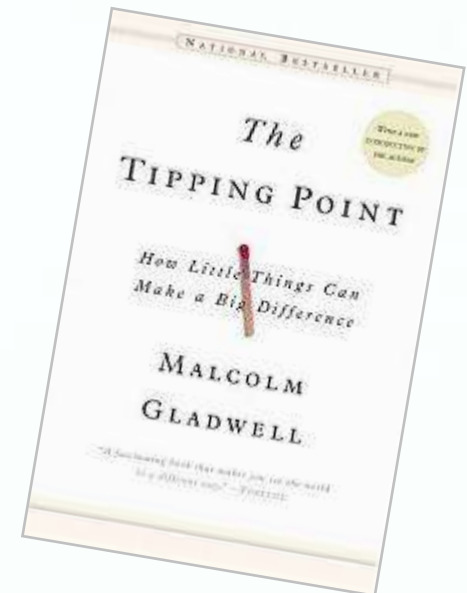




# Virtual Microscopy: A Tipping Point in Tissue Based Research and Education

**James Diamond**  
**Peter Hamilton**  
Queen's University of Belfast

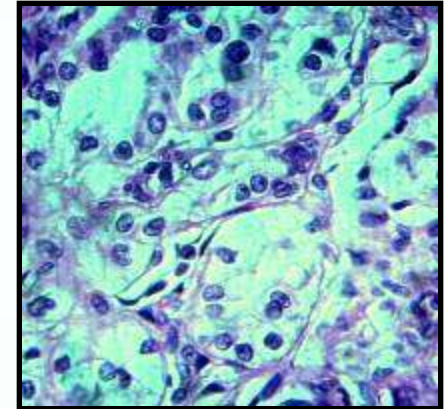
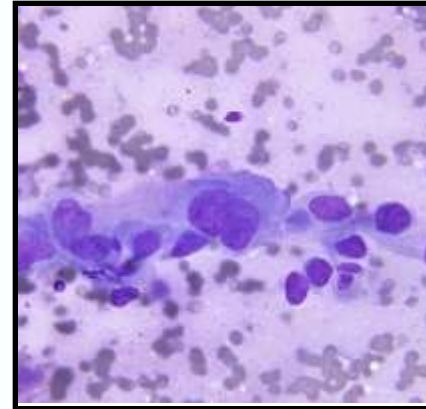
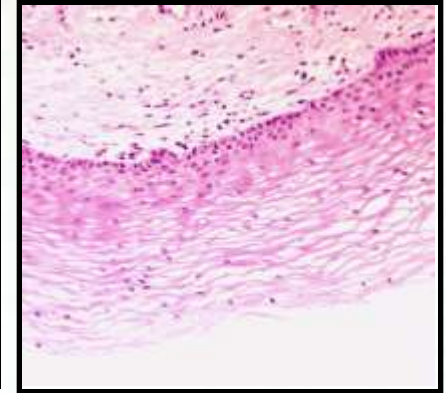
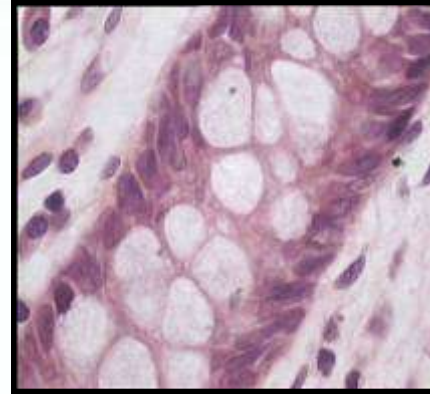


# Pathology



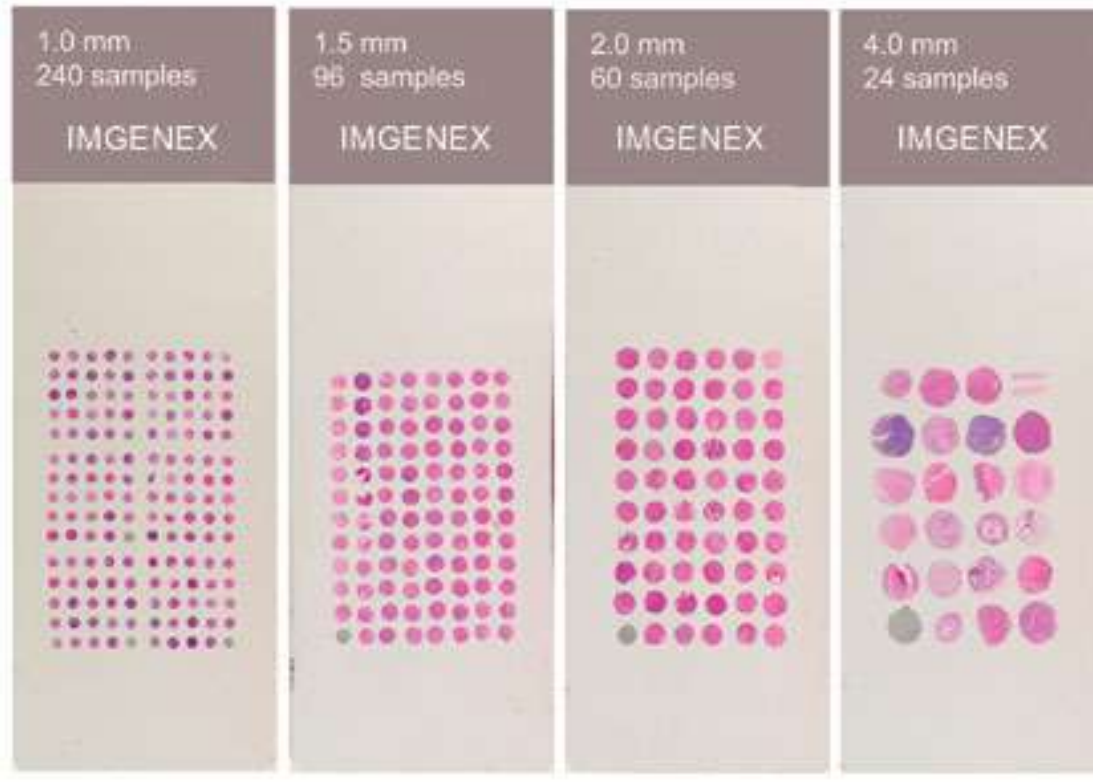
# Skilled interpretation of tissue morphology

## Pathology diagnostics is central to patient care

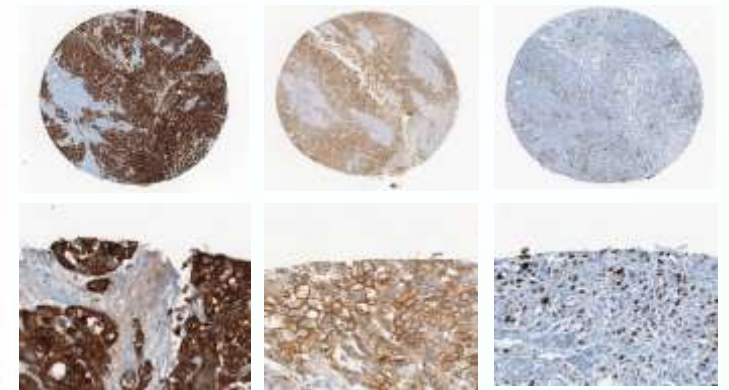




# Pathology diagnostics is central to translational research and biomarker discovery



(Courtesy of Imgenex)



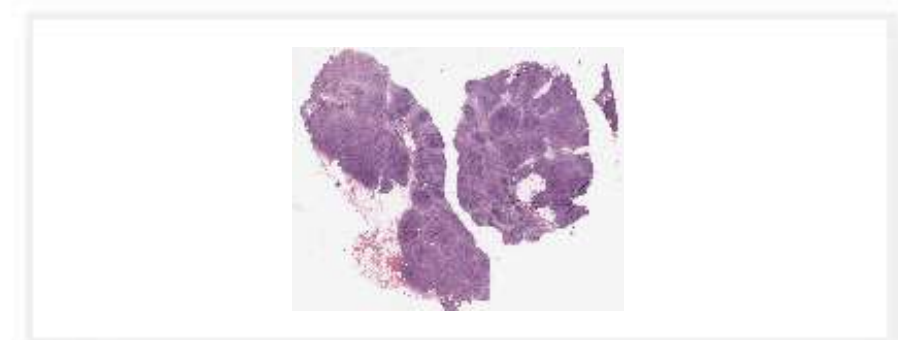
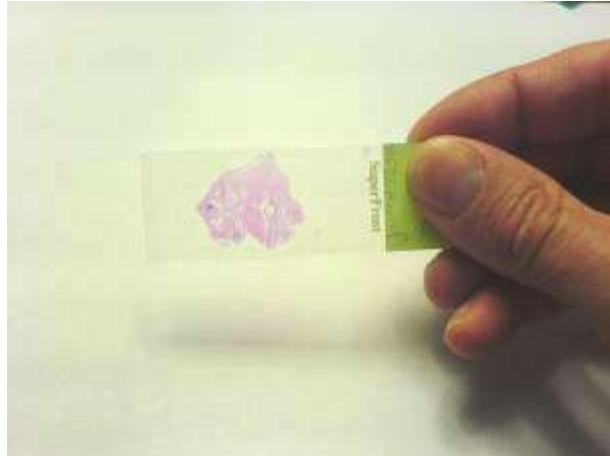
AE1/AE3

E-Cadherin

Ki67

# The Tipping Point ?

## Turning glass slides into bits and bytes



# Hardware – a competitive market

**HAMAMATSU**



**ai** APPLIED IMAGING



**Olympus .slide**



**Nikon CoolScope & BLISS**



**Aperio**  
TECHNOLOGIES

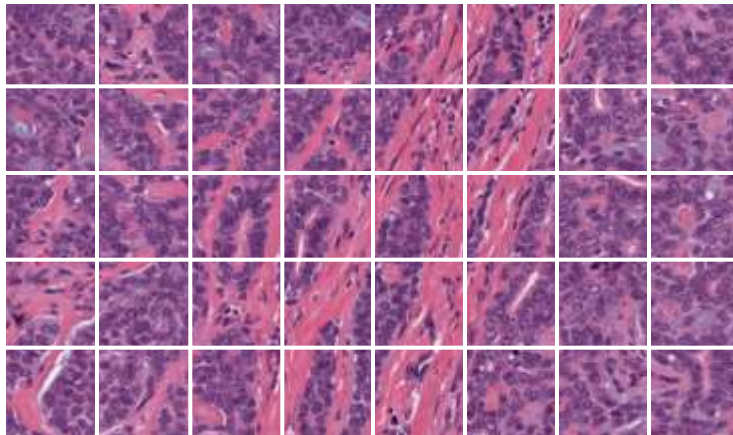


**ZEISS**

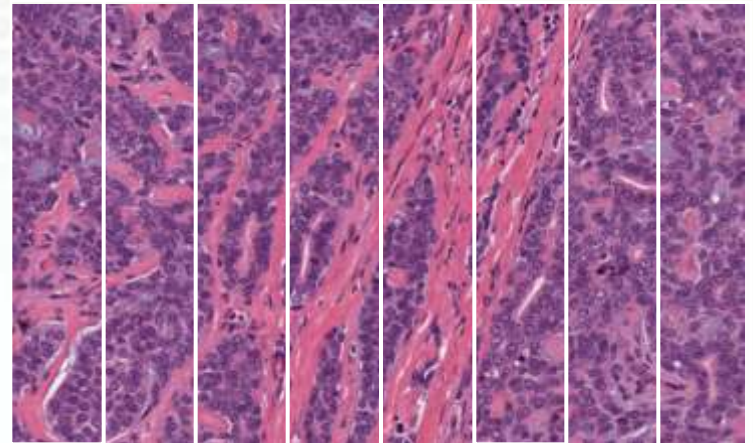


# Single objective

## Image tiling



## Line scanning

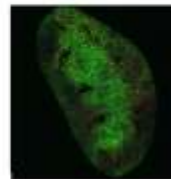
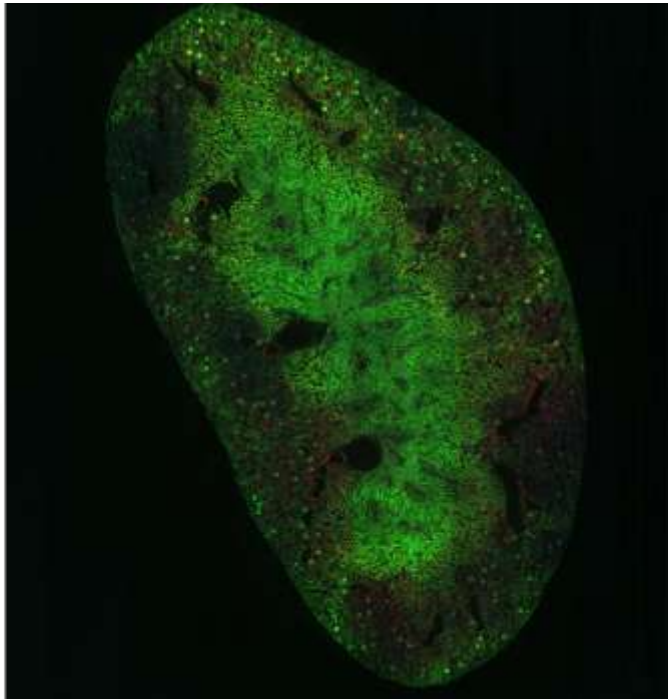


# The array microscope





## Virtual Fluorescence Slides



**HAMAMATSU**

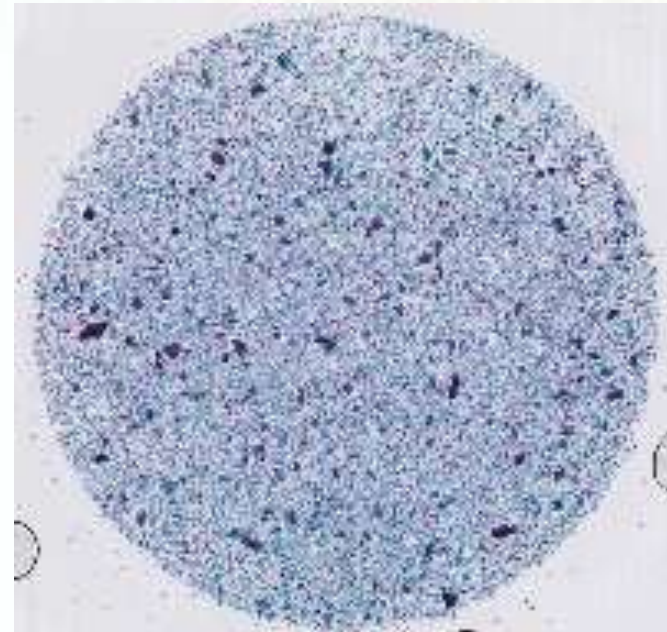




# Z-axis scanning and virtual focus

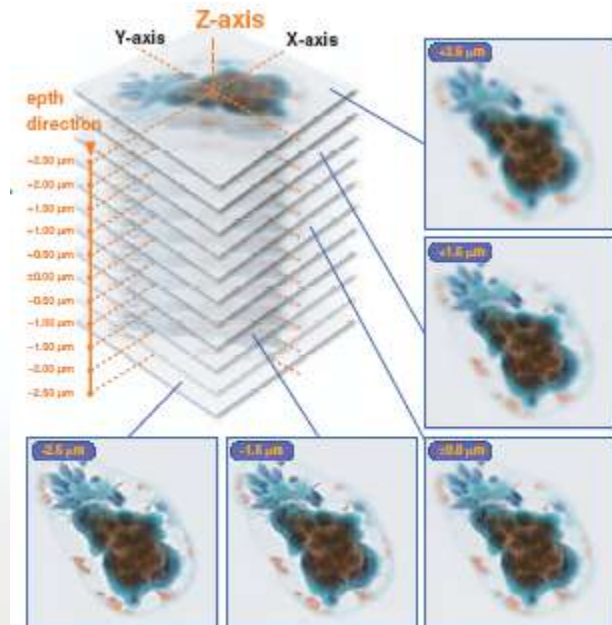


Cervical cytology

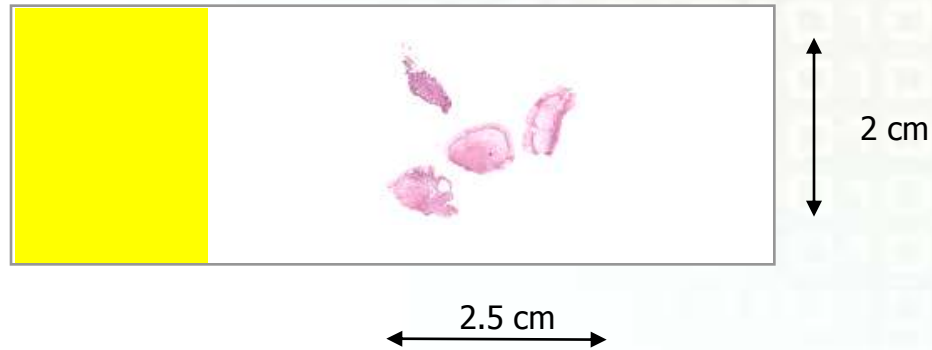


17 Gigabyte : TIFF JPEG compressed

**25 slides = 1 Terabyte**



# These are large images !



At x40 objective magnification the required resolution is  
0.24 microns per pixel = 104,000 x 85,000 linear pixels

**25 Gigabytes**

JPEG2000 compression 25:1

**RVH Belfast: 300 cases a day > 100 Terabytes of data a year**

# Technical challenges

- ◆ Defining standards ◆
- ◆ Storage of Virtual Slides ◆
- ◆ Enhancing on-line delivery of gigapixel imagery ◆
- ◆ Tools for viewing and interacting with virtual slides ◆
- ◆ Algorithms for automated machine vision of gigapixel images ◆
- ◆ High performance computing for tissue imaging ◆

**It's not the virtual slide that counts – it's what we do with it.**

# Application opportunities

## Making virtual microscopy work

Education and Training

Quality Assurance

Tissue archiving

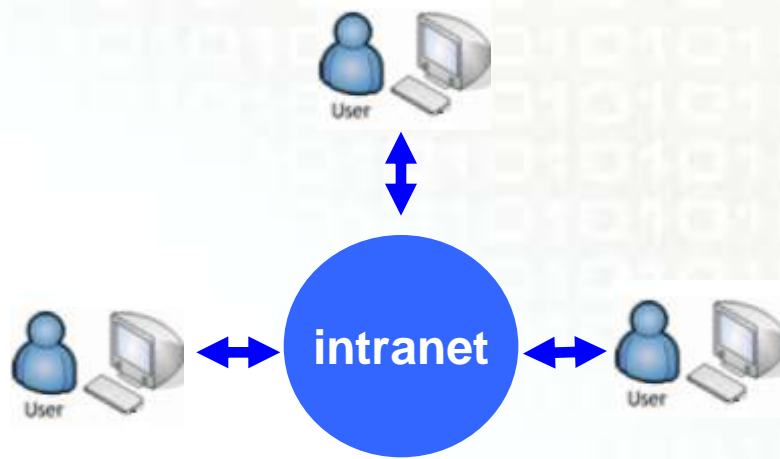
Tissue Research



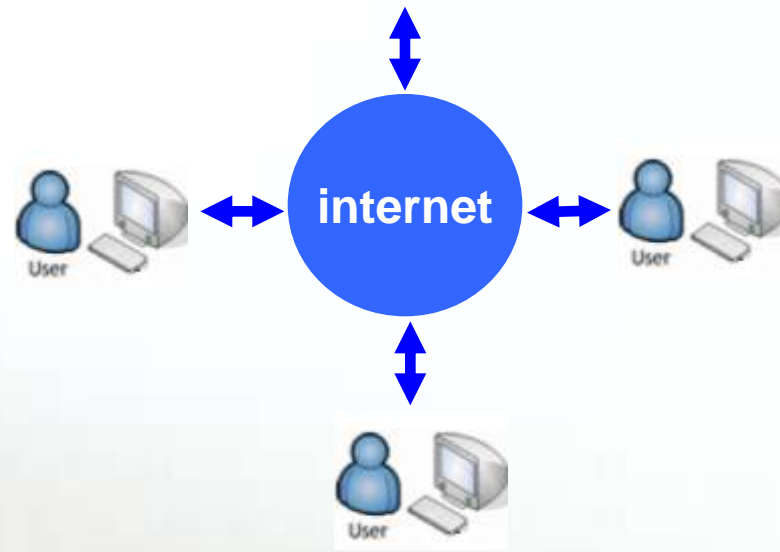
The screenshot shows the PathXL website's login interface. At the top, there is a navigation menu with links for Home, Services, About, Contact, Data, and a dropdown menu for Actions. Below the navigation is a 'Login to your account' section with a form for Username and Password, and a 'Sign in' button. To the left of the form is an image of a person using a microscope. The footer contains copyright information for Path Diagnostics Ltd.

The screenshot shows the Virtual Slide Viewer application running in Mozilla Firefox. The main window displays a histological slide of tissue. A toolbar at the bottom provides controls for Path Recording (Playback and Record buttons), Zoom (26x magnification with plus and minus buttons), Pan (directional arrows and a refresh button), and a Notes button. The PathXL logo and 'VIRTUAL SLIDE VIEWER' text are in the bottom right corner. A status bar at the very bottom indicates 'Transferring data from path.md.qub.ac.uk...'

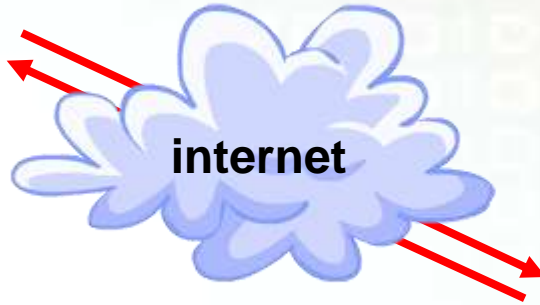
http://  
PATH R  
PLA  
Transferring



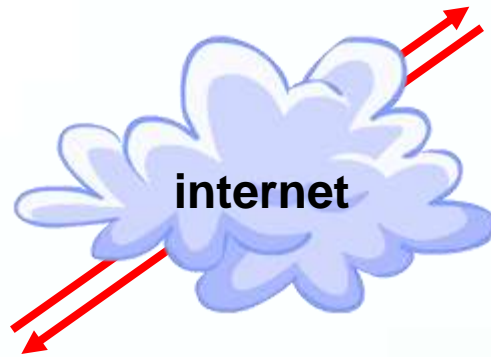
Virtual slide server



# User/student/trainee/researcher



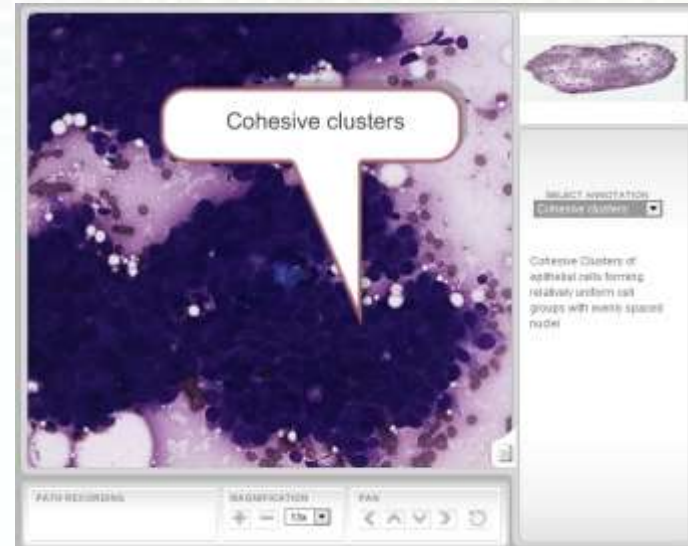
Virtual slide server



Author/Administrator



# Slide Annotations for Training and Education





# Virtual Slides for Examination and Proficiency Testing

MCQ (30 Marks): BC063

**Question 16**

How can you manage a patient with this stage of metastatic colorectal cancer?

Your Answer

- A. 5-FU
- B. cytotoxic combined with support therapy
- C. palliative cytotoxicity
- D. irinotecan

Navigation: 16 of 30, PLAYBACK, RECORD, SWITCH TO ADMIN VIEW, END TEST

MCQ (30 Marks): BC063

**Question 15**

What would be the best treatment for the patient with this histological colorectal cancer?

Your Answer

- A. cytotoxic alone
- B. removal of all lymph nodes
- C. palliative treatment
- D. irinotecan

Navigation: 16 of 30, PLAYBACK, RECORD, SWITCH TO ADMIN VIEW, END TEST

Setting virtual slide questions on-line  
Recording responses centrally on-line  
Setting examinations on-line



e-LEARNING & QUALITY ASSURANCE in PATHOLOGY



Contact Sitemap Help Your Account Logout

welcome news content demonstration subscription glossary

- Breast Cytopathology
- Cervical Histopathology
- Breast Histopathology
- Urine Cytopathology
- Cervical LBC
- UKNEQAS ICC
- Tissue Microarrays
- Melanocytic Skin Lesion Study
- MDT Meeting
- Lectures

you are here: home > modules > breast histopathology > case 9

### Case 9

< previous case | next case >

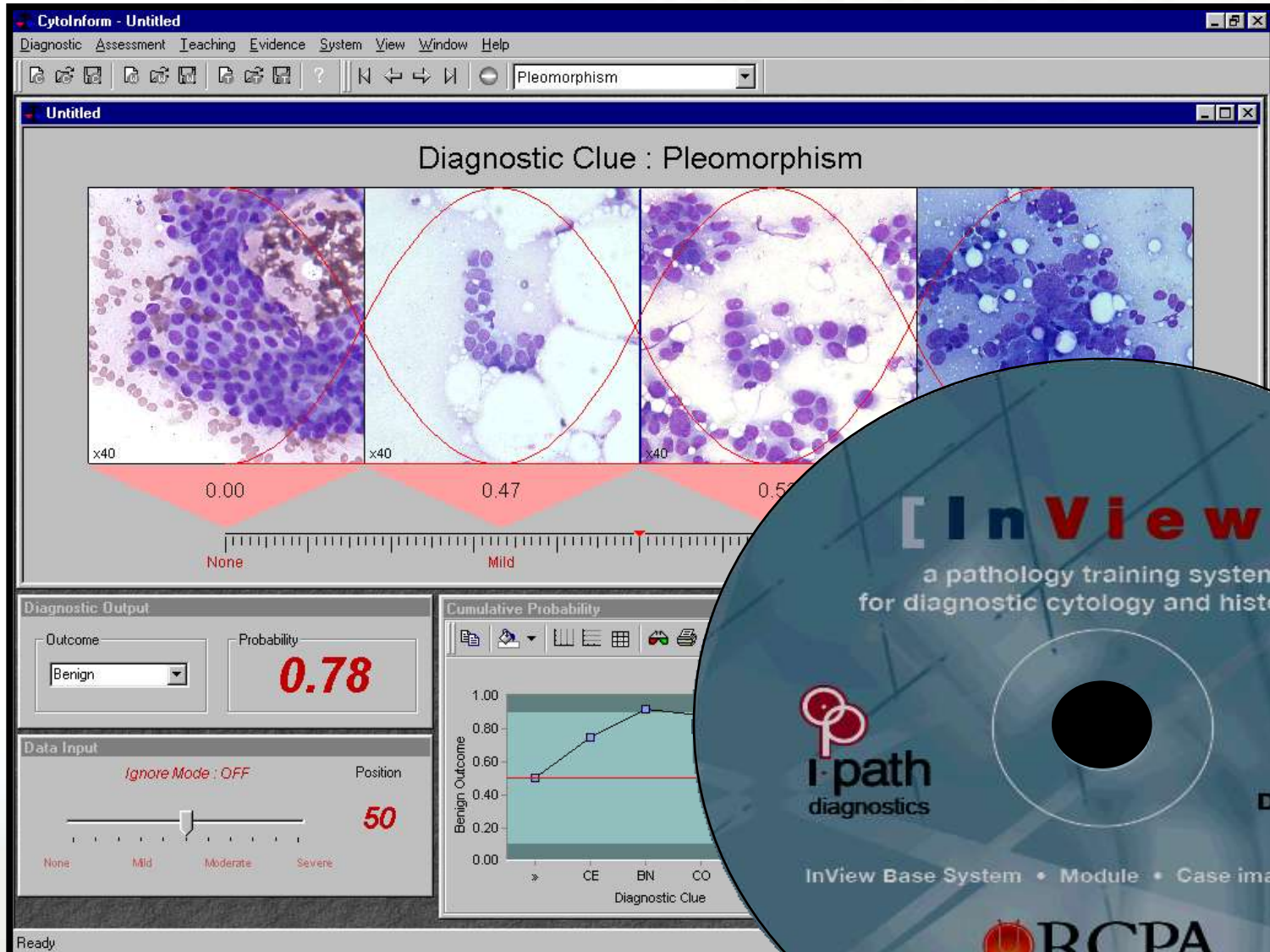
Breast Histopathology Case 9



- View in PathXL slide viewer
- View in standard slide viewer
- View instructional video with PathStream



## “Learning by diagnostic simulation”



**Diagnostic Clue : Pleomorphism**

0.00 0.47 0.50

None Mild

**Diagnostic Output**

Outcome: Benign Probability: **0.78**

**Data Input**

Ignore Mode: OFF Position: **50**

None Mid Moderate Severe

**Cumulative Probability**

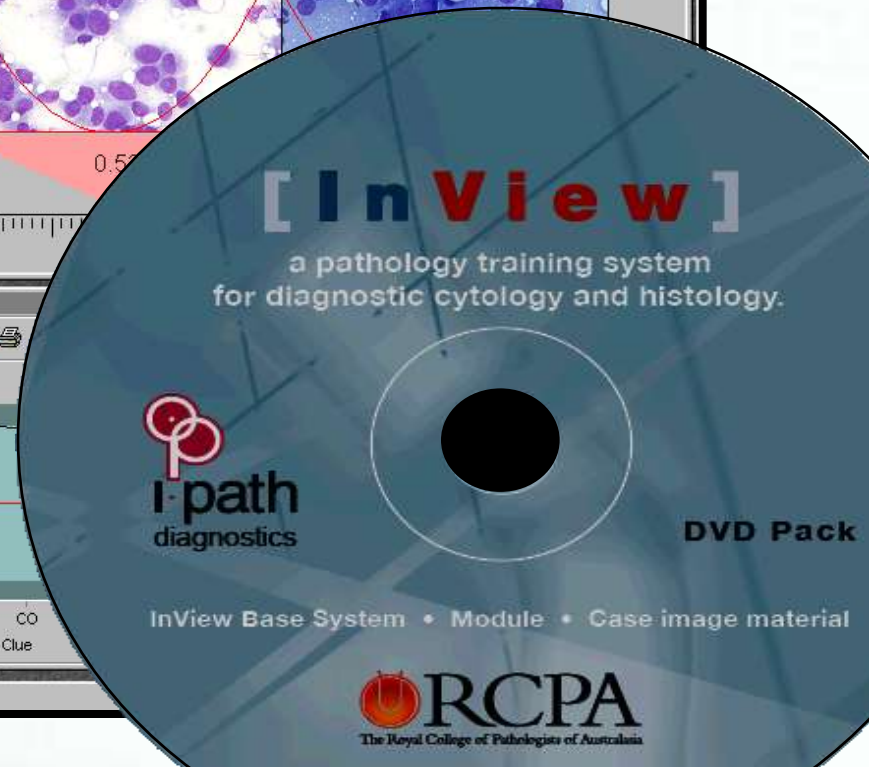
Benign Outcome

1.00 0.80 0.60 0.40 0.20 0.00

CE BN CO

Diagnostic Clue

Ready



# Unique approach to training in pathology

Stepping through the diagnostic clues



Visual comparison with template images

Diagnostic probability

Diagnostic map



# InView™

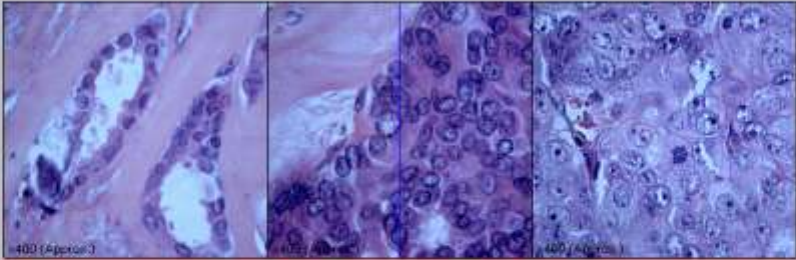
InView - Unfiled

Assessment Evidence View Help

Nuclear Atypia

Unfiled

### Assessment Clue : Nuclear Atypia



400 (Apex) 400 (Apex) 400 (Apex)

0.00 1.00 0.00

Low Intermediate High

Virtual Slide Tray

WHS011: Tubular Differential Diagnosis

Access

Virtual Slide

Instructional Video

Finish Close

Clinical Information

CASE 01: Female, 66 year old. Hard mass in left breast.

Diagnostic Route

Tubular Outcomes

Diagnostic Clue

NA

PRIOR

Data Input

Ignore Mode: OFF

Position

50

Low Intermediate High

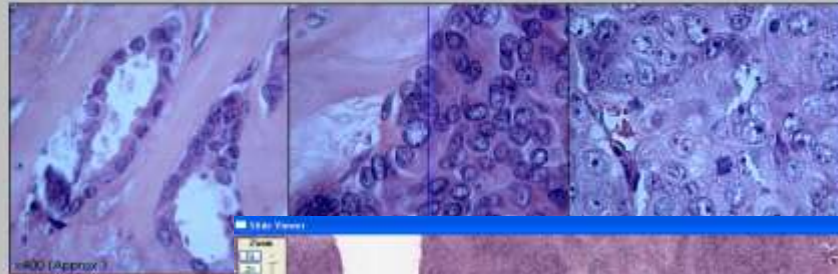
Diagnostic Output

Outcome

Probability

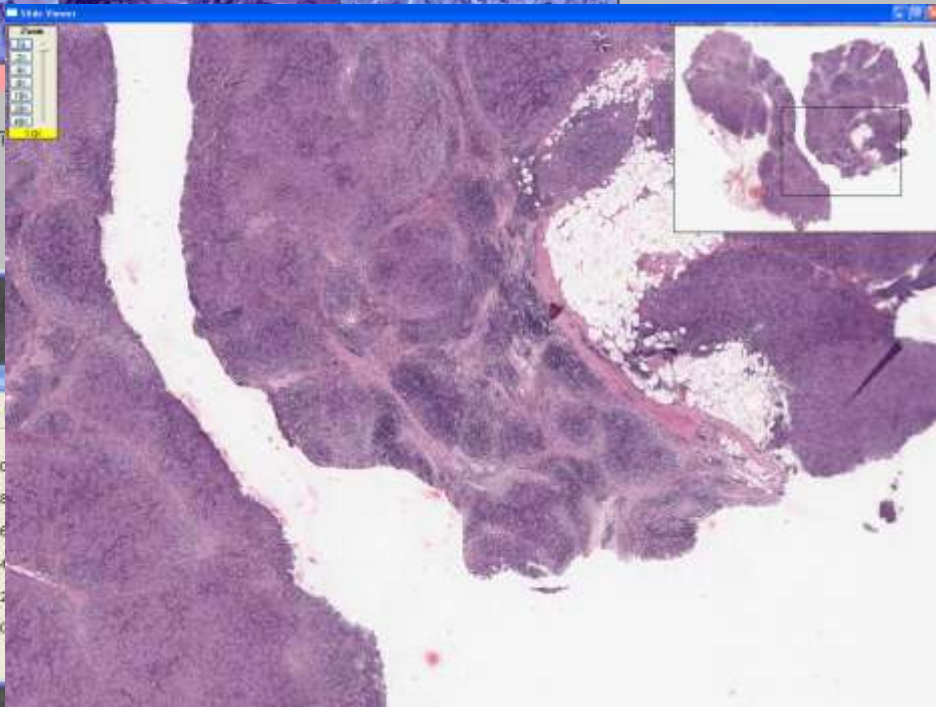
0.05

### Assessment Clue : Nuclear Atypia



0:00

Low



Virtual Slide Tray

BREAST: Tumour Differential Diagnosis

Access

Virtual Slide

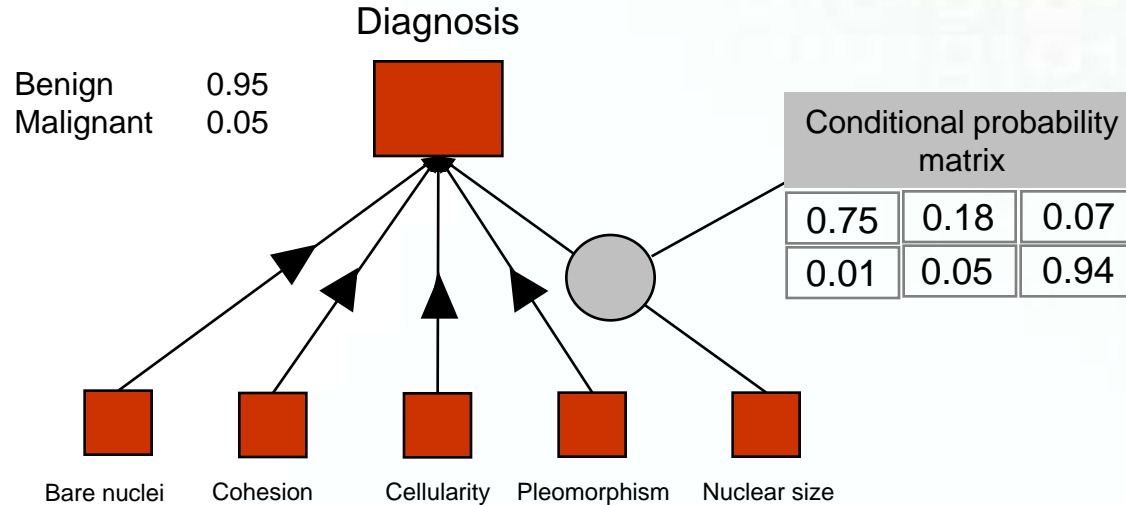
Instructional Video

Finish Close

Clinical Information

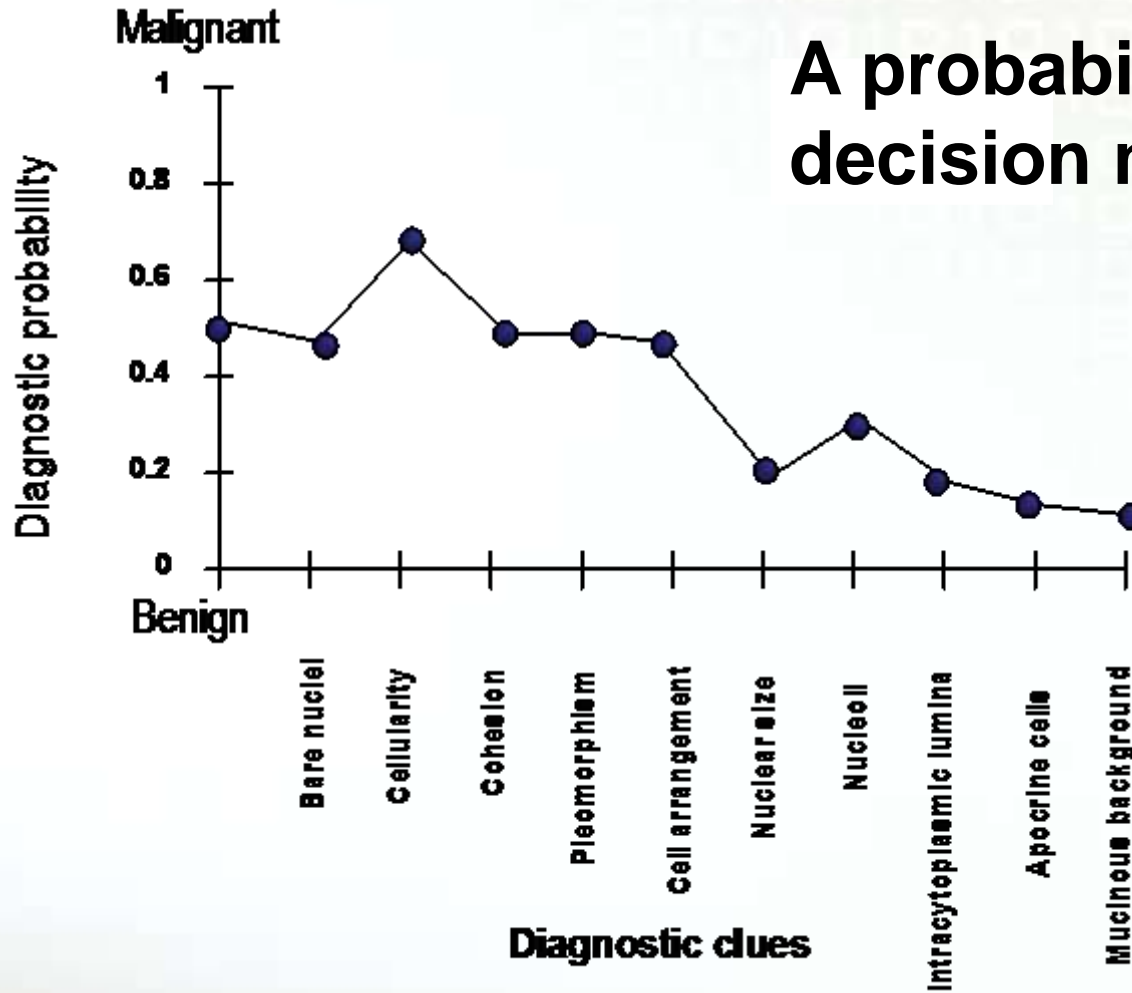
CASE 01: Female, 56 year old. Hard mass in left breast.

# Bayesian belief network engine with fuzzy logic representation of language



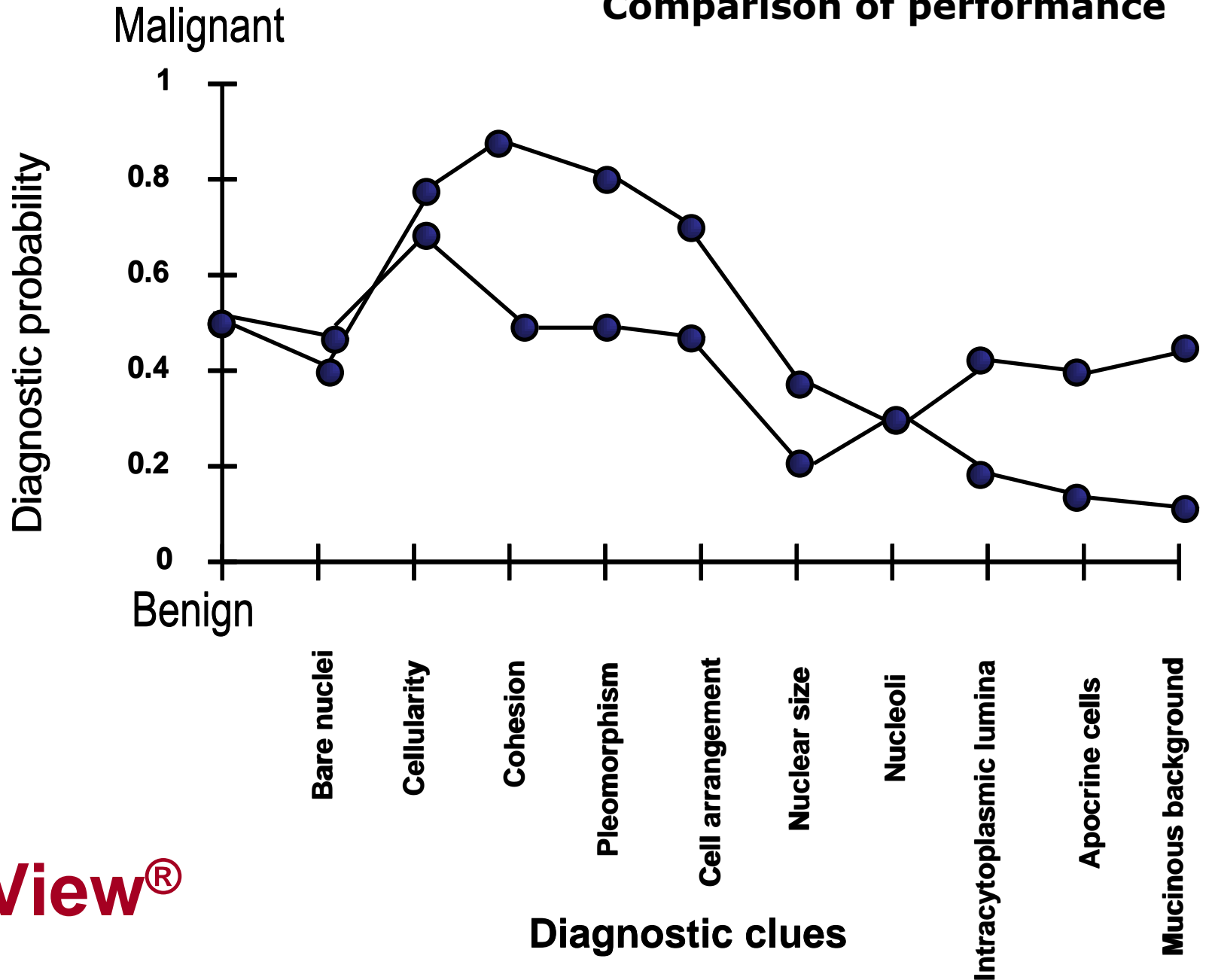
Allows us to model and record diagnostic knowledge from experts

# A probability decision map





# Comparison of performance



InView from i-Path Diagnostics Ltd Help ?

Annotations Pathway **Image** Additions User
Case Notes Video .pdf .ppt

+ - scope ▲

H&E Immuno 1 Immuno 2

Now Playing

**Diagnostics Analysis**

Diagnostic Route Most Likely All **Correct**

Feature	Benign Probability
Cellularity	0.95
Bare/Nuclear	0.90
Cohesion	0.95
Pleomorphism	0.85
Cell Arrangement	0.95
Nuclear Size	0.95
Nuclear	0.95
Cellularity	0.95

**Benign**

Probability: **0.95**

Ignore Clue Finish Assessment

« Pleomorphism »

x4

None

x4

Mild

x4

Moderate

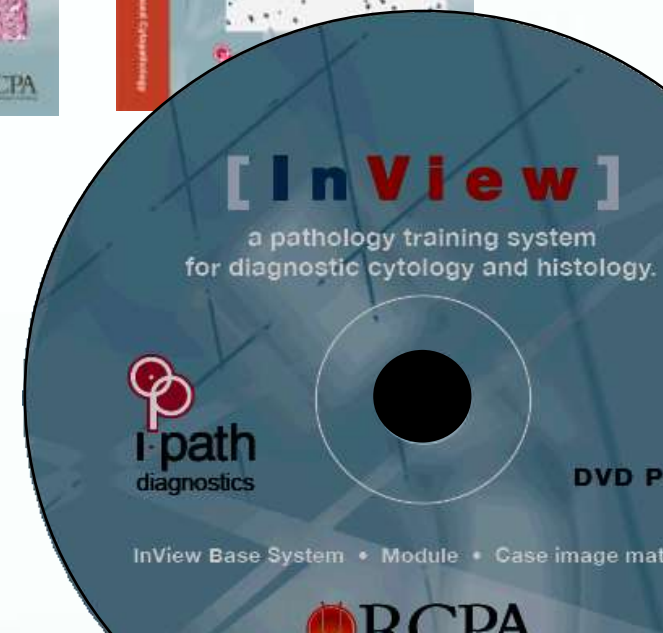
x4

Severe

“Learning by diagnostic simulation”



Endometrial Neoplasia  
Grading in Prostate Cancer  
Soft Tissue Tumours  
Salivary Gland Tumours  
Melanocytic Skin Lesions



# Virtual EQA

External Quality Control and Proficiency Testing



# Virtual Slides for EQA

## Melanocytic Skin Lesion Diagnosis in UK and Japan

**pathVZ**  
Virtual Slides for EQA

**Melanocytic Skin Lesion Study**

**Introduction**

This series of melanocytic lesions has been provided by 10 digital histology laboratories from the United Kingdom and Japan. The study is designed to assess interobserver variation in the assessment of the sites of melanocytic lesions.

Please review each of the slides and enter your diagnosis using the online form available for each case. While the 10 digital histology laboratories are available, a confidential, web-based method of review.

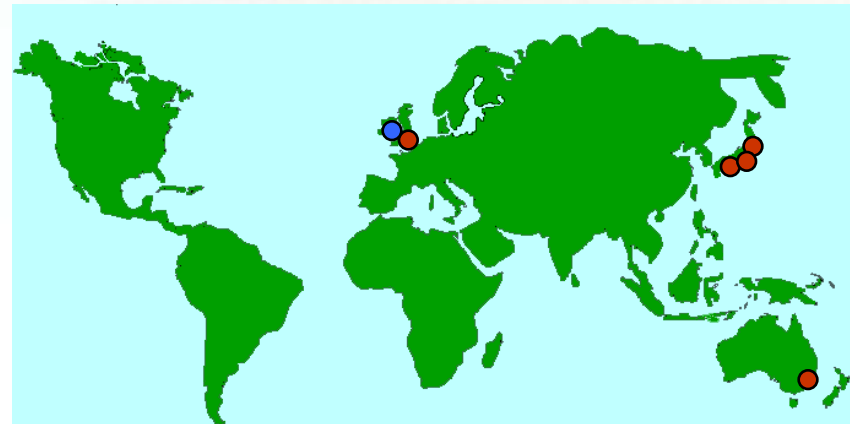
**Notes about slides**

After reviewing each case, the digital slides should automatically display when you click on the next button. If you are having difficulty viewing the slides, be assured that your institution has configured appropriate access to digital slide hosts. The 10 countries health care regulations where detailed access can be searched indicates. Please contact your IT department and request that they allow traffic on ports 80 and 80. This should resolve the problem. Alternatively virtual slides can be viewed at the computer connection to a standard internet or a local area network (LAN) broadband connection.

The more information on using virtual slides for training, education and research please contact: [PathVZ@path.vz](mailto:PathVZ@path.vz)

**Cases**

- Case 1: Female 40 years - right leg
- Case 2: Female 40 years - right shoulder
- Case 3: Female 40 years - left breast
- Case 4: Female 40 years - back
- Case 5: Male 40 years - chest



**PathVZ - Advanced Education and Learning - Mozilla Firefox**

http://www.path.vz.net/forms/default.asp?caseid=1\_10&id=329&case=module\_Melanocytic%20Lesions%20in%20UK&id=329

**Case 2**

Female 40 years - right shoulder

**Diagnosis**

- Acral-lentiginous melanoma
- Blue nevus
- Cellular blue nevus
- Combined nevus
- Compound melanocytic nevus
- Congenital melanocytic nevus
- Deep penetrating nevus
- Desmoplastic melanoma
- Dysplastic nevus
- Genital nevus
- Halo nevus
- Hyperpigmented junctional melanocytic nevus
- Intradermal melanocytic nevus
- Intracutaneous Spitz nevus
- Junctional melanocytic nevus
- Lentiginous melanocytic nevus
- Lentigo maligna
- Lentigo maligna melanoma
- Melanocytic carcinoma in situ
- Acral nevus

Please enter your ID and then your Diagnosis below. Click on the Confirm when finished. If you please review the entered diagnosis with your colleagues. Confidentiality of the

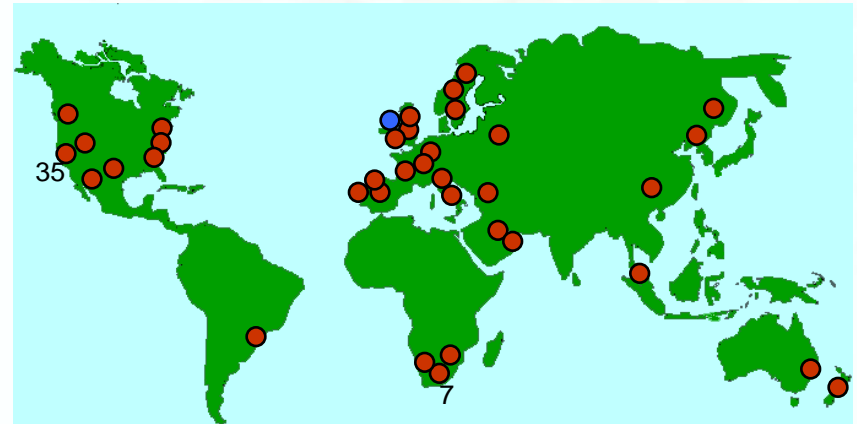
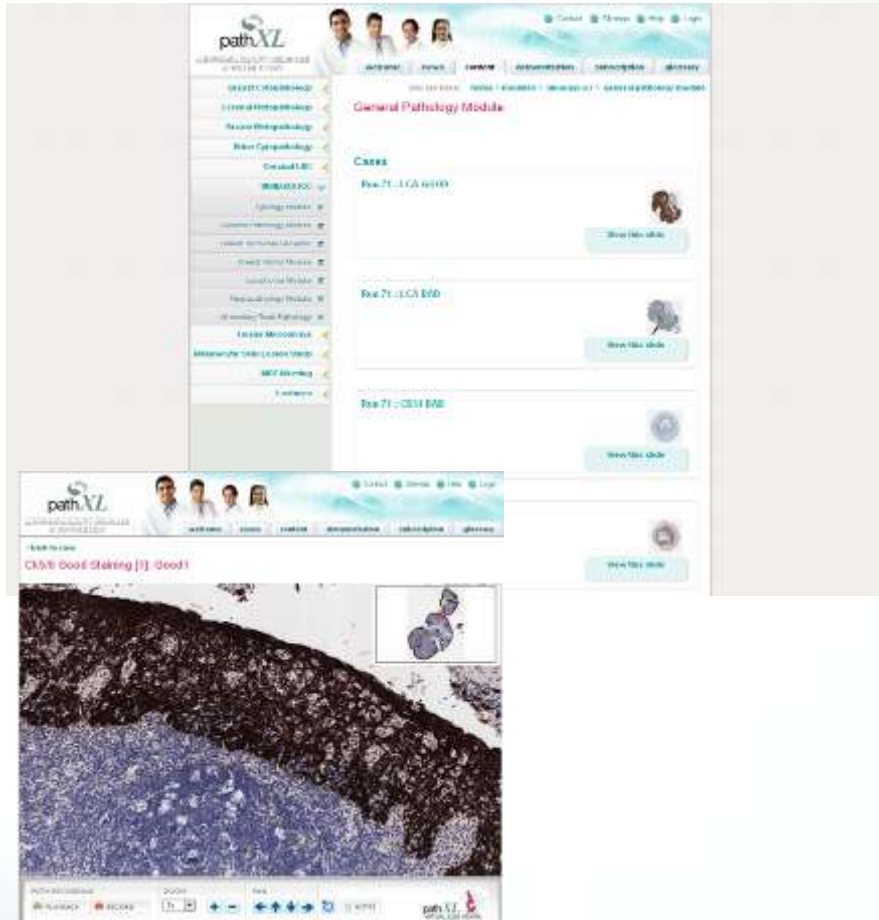
ID:

Diagnosis:

Other diagnosis:

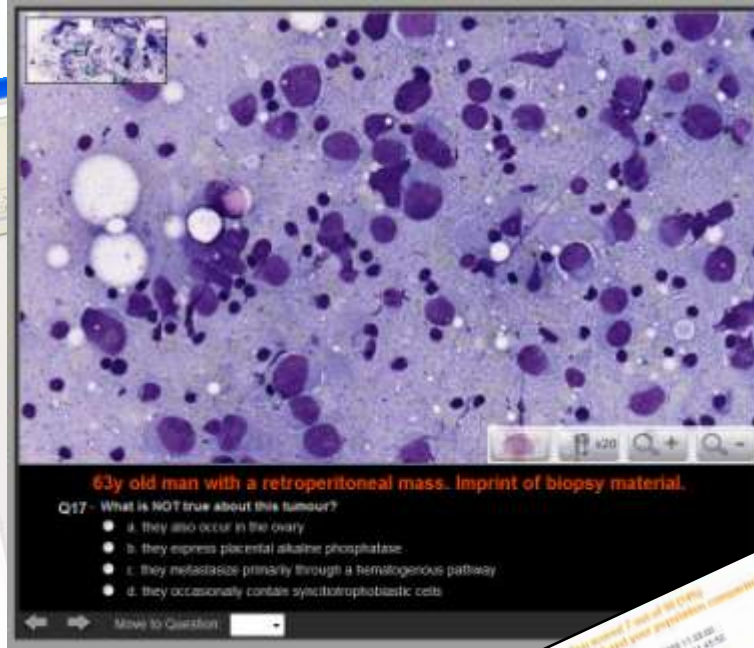
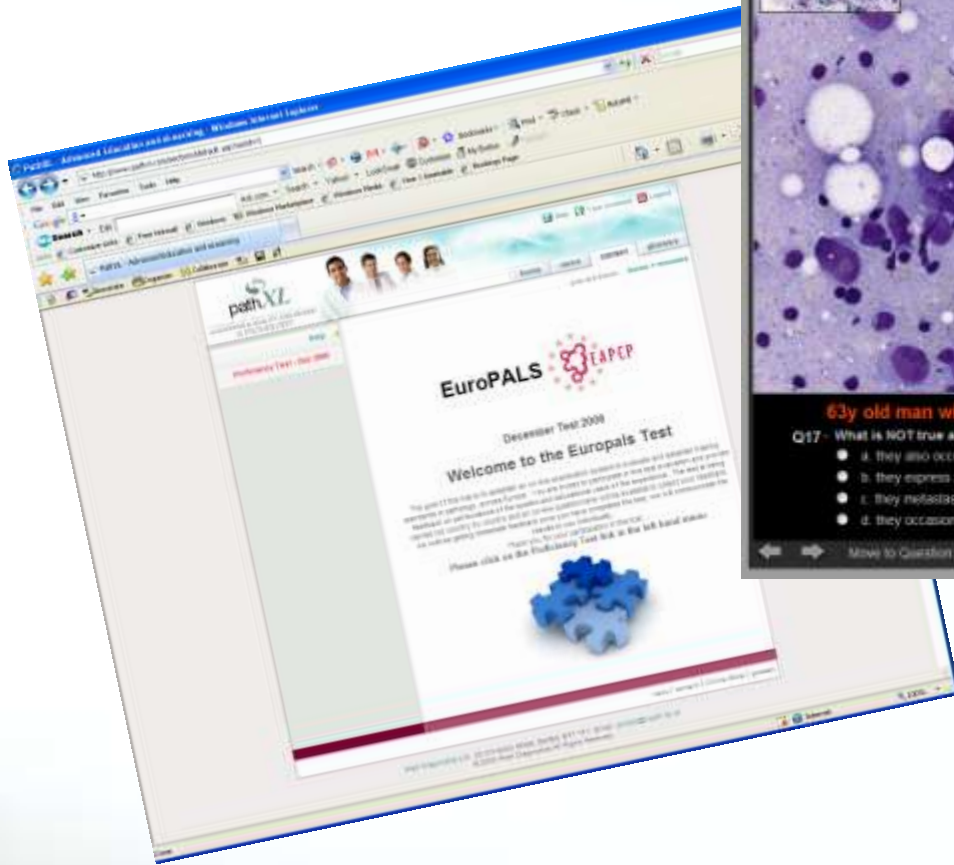
Clear Cancel Submit

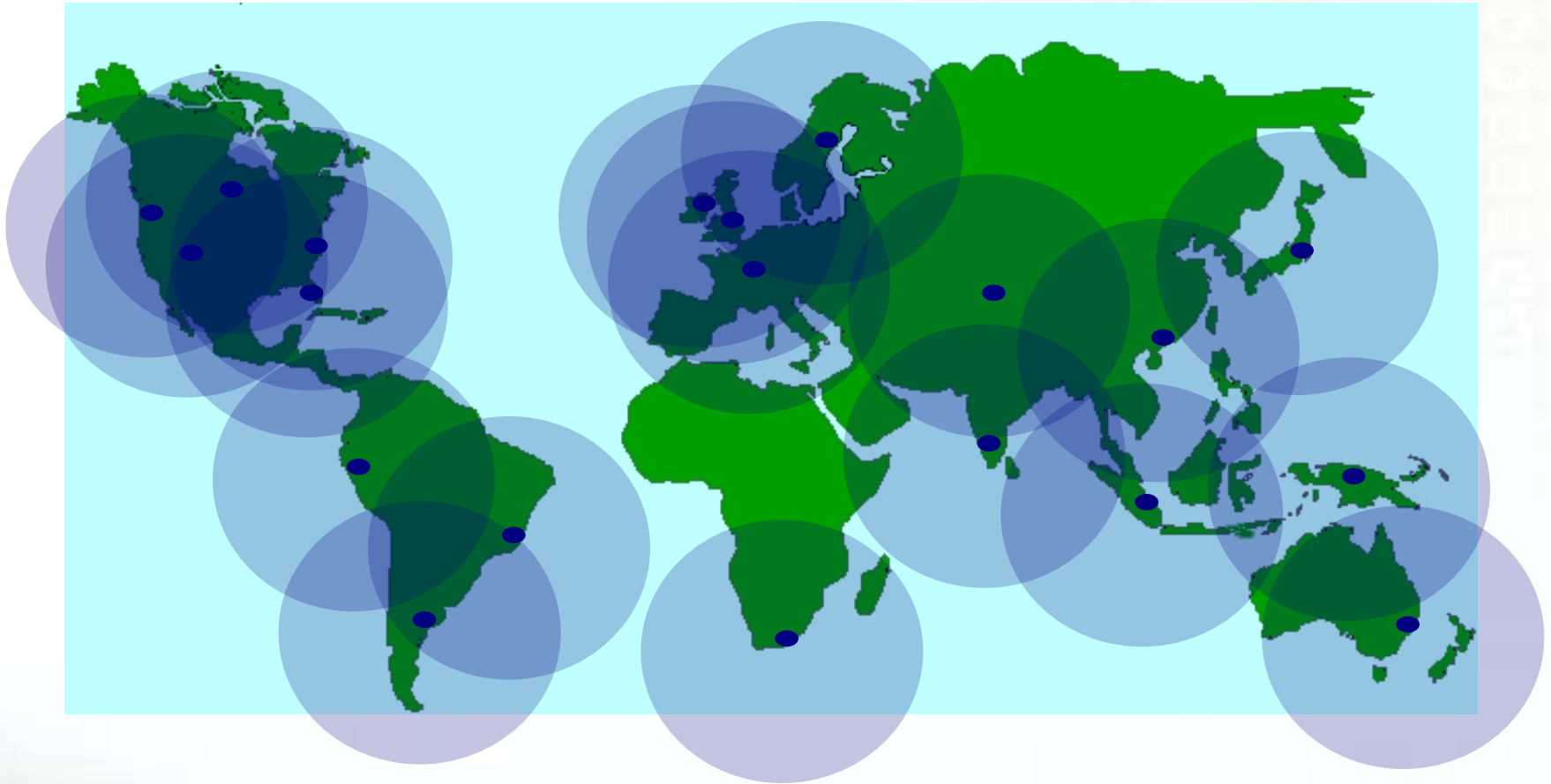
# UKNEQAS IHC Programme



The need for worldwide VM service network of data centers with rapid access to image data globally

# Proficiency Testing





**Worldwide server architecture for delivery of high resolution imagery**



# Virtual Tissue Banking



**OBBR**

Office of Biorepositories  
and Biospecimen Research



**Glasgow Biobank**  
**onCore UK**  
**Tayside Tissue Bank**  
**Wales Cancer Bank**  
**Northern Ireland Tissue Bank**

# Northern Ireland Virtual Tissue Bank (NIVTA) Bioimaging CTU, QUB



The screenshot displays the pathXL website interface. At the top, it features the pathXL logo and navigation links for Contact, Services, Help, and Login. Below the header, there are tabs for website, news, content, demonstration, subscription, and glossary. The main content area is titled "Tissue Microarrays" and includes a "Cases" section with a "Prostate TMA" entry. A "Virtual Slide Viewer" window is overlaid on the page, showing a large, circular histological slide with a grid overlay. The viewer includes a toolbar with navigation controls and a pathXL logo in the bottom right corner.



# Virtual slides for Tissue Archiving and Research

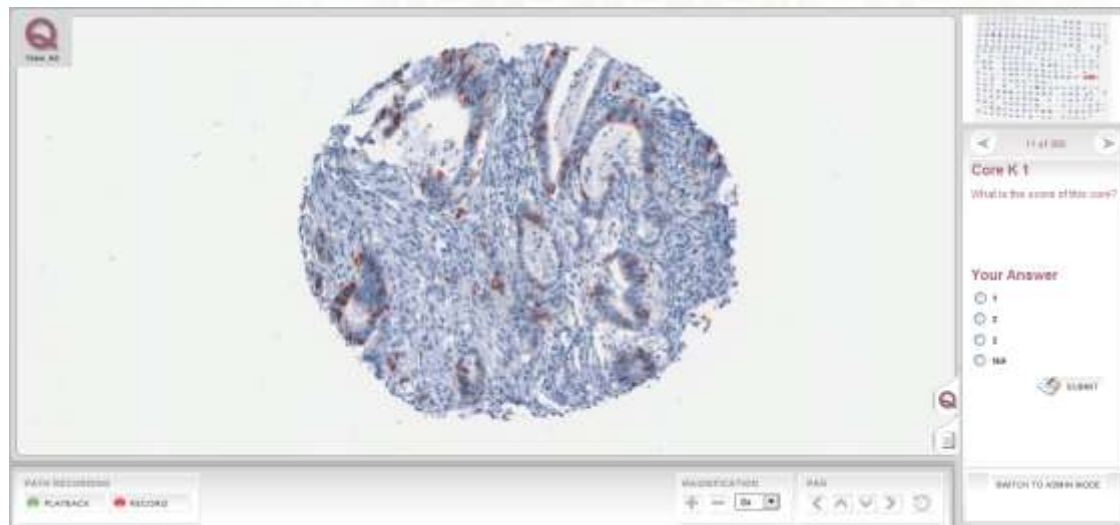
## On-line scoring of TMAs and tissue samples

### Enhancing biomarker evaluation



The screenshot shows the pathXL website interface. The top navigation bar includes links for 'welcome', 'news', 'contact', 'demonstration', 'subscription', and 'glossary'. A sidebar on the left lists various pathology categories such as 'Breast Cytopathology', 'Cervical Histopathology', 'Oral Cytopathology', 'Cervical LBC', 'Hepato-pathology', 'IBD/IGAS ICC', 'Pathological Society', 'Tissue Microarrays', and 'Melanocytic Skin Lesion Study'. The main content area is titled 'Tissue Microarrays' and lists several cases with 'View this slide' buttons:

- Prostate TMA** (Prostate TMA)
- Prostate TMA: Methylation marker** (TMA)
- Prostate TMA: H&E** (Prostate TMA)
- Dean Fennell Tissue Microarray** (Tissue Microarray: 9 May 2006)



The screenshot shows a virtual slide viewer interface. The central area displays a histological image of a tissue microarray core. The interface includes a navigation bar at the top with 'Home', 'About', 'Help', and 'Login' buttons. On the right side, there is a 'Core K 1' section with a question 'What is the score of this core?' and a 'Your Answer' section with radio buttons for '1', '2', '3', and 'NA'. A 'SUBMIT' button is located below the answer options. At the bottom, there are controls for 'PATH RECORDING' (PLAYBACK, RECORD) and 'IMAGE/FORM' (ZOOM, PAN, etc.).

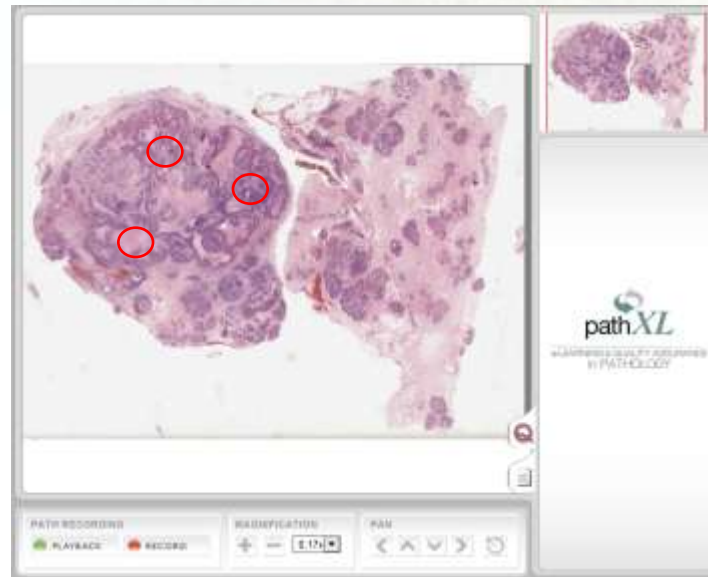
**Leeds University (Gastric TMAs)**  
**Cambridge University (Bladder BOXIT trial)**  
**Queen's University (Mesothelioma)**  
**Queen's University (Prostate response TMA)**  
**Fusion Antibodies Ltd**

**Controlled vocabularies**  
**Compatibility with Data Exchange Standards**  
**(Berman et 2003, CaBIG, CancerGrid, Dublin Core Header)**

# Virtual slides for Tissue Archiving and Research

## On-line evaluation of TMA

TMA generation



After

Before



TMA context mapping



# Shortfalls in visual interpretation

**Making the diagnosis**

**Scoring the biomarker**

Subjective criteria

Language – associated with uncertainty

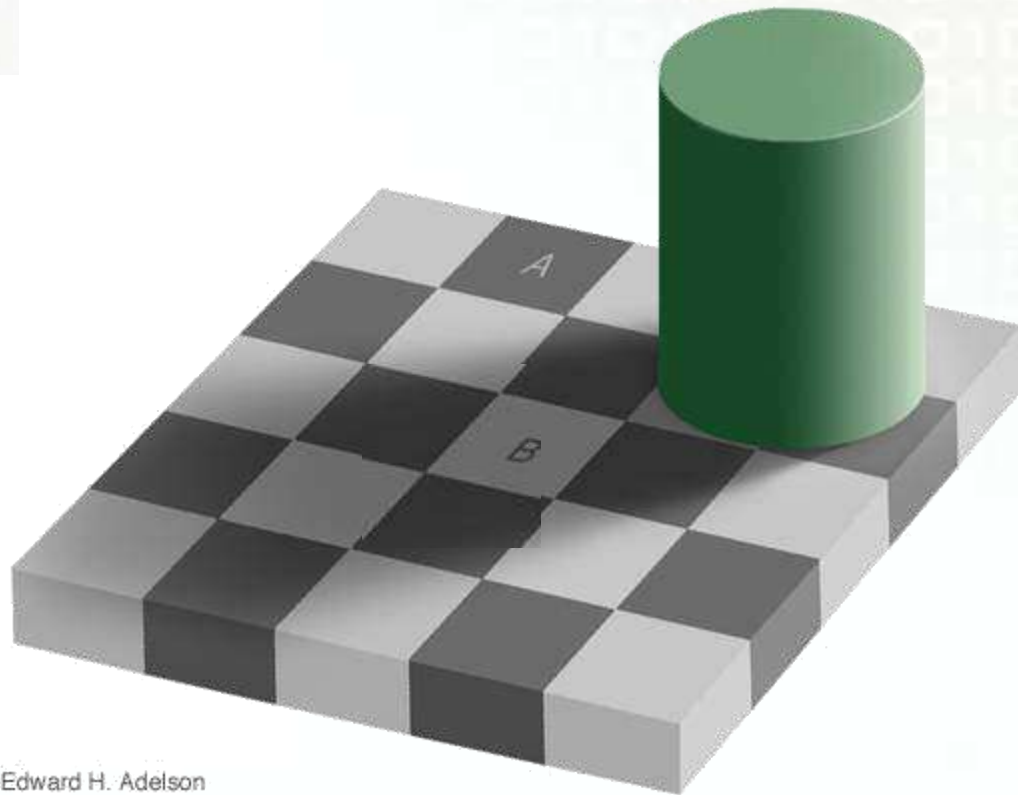
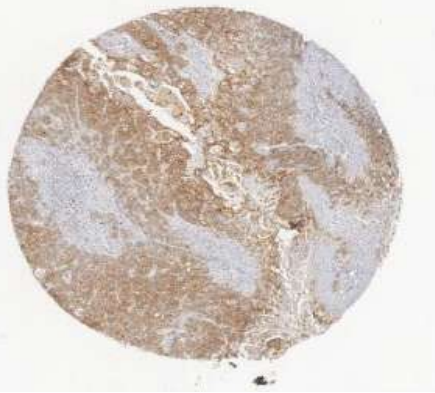
Disease “patterns” are based on predefined mind sets

Not good at detecting quantitative differences

Open to interpretation

Poor reproducibility

Inaccuracy



Edward H. Adelson

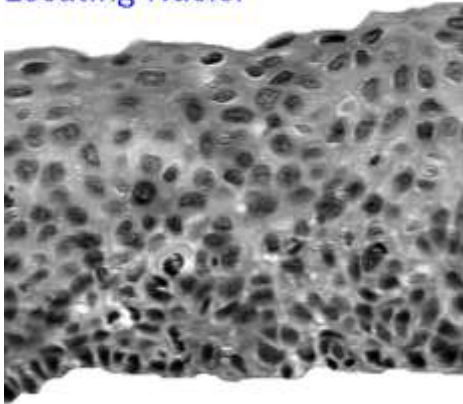
# Disagreement in pathological diagnosis

	<u>Kappa</u>
<b>Pre-invasive lesions of the bronchus</b> (Nicholson – <i>Histopathology</i> 2001;38:202-208)	0.55
<b>Cervical cytology</b> (Stoler – <i>JAMA</i> 2001;285:1500-1505)	0.46
<b>Cervical Histology</b> (McCluggage – <i>Br J Obs Gynae</i> 1998;105:206-210)	0.15 – 0.62
<b>Prostate Cancer</b> (Egevad – <i>Urology</i> 2001;57:291-295)	0.58
<b>Oral Dysplasia</b> (Warnakulasuriya – <i>J Pathol</i> 2001;194:294-297)	0.27 – 0.45
<b>Variation in interpretation of renal transplant biopsies</b>	Furness et al. 2001
<b>Aberrant diagnoses by surgical pathologists</b>	Wakely 1998
<b>Dysplasia classification: pathology in disgrace</b>	Bosman. 2001
<b>“Individuality” in the specialty of surgical pathology</b>	Ackerman 2001

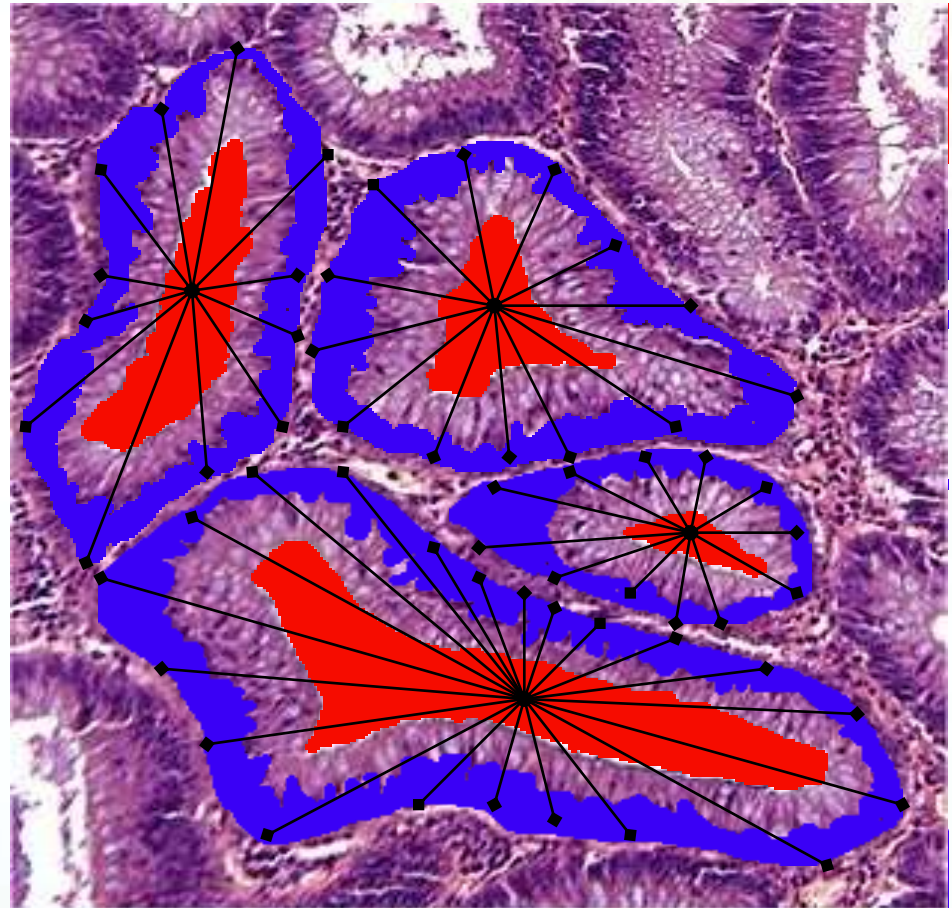
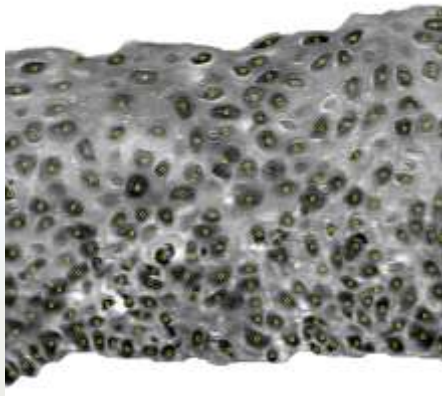
# Enormous opportunities to explore improved methods of tissue diagnostics that are objective, reproducible and reliable

Image analytics   Machine vision   Quantitative evaluation   Tissue classification

Locating Nuclei



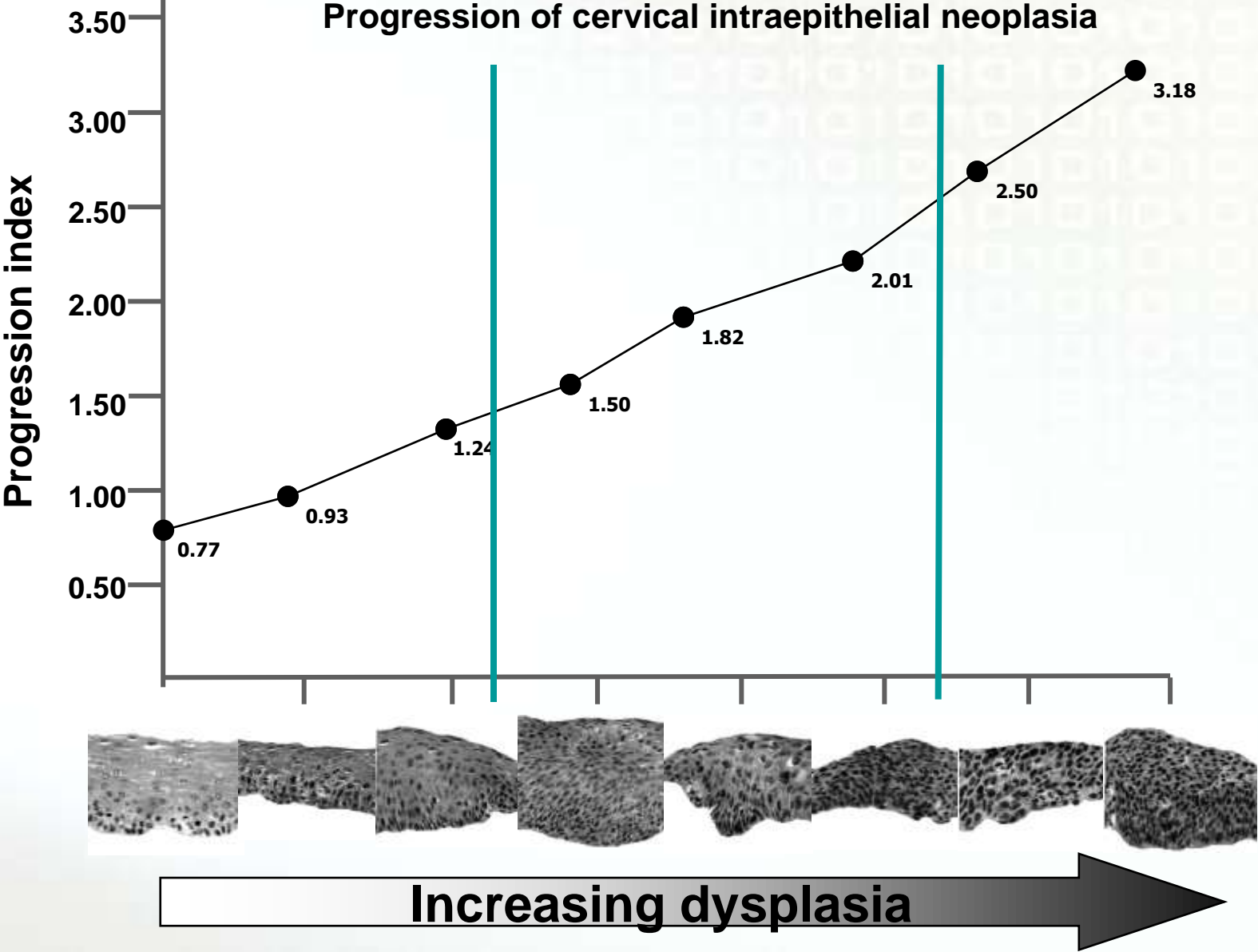
Cervical preneoplasia



Colorectal adenomas



# Progression of cervical intraepithelial neoplasia



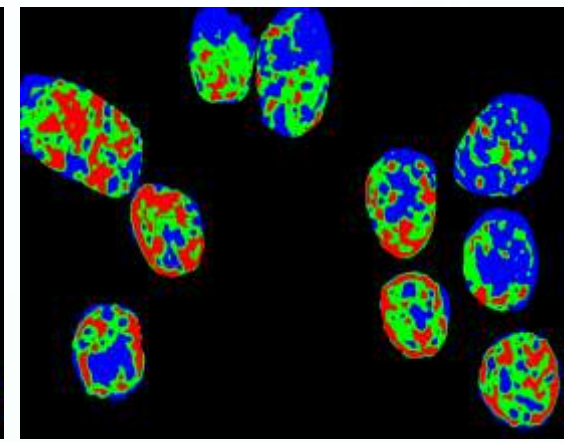
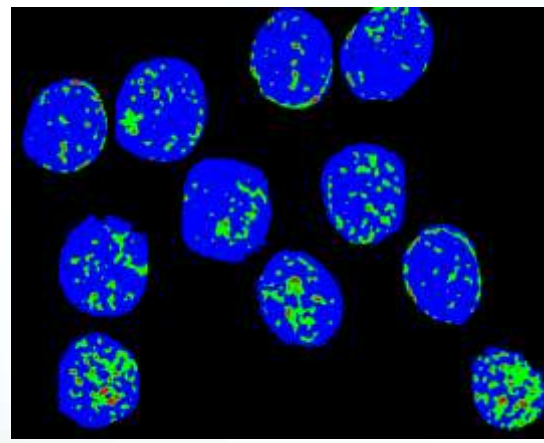
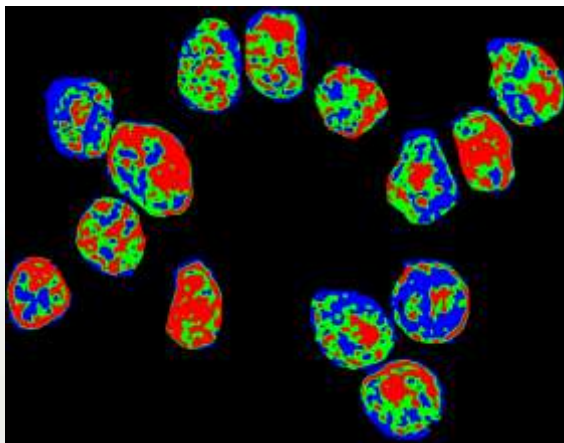
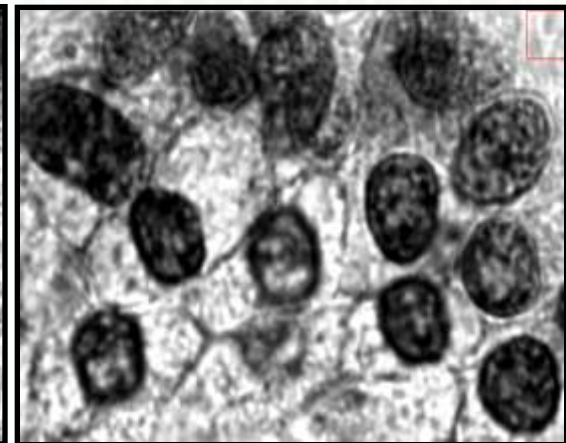
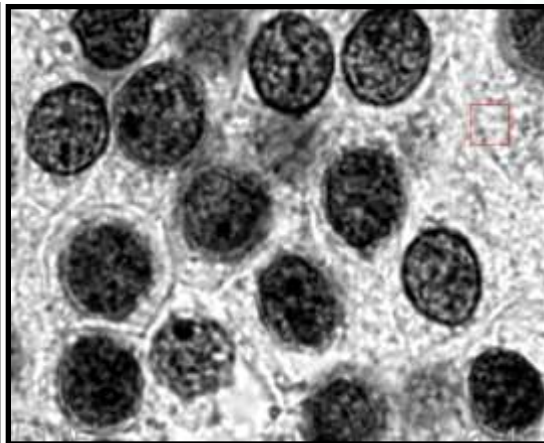
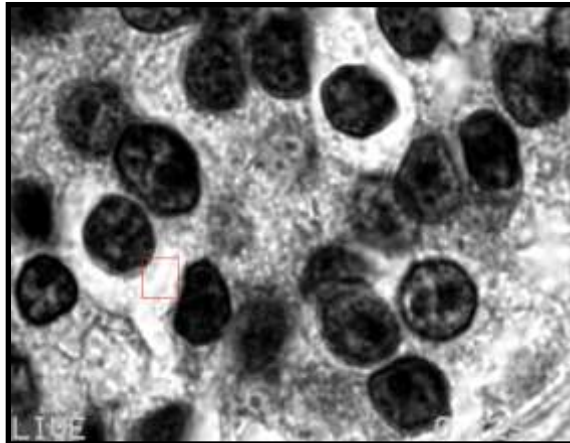
# Augmented Visualisation in Pathology

## Prostatic neoplasia

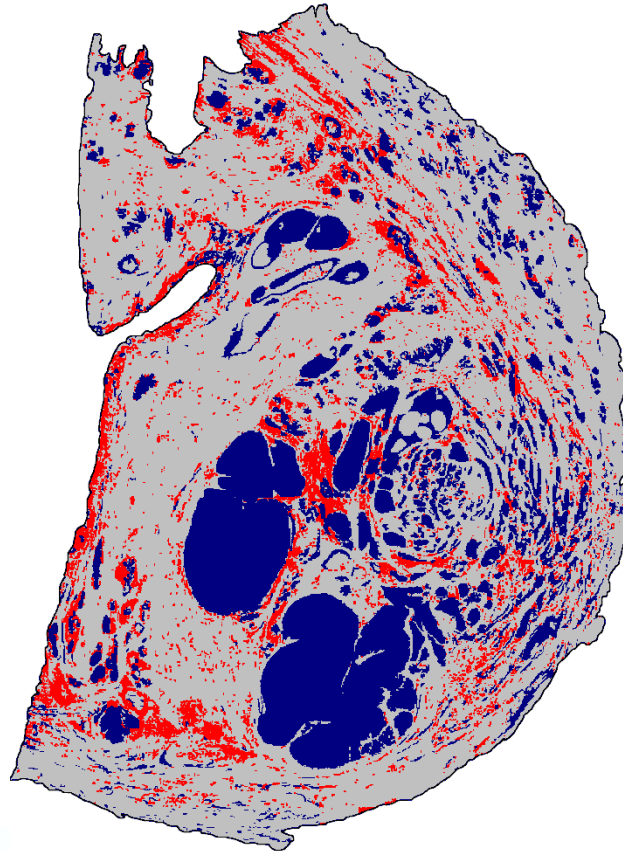
BPH

PIN

Prostate Cancer

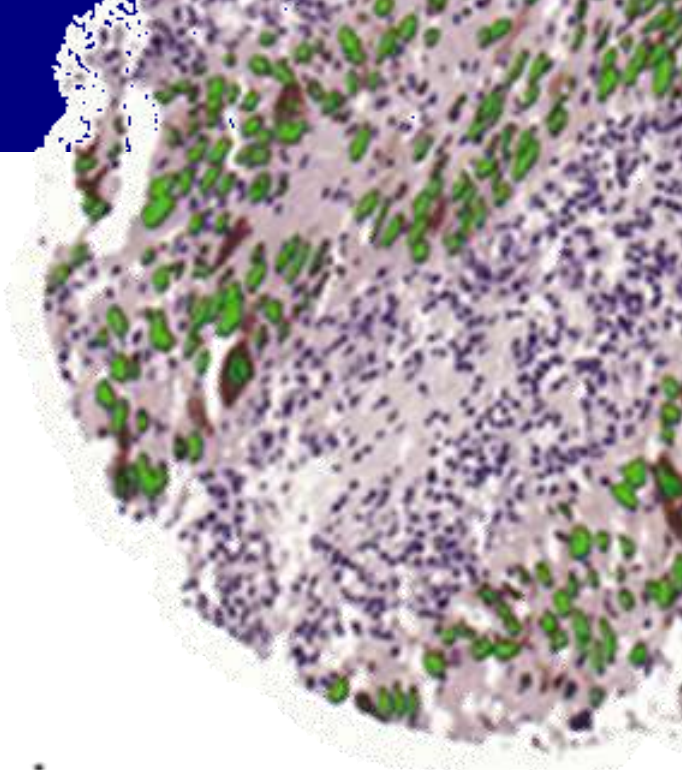
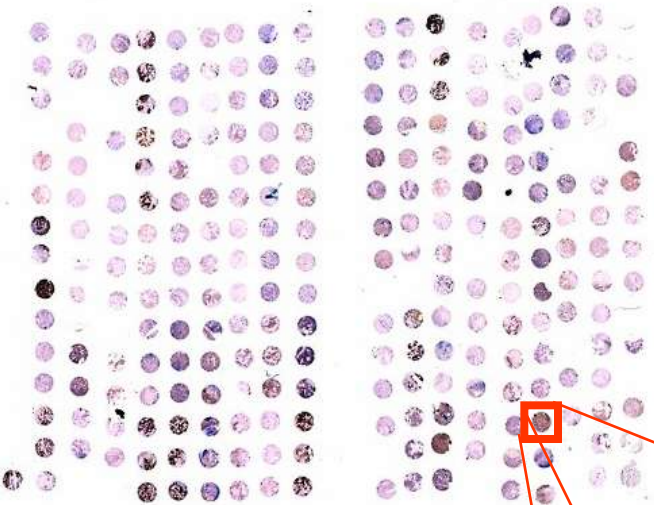


# Machine vision on virtual slides

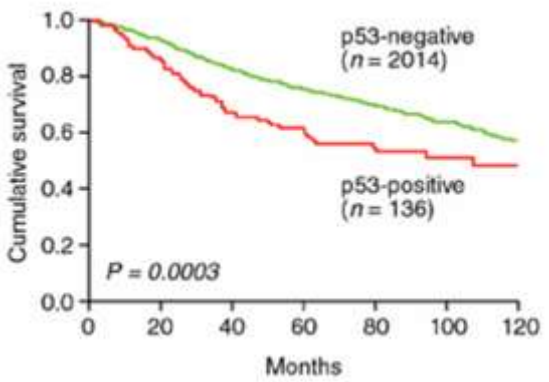
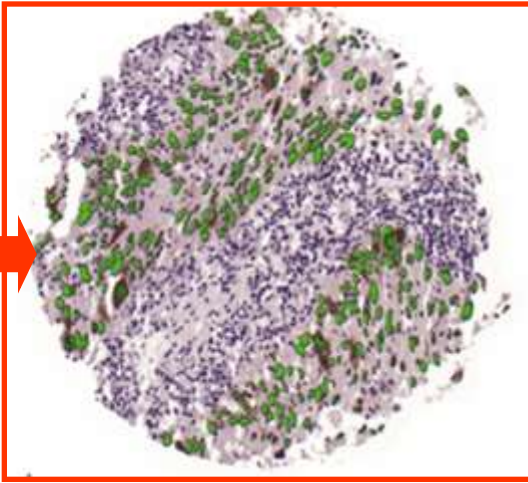
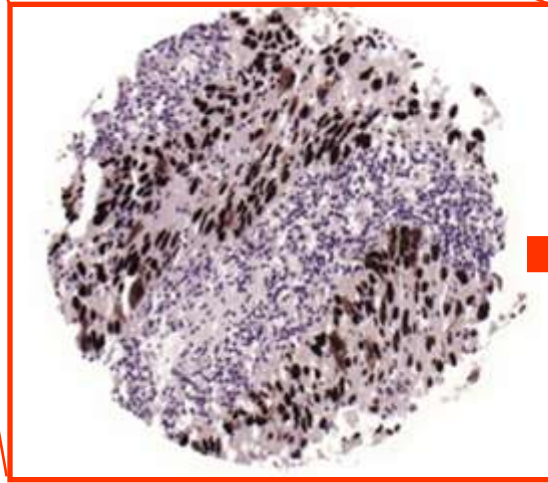




# Computer vision analysis of Tissue Microarrays

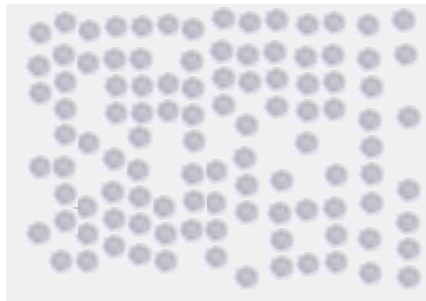


IOD=1.75 = Score 3

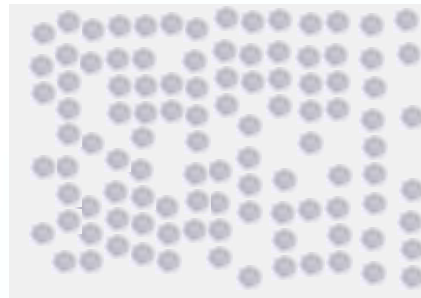




# High Performance Computing (HPC) using Clusters

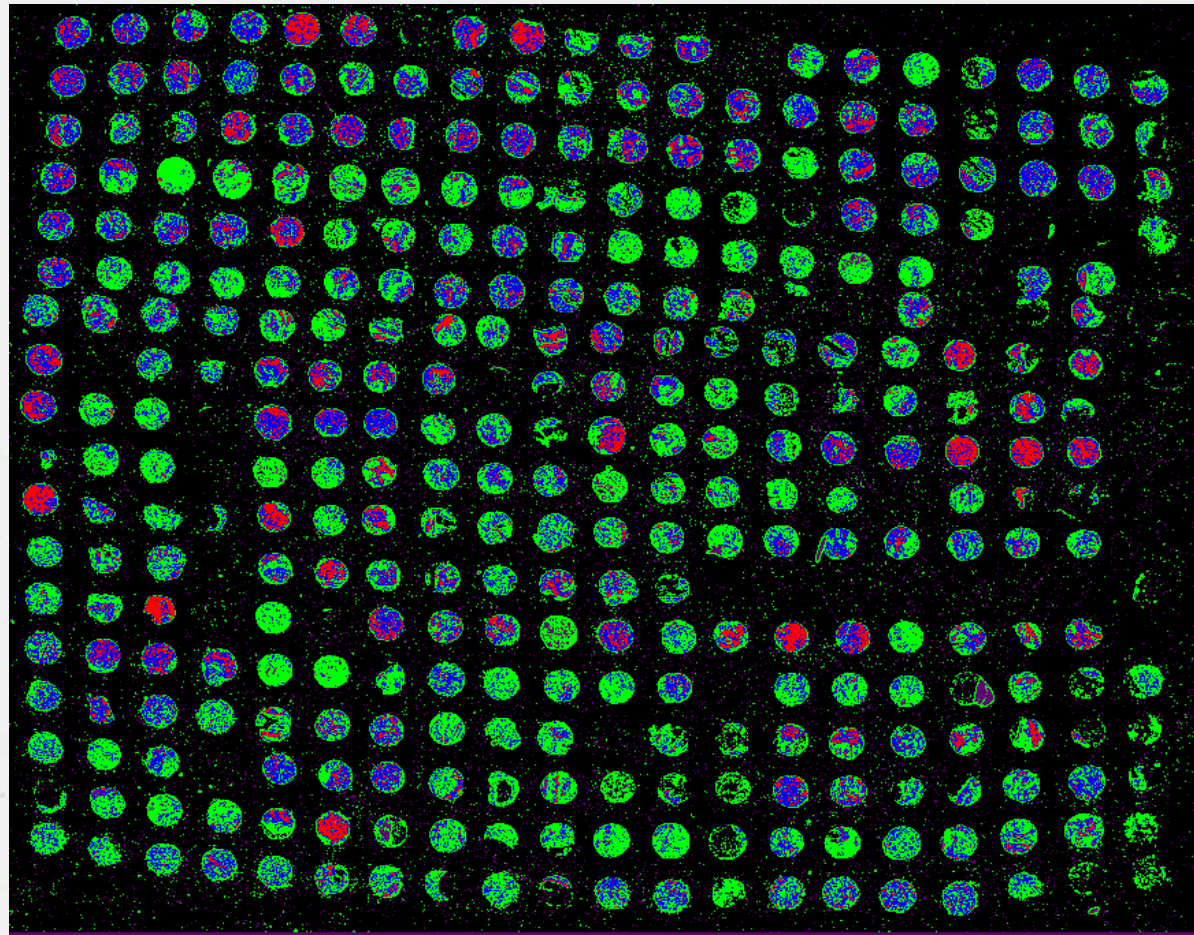


**Significant speed enhancements  
for densitometric analysis  
of gastric TMA**



**David McCleary**

# Augmented Visualisation in TMA assessment



# High Throughput Analysis of TMAs

**Speeding analysis**

**Providing objectivity and reproducibility**

**For algorithm development and evaluation**

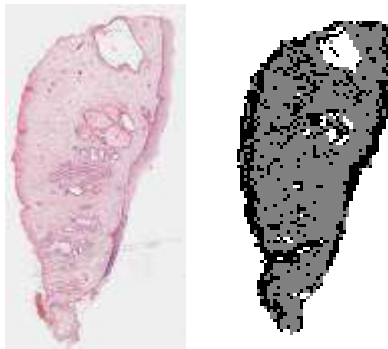
**For pre-selection of markers for manual analysis**

**For more sensitive scoring of markers for clinical evaluation**

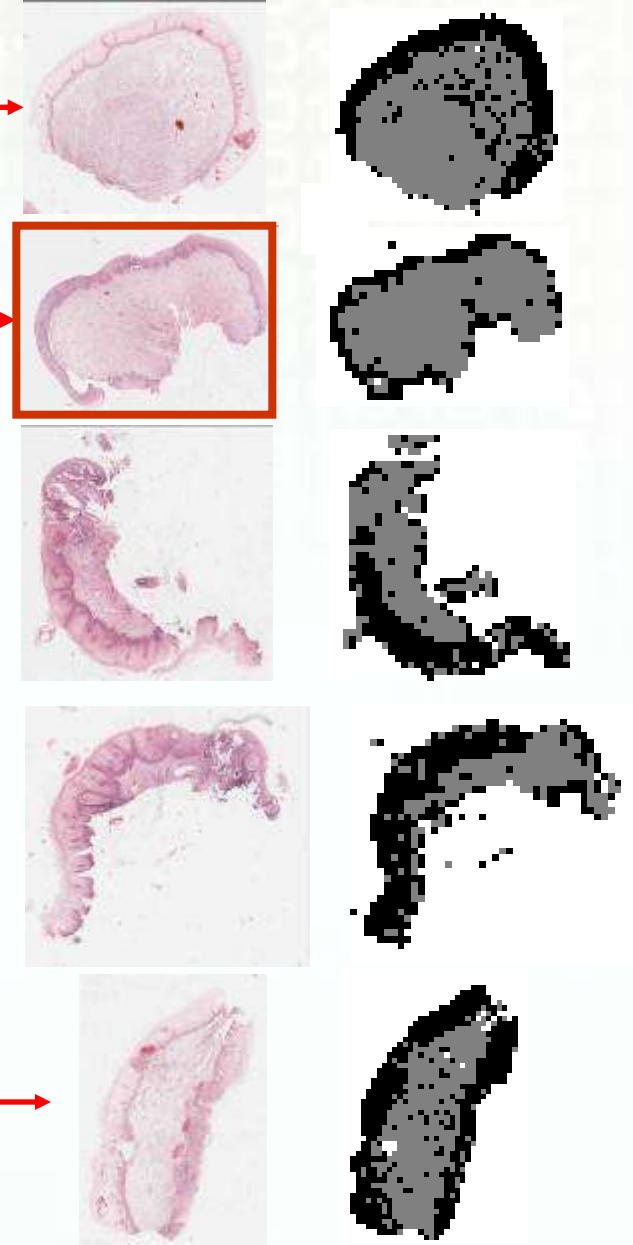
# Multiresolution texture analysis, CBIR and CBR

## Tissue archive

Similarity metric  
(Hamming Distance)



Test case



Case-based reasoning

TABLE I  
Texture Feature Descriptions

Texture Feature	Expression
Average intensity	$m = \sum_{i=1}^{L-1} z_i p(z_i)$
Average contrast	$\sigma = \sqrt{\mu_2(z)}$
Smoothness	$R = 1 - 1/(1 + \sigma^2)$
3rd Moment	$\mu_3 = \sum_{i=1}^{L-1} (z_i - m)^3 p(z_i)$
Uniformity	$U = \sum_{i=1}^{L-1} p^2(z_i)$
Entropy	$e = -\sum_{i=1}^{L-1} p(z_i) \log_2 p(z_i)$
Contrast	$F_{cov}^{Hor} = \sum_{i,j}  i-j ^2 p(i,j)$
Covrelation	$F_{cov}^{Hor} = \frac{\sum_{i,j} (i-\mu_i)(j-\mu_j) p(i,j)}{\sigma_i \sigma_j}$
Angular second moment	$F_{ASM}^{Hor} = \sum_{i,j} p^2(i,j)$
Inverse difference moment	$F_{IDM}^{Hor} = \sum_{i,j} \frac{p(i,j)}{1+ i-j }$

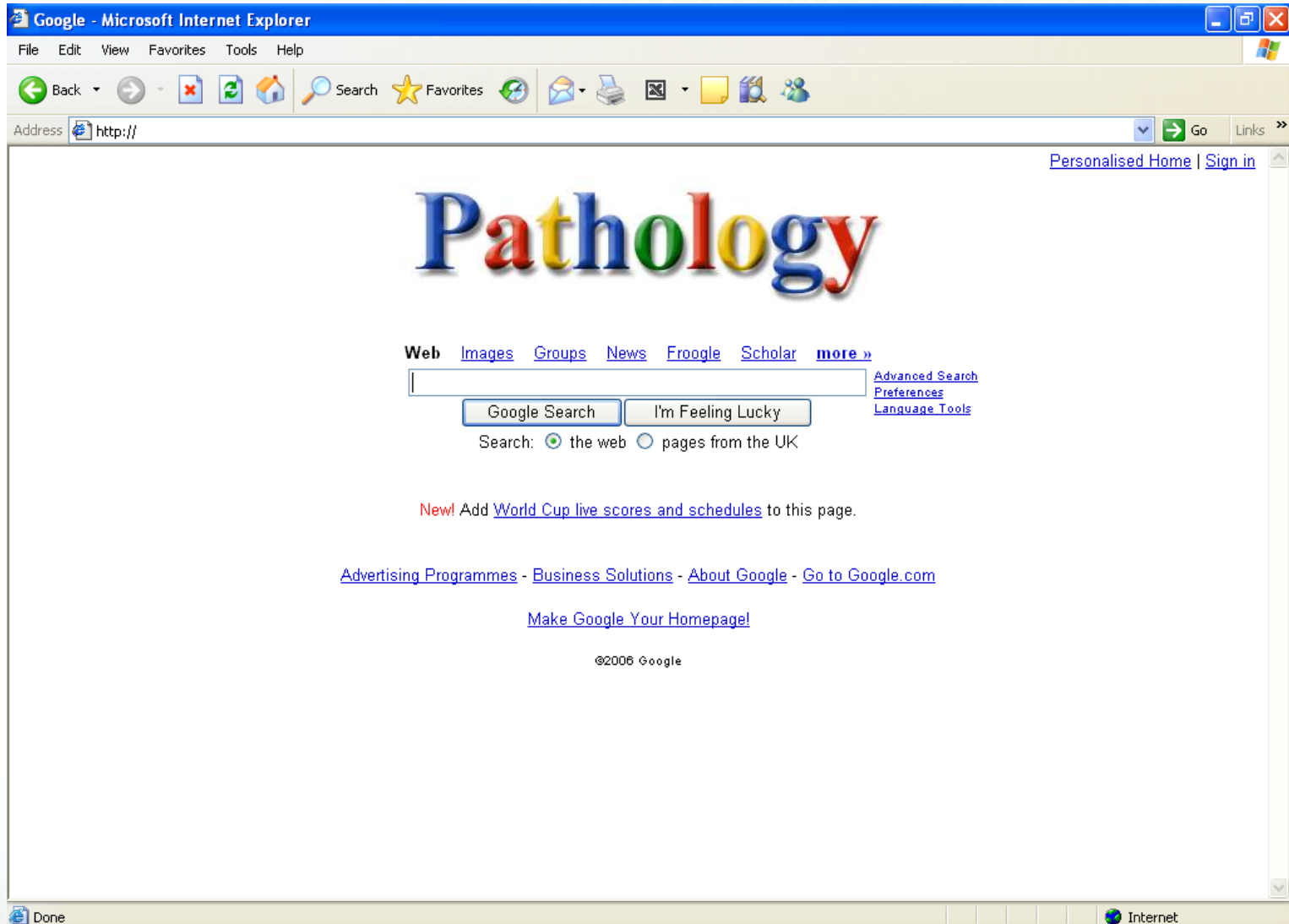
Where  $z_i$  is a random variable indicating intensity,  $p(z)$  is the histogram of the intensity level in a region,  $L$  is the number of possible intensity levels,  $\mu_2(z)$  is the variance,  $p(i,j)$  is GLCM,  $\mu_i = \sum_{i,j=0}^{N-1} i p(i,j)$  and  $\mu_j = \sum_{i,j=0}^{N-1} j p(i,j)$  are GLCM means, whereas  $\sigma_i = \sqrt{\sum_{i,j=0}^{N-1} p(i,j)(i-\mu_i)^2}$  and  $\sigma_j = \sqrt{\sum_{i,j=0}^{N-1} p(i,j)(j-\mu_j)^2}$  are GLCM STD.



## Growing numbers of virtual tissue archives



**Storage and Search?**



## Image Search and content-based image retrieval

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