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Invited Review

Clinical applications of image cytometry to human tumour analysis

C. Millot and J. Dufer

Laboratory of Cell Physiology and Hematology, UFR of Pharmacy, Reims, France

Summary. Image cytometry (ICM) is widely applied to the automated screening, the detection, the diagnosis, the classification, the prognosis and the therapeutic follow-up of different types of cancers (breast, bladder, cervix,...). This review describes the analysis methods and the applications of nuclear image analysis, the determination of DNA content and the analysis of morphometry and of nuclear texture. DNA content analysis can contribute to a prognostic information in addition to other prognostic factors for breast, renal and prostate cancers. For ovarian cancer, aneuploidy seems to be related to prognosis. Bladder tumours with DNA aneuploidy were frequently of high malignancy while ploidy was significantly correlated to relapse risk. For digestive cancers, patients presenting DNA diploid tumours show a better survival than patients with aneuploid ones. Morphometry seems to be a more important criterion than other conventional prognostic factors of invasive breast and digestive carcinomas. A differential diagnosis between normal and neoplastic thyroids is more precise when based on a quantitative evaluation of texture associated to morphometry. Textural parameters permit the discrimination of two populations of patients having a different prognosis and could thus be an aid for prognosis in prostatic cancers. Morphonuclear parameters contribute to separate low and high grade bladder carcinomas. Although ICM was frequently reported, results from the reported examples were not always obvious. In conclusion, the measurements obtained with ICM could be helpful for a decision in several cancers but could not be a substitute for the classical approach of the pathologist.

Key words: Image cytometry, Cancer cells, DNA content, Morphometry, Texture

Offprint requests to: Christine Millot, Laboratoire de Physiologie Cellulaire & Hématologie, UFR de Pharmacie, 1, rue du Maréchal Juin, 51100 Reims, France. e-mail: christine.millot@univ-reims.fr

Introduction

Cell analysis tools have led to remarkable progress for studying the clinical development and the progression of tumours. Indeed, the increased sophistication in automation and computer technologies has improved the visualisation and the quantification of human tumour cell parameters. The generalisation of quantitative microscopy approaches has provided new tools such as image analysers. These systems are equipped with interfaces able to control microscope functions and with software to perform the image analysis of cells (Meijer et al., 1997). The development of automated devices to screen slides and to characterize lesions has been the purpose of a number of studies.

Therefore, cell image analysis will be widely applied to different medical applications such as the automated screening, the detection, the diagnosis and classification, the prognosis and the therapeutic follow-up of different cancers (cervix, bladder, breast,...).

Other applications study the experimental carcinogenesis process and the cell response or resistance to chemotherapy.

Biologists, cytopathologists and histopathologists have to perform quantitative, reproducible and objective observations. The microscope is coupled with a computer to characterize the parameters from images of whole cells, nuclei or tissues. In medical and pathological research laboratories, the development and the existence of exploratory methods in cytology is associated with increasing needs in automatic treatment of microscopic images. The measurement objectivity of biological samples requires a large number of analysed cells in order to compensate for the often important intrinsic variability within samples and to ensure a maximal accuracy. The requirements for cell analysis are: 1) An effective representation of the information contained in the microscopic image. 2) A quantitative description of cells and tissues (size, shape, texture,..) in addition to the conventional qualitative evaluation. 3) An objective evaluation of the observed objects (malignant cells, mitosis, blasts,...) in addition to the human subjective observations. 4) A rational approach to a

phenomenon represented by their images (cell kinetics, ploidy, differentiation,...) (Brugal, 1995).

Thus, the quantitative microscopy instrumentation must perform both image acquisition, take into account several kinds of parameter extractions and evaluate their statistical significance with suitable statistical tests.

Image cytometry (ICM) and flow cytometry (FCM) are two measuring techniques in the field of analytical pathology. The possibility of combined FCM and ICM are illustrated in typical examples in the field of cervical, bladder and mammary cytology (Tanke et al., 1983). The main advantage of ICM that it is a more selective analysis of tumour cells, than the FCM analysis. In the oesophagus for example, a relatively small amount seems to be sufficient to represent the DNA content of the whole tumour. Results from both methods are equivalent concerning DNA ploidy with degree of tumour malignancy and local tumour spread stage (Papadopoulous et al., 1995).

ICM is applied in surgical pathology to quantify DNA content and nuclear and cytoplasmic immunostains (Cohen, 1996). Another interesting thing about ICM consists of the malignancy detection by classification approaches. Dysplastic lesions are pre-cancerous, and can either regress, persist or progress towards an invasive carcinoma. In dysplasias of the lung, by rapid DNA ICM, the early detection of pre-cancerous lesions has been performed (Auffermann and Böcking, 1985).

An automatic screening method by ICM can be applied to individually classify all the cells of the sample into defined categories and could represent a useful approach to detection of malignancy (Seigneurin, 1995).

The purpose of this review is first to describe some of the methods used for nuclear image analysis including sample preparation, DNA staining, image acquisition, determination of DNA content and analysis of morphometry and texture. Second, several clinical applications of ICM will be reported which analysed either the DNA content, the nuclear morphometry, or the nuclear texture.

Methods for nuclear image analysis

Sample preparation

Different methods of cell preparations have been reported : cell culture monolayers, imprints, smears from Fine-Needle Aspiration Biopsies (FNAB), smears from exfoliated cells, cytocentrifuged preparations from body fluids, cell separation specimens (after mechanic or enzymatic dispersion), other biopsies or from paraformaldehyde-fixed, paraffin-embedded formalin-fixed tissues (van Driel-Kulker et al., 1985; Frostad et al., 1999; Gherardi and Marveggio, 1999). The advantages and limits of each method have been previously described (Ottesen et al., 1997; Mainguene et al., 1997).

Fixation with paraformaldehyde is required before Feulgen staining with pararosaniline or thionine. The samples are air-dried at room temperature for at least 1 hour, fixed in 4% paraformaldehyde for 30 minutes and rinsed with distilled water.

However, the results from these preparations cannot always be compared (Hanselaar et al., 1992). Technical conditions are important and must be well-controlled due to their potential influence on the measurements. The reproducibility of the preparation methods of biological samples for staining and cell fixation is required. For example, in breast cancer, the freezing of the samples prior to fixation influences the morphometrical measurements when compared to unfrozen samples (Kronqvist et al., 1995). In addition, nuclear volumes are larger when samples are included in metacrylate as opposed to paraffin. During cytocentrifugation, unfixed cells are compressed (van Meir, 1991). Only in morphometrical studies, do fixation conditions by formaldehyde not influence the variability of ICM measurements in breast cancers (Ladekarl, 1994).

DNA staining

For absorption measurements of nuclear DNA, standardised nuclear staining with either Giemsa, Papanicolaou, hematoxylin/eosin, toluidin blue or Feulgen have been reported (Schulte, 1991; Christen et al., 1993; Bartels and Bibbo, 1993; Ferrer-Roca et al., 1998; Zeppa et al., 1998). Staining by the standardised Feulgen reaction is the most widely quantitative technique for nuclear image analysis, due to its DNA specificity and its stoechiometry (Schulte, 1991). This reaction must be carried out under carefully controlled conditions for the stoechiometric determination of DNA. Hydrolysis conditions for Feulgen have to take into account the tissue type, the time of the staining and the method of cell preparation. The optimal staining conditions, under controlled temperature and time conditions, are determined from hydrolysis curves (time versus Integrated Optical Density, IOD). The control of specificity of the Feulgen reaction may be performed by the staining of an un-hydrolysed specimen. Then, nuclear DNA content and textural parameters can be quantified. For analysis of fluorescence emission, DNA can be stained with fluorochromes such as DAPI, Hoechst 33352, propidium iodide (Böcking et al., 1995).

Nuclear image acquisition

Nuclear image analysis is based on the measurement of the light scattered or absorbed from each point of the observed cell. To obtain the most accurate image representation, the analyser must associate a conventional microscope with a captor which digitalizes the image. Each pixel is represented by its coordinates associated with numerical values with grey levels from one or several wavelengths.

The data analysis can be performed through the following steps (pre-treatment, segmentation, parametrisation, data treatment) (Brugal, 1984).

In ICM, the total number of measured cells is generally relatively low, the random cell selection and

the measurement selection may be used (Mesker et al., 1989). To avoid artefacts due to the cell selection method (Kronqvist et al., 1995; Jannink et al., 1995a), a systematic random sampling selection of nuclei has been proposed and a better reproducibility of results and of the prognostic value for patients with invasive breast cancer has been reported (Jannink et al., 1995b).

Among the recommendations is the shading and the densitometric linearity over the time required to be checked. Moreover, external reference cells (such as lymphocytes, granulocytes, hepatocytes from rat or mouse) fixed and stained in the same conditions as analysed cells (same staining bath,...) are required. These reference cells are used to transform the observed results from arbitrary units to reference units (for example 2c, 4c,...) (Böcking et al., 1995). All tumour values are expressed in relation to their corresponding staining control (2c value), denoting the normal diploid DNA content. All DNA measurements can be performed by the same investigator with no previous knowledge of coded clinical data, which can be broken after completion of all measurements.

Determination of nuclear DNA content

From the absorbance of stained DNA by Feulgen reaction, results are expressed in terms of DNA ploidy or in terms of DNA content, both considered as equivalent. DNA ploidy corresponds to DNA content in the case of a specific, stoechiometric and linear DNA analysis.

An increasing number of reports have described DNA measurements in a variety of human cancer tissues. Tumours with numeric chromosomal aberrations (different from the normal set of 2 x 23 chromosomes) can be characterized by their DNA histogram. Cytometric measurements of nuclear DNA content from individual tumour cells are derived from this DNA histogram which provides three parameters (Herman, 1992): 1) The presence or the absence of an aneuploid cell population. 2) The degree of aneuploidy of this population. The DNA Index (DI) corresponds to the ratio of the DNA amount in the tumour cell population to diploid cells. Thus, diploid cells have a DI = 1 whereas DI = 2 for tetraploid ones. 3) The determination of the Proliferation Fraction (PF) of the tumour cell population. Data are expressed as the percentage of cells with a DNA content between the G0/G1 and G2+M amounts (in S-phase of the cell cycle) (Herman, 1992).

From a DNA histogram, the measurement of the first parameter appears reproducible. However, the presence of an aneuploid cell population is less obvious because of the existence of an interaction between the DI and the percentage of aneuploid cells. The clinical application of DI and PF measurements is limited in cancer management of patients due to the lack of inter-laboratory reproducibility (Herman, 1992). To consider the clinical relevance of these measurements, it would be interesting to assess their reliability. A possible interaction has been described between the DI and the cell sample composition. Among the numerous factors which may interact with the determination of the S-phase fraction, the cell composition of clinical samples precludes the simple application of these measurements to human tumours. Due to the importance of proliferation activity in predicting the behaviour of tumours, other approaches have been shown to circumvent the problem of S-phase (Herman, 1992).

Analysis of nuclear morphometry and texture

Morphometry is the quantitative description of geometrical features of structures of any dimension (Baak, 1991). The morphometrical parameters are derived from the spatial distribution of the OD which belongs to the object inside the image. After the object has been designed by the observer, different geometrical information about the object are calculated. Among the particularities recognised inside the cells, most of them result from a detailed description of entities organized in the cell nucleus. The morphonuclear parameters are related to the size and shape (nuclear area, form factor, perimeter, roundness, maximum diameter,...) or to the chromatin aspect of stained nuclei (texture). These morphological data are calculated to discriminate malignant cells and to include an objective determination of their characteristics. ICM is the only method allowing the quantitative description of the textural parameters of a stained structure in a cell. The nuclear texture notion which reflects the global chromatin structure would evaluate the functional status of the nucleus (Giroud, 1987). Variations of the nuclear texture can be followed, such as heterogeneity, granularity, condensation or radial distribution related to the cell cycle progression (Rousselle et al., 1999). The chromatin profile of each tumour cell includes the condensation degree, the distribution and the chromatin organisation (Brugal, 1984; Giroud, 1987). The texture is then defined as the spatial repetition of the same pattern in different directions of the nucleus. For measuring nuclear texture, smears or cytocentrifuged cells can be analysed, mostly by absorption microscopy. To quantify textural parameters, a grey level image of the object is analysed in terms of the spatial distance between intra-nuclear optical densities that correspond to the texture notion. Many textural parameters can be analysed; contrast, entropy, local mean of grey levels, ... (MacAulay and Palcic, 1990).

Statistical analysis

The ESACP (European Society of Analytical Cellular Pathology) consensus on image cytometric DNA measurement provides a basis for standardisation of the measurements and of several details of the measurement conditions, preparation of samples and interpretation (Böcking et al., 1995; Haroske et al., 1998b; Giroud et al., 1998). However, the diagnostic and prognostic interpretation of ICM results remains difficult. So, appropriate statistics may provide a sufficient reliability for DNA image cytometry

interpretation. A set of these statistical methods is given to interpret a DNA histogram objectively, without any human interaction (Shapiro and Wilk's-W test) (Haroske et al., 1997, 1998a).

Differences in terms of morphometrical or of textural parameter values can be analysed with either the 'Student-t test or the Wilcoxon rank-sum test for normal and abnormal distributions, respectively.

To separate two cell populations in term of prognosis or survival with the best combination of morphonuclear parameters, several multivariate approaches are proposed, of which the discriminant analysis or the logistic regression. The resulting linear function of parameters allows the classification of the observations in different groups. Results of the classification can be evaluated in terms of sensitivity or of specificity percentages.

Nevertheless, the accuracy of analysis depends on the quality of image analysis systems, which could differ widely in their characteristics (Thunnissen et al., 1997; Haroske et al., 1998).

DNA content analysis

A growing number of reports describe the great interest of DNA measurements in a variety of human tumours. Rapid DNA evaluation appears useful in clinical diagnosis (Weid et al., 1983). Cytometric measurements of the individual tumour cell DNA content (ploidy and PF) are at once applied in the determination of tumour prognosis (Herman, 1992), more for low stage tumours than high stage tumours. The DNA content of tumour cells would influence the length of patient survival (Bottger et al., 1991). A tumour exhibiting DNA values within the limits of normal tissues (DNA euploidy) correlates with a favourable prognosis. In contrast, a tumour with increased or scattered DNA values (DNA aneuploidy) is an indication of poor prognosis (Fallenius et al., 1988). For most tumours, aneuploidy appears as an indicator of higher aggressiveness. DNA ploidy, among other prognostic indicators, seems to be the most significant. Besides, PF may also be important as a criterion of tumour prognosis, as a marker of the simple presence of aneuploidy.

In the study of DNA content profile, FCM and image cytometry (ICM) are complementary methods. FCM is a representative method using all the components of the sample (tumour, tissue origin) and is statistically reliable due to the important number of analysed cells. Besides, ICM is essentially focused on the only tumour component of the sample, where some hundreds of identified cells are analysed.

Variables derived from the DNA histogram (Auer et al., 1980).

Various and numerous criteria have been used to define euploidy and aneuploidy (Auer et al., 1980; Thorud et al., 1986) and to correlate nuclear DNA content and prognosis (Fallenius et al., 1988). The values of OD determined from the nuclear image are correlated with the specific staining of the DNA content in the nucleus. After normalisation, the distribution of staining intensities that corresponds to the DNA histogram can be defined. The Auer classification allows the interpretation of this histogram, as such (Auer et al., 1980):

- The type I histogram, displays a single distinct modal DNA value in the diploid or near-diploid region of normal cells (2c).
- The type II histogram has either a distinct peak in the near tetraploid region (4c) or two well-defined peaks in the 2c and 4c regions.
- The type III histogram often shows two peaks in 2c and 4c but displays a sizeable number (>5%) of cells with DNA amounts similar to those of normal cells during DNA synthesis, in the 3c region.
- The type IV histogram is distinctly aneuploid with increased and scattered DNA values exceeding the normal 4c region such as 5c,....

Due to the relative subjectivity of this classification, other parameters have been proposed among which are: the "DNA Index" (DI) (Herman, 1992); the "Exceeding Rate" (ER) (2,5cER, 5cER,...i.e the percentage of cells for which the DNA content is higher than the considered value); the "2c Deviation Index" (2cDI i.e the variance of the DNA staining intensity near the normal diploid peak) (Böcking et al., 1984); the "DNA Malignant Grade" (DNA-MG), i.e the logarithm value of the 2cDI parameter) (Böcking et al., 1985, 1989); the "DNA Regression Index" for survival (DNA-RI) (Böcking et al., 1985, 1995); and the "entropy" measuring the distribution heterogeneity of staining intensities (Stenkvist et al., 1986).

Breast cancer

Traditional staging and grading have failed to provide a sufficiently accurate prognosis in individual cases of breast cancer (Fallenius et al., 1988). Clinical staging is the determination of the tumour extent at a given moment providing little information about the growth rate of tumours or for the possible presence of metastases in apparently early stages of the disease. Morphological grading is based on the subjective evaluation of some histological and cytological criteria, such as mitotic activity or tubular differentiation. However, this analysis appears to be of limited prognostic value in the majority of breast cancer cases (Schiödt, 1966; Bloom and Field, 1971; Delides et al., 1982). Thus, some reports investigate whether DNA image analysis can contribute to a prognostic information in addition to other prognostic factors such as the node status, the tumour size or other morphological characteristics (Atkin, 1972; Auer et al., 1984).

A relationship between nuclear DNA content and mammary carcinoma survival has been reported in some

works (Atkin, 1972; Auer et al., 1980). Women with near diploid tumours present an increased survival as compared to women with aneuploid tumours. ICM measurements of tissue sections can discriminate DNA diploid from non-diploid clones except for near diploid sub-populations, and allows the analysis of very small lesions (Ottesen et al., 1997). The increase in DNA content was associated to a higher histological grade, a more important tumour size, a node invasion, a disappearance of hormonal receptors and an over-expression of c-erbB2 oncogene (Visscher et al., 1991) or anti-oncogenes (p53).

This strong relationship between nuclear DNA content and breast cancer prognosis has been confirmed by analyses of pre-invasive lesions and invasive breast tumours (Visscher et al., 1991; Opfermann et al., 1987; Fallenius et al., 1988; Mir et al., 1992). Tumours exhibiting DNA values within the limits of normal tissues (DNA euploidy) are correlated with a favourable prognosis, whereas an aneuploid DNA histogram could be considered as a bad prognostic factor. Tumours with abnormal DNA content expressed c-erbB2 more frequently: 31% for aneuploid versus 11% for diploid in invasive carcinomas, and 35% for aneuploid versus 14% for diploid in intraductal carcinomas (Visscher et al., 1991). These oncogene expressions and variations in DNA content may be related to biological parameters and to cytological genetic abnormalities (Wright et al., 1989). Progressive genetic aberrations may be one of the mechanisms of oncogene expression in breast cancer. According to DNA cytometric measurements of S/G2+M fraction (proliferation index), women with breast cancers classified as "Auer type IV" can be divided into subgroups with different tumour characteristics and prognoses (Baldetorp et al., 1998). The prognostic value of DNA histogram patterns may be independent of the nodal status (Mir et al., 1992).

In another study, no association between DNA measured parameters (Auer type, measurements of PF, 5cER and 2cDI) and survival was found in 168 cases of invasive ductal carcinomas (Wiesener et al., 1998).

A correlation between ploidy and oestrogen receptor expression has been described (Cornelisse et al., 1984). In a study of 166 breast cancers, near-diploid tumours were more frequently (70%) oestrogen-positive receptor than aneuploid tumours (50%) (Cornelisse et al., 1984). The relation between DNA content and prognosis does not persist when women are stratified according to hormonal receptor expression (oestrogen and progesterone) (Gilchrist et al., 1993). Moreover, the DNA ploidy does not enable a characterisation of pregnancy-associated breast cancers (hormonal sensitivity level) (Budel et al., 1997). The DNA profile is an independent factor of prognosis (Fallenius et al., 1988; van Rosen et al., 1986).

A combination of the cytological score evaluation and the DNA image analysis appears very useful to discriminate low risk from high risk cases in the field of breast borderline lesions (Gherardi and Marveggio, 1999). ICM combined with cytological grading might offer additional information for the characterisation of 28 breast carcinomas. These observations are of particular interest with the introduction of preoperative chemotherapy (Dey et al., 1999).

Ovarian cancers

In ovarian cancer, DNA aneuploidy is frequently reported and seems to be related to prognosis in the early stages (FIGO stages I and II/A). Women with less than 0.2% of cells with high DNA rate will present an 87% six-year survival versus 49% for patients with more than 0.2% of high DNA cell content (Wagner et al., 1994).

Cervical cancers

In cervical cancer, the frequency of aneuploid DNA cells increases with cervical dysplasia grading (Bibbo et al., 1985). However, the results are contradictory in terms of prognosis (Nasiell et al., 1979; Bibbo et al., 1989). Finally, different molecular or genetic changes associated to malignant transformation of the endometrium (p53, HER-2/neu proto-oncogene expressions, grade, myometrial invasion, oestrogen or progesterone receptor expression or FIGO stage) are known to be prognostic factors. Among them, DNA ploidy is the most strongly predictive and useful factor in persistent or recurrent diseases (Lukes et al., 1994).

Thyroid cancer

Ploidy levels have been determined in non-neoplastic and neoplastic thyroid cells (Auer et al., 1985). Benign tumour lesions often present a DNA aneuploidy level similar to that of neoplastic tissues. This criterion alone is not sufficient to assess a diagnosis of malignancy (Liautaud-Roger et al., 1992a; Salmon et al., 1992a).

Lung cancer

A relationship between DNA histogram data and clinical course of Small Cell Lung Cancer (SCLC) has been reported (Kimura et al., 1993). Indeed, DNA ploidy correlates with the regional lymph node changes in lung cancer (Ogawa et al., 1992). SCLC classified into peripheral and proximal types are compared in terms of clinical malignancy and nuclear content. All patients presented a stage III or a more advanced disease and received chemotherapy (cis-platin, etoposide). Nuclear DNA histogram patterns were classified into four types: A (a single peak at an euploid position); B (a single peak at position other than euploid); C (multiple peaks); and D (without peak). These histogram types allow the prediction of the response to chemotherapy, which has been reported to be 100% for A type, 82% for B type, 38% for C type and 17% for D type (Kimura et al., 1993). Most aneuploid DNA tumours had higher

frequency node invasion and metastasis (Ogawa et al., 1992). Metastases rates were significantly different between high (when the maximum DNA content was 8c in each type of histogram) and low grades (less than 8c) 81% and 7% respectively, and were closely related to the grade (sensitivity, 81%; specificity, 93%) (Kimura et al., 1993). To evaluate the relationship between aneuploidy and metastasis, histological studies could also be informative. Peripheral SCLC exhibits a significantly lower response rate (20%) than proximal SCLC (65%). Nuclear DNA measurements may be potentially useful for the prediction of distant metastasis and the response to chemotherapy. Rapid ICM of DNA would be able to separate squamous dysplasias of the lung into pre-cancerous and non pre-cancerous lesions (Auffermann and Böcking, 1985).

Digestive cancer

In oesophagus cancer, patients presenting DNA diploid tumours show a better survival (median survival time of 32 months) than patients with aneuploid tumours (22 months with hypotriploid and 6.5 months with hypertriploid tumours) (Bottger et al., 1991). This information is preoperatively of greater interest because it can influence the decision about the type of applied therapy (Bottger et al., 1991). Similar results have been reported in gastric cancer with no extra-nodal metastasis (Rugge et al., 1994). However, the DNA content of the tumour cells in stomach does not influence the prognosis (Bottger et al., 1992).

Hepato-cellular carcinomas include heterogeneous diseases with considerable differences in malignant behaviour. The best prognosis was reported for patients with diploid, hypotriploid and tetraploid tumours with a median survival time of 41 months in contrast to 3 months for patients with triploid, hypertriploid or aneuploid tumours. There was a strong correlation between histo-morphological parameters and the DNA content. This parameter may be of considerable clinical relevance in hepato-cellular carcinoma regarding the decision to perform a resection. In patients with prognostically unfavourable parameters, adjuvant oncological therapy may improve the prognosis (Bottger et al., 1996). DNA ploidy evaluation may be useful in the diagnosis on cytological samples and could represent an independent prognostic parameter in predicting the survival outcome of patients with hepato-cellular carcinoma (Zeppa et al., 1998).

The diagnosis of malignancy in endocrine tumours of the pancreas is difficult. The DNA content of malignant endocrine pancreatic tumours has an influence on the long term survival. Hypertriploid tumours have a statistically significantly worse prognosis than diploid, hypotriploid or triploid tumours (Bottger et al., 1997).

In a study on 168 colorectal cancers, 43% were classified as diploid and 57% as non-diploid. After a follow-up of six to seven years, a significant survival advantage was found for diploid compared with non-diploid cases. A long-term (8 years) survival rate was reported in 70% patients with a diploid DNA tumour versus 46% patients with non-diploid tumour (Kay et al., 1996). Tumour ploidy status measured by ICM might be useful in determining risk of colorectal cancer recurrence and death in patients following the resection of early colorectal cancer. However, no correlation appeared between ploidy and histological features (Sampedro et al., 1996). Finally, ICM analysis may be considered as highly efficient in the detection of aneuploidy in colorectal carcinomas and may prove useful for guiding adjuvant therapy.

Renal cell carcinoma

The course of patients suffering from renal cell carcinoma depends on various factors. The measurement of the nuclear DNA content has been considered as a prognostic indicator in addition to morphological clinical parameters. DNA parameters allow us to divide the patients into high-risk and low-risk groups. A significant correlation between the size of the tumour, lymph node metastases and distant metastases was obtained (Nenning et al., 1996). In another study of 70 renal cell carcinomas, diploid stem lines tended to be characterized by local growth, whereas tetraploid or aneuploid tumours showed a tendency toward venous invasion and perirenal spread (Papadopoulos et al., 1995).

Prostate carcinoma

The prognostic value of the DNA-MG and of the DNA-RI for survival has been tested in 19 patients with prostate carcinoma under hormone therapy. The DNA-MG was demonstrated to be an objective index of high prognostic value for the individual patient and for prediction of the prospective tumour response to hormone therapy. The DNA-RI gave additional prognostic values concerning tumour regression and cancer patient survival under therapy, independently of the DNA-MG. The DNA-RI allows the early identification of an ineffective therapy (Böcking et al., 1985).

The relative number of tumour cells in the proliferation phase of the cell cycle (S-phase) has been reported as an index of aggressiveness in prostate cancer. This cancer frequently includes a fairly low relative number (inferior to 10%) of cells in S-phase, even in aggressive tumours. DNA content is strongly related to tumour grade and stage, which allows a prognostic value with respect to overall or disease-specific survival. However, in localised tumours, the additional prognostic value of DNA content is less convincing when analysed with traditional prognostic factors (tumour grade and stage) (Adolfsson, 1994). DNA histograms show a single peak in the diploid range for the hyperplasia and Prostatic Intraepithelial Neoplasia (PIN). For well-differentiated carcinomas, two peaks can be observed both in the diploid range and in the tetraploid range. In poorly-differentiated carcinomas, an aneuploid distribution has been observed (Irinopoulou et al., 1997).

Bladder tumour

Tumours with DNA-aneuploidy were frequently of high malignancy, while DNA-diploid tumours were of low malignancy, as demonstrated by FCM (Klein et al., 1982). ICM on Feulgen-stained imprints of bladder biopsies is a simple and reliable procedure for assessing DNA ploidy in urothelial carcinomas. This analysis provides an important sensitivity for detecting small aneuploid peaks and multiploid tumours (Mainguene et al., 1997). Moreover, DNA analysis permits the increase in the detection sensitivity of relapses (Amberson and Laino, 1993; Seigneurin et al., 1993). By combining both conventional cytology and DNA cytometry, the overall sensitivity increased to 92% (instead of 72% with cytology alone or 84% with ICM alone) and 96% were reported with an additional immunocytological analysis (Lewis X antigen), which was associated with a decrease in specificity from 100 to 80% (Planz et al., 1998). It would be interesting to determine whether ICM associated with the Lewis X antigen detection could help to signal the relapse and the progression of transitional cell carcinoma of the bladder (Planz et al., 1998). Similarly, the combination of ICM and Fluorescence In Situ Hybridization (FISH) measurements of chromosome 9 aberrations provides an increase in sensitivity to transitional cell carcinoma either individually (55% for ICM and 42% for FISH) or in combination (92 to 97%) (Reeder et al., 1998).

For prognosis, DNA ploidy was significantly correlated to the relapse risk and the progression in superficial and invasive tumours (Collombel et al., 1995). In superficial tumours, 2cDI appears as the most important predictive survival factor (Schapers et al., 1993) and the relapse rate of non invasive tumours is correlated to the percentage of cells with a DNA content higher than 5c (Hemstreet et al., 1991).

Cutaneous tumours

A correlation exists between the DNA histogram type (according to Auer) and the lesion grade (atypic degree nevi) of cutaneous tumours (Schmidt et al., 1994). Using both ICM and FCM, Sangueza et al. (1993) analysed 38 dysplastic nevi to assess the DNA content of this type of nevus. These dysplastic nevi have been described to be associated with increased risk of melanoma (Sangueza et al., 1993). All cases demonstrated diploid populations by both methods.

In another study the DNA content in 61 different types of melanocytic nevi was determined and aneuploidy appears as a common feature of malignant melanoma (Talve et al., 1997).

Brain tumours

Treatment of most brain tumours by chemotherapy is ineffective, apart from some cases. For the development of new concepts in therapy, it would be interesting to characterize the proliferation properties of tumours such as gliomas (Jennemann et al., 1990).

DNA ploidy has no diagnostic value in the central or peripheral nervous system tumours (Salmon et al., 1995). For prognosis, medulloblastomas and astrocytomas represent particular cases because the forms with marked aneuploid DNA present a more favourable clinical evolution (Schofield et al., 1992) and an increased survival of patients, notably in the case of triploid tumours (Salmon et al., 1992a). The determination of DNA ploidy represents a valuable parameter in the definition of survival prognosis of children with neuroblastoma. For this disease, DNA aneuploidy was correlated with an increased patient survival time compared with patients with diploid or near diploid neuroblastomas (Bourhis et al., 1991). The benefits of histo-pathological grading and of DNA ploidy characterisation, with respect to the patient survival, have been studied in astrocytomas with complete clinical follow-ups (Salmon et al., 1995). Together with the degree of differentiation of astroglial tumours, the appearance of cell lines with abnormal DNA value and higher S-fraction have a prognostic value (Zaprianov and Christov, 1988). The prognostic value of both N-myc gene amplification and the DNA ploidy index was investigated in patients with neuroblastoma (Bourhis et al., 1991). The combination of N-myc and DNA index should be included in routine management of neuroblastoma.

Nuclear morphometry and texture analysis

In human oncology, textural parameters alone, or associated to the nuclear morphometry or to the DNA quantification can provide a real help for diagnosis, prognosis, therapeutic follow-up and to the understanding of cellular physio-pathology. This analysis would also detect DNA "chromosomal translocations" and could be considered as a real tumour marker.

Applications of nuclear morphometry analysis in oncology concern information in terms of diagnosis and tumour prognoses. However, the analysis of morphometry and DNA rate are not always associated to a significant diagnostic value for all locations (breast, melanomas,....). Then, the association of the textural parameters would improve the performances of ICM for the diagnosis. These data would provide an objective point of view on the grading and a differential diagnosis to influence the therapeutic strategy (Oberholzer et al., 1991).

Generally, this application to grading is not performed in routine analysis in addition to the well-known histo or cytopathological classification. A differential diagnosis between benign and malignant state and between the different degrees of malignancy is often difficult to realise. This diagnosis would be very useful due to its influence on the therapeutic strategy. However, careful statistical analyses are required due to the important number of morphological discriminant

parameters demonstrating an obvious correlation between themselves. Whatever the parameter considered, this would provide an aid to diagnosis and also when associated with other classical parameters. Several authors use only textural parameters to realise a differential diagnosis in routine examinations (Christen et al., 1993).

For prognosis, ICM allows prospective studies which have been described in prostatic carcinoma (Jorgensen et al., 1997).

Breast cancer

In order to understand the subjective grading, the histo-cyto-pathological classification has been shown to be applicable with objectivity in the case of breast cancer. Nuclear pleomorphism, mitotic index and tubular differentiation degrees allowed the discrimination between different types of cancer according to the only textural parameters (Dufer et al., 1993).

Nuclear area has been analysed from breast cancer (stage I and II) with a follow-up of five years. This parameter is well-correlated to either the presence or to the absence of axillary metastases but not to the presence of hormonal receptors (Fregene et al., 1994). Thus, nuclear morphometry would allow the prediction of disease recurrence in the early stage of breast cancer (Fregene et al., 1994).

For invasive cancer prognosis, morphometry seems a more important criterion than other conventional prognostic factors like tumour size (Wolberg et al., 1995). The prognostic value of assessments (mean and standard deviation of nuclear area) in invasive breast cancer of patients with long-term follow-up was described to be dependent on the sampling methods. Indeed, a systematic random sampling of nuclei (compared to convenience method) provides an increased prognostic value for patients with invasive breast cancer (Wittekind and Schulte, 1987).

The chromatin pattern in breast cancers is related to the amounts of oestrogen receptors. Indeed, the quantitative chromatin pattern description (morphometrical, densitometric and textural features) allows the identification of pregnancy-associated breast cancer from hormone-insensitive ones. Besides, the DNA ploidy level determination did not allow this characterisation to be carried out (Budel et al., 1997). Textural parameters alone have been proposed to predict the behaviour of intraductal lesions (Palcic et al., 1993).

Ovarian cancers

For ovarian granulosa cell cancers, both area and nuclei perimeter increase significantly for women showing relapse or death during their follow-up period (Haba et al., 1993). In addition, nuclear area has been confirmed as a predictive factor for women's survival (Katsoulis et al., 1995). In a study concerning sixty women with advanced epithelial ovarian cancer (FIGO III and IV), morphometry (mitotic index, nuclear perimeter, shortest and longest nuclear axis,...) can contribute to the diagnosis and to therapeutic decisions (Baak et al., 1985; Katsoulis et al., 1995). Indeed, the parameters related to nuclear dimensions but also the residual tumour size have been described as important predictors of the tumour response to cis-platin chemotherapy (Baak et al., 1988; Katsoulis et al., 1995). A differential diagnosis between dysplastic and cancer cells is also more precise when based on a quantitative evaluation of texture according to nuclear area (Deligdisch et al., 1993).

Cervical and endometrial cancers

Automated devices have been developed to screen cervical cytology slides for the detection of pre-invasive lesions (Anderson et al., 1997). For cervical cancer, results are similar to those of ovarian tumours. Nuclear morphometry (area, perimeter, roundness, ...) is actually considered as a predictive factor of the response to neo-adjuvant chemotherapy associated with radiotherapy in locally advanced cervical cancer (Yacoub et al., 1994). A combination of different nuclear texture features has been the most frequently studied for the quantitative assessment of both abnormal cervical cells and apparently normal intermediate cells (Anderson et al., 1997).

In cervical dysplasia, ICM could represent an important tool, considering the difficulty in the classification of these dysplasia. Differential diagnosis appears more accurate when based on the nuclear texture analysis (Deligdisch et al., 1993). For example, a discriminant analysis based on three textural parameters already gives a classification rate of 74.3%. When the surface area and IOD were added, this correct classification reaches 84.6% (MacAulay, 1990). In Cervical Intraepithelial Neoplasia (CIN) of women with a cytological follow-up only, features of chromatin organisation can potentially be used to predict the malignant or the progressive potential of these lesions, according to the extent of nuclear atypia as reflected in the CIN category (Hanselaar et al., 1998).

In endometrial lesions, the combination of textural parameters, nuclear area parameter and anisonucleosis degree improves the important discrimination between benign and malignant cases (Tezuka et al., 1995).

Thyroid cancer

A differential diagnosis between normal and neoplastic thyroids is more precise when based on a quantitative evaluation of the texture associated with the nuclear area (Unger et al., 1992). Morphometrical measurements showed a significant and a positive correlation between an increase in nuclear area and the development of thyroid pathology (normal to neoplastic stages) (Liautaud-Roger et al., 1992b; Salmon et al., 1992b).

However, for the follicular carcinoma of the thyroid

gland, no correlation has been displayed between morphonuclear parameters and patient prognosis (Palestrini et al., 1994).

The differential diagnosis of thyroid neoplasms by routine cytology presents major difficulties. A comparison of features in normal (from multi-nodular goiters), benign (from adenomas) and neoplastic (from carcinomas) nuclei of thyroid tissue was realised by digital cell image analysis. These quantitative features described the nuclear size, the DNA content and the chromatin texture (Salmon et al., 1992b). The nuclear area and the significant decreased condensed chromatin percentage associated to an increased heterogeneity have also been demonstrated to separate benign from malignant stages (Salmon et al., 1992b).

For an efficient contribution in diagnosis, a prospective study can be performed and a data bank can be established (Liautaud-Roger et al., 1989, 1992b). Textural features are very precise parameters because they can detect a simple chromosomal translocation in thyroid carcinomas. Textural parameters can have their own prognostic value in medulla thyroid cancers. When studied individually or associated with other morphological data and according to the histology and the disease type (sporadic or hereditary), the multivariate analysis (Cox model) shows that only age, stage, sex and percentage of cells with DNA > 5N have a predictive value (Galera-Davidson et al., 1990). One densitometric parameter and two textural parameters can well separate adenomas and carcinomas. Moreover, tumours with or without chromosomal translocation are discriminated with three geometrical parameters (Liautaud-Roger et al., 1989, 1992b; Teyssier et al., 1990).

Cell lung carcinoma

No correlation between the nuclear morphometry (nuclear area, nuclear perimeter, diameters,...) and the prognosis in stage I Non-Small Cell Lung Carcinoma (NSCLC) has been observed (Cagle et al., 1992). In Large Cell Carcinoma (LCC), morphometry may therefore be helpful in the differential cytological diagnosis of adenocarcinoma and LCC (Burns et al., 1989).

Digestive carcinomas

Nuclear morphometry has been described as a prognostic factor of laryngeal squamous cell carcinomas (Panayiotides et al., 1993). The perimeter and the minimal diameter of nuclei have lower values in patients with bad prognosis (Panayiotides et al., 1993).

For oesophageal cancer, nuclear area and circularity factor values were higher in cancer tissues than in the healthy epithelium. This increase seems more important in patients with short survival or exhibiting metastases (Wang et al., 1994).

A fitted combination of several parameters (related to the densitometry or to the texture) was reported to be correlated with the malignant score and the histological grading in different pancreas tumours (Rickaert et al., 1992). From pancreatic lesions, this morphological description offers the same information as the conventional histo-pathological grading (Rickaert et al., 1992). Chronic pancreatitis and pancreatic adenocarcinoma were distinguished with 82% of sensitivity and specificity using DNA quantification, morphometry and texture analysis (Sears et al., 1998). Finally, morphonuclear features were used to classify ampullary and biliary adenocarcinomas (with for example, 71% of sensitivity) and were well correlated to pathological classification and survival data (Yeaton et al., 1998).

For colorectal cancer, morphonuclear values strongly differ between normal and carcinoma tissues (Deans et al., 1993). However, no correlation has been described between the nuclear morphometry and the tumour prognosis. The same analysis was performed in colorectal polyps (Verhest et al., 1990). Five parameters (nuclear area, IOD and three parameters of texture) provide the best discrimination between normal cells and borderline cells. IOD and nuclear area alone are able in several cases to well separate normal and tumour cells.

Renal cell carcinoma

In the early stage of renal carcinomas, the parameters associated with the nuclear shape enable a correct identification of patients at high risk for relapse after surgery as candidates for adjuvant therapy. These parameters appear as predictive markers with a confidence higher than 73% and improve significantly the prognostic value derived from the stage and the grade of the tumour (Carducci et al., 1995). Nuclear shape parameters are also well related to the patient's survival (Delahunt et al., 1994) and may be the best discriminant diagnostic parameter, with an 80% correct classification between oncocytomas and carcinomas (Castren et al., 1995). Thus, classification strategies, based on nuclear morphometry and densitometry could contribute to a significant diagnostic information for renal cell carcinomas and would reduce the problem of inter-observer reproducibility (François et al., 1997). The chromatin pattern of cell nuclei could be of prognostic value. This was investigated on a series of 105 renal cell carcinomas, generating thirty morphonuclear and eight nuclear DNA content-related variables. This analysis was compared to conventional diagnostic and/or prognostic markers (including histo-pathological grades, tumour invasion levels and the presence or the absence of metastases) and allows more correct predictions (François et al., 1998).

Prostate carcinoma

At present, no histo-pathological grading system has been widely accepted for this type of carcinoma. Patients at a given stage and grade have considerable individual

variations in their response to treatment, disease progression and survival. Therefore, a study was performed to evaluate the relationship between morphometrical features and the prognosis in patients with clinical stage B prostatic cancer. As the histological and conventional grading in prostate adenocarcinomas is poorly reproducible, Schultz et al. (1990) developed a classification based on morphology (nuclear area, perimeter....), cellularity, or texture criteria which was compared to histology. This classification allows the separation of low grade cancers (well differentiated) and high grade (poorly differentiated) with a rate of 81%. However, moderately differentiated cancers cannot be classified as a distinct group (Schultz et al., 1990). In PIN and carcinomas, a considerable variation in the volume of nuclei has been reported (Irinopoulou et al., 1997). The changes described may lead to the monitoring either of the lesion progression or the response to treatment or the chemopreventive intervention (Bartels et al., 1998b). The findings of characteristic changes in nuclear chromatin texture of nuclei from histologically normal tissue in prostates with PIN or adenocarcinoma offers the potential for a higher sensitivity of detection of such lesions and for an earlier detection of changes that precede the development of a clinically significant disease (Bartels et al., 1993, 1998a). The mean nuclear area allows the separation between benign prostatic hyperplasia and adenocarcinoma (Montironi et al., 1990).

Textural parameters can discriminate two populations of patients having a different prognosis and could then be a help for prognosis. Among a population of twenty-three patients with stage B (US clinical staging) prostatic carcinoma, two classes have been separated according to the absence or the presence of metastases three years after surgery (Irinopoulou et al., 1993). In this report, a linear combination of five textural parameters allows a complete separation (100%) between the groups with a good or a poor prognosis (Irinopoulou et al., 1993). In this same type of metastatic cancer, the prognostic value of texture has been compared with classical clinical parameters. In this way, it has been demonstrated that the texture is the most important prognostic factor using multivariate analysis with the Cox model (Jorgensen et al., 1997). Textural parameters are able to predict the possible biochemical progression of these cancer forms. The post-surgery score of Gleason presents an important predictive value (sensitivity of 73% with 84% of specificity) (Veltri et al., 1996). The association of parameters and Gleason score increases the sensitivity to 89% without changing the specificity. This will be important in adapting treatments and to stratify patients in function of their relapse (Veltri et al., 1996).

For prognosis, the main interest of ICM concerns prospective studies (Jorgensen et al., 1997). In advanced prostatic carcinomas (D2 stage) with bone metastases, there is no prognostic factor. Two populations of patients have been analysed and compared, with an association of four textural parameters; 95% of good classification was obtained after surgery. These two classes are separated according to the presence or the absence of the lesion progression for three years. This combination of parameters can make the separation of populations with or without relapse possible (Jorgensen et al., 1997).

Bladder cancer

The possibility of identifying urothelial neoplasia and normal urothelium by ICM has been investigated from urinary sediments. Cell profiles using 18 parameters related to size, shape, densitometry and chromatin texture were created. The results suggested that the cell population features may be of diagnostic values in designing a classifier dedicated to the prescreening of urinary sediments for the detection of bladder cancers (Brugal et al., 1986). Using three nuclear texture features, this method may be become clinically relevant as a supplement to conventional cytological examination (Gschwendtner et al., 1999).

Bladder cancers are often classified in I, II or III stages. Types I and III are easily recognised but type II is less obvious due to its continuity with I and III. Three parameters related to the densitometry can separate better benign versus malignant tumours (I, II and III different stages) and superficial versus invasive tumours (van Velthoven et al., 1994).

Texture can also be considered as a marker and can predict a relapse (van Velthoven et al., 1995). In two populations of in situ bladder carcinomas, a score based on the measurement of five textural parameters and nuclear area parameter predicts 91% of relapse (Wheeless et al., 1993; van Velthoven et al., 1995). Besides, the ploidy level only predicts the relapse in 41% of cases. Finally, nuclear morphometry allows the classification of carcinomas with transitional cells with important reproducibility and objectivity (Blomjous et al., 1989; Seigneurin, 1996). Tumour recurrences are predicted with measurements of morphonuclear parameters (Borland et al., 1993). The most informative are: the nucleus/cytoplasm area ratio, the area and circularity of the nucleus (Wehner, 1986; Fukusawa et al., 1995). These criteria contribute the discrimination of low and high grade bladder carcinomas (Pich et al., 1994).

Cutaneous tumours

Chromatin texture analysis was used to discriminate between nevi and melanomas (Stolz et al., 1991). This analysis allowed individual nuclei to be classified as malignant or benign with 79.2% accuracy, and the lesions themselves to be classified as common nevi or malignant melanomas with 100% accuracy (Stolz et al., 1991). However, this study did not evaluate dysplastic nevi which present greater diagnosis difficulties than common nevi. Fleming and Friedman (1993) have reported that textural parameters and nuclear perimeter

can help in discriminating benign cells from malignant cells (with 81.8% good classification) but not lesions into nevi or into melanomas (with only 93% accuracy) (Fleming and Friedman, 1993). However, morphonuclear characteristics of melanomas from women and men were very distinct and depend on sex steroids (Lorea et al., 1997).

Brain tumours

The classification of tumours of the central nervous system (astrogliomas and astrocytic tumours) has been recently revised (Kleihues et al., 1993). The histological and cytological aspects are very pleiomorphic, which increases the difficulty to realise a differential diagnosis. A discriminant analysis based on two textural parameters separates all astrogliomas with low grade and glioblastomas. When a parameter is added (for example IOD), the discrimination rate between low and high grade can also be improved (Scarpelli et al., 1997).

Tumour physio-pathology

The existence of malignancy-associated changes (MACs) has been described in various locations (in blood cells, in bone marrow cells, in bronchial epithelial cells,...) in patients with malignant tumours (Nieburg et al., 1967; Ikeda et al., 1998). These changes in the nuclear texture which appear in the normal cells which are near neoplastic cells have been reported as useful criteria to determine biological behaviour of intra-epithelial (pre-invasive) neoplasia (Ikeda et al., 1998). Indeed, in bronchial epithelial cells, the frequency of cells expressing MAC increased as the degree of abnormality of the groups increased (Ikeda et al., 1998).

In colorectal system, these local and diffuse changes (genetic alterations,...) could be an epiphenomenon which accompanied a progression towards a malignant transformation of the cells (Vogelstein et al., 1988). The same results were obtained in the cervix (Hanselaar et al., 1991). A system based on the changes of chromatin texture has been reported as a new biomarker (Palcic et al., 1993). Nevertheless, this interesting concept must be validated with reliable data bases probably different according to each pathology.

Phenomenon related to diagnosis and to prognosis of cancers, other than nuclear morphometry can be analysed such as tumour breast carcinoma vascularisation (Visscher et al., 1993) or counting of mitosis using proliferation markers such as Mitotic Activity Index (which accounts for the total number of mitosis in the most cellular area at the periphery of the breast tumour) (Jannink et al., 1995b; Biesterfeld et al., 1995).

Conclusion

Due to improved tools of image acquisition and treatment, diversified tumour types have to be analysed with a new objective classification. Compared with FCM measurements, ICM also has its own advantages and limits. When the tumour area represents a small proportion of the analysed tissue section, ICM allows a DNA content analysis, which would better correspond to the tumour cell component of the sample than FCM (Mesker et al., 1989).

A comparative study with ICM or FCM was performed on 166 cases of breast cancer and displays a correlation between the DNA indices measured with each technique (Cornelisse et al., 1984). FCM has become a routine method in breast cancer diagnosis for evaluation of ploidy and proliferation kinetics. In such cancer, ICM appears less practicable and provides less information than FCM (Sinn et al., 1997). If possible, FCM could be combined to ICM (Ottesen et al., 1997). ICM appears to be supplementary to FCM for the study of DNA ploidy abnormalities and combined results of these methods have a major influence on the clinical outcome (Rodenburg et al., 1987).

DNA image analysis is frequently performed in clinical practice as a prognostic tool and to improve diagnosis (Puech and Giroud, 1999). An important correlation has been frequently reported between a prognostic survival factor and aneuploidy. Moreover, this analysis could be improved by combining ICM with immunocytological analysis. The precision of prognosis and diagnosis depends on the accuracy of analysis and particularly on the quality of image analysis systems. Tools, procedures and criteria for evaluation of system quality of image analysis have been described to propose limits of accuracy. According to the conclusions of European projects called PRESS (Prototype Reference Standard Slide) and EUROPATH (European Pathology Assisted by Telematics for Healthcare) which control accuracy limits, some image analysis systems are not qualified to deliver sufficiently precise DNA measurements for cancer case analysis (Giroud et al., 1998; Haroske et al., 1998a,b; Puech and Giroud, 1999). In DNA image quality, the standardisation is obligatory and the use of an internal control for determining the diploid peak in a histogram of samples is recommended (Thunnissen et al., 1997)

Although the DNA ploidy measurement for diagnosis was frequently reported, results from the reported examples above were not always obvious. These measurements appear to present a higher interest in prognosis in terms of survival, relapse prediction, metastatic dissemination and response to chemotherapy. Besides, the main difficulties of these analyses would be related to a weak inter-laboratory reproducibility of these measurements. Thus, the interpretation of these results in daily routine clinical practice would become optimal with the setting of an adequate quality control (Giroud, 1987; Bacus and Bacus, 1994). Consensus documents have therefore been elaborated (Böcking et al., 1995) and validations (e.g. Euroquant server) are in process of setting at an european level (European Society of Analytical Cellular Pathology, ESACP) consensus on

diagnostic DNA (Giroud et al., 1998; Haroske et al., 1998a,b).

Nevertheless, ICM would help for the diagnosis between benign and malignant lesions (Seigneurin, 1995) and nuclear morphometry and chromatin texture analyses have been proposed to realise a differential diagnosis in routine examinations (Oberholzer et al., 1991).

Finally, it should be concluded that the measurements obtained with ICM could be helpful for a decision but not substitute the classical approach of the pathologist (De Meester et al., 1991).

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