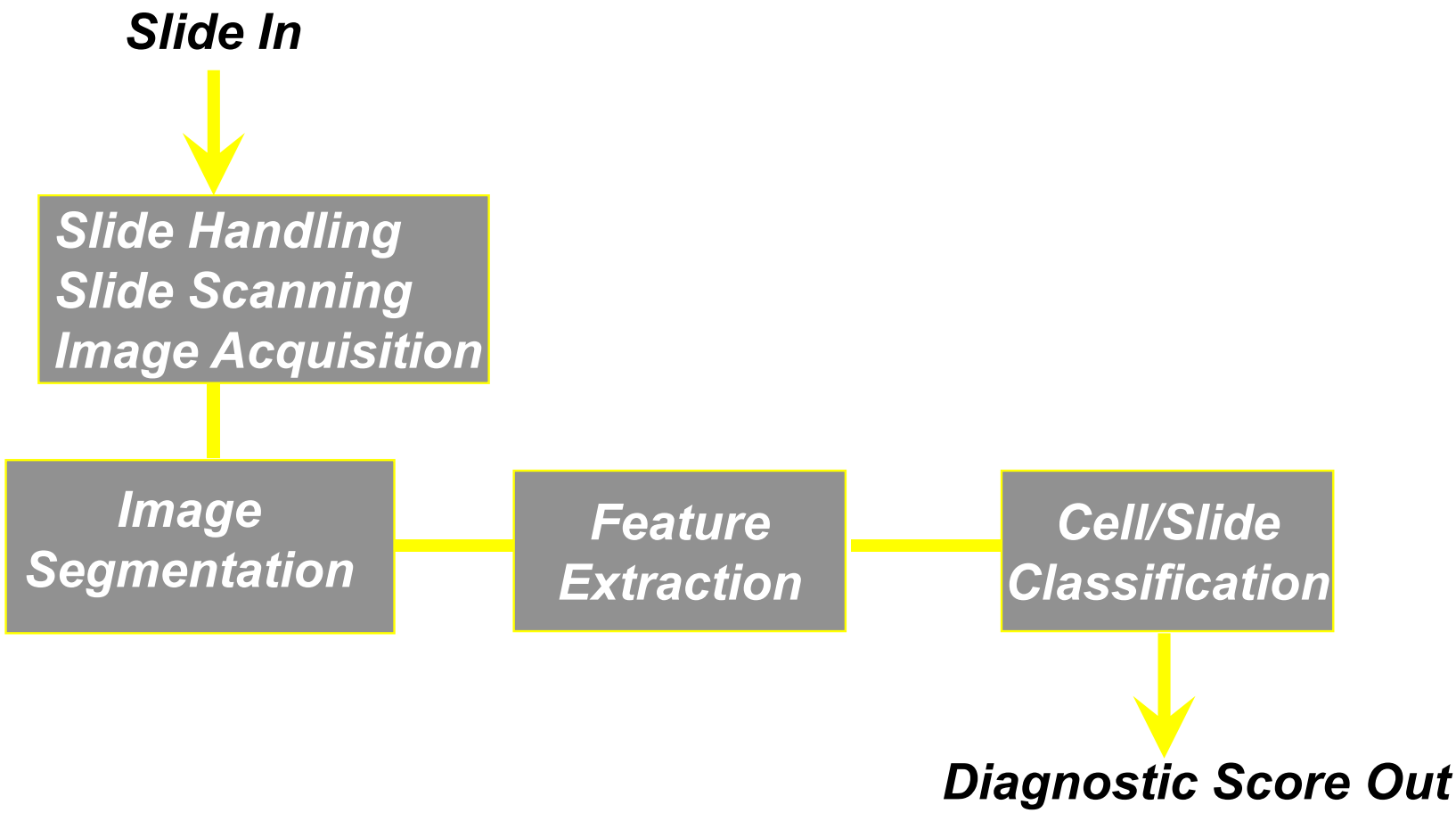
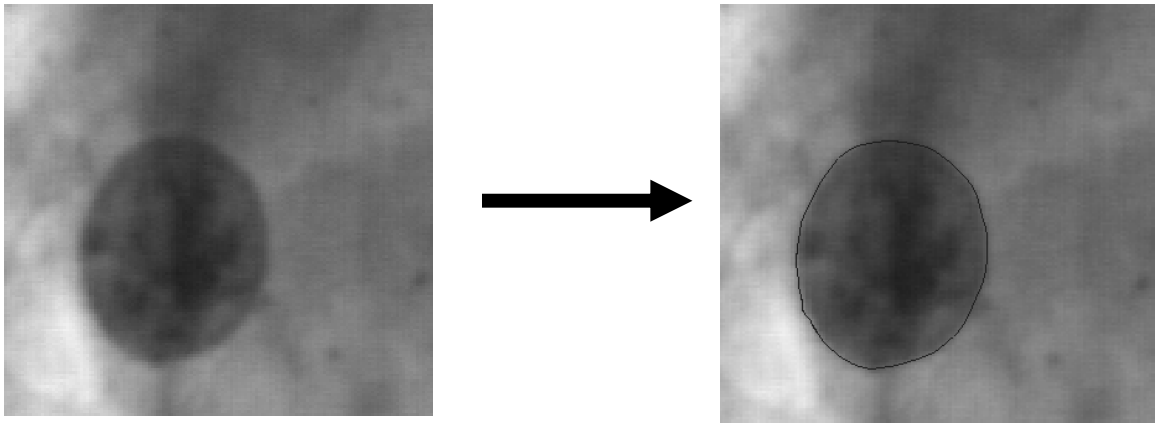
Case Study: A Methodology for Quality Control in Cell Nucleus Segmentation

Slides courtesy Pascal Bamford

Cytometric Image Analysis System



Cell Nucleus Segmentation



The images:

- **are of the order 128x128 pixels, 256 graylevels**
- **contain one desired object per image (maybe doublets)**
- **often contain many artefacts**
- **are highly variable due to staining and illumination**

Cost (Energy) of a Contour

$$\sum_i C(i,\lambda) = \sum_i \lambda \,\mathrm{curv}(v_{i-1}, v_i, v_{i+1}) + (1-\lambda)(1-\vec{\nabla} I(v_i))$$

Only one parameter

Where:

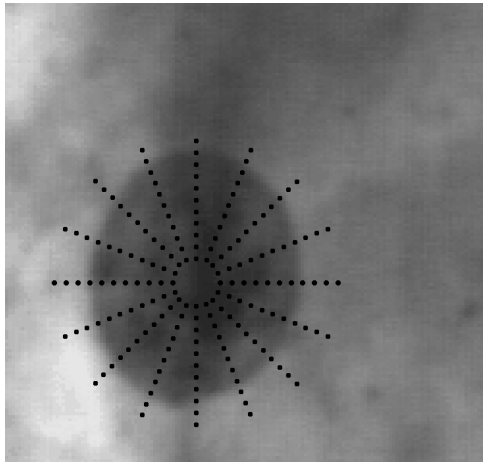
$$\mathrm{curv}(v_{i+1,j}, v_{i,j}, v_{i-1,j}) = \left(\frac{v_{i+1} - 2v_i + v_{i-1}}{v_{i+1} - v_{i-1}} \right)^2$$

is the curvature of the contour calculated locally over three adjacent points

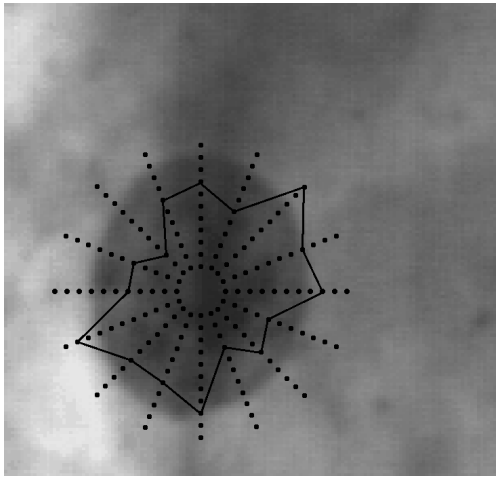
and

$\vec{\nabla} I(v_i)$ **is the directional gradient along a radius towards the centre of the object**

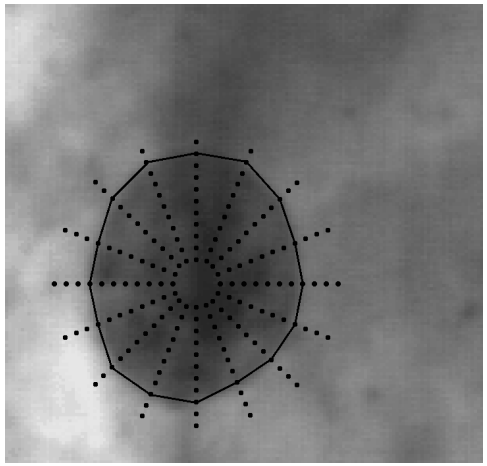
Method



Superimpose discrete search space on image



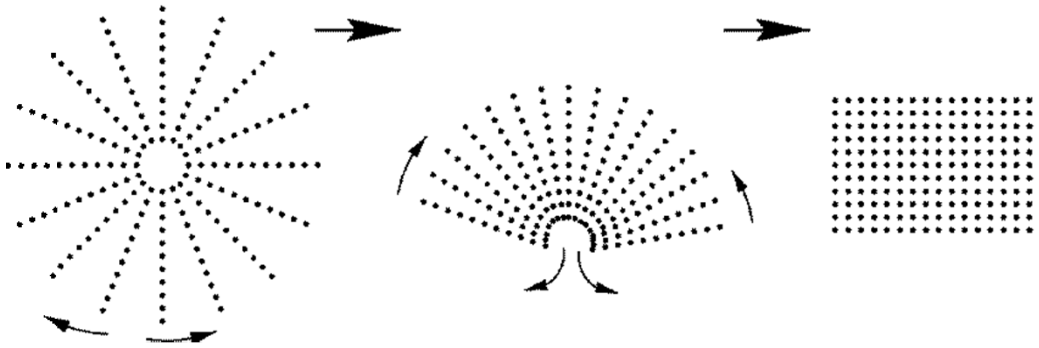
Evaluate every possible contour lying on the search space by choosing one point on each radius

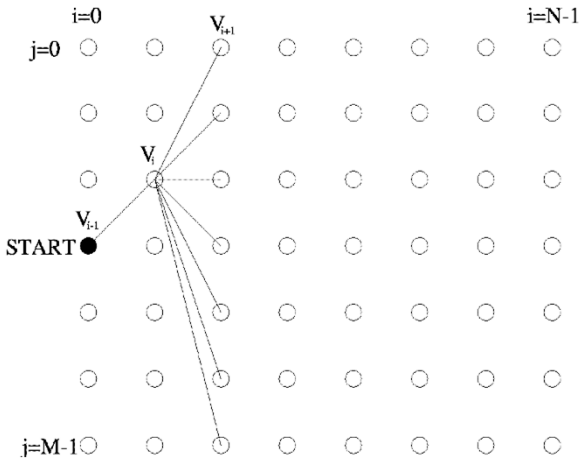


Choose contour that minimises cost function below

Viterbi Trellis Search Space

$$S_i(v_{i+1}, v_i) = S_{i-1}(v_i, v_{i-1}) + \min[C(i, \lambda)]$$

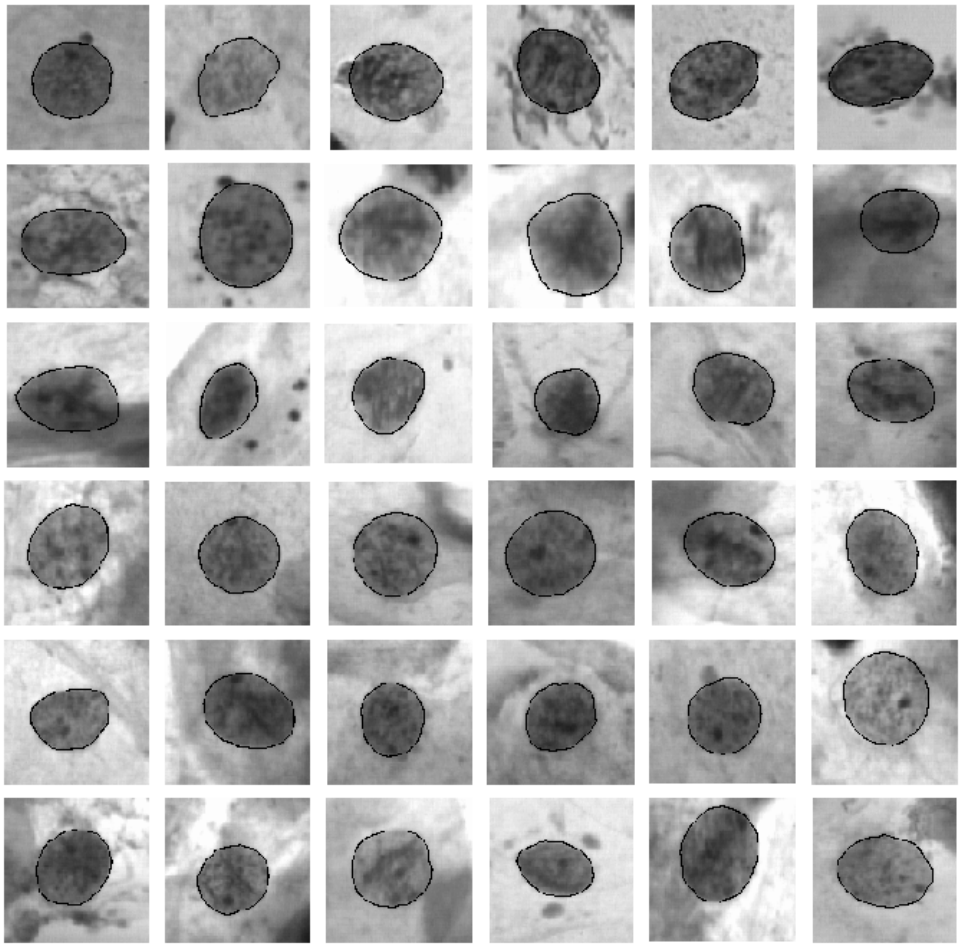




Segmentation Performance

- The algorithm was run upon a dataset of 20,130 images with λ=0.8
- Each image was examined (by a human) and the segmentation classified as ‘pass’ or ‘fail’
- 20,057/20,130 (99.64%) of images were correctly segmented
- Of the 73 failures:
  - –44 could be rectified by a better marker,
  - –26 could be rectified by using another value of λ,
  - –3 were unsegmentable and failed at all attempts

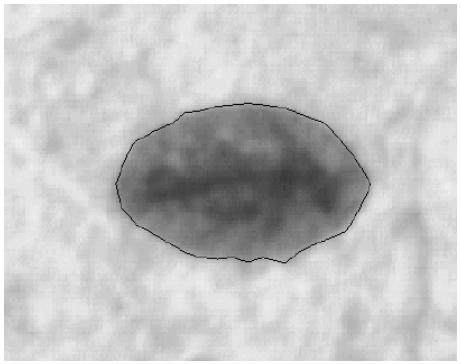
Typical Segmentation Results



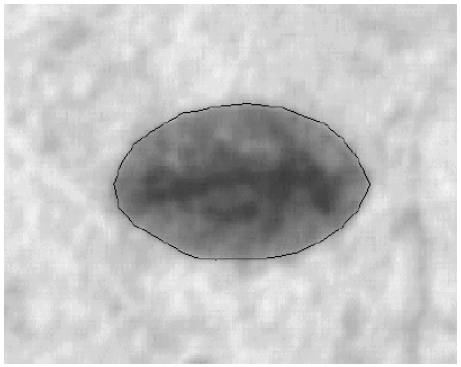
Interpretation of Parameter Lambda

- This parameter determines the weighting between smoothness and requirement for contour to lie on high gradient edges
- $\lambda=0$, segmentation based on gradient information alone; sensitive to “noise”
- $\lambda=1$, segmentation based on smoothness constraint alone; produces circle and ignores image

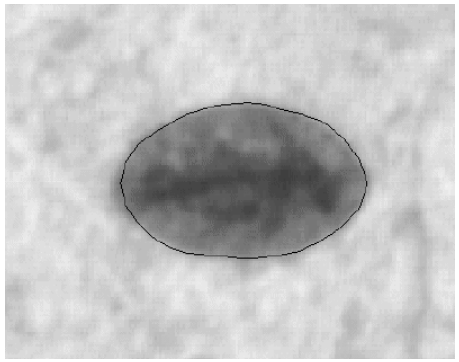
Lambda Sensitivity



λ=0.1

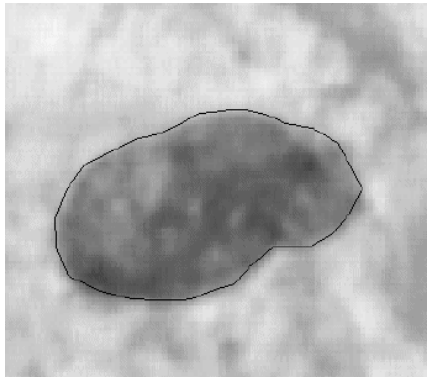


λ=0.5

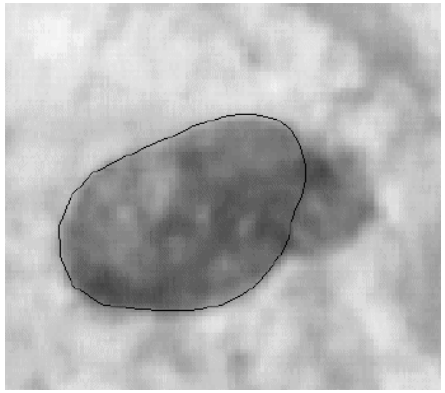


λ=0.9

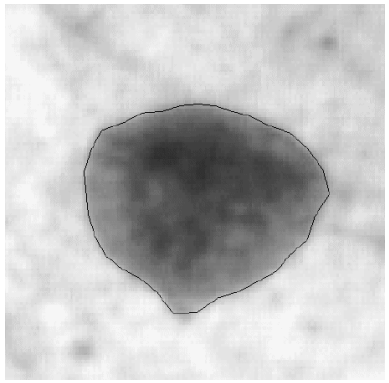
Observation: 'Easy' to segment images are stable over a wide range of λ



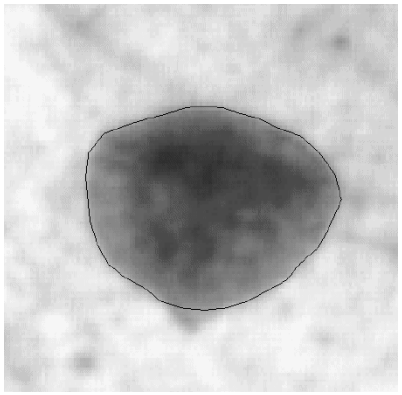
λ=0.5



λ=0.7



λ=0.5



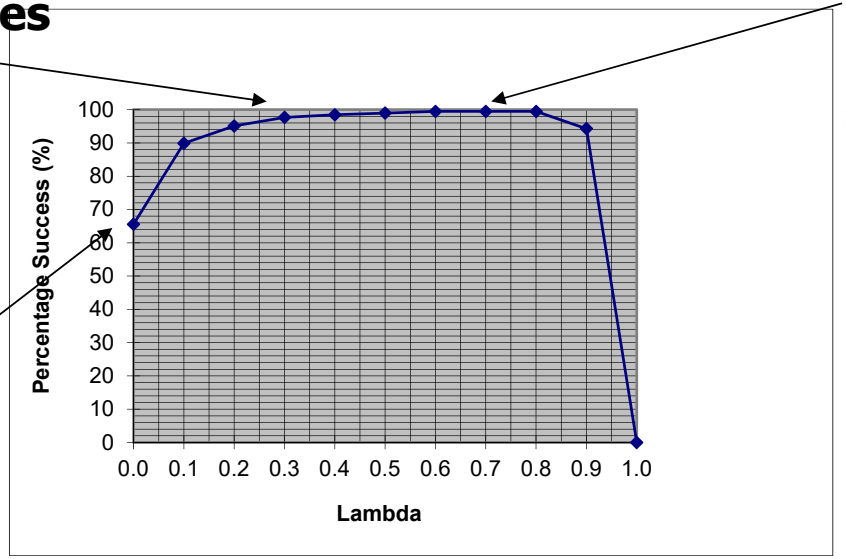
λ=0.8

Observation:'Difficult' to segment images are not stable over a wide range of λ

Probability of Correct Segmentation

Harder images segmented correctly

Easy images segmented correctly



Overall highest probability of correct segmentation 99.6%

In order to gain an *a priori* estimate of the probability of a correct segmentation, the above graph was obtained by segmenting a subset of 772 images with values of λ in the range $\lambda = 0.1k$ where $k = 0,1,2,\ldots,10$.

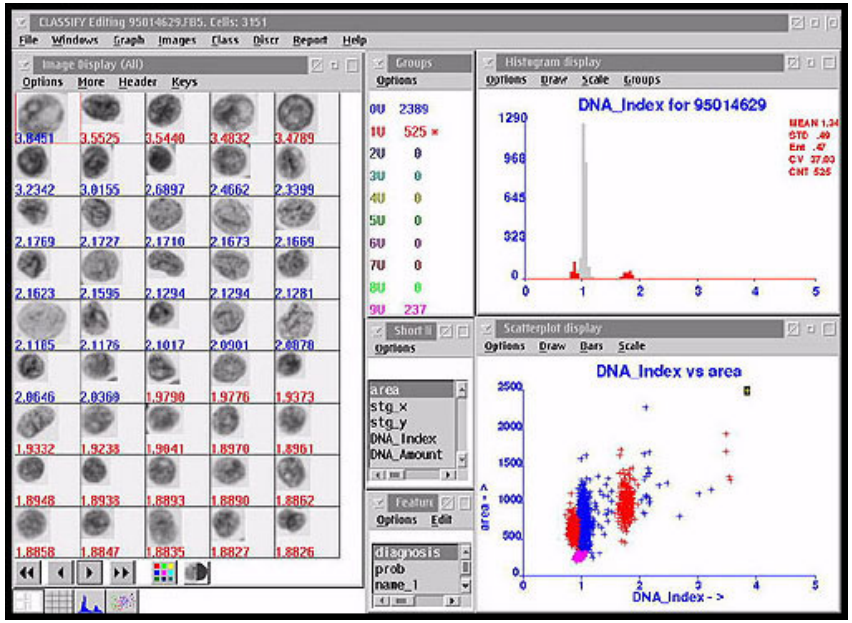
Classification into “Easy” and “Hard” Images

1. Segment all images with $\lambda = 0.7$ say
2. Resegment at $\lambda = 0.0, 0.1, 0.2, \ldots$
3. Observation: Cell Images correctly segmented for low λ (<0.5) are strict subsets of the images correctly segmented at $\lambda = 0.7$
4. If segmentation at $\lambda = 0.7$ and $\lambda = 0.0$ are the same, image was “easy” and probability of segmentation error at $\lambda = 0.7$ is extremely low. Just about any method would work for these.
5. Similarly, if segmentation at $\lambda = 0.7$ and $\lambda = 0.1$ are the same, but different from $\lambda = 0.0$ segmentation, image is “harder” with higher probability of segmentation error at $\lambda = 0.7$.
6. Achieve 100% correct segmentation by rejecting the images classed as “hard.” (about 10% of the database)

AcCell-SavantTM

The AcCell-SavantTM is a fully-automated, high-resolution, absorbance microscopy-based image cytometer that processes Thionin-Feulgen-stained cytology preparations and presents analytical results regarding the cellular DNA content of processed samples.

Cell Analysis Interface



Typical digital images of DNA-stained nuclei and the associated ploidy distribution from an aneuploid population of cells from a mesothelioma. The AcCell-SavantTM also displays in this sample the DNA index as a function of the area of the sampled nuclei.

Outcomes

- We now have a reliable method of grading images into “easy” and “hard” classes without human intervention
- Can achieve 100% correct segmentation by automatically rejecting a small percentage of the images collected.

Malignancy Associated Changes (MACS)

- MACS - specific sub-visual changes in cell morphometry, optical density and chromatin distribution in a proportion of visually normal cells in smears from or near malignant and pre-malignant lesions
- MAC analysis is a new way of looking at cells on a smear
- Made possible due to CCD camera development, advances in computing power and modern image analysis algorithms

Special Features of MACS

- MACs can be detected in cells distant to dysplastic and malignant lesions, thereby reducing false negatives which result from inadequate sampling of lesions (no requirement for “diagnostic” cells in smear)
- MAC analysis can detect very subtle sub-visual cellular changes and therefore can detect cancer at an early stage allowing early intervention

MACS

- Objective MAC scoring eliminates the subjectivity of visual smear assessment
- The MAC score gives an indication of the likely behaviour of lesions and therefore has prognostic value
- Needs less expensive hardware
- May enable screening for other cancers *e.g.,* lung, oral, bladder.