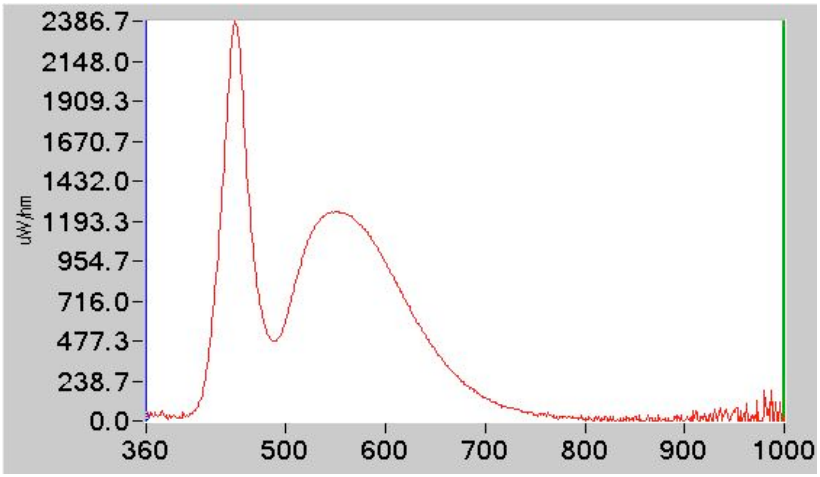
Supplementary Material for: Robertson et al., "Light Emitting Diode Flashlights as Effective and Inexpensive Light Sources for Fluorescence Microscopy"

Spectrum of LED light emission

The spectrum from the Inova Bolt 4.6 watt/6 volt flashlight in Fig. 1A was measured using a Quantamaster QM-7/SE spectrophotometer with the light source introduced into the black chamber of the device through a 3 mm aperture at a right angle from the detector in order to minimize risk to the sensitive photomultiplier. As a result of unequal reflection, parts of the spectrum in Fig. 1A may be underrepresented. Fig S1 shows the spectrum form the Inova Bolt provided by the manufacturer.

Supplemental Figure 1. This is the spectrum of the LED in the flashlight as supplied by the manufacturer:



Wavelength (nm)

Relative light intensities for LED vs. Mercury Sources

Supplemental Table 1. Light intensities from the LED and mercury light sources measured through the Olympus IX71 microscope at the position of the sample using the excitation filters in the microscope. Light intensity was measured at the position of the sample with a LI-COR 185B Quantum Radiometer/Photometer by attaching the probe to a microscope slide mounted on the microscope. The filter sets used for these measurements were the Chroma YFP filter set #410290 with excitation at half band pass

500/20, and Chroma ZsGreen filter set #42002 with excitation at half band pass 470/30. Light intensity was measured in μEinsteins/m^2/s^2. Four different microscopic objectives were used (4X, 10X, and 20X), with similar results. Note that the intensity of the LED source compared more favorably with the mercury source for blue excitation (470/30 nm) than for blue-green excitation (500/20 nm), presumably because of the large peak emission of the LED in the 450-475 nm range.

Source	LED		Mercury		Ratio (Mercury:LED)	
Excitation Filter	500/20	470/30	500/20	470/30	500/20	470/30
4x Objective	25	71	115	160	4.6	2.3
10x Objective	28	83	110	155	3.9	1.9
20x Objective	24	72	100	140	4.2	1.9
Average	25.7	75.3	108.3	151.7	4.2	2.0

Filter Sets Used

Two different filter sets were used in this study. Chroma ZsGreen1 #42002 has an excitation filter HQ470/30x, a dichroic Q495LP, and an emission filter HQ520/40m. The spectrum and specifications for this filter set can be seen at Chroma's website: http://www.chroma.com/products/catalog/42000_Series/42002

The other filter set Chroma Yellow GFP LP (10/c Topaz) #41029 has an excitation filter HQ500/20x, a dichroic Q515LP, and an emission filter HQ520LP. The spectrum and specifications for this filter set can be seen at Chroma's website: http://www.chroma.com/products/catalog/41000_Series/41029