<u>Reducing the Risk of Mercury Exposure for a Sustainable Future</u>

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Research labs generally use high-pressure mercury or xenon light sources for fluorescence microscopy. These lamps are bright and produce ample light for most work, and, as a result, have been widely used in research for several years. But the increased brightness afforded by these light sources comes at a substantial monetary and environmental cost. Mercury-containing light sources present both a personal safety hazard and an environmental hazard. In addition, the cost of ownership of these light sources is high, with replacement bulbs costing several hundred dollars each. And, as experiments become more sophisticated, mercury-containing light sources often no longer meet the experimental needs of many modern laboratories.

Safety Hazard

The two most commonly used mercury-containing light sources are mercury arc lamps and metal halide bulbs. Mercury arc lamps contain a precisely measured amount of metallic mercury within an envelope in each bulb. When the lamps are cold, small droplets of mercury can often be observed on the inside walls; once the lamp is ignited, the mercury vaporizes over the course of a 5 to 10 minute transition phase. It is at this time that the pressure in the envelope starts to build. These lamps operate under very high pressure; explosions are not uncommon.

The microscope arc-discharge lamp external power supply is usually equipped with a timer to track the number of hours the burner has been in operation. Arc lamps lose efficiency (i.e. intensity) quickly and are more likely to shatter if used beyond their rated lifetime (200-300 hours).

Metal halide bulbs contain more mercury per bulb than mercury arc lamps (11mg v 34mg/bulb). While these bulbs are not under as much pressure as mercury arc lamps, when they do explode the potential safety hazard is quite high. These light sources are rated for 2000 hours. Like mercury arc lamps, they are more likely to shatter if used beyond their rated lifetime.

Mercury is not the only hazard associated with these bulbs. Mercury bulbs are rated at the highest ANSI/IESNA Standard RP-27 Risk Group 3, meaning hazardous even with momentary exposure, because of their strong ultra-violet emission. This radiation can cause permanent eye damage (including blindness) and serious skin injury (including burns and blistering).

To avoid eye damage, other personal injury, and/or property damage due to either the mercury contents or the UV-emission mercury arc lamps MUST be operated within a suitable fixture. A suitable fixture is one that will prevent the arc from being viewed directly during operation, and, in the event of a lamp rupture, will prevent hot (up to 800˚C/1472˚F) flying fragments of quartz and/or metal from escaping into the area.

Should one of the bulbs explode, this vaporized mercury would be lost into the room, directly exposing any individuals in the room or indirectly exposing them later by contaminating the work surface. Mercury is highly toxic and exposure can lead to serious liver, kidney or CNS damage. The National Institute of Occupational Safety and Healthy (NIOSH) for mercury is 0.05mg/m^3 (8hr, Time Weighted Average). However, air saturated with mercury vapor at 20˚C exceeds this level by 50-fold. Therefore, if a bulb were to explode, the potential exposure would be significant.

The following is adapted from the University of Oxford Medical Sciences Division regarding mercury bulbs used with microscopes:

Explosion risk

• Leave the room immediately and seek assistance e.g. Supervisor, Departmental Safety Officer, Area Safety Officer.

• Isolate the room and ensure appropriate warning notices are prominently displayed to restrict access. If necessary, lock doors to restrict entry into affected areas.

• If you believe someone has been exposed to mercury and only if it is safe for you to do so, remove them from the area. Normal emergency procedures apply, although Medical Assistance should be obtained.

• Leave the room for at least one hour before re-entering.

• Contact the Safety Office for advice.

• When it is safe to re-enter the room, remove the lamp housing to a fume cupboard and leave it open to fully dissipate for a further 24 hours.

• Collect all potentially contaminated glass and bulk fragments for disposal via the hazardous chemical waste stream.

• Record the incident in the Accident Book.

Critical Symptoms to be aware of for Mercury Exposure are:

Skin – There may be only mild irritation at the site of contact, but severe burns with blister formation could occur. Mercury can be readily absorbed by the skin and could therefore cause symptoms similar to inhalation or ingestion.

Eye – There may be only mild irritation, but corneal burns could occur.

Inhalation – Mild poisoning causes vomiting, diarrhea and a metallic taste in the mouth. Severe poisoning causes abdominal pain, shock, bloody diarrhea and damage to the kidneys.

Ingestion – There may be a headache with a metallic taste in the mouth, dizziness, slurred speech and a staggering walk. Increased salivation and painful red gums could occur. Severe poisoning could cause convulsions and kidney damage.

Not only are mercury-containing light sources potentially hazardous to scientists working in labs, but they are also potentially hazardous to the people responsible for properly disposing of the bulbs. OSHA has written a white paper describing how to protect employees from mercury exposure. It is recommended that workers be given disposable or protective clothing while on the job. This clothing must be removed and decontaminated prior to leaving the work site. It is also recommended that the air of the job site be regularly monitored and that workers exposed to mercury be medically monitored.

Environmental Hazard

Given the toxicity of mercury in people, it is not surprising that mercury is also toxic for the environment. In fact, mercury has long been recognized as being hazardous to the environment. While proper disposal of mercury bulbs should mitigate the effect of this toxin, proper disposal is not always guaranteed. Bulbs may be thrown away unknowingly in the trash, for example. And even if the bulbs are given to a disposal site, bulbs may break during the disposal process.

According to the EPA, mercury in the air or mercury that has leaked into the ground may find its way into bodies of water, affecting aquatic life and drinking water. Through microbial activity mercury is converted into methylmercury, a compound that, when accumulated in fish, may harm the fish and the animals that eat them. Methylmercury has been shown to cause reduced fertility, retarded development, abnormal behavior, and even death. In fish, methylmercury has been implicated in affecting reproduction by altering the endocrine system.

The effects of mercury on humans and the environment have been deemed significant enough to warrant the founding of a UN program in 2003 aimed at "reducing human-generated mercury releases". In 2013 over 140 governments agreed to a global, legally-binding treaty to reduce mercury pollution.

Cost of Ownership

The cost of ownership of mercury-containing light sources is high, both in terms total dollar outlay for bulbs and in terms of energy costs.

The rated lifetime of mercury arc lamps depends on how they are used, and the usual 200-hour limit can be compromised by an excessive number of starts (ignitions) or by repeated ignition of warm or hot lamps. Normal operation requires burn periods for a minimum of 30 minutes with a total number of ignitions not to exceed one-half the total number of rated hours (around 100 maximum). Therefore, a typical HBO 100 lamp should be ignited no more than 100 times and burned for an average of two hours per ignition. This is not a hard-and-fast rule because some burn cycles are much longer (lasting, for example, an 8-hour day). As mercury arc lamps age, they blacken and become increasingly more difficult to ignite due to degeneration of the cathode and anode. Each replacement bulb costs approximately $150. Over the lifetime of microscope system, the total number of replacement bulbs averages 150, meaning that $22,500 is spent on mercury bulbs.

The rated lifetime of metal halide light sources also depends on how they are used, but typically the bulb should be replaced after 2000 hours. The cost of a metal halide bulb is approximately $700. Therefore, over a similar lifetime, $10,500 will be spent on bulbs. Moreover, metal halide light sources require a liquid light guide to couple the light source to the microscope. The liquid light guide should be replaced every 4000 hours, or every other bulb change. The cost of a liquid light guide is also approximately $700. Taken together, the total cost of ownership for a metal halide light source is $10,500 for the bulbs + $5600 for the liquid light guide = $16,100.

The table below shows the costs described above.

Cost of Ownership of Mercury-Based Light Sources

	MERCURY ARC	METAL HALIDE
Replacement Bulbs	150	15
Total Bulb Costs	$22,500	$10,500
Total Liquid Light Guide Costs	$0	$5600
Total Disposal Costs ($5/bulb)	$750	$75
Total Management Costs (15min @ $10/hr)	$375	$38
Cost of Ownership	**$23,865**	**$16,213**

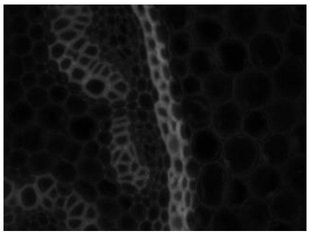
Alternatives

Until a few years ago, mercury-based light sources, in spite of their many flaws, were the best choice for scientists. They provided the brightest light at wavelengths needed to excite fluorescent probes in biological samples. However, new high-power light emitting diodes (LEDs) generate sufficient intensity to provide useful illumination for a wide spectrum of applications in fluorescence microscopy. Note that for the purposes of this paper all LED-based solid state devices will be termed 'LEDs', however there are several different types of solid state light sources that employ enhanced LED technology and these have various commercial names. The wider bandwidth featured by LEDs is more useful for exciting a variety of fluorescent probes. In addition, compared with the excessive heat and continuous spectrum emitted by arc lamps, LEDs are cooler, smaller, and provide a far more convenient mechanism to cycle the source on and off, as well as to rapidly select specific wavelengths. Currently available high-performance LEDs are sufficiently bright to function individually as a highly effective source of monochromatic light having low spatial coherence for observations in fluorescence epi-illumination or polychromatic light in transmitted microscopy. In other words, LED light sources provide a better light source for the kinds of experiments modern scientists do. For live cell experiments, LEDs are gentler and less toxic to the specimen. For experiments involving imaging over time, LEDs are capable of switching wavelengths within microseconds, something mercury-based light sources cannot do.

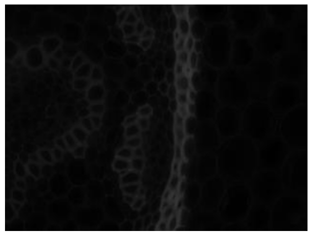
Intensity Comparison

A comparison of the 'brightness' of an LED light source versus a metal halide light source are shown below. The metal halide bulb had approximately 1800 hours on it.

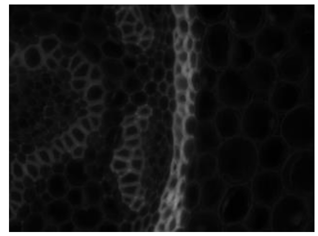
DAPI comparison



metal halide

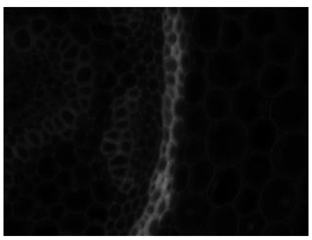


LED 1

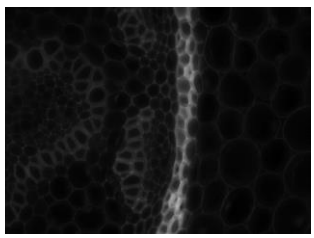


LED 2

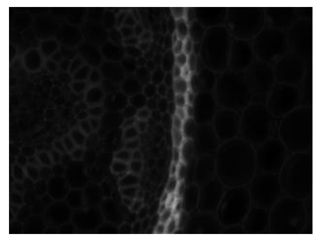
GFP comparison



metal halide

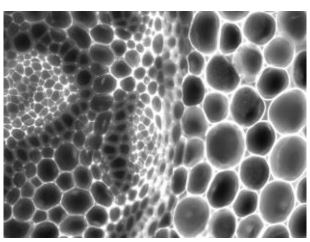


LED 1

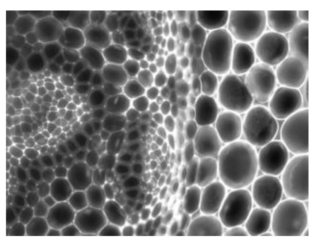


LED 2

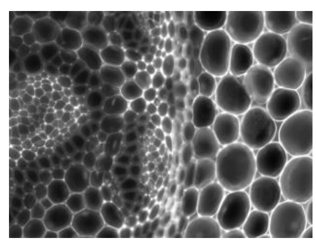
CY3 comparison



metal halide

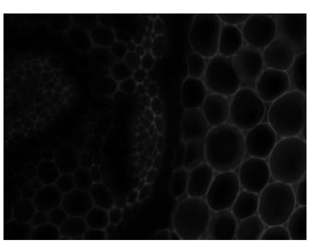


LED 1

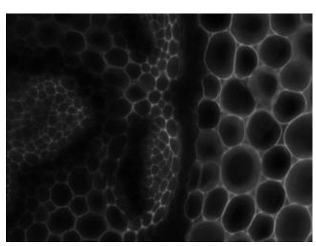


LED 2

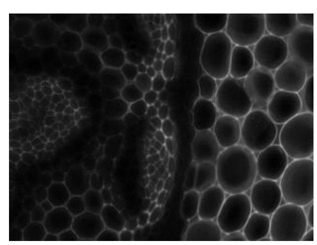
CY5 comparison



metal halide



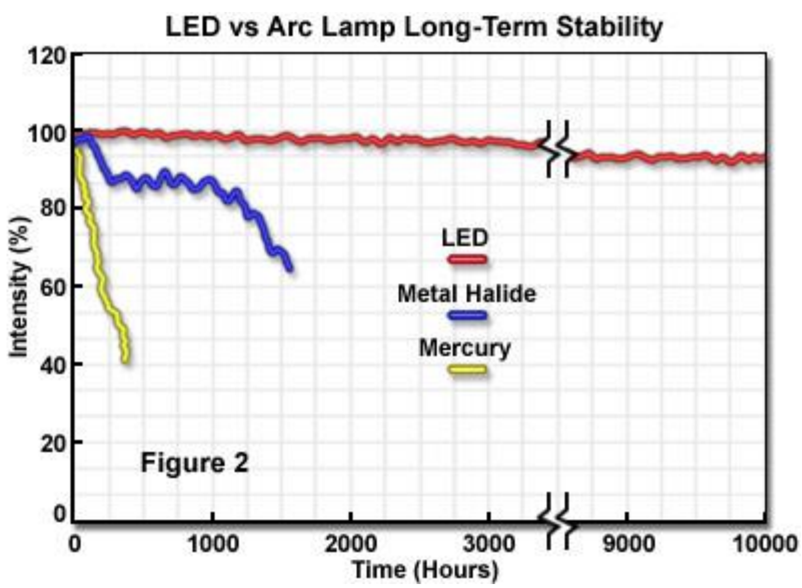
LED 1



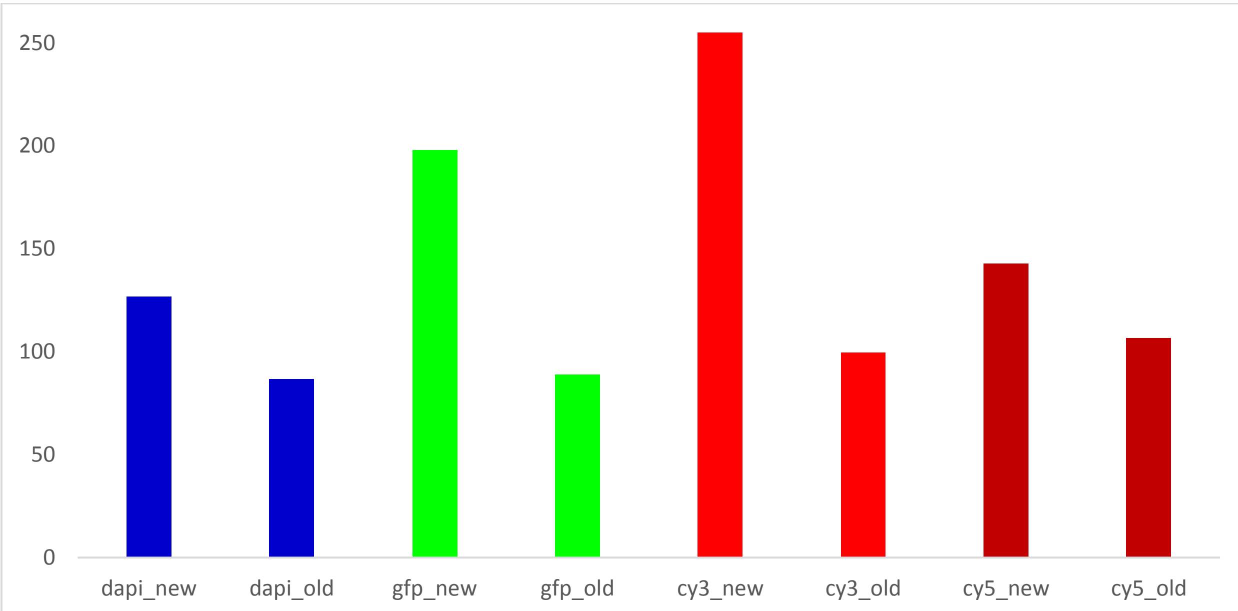
LED 2

All lamps that produce a significant level of heat, including LEDs, also exhibit a dependence of emission output on the source temperature. For incandescent and arc lamps, a period of up to one hour is required for the illumination source to become sufficiently stable to enable reproducible measurements or to gather time-lapse video sequences without significant temporal variations in intensity. A unique aspect of LED illumination is the outstanding spatial and temporal stability observed over time. This stability allows for highly accurate data over extended periods of time that can be used reliably for quantitative analysis. LEDs are governed by the fully reversible photoelectric effect during operation. As a result, LEDs feature the lowest operating temperatures of all light sources in optical microscopy.

Furthermore, provided LEDs are operated at the proper voltage and current, they feature significantly longer lifetimes than any of the other currently available light sources. Mercury and xenon arc lamps have a lifespan of 200 to 400 hours (respectively), whereas metal halide sources last approximately 2000 hours. Tungsten-halogen incandescent lamps have lifetimes ranging from 500 to 2000 hours, depending on the operating voltage. In contrast, many LED sources exhibit lifetimes exceeding 10,000 hours without a significant loss of intensity, and some manufacturers guarantee a lifetime of 100,000 hours before the source intensity drops to 70 percent of the initial value. The chart below, taken from work done by Michael W. Davidson at Florida State University and published on 'Molecular Expressions', demonstrates the long-term stability of LEDs.



The intensity instability seen in mercury and metal halide bulbs is often not accounted for in scientific experiments, making it nearly impossible to quantify images taken at different times. Below are data collected by My Green Lab demonstrating the intensity difference between a metal halide bulb at 305 hours (designated 'new' below), and the same bulb at 1895 hours (designated 'old' below).



Energy Consumption

Other light sources have a recommended 30 minute cool-down period before the lamp can be reignited. But this necessitates most microscope operators keep the lamp lit even when no illumination is needed, drawing a significant amount of power unnecessarily. This long waiting period is not required for LEDs, which are capable of reacting extremely fast (within a few microseconds).

My Green Lab tested the energy consumption of various light sources. The data show that a 120W metal halide lamp uses an average of 3.5 kWh/day when operated for 24 hours, such as during a time lapse experiment. In contrast, an LED light source operates at an average of less than 0.015 kWh/day. If an experiment requires illumination for one second every hour over 3 days, the arc lamps will be on for the entire 72 hours; the LED will be on only 72 seconds. The table below shows that because of the operational conditions of each light source an LED can use several hundred times less energy and dramatically lower the carbon footprint of the instrument.

Sample Experiment: One second illumination every hour for three days

For the purpose of illuminating the quantitative differences between the various types of scientific lighting available, consider the following experiment. One second of illumination is required every hour for three days. This experiment can only be performed 25 times with a typical metal halide doped mercury arc lamp, assuming the lamp is only used for experiments. The standard mercury arc lamp lifetime is ten times shorter, meaning this experiment would only be successful twice; the bulb would fail during the third experiment. An LED light source can support this experiment one million times at a fraction of the cost. The need for frequent and expensive replacement of mercury arc lamps due to the relatively short lifetimes, and the requirement to leave the lamp on even when not in use because of stability constraints, adds to the cost of ownership and unreliability of the microscope. The table below demonstrates the environmental impact of running a 3-day timelapse experiment with a metal halide light source versus running the same experiment with an LED light source.

STATISTIC	120W METAL HALIDE	LED	DIFFERENCE
ON Time	72 hours	72 seconds	3,600x
Energy Used	10.5 kWh	0.038 kWh	276x
CO_2 (2 lbs/kWh)	21 lbs	0.076 lbs	276x
Unit Lifetime Used	4%	0.0001%	40,000x

Taking into account the energy data provided in the table above, it is possible to calculate the total cost of ownership for the two different types of light sources. This is shown below.

Cost of Ownership: Metal Halide Light Sources v. LEDs

	METAL HALIDE	LED
Replacement Bulbs	15	0
Total Bulb Costs	$10,500	$0
Total Energy Costs ($0.1/kWh)	$438	$1.90
Total Disposal Costs ($5/bulb)	$75	$0
Total Management Costs (15min @ $10/hr)	$38	$0
Cost of Ownership	**$11,051**	**$1.90**

Mercury Comparison

The table below, which includes data from Osram product safety data sheets, shows the amount of mercury contained within these sources. The mercury arc lamp is equivalent to 9762 CFL bulbs and the metal halide lamp is equivalent to 1242 CFL bulbs.

Total Mercury Levels of Hg-containing bulbs and Hg-free LEDs

	MERCURY ARC	METAL HALIDE	LED
Bulbs	150	15	0
Mercury/bulb (mg)	11	34	0
Total Mercury (mg)	**1650**	**510**	**0**

In conclusion

Advancements in LED technology have made it possible to eliminate mercury bulbs from research labs. Mercury-containing light sources are hazardous to personal health and are toxic for the environment. Moreover, they cost over $1000/year in ownership costs in the form of bulbs and electricity. LEDs pose none of the health risks, and are more beneficial for scientific research. They are also less expensive to own, having little operating cost over the lifetime of the system.

Methods

Imaging data were acquired on a Leica DM5000B microscope with a DFC 365 camera. The objectives used were a 10x Apo NA 0.3, a 20x Apo NA 0.7, and a 40x Apo NA 0.85. Data were acquired and analyzed using Metamorph software. In all cases camera exposure times and light intensities were kept constant across light sources. Intensity measurements were taken using Fluorescence Reference Slides.

Energy consumption data were acquired using a Watt's Up Pro meter.

Light sources tested include the Leica EL 6000, the Lumen Dynamics X-Cite 120Q, the Lumencor Sola II (manual version), and the Lumen Dynamics X-Cite 120LED.

Special thanks to JH Technologies for generously providing the test space and the equipment.