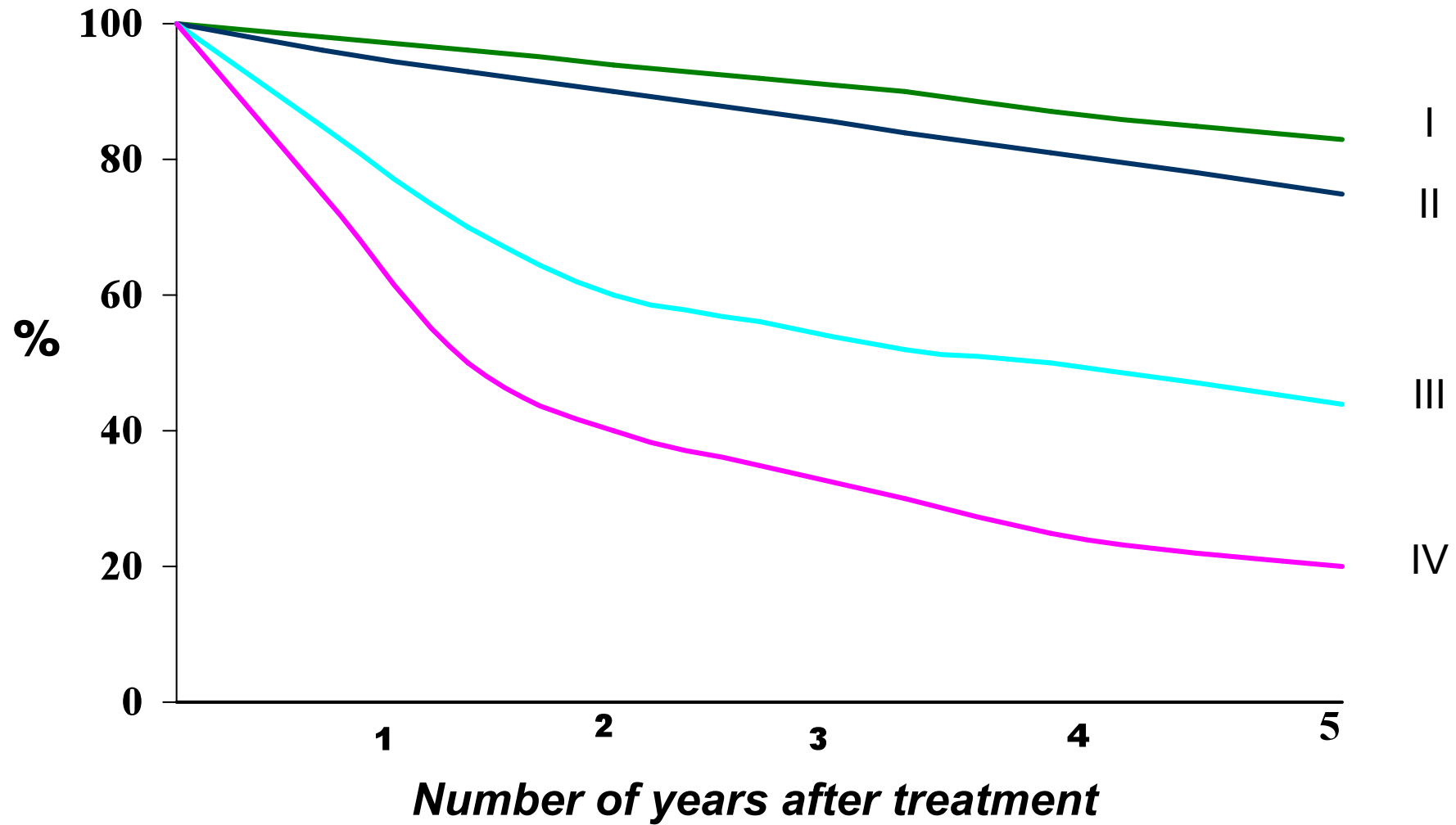
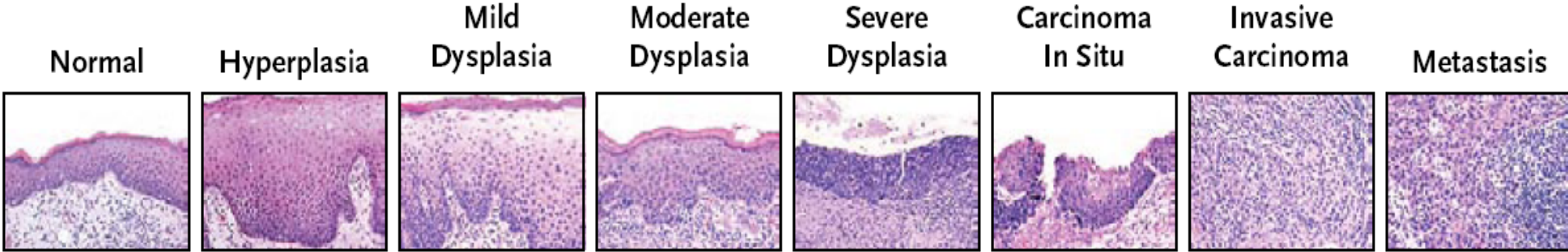


Improvements in biomarker labeling and analysis in tissue sections

Assessing the risk of progression of oral premalignancies

5 year survival for oral cancers

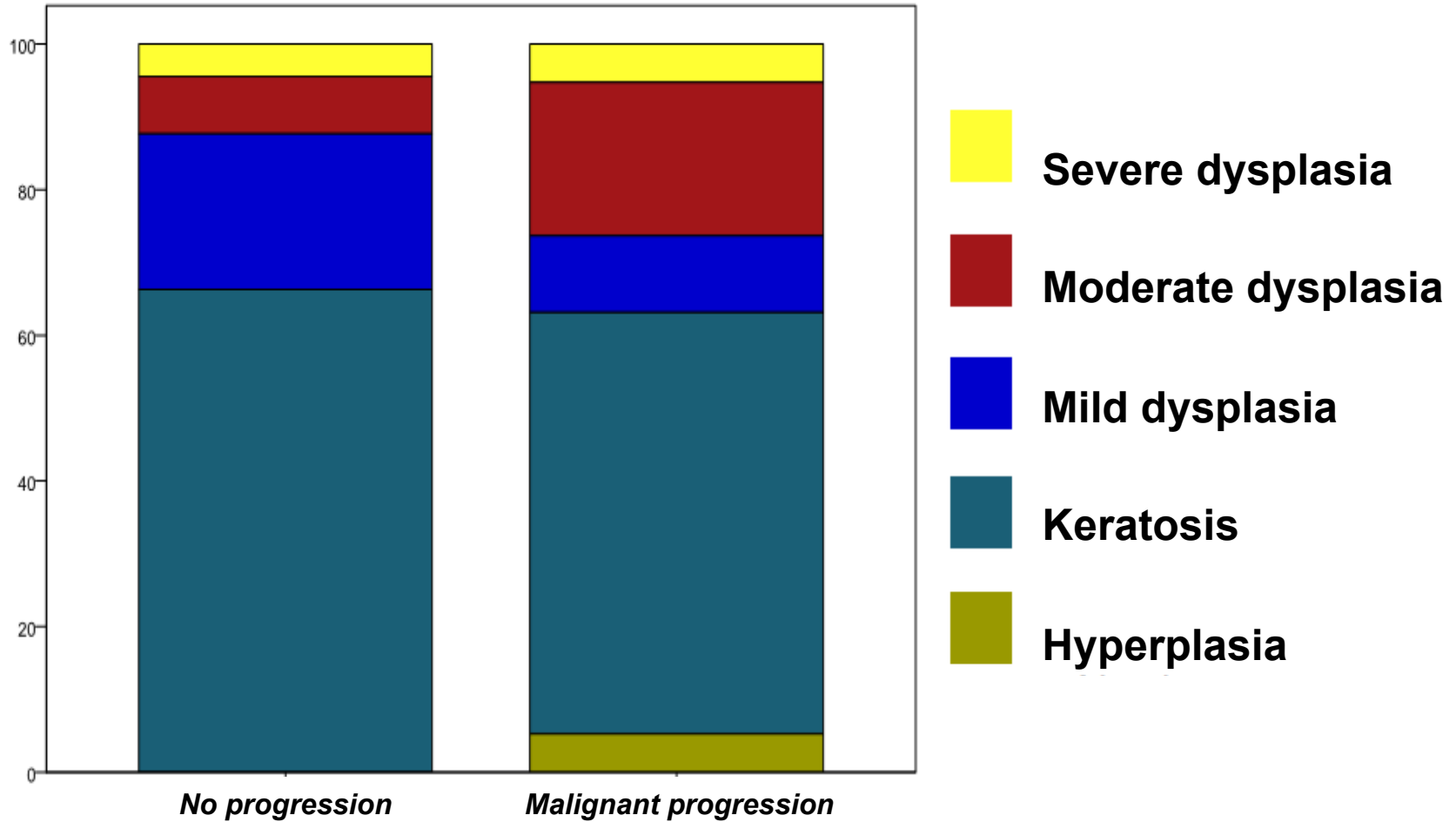




WHO	SIN	SIL
normal	normal	normal
inflammation	inflammation	inflammation
hyperplasia		squamous cell (simple) hyperplasia
		basal/parabasal cell hyperplasia
mild dysplasia	SIN 1	atypical hyperplasia
moderate dysplasia	SIN 2	
severe dysplasia	SIN 3	carcinoma in-situ
carcinoma in-situ		
squamous cell carcinoma	squamous cell carcinoma	squamous cell carcinoma

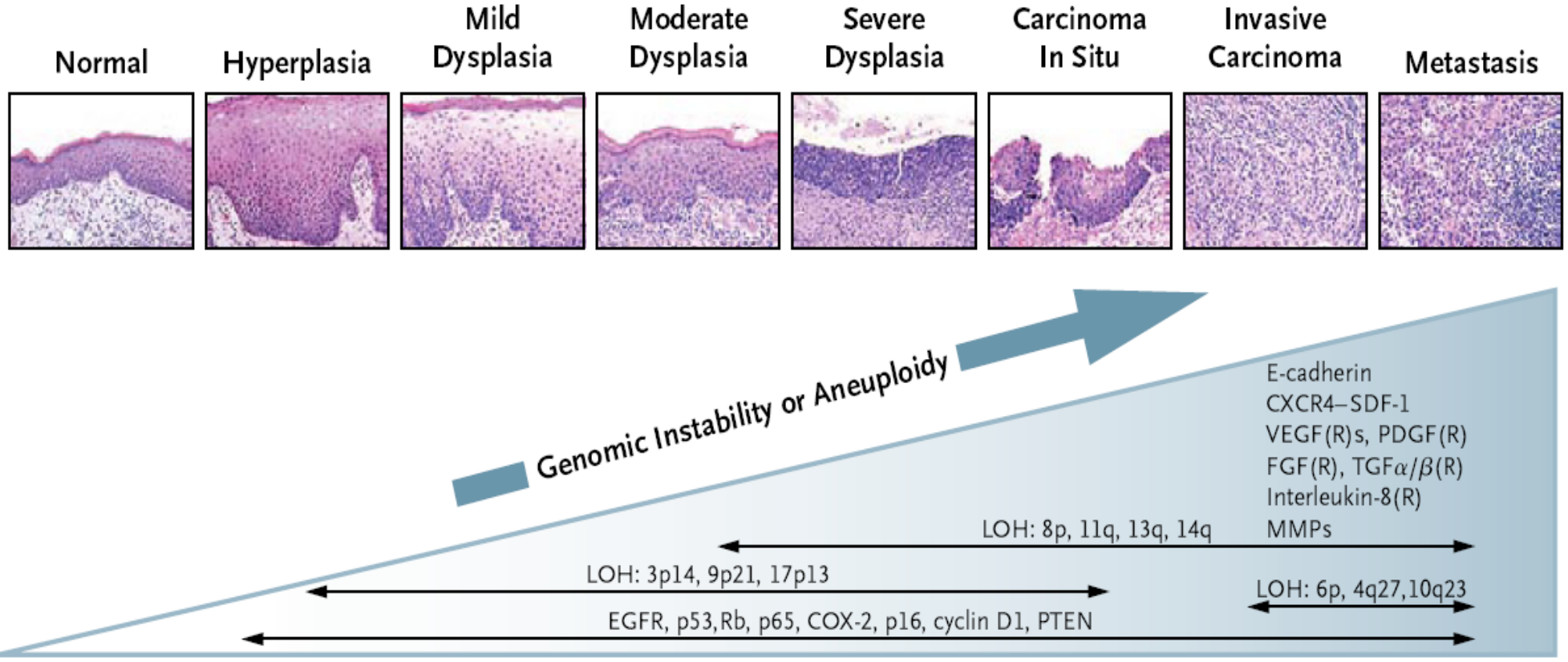


Follow-up of oral cancer patients



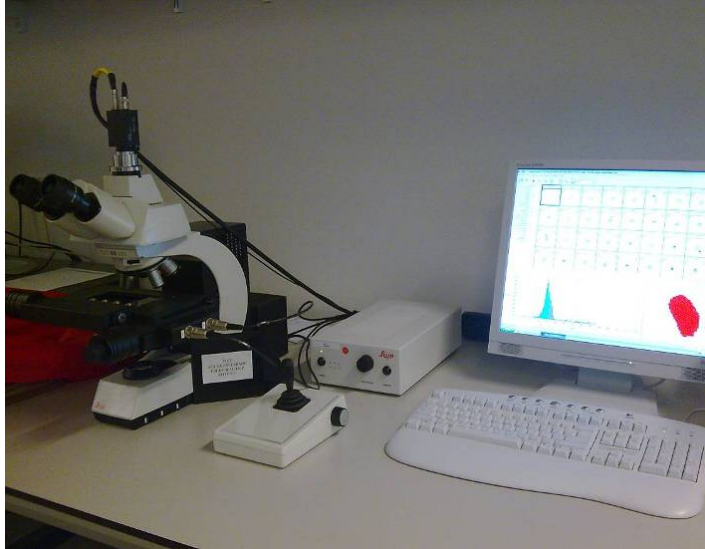
Unweighted κ -values with 95% confidence intervals

	All options	Two-grade	Three-grade
WHO			
<i>Overall κ-value</i>	0.21 (0.17-0.26)	0.57 (0.46-0.68)	0.35 (0.27-0.43)
SIN			
<i>Overall κ-value</i>	0.28 (0.23-0.33)	0.60 (0.49-0.71)	0.40 (0.32-0.48)
Ljubljana			
<i>Overall κ-value</i>	0.19 (0.14-0.24)	0.50 (0.39-0.61)	0.30 (0.22-0.39)

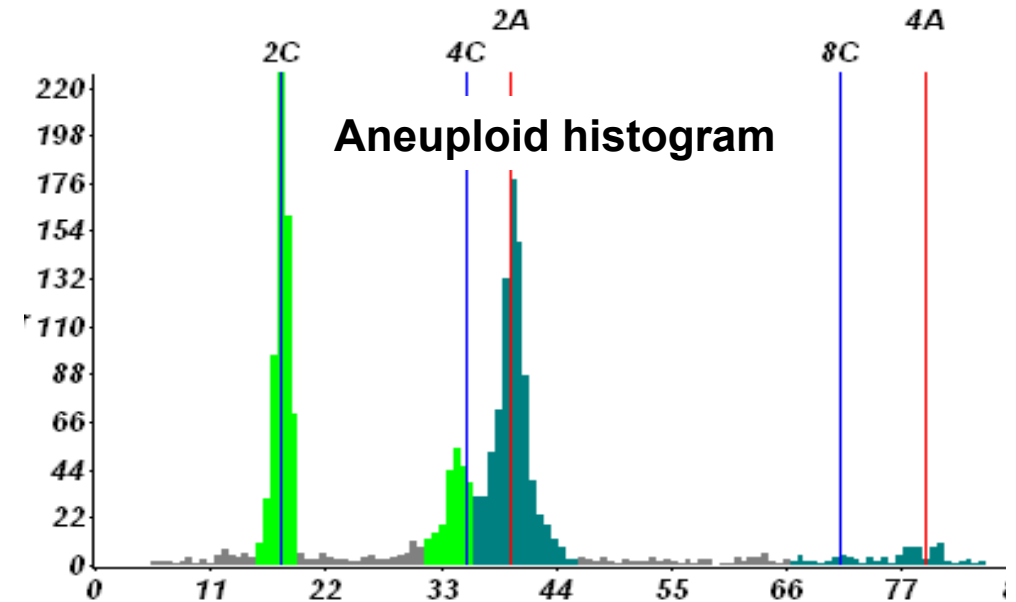
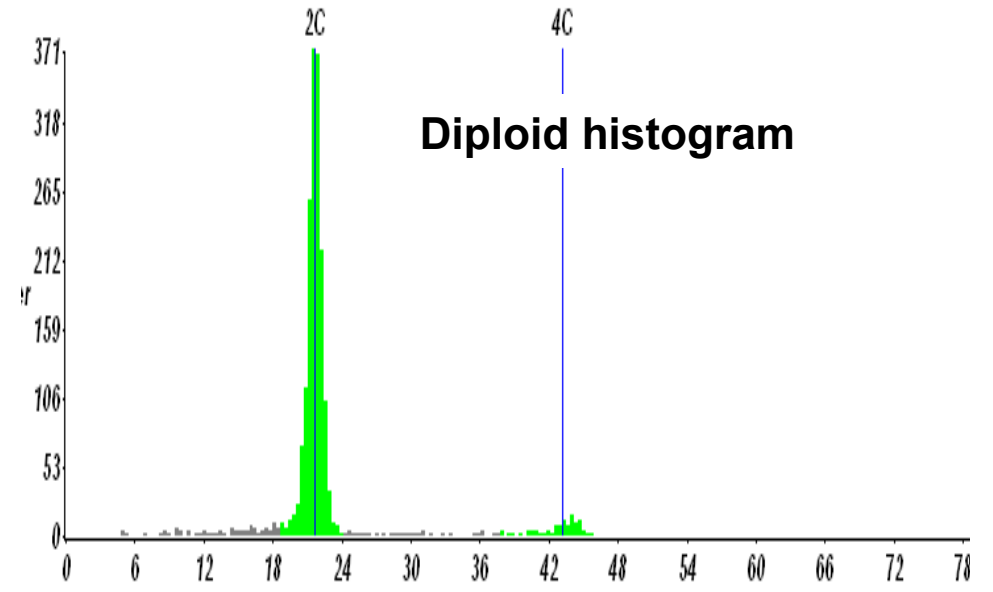


Haddad *et al* NEJM 2008

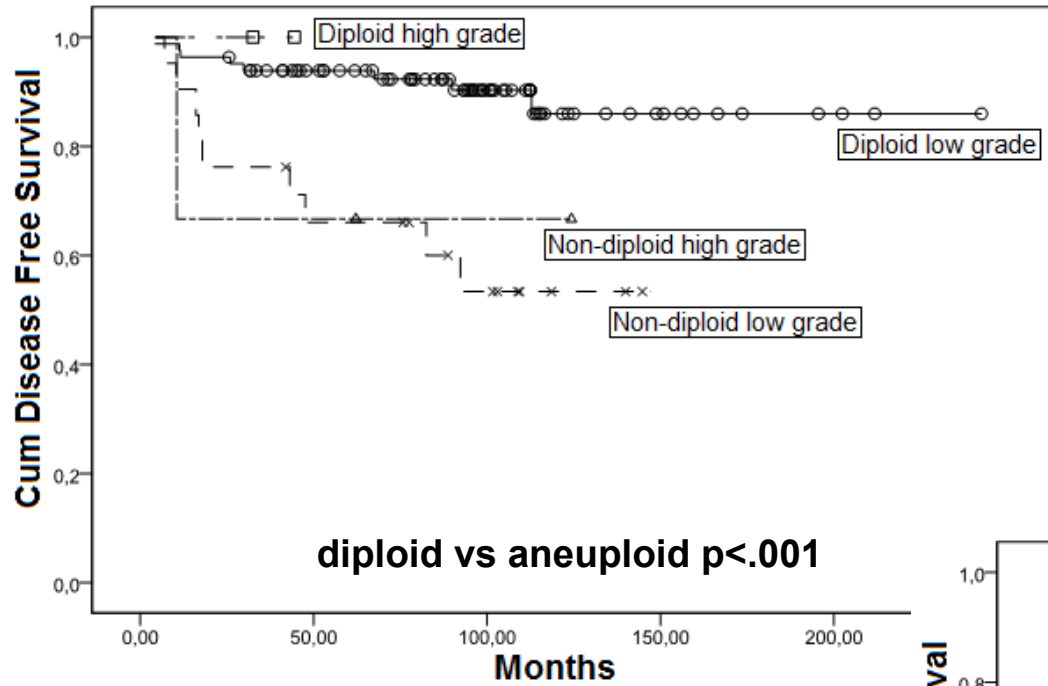
Gale *et al* Histopathol 2009



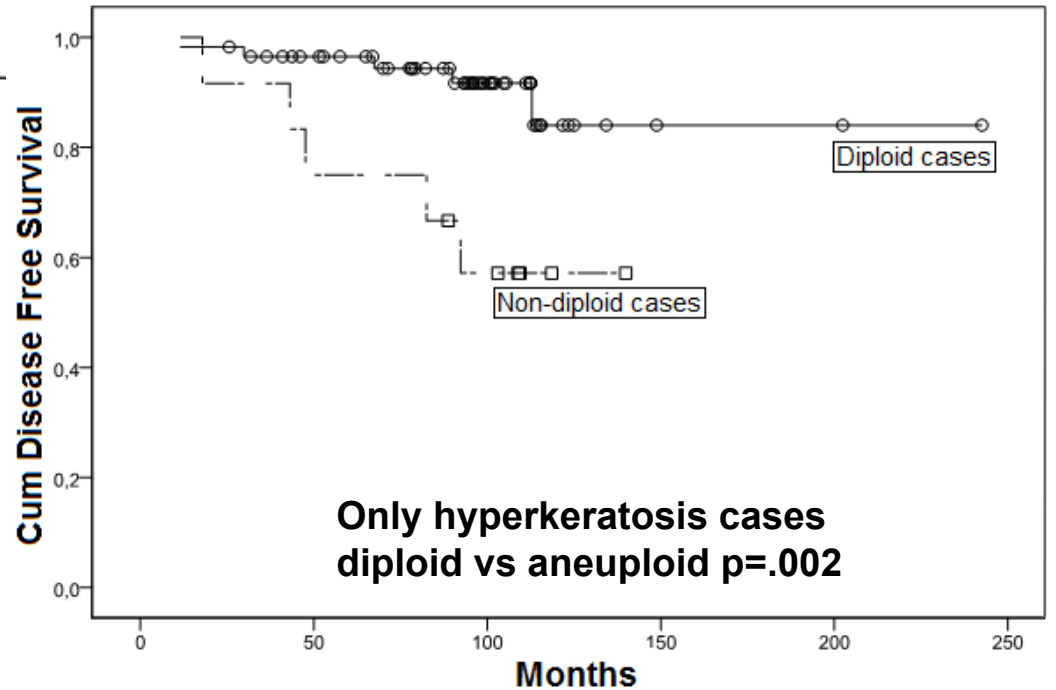
79.60 ✓	79.79 ✓	79.87 ✓	79.87 ✓	79.92 ✓
80.39 ✓	80.42 ✓	80.48 ✓	80.55 ✓	80.59 ✓
80.95 ✓	80.95 ✓	80.97 ✓	81.34 ✓	81.42 ✓
83.17 ✓	83.27 ✓	83.38 ✓	84.40 ✓	84.43 ✓



Kaplan-Meier Survival Curve



Kaplan-Meier Survival Curve



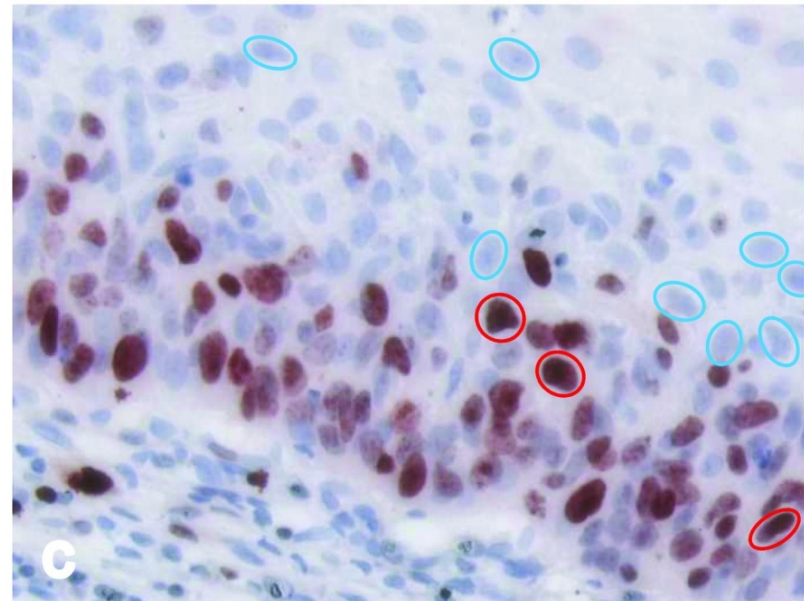
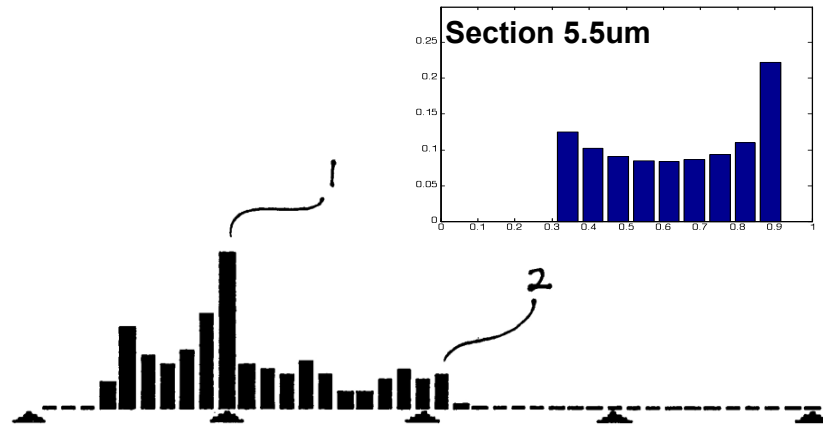
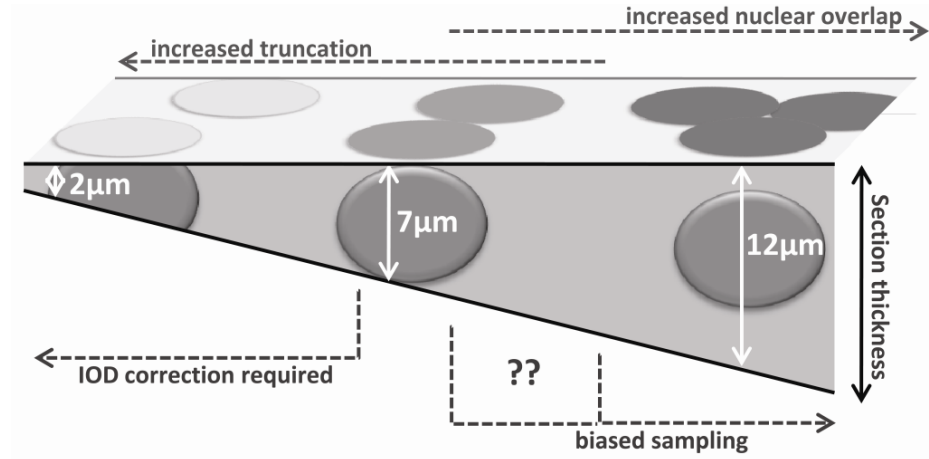
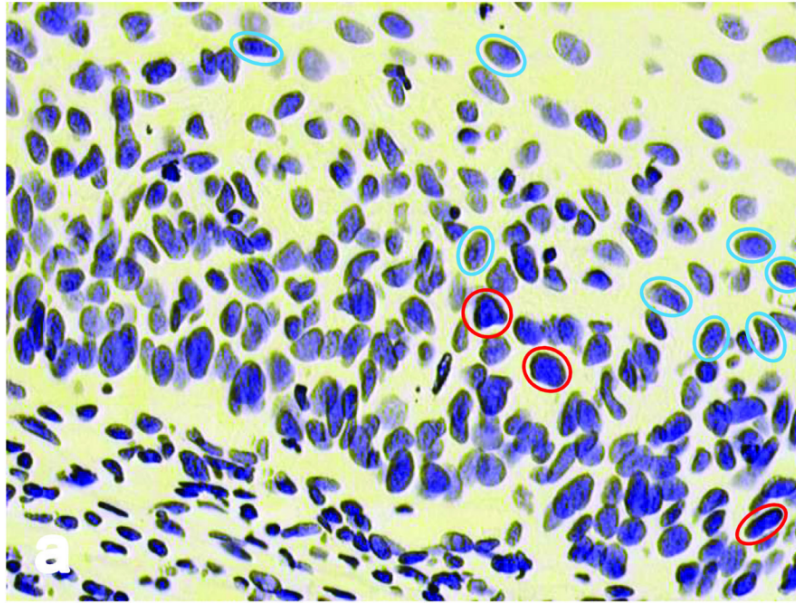
Reinforcements

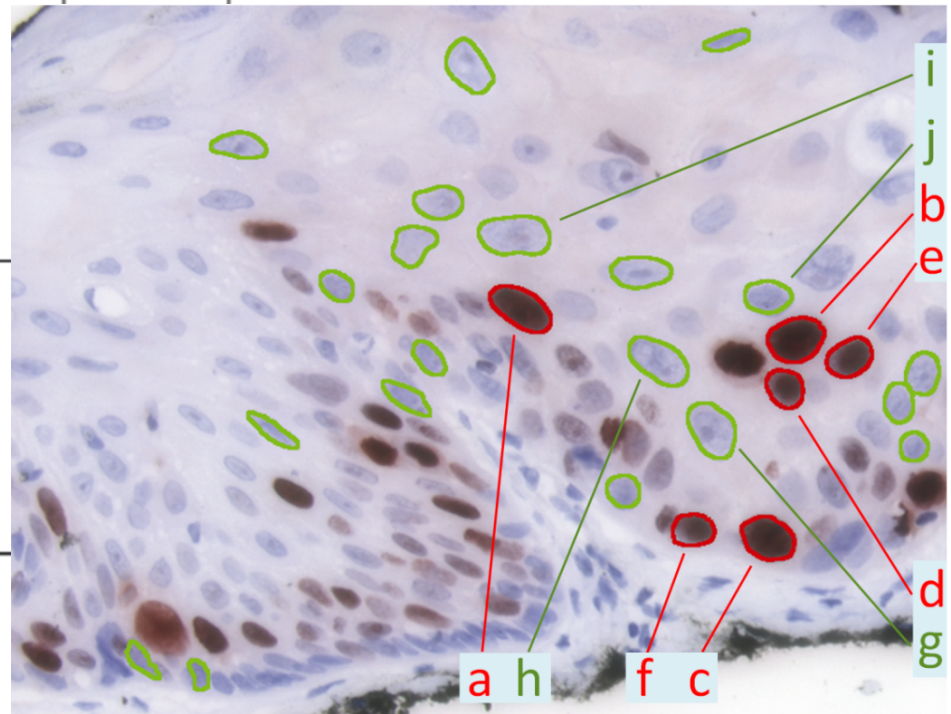
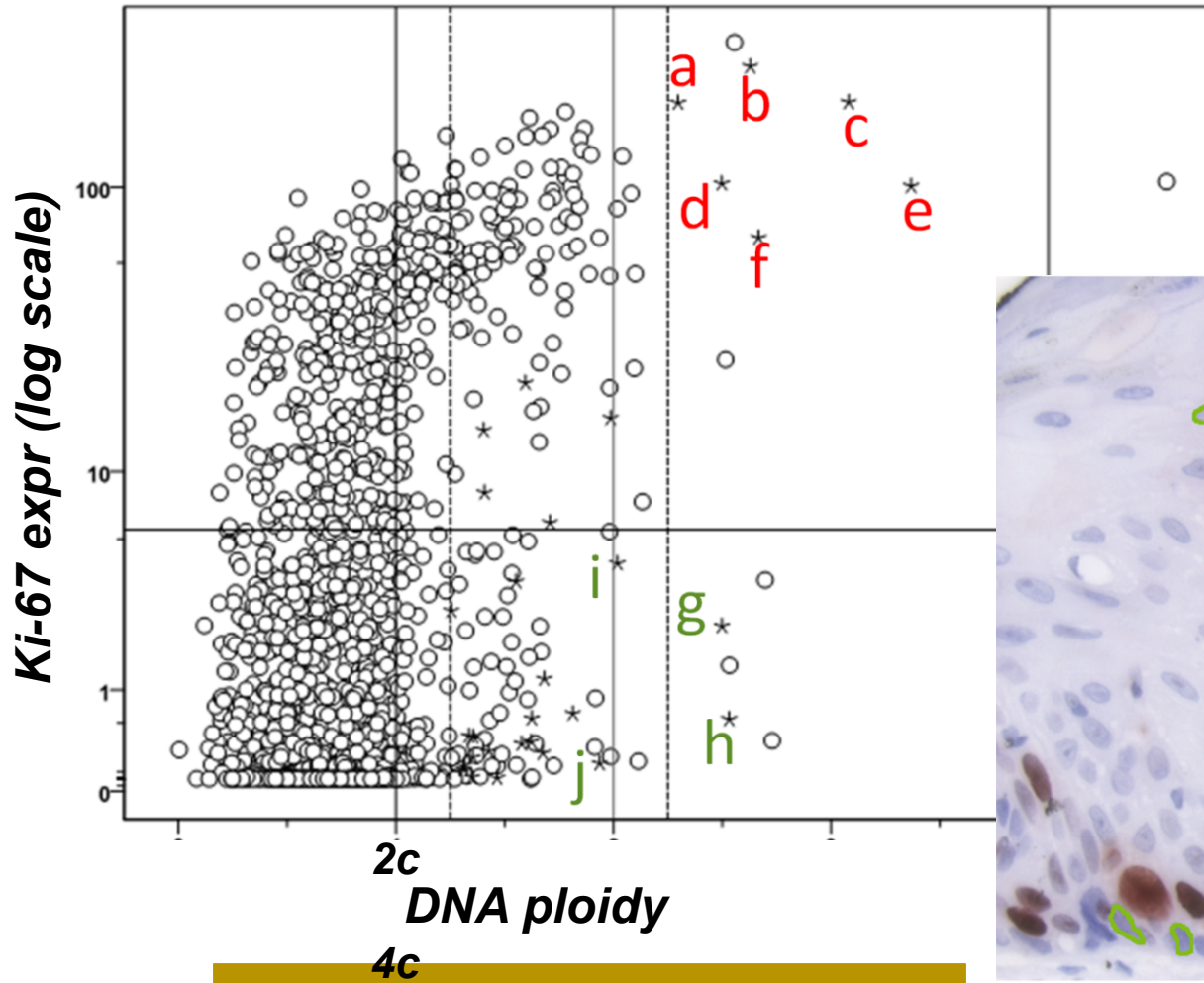
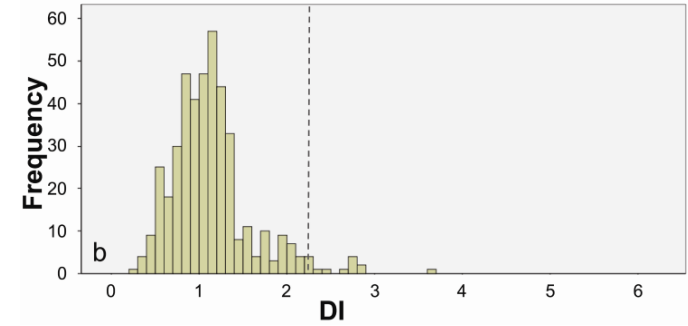
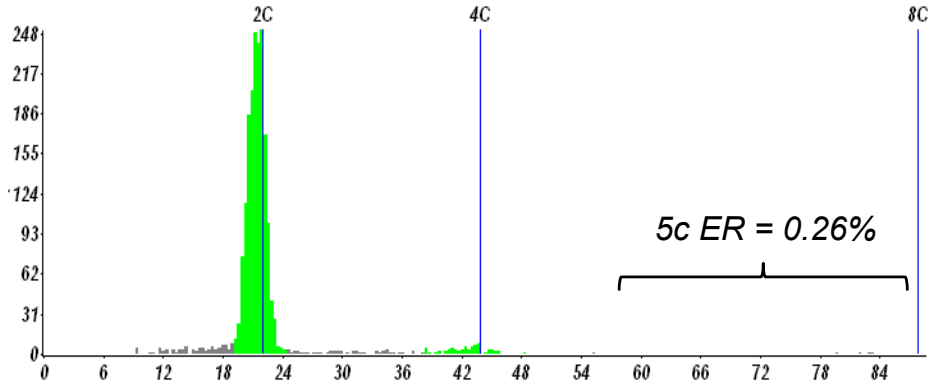
- Sample enrichment (dilution with non aberrant tissue decreases sensitivity)
- Combination with other relevant biomarkers (e.g. $\gamma\text{H}_2\text{AX}$, p53, p16 etc)
- Combination with histopathology
- Applicability in small (biopsy) samples with limited tissue availability (e.g. larynx)

- ***HYPOTHESIS***

Combined assessment of DNA ploidy and expression of other relevant biomarkers *in situ* in tissue sections allows accurate identification of subpopulations of cells at risk for malignant progression.

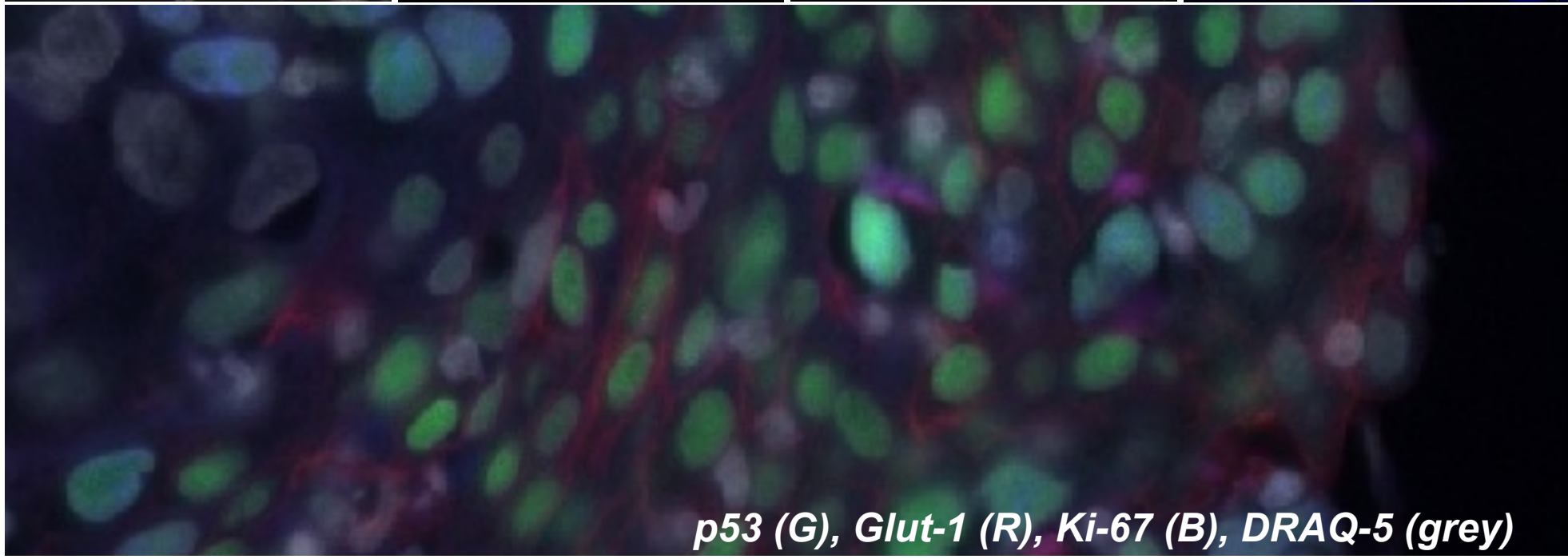
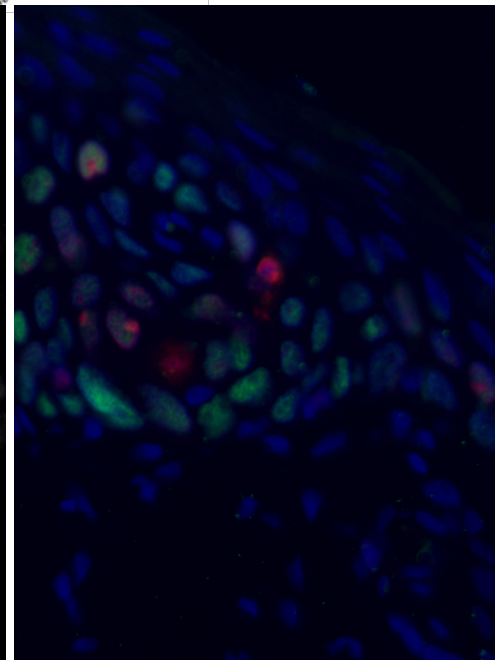
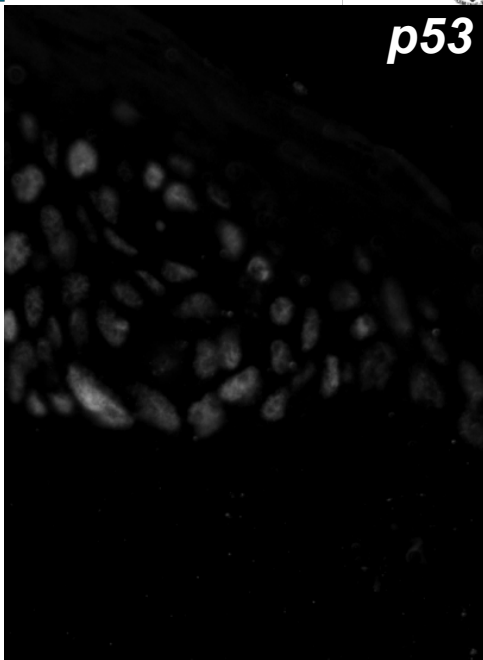
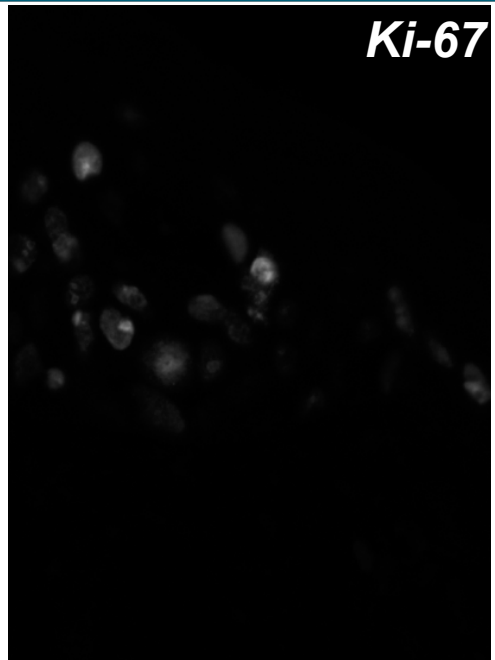
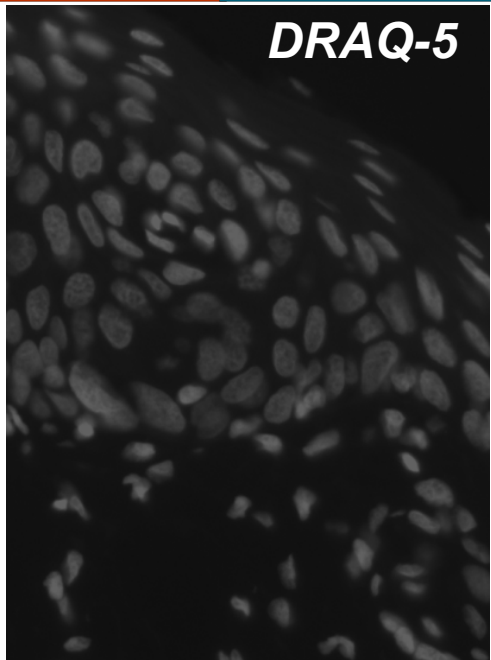
- **AIM**
Development of a method for *in situ* ploidy analysis, which may be combined with assessment of other relevant biomarkers at the single cell level
 - Ploidy analysis in tissue sections
 - Multiplexing of fluorescent signals
 - Automated recognition of nuclear profiles in sections

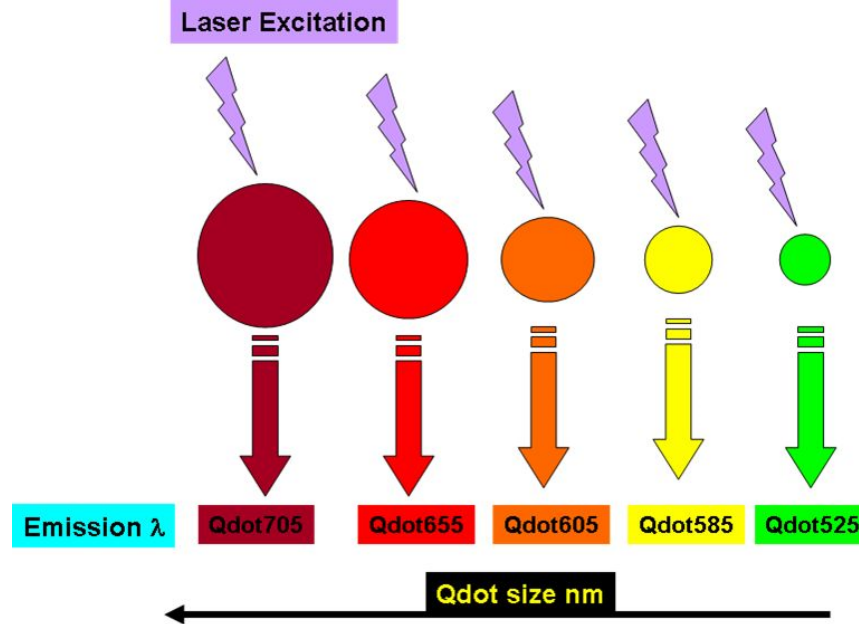
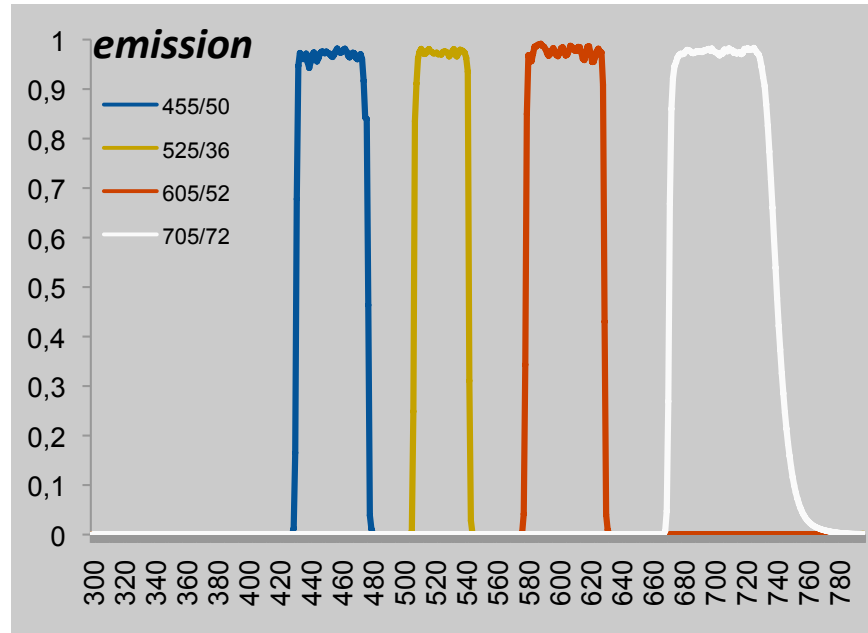
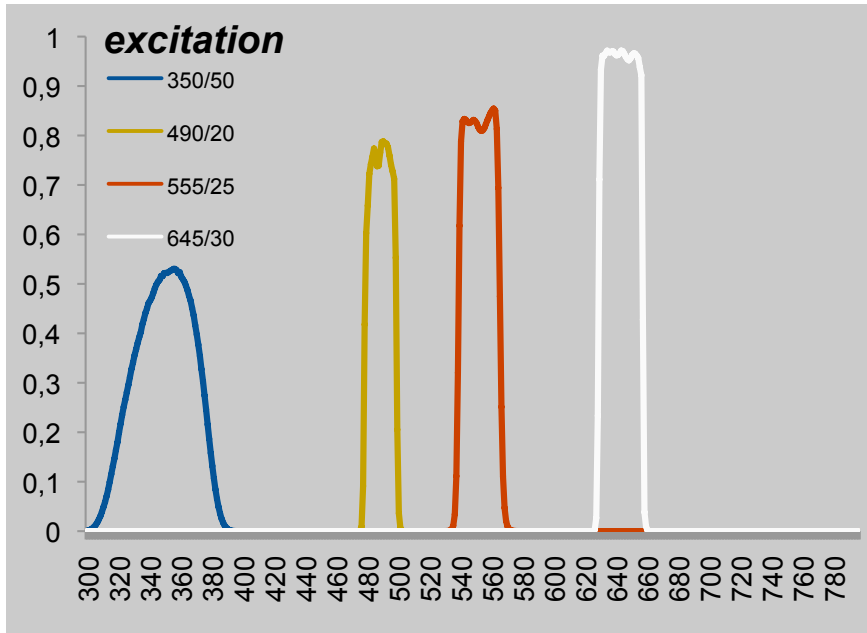




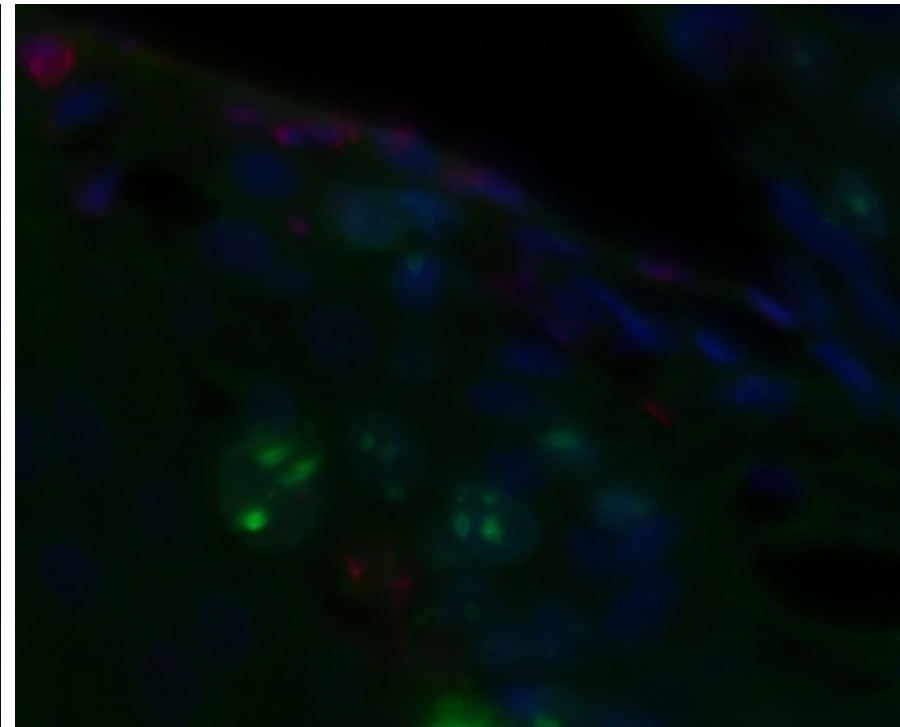
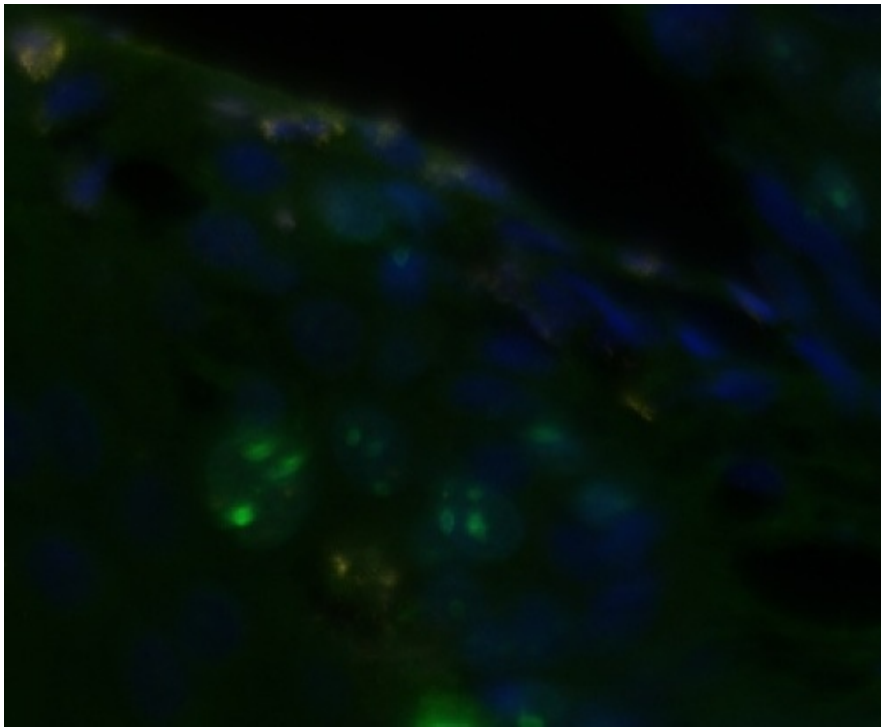
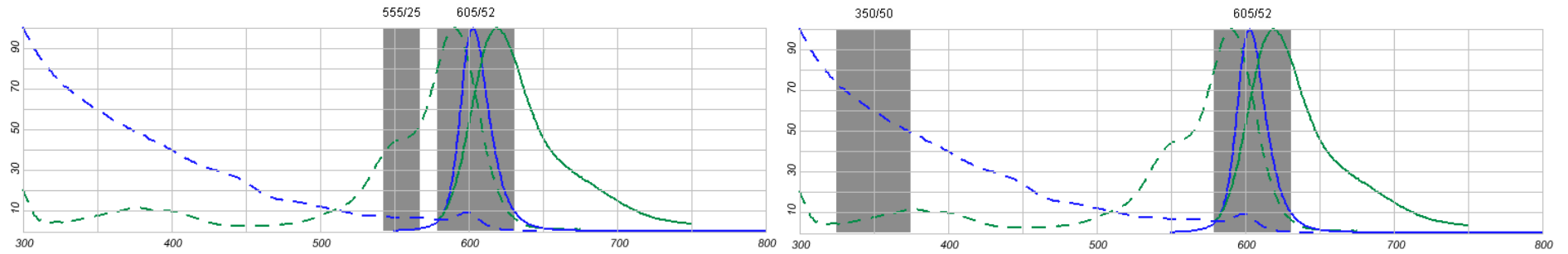
Tissue ploidy analysis	DNA ploidy measured in isolated nuclei		
	Diploid	Tetraploid	Aneuploid
<i>Transmitted light microscopy (n=22)</i>			
Diploid	11	0	0
Non-diploid	2	4	5
<i>Fluorescence microscopy (n=5)</i>			
Diploid	3	0	0
Non-diploid	1	1	0

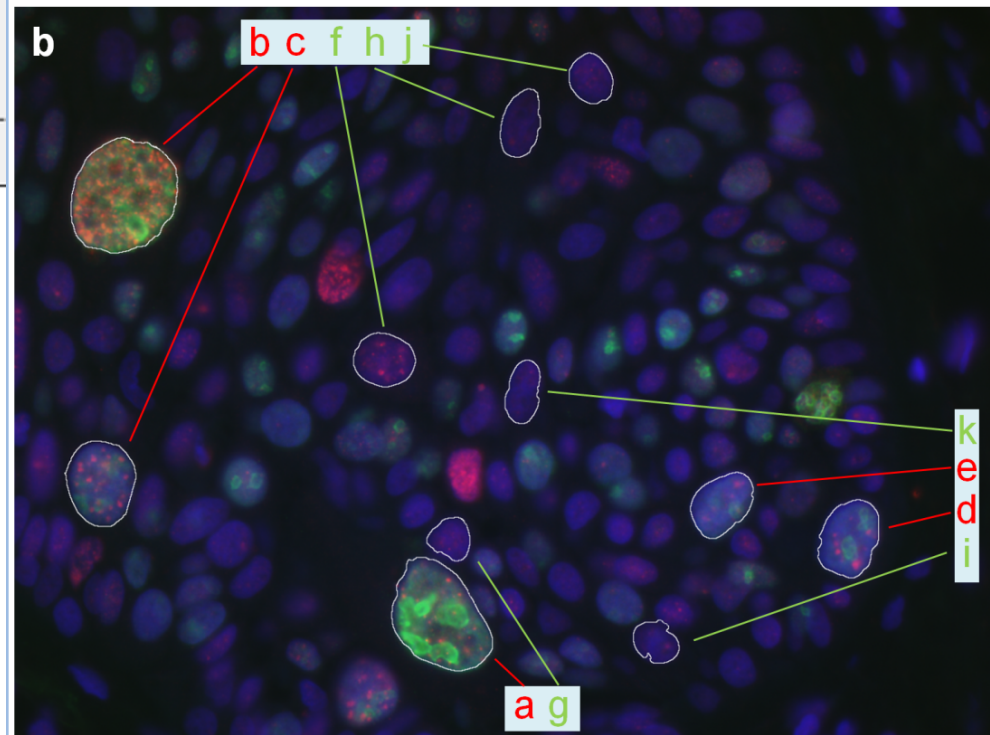
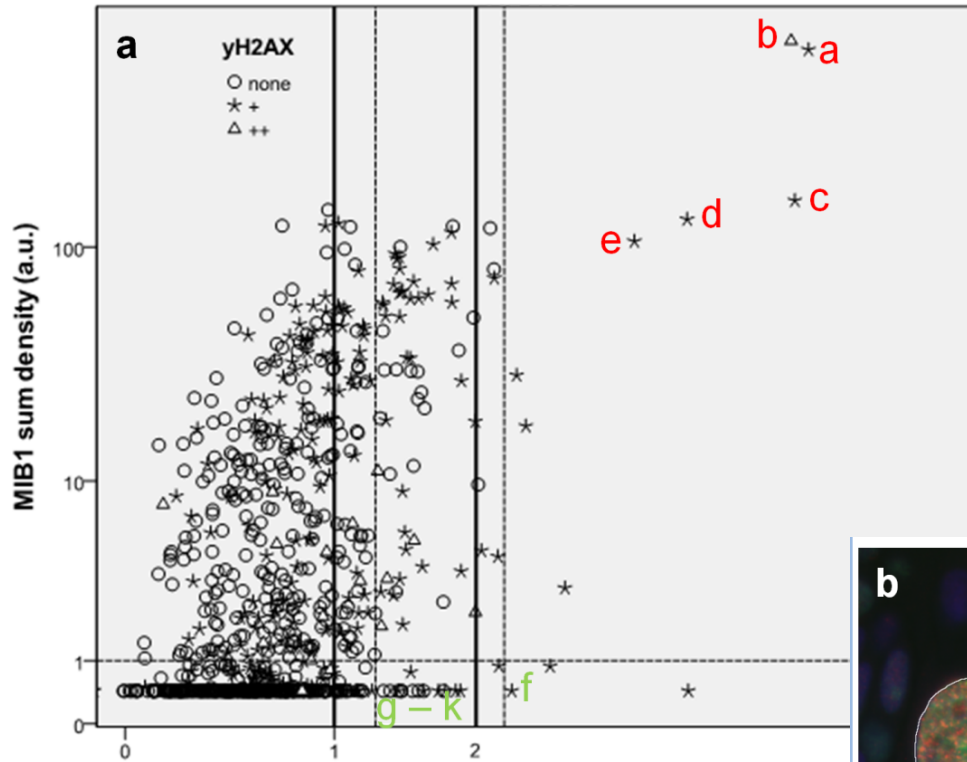
Confirmed by double target FISH (c#1/c#7)

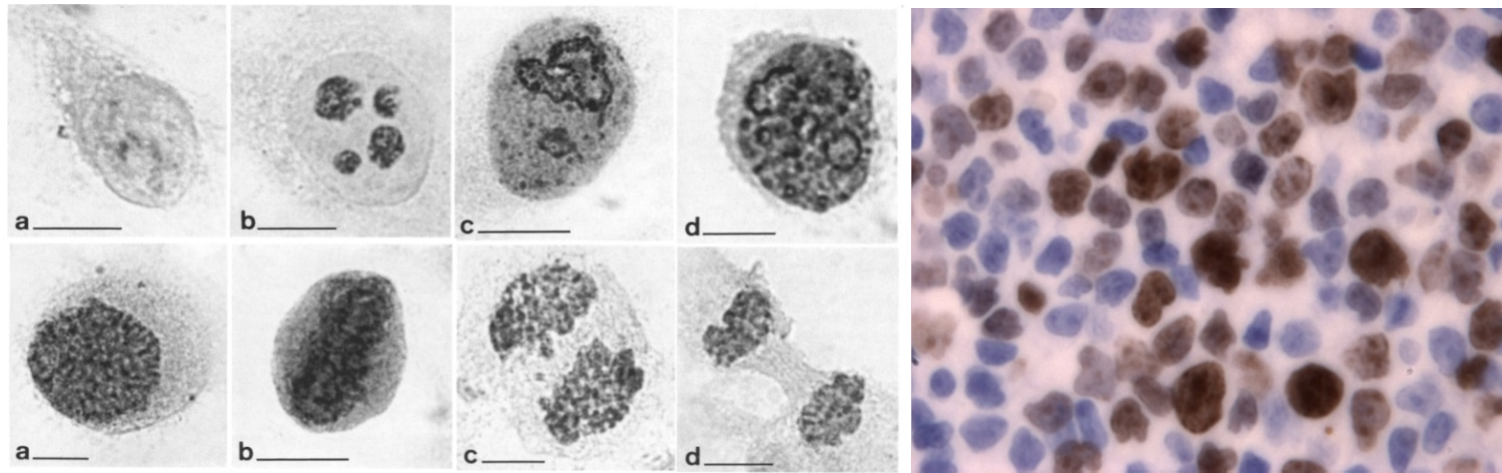




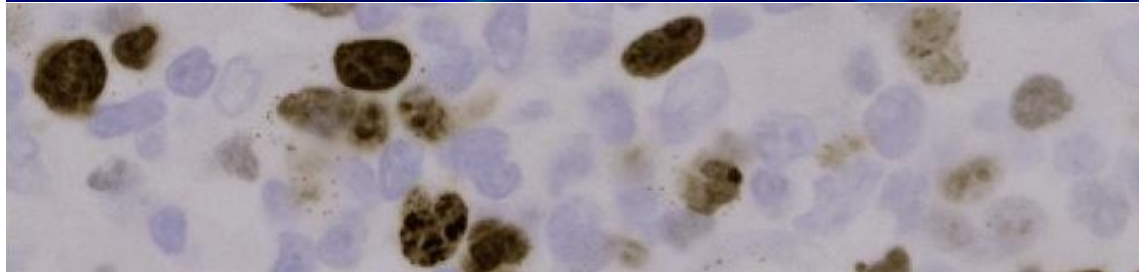
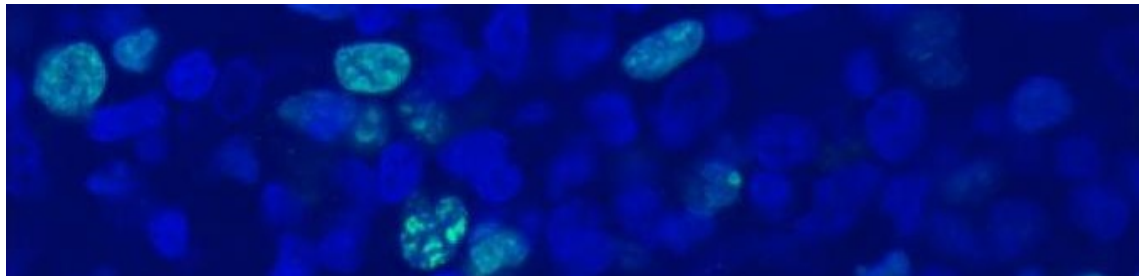
p53 – Qdot 605 (in red); Ki-67 (sp6) – Alexa Fluor 594 (in green); DRAQ-5 (in blue)







G0 G1 early S late S G2 M



Characterization of Chromatin Distribution in Cell Nuclei

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Pattern Recognition Group, Department of Applied Physics, Delft University of Technology, 2600 GA Delft, The Netherlands (I.T.Y., P.W.V.) and Biomedical Sciences Division, Lawrence Livermore National Laboratory, University of California, Livermore, California 94550 (B.H.M.)

Received for publication July 18, 1985; accepted April 7, 1986


In this paper we develop four measures to describe the distribution of nuclear chromatin. These measures attempt to describe in an objective and meaningful way the heterogeneity, granularity, condensation, and margination of chromatin in cell nuclei. Starting with a high-resolution digitized image of a cell where the nuclear pixels have been identified, the four measures may be rapidly estimated. The range of each is derived and the

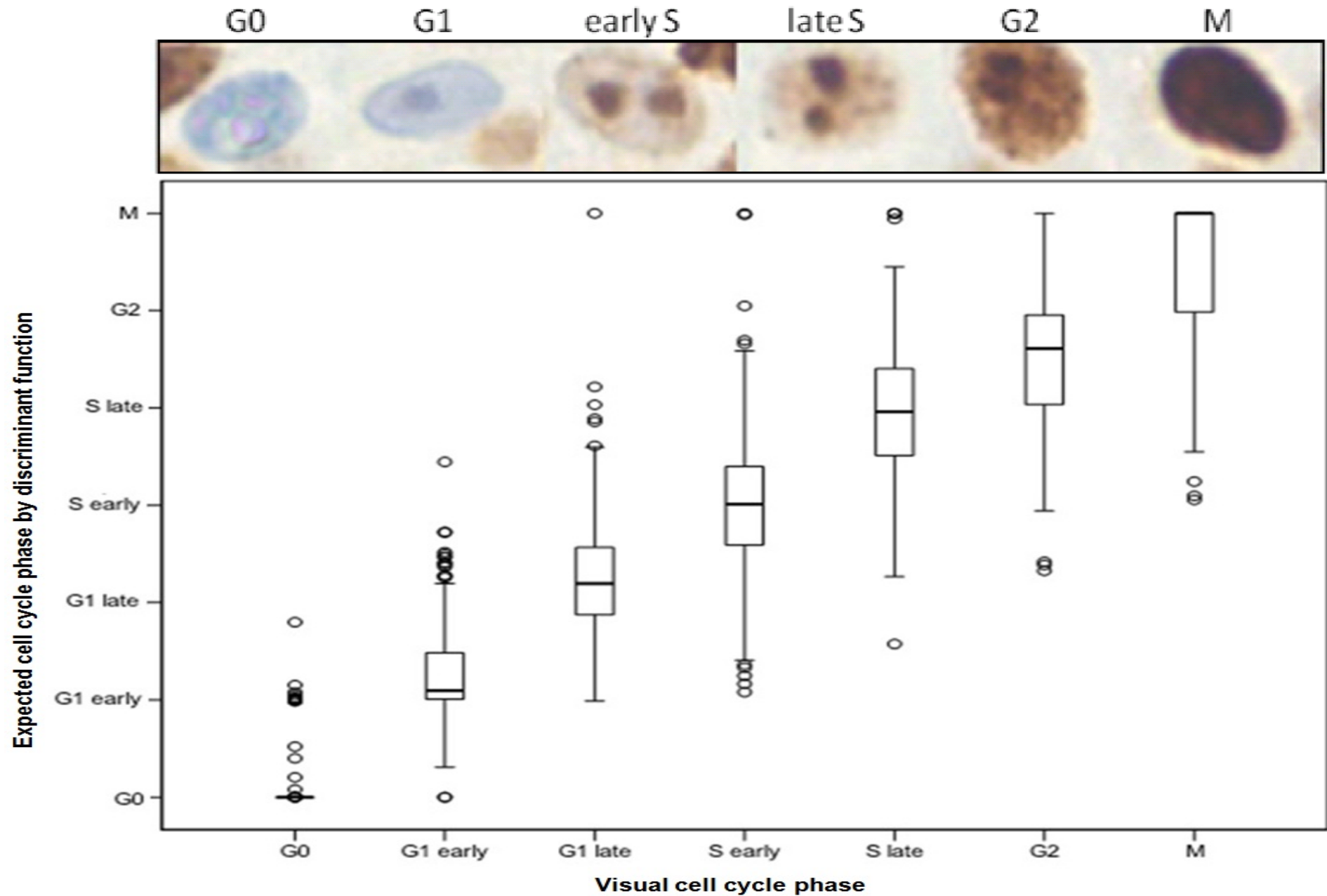
interpretation of the measures in the context of chromatin compaction and distribution is developed. Implementation issues such as sampling density, thresholding and subsequent pre-processing, and algorithmic complexity are discussed.

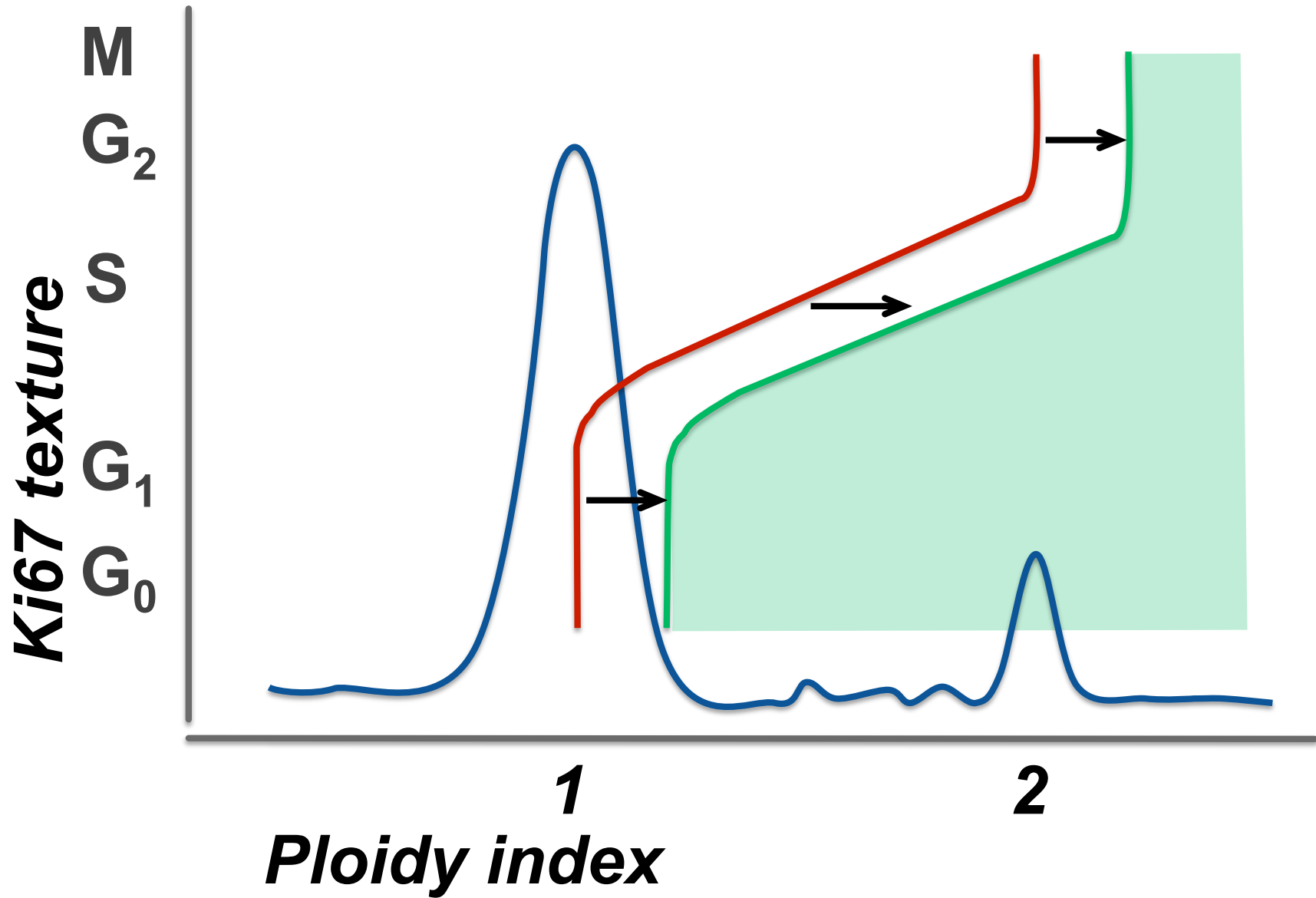
Key terms: Quantitative microscopy, image processing, texture measures, pattern recognition, image measurement

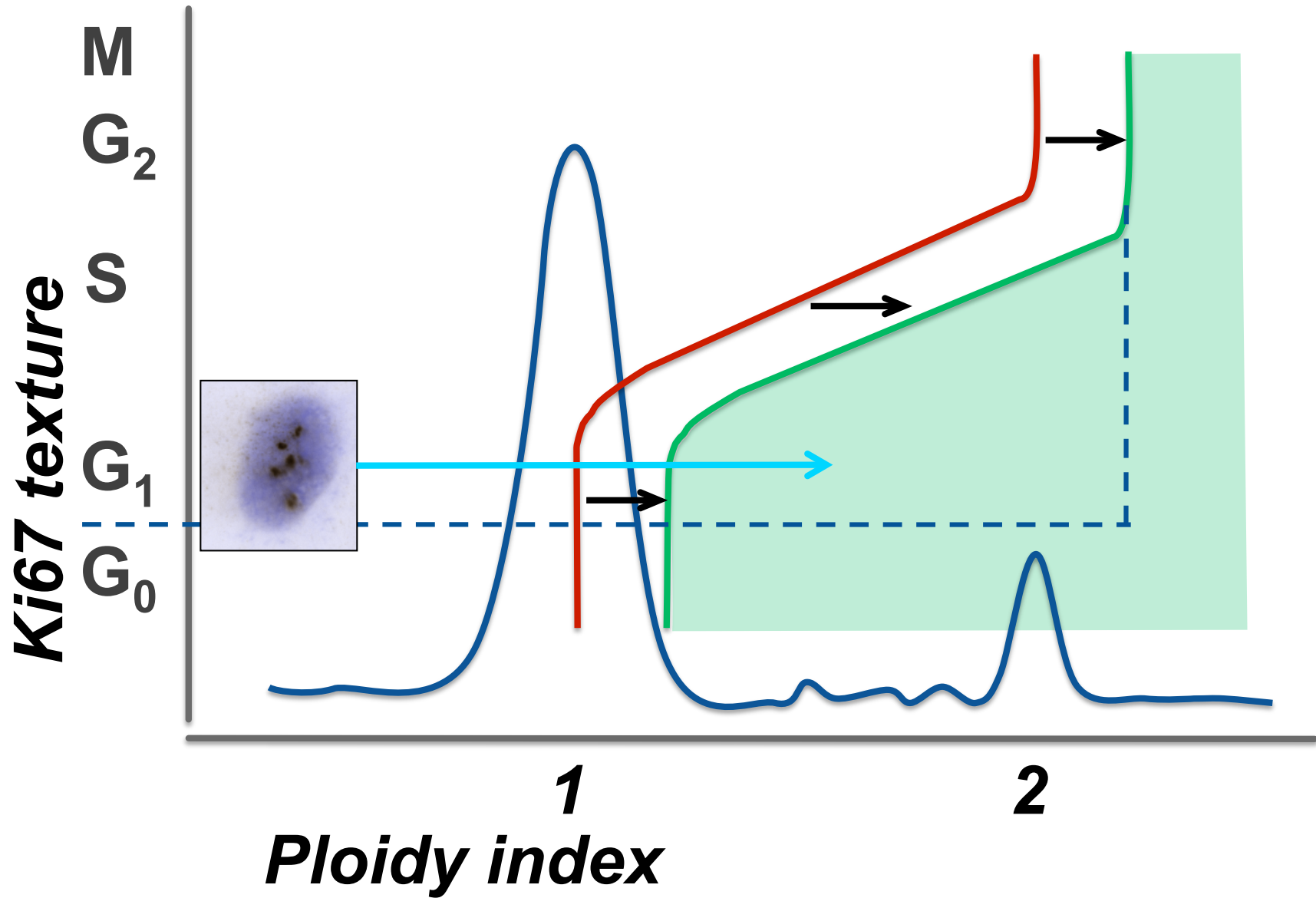
In this paper we present four measures that we have developed to quantify and characterize the distribution

the chromatin within the nucleus. This is illustrated in Figure 2A-D where all four cell images have precisely



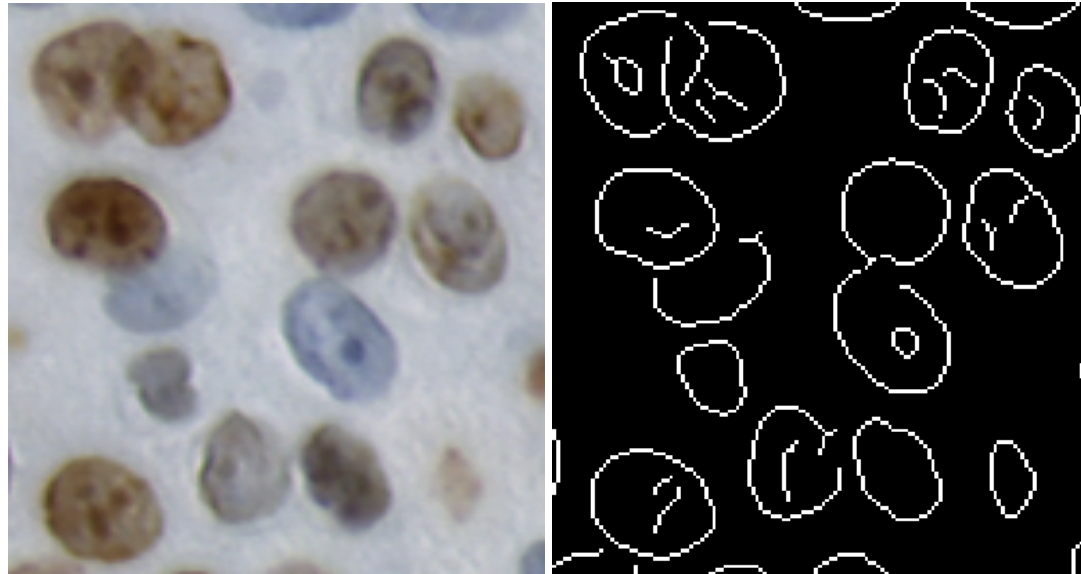






(Partly) restricted randomized Hough transform for ellipse detection

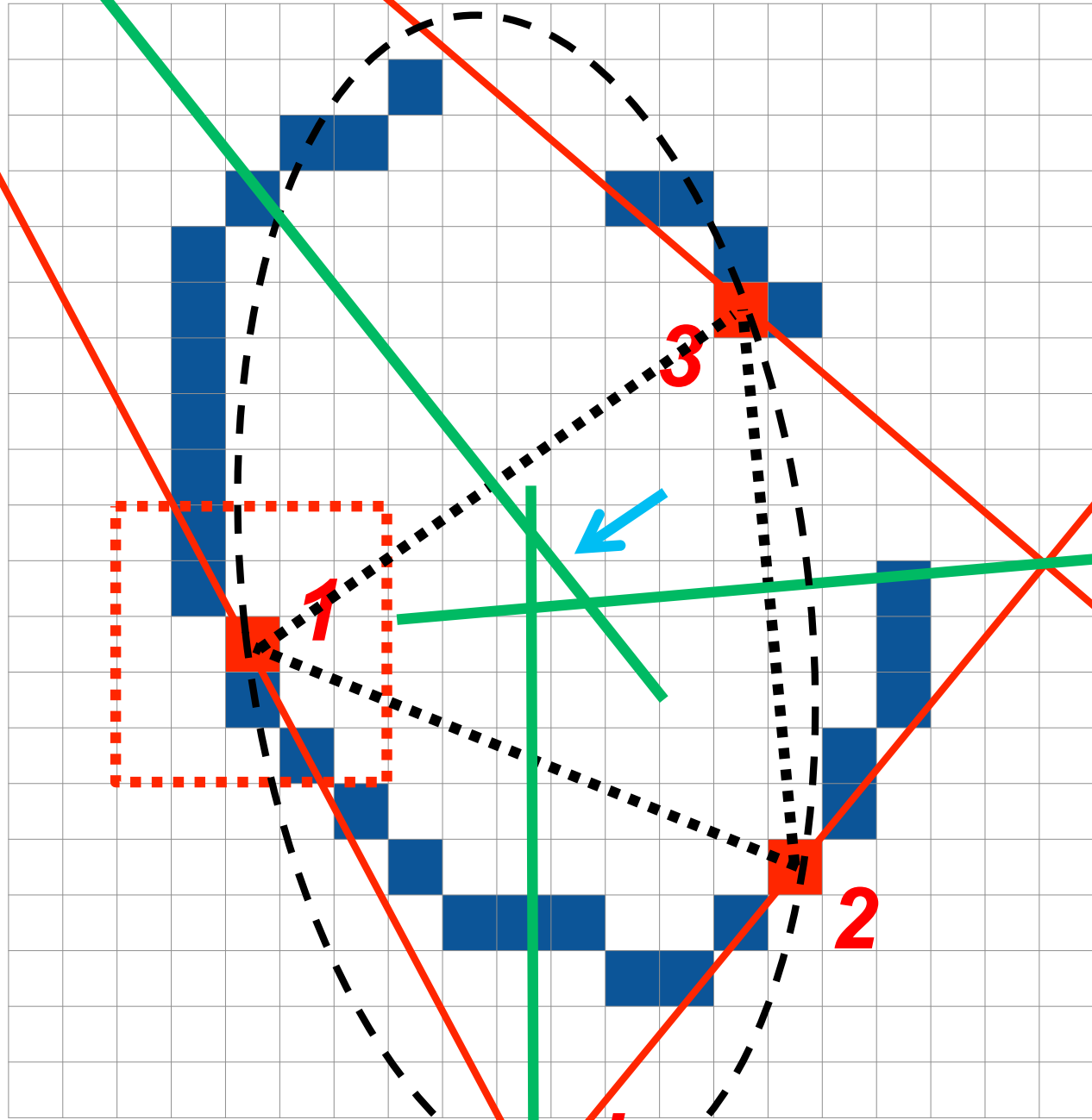
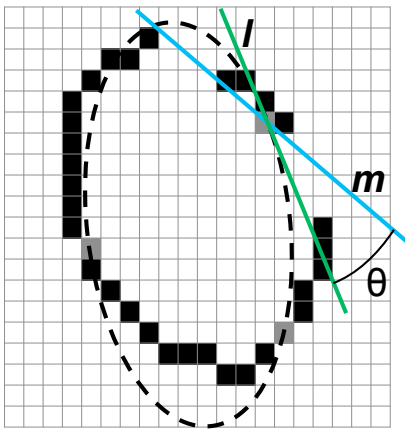
- Canny edge detection



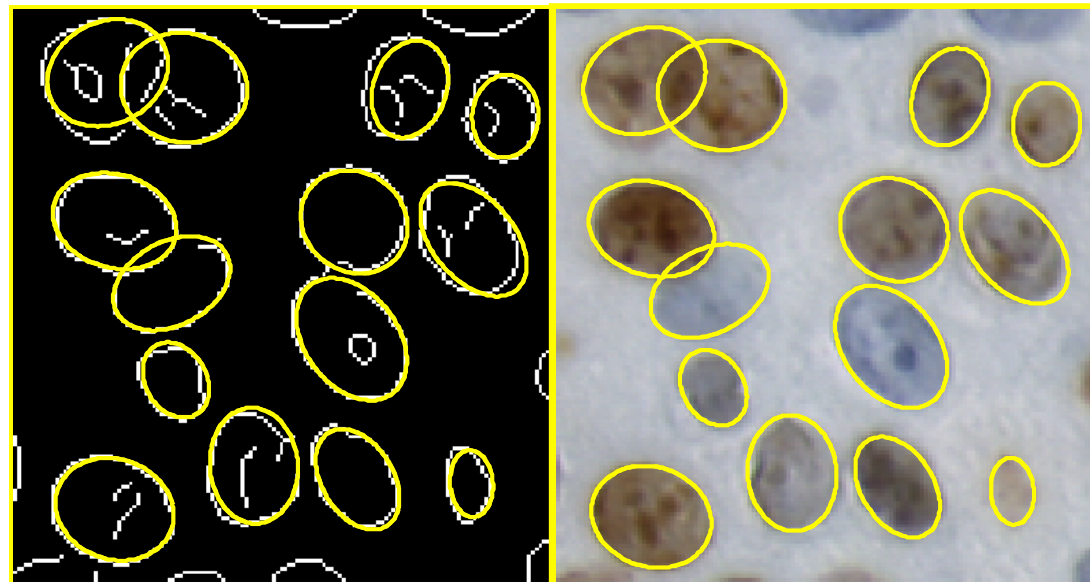
pRRHTell

a

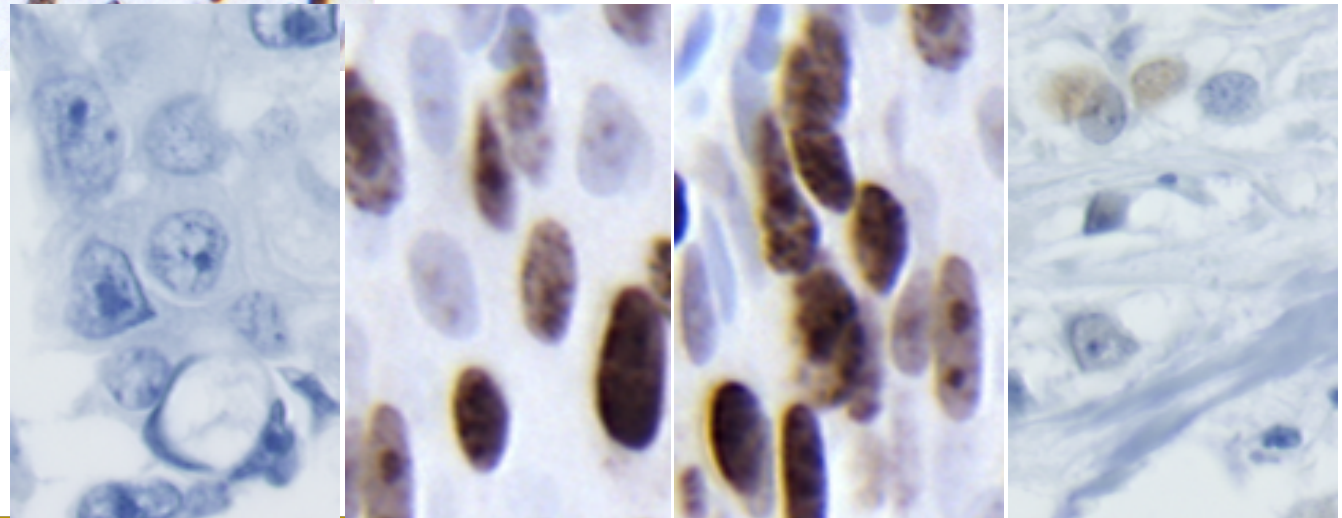
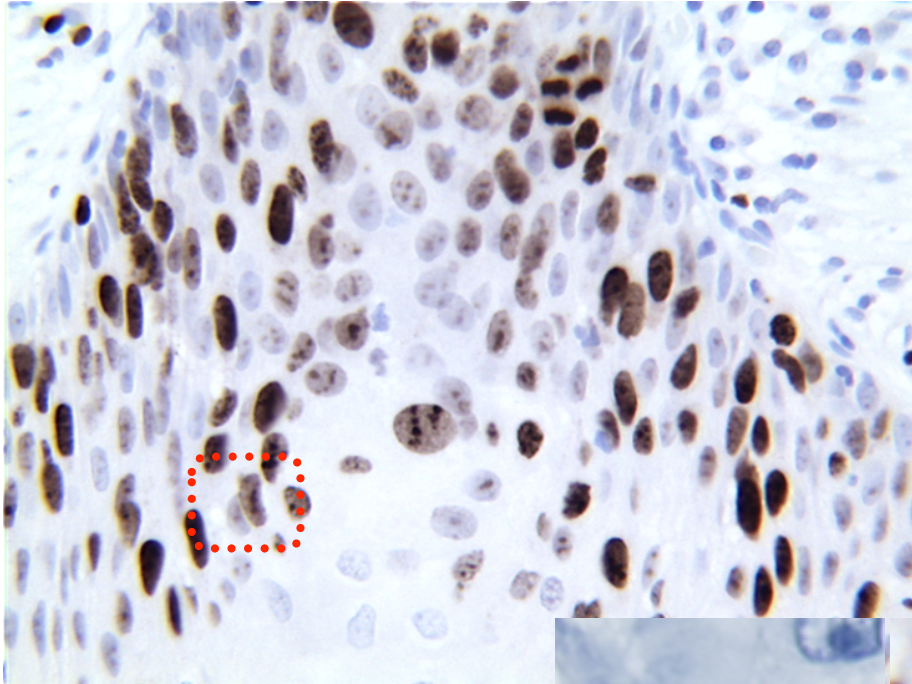
$$\frac{dy}{dx} = -\frac{ax + by}{bx + cy}$$



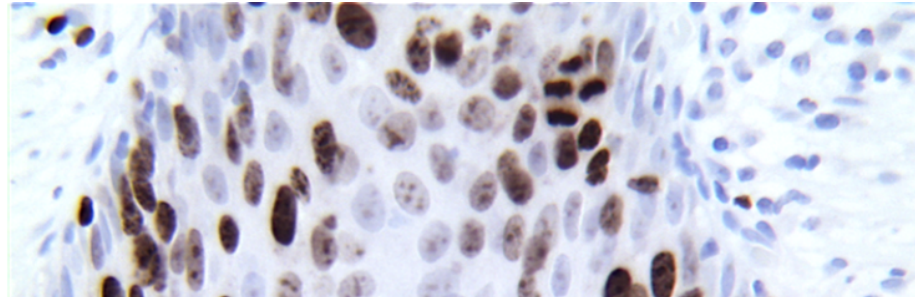
Restricted randomized Hough transform for ellipse detection



Globally optimal active contour model



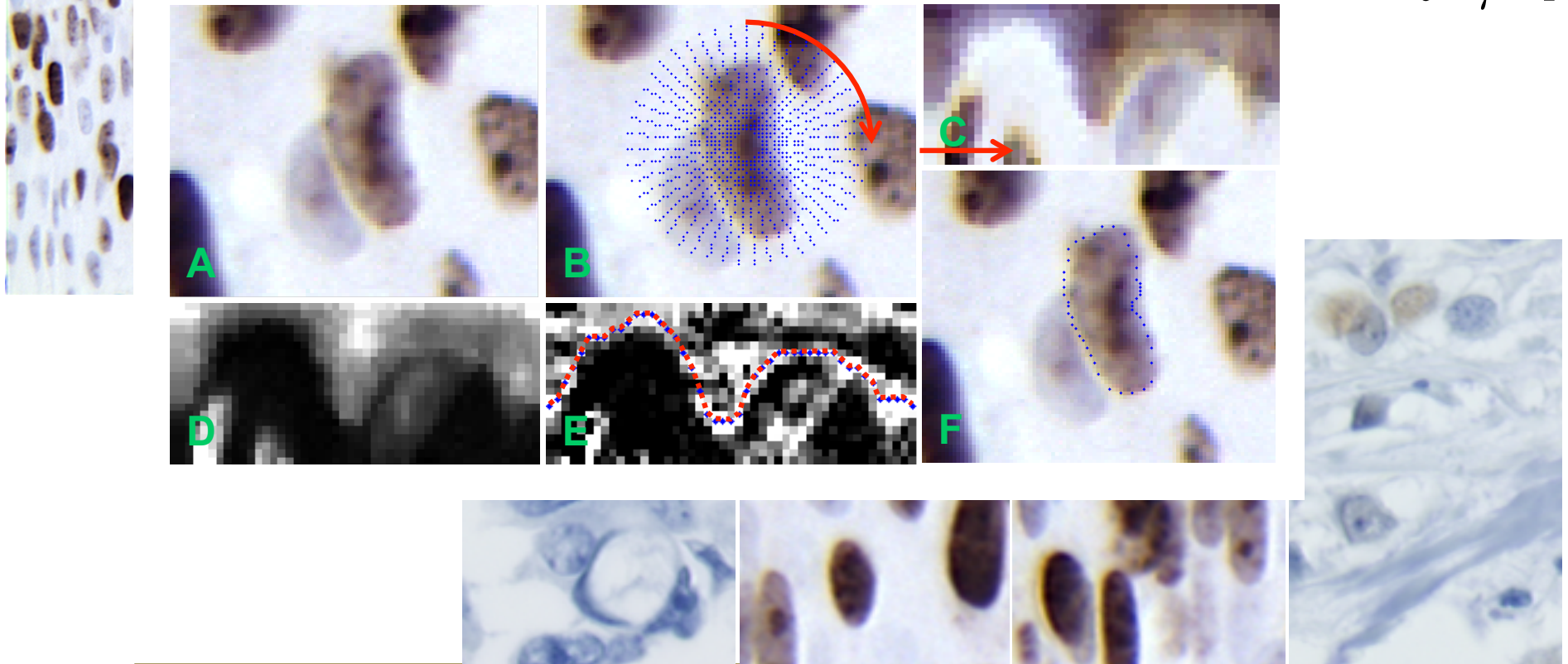
Globally optimal active contour model



$$E(\bar{v}(s)) = \int_{s=0}^1 \alpha(s) \left| \frac{d\bar{v}}{ds} \right|^2 + \beta(s) \left| \frac{d^2\bar{v}}{ds^2} \right|^2 - \gamma |\nabla I(\bar{v})| ds$$

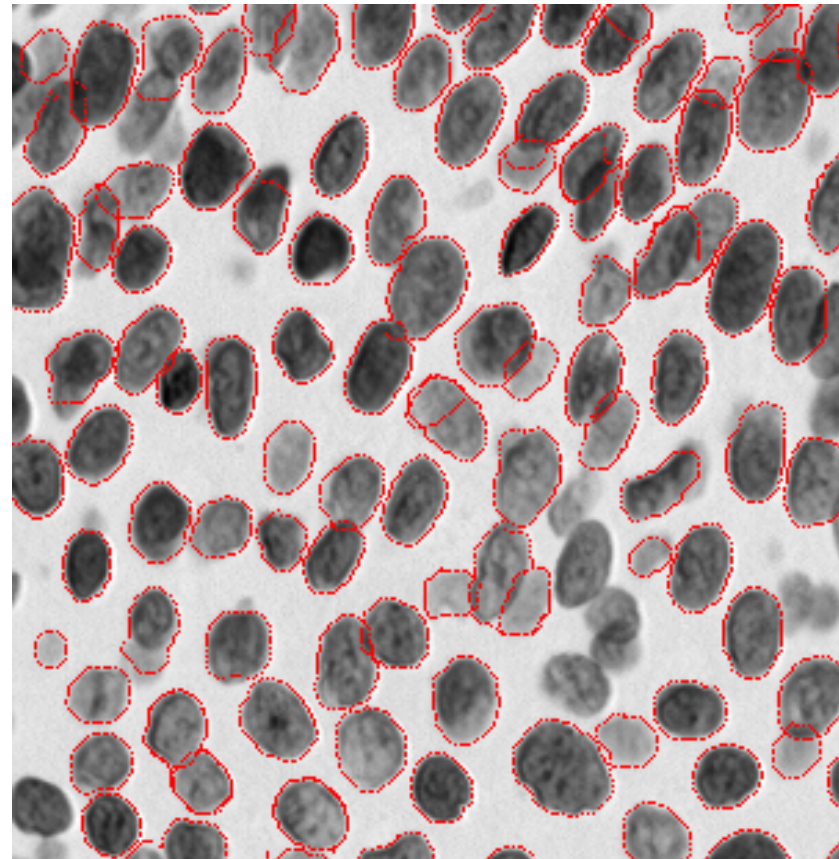
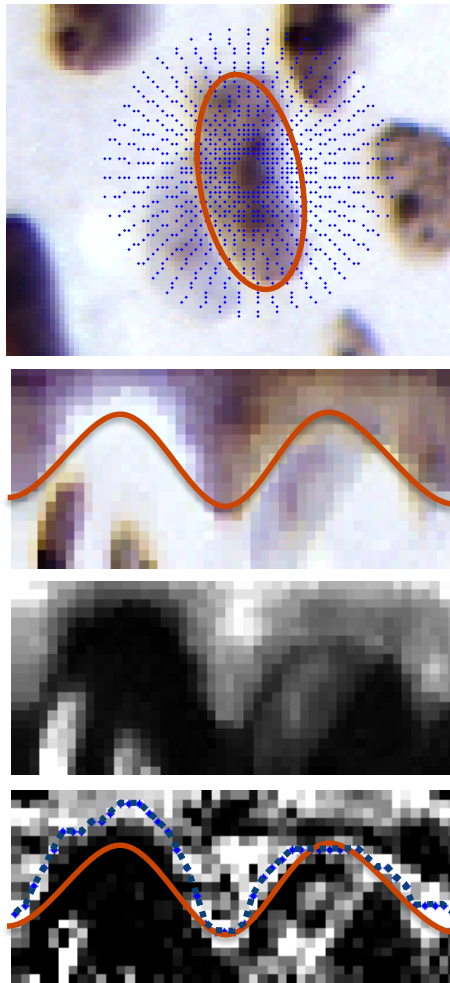
$$E(\bar{v}(s)) = \int_{s=0}^1 \gamma \left| \frac{d^2\bar{v}}{ds^2} \right|^2 + (1-\gamma) f(\nabla I(\bar{v})) ds$$

$$0 \leq \gamma \leq 1$$



Globally optimal active contour model combined with Hough transform

$$E(\bar{v}(s)) = \int_{s=0}^1 \gamma \left| \frac{d^2 \bar{v}}{ds^2} \right|^2 + (1 - \gamma) f(\nabla I(\bar{v})) + \varepsilon (\bar{v} - E(s))^2 ds$$



- ***CONCLUSION***

A method was devised enabling detection of aneuploid cells in tissue sections, while at the same time yielding information regarding cell cycle phase. This method can be combined with assessment of other relevant biomarkers, employing fluorescence multiplexing.

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