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Computer-Assisted Microscopy

The Measurement and Analysis of Images

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Preface

The use of computer-based image analysis systems for all kinds of images, but especially for microscope images, has become increasingly widespread in recent years, as computer power has increased and costs have dropped. Software to perform each of the various tasks described in this book exists now, and without doubt additional algorithms to accomplish these same things more efficiently, and to perform new kinds of image processing, feature discrimination and measurement, will continue to be developed. This is likely to be true particularly in the field of three-dimensional imaging, since new microscopy methods are beginning to be used which can produce such data.

It is not the intent of this book to train programmers who will assemble their own computer systems and write their own programs. Most users require only the barest of knowledge about how to use the computer, but the greater their understanding of the various image analysis operations which are possible, their advantages and limitations, the greater the likelihood of success in their application.

Likewise, the book assumes little in the way of a mathematical background, but the researcher with a secure knowledge of appropriate statistical tests will find it easier to put some of these methods into real use, and have confidence in the results, than one who has less background and experience. Supplementary texts and courses in statistics, microscopy, and specimen preparation are recommended as necessary.

This text was originally created for use in teaching both a regular semester course and a one-week summer short course in image analysis. Although aimed initially at students in materials science and engineering, the courses have consistently attracted people from the life sciences, veterinary and medical schools, food sciences, forest products, geology, and archaeology, and so more examples and terminology from those fields have been incorporated. Many of the same methods, and indeed the same computer systems, can be used for macroscopic applications ranging up to astronomy and remote sensing, but the terminology used here is primarily that of the microscopist.

Of course, there are many kinds of microscopy. These include not only the familiar light and electron microscopes, but also ion, acoustic, X-ray, magnetic resonance, and other devices, and even analytical instruments not usually thought of as microscopes that nevertheless produce two- or three-dimensional arrays of data that can be treated and understood as images. Not all of the techniques covered here are appropriate to all of these kinds of images, but most of the useful methods are covered.

There is no substitute for actually using these methods, and no incentive better than the need to perform a real task. The reader or student with access to a source of images of specimens that are of real interest, and some computer-based image analysis system, should "try out" as many of the various operations as possible to better understand their consequences, as each subject is considered.

Finally, the user of these systems and methods should be alert to an important side-effect of studying this material - it should also make you a better observer. As you learn what the computer "sees" in images, you will learn to see some of it yourself. This will assist in selecting the proper algorithms for processing, discrimination and measurement, as well as forcing you to be a more careful microscopist, producing the best possible images for analysis.

This text, with all its figures and tables, was prepared on a Macintosh computer and printed directly on a Laserwriter, so any errors are solely my own responsibility. Special thanks are due to Chris Russ (Analytical Vision, Inc., Raleigh, NC) who has helped to develop methods and write many of the programs that execute these image analysis algorithms (also on the Macintosh), to John Matzka (Plenum Publishing Corp.) who has patiently tried to educate me in the preparation of book manuscripts, and to Helen Adams, who has long understood and tolerated my compulsion to undertake writing projects like this one.

John C. Russ
Raleigh, NC
February, 1990

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Chapter 1

Introduction

The importance of images

Mankind's principal means of interacting with his environment is visual. In teaching students, I sometimes encounter those who express themselves by saying "I hear what you're telling me" or "I grasp that idea," but most of the time the expression is "I see what you mean." In fact, students who are principally auditory or tactile learners sometimes have real problems in dealing with engineering or scientific material that is presented in textbooks heavy with diagrams and graphs. Most of us learn visually. As age diminishes the acuity of our senses, we use eyeglasses commonly, hearing aids occasionally, and practically never any prosthetic aids for any of the remaining senses. The Chinese proverb that a picture is worth 1000 words probably underestimates.

This affects the kinds of scientific research we do, as well. For instance, the recent space probes to Comet Halley carried a number of sophisticated and important instruments, ranging from magnetometers to mass spectrometers. But it was by the pictures they returned that we judged their success (and this has been true of most of our other space projects as well). Many scientific instruments directly produce pictorial images (such as electron microscopes); others that do not usually have some type of graphics display (for instance to show a spectrum), expecting the operator to be able to extract meaning more readily from this than from a list of numbers.

This influence is even being felt in unexpected places. The "hottest" recent development in computers, popularized by the Macintosh, is the use of "icons," little pictures representing programs or data, which the user can recognize and point to instead of having to read words from the screen (Figure 1-1).

The evolution of man's visual apparatus has made it our most important and relied-upon sense by tailoring it to extract meaning from images. Approximately 60 percent of the sensory inputs to the brain come from the visual system. Not all animals have that reliance: bats use sound echolocation, fish have a pressure sensing organ we don't even possess, some snakes sense heat, birds and some bacteria respond to magnetic fields, and many animals have a sense of smell that communicates important information about the world around them. We find it hard to imagine what the world "looks like" to such an animal; in fact the word "imagine" itself carries with it an implicit visual metaphor.

Why measure images?

The fact that humans can easily interpret images does not mean we should not - or do not need to - use computerized methods for image measurement. In fact, it increases our desire to do so. One purpose can be to better understand the visual process itself, by duplicating or emulating its responses. That is not our goal here.

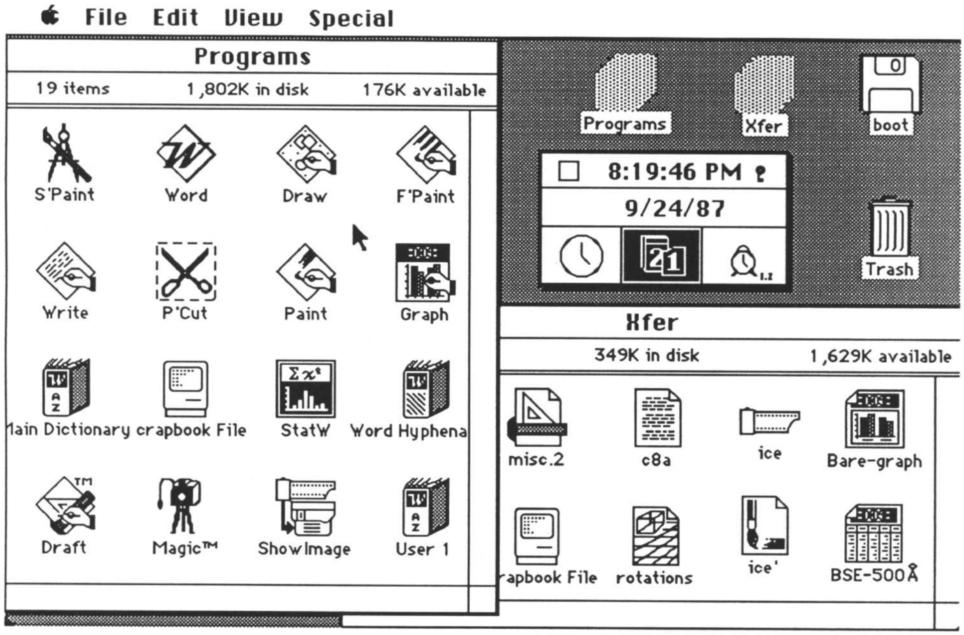

Figure 1-1: Example of a Macintosh screen with icons.

Computerized "understanding" of images, for instance the kind of real-time interpretation of a changing scene that allows us to guide a car down a road, requires a massive investment of computer resources and even so can deal with only very simplified situations. That is not our goal, either.

But recognizing, counting and measuring the size, shape, position, density, and other similar properties of particular objects in an image is something that is well within the power of mini- and microcomputers, and can be done by the computer relatively quickly with excellent reproducibility. Images in a form suitable for acquisition and analysis are produced by a variety of instruments, and computerized measurement can be used to extract specific information from the images much more accurately and reproducibly than a human can without such aid. In fact, human observers tend not to do this very well, with results that vary from observer to observer and from time to time. This perhaps reflects the fact that in normal situations, humans rarely need to exactly measure an object in an image. Instead, they can interact with their environment, to bring a comparison object or ruler into play, for example.

Computer image measurement is less easily distracted from what is important by trivia in the image, and is also better than a human observer at paying attention to all of the details present. It doesn't get bored, and it makes no (or at least very few and usually explicit) assumptions.

On the other hand, humans are very good at recognizing objects, often based on very incomplete or unconventional images, and this capability is much harder to program into the computer. There is considerable evidence that humans literally "turn things over" in the mind, to obtain the best viewpoint for examination or comparison, as shown in Figure 1-2. This is beyond the capability of most computer methods now.

The process of image measurement involves an enormous reduction in the amount of data, by selecting from the original image those objects and parameters that are important. An original image may represent a million separate points stored in the computer (in the human eye, there are more than 150 million individual receptors on the retina). But the desired information may be as simple as (e.g.) the number of white blood cells on a slide, the size (width, etc.) of a device in an integrated circuit, the variation in the amount of a phase near the surface of a metal, or even just the presence of a tumor in an X-ray image. This selection and reduction is at the heart of image analysis and measurement. It is achieved by ignoring irrelevant information.

Computer methods: an overview

Most of the images that we will deal with here are single, two-dimensional ones, much like a single-eye look at of some real world view. In many cases we will further limit ourselves to a monochrome (black, shades of grey, and white) image rather than full color. The later chapters will deal with the additional information that can be obtained from multiple images, either used in a stereo (two-eyed) sense, or a series of parallel sections through an object, or projections in many directions as used in tomography. But even when several images are involved, each single one is usually dealt with to some extent separately so we are justified in first considering how to work with an individual image.

Many of the computer methods use algorithms that either consciously or accidentally mimic many aspects of human vision. For convenience, it is usual to separate the human

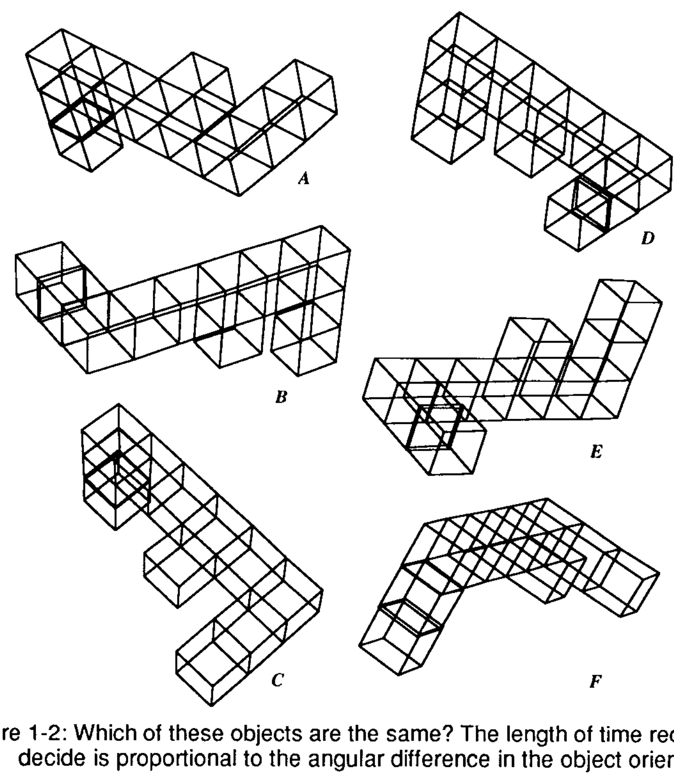

Figure 1-2: Which of these objects are the same? The length of time required to decide is proportional to the angular difference in the object orientation.

visual process into "early" and "late" vision. The former roughly corresponds to the processing of information in the retina and the neural networks close to it, before the higher level data is transmitted to the brain, while the latter refers to the further unravelling of the information in the brain, where more "learned" facts about the world can be brought into play. The distinction also has a rough correspondence to the distinction between extracting low level information from the image, such as the presence, location and orientation of edges, boundaries, and perhaps objects ("early" vision), as compared to the use of this information to "understand" a scene and the relationships between the objects present in it.

Most of our computer methods for image analysis and measurement use algorithms related to "early" vision. There is another large and active field, for instance connected with research in robotics and artificial intelligence, that seeks to understand and describe scenes, but we will not be dealing with it here. Fortunately, most of the images that are important for analysis and measurement are not "general" in nature, but are obtained in highly controlled situations where much is known about the specimen and the viewing conditions.

The examples are generally taken from the field of microscopy, but we will see that the same techniques apply (with a few additions and restrictions) to astronomy, remote sensing (satellite photos), and so forth. In all of these cases, we generally know beforehand that the subjects are (for example) flat surfaces cut through a material, or projected images through a thin section of the sample, and that the image contrast is primarily produced by some particular interaction such as light scattering or absorption, secondary electron or X-ray production, and so forth. This greatly simplifies the interpretation of the image. It also permits some computer modelling to predict the images that should be obtained from particular structures and objects.

The reproducibility of computerized image analysis and measurement methods can be far better than that of a human observer because the algorithms overtly ignore much of the content of the image, and the sensors and discriminators respond only to the image itself. Humans are influenced by many other things (hunger, emotional response to stress, etc.) that clog up the neural pathways and "take our minds off" the job at hand. This causes us to miss things in images that would otherwise be obvious (just after an argument, I might drive through a stoplight because I didn't "see" it). Likewise, we respond to information in the image other than that which we need to see (I might also drive through the stoplight because I was busy watching the bikini-clad blonde on the corner).

{As another example, I might see the stoplight, but knowing that it was 2 a.m., that there were no headlights visible in either direction, and that I was in a hurry, I might go through the light anyway. That isn't computer vision, it's Artificial Intelligence (AI).}

The often observed result is that reproducibility tests on the same images show large variations between different persons, or the results from a single person at different times of the day or week. Computer–based measurement does not show this pattern, and the variations are more or less directly tied to simple statistical patterns of fluctuation, so that the errors can be predicted and in many cases controlled.

On the other hand, this reproducibility does not imply accuracy. The speedometer in my car always reads 55 at a particular speed (reproducibility), but that is of little value in arguing with the trooper who pulls me over and says his radar clocked me at 63 (accuracy, presumably). Some of the techniques we employ require calibration against some other external source, such as known standard specimens or mathematical simulation, to produce accurate results.

Implementation

It is easy to be distracted from the real purpose of image analysis and measurement by the hardware and software used for its implementation. There are computer-based systems in existence, some of them commercially available, that employ many widely different approaches to similar problems. Some of the more obvious differences are sequential vs. parallel processing, hardware vs. software calculations, and various computer languages.

These differences are all unimportant compared to the choice of appropriate algorithms to carry out the desired method. In principle, any result obtained by a massively parallel computer employing hardware array processors and programmed in LISP can also be obtained using a conventional sequential computer with only software calculations and using Basic. There may be a noticeable difference in the ease with which the programmer initially implemented the method or the convenience with which it can be modified, and there may be a significant difference in the speed with which it can be applied, but the result should be indistinguishable.

Serial computers (the classic "von Neumann" architecture) perform one operation at a time, albeit at the rate of a few million per second. Data values are fetched from memory to a central processor, combined with other values, and written back to memory. This produces a bottleneck that limits the number of calculations that can be performed per unit time. Particularly for images, in which we very often want to perform the same operation or series of operations for every point in the picture, or perhaps to perform the same measurement for every object, it is particularly attractive to find some way to bypass this bottleneck.

Parallel computer architectures are actively being developed which allow this. Generally, they employ a fairly large number of identical processors (often single chips, in this era of large scale integrated circuits), each with its own program memory and perhaps its own data memory, and some means of communicating with the other processors. Two particular arrangements have been especially used: the processor array and the cosmic cube (Figure 1-3). In both cases, each processor might, for example, be

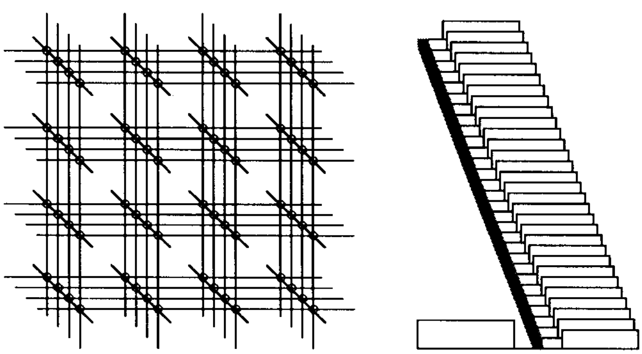

Figure 1-3: Two different parallel computer architectures.

given a portion of the image to work on. The image itself might either be written into the dedicated data memory of the processors, or each processor might also be able to access the main memory holding the image.

As the processors do their work simultaneously, they sometimes need results from the work of other processors (for instance to get information on neighboring points that lie in a different segment of the image belonging to another processor). This information is provided by communication between processors. In the cosmic cube architecture, each processor has a direct communication link with its neighbors. In the processor array case, there is another computer, nominally the boss who assigns the tasks and sends the data, but in actual practice more the mailman who carries messages back and forth. In either case, the total computation time is reduced nearly in proportion to the number of processors assigned to the task.

Assigning each processor a different portion of the image is not the only way that parallel computation can be organized, of course. In some cases it is more attractive to assign each object to a different processor, to classify it and measure its parameters and to determine inter-object comparisons, spacings, etc. from its neighbors. This method is especially attractive when object recognition is required, or when periodicities or other relationships between objects are to be found.

It is difficult to apply these parallel methods to general purpose computing, where tasks are generally quite varied and non-repetitive. But for image analysis, as for a few other problems such as some simulations, the application of parallelism is more direct and the results easier to achieve. For the particular case of image processing (to be more fully defined shortly), there are two other "parallel" cases that can be mentioned.

First is the array processor (distinct from the processor array). This is a special purpose arithmetic unit that works under the direction of the central processing unit (usually a conventional sequential computer) to carry out particular repetitive tasks at very high speed. It is often used for images, because the data can be sent to the array processor as a sequence of values (for instance, the brightnesses along lines in the image) to perform simple operations like addition, multiplication, subtraction, etc. and the results written back to memory. The specialized nature of the array processor and the low level of its operations allows it to be much faster than the main general purpose processor for this purpose. However, the array processor is rather inflexible and hard to program for any but the simplest operations. While it can speed up some image operations, it is inapplicable to many others.

Another rather new development is the use of so-called neural nets within the image sensor itself. This corresponds in a very simplified way to the operation of the human visual system (which we will encounter in the final chapter), in which there are many additional cells connected to the outputs of each few retinal receptors to compare and combine their responses to light in ways that extract specific information (such as the location, orientation and motion of edges), so that only the reduced data is sent on to the brain. This allows the human to respond very quickly to particular types of stimuli, and using electrical devices to form the same types of connections in the image sensor can also produce simple computerized devices that recognize and respond to certain features.

Both of these types of devices are rather specialized for our needs in general image analysis and measurement, although it is quite possible that applications research using a flexible general purpose system on a particular problem will result in a design that successfully applies these tools to individual situations.