Analysis of Kinetics of FRAP

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Outline

- Introduction of FRAP
- The Goal of the research
- Theoretical modeling of FRAP
  - Assumptions of the Analysis
  - The process of photobleaching
  - The intensity profile of laser beam
  - The recovery of photobleaching
- Data Processing using MATLAB
  - The main structure of MATLAB codes
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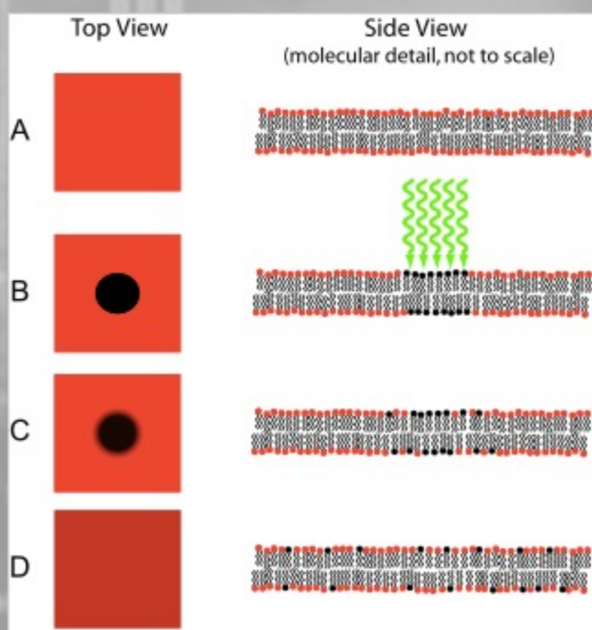
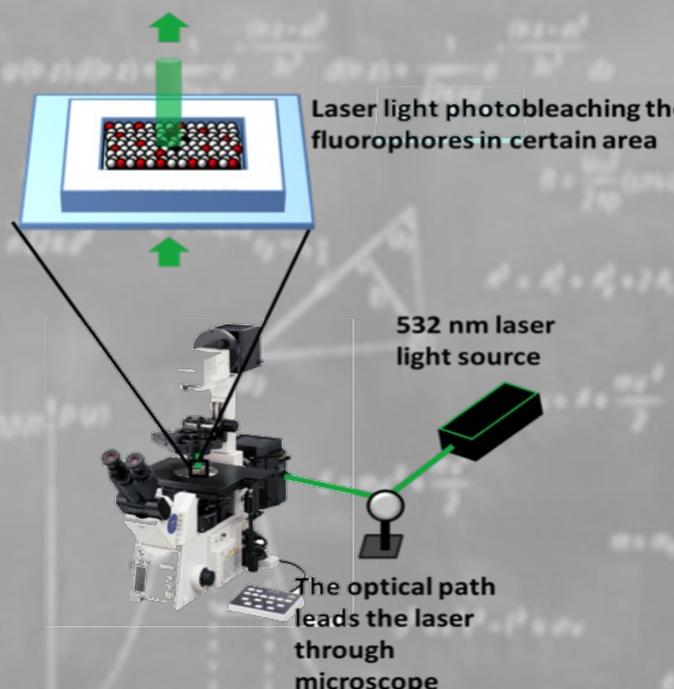
- Reference

Introduction of FRAP

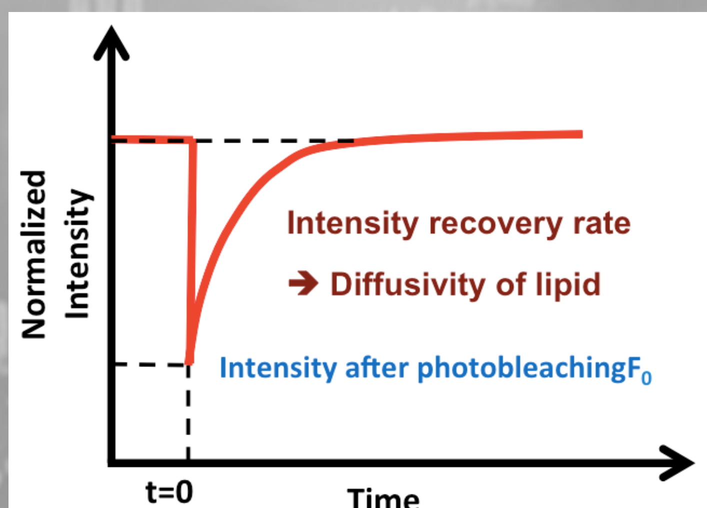
- FRAP (fluorescence recovery after photobleaching) an optical technique capable of quantifying two dimensional lateral diffusion of
  - a fluorescently labeled thin film
  - a single cell



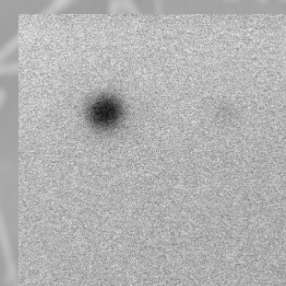


Introduction of FRAP

- An FRAP experiment provides information including:
  1. transport process type, i.e. the admixture of random diffusion and uniform directed flow
  2. the diffusion constant and/or flow velocity
  3. the fraction of total fluorophore which is mobile.

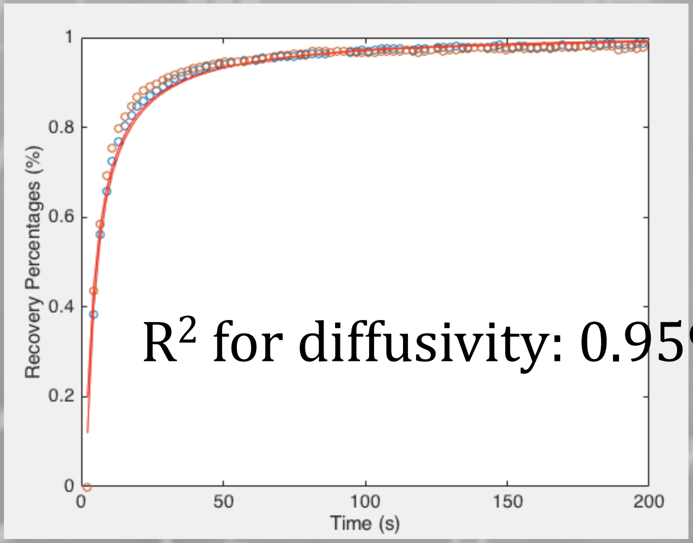


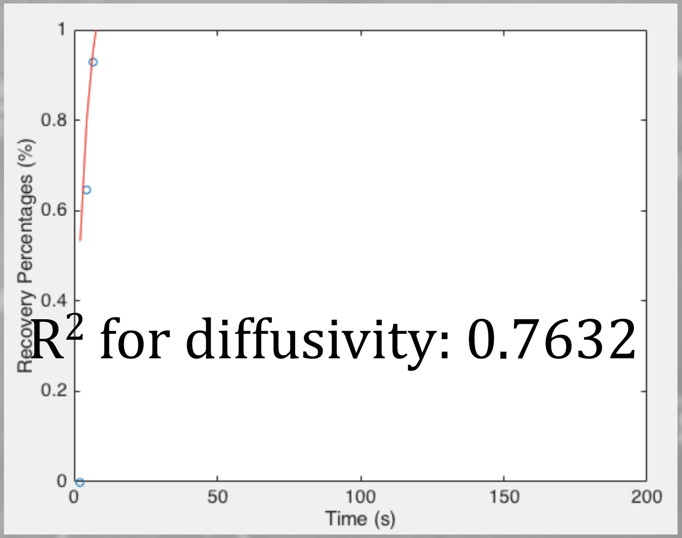
Recovering Intensity F(t)



The Goal of The Research

- Our goal is to derive the model of FRAP, processing data to obtain parameters we want using MATLAB.
- In the earliest work, a fitting formula for the FRAP of DOPC has been developed. However, when fitting the data based on GPMVs, the equation does not fit very well.





- Possible Solution
  - Adjustment of FRAP theory
    ex. differences of diffusivity between two layers of GPMVs

$$F(t) = F_i \sum_{m=0}^{\infty} \frac{(-K)^m r_e^2}{m!\,[r_e^2 + m(8D_e t + r_n^2)]} \rightarrow \text{Modified Equation}$$

  - Adjustment of MATLAB code
    ex. the adjustment of the way of approximation

- Theoretical modeling of FRAP
  - Assumptions of the Analysis
  - The process of photobleaching
  - The intensity profile of laser beam
  - The recovery of photobleaching

Biomimetic Lipid Membrane

Assumptions of the Analysis

When analyzing the kinetics of FRAP, we made following assumptions:

1. Laser beam: paraxial and Gaussian distributed
2. The intensity and power of laser is constant with time
3. Diffusion occurs only after the laser beam stops acting.
4. Photobleaching is a irreversible 1^{st} order reaction.
5. The recovery of photobleaching is pure diffusion and circular symmetric.
6. The fluorescence intensity of fluorophore is proportional to its concentration.

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The Process of Photobleaching

1. Assume photobleaching is an irreversible 1st reaction, we have:

$$\text{rate} = -\frac{dC}{dt} = k_r C\,,\, k_r = \alpha I(r) \Rightarrow \frac{dC(r,t)}{dt} = -\alpha I(r) C(r,t)$$

with initial condition $C(r,0)=C_0$

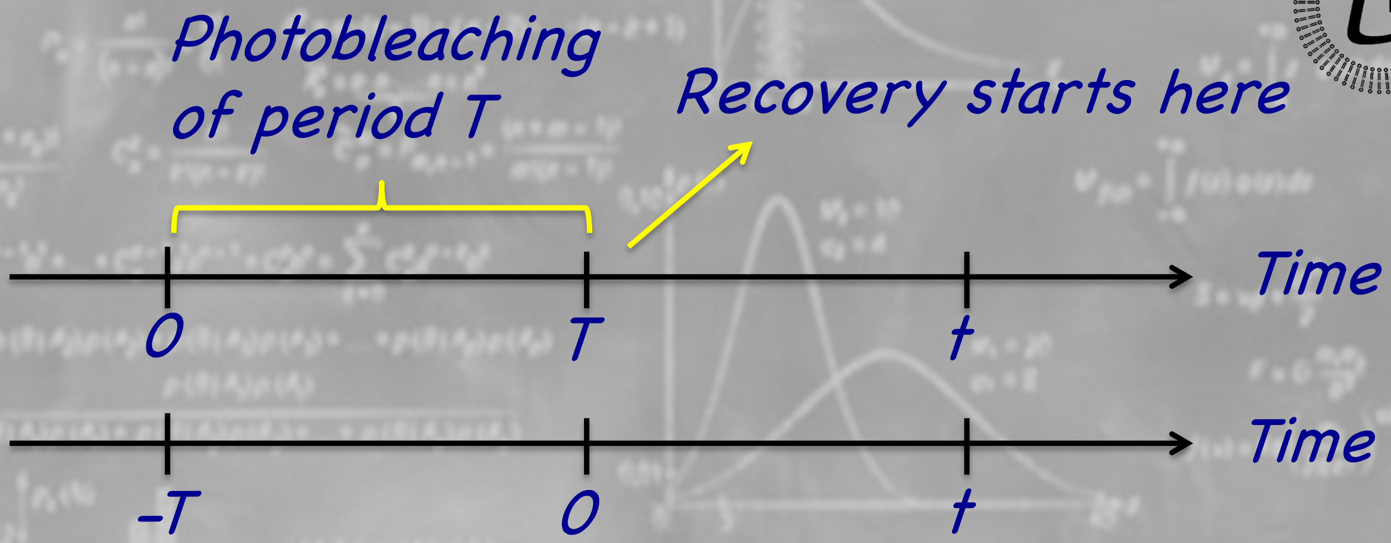
Solving the differential equation, we have:

$$C(r,t) = C_0 e^{-\alpha I(r) t}$$

If the photobleaching last for a period T

$$C(r,T) = C_0 e^{-\alpha I(r) T}$$

Theoretical Modeling of FRAP



Therefore, we obtain $C(r,0) = C_0 e^{-\alpha I(r) T}$

Also, we define the bleaching parameter as $K = \alpha I(0)$

Now, we are going to find I(r), i.e. the intensity profile to see the importance of K on C(r,0).

The Intensity Profile of Laser Beam

According to the Gaussian distribution, we could assume the intensity profile as $I(r)= A\exp(-Cr^2)$

We solve the coefficient A and C for we know that I(r) must satisfy conditions as follows:

1. Definition of intensity: (P_0 is the power of laser beam.)

$$\int I(r)dA = \int_0^{2\pi}\int_0^{\infty} A\exp(-Cr^2)rdrd\theta = P_0$$

$$\Rightarrow A = \frac{CP_0}{\pi} \Rightarrow I(r) = \frac{CP_0}{\pi}\exp(-Cr^2)$$

2. Paraxial Approximation:

For a paraxial ray with radius r, its power can be expressed as:

$$P(r,z) = P_0(1 - e^{-\frac{2r^2}{w^2(z)}})$$

where w(z) is the radius where the intensity is $1/e^2$ of that of axis.

Consider the maximum of the intensity:

$$I(0,z) = \lim_{r\to 0} \frac{P_0(1 - e^{-\frac{2r^2}{w^2(z)}})}{\pi r^2} = \frac{2P_0}{\pi w^2} = \frac{CP_0}{\pi} \Rightarrow C = \frac{2}{w^2}$$

Therefore, we have

$$I(r) = \frac{2r^2}{\pi w^2}\exp(-\frac{2r^2}{w^2})$$

Theoretical Modeling of FRAP

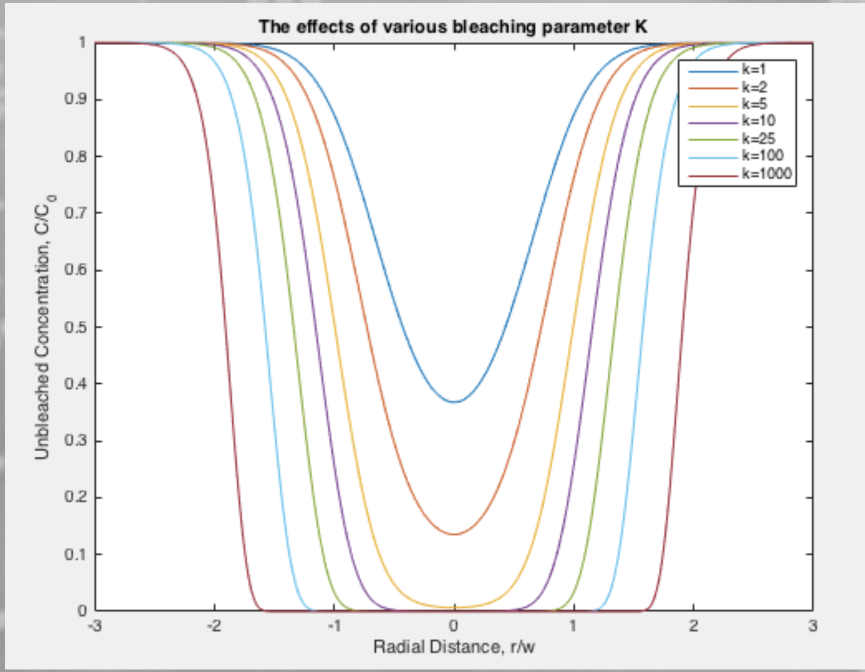
Recall that

$$K = \alpha I(0) = \alpha T\left(\frac{2P_0}{\pi w^2}\right) \Rightarrow \alpha T = \frac{K\pi w^2}{2P_0}$$

Substitute into the expression of C(r,0), we obtain

$$\frac{C(r,0)}{C_0} = \exp\left(-K \exp\left(-2\frac{r^2}{w^2}\right)\right)$$

Plot $C(r,0)/C_0$ v.s. r/w as follows:



And from the figure we can know that for larger K, C/C_0 is smaller, which means more fluorophores have been photobleached.

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The Recovery of Photobleaching

The essence of the recovery is actually a diffusive motion. Thus, we can write:

$$\frac{\partial C(r,t)}{\partial t} = D\nabla^2 C(r,t) - V_0[\frac{\partial C(r,t)}{\partial x}] = D[\frac{\partial^2 C}{\partial r^2} + \frac{1}{r}\frac{\partial C}{\partial r} + \frac{1}{r^2}\frac{\partial^2 C}{\partial \theta^2}] - V_0\frac{\partial C}{\partial x}$$

As a result of pure diffusion and circular symmetry, the equation reduces to:

$$\frac{1}{D}\frac{\partial C}{\partial t} = \frac{\partial^2 C}{\partial r^2} + \frac{1}{r}\frac{\partial C}{\partial r}$$

$$\text{with B.C.} \begin{cases} C(0,t) = \text{finite} \\ C(\infty,t) = C_0 \end{cases} \text{and I.C.}: C(r,0) = C_0 e^{-\alpha T I(r)}$$

Nonhomogenous

Theoretical Modeling of FRAP

Define fluorescence $F_k(t)$ as

$$F_k(t) = \left(\frac{q}{A}\right)\int_0^\infty I(r)C_k(r,t)d^2r = \left(\frac{q}{A}\right)\int_0^\infty I(r)C_k(r,t)2\pi r dr$$

(q: overall quantum efficiency; A: attenuation factor)

Solving $C_k(r,t)$ based on the previous PDE, we obtain $F_k(t)$

$$F_k(t) = \frac{qP_0C_0}{A}\sum_{n=0}^{\infty}\frac{(-K)^n w^2}{n!\,[w^2 + n(w^2 + 8Dt)]}$$

and its fractional form as $$f_k(t) = \frac{F_k(t) - F_k(0)}{F_k(\infty) - F_k(0)}$$

$$F_k(0) = \frac{qP_0C_0}{A}\sum_{n=0}^{\infty}\frac{(-K)^n w^2}{n!\,(w^2 + nw^2)} = \frac{qP_0C_0}{A}\sum_{n=0}^{\infty}\frac{(-K)^n}{(n+1)!}$$

Theoretical Modeling of FRAP

Biomimetic Lipid Membrane

$$F_k(0) = \frac{qP_0C_0}{A}\left(-\frac{1}{K}\right)\sum_{n=0}^{\infty}\frac{(-K)^{n+1}}{(n+1)!}$$

$$= \frac{qP_0C_0}{A}\left(-\frac{1}{K}\right)\left[\sum_{n=0}^{\infty}\frac{(-K)^n}{(n)!} - (-K)\right] = \frac{qP_0C_0}{A}\left(\frac{1-e^{-K}}{K}\right)$$

On the other hand, $as\ t \to \infty, C_k(r,t) = C_0$

and $I(r) = \left(\frac{2P_0}{\pi w^2}\right)\exp\left(-\frac{2r^2}{w^2}\right)$

$$F_k(\infty) = \frac{qC_0P_0}{A}\left(\frac{4}{w^2}\right)\int_0^{\infty}\exp\left(-\frac{2r^2}{w^2}\right)rdr$$

$$= \frac{qC_0P_0}{A}\left(\frac{4}{w^2}\right)\left(-\frac{1}{4}w^2e^{\frac{-2r^2}{w^2}}\Big|_0^{\infty}\right) = \frac{qC_0P_0}{A}$$

By substitution, we may obtain

$$f_k(t) = \frac{\sum_{n=0}^{\infty}\frac{(-K)^n w^2}{n!\,[w^2 + n(w^2 + 8Dt)]} - \left(\frac{1-e^{-K}}{K}\right)}{1 - \left(\frac{1-e^{-K}}{K}\right)}$$

- Data Processing using MATLAB
  - The main structure of MATLAB codes
  - Results obtained by MATLAB
  - Discussion

The Main Structure of MATLAB Codes

Step 1: Set the route of file reading

Step 2: Plot the intensity profile at t=0

Han_modified_FRAP4_Axelrod_K_frac_loop_newout.m | FRAP_modeling_modified_HWT.m

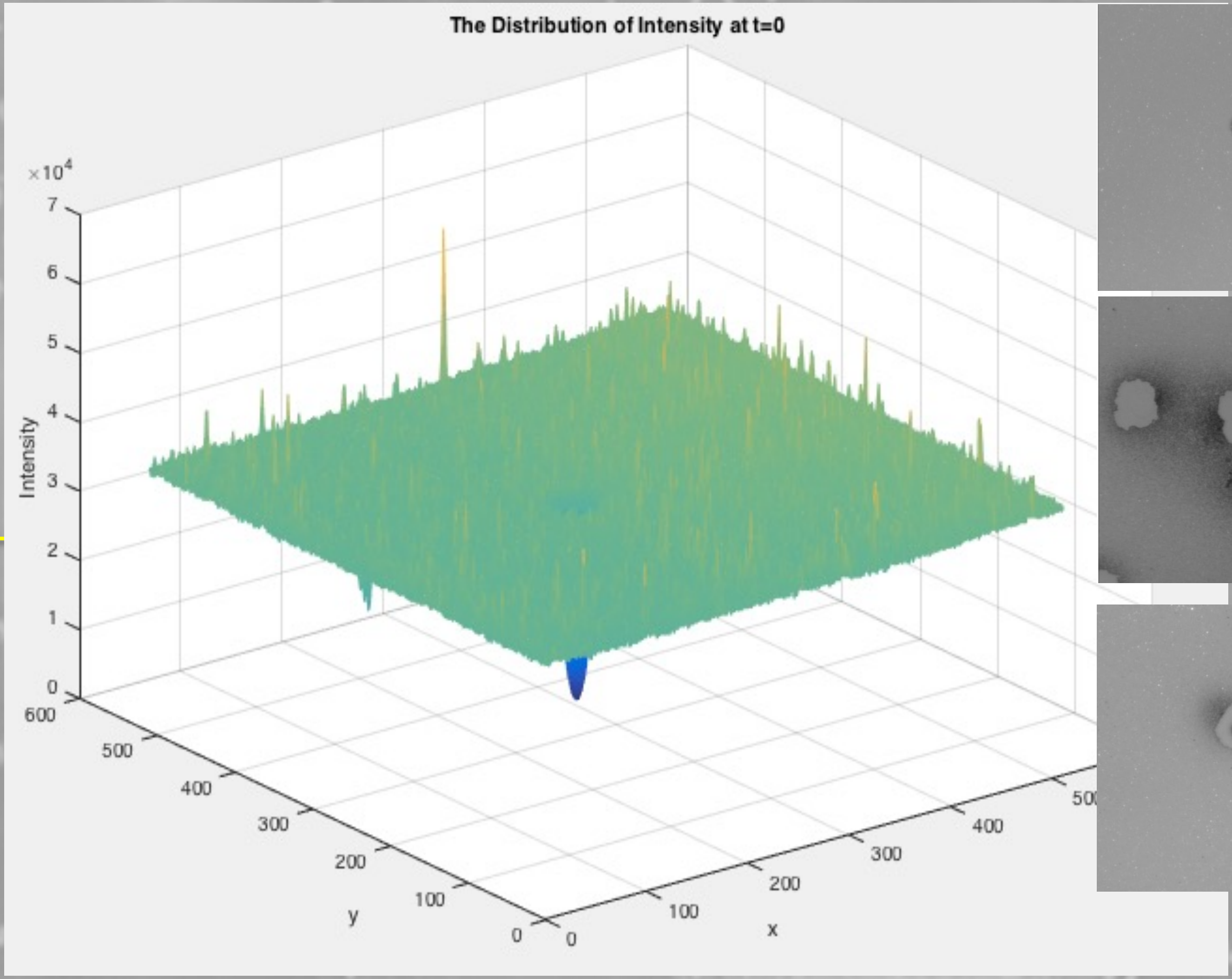
```
clear
clc
image_load_route='/Users/William/Documents/Laboratory Projects/FRAP modeling/GPMV patch/';
%(此為FRAP圖檔的所在位置)

for m=1:2%迴圈數用以一次分析多組圖檔
%Part1: 設定檔案讀取路徑
    ab=num2str(m);
    filename=['GPMV_frap_cy3(0.2)_00' ab '.tif'];
    bfilename=['GPMV_frap_cy3(0.2)_00' ab 'b' '.tif']; %打FRAP之前的照片
    animation_load=[image_load_route,filename];

%Part2:畫出t=0(打完FRAP的第一張)時Intensity在位置上的分佈
    I_1st=double(imread(animation_load,1));
    [x_length,y_length]=size(I_1st);
    [xx,yy]=meshgrid([1:x_length],[1:y_length]);
    figure(1)
    mesh(xx,yy,I_1st') %記得a*b的矩陣畫出的圖為b*a,故要轉置dimension才相符
    xlabel('x');ylabel('y');zlabel('Intensity')
    title('The Distribution of Intensity at t=0')
```

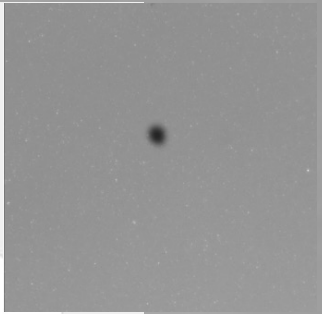
Data Processing Using MATLAB

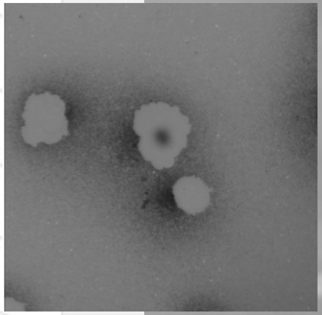
The Main Structure of MATLAB Codes

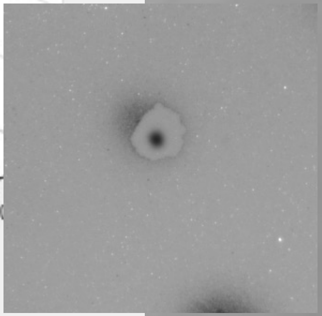
Step 1: Set the route of file reading

Step 2: Plot the intensity profile at t=0







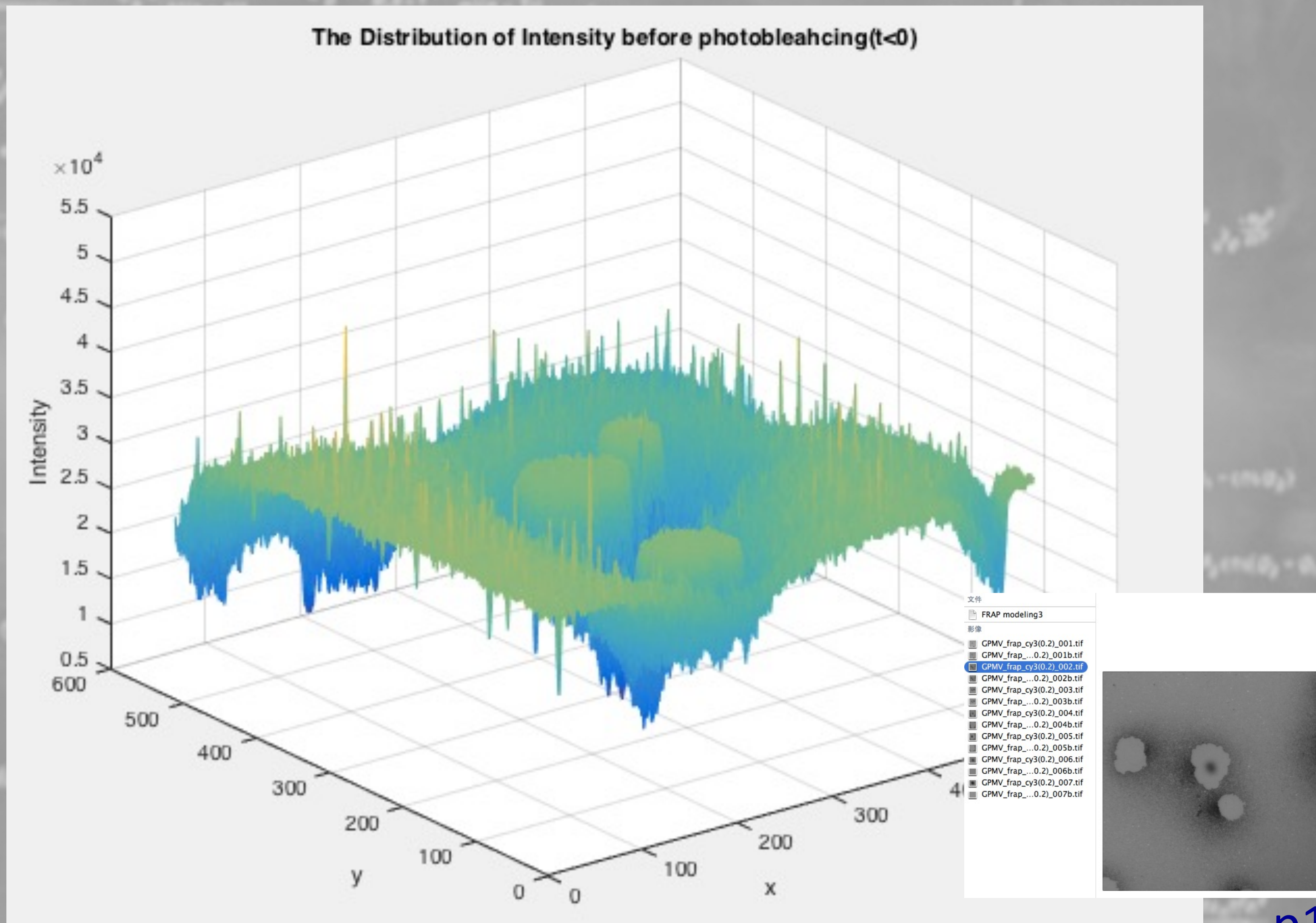


Step 3: Plot the intensity profile before bleaching
Step 4: Plot the normalized intensity profile at t=0

emporary_test.m | Han_modified_FRAP4_Axelrod_K_frac_loop_newout.m | FRAP_modeling_modified_HWT.m*

```
%Part3:畫出打FRAP前的Intensity分佈
    preimages_file_in=[image_load_route,bfilename];
    pre_image=double(imread(preimages_file_in));

    figure(2)
    mesh(xx,yy,pre_image') %同樣記得轉置
    xlabel('x');ylabel('y');zlabel('Intensity')
    title('The Distribution of Intensity before photobleahcing(t<0)')

%Part4:畫出t=0時的Normalized Intensity Distribution
    temparray=size(imfinfo(animation_load)); %temporary array 只是把一行拆開,n*1
    number=temparray(1); %imfinfo:one element for each image,此即分解圖的數量,n
    bg1=min(min(I_1st));bg2=min(min(pre_image));
    bg(m)=min(bg1,bg2); %Background的值

    for i=1:number
        I_original(:,:,i)=double(imread(animation_load,i)); %第i張的I,orignial指的是還沒normalized
        I_normalized1(:,:,i)=(I_original(:,:,i))./(pre_image); %沒扣背景值的版本
        I_normalized2(:,:,i)=(I_original(:,:,i)-bg(m))./(pre_image-bg(m)); %有扣背景值的版本
    end
```

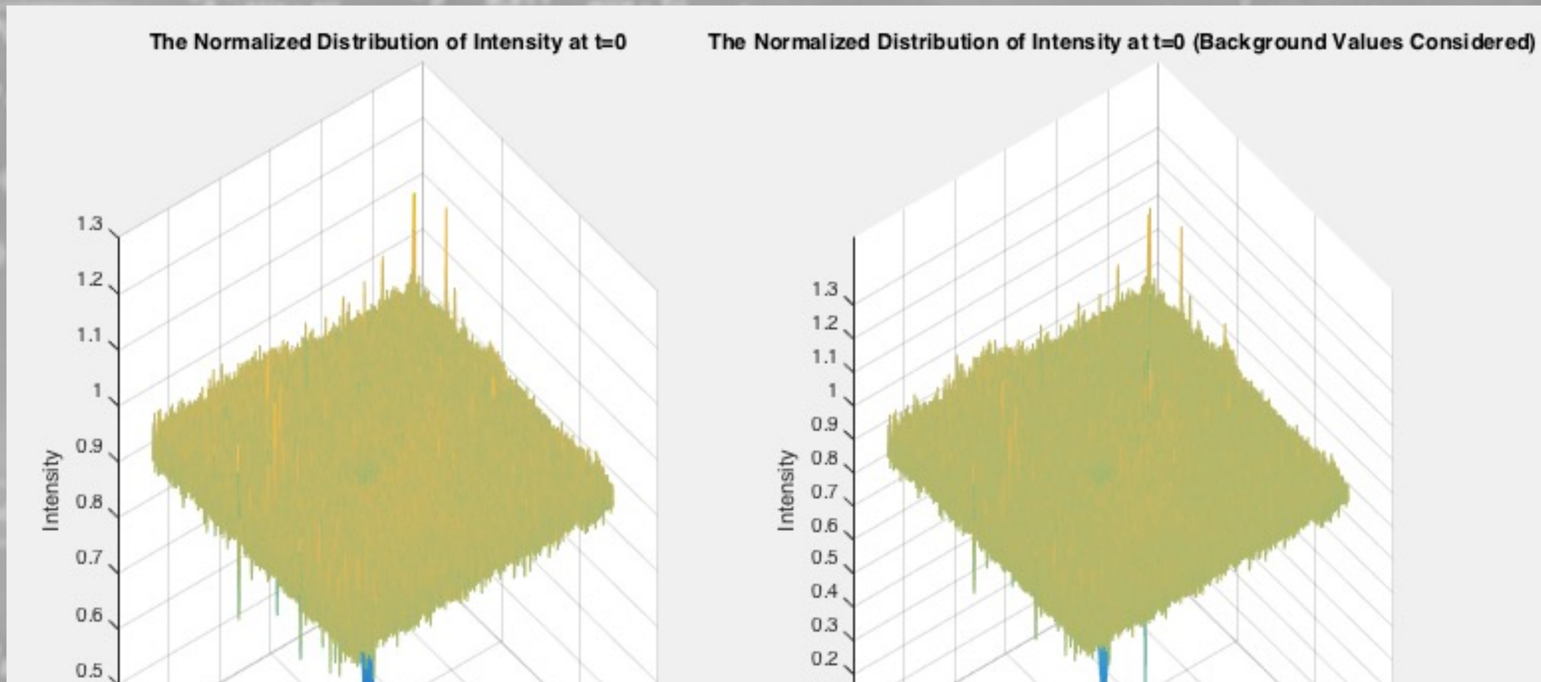
Step 3: Plot the intensity profile before bleaching
Step 4: Plot the normalized intensity profile at t=0

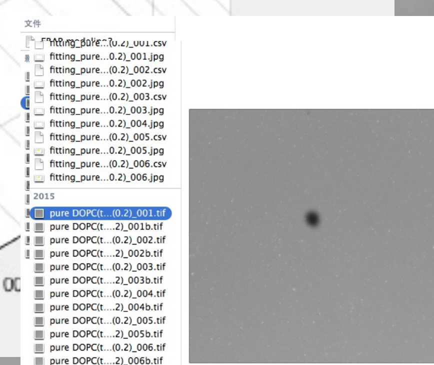


Data Processing Using MATLAB

Step 3: Plot the intensity profile before bleaching

Step 4: Plot the normalized intensity profile at t=0





Step 5: Define the photobleached area and determine its centroid

temporary_test.m | Han_modified_FRAP4_Axelrod_K_frac_loop_newout.m | FRAP_modeling_modifie

```
%Part5: 定義受到光漂白的區域並算出其質心
    bleaching=1-I_normalized2(find(I_normalized2(:,:,1)<1));

    %這裡的bleaching指的是被光漂白掉的量,包含微量的photobleaching和被雷射影響的區域
    %(由此開始考慮背景值).由於對於近軸光有個特徵半徑是軸向光強度1/e^2,我們仿照此做法定出
    %threshold,threshold,I_normalized小於threshold的即為受雷射影響區域

    threshold=1-max(bleaching)/(exp(1))^2 %剩下的螢光強度
    %threshold=0.8;
    X_sum=0;Y_sum=0;
    inside_I=(I_normalized2(:,:,1)<=threshold); %受影響區域
    outside_I=((I_normalized2(:,:,1)>threshold)&(I_normalized2(:,:,1)<1));
    %I_normalized<1但被歸類在受影響區域之外

    %以下用以計算質心的位置
    for i=1:x_length
        for j=1:y_length
            X_sum=X_sum+inside_I(i,j)*i;
            Y_sum=Y_sum+inside_I(i,j)*j;
        end
    end

    Xcenter=round(X_sum/sum(sum(inside_I)));
    Ycenter=round(Y_sum/sum(sum(inside_I)));
```

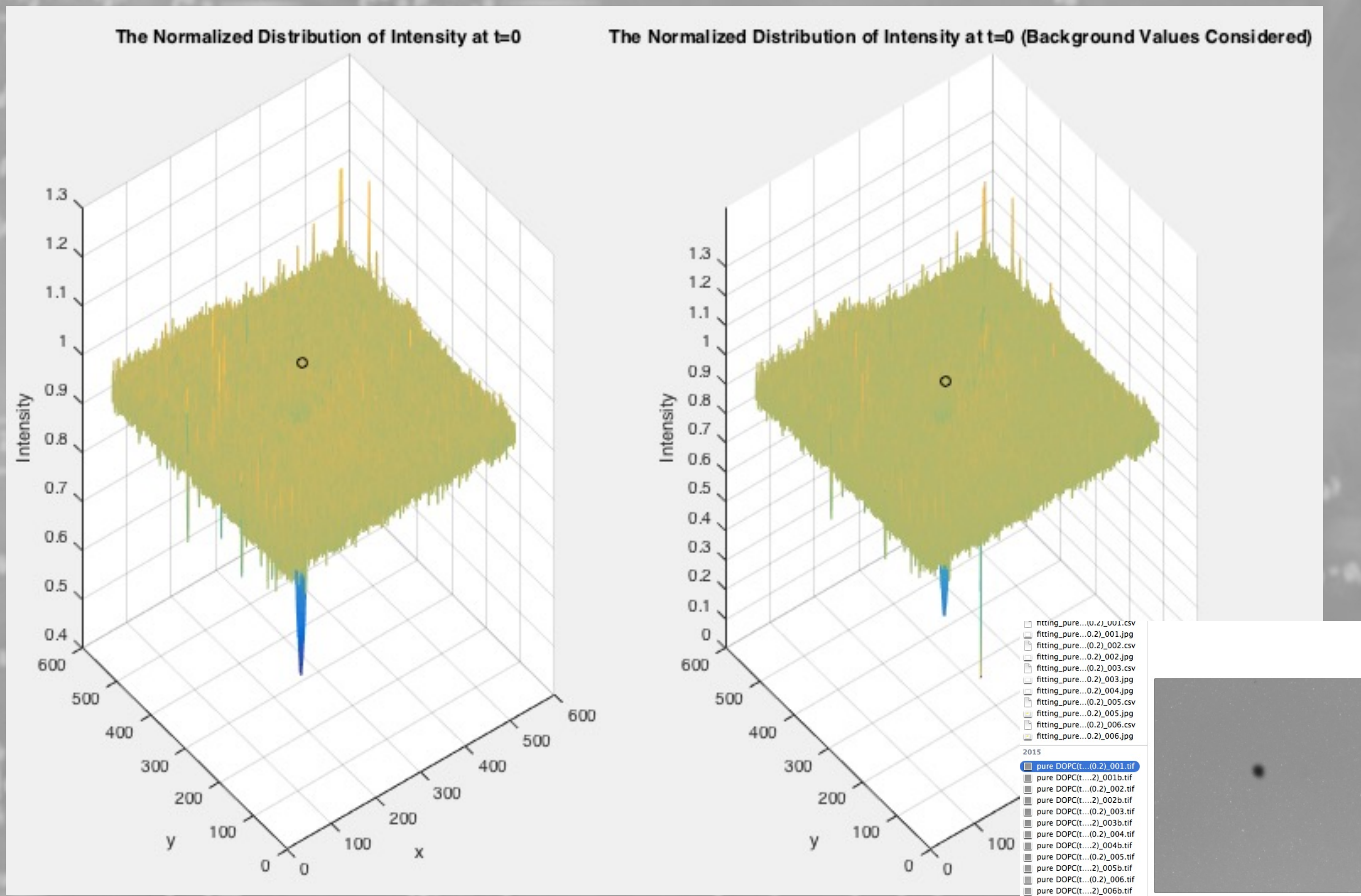
Step 5: Define the photobleached area and determine its centroid

Biomimetic Lipid Membrane

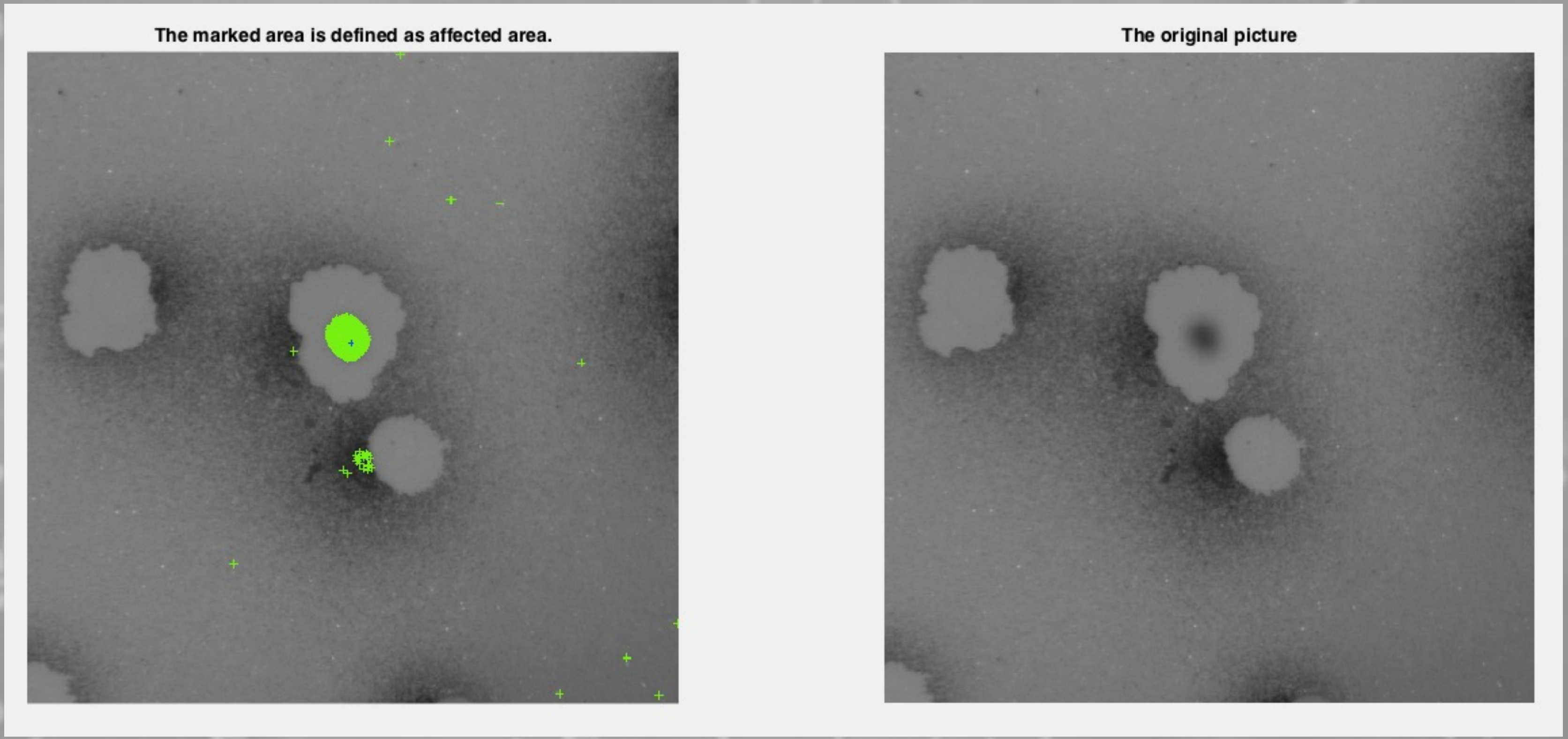
temporary_test.m | Han_modified_FRAP4_Axelrod_K_frac_loop_newout.m | FRAP_modeling_modified_HW

```matlab
%以下在圖片上做標記
[row_i,col_i]=find(inside_I); %row and column of nonzero of logic_I (受影響區域)
[row_o,col_o]=find(outside_I);
pos_i=zeros(length(row_i),2);pos_o=zeros(length(row_o),2);

for i=1:length(row_i)
    pos_i(i,2)=row_i(i);
    pos_i(i,1)=col_i(i);
end

for i=1:length(row_o)
    pos_o(i,2)=row_o(i);
    pos_o(i,1)=col_o(i);
end
k1=insertMarker(imread(animation_load,1),pos_i); %標示出受影響的區域
%k2=insertMarker(imread(animation_load,1),pos_o); %受影響區域之外
k3=insertMarker(k1,[Ycenter,Xcenter],'color','blue','size',2); %再標示出受影響區域的質心
%記得insertMarker讀進去的時候x,y會對調
%在這裡我們最後採取的做法是指標出受影響區域外的地方,則結果中環撞區域內即是受影響區域
figure(3)
subplot(1,2,1)
plot3(Xcenter,Ycenter,1.1,'ko'),hold off
subplot(1,2,2)
plot3(Xcenter,Ycenter,1.1,'ko'),hold off

figure(4)
subplot(1,2,1)
imshow(k3)
title('The marked area is defined as affected area.')
subplot(1,2,2)
imshow(animation_load)
```

Step 5: Define the photobleached area and determine its centroid



Step 5: Define the photobleached area and determine its centroid



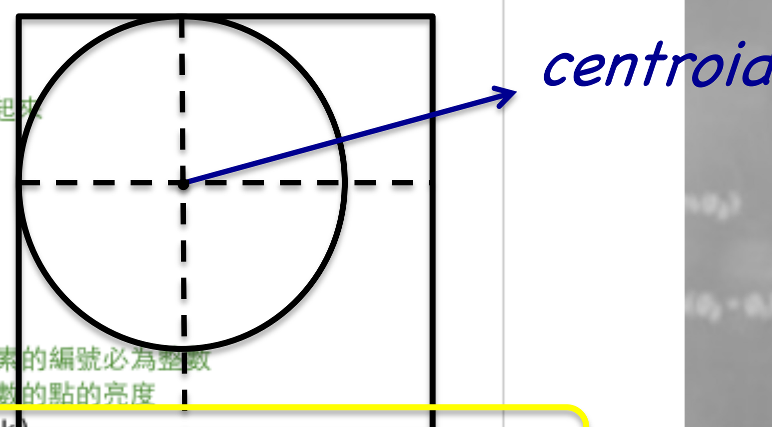
Data Processing Using MATLAB

Biomimetic Lipid Membrane

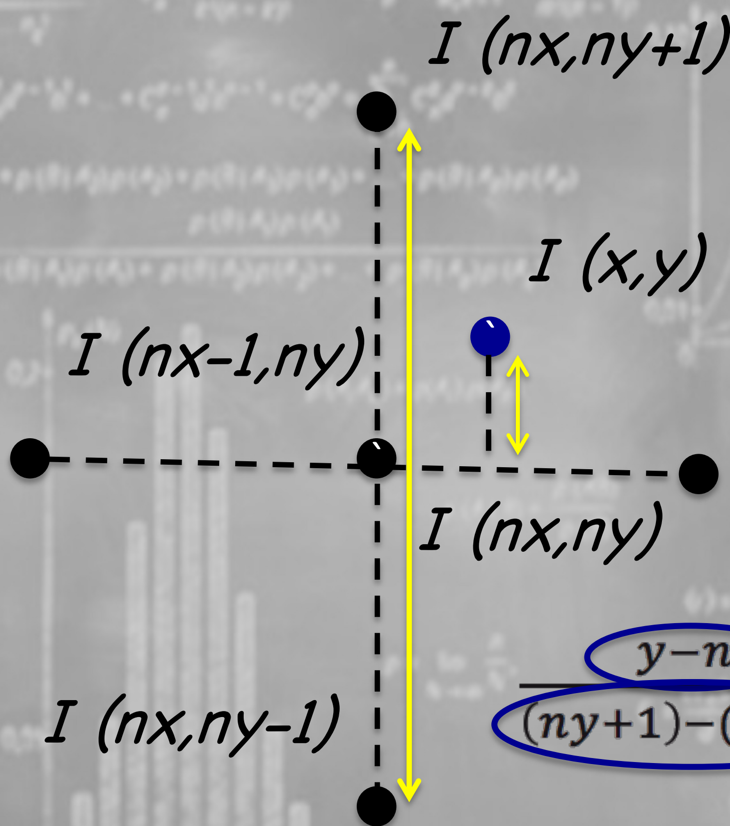
Step 6: Coordinate transformation and computation of intensity at radius r

temporary_test.m | Han_modified_FRAP4_Axelrod_K_frac_loop_newout.m | FRAP_modeling_modified_

```
%Part6: 將座標系轉換成 r-theta 座標系後算出與質心距離r所有點的平均亮度
    %先算出可以作座標轉換的最大範圍
    dist_leftbound=Xcenter-1;
    dist_rightbound=x_length-Xcenter;
    dist_upperbound=Ycenter-1;
    dist_lowerbound=y_length-Xcenter;
    r_length=min([dist_leftbound,dist_rightbound,dist_upperbound,dist_lowerbound])-1;
    %由於後面在積亮度的時候利用到(nx,ny)上下左右四個點,為了避免在邊界的時候I的index為0
    %在這裡減1縮小轉換範圍
    r=[1:1:r_length];

    %以下轉換座標的同時,將距離質心r的所有點的亮度平均起來
    for k=1:number
        for i=1:r_length
            I_sum=0; j_sum=0;
            for j=0:0.01:2*pi
                x=Xcenter+r(i)*cos(j);
                y=Ycenter+r(i)*sin(j);
                nx=round(x); ny=round(y); %像素的編號必為整數
                %以下用內插法的方式來估計座標非為整數的點的亮度
                I_sum=I_sum+I_original(nx,ny,k)...
                    +(I_original(nx-1,ny,k)-I_original(nx+1,ny,k))/2*(x-nx)...
                    +(I_original(nx,ny+1,k)-I_original(nx,ny-1,k))/2*(y-ny);
                j_sum=j_sum+1;
            end
            Ir(i,k)=I_sum/j_sum;
            %在這裡我們是假設與質心距離r的所有點亮度皆為Ir
        end
    end
```



Step 6: Coordinate transformation and computation of intensity at radius r



$$\frac{y-ny}{(ny+1)-(ny-1)} = \frac{I(x,y)-I(nx,ny)}{I(nx,ny+1)-I(nx,ny-1)}$$

2

$$I(x,y) = I(nx,ny) + \left(I(nx,ny+1) - I(nx,ny-1)\right)(y-ny)/2$$

```
I_sum=I_sum+I_original(nx,ny,k)...
      +(I_original(nx-1,ny,k)-I_original(nx+1,ny,k))/2*(x-nx)...
      +(I_original(nx,ny+1,k)-I_original(nx,ny-1,k))/2*(y-ny);
```

Data Processing Using MATLAB

Step 7: Find the optimized radius for Gaussian fitting and the normalized intensity profile

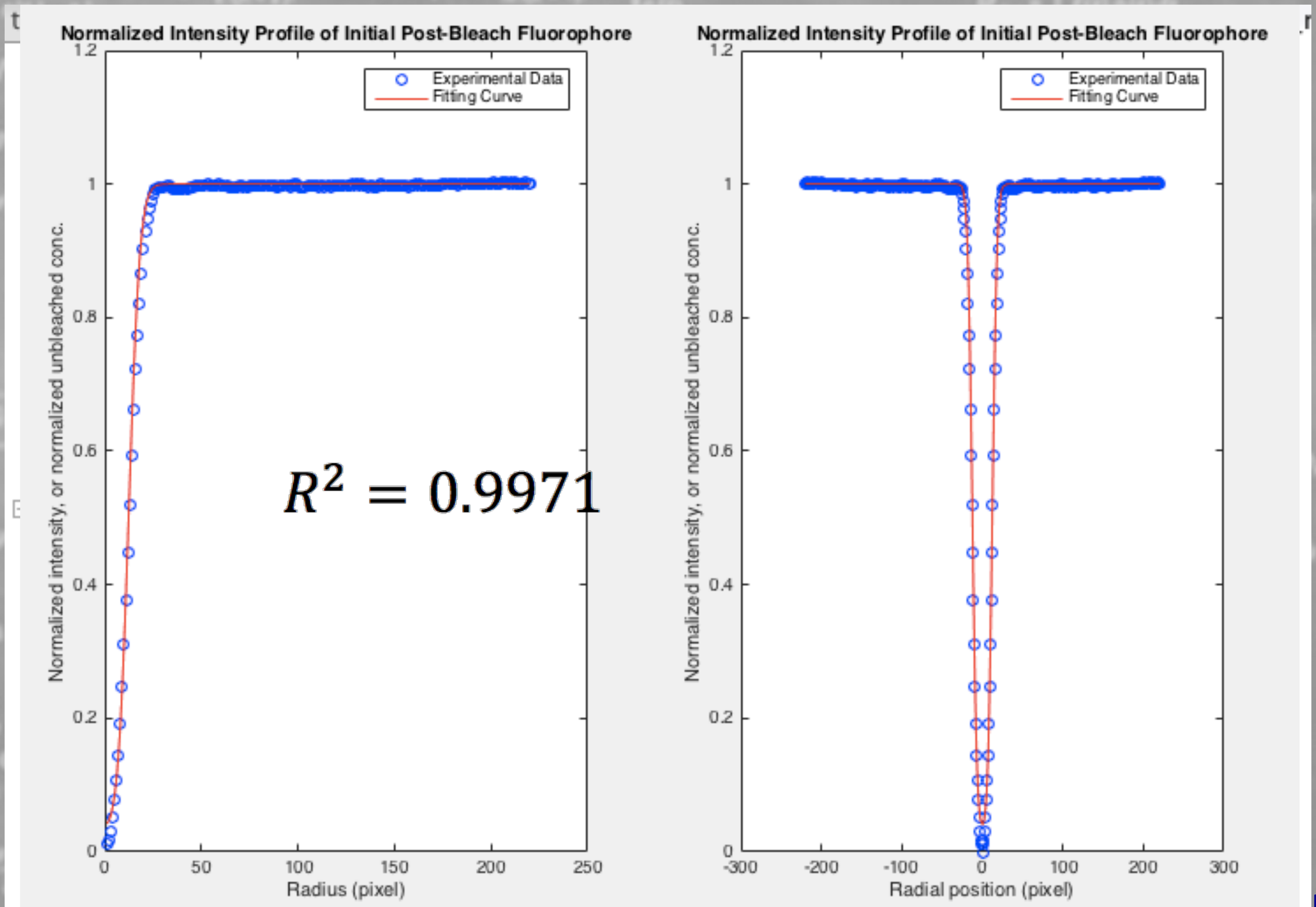
Biomimetic Lipid Membrane

Han_modified_FRAP4_Axelrod_K_frac_loop_newout.m | FRAP_modeling_modified_HWT.m*

```
%Part7: 找出Gaussian fitting的最佳範圍,並出normalized intensity的分佈圖
    for Gaussian_Radius=1:r_length
        for k=1:number
        I_outside=mean(Ir([Gaussian_Radius:r_length],1));
        It=[(Ir-bg(m))/(I_outside-bg(m))];
        %即intensity 的比值 C(r,0)/C0,轉置是為了在nlinfit時使其和r的dimension相同

        frapfunc=inline('exp(-beta(1).*exp(-2*r.^2./beta(2).^2))','beta','r');
        opts=statset('TolX',1e-12); %設定可以容忍的最大誤差值
        [beta,R,J,COVB,mse]=nlinfit(r,It(:,k)',frapfunc,[1,5],opts);
        % nlinfit 用以作非線性迴歸求出beta矩陣(即K和w),R為所有圖的誤差(residuals),
        % J: Jacobian of frapfunc, COVB: estimated variance-covariance matrix
        % for the estimated coefficients, mse: an estimate of the variance
        % of the error term,而[1,5]則是在迴歸過程中迭代所需的initial values

        K_testarray(k,Gaussian_Radius)=beta(1);
        w_testarray(k,Gaussian_Radius)=beta(2);
        rsq2_testarray(k,Gaussian_Radius)=1-sum(R.^2)/sum((It(:,k)'-mean(It(:,k)')).^2);
        % 每換一個Gaussian_Radius的值,I_outside和Ir_1st的範圍就不一樣,迴歸後得到的K
        % 和w丟進testarray,以下接著找出有最大R^2值的地方,即找出最佳fitting結果的範圍
        % 這裡很多矩陣轉置的原因很簡單,都是為了使dimension相符合
        end
    end
    %以第一張的為主找到最佳範圍
    [max_rsq2,best_fit_radius]=max(max(rsq2_testarray(1,:))); %[a b]=max(A), a為max, b為index
    max_rsq2(m)=max_rsq2; %轉成矩陣
    K(m)=K_testarray(1,best_fit_radius);
    w(m)=w_testarray(1,best_fit_radius);
    I_outside(m)=mean(Ir([best_fit_radius:r_length],1));
    % 取平均,在這裡假設了outside的intensity為均質
```

$$\frac{I(r,0)}{I_0} = \frac{C(r,0)}{C_0} = \exp\left(-K\exp\left(-2\frac{r^2}{w^2}\right)\right)$$

Step 7: Find the optimized radius for Gaussian fitting and the normalized intensity profile

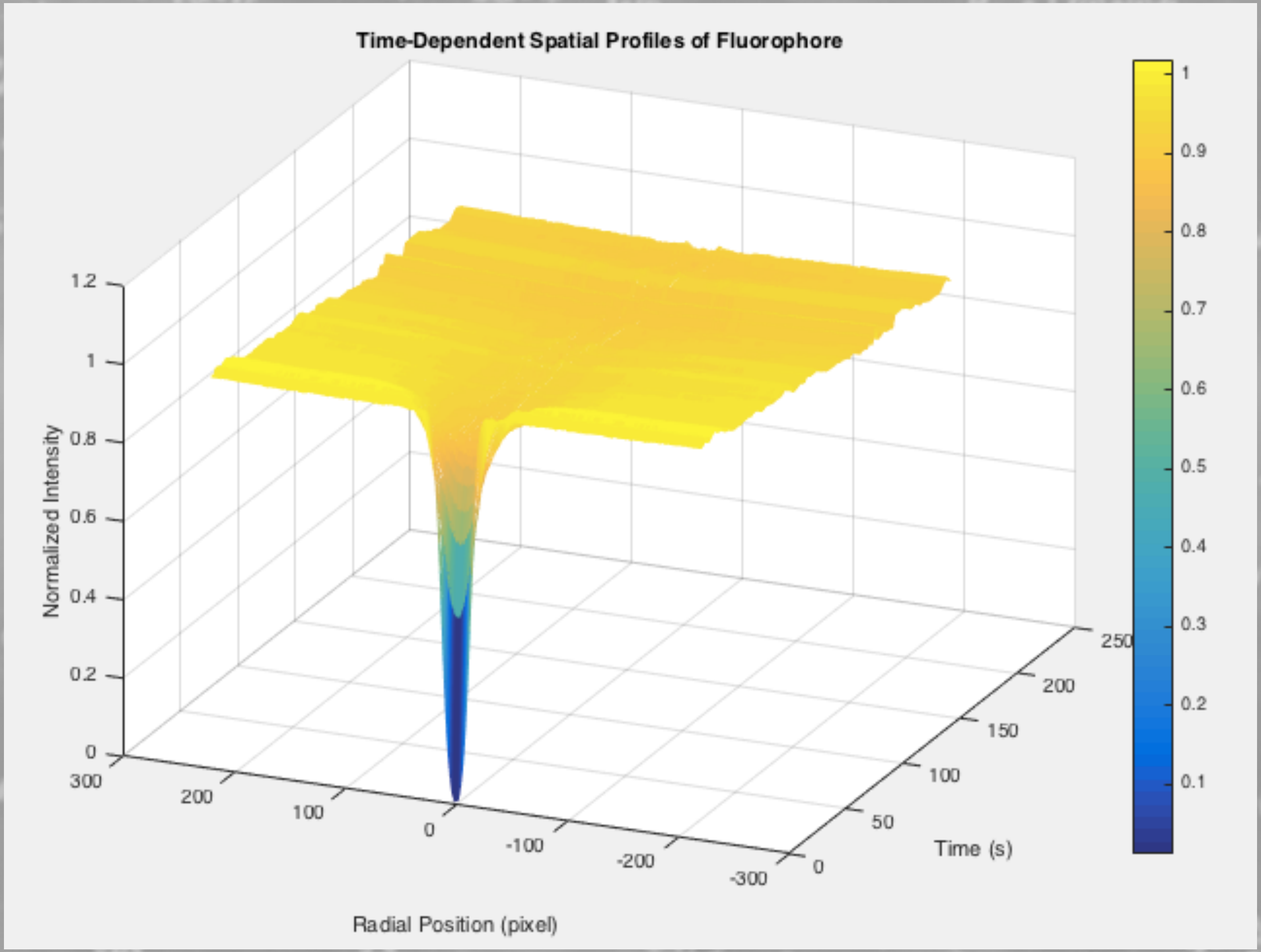


Step 7: Find the optimized radius for Gaussian fitting and the normalized intensity profile

porary_test.m | Han_modified_FRAP4_Axelrod_K_frac_loop_newout.m | FRAP_modeling_modified_HWT

```
%以下我們試著用time-radial position-intensity為三軸,畫出intensity在不同位置和時間下的變化
duration_each_frame=2.2;
time=[0:number-1]*duration_each_frame;

[x_plot,y_plot]=meshgrid(time,xxx);
for k=1:number
    for i=1:length(r)
        Z(k,i)=It(length(r)+1-i,k);
    end
end
z_plot=[Z';ones(1,100).*It(1,:);It]; %在這裡r=0的intensity用r=1代替
% Z要轉置才相符,注意跟上面不同的是三者用分號而非逗點隔開,如此一來x_plot,y_plot,z_plot
% 的dimension都是441*100

figure(10*m+6)
mesh(x_plot,y_plot,z_plot),colorbar;
title('Time-Dependent Spatial Profiles of Fluorophore')
xlabel('Time (s)'),ylabel('Radial Position (pixel)'),zlabel('Normalized Intensity')
```

Step 7: Find the optimized radius for Gaussian fitting and the normalized intensity profile



Step 8: Plot the FRAP recovery curve

Before coding, recall the definition of fluorescence

$$F_k(t) = \left(\frac{q}{A}\right)\int_0^\infty I(r)C_k(r,t)d^2r = \left(\frac{q}{A}\right)\int_0^\infty I(r)C_k(r,t)2\pi r dr$$

and its fractional form $$f_k(t) = \frac{F_k(t) - F_k(0)}{F_k(\infty) - F_k(0)}$$

For $$I(r) = \left(\frac{2P_0}{\pi w^2}\right)\exp\left(-\frac{2r^2}{w^2}\right)$$

we have

$$F_k(t) = \left(\frac{q}{A}\right)\int_0^\infty \left(\frac{2P_0}{\pi w^2}\right)\exp\left(-\frac{2r^2}{w^2}\right)C_k(r,t)2\pi r dr$$

$$= \left(\frac{q}{A}\right)\left(\frac{4C_0P_0}{w^2}\right)\int_0^\infty \exp\left(-\frac{2r^2}{w^2}\right)\left(\frac{C_k(r,t)}{C_0}\right)r dr$$

Step 8: Plot the FRAP recovery curve

Here, note that before bleaching

$$\lim_{t\to 0^-} F_k(t) = F_k(0^-) = \lim_{t\to 0^-} \left[q/A \int I(r) C_k(r,t) d^2 r\right]$$

$$= \left(\frac{qC_0}{A}\right) \int I(r) d^2 r = \frac{qP_0 C_0}{A}$$

Therefore,

$$F_k(t) = \left(\frac{qC_0P_0}{A}\right)\left(\frac{4}{w^2}\right) \int_0^\infty \exp\left(-\frac{2r^2}{w^2}\right)\left(\frac{C_k(r,t)}{C_0}\right) r dr$$

$$\Rightarrow Let\ \mathrm{trapz}(AA) = \frac{F_k(t)}{F_k(0^-)} = \left(\frac{4}{w^2}\right) \int_0^\infty \exp\left(-\frac{2r^2}{w^2}\right)\left(\frac{C_k(r,t)}{C_0}\right) r dr$$

Step 8: Plot the FRAP recovery curve

Similary, $as\ t \to \infty, C_k(r,t) = C_0$

$$\text{Let trapz}(BB) = \frac{F_k(\infty)}{F_k(0^-)} = \left(\frac{4}{w^2}\right)\int_0^\infty \exp\left(-\frac{2r^2}{w^2}\right) r dr$$

hw14.m | temporary_test.m | FRAP_modeling_modified_HWT.m | +

$$\text{trapz}(AA) = \frac{F_k(t)}{F_k(0^-)} = \left(\frac{4}{w^2}\right)\int_0^\infty \exp\left(-\frac{2r^2}{w^2}\right)\left(\frac{C_k(r,t)}{C_0}\right) r dr$$

```matlab
%Part8: 定義出f(t)對時間作圖,即 Frap Recovery Curve

    %[illegible]照片Outside的平均
    for k=1:number
        Ir_outbg=0;
        for i=best_fit_radius:r_length
            Ir_outbg=Ir(i,k)+Ir_outbg;
        end
        Ir_out(k)=Ir_outbg/(r_length-best_fit_radius+1);
    end

    %接著我們算出F(t)和f(t)
    clear AA BB
    %不知道為什麼,發現跑多個迴圈的時候AA跟BB的dimension會有問題,故在這裡先把前一次的變數清除
    for k=1:number
        AA(:,k)=exp(-2.*r'.^2/w(m)^2).*Ir(:,k)./Ir_out(k)*4.*r'/w(m)^2;
        Fe(k)=trapz(r,AA(:,k));
        BB(:,k)=exp(-2.*r'.^2/w(m)^2)*4.*r'/w(m)^2;
        Fe0(k)=trapz(r,BB(:,k));
        f(k)=(Fe(k)-Fe(1))./(Fe0(k)-Fe(1));
    end
```

$$\frac{F_k(t)/F_k(0^-) - F_k(0)/F_k(0^-)}{F_k(\infty)/F_k(0^-) - F_k(0)/F_k(0^-)} = \frac{F_k(t) - F_k(0)}{F_k(\infty) - F_k(0)} = f_k(t)$$

Data Processing Using MATLAB

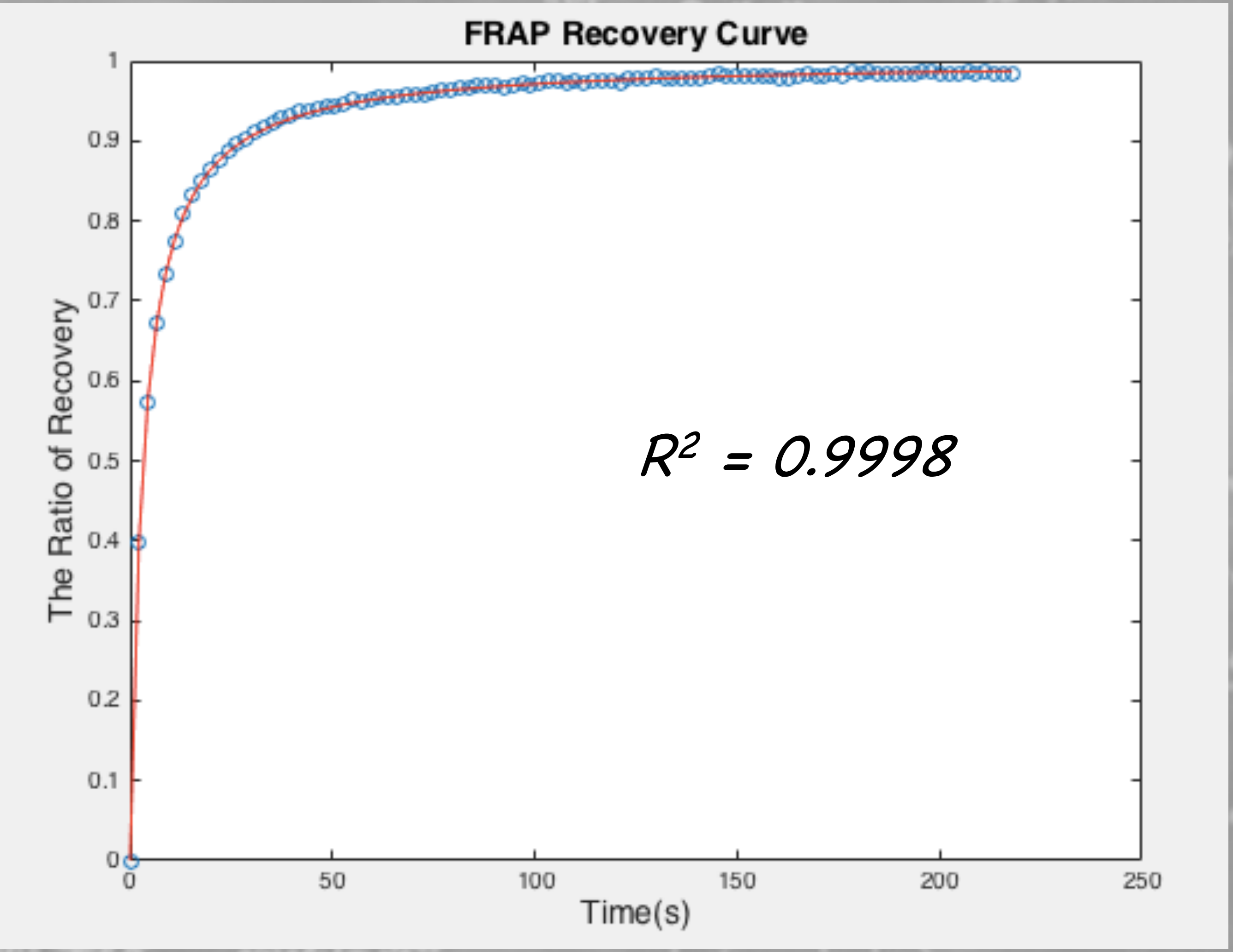
Biomimetic Lipid Membrane

Step 9: Determine the diffusivity and the mobile fraction of fluorophore

```
hw14.m    temporary_test.m    FRAP_modeling_modified_HWT.m    +
%Part9: 用非線性迴歸求出fluorophore的diffusivity和 mobile fraction

    %由於 inline 不能放變數,在這裡我們使用 anonymous function
    frapfunc2=@(beta,t)(beta(1)*...
    ((((-K(m))^0)*w(m)^2./factorial(0)./(w(m)^2+0*(8*beta(2)*t+w(m)^2))...
    + ((-K(m))^1)*w(m)^2./factorial(1)./(w(m)^2+1*(8*beta(2)*t+w(m)^2))...
    + ((-K(m))^2)*w(m)^2./factorial(2)./(w(m)^2+2*(8*beta(2)*t+w(m)^2))...
    + ((-K(m))^3)*w(m)^2./factorial(3)./(w(m)^2+3*(8*beta(2)*t+w(m)^2))...
    + ((-K(m))^4)*w(m)^2./factorial(4)./(w(m)^2+4*(8*beta(2)*t+w(m)^2))...
    + ((-K(m))^5)*w(m)^2./factorial(5)./(w(m)^2+5*(8*beta(2)*t+w(m)^2))...

    clear beta %清除上次作nlinfit的beta,或可改名
    opts=statset('TolX',1e-12);
    [beta,R,J,COVB,mse]=nlinfit(time,f,frapfunc2,[1,1],opts);

    M_Frac(m)=beta(1);
    Diffusivity_pixel(m)=beta(2);
    rsq2_for_Diffusivity(m)=1-sum(R.^2)/sum((f-mean(f)).^2);

    fitting_result=(beta(1)*...
    ((((-K(m))^0)*w(m)^2./factorial(0)./(w(m)^2+0*(8*beta(2)*time+w(m)^2))...
    + ((-K(m))^1)*w(m)^2./factorial(1)./(w(m)^2+1*(8*beta(2)*time+w(m)^2))...
```

$$f_K(t) = \frac{\sum_{n=0}^{\infty} \frac{(-K)^n w^2}{n!\,[w^2 + n(w^2 + 8Dt)]} - \left(\frac{1-e^{-K}}{K}\right)}{1-\left(\frac{1-e^{-K}}{K}\right)}$$

Step 9: Determine the diffusivity and the mobile fraction of fluorophore



Step 10: Output the results to an Excel file and command window of MATLAB

Biomimetic Lipid Membrane

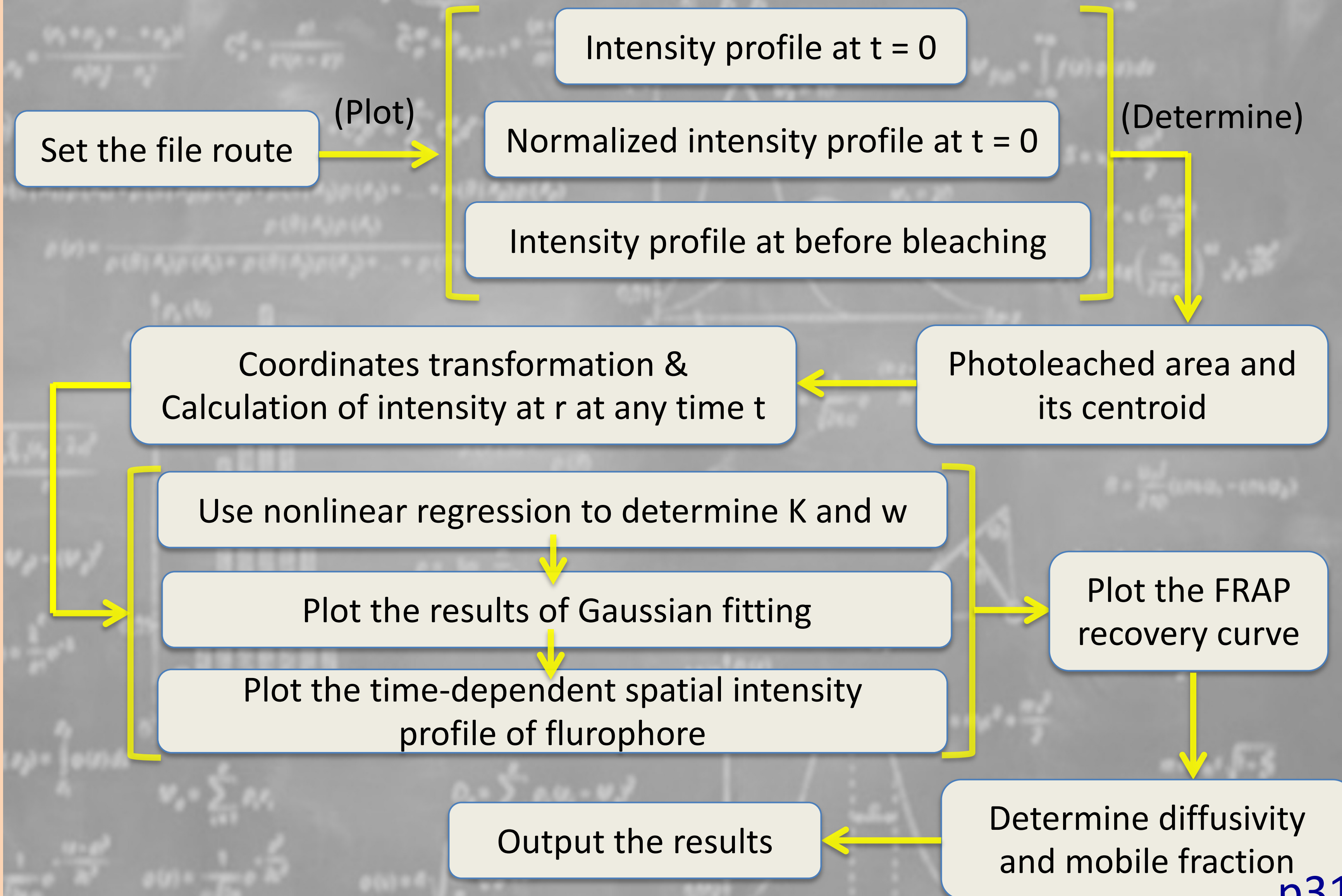
hw14.m | temporary_test.m | FRAP_modeling_modified_HWT.m

```
%Part10: 將結果寫到command window, 並輸出成excel檔
    fileroute= [image_load_route,'/fitting_',name1,ab,'.csv']
    sheetname='FRAP Modeling';

    xlswrite(fileroute,'Sample',sheetname,'A1')
    xlswrite(fileroute,'Time interval(s)',sheetname,'B1')
    xlswrite(fileroute,'Diffusivity',sheetname,'C1')
    xlswrite(fileroute,'R squared value',sheetname,'D1')
    xlswrite(fileroute,'K',sheetname,'E1')
    xlswrite(fileroute,'w',sheetname,'F1')
    xlswrite(fileroute,'mobile fraction',sheetname,'G1')

    column=num2str(m+1);
    xlswrite(fileroute,ab,sheetname,['A' column]);
    xlswrite(fileroute,duration_each_frame,sheetname,['B' column]);
    xlswrite(fileroute,Diffusivity_um,sheetname,['C' column]);
    xlswrite(fileroute,rsq2_for_Diffusivity,sheetname,['D' column]);
    xlswrite(fileroute,K,sheetname,['E' column]);
    xlswrite(fileroute,w,sheetname,['F' column]);
    xlswrite(fileroute,M_Frac,sheetname,['G' column]);
end

clc %清掉煩人的Warning哈哈

A0='Information you may be interested:';
A1='The name of the file analyzed: ';
A2='The number of samples analyzed (number of loops): ';
```

Step 10: Output the results to an Excel file and command window of MATLAB

```
Command Window
New to MATLAB? See resources for Getting Started.
Information you may be interested:
The name of the file analyzed: pure DOPC(tr0.5)_frap_cy3(0.2)_001.tif
The number of samples analyzed (number of loops): 1
The duration of each frame: 2.2 second
The background values near the location interested: 9341
The threshold normalized intensity: 0.8
The bleaching parameter K is: 3.0102
The estimated value of half-width at e^(-2) height: 4.549 micrometer
The R squared value of Gaussian fitting: 0.99898
The diffusivity of the fluorophore: 2.0388e-12 m^2/s
The R squared value of the diffusivity: 0.99975
The mobile fraction of the fluorophore: 1.0017
Elapsed time is 27.205925 seconds.
fx >>
```

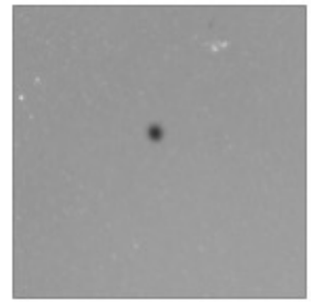
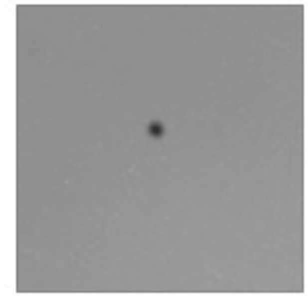
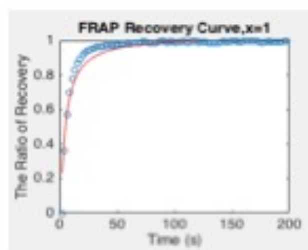
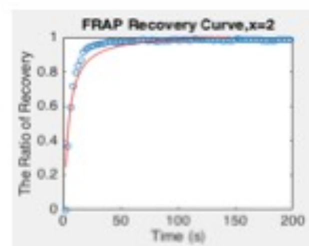
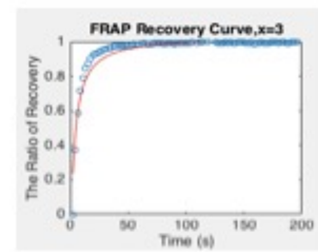
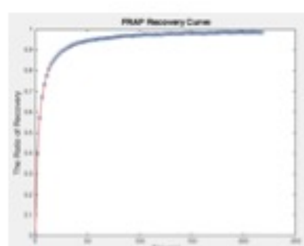
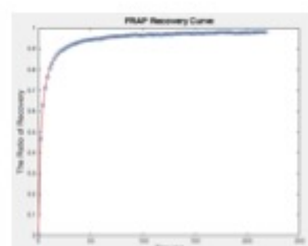
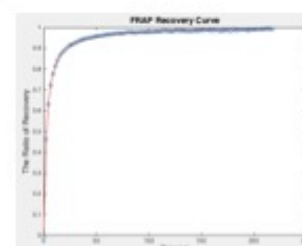
Main Structure of MATLAB codes



Biomimetic Lipid Membrane

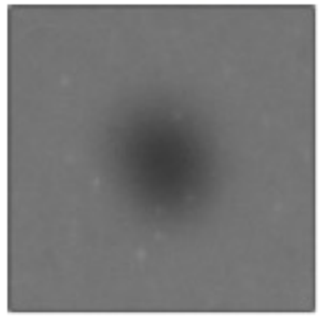
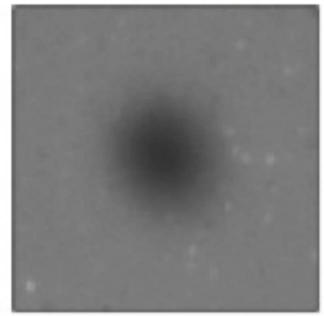
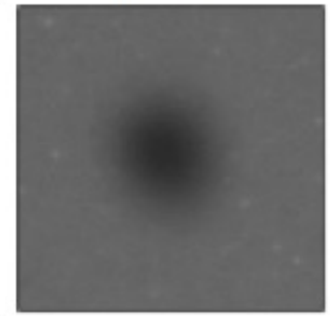
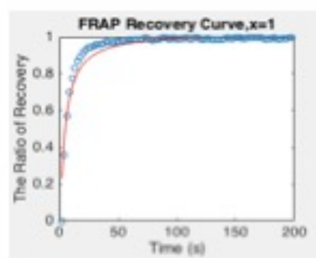
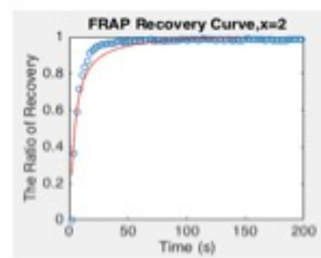
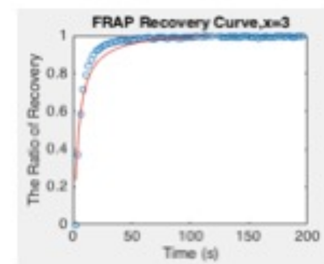
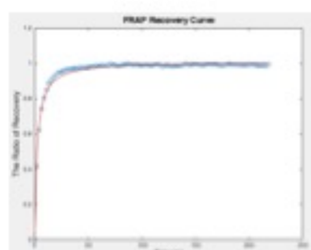
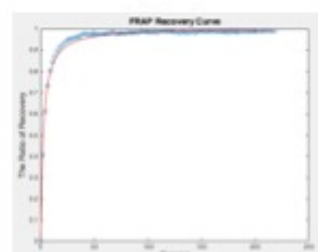
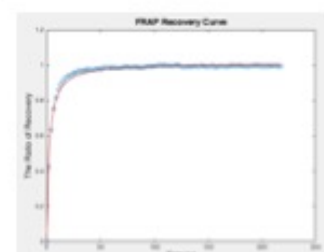
Results obtained by MATLAB

Pure DOPC

pure DOPC(tr0.5)_frap_cy3(0.2)_00x				
		Sample1 (x=1)	Sample2 (x=2)	Sample3 (x=3)
The 1st Picture taken after photobleaching begins				
Original Codes	Recovery Curves			
	R^2	0.9856	0.9598	0.9677
Modified Codes	Recovery Curves			
	R^2	0.9998	0.9997	0.9997

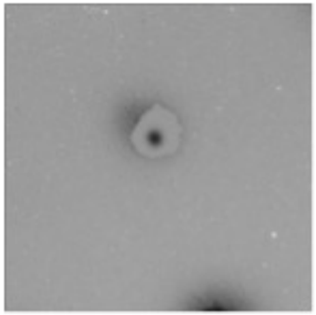
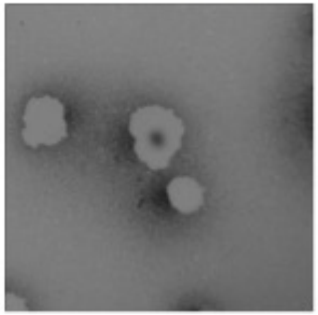
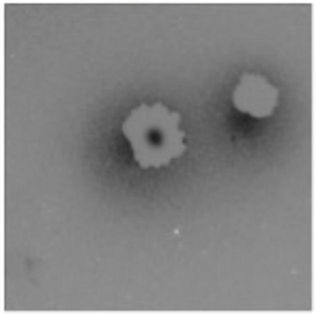
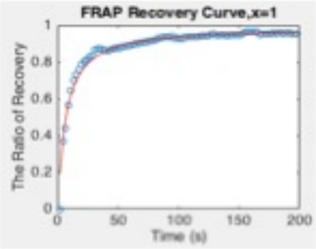
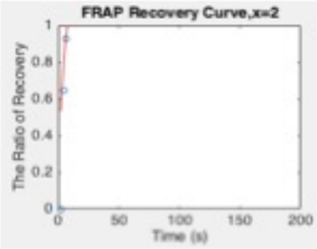
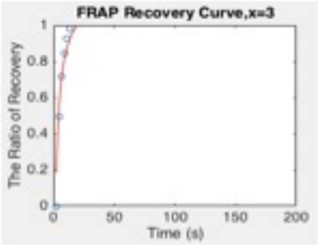
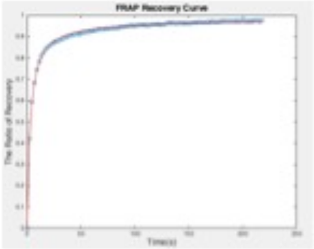
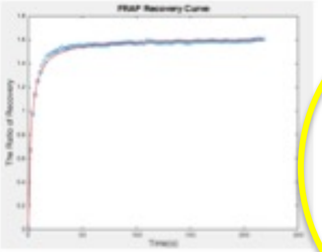
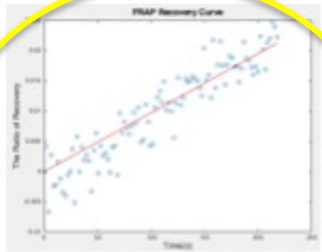
Biomimetic Lipid Membrane

DOPC with GPMVs aside

frap-pDOPC(tr0.5)_frap_cy3(0.2)_00x				
		Sample1 (x=1)	Sample2 (x=2)	Sample3 (x=3)
The 1st Picture taken after photobleaching begins				
Original Codes	Recovery Curves			
	R^2	0.9232	0.9073	0.9219
Modified Codes	Recovery Curves			
	R^2	0.9864	0.9881	0.9891

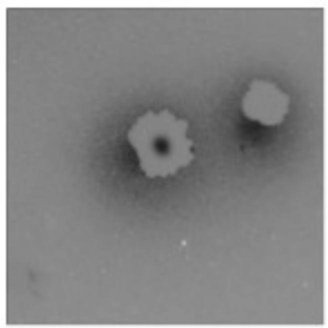
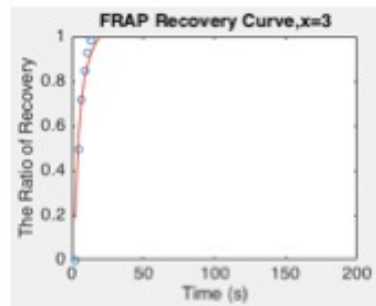
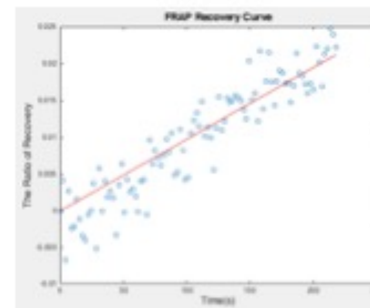
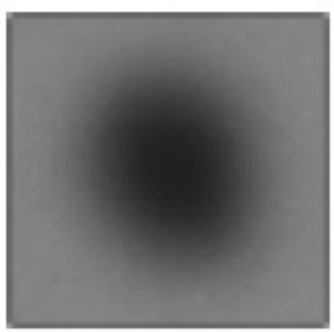
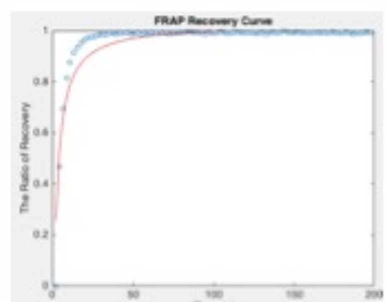
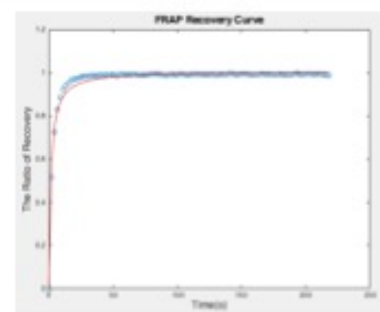
Data Processing Using MATLAB

GPMVs

<table>
<tr><th colspan="5">GPMV_frap_cy3(0.2)_00x</th></tr>
<tr><td colspan="2"></td><td>Sample1 (x=1)</td><td>Sample2 (x=2)</td><td>Sample3 (x=3)</td></tr>
<tr><td colspan="2">The 1st Picture taken after photobleaching begins</td><td></td><td></td><td></td></tr>
<tr><td rowspan="2">Original Codes</td><td>Recovery Curves</td><td>
</td><td>
</td><td>
</td></tr>
<tr><td>R^2</td><td>0.9690</td><td>0.7632</td><td>0.9496</td></tr>
<tr><td rowspan="2">Modified Codes</td><td>Recovery Curves</td><td>
</td><td>
</td><td>
</td></tr>
<tr><td>R^2</td><td>0.9974</td><td>0.9927</td><td>0.8465</td></tr>
</table>

Dealing with the huge deviation of Sample 3 in the case of GPMVs, we considered that the outcomes may be influenced by other GPMVs.

⟶ We cropped the photo and reanalyzed it as follows:

GPMV_frap_cy3(0.2)_003		Original Codes	Modified Codes
Before Cropping			
		R^2 =0.9496	R^2 =0.8465
After Cropping			
		R^2 =0.8822	R^2 =0.9821

Discussion

1. Diffusivity

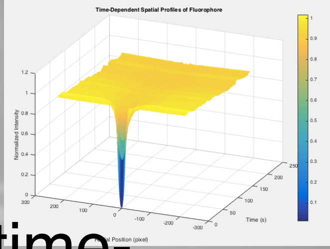
Diffusivity and Mobile Fraction of Fluorophores on Different Substances			
Susbstance		Diffusivity ($\mu m^2/s$)	Mobile Fraction
Pure DOPC	Sample 1	2.0388	1.0017
	Sample 2	2.1061	0.9910
	Sample 3	2.0590	1.0037
	Avg	2.0380	0.9988
DOPC with GPMVs aside	Sample 1	2.6376	1.0142
	Sample 2	2.6253	1.0090
	Sample 3	2.7278	1.0166
	Avg	2.6636	1.0133
GPMVs	Sample 1	1.8732	1.0618
	Sample 2	1.4501	0.9807
	Sample 3	1.5581	1.0099
	Avg	1.6271	1.0175

2. R^2 for Diffusivity

R^2 for Diffusivity of Fluorophores on Different Substances			
Susbstance		Original Codes	Modified Codes
Pure DOPC	Sample 1	0.9856	0.9998
	Sample 2	0.9598	0.9997
	Sample 3	0.9677	0.9997
	Avg	0.9710	0.9997
DOPC with GPMVs aside	Sample 1	0.9232	0.9864
	Sample 2	0.9073	0.9881
	Sample 3	0.9219	0.9891
	Avg	0.9175	0.9879
GPMVs	Sample 1	0.9690	0.9974
	Sample 2	0.7632	0.9927
	Sample 3	0.9496	0.8465
	Avg	0.8939	0.9455

Future Work & Conclusion

From the presentation above, we know that the modified MATLAB codes can analyze the diffusion of fluorophore satisfactorily. Still, there are some improvements can be made:



1. Incorporation of self-cropping function
2. Time shortening when dealing with the time-dependent spatial profile of fluorophore
3. A more appropriate determination of the photobleached area.
4. Investigation of influences caused by domination of reaction or diffusion
5. Trial of different kinetics of photobleaching

References

Reference

Mobility Measurement by Analysis of Fluorescence Photobleaching Recovery Kinetics , D.Axelrod, D.E, Koppel, et al. (1976)

Thank you !