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The Addition of a Propel Labs Co-Lase tower to a MoFlo* Legacy Cell Sorter for use in a Stem Cell Laboratory

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Introduction:

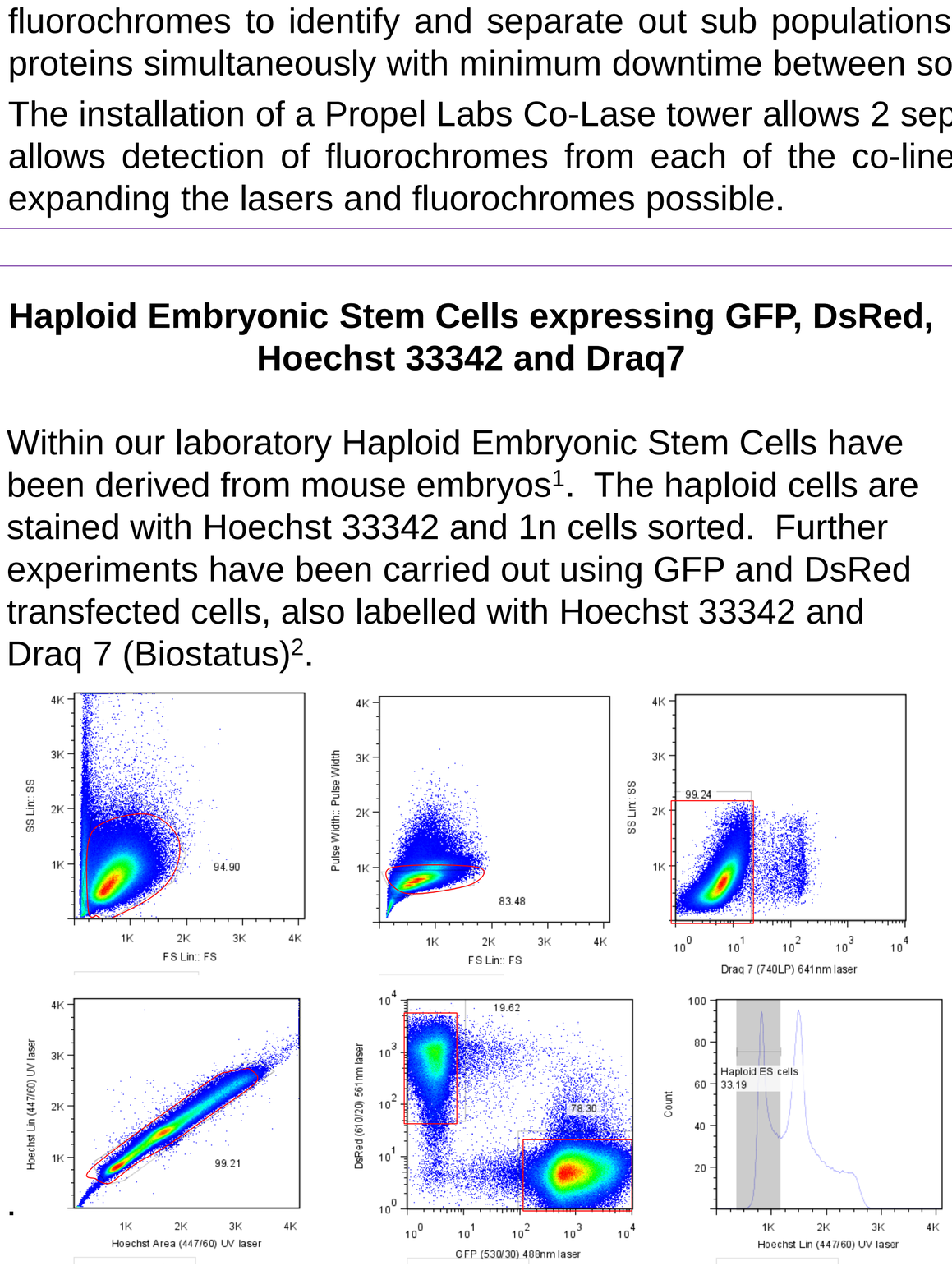
MoFlo Legacy sorters are commonplace in many flow cytometry laboratories as a reliable robust sorter, however their limiting factor is the 3 laser pinholes. With the advancement of lasers and fluorochromes in the past few years, this has meant that either lasers have to be physically moved or scientists have to adapt their experiments to use only 3 laser lines and often compromise the excitation/selection of their fluorochromes.

Running a busy sorting facility within a stem cell laboratory means that the machine has to be able to detect multiple fluorochromes to identify and separate out sub populations and it is important to be able to identify a number of fluorescent proteins simultaneously with minimum downtime between sorts.

The installation of a Propel Labs Co-Lase tower allows 2 separate laser paths to go through a pinhole as co-linear beams. This allows detection of fluorochromes from each of the co-linear lasers in the existing detection pathway of the MoFlo Legacy expanding the lasers and fluorochromes possible.

Haploid Embryonic Stem Cells expressing GFP, DsRed, Hoechst 33342 and Draq7

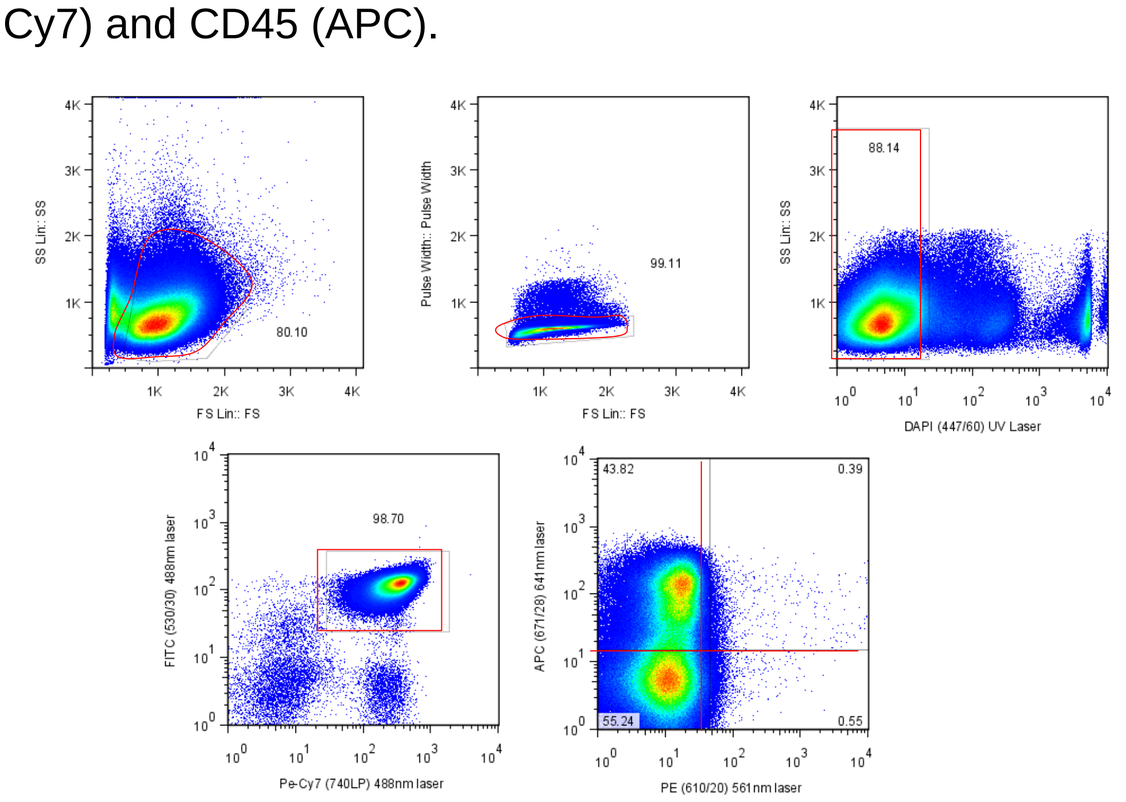
Within our laboratory Haploid Embryonic Stem Cells have been derived from mouse embryos[1]. The haploid cells are stained with Hoechst 33342 and 1n cells sorted. Further experiments have been carried out using GFP and DsRed transfected cells, also labelled with Hoechst 33342 and Draq 7 (Biostatus)[2].



By using the Co-Lase tower it has been possible to minimise any spill over between the DsRed and GFP as they are detected by different lasers (561nm and 488nm respectively). It also allows identification of the Haploid cells based on their DNA content using Hoechst 33342 exicted by the UV laser). Draq 7 is used for the elimination of dead cells (essential after transfection of cells), using a 740LP filter to avoid any red emission from the Hoechst.

Peripheral Blood Cells stained with FITC, PE, PE-Cy7, APC and DAPI.

CD4 pre-enriched human peripheral blood mononuclear cells were stained for, CD4 (FITC), CCR7 (PE), CD3 (Pe-Cy7) and CD45 (APC).



This experiment utilised the 4 lasers on the MoFlo, using the 488 nm laser to excite the FITC and the 561nm laser to excite the PE which reduced spectral overlap and the amount of compensation needed. Pe-Cy7 was read using the 488nm laser. The Co-Lase tower allowed DAPI to be used as a live/dead dye as well APC as a marker. The cells were sorted according to the gating scheme above and post-sort analysis by PCR was performed.

Other combinations of dyes using the 4 lasers include: GFP, RFP, APC and DAPI.

Conclusions:

By fitting a Co-Lase tower to our MoFlo Legacy this have increased the number of laser lines we can use and increased the versatility of the machine. The Co-Lase tower has allowed us to increase our range of flurorochromes and is now essential for our stem cell research.

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1= Leeb et al, Nature, 479 131-134, 2011
2= Leeb et al, Development, 2012