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## 1 Ticklers

- 1.1 Consider re-uping maintenance agreement for IDL Quote No. QTO-042209
- 1.2 **DONE** FU: Don Bers Could we set up a call?

## 2 Meetings

### 2.1 Phone call with Sabine Van Dijk

<2018-01-15 Mon>

### **2.1.1 Pre-call questions/notes**

1. I assume these are cardiac myocytes? How well was AKAP over-expressed?
  - (a) Yes. Don't really know - nothing to compare it to.
2. What worries me is that all of the taus in the grant are lower except the H89. Its as if the forskolin wasn't working.
3. Do the cells respond physiologically to the forskolin?
  - (a) She will check this.
4. What is your data acquisition rate? Can you send me a data set?
  - (a) She talked about sending me one but I didn't re-enforce it so it may not happen.
5. Can we try photobleaching more of the cell?
  - (a) Will try
6. What happens with PLB over-expression? Can we do a double expression experiment?
  - (a) Maybe for the future
7. myotubes are bigger... Should we try those?
  - (a) Maybe later
8. Should we talk again?
  - (a) Yes. Circumstances dictated 3 weeks to 1 month
9. Do you have other projects?
  - (a) She does. They are AKAP related and they aren't going well either but all of her eggs aren't in this basket

## **2.2 Phone call with Don Bers (530) 752-6517 2018-05-22**

### **2.2.1 Struggling**

1. We weren't getting adequate temporal resolution but now measuring time to 50% decline for every transient so we think that issue is solved.
2. Cells tend to die upon rapid switch (Did Lipsius do rapid switch?)
3. Cells tend to stop contracting.
4. Not at all sure Dan has the most efficient set up - sometimes misses cells and we get no response.

### **2.2.2 Wanted to talk before Friday so that a plan would be in place**

### **2.2.3 Would suggest that Dan visit California for at least 2 weeks in July**

1. Would like for him to see how you guys set this experiment up.
2. Might be helpful for you to see what's going wrong
3. Forces separation from other lab and allows Dan to concentrate on the experiments.

2.2.4 Don agreed with this plan. Suggested I come out for a couple days toward the end of the visit.

### 2.3 AKAP Meeting

2.3.1 Ken and Sabine doing ISO and epinephrine kinetics on AKAP KO.

2.3.2 Interfering peptide with FRAP

2.3.3 Tom - FRAP experiments

2.3.4 Sabina is seeing striations with AKAP expression - I never did.

2.3.5 She will try decreasing exprssion time.

2.3.6 Sending Dan to Bers Lab in July to work with Ken. I will join the last couple days

2.4 CRC Laboratory Representative Meeting; Cohn Auditorium <2019-04-05 Fri 11:00>-<2019-04-05 Fri 12:00>

## 3 Notes

3.1 You were having trouble with 2019-06=19-12. You were just about to reanalyze with new parameters for 25 micron band. Try these.

### 3.2 Ideas for Dan

3.2.1 TG to inhibit phosphorylation of some of the PLB

3.2.2 Partial Ca channel block

3.2.3 Even slower stimulation rate

### 3.3 The Biophysicist?

Announcing The Biophysicist: A New Journal from BPS

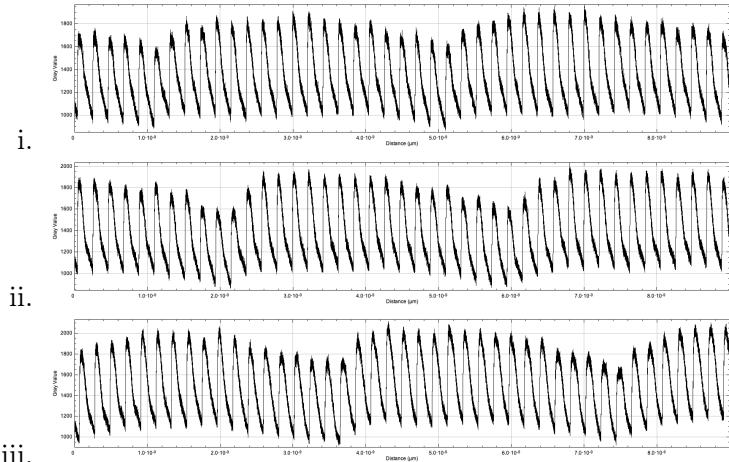
### 3.4 2019

3.4.1 963.64 us/line for this data

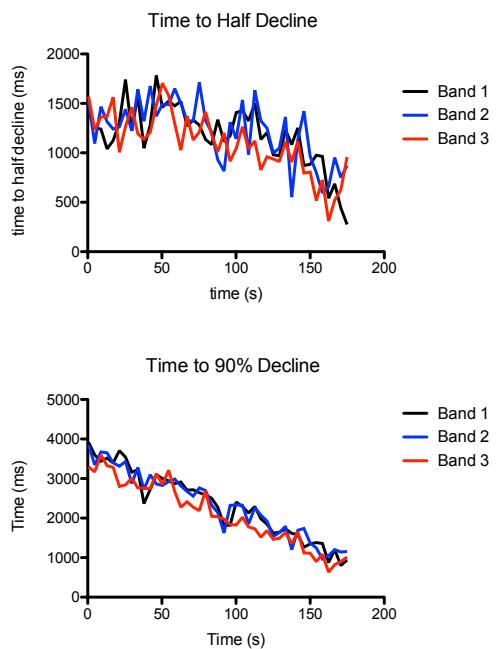
3.4.2 2019-05

1. 2019-05-31

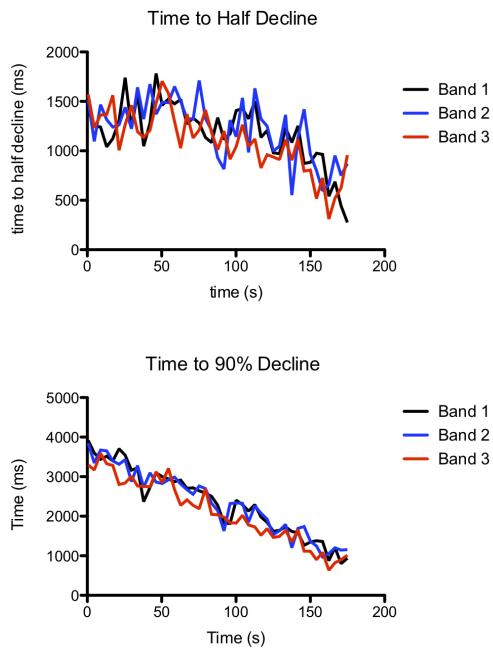
(a) 2019-05-31-4



- iv. Supper noisy transients. Even if there was something there I don't think I'd pick it up.
- v. Did not analyze B and C



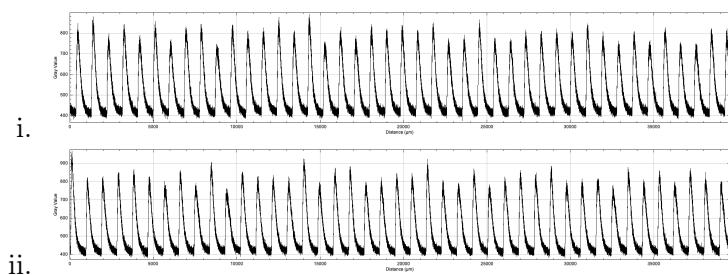
A.

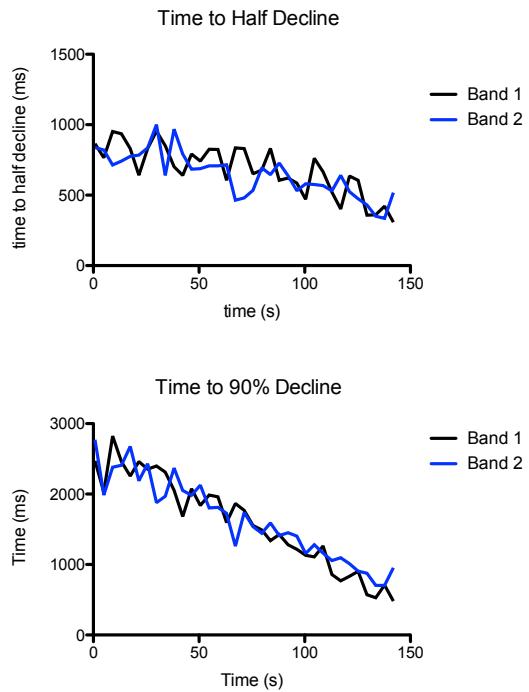


B.

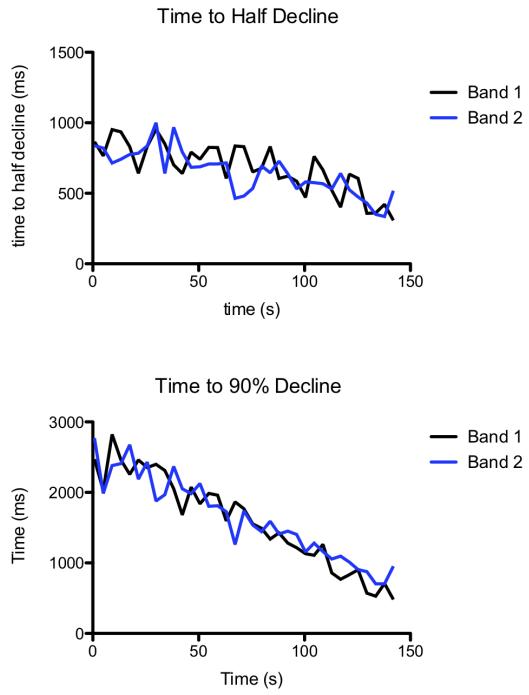
C. Rhod2-LineScanISO0531194.pzf

(b) 2019-05-31-6





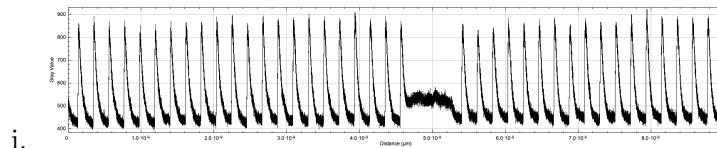
iii.



iv.

v. Rhod2-LineScanISO0531196.pzf

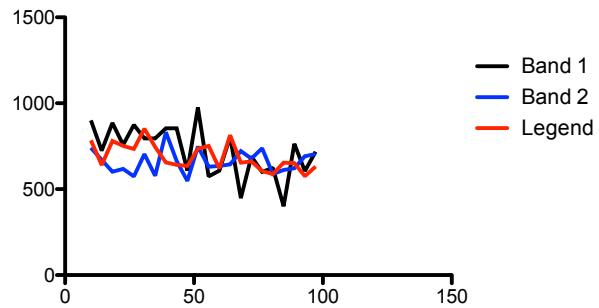
(c) 2019-05-31-7



i.

ii. This one didn't have transients that appeared to shorten that much.

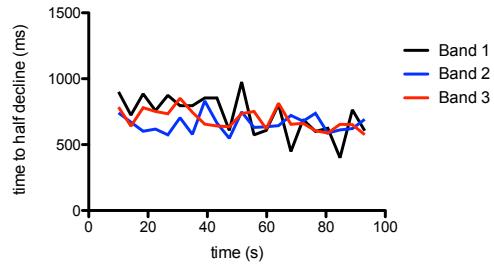
Data 1



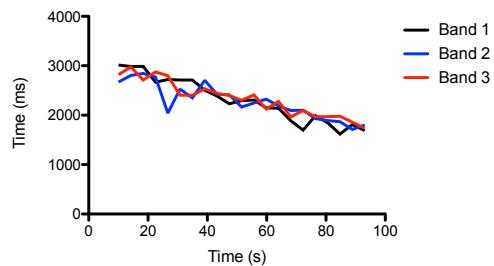
iii.

iv. This analysis didn't go well.

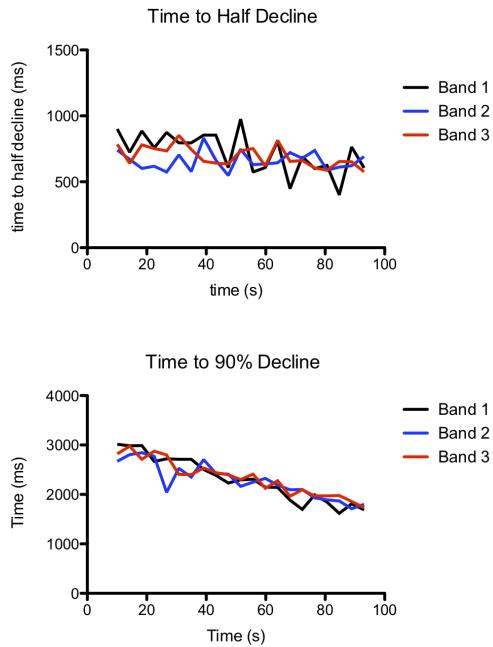
Time to Half Decline



Time to 90% Decline



A.

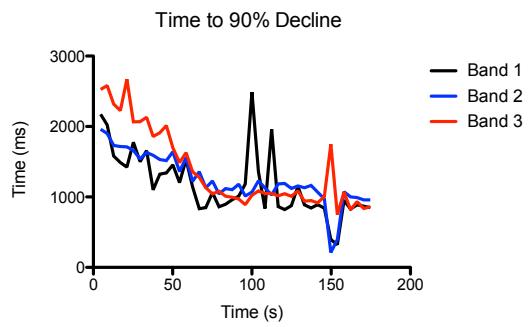
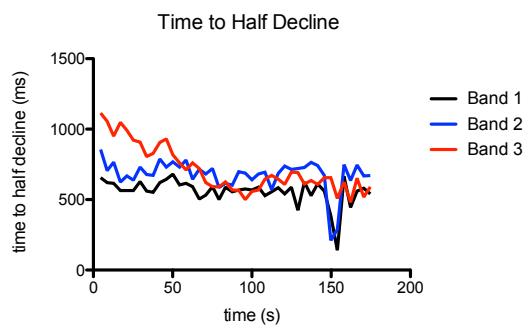
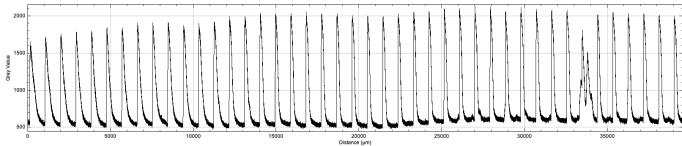


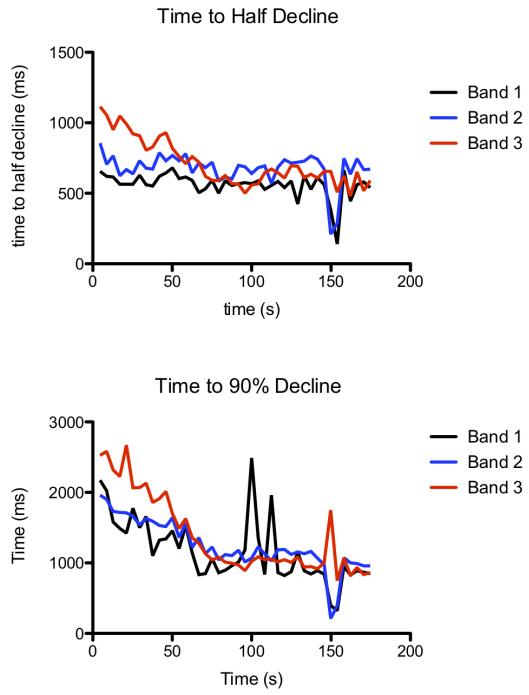
B.

- C. Rhod2-LineScanISO0531197.pzf
- D. The x-axis appears to be messed up so I was guessing as to how many lines in I should make the first transient.
- E. Had to round the interval between beats to an integer which probably introduced a small time error that accumulated over many beats.
- F. It also appears that the stimulation rate wasn't exactly 0.5 Hz.

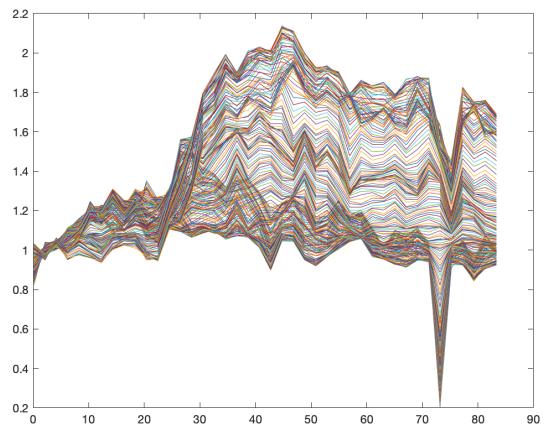
### 3.4.3 2019-06

1. 2019-06-04
  - (a) 2019-06-04-1
    - i. Initial Analysis



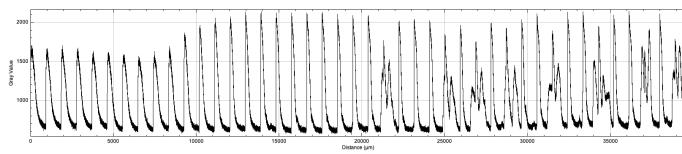


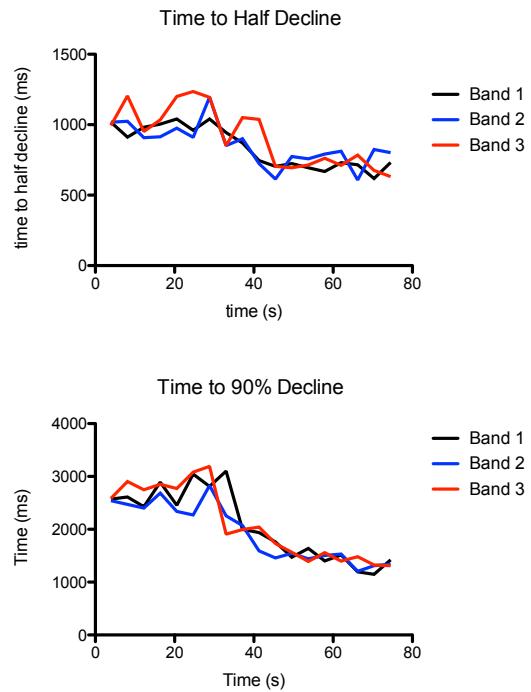
- A. Rhod2-LineScanISO0604191.pzf  
 ii. Reanalysis 2019-06-19  
 A. 2019-06-04-1 and -2 This data's no good. It looks like there might be a gradient in the transients but the peaks are almost random as a function of space.

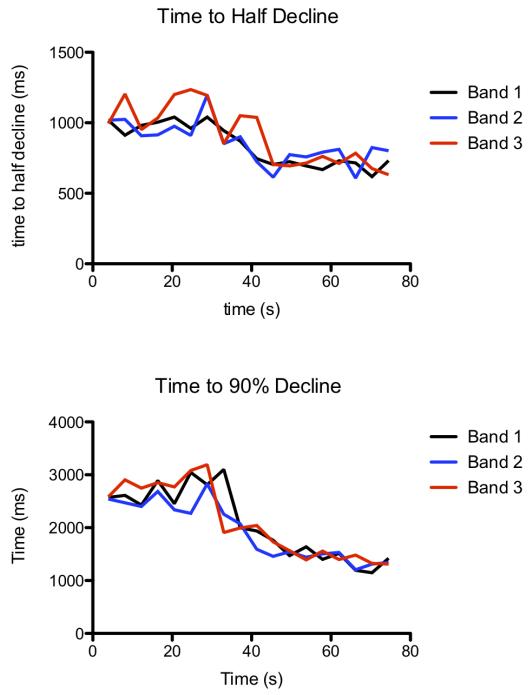


(b) 2019-06-04-02

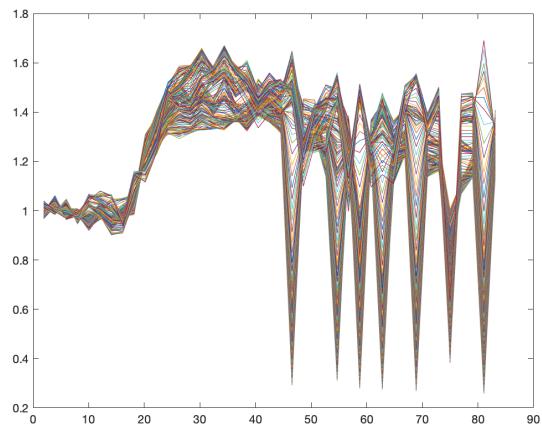
i. Initial Analysis







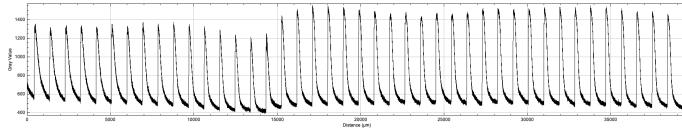
- A. Rhod2-LineScanISO0604192.pzf  
 ii. Reanalysis 2019-06-19  
 A. 2019-06-04-1 and -2 This data's no good. It looks like there might be a gradient in the transients but the peaks are almost random as a function of space.



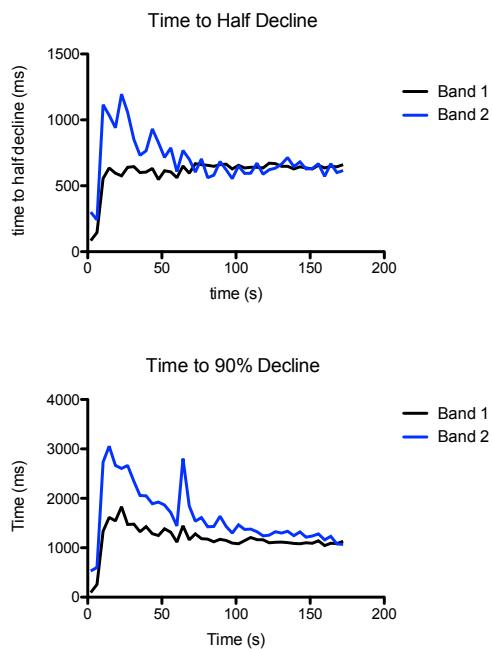
2. 2019-06-06

(a) 2019-06-06-5

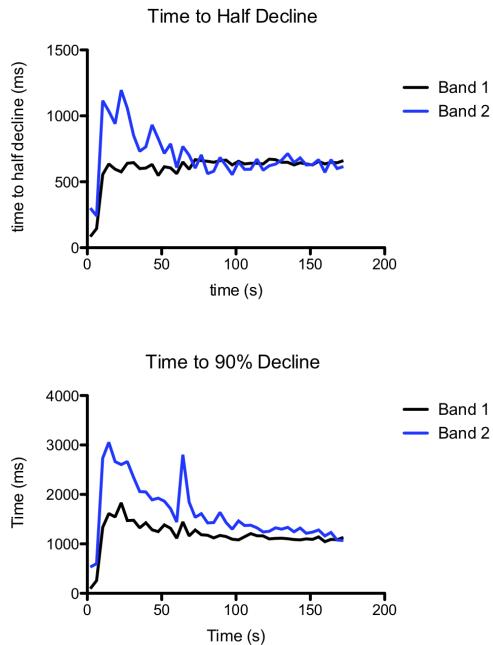
i. Initial Analysis



A. Seems to get shorter



B.

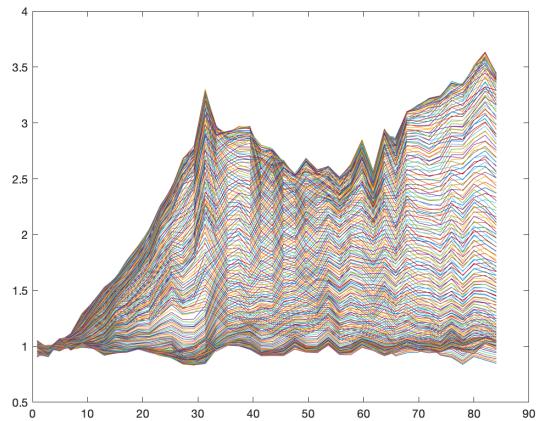


C.

- D. Rhod2-LineScanISO0606195.pzf
- E. Hmmmm... BAnd 2 may have shortened a bit but no response from Band 1 at all. Odd cell.

ii. Reanalysis 2019-06-19

- A. pretty noisy. The peak transient data migh tbe useful.



### 3. 2019-06-07

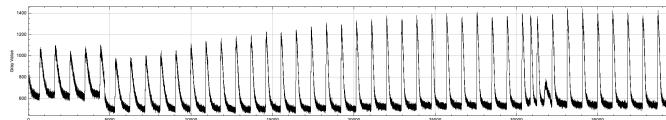
#### (a) Initial Analysis

- i. The analysis of these cells indicated a possible affect on the decline to 50%. I'm not sure , though. The linear nature of the decline makes me think that as the transients gradually become more off center the time to 90% gets shorter just because the transient never gets to 90% of the pak i.e. the "time to 90%" is actually the time to the end of the segment. Still, its always band 3 that seems to be hitting this limit. So the decline must still be slower...

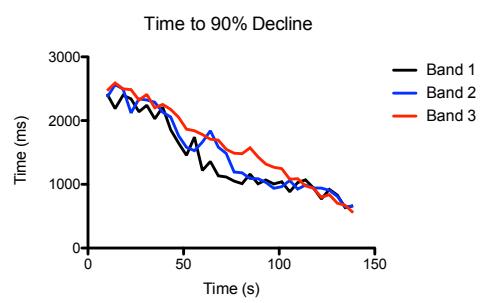
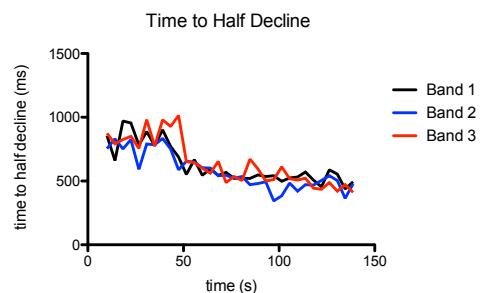
#### ii. **I think we need some controls**

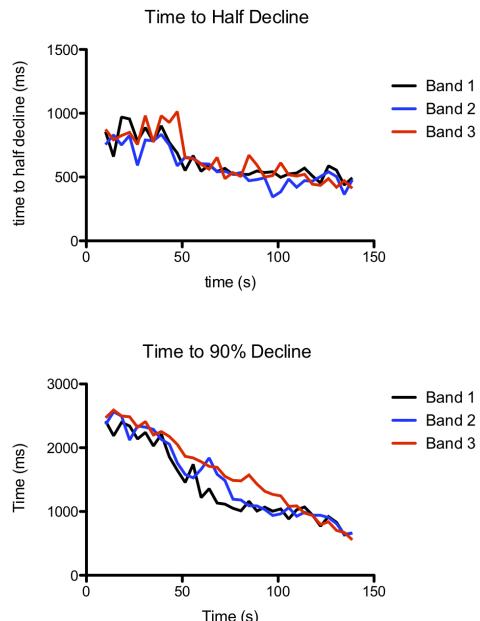
#### (b) 2019-06-07-2

##### i. Initial Analysis

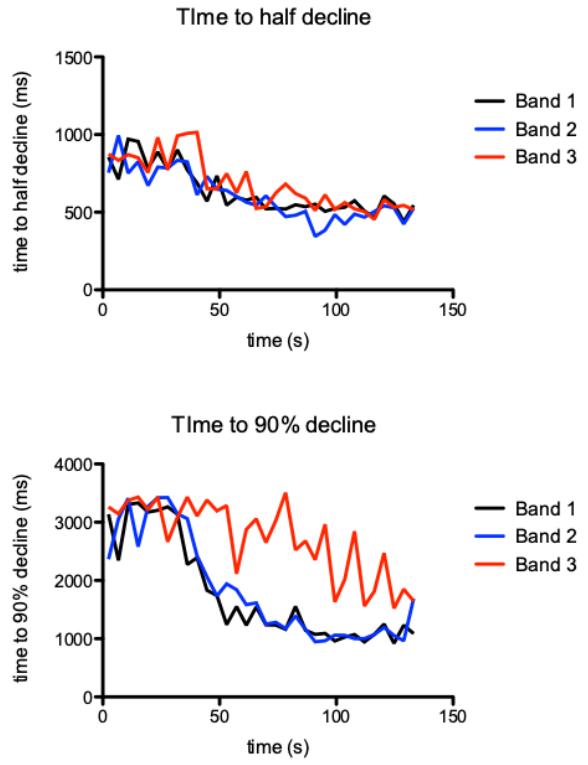


- A. Definitely shorter
- B. *[2019-06-10 Mon]* Analysis indicated no difference in time to half decline but there might be a difference in the time to 90% decline. I may have to smooth this data a bit.



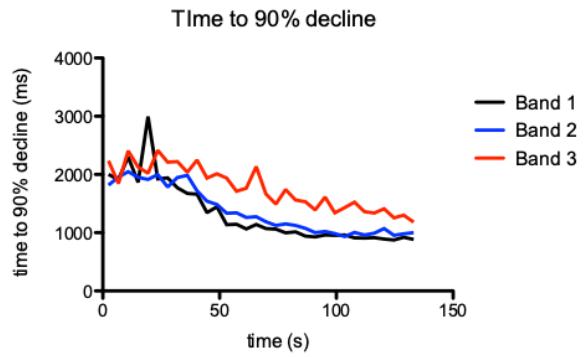
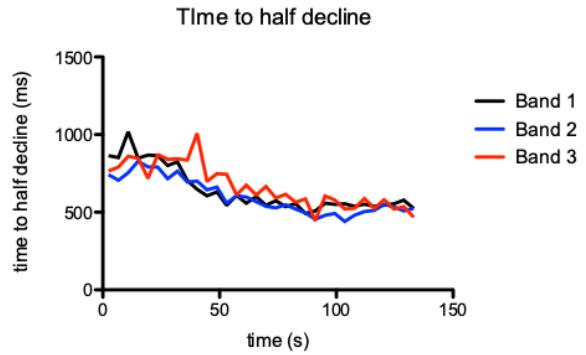


- C. Rhod2-LineScanISO0607192.pzf
- D. *[2019-06-11 Tue]* Reanalysis
- E. Reanalysis 1 was simply playing with the times to center the transients
- F. Rhod2-LineScanISO0607192R1.pzf



G.

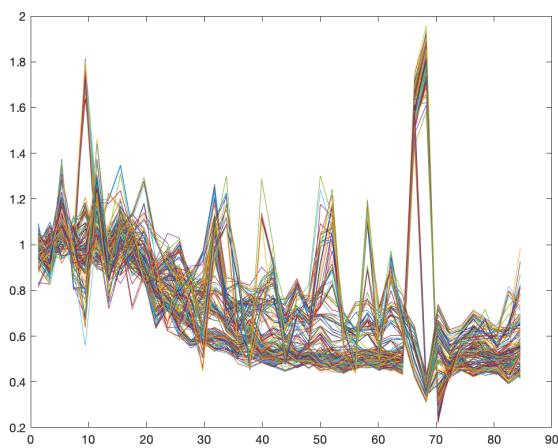
H. Reanalysis 2 was reanalysis 1 with a 9 point box car smooth

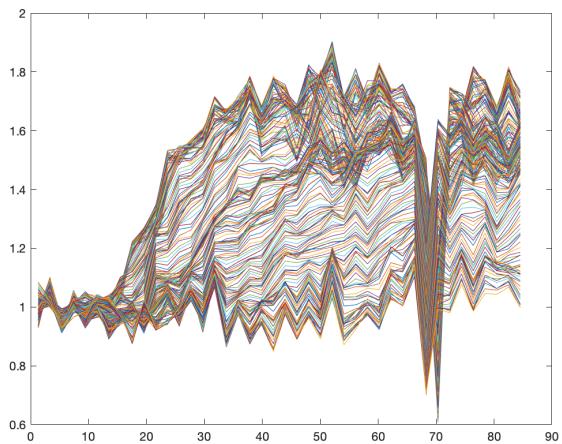
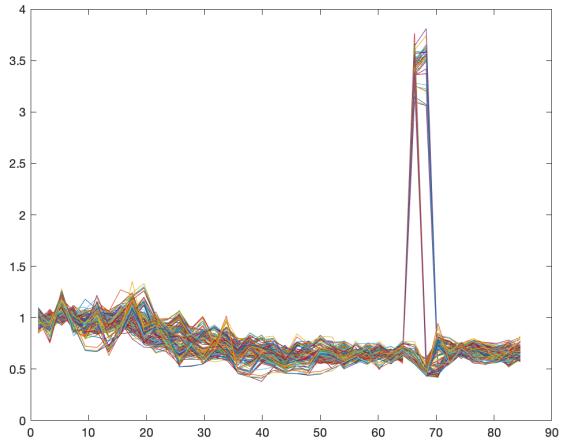


I.

J. Rhod2-LineScanISO0607192R1.pzf

ii. Reanalysis 2019-06-18



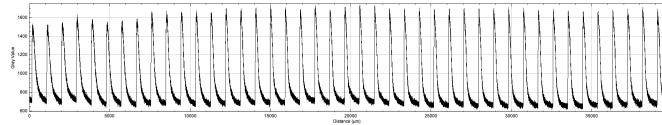


- A. There might be a gradient in the tt90 data but I wouldn't bet that it will amount to anything. Nothing in the tt50 data.

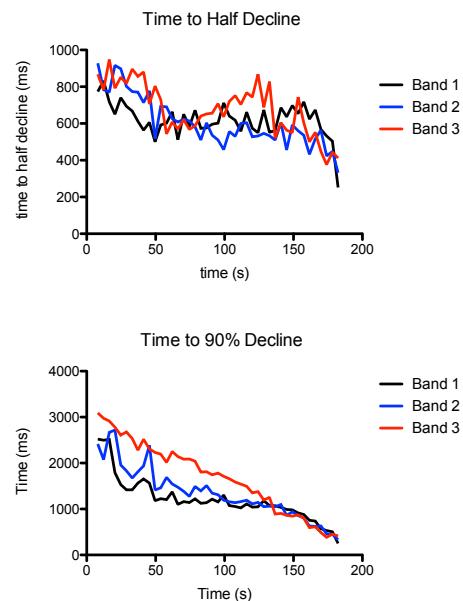
The transient peak data shows a definite gradient. The 25 um band data looks better.

(c) 2019-06-07-3

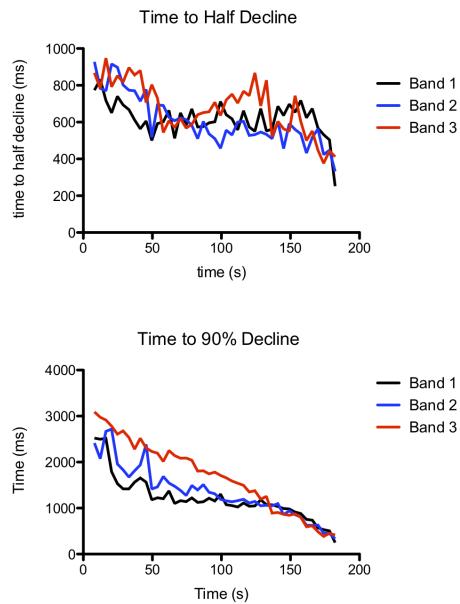
i. Initial Analysis



- A. Reaction to ISO not as apparent as 2
- B. Couldn't quite get all of the cell into the analysis. It looked like there was still some fluorescence left at the very end of the spacial profile.



C.

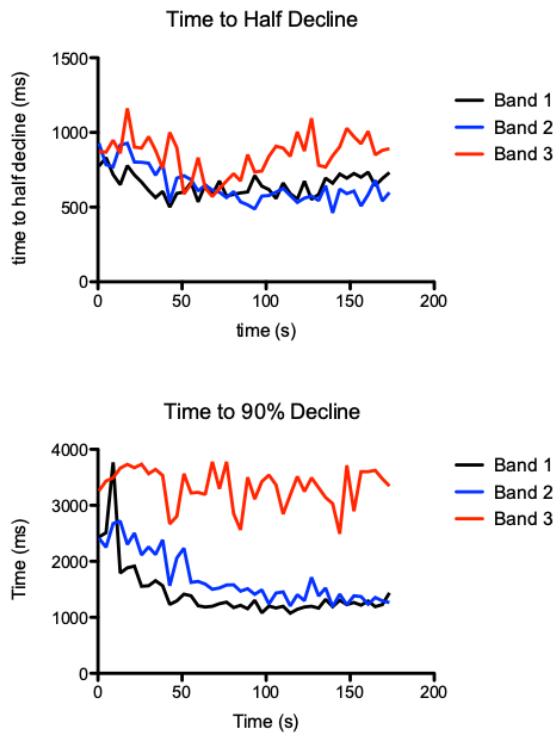


D.

E. Rhod2-LineScanISO0607193.pzf

F. Again, it looks like the difference is in the time to 90% decline. I know that some of the transients at the end of this must be messed up because I was only getting half of the transient to analyze. Still the difference looks like its there in the early data when I know the analysis is good.

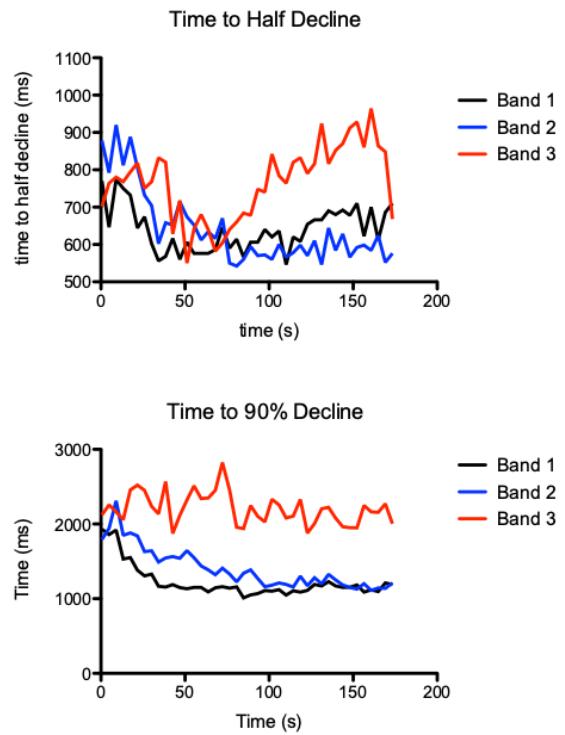
G. Reanalyzed this one twice as with 2019-06-07-2



H.

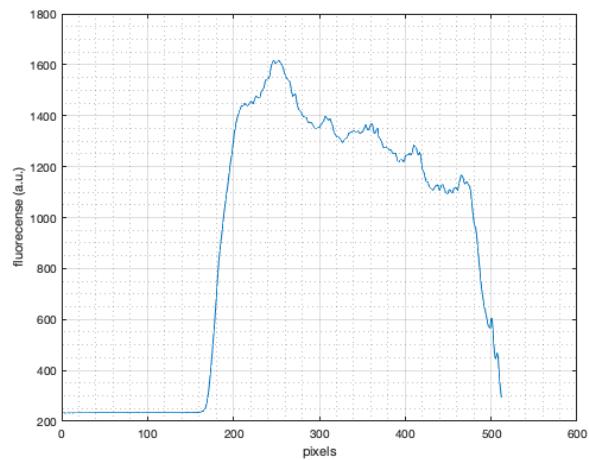
I. Rhod2-LineScanISO0607193R1.pzf

J. Rhod2-LineScanISO0607193R2.pzf

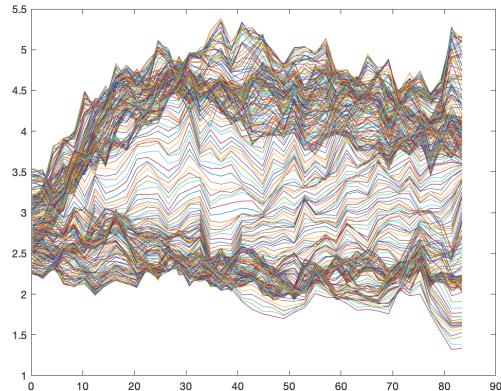


K.

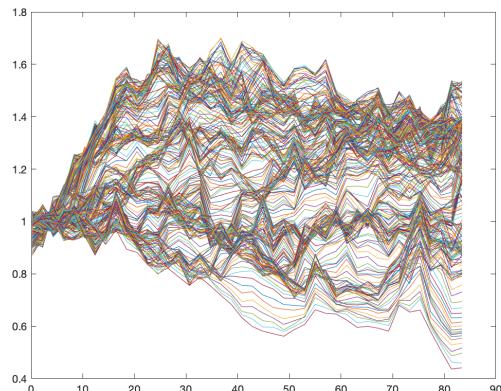
ii. Reanalysis 2019-06-18



A. Note that the profile for this cell went all the way to the edge. Used 500.

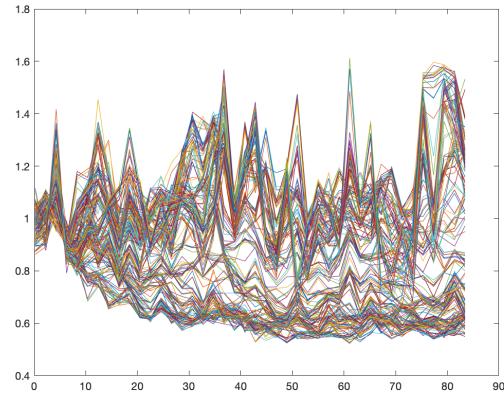


B.

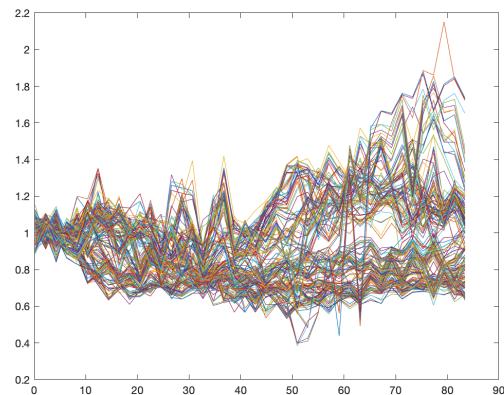


C.

D. This data is noisy but there's a gradient there. Unfortunately the peaks were unsteady and a bit up and down. I don't know how usable this data will be.

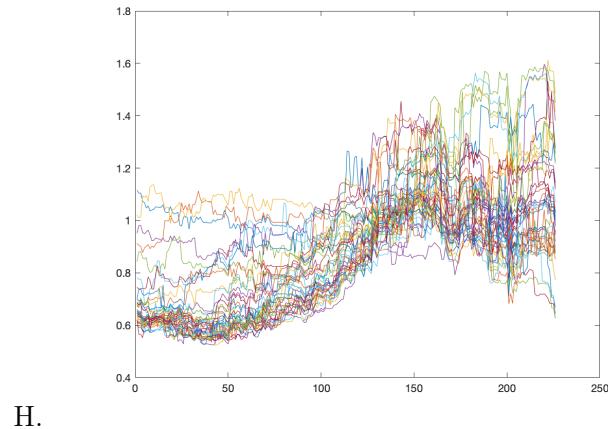


E.



F.

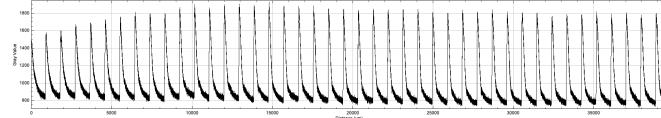
G. Can probably use the tt90 data but not the tt50.



I. ISO apparently hit the cell about halfway up.

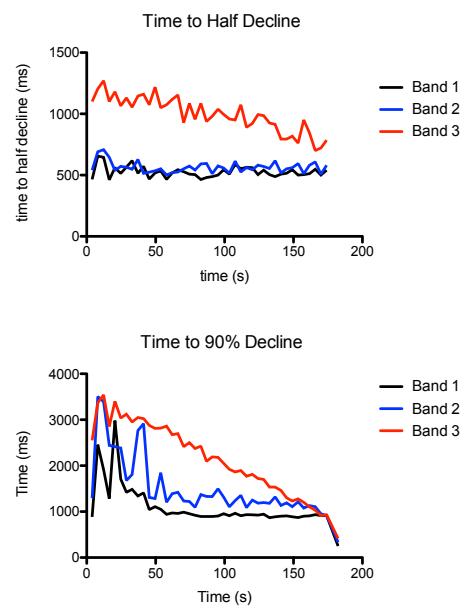
(d) 2019-06-07-4

i. Initial Analysis

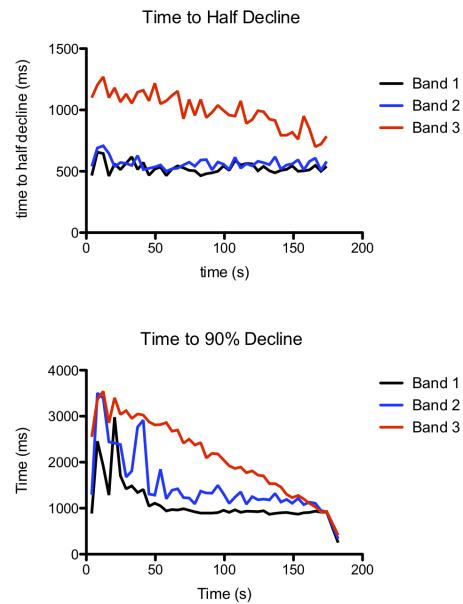


A. Again reaction not as apparent as 2.

B. Upon analysis, I don't think anything is here.



C.

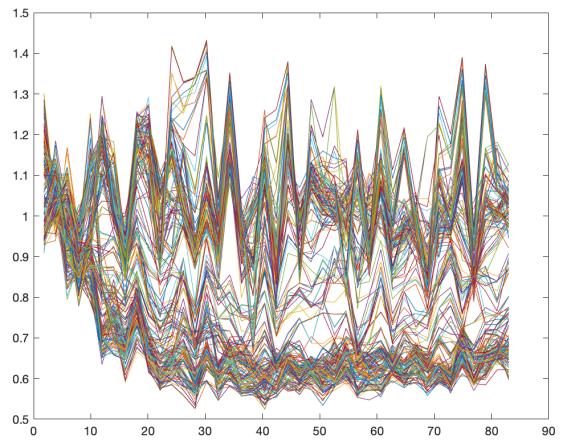


D.

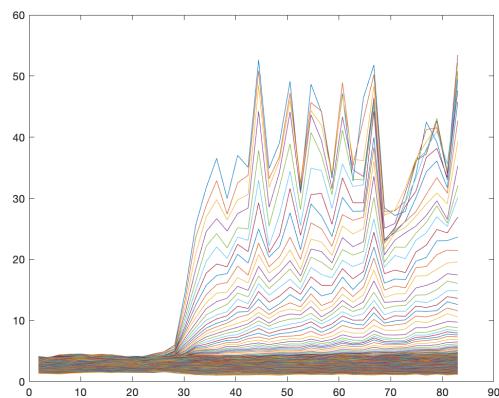
E. Rhod2-LineScanISO0607194.pzf

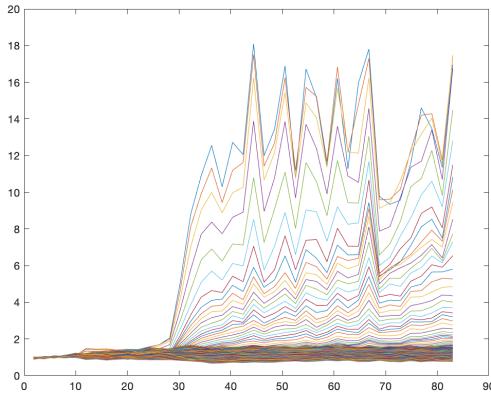
F. Maybe a slower decline to 90% in band 3 as with the other cells. But the linear nature is suspicious.

ii. Reanalysis 2019-06-18



A. The gradient is now evident here. There's nothing  
thee in the time to hald decline state.





- B. It appears that there is also a gradient in the peak transient.
- C. Both of these seem to show no response high up in the cell near the top. The ISO response doesn't seem to propagate all the way throughout.

#### 4. 2019-06-11

- Note taken on */2019-06-16 Sun 09:13/*

These are all control cells where the ISO simply wasn't turned on. So they are all just being stimulated at steady-state. I just wanted to confirm that there aren't any relevant artifacts being added by the analysis.

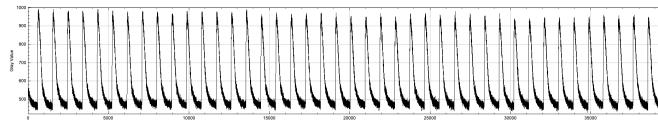
##### (a) Re: More analysis

I got 3-5 Control-Tyrode cells today, some better than others but it is clear that there was no increase in transients over the course of the run. There was a shift with some either up or down a little at the start of the perfusion but no sign of any increase and in contrast for some the transient actually decreases toward the end which is not seen with the ISO cells. So you can get the data tomorrow if possible I think that these will suit your purpose.

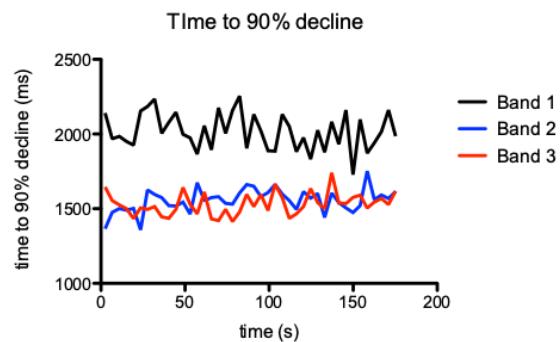
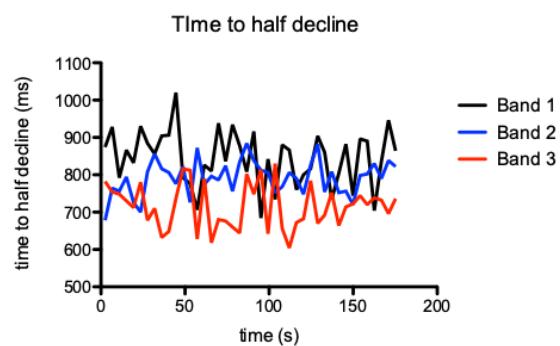
Also, I'm doing a cell isolation again tomorrow and Thursday since I can not do one Friday ( Jiajie needs to use the confocal ) and will attempt to get more ISO perfused cells.

(b) 209-06-11-05

i. Initial Analysis



A. Rhod2-LineScanCTRL0611195.pzf

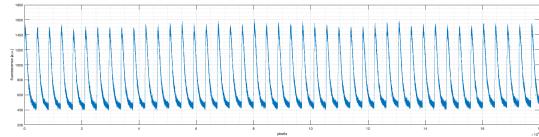


ii. Reanalysis

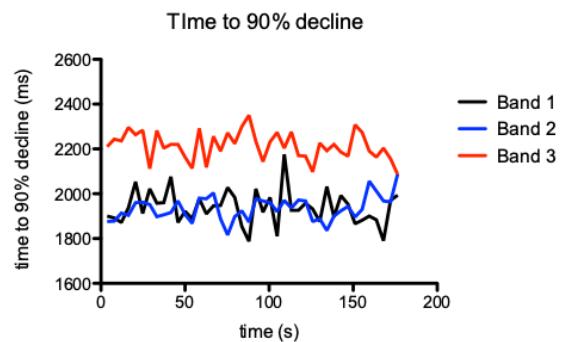
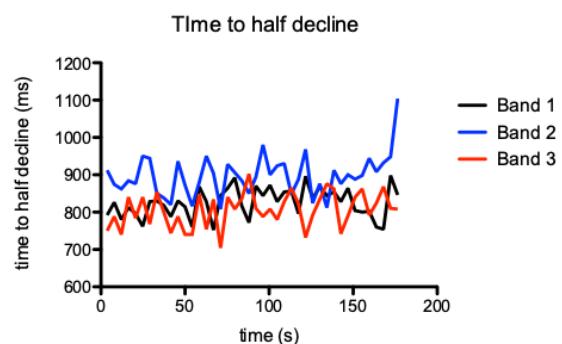
A. The reanalysis of this data using the moving average still showed no artifacts due to analysis.

(c) 2019-06-11-06

i. Initial Analysis



A. Rhod2-LineScanCTRL0611196.pzf

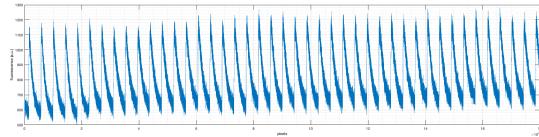


ii. Reanalysis

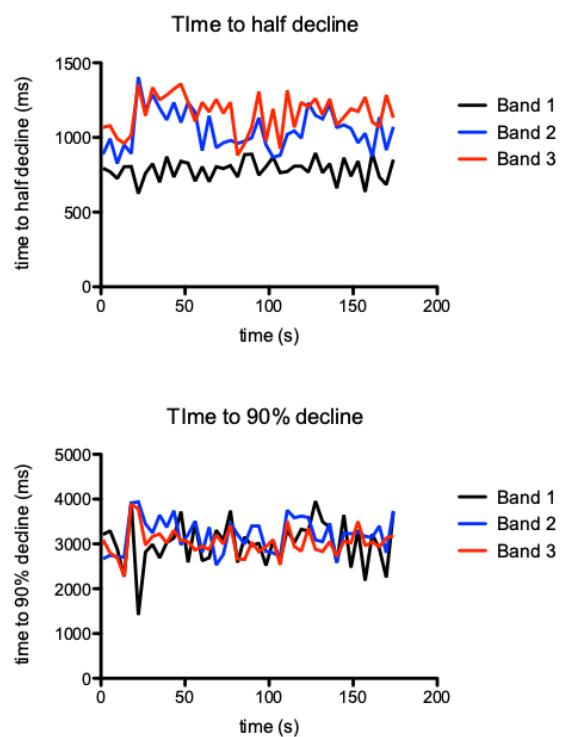
A. The reanalysis of this data using the moving average still showed no artifacts due to analysis.

(d) 2019-06-11-07

i. Initial Analysis



A. Rhod2-LineScanCTRL0611197.pzf

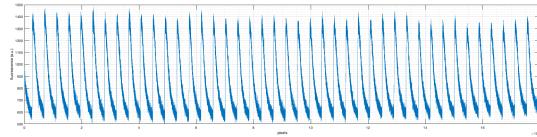


ii. Reanalysis

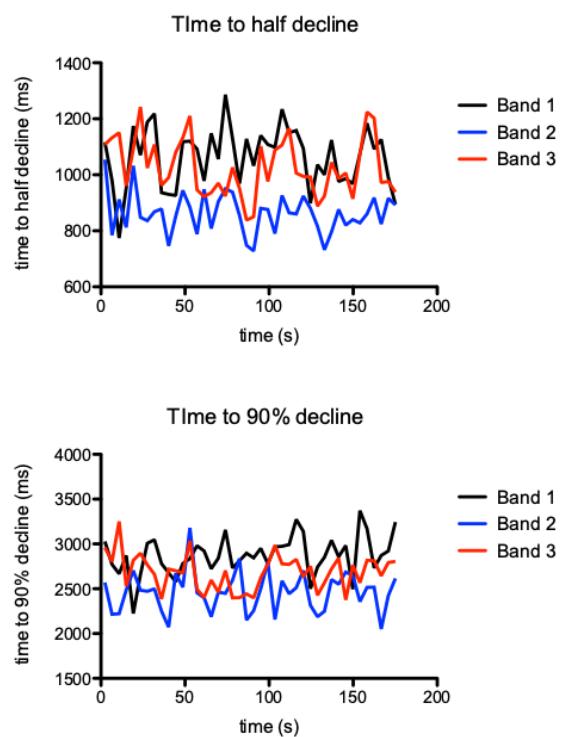
A. The reanalysis of this data using the moving average still showed no artifacts due to analysis.

(e) 2019-06-11-08

i. Initial Analysis



A. Rhod2-LineScanCTRL0611198.pzf



## ii. Reanalysis

- A. The reanalysis of this data using the moving average still showed no artifacts due to analysis.

(f) 2019-06-11-09

### i. Initial Analysis

- A. Very weird transients in band 3.

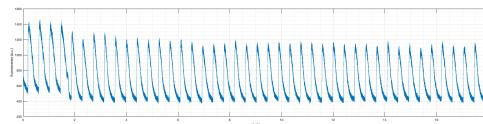
ii. Reanalysis

A. The reanalysis of this data using the moving average still showed no artifacts due to analysis.

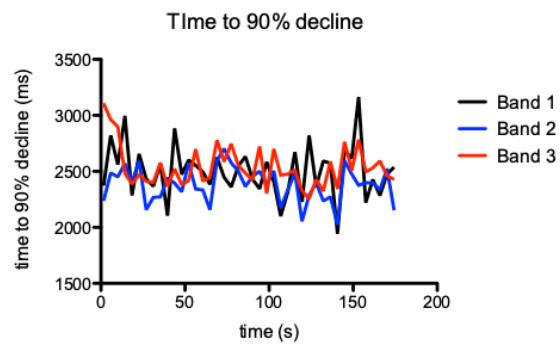
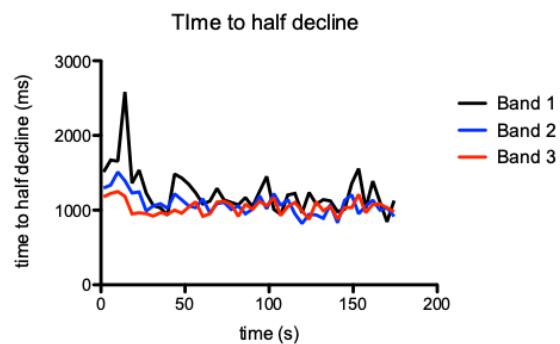
(g) 2019-06-11-10

i. Initial Analysis

A. Big drop in baseline after fourth beat for some reason.



B. Rhod2-LineScanCTRL06111910.pzf

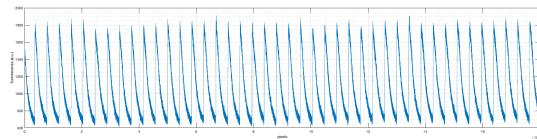


ii. Reanalysis

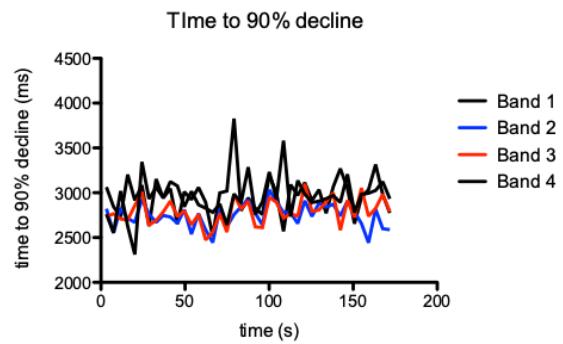
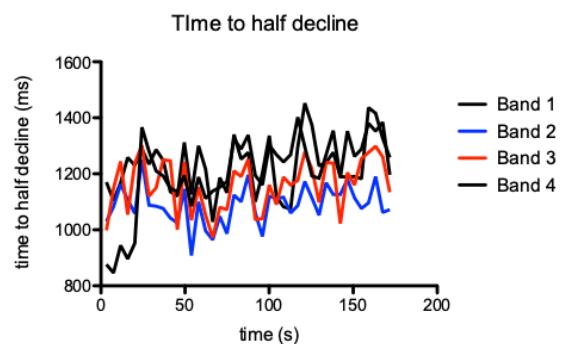
A. The reanalysis of this data using the moving average still showed no artifacts due to analysis.

(h) 2019-06-11-11

i. Initial Analysis



A. Rhod2-LineScanCTRL06111911.pzf



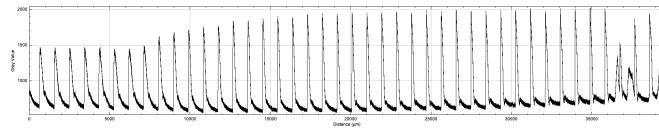
ii. Reanalysis

A. The reanalysis of this data using the moving average still showed no artifacts due to analysis.

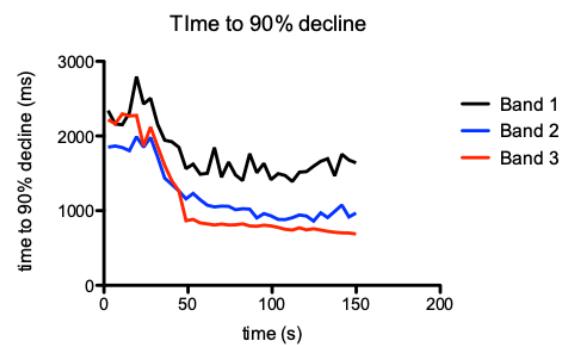
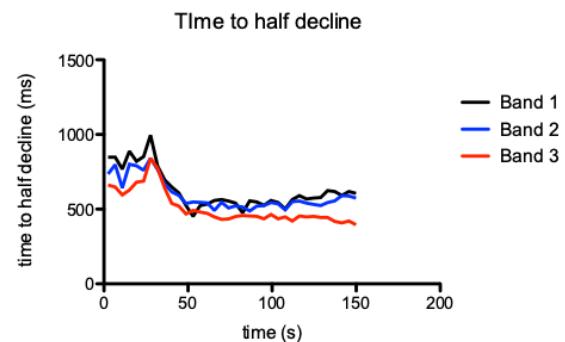
5. 2019-06-12

(a) 2019-06-12-01

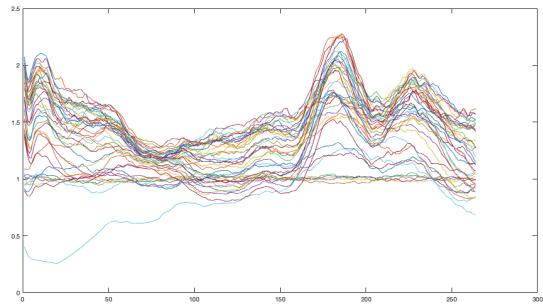
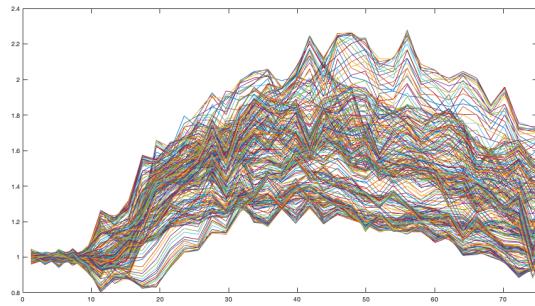
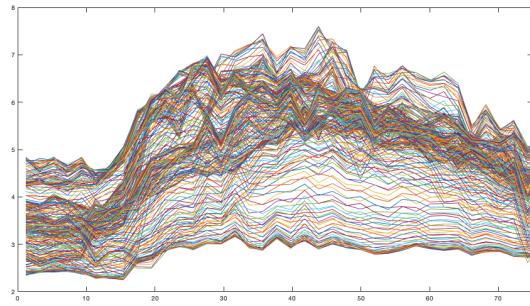
i. Initial analysis



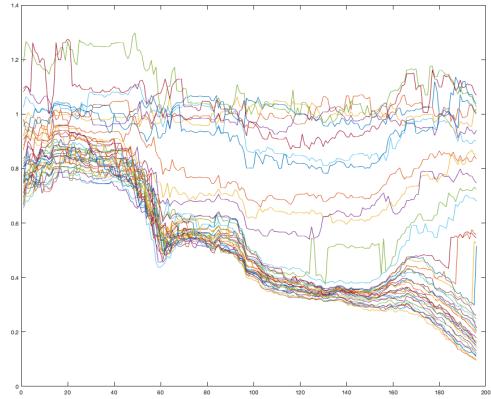
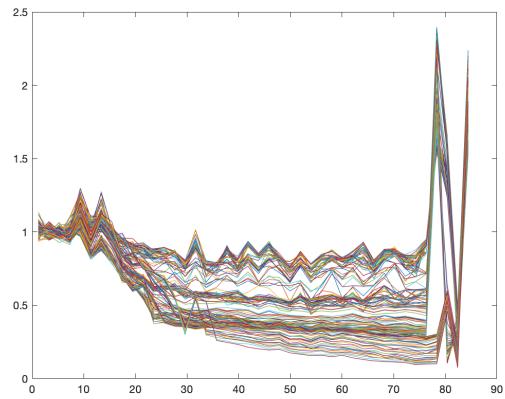
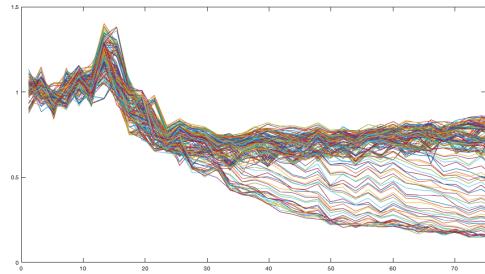
A. Rhod2-LineScanISO0612191.pzf



ii. Reanalysis 2019-06-18



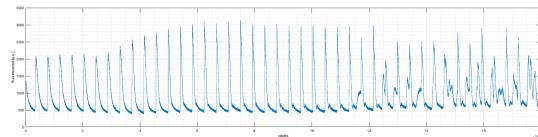
A. Transient peak data shows a gradient which is uncovered after normalization to initial level. Band data seem to indicate the initial increase is taking place pretty far up the cell in the middle.



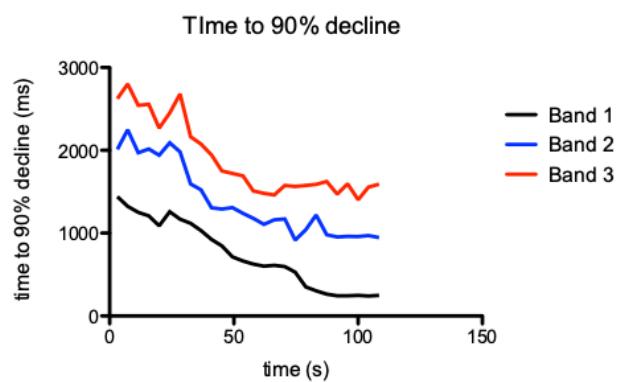
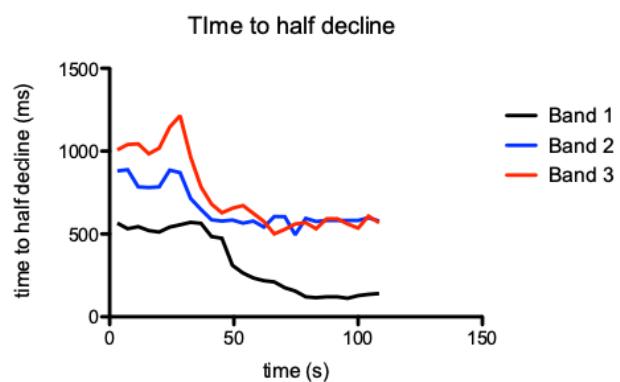
B. Decline data is showing a gradient but only at the very top of the cell. He hit this one high.

(b) 2019-06-12-03

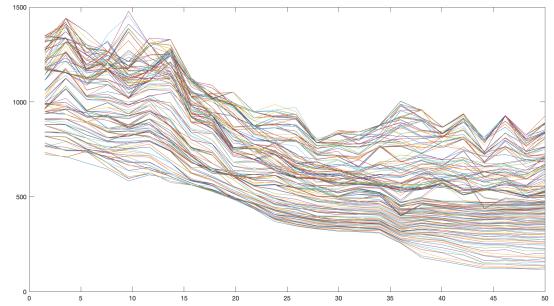
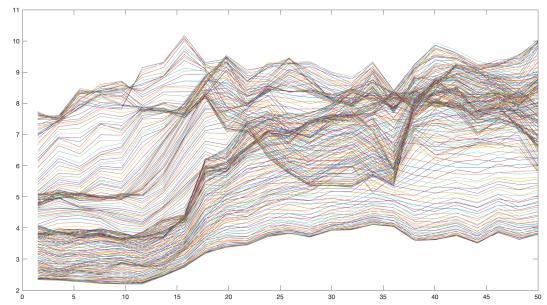
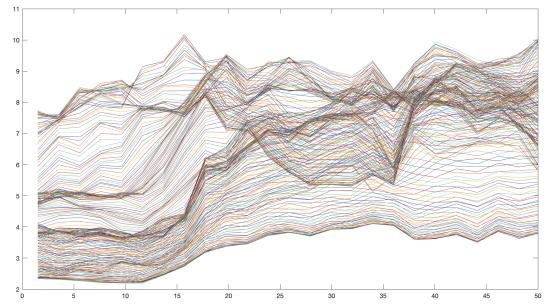
i. Initial Analysis

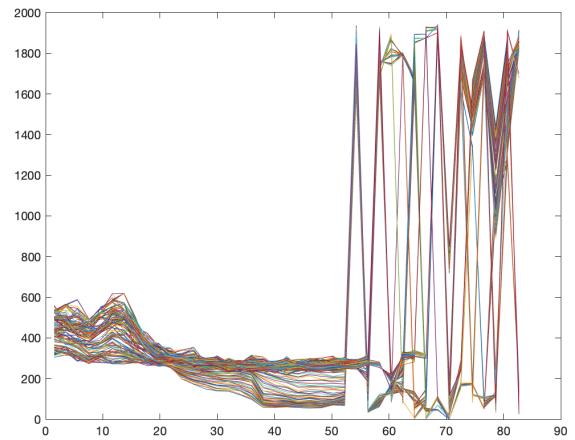


Rhod2-LineScanISO0612193.pzf



ii. Reanalysis 2019-06-17

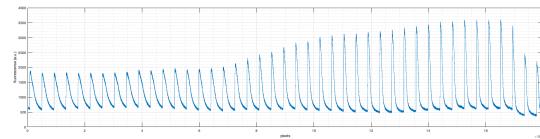




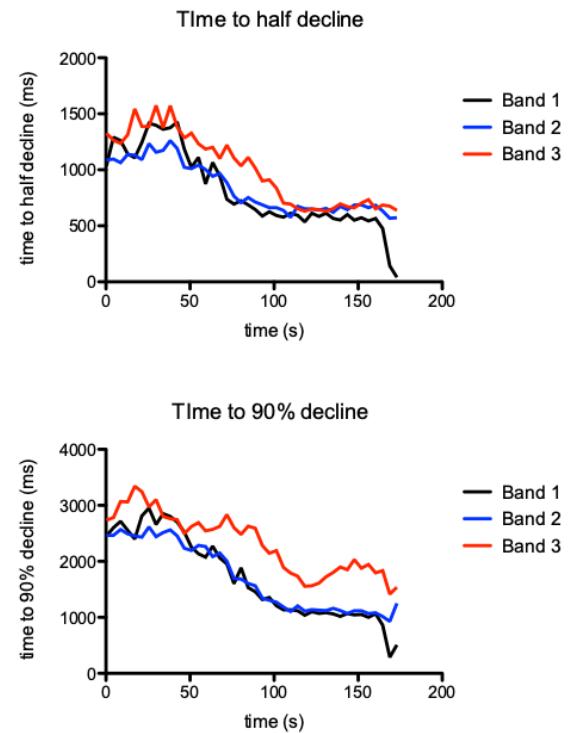
A. There may be a delay in the transient peak data. The rest will need further analysis. Probably needs to be normalized to initial level.

(c) 2019-06-12-04

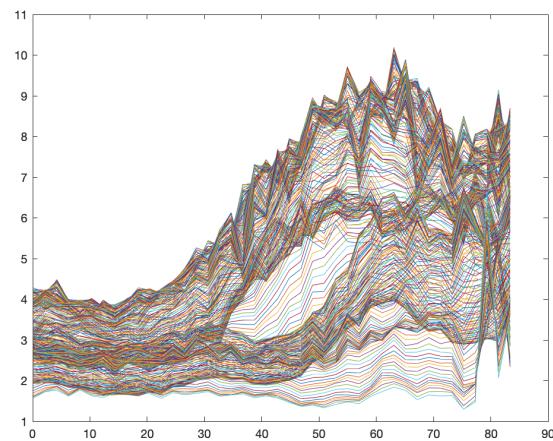
i. Initial Analysis

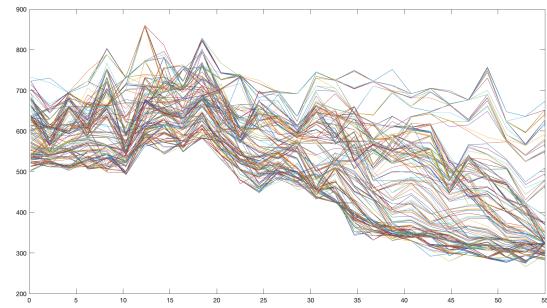
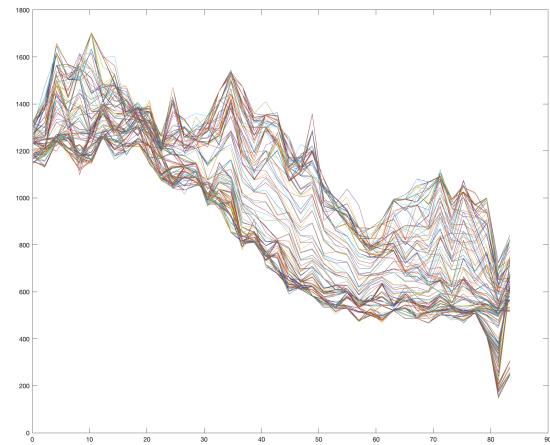
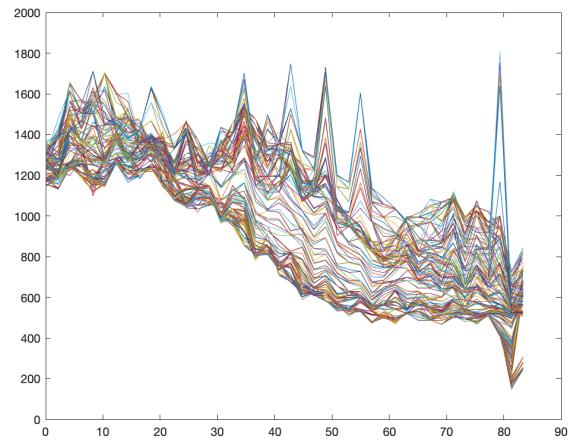


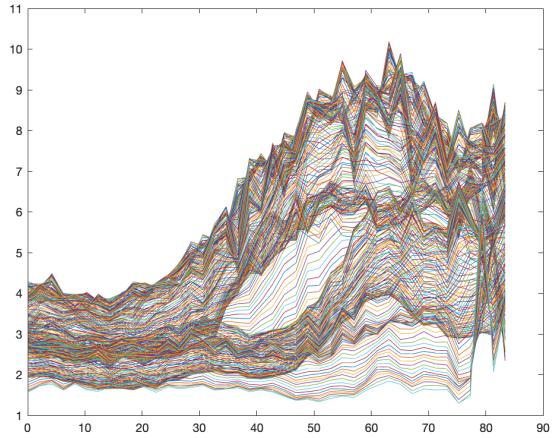
A. Rhod2-LineScanISO0612194.pzf



ii. Reanalysis 2019-06-17







- A. No surprise, this analysis confirms what we saw with the initial band analysis. Both the time to 90% and the time to half decline is delayed further up the cell. The increased detail in this data should help me to make better calculations.
- B. The 10 m band gives OK results for the peak - which also shows a gradient. I felt the 25 m band gave better results for the declines.
- C. Judging from the transient peak spatial data it looks like the ISO hit about half way up the cell. This needs to be looked at further.

6. 2019-06-19

(a) DAN'S NOTES Best cells

***Rank Order -First to Last;*** Analysis Priority

Cells 21, 14, 12, 19, 25, 18, 9

***Irregular Effects***

Cells 3, 4, 5, 7, 10, 11, 17, 23, 24

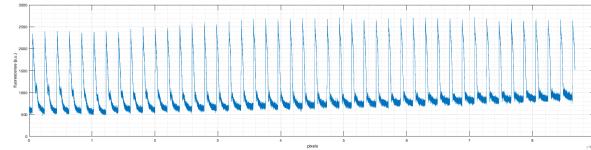
***Waves***

Cells 1, 2, 6, 8, 13, 15, 16, 22

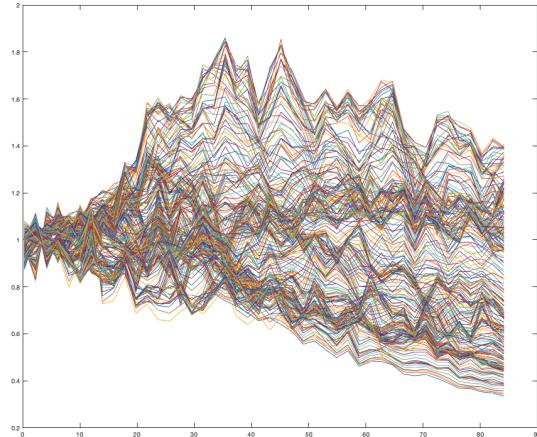
*No effect*

Cell 20

(b) 2019-06-19-09

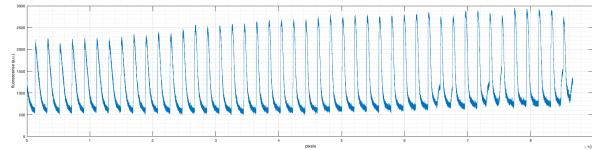


- i. The bands near the top of the cell away from the pipette are very weird looking. A lot of the bands have a little hitch in the decline but its exaggerated on that end relative to the peak of the transient. Movement artifact? Those bands will likely have to be cut out of the analysis.

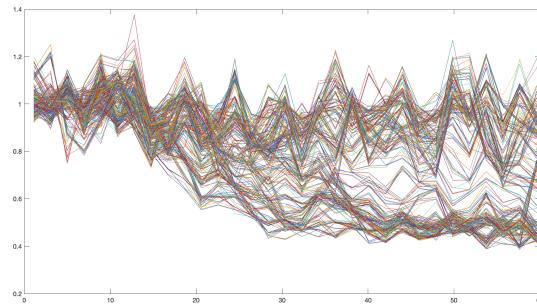
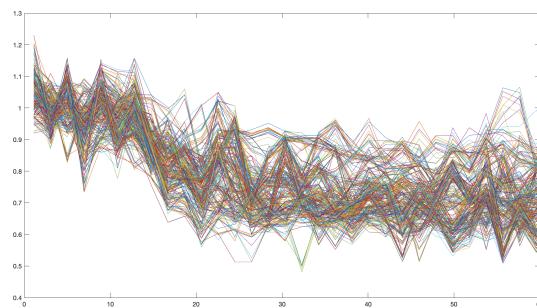


- ii. Looks like there's a moving baseline here but there's definitely a gradient. I can probably find a better way to analyze this to make it usable. This one is 10 m bands.

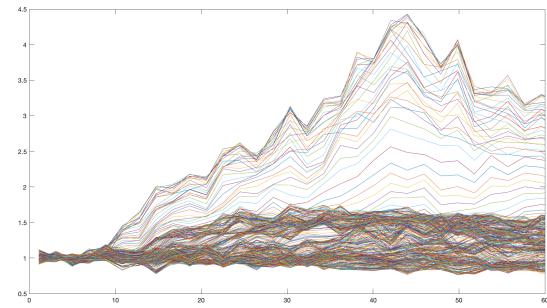
(c) 2019-06-19-12



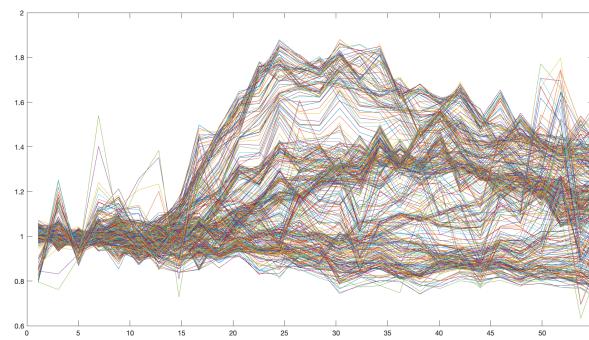
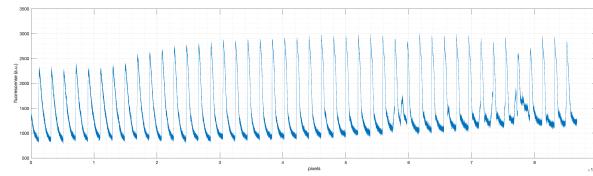
- i. Definitely a gradient in time to 90% but not time to 50%  
(25 micron band)

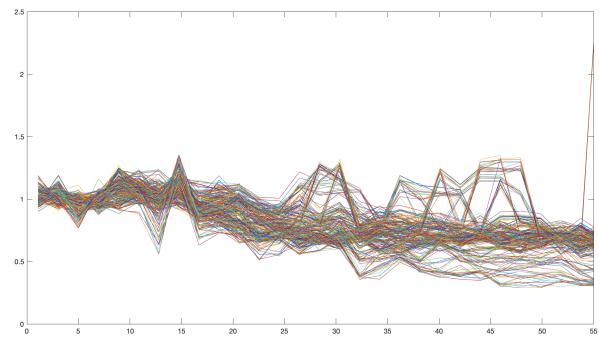
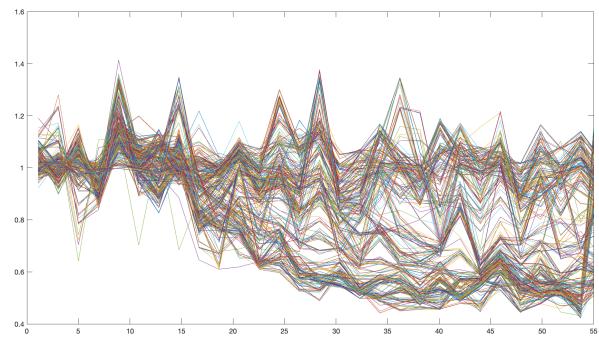


- ii. Gradient in time to peak, which definitely comes earlier than time to 90% but there's a "pause" in the middle. I'm not sure what that is. (10 micron band)



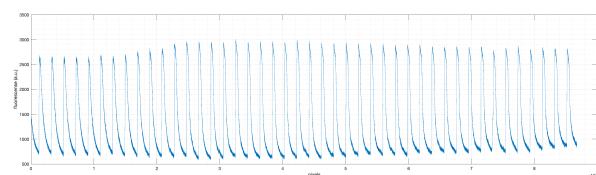
(d) 2019-06-19-14

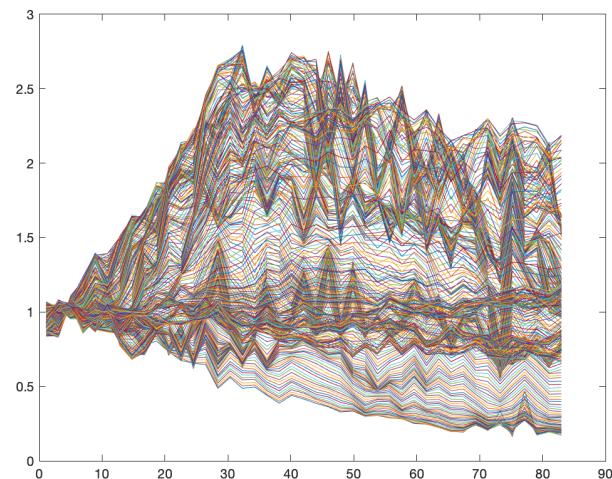
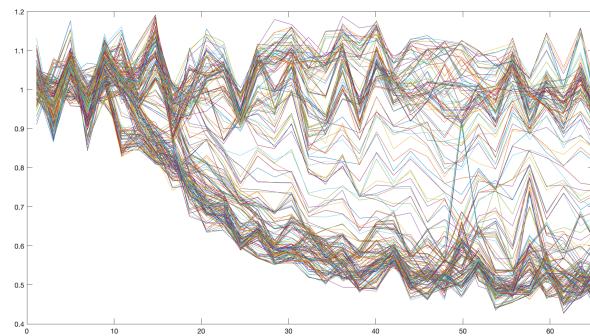




i. Really the same as 12 in almost all ways.

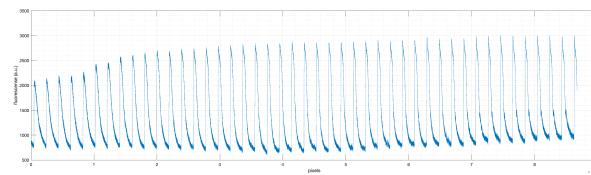
(e) 2019-06-18-18

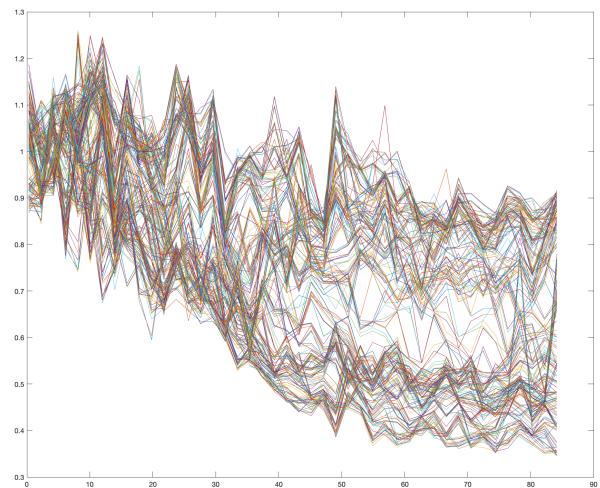
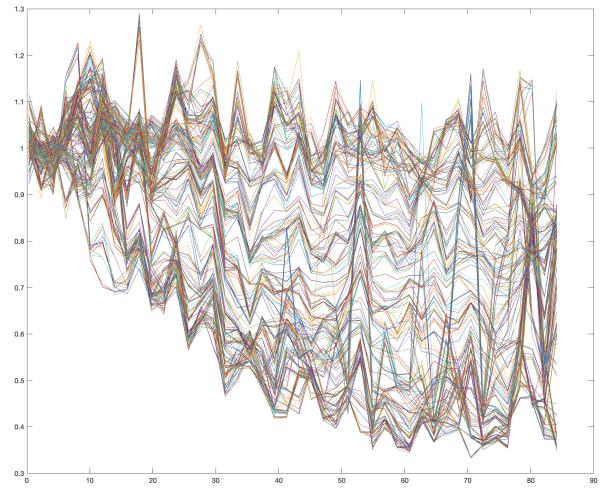


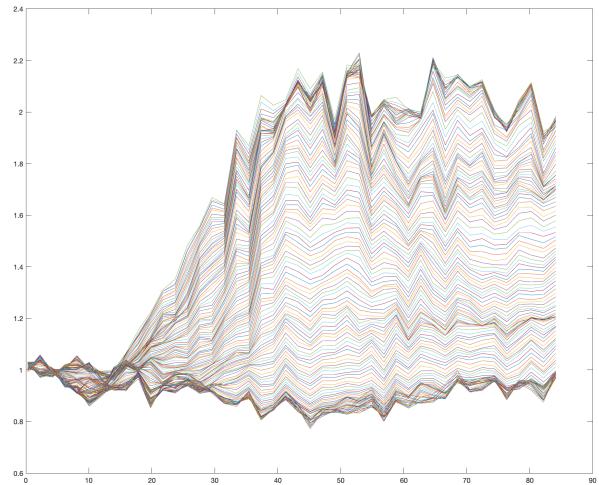


i. Just like 12

(f) 2019-06-19-19

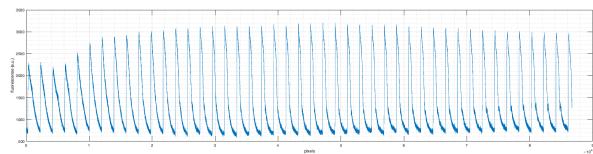


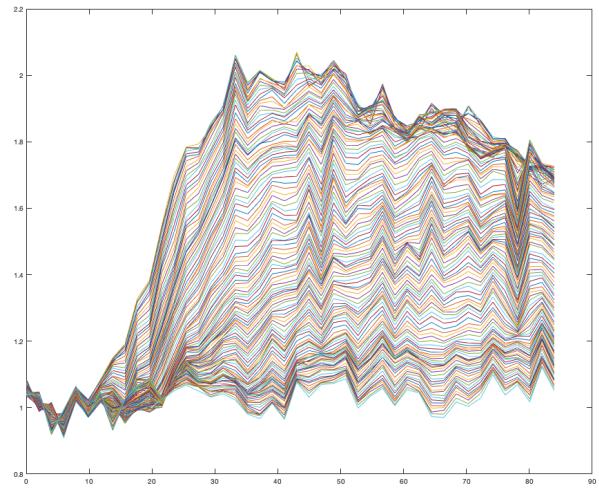
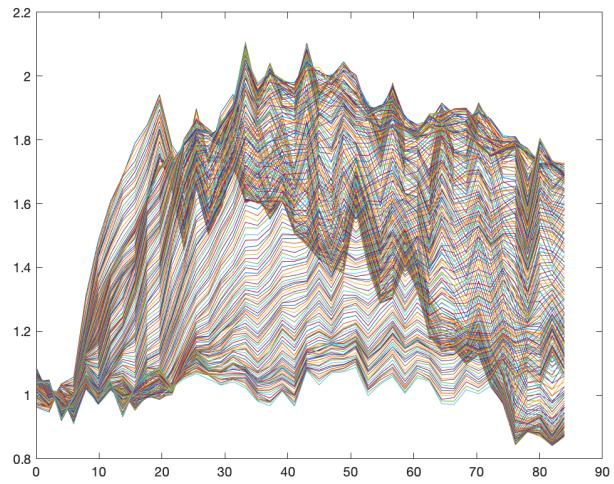


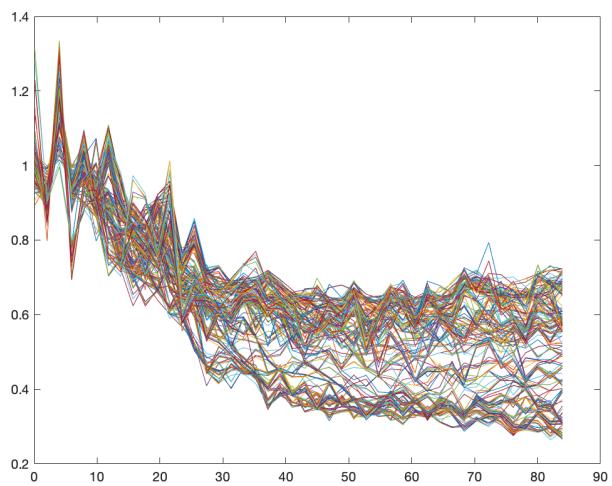
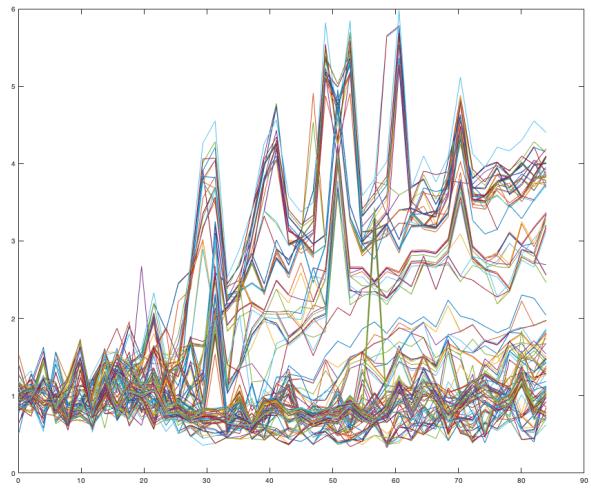


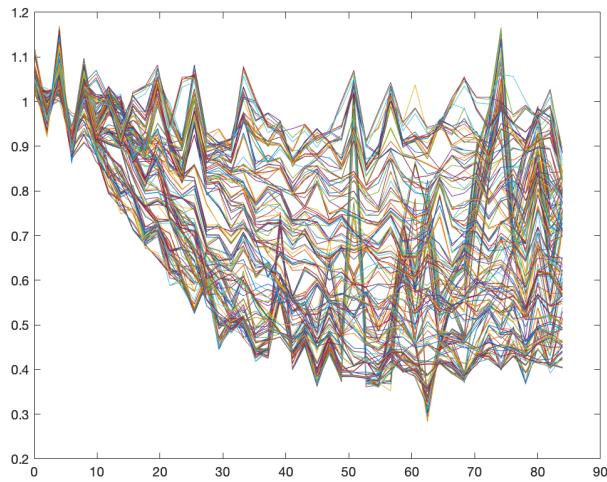
- i. This one showed a change in both the time to 90% and the time to 50%. All of these are 25 micron band analysis. I think I should probably just use This from now on. It looks better.

(g) 2019-06-19-21



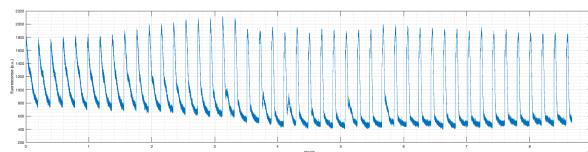


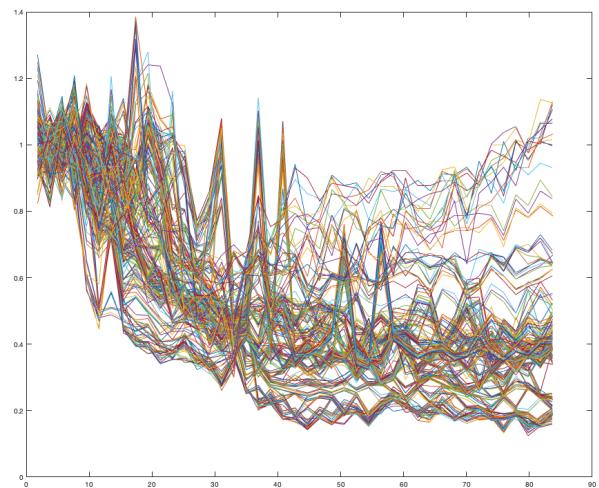
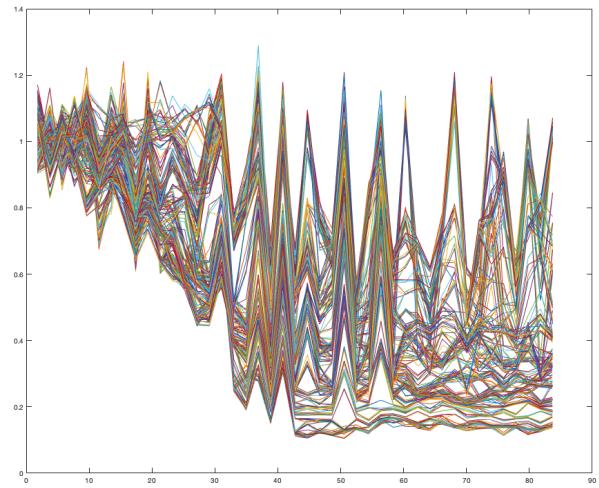


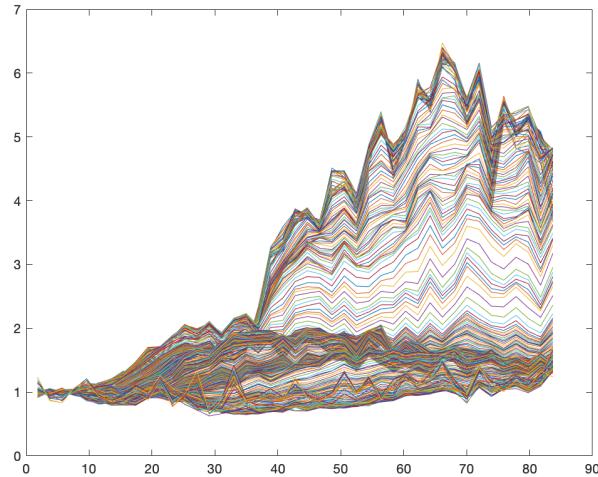


- i. The time to 90% and 50% decline are almost perfect
- ii. The time to peak showed usable data. Surprisingly This increased closest to the pipette not decreased
- iii. The transient peak data looks good but I had to cut a lot of bands on the ISO edge of the myocyte. These increased, then decreased in an exaggerated way. The figues above are with and without filtering.

(h) 2019-06-19-25







- i. This data is really noisy. Maybe the transient peak data is usable.

## 7. 2019-06-10 Analysis

- Note taken on *[2019-06-10 Mon 10:29]*  
Analyzed 2019-05-31 to 2019-06-07. I'm concerned about the analysis.

It looks to me like the "best" cells from Dan showed a difference in the time to 90% decline. These were 2019-06-07-2 and 2019-06-07-3. These were collected after Dan upped the ISO concentration to 2 uM.

What is concerning is that band 3 shows a linear decline in these cells. This might be due to a gradual drift in the analysis due to the fact that the stimulation rate wasn't exactly 0.5 Hz. The "time to 90%" may have actually been the time to the end of the segment that I was analyzing. This should, of course have been a window where the fill transient could be visualized. However, it was obvious that it was cutting off part way down the transient late in the analysis at the later time points.

- The other thing that worries me is that I think we need control data to make sure that even the data that we didn't think came

from "good" cells showed a decline in both time to peak and time to 90%.

- I think we should keep the ISO at 2 uM
- **I think we need control data to make sure that what I'm seeing isn't an artifact.**

#### 8. 2019-06-11 Reanalysis

- Note taken on *[2019-06-11 Tue 08:12]*  
Reanalyzed 2019-06-07-2 and 2019-06-07-3.

Reanalysis 1 was simply playing with the begin and end transient times to center the transients since we weren't at exactly 0.5 Hz.

Reanalysis 2 was reanalysis 1 with a 9 point box car smooth.

Both sets of data look better.

Still no differences in either cell in the time to half max.

Cell 2 seems to definitely have a slower response in band 3 than bands 1 and 2 in the time to 90% decline. This confirms what the first analysis showed but the data look better and less like it might be artifact.

Interestingly, cell 3 had no shortening at all in band 3 for time to 90% decline. And there appears to be a real difference between bands 1 and 2 (unlike cell 2).

Reanalysis 2 has all of the parameters hard coded in so I'll know exactly what I used.

#### 9. 2019-06-15 Analysis of 2019-06-11 and 2019-06-12

- Note taken on *[2019-06-16 Sun 09:17]*  
2019-06-11 was all control data with no ISO perfusion. I did some analysis of the 2019-06-11 data but didn't finish. The analysis of the cells up to number 10 was wrong because I wasn't using the right spatial parameters. I'll have to redo cells 5-9.

Cells 10 and 11 were done correctly and showed no decline in the time to half decline or time to 90% decline. So I'm reasonably confident that the analysis isn't adding any artifacts.

2019-06-12 was all ISO data. All bands in Cells 1,3 and 4 declined but cells 1 and 3 didn't show any apparent difference in the decline of the times to half and 90%.

Only cell 4 showed the response we are looking for. Band 3 definitely responded late and possibly more slowly to the ISO for both the time to 50% decline and time to 90% decline.

Interpretation:

- The mathematical analysis isn't adding an artifact.
- I'm a bit concerned about the "time to declines" that I'm getting in terms of the numbers. Given that the whole transient should be only 2000 ms long, a time to 90% decline of 3000 seems wrong. I'll have to take a further look at the code to see where this discrepancy is coming from.
- I think Dan might be hitting too much of the cell on some of these so that the ISO effect disappears. Given the amount of trouble he's having controlling this, I may have to do some further analysis in order to make this data quantitative.

I'm thinking of keeping the 50 pixel banding but moving down the cell line by line (average of 25 pixels on each side). I'll look at each line and determine when they hit some mark, let's say a time to 25% decline in time to 90%. I'll determine more or less where along the length of the cell the first delay in this time seems to be and figure that's where the ISO stops hitting the cell. From there, determining the rate at which the response propagates up the cell shouldn't be hard.

#### 10. 2019-06-17 Re-analysis of 2019-06-12

- Note taken on *[2019-06-18 Tue 07:28]*  
Only analyzed cells 3 and 4.

This revised analysis is a moving spatial average of the fluorescence. So, for instance, the first "band" of the 25 m is actually centered on a line 12.5 m from the edge with a total width of 25. The analysis then moves 1 pixel over and repeats for the next band. This gives filtered data over the entire length of the cell.

I also generated figures which have band number on the x axis. In this case, each line represents a transient. My hope is that I can better localize exactly where the ISO is hitting the cell by looking at which band immediately respond to the ISO (those that are being perfused) and which are delayed and by how much (the longer the delay, the further from the region being perfused).

This led to some interesting results.

It looks like the ISO hit cell 4 about half way up. This needs to be looked at further. The cell responded well and You can definitely see a spatial gradient in the response in terms of time to half decline, time to 90% decline and transient peak.

There might be something there in cell 3. The transient peak data seems to show a gradient but its hard to tell. This data really needs to be normalized to the intial level.

Note also that I fixed the time bug. The times to half and 90% decline should be accurate now.

#### 11. 2019-06-18 Reanalysis of 2019-06-12-01, 2019-06-11-09 to 11 and 2019-06-07

- Note taken on *[2019-06-18 Tue 14:36]*

I also reanalyzed 2019-06-12-03 and 2019-06-12-04 so that the normalized graphs would be generated. Should make it easier to evaluate this data, especially 03.

- Note taken on *[2019-06-18 Tue 08:37]*

Added a normalization protocol to the analysis so that now we have graphs of data normalized to the initial levels.

2019-06-12-01 This cell actually did respond to ISO but the transient data kind of looks like it responded in the middle and them the response diffused out to the ends. The time to declines who a gradient only on the top end. Note the band data where the final decrease in time to 90% takes place very lat at the very top 20% or so. Dan hit this one really high be there might be something we can get out of it.

2019-06-11-09 to 11 These were the only control cells that I analyzed. I'll go back and reanalyze later. Suffice it to say that

there was no apparent change due to the analysis and there do not appear to be any artifacts added on that account.

2019-06-07-4 There definitely is a gradient here in the time to 90% decline. There's nothing there in the time to half decline. The gradient is there in the peak transients as well.

Both seem to show that the response basically stops and doesn't make it all the way up the cell.

2019-06-07-3

Note that the profile for this cell went all the way to the edge. Used 500.

There's some unsteadiness in the peak transient data over time but it looks like there's a gradient there. When You look at the raw transient traces over time it does look like the peaks go up and down a bit. May not be able to use this.

The band Vs. tt90 data seems to show that this cell got hit about halfway up. Definitely a gradient in this data but its very noisy. There might be something in the tt50 but its too noisy to tease out.

2019-06-07-2

There might be a gradient in the tt90 data but I wouldn't bet that it will amount to anything. Nothing in the tt50 data.

The transient peak data shows a definite gradient. The 25 um band data looks better.

## 12. 2019-06-19 Reanalysis of 2019-06-11-05 to 08

- Note taken on *[2019-06-19 Wed 13:48]*

2019-06-04-1 and -2 This data's no good. It looks like there might be a gradient in the transients but the peaks are almost random as a function of space.

- Note taken on *[2019-06-19 Wed 13:18]*

2019-06-06-05 Was pretty noisy. The peak transient data might be useful.

- Note taken on *[2019-06-19 Wed 07:27]*

Reanalyzed 2019-06-11-05, 06, 07 and 08 as on 2019-06-18 and 19.

There was, as expected, no artifacts associated with the analysis in these control cells.

I did add a line to the script to save the workspace. This may be a good idea as having the data saved may help with the inevitable further analysis that Will need to take place with this data.

### 13. 2019-06-22,23 & 24 Analysis of 2019-06-19 data

- Note taken on *[2019-06-24 Mon 07:06]*

Only analyzed Dan's "best" cells. I'm going to have to get around to analyzing soe of the more iffy cells eventually to make sure there isn't something significant hiding in there.

Some of This data was analyzed with 10 micron moving averages for the transient peaks but 25 micron moving averages seem to be giving the best analysis and I think I'll stick to that from now on.

This was for the most part usable data. 2019-06-19-21 was, indeed, the best and This might make a good sample cell.

Generally speaking the Peak transient data is the least noisy but it often seems to go up and down and the peaks most downstream are sometimes lower than the ones more toward the middle of the cell. It's possible that Dan is hitting the cells high enough up to where the ISO is diluting out as it is washed downstream over the cell. However Note that the transient decline data does not show This tendency towards dilution of effect downstream.

The time to half decline remains unreliable but it is definitely there on occasion. For whatever reason it doesn't appear to be as sensitive as the time to 90% decline.

All of the effects tend to stop short rather than propagate all the way up the cell.

- Could be the SR Ca pump is so cranked up that it's taking up the Ca faster than it can diffuse (both inside and outside the SR).
- Could be that some essential element is not diffusible. What is the role of the cytoskeleton? Perhaps some experiments with cytochalasins?

## 4 Tasks

- 4.1 **TODO** Read Wayne's new article
- 4.2 **TODO** Read Mike/Xun article
- 4.3 **TODO** Another Fill article
- 4.4 **DONE** Analyze Dan's latest data before phone call with Don at noon.
  - 4.4.1 Looks to me like he's overdosing with ISO.
  - 4.4.2 Check solutions
  - 4.4.3 Decrease dose to 100 nM
- 4.5 **DONE** Renew IDL Maintenance by July 1
- 4.6 **DONE** TRAVEL FORMS!
- /2018-07-17 Tue/*
- 4.7 **DONE** Renew Matlab (90 day notice)
- 4.8 **DONE** Call Dan
- 4.9 **DONE** Do work for Don on AKAP stuff
- 4.10 **TODO** Work on methods paper
- 4.11 **DONE** FU: Dan (due June 12) Annual Renewal Notification IACUC #17-040
- 4.12 **TODO** Prepare a PowerPoint for call with Don Re: Could we set up a call?