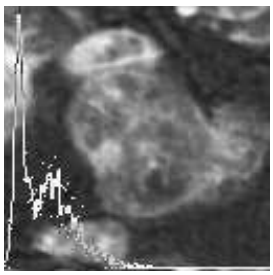
How does IDL rate?	
Flexibility	*5*
Simplicity	*3*
Time saved	*5*
5=highest	

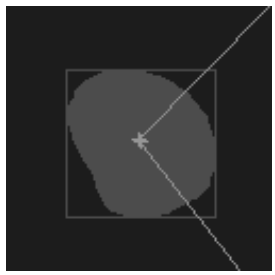
Microscope Image Analysis

Quantitative Microscope Image Analysis for Improved Diagnosis and Prognosis of Tumours in Pathology

In addition to clinical, cytological and histological parameters morphometric parameters of cell nuclei are obtaining increasingly more importance for objective diagnosis and prognosis assessment. Different research studies prove the worth of image analytical procedures. The distribution of DNA content (Deoxyribonuclein acid) of cells from an organ or tumour area is already being routinely measured in some clinics and applied for assessment (DNA-analysis). In the meantime it is becoming apparent that quantitative evaluation of chromatin arrangement in cell nuclei leads to comparable results with DNA analysis and allows additional questions. An essential advantage of the analysis of chromatin arrangement is its applicability on histological sections. DNA analysis normally needs the whole cell nucleus for evaluation the so-called cytological measurement. In fact histological material exists in archives in large quantities and hence it is possible to perform large (retrospective) studies. The procedure of mea-suring stained cell nuclei is briefly described.



Nucleus in extinction with frequency distribution



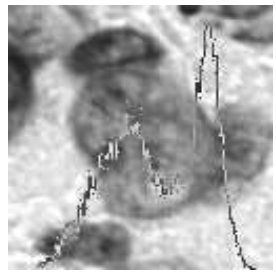
Mask of nucleus with surrounding box and principal axes

After selection of the cell nucleus and its digitisation with a CCD-camera, the cell nucleus is segmented in the image and (at least during the development step) examined with a multitude of analysis procedures. Each procedure delivers quantitative features. These features are then correlated with external criteria such as survival time or diagnosis. The features with highest correlation or most discriminatory power are selected. This procedure is called "supervised learning". In later routine work the calculation of the chosen features only is necessary.

Which features are finally calculated ?

For the training phase extinction features like total sum (IOD = integrated optical density), mean value, standard deviation, entropy etc. from different areas of the cell nucleus are calculated. Extinction is physically related with mass of stain and hence its distribution reflects the arrangement of stain. E.g. with Feulgen stain the amount of DNA in the nucleus can be measured. The selection of different areas follow the procedures of visual observation of the pathologists to

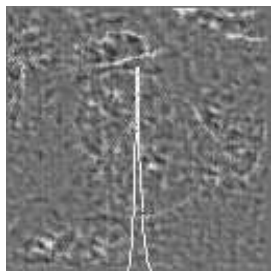
- discriminate chromatin between margin and centre of nucleus
- discriminate the total arrangement of extinction
- observe the arrangement of eu- (dark) and hetero- (bright) chromatin areas or particles inside the nucleus.



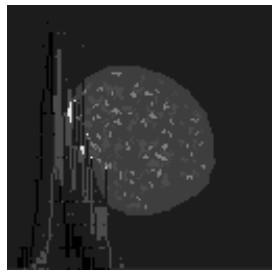
Section of a nucleus in transmission with frequency distribution

The features mentioned describe in a statiscial manner the shape of frequency distributions. This is the reason that in the illustrations frequency distributions are mostly outlined.

In addition different procedures derived from pattern recognition for texture analysis are used. For a reproducible generation of border, eu- and hetero-chromatin areas, methods from the field of mathematical morphology are applied. The evaluation of images and their representation are carried out completely with IDL including several user written C routines.



Flat texture image of the nucleus after 2D median filtering with frequency distribution



Eu- and hetero chromatin particles with extinction frequency distribution

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