Discriminating Multiplexed GFP Reporters in Primary Articular Chondrocyte Cultures using Cellometer Image Cytometry

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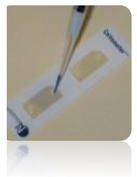
1. ABSTRACT

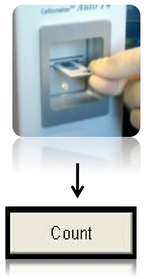
Flow cytometry has become a standard tool for defining a heterogeneous cell population based on surface expressed epitopes or GFP reporters that reflect cell types or cellular differentiation. The introduction of image cytometry raised the possibility of adaptation to discriminate GFP reporters used to appreciate cell heterogeneity within the skeletal lineages. The optical filters and LEDs were optimized for the reporters used in transgenic mice expressing various fluorescent proteins. In addition, the need for compensation between eGFP and surrounding reporters due to optical cross-talk was eliminated by selecting the appropriate excitation and emission filters. Bone marrow or articular cartilage cell cultures from GFP and RFP reporter mouse lines were established to demonstrate the equivalency in functionalities of image to flow cytometry analysis. To examine the ability for monitoring primary cell differentiation, articular chondrocyte cell cultures were established from mice that were single or doubly transgenic (Dkk3eGFP and Col2A1GFPcyan), which identify the progression of superficial small articular cell to a mature chondrocyte. The instrument was able to rapidly and accurately discriminate cells that were Dkk3eGFP only, Dkk3eGFP/Col2A1GFPcyan, and Col2A1GFP, which provides a useful tool for studying the impact of culture conditions on lineage expansion and differentiation.

2. CELLOMETER IMAGE CYTOMETRY

Pipette 20 µL of sample into disposable counting chamber

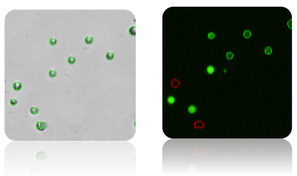


Insert chamber in Cellometer



Output data generated instantly

Bright-field (BR) and fluorescent (FL) images



Assay: CBA_GFP+RFP Dual Expression Date: 05/12/2014 11:52:58

Cell Type F1: CBA_Dual Expression GFP
Cell Type F2: CBA_Dual Expression RFP

Sample ID: CBA_GFP+RFP-2
Dilution Factor: 1.00

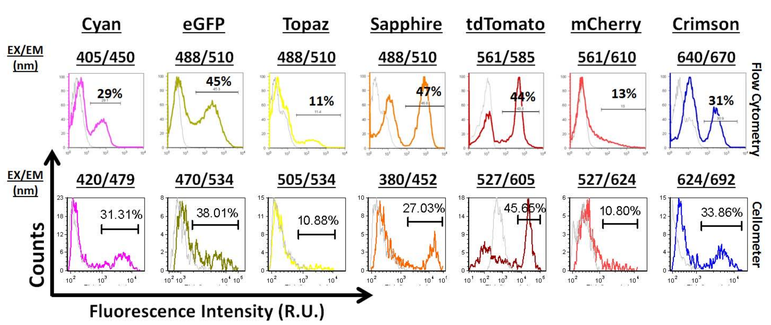
Results:

Count	Concentration	Mean Diameter
Total cells: 1316	1.85x10^6 cells/mL	8.7 microns

Please export data to Nexcelom Data Package to further analyze

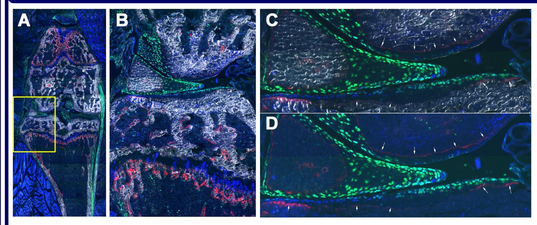
1. Click Export
2. Select option: Export data to Nexcelom Data Package
3. Have option checked on for "When done open Nexcelom Data Package with FCS Express 4 Flow"
4. Click Continue...
5. Select location to save file
6. Click Save

3. DETECTION OF FLUORESCENT PROTEINS USING CELLOMETER



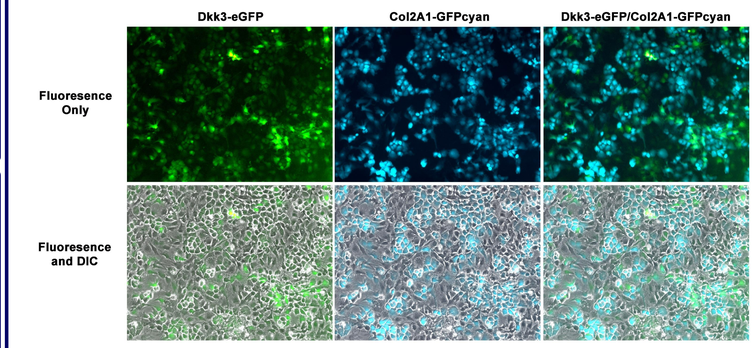
- Comparison of flow and Cellometer image cytometry detection of cells expressing different fluorescent proteins, such as Cyan, eGFP, Topaz, Sapphire, tdTomato, mCherry, and Crimson

4. OBSERVING DKK3-EGFP AND COL2A1-GFPCYAN IN FL MICROSCOPE



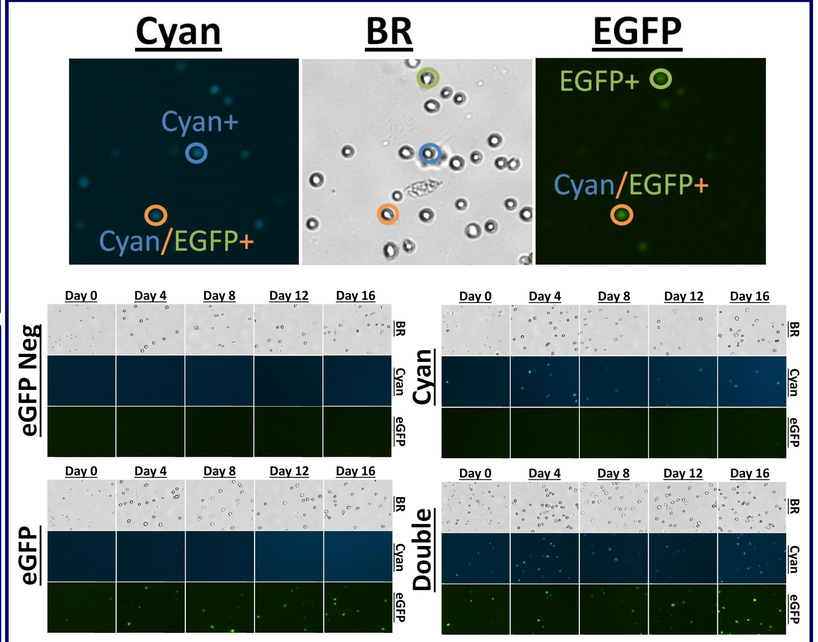
A. Fluorescent microscope of full view of ligament
B. Enlarged section from yellow square in (A)
   1. Silver is mineral
   2. Dkk3 is green
   3. Col2A1 is blue

C. and D. shows mostly green Dkk3 expression around the ligament, with weak blue Col2A1



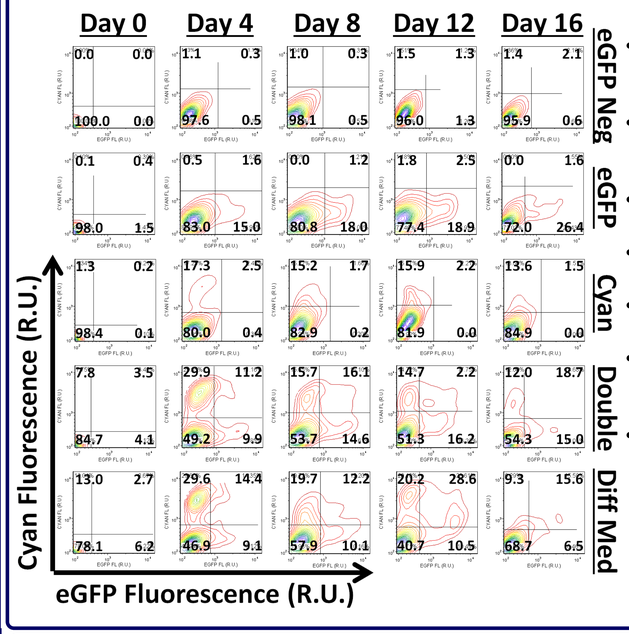
- Dkk3-eGFP and Col2A1-GFPcyan in primary culture of articular chondrocytes are examined using a fluorescent microscope
- The composite of the 2 colors were revealed by the DIC image, which shows the complexity of the sample
- In order analyze the cell population, FACS or Cellometer Image Cytometer can be utilized

5. CAPTURING FL IMAGES USING CELLOMETER IMAGE CYTOMETER



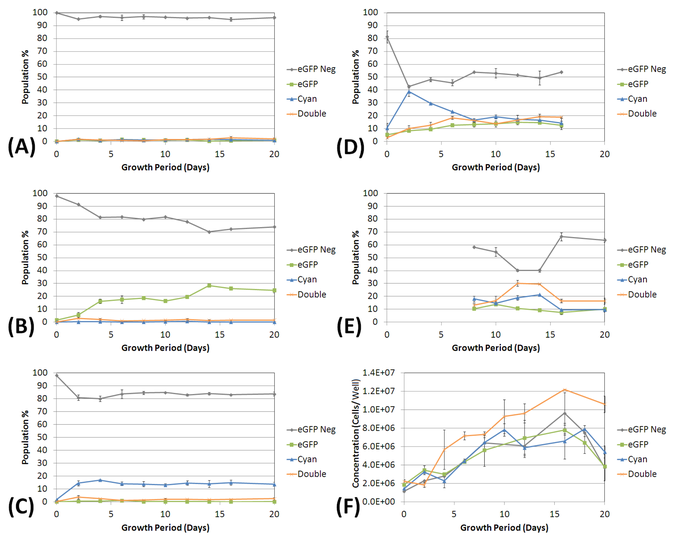
- Single and double colored articular chondrocyte captured by Cellometer Image Cytometer
- Optimized filter selection minimized optical cross-talk between the 2 colors
- Dual FL images were captured for 21 days of articular chondrocyte cultures for control (eGFP negative), Dkk3-eGFP only, Col2A1-GFPcyan only, and dual expression

6. FLUORESCENCE ANALYSIS USING CELLOMETER AND FCS EXPRESS EXPORT



- Time-course fluorescent scatter plots for each articular chondrocyte sample
- eGFP negative sample did not show any increase in cell population for green nor blue
- eGFP only showed increase of Dkk3-eGFP up to 26.4%
- Cyan only showed increase of Col2A1-GFPcyan up to 15.9%
- Double color showed increase of cell population up to 18.7%
- An articular chondrocyte sample was treated with a growth media
- It showed that cultures with a growth differential media added, increased the population with dual colors

7. CONTROLLED HEAT-KILLED VIABILITY COMPARISON



- Time-course cell population analysis from FCS Express: A, B, C, D, E are eGFP negative, eGFP only, Cyan only, double fluorescence, and differential media treated sample, respectively
- In F. the cell concentration increased the most for the double color, in comparison to the other samples

8. CONCLUSION

In summary, the use of GFP reporters, as markers of lineage differentiation, can be significantly augmented by applying flow cytometric principles to cell proliferation and differentiation, which are the hallmarks for describing lineage regulation. Establishing stable and reproducible culture models are an essential first step before growth factors, scaffolds, 3D hydrogels or other manipulation are explored as potential agents to promote lineage expansion and differentiation, or to study the impact of a mutation that compromises lineage health. The modifications to image cytometry system described here can provide an efficient and simple method for the cytometry community and can conform within the daily workflow of a cell culture based laboratory.