Determination of structural parameters of mitochondrial DNA of *Trypanosoma cruzi*: a multidisciplinary fluorescence approach

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Abstract

Chagas disease is a life-threatening neglected disease that affects millions of people in Latin American countries. Its etiological agent, the *Trypanosoma cruzi*, has a unique structure, called kinetoplast, that harbor mitochondrial DNA (kDNA) organized in a peculiar arrangement of circular DNA molecules. This structure is very dynamic during the parasite life cycle exhibiting different shape and volume during the infective and non-infective stages of the protozoan. Because it unique characteristics, the kinetoplast is considered a potential target for drug development. The aim of the present work is to develop an image analysis tool to process confocal microscopy data in order to overcome the challenge of a structural characterization of *T. cruzi* kinetoplast. Confocal microscopy has a great advantage compared to other techniques because it allows cell observation without severe pretreatment like sample dehydration. In this multidisciplinary approach, 3D confocal images of kDNA labeled with the fluorescent stain DAPI were subjected to an image analysis tool that determines the shape of this structure and its total volume. Three dimensional reconstructed images were also produced allowing a unique visualization of this structured DNA. For validation of our image analysis tool and our numerical results, we compared the total volume determined at different stages of T. cruzi with a well-established technique, the transmission electron microscopy (TEM). Our data suggest that the dehydration step during the processing of sample for TEM potentially reduces the total volume of kinetoplast. Our results also indicate that confocal microscopy has the potential to unveil structural detail with unprecedented precision.

Keywords: *Trypanosoma cruzi*, kinetoplast, Chagas disease, confocal microscopy.

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