Statistical Analysis of Phototoxic Effects of Fluorescent Markers on Cell Motility

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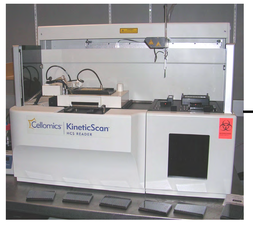
Abstract:

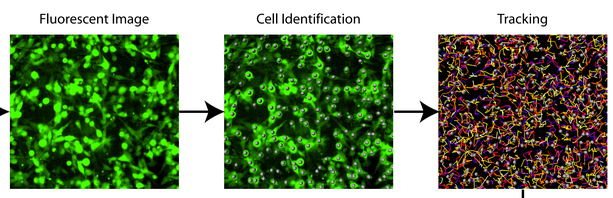
Fluorescent stains are widely used to detect cell proliferation and position, as well as morphology and internal structure. With the advent of new imaging systems, complete with environmental chambers, it is now possible to image cells over hours, or even days. This long time-lapse data allows us to gather dynamic information about cell motility, but is limited by two problems, photobleaching and phototoxicity. Photobleaching is the gradual fading of the fluorescent dye due to light induced oxidative damage accumulated over time. The far more serious problem is phototoxicity – the effect that these bleached dyes cause in the cell. We wanted to quantitatively determine the effect of phototoxicity on cell motility, comparing different cell types and anti-oxidant additives.

It has been suspected for years that these molecules may perturb the cell state – even producing toxic effects, through the combination of intense light and photoreactive species. Effectively, the fluorescent stains are generating free radicals and other oxidative and reactive species after their bombardment with intense light. These free radicals then cause random damage throughout the cell. Different cell types demonstrate different degrees of susceptibility to photodamage. Anti-oxidants are able to ameliorate this effect, allowing the fluorescent stains to be used.

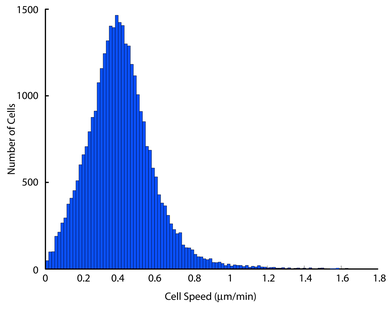
We demonstrate this through an analysis of cell speed, calculated using a long term time-lapse scan on a microscope with an environmental chamber. We used brightfield images as a control, assuming that brightfield is the least destructive method of imaging. We then performed the same analysis on the cells with various fluorescent chemical stains, as well as with GFP transfection. Finally, we used the same fluorescent stains in conjunction with anti-oxidants such as Trolox and vitamin C.

KineticScan Instrument(Cellomics)





Analyzed Data



In order to collect the data for these experiments, we used a KineticScan instrument from Cellomics. Briefly, the KinteticScan is a Zeiss 200M microscope surrounded by a temperature and CO_2 controlled enclosure. It has a motorized stage which allows 96-well plates to be inserted for high data throughput. We used this scope for all data except the transmitted light data provided as a control - for that scan an Applied Precision DeltaVision scope was used, with Solent chamber enclosure for temperature and CO_2 control. All images were taken at 10X magnification(.5 NA)

After data collection, we processed the data in Imaris(Bitplane). The data was analyzed to first find the cell location, and then track the positions over time. This data was extracted from Imaris and further analyzed in MATLAB to find velocity trends over time.

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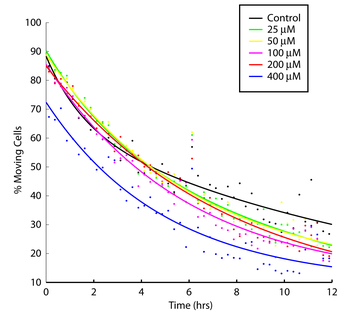
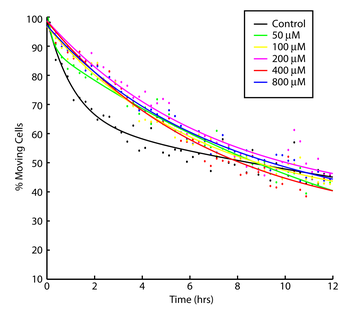
Antioxidant Additives

Ascorbic Acid(Vitamin C)



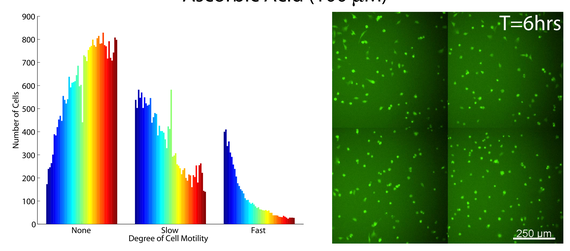
Trolox(Vitamin E)



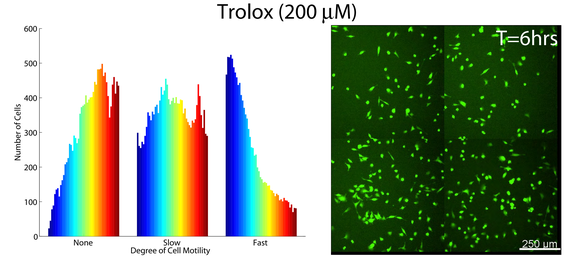
The above data was analyzed using .1 µm/min as a threshold for moving cells. The overall percentage of moving cells was then calculated for several different concentrations of antioxidant additives. It is clear from this data that ascorbic acid does not ameliorate the phototoxic affects of the stain, but Trolox seems to have some effect. The protective effects of Trolox only seem to last for approximately 6 hours, before the cells mostly stop moving and round-up.

Control (No Antioxidant Added)

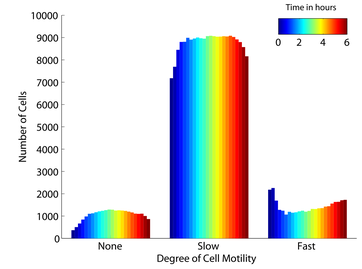
Ascorbic Acid (100 µM)

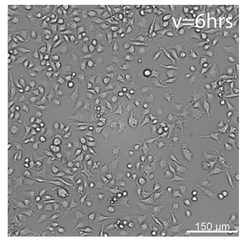


Trolox (200 µM)

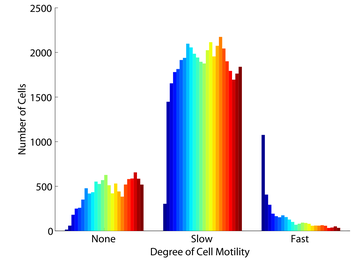


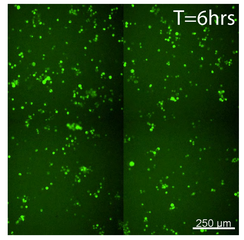
Transmitted Light Control



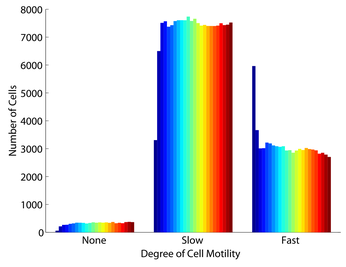


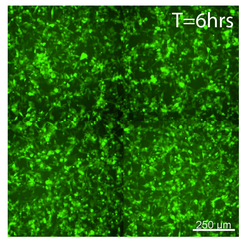
CMFDA Stained Cells





GFP Transformed Cells





The data on the left shows the breakdown of the experimentally determined optimal antioxidant concentration. The accompanying images show the cell condition at T=6hrs - it is clear that the Trolox is having a beneficial affect on these cells.

The above data demonstrates the differences between transmitted light imaging, CMFDA staining, and GFP transformation for imaging. Using transmitted light as a control, it is clear that the CMFDA is damaging the cells ability to move. However, GFP cells seem to be able to move quite well, with their group speed staying constant throughout the experiment