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; press `T` to save the selection in the ROI manager, and press `M` to measure it's area
4. Make a circular selection of a part of the plate that is free of writing and edges (aim for roughly 40–75% of the whole plate); again, press `T` and then `M`; make sure to maintain this 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); if the colonies are white on a black background, do `Edit` → `Invert`
7. Do `Process` → `Binary` → `Watershed` to separate overlapping colonies into individual spots
8. Go to `Analyze` → `Analyze Particles...`
9. Leave `Circularity` at 0–1, select `Overlay Masks` from the `Show:` dropdown menu, and tick the `Summarize` and `In situ Show` boxes

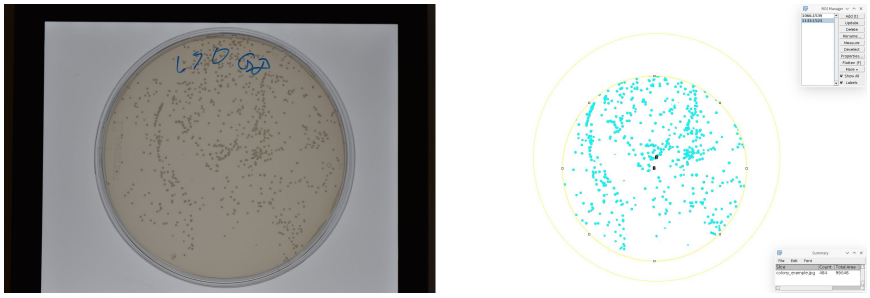

Figure 1: Example image of a bacterial plate (left), and the same plate after processing (right). In the right image, the large yellow circle is the selection created in step **3**, and the smaller yellow circle the selection created in **4**

10. Click `OK`

11. 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 (see figure 1)

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

Note: This tutorial covers only simple counting and area measurements. If you want to characterise additional parameters of the colonies such as colony shape, colour or heterogeneity, we just need to tweak a handful of steps. Let me know, and we can go through that together.