

Intravital microscopy of cancer cell invasion and therapy response

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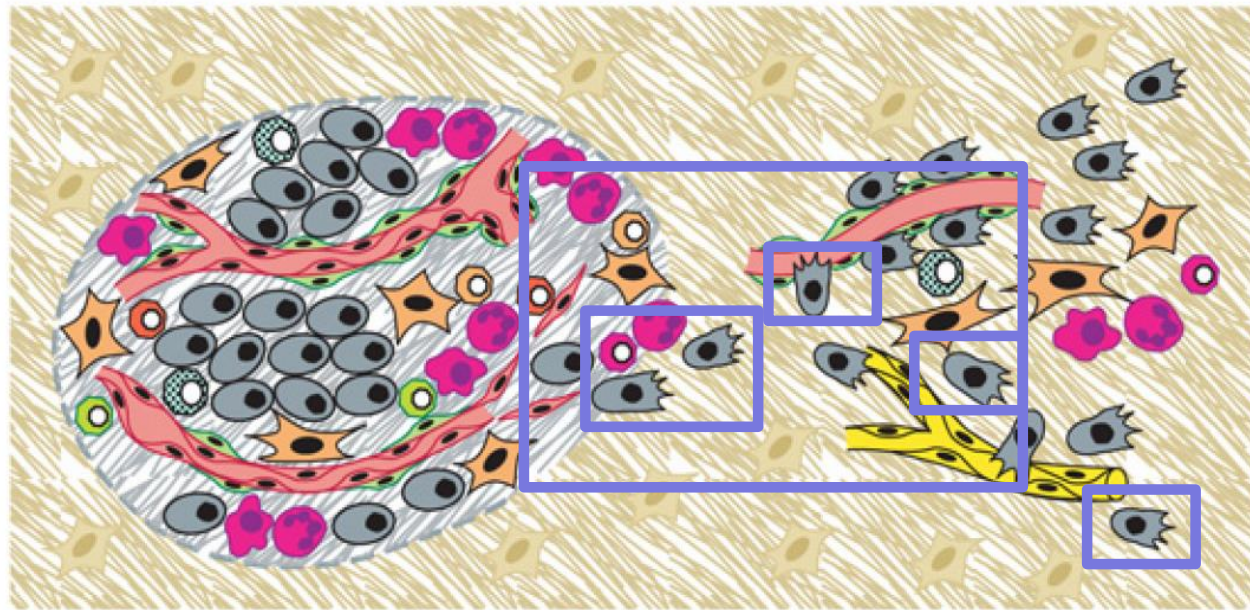
David H. Koch Center
The University of Texas MD Anderson Cancer Center



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university medical center

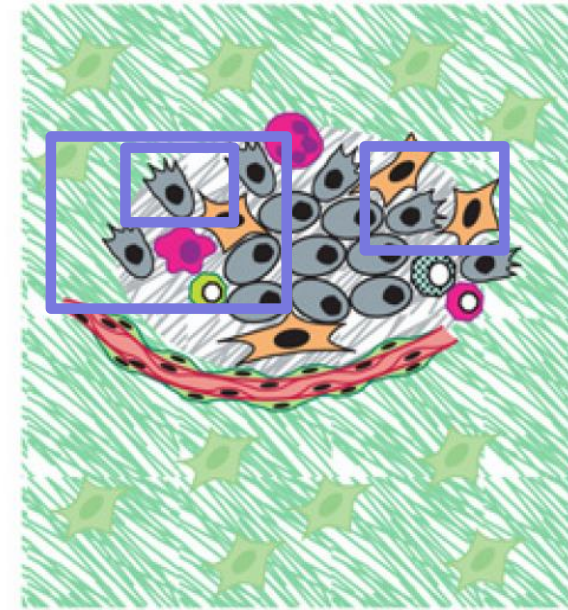
THE UNIVERSITY OF TEXAS
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~~Cancer Center~~
Making Cancer History®

Cancer progression and metastasis: niche business



Core of Primary Tumor
microenvironment

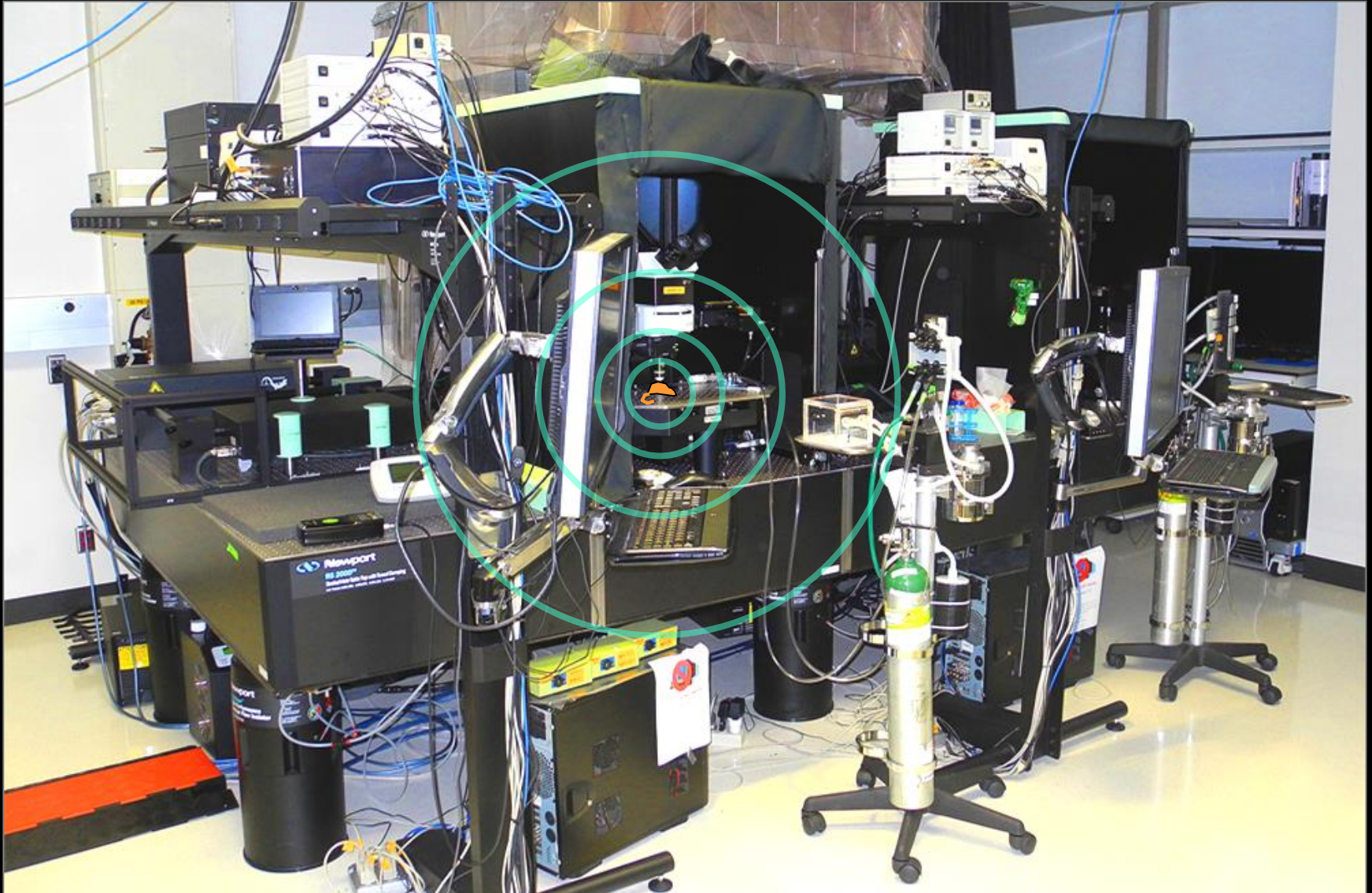
Invasive Tumor
microenvironment



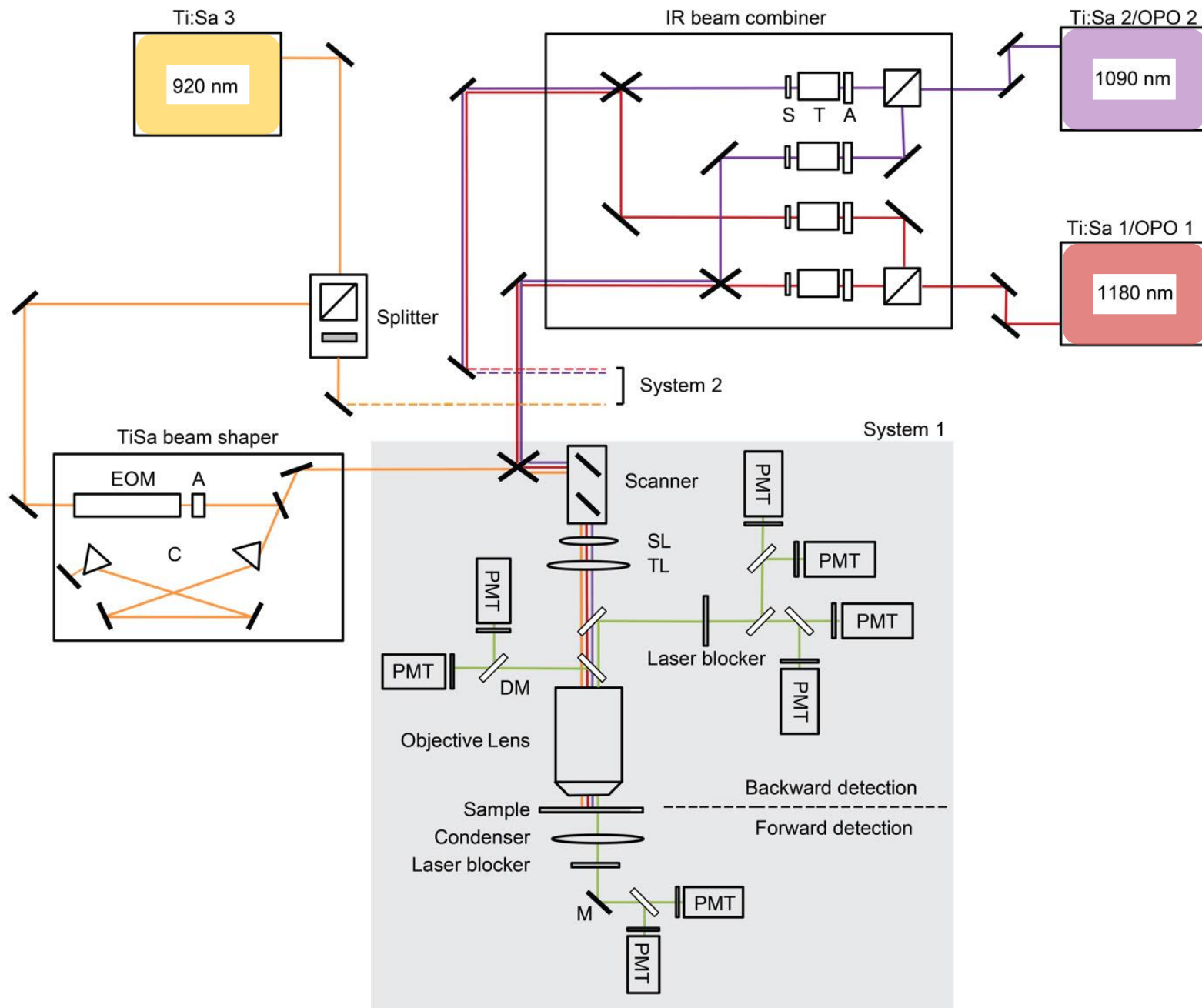
Metastatic Tumor
microenvironment

Hanahan & Weinberg, Cell, 2011

Near-infrared and infrared multiphoton microscopy



3 Titanium:Sapphire lasers (700 – 1060 nm)
2 Optical Parametric Oscillators (1060 – 1400 nm)

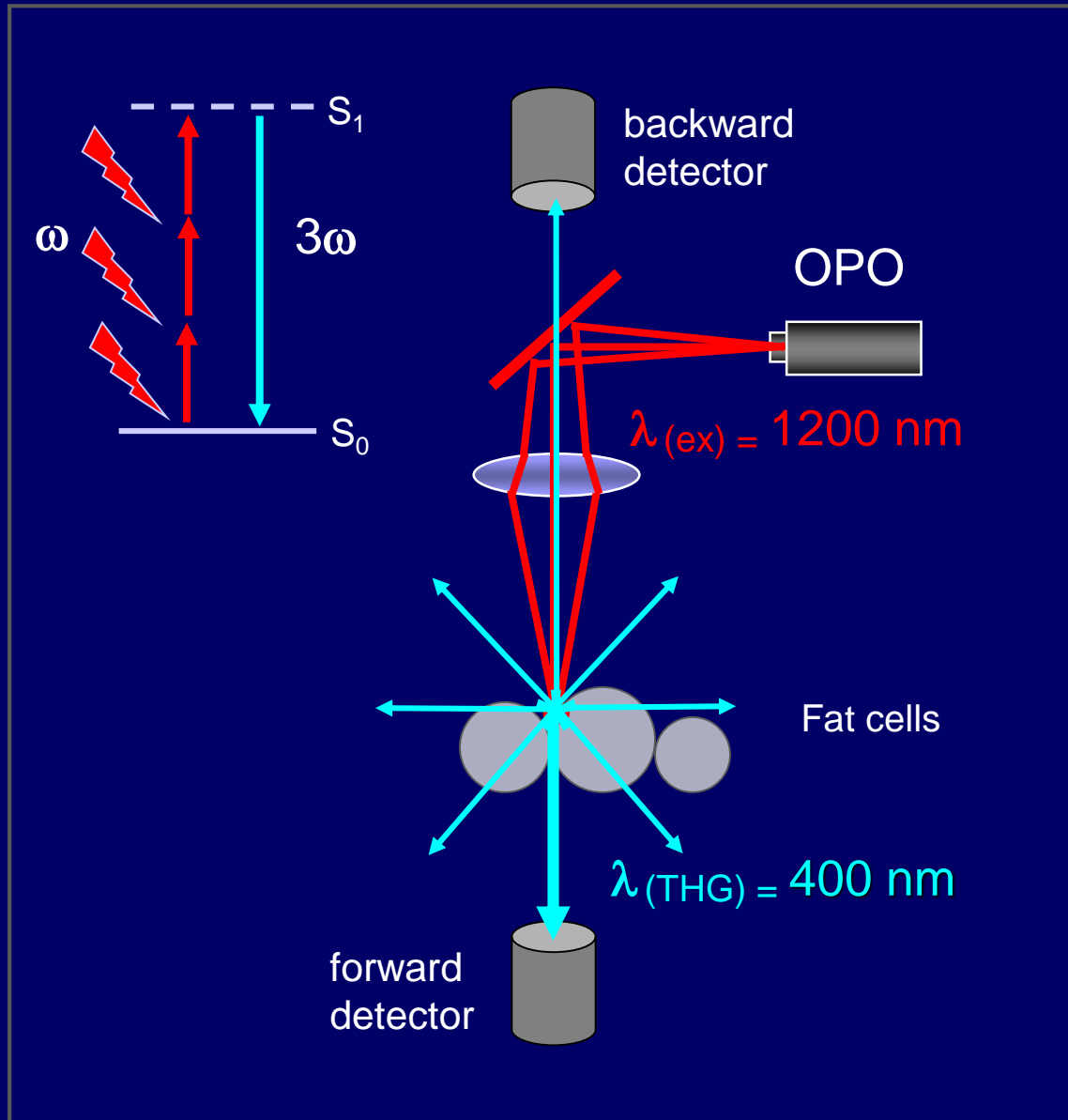


Nearinfrared / infrared-excited multiphoton microscopy

- ▶ Efficient excitation of red-shifted fluorophores (RFP, mCherry, Neptun, E2-Crimson, AlexaFluor 660...)
- ▶ 80 - 100% deeper tissue penetration
- ▶ Low phototoxicity (<100 mW)
- ▶ **Third harmonic generation** to visualize interfaces
- ▶ Combined fluorescence intensity with lifetime detection
- ▶ *In development:* Multiphoton endomicroscopy of deep tissue regions

Andresen et al., Curr. Opin. Biotechnol. (2009); Weigelin et al., IntraVital (2012)
Bakker et al., Meth. Enzymol. (2012); Alexander et al., Curr. Opin. Cell Biol. (2013)

Third harmonic generation (THG)



Scatter process:

unification of 3
photons

→ triple frequency

→ scattered by tissue

Generated by:

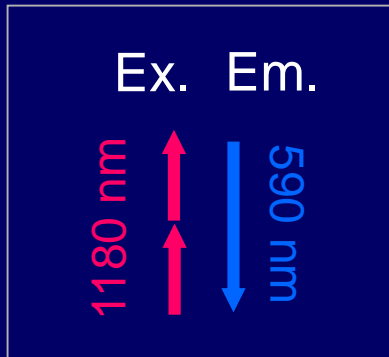
Interfaces

Inhomogeneities

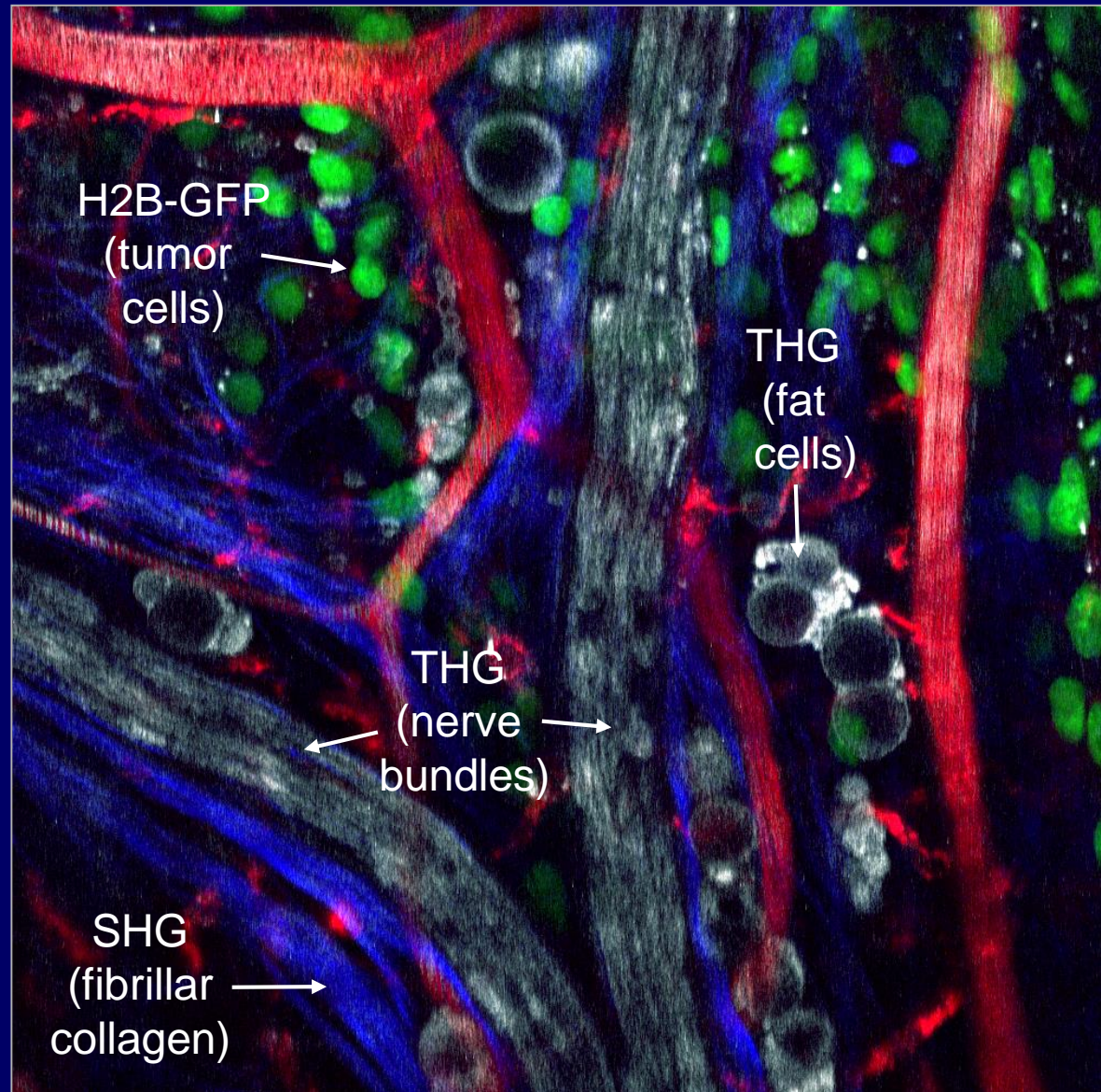
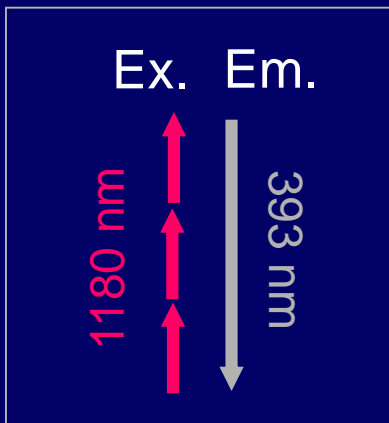
Dependent on size and
orientation of
heterogeneities relative
to light path

Combined THG / SHG / fluorescence intensity imaging

Second harmonic generation

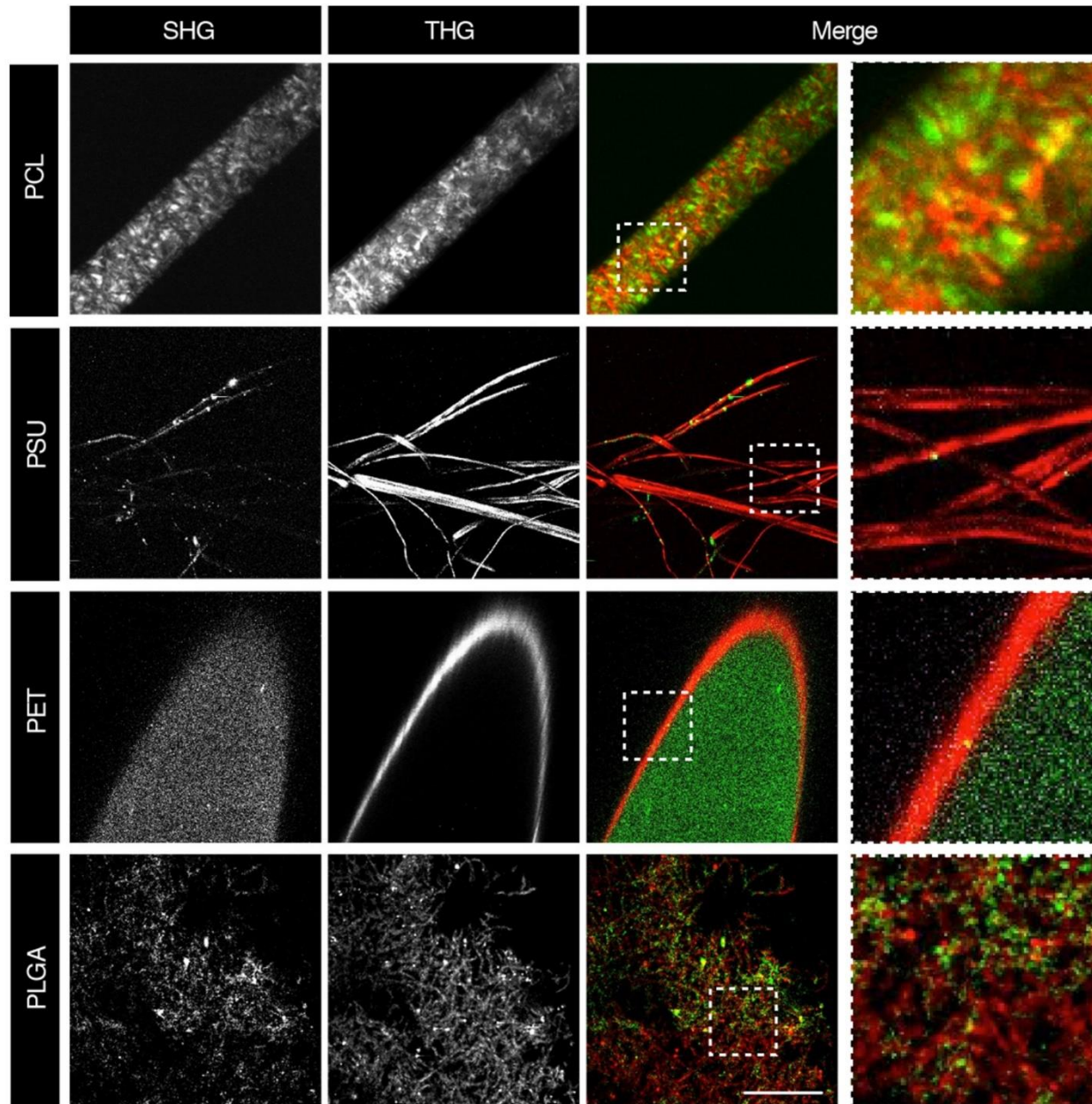


Third harmonic generation

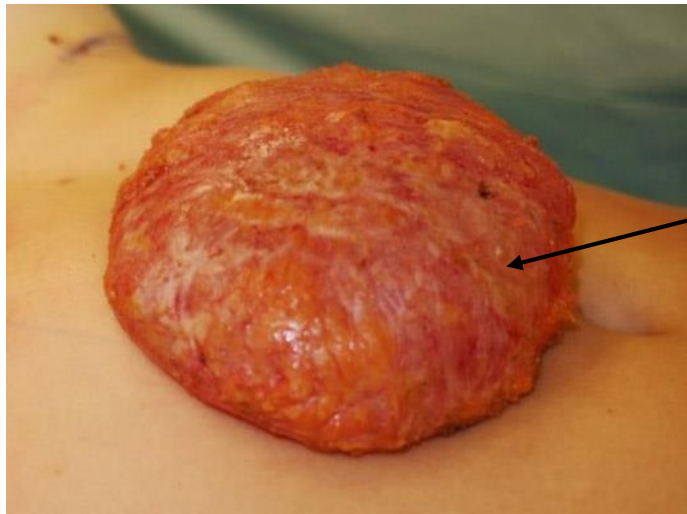


Nonlinear
microscopy of
biomaterials
in vivo

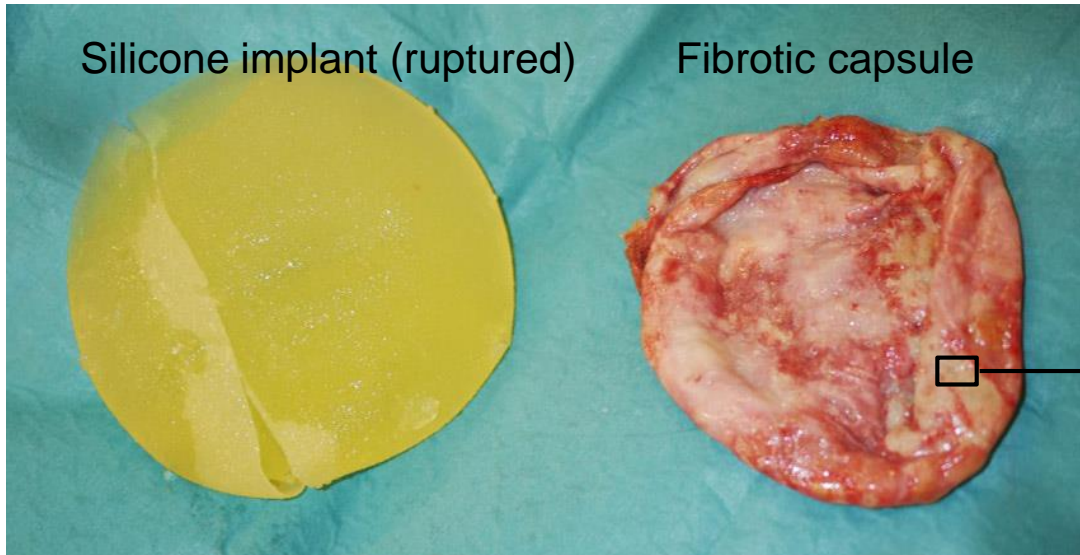
Higher harmonic microscopy of medical implant materials



Foreign body response: fibrotic encapsulation of implants

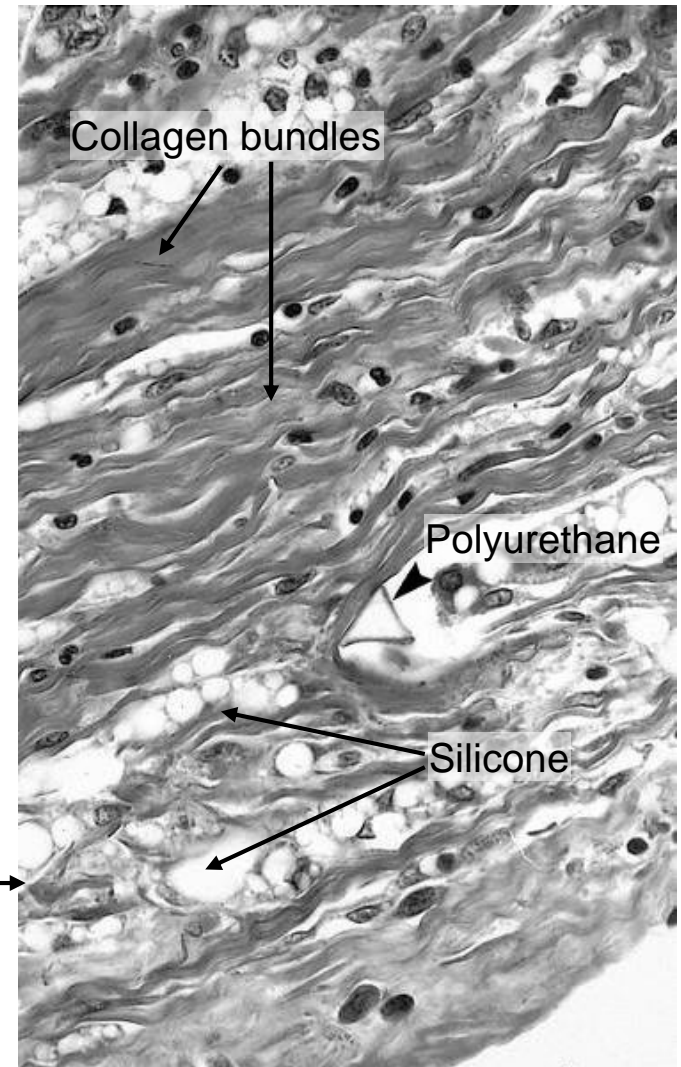


Fibrotic
silicone
breast
implant
(after
surgical
removal)



Silicone implant (ruptured)

Fibrotic capsule

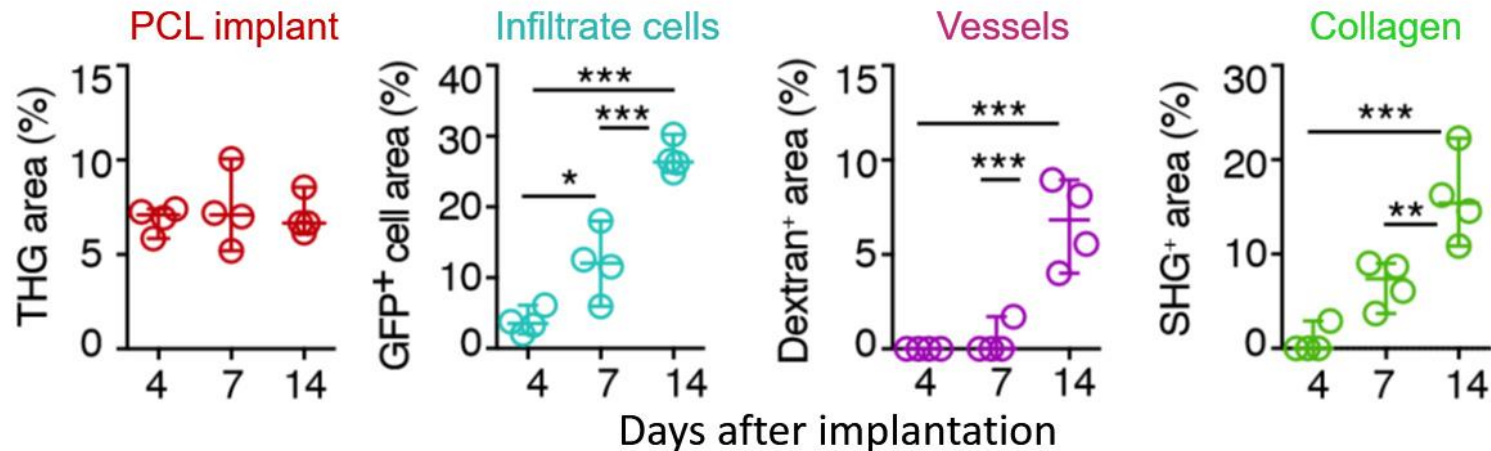
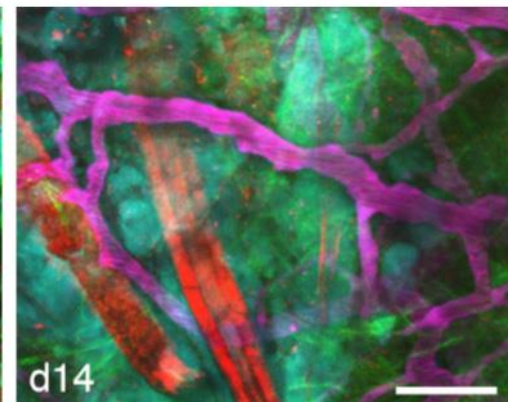
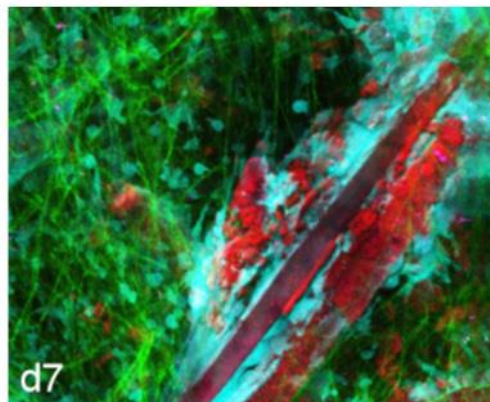
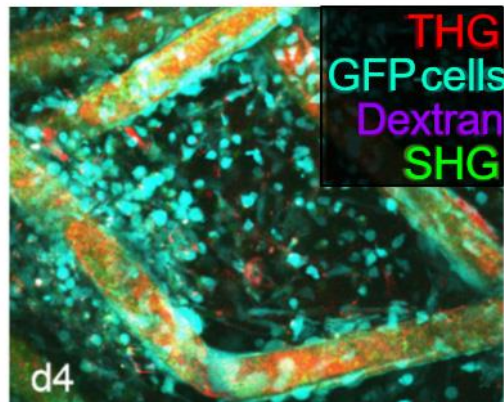
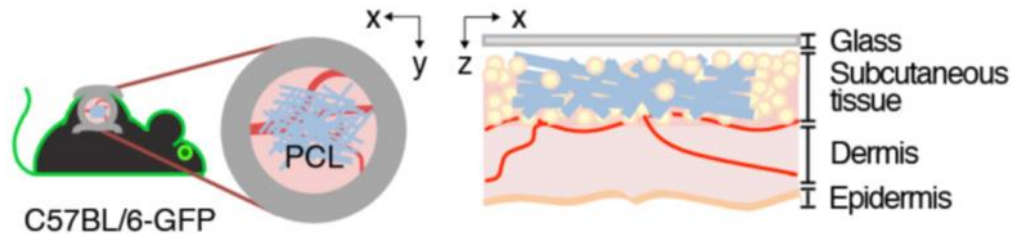


Collagen bundles

Polyurethane

Silicone

Higher harmonic microscopy of the FBR response



nature biomedical engineering

Monocyte/
macrophage

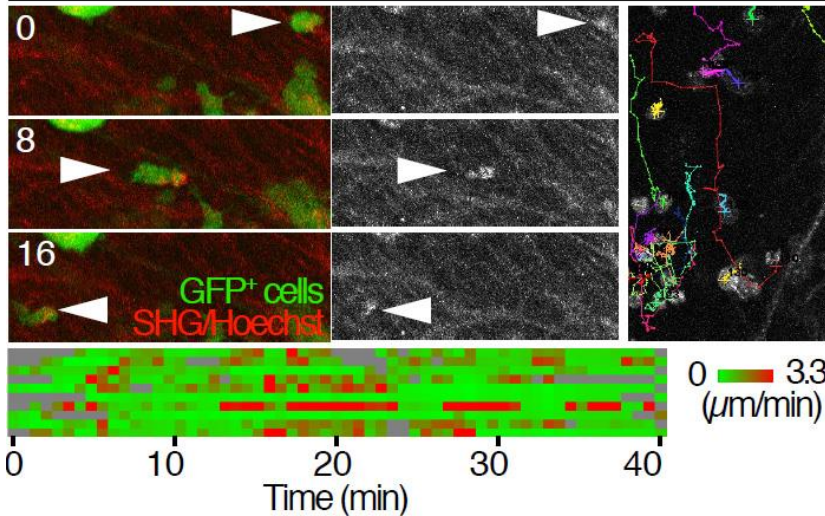
Giant
cell

PCL fiber

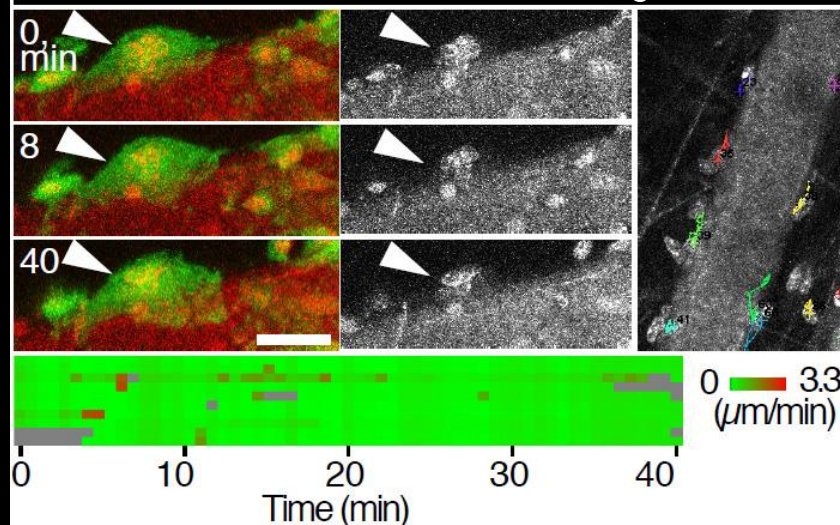
Neovessel

Fibrotic
capsule

Interstitial cells (monocytes/macrophages)



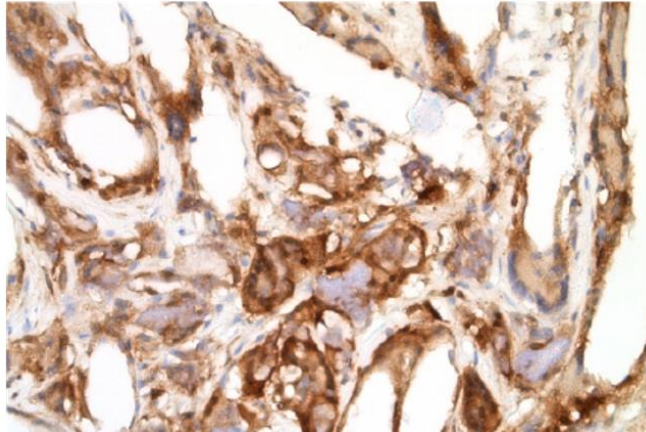
Scaffold-associated multinucleated giant cells



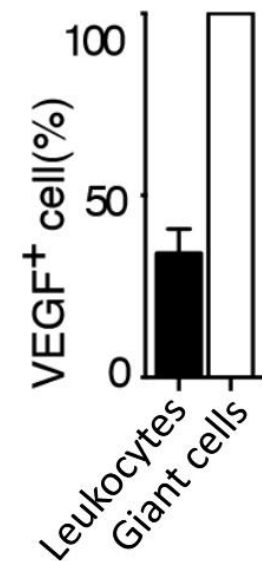
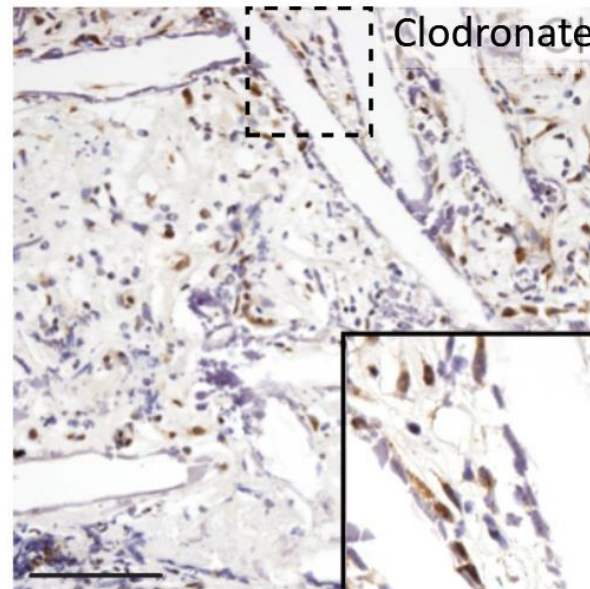
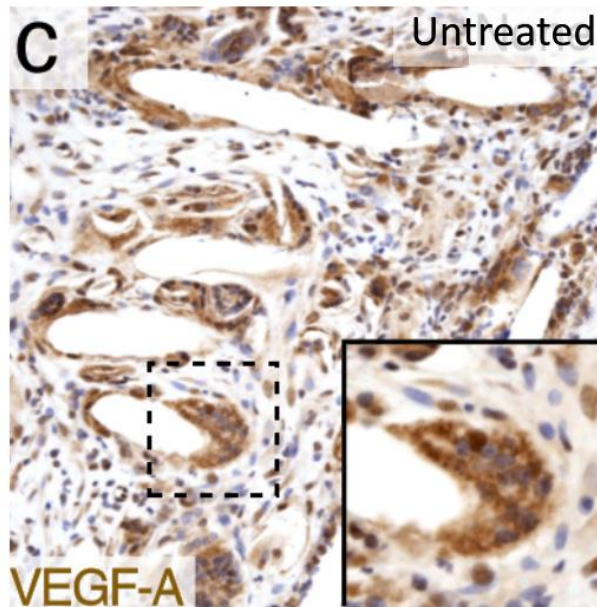
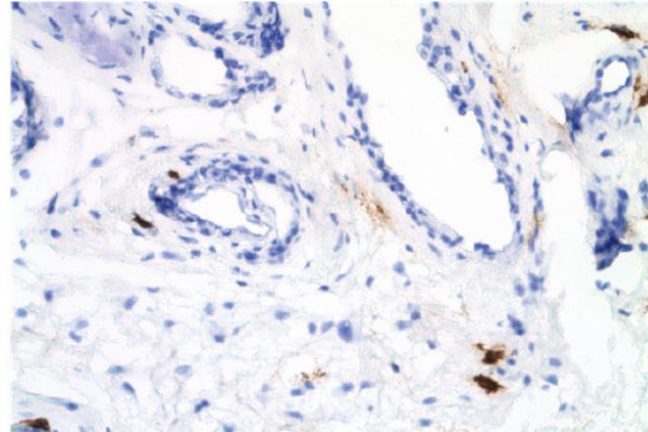
Macrophages and giant cells:
friends or foes?

VEGF producing M1 macrophages and giant cells

IRF5 (M1)



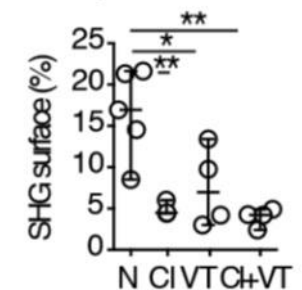
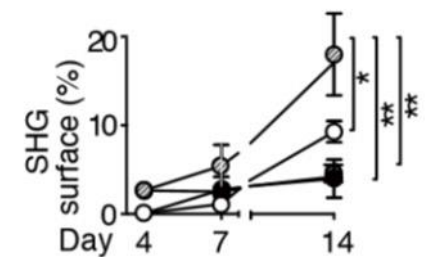
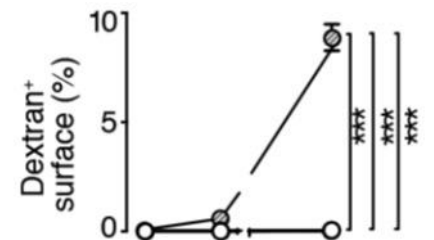
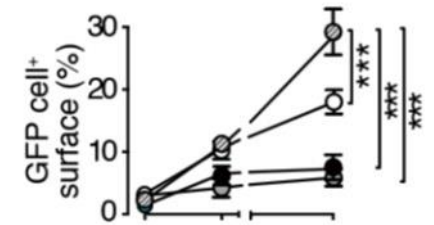
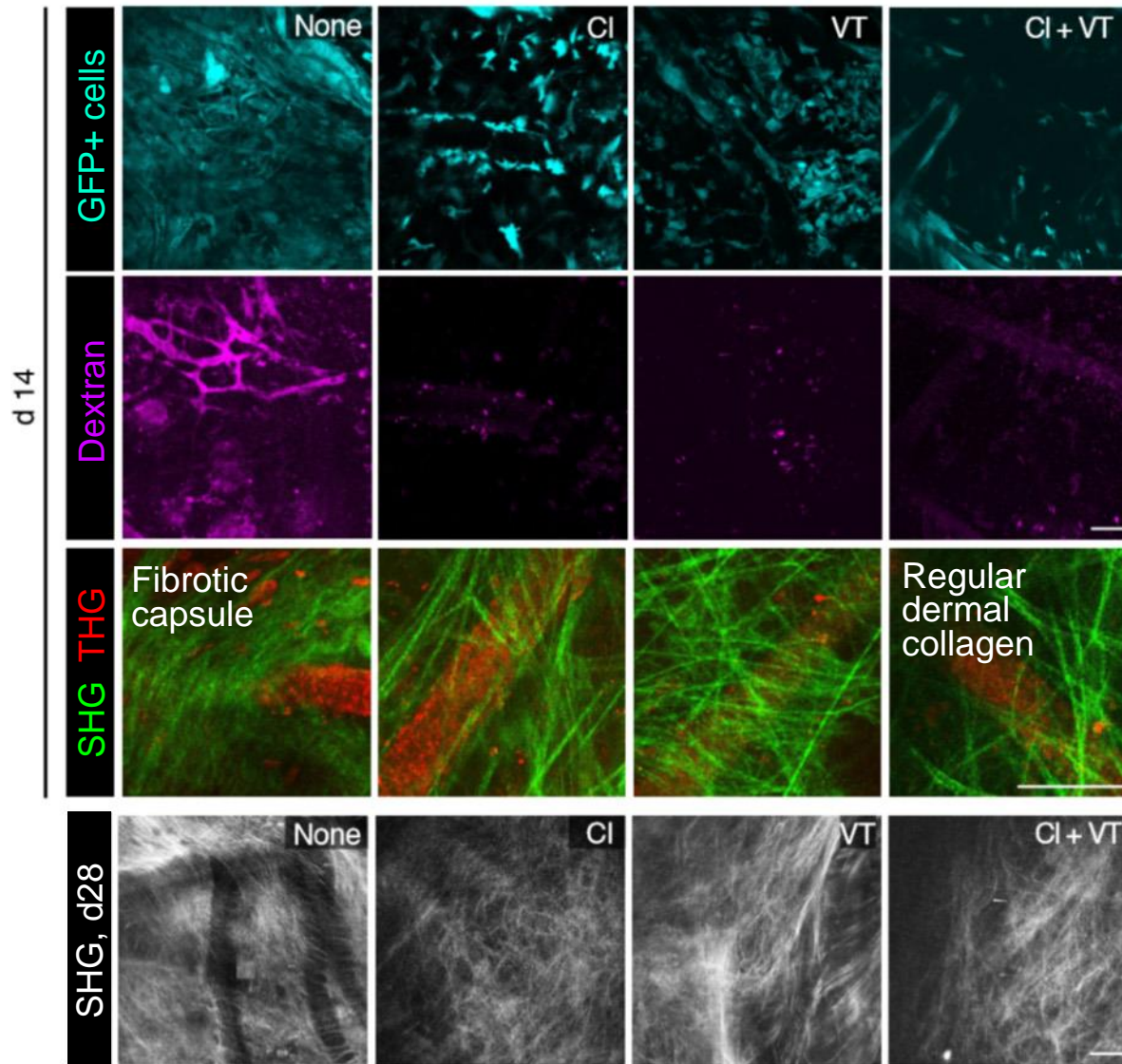
CD163 (M2)



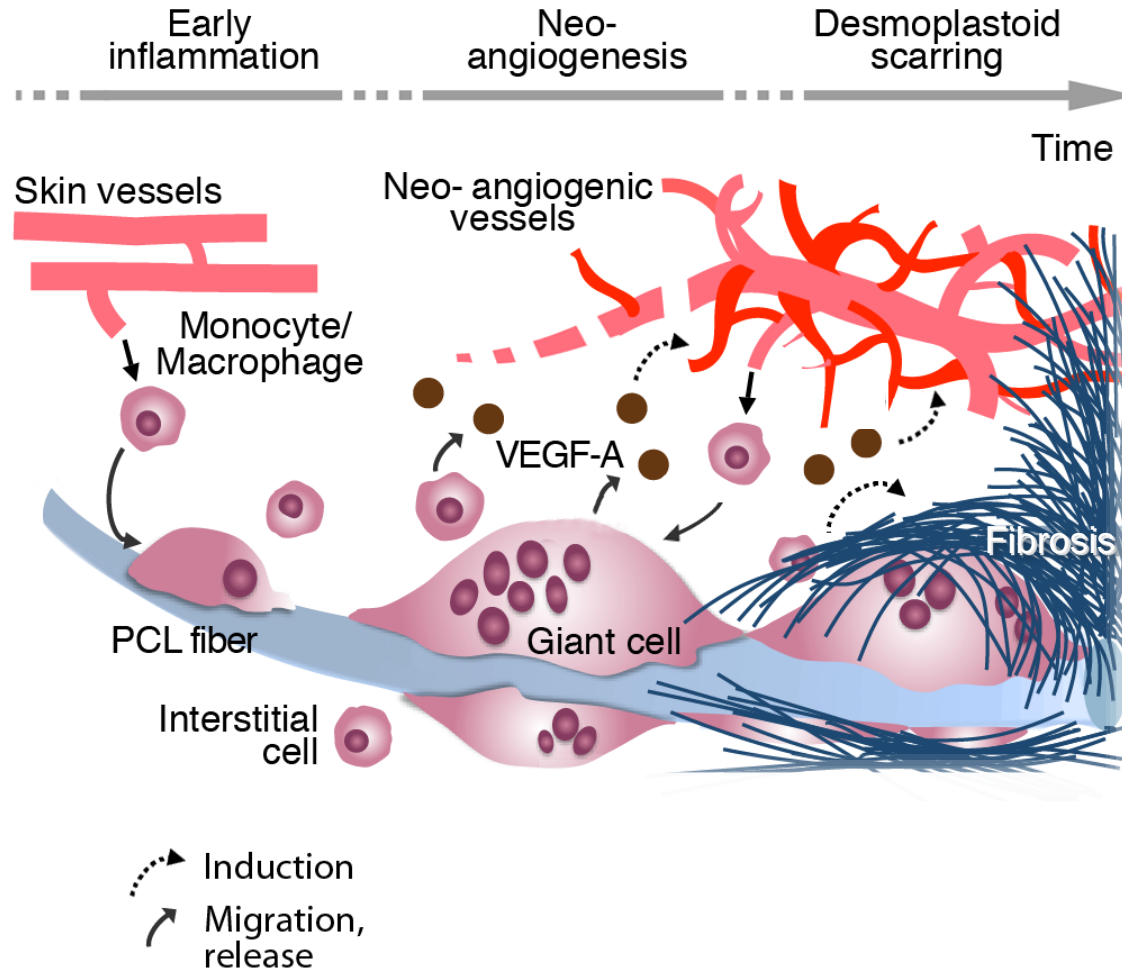
Interfering with the foreign body response



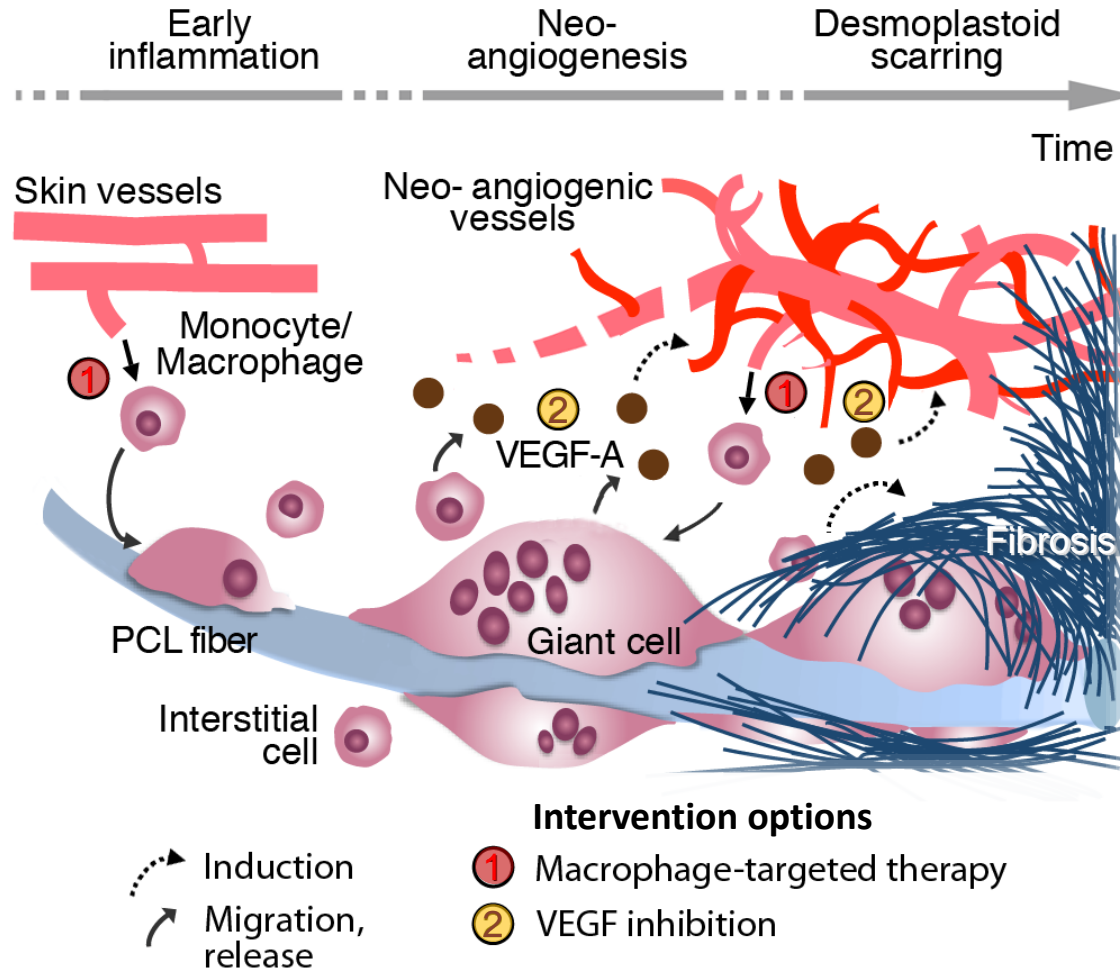
- Legend for graphs:
- Untreated
 - VEGF Trap
 - Clodronate
 - Clodr.+VT



Foreign body response: desmoplasia-like progression

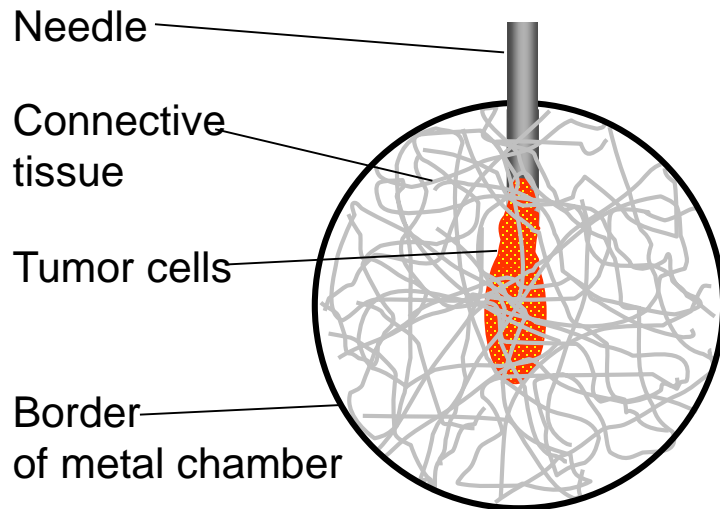


Foreign body response: desmoplasia-like progression

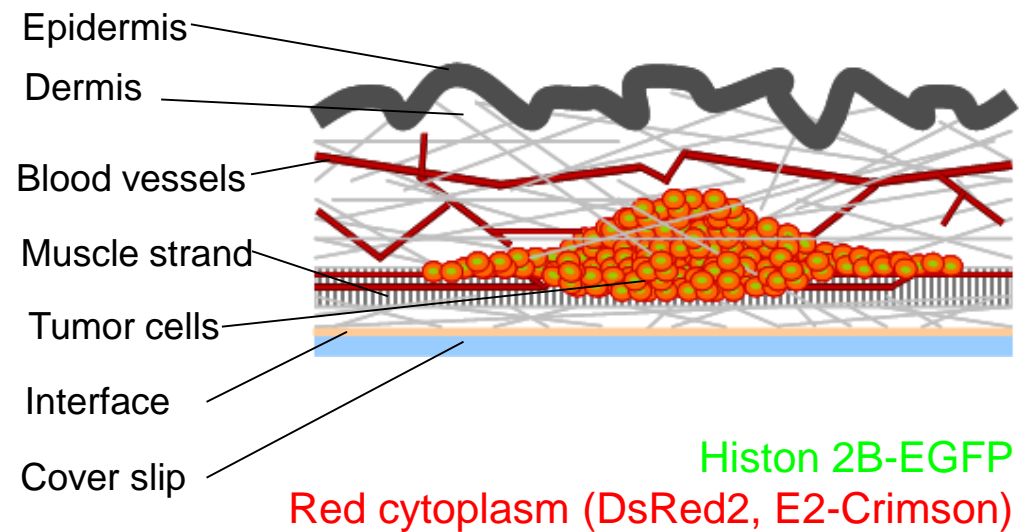


Monitoring tumor invasion and metastasis

Modified dorsal skin-fold chamber model



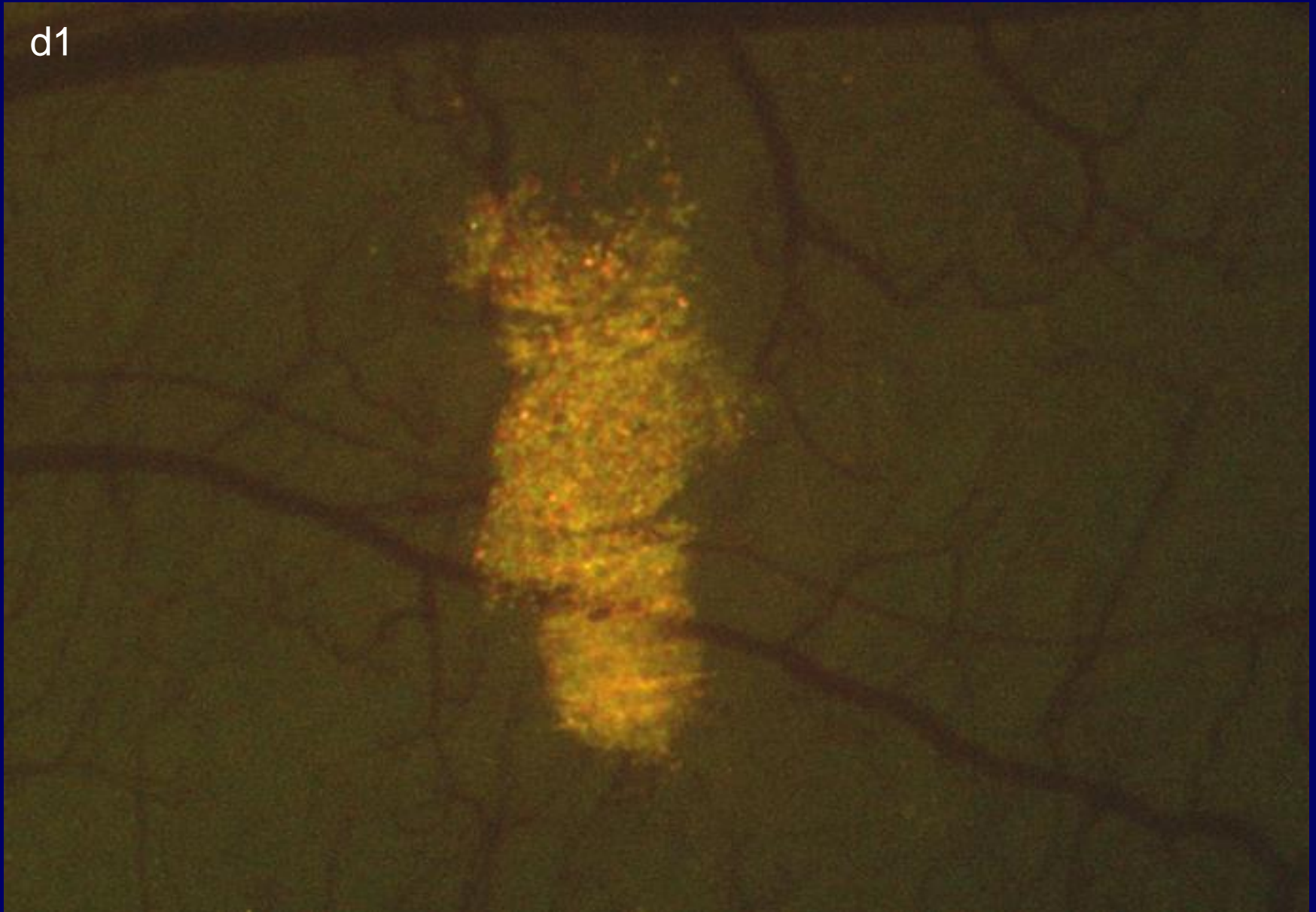
Xenograft tumors: HT-1080, MV3
Syngenic tumors: B16/F10, MFS



Alexander et al., Histochem. Cell Biol. (2008)

Dynamics of tumor neoangiogenesis and invasion

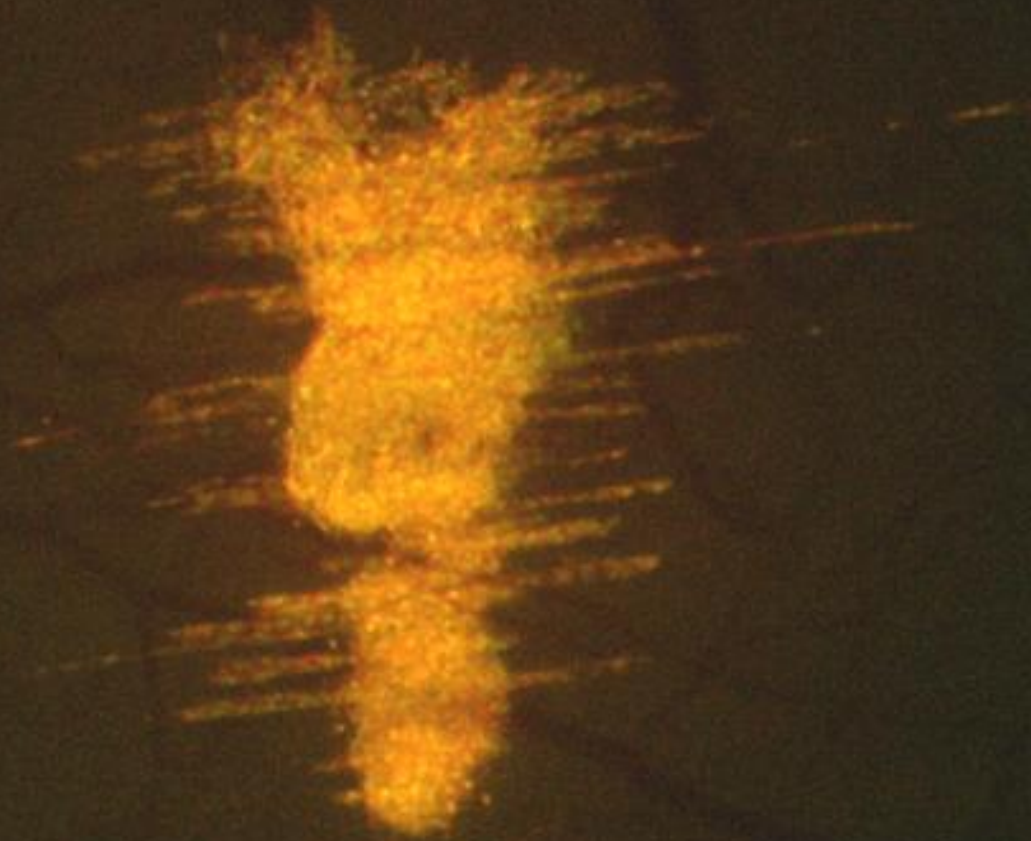
d1



Dynamics of tumor neoangiogenesis and invasion

d3

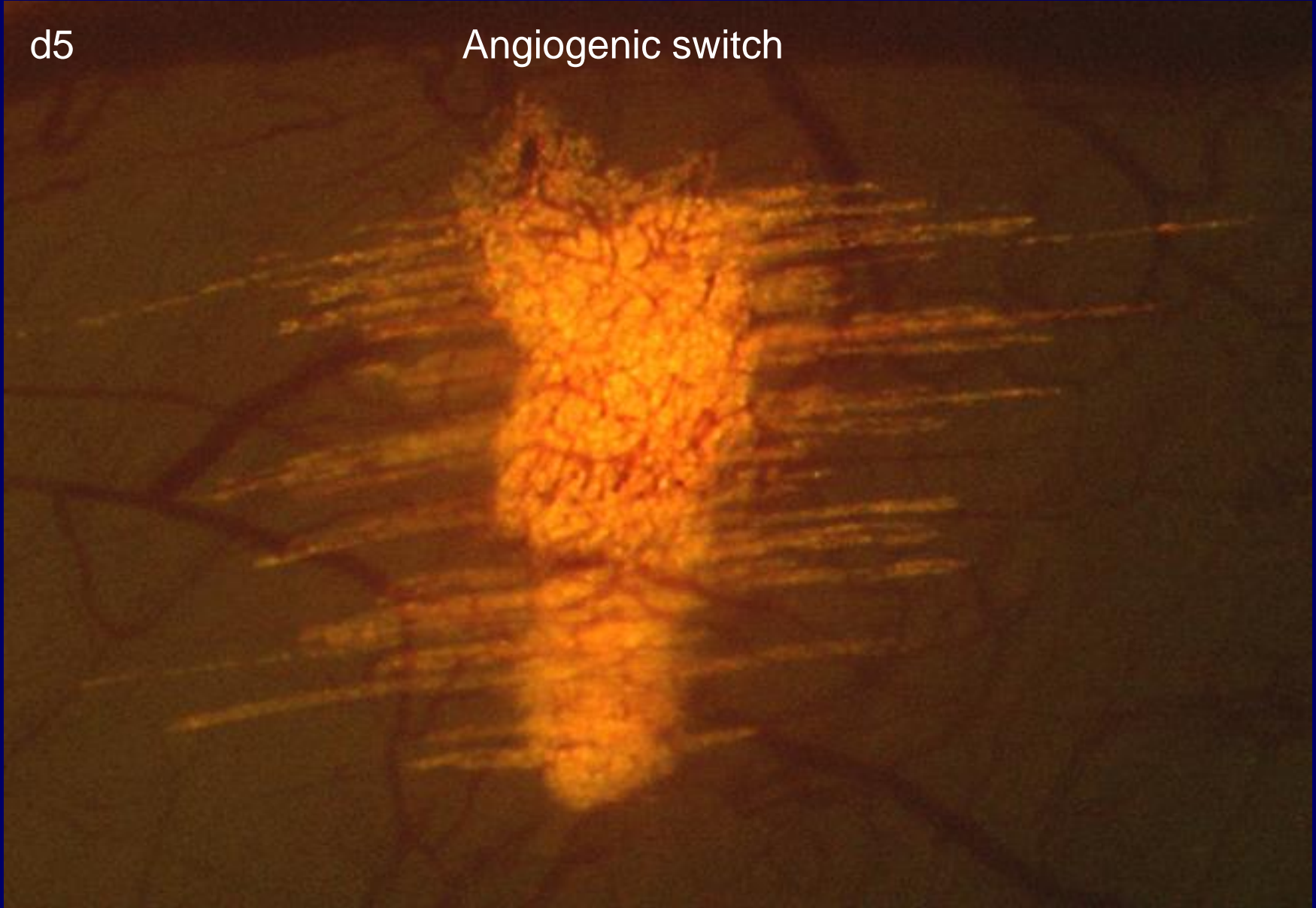
Onset of invasion



Dynamics of tumor neoangiogenesis and invasion

d5

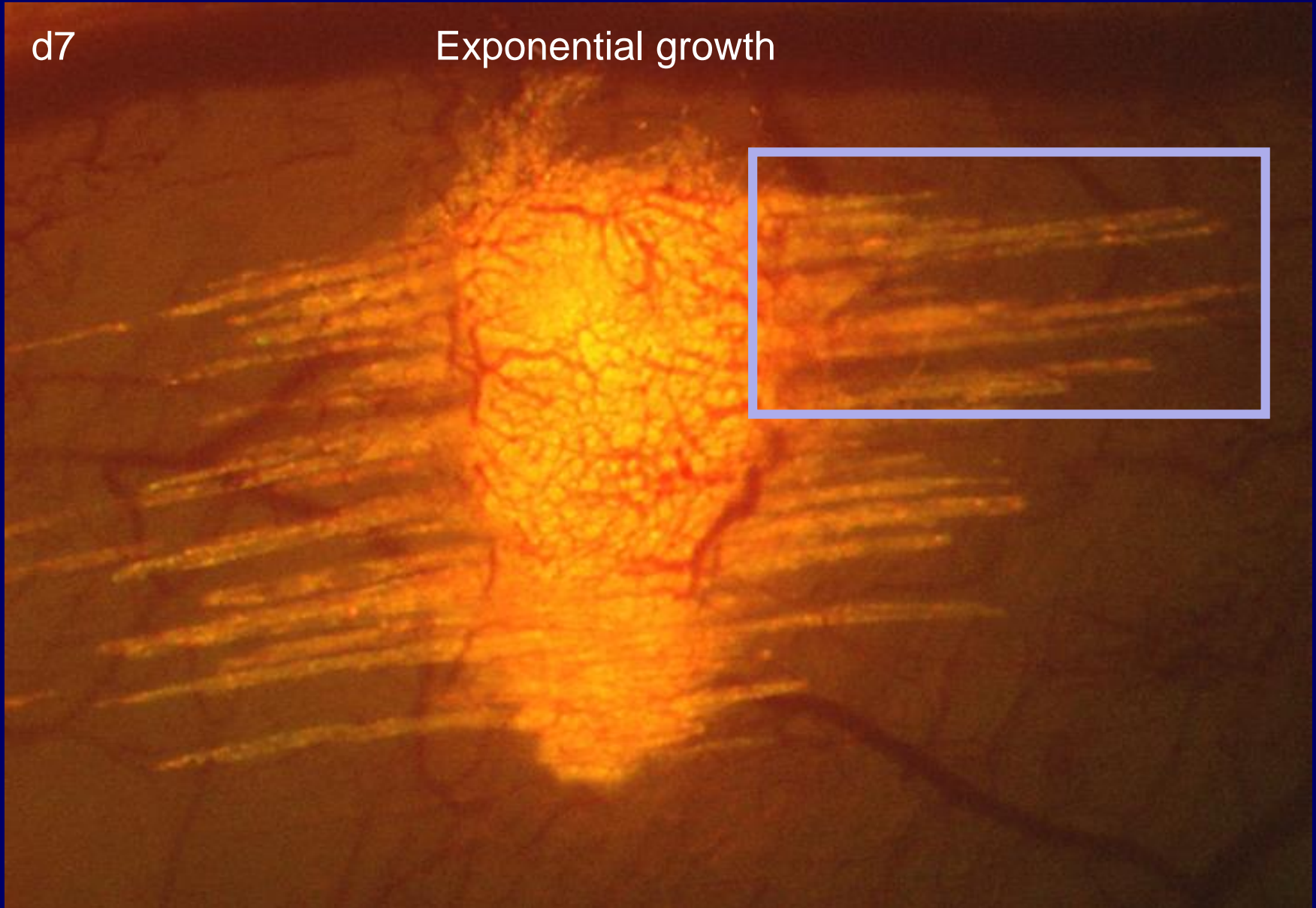
Angiogenic switch




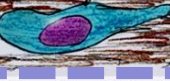





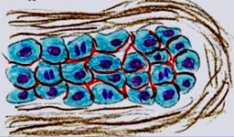

Dynamics of tumor neoangiogenesis and invasion

d7

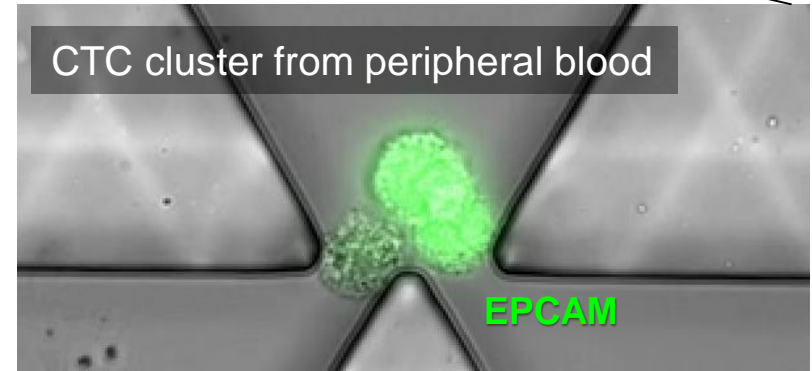
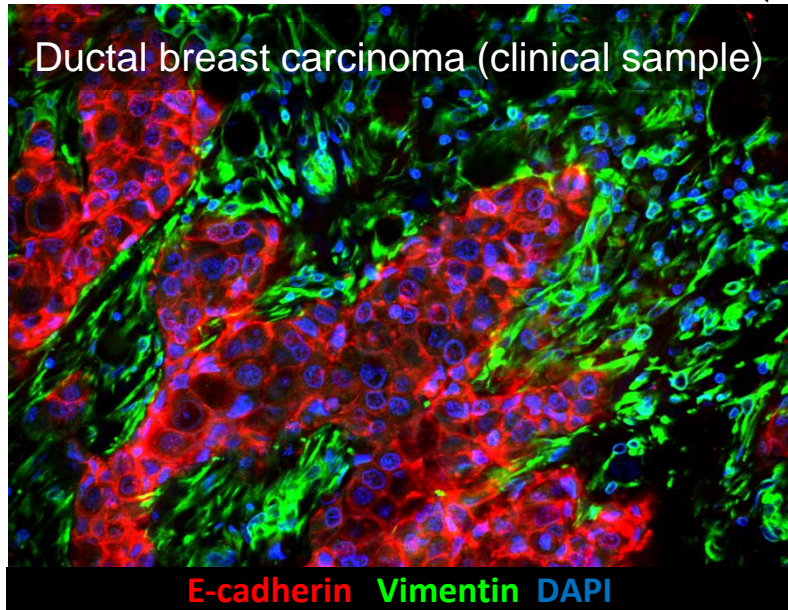
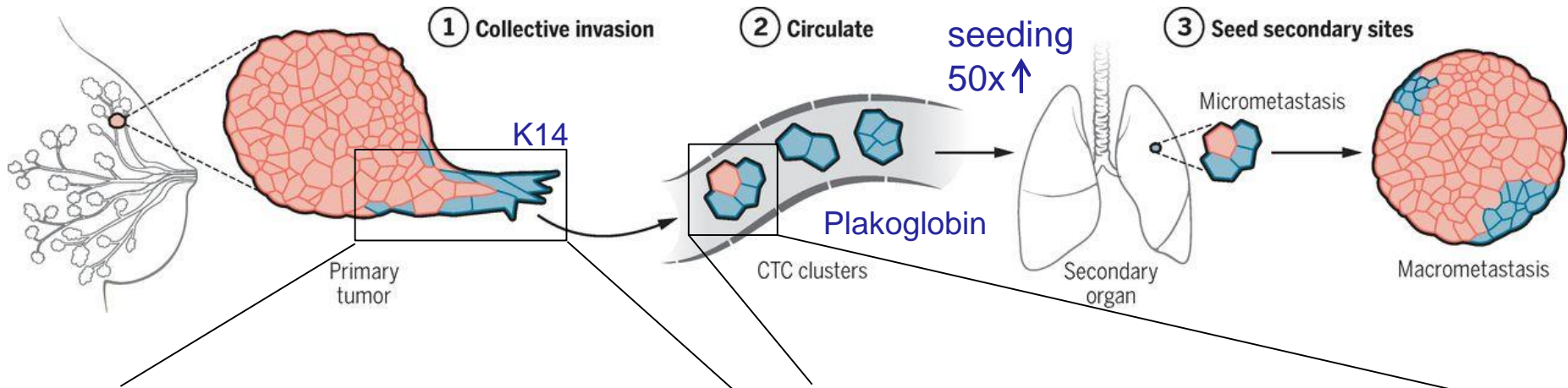
Exponential growth



Diversity of invasion programs *in vivo*

		Cell-cell junctions	Tumor type	
Individual-cell migration	Single-cell migration			
	Amoeboid		-	Leukemia, lymphoma cell subsets (all tumors)
	Mesenchymal		-	Stromal tumors, epithelial tumors after EMT
	Multicellular streaming			
Multicellular migration	Amoeboid (multicellular)		?	All tumors developing amoeboid single-cell dissemination
	Mesenchymal (multicellular)		(+)	Tumors with mesenchymal invasion; fibroblasts leading tumor cells
	Cluster		++	Moderately differentiated epithelial tumors
	Solid strand		++	Moderately differentiated epithelial tumors with subregions after EMT; basal and squamous cell carcinoma
	Strand (with lumen)		++	Differentiated epithelial tumors; vascular neoplasia
	Strand (protrusive)		++	Moderately differentiated epithelial tumors lacking EMT
Growth	Expansive growth		++	All solid tumors

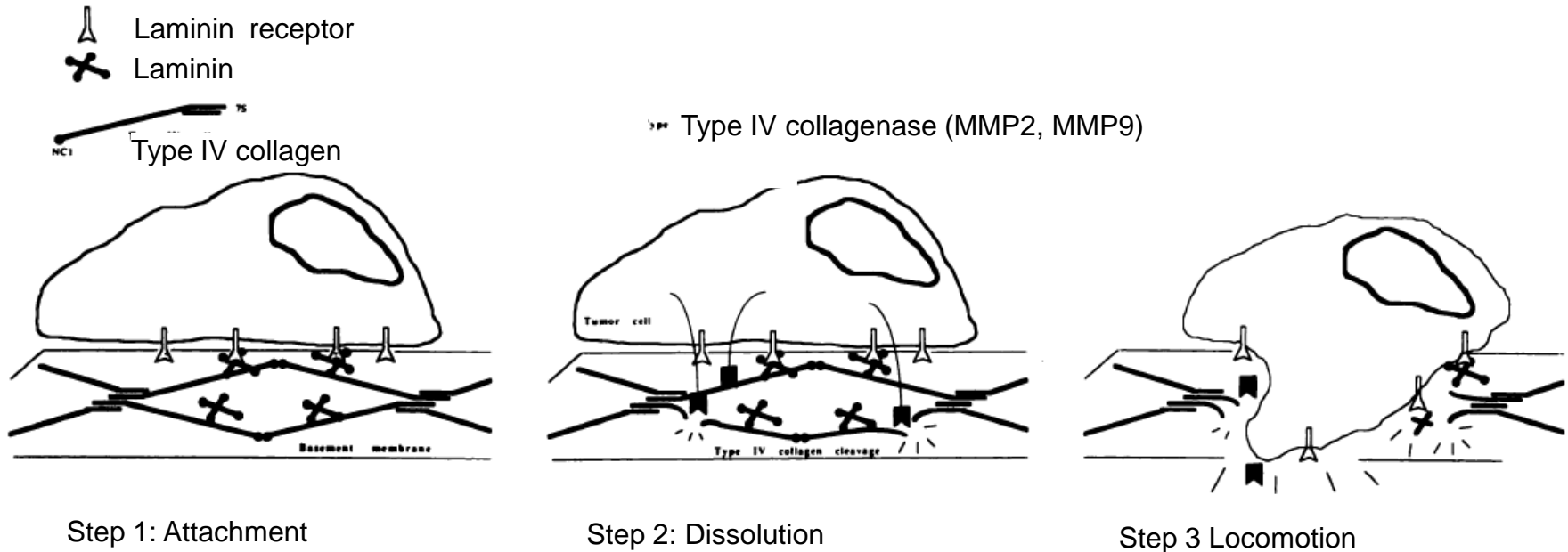
Collective cancer invasion and metastasis



Wolf et al., Nat. Cell Biol. (2007)
Friedl et al., Nat. Cell Biol. (2012)
Aceto et al., Cell, 2014
Cheung et al., PNAS, 2016
Cheung & Ewald, Science, 2016

Microenvironmental
context:
principles of
guidance by tissue
structures

First three-step model of invasive cell migration



Liotta, LA, Trygvason K, et al., Nature (1980)

Liotta, LA Rhodes Memorial Award Lecture, Cancer Res. (1986)

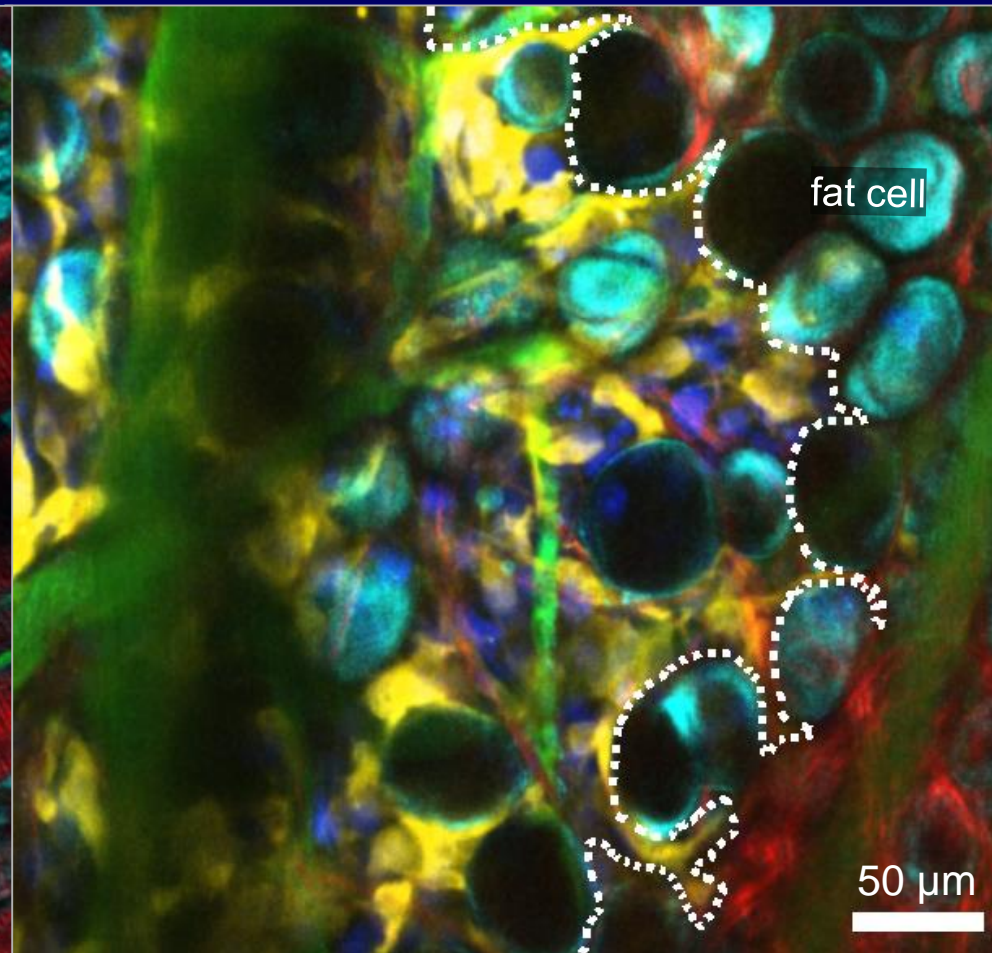
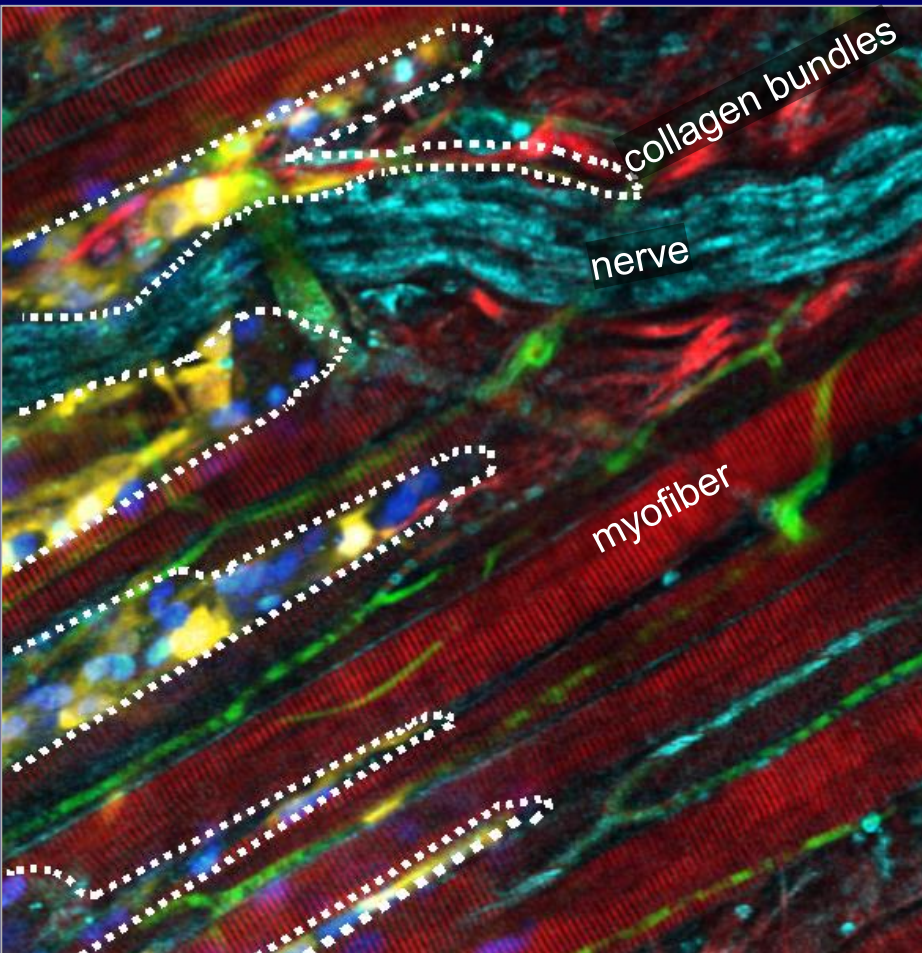
Deduced paradigm:

Proteolytic degradation of extracellular matrix is required for cell migration into tissue

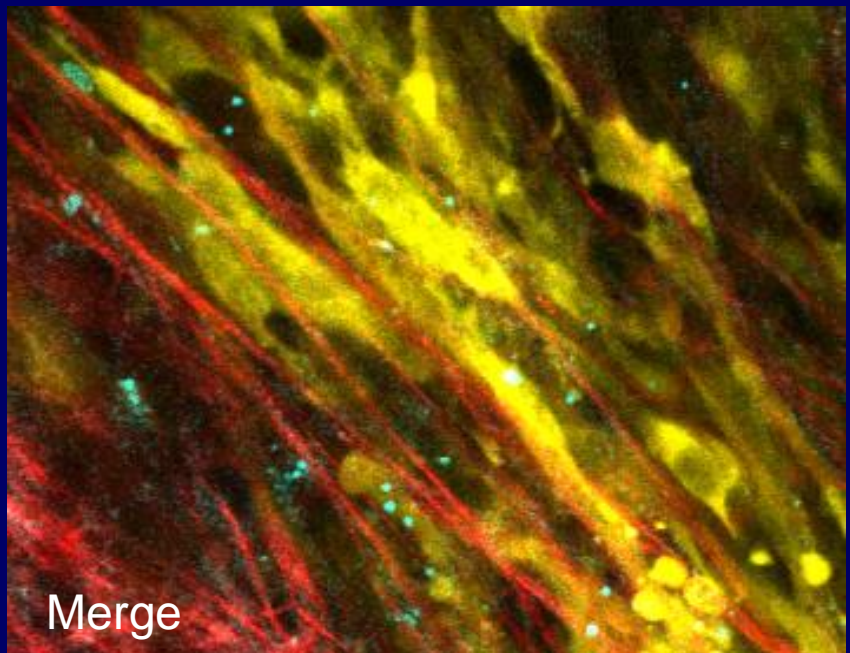
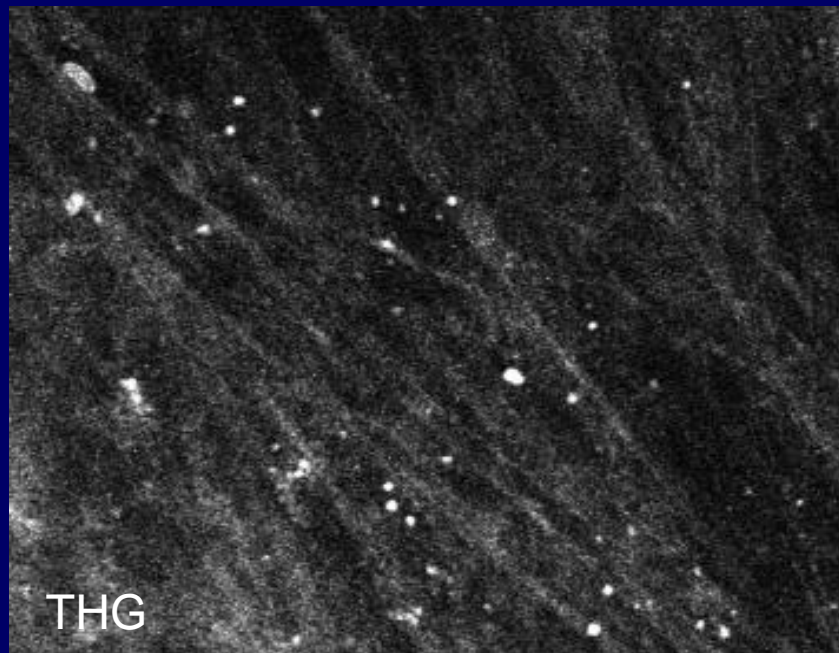
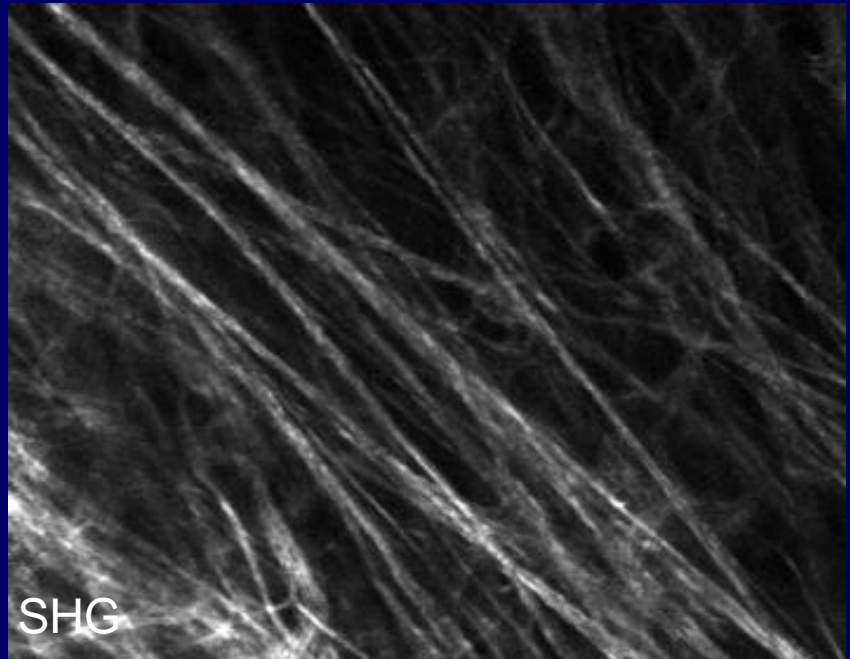
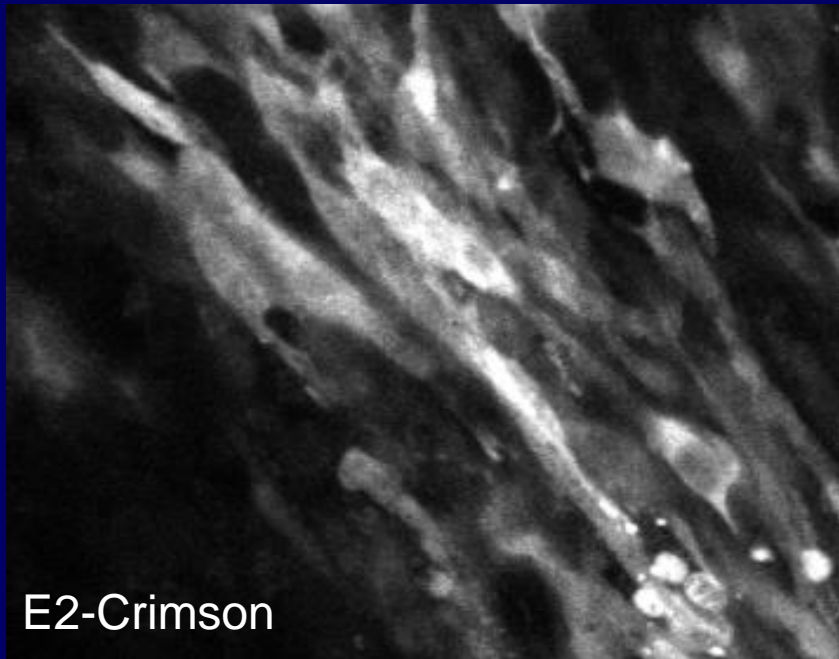
Two types of collective invasion patterns in response to connective tissue organization

Linear / confined

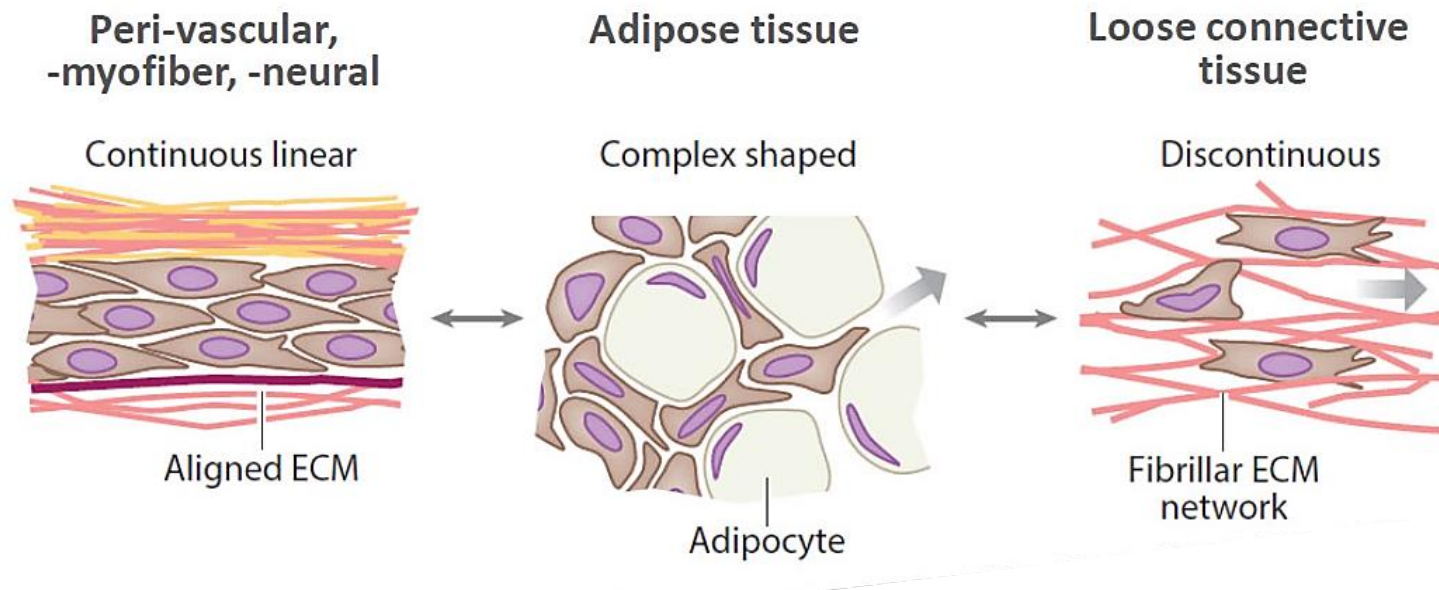
Broad / diffuse



Multicellular streaming along aligned fibrillar collagen



Tissue geometry dictates invasion pattern



→ Non-destructive migration along preexisting interfaces

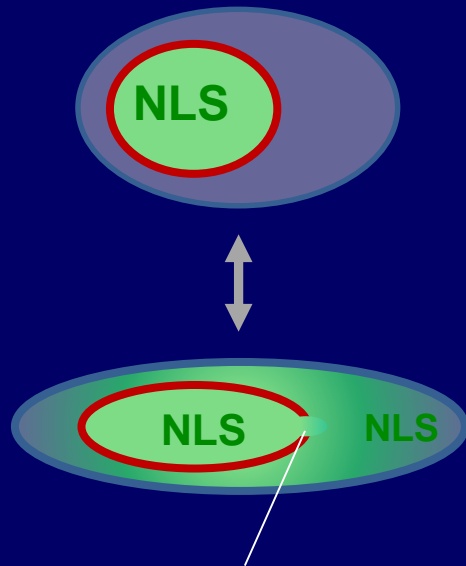
Weigelin et al., Intravital (2012)

Te Boekhorst et al., Annu. Rev. Cell Dev. Biol. (2016)

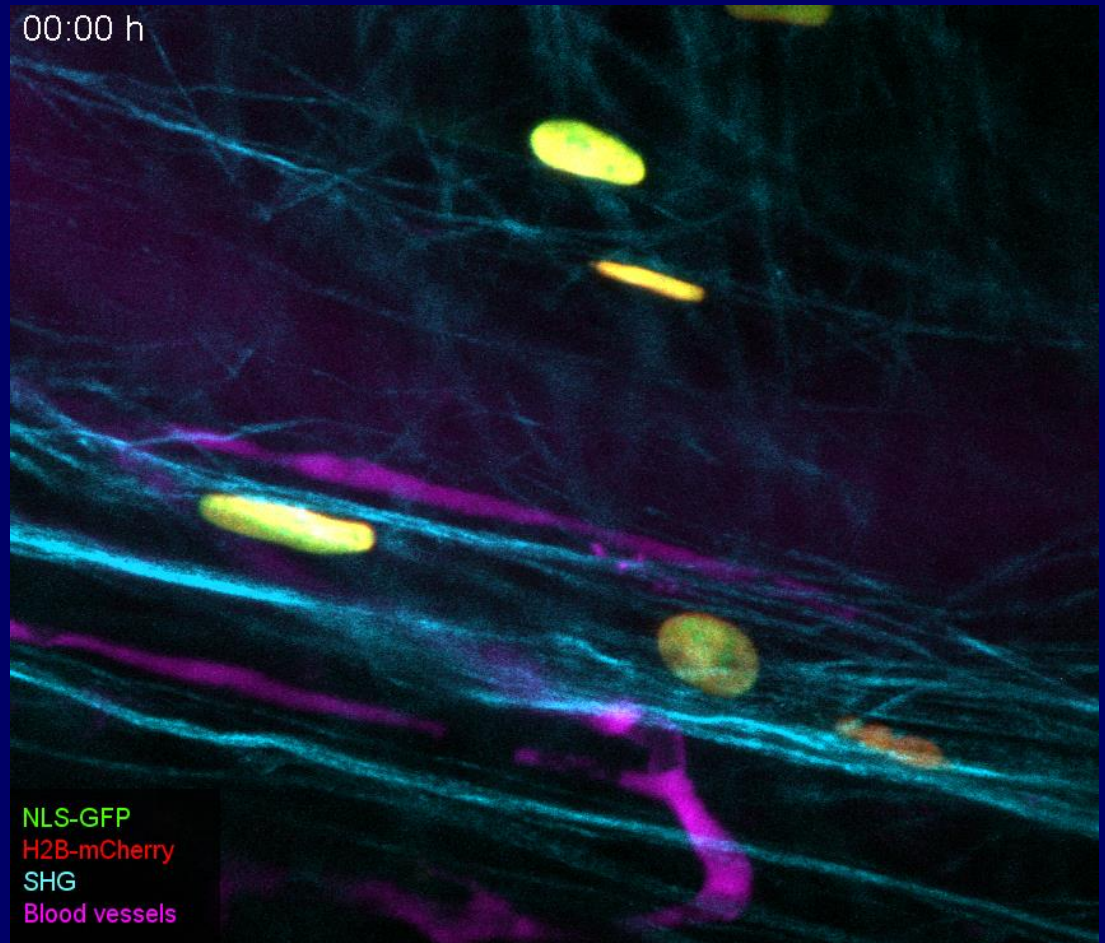
Collective invasion
protects moving cells
from mechanical
assault

Identification of mechanical challenge in vivo

Mechanical stress
sensing: translocation
of NLS-GFP



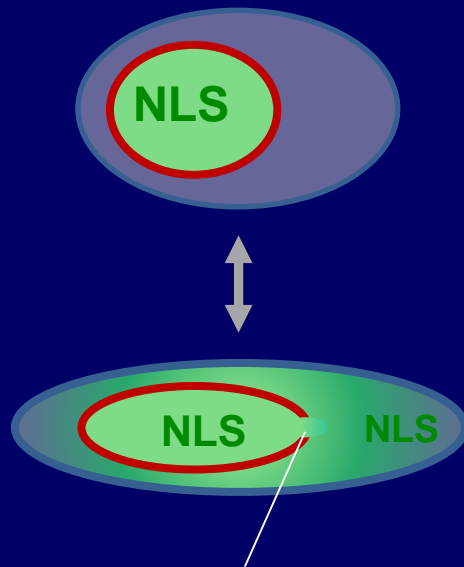
Transient rupture of
the nuclear envelope



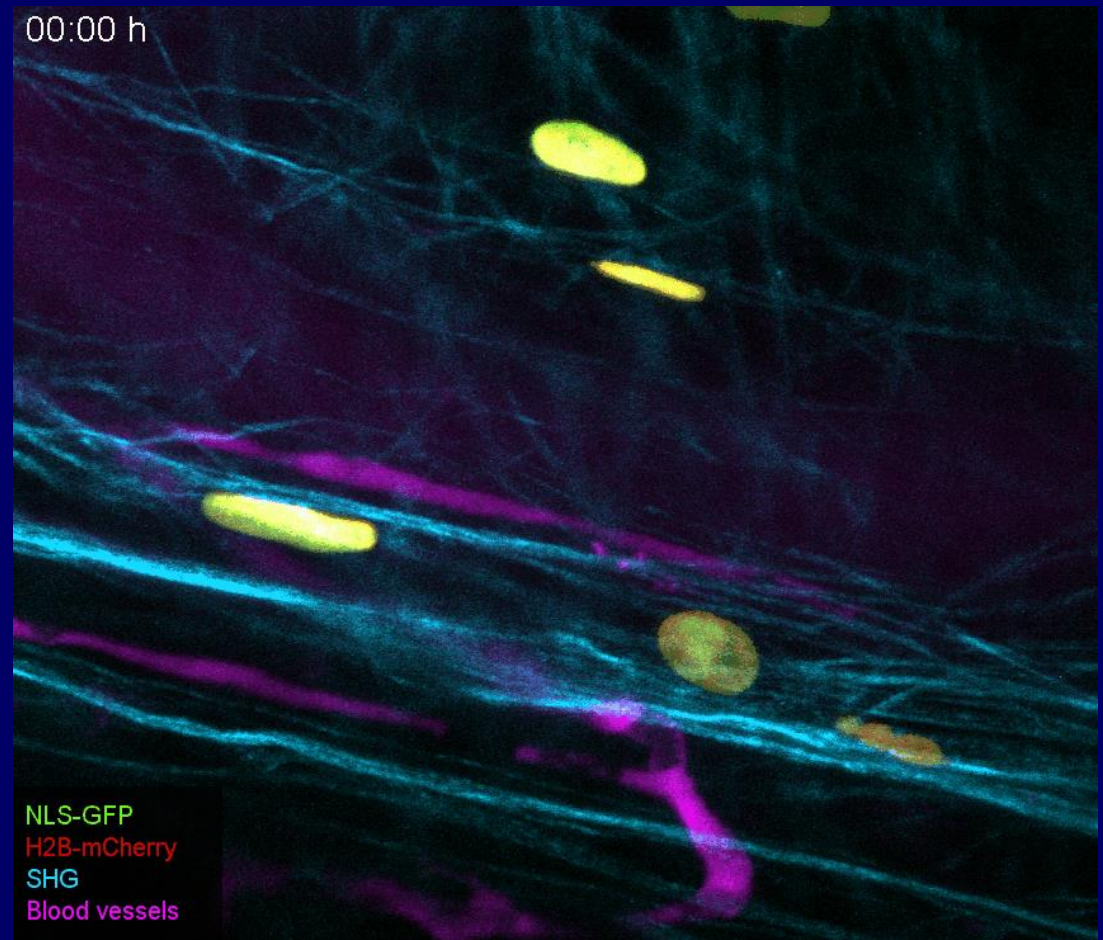
Collaboration with Jan Lammerding, Cornell Univ.
Denais et al., Science (2016)

Identification of mechanical challenge in vivo

Mechanical stress
sensing: translocation
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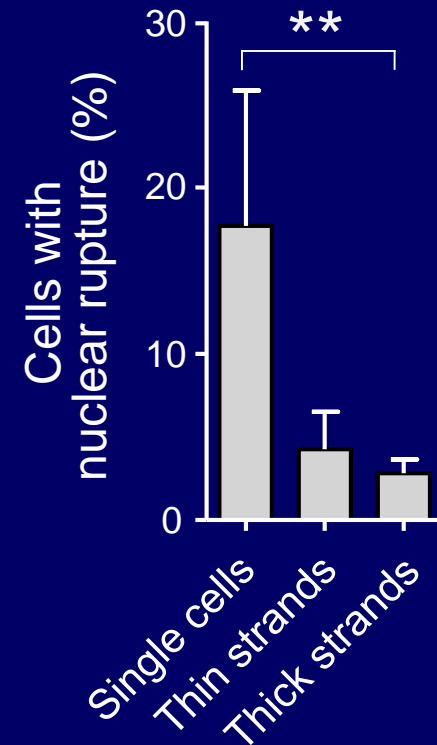
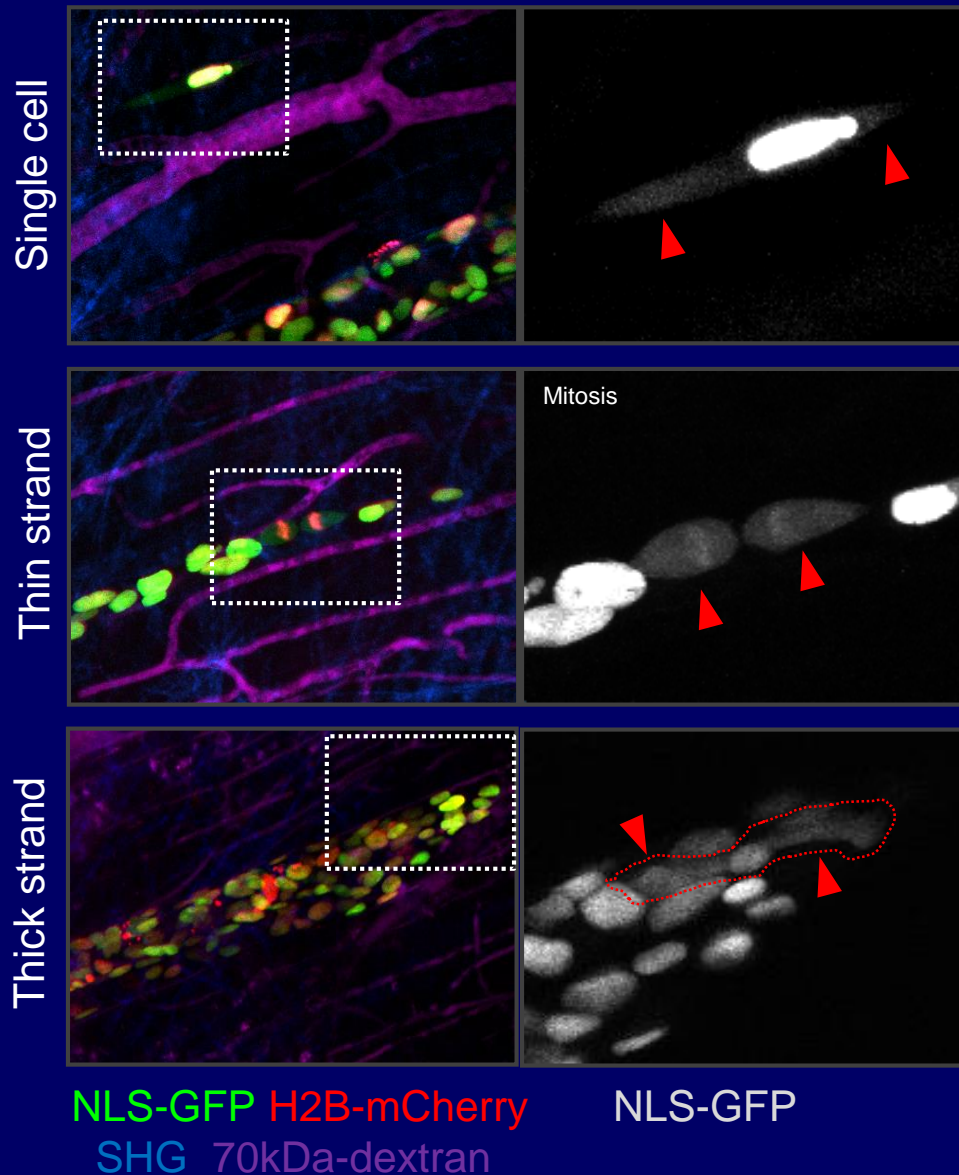


Transient rupture of
the nuclear envelope



Collaboration with Jan Lammerding, Cornell Univ.
Denais et al., Science (2016)

Tissue regions imposing mechanical challenge in vivo

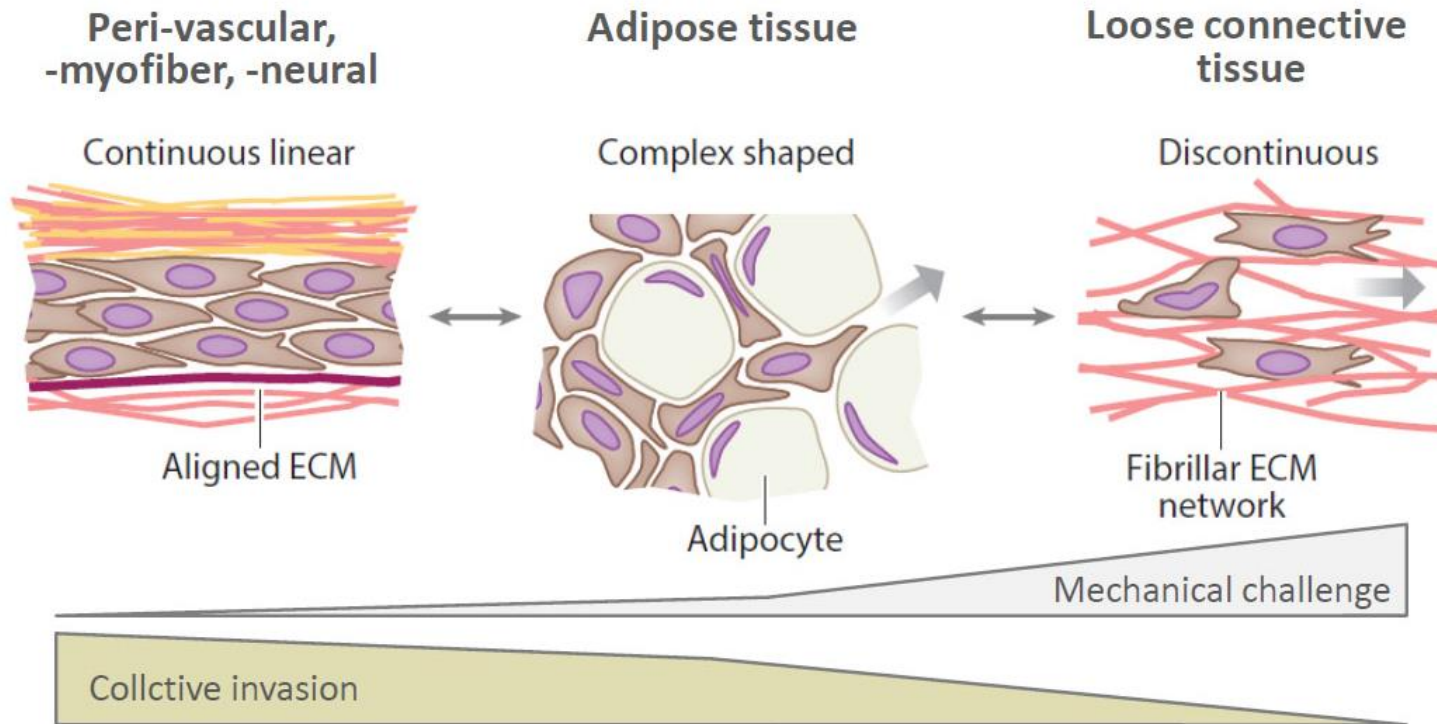


Number of cells observed:

Single cells: 22
Thick strands: 417
Thin strands: 82

4 independent mice

Adaptation of migration mode and tissue patterns

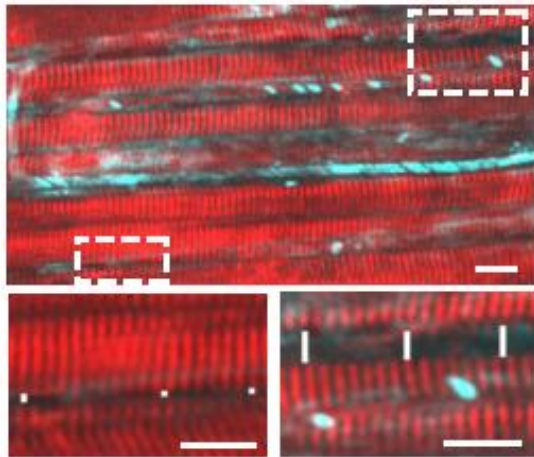


Weigel et al., Intravital (2012)

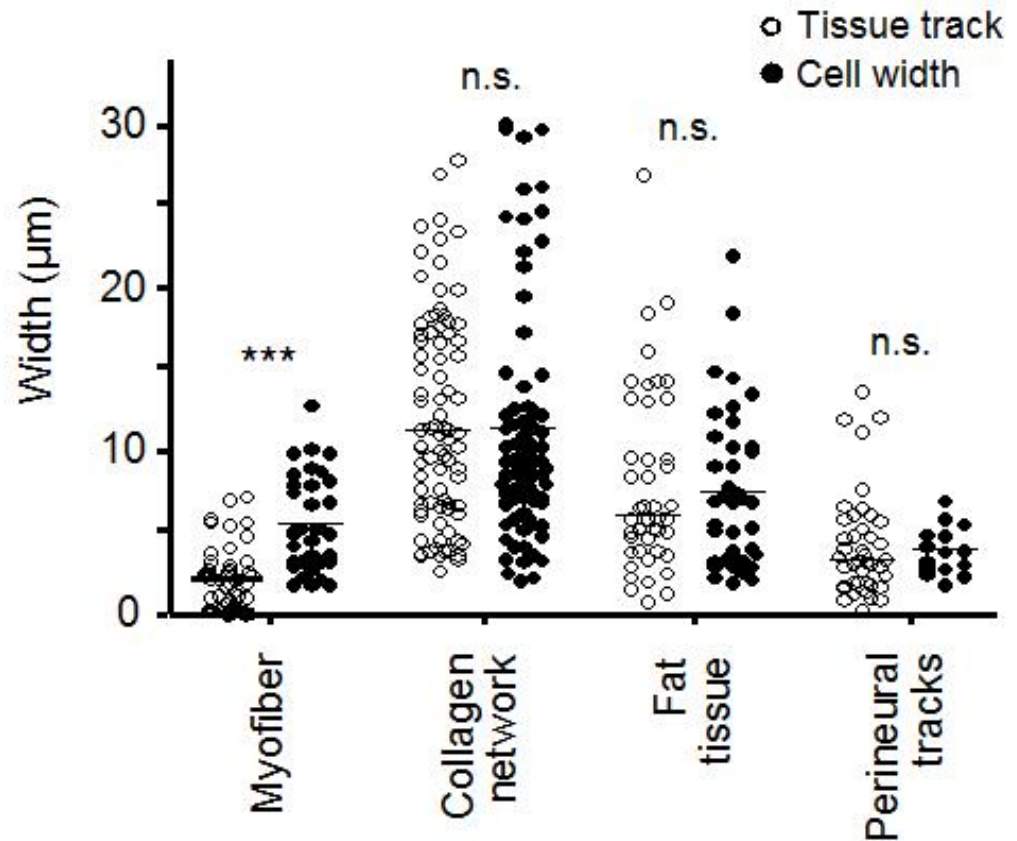
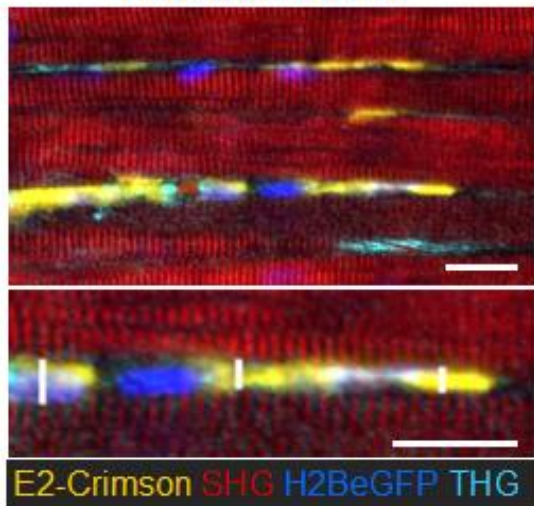
Denais et al. Science (2016)

Track widening or not widening

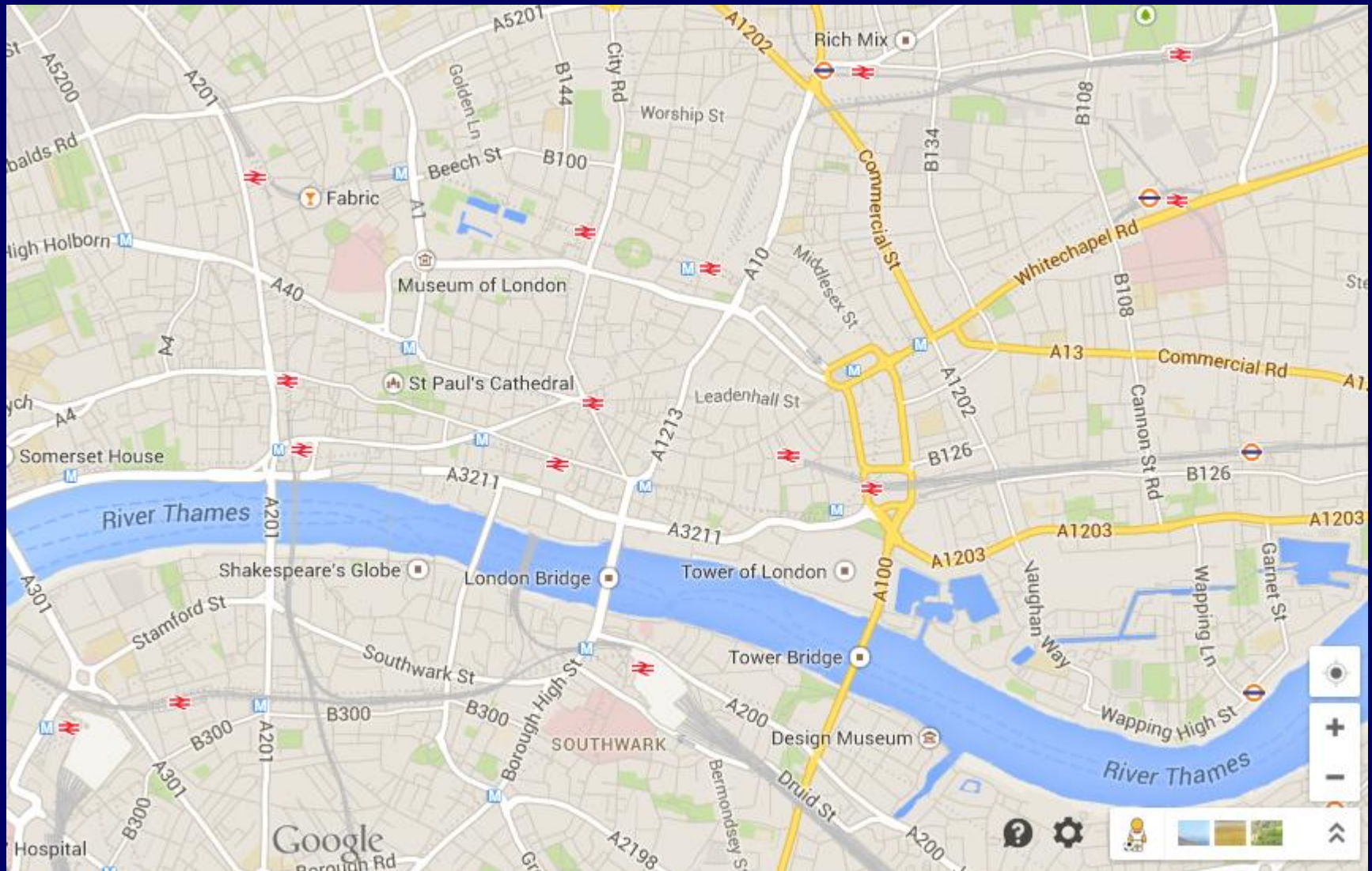
Tumor-free tissue



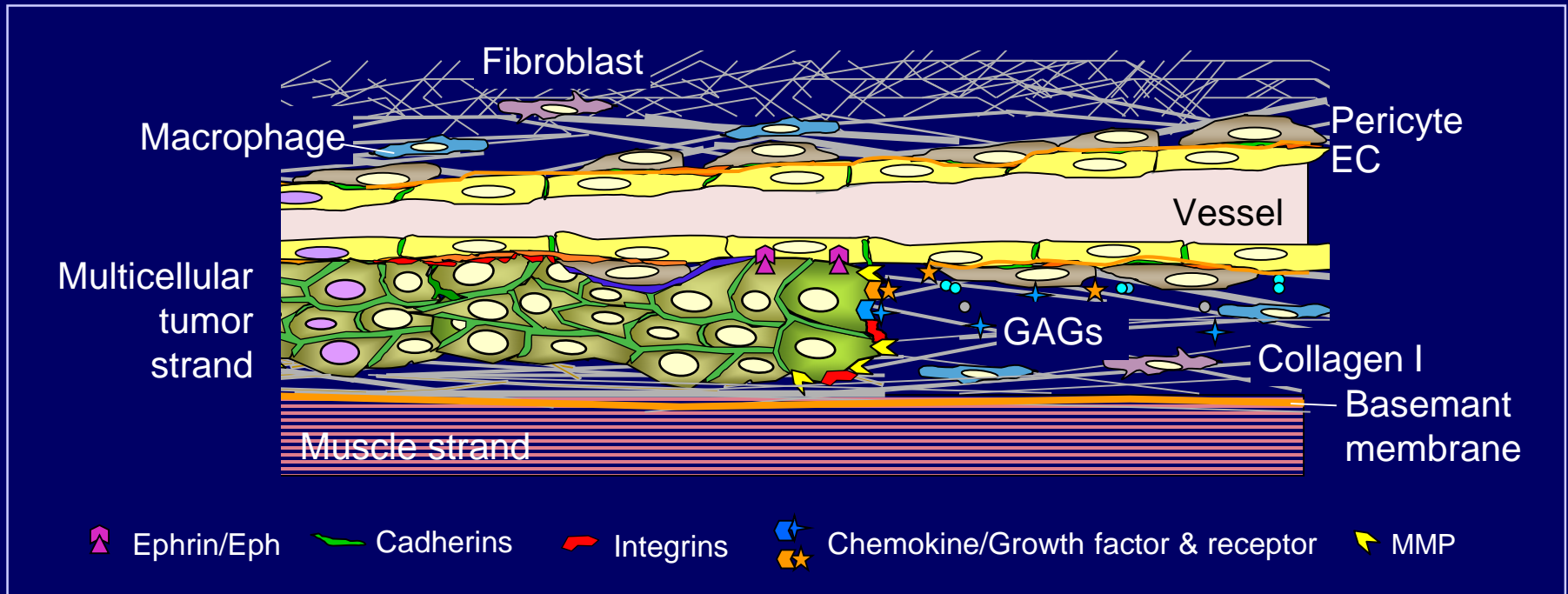
Invasion zone



Infrastructure enabling mobility: tubes and tracks



The invasion niche



- Aligned tracks of least resistance (microchannels)
- Multiple interfaces: collagen fibers, BM, cell surfaces, GAGs
- Nearby nutrient / oxygen supply
- Constitutive trafficking routes for passenger cells

→ Guided migration along "tissue highways"

Conclusions

Intravital microscopy has enabled understanding of key questions:

- Tissue building blocks and interfaces enabling nondestructive guidance for cancer cell invasion
- Invasion is coupled to accelerated DNA damage response and enhances cancer cell survival during radiation therapy
- Tumor cell elimination by cytotoxic T lymphocytes is greatly enhanced by CTL cooperation and additive cytotoxicity along migration-supporting tissue tracks



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