PLOIDY WORK STATION

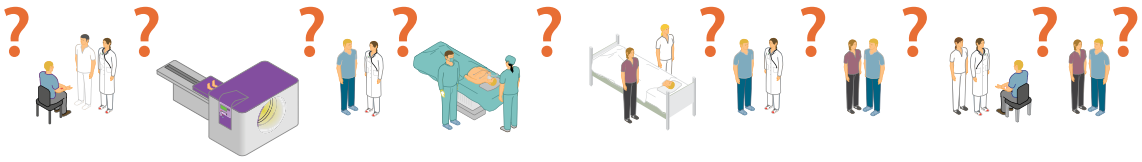
An automated system for DNA Ploidy - A diagnostic and prognostic marker for cancer

"Prognostic markers help identifying patients with varying risks of clinical outcome."

PROGNOSTIC MARKERS

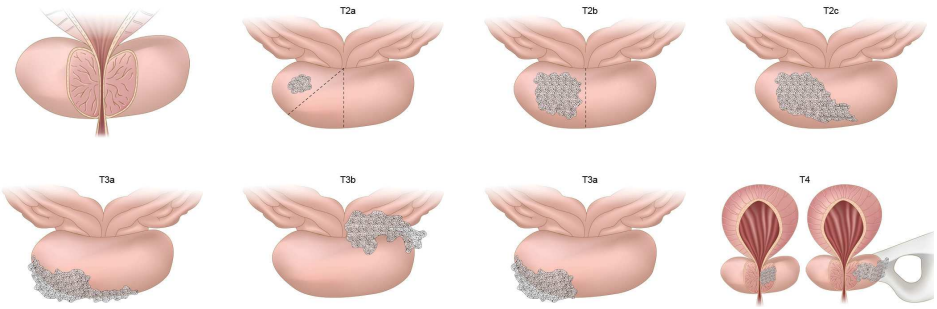
The main challenge for any oncologist is to predict the outcome of any chosen treatment for a cancer patient.

Epidemiologically and statistically, the probability of 5 year survival for treated cancer



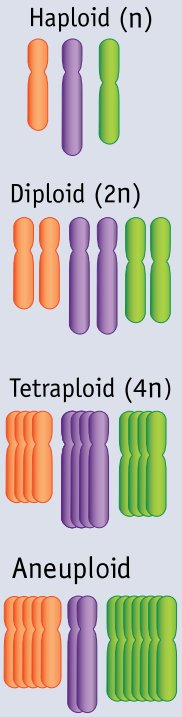
patients is estimated based on clinical staging, where survival is ranging from 60-95% in stage I to 0-15% in stage IV. With few exceptions, there is no method to predict if a single patient belongs to the non-surviving or surviving (good prognosis) group. A good prediction (a good prognostic marker) should allow the oncologist to choose the optimal treatment (or no treatment) for each patient, and hence either cure the patient with reduced long term complications and side effects, or improving life quality for those who can not be cured. Reducing the overall financial costs of treatment is also an added feature.

MARKERS IN RESEARCH



Clinical stages in prostate cancer

In many cancer types it is at the time of diagnosis difficult for the clinician to predict the tumors growth and behavior. The predictions made at this time affect the type of treatment for the patient and can therefore have large consequences for the future outcome and his or her life quality. Cancer treatment can be a hard stress, and if the patients tumor is indolent, an aggressive treatment might actually cause more pain and discomfort than the cancer itself. To separate the aggressive tumors from the indolent ones at the time of diagnosis, and further to choose the right treatment, is a main challenge in cancer care.



Ploidy is a cytogenetic term used to describe the number of chromosome sets, or deviation from the normal number of chromosomes in a cell. In cytometry, the expression DNA ploidy is used either to describe the DNA content in a cell or the total DNA distribution in a cell population. Normal human somatic cells contain 46 (2 × 23) chromosomes, and are called diploid (2n). The corresponding amount of DNA measured with cytometry is named DNA diploid (2c). Tetraploid cells have 2 times the diploid number of chromosomes and are named tetraploid (4n), and the corresponding DNA amount is classified as DNA tetraploid (4c). Cells with a chromosome number that is exact multiple of the normal diploid number (4n, 8n, 16n) is also called euploid, and cells with either fewer or more than the normal (or multiple) number of chromosomes are defined as aneuploid. The corresponding classification in cytometry is DNA aneuploid. Aneuploidy is an abnormal and unstable situation and is often related to a poor prognosis for cancer patients.

DNA PLOIDY

An image cytometry system has been developed, consisting of PWS (Ploidy Work Station) Grabber and PWS Classifier for analysing the DNA distribution in a given tumour sample. DNA is stained in situ by Schiff's reagents by the Feulgen technique (See detailed specimen preparation on the next page). The method results in stained nuclei were the staining intensity is proportional with the DNA content.

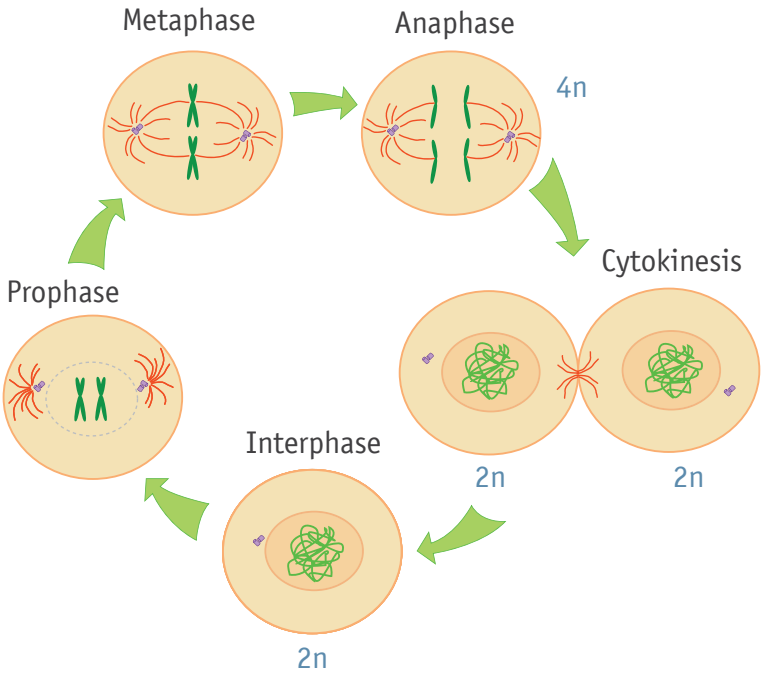
The DNA ploidy analyses give an objective measure of large-scale genomic instability which discriminates between specimen with a normal DNA content and nuclei with an abnormal and often unstable DNA content.

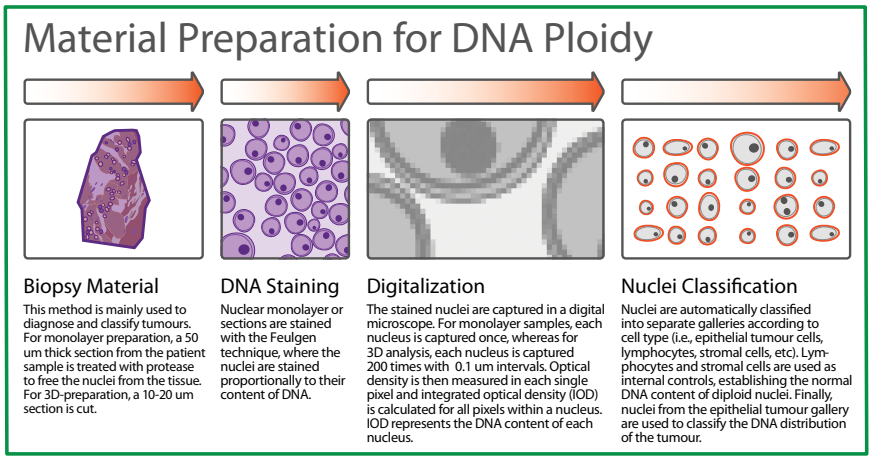
DNA ploidy has been shown in multiple studies to be a diagnostic and prognostic marker for patients with different cancer types, and in particular for patients with gynaecological cancers. By performing DNA ploidy analyses on tumour material one can therefore obtain valuable information that can contribute in the process of determining the best treatment scheme for each patient.

In simple words, the basic concept is that tumours with abnormal DNA content and/or unstable chromatin structure is in a more advanced stage and is more likely to have micro metastases (spreading of the cancer). This "large scale genomic instability" can be measured on samples from routine biopsy specimens by computerized analysis of images from the microscope.

DNA PLOIDY IN THE CELL CYCLE

DNA ploidy is a cytogenetic term describing the number of chromosome sets (n) or deviations from the normal number of chromosomes in a cell. In cytometry, the expression is used either to describe the DNA content in a cell (c) or the total DNA distribution in a cell population.



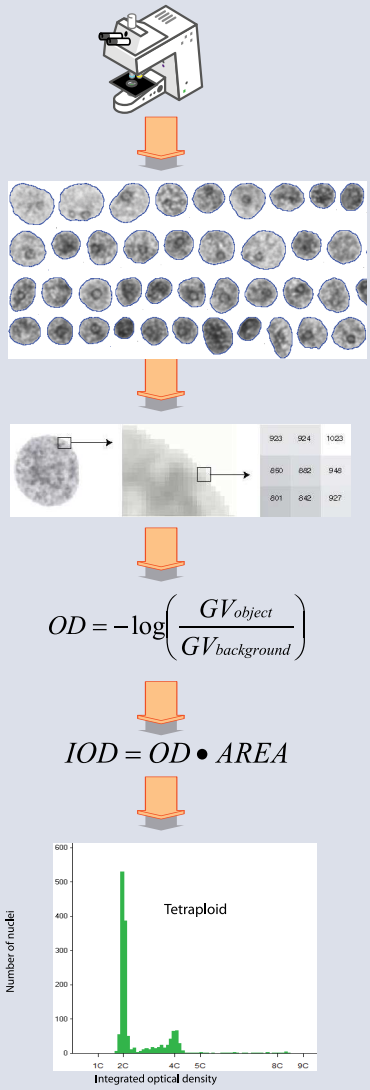


MONOLAYERS

A suspension of isolated whole nuclei are centrifuged to a specially prepared slide such that the nuclei lie in a single layer (monolayer). This preparation method is used for DNA ploidy analyses. The benefit of using such a method is that one can measure intact nuclei, which can be identified automatically aided by simple image analyses methods (threshold methods). By using a DNA specific staining method such as the Feulgen-Schiff method, one can also measure the amount of absorbed light in the whole nucleus to find a precise measurement for the amount of DNA.

DNA ploidy investigation is defined here as the sequence of operations originating from paraffin embedded tumor blocks consisting of selection of tumor area, sectioning for preparation, preparation, Feulgen staining, measurement, editing, and classifying.

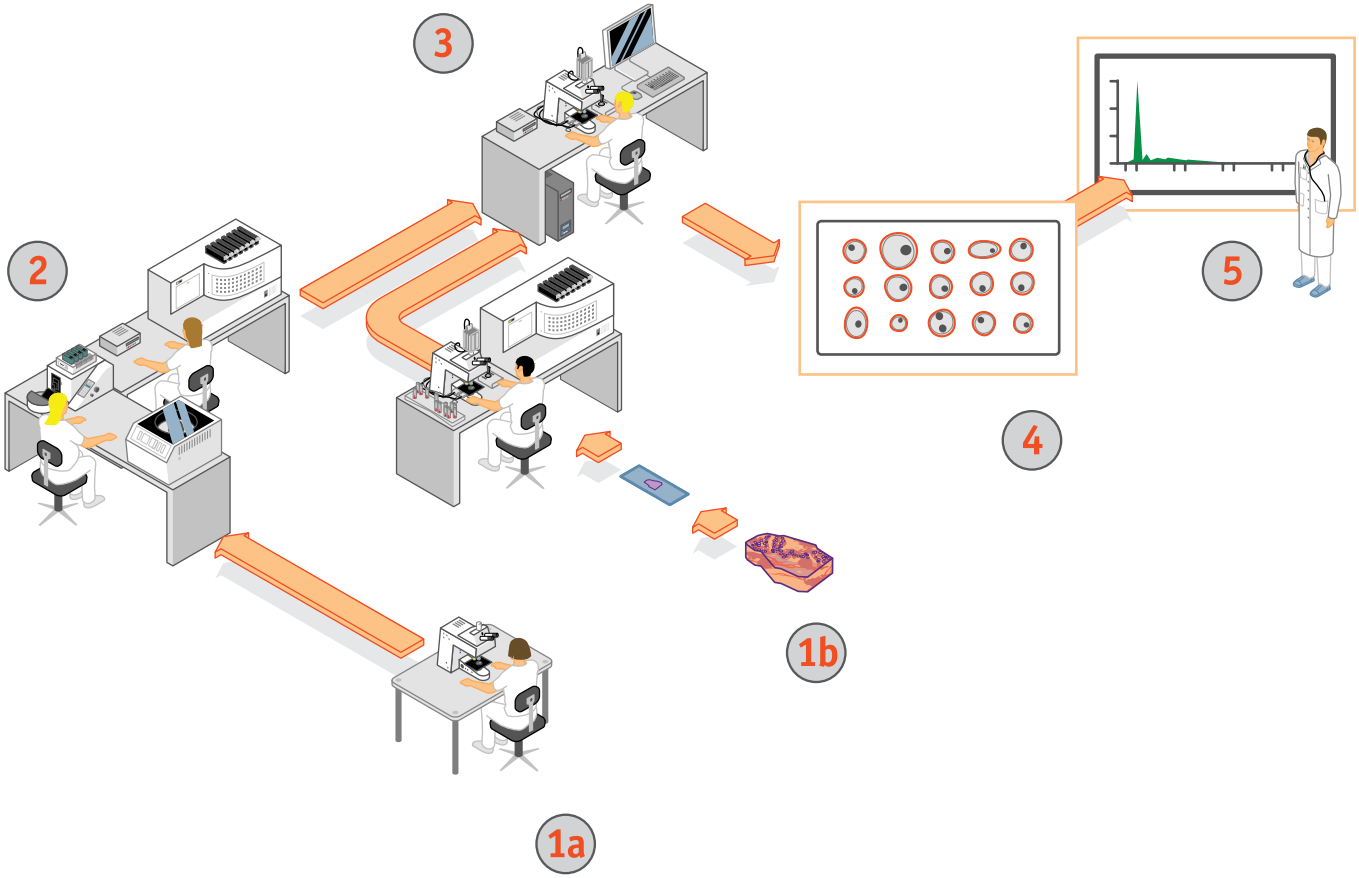
Image cytometry is based on the Feulgen technique which is a widely used staining procedure within biomedicine. The Schiff or Schiff-related reagent is used to bind to aldehydes released after the DNA is hydrolyzed with acid. This allows for staining of DNA in situ. The staining intensity is proportional to the DNA concentration and the amount of DNA in the nucleus is expressed by light absorbed by the Feulgen stain in the whole nucleus.



The Feulgen reaction is used to quantify the amount of DNA in a tumor nucleus. A digital camera captures images of individual nuclei in the specimen. The images are divided into image elements (picture elements - pixels). The gray tone value for each pixel represents the intensity of DNA specific staining. The value is saved in the computer which numerates between 0 (black) and 1023 (white). The relative amount of DNA in each nucleus (DNA ploidy) is yielded by summing the optical density of all the pixels in the nucleus. DNA ploidy measured in this way is a diagnostic and prognostic marker for patients with different cancer types.

During ploidy measurement, the IOD values of all of the nuclei in the specimen are registered and can be graphically developed into a histogram (x-axis: IOD value, y-axis: number of nuclei).

DNA PLOIDY WORKFLOW



1a The test material from the patient is evaluated by a pathologist, outlining the area of interest (e.g. the tumour) under the microscope.

1b A new 3D method allows for DNA Ploidy measurements directly in the DNA stained tissue.

2 Traditionally the tissue sample from the patient is prepared into a monolayer of cell nuclei and then stained specifically for DNA.

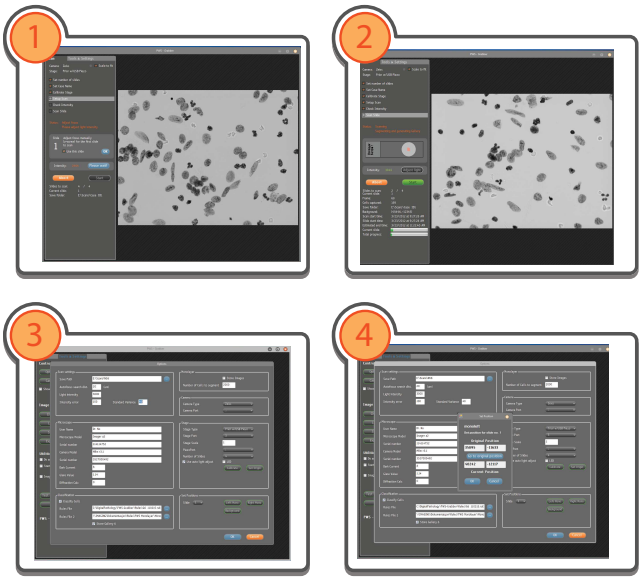
3 Nuclear images from DNA stained samples are digitized and transferred to the computer for automatic segmentation and measurements.

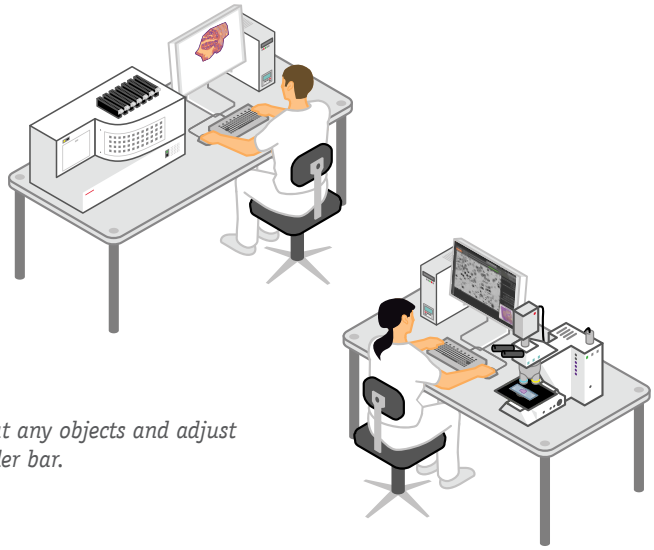
4 The computer displayes classified nuclear galleries for interactive confirmation by the operator.

5 Integrated optical density (IOD) of each DNA stained nucleus is measured by the computer and displayed in a DNA histogram for final manual DNA ploidy classification.

PWS GRABBER

An application for automatic microscope scanning of monolayers.





Simple Operation

1. *Slides and case ID are specified.*
2. *Adjust focus for slide 1. Set background position to an area without any objects and adjust light intensity. Adjust focus for slides in position 2-4 using the slider bar.*

Controlled Setup Procedure

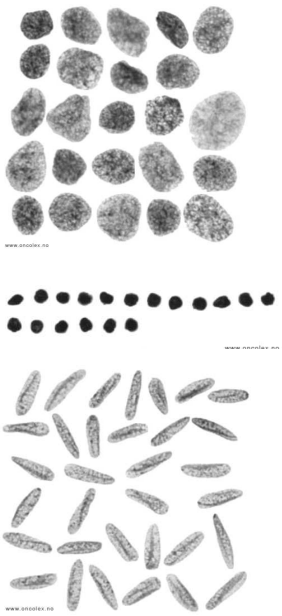
3. *After logging in, tools and settings may be altered.*
4. *The options panel let's you alter scan specifications, specify microscope system, camera and stage details, cell classification rules can be loaded, and positions to be scanned can be specified. Here are also possibilities to correct for the optical challenges like dark current, glare*

The PWS Grabber is employed to focus, grab, and segment cancer nuclei stained with the Feulgen-Schiff method. The absorbed stain is proportional to the DNA content in each nucleus.

MAIN FEATURES OF THE SOFTWARE:

- Automatic high resolution scanning of monolayers
- Supports multiple slide scanning
- Automatic control of microscope, (navigation, focusing, image grabbing)
- Saves high quality images (TIF) and .nuc files
- Automatic segmentation and classification

Cell classification is completed using a rules set file, that is made based on results from manual editing. Different rules are used for different cell types and for monolayer and sections. The rules set makes it possible to store only the correctly segmented cells, and classify them in to galleries containing different cell types.



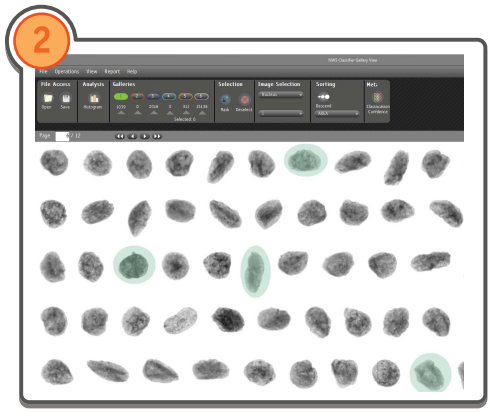
From top: Epithelial nuclei, lymphocytes and fibroblasts.

PWS CLASSIFIER

Automatic classification of cell types

PWS Classifier handles the images of the objects grabbed and segmented by the PWS Grabber. The software enables manual editing (sorting of objects), it displays DNA ploidy histograms, and performes calculations and statistics of selected objects. Finally, PWS Classifier enables manual and automatic DNA ploidy classification that could be used in diagnosis and prognosis of cancer patients.

1. *After opening a new case one may select the wanted image selection and start sorting the nuclei by selected parameter.*



2. *Nuclei are sorted into the galleries by area and the editing of each case by selecting nuclei may start. Misplaced nuclei can be put in other galleries.*

3. *After editing the histogram can be opened. The nuclei in the different galleries are indicated by different colors. The selection and setting of the 2c peak is done.*

4. *Tags with statistical information of the selected peak may be added and it is possible to select and view the selected objects. The whole process can also be done automatically.*

5. *The results are added to a report where the histogram and the related statistical information is saved.*

6. *A scatter plot enables a plot with selected parameters. During setup it is possible to adjust for the optical features glare, dark current and diffraction.*

DNA ploidy analyses can give diagnostic and prognostic information that can contribute in the process of determining the best treatment scheme for cancer patients.

PWS (Ploidy Work Station) is a high resolution DNA ploidy image cytometry system – Consisting of two elements PWS Grabber and Classifier – it enables fully automated focusing, scanning, grabbing and segmentation of high quality images of a high number of nuclei.

PWS automatically sorts the nuclei into galleries containing different cell types. Visual control of the images ensures that only intact tumor nuclei are analyzed.

PWS Classifier handles objects grabbed by the PWS Grabber. The software enables manual editing (sorting of objects), it displays DNA ploidy histograms, and performs calculations and statistics.

PWS enables manual and automatic DNA ploidy classification that could be used in diagnosis and prognosis of cancer patients or patient stratification.

RECENT PUBLICATIONS ON PLOIDY

1. The prognostic value of DNA ploidy in a total population of uterine sarcomas.
Kildal W, Abeler VM, Kristensen GB, Jenstad M, Thoresen SO, Danielsen HE
Ann Oncol. Jun;20(6):1037-41. 2009

2. Gross genomic alterations differ between serous borderline tumors and serous adenocarcinomas - An image cytometric DNA ploidy analysis of 307 cases with histogenetic implications.
Pradhan, Manohar; Davidson, Ben; Tropé, Claes; Danielsen, Håvard; Abeler, Vera; Risberg, Björn
Virchows Arch. Jun;454(6):677-83. 2009

3. Large Scale Genomic Instability as an Additive Prognostic Marker in Early Prostate Cancer.
Maria E. Pretorius, Håkon Wæhre, Vera M. Abeler, Ben Davidson, Ljiljana Vlatkovic, Ragnhild A. Lothe, Karl-Erik Giercksky and Håvard E. Danielsen.
Cell Oncol.;31(4):251-9. 2009

4. Large-scale genomic instability in colon adenocarcinomas and correlation with patient outcome.
Bondi J, Pretorius M, Bukholm I, Danielsen H.
APMIS. Oct;117(10):730-6. 2009

5. DNA ploidy heterogeneity in endometrial carcinoma: comparison between curettage and hysterectomy specimens.
Pradan M, Abeler VM, Davidson B, Kildal W, Nybøen Å, Trope CG, Risberg B, Danielsen HE.
Int J Gynecol Pathol., 29(6):572-8, 2010

6. Comparison of nuclear texture analysis and image cytometric DNA analysis for the assessment of dysplasia in Barrett's oesophagus.
Dunn J.M, Hveem T, Pretorius M, Oukrif D, Nielsen B, Albregtsen F, Lovat L.B, Novelli M.R, Danielsen H. E
British Journal of Cancer, 105(8):1218-23, 2011.

7. Prognostic importance of DNA ploidy and DNA index in stage I and II endometrioid adenocarcinoma of the endometrium.
Pradhan M, Abeler VM, Danielsen HE, Sandstad B, Tropé CG, Kristensen GB and Risberg BÅ.
Ann Oncol 2011. sep 30 (Epub ahead of print)

8. Multiple chromosomal monosomies are characteristic of giant-cell ependymoma.
Dahlback H-SS, Brandal P, Krossnes BK, Fric R, Meling TR, Meza-Zepeda L, Danielsen HE, Heim S.
Hum Pathol. 42(12):2042-2046, 2011.

9. Genomic imbalances in type I endometrial carcinomas – comparison of DNA ploidy, karyotyping and comparative genomic hybridization.
Kildal W, Micci F, Risberg B, Abeler VM, Heim S, Danielsen H
Mol Oncol 6(1):98-107, 2012.

10. Large scale genomic instability is a sensitive predictor for relapse in patients with stage II colon cancer.
Danielsen H. E, Hveem T. S, Pretorius M.E, Clinch N, Merok M, Sjo O, Liestøl K, Lothe R and Nesbakken A.

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