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MICROSCOPY, MORPHOLOGY AND MICROCOMPUTERS

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ABSTRACT

Two microcomputer programs for morphological analysis are described. IMPS is a program for setting up and carrying out a point-counting stereological analysis. GRIDSS is a set of routines for serial section reconstruction and viewing.

INTRODUCTION

The relatively low price of powerful microprocessor-based microcomputers has made it possible for individuals to have access to their own dedicated computing facilities. Such machines are most suitable for data capture and handling in the analysis of structure (Hoppeler et al. 1980) and two interactive microcomputer programs which can be used for structural analysis are described below. The first of these (IMPS) is a general-purpose program for stereological analysis, on random sections, of shape-independent parameters using point and intersection counting. It has been described in detail before (Briarty and Fischer 1981) and is based on an earlier mainframe program (Gnägi et al. 1970) but is included here as an example of the way in which a general-purpose microcomputer can be used for a specific aspect of structural analysis without any extra or specialised peripheral equipment.

The second program (GRIDSS) exemplifies a complementary approach to the analysis of structure, being used for the reconstruction of three-dimensional objects from serial sections. While this process can be carried out by model construction or similar methods (Gaunt and Gaunt 1978) these techniques have drawbacks, and now that graphics software is readily available such reconstruction can be carried out using a computer.

The program uses a digitizer tablet as an input device to enter tracings of serial sections, and output is to a monitor screen or whatever devices are available. It differs from previously published programs (e.g. Levinthal et al. 1974, Veen and Peachey 1977) in that it requires no more specialised input devices than a (commercially available) digitizer tablet and that it is written using the device-independent graphics package GINO (CAD Centre, Cambridge) which runs with FORTRAN. These factors mean that the program is portable and, since GINO is now available on a microcomputer, may be run on such a small system as well as on the larger machine on which it has been developed.

IMPS: an interactive stereology program

This is written in PolyMorphic disc BASIC and is stored as three sub-programs which are automatically CHAINed in; it requires at least 48Kbytes of free RAM in which to run, and the system needs at least two disc drives and a printer for output.

The program has been written in an interactive form; its use requires no programming experience on the part of the operator who, having designed a particular experiment, sets it up on the computer. To this end record sheets are available which should be used in conjunction with the manual (Briarty and Fischer 1981). This imposes a definite structure on the experiment and aids the user in identifying what he wants to measure, and more importantly it means that the process of entering the experimental design data into the computer involves simply entering numbers from a sheet.

The structure of an experiment, which must be adhered to, is taken from that developed by Gnägi et al. (1970). An experiment is divided into a number of experimental groups. This could represent the number of developmental stages being compared in an organism (Briarty, Hughes and Evers 1979) or different dosage rates of a drug being administered (Staubli, Hess and Weibel 1969). Each group is further divided into a number of experimental objects, for instance the number of organisms used at each developmental stage or the number of animals receiving a particular dosage of drug. Each object, in turn, is comprised of a number of representative samples. Finally, each sample is composed of a number of pictures, light or electron micrographs depending on the level of magnification being used.

After defining and entering the experimental design picture data are entered using previously defined keyboard keys corresponding to compartment point counts, object and intersection counts; during data input a number of display and correction options are available before counts from a picture are finally entered. The picture data are subsequently summed to give sample data for each sample and sample parameters are calculated when all the data for a sample has been entered. At the next level, object data are calculated; object parameter means and standard errors are calculated using two separate methods. In method I, each sample is considered to be a representative statistical sample and object means are calculated as means of sample means, and object standard errors are calculated in the usual manner. In method II, all the picture data for a complete object are summed and taken to be a representative statistical sample.

At the object level, absolute values of

volume fractions and surface areas are also calculated using an externally supplied value for cellular volume or a value calculated from the numerical density of nuclei (NVnuc). If the latter option is selected, cellular volume is taken to be equal to the reciprocal of NVnc on the assumption that cells are mononuclear. Finally, when all the group data has been calculated, Student's t test is applied to group parameters for any two groups selected. Significance tests are performed using three levels of significance: $P<0.05$, $P<0.01$, $P<0.001$.

During the data input stage the program may be halted and all the data and variables are saved as disc files; similarly data files for each object and group are saved automatically on completion. The data files can subsequently be used to re-run data through a different set of experimental calculations.

GRIDSS: a serial section reconstruction program

This set of three programs carries out the storage of graphic data from serially sectioned material (GRIDSAVE), alignment of the individual sections (GRIDLINE), and then the display of part or all of the series of aligned sections from any chosen viewpoint (GRIDDISP). The programs are written in BASIC and FORTRAN; currently GRIDSAVE (BASIC) and GRIDLINE (FORTRAN) are run on a RML 380Z microcomputer (Research Machines Ltd., Oxford) with 56Kbytes of RAM and two eight inch disc drives. The reconstruction program (GRIDDISP; FORTRAN) uses GINO graphics software and is available in two forms, one of which runs on the ICF Prime 400 computer on which it was developed, and the other which runs on the 380Z; thus the whole set of routines can be run, albeit in a slightly limited way, on the microcomputer.

Input of primary graphical data is via a 'Bitpad' digitizer tablet, a cursor being used to trace around the shapes to be recorded. Thus any image which can effectively be positioned on the 280 mm square surface of the pad can be used;

photographs or projections of slides (35 mm transparencies or microscope slides) are satisfactory, or the Bitpad can be positioned under a camera lucida so that a microscope preparation can be traced around directly, eliminating the need for an intermediate photograph.

GRIDSAVE when run requires the operator to enter experiment and section identification data and z axis position and then prompts for the input of graphical data from a section. A number of types of such data may be entered; registration marks, an outline (e.g. the external outline of an organ or other structure), internal outlines (e.g. profiles of internal structures of one or two different types) and one set of point positions. Keyboard entries are used to define the type of data and then intermediate and termination information is input from the Bitpad. When all entries from a section have been made the data are saved as xy coordinates in a named disc file.

GRIDLINE, the second of the programs, is used to align the primary section data in space and to produce a new set of aligned data files. One of the sections is chosen as a template and this and the section to be aligned are displayed together on the monitor screen. Using the Bitpad cursor the operator can shift and rotate the second section in relation to the template until the registration marks (or other cues) are aligned, after which the aligned section is finally saved. Subsequent sections can then be aligned in relation to this, or to the original template section.

The provision of registration marks on serially sectioned material is not always convenient or even feasible (see discussion in Gaunt and Gaunt, 1978), and where it is not possible this manual alignment procedure, using the section data itself, is satisfactory. However, where three reference marks can be provided on each section, a computer routine is available in the Prime implementation which will shift and rotate the section to give the best alignment of the two triangles produced by the reference marks.

The aligned data are converted via a small file conversion program into pseudofiles, that is to say files which can be accessed by the GINO-graphics routines. They can be displayed using GRIDDISP.

GRIDDISP allows the user to define first of all which of the total set of files (i.e. sections) making up the object he wishes to be displayed, and which segments of each section are to be shown. Thus it might be desirable to display only one of the sets of internal outlines of a particular object, with the external outline from every fifth section displayed to give an overall form.

The user now defines the viewpoint from which the object is to be displayed, this is done by determining first a 'centre of interest', usually within the object. This is in effect the origin of the Cartesian axes about which the object is to be rotated, and the user defines the angle (positive or negative) of rotation for the x,y and z axes, in any order. Scale factors, both overall and on the z axis, can be applied at this stage to change the overall image size. All these data are used to define a transformation matrix which is used by the program to display each section as though viewed from the defined viewpoint; the final image of the object is produced by the superimposition of all the section segments requested, spread out along the (transformed) z axis.

A suitable hidden line removal routine has not yet been developed, and the display of many sections containing large amounts of data can produce a confusing image. Depending on the output devices available this confusion can to some extent be resolved; picture segments of different types can be displayed in different colours (on a plotter or colour monitor) or the final image can be saved as a file and used to produce stereo pairs (Fig. 1). Using the Prime 400 implementation of the program files can be sent to an FR80 film plotter and recorded on 35 mm colour film as stereo pairs or anaglyphs (red/green superimposed images), or as animated 16 mm film sequences where the object can be rotated 360° around the z axis. The stereo effect thus produced allows a better understanding of the spatial relationships within the object.

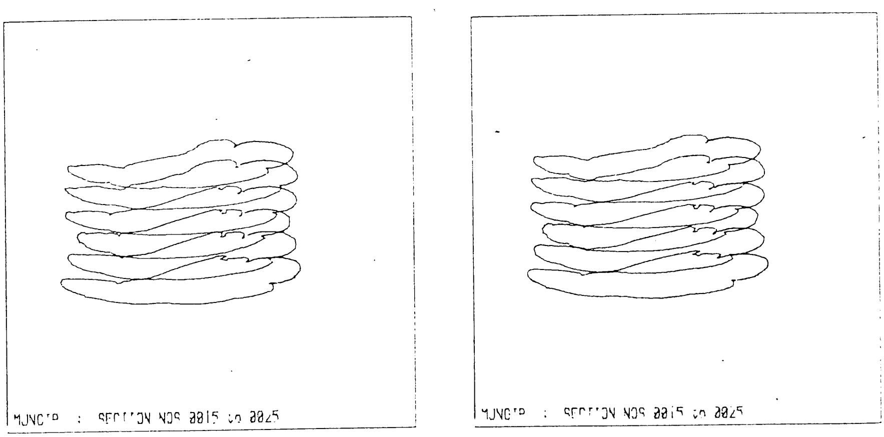

Fig. 1. Stereo-pair of outlines only from serial sections of part of a broad bean cotyledon. Most people should be able to fuse the images visually without difficulty.

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