



**BioRad/UMAX GS-800 Scanner
and Bio Rad Quality One Software**



**Darkfield
Viewers**

Colony Counting SOP and Description

Prepared by: Bob Morrison
FVCC, Instrumentation Specialist
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Colony Counting: Using GS800 Scanner, Quality One Software

SLCC - Florissant Valley



1. Turn on Scanner and wait for both lights to show steady green- this is the ready state.
2. Turn on Host computer, logon as Operator/SMET
3. Select Quality One to scan (acquire) and image

Internet Explorer



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- Students must be aware of and comply with the licensing and copyright restrictions applicable to software and data files they may access. Copying software is strictly forbidden unless specifically authorized by an appropriate College authority.
- Users must respect the privacy of others; they may not access private files or communications of others, even if those files are unprotected.
- Federal law prohibits the transmission of certain software into certain foreign nations. When in doubt, students should not send software.
- Game-playing within College computer laboratories is prohibited unless assigned as part of a course.
- Use of College computer systems to listen to Internet-provided music (MP3, Streaming Audio) or Internet-provided video (Streaming Video) is prohibited unless as assigned as part of a course.

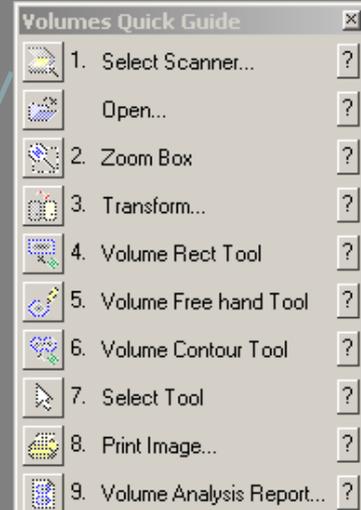
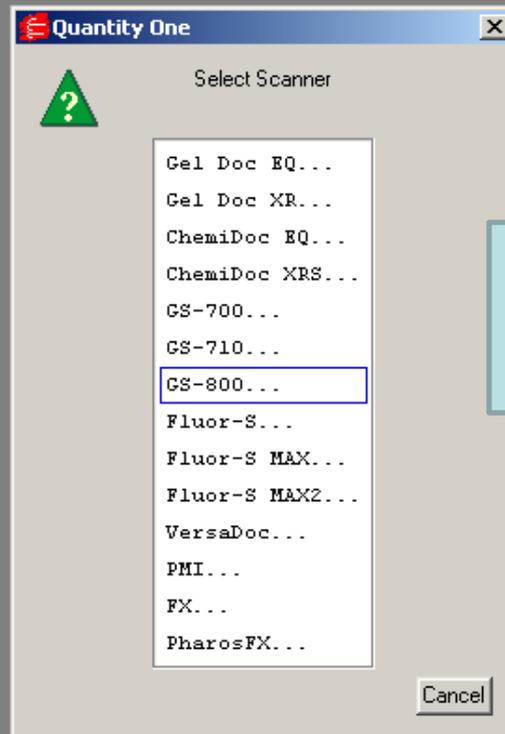
al or pornographic (as
nts or images is strictly

any abusive conduct
damaging any College
oducing a computer

[**Link to BioRad Quantity One 1-D Analysis Software Users Manual \(pdf\)**](#)

- Under Missouri law, unauthorized access or interference with computer systems, computer data and other computer users is a felony. Page 2

Colony Counting: Quality One, Select Scanner



1. Choose Select Scanner on the Volume Quick Guide menu
2. Select GS-800 as scanner type

Colony Counting: Set Light, Preview, Acquire Image

File Edit



Step I - Select Application

Select...

X-ray film

Gray film

Filter Red Green Blue White

Light Reflective Transmissive

Step II - Select Scan Area

Preview scan...

Click and drag in diagram to set scan area

Top: Left:

Bottom: Right:

Step III - Select Resolution

Select...

X resolution Microns

Y resolution Microns

Image file size: 4.17 Mb



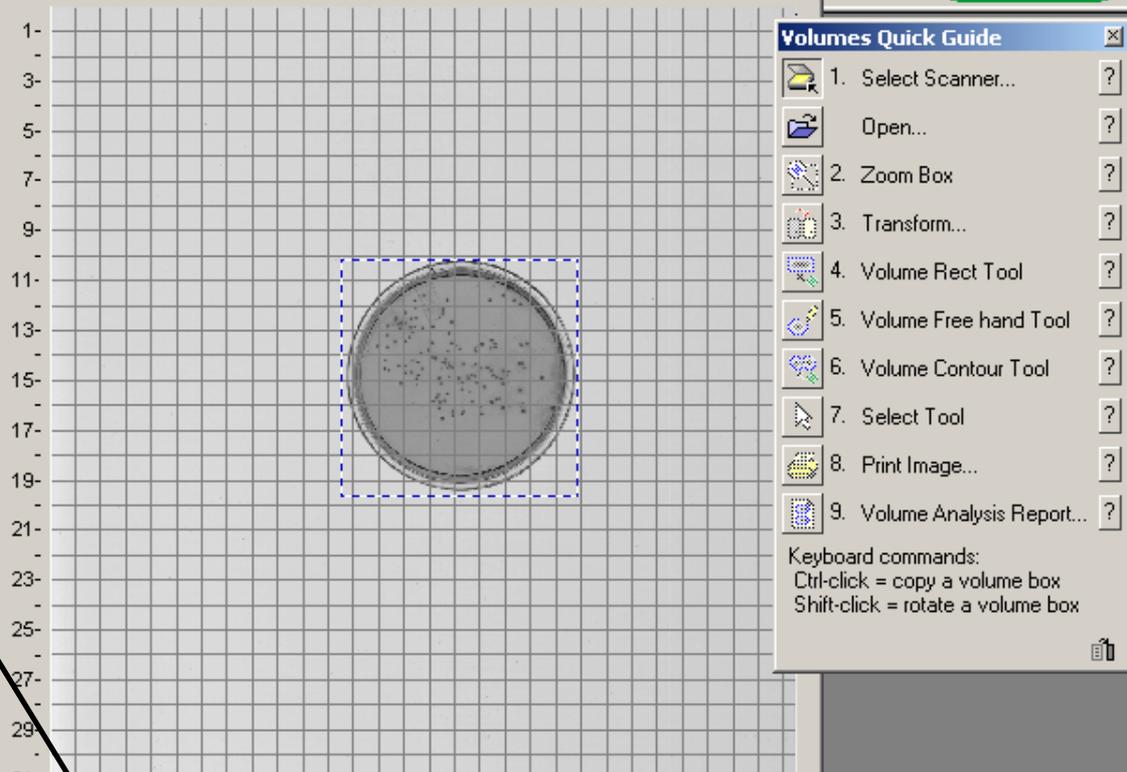
Options

Auto Save After Scan Hide Grid

Make Backup Copy

Highlight Saturated Pixels

Options... Help



1. Select image and then Photograph
2. Change Light from Reflective to Transmissive to best see your specimens
3. Preview Scan and wait for image outline to appear
4. Adjust "crop box" to contain just your specimen
5. Select "ACQUIRE" to capture the image
6. Hit STOP if image preview fails to appear

Colony Counting: Scanner, Examine Acquired Image

Quant File Edit

Step I - Select Application
Select...
X-ray film

Operator 2010-07-09 10hr 03min (Raw 1-D Image, New)



1-
3-
5-

1
3
5
7
9
11
13
15
17
19
21
23
25
27
29

35-
37-
39-

Acquire Stop

Options

- Auto Save After Scan
- Hide Grid
- Make Backup Copy
- Highlight Saturated Pixels

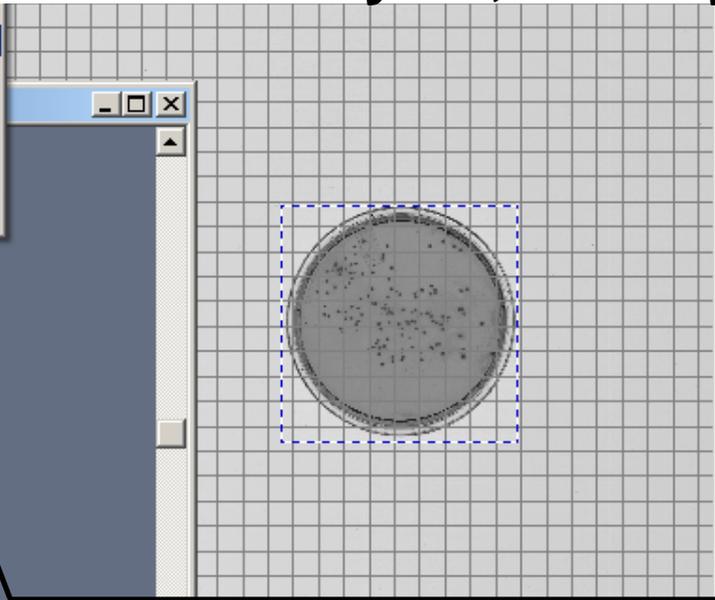
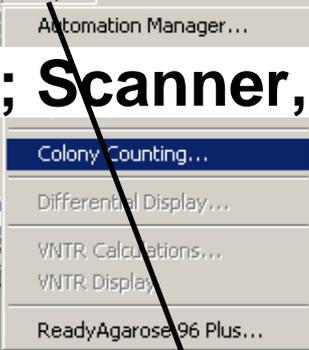
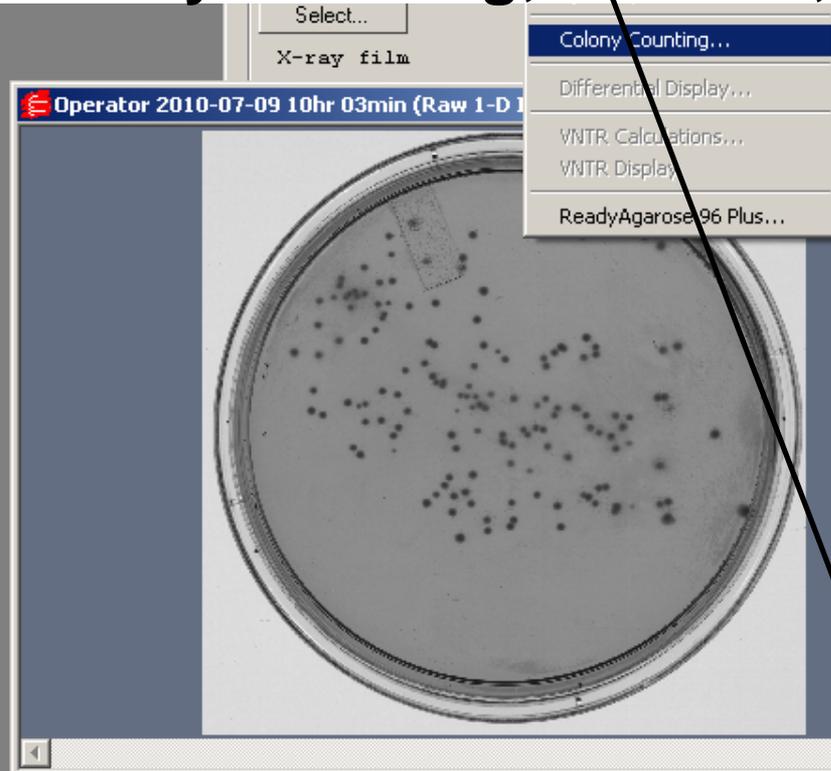
Options... Help

Volumes Quick Guide

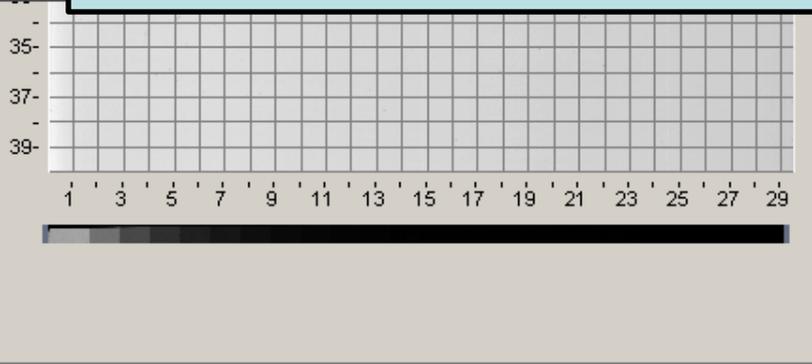
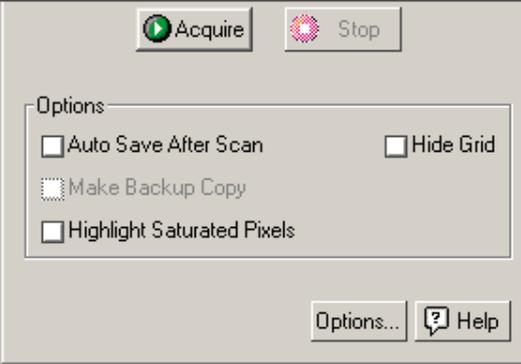
1. Select Scanner... ?
- Open... ?
2. Zoom Box ?
3. Transform... ?
4. Volume Rect Tool ?
5. Volume Free hand Tool ?
6. Volume Contour Tool ?
7. Select Tool ?
8. Print Image... ?
9. Volume Analysis Report... ?

Keyboard commands:
Ctrl-click = copy a volume box
Shift-click = rotate a volume box

Colony Counting; Scanner, Select Analysis, Colony Counting



- 1. Select Analysis
- 2. Select Colony Counting from Drop Down Menu



Colony Counting: Adjust Area of Colony

Step 1: Define Region and Count

Define Counting Region.
Drag the cursor from center to edge of dish image.

Count Sensitivity
Averaging 1 3 5 7 Plaque

Step 2: Adjust Results

White Blue
Colony count
Adjusted count

Count vs.
Peak density



Cutoff
White/Blue

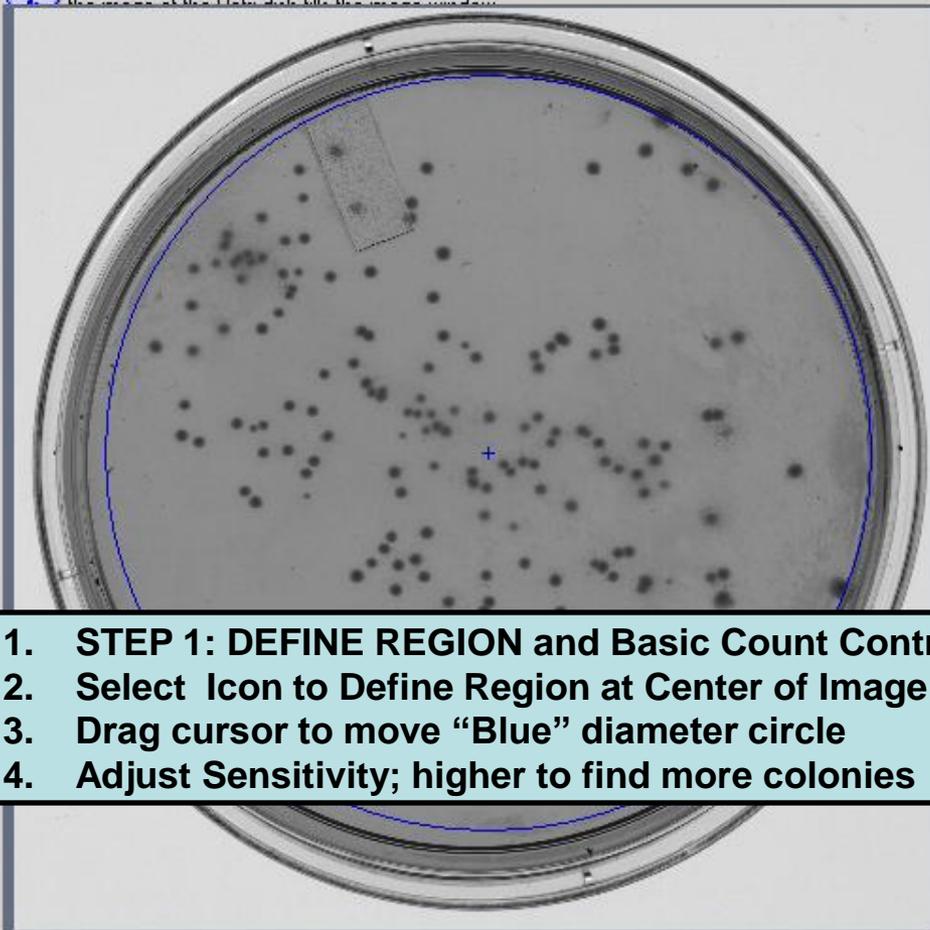
Step 3: Tools/options

Ignore region Show data area
 Make Colony Mark white colonies
 Erase Colony Mark blue colonies

Step 4: Save To Batch File

Batch mode...
Batch File Becky.GFP.Neg.03.batchcount.1.xls
Count Name Operator 2010-07-09 10hr 03min/10:04:54
Count Comment

For best results, adjust the Gel Doc zoom lens so that the image of the Petri dish fills the image window.



1. **STEP 1: DEFINE REGION** and Basic Count Controls
2. Select Icon to Define Region at Center of Image
3. Drag cursor to move "Blue" diameter circle
4. Adjust Sensitivity; higher to find more colonies



Colony Counting: Adjust Count vs. Density

Step 1: Define Region and Count

Define Counting Region.
Drag the cursor from center to edge of dish image.

Count Sensitivity
Averaging 1 3 5 7 Plaque

Step 2: Adjust Results

	White	Blue
Colony count	<input type="text" value="0"/>	<input type="text" value="75"/>
Adjusted count	<input type="text" value="0"/>	<input type="text" value="75"/>

Count vs.
Peak density



Cutoff

White/Blue

Step 3: Tools/options

<input checked="" type="checkbox"/> Ignore region	<input checked="" type="checkbox"/> Show data area
<input checked="" type="checkbox"/> Make Colony	<input checked="" type="checkbox"/> Mark white color
<input checked="" type="checkbox"/> Erase Colony	<input checked="" type="checkbox"/> Mark blue color

Step 4: Save To Batch File

Batch mode...

Batch File

Count Name

Count Comment

1. If there is a clear peak on the left end of the colony histogram, it is probably due to background intensity or noise in the image.
2. Use Cutoff slide to adjust per notes below

If background is being detected as colonies, you can use the histogram and the **Cutoff** slider to correct this.

Drag the **Cutoff** slider to the right until it is centered on the right edge of the background peak.

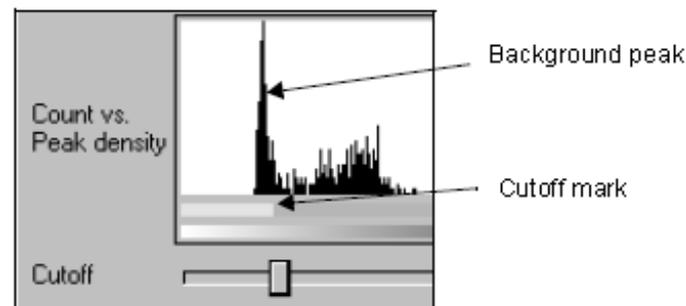


Fig. 8-5. Using the Cutoff slider.

The yellow portion of the bar beneath the histogram marks the range of image data has been designated as background noise, and is not being considered for colony counting purposes. The gold portion of the bar marks white colony data range.

Colony Counting: Adjust White vs. Blue

Step 1: Define Region and Count

Define Counting Region.
Drag the cursor from center to edge of dish image.

Count Sensitivity
Averaging 1 3 5 7 Plaque

Step 2: Adjust Results

	White	Blue
Colony count	<input type="text" value="0"/>	<input type="text" value="75"/>
Adjusted count	<input type="text" value="0"/>	<input type="text" value="75"/>



Cutoff
White/Blue

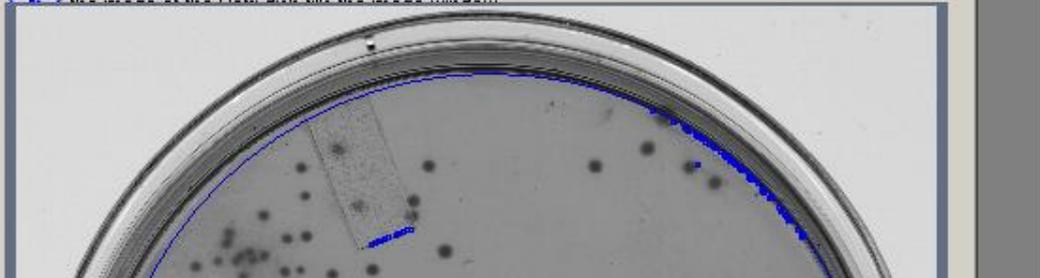
Step 3: Tools/options

Ignore region Show data area
 Make Colony Mark white colonies
 Erase Colony Mark blue colonies

Step 4: Save To Batch File

Batch mode...
 Batch File
 Count Name
 Count Comment

For best results, adjust the Gel Doc zoom lens so that the image of the Petri dish fills the image window.



White and Blue Colonies

If you know you have white and blue colonies in the image, and there are two clear peaks on the histogram to the right of the background peak, you can use the histogram to distinguish between these types of colonies.

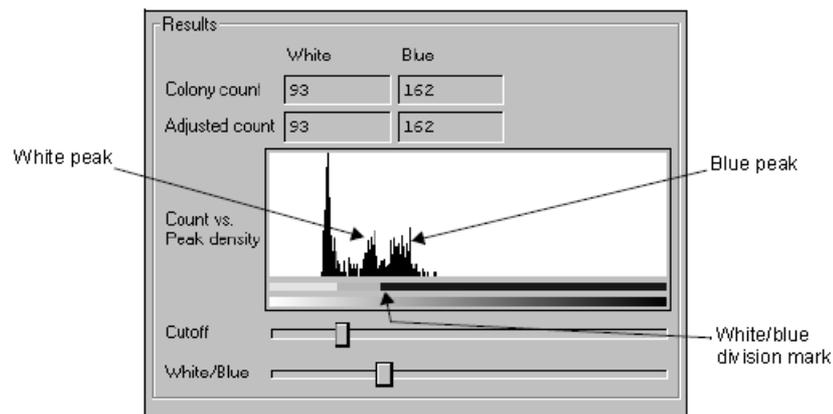


Fig. 8-6. Using the White/Blue slider.

Drag the **White/Blue** slider to the left until it is positioned between the two peaks. The white colony data range is indicated by gold on the bar beneath the histogram, and the blue colony data range is marked with blue.

As you drag the slider, the numbers of white and blue colonies will change in the dialog and in the text box on the image. Also on the image, you should see the marked white colonies (gold triangles) change to blue colonies (blue squares).

Note: If the blue colonies are not marked on the image, check to make sure that the **Mark Blue Colonies** checkbox at the bottom of the dialog is checked.

Totals
White 0
Blue 75

Colony Counting: Adjust counted items

Step 1: Define Region and Count

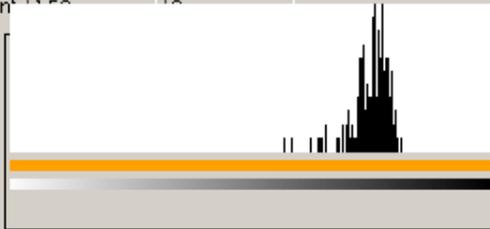
Define Counting Region.
Drag the cursor from center to edge of dish image.

Count Sensitivity
Averaging 1 3 5 7 Plaque

Step 2: Adjust Results

	White	Blue
Colony count	153	0
Adjusted count	153	0

Count vs.
Peak density



Cutoff

White/Blue

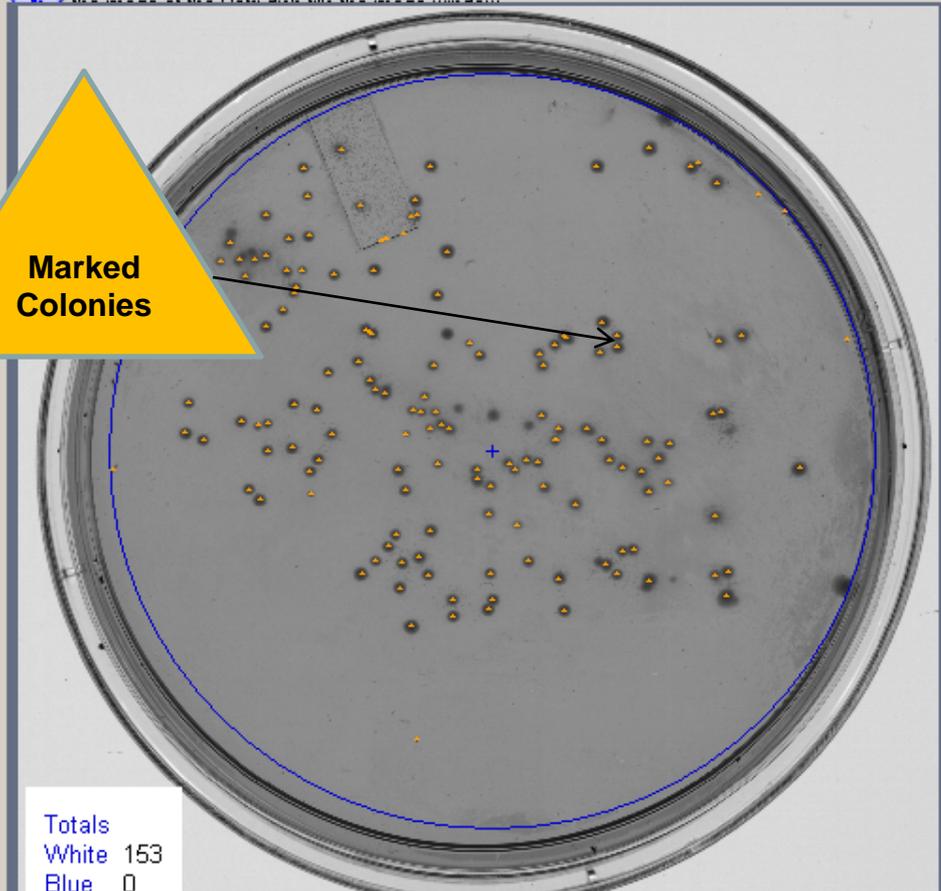
Step 3: Tools/options

Ignore region Show data area
 Make Colony Mark white colonies
 Erase Colony Mark blue colonies

Step 4: Save To Batch File

Batch mode...
 Batch File
 Count Name
 Count Comment

For best results, adjust the Gel Doc zoom lens so that the image of the Petri dish fills the image window.



Totals
White 153
Blue 0

1. Use STEP 3: to Adjust items in/out of the counts
2. Erase colony; then pick items from image that are NOT part of the colonies
3. Make Colony; then pick items to be added as colonies (yellow markers)

Colony Counting: Save Count Data to File (Excel, other)

Step 1: Define Region and Count

Define Counting Region.
Drag the cursor from center to edge of dish image.

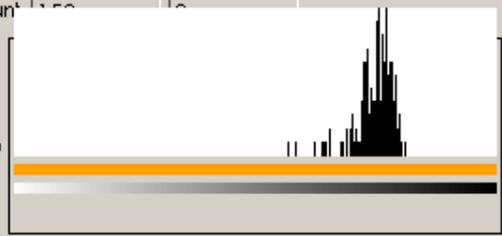
Count Sensitivity

Averaging 1 3 5 7 Plaque

Step 2: Adjust Results

	White	Blue
Colony count	<input type="text" value="152"/>	<input type="text" value="0"/>
Adjusted count	<input type="text" value="152"/>	<input type="text" value="0"/>

Count vs. Peak density



Cutoff

White/Blue

Step 3: Tools/options

Ignore region Show data area

Make Colony Mark white colonies

Erase Colony Mark blue colonies

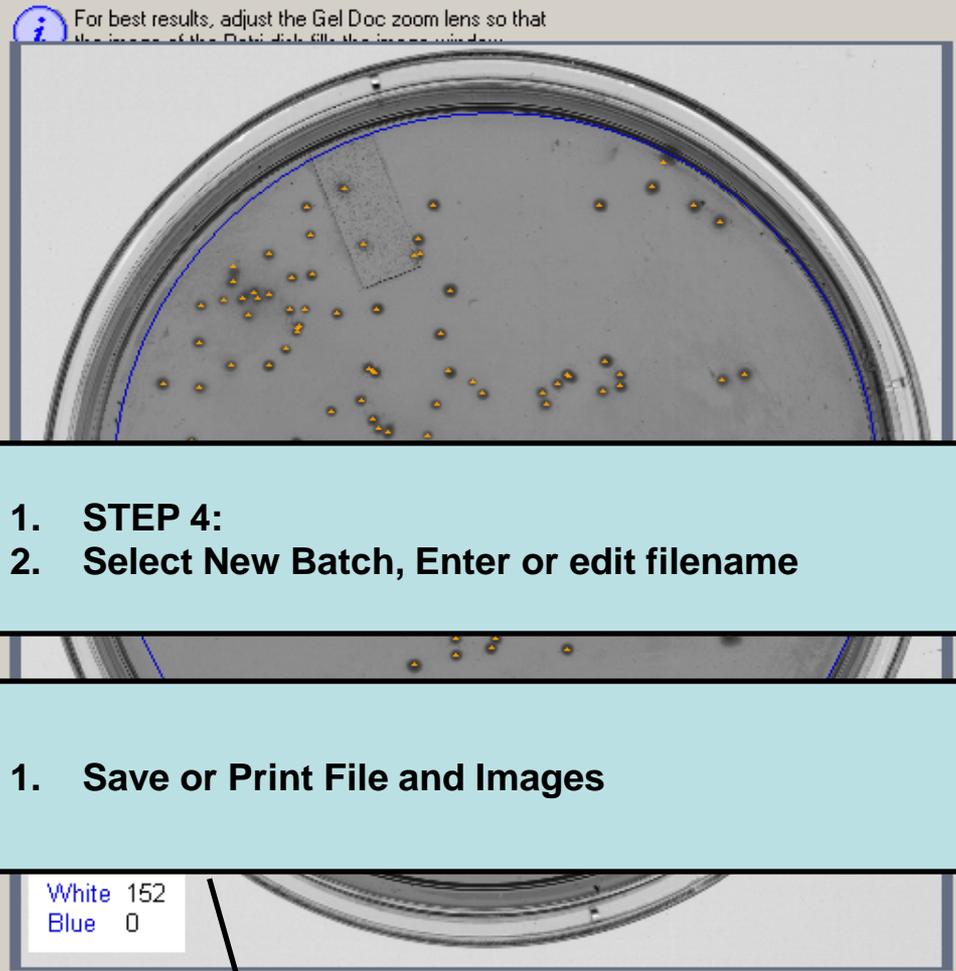
Step 4: Save To Batch File

Batch mode...

Batch File

Count Name

Count Comment



1. STEP 4:
2. Select New Batch, Enter or edit filename

1. Save or Print File and Images

White 152
Blue 0



Colony Counting: Viewers, Counters at FV

Key Features:

- Even, glare-free illumination
- Light is spread uniformly over the entire culture plate
- Colonies are bright and easily distinguished
- Adjustable dish holder for centering both round dishes and square culture plates
- Adjustable focusing rod
- Lens rotates a full 360°
- Built-in tilt leg
- Optional 1.5X auxiliary lens fits over standard lens, increasing magnification to 3X
- Footprint: 12.5in wide x 14 in high x 12 in depth



Quebec Darkfield Model 3330

1. An adjustable dish holder for centering round dishes with diameters up to 100mm and square culture plates up to 100mm x 100mm.
2. An optional 1.5X auxiliary lens fits over the standard lens increasing magnification to 3.0X.
3. The adjustable focusing rod allows the 1.5X standard lens to be raised or lowered. The lens also rotates a full 360° for ready access to culture plates.
4. A built-in tilt leg may be mounted in the front or rear of the instrument allowing a convenient tilt angle, or it may be locked flat to the instrument base.
5. A white-ruled Wolffheugel counting plate is included.
6. Internal standard light bulb, 110V plug connection, On/Off switch.

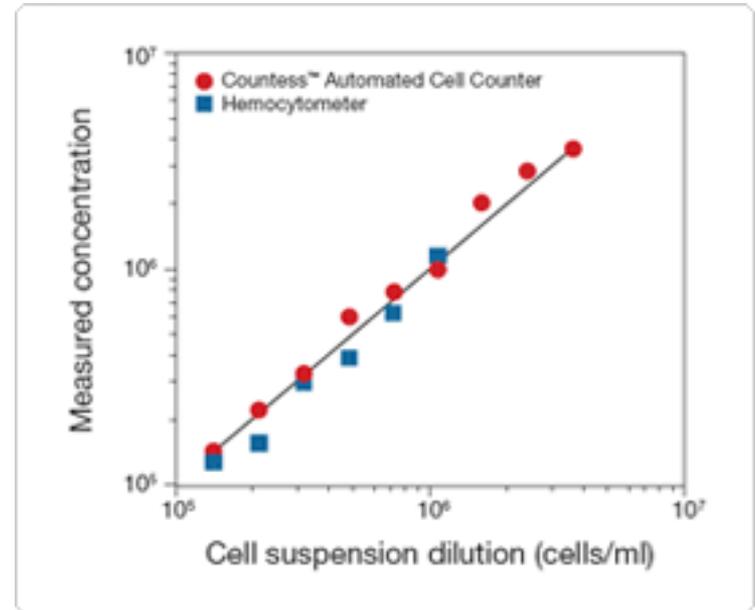


Quebec Darkfield Model 3325, 3327

Counter: Automated, Invitrogen “Countess” ; (Example Only , Presently Not at FV)



The Countess™ Automated Cell Counter uses trypan blue staining combined with a sophisticated image analysis algorithm to produce accurate cell and viability counts in just 30 seconds. The algorithm also measures average cell size of live, dead, and total cells to give you all the data you need to proceed with your experiments. The measurement range extends from 1×10^4 to 1×10^7 cells/ml, with an optimal range from 1×10^5 to 4×10^6 cells/ml, broader than that of a hemocytometer (view technical notes for more comparison data). The optimal cell size is between $5 \mu\text{m}$ to $60 \mu\text{m}$ (view validated cell lines). A handy dilution calculator even helps you determine how to prepare your sample for your next passage or experiment.



The Countess™ Automated Cell Counter eliminates the tedium and subjectivity of manual cell counting. Automated counting frees up your time, reduces eye strain, and minimizes subjective judgments that can lead to error. It takes 3 simple steps:

1. Mix $10 \mu\text{l}$ of sample with $10 \mu\text{l}$ of trypan blue, and pipet into Countess™ chamber slide
2. Insert slide into the instrument
3. Press the "Count cells" button, results are displayed in 30 seconds

: Other Protocols or Notes

- Future home of other or more details protocols....