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# Computer-Assisted Pathology of Intraepithelial Adenocarcinoma and Related Lesions: 3-D Distribution, Structural Aberration and Discrimination

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**Abstract** To discriminate among intraepithelial neoplasms, we have been relying on tissue microscopy, but pathologists' subjectivity sometimes impairs diagnosis. Even an individual pathologist is sometimes unable to reproduce exactly his or her own previous diagnosis. Are various atypical lesions classifiable in a reproducible way, and if they are, how? The reliability of a diagnosis will be strengthened if we can define the "natural" categories inherent in cells or tissues. Morphometry and statistical analysis using a computer can provide answers.

Atypia, a morphological feature of carcinoma, is essentially multivariate. Quantification of a tissue feature requires reducing it to a set of ten or more quantities, including size, shape and position of the nucleus, nucleolus, and the cell itself. The grade of aberration from the norm can be assessed only by a synthetic approach, using a computer for multivariate cluster analysis. This classification has been attempted in adenocarcinoma and related lesions of the lung and pancreas. The categories thus established are reproducible, because the lesions fall into distinct divisions according to their forms. We can also examine the organ distribution of intraepithelial neoplasms by three dimensional (3-D) computer-assisted mapping.

To reach a higher level of reliability, as many meaningful features as possible should be taken into account. Particularly, we emphasize the significance of architectural pattern as a biomarker for intraepithelial glandular neoplasms. Computer-aided 3-D structural analysis visualizes the basic skeleton of these neoplasms around which the cells adhere. Instead of the dichotomous tree pattern of normal glands, the tumors basically harbor a 3-D network, tubular or porous, which increasingly deviates from the norm along with the transition from adenoma to well to moderately to poorly differentiated adenocarcinoma. This structural aberration, if recognizable on 2-D sectional images, will serve as a surrogate endpoint biomarker for glandular tumors. 

**Key words:** Computer-assisted 3-D structural analysis, morphometry, intraepithelial glandular neoplasia, multivariate analysis

Our understanding of the mechanism of carcinogenesis has been getting more profound and far-reaching with recent progress in molecular oncology. However, microscopic evaluation by histopathologists and cytopathologists is needed to determine whether the lesion in question is a cancer already in the final stage of carcinogenesis, or in an intermediate stage. In fact, routine

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biopsies often find cells with features intermediate between normal and cancerous. The degree to which these cells deviate from the norm is so widely variable that even the most experienced pathologist may sometimes be unable to reproduce exactly and without fail his or her previous evaluation. The evaluation is likely to be much more difficult to reproduce when different pathologists are involved. In these circumstances, efforts will have to be made to improve the accuracy of microscopic evaluation on cells with various degrees of aberration; this is necessary to correlate the steps of molecular change with corresponding cell morphology. A reliable set of diagnostic criteria for various forms of cells, if established in a reproducible way, will serve as a surrogate endpoint marker for chemoprevention trials.

In this article, we will introduce our new tools and ways of thinking about the morphology of cancer, using adenocarcinomas and related tumors as basic materials. First, we show by 3-D mapping of various organs how adenocarcinoma and its preceding lesions are distributed. This will help us gain insight into the way cancer develops and extends in such organs. Second, we discuss which principles we can use to classify various atypical cells in terms of cell morphology, and in an analytical way, for instance, into carcinoma *in situ* (CIS) and dysplasia. This is a basic requirement if one intends to improve the quality and reproducibility of morphological discrimination. Also, algorithms necessary for categorization and computation will be outlined. Finally, it will be shown that an evaluation should rely not only on abnormal cells, but also on the structure, since cancer is associated with aberrations in the way cells are assembled. This is an essential feature particularly in adenocarcinoma, although little attention has been paid so far. Computer assistance is indispensable in all these attempts. Future studies of tumor pathology will rely on computers.

## DISTRIBUTION OF CANCER AND ITS PRECEDING LESIONS REVEALED BY COMPUTER-AIDED 3-D MAPPING

It is well known that in the colon, adenocarcinoma usually arises from a preformed adenoma in a sequence designated as cancer in adenoma [1]. However, we experience a wide variety of colonic adenomas with different atypia of cells, from mild dysplasia, a lesion still considered benign, to severe dysplasia, which includes lesions consistent with a diagnosis of intraepithelial adenocarcinoma. We performed computer-assisted 3-D mapping of colonic adenomas with focal malignancy, assessing cellular atypia according to the grading rule used in Japan, and demonstrated that there is often a multizonal distribution where an overt cancer is surrounded by zones of less atypical cells, the grade of atypia appearing to increase toward the center [2]. This is likely to be a morphological expression of multistep carcinogenesis as surmised from DNA analysis of colonic adenoma and adenocarcinoma [3]. Thus, the concentric zones around the overt cancer may possibly reflect the corresponding steps in a series of DNA aberrations.

A mantle of a less atypical zone may be hard to confirm in adenocarcinomas arising in organs other than the colon or possibly the stomach. This is because most of the other adenomatous tumors arise in a ductal system which spreads in three dimensions as a finely arborizing tree. Therefore, if one attempts to visualize the distribution of atypical areas by mapping, 3-D reconstruction cannot be avoided. Thanks to the recent development of computers and software techniques, 3-D mapping has become much less time-consuming [4], allowing us to access various organs. For example, in liver lobes resected from patients with hepatohilar bile duct carcinoma, a CIS is often located within a zone of dysplasia, as shown in Fig. 1 [5]. Three-D mapping disclosed that not only multizonality but multiplicity of cancer is quite common. In the breast, computer-aided mapping was helpful in detecting minute cancers which proved to arise either in multiple intraductal papillomas growing in the ducts, or in foci of atypical ductal hyperplasias [6]. Scanning serial sections of breast, cancers as small as 0.2 mm in diameter were often detected; a minute cancer is definable as such when and only when it has been encountered incidentally while performing 3-D space scanning. In pancreases resected for multicystic papillary adenoma (also called cystadenoma or mucus hypersecreting tumor), the inner lining of cysts presents as a mosaic of patchy areas of various grades of atypia; here too, *in situ* or invasive carcinomas develop in areas of dysplasia [7]. Except in adenocarcinoma of the lung where

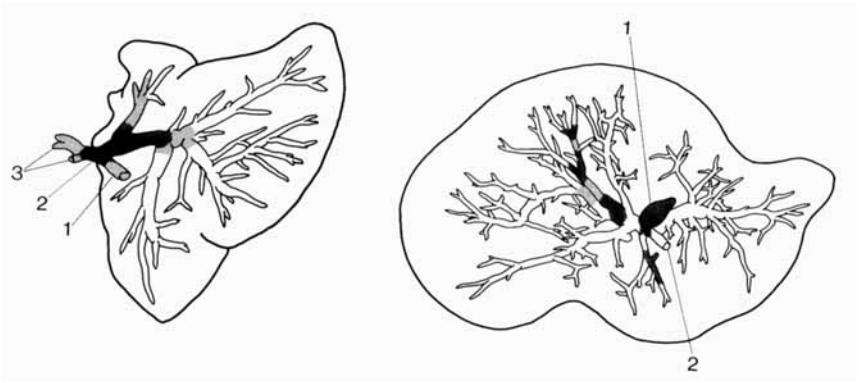



**Fig. 1.** Two examples of 3-D mapping of bile ducts showing the distribution of atypical lesions. Left: In a surgically resected left liver lobe, CIS is involving the hepatic duct (2) which, however, is surrounded by ducts with dysplasia (1,3). Right: An autopsy liver with carcinoma of the gallbladder (1). There are four skip areas of CIS in the intrahepatic biliary tree, each associated with dysplastic zones. No tumor at the stump of the common bile duct (2). Re-drawn based on a paper by Suzuki *et al.* [5].

often a surrounding zone of less atypical cells was already visible on 2-D sections, zonal distribution in all these examples was visualized only by 3-D mapping from serial sections [8]. Thus it appears to be a rule that a cancer which has not advanced very far has a multizonal distribution pattern. Pathologists may find this quite significant since the outer, less atypical zone is likely to correspond to a step preceding the central, overt cancer.

In this context, however, a question arises about the reproducibility of evaluations we make for atypical cells. In the above mappings, discrimination among CIS, dysplasias, and other related changes was made on a subjective microscopic basis. Therefore, we next have to reconsider, briefly and in accurate morphological terms, the technical basis for discrimination. This is synonymous with attempting to establish an objective and reproducible categorization of atypical cells.

## CATEGORIZATION OF ATYPICAL CELLS BY MORPHOMETRY AND STATISTICAL ANALYSIS

Figure 2 illustrates conditions under which we can classify a variety of lesions. Let us assume that in a large population, we measure a quantity X, for example, blood pressure. If, as a consequence of these measurements, we obtained a bimodal distribution in the upper figure, then we

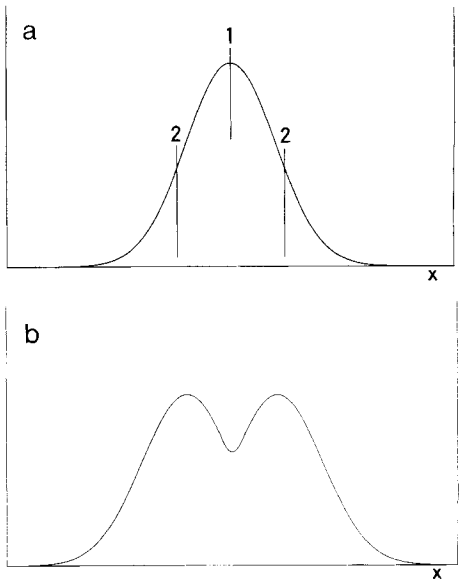



**Fig. 2.** A population is classifiable with regard to X when there is a bimodal distribution (b), but not a monomodal distribution (a).

can say the population is classifiable into the high pressure and low pressure groups. But, in case of monomodal distribution as in the lower frame, we have to say the object itself is not susceptible to classification. What can be done in this case is nothing more than to make an arbitrary division, *e.g.*, into two groups (high and

low), into three groups (high, medium and low), or into any number of groups and in any way. The presence of indefinite ways of arbitrary division implies that the object itself is not susceptible to classification. Thus, classifiability of the object of analysis depends on whether it belongs to one or the other of these two types, which appears to be profoundly significant in pathology, where classification has been one of the most important methods of study. Also, we have been discussing a typical problem: the classifiablity of various atypical cells. Using a computer-assisted approach, we have attempted to examine whether or not atypical lesions are classifiable, and if they are, how we can define the most appropriate categories. We applied morphometry and multivariate statistical analysis using adenocarcinoma of the lung as an example [9].

Subtypes of pulmonary adenocarcinoma are defined according to cell origin [10]. Of these, Clara cell type and Type II alveolar cell adenocarcinomas often have a surrounding zone of less atypical cells, which are regarded as a forerunner of these cancers and called atypical adenomatous hyperplasia (AAH) [11]. Clara cell carcinoma cells have some features of bronchiolar cells; those of Type II alveolar cell carcinoma have some features in common with surfactant-producing alveolar epithelia. The problem is whether or not there is a firm morphological definition that discriminates AAH from adenocarcinomas. In reality, lesions we diagnosed as AAH appear to range over a wide variety of cellular atypia, from slightly more atypical than adenomatous hyperplasia to severe atypia seemingly bordering on CIS.

Although morphometry is a powerful weapon for objective assessment, certain considerations are necessary for its application to cell form. It is well recognized that features of malignant cells cannot be reduced to a single quantity, but require a combination of several quantities. In discriminating among the lung tumors in question, we found that at least 12 quantities are involved, including the nuclear area, the form factor of the nucleus, the height of nucleus and so on, together with their variances. This implies that we have to resort to a method which somehow synthesizes the 12 quantities and induces a classification. For this sort of problem we rely on cluster analysis, a technique of multivariate statistics, which is outlined briefly below.

Suppose that, as in Figure 3, we examine five patients with two quantifiable properties X and Y. Let us imagine, for instance, that we are examining cells from the five patients, where X is nuclear area and Y is pleomorphism. Now, we instantly recognize two groups, one comprising Cases 1, 2 and 3 and the other Cases 4 and 5. They appear so clear because we see the separation of groups as defined by the distances among the subjects. For example, Cases 1 and 2 form a group because their distance is very small. Therefore, if we calculate distances for all possible pairs of the subjects by round robin, the nearest pair turns out to be 4 and 5, the next 1 and 2, and so on. Thus, classification is a process

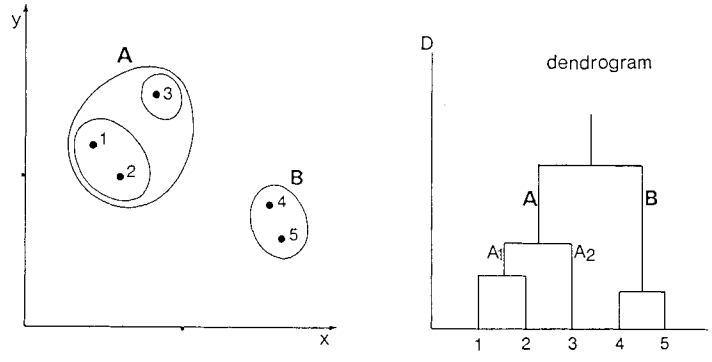



**Fig. 3.** The principle of cluster analysis. Left: Cases 1 to 5 are separate and classifiable into Clusters A and B by putting together the pair with the shortest between-subjects distance. Right: The process of clustering is expressed as a dendrogram where the hierarchical relation among the subjects is clearly shown.

of putting together the nearest pairs to create a hierarchy in the form of a dendrogram, shown in the right half of Figure 3. In this example we are dealing with 2-D, XY distances. Therefore, the distance D(i,j) between a pair of cases [Case i, Case j] is given by $D^2(i,j) = (x_i - x_j)^2 + (y_i - y_j)^2$. In a space of more than three dimensions, D is no longer visible. However, in mathematical terms, it is definable in any dimension, as:

$$D^2(i,j) = (x_i - x_j)^2 + (y_i - y_j)^2 + (z_i - z_j)^2 + (w_i - w_j)^2 + \ldots$$

Since, generally, parameters are measured on different units, D(i,j) is given as below where each parameter value is standardized with the corresponding variance $s$:

$$D^2(i,j) = (x_i - x_j)^2/s_x2 + (y_i - y_j)^2/s_y^2 + (z_i - z_j)^2/s_z^2 + (w_i - w_j)^2/s_w2 + \ldots$$

In lung tumors where the form of cells was expressed by a combination of 12 parameters, $D^2(i,j)$ is a 12-D distance which is, of course, no longer visible. There are several methods for defining between-cluster distances, but we employed the widely applied Ward method [12]. All computations were performed using a mainframe computer.

Figure 4 is a dendrogram showing the result of cluster analysis for a total of 97 atypical areas measured in lung sections selected from 303 patients. Of the 97 areas, 35 were diagnosed as Clara cell carcinoma, 22 as Type II alveolar cell carcinoma, and 40 as AAH. It should be emphasized that these are premorphometry subjective diagnoses, and are to be compared with the result of cluster analysis. The dendrogram demonstrates that the 97 lesions are classifiable into three groups. What is presented here is not an artificial, but a natural classification inherent in the lesions themselves. Of these, Cluster 3 consists almost purely of lesions evaluated prior to morphometry as Clara cell carcinoma. Likewise, Cluster 2 is dominated by lesions diagnosed as AAH. This implies that AAH and Clara cell carcinomas are definable as independent lesions with certain cell features. On the other hand, Cluster 1 poses a problem. Since lesions diagnosed as Type II alveolar cell carcinoma were mostly assigned here, it appears to be a cluster for this type of adenocarcinoma. However, it is striking that the lesions we considered AAH were split, and more than one third were assigned not to Cluster 2 but to this group. Their placement with Type II alveolar cell carcinoma does not justify our premorphometry diagnosis, and strongly suggests that they must have been adenocarcinoma in the first place. In this way, cluster analysis serves as a good lesson, and often makes us update our own diagnostic standards. How far the clusters are separated cannot be shown visually in the 12-D space in which the 97 lesions are positioned, but we proposed a

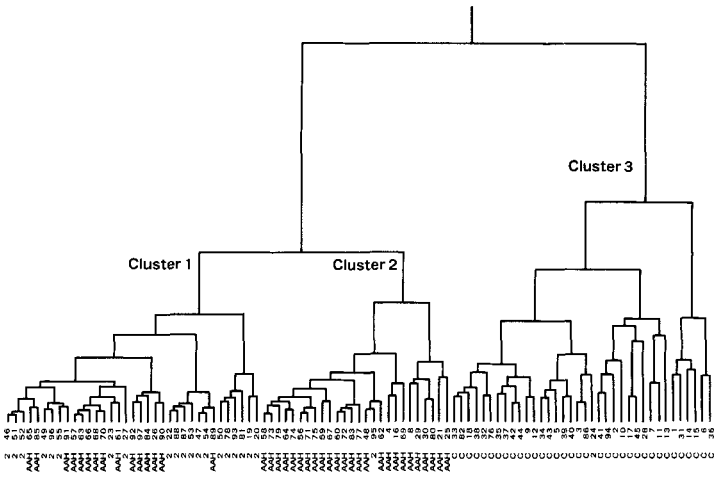


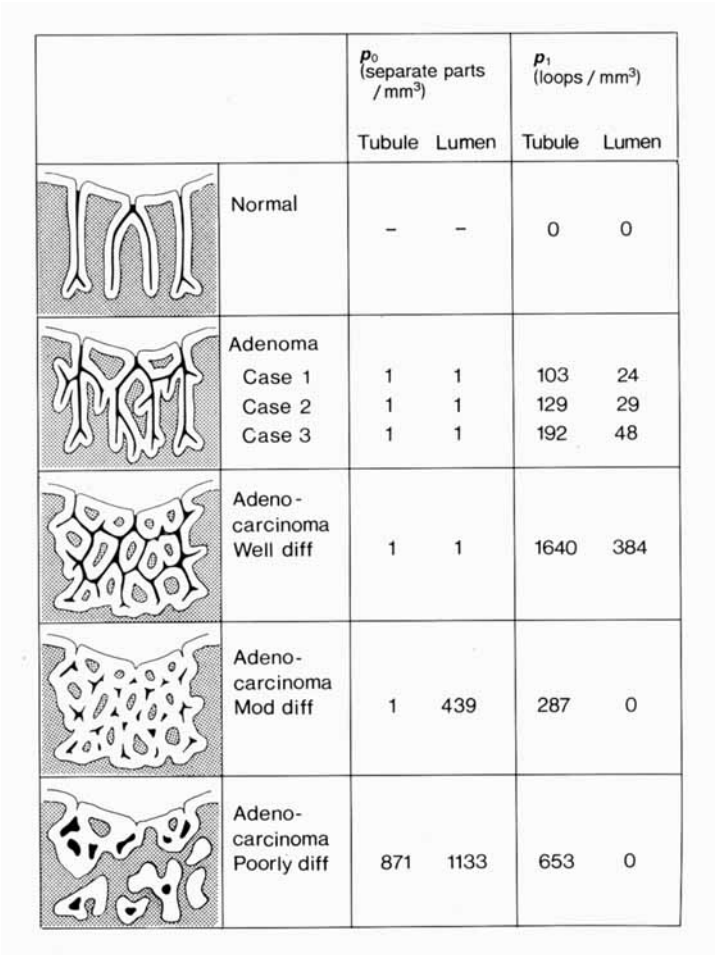

**Fig. 4.** A dendrogram showing the result of cluster analysis from 97 atypical lesions of lung. Three clusters are formed. C, Clara cell carcinoma; II, Type II alveolar cell carcinoma; AAH, atypical adenomatous hyperplasia. Reproduced from Mori *et al.* [9].

method of visualization in a 2-D scattergram with the first and second canonical discriminant functions [9].

In pancreata resected for papillary intraductal adenoma and adenocarcinoma, we again experienced division into malignant and premalignant clusters of what we used to diagnose as dysplasia [13]. Cluster analysis of cells lining the neoplastic cysts gave rise to the formation of three clusters: normal epithelia, mild dysplasia, and severe dysplasia. The second group was comprised of lesions diagnosed as regenerative hyperplasia, and the third, CIS and invasive adenocarcinoma, justifying the designation of the former as a benign, and the latter as a malignant cluster. Here too, lesions we evaluated as dysplasia before morphometry split into the benign and malignant clusters. The result makes us realize that what we used to put under the common diagnosis of dysplasia should instead be assigned to either a benign or malignant group. Also, ascending atypia from the normal to mildly to severely dysplastic clusters was shown to closely correlate with changes in the immunocytological histochemical behavior of the cells, for example, the distribution pattern in and around the cells of carcinoembryonic antigen [13].

## STRUCTURAL ATYPIA AS AN ENDPOINT MARKER

The more parameters we employ as measures of cell form, the more reliable cluster analysis



| | | $p_0$ (separate parts / mm$^3$) | | $p_1$ (loops / mm$^3$) | |
|---|---|---|---|---|---|
| | | Tubule | Lumen | Tubule | Lumen |
| | Normal | – | – | 0 | 0 |
| | Adenoma | | | | |
| | Case 1 | 1 | 1 | 103 | 24 |
| | Case 2 | 1 | 1 | 129 | 29 |
| | Case 3 | 1 | 1 | 192 | 48 |
| | Adeno-carcinoma Well diff | 1 | 1 | 1640 | 384 |
| | Adeno-carcinoma Mod diff | 1 | 439 | 287 | 0 |
| | Adeno-carcinoma Poorly diff | 871 | 1133 | 653 | 0 |

**Fig. 5.** The change of 3-D architectural framework from normal gastric gland to adenoma to adenocarcinoma of the stomach. $p_1$, the number of loops (1st Betti number of network) per mm$^3$ tissue; $p_0$, the number of separate parts of network (0th Betti number).

becomes. For example, the chromatin pattern of nuclei is an important factor of atypia, and one of us (F.T.) is attempting to quantify the nuclear texture of endometrial tumors [14] by applying the technique of Young *et al.* [15]. Here we briefly sketch another important feature of adenoma and adenocarcinoma—the aberrant structural pattern. It has been said in Japan that in evaluating glandular tumors, particularly of the stomach, one should pay attention not only to cellular atypia (CAT), but to abnormal glandular patterns which are called "structural atypia (SAT)". In reality, the latter has been more talked about than actually defined, probably because SAT may be far more difficult to define than CAT due to its 3-D character. With a computer, one of us (T.T.) managed to visualize how the skeleton differs among various types of gastric adenocarcinoma and adenoma [16]. We think this will serve as an important endpoint marker.

The study disclosed an obvious deviation of the skeleton in adenomatous tumors of the stomach from that of ordinary gastric glands. The pattern proved to vary among carcinomas with different grades of differentiation, and there was a continuous transition of the skeleton from ordinary glands to adenoma to well to moderately to poorly differentiated adenocarcinoma (Fig. 5). Normal glands are small but independent trees. In gastric adenoma where carcinoma sometimes develops, anastomosis between contiguous glands begins to occur, forming a loose network. Well-differentiated adenocarcinoma may sometimes look quite like an adenoma on microscopic section, while 3-dimensionally, the tree structure of normal glands is lost and replaced by a dense network of tubules. In this tumor, however, the lumina are still open and totally continuous, ensuring drainage of secretory products to the exterior. In a moderately differentiated tumor which presents as the most common picture of adenocarcinoma, nests of cancer cells remain as a 3-D network, but their lumina have lost continuity and split into separate vesicles. This, betraying the term "tubular adenocarcinoma," may better be expressed as a state of porosity. The so-called "cribriform" pattern, a well-known feature of adenocarcinoma, corresponds to a sectional picture of porous nests. Accumulation of secretory products in disconnected vesicles of porous nests often causes luminal rupture, creating another feature of structural atypia. In poorly differentiated tumors, not only the lumina but the nests lose continuity and begin to break loose. This corresponds to a stage of complete disintegration of structure.

It is clear in this serial transition that not only the form of the cells, but also the supercellular structure changes continuously with the grade of differentiation. For instance, it implies that in well-differentiated adenocarcinoma, not only are the cells mature, but also that the structure is well organized. The deviation of glandular structure from the norm is expressed in various findings in 2-D sectional pictures, *i.e.*, network formation, cribrous lumina in a single nest, rupture of distended lumina, irregular dispersion of nests, and so on. A set of these aberrant patterns, if kept in the pathologist's mind, will greatly serve as an endpoint marker for evaluation of glandular tumors.

## CONCLUSIONS

Three-D mapping of organs harboring carcinoma is often visualized as a multizonal distribution where an overt cancer is surrounded by a less atypical zone, thus reflecting the multistep progression of cancer. Morphometry and cluster analysis of cells, if combined, serve as a powerful weapon in defining categories of atypical lesions, and help us to define the steps of carcinogenesis. Not only cellular, but 3-D structural abnormality of tumors may serve as biomarkers. In all these aspects, computer assistance is essential.

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