Photo-transfection of mouse embryonic stem cells with plasmid DNA using femtosecond laser pulses

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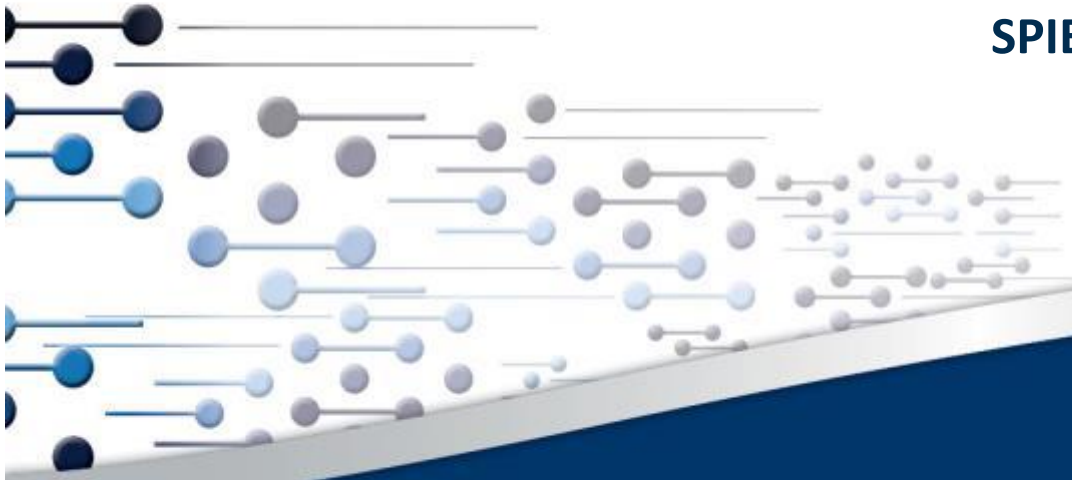

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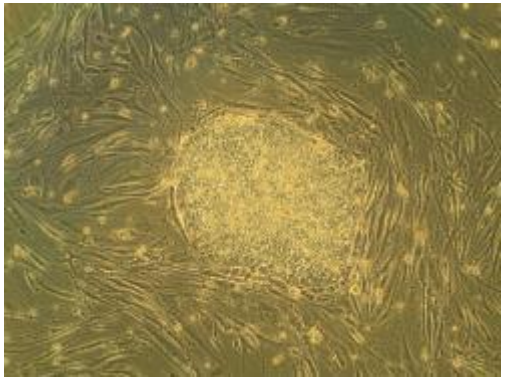
Outline

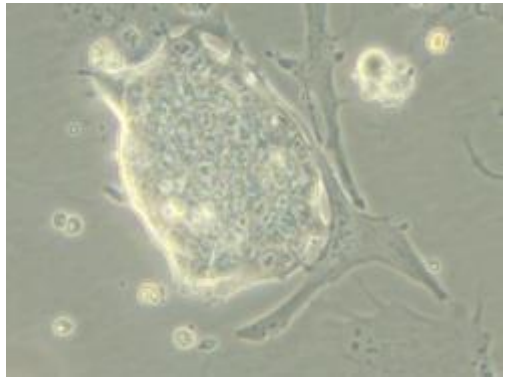
- Background on embryonic stem cells (ES)
- Phototransfection
- Objectives of the study
- Results
- Discussion and Conclusion

Background on embryonic stem cells (ESCs)

- ESCs are non-specialized cells, that are capable of producing all cell types in a multicellular organism



Human ES



Mouse ES

- Properties: Self-renewal, Potency, Differentiation

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Potency levels of ES

Totipotent- all types, zygote

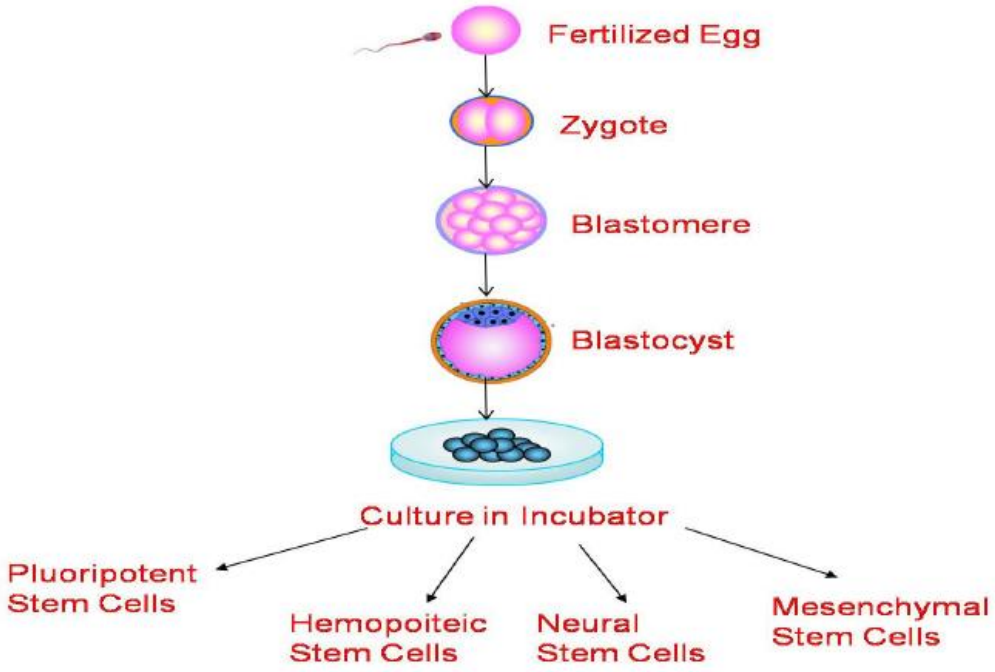
Pluripotent- blastocyst, germ layers

Multipotent- related cell types, adult stem cells, red and white blood cells

Oligopotent- Adult, lymphoid, myeloid

Unipotent- Only one type, muscle

Induced pluripotent- Adult ES genetically reprogrammed to pluripotency



- Karla K et al. Stem Cell: Basics, Classification and Applications. American Journal of Phytomedicine and Clinical Therapeutics.2014,27, pg 919-930

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Therapeutic applications of ES

Regeneration therapy

- Diabetes- pancreatic β cells Fibroblasts, DiPS
- Parkinson's disease- iPS, dopaminergic neurons

Transplantation

- Autologous- bone marrow, tissue defects, leukemia
- Haematopoietic- blood dieases, autoimmune disorders
- Mesenchymal- neurological disorders

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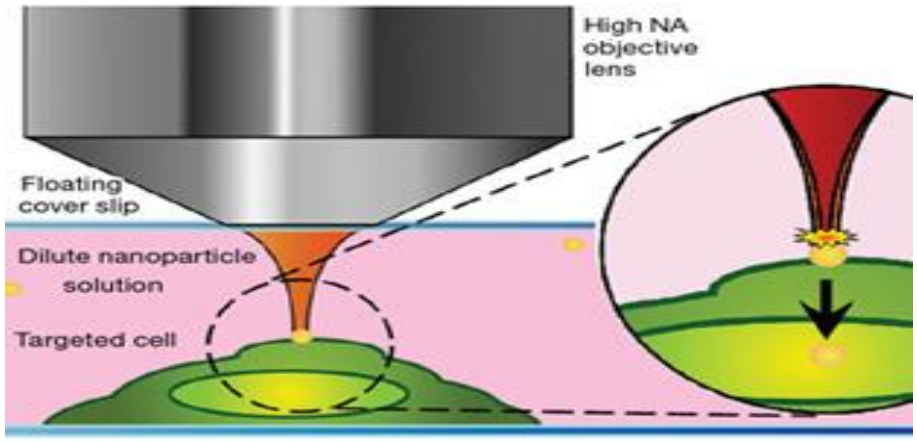
Phototransfection

- Transfection refers to the delivery of genetic material (DNA, RNA) into live cells to induce a change in phenotype or functionality.



An illustration of photo-transfection using laser pulses. *K. Dholakia et al. "Optical Micromanipulation," Chem. Soc. Rev. 37, 42 (2008)*

- Photo-transfection is specific DNA/RNA delivery using photons. Non-invasive. No latent chemical or viral side effects.

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Objectives of the study

- Design and build an optical set up for phototransfection
- Porate mES in the presence of fluorescent plasmid GFP.
- Image and compare mES post transfection
- Analyze cell viability using molecular assays: ATP and LDH

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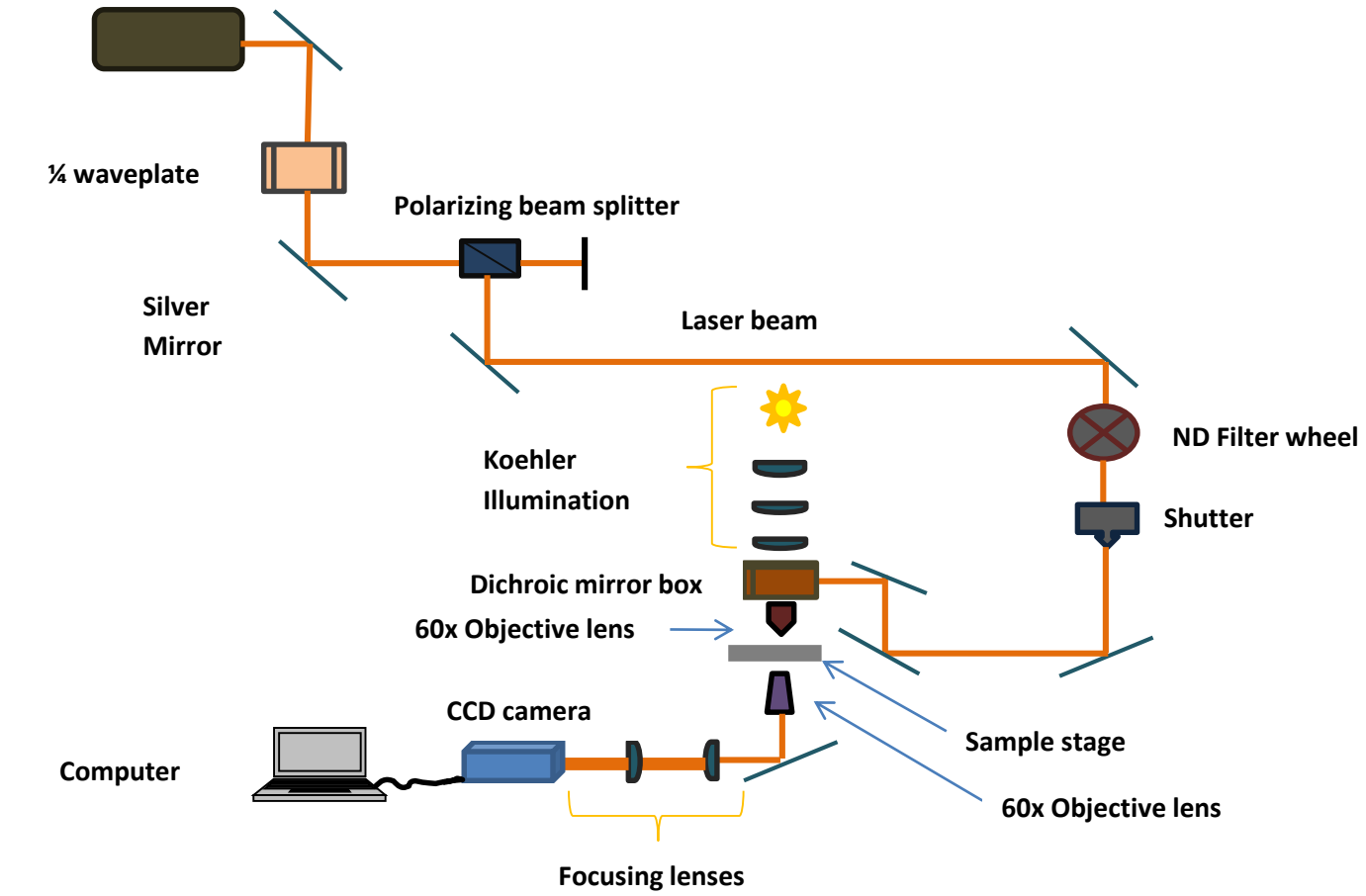
Results: Optical set-up design

It: sapphire Femtosecond laser, 800nm



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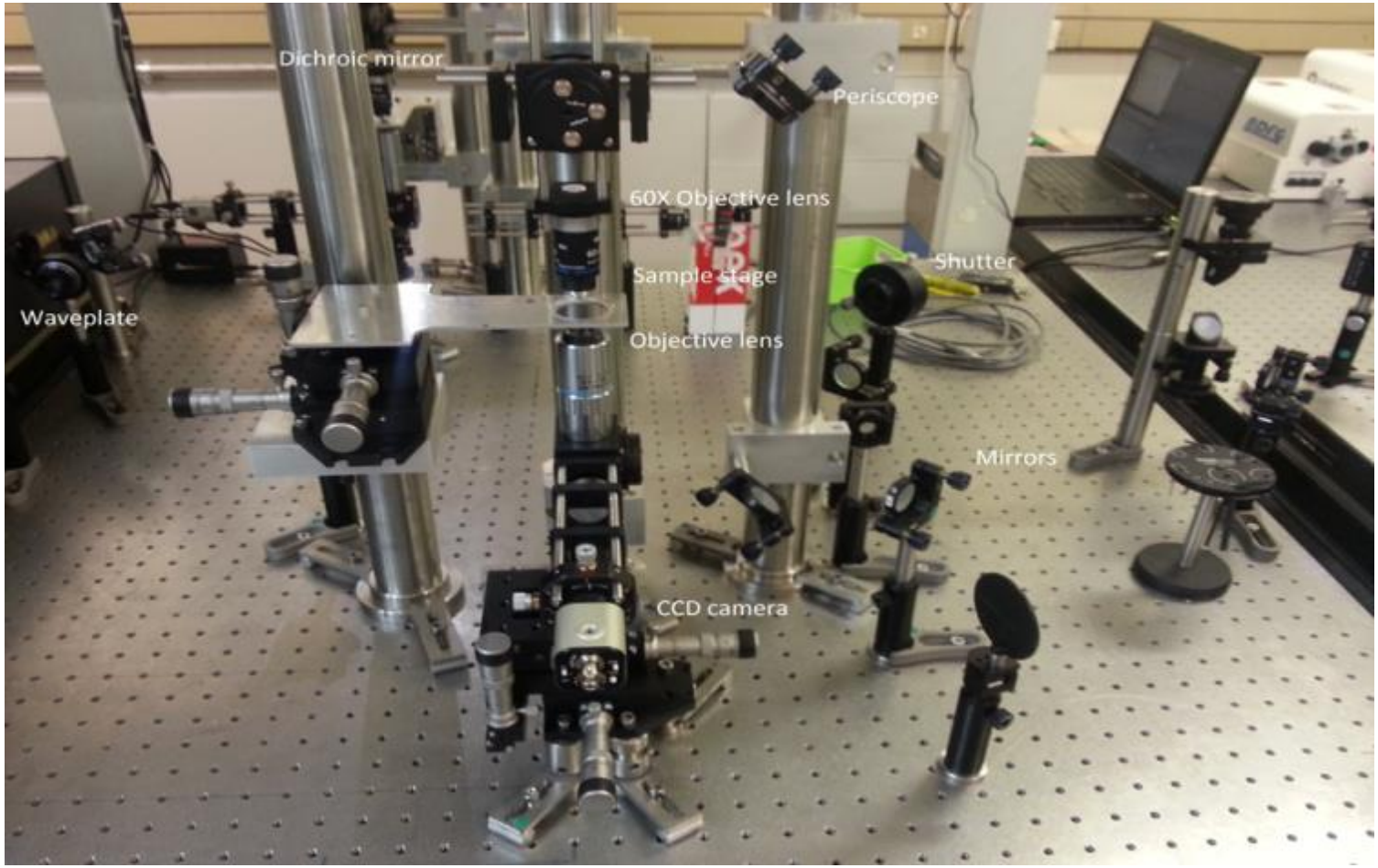
Femtosecond laser set up



1 kHz Ti: sapphire laser, Pulse duration <130 femtoseconds, Beam diameter 10-15mm. Average power 1 Wattz. 800nm

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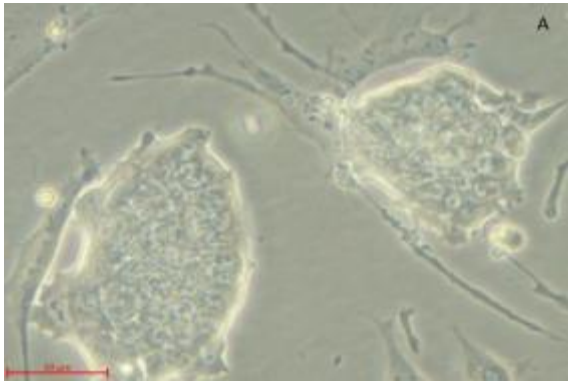
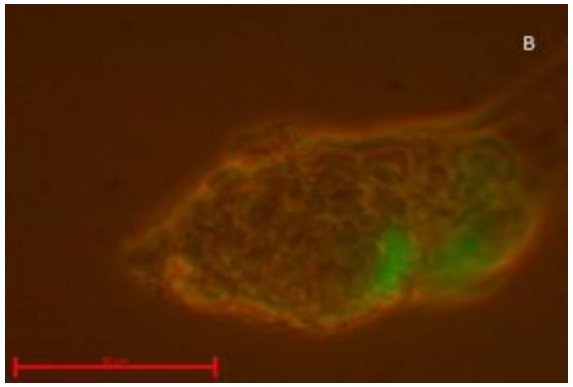
Imaging post irradiation

Phototransfection: mES porated using laser powers from 2-20uW. 15ug/ml pGFP in cell media. 24 hours post irradiation

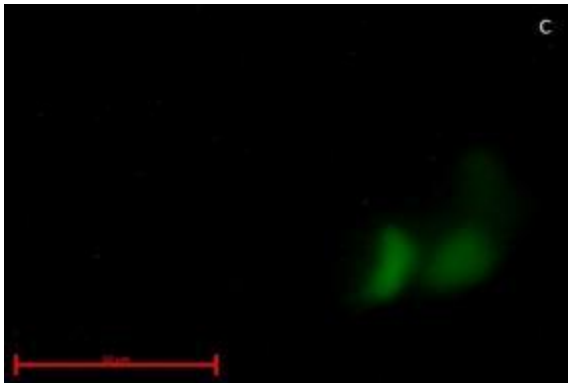
Control



Chemical transfection,2ug/ml pGFP



Fluorescent image



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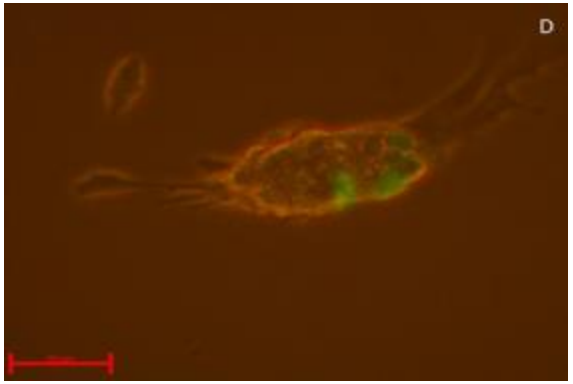
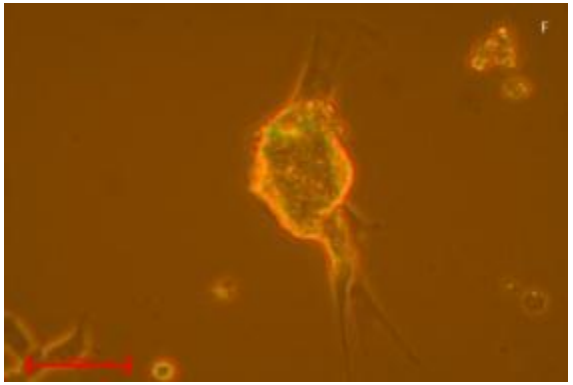
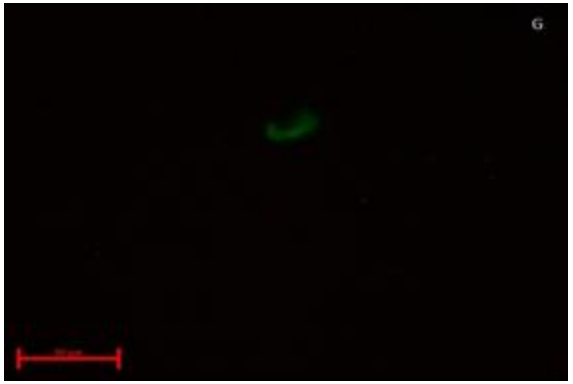
2uW, 10ms



6 uW, 10ms





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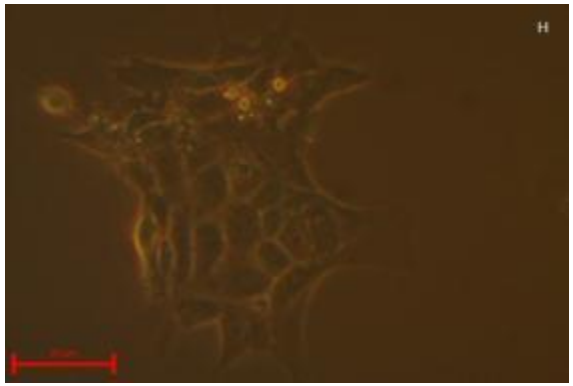
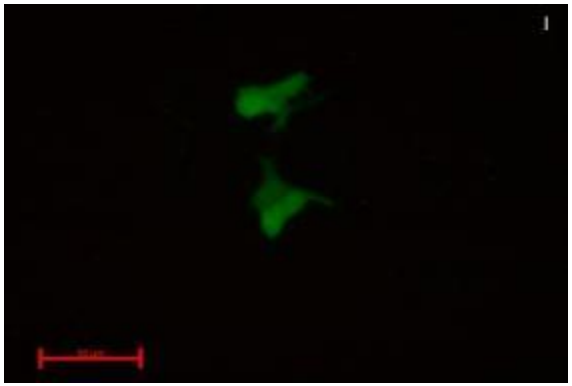
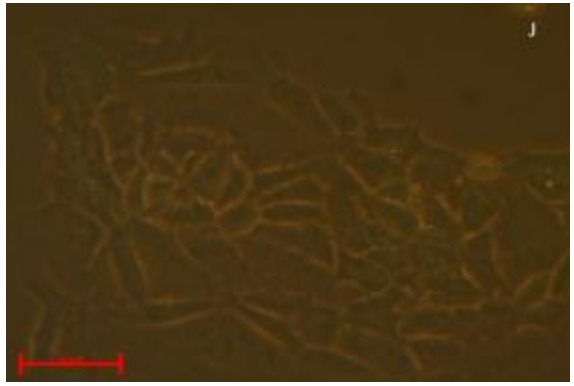
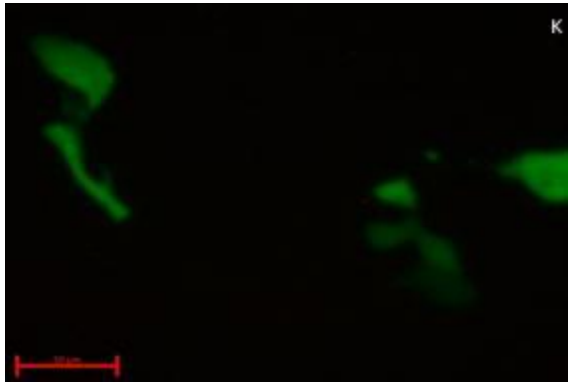
8uW, 10ms





20uW,10ms





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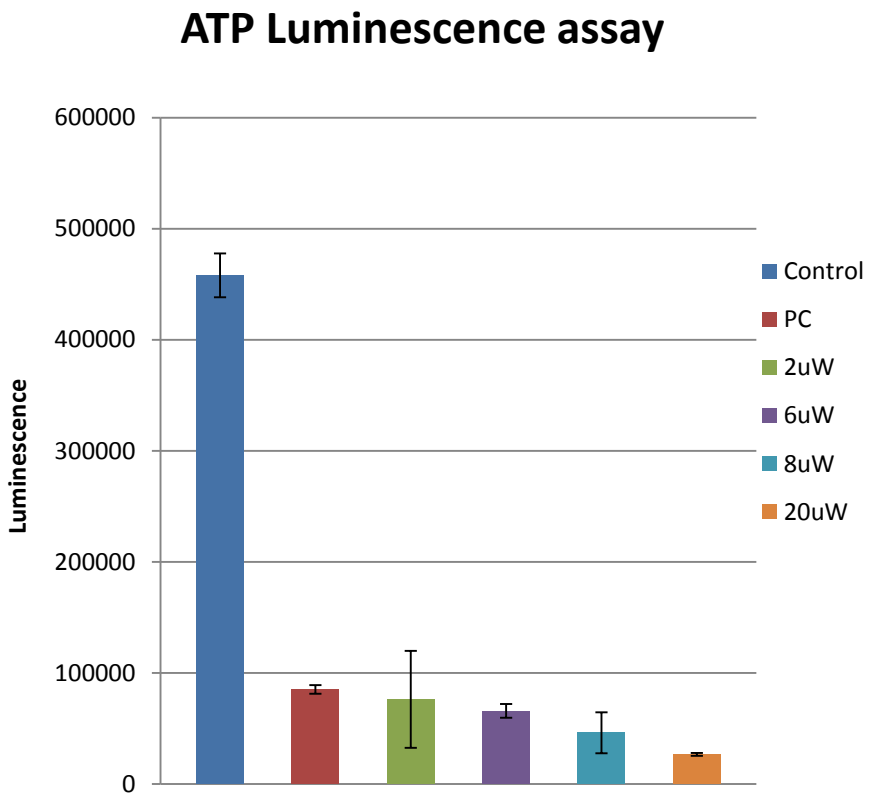
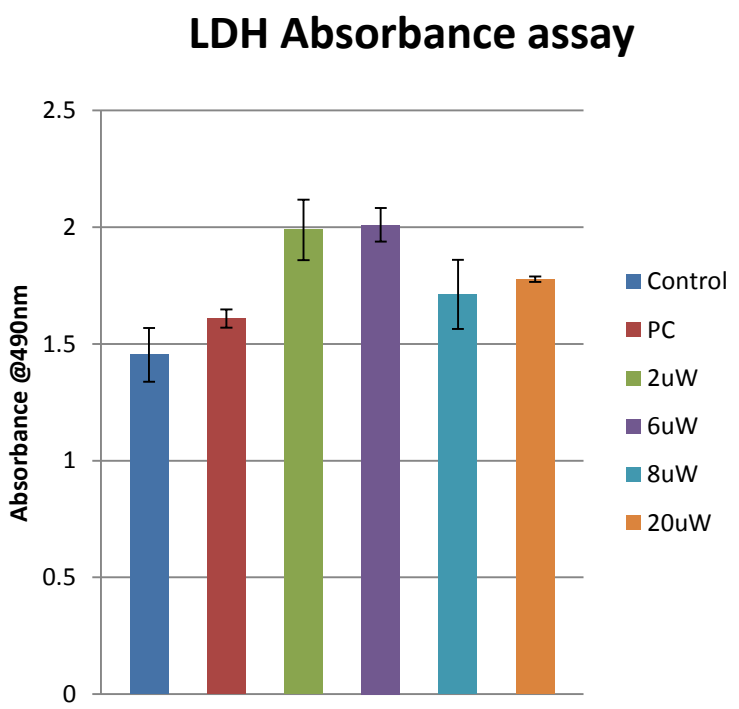
Cell viability assay

ATP: cell well being



ANOVA test n= 3 : F(5,12)=59.7, p<0.025.

LDH : cell death, necrosis



n= 3 F(5,12) = 4.79, p<0.025

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Discussion and Conclusion

- **Fluorescence imaging**: Control mES morphology intact after 24hrs, no fluorescence. Phototransfection causes physical changes to cells more than chemical transfection, the latter shows similar fluorescence to the irradiated cells. Increase in laser power induces more drastic changes to the mES with complete differentiation seen at 8uW and 20uW. Highest fluorescence seen at 20uW due to relatively increased entry of pGFP.

- **ATP and LDH**: Control has highest ATP and lowest LDH, consistant with relatively good cell health. Transfection experiments lowered ATP and increased LDH, with chemical transfection values being less than phototransfection in LDH, higher in ATP. High laser powers (8 and 20uW) show less ATP and less LDH, this may be caused by poration of floating cells.

- **Conclusion**: Phototransfection was successful in delivery of pGFP into mES. Poration of monolayer cells at powers between 2-6 uW may lower extent necrosis and improve viability.

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Acknowledgements

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