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Morphology of Nurse Cell Nuclei Induced by *Meloidodera floridensis*: A Computer Graphics Application

MANUEL MUNDO-OCAMPO,[1] MARIE GREENE,[2] MICHAEL FLAXMAN,[2] AND JAMES G. BALDWIN[1]

Abstract: The highly irregular distribution of nuclear material in the host nurse cell induced by *Meloidodera floridensis* has made it difficult to interpret the number of nuclei from two-dimensional micrographs alone. The primary goal of this investigation was to determine the distribution of nuclear material from a three-dimensional solid surface model of the nurse cell nucleus. This model demonstrated the continuity of nuclear material as a single highly irregular nucleus. Custom computer graphics programs were written to accept digitized tracings of nuclear material. From these digitized tracings, a wireframe or polygonalized mesh model was generated. The model was shaded, colored, rotated, and analyzed. This technique provides controlled transparency of the model to display nucleoli within the nucleus. Photographs of the computer screen, color printouts, and video recordings were used to record final results. These refined computer graphic tools have a range of applications in nematode host–parasite relationships, ontogeny, and morphology.

Key words: digitized image, giant cell, graphics modeling, method, morphology, nematode, serial reconstruction, surface imaging, tridimensional graphics, visualization, volume visualization.

The number and morphology of nuclei in nurse cells induced by Heteroderoidea are often difficult to determine by microscopy alone. Yet nuclear changes and proliferation have been considered crucial to understanding the mechanism by which these cells form, as well as to understanding parasite phylogeny. Huang and Maggenti (3) argued that 4n, 8n, 16n, 32n, or 64n nuclei in nurse cells induced by *Meloidogyne javanica* (Treub) Chitwood, 1949 indicated formation by karyokinesis without cytokinesis. Numbers of nuclei were also treated as character states useful for phylogenetic analysis of Heteroderidae (1). Specifically, Mundo and Baldwin (4) argued that certain genera, including *Meloidodera*, share the pleisiomorphic character state of a single uninucleate nurse cell, in contrast to other genera, such as those forming cysts, which induce multinucleate syncytia.

The number of nuclei in the nurse cells of *Meloidodera* has been difficult to confirm, because the nuclear material is highly irregular in shape and has multiple nucleoli. In a given two-dimensional section, the nuclear material appears to be discontinuous between separate lobes, and in some cases small islands of cytoplasm appear to be surrounded by nuclear material. It often is not clear if this cytoplasm and nucleoplasm is continuous in other planes. Competing hypotheses that the nurse cell induced by *Meloidodera floridensis* Chitwood et al., 1956 has a single nucleus, versus multiple nuclei, were tested by developing a computer graphic program for reconstruction of the three-dimensional image from serial light microscope sections. A similar program developed in our laboratory for botanical morphology provided a starting point for the present investigation (2).

MATERIALS AND METHODS

Preparation and collection of the serial sections: Pine roots (*Pinus taeda* L.) infected with *M. floridensis* were fixed with Karnovsky's fixative, postfixed with 4.0% osmium tetroxide for 2.0 hours, and rinsed with 0.2 M sodium cacodylate buffer at pH 7.4. Root pieces with a length of ca. 1.0 mm were dehydrated in a methanol series and embedded in Spurr's resin. Serial sections of ca. 2.0 μm were stained in 1% aqueous Toluidine Blue, mounted on glass slides,

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[1] Department of Nematology, University of California, Riverside, CA 92521.
[2] Academic Computing Graphics and Visual Imaging Lab, University of California, Riverside, CA 92521.

and sealed under glass coverslips with Permount. Serial sections of a representative nurse cell including nuclear material were observed under interference contrast light microscopy and photographed.

Data entry and alignment of sections: Computer hardware included on Evans and Sutherland PS390 linked to a VAX 8700 and Silicon Graphics 320VGX Unix Workstation. Communication between computer systems was through an ethernet network. Boundaries of the nuclear material were hand-traced from serial photographs directly onto acetate transparencies, aligned with fiducial marks, and digitized into the Evans and Sutherland computer using the program ANATOGRAPH (7). The distance between sections was carefully calculated to include magnifications of the micrograph and entered into the program before tracing. This was imperative to reconstruct a final model with accurate overall dimensions. The distance between sections can vary during the tracing to allow skipping of sections for very regular data or every section to show irregular data. In this investigation we entered in as many sections as possible, because the nucleus has a highly irregular shape. ANATOGRAPH records each click of the mouse as a vertex point to be used for future calculation of a wireframe model. Fifty different models can be traced per section.

Creation of models: After all the models of each section were traced, a binary file containing traced information (the number of sections, distance between sections, number of models, number of vertex points per model) was created. These data were converted for use in other programs to display and manipulate the final reconstructed model.

Display of models and data analysis: Model data were input into the software packages SYNU (6), Explorer (5), and AVS (8). Solid

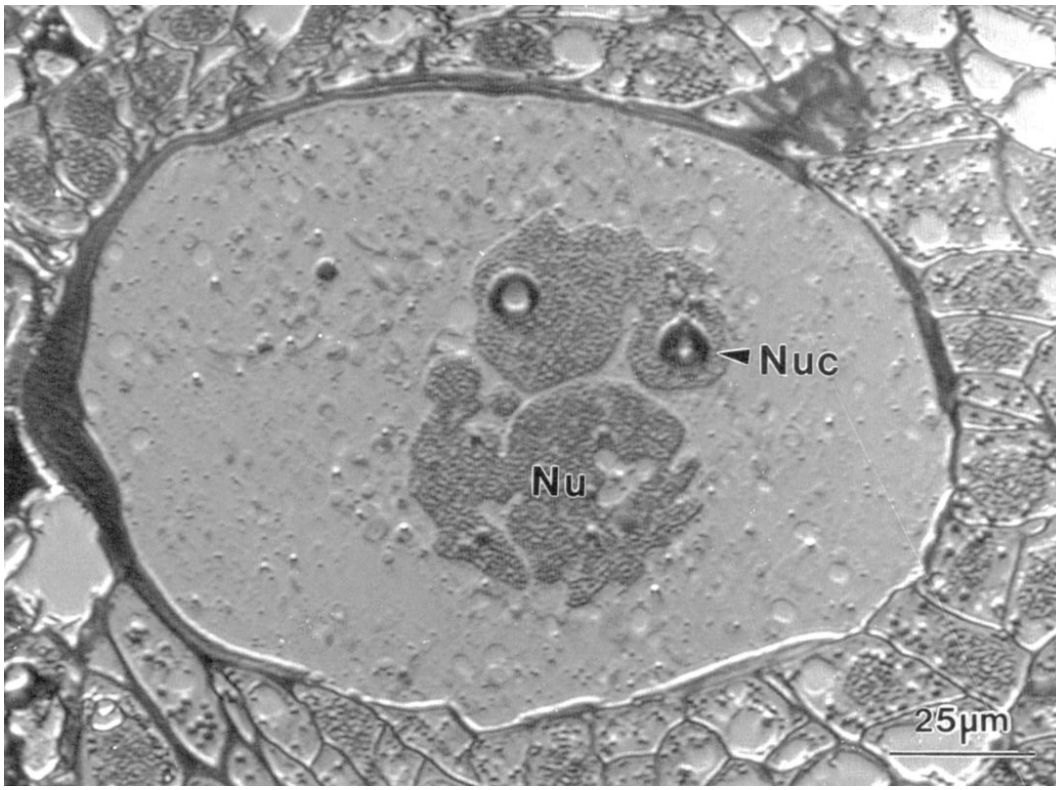

FIG. 1. Interference contrast micrograph of nurse cell induced by *Meloidodera floridensis* in *Pinus taeda*. Nuclear material (Nu) appears discontinuous. Nuc = nucleoli.

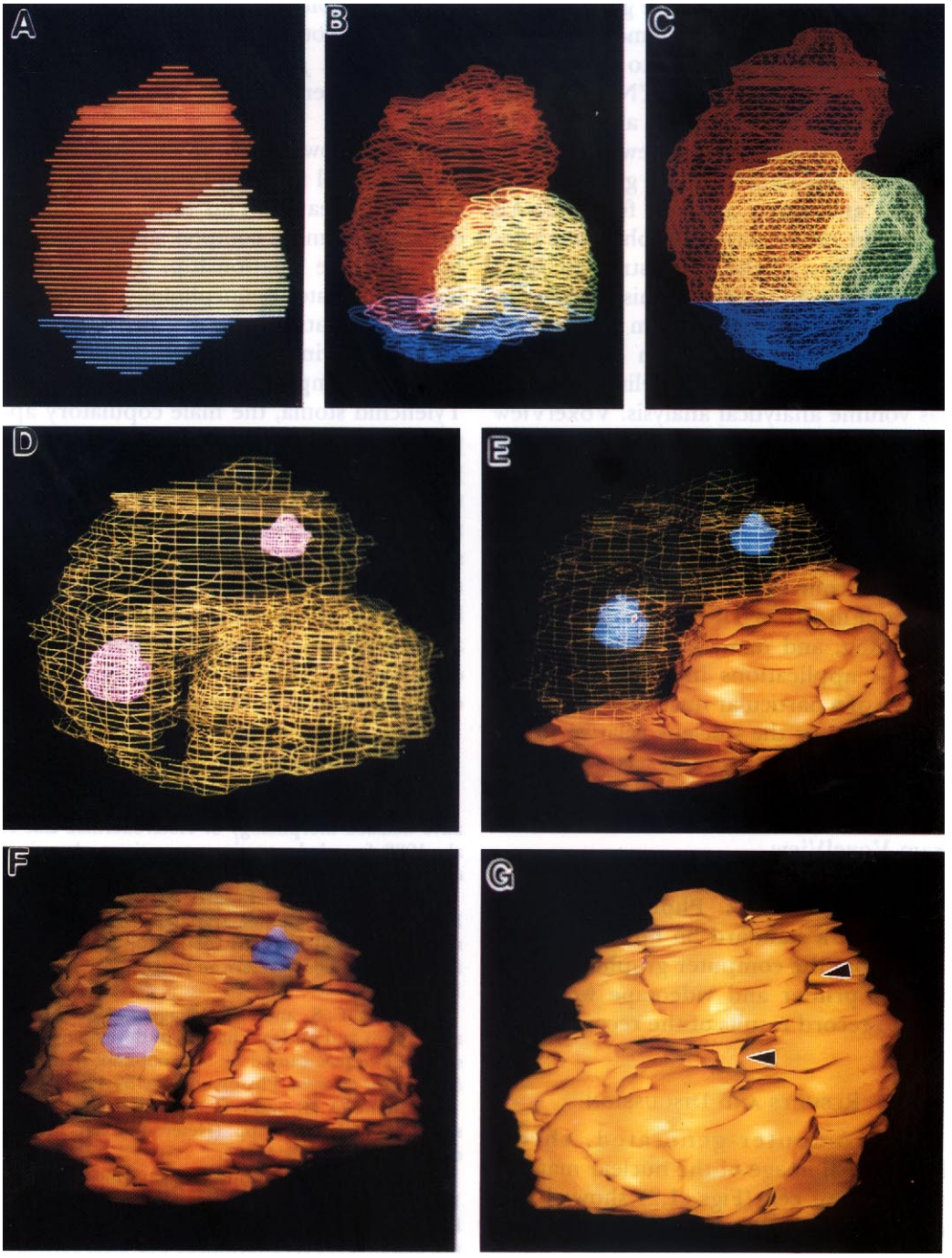

FIG. 2. Computer graphic models of the nucleus of the nurse cell induced by *Meloidodera floridensis* in *Pinus taeda*. Reconstruction is from sections including that shown in Fig. 1. Different colors in A through C represent different model components of the nucleus. A) Reconstruction from ANATOGRAPH showing perspective perpendicular to the plane of the sections. B) Tilt of reconstruction shown in Fig. 2A, revealing outlines of component sections. C) Wireframe three-dimensional reconstruction of the nucleus, rotated 180° from Fig. 2B. D) Wireframe reconstruction of the nucleus, including nucleoli. E) Solid plus wireframe reconstruction showing nucleoli inside wireframe region. F) Solid reconstruction with transparency showing positions of nucleoli. Model rotated 180° from Fig. 2E. G) Solid reconstruction showing surface texture of nucleus, with deep invaginations (arrows). Model rotated 90° from Fig. 2E.

and transparent models were generated by each of these programs. We mention three because each can be used to display the final data. The program SYNU provides very smooth transparency and was the only program with stereo viewing capabilities. AVS and Explorer have good lighting control and many analysis features. All three run on a Silicon Graphics workstation. Figures of the reconstructed solid and transparent models in this paper were generated using the program SYNU. Another valuable tool used in conjunction with the solid surface modeling program was volume analytical analysis. VoxelView (9) is a powerful program that uses all of the micrograph data, not just the surfaces. It provides tools such as slicing through the sections from any angle, volume and area measurements, histograms, color gradient determination, image analysis, image enhancement with sharpening and smoothing, and many other applications. Specific applications include measuring the size of a particular nucleolus relative to the nucleus or the nucleus relative to the cell for one section, several sections, or the entire data. Micrograph sections were entered directly via a scanner into the program VoxelView.

Recording results: Models were saved as image files on disk. Photographs were taken with a standard 35 mm camera directly from the computer graphics screen. A color printer and videotape were also used to record results of the reconstructed model.

Results and Discussion

The computer-generated, three-dimensional image of the nuclear material in the nurse cell (Figs. 1,2) showed that what appeared to be discontinuous nuclear material or independent nuclei in a single plane (Fig. 1) was actually interconnected lobes of one large nucleus with deep channels and several nucleoli (Fig. 2F,G). Computer graphics also permitted varying degrees of transparency and coding of internal structures with contrasting color to determine the shape and relative positions of the nucleoli (Fig. 2A–F). Findings support the hypothesis that the nurse cell induced by *M. floridensis,* contrary to cyst-forming Heteroderidae, has only one nucleus.

Results show that computer graphics is a powerful tool that can be useful in nematological research. This technique can be extended to more complex applications in host–parasite relationships and particularly in nematode morphology. These include elucidating complex cell interrelationships during ontogeny as well as reconstructing complex structures such as the Tylenchid stoma, the male copulatory apparatus, and sensory organs. With the explosion of computer graphics applications, many research institutions already have, or may soon have, centralized shared facilities similar to those used in the present investigation (5). Solid surface modeling combined with three-dimensional image data analysis provides nematologists a powerful computer graphics tool that may enhance existing research techniques.

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