Focal Points

щUVP

Application Note FP-142

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Colony Counting GFP Bacteria
With the ColonyDoc-It™ Imaging Station

Introduction

Originally extracted from jelly fish, GFP (Green Fluorescent Protein) is a fluorescent protein that emits a green glow. GFP absorbs the energy of blue light and then emits the partially-consumed energy as green light.

GFP is employed as an easily detectable marker protein. When a bacterial colony is labeled with GFP for comparative study, incubated under protocol conditions, and viewed under blue light, the GFP bacteria emits green light. The green light emission allows the researcher to visualize and distinguish the GFP-labeled bacteria, which makes the distinction of GFP labeled cells from unlabeled cells simple and effective.

Fig. 1 – ColonyDoc-It Imaging Station

The ColonyDoc-It™ Imaging Station (Fig. 1) is designed with a epi blue light (~470nm) excitation source (Fig. 2) and a GFP filter to optimize enumeration of colony growth of bacterial and yeasts utilizing GFP.


Fig. 2 – Blue Light Excitation

Sample Preparation

- Generate GFP expressing E. Coli. colonies (Fig. 3) by using a pGLO bacterial transformation kit.

- Introduce the non-fluorescent HB101 strand E. Coli. to a pGLO plasmid by heat-shocking.

- Encode the pGLO plasmid with the arabinose promoter, GFP expression and ampicillian resistance.

- Grow the transformed E. Coli. on LB agar plates containing ampicillin (100ug/mL) and arabinose (6mg/mL).
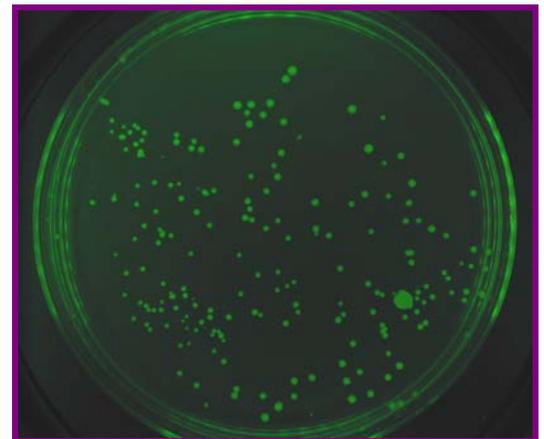
■ Incubate the agar plates overnight at 37°C.   **Fig. 3 – GFP Colonies**

Imaging Methods

■ Place the GFP sample in the ColonyDoc-It Imaging Station.

■ Close the two doors on the ColonyDoc-It to limit the ambient light from entering the ColonyDoc-It Imaging Station during the count process.

■ From the ColonyDoc-It software (Fig. 4) camera control panel, select the **plate diameter** size for the GFP sample.

■ Select the **smallest colony** size in the GFP plate to be counted.

■ Select **fluorescent** colony lighting to turn on the epi blue light.

■ Select **preview** to insure that the plate fits inside the region of interest.

■ Once positioned correctly, select **capture** to take a picture of the colonies.

■ Click **start colony count** and **automated count** to enable the software to count the colonies. Within seconds, the software will produce a total colony count.
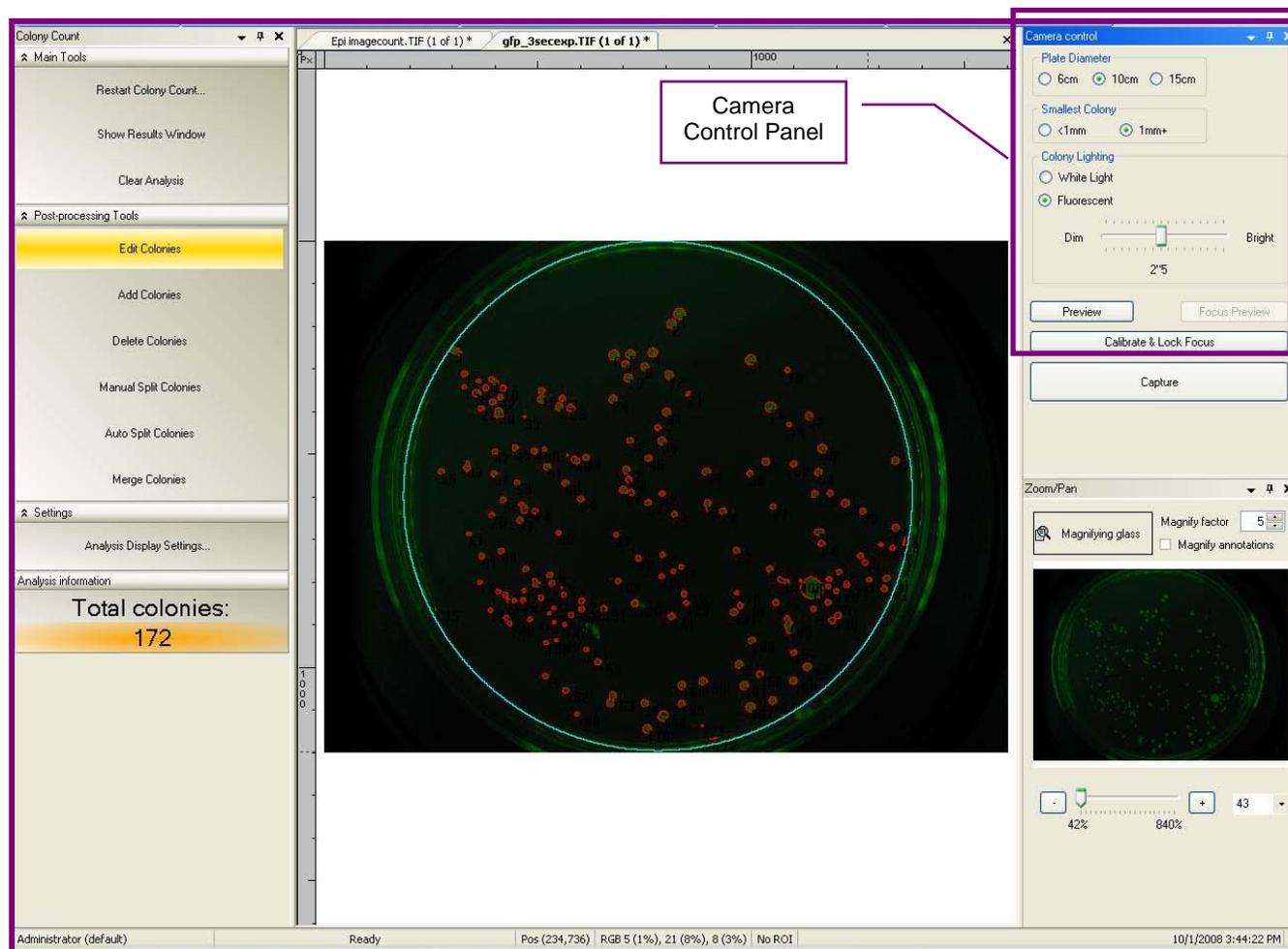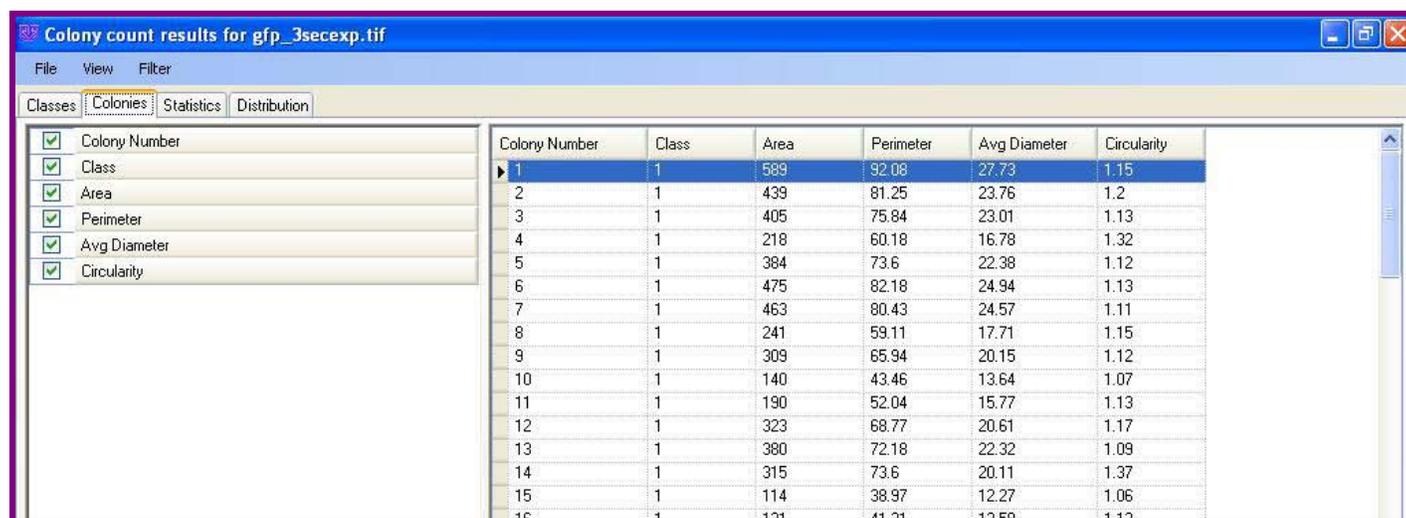

Fig. 4 – ColonyDoc-It Software Interface

Results

The software found 172 GFP colonies and created a detailed report of each colony counted. Results of the colony count are automatically entered into a tabular format and can be exported into Excel. The reported parameters are: classes, colonies, statistics and distribution of colony area.

- The classes parameter of the report details what number and type of classes were detected in the count.
- The colonies parameter lists each colony by number and provides information related to the class, area, perimeter, average diameter, and circularity of each colony counted.
- The statistics parameter of the report presents the numerical value of each value in a statistical format.
- The distribution parameter provides a bar graph of each colony area.

Fig. 5 - Results

Conclusion

The ColonyDoc-It Imaging Station provides a simple tool for capturing, counting, and reporting GFP-labeled colonies. By providing researchers with built-in epi blue lighting for fluorescence and an optimized GFP filter, colonies are detectable and counted with ease and accuracy.

ColonyDoc-It is a trademark of UVP, LLC