Using time-lapse video technology to understand satellite cell activities, etc

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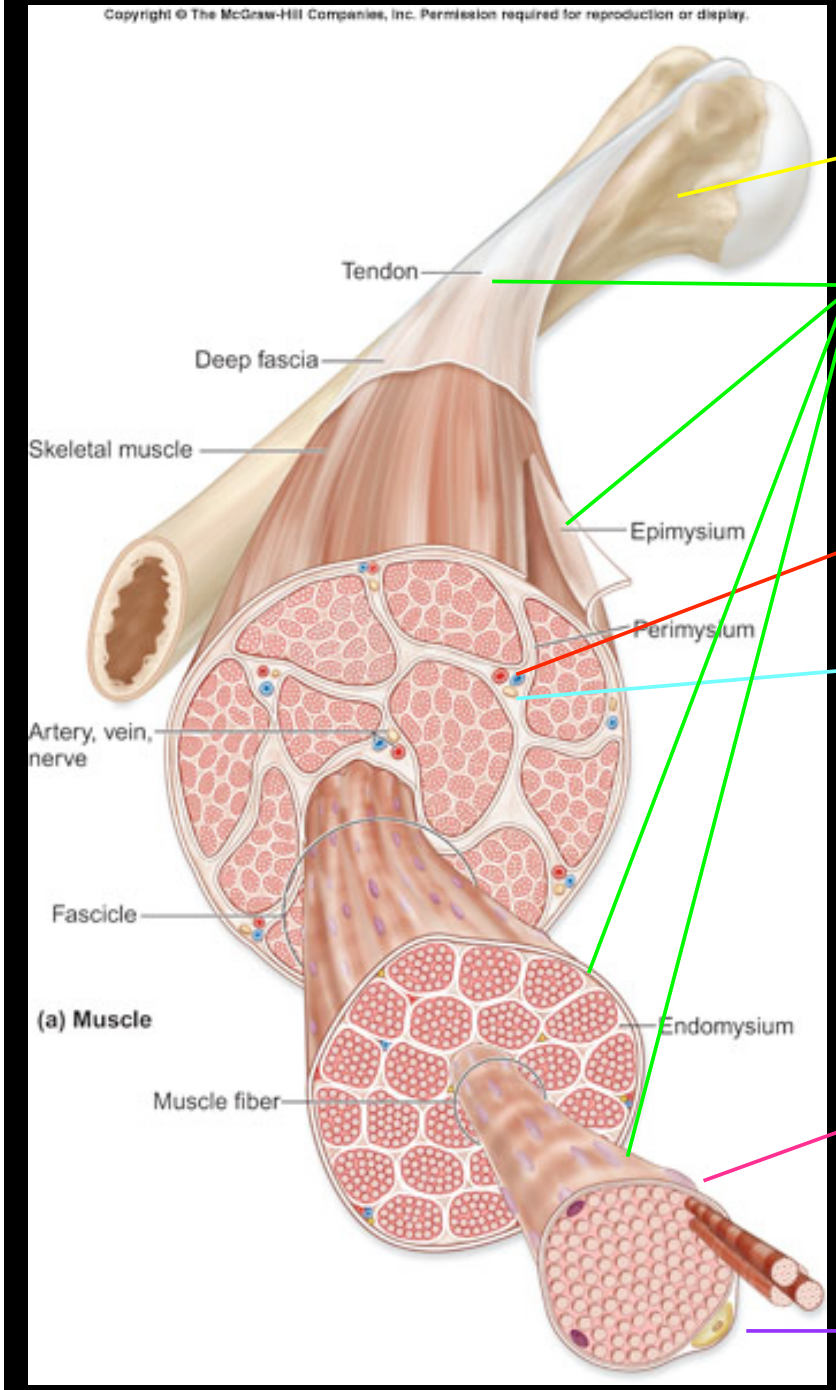
02/28/11
Mentor: D Cornelison
Graduate Student: Ashley Siegel



bone

connective tissue/ECM

vasculature

neurons

muscle fiber

satellite cell

Stem and progenitor cells in skeletal muscle development, maintenance, and therapy.
Péault B, Rudnicki M, Torrente Y, Cossu G, Tremblay JP, Partridge T, Gussoni E, Kunkel LM, Huard J.
Mol Ther. 2007 May;15(5):867-77. Epub 2007 Mar 27. Review.

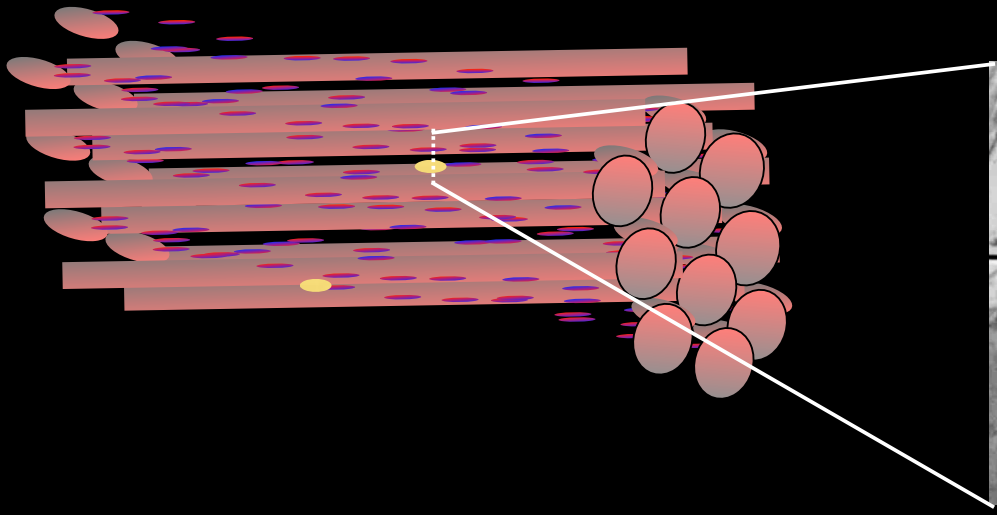
Netrins and neogenin promote myotube formation.
Kang JS, Yi MJ, Zhang W, Feinleib JL, Cole F, Krauss RS.
J Cell Biol. 2004 Nov 8;167(3):493-504. Epub 2004 Nov 1.

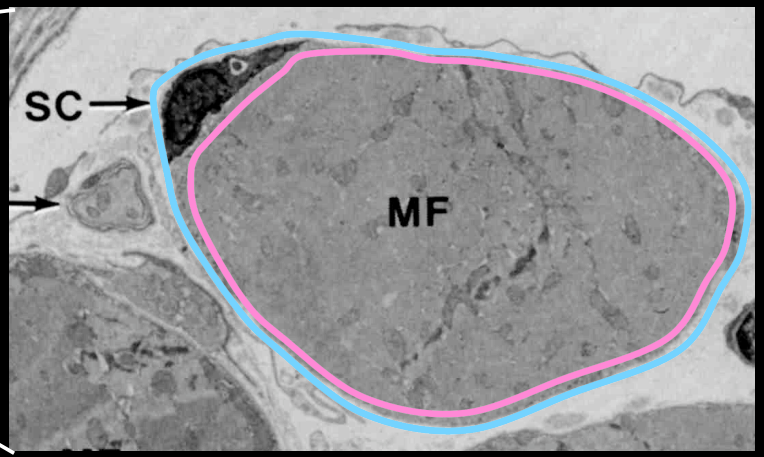
Purpose/Importance

- Understanding satellite cells will help improve current stem cell therapy for muscular diseases
- The ultimate goal of this research is to understand the mechanisms of satellite cells enough to find cures for muscular dystrophy

First test of a "high-density injection" protocol for myogenic cell transplantation throughout large volumes of muscles in a Duchenne muscular dystrophy patient: eighteen months follow-up.
Skuk D, Goulet M, Roy B, Piette V, Côté CH, Chapdelaine P, Hogrel JY, Paradis M, Bouchard JP, Sylvain M, Lachance JG, Tremblay JP.
Neuromuscul Disord. 2007 Jan;17(1):38-46. Epub 2006 Dec 4.

Dystrophin expression in muscles of duchenne muscular dystrophy patients after high-density injections of normal myogenic cells.
Skuk D, Goulet M, Roy B, Chapdelaine P, Bouchard JP, Roy R, Dugré FJ, Sylvain M, Lachance JG, Deschênes L, Senay H, Tremblay JP.
J Neuropathol Exp Neurol. 2006 Apr;65(4):371-86.

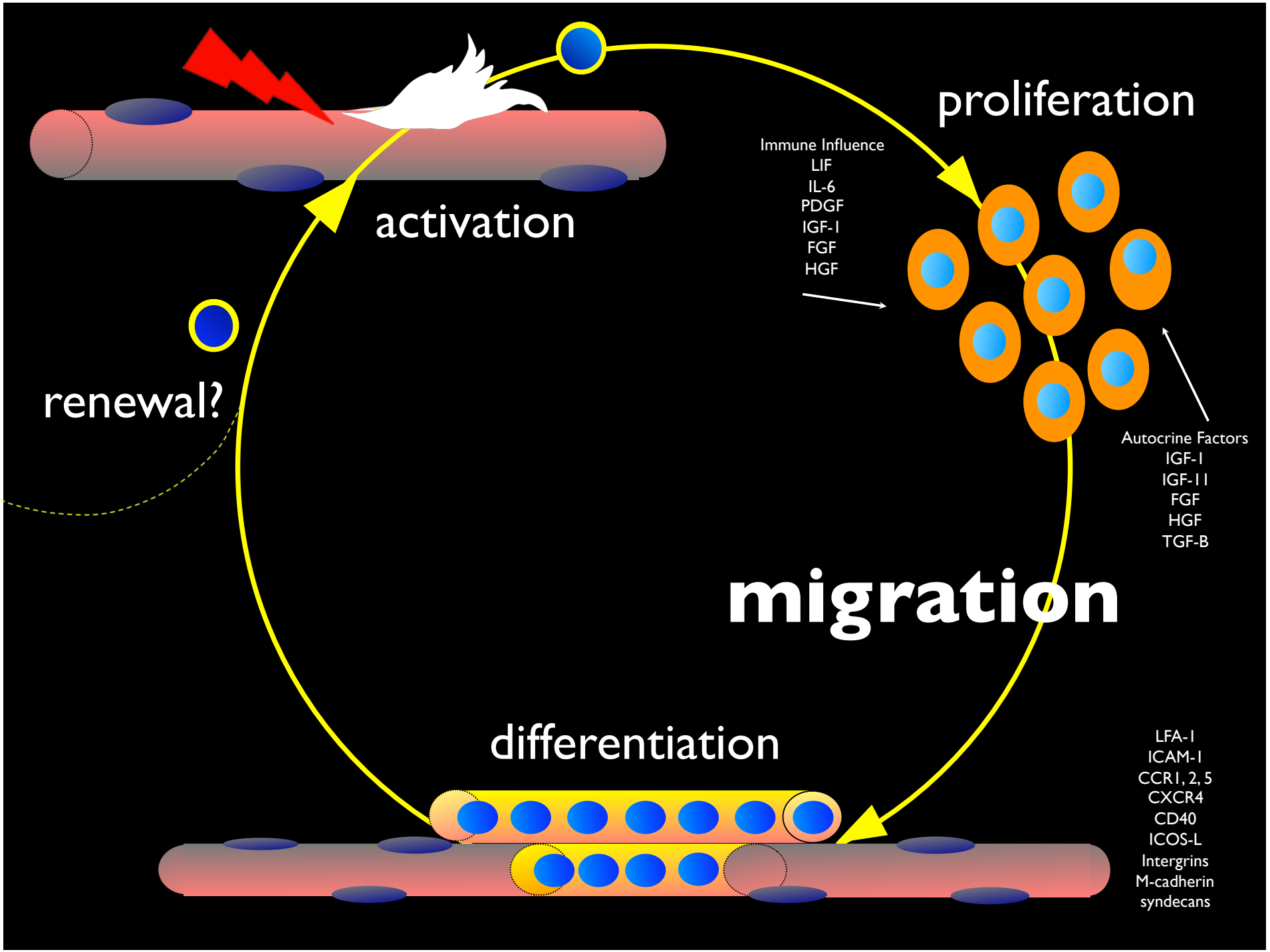




- Very rare- 1-6% of muscle-associated nuclei
- Located between the basal lamina and sarcolemma of the host fiber in uninjured muscle
- Quiescent in uninjured muscle (nonproliferative, minimal cytoplasm, minimal metabolism)
- Quiescence is maintained by isolation in the sublaminar compartment; activation must involve direct transmembrane signaling

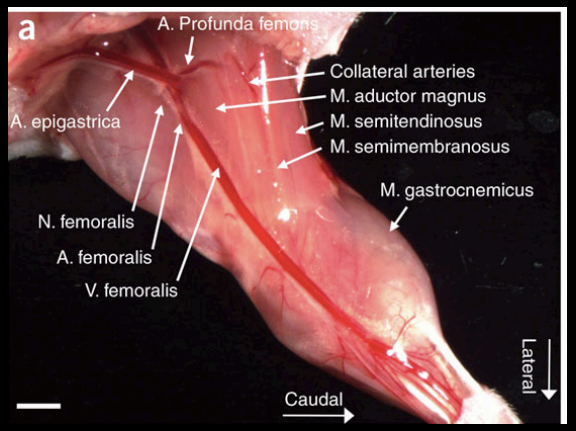
- Skeletal muscle is capable of many rounds of complete satellite-cell mediated regeneration

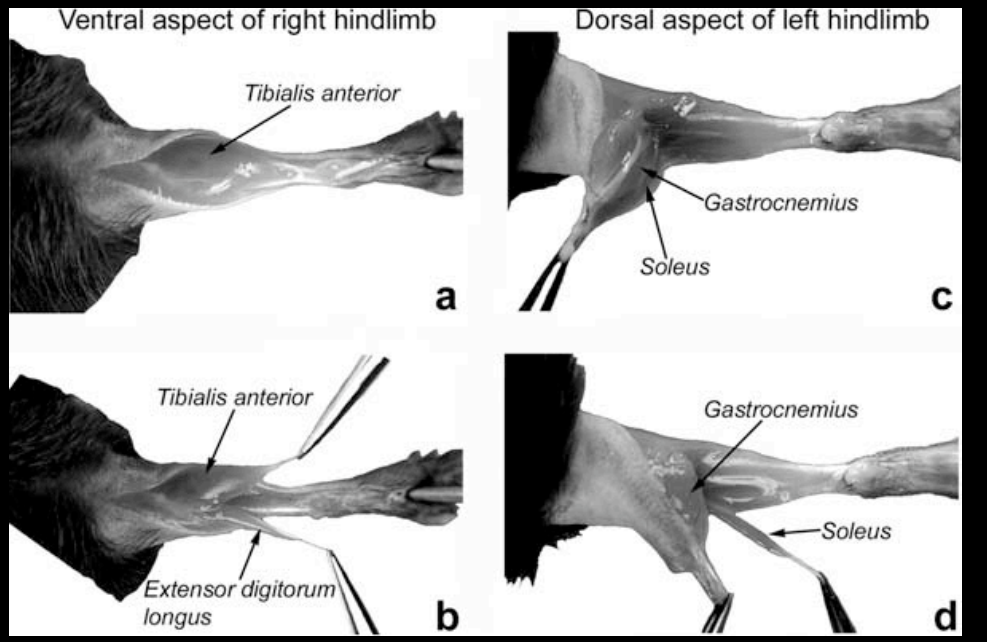
Muscle stem cells in development, regeneration, and disease. Shi X, Garry DJ. Genes Dev. 2006 Jul 1;20(13):1692-708. Review.


proliferation
Immune Influence
LIF
IL-6
PDGF
IGF-I
FGF
HGF
activation
renewal?
Autocrine Factors
IGF-I
IGF-II
FGF
HGF
TGF-B
migration
differentiation
LFA-1
ICAM-1
CCR1, 2, 5
CXCR4
CD40
ICOS-L
Intergrins
M-cadherin
syndecans

Fibers

- Mouse hindlimbs are dissected the soleus, plantaris, and extensor digitorum longus are carefully removed and digested with collagenase
- Fibers float free and are then used in collagen gel to film the satellite cells

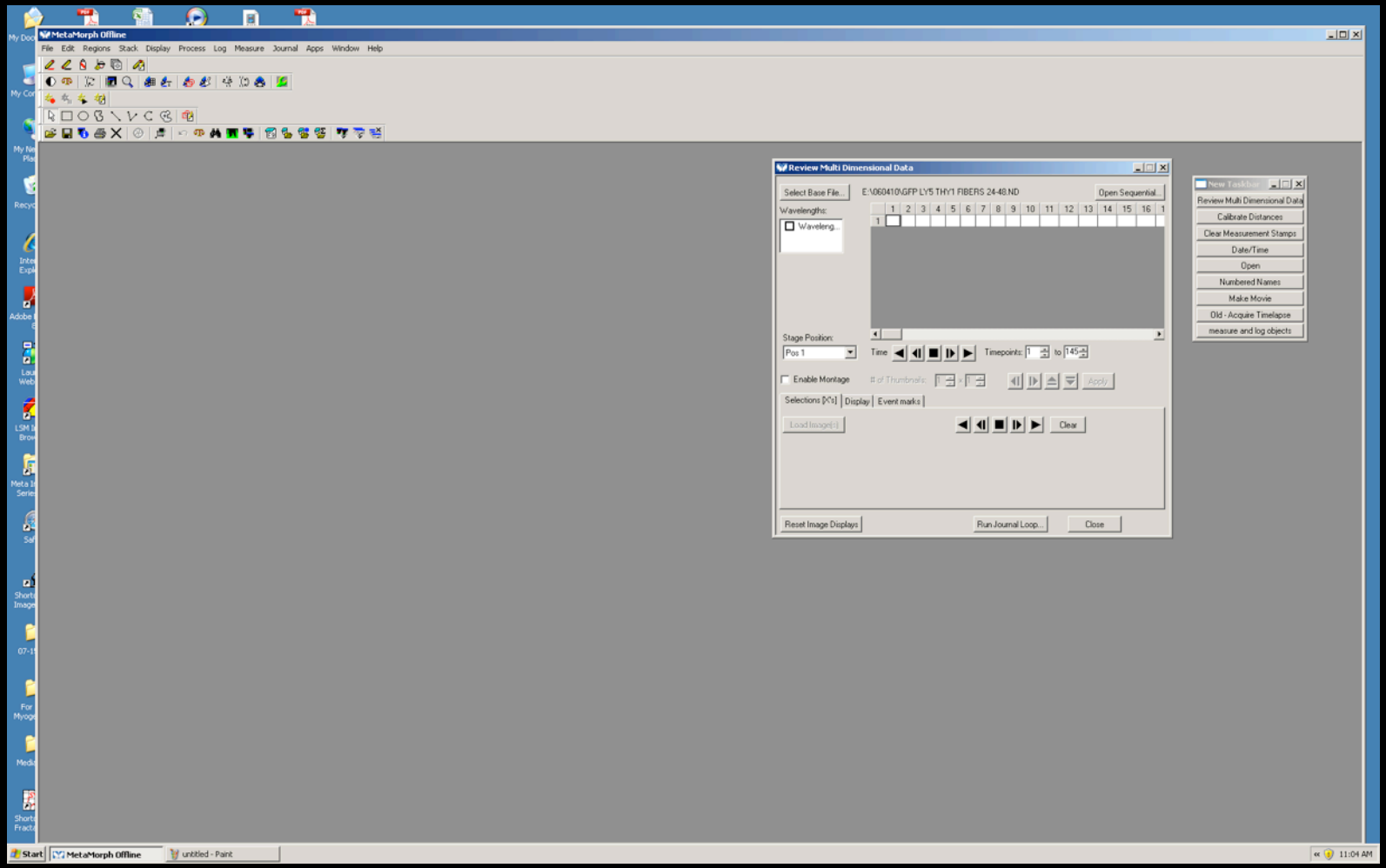




Scope Collection

- Leica Inverted Scope
- Incubated chamber
- Images taken at same x y z every ten minutes
- stored as tif with data from program

Metamorph


MetaMorph Offline
File Edit Regions Stack Display Process Log Measure Journal Apps Window Help
Review Multi Dimensional Data
Select Base File...
E:\060410\GFP LY5 THY1 FIBERS 24-48.ND
Open Sequential...
Wavelengths:
Stage Position:
Pos 1
Time
Timepoints:
Enable Montage
Selections
Display
Event marks
Load Image(s)
Clear
Reset Image Displays
Run Journal Loop...
Close
New Taskbar
Review Multi Dimensional Data
Calibrate Distances
Clear Measurement Stamps
Date/Time
Open
Numbered Names
Make Movie
Old - Acquire Timelapse
measure and log objects
Start
MetaMorph Offline
untitled - Paint
11:04 AM

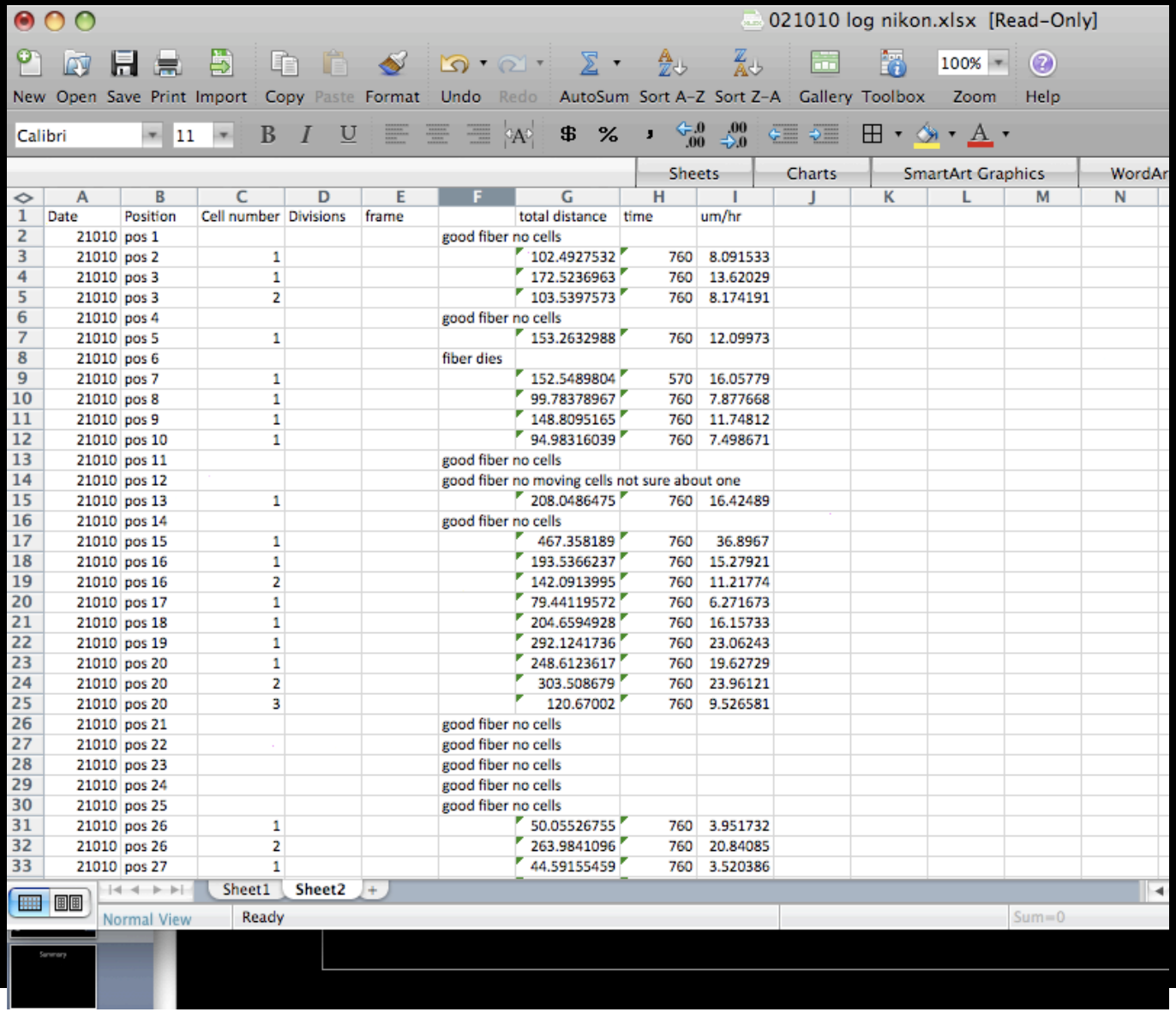
Metamorph


MetaMorph Offline
File Edit Regions Stack Display Process Log Measure Journal Apps Window Help
Review Multi Dimensional Data
Select Base File...
E:\042110\042110 WT.ND
Open Sequential...
Wavelengths:
BF
Stage Position:
pos 9
Time
Timepoints: 1 to 115
Enable Montage
# of Thumbnails:
Apply
Selections (XYs)
Display
Event marks
Load Image(s)
Clear
Reset Image Displays
Run Journal Loop...
Close
New Taskbar
Review Multi Dimensional Data
Calibrate Distances
Clear Measurement Stamps
Date/Time
Open
Numbered Names
Make Movie
Old - Acquire Timelapse
measure and log objects
Start
MetaMorph Offline
Microsoft Excel
11:09 AM

Cell Rules

- Must be visable the whole movie
- Must not die nor the fiber
- Must be able to be defined the entire time
- Track all that meet these requirements
- If it divides randomly choose one-or sometimes only one stays in frame the whole time

Excel Sheet



021010 log nikon.xlsx [Read-Only]

New Open Save Print Import Copy Paste Format Undo Redo AutoSum Sort A-Z Sort Z-A Gallery Toolbox 100% Zoom Help

Calibri 11

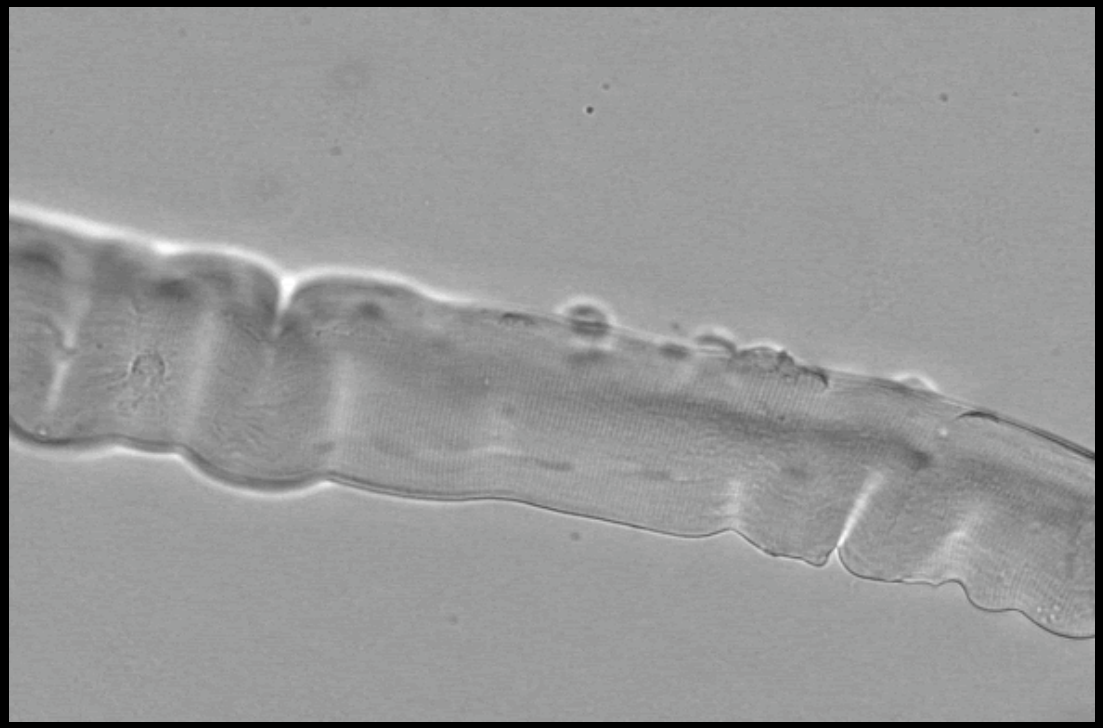
Sheets Charts SmartArt Graphics WordAr

	A	B	C	D	E	F	G	H	I
1	Date	Position	Cell number	Divisions	frame		total distance	time	um/hr
2	21010	pos 1				good fiber no cells			
3	21010	pos 2	1				102.4927532	760	8.091533
4	21010	pos 3	1				172.5236963	760	13.62029
5	21010	pos 3	2				103.5397573	760	8.174191
6	21010	pos 4				good fiber no cells			
7	21010	pos 5	1				153.2632988	760	12.09973
8	21010	pos 6				fiber dies			
9	21010	pos 7	1				152.5489804	570	16.05779
10	21010	pos 8	1				99.78378967	760	7.877668
11	21010	pos 9	1				148.8095165	760	11.74812
12	21010	pos 10	1				94.98316039	760	7.498671
13	21010	pos 11				good fiber no cells			
14	21010	pos 12				good fiber no moving cells not sure about one			
15	21010	pos 13	1				208.0486475	760	16.42489
16	21010	pos 14				good fiber no cells			
17	21010	pos 15	1				467.358189	760	36.8967
18	21010	pos 16	1				193.5366237	760	15.27921
19	21010	pos 16	2				142.0913995	760	11.21774
20	21010	pos 17	1				79.44119572	760	6.271673
21	21010	pos 18	1				204.6594928	760	16.15733
22	21010	pos 19	1				292.1241736	760	23.06243
23	21010	pos 20	1				248.6123617	760	19.62729
24	21010	pos 20	2				303.508679	760	23.96121
25	21010	pos 20	3				120.67002	760	9.526581
26	21010	pos 21				good fiber no cells			
27	21010	pos 22				good fiber no cells			
28	21010	pos 23				good fiber no cells			
29	21010	pos 24				good fiber no cells			
30	21010	pos 25				good fiber no cells			
31	21010	pos 26	1				50.05526755	760	3.951732
32	21010	pos 26	2				263.9841096	760	20.84085
33	21010	pos 27	1				44.59155459	760	3.520386

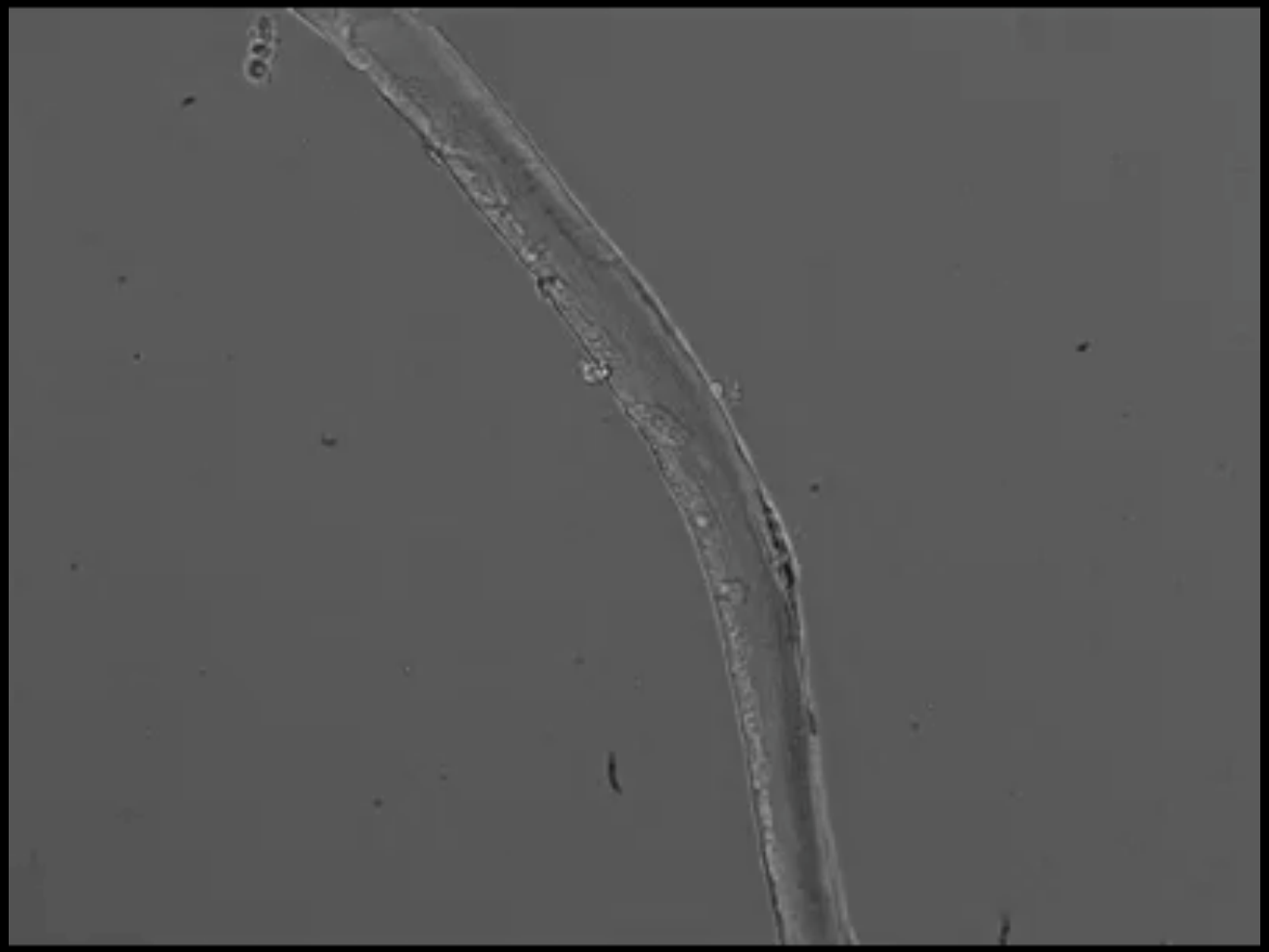
Sheet1 Sheet2

Normal View Ready Sum=0

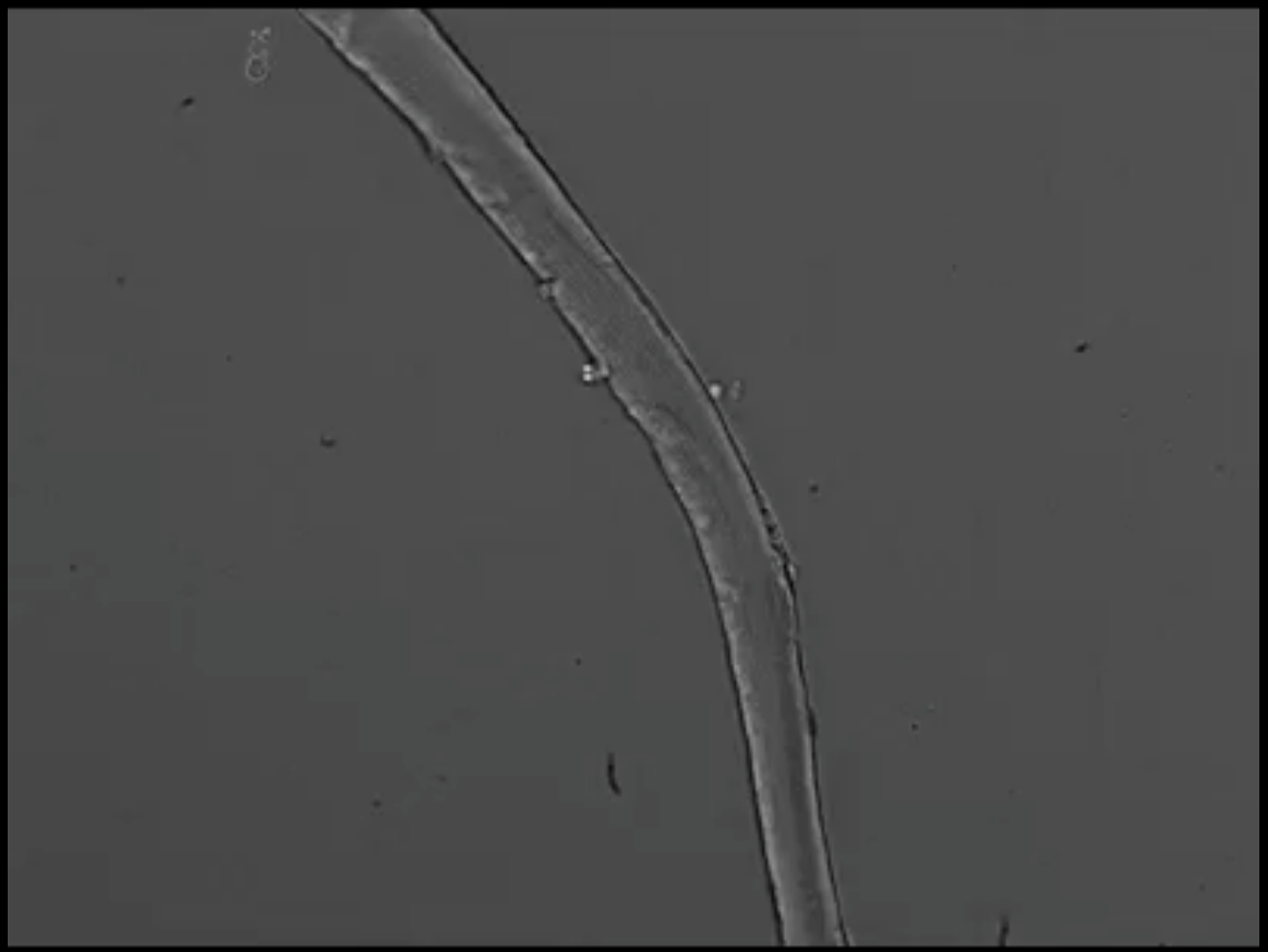
Images



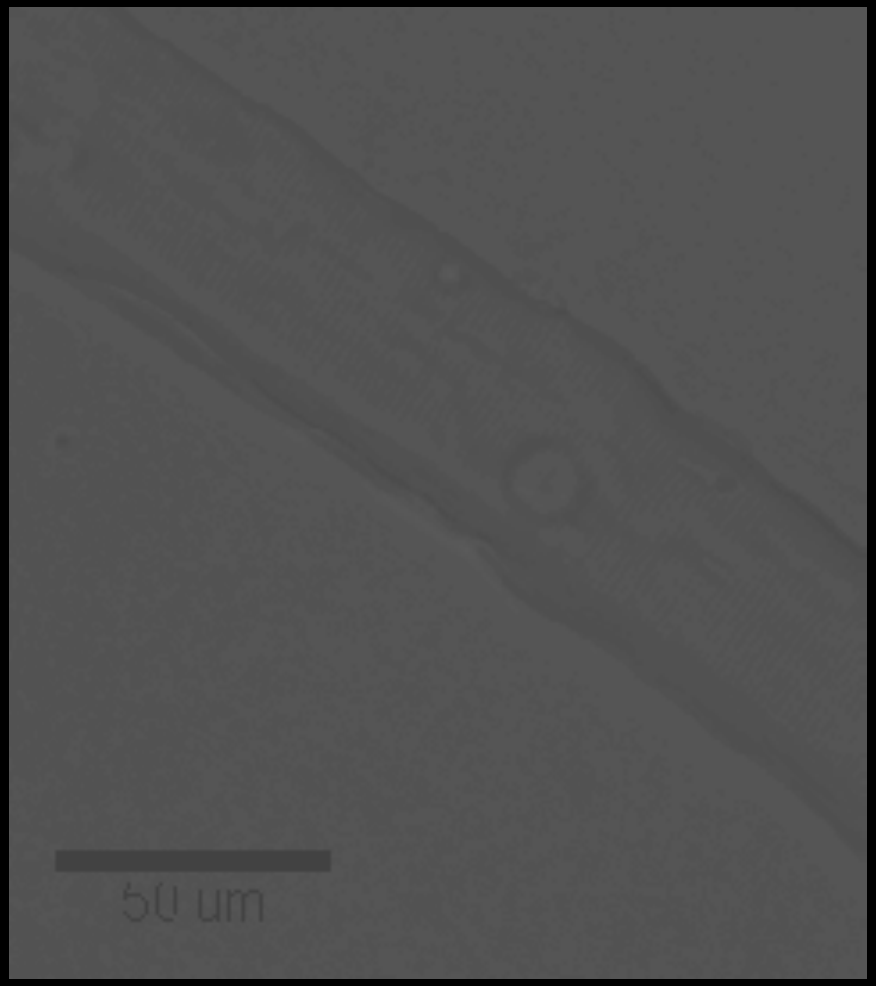
More images…

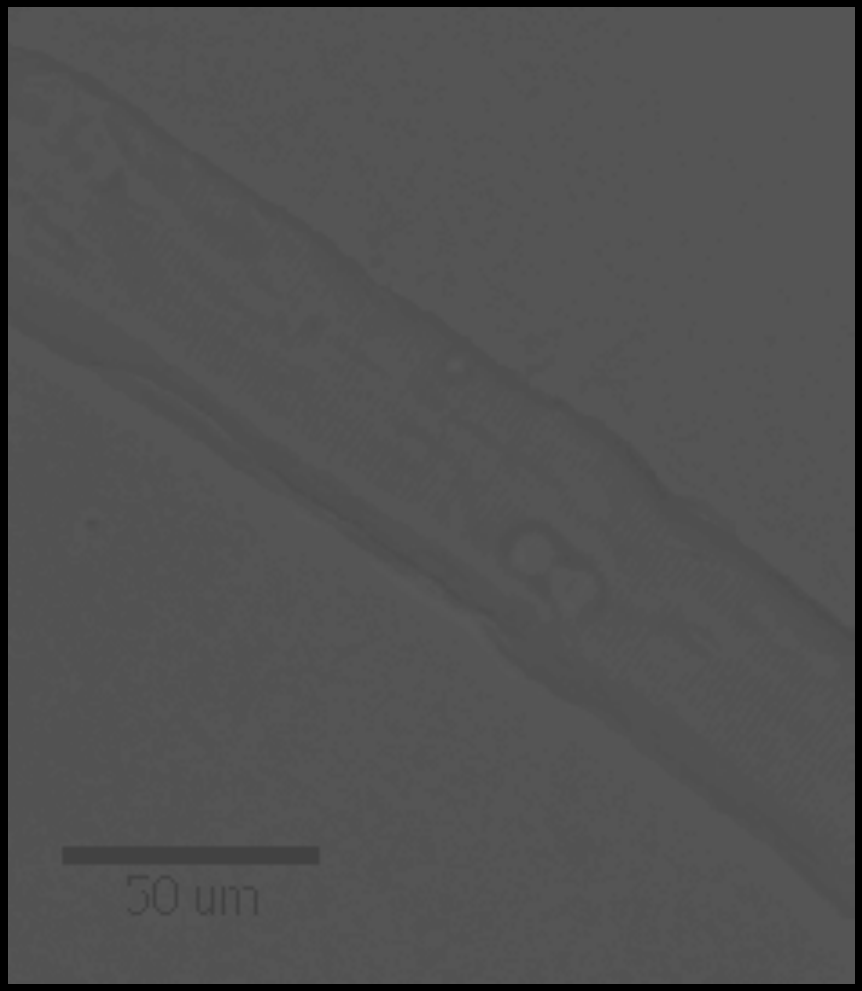


And more…

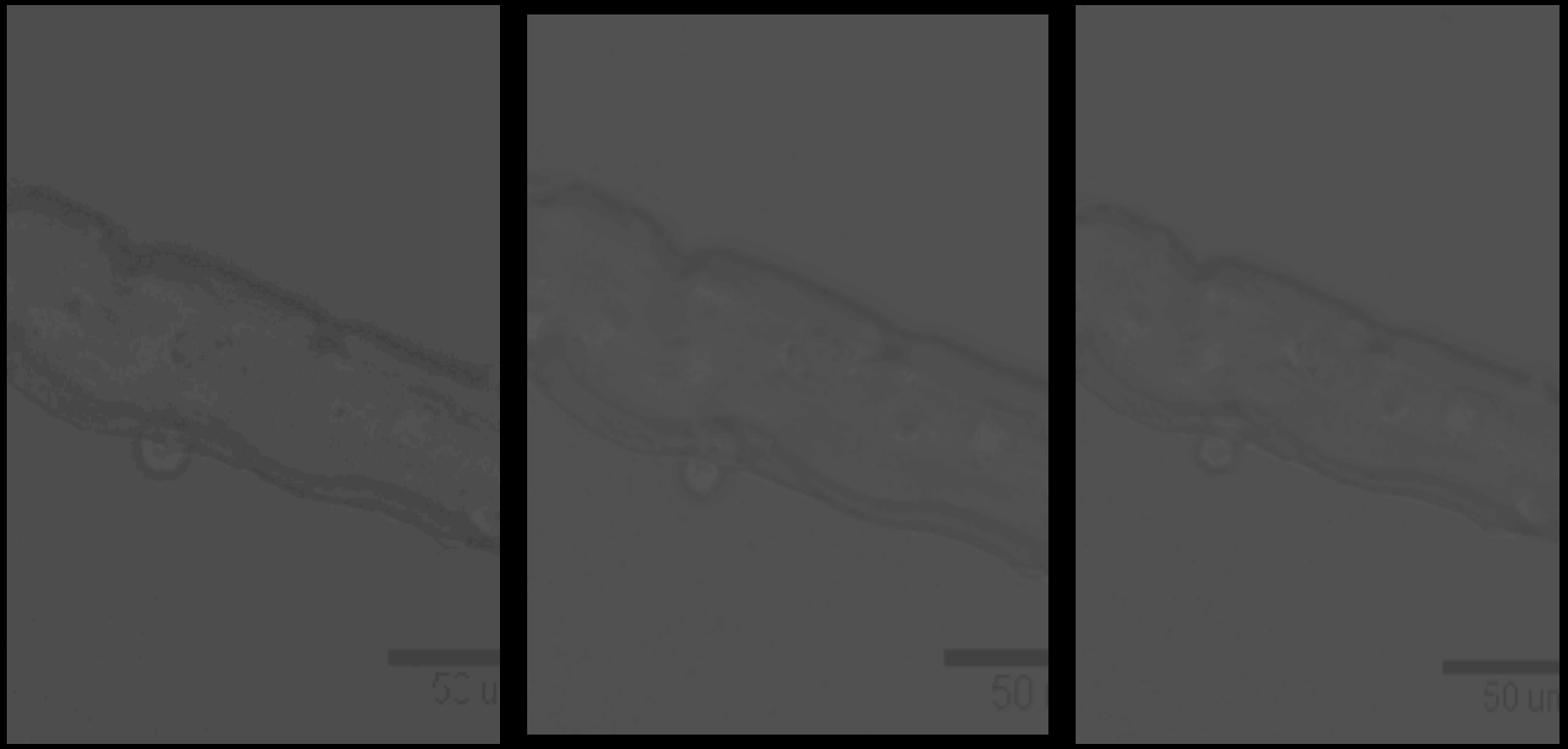


Horizontal Division

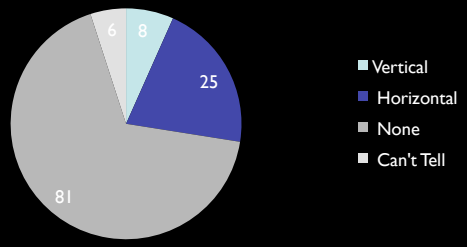




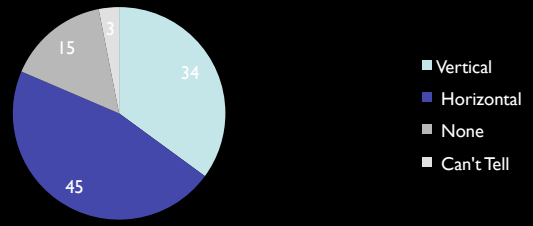
Vertical Division



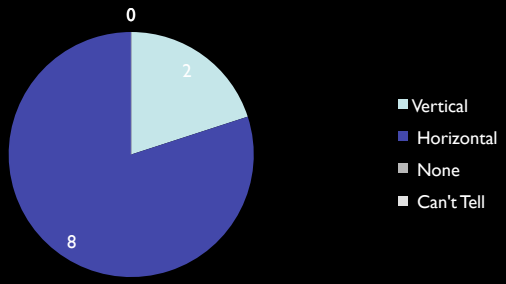
Myf-5 Division 1 (24-48 hrs)



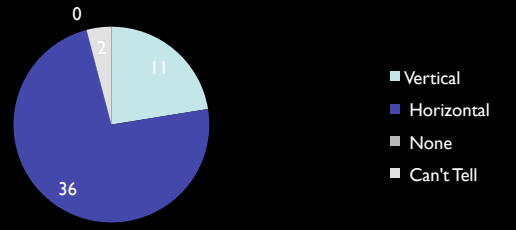
WildType Division 1 (24-48 hrs)



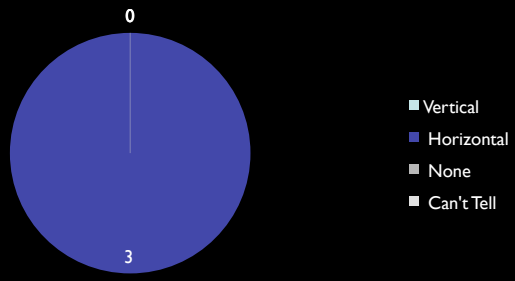
Myf-5 Division 2 (24-48 hrs)



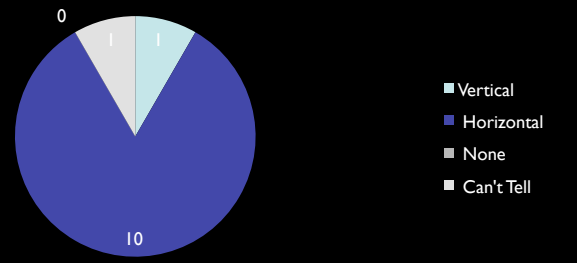
WildType Division 2 (24-48 hrs)



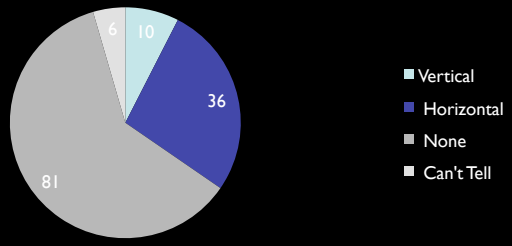
Myf-5 Division 3 (24-48 hrs)



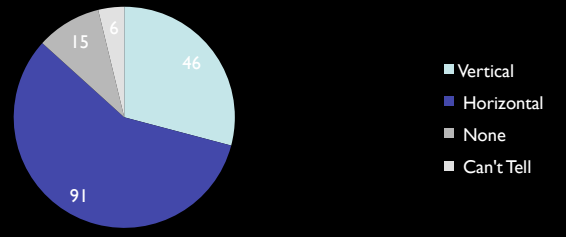
WildType Division 3 (24-48 hrs)

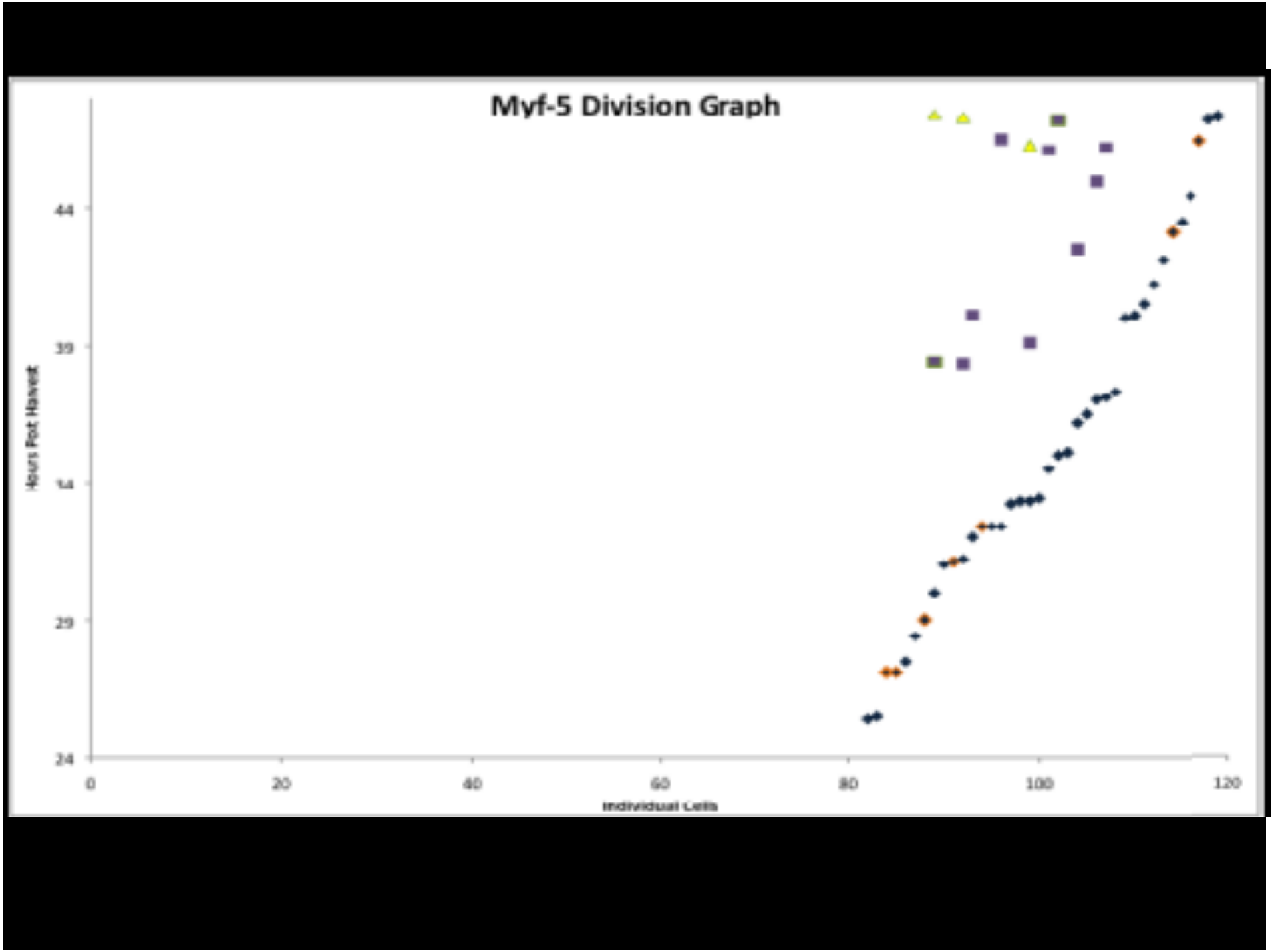


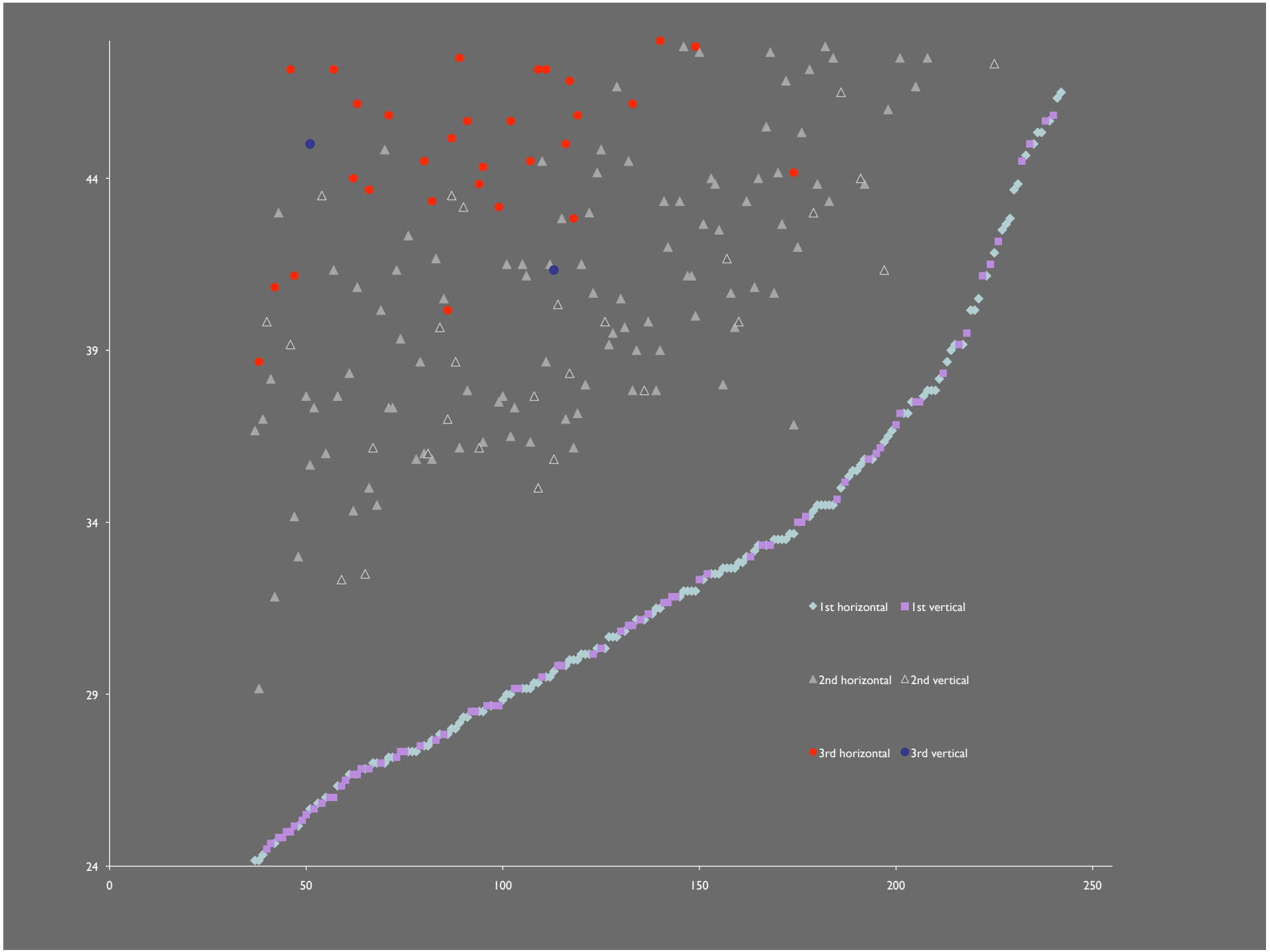
Myf-5 All Divisions (24-48 hrs)



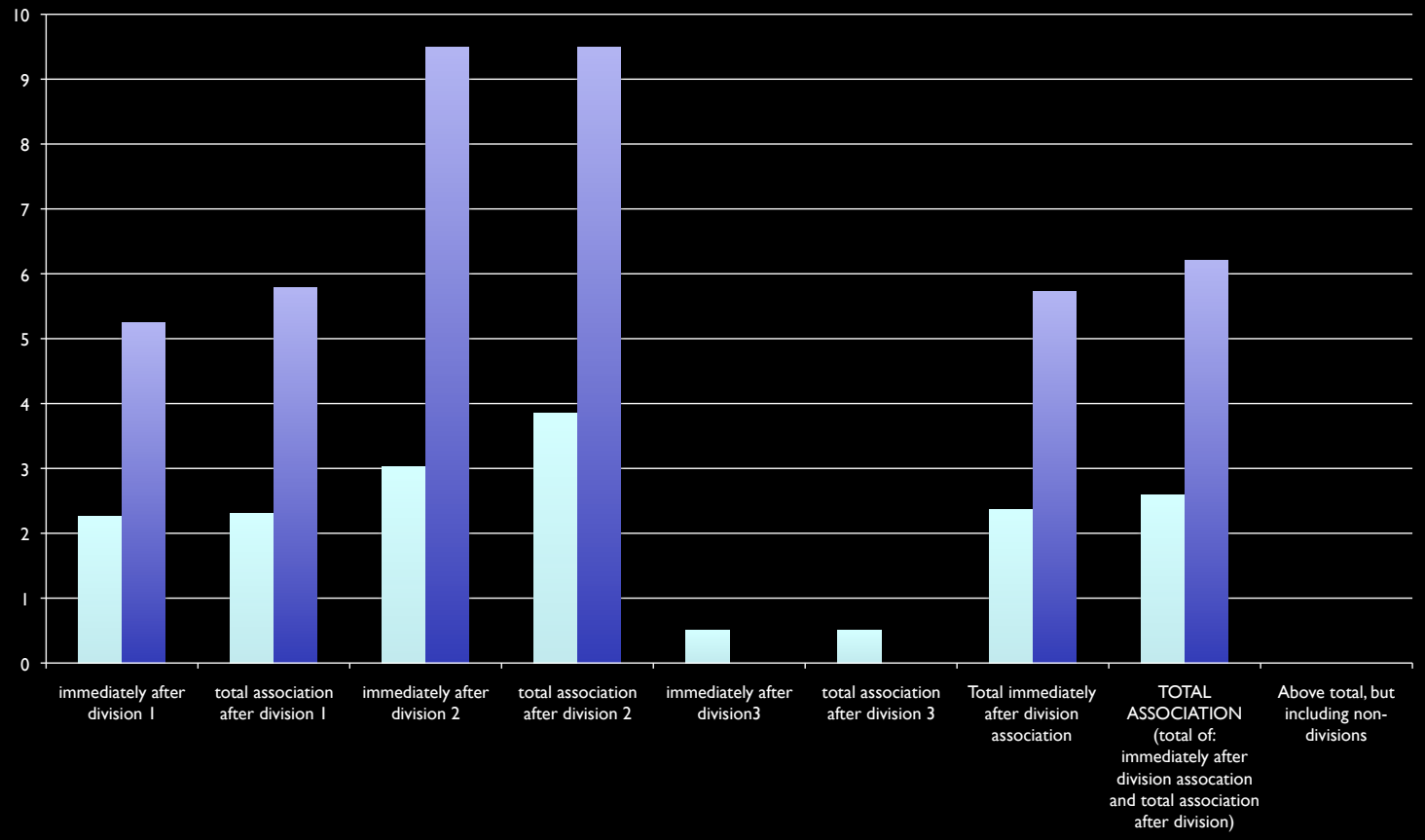
WildType All Divisions (24-48 hrs)

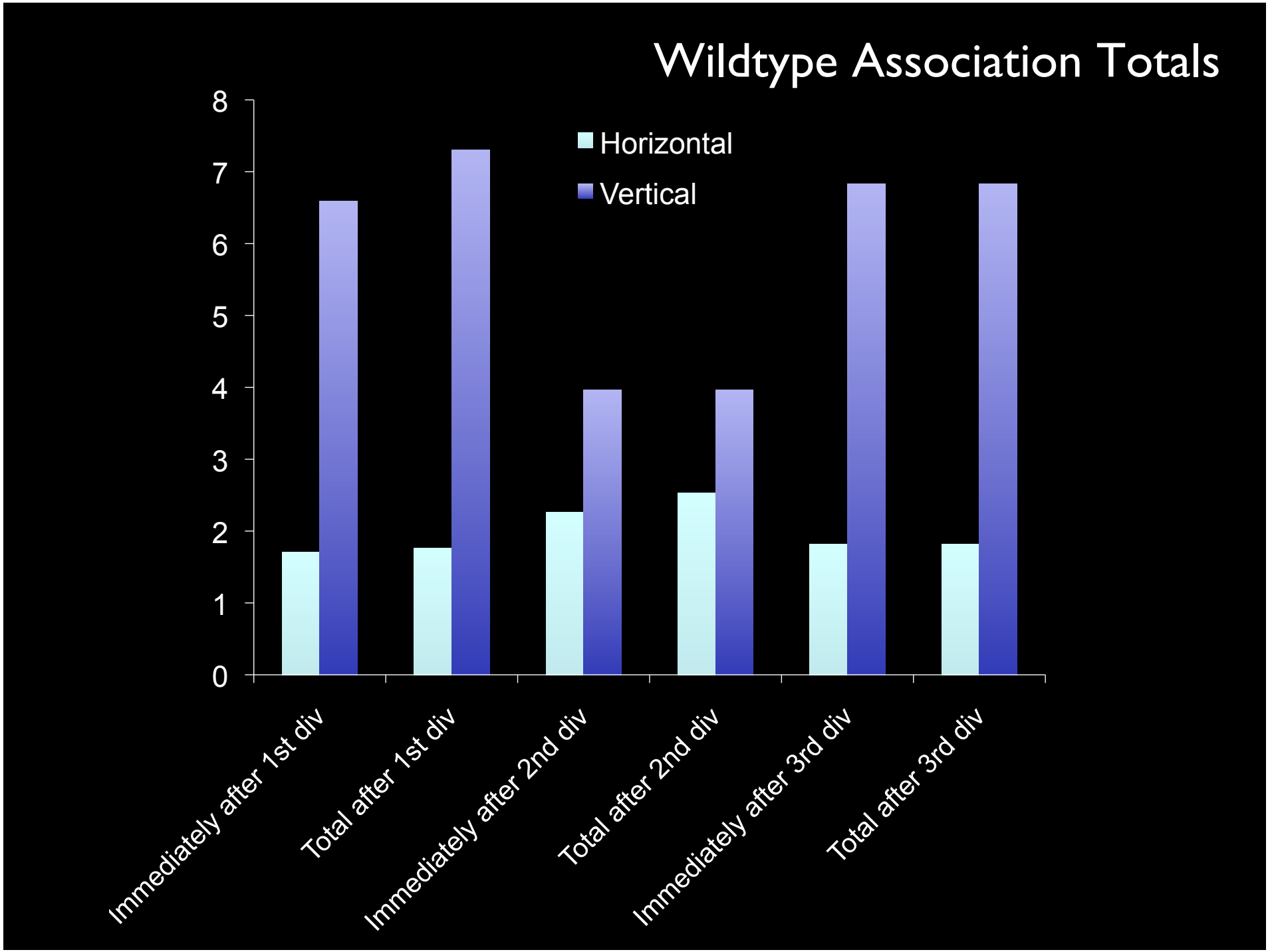



Myf-5 Division Graph
Hours Post Harvest
44
39
34
29
24
0
20
40
60
80
100
120
Individual Cells


44
39
34
29
24
0
50
100
150
200
250
1st horizontal
1st vertical
2nd horizontal
2nd vertical
3rd horizontal
3rd vertical

Myf-5 Association Totals




Wildtype Association Totals
8
7
6
5
4
3
2
1
0
Horizontal
Vertical
Immediately after 1st div
Total after 1st div
Immediately after 2nd div
Total after 2nd div
Immediately after 3rd div
Total after 3rd div

Conclusion

- Myf-5 and wildtype do not act the same (Myf-5 = less divisions, later division times, longer association, less movement)
- More horizontal divisions than vertical
- Vertical divisions typically happen earlier (chronologically)
- Divisions are not synchronous, and do not correlate with later divisions
- Association (both sister and non-sister) actually occurs
- Vertical divisions stay associated longer

Goals

- **Understanding movement patterns**
- Extend the study of satellite cells in the 3D system
- Better assess the pattern with new kinds of measurements
- How to best use the data

Agent Based Modeling

- computer modeling can be seen as a means of dynamic knowledge representation that can form a basis for formal means of testing, evaluating and comparing what is currently known within the research community.
- ABMs are not appropriate if the starting point is a mass of raw data; rather, one must have already had some idea of potential mechanisms that lead to the generation of the data.
- envision an iterative process by which inductive models are applied to large data sets, wet lab experiments are carried out to evaluate and refine the mechanisms inferred from the inductive model, and the experimentally confirmed mechanisms are used as a basis of an ABM which would close the discovery loop by recapitulating the original data set
    - ABM's incorporate space, parallelism, stochasticity
    - Possible platforms MASON, NetLogo, Repast, Swarm or can be state-space model

New Direction

- Satellite cells on muscle fibers from the tibialis anterior of mice *in vitro* were observed to move extensively, divide frequently, and associate with other satellite cells by way of timelapse microscopy
- Limited success in satellite cell therapies due to engrafted cell death, rejection of new myoblasts, and minute migration of the injected stem cells
- Remains uncertain whether migration contributes to regeneration, but it seems directional motility is necessary for fixing localized injury because of the sparse distribution of quiescent satellite cells
- Previous data suggests that *in vivo* satellite cells do indeed move along and even between muscle fibers, but none of this data was quantified, only descriptional

Siegel, Ashley L, Kevin Atchison, Kevin E Fisher, George E Davis, and D DW Cornelison. "3D Timelapse Analysis of Muscle Satellite Cell Motility." *Stem Cells*. 27. (2009): 2527-2538. Print.

Siegel, Ashley L, Paige K Kuhlmann, and D DW Cornelison. "Muscle satellite cell proliferation and association: new insights from myofiber time-lapse imaging." *Skeletal Muscle*. 1.7 (2011): Print.

Future Goals

- Re-examine satellite cell movement *in vivo*
- Quantify cell movement and gain further understanding into why and how they move
- Solve problem of stationary injected satellite cells, leading to more effective stem cell therapy

Future Methods

- ROSA26 LacZ mice (Protein and promoter – LacZ STOP protein surrounded by two loxP sites located behind ROSA promoter)
- Inject adenovirus containing Cre gene – cuts out LacZ STOP to express LacZ gene (codes for B-galactosidase enzyme - expression detected with substrate X-gal)
- 26 days later: injure muscle with chemoattractive injection and use X-gal to determine movement of cells expressing B-gal (stain before sectioning) by sectioning the muscle
- Section tibialis anterior using cryostat and count sections expressing B-gal to measure length of injury and distance travelled by cells
- Stain with antibodies after sectioning for different purposes: recognize extent of injury and presence of satellite cells

Expected Results/Significance

- Find out if native satellite cells will move within the muscle in a natural situation when injury occurs
- Purpose behind the movements
- Proceed in answering fundamental questions about the downfalls of satellite cell therapy if cells *in vivo* behave like the cells *in vitro*
- If not, pinpoint the extracellular signaling and pathways that are causing them to behave differently
- Once we can understand how and why satellite cells move and behave the way they do, we can begin to apply those understandings to fixing problems with stem cell treatment