1. Question 1

These exercises are meant to give you practice with the MATLAB programming environment and are designed for you to demonstrate your competence performing basic computations using this software.

Part 1: Array computations for simple data analysis

For this question you will read in data that were obtained experimentally using a laser scanning confocal microscope operating in line-scan mode. The manipulations required in the assignment include:

1) accessing only a particular portion of the data (a defined spatial position),

2) spatial averaging to reduce noise,

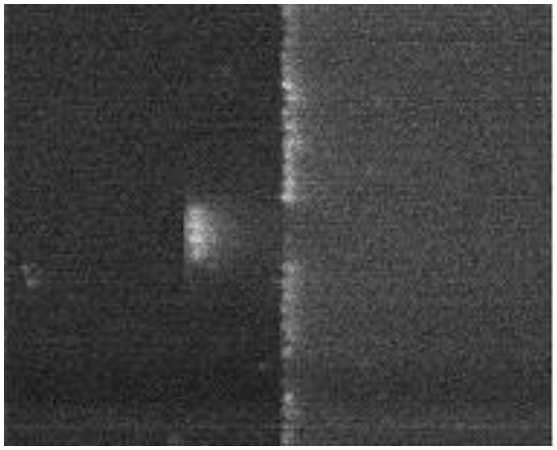
3) normalization, and

4) converting the data into different units.

First, for those of you who are interested, a few words about what the data represent. In this experiment a rat ventricular myocyte was loaded with the Ca^{2+} sensitive indicator fluo-3, and a confocal microscope was used to record changes in intracellular [Ca^{2+}]. The microscope was operated in "line scan" mode such that one dimension of the resulting "pseudo-image" is space and the other is time. This cell was also loaded with the Ca^{2+} buffer NP-EGTA. This buffer has unique properties such that when it is exposed to high-intensity ultraviolet light, it loses its ability to bind Ca^{2+}. Thus, delivering a pulse of UV light to a cell loaded with NP-EGTA provides a means to rapidly increase [Ca^{2+}] by "uncaging" it from the NP-EGTA.

The initial, local increase in [Ca^{2+}] in this image results from a spatially localized flash of UV light. The second increase results from electrical stimulation of the cell. The goal is to determine how the increase in [Ca^{2+}] due to the flash affects the later increase due to the electrical stimulus. The regions of the cell not exposed to the UV light serve as internal controls.

The data appear as follows:



To perform this analysis, you have to take the following steps:

a) Download flash4.jpg from here.

Read in the file: data = imread('flash4.jpg','jpg') ;

b) Take a look at it using the imagesc function. Orient the data so that time runs from left to right. Transpose the matrix if necessary.

c) Average over the region of the UV flash. Store this in the variable flash. This should have dimensions 1 x 634.

d) Average over a control region that does not contain the flash. Store this in the variable noflash

e) The fluorescence units are arbitrary, since the number depends on laser intensity, dye concentration, microscope detector gain, etc. Thus, we are interested in relative changes in fluorescence (F) over the baseline value (F_O) in a resting cell. Convert from raw fluorescence to units of F/F0 by normalizing *flash* and *noflash* to the average fluorescence in a region with no activity. (Hint: between lines 70 and 100 is a good region).

f) To a first approximation, one can assume that the dye used in this experiment, fluo-3, only emits fluorescence when it is bound to Ca^{2+}. Thus, where K_D is the dissociation constant of the dye, and [Fluo3]_TOTAL is the dye concentration, fluorescence is proportional to:

$$F \sim \{ \frac{[Ca^{2+}][Fluo3]^{TOTAL}}{[Ca^{2+}]+K_D} \}$$

If one makes a reasonable assumption for baseline [Ca^{2+}] (e.g. 100 nm), the following equation can be used to convert from a ratio R (units of F/F_0) to [Ca^{2+}] in units of concentration:

$$[Ca^{2+}] = \{ \frac{RK_D}{\frac{K_D}{[Ca^{2+}]_{baseline}} - R + 1} \}$$

Implement this equation to convert flash and noflash from units of F/F_0 to units of [Ca^{2+}], in nM.

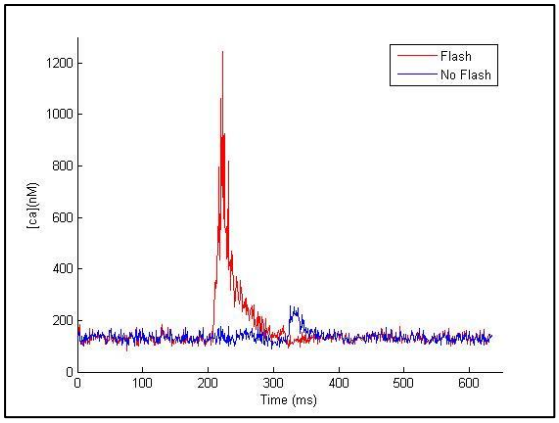
You can assume $[Ca^{2+}]_{baseline}$ = 100 nM, and K_D= 700 nM. Keep in mind that your variables flash and noflash are not just scalars (numbers) but are defined at many time points.

g) Plot both versus time, on the same scale, in different colors.

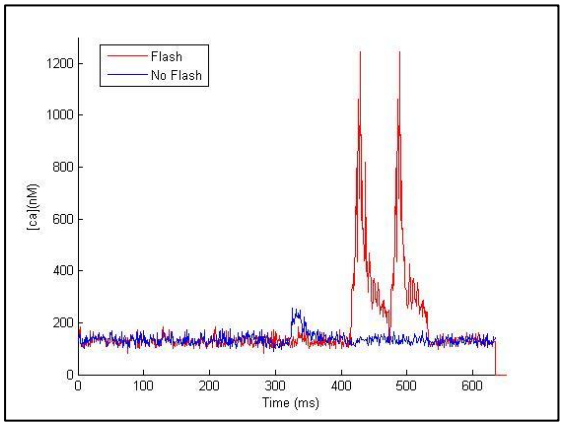
Which of the following plots can represent the output plot if you assume $[Ca^{2+}]_{baseline}$ = 150 nM and K_D=1000 nM?

CHOOSE THE CORRECT OPTION

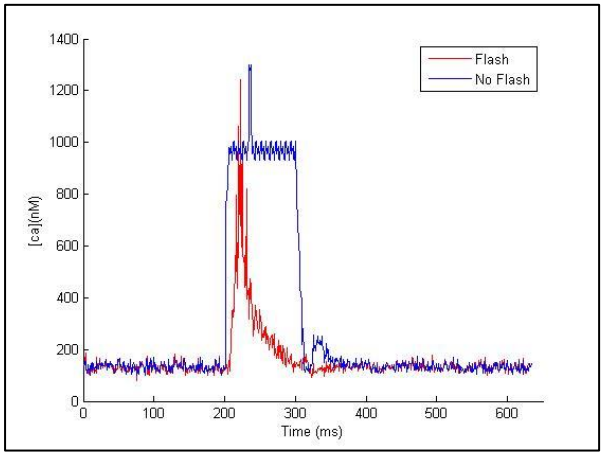
(a)



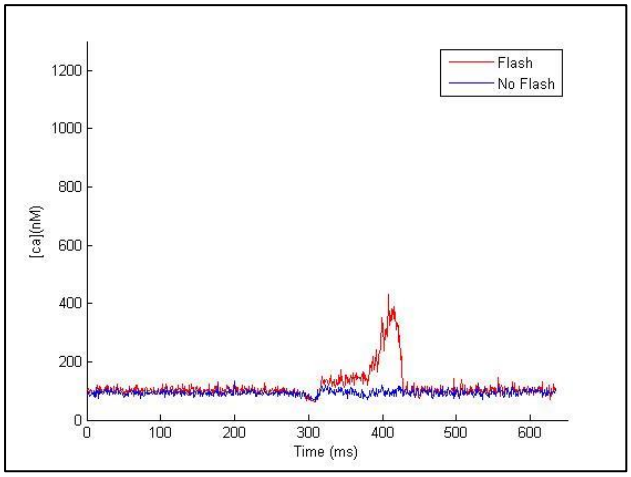
(b)



(c)



(d)


Flash
No Flash
[ca](nM)
Time (ms)
0
200
400
600
800
1000
1200
0
100
200
300
400
500
600