**Calibration protocol**

1. Ensure you have the following files
   1. InitProcessImageGUI\_revised.m
   2. InitProcessImageGUI\_revised.fig
   3. Neon.fig
   4. Tylenol.fig
   5. Folder with data
      1. Within folder you should have
         1. Darkspec.mat
         2. Darkspec\_cali.mat
         3. Tylenol.mat
         4. Neon.mat
         5. Whitelamp.mat
         6. Throughput.mat
         7. Other data files that will be calibrated
2. Run “InitProcessImageGUI\_revised.m”
3. Under Initial Processing there is a File Directory input. Select folder with data
4. Press Process
   1. This part will calibrate for pixel number to wavelength, wavelength to Raman shift, readout noise, cosmic ray removal, aberration, throughput, and fixed pattern
5. After a few moments the top figure will have black which is a neon reference measurement. The bottom will be blue which is the data saved in the data folder.
   1. Ensure all peaks in measured (blue) data match reference (black data)
   2. Edit as need be and press OK

Diagram

Description automatically generated with low confidence

1. Same for Tylenol
   1. Edit and OK

Graphical user interface, diagram

Description automatically generated

1. Status will change to Ready once this part is complete
2. Within Final Processing select Initial Processed Directory as folder within your original that has been saved in the previous step called “initprocess”. Press Process
3. This will do the fluorescence removal via a polynomial. Data will then appear in the windows and a prompt will pop up on where to save the data.

**What is saved from calibration?**

After calibration a “process structure” is saved. Once the data is loaded into matlab the following are seen in the structure. The number of cells = number of locations or samples measured. (In this case 4 locations)

Graphical user interface

Description automatically generated with medium confidence

* Anitaspec: processed Raman data.
  + Each cell contains a 499x3 matrix
    - 3: spectra for each leg. (i.e. column 1 corresponds to spectrum from 0 mm leg, column 2 corresponds to spectrum from 3 mm leg, column 3 corresponds to 6 mm leg.
* List: tells you file names that correspond to which cell
  + Allows you to organize data
* Meananitaspec: do not use. This is from old bundle.
* Mean spec: do not use.
* Spec: Fluorescence + Raman data
  + Each cell contains 499x3 matrix
* Wavenum: Raman shift