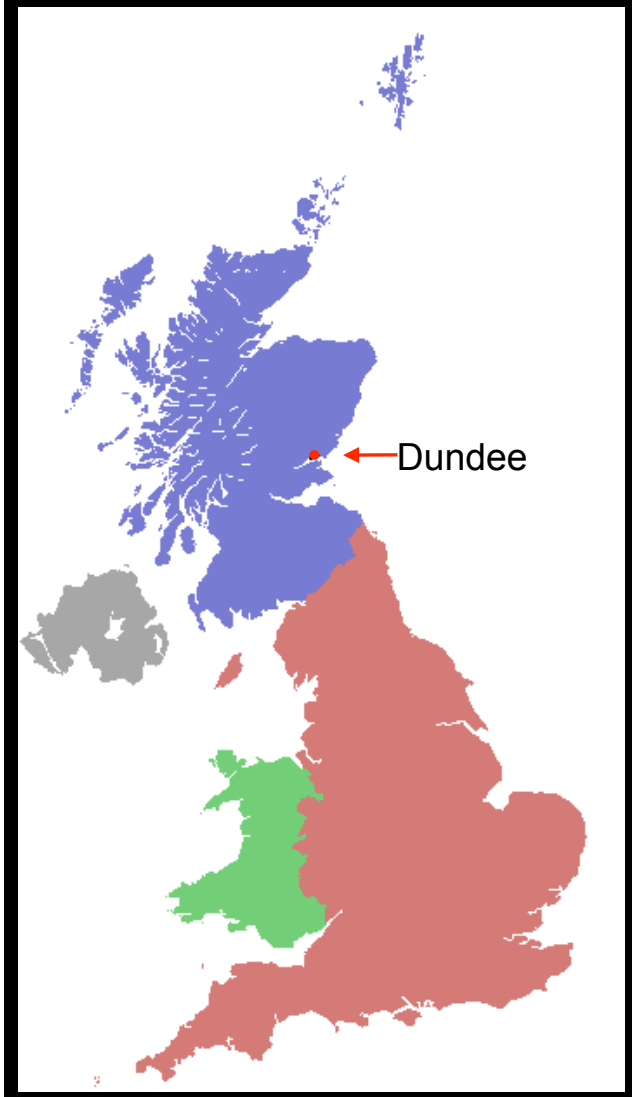
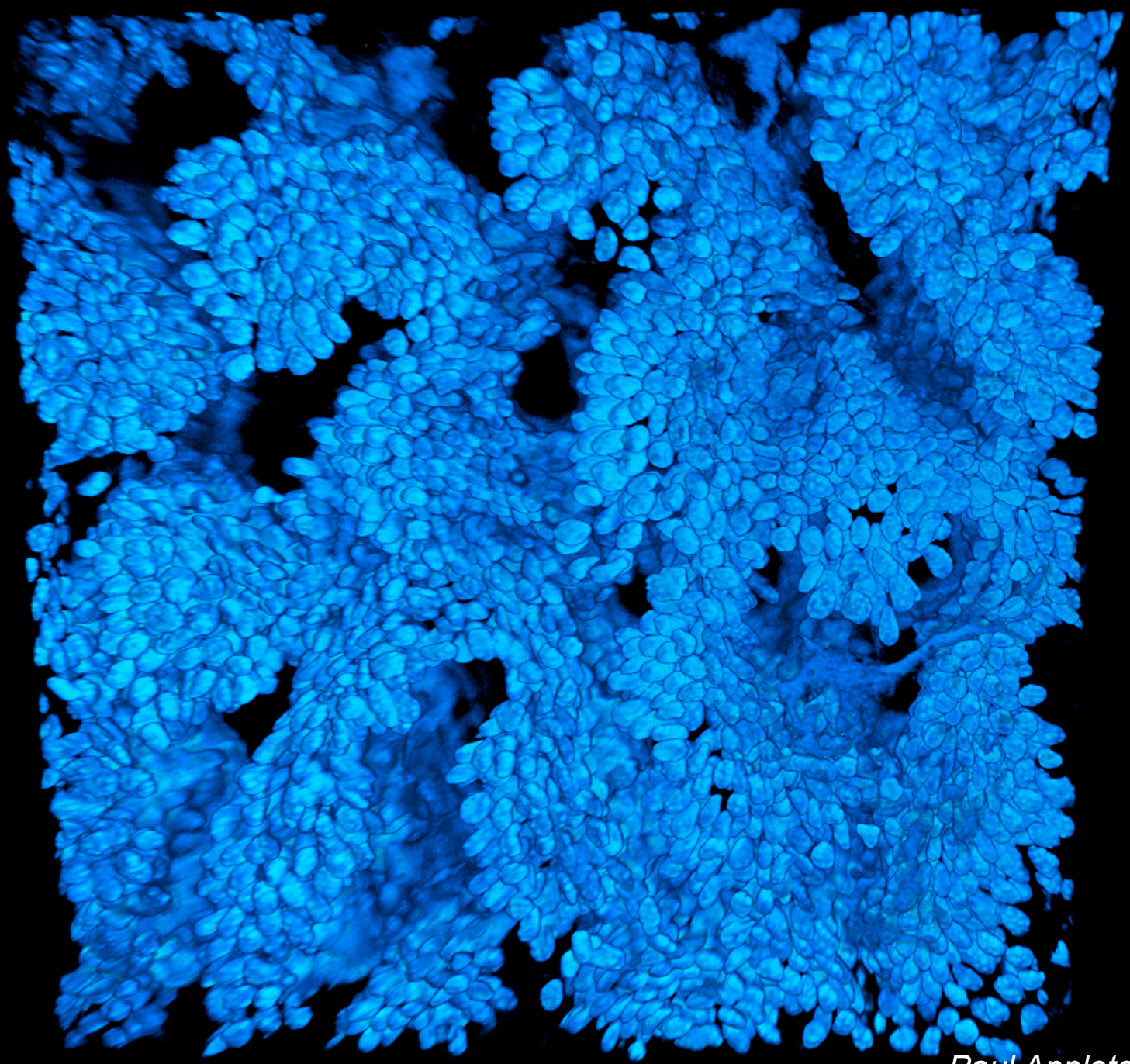
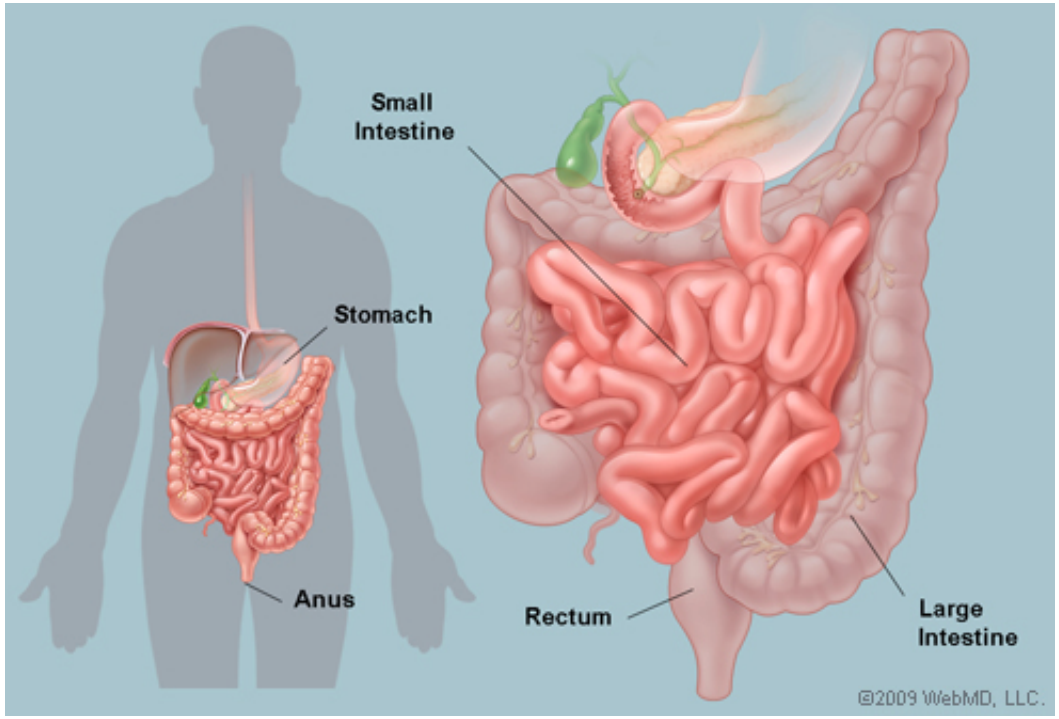
Inke Näthke

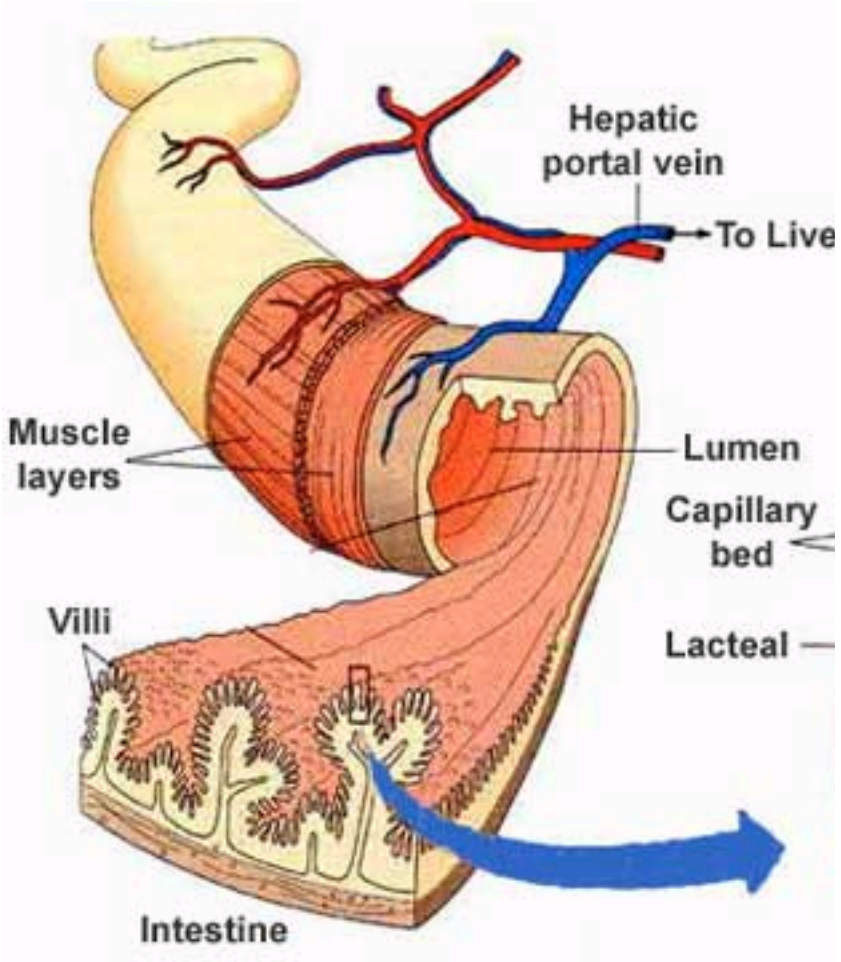
Cell & Dev. Biology
University of Dundee





Paul Appleton

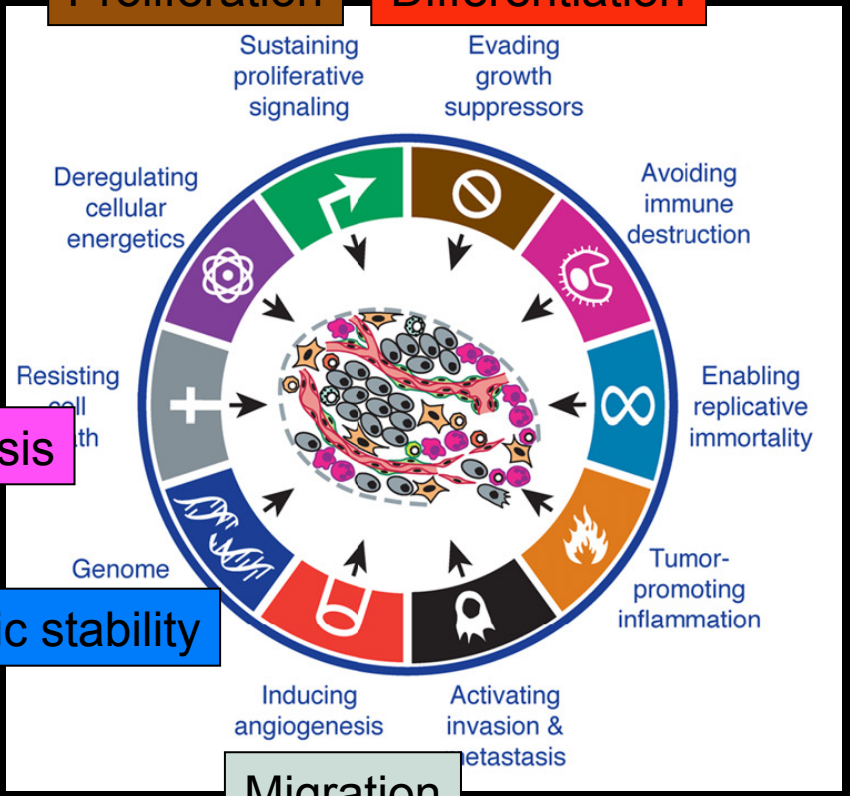

Small Intestine
Stomach
Anus
Rectum
Large Intestine
©2009 WebMD, LLC.


Hepatic portal vein
To Live
Muscle layers
Lumen
Capillary bed
Villi
Lacteal
Intestine

Tissue depends on constant migration, while differentiating and maintaining cell-cell and cell substrate adhesion. Proliferation has to be balanced by exfoliation/apoptosis.

Proliferation

Differentiation

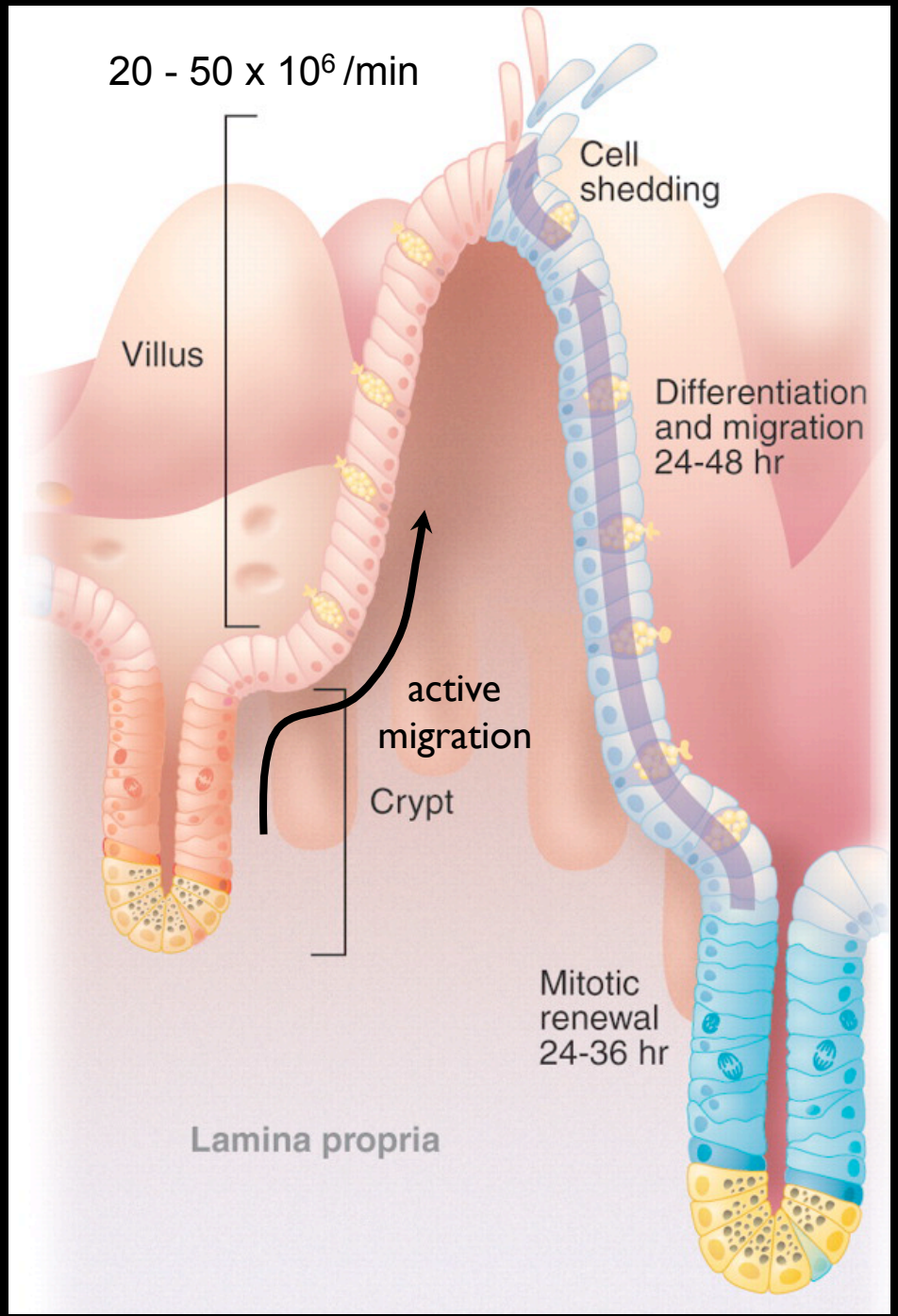


Apoptosis

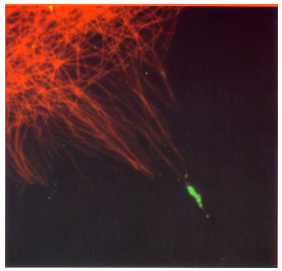
Genetic stability

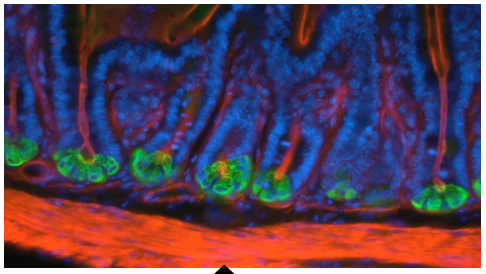
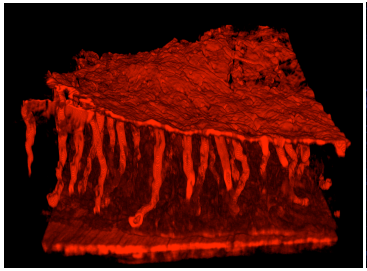
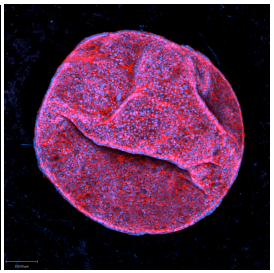
Migration

Hanahan & Weinberg, 2011



Epithelial tissue biology in health and disease



Migration

Cytoskeletal regulator
Centrosomes
Epithelial migration
Crypt shape & cell number
Dominant effect of APC fragments

Differentiation

Adhesion

β-catenin/Wnt
Hypoxia
Cytoskeleton

Proliferation

β-catenin/Wnt
Hypoxia

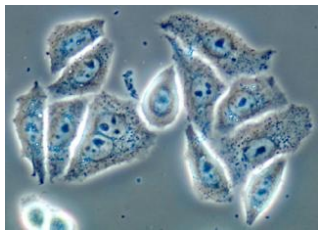
Genetic stability

Cytoskeletal regulation
Mitotic spindle
Spindle checkpoint
Stem cell maintenance
Chromatin compaction

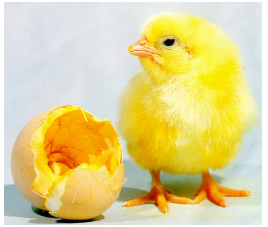
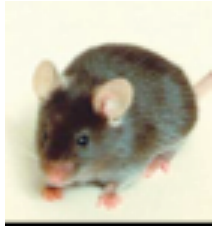
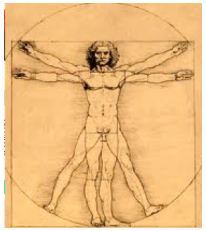
Apoptosis

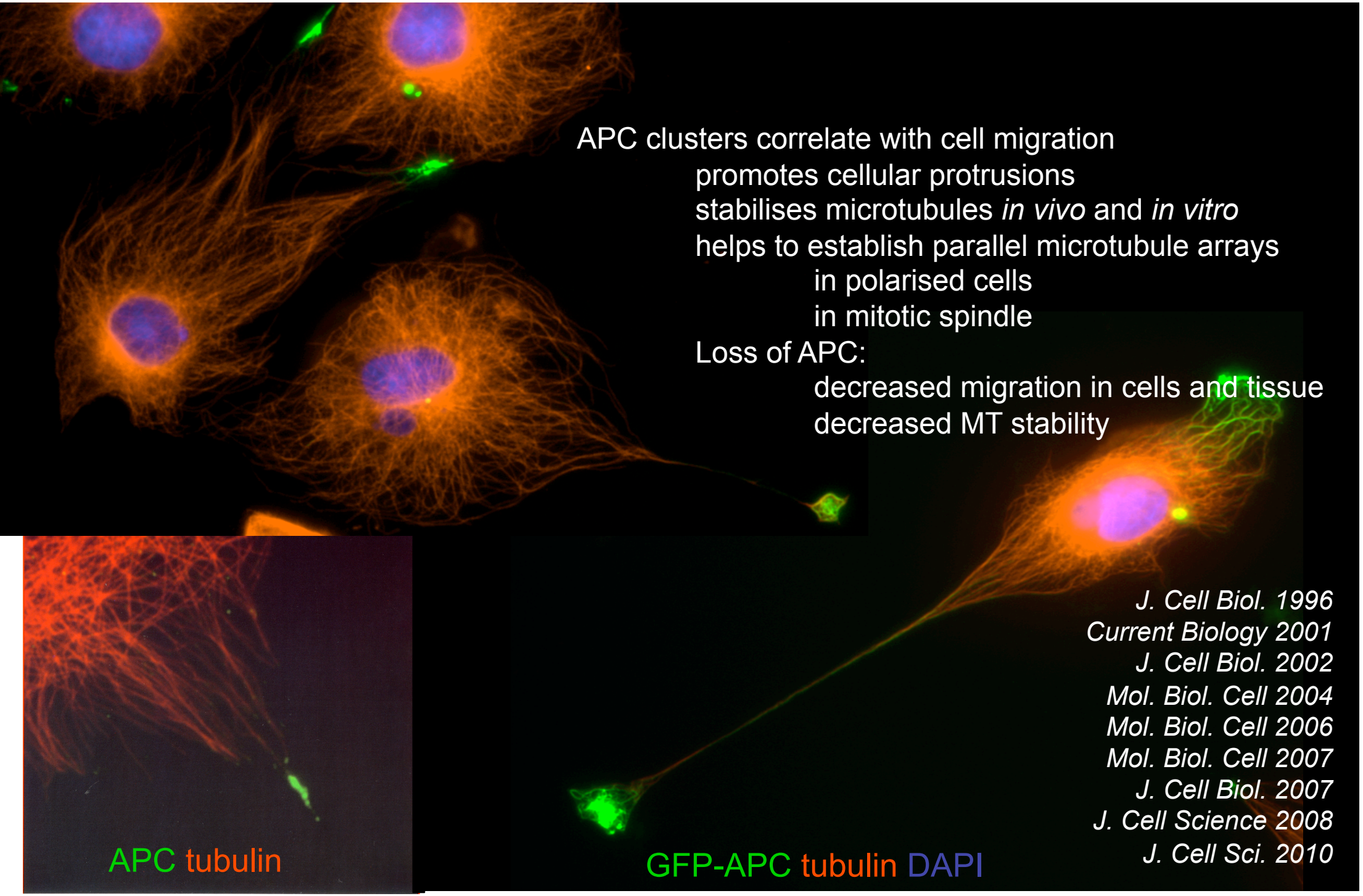
Bcl2 upregulation
BIM at mitochondria
Selective sensitivity to MT poisons + Bcl2-inhibitors

Adenomatous Polyposis Coli (APC)

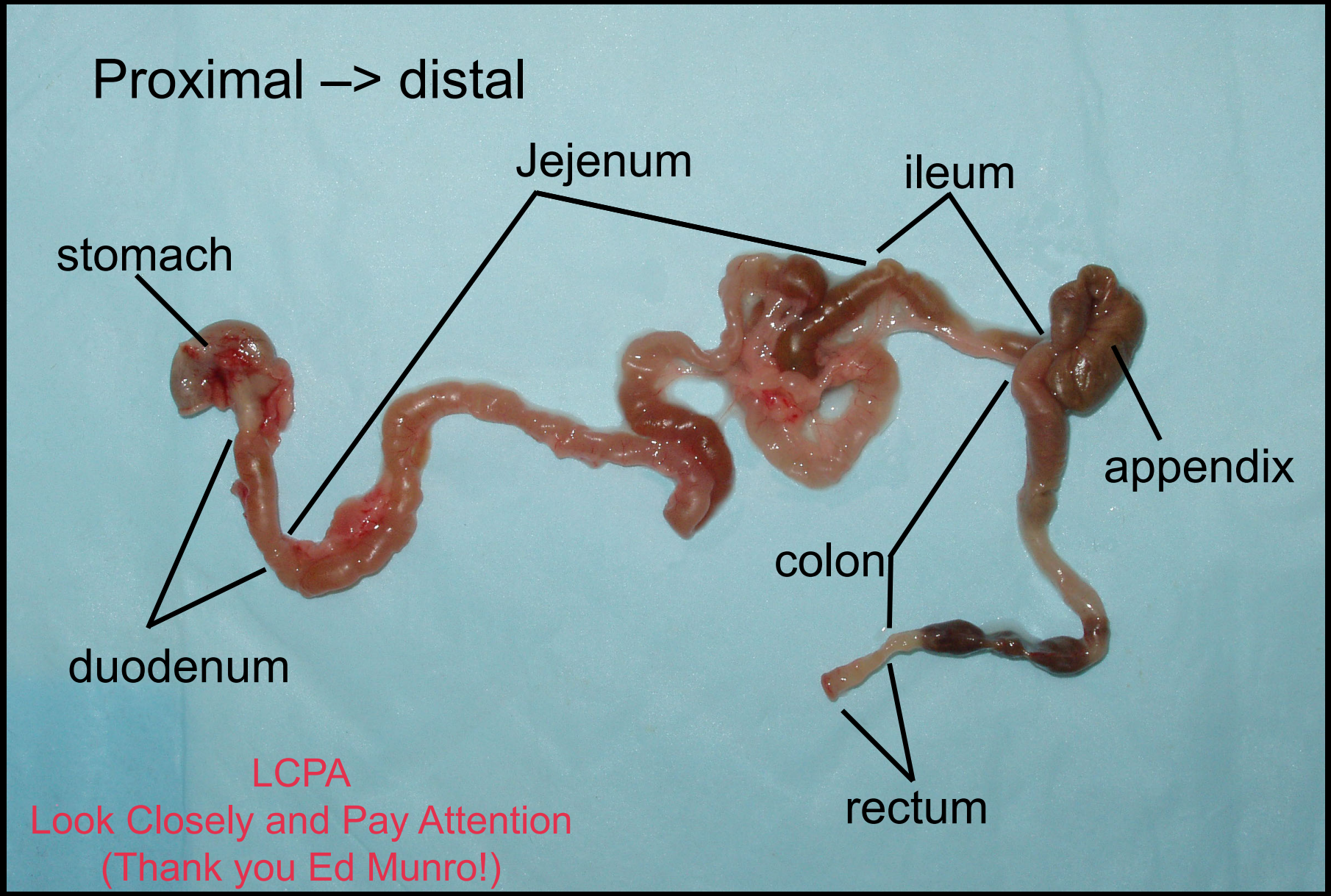





APC clusters correlate with cell migration
promotes cellular protrusions
stabilises microtubules *in vivo* and *in vitro*
helps to establish parallel microtubule arrays
in polarised cells
in mitotic spindle
Loss of APC:
decreased migration in cells and tissue
decreased MT stability
J. Cell Biol. 1996
Current Biology 2001
J. Cell Biol. 2002
Mol. Biol. Cell 2004
Mol. Biol. Cell 2006
Mol. Biol. Cell 2007
J. Cell Biol. 2007
J. Cell Science 2008
J. Cell Sci. 2010
APC tubulin
GFP-APC tubulin DAPI

Mouse Intestinal Tract



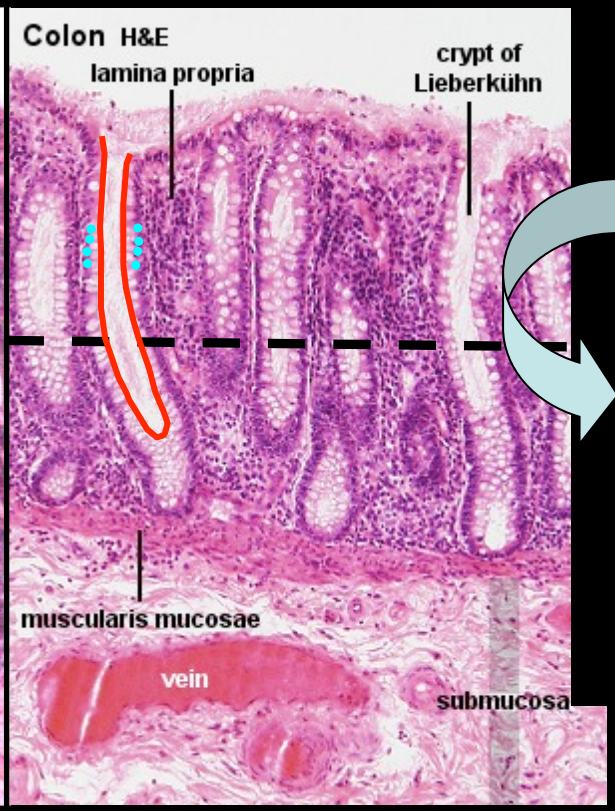
Aaron Quyn/Paul Appleton/Scott Nelson

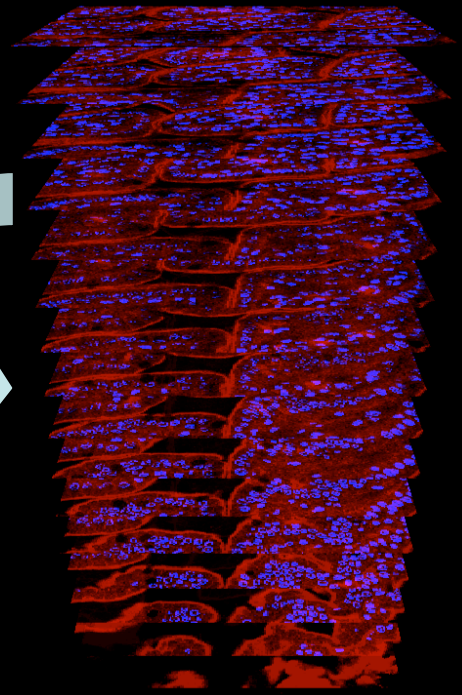
Conventional tissue staining, 2-D

Imaging same tissue in 3-D

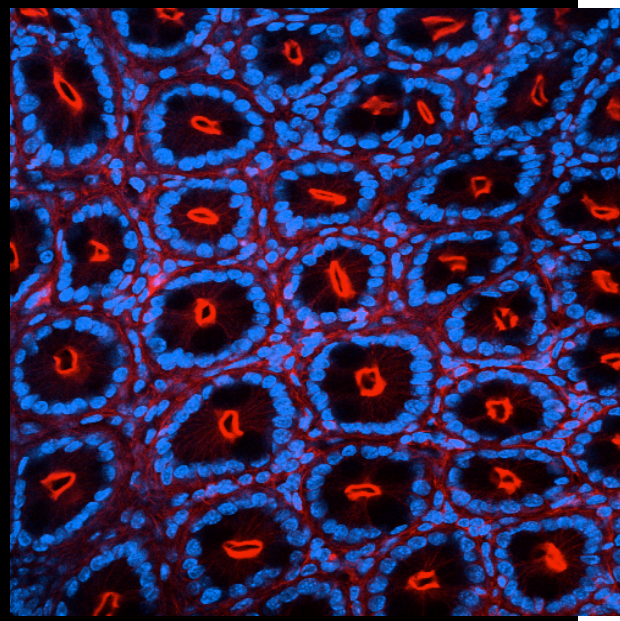
Intestine

Colon

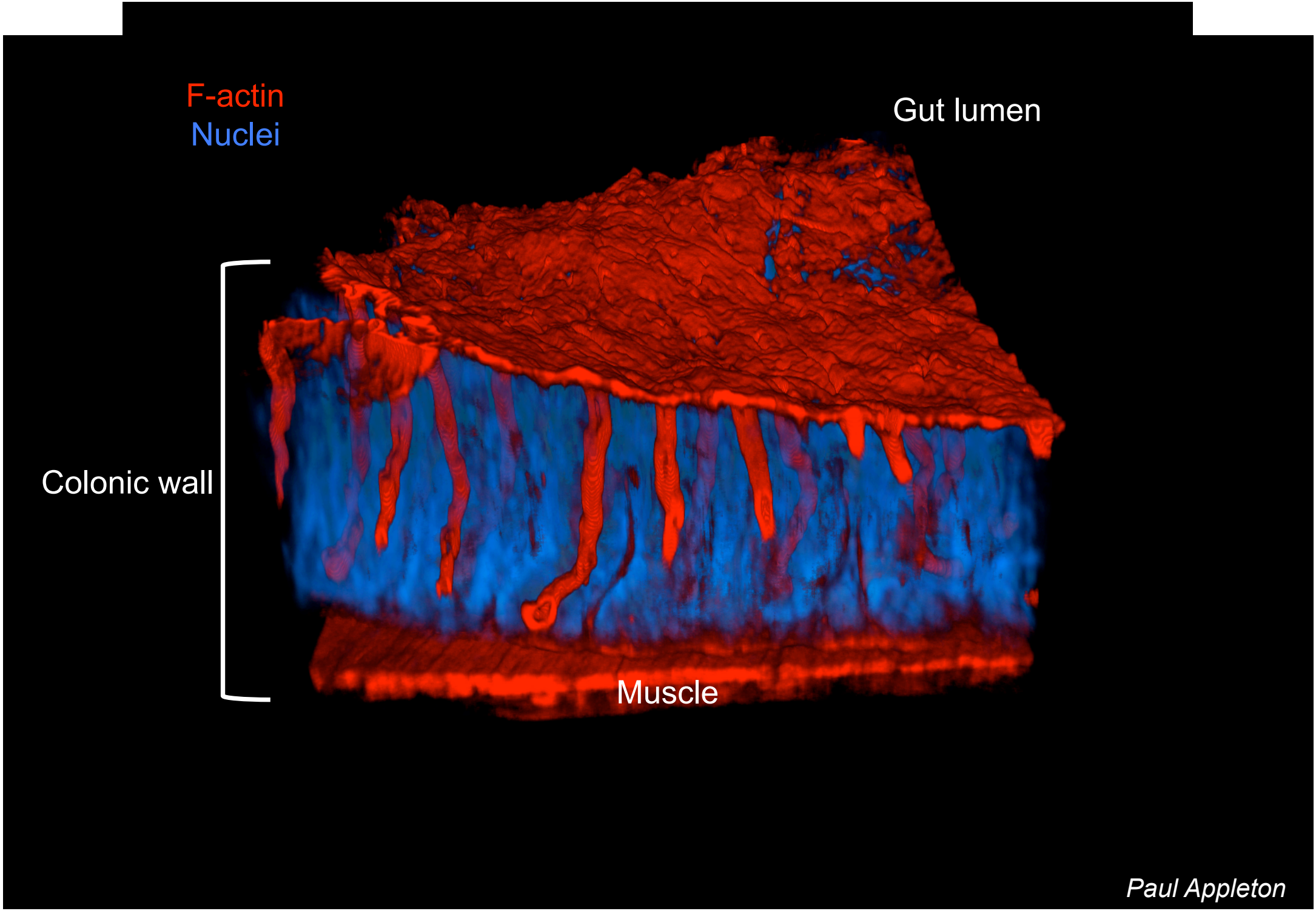




F-actin DNA

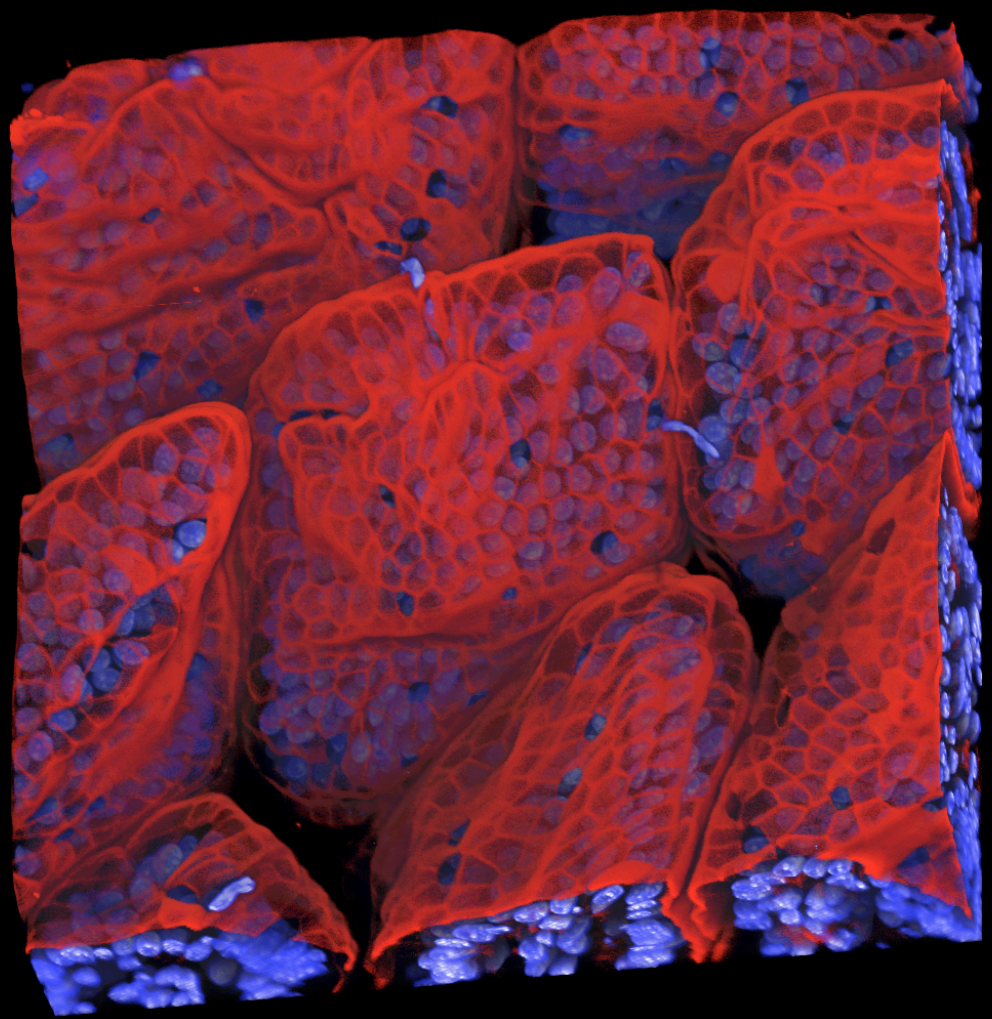


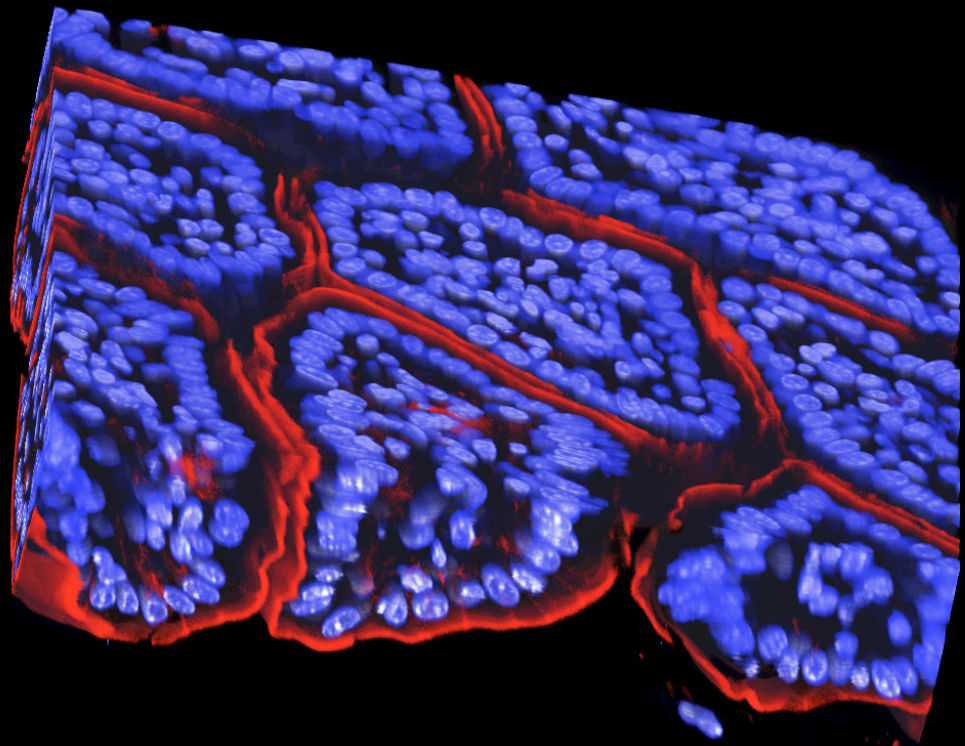
Paul Appleton


F-actin
Nuclei
Gut lumen
Colonic wall
Muscle
Paul Appleton

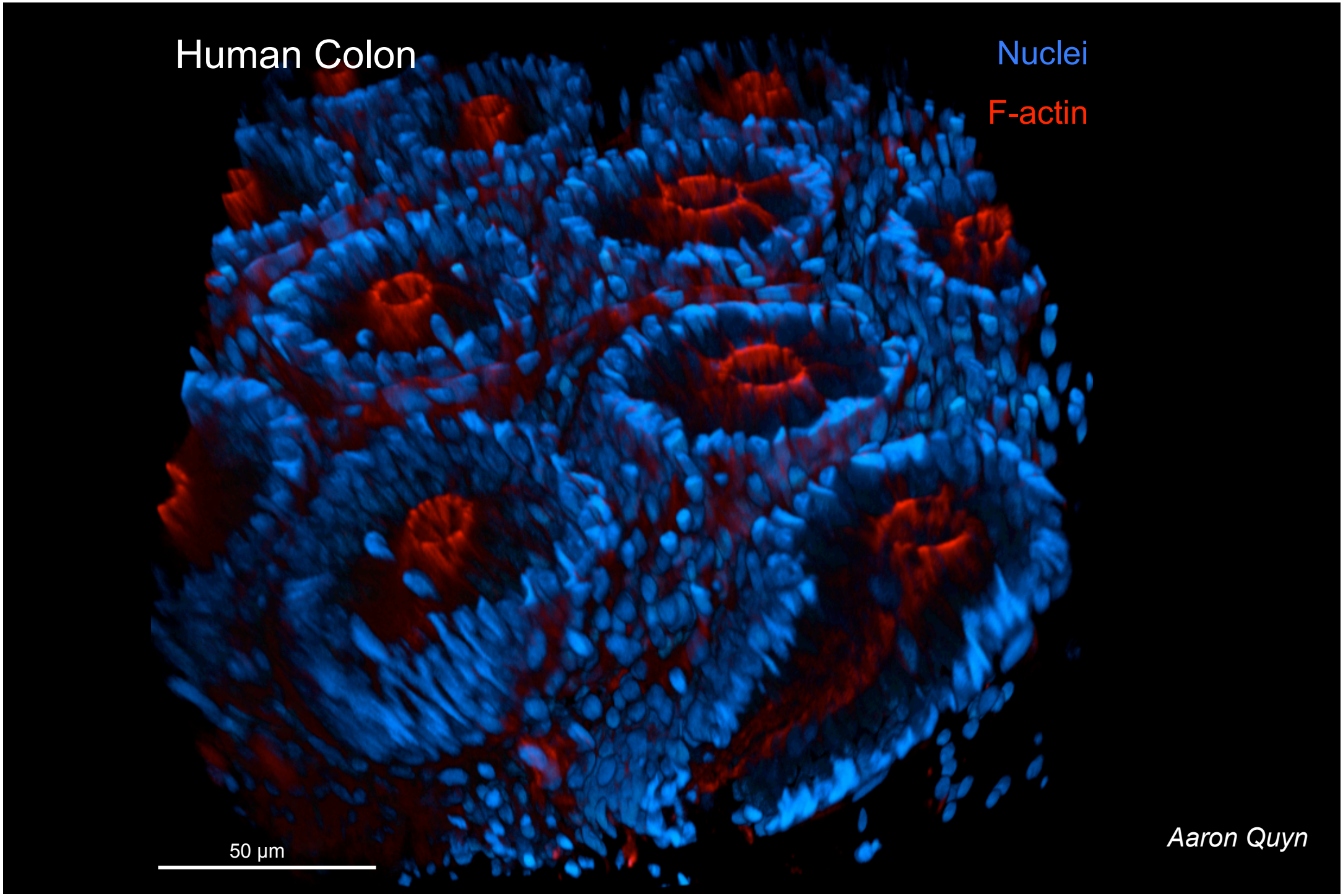
F-actin
Nuclei

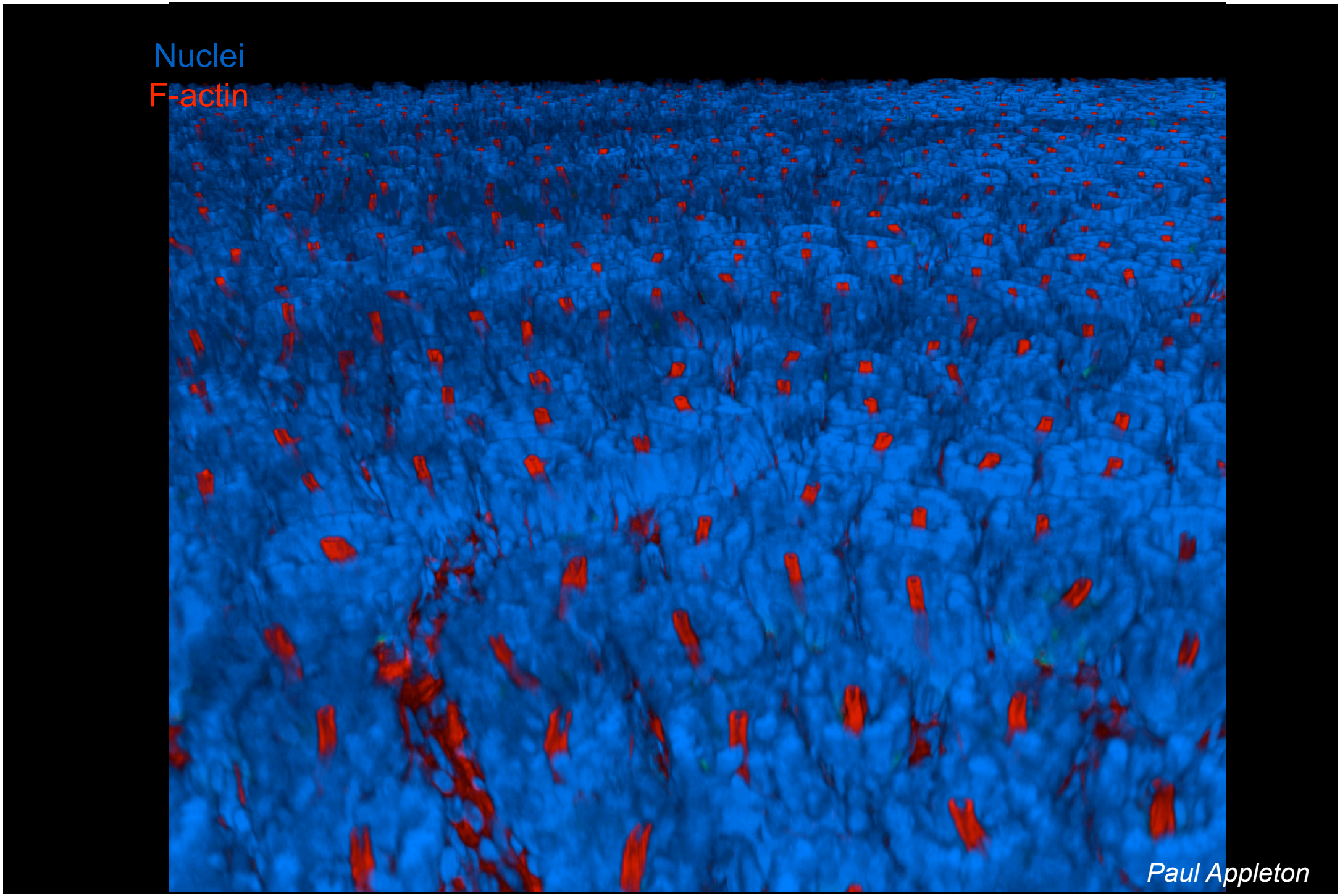
Villi in small intestine

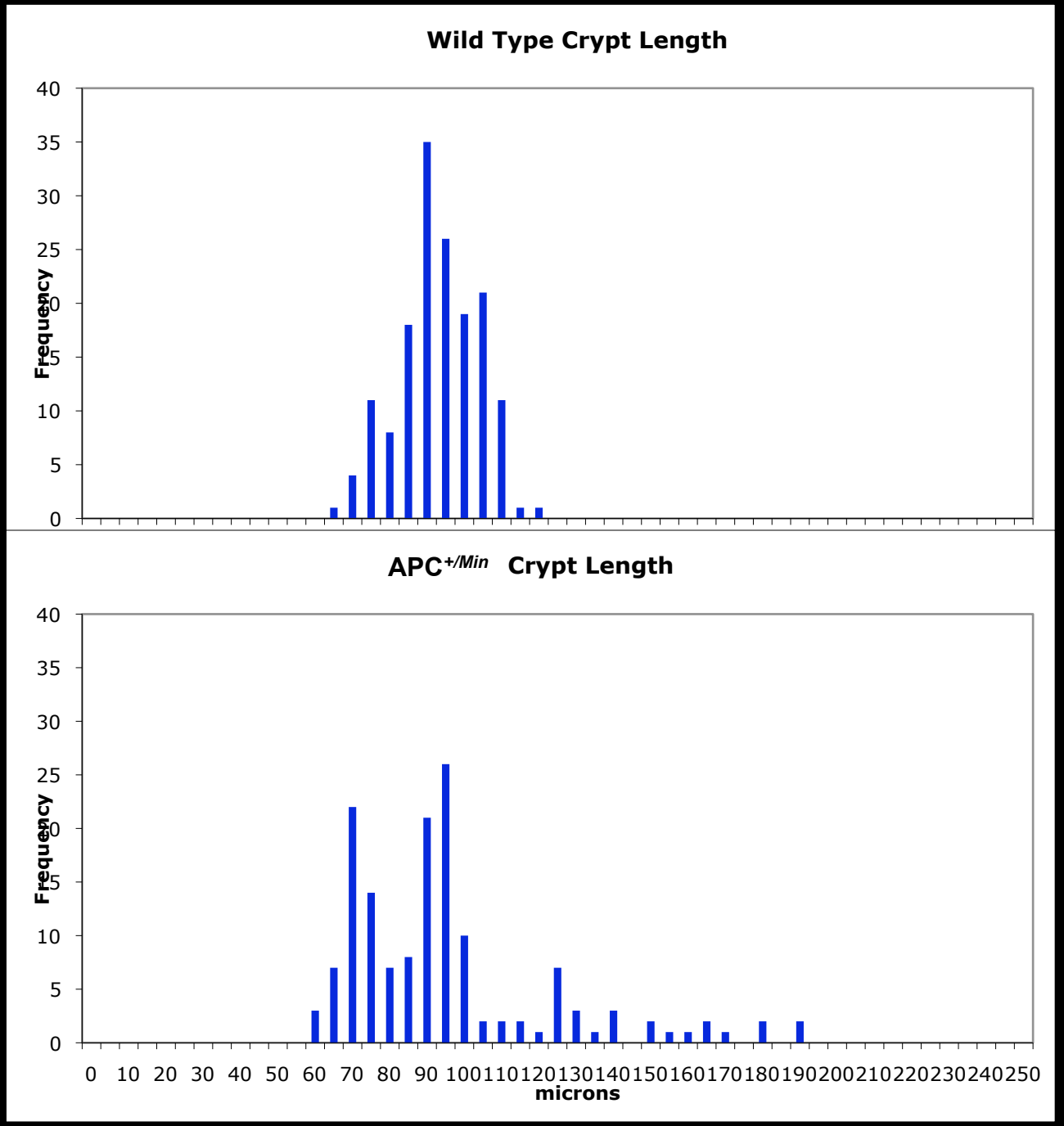




Paul Appleton

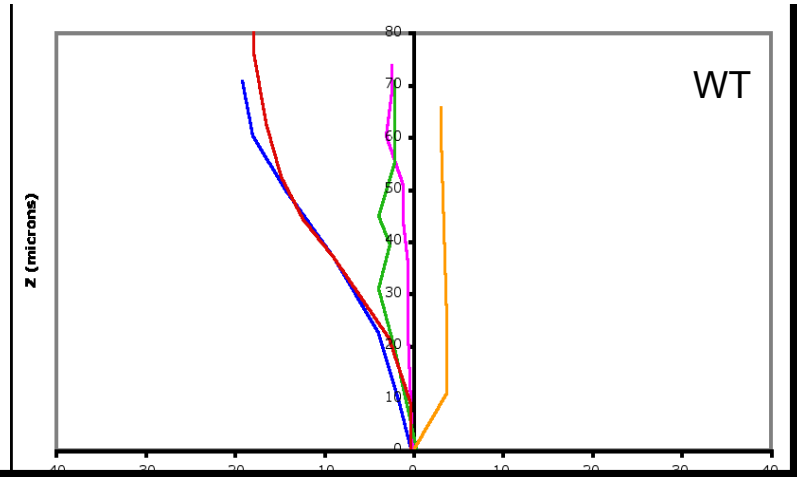

Human Colon
Nuclei
F-actin
50 µm
Aaron Quyn

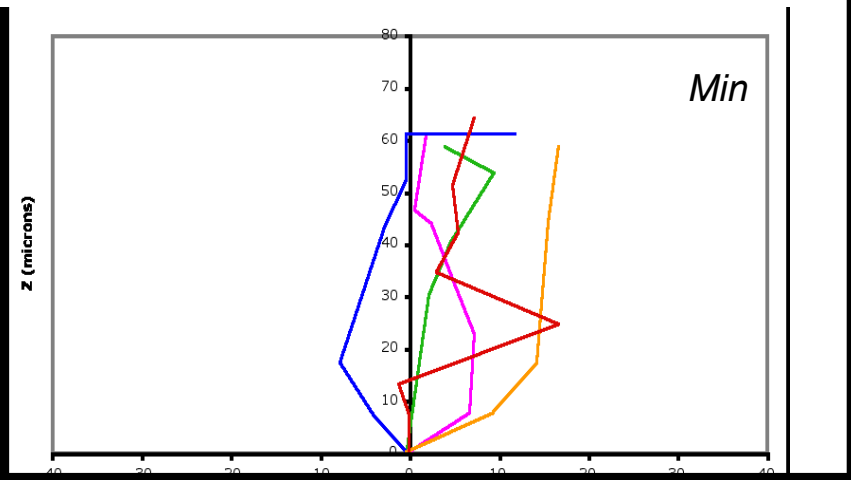

Nuclei
F-actin
Paul Appleton

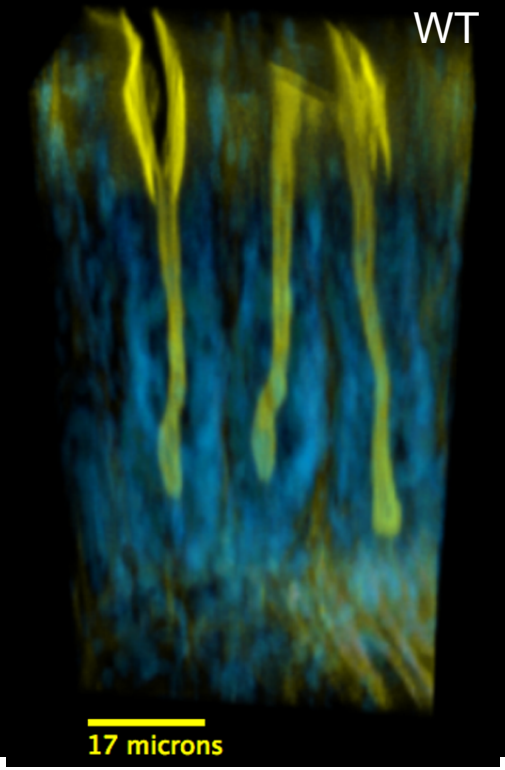


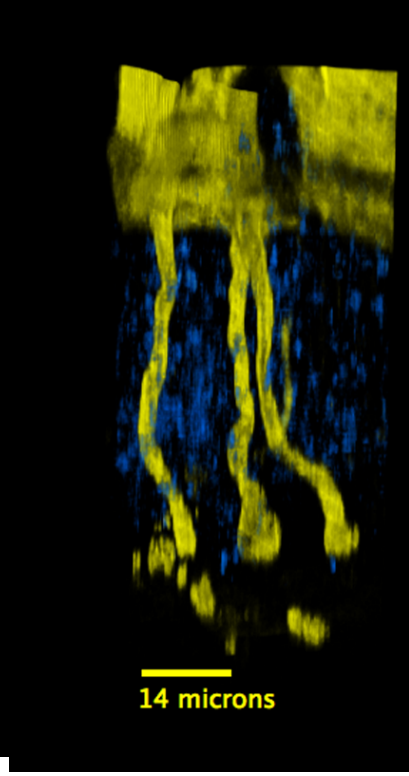
Scott Nelson

Crypts are more convoluted and less regularly packed in *APC* heterozygous, pre-cancerous tissue



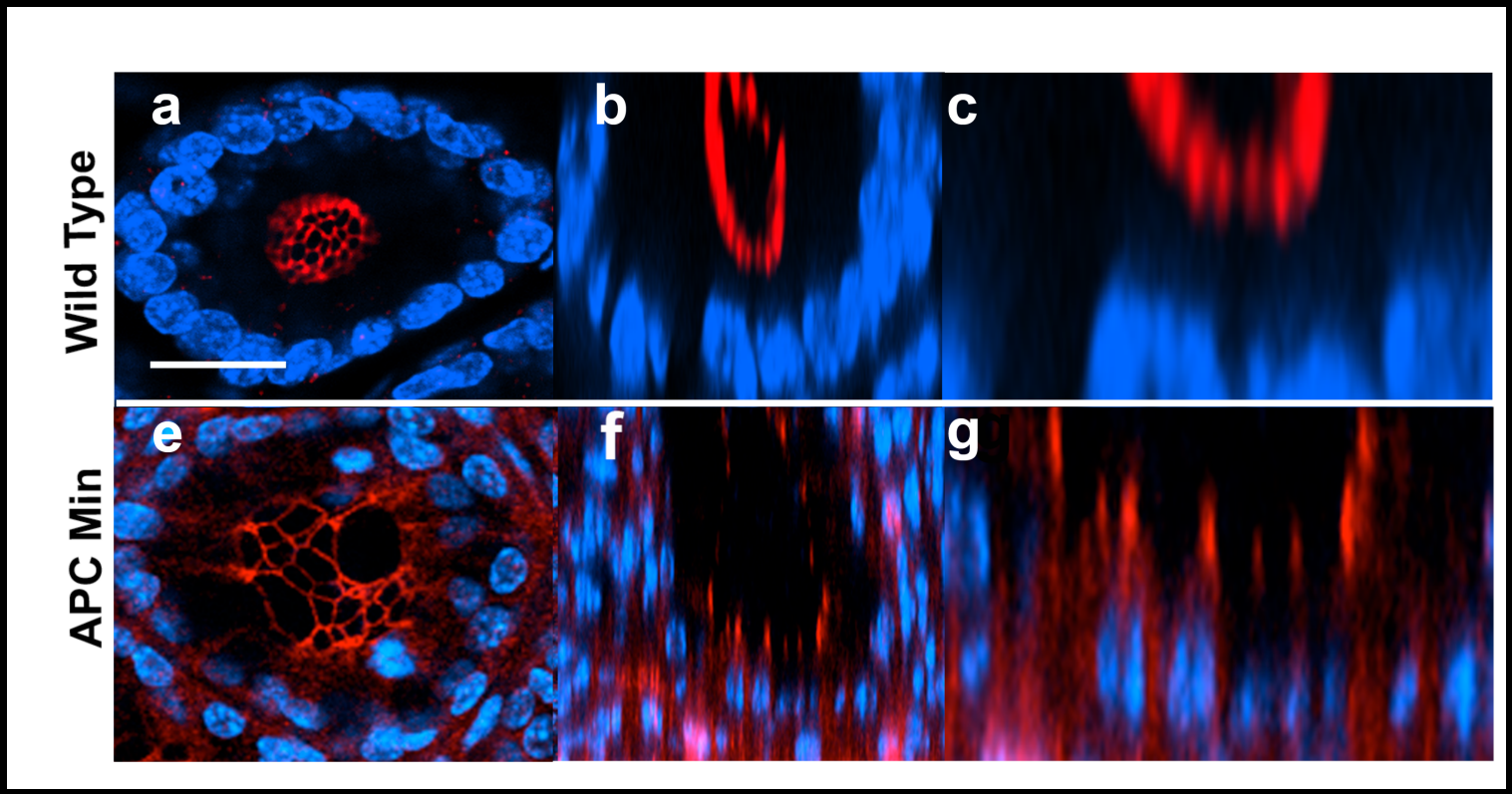


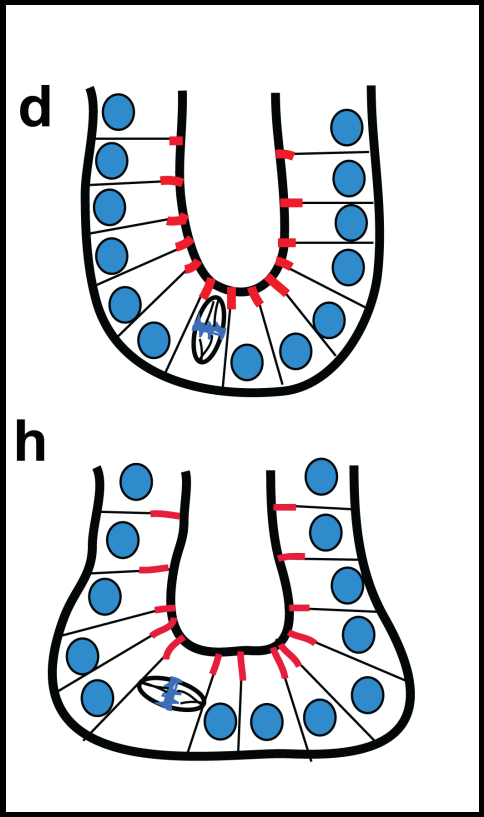




Scott Nelson

Crypts are wider at the bottom in *APC* heterozygous, pre-cancerous tissue

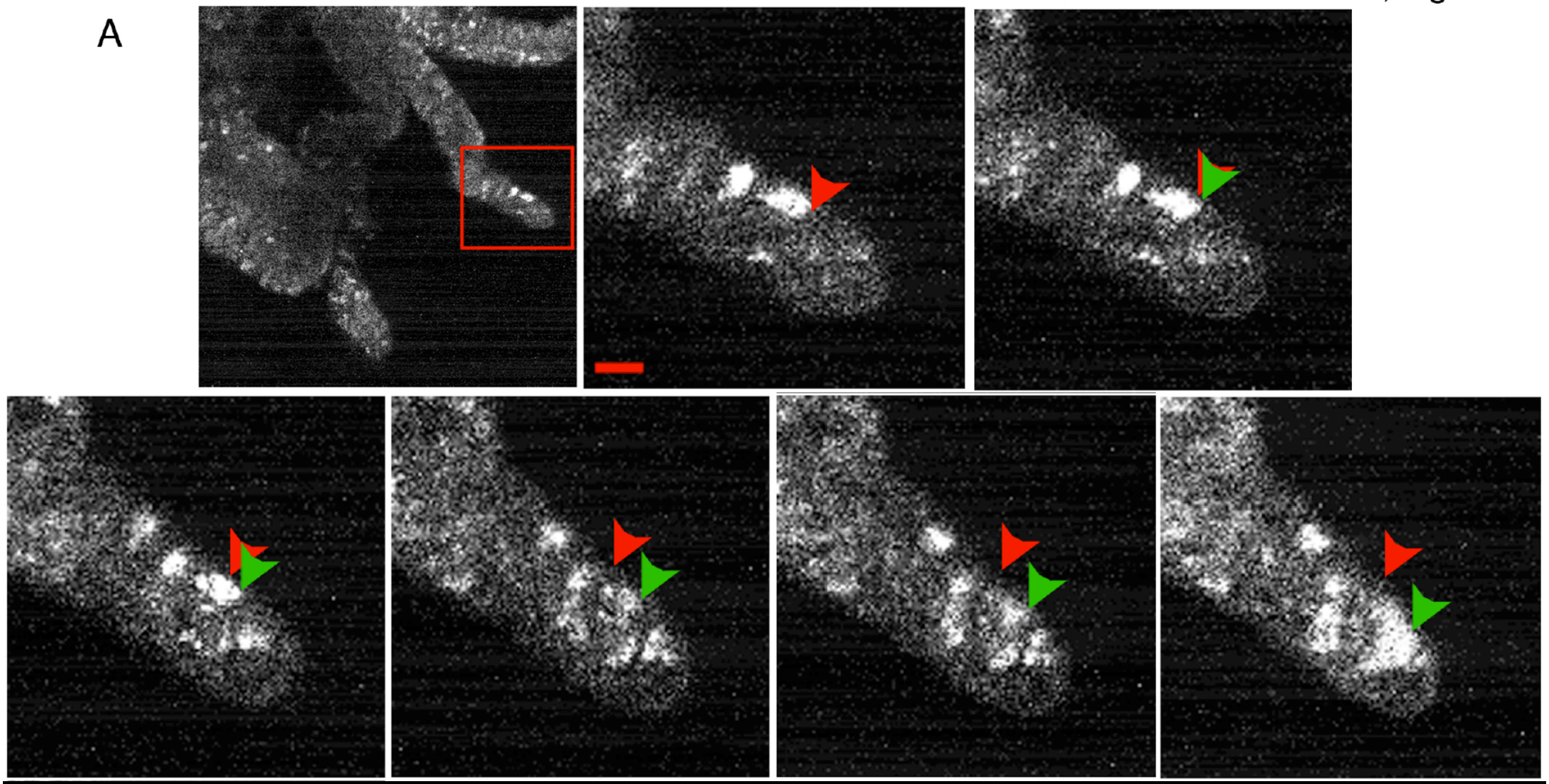




What drives cell migration in gut epithelium?

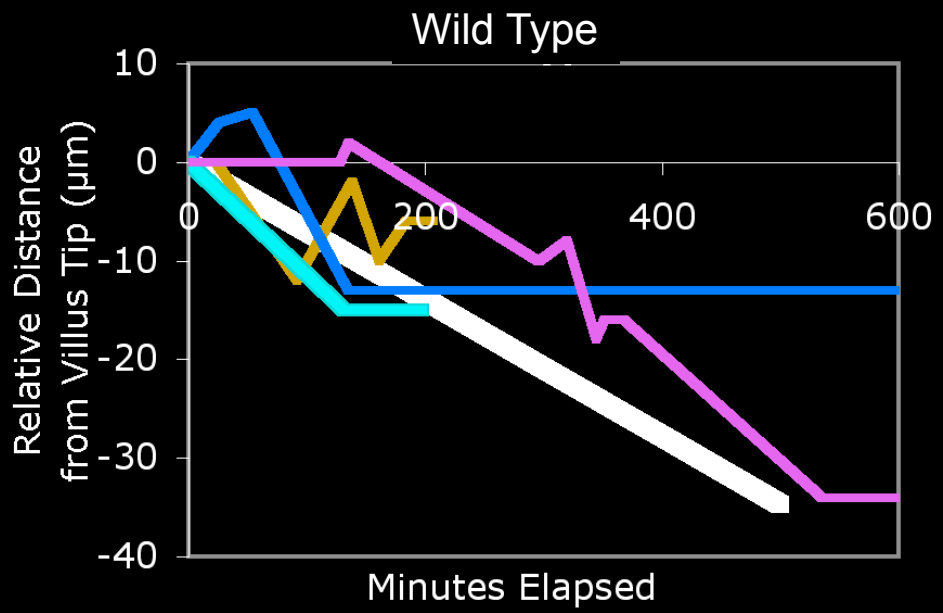
- Apoptosis?
- Chemical factors?
- Mechanical forces?
- Proliferation?
- Gradients in some or all of the above?

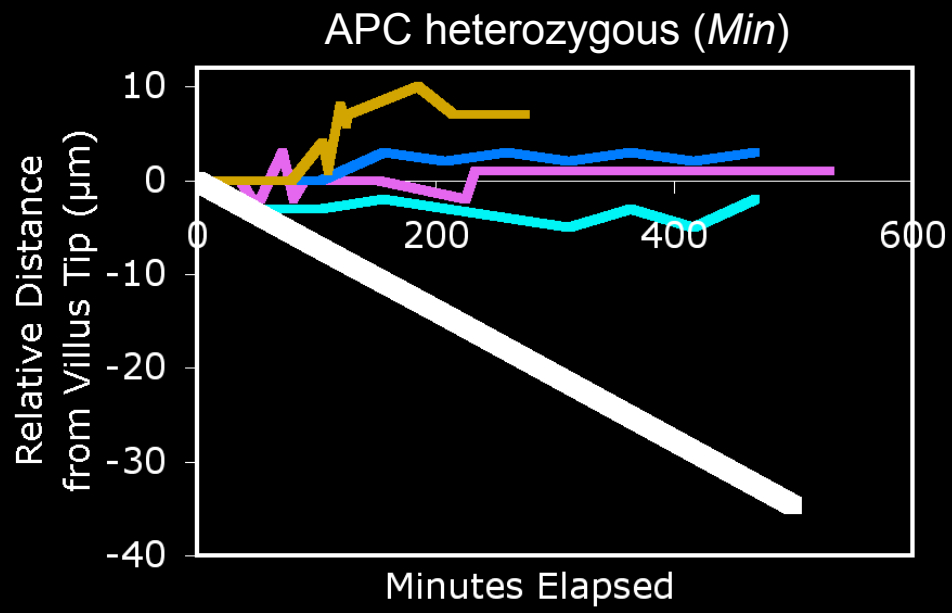
Cell migration in live gut tissue



Scott Nelson

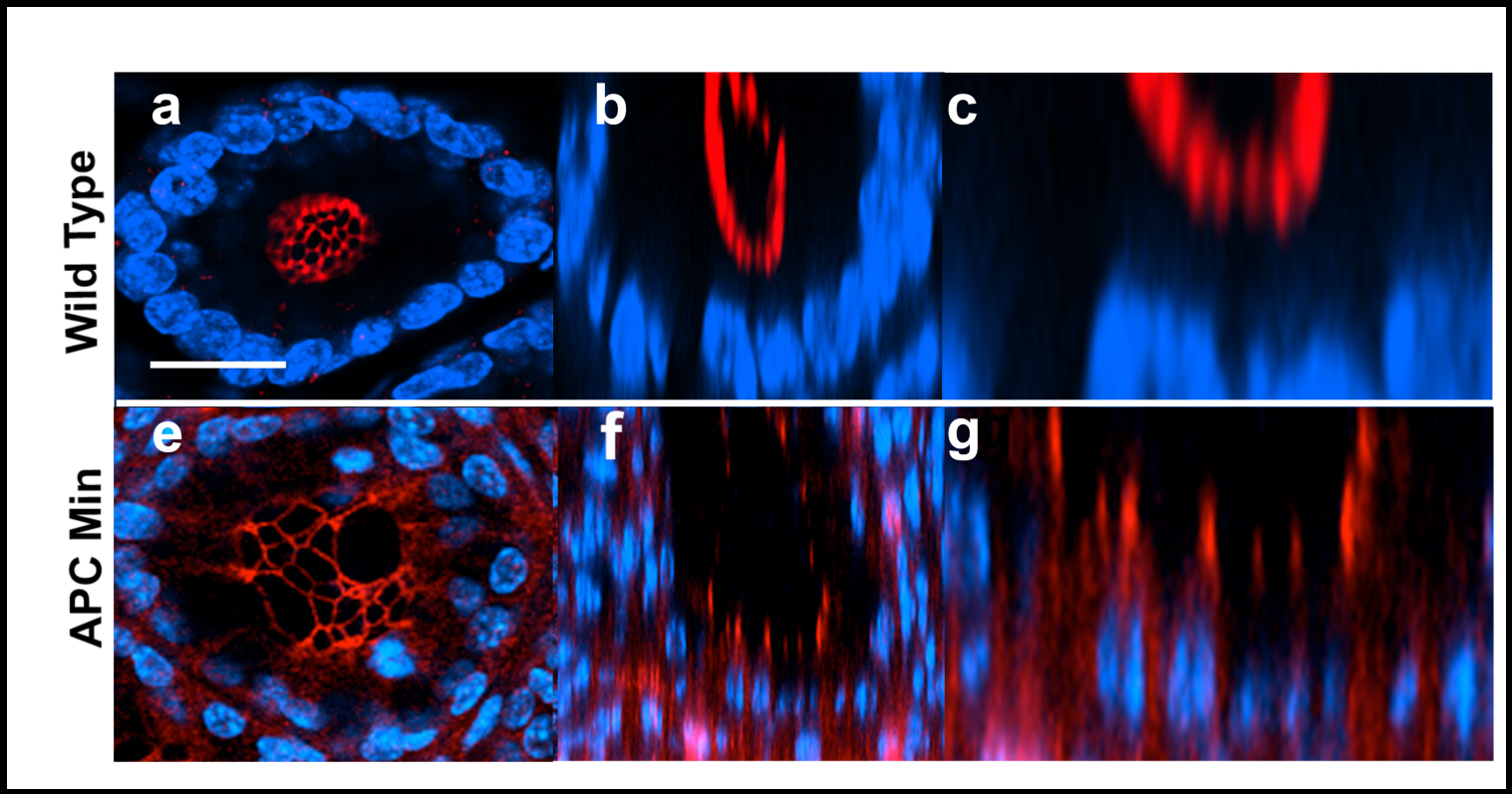
Epithelial migration in WT vs. APC-heterozygous, precancerous tissue



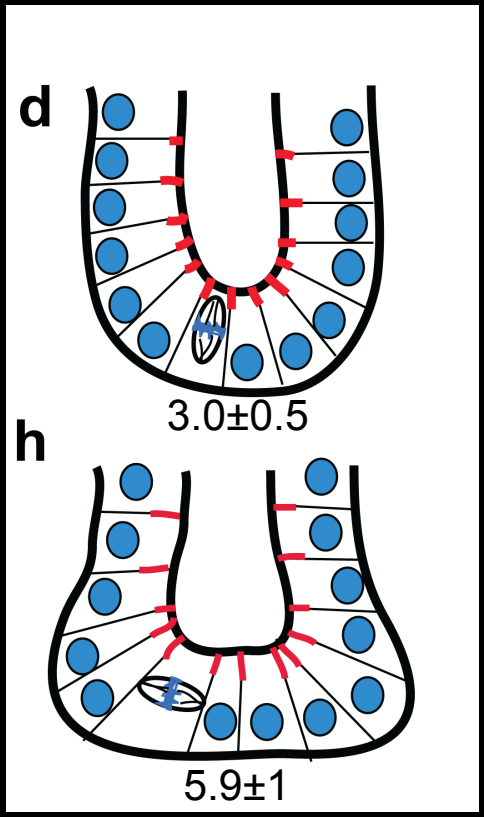


Scott Nelson

There are more cells in the bottom of crypts in *APC* heterozygous, pre-cancerous tissue



Par-3 DAPI



What drives cell migration?

- Apoptosis?

  Increasing apoptosis everywhere (removing the gradient of apoptosis) decreases directionality.

- Chemical factors?

  Changing activity of signaling pathways, i.e. BMP increases crypt fission; altered ephrin expression changes cell sorting.

- Mechanical forces?

  Muscle layers have specific orientation that predicts oriented forces along crypt-villus axis. No migration in organoids lacking muscle.

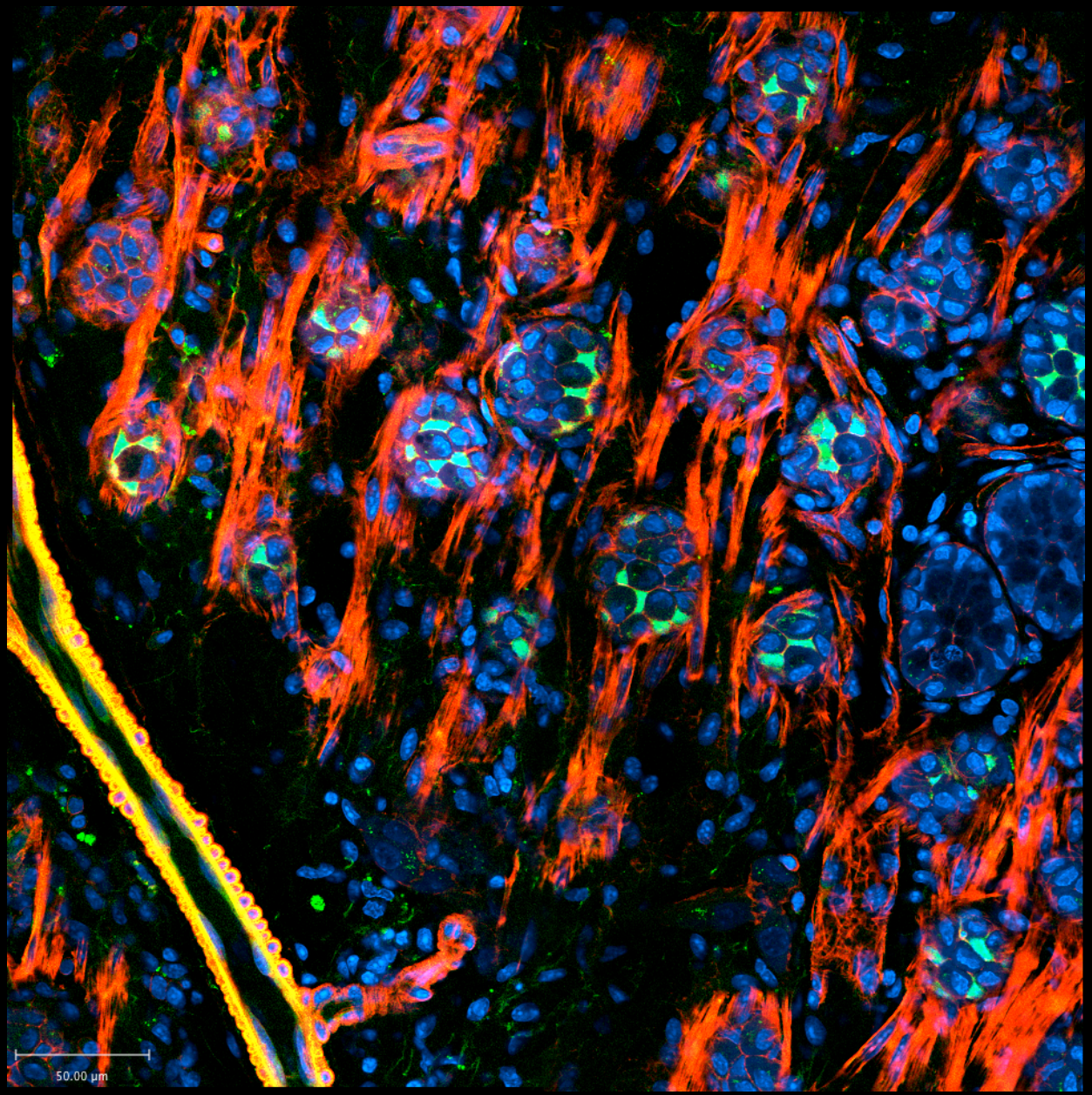
- Proliferation?

  Randomising the preferred asymmetric orientation of dividing stem cells correlates with decreased migration and accumulation of cells in crypts

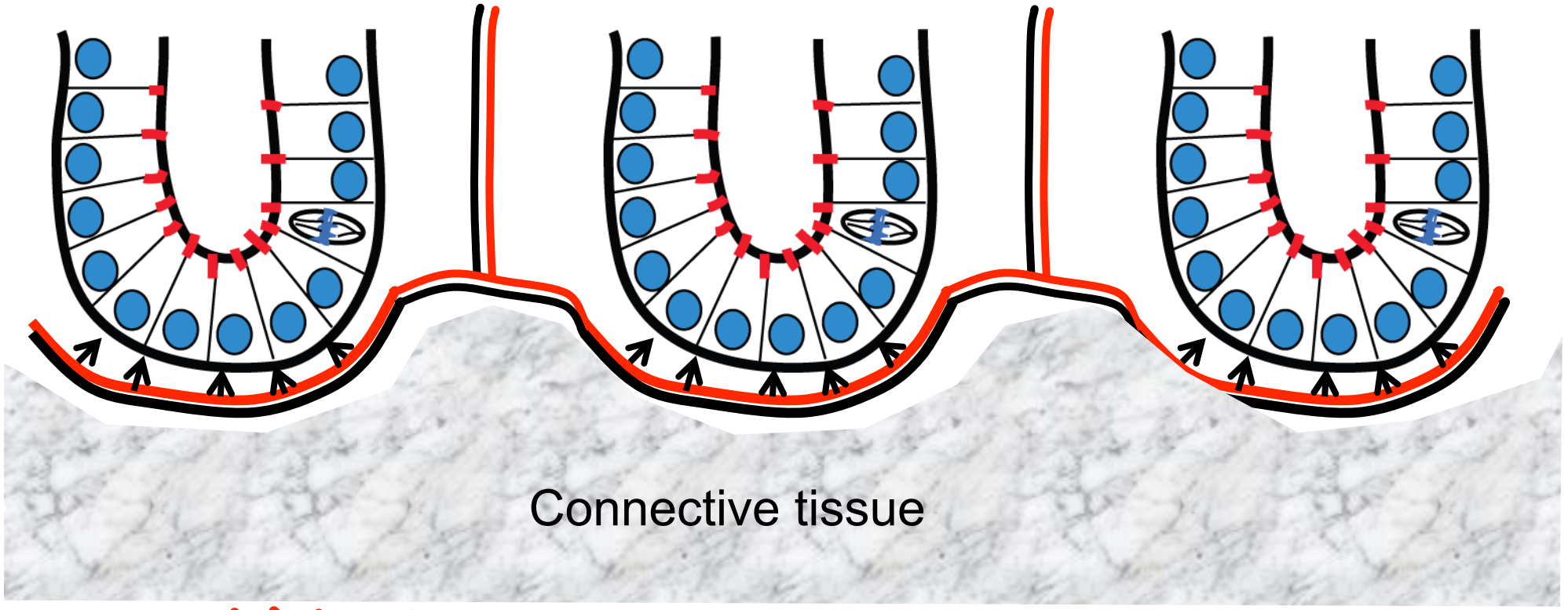
- Gradients in some or all of the above?

  Signaling molecules and their receptors are expressed in gradients along the crypt-villus axis. There is no migration in organoids, which grow in uniform concentrations of signaling molecules.

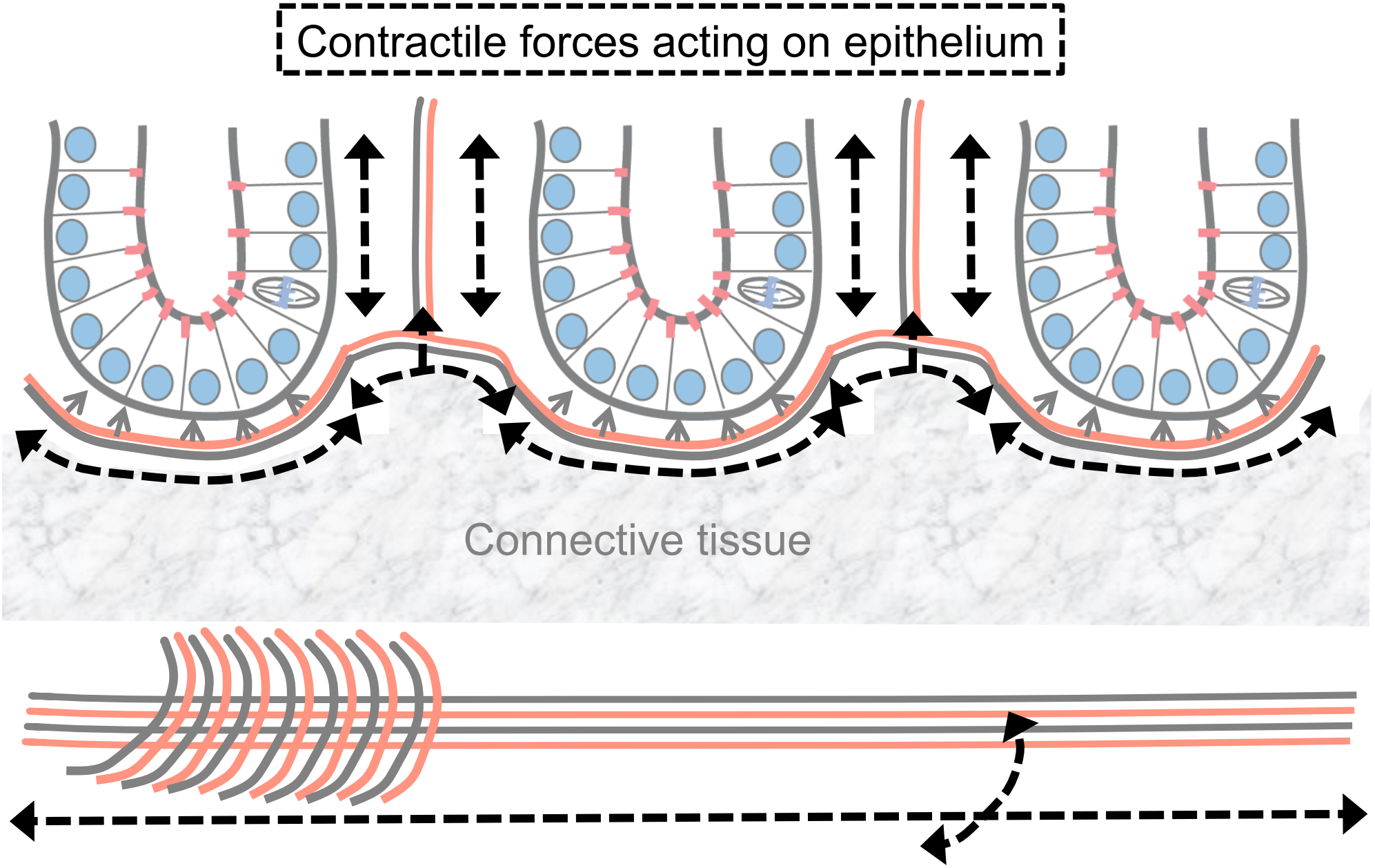
Crypt bases
Lgr5 (Stem cells)
F-actin
DNA



Paul Appleton



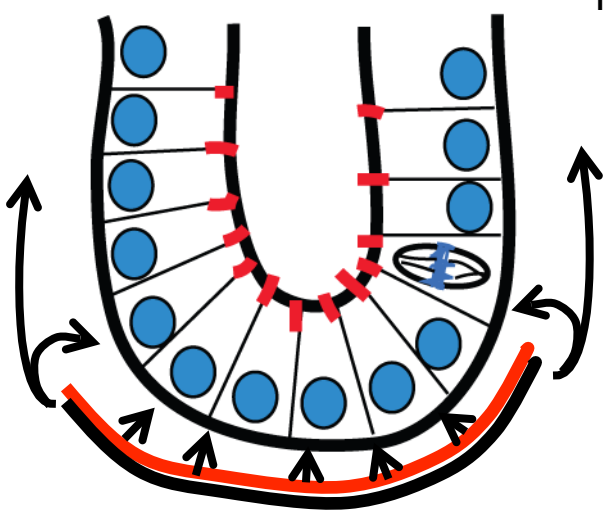
Two orthogonal muscle layers

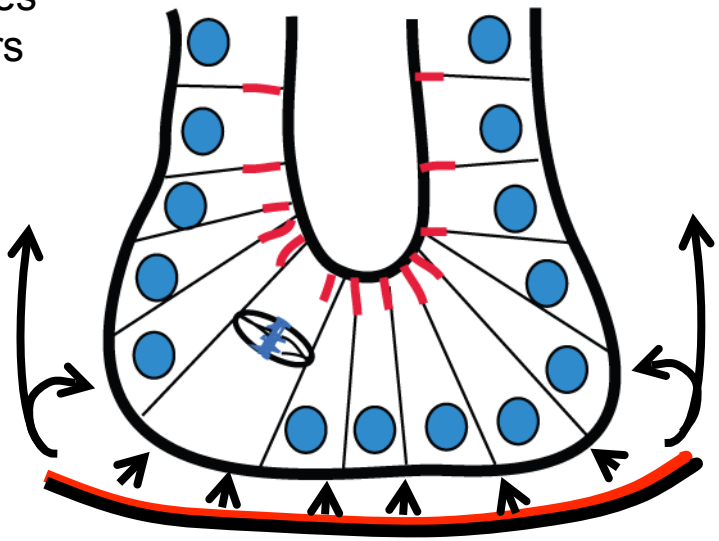

Contractile forces acting on epithelium
Connective tissue

Wild type

Min

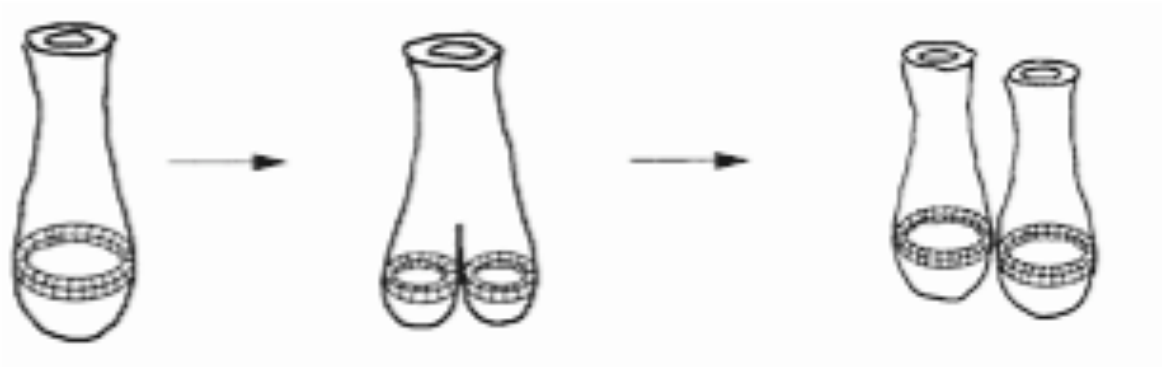
Mechanical forces
Chemical factors

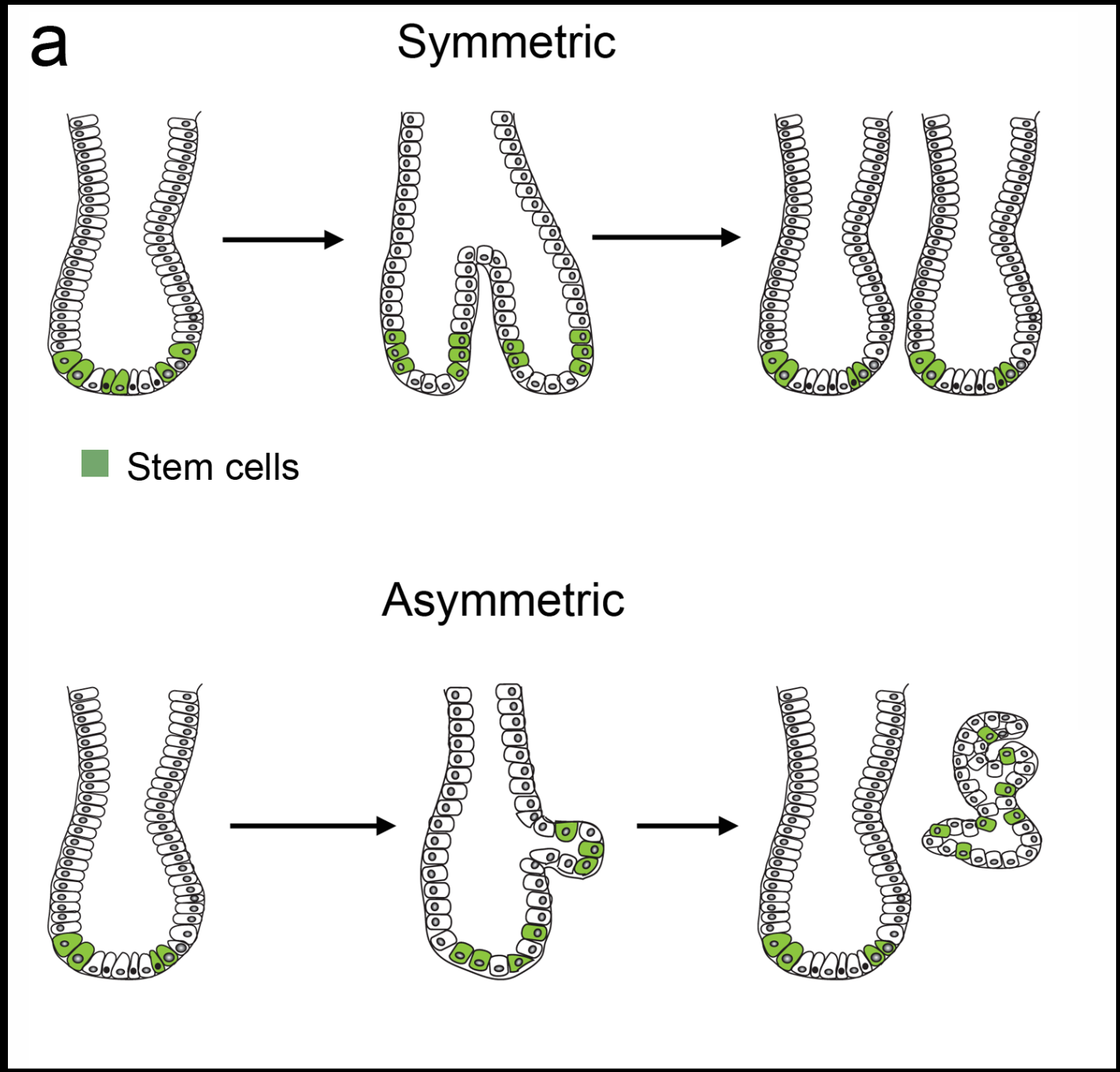




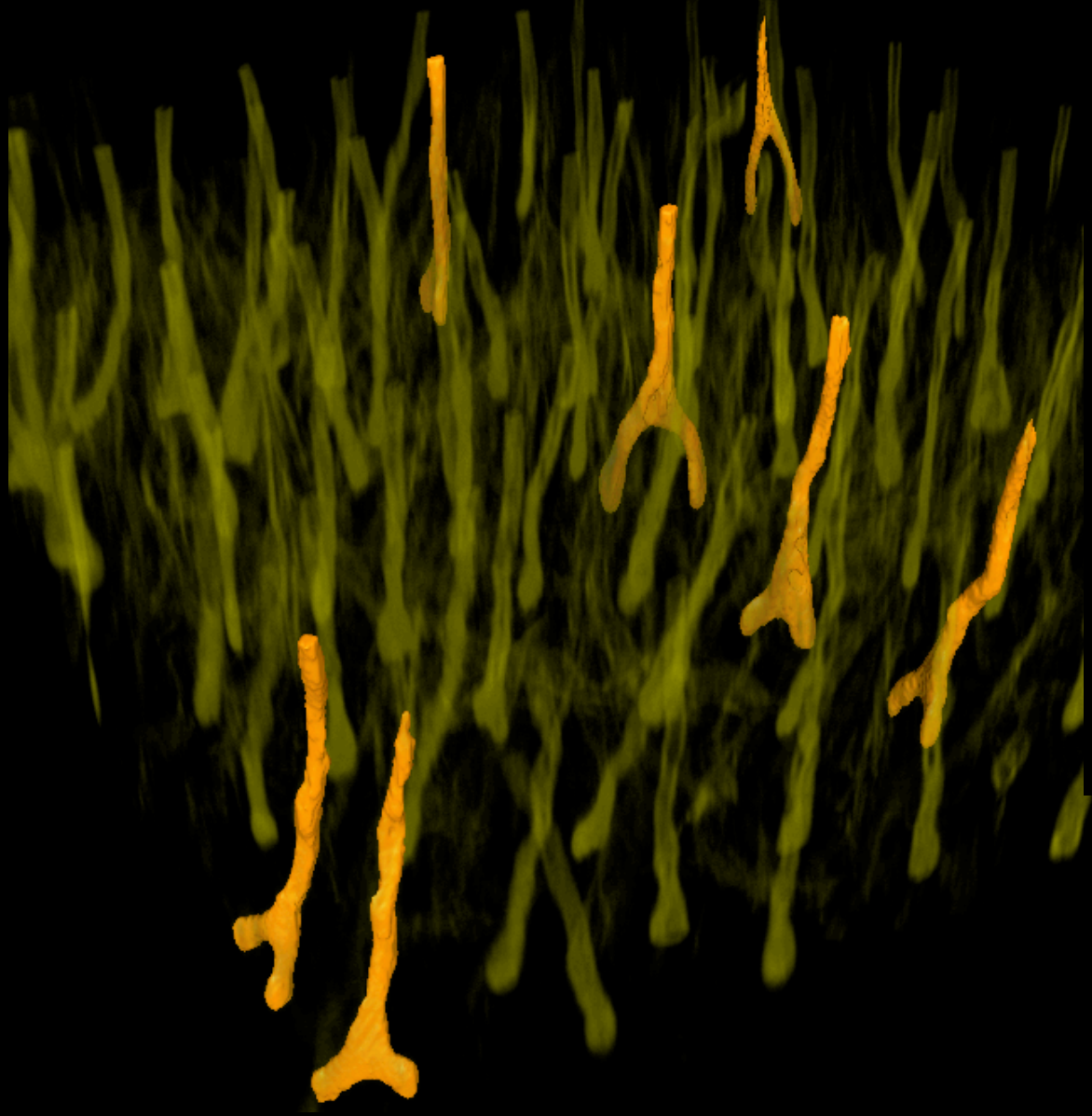
Crypt Fission

- First observed as bisecting base of parental crypt that ascends longitudinally producing two symmetrical new daughter crypts
- Elongation of intestinal tract
- Drives adenoma growth




a
Symmetric
Stem cells
Asymmetric

Crypts undergoing fission highlighted in 3-D specimen

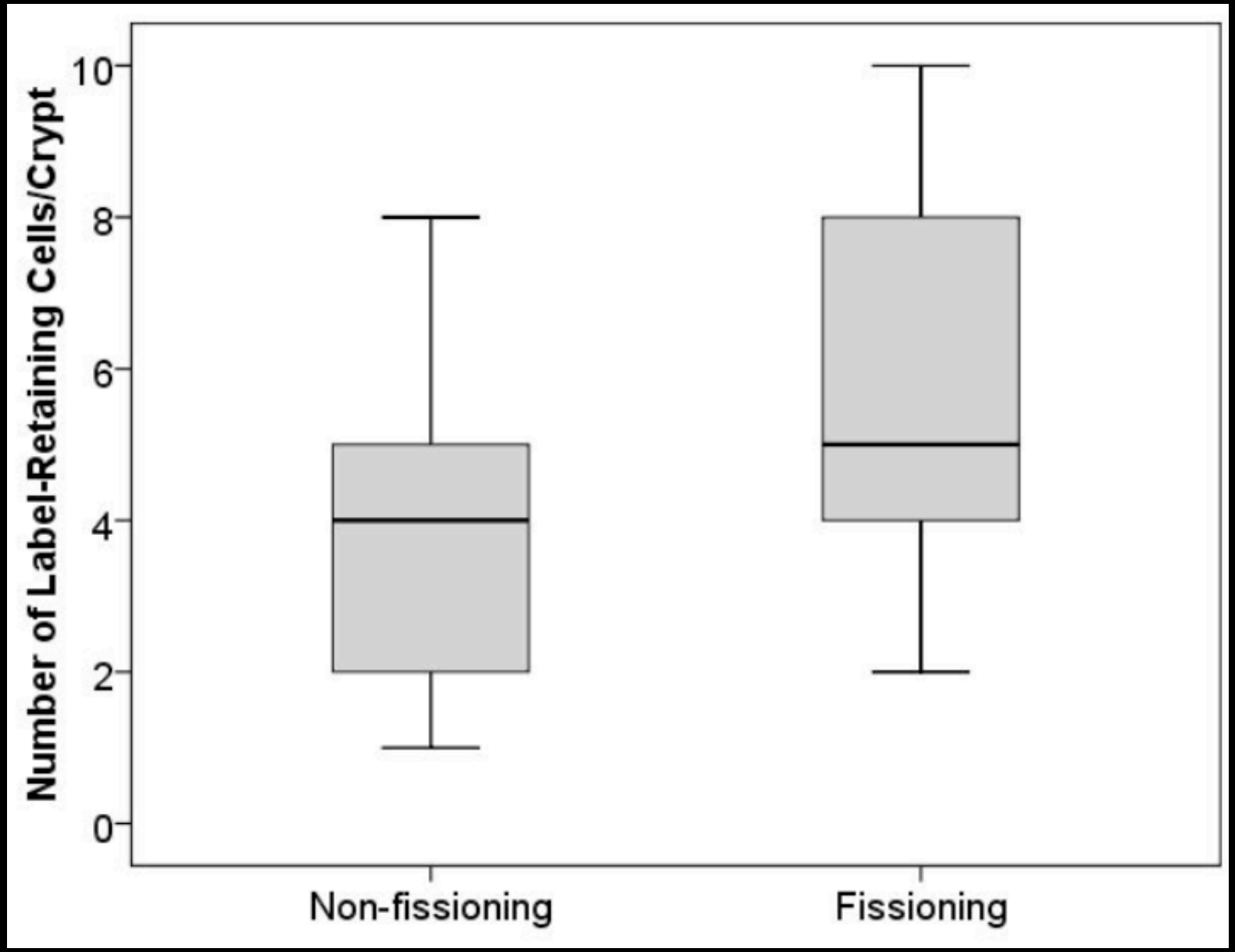


Budding

Symmetric

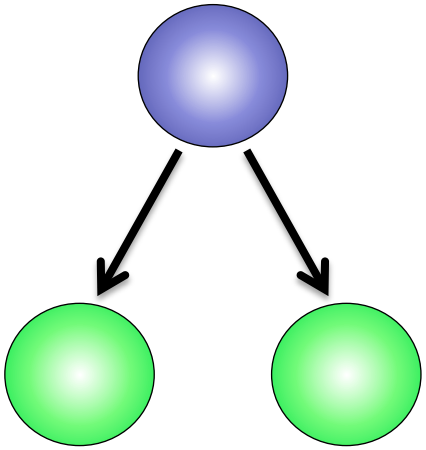
Asymmetric

Aaron Quyn/Paul Appleton


Number of Label-Retaining Cells/Crypt
10
8
6
4
2
0
Non-fissioning
Fissioning

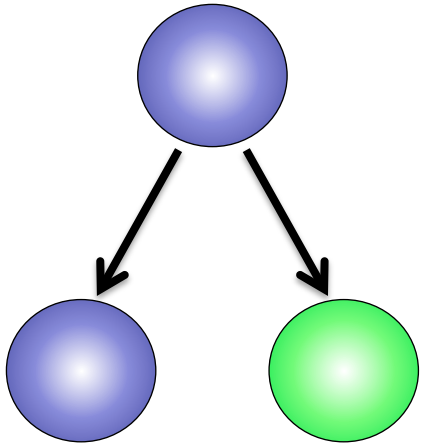
Controlling stem cell number

Symmetric



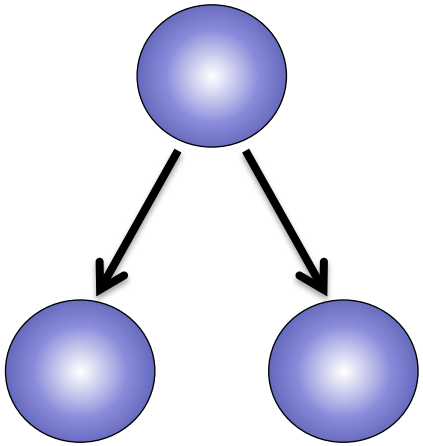
Depletion

Asymmetric



Maintenance

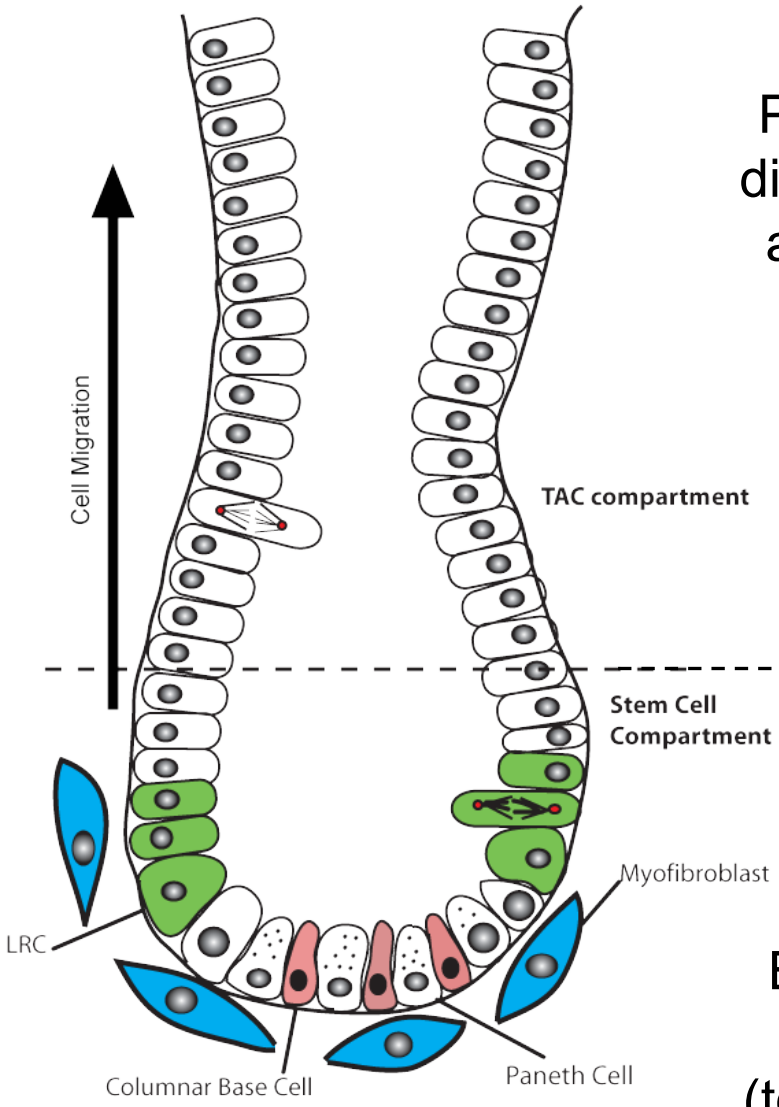
Symmetric



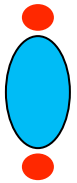
Expansion

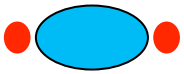
↓

Crypt fission
Adenoma growth



Preferred orientation of division differs between transit amplifying and stem cell compartments in mouse and man





Bias is lost in pre-cancerous, APC heterozygous tissue
(together with changes in cell shape)

Aaron Quyn/Paul Appleton, Cell Stem Cell 2010

What initiates and drives crypt fission?

How are stem cell numbers and crypt fission co-ordinated?

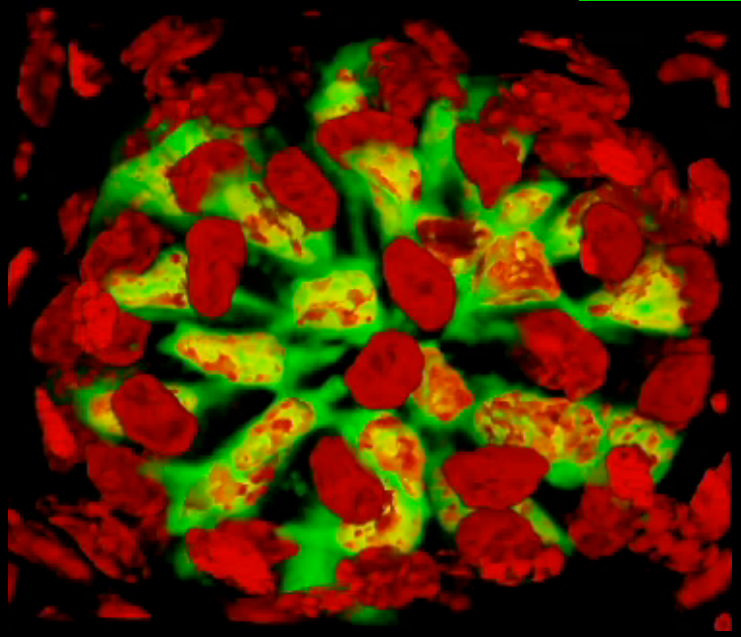
One possibility is that stem cell number increases stochastically and once beyond a certain threshold, fission is initiated. What is this threshold?

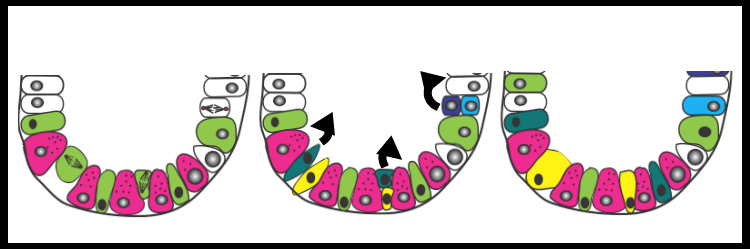
How often does a new crypt form?

Why is this different in different regions of the gut?

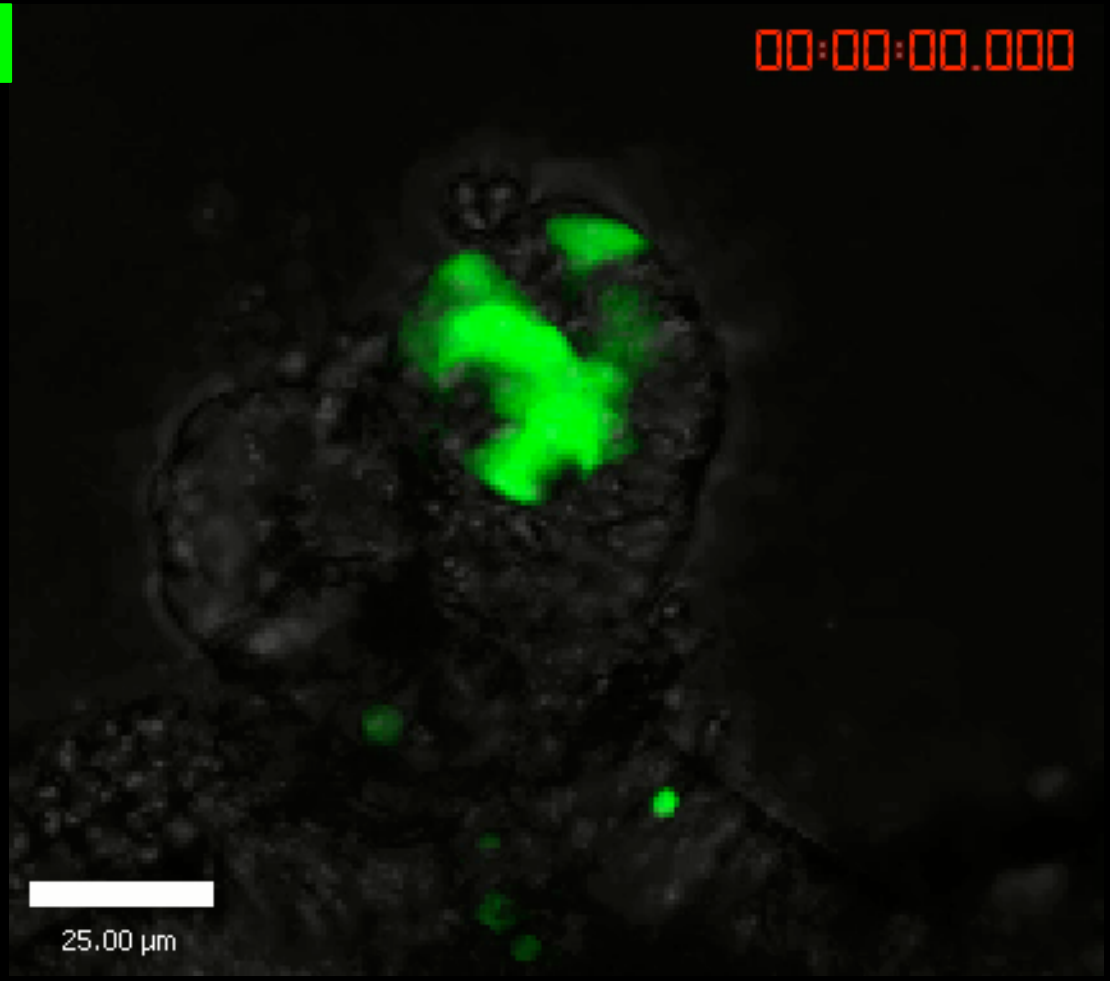
What is deregulated in adenoma and tumours?

How is the alternate stem cell – non-stem cell arrangement achieved?



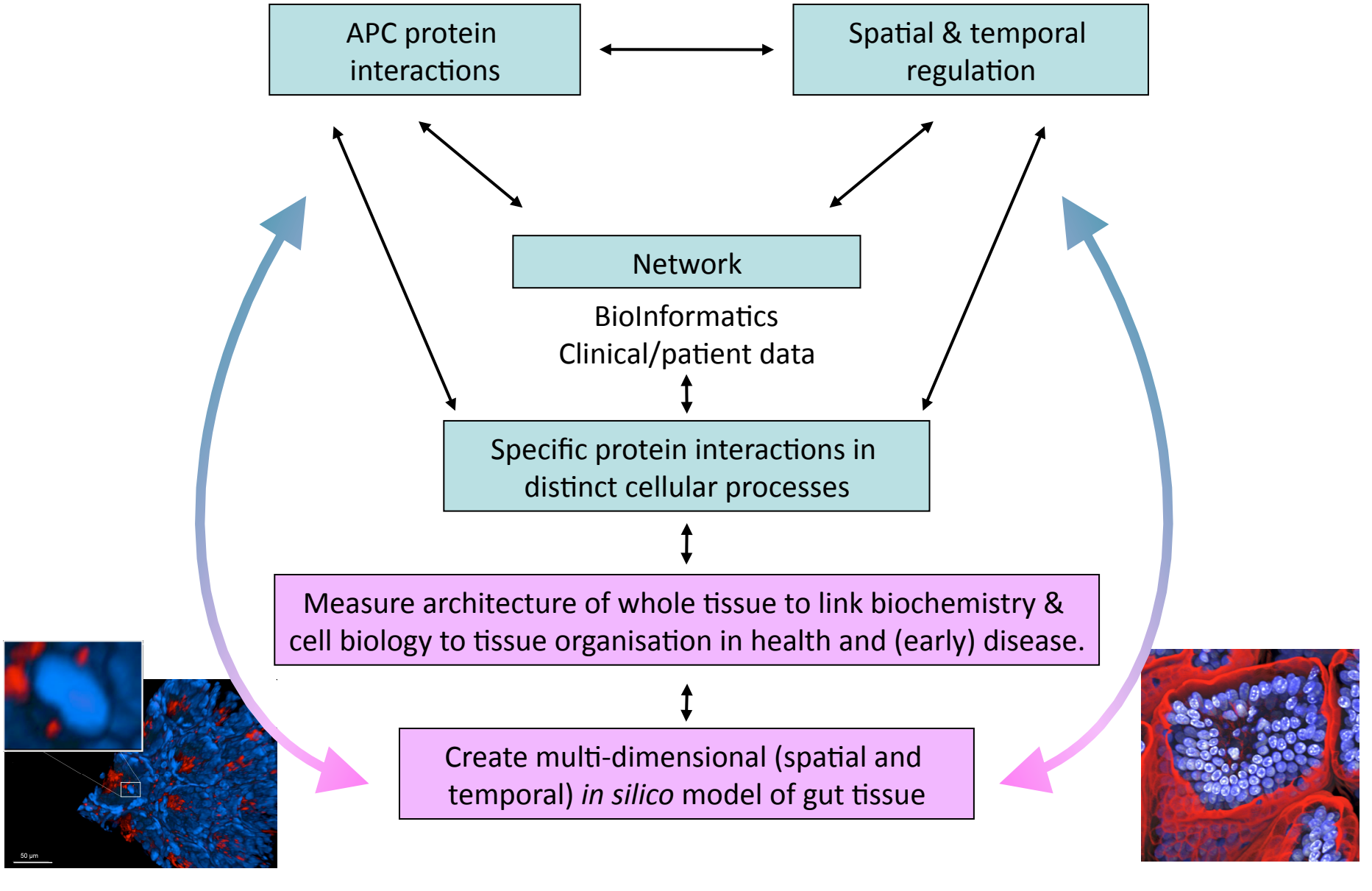


Stem cell divides in crypt organoid in culture; one daughter cell dies the other daughter divides again.



Cytoskeletal proteins in gut epithelium

F-actin microtubules nuclei


APC protein interactions
Spatial & temporal regulation
Network
BioInformatics
Clinical/patient data
Specific protein interactions in distinct cellular processes
Measure architecture of whole tissue to link biochemistry & cell biology to tissue organisation in health and (early) disease.
Create multi-dimensional (spatial and temporal) *in silico* model of gut tissue
50 µm