Images Of Serial Sectioning In Electron Microscopy : 3D Visualisation Of Objects Of Biological Interest

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Abstract

In the case of serial sections observed by the means of an electron microscope, it is possible to rebuild an image of an object, using the local intelligence of an image work-station without the need of a powerful computer. We will explain the basic principles of a program that we have written and explain its further developments.

The problem

Serial sectioning at the ultrastructure level provides information about the 3D organization of the cell and subcellar components. Out of the experimental difficulties which will not be discussed in this paper, the problem is that one obtains a 2D information for each serial section. Then, from a series of images, one must try to rebuild a 3D image [1-3].

Various techniques have been used to solve this problem. Each image in each section is reduced to a set of contour lines but this is a slow and tedious process. Then with the help of a computer one may plot either stereographic projections of the sets of contour lines [4-7] or draw a perspective image as this is usually done for the representation of block diagrams [8-10]. More sophisticated methods using a wire frame reconstruction of the object have been developed [11].

The appearance of the object is rather crude since it is reduced to a set of black lines on the paper. The use of a TV monitor allows to rebuild shaded images : starting from a mesh of the surface, it is possible to fill the facets with colors and vary the intensity of the light according to the inclination of the facets. The 3D appearance is drastically improved [12].

The problem of the image reconstruction may be summarized in the following scheme (fig.1) :

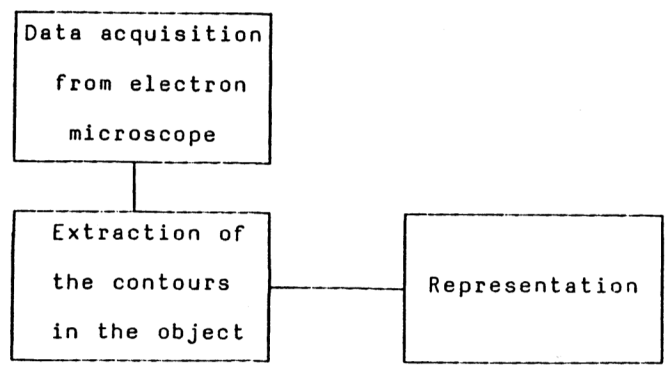

Figure 1. Principle of the image reconstruction.

Difficulties appear at each stage of the process. We will briefly discuss the different steps :

- The acquisition of the image may be done either using photographic emulsions or TV cameras. The greatest advantage of this method is that it allows to directly transfer the data into a picture system. The main limitation is that it is not possible, in the same time, to get a high resolution and a large field of view, since the number of pixels along a line or a column, is fixed in all TV systems.

- The definition of the contours for the object is usually done by hand : this is a long and tedious work. Various methods have been developed to define these lines automatically [10] [12-13] ; however it always required manual corrections :

1) The section may be damaged in the microscope and locally distorted : since this is not a systematic deformation of the image there is no way to avoid manual corrections at this stage.

2) Optical aberrations in the microscope must be corrected. This problem has been already solved in computer simulation of electron micrographs [14].

3) The contrast may be too weak to let an automatic system determine a contour line. Pattern recognition methods are of great help and may be included in the workstation. However the most sophisticated one may need a fast link to a main frame.

- An object may be represented either as a wire frame or by its surface with colors and lights. A wire frame representation needs rather sophisticated calculations to determine the polygons. Moreover, when the distance between following serial sections is not constant, using an interpolation method to determine the missing lines may lead to wrong representations especially for biologic objects which shape may be rather complicated. The representation of an object as a wire frame may be quite obscure, especially when one is interested in looking to secondary structures inside the object : it is necessary to use stereographic images or sophisticated 3D graphic systems which allow to rotate the image in real time.

The rapid sophistication of computer systems and workstations now allows a complete different approach : solid modelling permits 3D representations of excellent quality which are easy to observe and provide a lot of details in a single image. However it may need very large computers to compute an image. Recently, simple techniques have been developed [15] which allow to represent images of good quality using only an image workstation.

We have adapted such a technique to visualize the details of 3D organization of some nuclear organelles. Our results show that this method is very well suited for the observation of serial sections in electron microscopy.

Principles

The aim is to present a 3D image of an object using an information acquired through the digitalization of a series of 2D images, each of them beeing a cross section at different altitudes through the object (fig. 2).

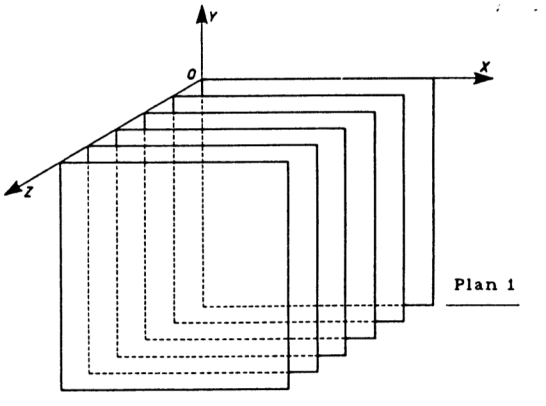

Figure 2. Stacking of the sections

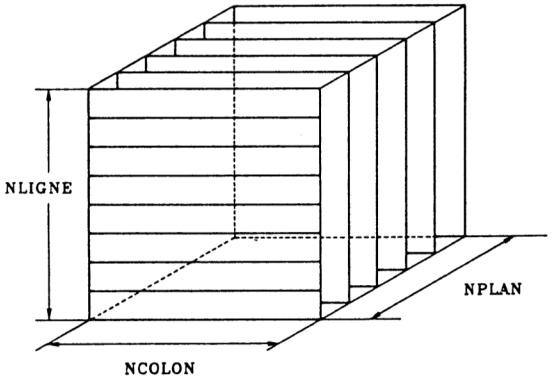

Figure 3. Continuity of an object

In each section different areas must be shown in the image. They are recognized by their contrast ; image filtering permits to enhance the contrast when it is too weak. This is quite well known and may be implemented in the workstation [16]. We will not discuss this point since it does not require special developments for image representation.

We assume that the distance between two following sections is constant. When some of them are missing it is easy to introduce additionnal sections by a simple interpolation : this is directly included in the basic software of most workstations and does not need a special software. This interpolation does not create additionnal information : if an organelle exists in a given cross-section and disappears in the next one, the interpolation will smooth the intermediary section but there is no way to rebuild the exact shape of the object in the volume where we do not have any 2D image : the interpolation is just a process to smooth the image.

In each cross-section the data are coded on one byte, each value corresponding to a different level of grey in the original image. These data are structured into segments. A segment corresponds to all data which values are included between two limits S_{min} and S_{max}. A given segment is represented on the screen with a given hue, choosen by the operator.

The 3D image is obtained, drawing each cross-section starting from the back size of the object, using a cavalier perspective (fig. 2). It is possible to define a window in the object, limiting the number of lines (NLIGNE) and columns (NCOLON) in each cross-section and the number of sections (NPLAN) as schemed in fig. 3. It is very easy to eliminate the hidden parts of the object : since we start from the back, each lighted pixel, in the cross-section to be drawn, will replace any preceeding information from another pixel belonging to a preceeding cross-section. This means that the limits S_{min} and S_{max} of the segments belonging to a given cross-section must not be continuous, otherwise all the preceeding cross-sections would be automatically hidden.

The impression of depth is increased by varying the intensity of the light without changing their hue and saturation : the cross-sections in the back are darker than the ones in the front. Figures 4 and 5 show computed images drawn according to these principles.

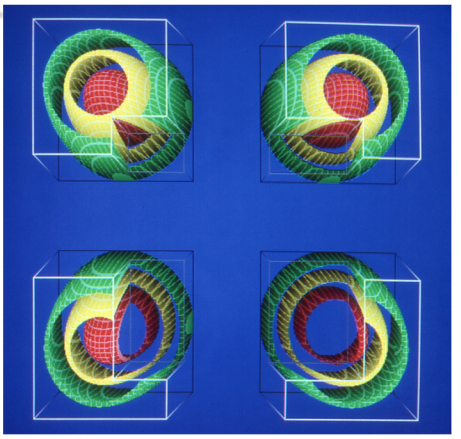

Figure 4. Representation of the contents of a sphere.

Fig. 4 is the representation of data inside a sphere : the values are proportionnal to the distance to the center. Red means low values, blue large ones. Four different points of view are drawn in the same picture. Other are available : by reversing the front and back cross-section the back size of the object may be easily seen. Fig. 5 is the representation of a theoretical electronic density around a tetramethylmethane molecule, computed from cristallographic data. The high density of electrons around the carbon atoms is shown in red. The choice of the window allows to have a look inside the molecule. An additionnal part is removed on the top right of the image. This permits to look very easily to new details inside the molecule. This cut may be introduced at any corner of the image to look at details of interest when they would normally be hidden by other parts of the object in the front. Contour lines have been drawn to increase the depth impression but this feature is optional.

An important point must be noticed, in this image : the quality of the 3D representation is good because one represents shells of a certain thickness : this means that the limit values of a segment S_{min} and S_{max} must be wide enough to obtain a correct representation.

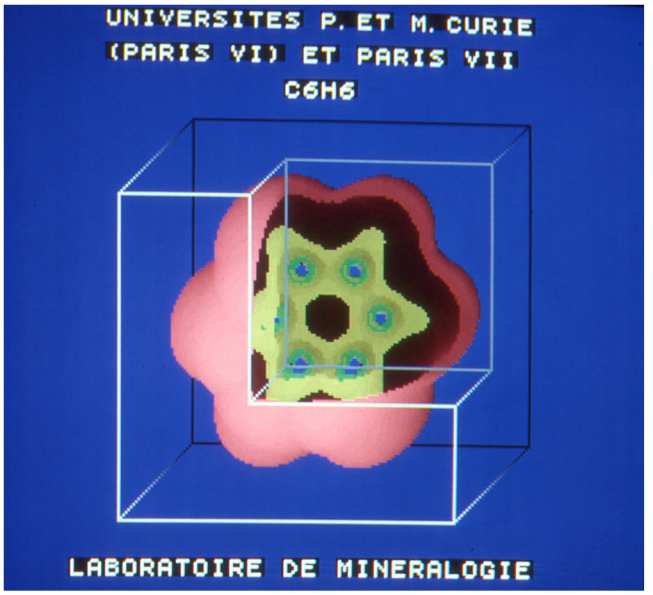

Figure 5. Electronic density around the atoms of a ~~C_6H_{12} (tetramethylmethane)~~ C_6H_6 Benzine molecule.

Application to biology

In biological electron microscopy the preceeding technique may help to clarify the relationship between the different elements of the structure. This is of the utmost importance in spatial biology where one is interested in the modification of the cells when submitted to low gravity [17]. As a test we have drawn the image of a cerebellar explant from a 5 days-old newborn hamster [18]. Since, at that time, we did not possess a television camera to digitalize the images, the main features of the cell have been drawn on acetates, then digitalized on a plotter. This explains why the segments, in the image of fig. 6 are reduced to thick lines.

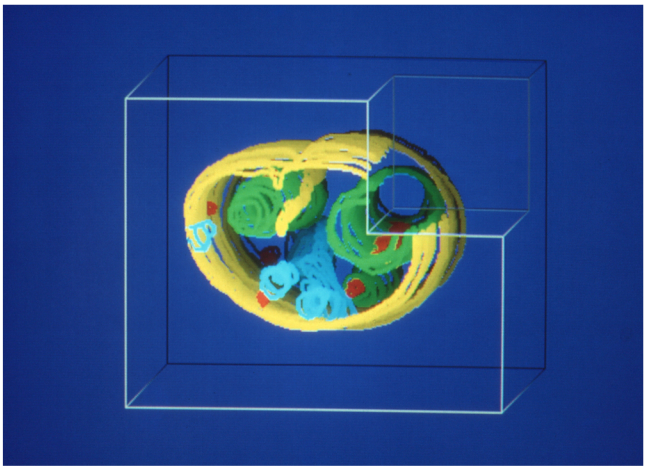

Figure 6. Nucleus of a cell of cerebellar explant of hamster.

The nuclear envelope is yellow, the nucleoli are blue and the nuclear bodies are red. The cell was infected by measles virus and the nuclear viral inclusions are drawn in green. The examination of this 3D image has revealed features which were not visible by the means of the techniques usually employed in cytology. Two nucleolar channels are clearly visible and were not known before.

Conclusion

Fig. 6 was just a test image to know if 3D representations could be of interest for the study of serial sections in electron microscopy.

The conclusion has been that such a technique may be employed as long as the contrast of the features, one is interested, is clearly visible in the images. More sophisticated techniques may be used [19], but need long calculations. The great advantage of this representation is that it can be easily included in the basic package of an image workstation. This will be the next step of our work.

Using a workstation will help the experimentator at each step of his work : acquisition may be done automatically. A graphic tablet and the software included in the station allow a better and faster work than drawing contour lines on acetate sheets. 3D images may be drawn very quickly and speed up the work since it is possible to draw a first image with a limited number of serial sections.

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