Digital cytology: an introduction

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Seminar on Digital Cytology (A.Bondi, M.Boon, V.Della Mea, U.Schenk)

Outline

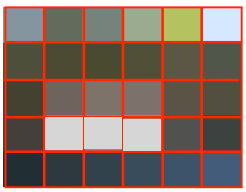
- Basic imaging concepts
- The digital image
- The digital slide
- Practical aspects

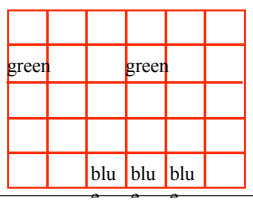
Basic imaging concepts

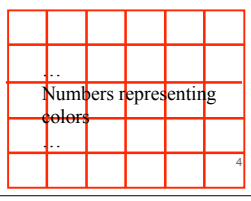
Bitmap coding

Resolution

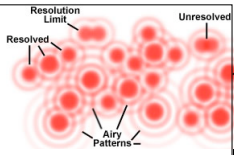
Bitmap coding of images







Resolution



- Resolution: least measurable distance between two points
- Depends on wavelength ν objective' numerical aperture (NA), which is related to magnification:

$$R \cong \frac{\nu}{2 \cdot NA}$$

- If ν=550 nm (by convention, green light), NA=1.4 (maximum, oil immersion), then **R = 0.196 µ**

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Optical resolution

- In practice 0.2 µ is the least detail visible at the microscope
  - NA=1.4 means 100x, oil immersion
- Other magnifications?

Magnification	NA	Resolution (µ)
5x	0.1-0.2	2.8-1.4
10x	0.25-0.45	1.1-0.6
20x	0.40-0.75	0.7-0.37
40x	0.65-0.95	0.42-0.29

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Resolution and sampling

- When acquiring, we should take into account the Shannon-Nyquist theorem
  - (sampling frequency at least double than signal frequency of interest)
- In practice: at least two pixels per resolved point
- I.e., 1 pixel every 0.1 µ (~, at 100x oil immersion)

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Other magnifications: an example

- Magnification: 40x
  - Typical NA 0.70,
  - Field of View : 200x150 micron
- How many pixels do we need?
  - Optical resolution: 0.550 / 2 * 0.70 = 0.393 µ
  - Acquisition resolution: 0.196 µ ~ 0.2 µ
  - Minimum number of needed pixels:
  - 200/0.2, 150/0.2= **1000 * 750** = 0.75 Mpixel camera

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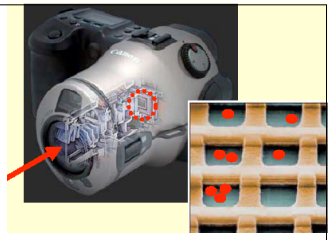
The digital image

Acquisition

Sensors

Acquisition: CCD



- Matrix of light-sensitive elements
- Each element (well) collects photons over a fixed time; their count is proportional to the amount of light hitting the well
- The number of photons is then sent to the computer (which could be the internal camera processor)

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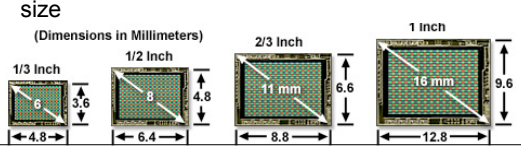
CCD: size

- CCD size:
  - Measured in inches (fraction of), which do not correspond to the real size of the sensor
  - 1/8" - 1"
- Element size: some square micrometer
- Number of elements: the well known MegaPixels
  - Millions of elements, related to CCD and element size



CCD and noise

- Many noise sources
- **Thermal noise**: the sensor body at temperature > 0°K, emits moving electrons that hit the wells as if they were photons
  - Dark areas will not be really black
- electronics
- If the element is very small, it will not receive many photons so small dynamic range
- **Element size (CCD size) is important!**

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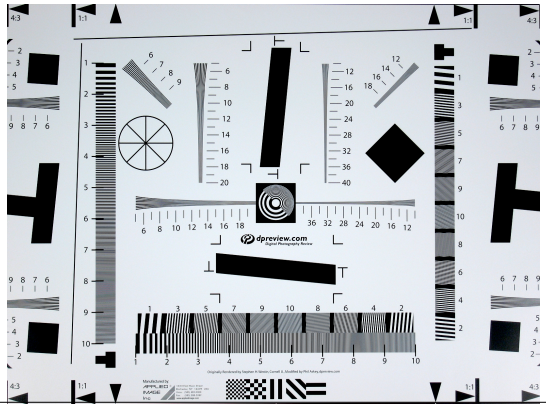
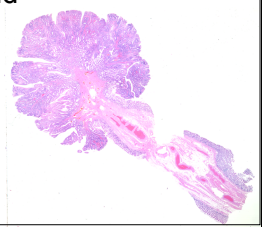
Real world resolution



The digital slide

Digital slide

- Is a digital copy of the traditional glass slide,
- Obtained acquiring the whole slide, or just parts of area substantially larger than the view field
- **Aim**: to capture all diagnostic information available/useful



An example: eSlide

- We developed a digital slide acquisition system: eSlide
  - MITEL, University of Udine, Italy
- The software part is freely available at http://www.eslide.net
  - Sample cases too
- Hardware supported until now:
  - analog cameras + Scion Firewire cameras
  - Märzhäuser LSTEP and Prior Optiscan stages
- Let's look at the eSlideScope…

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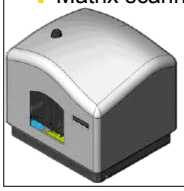
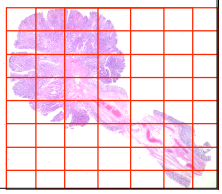
Acquisition

- Slide scanners: fully automatic devices
  - Aperio, Olympus, Zeiss, …
  - Linear CCD sensor (like flatbed scanners)
- "traditional" robotized microscopes
  - Bacus, eSlide, …
  - Matrix scanning of the slide





Acquisition techniques

- *Top-down* systems:
  - A low magnification overview of the slide is acquired, on which interesting areas are acquired at higher magnification. Inside the latter, further interesting areas may be acquired at even higher magnification.
- *Bottom-up* systems:
  - All the slide, or a significant part of it, is acquired at high magnification; from this, lower magnifications are automatically calculated

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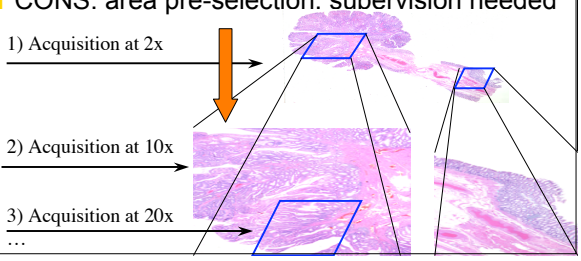
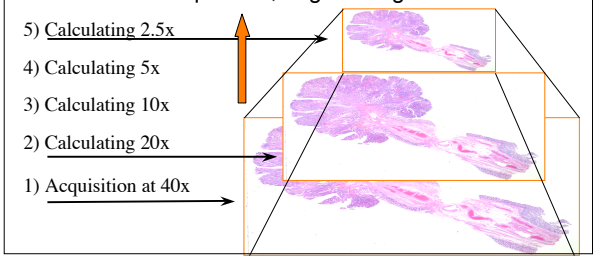
Top-down systems

- PROS: slightly less storage needed, faster acquisition
- CONS: area pre-selection, supervision needed



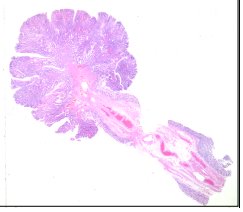
Bottom-up systems

- PROS: complete glass slide, non supervised acquisition
- CONS: slow acquisition, large storage needs



Storage needed

- Let's consider a 1 cm^2 sample (=1x1 cm = 10.000x10.000 µ)
- To store it at maximum optical detail: **100.000x100.000** pixel
  - ~ thousands of traditional images
  - However, 100x, oil immersion
- 40x, 0.70 NA:
  - **50.000x50.000** pixel
  - 7.5 GB, uncompressed
  - <500MB, safely compressed



Storage needed/2

	Area	100x	40x
Small biopsies	10 mm^2	200 MB	50 MB
Surgical sample	1 cm^2	2 GB	500 MB
Cytology	3 cm^2	3 GB	750 MB
PAP	9 cm^2	9 GB	2.25 GB

* Compression: JPEG 15:1, according to Foran & Meer guidelines;
** Compression: JPEG 30:1

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Unsupervised acquisition

- Acquisition is unsupervised, so:
- Recognition of non-blank fields
  - The user selects areas of interest,
  - The system acquires non blank fields inside that areas *(tissue finding)*
- Autofocus:
  - The system should focus in some way
    - Real autofocus: used on microscope-based systems, sometimes can fail
    - Interpolated autofocus (i.e, focus on some points, interpolation on other): used on most slide scanners, good mainly for histology, fails when specimen has variable thickness

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Time needed

- Slide scanners:
  - 20 minutes /1 cm^2
- Robotic systems:
  - Up to 2 hours / cm^2
- Where the speed comes from?
  - Mainly but not only in focus system
    - Slower but apparently more precise on robotic systems
  - European Congress on telepathology: "scanners for routine, robotic microscopes when more precision is needed"

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Practical aspects

Diagnostic performance

Use in cytology

Digital images in pathology

- "Traditional" telepathology:
  - Dynamic telepathology (realtime)
    - Robotized microscope driven at distance
  - Static telepathology (store-and-forward)
    - Selection of microscope images representing a slide
- Digital pathology:
  - *Virtual Microscopy*, or *whole slide telepathology*
    - Entire slide acquisition, storage and esamination at distance

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Can we use digital images/slides?

- The question can be translated as:
- Are digital images/slides "equivalent" to those we see at the microscope?
  - I.e., do they bring the same diagnostic information?

?

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The answer for static images

- Yes (provided that images are technically good)
- Many papers in the last 10 years,
  - Recently: on 1255 cases, AFIP reported 97.3% diagnostic agreement (TP vs. microscope)
    - Williams BH, et al. Clinical evaluation of an international static image-based telepathology service. Hum Pathol. 2001 32:1309-17.
  - *(…) all the necessary technology for telepathology is available, there is strong published evidence for a diagnostic accuracy comparable with glass slide diagnosis, in many contexts there is a clear-cut economic argument in favour of telepathology, and that the technique should now be integrated into mainstream diagnostic histopathology.*
    - Cross SS, Dennis T, Start RD. Telepathology: current status and future prospects in diagnostic histopathology. Histopathology 2002;41:91-109

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Selected images and digital slides

- At first glance, it seems that if selected images bring diagnostic information, the whole digital slide will be even more adequate
- … but there is a subtle difference:
  - Selected images are manually and carefully acquired by an expert pathologist,
  - While the digital slide is automatically acquired
  - Focus is not guaranteed on every image!

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The answer for digital slides

- Looking at diagnostic agreement: very good
- Looking at details: reports of local failures
  - Gilbertson JR, et al. Primary histologic diagnosis using automated whole slide imaging: a validation study. BMC Clin Pathol. 2006
    - *(…) very positive result; however, **this does not mean that WSI is as good as a microscope**. Virtually every slide had focal areas in which image quality (focus and dynamic range) was less than perfect.*
    - *(…) 10 of 210 slides showed evidence that "tissue finding" function had failed and that **significant areas of the tissue had not been imaged** (this usually involved immunohistochemistry slides with light counter staining). In those cases, the technician manually adjusted the imaging window and let the system re-imaged the slide.*
- **So: automatic, but with human check!**

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Digital slides in cytology

- First proposal and experimentation:
  - R.N. Taylor, M. Gagnon, J. Lange, T. Lee, R. Draut, E. Kujawski, CytoView. A prototype computer image-based Papanicolau Smear Proficiency Test, Acta Cytologica (1999) 43: 1045-1051.
- More works on histology than on cytology
  - Due to extra difficulties…

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Cytology features

- Some (technical) features of cytology influence its acquisition and use:
- Focus problems:
  - No section -> variable focus plane
  - Specimen thickness -> information spread on more than one focus plane (fine focusing)
- Very large samples
  - To be thoroughly examined

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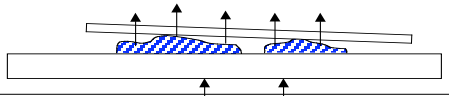
Focus

- Some slide scanners do autofocus on just some points
  - Then interpolate focus values for the other fields
  - This covers only regular trends due to coverslip being not parallel to slide, or regular variations in section thickness
  - Focus can be maintained increasing depth of field (i.e., closing diaphragm)
    - **But this decreases resolution!**
- Much, much easier on thin layer



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Sample size

- Visualization time depends on
  - Training in the use of the software
  - Screen size
    - Field of view smaller than at the microscope
- Time needed for diagnosis: higher than at the microscope (PAP average: 26 min.)
  - Della Mea V, et al. User attitudes in analyzing digital slides in a quality control test bed: a preliminary study. Computer Methods and Programs In Biomedicine. 2006; 82 (2)
  - Inadequate for routine diagnosis, acceptable for training and test

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The most promising applications

- Teaching and Quality assurance:
- Implementation of QUATE-like tests
  - Possibility of replicating the same slide as many times as needed
    - Taylor RN et al. CytoView. A prototype computer image-based Papanicolau Smear Proficiency Test. Acta Cytologica 1999; 43:1045-1051.
    - Demichelis F et al. Digital storage of glass slides for quality assurance in histopathology and cytopathology. J Telemed Telecare 2002;8:138-42.
- Tracking of diagnostic path
  - Where the user looked at? Where not?
    - Costello SS et al. Development and evaluation of the virtual pathology slide: a new tool in telepathology. Journal of Medical Internet Research 2003; 5:e11.

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Let's go!