Virtual Reality in Biological Microscopic Imaging

R. v Liere, W. de Leeuw, J. Mulder
Department of Information Systems
Center for Mathematics and Computer Science
Amsterdam, Netherlands

P. Verschure, A. Visser, E. Manders, R. v Driel
Swammerdam Inst. for Life Sciences
University of Amsterdam
Amsterdam, Netherlands

Abstract

Confocal microscopes have recently allowed biologists and biomedical researchers to obtain time dependent 3D data sets of biological objects, such as cells and tissues. Scientific visualization can provide visual presentations of structural characteristics of these data sets. This paper addresses the role of virtual reality in gaining insight in these presentations. The understanding of structural characteristics of time dependent 3D confocal biological data requires spatial judgments. Perceiving these characteristics is enhanced by using virtual reality technology. The advantage of virtual reality is particularly apparent in the exploration phase of the analysis when the behavior of the underlying biological processes is not a priori known.

Keywords*: cell biology, living cells, biomedical imaging, virtual reality.*

CR Categories and Subject Descriptors: *H.5 [Information Interfaces and Presentations]: User Interfaces; I.3 [Computer Graphics]: Three-Dimensional Graphics and Realism; I.3*

1 Introduction

Microscopy has always been an essential component in biological and biomedical research. In the past decade particularly, new developments in digital light microscopy have progressed at a rapid pace. Until recently, most structural analysis on nuclear organization was done on fixed, dead cells. However, many cell components in general and nuclear components in particular display a highly dynamic behavior. In the past decade new fluorescent labeling techniques have become available which allow visualization of the dynamics of proteins and other molecules in living cells. These developments are still progressing rapidly, and there is no doubt that this is opening a new chapter in our understanding of the combination of processes that we recognize as 'life' at the molecular and cellular level.

The field of biomedical imaging and visualization is also expanding rapidly, [1, 2]. Advanced image processing techniques, like 3D image restoration of confocal data, have been accepted by biologists. The need for these techniques is driven by their capability to provide new information about spatial organization at the sub- and supra-cellular level. Unfortunately, when studying time dependent 3D visualizations, biology researchers still struggle with the extraction of usable information from the images. For example, time dependent 3D distributions of two or more different nuclear components are usually highly convoluted and therefore the dynamics is difficult to comprehend.

Virtual reality is often referred to as the technology that gives a user the experience of being immersed in a computer-generated virtual world. In this paper we address the role of virtual reality in understanding structural information from 4D biological data. In doing so, we discuss how virtual reality device technology (i.e. display technology, tracking hardware and input devices) and abstract techniques (i.e. visualization algorithms, interaction metaphors) effect the perception of structural information in 4D biological data. We postulate that the understanding of biological processes requires spatial judgments. Perceiving structural information is enhanced by using virtual reality.

The paper is organized as follows. First, we discuss how the notion of 'structure' in 4D biological data is related to 'function' of biological processes. We give two concrete examples of our research in which virtual reality played a vital role in perceiving and understanding the structural information. In Section 3, we enumerate various depth cues which are important in perceiving structure. We show which VR device technologies can be used to implement these cues. Section 4 sketches the Proteus system, a virtual environment developed for studying 4D confocal biological data. Proteus has mainly been used in an environment consisting of a large head-coupled display. We discuss the pros and cons of using Proteus in other virtual environments, such as the CAVE, desktop workstations, and with different input devices. Finally, in section 5, we discuss the biological re-

sults of using the Proteus system and evaluate the usage of the system.

2 Structure in Cell Biology

'Structure' is an extremely important concept in biology. This includes the 3D folding of the polypeptide chain in proteins, the spatial organization of the cell, and the arrangement of cells in a tissue. Throughout biological sciences 'structure' is the determinant for 'function' at all levels of organization, i.e. from macro-molecule up to organism. Spatial distributions of objects, spatial object correlations, object trajectories, and the definition of object shape as concentration variations are important characteristics of structure. To study structure, a broad range of microscopy techniques are available. For example, modern confocal microscopes in combination with labeling techniques can capture multi-channeled 4D data sets of cellular components, cells and tissues.

The focus of our research is aimed at gaining insight into the functional organization of the cell nucleus in relation to the regulation of gene expression, RNA processing and transport, and DNA replication and repair. Here we give two examples that illustrate our work:

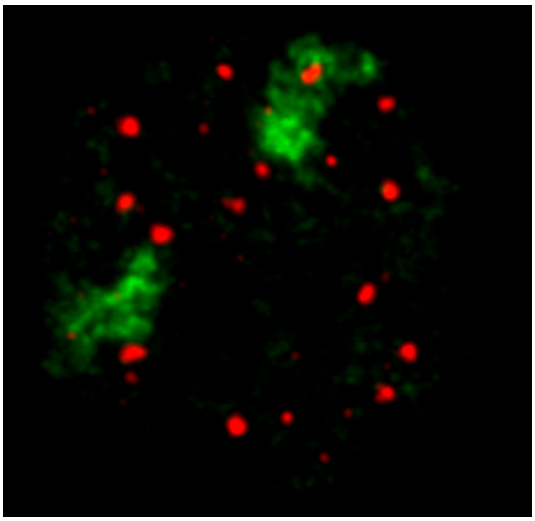

Figure 1. Female human fibroblast cell nucleus: X-chromosomes are shown as semi-transparent green volumes and the PML bodies are shown as red dots.

1. We have studied the spatial relationship between chromosomes and a specific type of nuclear domains, called PML bodies, in interphase nuclei of cultured human fibroblasts. In nuclei, each chromosome occupies its own chromosome territory, a domain not invaded by chromatin from other chromosomes. Chromosome territories are rather open structures consisting of domains that contain highly compacted chromatin and of a considerable volume of interchromatin space. PML bodies are spherical nuclear domains with a diameter of up to 1 micrometer. Fibroblasts contain in the order of 20 of such domains. The function of PML bodies is not fully understood though they seem involved in viral replication and gene regulation.

   An important question is whether nuclear domains have a specific spatial relationship with chromosome territories. These and related questions about the functional organization of the cell nucleus have been addressed by dual labelling 3D confocal imaging. Chromosomes were fluorescently labelled by in situ hybridization, while PML bodies were labeled by indirect immunofluorescent labeling techniques.

   Figure 1 shows an image from this study [1]. The image is of a female human fibroblast cell nucleus. Volume rendering is used with a semi-transparent green transfer function to visualize the chromatin, the red points represents the PML bodies in the nucleus.

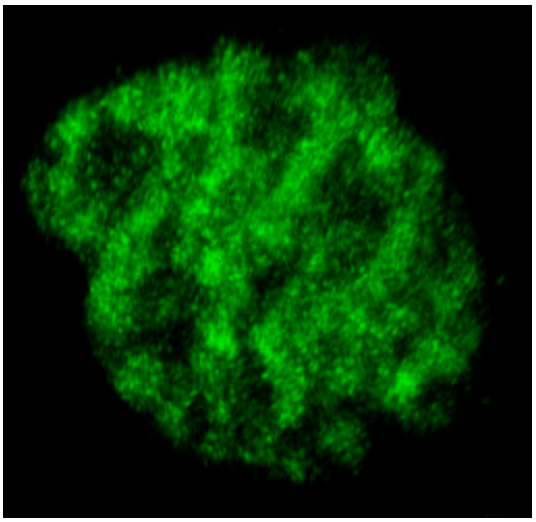

Figure 2. Chromatin data for one time step in the decondensation process. Bright green domains in the image indicate compact chromatin regions.

2. We have studied the decondensation of chromosomes during the formation of the new G1 nucleus after cell

[1] http://www.cwi.nl/ robertl/vr has two mpeg animations of figure 1 and 2.

division. In these studies we use HeLa cells that express histone H2B-GFP, thereby fluorescently labeling all chromatin in these cells. A time series of 135 3D data sets were obtained over a three hour time period, monitoring chromatin decondensation and chromosome movement during G1 nucleus assembly in a single cell.

The spatial distribution of chromatin is highly complex, as is its dynamic behavior. The aim of this study was to obtain a general understanding of what type of dynamical biological processes occur. The goal of the visualization was to perceive and find patterns in the movement of chromatin.

Figure 2 shows the final G1 nucleus. Bright green spots in the semi-transparent green image indicate compact chromatin regions.

3 Perceiving Biological Structures

Scientific visualization techniques, such as volume rendering, iso-surfacing, object annotation, path tracing and editing, and object tracking have been used to render time dependent confocal data sets, [3, 4]. There are various factors that make the visualization of these data sets challenging. First, in many cases the autonomy of cell components is not well understood and cannot be determined. This makes (semi-automatic) classification, extraction and segmentation of the data more difficult. Second, confocal data are very noisy which can lead to difficulties in the segmentation and tracking of objects. Third, object sizes often approach the resolution of the microscope. This leads to ambiguities and visual artifacts when objects are extracted from the data. Finally, the cell can translate, rotate and expand during the scanning of the cell. This results in additional external movements of the cell components due to movement of the cell. In our examples, these external movements must be corrected through global non-affine transformations.

Obtaining accurate and robust depth information from visualizations of biological data is an important aspect in perceiving their structure. Computer graphics practitioners have used various depth cues to make objects appear 3D on a 2D display. Many of these cues are based on psychological research about human perception and the cognitive processing of visual information. Often depth cues are placed in two categories: primary cues (binocular disparity, convergence and accommodation) and secondary cues (perspective, texture, shading and shadows, reference frames and motion). The usage of secondary cues has been standard in 3D computer graphics. However, it is not well understood which cue is essential for the correct interpretation of spatial information in the image. Moreover, it is not well understood what the importance of a particular cue is when used in combinations with other cues.

Despite the above mentioned secondary cues, biology researchers still struggle with the perception of the complexities of structure. As the examples in section 2 show, objects are highly convoluted and evolve over time. Furthermore, object distributions tend to be dense resulting in spatial object relationships that are difficult to perceive.

Virtual reality can help in perceiving these dynamic structures. We list a number of virtual reality technologies which have a positive effect on the perception of structure:

- head tracking

  Motion parallax based on head tracking (the apparent angular velocity of objects which is inversely proportional to real distance) is a very powerful cue to generate depth and shape information. Although we lack formal evidence, we believe that motion parallax aids the spatial resolution of object distributions and object correlations in 4D biological data. There is formal evidence that stereo and motion cues do increase task performance in understanding the structural complexities of information nets in three dimensions, [5], and for blood vessel path tracing in 3D brain scan images, [6]. Many aspects of these formal studies also apply to cellular structures, even though these studies were conducted with non-time dependent data. We surmise that motion parallax provides crucial depth information when the cellular structures change over time.

  It has been argued that motion parallax can also be obtained by rocking the scene back and forth about a vertical axis. For example, it has been shown that structure of moving scenes can be interpreted correctly even when the static view of the objects does not contain information about structure at all, [7]. Rocking techniques are frequently found in molecular graphics and statistical analysis packages. Although we do not disagree that motion parallax can be obtained by scene rocking, head coupled displays are more natural and transparent to use. Furthermore, using rocking for motion parallax may cause visual ambiguities in the case when objects are in motion.

- stereo vision

  Binocular disparity allows depth information to be obtained from two slightly different projections of an object. This provides the basis for stereo vision. Binocular disparity can only be used for objects at distances closer than a few meters.

  The human visual system also uses accommodation, convergence and their interrelation as cues for depth perception. However, there are no displays available which can maintain the accommodation cue in stereo

vision. Depth of field rendering techniques have been developed that may reduce this problem, [8].

- large display

  With a large display, the cellular structure can be scaled so that the foreground objects near to the biologist are perceived to be very far from those objects in the background. This induces a feeling of spaciousness when the biologist gazes at foreground objects. Furthermore, large displays enhance the sensation of 'presence' which aids spatial judgments, [9].

- spatial input

  Interaction can support perception by increasing the efficiency in which the biologist can extract meaningful information from data. Interaction compromises, for example, on-the-fly data cutting and slicing, line, surface and volume measurements, placement of annotation markers, and the interactive tracing of line structures.

  Unfortunately, spatial input techniques still suffer from technical drawbacks. However, we believe that spatial input is a natural and transparent way of directly manipulating a biological object, and thereby can contribute to the perception of the object. The use of 2D input devices for 3D interaction is more indirect and less transparent.

4 The Proteus System

The Proteus system is a virtual environment for the exploration multi channel, time dependent, 3D images obtained by confocal microscopy. The goal of Proteus is to provide an environment in which a biologist is seated in front of a data set of three dimensional cellular structure which is scaled, probed and manipulated as if it were a real cell. Proteus provides a number of visualization techniques which are useful for the display of structural characteristics of 4D biological data. These techniques include the interactive control over volume and iso-surface rendering parameters (e.g. transfer functions and iso-values), data selection tools to cut and paste data regions, free hand drawing tools for path tracing, distance measuring tools, and tools for tracking features in the data.

Proteus has been written using PVR, an in-house system for portable virtual reality applications, [10]. PVR has been used as a test-bed for other interactive applications [11] and our VR related research, [12]. PVR is also used in various environments, such as a desktop workstation, a large fish tank display and the CAVE.

The Proteus user interface is based on the metaphor of a virtual hand which manipulates the data (see figure 3). Due to technical constraints, the virtual hand is currently defined

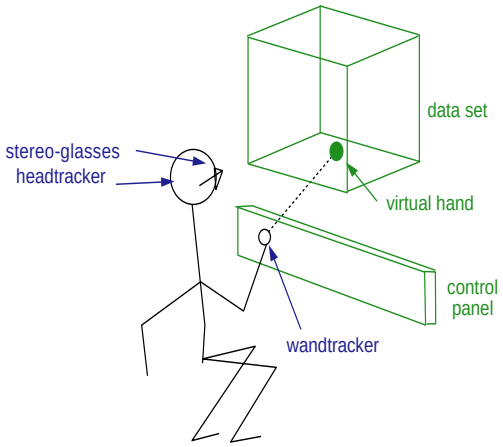

Figure 3. The Proteus system: a biologist is seated in front of a three dimensional cellular structure.

by a 3D point. All interaction is performed with the virtual hand. For example, the virtual hand is used to grab and directly manipulate data, press buttons on the control panel to activate operations, etc. Proteus has been extensively used on a large fish tank display with a 6 DOF wand for interaction. The virtual hand has been implemented with the wand and a 'stick' that offsets the wand position. Figure 4 shows the Proteus user interface.

Using Proteus with different virtual reality hardware would require alternative mappings of the data region and virtual hand. We discuss a few considerations:

- semi- vs full- immersion

  The concept of Proteus is that of a biologist seated in front of and interacting with a virtual 4D cellular structure rendered in a data region. A semi-immersive "outside-to-inside" viewing mode is appropriate to realize this vision. Although the biologist often translates, rotates and scales the data region, it is our experience biologists do not place their head in this region; i.e. biologists do not view cell structures by "inside-to-outside" viewing. Hence, a large fish tank display is sufficient for implementing the "outside-to-inside" view.

  Despite the absence of full-immersion, we found that biologists still have a sensation of presence in "outside-to-inside" viewing mode.

- the virtual hand

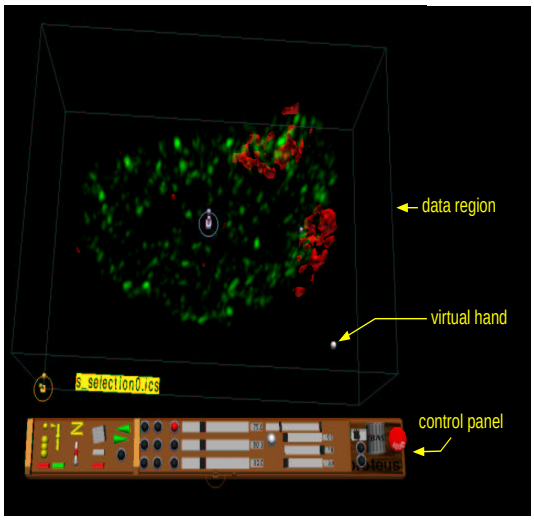

Figure 4. The Proteus user interface: a virtual hand is used to directly manipulate data in the data region. On the bottom is the control panel to select operations and adjust parameters. The data region shows transcription sites (green volume rendered) and X-chromosomes (red iso surface) in a cell nucleus.

The virtual hand metaphor that directly manipulates the data is easy to understand. However, with the current implementation a number of problems were encountered which prevent precise interaction and thereby decreasing the sensation of presence. First, by using the wand as a remote controller of the virtual hand, the biologists did not know the exact position in the data they are probing. Second, the accurate positioning of the remote virtual hand is difficult due to tracker noise, since the noise is amplified by the length of the stick.

A more natural implementation would be to have the virtual hand at the exact same position as the biologist's dominant hand. However, when used with back projected displays, occlusion will occur. Also, the work space will be limited to arms reach and the biologist cannot manipulate objects behind the display.

- volume rendering performance

  Biologists prefer the volume rendered images more than extracted iso-surface rendered images. In general, cell components are best represented as amorphous objects with compact regions. Iso-surfaces are confusing because they suggest clearly bounded objects, while the data contains only concentration information.

  Unfortunately, volume rendering time dependent confocal data sets at 20 frames per second (10 frames for each eye) is still very challenging. A single pipe SGI Infinite Reality Onyx2 system cannot realize these frame rates for the data sets discussed in section 2.

  Adaptive volume rendering strategies may be used to speedup rendering. For example, the user's gaze direction could be used to render regions with high accuracy. An alternative speedup strategy would be to use a data base of pre-computed perspective images and select a best image based on the tracked user. A similar approach was suggested by Venolia and Williams, [13]. However, this strategy does not allow on-the-fly interaction and the data base of pre-computed images will become prohibitively large for time dependent data sets.

5 Results and Evaluation

The Proteus system has been used by biologists in their research. Using Proteus they were able to visually explore the 4D data sets of the examples given in section 2:

- In the first example specific spatial relationships between X chromosome territories and PML bodies were delineated, [14, 15]. It was found that PML bodies occur predominantly in the interchromatin space inside and around chromosome territories. Furthermore, it was found that PML bodies occur only at the periphery of sub-chromosomal chromatin domains and never coincide with chromatin.

  The data in this study was initially explored on a desktop workstation using standard 3D image processing and visualization techniques. The mentioned results were not found, primarily because the locations of PML bodies with respect to the highly convoluted substructure of X chromosomes territories are difficult to perceive.

- In the second example it was initially not clear what type of chromosome movement and chromatin decondensation was in the data. After using Proteus, it became clear, for example, that what looked like chromatin decondensation in fact was rather the formation of chromatin-poor regions in the nascent nucleus between more condensed chromatin domains, [16].

  Perceiving these formations on a desktop workstation is difficult, primarily because the dynamics of the spatial relationships. The definition of compact chromatin domains as concentration variations varies over time and the spatial correlations between convoluted chromatin domains are difficult to follow. Based on the general understanding of the process we gained using

virtual reality, we were able to ask specific questions which were then translated into specific 4D image analysis algorithm. An example is the development of a tracking algorithm of recognizable condensed chromatin domains, [17].

The major problem encountered with Proteus is that the ergonomics of the system is poor. Often heard complaints from biologists are that they must use the system in a dark room, the wand causes considerable arm strain when used for an extensive period, wired tracking systems are tedious, some users encounter eye strain when using the system for a long period of time, and the rendering lag caused by volume rendering is too high. Although many of these issues will improve as device technology progresses, others will require more thought on the abstract technique level. If virtual environments are to be really useful for research in biological microscopic imaging, then the environment must be integrated into the daily work environment and be usable in normal working conditions.

Structural biologists at the University of Chicago have reported the use of the Crumbs system, [18, 19]. The motivation, goals and functionality of Crumbs and Proteus are similar, but the system designs differ. In contrast to Proteus, Crumbs is designed for "inside-to-outside" viewing, allowing the user to be fully immersed in the data. Also, Crumbs uses more modalities, such as audio and video. Audio is used as a natural command interface to the Crumbs system. In addition, the developers of Crumbs have invested significant effort to accelerate volume rendering. This results in significant improvements in rendering times and less overall system lag when compared to Proteus.

6 Conclusion

In a recent progress report on VR for scientific visualization, van Dam et al. convey the hope that within a decade there is a list of "important scientific discoveries or designs that would not have happened without the use of immersive virtual reality based scientific visualization as an integral part of the discovery or design process". This paper discusses the role of virtual reality in biological microscopic imaging. Time dependent microscopic biological data requires spatial judgments in order to obtain a general understanding of the underlying biological processes. Virtual reality systems offer depth cues which enhance the perception of structural information.

Biologists emphasize that the advantage of virtual reality is particularly apparent in the exploration phase of the analysis, when the behavior of the underlying biological processes is not a priori known. Based on the knowledge gained from the exploration phase more concrete questions about the data are formulated. These questions can be addressed through, for instance, specific 3D image processing and analysis techniques.

We have found that virtual reality has helped our research. Most probably, the discoveries in the analysis of chromatin decondensation in living cell would not have occurred without the use of virtual reality.

References

[1] Advances in computational image processing for microscopy (special issue). *Journal of Structural Biology*, 116(1):1–248, January/Feburay 1995.

[2] R. Rizzuto, W. Carrington, and R.A. Tuft. Digital imaging microscopy of living cells. *Trends in Cell Biology*, 8:288–292, 1998.

[3] A. Kaufman, R. Yagel, R. Bakalash, and I. Spector. Volume visualization in cell biology. In A. Kaufman, editor, *Proceedings IEEE Visualization'90*, pages 160–167. IEEE Computer Society Press, October 1990.

[4] R.A. Robb. *Biomedical Imaging, Visualization and Analysis*. Wiley-Liss, 2000.

[5] C. Ware and G. Franck. Evaluating stereo and motion cues for visualizing information nets in three dimensions. *ACM Transactions on Graphics*, 15(2):121–140, 1996.

[6] R. L. Sollenberger and P. Milgram. A comparative study of rotational and stereoscopic computer graphic depth cues. In *35th Annual Meeting of the Human Factors Society*, pages 483–499, 1991.

[7] H H. Wallach and D.N. O'Connell. The kinetic depth effect. *Journal of Experimental Psychology*, 45:205–217, 1953.

[8] J.D. Mulder and R. van Liere. Fast perception-based depth of field rendering. In K. Y. Wohn, editor, *VRST 2000: Proceedings of ACM Virtual Reality Software Tools 2000*, pages 129–133. ACM Press, 2000.

[9] A. van Dam, A.S. Forsberg, D.H. Laidlaw, J.J. LaViola, and R.M. Simpson. Immersive VR for scientific visualization: a progress report. *IEEE Computer Graphics and Applications*,, 20(6):26–52, 2000.

[10] R. van Liere and J.D. Mulder. PVR - an architecture for portable VR applications. In M. Gervautz, A. Hildebrand, and D. Schmalstieg, editors, *Virtual Environments '99, Proceedings of the Virtual Environments Conference & 5th Eurographics Workshop*, pages 125–135. Springer Verlag, 1999.

[11] H. Huitema and R. van Liere. Interactive visualization of protein dynamics. In T. Ertl, B. Hamann, and A. Varshney, editors, *Proceedings IEEE Visualization 2000*, pages 465–468. IEEE Computer Society Press, 2000.

[12] J.D. Mulder and R. van Liere. Enhancing fish tank VR. In S. Feiner and D. Thalmann, editors, *Proceedings of the IEEE Virtual Reality 2000 Conference*, pages 91–98, 2000.

[13] D. Venolia and L. Williams. Virtual integral holography. In *Extracting Meaning from Complex Data: Processing, Display, Interaction, Proc. SPIE*, volume 1259, pages 99–105, Santa Clara, 1990.

[14] A.E. Visser and J.A. Aten. Chromosomes as well as chromosomal subdomains constitute distinct units in interphase nuclei. *Journal of Cell Science*, 112:3353–3360, 1999.

[15] P.J. Verschure, I. van der Kraan, E.M. Manders, and R. van Driel. Spatial relationship between transcription sites and chromosome territories. *Journal of Cell Biology*, 147:13–24, 1999.

[16] E.M. Manders, A.E. Visser, A. Koppen, W.C. de Leeuw, R. van Liere, G.J. Brakenhoff, and R. van Driel. Chromatin dynamics during the formation of the interphase nucleus. 2001. Submitted for publication.

[17] W. de Leeuw and R. van Liere. Chromatin decondensation: a case study of tracking features in confocal data. In *Proceedings of the 2001 IEEE Visualization Conference)*, 2001. Accepted for publication.

[18] T. Karr and R. Brady. Virtual biology in the CAVE. *Trends in Genetics (TIG)*, 16:231–232, May 2000.

[19] R. Brady, J. Pixton, G. Baxter, P. Moran, C.S. Potter, B. Carragher, and A. Belmont. Crumbs: a virtual environment tracking tools for biological imaging. In M. Loew and N. Gershon, editors, *Proceedings of the IEEE Symposium on Frontiers in Biomedical Visualization*, pages 18–25, Atlanta, GA, 1995. IEEE Computer Society Press.