

TECHNOLOGY OFFER

Dynamic Background Compensation for Fluorescent Microscopy

Background light that typically contaminates a fluorescent image is removed.

BACKGROUND

In confocal, two-photon, and nonlinear optical microscopy, light of one wavelength induces light of a different wavelength. By collecting this returned light from multiple sampling locations across a sample, an image can be formed, but background light contaminates the image.

Therefore, such microscopy is typically done in dark conditions. With this invention, it is possible to measure in conditions with ambient light.

For applications like optogenetics where background light is intense, temporal or spectral separation is required to separate the light for optogenetic activation from fluorescence measurement. With this invention, simultaneous activation and measurement is possible. For the same reason, the invention allows visual stimulation of the retina or other light sensitive tissue during measurements.

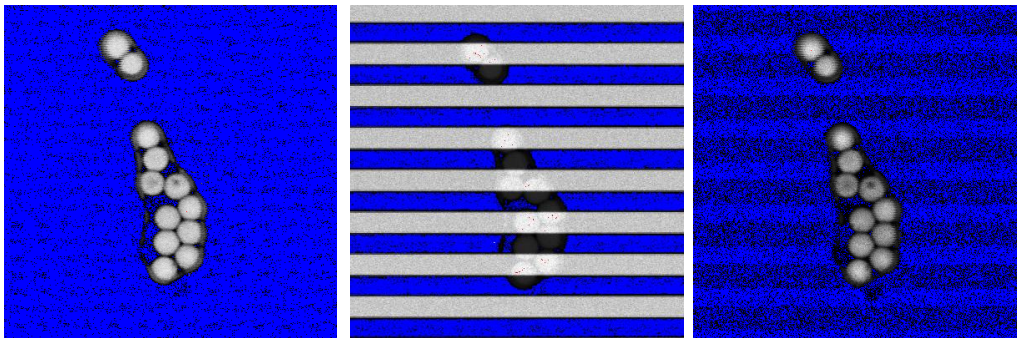


Figure Left: undisturbed image, Middle: image disturbed by a pulsed optogenetic LED during acquisition, Right: compensated image

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The invention utilizes the fact that (nonlinearly excited) fluorescent signals (and other signals generated through nonlinear optical effects) have a very low duty cycle. That is, the time when no signal is generated is much longer than the time when meaningful signal is generated. Thus, when sampling the signal many times in-between different excitation pulses, only one or very few samples contain fluorescent signal (or signals generated through other nonlinear optical effects). The signal detected in the other samples contains information on a possibly time-varying background, or noise. Thus, by using only the samples containing the intended signal, one can improve the signal to noise ratio. By registering all samples, one also obtains information about the nature of the noise or background. If there is only electrical noise present in the samples that do not contain signal, subtraction of the measured value does not influence the image quality. If there is a time varying background (which varies only on a time scale much longer than the time between two excitation pulses, which is typically the case), this background can be subtracted and the image quality is largely improved.

BENEFITS

- Fluorescence microscopy in conditions with ambient light
- Simultaneous optogenetic activation and fluorescence measurement
- Measurements during visual stimulation

REFERENCE:

M036/2018

APPLICATIONS:

Fluorescent microscopy

DEVELOPMENT STATUS:

Proof of concept
Prototype

KEYWORDS:

Background light,
Ambient light,

OPTIONS:

R&D Co-operation
Expertise

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