Vi-CELL® XR

Cell Type Tutorial

INNOVATE ▪ SIMPLIFY ▪ AUTOMATE

PN A62133 Rev AB

Purpose of the Tutorial

- Introduce users to the Cell Type Feature in the Vi-CELL XR software.
- Identify the default cell types provided with the software.
- Demonstrate the ability to create, modify and add cell types
- Identify the available features in the Cell Type Dialog.
- Explain the purpose of the various options for defining a cell type.
- Provide scenarios where the default settings may need to be adjusted.

What is a Cell Type?

- **A Cell Type is a collection of settings that control the sample preparation and analysis.**

Cell Size Thresholds

Sample Prep

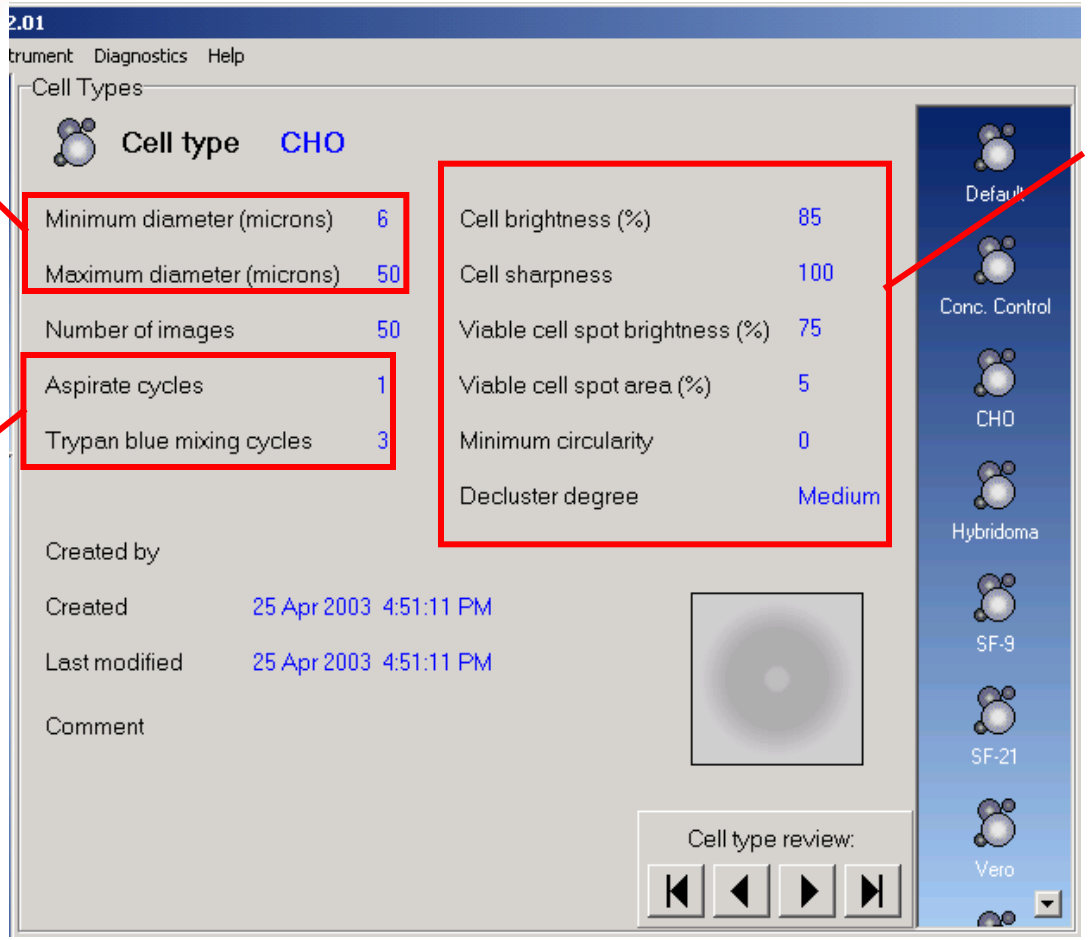
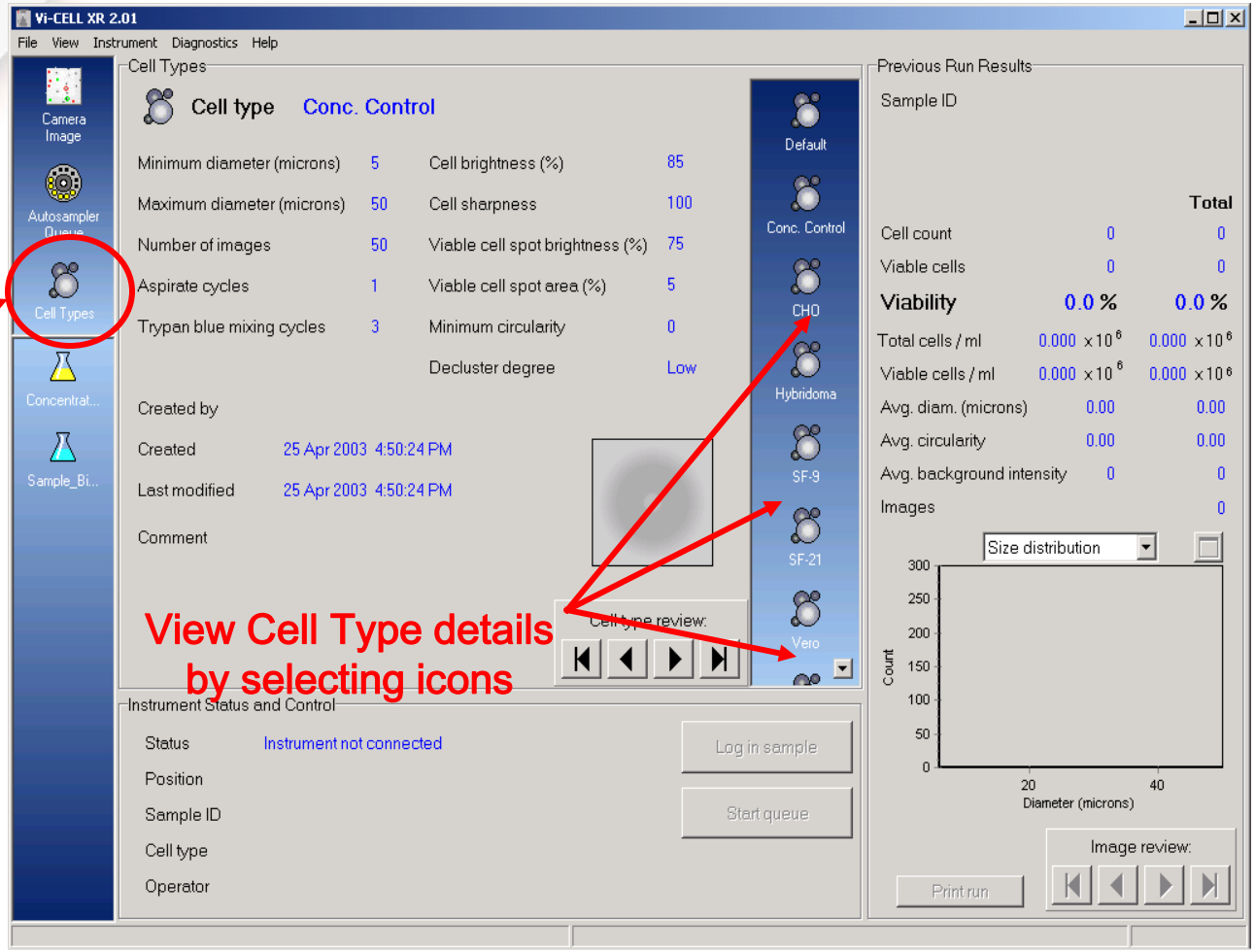


Image Analysis Settings

Where are the Cell Types?

- The Cell Types can be viewed by selecting the icon.



Select Cell Types Icon

View Cell Type details by selecting icons

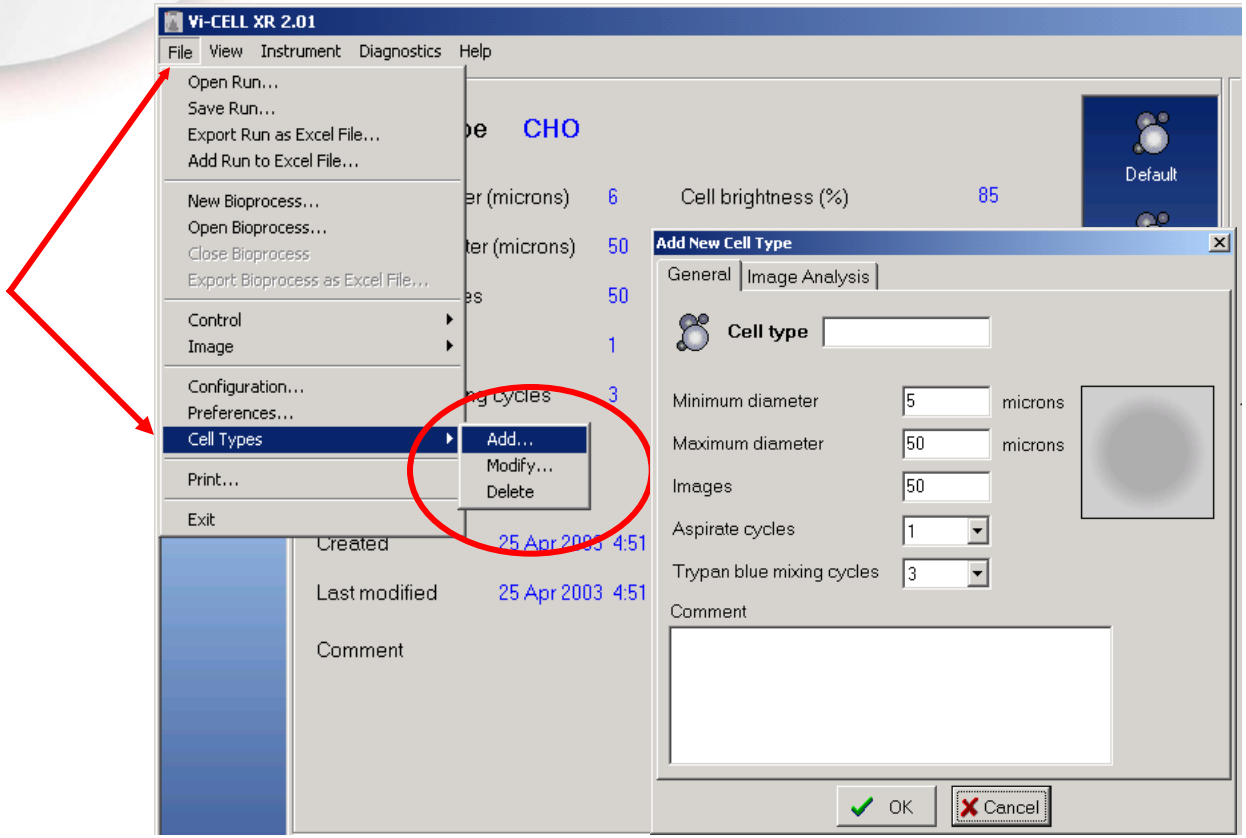
Why Change the Cell Type?

- In most cases, there is no need to change the cell type. The supplied Cell Types - CHO, Hybridoma, SF9, SF21, Vero, and Default - provide good results.
- In cases where the cell line used is not part of the Cell Type list, or customers want to change the sample preparation or results, a new Cell Type can be created or an existing Cell Type can be modified.
- In cases where the cells are the same type as the defaults, but have been treated or genetically altered, the cell type settings may need to be modified.

How Do I Change the Cell Type?

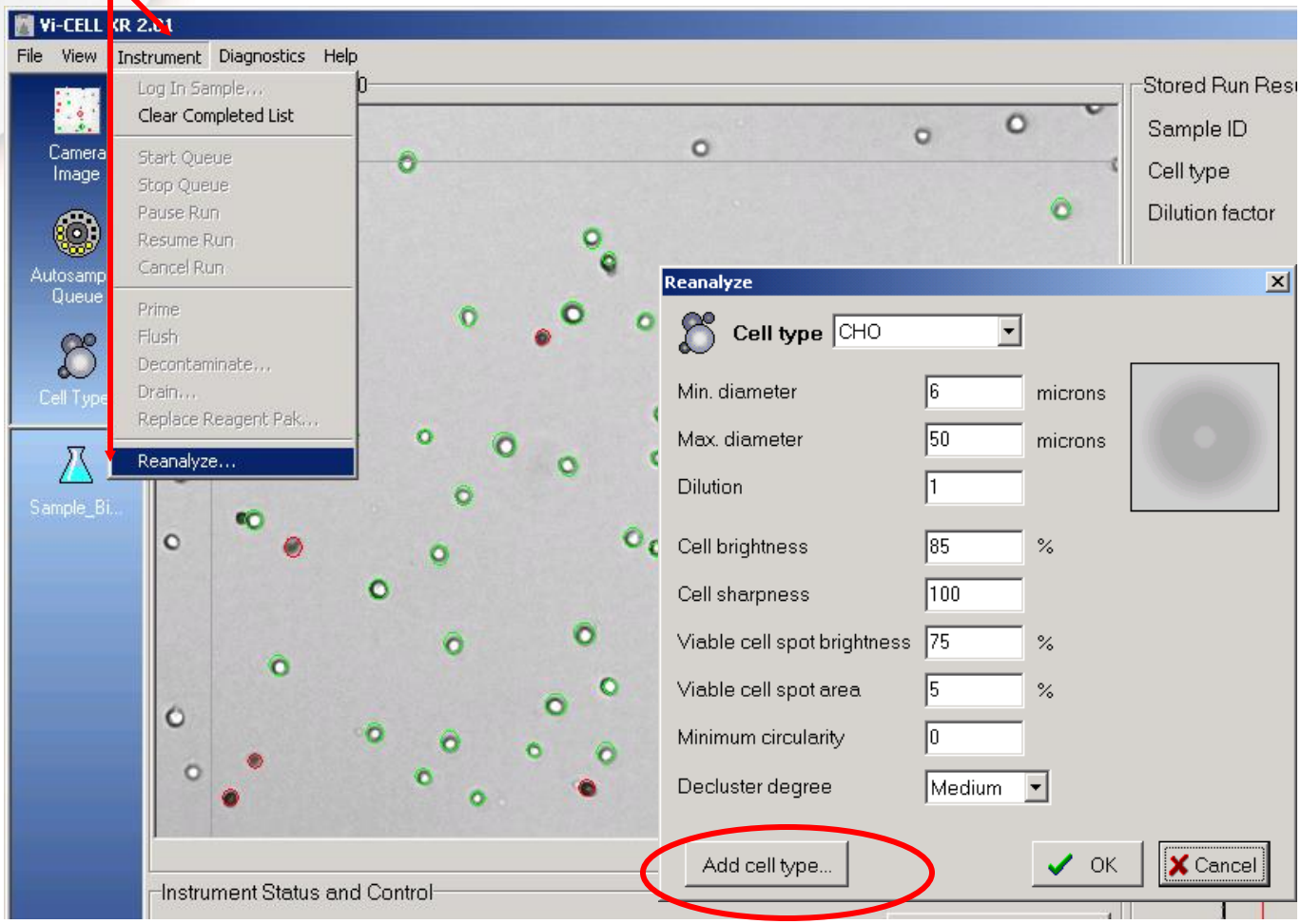
- There are several ways to do this.

1. Add, Modify, or Delete a Cell Type from the “File” menu…



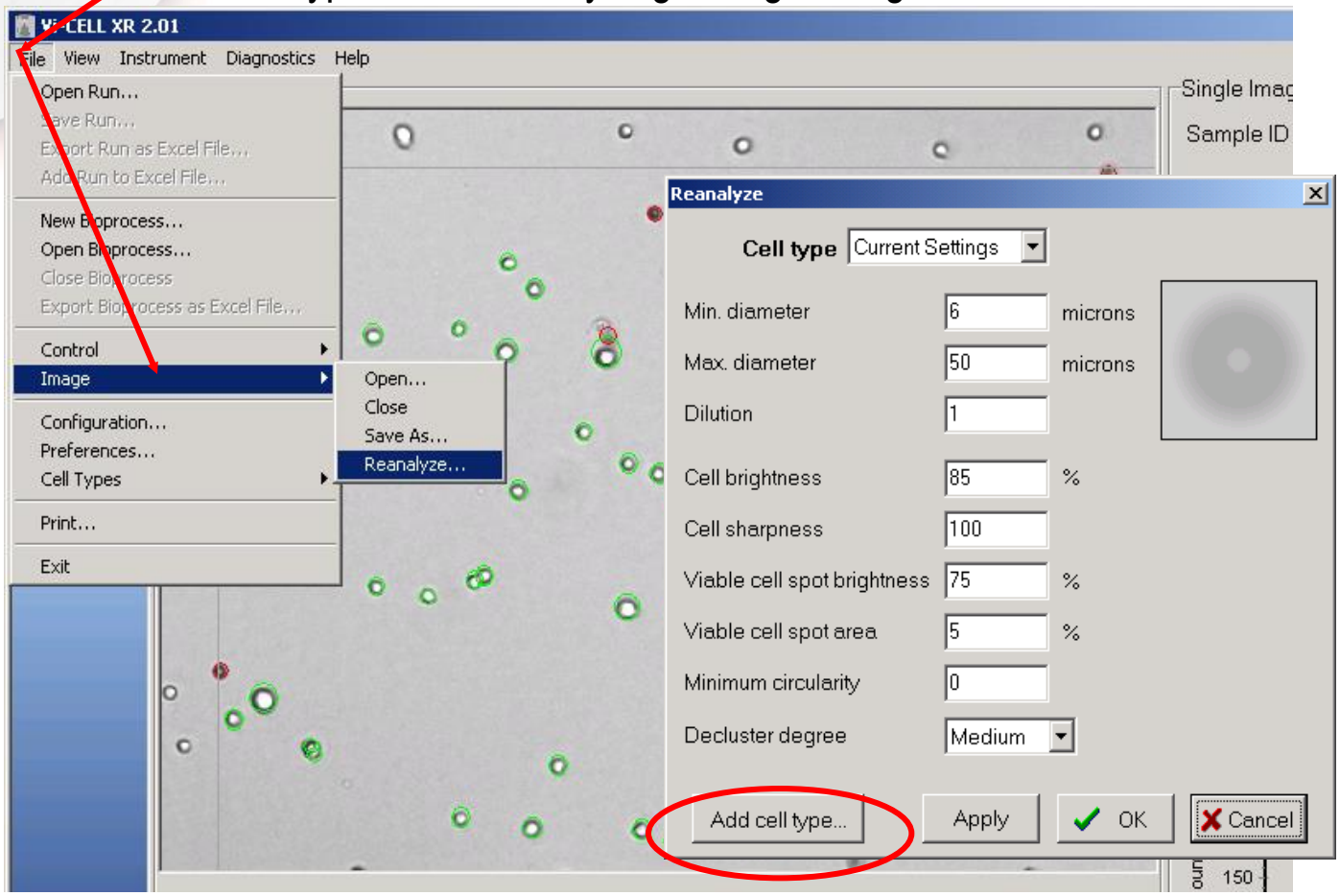
How Do I Change the Cell Type?

2. Add a Cell Type after Reanalyzing an entire run.



How Do I Change the Cell Type?

3. Add a Cell Type after Reanalyzing a single image.



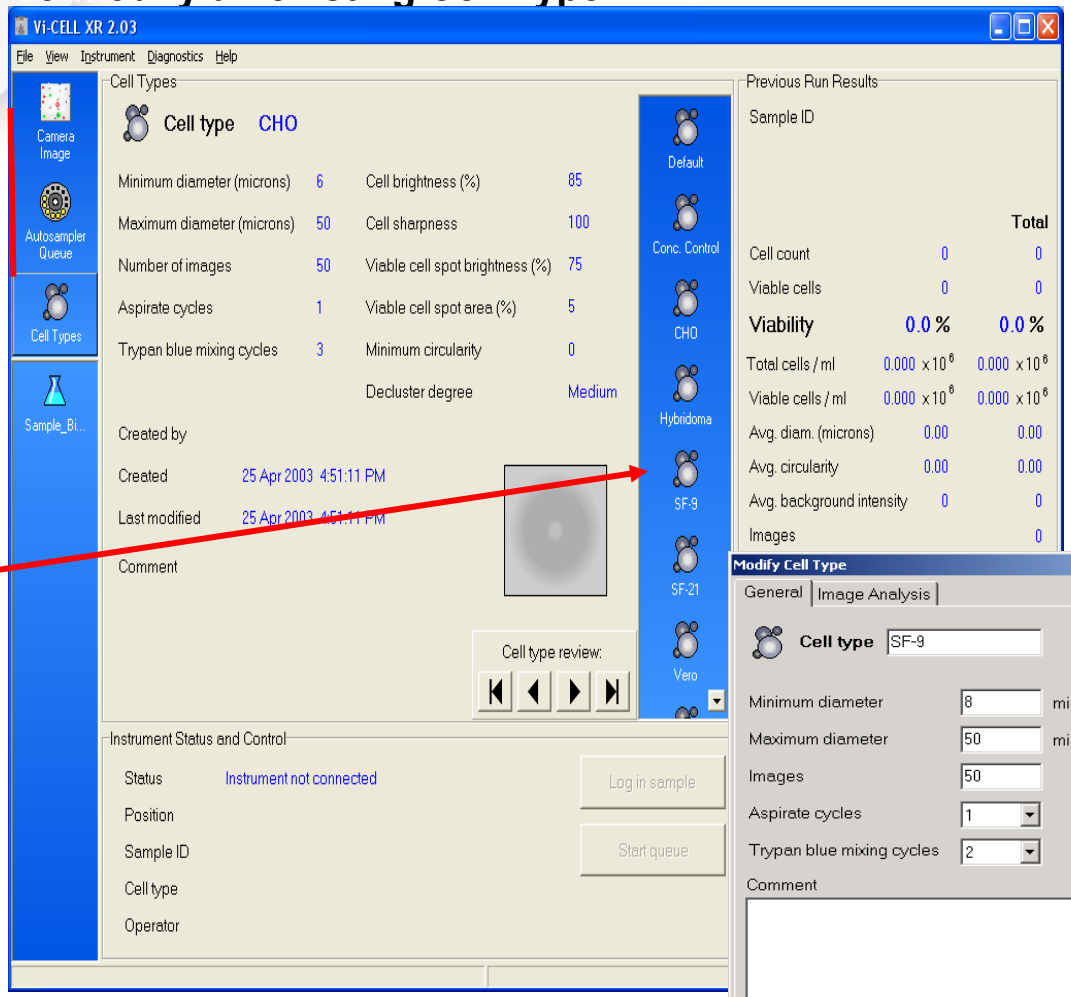
How Do I Change the Cell Type?

4. **To modify an existing Cell Type:**

1 - Select the Cell Type Icon from the left menu

2 - Select the Cell Type to be modified

3 - Select File>>Cell Types>>Modify from the main menu to obtain the Modify Cell Type dialog

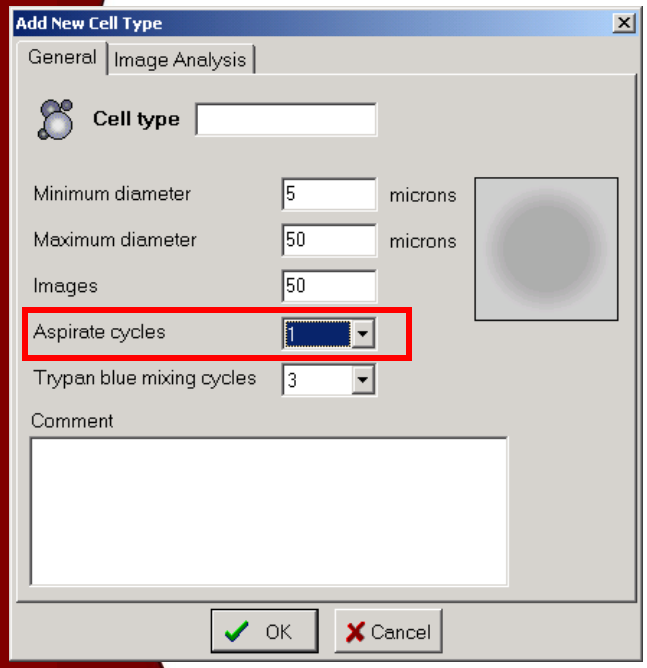


What are the Functions of the Cell Type Parameters?

- The Cell Type parameters are used to define the following:
  1. Sample Preparation Options
  2. Cell Size Thresholds
  3. Image Analysis Parameters
  4. Number of Images to Collect
- Let's review each area in more detail…

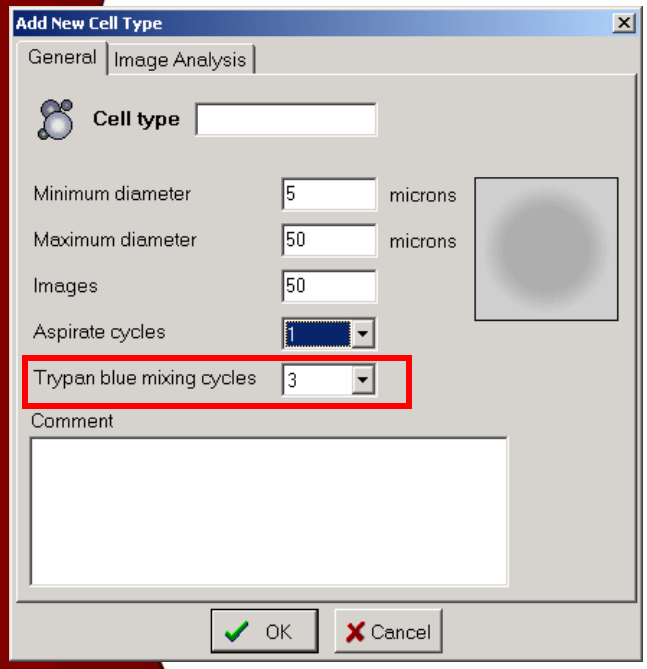
Sample Preparation Options

- Aspirate Cycles
  - Controls the number of sample mixing cycles before the precise volume is dispensed and Trypan Blue is added.
  - This is important to ensure sample is re-suspended in the sample cup prior to sampling. It ensures the Vi-CELL is presented with a representative sample of the cell suspension.
  - Aspirate Cycles are also useful in de-clustering clumps of cells, so increasing this value can often improve the analysis of CHO and other “clumpy” cells.
  - The default value and recommended minimum number of aspirate cycles is 1, but this number can be increased to help break up cell clusters.



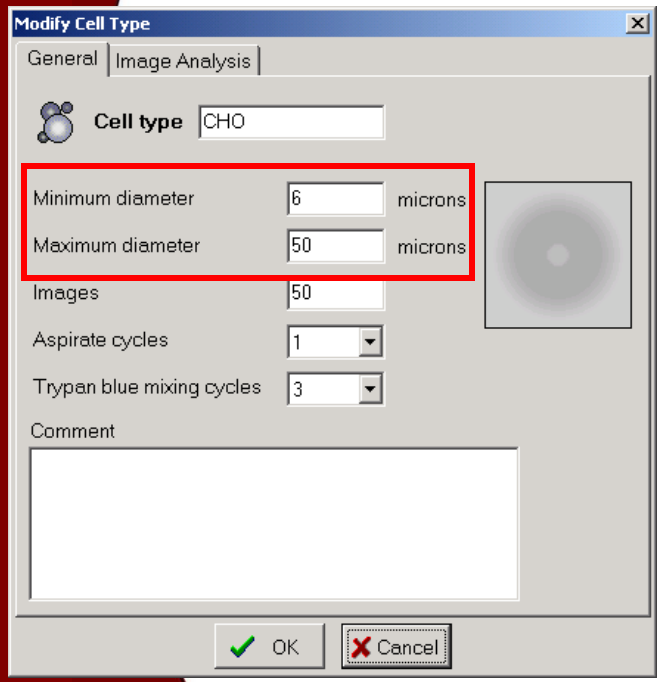
Sample Preparation Options

- Trypan Blue Mixing Cycles
  - Controls the number of sample mixing cycles after the addition of Trypan Blue.
  - For mammalian cells, the default value is 3 cycles.
  - For insect cells, the default value is 2 cycles, as these cells are generally more fragile.
  - Since increasing the number of mixing cycles also increases the staining time prior to analysis, this value can also be increased if customers would like longer staining time.



Cell Size Thresholds

- Minimum and Maximum Diameter
  - Excludes live and dead cells and also debris from the analysis that is less than the minimum diameter or greater than the maximum diameter.
  - The maximum diameter value should be set to 40 um or higher for the best cell de-clustering performance for mammalian cells.
  - The minimum diameter value can be adjusted to help exclude small debris or apoptotic bodies from the analysis.
  - To provide help in setting these values, size information can be obtained for every cell found in an image. The following slide provides details…



Viewing Size of Individual Cells

- The size, viability, and circularity determination of individual cells can be viewed in the software as shown below.

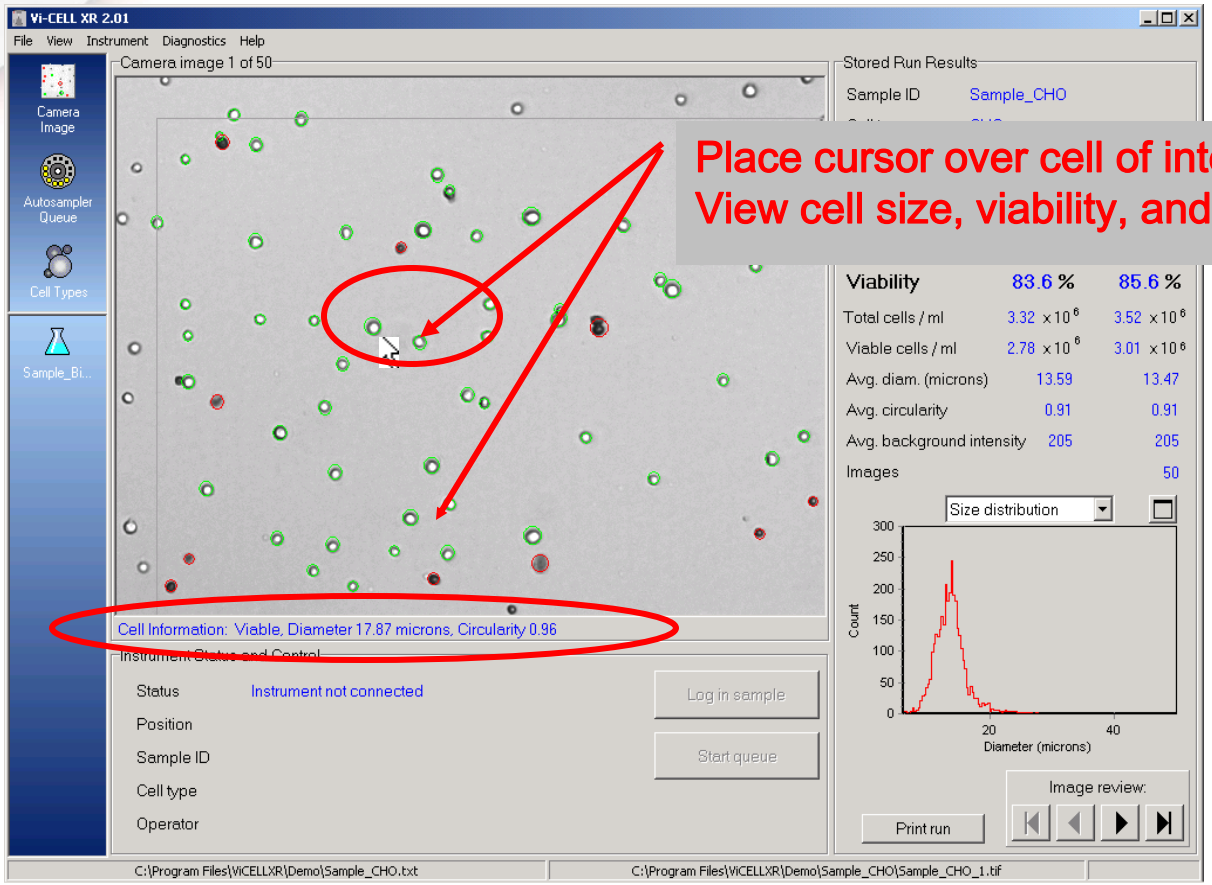
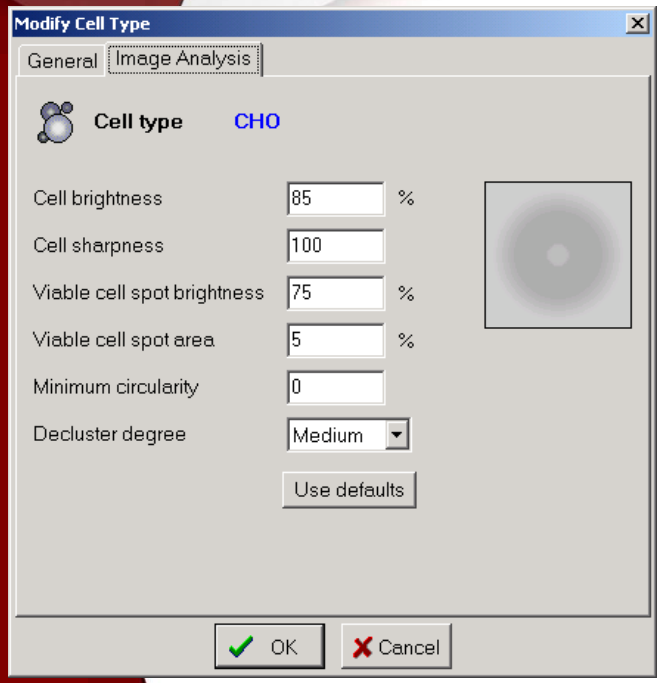


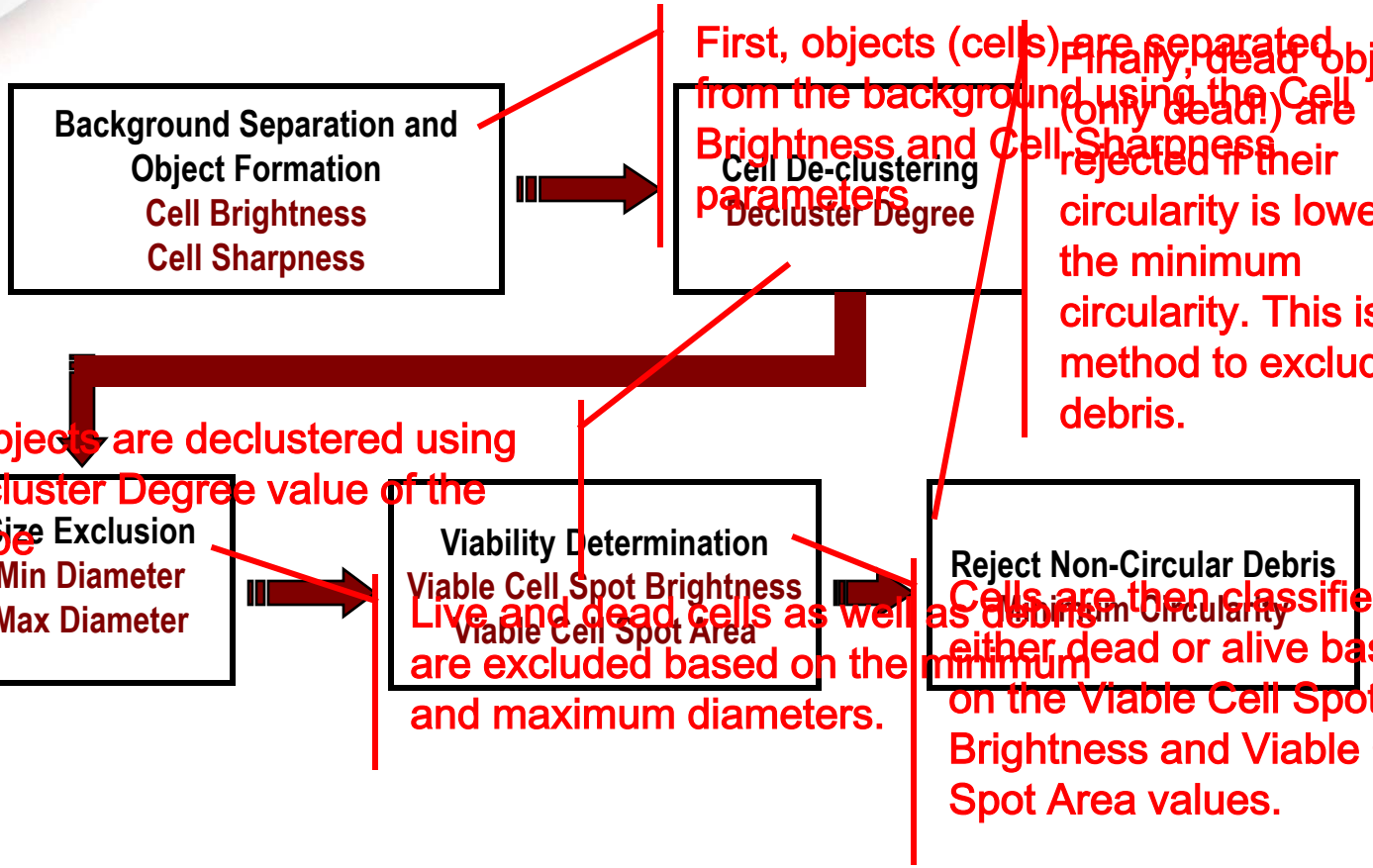
Image Analysis Parameters



- Cell Brightness (% of background)
- Cell Sharpness (unit less)
- Viable Cell Spot Brightness (% of background)
- Viable Cell Spot Area (% of total cell area)
- Minimum Circularity (1 = perfect circle)
- Decluster Degree
- Let's review each parameter in detail…

Image Analysis Flowchart

- Vi-CELL XR Image Analysis Flowchart (simplified)

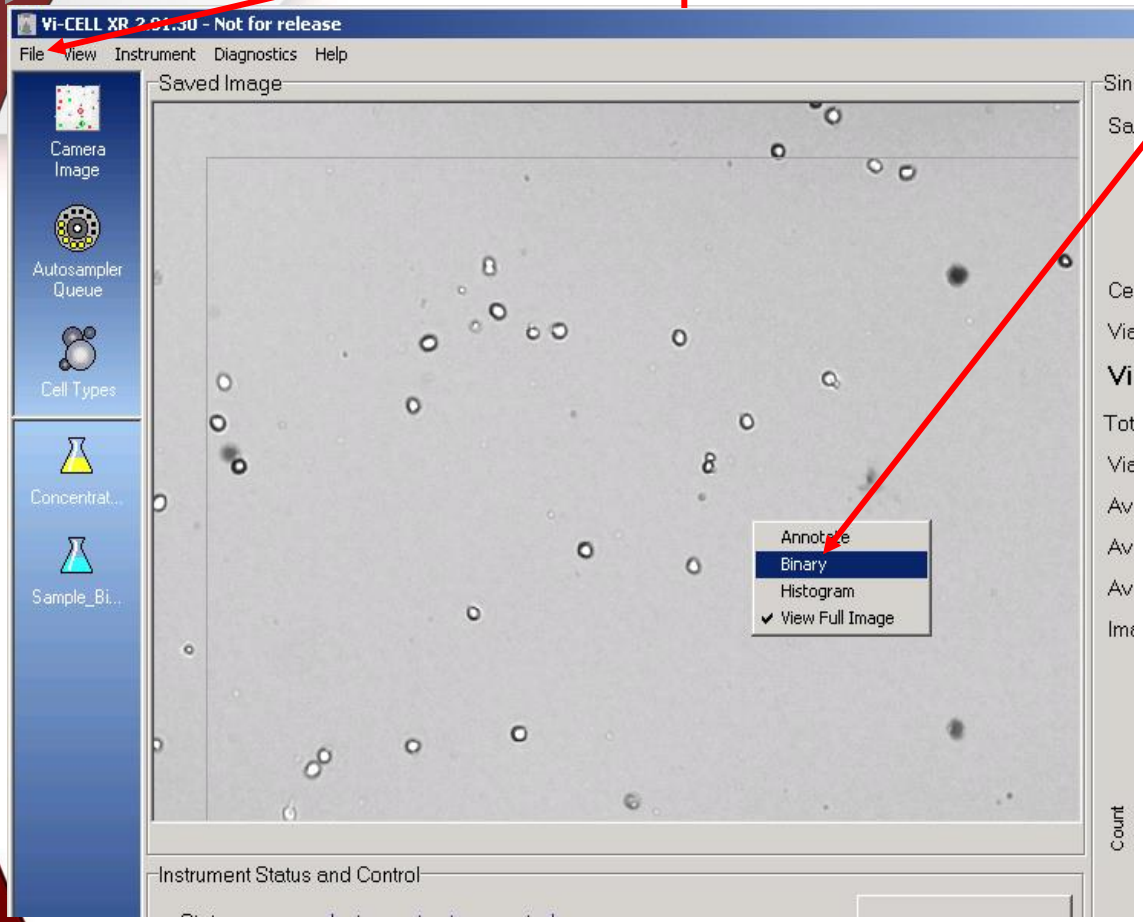


Parameters – Cell Brightness

- Adjusting the Cell Brightness value changes the threshold at which dark pixels are included as objects.
- Higher values of Cell Brightness include light grey pixels in the object separation.
- Lower values include only very dark pixels in object separation.
- Use Binary Image mode to see the changes.
- Let's practice making some changes…

Parameters – Cell Brightness

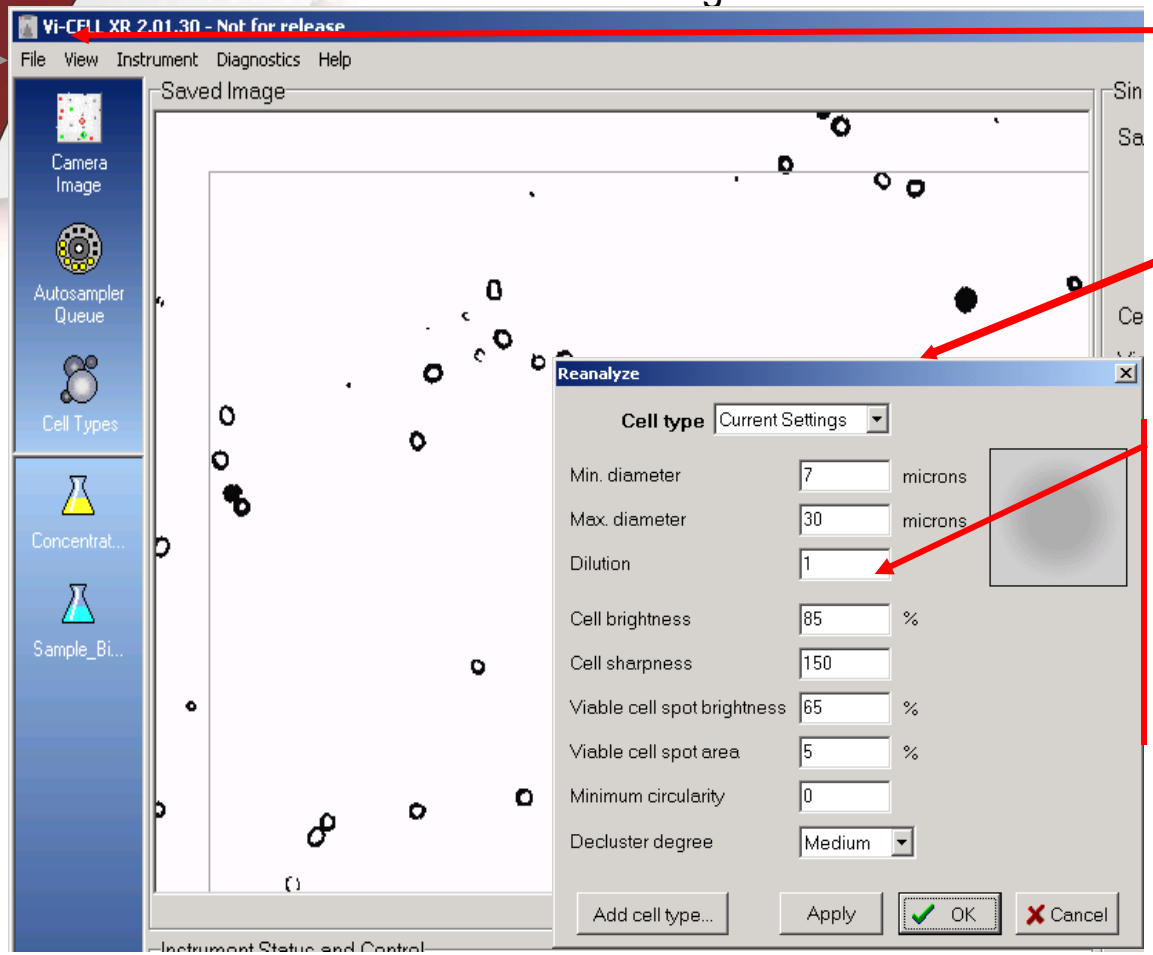
First, open a single image using the "File", "Image", "Open" menus…



Then, right click the mouse on the image to bring up this menu. Turn "Annotate" off and turn "Binary" on to get a black and white image.

Parameters – Cell Brightness

- You should now have an image like this…

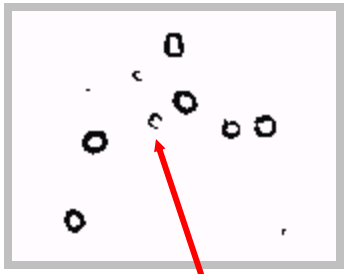


Select “File”, “Image”, “Reanalyze…” to get this menu

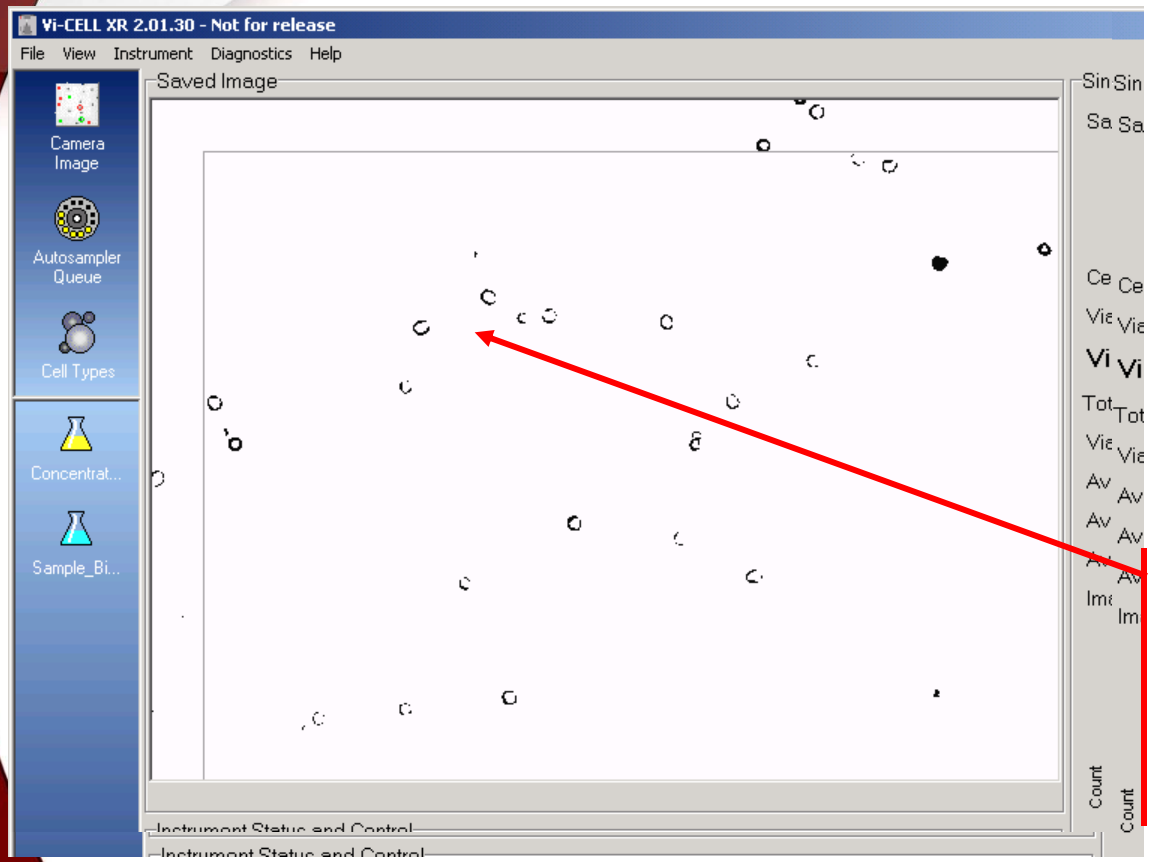
Change Cell Brightness to 50, select Apply, and see changes to the image. You will notice there are fewer dark pixels and fewer cells are found.

Parameters – Cell Brightness

- **Notice the difference in the binary image…**



Notice the debris and small grey cells in the image, when Cell Brightness is set to 85



With Cell Brightness = 50, notice how those areas are gone. This shows how Cell Brightness can be used to remove debris or faint cells.

Parameters – Cell Brightness

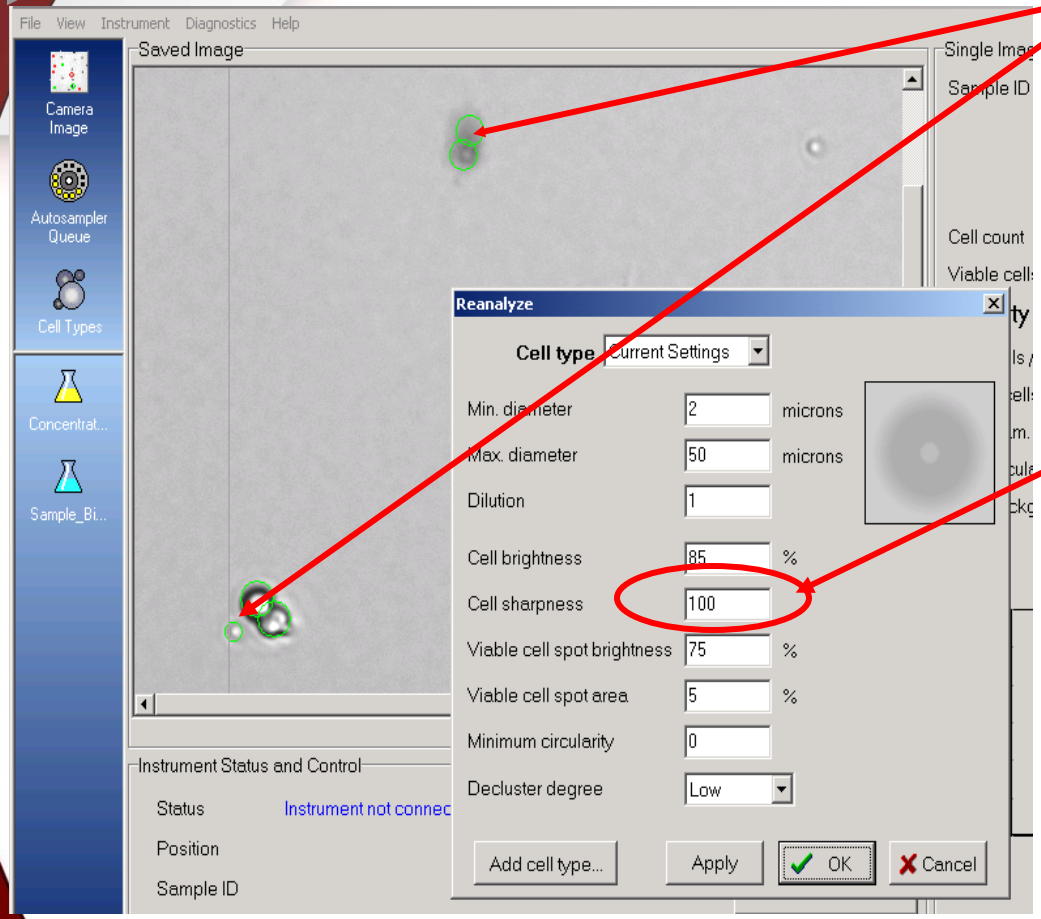
- Although we used Cell Brightness of 50 for illustration, you will most likely never want to use such a low value.
- A value of 85 is best, and the normal range of values is 75 (to exclude debris and apoptotic bodies) to 90 (to include all objects in the analysis)
- Remember, normal values are 75 – 90!

Parameters – Cell Sharpness

- Cell Sharpness is also used to include or exclude objects from the analysis.
- Cell Sharpness describes how quickly an object boundary transitions from light to dark.
- Lower Cell Sharpness values will exclude “fuzzy” edged objects or cells from the analysis.
- Higher values include more objects.
- Let’s try it…

Parameters – Cell Sharpness

- **Large fuzzy cell and small apoptotic body are included in this image, to exclude them…**

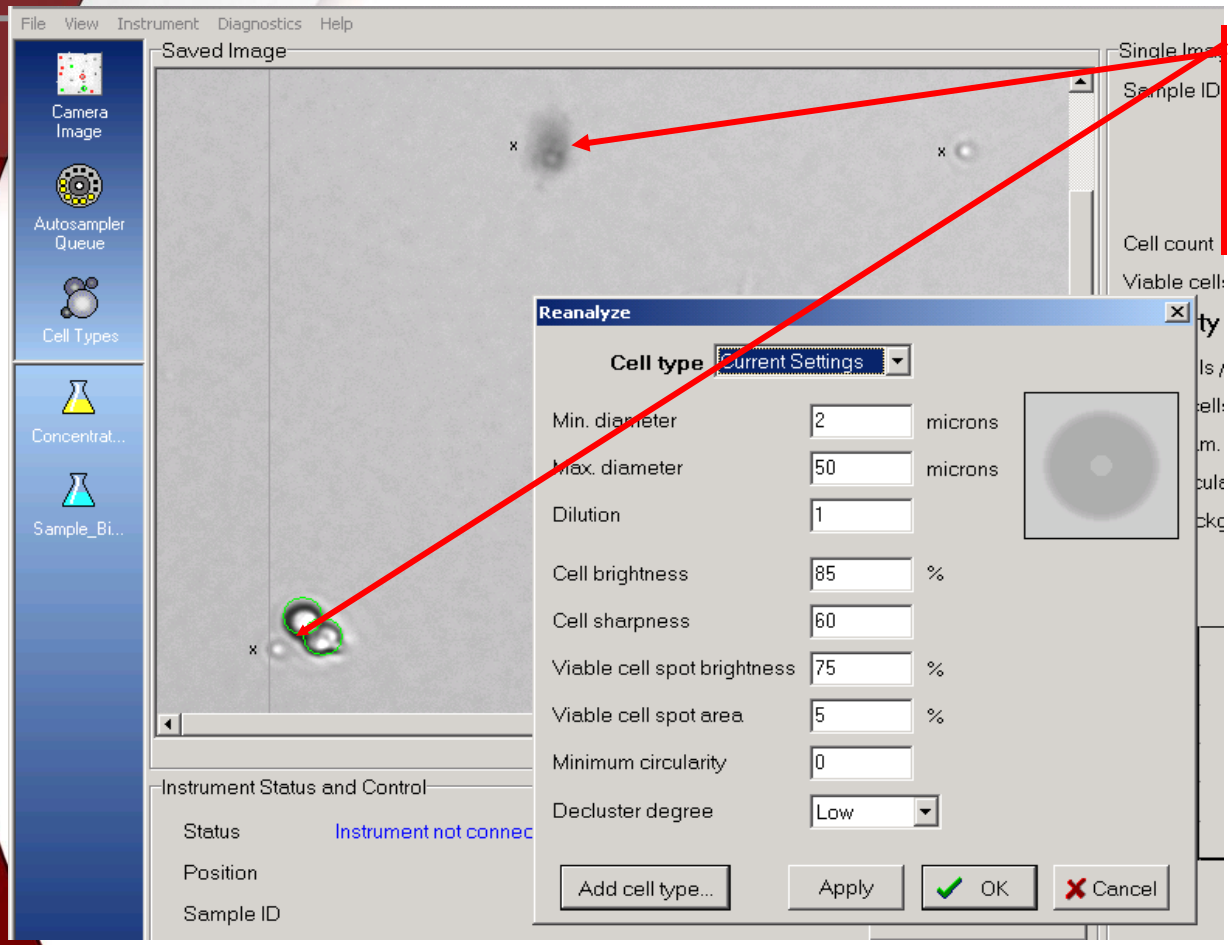


Notice large "fuzzy" cell and small object. We can adjust the cell sharpness parameter to exclude them from the analysis.

Change Cell Sharpness value to 60 and select "Apply" to view the change.

Parameters – Cell Sharpness

- Notice the cells are no longer annotated as live cells and the small ‘x’ to the left of the cell, indicating sharpness rejection.



Fuzzy cell and apoptotic bodies now excluded from analysis with small ‘x’ indicating rejection for Cell Sharpness.

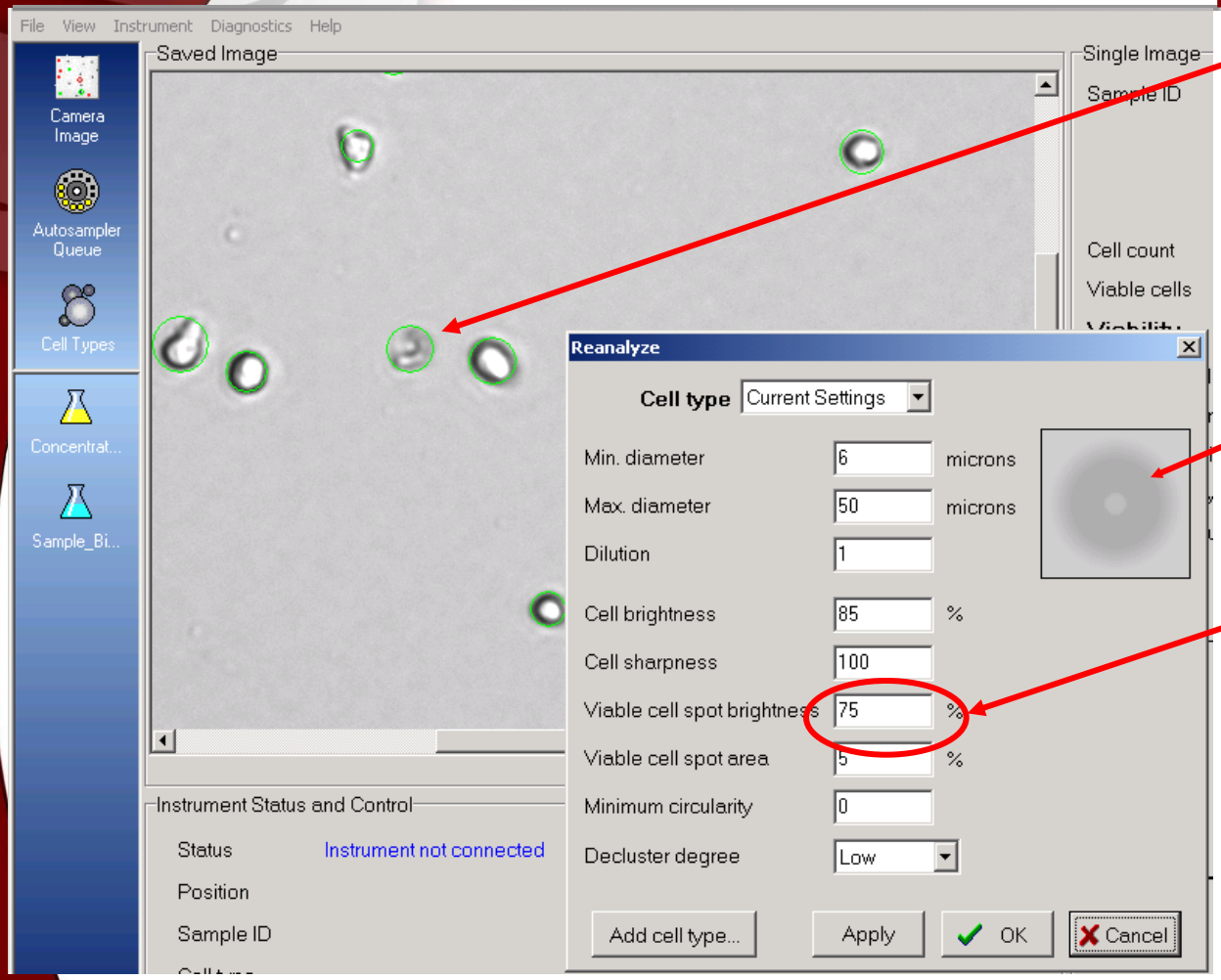
Parameters – Cell Sharpness

- Cell Sharpness is an effective way to exclude “fuzzy” objects and apoptotic bodies because they generally do not have a well defined cell membrane.
- Be careful to check that a lower setting does not exclude normal cells.
- Default setting is 100, typical settings can go as low as 60.
- Normal range of settings is 60 – 200.

Parameters –
Viable Cell Spot Brightness

- After the cells are separated from the background by the Cell Brightness and Cell Sharpness values, and the declustering and size thresholds are applied, the cell viability is determined using the Viable Cell Spot Brightness and Viable Cell Spot Area settings.
- Viable Cell Spot Brightness defines how ‘white’ the cell center spot must be to be classified as viable.
- It is a percentage of the full scale white value.
- Let’s look at an example…

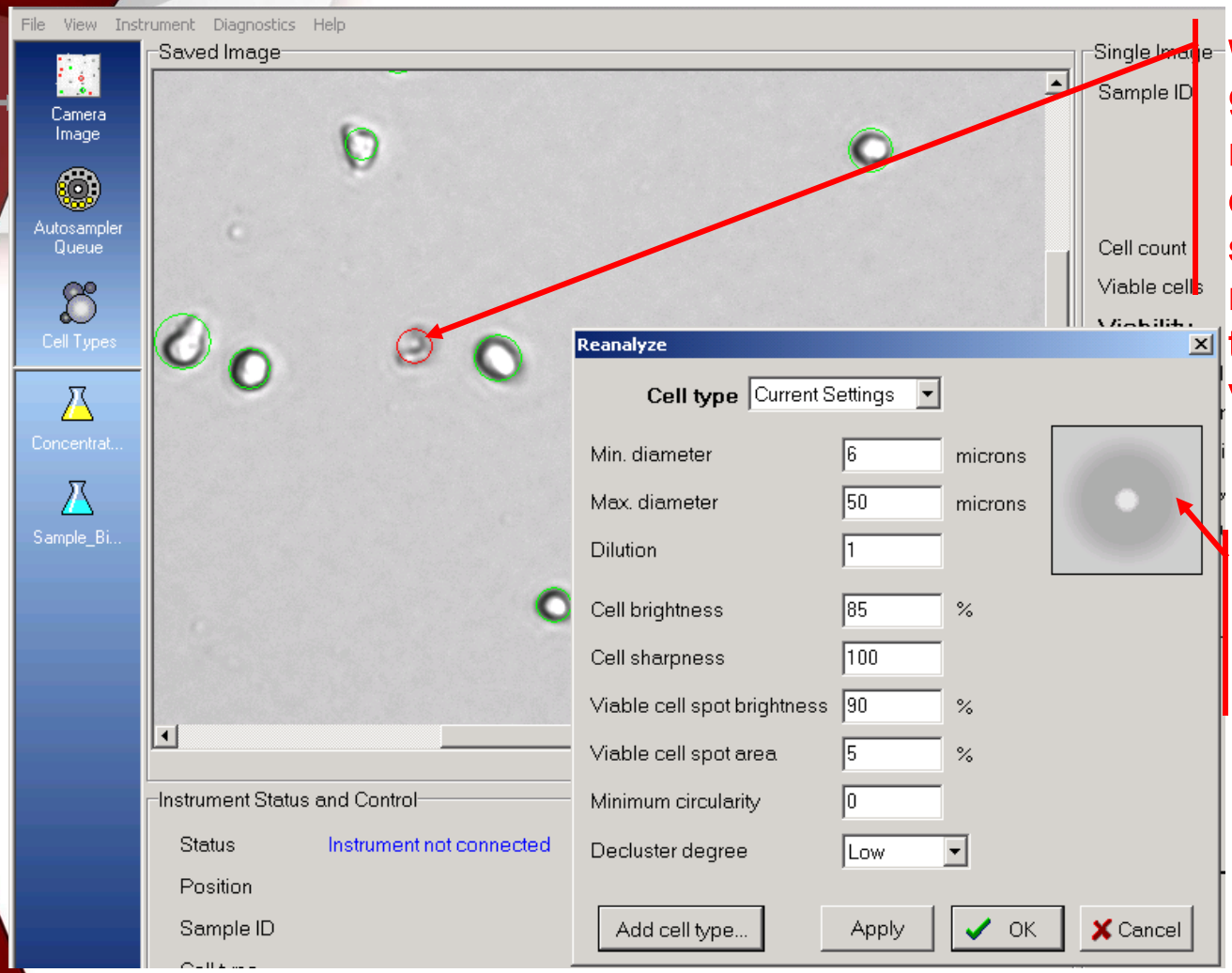
Parameters –
Viable Cell Spot Brightness



Notice this cell, which has a light grey center spot, is classified as live with the default Viable Cell Spot Brightness value of 75. Notice the preview image in the Reanalyze dialog

Change the value to 90 and select Apply to view the change.

Parameters –
Viable Cell Spot Brightness



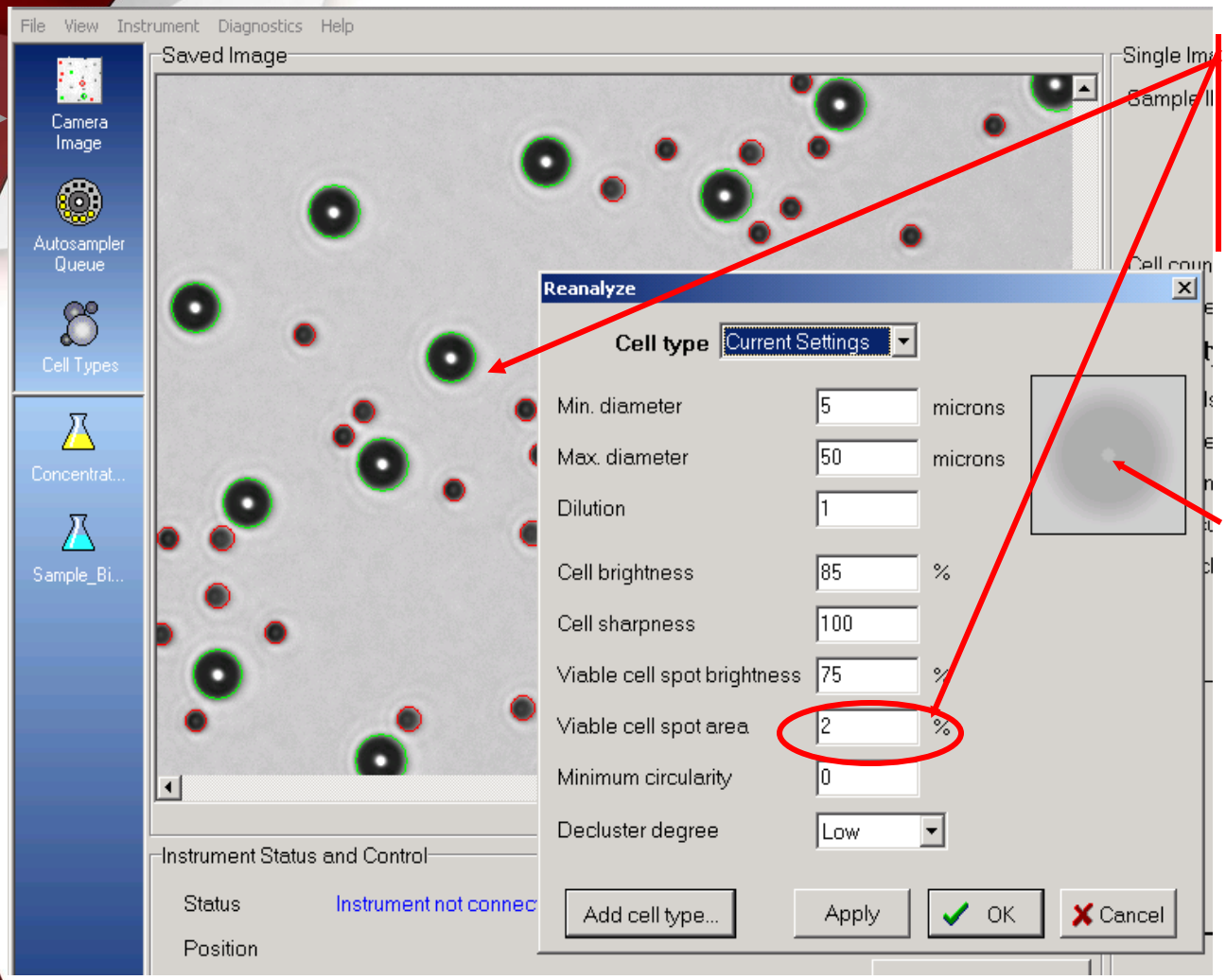
With a value of 90%, this cell is now classified as dead. The center spot of the cell is not bright enough to meet the viability criteria.

Notice the image preview in the Reanalyze dialog.

Parameters –
Viable Cell Spot Area

- Viable Cell Spot Brightness and Viable Cell Spot Area work together to classify cells as dead or alive.
- Viable Cell Spot Area is the required size of the center bright spot to meet the viability threshold.
- Default value is 5% of total cell area, but values of 1% to 20% could be used.
- Be careful though. Let's take a look…

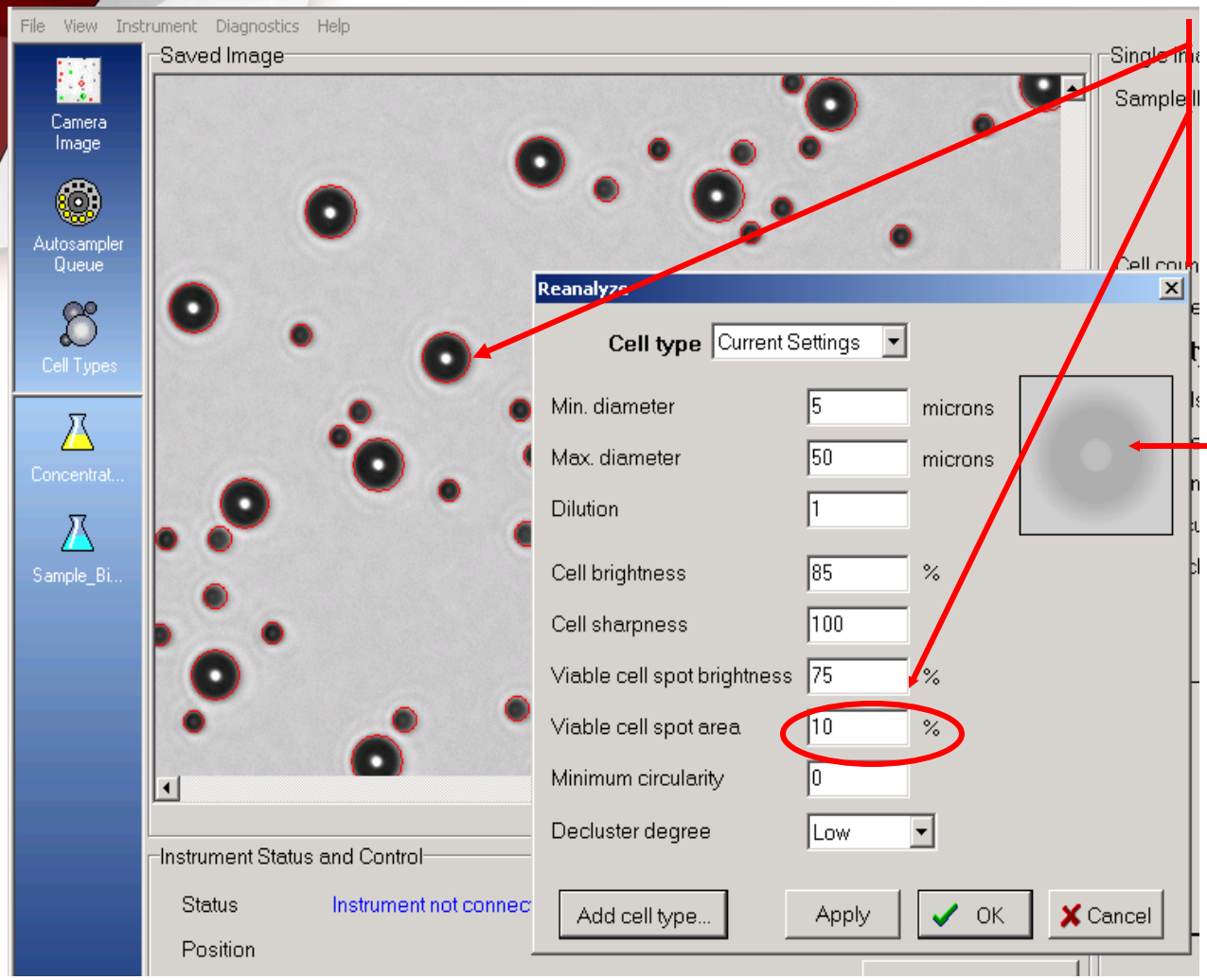
Parameters –
Viable Cell Spot Area



With a 2% center spot area setting, these 20 um beads are classified as live.

Notice the preview graphic, and the size of the cell spot area here

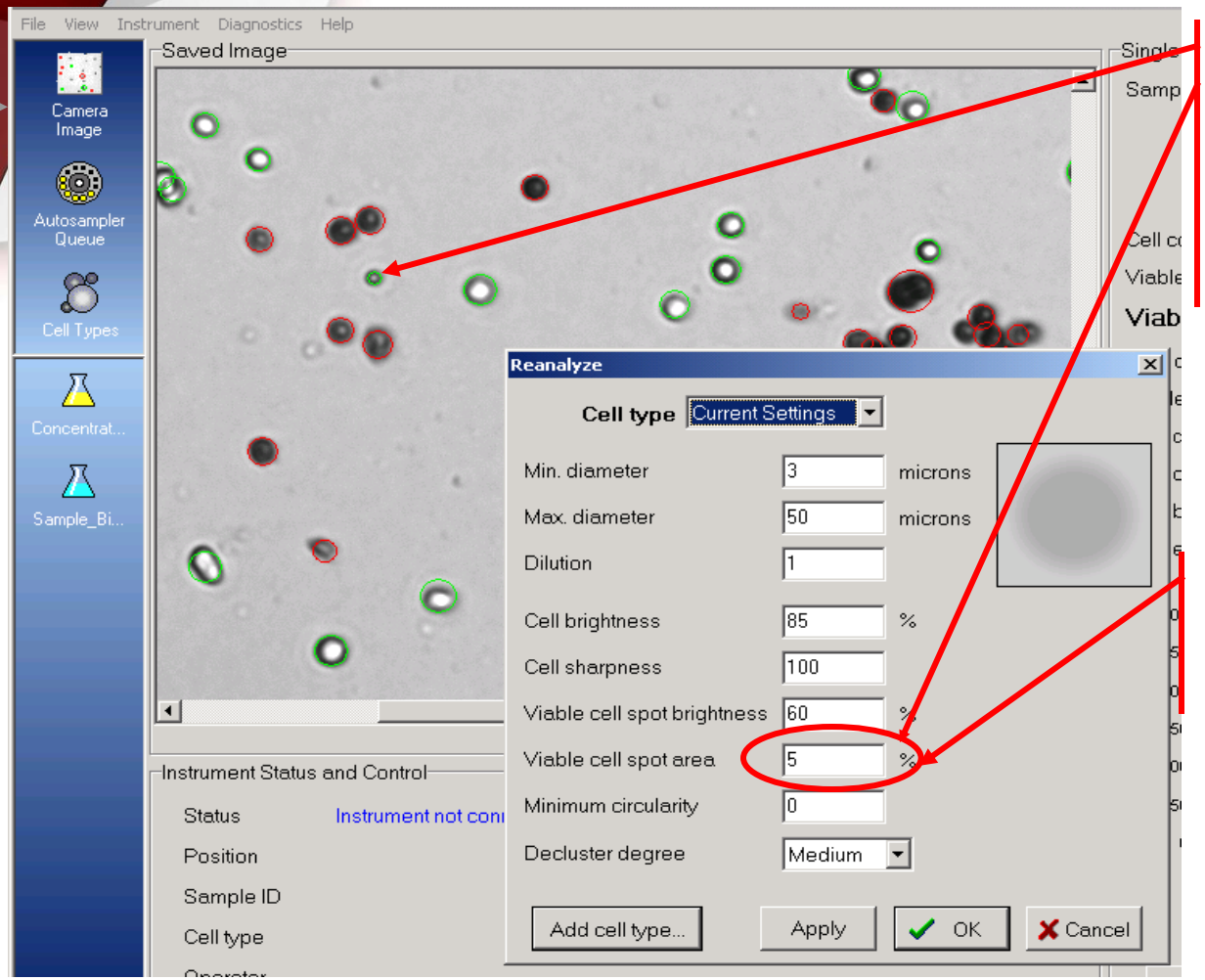
Parameters –
Viable Cell Spot Area



But with a 10% requirement, they are classified as dead. The cell spot is less than 10% of the total cell area.

Graphic preview

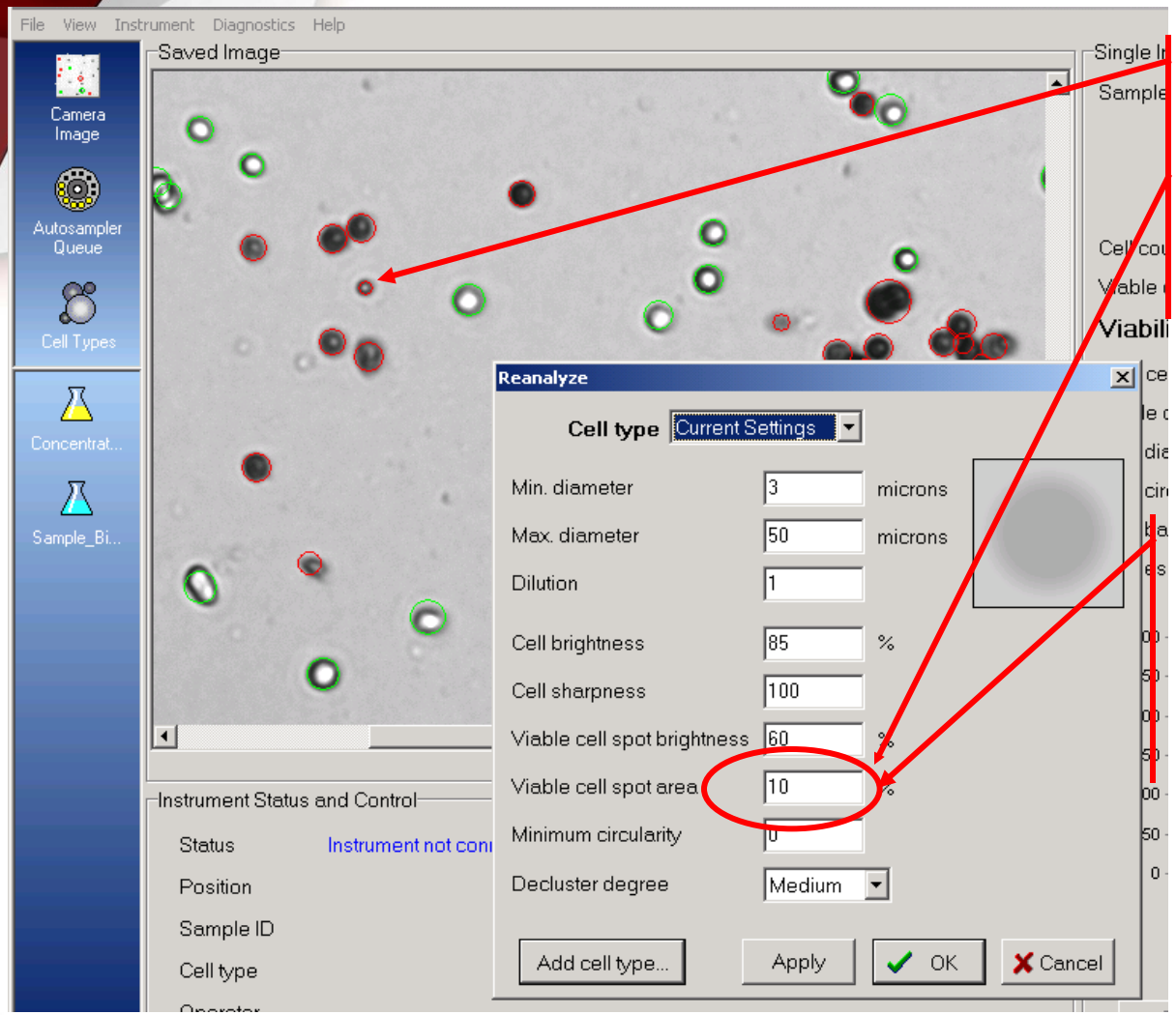
Parameters –
Viable Cell Spot Area



With a 5% center spot area requirement, notice that this small cell is classified as live

Changing the value to 10%, will make this cell dead

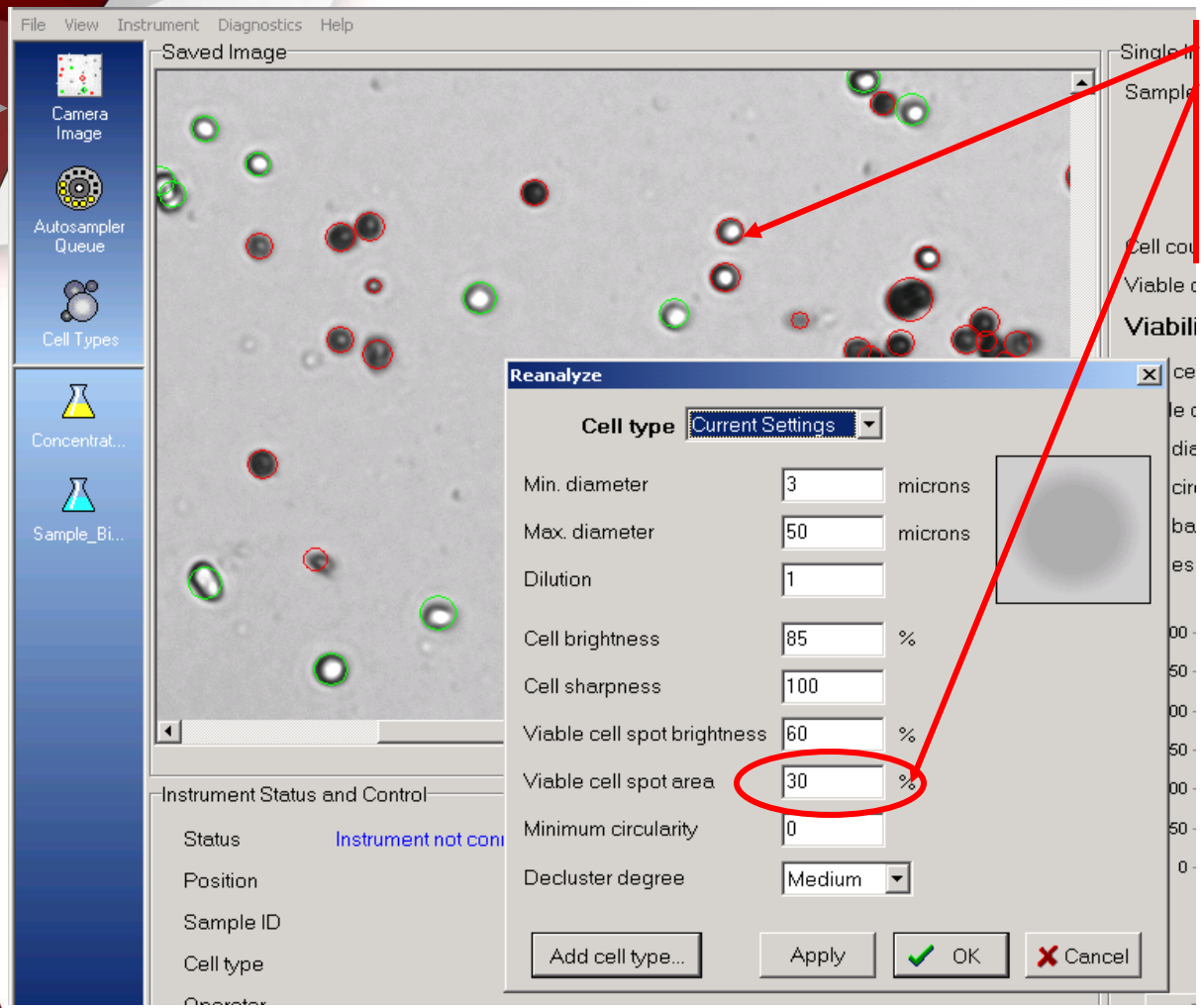
Parameters –
Viable Cell Spot Area



The bright center spot is less than 10% of the total cell area, so is classified as dead.

Be careful though, because changing the value to 30% causes live cells to be misclassified.

Parameters –
Viable Cell Spot Area



A value of 30% causes these cells to be classified as dead, as well as the small cell!

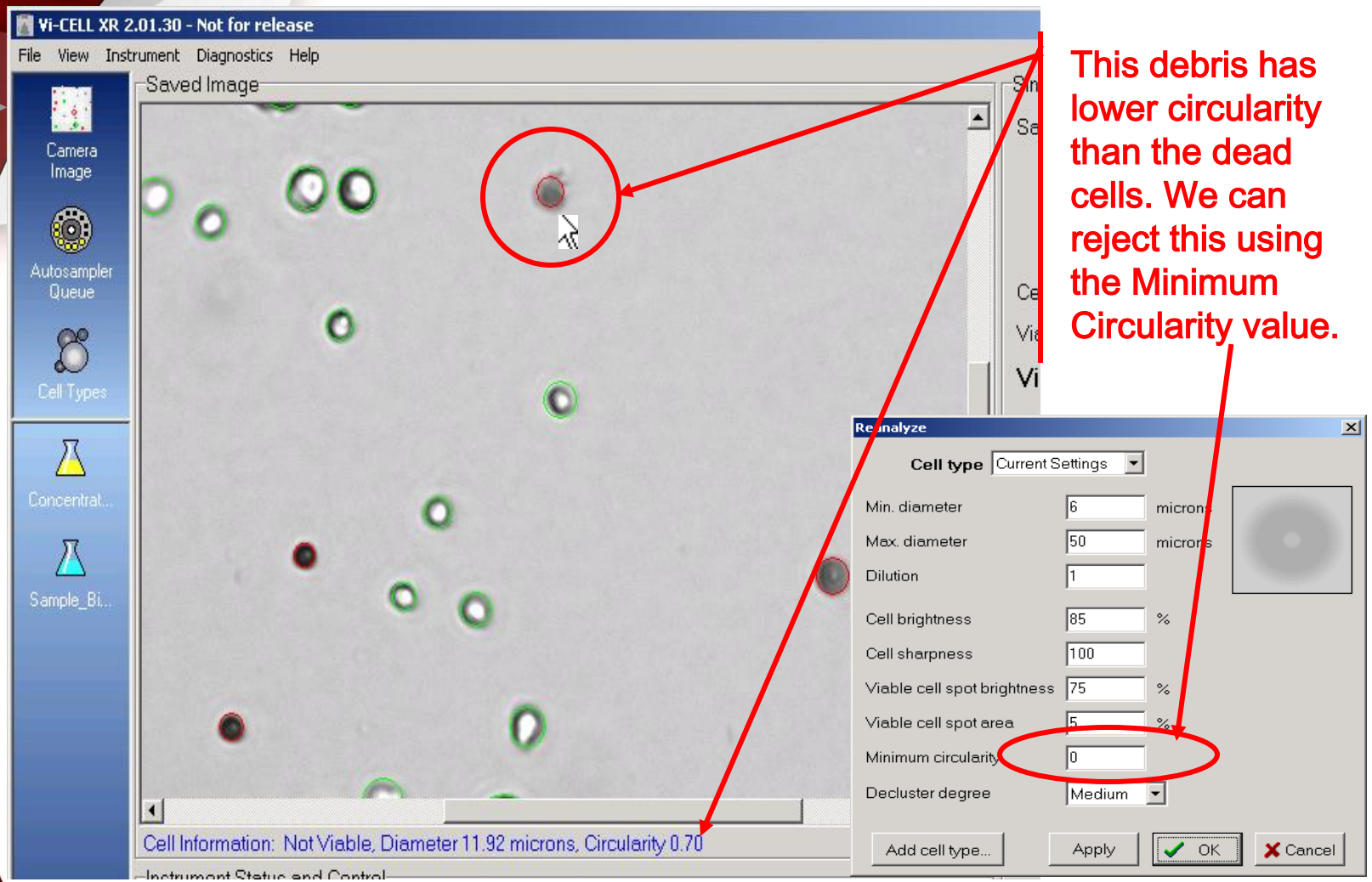
Parameters –
Viability Review

- Remember, the viability determination is made after the cells are separated from the background, the declustering is applied, and the size thresholds are applied.
- Viable Cell Spot Brightness and Viable Cell Spot Area work together to determine viability.
- Increase both values to reduce viability, decrease both values to increase viability.
- Default values are generally the best.

Parameters –
Minimum Circularity

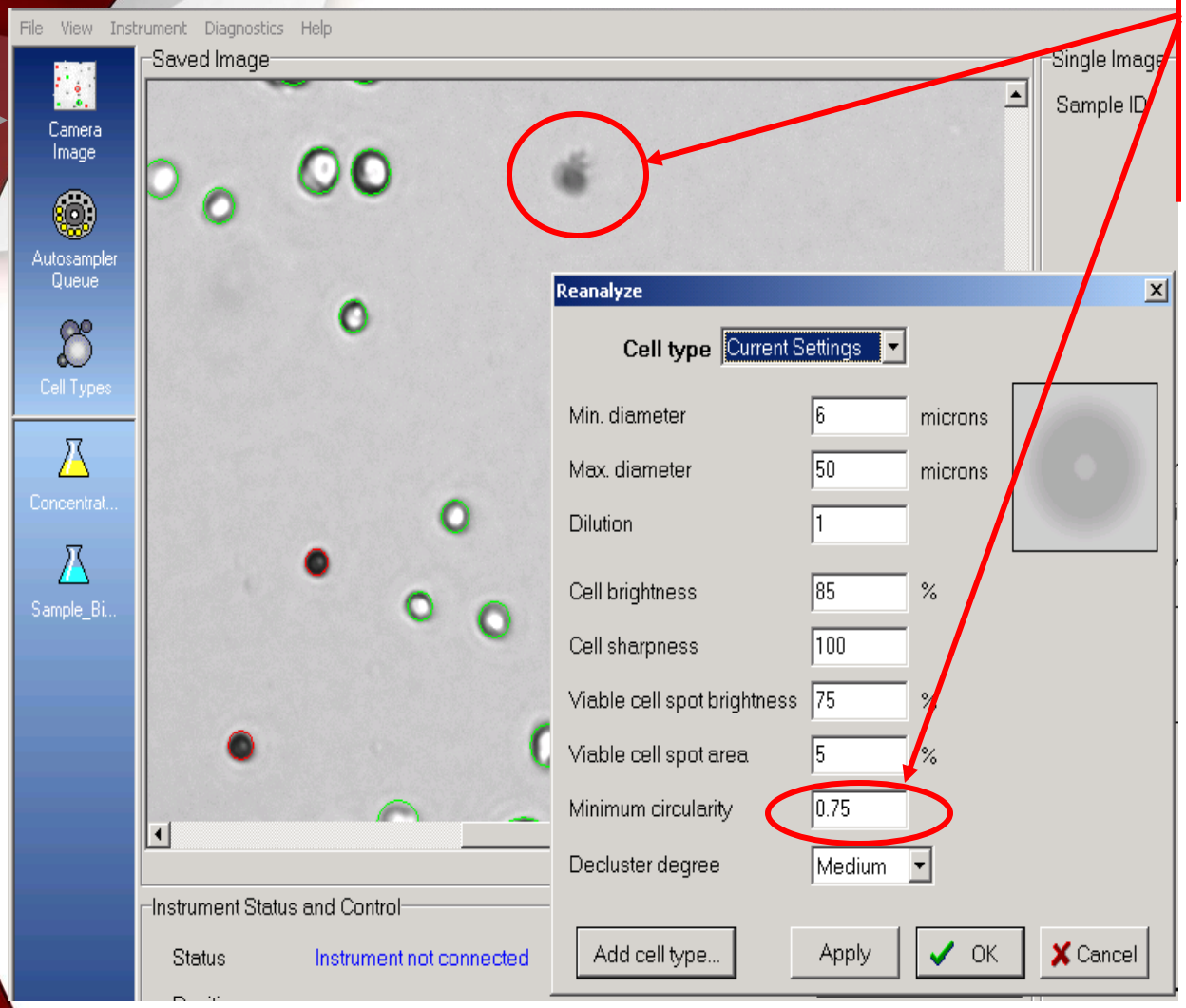
- Minimum circularity excludes **only dead** cells from the analysis.
- Dead cells or debris that are oblong in shape can be excluded using this parameter.
- Default value is 0 which includes all dead cells and oblong debris.
- Be careful using this parameter because a value of 1 will exclude all dead cells!!
- Let's have a look…

Parameters –
Minimum Circularity



This debris has lower circularity than the dead cells. We can reject this using the Minimum Circularity value.

Parameters –
Minimum Circularity



With a Minimum Circularity value of 0.75, this cell is no longer included in the analysis.

Parameters –
Minimum Circularity

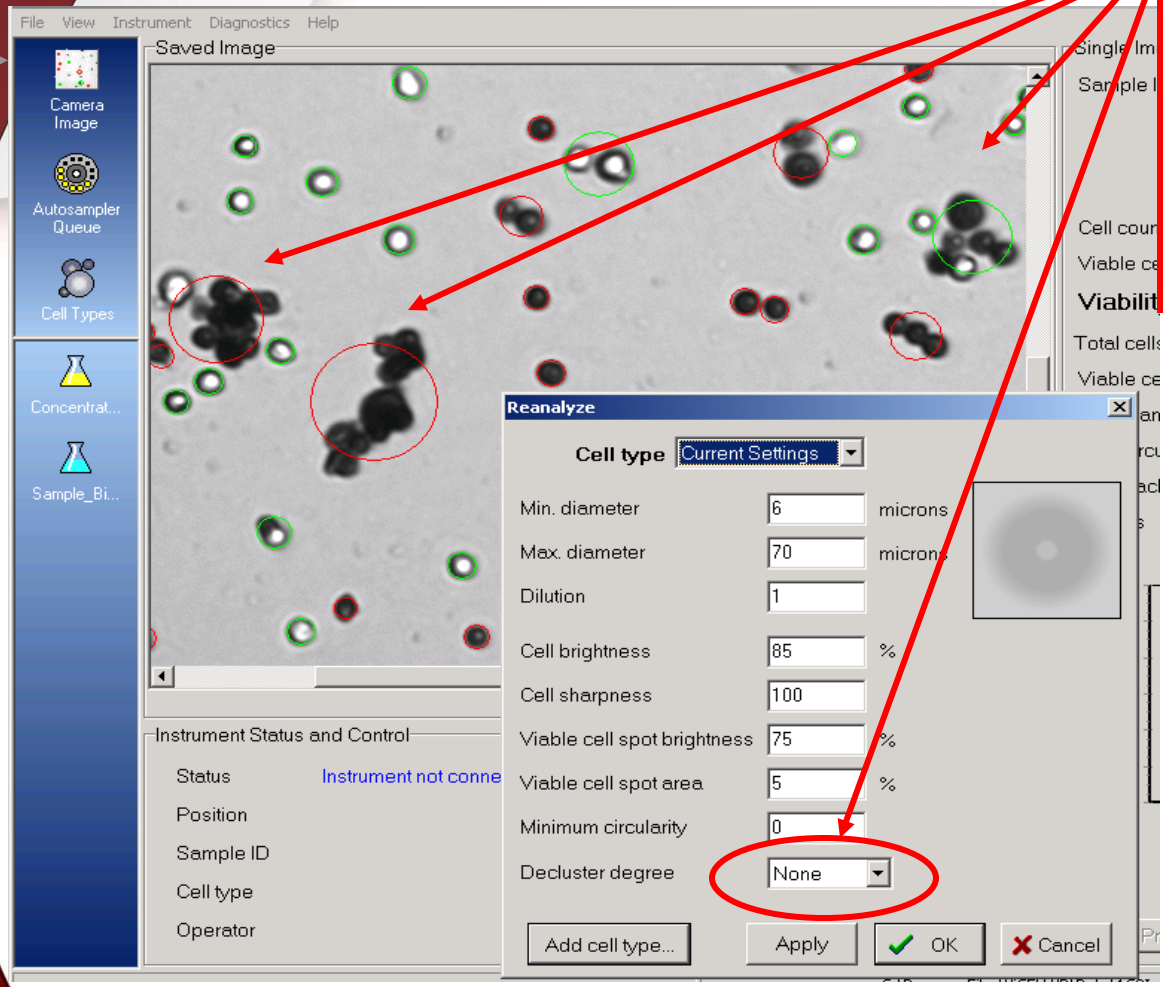
- Remember, minimum circularity excludes **only dead** cells from the analysis.
- The Default value is 0 which includes all dead cells and oblong debris.
- Normal values are in the range of 0 to 0.5.
- Be careful using this parameter because a value of 1 will exclude all dead cells!!

Parameters – Decluster Degree

- Decluster Degree includes 4 settings for how aggressively clumps of cells should be declustered.
- Setting options are:
  - **None** – Clumps are ignored (Version 2.01) or counted as one large cell (Version 2.02)
  - **Low** – Analysis looks for arcs in the clumps and closes larger radius arcs.
  - **Medium** – Analysis looks for arcs in the clumps and closes large and small radius arcs
  - **High** – Analysis looks for arcs in the clumps and closes even smaller radius arcs (note: sometimes cells in clusters get excluded by size with High degree.
- Setting applies to live cells and dead cells, although Version 2.02 of Vi-CELL XR has much improved declustering of dead cell clumps.
- Let's give it a spin…

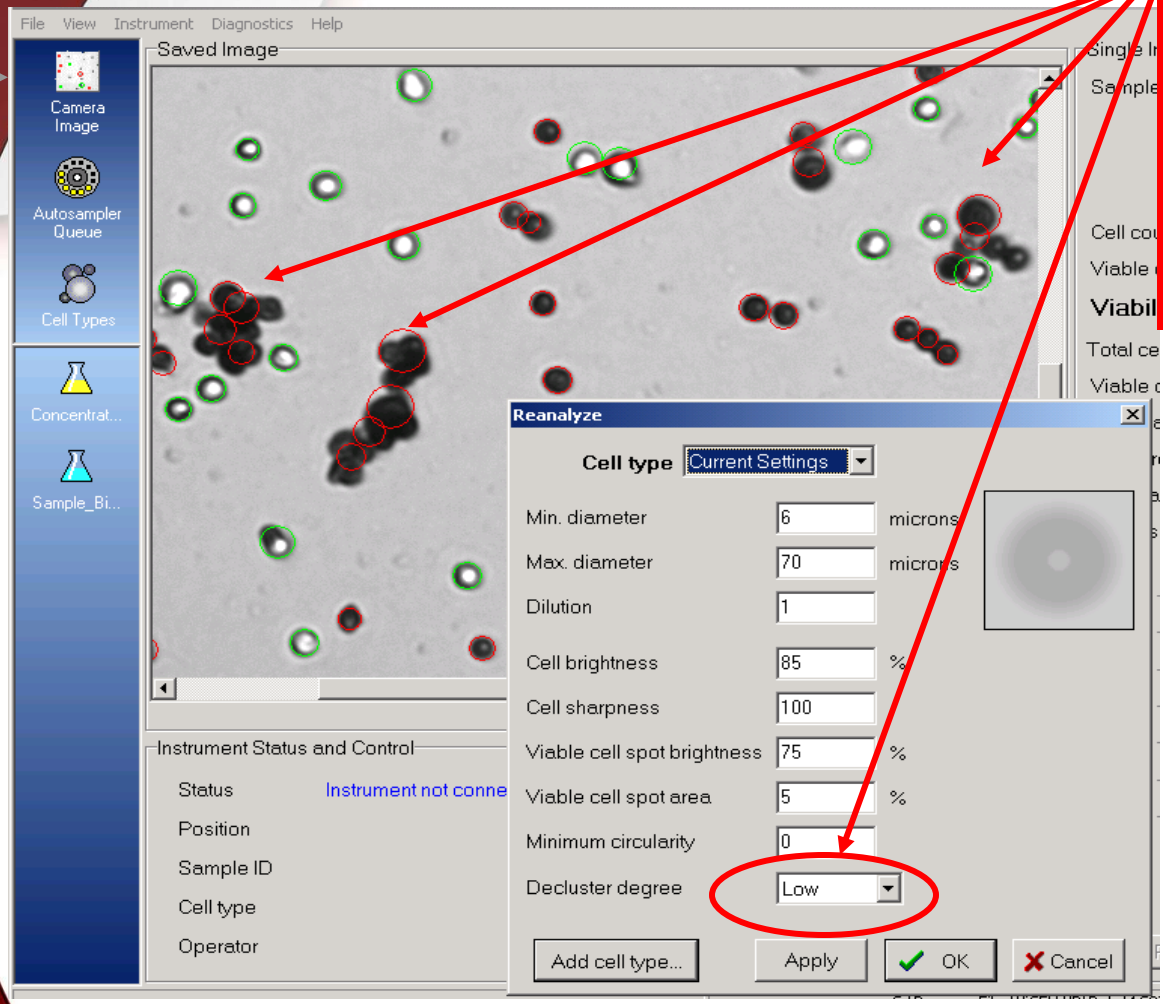
Parameters – Decluster Degree

With Decluster Degree = None, clumps are classified as one big cell. Note that the Max. Diameter setting must be large enough not to exclude these clumps

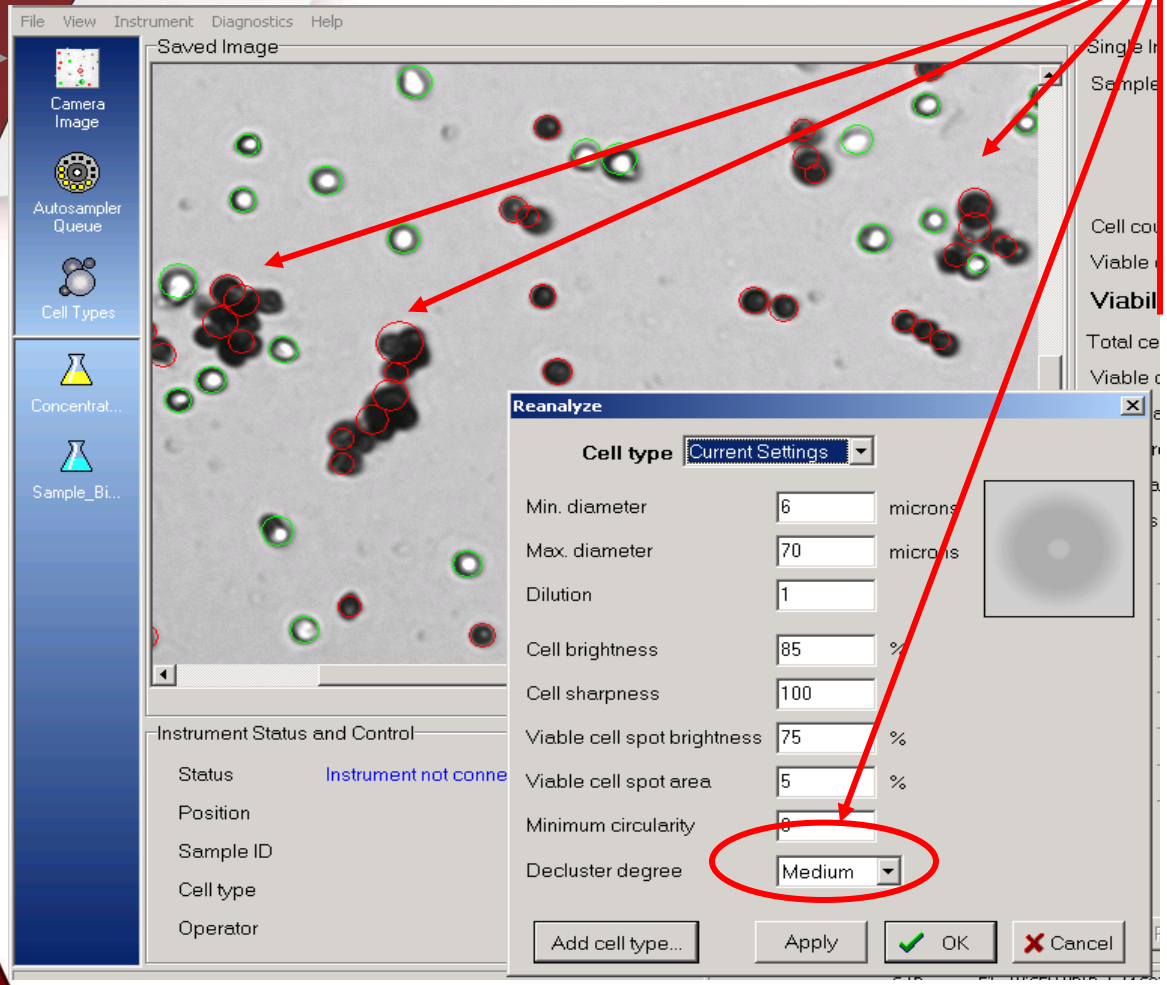


Parameters – Decluster Degree

With Decluster Degree = Low, clumps are declustered. Notice the large dead cell clumps are classified as 4 and 4 dead cells respectively.



Parameters – Decluster Degree



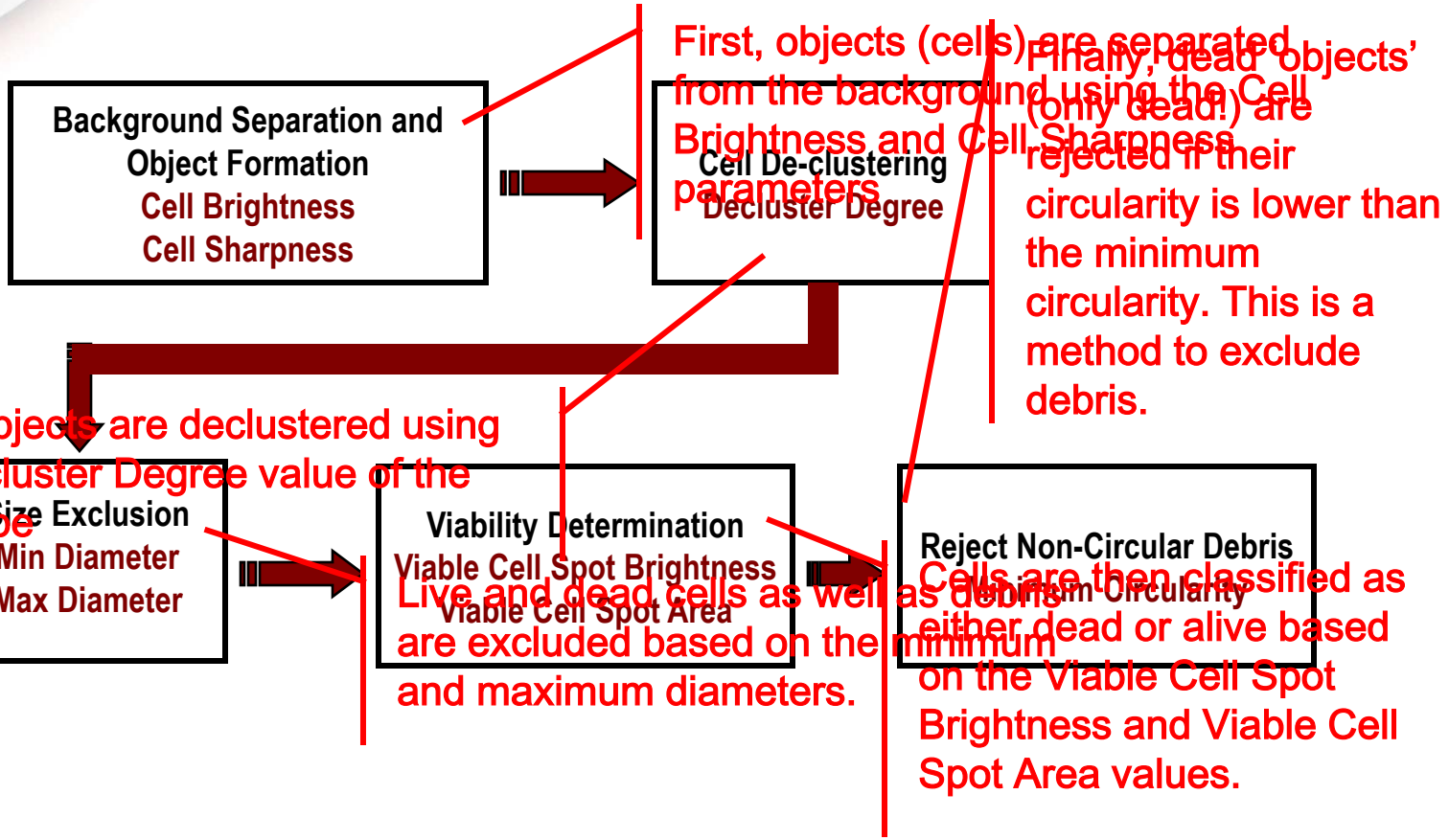
With Decluster Degree = Medium, clumps are declustered more aggressively. The large dead cell clumps are classified as 4 and 6 dead cells respectively.

Parameters – Decluster Degree

- Decluster Degree selection should be based on how the user typically counts cell clumps manually. Review a sample of images and decide on the most appropriate setting.
- ‘Low’ setting is typically used for Hybridoma and insect cells, as these are not cluster prone.
- ‘Medium’ setting is typically used for CHO and Vero cells as these tend to be clumpy.
- ‘None’ setting is useful for myocytes or other none circular cells (Version 2.02 software and later).

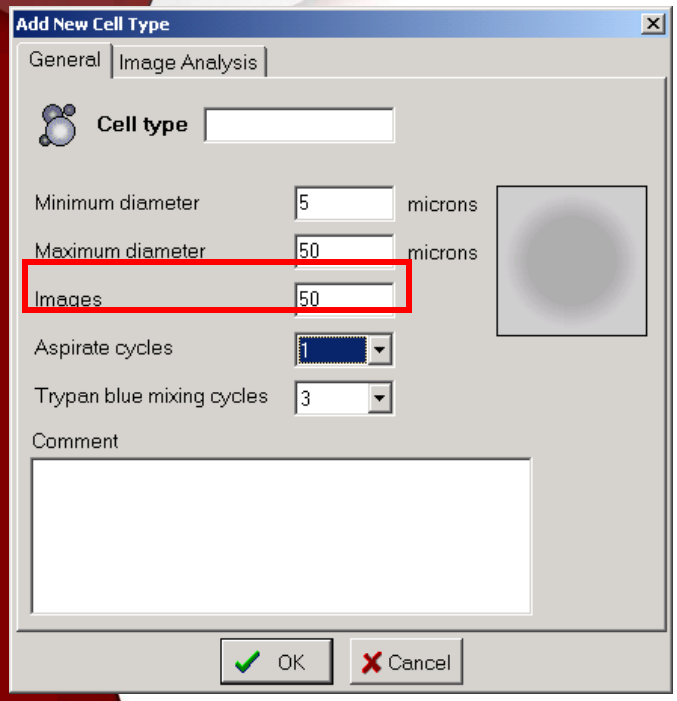
Image Analysis Review

- Vi-CELL XR Image Analysis Flowchart (simplified)



Number of Images Setting

- **Number of Images**
  - The number of images collected can be adjusted between 1 and 100, depending on speed and accuracy requirements.
  - Increasing images to 100 helps improve statistical accuracy of low concentration samples.
  - Decreasing number of images reduces sample analysis time by approximately 1 second per image.
  - Default value is 50.



Cell Types – Recommendations

- Start with the supplied Cell Types or Default values.
- To exclude debris or other objects, first try to adjust the size thresholds to eliminate.
- Don’t get frustrated on problems in a single image, look at the overall run result accuracy.
- When in doubt, call your Beckman Coulter Rep.
- Practice re-analyzing runs and images!!

Good Luck

and

Enjoy your Vi-CELL™ XR Cell Viability Analyzer