IIB Project Technical Milestone Report
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 used to be able to train neural networks for the segmentation task, 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 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 [16].

These experiments produce a lot of data in the form of images which then need to be analysed. Classification and segmentation are important steps in the analysis process. Classification is used to identify whether there is a cell in an image, which can be done on sections of an image as well as the whole [12]. Segmentation creates a mask out of the image, separating background from foreground and foreground objects from each other, and attempting to classify what object or section of the image individual pixels are part of [8].

Segmentation and classification algorithms perform better on higher resolution images than lower resolution images in general, but this creates an issue for the throughput of the experiment. A screening experiment might be tracking 10^5 linear colonies, but if each image must be taken at a high resolution to allow for good classification/segmentation performance, more images must be taken to cover every colony, and each individual colony will be imaged less frequently. This causes a three-way trade-off in these screening experiments, whereby picking two of spatial resolution (magnification of the image), temporal resolution (frequency of imaging of a particular colony), and data throughput (number of colonies in the experiment) to be high means the other must be lower.

2.2 Mother Machine

The mother machine is a microfluidic device which contains bacterial growth channels about one micron in diameter, 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 [14]. Each channel is about the width of the bacteria so as the

bacteria grow they push the other bacteria in front of them out of the trench, so there are only ever a few bacteria at one time. This means that there are none of the issues often associated with agar pad experiments, where after a certain amount of time the colony either grows too large to keep track of or it consumes all of the food supply and becomes dormant [13].

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.

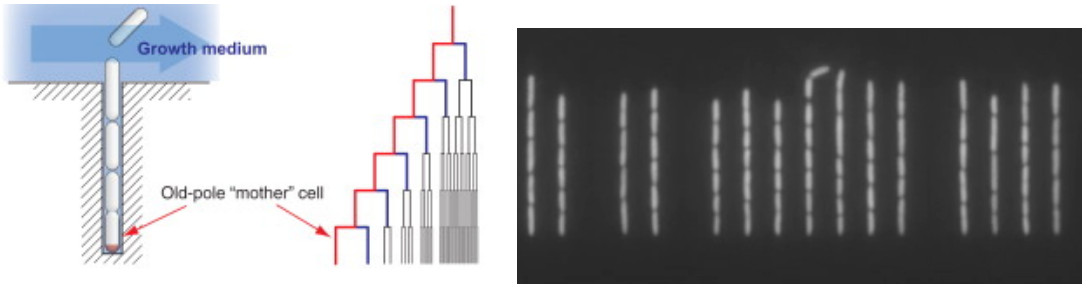

Figure 1: Left image shows a single mother machine trench and the lineage of the 'mother' cell [14], right image shows a typical image from a mother machine [14]

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 [6]. 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 [3]. 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 [7], 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 [15] to generate a similar image to what is observed in reality. This process is shown in figure 2 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.

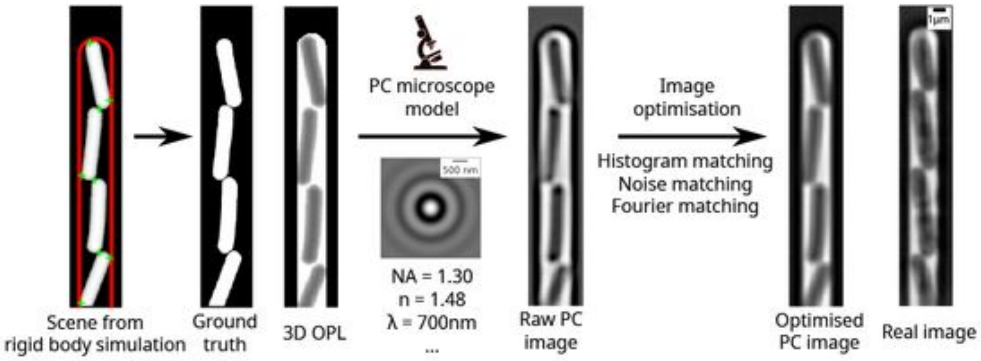

Figure 2: SyMBac image generation pipeline [7]

2.4 Imaging modalities

Two of the main imaging modalities for bacterial images are fluorescence and phase-contrast, shown in figure 3. 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 [6], so to take full advantage of low magnification images phase-contrast will need to be used.

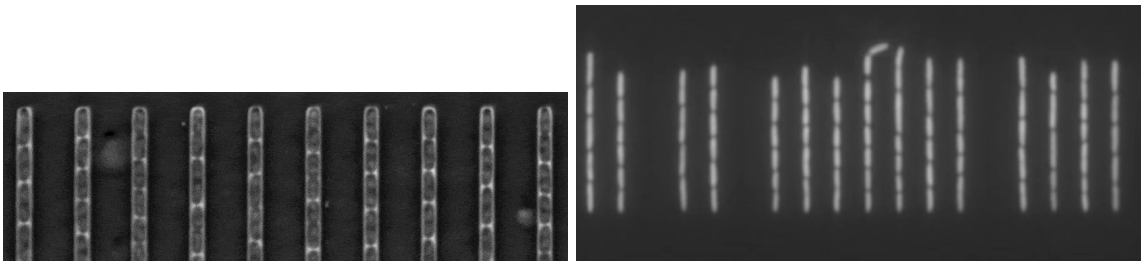

Figure 3: Left image shows a mother machine imaged with phase-contrast [9], right image shows a mother machine with fluorescence imaging [14]

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) or Jaccard index [11] 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 super-resolution algorithm can improve the relationship. Single image super-resolution 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 [17]. If using a network like this can introduce even a small super-resolving 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 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). This leaves a lot of room for cells that are incorrectly merged and incorrectly split to cancel out error wise but it still gives a good picture of how identification might be expected to behave. 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 centre of 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.

The four methods shown on the graphs in figure 4 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 [10]. 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.

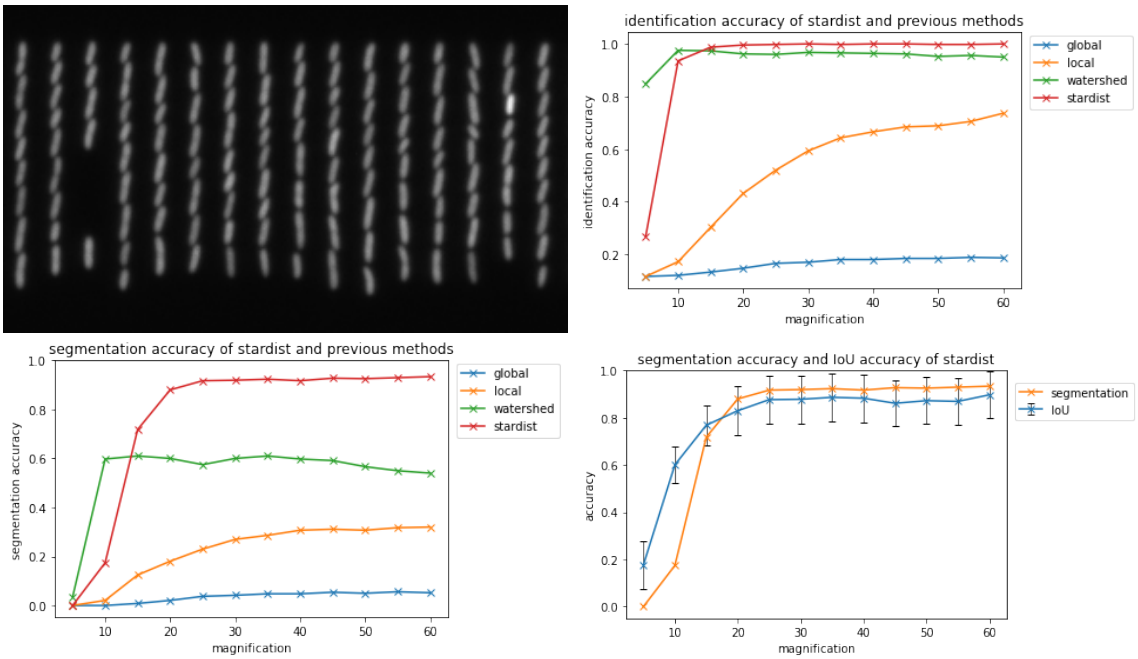

Figure 4: 1. top left image shows a section of the image used for this experiment, 2. top right image shows identification accuracy for the four identification methods, 3. bottom left image shows segmentation accuracy for the four segmentation methods, 4. bottom right shows segmentation accuracy calculated as before against segmentation accuracy calculated with IoU, along with error bars showing ±1 standard deviation

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 [1]. We can see 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 [2]. This can help to further distinguish cells that might be difficult to otherwise, and we can see that 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 5. 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 mitosis 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×.

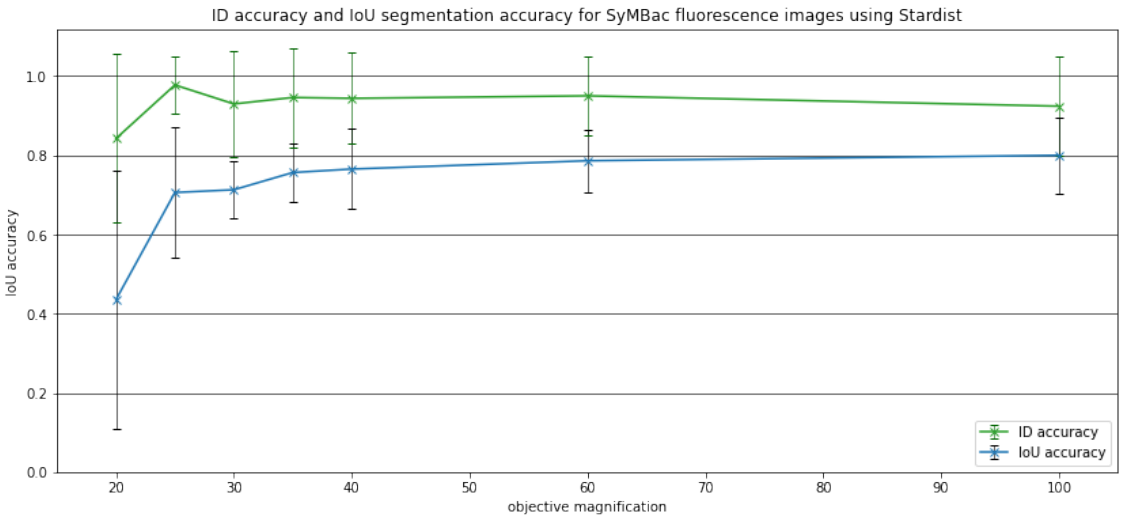

Figure 5

5 Future plans

Building on the results of the experiment in section 4.2, a neural network will be trained on the SyMBac-generated data using Omnipose [4], which is optimised for cells in mother machine images. This model can then be compared to Stardist to see the improvement offered by using SyMBac to train a network compared to a pretrained network which works generally on fluorescent shapes. Then the experiment can be repeated using phase-contrast images, and the results compared to the fluorescent experiment. Some minor tweaks may be made to SyMBac along the way as some bugs cropped up when very low resolution images were being generated. Once this is completed, investigations can begin on the impact of super-resolution networks. The first step will be to test images which have simply been upsampled, for example using bicubic interpolation, and use this as a control to gauge the impact of any super-resolution. The next step will be to train a version of SRCNN, which was one of the earlier super-resolution architectures [5], to use as a benchmark for other

attempts. From here, various other more recent architectures can be tested, to see whether significant gains can be made. If it is found that significant gains are expected, this can then be tested on real image data to see if the synthetic images serve as a good proxy for real data in this application.

References

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