Fluorescence and Laser Scatter Imaging of Mouse Embryonic Stem Cells and Embryoid Bodies

Embryonic stem (ES) cells have the potential of forming every type of cell and tissue in the body and are an important tool for understanding the molecular basis of embryonic development and cellular differentiation. The most common method for initiating differentiation from ES cells is the formation of aggregates called embryoid bodies (EBs) in suspension cultures. Groups of cells within the aggregates have been shown to differentiate into a variety of different tissues. The signals for self-renewal versus differentiation are the subject of intense research efforts and protocols have been developed to activate specific differentiation pathways. The ability to develop methodologies to isolate, characterize, and purify differentiated cell lineages is dependent on the ability to identify the cells. In this report we demonstrate that the ImageXpress® Velos System with its unique optics for laser scatter and fluorescence imaging is well suited for this application.

Materials and Methods

Mouse E14 ES cells and EBs were obtained from Dr. R. Pera’s laboratory (UCSF) for evaluation with the ImageXpress Velos System (Blueshift Biotechnologies, Inc.). The E14 ES cells were cultured in E14 media (Glasgow MEM) with 2mM glutamine, 1mM sodium pyruvate, 1x non-essential amino acids, 10% FBS, 1x BME, and supplemented with 500 µl ESGRO (LIF) per 500 ml of media. The E14 ES line was grown in gelatin–coated T-25 flasks and the XX-ES line was cultured on irradiated mouse embryo fibroblasts (mEFs). The E14 ES line designated PmaxGFP (E14-GFP) expressed GFP from the CMV promoter. The XX-ES line contained a Dazl-GFP lineage-specific construct that is expressed in mouse EBs. The EBs formed using the E14 control, E14-GFP, and XX-line (Dazl-GFP) were scanned using the ImageXpress Velos™ in suspension using 6-well TC multiwell plates.

ImageXpress Velos. The ImageXpress Velos System laser scanning platform was setup for 3-channel acquisition with the following band pass filters: Ch1: 510-540nm (green); Ch2: 450-490nm (laser scatter); Ch3: 560-610nm (orange). The initial image acquisition was done at 10 x 10 micron sampling (pixel) size and an entire 6-well plate was scanned. Selected wells with defined scanned areas were scanned at both 5 x 5 micron and 2.5 x 2.5 micron sampling. Images were analyzed using BlueImage software for object identification and enumeration.

Results

The Dazl-GFP ES line and negative control ES cells were grown on mEFs and scanned. The negative control ES cells showed very good colony morphology using scatter and a very weak signal in the green channel. The Dazl-GFP cells showed a similar morphology in the scatter image and a strong signal in the green image. Notably, the scatter image was of sufficient resolution for the detection of growing colonies (Figure 1).

The E14-GFP ES cells were suspended in differentiation media, 2 ml were added to each well of a 6-well plate, the suspension culture of EBs were allowed to differentiate for 3 days, and then scanned on the ImageXpress Velos System. Figure 2 shows the 5 x 5 micron scatter image of the E14-GFP day 3 EBs. The laser scatter image shows the optically dark areas in EBs and the typical image quality obtained in TC grade 6-well plates. A comparison of the green emission with the scatter image demonstrates that the unique optics of the ImageXpress Velos System enables the detection of fluorescence signals within EBs. This is due to the fluorescence signal being generated along the focus of the laser beam. The results suggest that the combination can be used for the identification of EBs containing cells with the desired fluorescence signals. A comparison of the green GFP with the orange image demonstrates that the GFP emission is also observed in the orange channel.
To evaluate the ability to discriminate GFP expression from autofluorescence signals and to provide for a direct comparison, the negative control EBs (no GFP) were added to a well containing day 3 EBs with the Dazl-GFP transgene. The no GFP negative control EBs showed a weaker green emission than the Dazl-GFP EBs with the mean fluorescence approximately 2.5 times brighter than the negative control. In the orange emission channel the Dazl-GFP EBs were approximately 1.9 times brighter than the negative control EBs. A scatter plot of the green mean fluorescence versus the orange mean fluorescence demonstrated that the ratio distinguished EBs with background autofluorescence from the GFP expressing EBs (Figure 3). Notably, there are outliers; more work is needed to evaluate the ability to discriminate weak GFP expression from the autofluorescence signal.

A 1:1 mixture was made of E14-GFP and E14 control EBs from cultures differentiated for 8 days. Images were acquired at 2.5 micron sampling. The Bluelmage analysis results using these images and thresholding on the scatter signal identified the EBs as objects and generated EB specific fluorescence parameters. The green versus orange EB analysis was plotted using maximum fluorescence values (Figure 4). A comparison of the plots demonstrated that the fluorescence maximum values clearly defined the no GFP from the E14-GFP EBs. The data was generated using a defined region of interest (ROI) and a total of 64 EBs were identified and classified as objects. Out of 64 total EBs 28 had a maximum green fluorescence value greater than 10,000 relative fluorescence units (RFU).

Next, EBs were formed by mixing the E14-GFP with the E14 control ES cells in a 1:1000 ratio and differentiated for 8 days. The EBs were scanned at 2.5 micron sampling with the aim of using the Bluelmage analysis program to identify EBs by scatter and then enumerate the fluorescent objects within the EBs. The green versus orange maximum fluorescence values were plotted and one object was identified for further analysis (Figure 5 top; data point in green box). The x,y location parameters were used to locate the object in the scatter image and the corresponding green and orange images (Figure 5 bottom).