

MBR Manual

Harvard School of Public Health
Dana-Farber Cancer Institute

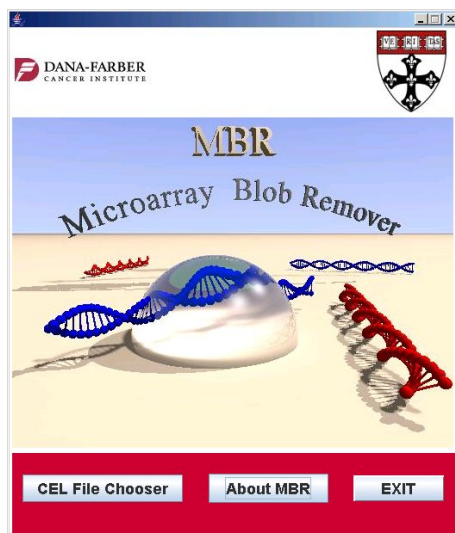
1 Installation

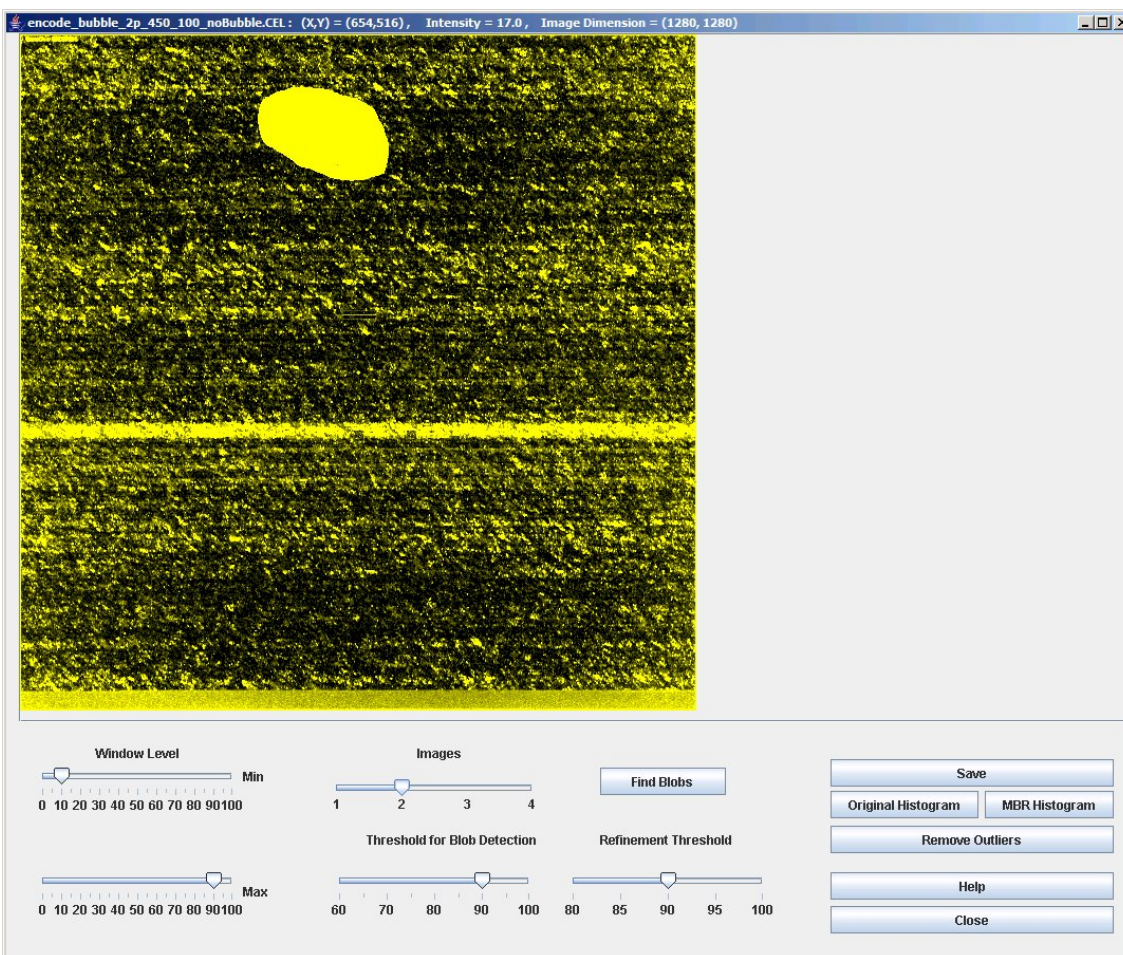
- **REQUIREMENT:** MBR requires Java Runtime Environment (JRE) 5.0 or higher, which you can download from <http://java.sun.com/>. In some cases, you might need to update JRE several times to install the latest version of JRE; this comment is particularly pertinent to Mac owners.
- Download the package MBR.zip and uncompress it using your favorite method.
- Windows: Double-click on MBR\dist\MBR.bat to launch the program. By default, the script allocates 400 MB of memory; you can change this amount by editing the script with a text editor.
- Linux, Unix or Mac: change directory to MBR/dist/ and execute the command
java -Xmx400m -jar MBR.jar

On Mac, double-clicking on MBR.jar should also start the program automatically; but if you want to pre-allocate memory, then use the above command.

- To modify and recompile the source code, download the JFreeChart class library from <http://www.jfree.org/jfreechart/>.

2 Loading and Processing Files





1. Upon launching the program, click on the “CEL File Chooser” button and choose the CEL files that you wish to analyze. MBR will then display the hybridization intensities as a stack of 2D images in the main interaction panel. **N.B.: The CEL files must be in Affymetrix’s “Version 4 Format” which is binary. If you are loading multiple files, then all of them must have the same array dimension.**
2. If necessary, adjust the “Window Level” sliders to set the intensity interval for display.
3. Click on “Find Blobs” and try to detect any visible blobs using default parameters.
4. If necessary, adjust the “Threshold for Blob Detection” and “Refinement Threshold” sliders and click on the “Find Blobs” button again. **To detect low intensity blobs, try lowering the “Threshold for Blob Detection” value. Raising the “Refinement Threshold” value will tighten the boundary around detected blobs.** See Figure 1.
5. If you wish, click on the “Original Histograms” and “MBR Histograms” buttons to check the effect of blob removal on the distribution of intensities.
6. Click on the “Save” button to create a new CEL file with the coordinates of detected blobs in its “Outlier Entries” section.

7. Click on the “Remove Outliers” button if you want to create a new CEL file with an **empty** “Outlier Entries” section.

N.B. MBR changes only the “Outlier Entries” section of CEL files and does not modify the original hybridization intensities.

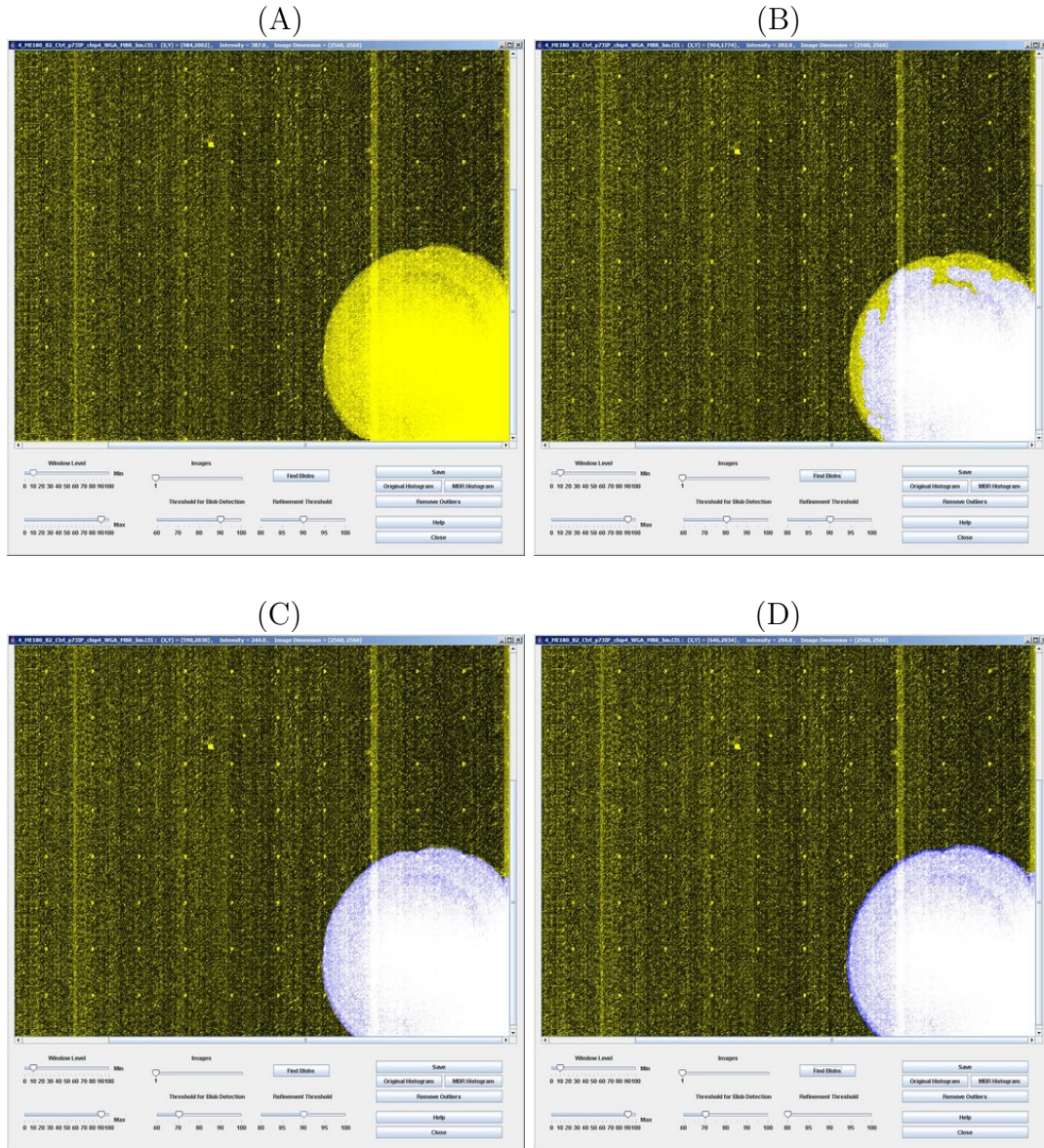


Figure 1: (A) Original image with an apparent defect. (B) Partial detection of the blob at 80% "Threshold for Blob Detection". (C) Full detection of the blob at 70% "Threshold for Blob Detection". (D) The boundary of the detected blob can be expanded by lowering the "Refinement Threshold" value.

3 Summary of MBR Sliders and Buttons

SLIDERS

- **Window Level:** Adjusts the intensity interval to be displayed.
- **Images:** Scrolls through multiple images.
- **Threshold for Blob Detection:** Quantile cutoff for the first scan. At least half of the pixels in a 100x100 sliding square must have intensity higher than this cutoff in order for the square to be considered as a candidate blob. If the program doesn't detect what you suspect to be a blob, try lowering this threshold.
- **Refinement Threshold:** The candidate square regions are rescanned with small circles; and the percentage of high intensity pixels in a circle must be greater than this threshold in order for the circle to pass as a blob.

BUTTONS

- **Find Blobs:** Detects blobs using the selected parameter values.
- **Save:** Writes a new CEL file with the coordinates of all detected blobs in “Outlier Entries.”
- **Original Histograms:** Displays the histograms of probe intensities from the original CEL file.
On the histograms panel, Left-click and drag to zoom in, and Right-click to get options.
- **MBR Histograms:** Displays the histograms of probe intensities, with MBR detected blobs as outliers.
- **Remove Outliers:** Writes a new CEL file with an empty “Outlier Entries” section.

4 Contact Information

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