

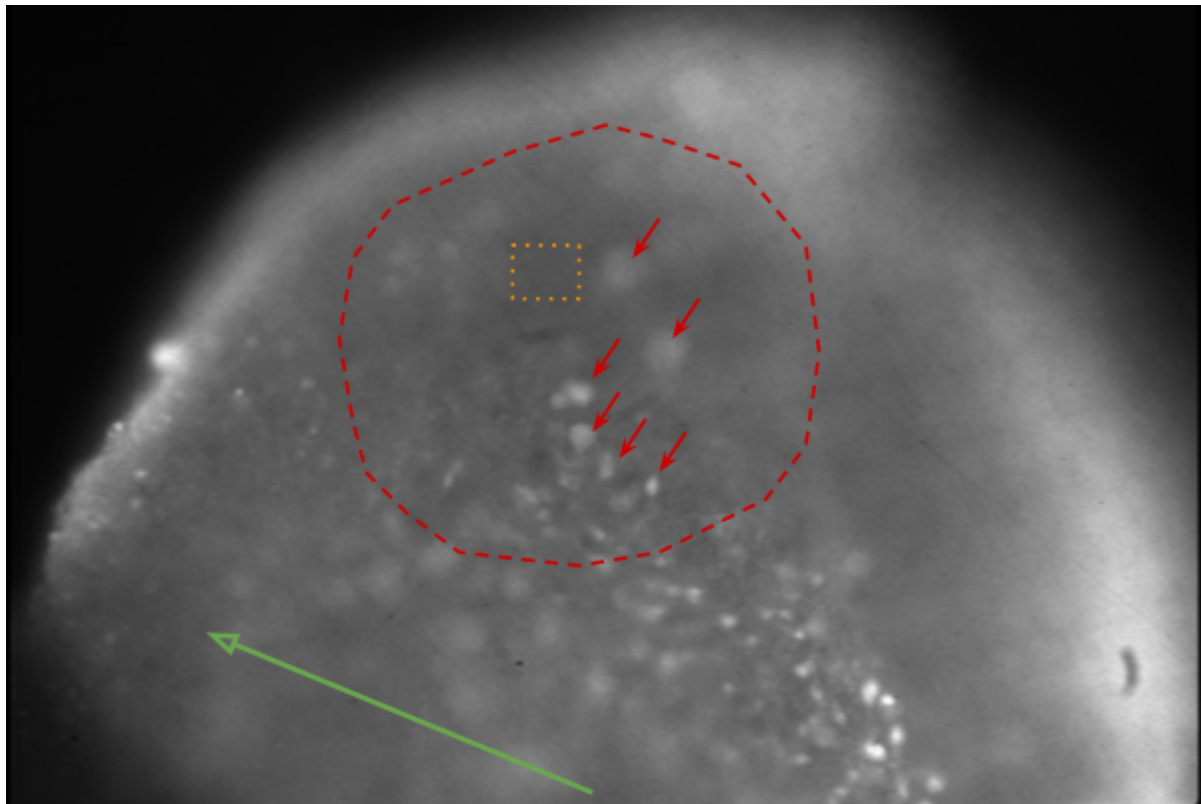
Topic: pH Analysis from SBFI_Con7
Keywords: SBFI, pH

Introduction

I conducted a calibration Na imaging experiment using SBFI AM. Before beginning of the experiment, I conducted a series of pH tests to examine how the dye changes with pH at the standard extracellular [Na] of 155mM. The membrane was not perforated for these tests.

Imaged Cells

I used the six motor neurons shown below across all images. For background subtraction, I used the area indicated by the orange dotted line.



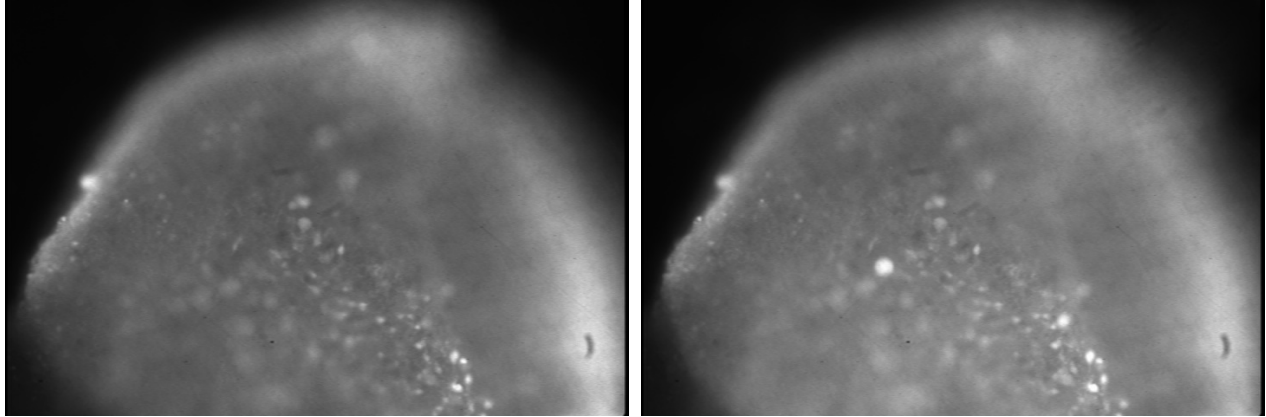
AVG_con7_pH_1500_340 (SBFI_Con7, images from pH test, 15:00, 340nm)

Top of image is lateral, bottom is medial. Motor column is indicated by red dotted line. Motor neurons indicated by arrows (though two in the upper right are in a more shallow focal plane than the other four). Dorso-ventral axis is indicated by solid green arrow.

Note that there are some small, brightly-labeled cells in the dorsal aspect of the cord. Some more medial neurons (probably interneurons) are also labeled but out of focus. In this image, pH was at 7.01 and extracellular solution was a low-Cl version of Tyrode's with [Na] = 155mM. Bath temperature was 29.7 °C. There were no drugs.

(a)

(b)

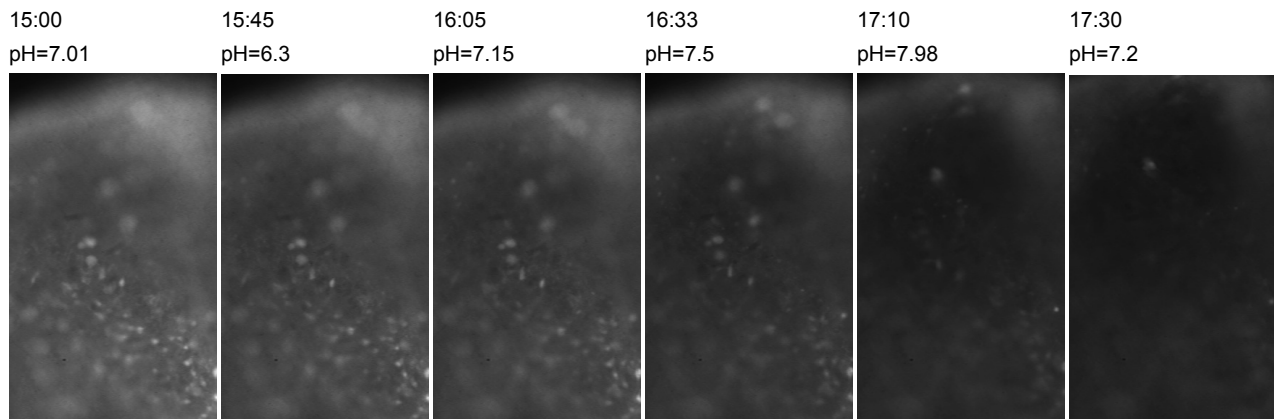


AVG_con7_pH_1500 (SBFI_Con7, images from pH test, 15:00)

Slice from lumbrosacral cord of E10 chick embryo loaded with SBFI AM and fluorescently imaged with excitation wavelengths of (a) $\lambda=340\text{nm}$ and (b) $\lambda=380\text{nm}$.

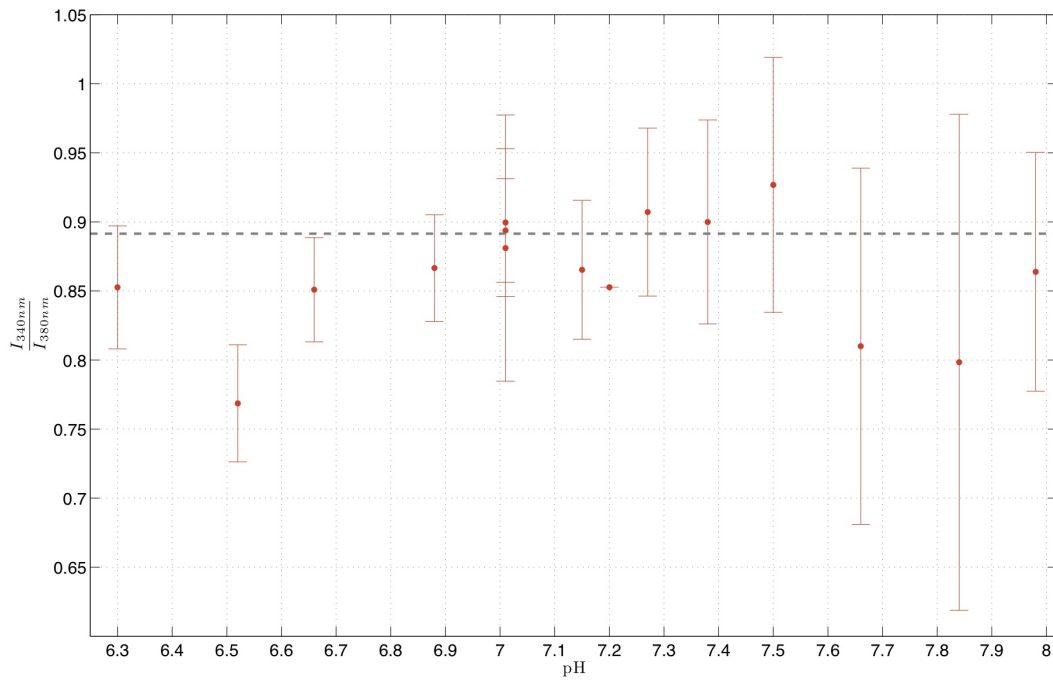
Analysis

Some representative images are shown below.

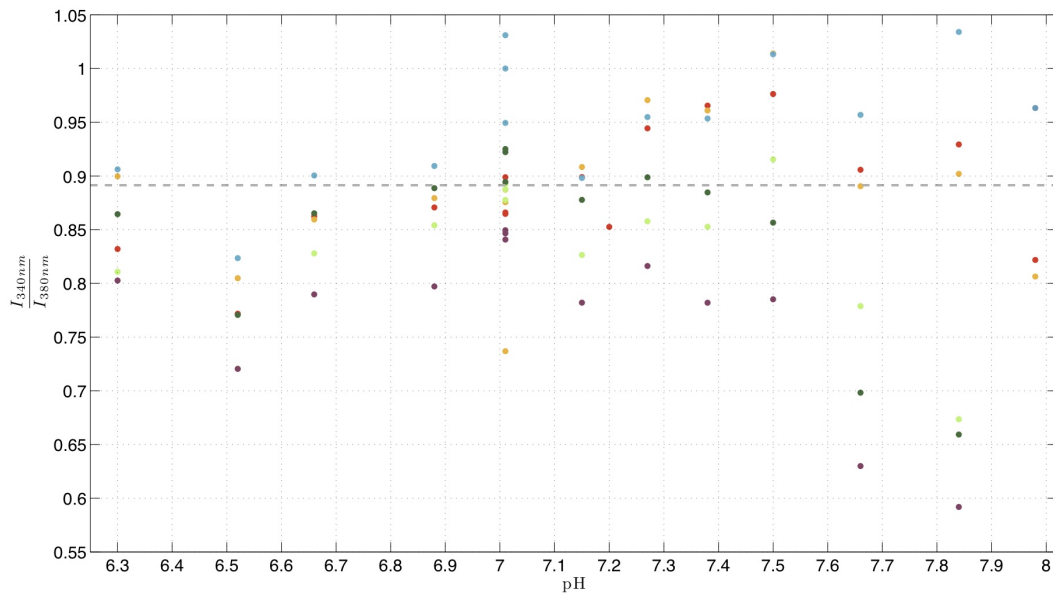


After pH is brought nearly to 8, it becomes nearly impossible to see the cells anymore, and fluorescence doesn't recover even when pH is brought back to a reasonable level. I suspect this is because the pH changes were so radical. To be sure of that the loss of efficacy of the SBFI isn't simply due to time, I need to conduct a similar experiment where I do NOT mess around with pH but instead leave the perfusion on for ~3 hours and check fluorescence every 15-20min.

Plot of Ratio as a function of pH:



This is not really as informative as I would have liked (or expected). A little more information is available when looking at individual cells (indicated by color):



Finally, the most information is available here:

time	pH	temperature	chemical recently added	mean ratio	std ratio	diff from 0.8914
15:00	7.01	29.7	--	0.8810	0.0964	0.0104
15:24	7.01	29.7	--	0.8995	0.0535	-0.0081
15:31	7.01	29.8	--	0.8937	0.0374	-0.0023
15:45	6.30	29.9	HCl	0.8526	0.0445	0.0388
15:50	6.52	30.0	KOH	0.7686	0.0424	0.1228
15:55	6.66	29.9	KOH	0.8509	0.0378	0.0405
16:00	6.88	29.7	KOH	0.8665	0.0386	0.0249
16:05	7.15	29.8	KOH	0.8653	0.0503	0.0261
16:15	7.27	29.9	KOH	0.9071	0.0608	-0.0157
16:25	7.38	29.8	KOH	0.8999	0.0738	-0.0085
16:33	7.5	29.9	KOH	0.9268	0.0924	-0.0354
16:45	7.66	29.8	KOH	0.8100	0.1289	0.0814
16:55	7.84	29.9	KOH	0.7983	0.1795	0.0931
17:10*	7.98	30.0	KOH	0.8638	0.0864	0.0276
17:30**	7.2	29.9	HCl	0.8526	n=1	0.0388

*recording at 17:10 only included three cells

**recording 17:30 only included one cell

Ratios do seem to stay fairly stable in the 7.0-7.4 range, which is good.

They start looking pretty strange, though, outside of this. Superficially it appears that acidic pH reduces ratios and basic pH increase ratios, but at 7.66 and higher this rule seems to break down.

some additional handwritten notes...

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<u>Real Time</u>	<u>Video Time</u>	<u>pH</u>	<u>temp</u>	<u>[Na]</u>	<u>notes</u>
15:00	0:02 - 0:19	7.01	29.7°C	155mM	no drugs
15:24	0:19 0:19 - 0:41	7.01	29.7°C	155mM	no drugs
15:31	0:56 - 1:13	7.01	29.8°C	"	"
15:45	1:13 - 1:33	6.30	29.9°C	"	add several pipette-fuls of 0.1 M HCl to lower pH a lot 15:35
15:50	1:33 - 1:53	6.52	30.0°C	"	added a few drops of 1 M KOH to slowly raise pH betw each measurement
15:55	1:53 -	6.66	29.9°C		
16:00	- 2:28	6.88	29.7°C		
16:05	2:28 - 2:46	7.15	29.8°C		
16:15	2:46 - 3:02	7.27	29.9°C		
16:25	3:02 - 3:19	7.38	29.8°C		
16:33	3:19 - 3:34	7.5ish 7.5	29.9°C		
16:45	3:34 - 3:50	7.66	29.8°C		
16:55	3:50 - 4:07	7.84	29.9°C		
17:10	4:07 - 4:27	7.98	30.0°C		
17:30	4:27 - 4:44	7.2	29.9°C		added a lot of HCl to bring back to normal pH (looks like cells are gone)

pH takes FOREVER to stabilize ... sti varies sometimes after waiting 10 m

when I removed the slices, there was a ton of precipitation. I cleaned this a lot before putting in new slices.

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30 May 2010