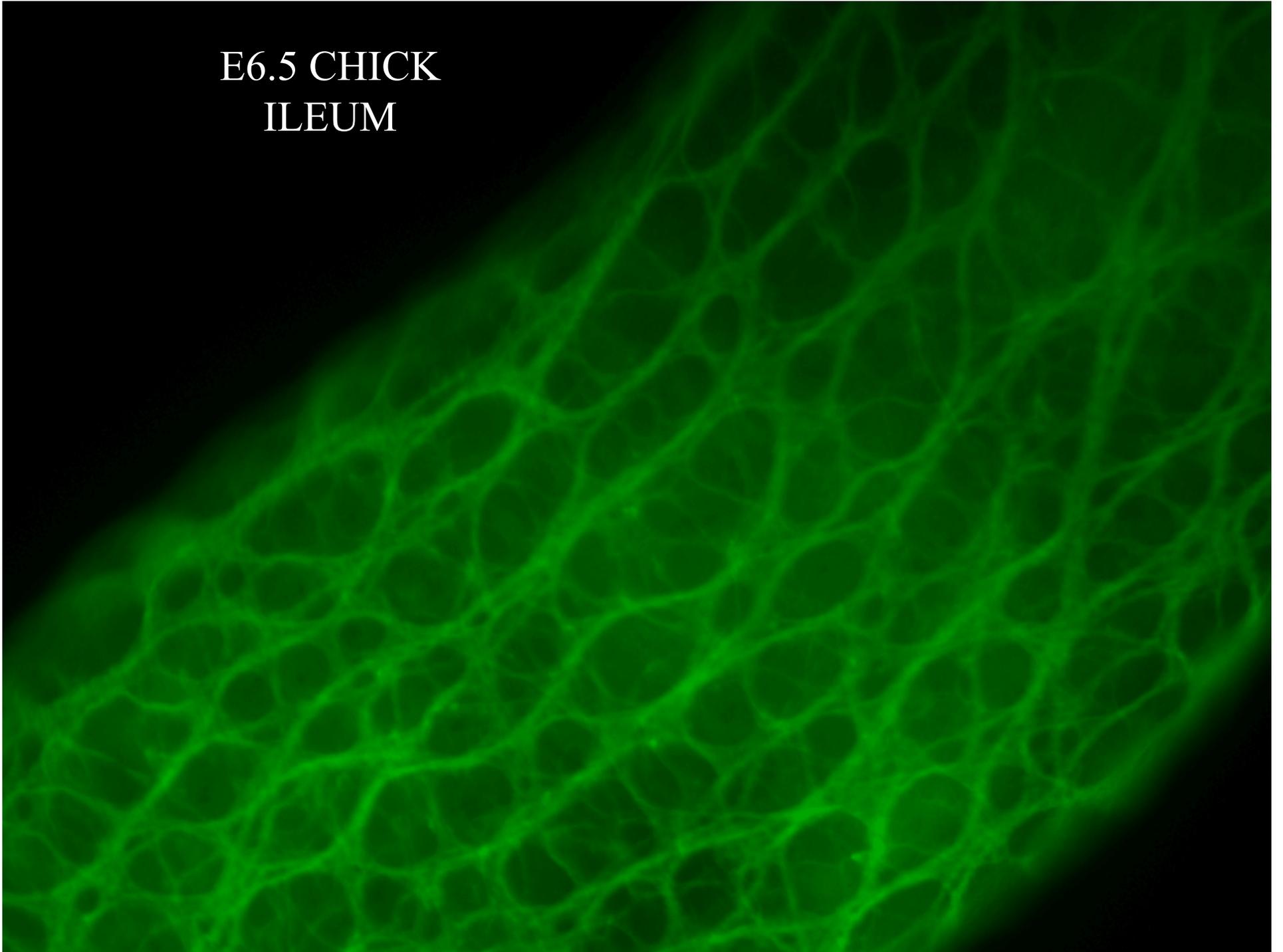


Formation of the ENS involves  
migration  
proliferation  
differentiation

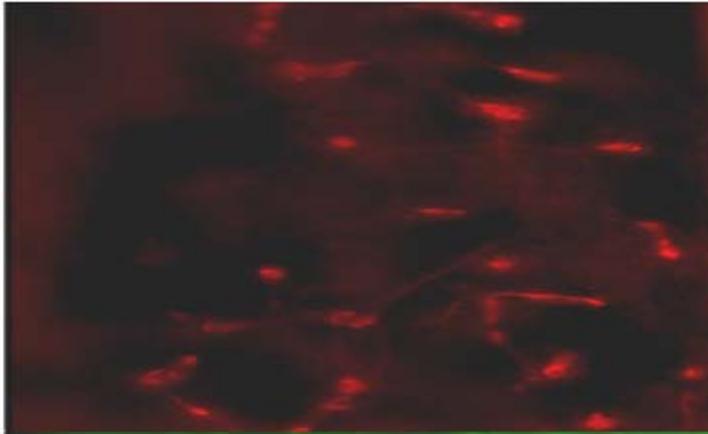
E6.5 CHICK  
ILEUM



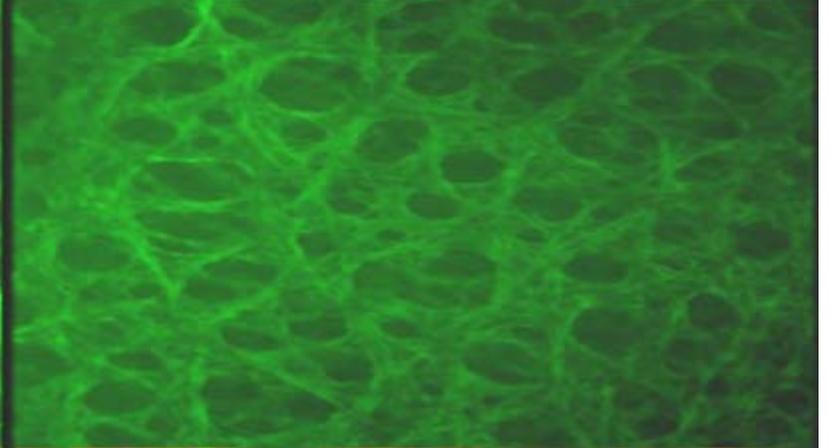
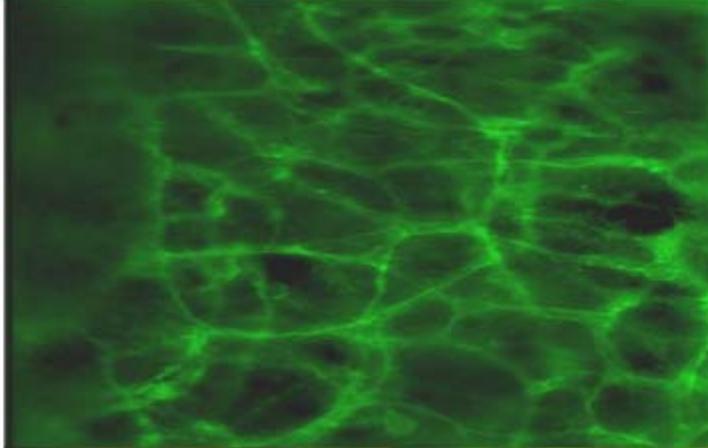
**E4.5C**

**E7.5C**

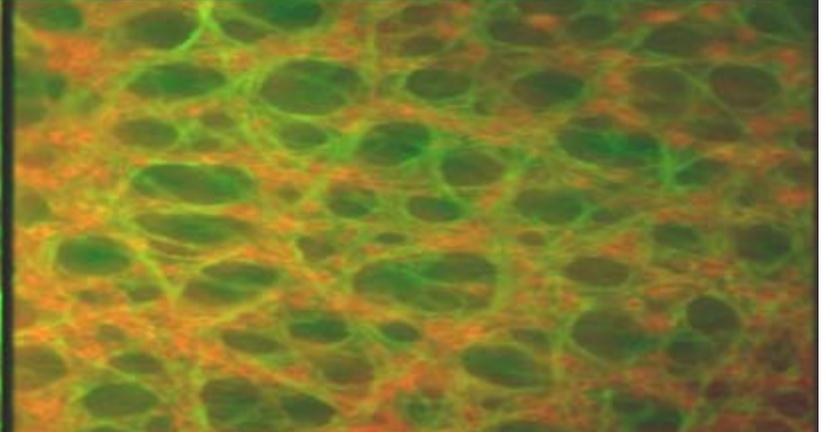
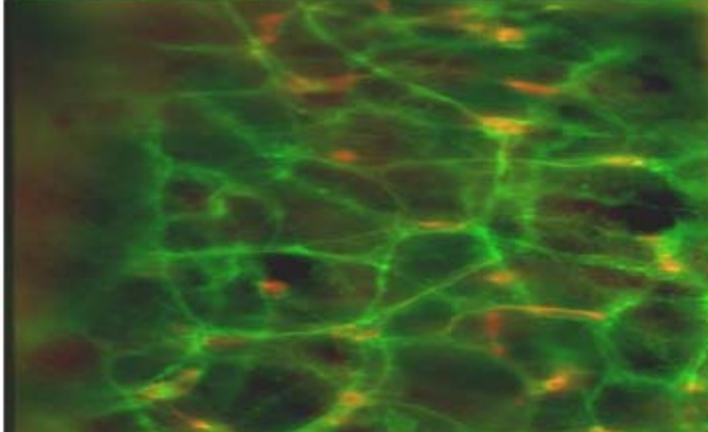
**Hu**



**Hnk**



**Hu /  
Hnk**



## QUESTIONS :

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- CELLS FOR INSERTION ARE PRODUCED JUST BEHIND THE WAVEFRONT.

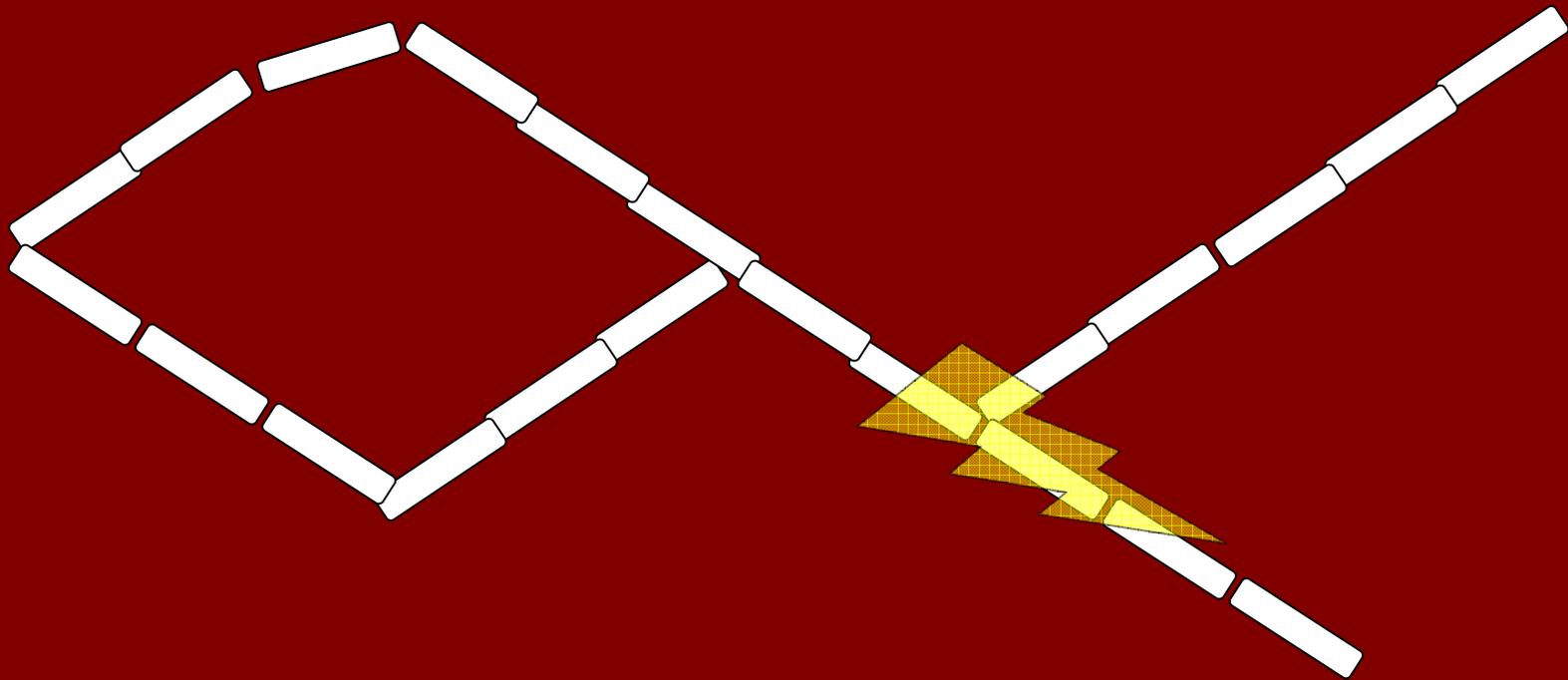
EXTENTION

MOVEMENT

PROLIFERATION

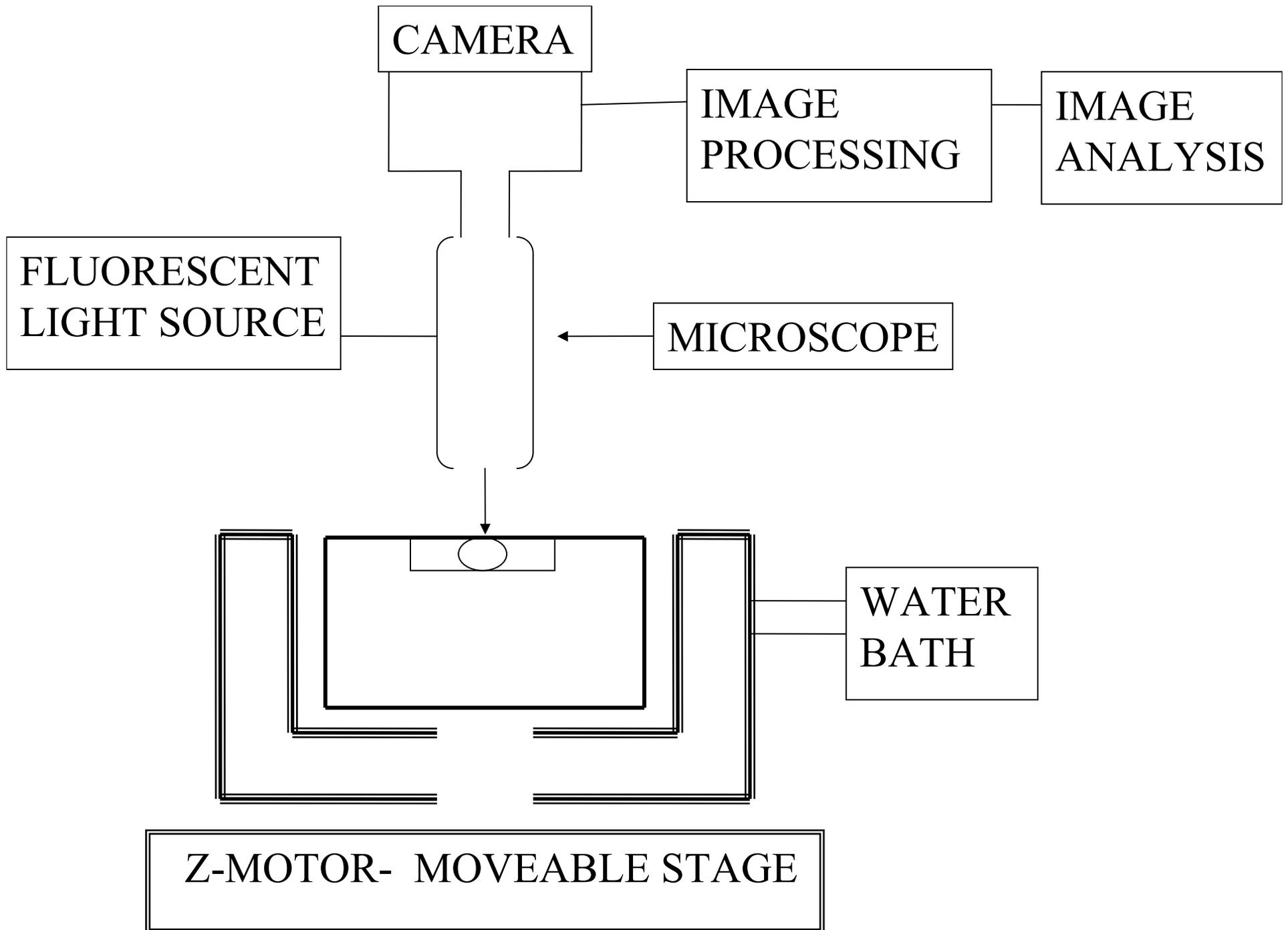


# FORMATION OF NEW STRANDS BY DIVERGENCE



## GENERATION OF FLUORESCENT CREST CELLS

- *WNT-1 CRE MICE ARE MATED WITH ROSA 26 FLOXED -GFP MICE OR ROSA 26R FLOXED YFP MICE*
- *THE ENTIRE GUT FROM E10.5-12.5 GFP+ EMBRYOS WAS MOUNTED AS A CATANARY EXPLANT (C. J. HEARN ET AL. ) ON A GLASS COVERSLIP THAT FORMS THE BOTTOM OF PLASTIC CULTURE DISH .*
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## PATTERN OF CELL MOVEMENT IN MOUSE ILEUM

- STRANDS OF CELLS (FREIGHT CARS IN A TRAIN)
- DISTAL STRANDS ADVANCE INTO UNCOLONIZED GUT
- CELLS ADVANCE ON THE STRANDS (RIDER CELLS)
- A FEW CELLS ARE AHEAD OF STRANDS (PIONEERS).
- STRANDS INTERSECT , THEN DIVERGE AND INTERSECT AGAIN

E10.5 Mouse Ileum

Ileum  
Ileum

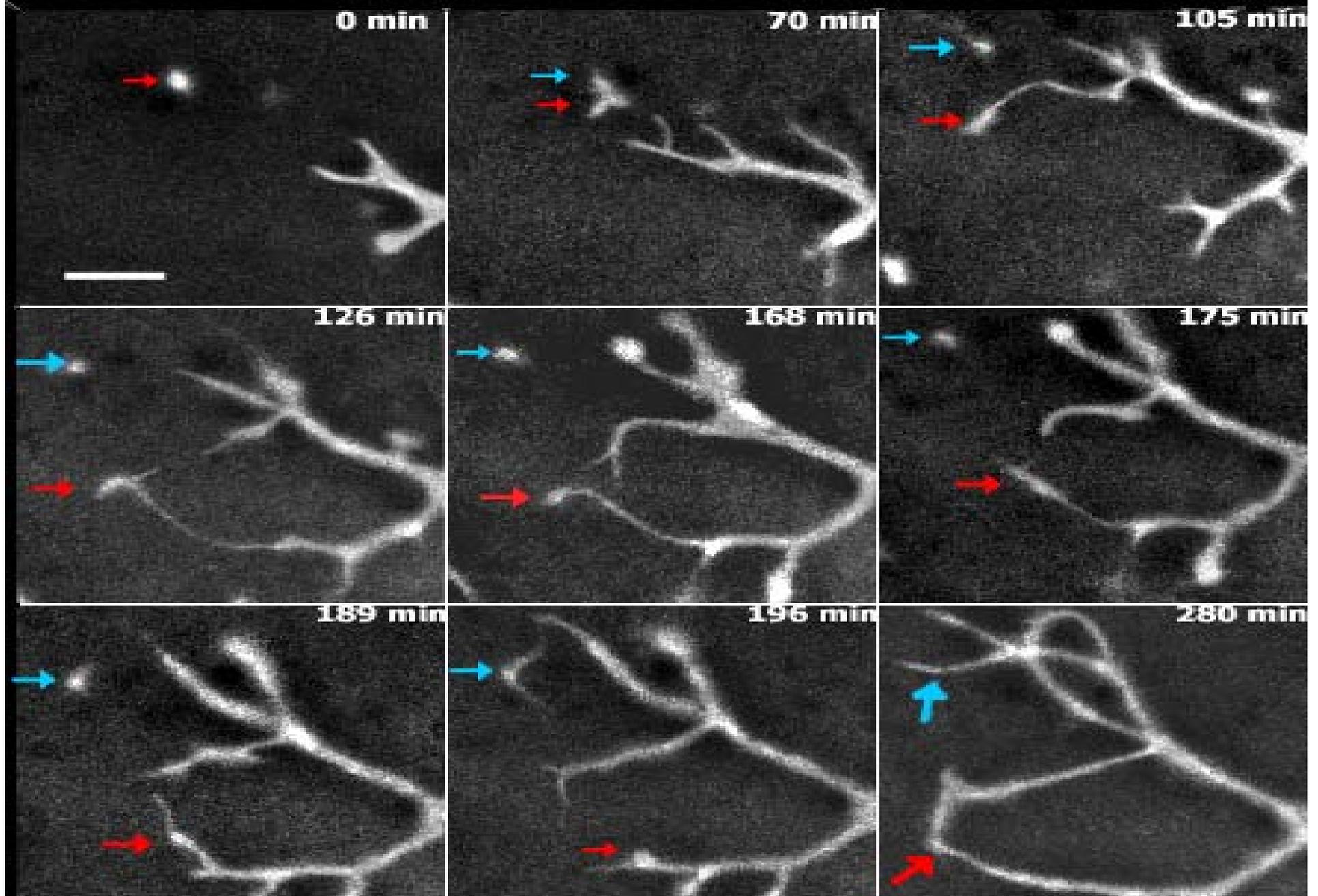
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format.

pioneer cells

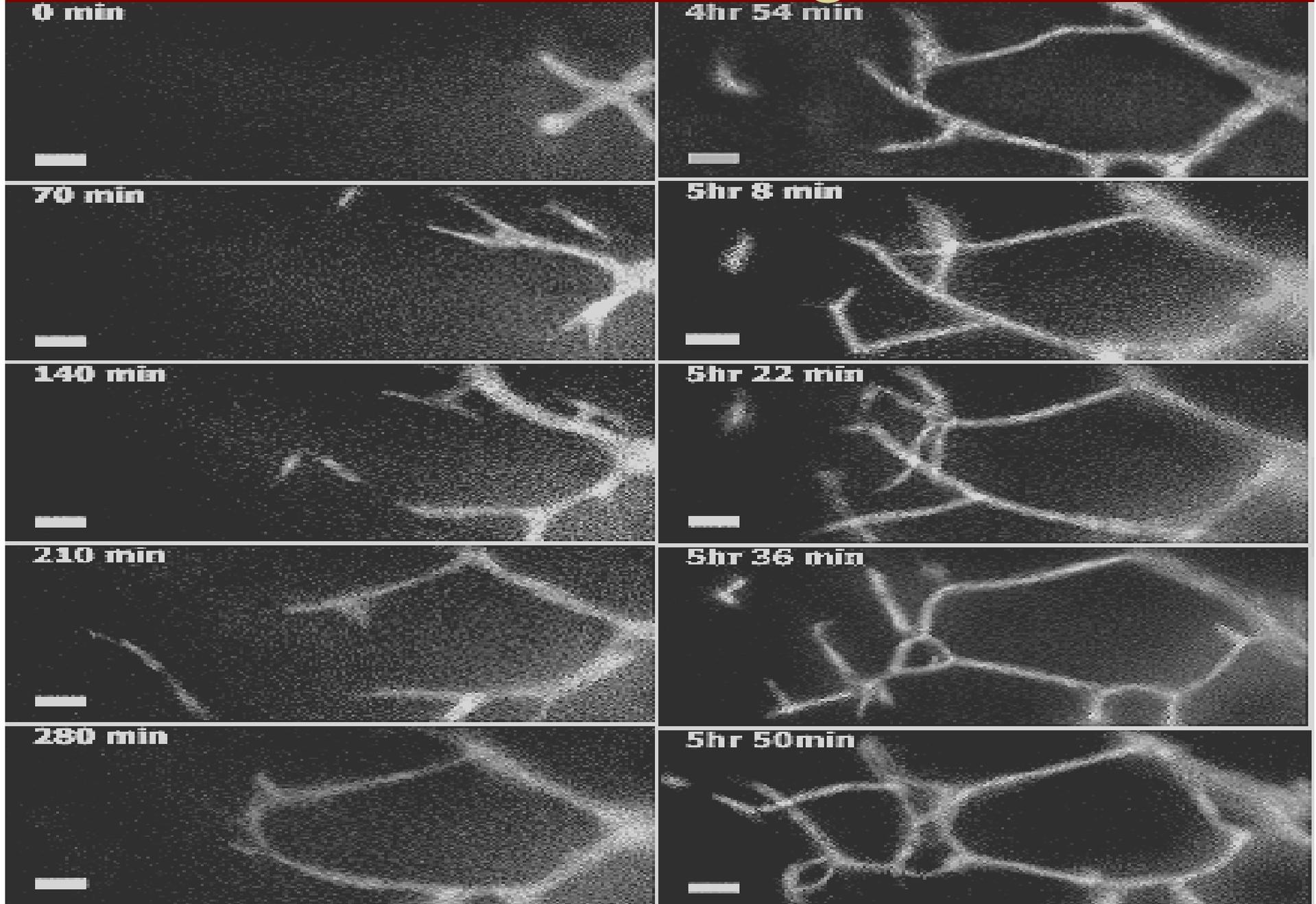
distal cells  
of distal  
strands

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format.

# Pioneers



# Strand Convergence

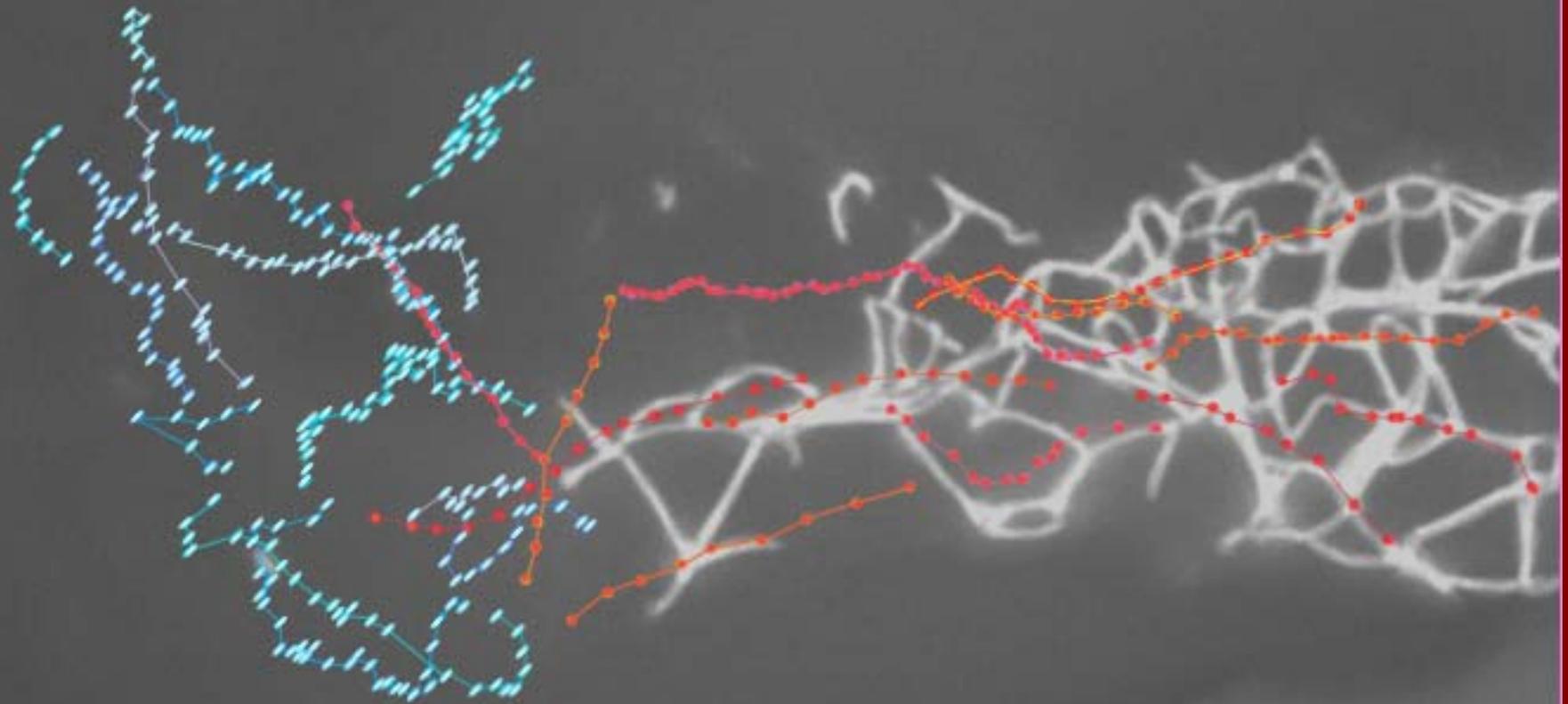


## Average Velocity

**Distal Strands:** 1.54 microns/min  $\pm$  .41

**Riders:** 2.10 microns/min  $\pm$  .30

(N=4 films)



## Cell Movement

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## What We Learned From the Mouse Ileum

- Single Cells (“Pioneers”) are found ahead of and interact with the Distal Strands.
- The movement of the Pioneers and Distal Strands is not continuous.
- Distal strands extend by both convergence and divergence.
  - Cells move along the strands.

# Electroporation of E1.5 Chick Embryos With GFP Plasmid

Only Neural Crest Cells Are GFP+ in the Gut

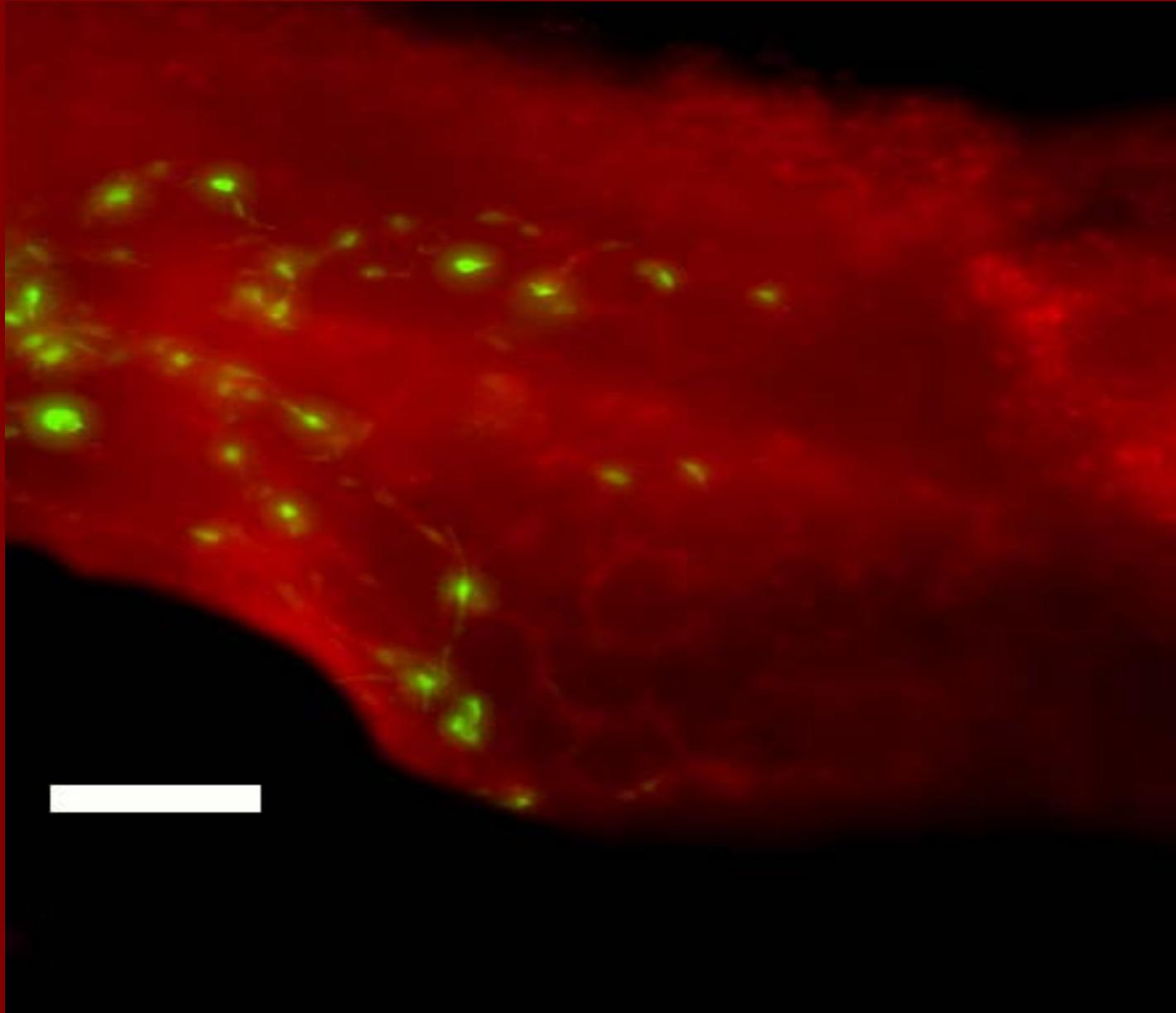
## Advantages:

- Visualize the movement of single cells

## Disadvantages:

- Restricted duration of expression
- Location of expression with respect to the wavefront is unknown

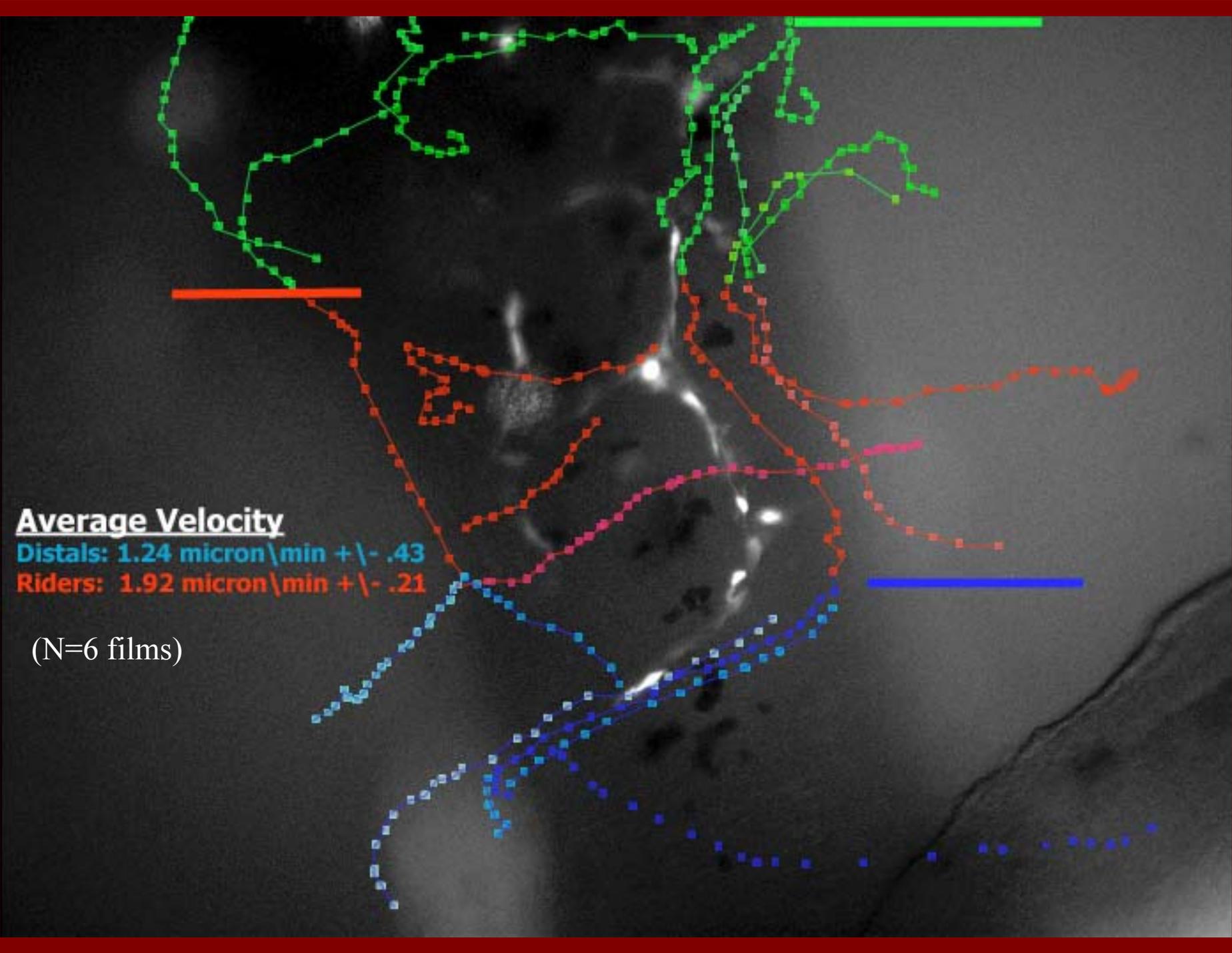
Pre-umbilical Chick GFP/HNK



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format.

# E4 Chick Ileum

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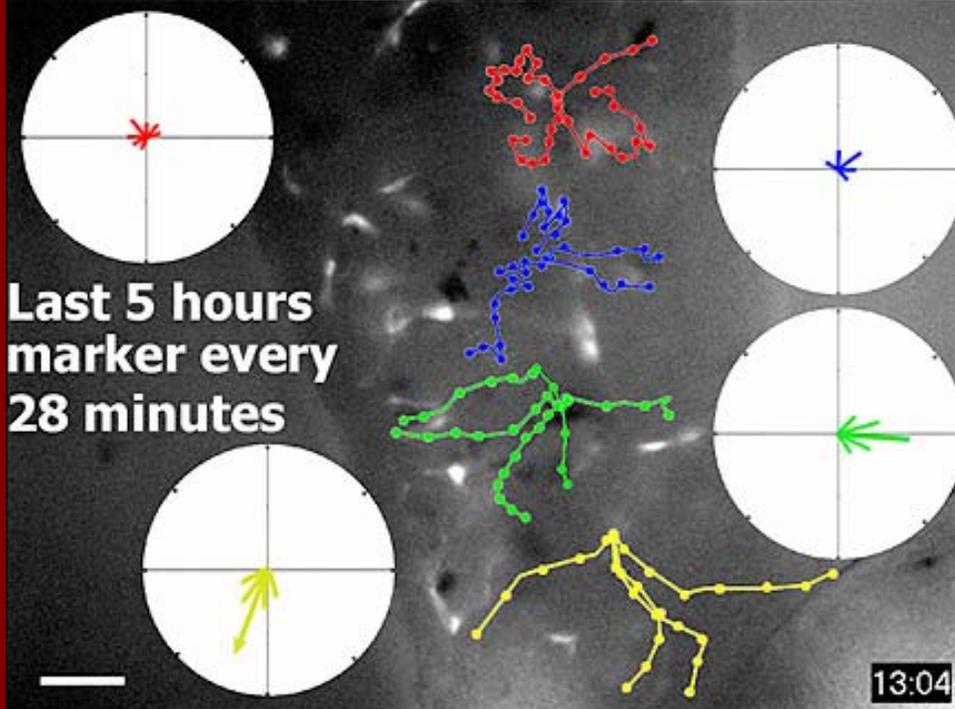
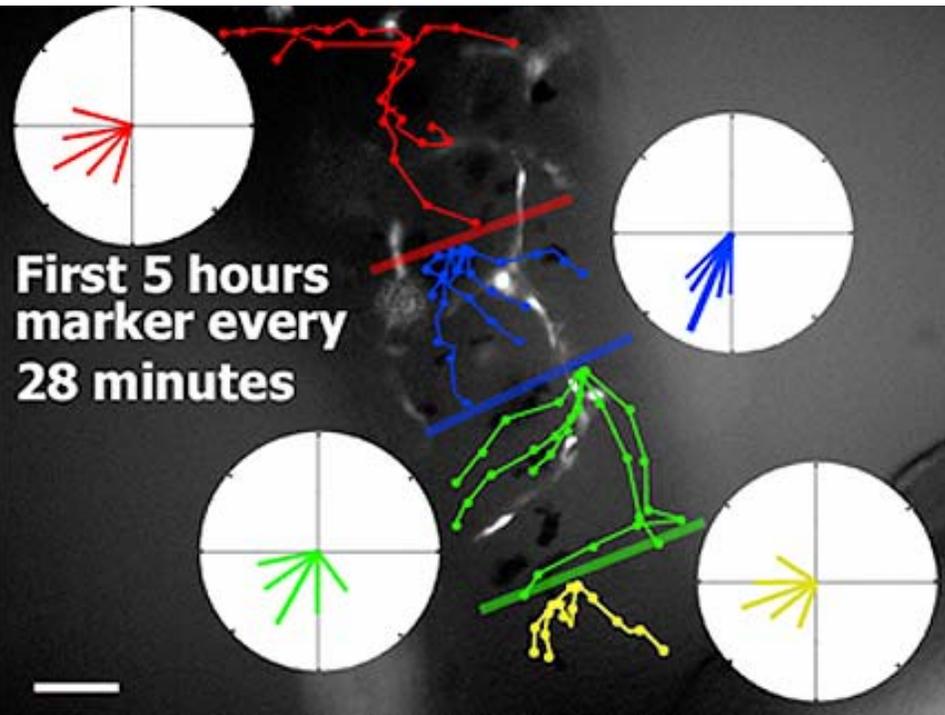
A grayscale microscopy image of a cell, possibly a yeast cell, showing its internal structure. Overlaid on the image are several colored tracks (green, orange, pink, blue) representing the movement of particles. The tracks are composed of small squares connected by lines. There are four horizontal scale bars: a green one at the top right, an orange one on the left, a pink one on the right, and a blue one at the bottom right. The tracks show various paths, some more linear and others more convoluted.

**Average Velocity**

Distals: 1.24 micron/min  $\pm$  .43

Riders: 1.92 micron/min  $\pm$  .21

(N=6 films)



## What we learned from the chick:

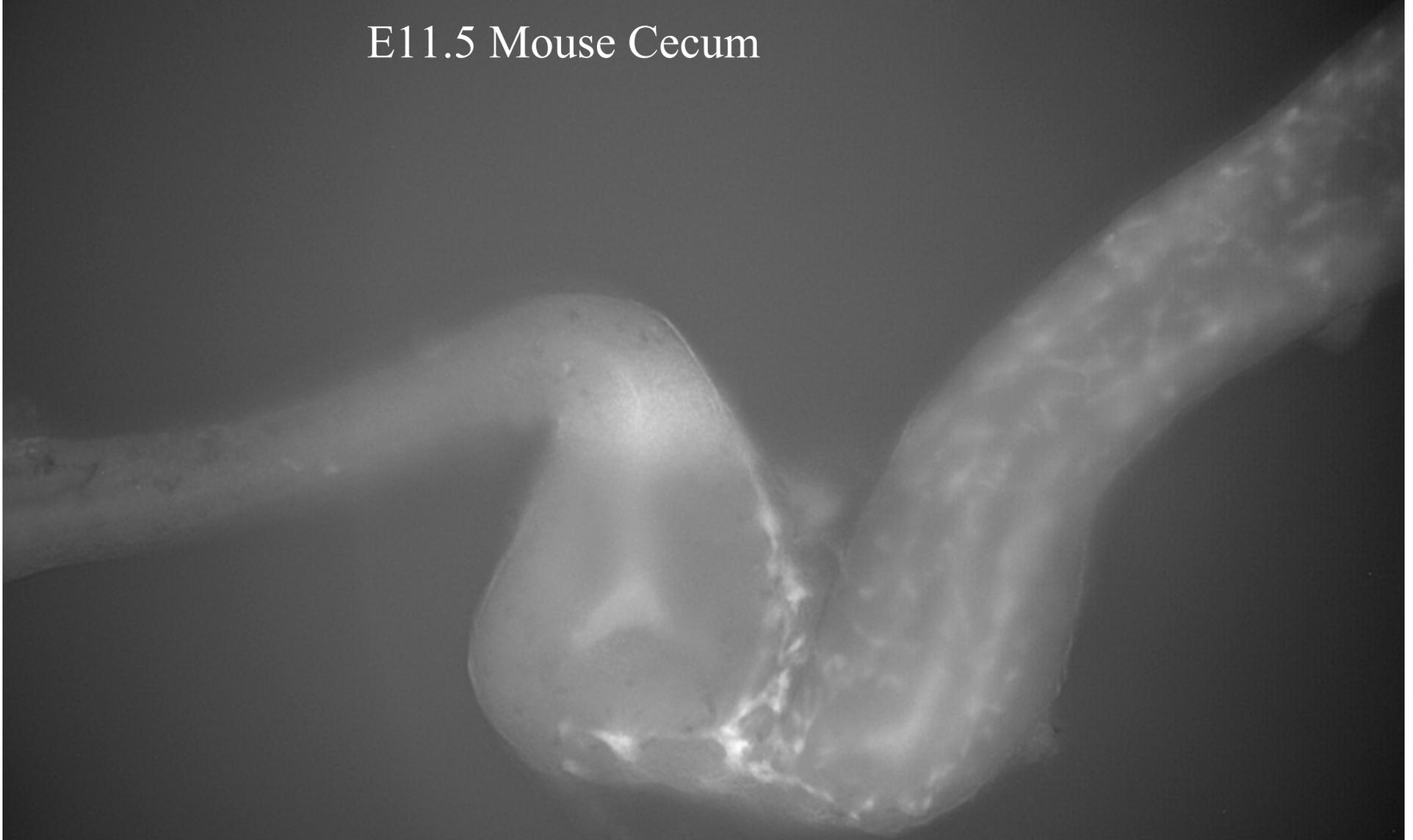
- Individual cells extend small processes.
- The velocity of cells varies with time and location.
- Individual cells appear to follow the same path.

Mouse Cecum has a unique pattern of colonization.

-Restricted pathway followed by massive ingress of individual cells.

-Occasional trans-mesenteric movement.

# E11.5 Mouse Cecum



200um



# Cecum 2

two photon

E11.5M

Cecum

The video cannot be displayed in this  
format.

## Conclusions

- Strands made up of individual cells extend into uncolonized territory.
- Isolated cells (pioneers) are found ahead of the most caudal strands.
- Individual cells behind the most caudal cells move along and extend the strands.
- As colonization progresses proliferation occurs just rostral to most caudal strands.

NOAH DRUCKENBROD

PAUL CONNER

PAUL FOCKE

STACY STAFNE

LYNN VANDERWIELEN

MICHAEL REINBERG

SUZY LIN

BILL OLDENHOFF

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format.

## E3.5C Ileum

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## E11.5 M Cecum

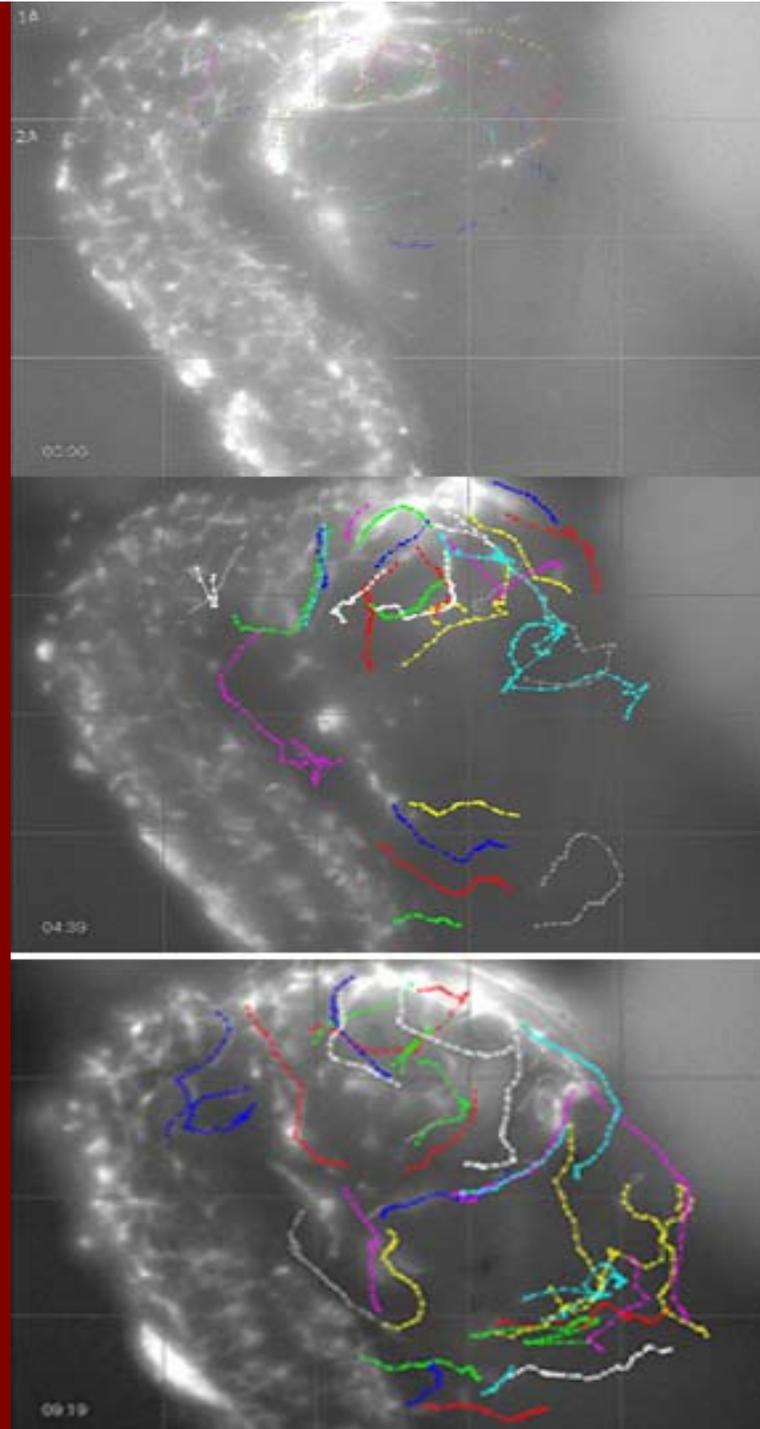
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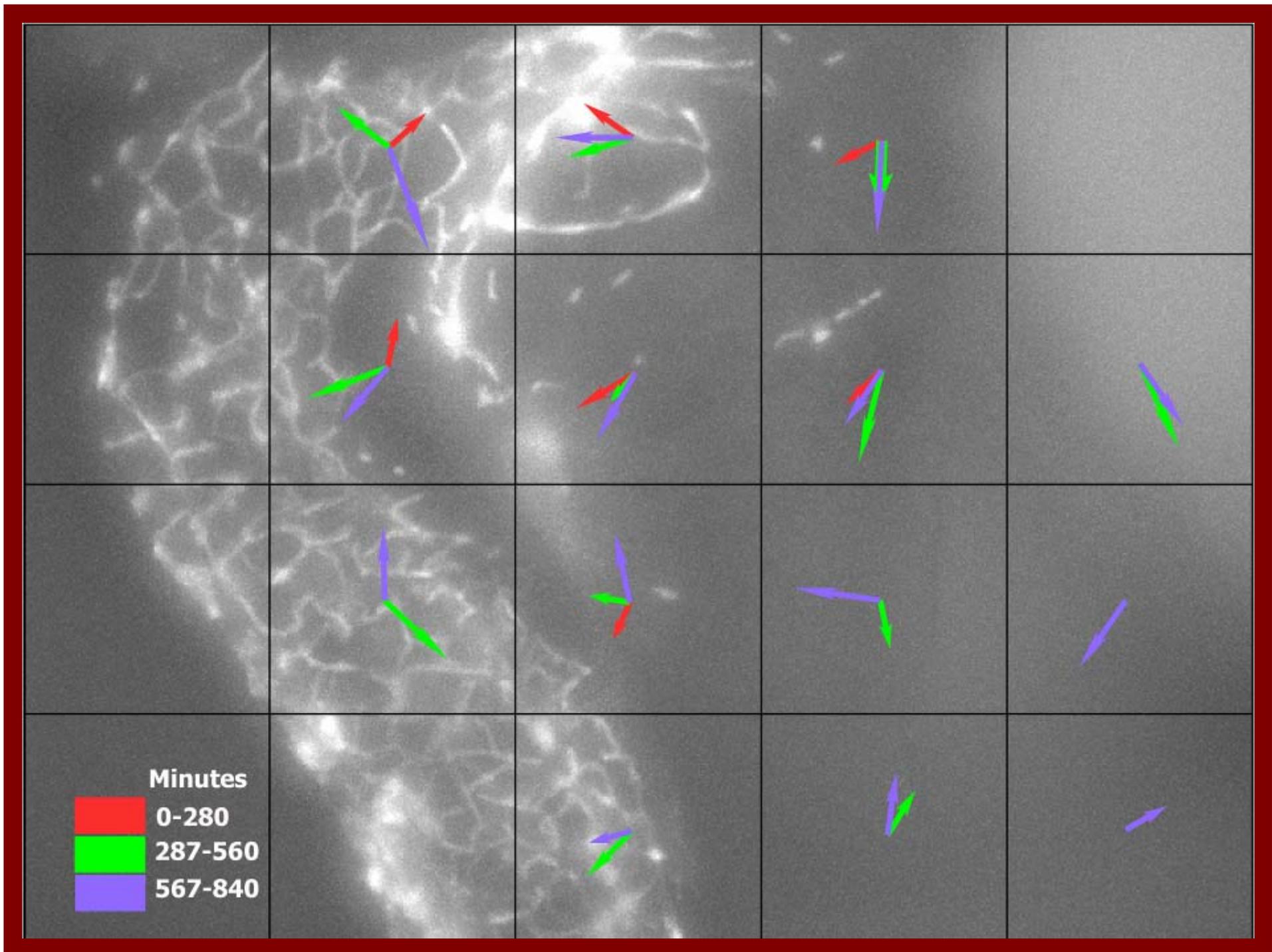
# Cecum 1 Paths

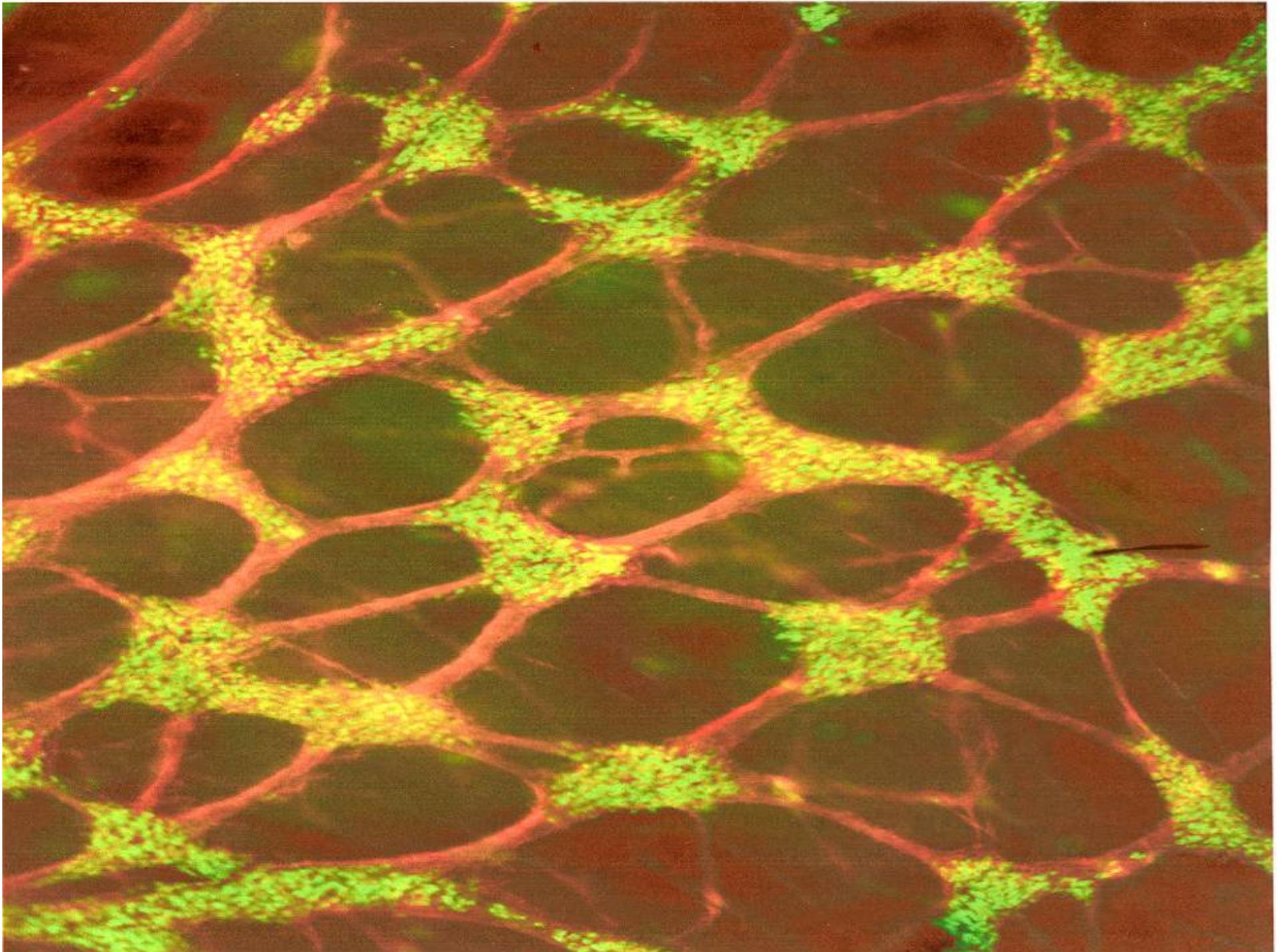
Cecum1

Each panel shows

280 minutes







# Functions of Enteric Nervous System

Motility of Gut

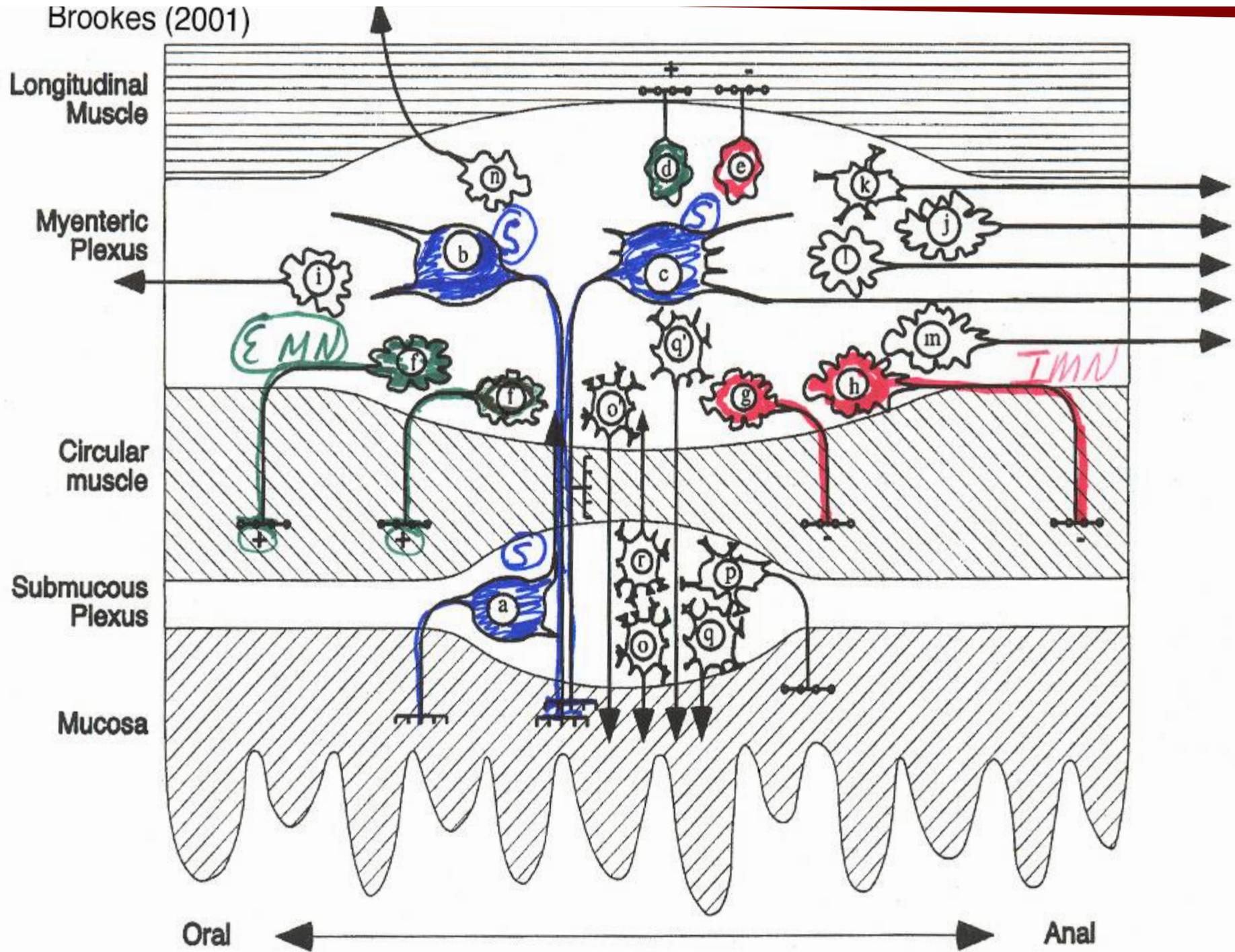
Secretion from gut

Blood Flow to gut

## PROPERTIES OF ENS

- WORKS INDEPENDENTLY OF CNS
- CONTAINS SENSORY-, INTER-,MOTORNEURONS
- NEURONS PRODUCE DIFFERENT NEUROTRANSMITTERS
- GLIA RESEMBLE ASTROCYTES OF CNS

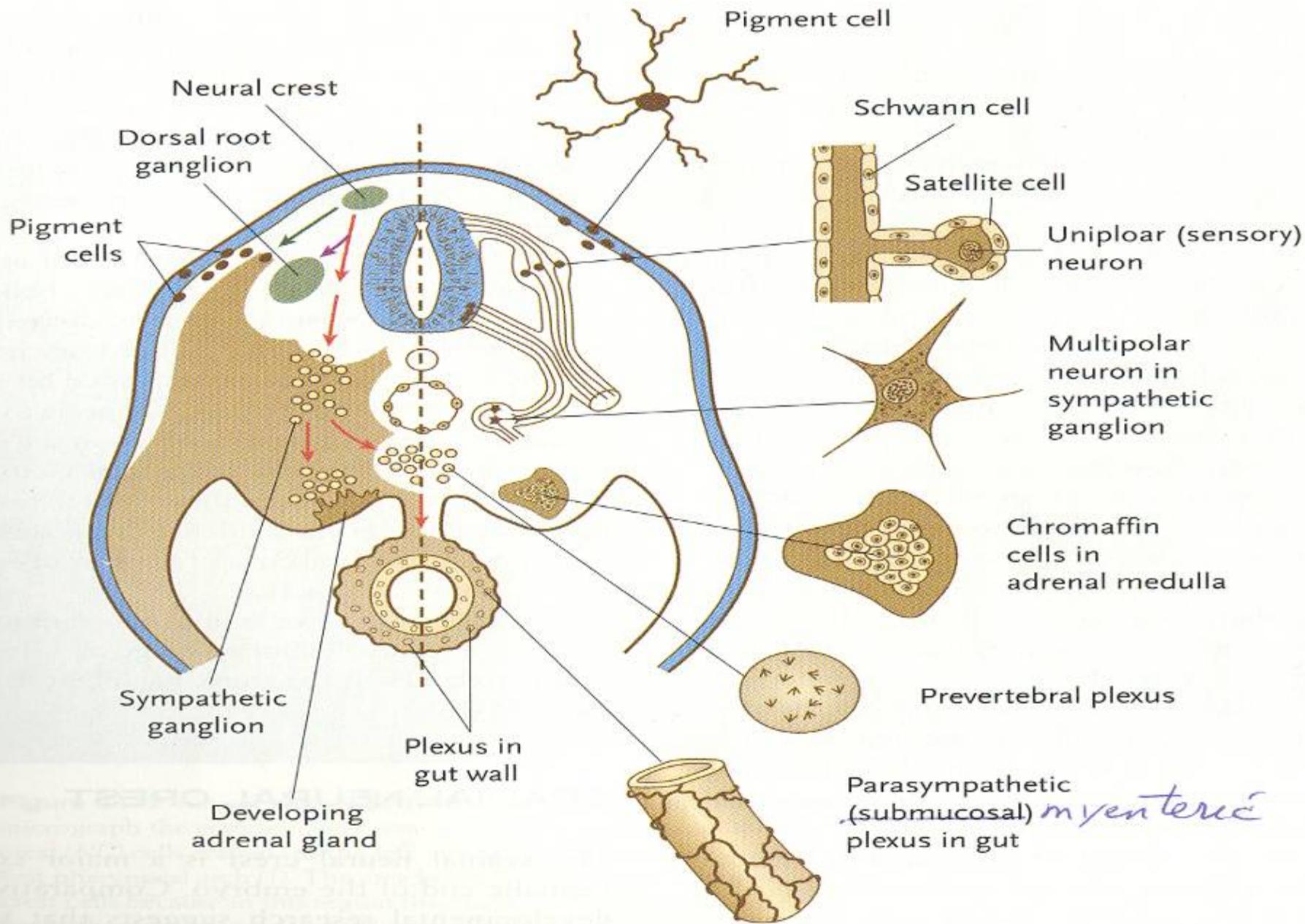
Brookes (2001)



## DEVELOPMENT OF ENS

CREST CELLS ENTER THE PHARYNX, MIGRATE, PROLIFERATE , DIFFERENTIATE AND ADVANCE TO THE END OF THE GUT.

THESE PROCESSES OVERLAP SO THAT IN A REGION OF GUT MIGRATION, PROLIFERATION, AND DIFFERENTIATION APPEAR CONCURRENTLY.



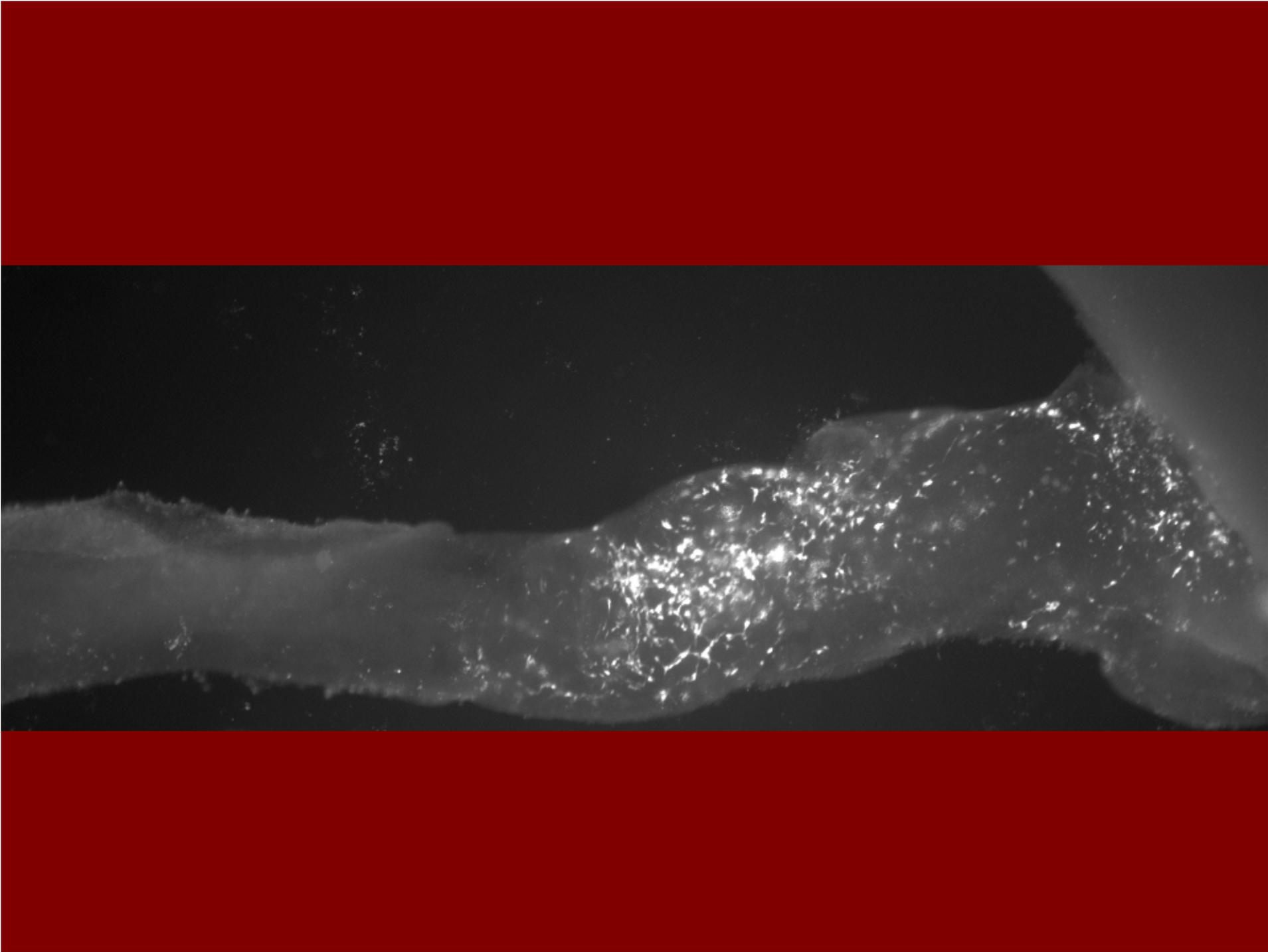
**Figure 11-4** Major neural crest migratory pathways and derivatives in the trunk. *Left*, Pathways in the early embryo. The dorsolateral pathway is indicated by the green arrow; the ventral (sympathoadrenal) pathway is indicated by the red arrow; and the ventrolateral pathway is indicated by the purple arrow. *Right*, Derivatives of the trunk neural crest.

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Average Angle ( $A'$ ) of Individual Cells ( $a$ ):

$$A' = \tan^{-1} Y/X \quad Y = \sum \sin a/N \quad X = \sum \cos a/N$$

Dispersion of Cells ( $S$ ):

$$S = \sqrt{-2 \ln r} \quad r = \sqrt{x^2 + y^2}$$

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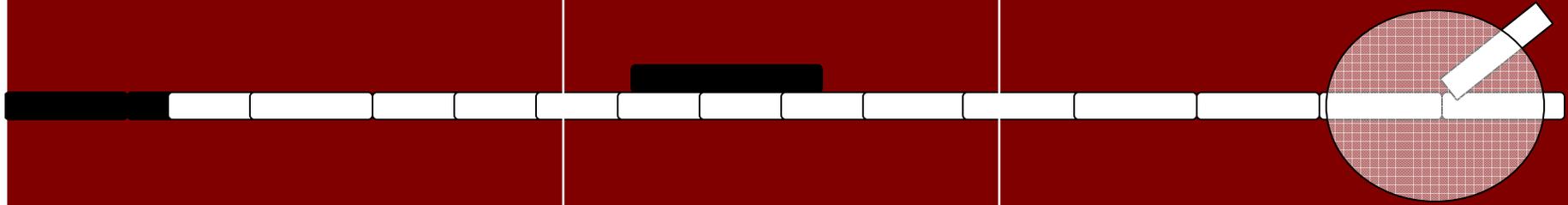
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MOVEMENT

PROLIFERATION



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