

Focal plane selection in microscopic embryo images

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Abstract

The most critical quality property for an image is its focus. Without a good sharpness, the image will be meaningless for both human eyes and for computerised analysis. As the use of camera recordings increase around us, both in our daily life, for the purpose of documentation, communication, surveillance or for recreational purposes, so it also increases in the laboratory and in the science communities. More often, images are captured as part of the scientific process. Often as a method of documentation, but more and more also as a part of the analytical process itself. The number and complexity of available algorithms for image segmentation, computer vision or pattern recognition continues to grow and is likely to play a large role in how we handle data in the future. When we hand over more and more of medical surveillance and diagnostic tasks to automation, it is crucial that we can rely on the accuracy of these automatic procedures. One way to ensure a level of quality, and to make sure we do not waste time trying to analyse material of poor standard, is to make sure that the images we introduce to an analytical pipeline hold a high enough quality. Image quality may depend on a number of properties such as focus, level of noise and a number of spherical and chromatic aberrations. Here we propose a simple, adjustable software framework for detection of image focal planes, making it possible to extract image sections for further analysis.

Categories and Subject Descriptors (according to ACM CCS): I.4.8 [Image Processing and Computer Vision]: scene analysis—Depth cues

1. Introduction

Many algorithms for automatic focus rely on computation of the power spectrum[1]. In cameras with moving lenses, it is also possible to adjust the focus based on the image contrast, a method often referred to as contrast detection autofocus. Several images are then captured in sequence, while searching for a local maxima of the image contrast or the gradient of the image contrast. This is usually not done for the entire image but, for a selected area of interest. Several autofocus algorithms have also been evaluated for microscopy[2], [3]. In optical microscopy[4], where the depth of field is usually very short, and when studying human embryos, the entire embryo is rarely in focus at one optical setting. Instead, the user may be required to adjust the focus back and forth in order to get a complete view of the sample. Here, we implement a variety of the contrast detection, adapt it to Hoffman Modulation Contrast microscopy[5], and use it as a filter to

select regions of interest within an already captured image of a human embryo.

2. Materials and methods

2.1. Sample preparation

Human tissue sample material was anonymously donated by patients and the project has been approved by Coventry Research Ethics Committee (04/Q2802/26) and the Human Fertilisation and Embryology Authority (R0155). Fresh embryos unsuitable for transfer or cryopreservation and thawed embryos no longer required for treatment were cultured using Medicult media (Origio, Redhill, UK) for up to 7 days and incubated in 37°C 5%CO₂ in air. Embryos were otherwise untreated or undisturbed during the imaging process.

2.2. Image capture

During image capture, embryos were cultured in an Embryoscope (Fertilitech, Copenhagen, Denmark), and images were captured at up to 7 focal depths and 5 recordings per hour using a Hoffman Modulation Contrast optical set-up, with a 635nm LED as light source. Image capture ran for up to 95 hours.

2.3. Algorithms

All image processing was done using standard libraries from Matlab. Prior to handling, captured images, $I(x,y)$, were filtered with a Gaussian filter to remove speckle noise. For most images, the gaussian filter had a size of 5x5 pixels, and a standard deviation of 1. To detect the image contrast variation, a Laplacian filter,

$$L(I) = \frac{\nabla^2 I}{4} = \frac{1}{4} \cdot \left(\frac{d^2 I}{dx^2} + \frac{d^2 I}{dy^2} \right) \quad (1)$$

was applied. Note that due to the asymmetrical nature of Hoffman Modulation Contrast imaging[6], the symmetrical Laplacian was chosen, rather than the direction-dependant gradient. To detect areas of high sharpness, an H-maxima transform, H , was applied to the result $L(I)$, using an 8-connected neighbourhood, and suppressing maxima lower than 15% of the image maximum. The recovered maxima were then extended using a close transform with a 7 pixel diameter circular structure element, and the resulting image was thresholded and converted to a binary mask, M . Holes were removed from the mask using a filling function, and the mask was then used to extract the corresponding region from the original image, I .

3. Results

Figure 1 shows the extraction of regions in focus, based on the contrast detection algorithm, from an image of a human embryo. The embryo is in its blastocyst stage and has the shape of a hollow sphere, with cells covering its walls. Due to the varying distribution of cells, the spherical shell of the embryo may vary in tissue content. The stack moves from slightly above the horizontal embryo central plane and downwards until it reaches the embryo base, where structures on bottom of the embryo container are also encountered, and can be seen outside the embryo circumference.

4. Discussion

A reliable and robust algorithm for focus level detection may be useful not only as a pre-processing step for image analysis, but also as method for automatic focus of microscope hardware[7], when the images are being captured during

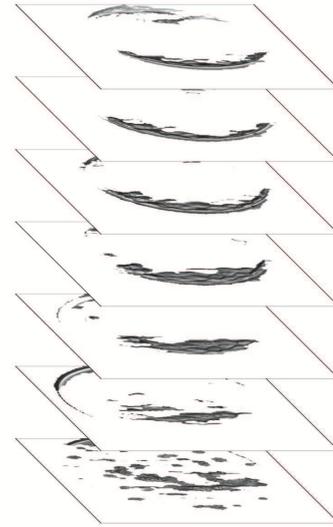


Figure 1: A human embryo, captured at seven different focal planes: extraction of regions of interest based on image contrast.

long periods of time, when the capturing process is automatic, or when the microscope is placed in a climate chamber, as is often the case in time-lapse sequencing. Here we present the construction of a simple algorithm for focal plane detection in Hoffman Modulation Contrast microscopic images. We intend to use this algorithm to facilitate future analysis of these images, by selecting images, or regions of images, as appropriate for segmentation, pattern recognition or other computerised analysis. With this type of quality detection as a basis, we hope to be able to quantify the image sharpness, thus being able to exclude regions of an image, or whole images, from further treatment, if their quality is deemed as being too poor. For the future, we propose to generalise the quality detection to cover several types of images. Only when more than one image set can be evaluated against the same standardised criteria can we have a true comparison of the image quality.

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6. References

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