IIB Project TMR: Limits of spatial resolution for accurate segmentation and lineage reconstruction

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1 Abstract

This report outlines the background and motivations, objectives, current progress and future plans for this project. The limits of objective magnification at which segmentation can still be performed successfully are investigated, to better design experiments to maximise data throughput and temporal tracking of cells, which allows the study of gene expression in cells, among other things. Synthetic images are preferred to real data to train neural networks for segmentation, since labeling real images is infeasible and inaccurate. So far, experiments have been down with fluorescence mother machine images, but soon this will move onto phase-contrast images as these are more applicable for high temporal frequency imaging. Finally, the effect of super-resolution (SR) neural networks will be investigate to see if these can offer any improvement in performance for low resolution images.

2 Background

2.1 High throughput microscopy

High-throughput microscopy allows researchers to acquire images automatically from thousands of different treatments overnight or over several days. This makes it possible to conduct large-scale, image-based screens to discover novel genes and novel functions of familiar genes [1]. These experiments produce a lot of image data which needs to be analysed. Classification and segmentation are important steps in the analysis process. Classification is used to identify where cells are in an image, while segmentation attempts to classify what object or section of the image individual pixels are part of. Segmentation and classification algorithms perform better on higher resolution images in general. This creates a trade-off between the spatial resolution (magnification of the image), temporal resolution (frequency of imaging of a particular linear colony), and data throughput (number of colonies in the experiment) where having two of these high means the third must be lower, as is shown in Figure 1.

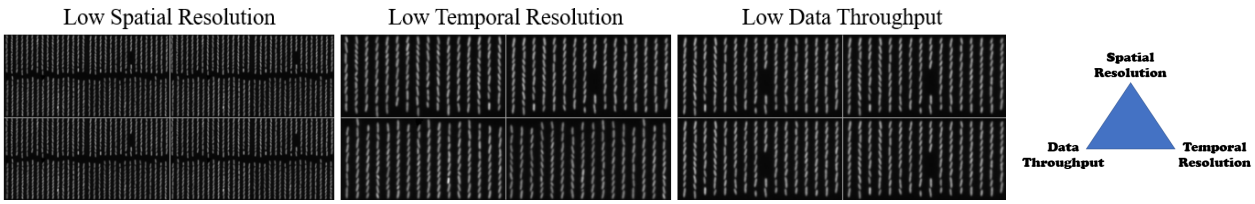

Figure 1: The three-way trade-off for screening experiments. For the images shown, each quadrant represents a single image with the specified setting lowered. Right image shows the three facets of the trade-off.

2.2 Mother Machine

The mother machine is a microfluidic device which is commonly used for these types of screening experiments. It contains bacterial growth channels, with each channel being closed on one end and open to a stream of media, containing food and other necessary chemicals, at the other end [2]. Each channel is about the width of the bacteria (around 1 micron) so the colonies remain linear, which makes analysing these images much easier. The mother machine also allows a long term study of an individual cell, since the cell at the closed end of the channel (the 'mother' cell) will always remain. This is important for studying how individual cells may evolve or react to changes in their environment over time, or what factors can lead to cell death.

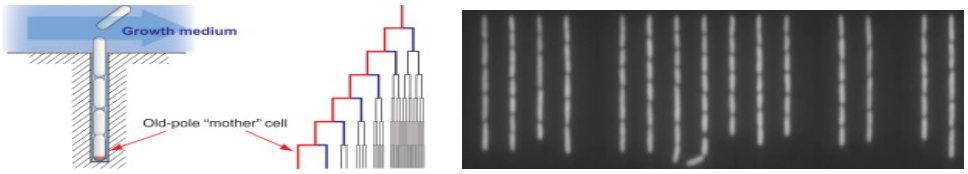

Figure 2: Schematics of a mother machine. Left image shows a single trench and the lineage of the 'mother' cell [2], right image shows a typical image from a mother machine [2].

2.3 SyMBac

Using the mother machine for large scale screen experiments to analyse single cells is quite an attractive prospect, but there are a few problems that must be dealt with [3]. Segmentation and identification in images are usually carried out using neural networks, but these require a lot of training data to become effective. One possibility is to capture real images of bacteria, but these need to be labeled with correct answers to be used to train the network. This is very time consuming for a human to carry out, and in the case of segmentation it can be quite inaccurate. The size of bacteria is on the scale of microns, which is similar to the wavelength of light so imaging bacteria with a microscope is a diffraction limited process [4]. This means that humans cannot accurately segment images of bacteria, especially at lower magnifications, which causes issues for the training process. Instead, a synthetic image pipeline, SyMBac [5], will be used. This pipeline generates a ground truth image by simulating bacterial growth in a mother machine and then convolves the image with the point-spread function of the microscope [6] to generate a similar image to what is observed in reality. This process is shown in Figure 3 This ground truth and realistic image pair can then be used to train a neural network to segment images of bacteria in a mother machine.

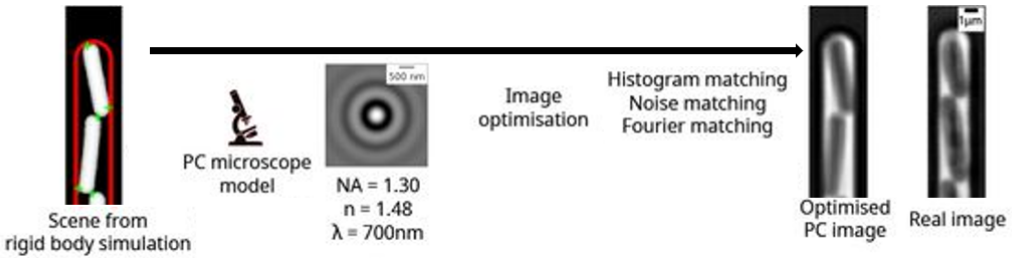

Figure 3: SyMBac image generation pipeline (adapted from [5]).

2.4 Imaging modalities

Two of the main imaging modalities for bacterial images are fluorescence and phase-contrast, shown in Figure 4. Fluorescence images are much more desirable for segmentation, since cells appear as bright objects on a dark background, while cells in phase-contrast appear with an intermediate intensity compared to the dark mother machine and the light media. Fluorescence images are achieved by introducing a fluorescent expression marker into the cells, which activates when an image is taken. However, if images are taken too frequently this causes photo-toxic damage to the cell [3], so to take full advantage of high temporal resolution phase-contrast will need to be used.

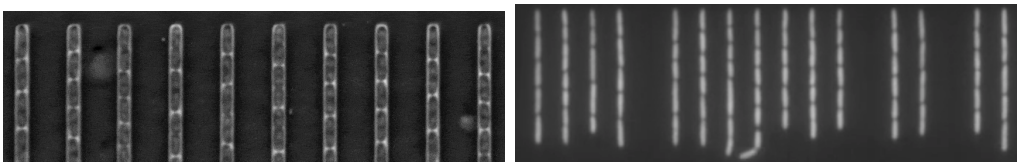

Figure 4: Left image shows a mother machine imaged with phase-contrast [7], right image shows a mother machine with fluorescence imaging [2]

3 Objectives

3.1 Limits of spatial resolution

We see that higher spatial resolution either leads to reduced temporal resolution for each linear colony or causes lower data throughput for the experiment. Therefore, a thorough investigation into the performance of classification/segmentation algorithms with respect to image resolution will be carried out to get a good idea of how to navigate this trade-off effectively. For low resolutions, this will allow experiments to be undertaken with as low a resolution as is possible while still achieving some desired level of segmentation and identification accuracy. On the other end, if there is an objective magnification above which accuracy plateaus, experiments needing high accuracy will be able to take advantage of this to not overdo the microscope magnification.

The aim of this investigation is to produce charts with some accuracy score for the algorithm, such as the intersection over union (IoU) for segmentation, on the y-axis against the image resolution/magnification on the x-axis, for both fluorescence and phase-contrast images. Once this has been completed, the performance of these algorithms can be tested on real data at different resolutions to see whether the use of synthetic images for training is effective.

3.2 Super-resolution

Once this accuracy/magnification relationship has been established, we wish to investigate to what extent using a deep-learning SR algorithm can improve the relationship. Single image SR is a challenging ill-posed problem in computer vision which aims to obtain a high-resolution output from a low-resolution version as an input [8]. If using a network like this can introduce even a small SR effect, it could lead to big increases in temporal resolution or data throughput for an experiment for a small cost of extra image processing time after the images have been collected.

4 Progress

4.1 Initial experimentation

To begin the investigation, several classical algorithms were tested along with a pretrained neural network model (Stardist) on a real fluorescence mother machine image. The base image had an objective magnification of 60×, and lower resolution images were created by downsampling by the relevant scale and then upsampling the image back to full size. This does not recreate the blurring effect of lower magnifications from a microscope, but it does give an insight into the effect of the pixelation of the images on its own. A ground truth image was created by manually painting over the image to create larger contrasts in areas of ambiguity and then running a segmentation algorithm to create a sufficiently good mask. Identification accuracy was roughly calculated as the number of cells identified over the number of cells in the ground truth image (or the reciprocal if that number ended up greater than 1), which can allow for different errors to cancel each other but still gives an indication of the expected performance. Segmentation accuracy was essentially calculated as the fraction of cells in the ground truth image that had a reasonable mask representation in the segmented image. This was done by calculating the closest mask to the position of each actual cell (from midpoint to midpoint) and the area of that mask. If the mask was within a 5 pixel radius and the area of the mask was within 0.8-1.2 times the area of the cell, it counted as a reasonable representation. This method of calculating segmentation accuracy was used because it allowed the use of the "Analyse Particles" tool in Fiji which made the segmentation process easier, but to check whether it is representative of a more standard measure this metric was compared to IoU for the Stardist results, and the trends were similar enough that it is a useful metric. In particular, IoU was a bit higher at lower resolutions and a bit lower at higher resolutions.

The four methods shown on the graphs in Figure 5 are: global thresholding, local thresholding, local thresholding with watershed, and Stardist. Global thresholding is a very basic method of segmentation, where a single global intensity value is calculated. For Otsu's method, the cutoff between foreground and background is calculated to maximise the inter-class variance between the two groups of pixels [9]. Due to bleed-through effects of neighbouring cells in the same trench, this method is unable to distinguish cells in the same trench so its performance is expectedly bad.

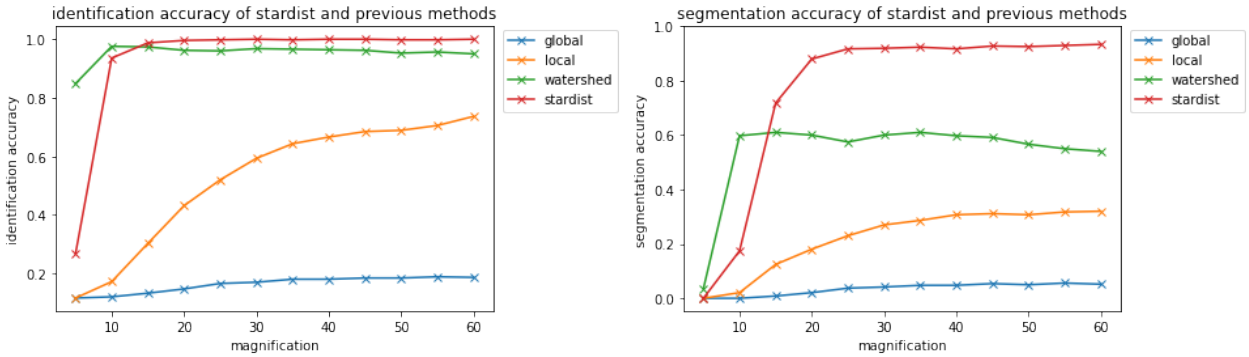

Figure 5: Experimental results

Local thresholding works in a similar way to global thresholding, except it calculates a separate threshold for each W×W window of pixels, so it can distinguish nearby cells more effectively. The method used for this experiment was Bernsen's method [10]. It performs much better than global thresholding but still has much room for improvement. One way to get this improvement is to use the watershed algorithm after the local threshold algorithm. Watershed is built on a topographical analogy of the pixel intensity map, whereby low intensity regions are 'flooded' from regional minima until different water sources meet, and then create intensity barriers at these meeting points [11]. This can help to further distinguish cells, and this leads to large improvements, especially in terms of identification accuracy. As expected, the pretrained neural network was able to outperform the other methods in most cases, apart from the very low magnification images. However, since the effect of blurring from the microscope would have most effect in these cases, we should be cautious reading too much into these results.

4.2 SyMBac for Fluorescence

Using SyMBac, fluorescence mother machine images were produced for a range of objective magnifications. The images were segmented using Stardist, and identification and IoU accuracy was calculated for the images, then averaged to produce Figure 6. The IoU is a bit lower than we would expect, especially for a fluorescence image, but this can be explained partially since SyMBac counts cells which are in the process of splitting as one all the way until they are fully separated, whereas Stardist separates them much earlier. However, this result does show that a model trained on the SyMBac data would probably be able to perform very well on the data, even down to resolutions as low as 25×.

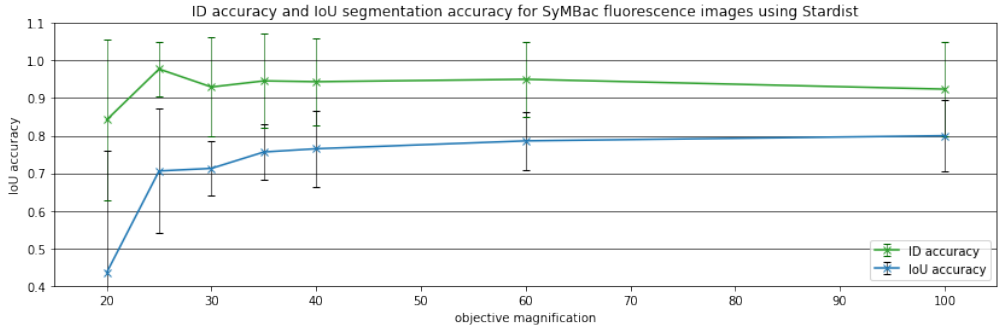

Figure 6: Segmentation accuracy (using IoU) and ID accuracy for Stardist on SyMBac images. Error bars show ±1 standard deviation.

5 Future plans

Following on from what has been done so far, the next steps in this project will be:

- to build on the results from section 4.2 by using Omnipose [12] to train a segmentation model for fluorescence images and document any improvements over using Stardist;
- to use SyMBac to generate phase-contrast instead of fluorescence images and train a model on this data, using Omnipose again;

- to collect some real data at various objective magnifications, since SyMBac works best when it has real images at that magnification to work with;
- to begin investigation on the impact of SR networks. This would include using basic upsampling using bicubic interpolation as a control and SRCNN [13] as a benchmark SR network, after which various architectures and techniques can be investigated to determine any performance improvements.

References

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