Processing SpecPhone Images and Wavelength Calibration

Materials

- iPhone 5
- USB lightning connection cable
- Computer or laptop equipped with ImageJ and Microsoft Excel
- Blue diode laser
- Red diode laser
- Green diode laser
- Avantes SensLine AvoSpec-HS-TEC (or other commercial spectrometer)
- Computer or laptop equipped with Excel
- ImageJ software available at: http://imagej.nih.gov/ij/

Introduction

The spectrogram recorded by the SpecPhone contains wavelength and intensity information. In order to analyze the data quantitatively, the intensity information needs to be read out from the digital image file and converted from pixel position to wavelength. This protocol describes these two activities.

The first activity is to convert the photographs to intensity and absorbance spectra. The images need to be transferred from the iPhone to a computer using a USB connection or email. The second activity uses laser pointers of known wavelength to calibrate the spectrum by converting pixel position to wavelength.

Image Processing

If ImageJ is not already on the computer you are using, download and install it using the link above. ImageJ is a free, open-source, platform-independent program developed by the National Institutes of Health for displaying and analyzing medical images. It reads many image formats and is ideally suited for extracting spectral data from the photos.

Procedure

1. Open ImageJ and import the photos to the program:
2. Click 'File', Scroll to 'Import', select 'Image Sequence' and choose the renamed images.
3. View the slideshow to confirm all photos are present.
4. The images will need to be cropped and sometimes rotated in order to better visualize and analyze the spectra:
5. Draw a box around the image (leaving plenty of blank space but positioning the spectrum in the middle), Click 'Image' and select 'Crop'.
6. At this point it may be helpful to save the stack of images as a *.tiff file. This will maintain the stack order and crop area for later analysis.
7. Rotate as needed to align the spectrum horizontally: Click 'Image', scroll to 'Transform' and select 'Rotate'.
8. Check preview and insert desired degree of rotation so that the spread of colors is parallel to the rows of pixels.
9. Draw a box around the spectrum of the first image (careful to remove as much blank space as possible on all edges, see below
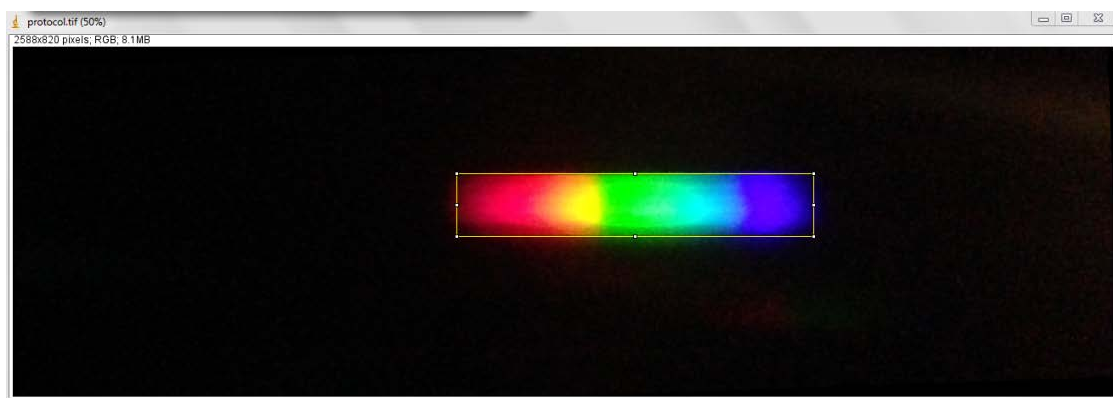
10. Click 'Analyze' and select 'Plot Profile' (Ctrl+K) and the following curve of intensity versus pixel position will appear in a new window:
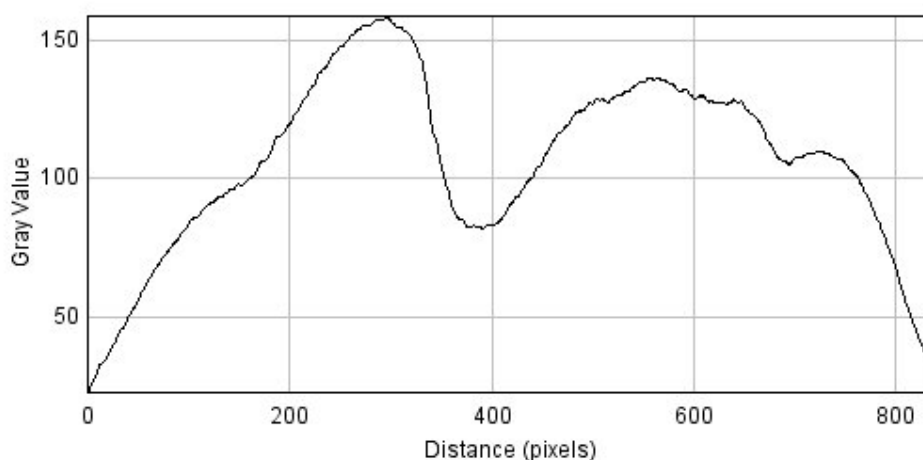
11. From the pull down menu, select copy data and then paste the data in an open Excel spreadsheet.
12. The values copied to Excel are the data for the displayed image, in this case the first image in the sequence. The box drawn around the spectrum will remain on all images as you scroll and view the slideshow. To analyze all the photos simply scroll to the next image in the series, follow the same steps: click 'Analyze', select 'Plot Profile' and copy the data into Excel.
13. The values in Excel represent pixel position and intensity of the transmitted light. Convert these intensity values into absorbance values using the following relationship (where I_o is the intensity of the blank cuvette):

$$A = -\log(\frac{I}{I_0})$$

Wavelength Calibration

The wavelength calibration procedure is essentially finding a functional relationship between pixel position and wavelength. In general one needs to find a light source with defined spectral features of known wavelength. Here we use inexpensive laser pointers, but other sources like commercial fluorescent lighting can be used as well. If a commercial spectrometer is not available, then the first section below can be skipped and the spec wavelengths from the laser pointer manufacturer can be used. Diode laser wavelengths are sensitive to processing defects, so this may add some error as seen in the table below.

Evaluate lasers with the AvoSpec

1. Set up, turn on and connect the AvoSpec to the computer.
2. Open Avasoft on the computer only after the spectrophotometer is connected.
3. Set an integration time of 100ms with 5 scans. Once additional light sources (i.e. overhead lighting) have been minimized, press 'Start'.
4. Direct a blue laser (405nm +/- 10nm) near the sensor of the AvoSpec. Do not shine directly into the sensor to avoid damage and saturation.
5. The resulting plot shows peaks at the wavelength of max absorption. Once the plot peaks have stabilized, press 'Stop'. The picture below shows the AvaSoft plot of fluorescent light as an example.
6. Save this data as an ASCII file.
7. Repeat steps 4-6 with a red laser (650nm +/- 10nm) and a green laser (532nm +/- 10nm).
8. Open this data in Excel and save as an Excel workbook.
9. Since the data from an ASCII will present as delimited, check 'Delimited' and click 'Next. Check the 'Semicolon' box as the delimiter, click 'Next' and then click 'Finish'.
10. Locate and record the wavelength of maximum absorption (corresponds to highest Sample value) for all 3 lasers.
11. To normalize the sample values, divide all sample values of one laser by the sample value at the corresponding wavelength of maximum absorption. Do this for all three lasers.
12. Plot all three sets of normalized sample values against the wavelength on one graph. One peak for each laser displays the wavelength of maximum absorption as seen below.

| Laser	Theoretical Wavelength (nm)	Experimental Wavelength (nm)
Blue	405	401.37
Green	532	531.02
Red	650	657.37
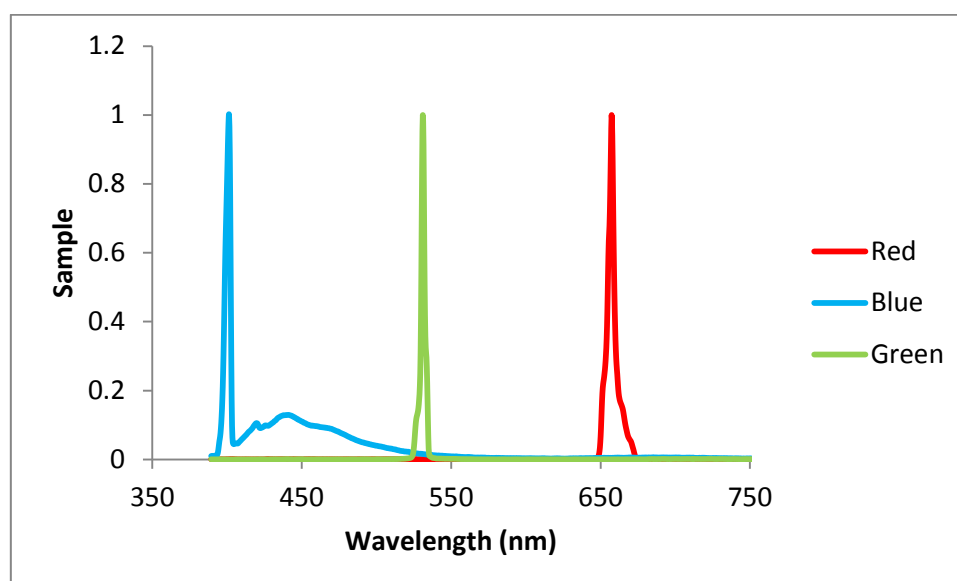
Record laser spectra with the SpecPhone

1. Make sure there is no cuvette in the device and minimize outside light sources. A dark room is best.
2. Place a Kim-wipe in front of the SpecPhone slit to disperse the light from the laser.
3. Shine the laser into the SpecPhone and take a photo of the resulting spectrum using the iPhone camera application.
4. Repeat this process for all three lasers. A merged photo of the resulting spectra of the three lasers can be seen below.
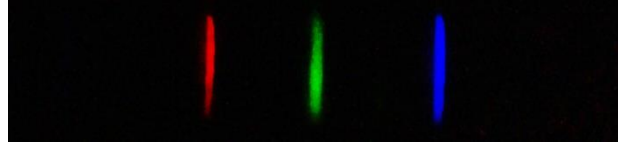

5. Upload and analyze these photos using the same procedure in the **Image Processing Protocol**.
6. Locate and record the pixel value at which the intensity is highest for each laser.
7. To normalize the intensity values, divide all intensity values of one laser by the greatest intensity value at that corresponding pixel value. Do this for all three lasers.
8. Plot all three sets of normalized intensity values against the wavelength on one graph. One peak for each laser displays a pixel number of maximum intensity as seen below.
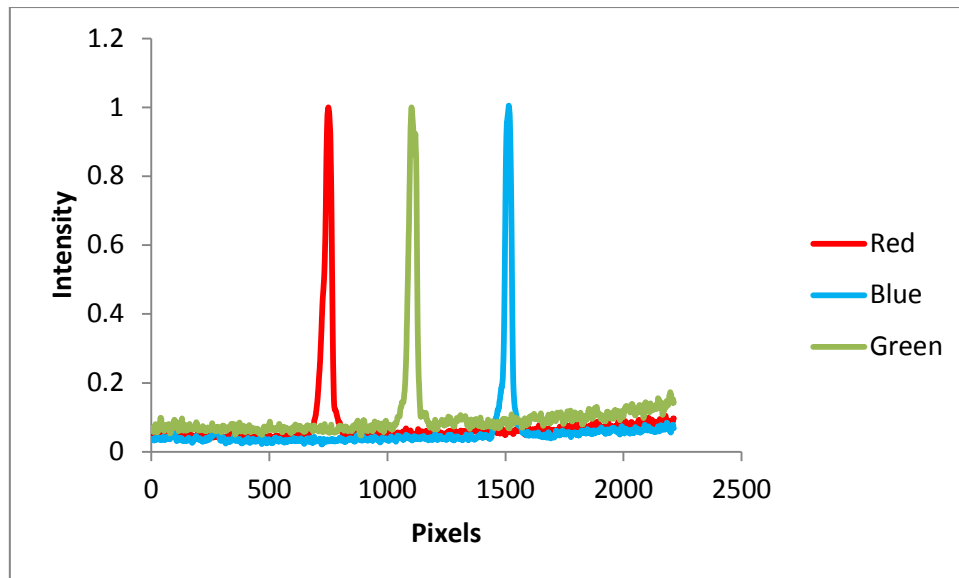
Calibration of SpecPhone Wavelength

1. Plot the wavelength of maximum absorption (y-axis) versus the pixel value of maximum intensity (x-axis) for the three lasers. This will yield a graph with three points as seen in the plot below.
2. Fit the data with a 4th degree polynomial trend line which conveys the relationship between wavelength (y) and pixel position (x).

Wavelength (nm) vs Pixel Number

$$y = 6\text{E-}05x^2 - 0.468x + 975.23$$
$$R^2 = 1$$
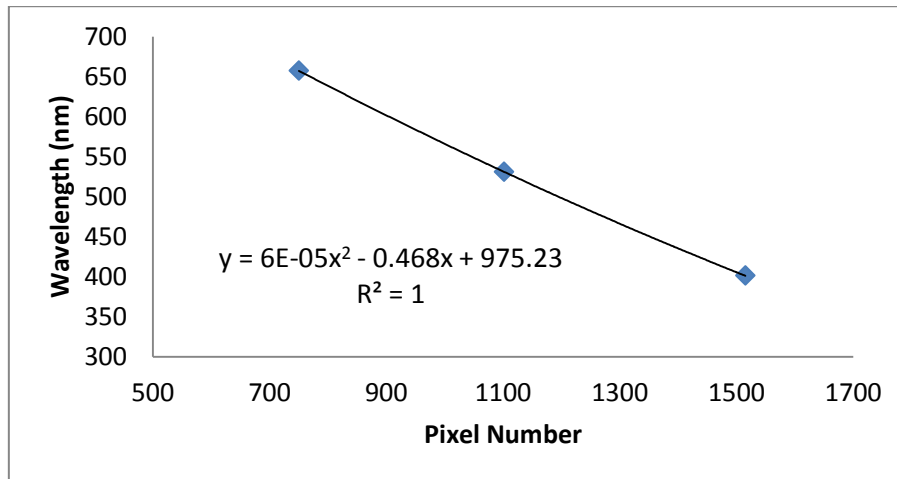
3. Calculate the calibrated wavelength using the trendline from your pixel versus wavelength plot. Plot this calculated wavelength versus the intensity from the SpecPhone pictures to confirm the relationship. This can be seen below:

Normalized Intensity vs Calibrated Wavelength (nm)

Red
Blue
Green