Image Analysis and 3D Reconstruction: An Innovating Methodology in Microscopy

R. Cabado[1] and G.M. Machado-Santelli[1]
[1] *Departamento de Histologia e Embriologia*
Instituto de Ciências Biomédicas - Universidade de São Paulo
Av. Prof. Lineu Prestes, 1524 - ICB 1 – CEP 05508-900 - São Paulo – SP , Brasil
rcabado@usp.br - gmmsante@usp.br

Abstract

The precise interpretation of the results in Cell Biology is crucial for a deeper understanding of cellular phenomena. The technological development of other areas introduced new approaches in biological research. New reagents make possible the identification of specific structures and molecules inside the cell. Their visualization with new equipments such as laser scanning confocal microscope generates growing amount of information that must be. The interpretations of these results depend on new hardwares and softwares in constant evolution. Optical section of a single salivary gland polytene nucleus were used to explore the potential of several image and 3-D reconstruction softwares. The results showed that the diversification of techniques improves the understanding of conventional data in Cell Biology.

Keywords: image analysis; 3-D reconstruction; laser scanning confocal mieroscope; polytene nucleus; *Rhynchosciara americana*

Introduction

The modern light microscopy has become a powerful tool in the study of the cellular biology including the molecular processes in live cells. The construction of the new microscopes has been consequence of the great technological progress that counts with resources of last generation of the Physics coupled to the support of the computer science. The techniques of preparation of the biological sample and the availability of tracer for specific structures have also developed. The synthesis of new fluorochromes, more stables and with a narrow emission spectrum improves the sensibility, the quality and size of the image, reducing the background (or noise) and increasing the quality and final resolution of the digitalized image. The use of chimerical constructions with the GFP protein (green fluorescent protein) viabilizes the study of the dynamics of different proteins directly in live cells. Additional tools have been proposed for the quantification of different parameters in image analysis (1,7).

Kam et al. (4) proposed the term "multidimensional microscopy" to include the acquisition, processing and presentation of multiple 2 and 3 dimensional images of a same specimen. The 3rd dimension can include several parameters, from transmitted light to spectrophotometric methods. The generated images are complex and it is necessary the establishment of a new methodology to extract correctly all the information they contain.

Starting from a collection of traditional images of light or fluorescence microscope, or gallery of optical sections obtained with laser scanning confocal microscope, it is possible to perform morphometric 2D evaluations, that has already been used for several years. The quantification of the images is made by the determination of the dimensions, morphology, orientation and distribution of cellular structures associated to the amount of specific molecules. Usually, the analyzed structures are separate from the background through a segmentation process of the image that is actually simpler for images of light microscope than for those of fluorescent microscope. However, the procedures for the segmentation and quantification should be adequated for each cell type, structures or even molecule to be analyzed (4).

More recently it has been suggested the use of 3D reconstruction starting from the usual data to reach deeper level of information and even, for more precise interpretation of new data. Software of 3D reconstruction can be applied to galleries of images of confocal microscope, mainly by studying small specimens in whole preparations (whole-mount preparations), cultured cells or

thick histological sections. The 3D reconstruction of "in situ" hybridization confocal images was proposed by Hecksher-Soerensen and Sharpe (3) for the analysis of gene expression in embryos. This same approach allows the study of the gene expression in single cells in intact tissue.

The aimed of the present study was to use image analysis and three-dimensional reconstructions techniques of optical sections obtained with laser scanning confocal microscopy as a tool to improve the potential of conventional approaches in Cell Biology.

Materials and Methods

Study Model- Salivary glands of *Rhynchosciara americana* were mounted with anti-fading solution on slides. The chromosomes were stained with Sytox® green.
Obtaining Images- A laser scanning confocal microscopy system (Zeiss LSM 510) with three laser sources (Argon-488nm, Helium-Neon 1- 543nm and Helium-Neon 2-633nm) and a fluorescence inverted microscope Zeiss Axiovert 100M was used to obtain series of images.
Analysis and 3D Reconstruction- For the image processing we used several software in distinct operating systems and two types of graphic workstations:
Softwares - Windows NT - Zeiss LSM 510 (Figures 1 and 2), 3D for Zeiss LSM 510 (Figure 3), Photoshop, Quick Time (Figure 6); UNIX/IRIX – Bitplane Imaris 2.7, Bitplane Imaris 3.0 (Figure 5), Open Inventor (Figure 4)
Hardwares - Graphic Workstation PC/Intel (Windows NT (IRIX 6.5)

Results and Discussion

Salivary gland polytene nuclei images were obtained with a laser scanning confocal microscope after staining the DNA with Sytox® green. Figures 1 and 2 show, respectively, a single section and a gallery of 63 sections of 0.51µm. This database allowed us to reconstruct the polytene chromosomes and analyze new aspects of salivary gland nucleus architecture. Some of the possible approaches in image treatment are presented in this paper. Three-dimensional surface reconstructions are shown in figures 3 and 4. The image in figure 3 was obtained by using the 3D for Zeiss LSM 510 software itself, meanwhile that in figure 4 was from Bitplane Imaris 2.7 module. Both modules are interactive, however the result from Imaris is much better since the reconstruction is based on a wireframe composed by thousand of triangles, with colour, reflection and shadow data. 3D volumetric reconstruction of the same nucleus was performed using the easy 3D of the Bitplane Imaris 3.0 (figure 5). Light and shadow projection are essential for this easy and fast visualization of volume in static reconstruction. Animation of these Imaris volumetric 3D reconstruction can be done in several ways. One of them is presented in figure 6, QuickTime format.
The 3D reconstruction represents, therefore, a powerful technique in the elucidation of problems in cellular and molecular biology, through the analysis gene expression, intra and extra-cellular structures and of the dynamics of cellular processes. However, in each step technical adaptations are necessary, such as the appropriate staining, the use of tracer like GFP, computerized tomography in electronic microscopy or laser scanning confocal microscopy (6).

The use of several fluorochromes provides the best spatial relationship of the several structures in the specimen, inside the cell among them (2, 5).

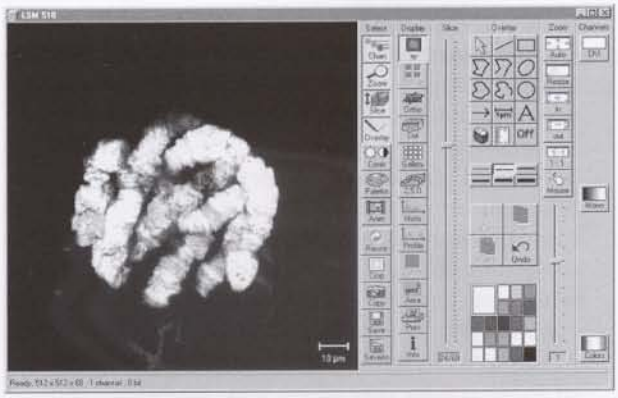

Figure 1 – Single image of a polytene nucleus obtained by laser scanning confocal microscopy. DNA was stained with SYTOX® green.

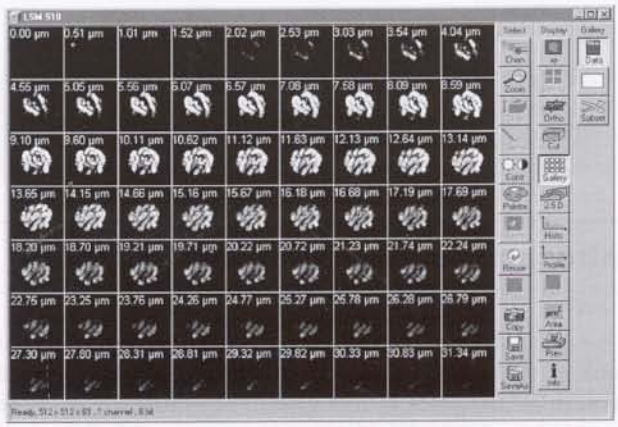

Figure 2 – Gallery display with a stack of images obtained by optical sections of the same nucleus in figure 1. The digital image and the database are available for the different types of analysis. They are the base for all types of 3D reconstructions.

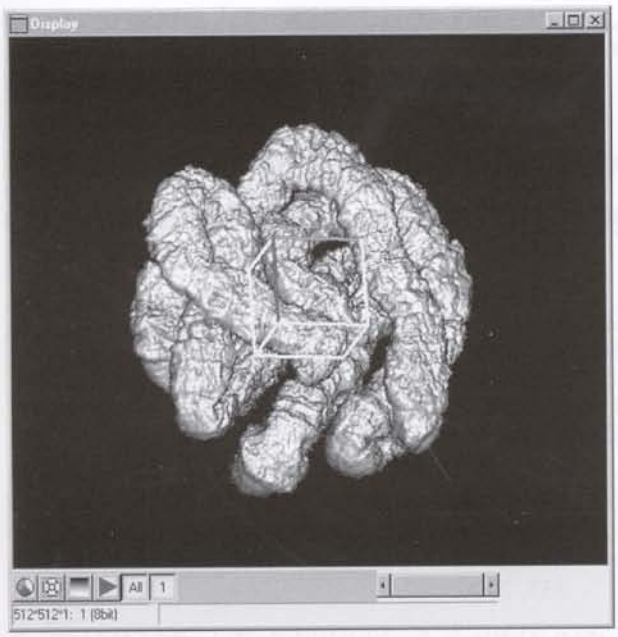

Figure 3 – 3D surface reconstruction performed with 3D for Zeiss LSM 510 software.

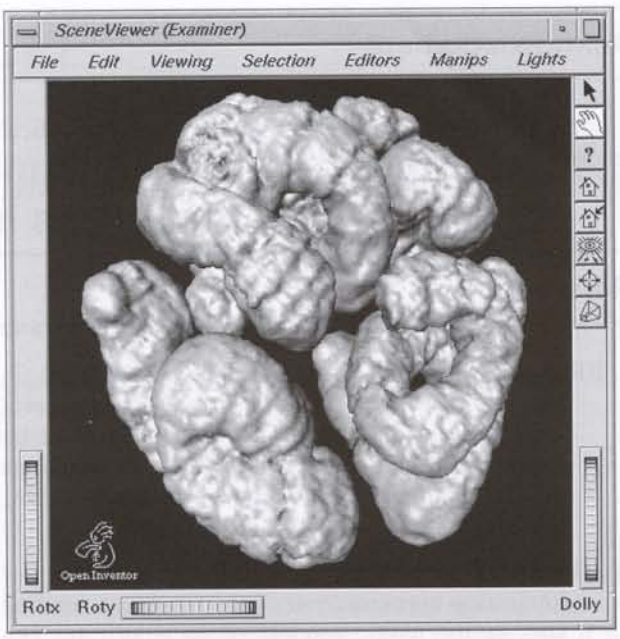

Figure 4 – 3D surface reconstruction from the same stack of image, carried out with Bitplane Imaris 2.7 software.

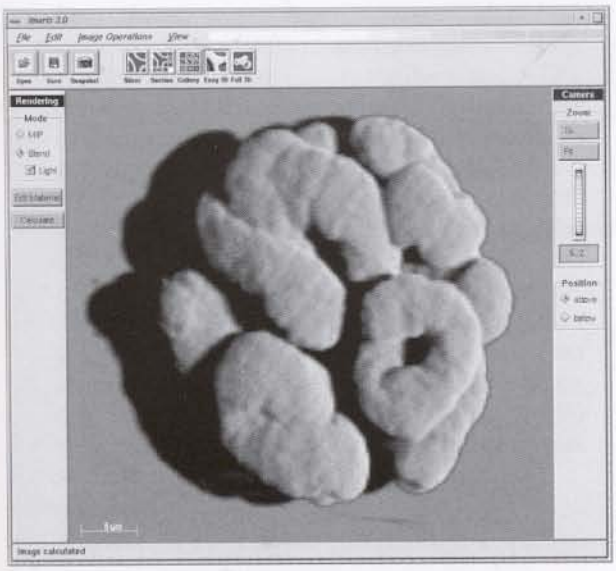

Figure 5 – By using shadow and light projection, the volumetric 3D reconstruction enhance the chromosome visualization. (Bitplane Imaris 3.0)

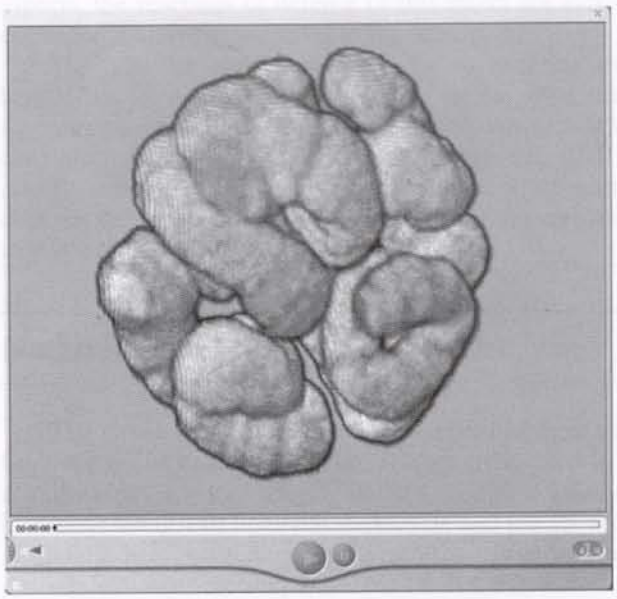

Figure 6 – Shows the use of QuickTime software in animation of the former reconstruction.

Conclusions

- The diversification of the techniques in Cell Biology studies can be a decisive element for deeper interpretation of the results.
- Image analysis of optical sections, three-dimensional reconstructions, two and three-dimensional animations, interactive visualizations are some of the techniques that we are suggesting as components of an innovating methodology.
- The technological advance and the interaction of the different scientific activities (Information Technology, Computation, Computer Graphic) are important contributions to Cell Biology.

Acknowledgment

We would like to thank Dr. Patricia Gama for critical reading of this manuscript.

This work has been supported by FAPESP and CNPq.

References

1. BROCKS, R.; VAMOSI, G.; VEREB, G.; JOVIM, T. M. Rapid characterization of green fluorescent protein fusion on molecular and cellular level by fluorescence correlation microscopy. **Proceedings of the National Academy of Sciences USA**, Washington, v. 96, p. 10123-10128, 1999.

2. GAMA, P.; ÁLVARES, E. P. Localization of luteinizing-hormone releasing hormone binding sites in the gastric mucosa of suckling rats. **The Anatomical Record**, Philadelphia, v. 264, p. 43-50, 2001.

3. HECKSHER-SOERENSEN, J.; SHARPE, J. 3D confocal reconstruction of gene expression. **Mechanisms of Developmental**, Limerick, v. 100, p. 59-63, 2001.

4. KAM, Z.; ZAMIR, E.; GEIGER, B. Molecular probing process in live cells by quantitative multidimensional microscopy. **Trends in Cell Biology**, Cambridge, v. 11, p. 329-334, 2001.

5. MANELLI-OLIVEIRA, R.; MACHADO-SANTELLI, G. M. Cytoskeleton nuclear and alterations in human lung tumor cells: confocal microscope study. **Histochemistry and Cell Biology**, Berlin, v. 115, p. 403-411, 2001.

6. MIRONOV, A. A.; POLISHCHUK, R. S.; LUINI, A. Visualizing membrane traffic in live by combined video fluorescence and 3D electron microscopy. **Trends in Cell Biology**, Cambridge, v. 10, p. 349-353, 2000.

7. WOUTERS, F. S.; VERVEER, P. J.; BASTIAENS, P. I. Imaging biochemistry inside cells. **Trends in Cell Biology**, Cambridge, v. 11, p. 203-211, 2001.