Part I

Introduction

1 Medical Imaging

2 Sub-Diffraction-Limit Imaging

Imaging objects becomes more difficult as they get smaller because of the wavelength of light. Once two objects are separated by a distance of an order similar to that of the wavelength (λ) of the light used to view them, it is no longer possible to resolve these two objects apart, instead all that can be seen is a blur of the two objects together.

There have been several techniques developed for distinguishing objects apart on smaller and smaller scales. Many of these involve using different wavelengths of light. For example, instead of being limited by visible light, $\lambda \approx 5 \times 10^{-7}$m, x-ray radiation ($\lambda \approx 10^{-10}$m) or even electrons ($\lambda \approx 10^{-11}$m) can be used to resolve smaller scales in x-ray and electron microscopy respectively. These, however, have the issue that, because the smaller wavelengths imply higher energies, there is the danger of damaging the sample. When imaging biological samples, this can be unreasonable.

2.1 Image Manipulation

Other techniques employ different methods of actually capturing the image, or cleaver manipulation of the images that are produced, to get around the limitations of the diffraction problem.

For example the STORM method [Rust et al., 2006] uses a technique where the objects to be imaged are molecules of a fluorescent dye. The type of dye molecule used allows the fluorescence to be switched on and off, allowing some markers to be imaged separately to others, effectively increasing the distance between points. Once an image is captured, the point spread function (PSF) of the point is used to locate the single marker, the "on" markers are changed and the image retaken.

Even using exotic types of lenses to reduce or remove the problem of diffraction [Fang et al., 2005].

3 Benchmarking

Throughout the project, a set of files wil be used to test the algorithms that are developed, their correctness and effectiveness, speed and resource use. These files contain real data formated in the same way as would be expected for data given to the plugin in general use. The four files that will be used are detailed in table 3.

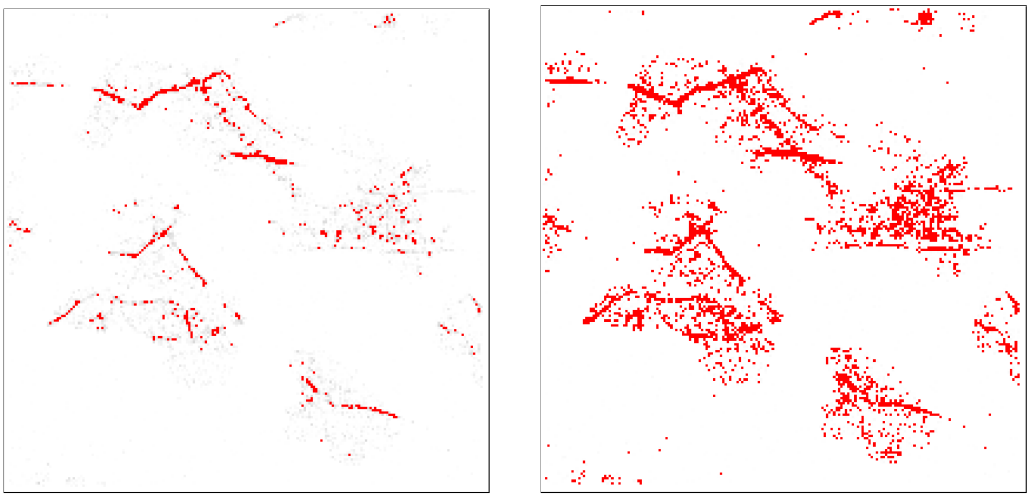
File Name	Size	No. of points
`palm-1.txt`	12 MiB	65572
`palm-2.txt`	6.4 MiB	36672
`palm-3.txt`	5.8 MiB	33342
`palm-3-small.txt`	176 KiB	1000

Note that `palm-3-small.txt` is a subset of `palm-3.txt` which is used for simply checking correctess of alogorithms. A summary of the columns that are included in the files, used and unused fields, is included in Appendix **??**.

4 Simple Grid Method

The simplest method for analysing the distribution of points is to use a regular grid of cells and place the points into the cells one at a time. Once all points have been added, the number of points per cell can be treated as a greyscale birghtness value. A thresholding filter can then be applied to remove the points that are isolated. The implementation to acheive this outputs a single pixel value for each of the cells of the grid. If written to a file, this can easily be viewed by treating the resulting file as an pnm image format.

Though the resolution of this method can be easily changed by altering the size of the cells, it performs badly when presented with data that is even slightly noisy. If the clusters themselves have a density that is not significantly above the background noise level, the thresholding step is prone to either exclude much of the real data, or to increase the size of the clusters by including too much noise. These two effects can be seen clearly in figure 1, where `palm-1.txt` was used with a cell size of 200.



(a) **(b)**

Figure 1: *Setting a low threshold, (a), means that many of the points in the clusters are lost. Setting it higher, (b), includes too many of the points deemed to be noise.*

References

[Fang et al., 2005] Fang, N., Lee, H., Sun, C., and Zhang, X. (2005). Sub–diffraction-limited optical imaging with a silver superlens. *Science*, 308(5721):534–537.

[Rust et al., 2006] Rust, M. J., Bates, M., and Zhuang, X. (2006). Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). *Nature methods*, 3(10):793–796.

A Data File Structure

The data files that are produced from the initial analysis of the images have a standard format.

1. Tab separated fields.
2. Single header line with names of fields.
3. One or more item of data, separated by newlines.

The columns that represent fields in the file are as follows.

Header	Meaning	Used?
`Channel Name`	Wavelength channel that was used to capture data. First value, I, is the incident wavelength of the light used to excite the dye and the second, E, is the wavelength emitted that was imaged.	no
`X`	x-coordinate of the point	no
`Y`	y-coordinate of the point	no
`Xc`	centered, normalised x-coordinate of point	yes
`Yc`	centered, normalised y-coordinate of point	yes
`Height`	the height of the fitted gaussian peak used to extract the point from the original image	not yet
Area	area of the point	not yet
`Width`	full width half maximum of the point	not yet
`Phi`	?	no
`Ax`	?	no
`BG`	?	no
`I`	?	no
`Frame`	?	no
`Length`	?	no
`Valid`	?	no
`Z`	?	no
`Zc`	?	no
`Photons`	?	no
`Lateral`	?	no
`Localisation`	?	no
`Accuracy`	?	no
`Xw`	?	no
`Yw`	?	no
`Xwc`	?	no
`Ywc`	?	no