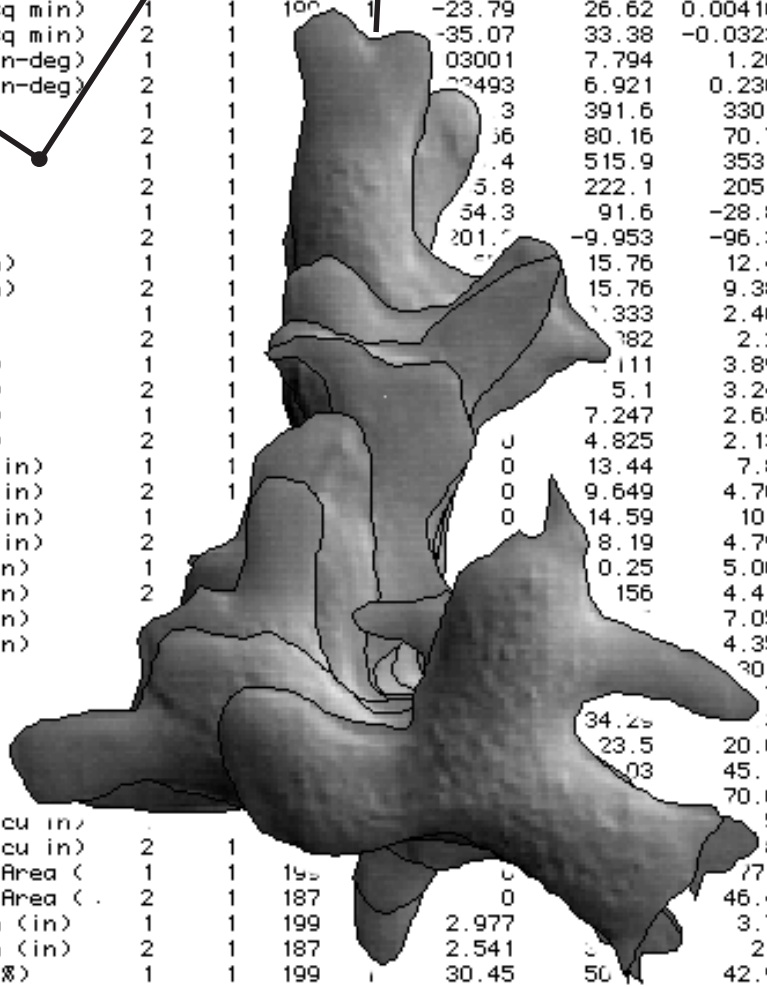
DIAS® 3.0

The Dynamic Image Analysis System

	Item	Obj	First	Last	Inc	Min	Max	Mean	StDev
1	Index	0	1	199	1	1	199	100	57.45
2	Time (min)	0	1	199	1	0	39.6	19.8	11.49
3	Speed (in/min)	1	1	199	1	0.1135	13.03	2.671	1.655
4	Speed (in/min)	2	1	187	1	0.008468	14.34	0.6278	1.453
5	Direction (deg)	1	1	199	1	-541.8	72.96	-244.9	196.4
6	Direction (deg)	2	1	187	1	-409.6	59.47	-137.3	105
7	Direction Change (deg)	1	1	199	1	0	132.5	14.24	21.07
8	Direction Change (deg)	2	1	187	1	0	174.9	26.58	41
9	Acceleration (in/sq min)	1	1	199	1	-23.79	26.62	0.004165	4.892
10	Acceleration (in/sq min)	2	1			-35.07	33.38	-0.03238	5.022
11	Persistence (in/min-deg)	1	1			03001	7.794	1.209	1.127
12	Persistence (in/min-deg)	2	1			03493	6.921	0.2366	0.5336
13	Centroid: X (pix)	1	1			3	391.6	330.9	31.86
14	Centroid: X (pix)	2	1			6	80.16	70.74	6.984
15	Centroid: Y (pix)	1	1			.4	515.9	353.4	94.87
16	Centroid: Y (pix)	2	1			5.8	222.1	205.3	7.254
17	Axis Tilt (deg)	1	1			54.3	91.6	-28.82	74.12
18	Axis Tilt (deg)	2	1			201.	-9.953	-96.33	57.99
19	Maximum Length (in)	1	1				15.76	12.49	1.503
20	Maximum Length (in)	2	1				15.76	9.388	2.529
21	Mean width (in)	1	1				.333	2.461	0.3022
22	Mean width (in)	2	1				82	2.22	1.301
23	Maximum width (in)	1	1				111	3.895	1.115
24	Maximum width (in)	2	1				5.1	3.248	1.91
25	Central width (in)	1	1				7.247	2.652	1.374
26	Central width (in)	2	1			0	4.825	2.137	1.745
27	X Bounding width (in)	1	1			0	13.44	7.88	2.802
28	X Bounding width (in)	2	1			0	9.649	4.707	3.079
29	Y Bounding width (in)	1				0	14.59	10.5	1.758
30	Y Bounding width (in)	2					8.19	4.794	3.031
31	X Maximum width (in)	1					0.25	5.068	1.734
32	X Maximum width (in)	2					156	4.412	2.921
33	Y Maximum width (in)							7.055	2.4
34	Y Maximum width (in)							4.357	2.85
35	Area (sq in)							30.7	2.82
36	Area (sq in)							78	1.433
37	Perimeter (in)						34.25	37	2.657
38	Perimeter (in)						23.5	20.67	1.559
39	Roundness (%)						03	45.16	4.883
40	Roundness (%)							70.61	7.809
41	Predicted Volume (cu in)							59	30.85
42	Predicted Volume (cu in)	2	1					88	39.27
43	Predicted Surface Area (	1	1	199		0		77.2	8.903
44	Predicted Surface Area (	2	1	187		0		46.45	27.07
45	Mean Radial Length (in)	1	1	199		2.977		3.78	0.3251
46	Mean Radial Length (in)	2	1	187		2.541		2.9	0.1419
47	Radial Deviation (%)	1	1	199	1	30.45	50	42.96	3.3
48	Radial Deviation (%)	2	1	187	1	13.2	40.61	29.18	6.088
49	Mean Convexity (deg)						688.1	526.2	52.67
50	Mean Convexity (deg)						528.9	453.4	26.35
51	Mean Concavity (deg)	1	1	199	1	69.88	364.6	168	53.27
52	Mean Concavity (deg)	2	1	187	1	31.8	168.9	93.36	26.35
53	Positive Flow (%)	1	1	199	1				
54	Positive Flow (%)	2	1	187	1				
55	Negative Flow (%)	1	1	199	1				
56	Negative Flow (%)	2	1	187	1				
57	Area Sec 1 (sq in)	1	1	199	1				
58	Area Sec 1 (sq in)	2	1	187	1				
59	Perimeter Sec 1 (in)	1	1	199	1				
60	Perimeter Sec 1 (in)	2	1	187	1				
61	Pos Flow Sec 1 (%)	1	1	199	1				
62	Pos Flow Sec 1 (%)	2	1	187	1				



United States Patent 5,655,028

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I
Introduction

Introduction

Most animal cells exhibit some form of motile behavior in the form of a change in cell shape or cellular translocation. For years, cell biologists were limited to assessing these behaviors qualitatively, which severely restricted their capacity to discriminate subtle changes. We now know that the motile behavior of animal cells is quite complex and involves surprisingly large numbers of molecules involved in signal reception, signal transduction, adhesion and cytoskeletal reorganization. Cellular translocation and changes in cellular morphology are basic to embryogenesis as well as the maintenance of the mature organism and the cellular immune response. Abnormalities in motility and cell shape can play key roles in neoplasia and metastasis, as well as in congenital defects. It is, therefore, critical that methods are developed which allow researchers to quantitate subtle as well as major changes in cell shape and cellular translocation. The need for refined quantization has proved to be crucial in recent studies of cells with mutations in specific cytoskeletal elements. These mutants are capable of growing, developing and translocating. However, refined analysis of their motile behavior has revealed subtle defects which have been crucial in interpreting the role of each cytoskeletal element in normal motility.

The methods which have evolved for analyzing how cells move and change shape are assisted by computers, and the system we have developed at Solltech, Inc. represents the third generation of a series of motion analysis systems which began to be developed in 1985 at the University of Iowa. The first system, the Dynamic Morphology System (DMS), ran on a SUN computer, and a second version was developed for the IBM-PC (AT). However, in 1989, the advantages of the Macintosh® platform led to the development of a completely revised and advanced system for this platform, which is the present Dynamic Image Analysis System (**DIAS**®). This system employs both QuickTime® and other framegrabbers for image acquisition, and provides both automated and manual modes of digitization for obtaining the perimeter of a moving cell. It includes image processing capabilities and a unique method for generating a smoothed, continuous perimeter which is crucial for quantization. Most importantly, **DIAS**® then computes more than 30 parameters of motility and dynamic morphology, provides a mechanism which allows the user to generate customized parameters, and computes any or all parameters every thirtieth of a second for up to 50 cells moving in parallel. **DIAS**® provides a number of options for printing out data in tabular or graphic form, and will generate computer movies of the digitized cell in a number of dynamic and, in some cases, unique formats. **DIAS**® is the culmination of 12 years of programming. We believe it is the most unique dynamic morphology system available.

A Brief Description of How a Biologist Uses DIAS®

DIAS® has been employed to analyze how microtubules, cells, larvae, worms and the left ventricular cavity of the mouse and human heart move and/or change shape with time. Although we will limit our discussion to its application to cell motility, it should be kept in mind that **DIAS**® can be used for any object which changes shape and/or translocates, and which can be videorecorded.

In most cases, a cell biologist will employ **DIAS**® to characterize how a normal cell moves, or to compare the behavior of an abnormal cell (e.g., a neoplastic or mutant cell) with its normal counterpart. The cell image is videorecorded through a microscope in real time onto videotape or other video recording device. If the cell or cells of interest are not in association with other cells, their perimeters will be intact and distinguishable, and the user will have no problem manually digitizing the

perimeter into the **DIAS**® data file. If the entire edge of the image contrasts with the substratum, the automatic digitizing system can be employed. Therefore, the user should spend some time optimizing the microscope format and illumination for recording the behavior of the cell or cells of interest. If a cell of interest is in contact with other cells of equal contrast, it is unlikely that the automatic digitizing mode of **DIAS**® can be employed.

To initiate an experiment, the user simply records the behavior of a cell through a videocamera attached to a microscope. The microscope can be standard or inverted. The cell can be on any substratum, on a slide, or in a chamber. As long as the user can distinguish the outline of the cell in the recording, he or she will be able to analyze it. The recording is then frame-grabbed into **DIAS**®. If the user wishes to analyze several cells in a single field, a lower magnification may be necessary. The user must be aware that a cell may move out of the recording area, and the user may, therefore, wish to initially decrease magnification, or move the recording area at some point during the recording period. If the user wishes to use the automatic mode for digitization, the user will have to perform initial experiments to adjust illumination in order to obtain high contrast cell substrate interphases which can be identified by the **DIAS**® system. This usually involves illumination which results in a dark cell image on a light substratum, but the reverse can also be effective.

Once the video is frame-grabbed into **DIAS**® as a **DIAS**® or Quick Time® movie, the user either directs **DIAS**® to automatically digitize the perimeter of the cell(s) of interest, or manually traces those perimeters. For automatic outlining, **DIAS**® provides contrast and smoothing algorithms to facilitate automatic edge detection. Once a sequence of images is digitized into **DIAS**®, the user can then generate a centroid (cell center) path, which appears as a spatial sequence of dots attached by thin lines, or a perimeter plot, which appears as a spatial sequence of overlapping cell perimeters. The user can also generate difference pictures, which are really double images containing the overlapped perimeters of 2 sequential images in which expansion zones (areas only in the later perimeter) and contraction zones (areas only in the earlier perimeter) are coded. This provides the user with a unique view of cellular dynamics. Once the images are digitized, the user can then direct **DIAS**® to compute parameters based on the dynamics of the cell centroid, such as velocity, direction change and persistence, or on the dynamic changes in contour, such as width, length, area, perimeter, roundness, convexity, concavity, etc. A particular region of a cell can also be windowed and individually analyzed, such as a pseudopod, lamellopod, or uropod. The quantitative capabilities of **DIAS**® are quite large, and can only be appreciated by actually using the system. If there is a unique computation which the user desires, the user can use some of the options in **DIAS**® to generate customized parameters, and, if necessary, can interact with our staff to accomplish this. If desired, the user can generate computer movies of the cell centroid, the cell perimeter or the differenced image, which can be viewed at any speed, or transferred back to videotape for presentation. The quantitative data can be viewed or printed in tabular or graphic form, and **DIAS**® includes a number of styles for publication-ready presentation.

Although **DIAS**® has been fine-tuned to digitize images, generate unique dynamic representations of the digitized image and quantitate many aspects of cell motility and the dynamic changes in cell shape, there are certain aspects of motion analysis which the user must be acutely aware of if the user wishes to obtain full and effective use of the system. Some of these points are considered in the remaining portions of this Introduction.

The Biological Preparation

There are many options which the user can employ to obtain analyzable recordings. The most important thing to keep in mind is that if you can follow the object in your recording, you can use **DIAS**® to analyze it. For instance, if you record the behavior of a cell <u>in tissue</u> or in cell aggregates and can discriminate some or all of its perimeter, you can hand outline its behavior and obtain meaningful

data. On the other hand, if you are interested in the refined behavior of a pseudopod which is less than 3 um in length, you may need to isolate that cell from other cells so that the perimeter is intact, and fine tune the illumination in your microscope in order to obtain the best outline of the cell without affecting cell behavior as a result of light or heat.

When analyzing cell motility in vitro, one must test whether the conditions employed support continued motile behavior. For instance, many cells will stop moving when constantly illuminated or when starved for oxygen. Some cells will not move because they can not properly adhere to the substratum. One must, therefore, be conscious of temperature, light, substratum, composition of the medium and genesis of microenvironments. In other words, the biology of the system being analyzed is the most important initial aspect of a motility experiment. Optimize your system. Be careful of the composition of the supporting medium. Test motility on different substrates. Make sure your cell is not glued to the substratum, and make sure the motility you are measuring is not due to flotation. Make sure your cell is not being pushed backwards by an extending pseudopod because the cell body is inadequately attached to the substratum. Check the temperature of your preparation on the microscope stage. Make sure your preparation is not losing motile activity with time because it is overheating. Check for pH. Check for light sensitivity. If necessary, use perfusion chambers, which will inhibit the genesis of microenvironments, provide continuous nourishment and help maintain the proper temperature.

Lighting, Magnification and Recording

As noted, **DIAS®** has both a manual and automatic mode for digitizing the perimeters of cells. The manual mode is slower than the automated mode, but provides greater detail and more refined perimeters. In cases of cell crowding and overlap, or in cases in which a cell is imbedded in tissue or is on top of a monolayer, the manual mode is in many cases necessary. Remember that when one is following the path of a cell in a complex setting, one can interpret the perimeter even when it is not clearly exhibited if one has viewed the cell perimeter in previous and subsequent frames. The work which would be necessary for a computer program to achieve the same level of discrimination is beyond the size and speed of almost any platform. However, manual digitization is not really that slow when one considers the time it takes for hand tracing in relation to the total time of the experiment.

In some cases, flat cells like fibroblasts may not provide a distinct enough, or contrasted, edge in relation to the substratum, and will require hand digitization. In addition, certain kinds of lighting such as phase contrast will generate a black/white edge on one side of a cell, and a white/black edge on the other side. In addition, the phase halo may interfere with automatic digitization. The best way to test whether automatic digitization is feasible is to simply test your lighting conditions, and play with the light source, magnification, type of optics and condenser. In many cases, a high contrast image which loses intracellular detail provides the best edges for automatic digitization of the cell perimeter. If your project requires that large numbers of cells are analyzed, and you are not that interested in high resolution detail of the perimeter, then high contrast, low magnification conditions can easily be developed for automated digitization. Since **DIAS®** will analyze in parallel as many as 50 cells moving on a substratum, magnification must be set so that the necessary number of cells are contained within the field recorded.

Although recording on half inch tape provides an adequate image, three-quarter inch tape and direct digital recording provides higher resolution. Since many cameras provide the user with the capacity to process the image as it is recorded, the user should experiment with the system employed, especially if a high contrast image is needed for automatic digitization. It should also be kept in mind that under certain circumstances, the video signal from the camera can be hooked directly into the framegrabber. However, possessing a recording of your preparation has a number of advantages and provides a backup in case a computer problem arises.

Adequately utilizing the Quantization and Dynamic Representation Provided by DIAS®

The quantitative capabilities of the original DMS and the new **DIAS®** programs have had a history of underutilization. The user must realize that once your cells have been digitized into the **DIAS®** database, you can apply virtually every capability of the program in your analysis of that cell. If you originally only need a velocity or roundness measurement with time, compute some of the other parameters at your disposal. You may very well be surprised to find that an unexpected parameter provides new insights into the behavioral phenotypes that you are defining. This is especially true when you are comparing a normal and neoplastic cell, or a wild type and mutant cell.

In addition, generate some of the dynamic representations provided by **DIAS®**. Play them at different speeds. Sometimes, speeding-up a movie exposes cyclic behaviors missed in slow view (real time) movies. Generate a dynamic difference picture. It will give you insight into the expansion and contraction zones of your motile cell. These informative movies can then be videorecorded and used in presentations and seminars.

Problems of Three Dimensionality

We tend to view a cell as a 2 dimensional object primarily because in conventional microscopy we view the cell from the top or bottom, and tend to perceive behavior in a single focal plane. Remember that your cell is, in fact, 3 dimensional and may not always be in contact with the substratum at all points on the cell surface opposing the substratum. Unfortunately, **DIAS®** is a two dimensional system. Usually, analyses are performed at a relatively constant focal plane at a set distance from the substratum. Therefore, **DIAS®** provides no direct information on z-axis changes. Contour changes and motility parameters are all limited to the x,y-axes. In most cases, x,y-axis information is useful and provides valuable information concerning a behavioral phenotype, but the user must remember that without z-axis information, the description may be incomplete. There are two indicators of z-axis behavior which the user can assess. First, if the area of an image changes significantly, and, especially, if it cycles, there may be a strong z-axis component to the behavior of the cell of interest. Second, if parts of the cell move out of the focal plane and, therefore, become blurred, it is probably due to behavior in the z-axis. The user can adjust the focal plane to continue monitoring that portion of the cell. In most cases, z-axis information would add to the description of phenotype, but is not essential for extracting relevant and useful 2D data.

Time to Begin

With the above points in mind, the best way to begin using **DIAS®** is to simply turn it on and become acquainted. Once you achieve initial familiarity with the system, take one of your videotapes and move through the manual. This initial test of the system will help you optimize your conditions for obtaining videotapes for analysis. If you experience problems, feel free to contact us at Solltech. We will be more than happy to work with you in the initial stages of learning the system.

II
Installation

It does not require a computer expert to use **DIAS**®. However, we do recommend that if you are a new user, you begin by mastering the basics of your Macintosh® computer. Tutorials and manuals included with your Macintosh® will help you to become better acquainted with how a Macintosh® computer works. Before continuing, please be sure you have familiarized yourself with the basic terminology of the Macintosh® Operating System, how to use a mouse, etc.

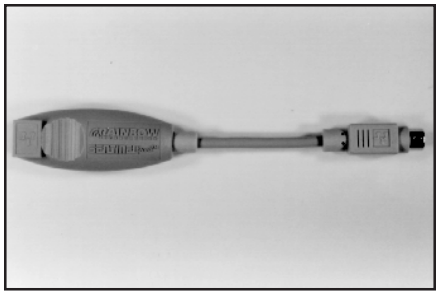

Fig 2.1 A photograph of the SentinelEve3®.

The Sentinel protective device

The **DIAS**® system includes three separate parts; the **DIAS**® manual, the **DIAS**® program diskette, and the Sentinel® protective cable (Fig 2.1). The SentinelEve3® cable must be connected between your keyboard and the cable connecting the keyboard to your Macintosh® CPU in order for the **DIAS**® software to start up and run. If you try to start or run **DIAS**® without attaching the SentinelEve3® device, you will be asked to check the connection and try again (Fig 2.2). After hooking up the SentinelEve3® for the first time, the computer must be restarted. With the SentinelEve3® attached, you are still able to back up both the **DIAS**® program as well as all of your program files. The SentinelEve3® is only designed to keep multiple copies from being run on other computers, simultaneously.

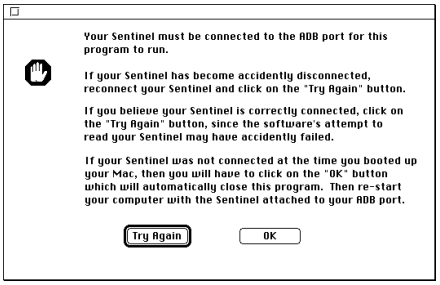

Fig 2.2 The message you will see if you start or run **DIAS**® without the SentinelEve3® attached.

Note: Any text, graphics, or QuickTime® files which are created in **DIAS**® may be opened and used by other programs, without the need for the SentinelEve3®.

System settings

When running **DIAS**®, you need to have your monitor control panel set for at least 256 colors, but the 'Thousands' or 'Millions' settings will give you both better (visually) and faster (screen refresh) results. Also, **DIAS**® is set to run at a minimum of 12 Mb of RAM, however, it is advisable that you run it at 24Mb if possible. In both cases, this refers to the amount of RAM memory available after subtracting the amounts used by the System, and any other currently running programs, not to the total system memory. In order to change the amount of memory allocated to **DIAS**®, single-click on the **DIAS** 3.0 program icon (to highlight it) and then, while in the Finder, select **Get Info** from under the **File** menu (or type'⌘I'). Doing so will bring up

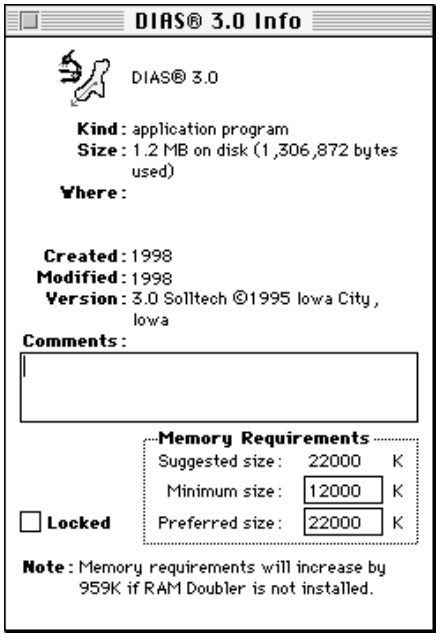

Fig 2.3 The **DIAS**® program 'Info' box.

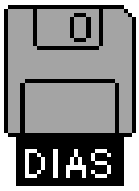

Figure 2.4. The DIAS disk.

Figure 2.5. The DIAS folder.

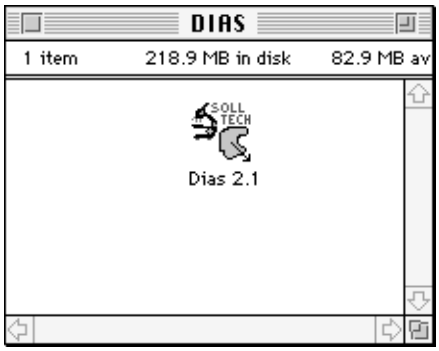

Figure 2.6. The **DIAS**® program.

Figure 2.7. Examples of movie file icons.

Figure 2.8. An example of a path file icon.

Figure 2.9. An example of a text file icon.

a dialog box similar to the one shown in Figure 2.3. To increase the memory allocation to the **DIAS**® program, highlight and change the number listed in the 'Preferred size' box in the lower right hand corner of the dialog box. We recommend setting it within the range of 12000 to 24000, although you can increase it even higher if you have enough memory.

Installation

To install **DIAS**®, insert the **DIAS**® disk into the floppy drive of your computer (Fig. 2.4). Double-click on the icon of the floppy disk to open it. You will see a folder called "DIAS" (Fig. 2.5). Drag (copy) the DIAS folder to your hard drive.

The folder contains the **DIAS**® program (Fig. 2.6).

You can begin using **DIAS**® by double-clicking it's icon. This will take you into the program and load the **DIAS**® menus into the menubar.

Descriptions of DIAS® icons

The following is a list of the different types of icons you may generate in **DIAS**®, what they represent, and where you can find more information about them.

Movie files (Fig. 2.7). These icons represent movies, either **DIAS**® or QuickTime® movies, which have been either captured or processed by **DIAS**® (Chapters III and XVI). QuickTime® movie file icons will have a small 'QT' in the upper right hand corner of the icon.

Path file (Fig. 2.8). This icon is for path files and when double clicked, opens up the **ViewPaths** and **EditPaths** sections of **DIAS**® (Chapter ***VI***).

Text file (Fig. 2.9). This icon can represent any type of text file, but will primarily be used when you save summaries of computed output.

Database file (Fig. 2.10). This icon represents a specifically formatted **DIAS**® database text file. Double-clicking on this icon will open up the **Use database** section of the program (Chapter X).

Graph file (Fig. 2.11). This icon represents graphs created while in either the **Compute parameters** or **Use database** sections. Double-clicking on this icon will open up the **Graphics Manager** (Chapter IX).

How to proceed

In order to analyze an experiment in **DIAS**®, you must first have made a digital recording of that experiment (Chapters III and XVI). Once you have done so, you should proceed to tracing the objects of interest in the movie, and making paths of those objects (Chapter IV). With paths made, you may analyze

the motility and dynamic morphology of the traced objects using graphing and numeric methods (Chapter VIII), by creating and studying dynamic movies of movement and shape change (Chapter VI), or by studying static images of changing shape (Chapter XI).

> Note: Throughout the **DIAS**® program, it is always important to read the title bar of the open window, as it will often instruct you on how to proceed during a current step.

III
Making Movies for Analysis

What types of movies can DIAS® use?

DIAS® is designed to analyze how cells move and change shape over time. In order to do so, it is necessary to translate analog movies (standard video) into a digital format which **DIAS®** can open, read and analyze. **DIAS®** can analyze movies in two types of digital formats, QuickTime® movies and its own **DIAS®** movie format.

DIAS® analyzes and outlines movies in its own format significantly faster than it can QuickTime® movies of the same window size and number of frames. As movies increase in size, the difference in speed will grow geometrically. This is due to how QuickTime® movie files are stored. They store a significant amount of information in the resource fork of the file. While it is not important for you to understand this, it is important that you understand that this is the same section of the file which **DIAS®** uses to store traces and slots (Chapter IV).

If you already have movies saved as QuickTime® files, it is easy to convert them to **DIAS®** movies. That process, and the various options involved will be described in the next section of this chapter.

DIAS® will also create movies in its own format using some QuickTime® Framegrabbers. That process will be covered in Chapter XVI.

Saving and converting between movie types

The following process will be used for a number of purposes. Use the following process if you wish to change a **DIAS®** movie to the QuickTime® format for use in another program. These are also the steps to use if you need to edit the size, shape or image quality of a movie file, or if you wish to convert a QuickTime® movie file to the **DIAS®** format for use in outlining. In any of the above cases, the process will be similar.

In order to convert between formats, you must first start the **DIAS®** program, and open the applicable movie, using the **Open** command, from under the **File** menu.

With the movie open and on the desktop, select **Save** from under the **File** menu. Doing so will bring up the 'Set Frame Parameter' dialog box (Fig. 3.1). This is the first of many dialog boxes which will give you

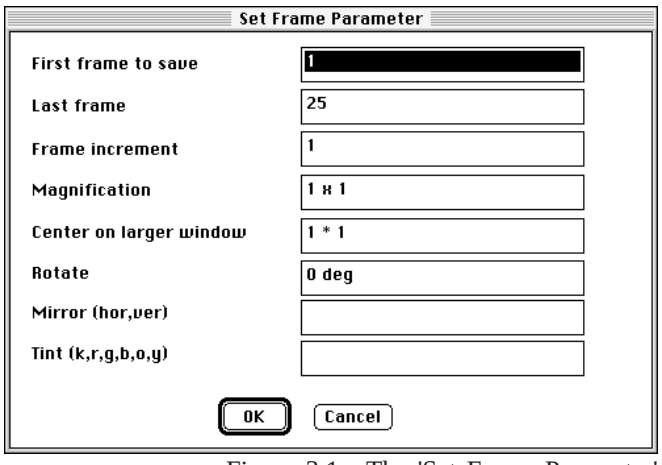

Figure 3.1. The 'Set Frame Parameter' dialog box.

extensive control over, and the ability to change and process, your movie.

Many of the options in the Parameter dialog box are self-evident. You can select to save only a subsection of the total number of frames in your movie (using 'First frame to save' and 'Last frame'). You can even skip among the frames within the subset (using 'Frame increment'). There are also several methods of magnifying the movie, some of which may be less self evident.

By making entries into the 'Magnification' section of the 'Parameter' dialog box, you can enlarge or shrink your movie. To enlarge or reduce the movie uniformly, simply enter a single number, followed by an 'x'. For instance, entering '2x' will double the size of the movie, and entering '.5x' will reduce its size by 50%. You can also use this section of the dialog box to change the relative sizing of the movie. Entering '1 x 1.5' will stretch the height of the movie window to 150% of the original size, while leaving the width unchanged.

You may also use the 'Center on larger window' command in the dialog box to change the overall window size of the movie, while leaving the actual image size intact. If you are putting together a video presentation, and some of the movie clips vary in size, you may wish to size and center them all to the same window size. For instance, if you are putting together a presentation, and most of the movies are 640 x 480 resolution, but your current movie is only 512 x 400, you can either use the above described method to increase the image size of the movie, using 'Magnification', by entering '1.25 x 1.2' which will distort your movie image, or you can enter '1.25 * 1.2' in the 'Center on larger window' section. Using the centering command, instead of the magnification command, adds a white border around the original movie, leaving the original movie image intact in size and relative resolution, but changing the size of the encompassing movie window. This is helpful when creating movies that will be combined in other applications, such as MoviePlayer or Premiere, as those programs will not center movies of differing sizes, and may actually change the size and relative resolution of the movie.

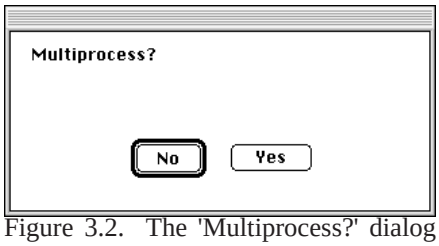

Figure 3.2. The 'Multiprocess?' dialog box.

Besides resizing the movie image, you can also rotate it using either the 'Rotate' command, which allows you to enter a degree of rotation, or the 'Mirror' command, which allows you to mirror the image along either the 'hor'izontal or 'ver'tical planes.

Finally, the 'Parameter' dialog box allows you to 'Tint' the greyscale images using either blac'k', 'r'ed, 'g'reen, 'b'lue, 'o'range, or 'y'ellow.

Once you have made all of your choices in the 'Parameters' dialog box and clicked on 'OK', you will then be asked whether you wish to 'Multiprocess?' (Fig. 3.2). Multiprocessing will be covered later, in Chapter XVII. If you wish to image process your movie at this time, proceed to that part of the manual for

more information. Otherwise, click 'No'.

You will now be given the opportunity to 'Select (a) clip rectangle?' (Fig. 3.3). Answering 'Yes' will allow you to select a subsection of the window size for saving. Doing so will decrease the movie window size, as well as decreasing the amount of memory which the movie will take up on your hard drive. If you answer 'No', the movie will be saved at the same size. If you select 'Yes', the title bar of the movie window will instruct you to select a clip rectangle. At this time, you may either select an area by freehand, simply using the mouse to draw a bounding box around the area of choice, or you may select an area of exact size, by typing 'f' for frame size.

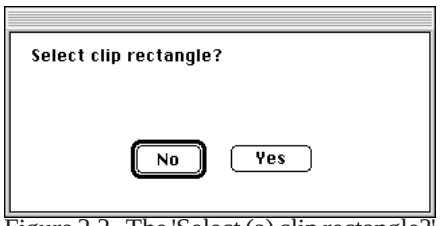

Figure 3.3. The 'Select (a) clip rectangle?' dialog box.

Typing 'f' brings up a dialog box which will ask you to 'Set (the) Window Size' (Fig. 3.4). Type in an exact size for your bounding box and click 'OK'. You will then see a moving bounding box of that size, which you may use to select a subsection of your movie image window. You may want to use this option if you wish all of your movies to be the same, smaller, size for later presentation.

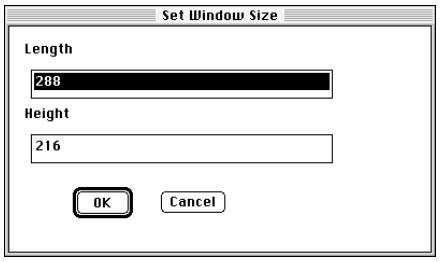

Figure 3.4. The 'Set Window Size' dialog box.

Upon making a movie area selection, you will be asked to name your new movie, using a standard Macintosh Save dialog box. Do not attempt to rename the movie with the current name, as that will cause system problems. After picking a new name, you will be presented with a dialog box giving you different types of 'Movie (file) format(s):' for your final movie (Fig. 3.5). The 'BIN' format should only be selected if you wish to use the saved movie with an older version of **DIAS**®. The 'DIAS' format should be selected if you wish to use the movie for analysis, and particularly if you wish to trace on the movie. The 'QuickTime®' format would be appropriate if you wish to use the movie as part of a presentation, and wish to use it, or edit it, with other programs such as MoviePlayer® or Premiere®. The 'PICT Stack' format is useful if you wish to break up your movie into individual PICT images, for use in a paper, or other static presentations. The 'ECON' format should only be used with movies which already contain traces. This format saves only the areas of the image within the traced regions. Discarding the background of the image.

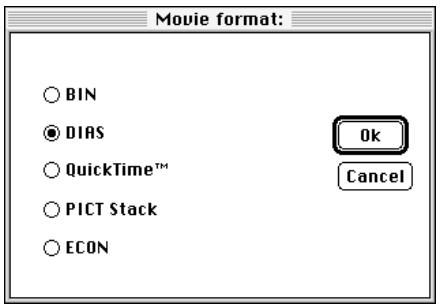

Figure 3.5. The 'Movie Format:' dialog box.

> Note: If you select the QuickTime® format, you will be presented with a standard QuickTime® Compression Dialog box. We will not attempt to discuss those options in this manual, as it is an Apple designed selection, and well beyond the scope of this manual.

After selecting a 'Movie format:' and selecting 'OK', you will then be asked if you wish to compress the movie (Fig. 3.6).

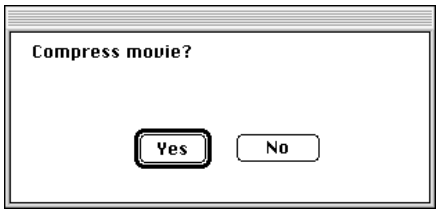

Figure 3.6. The 'Compress movie:' dialog box.

Doing so will shrink the amount of memory your movie will take on your hard drive. However, doing so will also convert your movie to greyscale, removing all color or tint.

Once you have made all of your selections, you will see a new window open up, and your movie will be saved in that new window, several groups of frames at a time.

Using a stack of PICT images

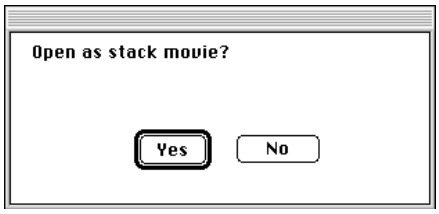

Figure 3.7. The 'Open as stack movie?' dialog box.

A **DIAS**® movie may also be created by converting a stack of sequentially numbered 'PICT' images. 'PICT' stack conversion is designed for those people who use NIH 'Image', or other, similar programs, and a frame grabber to grab individually numbered frames. The frames of the stack must be stored in a separate folder, and numbered sequentially, using no letters or punctuation (i.e., 1,2,3; not 01, 02, 03). The frames can then be converted into a movie by opening the first 'PICT' image in **DIAS**®. If the file is in a folder as described above, when opening the first image, a dialog box will ask whether you wish to 'Open as a stack movie?' (Fig. 3.7). If you click on 'Yes', the images will be opened as a movie. You should then save the movie, following the steps designed above. If you click on 'No' in the dialog box, the file will open as a simple PICT image.

IV
Tracing Cells and Making Paths

Why Trace Cells?

For **DIAS**® to analyze the motility and dynamic morphology of the objects in your movie, those objects must be outlined and identified within **DIAS**®. **DIAS**® provides two automated, **Auto Trace by Threshold** and **Auto Trace DIC**, and two manual, **Trace on Movie** and **Trace on Movie with Splines**, methods for tracing/outlining your cells (Fig. 4.1).

Both of the automatic tracing methods, which will be covered in more depth later in this chapter, include various controls to allow for the best outlines possible. They both utilize powerful algorithms for fast and easy outlining. However, there will be circumstances when the automatic outliners are not enough, and complete, manual control is needed.

One instance may be when the lighting and/or resolution of the images in the movie are poor or uneven, making accurate, automatic tracing difficult. Another, less obvious instance, is when analyzing large numbers of cells which collide or cross paths. While the automatic outliners may work well for these types of movie images, constructing paths may be extremely difficult because of collisions and complex path crossings. If this is the case, manually outlining individual cells, through the length of the movie, and placing the traces of individual cells in separate slots (explained below), may be the best answer.

In any case, because outlining, using any of the methods, is so important to performing an accurate analysis, it is important that you understand all of the capabilities of the various methods.

Figure 4.1 The **DIAS** menu.

> Note: You will have to use the **Trace on Movie** command in order to create a scale factor for your movie, unless your are only interested in relative movement and shape change, or know the scale factor from other experiments.

What are Trace Slots?

Even though outlining cells is essential to your work with **DIAS**®, it is also important that those tracings do not permanently change or mark over the original movie images. **DIAS**® saves traces as a separate part of your movie, in the resource fork of the movie file. Because there will be times, as described above, when there are multiple traces needed for the same file, **DIAS**® allows you to save up to 61 different sets of tracings for each movie. Each tracing is a transparent overlay for each individual

frame of your movie file. Selecting a specific trace slot displays, and allows you to edit, the traces saved in that slot only. So, it is important that a trace slot is selected before you begin tracing on your movie file. The default slot is always slot '1', and it may be that you never need to change that selection.

If you do need to select a different slot in order to trace a separate object, try a different method of tracing, or for whatever reason, simply select **Current Trace Slot:** from under the **DIAS** menu. Doing so will bring up the 'Set Trace Slot' dialog box (Fig. 4.2). You may select trace slots 1 thru 9, a thru z, and capital A thru Z. Once you have selected a slot, or elected the default, you are ready to begin tracing.

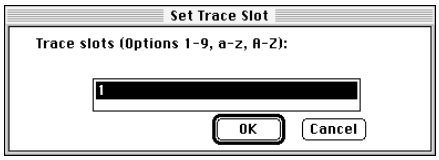

Figure 4.2 The 'Set Trace Slot' dialog box.

Note: The menu will always display the current slot for the open movie.

Note: You may select to display two or more slots by listing them in the dialog box, separated by commas. In fact, it is often helpful to do so when tracing images crowded with multiple objects. However, you may only trace or edit the traces in one slot at a time. It will be the slot listed first, and it will be the only one whose outlines are not red.

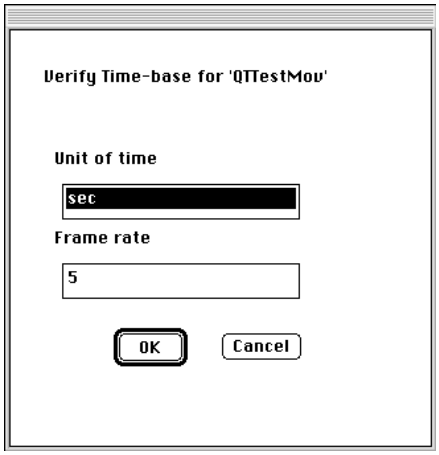

Figure 4.3. The QuickTime® time-base verification dialog box.

Auto Trace by Threshold

Before selecting any of the trace functions, you must have first opened either a **DIAS**® or QuickTime® movie. If you are opening a QuickTime® movie for the first time, **DIAS**® will ask you to verify the movie's frame rate. A dialog box will appear on screen, asking for a 'Unit of time' and 'Frame rate' (Fig. 4.3). 'Unit of time' refers to the time units which will be used on all graphs and charts. **DIAS**® recognizes 'us' (microseconds), 'ms' (milliseconds), 'sec', 'min', 'hr', and 'day'. Do not use periods '.' when typing in the time unit. 'Frame rate' refers to the number of frames captured during a single passage of the time unit. The example in Figure 4.3 gives the current movie a frame rate of 5 frames per second. You could also enter this as 300 frames per minute. If you make a mistake, you can change the time unit and rate later by editing the file's header.

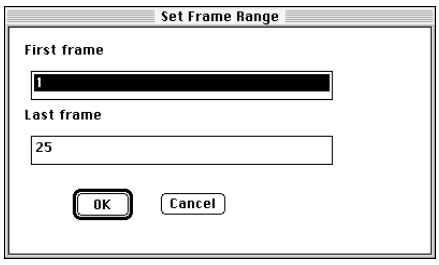

Figure 4.4. The 'Set Frame Range' dialog box.

After the movie has opened on the desktop, you may then select **Auto Trace by Threshold** from the **DIAS** menu. Doing so will bring up a dialog box which will ask you the range of frames which you wish to trace (Fig. 4.4). You may pick any range you wish. You may outline different sections of the same movie, using different settings. It is to theses frames that the automatic tracing algorithms will be applied. Do not worry if

some of the frames within the range do not trace well. All of the traces made during the automatic processes may be edited later using the **Trace on Movie** command.

After you have selected a frame range, the title bar of the movie window will instruct you to select an area to outline. Again, in similar fashion to the frame range, your selection tells **DIAS**® if you wish to select a specific region of interest to outline, instead of the entire movie window. This is occasionally useful if the cell of interest only moves around within a small area of the total window, or if there are debris or objects in the perimeter of the image which you do not wish to outline. Cutting those objects out of the outlining process now, means that you will have to do less editing of the traces and/or path files, later. To select an area, use the mouse to draw a rectangle around that area. If you wish to trace the entire movie window, click the return key.

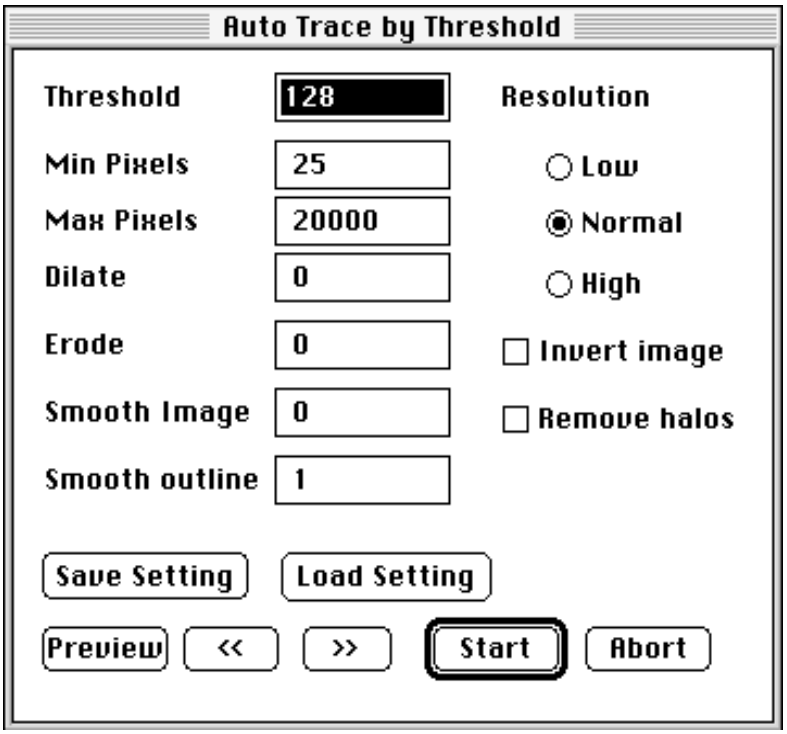

Figure 4.5. The 'Auto Trace by Threshold' dialog box.

At this point, the 'Auto Trace by Threshold' dialog box will open on the desktop (Fig. 4.5). There are several entries which you may make in this dialog box. All of them designed to make automatic outlining easier.

Threshold

Greyscale images run from black (0), to white (255). The number which you enter here determines at what cutoff level to outline above. While you do not see this on the screen, **DIAS**® will convert everything above that number to white, and everything below that number to black. The black areas are then outlined. In the example above, any objects which have a greyscale level above 128 will be outlined. Changing these numbers will change the areas being outlined. Assuming that your cells are dark on a lighter background, increasing these numbers will shrink or remove outlines, while lowering the numbers will include a greater area of outline.

Min and Max Pixels

The pixels that these commands are referring to are the number of pixels making up the outline of the object(s). Use the 'Min' number to eliminate small debris from the outline. Use the 'Max' number to eliminate large areas, for instance cover slip edges, from the tracings.

Dilate and Erode

'Dilate' provides a powerful tool for repairing objects that have gaps in their outlines which would normally prevent the

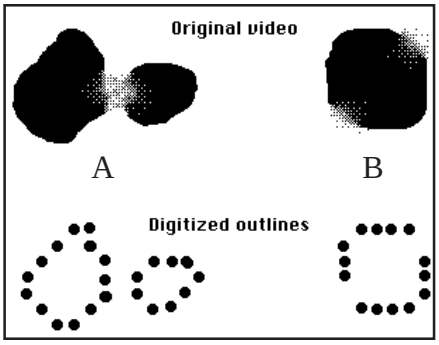

Figure 4.5. Two objects needing dilation to close the boundary

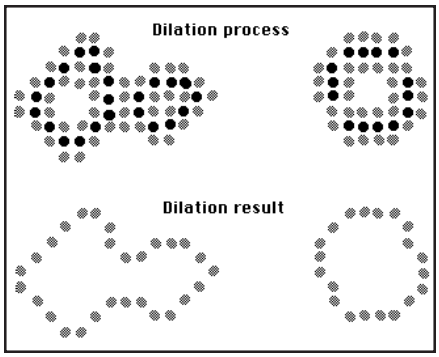

Figure 4.6. The process of dilation

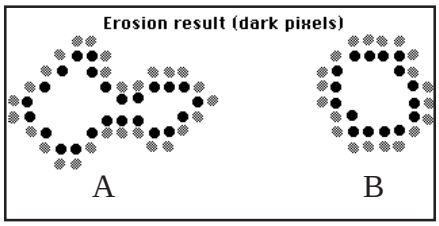

Figure 4.7. The erosion process

outline from being closed, or for objects with small, thin extensions.

In figure 4.5, the center of the image of object A was so diffuse in one section as to appear transparent. Object A therefore, digitized as two objects. The result of a 'dilation' of 1 is shown in Figure 4.6. Note that for each pixel, the four horizontal and vertical neighbors are added. The result is then outlined. Therefore, only the outermost portions of the dilated object are used. Higher dilations simply repeat this process the desired number of times. Dilations of 3 or higher significantly distort the shape of the object. The default is no dilation (0).

The above dilation process ‘fattens’ the object by the amount of 'dilation'. Erosion ‘thins’ the object the required number of times so that the original size is approximately preserved (Fig. 4.7). One round of erosion moves each boundary pixel inward a distance of one pixel. As a rule, if the object was 'dilated' n times, the 'erosion' should be n or n-1. High erosion numbers can create bizarre results.

Smooth Image and Smooth Outline

Both of these commands allow you to make subtle changes to the outlines of your cells. Occasionally, because of sharp contrast or lighting, the edges of an outline will appear jagged. In order to smooth those outlines, you can smooth either the image or the outlines directly any number of times.

> Note: Any smoothing which you perform here is temporary, and only effects the outlining process, and not the image itself.

Resolution

Resolution refers to the resolution of the outlines, and not the original image. Changes in these selections will effect how tightly or loosely the outlines adhere to fine detail of the objects.

Invert image

In order to automatically outline the cell(s) in your movie, they should be uniformly dark on a light and even background. If they are not, clicking on 'Invert image' flips the image from light cells on a dark background, to dark cells on a light background, making the threshold process work correctly. Again, this does not permanently effect the movie image.

Remove halos

Clicking on this option attempts to remove the double-traced halos which occasionally occur in some types of microscopy.

Save and Load Setting

Once you have optimized all of your settings, clicking on the 'Save Setting' button brings up a standard Macintosh® Save dialog box, which will allow you to name and save a formatted

text file containing the settings which you have entered. Once you have done this, you can click on the 'Load Setting' dialog box to load those settings when working on other movies.

> Note: These capabilities will appear in many dialog boxes throughout the program.

Preview, <<, and >>

After you have made any changes in the above options, clicking on the 'Preview' button shows the effects the changes have made on your outlines.

If you think that the lighting or quality of your movie images changes significantly over time, you can single frame step through your movie, seeing the effects of your options on each frame, before beginning the actual outlining process.

> Note: None of the outlines you see during the 'Preview' or single frame advance process are permanent. It is not until you click on 'Start' that all of the frames are outlined.

After clicking on 'Start', you will be able to see **DIAS®** as it outlines each frame. You may stop the process early by pressing and holding the Apple and '.' keys simultaneously, until the process stops. Remember, the outlines do not have to be perfect at this point, as you can edit them with ease using the **Trace on Movie** command.

Auto Trace DIC

Auto Trace DIC is another automatic outlining method offered by the **DIAS®** program. This method uses a pixel complexity method for finding the outlines of in-focus cells. While it may be used for fields of cells, because of the methods it uses for targeting cells, it is used primarily for movies of single cells. When you first select this command, you will be asked for a range of cells, and to select an area of the image window, just as you were with **Auto Trace by Threshold**.

Once the dialog box opens, you will notice that there are four basic controls to **Auto Trace DIC** (Fig. 4.8) All four of the options are percentage based, so numbers between 1 and 100 should be entered for each.

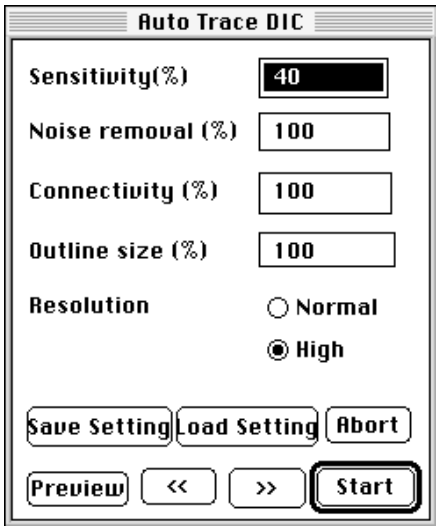

Fig. 4.8. The 'Auto Trace DIC' dialog box.

Sensitivity

This control refers to the sensitivity of the method in looking for changes in pixel complexity around the cell. The more you increase sensitivity, the tighter the outline will generally adhere to the cell boundary.

Noise removal

This command is similar to smoothing in the case of the automatic threshold outliner. As you increase this number, small variations will be lessened.

Connectivity

Variations in this number will effect the attachment of small, barely attached objects.

Outline Size

This command is similar to erode command, with greater variability. Varying this number will effect the general tightness of the outline.

The other options in this dialog box are identical to those already covered under the **Auto Trace by Threshold** command.

Trace on Movie

Some of the various reasons for hand tracing have been covered earlier in this chapter. Two other important reasons will be covered here as well. First of all, the **Trace on Movie** command allows you to edit the automatic traces made using either of the previously described methods. Opening a movie, as described earlier, and selecting **Trace on Movie**, will allow you to erase whole traces, or parts of outlines, making it easy to make simple corrections to the previously saved outlines. Most importantly, it is while under this command that you may set the scale for your movie. Doing so will allow you to compare the motility and morphology measurements between various cells and experiments. Measuring and setting scales will be covered later in this section.

In order to trace/outline manually on your movie, simply open it on the desktop and select **Trace on Movie**. Because everything at this point is manual, there is no need to select a range of frames, you may stop and skip around frames as you wish. There is no need to select an area, as you will do so by drawing only in the areas that you wish.

The **Trace on Movie** command gives you a cross-hair pointer which you may use to draw freehand on top of your movie frame image. To outline a cell or object, simply click and draw around the desired object, making sure that the outline is closed, without gaps in the outline. It is essential that an outline is closed. If it is not, that object will not be included in future paths and analysis. While in either of the manual trace modes, if an outline is not closed, **DIAS**® will warn you of that fact, giving you the opportunity to make a correction now, instead of waiting until you discover the problem later, while making paths.

While in the **Trace on Movie** command, you will notice that the title bar of the movie list the usual file name and frame number information. However, it also lists 'Type ? for Help'. This will appear in both of the manual trace title bars. For a list of additional control and key commands appropriate for the

current trace method, type '?'. Doing so will bring up a window listing those options (fig 4.9).

Most of the options make your manual tracing experience easier, and are self-explanatory. The one option which is more involved, and necessary for most users is the 'measure/set scale factor' command, accessed by typing 's'.

In order to make accurate and comparable calculations, a scale factor must be entered into the **DIAS**® header for each movie. To enter a scale, you must have recorded something of known length at some time during the making of the movie (see Chapter I). Proceed to the frame of the movie which contains the measurable item by typing 'g'. This will bring up a dialog box asking for the desired frame number. Enter that frame number and click 'OK'. You are now ready to type 's' and measure/set your scale.

```
      Trace on Movie Commands


1-6          magnify the image
q, <cr>      done
Q, cmd-.     abort (changes not saved)
+, =         increase the eraser size
-            decrease the eraser size
u            undo all drawing
v            finish and close current curve
c            clear all current tracing

Hold option key down while drawing to erase

       For movies only:

b            previous frame
n            next frame
g            goto frame
y            set trace slot
h            view/edit DIAS header
s            measure/set scale factor
f            save the trace and read it back
```

Fig. 4.9. The **Trace on Movie** help screen.

When you first select the measure scale factor command, a dialog box will list the current scale factor, and that you may change the scale factor by typing '?' in the top line of the dialog box (Fig. 4.10). To change the scale factor, type '?' in the top line. The second part of the dialog box will allow you to enter the increment of measurement which you wish to use. **DIAS**® recognizes 'um', 'mm', 'cm', 'm', 'in', and 'ft' as units of measurement. When typing in a unit of measurement, do not use a period '.'.

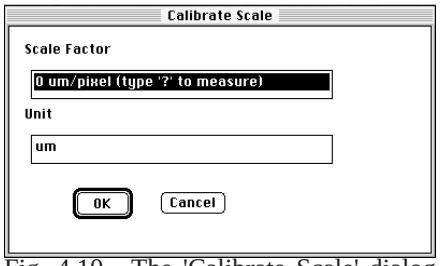

Fig. 4.10. The 'Calibrate Scale' dialog box.

After you have typed a '?' in the top line of the dialog box, and a unit of measurement in the second, click 'OK'. At this time, the title bar of the movie window will ask you to draw a line of know length. After you draw the line, by clicking and dragging the mouse a known length, a dialog box will ask you to enter the 'Length of (the) line', and to verify the 'Unit' of measurement which you wish to use (Fig 4.11). After you have entered the correct information and clicked 'OK', you are done.

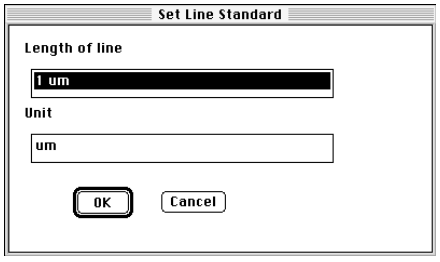

Fig. 4.11. The 'Set Line Standard' dialog box.

Because of the flexible nature of the both of the manual tracing commands, you may work on as many or few frames at a time as you wish. You may always come back and reedit or finish tracing later.

Trace on Movie with Splines

Trace on Movie with Splines is very similar to the **Trace on Movie** Command. It is designed for manually tracing cells. However, in this command, instead of freehand drawing lines and curves, you use the mouse to place points around the curves of the objects in which you are interested, and then use **DIAS**® to finish out those curves. As in the other manual tracing command, there is a help list available by typing '?' (Fig. 4.12). You may also use this command to measure/set your scale factor.

```
      Trace on Movie with Spline Commands

<space>      finish current curve
1-6          magnify the image
q, <cr>      done
Q, cmd-.     abort (changes not saved)
<del>        delete the last point
u            undo all drawing
v            finish and close current curve
c            clear all current tracing

             For movies only:

b             previous frame
n             next frame
g             goto frame
y             set trace slot
h             view/edit DIAS header
s             measure/set scale factor
f             save the trace and read it back
```

Fig. 4.12. The 'Trace on Movie with Splines' help list.

Again, most of these key commands are self explanatory, and will not be covered here.

Show Tracing

This command allows you to display or remove the image of the traces from the foremost movie file. This command does not permanently effect the trace files.

Clear Tracing

This command allows you to erase, or clear, all of the traces from your current trace slot. After selecting this command, you will be asked for a range of frames to clear.

Note: Instead of clearing traces, you can always change to a different trace slot. That way, you do not lose your work if you are unsure as to whether you will want to use it again in the future.

Backup Tracing

This command allows you to backup your traces to a separate file. This is a good safety feature, and should be used often, especially if you are doing a great deal of time-consuming manual tracing. This command backs up the traces in all of the slots in the current movie.

After selecting this command, you will be prompted for the name of the backup file, using a standard Macintosh save dialog box.

The file created with this command is only useful when paired with the **Restore Tracing** command.

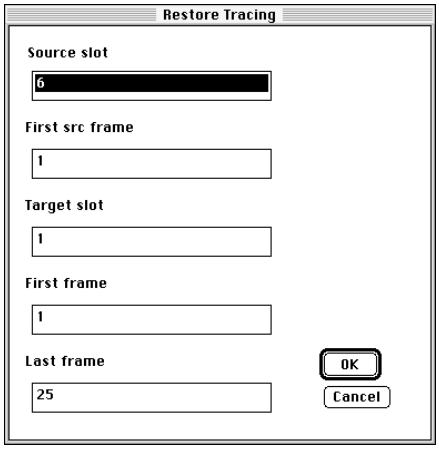

Fig. 4.13. The 'Restore Tracing' dialog box.

Restore Tracing

This command allows you to restore a set of traces to a movie, assuming that you had backed them up, using the **Backup Tracing** command.

You should have the movie open which you wish to restore traces to, before selecting this command. After doing so, and selecting the command, you will be prompted for the backup file you wish to use. When you have selected the backup file, the 'Restore Tracing' dialog box will open (Fig. 4.13). In this dialog, you must enter which slot from the backup file you with to use (the 'Source slot'), which frame you wish to start with from the source file (the 'First src frame'), which slot you wish to place the traces into in the current movie ('Target slot'), and where in the current movie you wish to start and finish the traces.

The variation in the start frames between the source file

and the target movie allows you to copy traces from one movie, to a much longer version of the same, without having to replicate all of the actual tracings. In this way, you can merge short sections of traces into a longer movie, or add frames to the beginning or end of a shorter movie.

Save Tracing as Movie

This command allows you to make a movie of just the outlines of your objects. This is occasionally useful for presentations. Similar types of movies may be created using the commands under the **ViewPaths** menu (Chapter VI).

Merge Tracing into Movie

This is another command which is useful for some types of presentations. If you wish to show a colleague what you have outlined, but they do not have **DIAS®**, you can **Merge the Tracing into Movie**, save the movie as a QuickTime® file, and let them view both your original data, as well as your traces.

Join Stage Movement

This command is designed to remove the jumps in cells and data which occur when slides or stages are moved during an experiment. **DIAS®** may determine that there are in fact two different objects or cells, the second one appearing after the jump while making paths because of large, sudden movements. This command allows you to take the jump out of the movie, thus eliminating the problem.

DIAS® has several commands designed to help with the same problem. There are the two join path commands under the **EditPaths** menu (Chapter VI). However, these commands may slightly distort the motility for the frame where the jump occurs. This is the case, because it cannot always accurately discount the movement of the cell from the movement of the stage. Under the **Movie** menu is the **Stabilize Movie** command. This command is very similar to **Join Stage Movement** feature, but designed for more continuous movement, as seen in poor videos, or in equipment with poor tracking. Moreover, this command can only be used on raw movies. It will erase any traces in a movie.

The **Join Stage Movement** command is the only one that allows you to subtract the movement of the stage only, and still retain all of your traces.

If you decide to use the **Join Stage Movement** command, you must first have a movie open and on the desktop. Selecting the command will bring up the standard Frame Range dialog box. Since this command will create a new, stand alone movie, make sure to include all of the movie which you wish to analyze. After selecting the desired frame range, a dialog box will ask you

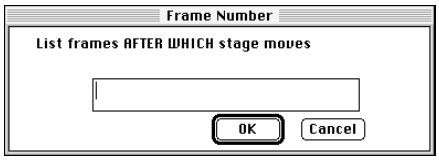

Fig. 4.14. The 'Frame Number' dialog box.

to 'List (the) frames AFTER WHICH the stage moves' (Fig 4.14). Enter here the first shifted frame number.

After you have done so and clicked 'OK', you will be taken to the frame previous to the shift, and the title bar of the movie will instruct you to select a known object before the stage shift. You should click the mouse on a stationary object. Use any spot or object which remains stationary throughout the current section of the movie. If you select the moving cell itself, the correction in stage shift will also subtract any motility for the cell between those two frames.

After choosing and clicking on your reference point, the movie will advance to the next frame, and you will again be instructed to click on the same point. Following this will be save and movie type dialog boxes, identical to those described in the previous chapter. After those dialog boxes, you will be asked if you wish 'Economize' the new movie. Economizing was also covered in the last chapter, but in essence is the process of removing all of the background in the movie, and leaving only the outlined areas.

Once you have made your choices, you will be asked which trace slots to preserve. You should preserve any slots in which you currently have traces.

Upon making this final selection, a new window will appear, and you will see the new frames saved. Notice that the new window is larger than the old movie window. This is to accommodate the new larger area of analysis, caused by the inclusion of the shifted area. When the movie gets to the shifted frame, you should see how the rest of the movie is shifted in response to your target points.

You may now proceed to further tracing, or to making paths.

Make Path From Trace

Tracings importance was partially covered at the beginning of this chapter. It was explained that accurate traces provide accurate morphometric information motility analysis. While the traces are all that is needed for the morphometric analysis, before motility may be measured, paths must be made for each of the cell/objects you wish to analyze.

Without making accurate paths, **DIAS®** won't be able to asses which cells are linked, and how to calculate any of the motility based parameters. The **Make Path From Trace** command is the first part of the path creation process.

Before beginning the path making process, make sure that the desired movie is open and on the desktop, and that the desired trace slot is selected.

After selecting the movie and trace slot, and clicking on **Make Path From Trace** from under the **DIAS** menu, the 'Make

Paths From Traces' dialog box will open (Fig 4.15).

The first three items in the dialog box are self explanatory and will not be covered here.

'Max movement/frame' helps determine how objects are connected. When an object is detected, the X and Y coordinates of the pixels are averaged, respectively, to calculate the **centroid** of the object. When making paths, the position of an object is considered to be its centroid. Paths are constructed as follows:

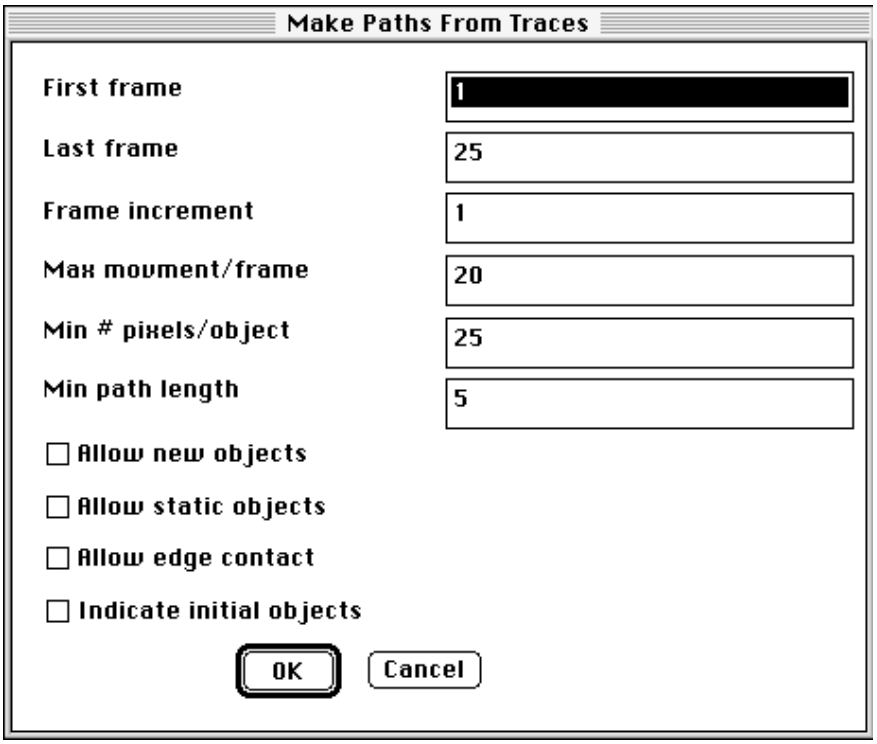

Fig. 4.15. The 'Make Paths from Traces' dialog box.

a) Objects and their centroids in the current frame and the next frame are found.

b) Pairs of objects, one in the current frame, the other in the subsequent frame, are found, whose centroids differ in position by <=5 pixels.

c) The closest such pair is considered to represent the position of an object in the current and subsequent frame. This portion of its path is noted and the object is removed from consideration when matching other objects in the frame. Return to step (b) unless no pair differ by <=5 pixels.

d) The distance value 5 above is called the 'current distance threshold'. This threshold is incremented by 5 and step (b) is repeated now using 10. That is, objects that have moved <=10 pixels are sought.

This process continues using 5,10,15,... until the current threshold exceeds the 'Max movement/frame' parameter. Objects that move more than this distance in one frame will have their path terminated. Any objects still remaining after this path extension process for a frame are considered to be new objects and new paths will be started with new object numbers.

Note: If the maximum movement parameter is too large, one object will be associated with several paths. Therefore, use the smallest workable number. If an object moves so fast that its path is segmented, the commands described in the **ViewPaths** and **EditPaths** chapter (X), can be used to splice the paths together.

Note: If the maximum movement parameter is too small, two objects that have moved too close together will have their paths confused. Again this could be repaired by cutting and splicing paths using **ViewPaths** and **EditPaths.**

'Min # pixels/object' is similar to the minimum and maximum controls available under the automatic trace commands. Use this entry to exclude small objects which have been automatically outlined. The larger you set this number, the more objects you will exclude. However, make sure to set it smaller than the smallest object which you are interested in, in any frame.

The 'Min path length' command allows you to exclude objects which may have passed through the movie image for a short period of time. If you do not wish to include these objects in future analysis, they may be excluded by making the path length longer than they were in view. However, if objects move more than the 'Max movement/frame', such as in a stage shift, short, separate paths may be created for the same object. Make sure that this does not happen by varying this number.

If you do not check the 'Allow new object' box in the dialog box, then only objects whose paths appear in the first frame will be included. Check this box if you wish to analyze the paths of objects which appear later in the movie, or if a single objects path may be truncated because of jumps in movement, as described above.

The 'Allow static object' checkbox allows you to include cells which periodically do not move. Not checking this box is any easy method of eliminating debris which may have been automatically outlined, but which will generally not move.

Generally, **DIAS**® will not include any object which runs off of the edge of the screen. It does so because the morphology of the object may be obscured by the loss of part of its screen area. Clicking the 'Allow edge contact' command will keep such objects as part of the path. However, realize that such data may be incomplete because of the loss of part of the objects area.

The last command, 'Indicate initial objects', allows you to select among the outlined objects in the first frame, in order to determine which objects to include. If you select this option, when you click 'OK' in the dialog box, the title bar of the movie window will ask you to select the initial objects by clicking on them. Doing so will add a dot to the object. When you have selected all of the objects of interest, press the return key.

After making all of your selections, and clicking 'OK', a dialog box will ask you if you wish to 'Include interior pixel data in the path file?' (Fig. 4.16). This option gives you the ability to include the actual interior image of each cell in later parts of the

analysis, including viewing and editing dynamic paths (Chapter VI), and shape analysis (Chapter XI). If you click 'No', only the outlines will be included in those two analysis areas. However, you should not include interior pixel data if analyzing more than a few cells, and 100 or so frames, otherwise, you may overload the resource fork of your file and damage or lose your file. Either way you answer at this time will not effect the path making process, visually.

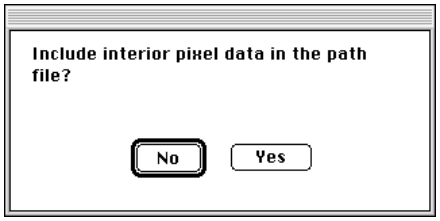

Fig. 4.16. The interior pixel data dialog box.

After you have made your selection, **DIAS**® will outline the objects in the first frame, and show the paths of movement in a different color. Any new objects which appear after the first frame will also have their shape in their first frame outlined, assuming that you checked to allow new objects. As the program is working, a box will display the frame numbers and number of objects found as **DIAS**® works through the movie.

Before continuing, it is important for you to realize how much editing should be done during this step. The most important thing is that all of the cells/objects in which you are interested are outlined and included. If the path of an object is broken, you can tell because an outline of the object will appear somewhere besides the first frame. You want to make sure that is because the maximum movement allowance has been overrun, and not because the outline of the object is open in some frame. If the reason has to do with movement, it is easier reattach the paths later, using commands under **EditPaths**. However, if an object is not included in a certain frame because it was not traced, because the trace was not closed, or for any other reason, there is no other point in the program in which you can make that change. **DIAS**® offers an array of other path display and editing capabilities, and they will be explained later in chapters VI.

Understanding the above, notice that when **DIAS**® has worked through the entire movie to find objects and paths, the title bar will allow you to either accept or edit the paths. The title bar will tell you to 'Click to Edit; Type r:redo and <cr>: done'.

You may click anywhere along the path lines. Doing so will take you to that frame and place you temporarily in the **Trace on Movie** mode. This is to allow you to make changes to the traces in that particular frame. Again, this is designed primarily to allow you to edit traces, add traces, or erase objects which have been traced, which you wish to change for further analysis. You can make these changes, using the capabilities explained earlier, including moving to different frames. When you are through editing, press the return key to take you back to the make paths controls.

The other option you have is to type 'r'. Doing so will bring you back to the original 'Make Paths From Traces' dialog box, and you may change your options.

Once you have made all of your decisions and are

relatively happy with the paths created, press the return key. You will be asked to name the new path file created.

Merge Parallel Path Files

This command allows you to merge already created path files together over the same time points. This is primarily directed towards the situation described earlier, when there are several cells in the same movie. Because the cells were traced in separate slots, different paths had to be made for each cell. After each of the paths is made, they may be combined using the merge parallel command.

After selecting this command, you will be prompted to open each of the paths you wish to merge, using a standard Macintosh open dialog box. Keep selecting relevant path files until you are done, then click the cancel button. You will be prompted for the name of the new path file.

> Note: You may merge path files directly after making them, or after editing them using the commands under **ViewPaths** and **EditPaths**.

Merge Serial Path Files

This command is similar to the one described above, except the it adds the paths one after another, sequentially. This is used when short sections of a movie have been traced and separate paths made. Follow the same directions as given above for merging the path files into a single, longer, path.

Make Path File Segment

This command allows you to select a subsection of a path file and to use it to create a new path file. You may wish to use this if you want to analyze a single cycle, or other periodic section of a path.

When you select this command, you will be prompted for the name of the longer path file, and then for the frame range you wish.

The rest of the DIAS menu

The remaining items under the **DIAS** menu pertain to whole other sections of the program, and will be covered later in the manual, under their own chapters.

V
Editing the file header

What is the file header for?

The file header contains information which **DIAS**® uses when calculating parameters, drawing graphs and charts, and displaying and printing the outlines of your digitized objects. The file header was originally created when paths were made.

Editing the file header

You can open and edit the file header for any path file. To open and edit a file header, select **Edit Path File Header** from the **DIAS** menu (Fig. 5.1). When the Macintosh® Open dialog box appears, select the path file which you wish to edit. The header for that file will appear on screen (Fig. 5.2).

Figure 5.1. Selecting **Edit Path File Header** from the **DIAS** menu.

Title

This can be anything. It will be printed out at the top of graphs, etc.

Date

Automatically entered as the current date when outlining first begins.

Time Unit

Used as the time unit in subsequent calculations and as the time designation in charts and graphs.

Distance Unit

This was automatically determined when the scale factor was measured. This unit of measurement will be used in subsequent graphs and charts. If you change the unit, you must also change the measurement. For example, if the distance unit is 'um' and the scale factor is '1.2', to change the unit to 'mm', the scale factor must be changed to '1200'.

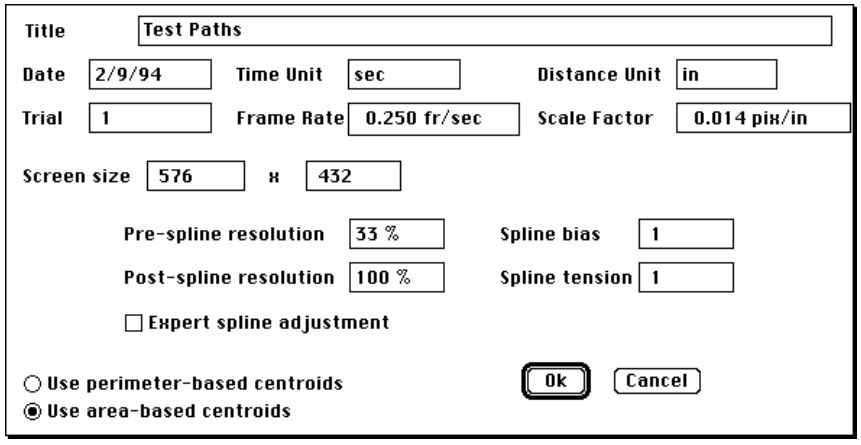

Figure 5.2. A path file header.

Frame Rate

This rate defines the time unit between frames. Specify

the number of frames per time unit (may be fractional). If the time unit is changed, the frame rate must also be changed. For example, if the time unit 'min' and frame rate '12' is changed to 'sec', the frame rate must be changed to '0.2'.

Scale Factor

Entered automatically during the 'Measure/set Scale' procedure. For more information, see 'Distance Unit'.

Screen Size

The window size in pixels. The default for this entry is the size, in pixels, of the movie from which the path was created. This may be changed here, or by using the **Change window size** command under the **ViewPaths** menu.

> Note: Even after you change the screen size, you may not be able to fit an entire path on screen at once if your actual monitor screen is too small. However, you will be able to scroll around the window after changing the screen size, and, in that way, you will be able to view all parts of the path.

Splines, resolution, bias and tension

The initial shape of the outline is determined during the digitization process. It consists of a connected circuit of pixels. The final shape is the mathematically precise shape of the object after the following parameters have been applied to the initial shape, resulting in a polygon with vertices specified by 6 significant digits of precision.

Beta splines replace the object's outline with curves that approximate the original pixels. The idea is similar to using a 'French Curve' in drafting. It is beyond the scope of this manual to define beta splines or to precisely define 'bias' and 'tension'. A good reference is Computer Graphics and Geometric Modeling Using Beta Splines by Brian A. Barsky, published by Springer-Verlag 1988.

Pre and Post-spline resolution

Resolution determines how carefully the curve segments are drawn. 'Pre-spline resolution' refers to the number of pixels to be used from the original outline. Because computer video is made up of a series of pixels, your original outline will probably have a jagged, or stair-stepped outline. That is only if you use every pixel (100%). This has the effect of shutting off the splines completely. Setting 'Pre-spline resolution' to 33% bases the outline splines on every third pixel and allows splines to be used.

You can experiment with what works best for you, but we have found 33% to be the best 'Pre-spline resolution'. 'Post-spline resolution' is similar in that you do not want to use all of the pixel points when reconstructing your outline, since your splines will be based on the stair steps. We have found 50% to be the best setting. These settings will retain the correct shape for the outline, while removing the jagged outline.

Bias and tension

'Bias' determines how the tangent line at a point on the object's outline affects either side of the curve extending from that point. A 'bias' of 1 is considered to be 'equal bias'. 'Tension' determines how tightly the curve 'adheres' to the pixels. If the tension is large, it is as if a tight rubber band is stretched around the pixels. The default bias and tension is 1.0.

The easiest way to see the affect of entering different values in the above four categories is to click on 'Expert spline adjustment'.

Expert spline adjustment

The 'Expert spline adjustment' control panel allows you to adjust all of the above settings simultaneously, and to see the changes instantly. After selecting 'Expert spline adjustment' and clicking 'OK', a dialog box will ask you which frame and object in that frame you wish to see the effects of the various settings on (Fig. 5.3). After making that selection, **DIAS**® opens up a window and control panel (Fig. 5.4). In the window is the current outline and original pixel points of the object. The red points represent the outline created either during automatic or manual outlining. The blue outline is the mathematical representation of those points as determined by the settings in the control panel below. Experimenting with these settings is the easiest way to see the effects of the various settings and combinations.

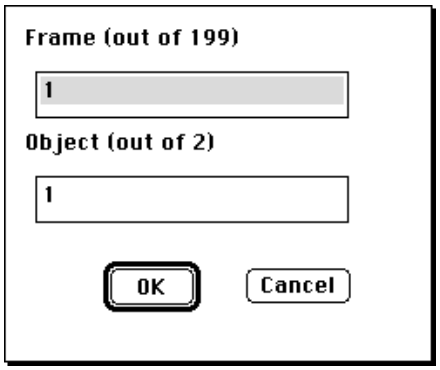

Figure 5.3. When selecting 'Expert spline adjustment', **DIAS**® gives you the ability to select a frame and object to work with.

Area and perimeter based centroids

If the **perimeter-based** method is selected for determining the centroid (center) of a cell, the average of the x and y coordinates, respectively, of the vertices is used. If the **area-based** method is selected, the mathematical center of mass is computed for the polygonal shape, assuming uniform density. This is also called the **center of area**.

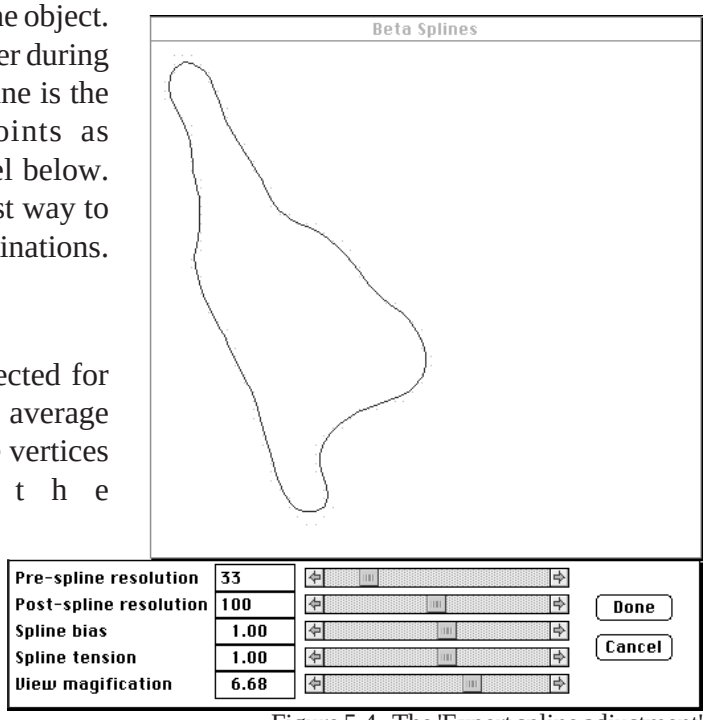

Figure 5.4. The 'Expert spline adjustment' control panel.

Note: If the object has holes, vertices on the boundaries of holes count

towards the centroid computation with the **perimeter-based** method. With the **area-based** method, the interior of a hole does not contribute to the center of area.

VI
View/Edit paths

The View/Edit Path menu

While in the **View/Edit Path** mode, you may analyze and edit paths created by **DIAS**®. You can delete, cut, and join various paths, as well determine how the paths and outlines appear on screen and on the printed page. You can use this process to view the path of your cell's centroid or its outline displayed over time. **DIAS**® also gives you the ability to difference your images, allowing you to see expansion and contraction zones. You can print the static pictures of these representations or make dynamic digital reconstructions, or digital animations, of these unique outlines, including QuickTime® movies.

To begin the process, select **View/Edit Path** from the **DIAS** menu (Figure 6.1). A standard Macintosh® Open dialog box will ask you to select a path file you wish to view, and after doing so, a window will open, showing you the file (Fig. 6.2). You may also enter these processes by opening a path file using the **Open** command under **DIAS**®'s **File** menu. Opening a path file automatically takes you the **ViewPaths** and **EditPaths** menus.

Figure 6.1. Selecting **View/Edit Path** from the **DIAS** menu.

> Note: Any changes made using the options under the **ViewPaths** menu affect only how paths and shapes are viewed on screen and printed, and do not affect parameter calculations. Any changes made using the options under the **EditPaths** menu permanently affects the file and how parameters will be calculated later.

The View/edit window

When the path file you have selected first opens, you will notice several things. The title bar will list the path file's name, the number of objects in the file, the current frame number, and the total number of frames in the file. In the window itself, you will see the outline of the first object in it's first frame, and the centroids and centroid paths of all of the objects in the file. Under the **ViewPaths** menu (Fig. 6.3) you will find several commands which will allow you to alter the current view in the window and to make and save movies. Under the **EditPaths** menu (Fig. 6.24) you will find several commands which allow you to edit and change the paths themselves. The commands under these two menus will be explained below.

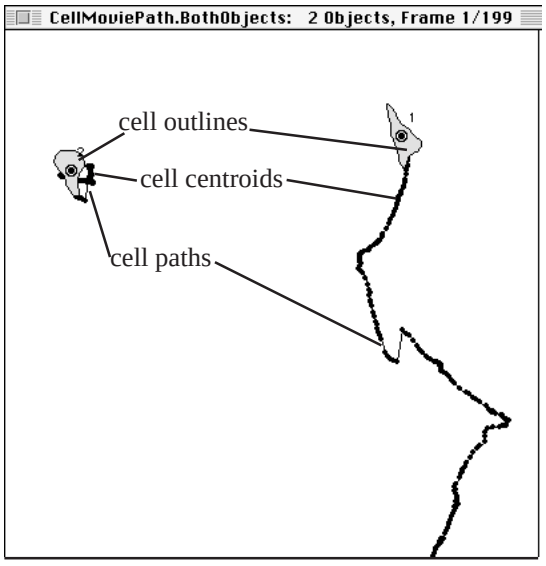

Figure 6.2. An open path file, showing cell outlines, centroids and paths.

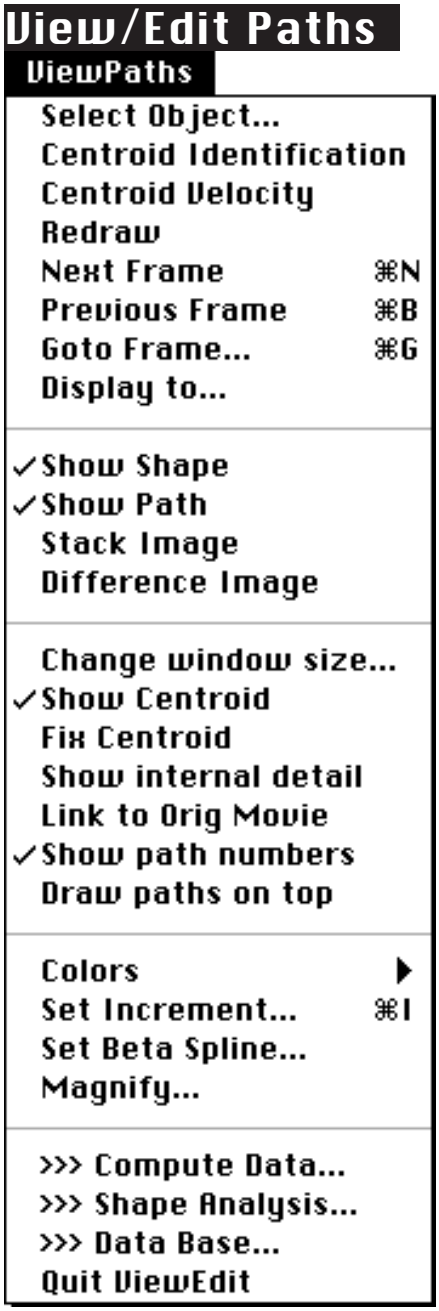

Figure 6.3. **DIAS® ViewPath**s menu.

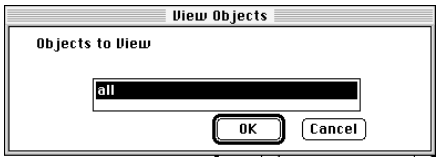

Figure 6.4. The 'View Objects' dialog.

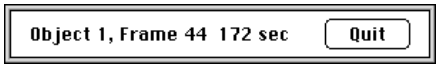

Figure 6.5. The 'Centroid identification' message window.

Figure 6.6. The 'Centroid Velocity' message window, asking you to select a object position.

Select Object

Upon selection of this option, a dialog box will prompt you for the objects (by number) which you wish to display (Fig. 6.4). The default is to display 'all' of the objects. To view any subset of all of the objects, type in the numbers of the objects you wish to keep on screen.

> Note: In this step, and any other that asks for a list of numbered choices, you can specify ranges ('4-8'), or type in a list separated by commas ('4, 5, 6, 7, 8'). Do not separate items by spaces, since **DIAS®** will not accept the list in that format.

> Note: Objects not selected for view are not deleted. To view objects not currently being viewed, click on **Select objects** and type in their number, or 'all' (to restore the window to the default settings).

Centroid identification

After selecting this command, a message window will prompt you to select a centroid with the mouse (Fig. 6.5). To do so, position the cursor near a node (or centroid) and click the mouse button. The number of the object, its frame number and time will be displayed in the message window. If you do not click directly on a node, the closest one is used. Repeat this process as desired. To stop the process, click on 'Quit' in the message window.

Centroid Velocity

This command will allow you to find out the velocity (speed) for any specific object at any one time point. To find the velocity of an object, select **Centroid Velocity** from the **ViewPaths** menu. A dialog box will ask you to 'Select (a) position with (the) mouse' (Figure 6.6). As you click on centroids, shape outlines or paths, the dialog box will change and list the object number, the frame number and the speed of the object at that time point (Figure 6.7). When you are through checking velocity, click on 'Quit' in the dialog box.

> Note: Speed is calculated based on the definitions listed in Chapter VIII.

> Note: If you do not click directly on a centroid, outline or path, **DIAS®** will calculate velocity for the centroid closest to the spot you have chosen.

Redraw

You should select this command if the screen becomes cluttered. For example, if you have been drawing or typing in the window, selecting **Redraw** undoes the work.

> Note: You should select redraw after making changes in the increment and magnification settings.

Next frame

This command displays the outlines of the objects in the next frame or at the next time point. If only the path is visible (**Show shapes** is deselected) this will have no visible effect.

Previous frame

This command displays the outlines of the objects in the previous frame or time point. If only the path is visible (**Show shapes** is deselected) this will have no visible effect.

Go to frame

Selecting this command brings up a dialog box asking you which frame to go to. The objects in that frame will be displayed.

Display to

Display to is similar to **Go to Frame** except that all the frames between the current frame and the one specified are flashed onto the screen as a movie. This is a powerful display tool when paired with stacking and differencing. When this option is selected, a dialog box will ask you which frame to display to and whether you wish to make a movie of the display. To view the display, simply click 'OK'. To make a movie, type 'y' or 'yes' in the dialog box and click 'OK'. You will then be prompted to name the movie, for a movie type, and whether you wish to compress your movie, as described in chapter III.

After you have chosen a movie format, you will be given the option of adding additional information to the movie (Fig. 6.8). A dialog box will give you the ability to 'Title' your movie. That title will appear in the upper left hand corner of your movie as it displays. You will also be able to mark any single frame in the movie (which may be used to signal a change in the experiment) by having **DIAS**® outline the frame of the movie for that time point. You can also show a continuous time change throughout the frames ('Frame interval (sec)'), even starting by counting backwards to a specific frame ('Time 0 frame'). These are all options which can enhance your movie for a presentation, but which do not permanently change your path file.

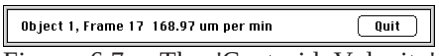

Figure 6.7. The 'Centroid Velocity' message window, listing object number, frame number and speed.

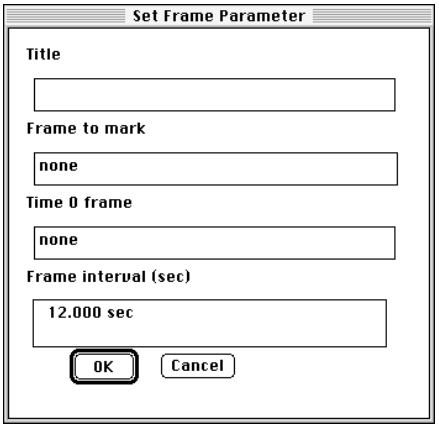

Figure 6.8. The 'Set Frame Parameter' dialog box.

Once you have made all of your choices, you will see your movie play on screen. It is only at this point that the movie is saved, so do not interrupt the process unless you do not want to save the movie.

Show shapes

With this option selected (selection is denoted by a checkmark next to the item on the menu) the outlines of all the cells (or those selected by **Select objects**) appear in the window (Fig. 6.9). The color, resolution, and size of the shapes may be modified by selecting the **Show internal detail, Link to Original Movie**, **Make B/W**, **Object edge color**, **Object interior color**, **Vary object color**, **Set beta splines**, and **Magnify** commands, described later. You can use the **Next frame** and **Previous frame** commands to display the shapes for different or multiple frames.

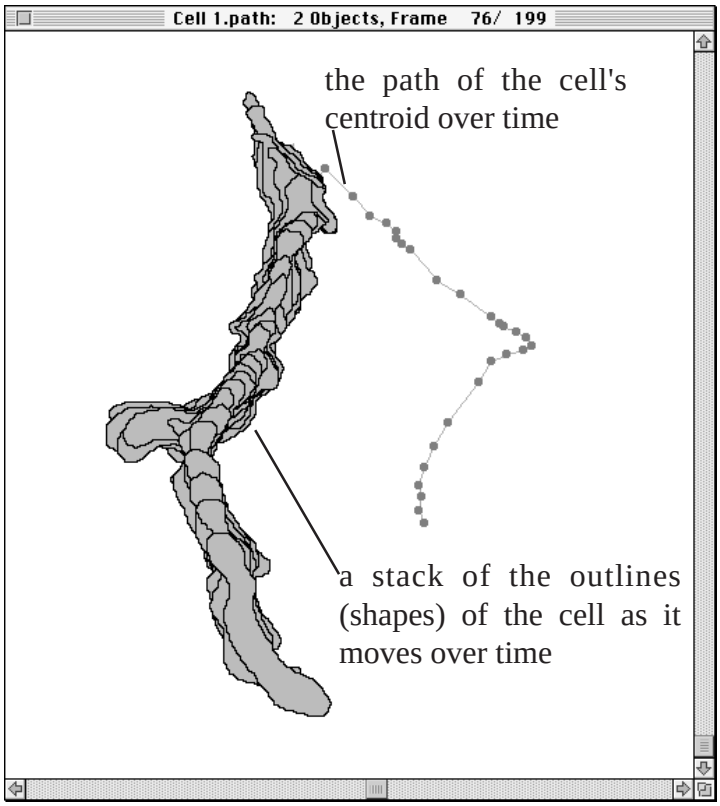

Figure 6.9. Examples of **Show shapes**, **Stack images**, and **Show paths**.

Show paths

With this option selected, the paths of all the cells (or those selected by **Select objects**) appear in the window. The color, time intervals, and size of the paths may be modified by selecting the **Make B/W**, **Path color**, **Path node interior color**, **Vary path color**, **Set increments**, and **Magnify** commands, described later. When you first select **View/edit paths**, the paths will be shown as a default. Selecting **Show paths** from the **ViewPaths** menu will turn off the paths. After deselecting **Show paths**, the next time you select to **Show paths**, you will be given the option to show a subsection of a path. A dialog box will ask you whether you wish to 'View paths for a range of frames (yes) or a single frame (no)?' (Fig. 6.10). If you wish to view the entire path, select 'No', if you wish to view a subsection of the path, select 'Yes'. You will be prompted for the frame numbers that you wish to view. This is helpful if you are analyzing crowded fields with overlapping paths.

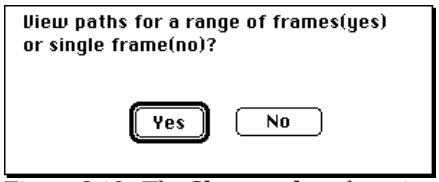

Figure 6.10. The **Show paths** subsection dialog box.

Stack images

When this option is selected and **Display to** is used, the outlines of the objects in the current frame are not erased when the outlines of the next frame are drawn. Figure 6.9 gives an example of stacked images on the left and a centroid path (no shapes) on the right.

Difference images

With this option selected, the outline of the object in the current frame is superimposed on the outline of the object in a previous frame (Fig 6.11). Using the **Set increments** option (described later), the number of frames, or time points, separating the two outlines may be changed. The regions of the later cell image not overlapping the earlier cell image are in green, and are considered 'expansion zones,' the regions of the earlier cell image not overlapping the later cell image are in red, and are considered 'contraction zones'. Areas common to the cell image at both time points are in grey.

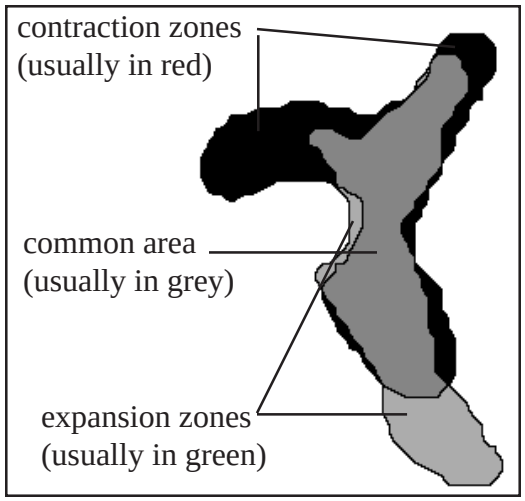

Fig. 6.11 An example of a difference picture.

Change window size

This command allows you to change the size of the window, in pixels, in order to fit more or less of the image into the window. Making a change here will also change the 'Screen size' in the file header. This may be very useful, especially after joining paths into a longer path.

Show Centroid

When this option is selected, the centroid of the object is shown as a circle in the interior of the object.

Fix centroids

When this option is selected, the objects will be locked into one location (taking out the factor of motility) when drawing the objects outline. This is accomplished by superimposing centroids in sequential images. With this option and either **Stack images** or **Difference images** selected, **Display to** provides a view of pseudopodal extension and shape change in the absences of centroid translocation.

Note: **Show shapes**, **Show paths**, **Stack images**, **Difference images**, **Show centroids**, and **Fix centroids** can all be used in varying combinations. For instance, difference pictures may be stacked, and the result saved as a QuickTime® movie.

Show internal detail

If you chose to 'Include interior pixel data in the path file?' when making the current path, you may now choose to display that internal detail, instead of using the standard colors. To do so, select this option. You can also still stack and display to images. However, because of the multi-color basis of differencing, internal detail will probably be obscured.

Because the internal detail of the movie has actually been saved to the path file, editing paths, magnification, and other path

file changes should not effect the ability to display this internal information.

Link to Orig(inal) Movie

Link to Orig Movie is similar to **Show internal detail**, in that they both allow you to view the object's actual, original interior while in **View/Edit Path**. However, there are several differences between the two methods. As described in Chapter IV, you should only include internal detail with a path file if the movie/path is short, and there are very few objects. These restrictions are memory based. However, including the pixel data with the file is very robust in terms of later imaging. As noted above, you can cut and paste paths, magnify, etc. With **Link to Orig Movie**, no additional information is included with the path file, so there are no memory limitations. This command allows you to manually pick the movie to link it to. This automatically and dynamically cuts and pastes greyscale data from one file to the other. It reads the x & y coordinates from the open path file, and captures the data within the same coordinates from the selected movie file, showing it within the path file shapes.

While this procedure allows you to link paths of a significantly larger size, and including many more objects per frame, there are limitations. Because of the exactness of the x,y coordinate matching, this command will not work if you **Magnify** within **ViewPaths**, if you **Join Paths end-to-end**, **Change the window size** under **ViewPaths** or the 'Screen size' within the header or make any other placement changes in either the path file or movie file. You must also make sure to save and keep the original movie file on your hard drive.

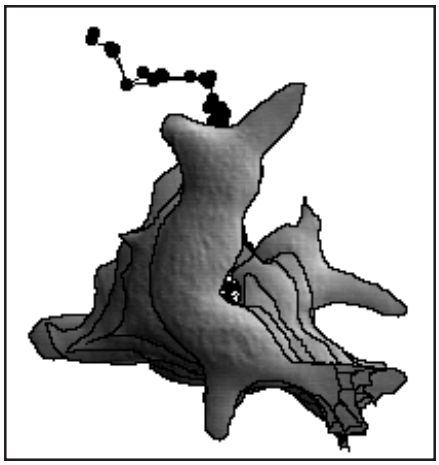

Figure 6.12. An example of a stack of outlines with actual object interiors linked to the original movie.

If you can work within these limitations, and did not save the internal pixel data to the path file, you may use **Link to Orig Movie** to display actual cellular information. To do so, select **Link to Orig Movie** from the **ViewPaths** menu. A standard Macintosh® Open dialog box will appear. Use this dialog to select the original movie from which the path file was created. After doing so, **DIAS®** will read the movie into memory and replace the objects colored interiors with digital information taken from the original movie (Fig 6.12).

Show path numbers

This command allows you to display, or turn off the display of cell/object numbers.

Draw paths on top

Generally, when showing shapes, especially in conjunction with **Display to** and **Stack Image**, the shapes are shown over top

of the path files. Selecting this command will reverse that layering. This is sometimes useful for publications and other figures.

Colors

Under **Colors** there appears a submenu (Fig. 6.13) which gives you control over the colors of various parts of the paths and shapes. Most of them are self-explanatory. The **Vary object color** and **Vary path color** are useful when viewing multiple objects whose paths and outlines intersect and overlap. It makes it easier to distinguish separate objects.

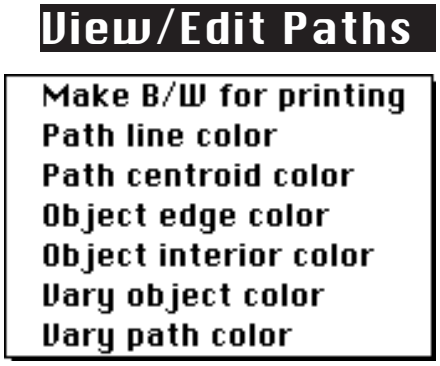

Figure 6.13. The **Colors** submenu.

> Note: If you are going to select **Make B/W for printing**, do so before using **Display to**, as invoking **Make B/W** will erase all frames shape outlines except for the current frames.

Set increments

After selecting this option, a dialog box will appear, providing two types of increments which can be changed (Fig. 6.14). The first change you can make is in 'Frame inc.' The default is '1', which means the nodes and shapes for every frame will be displayed. A frame increment of 1 draws the centroids and shapes for all frames. An increment of 2 draws centroids and outlines in frames 1, 3, 5, ...etc. Changes in the frame increment primarily affect the density of centroids and image outlines in a path (Fig. 6.15). 'Difference increment' defines the meaning of previous frame when showing difference pictures. The previous frame number equals the current frame minus the 'Difference inc(rement)'. If a 'Difference inc(rement)' is set higher than the available number of previous frames available, no differencing will immediately occur. Hence, if the 'Difference inc(rement)' is set at '3', and you are currently one frame past the earliest frame when you select **Display to**, no differencing will occur. Differencing will begin with frame four. Figure 6.16 is an example of how different 'Difference inc(rements)' can affect your difference picture.

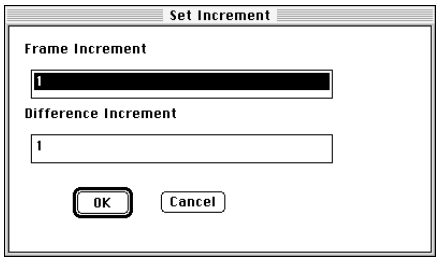

Figure 6.14. The 'Set Increment' dialog box.

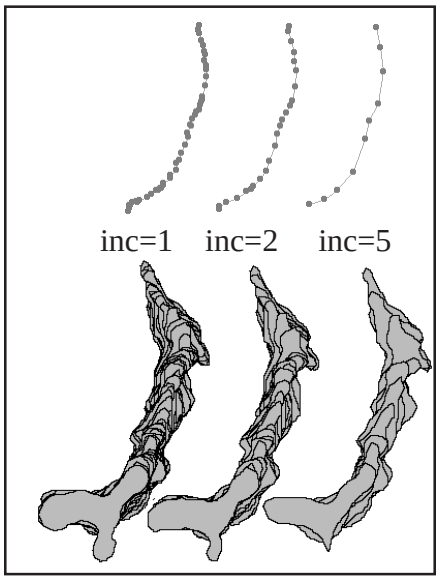

Figure 6.15. Examples of 'Frame inc(rements)' of 1,2 and 5 on both paths and stacks of images.

> Note: You should select **Redraw** and re-select **Display to** after making changes in the increments settings.

> Note: Changes in increments do not affect parameter calculations, or the time points which will be used in graphs and charts. The changes are for viewing and printing only.

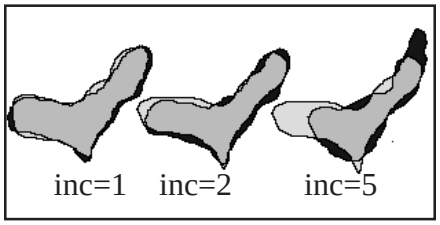

Figure 6.16. Examples of 'Difference inc(rements)' of 1,2 and 5 on difference pictures.

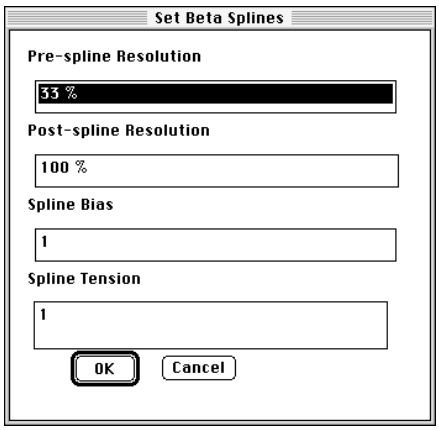

Figure 6.17. The 'Set Beta Splines' dialog box.

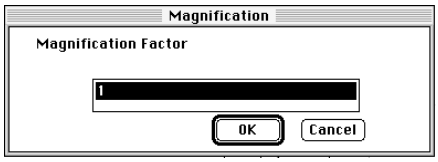

Fig. 6.18. The magnification dialog box.

Set Beta Splines

Selecting this option from the **ViewPaths** menu brings up a dialog box with several bias, resolution and tension options (Fig. 6.17). These were originally covered in chapter V and will not be repeated here except to point out that the changes you make here do not affect parameter calculations, but are designed to affect viewing and printing only.

> Note: If you made bias, resolution and tension changes in the header, those changes are already incorporated in the **ViewPaths** section, and do not have to be repeated.

Magnify

This command allows you to magnify (or reduce) a portion of the image. When selected, you are prompted for a magnification factor (Fig. 6.18). This is always based upon the original screen size (not the current magnification-if changes have already been made). The magnification may be fractional or as high as 600. After entering the magnification factor, the user is prompted to select the center of magnification by clicking the mouse. This will be the new center of the magnified screen. To disable any magnification and return to the original screen size, use a magnification factor of 0. If the new, magnified images do not fit within the window, you may still **Change window size**. When you leave **ViewPaths**, the current magnification is optionally saved (via a prompt) so that when you reenter **ViewPaths** later, the view will be the same.

> Note: You should always select **Redraw** and reselect **Display to** after magnifying.

Compute Data, Shape Analysis and Database

These options will be covered in subsequent chapters.

Quit ViewEdit

Select this to exit the **View/Edit Path** section. If changes have been made, you will be asked whether you wish to save them. If you simply close the window containing the paths and outlines, the changes you have made will not be saved. You must instead quit the **View/Edit Path** section of the program. This is not the same as quitting **DIAS®**.

> Note: In the **EditPaths** menu (Fig. 6.19), there are several commands which affect not only how the paths are viewed on the screen, but how objects

and paths are treated later during the calculation of parameters. When you select **Quit ViewEdit** from the **ViewPaths** menu, you will be asked if you wish to save any changes you have made. Remember, the changes also include changes made in the **EditPaths** menu, and can affect which paths (objects) are included when calculating parameters.

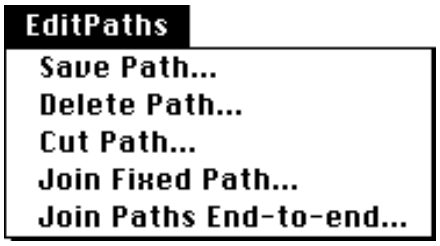

Fig. 6.19. The **EditPaths** menu.

Save Path

Selecting **Save Path** from the menu brings up a dialog box asking you which objects (paths) to save (retain) (Fig. 6.20). You may want to select this option if there are multiple paths, and there are fewer you wish to save than delete. When typing in a list of multiple paths, separate the numbers with commas; do not use spaces. The default for this option is 'all'.

After you have typed in your selections and clicked on 'OK', the appropriate paths will be permanently removed, and those remaining will be renumbered.

Fig. 6.20. The 'Retain objects' dialog box.

Delete Path

Selecting the **Delete path** option brings up a dialog box asking you which objects (paths) you wish to remove (Fig. 6.21). When typing in a list of multiple paths, separate the numbers with commas; do not use spaces. The default is 'none'.

After you have typed in your selections and clicked on 'OK', the appropriate paths will be permanently removed, and those remaining will be renumbered.

Fig. 6.21. The 'Remove Object' dialog box.

Cut Path

Selecting the **Cut Path** option brings up a dialog box asking you which object (path) you wish to cut, and after which frame. After you make this selection, you will have two paths (instead of one). The second path will start with the frame entered in the dialog box in Figure 6.22 and be renumbered.

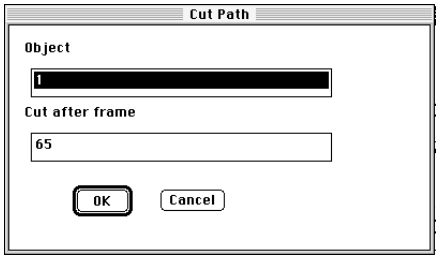

Fig. 6.22. The 'Cut Path' dialog box.

Join Fixed Path

If the path of an object you are tracking shows up in **DIAS**® as two or more paths, you will need to join paths in order to calculate the correct parameters. **DIAS**® gives you two different methods to do so.

The **Join fixed paths** command joins together two or more paths without moving them, interpolating centroids if there is a gap in time between the end of the first path, and the beginning of the second. This is useful if a path was broken due to digitization problems or collisions with other objects, or because settings in the **Max movement/frame** number were too low

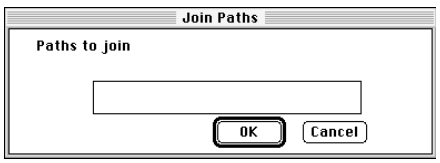

Fig. 6.23. The 'Join Paths' (both fixed and end-to-end) dialog box.

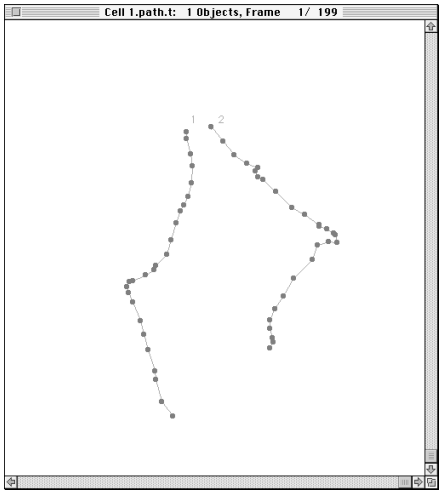

Figure 6.24. The original path file with two paths shown.

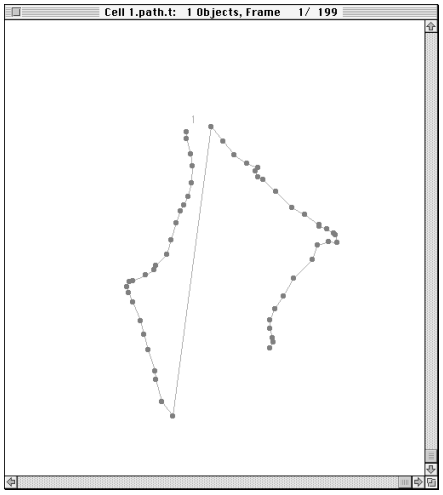

Figure 6.25. The same paths joined using the **Join Paths Fixed** command.

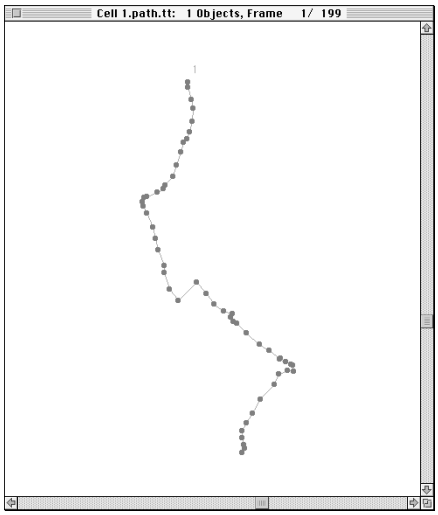

Figure 6.26. The same paths joined using the **Join paths end-to-end** command.

when making the path (Chapter IV). When the **Join Fixed Path** dialog appears, list the paths you wish to join (up to 25 at a time). They need not be in order, since the computer will automatically sort them in the order of the paths starting times. If paths overlap, the one that started earlier has precedence. When you type in the numbers and click 'OK', a standard Macintosh® Save dialog box will appear, asking you for the name of the new path file. When you have typed that in and selected 'OK', the new path will be drawn, and all the remaining paths will be renumbered. When you first select either **Join Fixed Paths**, or **Join Paths End-to-End**, a dialogue box will ask you which paths to join (Fig. 6.23).

Figures 6.24-26 are examples of original and joined paths.

Join paths end-to-end

The **Join Paths End-to-End** option differs from the **Join Fixed Paths** in that the second path being joined is moved so that its starting point is at the predicted (using linear interpolation) location of where it would be if the object in the first path had continued moving. This type of joining is generally used if an object being viewed under a microscope keeps moving out of view, and the observer has to adjust the microscope stage to re-center the object. The result of this type of action is the creation of several paths beginning near the center and moving towards the edge of the screen. The **Join Paths End-to-End** command will allow you to reconnect the paths correctly, so that the object's full path is viewable.

When the **Join Paths End-to-End** dialog appears, list the paths you wish to join (up to 25 at a time). They need not be in order, since the computer will automatically sort them in the order of the paths starting times. If paths overlap, the one that started earlier has precedence. When you type in the numbers and click 'OK', a standard Macintosh® Save dialog box will appear, asking you for the name of the new path file. When you have typed that in and selected 'OK', the new path will be drawn, and all the remaining paths will be renumbered.

Because the new path may be much larger than the original, the entire path may not fit in the old window (and you cannot scroll around the window to see the rest). If this is the case, you will need to increase the 'Screen size' in the path file header (Chapter VII). To do so, **Quit ViewEdit**, saving the changes, open the file header for the path file you just created, and increase the numbers in the 'Screen size' section of the dialog box. When you reselect **View/edit paths**, you will be able to either see the entire path on screen, or at least be able to scroll around the window to see the entire path. When you first select either **Join fixed paths**, or **Join paths end-to-end**, a dialogue box will ask you which paths to join (Fig. 6.23).

Figures 6.24-26 are examples of original and joined paths.

Note: Attempting to join paths of a single timepoint may not work. If you are having problems with broken paths, you may want to change the settings during path construction, specifically allowing for a larger movement per frame.

Quit(ting) ViewEdit

Remember, you must quit the **View/Edit Path** section in order to save any changes made to your path file.

View/Edit Paths

VII
Set Graph Size

Which graphs

This option is designed to allow you to vary the size of the graphs generated during the **Compute Parameters** and **Use Database** chapters (VIII & IX). When you select **Set graph size** from the **DIAS** menu (Fig. 7.1), you will get a dialog box asking you for the size of the graphs you wish to create, measured in pixels (Fig. 7.2). As a reference, a standard Macintosh® 13" monitor measures 640 (length) x 480 (height) pixels. The default for a graph size is 500 by 400.

> Note: You can set these limits larger than your actual monitor, shrink the window later and use the scroll bars to move around the graph if you want to see more detail. You can also use the **Magnify** command in the **Graphics Manager**. However, magnifying the graph does not magnify the window size.

Figure 7.1. Selecting **Set graph size** from the **DIAS** menu.

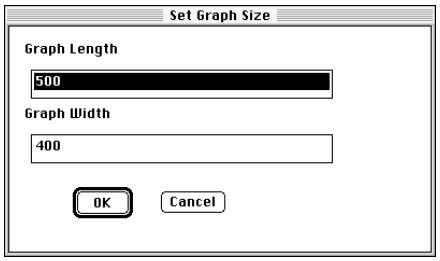

Figure 7.2. The **Set graph size** dialog box.

Set graph size

VIII
Compute Parameters

Computing DIAS® parameters

In order to perform graphical and numberical analysis of the motility and dynamic morphology for the cells you have outlined, select **Compute parameters** from the **DIAS** menu (Fig 8.1). A standard Macintosh® Open dialog box will ask you which path file you wish to compute parameters for. After typing in the name of the path file and selecting 'OK', the **DIAS®** 'Compute Parameters' dialog box will appear. You may also go directly to **Compute Parameters** by selecting that command from under the **ViewPaths** menu, when you have a path file open. There are several options and parameters (Fig. 8.2) available under this command, and they will be described in detail below.

Figure 8.1. Selecting **Compute Parameters** from the **DIAS** menu.

Objects

Enter the list of objects (paths) for which you wish data to be computed. Entries must be separated by commas and ranges are allowed. For example, '1,3-8,9' means the same as '1,3,4,5,6,7,8,9'. Enter 'all' if you wish all objects to be analyzed.

First Frame

Enter the number of the first frame you want analyzed.

Last Frame

Enter the number of the last frame you wish analyzed.

Frame Increment

This is the increment added to the first, and all subsequent frames to get the subsequent frame number. A frame increment of 2 will process every other frame. The frame rate is automatically corrected to take into account the frame increment.

For example, assume that the following values are entered (with 8 frames/min being the frame rate):

First Frame: 7

Last Frame: 32

Frame Increment: 3

The result would be as if the path file had 9 frames with a frame rate of 4=(8/3) frames/min, the 9 frames being (in the original frame

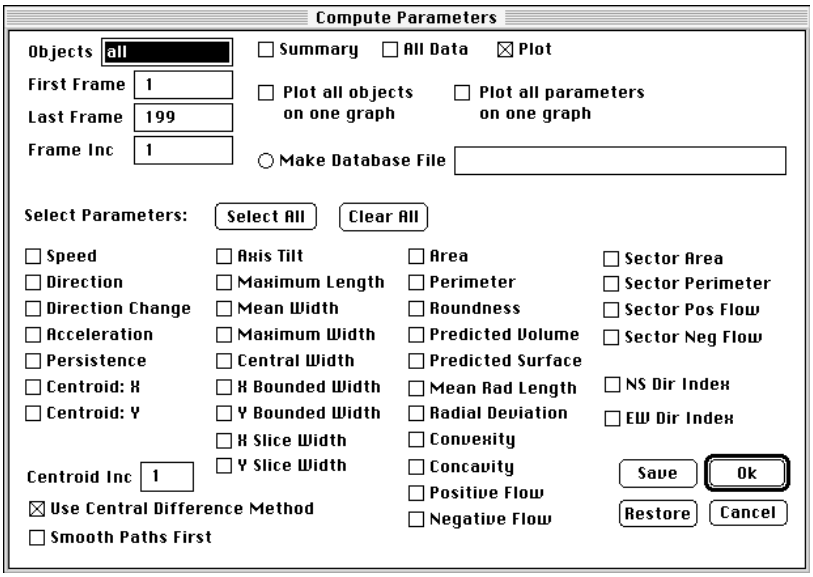

Figure 8.2. The **DIAS®** 'Compute Parameters' dialog box.

numbers) 7,10,13,16,19,22,25,28,31.

Summary

A data summary will be displayed if this option is checked. The means and standard deviations of the selected parameters will be shown. A summary will be generated for each selected object. A final summary, with data averaged over all objects, will also be generated.

data.text

```
Title: Test Paths
2/9/94,  Trial 1,  199 Frames,  2 Objects
Frame Rate: 0.25 fr/sec  Scale: 0.014 in/pixel
Pre-spline resolution: 33 %Post-spline resolution: 100 %Spline bias: 0  Spline tension: 1
Centroid Method: Center of area

Object 1,  Frames: 1 to 199
Time: 0 to 792 sec, Total Elapsed Time: 792 sec
Total path length: 7.22194 in  Net path length: 5.24486 in
Directionality -  Total: 0.726241  Upward: -0.726239  Rightward: -0.00136096
Speed (in/sec): 0.00851617 +/- 0.00465043
Direction (deg): 265.978 +/- 37.0801
Area (sq in): 0.151451 +/- 0.0198516


Object 1:
  Frame    Time    Speed   Direct     Area
          (sec)  (in/sec)   (deg)   (sq in)
    1     0.000    0.007  -95.366    0.114
    2     4.000    0.004  -96.478    0.114
    3     8.000    0.003  -82.836    0.109
    4    12.000    0.010  -73.622    0.122
    5    16.000    0.011  -69.356    0.110
    6    20.000    0.010  -76.184    0.133
    7    24.000    0.009  -82.834    0.133
    8    28.000    0.008  -90.690    0.130
    9    32.000    0.010  -82.231    0.137
   10    36.000    0.008  -74.418    0.135
```

Figure 8.3. A sample data output.

All Data

For each selected object and parameter, a list of the data values of that parameter will be displayed, indexed by frame number (Fig. 8.3). This will be covered in more depth later.

Plot

For each selected object and parameter, a graph is generated, with time plotted on the x-axis and the data parameter on the y-axis (Fig. 8.4). Two new menus with graphics functions is provided with each graph, and the **Graphics Manager** (Chapter IX) is entered. The number of graphs that may be displayed at one time is limited only by the computer's RAM memory. The number of graphs generated can be greatly reduced by selecting one or both of the 'Plot all objects on one graph' or the 'Plot all parameters on one graph' options. For example, assume that you have selected 3 objects and 5 parameters. Normally this results in 15 graphs. The computer will plot as many as possible, given the amount of memory available. If 'Plot all objects on one graph' is selected, only 5 graphs will be generated: each graph will contain 3 plots, one for each object. On the other hand, if 'Plot all parameters on one graph' is selected, there will be 3 graphs, one for each object, with 5 parameters plotted on each graph. If both are selected, only one graph will result, with 15 plots. Too many plots on one graph may seem awkward, but it is possible to use the **Plot** and **Axes menus** to selectively view only the desired plots.

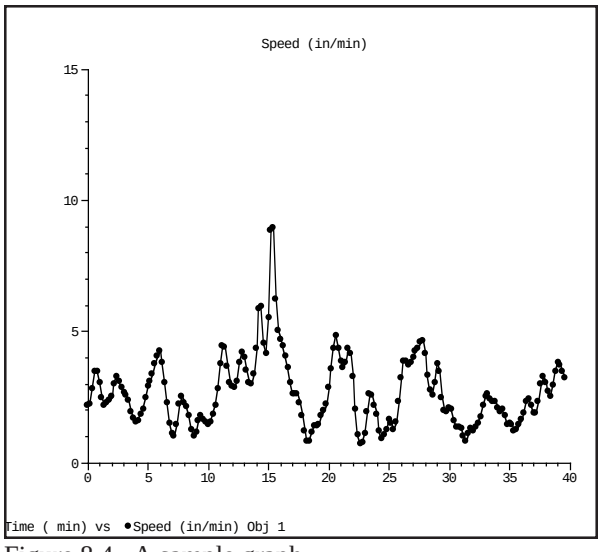

Figure 8.4. A sample graph.

Make Database file

The numerical data generated by 'Summary' or 'All Data' appears in a standard Macintosh® text window. You can save and edit the text in this window with many standard Macintosh® text applications. If you chose 'Make Database file' from the diaog box, you can open this file in a format that makes cutting and pasting between other applications (spreadsheets and graphing programs) possible. To do so, use the **View as text** or **Export Data Files** command under the **Data** menu (see Chapter X).

Figure 8.4. An example of the 'DATA' icon.

DIAS® also supports a more structured database file, called a Data Text file, (of type 'DATA') with its own icon (Fig. 8.4). The database file is used in conjuction with the **Use Database** command described in Chapter X. All of the 'DATA' files collectively act as a database. The **Use database** command is capable of manipulating several files from within the database at one time. To create a DATA file, select 'Make Database File'. A database file is created with the same name as your path file, but with '.data' appended. If you wish to use a different name, highlight the current name and type in your new choice**.**

> Note: You can also open these files (using **Open** under the **File** menu) and cut copy and paste between other applications.

The Parameters

The user may check up to 33 parameters to be computed. The definitions of the parameters are given at the end of this chapter. 'Select All' and 'Clear All' buttons are provided for convenience when selecting parameters.

The computation of the parameters is modified by three items found at the bottom right of the dialog: **Centroid Inc**, **Use Central Differences**, and **Smooth Paths First**. These are discussed later in this chapter under the definition of the parameter **Speed**. They affect only those parameters determined by centroid movement. If either **Positive Flow** or **Negative Flow** are selected, the user will be prompted for more information concerning the calculation of these parameters.

Computing the data

Once the objects, frames, output mode, and parameters have been selected, click 'Ok' to continue. As the computation proceeds, a visual meter shows the progress. Type 'command-.' to abort the computations, if desired. If 'Plot' is selected, graphs will appear first, one by one, on screen. Finally, a text window will appear with the 'Summary' and (if selected) the numerical ('All') data. If a graph is brought to the foreground (by clicking

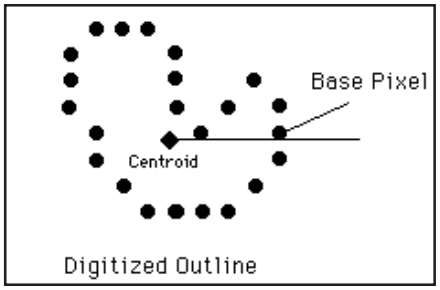

Figure. 8.5. Finding the base pixel.

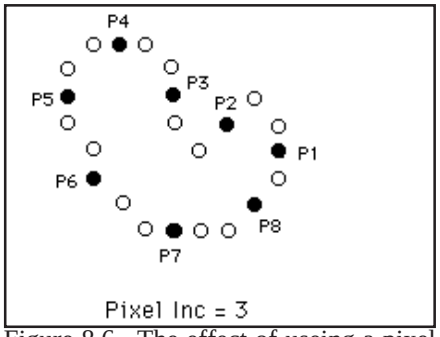

Figure 8.6. The effect of useing a pixel increment.

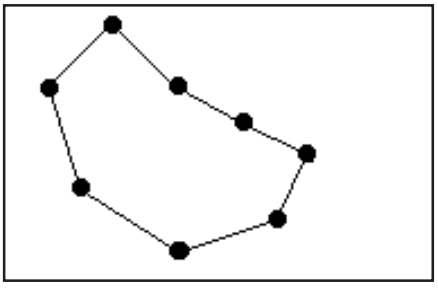
Figure 8.7. Splines disabled.

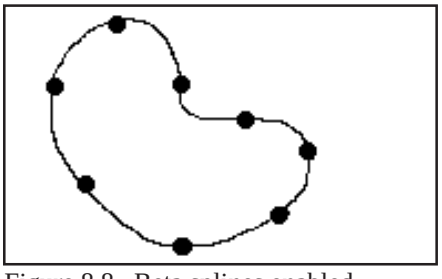
Figure 8.8. Beta splines enabled.

on it with the mouse), the **Graphics Manager** (Chapter IX) menus will appear on the menubar. These menus will be covered later in the manual. A graph can be saved to disk using the **Save** command located under the **File** menu. It is saved in either a native **DIAS®** graph format, or in the'PICT' image format. If saved in the 'PICT' format, **Plot** and **Axes** functions must be applied prior to saving - they will not be available when the image is reopened. However, all drawing and painting options will still be available. If you plan to work with data over a period of time, it is best to have the data in a database file and select **Use database** to invoke the **Graphics Manager** menus**.**

Object Shape and Centroid Definition

Based on the original digitized outline of the object, certain settings found in the Path File Header determine precisely the meaning of 'centroid' and 'shape'. Shape is determined by

1) Pixel Increment, i
2) Resolution, r
3) Bias, b
4) Tension, t

Bias and tension are beta spline parameters and are described in Chapter V, **Edit Path File Header**. To make changes in how shape is computed, make changes in the path file header before selecting **Compute parameters**. The settings made under the **ViewPaths** menu (See Chapter VI) only affect how the objects look and are printed. Centroids are based on shape and are computed by either the **Boundary** or the **Area** method (see Chapter V for original selection of methods).

The **initial shape** is the outline produced by the original digitization process. It consists of a connected circuit of pixels. The **final shape** is the mathematically precise shape of the object after parameters 1-4 have been applied to the initial shape, resulting in a polygon with vertices specified by 9 significant digits of precision. The final shape is determined from the initial shape by the following steps:

1) Determine the base (or starting) pixel. A preliminary centroid is calculated by averaging the x and y coordinates, respectively, of the pixels of the inital shape. Now, starting at the preliminary centroid, a scan is made towards the right until a pixel of the initial shape is reached. This is the base pixel (Fig. 8.5)

2) Prune out pixels using the pixel increment **i**. Let the pixels be labelled (in a counterclockwise direction) **p1,p2,...** with **p1** being the base pixel. The pixels retained after pruning are **p1,p(1+i),p(1+2i),... .** If the pixel increment is 1, all pixels are retained. If the pixel increment is 2, every other pixel is retained, etc (Fig. 8.6).

3) Determine the mathematical equations of the beta splines.

If they are enabled. This results in a mathematically defined curve segment associated with each pair of adjacent pixels (after pruning from step 2). If splines are disabled, the curve is taken to be the line segment joining adjacent pixels (Figs. 8.7 & 8.8).

4) The resolution **r** is used to convert each curve segment from step 3 into a sequence of line segments: **r+1** vertices are evenly spaced (by curve length) along the curve segments. The vertices are connected by **r** line segments. This is the **final shape** (Fig. 8.9).

The final centroid is computed: If the **perimeter-based** method was selected during the **Edit path file header** process, the average of the x and y coordinates, respectively, of the vertices from step 4 is used. If the **area-based** method was selected, the mathematical center of mass is computed for the polygonal shape from step 4, assuming uniform density. This is also called the **center of area**. All Time Series calculations use this centroid for speed, acceleration, etc.

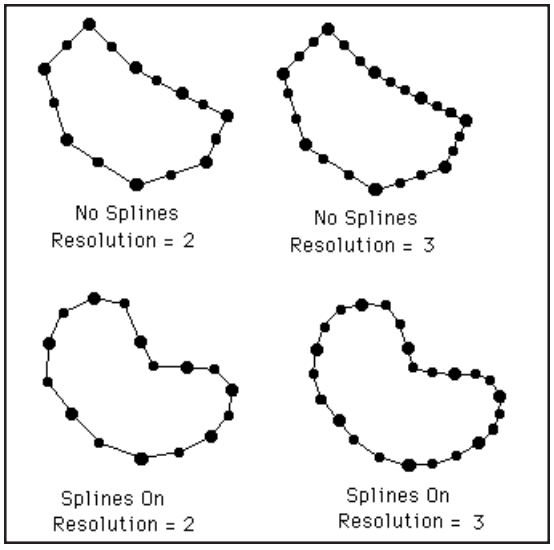

Figure 8.9. How various settings effect of the final shape.

Note: If the object has holes, the above process is repeated for each hole. Vertices on the boundaries of holes count towards the centroid computation with the **perimeter-based** method. With the **area-based** method, the interior of a hole does not contribute towards the center of area.

Definition of the parameters

So that we may be precise in the descriptions, some notation must be given. When we refer to the pixels **P**1,...,**P**n of a shape or its centroid we are referring to the **final shape** and **final centroid** (Step 5 above).

Note: If the first frame, last frame, or frame increment are other than the default, reread the discussion above under **Frame Increment**. If, for example, the selected frames were 7, 10, 13, 16, 19, 22, 25, 28, 31 (frame inc =3), the computations proceed as if the file had 9 frames numbered 1, 2, 3, 4, 5, 6, 7, 8, 9 with frame rate 1/3 of the original frame rate. In the following discussion, 'frame' refers to the renumbered frames.

Notation:

F is the total number of frames. f is the 'current' frame.

(**x**[f],**y**[f]) are the coordinates of the centroid of an object in frame f, 1<=f<=**F**.

I is the centroid increment.

n is the number of (final) pixels for a shape. **P**1,...,**P**n are the pixels.
frate is the frame rate in # of frames per unit time.

scale is the scale factor in distance units per pixel.

sqrt is the square root function.

abs is the absolute value function.

angle(x,y) is the angle (in degrees) that the vector from the origin to the point (x,y) makes with the x axis. Positive angles are measured counter-clockwise, as in trigonometry.

NAN stands for 'not a number'. **NAN**s do not appear as a point on a graph and are not counted in an average.

x is sometimes used for multiplication

Speed

When the central difference method is selected, centroids before and after the current frame are used to compute speed, direction, etc. This gives a slightly smoother result, less influenced by sudden 'jumps' of the centroid. Without the central difference method, only the current and previous centroids are used.

Another way to reduce the effect of 'jumps' or 'noise' is to increase the **Centroid Inc(rement)**. By default it is 1. It defines what is meant by 'previous' and 'subsequent' frames during calculations. Increasing it will tend to emphasize more general movement and will minimize small local movement. The centroid increment is applied after the frame increment. If the frame increment is 3 and the centroid increment is 2, centroids in 6-frame time lags will be used for calculations. They will be computed, however, every 3 frames.

Definition of speed at frame f

Without Central Difference Method:
Speed[f] = 0 when f-**I**< 1

Speed[f] = (**scale**_**x**_**frate**)_**x**_**sqrt**(((**x**[f]-**x**[f-**I**])/**I**)^2 + ((**y**[f]-**y**[f-**I**])/**I**)^2) when 1<=f-**I**

With Central Difference Method:
Speed[f] = (**scale**_**x**_**frate**)_**x**_**sqrt**(((**x**[f+**I**]-**x**[f-**I**])/2**I**)^2 + ((**y**[f+**I**]-**y**[f-**I**])/2**I**)^2)
when 1<=f-**I** and f+**I**<=**F**

Speed[f] = (**scale**_**x**_**frate**)_**x**_**sqrt**(((**x**[f+**I**]-**x**[f])/**I**)^2 + ((**y**[f+**I**]-**y**[f])/**I**)^2) when f-**I**< 1 and f+**I**<=**F**

Speed[f] = (**scale**_**x**_**frate**)_**x**_**sqrt**(((**x**[f]-**x**[f-**I**])/**I**)^2 + ((**y**[f]-**y**[f-**I**])/**I**)^2) when 1<=f-**I** and f+**I**>**F**

Speed[f] = 0 otherwise

Direction

Without Central Difference Method:
Dir[f] = **angle**((**x**[f]-**x**[f-**I**]), (**y**[f]-**y**[f-**I**])) when 1<=f-**I**

Dir[f] = **angle**((**x**[f+**I**]-**x**[f]), (**y**[f+**I**]-**y**[f])) when f-**I**< 1 and f+**I**<=**F**

Dir[f] = 0 otherwise

With Central Difference Method:
Dir[f] = **angle**((**x**[f+**I**]-**x**[f-**I**]) , (**y**[f+**I**]-**y**[f-**I**]))
when 1<=f-**I** and f+**I**<=**F**

Dir[f] = **angle**((**x**[f+**I**]-**x**[f]), (**y**[f+**I**]-**y**[f]))
when f-**I**< 1 and f+**I**<=**F**

Dir[f] = **angle**((**x**[f]-**x**[f-**I**]), (**y**[f]-**y**[f-**I**]))
when 1<=f-**I** and f+**I**>**F**

Dir[f] = 0 otherwise

Note: Multiples of +/- 360 degrees are added to the direction to make the graph continuous. For example, an object moving in a spiral would have directions: 0, 45, 90, 135, 180, 225, 270, 315, 360, 405, etc.

Direction Change

DirCh[f] = 0 when f-**I**< 1 **DirCh**[f] = **abs**(**Dir**[f] - **Dir**[f-**I**]) otherwise

> Note: if the direction change is greater than 180 degrees, it is subtracted from 360. This always gives values between 0 and 180 degrees.

Acceleration

Without Central Difference Method:

Acc[f] = **Speed**[f] - **Speed**[f-**I**] when 1<=f-**I**
Acc[f] = 0 otherwise

With Central Difference Method:

Acc[f] = (**Speed**[f+**I**] - **Speed**[f-**I**])/2 when 1<=f-**I** and f+**I**<=**F**
Acc[f] = **Speed**[f+**I**] - **Speed**[f] when f-**I**< 1 and f+**I**<=**F**
Acc[f] = **Speed**[f] - **Speed**[f-**I**] when 1<=f-**I** and f+**I**>**F**
Acc[f] = 0 otherwise

Persistence

Persis[f] = **Speed**[f]/(1 + (100/360)***x***DirCh**[f])

> Note: Persistence is essentially speed divided by the direction change (given in grads instead of degrees). One is added to the denominator to prevent division by 0. If an object is not turning, its persistence is the same as the speed.

Centroid: X

CenX[f] is the x coordinate of the centroid at frame f. It is given in pixel coordinates. It must be multiplied by **scale** to get real world coordinates.

Centroid: Y

CenY[f] is the y coordinate of the centroid at frame f. It is given in pixel coordinates. It must be multiplied by **scale** to get real world coordinates.

Axis Tilt

To calculate this parameter we must first define the **major axis** of an object. First, the pixel on the shape's outline farthest from the centroid is found. If there is more than one such pixel, the one closest to the base pixel in counter-clockwise order is used. This pixel becomes one end point of the major axis. Now the pixel on the shape's outline farthest from that end point is found. Again, if there is more than one, the closest such pixel to the base pixel is used. This is the other end point of the major

axis.

The axis tilt **Tilt**[f] is the angle (in degrees) that the major axis makes with the horizontal (always less than 180 deg). See Figure 8.10.

Ideally, the major axis should be defined to be the line connecting the two farthest points on the boundary. If there were 200 pixels on the boundary, about 20,000 distance calculations would be required. This would vastly slow down the computation of axis tilt. The method described above requires only 400 distance calculations.

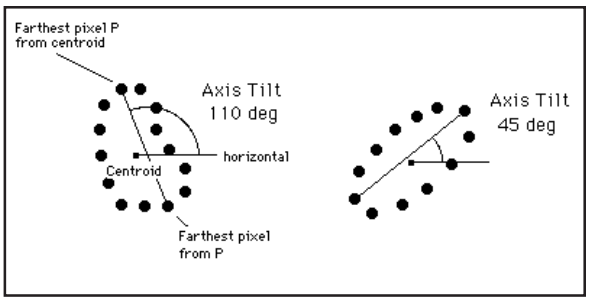

Figure 8.10. Axis tilt.

Note: Multiples of +/- 180 are added to the axis tilt to give continuity. Divide by 180 and take the remainder to get the actual tilt. If an oblong object is spinning at a constant rate, the axis tilt graph will be a line of constant slope. The slope will be positive if the object is spinning counter-clockwise and negative if clockwise.

Mean Width

The Mean Width **MeanWid**[f] is defined as the area (defined below) divided by the maximum length: **MeanWid**[f] = **Area**[f]/**MaxLen**[f] . This turns out to be the same as the average length of all chords (not counting the portions of the chords that leave the object) perpendicular to the major axis. See Figure 8.11.

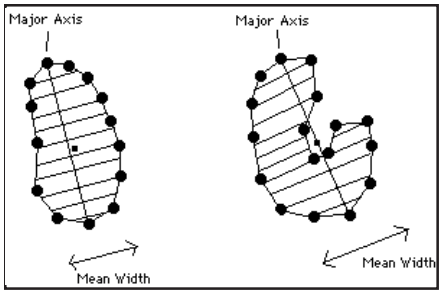

Figure 8.11. Mean width.

Maximum Width

The Maximum Width **MaxWid**[f] is defined as the length of the longest chord perpendicular to the major axis. See Figure 8.12.

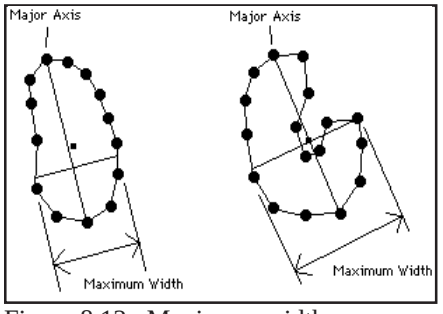

Figure 8.12. Maximum width.

Central Width

The Central Width **CenWid**[f] is defined as the length of the chord through the centroid perpendicular to the major axis. See Figure 8.13.

X Bounded Width

The X Bounded Width **XWid**[f] is defined as the width of the smallest enclosing rectangle (with horizontal and vertical sides). See Figure 8.14.

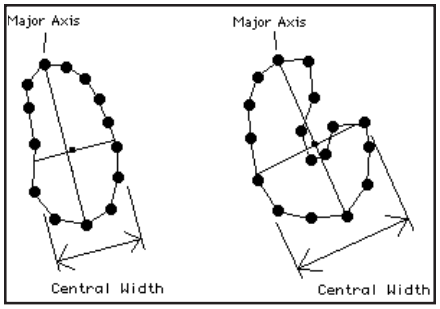

Figure 8.13. Central width.

Maximum Length

The Maximum Length **MaxLen**[f] is defined as the length of the major axis defined above.

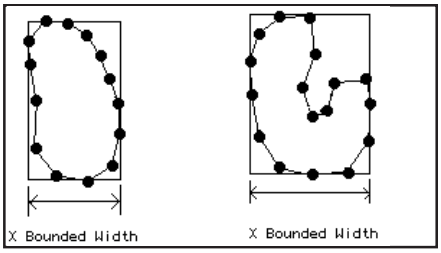

Figure 8.14. X Boundedwidth.

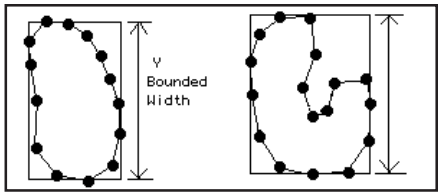

Figure 8.15. Y Bounded Width.

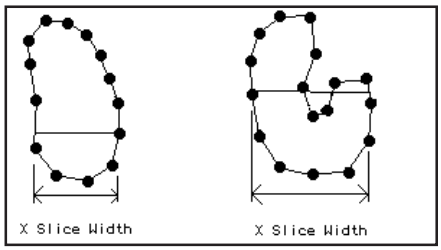

Figure 8.16. X Slice Width.

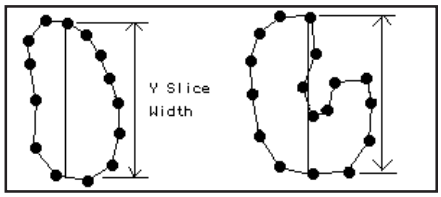

Figure 8.17. Y Slice Width.

Y Bounded Width

The Y Bounded Width **YWid**[f] is defined as the height of the smallest enclosing rectangle (with horizontal and vertical sides). See Figure 8.15.

X Slice Width

The X Slice Width **XSWid**[f] is defined as the length of the longest horizontal chord. See Figure 8.16.

Y Slice Width

The Y Slice Width **YSWid**[f] is defined as the length of the longest vertical chord. See Figure 8.17.

Area

Area[f] is defined as the area of the **final shape** minus the area of any holes. Let (**x**[i],**y**[i]) for i=0,...,n be the vertices of the final shape, such that **x**[0]=**x**[n] and **y**[0]=**y**[n] (so that it ends where it starts). Let **dx**[i]=**x**[i+1]-**x**[i] and **dy**[i]=**y**[i+1]-**y**[i]. Then area is computed (using Green's Theorem) by:

Area[f] = 0.5$\underline{x}$**abs**{ (**x**[0]$\underline{x}$**dy**[0]-**y**[0]$\underline{x}$**dx**[0]) + ... + (**x**[n-1]$\underline{x}$**dy**[n-1]-**y**[n-1]$\underline{x}$**dx**[n-1]) }

Perimeter

The perimeter **Perim**[f] is defined as the perimeter of the **final shape** plus the perimeter of any holes. Let (**x**[i],**y**[i]) for i=0,...,n be the vertices of the final shape, such that **x**[0]=**x**[n] and **y**[0]=**y**[n] if the shape is closed. Let **dx**[i]=**x**[i+1]-**x**[i] and **dy**[i]=**y**[i+1]-**y**[i]. Then perimeter is computed by:

Perim[f] = **sqrt**(**dx**[0]^2 + **dy**[0]^2) + ... + **sqrt**(**dx**[n-1]^2 + **dy**[n-1]^2)

Roundness

Round[f] = **100** $\underline{x}$ **4**π $\underline{x}$ (**Area**[f]/**Perim**[f]^2)

Roundness is a measure (in percent) of how efficiently a given amount of perimeter encloses area. A circle has the largest area for any given perimeter and has a roundness parameter of 100%. A straight line encloses no area and has a roundness parameter of 0%. The factor **4**π in the formula ensures that a circle has 100% roundness. The perimeter is squared to make the roundness scale invarient (i.e. dimensionless).

Predicted Volume

The predicted volume **Vol**[f] is the volume of the ellipsoid, with circular cross-section, having length **MaxLen**[f] and width **MeanWid**[f]:

Vol[f] = (4π/3)$\underline{x}$(**MaxLen**[f]/2)$\underline{x}$(**MeanWid**[f]/2)^2

Predicted Surface

The predicted surface area **Sur**[f] is the surface area of the ellipsoid, with circular cross-section, having length **MaxLen**[f] and width **MeanWid**[f]:

Sur[f] = **CF** $\underline{x}$ π $\underline{x}$ **MaxLen**[f] $\underline{x}$ **MeanWid**[f]

where **CF** is the ellipsoidal surface correction factor defined by

CF = Ú **sin**(x)$\underline{x}$**sqrt**(**sin**^{2}(x)+r^2**cos**^{2}(x)) dx

where r=**MeanWid**[f]/**MaxLen**[f].

Using Simpson's Rule with N=10000, the computer approximates this via the polynomial

CF = $0.15r^2 + 0.065r + 0.785$

Mean Radial Length

The mean radial length **RadLen**[f] is the average distance of boundary pixels of the object to the centroid (holes are ignored). Let n be the number of vertices of the final shape, indexed from 0 to n-1. Let **L**[i] be the distance from the i'th vertex to the centroid. Then: **RadLen**[f] = (**L**[0] + ... + **L**[n-1])/n

Radial Deviation

The radial deviation **RadDev**[f] is the ratio of the standard deviation of the above average to that average itself, written as a percent. Let **SD** be the standard deviation of **L**[0],...,**L**[n-1]. Then:

RadDev[f] = 100 $\underline{x}$ **SD**/**RadLen**[f]

Convexity and Concavity

To compute **Convex**[f] and **Concav**[f], line segments connecting the vertices of the final shape are drawn. The angles of turning from one segment to the next are then measured. Counter-clockwise turning represents a positive angle, while clockwise turning a negative angle. For a closed outline, these angles always add up to 360 degrees. This proceedure is repeated for holes.

The total sum of the angles is 360$\underline{x}$(1 + Number of holes). **Convex**[f] is defined to be the sum of the positive turning angles, while **Concav**[f] is the absolute value of the sum of the negative angles (See Fig. 8.18). The following equation holds:

Convex[f] - **Concav**[f] = 360$\underline{x}$(1 + Number of holes)

Convexity and concavity measure the complexity of a shape. A circle has convexity 360 and concavity 0. A shape (such as in Fig. 8.19) with K 'arms' will have convexity approximately K$\underline{x}$180 and concavity K$\underline{x}$180-360 = (K-2)$\underline{x}$180. Each arm contributes about 180 degrees to the convexity.

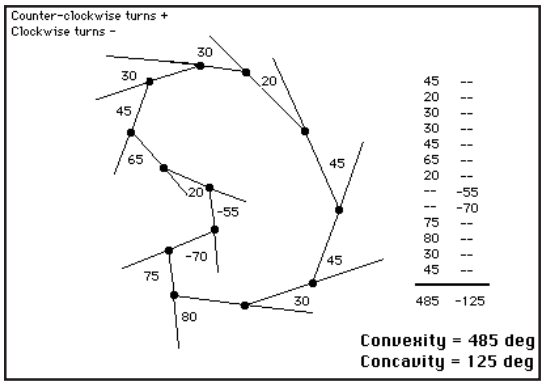

Figure 8.18. Computation of Convexity and Concavity.

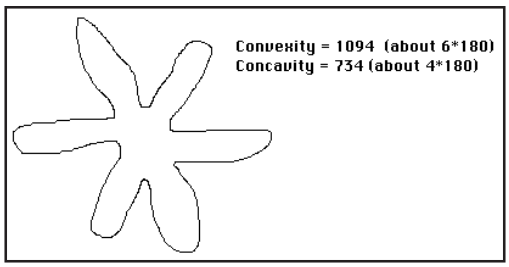

Figure 8.19. A figure with arms.

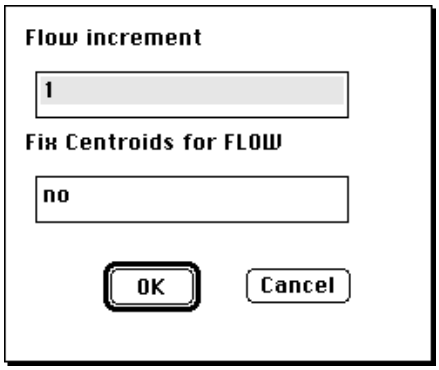

Figure 8.20. 'Positive' and 'Negative Flow' options dialog box.

Positive and Negative Flow

Positive flow is essentially the amount of new area formed in a certain amount of time (determined by the 'Flow Increment' which is prompted for below). It is expressed as a percent of the total area. Negative flow is the amount of area lost in the same amount of time, written as a percentage. In other words, positive and negative flow measure the percent of area expansion and contraction of an object per unit of time. If a cell has no z axis change and no significant volume change during the **Flow** increment, then the **Positive** and **Negative Flow** values should be similar.

To make this more precise: Let f be the current frame and **FI** be the Flow Increment. Let **A** be the interior of the shape at frame f-**FI,** minus any holes. Let **B** be the interior of the shape at frame f, minus holes. (If f-**FI** is less than 1, the positive and negative flow are undefined). Let **P** be the area in **B** that is not in **A** (**P**=**B-A** in Set notation). Let **N** be the area in **A** that is not in **B** (**N**=**A-B** in Set notation). Then define:

PosFlow[f] = 100 $\underline{x}$ **Area**(**P**)/**Area**(**A**) and **NegFlow**[f] = 100 $\underline{x}$ **Area**(**N**)/**Area**(**A**)

If you select either **Positive** or **Negative Flow** from the Time Series Parameters dialog box, you will see a dialog box giving you options for how the flows will be measureed (Fig. 8.20). Flow increment refers to the time points between flow measurements. For example, if each frame represents 5 seconds, a Flow increment of 1 measures the % expansion/contraction per every 5 sec. A Flow increment of 2 measures the % expansion/ contraction per every 10 sec. The centroids may be fixed before comparing the shapes. This means that **B** is moved so that its centroid matches that of **A**, before computing **Positive** and **Negative Flow**. This has the effect of 'subtracting out' centroid movement from shape change. As computations proceed, for each object and each frame selected a window showing the positive and negative regions appear.

Sectors

Sector Area, **Perimeter**, **Pos(itive) Flow**, and **Neg(ative) Flow** are derivative measurements of the standard parameters **Area**, **Perimeter**, **Positive Flow**, and **Negative Flow**. These parameters allow you to calculate the original parameters for 4 sections of the cells shape. When you select these options, you will be presented with two dialog boxes which will help you to determine how these parameters are calculated.

The first dialog box will ask you where to begin and end your sectors (Fig. 8.21). The default is from 0 degrees to 360 degrees. This will give you 4 equal measurements of the entire

shape of the cell. You need to enter a range of degrees that is meaningful for your cells. 0 degrees is considered the topmost point of the cells shape. Whatever range you enter will be repeated all the way around with the second sector starting at the end point of the first. For instance, if you entered 0 and 90 in the dialog box, the sectors would consist of the sections measured 0-90 degrees (sector 1), 91-180 degrees (sector 2), 181-270 degrees (sector 3), and 271-360 degrees (sector 4). Four sectors will always be measured, so there may be some overlap in the later sectors (if the initial sector is more than 90 degrees), or the entire shape may not be measured (if the initial sector is less than 90 degrees). You can also start at any point. You do not have to start at 0 degrees.

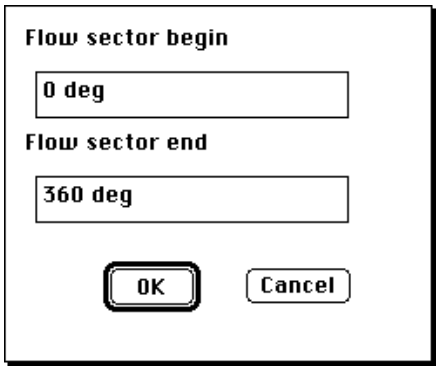

Figure 8.21. 'Sector' definition dialog box.

The second dialog box will ask you whether you wish to 'Use dir(ection) of travel for 0 deg(rees)?' (Fig. 8.22). The default is 'No', setting 0 as the topmost point of the cells shape. If you select 'Yes' here, 0 degrees becomes the point where the line showing the direction of the cells movement crosses the cells outline. The degree measurement calculated for the cell in a particular frame now becomes degree 0 for these calculations.

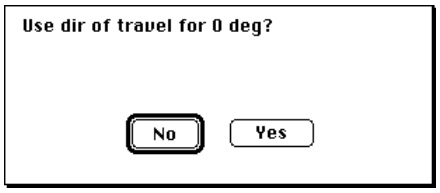

Figure 8.22. O degree dialog box.

After making your choices, a window will open displaying the shape and/or flow for each object chosen. This will be followed by the appropriate graphs and data.

NS and EW Dir Index

The 'NS' and 'EW Dir' parameters are each a chemotactic index for general directionality. The 'NS' parameter measures directionality in an up and down plane, with '1' denoting movement straight northward, or upwards. The 'EW' parameter is the same measurement with '1' being straight right, or eastward movement. Neither of theses parameters are graphed, and only show up in the summary.

Summary and Data Output

The summary (Fig. 8.23) gives the following information:

Time: The time of the beginning and the end of the path for this object.

Total Elapsed Time: The ending time minus the beginning time for the path.

Total Path Length: The lengths of the line segments connecting centroids of the path are summed.

Net Path Length: The distance directly from the path starting point to the ending point.

Directionality:

Total: The net path length divided by the total path length. This gives 1.0 for a completely straight path and a smaller value for a meandering path.

Upward: This is defined to be: (**Y** coordinate of the end of the path minus **Y** coordinate of the beginning) divided by the total path length. This gives 1.0 for an object moving directly upward and -1.0 for an object moving directly downward.
An object whose net movement is only horizontal gives 0.0 .

Rightward: This is defined to be: (**x** coordinate of the end of the path minus **x** coordinate of the beginning) divided by the total path length. This gives 1.0 for an object moving directly towards the right and -1.0 for an object moving directly towards the left. An object whose net movement is only vertical gives 0.0 .

Finally, the summary gives the means +/- the standard deviations of the selected parameters.

Note: Sometimes parameters such as **Area** are so large that the data printout is obscure. The best way to handle this is to edit the File Header (Chapter V) to change the units of distance and/or time to be more convenient. For example, change **um** to **mm** or **in** to **ft,** etc. Make sure you adjust the frame rate and scalefactor accordingly.

data.text

```
Title: Test Paths
2/9/94,  Trial 1,  199 Frames,  2 Objects
Frame Rate: 0.25 fr/sec  Scale: 0.014 in/pixel
Pre-spline resolution: 33 %Post-spline resolution: 100 %Spline bias: 0  Spline tension: 1
Centroid Method: Center of area

Object 1,  Frames: 1 to 199
Time: 0 to 792 sec, Total Elapsed Time: 792 sec
Total path length: 7.22194 in  Net path length: 5.24486 in
Directionality -  Total: 0.726241  Upward: -0.726239  Rightward: -0.00136096
Speed (in/sec): 0.00851617 +/- 0.00465043
Direction (deg): 265.978 +/- 37.0801
Area (sq in): 0.151451 +/- 0.0198516


Object 1:
  Frame    Time     Speed    Direct      Area
          (sec)  (in/sec)    (deg)   (sq in)
     1    0.000     0.007  -95.366    0.114
     2    4.000     0.004  -96.478    0.114
     3    8.000     0.003  -82.836    0.109
     4   12.000     0.010  -73.622    0.122
     5   16.000     0.011  -69.356    0.110
     6   20.000     0.010  -76.184    0.133
     7   24.000     0.009  -82.834    0.133
     8   28.000     0.008  -90.690    0.130
     9   32.000     0.010  -82.231    0.137
    10   36.000     0.008  -74.418    0.135
```

Figure 8.23. A sample data output.

IX
The Graphics Manager

Using the Graphics Manager

The **Graphics Manager** is accessed whenever a graph is created while in the **Compute Parameters, Use Database** or **ViewPaths** sections. The **Graphics Manager** adds two menus to the **DIAS**® program, the **Plot** and **Axes** menus (Figs. 9.1 and 9.14). Definitions and descriptions of the options under the **Plot** and **Axes** menus follow.

Figure 9.2 shows a typical graph with 3 items. Each parameter in the graph has a color and a point style. At the bottom of the graph, the x-axis item name is given followed by 'vs' and the y-axis items. Each item name is proceeded by its point style and is in the color of its associated graph. The style, size, and font of the text can be changed by making the appropriate selections under the **Option** menu before creating the graph. The graph can be annotated by using various text and draw features located under the **Edit** menu. The graph can also be saved as either a 'PICT' file or as a **DIAS**® graph file by selecting **Save** under the **File** menu.

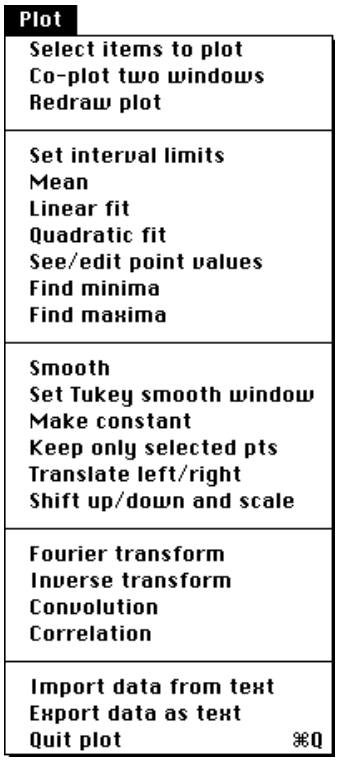

Figure 9.1. The **Plot** menu.

Select Item to Plot

This command allows you to change which items to plot, and the color and point style of the items you wish to plot. When **Select Item to Plot** is selected, a dialog box gives you various style and item choices (Fig 9.3). On the left are the colors for each item. These colors refer to the color of the line segments joining the points. Click on the desired color to make a change. The '**?**' color will display a standard Macintosh® color wheel, from which the you can select any custom color desired. To the right of the colors selections are the point styles. The 'dash' represents the use of no point. The items available to plot are listed on the right. Click in the check boxes to select or deselect the desired items. The 'Select All' and 'Clear All' buttons let you select all of the items or clear all of the items with one mouse click. Click 'Ok' when you have made your choices. The graph will be redrawn according to your

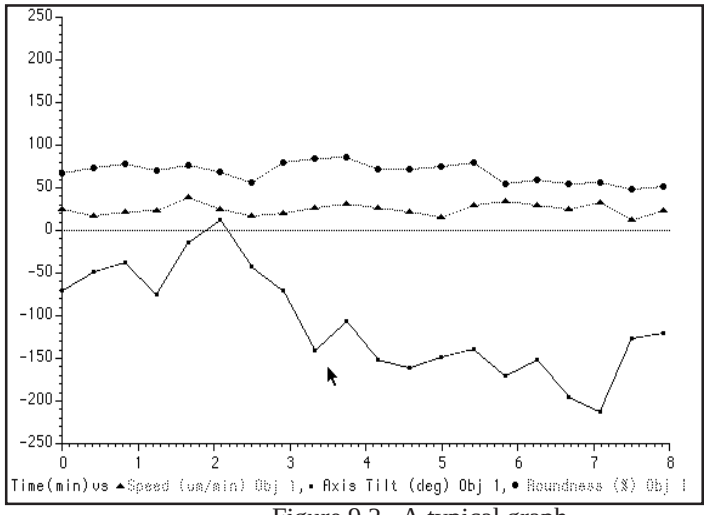

Figure 9.2. A typical graph.

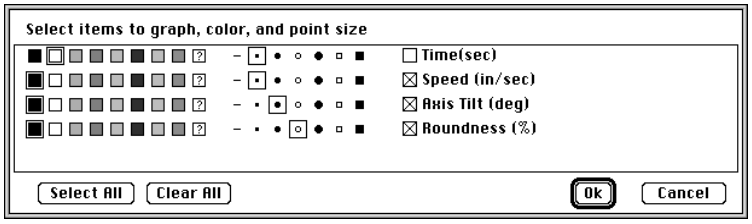

Figure 9.3. The **Select Item to Plot** dialog box.

selections.

> Note that the X-axis item (here: Time) is not selected. If it were, a diagonal line plotting time vs itself would appear on the graph.

Co-plot Two Windows

This option will co-plot the y-axis items of the last two graphs (windows) selected. To select a graph (window), click on it or select it from the **Windows** menu. This will bring that graph to the foreground. After selecting the two desired graphs (windows), clicking the co-plot command creates a new graph with all of the y-axis items from the two graphs plotted.

Redraw Plot

If for some reason the graph becomes cluttered or marred, select this command to redraw the graph. It takes into account the settings from **Select Item to Plot** and **Graph Type**. Annotations made from the **Edit** menu (including all draw and paint commands) will be lost.

Set interval limits

This option allows you to select a limited range for the x-axis, where means, smoothing, etc. are applied. After selecting this option, instructions appear in the title bar asking you to click on the left side of the range. You may click directly on the x-axis or on a plotted point. Next, the title bar prompts you to click on the right side. Select **Redraw Plot** if you wish to see the limits drawn on the graph. These limits apply to the following commands: **Mean**, **Linear Fit**, **Find Maxima**, **Find Minima**, **Smooth** and **Make Constant**. To reset the limits, select **Set Interval Limit** again and click on the extreme ends of the graph to set the limits to the widest possible range. Then select **Redraw Plot** to update the graph**.**

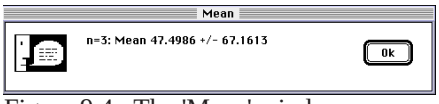

Figure 9.4. The 'Mean' window.

Mean

This option computes the mean and standard deviation for all the data points of all 'selected' items between the limits (from **Set Interval Limits**). The mean and standard deviation are shown on the graph as horizontal lines. The numerical values are displayed in a small window (Fig. 9.4). Click 'Ok' in the window to continue.

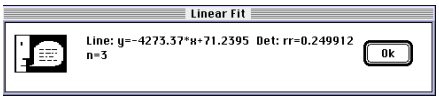

Figure 9.5. The 'Linear Fit' window.

Linear Fit

This option performs a least squares fit to all the data points of all 'selected' graphs between the limits (from **Set interval**

limits). All numerical values are displayed in a window: **rr** is the coefficient of determination r^2, **n** is the number of data points, **sx** is $\sum x$, **sy** is $\sum y$, **sxx** is $\sum x^2$, **sxy** is $\sum xy$, and **syy** is $\sum y^2$ (Fig. 9.5).

Quadratic Fit

Quadratic Fit is defined as a linear fit using 2ND degree equations instead of lines (Fig. 9.6).

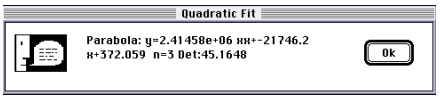

Figure 9.6. The 'Quadratic Fit' window.

See/Edit Point Value

When selected, you are prompted to click the mouse at any point in the graph. The x and y values of that point are displayed in the prompt window along with the name of the item. If you wish to change the value, enter the new y value. Click on 'Done' when you are finished examining points.

Find Minima

After choosing this option, the local minima of all 'selected' points between the limits (from **Set interval limits**) are shown as vertical lines. A window displays the mean distance between minima. Click 'Ok' in the window to continue.

Find Maxima

After choosing this option, the local maxima of all 'selected' points between the limits (from **Set interval limits**) are shown as vertical lines. A window displays the mean distance between maxima. Click 'Ok' in the window to continue.

Smooth

This option smooths the portions of the 'selected' items between the limits (from **Set interval limits**). You are prompted for the number of times you wish the smoothing to be done. A Tukey window (described below) is used to determine the method of smoothing.

Set Tukey Smooth Window

This selection determines the method of smoothing. The Tukey window is a list of weights to be applied to each data point and its immediate neighbors (Fig. 9.7). The default Tukey window is '**5, 15, 60, 15, 5**'. Here each point of the graph is averaged with the two points on either side for a total of five points. A Tukey window may have any odd number of weights that add up to 100. Denote 5 successive points by

(**x**[i-2],**y**[i-2]) , (**x**[i-1],**y**[i-1]) , (**x**[i],**y**[i]) , (**x**[i+1],**y**[i+1]) , (**x**[i+2],**y**[i+2]) .

Figure 9.7. The 'Set Tukey Window' window.

Then the point (**x**[i],**y**[i]) is altered by smoothing (with window

'**5 15 60 15 5**') to

x[i]= 0.05***x*****x**[i-2] + 0.15***x*****x**[i-1] + 0.60***x*****x**[i] + 0.15***x*****x**[i+1] + 0.05***x*****x**[i+2] and

y[i]= 0.05***x*****y**[i-2] + 0.15***x*****y**[i-1] + 0.60***x*****y**[i] + 0.15***x*****y**[i+1] + 0.05***x*****y**[i+2] .

Points at the beginning and end of the graph are handled in the following manner:

x[1]= (0.05 + 0.15)***x*****x**[0] + 0.60***x*****x**[1] + 0.15***x*****x**[2] + 0.05***x*****x**[3] and

y[1]= (0.05 + 0.15)***x*****y**[0] + 0.60***x*****y**[1] + 0.15***x*****y**[2] + 0.05***x*****y**[3] .

Make Constant

After making this selection, you are prompted for a value. All 'selected' items have their **y** values changed to the number you entered. This affects only the points within the limits determined by **Set internal limits**.

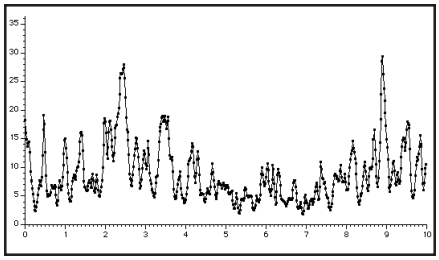

Figure 9.8. The original data.

Keep Only Selected Pts

With this option, you are prompted to click on points from the 'selected' items. Click on as many as desired. Click 'Done' when finished. All points on the graphs *not* clicked have their **y** value set to zero.

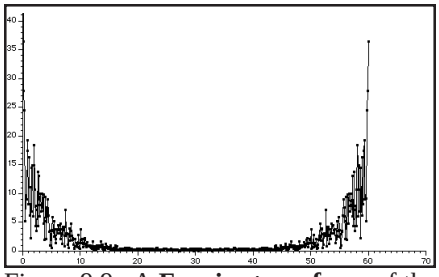

Figure 9.9. A **Fourier transform** of the data.

Translate Left/Right

After selecting this option, you are prompted for a value. If the value is positive, all 'selected' points are translated (i.e. moved) to the right by the amount specified. A negative value indicates left translation.

Shift Up/Down and Scale

Choosing this selection gives you a dialog box asking for three values: **Shift1**, **Scale**, and **Shift2**. The **y** values of all **selected** points are modified by the following formula: new **y** = (**y** + **Shift1**)***x*****Scale** + **Shift2** .

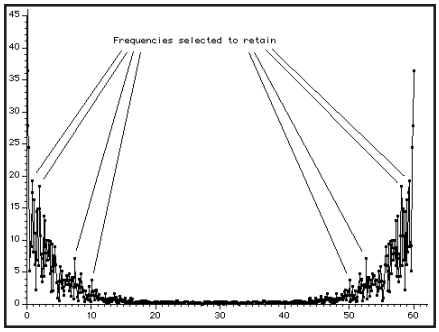

Figure 9.10. A selection of frequencies.

Fourier Transform

This option computes the Fast Fourier Transform (FFT) of the currently selected parameters. The x-axis of the transform becomes frequency - the number of cycles per time unit. Peaks indicate a strong fundamental frequency component in the data. A feature of the FFT is that the result is always symmetrical about the vertical line dividing the x-axis into halves. The frequency labels on the x-axis are significant only below the midpoint. Lowest frequencies are on the left, while the highest

frequencies are at the center. The user is prompted to optionally select certain frequencies. If 'Yes' is selected, the user is prompted to click the mouse at those frequencies to be retained (all others are removed). Both left and right counterparts of the desired frequencies should be selected (otherwise, the reconstructed amplitude will be half the correct value when **Inverse transform** is later performed). The result will show the selected frequencies and the component waveforms plotted in various colors. Figures 9.8 and 9.9 show original data and the **Fourier transform**. Figures 9.10 and 9.11 give an example of frequency selection.

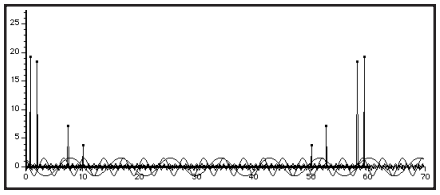

Figure 9.11. The result of selection.

Inverse transform

From the frequency graph (and internally stored amplitude and phase information), the original data is reconstructed. If an entire range of frequencies is to be zeroed out (for high or low frequency filtering), use **Set interval limits** followed by **Make constant** to zero the FFT plot in a selected range; then apply **Inverse transform**. Figure 9.12 shows the FFT with the high frequencies removed. Figure 9.13 shows the Inverse FFT, resulting in a high-frequency filter of the original data from Figure 9.8.

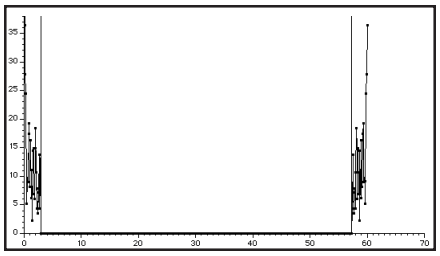

Figure 9.12. The result of high frequencies being zeroed out.

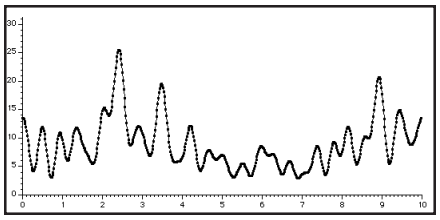

Figure 9.13. The **Inverse transform** of Fig. 9.11.

Convolution

Using the FFT, two parameters may be convolved or one parameter may be self-convolved. Upon selecting **Convolve**, you are prompted to choose one or two parameters. The convolution of two functions f and g is defined to be:

$$\text{Conv}(s) = \int f(x)g(x-s)\,dx .$$

Intuitively this means:

a) Pick a shift number s.
b) Take the mirror image of g about the y axis (called folding)
b) Shift the graph of the mirrored g to the right by s units.
c) Multiply the graph of f with the shifted mirrored graph of g.
d) Find the total area (regions below the axis count as negative) of the result. This is the convolution value associated with shift s.

Convolution is included in **DIAS®** for the sake of completeness only. Normally, correlation is used.

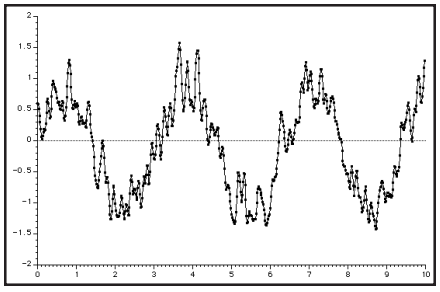

Figure 9.14. The original data.

Correlation

Using the FFT, two parameters may be correlated, or one parameter may be self-correlated. Upon selecting **Correlate**, you are prompted to choose one or two parameters. The correlation of two functions f and g is defined to be:

$$\text{Corr}(s) = \int f(x)g(x+s)\,dx .$$

Intuitively, this means:

a) Pick a shift number s.
b) Shift the graph of g to the left by s units.

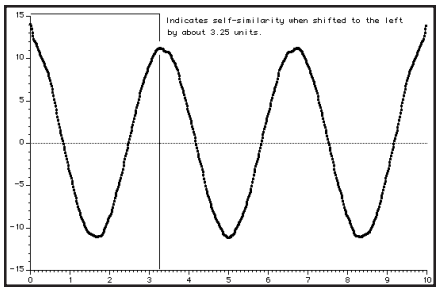

Figure 9.15. The result of self-correlation.

c) Multiply the graph of f with the shifted graph of g.
d) Find the total area (regions below the axis count as negative) of the result. This is the correlation value associated with shift s.

A peak on the left side of the correlation plot at x=K indicates that the two parameters are similar when the second parameter is shifted to the left by K units. Figures 9.14 and 9.15 show original data and the result of self-correlation.

*name	**Example**
value	
value	*speed
.	9.4
.	9.2
value	14.65
	9.78
*name1	
*name2	*length
value value	*width
value value	20.64 14.23
.	21.45 15.3
.	19.32 9.45
value value	22.34 9.65
etc.	

Figure 9.16. An example of the format needed for imporatation of text files.

Import Data From Text

This command reads new data items from a text file. A standard Macintosh® dialog prompts the user for the text file name. The format of the text file is shown in Figure 9.16. If the data is listed as a single column of numbers, it is immediately preceded by an item name beginning with a '*'. A blank line should follow the last number. Data may appear in any number of columns. There must be as many item names as there are columns. Those names appear immediately before the data and begin with '*'.

When the data has been read in, click on the **Select Item to Plot** command to verify that the new items are there. To plot them against the previous x-axis item, select the new items from the selection dialog. If you wish one of the new items to be the x-axis, you must select the **X-Y Crossplot** command under the **Axes** menu. You are prompted first for the new x-axis item, then for the items to be plotted on the y-axis. At first, only the data points will be visible. To make the lines connecting the points visible, go to **Select Item to Plot** and choose a color for the (new) x-axis item. This will be the color of the lines connecting the points. You will probably also have to adjust the x and y axis ranges using the **Set X Axis** and **Set Y Axis** commands located under the **Axes** menu. They are described in detail below.

```
Title: Exported files from graph

Param: first item name
value
 .
 .
value

Param: second item name
value
value
 .
 .
value

etc.
```

Figure 9.17. An example of the output type created using **Export data as text**.

Export data as text

This command creates a text file that is compatible with the **Use database** command (Chapter X). The data points for the currently selected graphs are written to a file (a standard Save File dialog prompts you for the name of the new file) in the format shown in Figure 9.17. This is not the same format for importing files (see above), but could easily be made so by making the following changes (using any text editor) to the file:

1) remove the title line
2) replace 'Param' with '*'

Note: This data is also in a form that may be easier to cut and paste from if you want to use this data with other programs.

Quit Plot

Click this to exit from both the **Graphics Manager**. Changes in the data, made while using this program, have no effect on data files.

Histogram

This command, under the **Axes** menu (Fig. 9.18), makes frequency histograms of all currently selected items. After making this selection, you are prompted for the histogram class width (the width of a bar). The y-axis values are distributed into classes (starting at 0) with this width. For example, a width of 0.5 gives classes (as half open intervals):

... [-1,-0.5) [-0.5,0) [0,0.5) [0.5,1) [1,1.5) ...

The frequency of y values in each class is counted and plotted as bars, the width of the bar corresponding to class width and the height being the frequency (Fig. 9.19). Keep in mind that the class width refers to the y-axis and not the x-axis. Use **Select Item to Plot** to alter the color of the bars. The color of the (now unused) x-axis item determines the color of the bar outlines.

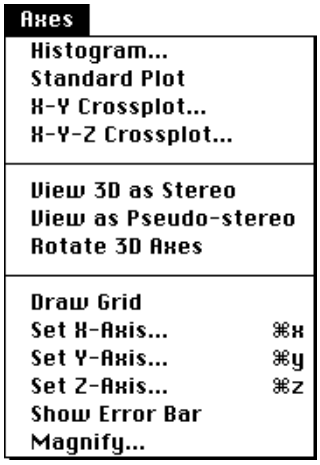

Figure 9.18. The **Axes** menu.

Standard Plot

Click on this command to return to the original graph, after having selected **Histogram.**

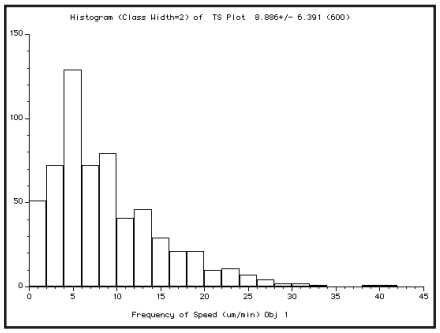

Figure 9.19. A **Histogram**.

X-Y Crossplot

This command lets you choose a new x-axis item and also prompts for new y-axis items. Any parameter may be the x-axis. Only points are initially shown (as is usual for scatter plots). If you wish to connect the points, use **Select Item to Plot** to change the color of the x-axis item. The x-axis color determines the color of the line segments joining the points. Usually the ranges of the x and y axes must be changed when doing a crossplot. See **Set X-Axis** and **Set Y-Axis** below. Figure 9.20 shows a scatter plot of speed vs roundness done with **X-Y Crossplot**.

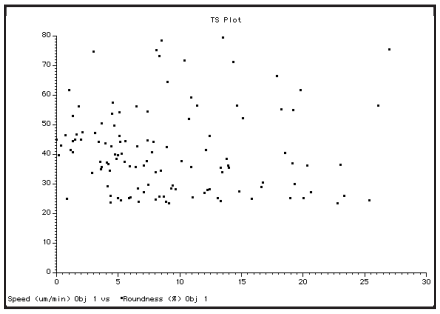

Figure 9.20. A scatter plot made with **X-Y Crossplot**

X-Y-Z Crossplot

This option allows you to plot parameters in three dimensions. You are prompted to select one item for the x-axis and one item for the y-axis. Then you are prompted to select any number of items for the z-axis. As in **X-Y Crossplot**, only the points are shown initially. All three axes may need their ranges adjusted to see anything. See **Set X-Axis**, **Set Y-Axis** and **Set Z-Axis** below. As before, the color of the line segments joining the points is determined by the color of the x-axis item. Use **Select Item to Plot** to change it.

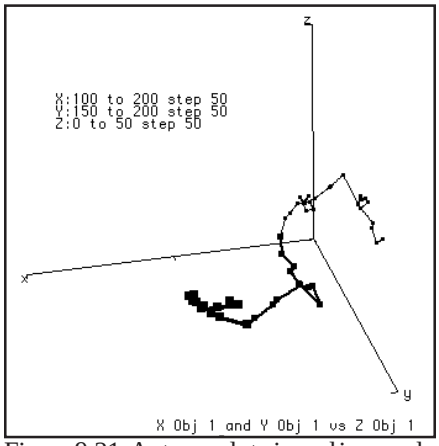

Figure 9.21. A stereo plot viewed in pseudo 3D.

View 3D as Stereo

When you have made an **X-Y-Z Crossplot**, it is possible to view it using blue-red filtered glasses (left eye:blue, right eye:red). Select this command to view the image with these glasses. (A check mark appears beside the menu, indicating that this view mode has been selected.) Select this command again to turn off the stereo view mode.

View as Pseudo-stereo

This command draws the **X-Y-Z Crossplot**, varying the thickness of points and lines to simulate distance. It does not require special glasses and may be printed. Select this command a second time to turn off this mode. Figure 9.21 shows a pseudo-stereo plot.

Rotate 3D axes

Select this command to rotate your **X-Y-Z Crossplot** (in any view mode). Rotation is about the z-axis. Rotation continues until the mouse button is clicked.

Draw Grid

Select this command to have grid lines drawn on the graph (either 2D or 3D). They are spaced at the same intervals as the axis numerical labels. Select this command a second time to turn off the grid.

Set X-Axis, Set Y-Axis, and Set Z-Axis

These commands allow you to determine the range and labelling of the specific axes. Figure 9.18 shows a sample dialog prompting for new x-axis values (The y and z axis dialog boxes are similar). The minimum and maximum values determine the range of the axis. The axis need not begin at 0. The increment determines the spacing of the axis divisions which are labelled numerically. The 'divisions' parameter determines the number of minor marks between each pair of labelled ones. In the figure, the labelled main divisions will be **0.0,0.1,0.2,...,1.0** . There will be 10 subdivisions between each of these.

Show Error Bar

This command plots a second parameter relative to a given parameter. For example, if the given parameter has value 5 and the second (relative) parameter has value 2, a line from y=5-2 to y=5+2 will be drawn. Generally, the relative parameter is an error or standard deviation associated with the given parameter.

Before using this command, select one parameter to graph. Then select **Show Error Bar**. You are asked which parameter is to be plotted relative to the initial parameter. The color of that parameter determines the color of the error bars. Figure 9.22 shows a sample error bar plot.

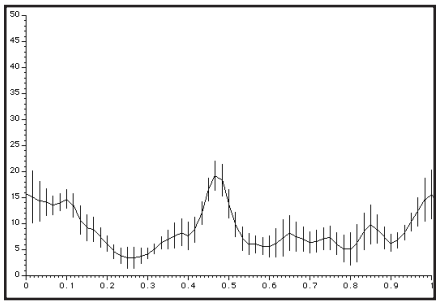

Figure 9.22. A plot with error bars

Magnify

After selecting this option, you are prompted for a magnification factor (use **0** to return to the original view). You are then prompted to click the mouse at what will be the center of the new view. This can be used on any type of graph. The **See/ Edit Point Value** command works properly on a magnified view.

Saving

Graphs can be saved as either **DIAS®** graphs or as 'PICT' files. If you select **Save** from the **File** menu, a dialog box will give you these two options (Fig. 9.23). If you elect to save the graph as a 'PICT' file, you can still use the draw and paint options under the **Edit** menu, but you cannot access the **Graphics Manager**. If you save the graph as a graph file (Fig. 9.24), when you open it, you can still manipulate the data using the commands in the **Graphics Manager**.

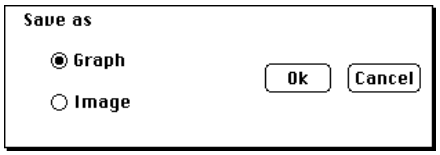

Figure 9.23. The graph 'Save as' choices.

Figure 9.24. An example of a graph icon.

X
Use database

Accessing the database

The **Use Database** command may be selected from under the **ViewPaths** or **DIAS** menu (Fig. 10.1), and allows you to view, plot and define functions based upon the data computed and saved during the **Compute Parameters** step (Chapter VIII). Since database files (Fig 10.2) are made up of specially formatted text, the user may enter data directly into a file using many ASCII text editors (including **DIAS**®). The format of the data file is given at the end of this chapter.

After selecting **Use Database** from the **DIAS** menu, a standard Macintosh® Open File dialog box appears prompting you to choose a file. If a text file is selected that is not in the proper format, an error message will appear. **Use Database** will read a correctly formatted data file and display a summary of the available items in a text window (Fig. 10.3).

The name of the file along with its title, date, etc. is displayed. Then, each available item is listed. **Obj**(ect), **First** (frame), **Last** (frame), and (frame) **Inc** are the same as in **Compute parameters** (Chapter XI). **Index** and **Time** have the same meaning for all objects and are not given an object number. The **Index** counts from 1 to the total number of frames. A **Data** menu also appears on the menubar (Fig. 10.4).

Figure 10.1. Selecting **'Use Database'** from the **DIAS** menu.

Figure 10.2. An example of a database file icon.

Append Data File

Any number of data files may be present at one time in the display window. To add another, select **Append Data File** and choose the desired file when the Macintosh® Open File dialog box appears. New files continue numbering the items where previous files left off, so that each item from any file has a unique number. Most commands described below use these item numbers to specify the desired parameters.

Clear Board

Clear Board removes all current files from the display window (the files themselves are not altered). Select **Append Data File** to enter a new file.

Text

Cell 1.data
' Test Paths' Date: 2/9/94 Trial: 1
Frames: 199 Objects: 2 Frame Rate:0.25 fr/ sec Scale Factor:0.014 in/pixel

	Item	Obj	First	Last	Inc	Min	Max	Mean	StDev
1	Index	0	1	160	1	1	160	80.5	46.19
2	Time (sec)	0	1	160	1	0	636	318	184.7
3	Speed (in/sec)	1	1	76	1	0.0001356	0.01971	0.00886	0.004258
4	Speed (in/sec)	2	77	160	1	0.0002207	0.08054	0.009491	0.009569
5	Area (sq in)	1	1	76	1	0.1028	0.197	0.1497	0.02496
6	Area (sq in)	2	77	160	1	0.1232	0.201	0.1562	0.01555
7	Perimeter (in)	1	1	76	1	1.53	2.564	2.097	0.2585
8	Perimeter (in)	2	77	160	1	1.714	2.609	2.141	0.1818

Figure 10.3. A data summary file.

Figure 10.4. The **Data** menu.

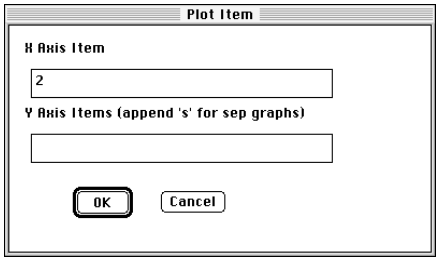

Figure 10.5. The **Plot Items** dialog box.

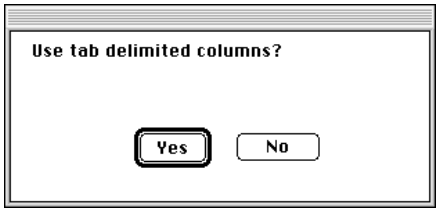

Figure 10.6. The **View as Text** dialog box.

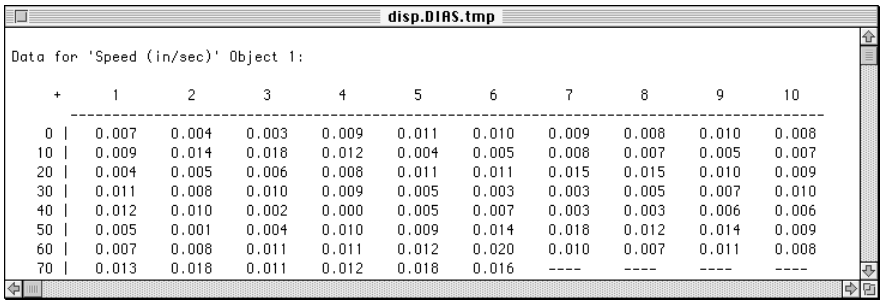

Figure 10.7. An example of the **Display Items** tabular format.

Plot Items

Plot Items allows you to plot any number of items (on the y-axis) against one x-axis item. When the item selection dialog box appears (Fig. 10.5), specify one item for the x-axis and any number of items for the y-axis. Separate these items by commas or dashes. When this data is plotted, the **Graphics Manager** is accessed. It can be used as described in the previous chapter.

Note: Any changes made to the data while in the **Graphics Manager** does not affect the 'DATA' files.

Note: To get back to the **Use database** program after plotting, either select **Quit** from the **Plot** menu, close the graph's window, or click in the 'DATA' file display window (Fig. 10.3). The latter option does not erase the graph.

View as text

This command allows you to view the data as a text file. After you have selected this command, you will be prompted as to whether you wish to 'Use tab delimited columns' (Fig 10.6). If you select 'Yes', the text will be shown in a column form, which can then be saved as a text file and imported by many spreadsheet programs. If you select 'No', the text file will appear as a single column, from which you can easily cut and paste into the same programs.

Set Plot Size

By default, the size of a graph's window is 500x400. Use this command to change the graph window size (before selecting **Plot Items**). A dialog asks you for the new length and height.

Display Items

This command lists the numerical data of selected parameters. After selecting this command, you are asked for the list of items to be displayed. You are then given the option to display the data in either columns or tables. The result appears in a text window. Figure 10.7 is an example of the data in a tabular form. Each row lists 10 consecutive frames. In this example, the speed at

frame 47 is 0.003 inches per second.

Show Mean

Upon selecting this option, you are prompted for a list of items (Fig 10.8). All the values associated with each item are lumped into an aggregate. The mean and standard deviation of that aggregate are computed and displayed in a small text window (Fig. 10.9). The number of aggregate values and the min-max values are also displayed. For example, if three items are selected, each having 200 values, the mean is computed for a total of 200x3=600 numbers.

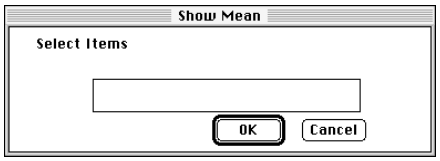

Figure 10.8. The **Show Mean** 'Select Items' dialog box.

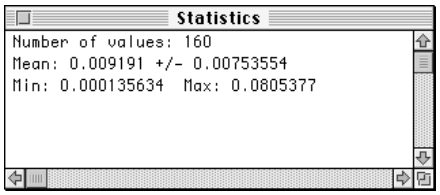

Figure 10.9. A **Show Mean** 'Statistics' dialog box.

> Note: The commands so far described have no effect on the data file contents. Except for **Quit**, the remaining commands in some way alter the 'DATA' files.

Change Item Name

This command allows you to change the name of an item (except item #1), it's object number, and the first and last frame and increment of items. You are asked for a list of items to be changed (Fig 10.10). For each selected item a dialog box appears (Fig. 10.11). Make the desired changes and click 'OK'. The changes are written into the data file containing the item. Generally you will not want to change the frame limits or increments.

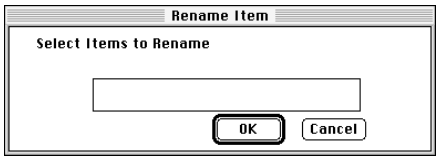

Figure 10.10. The 'Rename Item' dialog box.

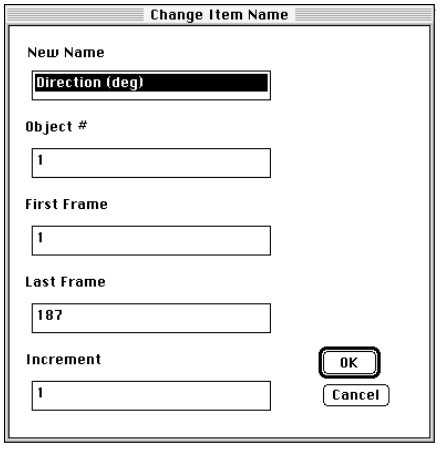

Figure 10.11. The **Change Item Name** dialog box with the original options listed.

Duplicate Items

After selecting this option, you are asked for a list of items to be copied (Fig. 10.12). The duplicates have the same name as the originals except that an '@' is inserted at the beginning of the items name. If there is more than one 'DATA' file current, you will also be prompted for the intended data file. The original items are not affected by this command. This command is useful when you want to smooth an item, while still retaining the original.

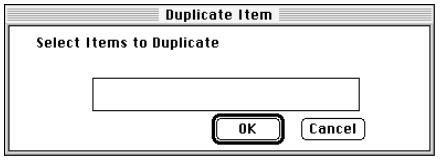

Figure 10.12. A dialog box asking which **Items to Duplicate**.

Erase Items

Upon selection of this option, you are prompted for a list of items to remove from the 'DATA' file(s). This command cannot be undone. Important 'DATA' files that cannot easily be remade should be backed up by duplicating them, using the Finder.

Interpolate Items

Items that have a frame increment other than 1 have NAN's (Not A Number) as their values for intermediate frames.

When plotted, the NAN's are ignored. When a table of values is displayed, the NAN's appear as —'s. Use this command to fill in the NAN-gaps using linear interpolation. Only the NAN's between the first and last frames are interpolated. You are prompted for a list of items to interpolate. This command is useful if you wish to plot or define a function involving two parameters with different increments.

Collapse Item

This command removes all NAN's, including those before the first frame, for a parameter. If the original item started at frame 25 and ended at frame 180 with a frame increment of 5, the item will be listed starting at frame 1, end at frame 31 and have a frame increment of 1. You are prompted for the list of items to collapse.

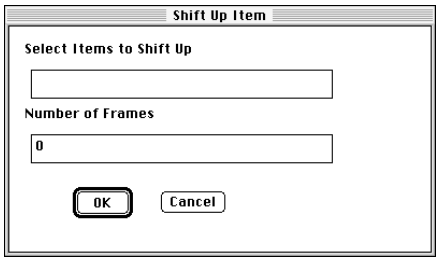

Figure 10.13. The **Shift Up Item** dialog box.

Shift Up Item

This option prompts you for the list of items to shift, along with the number of frames (Fig. 10.13). If the original parameter has values v1,v2,... , the result will have values NAN,..., NAN, v1, v2,... , where the number of NAN's inserted is the number of frames to shift. Values pushed beyond the maximum index are lost.

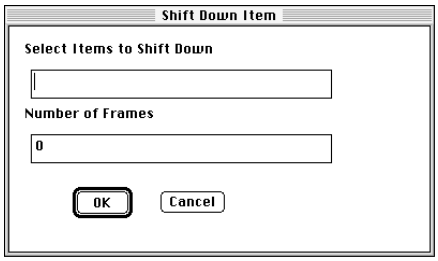

Figure 10.14. The **Shift Down Item** dialog box.

Shift Down Items

With this option, you are prompted for the list of items to shift, along with the number of frames to shift (Fig. 10.14). If the original parameter has values v1,v2,... , the result will have values vN+1, vN+2,... where N is the number of frames to shift. The first N values are therefore lost.

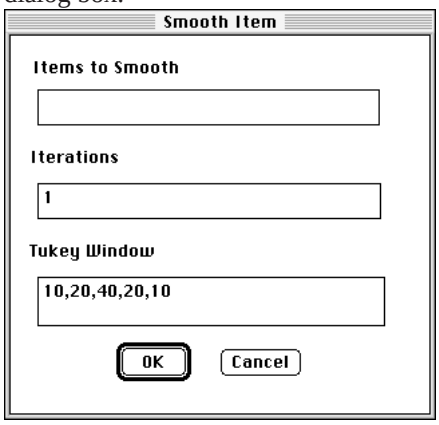

Figure 10.15. The **Smooth Item** dialog box.

Smooth Items

After selecting this option, a dialog appears (Fig. 10.15) prompting you for a list of items to smooth, the number of iterations (the number of times smoothed), and the Tukey window. Tukey windows were described in Chapter IX.

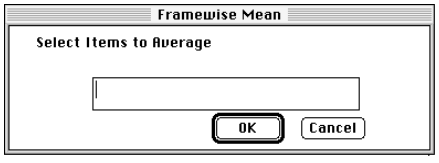

Figure 10.16. The **Framewise Mean** dialog box.

Framewise Mean

This option averages several parameters, frame by frame, in parallel. For example, take five parameters A,B,C,D,E. Let A1,A2,... be the values of A at frames 1,2,... etc. , and similarly for B,C,D and E. The resulting framewise mean parameter, M, and framewise standard deviation parameter, S, are formed as follows:

M1= Mean of {A1,B1,C1,D1,E1} S1= Standard Deviation of {A1,B1,C1,D1,E1}

M2= Mean of {A1,B1,C1,D1,E1} S2= Standard Deviation of

{A1,B1,C1,D1,E1} , etc.

You are prompted for the list of parameters to be averaged (Fig.10.16). Two new items, 'Framewise Mean' and 'Framewise Deviation', are created. As described in Chapter XI, the framewise mean may be plotted, with the framewise deviation shown as error bars (Fig. 10.17).

Itemwise Mean

After selecting this option, you are prompted for a list of items. Assume that 5 items (parameters) are selected. Let A, B, C, D, E be those five parameters. Let M(A), S(A) be the mean and standard deviation of the values of parameter A, and similarly for B, C, D and E. Two new items, 'Itemwise Mean' and 'Itemwise Deviation', are created. 'Itemwise Mean' will (in this example) contain 5 values: M(A), M(B), M(C), M(D) and M(E); 'Itemwise Deviation' will contain the 5 values: S(A), S(B), S(C), S(D) and S(E). In a sense, **Itemwise Mean** is perpendicular to **Framewise Mean**. The number of frames in the 'Itemwise Mean' and 'Deviation' parameters is the same as the number of items selected.

The following is a situation where **Itemwise Mean** might be used. Digitize 10 different cells and compute the speed for each. Read the data file for each cell into **Use Database**. Perform **Itemwise Mean** on the speed parameter for each cell (10 parameters will be selected). Plot the resulting 'Itemwise Mean' with 'Itemwise Deviation' as error bars. The x-axis indexes the cell number. The y-axis give the mean alone with standard deviation bar speed of each cell (Fig. 10.18).

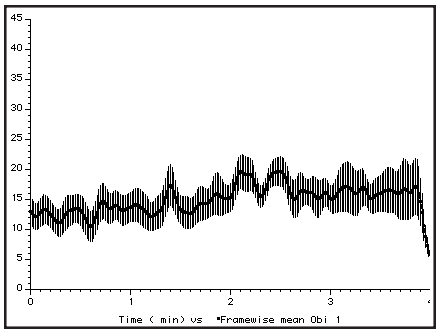

Figure 10.17. The **Framewise Mean** of the speed of 10 different objects showing error bars.

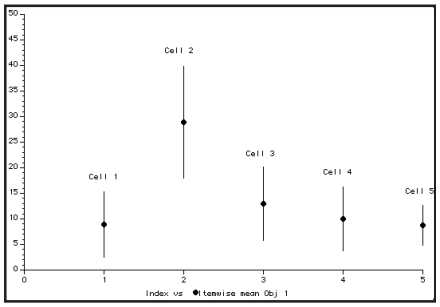

Figure 10.18. The mean speed with standard deviation bars for five objects

Apply Function

This command allows you to define new functions based on the items appearing in the **Use Database** window. When selected, the 'Function Calculator' (Fig. 10.19) appears.

Functions are defined using RPN notation (Reverse Polish Notation). The Function Calculator allows up to 6 variables: A,B,C,X,Y,Z. Three things must be done to define a function:

1) Enter an item name in the **New Item Name** slot.

2) Associate actual item numbers with the variable letters A,B,C,X,Y,Z . Not all of the variable letters need be used.

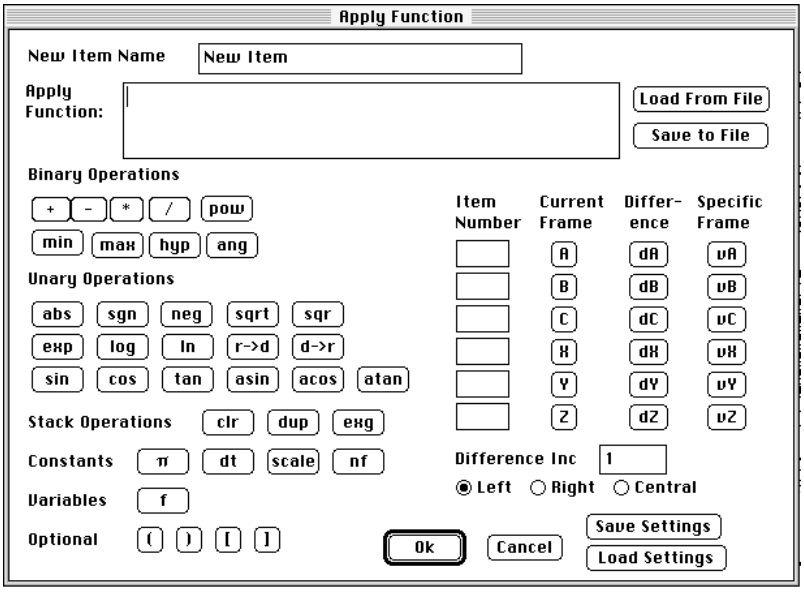

Figure 10.19. The mean speed with standard deviation bars for five objects

3) Enter a function in the **Function** slot, either by typing it in directly or by clicking on the convenient symbol buttons.

The function is then applied to the selected parameters on a frame by frame basis.

Let's define a sample function. We wish to compute (for each frame): newpar=ln(speed^2 + area)/(speed+perimeter) .

In this example, assume that speed is item 3, area is item 5, and perimeter is item 7. Use the following steps:

1) Select **Apply Function** from the **Data** menu.

2) Enter 'NewPar' in the 'New Item Name' slot of the dialog.

3) Next to the 'A' button, enter 3 for 'Item Number'. This associates the variable letter 'A' with item 3, speed.

4) Next to the 'B' button, enter 5 for 'Item Number'. So 'B' is area.

5) Next to the 'C' button, enter 7 for 'Item Number'. So 'C' is perimeter.

6) Now enter 'A sqr B + ln A C + /' in the 'Function' slot. Either type it in directly, or click on the buttons with the appropriate symbols.

When finished, the new item 'NewPar' appears in the display window. Intermediate computations are held in a 'stack'. Unary operations take the top number on the stack. Apply the operation, and replace it back on to the top of the stack. Binary operations take the first two numbers on the stack. Apply the operation, and put the result on to the top of the stack (the original two numbers are removed from the stack). When new items are added to the stack, the stack contents are pushed down. Figure 10.20 shows the stack contents for each step of calculation for the above example.

operation	stack top	rest of stack —>	
A	**A**	—	—
sqr	**A^2**	—	—
B	**B**	**A^2**	—
+	**A^2+B**	—	—
ln	**ln(A^2+B)**	—	—
A	**A**	**ln(A^2+B)**	—
C	**C**	**A**	**ln(A^2+B)**
+	**A+C**	**ln(A^2+B)**	—
/	**ln(A^2+B)/(A+C)**	—	—

Fig. 10.20 Contents of the stack in a computation

Other features of the Function Calculator:

Save to file

After defining a function, click here to save the function definition (but not the item numbers or the item name). You will be prompted for the file name.

Load from file

This command is used to retrieve a function saved with **Save to file**. You are prompted for the file name.

Difference variables

The difference variables are 'dA', 'dB', 'dC', 'dX', 'dY', and 'dZ'. Their values are affected by the difference increment and the type (left, right or central). Here is the definition of 'dA' (the others are analogous): Let **A**[1], **A**[2], ..., **A**[F] be the values of parameter **A** for frames 1, 2, ..., F, where F is the number of frames. Let **I** be the frame increment. The derivative of **A** would be written as '**dA dt /**'

1) Left differences
dA[f] = 0 when f-**I**< 1
dA[f] = (**A**[f]-**A**[f-**I**])/**I** when 1<=f-**I**

2) Right differences
dA[f] = 0 when f+**I**>**F**
dA[f] = (**A**[f+**I**]-**A**[f])/**I** when f+**I**<=**F**

3) Central differences
dA[f] = (**A**[f+**I**]-**A**[f-**I**])/2**I** when 1<=f-**I** and f+**I**<=**F**
dA[f] = (**A**[f+**I**]-**A**[f])/**I** when f-**I**< 1
dA[f] = (**A**[f]-**A**[f-**I**])/**I** when f+**I**>**F**

Specific frame variables

They are 'vA', 'vB', 'vC', 'vX', 'vY', 'vZ'. This allows you to specify a value at a specific frame, other than the value at the current frame. This is best described by an example. Let's normalize area (assume it is item 5) so that the first frame has area 100. We wish to add (100 - first frame area) to the area in each frame. If we associate the letter 'A' with item 5, so that 'A' is area, the function definition is '**A 1 vA - 100 +**'.

vA looks in the top of the stack for a frame number and replaces it with the actual value of the parameter at that frame (0 results if the frame number is out of bounds). **f** is a special symbol that stands for the current frame number. If we wanted to define the function:

fun[f]= **A**[f]+**A**[f-1] when f>2
fun[f]= **A**[f] when f=1

we would use '**A f 1 - vA +**' for the definition. Optional parentheses (totally ignored by the computer) may be used to clarify the definition: '**A (f 1 -)vA +**'.

Another equivalent definition is '**(f)vA (f 1 -)vA +**'. In fact the letter 'A' is equivalent to **(f)vA**.

Function calculations

Other 'Function Calculator' operation definitions are as follow.

Funtion Calculator Summary

Binary Operations

+	Takes x,y off the stack (x at the top). Pushes y+x onto the stack.
-	Takes x,y off the stack (x at the top). Pushes y-x onto the stack.
*****	Takes x,y off the stack (x at the top). Pushes y*x onto the stack.
/	Takes x,y off the stack (x at the top). Pushes y/x onto the stack.
min	Takes x,y off the stack (x at the top). Pushes the smaller of x,y onto the stack.
max	Takes x,y off the stack (x at the top). Pushes the larger of x,y onto the stack.
pow	Takes x,y off the stack (x at the top). Pushes y^x onto the stack.
hyp	Takes x,y off the stack (x at the top). Pushes sqrt($x^2 + y^2$) onto the stack.
ang	Takes x,y off the stack (x at the top). Pushes the (radian) angle the vector (y,x) makes with the horizontal onto the stack.

Note: values come off the stack in the reverse order as they were pushed. So: "**A B -**" is **A-B** "**A B ang**" is the angle made by (**A**,**B**).

Unary Operations

abs	Takes x off the stack. Pushes |x| onto the stack.
sgn	Takes x off the stack. Pushes sgn(x) onto the stack. sgn(x)= -1 if x<0 ; 0 if x=0 ; 1 if x>0 .
neg	Takes x off the stack. Pushes -x onto the stack.
sqrt	Takes x off the stack. Pushes sqrt(x) , the square root, onto the stack.
sqr	Takes x off the stack. Pushes x^2 onto the stack.
int	Takes x off the stack. Pushes the integer part of x onto the stack.
exp	Takes x off the stack. Pushes e^x onto the stack.
log	Takes x off the stack. Pushes $\log_{10}(x)$ onto the stack.
ln	Takes x off the stack. Pushes ln(x) onto the stack.
r->d	Takes x off the stack. Pushes (180/π)x onto the stack.
d->r	Takes x off the stack. Pushes (π/180)x onto the stack.
sin	Takes x off the stack. Pushes sin(x) onto the stack.
cos	Takes x off the stack. Pushes cos(x) onto the stack.
tan	Takes x off the stack. Pushes tan(x) onto the stack.
asin	Takes x off the stack. Pushes $\sin^{-1}(x)$ onto the stack.
acos	Takes x off the stack. Pushes $\cos^{-1}(x)$ onto the stack.
atan	Takes x off the stack. Pushes $\tan^{-1}(x)$ onto the stack.

Stack Operations

clr	Takes x off the stack. Pushes nothing.
dup	Takes x off the stack. Pushes x back on twice.
exg	Takes x,y off the stack. Pushes y,x back on.

Special Constants

π	3.10159...
dt	the time increment between frames = item2[2]-item2[1] , since item2 is Time.
scale	The scale factor in spatial units/pixel.
nf	Total number of frames

Special Variables

f	The current frame during function calculation

Optional

( ) [ ]	Completely ignored. Used to improve readability.

Special operations

sA,**sB**,**sC**	Integral sum. **sum**[f]= **A**[1]+...+**A**[f]
sX,**sY**,**sZ**	"**sA dt ***" gives the integral.
scon	Simple condition. Takes v,u,c,x off the stack (v at top). if (x<=c) pushes u onto the stack ; if (x>c) pushes v. The form of the command is: (x c u v)**scon** since they come off the stack in reverse order.
ccon	Complex condition. Takes x,c,d,u,v,w off the stack (x at top). if (x<=c) pushes u onto the stack ; if (x>c & x<=d) pushes v. if (x>d) pushes w. The form of the command is: (x c d u v w)**ccon** since they come off the stack in reverse order.

Function definition examples

1) Define the left difference of **A** directly, without using **dA**:

ldiff[f] = 0 when f<=1

ldiff[f] = **A**[f]-**A**[f-1] when f>1

The function definition is: **f 1 0 [(f)vA (f 1 -)vA -] scon**

So when **f<=1** we get **0** and when **f>1** we get **[(f)vA (f 1 -)vA -]**

Note: **(f)vA** could be more simply written as 'A'.

2) Restrict parameter **A** to a maximum value of 100:
Function definition: **[A 100 A 100]scon**

3) Define a "20 60 20" Tukey window smoothing of parameter 'A':
Function definition:

f 1 nf A [(f 1-)vA 0.20* A 0.60* (f 1 +)vA 0.20* + +] A ccon

The DATA file format

We will indicate the format by giving an example. Explanations are in **bold** type - they are not part of the file.

```
* DIAS TS Data File      Any comment can follow a *
Title: Sample cell      The title of the file (required)
Date: 7/16/86           The date in month/day/year format (optional)
Trial: 1                Trial or experiment number (optional)
Frames: 10              Total number of frames (optional)
Objects: 1              Number of objects (optional)
TUnit: min              Unit of time (3 letters max)  (optional)
SUnit: um               Unit of distance (3 letters max) (optional)
FRate: 12               Frame rate in frames/unit time (optional)
Scale: 0.74             Scale factor in units/pixel (optional)
SLen: 512               Raw data horizontal resolution (optional)
SWid: 512               Raw data vertical resolution (optional)
Pinc: 2                 Pixel increment (optional)
Res: 3                  Spline resolution (optional)
Bias: 0.5               Spline Bias (optional)
Tension: 2              Spline Tension (optional)
CenMethod: Area         Method of centroid calculation (optional)
```

> Note: Frame rate and scale factor must be specified if you intend to use the constants **dt** or **scale** in function **definitions.**

```
Param: Speed (um/min)   Item name - must preceed each item

Obj: 1                  Object number (assume 1 if unspecified)
Start: 1                Starting frame number (assume 1 if unspecified)
End: 10                 Ending fr. number (use number of values if unspecified)
Inc: 1                  Frame increment (assume 1 if unspecified)
25.3103                 The values - one per line.  NAN means Not A Number
22.2739
15.4888
8.00085
9.49656
17.6906
31.4252
37.424
32.832
24.7711
```

Param: Area (um-um) **Next Item name**

Obj: 1
Start: 1
End: 10
Inc: 1
25.3103
22.2739
15.4888
8.00085
9.49656
17.6906
31.4252
37.424
NAN
NAN

Carriage return must follow last number in the file!

Export Data File

This command allows you to export the currently open data file as a tab delimited text file. This type of file may be opened in programs such as Excel for additional analysis.

This command is similar to selecting **View as text**, and then saving the new file in a tab delimited format. However, this command exports the information in the data file, instead of the frame by frame data sets.

XI
Shape analysis

The **Shape Analysis** section of **DIAS**® gives you the ability to view an objects morphology, and its morphological changes over time, using a variety of unique, static, images.

After selecting **Shape Analysis** from the **DIAS** menu (Fig. 11.1), a standard Macintosh Open dialog box will ask you which file to use. You must choose a path file. When you have selected a path file, the **Shape Analysis** dialog box will appear (Fig. 11.2).

The **Shape Analysis** dialog is divided into five sections.

1) Select objects and frames
2) Type of picture
3) Display mode
4) Size and direction
5) Color choice

Each section will be described below.

Figure 11.1. Selecting **Shape Analysis** from the **DIAS** menu.

Select objects and frames

In this section, you may select which objects to analyze (by number), the starting frame, ending frame and the frame increment for the objects. The frame increment is only used to determine the frames for which information is drawn. It does not affect flow increment, centroid increment, or difference increment: these are always based on the original frame numbers.

Type of Picture

There are three picture types which may be generated during **Shape analysis**. They are 'Shape Pictures', 'Curvature Pictures', and 'Flow Pictures'.

'Shape Pictures' consist of the actual digitized outline of the object (as defined by the settings under the path file header). In addition to the outlines, you may 'Show centroids', 'Show direction', and/or difference the pictures.

'Curvature Pictures' consists of the amount of curvature along the points of the boundary. Curvature is the rate of turning of the tangent line as it moves along the boundary of the object (in

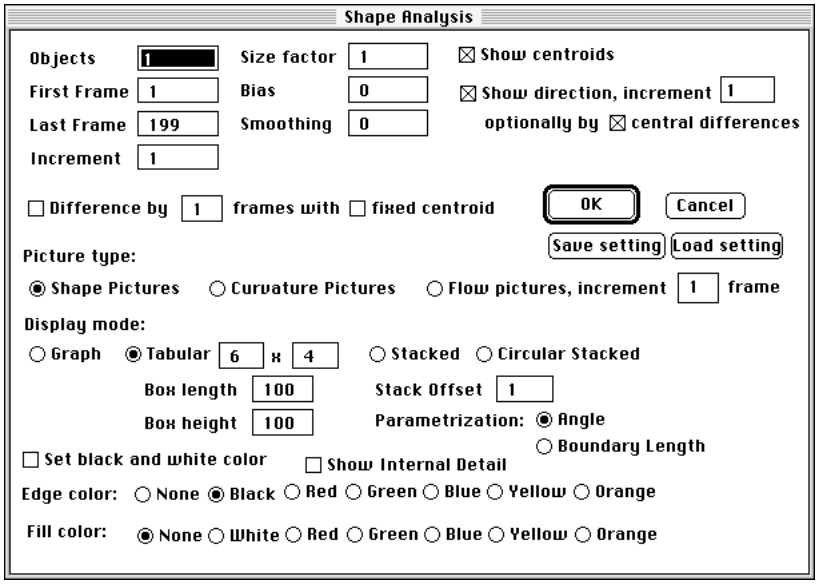

Figure 11.2. The 'Shape Analysis' dialog box.

degrees per unit of length). Counterclockwise turning is positive curvature, while clockwise turning is negative curvature.

'Flow Pictures' consist of the amount of flow outward or inward along the points of the boundary. Flow is computed as follows: A flow increment **FI** is given. The shape at the current frame is compared with the shape at the current frame **- FI** (if not a valid frame, the flow is zero). For each point on the boundary of the current shape, a vector is drawn from the centroid through that point. The place where this vector intersects the earlier shape, closest to the original point, is found. The directed distance between these two points is the flow (See Fig. 11.3). Positive flow indicates outward movement, while negative flow indicates inward movement.

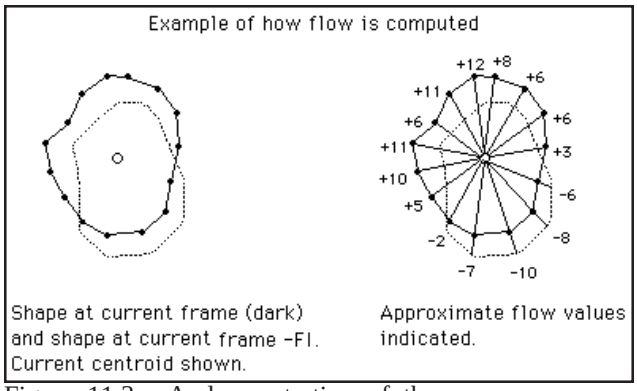

Figure 11.3. A demonstration of the computation of flow

Curvature and flow yield a shape in the following manner: Curvature and flow are computed at each point along the boundary of the original object. The boundary pixels are determined (taking into account the spline settings and pixel increment) as described in the **Compute parameters** and **Edit path file header** chapters. The vector from the centroid to each boundary point is noted. The length of that vector is changed to be the amount of curvature or flow (if negative the vector points in the reverse direction). The point of the resulting shape is drawn at the end of this vector (See Fig. 11.4). To avoid negative numbers, a **bias** can optionally be added to all curvature and flow values, and the 'Size factor' may be varied. Bias is described in section 4, under 'Size and direction'.

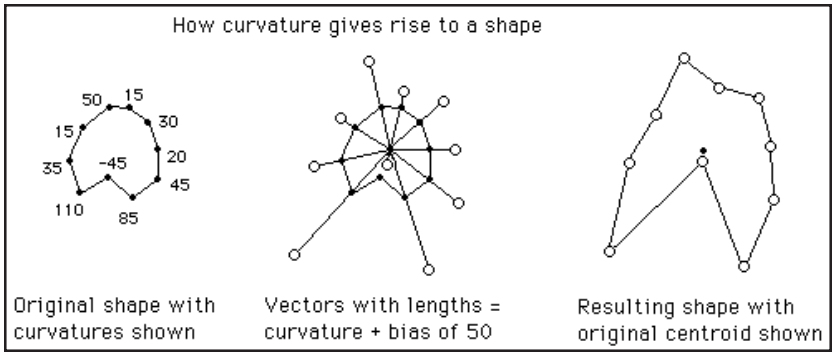

Figure 11.4. How curvature (and flow) determine a shape.

Differencing

Any of the three types of pictures may optionally be differenced. A difference increment (frames), **DI**, is specified in the 'Shape Analysis' dialog box. The pictures (of whatever type) are computed for the current frame and the current frame **-DI**. They are then superimposed, either with centroids at their actual position, or optionally, with their centroids fixed in the same place (check 'fixed centroid' to the right of 'Difference by'). Regions of the interior of the later picture, not contained in the earlier picture, are shown in green. Regions of the interior of the earlier picture, not contained in the later picture, are shown in red. The intersection of both regions is shown in gray. This type of differencing is the same as that described in the chapter **ViewPaths** (Chapter VI).

Modes of display

There are four modes that determine the way pictures are drawn and displayed:

1) Graph
2) Tabular
3) Stacked
4) Circular stacked

In the 'Graph' display mode, the shapes are 'unwrapped' either by angle or by boundary length (see below) and plotted (Fig. 11.5). Because the shape of each frame is graphed, limit the number of frames to 10 for this display mode. When unwrapping does not result in a true function, the largest of several values with the same x-coordinate is used. After the graph is drawn on the screen, the **Graphics Manager** will become active (Chapter IX).

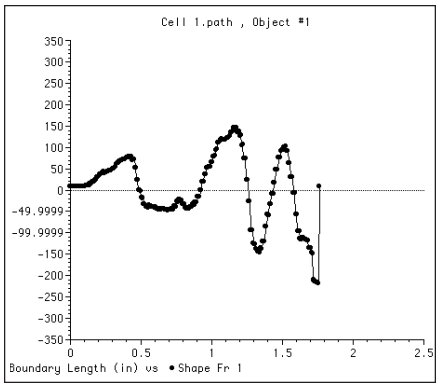

Figure 11.5. The shape of an object graphed.

In the 'Tabular' display mode, pictures are displayed in a matrix of subwindows (Fig. 11.6). You can specify the size of each subwindow (in 'Box length' and 'Box height') and the number of subwindows across and down (by changing the '6 x 4' to the right of 'Tabular').

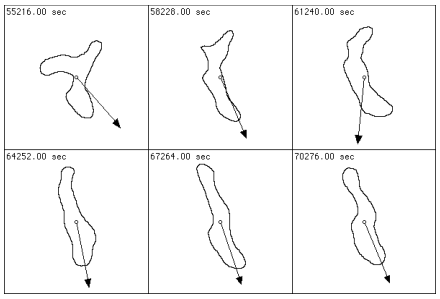

Figure 11.6. 'Shape' pictures shown in a tabular form with centroids and direction shown.

In the 'Stacked' display mode, the shapes are 'unwrapped' either by angle or by boundary length (see below) and plotted, each plot offset lower than the last (Fig. 11.7). The stack offset determines the amount of offset (in pixels).

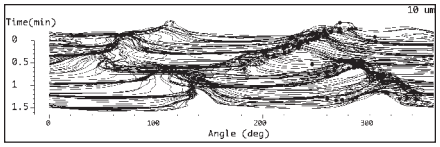

Figure 11.7. 'Stacked' pictures.

In the 'Circular stacked' display mode, the shapes are drawn in one window, centroids fixed at the center of the window, with the scale expanding at a constant rate, frame by frame. The shape for the first frame is drawn smallest. The shape for the next frame is drawn slightly larger, etc. (Fig. 11.8). The stack offset determines how much larger (in pixels) one picture is than the last. This is done by adding successive multiples of the offset to the radius vectors determining the shape.

Both of the stacking modes produce images which are helpful in displaying global shape change over time, as well as rhythms in those changes.

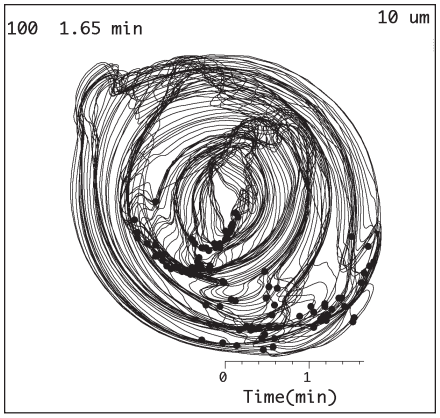

Figure 11.8. 'Circular stacked' pictures.

Unwrapping

Unwrapping by angle is a conversion from polar to rectangular coordinates (with the centroid as the polar coordinate origin). Let P1, P2, ... be the points of the boundary of the shape to be unwrapped. Let (R1,T1), (R2,T2), ... be the polar coordinates of the points (R1=distance to the centroid and T1=the angle of the vector connecting the centroid to the boundary point). The unwrapped points are plotted as (T1,R1), (T2,R2), ... in (x,y) rectangular coordinates. The x-axis is the angle and the y-axis is the distance to the centroid. The result is not always a simple function, since a complex shape may have more than one boundary

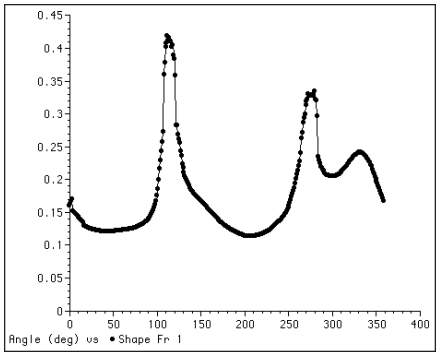

Figure 11.9. A 'Shape' picture unwrapped using the 'Angle' method, and graphed.

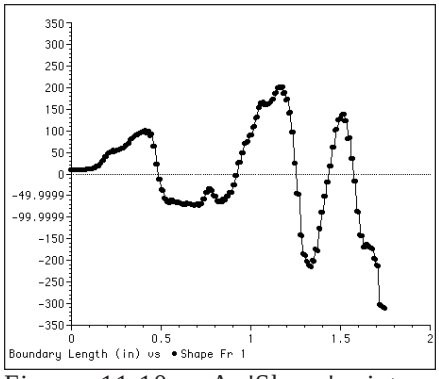

Figure 11.10. A 'Shape' picture unwrapped using the 'Boundary Length' method, and graphed.

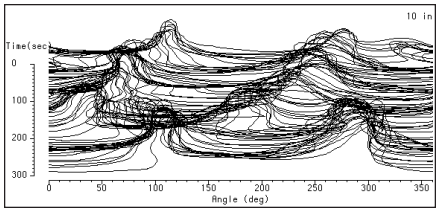

Figure 11.9. A 'Shape' picture unwrapped using the 'Angle' method, and 'Stacked'.

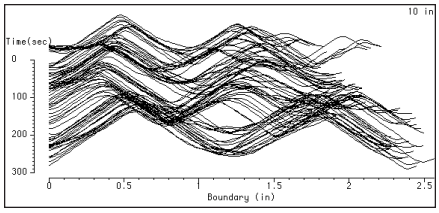

Figure 11.10. A 'Shape' picture unwrapped using the 'Boundary Length' method, and 'Stacked'.

point at the same angle.

Unwrapping may also be done by boundary length. Let P1, P2, ... be the points of the boundary of the shape to be unwrapped. Let (R1,L1), (R2,L2),... be the coordinates of the points parametrized by boundary length: R1, R2,... are the distances from the points to the centroid and L1, L2,... are the boundary length distances (length measured along the arc of the boundary) of the points from the first point P1. Hence L1=0, L2=distance along the shape's boundary from P2 to P1, etc. The L1, L2,... are always increasing, as no two points have the same boundary length from P1. The unwrapped points are plotted as (L1,R1), (L2,R2),... in (x,y) rectangular coordinates. The x-axis is the boundary length and the y-axis is the distance to the centroid. The result is a function, since no two points have the same L value. Figures 11.9-11.12 illustrate the difference between unwrapping by angle and unwrapping by boundary length.

Size and direction

'Size Factor' gives you the ability to increase the size of any of the picture type outputs. Increasing the 'Size factor' only works in the 'Tabular', 'Stacked', and 'Circular stacked' modes, and if in the 'Tabular' mode, you may want to increase the length and height of the boxes in order to fit the entire shape picture. A size of 1 is the original digitized size. To double the size use 2, etc. Fractional sizes are allowed.

'Bias' refers to a constant that is added to the 'radial' lengths of whatever object is being plotted. This should only be used when creating curvature or flow pictures. This is done to avoid negatives.

'Smoothing' is primarily for the 'Curvature' and 'Flow Pictures' and 'Stacks'. The number entered refers to the number of rounds of 'Smoothing'.

'Show Centroids' displays the centroids of the objects, provided it makes sense to do so, given the display mode.

'Show Direction' shows the direction of centroid movement. In tabular form, the direction is shown as an arrow emanating from the centroid. In the stacked modes, the direction is shown as a red mark on the objects boundary where the direction vector would intersect. Figure 11.6 shows both centroids and direction as part of a 'Tabular' 'Shape Picture'. The next two items allow you to specify exactly how direction is to be computed:

Centroid Inc:

This is the frame increment used for the computation of direction. See the chapter on **Computing Parameters** (VIII) for the definition of direction using a frame increment. Increasing this increment tends to smooth out local variations of centroid movement. This increment is totally independent of the frame

increment or the difference increment, described earlier in this chapter.

Central Diff Method:

Check this to use the central difference method for computing direction (described in the **Computing Parameters** chapter-VIII).

> Note: If you are drawing difference pictures and want the direction arrow to actually connect the centroids, set the 'Centroid Inc' to be the same as the 'Difference Inc' and un-check 'Central Diff Method'.

Color choice

Select the desired edge and fill color for objects. When differencing, edge color is the expanding portion and fill color is the contracting portion while the current drawing color (set from the **Option** menu) is the intersection color. If 'None' is selected for the fill color, green, red, and gray will be used for differencing.

You may also choose to set all colors to black and white by checking the 'Set black and white color' box, in order to match output to your printer. If you selected to include internal pixel detail when constructing paths (Chapter IV), you may include that detail here.

To start drawing once selections have been made

Click 'OK' when you have made all the desired selections. Unless you chose 'Graph', a new window appears and the program begins to draw in it. If you wish to stop the drawing, click the mouse button to interrupt. You are then asked whether you wish to save what has been drawn so far. If you choose 'Yes', the **Shape Analysis** program quits, leaving the window on the screen. If you select 'No', you are returned to the **Shape Analysis** dialog box so that you may alter the settings and try again. This is especially useful for correlating the 'Size Factor' with other settings.

> Note: Because the **Shape Analysis** dialog box is complex, once you have determined working settings for your objects, you may want to save those settings, using the 'Save setting' button, so that they may be recalled later, using the 'Load setting' button.

Shape Analysis

XII
Measure Relative Flow

What can this tell me

The **Measure Relative Flow** command gives you the ability to measure, as percentages, the changing morphology of cells. This is done by comparing the percentages of area represented by the three different colors displayed in a difference picture.

Measuring relative flow using difference pictures

The **Measure Relative Flow** command is always available under the **DIAS** menu, but before selecting it (Fig. 12.1), you must have a 'PICT' image file open on the desktop. This command is designed to work with the 'Tabular' difference pictures you created using the commands in the **Shape Analysis** dialog box (Chapter XI). You may either have just finished creating the pictures, or you can have created and saved them previously.

After opening the appropriate file and selecting **Measure Relative Flow**, the title bar will instruct you to 'Click on the 1st color representing the total', then to click on the second and third colors making up the total of the difference picture (red, green, and grey). For each color, simply place the mouse pointer anywhere the color appears in the shape and click.

After picking the colors, you are then prompted as to whether you wish the flow measurement values to be placed automatically. If you answer 'Yes', after measuring flow, **DIAS**® will automatically place the percentage measurement numbers within the image. This is generally the easiest method. However, if you are planning to publish the image, or wish for more control over the presentation of the image, select 'No'. After selecting an area to measure, you will be able to pick where the measurement number is to be placed, using the cursor.

Once you have answered the placement question, the title bar will prompt you to 'Box in the desired region (difference picture)'. To do so, draw a bounding box around the entire difference picture, but within the bounds of the 'Tabular' box (Fig. 12.2).

Note: Total area is defined to be the number of pixels having one of the 3 colors you have chosen. If you wish to use less than 3 colors, click on one color more than once, so that a total number of 3 clicks have been made. For example, a difference picture is comprised of red, gray and green. The

Figure 12.1. Selecting **Measure Relative Flow** from the **DIAS** menu.

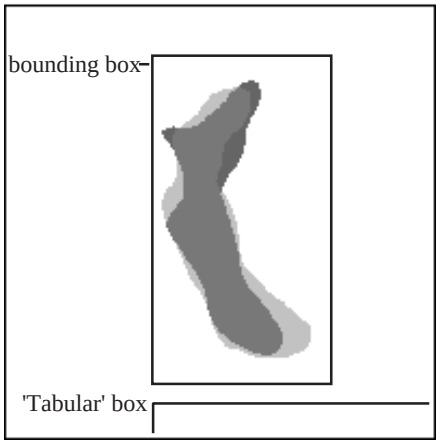

Figure 12.2. An example of how to draw a bounding box.

area of the earlier image is the red and gray parts, and the area of the later image is the green and gray parts. If you wish total area to be that of the earlier object, click on red twice.

Once this is done, the title bar will ask you to 'Click to measure (the area of interest)'. You may click on any red, gray or green portion within the enclosed region. The percent area of that portion (equal to the number of all pixels of the same color, *connected* to the portion you selected) relative to the total area (as defined by the 3 clicks above) is computed. If you selected automatic placing, the % ratio will automatically be placed next to the selected portion. If not, the cursor becomes a text insertion bar and the title bar asks you to 'Click to position (the % measurement)'. Repeat clicking on portions to measure as needed. When done with one picture, click on the return key.

You are again prompted to select a rectangular region enclosing another difference picture. If you are completely finished, click the return key again to exit this section of the program.

Subwindows

Before clicking on a portion to measure, you may optionally type the 's' key to select a subwindow in which to restrict a flow measurement. Use the mouse to draw the rectangular subwindow. Now, when you click on a portion inside the subwindow, all pixels of the same color *only within the subwindow* are counted. Figure 12.3 shows an examples of flow measurements and windowing.

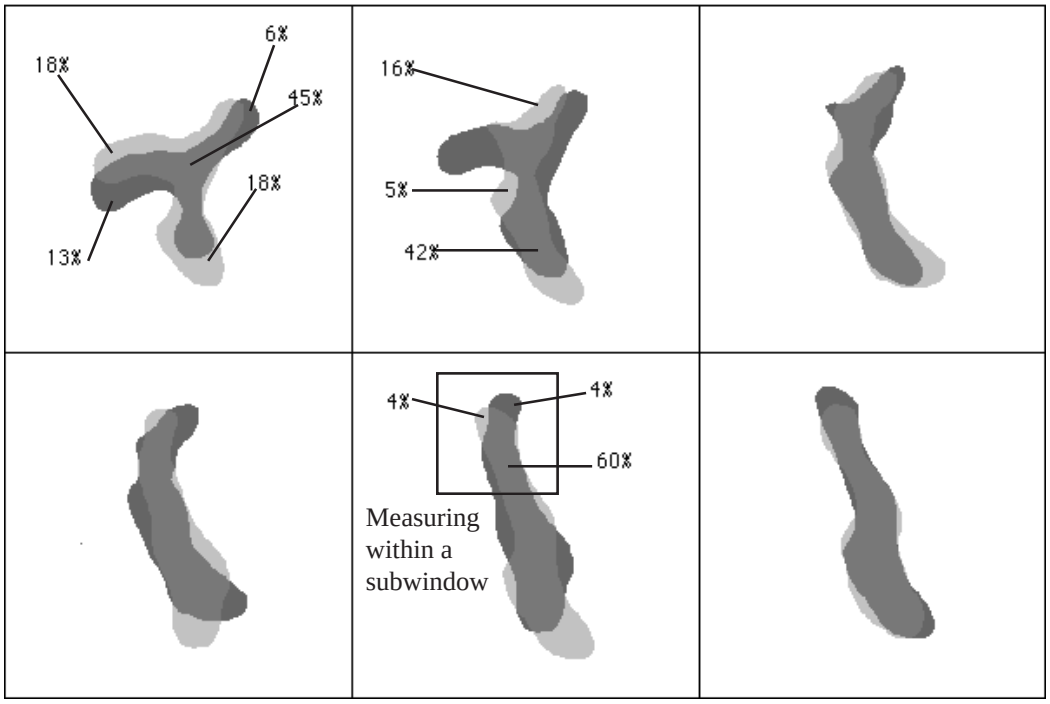

Figure 12.3. Examples of relative flow measurements.

XIII
The File menu

File

The **File** menu (Fig 13.1) contains standard commands for opening and closing files, for printing, and for saving various files, images and movies.

Fig. 13.1 The **File** menu

New Image

This command creates a new, empty graphics window you can use to copy figures into, or draw in. When selecting this option from the **File** menu, you will get a dialog box asking you to define the **New Image** window (Fig 13.2).

'Title' is self-explanatory. 'Horizontal Length' and 'Vertical Width' are asking for the size of the image in pixels. As a reference, a standard Macintosh® 14" monitor measures 640 x 480 pixels. If you create a new window larger than this, you will have to use the scroll arrows to move around in it.

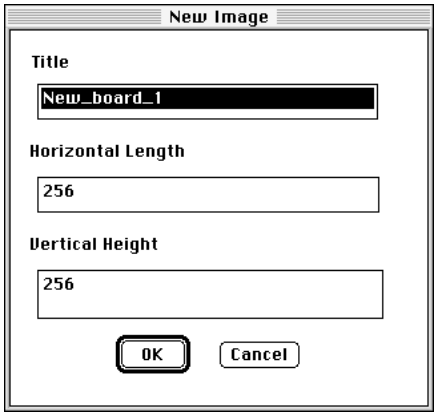

Fig. 13.2 The **New Image** dialog box

New Text

This command creates a new text window which can be used with the various options under the **Option** menu.

Open and Close

These commands are standard to the Macintosh® operating system and so will not be covered here accept to say that you can use the **Open** command to open any standard 'PICT' or QuickTime® file (from any program) or any **DIAS**® files.

Save

This command may offer several options before getting to the actual 'Save' dialog box, which prompts you for the name you wish to save the file under. Those other options have been covered throughout the manual.

We recommend using this command often.

> Note: Saved **DIAS**® text files should be openable by many standard text processors. **DIAS**® image files are saved as high-resolution PICT files, and should be openable by many image processors (make sure to set them for 300dpi). **DIAS**® data files may exported for use in programs such as Excel. **DIAS**® movies may not be opened in any other programs, but may be exported as QuickTime® movies for use in other programs.

Memory Map

The 'Memory Map' is a window which contains a visual display of memory allocation and usage for **DIAS®** (Fig. 13.3). When this command is selected, a new window will open, displaying the total memory allocated for the program, and the current usage of memory. Memory usage will be displayed by blocks of color. As the window fill up with color, you are using more memory. You may leave this window open, and in the background while using the program to check for low memory.

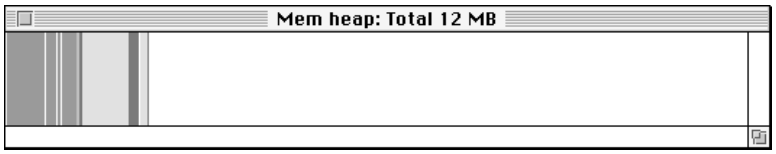

Fig. 13.3 The 'Memory Map' window

Page Setup

This command allows you to set the printer options that are available for your printer. Figure 13.4 shows the **Page Setup** dialog box for the Apple LaserWriter Pro 600 using the LaserWriter 8.0 driver. Because the dialog box you get, and the various options involved, will differ by printer and driver, the specific options will not be covered here. If you wish to learn more about them, you can get the documentation from Apple, or turn on and use the 'Help Balloons', using the **Show Balloons** command under the '**?**' menu at the right of your screen (Fig 13.5).

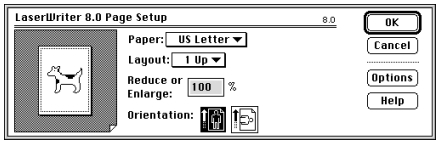

Fig. 13.4 The **Page Setup** dialog box for the Apple LaserWriter Pro 600 and the LaserWriter 8.0 driver

Fig. 13. 5 How to activate Balloon Help

Print Preview

This command will open up a new window, displaying a low resolution copy of the current window. This is done to preview placement of images for printing. Do this to make sure that the current image fits on one page. If it does not fit, part of the image will be cut off in the preview. If that is the case, go back to **Page Setup** and either reduce the image or change its orientation.

Close this window before actually printing.

Print

After selecting this command, a printer dialog box will appear. Because different printers and drivers use different dialog boxes, this dialog box will not be covered, except to suggest using Show Balloons if you have a question.

After making your selections, click on 'OK' to print the document.

Note: A printing trick. If you wish **DIAS®** to use thinner lines to print out shapes, such as in **ViewPaths** and **Shape Analysis**, double the size of the image using the **Enlarge** command under the **Image** menu, then reduce the printed image

using a setting of 50% in the 'Reduction' section of the Page Setup dialog box. This will give you an image of the same size, with thinner lines.

Quit

This command returns the user to the **Finder**. If there are any **DIAS**® windows open and changed, the user will be asked for confirmation before quitting.

XIV
The Edit menu

The Edit menu

The **Edit** menu contains standard **Cut**, **Copy** and **Paste** commands, as well as other, related, capabilities.

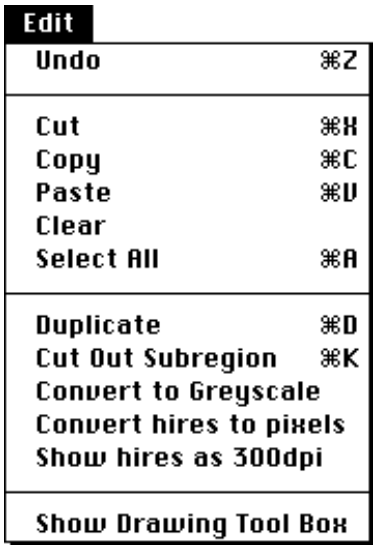

Fig. 14.1 The **Edit** menu

Undo

The **Undo** command undoes the effects of the last **Edit** or **Image** menu command. **Undo** does not affect changes in contrast.

Cut

The **Cut** command deletes any highlighted text if you are in a text mode. If you are working on an image, first select **Cut** from the **Edit** menu, then click and drag an enclosing rectangle around the area you wish to **Cut** from the image. The part of the image contained within the rectangle will be removed from the current image and placed in the clipboard for copying.

Copy

The **Copy** command is similar to **Cut** in that it places the selected text or image in the clipboard. Unlike **Cut**, the selected information is not removed from the current window. Instead, a duplicate is created and placed in the clipboard.

Paste

When working with text, **Paste** places the text captured during the **Cut** or **Copy** commands in-line with the other text. The insertion point is the current location of the cursor. When working with images, selecting **Paste** places a rectangle the size of the image captured during **Cut** or **Copy** on top of the current image. You can move the rectangle by moving your mouse. Clicking the mouse button places the actual image in place of the rectangle. If you do not like the placement of either text or image, selecting **Undo** undoes the current **Paste** action but leaves the information in the clipboard.

Clear

This command erases the entire contents of an image window.

Select All

This command encloses the entire contents of a window. This is often used for copying contents of window to another.

Duplicate

Duplicate creates a new window with exactly the same image as the one in the current window, and gives it the same name with a suffix of 'copy'.

Cut out subregion

The **Cut out subregion** command is similar to **Copy** in that you select a region (rectangle) of interest in a current image without deleting it from the current image. It differs because instead of copying the image to the clipboard, the image section becomes a new window with the original windows name with the suffix 'cut out'.

Convert to Greyscale

This command converts any colors in the current image to grey. Useful if an image is going to be printed on a black and white printer.

Convert hires to pixels

All lines, text, shapes, and anything created with the 'Drawing Tool Box' are actually high resolution objects. As with most drawing programs, they are designed to be dealt with as whole objects. It is difficult to cut or paste part of an object created in the above manner. It is easier to work with the entire object. If you wish to edit such an object, it may be easier to invoke this command. Doing so will allow you to edit the objects on a pixel by pixel basis. However, when printed, the lines may not be as smooth as before, they may appear jagged.

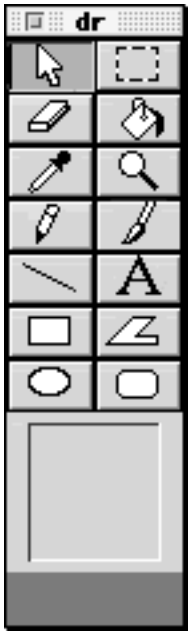

Fig. 14.2 The **Show Drawing Tool Box**

Show hires as 300dpi

If you wish to edit drawn objects as described above, you may want to show the high resolution image at 300dpi (dots per inch). Saving and working with this type of image will retain the smoothness of the lines.

> Note: This command enlarges the image up 400% in order to retain the smoothness of the lines in the image. When printing, set the 'Reduction' factor in the **Page Setup** dialog box to 25% in order to print the image at the original size.

Show Drawing Tool Box

This command brings up the **DIAS®** 'Drawing Tool Box' (Fig. 14.2) The options under this tool box are standard to most drawing programs. They will not be covered here. If you would like additional information, you may use the 'Help Balloons'.

XV
The Option menu

The Option menu

The **Option** menu (Fig 15.1) contains commands which will give you different text and drawing options.

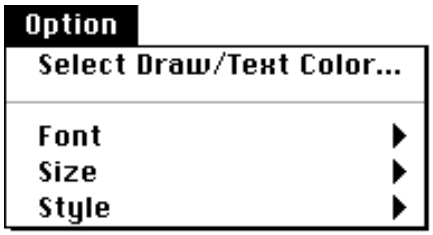

Fig. 15.1 The **Option** menu

Select draw/text color

This command presents several color options to use with the various drawing and text tools under the **Edit** menu (Fig. 15.2). To change colors, select that color with the mouse. The selected color will have a box around it. For more choices, double-click on the right most color. Doing so will bring up a standard Macintosh® color choice system.

Fig. 15.2 A greyscale version of the color choice palette.

Font

Selecting this command brings up a submenu listing all of the PostScript® and TrueType® fonts available to your system. For some of the text files in **DIAS**®, selecting a different **Font**, **Size** or **Style** from the menu with a window open will make those changes to text in the window. You do not have to highlight the text first. For other windows, the changes must be made before creating the data.

Size

Selecting the **Size** command brings up a submenu (Fig 15.3) which gives you control over the character size in various text windows. A checkmark shows the current settings.

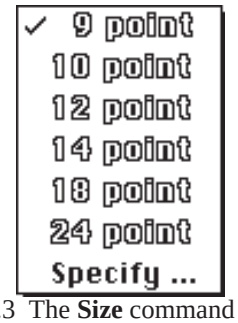

Fig. 15.3 The **Size** command submenu

Style

The **Style** command works the same as the **Font** and **Size** command, with a submenu (Fig 15.4) showing you the current settings.

Fig. 15.4 The **Style** command submenu

Option

XVI
The Movie menu

The **Movie** menu (Fig. 16.1) includes commands for controlling movie play, for editing and combining movies, and for processing movies. These commands will work with both QuickTime® and **DIAS®** movies, but will work fastest with **DIAS®** movies.

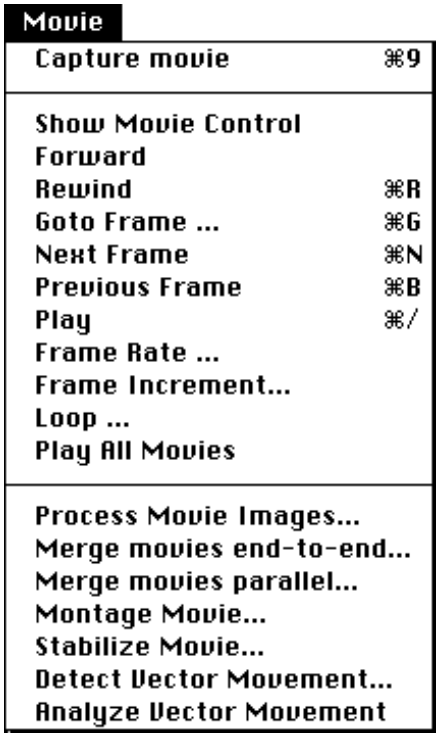

Figure 16.1. The **Movie** menu.

Capture movie

As stated in the **Making Movies** chapter (III), **DIAS®** may be used to analyze movies captured with other types of software, or using its own, built-in, capabilities. **DIAS®** supports most frame-grabbers which include their own, QuickTime® V-Dig file in the extensions folder. These include several Data Translation frame grabbers and those models which come built-in to some Macintosh AV models.

In order to see if your model is supported, and to begin the process, select **Capture movie** from the **Movie** menu. Doing so will open a control panel listing those frame-grabbers available to the program (Fig. 16.2). In this case, only the built-in AV model frame grabber is listed.

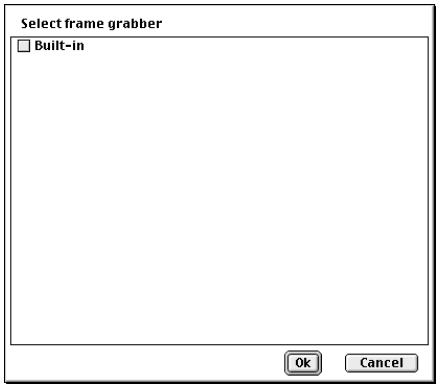

Figure 16.2. The **frame-grabber selection dialog box.**.

After clicking on the desired model, a dialog box will give you control over the capture process (Fig. 16.3). In this dialog box, you will need to enter the time 'Interval', or length of recording time, the number of frames you wish to record, and the size of the recording window.

DIAS® also allows you to average frames, and to only grab frames based on an amount (percentage) of motion detection/ change. If you are grabbing frames in a nonconsistant manner, either by manually selecting frames, or by selecting frames based on motion, you should choose to 'Time stamp frames' for periodicity accuracy.

After making all of those choices, a window will appear on your desktop with the movie playing within it. You will be asked to select a region of interest, and to press the return key when you are ready to begin recording.

The recording process will continue through the end of the selected cycle, or until you press the Apple and period '.' keys simultaneously. Once your movie has been recorded, you will go through the same saving process as covered in the **Making Movies** chapter (III).

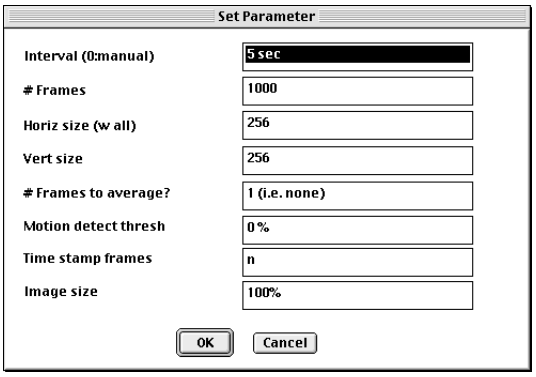

Figure 16.3. The 'Set Parameter' dialog.

Show Movie Control

When you **Open** a movie from the **File** menu, a

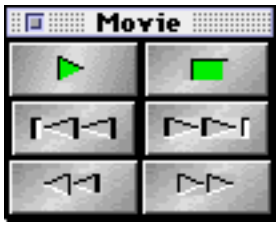

Figure 16.4. The movie player control panel.

window containing buttons for controlling the playback of a movie will automatically appear in the lower right hand corner of your computer screen (Fig. 16.4). You may close that control panel at any time. If you wish to open this window again later, select **Show Move Control** from the **Movie** menu.

To play a movie, select the|> button. This will play all open movies at the same time. To advance forward or backward frame by frame select the |>|>| or |<|<| buttons, respectively. To stop the movie from playing, click the square button; to advance the movie to the last frame or rewind it to the beginning, select the |>|> and <|<| buttons, respectively.

Forward, Rewind, Goto Frame, Next Frame Previous Frame and Play

These commands are self-evident, and will not be covered here.

Frame Rate

The **Frame Rate** command allows you to view your movie(s) at different rates based on frames per second. A dialog box will give you the option to change frame rates. Enter an integer value or 'max' for the fastest.

Frame Increment

This command allows you to skip frames while viewing your movie(s). After selecting the menu item, you are presented with a dialog box asking for a new increment. An increment of 3 means you would view frames 1, 4, 7, 10, etc. The default is 1.

Loop

This command allows you to play the movie continuously, over and over again, through a desired number of frames. When first selecting this command, a dialog box will ask for the desired first and last frames of the loop. Make sure the first frame number is greater than or equal to the last frame number.

Play All Movies

Play All Movies allows you to simultaneously play multiple movies at once. This works for any movies opened by **DIAS®**.

Process Movie Images

This command allows you to use the **Quick Processing** image processing capabilities explained in the next chapter on whole movies.

Merge movies end-to-end

This option allows you to combine two movies into one, longer movie. When you select this option, a series of standard Macintosh® Open dialog boxes will appear in order, asking you for the names of the movies to open. The first movie you select will become the first part of the movie, and the second part of the movie will be constructed from the second movie you choose. After you have chosen the two movies, a dialog box will ask you for the name of the new movie. When you have made your selection, the two movies will appear on screen, and play, one at a time, end to end. It is only at this point that the merging takes place, so do not attempt to stop the process. After both movies have played through, you can open the new movie and play it, or merge it again with another movie.

> Note: The first movie you pick determines the size of the total movie. If the first movie is smaller, in screen size, than the second. The second will be cropped to match.

Merge movies parallel

This command allows you to merge the paired frames of two movies. For each movie, each paired, matched number of frames is combined. For instance, frame 1 from movie 1 is merged with frame 1 of movie 2, etc. This command is designed to combine greyscale and color movies. This is useful for combining movies made with the commands under **ViewPaths**, with the original greyscale background.

After selecting this command, two dialog boxes will open, asking you for the names of the movies. Select the greyscale movie first, as that becomes the background image, and the color information from the second movie will appear on top of those original images.

Montage Movie

This command allows you to view individual frames from your movie as a table of single frames. After selecting this option a dialog box will ask you for the number of frames across and down to display, followed by another dialog box asking which frame numbers to include. Once these options are selected, another dialog prompts you to choose whether or not to draw the frame numbers on the frames displayed.

Having made those choices, the frames will be displayed.

Stabilize Movie

This option allows the user to correct a movie which was recorded with continued, undesired movement or jitter. If your

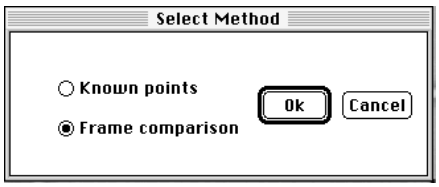

Figure 16.5. The first 'Select Method' dialog box.

Figure 16.6. The second 'Select Method' dialog box.

movie contains only occasional jumps, due to stage movements, use the **Join Stage Movement** command, described in chapter IV. To 'Stabilize (a) movie' make sure that it is open on the desktop, and select the command from the **Movie** menu.

There are two major methods for stabilization, picking known points, or frame comparison. Using the known points method, you click on a known, non-moving point on every frame of the movie. **DIAS**® then realigns all of the frames in the movie, based on the matching of those points and clicks. The second method gives you more control over each frame, and involves comparison of each pair of frames in the movie. After first selecting **Stabilize Movie**, a dialog box will ask you to select from these two different methods (Fig. 16.5).

If you select the 'Known points' method, you will click on those known points throughout each frame of your movie. If you select the 'Frame comparison' method, a second dialog box will allow you to select between two variations in that method (Fig. 16.6). Each of the methods allows you to select an original rotational point, and then to use your up, down, right and left arrow keys to move the frames, as they jump back and forth between sequential pairs of frames. The idea is to look at the movement of items as the frames shift, and then fine-tune the stabilized matching. You move to the next set of frames by pressing the return key.

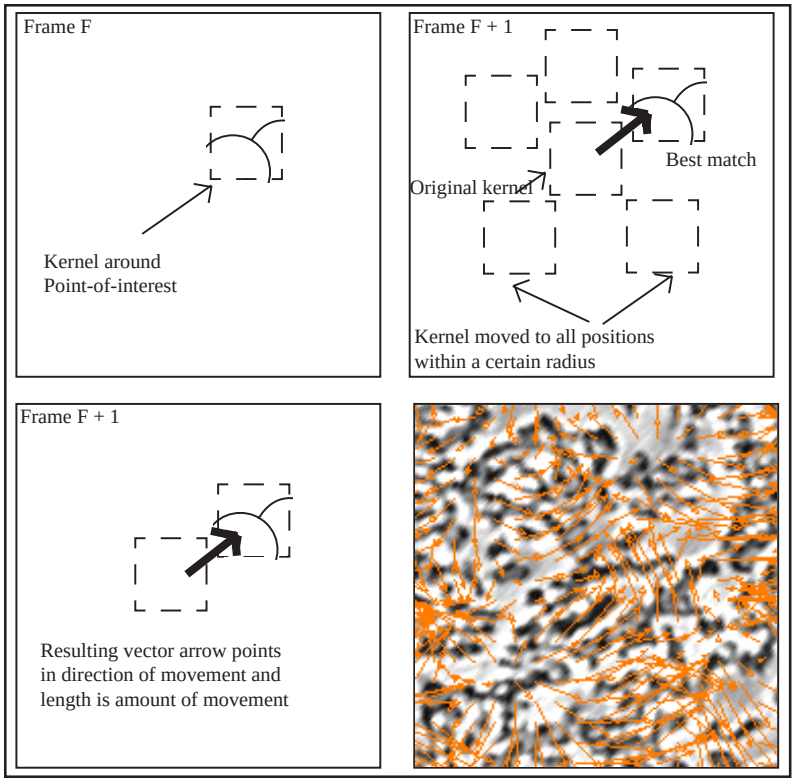

Figure 16.7. A vectoring example.

Detect Vector Movement

It is not always possible to adequately outline cells. This is particularly true in cases of aggregation, waves, or other phenomenons that involve large crowds of moving cells or objects. In those cases, vectoring may be helpful.

Vectoring is the process of analyzing moving and changing grey values. No outlines or traces are needed. In this procedure, a grid of points spaced at 5-pixel intervals was superimposed on the cells. The program then connected a point in one frame with a point in the next frame by forming a kernel square around the first point and then finding the “best fit” of video pixel data within the kernel by moving it to all positions within a certain radius from the original position and comparing the video data to the

original. When the best fit is found, a vector is drawn connecting the center of the original kernel to the center of the moved kernel (Fig 16.7). You may regulate the size of the kernel and radius. Thus, **DIAS**® generates vectors, the length of which are proportional to the net distance the kernel has traveled and the direction of which represents net direction of travel.

It takes two steps to completely analyze vectors. First you must create a vector movie, using the **Detect Vector Movement** command. Once selected, a dialog box (Fig. 16.8) allows you to define the 'Kernel size', or the area, in square pixels, over which the comparison will be made for vectoring. You may also define the maximum area of movement allowed before a vector will be disallowed. The rest of the dialog box allows you to define look of the vectors drawn. After you have entered the settings, press the return key, and you will be asked to choose a region for which to detect vector movement. The process then begins, creating and displaying the vectors for each new frame. It works by comparing each frame with its previous frame and calculating the change in position, as described above. This results in one less frame than your original movie because the first frame does not have vector movement, only the second frame in comparison to the first frame has a change in position. When this step is finished, the result is a new movie with the vectors of motion marked over the body of each cell within the region you selected. You may play this movie to show overall movement, or you may analyze the vectors themselves, using the **Analyze Vector Movement** command.

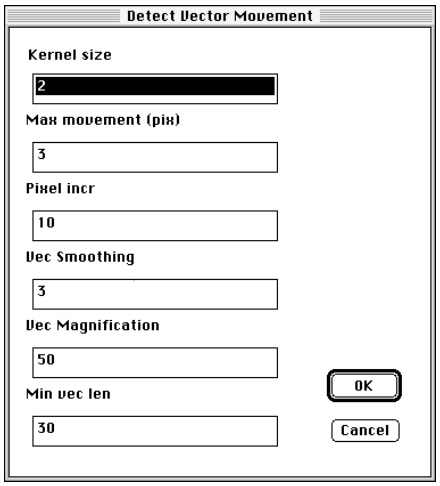

Figure 16.8. The 'Detect Vector Movement' dialog box.

Analyze Vector Movement

This command is designed to analyze, for motility, the vectors created using the previous command.

To do so, with the newly created vector movie open, select this command from the **Movie** menu. Doing so will place the movie in a larger window, with a small amount of white, bordering the new movie. The vector movie will play, giving you a chance to view the movie. In the titlebar of the movie window, it will tell you to type 'w' in order to window. Do this now, and select an area of the vector movie to analyze. After doing so, the titlebar will ask you to indicate a direction. To do so, draw a line within the rectangle in the direction of general movement. This will tell the program how to analyze positive and negative directionality. Finally, the title bar will ask you to 'place' the graph. Move the mouse pointer somewhere outside of the rectangle of interest, but within the movie window.

After doing so, the titlebar will again ask you to select a window. If you wish to analyze more than one area of the movie, continue on as described above. Once you have made all of your

choices, click the return key, and **DIAS**® will create a small graph, showing directionality of movement.

XVII
The Image menu

The Image menu

The **Image** menu (Fig 17.1) contains image processing and correction commands designed to work with static images. Before selecting any of these commands, you must have an image open on the desktop.

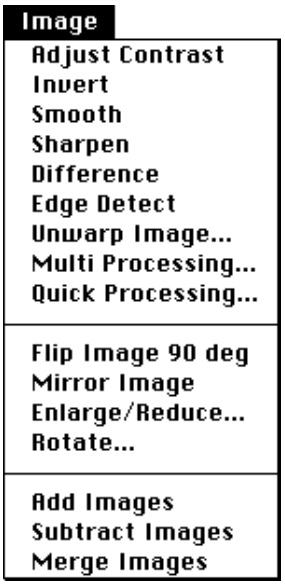

Fig. 17.1 The **Image** menu

Adjust contrast

Selecting **Adjust Contrast** adds the current image's gain and brightness settings and a '?' to the menubar of the currently open image (Fig. 17.2). Typing '?' shows you the contrast adjustment commands (Fig. 17.3).

Click on the up key on your keyboard up to increases the image's contrast, moving it down to decreases contrast, pressing the left arrow key decreases brightness and right increases brightness. The image and numbers will change as you move the mouse. When you have finished adjusting the contrast, press the return key.

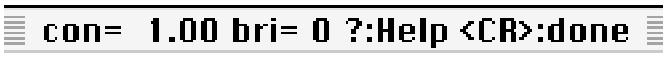

Fig. 17.2 The current settings listings.

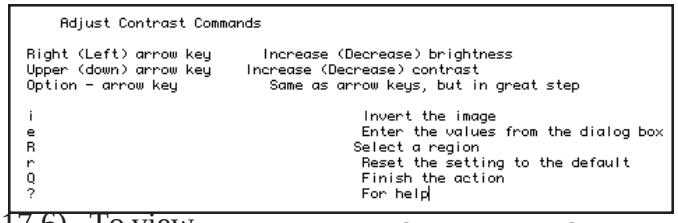

Fig. 17.3. The contrast adjustment options.

Invert

This option will create a negative (Fig. 17.5) of your original image (Fig 17.4), or if you have scanned in a photographic negative, **Invert** will reverse it into a standard image.

Smooth

This command automatically smooths the entire image; it softens edges of lines and blurs them so that edge detection is less sensitive (Fig. 17.6). To view further options available under this menu item, hold down the 'option' key while selecting **Smooth** and it will bring up a dialog box with two more options. The first text field asks for the number of times you would like the image smoothed; more numbers result in more smoothing. The second text field is labeled 'Kernel' and gives you a choice of 4 numbers (3,4,5,7). It results in a heavier smoothing factor with higher numbers.

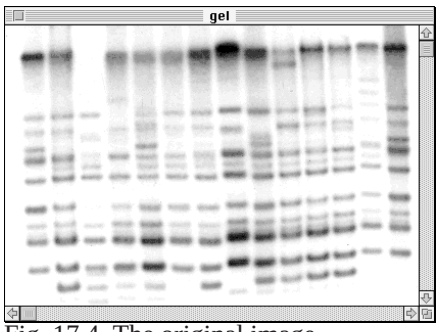

Fig. 17.4 The original image

Sharpen

Figure 17.7 is an example of sharpening. Holding down the 'option' key provides additional sharpening controls.

Difference

In differencing, the image is subtracted from itself, which

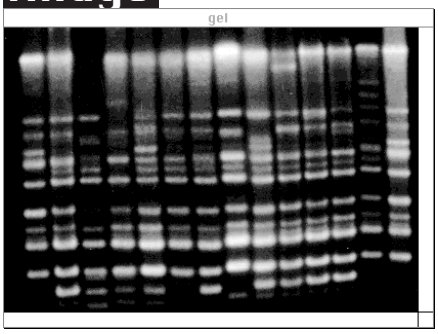

Fig. 17.5 The inverted image

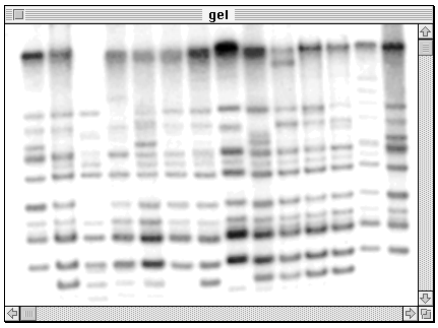

Fig. 17.6 The smoothed image

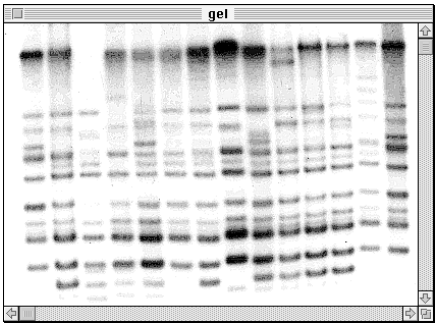

Fig. 17.7 An example of sharpening

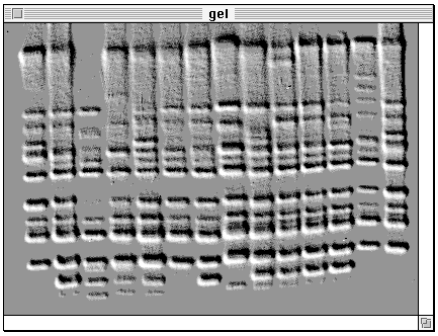

Fig. 17.8 The original image differenced

gives it a 3-dimensional appearance (Fig 17.8). The amount and direction of the differencing may be controlled by the arrow keys on your keyboard.

Edge Detect

This filter finds the minimum intensity in each defined kernel and subtracts that intensity from the center pixel's intensity. This is done for all pixels. The result may enhance the edges of the image (Fig. 17.9).

Unwarp Image

Unwarp image has two different components, unwarping based upon a set of guiding lines, and aligning. When first selecting this command, a dialog box will ask you if you wish to 'Slide adjust?' (Fig 17.10). If you select 'Yes' the title bar will ask you to 'Select region to adjust' (Fig 17.11). After selecting a region, or pressing the return key which selects the entire image, a dialog box will ask you for an 'Orientation' for aligning (Fig 17.12). Selecting 'Horizontal' means that you will be able to stretch, slide and compress the image (or section of the image) to the right and left. 'Vertical' gives you the same ability, moving up and down. Once you have selected an orientation and section, to unwarp your image, click on the image and move it. If you click on the center of the image and move, you will slide the entire selected area. If you click on an edge (right or left for horizontal orientation-top or bottom for vertical orientations) and move in the direction of that edge (you click on the right side and move farther right), you will stretch the image, if you move opposite of the side you click on, you will compress your image (or section of the image).

If you answer no to the 'Slide adjust?' question, you will be shown another dialog box asking you wether you wish to 'Use simple line segments?'. The type of line segments you use will depend on the types of distortions in your image. Simple line segments, which are straight, unkinked lines, are used in cases of continuous skews in an image. To draw simple line segments, click and drag the mouse to draw horizontal lines along the known skews in the image. Because simple line segments are straight, unkinked lines, when drawing them, you need only click and drag the mouse a short distance. **DIAS**® will finish the line for you, extending it the entire horizontal or vertical distance of the image. If the distortions in the image involve kinking, simple line segments would not effectively straighten the image.

Complex line segments are a series of short, connected lines (Fig 17.13). To draw 'Complex line segments', click and drag along the known skews as you would with 'Simple line segments', but when you reach a kink or bend, release the mouse

button to end a segment and then re-click and continue to create the 'Complex line segment'. Because of their complexity, after finishing each horizontal or vertical line segment, you must press the return key to finish the line before continuing to the next, or you will create a series of connected, zig-zagging lines.

When using either simple or complex line segments, it does not matter in what order you draw the lines. You can start with horizontal or vertical lines, and continue either right to left or vice-versa. The same is true when starting at the top or bottom. **DIAS**® is flexible. As long as the lines you draw are relatively horizontal *or* vertical, **DIAS**® can interpret the guide lines. The only caveats are that you can not 'box' your image with one continuous line; you must box in your gel with at least two horizontal, and two vertical lines. The more horizontal and vertical lines you draw, the better unwarping job **DIAS**® will be able to do. This is true because **DIAS**® works the with sub-blocks of the image created by the intersecting horizontal and vertical lines, and the more blocks the image is broken down into, the more control **DIAS**® has over the image.

Looking at the title bar, it tells you to type 'd' when you have finished. After doing so, a new blank window will open, and your image will start to rebuild itself piece by piece (these pieces are based upon the cross sections created by the intersecting horizontal and vertical unwarping lines) (Fig 17.13).

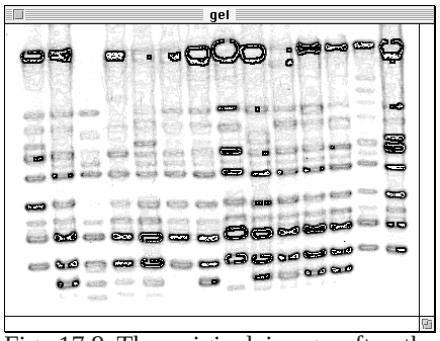

Fig. 17.9 The original image after the edge detect filter is applied.

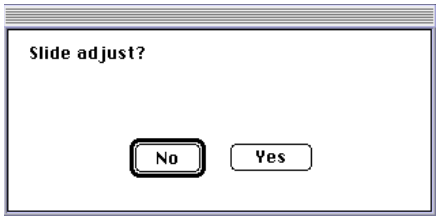

Fig. 17.10 The first dialog box after selecting **Unwarp**

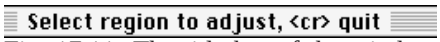

Fig. 17.11 The title bar of the window during **Unwarp**

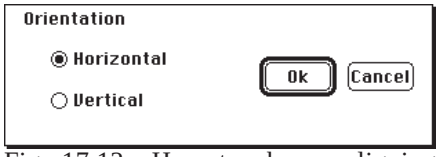

Fig. 17.12 How to choose aligning techniques during **Unwarp**

Multi Processing

When you first select **Multi Processing**, a rectangle appears on your image. You use this rectangle to select an area to use as a test region. Once you have done this, two copies of the test region and the multiprocess control panel appear on screen. The **Multiprocess** command allows you to use several image processing filters at the same time and in progression. Since the filters have already been explained, they will not be covered here. To use a filter, click on the box to the immediate left of it. For some of the filters, you can use multiple 'Iterations'. There are 4 'Processors' so that the filters can be applied in progression, instead of all at once, which may cause problems with the image. The image on the left is the original, and the image on the right represents the original after all of the filters have been applied. Once you have chosen specific filters, you can see their effect on other parts of the image by using the various scroll buttons. You can also save and load filter settings.

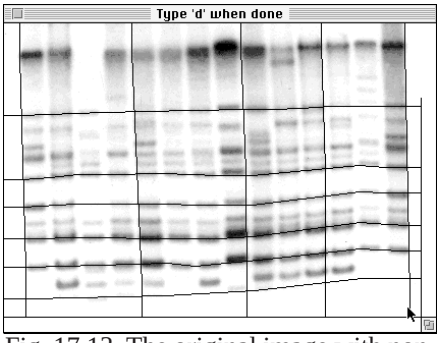

Fig. 17.13 The original image with non-simple (complex) line segments drawn for unwarping

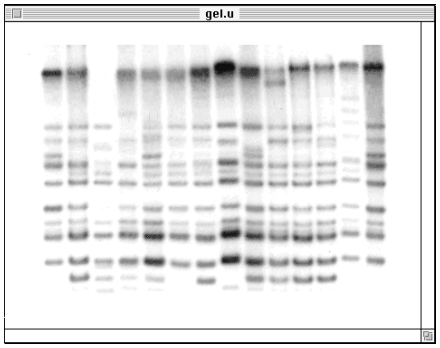

Fig. 17.14 The unwarped image

Quick Processing

Quick Processing allows the user to adjust the brightness and contrast for an entire image. It is similar to **Multi Processing** in this respect, except that is displays various processing outcomes

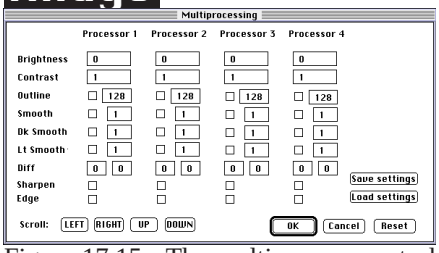

Figure 17.15. The multiprocess control panel.

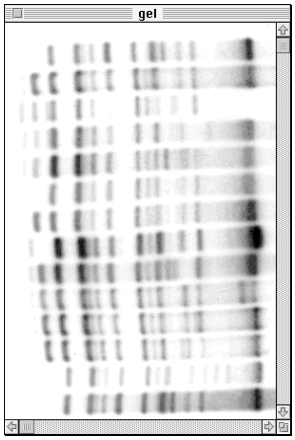

Fig. 17.16 The image rotated 90 degrees

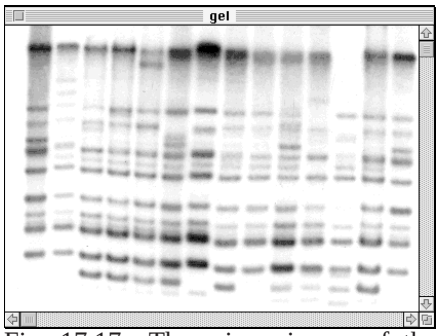

Fig. 17.17 The mirror image of the original gel

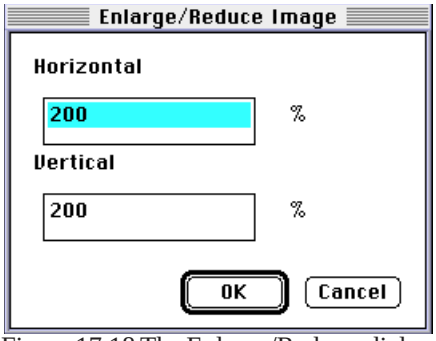

Figure 17.18 The Enlarge/Reduce dialog box.

simultaneously, for comparison. Brightness is decreased as the choices go to the right, and contrast is decreased as the choices go down the columns.

There are several other options available to the user at this point. To see them while the program is running, hit '?' at this time and a legend will appear. 'B' results in all of the available choices becoming brighter than they currently are, 'd' results in the choices becoming darker , and 'i' results in the image becoming inverted.

If further choices modeled after a particular contrast and brightness selections are desired, that selection's number may be typed and other filters will be derived from that choice. For example, if the user desires a filter similar to choice number 3, he or she can type '3' and 12 new filters based on choice 3 will be created. This allows for finer adjustments in shading and contrast than were presented to the user at first.

After a filter is selected, a dialog box asks the user whether to apply the processing to the entire image or not. 'Yes' results in the whole image being affected; 'no' results in just the processing box which was initially selected being processed.

Flip Image 90 deg

This command takes the original image and flips the window 90 degrees to the right (Fig 17.16).

Mirror Image

This option reverses the image from right to left (Fig 17.17).

Enlarge/Reduce

Selecting this command brings up a dialog box (Fig 17.18) asking you for percentages of reduction or enlargement. Be sure to use the same percentage for both the 'Horizontal' and 'Vertical' settings, or you may distort your image.

Rotate

The **Rotate** command is similar to the **Flip Image 90 deg** command in that it allows you to rotate your image. There are, however, two very important differences between the two commands.

The first difference is that this command gives you control over how much to rotate the image. This is useful if the image is slightly skewed, and not completely turned around. When first selecting this command (with the desired image open and on the desktop), a dialog box (Fig 17.19) will ask you to 'Select center of rotation'. This is the point around which the rest of the image

will be rotated. This will generally be center, but you can pick any point in the image. After making your selection, a second dialog box will ask you the 'Angle', in degrees, you wish to rotate the image (Fig. 17.20).

This ability brings up the second important difference between the two commands. Because the rotation angle need not be in increments of 90 degrees and the image can rotate on any point, the image may no longer be rectangular in nature. This being the case, **DIAS**® cannot create a new non-rectangular window, so it rotates the new image within the existing window. Thus, portions of the image may be cut off. To eliminate this problem, we recommend creating a **New Image** window much larger than the original, and copy the image to the center of the new window. Now, when you rotate the image, there should be enough room to fit the entire image in the window, and after you are done, you can **Cut out (a) subregion** of the new image to eliminate any leftover white area.

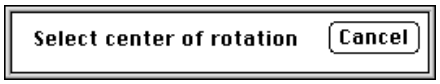

Fig. 17.19 The rotation center dialog box

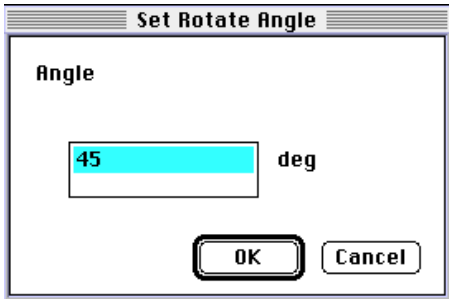

Figure 17.20 The Rotate dialog box.

Add, Subtract, and Merge images

These three commands are all designed to allow you to combine images. With most cut and paste commands, anything under the area of the image you paste is erased. With these three commands, you may combine images without losing the information below.

Before you can use these commands, you must have two images open on the desktop. **Add Images** transparently pastes the background image on top of the foreground image. **Subtract images** transparently pastes the negative of the background image on top of the foreground image. Both of these commands are designed for use with greyscale images because the backgrounds of the images may blur when the actual images are combined. With the **Merge images** commands, the backgrounds are not combined, so less of the blurring occurs. The choice of which merge command will depend on the background of the images being merged. The merge command is similar to **Add Image**, but is better for images with high-resolution lines and drawings, such as graphs.

Experiment with these three commands in order to obtain the best results.

Image

XVIII
Measure Menu

Measure Menu

The **Measure** menu (Fig. 18.1) contains commands allowing you to make measurements of static images, based on the relative lightness and darkness of the pixel data composing the image. An image must be open before these options become available.

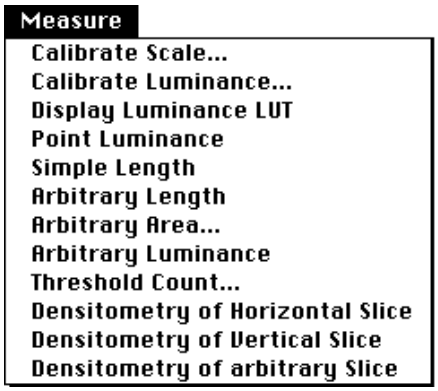

Fig. 18.1 **Measure** menu.

Calibrate Scale

This command allows you to change the length scale of measurement from pixels to another unit. The user is prompted for the scale factor and for the unit (Fig. 18.2). If the scale factor is known, it may be directly entered. If '?' is entered for the scale factor, the user is prompted to draw a line of known length on the image and is then asked for the length of that line. The scale factor is then automatically computed. Each window has its own scale factor.

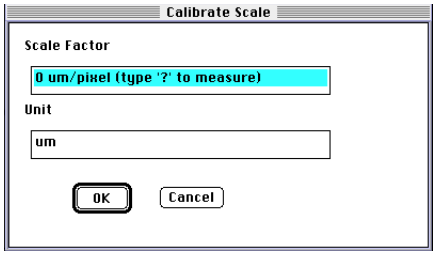

Fig. 18.2 'Calibrate Scale' dialog box.

Calibrate Luminance

With this option you may change the luminance scale from 0(black) - 255(white) to a custom scale. The title bar of the open window will prompt you to select points on the image and enter the desired luminance value (Fig. 18.3). Up to 16 sample points may be given. Type the 'return' key to exit. Luminance is now computed by linear interpolation from the given values.

Fig. 18.3 'Calibrate Luminance' dialog box.

> Note: Even in a custom scale, darker intensities should have lower values than lighter ones.

Display Luminance LUT

Selecting this command shows the graph of the LUT (look up table) where output luminance (in the calibrated scale) is given as a function of input luminance (Fig. 18.4).

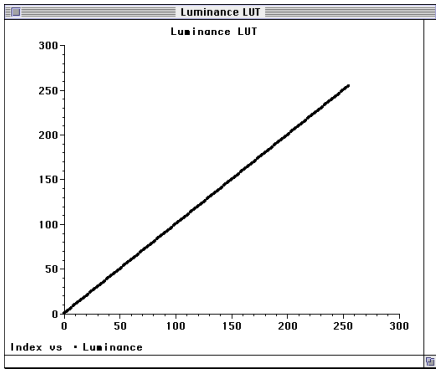

Fig. 18.4 'Display Luminance LUT'.

Point Luminance

After selecting this command, you may click on any point with the mouse and the luminance value appears in the titlebar of the window. A text insertion bar appears, allowing you to indicate where the value will be displayed. Press the return key to exit.

Simple Length

This command gives the length of a line drawn using the mouse. It also gives the angle of the line in degrees. This information will appear in the lower left hand corner of the open window. Press the return key to exit. This option may serve to estimate lengths of interest, such as the cell body or nucleus length.

Arbitrary Length

This command measures the length of curves traced with the mouse. Draw a curve by holding the mouse button down, while moving the mouse, or by point-to-point clicking. Press the spacebar when done. A text insertion bar appears; position it and click to have the length of the curve tagged to the image. More curves may now be drawn. Press the return key to exit. This option allows the calculation of the total length of a curved surface.

Arbitrary Area

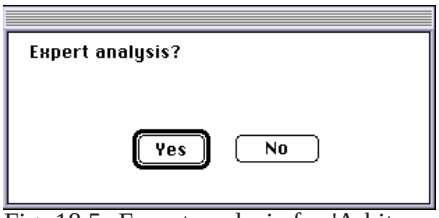

Fig. 18.5 Expert analysis for 'Arbitrary Area' dialog.

Arbitrary Area measures the area of simple regions drawn with a mouse. When first selecting this command, a dialog box prompts you to choose 'Expert analysis' or not (Fig 18.5). Expert analysis measures perimeter length, area, roundness as well as several other shape parameters, and presents these results in a tabular form. Once the region is drawn, the area parameters are calculated and then you may draw another region to be analyzed or press the return key to exit this mode.

Arbitrary Luminance

The instructions to using this command are displayed in the window's title bar when this option is selected. After drawing a shape of any size and pressing the spacebar to close the region, the mouse places the text which contains information about the luminance of the region. Press the return key again to finish with this option.

Threshold Count

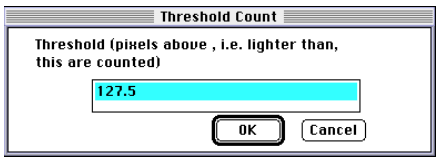

Fig. 18.6 'Threshold Count' dialog.

This command allows the user to enter a threshold value for which pixels are to be counted within a region (Fig. 18.6). The result is a fractional number representing the number of pixels at or above the threshold value over the total number of pixels. You may again select where the notation should be printed.

Densitometry of a Horizontal Slice

After selecting this command, box in a horizontal slice of

your image. For each horizontal position within the rectangle, the intensities of the pixels (on the vertical line) in the rectangle with the same horizontal position are averaged. If background subtraction is requested, a duplicate of the first rectangle replaces the arrow cursor. You are then asked to position this rectangle to sample the background luminance. These luminance values are then subtracted from the luminance values of the original slice. If the background is darker than the original slice in over 20 locations, the subtraction is canceled. After the luminance graph appears, areas under the graph can be computed. The horizontal line across the bottom of the graph is the base line based on the lowest point of the luminance graph. Dialog boxes prompt the user to indicate the left and right boundaries with the mouse. Once the area of the bounded region has been found, you place it in the window with the mouse.

Densitometry of a Vertical Slice

This command works similarly the horizontal densitometry, except the slice is oriented vertically.

Densitometry of arbitrary slices

When choosing this command, you can type any of the number keys 1-9 in order to increase the thickness of the slice. You then draw the line (slice) with the mouse. The rest of the steps are similar to those described above.

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