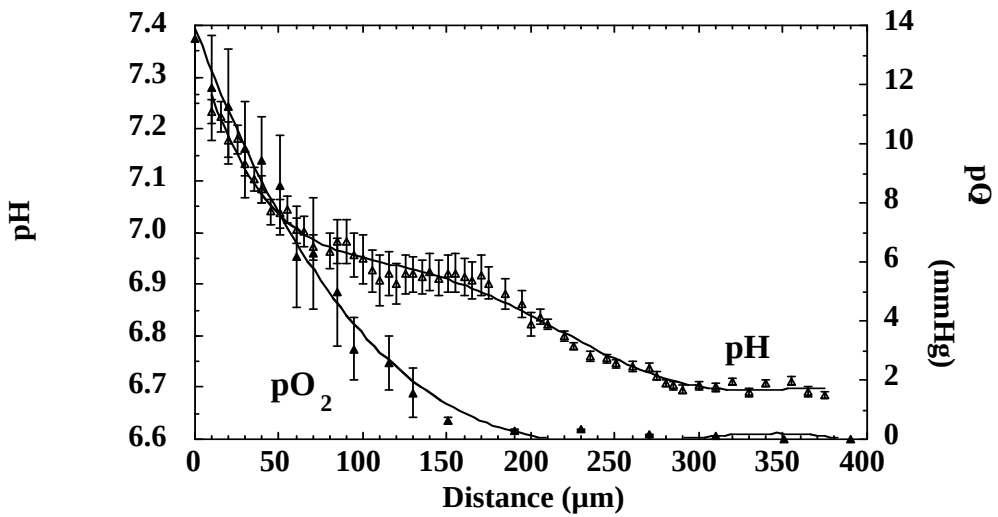



Reference: *Netti et al., Microvascular Research, (1996)*

# Metabolic Microenvironment



| Tumor Region | Perfusion | Interstitial Pressure | $pO_2$ | Extracellular pH | Drug Delivery |
|---|---|---|---|---|---|
| Proliferative | ++ | ++ | ++ | <7.4 | ++ |
| Quiescent | + | +++ | + | ~6.5 – 7 | + |
| Necrotic | - | +++ | - | ~7 - 8 | +/- |



# Summary – I

- **Tumor blood flow is important in tumor growth, detection and treatment.**
- **Various microcirculatory preparations permit an *in vivo* look at the tumor blood flow.**
- **Tumor blood flow is spatially and temporally heterogeneous, and depends on the host-tumor interaction**
- **Tumor perfusion rate may decrease with growth.**
- **Local imbalance of positive and negative regulators of angiogenesis may contribute to focal necrosis.**
- **Blood flow is proportional to arterio-venous pressure difference and inversely related to geometric and viscous resistances.**
- **Both geometric and viscous resistances in tumors are higher compared to several normal tissues.**

# Summary – II

- **Geometric resistance offered by tumor vessels is higher due to their peculiar geometry and branching patterns.**
- **Tumor vessels may be compressed by growing cancer cells and/or interstitial matrix.**
- **Viscous resistance in tumors is elevated due to hemoconcentration.**
- **Tumor blood flow may be impaired due to high vascular permeability coupled with interstitial hypertension.**
- **Impaired microcirculation can compromise the metabolic microenvironment of tumors. Hypoxia and acidosis can induce drug resistance.**