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IMAGE PROCESSING IN BIOLOGICAL SCIENCE

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to be interested in and funded for biomedical image processing are assembled here. It is my further, but not pejorative observation, that about half these people are interested in chromosome analysis. However, there are many other problems of medical importance.

My final point is that I do not have a sense that the technology, the hardware, is really lacking at this juncture to perform many of the analyses that I have described. The developments in technology are exciting. I can think of some things I would like to do that I understand are not feasible, and we have heard some things that really take sophisticated development and application of engineering. However, much can be done now with existing hardware. Most of the work that I have been doing has been done with a $60 slide projector and a $60,000 PDP-7 computer. That is not the final answer to all the problems, but one can make a beginning that way.

Lederberg: Dr. Ervin has anticipated many of the things I was going to say. Dr. Vastola's presentation struck a responsive chord on my part. Having most of my roots in biochemical genetics, I am very enthusiastic about developmental anatomy as an answer to many questions about the central nervous system. In our analysis we must realize that the brain is very complex and in our present state of knowledge we know very little about its structure.

It is unnecessary to pose the ultimate difficulty as Dr. Glaser did, i.e., that to record the distribution of all the elements in a brain would tax our image storage capability. Beginning to know something about the brain does not require knowing everything there is to know about it. Elementary advances in our knowledge of interconnecting pathways within the brain would be worthwhile; any quantitative information would be helpful. If cell counts can be obtained, regardless of the types of cells, there will be some discrepancy between our naive expectations as to what those numbers are thought to be and what they really are. At this point our investigations leave the realm of speculation and begin to assume the proportions of a true science.

An organ as complex as the brain will show deviations in its structure as a result of developmental variations. With a single nucleotide, a difference in the DNA or a small developmental perturbation is likely to give rise to major structural variations. Of course, what should astonish us is that we are able to communicate with one another with brains that must have great differences in detail and internal structure from the very complexity of the way in which they have developed. Most of the perturbations that we find in neurological performance will be found to have some anatomical variation behind them.

These variations may be too subtle for us ever to discover. They may overlap with the range of what we call normal variation to such an extent that we may never be able to perceive them. However, I do not see how we can offer anything but maximum encouragement to research approaches

which promise some hope of contributing to our knowledge of the structure of the brain.

On the other hand, I wonder if we would not be putting too much reliance on the very specialized technology proposed by Dr. Vastola. Concerning the very particular question of how many neurons there are in the brain, I would not use cell counting *per se* as the most likely approach. I certainly would not take samples of sections as a means of doing that. Instead, estimates of total brain DNA, together with measurements which can be done on a cytochemical basis of DNA turnover in different categories of cells, or estimates of the ratio of neurons to other cell types, could give rise to a very much quicker count of brain neurons. Zamenhof is among the very few employing this very direct approach (5).

My general reaction to this symposium echoes Dr. Ervin's. This has been a conference on the uses of information processing, and I had something of the impression that I had walked into the stone chippers' convention where Praxiteles, Michelangelo, Epstein and Moore were arguing about good ways of using a hammer and chisel to hit a piece of marble.

We do know that magnificent results are possible. We can even see the end product. However, for me to try to evaluate the relative virtue of one work of art compared to another would be rather arrogant. No one would take on the burden of telling me as a scientist what I am going to be interested in pursuing. No one can give me my research passions, and really no one knows what is going to be important, three, four, or five years or even decades ahead in terms of the individual research paths to be pursued. In an area that is so full of craftsmanship, how really effective can our communication with one another be? This is perhaps the most disturbing residual of this conference.

We have heard some rather general ideas about how some other scientist has approached a particular problem. But how useful are these general ideas in such a complex field when we go back to our own laboratories and attack some slightly different problem?

Behind this complaint is that Tower of Babel of computer programming languages. I do not want to put undue stress on the programming problem, but surely we need to be able to exchange subroutines with one another over a wire in order to try out a new approach with minimum initial effort. At the present time it is far too cumbersome for us to overcome the barrier presented by differences in computers and computer languages.

It is our responsibility to practice self-discipline, so that when we make scientific advances our methodology can be easily communicated to our colleagues. Sometimes this may be more painful because of the necessity of documentation. However, it is the only way we are going to avoid redundancy of effort in working on the same problem from virtually the same standpoint, no one really being able to tell whether one effort is better or worse than the other.

Dr. Ervin made a point I would echo. I was sorry, Dr. Macy, that you gave up so soon in finding out what your radiologist was doing. The only way one can discover how some obscure hidden mental process in in fact accomplished is to insist that it be programmed. No other simulation will do it quite as well.

Surely, there are some radiologists who will be willing to make one more iteration. If skin thickening was not the cue, some other cue in the rest of the section is making it seem that the skin is thicker.

Generally speaking, we should think first about sponsoring applications anywhere that photographs or image representations are now used in medicine. The research applications can be classified as extensive and intensive. Extensive applications try to reduce the work involved in looking at a great many different photographs and picking out the ones that are interesting by whatever cues are available. In this case, a reasonably automated system would be desirable. However, I think we may not be doing ourselves full service by trying to get the entire process completely mechanized too quickly. For example, I was simply delighted when I finally realized why Dr. Wald used a laser beam in his cell finder. That sort of specific technology may end up being very much more useful to the actual and immediate support of work in the biological laboratory than the very much larger effort that has gone into trying to build a completely automated processing system.

There are other forms of image processing that should be a part of the laboratory routine. Pictures are used in biology and medicine for obtaining spectra. One may have an absorption spectrum, giving great detail. Even denser with information would be the photographic rendition of a mass spectrum from a mass spectrometer, possibly the only way that all the information that the mass spectrometer is able to offer can be extracted efficiently. The next problem, then, is one of reading this information.

It would be very easy to do this on a much larger scale if we had smart densitometers: something that looks at a set of blackenings on a film. Instead of spending a uniform amount of time at each micron of the negative, which is not related to the problem we are trying to solve, it analyzes the information on that film in relation to what it is we are trying to learn.

This will vary in different circumstances. In analyzing a mass spectrum, some of the peaks have to be defined with much higher precision than others; the location of the peak maximum is often more important than a great deal of detail about the wings.

This capability does not represent an enormous challenge to image processing technology, but if the technique is routinely available in the laboratory, not only would a great deal of biological work be speeded up, data would be much more closely examined than they are now.

Another candidate for automatic image processing is electron microscopy. An electron micrograph, whether of a section, a shadowgraph, or a particle,

contains a great deal of information about some construct in nature that we are trying to model. This is a painful process for human beings. Generally it is not done at all except by the application of intuition. Any electron micrograph that was worth taking should be processed in such a way that some equivalent three-dimensional representation results from the analysis.

Most of us have learned the rules for translating what is seen on an electron micrograph into its three-dimensional equivalent, but we do not always apply these rules successfully. We sometimes fail to apply all of the quantitative criteria to evaluate the material we have. When we obtain a virus suspension that has been spread out over an electron micrograph width, we are really trying to build a model of a three-dimensional shape that will account for all of the micrographs that were made on a given spread.

It would be desirable to have a routine facility whereby individuals who are interested in virology could get their electron micrographs processed so that they would achieve the best possible approximation of what the actual shape of an individual virus particle has to be to account for the 30 or 40 representations of it appearing on a particular film.

Similarly, for histological sections—and particularly for serial sections—we are interested in some three-dimensional percept of what the shape of an object actually was. We go to a great deal of effort, some of it misapplied, to reconstruct the shape from a single section. Actually, we hardly know how to do it from serial sections by manual operation. It is not that it is fundamentally very difficult; it is simply that it is extremely tedious. These are the kinds of operations in which the computer can be used very, very effectively.

The facility I have in mind should be a routine facility where researchers doing photomicrographic work could go, so that they would not have to be engineers or photo experts in order to obtain the benefit of automatic interpretation of the data they are producing.

The recognition of faces was mentioned during the conference. I think the genetics of somatotype, of body constitution, for which data could be collected on a very large scale, might prove to be very useful for understanding human polymorphism. The same thing that can be said about human faces can be said about plant leaves or about fly bristles. There might even be a resurgence of interest in Drosophila genetics if the bristles on their backs could be counted by computer instead of by hand.

A relatively small advance in technology would make it possible to squash cells on an individual basis. If we did not like the squash because two chromosomes had overlapped, we would tap it again. The two chromosomes would be pulled apart until they could be examined separately and a good analysis of that particular plate became possible.

Some of the image processing problems that we are trying to alleviate by very large-scale screening could be done very much more directly if hardware were introduced early in the processing. We have to reorient our think-

ing about what kinds of images we should be looking at when we do have an automatic processing tool. This conference has vividly reminded me of some work that has lain fallow for a long time. In 1949, Schultz & St. Lawrence (4) published a note entitled, "A Cytological Basis for a Map of the Nucleolar Chromosome in Man", a chromomere analysis of spermatogonial prometaphases (see Figure 128). This is a rough analog of the salivary band analysis that has been so successful in uncovering some details of chromosome structure in fruit flies. Both are laborious procedures if they have to be done by hand.

We hope to unravel other forms of human chromosomes which have much richer structural detail, but whose analysis by conventional techniques would be too clumsy. I would remind you that we know a great deal about the fine structure of Drosophila chromosomes because stages have been detected which exhibit much fine structure.

Figure 129 is a UV-micrograph of salivary band chromosomes which shows the rich detail of information that is available in chromosome

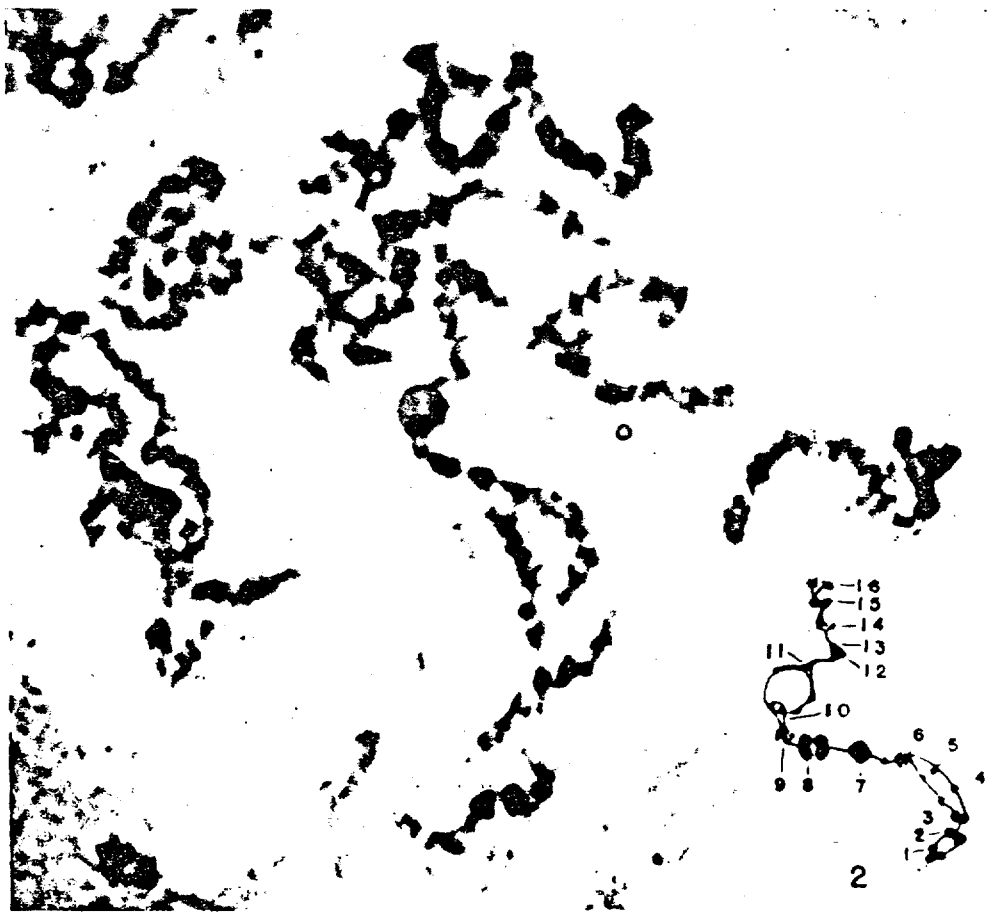

Figure 128. The human nucleolar chromosome. (From Schultz & St. Lawrence, 4.)

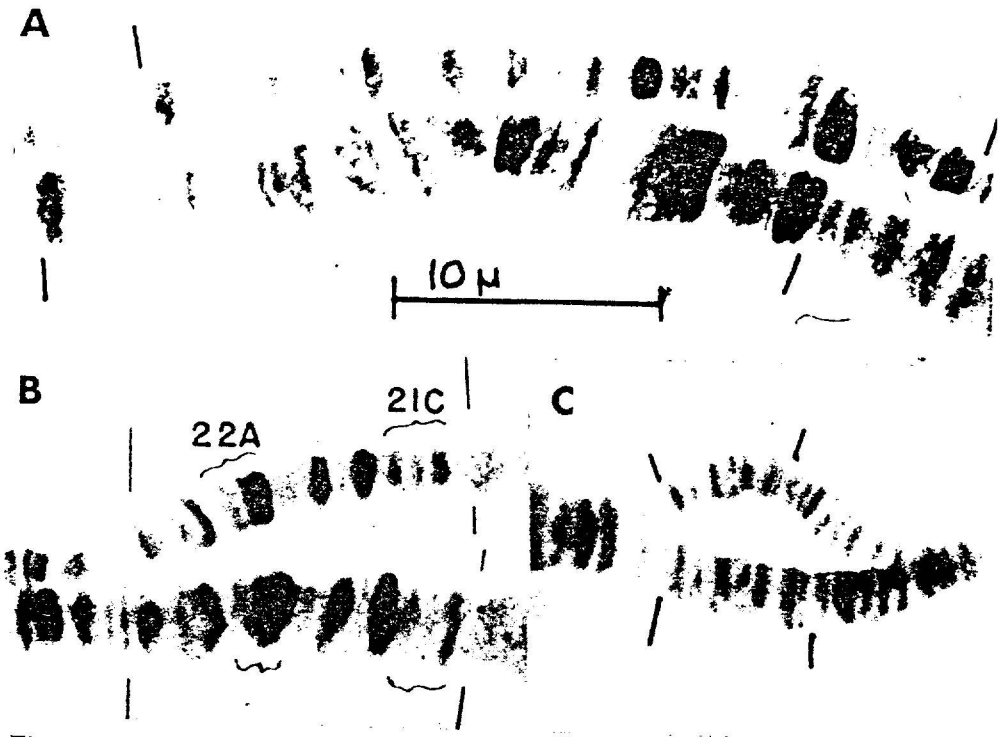

Figure 129. Ultraviolet absorption of haploid and diploid chromosomes. (From Rudkin, Aronson, Hungerford & Schultz, 3.)

structures. The analysis of these structures involves consideration of topological distortions of a pattern of very simple order. Bands are moved apart or folded together with the sequencing of light and heavy bands. The transformations of the lists of bands are really not that complicated to enumerate. There is a very large amount of redundant information available for matching segments. We may begin to understand something about human chromosome structure when we can automatically manipulate the chromosomes which have that kind of information to offer. This area of endeavor has been totally overlooked because of technical difficulties that in large part automatic processing would overcome.

This mapping problem also applies to DNA molecules (see Figure 130). Kaiser & Inman (1) have measured the length of DNA molecules taken from a bacteriophage. They spent hours making tracings of shadow electron micrographs of these DNA molecules on large sheets of paper and then measuring DNA length manually with a planometer.

It would be very desirable to measure DNA length with a precision of one per cent error. Because of mutations, however, we expect to have discrepancies in total DNA information, in the total length of DNA, and we would like to make more precise correlations of their genetic behavior. One might call this determining their chemical composition by morphology.

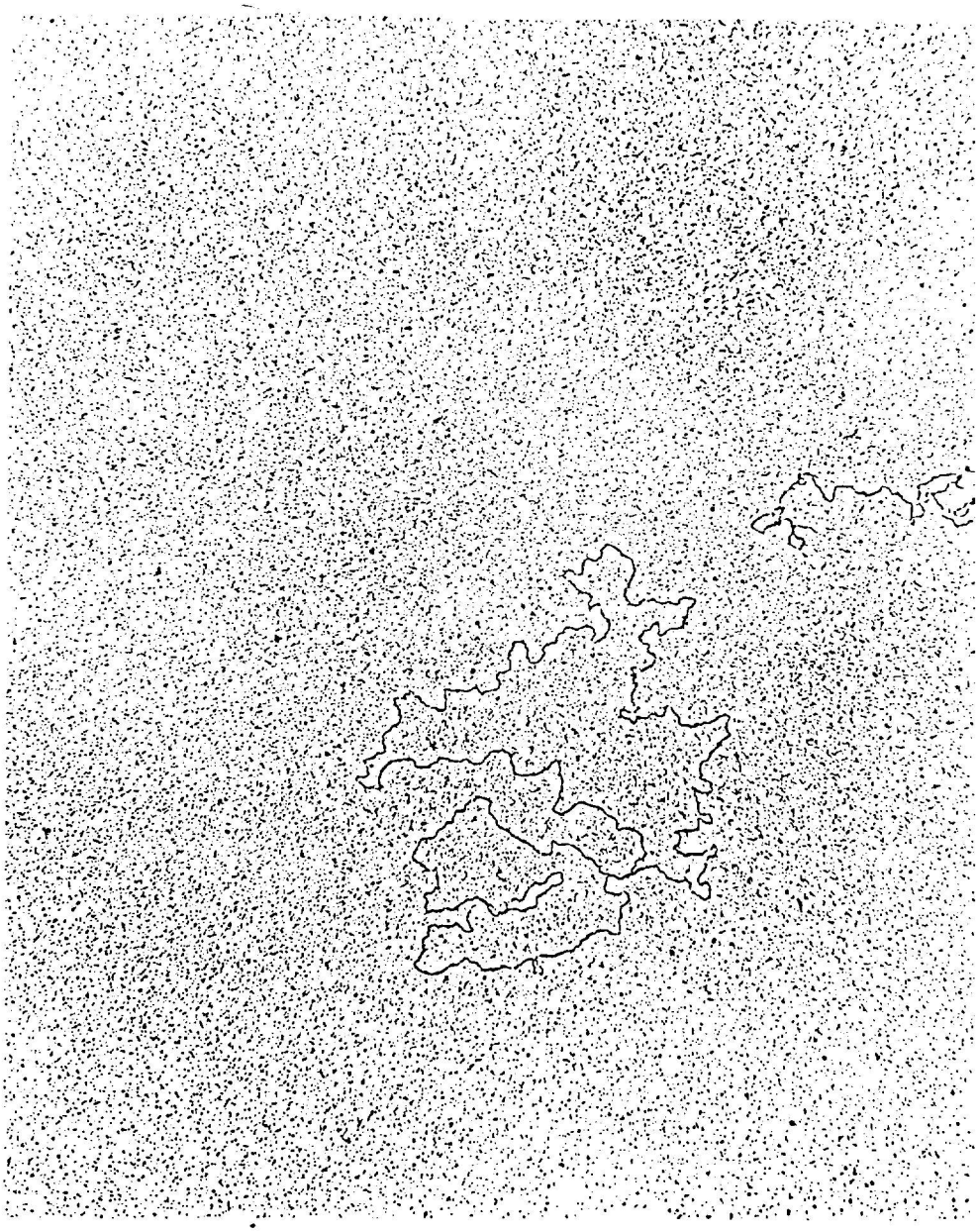

Figure 130. Electron micrograph of a λ DNA molecule taken from a bacteriophage. (From Kaiser & Inman, 1.)

Dr. Minsky has wisely inquired if the "kinks" in the DNA molecule have any significance. The answer I gave him was, "I wish I knew." That reply poses some of the same problems I mentioned with respect to band analysis of salivary chromosomes. We are not going to find the answer without some assistance from image processing technology. It is essential to be able

to achieve some elementary topological reformation of these representations in order to look for similarities from one molecule to another. Of course, we do not have to rely on analysis of the "kinks" exclusively. We also could do differential melting.

It should be true that some regions of the DNA molecule have differences in local base composition, different enough from other regions that they could be melted out. This melting out could be detected by their appearance, and some fairly elementary image processing technique might make it possible for the biochemist to rekindle his interest in this kind of analysis, whereas formerly the sheer tedium of manually comparing the chromosome sets deterred him from any further karyotyping.

In connection with cell handling from examination, I suspect that one of the really creative applications of image processing is going to be in the recognition of cell types for the purposes of cell separation. There are going to be a great many applications where it will be very desirable to take a patient's cells, sort them, and give back his normal leukocytes, throwing away his cancer cells, for example, if he has leukemia. There is no way that this can be done fast enough without the application of some highly sophisticated image processing technique.

My last plea is that, as soon as possible, we take the responsibility away from the hardware developers and give it to the researchers in the laboratories, because they are the ones who are going to find the useful applications for the hardware. If we had kept the microscope in the hands of the opticists, we would never have discovered its creative applications.

For Hardware

Clark: Dr. Minsky's and my task of summation is very much easier than Dr. Ervin's and Dr. Lederberg's, because so little was said about "hardware" in the course of this conference, with the notable exception of Dr. McCormick's very imaginative Illiac III presentation.

Even though I am wearing my hardware designer's hat, I find this actually very refreshing, because (and here I agree with Dr. Lederberg) I think that the right approach to the solution of our image processing problems is not to be found in terms of particular hardware features, but rather in the imaginative use of apparatus which is now available or foreseeable.

As I look out on your smiling faces, it grieves me to think that almost to a man, for various reasons, you will not be using the Illiac III for your work in image processing. This is probably all right. Powerful as the Illiac III will be (and perhaps a conservative guess is that when that machine is "up", it will double the world's image processing capability), it will probably best be suited to the very large problems. I think you will agree that there are many small problems that have to be solved as well.

The Illiac III is a very large undertaking and is now in its third year, with another year or so to go before it is fully operational. In the meantime, the

optical scan (which is similar to using many vidicons.) Vidicons cost only $100 for one with 500-line resolution, but I suppose they cost much more for 1500-line resolution.

McCormick: That is developmental cost. It is the same price as the 500-line vidicon.

Minsky: How much does the deflection circuit for a 1000-line instrument cost?

McCormick: You can buy it off the shelf with no intermediary.

Minsky: D-to-A converters of more than ten bits rise very sharply in price.

McCormick: There is no D-to-A converter in it. It is a straight TV unit.

Minsky: There are disadvantages in that. You have to have a scan converter tube, a storage device, or a large memory in your computer. There are many trade-offs and it may be that the economical instrument is one with low resolution but with a good mechanical stage.

Preston: One more comment on the input device on the scanner. I think the reason that people build their own instruments is not so much that they are not commercially available to a certain extent, but that the requirements for one person's scanner are so different from the next person's. The scanner needed to handle the 10 inch X-ray films is not necessarily of very much use for microscope slides, whereas there is more and more standardization in computer componentry.

Minsky: I do not think so. You should be able to have a sort of eye which can be put on the microscope or centered on an X-ray image like a camera with interchangeable lenses. I simply do not see any large difference in the requirements for any of these tasks.

Preston: An input device which is a factotum for handling large X-ray films all the way down to the most micro of the microscope slide formats would be a very elaborate and expensive device.

Minsky: Only if you want to buy all the lenses with it.

Lederberg: I would like to make a final remark about a trivial application. Several of you have commented that it would be desirable to know which nerves were connected to which when a Golgi preparation is made. Try to figure out what is connected to what some time with an actual microscope specimen. It requires a great deal of manipulative finesse. Most of the resolution is achieved with a very shallow depth of focus, and one usually decides whether two fibers are derived from the same original one, or if in their crossing are joined to one another, by a rather elaborate scan around the region of intersection. This is a time-consuming operation for the manual observer, not because the visual information is so difficult to process, but because there is a problem of manual dexterity in controlling the dual positioning micrometers. For some of the more elementary operations involved in determining which cells in the cerebral cortex are connected to which other ones, it would be highly desirable to have some kind of automatic me-

chanical control of the scanning procedure, with some degree of manual intervention also possible. Image processing alone is not going to solve this problem.

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