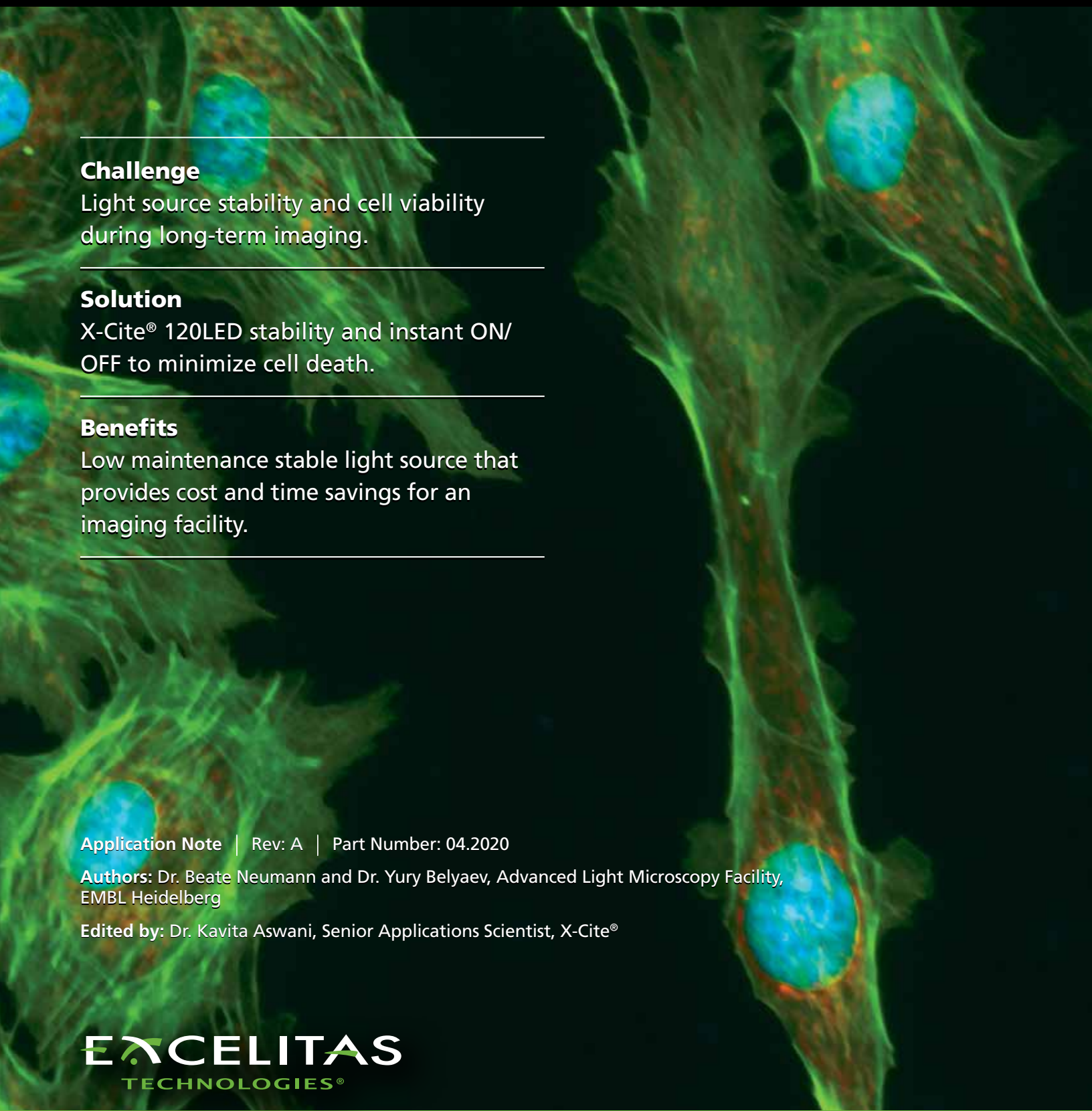
X-Cite®
Fluorescence Illumination • In Control

Use of White Light Fluorescence LED in Long-term Live Cell Imaging Applications



Challenge
Light source stability and cell viability during long-term imaging.

Solution
X-Cite® 120LED stability and instant ON/OFF to minimize cell death.

Benefits
Low maintenance stable light source that provides cost and time savings for an imaging facility.

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Authors: Dr. Beate Neumann and Dr. Yury Belyaev, Advanced Light Microscopy Facility, EMBL Heidelberg

Edited by: Dr. Kavita Aswani, Senior Applications Scientist, X-Cite®

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INTRODUCTION

The use of fluorescence light microscopy for live cell imaging has increased dramatically since the discovery of fluorescence proteins[1] (Tsien et al.). Currently, we are able to fluorescently tag most proteins in order to study their localization and dynamic behavior. Despite these developments, long-term live-cell imaging can still be challenging as extensive light exposure might lead to cytotoxic stress.

Research involving fluorescence imaging is no longer a simple "is it present or absent" question. It is now more quantitative, looking for small changes of fluorescence intensity in the image over long time periods, influenced by drugs or genetics. Imaging should be performed in physiological conditions, thus one needs to reduce sample exposure to light as much as possible, to exclude any effect on cell proliferation.

Our microscopy facility conducted a range of high-throughput screening projects. For example, we studied the effect of genome-wide siRNA treatment on cell proliferation typically over a 48-hour time span, acquiring images every 30 min throughout the imaging period[2] (Neumann et al.).

For these studies, it is very important to have a uniform and stable light source to ensure that the images and data are accurate not only over the field of view but also between the time points. We also require the ability to turn the light off and on quickly, thereby limiting sample exposure to light when images are not being acquired. Sample exposure to light leads to unnecessary photo-bleaching and unwanted cell toxicity[3] (Schneckenburger et al.).

Traditionally, we have used Xenon or HBO lamps on our high-throughput screening systems, with automated time-lapse experiments up to 48 hours. In this note, we evaluate the feasibility of using an X-Cite® 120LED system in our high-content time-lapse studies.

MATERIALS AND METHODS

Imaging was conducted using a Zeiss Axiovert 200M equipped with an AxioCam MRm camera. The microscope was controlled by Zen 2012 (blue edition) software. HeLa cells stably expressing H2B-mCherry were imaged with EC Plan-Neofluar 10x/0.3 Ph1 objective in 8 positions of an 8-well LabTek over 48 hours with a temporal resolution of 15 minutes. We used fluorescence filter sets with 565/24 excitation and 620/52 emission. Nuclei segmentation and counting were performed in Volocity software (PerkinElmer). The number of cells per position at the beginning of the experiment was between 34 and 74 (X-Cite 120LED) and 52 and 114 (for HBO). Obtained proliferation curves were averaged over all 8 positions. Details on the imaging conditions are presented in the table below.

IMAGING PARAMETERS	HBO	X-CITE® 120LED
Filter	mCherry	mCherry
Exposure	400 ms	400 ms
Power	3.1 mW	3.1 mW

For the illumination stability data with X-Cite 120LED and with HBO, we imaged a green Chroma slide with 20 ms exposure for a total of 3 minutes, generating 2258 images and measured average intensity over time. A GFP filter cube was used with excitation 470/40 and emission 525/50. The light intensity was set to 50 µW at the sample plane. Illuminated area was 90 µm in diameter.

Data for the illumination uniformity was generated with the same conditions as above except for the field diaphragm size which was set to illuminate a sample area of 300 µm in diameter. For stability and uniformity of illumination we used LD Plan-Neofluar 40x/0.6 Corr objective. The fluorescence light intensity after the objective was in every case measured with X-Cite Optical Power Measurement System. Produced images were analyzed with FIJI software.

RESULTS – Proliferation

Our proliferation studies indicated that in our lab, when imaging HeLa cells over 48 hours with a traditional HBO lamp vs. the X-Cite 120LED, X-Cite 120LED allowed slightly larger cell proliferation when compared with HBO. This increase is not statistically significant, and also may be due to the fact that the starting cell count was different in the two instances (Figure 1).

Cell Proliferation with X-Cite 120LED vs. HBO lamp

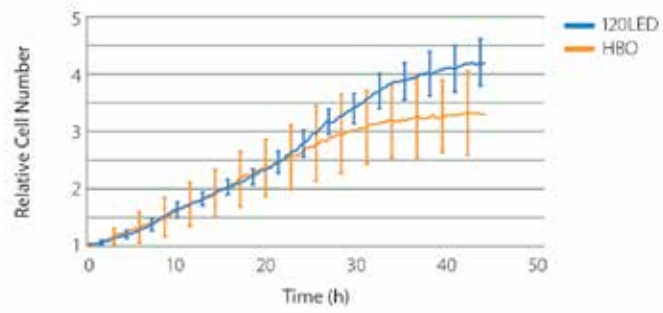

FIGURE 1: HeLa cells proliferation when imaged with X-Cite 120LED vs. HBO. Error bars represent standard deviation. The light intensity was set up to have the same excitation power for 120LED and HBO. To avoid bleaching of non-imaged part of the sample, field stop was set to limit camera field of view only.

Stability of X-Cite 120LED vs. HBO lamp

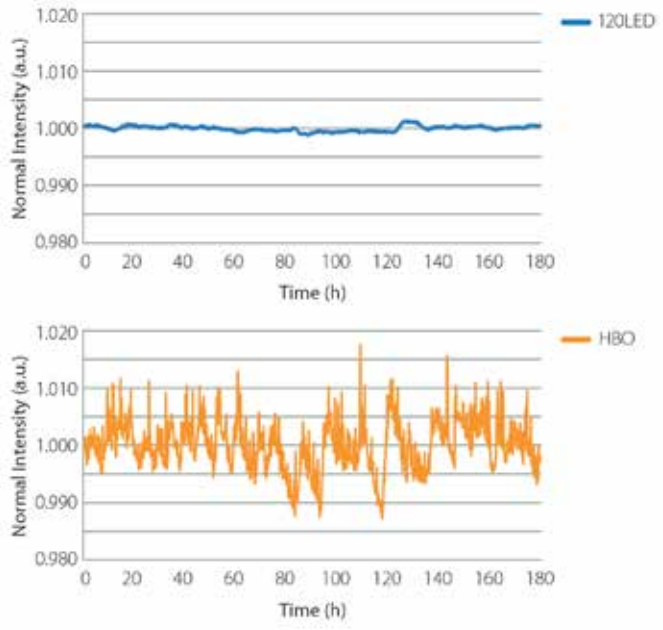

FIGURE 2: Normalized intensity of signal generated from a fluorescent sample imaged by X-Cite 120LED vs. HBO lamp; 2258 images acquired over 3 minutes (20 ms exposure). The curves have been corrected for photo-bleaching.

Field Uniformity of X-Cite 120LED

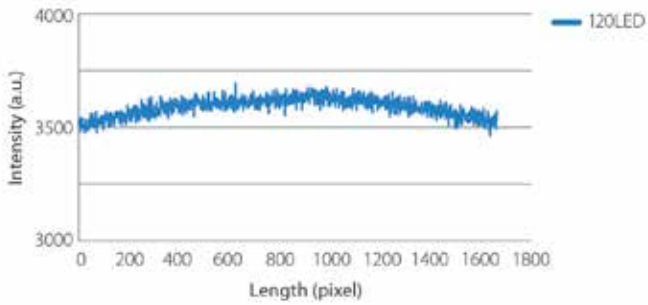

FIGURE 3: Intensity profile across green Chroma slide imaged with 40x objective with X-Cite 120LED.

Figure 2 demonstrates how the LED system tested in this study provides better stability for imaging when compared with an HBO lamp. The short-term stability of 120LED is in the range of 0.2% whereas the HBO lamp stability is almost 10 times lower and amounts at 2%.

We also found that 120LED shows very good long-term stability. We have not observed any significant illumination intensity change for LED after 48 hours, while HBO intensity was found to be about 20% less than at the beginning of the experiment.

Additionally, 120LED shows excellent uniformity of illumination. An intensity variation over whole field of view is less than 5% (Figure 3).

DISCUSSION

Traditional HBO lamps are being replaced in labs by either pre-aligned bulb systems, or by newer LED technology. In this application note, we demonstrate some of these benefits through our time-lapse experiments. We have shown that LEDs allow for slightly higher proliferation of cells when compared with HBO – this could be due to the fact that the LED is off when images are not being acquired. HBO lamps cannot be turned on and off due to their long warm-up times. This may lead to UV/IR filter leakage, leading to cell stress. When the mechanical shutter is used, it also produces significant temporal overhead due to limited speed of shutter opening and closing and subsequently the lamp is not used efficiently during long time-lapse experiments. We have also demonstrated that LEDs provide higher stability when compared to HBO. This is inherent to each technology, as LEDs are more stable, vs. HBO lamps that are prone to flicker and arc wander. LEDs also produce extremely uniform illumination which allows for accurate quantitative image evaluation without performing flat field correction.

WHY SHOULD USERS CONSIDER A SWITCH TO X-CITE® 120LED OR SIMILAR TECHNOLOGY?

1. **Time savings**: No maintenance, no bulb changing or aligning;
2. **Short-term stability**: X-Cite 120LED shows a much higher stability of 0.2% compared to HBO showing 2%;
3. **Long-term stability**: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;
4. **Costs**: If the price is comparable to a normal XBO/HBO, X-Cite 120LED has lower running costs, no consumables and longer operation time. The LEDs are warrantied for 25,000 hours or 3 years, and will last longer than a lamp ever would;
5. **Triggering capabilities**: Better for live cell imaging, as the camera exposure can be synchronized with illumination as opposed to the HBO lamp with mechanical shutter, which might have vibration and significant timing overheads;
6. **Proliferation rate:** The long-term proliferation rate is slightly higher than that obtained with the HBO lamp.

CONCLUSION

Our results indicate that LEDs provide more stable and uniform illumination for imaging conditions, and also allows for slightly higher cell proliferation when compared with a traditional HBO lamp.

REFERENCES

1. Tsien et al., Annu Rev Biochem. 1998;67:509-44.
2. Neumann et al., Nature. 2010 Apr 1;464(7289):721-7.
3. Schneckenburger et al., J Microsc. 2012 Mar;245(3):311-8.

EXCELITAS TECHNOLOGIES®

www.excelitas.com
x-cite@excelitas.com

2260 Argentia Road
Mississauga, Ontario
L5N 6H7 CANADA

Telephone: +1 905 821-2600
Toll Free (USA and CAN): +1 800 668-8752
Fax: +1 905 821-2055

For a complete listing of our global offices, visit www.excelitas.com/locations