IntelliCal FAQ – Revised 9/27/12

IntelliCal™—Intelligent spectral calibration routine from Princeton Instruments, available with 64-bit LightField™ data acquisition software.

Figure 1. IntelliCal light sources.

Q. What is IntelliCal?

A. IntelliCal is an option to Princeton Instruments' 64-bit data acquisition software, LightField™. IntelliCal provides the most accurate calibration available of both the X and Y axes in optical spectroscopy.

Q. How does the X axis calibration work?

A. IntelliCal wavelength calibration is a patent-pending routine that uses an emission lamp to fit *the wavelength of each pixel simultaneously across the entire focal plane* of an array detector mounted on a dispersive spectrograph. IntelliCal will report and store the calibration accuracy at every point in a spectrum. Conventional calibration routines use only a few emission lines or Raman peaks and interpolate between them. The calibration accuracy at points other than those used in the fit is not known.

Q. How much more accurate is IntelliCal than conventional wavelength calibration routines?

A. Depending on the instrumentation used, IntelliCal is 3 to 10X more accurate.

Q. I'm a Raman spectroscopist and prefer to calibrate my spectra with an internal standard.

A. IntelliCal wavelength calibration can calibrate in nm or Raman shift cm^{-1}. A future release will accommodate secondary standards. Just calibrate your instrument once using a line source, collect your reference spectrum, and you're done. You may use your secondary standard hereafter.

Q. How much does wavelength calibration accuracy matter?

A. Quite a lot. When comparing Raman or LIBS spectra taken with different instruments, or by different labs, it's really important to agree on where the peaks are. This is particularly important when taking difference spectra to monitor changes in a sample, as shown below in Figure 3 and 4. Frequent, accurate and traceable calibrations are essential in QA/QC and GMP environments. IntelliCal stores the data and accuracy of calibration in the data file.

Other areas where wavelength accuracy is critical include process analytical, forensic and hazmat identification, where a spectral fingerprint serves to identify the unknown material. Search-match algorithms compare a measured spectrum to spectral libraries using pattern recognition routines. Accurate knowledge of the Raman shift of a given band, or the precise emission wavelength of a line in a LIBS spectrum, ensures the success of the match, while inaccurate spectra increase the risk both of false positives and negatives.

Q. How do I use IntelliCal wavelength calibration?

A. A dual source emission lamp, powered by USB, is included with every purchase. It contains Hg and Ne/Ar lamps and is easily attached to the entrance slit of spectrometers from Princeton Instruments such as the Acton SP series and IsoPlane SCT 320. Mount it outside the entrance slit (or use it to illuminate your fiber or collection optics), connect it to a computer or the spectrometer USB hub with a USB cable, turn the lamp on with the rocker switch, select the appropriate source in software,

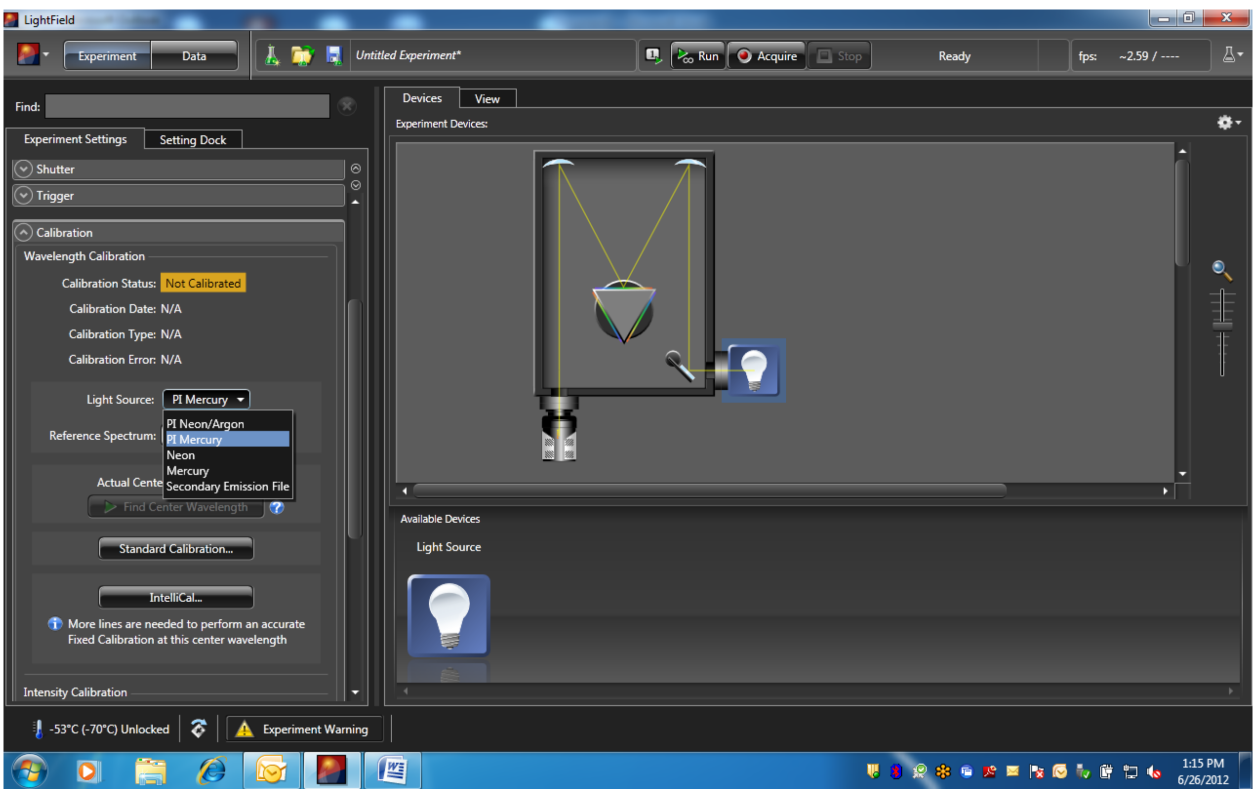


Figure 2. Screen shot of PI's LightField software showing IntelliCall light sources available.

Acquire a background, click the Calibrate button and let the software do the rest. The routine records the emission intensity at every pixel, compares the spectrum to a table of lines from NIST (Figure 5), and refines the model spectrometer parameters to fit the observed spectrum. For best results, the intensity of the spectral lines acquired should be between 200 and 50,000 counts.

Q. What's the difference between IntelliCal Fixed and Broad routines?

A. The Fixed routine calibrates the spectrum acquired at the current grating position. It's the most accurate implementation of IntelliCal and should be used whenever possible. The Broad routine is useful in cases where the density of emission lines is insufficient for a Fixed calibration. Broad calibration rotates the grating to record spectra at four different regions, performing a Fixed calibration at each point. Because uncertainty is generated every time the grating is moved, this routine will be slightly less accurate in general, although it is still more accurate than interpolation-based routines. Always perform a Find Center routine (unique to LightField) after moving the grating for best accuracy.

Q. Can I use IntelliCal with other instruments or software?

A. No. Both IntelliCal routines rely on information about the Princeton Instruments cameras and spectrometers that is stored in firmware. The routines cannot accommodate 3rd party hardware where key parameters are unknown. LightField with IntelliCal can be controlled by packages such as LabView and Matlab via the Automation functionality, however.

Q. I'd like to know more about how IntelliCal wavelength calibration achieves its results.

A. See our tech notes at www.princetoninstruments.com:

Fully automated wavelength calibration method optimizes data accuracy

Automated Wavelength and Intensity Calibration Routines

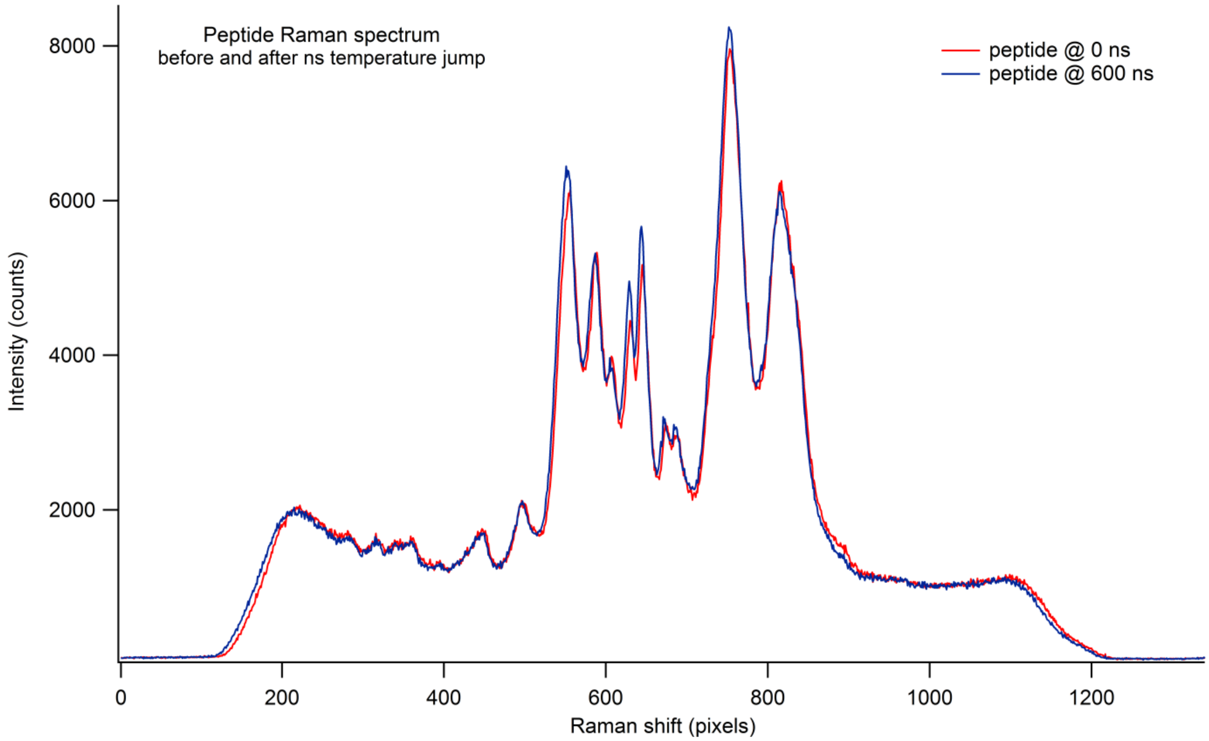


Figure 3. Raman spectrum of a peptide showing time-dependent changes.

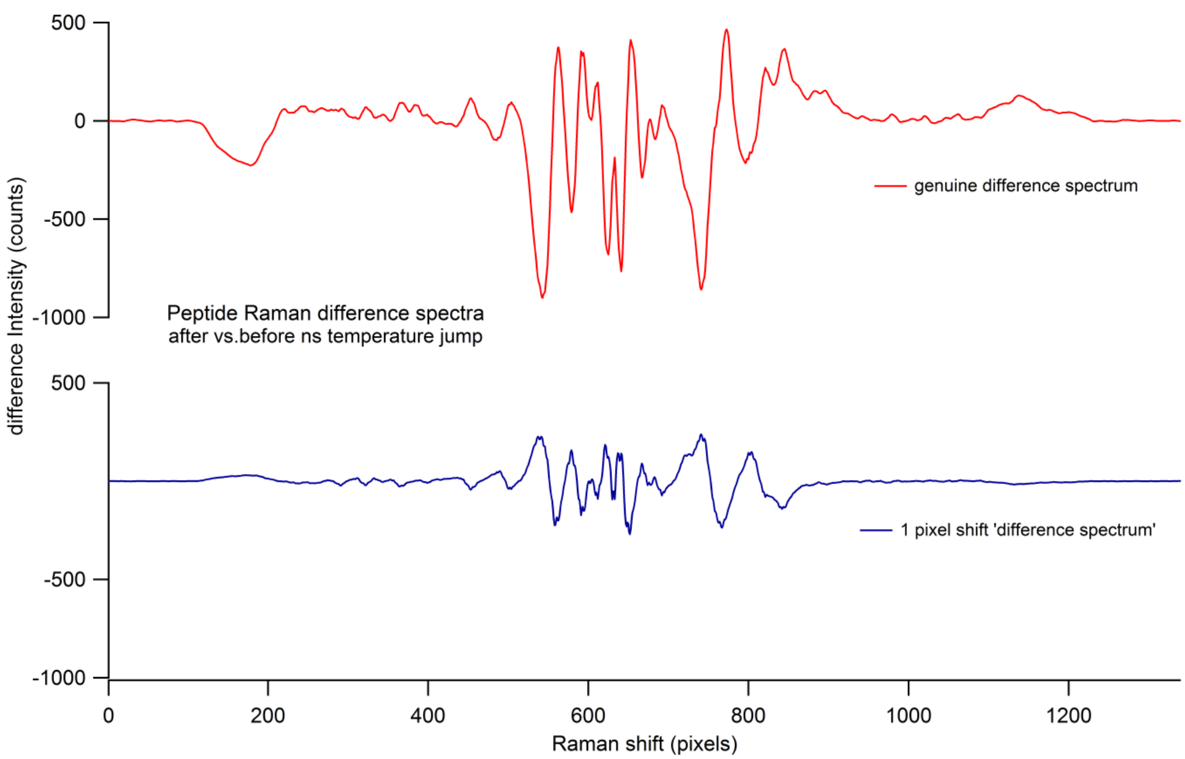


Figure 4. Genuine Raman difference spectrum compared to a fictitious difference spectrum created by an X axis shift of one pixel.

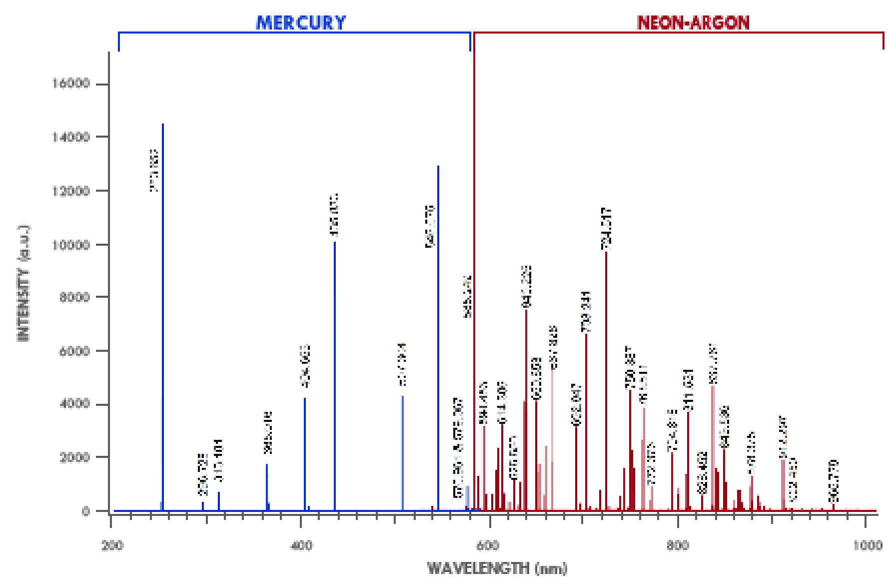
Q. How often should the wavelength source be recalibrated?

A. As atomic emission lines are used, there is no need to recalibrate the wavelength source itself. While the *intensities* of the spectral lines may change, their *positions* do not.

WAVELENGTH CALIBRATION SPECTRUM

* INDICATES STRONG LINE WITHIN WAVELENGTH GROUP ** INDICATES STRONGEST LINE FOR THE ELEMENT () INDICATES 2ND OR 3RD ORDER

Princeton Instruments
USB Hg and Ne-Ar Source



	100	200	300	400	500	600	700	800	900
MERCURY	184.950 194.227	226.223 237.832 248.200 253.652** 265.204 280.346 289.360 296.728	302.15 312.567 313.155 313.184 334.148 365.016* 365.484 366.328	404.656* 407.784 435.833*	507.304* (2x253.65) 546.075* 576.961 579.067	625.14 (2x312.57) 626.37 (2x313.184)	730.03 (2x365.016) 760.95 (3x253.65)		
ARGON				404.442 415.859* 416.418 418.188 419.103 420.067* 425.936 427.217 430.010 433.356		696.543*	706.722 727.294 738.398 750.387* 751.465 763.511* 772.376 794.818	800.616 801.479 810.369 811.531* 826.452 840.821 842.465 852.144 866.794	912.297* 922.450 965.779 978.450
NEON			336.99 341.790 344.770 346.658 347.257 352.047* 359.353		533.078 534.109 540.056 585.249** 588.190 594.483 597.553	603.000 607.434 609.616* 614.306* 616.359 621.728 626.650 630.479 633.443 638.299* 640.225* 650.653* 653.288 659.895 667.828* 671.704 692.947	702.405 703.241* 705.911 717.394 724.517* 747.244 748.887 753.577 754.404 783.906 794.318	808.246 811.855 813.641 825.938 826.608 830.033 836.575 837.761* 849.536 863.465 865.438 878.062 878.375 885.387	

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Figure 5. Wavelength calibration chart for use with IntelliCal.

Q. How does the Y axis calibration work?

A. IntelliCal intensity calibration uses a solid-state LED emission lamp to correct for instrumental artifacts in the recorded intensity of a spectrum.

Q. Instrumental artifacts? That sounds bad! What are they and where do they come from?

A. Every optical spectral measurement requires three things: light collection, light dispersal, and light detection. Light collection is accomplished by optics such as fibers, lenses or mirrors, which display chromatic aberration or spectrally-dependent absorption and reflection. Light dispersal is accomplished by prisms or gratings, each of which has its own spectral signature. Light detection is accomplished by single channel or array detectors, which are sensitive over only a portion of the spectrum. Each of these elements adds its own spectral signature to the data that you record. **See Figure 6 and Figure 7 below**.

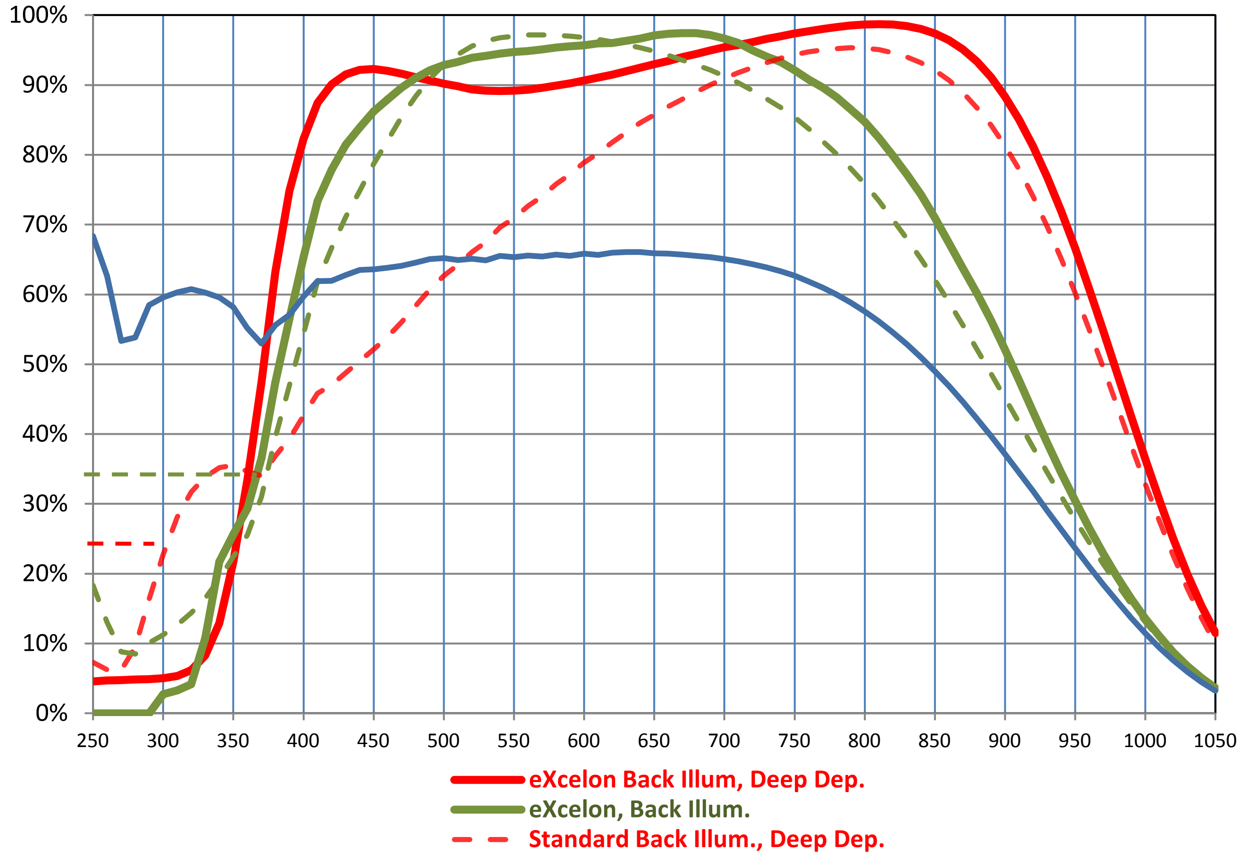

Figure 6. Quantum efficiency of selected Princeton Instruments detectors.

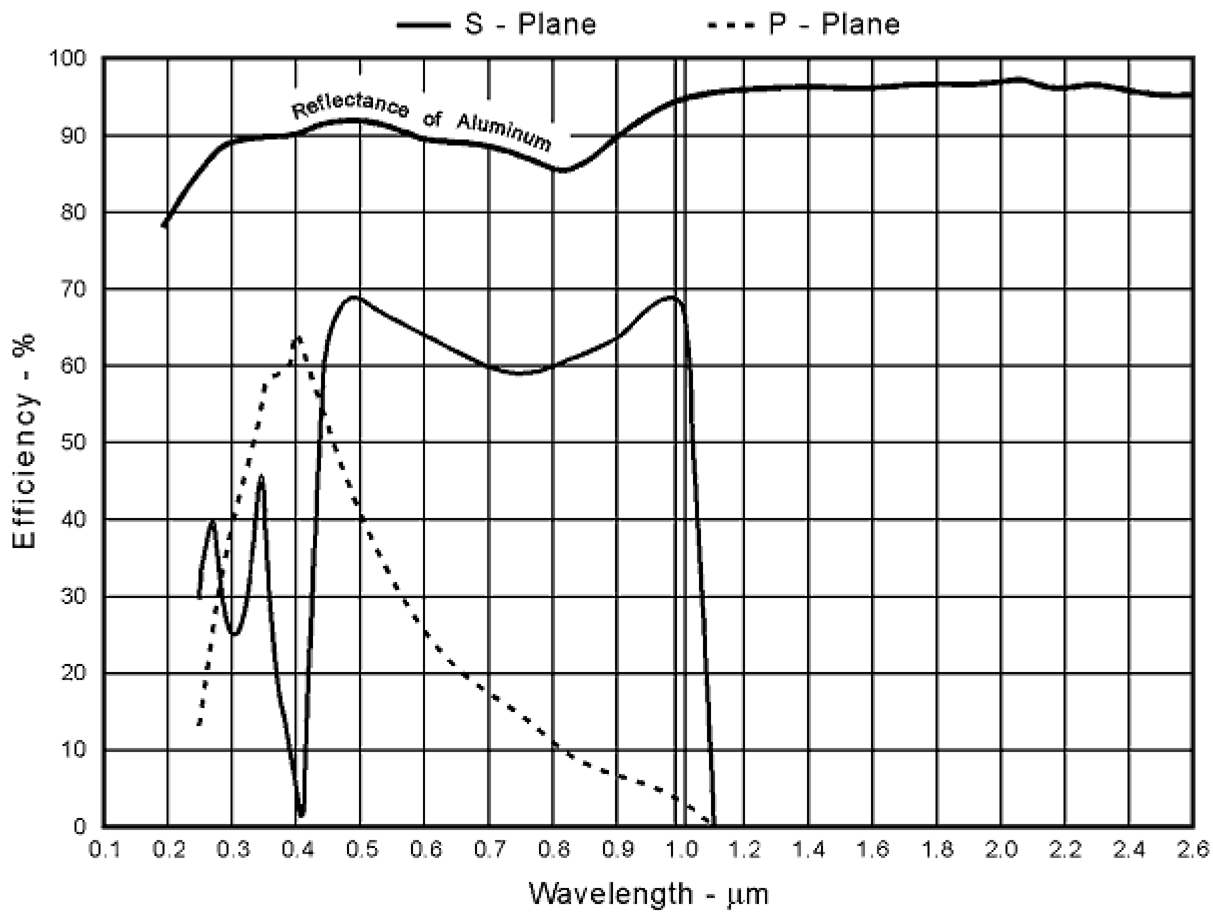

Figure 7. Diffraction efficiency curve of a typical holographic grating. Note the sharp features with S polarized radiation.

Q. What do these instrumental artifacts do to my spectrum?

A. Suppose that you measure the output of a QTH (Quartz Tungsten Halogen) lamp with three different CCD cameras. Each detector has its own spectral response, termed the Quantum Efficiency or QE. The measured spectral intensity is modified by the product of each of the elements in the optical path. If the three cameras have QE peaks in different regions of the spectrum, so will your recorded spectra (Figure 8).

Q. OK, it's easy to understand how camera response affects my spectrum. That shouldn't be too difficult to correct for. How about gratings?

A. Gratings also modify the measured spectrum with their own diffraction efficiency curves. These curves often show sharp features, like Woods' anomalies, that are difficult to correct for (see Figure 9). In addition, using gratings in conjunction with an array detector like a CCD camera creates a more subtle problem. Acquiring a spectrum with both high resolution and broad spectral range requires the use of a grating with high groove density and rotating it stepwise over the desired range, acquiring a spectrum at each step. The individual spectra are then glued together. At each step, however, there is a finite change in diffraction efficiency, manifested by a sharp change in the slope of a broadband spectral feature. In many cases, this step-and-glue procedure can even create spurious spectral peaks (Figure 10).

Q. Does this mean that my recorded spectrum is not the real spectrum of my sample?

A. Yes.

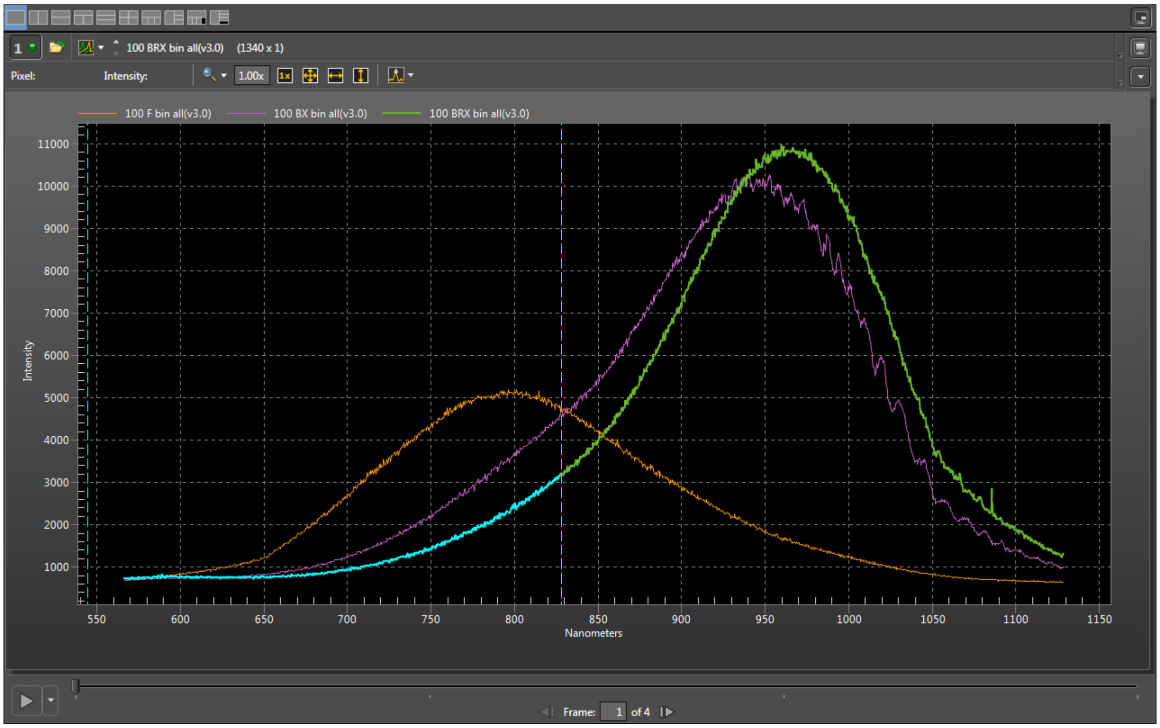

Figure 8. Emission spectra of a QTH lamp measured with three different CCD cameras.

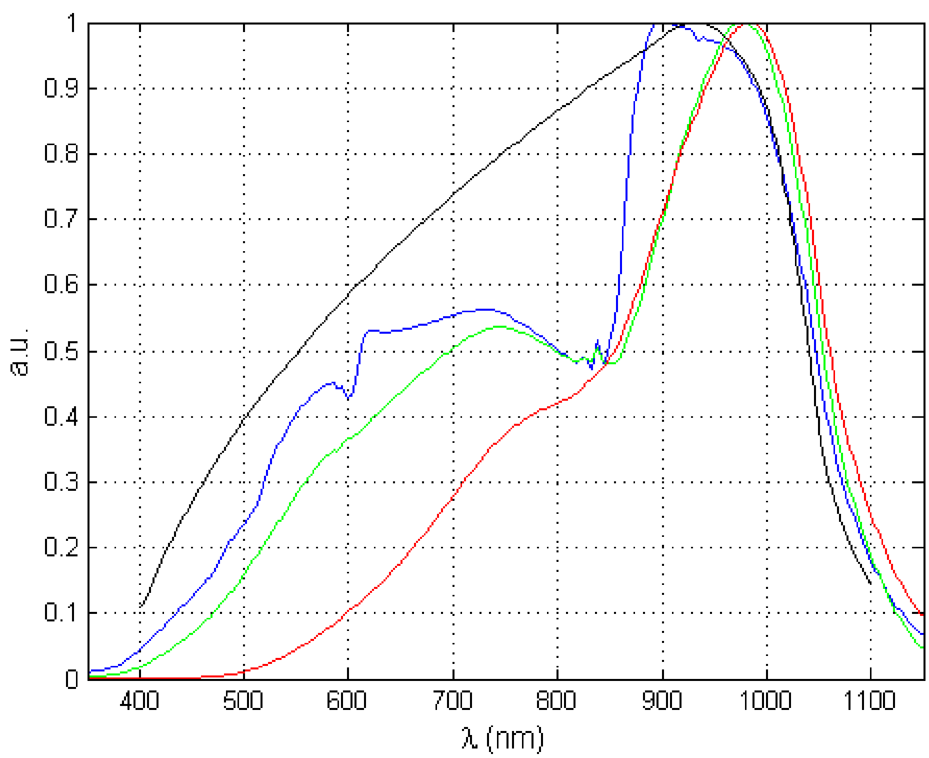

Figure 9. Blackbody spectra acquired with different diffraction gratings. The black trace shows the true spectrum; colored traces are from actual measurements.

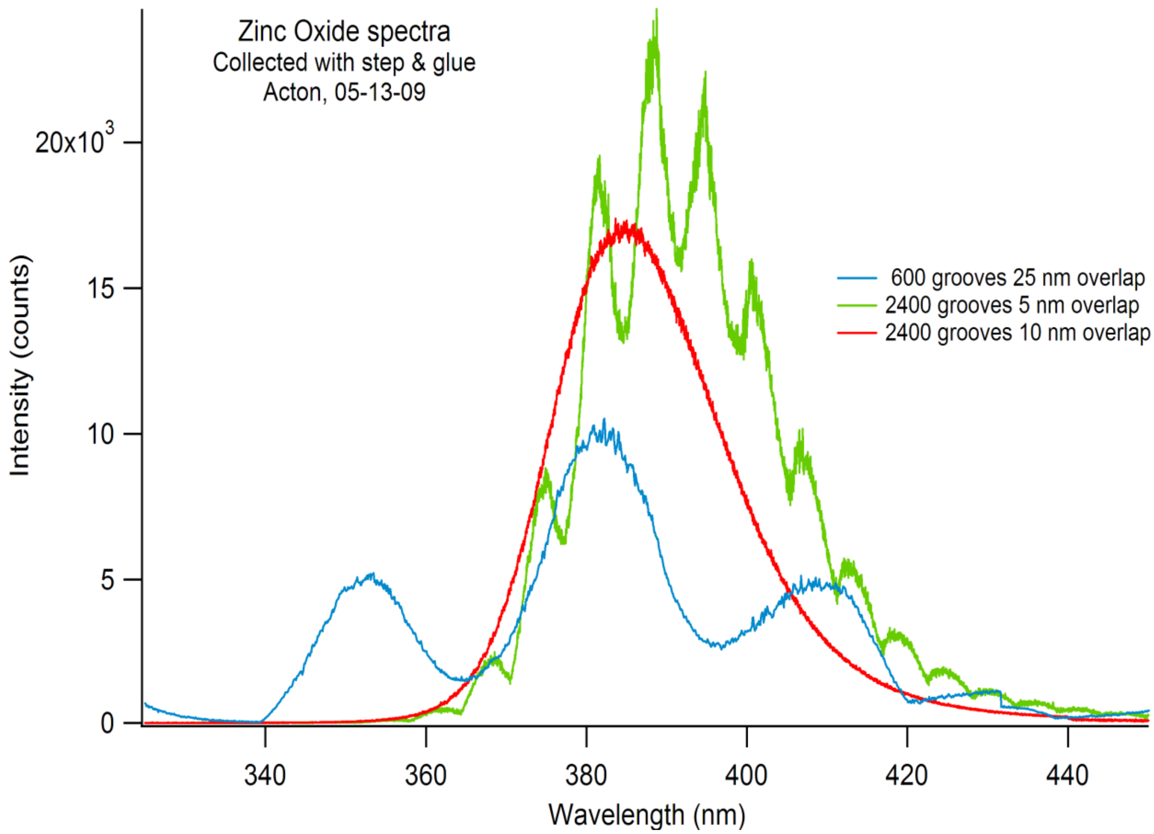

Figure 10. Spurious peaks in a step-and-glue spectrum (green and blue traces). The true spectrum is in red.

Q. Spurious peaks, Woods anomalies, detector responsivity…how do I separate my spectrum from the instrumental artifacts?

A. IntelliCal 2.0, an option to LightField 4.0 and above, includes both wavelength and intensity calibration routines. The intensity calibration engine is a USB powered multi-LED light source with emission from 400 to 1025 nm. Each highly stable source is individually calibrated against a NIST-traceable standard and the spectrum recorded in the device's firmware. To calibrate a spectrometer, a researcher illuminates the entrance slit (directly or indirectly) with the intensity source and records the spectrum (Figure 11, left). The software compares the acquired data to the stored spectrum, derives a correction factor at each wavelength, and applies the correction factors. The uncorrected spectrum shows numerous spectral gluing artifacts at 550 and 910 nm and elsewhere and etaloning above 800 nm that arises from interference fringes in the back-illuminated sensor used to collect the data. The corrected spectrum is at right in Figure 11. The spectral gluing and etaloning artifacts are gone, and the relative peak heights have also been corrected.

In this example, the intensity calibration light source has been used to correct its own spectrum, but the routine is entirely general, and a Raman, photoluminescence, fluorescence, absorption or LIBS spectrum can be corrected as easily as that of the light source itself. For example, when acquiring spectra using an optical fiber, simply illuminate the end of the fiber with the calibration source. Similarly, Raman or other optical microscopies can be intensity calibrated by illuminating the objective with the calibration source.

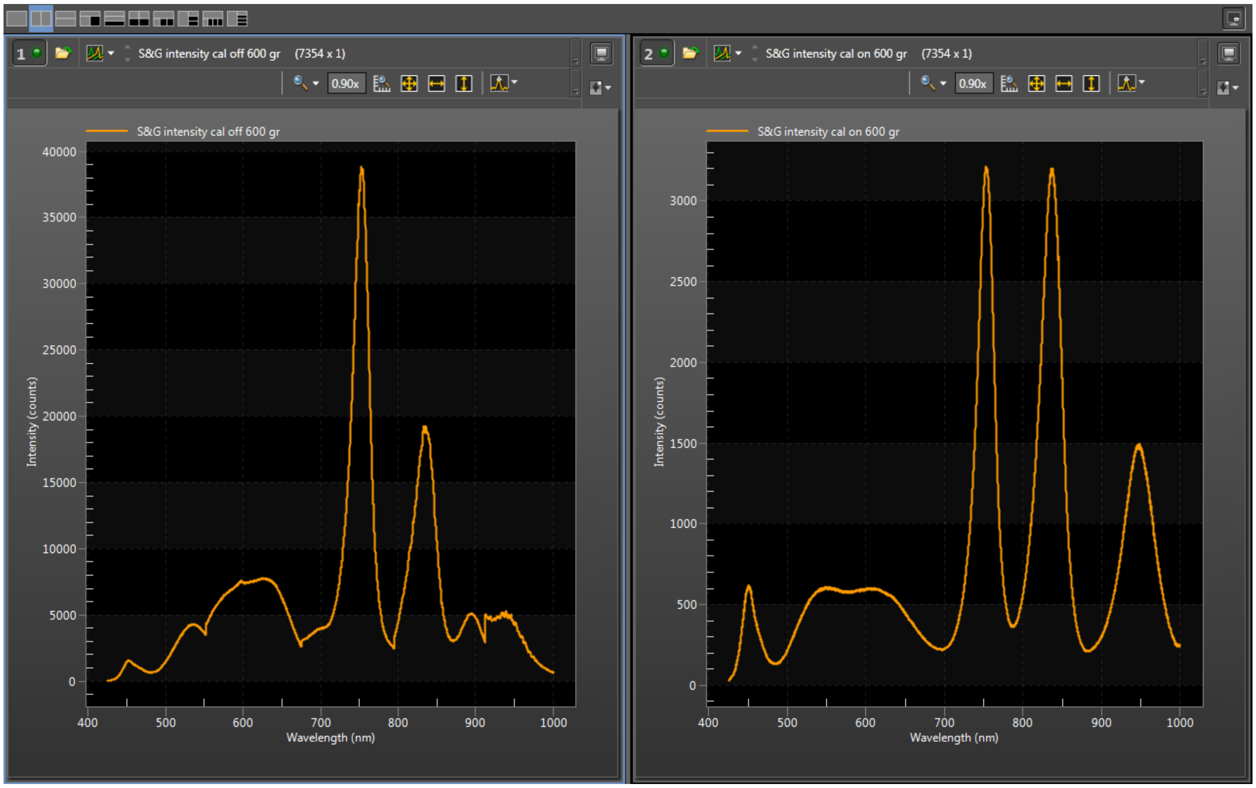

Figure 11. Spectrum of IntelliCal intensity calibration lamp. Left: uncorrected spectrum showing step-sand-glue artifacts and etaloning. Right: corrected spectrum.

Q. Speaking of QTH standards, I think that it should be very easy to correct my spectrum with a blackbody source. Why are you making such a big deal out of it?

A. Try it and let us know how you get on. It is more difficult than it seems for several reasons, including the necessity to uniformly illuminate the entrance optics with a large source, as well as the changes over time in the emissivity of the tungsten filament. Choquette has outlined the technique in Applied Spectroscopy (2007, 61: 117).

Q. What does an intensity-corrected Raman spectrum look like?

A. Figure 12 (following page) shows the Raman spectra of stearic acid, a common component of pharmaceutical tablets. The left hand spectrum represents raw data, while the right hand spectrum has been corrected. The excitation wavelength was 785 nm, and the C-H stretching peaks at 2900 cm^{-1} are at 1015 nm, where the detector quantum efficiency is rapidly decreasing to zero. Intensity calibration restores the proper peak height ratio while flattening the baseline, allowing rigorous quantification of the fraction of stearic acid in a mixture. Without intensity calibration, the ratio of any two peak heights would change depending on the instrumentation used.

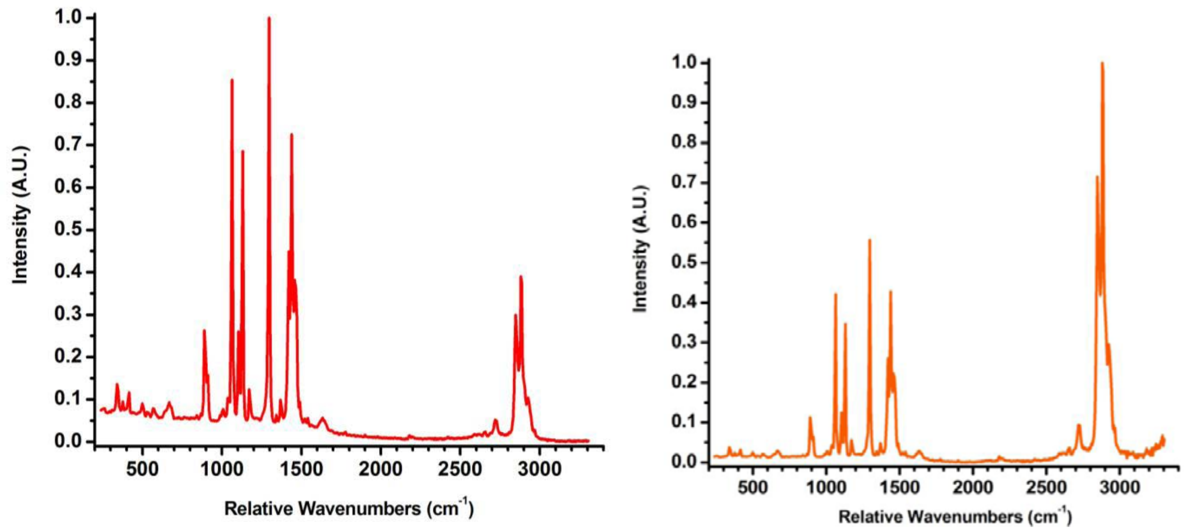

Figure 12. Raman spectra of stearic acid at 785 nm excitation, with (right) and without (left) intensity calibration.

Q. I use my own software, via PICAM, rather than LightField, to control my system. Can I use IntelliCal?

A. Yes and no. IntelliCal is not currently available as a PICAM routine, and the intensity calibration source can only be turned on and off from the LightField GUI. The lamp calibration data, which are different for each lamp, are stored in firmware, but are also included in an Excel spreadsheet that is shipped on a CD with every lamp sold.

Q. How do I use the Intensity calibration source?

A. Unlike the wavelength calibration source, this instrument is controlled by software.

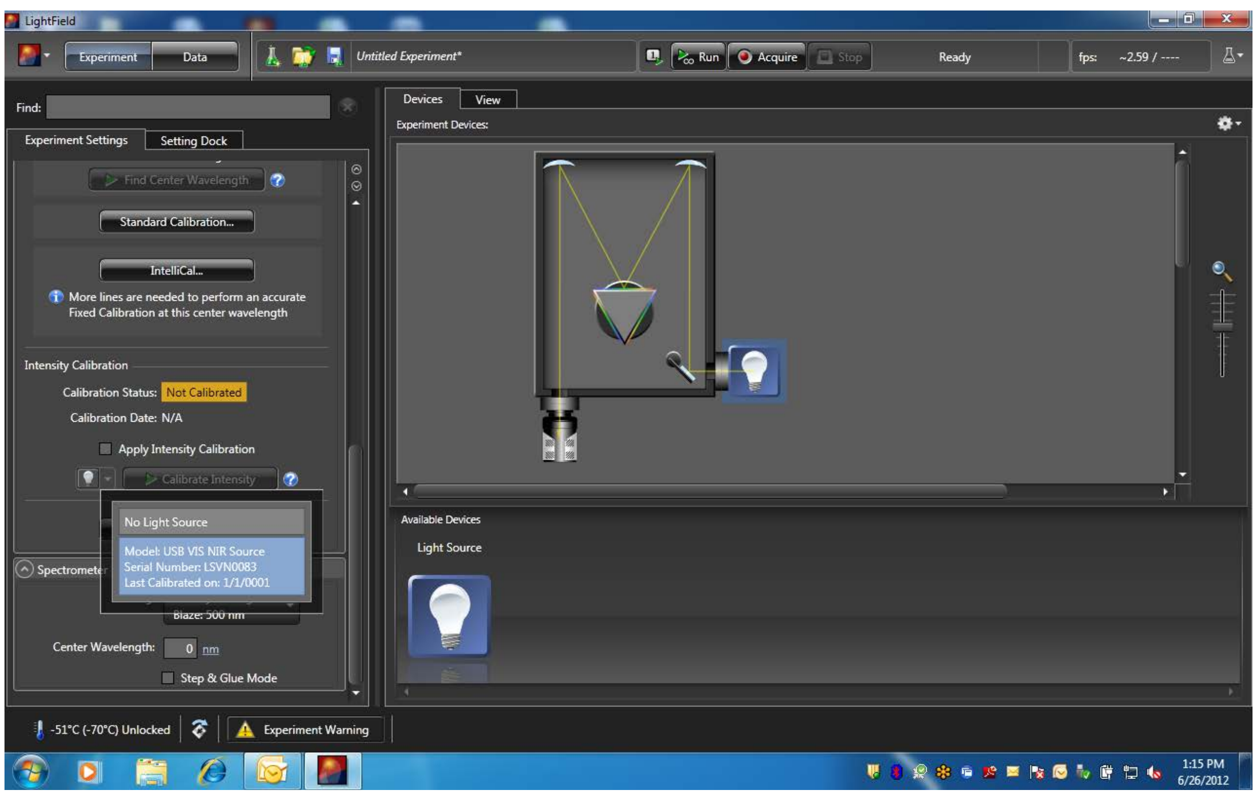

Figure 13. Screen shot of LightField indicating that an intensity source is available.

Mount it outside the entrance slit (or use it to illuminate your fiber or collection optics), connect the source to your computer or the USB hub port of the spectrometer with a USB cable, turn the lamp on in software (there is no hardware switch)[1], acquire a background, and click the Calibrate Intensity button. The software will acquire a spectrum and check the Apply Intensity Calibration button. Subsequent spectra will be calibrated (however, the raw data are also saved by default). Changing the range over which the spectrum is collected will require a new calibration to be done. A good signal-to-noise ratio in the calibration spectrum will give the best results, for example, one can average the calibration acquisition over 10-20 exposures of 100-500 ms each, ensuring that the spectrum is neither saturated nor too noisy.

Q. Will the intensity calibration work outside the source emission window of 400-1025 nm?

A. No.

Q. Why does the intensity calibration source come with a DC power supply?

A. It powers a heater, which maintains the source at constant temperature. Over the range of the emission (400-1025 nm) the accuracy is ±3% or better, which compares very favorably to expensive QTH NIST–traceable emission standards.

Q. How often should I recalibrate my source?

A. It's a good idea to have the source recalibrated once a year. Contact your PI sales engineer for details.

Q. Why did the intensity scale of my spectrum change after intensity correction? In Figure 11, the raw data has a peak height of 39,000 counts vs. 3200 counts for the corrected data. Did you just steal 90% of my data?

A. The absolute signal strength of an intensity-corrected spectrum expressed in counts is no longer very meaningful. That is because the correction factors depend on the exact wavelength range and instrumental setup used. No data are lost; the intensities are simply normalized to the stored calibration file. However, the instrument will still function normally with intensity correction applied, so that, for example, doubling the acquisition time will double the recorded intensity. Go ahead and publish your data with confidence that the spectrum you see is the spectrum that is true.

In summary, Princeton Instruments, known as the industry leader in sensitive cameras and accurate spectrometers, has taken the next step and added ease of use to the list of instrumental criteria. Instead of spending valuable laboratory time on developing their own correction routines, spectroscopists can now use the one-click IntelliCal software with 100% confidence in both axes of their recorded spectra.

Q. Where can I get these great products?

A. Princeton Instruments

[1] Occasionally the lamp will not be immediately recognized by LightField. Try reconnecting the USB cable if this happens. There are a few laptops that have USB ports with insufficient voltage to power the lamp.