Quantification of Live/Dead Staining Using Fiji Software

Analyze your Cell-Mate3D™ Live/Dead staining images with FIJI open source image analysis software. Use this protocol as a guide to quantify the number of live and dead cells in an image.

To access FIJI software, visit: http://fiji.sc/Fiji

Workflow:
1) Split channels: live cells are in the Green Channel and dead cells are Red Channel. Discard the Blue Channel. Channels must be analyzed separately for live and dead fluorescent channels
   (Image > Color > Split Channels)
2) Work with one channel at a time. Convert the channel to 8-bit (if they are not yet in that color graphic format). This is done so that the images may be threshold based on intensity
   (Image > Type > 8-bit)
3) Select the Find Maxima function from each channel to count number of dead or live cells. Select the "Point Selection" output type and check the box for "Preview Point Selection". Adjust the "Noise Tolerance"* values by increments of 5 or 10 until background staining is excluded. The number of points will be the total number of cells positive for the stain of interest (numbers are based on user's definition of Live/Dead cells)
   (Process > Find Maxima)
4) Use the following formulas for quantification:
   - Total Cell Number = Live Cells + Dead Cells
   - Percentage of Live Cells = (Live Cells/Total Cell Number)*100
   - Percentage of Dead Cells = (Dead Cells/Total Cell Number)*100

*Note:
Be mindful of adjusting the tolerance value so the red and the green "points" do not overlap. However, some overlap may occur and it is dependent on the user to determine how much overlap is acceptable.

Disclaimer:
Bioactive Regenerative Therapeutics, Inc. shall not be held liable for any loss, injury or damage as a result from the use of this protocol.

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