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REVIEW ARTICLE

# SOFTWARE SOLUTIONS TO PROBLEMS IN STEREOLOGY

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## ABSTRACT

A compilation of essential details about computer programs developed by stereologists is presented. They are classified under the headings: classical point counting methods, size distributions, serial sections and 3-D reconstruction, statistics, educational aids, image analysis and special applications. It is intended to provide information adequate for the establishment of contact between stereologists with computing problems in common and to promote the free exchange of software. A classification of the programs by language and by machine is also given.

## INTRODUCTION

The computer program committee of ISS was formed at the request of the ISS president and board in 1980, under the joint chairmanship of the authors. Various scientists were approached, and the final constitution of the committee was as follows:

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|---|---|
| Dr D. Braggins | United Kingdom |
| Dr. L.G. Briarty | United Kingdom |
| Dr. S. Eins | F.R.Germany |
| Dr. S. Griph | Sweden |
| Dr. H. Hoppeler | Switzerland |
| Dr. V. Howard | United Kingdom |
| Dr. T. Jakeman | Australia |
| Prof. P.J. McMillan | United States |
| Dr. F. Meyer | France |
| Dr. L.D. Peachey | United States |
| Dr. L. Scales | United Kingdom |
| Dr. H. Schwartz | F.R. Germany |

One of the first acts of the committee was to send a questionnaire to each member of ISS inviting participation in a software information scheme, of which this report is the result. 320 questionnaires were mailed, 39 positive and 13 negative replies have been received to date. In addition, members of the committee have helped to compile a list of relevant citations.

We aim to give enough information for you to decide if a particular program may be of use. Thereafter, personal contact should be made with the author. Co-operation by authors with other ISS members is implicit with inclusion in this report and those who answered the questionnaire are marked with an *. Other inclusions in the report are gleaned from the literature and although it is hoped that those authors will co-operate in releasing software, this cannot be guaranteed.

Professor Paul McMillan conducted a poll of members of the 1st North American Symposium for Stereology in September, 1981. 45% of participants responded to that questionnaire, and although the sample was small, the following results were obtained:

1. Of those who utilised computers, 8% only had access to main-frame or time-share systems, while 39% only had access to an in-house microcomputer. 53% had access to both sorts of systems.

2. Therefore 66% of respondents to the questionnaire had access to mini- or microcomputers, and all of these used some software written "in-house".

3. 44% would have liked the opportunity to discuss software problems with other stereologists.

4. 37% of respondents were actively engaged in acquiring a new machine.

The majority of the committee managed to meet in Sheffield at "Stereology '82", and we decided to publish this report under the seven main headings given below. It was difficult to arrive at an ideal classification, but this one seems to work and it is open to modification at a later date. At the end of the report the programs are re-classified, using their code numbers, both by machine and by language.

Software development is so time consuming that it is hoped to extend this service to ISS members by publishing regular supplements to this initial report which will include recently developed programs written by stereologists.

The committee has also considered other areas in which it could usefully work. Among these are the standardisation of data formatting, to facilitate portability, which was thought to have high priority. However, apart from recommending paper tape as a reasonably fool-proof method, we decided that the problem could not be tackled in the time available before VI International Congress for Stereology in Gainesville, Florida. It remains a problem area for the future work of the committee.

Over the next decade, we expect an increase in the production of in-house software, particularly in the field of 3-dimensional reconstruction. The structure of ISS provides an excellent vehicle for the dissemination of these programs to other interested users, and we would like to encourage those of you with complete packages to communicate with us at the earliest opportunity.

The report is organised under the following headings:

A - Classical point counting methods.
B - Size distributions.
C - Serial sections and 3-D reconstruction.
D - Statistics.
E - Educational aids.
F - Image analysis.
G - Special applications.

## A. CLASSICAL POINT COUNTING METHODS

1.* **Author:** L.G.Briarty and P.J.Fischer.
**Program:** IMPS, a BASIC (PolyMorphic) program designed for the collection of point counted stereological data. Allows flexible equation construction using standard stereological formulae. Interactive mode. Size 46 kbytes. Any suitable micro. Now available in Microsoft BASIC.
**Hardware:** Requires 25 kbytes of free RAM.
**Reference:** J Microsc 1981; 124: 219.

2.* **Author:** H.J.G. Gundersen.
**Program:** Point counting of up to 9 items and construction of two size distributions of up to 10 classes (max. 1799 items in each distribution).
**Hardware:** Texas TI 59 calculator with PC 100C printer.
**Reference:** Stereological and Electronmicroscopical Diabetes Research Laboratories, Second University Clinic of Internal Medicine, University of Aarhus, Denmark.

3.* **Author:** H. Haug and A. Schliesser.

**Program:** A series of 5 Wang BASIC programs of between 12 kbytes and 20 kbytes to control and collate: a) data-input from KONTRON MOP-AM01 and 03, and a Videoplan, b) data review and correction, c) primary-data protocol on printer, d) creating data files on floppy-disk, e) differentiate cell types and collate information about cell positions, shapes and orientations.

**Hardware:** Wang 2200 28K machine with triple floppy disk.

**Reference:** Microscopica Acta 1979; 82: 147.

4.* **Author:** H. Hoppeler and K.R. Tyler.

**Program:** Simultaneous point, intersect and number counts of up to 10 variables by a fast machine code program specific for Tandy TRS-80. Primary data is then handled by a BASIC program which allows for the flexible use of equations,test systems and statistics.

**Hardware:** Tandy TRS-80. BASIC program can be run on any suitable micro.

**Reference:** Anatomical Institute, University of Bern, Switzerland.

5.* **Author:** M. Oberholzer.

**Program:** A BASIC program in two parts: 1) to collect and manage primary stereological data - area, circumference, length, orientation and co-ordinates. 2) is a transformation program allowing the data to be manipulated to produce stereological results.

**Hardware:** Apple II Europlus or IIe (48 kbyte RAM). 2 Apple disk drives. Apple graphics tablet. Printer.

**Reference:** Dept. of Pathology, Schoenbeinstr. 40. CH-4003, Basel, Switzerland. See also 'Morphometrie in der Klinischen Pathologie, M. Oberholzer, Springer Berlin, 1983.

6.* **Author:** L.D. Peachey.

**Program:** An inexpensive digital planimeter which can measure surface area and length on micrographs and costs about US $2000. Written in Z-80 assembly language "burned" onto 2716 EPROMS.

**Hardware:** Z-80 Starter kit, SD Systems Inc., Dallas, USA. 2 MHz clock, two bidirectional parallel I/O ports, 1 kbyte RAM, provision for three 2 kbyte PROM (or EPROM) modules, LED display, BITPAD-ONE (Summagraphics Inc., Fairfield, CT, USA) digitizer.

**Reference:** Ultramicroscopy 1982; 8: 253.

7.* **Author:** L.E. Scales, C.V. Howard and S. Griph.

**Program:** SAPS a series of four ALGOL 68 programs for simultaneous point, intersect and profile counting up to ten variables in each category. Allows flexible application of stereological formulae to primary data. The format of each experiment is stored in its own file. Each program requires about 60 kbytes of memory. Could be made available in PASCAL if there is suitable demand.

**Hardware:** Any machine offering ALGOL 68 and interactive facilities.

**Reference:** J Anat 1978; 127: 219. (short abstract)

8.* **Author:** C.A. Squier.

**Program:** Point counting of up to 10 variables simultaneously. Elementary (means) statistics on up to 6 data sets.

**Hardware:** Texas TI 59 with PC 100C print cradle.

**Reference:** College of Dentistry, University of Iowa, Iowa 52242, U.S.A.

9.* **Author:** G. Wassilew and K. Frohlich.

**Program:** A collection of formulae used in stereology and morphometry is stored in a computer program (PL/1). According to the class of entered data, relevant parameters are calculated together with corresponding statistics.

**Hardware:** The program needs 100 kbyte memory and is implemented on an EDVA EC 1040.

**Reference:** Acta Stereol 1982; 1: 73.

## B. SIZE DISTRIBUTION

1.* **Author:** William G. Fricke.

**Program:** GRSZ. This is a BASIC program of 150 lines of code which runs interactively. It uses the methods of ASTM standard E112 to calculate grain size from lineal intercept count in three principal directions (2 directions for axi-symmetric rod). Types report for specimen, giving a series of parameters based on the grain size. Should work for any space-filling polyhedra.

**Hardware:** Running on PDP11, but will run on any system supporting BASIC.

**Reference:** Alcoa Labs., Alcoa Centre, PA 15069, U.S.A.

2.* **Author:** K. Friederich.

**Program:** SEPAR. BASIC program occupying 550 lines of code which combines linear intercept measurements made at two different magnifications and fits two superimposed log-normal size distributions to the experimental curve by iteration, selecting a minimum Chi-square value. It prints the parameters of the distribution (means and standard deviations) and the minimum Chi-square.

**Hardware:** Minicomputer (KONTRON Videoplan), 64 kbytes, 2 dual density minifloppies, TV monitor, digitizing tablet with interface.

**Reference:** Max Planck Institut fuer Metallforschung, Seestr. 92, D7000 Stuttgart, F.R.G.

3.* **Author:** A.J. Jakeman.

**Program:** A package written in UNIVAC FORTRAN V for the solution of Abel equations in the estimation of size distributions and some of their linear functionals. The whole package is well described in the reference below.

**Hardware:** Any mainframe supporting UNIVAC FORTRAN. Will run in the batch or interactive mode.

**Reference:** Mikroscopie 1980; 37 (Suppl), 458.

4.* **Author:** L.E. Scales and C.V. Howard.

**Program:** Parametric and distribution free unfolding of unimodal distributions. Parametric unfolding to give individual components of polymodal distributions. Fitting of distributions without unfolding. Error analyses in all cases with graphical display of results using GHOST.

Under development to implement distribution free unfolding by Cruz-Orive (1983). To implement parametric unfolding with truncation according to Coleman (1982). To allow choice of parameterised model distribution. Language: ALGOL 68 (possibility of PASCAL soon). Also possibility of GKS international standard graphics.

**Hardware:** Batch mode, mainframe, approx. 1,500 lines of code, approx. 120 kbytes memory.

**Reference:** Dept. of Anatomy, University of Liverpool, PO Box 147, Liverpool L69 3BX, U.K.

5.* **Author:** A.E. Stacey.

**Program:** Field and Feature data, a BASIC (Hewlett-Packard) program running in 15.4 kbytes which provides structural information of the porous structure of coke with specific relation to strength properties. Type of data obtained includes porosity per cent, mean intercept sizes of pores and solid and their size distributions, perimeter, number of pores, and in the feature data mode; mean area, mean perimeter, mean convex perimeter and Feret's diameters of pores. Program gives standard errors of determination of mean values and print-outs of distributions.

**Hardware:** Hewlett-Packard 9820B combined with Quantimet 720 with Calculator Field/Feature Interface, Co-ordinate transform module, etc.

**Reference:** British Carbonisation Research Association, Mill Lane, Wingerworth, Derbyshire, U.K.

6. **Author:** R. Warren and M.-C. Durand.

**Program:** A suite of programs in BASIC to model 2-D sections through 3-D microstructures consisting of particles with arbitrary size and shape distributions chosen by the user. The two main programs make repeated random cuts through a single solid body of chosen shape, which are analysed for section area, perimeter length, Feret diameters, and number of edges (for polyhedra). Size distributions may also be created. Modifications are available for ellipsoids. The number-frequency distributions for these parameters are then stored and may be used subsequently to model real data.

**Hardware:** Runs on a Nordata ND 100 . The programs occupy 13 kbytes. Should be adaptable to any system supporting BASIC.

**Reference:** 'Swedish symposium on non-metallic inclusions in steel'. Published by Uddenholm, Hagfors 1981.

## C. SERIAL SECTIONS AND 3D-RECONSTRUCTION

1.* **Author:** B.Bauer.

**Program:** Digitizing stereopairs (T.E.M.) allows the calculation of x y z co-ordinates of particle centroids, their volume and nearest neighbour distances. Approximately spherical particles are assumed. FORTRAN, 16 kbytes.

**Hardware:** Digitizing tablet, parallax transducer, mirror stereometer, Videoplan (Kontron), 2 dual minifloppies, TV monitor, printer.

**Reference:** Stereol Iugosl 1981; 3: (Suppl. 1) 255.

2.* **Author:** B. Bauer.

**Program:** x y z co-ordinates of rough surfaces are calculated by digitizing stereopairs.

**Hardware:** Digitizing tablet, parallax transducer, mirror stereometer, Videoplan (Kontron), 2 dual minifloppies, TV monitor, printer.

**Reference:** Prakt Metallogr 1981; 18: 327.

3.* **Author:** L.G.Briarty and P.H.Jenkins.

**Program:** GRIDSS. A program for serial reconstruction using computer graphics. Individual section data are input from a digitizer pad and their reconstructed composite image can be displayed as if viewed from any direction. Stereo pairs may be produced. The system has been developed to run under COS 3.4 C/M and CP/M V1.4B. Extended BASICSG2 (V5.0H), a text editor, Microsoft LINK-80 and FORTRAN-80 together with the high resolution graphics package GINO-F (Computer Aided Designs Centre, Cambridge) are required. Total cost ca. £5000.

**Hardware:** Research Machines 380Z with 56 kbytes RAM, high resolution graphics and 2 double sided 8" floppy disk drives. Summagraphics Bitpad One with single button cursor. Houston Hiplot A4 graphics plotter.

**Reference:** J Microsc 1984; 134: 121.

4. **Author:** J.J. Capowski and W.L.R. Cruce.

**Program:** Spatial structures (neuronal trees) are computed using their x y z (focus)-co-ordinates in a microscope slide. Methods to correct artifacts of section handling and to correctly align adjacent sections are included in the data acquisition part of this interactive program. Finally, different aspects of a tree may be seen on the graphic display. FORTRAN.

**Hardware:** Stage equipped with stepping motors for x y z. Any micro- or minicomputer, graphic display, plotter.

**Reference:** Comput Biomed Res 1979; 12: 569.

5. **Author:** E. Dykes and F. Afshar.

**Program:** Traced x y co-ordinates from serial sections are used to reconstruct the original 3-dimensional structures. There are facilities to remove hidden lines and to inspect the specimen from different directions. The production of stereo pairs is also possible. Program is in FORTRAN.

**Hardware:** Plotting microscope with co-ordinate storage on paper tape, CDC 6600 computer.

**Reference:** Acta Stereol 1982; 1: 289.

6.* **Author:** S. Eins, J. Lessmann and J.R. Wolff.

**Program:** Visible parts of blood vessels in serial sections are digitized and superimposed. The program finds matching parts of vessels in different levels of sectioning and draws a positional map of an interactively selected vessel class. BASIC, 64 kbytes.

**Hardware:** Digitizer, microcomputer (HP 9845) with a graphic display, optional plotter.

**Reference:** Acta Stereol 1983; 2: (Suppl. 1), 149.

7.* **Author:** G.O. Fior.

**Program:** Data obtained by digitizing stereopairs (S.E.M.) are stored on a Silent 700 (Model 765, Texas Instruments). 10 kbyte processing program.

**Hardware:** Digitizer (1924 Numonics). Processing of stored data in a computer centre on a UNIX-system to obtain graphical representation as well as topographical and statistical data.

**Reference:** Lawrence Berkely Lab, Univ California, Berkley, CA 94720.

8.* **Author:** R. Gordon.

**Program:** An interactive FORTRAN program (75 kbytes) to prepare surface displays of 3-dimensional objects. Data from serial sections.

**Hardware:** CDC Cyber.

**Reference:** Quantitative Morphology Unit, Univ Manitoba, Winnipeg Manitoba, R3E OW3.

9. **Author:** P.M. Kaufmann, F.A.L. Dullien, I.F. MacDonald and C.S.Simpson.

**Program:** Contour data obtained by digitization of serial sections are used for 3-dimensional reconstruction. The resulting image may be displayed with or without hidden lines and - for better visual inspection - image rotation is possible. Designed for geological applications (sandstone pore structure).

**Hardware:** Digitizer, Apple II+, graphic terminal and disk. (Mainframe only for calculation of other pore structure parameters).

**Reference:** Acta Stereol 1983; 2: (Suppl. 1), 145.

10 **Author:** J.L. Paul.

**Program:** Contour data of serial sections are used to display objects as solid bodies. Changing of viewing direction and zooming are possible. Twisted objects and bifurcations are allowed. Section distance need not be equal to contour units.

**Hardware:** PDP 11/23 with 192 kbytes memory, 15 M disk. Input: Houston Instruments Hipad; output: DEC VT 100 upgraded with Selanas Corp. Graphics 100.

**Reference:** Graphics Interface '83, Edmonton, Alberta, Canada, 1983.

11. **Author:** A. Schleicher, L. Doering, A. Wree and K. Zilles.

**Program:** The existence and position of cortical barrels (features of differentiation in the central nervous system) is reconstructed from serial sections. Volume densities are measured automatically and the resulting data matrices are processed by filter operations. Special attention is given to the alignment of curved laminar data profiles in single sections and the correction of the section thickness effect on volume density.

**Hardware:** WANG 22100 MVP, Digitizer Bit PAD 1 (Summagraphics). Disk or floppy disk.

**Reference:** Acta Stereol 1983; (Suppl. 1), 85.

12.* **Author:** H. Schwarz.

**Program:** FRACTAL, a FORTRAN program for calculation of fractal dimensions of any boundary defined by up to 2000 co-ordinate pairs. Size 14 kbytes. Interactive semiautomatic mode.

**Hardware:** Minicomputer (KONTRON Videoplan) 64 kbytes, digitizing tablet, 2 dual floppy disks. TV monitor, printer.

**Reference:** Powder Technol 1980; 27: 207.

13. **Author:** J.C. Tipper.

**Program:** Fully or semiautomatic mode of data collection of profiles from serial sections. The reconstructed model is displayed as a wire-frame body. Alignment of structures is done by point-to-point matching of profile points in adjacent sections. FORTRAN. Required memory is 20 kbytes.

**Hardware:** Honeywell 66/60, incremental plotter.

**Reference:** Computer & Geosciences 1977; 3: 579.

14.* **Author:** A Veen and L.D. Peachey.

**Program:** Reconstruction by manual tracing of structural boundaries in serial sections. Interactive alignment of sections. Removal of hidden lines from a dynamically changing scene. Subprograms for tracing, editing, alignment and reconstruction. Generation of stereopairs. FORTRAN.

**Hardware:** Digitizer. Paper punch for primary data storage, teletype. Data collection with PDP 8L (4 kbytes of memory), data handling and reconstruction on PDP 10, interactive graphics terminal (Tektronix 4010), plotter. Rotation of the 3D-scene is accomplished by using a refresh-type display in connection with a special 3D-transformation hardware and PDP 11/40 (32 kbytes).

**Reference:** Comp. & Graphics 1977; 2: 135.

## D. STATISTICS

1.* **Author:** H.J.G.Gundersen.

**Program:** A series of statistical programs in TI-59 machine code. Performs Pearson product moment correlation coefficient, weighted regression analysis and calculates the variation of ratio estimators.

**Hardware:** Texas TI-59 calculator and PC-100 print cradle.

**Reference:** see A3

2* **Author:** Vyvyan Howard.

**Program:** OPTIMAX. A program in TI-59 machine code for performing nested analysis of variance (up to 10 levels) and optimisation of sampling effort, within animal, by "costings" at each sampling level.

**Hardware:** Texas TI-59 calculator and PC-100 print cradle.

**Reference:** see: J Micros 1981; 121: 65. For address see B4.

3* **Author:** A.Schmassmann and H.P.Rohr.

**Program:** An interactive statistics package accepting data either automatically from a Kontron MOP-AM2 or manually. Gives a very comprehensive analysis of the data, including: T-Test (paired and unpaired), Pearson, Mann Whitney, Wilcoxon, Spearman, Sign-Test, Kendall, F-Test and AOV. It is written in HP 9815A system language and occupies 2008 lines of code.

**Hardware:** Hewlett Packard HP 9815A and printer.
**Reference:** Institute for Pathology, Schoenbeinstr. 40, CH-4031, Basel, Switzerland

## E - EDUCATION

1.* **Author:** S. Griph.
**Program:** "Tomato Salad". A 20 K FORTRAN program which runs in the interactive mode and performs simulated sectioning through several populations of spheres using the Monte Carlo method. 3-D size distributions can be generated by the program or introduced by the operator. The sample size of 2-D profiles can be selected and also the section thickness. Polymodal size distributions can also be used. The program gives the operator a feel for the effects of sample size and section thickness on the process of "unfolding".
**Hardware:** Any mainframe supporting FORTRAN.
**Reference:** Computer Laboratory, Department of Technical Sciences, Uppsala, Sweden.

2.* **Author:** S. Griph.
**Program:** Sample size. A 60 kbytes FORTRAN program (ca 400 lines of code) which can run in either the batch or interactive modes. Performs nested analysis of variance on a hierarchical experimental design. The operator is able to choose the sample size at any particular level (e.g. numbers of blocks, micrographs, fields and measurements etc.). Furthermore, he is able to assign costs to each level of the experiment and see how this affects the optimality of his design.
**Hardware:** Any mainframe supporting FORTRAN.
**Reference:** Nil but see Shay, Am J Path 1975; 81: 503.

## F. IMAGE ANALYSIS

1. **Author:** P.E. Danielsson and B. Kruse.
**Program:** PICAP II
**Hardware:** 16 processors, including TV input, graphic overlays and filters are connected with 16 memory modules (256 kbytes each) via a fast (40 Mbytes) time-shared bus. The system is controlled by a conventional host computer and designed to operate as a fast and flexible picture processing machine.
**Reference:** Mikroskopie 1980; 37: (Suppl.) 425.

2. **Author:** R. Gardette, A. Mallet and J.C. Bisconte.
**Program:** Automatic recognition and characterisation of tissues by diskriminant analysis applied to the measured grey level histograms. First a learning sample has to be processed. The result is a typical densitometric pattern given by the components of a certain region (application: cerebellum).
**Hardware:** Texture Analysis System TAS (Leitz) MITRA 15-35 computer.
**Reference:** J Neurosc Meth 1981; 3: 233.

3.* **Author:** P. Lemkin and L Lipkin.
**Program:** Interactive or batch mode FORTRAN program for distributed image processing in a biological environment. The package allows one to store, edit, compare, segment and measure image structures, and to arbitrarily combine these steps.
**Hardware:** Tape, film or TV image input. PDP 8e with 32 kbytes (12 bit word length), disk storage with 1-6 Mbytes capacity. A Quantimet 720 is part of the system if TV images are considered.
**Reference:** Computer Progr Biomed 1980; 11: 21.

4.* **Author:** J.L. Paul.
**Program:** The following image processing procedures are possible; median filtering, affine geometric transforms, setting of rey-level thresholds followed by particle analysis, distance mapping and skeletonization.
**Hardware:** PDP 11/23 with 192 kbyte memory, 15 Mbyte disk.
**Reference:** J.L. Paul, Dept. Pathol., Univ British Columbia, Vancouver, B.C., Canada

5.* **Author:** K. Rodenacker.
**Program:** General purpose image processing and analysing program in FORTRAN IV/ASSEMBLER ASS 300 /PASCAL, 270 kbytes, interactive or batch mode. Calculates single object properties and stereologic estimates. Computer controlled movement of the specimen in x, y and z.
**Hardware:** Minicomputer Siemens P 330 (128 kbytes), TV camera, image memory, array processor, automatic stage.
**Reference:** Mikroskopie 1980; 37: (Suppl.) 421.

6.* **Author:** H. Schwarz.
**Program:** A 48 kbyte FORTRAN program to generate, test or measure point distributions in a given frame. Check of randomness, characterization of clusters and interference of regular arrangements belong to the output of this program.

**Hardware:** PDP 11/04, plotter, dual floppy disk.
**Reference:** J Microsc 1983; 129: 155.

## G. SPECIAL APPLICATIONS

1. **Author:** P. Cau and J.L. Boudier.
**Program:** A suite of BASIC (Hewlett-Packard) programs occupying 32 kbytes. The purpose of the programs is to design an experiment for quantitative autoradiography. It calculates the Chi-square value between grain and test circle distributions and calculates for each item the crude relative specific radioactivity, volume and surface densities and grain per unit volume or surface densities. Finally, it can be used to analyse the distance between grains and the plasma membrane in the grain density method of Salpeter et al. (1969).
**Hardware:** Hewlett-Packard HP 85 microcomputer, dual floppy disk drive, printer and plotter.
**Reference:** Acta Stereol 1982; 1: 285.

2. **Author:** V. Cavallari, G. Basile and A. Maiorana.
**Program:** Acquisition and processing of typical parameters in peripheral nerve morphometry (fascicular area, size distribution of fibre diameter, myelin thickness, myelin-axonal ratio, number of myelin lamellae, number of neurotubules and filaments).
**Hardware:** Apple microcomputer, digitizer, dual disk.
**Reference:** Acta Stereol 1983; 2: 373.

3.* **Author:** S. Eins.
**Program:** A series of BASIC programs to control a HPIB-compatible microscope stage for different kinds of stage positioning in a given microscopic specimen including: systematic, random, systematic random, circular, straight or curved lines. 6 kbytes of memory.
**Hardware:** Microcomputer 9830 or 9845 (Hewlett-Packard), HPIB compatible automatic stage with 0.25 um minimal step size and 2.5 mm/s maximal speed. Optional plotter to show the positions of selected fields.
**Reference:** Acta Anatomica 1981; 111: 38.

4.* **Author:** S. Eins.
**Program:** This 6 kbyte BASIC-program has been designed for automatic measurement of inhomogeneous vascularization of the cerebral cortex of rats, but it may be used in other

applications too. The program controls the stage movement along a predefined line and registers the changes of basic stereologic estimators (e.g. lamination of tissue) as obtained from section measurements by a Quantimet image analyzer.

**Hardware:** Microcomputer HP 9830 or equivalent, Quantimet 720, microscope with automatic xy-stage.

**Reference:** Adv Anat Embryol Cell Biol 1980; 59: 1.

5.* **Author:** B. Erne and H.P. Rohr.

**Program:** 'Morphometric calculations prostata LM'. Calculation of corrected volume densities, surface densities, length density, mean diameter of acinus, mean epithelial height and mean distance between acinus from the primary counting parameters at light microscopical level. The program is written in HP 9815A System Language and occupies 2,008 lines of code. It is designed to run in combination with program D5.

**Hardware:** Hewlett-Packard 9815A.

**Reference:** For formulae see Romppanen, T. et al.: Investigative Urology 1980; 18: 59.

6. **Author:** A.H. Fabbri, and T. Kasvand.

**Program:** GIAPP. A geological analysis program package for estimating geometrical probabilities in microscopic specimens and geological map patterns.

**Hardware:** Binary image data are transferred to a general purpose computer using interactive display terminals.

**Reference:** Mikroskopie 1980; 37: (Suppl.) 431.

7. **Author:** M.H. Goldrosen, G.A. Miller and W.B. Warshaw.

**Program:** Control of an image analysis system as used for cell counting in immunological in-vitro assays. Cells are enumerated in 60-well microtest plates. The BASIC program includes stage control, focus adjustment, statistical analysis and data display on the monitor.

**Hardware:** Automatic microscope stage, Omnicon alpha image analyzer (Bausch & Lomb), microcomputer HP 9835 A.

**Reference:** Comput Programs Biomed 1982; 13: 121.

8. **Author:** B.J. Lay, C.E. Baudoin and J.C. Klein.

**Program:** Microaneurysms are automatically found and graded by this special software written in TASIC for the TAS image analyzer. The images (fluoroangiograms) are processed by different transformations to reject noise, artifacts and blood vessels and to detect microaneurysms only.

**Hardware:** TAS (Leitz), 2 floppy disks.

**Reference:** Acta Stereol 1983; 2: (Suppl. 1), 111.

9.* **Author:** A.L. Mackay.
**Program:** GENINV. A Microsoft BASIC program which calculates the generalised inverse of a matrix. Useful for all questions of linear algebra.
**Hardware:** Any system supporting BASIC.
**Reference:** Practical Computing, September 1981, p.108

10. **Author:** R.C. Manaka and H.H. Malluche.
**Program:** A BASIC program assisting diagnosis and treatment of bone disease. Numerical, surface and volume densities of all bone components and their relations are calculated using semiautomatically digitized images. Special attention is given to the osteocyte histology and dynamics of bone remodelling as observed with fluorescent labels.
**Hardware:** Digitizer (HP 9864 A), microcomputer (HP 9830), printer, plotter, floppy disk.
**Reference:** Comp Progr Biomed 1981; 13: 191.

11. **Author:** L.-E. Nordell.
**Program:** The PICAP (picture array processor, Kruse) machine has been programmed for automatic or semiautomatic (graphical interaction) of muscle fibre analysis. Local threshold, gradient and line filter operations on the grey picture give a sufficient segmentation for the calculation of areas, perimeters and diameters separately for type I and type II fibres in ATPase stained material.
**Hardware:** PICAP-system. Disk for storage of calculated contour data.
**Reference:** Mikroskopie 1980 37: (Suppl.) 448.

12.* **Author:** J.P.Rigaut, S.Margules, M.Boysen, M.T.Chalumeau and A. Reith.
**Program:** Automated karyometry in Feulgen stained sections of epithelial tissues by an image analyzer. Stereologic estimators and size distributions are interpreted as pathological gradings and ploidy peaks respectively. Interactive stage positioning and a special algorithm has been implemented to cover the morphology of epithelial cells, layered arrangement and prolate shaped nuclei. The image analysis part is written in TASIC, other routines in FORTRAN 4.
**Hardware:** Leitz TAS image analyzer and PDP 11-34
**Reference:** Path Res Pract 1982; 174: 342.

13.* **Author:** J.-P. Rigaut, P. Berggren and B. Robertson.
**Program:** This FORTRAN-80 program was specially designed for the IBAS image analyzer to automate stereology of

lung alveolar structure. Results are compared to those obtained by manual analysis.

**Hardware:** IBAS (Kontron).

**Reference:** Acta Stereol 1983; 2: (Suppl. 1), 103.

14. **Author:** R. Sammeck and W. Gibb.

**Program:** The nerve analysis program is a modified version of commercial distribution analysis software supplied by Kontron for the MOP-System. Number, size and form of nerve fibre and myelin sheath cross-sections may be analysed in light and/or electron microscopic (TEM) pictures. Parameters of other tissue components (blood vessels, different cell types and their nuclei) are correlated to the fibre data, including the distribution statistics. Program 20 kbytes, BASIC.

**Hardware:** Microcomputer HP 9825A with cassette memory, MOP AM02 (Kontron).

**Reference:** Ph.D. Thesis, W. Gibb, Goettingen 1983

15.* **Author:** H.E. Schroede

**Program:** CELLANAL. It is designed for analyzing free cells such as lymphocytes, neutrophilic granulocytes, monocytes, etc. at 3 levels of magnification (EM) in terms of volume, surface, and numerical density parameters, covering all structurally recognizable constituents. It serves for automatic data processing.

**Hardware:** HP 9830A with 8kbytes, R/W memory and thermoprinter, ROMs (Matrix operations, string variables, extended I/O, Infotek Systems ROMs (Fast BASIC I and II), HP external cassette memory, FD-30 memory system (Infotek).

**Reference:** Cell Tiss Res 1978; 92: 121.

16.* **Author:** H.E. Schroeder and W. Weilenmann.

**Program:** ANACON/AOV. A program for analysing the volume and surface density parameters of developing fibrocartilage in conjunction with autoradiographic labelling. Written in HP-BASIC plus, it occupies 3350 lines of code (16 kbytes).

**Hardware:** As in G.15

**Reference:** Dental Institute, Plattenstrasse 11, CH-8028, Zurich, Switzerland

17. **Author:** D. Schoevaert, D, M. Roch-Arveiller, P. Michel, A. Pompidou and J.P. Giroud.

**Program:** MACRAP. This is designed to analyse the image from a Zeiss microscope using a Plumbicon camera interfaced to a Quantimet 720 and PDP 11/34. Two binary images are built by thresholding and are analysed in parallel, the

first corresponds to the cytoplasm and the second to the nucleus. The histogram of the distribution of these ratios is produced.

**Hardware:** Quantimet 720 and PDP 11/34.

**Reference:** Laboratoire D'Histologie Embryologie Cryogenetique, Centre Hospitalier Kremlin-Bicetre, 94270 Kremlin-Bicetre, France

18. **Author:** W. Weilenmann.

**Program:** BASIC program for processing of point counting data, designed for application in dentistry (growth of Condylus mandibulae).

**Hardware:** HP 9830 (Hewlett-Packard), tape cassette as primary storage medium, disk, printer.

**Reference:** Thesis, Zurich 1980.

## CONCLUSIONS

The response rate to the ISS questionnaire was not particularly high and in view of the fact that two years have elapsed since the it was sent out, it is highly probable that new developments have taken place in the meantime. By producing this report we hope that there is now a mechanism by which news about new or existing software can be disseminated. The plan is to produce an up-date document every one or two years, depending upon demand, and any ISS members with useful contributions are urged to send details to Dr. Lewis G. Briarty, Department of Botany, Nottingham University, University Park, NG7 2RD, UK.

From a perusal of the report you will see that the largest section is for special applications. This is not surprising but there are some very notable gaps, particularly in the fields of statistics and educational aids. Our feeling is that one of the major roles for ISS is to provide educational facilities to fellow scientists who wish to embark upon serious quantitation of their results. The two programs submitted by Sverker Griph are very good teaching aids and there is a definite requirement for further developments in this direction. Considering the number of statistics programs that must be in existence we feel that Section D in this report is severely under-represented. Doubtless during the next decade there will be a significant increase in the number of programs dealing with three-dimensional reconstruction from serial sections.

A very major problem obviously exists concerning the portability of software between one operating system and another. There seems

little doubt that there will be a move away from mainframe machines towards personalised microcomputers. The evolution of BASICODE offers some hope for communication between machines with different dialects of BASIC. However, the subset of BASIC dealt with by BASICODE is still very limited although a more extended version is now being developed. The portability of graphics packages has always been a problem and will, no doubt, continue to be so. Perhaps authors should consider trying to do software according to the GKS International Standard Graphics in the future.

In the final analysis this project can only realistically be expected to facilitate contact between scientists with similar interests. Whether or not it would be quicker to modify an existing package or start from scratch to develop a new one will always be a matter of personal judgement. However, it would certainly be helpful if ISS members could decide upon two languages, say a Microsoft compatible BASIC and PASCAL. Furthermore, it would be extremely beneficial if software could be designed so that the main portion of the algorithm (i.e. the stereological section) was in its own separate segment while the file-handling and graphics packages, usually much less portable, were kept apart. This would make the translation of programs from one machine to another much more simple.

APPENDIX A -Classification of programs according to language.

| | |
|---|---|
| ALGOL 68 | A7, B4. |
| BASIC | A1, A3, A4, A5, B1, B2, C6, C10, G9, G10. |
| FORTRAN | B3, C1, C3, C4, C5, C8, C13, C14, E1, E2, F3, F5, F6, G12, G13. |
| HEWLETT-PACKARD | B5, D3, G1, G3, G4, G5, G7, G14, G16, G18. |
| PASCAL | F5. |
| PL/1 | A9. |
| TASIC | G8, G12. |
| TI-59 MACHINE CODE | A2, A8, D1, D2. |
| Z-80 ASSEMBLER CODE | A6. |

APPENDIX B - Classification of programs by machine make.

| | |
|---|---|
| APPLE | A5, C9, G2. |
| DIGITAL | B1, C10, C13, F3, F4, F6. |
| HEWLETT-PACKARD | B5, C6, D3, G1, G3, G4, G5, G7, G10, G14, G15, G18, G16, G17. |
| KONTRON | B2, C1, C2, C12, G13. |
| LEITZ TAS | F2, G8, G12. |
| MAINFRAME | A9, C5, C8, E1, E2. |
| OMNICON | G7. |
| QUANTIMET | B5, F3, G4, G17. |
| RESEARCH MACHINES | C3. |
| SIEMENS | F5. |
| TANDY | A4, |
| TEXAS TI-59 | A2, A8, C7, D1, D2. |
| WANG | A3, C11. |
| ZILOG | A6. |

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