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;

prompstr={'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(prompstr,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);

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

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;

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

% 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

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');

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