

Readme for details of .traces files

Related to dryad file <https://doi.org/10.5061/dryad.612jm641d>

For publication <https://doi.org/10.1073/pnas.1918517117>

.traces files contain the time record of intentisites of donor and acceptor molecule pairs extracted from movies acquired in a microscope. They are easily opened with the MATLAB script below.

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%
%   runs through all .traces files in a folder
%   also allows user to save selected traces in IGOR PRO .itx format
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function keith_Folder_view_tir;
promptstr={'path (dont end in \) to folder with .traces:', 'Enter prefix of file being
analyzed (rib):', 'Enter # for first movie', 'Enter # for last movie'};
initstr={'C:\keith\analysis\', 'cascade', '1', '1'};
titlestr='DO NOT PRESS CANCEL!';
nlines=1;
result=inputdlg(promptstr, titlestr, nlines, initstr);

Initialdir = result{1};
fileprefix= result{2};
startNum = str2num(result{3});
endNum = str2num(result{4});

dir=[Initialdir '\\'];
display (dir)
cd(dir);
close all;
    counter = 0

keithswitch = 1

for j = startNum :endNum ,                               %iterate on the movie number
close all;
    counter = 0 %reset counter each movie if want to
file = [fileprefix num2str(j) ];
to_open = strcat(file, '.traces');
fid=fopen(to_open, 'r');
disp('working on');
disp(file);
len=fread(fid,1, 'int32');
disp('The length of the time traces is: ')
disp(len)
Ntraces=fread(fid,1, 'int16')
disp('The number of molceules is:')
disp(Ntraces/2);
raw=fread(fid,Ntraces*len, 'int16');
disp('Done reading data. ');
index=(1:Ntraces*len);
time1 = zeros(1, len);
Data=zeros(Ntraces+1, len);
donor=zeros(Ntraces/2, len);
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acceptor=zeros(Ntraces/2,len);
fretE=zeros(Ntraces/2,len);
time_1 = zeros(len,1);
donor_1 = zeros(len,Ntraces/2);
acceptor_1 = zeros(len,Ntraces/2);
time=(0:(len-1));
Data(index)=raw(index);
time1(1,:) = Data(1,:);

for i=1:(Ntraces/2),
    acceptor(i,:)=Data(i*2,:);
    donor(i,:)=Data(i*2+1,:);
end

axis_x = len*1; %for time lapse need to multiply by spacing (usu 350)
m1=1;
leakage = 0.06; %for cy3 leakage into cy5 factor 0.09, set to zero
AnotherPrevLastGood=m1
prevlastgood=m1 %to allow user to go back 2 traces
lastgood=m1 %to allow user to go back 2 traces
m=m1

while m < (Ntraces/2 + 1)
    time_1 = time1';
    donor_1 = donor(m,:);
    acceptor_1 = acceptor(m,:)'-leakage*donor_1; %9% leakage Cy3 into Cy5
    cy5max=-10000
    for i = 1:len
        if acceptor_1(i,1)>cy5max
            cy5max = acceptor_1(i,1);
        end
        total = donor_1(i,1)+acceptor_1(i,1);
        if total <= 0
            fret_1(i,1) = 0.01;
        else
            fret_1(i,1) = acceptor_1(i,1)/(donor_1(i,1)+acceptor_1(i,1));
        end
    end
end

keithswitch=1;
if keithswitch > 0 %show all traces
    counter = counter +1
    subplot (2,1,1)
    plot(time_1(1:len-5),donor_1(1:len-5),'b',time_1(1:len-5),acceptor_1(1:len-5),'r');
    grid on;
    temp=axis;
    temp(1)=0;
    temp(2)=axis_x;
    axis(temp);
    title(['Molecule ' num2str(m) ' ' file]);
    zoom on;
    subplot (2,1,2)
    plot(time_1(1:len-5),fret_1(1:len-5),'m');
    grid on;
    axis([0 axis_x -0.1 1.1])
    zoom on;

ans = input('press k to smooth, b to back up, any other to pass ','s');
if isempty(ans)== 1
    ans=' '
    AnotherPrevLastGood=prevlastgood;
    prevlastgood=lastgood;
end

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    lastgood = m;
    m=m+1;
end

if ans== 'b'
    m=lastgood-1;
    lastgood=prevlastgood;
    prevlastgood=AnotherPrevLastGood;
end

if ans == 'k'
    A = 'q';
    while (strcmp(A,'k')==0)
        donor_bg = 0;
        acceptor_bg = 0;
        donor_1 = donor(m,:)'-donor_bg;
        acceptor_1 = acceptor(m,:)'-acceptor_bg-leakage*donor_1;
        for i = 1:len
            total = donor_1(i,1)+acceptor_1(i,1);
            if total <= 0
                fret_1(i,1) = 0.01;
            else
                fret_1(i,1) = acceptor_1(i,1)/(donor_1(i,1)+acceptor_1(i,1));
            end
        end
    end

    % smooth the data with n points average
    n = input('n of points to average over = ');

    len_n = floor((len-5)/n);

    time_n = zeros(len_n,1);
    donor_n = zeros(len_n,1);
    acceptor_n = zeros(len_n,1);
    fret_n = zeros(len_n,1);

    for i = 1:len_n
        time_n(i,1) = sum(time_1(n*i-n+1:n*i,1))/n;
        donor_n(i,1) = sum(donor_1(n*i-n+1:n*i,1))/n;
        acceptor_n(i,1) = sum(acceptor_1(n*i-n+1:n*i,1))/n;
        total_n = donor_n(i,1)+acceptor_n(i,1);
        if total_n<=0
            fret_n(i,1) = 0.01;
        else
            fret_n(i,1) = acceptor_n(i,1)/(donor_n(i,1)+acceptor_n(i,1));
        end
    end

    end

    subplot (2,1,1);
    plot(time_n,donor_n,'b',time_n,acceptor_n,'r');
    zoom on;
    temp=axis;
    temp(1)=0;
    temp(2)=axis_x;
    axis(temp);
    title (num2str(m));
    grid on;
    subplot (2,1,2);
    plot(time_n,fret_n,'m');

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        zoom on;
        axis([0 axis_x -0.1 1.1]);
        grid on;

        A = input('satisfied with the smoothing? k = ok, others = not','s');
    end

    ans = input ('to save the trace press k == ' , 's');
    if isempty(ans)== 1
        ans=' '
        AnotherPrevLastGood=prevlastgood;
        prevlastgood=lastgood;
        lastgood = m;
        m = m+1;
        end

    if ans == 'k'
        fname=[file 'trace' num2str(m) '.itx'];
        igorname=[file 'trace' num2str(m)];
        [fid2,message] = fopen(fname,'w');
        fprintf(fid2,'IGOR\nWAVES/D ');
        fprintf(fid2,'Cy3');
        fprintf(fid2,igorname);
        fprintf(fid2,' Cy5');
        fprintf(fid2,igorname);
        fprintf(fid2,'\n');
        fprintf(fid2,'BEGIN\n');

    for i = 1:len
        fprintf(fid2, num2str(donor_1(i,1)));
        fprintf(fid2, ' ');
        fprintf(fid2, num2str(acceptor_1(i,1)));
        fprintf(fid2, '\n');
    end

        fprintf(fid2,'\nEND\n');
        fclose(fid2);
        AnotherPrevLastGood=prevlastgood;
        prevlastgood=lastgood;
        lastgood = m;
        m=m+1
    end

    end
else
    m=m+1
end
end
display(counter);
end % end iterate on movie number in a given folder

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