A quick guide to colony counting in Fiji

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Considerations before you start

This quick step-by-step guide will allow you to count colonies from pictures of agar plates. As with most automated methodology, the precision and accuracy depends primarily on the quality of the input. Make sure that the colonies are large enough to be clearly visible, sparse enough to prevent adjacent colonies from overlapping, and that the picture has an even illumination and background.

The counting procedure

Once you have appropriate pictures, the procedure is as follows:

1. If not already done, download the ImageJ distribution Fiji

2. Start Fiji and open the plate image

3. Make a circular selection of the whole plate and press `M` to measure; note down the area of the plate

4. Make a circular selection of a part of the plate that is free of writing and edges (aim for roughly 10–75% of the whole plate); press `M` to measure and note down the area; make sure to maintain the selection for the next two steps

5. Do `Edit → Clear Outside` to remove everything from the picture except the selected region of the plate

6. Do `Process → Binary → Make Binary` to transform the picture into black (colonies) and white (background)

7. Do `Process → Binary → Watershed` to separate overlapping colonies into individual spots

8. Make a new circular selection the size of a single colony; press `M` to measure and note down the area

9. Go to `Analyze → Analyze Particles...`

10. In the newly opened window, set the expected size of the colonies (`Size (pixel^2)`) according to the size of the colony you measured in step 8; set the range generously, *e.g.* if you measured an area of 300 px, set the range to 100–1500 px

11. Leave `Circularity` at 0–1, select `Overlay Masks` from the `Show:` dropdown menu, and tick the `Summarize` and `In situ Show` boxes

12. Click `OK`

13. In the new `Summary` window, you see the number of counted colonies under `Count`; the counted colonies will appear coloured in the image, pixels that were ignored in the counting remain black

14. Finally, extrapolate the number of total colonies on the plate using the areas measured in steps 3. and 4.