

Visualizing intestinal immune homeostasis:
T cells patrol independent of a mucosal dendritic cell network

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a partnership with Notre Dame

Outline

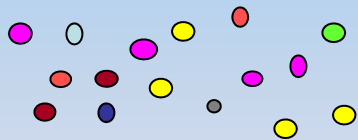
- Background
- Mucosal Intravital Imaging Validation
- Description of Dendritic Cell Network
 - Mucosal T cell Patrolling

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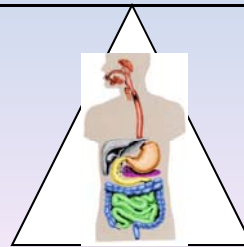
Immune homeostasis in gut

- 10^{14} Bacteria Reside in Normal Gut (approx. 2000 species)
 - Aids in fiber digestion
 - Produces some vitamin K and B
- Immune inflammation is controlled
- Protective immunity is preserved
- ~80% of total leukocytes are in the gut
- More effector lymphocytes in the gut than anywhere else (ex. Th17)



Quiescence and
Protective Immunity

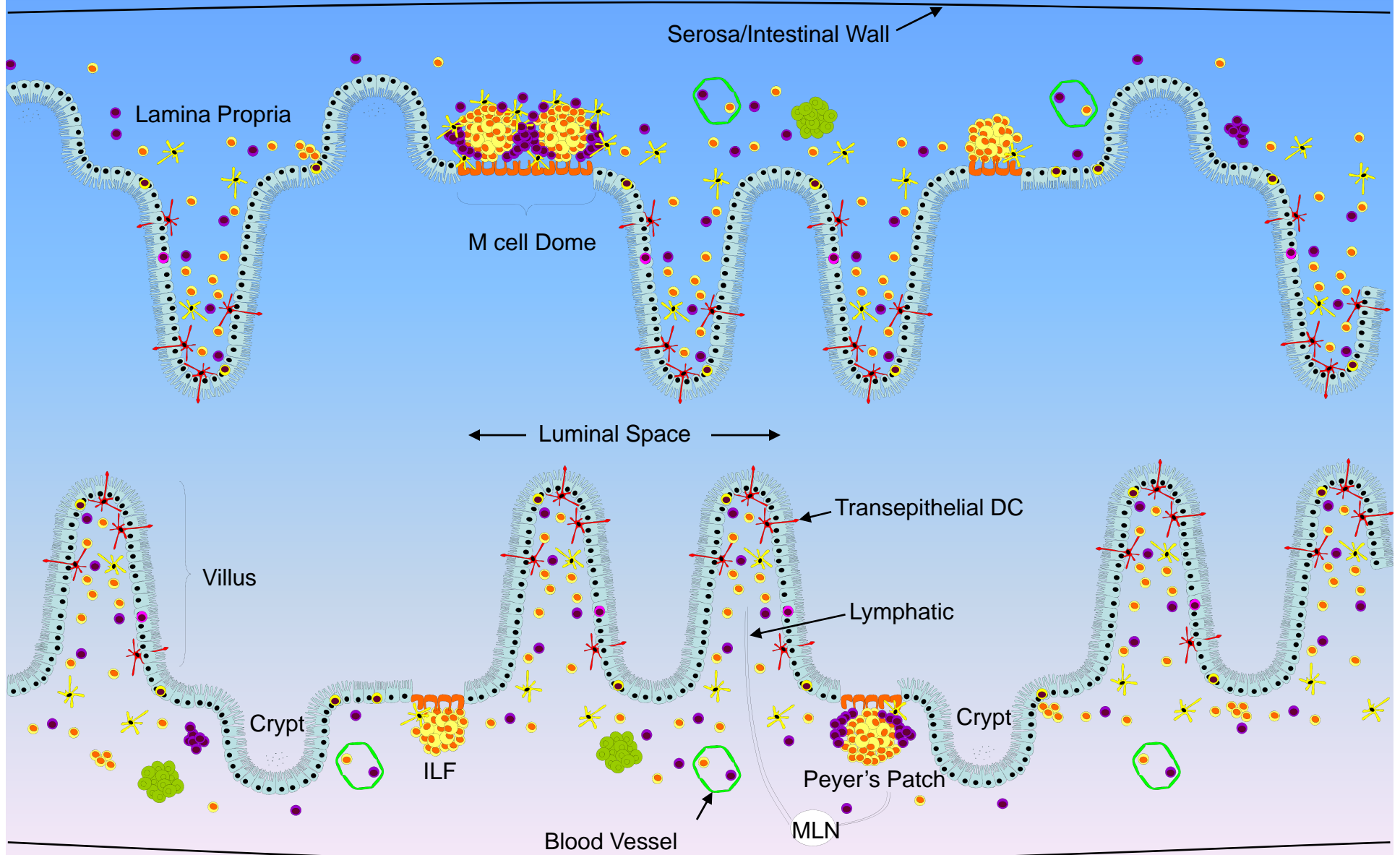
Enteric Flora



Inflammation
Tumorigenesis

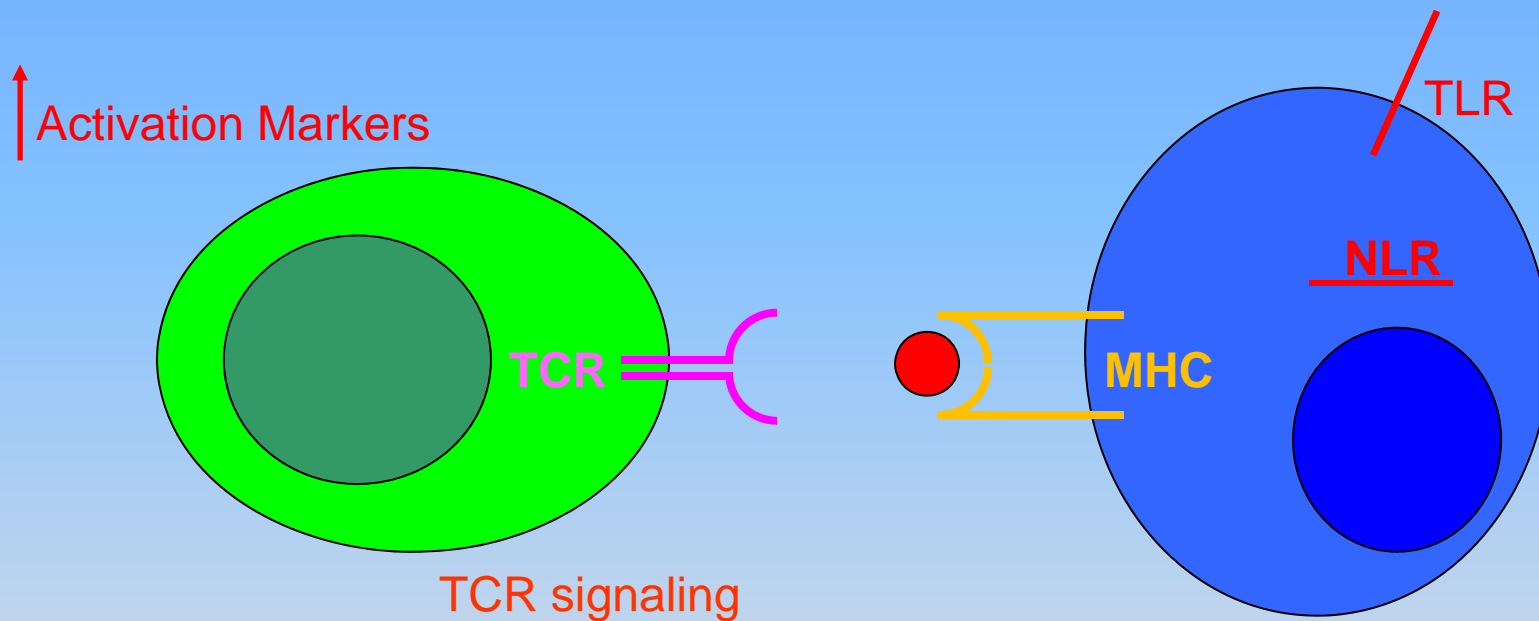
Host Genetics

Immune microanatomy of small intestine



Not to scale

T cell patrolling and activation



Overall hypothesis: The study of the modulation of T cell patrolling will provide basic insights into regulation of T cell activation in the intestine

Why IVM to understand gut immunobiology?

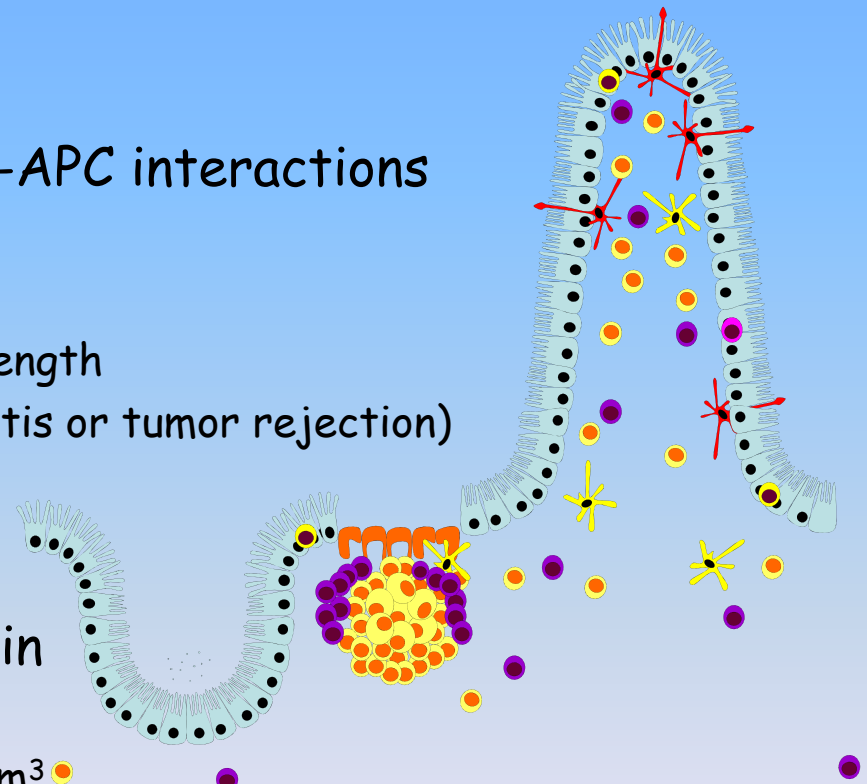
- Where do the important T cell-APC interactions occur?

- What is the physical nature of T cell-APC interactions in the intestine?

- Stable versus scanning
- Biochemistry of TCR-pMHC and signal strength
- Does this change during inflammation (colitis or tumor rejection)

- How does a single T cell find antigen in volume $\sim 50,000X$ its own size?

- Volume of a T cell: $\pi r^3 = 3.14 \times 5^3 \sim 400 \mu\text{m}^3$
- Volume of a Villus: $\pi r^2 h = 3.14 \times 25^2 \times 100 \sim 200,000 \mu\text{m}^3$
- Volume of lamina propria in intestine: Vol. of villus \times number of villi $\sim 200,000 \times 1,000 \sim 2e8 \mu\text{m}^3$



Application of LSM intravital microscopy

- Intact microenvironment (blood, O_2 , endocrine etc.)
- Conserved microanatomy
- Identify what cellular interactions and migratory behavior are important to maintain homeostasis and immunity
- Other unpredicted insights
- Many intravital immunologic studies have focused on secondary lymphoid organs, not effector sites like the gut

Challenges in applying fluorescence IVM

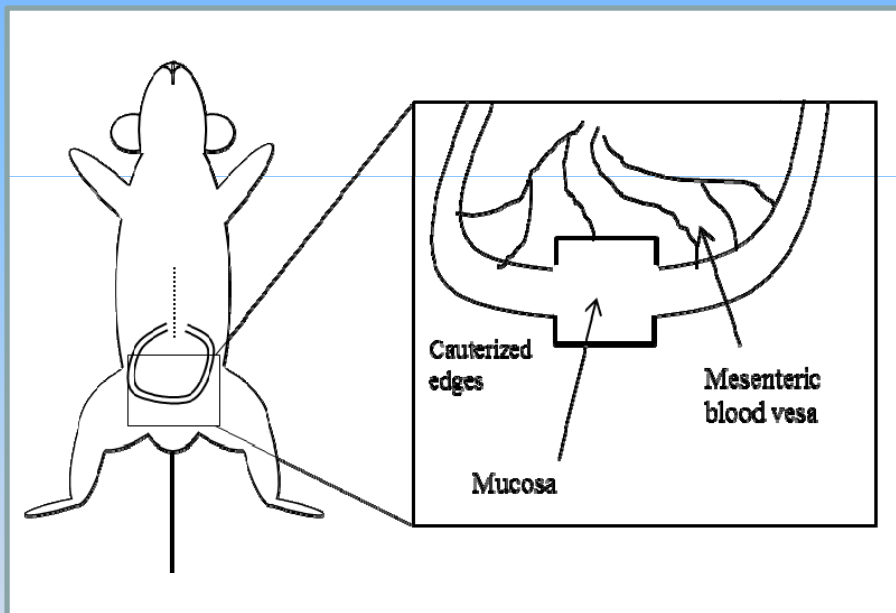
- Temperature
 - $<35^{\circ}\text{C}$, cells stop migrating
 - $>39^{\circ}\text{C}$, cells stop migrating
- Perfusion
 - Lack of blood flow, cells stop migrating and disrupted para/endocrine systems
- Photodamage and phototoxicity

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Intravital Microscopic Set-up

Surgical Exposure of Small Bowel



Imaging Platform



- A. Oxygen
- B. Temperature monitoring and modulation
- C. Environmental chamber

Visualizing Gut Leukocytes via the Mucosal Surface

Olympus FV1000

Ti:Sapphire fs pulsed w/neg. chirp (MaiTai DeepSee HP)

4NDD

Inverted

Typically use 25X 1.05NA, hi transmission near IR

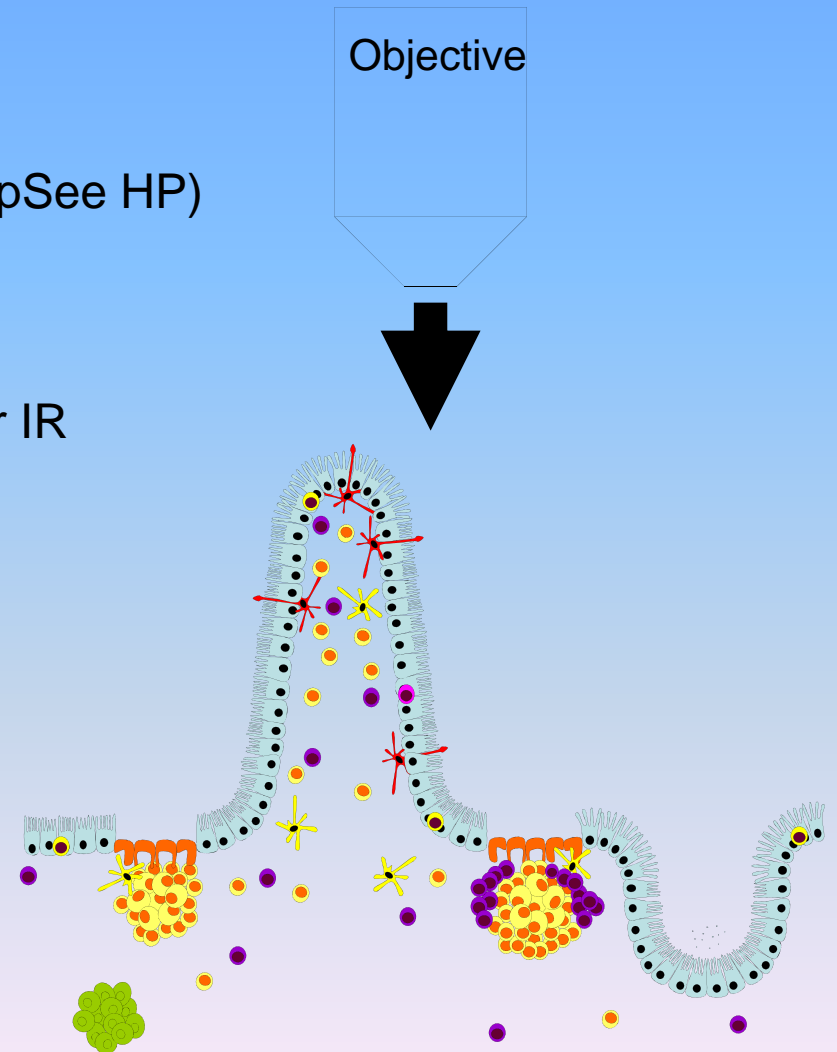
Typical acquisition parameter

~620 x 620 pixels

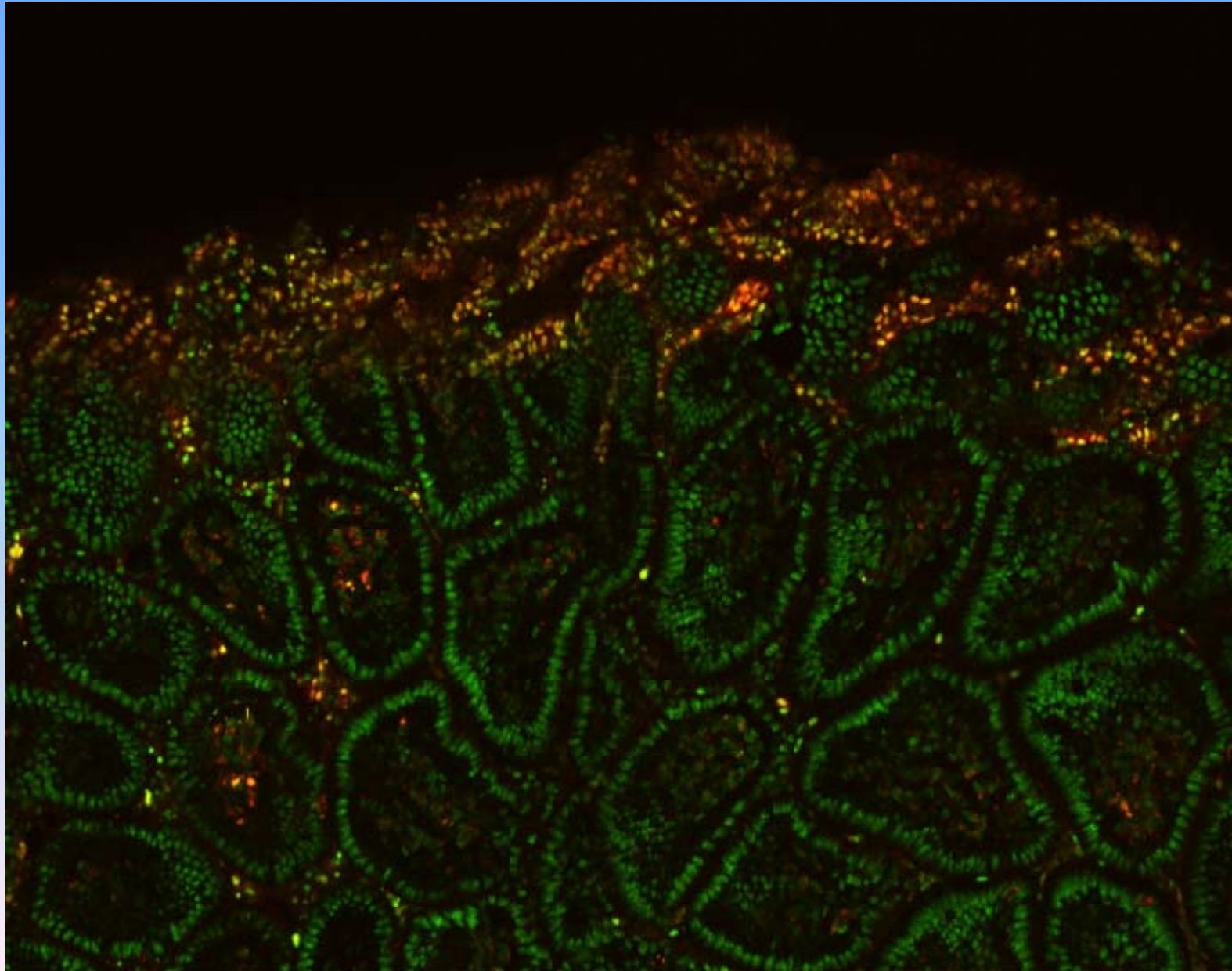
2 microsecond dwell

15 z @ <30sec intervals

Playback: 5fps



Localized tissue damage

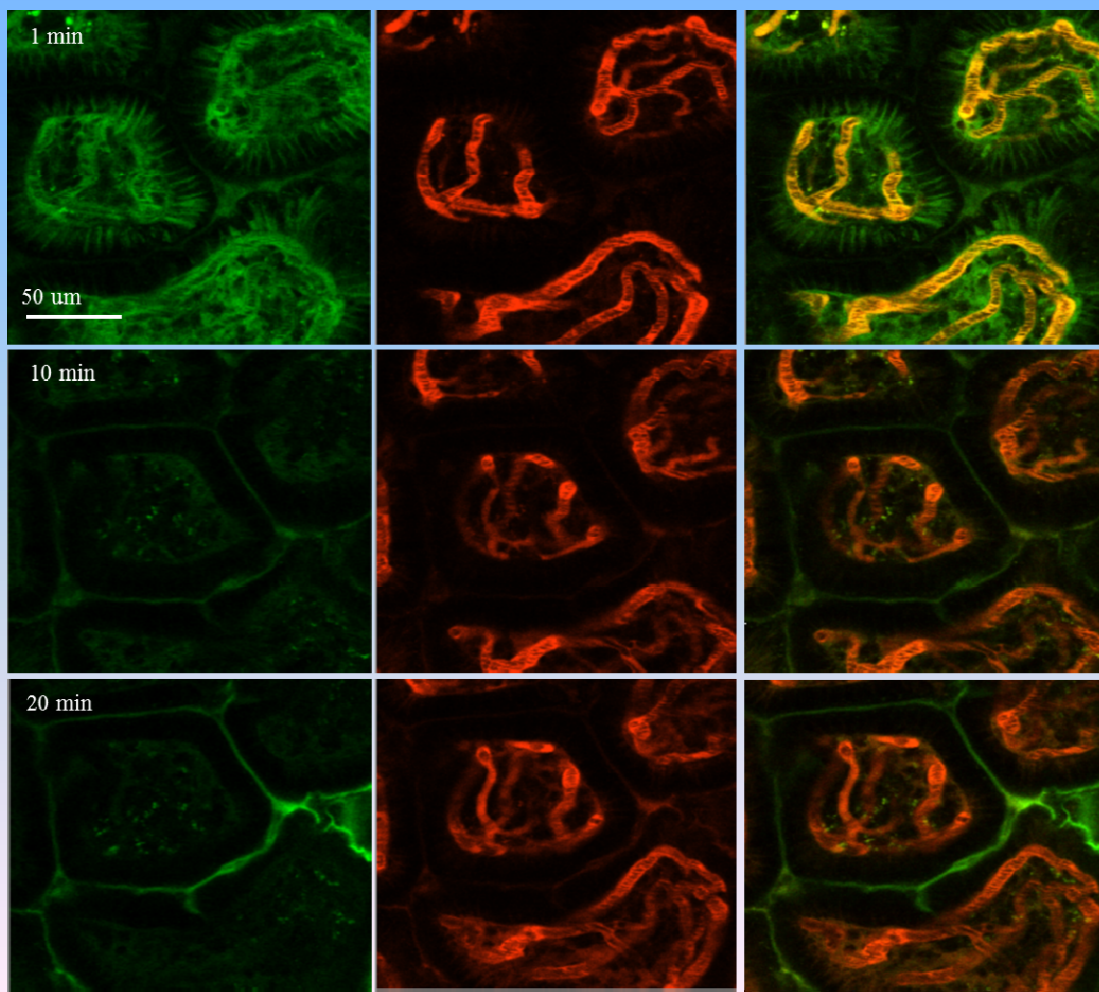


Clearance of dextran from vasculature

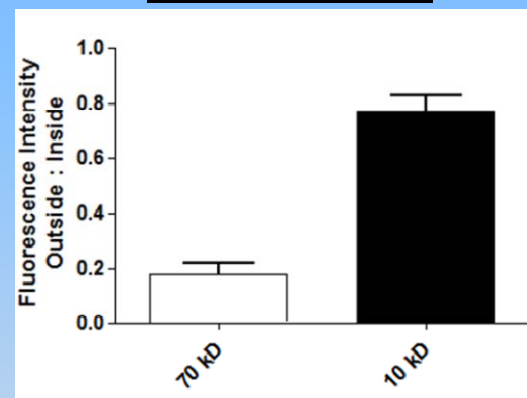
10KDa Dextran – FITC
70KDa Dextran – TR



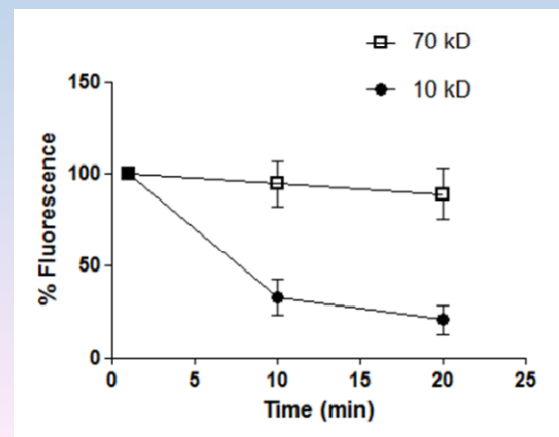
Analyze small bowel
blood vessel fluorescence



Permeability



Clearance



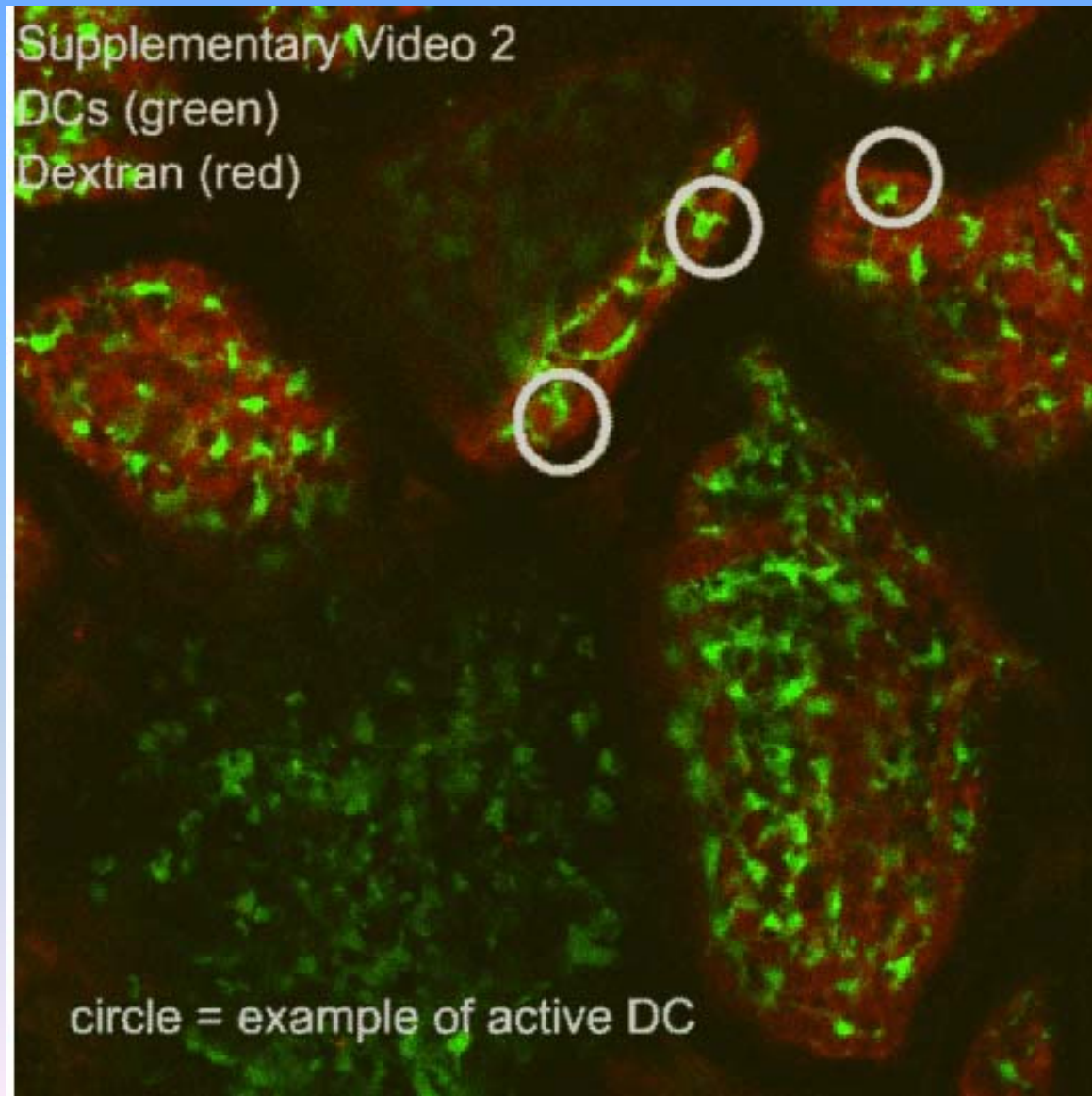
Validation Summary

- Limited tissue damage due to surgical procedure
- Tissue perfusion is intact
- Blood filtration from kidney is intact

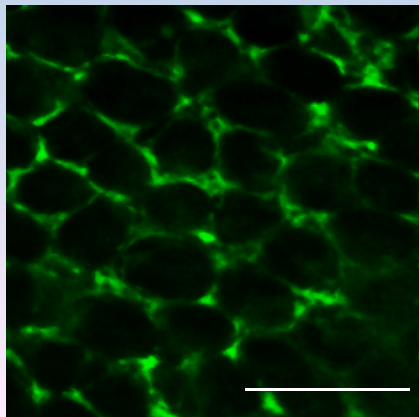
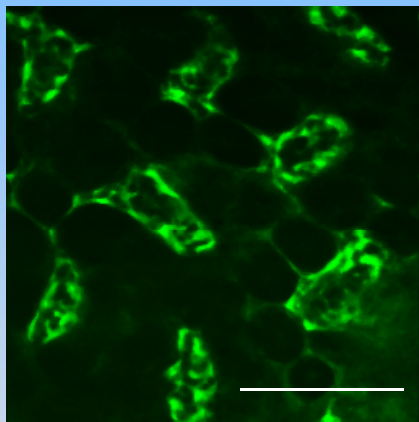
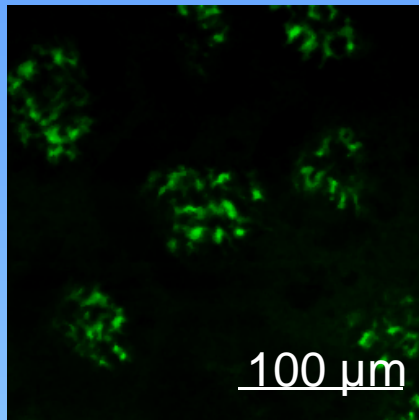
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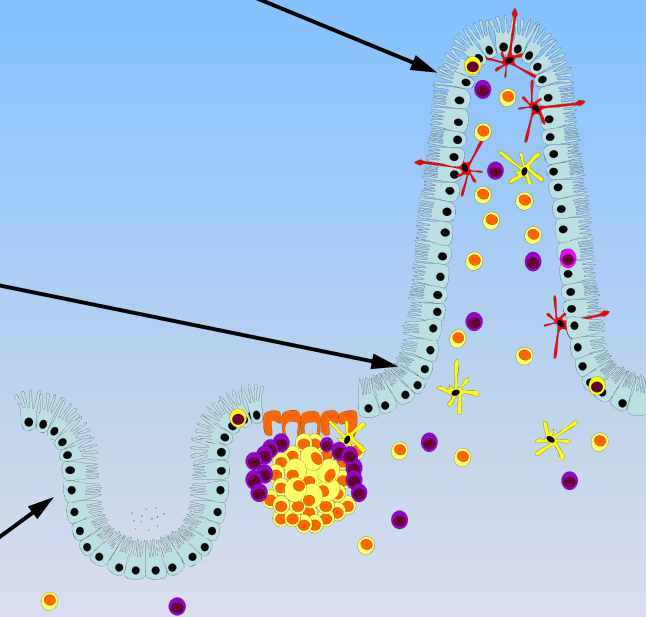
Small intestine villus DC activity and intact blood flow



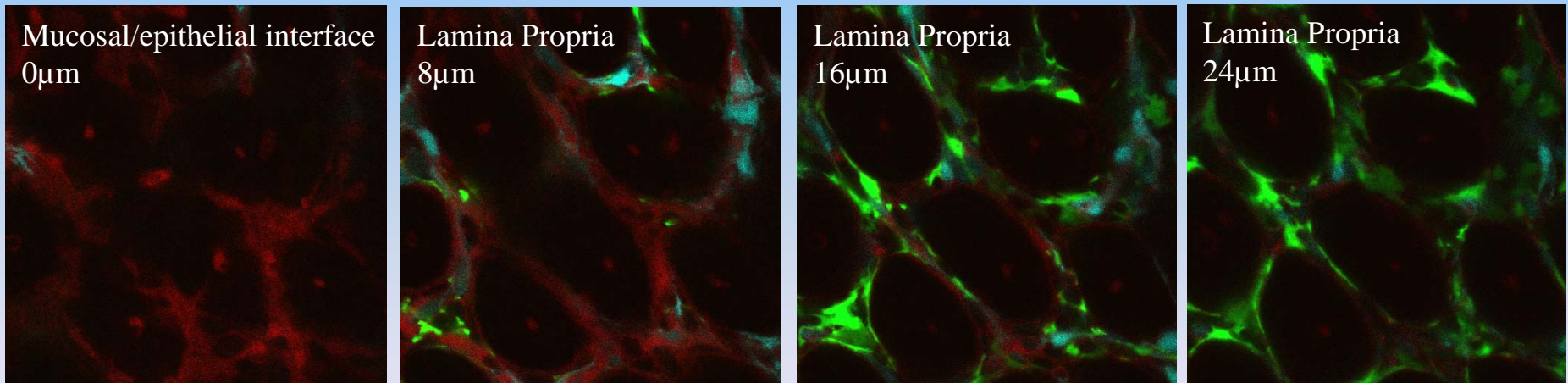
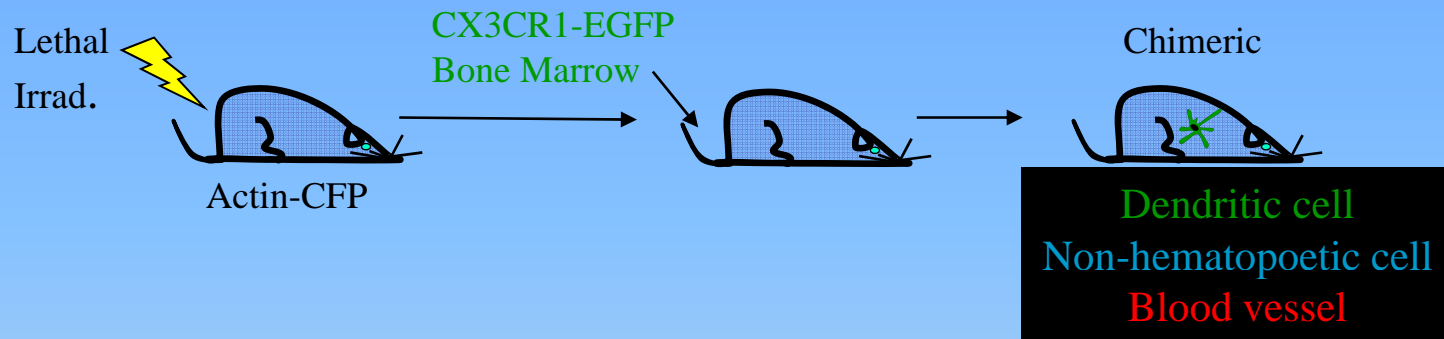
Visualizing Gut Leukocytes



Objective



Overlapping non-hematopoietic and DC networks



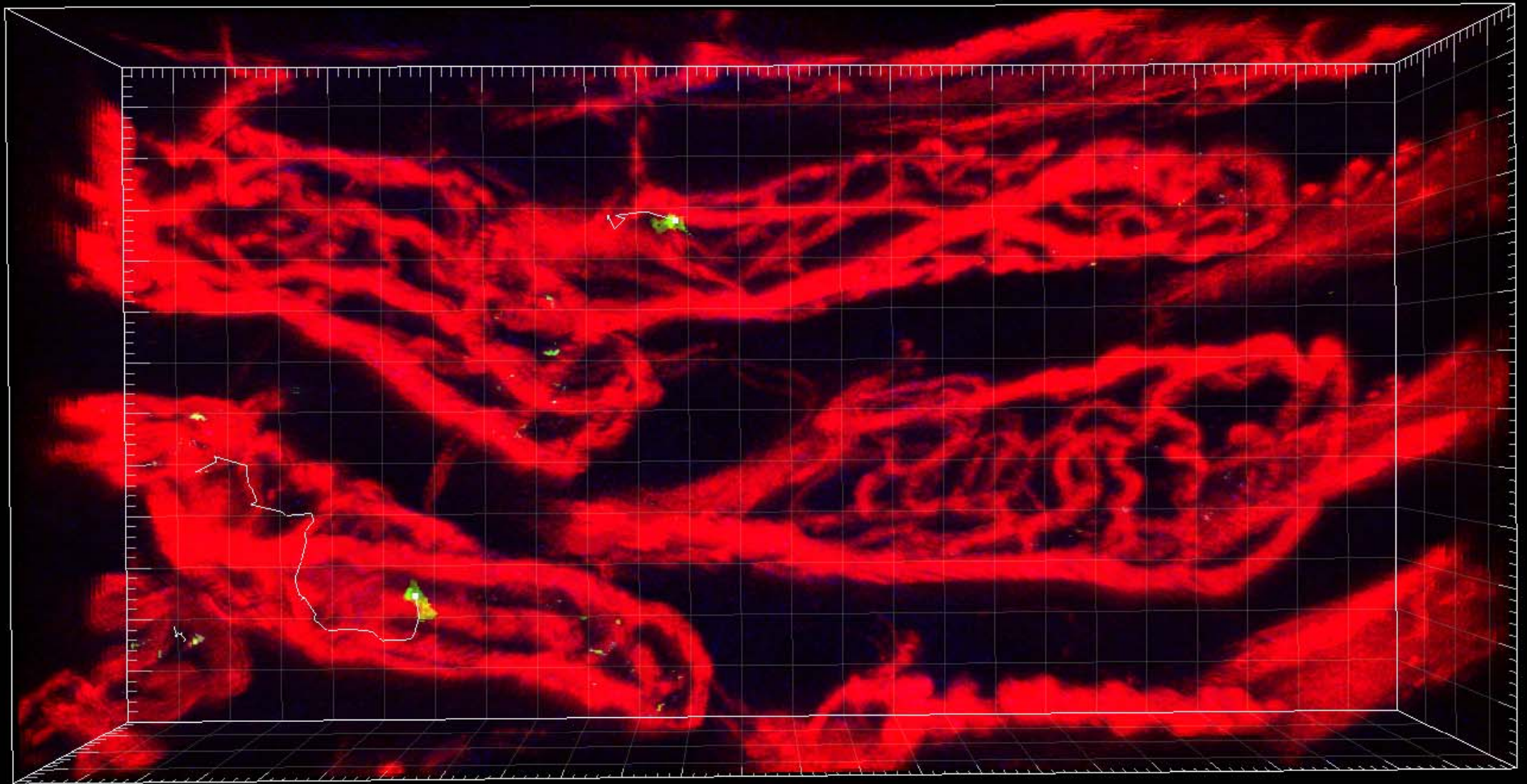
DC network summary

- DCs form a continuous network in both small and large bowel
- Network spans from villi to crypt lamina propria
- Mucosal DCs express low amounts of gap junctions
- DC network overlaps a non-hematopoietic cell network

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Villus T cell migration



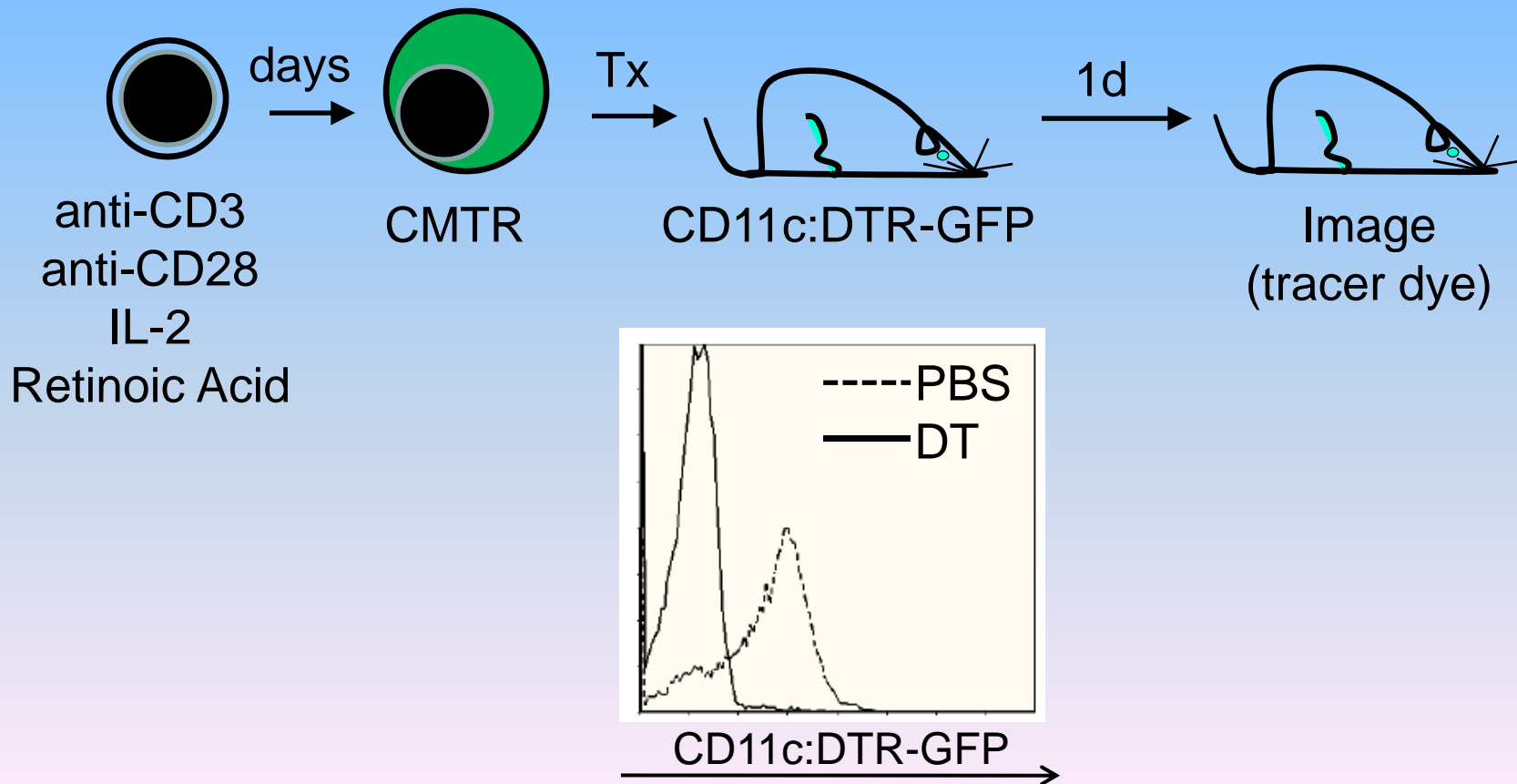
50 μm
0d00:00:00.000

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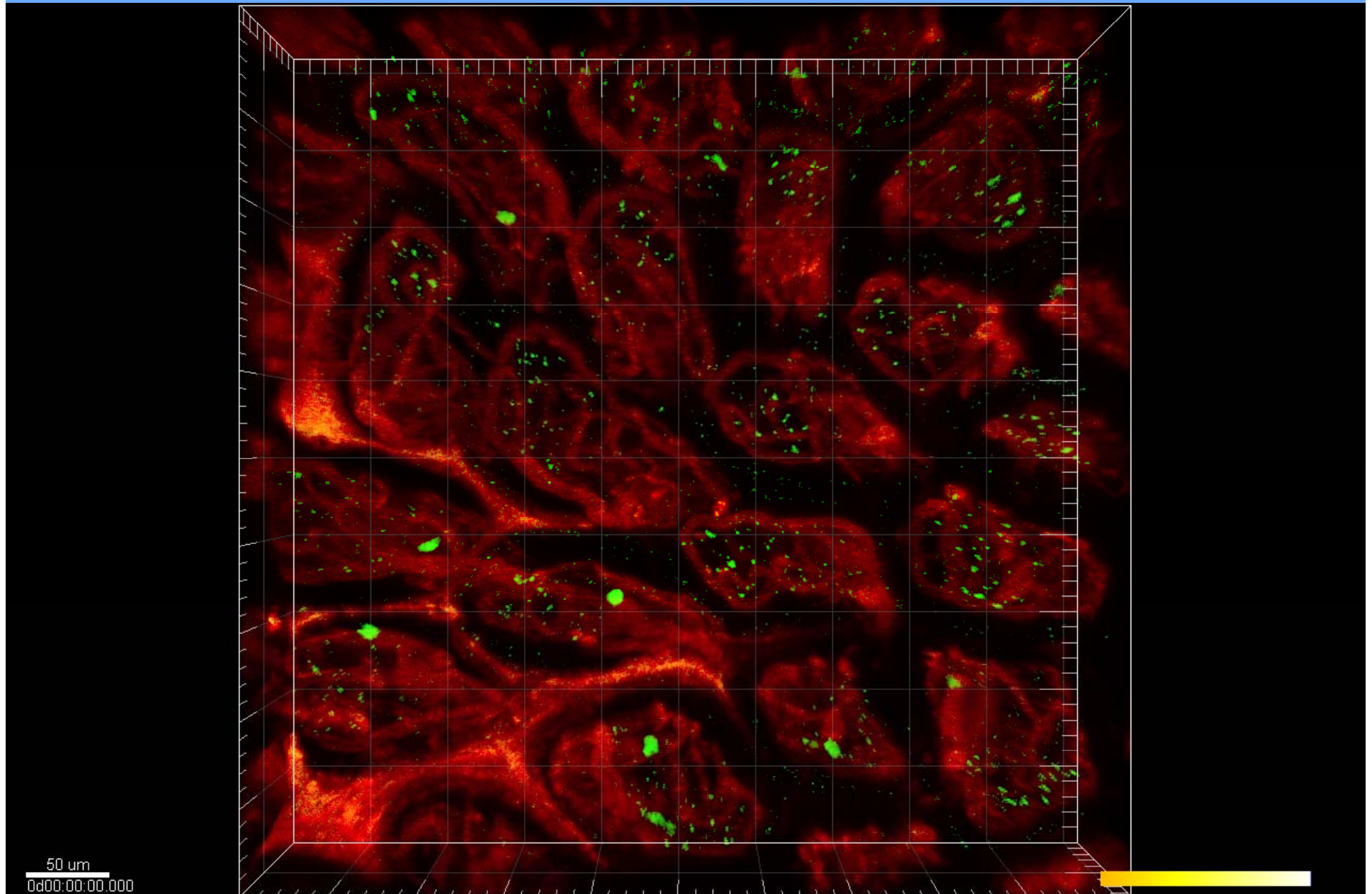
The role of DCs in homeostatic T cell patrolling

Role of dendritic cells in homeostatic T cell patrolling

Hypothesis: T cells do not require DCs for homeostatic patrolling

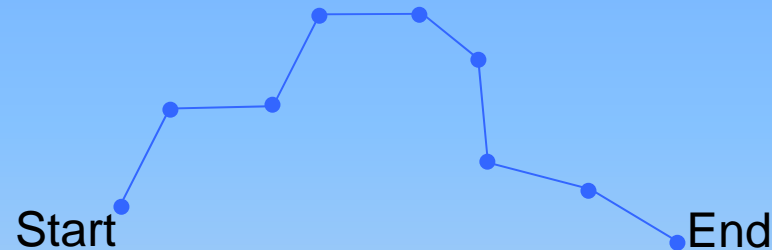


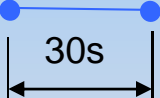
Homeostatic T cell patrolling



How is cell migration quantified?

- This diagram represents a cell whose migration that is observed over time



- Each blue dot represents an observation
- An observation is made every 30 seconds 
- Every cell in the imaging field is monitored and migration characteristics quantified

Dendritic cells in T cell patrolling

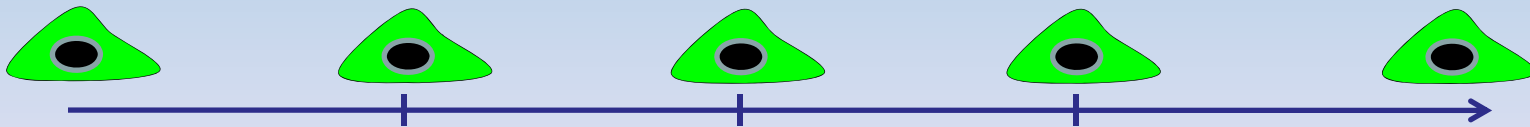
Question 1: Do T cells *scan* or *stop-and-go* on dendritic cells during homeostasis?

Scanning



If T cell scan DCs,
speed and arrest coefficient will remain unaffected in the absence of DCs

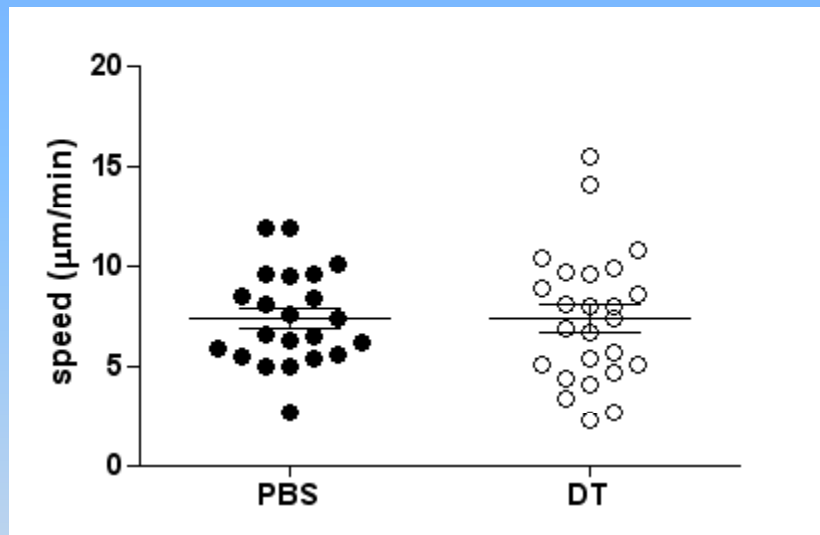
Stop-and-go



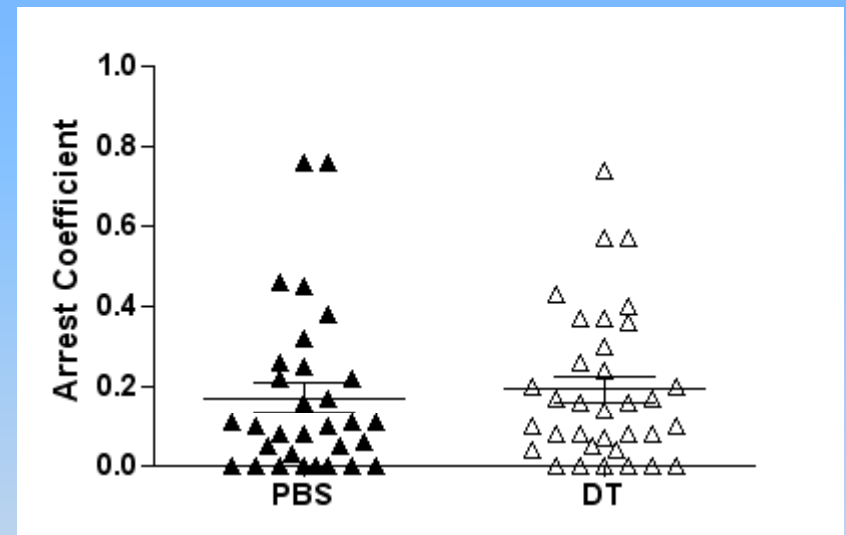
If T cell stop-and-go on DCs,
they will migrate faster and/or arrest less often in the absence of DCs

Dendritic cells in T cell patrolling part I

$p=0.29$



$p=0.50$



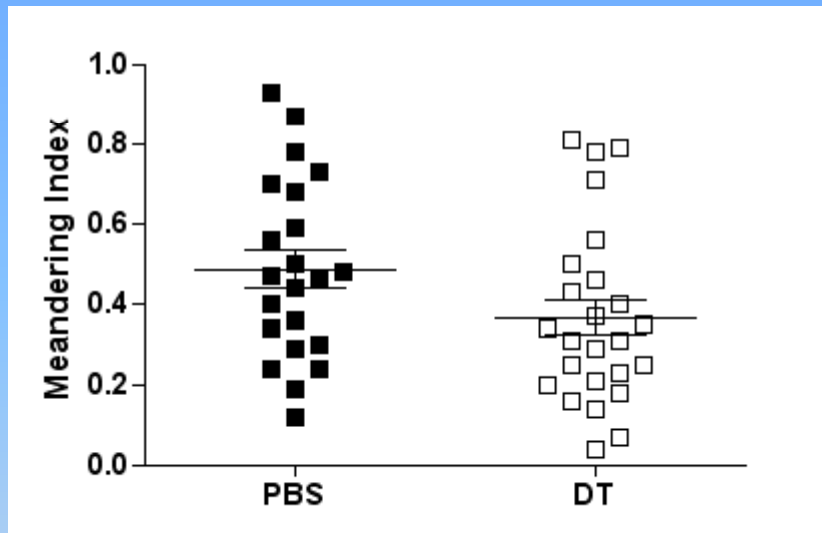
Speed – mean rate of migration per cell

Arrest coefficient – percent of time a cell is migrating $<2\mu\text{m}/\text{min}$

T cell scan DCs as they migrate through tissue during homeostasis

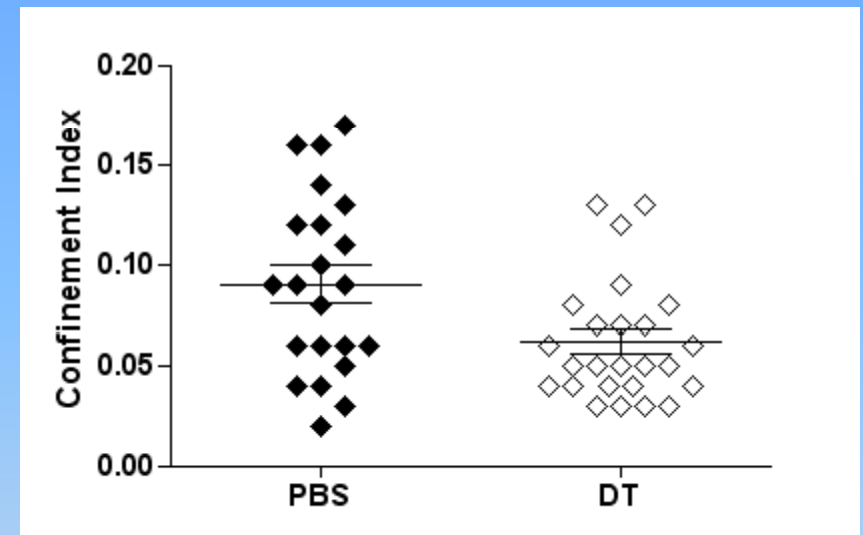
Dendritic cells in T cell patrolling part II

$p=0.08$



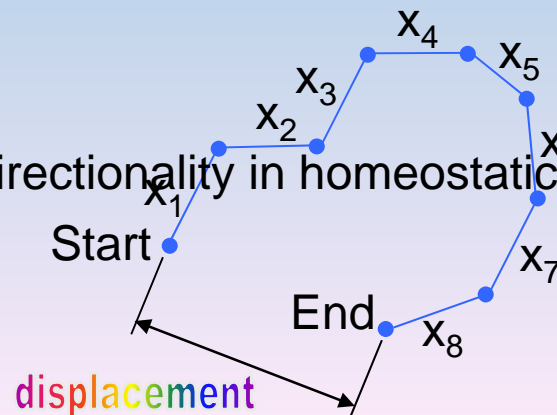
MI – displacement / track length

$p=0.02$



CI – max. displacement / track length

DCs are required for directionality in homeostatic mucosal T cell patrolling



Summary and conclusion

- We established a system for studying mucosal biology
 - Tissue damage is limited to incision area
- Perfusion is intact and dextran clearance is consistent with intact kidney filtration
- Vasculature is permeable to low MW dextran
- Mucosal dendritic cells form a highly organized network
- Dendritic cells are sessile and actively probe during homeostasis, reminiscent of DCs in secondary lymphoid organs
- T cells patrol independent of mucosal DCs, scanning as they migrate through tissue
- T cells require mucosal DCs to provide directionality

Single- and MP- Excitation IVM

Quantification

- Cell migration
 - Chemotaxis
 - Confinement
 - Speed
 - Arrest
 - Randomness
- Perfusion
- Vascular permeability
- Cell viability

Application

- Homeostasis
- Infectious Disease
- Tumor formation/rejection

Organs

- Small bowel
- Viscera (skin)
- Parietal peritoneum
- Liver
- Footpad
- Spleen
- Inguinal Lymph Node

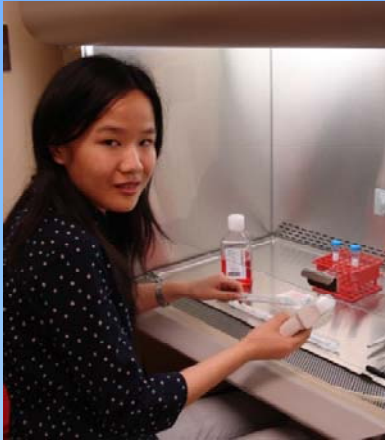
Labels

- Organic dye
- Soluble dye
- Antibody
- Genetic
- SHM

Acknowledgements

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