Characterization of High Power LED Replacement for Halogen Sources in Microscopy

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Much has been written on the conversion of older incandescent and/or halogen sourced microscopes to new LED sources. Many of these conversions have required substantial rework of the lamp houses to accommodate the new LEDs and wiring. In addition, evaluation of these conversions have been based primarily on visual accounts of what is seen, which arguably is the most important conclusion. What appears to be missing in these evaluations however, is the addition of spectral-based comparisons to better characterize differences from the various sources.

Here, spectra, color space, and visual analysis were utilized to compare five LEDs against an original 12 volt, 100-watt halogen lamp source. In this investigation LEDs were selected that could be used as a direct replacement to the original halogen lamp, and required no alteration to the original illuminator or microscope optics. (Note: Three of the five LEDs tested required a G6.35 to G4 socket change which were identical in height and did not change the illuminator or microscope optics.)

Instrumentation:

The microscope used was a Zeiss Photomicroscope III equipped with two Zeiss Illuminator 100 housings mounted to a Zeiss lamp house adapter, Photo-1 and 2. In this fashion either the halogen illuminator or the LED illuminator could be brought into the light path with a turn of the selector knob. Alignment of both Illuminator 100 housings was optimized per instructions in the Illuminator manual. All LEDs contained a solid backplane negating any option to optimize the rear collection mirror.

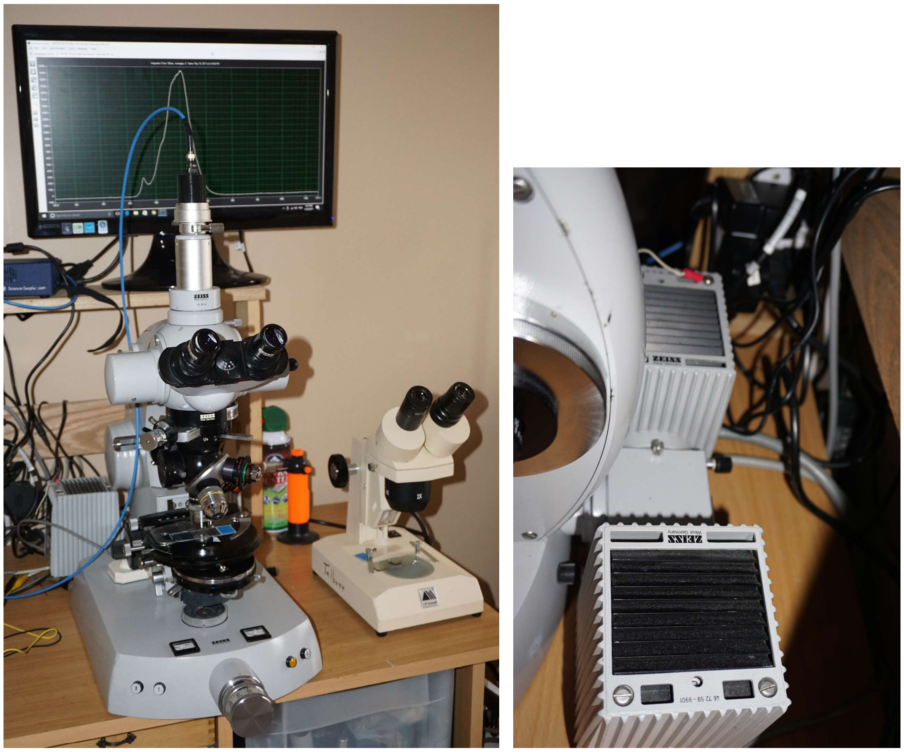


Photo-1 and 2: Zeiss Photomicroscope III and dual lamp house adapter

For spectral acquisition, the microscope was set to transmission with a Type III C Epi-condenser, H-PL reflector, Zeiss 40X HD objective focused on the top surface of a glass slide, and a Z Pol 0.9 NA condenser focused for Kohler illumination. The condenser and field diaphragms were set to full open. The polarizer, analyzer, and retardation plates were removed from the light path during acquisition. The photo head had an Olympus FK 2.5X photo eyepiece and was set for 100% transmission.

The halogen illuminator was powered at 12 volts using the Photomic's power supply. The illuminator with the three low power LEDs was powered by the combined 12 volt leads of a 250W computer power supply. For the high power LEDs, a DROK LN2956 constant voltage/current converter was added between the power supply and the illuminator and fixed at 3.1 volts.

A Science Surplus CCD spectrophotometer with ruled 600 lines/mm and 500 nm Blaze grating and 25 um slit was used to acquire the spectra. A 400 µm optical fiber (wavelength range from 200 to 1100 nm) connected the spectrophotometer to a fiber coupler (360 nm to 1100 nm wavelength range). The coupler connected the microscope through a 3D printed adapter with focal point positioned at the positioned at the height of the Ramsden disc. Calibration of the spectrophotometer was with halogen, LED, Xenon, and LASER sources verified with Holmium solution and didymium glass.

For spectral acquisitions, the Photomic's in-base neutral density filters were used to attenuate the lamp and LED output to achieve counts below 60000 by the spectrometer. Each acquisition was the average of three scans, each 100 msec in duration. A dark spectrum, acquired at the beginning of the tests, was subtracted from each spectrum acquired. Stability of the spectrometer was confirmed by blank spectra acquired at the beginning and end of the testing.

Emission spectra were acquired of each source with and without the in-base Zeiss blue CB12 filter. Additional spectra were acquired with the microscope setup for Polarized work with the red tint retardation plate. Unless stated below, the in-base CB12 blue conversion filter was inserted for all Halogen testing and not used for LED evaluations. Spectra were also acquired of the in-base green filter.

All spectra were normalized to a maximum value of "1" and submitted to a Color Software package (Shimadzu) for calculation of CIE x, y, Y and L*a*b* color space values using a D65 illuminant reference. Dominant wavelength and color temperature were also determined.

For photo acquisition, the Olympus FK 2.5X photo eyepiece was inserted into a Canon F Microscope Camera photo adapter. A "FOTGA" Canon FD to E mount adapter (no optics) attached a Sony A6000 camera to the microscope. This setup gave a field of view equivalent to that observed with Zeiss CPL 10X/18 oculars. Photos were acquired in Aperture priority fixed ISO of 200. A wireless trigger with 10 second delay reduced vibration during image capture. An HDMI monitor was connected to the Camera to assist in fine focus of the images. For photo imagining, a quintuple nosepiece (Zeiss 47 31 56) was inserted and housed Neofluar objectives of 25X, 40X, 63X, a Zeiss 40X Phase Planachromat, and a 40X Hoffman Modulation Achromat. For photos, the condenser optimum resolution (between 1/3 and ¼ closed diaphragm) and not altered between images.

Two power LEDs tested required the addition of an anode and cathode wire that also served as support in the G6.35 socket. Aluminum heat sinks were attached to the high power LEDs to facilitate heat removal, Figure-3. . All LEDs were selected to position the LED in the same position as the halogen lamp filament, Figure-1 and Figure-2.

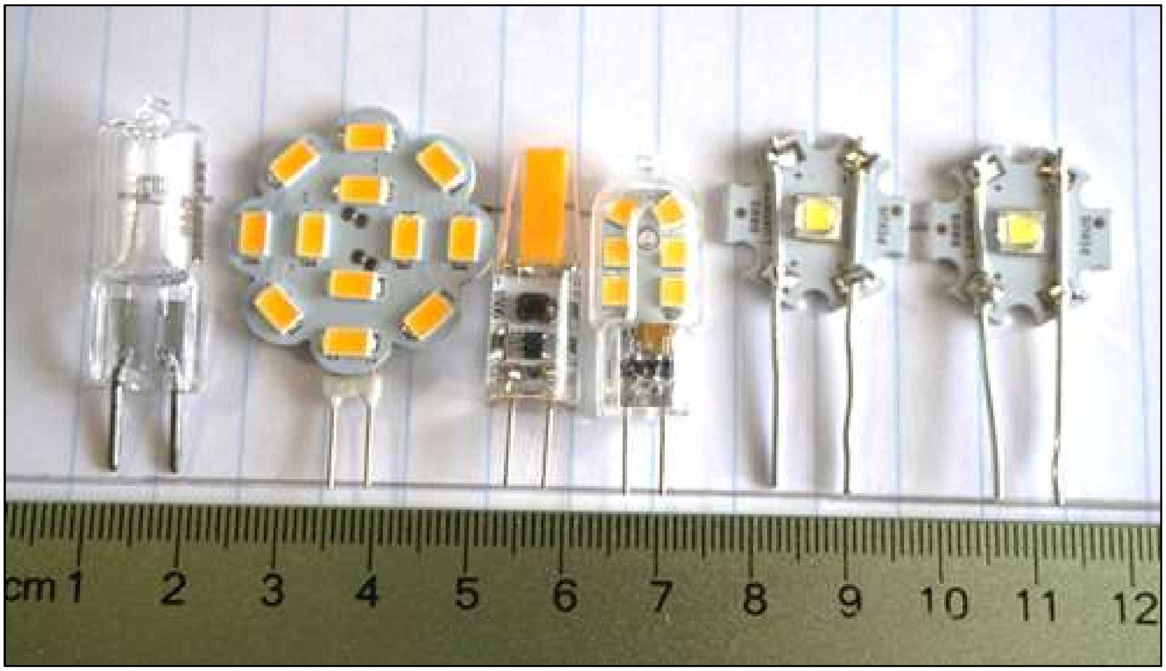
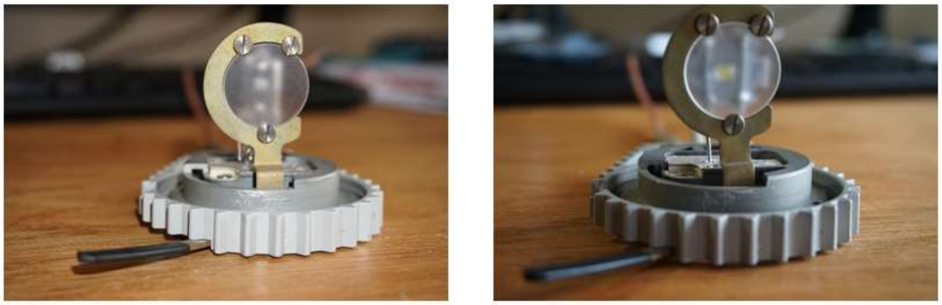


Figure-1: Microscope sources: (left to right) Original 100W halogen, 2.9W Warm White LED, 6W COB Warm White LED, 3W Warm White LED, High Power Neutral White LED, and High Power Cool White LED.

6W LED 3W LED 2.9W LED



100W Halogen Cool White LED

Figure–2: Each LEDs and Halogen lamp positioned in the socket of the illuminator housing base.

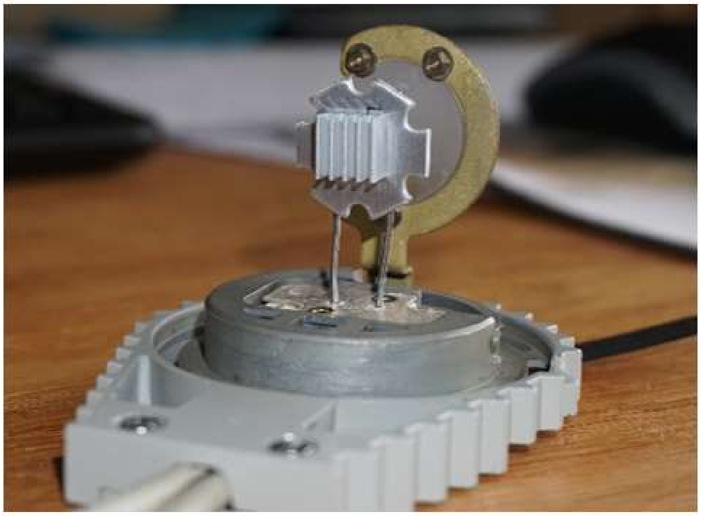


Figure-3 Heat sink attached to the back of the High Power LEDs

Relative lamp output through the microscope was measured using the luminous flux meter of a DVM. The lux mmeter was placed directly over the photo eyepiece and adjusted to obtain the maximum Lux reading for each source.

Results: Low Power LEDs

Figure-4 shows emission that the spectra of the three low power LEDs, all classified as “warm white” and color temperatures between 2700K and 3500K, were very similar, with only minor differences in the phosphor showing between 500 and 600 nm. The spectra show the 453 nm emission from the blue excitation die used to excite the white phosphor coating. All LEDs gave a working range between 500 and 680 nm. Contrary to some reported information, no LED provided emittance in the UV nor in the NIR above 720 nm.

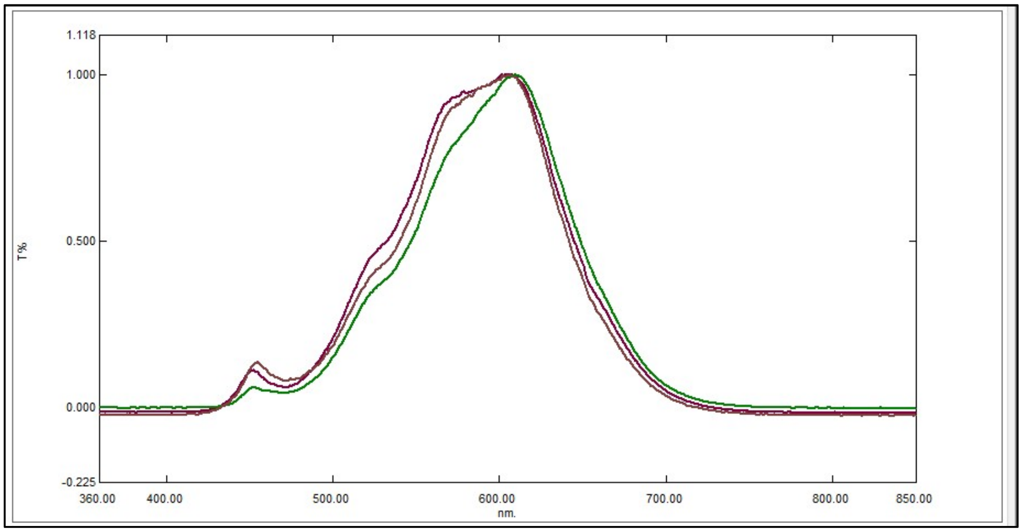


Figure-4: Comparison of emission spectra from LEDs, 3W (brown), 6W (dark purple), and 2.9W (dark green)

Emission spectra were also acquired with the Zeiss CB12 conversion filter in place and are shown in Figure-5. The blue conversion filter modified all LED output in overall intensity and in the 650 – 700 nm range.

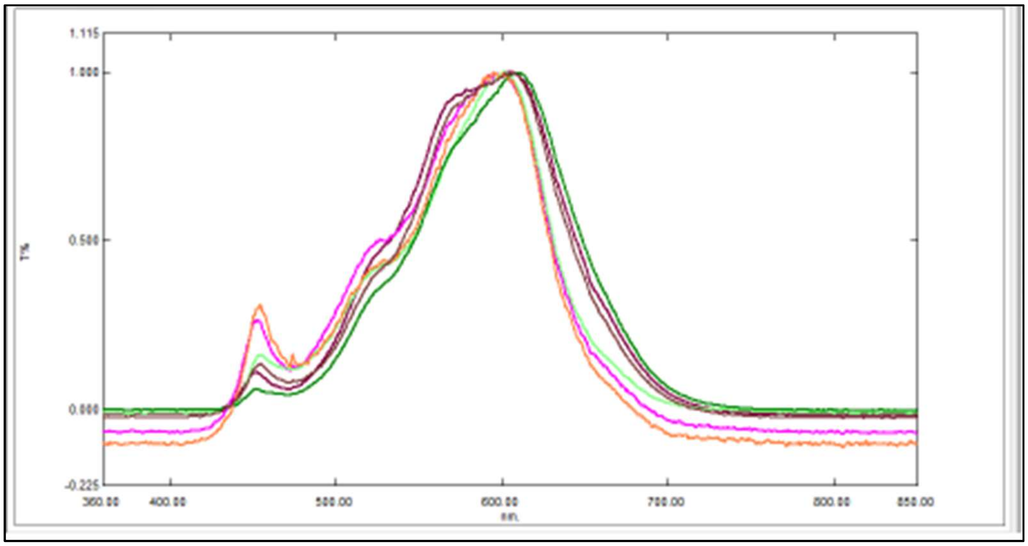


Figure-5: Comparison of LED emission spectra with and without the CB12 blue conversion filter. 3W (brown), 3W with blue (orange), 6W (dark purple), 6W with blue (light magenta), 2.9W (dark green), and 2.9W with blue (light green).

Figure-6 compares the halogen emission spectra with and without the CB-12 filter against the low power LED spectra without the CB12 filter. The purpose of the blue filter is to adjust the color temperature of the halogen source from 3300K (100W at 12 Volts) to 5500K (daylight film) (Incident-light Photomicroscope III, Operating Instructions, G41-656-e, Zeiss, page 13.) The spectra in figure-6 shows clearly how the CB-12 filter attenuates the red and infrared portions of the spectrum ultimately increasing the color temperature. Figure-6 also shows that the warm white low power LEDs tested have spectra on the red side that are more in line with that of the CB-12 converted halogen spectrum. However, the LED emission spectra lack intensity on the blue side which the halogen sources has in abundence.

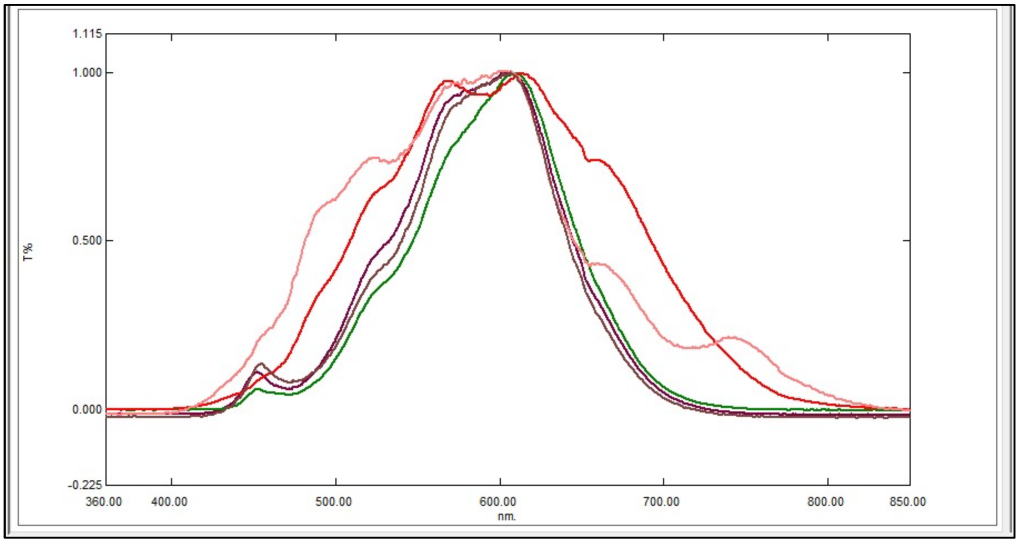


Figure-6, Comparison of LED emission spectra (no CB12 blue filter) with Halogen spectra (with and without CB12 blue filter). 3W (brown), 6W (dark purple), 2.9 W (dark green), Halogen (dark red), and Halogen with CB12 blue filter (light red).

The lack of blue intensity in the “warm white” LED spectra would suggest lower color temperatures than observed by the halogen source, and this is seen in the color space analysis in Figure-7 and table-1.

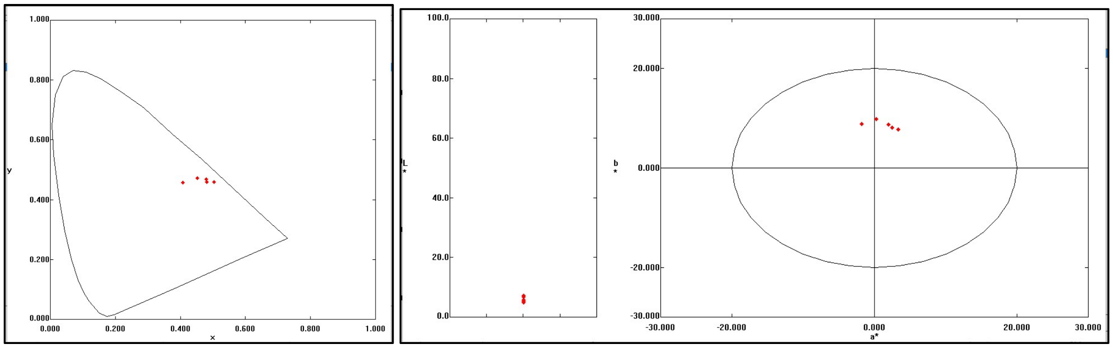


Figure-7, The CIE x,y,Y and L*a*b* color space analysis of the spectra in figure -6.

The table shows that all calculated color temperatures for the low power, warm white, LEDs were significantly lower than the reported values and even farther below the halogen color temperature both with and without the CB12 conversion filter. Thus, even though the LED spectra more resemble the color temperature of the blue converted halogen spectrum, the significant differences on the blue side of the spectrum provide differences in the perceived visual color.

Table-1

WI versus LED Testing

Source	Volts	mA	Type	Emitters	Base	Beam	Rpt Lumens	Style	Rpt K	mea Lux	Y	x	y	L*	a*	b*	DWl nm	Calc K
WI 100W	12	833.0	W	1	G6.35	360 Deg	N/A	N/A	3400	656	0.74	0.452	0.4723	6.69	0.27	9.81	571.7	3107.4
WI 100W B	12	833.0	W	1	66.35	360 Deg	N/A	N/A	5500	N/A	0.78	0.4076	0.4562	7.08	-1.76	8.81	567.9	3889.7
6W SMD12	12	131.0	COB	2	G4	360 Deg	480	warm white	3000	9	0.63	0.4793	0.4678	5.68	1.97	8.75	574.8	2843.8
3W 2835LED	12	243.0	12 SMD	12	G4	360 Deg	280	warm white	2800-3500	8	0.59	0.4813	0.4592	5.37	2.44	8.14	575.9	2759.8
2.9W	12	147.5	12 SMD	12	G4	180 Deg	285	warm white	2700	6	0.54	0.5036	0.4589	4.90	3.33	7.81	577.8	2499.4

This difference is both spectrally and visually seen when investigating a cross polarizer with sensitive red tint retardation plate. Spectra were acquired of the 6W LED and Halogen with CB12 blue modification with a Polarizer-Analyzer-Ret Tint combination in the light path, Figure-8, and shows significant spectral differences for all three sources. Color space analysis of the three spectra, Figur-9, shows that when using the Polarizer-Analyzer-Ret tint combination, the halogen lamp without the blue filter and the 6W LED are more similar in perceived color, however. Calculated dominate wavelength for the three are Halogen – 606nm, Halogen with blue filter – 500 nm, and 6W LED – 609 nm.

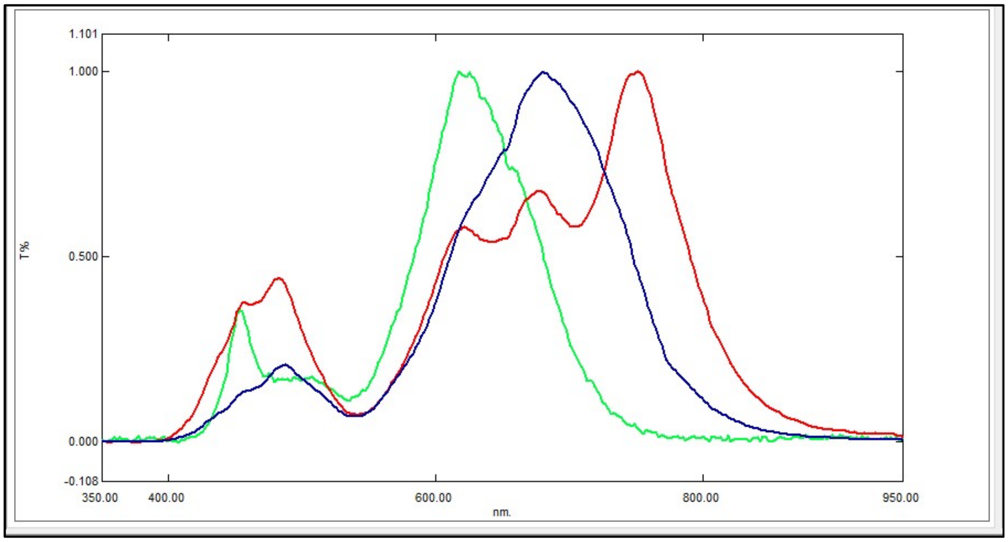


Figure 8, Emission spectra of 6W LED (green), Halogen (red), and Halogen with blue (blue); all with a polarizer, analyzer, and red tint compensator inserted into the beam path.

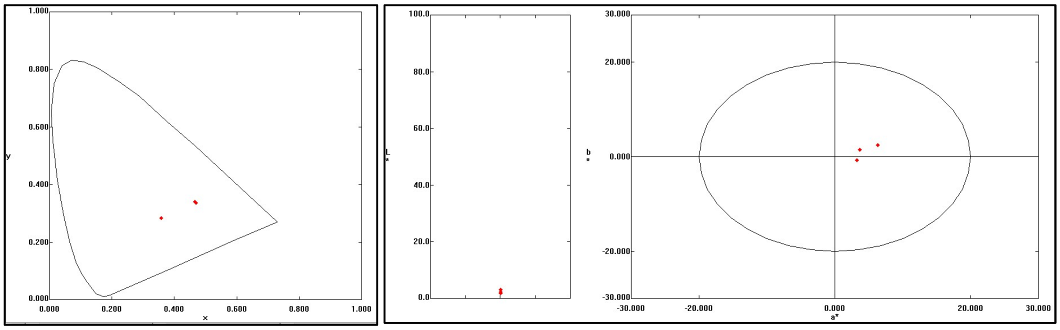


Figure 9, CIE x, y, Y and L*a*b* color space analysis of spectra in Figure 6

In a similar way, the in-base green filter was analyzed spectrally using the Halogen with blue CB12 filter source and the 2.9W LED, Figure-10. Color space analysis shows only very minor differences in the dominant wavelengths of 547 nm for the 2.9W LED and 541 nm for the Halogen with blue filter.

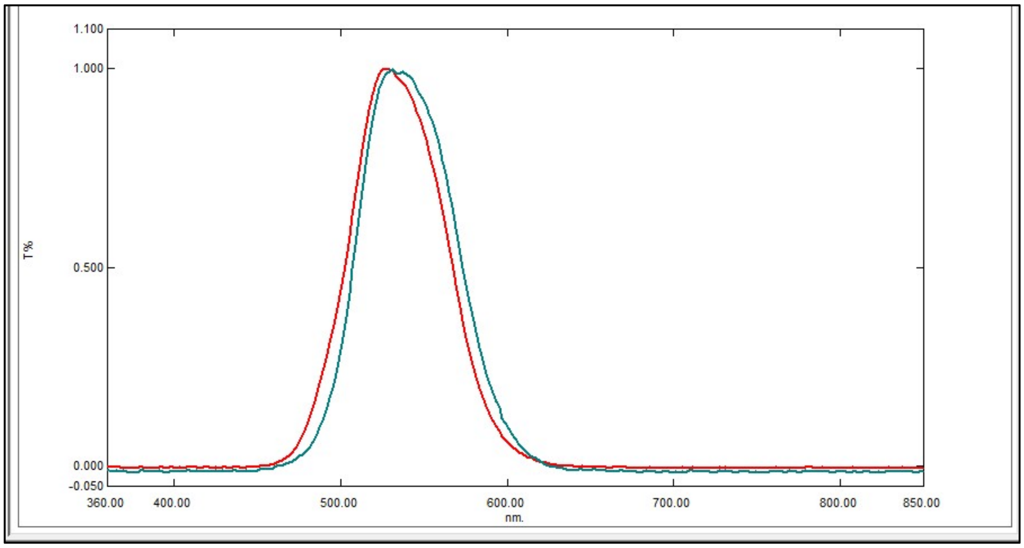


Figure-10, In-base green filter spectral tests for Halogen with blue filter (red) and 2.9W LED (blue)

Table-1 also shows the measured LUX values for each source through the microscope. The intensity of the halogen source was significant at 656 Lux where the LEDs were 9, 8, and 6 Lux respectively.

Conclusion for Low Power LEDs

The lower power LEDs with a “warm white” phosphor were ultimately a poor replacement for the halogen lamp. The low power LEDs offer significantly lower throughput and would only be suitable for the most optimum bright field conditions. In addition, even though the LED spectra were a close match to the converted halogen source on the red side, the significant differences on the blue side of the spectra made for very poor color temperature matching which was immediately visible in color intense applications such as cross polarization with compensation.

Results of High Power LED Testing

As an alternative option for Halogen replacement, two high power Cree LEDs were evaluated and included a “cool white” and a “neutral white”. Reported color temperatures of the LEDs were more in line with a CB12 converted halogen source at 6500K and 4000K, respectively. Figure-11 shows a 20X epi-view of the LED phosphor with no power applied. Figure-12 shows the same phosphor with only minimum voltage of 2.46 volts, and attenuated by a Zeiss 0.03 Neutral Density filter. Clearly in figure-12, the multiple blue excitation die pattern can be observed which ultimately gives the even “filament” for these LEDs.

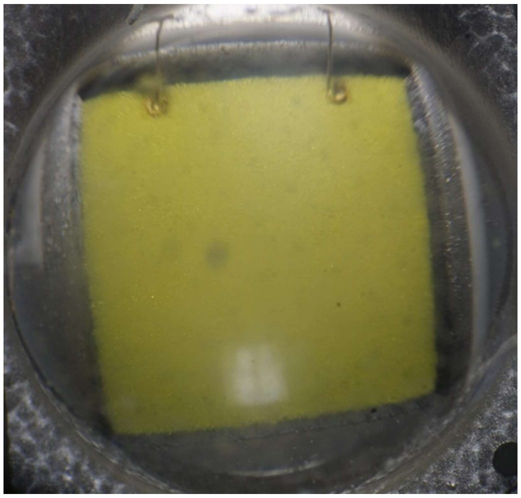


Figure-11: Cree High Power Cool White LED COB showing phosphor, 20X, no power.

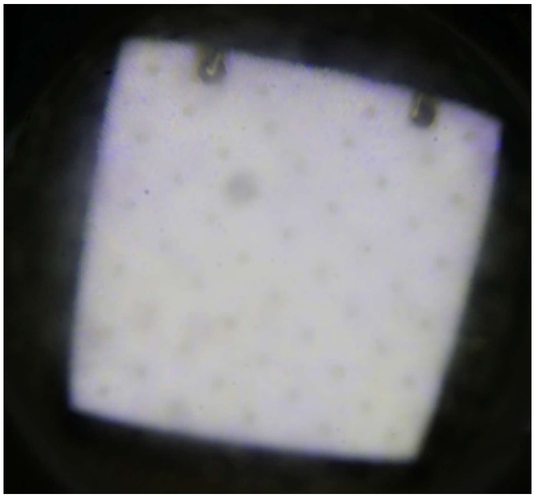


Figure-12: Cree High Power Cool White LED with 2.46 Volts and 0.03 ND filter showing array of excitation dies.

Emission spectra of the halogen source with and without CB12 conversion filter and the Cool White and Neutral White LEDs were acquired and are shown in Figure-13. The spectrum of the neutral white LED more closely matches that of the converted halogen spectrum on the red side, but again both the cool white and the neutral white LEDs lack blue intensity to match the halogen source.

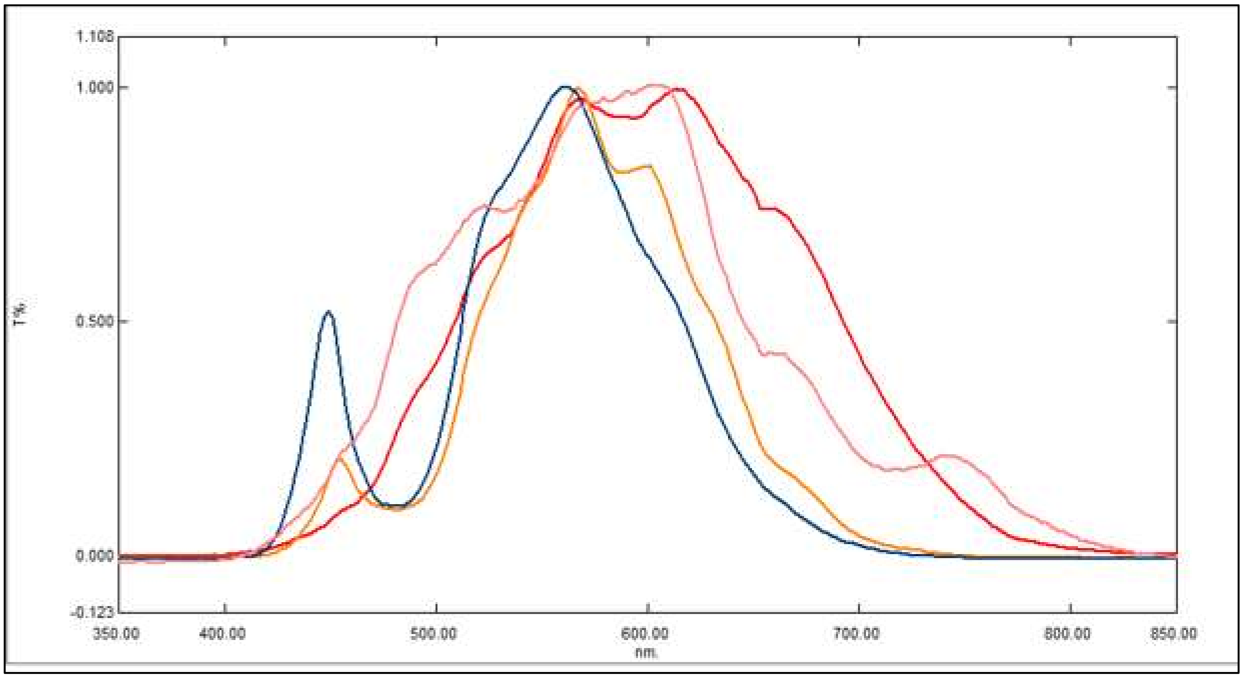


Figure-13: Spectra of sources (no Pols), Halogen (red), Halogen with blue filter (lt red), Cool White (blue), Neutral White (orange)

Color temperatures for the spectra were calculated to be NW – 3471K, CW – 4453K, and HalB - 3889K, table-2. In a similar fashion to the low power LEDS, examination of the high power light sources in a cross polarization with red tint retardation show significant spectra differences, figure-14.

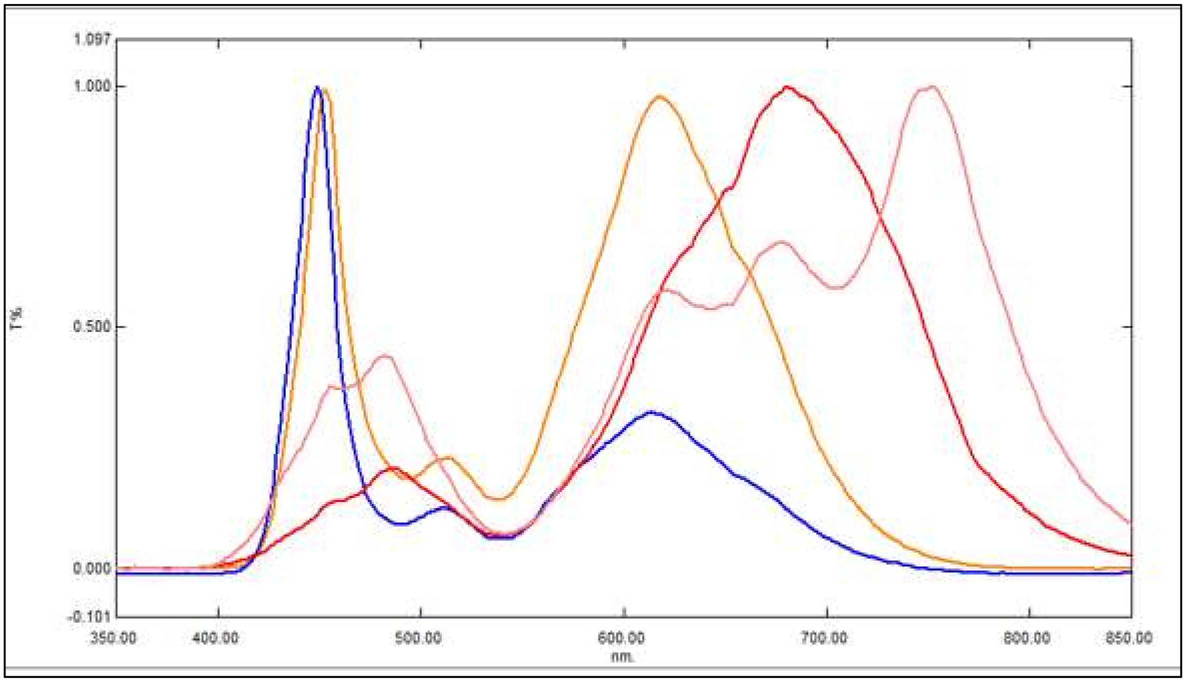


Figure-14: Comparison of CPR with Neutral White (orange), Cool White (blue), Halogen (red),

and Halogen with blue filter (lt. red)

Color space analysis of these spectra are shown in figure-15, and show significant differences in the color space values for the Halogen (converted), Cool White, and Neutral White LEDs. Table-2 gives the calculated values and shows dominant wavelengths for CW, NW, and Hal-B as of 560.7, 502.0, and 505.8 nm and respective color temperatures of N/A, 2768K, 3887K.

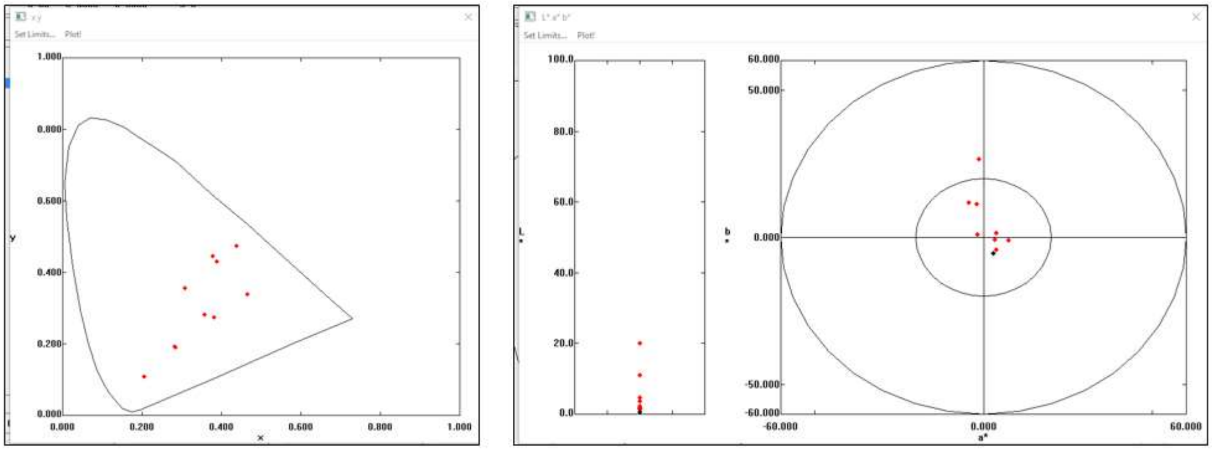


Figure-15: Color space analysis for Cree High Power LEDs showing large variance in color space values and color temperatures.

WI versus LED Testing

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WI 100W	12	833.0	W	1	G6.35	360 Deg	N/A	N/A	3400	656	0.74	0.452	0.4723	6.69	0.27	9.81	571.7	3107.4
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2.9W	12	147.5	12 SMD	12	G4	180 Deg	285	warm white	2700	6	0.54	0.5036	0.4589	4.90	3.33	7.81	577.8	2499.4
CREEXML2-W318 (2 to 10 W) Cool White	2.85 3.05 3.33	700 1500 3000	XM-L2	1 (T6)	N/A	125 Deg	385 541 679	Cool White	6500 (5000 to 8300)	127 (840mA)	1.28	0.3775	0.4455	11.14	-4.3	11.83	564.1	4453
CREEXML2-NW296 (2 to 10W) Neutral White	2.85 3.05 3.33	700 1500 3000	XM-L2	1 (T5)	N/A	125 Deg	357 502 631	Neutral white	4000 (3700 to 5000)	174 (920mA)	3.01	0.4376	0.474	20.08	-1.36	26.47	569.9	3471
CW CPR											0.17	0.283	0.1899	1.53	3.77	-4.19	560.7	N/A
NW CPR											0.40	0.3818	0.2742	3.63	7.34	-1.06	502.0	2768.40
WI CPR											0.21	0.4654	0.3393	1.89	3.64	1.51	605.8	2056.20
WI CPRB											0.25	0.3578	0.2824	2.22	3.22	-0.72	505.8	3887.40

Table-2: Results for LED testing

The throughput of the High Power LEDs were significantly better than the lower power units showing Lux values of 127 for the cool white and 174 for the neutral white. The LEDS were rated at 3000 mA, but were operated at a constant voltage of 3.1 volts and 840 mA and 920 mA respectively.

The high power LEDS were further examined in use by comparing several prepared slides for background color differences. It was obvious that the white balance of the camera could readily affect the results, so photographs were taken with various white balance settings including auto white balance, daylight, and custom settings established on the sources themselves of Halogen with CB12 (5700K), Neutral White (3700K), and Cool White (7100K) (color temperatures given by the camera during white balance.) The results follow in figures 16 to 23. All photographs were taken with ISO set at 200, aperture priority, and the shutter duration listed in each figure. For the case of the halogen lamps, suitable in-base neutral density filters were used to bring the shutter speed in line with that used in the corresponding LED trials.

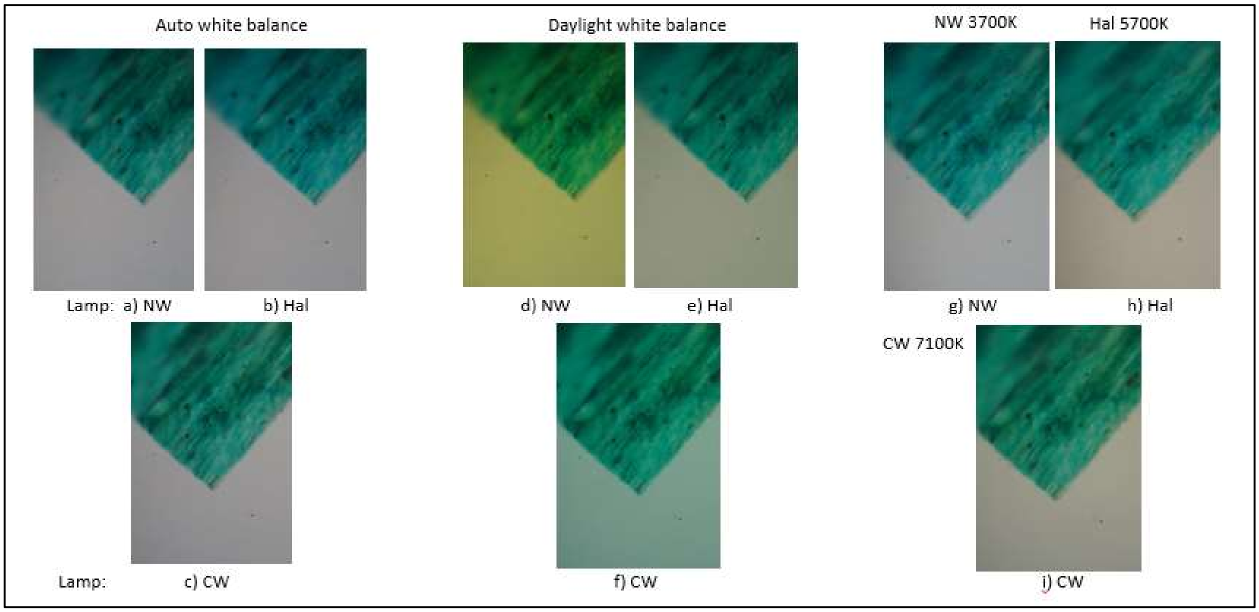


Figure-16: Leaf stalk of Garden Radish T.S., 25X Neofluar, ISO 200, neutral white (NW) and Cool White (CW) - no filters, halogen (Hal) CB12 and 0.5 ND: a) NW 1/350, b) Hal 1/250, c) CW 1/1000, d) NW 1/350, e) Hal 1/250, f) CW 1/1000, g) NW 1/1500, h) Hal 1/1500, and i) CW 1/1000.

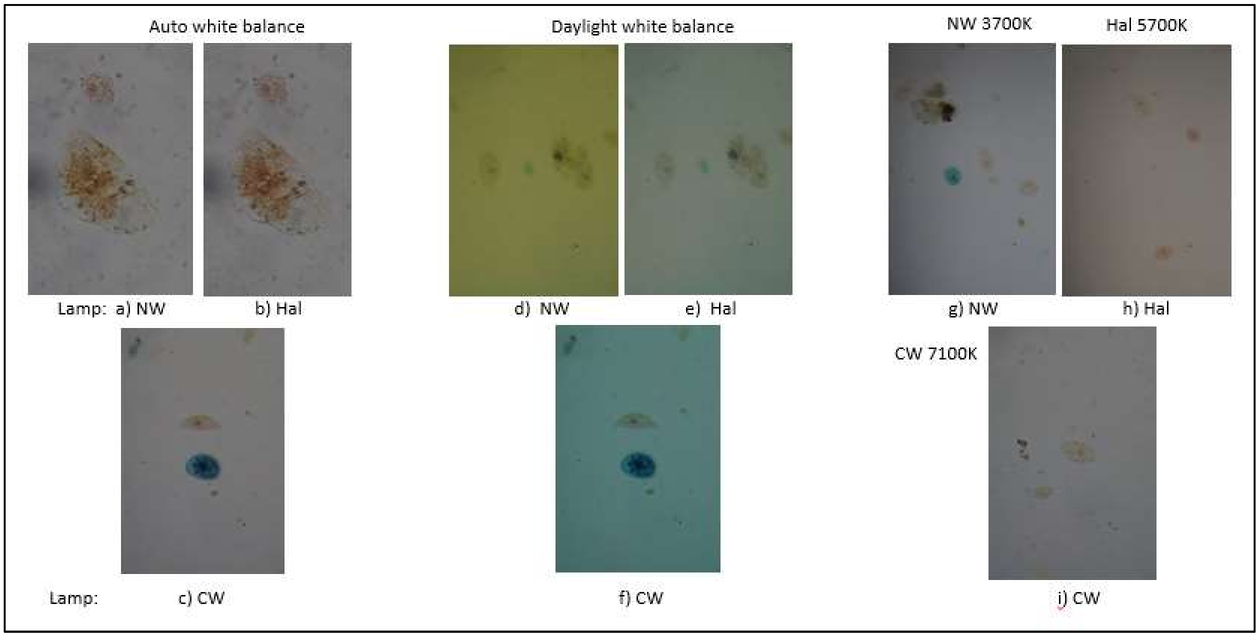


Figure-17: Euglena W.M., ISO 200, Neutral white (ND) and Cool White (CW) - no filters, Halogen (Hal) CB12 and 0.5 ND: a) 40X Neo NW 1/60, b) 40X Neo Hal 1/60, c) 40X Neo 1/750, d) 25X Neo NW 1/350, e) 25X Neo Hal 1/250, f) 40X Neo CW 1/750, g) 25X Neo NW 1/3000, h) 25X Neo Hal 1/3000, i) 40X Neo CW 1/350.

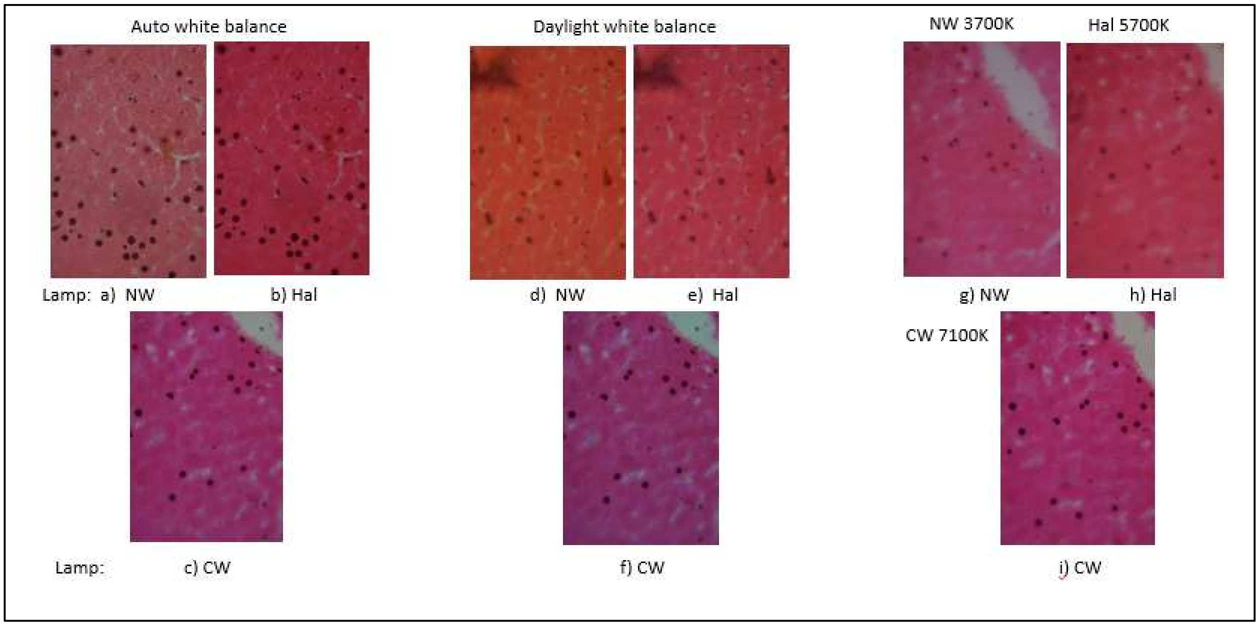
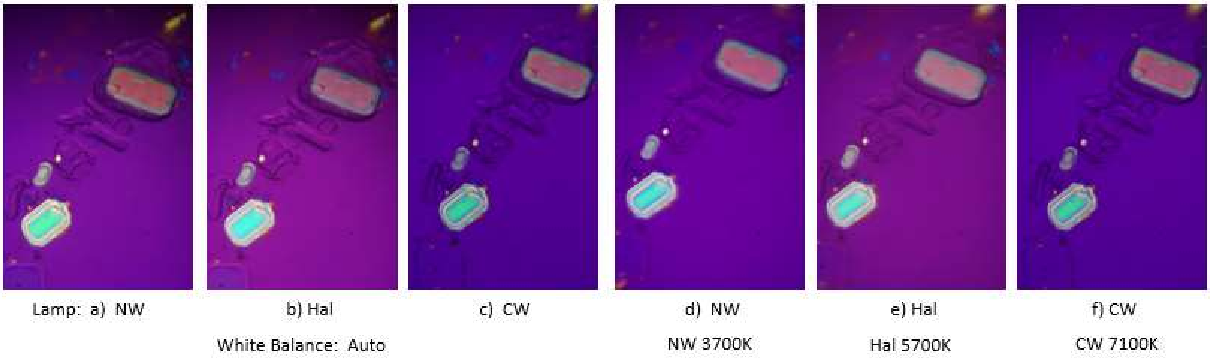


Figure-18: Liver T.S., 40X Neofluar, ISO 200, Neutral White (NW) and Cool White (CW) - no filters, Halogen (Hal) CB12 0.50 ND: a) NW 1/250, b) Hal 1/250, c) CW 1/500, d) NW 1/200, e) Hal 1/200, f) CW 1/500, g) NW 1/1500, h) Hal 1/1000, and i) CW 1/500.

Lamp: a) NW b) Hal c) CW d) NW e) Hal f) CW

Auto white balance Daylight white balance

Figure-19: Striated Muscle ISO 200, a) 63X Neofluar, NW 1/15, b) 63X Neofluar Hal 1/15, c) 40 X Neofluar CW 1/350, d) 40X Neofluar NW 1/200, e) 40X Neofluar Hal 1/125, and f) 40X Neofluar CW 1/350.



Lamp: a) NW b) Hal c) CW d) NW e) Hal f) CW

White Balance: Auto NW 3700K Hal 5700K CW 7100K

Figure-20: Strontium chloride crystal, 25X Neofluar, ISO200, Neutral White no filters, Halogen Blue and 0.5 ND, Cool White no filters Crossed Polars with red tint compensation: a) NW 1/30, b) Hal 1/30, c) CW 1/45, d) NW 1/60, e) Hal 1/50, and f) CW 1/45

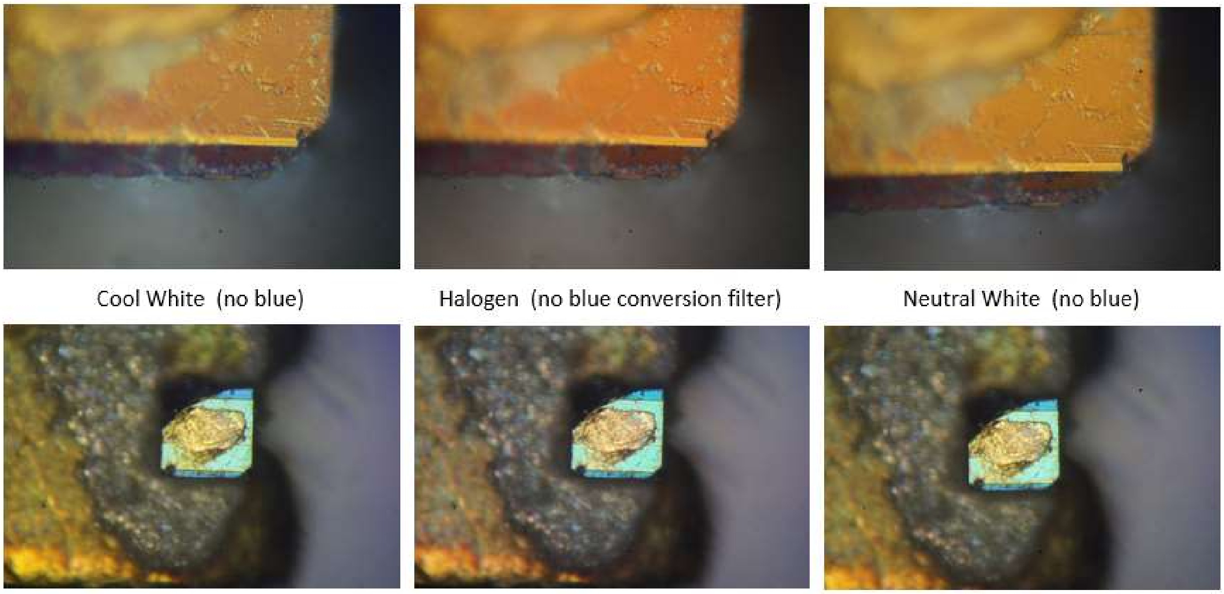


Figure-21: Epi-DIC images of a red laser die at 80X and 400X (Zeiss Pol 8X and Zeiss Pol 40X objectives) using sources Cool White and Neutral White LEDs and Halogen. No filtration or conversion filters were used in these photos. White balances was auto.

Figure-22: Euglena W.M., From left to right. Hoffman Modulation 400X, Hoffman modulation 400X and 2X Optovar, Phase Contrast 400X, and Phase Contrast 400X and 2X Optovar.

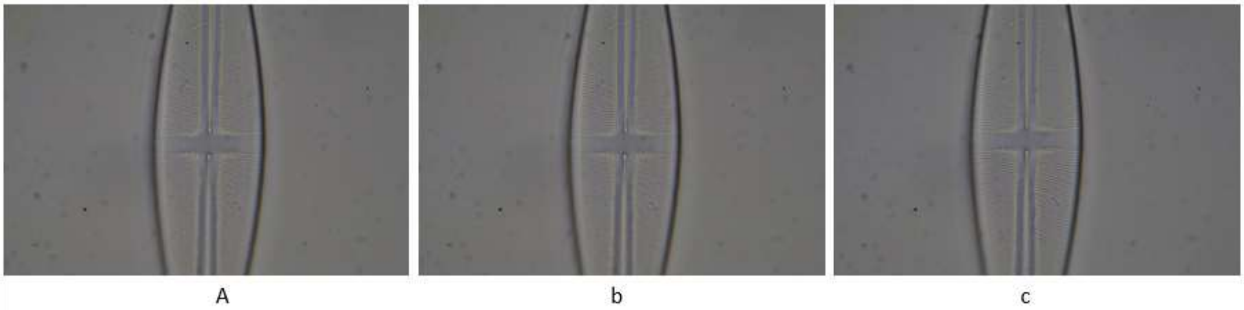


Figure-23: Diatom S. phoenicenteron, Zeiss 100X Planapochromat, N.A. 1.3, 1.3 N.A. Condenser, oiled condenser and objective, auto white balance, Neutral White Source a) Halogen CB12 and 0.5 ND filters 1/350, b) Cool white LED 1/250, c) Neutral white LED 1/350.

Conclusions for the High Power LED testing

In the above figures 16 to 23, it is well demonstrated that the auto white balance of the camera gave better unified results for all three sources than did other white balance settings. Exceptions to this was figure-18 the liver thin section, and figure-20 crystals under cross polarizers with red tint retardation plate. Both of these observations can be understood from the spectra in figures-13 and 14. For the predominantly red liver tissue samples, the spectra show different red components for each source, figure-13. It stands to reason, that for samples with a red tint, the three sources would give different observable red hues as is seen. Similarly, it is expected that the significantly different spectra in figure-14 for the pol/red tint tests, would also give different visual results which is exactly what is seen in figure-20. For all other test samples, there was very little visual difference observed between the cool white, the neutral white, and the converted halogen sources. Differential Interference Contrast images, figure-21, only showed minor visual difference in hue between the three sources.

The measured LUX values for throughput for the high power LEDs were one fifth of the value for the halogen source. However in no test, including the DIC and Pol testing, was there insufficient illumination for the task at hand. This fact is further supported by the 1000X diatom images shown in Fugre-23.

Final Conclusions

High Power LEDs have become a suitable replacement option for halogen lamps in microscopes. High Power LEDs are now available that can be a direct lamp replacement requiring no alteration to the microscope and microscope illuminator, allowing no change to the original optical engineering as designed by the microscope manufacturer. High Power LEDS provide sufficient intensity to work with all enhancement techniques including polarization, DIC, Hoffman Modulation and Phase contrast work. For most general work, working with LED replacements of the neutral white and cool white variety will provide no color difference than what would be observed with the original halogen lamp. However, it must be borne in mind that the LEDs do not have the same red-component specular intensities and also differ significantly from the halogen source. Therefore, samples that are primarily red in color or stain as well as techniques that modify the red portion of the observable spectrum may visually show significant color variation from LED to LED and to Halogen.