X-Cite®
Fluorescence Illumination • In Control

Time-Lapse Imaging with Lamp & LED – Stability vs. Repeatability

Challenge
Selecting which light source is most suitable to use based on optical stability

Solution
X-Cite® LED light sources for repeatable light exposure every time a sample is imaged

Benefit
Repeatable sample exposure for reliable data

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Application Overview

NEED FOR STABILITY

Fluorescence microscopy is a standard technique used in most laboratories around the world. With the invention of fluorescence proteins such as GFP, publications using fluorescence techniques have skyrocketed. HBO/mercury lamps were replaced by pre-aligned lamps such as X-Cite® in the early 2000s. LED light sources for microscopy were almost introduced too early when prices were high and output was still low, creating a poor impression of this technology in our industry. Only a few years later, LEDs are gaining popularity due to availability of higher power LEDs, influenced by the lighting and projection industries. This shift in technology has generated many questions about LEDs, and this article focuses on answering at least one of the common questions: Which light source is more stable - lamp or LED?

Time-lapse imaging involves taking several images over a course of time which can range from hours to days. The exposures of these images are in the range of milliseconds to seconds. When arc lamps are used in imaging, a physical shutter is required to allow light to reach the sample while the camera is exposing. The shutter also serves to block light when the camera stops the exposure, thereby eliminating unnecessary phototoxicity and photobleaching caused by light exposure to the sample when it is not being imaged. This works well as far as protecting the sample. However, the user needs to be aware of the degradation of lamp intensity when it is left on for the duration of the experiment (Fig. 1).

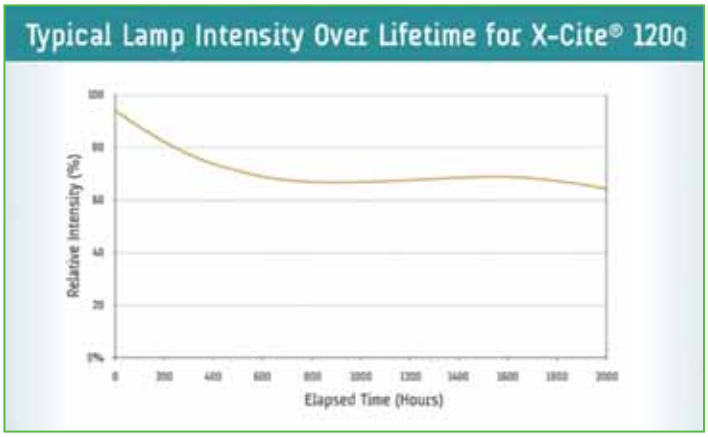

Fig. 1 – Intensity decay of an arc-lamp over 2000 hours

LAMP STABILITY

Most AC lamps demonstrate fluctuation in power intensity as shown in Figure 2a. For most routine imaging applications, this fluctuation of ±2% over two hours is not an issue. However, over a time-span of 100 hours, the intensity of the lamp drops by 10% when compared to a new lamp. This should be taken into account when repeating experiments after days or weeks, or proper controls should be imaged in parallel. In addition, lamps are also prone to flickering and arc wander. If this occurs in the middle of an exposure, data may be severely skewed, and potentially require repeating experiments.

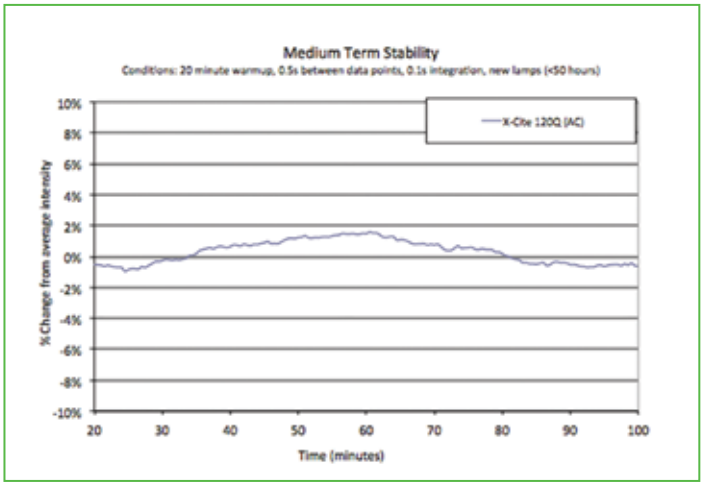

Fig. 2a – AC lamp stability over 120 minutes

DC lamps such as X-Cite 200DC or X-Cite *exacte* are most suitable options if the user requires higher stability for shorter imaging exposures (Fig. 2b, green curve).

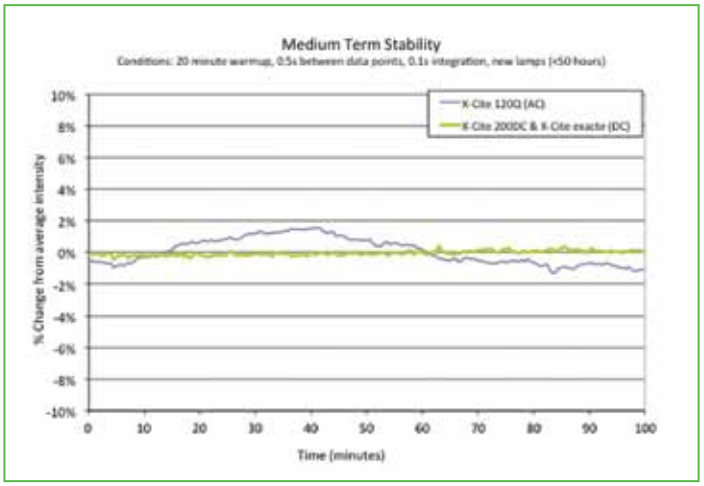

Fig. 2b – AC vs DC lamp stability over 120 minutes

In systems that have a feedback mechanism (e.g. X-Cite *exacte*), the light intensity can be maintained by engaging a Closed-Loop Feedback mechanism. This mechanism adjusts the iris in order to maintain the output light over the course of the experiment. Figure 3 depicts the output stability with and without Closed-Loop Feedback engaged. Once this mechanism is active, the variability of light output is reduced to less than ±1% over 100 hours. Figure 3 also demonstrates that DC lamps degrade by about 10% after 100 hours when compared to a new lamp for systems without Closed-Loop Feedback.

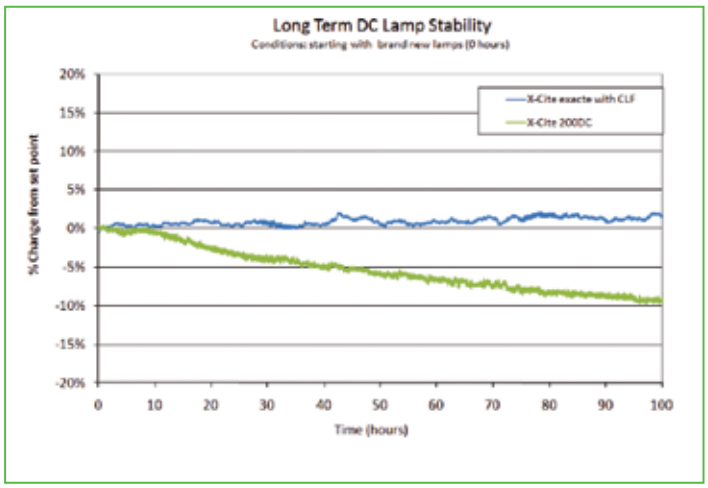

Fig. 3 – DC lamp intensity change with and without closed-loop feedback over 100 hours

LED STABILITY

Figure 4 shows the relative intensity of four different LEDs present in the X-Cite XLED1. An inherent property of LEDs is to have an intensity spike when first turned on until the LED reaches thermal equilibrium. It takes a few minutes for the LED to stabilize, and after this time, most LEDs are stable within a percent of intensity. All LEDs from any manufacturer will demonstrate this same behaviour. The important thing to note about this is the drop from ON time to when the LED is stable; the magnitude of this drop is related to thermal management.

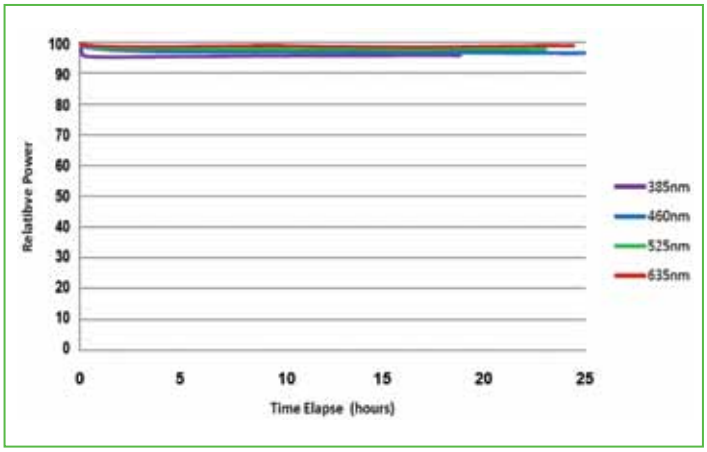

Fig. 4 – Typical intensity stability of LEDs

If an LED is thermally well-managed, the drop in this intensity will be lower than if the LED temperature is poorly maintained (Fig. 5). Poor thermal management will also translate to a shorter lifetime of the LED.

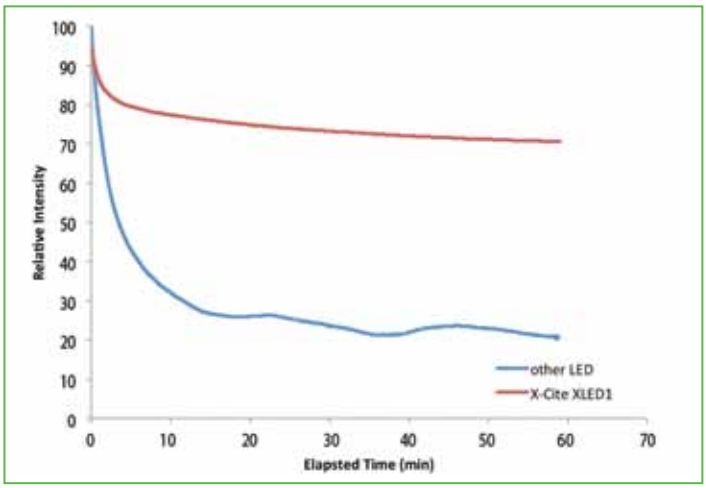

Fig 5 – Intensity drop and stabilization of X-Cite XLED1 vs. another LED unit

Repeatability is a better measure of a microscope system than simple lamp output stability when left on. Since we are discussing time-lapse microscopy applications with LED sources, there is also the capability of turning them on and off with every frame of exposure (something which cannot be done with arc lamps due to electrode heating, melting and cooling times). Due to the solid state design of LEDs, turning them on and off results in extremely consistent output shapes when the thermal design is precise enough, which can reduce the frame-to-frame variability in the experimental exposure to levels much better than even the stabilized arc lamp with Closed-Loop Feedback. The key to selecting an LED illumination system is to ensure that every time the LED is switched on, the intensity of light exposure is repeatable. LEDs do not offer an advantage when used as a lamp in which the unit remains on and the light is shuttered. Leaving LEDs turned on exhibits the temperature sensitivity of their design, while at the same time it negates the advantage of having a system that can switch ON and OFF rapidly only when the camera is exposing (through TTL or USB control). As long as every time the LED is switched ON and produces the same repeatable intensity of light, i.e., the sample receives the same amount of light each time, the data produced from these exposures will be more consistent and show less variability due to light source fluctuation than other methods.

Figure 6 attempts to reproduce real exposure times, showing relative intensity when an LED is turned on for 10 ms, 20 ms, 50 ms, and 100 ms. The stability in each case is better than 1% when the LED stabilizes.

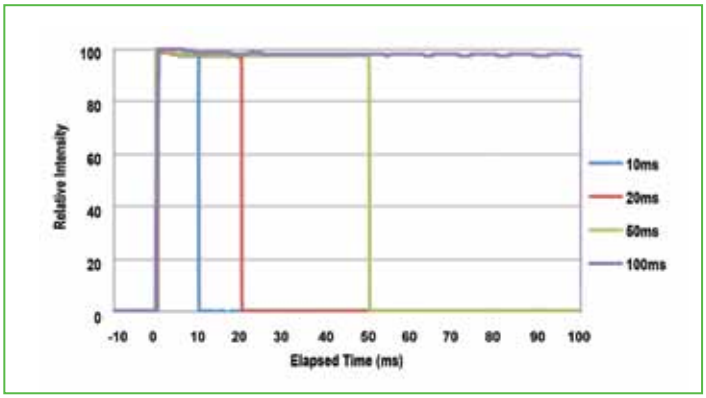

Fig. 6 – X-Cite XLED1 intensity stability over various exposure times

Fig. 7 depicts what is really important with regards to stability. This is ensuring that the amount of light reaching your sample each time is a repeatable event. Here, the LED is pulsed for 10 ms with an interval of 500 milliseconds, pulsed again, and this pattern is repeated for 10 minutes (Fig. 7a).

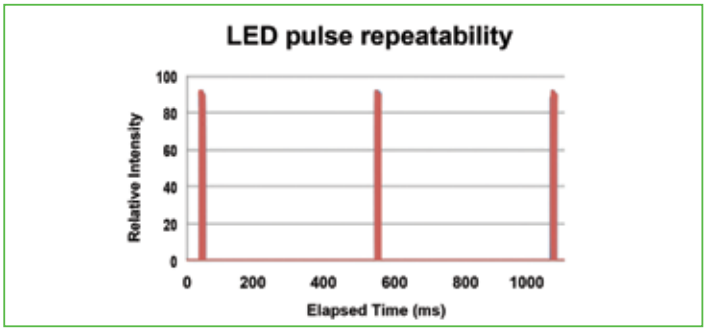

Fig. 7a - X-Cite XLED1 10ms pulse repeatability over 10 minutes.

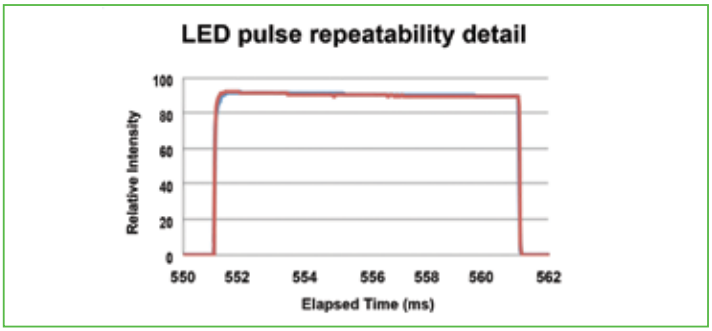

Fig. 7b - Intensity of each 10ms pulse (from fig. 7a) over 10 minutes, overlaid onto one graph.

If you then overlay every pulse over the 10 minutes each pulse overlaps to the point where separate lines cannot be seen (Fig. 7b). This means that every time the sample is exposed to light, the area under this inset curve is the same, meaning that the sample is receiving the same amount of light each time the LED is turned on. This translates to the fact that stability is important if the light is left on for shuttered exposures, but what is key is repeatability of sample exposure to ensure viable data.

Conclusion

LEDs have gained popularity in our homes as well as other industries, and are starting to migrate into research and hospital laboratory imaging systems. With the ease of use, long lifetimes and low waste, they are only destined to continue to take over the role of the traditional lamp. This application note attempts to explain the differences in stability between lamps and LEDs, and also to explain the importance of repeatability, as that is what counts when comparing images taken between time points, even if this is within the same short experiment or time-lapse experiment. In a recent application note published with the EMBL, the author stated: "X-Cite 120LED shows a much higher short-term stability of ±0.2% compared to HBO showing ±2%. The long-term stability is much higher compared to the tested HBO lamp. The X-Cite 120LED lamp showed no significant reduction of intensity after 48 hours of imaging whereas the HBO lamp that was used lost 20% of its initial intensity over the same duration."

Lamps, however still have a place in microscopy imaging until LED technology can fully replace their capabilities. They are still brighter at most wavelengths, and the only technology for useful images requiring fluorescence excitation under 350nm and over 680nm. Demand for LEDs in these wavelengths is low in large industries such as lighting and projection. It is these markets that force manufacturers to create solutions to compete for their business. Unfortunately, our microscopy industry is only a tiny drop in this ocean, and in order for microscopists to have an affordable solution for our needs, we have to rely on technology developed for the lighting giants.

Further Reading:

Barbara Foster. Light-Emitting Diodes: A New Solution for Fluorescence. American Laboratory, October 2011, 36-38

Feng Xu, James Jonkman and Kavita Aswani. X-Cite Application Note – Pulsed LED Illumination

Neumann Beate and Yury Belyaev. X-Cite Application Note - Use of White Light Fluorescence LED in Long-term Live Cell Imaging Applications.

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