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# Protocol (C): Automated segmentation of the otic vesicle and image analysis

In 1 collection

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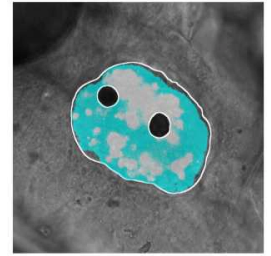
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**Protocol Integer ID:** 99200

**Keywords:** Danio rerio, multispecies bacterial infections, image analysis, automated segmentation, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*

## Abstract

This protocol details the automated segmentation of the otic vesicle and image analysis.



## Attachments



ImageAnalysis\_Script...

8KB

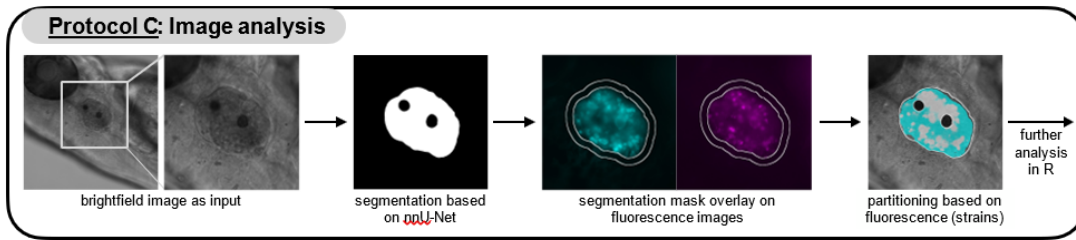
## Materials

For this protocol, you will need the following scripts and software:

- Docker and the docker image (docker pull branhongweili/dqbm\_cell\_seg:v3.1) to run the pre-trained segmentation model.
- Depending on the operating system, either use the segmentation.sh shell script (for MacOS/Ubuntu) or the segmentation.ps1 PowerShell script (for Windows).
- An installation of FIJI.
- The check\_measure.py script.
- An installation of R.
- The co\_localization.R script.

## Automated segmentation of the otic vesicle and image analysis

1



2 Organize your images in a folder with two subfolders:

- one for the brightfield images named 'Images',
- one for all fluorescence images named 'Fluor'.

The image file names have to contain the slice number with a Z as a prefix and the channel number (0-based) with a C as a prefix (e.g., PositionName\_Z00\_C00.tif). For example, for measuring two different fluorophores and the brightfield image, we have three channels, C00 (e.g., brightfield), C01 (e.g., GFP), C02 (e.g., mCherry). We include an example script to convert Leica image files accordingly (sort\_images\_lif.py), which can be used as an example of how to organize all images automatically as described.

3 For the segmentation of the otic vesicle, run the segmentation.sh (segmentation.ps1 for Windows) script with the previously created directory as input (e.g. for Mac: in Terminal). Make sure the shell script is executable (e.g. for Mac: by typing

```
chmod +x segmentation.sh
```

in Terminal). The script will start a docker container and the segmentation model on the specified brightfield images.

4 In the same directory that contains your brightfield (Images) and fluorescence (Fluor) images, a directory named output\_real\_value is created that contains the segmentation masks.

5 Start FIJI and open the check\_measure.py script.



- 6 Run the script and select the directory with your brightfield (Images) and fluorescence (Fluor) images and segmentation masks (output\_real\_value) as input.
- 7 FIJI will display the brightfield images and the corresponding segmentation. In the ROI Manager window untick the box "Show All" and select one of the ROIs in the list to only show the segmentation for a single ROI. Go through the ROI sequence and delete inaccurate segmentations.
- 8 Complete the manual review of the segmentations by pressing OK.
- 9 The script creates six files:
  - 9.1 an image with an overlay of the strain partitioning based on fluorescence (e.g. Sample1\_overlay.tif),
  - 9.2 a text file containing the log to check what has been done up until here (e.g. Sample1\_log.txt)
  - 9.3 a zip file with the ROIs (Sample1\_rois.zip)
  - 9.4 a table with fluorescence values within the whole otic vesicle (mean and integrated density for both GFP and mCherry; e.g. Sample1\_ov\_data.csv)
  - 9.5 A table with the size of the area occupied by tagged strains (e.g. Sample1\_strain\_count.csv) → e.g. needed to calculate co-localization
  - 9.6 A table with the fluorescence values of individual pixels within the otic vesicle (e.g. Sample1\_pixel\_data.csv).

**Note**

Since the zebrafish shows inherent auto-fluorescence, we introduced a threshold value to delineate bacterial occupation from auto-fluorescence. The threshold value for bacterial occupation is defined as twice the fluorescence value observed in the surrounding tissue of the otic vesicle (a layer of approximately 16  $\mu\text{m}$ , corresponding to 50 pixels).



- 10 Use the accompanying R-script to visualize the size of the area occupied by each tagged bacterial species and the overlap between the two species to quantify their co-localization (script: co\_localization.R).
- 11 Check whether the fluorescence images in FIJI correspond to the values in R by dropping the Fluor folder into FIJI and comparing it to the R plot.