Fluorescence Screening Platform for Embryonic Stem Cells and Embryoid Bodies

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Abstract

Embryonic stem (ES) cells have the potential of forming every type of cell and tissue in the body. In addition, human embryonic stem cells hold great promise for development of novel cell-based therapeutics. A common method for initiating differentiation from ES cells *in vitro* is the formation of aggregates called embryoid bodies (EBs). The intimate intercellular contacts within EBs provide three-dimensional microenvironments that are thought to emulate *in vivo* developmental niches required for the activation of specific differentiation pathways. Molecular Devices, Inc. has developed a powerful screening platform, the **IsoCyte™** Laser Scanning Cytometer, for multiparametric screening of cells, colonies, and EBs in multiwell tissue culture (TC) plates using laser scatter and fluorescence measurements. The platform addresses the need to i) monitor the growth of ES colonies and EBs, ii), detect wells containing fluorescent protein expressing cells, colonies, or EBs, and iii) provide object location parameters enabling picking of ES colonies and EBs.

Introduction

In this report the **IsoCyte™** laser scanning platform with its unique optics has enabled the ability to identify embryonic stem (ES) cell colonies and cells within embryoid bodies (EBs) on the basis of fluorescence and laser scatter imaging. Live cell detection in ES cells and EBs was demonstrated using ES cells with fluorescent protein-reporter transgenes, such as green fluorescent protein (GFP) expressed from a lineage-specific promoter. Researchers are using knowledge of protein-protein interaction networks coupled with Fluorescence Resonance Energy Transfer (FRET) for the identification of lineage-specific cells by protein-protein interaction screens. Molecular Devices has demonstrated a new strategy for detecting FRET from fluorescent protein-protein interactions based on depolarized sensitized emission (1). **IsoCyte™** platform with 2-color anisotropy capability allows adaptation of the FRET The fluorescent protein-protein interaction technology to screening applications.

Materials and Methods

Cells and Culture Conditions. Mouse E14 ES cells and EBs expressing GFP were obtained from Dr. R. Pera's laboratory (UCSF) for evaluation with the IsoCyte™. 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 ml ESGRO (LIF) per 500 ml of media. The E14 ES line was grown in 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 IsoCyte™ (Molecular Devices, Inc.) in suspension using 6-well TC plates.

IsoCyte™ Scanning Platform

Detection of GFP Expression by IsoCyte™. The **IsoCyte™** laser scanning platform (shown above) 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 dish was scanned. Selected wells with defined scanned areas were scanned at both 5 x 5 micron and 2.5 x 2.5 micron sampling.

Results

Detection of GFP Expression in ES Cells Grown on mEFs

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 (**Fig 1**). A schematic of image acquisition, ES cell colony identification by scatter signal, fluorescence measurement, and colony location process is shown in **Fig 2**.

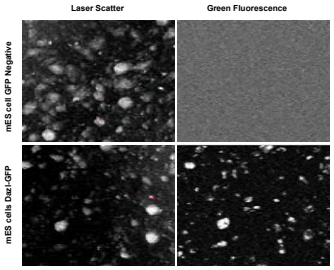

Figure 1. Mouse ES cells grown on mouse embryonic feeders (mEFs). Top panel: Ch 2 scatter (left) and Ch 1 green fluorescence (right) of GFP negative line. Bottom panel: Ch 2 scatter (left) and Ch 1 green fluorescence (right) of Dazl-GFP expressing line. Images are at 2.5 micron sampling and cells are in 6-well TC plates.

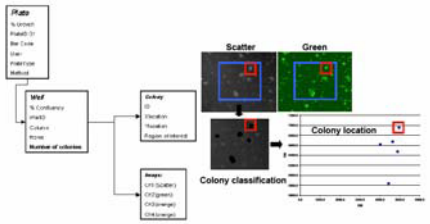

Figure 2. Schematic of image acquisition, ES cell colony identification by scatter signal, fluorescence measurement, and colony location process.

Results – con't

Detection of GFP expression in EBs formed from the E14-GFP ES line

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 **IsoCyte™**. **Fig 3** 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 **IsoCyte™** 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.

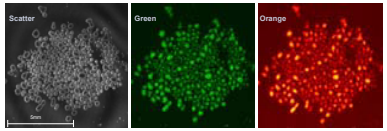

Figure 3. Cluster of embryoid bodies in a 6-well TC plate.

Discrimination of GFP Expression from Autofluorescence in 1:1 Mixtures of E14-GFP plus E14 EBs

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 BlueImage 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 (**Fig 4**). A comparison of the plots demonstrated that the fluorescence maximum values clearly defined the no GFP from the GFP expressing 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 (RFUs).

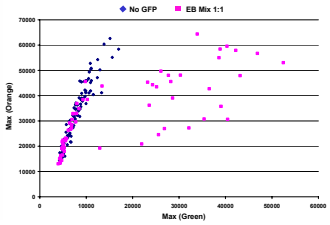

Figure 4. Scatter plot of Ch 1 green versus Ch 3 orange particle analysis using maximum fluorescence. The green to orange ratio distinguished the no GFP versus the E14-GFP EBs.

Results – con't

Detection of GFP Expressing Cells in Day 8 E14-GFP EBs formed from a 1:1000 ratio of E14-GFP to E14 control ES cells

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 BlueImage 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 (**Fig 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 (**Fig 5** bottom). The recovery of selected EBs by manual picking has been demonstrated.

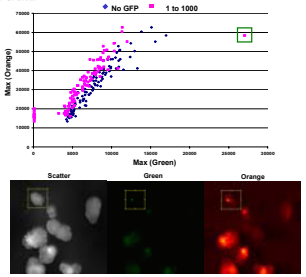

Figure 5. Scatter plot (top) of Ch 1 green versus Ch 3 orange particle analysis by maximum fluorescence. The data point in the green box defined the location of the EB (bottom images).

Conclusions

In this report the IsoCyte™ laser scanning platform with its unique optics has enabled the ability to identify embryonic stem (ES) cell colonies and cells within embryoid bodies (EBs) on the basis of fluorescence and laser scatter imaging. Significant features of this platform are:

- Live cell functional imaging of fluorescent protein expression in ES cells and EBs
- Optics with a large depth of field for imaging ES cell colonies and EBs by laser scatter and fluorescence in TC quality 6-well plates
- Collection optics with a confined detection region that reduces fluorescence background and provides ability to identify and enumerate cells and groups of cells expressing fluorescent protein transgenes directed by lineage-specific promoters in ES cell colonies and EBs
- Ability to do live cell protein-protein interaction screening by FRET

Acknowledgements

REFERENCES

1. Rizzo, M.A. and Piston, D.W. Biophys. J., L14, 2005.

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Molecular Devices invites you to contact us to discuss your specific applications for the IsoCyte™ platform.