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Which light source is best suited for my application?
Lamp or LED?

K K ASWANI, [1], T JINADASA[2], AND CM BROWN[3]

1. Senior Applications Scientist, Life Sciences, Lumen Dynamics, Canada,
2. Post Doctoral Fellow, Department of Physiology, McGill University, Montreal, Quebec, Canada,
3. Assistant Professor, Department of Physiology, and Life Sciences Complex Imaging Facility Director, McGill University, Montreal, Quebec, Canada

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Fluorescence microscopy techniques have become the standard in life sciences research. These techniques rely on the light emitted from traditional HBO sources, lasers, and now LEDs. These technologies have increased the choices available to scientists to conduct their research using fluorescence microscopy. So which one should you choose? Should you replace your trusted mercury lamp for one of these other technologies? Which light source should you use for your research - Lamp, Lasers or LEDs?

The answer lies in your specific application. Is photobleaching or phototoxicity an issue with your application? Are you conducting multi-colour imaging and how fast do you need to switch between colours? Is stability or noise a serious issue for you? How long are your imaging sessions? How much power do you need?

This presentation will cover the pros and cons of lamp vs. LED technology in the hope to assist in answering these questions relating to new and traditional fluorescence excitation sources.

HBO sources: these are still predominant in labs, but are being replaced by other technologies such as metal halide (X-Cite –like sources) and LEDs due to their longer lifetimes, convenience, and uniformity of illumination (Figure 1). Mercury lamps emit high power, but may also contribute to photobleaching and decreased cell viability when imaging live samples. Heat and shuttering may affect image stability.

Metal Halide X-Cite-like sources provide high intensity fluorescence excitation with a longer lifetime (guaranteed), pre-aligned bulbs, shuttering ability and light delivery via a liquid light guide providing uniform illumination.

LEDs entered the market many years ago with low powers which were disappointing. Recent advances in this technology are producing a wide variety of powerful LEDs to cover the entire UV and visible to the near-IR wavelengths. LED lifetimes are tremendous, and stability is unparalleled.

We will present the X-Cite XLED1 system with a wide range of high-power, narrow wavelength LED options. These wavelength modules are easily switchable by any user with a switching and ON/OFF speed that is unmatched. The system is entirely configurable, matching LEDs to user applications and supported by a wide variety of imaging software. The system can also be programmed to generate timed pulses (including advance/delay sync out pulses) in a specific order to allow for automated imaging without an external trigger.

The light technology required for an application depends on the needs of the specific experiment that will be summarized in this workshop in order to help the user make an informed decision.

References

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***Figure 1. Field illumination intensities and fluorescence images**. Images were taken on a Zeiss 200M microscope with an EGFP fluorescence cube (EGFP-ET, Chroma Technologies Corp.) and a 63x/1.4 oil immersion lens with a Zeiss Axiocam HRm CCD camera with no pixel binning. A) Images were taken **of a** yellow-green fluorescent slide in order to observe the excitation field illumination uniformity. B) The pixel intensities of a diagonal line across each image from A were measured using a 3 pixel average. The intensity data was normalized to the maximum intensity for each image in order to compare the field uniformity for the three light sources. C) CHO cells stably expressing paxillin-EGFP where stained with DAPI, fixed with 4% formaldehyde and imaged with each light source. EGFP was imaged with the same filter cube as in part A, while DAPI was imaged with a Carl Zeiss Filter Cube 49. For the LED illumination the FITC and DAPI LEDs were used. Images are of the same cells in the same field of view, they were background corrected, processed with a "sharpen" filter and displayed with a gamma factor of 0.45 in the Zeiss ZEN software (Carl Zeiss, Jena, Germany).*