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QUANTITATIVE HISTOPATHOLOGY
DISCOURSE

SCOPE AND CONCEPTS OF QUANTITATIVE HISTOPATHOLOGY

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ABSTRACT

Quantitative histopathology uses quantitative microscopy of tissues and cells for medical decisions on diagnosis and grading. The behavior of a method in the light of the diagnostic indices (sensitivity, specificity, efficiency, predictive value) is more important for the patient than the technical characteristics of the method. The variation in the methodology, and the biological relevance, determine the value of the above indices. In grading of neoplasms the performance of the tests can be estimated with the efficiency of grading after repeat experiments. Morphometric methods in diagnostic quantitative histopathology are not primarily aiming at stereologically correct interpretation, but also stereological applications are increasing, especially in research. The present expansion on computer and program development, and development of microscopic instrumentation, greatly support morphometric quantitative histopathology. Image analysis is based on the digitized microscopic image, and is also greatly dependent on the development in programs. Densitometric measurements e.g. DNA cytometry, or quantitation of immunohistochemical reactions, are typical applications. Completely automatic solutions are rare, and difficult to apply because of the great demands on laboratory performance. Flow cytometry is much used in association with histopathology because nuclei digested from paraffin embedded tissue can be studied with this method. Recent developments in molecular pathology and interphase cytogenetics have brought together quantitative histopathology and DNA hybridisation histochemistry. The prospects are that this development continues soon allowing diagnostic detection of genomic changes through microscopy.

Key words: Quantitative pathology, stereology, morphometry, image analysis, densitometry, cytometry, cytogenetics.

INTRODUCTION TO THE PRINCIPLES OF DIAGNOSTICS

Galen and Gambino (1975) published an important book which is applicable in any context in which one uses a test to decide on the presence or absence of a condition. They were not the first

in the field, but nicely collected the principles and added considerations of their own to what was known before. The principles are generally accepted in clinical chemistry and internal medicine, and applied in clinical cytology, but much less interest has been paid on them in other fields of medicine, e.g. in diagnostic histopathology. When a test is done, the result either suggests that the condition is present, or that it is absent, or the test result is inconclusive. On the other hand, the suggestion is either true or false. Good tests give a large fraction of true results, bad tests are those which give false results more often than other tests. Starting from this it is possible to create a number of indices which characterize a test. These include sensitivity, specificity, efficiency, and predictive value of a test result. These indices are fractions, and are usually expressed as percentages.

Sensitivity tells us the fraction of tests which are truly positive (number of true positive tests = TP) of all tests done in a group of patients who have the condition under investigation. So sensitivity is

$$\frac{TP}{TP+FN} , \qquad (1)$$

in which FN is the number of false negative test results. Note that TP+FN is the total number of patients with the condition.

Specificity tells us the fraction of true negative test results among patients who do not have the condition under study. So specificity is

$$\frac{TN}{TN+FP} , \qquad (2)$$

in which TN is the number of true negative test results, and FP the number of false positive test results among persons who do **not** have the condition under study. Note that TN+FP is the total number of persons without the condition under study.

Efficiency relates to a group of persons, among which there are patients with the condition, and persons who do not have the condition. Efficiency is the fraction of tests which characterizes the patients correctly. So efficiency is

$$\frac{TN+TP}{TN+TP+FN+FP} . \qquad (3)$$

Predictive value of a negative result is the fraction of true negative results of all negative results, i.e.

$$\frac{TN}{TN+FN} . \qquad (4)$$

The predictive value of a negative result evaluates the negative test results. If 95% of negative test results are true negatives, we know that among the negative results there is a fraction of 5% of tests which are false negative.

Predictive value of a positive result is the fraction of true positive results of all positive result, i.e.

$$\frac{TP}{TP+FP} . \qquad (5)$$

The indices above apply to any type of test system, or measurement method which is applied to detect a condition, or to characterize the state of disease. From the point of view of the patient the above indices are much more important than the type of method (histological, chemical) which is used. For the patient the type of method matters only to the degree the method affects the above indices. The indices are especially valuable because the same tests are not used all over the world. By comparing the tests with the help of the indices it is possible to find the best test within the capacity of the diagnostic organization (i.e. hospital, laboratory, health station in each country). The selected test should be able to perform the diagnostic evaluation with acceptable level of specificity, sensitivity, and efficiency.

METHODS OF QUANTITATIVE HISTOPATHOLOGY

Traditional histopathology is generally considered as qualitative in nature. However, lots of quantitation takes place also in routine diagnostics. This quantitation is subjective, e.g. in estimating the grade of carcinomas, the pathologist evaluates numerous features from the histological section. There are more obvious quantitative methods (Baak 1991); DNA cytometry, nuclear morphometry, and stereology. Subjective quantitation is a rather crude method of measurement, but not worthless - here the variation present must be reflected against the type of use the method is put in each area of application. Through small changes these methods can be made to approach quantitation in the true sense of the word (cf. Collan 1991). The medical value of these latter methods, however, is also dependent on the value of the diagnostic indices explained above, not primarily on the theoretical characteristics of the measurement method (cf. Collan 1989a).

VARIATION IN HISTOPATHOLOGY AND QUANTITATIVE HISTOPATHOLOGY

The distinction between cancer and benign lesions is generally made in a very reproducible fashion in histopathology (Linden et al. 1960). There are problem areas though. The diagnostic environment in these areas has developed so that the pathologist already after characterizing the lesion knows that histopathology will not be a good guideline for prognosis or clinical behavior. Here we are usually dealing with neoplasms which are benign in the majority of cases, but can occasionally behave as malignant tumors. One example is the granulosa cell tumor of the ovary (Scully 1979). Problems of distinction arise in association with endocrinological neoplasms in general, e.g. the distinction between a follicular adenoma and a follicular carcinoma of the thyroid gland may be problematic (Saxen et al. 1978). But variation is met more generally in grading, i.e. in placing the neoplasms or already diagnosed cancers into histological atypicality categories, which are related to prognosis (Ringsted et al. 1978). Grading can also be based on truly quantitative methods, including image and flow cytometry (Collan et al. 1992) or methods related to molecular pathology (Collan and Kuopio

1992). The value of the applied grading method depends on the variation present in the grading system (Collan et al. 1987). The fraction of correctly graded cases, as estimated on the basis of variation in the grading system, has been termed **grading efficiency** (Collan 1989b). Grading efficiency allows the estimation of the suitability of the method for grading, especially if the biological relevance of the measured variable is known. Such estimation can be made before the follow up which is necessary for estimation of the prognostic value of the method. The prognostic value as determined with multivariate analysis depends on the biological relevance and method variation (Cox 1972, Cox and Snell 1981, Cox and Oakes 1984).
There is evidence that methods based on true measurements are more reproducible than subjective methods in evalution of the same histological characteristics (Kosma et al. 1984, Kosma et al. 1985a, Kosma et al. 1985b, Kosma et al. 1987). This is why quantitative histopathology is intensively studied today. In these studies special weight is put on the correct use of statistics - in quantitative pathology some understanding of statistics is vital (James 1989).

STEREOLOGY

Stereology has a place in quantitative histopathology and for a long time there has been an interest among pathologists on stereology. Norio Suwa from Japan wrote a comprehensive book on stereology in Japanese, and he is a pathologist (Suwa 1977). Pathologists have edited books on stereology and morphometry (Aherne and Dunnill 1982, Collan and Romppanen 1982, Oberholzer 1983, Collan et al. 1984, Baak 1991). Generally the research applying the methods of stereology (Underwood 1970, Weibel 1979) has not been diagnostic in nature, but with new methods (Gundersen 1986, Gundersen et al. 1988a, Gundersen et al. 1988b, Weibel 1989, Cruz-Orive and Weibel 1990) lots of hope has been put in potentially valuable diagnostic applications. The potential applications associated with nuclear volume measurements were recently reviewed by Sörensen (1992) and some are also mentioned in the article by Whimster (in this issue). However, most of the practice and research on quantitative pathology is done by methods which are not always strictly stereological (Baak 1991). Some of the general principles of stereology are outlined in the article by Kalisnik (in this issue), and the use of the disector in neuropathology is exposed in the article by Collan et al. (in this issue). In research stereology is extremely informative, and 3-dimensional histopathology can be most helpful in making pathogenetic mechanisms clear and describing the changes reliably (Takahashi et al., in this issue).
The new stereological methods are new in the sense that attitudes to sampling strategies have changed. The general principle of stereologically unbiased isotropic sampling is presented in Fig. 1. The simple rule is that the cutting of the sample need be randomized around all three axes of the 3-dimensional space. When we speak about cutting planes it is enough to randomize within 0-180 degrees because the direction of the plane within the first

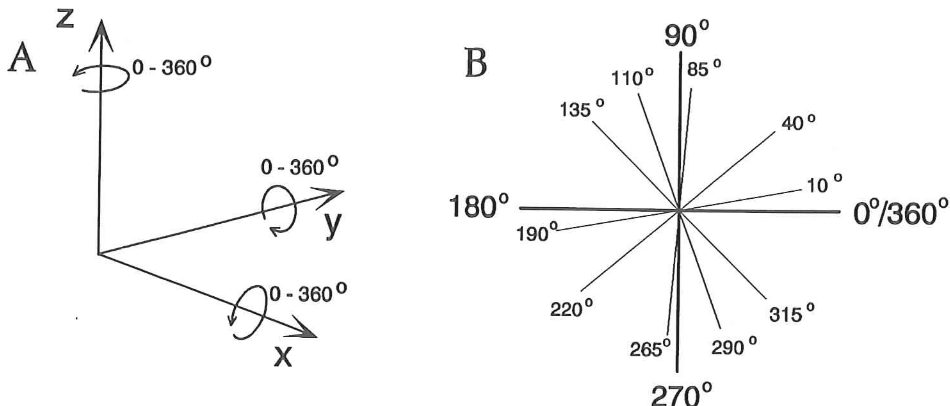


Fig.1. **A.** Optimal sampling for stereological measurements is based on randomization of the orientation of the cutting plane in respect to all three axes of the 3-dimensional space. Such sampling is unbiased, not dependent on the orientation of the structures studied. However, single samples do not give stereologically valid data, especially in clearly anisotropic tissues. The studied data need be based on several samples, each randomized in respect to the orientation of the cutting plane. **B.** Because the orientation within 0-180 degrees of the circle defines the orientation within 180-360 degrees, the randomization within the first 180 degrees is enough.

180 degrees also defines the direction within the next 180-360 degrees. This principle is applied in the orientator by Mattfeldt (1990). The vertical sections by Baddeley et al. (1986) is another solution to isotropic sampling. One has to realise that under these circumstances one sample cannot be considered enough for sampling but that valuable results can be got after generation of several isotropically selected section planes. Measurements should be made on all of these planes. This is far from the everyday practice in routine histopathology but has found lots of applications in research.
The disector (Sterio 1984), in principle based on the formula of Ebbeson and Tang (Eränkö 1955, Ebbeson and Tang 1965, Weibel 1979), is another method which no doubt has come to stay. It is reasonably fast in counting the total number of cells e.g. in brain nuclei. Because of its unbiased nature (the method is independent on the shape and size characteristics of the bodies counted) it is to be recommended for the purpose. Faster applications would need determination of the section thickness, which is problematic.

Stereology is today also practiced on images on the screen of television monitors. Suitable video-overlay options are available in many morphometry packages on the market (Fig. 2). These allow different types of grids to be selected, according to the needs of the investigator and the problem at hand.

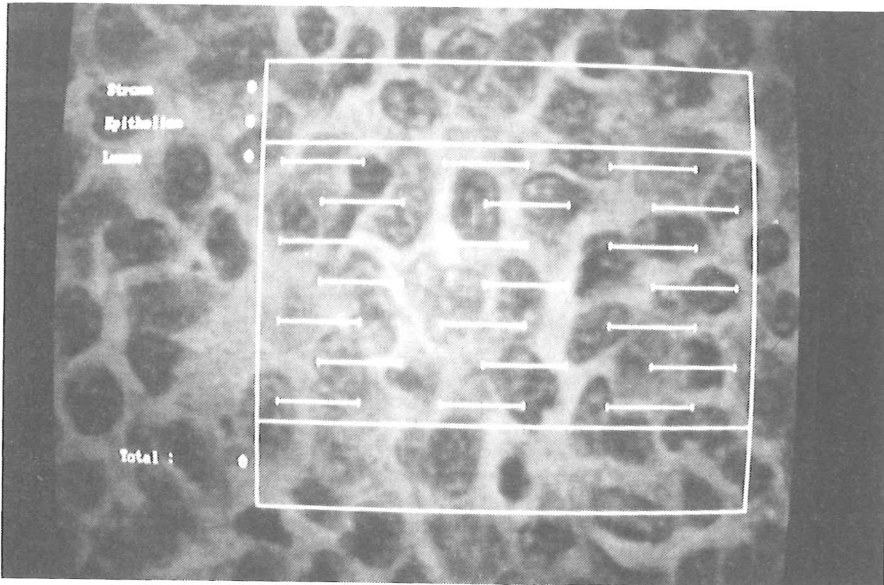


Fig. 2. Video-overlay on a television screen for collecting stereologically valid data. Different kinds of grids are available in different computer programs. For pointcounting or intersection counting separate channels are available. In optimal solutions different channels can be named and the keyboard used as the counter for each of the channels separately. The counted points are automatically totalled (lower left). The overlay shows distortion associated with optics, camera and monitor electronics, and computer hardware. In optimal solutions the distortion of the image and the overlay is absent, or the microscope image and the overlay are distorted in identical fashion.

MORPHOMETRY

Morphometry is a very general term, but usually used in histopathology to describe measurements made from 2-dimensional planar sections. Of course, the measurements done for stereology are also made from such sections. The methods can be called stereological if calculations are made to transform the measurements relevant to the 3-dimensional environment. Today instrumentation has much developed and various types of workstations are used to collect morphometric information. E.g. in collecting data from nuclei (Whimster, in this issue), nuclei can be outlined on the image screen by using a mouse connected to the workstation (Fig. 3). This outlining is not always easy, partly because the image is not as clear on the screen as it is in the microscope. One solution to the problem is the idea which the first author got in 1985 - the video overlay microscope.

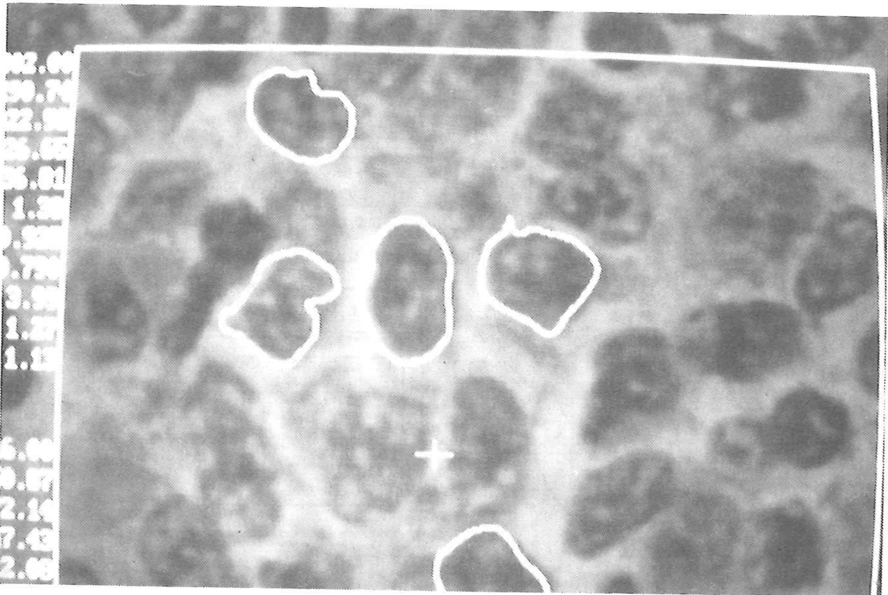


Fig. 3. Outlining of profiles on the television screen. The cursor is shown as a small cross, and can be operated by moving a mouse and pressing its buttons. The outlining is not always perfect (see the outlined nucleus right and up from the center of the figure), and it is important that measurement results can be deleted if errors occur. On the left, part of the measurement results are shown. The latter are immediately available after outlining each nucleus. The data from several nuclei are stored in computer memory. Such arrangement makes it possible to observe the data as histograms while the measurements are being made, and print the data with statistically relevant calculations after stopping the measurement.

There are numerous practical solutions to this principle, and Fig. 4 shows the most straightforward solution. Any morphometry programs which can be operated with a mouse and on black background of the screen, without the microscope image on the screen, are suitable. In the microscope the menu is shown through the side tube as an overlay on the microscope image. Movements of the mouse as they outline the nuclei, or draw other figures on the screen, are also shown on the overlay. In this solution the sharp image of the microscope, and the potential to focus the microscope on different levels of the section can be efficiently combined with morphometric measurements. The first author brought the principle to the attention of Dr. J.S.P. Ploem in 1986 in London. This principle is now applied in the Leitz Microvid-unit, and the HOME-project uses this principle in a microscope by Zeiss (v. Hagen 1991, Brugal et al. 1992). In the latter applications the video screen is built in or firmly combined with the microscope which makes the system compact and

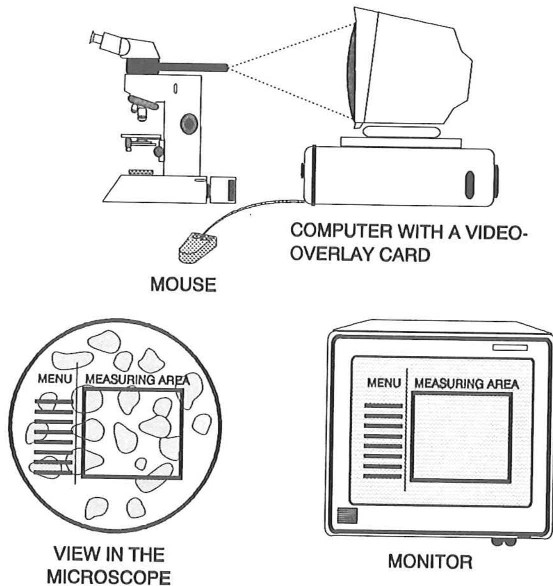


Fig. 4. Video overlay microscope is a microscope in which a video overlay is optically reflected on the microscope image. This can be done with a side tube from a computer monitor, or from a screen built in the microscope. It is essential that the microscope image is not seen on the computer screen, but only the overlay is shown. This solution allows the user to make measurements on the optical microscopic image with the mouse operating the morphometric computer program.

easy to handle. The practical performance of the compact system is also dependent on the programs written for the morphometry system. Also here the user-friendliness of the program determines the value of the system for the microscopist. It is not difficult to compare the available programs in this mode because anybody having a working computer program can create his or her own video-overlay microscope. This kind of solution is good when accurate outlining is desirable, when sections are thick, when overlaps occur. The easiest solution can be built with a side tube, computerized morphometry program, and a video screen (Fig. 5). For outlining of nuclei 100x objective is generally needed, because the nuclei to be outlined have to be large enough for the measuring system. Microscopes with an adjustable middle lens (zoom) might be helpful.
The morphometric tests are optimally based on measurements which show constancy within the sample, variation between samples, and are biologically significant e.g. in terms of presence or absence of disease, survival, response to therapy etc. A good example is the short diameter of the profile of a sectioned cylinder (Fig. 6), which is exemplified in the cut through a muscle fibre. The short axis is usually measured as the longest axis perpendicular to the long axis of the profile. Theoretically speaking this

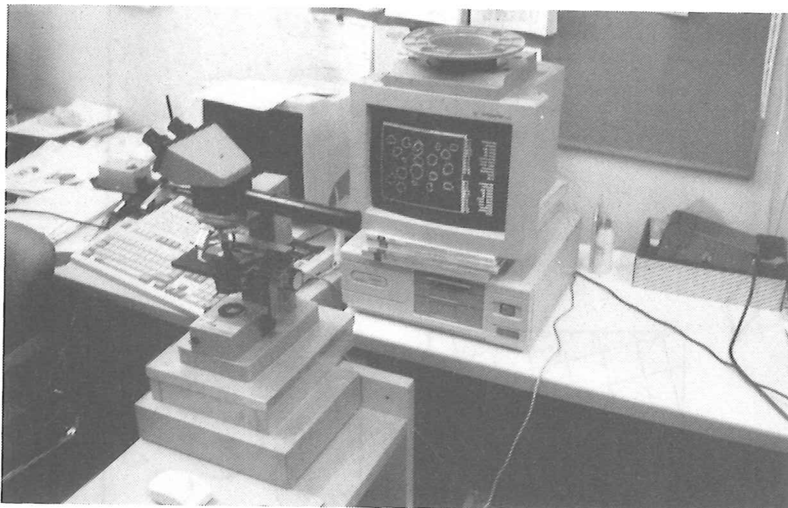


Fig. 5. A simple solution for video overlay microscopy. To make the movements of the cursor to match with the movements of the mouse in the XY-plane it is necessary to have the screen in a defined orientation. In the case shown such orientation was found when the monitor was turned upside down. If the reader tries this trick he should remember that the monitors are not necessarily built to function in abnormal positions.

variable is independent of the direction of the plane of sectioning, and is biologically meaningful (thin fibres: atrophy; thick fibres: hypertrophy). No wonder the quantitative side of the classification system by Dubowitz and Brooke (1973) is very much based on the short diameter of the muscle fibres.

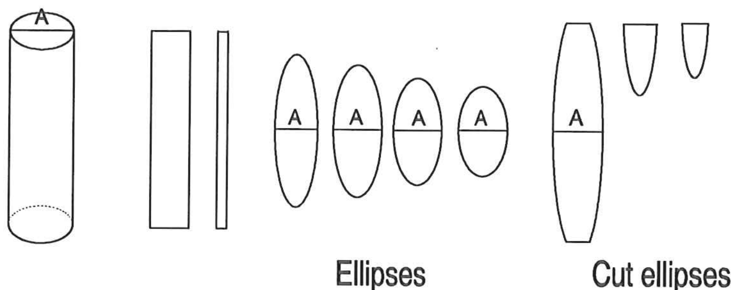


Fig. 6. Cuts from a cylinder. There are different types of cut. In the elliptical cuts the longest axis perpendicular to the long axis of the ellipse is called **the short axis**. The latter does not change with the orientation of the cut. Striated muscle fibres are cylindrical, and the measurement of the short axis creates a working diagnostic morphometry system for muscle fibers.

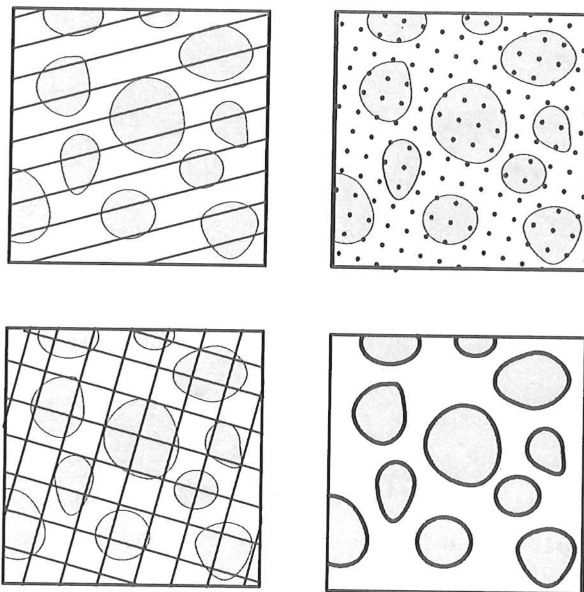
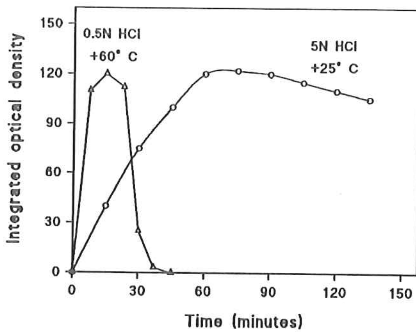


Fig. 7. Various ways of collecting information from a microscope image. Above and below left grids are superimposed on the image. Points falling on structures and intersections of image lines with the grid lines are counted. This approach is often used for collecting stereological data. Below righ is the morphometry system which applies outlining of the structures. In the latter, shape description, and length of line can be estimated more accurately with a smaller effort. Outlining also automatically counts the outlined structures. On the other hand the grids are generally applicable and need less instrumentation. Decision between the methods available need be made on the basis of the question under investigation. For the diagnostic purposes both approaches can be used, and the method selection should be based on the economy of effort and the performance of the system as evaluated by the diagnostic indices.

The measurements done by outlining with morphometry instrumentation and the measurements done with grids when collecting stereological information are not necessarily comparable. The outlining will be able to generate numerous planar variables, and be helpful also for collecting stereological information. The grids are not as good as outlining in shape features, and in line length measurements. For generation of values for many variables several counts are usually needed from the same sample. But grids are certainly more generally applicable, and need less instrumentation than the modern outlining systems (Fig. 7).
Numerous applications of morphometry are described in the present issue of Acta Stereologica. The paper by Whimster concentrates on nuclear measurements. Oberholzer et al. study the shape factors, Sisti et al. breast carcinoma prognostication with morphometry,



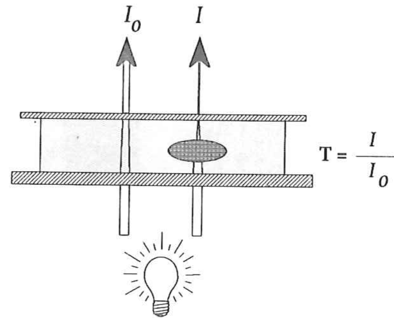


Fig. 8. **Left.** Integrated optical densities of cells hydrolysed with HCl and stained with Schiff's reagent. If hydrolysis is done at 60° C in 0.5N HCl, the process is fast and results in destruction of the aldehyde groups soon after the optimal level of hydrolysis. At room temperature and in 5N HCl the hydrolysis is slower, but so is also the destruction of the aldehyde groups. The latter method gives more consistent results in staining for DNA. **Right.** Transmittance is the ratio of the transmitted light I through the stained area (nucleus in DNA cytometry) and the transmitted light I_0 through the background.

Ruschoff, and Bearzi et al. estimate the value of counting the nucleolar organizing regions (AgNORs) in different applications. Haapasalo describes the M/V index for counting mitotic figures. The M/V index has proved to be of great value in the prognostication of ovarian cancer - in his studies it is the best histological prognosticator in the condition (Haapasalo et al. 1989a, 1989b, 1990, 1991). The M/V index also reflects the number of mitotic figures per volume of tissue (Collan 1992).

STATIC CYTOMETRY

Microscopic cytometry as a concept includes all types of measurements done on cells. In quantitative pathology it specially signifies quantitative histo- and cytochemistry, usually done with instruments (microscopic cytometers). Cytometers used with microscopes are called static cytometers (Fig. 11) - the cells studied are static (flow cytometers study cells suspended in fluid, and are generally not associated with microscopy). The most popular application is DNA cytometry - the estimation of the total amount of DNA in cell nuclei. The idea is to treat cells with HCl- solution to hydrolyze DNA, which creates aldehydes (Fig. 8), and demonstrate the aldehyde groups with Schiff's reagent - a process usually called the Feulgen reaction (Feulgen and Rossenbeck 1924, Mikel and Becker 1991). The hydrolysis is best done at room temperature with high HCl concentration rather than with the traditional method. The staining is stochiometric - the staining intensity is in linear relation with the total amount of DNA in the nucleus. The staining intensity is estimated by estimating the intensity of the transmitted light at the location of the nuclei measured. The

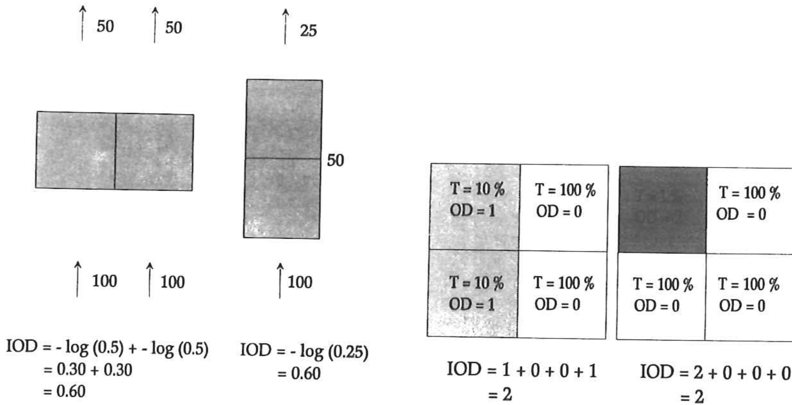


Fig. 9. Estimation of integrated optical density (IOD). **Left:** The IOD is the sum of optical densities (OD) in the region measured. If 50% of the light is absorbed at a unit of tissue, the IOD is the same when the units are measured side by side or on top of each other. So the IOD describes well the amount of stain present in the tissues. **Right:** Estimates from a region with 4 pixels. The optical densities of the four pixels on the left result in the IOD=2. On the right, only one pixel has stain, but because the pixel is stained intensively, the IOD is the same as on the left. Also, the amount of stain is the same in both stained areas.

intensity ratio of the transmitted light to the light through the background is called the transmittance (Fig. 8). The earliest instruments made these measurements on small areas over the nucleus. The transmittance (T) of light in each of these areas above the nucleus was estimated. Thereafter the optical density (OD) could be estimated:

$$OD = - \log T. \tag{6}$$

Fig. 9 demonstrates how the integrated optical density (IOD) then becomes estimated. IOD is the sum of the optical densities over an area (e.g. nucleus).
The measurements are today done on digitized images consisting of pixels (Oja and Collan 1982). Image cytometry is the term often applied in this context. Usually the computer is asked to outline the nuclei automatically, or they are outlined interactively by hand. Every pixel in the outlined area then gets its OD value. The mean of all these OD values, multiplied with the total number of pixels over the nucleus is IOD. The image to be analyzed is created under the illumination by 546 +- 10 nm light, because the absorption has a maximum at this wavelength.
The total mass of DNA present in the nucleus can be expressed in picograms. DNA is not directly measured in picograms, however. The use of picogram units is based on the measurements of the total amount of DNA within a cell or on calculations made on the basis of what we know of the size of the human genome

through cytogenetics and molecular biology. Such studies suggest that the diploid amount of DNA is about 7.1 picograms.
Usually, however, the amount of DNA is not expressed in gram units. Instead, the amount of DNA - as reflected in the IOD value over the nucleus - is compared with the amount of DNA in diploid cells. Normal human cells which are not in the proliferation cycle are usually diploid. At each measurement session diploid cells and the studied cells are measured separately from the same tissue sample and the IOD value of the studied cells is compared with the IOD value of the diploid cells. Lymphocytes or non-proliferating mesenchymal cells (such as fibrocytes) or benign non-proliferating epithelial cells can be used as diploid controls.
However, there are several problems. The IOD value of lymphocytes with condensed chromatin is not necessarily comparable with the IOD value of cells with less condensed chromatin texture. In fact, researchers have suggested that if small lymphocytes with condensed chromatin are used as controls, their IOD value should be multiplied by 1.15 before the values of studied cells are compared with the lymphocytes. Nondividing mesenchymal cells or benign nondividing epithelial cells do not generally need such correction factor. Controls are often measured first and the mode of their histogram placed at 2C on the scale (2C corresponding to the cellular content of DNA in normal diploid cells). Then the studied cells (usually tumor cells) are measured and their histogram placed at the correct location within the scale. The location, of course, is defined by the IOD values of the cells (Sandritter 1961, Sandritter 1981). Hereafter the "DNA histograms" can be compared. If the studied cells overlap with the controls, the cells are diploid. If the studied cells do not overlap with the controls they are aneuploid (euploid is usually used as a synonym for an exact multiple of the diploid amount of chromosomes and corresponding DNA, aneuploid as the term for chromosomes or DNA which are not an exact multiple of the diploid amount). Cells at the tetraploid region (cells with 2 x diploid DNA) are called tetraploid, slightly below that region hypotetraploid, and above hypertetraploid.
If the cell population is actively proliferating the proliferating cells are also shown in the histogram. When a diploid population is studied the proliferating cells are seen above the diploid region of the scale in the DNA histogram. Because of this the S phase fraction (fraction of cells in the DNA-synthesis phase) can be estimated from the histogram, also from the DNA histogram created with static cytometry (cf. Montironi et al. in this issue).

FLOW CYTOMETRY

Flow cytometry (FCM) is a technique which uses fluorescing probe molecules, and fluorescing stains to study cells in fluid. DNA is stained with fluorescing dyes and the amount of DNA in individual cells is estimated. The cells are passed fast through a laser beam - one by one - and the amount of light scatter and fluorescence recorded. Large number of cells can be studied in a short time. One of the reasons for the popularity of this

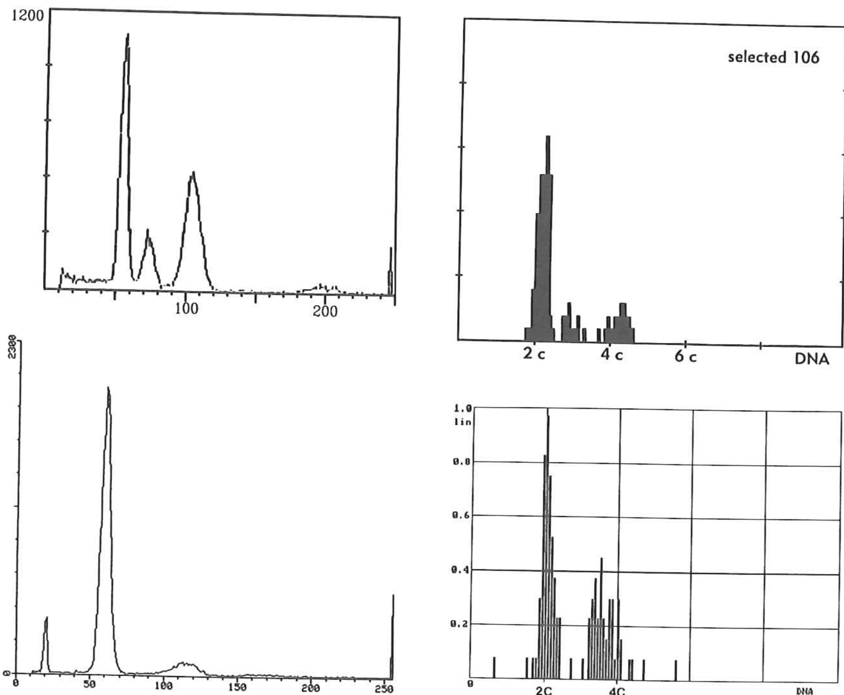


Fig. 10. Comparison of flow cytometry (histograms on the left) and static cytometry histograms from the same samples. **Above:** Both histograms show an aneuploid peak between the regions of 2C and 4C. In addition there is a tetraploid peak. The point in this pair of histograms is that the information contained in the histograms is identical with both of the methods. **Below:** Histograms are different: the aneuploid cells seen in the histogram from the static cytometer are not seen in the histogram from the flow cytometer. The difference may be caused by the selection of cells for measurement in the static cytometer, or destruction of the aneuploid cells by the methods used for flow cytometer. The most probable explanation is that neoplastic cells are not numerous in the sample studied. Most of the cells studied by flow cytometry are probably inflammatory cells. The neoplastic cells can be selected for static cytometry because they are seen in the microscope, whereas the flow cytometry histogram does not distinguish between neoplastic, and inflammatory or other non-neoplastic cells.

methodology in pathology departments is the finding by Hedley et al. (1983) that also paraffin embedded tissue can be studied with DNA flow cytometry. This immediately opened the way to prognostication studies with flow cytometry. The DNA histograms produced by flow cytometry are in about 80% of cases equal to the histograms from corresponding samples by static cytometry (Falkmer et al. 1990). When a larger number of cells is studied

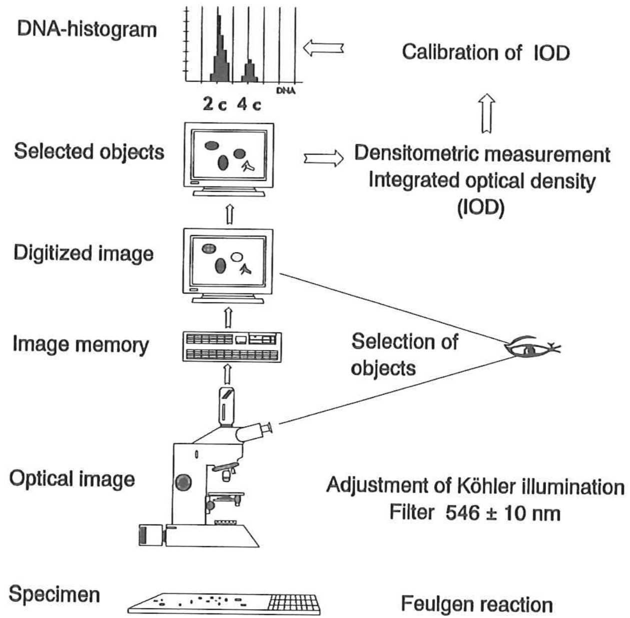


Fig. 11. Image analysis system for densitometric measurement of DNA. The specimen is stained with the Feulgen reaction and viewed in the microscope with light showing peak absorption by DNA. The microscope field is selected, digitized and stored in the image memory. The digitized image on the computer screen can be processed to make the measurement possible and biologically relevant (e.g. damaged cells are not measured, only neoplastic cells are measured). The DNA histogram is calibrated through the measurements done on diploid cells (small lymphocytes, diploid mesenchymal cells). In the calibration cells with condensed chromatin (small lymphocytes, granulocytes) may need a correction factor to be located correctly in the DNA scale.

in static cytometry, especially when the selection of cells is random, the histograms can be expected to be identical with both methods (Montironi et al., in this issue). Because of the greater number of cells studied in flow cytometry, the S phase fraction can be routinely estimated. However, the results are not perfectly consistent between different laboratories (Collan et al. 1992). Static cytometry seems to be sensitive in detecting abnormal cells when they are present in small numbers or are mixed with large numbers of diploid cells (Fig. 10). This is because the abnormality can be detected by the human observer, and abnormal cells selected for analysis. FCM, on the other hand has the advantage of being able to study a large number of cells in a reasonable short time. The interpretation of flow cytometry histograms, however, is not always reproducible (Joensuu and Kallioniemi 1989, Joensuu et al. 1992), and there is interlaboratory variation in estimating the same samples (Collan et al. 1992). Methods used in preparing the FCM samples influence

the results, and cell necrosis and autolysis may influence the interpretation of findings (Alanen et al. 1989a, 1989b).

Numerous papers on static and flow cytometry have described the principles of classification of DNA histograms, and have also shown the prognostic importance of these methods in clinical decisionmaking (Auer et al. 1980, Böcking et al. 1984, Auer et al. 1989, Eskelinen et al. 1989a, Eskelinen et al. 1989b, Eskelinen et al. 1990, Lipponen et al. 1990, Mellin 1990, Stenkvist and Strande 1990, Bibbo et al. 1991, Cornelisse and Tanke 1991).

IMAGE ANALYSIS

Morphometry and image analysis are often used as synonyms but certainly they are not. Morphometry can be done on any image, digitized or not, but image analysis is a computerized process which is based on the digitized image. The image is stored in the computer memory as pixels (Oja and Collan 1983). The digitized image is also a prerequisite to image densitometry which leads to the IOD value from an outlined area (static cytometry, Fig. 11). In histopathology, image analysis is performed with the intent of getting quantitative data corresponding to the data collected through interactive morphometry. The image analytic applications, however, especially try to apply automatic computer operations to make the measurements faster to perform. E.g. the analysis of the muscle fibres can be automated to a great degree (Collumbien et al. 1990, Zukowski et al. 1991). When automatic image analytic methods are applied in practice, however, human interaction is usually necessary because samples generally are not perfect. In the whole decision process involved human interaction is necessary anyway because the final diagnostic decisions cannot be left to a machine.

MOLECULAR PATHOLOGY

The development of quantitative pathology has been extremely fast, and is still much speeded by the development of computers, display systems, output devices, microscopes, telepathology, and new concepts and methods. Surprisingly enough, also the other present great line of development, molecular pathology, is fast approaching quantitative pathology, and uses quantitative pathology methods efficiently. We try to take the example from a special field of molecular pathology (Collan and Kuopio 1992). The proliferation associated genes, many of them called oncogenes, are often expressed in tumors, and this expression is in relation to prognosis in these tumors. Expression of oncogene proteins can be quantitated with image densitometry (van Diest et al. 1991) or simpler methods (cf. Lipponen and Collan, in this issue). Also when the protein, mRNA, or DNA is extracted, their bands in the electrophoresis gels can be quantitated with image densitometry. The specific in situ hybridisation histochemistry can be applied for detection of deletions in tumor suppressor genes. E.g. this was shown for the retinoblastoma gene RB1 recently (Joos et al. 1992). With suitable probes the deletions

can be detected - in the presence of deletions only one of the two alleles of the RB1 gene is shown in the interphase nuclei. By counting the stained dots representing the nonmutated gene one can find an abnormality at this locus. When tissue sections are studied, a little understanding of stereology will certainly be helpful.

INTERPHASE CYTOGENETICS

Aneuploidy in DNA cytometry generally means that the amount of DNA, and correspondingly the chromosome number, differs from the normal diploid values. However, also changes within individual chromosomes (e.g. deletions, see above) can cause aueuploidy. Small changes from normal, however, cannot be detected with classical DNA cytometry. E.g. the lack of one chromosome is generally not detectable. But with chromosome specific probes it is possible to locate the chromosomes in non-mitotic nuclei to estimate the chromosomal pattern of the stemline cells. This can be done by using radioactive chromosome probes or probes which can be stained with histochemical methods to make them detectable by light microscopy (Ambros et al. 1992, van Dekken et al. 1992, Fuzesi et al. 1992, Hopman et al. 1992, Looijenga et al. 1992). By counting the number of dots seen in the nuclei one can determine whether the cell type is diploid or has some of the studied chromosomes present in abnormal numbers. Some problems arise when sections of tissue are studied, but these problems can be solved with the help of stereology.

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