Methods to improve the understanding of microscopic embryo data sets using image analysis

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Abstract

In this work we propose a framework for visualisation of semi-automatically segmented microscopic images of human embryos. A large part of the education of biologists consists of learning to interpret the output from a variety of analytical methods and medical imaging modalities, which can be more or less abstract in nature. Even in visual microscopy, the optical setup and the different ways to increase contrast between the sample and background produce image artefacts which have to be taken into account when interpreting the image. In *in vitro* fertilisation, the correct evaluation of the quality of the embryo is crucial for successful future development of the implanted foetus. Embryos are selected for transfer based on a number of characteristics, such as blastomere symmetry, degree of fragmentation and number and size of blastomeres. Traditionally, this evaluation has to a large extent been done by manual observation through visual microscopy, and obtaining the necessary expertise takes years of training. Here we show how the output from different analytical methods may be combined and how creative visualisation and improved user interaction with large data sets may improve the understanding of the sample under study. We show how existing computer-aided tools can be used in embryo selection and discuss automation as a way to quantify the subjective bias of manual embryo selection. We use data from human embryos as a case study, but the methods may be applied to any type of biological or microscopic material.

Keywords: Human-computer interaction, medical visualisation, embryology, image segmentation, pattern recognition, computer-aided diagnosis

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1 Introduction

In several standard computer applications, computer vision algorithms are readily available, and in many fields of research there is a vast collection of tools for imaging and plotting, allowing the user to produce different visualisations of the data. In many cases these tools allow for multidimensional plotting, rescaling, the application of cross sections, the use of transfer functions, the addition of annotations, and the sorting and rescaling of data through various gates or histogram-based offsets. In the area of medicine, where the output from the method of detection is not visualised *a priori*, such as MRI, techniques for presenting the data have evolved for some time. Also, novel technique has made it possible to gather more and more data simultaneously in several dimensions and modalities. With this trend, the understanding of this data through a limited set of cross-sections has also become more difficult, and the interpretation of medical images has come to require more and more time in the education of the physician or the biologist. In microscopy, where data is directly visualised in two dimensions, little efforts have been done to present this data in any other way. Although the use of images from microscopic data has increased, the presentation tools available are usually two dimensional in nature, and lack interactivity. In microscopy, the same sample may look very different under different optical set-ups and the interpretation of microscopic images requires a high level of expertise. The aim of this paper is to give some insight into how standard computer vision techniques can be applied to microscopic data and how creative visualisation can help in the interpretation. We discuss how the physical process of image capture may influence the final image, and how knowledge of this process in some cases can be used to further improve the computer analysis. It is our hope that this article may further improve the microscopists understanding of the world of computer vision, and how they may use it for their benefit. We also believe that this article may be of interest to the computer scientists working in the field of computer vision, developing or studying algorithms for automated image analysis in the medical field.

The background section of this paper (2) first gives a short overview of a few in embryology commonly used, yet very different microscopic techniques (2.1), then gives an overview of the embryo selection process (2.2), time-lapse microscopy (2.3) and segmentation (2.4) and how these can be applied to embryology. Section 3 discribes the methods used and in the results section (4) we describe data extraction using two separate imaging techniques; HMC and confocal imaging. We apply three different methods of analysis to the data sets, using successively more refined methods of segmenting the image. Last, we discuss future work where we intend to extend the knowledge of human embryo development by combining information from several techniques. The beginning of each result section briefly describes the methods used.

2 Background and related work

2.1 Microscopic Imaging

Microscopic techniques can roughly be divided into quantitative or non-quantitative imaging, and destructive or non-destructive techniques. Non-descructive techniques is preferrable in many cases, where there is a need to keep interference with the sample at a minimum. In *in vitro* fertilisation (IVF), the sample under observation cannot be manipulated or disturbed in any way, but must be observed "as is", if it is to be used for implantation. With a few exceptions, most quantitative imaging is in some way destructive, so for research purposes, destructive techniques can sometimes be desirable.

The two destructive techniques most commonly used in the study of embryos are fluorescence microscopy [1] and confocal microscopy [2]. Microscopical techniques which can be counted as non-destructive include bright- and dark-field microscopy, phase-contrast microscopy (PC) [3], Hoffman Modulation Contrast (HMC) microscopy [4], Differential Interference Contrast (DIC) [5] microscopy and digital holography (DH) [6], [7]. For uses in cellular biology, see [8], [9]. Of these, bright- and dark-field microscopy produces an image of the amplitude of the transmitted (or reflected) light, as we are used to seeing it. However, cellular material is usually highly transparent, and for such objects, we can get a better sample-to-background contrast, if we study the phase of light instead of the amplitude. PC microscopy and HMC imaging are techniques where the phase information of diffracted light is optically converted to amplitude information. Microscopic imaging techniques such as these are very good for visualisation, but cannot be directly translated to quantitative data. DIC and DH are techniques where the sample phase-shift is imaged directly, and can therefore count as quantitative imaging techniques.

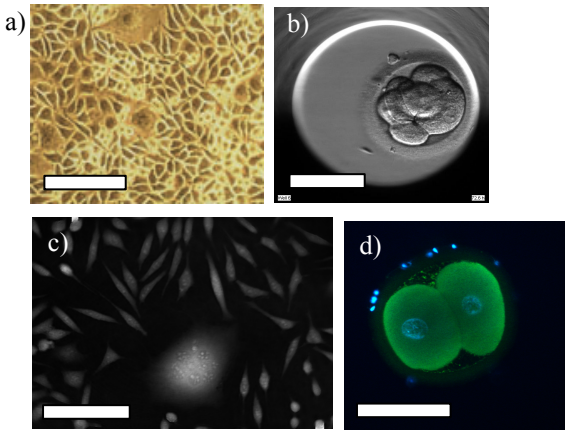


Figure 1: Examples of cellular images with common microscopic techniques. Phase contrast image of L-929 mouse fibroblast (a), HMC image of a human embryo (b), Digital holographic image (a) of mouse fibroblast L-929 (c) and Confocal image of a human embryo (d). Scale bars: 100µm.

2.2 The Embryo selection process

When selecting an embryo suitable for implantation, the embryologist may look at a number of criteria, such as pronuclear appearance and orientation [10], [11], number, size, shape of blastomeres, degree of fragmentation [12], degree of blastocoelic expansion, cellular composition and compactness of the inner cell mass and trophectoderm [13]. Discussions concerning the relevance of embryo morphology in quality assessment exists [14], but it is likely that embryo morphology will continue to play a large part in IVF embryo evaluation.

Traditionally, embryos have been studied using a microscope (commonly HMC) only at certain time points during the course of their development. It has been shown in time-lapse studies that the timing of key occurrences within the embryo can vary greatly between embryos that have similar morphologic appearance at the conclusion of the recording period, and correlation has been shown between the timing of key developmental events and embryo quality [15]. Some features, such as embryo fragmentation, which is usually connected with poor prognosis, have shown a high degree of variation in time-lapse studies [16], and embryos have also shown the capacity to reabsorb fragments [17]. It may be that the spatial and temporal pattern of fragmentation has higher impact on embryo quality than merely the presence of fragmentation [16], [18]. Such indication, in combination with new possibilities for time-lapse imaging of human embryos for an extended period of time with less negative effects to their health, makes it likely that the use of time-lapse recordings is going to increase in the future.

2.3 Time Lapse Microscopy

Time-lapse microscopy is the recording of an image sequence at intervals during a continuous period of time [17]. The length of the period and the time between intervals is determined as a trade-off between temporal resolution and potential sample deterioration. In fluorescence microscopy and confocal microscopy, which both count as destructive imaging techniques, the sample is usually fixed and no longer evolving, and it is rather fluorophore bleaching than potential damage to the sample itself, which limits sampling frequency. In non-destructive light microscopy on the other hand, imaging of live samples may be possible over several days, or even weeks. Long-term time-lapse imaging does not only require that the imaging technique causes low stress to the specimen. It also requires that the sample can be kept undisturbed in a favourable atmosphere for an extended period of time. Novel construction of incubators and cultivation chambers has recently made it possible to monitor embryos during the course of several days, without any registered severe consequences to their health.

There are difficulties other than the pure technical when combining automatic long term time-lapse imaging and microscopy. When examining embryos under the microscope, the three dimensional structure is very much of interest. In a traditional, manually handled microscope, much information can be gained by making proper use of the microscope controls, moving the sample around, scanning the focus, adjusting strength of illumination or making use of various filters and apertures in order to scan the three dimensional object in real time. In an automated time-lapse set-up, the possibility to manipulate optics is reduced when the optical set-up must incorporate a climate chamber to accommodate the living cellular material. If the microscope is instead meant to sit inside an incubator or other external chamber, the possibility to manipulate the optics is equally reduced, either because its operation requires the doors of the chamber to be opened, or because the optics is again shielded, to protect it from the high humidity of the chamber. In many time-lapse set-ups, the possibility to adjust image quality in real time has vanished, and the biologist is now limited to study the images some time after they are captured. This calls for new techniques to visualise this already captured data in creative ways, and possibly to regain some of the interactivity which was lost to the user in the process. Also, with the increased use of cameras and automated microscopic equipment, the amount of image data obtained has increased. Here is a possibility for more analytical material, but lots of data also means that time has to be spent interpreting the data. It would be beneficial to automatically point out features of interest in order to decrease the user workload.

2.4 Segmentation

There exists several examples of the segmentation of microscopic images in general [19]–[26], and segmentation of embryos in particular [27]–[31], but so far few attempts have been made to apply fully- or semi-automatic image treatment to the problem of selecting embryos. There are a number of potential benefits of automated image processing: Sampling time can be used for image processing, and the large amounts of stored image data available after capture will make the images available for further analysis and for validation by other experts. Automatic procedures will make the system less subjective, and the evaluation process will be more transparent, given that the automation process itself is made transparent. However, the differences between a standard camera image and microscopic images have a number of pitfalls, when applying standard image processing algorithms.

3 Materials and methods

The experimental section is divided into three parts. In the first, we apply a number of simple full image field transformations to a set of embryo images, to illustrate a common problem when working with the entire image. In the second, we restrict the region of analysis to regions of interest, and show how analysis of non-quantitative data still can give useful information. In the third and final section we illustrate how an embryo can be visualised in three dimensions given enough scans and a complete segmentation.

3.1 Asymmetric Imaging

Images of a human embryo at 72.6h after fertilisation were captured with the Embryoscope® system (Fertilitech, Copenhagen, Denmark), using HMC imaging at 635 nm. The raw images were plotted using Matlab, and a Canny edge and a one dimensional gradient was computed.

3.2 Embryo activity

The images in Figure 4 and 5 were captured in a 90h time-lapse series using the Embryoscope® system (Fertilitech, Copenhagen, Denmark), with a 0.2h interval between pictures. The embryos were mounted in wells in an EmbryoSlide® (Fertilitech, Copenhagen, Denmark) (Figure 3), one embryo per well, and the imaging of both wells (3 and 6 respectively) was done simultaneously, using a 635 nm LED. Both embryos are from the same patient. Three circular regions of interest were selected per image, one representing the total image field of the well (A), one selecting the body of the embryo within the zona pellucida (B), and one selecting the embryo centre at half the diameter of the embryo outline B (C) (Figure 4). The regions of interest have been manually

chosen from one image slide, and then applied to the rest of the image series. The variance of each region of interest was computed and plotted for each image in the series. The total image series of 448 images spans from 4.7h (4-6 blastomere stage) to 94.1h of development, at a focal plane located approximately half-way through the embryo.

3.3 Three dimensional Visualisation

Embryo nuclei were marked with DAPI, fixed on microscope slides and images were captured using the LSM510 confocal system (Zeiss, Hertfordshire, UK), using 400X magnification. The scans were captured with 1 µm between scans. The results were segmented using a combination of region growing segmentation filters (Neighbourhood Connected Thresholding and Confidence Connected Thresholding) and Watershed segmentation [32]. The segmented outlines where plotted as point clouds, and a Delauney triangulation was used to compute the surfaces. The resulting bodies were then put together in a three dimensional representation of the complete embryo. The complete experimental process has been described in [33].

4 Results

4.1 Asymmetric Imaging

In HMC imaging, light is passed through a pair of off-axis slits, converting gradients in sample optical path to bands of light and dark appearance, depending on the spatial sample direction (Figure 1b (raw image) and 2). Here, the slit pair is positioned so that image gradients appear symmetrical around a horizontal axis. This effect is most apparent when plotting the image as an isocontour (Figure 2a). When performing any kind of non-symmetrical image operations, this break in symmetry must be taken into account. Compare for instance the output of a derivative of the raw image taken along the horizontal and vertical axis, respectively (Figure 2c-d). In computer vision, a common approach is the application of an edge finding filter. Here too, the anti-symmetry of the HMC must be taken into account, because the angle of incident light will produce a shift in edges in the upper half of the image compared to the lower half. In Figure 2b, a Canny edge detection filter has been applied to the raw image. It is clear that the filter may find edges on both sides of the lighter and darker bands, resulting in an uncertainty when trying to determine the exact border of the embryo, or of a single embryo blastomere. However, since the direction of light depends on the azimuthal angle between splits and sample, this effect can be reduced by rotating the sample around a vertical axis, and combining information from several images along the rotation. Note also that the direction of change in optical path length is given by the polarity of the artefacts: Compare for instance the bright upper half of the sample well with the dark upper edges of the blastomeres themselves.

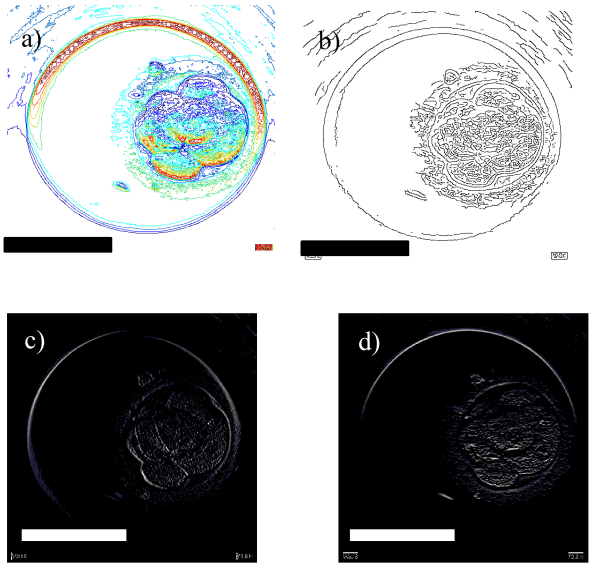


Figure 2: Contour plot of the raw HMC image given in fig 1b (a). Canny edge detector applied (b). Gradient of raw image taken in X (c) and Y (d) direction, respectively.

4.2 Embryo activity

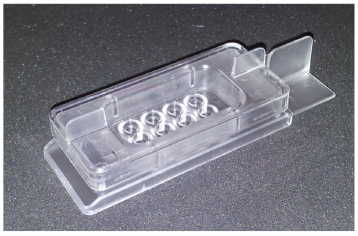


Figure 3: Cultivation slide used for embryos.

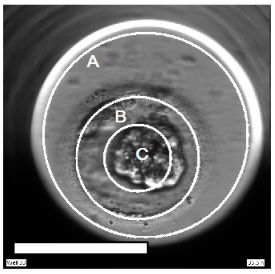


Figure 4: Regions of interest are a well (A), an embryo (B), and an internal region (C) of the embryo. The region C has been taken to be a region with centre equal to that of region B, but with half the radius. Scale bar: 100µm.

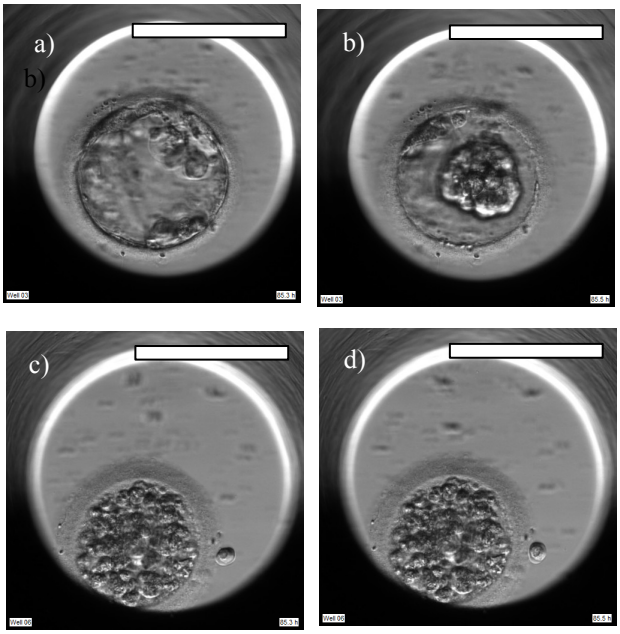
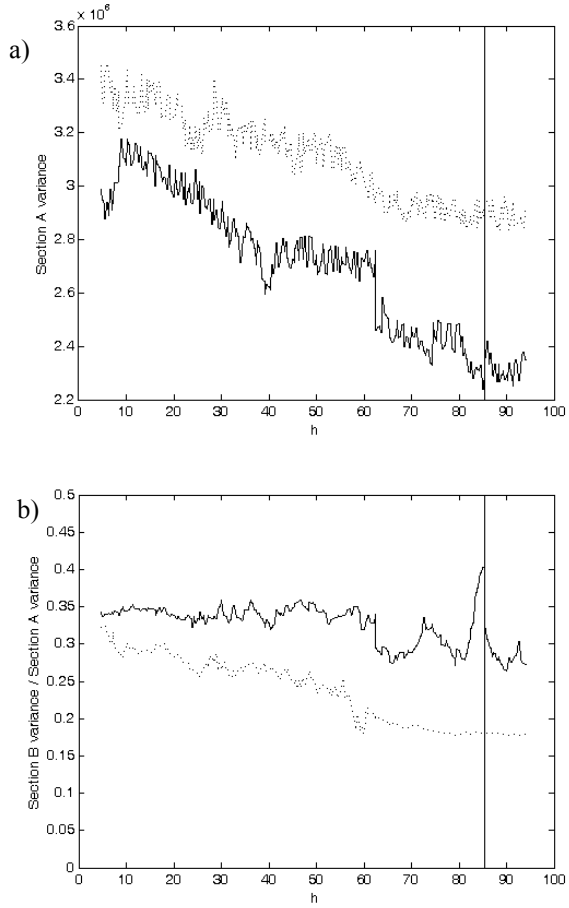


Figure 5: The embryo of well 3 at 85.3h (a) and 85.5h (b). The blastocoel starting to form. The embryo of well 6 at 85.3h (c) and 85.5h (d). Heavy fragmentation is visible, and the embryo activity is low. Scale bar: 100 µm.



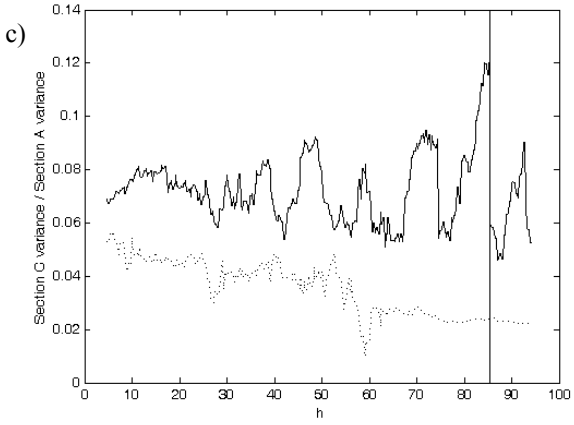


Figure 6: Variance (a) of region A. Arbitrary units. Image variance of region B (b) and region C (c) relative to mean of region A. Line represents position of example images (Figure 5). Solid: well 3, dashed: well 6.

The first embryo in well 3 experiences several cell divisions during the first hours of the series, after which it forms its first indication of a blastocoel at approximately 44.7h. After this, the embryo undergoes a series of morphological changes where it reverts back and forth between a blastocyst and a tight central cell structure (Figure 5). These changes are clearly reflected in the image amplitude and variance (Figure 6, solid).

The second embryo in well 6 experiences a reduction in division activity after approximately 34h, and clearly suffers from heavy fragmentation from image 54h and forward (Figure 5). Within the region of interest of the embryo, this is shown as a decreased image intensity, as well as a reduced image variance, when compared to the first embryo, even though for the image as a whole, the conditions are reversed. The effects are even more marked when the more restricted region of interest C is chosen (Figure 6, dashed).

4.3 Three dimensional Visualisation

Here we show that microscopic data from confocal images can generate enough data to form the basis for a three dimensional plotting with very little undersampling artefacts (Figure 7). The position and relative size of the blastomere nuclei are readily calculated. Note how the flattening of the embryo (a physical effect of being sandwiched between microscope slides) becomes apparent when applying a side view (Figure 7b).

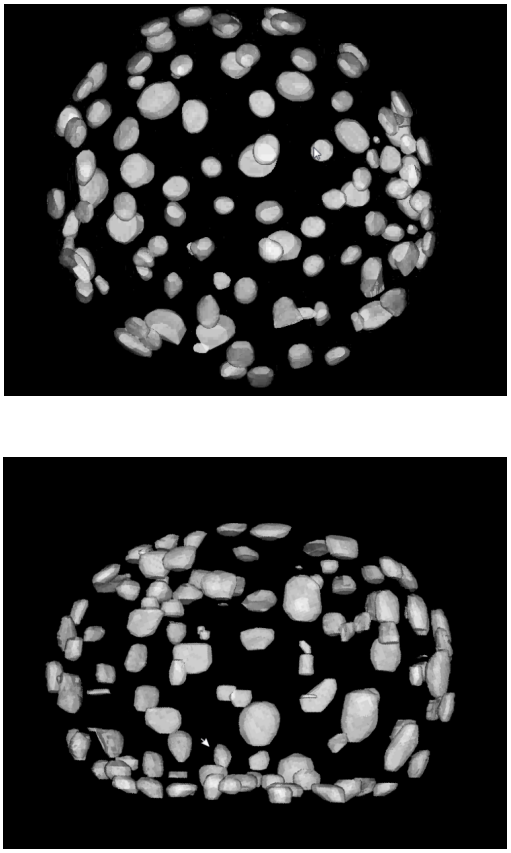


Figure 7: 3D rendering of the segmented data from a confocal image stack. Top view (a) and side view (b) of an embryo in blastocyst stage. The shapes shown are embryo nuclei (121 in total), spread along the inner wall of the blastocoel. The flattening of the embryo is due to the deformation caused by the imaging process. Blastocyst diameter is approximately 120 μm.

5 Conclusion and future work

Here we have shown how existing methods for image analysis may be combined to extract additional data from embryological data sets, and how computer analysis may be used to quantify results. Also, using 3D plotting, not only can we get a much more intuitive understanding of the embryo structure and the positioning of blastomeres relative to each other – it is also possible to get a measurement of cellular or nuclear volume, which is not possible with a single scan. With a three dimensional display, it is possible to view the sample from different directions, thus getting a clearer view of its spatial layout, and gaining a better interactivity with the sample. Here we show that the methods for a complete analytical chain from raw image to three dimensional vector plotting exists, and for future work we intend to put these methods together into a working one-piece semi-automatic framework for embryo evaluation, simulation and visualisation. Clearly, the accuracy of the 3D model depends on the amount of available data, the xy-resolution and the number of scans in z-direction. Confocal microscopy has been chosen here because the low depth of field allows us to separate the signal between images in the stack, thus obtaining cleaner data. Optical sectioning is also possible in some non-destructive techniques, in particular in HMC which has a relatively low depth of field compared to other types of light microscopy. If a successful segmentation of this type of image could be achieved, it would open new doors in the area of embryology, both for clinical purpose, but also for the understanding of early human development. An improved embryo selection can in turn result in a greater number of successful implantations, less need for multiple embryo transfer, which will in turn increase the chance of survival for the foetus and reduce the risk to both the foetus and the mother.

A computerised model of an embryo is useful for embryologists for training purposes, and would also in many ways be of great help when understanding the three dimensional dynamics of the embryological content, and may bring further insight into the early stages of human embryo formation. In the future we intend to further investigate the possibilities to extract data from microscopic images, in particular focusing on the non-destructive modalities, and using other techniques such as confocal microscopy as an endpoint and method for comparison.

6 Acknowledgements

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