```matlab
% for evaluating the synapse singal strength for each synapse position
% given in the pviot file
system('caffeinate -dims &');
stacks = {'-01-synapsinR_7thA.tif', '-02-synapsinGP_5thA.tif'};

%read in the positions
position = containers.Map('KeyType','char', 'ValueType','any');
fid = fopen('synapsinR_7thA.tif.Pivots-3.txt');
tline = fgetl(fid);
key = '50';
temp = [];

while ischar(tline)

    entry = strsplit(tline,',');
    val1 = entry(1); val2 = entry(2);
    val1 = val1{1}; val2 = val2{1};
    prev_key = key;
    key = entry(3);
    key = key{1};
    if ~isKey(position, key)

        if str2num(key) ~= 50
            position(prev_key) = temp;
        end


        temp = [str2double(val1), str2double(val2)];
    else


        temp = [temp; str2double(val1), str2double(val2)];

    end

    tline = fgetl(fid);

end

position(key) = temp;
fclose(fid);


%define for each posititition a quality metric based on area of the synapse
%or the sum of the edge pixel of the synapse
for image = 1 : 2

    file = stacks{image};

    for k = 1 : 41

        if ~isKey(position, num2str(k))
            continue
        end

        %threshold the image
        curr_position = position(num2str(k));
        [X,map] = imread(file,k);
        X(X < median(X(:))) = 0;
        X ( X <= mean(X(X>0)) + std2(X(X>0)) * 1.7) = 0;
        Area = zeros(numel(curr_position(:, 1)), 3);

        % or do edge detection, and use that as the metric
        Y = edge(X, 'Canny');
        figure(2)
        title('sample image after threshold')
        imagesc(X)


        for i = 1 : numel(curr_position(:, 1))

            entry = curr_position(i, :);

            %look at the neighborhood of the called synapse point, deduce
            %the size of the synapse
            upperx = entry(2) + 8;
            if upperx > size(X, 2)
                upperx = size(X,2);
            end
              lowerx = entry(2) - 8;
            if lowerx < 1
                lowerx = 1;
            end
            uppery = entry(1) + 8;
            if uppery > size(X, 1)
                uppery = size(X,1);
            end
              lowery = entry(1) - 8;
            if lowery < 1
                lowery = 1;
            end

            %set the size of the neighborhood
            integrate = X(lowery : uppery, lowerx : upperx);
            edge_sum = Y(lowery : uppery, lowerx : upperx);
            [columnsInImage, rowsInImage] = meshgrid(1:size(integrate,2), 1:size(integrate, 1));
            circlePixels = (int8(rowsInImage) -idivide(int8(size(integrate,1)), 2, 'floor')) .^2 +
  (int8(columnsInImage) -idivide(int8(size(integrate,2)), 2, 'floor')).^2 <= 15.^2;
            integrate(~circlePixels) = 0;
            edge_sum(~circlePixels) = 0;
            integrate(integrate > 0) = 1;

            %calculaet the area sum and the edge sum
            area = sum(sum(integrate));
            ed = sum(sum(edge_sum));

            Area(i, 1) =  entry(1);
            Area(i, 2) = entry(2);
            Area(i, 3) = area;
            Area(i, 4) = ed;

        end
        if image == 2
        position(num2str(k)) = [position(num2str(k)); Area];
        else
              position(num2str(k)) = Area;
        end


    end

end


temp1 = [];
temp2 = [];
for k = 1 : 41
    if isKey(position, num2str(k))
        temp = position(num2str(k));
        temp1 = [ temp1; temp(1: size(temp, 1) / 2, :)];
        temp2 = [ temp2; temp(size(temp, 1) / 2 + 1 : size(temp, 1), :)];

    end
end

%normalize to 0 to 1
syn1 = [];
syn2 = [];
syn3 = [];
syn4 = [];
syn1 = temp1(:,3) / max(temp1(:,3));
syn2 = temp2(:,3)/ max(temp2(:,3));
syn3 = temp1(:,4) / max(temp1(:,4));
syn4 = temp2(:,4)/ max(temp2(:,4));

disp(sprintf('We try to look at the actual synapsins channel image to get rid of the mislabeled sy
napse.\n It was not obvious as to how we can define a local bright spot on the image as qualified
candidate or not.\n As a result we tried to accomplish this feat by first thresholding the image a
nd then subjecting to the neighborhood of each labeled synapse in the pivot file to two different
metric.\n One based on the number of non-zero pixel in the neighborhod, and one based on the numbe
r of edge pixel in the neighbood.\n For the non-zero pixel metrix, it turned out that for most lab
eled synapse a value of 0 was obtained. We will look into better ways of quantifying this problem,
 but for now we just keep all the synapse position\n with values above a 0. '))
figure(1)
subplot(2,2,1);
xlim([0, 1]);
hist(syn1);
title('Synapsin-1 normalized')
xlabel('integrated area of brightness after threshold')
ylabel('counts of synapse')


subplot(2,2,2);
xlim([0, 1]);
hist(syn3);
title('Synapsin-1 normalized')
xlabel('sum of the edge pixels after threshold')
ylabel('counts of synapse')

subplot(2,2,3);
hist(syn2);
title('Synapsin-2 normalized')
xlabel('integrated area of brightness after threshold')
ylabel('counts of synapse')
```

```
subplot(2, 2, 4);
xlim([0, 1]);
hist(syn4);
title('Synapsin-2  normalized')
xlabel('sum of the edge pixels after threshold')
ylabel('counts of synapse')
```

We try to look at the actual synapsins channel image to get rid of the mislabeled synapse.
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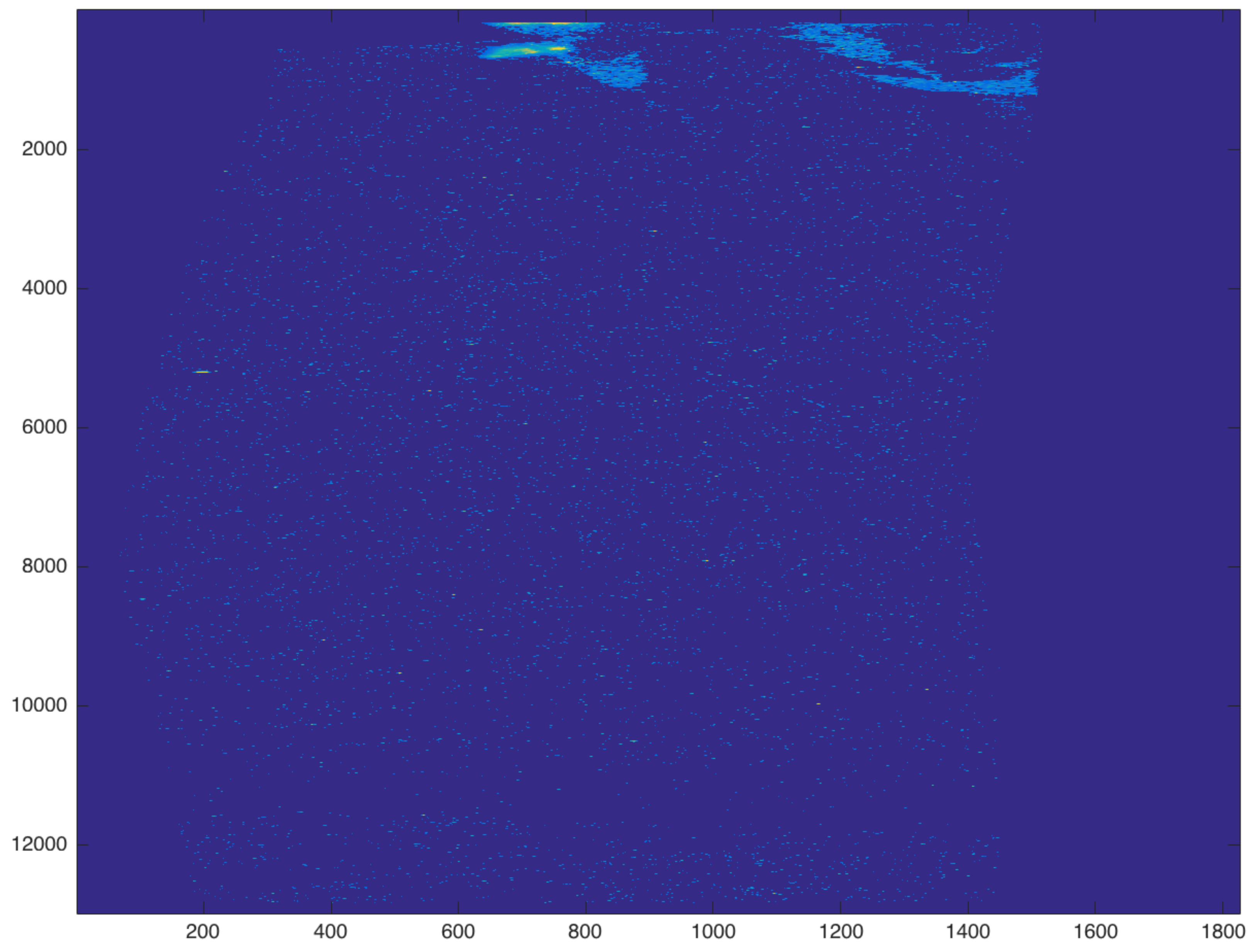
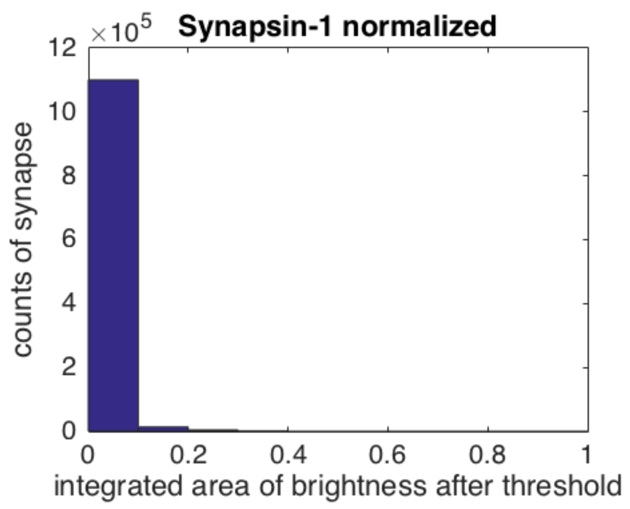
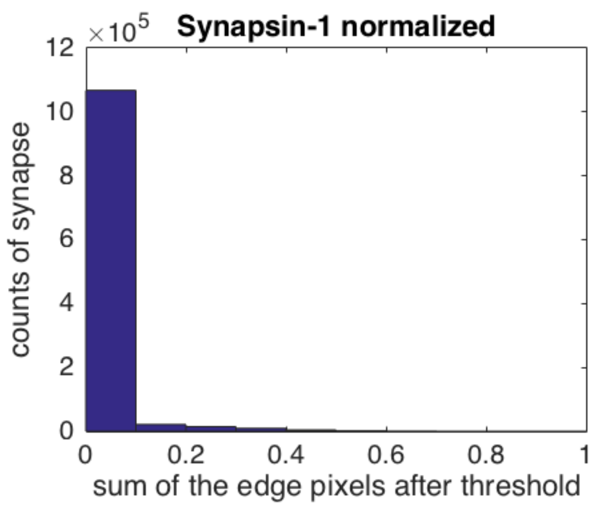
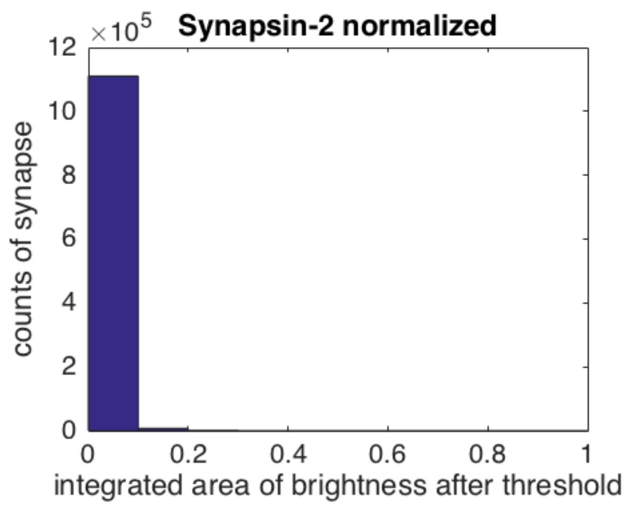
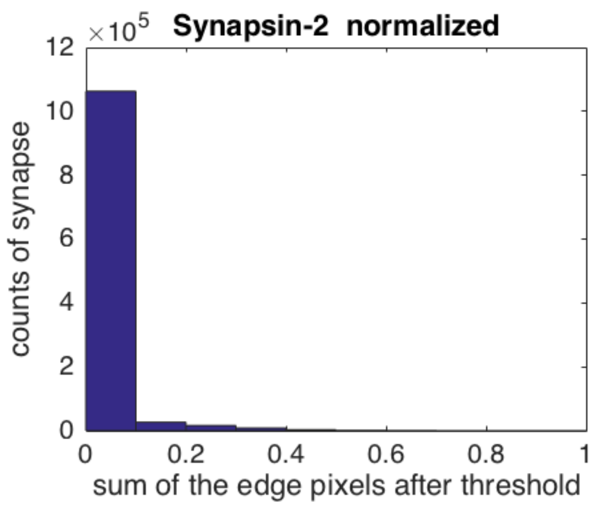



Synapsin-1 normalized
×10^5
counts of synapse
integrated area of brightness after threshold
0 2 4 6 8 10 12
0 0.2 0.4 0.6 0.8 1


Synapsin-1 normalized
×10^5
counts of synapse
sum of the edge pixels after threshold
0 2 4 6 8 10 12
0 0.2 0.4 0.6 0.8 1


Synapsin-2 normalized
×10^5
counts of synapse
integrated area of brightness after threshold
0 2 4 6 8 10 12
0 0.2 0.4 0.6 0.8 1


Synapsin-2 normalized
×10^5
counts of synapse
sum of the edge pixels after threshold
0 2 4 6 8 10 12
0 0.2 0.4 0.6 0.8 1