## Image analysis of object counts with ImageJ™

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## Using ADOBE Photoshop™ to get the best image for analysis.

- Take an image with camera of eggs. White background for black eggs and black background for white eggs. The eggs must be separate. Separation of eggs is easier with partial removal of the gel coat. see "Gel removal and extract"
- 2. Open image in Adobe Photoshop™
- 3. Image > Mode > Greyscale
- 4. Image > Adjust > Brightness/Contrast. Adjust the contrast and brightness to maximize separation of the eggs from the background.
- 5. Delineate the area with eggs with the Elliptic or Rectangular marque
- 6. > Cut
- 7. > Create new file > OK
- 8. Paste
- 9. Image > Levels Adjust to distinguish eggs
- 10. Filter > Noise > Despeckle
- 11. Save as tif for analysis in image analysis program.

## Analysis of image with Image J™

- Open in" ImageJ™" 1.33u: Wayne Rasband, National Institutes of Health, USA; http://rsb.info.nih.gov/ij/
- 2. process > sharper
- 3. Process > noise > despeckle
- 4. Image > adjust threshold goes red
- 5. Analyse > particles
- 6. Show "masks", "display results", "summarise" > OK
- 7. The "Image J™" program then produces a count of objects and a summary of average size in pixels, and distribution of size. It also produces image of counted objects