Bioimage informatics

BeadNet: deep learning-based bead detection and counting in low-resolution microscopy images

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Abstract

Motivation: An automated counting of beads is required for many high-throughput experiments such as studying mimicked bacterial invasion processes. However, state-of-the-art algorithms under- or overestimate the number of beads in low-resolution images. In addition, expert knowledge is needed to adjust parameters.

Results: In combination with our image labeling tool, BeadNet enables biologists to easily annotate and process their data reducing the expertise required in many existing image analysis pipelines. BeadNet outperforms state-of-the-art-algorithms in terms of missing, added and total amount of beads.

Availability and implementation: BeadNet (software, code and dataset) is available at https://bitbucket.org/t_scherr/beadnet. The image labeling tool is available at https://bitbucket.org/abartschat/imagelabelingtool.

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

Ligand-coupled beads are often used in in vitro experiments to mimic bacterial invasion processes (Braun et al., 1998; Hebert et al., 2017; Jung et al., 2009). In the experiments that led to the development of BeadNet, red fluorescent latex beads of 1 μm in size were chemically coupled with a bacterial surface ligand, and their internalization into human cells was investigated. To distinguish internalized beads and beads that remained outside of cells, the cells were fixed without permeabilization, and external beads were recognized using a ligand-specific antibody coupled to a green fluorophore (Fig. 1a).

The complexity of bead detection in low-resolution images may lead biologists to either use an inefficient and user-biased manual quantification or to use a user-friendly but inaccurate method. In addition, the parameters of sophisticated analysis pipelines need to be adjusted in case of changes in the experimental design. Even for experts, it is often easier to annotate new images instead of adjusting parameters for low-resolution and low signal-to-noise-ratio data. Thus, we designed the bead detection and counting software BeadNet to avoid parameter adjustments. For domain adaptation, biologists only need to create new training images using a built-in functionality and annotate them using our labeling tool. Despite its simple use, BeadNet enables a nearly error-free bead detection on a manually annotated low-resolution bead dataset. To our knowledge, this is the first annotated 2D dataset and software tackling the problem of counting beads in low-resolution images with high accuracy. The software combines a crucial upsampling preprocessing with a deep learning-based detection. In addition, BeadNet provides a graphical user interface for easy use, e.g. for generating new training data. The upsampling step and the training data generation are often missing in toolboxes, leaving users alone with the upsampling, the normalization and the creation of new training samples. BeadNet also provides the automatic calculation of evaluation measures and overlays of the detection results.

2 Materials and methods

BeadNet consists of an upsampling preprocessing step for low-resolution images and a deep learning-based bead detection with subsequent counting. The bilinear upsampling, hereby, enables distinguishing touching beads (Fig. 1b and c). Furthermore, it eases the annotation of a low-resolution dataset that is required for supervised learning (Fig. 1d). Due to the upsampling preprocessing, centers of touching beads do not share an edge anymore, and the bead detection can be treated as a semantic segmentation task. Thus, for the bead detection, an adapted U-Net trained with a combination of...
dividing seeded images very difficult. The application of a fourfold bilinear upscaling and the use of 2×2 px seeds enable to annotate beads quickly, to avoid touching seeds and to hit the center easily (Fig. 1c and d).

3 Low-resolution bead dataset

We annotated a low-resolution bead dataset consisting of 60 training, 15 validation and 25 test images with 2587 beads in total. The bead diameters range from about 2 px to 4 px (Fig. 1b). Neighboring seeds can touch each other and the center of beads is not well-defined. This makes manual annotation of seeds in the original images very difficult. The application of a fourfold bilinear upscaling and the use of 2×2 px seeds enable to annotate beads quickly, to avoid touching seeds and to hit the center easily (Fig. 1c and d).

4 Results

For performance measurements, the ground truth seeds are enlarged (Fig. 1d). Then, the normalized amount of missing (no predicted seed in the ground truth bead) and added beads (predicted seed in the ground truth background) can be counted. All shown metrics do not count added seeds at the border area (only fully visible beads are annotated, see Supplementary Information for the applied border correction).

Table 1 shows quantitative results of BeadNet, of the Laplacian of Gaussian-based seed detection of TWANG (Bartschat et al., 2016; Stegmaier et al., 2014), of a Hough-transform-based detection using MATLABs imfindcircles (Yuen et al., 1990) and of a simple Otsu thresholding (Otsu, 1979) with Euclidean distance transform on the (upsampled) test dataset. The median BeadNet prediction outperforms the other methods in nearly every metric. More detailed results including the single initializations, and a qualitative comparison with the FISH-quant software for the automatic counting of transcripts in FISH images (Mueller et al., 2015) is provided in the Supplementary Information.

5 Conclusion

BeadNet outperforms traditional bead detection methods, which need expert knowledge to adjust them. The high detection rate shows that no additional preprocessing toolbox is needed, e.g. a 2D adaptation of the image restoration toolbox CSBDeep for the denoising and isotropic recovery of 3D microscopy data (Weigert et al., 2018). Combined with the integrated training data generation enables the high detection rate a user-friendly end-to-end use of BeadNet for the detection of poorly resolved objects, e.g. of ligand-coupled beads or other spherical or non-spherical objects.

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