FREE CALCIUM CONCENTRATION IN NON-EQUILIBRIUM CONDITIONS

Giacomo Barzon
Dipartimento di Fisica e Astronomia "Galileo Galilei"
Università degli Studi di Padova
giacomo.barzon.1@studenti.unipd.it

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ABSTRACT

Calcium ions contribute nearly every aspect of cellular life. The most popular method for measuring the intracellular calcium concentration in mammalian cells is to monitor the fluorescence of an indicator. This is typically obtained using a mathematical model based on the assumption of chemical equilibrium between Ca^{2+} and the fluorescent dye and other reactants, but the reliability of this model breaks down during Ca^{2+} influx. Here a new model is derived, which goes beyond the equilibrium condition. With the aim of this model, $[Ca^{2+}]$ can be expressed as a function of the dye fluorescence, its time derivative and its extremal (maximum and minimum) values and the total dye concentration. The validity of the results is also confirmed by the numerical simulation shown in this work, which are obtained using realistic physiological parameters.

1 Introduction

Calcium ions (Ca^{2+}) contribute nearly every aspect of cellular life as a carrier of signals to a large number of cellular functions, such as the generation and degradation of metabolic products, the synthesis and release of hormones, muscle and non-muscle motility, and a variety of membrane-linked processes. The regulation of Ca^{2+} depends on the reversible complexation by specific intracellular proteins, which are either soluble in the cytosol, organized in non membranous structures, or intrinsic to membranes [3, 5]. The evaluation of the role of the calcium as an intracellular messenger requires quantitative measurement of cytosolic free Ca^{2+} concentrations, $[Ca^{2+}]_i$, and comparison with varied stimuli and cell responses. Currently the most popular method for measuring $[Ca^{2+}]_i$ in mammalian cells is to monitor the fluorescence of an indicator. Such indicators are Ca^{2+}-selective fluorescent dyes that are loaded into intact cells and their spectral properties are altered in a suitable manner by Ca^{2+} binding [7]. $[Ca^{2+}]_i$ is extrapolated from the linear combination of the fluorescence emission of free dye and dye bound to Ca^{2+}. This is typically obtained using a mathematical model based on the assumption of chemical equilibrium between Ca^{2+} and the fluorescent dye and other reactants (see Section 2.1). However the reliability of this model breaks down during Ca^{2+} influx due to the local non-equilibrium of the system [2].
So the aim of this work was to derived a mathematical model relaxing the assumption of chemical

equilibrium and to verify the model by simulating a Ca^{2+} influx from a point source in a unidimensional space initially filled by dye and Ca^{2+} at resting equilibrium. In order to simulate the interaction between the dye and Ca^{2+}, the reaction-diffusion equations were discretized both in time and space with the finite difference method.

2 Materials and methods

2.1 Fluorescence

After the fluorescent dye is loaded inside the cell, it interacts with Ca^{2+} as a bimolecular association reaction of the form:

$$Ca^{2+} + B \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} CaB \tag{1}$$

where B and CaB are free and bound dye respectively, k_{on} is the binding rate constant ($M^{-1}s^{-1}$) and k_{off} is the unbinding rate constant (s^{-1}). Eq.1 can be rewritten in a differential form, which is called *reaction equation*:

$$\frac{d[Ca^{2+}]}{dt} = k_{off}[CaB] - k_{on}[Ca^{2+}][B] \tag{2}$$

where $R = K_{off}[CaB] - K_{on}[Ca^{2+}][B]$ is the reaction term, which is null at chemical equilibrium [11].

The emission intensity F arising from an observation volume V (e.g., a single cell or a cell compartment loaded with a fluorescent dye) is proportional to the total dye concentration $[B]_{tot}$ in V:

$$F = S \cdot [B]_{tot} \tag{3}$$

where the proportionality coefficient S depends on the number of dye molecules, the illumination intensity, the dye absorption, the quantum yield of the dye, the photon-collection efficiency of the optical setup and the quantum efficiency of the detector. Since the dye properties differ in the free and the Ca^{2+}-bound indicator forms with respect to their quantum yield and their absorption, the concentrations $[B]$ and $[CaB]$ contribute with different factor S_f and S_b, respectively:

$$F = S_f[B] + S_b[CaB] \tag{4}$$

By assuming chemical equilibrium between Ca^{2+} and the indicator, the *law of mass action* is valid:

$$K_d := \frac{k_{off}}{k_{on}} = \frac{[Ca^{2+}][B]}{[CaB]} \tag{5}$$

where K_d is is the dissociation constant (M) of the indicator. So the measured fluorescence signal F can be converted to $[Ca^{2+}]_i$ by combining Eq.4 and Eq.5 and assuming $[B]_{tot} = [B] + [CaB]$ constant in time and space (since usually closed system are considered):

$$[Ca^{2+}]_i(t, \vec{x}) = K_d \frac{F(t, \vec{x}) - F_{min}(\vec{x})}{F_{max}(\vec{x}) - F(t, \vec{x})} \tag{6}$$

where $F_{min} = S_f[B]_{tot}$ is the fluorescence intensity at zero Ca^{2+} concentration and $F_{max} = S_b[B]_{tot}$ at saturating $[Ca^{2+}]_i$. These extremal fluorescence values have to be determined with a calibration measurement during the experiment [8].

2.2 Reaction-diffusion equations

Since calcium ions and dye molecules can move inside the cytoplasm [1, 13], a diffusive term has to be add to Eq.2, which lead to a system of so-called *reaction-diffusion equations*:

$$\frac{d[Ca^{2+}]}{dt} = R + D_{Ca}\nabla^2[Ca^{2+}] + J_{in} \tag{7}$$

$$\frac{d[B]}{dt} = R + D_B\nabla^2[B] \tag{8}$$

$$\frac{d[CaB]}{dt} = -R + D_{CaB}\nabla^2[CaB] \tag{9}$$

where D_{Ca}, D_B and D_{CaB} are the diffusion coefficients for free Ca^{2+}, free buffer and bound buffer, respectively, and J_{in} is the Ca^{2+} influx [4, 11].

2.3 Numerical algorithms

To simulate the model of reaction-diffusion dynamics and to evaluate the non-equilibrium model shown in Sec.3.1, a MATLAB (V. R2018b) program has been developed. The code is reported in Appendix A.2 and can be freely downloaded at `https://github.com/GiacomoBarzon/Non-equilibrium-calcium-fluorescence`.

Finite difference In order to find a numerical solution of the system of Eq.7, Eq.8 and Eq.9 a finite difference method was used. The finite difference method is one of the most powerful tools in the numerical analysis of partial differential equations and its simple implementation consists of discretizing the function's domain in order to approximate its partial derivatives [9, 12]. In particular for the following simulations a central FD in space and forward FD in time were used, also known as the Forward- Time-Central-Space or FTCS scheme. For instance, Eq.7 becomes:

$$[Ca]_j^{t+1} = [Ca]_j^t + \left\{ R_j^t + \frac{D_{Ca}}{\Delta x^2}([Ca]_{j+1}^t - 2[Ca]_j^t + [Ca]_{j-1}^t) + J_j^t \right\}\Delta t \tag{10}$$

Eq.10 has to be modified for the voxels at the ends ($j = 1, N_{tot}$) in accordance to the chosen boundary conditions. In the following simulation reflective boundary was considered (see Appendix A.2), so calcium and dye molecules cannot diffuse outside the cell and the total intracellular concentration is preserved.

Simulation parameters The 1-D space of $100\mu m$ in length (which is approximately the typical dimension of a mammalian cell) is discretized in voxels of $\Delta x = 1\mu m$ (≈ 100 voxels). For a simple diffusion simulation the timestep is required to satisfy the condition $p = \frac{2D\cdot\Delta t}{\Delta x^2} \leq 1$, where the probability p can be interpreted as the fraction of matter transferred to adjacent positions. Combining diffusion with computation of chemical reactions requires a smaller timestep in order to avoid generation of local abrupt concentration changes [2]. The timestep used in the simulation was $\Delta t = 0.1\mu s$, that is small enough for the solution to converge.
The simulation lasts $80ms$ of real time and during the first $10ms$ there is a Ca^{2+} influx $J_{in} = 50\frac{\mu M}{ms}$ entering in the middle cell of the discretized space. The space is initially filled with a intracellular calcium concentration of $[Ca^{2+}]_{t=0} = 0.1\mu M$, that is in chemical equilibrium with the dye. The total dye concentration is $[B]_{tot} = 100\mu M$ and is assumed to be a BAPTA molecule. All the

physiological parameters used for the computation are taken from [2]. So the free and bound dye initial concentration can be expressed as:

$$[B]_{t=0} = \frac{K_d}{K_d + [Ca]_{t=0}}[B]_{tot} \tag{11}$$

$$[CaB]_{t=0} = \frac{[Ca]_{t=0}}{K_d + [Ca]_{t=0}}[B]_{tot} \tag{12}$$

Also all the results are independent of the proportional coefficient S_f and S_b present in Eq.4 (except the trend of F and dF/dt obviously), so their values are assumed to be $S_f = 1A.U./\mu M$ and $S_b = 5A.U./\mu M$.

3 Results

3.1 Non-equilibrium model derivation

In this work a mathematical model was derived by relaxing the hypothesis of chemical equilibrium (i.e. R no longer negligible). Starting from the fluorescence intensity expression shown in Eq.4 and the reaction-diffusion equations Eq.7, Eq.8 and Eq.9, the cytosolic free calcium concentration $[Ca^{2+}]_i$ can be expressed as a function of the dye fluorescence F, its time derivative dF/dt and its extremal values F_{max} and F_{min} and the total dye concentration $[B]_{tot}$, which are all experimental parameters that can be easily measured. Also, the total dye concentration is considered fixed in space and time ($[B]_{tot}(\vec{x}, t) \approx const$): such hypothesis is clearly non true in principle since the free and bound dye can diffuse, but its goodness was confirmed by the numerical results.

Negligible dye diffusion There are generally two classes of Ca^{2+} indicators, genetically encoded fluorescent proteins and chemically engineered fluorophores. The first are fluorescent proteins derived from green fluorescent protein (GFP) or its variants, while the others are small molecules based on an EGTA homologue called BAPTA, with high selectivity for calcium ions [10]. So these indicators are compounds typically bigger than single calcium ions: this fact means that their diffusion coefficient is smaller compared to D_{Ca} (as the Stokes-Einstein equation predicts [6]) and in some experimental situation the diffusive term in Eq.8 and Eq.9 can be neglected (since $D_B \approx D_{CaB} << D_{Ca}$).

All the calculations are shown in Appendix A.1. Finally the free calcium concentration $[Ca^{2+}]$ can be expressed as:

$$[Ca^{2+}](\vec{x}, t) = \frac{1}{k_{on}(F_{max}(\vec{x}) - F(\vec{x}, t))}\left(\frac{dF(\vec{x}, t)}{dt} + k_{off}(F(\vec{x}, t) - F_{min}(\vec{x}))\right) \tag{13}$$

Non-negligible dye diffusion In the more general situation the diffusive term in Eq.8 and Eq.9 is relevant, so the assumption of non-diffusible dye is relaxed ($D_B \approx D_{CaB} \neq 0$). Then in Eq.13 an additional diffusive term appears, so the final result (as shown in Appendix A.1.2) is:

$$[Ca^{2+}](\vec{x}, t) = \frac{1}{k_{on}(F_{max}(\vec{x}) - F(\vec{x}, t))}\left(\frac{dF(\vec{x}, t)}{dt} + A\, D_B \nabla^2[B](\vec{x}, t) + k_{off}(F(\vec{x}, t) - F_{min}(\vec{x}))\right) \tag{14}$$

3.2 Simulation results

As mentioned in Sec.2.3, firstly the calcium and free and bound dye concentrations were simulated in all the space domain. In Figure 1 the temporal trend of the concentrations evaluated at the entry position of the external flow is reported. It can be noticed that the maximum is reached when the external flow is switched off, then the concentration values decrease due to the diffusion and the recombination, that are more important for free calcium ions.

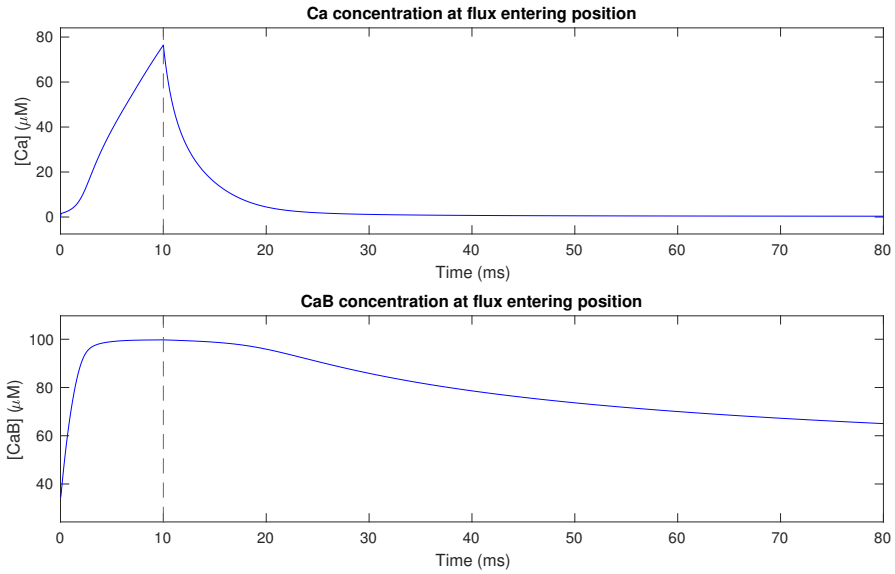

Figure 1: Temporal trend of free and bound calcium concentrations at fixed position (external flux entry position).

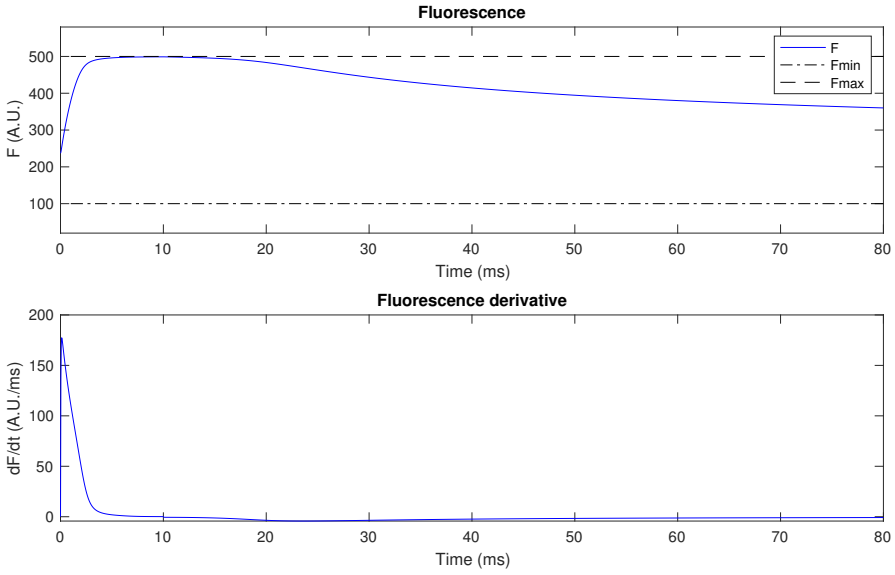

Figure 2: Fluorescence signal and its temporal derivative at the entry position of the external flux.

Then, the fluorescence signal is evaluated with Eq.4 starting from the concentration values. The fluorescence at flux entry position is reported in Figure 2 also with its temporal derivative, due to the fact that this term is present in the non-equilibrium model.

Finally in Figure 3 the simulated $[Ca^{2+}]$ (dotted curve) is compared with the trend derived with the mathematical models: the equilibrium model (Eq.6, blue curve), the non-equilibrium model with neglected dye diffusion (Eq.13, magenta curve) and the complete non-equilibrium model (Eq.14, red curve). As it can be noticed in Figure 3, the non-equilibrium model is in complete agreement with the simulated $[Ca^{2+}]$: this fact can be considered as the verification of the model validity.

Also the other two models are in accordance during the decreasing phase: this means that after the switch off of the external flux, the chemical equilibrium is rapidly reached. However, during the presence of the external flow, the $[Ca^{2+}]$ derived with these two model is underestimated, as it can better seen in Figure 4. The non-equilibrium model with neglected dye diffusion leads to good prevision in the initial millisecond, when the term of the fluorescence derivative is relevant (i.e. when there is a higher gradient in free and bound calcium concentration) as it can be noticed in Fig.2. After this initial part the model is similar to the equilibrium ones, while the diffusive term gains importance and is responsible for the discrepancy with the full model prediction.

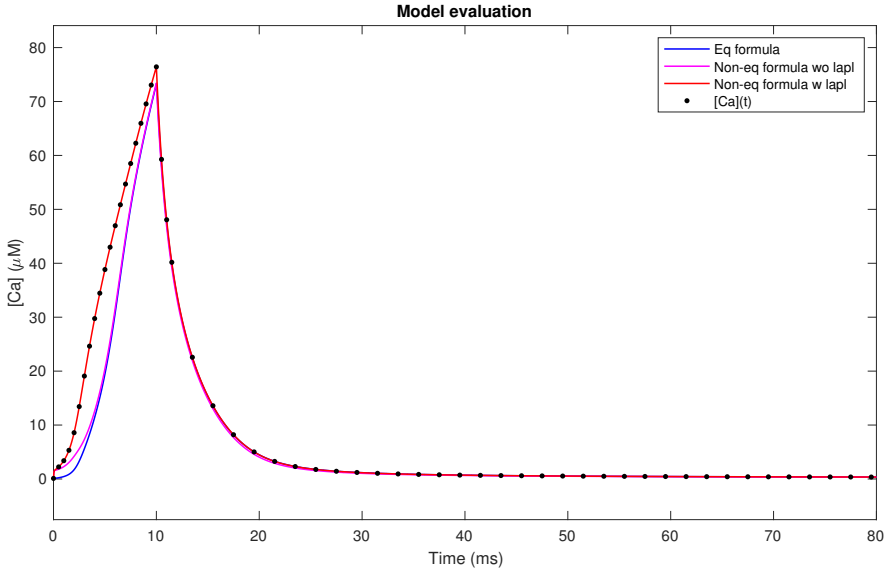

Figure 3: Comparison of $[Ca^{2+}]$ derived from the models and the simulated $[Ca^{2+}]$ in the entry position of the external flux.

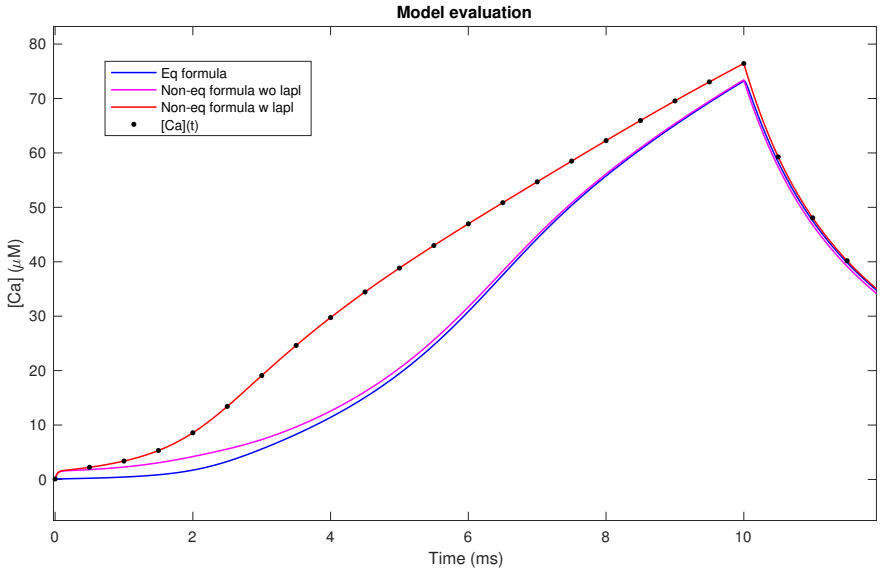

Figure 4: Comparison of $[Ca^{2+}]$ derived from the models and the simulated $[Ca^{2+}]$ during the action of the external flow (initial $10ms$).

4 Discussions

The above results show the validity of the non-equilibrium model here derived, that can be useful to obtain better estimations of $[Ca^{2+}]_i$ in situation where the system is far from the chemical equilibrium (i.e. higher concentration gradients due to external flux). However the diffusive term present in Eq.14 is usually unknown by the experimentalist, since it depends on the dye concentration at each position and at each timestep. For this reason the useful model is the one described in Eq.13, which depends only on variables that can be directly measured. Despite this model is an improvement of the equilibrium model shown in Eq.6, it's still an approximation and gets more correct expectation in presence of strong concentration gradients (that reflects in higher fluorescence derivative), while it becomes more incorrect as the diffusive term grows. Numerical simulation could be helpful in order to estimate this diffusive term and improve the estimation of $[Ca^{2+}]_i$.

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Appendix

A.1 Models computation

A.1.1 Negligible dye diffusion

Since the total buffer concentration $[B]_{tot} = [B] + [CaB]$ is fixed, Eq.4 can be rewrited as:

$$F = S_f[B] + S_b[CaB] = \frac{F_{min}}{[B]_{tot}}[B] + \frac{F_{max}}{[B]_{tot}}[CaB] \tag{15}$$

$$F = F_{min} + \frac{F_{max} - F_{min}}{[B]_{tot}}[CaB] = F_{max} - \frac{F_{max} - F_{min}}{[B]_{tot}}[B] \tag{16}$$

Now, deriving respect to time Eq.16:

$$\frac{dF}{dt} = A\frac{d[CaB]}{dt} = -A\frac{d[B]}{dt} \tag{17}$$

where

$$A = \frac{F_{max} - F_{min}}{[B]_{tot}} \tag{18}$$

So, Eq.17 can be compared with Eq.8 or Eq.9:

$$R = -\frac{1}{A}\frac{dF}{dt} \tag{19}$$

Now, by manipulating the term R:

$$R = k_{off}[B]_{tot} - (k_{off} + k_{on}[Ca])[B] \tag{20}$$

Using Eq.16 the dependence from [B] can be neglected:

$$\frac{F_{max} - F}{F_{max} - F_{min}} = \frac{[B]}{[B]_{tot}} \tag{21}$$

Inserting Eq.21 and Eq.20 in Eq.19:

$$\frac{[B]_{tot}}{F_{max} - F_{min}}\frac{dF}{dt} = -[B]_{tot}\left(k_{off} - (k_{off} + k_{on}[Ca])\frac{F_{max} - F}{F_{max} - F_{min}}\right) \tag{22}$$

$$\frac{dF}{dt} = (k_{off} + k_{on}[Ca])(F_{max} - F) - k_{off}(F_{max} - F_{min}) \tag{23}$$

$$\frac{dF}{dt} = k_{on}[Ca](F_{max} - F) - k_{off}(F - F_{min}) \tag{24}$$

$$\frac{dF}{dt} + k_{off}(F - F_{min}) = k_{on}(F_{max} - F)[Ca] \tag{25}$$

Finally the free calcium concentration Ca^{2+} can be derived as:

$$[Ca^{2+}](\vec{x}, t) = \frac{1}{k_{on}(F_{max}(\vec{x}) - F(\vec{x}, t))}\left(\frac{dF(\vec{x}, t)}{dt} + k_{off}(F(\vec{x}, t) - F_{min}(\vec{x}))\right) \tag{26}$$

A.1.2 Non-negligible dye diffusion

In the more general situation the diffusive term in Eq.8 and Eq.9 is relevant, so the assumption of non-diffusible dye is relaxed ($D_B \approx D_{CaB} \neq 0$).
So, in Eq.19 an additional term appears:

$$R + D_B \nabla^2[B] = -\frac{1}{A}\frac{dF}{dt} \tag{27}$$

By finishing the calculation as done in A.1.1 the final formula becomes:

$$[Ca^{2+}](\vec{x},t) = \frac{1}{k_{on}(F_{max}(\vec{x}) - F(\vec{x},t))}\left(\frac{dF(\vec{x},t)}{dt} + A\, D_B \nabla^2[B](\vec{x},t) + k_{off}(F(\vec{x},t) - F_{min}(\vec{x}))\right) \tag{28}$$

A.2 Code

```
%%
% FREE CALCIUM CONCENTRATION IN NON-EQUILIBRIUM CONDITIONS
%
% - discretization of the reaction-diffusion equations
% - Ca, CaB and B concentrations temporal and spatial evolution
% - Fluorescence estimation (and its temporal derivative)
% - Comparison of the model for the calcium concentration estimation
%   starting from fluorescence measurements
%
%
%%

clc; clear; close all;
disp('START SIMULATION');

% time evolution:
% - if true: show time-evolution of calcium and free dye spatial
    concentration
% - if false: hide time-evolution of calcium and free dye spatial
    concentration
timeEvolution = false;

tic;                                        % starting computational time

%% Physiological constants
Dca = 0.44;                                 % calcium diffusion coefficient (
    microm^2 / ms)

% BAPTA
Db = 0.27;                                  % dye diffusion coefficient (
    microm^2 / ms)
Kplus = 0.5;                                % dye binding rate constant (1 /
    microM / ms)
Kminus = 0.096;                             % dye unbinding rate constant (1 /
     ms)
Kd = Kminus/Kplus;                          % dye dissociation constant (
    microM)
```

```
%% Simulation variables initialization
Nvox = 101;                                % total number of voxels
vox = 1:Nvox;
dx = 1.0;                                  % voxel size (microm)
dt = 10^-4;                                % integration time (ms)
totalTime = 80;                            % total simulation time (ms)
steps = totalTime / dt;
fluxDuration = 10;                         % total influx time (ms)
fluxEnd = fluxDuration / dt;

J = 50.0;                                  % influx current density (microM /
    ms)
totalJ = J * fluxDuration;                 % total entering calcium
   concentration (microM)
n0 = ceil(Nvox/2);                         % influx position voxel

%% Concentration initialization
Cai = 0.1;                                 % initial free calcium
   concentration (microM)
Btot = 100;                                % total dye concentration (microM)

% concentration initialization for B and CaB -> steady state solution
Bi = Kd / (Kd + Cai) * Btot;               % initial free dye concentration (
   microM)
CaBi = Btot - Bi;                          % initial bound dye concentration
   (microM)

% array to store run-time concentrations
Ca = zeros(1,Nvox) + Cai;
B = zeros(1,Nvox)+ Bi;
CaB = zeros(1,Nvox) + CaBi;


%% Discrete reaction-diffusion model
% diffusion probability
pCa = 2 * Dca / dx^2 * dt;                 % fraction of calcium transferred
   to adjacent positions
pB = 2 * Db / dx^2 * dt;                   % fraction of dye transferred to
   adjacent positions

% kernel used to evaluate the laplacian
kernelCa = [pCa/2 -pCa pCa/2];
kernelB = [pB/2 -pB pB/2];

% array to store time evolution concentrations at fixed distance
% for istance, in flux entering position
Cat = zeros(1,steps);                      % temporal evolution of Ca at
   fixed distance
Cat(1) = Cai;                              % initial condition
CaBt = zeros(1,steps);                     % temporal evolution of CaB at
   fixed distance
CaBt(1) = CaBi;                            % initial condition
Bt = zeros(1,steps);                       % temporal evolution of B at fixed
    distance
Bt(1) = Bi;                                % initial condition

% array to store the laplacian of CaB
% used in the fluorescence formula evaluation
laplCaB = zeros(1,steps);
```

```
% create empty figures
if timeEvolution == true
    figure('Name','Concentration time evolution','NumberTitle','off','
       Position',[1 400 600 400]);
    ax1 = subplot(2,1,1);
    grid(ax1,'on');
    ax2 = subplot(2,1,2);
    grid(ax2,'on');
end

% LOOP OVER TIME STEPS
for k = 2:steps

    % Reaction-diffusion for Ca
    laplacianCa = conv2(Ca, kernelCa, 'same');

    % reflective boundary
    laplacianCa(1) = -pCa*Ca(1) + pCa*Ca(2);
    laplacianCa(Nvox) = -pCa*Ca(Nvox) + pCa*Ca(Nvox-1);

    R = Kminus .* CaB - Kplus .* Ca .* B;        % reaction term

    deltaCa = laplacianCa + R * dt;

    if k < fluxEnd
        deltaCa(n0) = deltaCa(n0) + J * dt;
    end


    % Reaction-diffusion for B
    laplacianB = conv2(B, kernelB, 'same');

    % reflective boundary
    laplacianB(1) = -pB*B(1) + pB*B(2);
    laplacianB(Nvox) = -pB*B(Nvox) + pB*B(Nvox-1);

    deltaB = laplacianB + R * dt;


    % Reaction-diffusion for CaB
    laplacianCaB = conv2(CaB, kernelB, 'same');

    % reflective boundary
    laplacianCaB(1) = -pB*CaB(1) + pB*CaB(2);
    laplacianCaB(Nvox) = -pB*CaB(Nvox) + pB*CaB(Nvox-1);

    deltaCaB = laplacianCaB -R * dt;


    % store laplacian of CaB
    laplCaB(k) = laplacianCaB(n0);


    % update concentrations
    Ca = Ca + deltaCa;
    B = B + deltaB;
    CaB = CaB + deltaCaB;
```

```
    % Plot
    if timeEvolution == true
        if (mod(k,10^4)==0) && (k*dt < 30)
            plot(ax1, vox, Ca);
            grid(ax1,'on');
            ylim(ax1, [0 100]);
            xlabel(ax1, 'X (\mum)');
            ylabel(ax1, '[Ca] (\muM)')
            if k < fluxEnd
                title(ax1, ['Ca concentration                Time: ',
                    num2str(k*dt),'ms, Flux: ON']);
            else
                title(ax1, ['Ca concentration                Time: ',
                    num2str(k*dt),'ms, Flux: OFF']);
            end

            plot(ax2, vox, CaB);
            grid(ax2,'on');
            ylim(ax2, [20 110]);
            xlabel(ax2, 'X (\mum)');
            ylabel(ax2, '[CaB] (\muM)')
            if k < fluxEnd
                title(ax2, ['CaB concentration               Time: ',
                    num2str(k*dt),'ms, Flux: ON']);
            else
                title(ax2, ['CaB concentration               Time: ',
                    num2str(k*dt),'ms, Flux: OFF']);
            end
            pause(0.4);
        end
    end

    % evaluate the constancy of total dye concentration in each position
    %if mod(k,500)==0
    %    temp = B + CaB
    %end

    % update temporal evolution in flux enter position
    Cat(k) = Ca(n0);
    CaBt(k) = CaB(n0);
    Bt(k) = B(n0);
end
% END LOOP OVER TIME STEPS


%% Concentration at fixed distance
figure('Name','Concentration at fixed distance','NumberTitle','off','
   Position',[800 400 700 400]);
ax3 = subplot(2,1,1);
ax4 = subplot(2,1,2);

% Free calcium concentration plot
plot(ax3, (1:steps)*dt, Cat, '-b');
xlabel(ax3, 'Time (ms)');
ylabel(ax3, '[Ca] (\muM)');
title(ax3, 'Ca concentration at flux entering position');
xline(ax3,(fluxEnd-0.5)*dt,'--');
ylim(ax3, [min(Cat)-max(Cat)/10 max(Cat)+max(Cat)/10]);
```

```
% Calcium bound concentration plot
plot(ax4, (1:steps)*dt, CaBt, '-b');
xlabel(ax4, 'Time (ms)');
ylabel(ax4, '[CaB] (\muM)');
title(ax4, 'CaB concentration at flux entering position');
xline(ax4,(fluxEnd-0.5)*dt,'--');
ylim(ax4, [min(CaBt)-max(CaBt)/10 max(CaBt)+max(CaBt)/10]);


%% Fluorescence simulation
Sf = 1;
Sb = 5;

% Fluorescence
Fmax = Sb * Btot;
Fmin = Sf * Btot;
F = Sf * Bt + Sb * CaBt;

% Fluorescence temporal derivative
kernelDer = [1 -1] / dt;
dF = conv2(F, kernelDer, 'same');   % discretization of temporal
    derivative
dFi = 0.0;                          % since initial configuration is in
    equilibrium
dF = [dFi dF];
dF(end) = [];

figure('Name','Fluorescence','NumberTitle','off','Position',[1 400 700
    400]);
ax5 = subplot(2,1,1);
ax6 = subplot(2,1,2);

% Fluorescence signal plot
plot(ax5,(1:steps)*dt, F, '-b', 1:totalTime, Fmin+zeros(1,totalTime), '-.k
    ', 1:totalTime, Fmax+zeros(1,totalTime), '--k');
xlabel(ax5, 'Time (ms)');
ylabel(ax5, 'F (A.U.)');
ylim(ax5, [Fmin-80 Fmax+80]);
title(ax5, 'Fluorescence');
legend(ax5, 'F', 'Fmin', 'Fmax');

% Fluorescence temporal derivative plot
plot(ax6,(1:steps)*dt, dF, '-b');
xlabel(ax6, 'Time (ms)');
ylabel(ax6, 'dF/dt (A.U./ms)');
title(ax6, 'Fluorescence derivative');


%% Models evaluation

% Calcium concentration from estimated formulae
CaEq = Kd .* (F-Fmin) ./ (Fmax - F);
CaNoLapl = (dF + (F-Fmin).*Kminus) ./ Kplus ./ (Fmax-F);
A = (Fmax - Fmin)/Btot;
CaLapl = (dF - laplCaB .* A ./ dt + (F-Fmin).*Kminus) ./ Kplus ./ (Fmax-F)
    ;
```

```
% Models comparing plot
figure('Name','Model evaluation','NumberTitle','off','Position', [400 1
    700 400]);
plot((1:steps)*dt, CaEq, '-b', (1:steps)*dt, CaNoLapl, '-m', (1:steps)*dt,
     CaLapl, '-r', 'LineWidth',1);
hold on;
plot((1:5000:fluxEnd+15000)*dt, Cat(1:5000:fluxEnd+15000), 'Linestyle', '
    none', 'Marker', '.', 'MarkerSize',10,'MarkerEdgeColor','k');
plot((fluxEnd+15000:20000:steps)*dt, Cat(fluxEnd+15000:20000:steps), '
    Linestyle', 'none', 'Marker', '.', 'MarkerSize',10,'MarkerEdgeColor','k
    ');
ylim([min(Cat)-max(Cat)/10 max(Cat)+max(Cat)/10]);
xlabel('Time (ms)');
ylabel('[Ca] (\muM)');
title('Model evaluation');
legend('Eq formula','Non-eq formula wo lapl','Non-eq formula w lapl','[Ca
    ](t)');


%% Evaluate computation time
computationTime = toc;
disp('END SIMULATION');
disp(['Total computation time: ', num2str(computationTime),' s']);
```