

Methods S1. Adaptive Local z-Projection Macro (MATLAB), Related to Star methods

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%Matlab macro building a local projection of two channels using one channel
as %reference
% Can be used to locally follow the curvature of tissue and project only a
% limited number of planes for the second channel.
% Ideally reference channel has to be E-cad:::GFP or any signal that is
% restricted in a single plane.
% Each z-stack has to be saved in a folder called 'zstacks' with the
% following structure : "C0_time.tif" and "C1_time.tif" with time being the
% frame number
%

clear all
close all
folder='zstacks';

%Main variables-----
zmax=40      % Number of z plane;
tmax=100;    % Number of time;
t0=1;        % Number of time;
METH=1;       % Choice for the method of local projection: 0=MEAN , 1=STDdev
dxy=40;      % Size of the analysis window/2
xystep=20;   % Sampling frequency (step size for shifting the window)
dz=3;         % Number of z planes that will be projected for the second
channel
dz0=6;        % Shift of the central position of the projection for the second
channel compared to the reference channel
gg=50;        % Size of the smooth window that will be used to smooth the z
profile
zcad=1;       % Range of max projection for the reference channel (here E-cad)
% ----

%%
name_ref= uigetfile( ['*.tif'], 'Open the first TIFF Zstack of the Reference
channel' );
name_ca= uigetfile( ['*.tif'], 'Open the first TIFF Zstack of the cic
channel' );
%%
mkdir local_zprojection
tic
% Loop for all the time (from t0 to tmax)
for t=t0:1:tmax

    name=[name_ref(1:size(name_ref,2)-7), '_t', num2str(t), '.TIF']
    name_CIC=[name_ca(1:size(name_ca,2)-7), '_t', num2str(t), '.TIF']
    I0=imread(name,1);
    mean_z=zeros(1,zmax);
    STANDARD_z=zeros(1,zmax);
    sum_all=zeros(size(I0));sum_all=double(sum_all);
    for z=1:zmax
        I0=imread(name,z);
        mean_z(z)=mean(I0(:));
    end
    % Smooth the profile
    mean_z=smooth(means, gg);
    % Compute the local projection
    if METH==0
        local_zprojection=mean_z;
    else
        local_zprojection=stddev_zprojection;
    end
    % Compute the standard deviation
    STANDARD_z=stddev_zprojection;
    % Compute the sum of all planes
    sum_all=sum_all+I0;
    % Compute the max projection
    max_projection=max(sum_all);
    % Check if the max projection is greater than the range of the reference channel
    if max_projection>zcad
        % If yes, set the max projection to the range of the reference channel
        max_projection=zcad;
    end
    % Save the local projection and the standard deviation
    save local_zprojection.mat local_zprojection STANDARD_z max_projection;
end

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STANDARD_z(z)=std2(I0(:));
sum_all=sum_all+double(I0);
end
sum_all=sum_all/zmax;
zcenter=find(mean_z==max(mean_z(:)));

%%
sx=size(I0,1);
sy=size(I0,2);
tic
XYZ=zeros(zmax, sx,sy);
X=[];Y=X;
z1=1;
z2=zmax;
for z=z1:z2

    I0=imread(name,z);
    i=0;
    for x=[1:xystep:sx sx] %take care if final 1:xystep:sx is not equal
to sx
        i=i+1;
        j=0;
        for y=[1:yystep:sy sy]
            j=j+1;
            X(i,j)=x;
            Y(i,j)=y;
            rect=[y-dxy x-dxy 2*dxy 2*dxy ];
            Icut=imcrop(I0, rect);
            if METH==0 %Calculate the mean intensity in the analysis
window
                XYZ(z,i,j)=mean(Icut(:));
            elseif METH==1 %Calculate the standard deviation of intensity
in the analysis window
                XYZ(z,i,j)=std2(Icut(:));
            end
        end
    end
    imax=i;
    jmax=j;
    toc
    pos_z=[];
    for i=1:imax
        for j=1:jmax
            if isnan(max(XYZ(z1:z2,i,j)))
                pos_z(i,j)=0;
            else
                pos_z(i,j)=min(find(XYZ(:,i,j)==max(XYZ(z1:z2,i,j))));%min to
remove singleton
            end
        end
    end
    x=X(:,1)';
    y=Y(1,:);
    [xq,yq]=meshgrid(1:sy,1:sx);
    NEW_pos_z=interp2(y,x,pos_z,xq, yq);

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%% Filter the z profile (gg is the size of the gaussian filter window)
h=fspecial('gaussian',gg,gg);
TEST=imfilter(NEW_pos_z,h);
NEW_pos_z2=round(TEST);
I0 imread(name,1);
TEMP=zeros(size(I0));
I0Z=zeros(zmax,size(I0,1),size(I0,2));
for z=1:zmax
    z;
    I0Z(z,:,:)=imread(name,z);
end
% Local projection of the reference channel based on the local position
% defined above
for x=1:size(I0,1)
    for y=1:size(I0,2)
        z0=round(NEW_pos_z2(x,y));
        z1=max(z0-zcad,1);
        z2=min(z0+zcad,zmax);
        TEMP(x,y)=max(I0Z(z1:z2,x,y));
    end
end
I_CAD=TEMP;

% Local projection on the second channel based on the local plane of
reference (New_pos_z2 matric)
I0 imread(name_CIC,1);
TEMP=zeros(size(I0));
I0Z=zeros(zmax,size(I0,1),size(I0,2));
for z=1:zmax
    z;
    I0Z(z,:,:)=imread(name_CIC,z);
end

for x=1:size(I0,1)
    for y=1:size(I0,2)
        z0=round(NEW_pos_z2(x,y))+dz0;
        z1=max(z0-dz,1);
        z2=max(z0+dz,1);
        z2=min(z2,zmax);
        TEMP(x,y)=max(I0Z(z1:z2,x,y));
    end
end
I_CIC=TEMP;
if t==1
    imwrite(uint16(I_CAD),['local_zprojection\cad_localproject.tif'])
    imwrite(uint16(I_CIC),['local_zprojection\yfp_localproject.tif'])
else
    imwrite(uint16(I_CAD),['local_zprojection\cad_localproject.tif'],'WriteMode',
'Append')
    imwrite(uint16(I_CIC),['local_zprojection\yfp_localproject.tif'],'WriteMode',
'Append')
end
toc

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%%
end
toc