Introduction to Raman spectroscopy measurement data processing using Igor Pro

This introduction is intended to minimally guide beginners to processing Raman spectroscopy measurement data, which includes loading raw data to PC, performing calibrations, and displaying spectrum, using Igor Pro. Igor Pro is a commercial software from Wavemetrics for data analysis and graphing, primarily used in USIL.

0 Getting familiar with Igor Pro

To begin with, you should get familiar with concept of a wave (a form of data) and user interface in IGOR Pro. Because instructing how to use it is beyond the scope of this introduction, learn it by yourself with Igor Pro manual, which can be downloaded from here (http://www.wavemetrics.net/doc/igorman/IgorMan.pdf). It is recommended to start with some guided tours in the getting started and to read the chapters listed below:

Getting started

I-1 Introduction to Igor Pro
I-2 Guided Tour of Igor Pro:
  Guided Tour 1 – General Tour
  Guided Tour 2 – Data Analysis

Chapters recommended to read

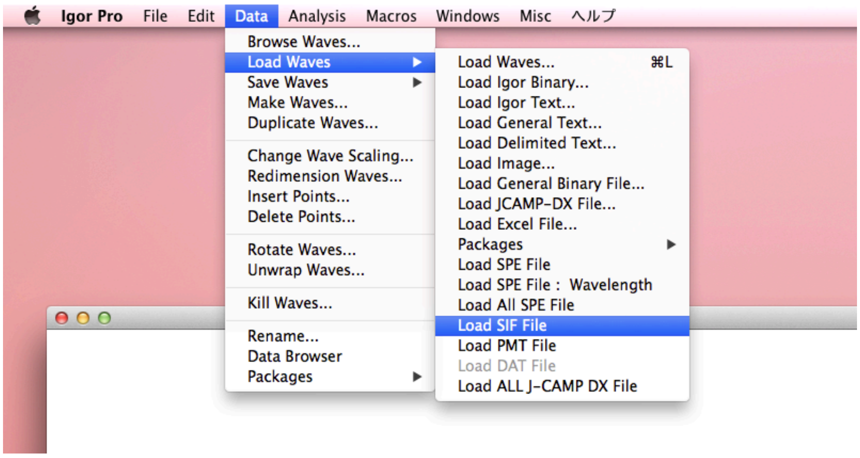
II-5 Waves
II-11 Tables
II-12 Graphs
III-8 Curve Fitting

1 Loading Measured Data in Igor Pro

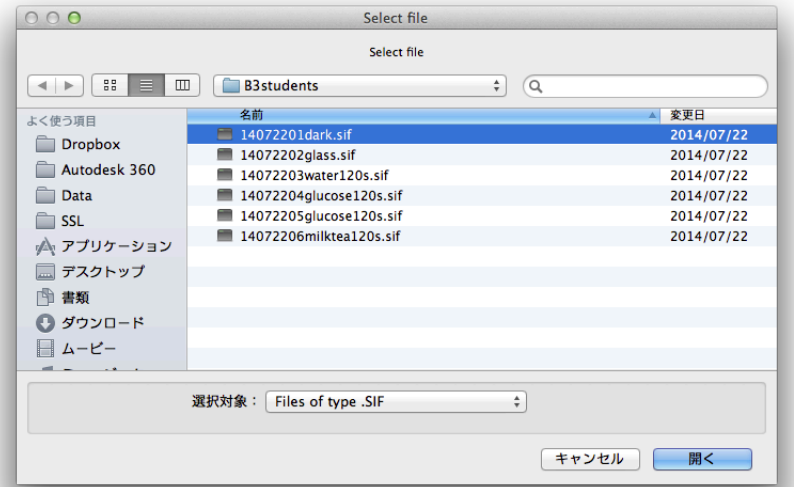
All the spectral data measured with the CCD cameras are saved in SPE file or SIF file depending on which maker of CCD camera they are from. SPE file is of Princeton Instruments, and SIF file of Andor Technologies. To load those data files, two extensions, called procedure in IGOR Pro, that are lab-made file loader, namely FileLoader.ipf and takadaShimadaExtension.ipf, are required. If those have not yet to be installed, place the procedure files in the folder described below and restart IGOR Pro: C:\Users\<user>\My Documents\WaveMetrics\Igor Pro 6 User Files\Igor Procedures\

Loading from SIF file

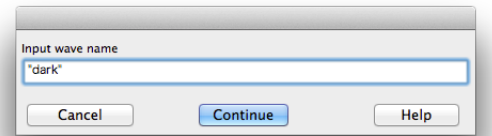
i. In the menu, select [Data] -> [Load Waves] -> [Load SIF file].



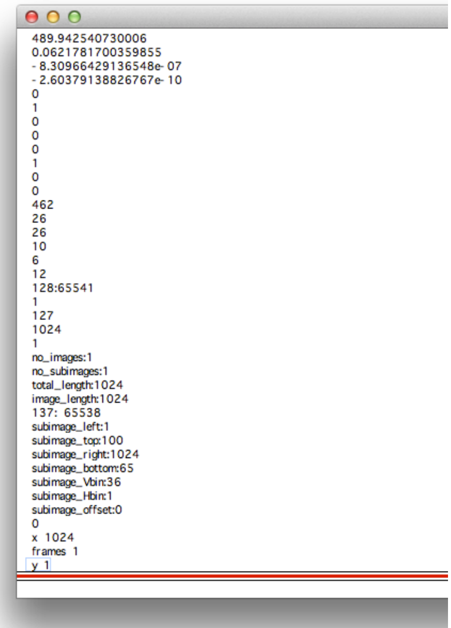
ii. Select a file to load in the window just appeared and click [Open] button.



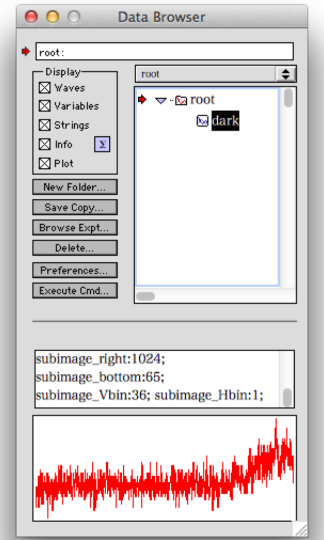
iii. Fill in the name of wave about to load and click [Continue] button.



iv. While loading, information about data is displayed in the history area. If no error is reported, the data is properly loaded as a named wave. You may find the loaded wave in the data browser.



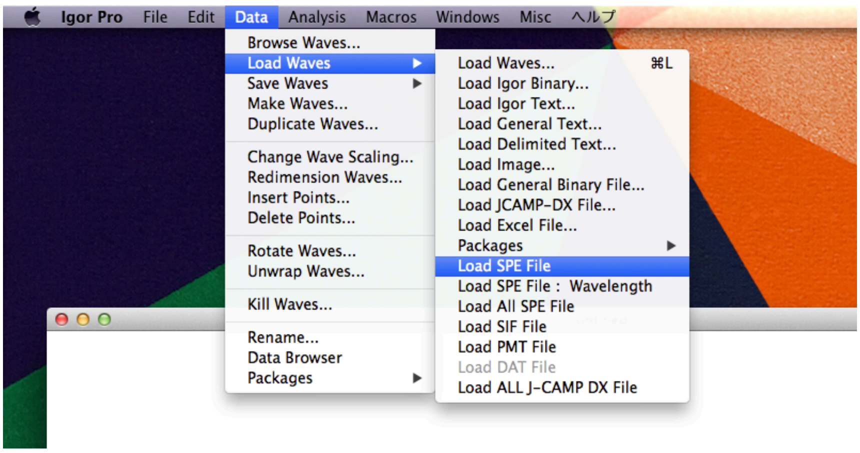
History area



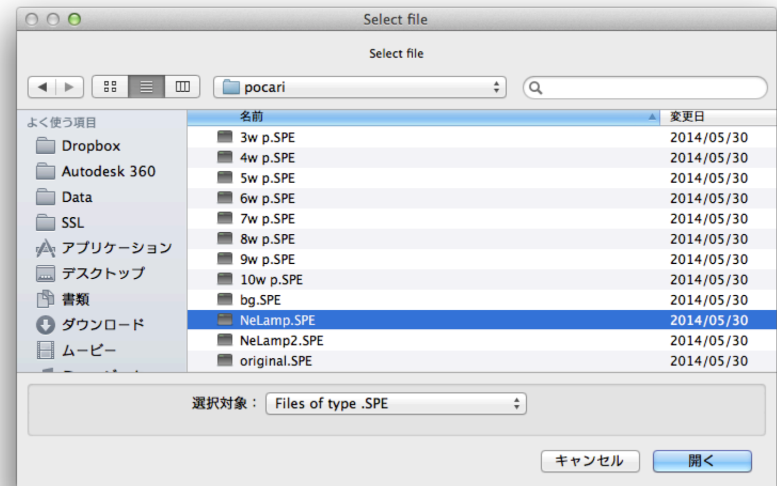
Data browser

Loading from a single SPE file

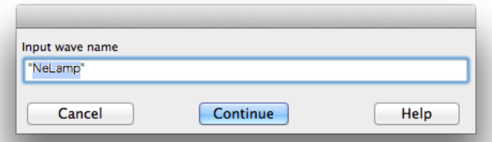
i. In the menu, select [Data] -> [Load Waves] -> [Load SPE file].



ii. Select a file to load in the window just appeared and click [Open] button.

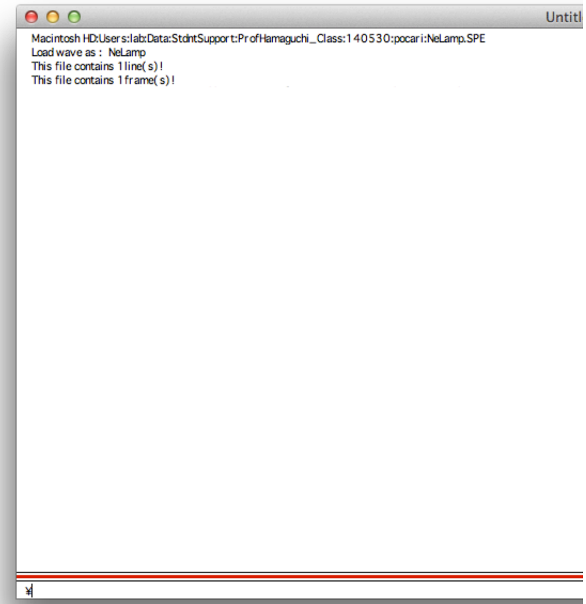


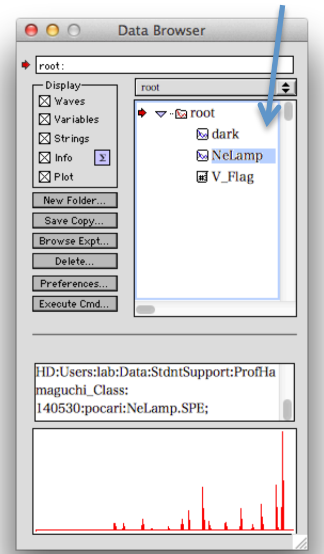
iii. Fill in the name of wave about to load and click [Continue] button.



iv. While loading, information about data is displayed in the history area. If no error is reported, the data is properly loaded as a named wave. You may find the loaded wave in the data browser.

Loaded data will be found in data browser





Information about data displayed in history area

2 Wavelength (Raman Shift) Calibration using atomic emission lines (Ne, Hg lamps)

Raw spectra data you measured have only CCD pixel index as the horizontal axis. (Even if it has wavelength in nanometer or Raman shift in wavenumber as horizontal axis, it may not be reliable so that you would need to determine the accurate horizontal axis by yourself.) To make a Raman spectrum, you need to convert the horizontal axis from pixel index to Raman shift by calibration. This is usually done in two steps: (i) Wavelength calibration, which connects pixel indices with wavelengths, and (ii) conversion from wavelength to Raman shift. This is because wavelength is supposed to be proportional to pixel index so that the calibration line of wavelength vs. pixel index will be close to straight line and easy to fit with polynomial curve. For wavelength calibration, atomic emission lines of, for example, Ne or Hg, wavelengths of which have been determined accurately and are available in the literatures, are usually used as standards. Once Raman shift axis is calibrated, you do not need to perform it unless the center wavelength or groove density of a grating in the spectrograph change no matter whether it is intentionally or not.

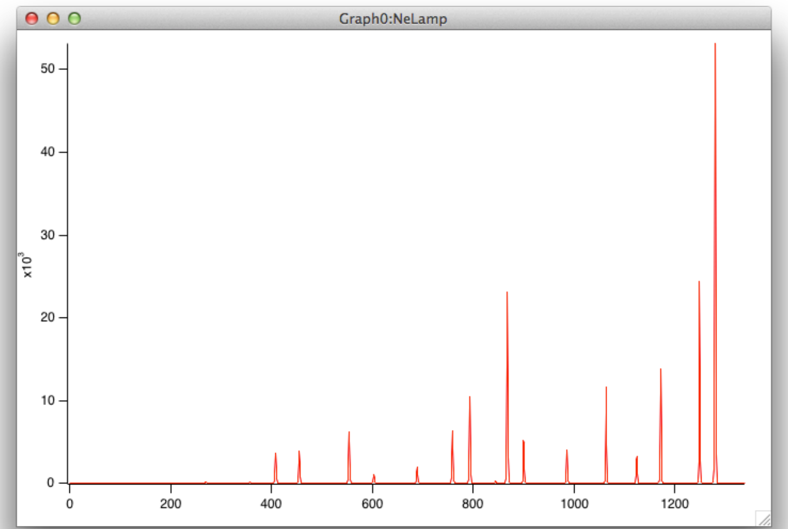
If your Raman-shift region of interest is limited in so-called "finger print" region, you can use indene as a direct Raman shift standard. This method is particularly useful when the wavelength of your excitation source has not been accurately determined or is variable depending on the ambient condition. Raman shift calibration using indene is to be described in the following chapter.

2-1

Display atomic emission spectrum of Ne or Hg lamps, etc.

Command:

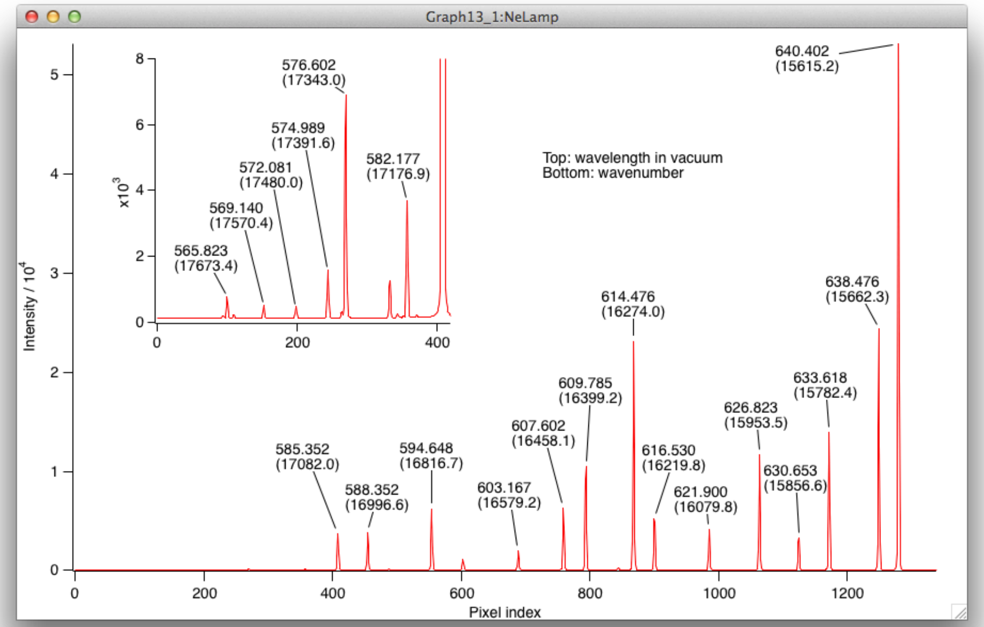
```
Display NeLamp
```



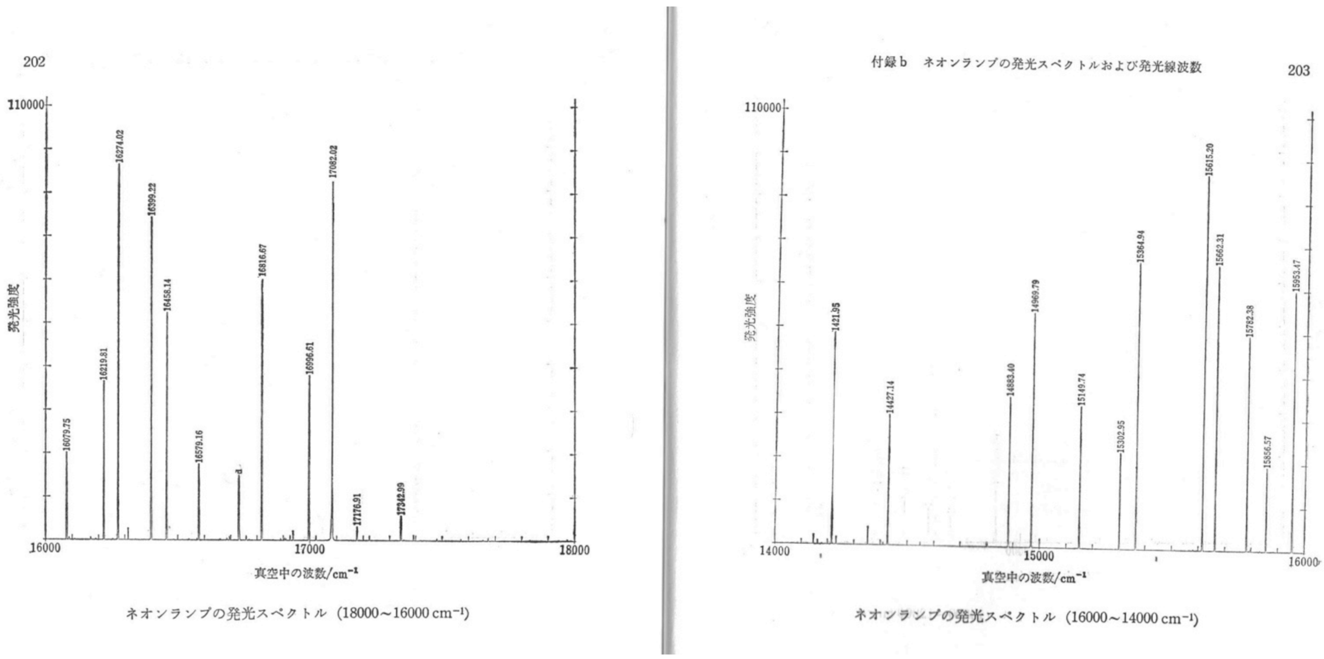
Displayed spectrum.

2-2

Compare the measured spectrum with that in a reference and assign each observed line to corresponding transition whose accurate wavelength is known. In the example, because lines below the 400th pixel are very weak, another spectrum with longer exposure time is measured (inset). Note that the resulting calibration line may be reliable only in the region covered by lines that are taken into account. Pick up as many lines from as wide area as possible for a better calibration.



By comparing with spectrum in the literature, assign each emission line and find accurate wavelength for it.



空気中の波長/nm	真空中の波長/nm	真空中の波数/cm⁻¹
540.05616	540.20628	18511.447
541.26490	541.41537	18470.107
541.85584	542.00645	18449.965
543.36513	543.51619	18398.716
544.85091	545.00234	18348.545
549.44158	549.59424	18195.242
553.36788	553.52161	18066.142
553.86510	554.01895	18049.924
556.27662	556.43110	17971.677
565.25664	565.41349	17686.171
565.66588	565.82284	17673.376
566.25489	566.41201	17654.993
568.98163	569.13951	17570.384
571.92248	572.08116	17480.037
574.82985	574.98926	17391.629
576.05885	576.21861	17354.525
576.44188	576.60175	17342.993
580.44496	580.60591	17223.386
581.14066	581.30182	17202.767
582.01558	582.17697	17176.908
585.24878	585.41103	17082.015
586.84183	587.00447	17035.645
587.28275	587.44556	17022.854
588.18950	588.35250	16996.614
590.24623	590.40980	16937.388
590.27835	590.44192	16936.467
590.64294	590.80660	16926.013
591.36327	591.52712	16905.396
591.89068	592.05469	16890.332
594.48342	594.64815	16816.667
596.16228	596.32741	16769.311
596.54710	596.71239	16758.492
597.46273	597.62825	16732.810
597.55340	597.71892	16730.272
598.79074	598.95663	16695.700
599.16532	599.33127	16685.263
600.09275	600.25899	16659.476
602.99971	603.16670	16579.165
604.61348	604.78090	16534.914
606.45359	606.62148	16484.744
607.43377	607.60198	16458.143
609.61630	609.78503	16399.222
612.84498	613.01462	16312.825
614.30623	614.47624	16274.022
616.35939	616.52995	16219.812
617.48829	617.65918	16190.159
618.21460	618.38565	16171.139
618.90649	619.07776	16153.060
619.30663	619.47800	16142.623
620.57775	620.74944	16109.559
621.38758	621.55950	16088.564
621.72813	621.90016	16079.752

λ in air 空気中の波長/nm	λ in vac. 真空中の波長/nm	ν̃ in vac. 真空中の波数/cm⁻¹
624.67294	624.84575	16003.950
626.64950	626.82284	15953.471
629.37447	629.54853	15884.399
630.47892	630.65326	15856.574
631.36921	631.54382	15834.214
632.81646	632.99145	15798.002
633.44279	633.61795	15782.381
635.18618	635.36178	15739.064
638.29914	638.47559	15662.306
640.22460	640.40157	15615.202
642.17108	642.34856	15567.872
644.47118	644.64930	15512.310
650.65279	650.83255	15364.935
653.28824	653.46874	15302.951
659.89529	660.07757	15149.735
665.20925	665.39296	15028.713
666.68967	666.87376	14995.342
667.82764	668.01202	14969.791
671.70428	671.88971	14883.395
692.94672	693.13787	14427.144
702.40500	702.59870	14232.876
703.24128	703.43523	14215.950
705.12937	705.32380	14177.885
705.91079	706.10545	14162.191
707.39380	707.58885	14132.501
724.51665	724.71629	13798.503
743.88981	744.09467	13439.150
747.24383	747.44961	13378.828
748.88712	749.09334	13349.471
753.57739	753.78489	13266.384
754.40439	754.61211	13251.841
772.46281	772.67538	12942.046
783.90550	784.12118	12753.131
792.71172	792.92975	12611.458
793.69946	793.91778	12595.763
794.31805	794.53651	12585.954
808.24576	808.46800	12369.073
811.85495	812.07815	12314.086
812.89077	813.11430	12298.394
813.64061	813.86433	12287.060
824.86812	825.09484	12119.819
825.93795	826.16497	12104.120
826.60788	826.83508	12094.310
826.71166	826.93888	12092.792
830.03248	830.26059	12044.411
836.57464	836.80456	11950.222
837.76062	837.99085	11933.305
841.71614	841.94741	11877.226
841.84265	842.07400	11875.441
846.33569	846.56824	11812.397
848.44424	848.67736	11783.041
849.53591	849.76932	11767.900

Spectra and tables in a literature. These are for 532-nm-excitation.

If you used different excitation wavelength, you need other region of spectra and tables.

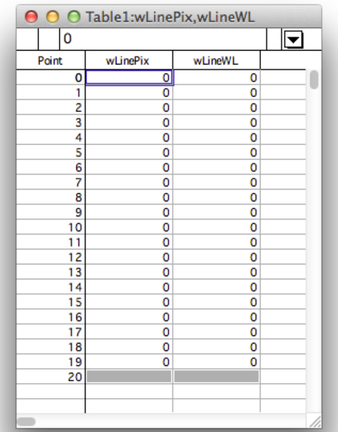
(*Raman spectroscopy* by H. Hamaguchi (written in Japanese))

2-3

Make two 1D waves, named as w*LinePix* and *wLineWL*, whose numbers of elements as large as the number of lines to take into account, and display them on a table. In this example, above-mentioned 20 lines are taken into account.

Command:

```
Make/N=20 wLinePix, wLineWL
Edit wLinePix, wLineWL
```

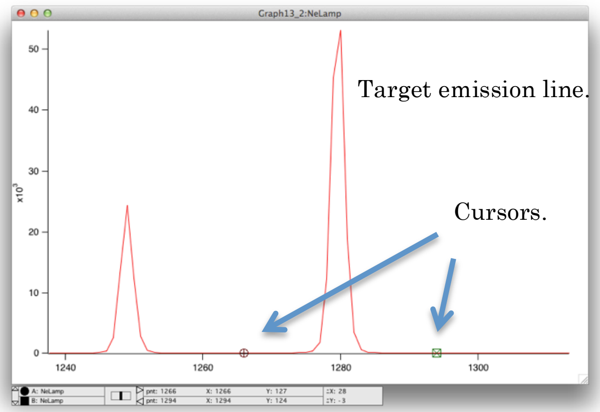


Point	wLinePix	wLineWL
0	0	0
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	0	0
16	0	0
17	0	0
18	0	0
19	0	0
20		

New table with wLinePix and wLineWL created.

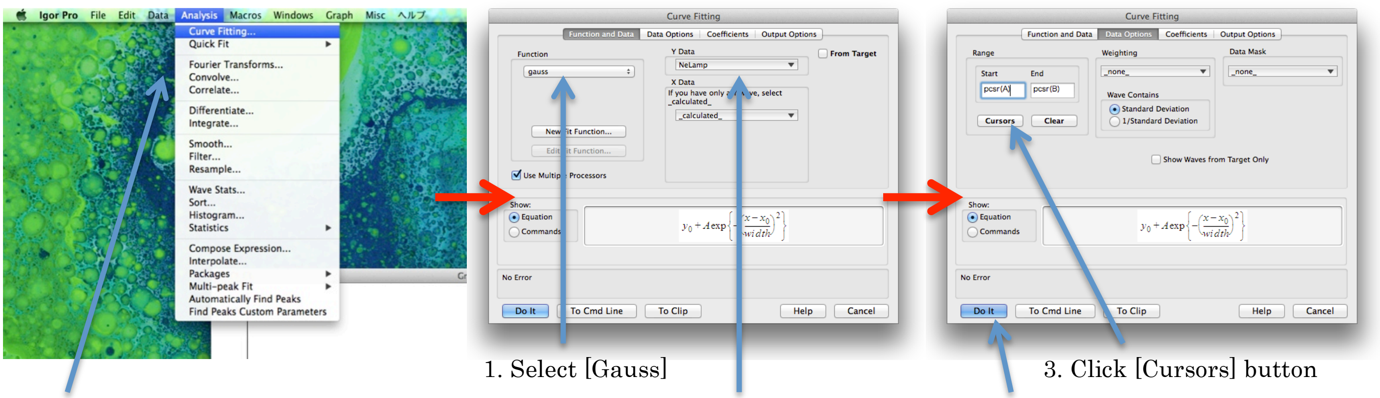
2-4

Zoom in on the first line and place cursors on the both left and right sides of line on the baseline and perform fitting analysis with Gaussian function within the region between the cursors.



First emission line to be analyzed is zoomed in.

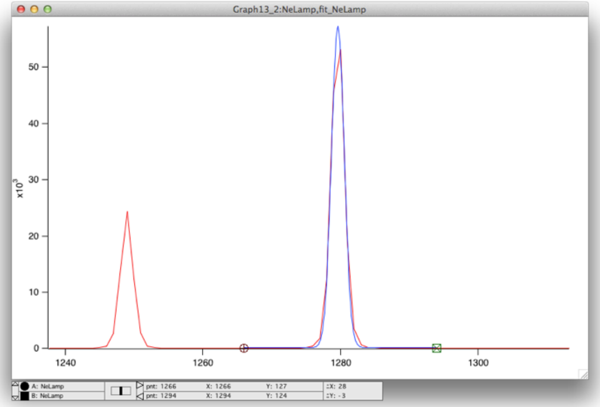
Curve fit using GUI:

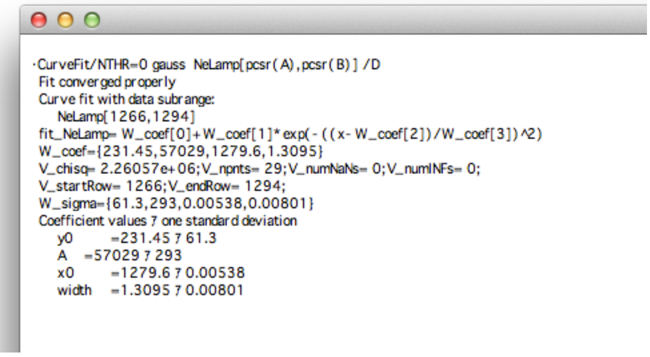


Or

Curve fit using command:

```
CurveFit/NTHR=0 gauss  NeLamp[pcsr(A),pcsr(B)] /D
```

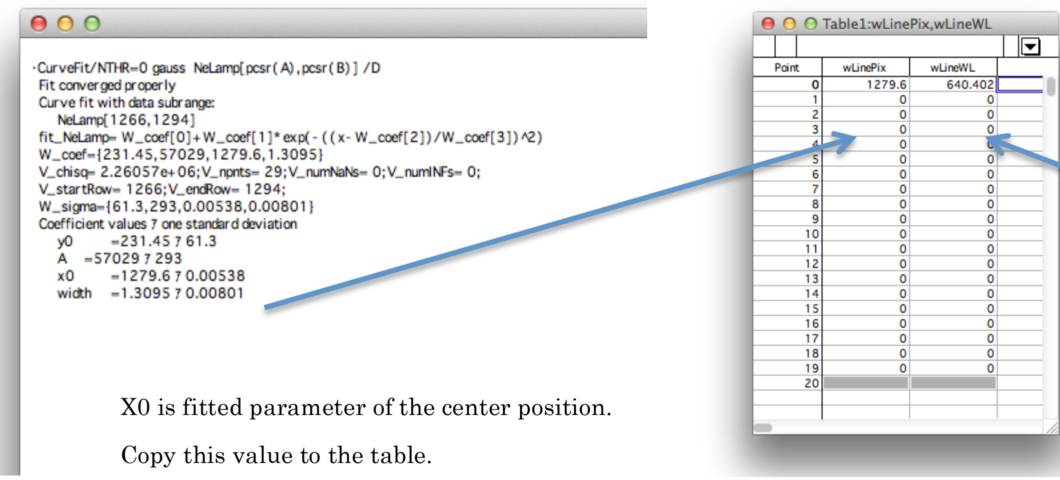




Fitting result is displayed in graph and history area

2-5

Input fitted peak position into the first row of the wave *wLinePix*. Also, input the wavelength of the present line into the corresponding row of the wave *wLineWL*. Note that the wavelength considered here must be that in vacuum.



Corresponding wavelength is taken from the literature

2-6

Repeat 2-4 and 2-5 for all the other lines to take into account, and then, plot *wLineWL* vs. *wLinePix*. If you find the plot is not smooth, your line assignment may be wrong.

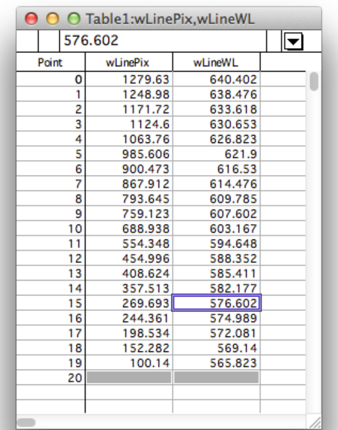
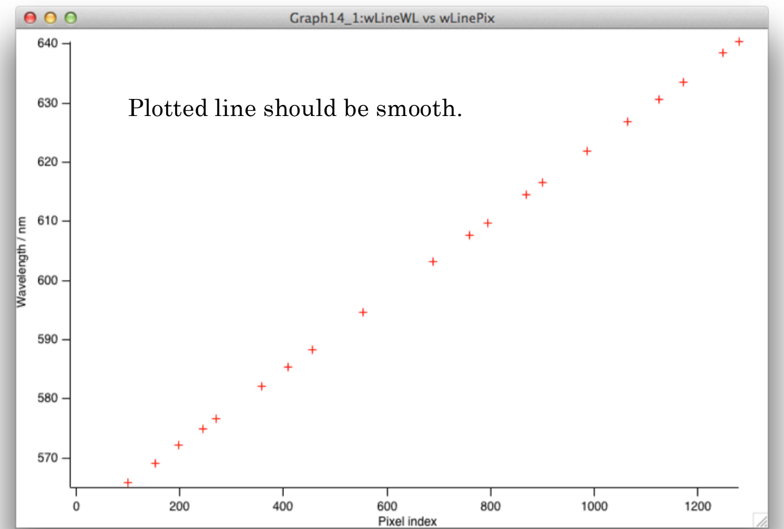


Table1:wLinePix,wLineWL

576.602

Point	wLinePix	wLineWL
0	1279.63	640.402
1	1248.98	638.476
2	1171.72	633.618
3	1124.6	630.653
4	1063.76	626.823
5	985.606	621.9
6	900.473	616.53
7	867.912	614.476
8	793.645	609.785
9	759.123	607.602
10	688.938	603.167
11	554.348	594.648
12	454.996	588.352
13	408.624	585.411
14	357.513	582.177
15	269.693	576.602
16	244.361	574.989
17	198.534	572.081
18	152.282	569.14
19	100.14	565.823
20		

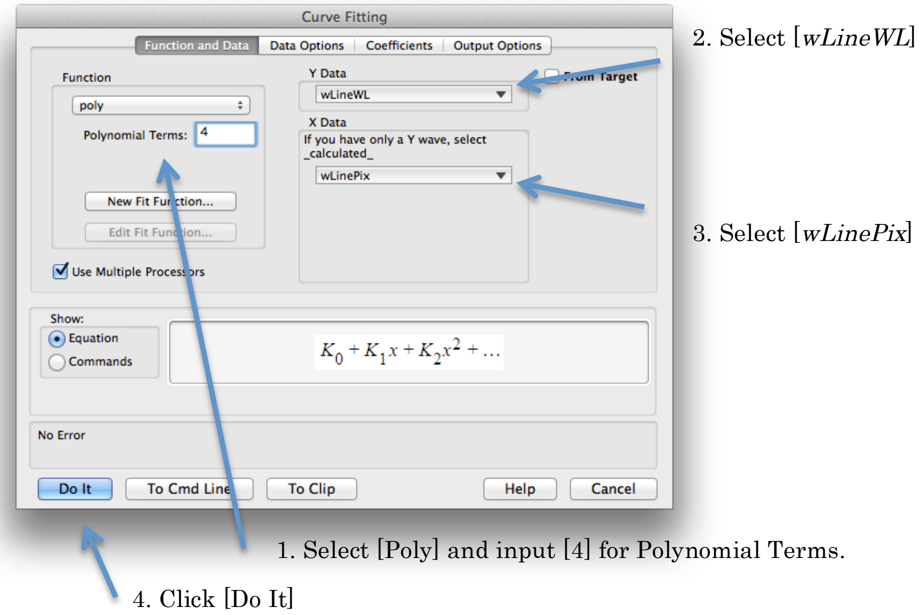
Completed table.



2-7

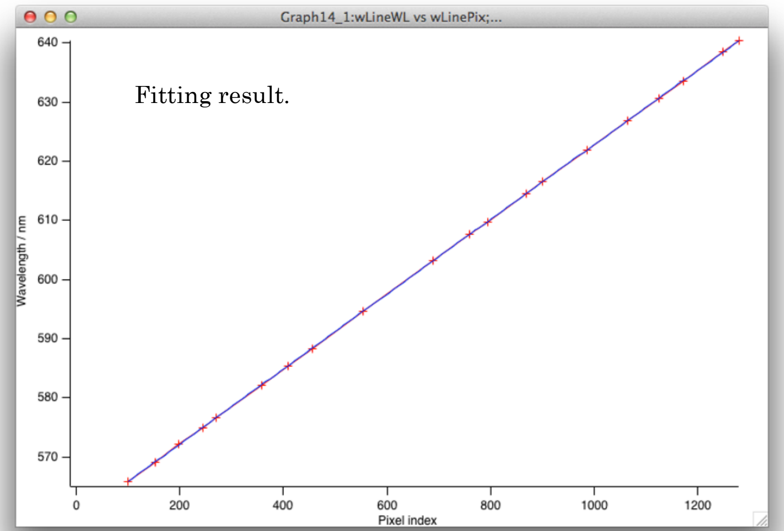
Fit the plot with 3rd-order polynomial. The fitting result gives the wavelength calibration curve, which connects CCD pixel indices and actual wavelengths in vacuum.

<u>Curve fit using GUI</u>:



<u>Curve fit using command</u>:

```
CurveFit/NTHR=0 poly 4,  wLineWL /X=wLinePix /D
```



2-8

Duplicate one of measured spectrum data waves as *wWavelength*, and input wavelengths at each pixel indices using fitted parameters just obtained above. Each fitted parameter is stored in a corresponding row of automatically generated wave, w_coef (0^{th}-term -> w_coef[0], 1^{st}-term -> w_coef[1], and so on).

<u>Command</u>:

```
Duplicate NeLamp, wWavelength
wWavelength = w_coef[0] + w_coef[1]*p + w_coef[2]*p^2 + w_coef[3]*p^3
```

2-9

Duplicate the wave *wWavelength* as *wRamanShift*, and input Raman shifts calculated from the Raman excitation wavelength (in vacuum) and wavelengths in *wWavelength* into *wRamanShift*. If the excitation wavelength is not definite, fit the Rayleigh line with Gauss function and determine it. In this example, suppose excitation wavelength was 532.22 nm.

<u>Command</u>:

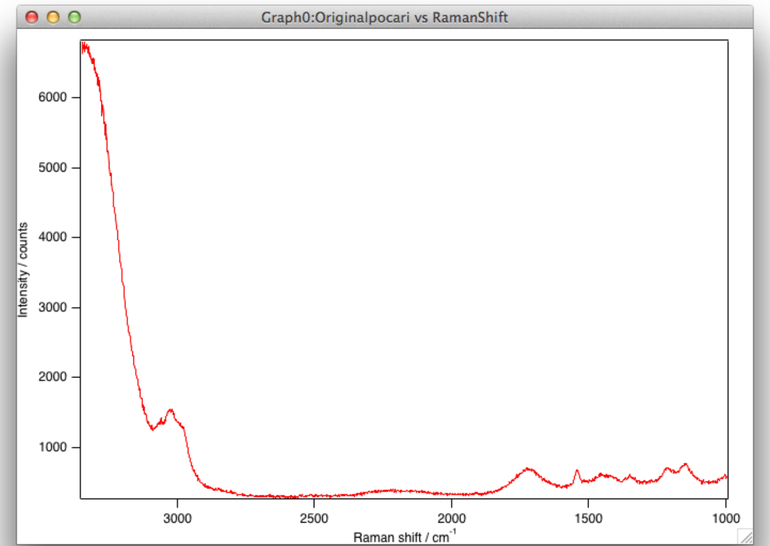
```
Duplicate wWavelength, wRamanShift
wRamanShift = 1E7 * (1/(Excitation wavelength) - 1/wWavelength)
```

2-10

Plot Raman spectra data, e.g. *wRamanSp* versus *wRamanShift*. Because Raman spectrum is conventionally plotted as wavenumber increases in a direction from right to left, switch the horizontal axis direction accordingly. Make the axes labels; Raman shift / cm^{-1} for horizontal axis, Intensity / counts for vertical.

<u>Command</u>:

```
Display wRamanSp, wRamanShift
SetAxis/A/R bottom
Label left "Intensity / counts"
Label bottom "Raman shift / cm¥¥S-1"
```



3 Raman Shift Calibration using Indene

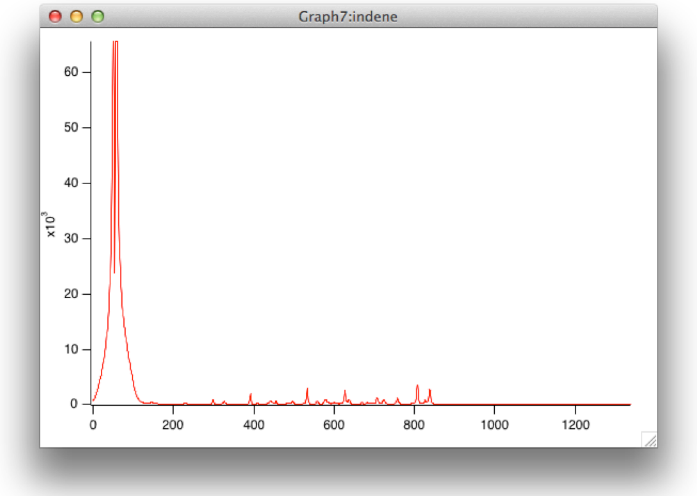
As mentioned above, it is useful to use indene as a standard for Raman shift calibration especially when your Raman shift region of interest is limited within the "fingerprint region" and the excitation wavelength has not been determined accurately or is variable with ambient condition. In contrast to the calibration using atomic emission from Ne and Hg lamps for example, that using Raman bands of indene directly assigns Raman shift value to each pixel index; not wavelengths but Raman shifts of bands are plotted against pixel index, so that no further conversion of calibration curve is required.

3-1

Display Raman spectrum of indene.

Command:

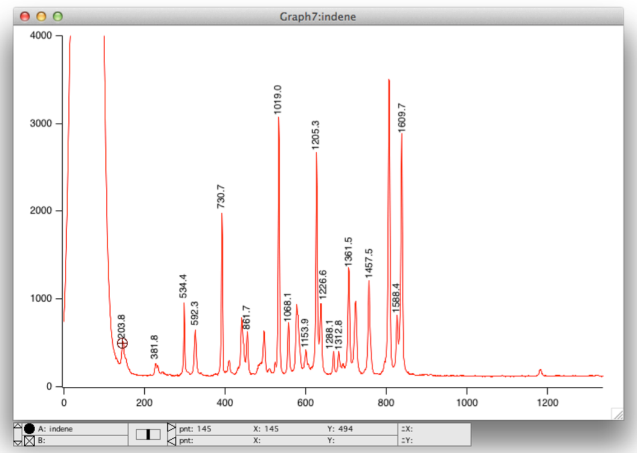
```
Display indene
```



Displayed spectrum.

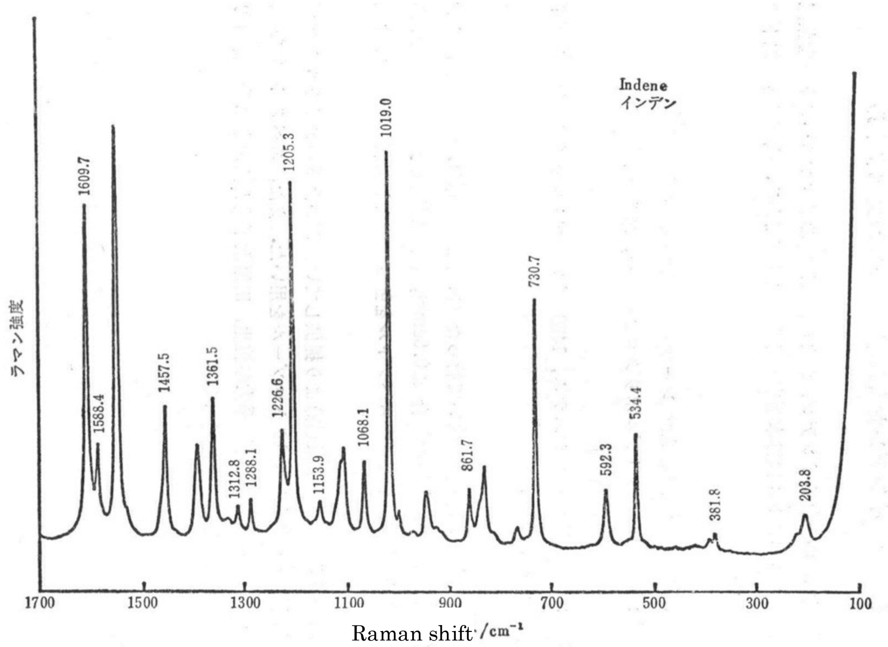
3-2

Compare the measured spectrum with that in a reference and assign each observed band whose accurate Raman shift is known.



By comparing with spectrum in the reference,

assign each band and find accurate Raman shift for it.



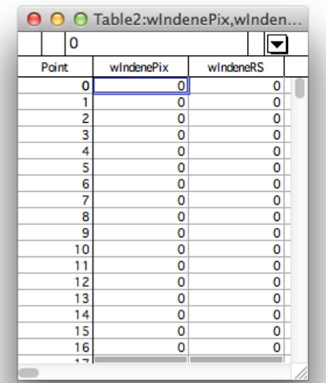
Reference

3-3

Make two 1D waves, named as w*IndenePix* and *wIndeneRS*, whose numbers of elements as large as the number of bands to take into account, and display them on a table. In this example, 17 bands are taken into account.

<u>Command</u>:

```
Make/N=17 wIndenePix, wIndeneRS
Edit wIndenePix, wIndeneRS
```

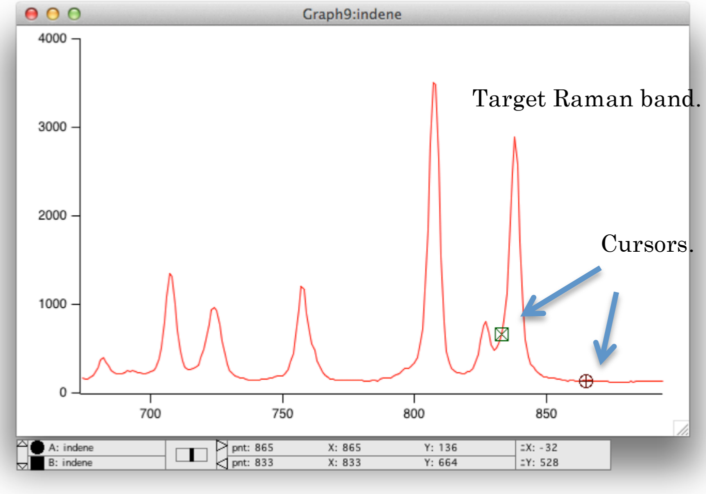


Point	wIndenePix	wIndeneRS
0	0	0
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	0	0
16	0	0

New Table with wIndenePix and wIndeneRS created.

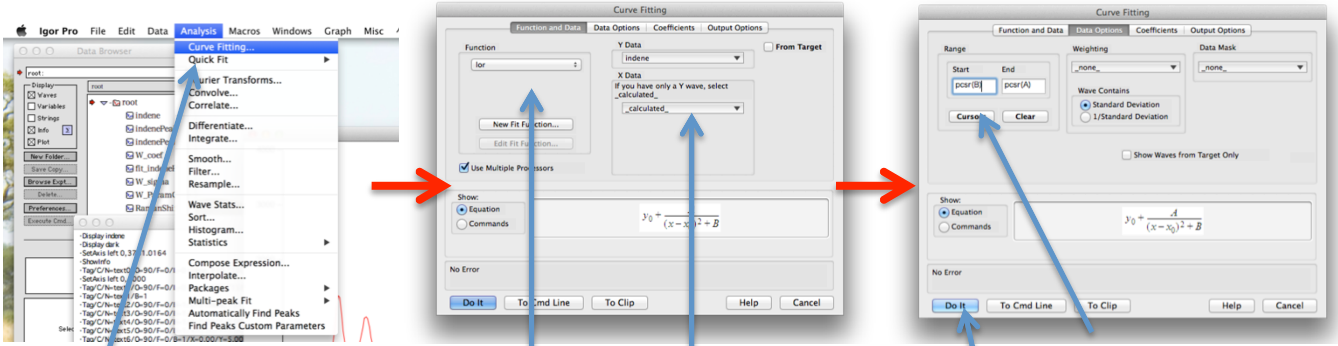
3-4

Zoom in on the first band and place cursors on the both left and right sides of the band on the baseline and perform fitting analysis with Lorentzian function within the region between the cursors.



First bands to be analyzed are zoomed in.

Curve fit using GUI:



Select [Analysis] -> [Curve Fitting…]

1. Select [Lorentz]

2. Select wave to be analyzed

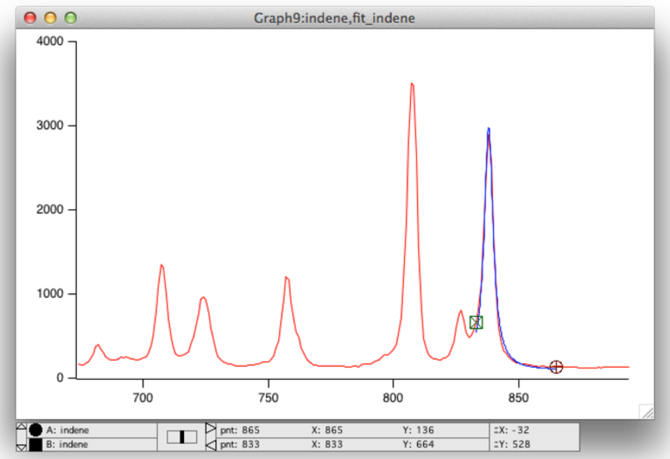
3. Click [Cursors] button

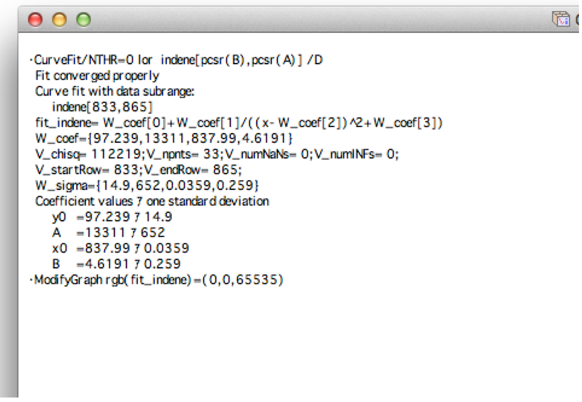
4. Click [Do It]

Or

Curve fit using command:

```
CurveFit/NTHR=0 lor  indene[pcsr(A),pcsr(B)] /D
```



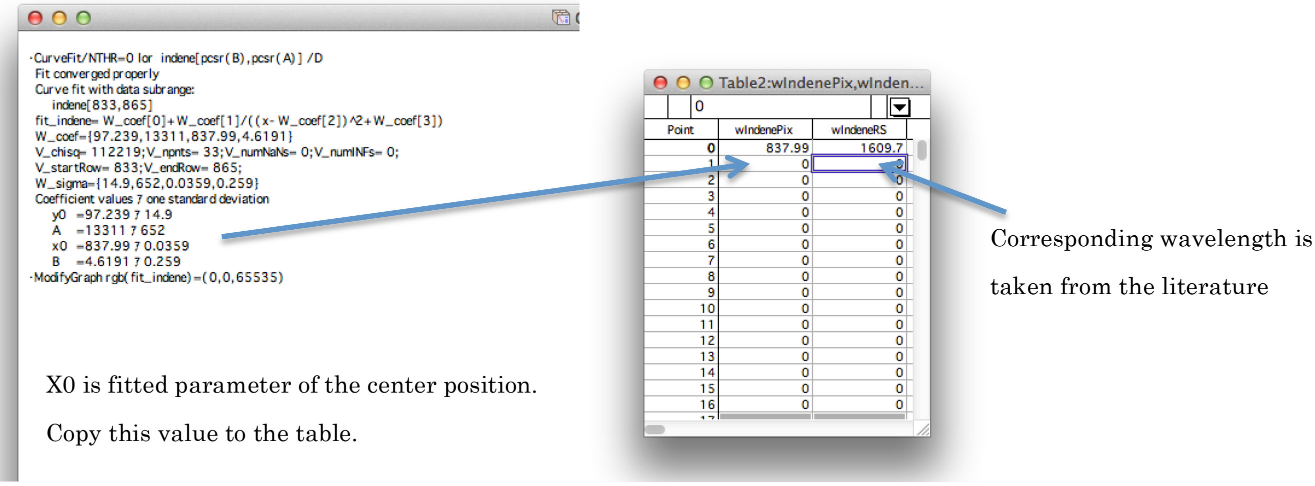


```
•CurveFit/NTHR=0 lor  indene[pcsr(B),pcsr(A)] /D
 Fit converged properly
 Curve fit with data subrange:
    indene[833,865]
 fit_indene= W_coef[0]+W_coef[1]/((x-W_coef[2])^2+W_coef[3])
 W_coef={97.239,13311,837.99,4.6191}
 V_chisq= 112219;V_npnts= 33;V_numNaNs= 0;V_numINFs= 0;
 V_startRow= 833;V_endRow= 865;
 W_sigma={14.9,652,0.0359,0.259}
 Coefficient values ? one standard deviation
    y0   =97.239 ? 14.9
    A    =13311 ? 652
    x0   =837.99 ? 0.0359
    B    =4.6191 ? 0.259
•ModifyGraph rgb(fit_indene)=(0,0,65535)
```

Fitting result is displayed in graph and history area

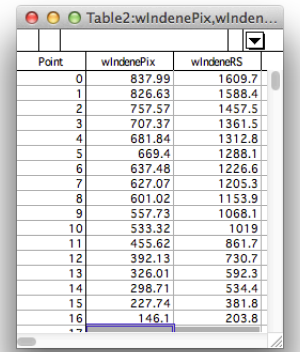
3-5

Input fitted peak position into the first row of the wave *wIndenePix*. Also, input the Raman shift of the present band into the corresponding row of the wave *wIndeneRS*.



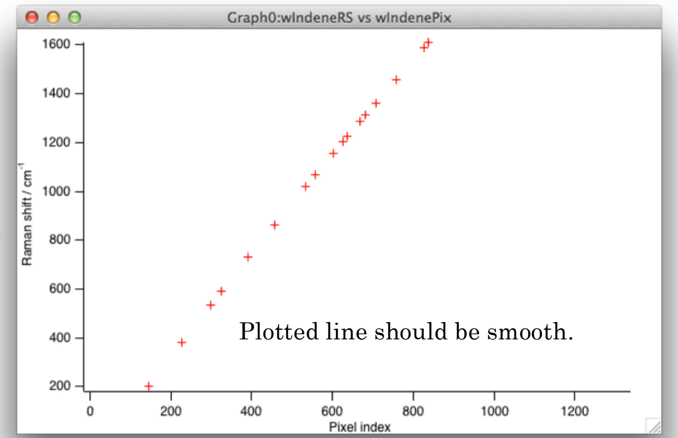
3-6

Repeat 3-4 and 3-5 for all the other bands to take into account, and then, plot *wIndeneRS* vs. *wInenePix*.



Point	wIndenePix	wIndeneRS
0	837.99	1609.7
1	826.63	1588.4
2	757.57	1457.5
3	707.37	1361.5
4	681.84	1312.8
5	669.4	1288.1
6	637.48	1226.6
7	627.07	1205.3
8	601.02	1153.9
9	557.73	1068.1
10	533.32	1019
11	455.62	861.7
12	392.13	730.7
13	326.01	592.3
14	298.71	534.4
15	227.74	381.8
16	146.1	203.8

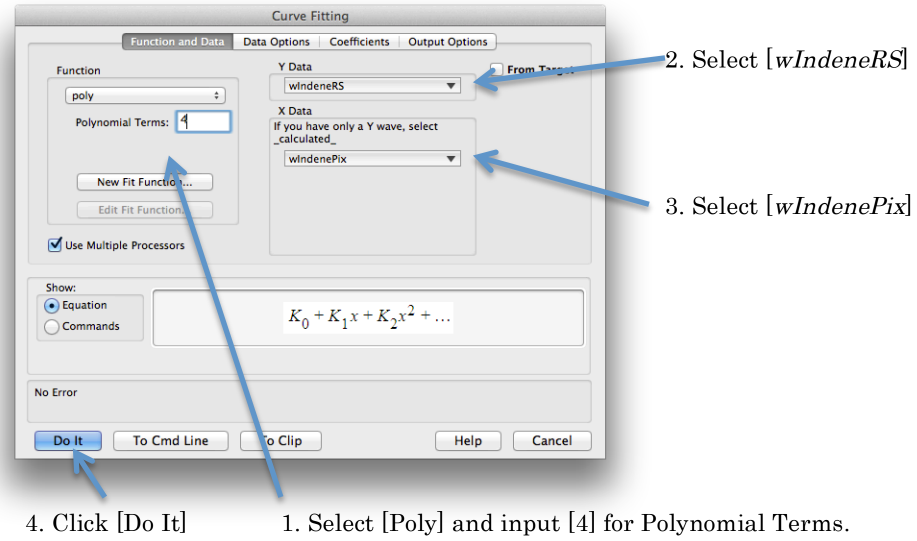
Completed table.



3-7

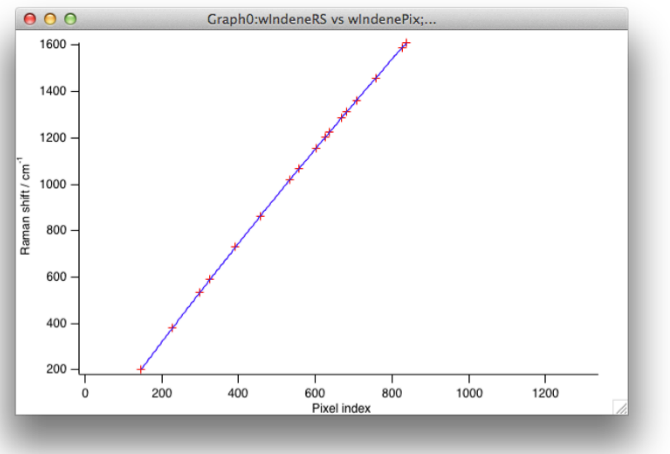
Fit the plot with 3rd-order polynomial. The fitting result gives the Raman shift calibration curve, which directly connects CCD pixel indices and actual Raman shifts.

<u>Curve fit using GUI</u>:



Curve fit using command:

```
CurveFit/NTHR=0 poly 4,  wLineWL /X=wLinePix /D
```



3-8

Duplicate one of measured spectrum data waves as *wRamanShift*, and input Raman-shift values at each pixel indices using fitted parameters just obtained above. Each fitted parameter is stored in a corresponding row of automatically generated wave, w_coef (0^{th}-term -> w_coef[0], 1^{st}-term -> w_coef[1], and so on).

Command:

```
Duplicate indene, wRamanShift
wWavelength = w_coef[0] + w_coef[1]*p + w_coef[2]*p^2 + w_coef[3]*p^3
```

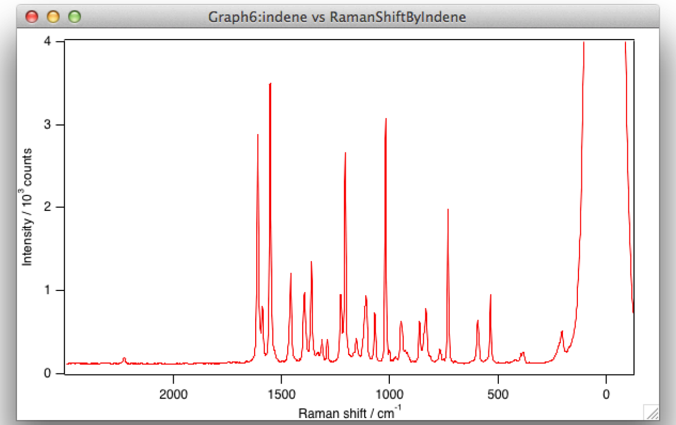
3-9

Plot Raman spectra data, e.g. *wRamanSp* versus *wRamanShift*. Because Raman spectrum is conventionally plotted as wavenumber increases in a direction from right to left, switch the horizontal axis direction accordingly. Make the axes labels; Raman shift / cm^{-1} for horizontal axis, Intensity / counts for vertical.

Command:

```
Display wRamanSp, wRamanShift
SetAxis/A/R bottom
Label left "Intensity / counts"
```

Label bottom "Raman shift / cm¥¥S-1"



4 Intensity Calibration

Relative intensity of a raw Raman spectrum along Raman-shift axis is not reliable as it is because every apparatus has specific non-flat sensitivity over spectral range, which mainly comes from sensitivity of the CCD camera, diffraction efficiency of the grating and transmittance of a long-pass edge filter dependent on wavelength. To compensate this variation in sensitivity, measured Raman spectra must be divided by a sensitivity curve. A normalized white-lamp spectrum measured with the same apparatus and supposed to be flat over the spectral range is usually used as a sensitivity curve. This treatment is called intensity calibration.

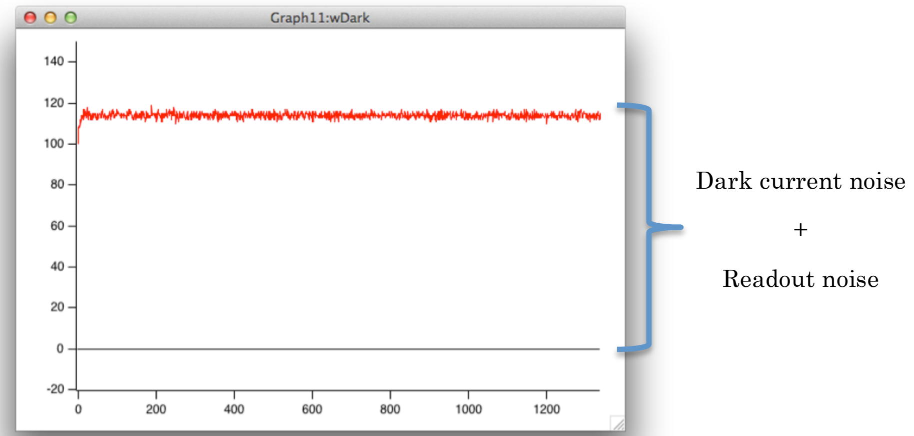
Before intensity calibration is performed, a part of intensity which comes from non-photon origins must be eliminated from an output of the CCD camera. Main contribution of this non-photon intensity is from readout noise and dark current noise. The readout noise is generated while a CCD camera is digitizing its charges, while the dark current noise is generated all the time and accumulated during an exposure and hence develops with time. To eliminate contribution from those noises, a dark spectrum, which is measured literally in the dark without excitation light and with the same exposure time as Raman spectra are measured with, must be subtracted from every raw Raman spectrum. Subsequently, the spectrum is divided by the sensitivity curve for intensity calibration.

4 - 1

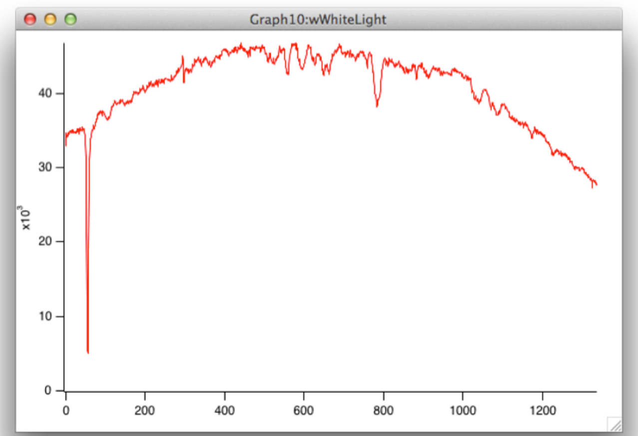
Display the dark and white light spectra.

<u>Command</u>:

```
Display wDark
Display wWhiteLight
```



Dark spectrum



White light spectrum

4-2

Subtract the dark spectrum form the white light spectrum. The dark spectrum must be measured with the same exposure time as the white light spectrum is measured with.

Command:

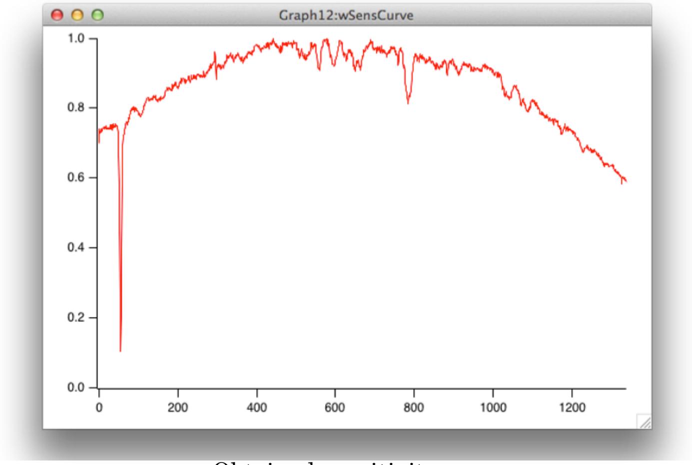
```
wWhiteLight -= wDark
```

4-3

Duplicate the wave *wWhiteLight* as *wSensCurve*. Normalize *wSensCurve* with an arbitrary value. The maximum or average value of the white light spectrum may be chosen for the normalization. Here, the sensitivity curve is normalized with the maximum value.

Command:

```
Duplicate wWhiteLight, wSensCurve
wSensCurve /= WaveMax(wWhiteLight)
```



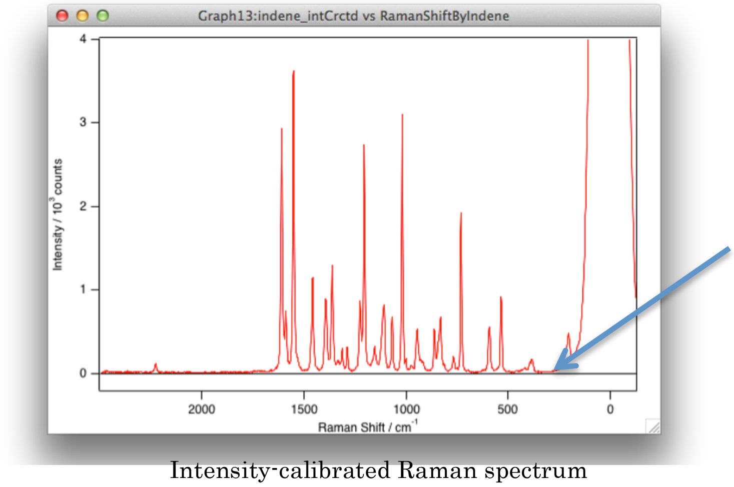
Obtained sensitivity curve.

4-4

Correct the intensity of a Raman spectrum by subtracting dark spectrum followed by dividing by sensitivity curve. The dark spectrum must be measured with the same exposure time as the Raman spectrum is measured with. Then, plot it.

Command:

```
wRamanSp -= wDark
wRamanSp /= wSensCurve
Display wRamanSp vs wRamanShift
SetAxis/A/R bottom
Label left "Intensity / counts"
Label bottom "Raman shift / cm¥¥S-1"
```



Baseline comes to zero.

Intensity-calibrated Raman spectrum