Digital Pathology and Tissue-based Diagnosis. How do they differ?

P. Hufnagl

Institute of Pathology (Rudolf-Virchow-Haus). Humboldt University, Berlin
Structure of the talk

- Possible workflow
  - routine diagnostic
    - diagnostic support
- Which scanner?
- Quantification / image analysis
- Conclusions
Structure of the talk

- Possible workflow
  - routine diagnostic
  - diagnostic support
- Which scanner can we trust?
- Quantification / image analysis
- Conclusions
The Conventional Workflow

Clinical request → HIS

Lab: Cutting, Staining, Coverslip

PLIMS

Physician: Assignment, diagnosing, consultation

Physician: Dictation, Images, Annotation

Report

Tissue
Institute of Pathology – Charité Berlin

The Digital Workflow

Tissue

Clinical request

HIS

Lab: Cutting, Staining, Coverslip

Slide Scanner: Registration, Digitalisation

PACS: Storage, Image streaming

PLIMS

Physician: Assignment, diagnosing, consultation

Image analysis: marker quantification

Report

Physician: Dictation, Images, Annotation

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The Digital Workflow

Missing link between cover slipper and scanner

Lab: Cutting, Staining, Coverslip

Missing link between scanner and PLIS

PACS: Storage, Image streaming

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Advantages of Virtual Microscopy

• No glass transportation, no glass archive (?
• Previous slides are always available
• Microscopic diagnostic - anytime – anywhere -
• Parallel viewing of different stainings, positions
• Viewing and handling parallel at different locations
• Facilitated second opinion - online
• Quantification and image analysis just in time
• Annotations are simple to be handled
• much more …..
Problems of the digital workflow

Disadvantages

• Additional process step: Digitalisation
• Huge and continuous hardware and software investments
• Training of personnel
• Diagnosis on the monitor is unfamiliar for pathologists
• Legal Problems
Strategy

• Start with a less critical application
  • Biobanking
• Introduce VM to applications with most effects
  • Tumour board
  • Second opinion in-house
• Solve all problems along the workflow
  • LIMS integration
  • Sure barcode identification
• Establishing of continuous testing
ZEBANC – CHARITÉ BIOBANK

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CURRENTLY OFFERED SERVICES

- Aliquotation
- Quality documentation (SPREC)
- WSI generation
- TMA generation and WSI* based analysis
- Automatic DNA- extraction
- DNA Sequencing

*whole slide image

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VALUE OF A SAMPLE

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Digitalisation established for samples from frozen section labs
Technical quality control implemented
Infrastructure for block-centric navigation established
Medical quality control in use (prototype tumor area detection)
TMA as sample array in CentraXX integrated
Several quantification procedures implemented to generate additional sample features
Virtual studies on virtual slides instead of real samples

→ Virtual microscopy has a huge potential for services in the context of a biomaterial bank
Clinical Pathology

Second Opinion, Studies, Marker Quantification
Workflow of Tumor Board Meetings

Set bookmarks and make annotations

Before meeting

Browse slides and move to annotations

On meeting
Quantification
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Requirements on Slide Scanning

- Correct slide information is present
- Completeness of tissue
- Image sharpness
- Color fidelity
VIRTUAL MICROSCOPY – SCANNER CONTEST

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2nd International Scanner Contest
technology meets pathology
MISSION

- Determination of the state of the art in slide scanning
- Support of pathologists and scientists to find the appropriate scanner for their applications
- Support of vendors to understand the needs of pathologists
- Determination of "quality of WSI" within the context of pathology
- Development of a set of standard features for the characterization and comparison of scanning devices
DISCIPLINES

- High Throughput
- Quality
- Fluorescence
- Image Analysis
- Technical
QUALITY | EVALUATION TERMINALS
VENDORS....
Aims:
- Measurement of colour fidelity and colour resolution of devices
- Determination of true effective pixel size
- Detection of image distortions

Material:
- IT8.7/1 Colour Target mounted on glass slide
  - 264 colour & 24 grey value fields
  - known absorption spectra and colourimetric coordinates
- Grid pattern glass slide
  - overall size: 1” x 3” (25mm x 75mm)
  - image area: 20mm x 50mm
  - clear aperture: 8.5 µm²
  - opaque lines: 1.5 µm²
  - pitch: 10 µm
2nd International Scanner Contest

Institute of Pathology – Charité Berlin

TECHNICAL – COLOUR & GEOMETRY | TASK

- General Conditions:
  - All participants had to scan the same slide
  - Any manual interaction was allowed
  - Rescan of slide was allowed
  - 1h time limit

- Evaluation:
  - Colour difference calculation to CIEDE2000
    - average inside middle 50% of each field
    - low-resolution scan
  - Measurements inside whole slide images
    - Inside sensor field (no stitching)
    - 9 sensor fields – 18 measurements each
**Fidelity test**: average dE over 144 fields
\[ dE_{avg} = \frac{\sum dE(c^*_{mes}, c_{ref})}{144} \]

Colour difference calculation to CIEDE2000

**mix-colours matrix for fidelity test**

**colour step wedges**
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COMPLETENESS OF SCAN
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technology meets pathology

2nd International Scanner Contest

HIGH THROUGHPUT |
AIMS & MATERIAL

30
- Sample identifikation (barcode / OCR)
- Sharpness assessment + Completeness of particles
FOCUS QUALITY ASSESSMENT

Green: quality sufficient
Red: not sharp, possible artefacts
VIEWING ON A VIRTUAL MICROSCOPE
Figure 1: Images of an enterobius parasite in an appendix are shown using a similar ×20 objective, but with different sensor pixel sizes. Images a and c are sampled with a 5.5 micron pixel size, whereas b and d were sampled with a 10 micron pixel size. The apparent magnification differs by ×1.7, the ratio of the pixel sizes.

Figure 2: Graphic depicting the added boost in magnification that comes from the workstation monitor.

MAGNIFICATION IS NOT RESOLUTION AND OPTICAL RESOLUTION IS NOT DIGITAL RESOLUTION!
Several Positions in Test

- Leap Motion over the table
- Leap Motion under acryl glass pane
Leap Motion

Stereo imaging based on infrared cameras (https://www.leapmotion.com/)
Handling

Possible workflow
Gesture Control

Next/ previous slide
Zoom in/ out
Histological Image Registration

• Goal
  – Inter-Modal Registration (Stain-To-Stain)

• Applications
  – WSI Navigation Support
  – Virtual Staining
  – 3D Reconstruction

• Approach
  – Intensity Based
  – Multi-Resolution

• Similarity Measure
  – Mutual Information

• Transformation Models
  – Rigid: Rotation + Translation
  – Affine: Linear Transformation + Translation
  – Free Form Deformation: B-Splines

• Optimization
  – Gradient Descent
Registration Of Renal Images: Reference Image (H&E) Template Image (SFOG)
Coarse to Fine Image Registration: Rigid, Affine, Free Form Model
Affine Registration
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The strong reputation of pathology is becoming weaker ….

January 4, 2008, 8:09 am

Breast Cancer Test Errors Cause Faulty Treatment
Posted by Jacob Goldstein

The era of personalized medicine won't work unless we can also find our way into the era of reliable diagnostic testing. And in the case of breast cancer, that means checking the accuracy of the drugs for detecting cancer very well.

... if trust is gone, you almost never get it back ....

Cervical cancer is almost entirely preventable with a new genetic test. Yet doctors still cling to the highly unreliable Pap smear. Something is very wrong here. Christine Baze and her husband of seven years were planning to start a family in 2000 when she found out she had cervical cancer. At 31 she underwent a hysterectomy followed by three months of drugs and radiation.

Baze was, as she describes it, "the girl who was doing everything right," getting annual Pap smears that screen for pre-cancerous cervical cells. But the Pap test missed the cancer that was growing inside her for a decade. Each test had returned a negative result. With early detection, Baze could have treated her cancer with chemotherapy and radiation. "I was devastated, and incredibly pissed at my doctor’s office. If they’d found the tumor three years earlier, I could have kept my uterus and had a child," says Baze, now 39 years old and executive director of the Yellow Umbrella, a cervical cancer prevention group she founded in 2002.

It borders on the scandalous that cervical cancer, among the few cancers that are preventable, kills 310,000 women a year worldwide. In 2007, 11,150 women in the U.S. were diagnosed with it. Half of them had not had a recent Pap test. Another third did get tested but got false negatives from the 65-year-old Papanicolaou biopsy. The Pap test is valuable, having cut the rate of cervical cancer by 70%, but it is archaic. It calls on a lab technician or machine to peer at a sliver of cervical cells under a microscope to spot the abnormal precancerous ones. This
An International Ki67 Reproducibility Study


Manuscript received April 2, 2013; revised September 3, 2013; accepted September 16, 2013.

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Background  In breast cancer, immunohistochemical assessment of proliferation using the marker Ki67 has potential use in both research and clinical management. However, lack of consistency across laboratories has limited Ki67’s value. A working group was assembled to devise a strategy to harmonize Ki67 analysis and increase scoring concordance. Toward that goal, we conducted a Ki67 reproducibility study.

Conclusions Substantial variability in Ki67 scoring was observed among some of the world’s most experienced laboratories. Ki67 values and cutoffs for clinical decision-making cannot be transferred between laboratories without standardizing scoring methodology because analytical validity is limited.

Nat J Inst., preprint November 2013
An International Ki67 Reproducibility Study

At the St. Gallen cutoff of 13.5% there are 32.3% high Ki67 by Lab A while Lab B would call the same cases low Ki67. ..

Figure 5. Degree of concordance (bolded box) between Laboratory B and Laboratory D at the St. Gallen–recommended Ki67 percentage of positively stained tumor cells cutoff of 13.5%. At a hypothetical 13.5% cutoff, there are 32.3% cases that Laboratory D would call high Ki67 but Laboratory B would call low Ki67. The plot is based on 96 cores (three cores were not scored by one of the labs, and one core was not scored by both labs). When more than one core obtained the same paired Ki67 measurements from the two labs, random jittering is used to displace the points vertically to aid visualization (the small amount of noise added to break ties was generated from a uniform distribution between -d/5 and d/5 where d is the smallest difference among the original values within each lab).
Optical Illusions

Not always funny, sometimes really critical…
Human brain:
Square a is lighter than square b!

**Reality:**
*Both are identical*
Simulated Ki67 15%
Estimation of variation of Ki67 scoring
FEATURE BASED MULTIRESOLUTION CORRESPONDENCE

Combined tumor annotations

- Annotated as tumor
  - by 10 pathologists
  - By 4 pathologists
  - By 2 pathologists
  - by no pathologist

Individual tumor annotations

- tumor
- non-tumor

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CLASSIFICATION RESULTS

Gastric Cancer (HER2)  Transmission to HE  Learning Sample

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Structure of the talk

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Summary on Relevance of VM

- Workflow
  - routine diagnostic
  - diagnostic support
- Which scanner?
- Quantification / image analysis
- Clinical-pathological tumor-conferences (tumor board)
- Biobanking

In routine path not yet active on a broad level
Excellent instruments exist, but they have to be integrated properly
Will become very important in personalized medicine
Is already very important
Is important in research institutions
Most important requirements on VM

- Clinical data (LIMS) are correctly connected to WSI
- Completeness of tissue
- Image sharpness
- Color fidelity
- Compression is adequate
- Resolution and quality of the monitor is sufficient

- Test continuously!
Let’s go virtual

13th European Conference on Digital Pathology


www.digitalpathology2016.org
Acknowledgement

Team
Norman Zerbe, Karsten Schlüns, Sebastian Lohmann, Mario Domhardt, Björn Lindequist, Daniel Heim, Stephan Wienert, Kai Saeger, Thorsten Knape, Arend Müller, Wolfram Schädel, Uwe Brunner, Thomas Schrader, Manfred Dietel