Multi-Dimensional Visualization and Quantitation of Molecular, Cellular and Anatomical Structures

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ABSTRACT

The function of visualization is to help the scientist explore relationships in scientific data. Effective exploration requires rapid cycling between visualization and additional experiments, to the point of even visual "steering" the experiment. Integration of visualization with acquisition and computational facilities encourages further exploration. While specialized visualization tools have been developed for specific imaging tasks, many of the problems encountered in one imaging realm are common to other imaging realms. Thus, tools developed for medical imaging of anatomy can be generalized for use at the cellular and molecular level. Visualization tools common to the areas of molecular, cellular and anatomical imaging are explored.

INTRODUCTION

Visualization and quantitation of three-dimensional structural information presents recurring problems independent of the source of information. These problems center on the need for efficient capabilities to display, manipulate, and measure relevant information present in multidimensional (e.g. 3D and time) images. In the last decade, imaging packages have evolved to address the specific imaging problems at hand. While these problems are often shared by other members of the scientific community in visualizing biological structure, the tools to interpret three dimensional images is hampered by incompatibilities and narrow scope in their visualization software and hardware. We have attempted to explore some of the requirements necessary to assist in the process of understanding the complex 3D structural information arising from molecular models derived from electron microscopy (EM), cellular organelles sampled by confocal light microscopy, and delineation, evaluation and therapeutic planning of anatomical structures as visualized by CT and MRI imaging. It is hoped that the integration of these tools into an efficient and synergistic platform to permit the facile interaction with the 3D models without relying upon specialized hardware or extensive software knowledge.

These tools address four major problem areas common to visualization of molecular, cellular and anatomical structures: (1) presentation of visual representation; (2) correlation; (3) quantitation and understanding; (4) data management. The tools addressing these problem areas are not intended to be exclusive and are indeed progressively dependent on integration of earlier modules. These tools should be brought together into an intuitive and interactive program package covering the four major modules stated above. This paper does not attempt to explore modes of implementation or application specific problems, but attempts to address general requirements for three dimensional biovisualization.

1. VISUALIZATION OF THREE DIMENSIONAL IMAGES

The requirements for visualization of multidimensional data have greatly increased in recent years. Both the quantity of data and the variation of data types have greatly increased. However, rapid 3D visualization provides a handle to understand and use the vast quantities and varieties of data. Visualization of 3D information encompasses many different aspects, ranging from static rendering, real-time orientation and interaction of specific volumes of interest, precise object discrimination, volumetric resectioning to present an optimal sectioning plane, reprojection of computed models for comparison with primary data, critique of segmentation methods, and evaluation of dynamic structures. Many of the requirements for visualization have been dominated by their high dependency on the type of data involved. This section will discuss common elements and attempt to specify basic requirements for visualization.

Three dimensional data is often collected from a series of contiguous two dimensional images or slices. The quality and inter-relationship of these two
dimensional images directly influences the resulting 3D volume. These image slices must be accessible in a rapid and flexible fashion. A display on arbitrarily selected regions of the display screen. Tools for magnifying, shrinking and panning the images, as well as dynamically mapping the data to the full range of screen intensities must be at hand. Image restoration, enhancement, and reconstruction modules [39] should be accessible at this level. Aspects of visualization have evolved that can be readily accessible. Specifying volumes of interest by region, voxel density or other segmentation criteria would be of great utility. The ability to view select volumes both in isolation and within the overall spatial context permits the user to focus on specific areas of interest free from overlapping and potentially obscuring structures. 3D rotation, translation, and scaling is necessary, as is the display of multiple objects controllable transparency (to see inside and behind objects) and color coding (for discrimination in complex displays). As before, mensuration, marking of groups of structures, general annotation and subsequent saving of the manipulated volumes is also necessary.

Three principal approaches to 3D visualization have evolved that can be grouped by their most primitive representational elements: contours, surfaces and volumes. Each of these methods have evolved from specific imaging environments. Historically, the selection of the appropriate approach has been dependent on characteristics of the input data and graphical environment of the user. Contours are formed by manual or semi-automated delineation of boundaries within the data. Surfaces can be obtained by placing tiles between previously specified contours or by tracking the edges of regions specified by boundary conditions. Approaches using contours or surfaces greatly reduce the amount of data needed to be stored, resulting in fewer computations and thus rapid manipulation and visualization. Widely used on small graphics systems, this method of visualization is dependent on extensive amounts of preprocessing by the user. The quality of the final visualization model is limited by the fidelity of the contouring process. Even with the highest fidelity, complex and convoluted objects may be very difficult to preprocess and visualize by either contours and surface based contours. Volumetric processing involves very little preprocessing, since the entire volume is accessed. Any part of the volume may be viewed without preprocessing. Routine volumetric imaging coupled with the necessary tools discussed here require substantial computer power and large bandwidth and closely coupled imaging capability. While it is not the method of choice for small, memory constrained systems, volumetric methods may turn out to be the most useful since they are largely independent of the characteristics of the primary data and make few assumptions about the data present in the volume.

A. Contours

Contours are piecewise linear elements usually delimiting boundaries within the image. Reducing a large volume of primary data to a few sets of contours results in a major data reduction. Subsequent manipulation of the 3D data, as presented by contours, can be performed with few computation and thus reasonable speed. Contours are readily obtained by simple thresholding of images with high signal-to-noise (S/N) ratios or ordered search strategies [34] and boundary gradient methods [29]. Automatic edge extraction of high contrast boney structures using an interactive, multiple windowing system [32] has been very effective used by Dev and Fellingham to examine musculoskeletal disorders. Pizer has developed a semi-automated contouring program with manual interaction whereby the user can control automatic contouring, edit contour segments, and manually retrace portions of the image [29]. Images with low contrast and poorly defined edges, such as the soft tissue of CT images, are often contoured by hand on a slice by slice basis. This tedious procedure of manual contouring can take several hours per 3D volume, depending on number of object, and complexity of each object. In addition, this type of display provides the extracted information obtained
from each two dimensional plane, but leaves gaps between the planes \([46,47]\).

B. Surfaces.

Surface approaches toward visualizing 3D data usually take the form of tiling and surface tracking. Tiling involves taking contours obtained by manual tracing or automatic methods and fitting polyhedral surfaces \([6,14,16,24,32]\) or other higher ordered structures \([41]\) spanning the gaps between the planes. These methods have problems with spanning branches in structure or accurately following convoluted webs of structure. As before, obtaining the contours is very time consuming and full of potential error. Complex and convoluted structures may be erroneously produced without interactive examining and editing of the tiling. Tiled surfaces have been found useful. Isodose contours and radiation beams have been displayed as colored lines and surfaces with shading \([2,6,32]\). Tiled surfaces have also found utility for the display of bone and prosthetic implants \([15,30]\).

C. Volumes.

Volumetric visualization does not explicitly extract contours or surfaces, but instead work on all of the voxel elements composing the entire volume of primary data. The amount of preprocessing is minimal in comparison with previously mentioned problems with manual or automatic tiling. While initially appearing computationally inefficient in having to handle all of the voxels, very simple visualization algorithms can be generated that may be suitable for hardware implementation \([17]\). All of the primary data remains present, permitting many different functions can be obtained from the same algorithm. One example would be the simultaneous oblique sectioning of shaded surface displays to reveal primary voxel densities inside of the specimen.

Three dimensional renderings can be generated by several methods, including thresholding or selecting a window of densities and rendering the voxels in a back-to-front \([BTF] \([13]\) or front-to-back \([FTB] \([33]\) order to remove hidden voxels from the resulting display. Other rendering schemes, using the entire dynamic range of the primary data, are called the shaded density or colored range method \([9]\). These methods have been reviewed by Goldwasser et al., \([17]\) and Farrell et al., \([10]\).

Reprojection of the 3D volume has proven useful in volume rendering for both generating new 3D views and for generating views for comparison with other data. Reprojection is usually accomplished by either parallel or orthographic projection \([19,20]\) or by some form of perspective ray tracing \([3]\). Source-site to check the reprojections assign a source strength and an attenuation coefficient to each voxel to allow for object translucency \([22,36]\). Depth shading algorithms that generate surfaces by tracing rays through the volume array until they hit a threshold defined surface and then assign an intensity inversely proportional to the distance from the eye \([46]\).

Buhle and Altschuler \([3]\) have developed a rapid and parallel method for generating perspective ray tracing through CT volumes to synthesize x-ray like images. These reprojected images can be directly compared with two dimensional planar radiographs obtained prior and during the delivery of therapeutic radiotherapy. This comparison permits the evaluation of treatment planning and verification of the delivered radiation dose.

Parallel or orthographic reprojection of a three dimensional molecular model obtained from of high resolution electron images of ordered arrays of proteins is a fairly standard proof of the fidelity of the reconstruction \([39]\). Tilted 2D images of crystallized proteins are obtained at different orientations. The Fourier transform of these tilted projections are aligned to a common phase origin and combined as central sections of a 3D Fourier transform. Once the 3D Fourier transform results in a 3D reconstruction of the structure. A simple comparison of orthographic projections of this reconstruction with the appropriate tilted primary images is frequently used to check the feasibility of the reconstruction.

Tools to evaluate and explore the resulting 3D rendering are quite important. Rapid rotation coupled with variably oriented sectioning planes permits features of interest to be explored within their 3D environment. Rapid rotation can also be used to create animation, achieving the perception of depth, shape, and relative location. Animation uses the following spatial clues; \(1\) by rotating data about its center, points in the front and back of the data can easily be distinguished by the eye since these points move in opposite direction in the 2D viewing plane.
(2) 3D perception is enhanced as multiple objects are moved in a different direction. (3) Disjointed fragments are seen as parts of the same structure since they move in a coherent fashion.

Stereoscopic display provides clear depth perception. However, implementation requires computation of two images at different orientation and specialized viewing hardware. In addition, approximately 10% of the population is unable to perceive depth from stereo pairs [35].

2. CORRELATION BETWEEN THREE DIMENSIONAL IMAGES

Comparison of 3D images with similar data taken at different time points or via other imaging modalities is an essential process in understanding the system under study. It is important for all analytical comparisons to be able to overlay images showing good structural resolution. This requires the 3D information to exist in a common spatial domain and dynamic range. It is customary for 3D images to be of different sizes, different orientations of acquisition, spacing, and section thickness. There are frequently corrections necessary that are specific to the data acquisition. In comparing 3D images obtained from different modalities, features seen in one modality may be difficult to see in another modality.

Images to be compared must be brought to a common spatial framework to allow for geometric alignment. Anamorphic scaling, rotation, and translation can be performed by manual examination of a low resolution volumetric rendering. This visual correlation is accomplished on the basis of reference landmarks common between the two sets of images. In images of the head, facial features such as the eyes, nose and mouth can be used to bring the volumes into a basic congruence. Internal landmarks, such as the mandibular fossa and the outline of the third ventricle, can also be used. More objective and analytical procedures discussed below can then be used to refine the alignment.

Assuming the two 3D structures are rigid and undeformed between measurements, alignment of 3D rendering on the basis of external fiduciary landmarks is clearly the simplest. Linear regression to obtain the appropriate transformation matrix relating the two structures can then be obtained [8]. Landmarks can also be obtained by obliquely sectioning one volume and comparing the oblique sections with the sections from the reference volume [27]. Each landmark on the reference image is cross-correlated with the corresponding landmark from the other image. Because of differences in sampling, as well as a number of reasons, distinct landmarks common to both 3D volume are often difficult to accurately identify.

Chen et al. [5] have devised a surface fitting scheme to parameterize the tiled surfaces from 3D contours, minimizing the volume between the two comparable surfaces. This method is based on correlating the 3D data in each scan plane of the first data set, forming a tiled surface. This surface is then transformed to the coordinate system of the second dataset. Transform contours are generated based on the intersection of the tiled surface from the first data with the primary sections composing the second dataset. This method has found some success in aligning intracranial lesions from CT data.

Other schemes take surfaces from the sample data and elastically deform it relative to the reference surface until a minimum of the potential energy is found [37]. Schwartz et al. [37] matches brain ventricles in the CT data of the head with those in the brain atlas, using an initial alignment based on first and second moments, followed by an iterative local matching. These techniques imply the ability to find distinct edges of structures. As discussed earlier with automatic contouring routines, edge detection techniques are dependent on the signal to noise characteristics of the images. Correlation of images has long been performed with Landsat data [1]. Unlike graphical data, where geometric transformation involves a matrix operation to move a vector or surface, image data can be manipulated by resampling the spatial continuum of the primary data.

3. QUANTITATION and INTERPRETATION

The ability to measure distance, area and volume have been suggested as the most important feature of 3D visualization [21]. From 3D volumetric data, one can also compute 3D shape descriptors, accurately define volumes, and perform 3D morphometry. The simultaneous use of multiple modalities to extract diagnostic information can synergistically address the problems encountered using a single imaging approach. By using parameters complementary to each other that are derived from images generated by multiple modalities, a more accurate quantitative and qualitative analysis of the images is possible.

Quantitation and image interpretation occurs at three levels, termed low,
intermediate and high level processing. Low level processing covers the extraction of image feature information, such as distance calculations, selection of areas and volumes of interest, local area statistics, edges, and gray level histograms from 2D or 3D images. Intermediate level processing involves segmentation, homogenous region growing, mathematical morphology [18] and information regarding motion between sampling studies [43].

High level processing is more of a Gestalt process, examining and understanding the three dimensional visual information in its totality. This higher level of understanding is necessary if the dynamics in three dimensions is to be explored. Examples of this level of processing in cell biology can found in typical 3D confocal images of fluorescently labelled receptors, endocytosed into the cell. Is the movement of these labels Brownian or vectorial? To understand the general pattern of organization of these labelled structures, data concerning both the global and local information regarding patterns of molecular distribution must be easily extracted and quantified over a statistically significant sample of cells [11].

Similar questions can be asked of structures examined by high voltage electron microscopy, serial section studies, or the high resolution structural analysis of supramolecular structures in different physiological states. These tools would be useful in further analysing the subunit arrangement in the sensitized and desensitize acetylcholine receptor [45], or the difference in structure between the nicotinic acetylcholine receptor with and without the 43-kD subunit [29]. Analysis of the position and functional results of induced crosslinking and other binding studies [25] could be examined of.

Rapid 3D visualization is essential to understand complex structural changes. For example, intracranial pathology frequently causes shifts of intracranial structures in any or all of the three planes (axial, sagittal, and caudal), making interpretation from serial 2D CT scans difficult. One of the advantages of 3D representation is the ability, in one image, to appreciate the relationship of pathology to normal cranial and intracranial structures. Shifts of anatomical structures in any or all planes can be rapidly appreciated in 3D representation by comparing the uninvolved side with the pathological side.

4. DATA MANAGEMENT

The large quantity of information that is visualized must be stored in a coherent and readily accessible fashion. Additional information, such as measurements from the visualized data and other pertinent knowledge should be stored in a similar way. Both the storage of the data and the mode of retrieval is clearly a database problem whose requirements are currently nebulous and therefore will not be discussed here. As more investigators begin to use 3D visualization on a routine basis, the questions they ask will be useful in designing the preliminary schema for the database. However, it is clear that the user should have access to some such database containing an interface free from the details of implementation and suitable for standardization. This database should be portable to allow sharing and cross-pollination of knowledge.

Summary

Visualization of three dimensional images encompasses common requirements for exploring relationships in the scientific data. Four general areas discussed here are: (1) presentation of visual representation, (2) correlation, (3) quantitation and understanding, and (4) data management. There are undoubtedly other areas which could have been included in this paper. The first topic has been extensively explored and is an active area of research. Correlation of deforming and elastic structures is an active area of research. Quantitation and understanding of the complex systems is being aided by the tools of visualization, but this area is still quite nascent. As the field of biomedical visualization evolves and matures, the information for designing the appropriate data structures for a database will evolve. It is an exciting area of work with a promising future.

REFERENCES


