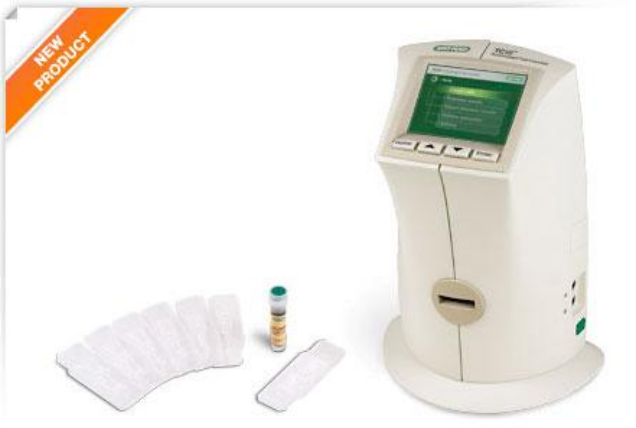
Colony Counting using BioRad/UMAX GS-800 Scanner and Bio Rad Quality One Software



BioRad TC10 Cell Counter With Thermal Printer

Colony Darkfield Viewers

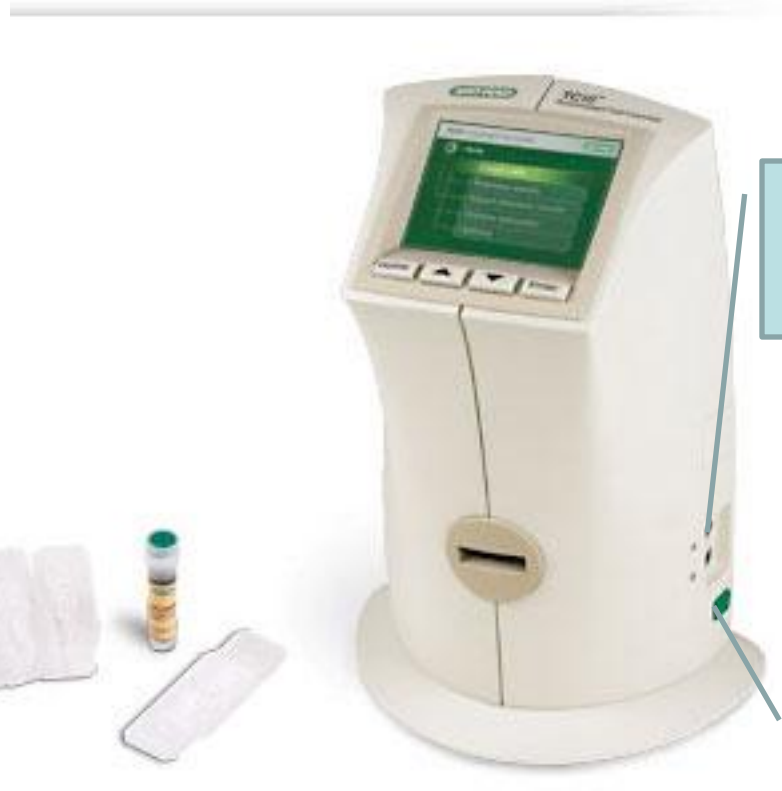
Counting; Cell and Colony SOP and Description

Prepared by: Bob Morrison
STLCC- CPLS, Instrumentation Specialist
August 2008 Initial, Latest Rev Mar 11

Counting: Cell Counter, Bio-Rad, TC10

TC10™ Automated Cell Counter
CAT# 145-0001
100–240 V, includes instrument
USB key,
30 TC10 dual-chamber counting slides (60 counts)
1.5 ml TC10 trypan blue dye

Slides: 145-0011 30 dual sides per box. (60 total counts)



Counting time 30 sec
Cell concentration range 5 x 10^4–1 x 10^7 cells/ml
Cell diameter range 6–50 µm
Sample volume 10 µl
Data storage 100 counts
Data export Via USB flash drive
Printer connectivity Yes
Dilution calculator Yes
Image of cells available Yes
Dimensions (W x D x H) 19 x 15 x 25.4 cm (7.5 x 6 x 10″)
Weight 2.2 kg (4.8 lb) (without the external power supply)

The TC10 cell counter is an automated device that provides a total count of mammalian cells and a live/dead ratio in one simple step with accurate, reproducible results. Count cells prior to cell culture or before starting processes and analytical techniques that require an accurate and consistent number of input cells. The TC10 automated cell counter helps you avoid the tedious task of counting cells with a microscope and hemocytometer which produce varying results. The TC10 automated cell counter uses multifocal plane analysis to assess cell viability. And, it demonstrates accurate cell counts of viable cells and across a range of cell concentrations and cell sizes.

Counting: Cell, Bio-Rad TC10, Loading Slides

Loading Slides

Handle the TC10™ counting slides using the edges and avoid touching the optical surface of the slides.

Important: When loading the sample, place the pipet tip at a 45-degree angle at the bottom of the sample loading area (half circle at outer end of the chambers). Slide the tip along the surface and carefully touch the apex of the half circle (Figure 7). Once the tip is stopped, depress the plunger to begin the capillary loading process. Care should be taken to avoid visible bubble formation or back splatter. Do not overfill or underfill the chamber.

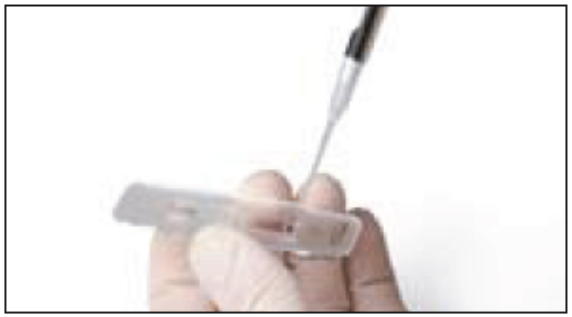


Fig. 7. Loading the TC10 counting slide.

Important: Load samples only into the outer ends of the counting chambers (Figure 8). The center areas are open to promote uniform cell distribution in the counting chamber. Do not use the center areas for sample loading to avoid an undercounting of the cells in the sample.

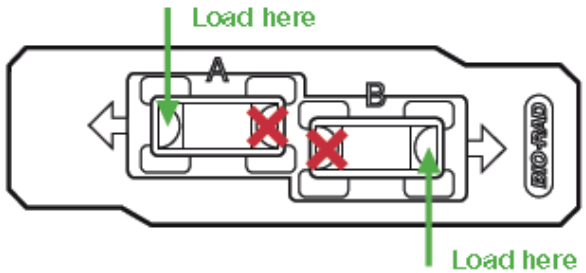


Fig. 8. Correct and incorrect loading are

TC10 cell counting slides cannot be reused. Dispose of used slides as biohazardous was according to your local environmental health and safety regulations.

Counting: Cell, Bio-Rad, TC10, User Interface

User Interface

The user interface contains a color LCD screen and the four navigation keys listed below:

- **Home** — for accessing the Home screen or for exiting any screen
- **Up** and **down** arrow keys — for moving to the desired setting within a screen
- **Enter** — for confirming a selection

Home Screen

The Home screen can be accessed from any screen by pressing **Home**. This screen (Figure 2) provides access to the following choices:

Count cells — initiates a cell count. When this option is selected, the instrument will automatically start counting if a slide is already inserted. If there is no slide in the instrument, the **Insert slide** screen will be displayed

Previous counts — for viewing results from up to 100 previous counts stored in the instrument; results are organized by date/time stamps, with the most recent count shown first

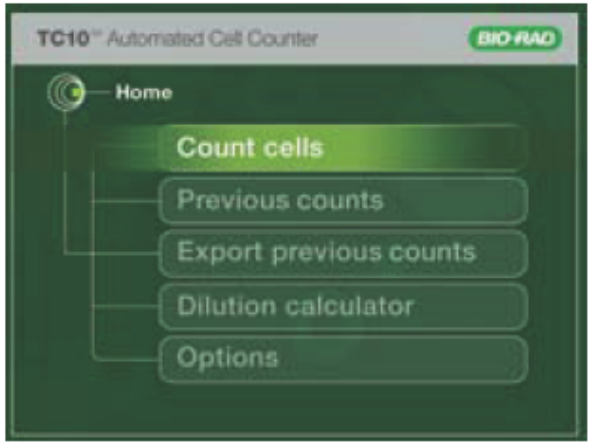


Fig. 2. Home screen.

Export previous counts — for exporting results from the instrument onto a USB flash drive

Dilution calculator — calculates volume adjustments required to achieve the cell concentration needed for the next experiment

Options — for selecting additional operations

Hot Link to Bio-Rad TC10 User Manual… pdf 36 pgs

Counting: Cell Counter, Bio-Rad, QuickGuide, pg 1/2

Hot Link to Bio-Rad TC10 User Manual… pdf 36 pgs

The TC10 automated cell counter counts mammalian cells in one simple step, with or without trypan blue staining, and assesses cell viability via trypan blue dye exclusion.

Important: Carefully read the safety information and instrument operating specifications provided in the TC10 Automated Cell Counter Instruction Manual before using the system.

Preparing Samples

The TC10 automated cell counter is designed to count suspended cells that have been grown as adherent or suspension cells. The TC10 counter demonstrates high reproducibility when counting cells within the ranges of 5×10^4–1×10^7 cells/ml and 6–50 µm cell diameter.

Important: Handle the TC10 counting slides using the edges and avoid touching the optical surface of the slides. TC10 counting slides cannot be reused. Dispose of used slides as biohazardous waste according to your local environmental health and safety regulations.

Preparing Samples without TC10 Trypan Blue Dye

Pipet 10 µl of the cell suspension into the **outer opening** of either of the two chambers of the counting slide (Figure 1).

Preparing Samples with TC10 Trypan Blue Dye

1. In a micro test tube, combine 10 µl of the cell suspension with 10 µl of TC10 trypan blue dye. When counting the sample in duplicate, combine 20 µl of the cell suspension with 20 µl of TC10 trypan blue dye. Gently pipet up and down ten times to mix the cells and dye.

2. Within 10 minutes of mixing, pipet 10 µl of the mixture into the **outer opening** of either of the two chambers of the counting slide (Figure 1).

Performing Cell Counts

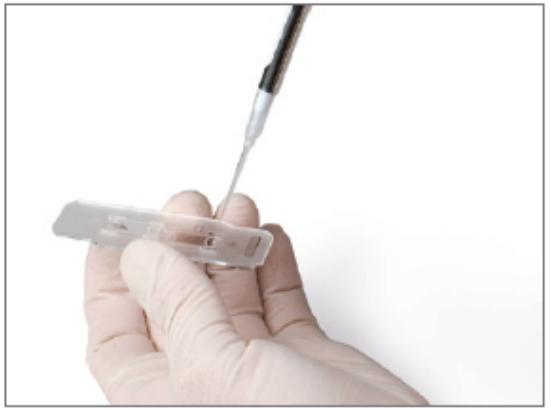


Fig. 1. Loading the sample onto a slide.

Fig. 2. Inserting the slide into the TC10 cell counter; counting automatically begins.

Counter: Cell Counting, Bio-Rad, Quickguide, Pg 2/2

1. Press Home and insert slide

2. The TC10 cell counter automatically detects the presence of the slide and initiates a count. The TC10 counter also automatically detects the presence of trypan blue dye.

Important: Do not remove the slide or interrupt the instrument while it is performing the count.

3. For samples without trypan blue dye — on the Current count screen, the TC10 counter provides the total cell count per ml (Figure 3).

4. For samples with trypan blue dye — on the Current count screen, the TC10 counter provides the total cell count per ml, live cell count per ml, and percentage of live cells (Figure 4). It also accounts for a 1:1 dilution with the dye and provides a dilution calculation based on live cells.

Important: If the number of cells is above or below the range detected by the instrument, a "Value out of range" message appears on the Current count screen. Press the down arrow key to select View image, then press Enter. View the image of the cells and determine whether the sample should be diluted or concentrated. Repeat the cell count.

5. Once the instrument completes the cell count, remove the slide from the slide slot.

Fig. 3. Current count screen showing a total cell count (without trypan blue dye).

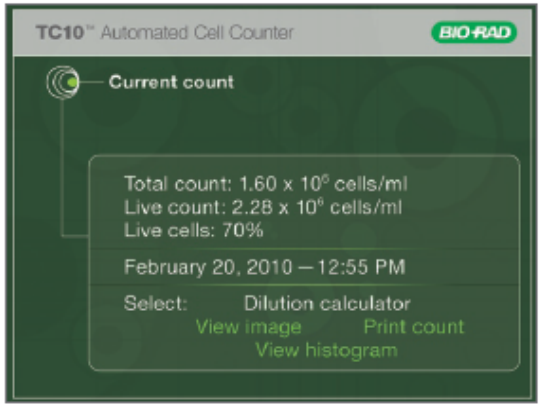


Fig. 4. Current count screen showing total cell count, live cell count, and percentage of live cells; cell viability is measured via trypan blue dye exclusion.

Additional Analysis Options

Additional analysis options are available in the Current count screen. Use the up or down arrow keys and press Enter to access the following screens: Dilution calculator, View image, Print count, and View histogram. Helpful tips for each screen are provided below.

Dilution Calculator

1. In the Dilution calculator, the current count is used as the starting cell concentration. The parameter that is ready to be modified is highlighted. Press the up or down arrow key to find the correct value, then press Enter to confirm the selection.

2. To print the Dilution calculator screen, press the down

View Image

Use the up arrow key to zoom in on an image and examine cells in detail. Use the down arrow key to zoom out.

Print Count

The TC10 automated cell counter allows you to print the cell count using the TC10 thermal label printer (catalog #145-0005) connected to USB port A or USB port B on the cell counter.

View Histogram

Counter: TC10, Exporting Images of Cells

Exporting the Image of Cells from the Current Count

The image from the most recent count can be downloaded onto a USB flash drive and viewed on your computer in JPEG format.

To export the image:

1. Press **Home** to go to the Home screen.
2. Use the **down** arrow key to go to **Options**, and press **Enter**.
3. Choose **Export current image**, and press **Enter**.
4. If there is no USB flash drive inserted into the instrument, "Insert USB flash drive into Port A to export current image. Press Home to cancel" is displayed on the screen. Insert a USB flash drive into the USB port A. If a USB flash drive is already inserted into the USB port A, the export starts automatically.
5. A bar showing the progress of the image download is displayed on the screen. When the image export is complete, "File saved as CELLxxx.JPG. Press Home to exit" is displayed.
6. To exit, press **Home**.
7. Remove the USB flash drive from the instrument.

   Note: If a new slide is inserted **while** the instrument is exporting an image, the instrument queues the count until it completes the export. The instrument then detects the slide and asks if it should start counting the sample in the new slide. If a new slide is inserted **after** the image is exported, the instrument starts counting the sample in the new slide.

Counting: Bio-Rad TC10™ Verification Kit

Bio-Rad part no: **145-0014**
Kit for validation of TC10 automated cell counter functionality, includes TC10 verification slide, protocol
Price: approx $360 retail.

Result of Verification Test:
March 28, 2011 - 8:41 AM
System verification performed
Side A: Pass (3886 of 3886, 0.50)
Side B: Pass (195 of 195, 0.50)
Rerun 9/30/11, same results as above

1. From the Home screen, select Options, then Enter
2. From the Options screen, select System Test and Enter
3. Follow instructions to insert side A of the verification slide, then side B, then a “blank” read is made.
4. Results of the test are displayed and can be exported to a flashdrive using the Export Previous counts function.
5. Complete instructions on the verification slide protocol can be found at the hotlink shown below.

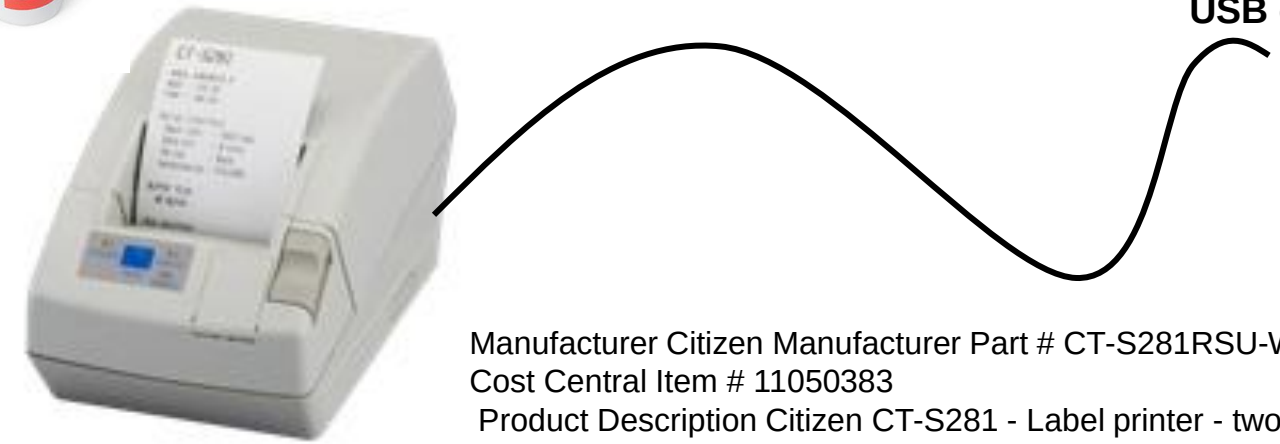
Hot Link to Bio-Rad TC10 Verification slide protocol… 2 pgs

Counting: Bio-Rad TC-10 Printer, Thermal

Bio-Rad Part#

145-0005 TC10 Thermal Label Printer, includes label printer, USB cable, 1 roll of 185 labels

145-0007 Thermal Printer Labels, 1 roll of 185 labels, for TC10 thermal label printer



USB connect to TC10 Counter

Manufacturer Citizen Manufacturer Part # CT-S281RSU-WH-P
Cost Central Item # 11050383
Product Description Citizen CT-S281 - Label printer - two-color - direct thermal - Roll (2.3 in) - 203 dpi - up to 189 inch/min –
Serial Printer Type Label printer - direct thermal - two-color (monochrome) Approximate Dimensions (WxDxH) 4.2 in x 7.1 in x 4.1 in
Approximate Weight 1.4 lbs
Localization United States Max
Media Size (Standard) Roll (2.3 in) Print Speed Up to 189 inch/min - max speed
Max Resolution (B&W) 203 dpi Interface Serial Barcodes Code 93, Code 39, QR code, EAN/JAN-8 , EAN/JAN-13 , UPC-A, UPC-E, Codabar, Code 128, PDF417, ITF\
Power AC 120/230 V Microsoft Certification Compatible with Windows 7

Hot Link to Bio-Rad (Citizen) TC10 Printer Manual… pdf 33 pgs

Counting: Colony, Using GS800 Scanner, Quality One Software

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

2100 expert

ND-1000 V3.5.1

Quantity One

nd-1000-v3....

Quantity One Manual

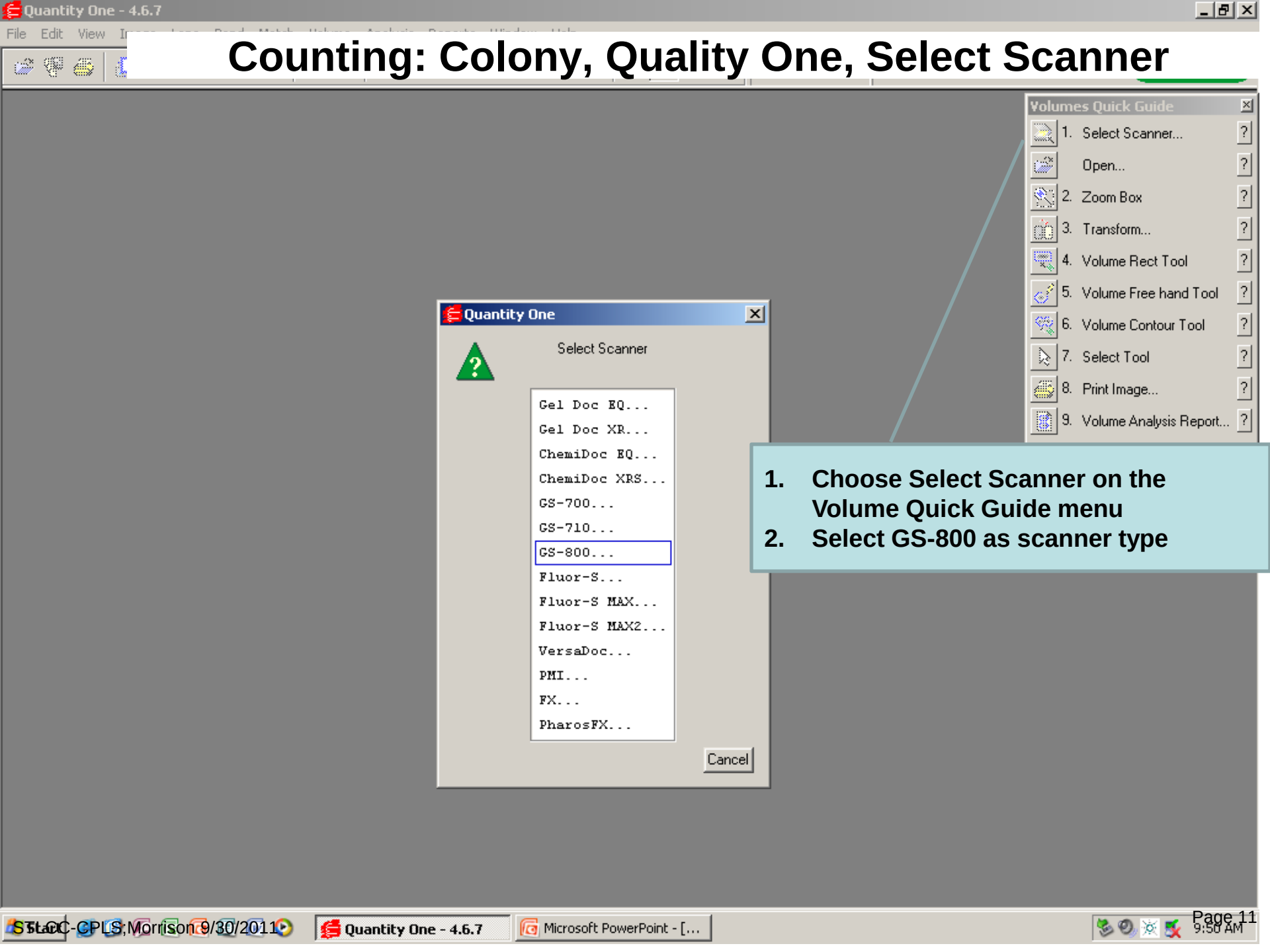
PDQuest

- Students may not use another person's account or lend their account to someone else.
- 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.

Link to BioRad Quantity One 1-D Analysis Software Users Manual (pdf)

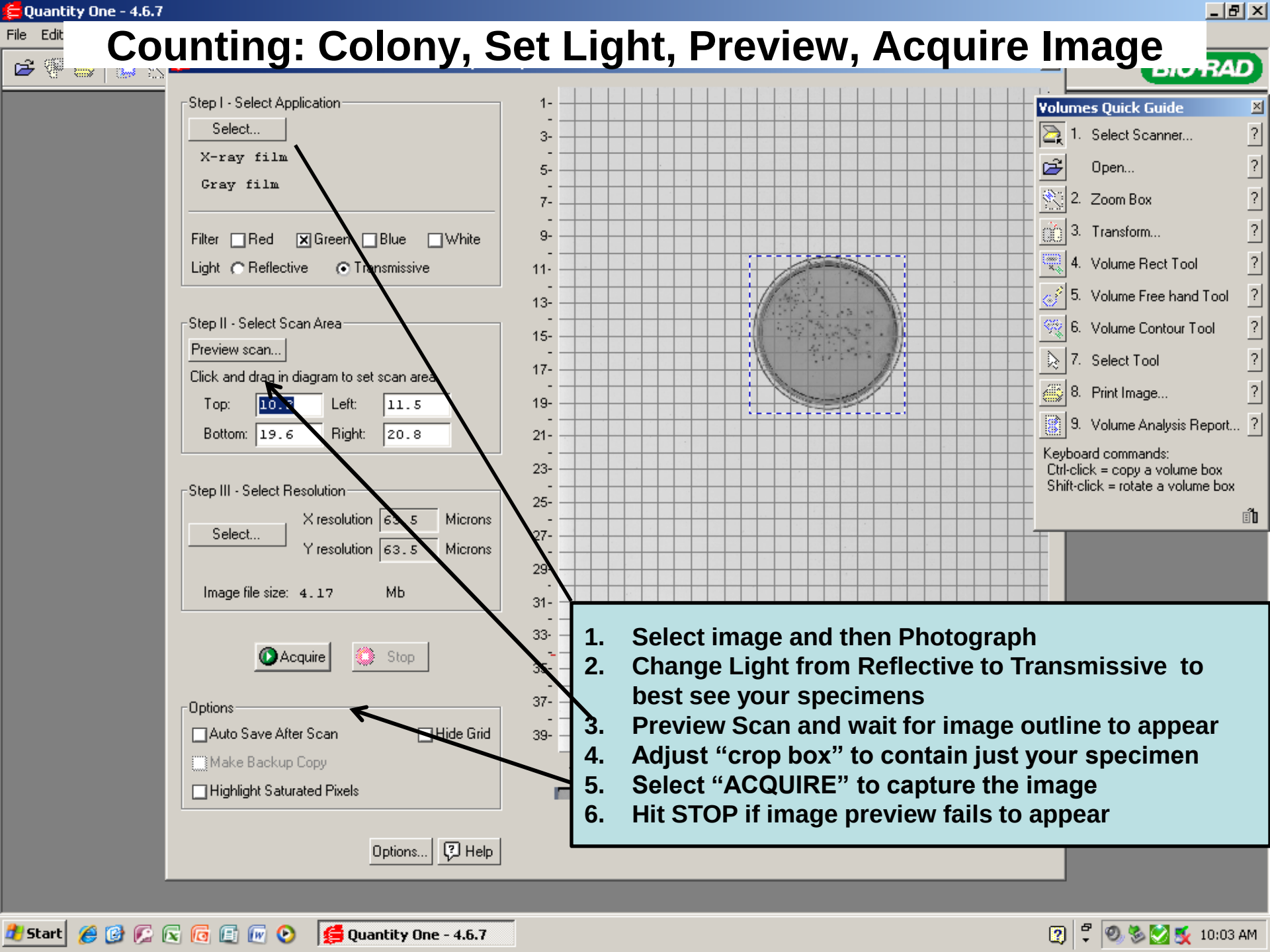
- Under Missouri law, unauthorized access or interference with computer systems, computer data and other computer users is a felony.

Counting: Colony, Quality One, Select Scanner



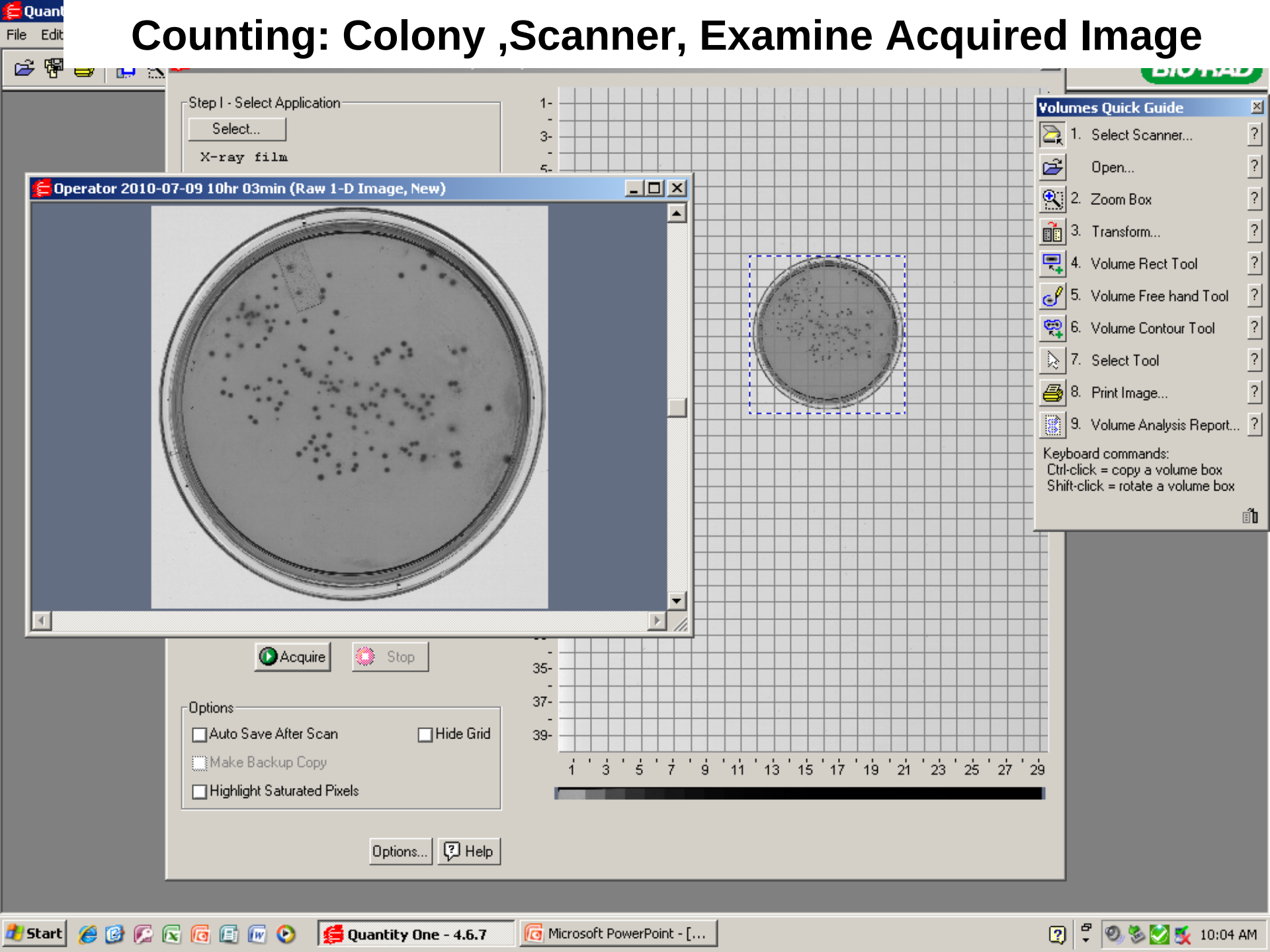
1. Choose Select Scanner on the Volume Quick Guide menu
2. Select GS-800 as scanner type

Counting: Colony, Set Light, Preview, Acquire Image

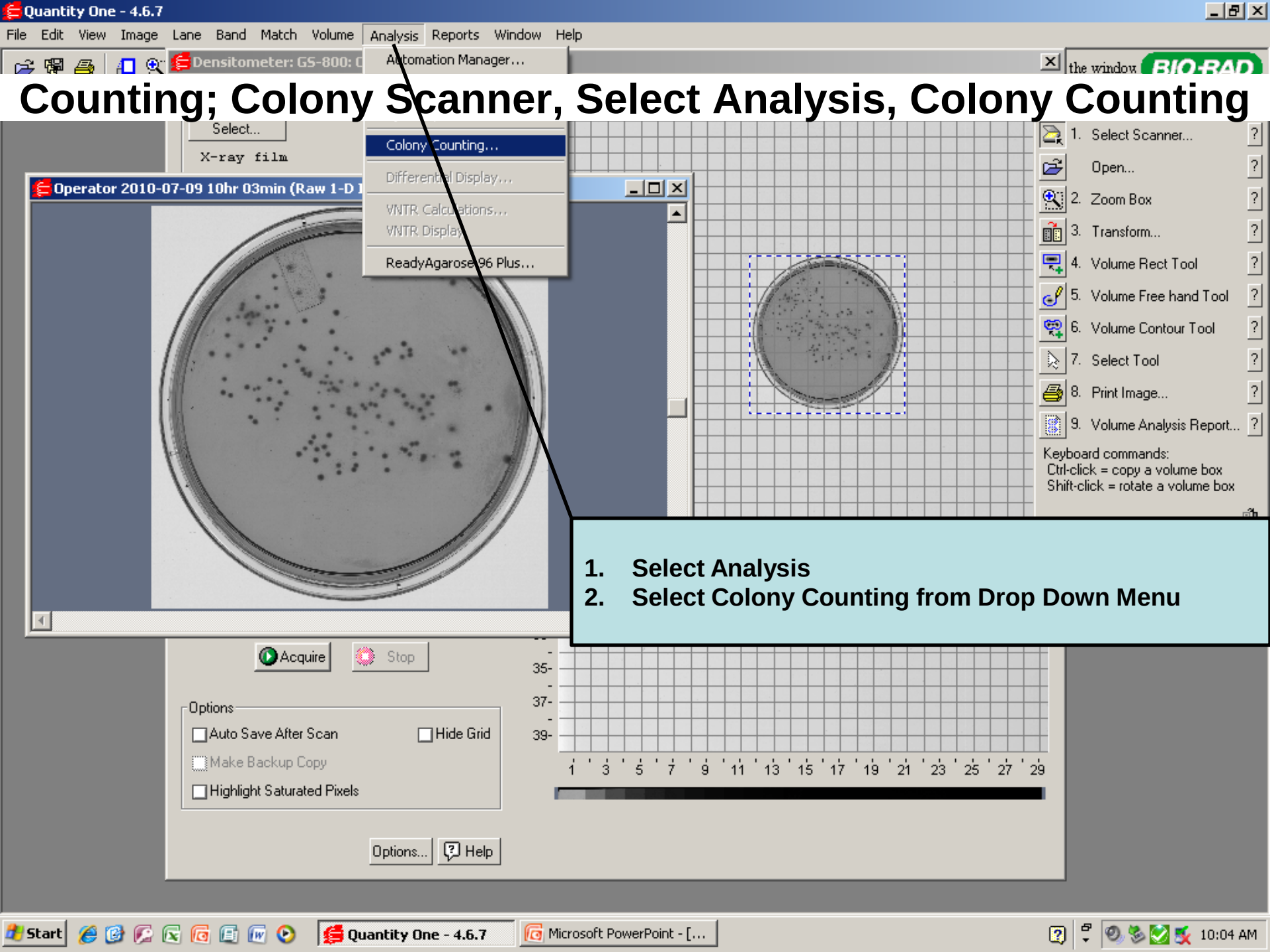


1. Select image and then Photograph
2. Change Light from Reflective to Transmissive to best see your specimens
3. Preview Scan and wait for image outline to appear
4. Adjust “crop box” to contain just your specimen
5. Select “ACQUIRE” to capture the image
6. Hit STOP if image preview fails to appear

Counting: Colony ,Scanner, Examine Acquired Image

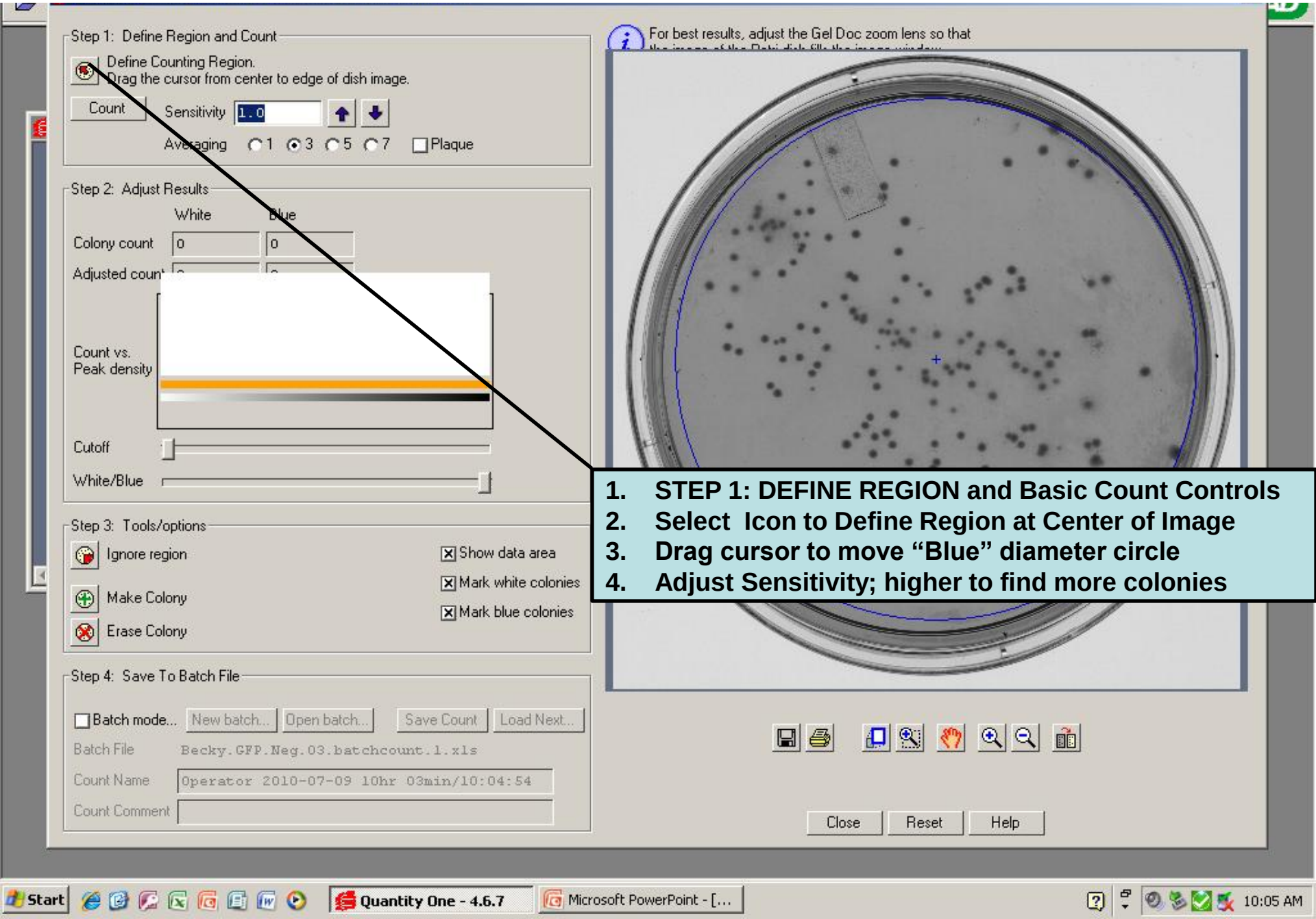


Counting; Colony Scanner, Select Analysis, Colony Counting



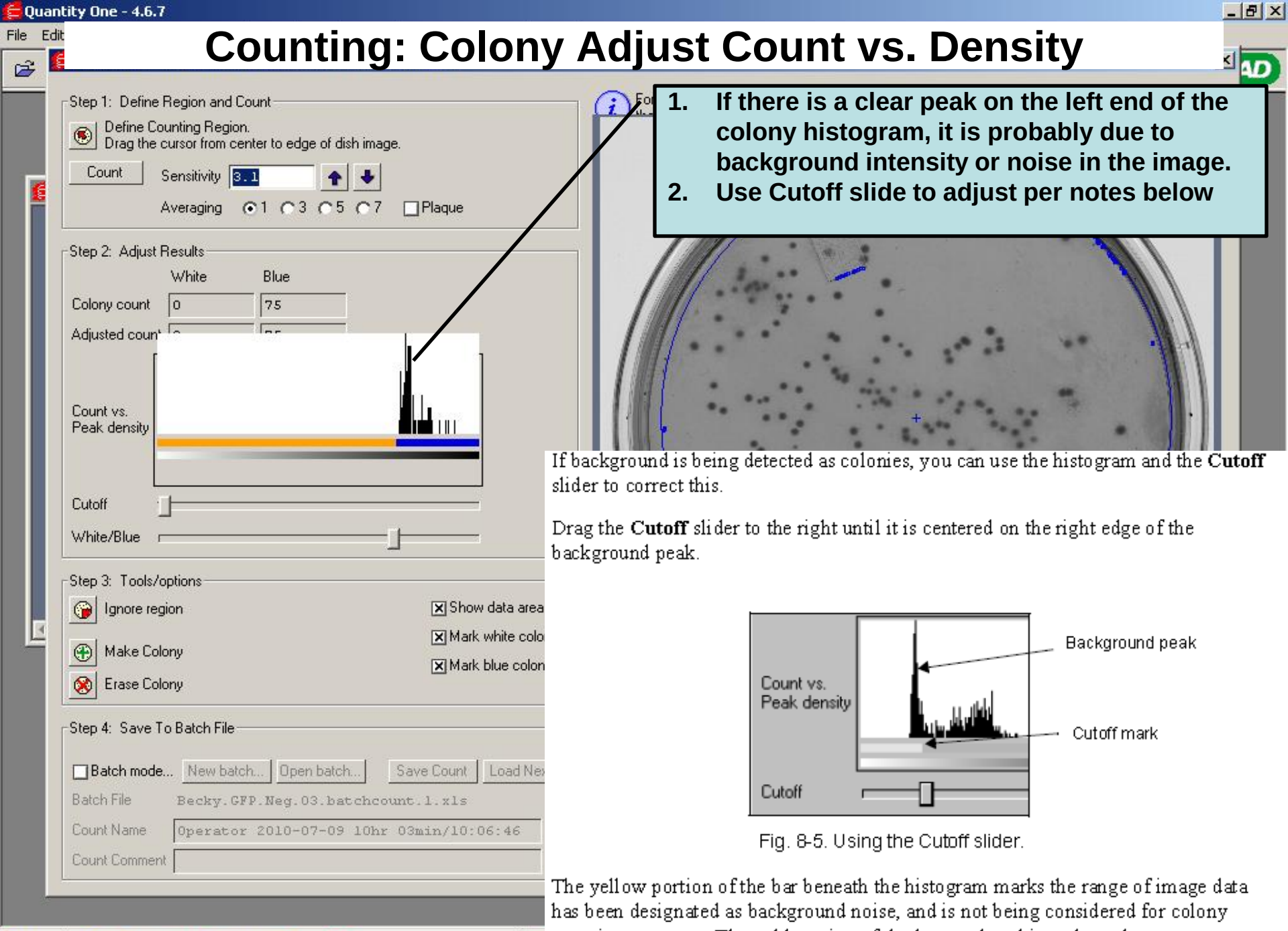
1. **Select Analysis**
2. **Select Colony Counting from Drop Down Menu**

Counting: Colony Adjust Area of Colony



Counting: Colony Adjust Count vs. Density

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.

Counting: Colony Adjust White vs. Blue

Quantity One - 4.6.7

File Edit

Step 1: Define Region and Count

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

Count | Sensitivity 3.1 | Averaging 1 3 5 7 | Plaque

Step 2: Adjust Results

	White	Blue
Colony count	0	75
Adjusted count		

Count vs. Peak density

Cutoff

White/Blue

Step 3: Tools/options

Ignore region
Make Colony
Erase Colony

Show data area
Mark white colonies
Mark blue colonies

Step 4: Save To Batch File

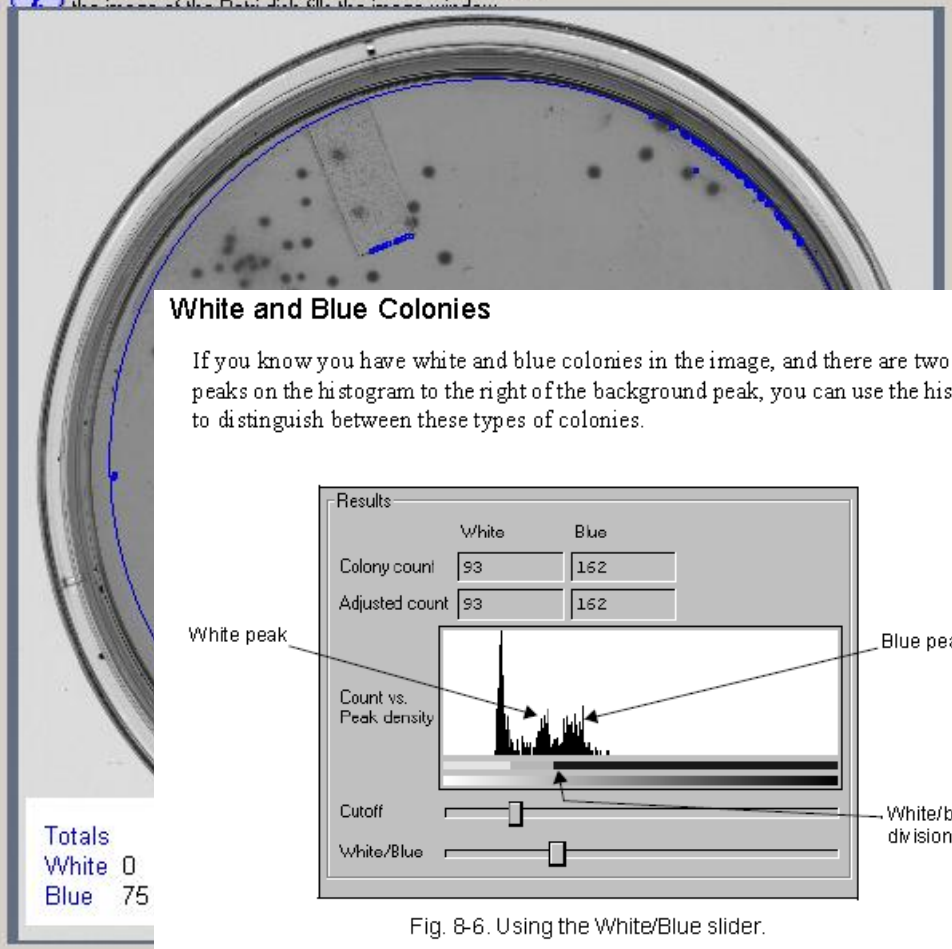
Batch mode... | New batch... | Open batch... | Save Count | Load Next...

Batch File: Becky.GFP.Neg.03.batchcount.1.xls

Count Name: Operator 2010-07-09 10hr 03min/10:06:46

Count Comment:

For best results, adjust the Gel Doc zoom lens so that



Totals
White 0
Blue 75

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.

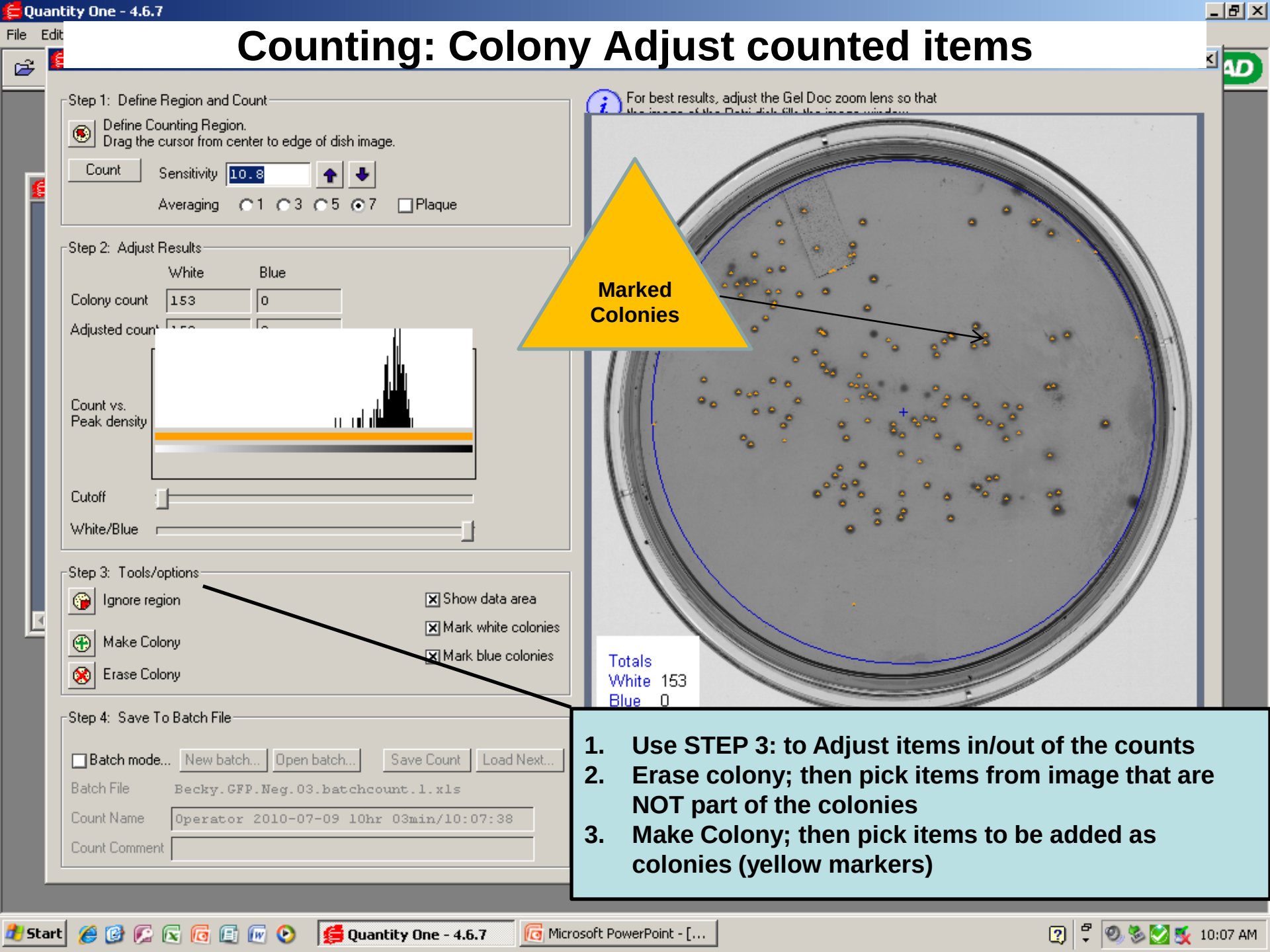
Drag the **White/Blue** slider to the left until it is positioned between the two peaks. The white colony data range is indicated by gold on the bar beneath the histogram, and the blue colony data range is marked with blue.

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).

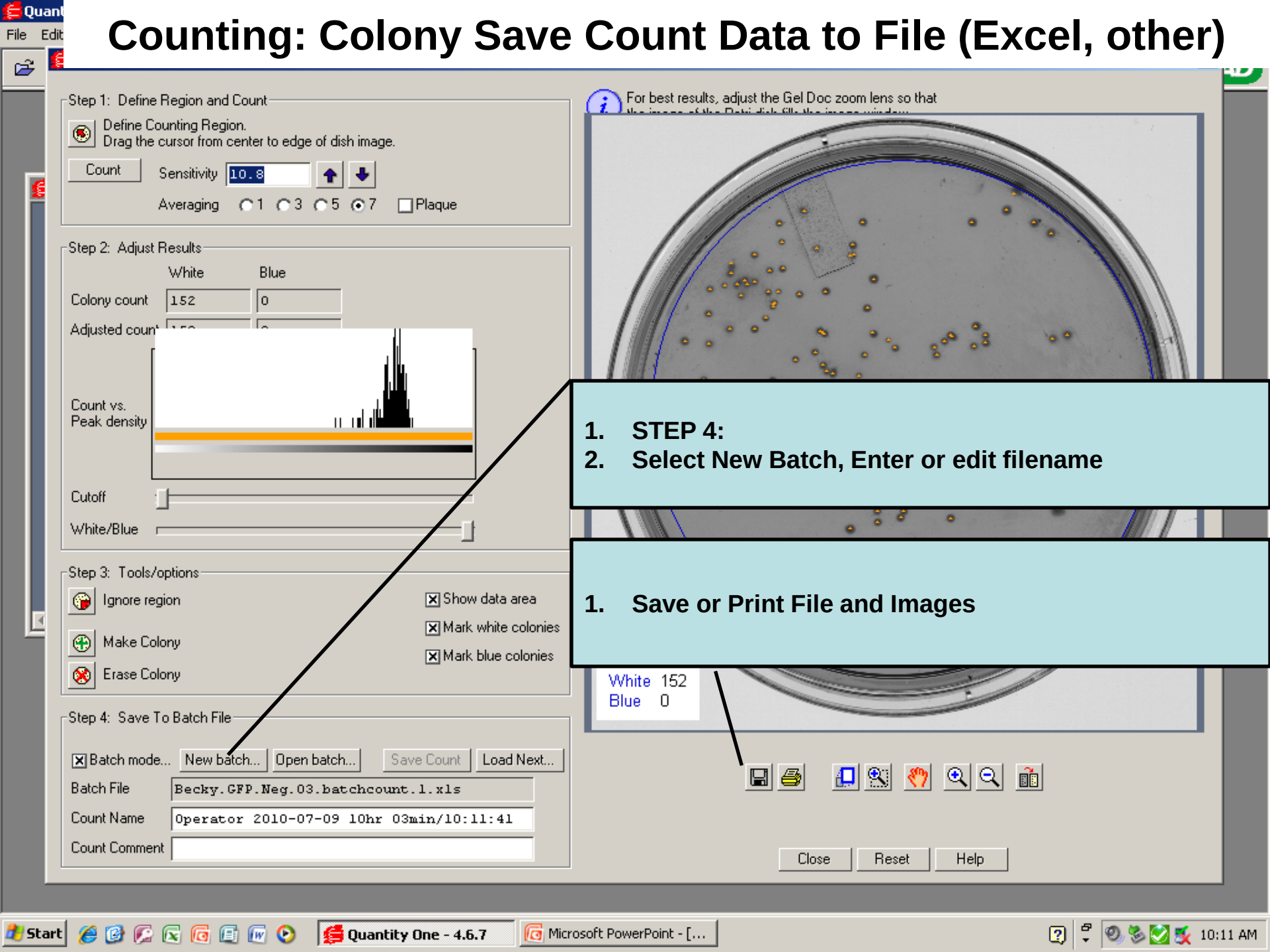
Note: If the blue colonies are not marked on the image, check to make sure that the **Mark Blue Colonies** checkbox at the bottom of the dialog is checked.

Start | Quantity One - 4.6.7 | Microsoft PowerPoint -

Counting: Colony Adjust counted items



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



Counting: Colony ,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 360º
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

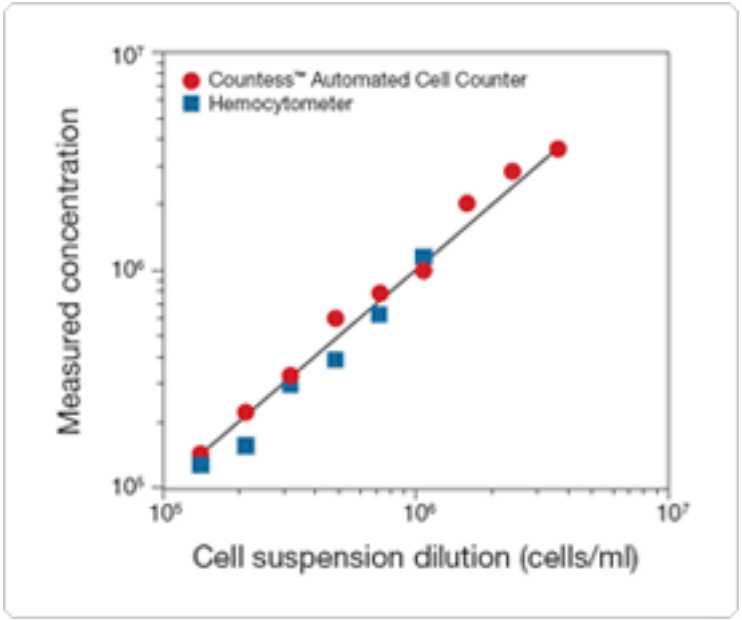
Quebec Darkfield
Model 3330

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 360º 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 x 104 to 1 x 107 cells/ml, with an optimal range from 1 x 105 to 4 x 106 cells/ml, broader than that of a hemocytometer (view technical notes for more comparison data). The optimal cell size is between 5 µm to 60 µm (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 µl of sample with 10 µl 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….