Supporting Information for

Multiplexed Detection of Epigenetic Markers Using Quantum Dot (QD)-Encoded Hydrogel Microparticles

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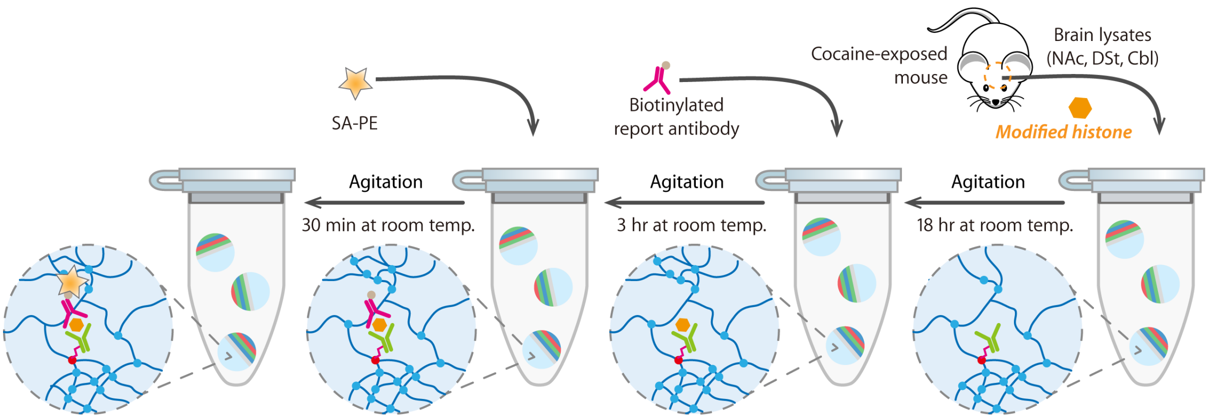

Figure S1. Detailed assay scheme for multiplexed detection of modified histones from mouse brain lysates. 1) incubation with a brain lysate per mouse containing 3 modified histones (NAc, DSt, or Cbl), 2) incubation with a biotinylated report antibody, and 3) incubation with a reporter fluorophore of SA-PE.

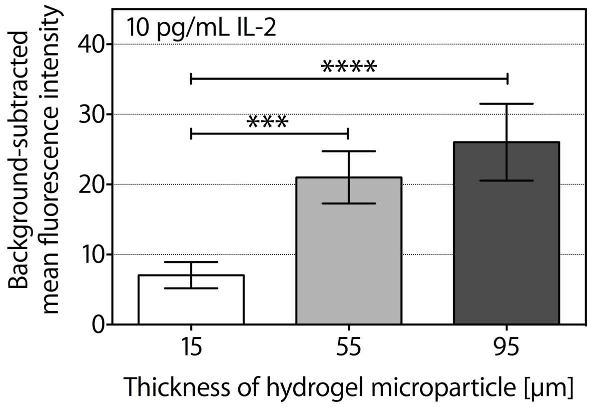

Figure S2. Optimization of thickness of hydrogel microparticles. Bar graphs presenting background-subtracted mean fluorescence intensity of IL-2 (10 pg/mL) from hydrogel microparticles with thickness of 15 (white), 55 (gray), and 95 (dark gray) μm. 5 hydrogel microparticles were analyzed for each data point. Error bars represent standard deviation. **** and *** denote significant differences of $p < 0.0001$ and $p < 0.001$, respectively.

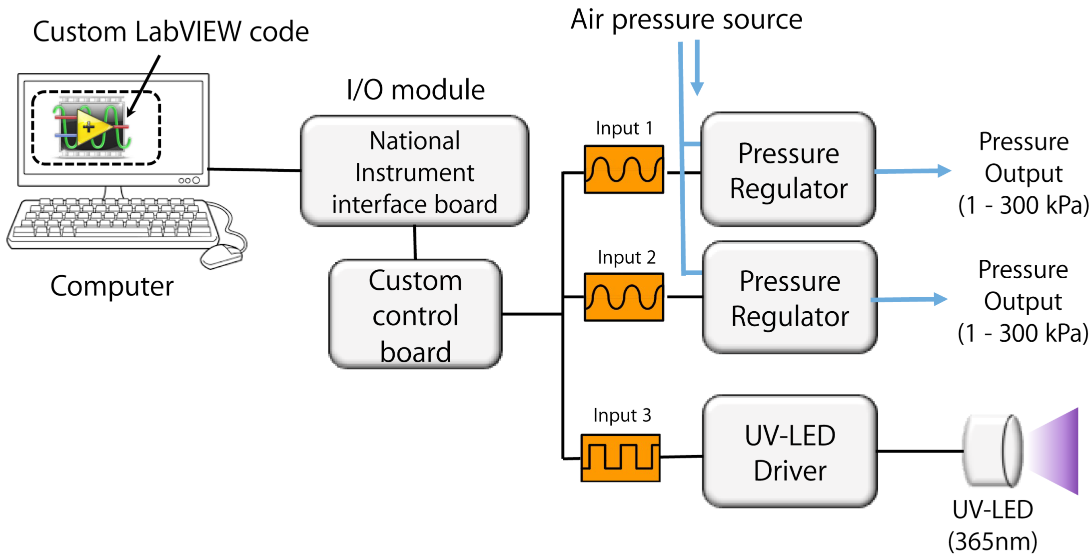

Figure S3. Block diagram of circuit design to control both pressure regulators and UV LED. Computer sends commands to a custom control board through the interface board to generate signals. Two analog signals and one digital signal are generated to control pressure regulators and UV-LED driver, respectively.

Table S1. Width of QD stripes by controlling pressure to probe and code regions

P_{probe}	P_{code}	P_{probe}/P_{code}	$w_{QD\text{-}stripe,mean}$ [μm]	$w_{QD\text{-}stripe,SD}$ [μm]	Coefficient of variation (CV) [%]
30	30	1	20.6	1.37	6.64
45	15	3	15.2	1.08	7.09
100	30	3.3	14.5	1.12	7.75
50	10	5	11.3	1.05	9.31
50.25	7.5	7	8.31	0.97	11.7
104	6	9	6.25	0.81	12.9

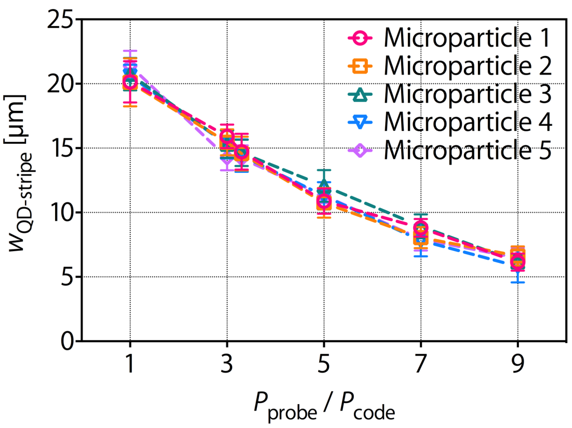

Figure S4. Inter- and intra-particle variations of width of QD-stripes by controlling ratio of pressure to probe and code regions. Red circle, organe square, green triangle, blue inverse triangle, and purple lozenge indicate mean of 5 QD-stripes per representative microparticle 1, 2, 3, 4, and 5, respectively. Error bars represent standard deviation.

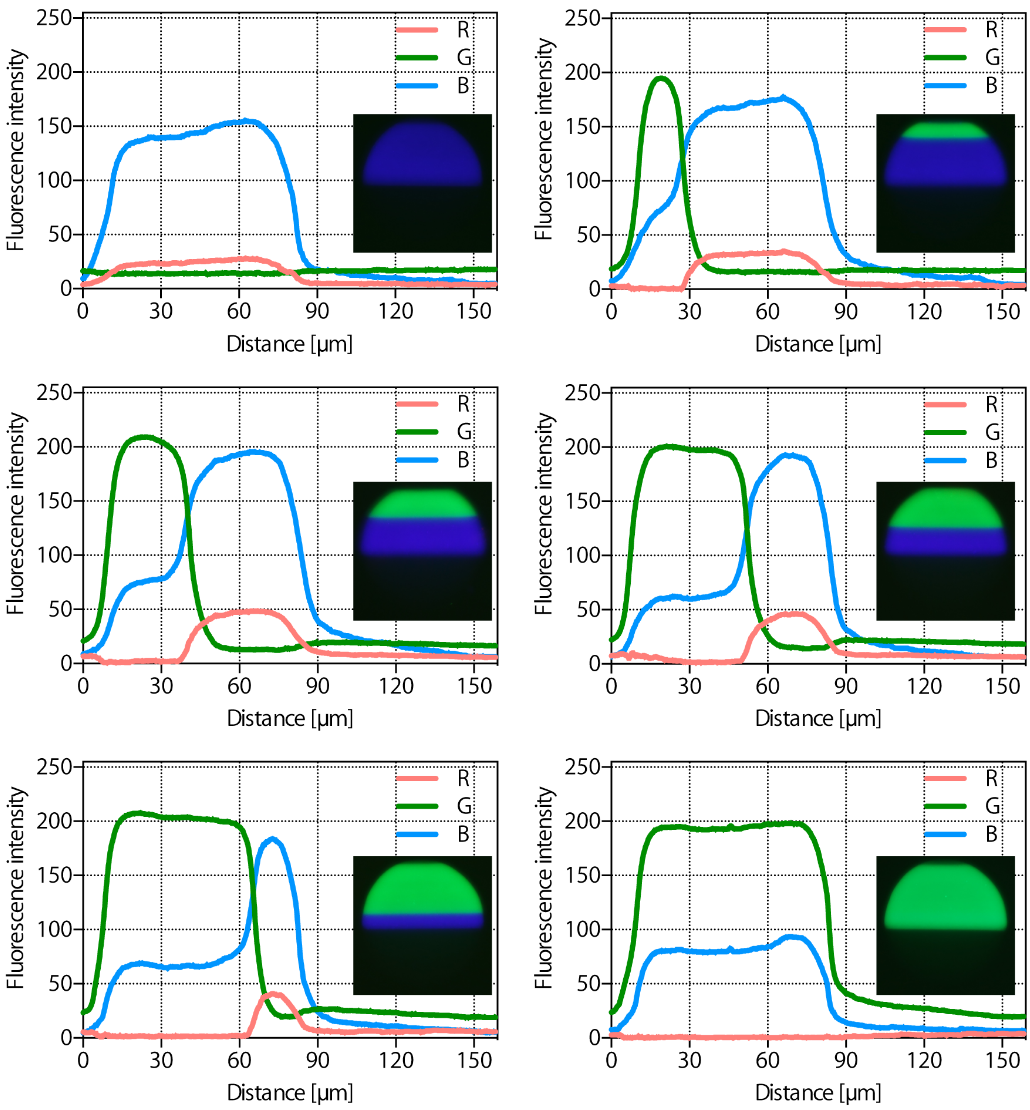

Figure S5. Spatial intensity profiles of red, green, and blue channels from representative microparticles (inset fluorescence micrographs) encoded with identical green and blue stripes consecutively located in the code region.

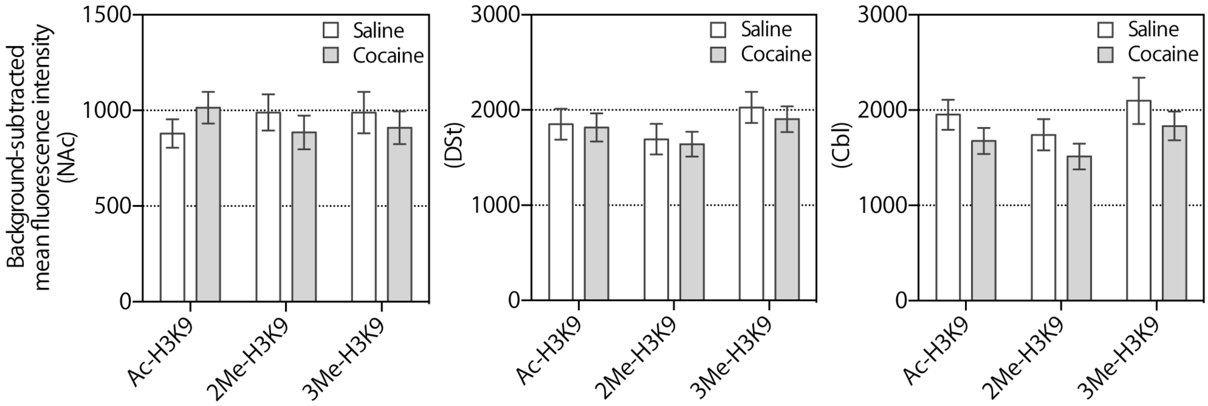

Figure S6. Representative example displaying detection of histone H3K9 modifications from mixture of multiple brain lysates. Bar graphs presenting background-subtracted mean fluorescence intensity of Ac-H3K9, 2Me-H3K9, and 3Me-H3K9 simultaneously detected from NAc (left), DSt (middle), and Cbl (right) of mice exposed to saline or cocaine for 7 days. White and gray bars are background-subtracted mean fluorescence intensity from saline- and cocaine-exposed groups, respectively. Error bars represent standard error of mean.

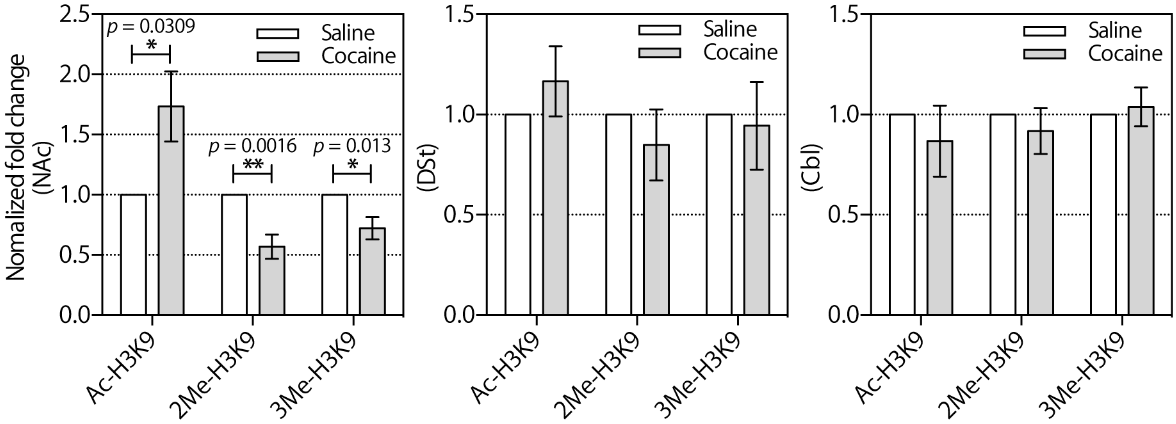

Figure S7. Western blot analysis from 50 μg of total proteins in the brain lysates of cocaine-exposed mice. Normalized fold changes in H3K9 modifications from NAc (left), DSt (middle), and Cbl (right). Cocaine-exposed mice display significant elevation in Ac-H3K9 and reduction in Me2- & Me3-H3K9 in response to cocaine. In both DSt and Cbl, no significant changes were observed. n ≥ 4 mice per group.

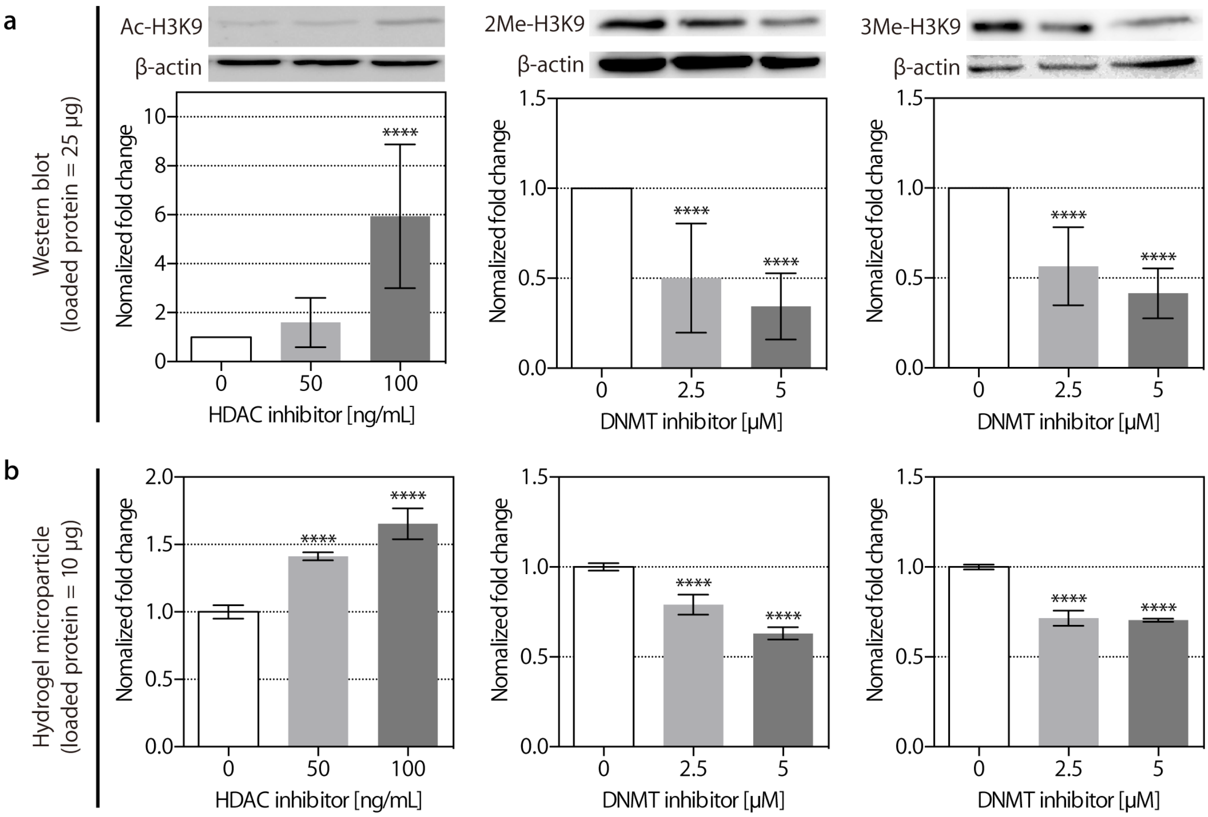

Figure S8. Quantification of histone H3K9 modifications from lysates of HEK cells treated with HDAC inhibitor or DNMT inhibitor. **a.** Western blot analysis with 25 µg of total proteins. HDAC inhibitor dose-dependently increased Ac-H3K9 status, while DNMT inhibitor also dose-dependently reduced 2Me-H3K9 and 3Me-H3K9. **** denotes $p < 0.0001$. n = 3 per group. b. Plots of normalized fold changes for hydrogel microparticle-based assay with the identical lysates (10 µg of total proteins). **** denotes $p < 0.0001$.

Video S1. Video clip showing microfluidic capture of hydrogel microparticles.

MATLAB code to rotate and crop acquired images

```
clc; %Clear Command Window
clear all; %Remove items from workspace, freeing up system memory
close all;

%Open standard dialog box for selecting directory
dname_fl = uigetdir('any desired location on a computer to find images' );

%List all tif files
cd(dname_fl);
img_path_fl=strcat(dname_fl,'/*.TIF');
file_fl = dir(img_path_fl);
file_num_fl = length(file_fl);
file_name_fl = {file_fl.name};
file_date_fl = {file_fl.date};

file_num_fl

clims_fl=[100 700];
rot_degree= [ ];
upperleftcorner = [ ];

img_w=620;
img_h=620;

probe_w=550;
probe_h=250;


for i = 1:file_num_fl
  %rotate image==================================
  figure(1)
  subplot(2, 1, 1)
  cd(dname_fl);
  img_fl=imread(file_name_fl{i});
  imagesc(img_fl, clims_fl)
  colormap(summer)
  axis equal
  grid on
  grid minor
  title(char(file_name_fl{i}))

  rot_degree_index=0;

  while rot_degree_index == 0
    prompt_fl={'Enter rotation degree:'};
    name_fl='Image rotation degree';
```

```
    numlines_fl=1;
    defaultanswer_fl={'90'};

    options.Resize='on';
    options.WindowStyle='normal';
    options.Interpreter='tex';

    rot_degree_dialog=inputdlg(prompt_fl,name_fl,numlines_fl,defaultanswer_fl,options);

    img_rot_fl=imrotate(img_fl, str2num(rot_degree_dialog{1}));
    %figure(2)
    subplot(2, 1, 2)
    imagesc(img_rot_fl, clims_fl)
    colormap(summer)
    axis equal
    grid on
    grid minor
    title(char(file_name_fl{i}))

    selectedButton = uigetpref(...
    'mygraphics',...                    % Group
    'savebeforeclosing',...         % Preference
    'Closing Rotation of Particle',...                  % Window title
    {'Are you satisfied?'},...
    {'Yes','No','Pass'},...       % Values and button strings
     'DefaultButton','No');          % Default choice
    switch selectedButton
       case 'no'
          rot_degree_index=0;
       case 'yes'
          rot_degree_index=1;
       case 'pass'
          rot_degree_index=2;
    end

    if rot_degree_index==0
       continue
    elseif rot_degree_index ==1
       rot_degree = [rot_degree; str2num(rot_degree_dialog{1})];
       break
    elseif rot_degree_index ==2
       break
    else
    end
end

%crop rotated image===========================================
```

```
    figure(2)
    %subplot(2, 1, 1)
    imagesc(img_rot_fl, clims_fl)
    colormap(summer)
    axis equal
    grid on
    grid minor
    title(char(file_name_fl{i}))

    crop_point_index=0;

    while crop_point_index == 0
      figure(2)
      %subplot(2, 1, 1)
      p1=impoint
      temp_upperleftcorner=getPosition(p1);

      img_crop_fl=imcrop(img_rot_fl, [temp_upperleftcorner(1) temp_upperleftcorner(2) img_w
  img_h]);
      figure(3)
      %subplot(2, 1, 2)
      imagesc(img_crop_fl, clims_fl)
      colormap(summer)
      axis equal
      grid on
      grid minor
      title(char(file_name_fl{i}))

      selectedButton = uigetpref(...
      'mygraphics',...                  % Group
      'savebeforeclosing',...          % Preference
      'Upper left corner of particle',...              % Window title
      {'Are you satisfied?'},...
      {'Yes','No','Pass'},...       % Values and button strings
       'DefaultButton','No');          % Default choice
      switch selectedButton
        case 'no'
          crop_point_index=0;
        case 'yes'
          crop_point_index=1;
        case 'pass'
          crop_point_index=2;
      end

      if crop_point_index==0
        delete(p1)
        continue
      elseif crop_point_index ==1
```

```
        upperleftcorner = [upperleftcorner; temp_upperleftcorner];
        break
    elseif crop_point_index ==2
        break
    else
    end

end

figure(3)
imsave


%crop rotated image==================================================

figure(3)

imagesc(img_crop_fl, clims_fl)
colormap(summer)
axis equal
grid on
grid minor
title(char(file_name_fl{i}))

crop_point_index=0;

while crop_point_index == 0
    figure(3)

    p1=impoint
    temp_upperleftcorner=getPosition(p1);

    img_crop_fl_probe=imcrop(img_crop_fl, [temp_upperleftcorner(1) temp_upperleftcorner(2)
probe_w probe_h]);
    figure(4)

    imagesc(img_crop_fl_probe, clims_fl)
    colormap(summer)
    axis equal
    grid on
    grid minor
    title(char(file_name_fl{i}))

    selectedButton = uigetpref(...
    'mygraphics',...                    % Group
    'savebeforeclosing',...          % Preference
    'Upper left corner of particle',...                 % Window title
    {'Are you satisfied?'},...
```

```
    {'Yes','No','Pass'},...       % Values and button strings
     'DefaultButton','No');          % Default choice
    switch selectedButton
      case 'no'
        crop_point_index=0;
      case 'yes'
        crop_point_index=1;
      case 'pass'
        crop_point_index=2;
    end

    if crop_point_index==0
      delete(p1)
      continue
    elseif crop_point_index ==1
       upperleftcorner = [upperleftcorner; temp_upperleftcorner];
      break
    elseif crop_point_index ==2
      break
    else
    end
  end

  figure(4)
  imsave

end

close all
```

MATLAB code to analyze fluorescence intensity from (rotated and cropped) images

```
clc; %Clear Command Window
clear all; %Remove items from workspace, freeing up system memory
close all;

%Open standard dialog box for selecting directory
dname = uigetdir('any desired location on a computer to find images');

%List all tif files
cd(dname);
img_path=strcat(dname,'/*.tif');
file = dir(img_path);
file_num = length(file);
file_name = {file.name};
file_date = {file.date};

file_num

%Determine row & column numbers for subplot
prompt1={'Enter row number:', 'Enter column number:'};
name1='Input for Limits of Fluorescence Intensity';
numlines1=1;
defaultanswer1={'5','1'};

options.Resize='on';
options.WindowStyle='normal';
options.Interpreter='tex';

rowcolumn_dialog=inputdlg(prompt1,name1,numlines1,defaultanswer1,options);

subplotsize = [str2num(rowcolumn_dialog{1,1}) str2num(rowcolumn_dialog{2,1})];

%Determine limits of fluorescence intensity
prompt2={'Enter the lower limit of fluorescence intensity:', 'Enter the upper limit of fluorescence
intensity:'};
name2='Input for Limits of Fluorescence Intensity';
numlines2=1;
defaultanswer2={'200','13000'};

options.Resize='on';
options.WindowStyle='normal';
options.Interpreter='tex';

clims_dialog=inputdlg(prompt2,name2,numlines2,defaultanswer2,options);

clims = [str2num(clims_dialog{1,1}) str2num(clims_dialog{2,1})];
```

```
probe_sig=[];

%Generate color-mapped images with the fixed limits for all images
figure()
for i = 1:file_num
   img=imread(file_name{i});
   colormap (jet);
   subplot(subplotsize(1),subplotsize(2),i);
   imagesc(img, clims)
   axis equal
   axis off
   %colorbar
   probe_sig=[probe_sig; mean(mean(img))];
end

mean_probe_sig=mean(probe_sig)

stdev_probe_sig=std(probe_sig)

CV_probe_sig=stdev_probe_sig/mean_probe_sig*100
```