Object Counter: Counting Cells

The Object Counter module allows a user to count the number of objects that occur on each slice of a loaded data set. The goal of this exercise is to count and measure cells from light microscopy data.

1. Load the **FatCells.avw** data set from the **$:\BIR\images\TutorialData** directory.

2. Open the **Object Counter** module (**Measure > Object Counter**).

3. Select the **PreProcess** tab. This tab allows for correction of intensity fall off near the edges of the image data by applying an Inhomogeneity Correction filter. Using the default settings, click **Apply**.

4. Select the **Connect** tab. This tab (figure 1) allows for the specification of region growing parameters used to segment objects, including whether the objects should be segmented in 2D or 3D (for this exercise, we will use 2D).

5. With 2D selected, set the following 'Connect' tab parameters:

   • **Max. Size: 3217**

   • **Min. Size: 50**

   • **Threshold Minimum: 106** (this will allow the cells to be separated from the background)
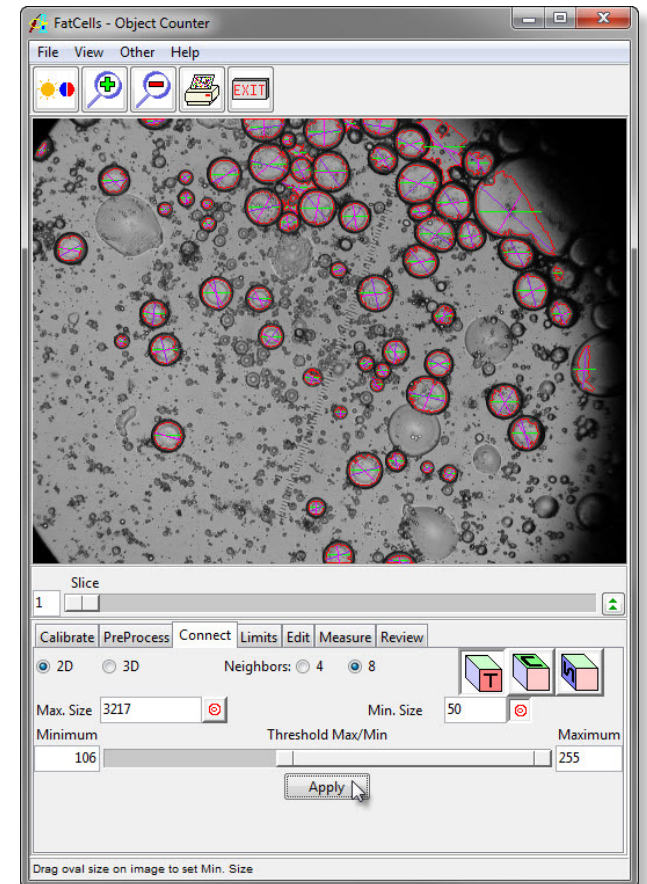
   note   The 'Max' and 'Min Size' parameters can be set by selecting the Interactive Size buttons next their respective text entry fields. Specify each by selecting either button and then drawing an ellipse around the largest/smallest object to be counted. Hold <Ctrl> while tracing forces the ellipse to be a circle.

6. Click **Apply**. All of the objects satisfying the size and threshold criteria will be identified and outlined in red on each slice of the volume.

7. Use the **Slice** slider bar to move through the slices in the data set. Return to slice 1 before proceeding to the next step.

8. Select the **Limits** tab. This tab allows interactive screening of segmented objects based on position and shape characteristics.

9. With the **Position** button selected, increase the **Y Min** to **11** using the arrow key found to the right. This will cause any objects with a Y coordinate less than 11 to be removed (objects along the bottom of the image display in the transverse orientation).

Figure 1

10. Reduce the **X Max** to **470** using the arrow key and observe the effect (seen along the right side of the image display).

11. By default, no shape screening is performed on the objects. Select the **Circularity** button, and check the **Restrict Circularity** option. Using the slider bar set the Circularity Maximum to 1.8.

12. Experiment with the effects of restricting Intensity, Diameter, Area, Perimeter, and Rectangularity.

13. Select the **Edit** tab. This tab allows objects to be added, deleted, and modified manually.

14. Select the **Measure** tab. This tab allows specification of the measurement statistics to be returned in the Object Counter Stats window.

15. To obtain a cell count on each slice click **Log Measurements** (without checking any of the options). The Object Counter Stats window will be returned, displaying a cell count for each slice (figure 2). Click **Done** to dismiss the window.

16. In the main Object Counter window select the **Measure** tab again. Check **Area** and then click **Log Measurements**. The Object Counter Stats window will be returned, displaying an object count for each slice and the average area of the objects on each slice.

17. To view the area for each individual object, select the **Detailed** option at the bottom of the Object Counter Stats window. When the 'Detailed' option is selected, click on any object in the Object Counter Stats window to highlight the object in the image display or vice versa (figure 3).

18. Experiment with other measurements available in the **Measure** tab.

19. Close the Object Counter module before proceeding to the next exercise.

tip To save the Object Counter Stats as a .stats file for use in another application (such as Microsoft Excel), right-click in the window and select Save Log.
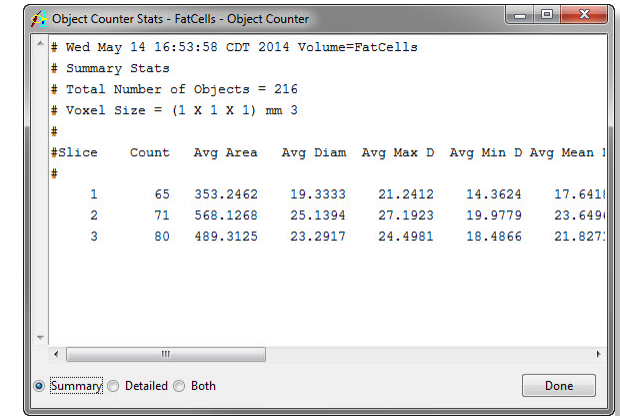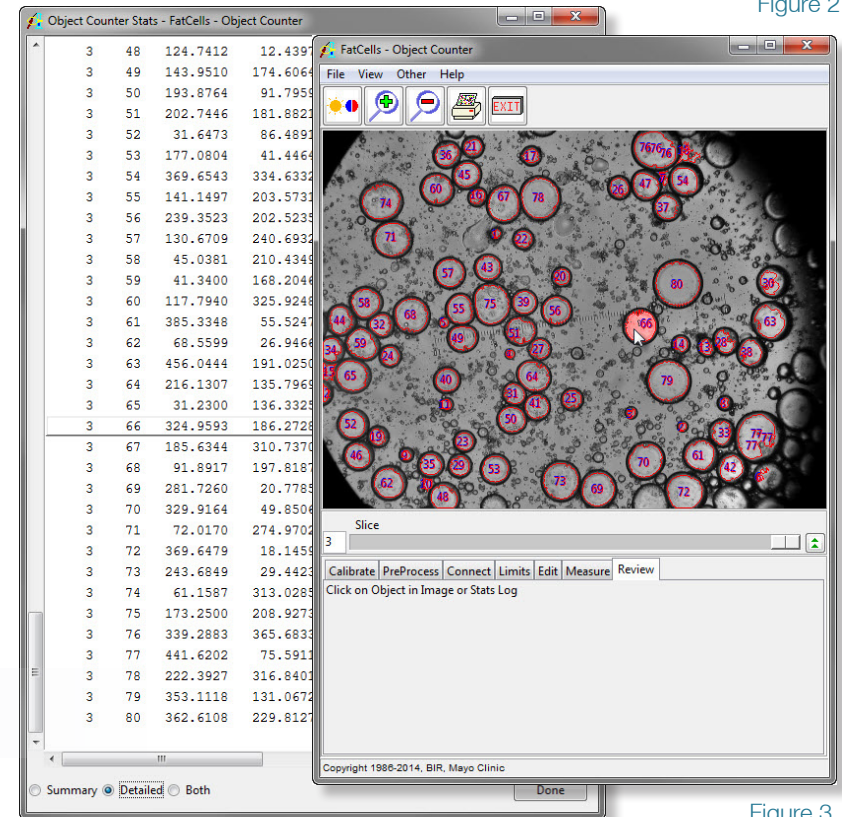

Figure 2

Figure 3