

High-throughput high-resolution image analysis of the trypanosome cell cycle

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A large proportion of biological research is dependent on the acquisition and interpretation of images. These images represent an enormous intellectual capital that is substantially underutilised if they are not captured, stored and analysed as part of an integrated research workflow. Moreover, such a workflow has become increasingly dependent on the quantitative analysis of large numbers of images [1]. As life science research evolves toward high-throughput biology such a workflow is fast becoming an integral part of experimental design. This evolution is fuelling the rapid development of automated microscopes and is promoting increased integration and cross disciplinary collaboration between life and computer scientists [2]. Here we present an interdisciplinary research on development and application of image processing methods for high-throughput analysis of the cell cycle of *Trypanosoma brucei*.

T. brucei is a unicellular parasite that causes a devastating disease across sub-Saharan Africa, affecting both the indigenous human populations and the livestock on which they depend. The parasite has two DNA containing organelles, a single nucleus and a mass of mitochondrial DNA called the kinetoplast (Figure 1(a)). Both of these organelles are readily imaged by staining with a DNA binding fluorescent marker (Figure 1(b) 1(c)). In the *T. brucei* cell cycle the kinetoplast duplicates first shortly followed by the nucleus. This temporal separation enables determination of the stage of each cell cycle by imaging and classifying the nucleus and kinetoplast numbers. Interrogation of mutant phenotypes and detailed study of the *T. brucei* cell cycle is critically dependant on this analysis. However, making these measurements in a quantitative and statistically significant manner is prohibitively time consuming hence there is a pressing need for automated image analysis methods developed.

The proposed image processing work-flow for trypanosome cell detection and analysis consists of the following procedures:

- segmentation:

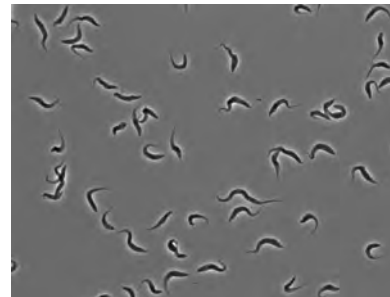
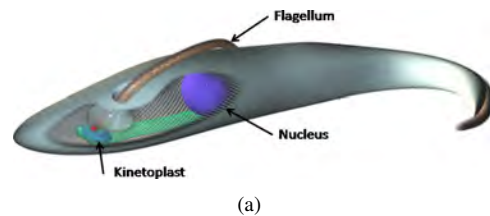


Fig. 1. a) A computer generated model based on electron microscopy of the *T. brucei* procyclic form [3], imaging of trypanosome cells: b) phase channel (trypanosome cells), c) DAPI channel (nuclei and kinetoplasts).

- cells, nuclei and kinetoplasts detection,
- removing cells that touch the image border,
- removing touching cells,

- analysis and classification:
 - cell centreline determination,
 - measuring the distance between nucleus, kinetoplasts and cell ends,
 - cell classification based on number of nuclei and kinetoplasts.

The segmentation of the cell body, nuclei and kinetoplasts is based on mathematical morphology methods. Cells are identified based on a shape compactness measure (Figure 2(a)). To ensure accurate classification, segmented cells that touch the image border or cells which overlap with other cells are ignored. Following segmentation of the cell body, the nuclei and kinetoplasts are identified, segmented and discriminated into two classes via k-means clustering of object area (Figure 2(b)). Individual *T. brucei* cells are then classified based on number of nuclei and kinetoplasts contained within the boundary of the segmented cell body (Figure 2(c)). The relative positions of the nuclei and kinetoplasts within the cell body. The following number of additional parameters are then determined (Figure 2(d) 2(e)):

- For each cell: Number of kinetoplasts and nuclei identified within the cell boundary, (x,y) coordinates of cell centroid, area and perimeter of cell, total intensity of cell pixel, length of central line for cell.
- For each nucleus/kinetoplast: (x,y) coordinates of each nucleus/kinetoplast centroid, area and perimeter of each nucleus/kinetoplast, total integrated fluorescence intensity of each nucleus/kinetoplast, distance from each object centroid to the posterior end of the cell end along the cell centreline.

In summary, we have proposed and developed high-throughput image analysis algorithms which facilitate the extraction of biologically meaningful information from simple microscopic images. These algorithms improve the accuracy and speed with which this information can be captured, while simultaneously reducing subjectivity and user error. This automation enables a whole range of biological experiments which would otherwise impossible due to intensive user interaction. Current work includes the validation of the measurements by comparison to manual analysis. Our goal is utilise these algorithms to obtain a more complete understanding of *T. brucei* cell cycle and motility, in order to postulate new ways to manage and treat the disease.

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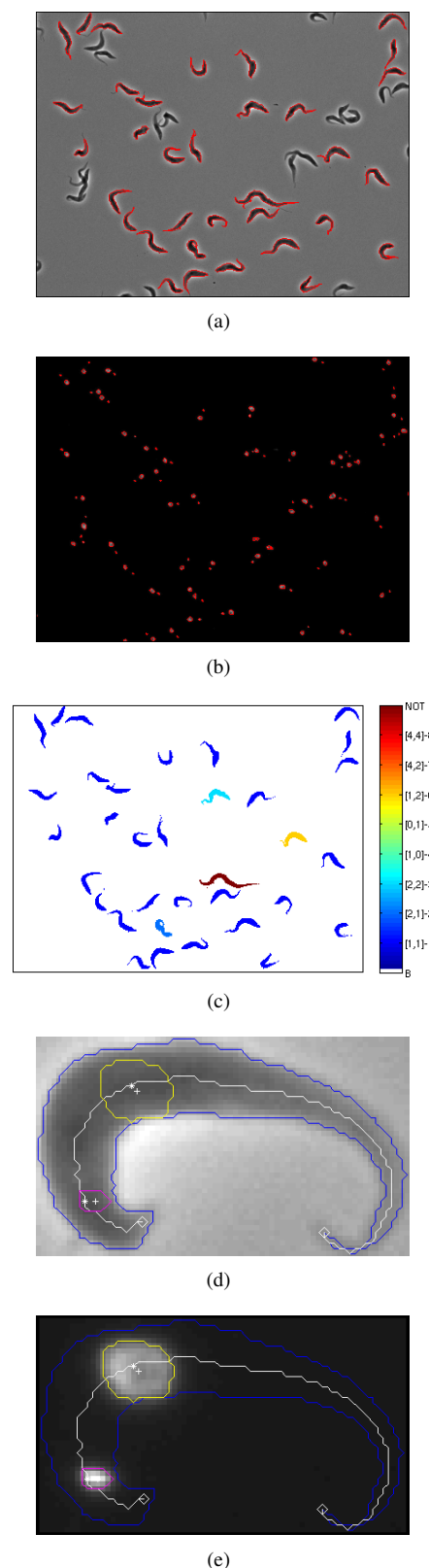


Fig. 2. Trypanosome cells, nuclei and kinetoplasts segmentation, analysis and classification: segmented cells after removing the cells that touch the image border and touching each other (a) and segmented nuclei and kinetoplasts (b) all outline in red, colour-indexed representation of cell classification (c). Measurement of spatial relations between cell, nuclei and kinetoplasts. All required positions are overlay on top of the phase (d) and DAPI (e) channel: cell border (in blue), cell centreline (in white), nuclei border (in yellow), kinetoplasts border (in red), nuclei, kinetoplasts centroid (as white cross), centroid's closest point on centre line (as white star), cell ends (as white rhombus).