Title: Quantitative Monitoring of Phototoxicity in Live-Cell Microscopy via Machine Learning–Based Synthetic Nuclear Labelling

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Abstract

In live-cell imaging, light exposure can induce phototoxicity that alters cell behaviour and compromises data reliability, yet quantitative tools to monitor these effects remain limited. Automated frameworks such as PhotoFiTT have demonstrated potential to assess phototoxic changes over time, but their applicability across different model systems is not yet established. Here, we adapt the framework to evaluate its transferability to MDCKII cells and explore its value as a quality assurance tool in a core facility setting.

Because mitotic progression and cell-cycle dynamics are particularly sensitive to light-induced stress, we focused on tracking mitotic timing as a primary readout of phototoxicity. A machine learning model was trained on paired phase-contrast and fluorescence images, enabling it to generate label-free, synthetic nuclear images from phase-contrast input. The resulting images were segmented and analysed to follow mitotic events in MDCKII cells exposed to controlled illumination at varying doses and wavelengths. Our workflow has the potential to distinguish dose- and wavelength-dependent alterations in mitotic behaviour, with its quantitative performance currently under evaluation.

By utilizing a part of the PhotoFiTT workflow, this approach presents a practical strategy for core facilities and their users to assess photodamage, optimize imaging protocols, and ensure reproducible live-cell experiments.