This is the step-by-step method for doing **exactly** what the macro does for each image.

1. Open *Fiji (ImageJ)*
2. Drag 'night_ski.gif' and drop it into *Fiji* as you would any other file. Alternatively open a .lif file as you normally would.
3. Apply a z-projection (Stk -> Z Project), be sure to set 'Projection Type' to 'Sum Slices'
4. Make a copy of your new image. (Image -> Duplicate)
5. DO STEPS 6 – 14 ON THE COPY ONLY, SAVE THE ORIGINAL (THE RESULT OF STEP 3) FOR MEASUREMENTS.
6. Apply Gaussian Blur (Process -> Filters -> Gaussian Blur) to slightly blur the image. This will have a negligible effect on your final numbers and helps to create a better selection mask.
7. (Image -> Adjust -> Threshold) Apply a threshold. Select the 'Huang' method. Press 'Auto'. Press 'Apply'. Press 'Convert to Mask'.
8. Press 'X' to close Threshold panel.
9. (Process -> Binary -> Dilate) to improve the selection outline by expanding it slightly.
10. (Analyze -> Tools -> ROI Manager) To open the mask/selection manager.
11. (Edit -> Selection -> Create Selection) Create a selection for the cells.
12. In the ROI Manager press 'Add'
13. (Edit -> Selection -> Make Inverse) To flip the selection inside-out and select the background.
14. In the ROI Manager press 'Add'
15. WHEW! Almost there! Take a breather after that.
16. Now, select the window of our original (the result from step 3).
17. In the ROI Manager, press 'Measure'
18. Voila, you have your cell-fluorescence measurement and you background measurement.

Note that this method will give you more accurate results than doing it by hand because it samples the *entire* background, not just aa small sample of it.