



# Focal Points



Application Note FP-143

**UVP, LLC** Upland, CA | (800) 452-6788 | (909) 946-3197 | info@uvp.com  
**Ultra-Violet Products Ltd.** Cambridge UK | +44(0)1223-420022 | uvp@uvp.co.uk  
**Web Site:** uvp.com

## Inhibition Zone Sizing with the ColonyDoc-It® Imaging Station

### Introduction

Inhibition zone sizing demonstrates the effectiveness of antibiotics on a given test organism. Disks soaked in antibiotic are placed in a Petri dish growing a test organism. If the test organism is susceptible to the antibiotic, the test organism's growth will be inhibited around the disk soaked in antibiotic. The size measurement of the inhibition zone is the clear region around the disk on the agars surface.

The ColonyDoc-It Imaging Station (Fig. 1) can be used for effective inhibition zone sizing.



Fig. 1 – ColonyDoc-It Imaging Station

### Materials

- E-Coli (Optional: transfected with pGLO plasmid)
- LB medium
- LB agar plates (Optional: 5mg/mL arabinose in the agar for GFP expression when using the pGLO plasmid)

### Methods

#### Preparation

- Grow the bacteria of interest in the suitable media to 0.5OD at 600nm.
- Spread 1mL of the bacteria in suspension on a 10cm diameter plate. (Dispense 2.25 mL if working with a 15cm diameter plate.)

**Note:** It is very important that the dispensed liquid fully covers the surface of the agar.

- Use a sterile tweezer to place the paper disc loaded with antibiotics on the surface of the agar. Ensure that the spacing between disks placed on the agar is even.
- Culture at 37°C overnight.



Fig. 2 - Plate demonstrating zone of inhibition

## Capturing the Image

- Place the plate inside the ColonyDoc-It. The plate can be upside-down if the medium is transparent.
- Turn on the switch for the transillumination light source on the front of the ColonyDoc-It unit.
- From the ColonyDoc-It software (Fig. 4) camera control panel, select the **Plate Diameter** size for the inhibition zone sample (Fig. 3).
- Select the **Smallest Colony** size in the plate.
- 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.

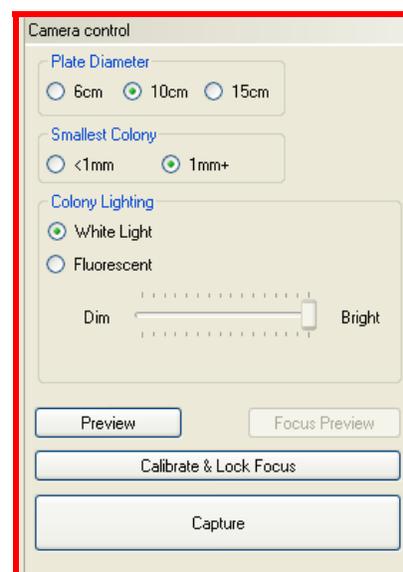


Fig. 3 - Camera Control Software Interface

## Generate Inhibition Zone Sizing

- To measure the size of the zones, open the Colony Counting plugin and select **Start Colony Count**. Click on the checkbox for **zone sizing** and then select **Manual count**. Click on a point within the zone. The software will automatically match all similar points in other zones. Adjust the sensitivity of the point selected until the entire zone area is highlighted. Click **Count**.
- Note: If performing inhibition zone sizing on similarly cultured (medium and bacteria) plates, save the parameters used in the manual count window as a template to speed the process for subsequent plates.

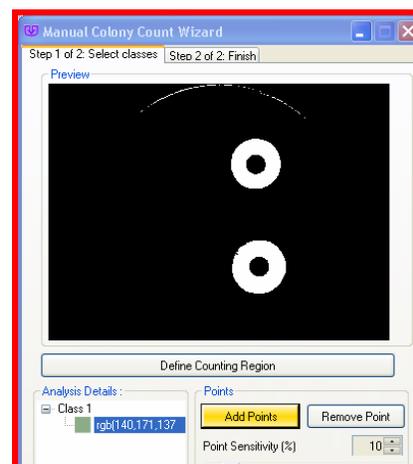


Fig. 4 - Manual Count Software Interface

## Results

The software found two inhibition zones and created a detailed report of each zone counted. Results 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 zones were detected in the count.
- The colonies parameter lists each zone by number and provides information related to the class, area, perimeter, average diameter, and circularity of each zone 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 zone area.

Colony Number	Class	Area(px)	Perimeter(px)	Avg Diameter(px)	Circularity
1	1	8865	311.25	93.93	1.12
2	1	8012	346.67	101.24	1.2

Fig. 5 - Results

## Conclusion

The ColonyDoc-It Imaging Station provides an easy method to capture, count, and report sizing information for inhibition zones. By providing researchers with a Colony Counting software module, zone sizing in antibiotic testing is achieved simply and accurately.