LED and Mercury Based Fluorescent Light Sources in FISH Imaging and Analysis: Tech Review

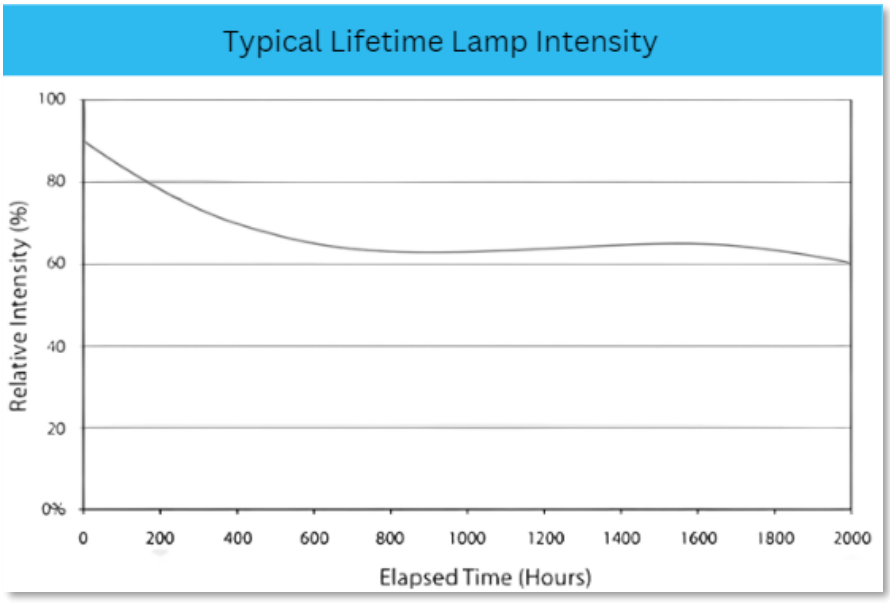
For over 25 years, BioView has been developing automated FISH imaging and analysis systems. Throughout the years, BioView has introduced new technological advancements to enhance both image quality and throughput while also reducing system maintenance and operating costs. LED fluorescent light sources allow us to do this. In this technical review, we share our insights on choosing the optimal fluorescent light source for your laboratory's needs.

Fluorescent in situ hybridization (FISH) imaging has long relied on mercury-based light sources. However, laboratories have started shifting to LED fluorescent light sources due to their energy efficiency, longer lifespan, and lower environmental impact. As the demand for LED fluorescent light sources increases, understanding the key performance differences between LED variants and the traditional mercury-based light sources is crucial.

Advantages of LED-based Fluorescent Light Sources Compared to Mercury-based Light Sources

1. Long Lifetime and Low Maintenance: LED fluorescent light sources deliver approximately 20,000 hours of operation, requiring no replacement of bulbs and/or light guides.
2. High Stability: LED fluorescent light sources show minimal decay or fluctuation in the light intensity output over time. This makes them an excellent choice for assays that rely on quantification of biomarkers. Additionally, automated imaging systems for FISH probes maintain control over the image quality and signal intensity of the captured images by dynamically adjusting the camera exposure to compensate for variability between samples and slow decay of the fluorescent light intensity.

   The diagram below describes the typical intensity of a mercury based light source over time.



3. No warm up time: LED fluorescent light sources provide immediate illumination without warm-up time, unlike mercury-based systems. They can be switched on and off frequently without compromising bulb longevity.
4. Energy Efficiency: LED fluorescent light sources consume less power compared to traditional light sources, resulting in lower energy costs and reduced heat generation (for example 120W vs. 40W).
5. Environmentally Friendly: LED Fluorescent light sources don't contain mercury, making them a more environmentally responsible lighting solution.
6. Intensity Control: Fluorescent light intensity is a crucial factor in the excitation of FISH signals. Fluorophores absorb energy from a light source at a specific wavelength and require certain level of light intensity to excite and produce a detectable signal. Higher light intensity results in a higher level of excitation, leading to stronger and brighter fluorescent signals. On the other hand, if the intensity of light is too high, it can cause fluorophores to degrade (also known as "photobleach"), leading to a decrease in signal intensity in matter of few seconds of exposure. Therefore, it is essential to balance the need for sufficient excitation with minimizing photobleaching. LED light sources enable precise software control over light intensity, allowing to reduce photobleaching of susceptible signals. BioView makes use of this software-level control by setting different fluorescent light intensities per filter and increasing camera exposure time to compensate when needed.

Differences in Various LED Fluorescent Light Sources

It is clear there are compelling advantages of LED fluorescent light sources. However, not all LED fluorescent light sources are made equal, and some should not be used for FISH analysis. This difference between these light sources becomes most apparent when comparing the intensity omitted by each.

Every fluorescent light source used for FISH analysis produces specific spectral peaks at specific wavelength ranges required for the excitation of FISH probes made by various manufactures. The fluorescent light intensity is measured by power output across each of the wavelengths omitted by the light source.

Here, an important distinction exists between effective output and "total" output omitted by the unit. While the fluorescent light source is set to provide high total power output, its power output at critical wavelengths, such as the FITC (green signals) channel, may be insufficient. This power deficiency impacts the visualization of subtle signals that are often associated with gene breaks or translocations. Furthermore, weaker fluorescent output necessitates increased camera exposure time, which reduces system throughput and prolonging the duration of imaging.

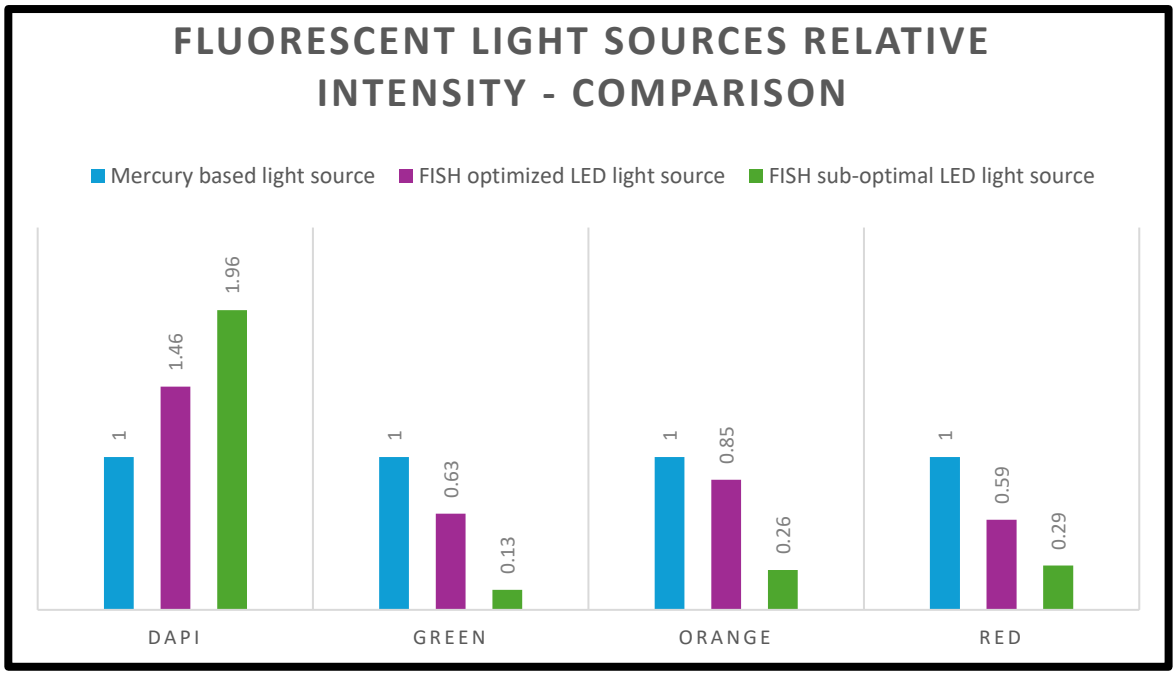
One would argue that sub-optimal excitation output can be compensated by the use of cutting-edge digital cameras introducing high sensitivity for signal pickup. However, even the most sophisticated cameras cannot detect signals that were not excited and are, thus, invisible.

Comprehensive Comparison of "FISH-Optimized" LED Fluorescent Light Sources

At BioView, we place image quality and authenticity at the top of our priorities. New technologies are carefully evaluated prior to their acceptance. The evaluation process combines rigorous assessment of specifications with hands-on performance testing. This process is designed to identify potential pitfalls and scenarios that could impact our systems' high quality and performance standards.

As part of this process, we conducted a comprehensive comparison of various "FISH-optimized" LED fluorescent light sources from different vendors.
The data was collected using an Olympus BX63 microscope, Semrock Spectrum Green, Spectrum Orange, Spectrum Red filters, and a x10/0.3 Olympus objective. The light was measured for each fluorescent channel using the PD300-UV photodiode sensor (Ophir, Israel) for the intensity power after the objective.

The bar chart below presents the fluorescent light intensity of two different light sources of two manufacturers, as measured by the BioView engineering department. We picked these two units as they demonstrate the vast range in quality one can expect using the most suitable vs. the least suitable LED fluorescent light source for FISH imaging and analysis purposes.



While comparing the relative intensity of different fluorescent light sources, our team uncovered a few key findings:

1. Mercury-based sources demonstrated a higher power output across all FISH-relevant fluorescent wavelengths, with the exception of DAPI.
2. There is a substantial difference in fluorescent intensities measured between the two different LED light sources:
    a. The fluorescent output used for the excitation of "Green" (Alexa 488, FITC) signals ranges from 63% to 13% of the mercury light source.
    b. The fluorescent output used for the excitation of "Orange" signals (Alexa 555, TRITC, Cy3) ranges from 85% to 26% of the mercury light source.
    c. The fluorescent output used for the excitation of "Red" (Alex 568, Texas Red) signals ranges from 59% to 29% of the mercury light source.

The selection of an appropriate fluorescent light source proves critical for both reliable FISH analysis and optimal automated imaging performance. While fluorescent LED light sources offer numerous advantages, we strongly recommend requesting detailed performance specifications for any fluorescent LED light source proposed as part of an imaging system package.

Whether you are a BioView customer or not, our engineers are happy to assist you in selecting a suitable fluorescent light source, which will ensure the accuracy of reported results and the maximization of your imaging system performance.

For expert consultation on light source selection, contact BioView's imaging experts at service-row@bioview.co.il