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ARTIGO DE OPINIÃO

Confocal fluorescence microscopy: a powerful tool in the study of Chagas' disease

Microscopia confocal de fluorescência: uma ferramenta poderosa no estudo da doença de Chagas

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Abstract *Confocal scanning fluorescence microscopy has become widely used in cell biology and pathology. In conjunction with monoclonal antibodies it may turn out to be a powerful diagnostic tool that also enables detailed studies of tissue forms of* Trypanosoma cruzi.

Key-words: Trypanosoma cruzi. *Chagas' disease. Confocal microscopy.*

Resumo *A microscopia confocal por varredura a laser vem se tornando extremamente útil na biologia celular e patologia. Com o uso de anticorpos monoclonais, pode ser uma poderosa ferramenta de diagnóstico assim como para estudos detalhados das diferentes formas do* Trypanosoma cruzi *em vários tecidos infectados.*

Palavras-chaves: Trypanosoma cruzi. *Doença de Chagas. Microscopia confocal.*

Confocal fluorescence microscopy has become a powerful tool that has enabled a tremendous improvement in morphology and cell biology studies[3 5]. Its potential application in the studies of Chagas' disease has recently been demonstrated by several publications using both *in vitro* samples[1 2 6] and also parasitized tissues from infected animals[7] or even chagasic patients[4].

The fundamental property of confocal systems that allow optical sectioning through the sample associated with image digitization are the basis of the potential of this technique. Very informative images regarding the state of parasite differentiation as well as a structural relationship with the host cell tissue in particular in the myocardium can be obtained by a combination of immunostaining with stage-specific monoclonal antibodies[1] and DNA probes such as DAPI (4',6'-diamino-2-phenylindole, dilactate) that intensely label the parasites' nuclei and kinetoplasts. Also, most confocal systems allow simultaneous transmitted image acquisition that in combination with either phase contrast or Nomarski differential interference contrast (DIC) can also aid the unequivocal identification of *Trypanosoma cruzi* amastigote as well as trypomastigote forms within the myocardial fibers.

With very simple procedures that are routinely used in pathology laboratories, paraffin-embedded specimens can be retrospectively examined[4] using standard immunofluorescence preparations. Deparaffinized 5-10µm thick sections are incubated with a primary antibody

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(that can be a specific monoclonal antibody), rinsed, and visualized by a fluorescence-conjugated secondary antibody in combination with DAPI staining. Slides are imaged directly in the confocal microscope that may be coupled with image processing and documentation accessories that can directly provide output of publication-standard print-outs.

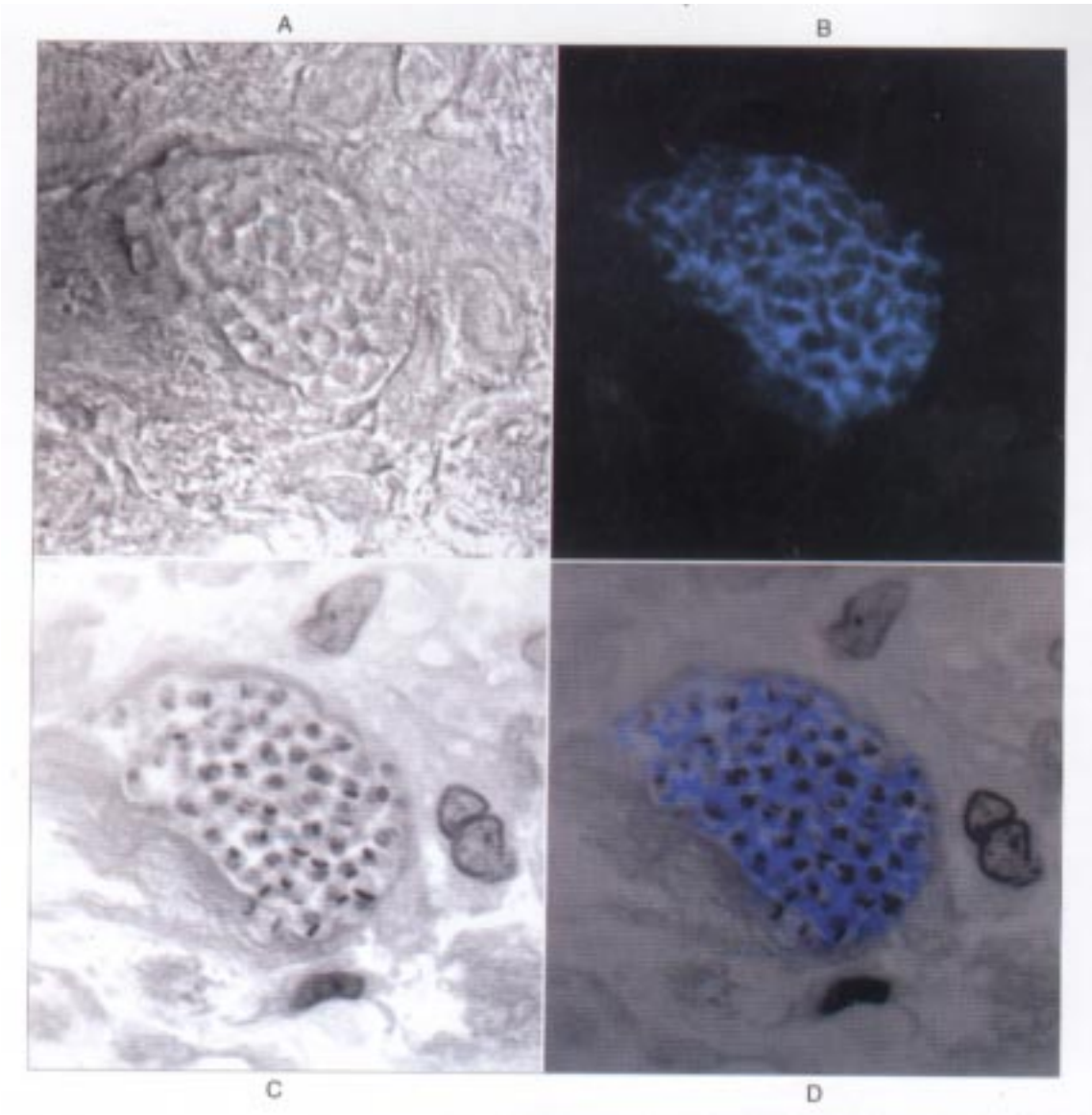

Figure 1 - Confocal imaging of myocardial tissue from Chagasic patient: A: Nomarski or differential interference contrast image of amastigote nest amongst cardiac myofibers. B: Confocal fluorescence image (single optical section) of same field labeled with 4D9, a monoclonal antibody that is specific for Trypanosoma cruzi *amastigotes[1]. C: DNA labeling with DAPI of same field where parasites' kinetoplasts and nuclei can be visualized with an inverted gray color table. D: overlay of immunofluorescence staining in blue on top of DAPI labeling. Magnification bar (A) in µm.*

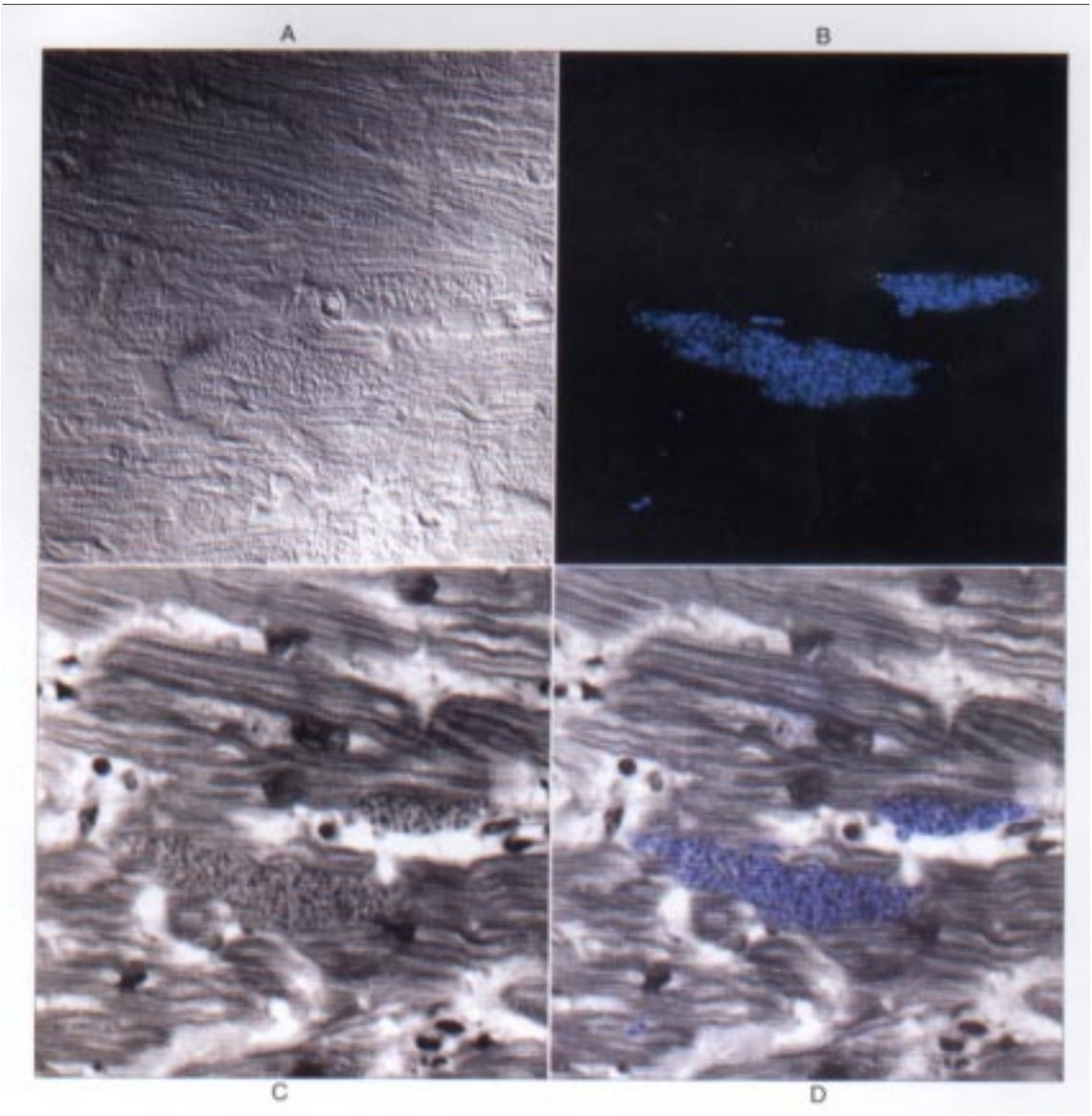

Figure 2 - Confocal imaging of myocardial tissue from chagasic patient: A: Nomarski or differential interference contrast

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