

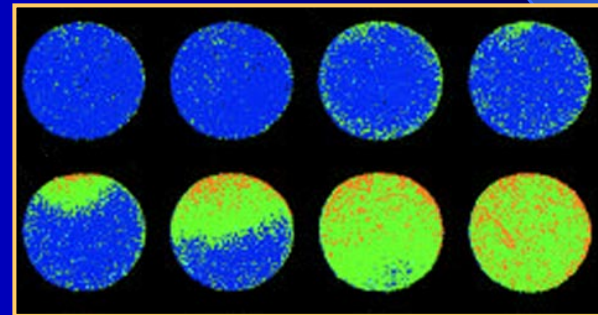
Calcium Wave Propagation in Mouse Eggs

Greg L Golden
Capco Lab
ASU CBS Program

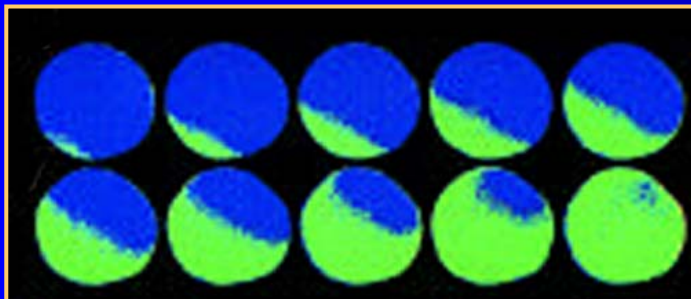
What Is A Calcium Wave?

- A sharp increase in intracellular calcium concentration, $[Ca_2^+]_i$, that spreads through an egg.

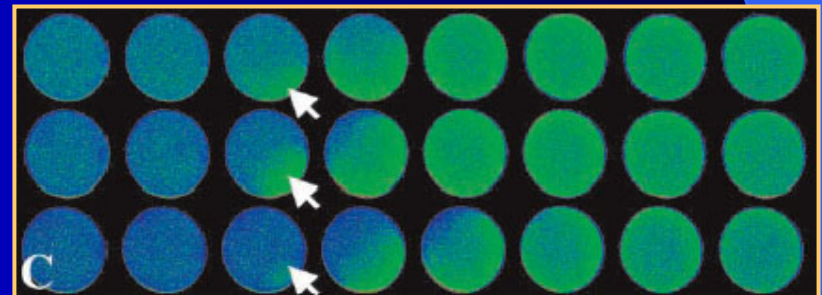
Sea urchin



Starfish



Marine worm



Experimental Goal

- Original goal was to compare the calcium waves from artificial vs. “natural” fertilization.
 - Artificial method is called parthenogenesis and can be caused by a pin prick, a calcium ionophore, or may just happen spontaneously.
 - Natural method is *in vitro* fertilization and uses sperm.

Experiment Overview

- Each experiment spanned 4 days
- A total of 22 separate experiments were conducted

DAY 1	DAY 2	DAY 3	DAY 4
4:30 PM PMSG Injection		4:30 PM hCG Injection	7:30 AM Main experiment

7:30 AM	8:00 AM	8:30 AM	10:00 AM – 1:00
Preparation	Mouse Dissection	Egg separation Ca Green added	10:00 AM Main experiment

Mouse Dissections

- Mice anesthetized and euthanized
- Abdominal incision made and oviducts removed

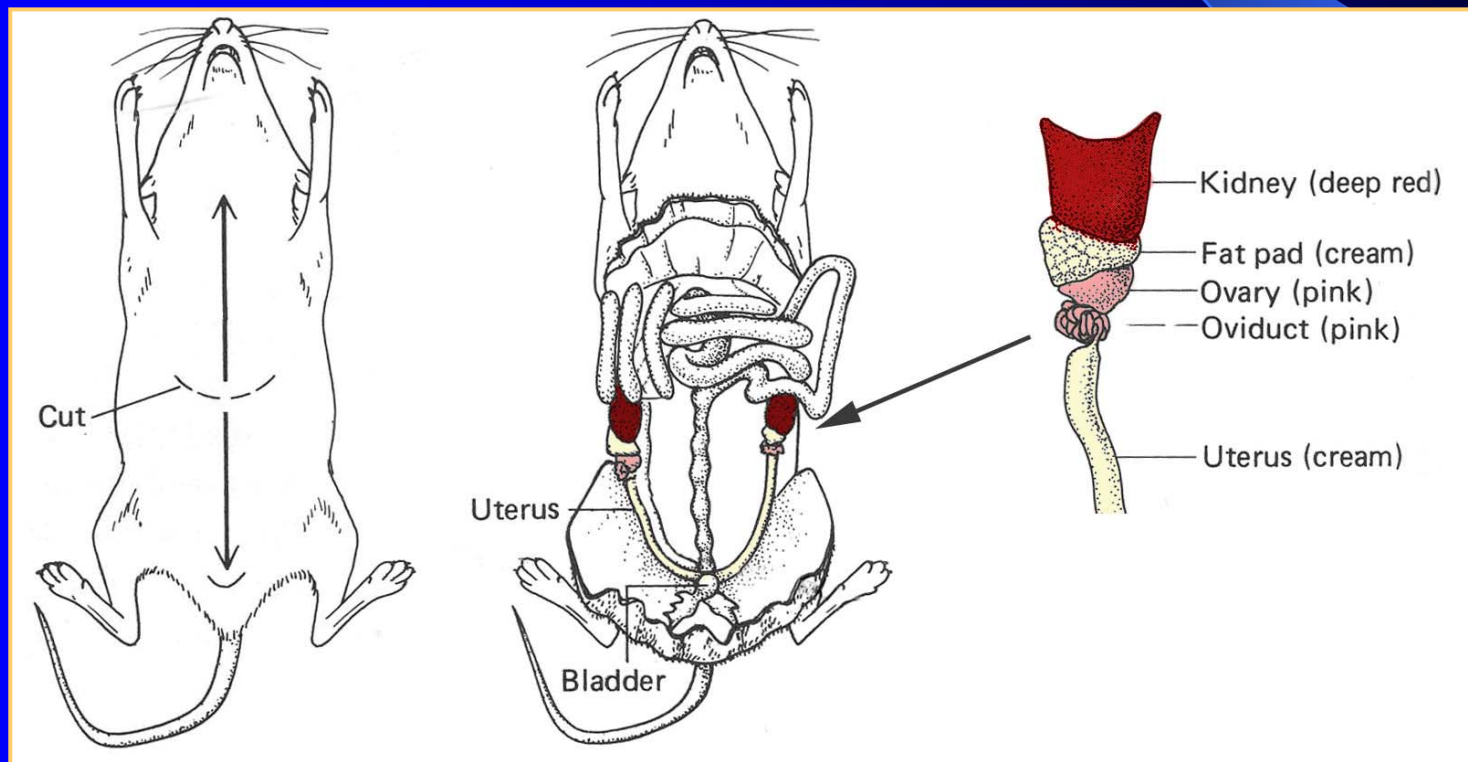


Figure from [3]

Egg Preparation

- Cumulus cell mass removed from oviducts under dissecting scope
- Eggs freed from cumulus cells with digestive enzyme called hyaluronidase

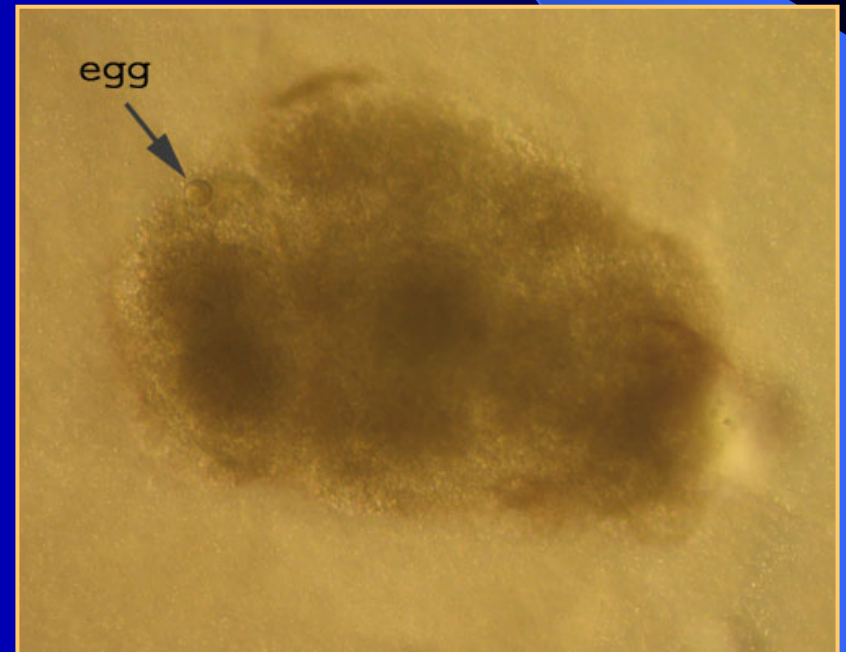
Tissue mass



Oviduct



Cumulus mass



Egg Preparation

- Eggs washed several times and then incubated for 20 minutes with Calcium Green dye.



Imaging

- Used Leica SP2 and Nikon C1si confocal microscopes



Imaging

- Movie process:
 - Position slide containing live eggs and media.
 - Locate egg(s) under scope.
 - Add calcium ionophore.
 - Take movie.

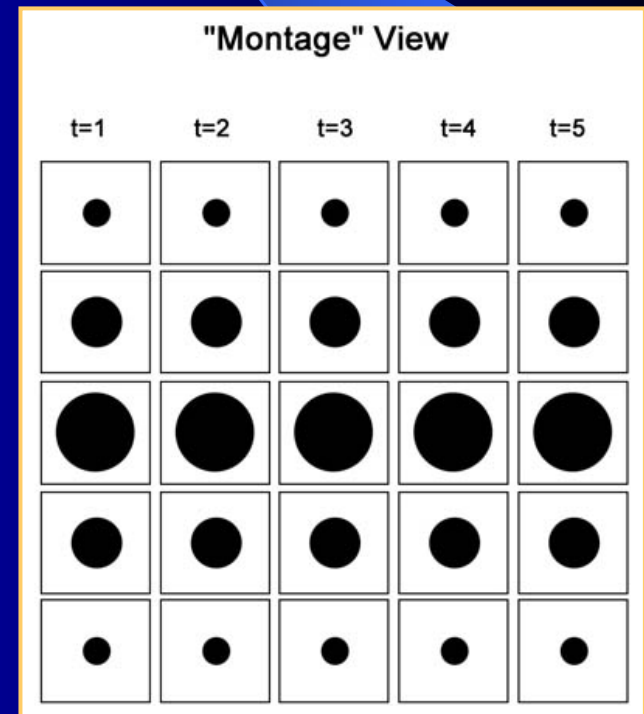
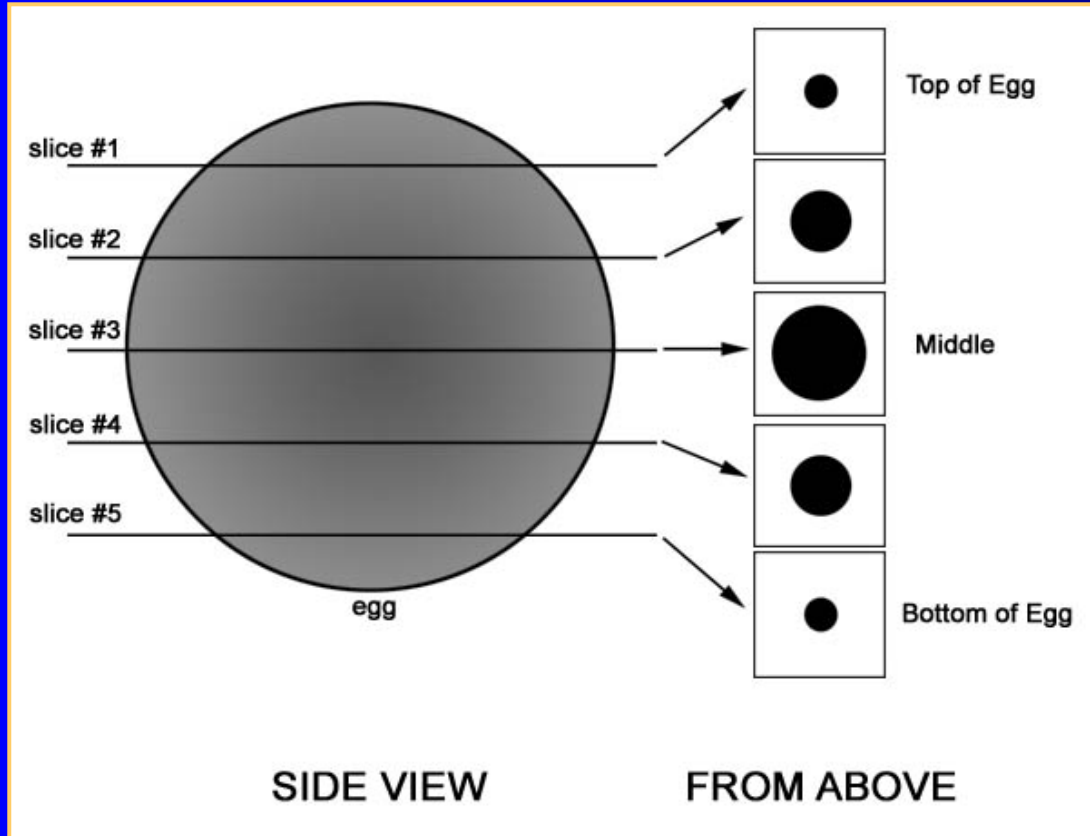


Movie of Imaging

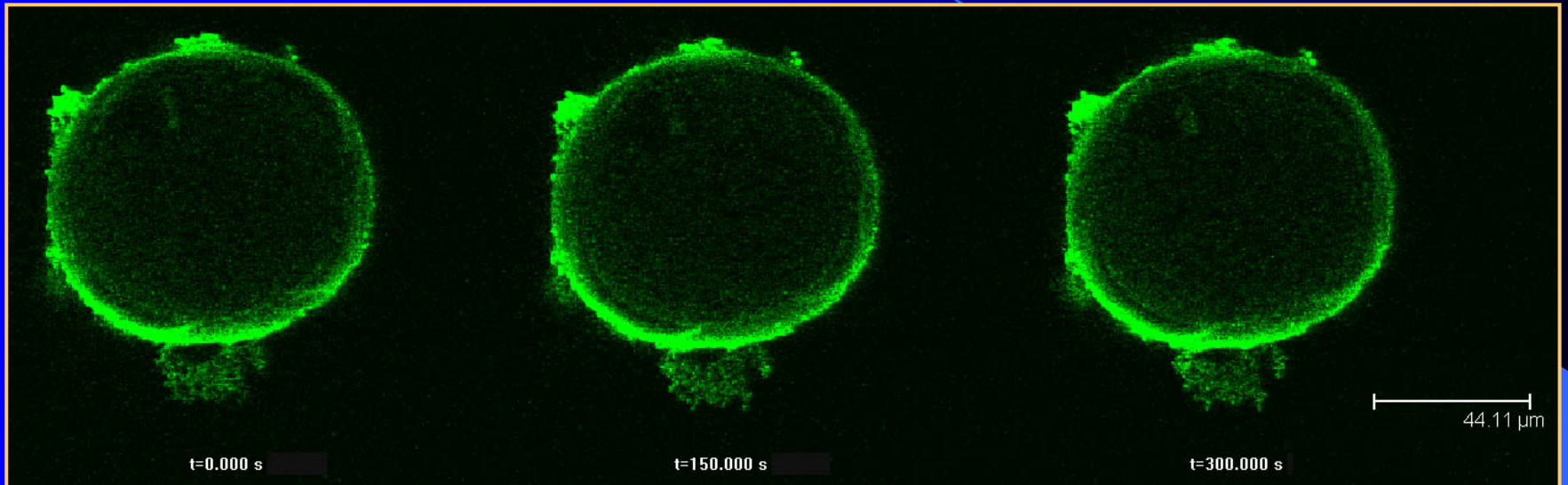
The background is a dark blue gradient. A thin, light blue curved line starts from the top left and arcs towards the right. On the right side, there is a light blue, semi-transparent shape that looks like a curved wedge or a portion of a circle.

Imaging

- Confocal can provide time series, z-axis slices, or both (zt-series)



Early Attempts



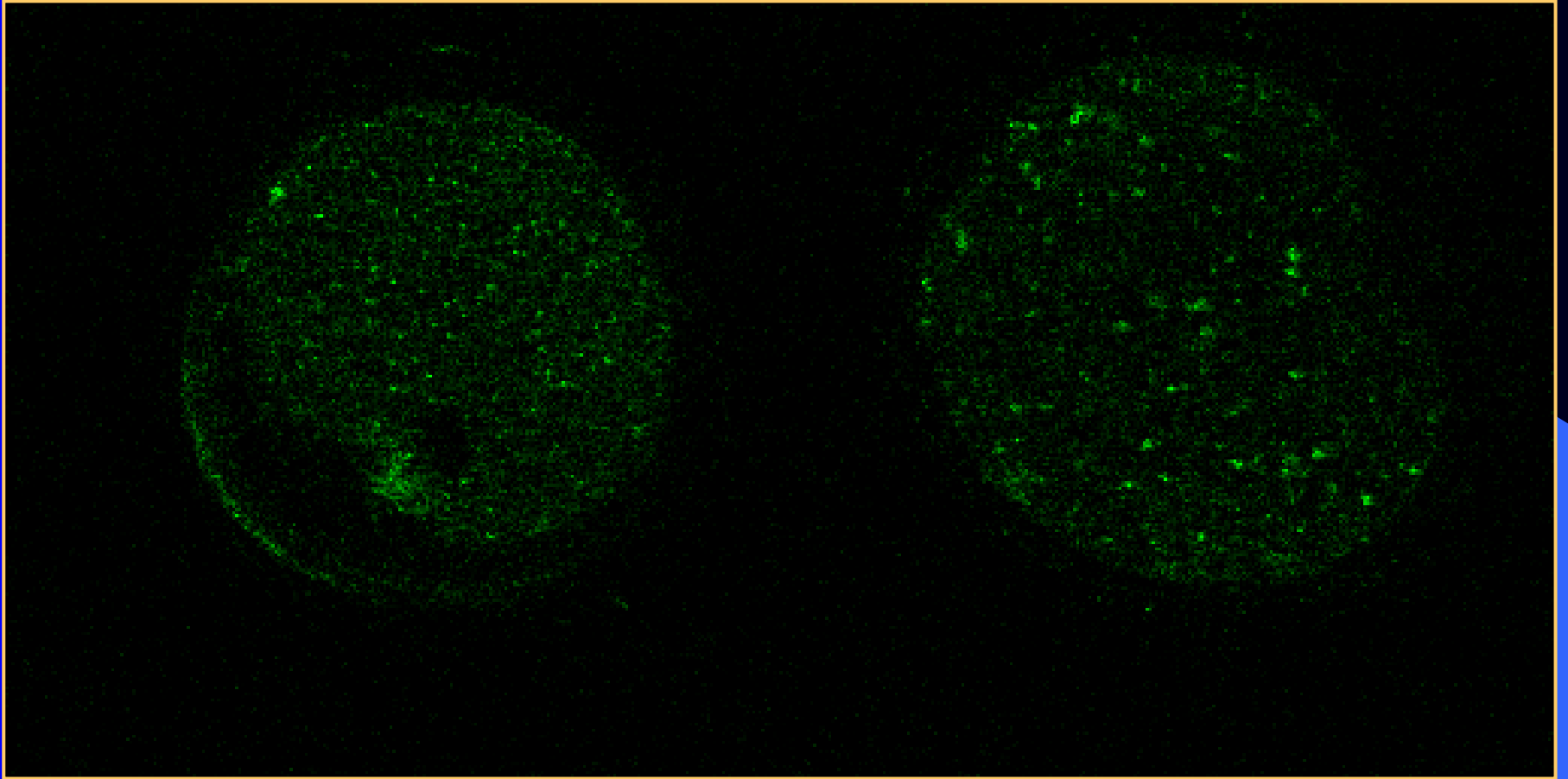
Variables

- Ionophore age (if too old it wouldn't activate the egg)
- Ionophore concentration (too low no activation, too high unhealthy for eggs)
- Calcium green age (if too old it would lose sensitivity)
- Calcium green concentration and incubation times (too low, no image. Too high, unhealthy for eggs)
- Hoechst stain may be interfering with activation
- Polylysine may be keeping the eggs from activating
- DMSO not working properly (we noticed heavy dye clumping around the eggs)
- Confocal microscope settings (were we using the correct laser frequencies?)
- Dish material (the plastic dishes were designed for cell culture, perhaps they were interfering with the eggs)

22 Experiments From May-Oct

Date	Summary	Results
5/15/2006	First attempt	No Ca wave, but dye showed up
5/18/2006	Changed dye incubation time.	No Ca wave, but dye showed up
6/8/2006	Using proven ionophore	No Ca wave, but dye showed up
6/13/2006	Test to see if ionophore is working	No Ca wave, but fixed eggs were later shown to have activated. Therefore ionophore is working
6/15/2006	Major experiment trying many variations	
6/21/2006	Trying without polylysine.	No Ca wave, but fixed eggs were later shown to have activated.
6/29/2006	Concentration curve for CaGreen	No Ca wave but everything else worked. Therefore chemicals not the problem.
6/30/2006	Incubation time curve for CaGreen	No Ca wave but eggs activating. CaGreen may not be working well.
7/13/2006	Problems with scope. Couldn't complete.	
7/24/2006	Testing CaGreen on 3T3 cell culture	
7/25/2006	24 hr. incubation on 3T3 cell culture	3T3 cells showed up very green
8/2/2006	Trying new CaGreen with Pluronic	Eggs were not sticking to bottom of plate
8/9/2006	1uM and 5uM conc. Of CaGreen	Some results!
8/10/2006	Repeat	Results
8/17/2006	Using RIM1 for PKC	Results
8/25/2006	1st male mouse	
8/30/2006	2nd male mouse. Also Ionophore	
9/13/2006	Ionophore with very young batch of mice	Results
9/20/2006	Verify Hoesht doesn't disturb ring formation	Realized we were getting spontaneous activation.
9/27/2006	Repeat	Results.
10/4/2006	3rd male mouse.	No fertilization. Tried for 2 1/2 hours
10/11/2006	Nikon scope	Best Results

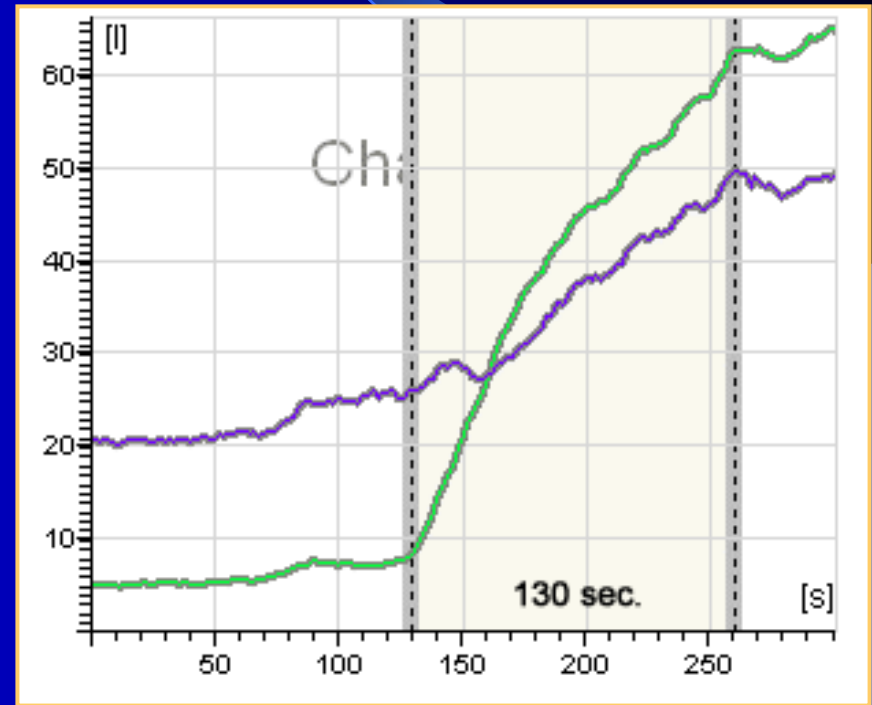
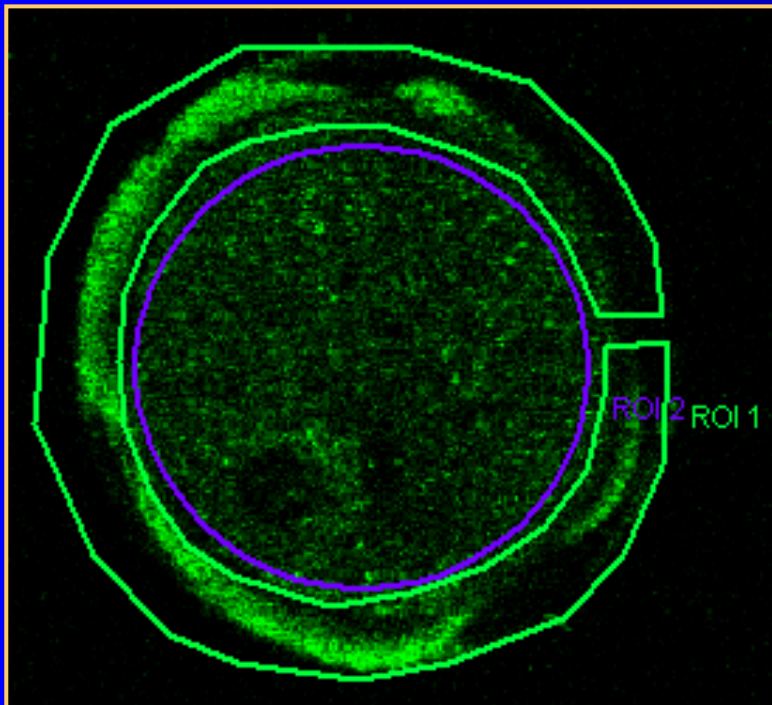
First Clear Activation



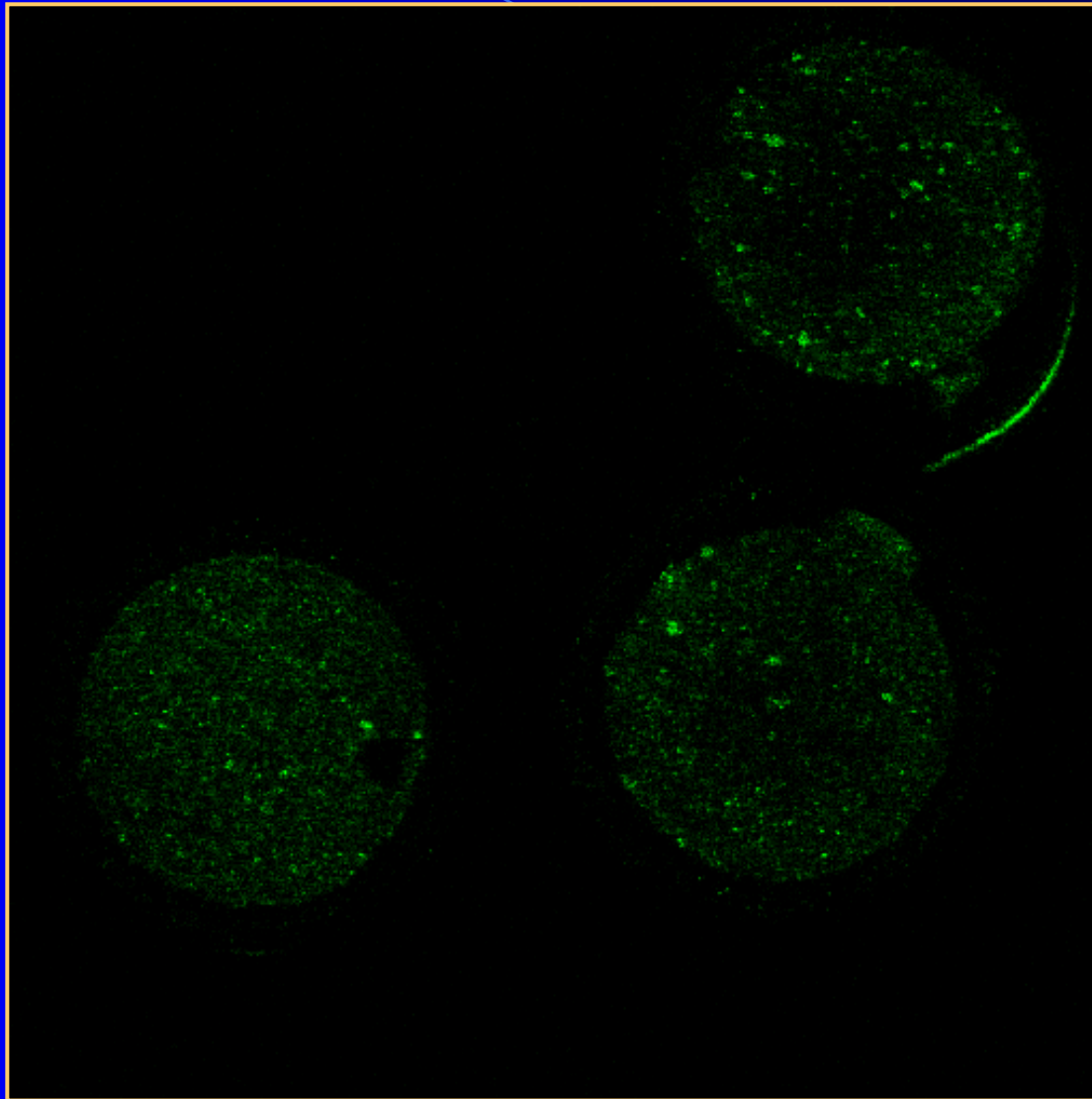
File: "9-13 Spindle 2Eggs Results part1"

First Clear Activation

- How do we quantify this?

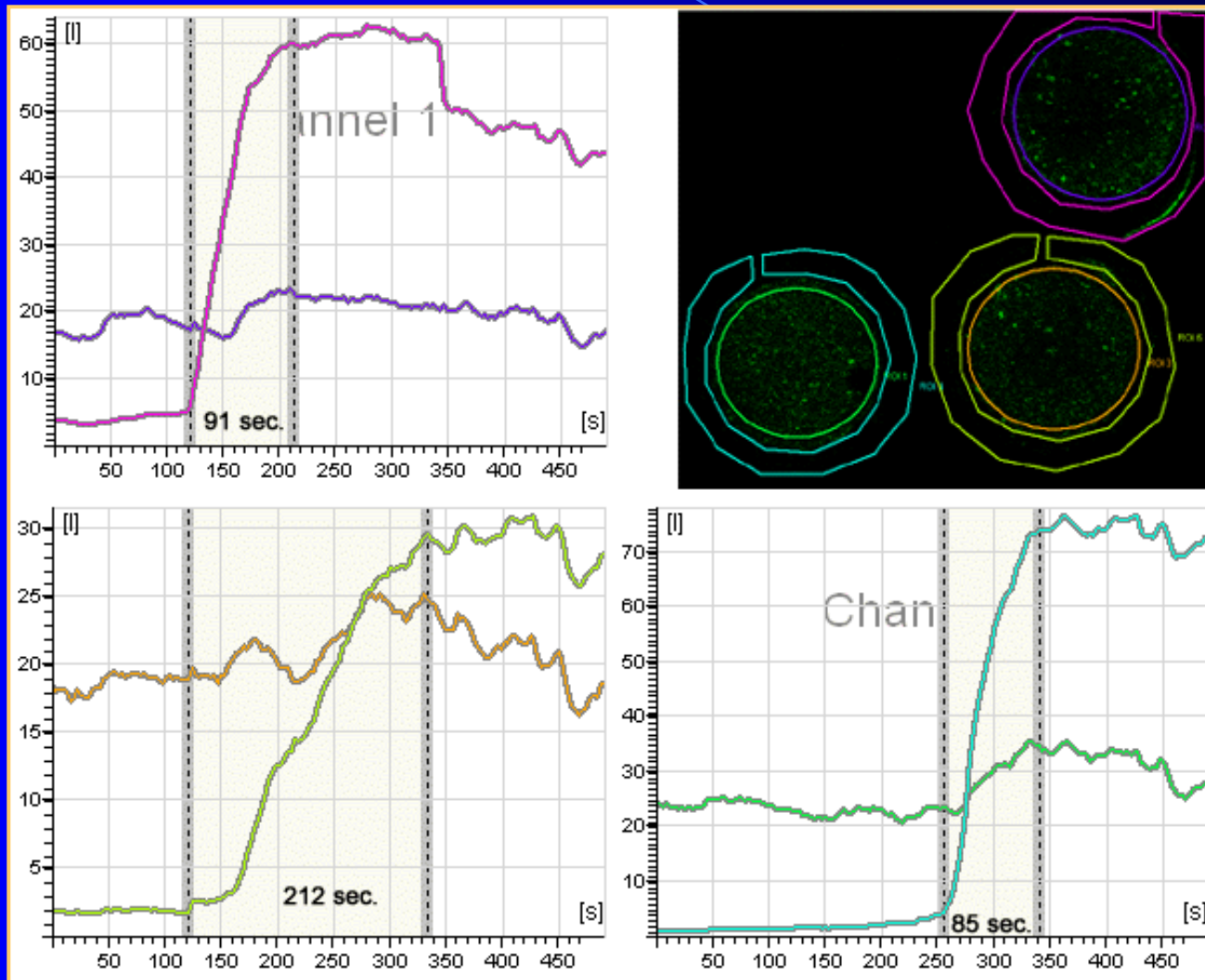


Three at Once

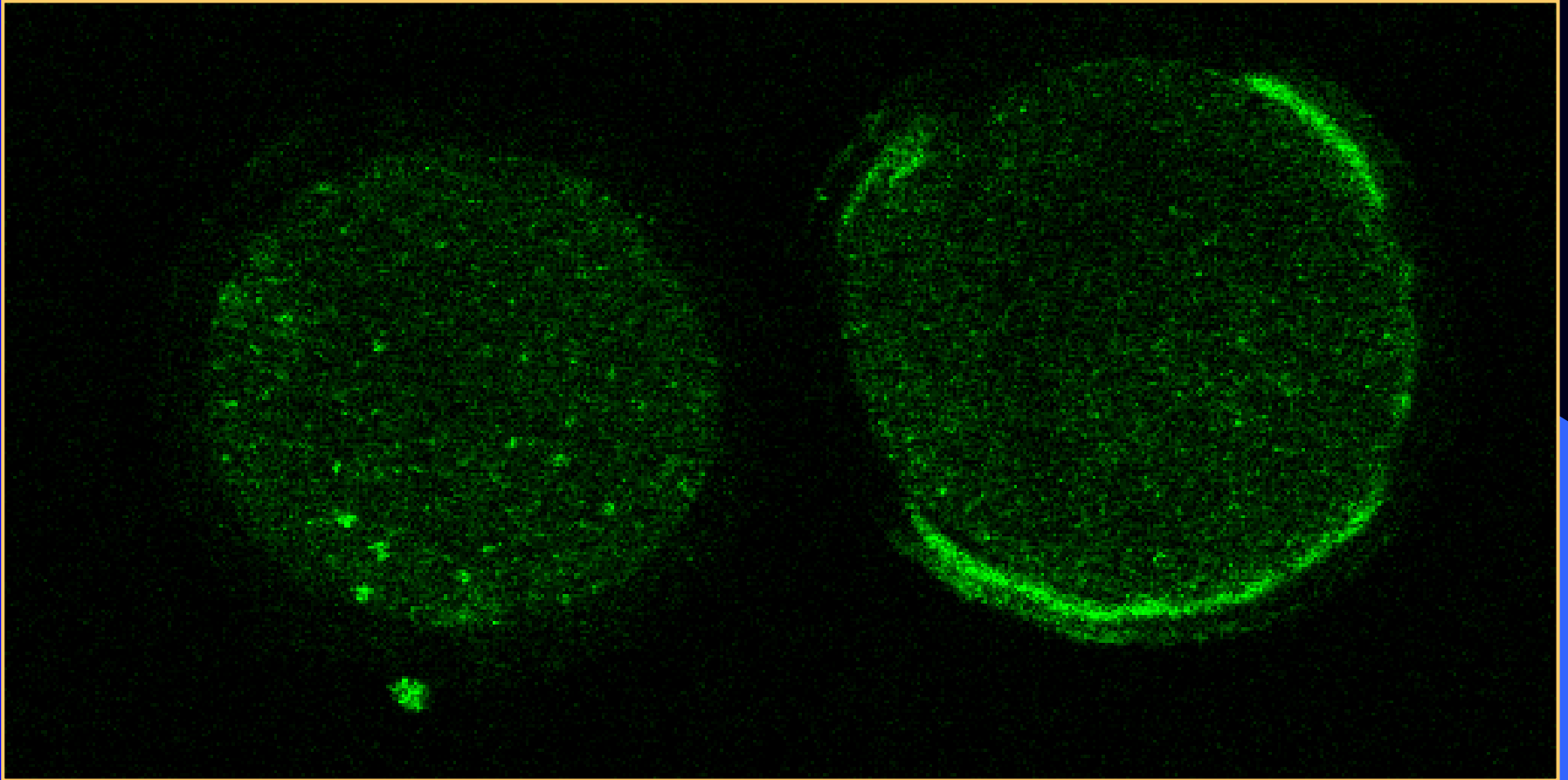


File: "9-27 num4 1st movie.avi"

Three At Once

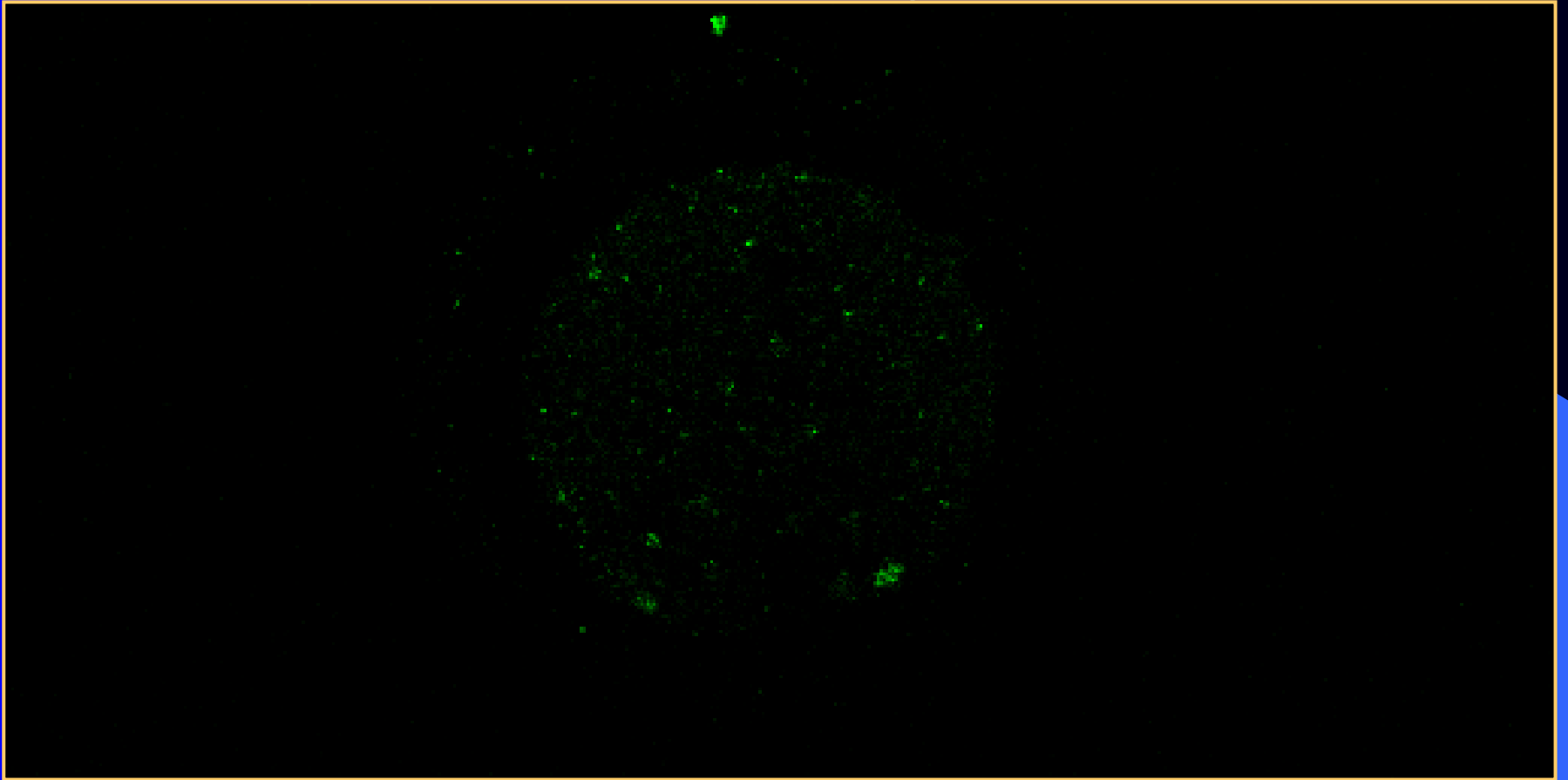


One Already Started



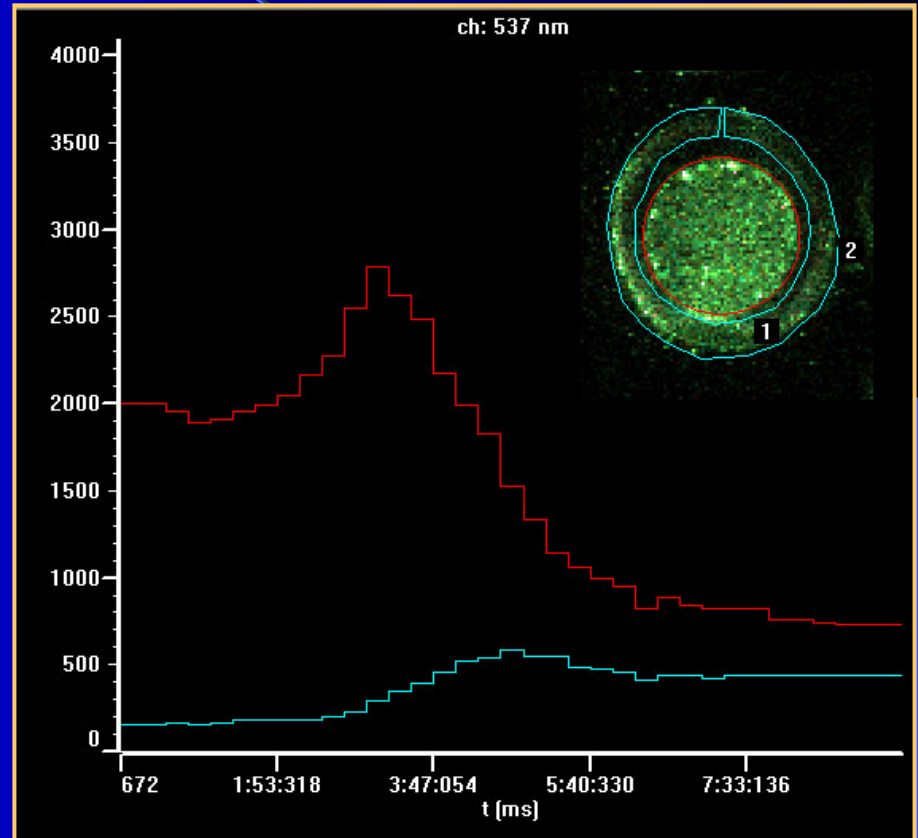
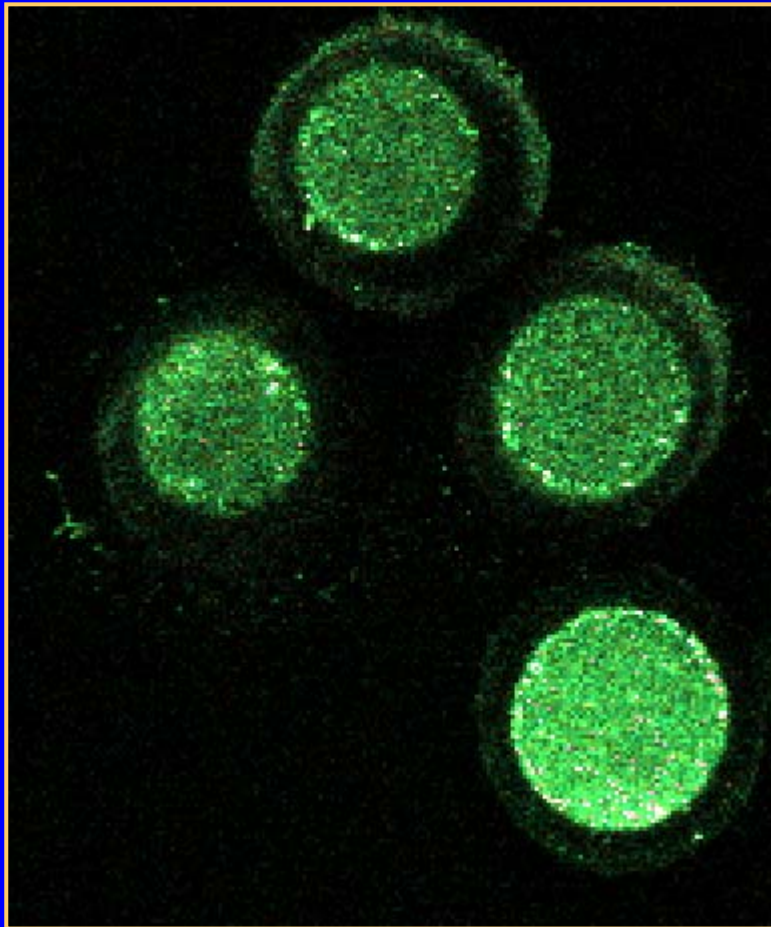
File: "9-20 2 eggs self activating.avi"

Smoothest



File: "9-27 num5 1 active egg.avi"

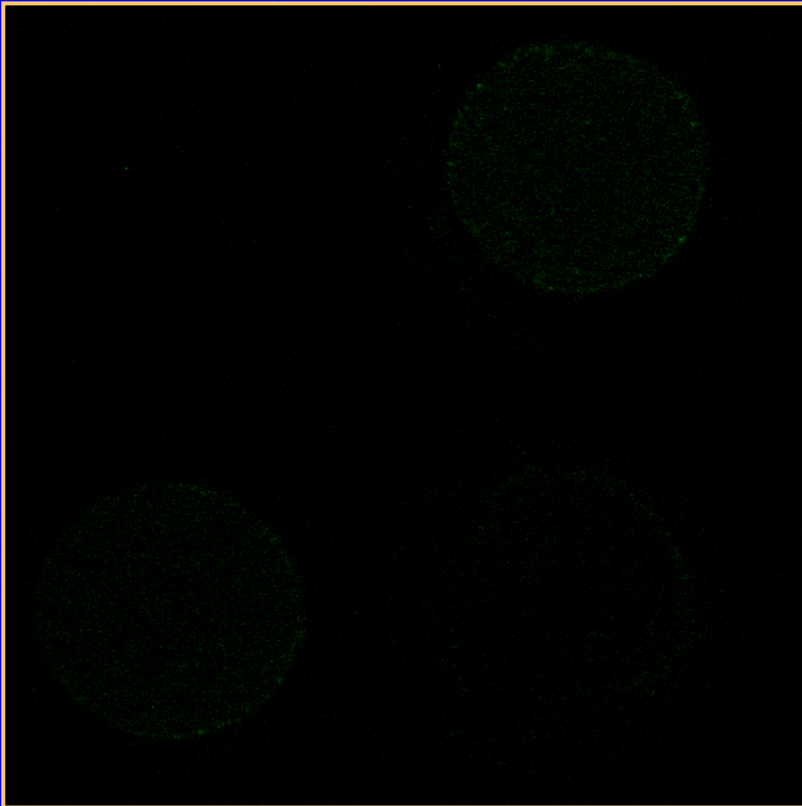
Final Movie From Nikon



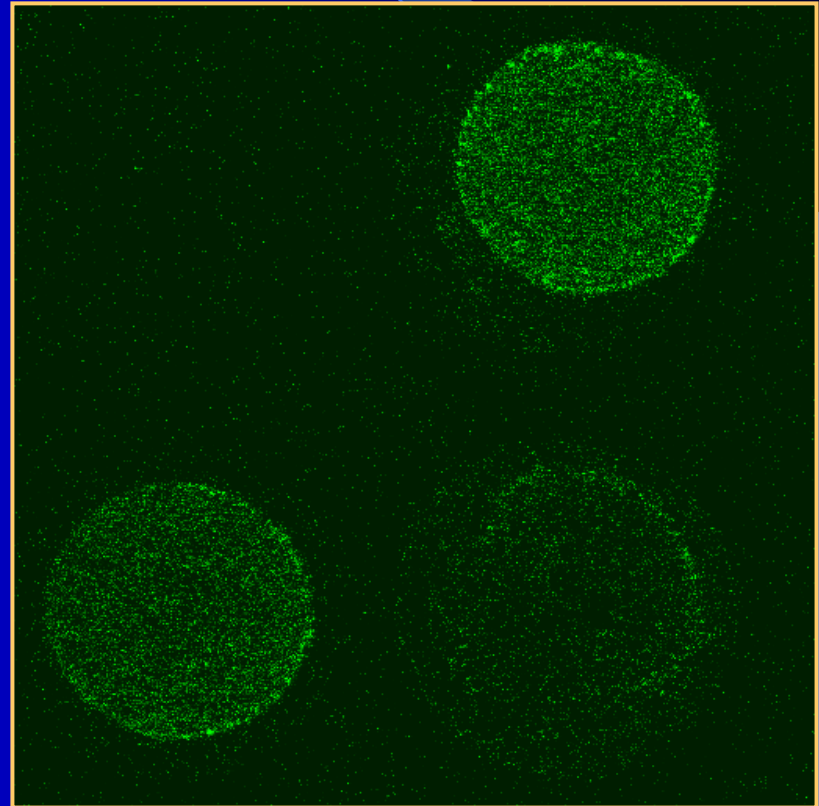
File: "10-11 Nikon.avi"

Contrast Adjustments

- Adjusting the contrast of some of our earlier experiments from August showed that we had actually achieved activation earlier than we thought.

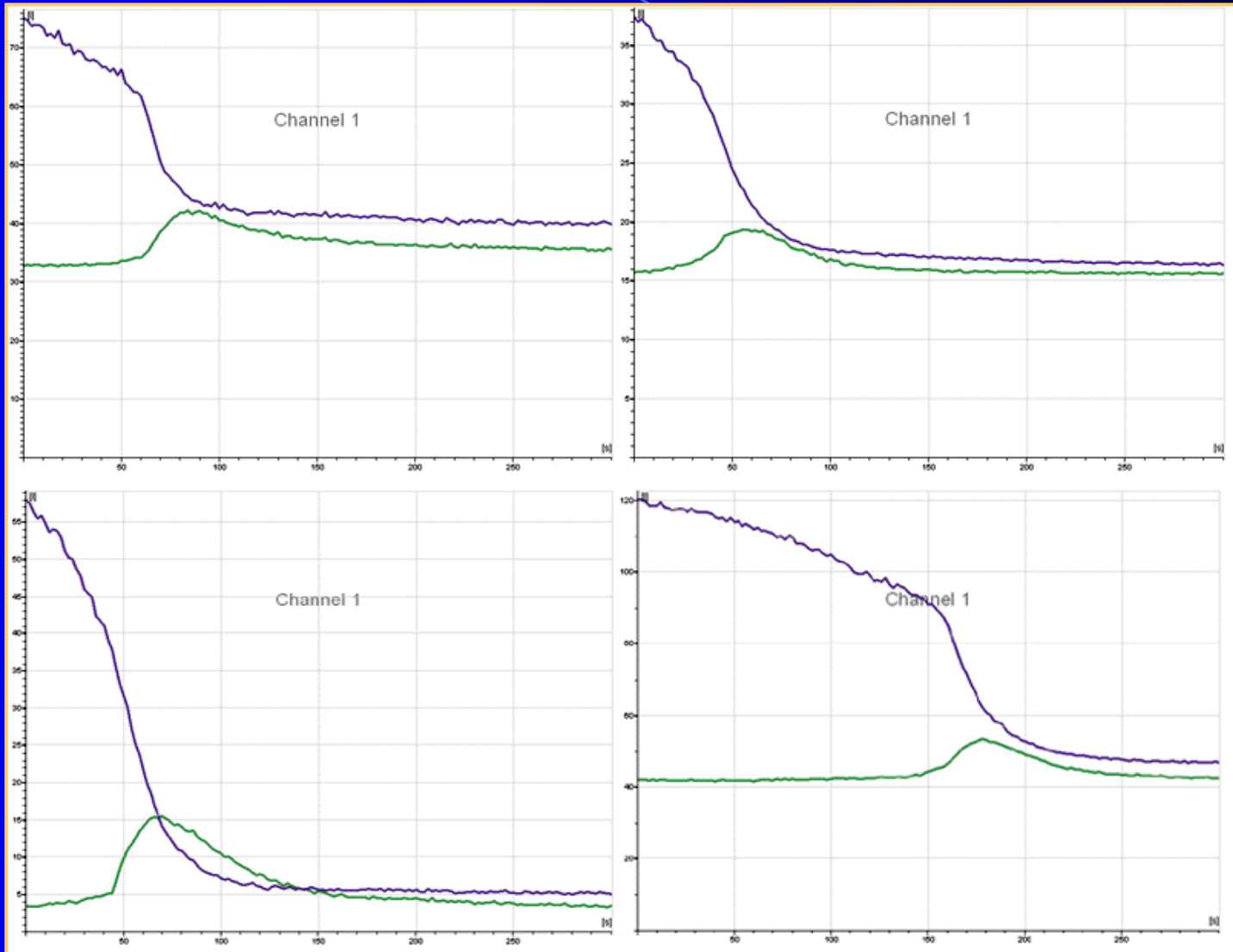


File: "8-9 First Success Dark.avi"



File: "8-9 First Success Bright.avi"

Contrast Adjustments

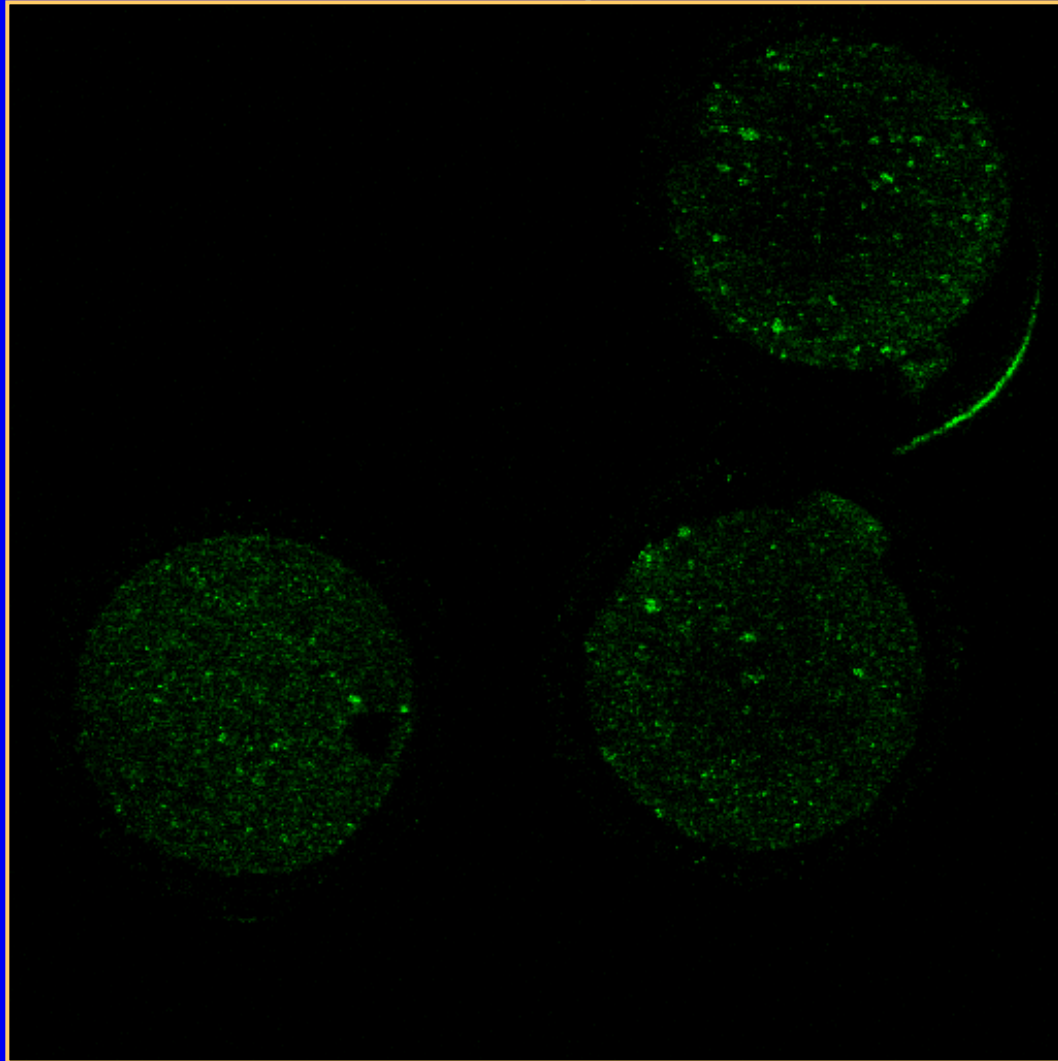


Summary of Activation Times

- Calcium waves lasted from just under 1½ minutes to over 4½ minutes
- Usually started within 1 to 3 minutes of adding eggs to plate (Uncertain due to spontaneous activations)

Date	Experiment #	Scope	Time (sec.)
9/20/2006	1	Leica	82
9/27/2006	4	Leica	85
8/9/2006	1	Leica	90
9/27/2006	2	Leica	91
8/17/2006	2	Leica	100
8/17/2006	1	Leica	110
8/10/2006	1	Leica	120
9/13/2006	1	Leica	130
9/27/2006	1	Leica	206
9/27/2006	3	Leica	212
10/11/2006	1	Nikon	260

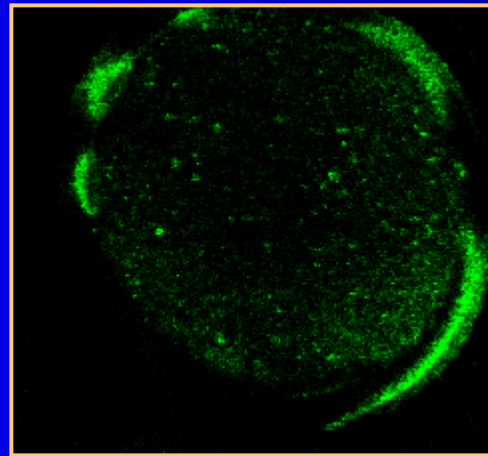
Spatial Characteristics




























File: "9-27 num4 1st movie.avi"

Spatial Characteristics

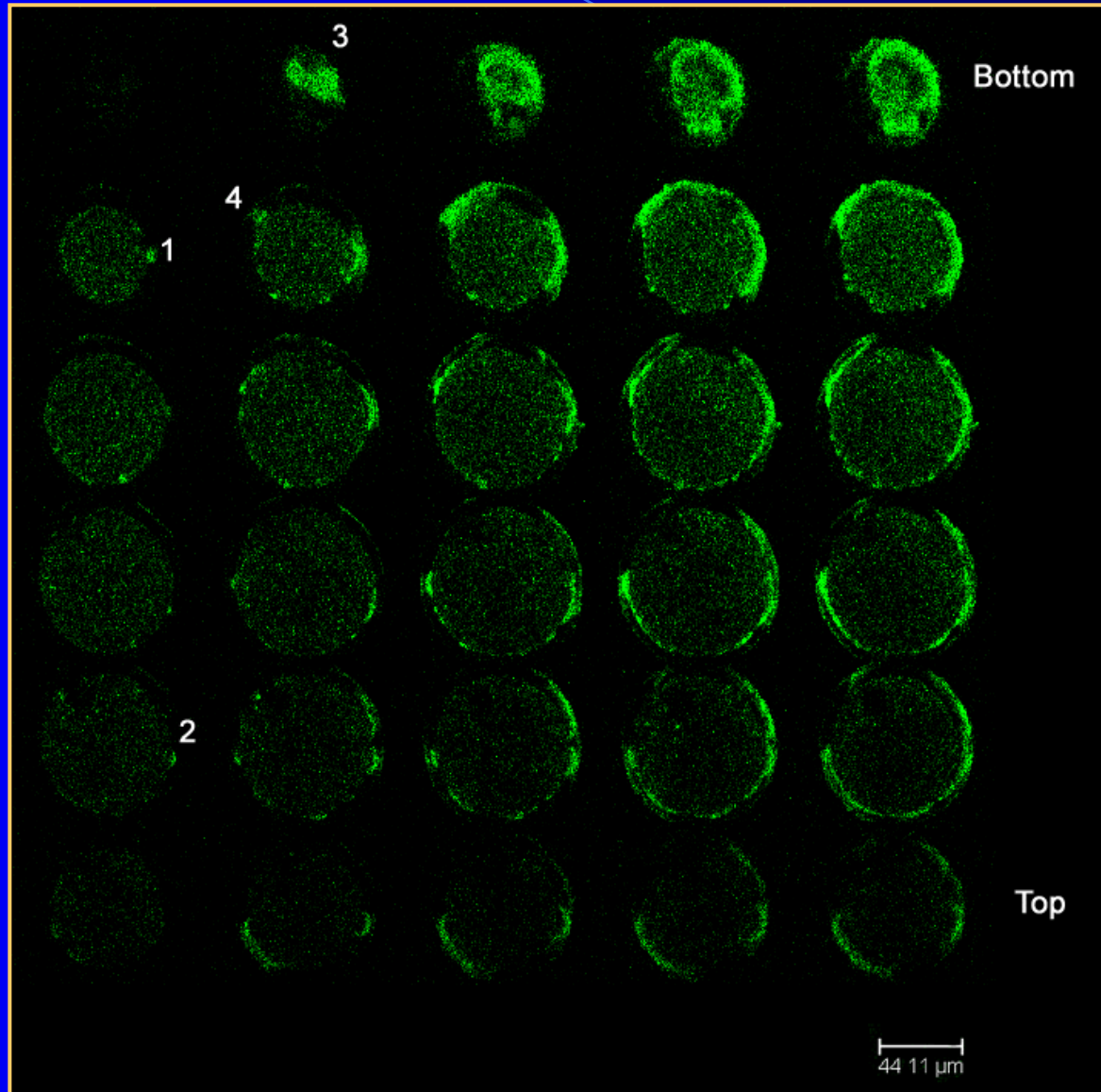
- Is the wave starting from one point or many?



"Montage" View

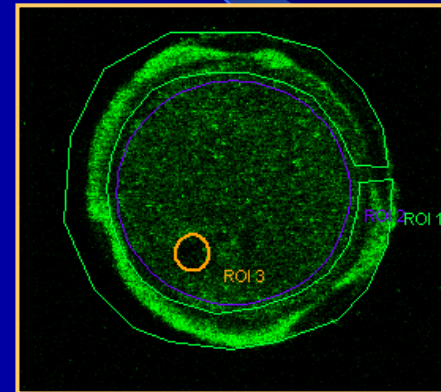
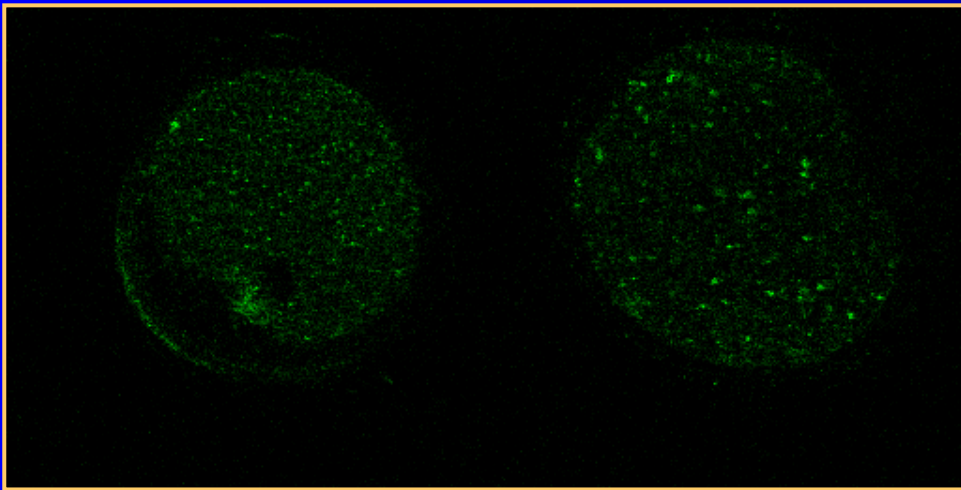
t=1	t=2	t=3	t=4	t=5
				
				
				
				
				

Spatial Characteristics

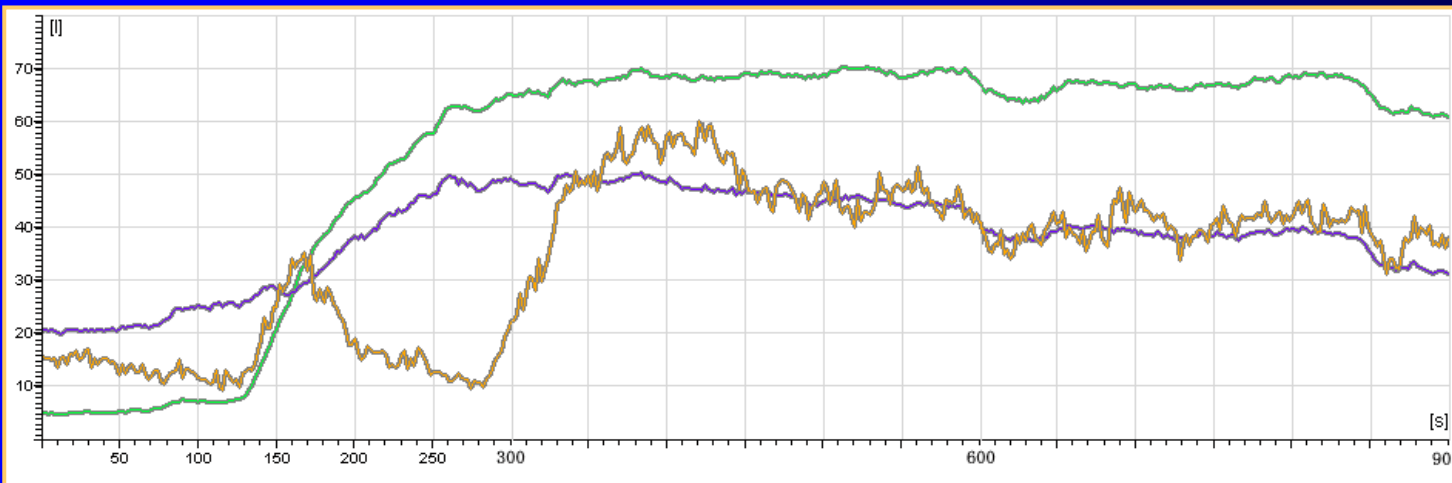


Future Research

- Why isn't calcium showing up in the spindle area?



File: "9-13 Spindle 2Eggs Results part1.avi"



Summary

- Successfully imaged several calcium waves.
 - Waves lasted from 82 to 260 seconds.
 - Waves can originate from multiple locations.
- Not able to achieve fertilization with sperm, probably due to the fact that the eggs were self-activating and keeping sperm from penetrating.

Questions?

References

1. Stricker SA, Smythe TL (2003). Endoplasmic reticulum reorganizations and Ca²⁺ signaling in maturing and fertilized oocytes of marine protostome worms: the roles of MAPKs and MPF. *Development*. Jul;130(13):2867-79.
2. Stricker SA. (2000) Confocal microscopy of intracellular calcium dynamics during fertilization. *Biotechniques*. Sep;29(3):492-4, 496, 498. Review.
3. *Manipulating the Mouse Embryo: A Laboratory Manual* by Brigid Hogan, Rosa Beddington, Frank Costantini, Elizabeth Lacy.
4. *Guide to Techniques in Mouse Development*. Edited by Paul M. Wassarman & Melvin L. DePamphilis.
5. Baluch, D.B., C.M. Pauken, and D.G. Capco. (2004). Cytoplasmic signaling and cell cycle control in the mouse egg and embryo, in "A Laboratory Guide of the Mammalian Embryo, edited by D.K. Gardner, M. Lane and A. Watson. Oxford University Press.