Insight demo 2018: Calcium signaling analysis

Initialization

Load the settings file and add relevant dependencies to path.

```
close all
clearvars
settings = prepareWorkspace();
```

```
Warning: Function psi has the same name as a MATLAB builtin. We suggest you rename the function to
avoid a potential name conflict.
```

```
Workspace prepared
```

Look at data

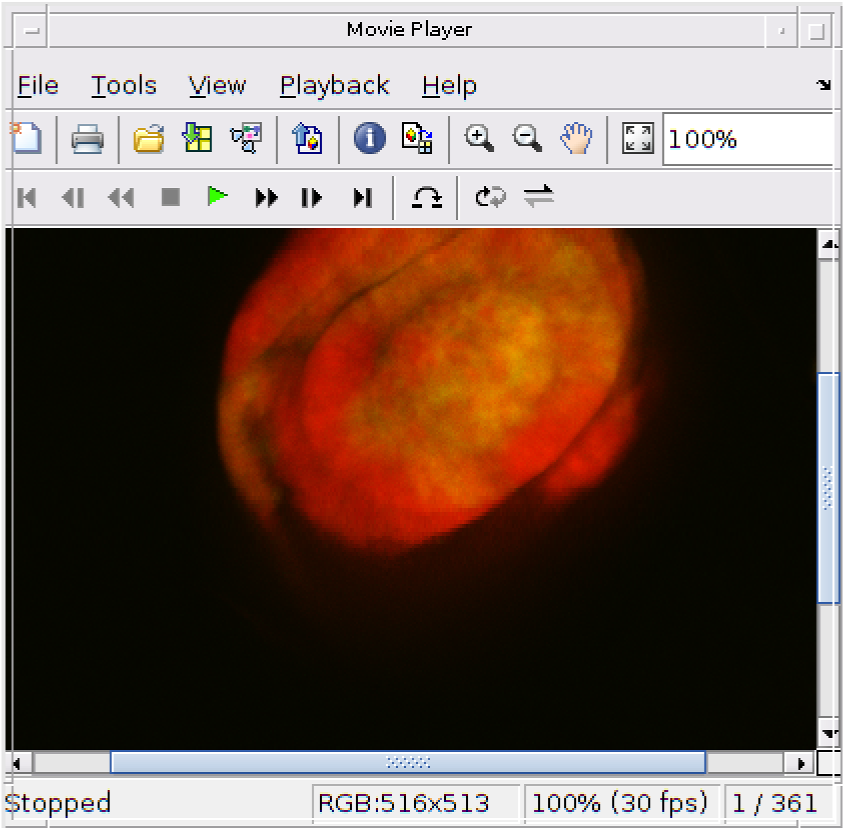
```
close all
demo_label = '2018_3_7 1346 x 2264 Day 6 Sample 13';
data = double(readTiff([settings.inExperimentalData demo_label '.tif']));
```

```
TiffDelegateReader initializing /media/pavel/PAVEL_LAB4/QualitativeAnalysisCalciumWaves/Quantitative/Data
Reading IFDs
Populating metadata
Checking comment style
Populating OME metadata
Reading series #1
    ........................................................................
    ........................................................................
    ........................................................................
    ........................................................................
    ........................................................................
    .
```

```
data_RFP = double(readTiff([settings.inRFP demo_label '.tif']));
```

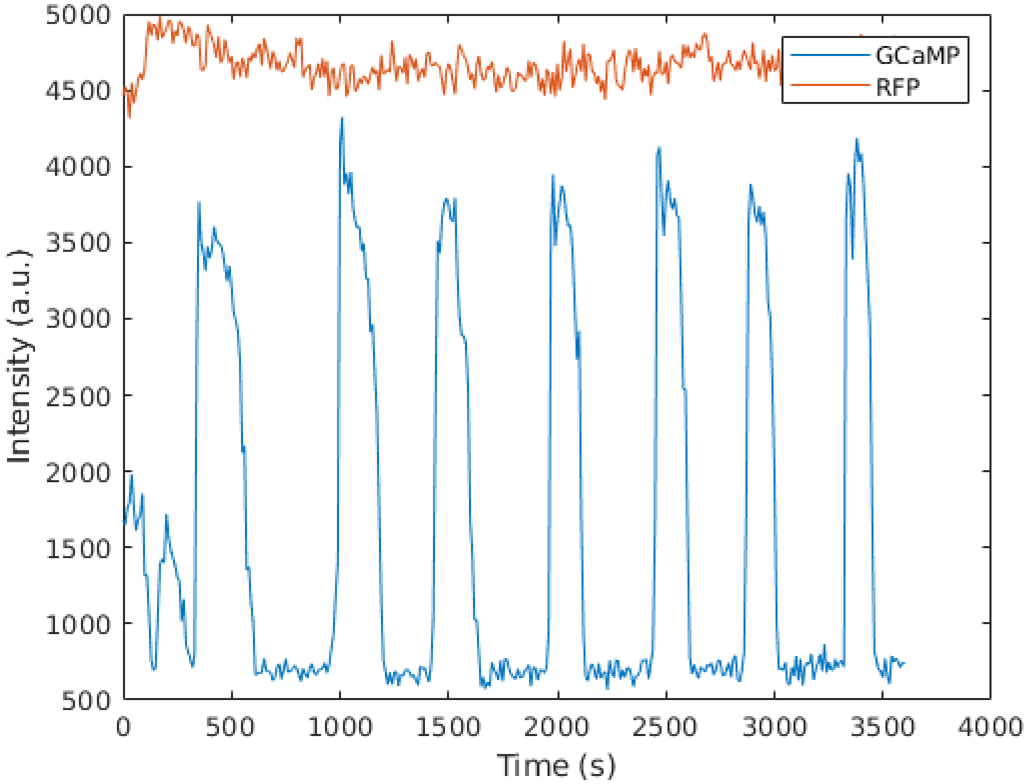
```
TiffDelegateReader initializing /media/pavel/PAVEL_LAB4/QualitativeAnalysisCalciumWaves/Quantitative/Data
Reading IFDs
Populating metadata
Checking comment style
Populating OME metadata
Reading series #1
    ........................................................................
    ........................................................................
    ........................................................................
    ........................................................................
    ........................................................................
    .
```

```
data_RGB = fuse_red_green(data_RFP, data);
implay(data_RGB, 30)
```



Look at intensity profile

```
I = squeeze(data(240,290,:));
I_RFP = squeeze(data_RFP(240,290,:));
ts = ((1:length(I)) - 1) * 10;
figure(1); clf;
plot(ts, I)
hold on
plot(ts, I_RFP)
xlabel('Time (s)'); ylabel('Intensity (a.u.)');
legend({'GCaMP', 'RFP'})
```



Filter signal

```
settings.dFoverFType = 'Conventional';
settings.tau = [10, 2, 400];
[dF_overF, F_0, F_bar] = dfOverF(I, settings);
```

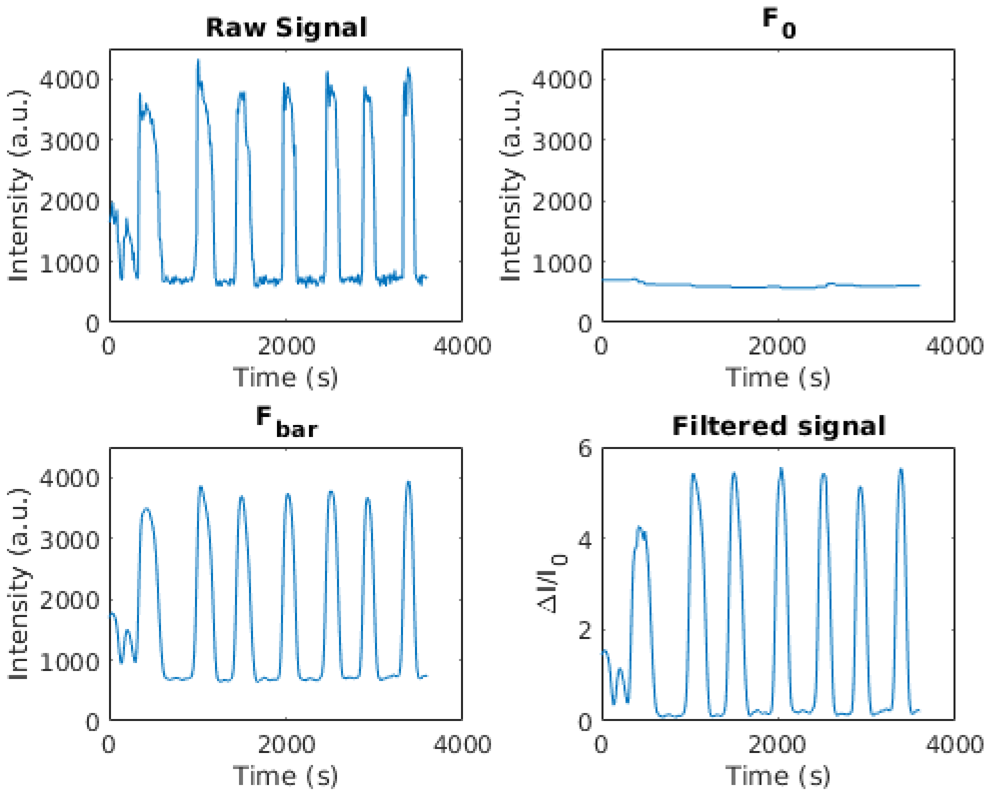
Demonstrate signal filtering

```
figure(2); clf;
subplot(2,2,1)
plot(ts, I);
xlabel('Time (s)'); ylabel('Intensity (a.u.)'); ylim([0,4.5e3]);
title('Raw Signal')
subplot(2,2,2)
plot(ts, F_0);
xlabel('Time (s)'); ylabel('Intensity (a.u.)'); ylim([0,4.5e3]);
title('F_0')
subplot(2,2,3)
plot(ts, F_bar)
xlabel('Time (s)'); ylabel('Intensity (a.u.)'); ylim([0,4.5e3]);
title('F_b_a_r')
```

Demonstrate filtered signal

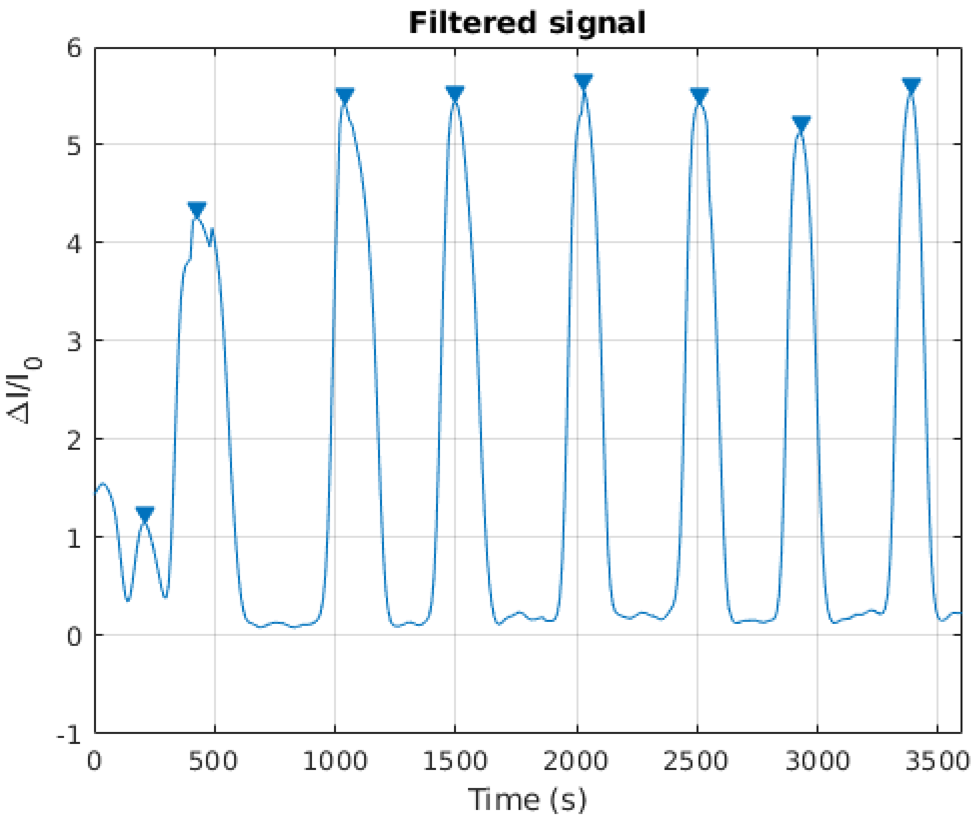
```
subplot(2,2,4)
```

```
plot(ts, dF_overF);
xlabel('Time (s)'); ylabel('\DeltaI/I_0');
title('Filtered signal')
```



Extract summary statistics

```
[stats, plotData] = extractStats(I, settings, I_RFP);
dt = 0.1;
figure(2); clf;
findpeaks(plotData.dF_overF, dt, plotData.findPeaksArgs{:})
xlabel('Time (s)'); ylabel('\DeltaI/I_0');
title('Filtered signal')
```




```
stats
```

```
stats = struct with fields:
                       mean_RFP: 4.6677e+03
                  min_I_over_RFP: 0.1209
               median_I_over_RFP: 0.1661
                 mean_I_over_RFP: 0.3492
    dfOverF_integrated_one_channel: 1.6731
                        median_I: 769
                          AmpRFP: 0.7698
                         AmpNorm: 5.2891
                      AmpNormDep: 3.9402
                             Amp: 3030
                             WHM: 135.4783
                        PeakRate: 2.2222
                            Freq: 2.1739
                          Period: 460
            Integral_by_dutyCycle: 4.5777e+03
                       DutyCycle: 3.1658
                          nPeaks: 8
           DutyCycle_by_df_over_f: 0
```

Obtain metadata and analysis

```
dataTable_raw = getDatatable(settings);
```

```
Warning: Directory already exists.
Measuring Times: 2018_3_10 1346 x 2264 Day 8 Sample 1
```

```
Extracting spatial maps: 16.08.08.1.2
Extracting spatial maps: 16.08.08.1.3
Extracting spatial maps: 16.08.08.2.1
Extracting spatial maps: 16.08.08.2.2
Extracting spatial maps: 16.08.08.2.3
Extracting spatial maps: 16.08.08.3.1
Extracting spatial maps: 16.08.08.3.2
Extracting spatial maps: 16.08.08.3.3
Extracting spatial maps: 16.08.08.4.1
Extracting spatial maps: 16.08.08.4.2
Extracting spatial maps: 16.08.08.4.3
Extracting spatial maps: 16.08.08.4.4
Extracting spatial maps: 16.08.08.4.5
Extracting spatial maps: 16.06.23.4.1
Extracting spatial maps: 16.06.23.4.3
Extracting spatial maps: 16.06.23.4.4
Extracting spatial maps: 16.08.09.2.1
Extracting spatial maps: 16.08.09.2.3
Extracting spatial maps: 16.08.09.2.4
Extracting spatial maps: 16.08.09.3.1
Extracting spatial maps: 16.08.09.3.2
Extracting spatial maps: 16.08.09.3.4
Extracting spatial maps: 16.08.09.3.5
Extracting spatial maps: 16.06.30.4.1
Extracting spatial maps: 16.06.30.4.2
Extracting spatial maps: 16.06.30.4.3
Extracting spatial maps: 16.06.30.4.4
Extracting spatial maps: 16.08.03.6.2
Extracting spatial maps: 16.08.03.6.4
Extracting spatial maps: 16.08.03.7.1
Extracting spatial maps: 16.08.03.7.2
Extracting spatial maps: 16.08.03.7.3
Extracting spatial maps: 16.08.03.7.4
Extracting spatial maps: 16.08.03.7.5
Extracting spatial maps: 16.08.03.8.1
Extracting spatial maps: 16.08.03.8.2
Extracting spatial maps: 16.08.03.8.3
Extracting spatial maps: 16.08.03.8.4
Extracting spatial maps: 16.08.03.8.5
Extracting spatial maps: 16.08.03.9.1
Extracting spatial maps: 16.08.03.9.2
Extracting spatial maps: 16.08.03.9.3
Extracting spatial maps: 16.08.03.9.4
Extracting spatial maps: 16.06.30.5.1
Extracting spatial maps: 16.06.30.5.4
Extracting spatial maps: 16.06.30.5.5
Extracting spatial maps: 2018_3_28 1346 x 1714 PTEN RNAi Sample 1 BR 1
Extracting spatial maps: 2018_3_28 1346 x 1714 PTEN RNAi Sample 2 BR 1
Extracting spatial maps: 2018_3_28 1346 x 1714 PTEN RNAi Sample 3 BR 1
Extracting spatial maps: 2018_3_28 1346 x 1714 PTEN RNAi Sample 4 BR 1
Extracting spatial maps: 2018_3_28 1346 x 1714 PTEN RNAi Sample 5 BR 1
Extracting spatial maps: 2018_3_28 1346 x 1714 PTEN RNAi Sample 6 BR 1
Extracting spatial maps: 2018_4_2 1346 x 331 UAS CycE Sample 1
Extracting spatial maps: 2018_4_2 1346 x 331 UAS CycE Sample 2
Extracting spatial maps: 2018_4_2 1346 x 331 UAS CycE Sample 3
Extracting spatial maps: 2018_4_2 1346 x 331 UAS CycE Sample 4
Extracting spatial maps: 2018_4_2 1346 x 331 UAS CycE Sample 5
Extracting spatial maps: 2018_4_2 1346 x 331 UAS CycE Sample 6
Extracting spatial maps: 2018_4_7 1346 x 2060 Arm S10 set 1 s1
Extracting spatial maps: 2018_4_7 1346 x 2060 Arm S10 set 1 s2
Extracting spatial maps: 2018_4_7 1346 x 2060 Arm S10 set 1 s3
Extracting spatial maps: 2018_4_7 1346 x 2060 Arm S10 set 1 s4
Extracting spatial maps: 2018_4_7 1346 x 2060 Arm S10 set 2 s1
Extracting spatial maps: 2018_4_7 1346 x 2060 Arm S10 set 2 s2
Extracting spatial maps: 2018_4_7 1346 x 2060 Arm S10 set 2 s3
```

```
Extracting spatial maps: 2018_4_7 1346 x 1308 Arm RNAi set 1 s2
Extracting spatial maps: 2018_4_7 1346 x 1308 Arm RNAi set 1 s3
Extracting spatial maps: 2018_4_7 1346 x 1308 Arm RNAi set 2 s1
Extracting spatial maps: 2018_4_7 1346 x 1308 Arm RNAi set 2 s2
Extracting spatial maps: 2018_4_7 1346 x 1308 Arm RNAi set 2 s3
Warning: Directory already exists.
Warning: Variable names were modified to make them valid MATLAB identifiers. The original names are saved in the VariableDescriptions property.
```

Remove samples with high laser power

```
dataTable = dataTable_raw(dataTable_raw.laserpower < 80,:);
```

Normalize integrated intensity to 50% laser power

```
dataTable.mean_I = dataTable.mean_median_I ./ dataTable.laserpower * 50;
```

Generate spatial map for demo video

```
figure(3); clf;
tblDemo = dataTable(demo_label,:);
t_demo = 100;
```

Show raw data

```
subplot(2,2,1)
imshow(data_RGB(:,:,:,t_demo))
title('Representative frame from raw data')
```

Show annotations on rotated and cropped image

```
load([settings.thruRotRFPFlipped demo_label '.mat'], 'croppedRFP')
load([settings.thruRot demo_label '.mat'], 'croppedVideo')
subplot(2,2,2)
imshow(fuse_red_green(croppedRFP(:,:,t_demo), croppedVideo(:,:,t_demo)))
hold on
scatter(tblDemo.APMat{1}(:,2),tblDemo.APMat{1}(:,1), '.w')
scatter(tblDemo.DVMat{1}(:,2),tblDemo.DVMat{1}(:,1), '.w')
title('Annotated frame')
```

Switch to painters renderer to avoid rendering artifacts with scatter plot

```
set(0, 'DefaultFigureRenderer', 'painters');
```

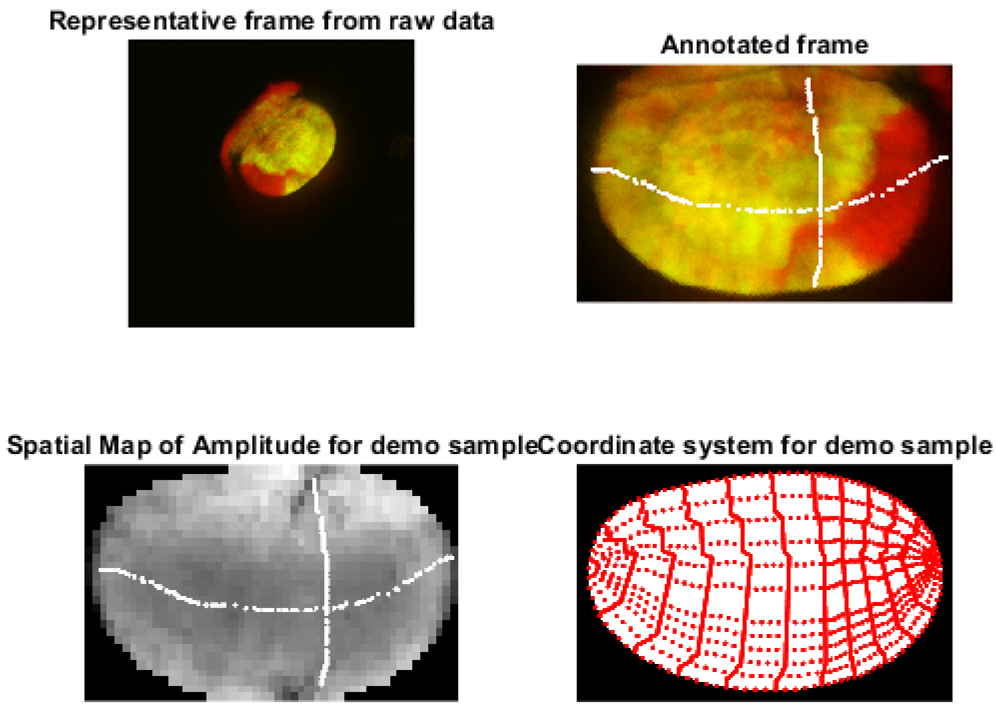
Show geometry on untransformed spatial map

```
subplot(2,2,3)
imshow(imresize(tblDemo.structMaps.AmpNorm, size(tblDemo.geometries.mask), 'nearest'),
hold on
scatter(tblDemo.APMat{1}(:,2),tblDemo.APMat{1}(:,1), '.w')
scatter(tblDemo.DVMat{1}(:,2),tblDemo.DVMat{1}(:,1), '.w')
```

```
title('Spatial Map of Amplitude for demo sample')
```

Show geometry on transformed spatial map

```
subplot(2,2,4)
imshow(tblDemo.geometries.mask, []); hold on;
showGeometry(tblDemo.geometries, 'AP', [1, 5], '.r');
showGeometry(tblDemo.geometries, 'AP', [10, 1], '.r');
title('Coordinate system for demo sample')
```



Show average spatial map of amplitude in controls of intermediate size. Compare anterior and posterior amplitude.

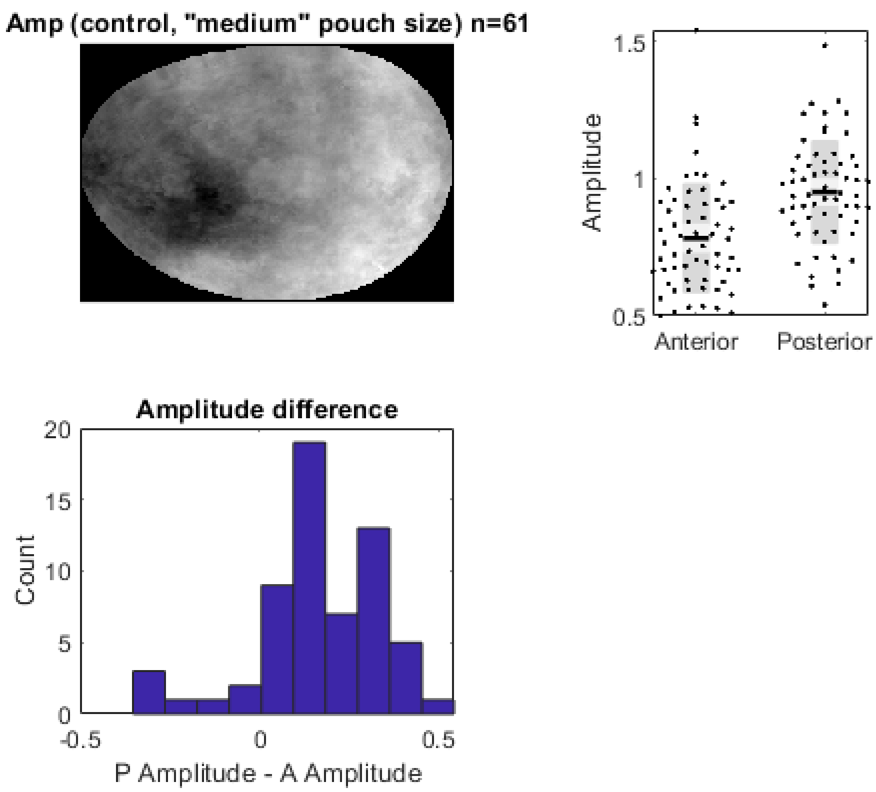
```
figure(2); clf;
subplot(2,2,1)
tblControl = dataTable(dataTable.category == 'control',:);
tblControl = tblControl(~cellfun('isempty', {tblControl.geometries.NCp}), :);
tblControl = tblControl(tblControl.pouchSizes > 1.5e4 & tblControl.pouchSizes < 2e4, :
transformed_AmpNorm = transformStatCoords(tblControl, 'AmpNorm');
```

```
Warning: Duplicate data points have been detected and removed.
The Triangulation indices are defined with respect to the unique set of points in
delaunayTriangulation.
Warning: Duplicate data points have been detected and removed.
The Triangulation indices are defined with respect to the unique set of points in
delaunayTriangulation.
Warning: Duplicate data points have been detected and removed - corresponding values have been
averaged.
```

```
imshow(nanmedian(transformed_AmpNorm, 3), [])
title(['Amp (control, "medium" pouch size) n=', num2str(size(tblControl, 1))])
subplot(2,2,2)
UnivarScatter(cat(2, tblControl.mean_A_AmpNorm, tblControl.mean_P_AmpNorm),'Label',{'A
```

```
ans = 61×2
    1.3324    2.1234
    0.7246    2.0000
    1.2754    2.2468
    0.7246    1.9296
    0.9449    1.9296
    1.0000    2.2111
    1.2493    2.2468
    1.0551    2.1662
    0.7246    1.7507
    1.2754    2.0704
       ⋮
```

```
ylabel('Amplitude')
subplot(2,2,3)
hist(tblControl.mean_P_AmpNorm - tblControl.mean_A_AmpNorm, 10)
xlabel('P Amplitude - A Amplitude'); ylabel('Count'); title('Amplitude difference')
```




```
[~, p] = ttest(tblControl.mean_A_AmpNorm, tblControl.mean_P_AmpNorm);
disp(['Paired t-test of Anterior vs Posterior amplitude. P value is ' num2str(p)])
```

```
Paired t-test of Anterior vs Posterior amplitude. P value is 6.7611e-10
```

Original interpretation affected by sampling bias

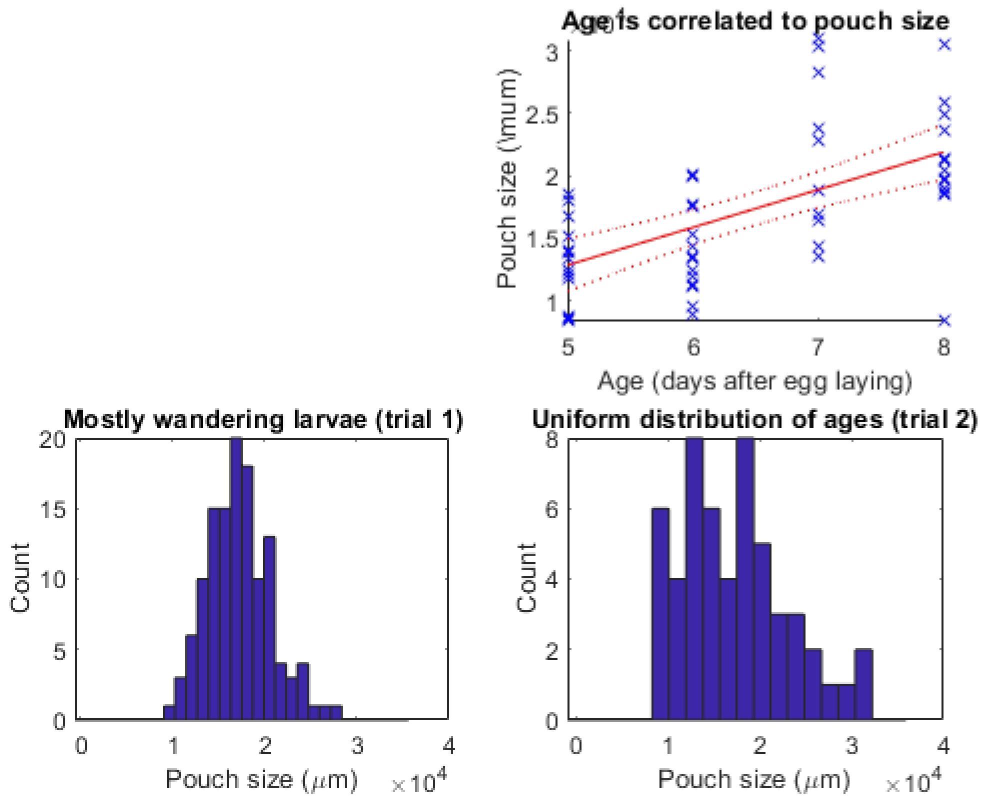
Age is highly correlated with pouch size

```
figure(1); clf;
subplot(2,2,2)
mdl_age_size = fitlm(dataTable.age_day_staging, dataTable.pouchSizes);
hold on; plot(mdl_age_size); title('Age is correlated to pouch size')
xlabel('Age (days after egg laying)'); ylabel('Pouch size (\mum)');
legend('off'); mdl_age_size
```

```
mdl_age_size =
Linear regression model:
    y ~ 1 + x1

Estimated Coefficients:
                   Estimate      SE        tStat        pValue
                   ________    ______    ________    __________

    (Intercept)    -2209.3     3708.1    -0.59578       0.55395
    x1              3014.4     567.23      5.3143    2.3772e-06


Number of observations: 53, Error degrees of freedom: 51
Root Mean Squared Error: 4.78e+03
R-squared: 0.356,  Adjusted R-Squared 0.344
F-statistic vs. constant model: 28.2, p-value = 2.38e-06
```

We were selecting early wandering larvae because they had oscilatory wave activity and we were analyzing wave activity. This biased our analysis and caused us to miss the important point that smaller disks had far more activity than larger discs, which is why they behaved in a qualitatively different way. Further, the lack of activity in very large discs was also missing from this story.

```
subplot(2,2,3)
hist(dataTable(dataTable.category == 'control',:).pouchSizes,linspace(0,3.5e4,30));
xlabel('Pouch size (\mum)'); ylabel('Count'); title('Mostly wandering larvae (trial 1)
subplot(2,2,4)
hist(dataTable(dataTable.category == 'control DS',:).pouchSizes,linspace(0,3.5e4,20));
xlabel('Pouch size (\mum)'); ylabel('Count'); title('Uniform distribution of ages (tri
```



Calcium activity is qualitatively different in discs of different ages

Load samples for different ages

```
demo_day5 = '2018_3_11_1346 x 2264 Day 5 sample 12';
demo_day6 = '2018_3_7 1346 x 2264 Day 6 Sample 13';
demo_day8 = '2018_3_10 1346 x 2264 Day 8 Sample 9';
dataTable.mean_median_I_over_RFP
```

```
ans = 759×1
   NaN
   NaN
   NaN
   NaN
   NaN
   NaN
   NaN
   NaN
   NaN
   NaN
    ⋮
```

```
data_5 = double(readTiff([settings.inExperimentalData demo_day5 '.tif']));
```

```
TiffDelegateReader initializing /media/pavel/PAVEL_LAB4/QualitativeAnalysisCalciumWaves/Quantitative/Data
Reading IFDs
Populating metadata
```

```
Checking comment style
Populating OME metadata
Reading series #1
    ......................................................................
    ......................................................................
    ......................................................................
    ......................................................................
    ......................................................................
    .
```

```
data_6 = double(readTiff([settings.inExperimentalData demo_day6 '.tif']));
```

```
TiffDelegateReader initializing /media/pavel/PAVEL_LAB4/QualitativeAnalysisCalciumWaves/Quantitative/Data
Reading IFDs
Populating metadata
Checking comment style
Populating OME metadata
Reading series #1
    ......................................................................
    ......................................................................
    ......................................................................
    ......................................................................
    ......................................................................
    .
```

```
data_8 = double(readTiff([settings.inExperimentalData demo_day8 '.tif']));
```

```
TiffDelegateReader initializing /media/pavel/PAVEL_LAB4/QualitativeAnalysisCalciumWaves/Quantitative/Data
Reading IFDs
Populating metadata
Checking comment style
Populating OME metadata
Reading series #1
    ......................................................................
    ......................................................................
    ......................................................................
    ......................................................................
    ......................................................................
    .
```

```
data_RFP_5 = double(readTiff([settings.inRFP demo_day5 '.tif']));
```

```
TiffDelegateReader initializing /media/pavel/PAVEL_LAB4/QualitativeAnalysisCalciumWaves/Quantitative/Data
Reading IFDs
Populating metadata
Checking comment style
Populating OME metadata
Reading series #1
    ......................................................................
    ......................................................................
    ......................................................................
    ......................................................................
    ......................................................................
    .
```

```
data_RFP_6 = double(readTiff([settings.inRFP demo_day6 '.tif']));
```

```
TiffDelegateReader initializing /media/pavel/PAVEL_LAB4/QualitativeAnalysisCalciumWaves/Quantitative/Data
Reading IFDs
Populating metadata
```

```
Checking comment style
Populating OME metadata
Reading series #1
    ........................................................................
    ........................................................................
    ........................................................................
    ........................................................................
    ........................................................................
    .
```

```
data_RFP_8 = double(readTiff([settings.inRFP demo_day8 '.tif']));
```

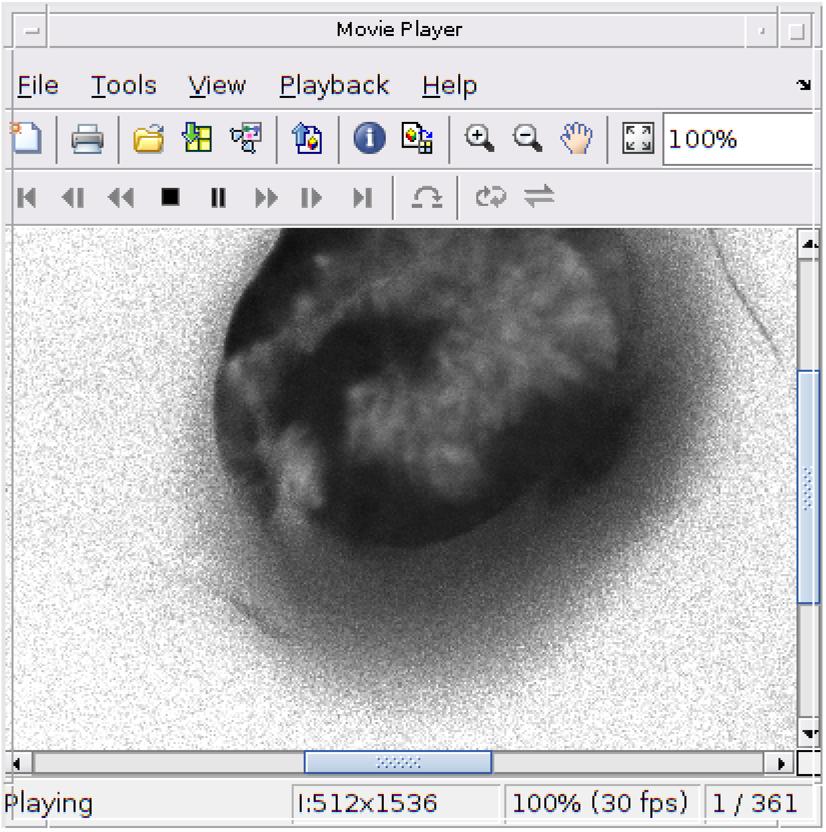
```
TiffDelegateReader initializing /media/pavel/PAVEL_LAB4/QualitativeAnalysisCalciumWaves/Quantitative/Data
Reading IFDs
Populating metadata
Checking comment style
Populating OME metadata
Reading series #1
    ........................................................................
    ........................................................................
    ........................................................................
    ........................................................................
    ........................................................................
    .
```

Generate normalized intensity videos and concatenate

```
data_5 = data_5(1:512,1:512,:) ./ data_RFP_5(1:512,1:512,:);
data_6 = data_6(1:512,1:512,:) ./ data_RFP_6(1:512,1:512,:);
data_8 = data_8(1:512,1:512,:) ./ data_RFP_8(1:512,1:512,:);

clear data_RFP_5 data_RFP_6 data_RFP_8

data_montage_norm = cat(2, data_5, data_6, data_8);

clear data_5 data_6 data_8
```

Visualize normalized videos

```
implay(data_montage_norm, 30)
```



Mean intensity decreases exponentially with pouch size

We observe a decrease in normalized median intensity with age.

```
figure(1); clf;
tblControlFig1 = dataTable(dataTable.category == 'control' | dataTable.category == 'co
tblControlFig1_RFP = dataTable(dataTable.category == 'control DS',:);
subplot(2,2,1)
scatter(tblControlFig1.pouchSizes, tblControlFig1.mean_mean_I_over_RFP, 'ok')
xlabel('Pouch size (\mum^2)'); ylabel('median(GCaMP/RFP)')
mdl_int_rfp_vs_size = fitnlm(tblControlFig1_RFP.pouchSizes, tblControlFig1_RFP.mean_mea
    , @(b,x) b(1).*exp(-b(2).*x),[0.65, 6e-5])
```

```
mdl_int_rfp_vs_size =
Nonlinear regression model:
    y ~ b1*exp( - b2*x)

Estimated Coefficients:
          Estimate        SE        tStat       pValue
          ________    __________    ______    __________

    b1     0.65944      0.079477    8.2972    6.7342e-11
    b2    6.66e-05    8.4035e-06    7.9253     2.486e-10


Number of observations: 51, Error degrees of freedom: 49
Root Mean Squared Error: 0.0642
R-Squared: 0.608,  Adjusted R-Squared 0.6
```

```
F-statistic vs. zero model: 352, p-value = 8.43e-30
```

```
title(['Normalized Ca^2^+ vs size R^2=' num2str(round(mdl_int_rfp_vs_size.Rsquared.Ord
tmdl = linspace(min(tblControlFig1_RFP.pouchSizes),...
    max(tblControlFig1_RFP.pouchSizes),200)';
[y, ci] = predict(mdl_int_rfp_vs_size,tmdl);
hold on
plot(tmdl, y,'-k'); plot(tmdl, ci,'--k');
```

We only have one dataset with RFP against which to normalize, so we test whether un-normalized median intensity is a good substitute for normalized median intensity.

```
subplot(2,2,2)
scatter(tblControlFig1.mean_I, tblControlFig1.mean_mean_I_over_RFP, 'ok')
xlabel('median(GCaMP)'); ylabel('median(GCaMP/RFP)');
mdl_int_vs_int_rfp = fitlm(tblControlFig1.mean_I, tblControlFig1.mean_mean_I_over_RFP)
```

```
mdl_int_vs_int_rfp =
Linear regression model:
    y ~ 1 + x1

Estimated Coefficients:
```

	Estimate	SE	tStat	pValue
(Intercept)	-0.0084442	0.019601	-0.4308	0.6685
x1	0.00035616	2.7935e-05	12.75	3.5165e-17

```
Number of observations: 51, Error degrees of freedom: 49
Root Mean Squared Error: 0.0494
R-squared: 0.768,  Adjusted R-Squared 0.764
F-statistic vs. constant model: 163, p-value = 3.52e-17
```

```
title(['Normalized Ca^2^+ vs Ca^2^+ R^2=' num2str(round(mdl_int_vs_int_rfp.Rsquared.Or
tmdl = linspace(min(tblControlFig1_RFP.mean_I),...
    max(tblControlFig1_RFP.mean_I),200)';
[y, ci] = predict(mdl_int_vs_int_rfp,tmdl);
hold on
plot(tmdl, y,'-k'); plot(tmdl, ci,'--k');
```

Now we look at all of our control data to see whether the RFP expression is changing the median intensity, because our genetically-perturbed conditions are all without the RFP background.

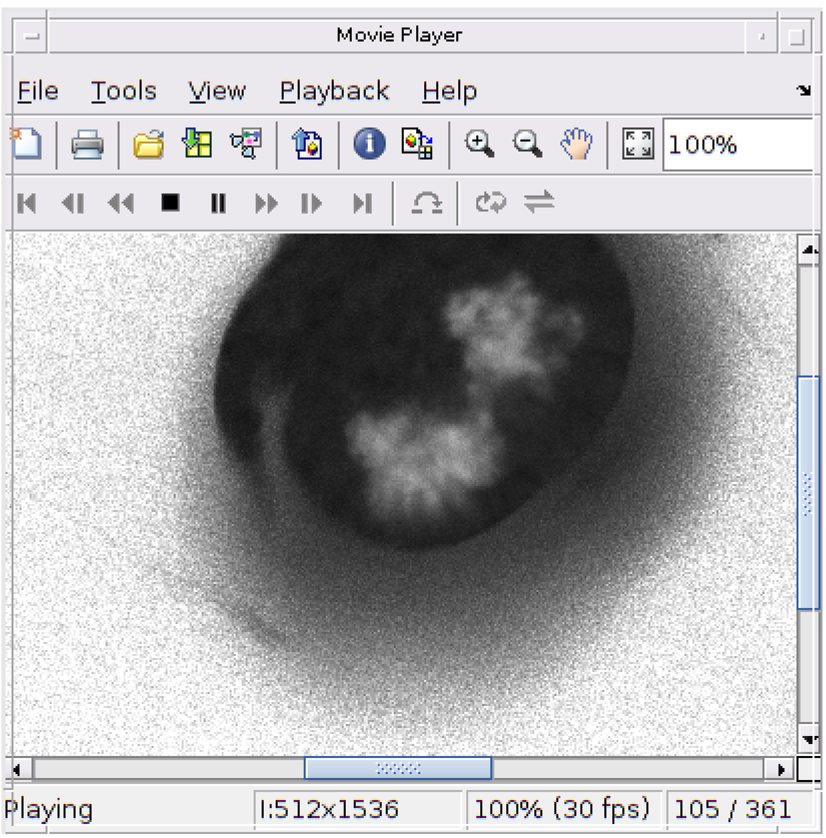
```
subplot(2,1,2)
scatter(tblControlFig1(tblControlFig1.category == 'control',:).pouchSizes, ...
    tblControlFig1(tblControlFig1.category == 'control',:).mean_I, 'or')
hold on
scatter(tblControlFig1(tblControlFig1.category == 'control DS',:).pouchSizes, ...
    tblControlFig1(tblControlFig1.category == 'control DS',:).mean_I, 'ok')
xlabel('Pouch size (\mum^2)'); ylabel('median(GCaMP)');
mdl_int_vs_size = fitnlm(tblControlFig1(~isnan(tblControlFig1.mean_I),:).pouchSizes,..
    tblControlFig1(~isnan(tblControlFig1.mean_I),:).mean_I, ...
    @(b,x) b(1).*exp(-b(2).*x),[3000, 6e-5])
```

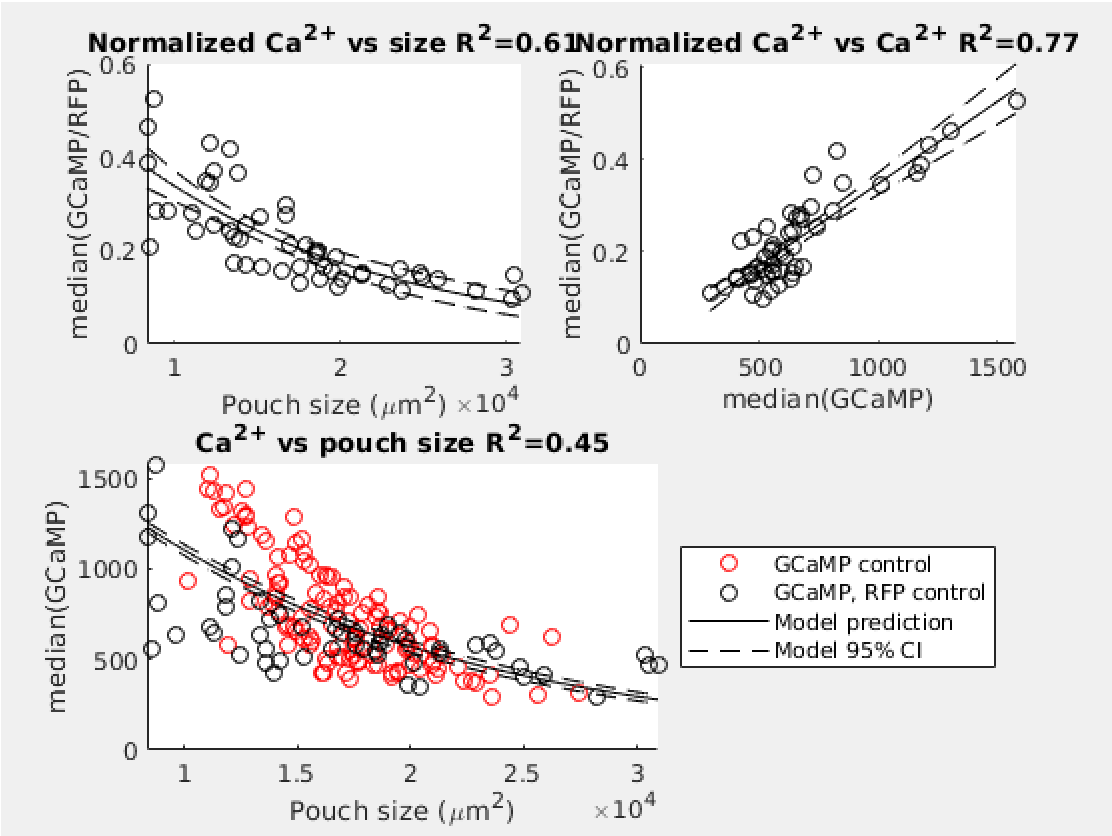
```
Warning: The Jacobian at the solution is ill-conditioned, and some model parameters may not be
estimated well (they are not identifiable). Use caution in making predictions.
mdl_int_vs_size =
Nonlinear regression model:
    y ~ b1*exp( - b2*x)

Estimated Coefficients:
```

	Estimate	SE	tStat	pValue
b1	2133.2	4.0401e-14	5.2801e+16	0
b2	6.5952e-05	1.3767e-06	47.905	1.5947e-102

```
Number of observations: 176, Error degrees of freedom: 175
Root Mean Squared Error: 210
R-Squared: 0.447,  Adjusted R-Squared 0.447
F-statistic vs. zero model: 2.19e+03, p-value = 6.18e-101
```

```
title(['Ca^2^+ vs pouch size R^2=' num2str(round(mdl_int_vs_size.Rsquared.Ordinary,2))
tmdl = linspace(min(tblControlFig1_RFP.pouchSizes),...
    max(tblControlFig1_RFP.pouchSizes),200)';
[y, ci] = predict(mdl_int_vs_size,tmdl);
hold on
plot(tmdl, y,'-k'); plot(tmdl, ci,'--k');
legend('GCaMP control', 'GCaMP, RFP control','Model prediction','Model 95% CI','Locati
```





Visualize with kernel density estimation

```
figure(9); clf
```

Switch to opengl renderer for smooth 3D manipulation

```
set(0, 'DefaultFigureRenderer', 'painters');
```

Assemble average pouch size, integrated intensity, and frequency for all control data

```
tblFig3 = dataTable(dataTable.category == 'control' | dataTable.category == 'control D
tblFig3 = tblFig3(:,{'pouchSizes', 'mean_I', 'mean_Freq'});
tblFig3(any(isnan(table2array(tblFig3)), 2), :) = [];
scatter3(tblFig3.mean_Freq, tblFig3.mean_I, tblFig3.pouchSizes,'.k')
xlabel('Frequency (mHz)')
ylabel('Integrated Intensity')
zlabel('Pouch Size (\mum^2)')
```

Generate uncertainty "range" with KDE.

First parameters are defined for the visualization including precision parameters, color and transparency parameters, and cutoffs for density.

```
interpolation_density = 100; pdf_gamma = 20; alpha = {0.3}; shade = {'blue'}; percenti

range_min = [min(tblFig3.pouchSizes), min(tblFig3.mean_I), min(tblFig3.mean_Freq)];
```

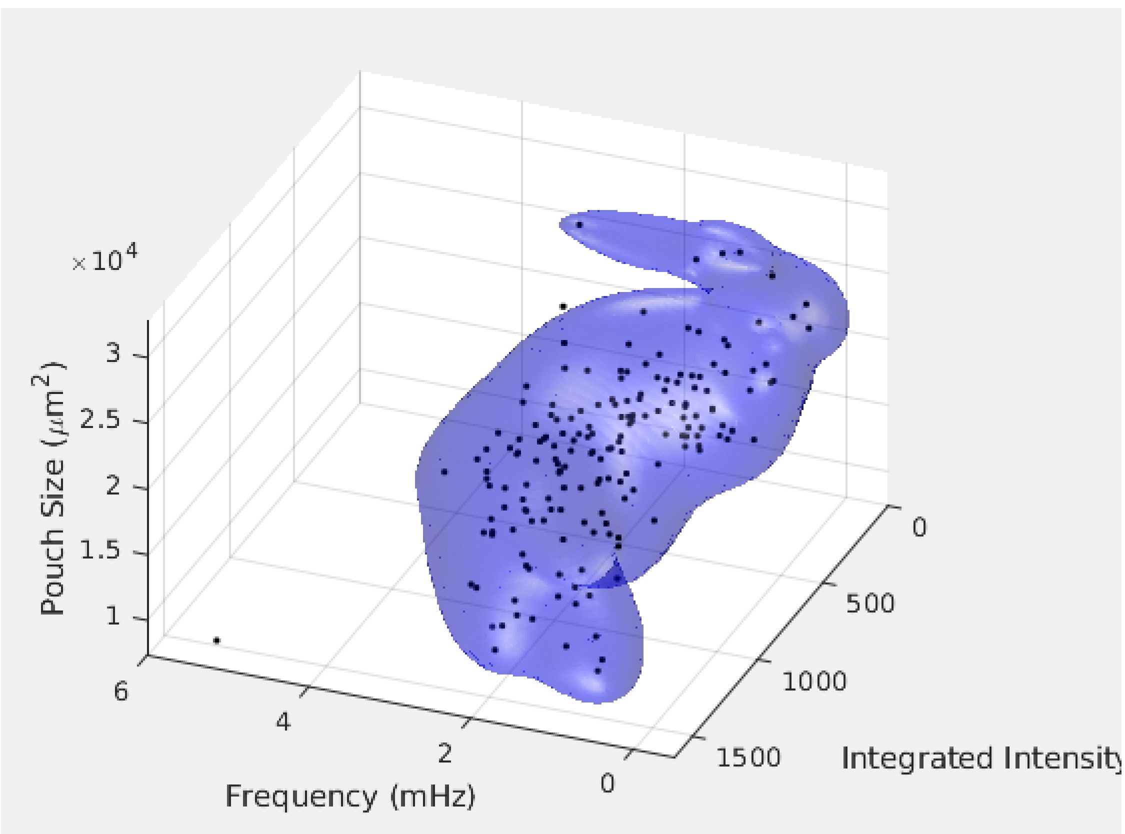
```
range_max = [max(tblFig3.pouchSizes), max(tblFig3.mean_I), max(tblFig3.mean_Freq)];
range_min = range_min - [std(tblFig3.pouchSizes), std(tblFig3.mean_I), std(tblFig3.mea
range_max = range_max + [std(tblFig3.pouchSizes), std(tblFig3.mean_I), std(tblFig3.mea
[X,Y,Z] = meshgrid(linspace(range_min(1),range_max(1),interpolation_density), ...
                   linspace(range_min(2),range_max(2),interpolation_density), ...
                   linspace(range_min(3),range_max(3),interpolation_density));
pdf = akde(table2array(tblFig3),[X(:),Y(:),Z(:)],pdf_gamma);
pdf = pdf / sum(pdf(:));
```

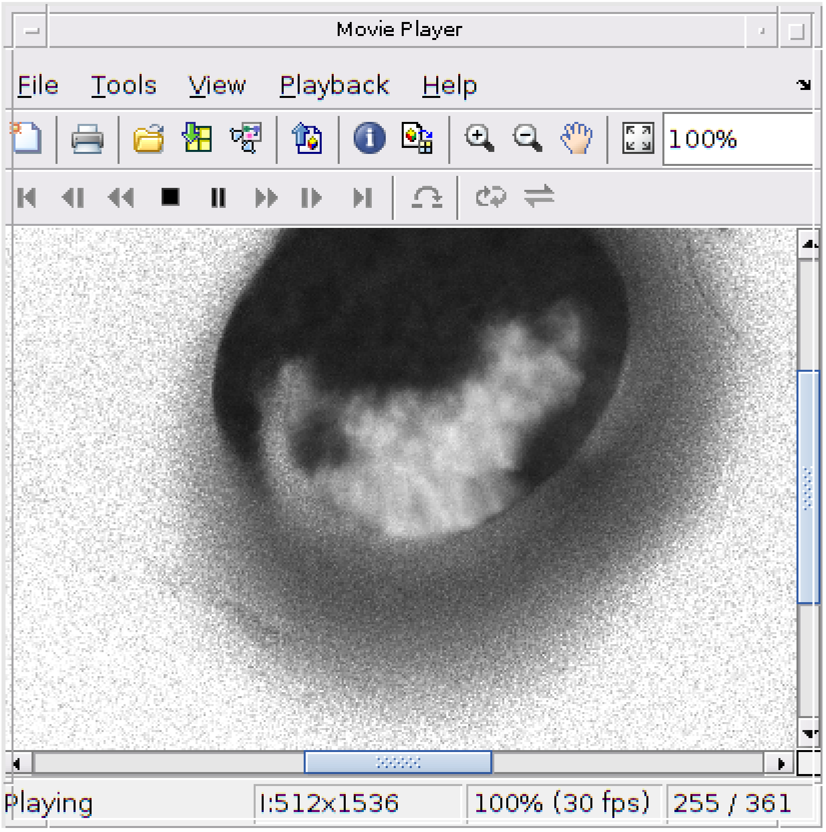
Visualize density estimates

```
for i = 1:length(alpha)
    [faces,verts,colors] = isosurface(Z,Y,X,reshape(pdf, size(X)),prctile(pdf,percenti
    patch('Vertices',verts,'Faces',faces,'FaceVertexCData',colors,'FaceColor',shade{i}
    hold on
end
```

Set up visualization lighting and angles

```
camlight
lighting gouraud
lightangle(0,180)
rotate3d
view(-158, 39)
```





Dimensional reduction

Switch to opengl renderer for smooth 3D manipulation

```
set(0, 'DefaultFigureRenderer', 'opengl');
```

Assemble average pouch size, integrated intensity, and frequency for all control data

```
tblFig3 = dataTable(dataTable.category == 'control' | dataTable.category == 'control D
tblFig3 = tblFig3(:,{'pouchSizes', 'mean_I', 'mean_Freq'});
X_raw = table2array(tblFig3);
X_raw(any(isnan(X_raw), 2), :) = [];
X = normalize(X_raw); % Obtain z-scores
```

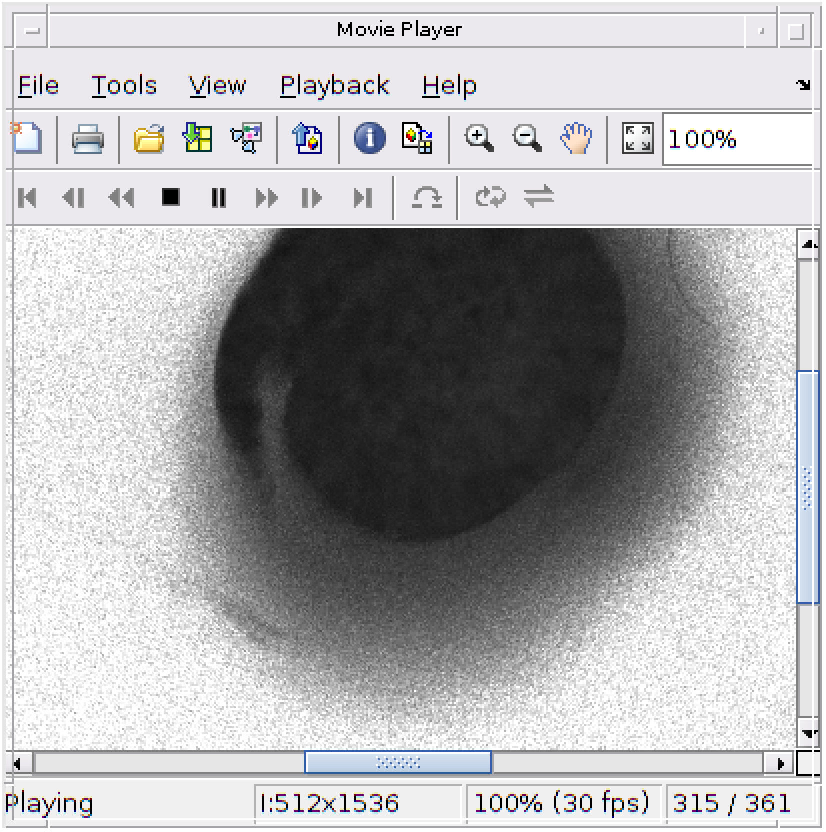
Perform dimensional reduction with PCA to show how poorly linear dimensionality reduction works with this data

```
[mapped_data, mapping] = compute_mapping(X, 'PCA', 1);
```

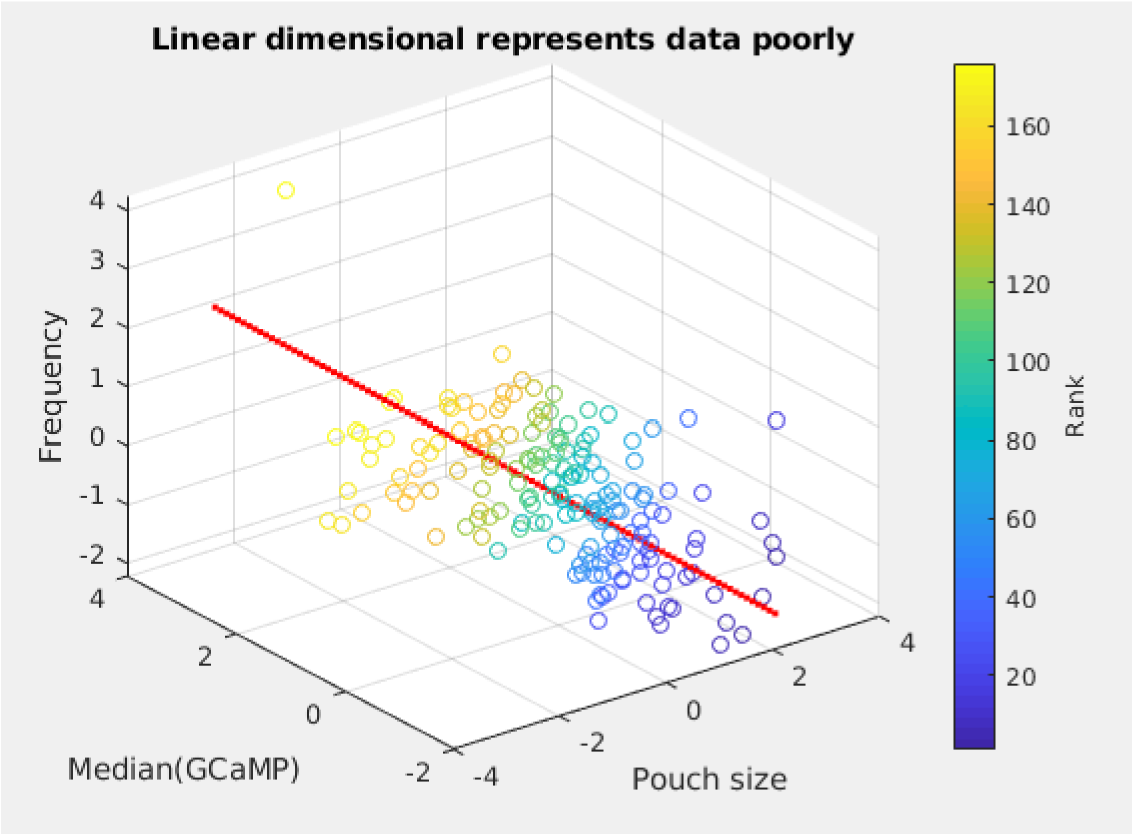
```
Welcome to the Matlab Toolbox for Dimensionality Reduction, version 0.8b (18-April-2012).
   You are free to modify or redistribute this code (for non-commercial purposes), as long as a refere
   to the original author (Laurens van der Maaten, Delft University of Technology) is retained.
   For more information, please visit http://homepage.tudelft.nl/19j49
```

```
mapped_rank = tiedrank(mapped_data);
```

```
figure(3); clf;
scatter3(X(:,1), X(:,2), X(:,3), [], mapped_rank, 'o');
hold on
recX = reconstruct_data(linspace(min(mapped_data), max(mapped_data), 100)', mapping);
scatter3(recX(:,1), recX(:,2), recX(:,3), '.r')
rotate3d
```




```
xlabel('Pouch size'); ylabel('Median(GCaMP)'); zlabel('Frequency');
title('Linear dimensional represents data poorly')
h = colorbar;
ylabel(h, 'Rank')
```



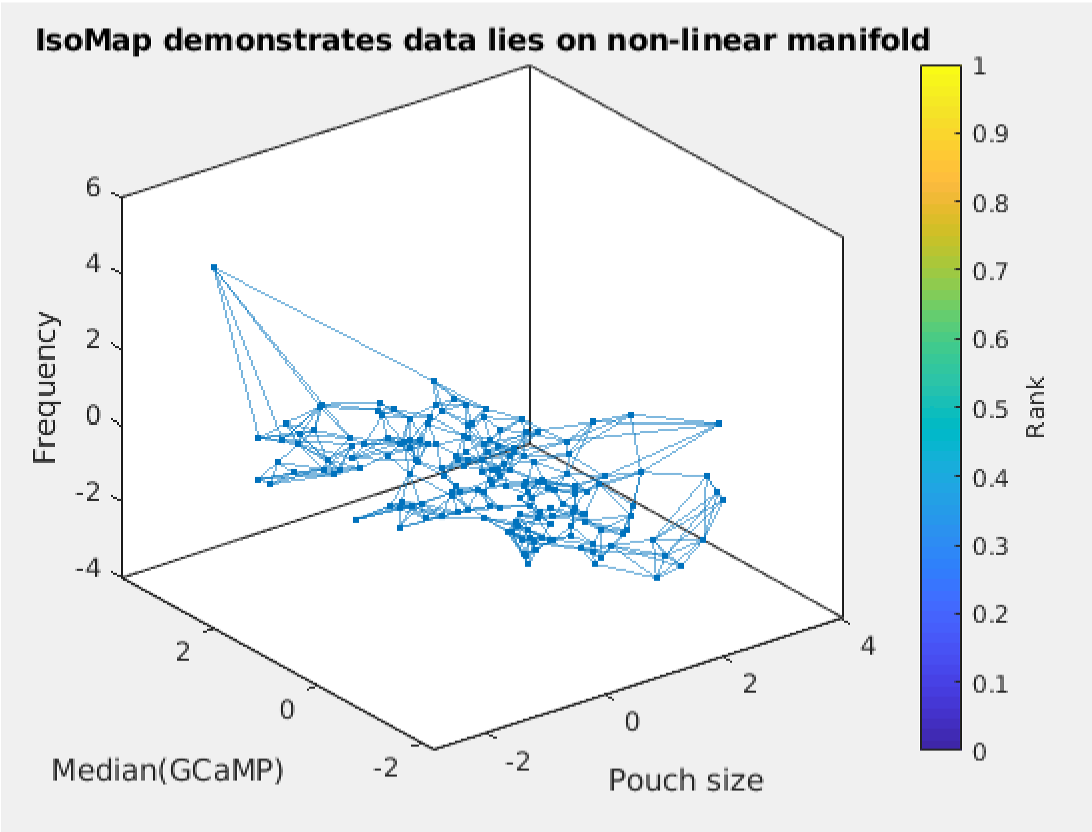
Generate IsoMap

```
options.dims = 1;
options.comp = 1;
options.display = 0;
options.overlay = 1;

[Y, R, E] = IsoMap(squareform(pdist(X, 'euclidean')), 'k', 5, options);
```

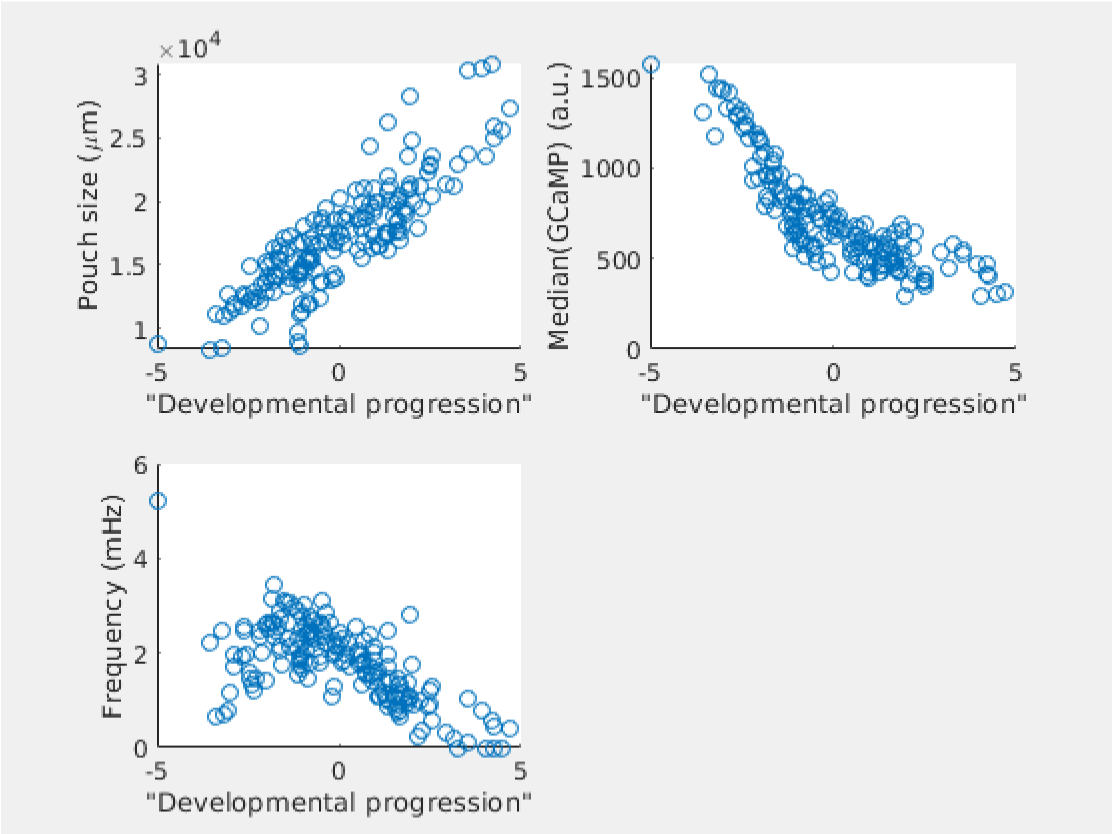
```
Constructing neighborhood graph...
Computing shortest paths...
 Iteration: 20     Estimated time to completion: 0.00070603 minutes
 Iteration: 40     Estimated time to completion: 0.00066005 minutes
 Iteration: 60     Estimated time to completion: 0.00046184 minutes
 Iteration: 80     Estimated time to completion: 0.0003363 minutes
 Iteration: 100     Estimated time to completion: 0.00024789 minutes
 Iteration: 120     Estimated time to completion: 0.00017489 minutes
 Iteration: 140     Estimated time to completion: 0.00010571 minutes
 Iteration: 160     Estimated time to completion: 4.4772e-05 minutes
Checking for outliers...
  Number of connected components in graph: 1
  Embedding component 1 with 176 points.
Constructing low-dimensional embeddings (Classical MDS)...
  Isomap on 176 points with dimensionality 1  --> residual variance = 0.16208
```

```
mapped_data = Y.coords{1};
mapped_rank = tiedrank(mapped_data);
figure(4); clf;
E(logical(eye(size(E)))) = 0;
```

```
plot(graph(logical(E)),'XData',X(:,1),'YData', X(:,2),'ZData',X(:,3))
hold on
xlabel('Pouch size'); ylabel('Median(GCaMP)'); zlabel('Frequency');
title('IsoMap demonstrates data lies on non-linear manifold')
h = colorbar;
ylabel(h, 'Rank')
rotate3d
```

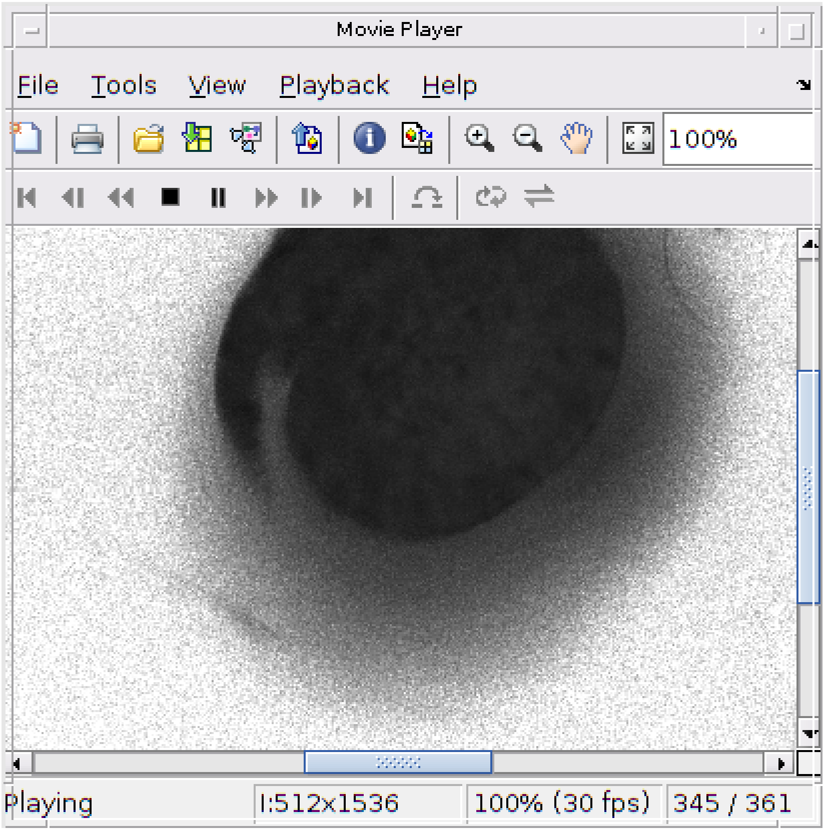



```
figure(5)
title('Projection onto "developmental space"')
subplot(2,2,1)
scatter(Y.coords{1}, X_raw(:,1))
xlabel('"Developmental progression"'); ylabel('Pouch size (\mum)');
subplot(2,2,2)
scatter(Y.coords{1}, X_raw(:,2))
xlabel('"Developmental progression"'); ylabel('Median(GCaMP) (a.u.)');
subplot(2,2,3)
scatter(Y.coords{1}, X_raw(:,3))
xlabel('"Developmental progression"'); ylabel('Frequency (mHz)');
```



Model ordinal Ca2+ activity variable

```
figure(10); clf;
```




```
tblControlFig1 = dataTable(dataTable.category == 'control' | dataTable.category == 'co
```

Modeling changes in size, intensity, and frequency over "developmental progression"

```
% tblDimReduced = table(Y.coords{1},X_raw(:,1),X_raw(:,2),X_raw(:,3),'VariableNames',{

% mdl_development =
```

Perturbing growth differentially changes integrated intensity

```
figure(8); clf
cats = {{'control','control DS'},...
     {'429 Smo RNAi','430 Smo RNAi','431 Smo RNAi',...
     '1230 Dpp RNAi','1233 Dpp RNAi'},...
     {'cycE',...
     '1714 pten RNAi','tkv ca'}};
catLabels = {{'nub>GCaMP6','nub>GCaMP6, RFP'},...
     {'nub>GCaMP6, 429 Smo RNAi','nub>GCaMP6, 430 Smo RNAi','nub>GCaMP6, 431 Smo RNAi'
     'nub>GCaMP6, 1230 Dpp RNAi','nub>GCaMP6, 1233 Dpp RNAi'},...
     {'nub>GCaMP6, cycE',...
     'nub>GCaMP6, 1714 pten RNAi','nub>GCaMP6, tkv*'}};
```

```
make_scatter_plot(dataTable, 'mean_I', cats, 'mean_Freq',[-inf,inf], catLabels, 'pouch
xlabel('Frequency (mHz)')
ylabel('Integrated Intensity')
zlabel('Pouch Size (\mum^2)')

rotate3d
```