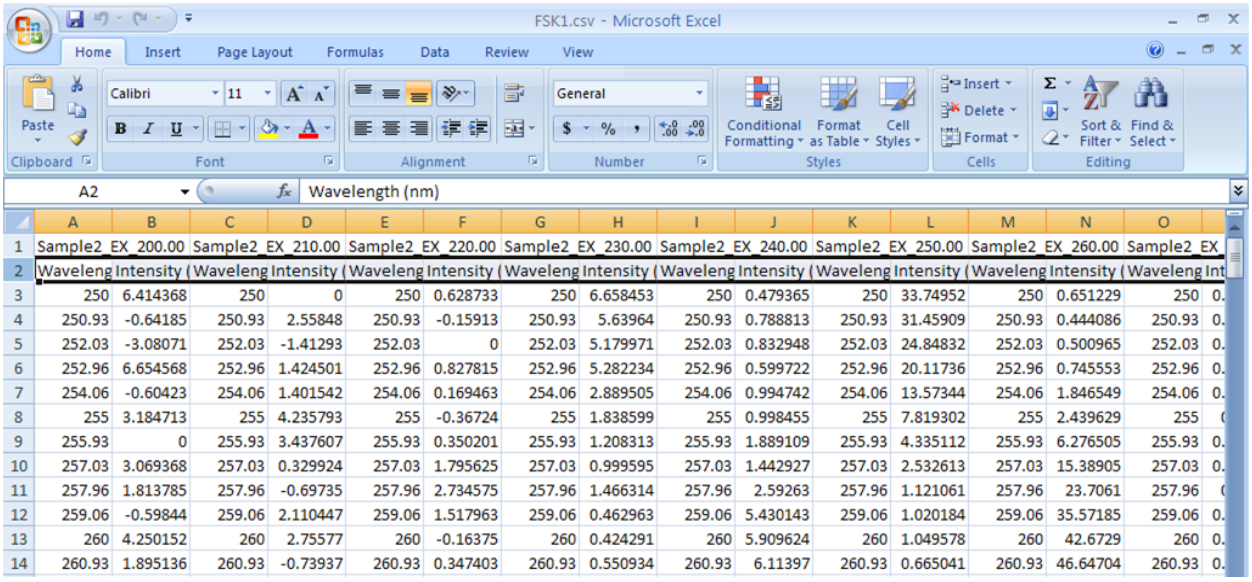
Fluorescence Resolution using PARAFAC (PARAlell FACtor analysis): A Guide

The following is a step by step procedure to resolve 3-D fluorescence data using PARAFAC. The 3D fluorescence spectra obtained from the spectrometer should be saved in the .csv format. The .csv file must be modified to fit the format as mentioned below. Once simplified, pre-processing of the data may begin.

Section 1: .CSV File PREP:

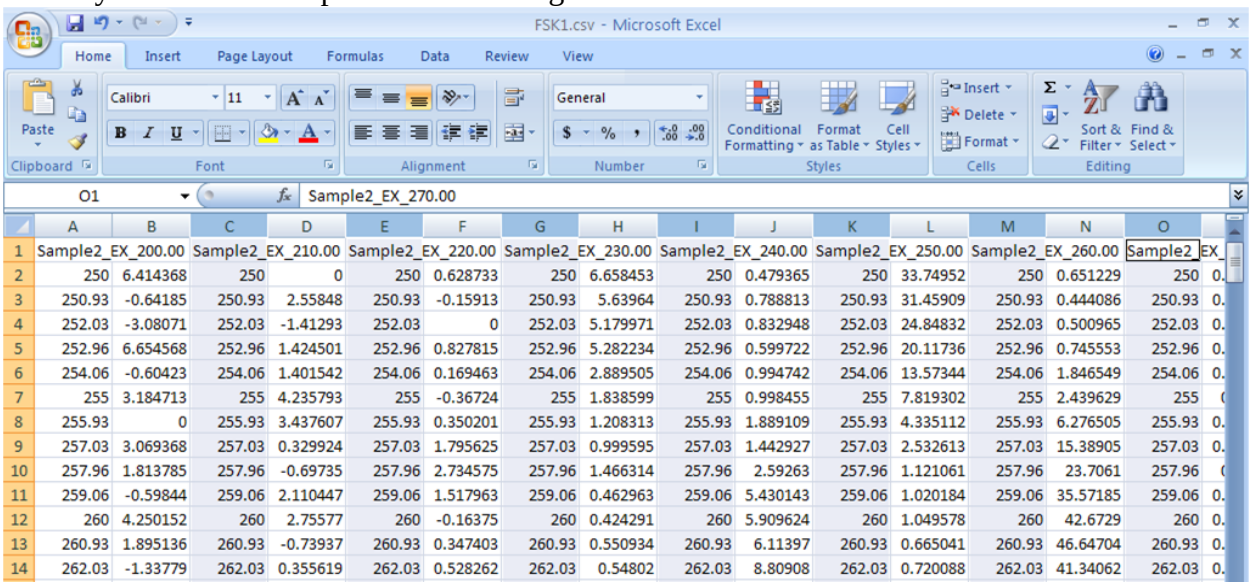
1. Open the .csv fluorescence file.

2. The second row contains headings for each column (wavelength and intensity). Remove the second row in the file.



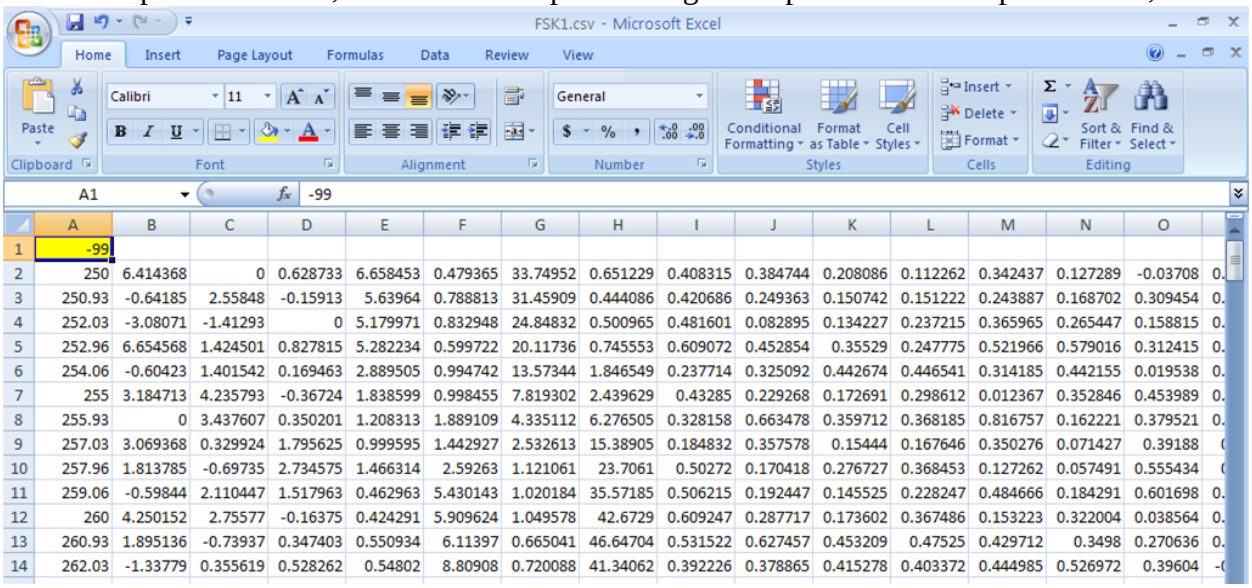
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Sample2_EX_200.00		Sample2_EX_210.00		Sample2_EX_220.00		Sample2_EX_230.00		Sample2_EX_240.00		Sample2_EX_250.00		Sample2_EX_260.00		Sample2_EX
2	Waveleng	Intensity (	Waveleng	Intensity (	Waveleng	Intensity (	Waveleng	Intensity (	Waveleng	Intensity (	Waveleng	Intensity (	Waveleng	Intensity (	Waveleng Int
3	250	6.414368	250	0	250	0.628733	250	6.658453	250	0.479365	250	33.74952	250	0.651229	250 0.
4	250.93	-0.64185	250.93	2.55848	250.93	-0.15913	250.93	5.63964	250.93	0.788813	250.93	31.45909	250.93	0.444086	250.93 0.
5	252.03	-3.08071	252.03	-1.41293	252.03	0	252.03	5.179971	252.03	0.832948	252.03	24.84832	252.03	0.500965	252.03 0.
6	252.96	6.654568	252.96	1.424501	252.96	0.827815	252.96	5.282234	252.96	0.599722	252.96	20.11736	252.96	0.745553	252.96 0.
7	254.06	-0.60423	254.06	1.401542	254.06	0.169463	254.06	2.889505	254.06	0.994742	254.06	13.57344	254.06	1.846549	254.06 0.
8	255	3.184713	255	4.235793	255	-0.36724	255	1.838599	255	0.998455	255	7.819302	255	2.439629	255 (
9	255.93	0	255.93	3.437607	255.93	0.350201	255.93	1.208313	255.93	1.889109	255.93	4.335112	255.93	6.276505	255.93 0.
10	257.03	3.069368	257.03	0.329924	257.03	1.795625	257.03	0.999595	257.03	1.442927	257.03	2.532613	257.03	15.38905	257.03 0.
11	257.96	1.813785	257.96	-0.69735	257.96	2.734575	257.96	1.466314	257.96	2.59263	257.96	1.121061	257.96	23.7061	257.96 (
12	259.06	-0.59844	259.06	2.110447	259.06	1.517963	259.06	0.462963	259.06	5.430143	259.06	1.020184	259.06	35.57185	259.06 0.
13	260	4.250152	260	2.75577	260	-0.16375	260	0.424291	260	5.909624	260	1.049578	260	42.6729	260 0.
14	260.93	1.895136	260.93	-0.73937	260.93	0.347403	260.93	0.550934	260.93	6.11397	260.93	0.665041	260.93	46.64704	260.93 0.

3. Every other column repeats the wavelengths used for the scan. Remove these columns.



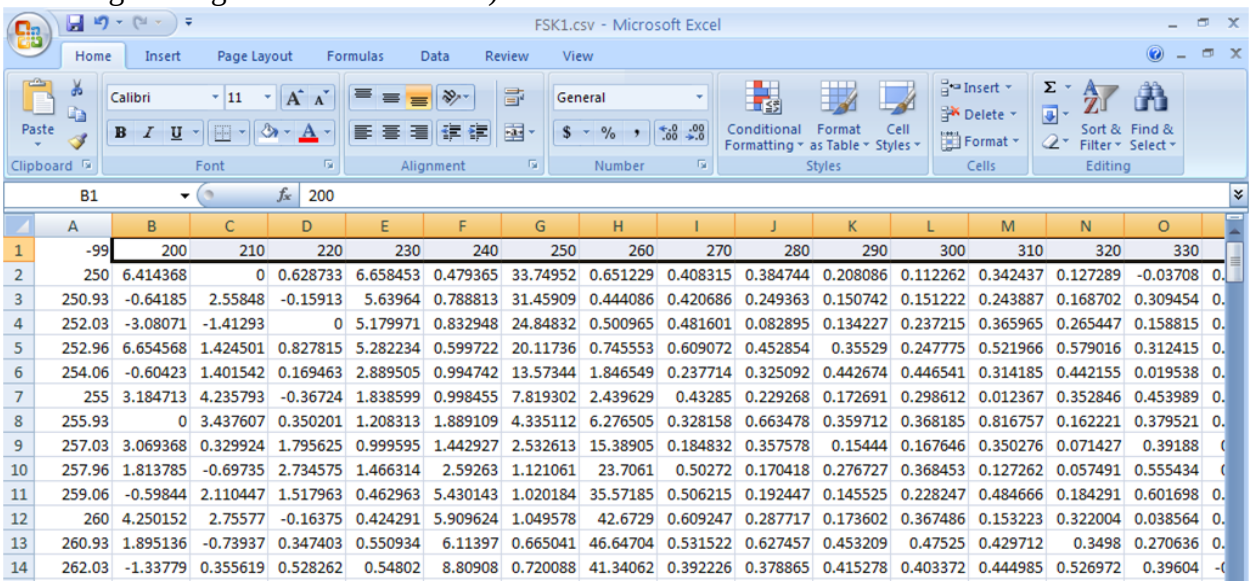
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Sample2_EX_200.00		Sample2_EX_210.00		Sample2_EX_220.00		Sample2_EX_230.00		Sample2_EX_240.00		Sample2_EX_250.00		Sample2_EX_260.00		Sample2_EX
2	250	6.414368	250	0	250	0.628733	250	6.658453	250	0.479365	250	33.74952	250	0.651229	250 0.
3	250.93	-0.64185	250.93	2.55848	250.93	-0.15913	250.93	5.63964	250.93	0.788813	250.93	31.45909	250.93	0.444086	250.93 0.
4	252.03	-3.08071	252.03	-1.41293	252.03	0	252.03	5.179971	252.03	0.832948	252.03	24.84832	252.03	0.500965	252.03 0.
5	252.96	6.654568	252.96	1.424501	252.96	0.827815	252.96	5.282234	252.96	0.599722	252.96	20.11736	252.96	0.745553	252.96 0.
6	254.06	-0.60423	254.06	1.401542	254.06	0.169463	254.06	2.889505	254.06	0.994742	254.06	13.57344	254.06	1.846549	254.06 0.
7	255	3.184713	255	4.235793	255	-0.36724	255	1.838599	255	0.998455	255	7.819302	255	2.439629	255 (
8	255.93	0	255.93	3.437607	255.93	0.350201	255.93	1.208313	255.93	1.889109	255.93	4.335112	255.93	6.276505	255.93 0.
9	257.03	3.069368	257.03	0.329924	257.03	1.795625	257.03	0.999595	257.03	1.442927	257.03	2.532613	257.03	15.38905	257.03 0.
10	257.96	1.813785	257.96	-0.69735	257.96	2.734575	257.96	1.466314	257.96	2.59263	257.96	1.121061	257.96	23.7061	257.96 (
11	259.06	-0.59844	259.06	2.110447	259.06	1.517963	259.06	0.462963	259.06	5.430143	259.06	1.020184	259.06	35.57185	259.06 0.
12	260	4.250152	260	2.75577	260	-0.16375	260	0.424291	260	5.909624	260	1.049578	260	42.6729	260 0.
13	260.93	1.895136	260.93	-0.73937	260.93	0.347403	260.93	0.550934	260.93	6.11397	260.93	0.665041	260.93	46.64704	260.93 0.
14	262.03	-1.33779	262.03	0.355619	262.03	0.528262	262.03	0.54802	262.03	8.80908	262.03	0.720088	262.03	41.34062	262.03 0.

4. In the top left hand cell, remove the sample heading and replace it with the placeholder, -99.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	-99														
2	250	6.414368	0	0.628733	6.658453	0.479365	33.74952	0.651229	0.408315	0.384744	0.208086	0.112262	0.342437	0.127289	-0.03708
3	250.93	-0.64185	2.55848	-0.15913	5.63964	0.788813	31.45909	0.444086	0.420686	0.249363	0.150742	0.151222	0.243887	0.168702	0.309454
4	252.03	-3.08071	-1.41293	0	5.179971	0.832948	24.84832	0.500965	0.481601	0.082895	0.134227	0.237215	0.365965	0.265447	0.158815
5	252.96	6.654568	1.424501	0.827815	5.282234	0.599722	20.11736	0.745553	0.609072	0.452854	0.35529	0.247775	0.521966	0.579016	0.312415
6	254.06	-0.60423	1.401542	0.169463	2.889505	0.994742	13.57344	1.846549	0.237714	0.325092	0.442674	0.446541	0.314185	0.442155	0.019538
7	255	3.184713	4.235793	-0.36724	1.838599	0.998455	7.819302	2.439629	0.43285	0.229268	0.172691	0.298612	0.012367	0.352846	0.453989
8	255.93	0	3.437607	0.350201	1.208313	1.889109	4.335112	6.276505	0.328158	0.663478	0.359712	0.368185	0.816757	0.162221	0.379521
9	257.03	3.069368	0.329924	1.795625	0.999595	1.442927	2.532613	15.38905	0.184832	0.357578	0.15444	0.167646	0.350276	0.071427	0.39188
10	257.96	1.813785	-0.69735	2.734575	1.466314	2.59263	1.121061	23.7061	0.50272	0.170418	0.276727	0.368453	0.127262	0.057491	0.555434
11	259.06	-0.59844	2.110447	1.517963	0.462963	5.430143	1.020184	35.57185	0.506215	0.192447	0.145525	0.228247	0.484666	0.184291	0.601698
12	260	4.250152	2.75577	-0.16375	0.424291	5.909624	1.049578	42.6729	0.609247	0.287717	0.173602	0.367486	0.153223	0.322004	0.038564
13	260.93	1.895136	-0.73937	0.347403	0.550934	6.11397	0.665041	46.64704	0.531522	0.627457	0.453209	0.47525	0.429712	0.3498	0.270636
14	262.03	-1.33779	0.355619	0.528262	0.54802	8.80908	0.720088	41.34062	0.392226	0.378865	0.415278	0.403372	0.444985	0.526972	0.39604

5. In row 1, add the wavelengths used for scan along the top of each column (In most cases, the wavelengths range from 200 – 450nm).



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	-99	200	210	220	230	240	250	260	270	280	290	300	310	320	330
2	250	6.414368	0	0.628733	6.658453	0.479365	33.74952	0.651229	0.408315	0.384744	0.208086	0.112262	0.342437	0.127289	-0.03708
3	250.93	-0.64185	2.55848	-0.15913	5.63964	0.788813	31.45909	0.444086	0.420686	0.249363	0.150742	0.151222	0.243887	0.168702	0.309454
4	252.03	-3.08071	-1.41293	0	5.179971	0.832948	24.84832	0.500965	0.481601	0.082895	0.134227	0.237215	0.365965	0.265447	0.158815
5	252.96	6.654568	1.424501	0.827815	5.282234	0.599722	20.11736	0.745553	0.609072	0.452854	0.35529	0.247775	0.521966	0.579016	0.312415
6	254.06	-0.60423	1.401542	0.169463	2.889505	0.994742	13.57344	1.846549	0.237714	0.325092	0.442674	0.446541	0.314185	0.442155	0.019538
7	255	3.184713	4.235793	-0.36724	1.838599	0.998455	7.819302	2.439629	0.43285	0.229268	0.172691	0.298612	0.012367	0.352846	0.453989
8	255.93	0	3.437607	0.350201	1.208313	1.889109	4.335112	6.276505	0.328158	0.663478	0.359712	0.368185	0.816757	0.162221	0.379521
9	257.03	3.069368	0.329924	1.795625	0.999595	1.442927	2.532613	15.38905	0.184832	0.357578	0.15444	0.167646	0.350276	0.071427	0.39188
10	257.96	1.813785	-0.69735	2.734575	1.466314	2.59263	1.121061	23.7061	0.50272	0.170418	0.276727	0.368453	0.127262	0.057491	0.555434
11	259.06	-0.59844	2.110447	1.517963	0.462963	5.430143	1.020184	35.57185	0.506215	0.192447	0.145525	0.228247	0.484666	0.184291	0.601698
12	260	4.250152	2.75577	-0.16375	0.424291	5.909624	1.049578	42.6729	0.609247	0.287717	0.173602	0.367486	0.153223	0.322004	0.038564
13	260.93	1.895136	-0.73937	0.347403	0.550934	6.11397	0.665041	46.64704	0.531522	0.627457	0.453209	0.47525	0.429712	0.3498	0.270636
14	262.03	-1.33779	0.355619	0.528262	0.54802	8.80908	0.720088	41.34062	0.392226	0.378865	0.415278	0.403372	0.444985	0.526972	0.39604

Section 2: PRE-PROCESSING DATA (Scatter Removal):

This next section describes how to preprocess the .csv files when absorbance corrections are not needed. If correcting for absorbance, disregard this section and refer to Section 3.

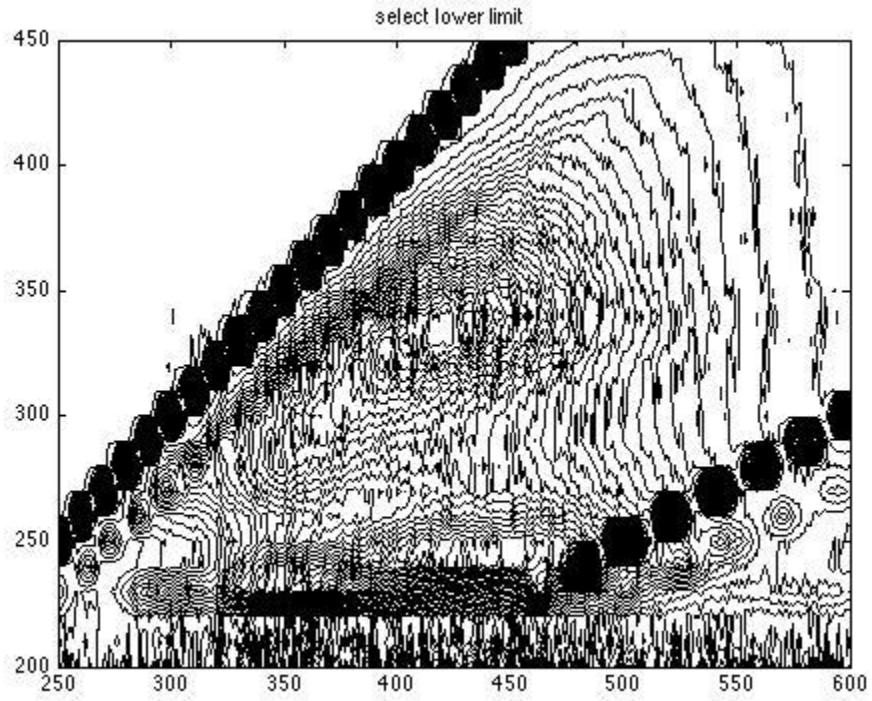
1. Open scatterremoval.m

2. Change the file name to match the .csv files being preprocessed.

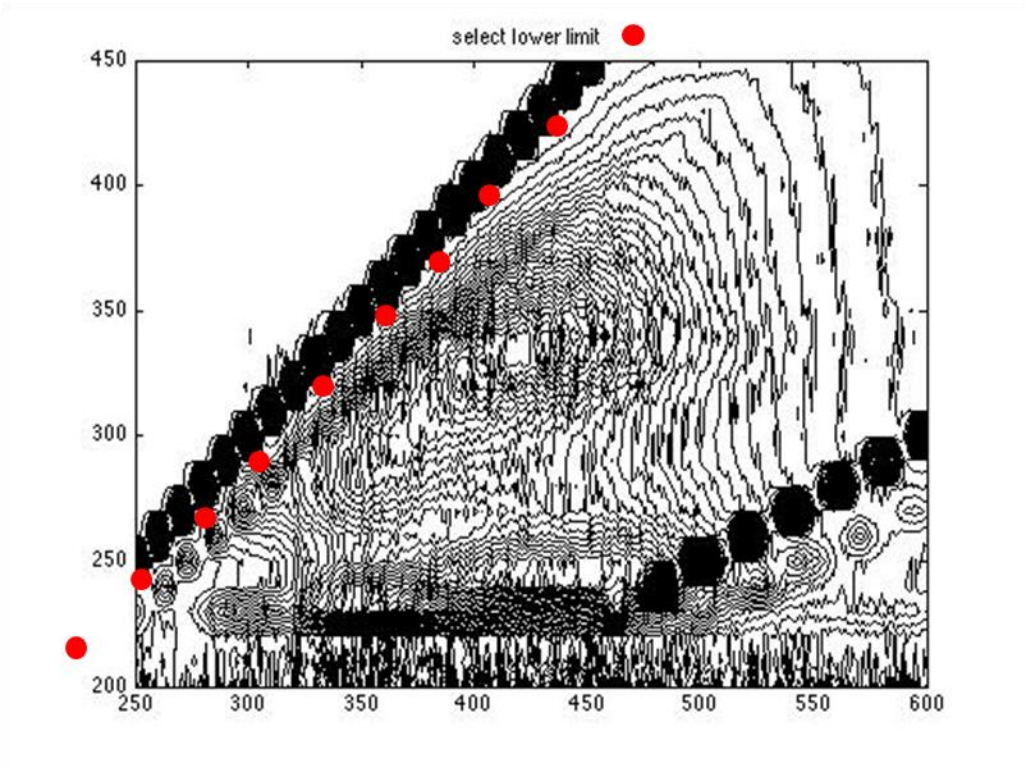
```
names=strvcat(...
'filename1', ...
'filename2', ...
'filename3');

for ii=1:size(names,1)
    name=names(ii,:);
    txt=['preprocess_fluor_data_csv_file ',name]; eval(txt);
end
```

3. Press the run m-file (green triangle) play button.

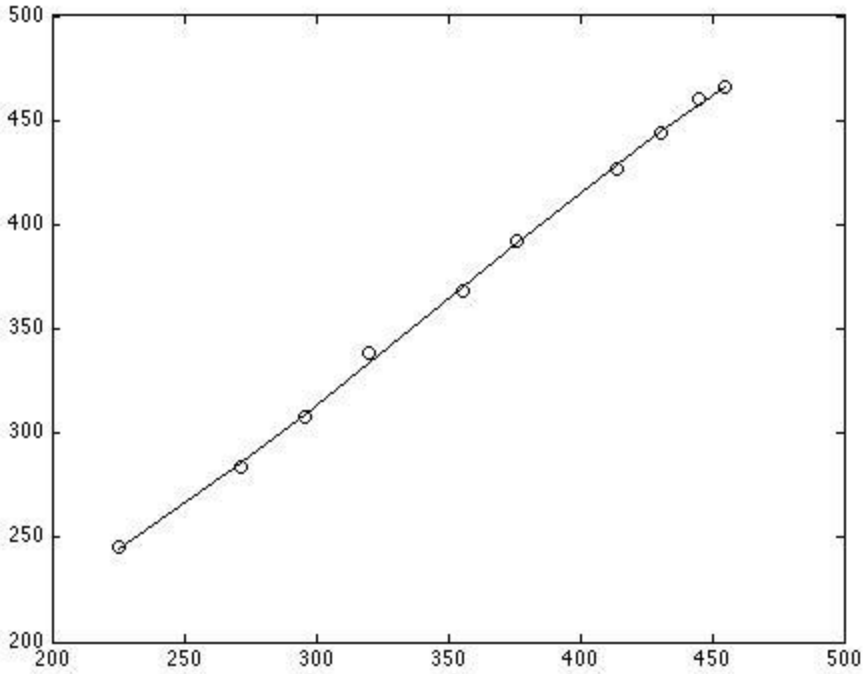
4. The following figure (1) will pop into a new window on screen



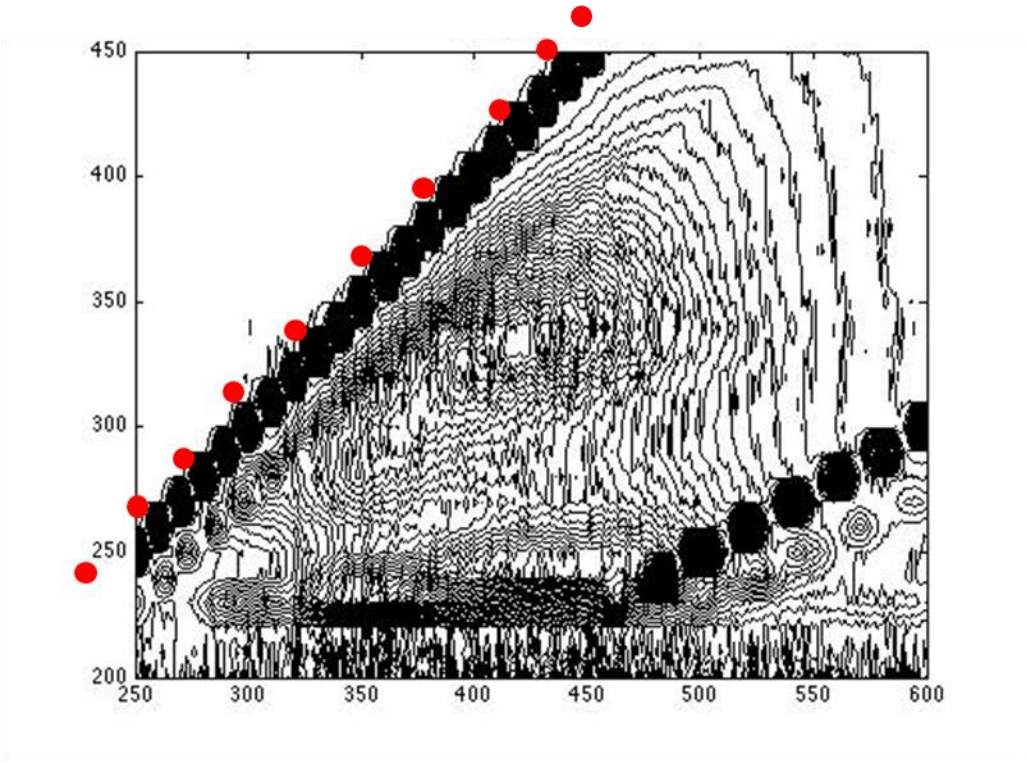
5. Select 10 points on the lower limit with mouse



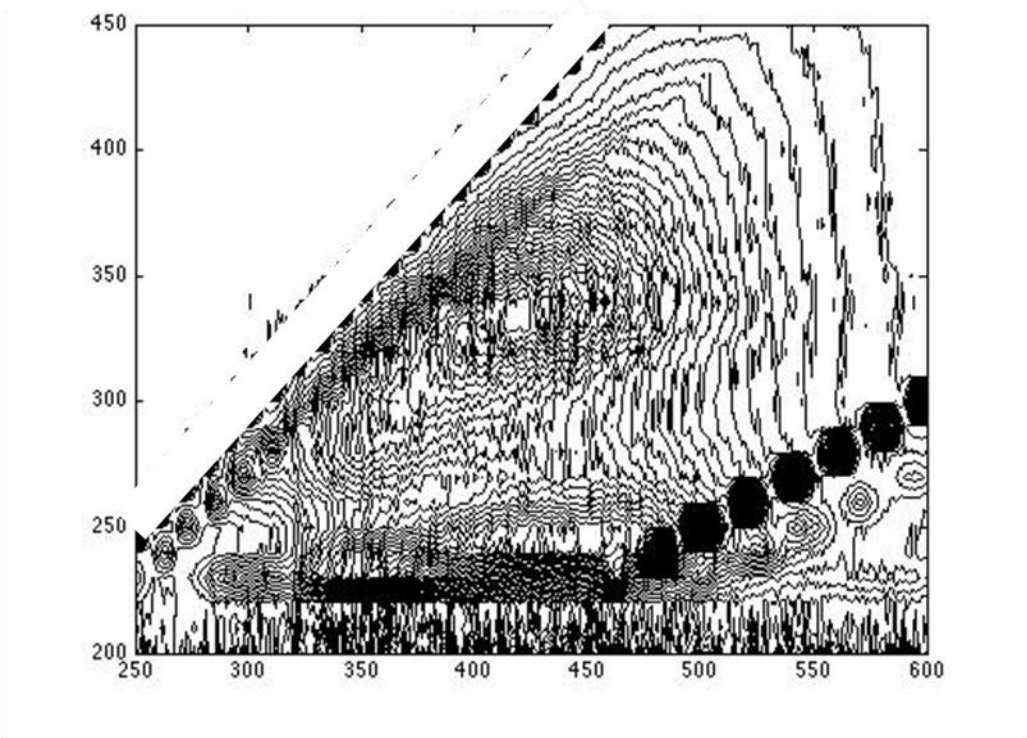
6. The following figure (2) will appear



7. Figure (2) will close and Figure (1) will reappear asking you to select the upper limit using the mouse. Again select 10 points.



8. Figure (2) will reappear, then close and figure (3) will appear.



9. Repeat steps 5 through 7 for the lower scattering.

10. Repeat process for all files. Once all files are complete, the figure of the last line to be fitted to remove the scattering will remain on screen.

11. Continue to Section 4.

Section 3: PRE-PROCESSING DATA (Scatter Removal) WHILE CORRECTING FOR ABSORBANCE

This next section describes how to preprocess the .csv files when correcting for inner filter affects. Absorbance spectra for each sample will need to be obtained (medium speed, 200 – 650nm).

1. Open the abs_correction.m and preprocess_fluor_data_csv_file_abs_corr.m MATLAB files.

2. In the abs_correction.m file, change the file name to match the .csv files being preprocessed.

```
preprocess_fluor_data_csv_file_abs_corr('filename', wavelength, A)
```

3. From the UV-Vis spectrum for the sample, copy and paste the wavelength and absorbance data into the data vector.

```
data=[...
      649.9916992 0.0023519103
      648.9721069 0.002460845
      647.9522705 0.0025235112
      646.9321289 0.0024569922
      646.0574951 0.0026021234
      645.0367432 0.0025036456
      644.0157471 0.0025101285
      642.9943848 0.0023687498
      641.9727783 0.0024299189
      640.9508057 0.002375413
      639.9285889 0.0025907694
      639.052124  0.0028715807
```

4. Press the run m-file (green triangle) play button.

5. Follow steps 4 – 9 from Section 3.

6. Repeat for each of the samples needing correction.

7. Continue to Section 4.

Section 4: PROCESSING DATA

1. Once the Scattering has been removed, open the m-file PARAFAC_process_mesocosm_samples_2009_justaugust.m

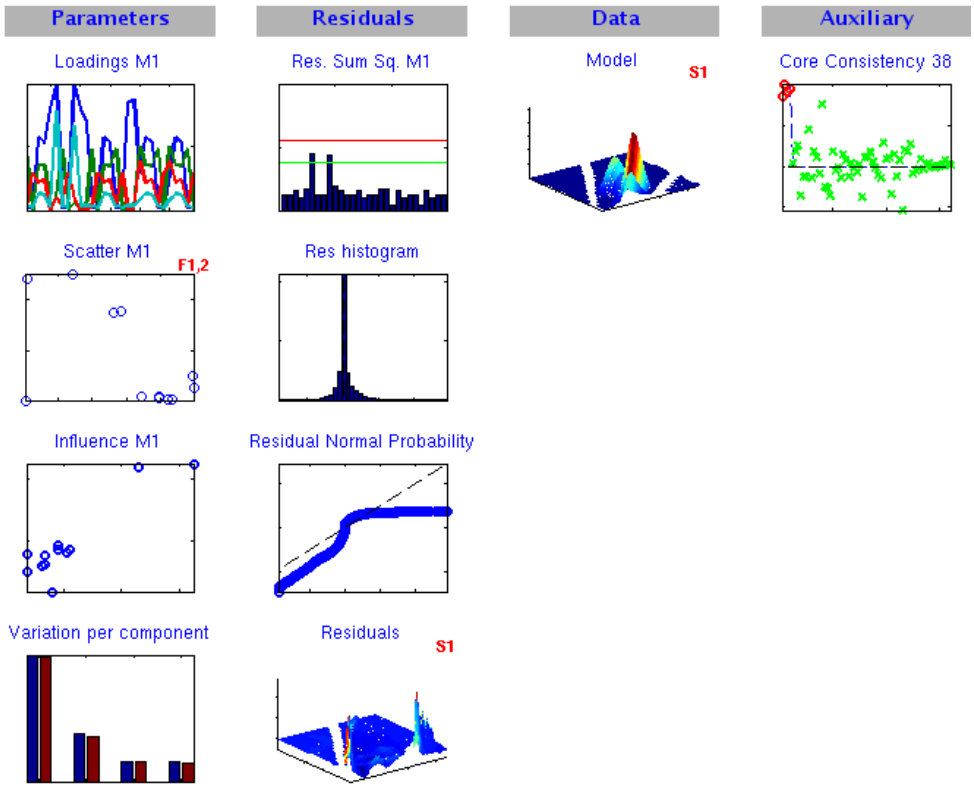
2. Change the file name to match the .csv files being preprocessed.

```
names=strvcat(...
'filename1', ...
'filename2', ...
'filename3');
```

4. If necessary, the number of components used to describe the data can be changed.

5. Press the run m-file (green triangle) play button. At this point a window will pop up which says “Fitting parafac. Please wait”. Below the text in the window is a red bar which monitors the progress of the program. Once the bar is completely red, PARAFAC is almost complete.

6. Once complete, the window will close and a new window will appear. This window is shown below. The percent of data explained by the fitting will be shown in the title header of the window.



7. To obtain a summary of the components for the samples, open the MATLAB file PARAFAC_summary_of_4_components.m.

8. On line 3 of the script, change the load name to match the number of components used to describe the data.

```
clear; clear global

load Hollypracticenumberofcomponents.mat
```

9. If only three components are used to describe the data, comment the final subplot used for component four.

```
%subplot(224); [C,h]=contour(em,EXX,surf4,3,'k'); set(h,'linewidth',2);
%h=xlabel('Emission (nm)');  set(h,'fontsize',12)
%h=ylabel('EXXcitation (nm)');  set(h,'fontsize',12)
%h=title('(d)');  set(h,'fontsize',12)
%set(gca,'linewidth',2)
%set(gca,'linewidth',2)
%set(gca,'fontsize',12)

print components.eps -depsc2

figure(1)

figure(2); clf

subplot(221); plot(conc1,'ko'); xlabel('site'); ylabel('conc');
title('component 1')
subplot(222); plot(conc2,'ko'); xlabel('site'); ylabel('conc');
title('component 2')
subplot(223); plot(conc3,'ko'); xlabel('site'); ylabel('conc');
title('component 3')
%subplot(224); plot(conc4,'ko'); xlabel('site'); ylabel('conc');
%title('component 4')
```

10. Press the run m-file (green triangle) play button.

11. To have a print out of the concentrations found in each sample for each component, type x = [conc1 conc2 conc3…concn] into the command window.

Section 5: SPECTRA FIGURES

1. Open the m-file spectra.m

2. Change the file name to match the .csv files being preprocessed.

```
simplereport ('filename',10,2);
```

3. To change the number of lines used to describe the data, change the first number after the file name

```
simplereport ('filename',10,2);
```

OR, in square brackets, list the values at which lines are needed to describe the data.

```
simplereport ('filename',[25, 50, 75, 100, 125, 150, 175, 200, 225,
250],2);
```

4. The last number in the command line alters the spectra to give you colour or black and white spectra (colour = 2, black and white = 1).

```
simplereport ('filename',10,2);
```

5. Press the run m-file (green triangle) play button.