TN429: Improvision Micro-Shutter performance

Introduction

In fluorescence microscopy a shutter is often used to limit the fluorescence exposure of the sample under investigation, as excessive exposure can cause photobleaching of the probes and damage to living cells. However, opening and closing the shutter each time an image is acquired adds a delay during a time-lapse experiment, thereby reducing the number of images that can be acquired in a given time interval.

The Improvision Micro-Shutter is a 6 mm diameter shutter, used in conjunction with the X-Cite 120 fluorescence light source (EXFO Photonics Solutions Inc.) and a standard fluorescence microscope. A test was performed in order to compare the performance of the Micro-Shutter against the performance of a built-in microscope shutter in terms of both speed of operation and ability to reduce exposure of the sample to fluorescence illumination.

Materials and Methods

Hardware

Axiovert 200M microscope (Carl Zeiss Inc.) with built-in fluorescence shutter

Improvision Micro-Shutter and Improvision Shutter Hub control unit

Orca ERG camera (Hamamatsu Photonics)

X-Cite 120 fluorescence light source (EXFO Photonics Solutions Inc.)

Software

Phylum Live Acquisition.

Method

An experimental protocol was set up in Phylum Live Acquisition to acquire 30 Z slices through a sample at five time points, therefore acquiring 150 images in total. The camera was set to 100 ms exposure, full frame, 1 x 1 binning. The time taken to acquire the complete image sequence was measured without using a shutter at all, using the Zeiss shutter and using the Improvision Micro-Shutter. Note: in both cases, the shutter is closed during the time that the Z focus drive is moving.

The time taken to open and close both types of shutter and the time taken to move the focus drive between images were measured using the “Measure Latencies” feature in Phylum Live Acquisition.

Results

Table 1. Shutter speeds

Shutter	*Time required to open and close (ms)*
Zeiss built-in shutter	65.0
Improvision Micro-Shutter	1.5

Time taken to move the Zeiss focus drive between images: 150 ms

Table 2. Total time taken to acquire image sequence

Shutter used	*Total acquisition time (seconds)*
No shutter	51
Zeiss built-in shutter	71
Improvision Micro-Shutter	51

Table 3. Total time sample exposed to fluorescence

This is defined as Total acquisition time – time taken to move focus drive 150 times.

Shutter used	*Total exposure time (seconds)*
No shutter	51.0
Zeiss built-in shutter	71 – (150 x 0.15) = 48.5
Improvision Micro-Shutter	51 – (150 x 0.15) = 28.5

Conclusion

When measured using Phylum Live Acquisition, the Improvision Micro-Shutter was found to open and close over 40 times faster than the Zeiss built-in shutter (1.5 ms compared to 65.0 ms).

The image acquisition test showed that using the Improvision Micro-Shutter reduces the length of the experiment by over 28% compared to using the built-in shutter (Table 2). This means that using a conventional shutter can slow down image acquisition by a considerable number of seconds or even minutes over the course of a time-lapse experiment.

Although the time taken to acquire the image sequence was the same as using no shutter at all, the Improvision Micro-Shutter was shown to reduce total sample exposure to fluorescence by over 44% (28.5 seconds compared to 48.5 seconds) (Table 3). Using the Zeiss built-in shutter was only marginally better than using no shutter at all in terms of sample protection.

In conclusion, this test shows that using the Improvision Micro-Shutter reduced both total acquisition time and sample exposure by a significant amount when compared to the Axiovert 200M microscope built-in shutter.