How to introduce virtual microscopy (VM) in routine diagnostic pathology: constraints, ideas, and solutions

Klaus Kayser, Stephan Borkenfeld, Gian Kayser

History
Workflow
Design of virtual microscopy
Constraints of implementation
Potential solutions
Perspectives
Definition and application of tissue based diagnosis

- Tissue based diagnosis is the interpretation of images obtained from the human body at light microscopy and higher magnification in combination with clinical data.
- Digital pathology works with digitized images & includes histology, cytology, molecular biology, cytogenetics, molecular genetics, electron microscopy, and biochemistry images, and gross specimen.
## Microscopy

### I. Pretherapeutic diagnostics

### II. Intra-operative diagnostics

### III. Post-operative diagnostics
Diagnosis types and computerized assistance (diagnosis assistants)

- Classic diagnosis: primary: not essential; secondary: partly

- Prognosis estimation (quantitative immunohistochemistry): partly

- Predictive diagnosis (quantitative immunohistochemistry, gene analysis): partly

- Risk estimation (array technique): essential
Components of digital tissue – based diagnosis

- Hospital (patients’) information system (HIS)
- Laboratory information system (LIS)
- Virtual microscopy (VM)
  - Interactive
  - automated
- Pathologist
What is virtual microscopy?

- Virtual microscopy is the diagnostic work on a completely digitized slide (independent from its stain).
- It includes slide digitalization, image presentation, image measurements, image storage, data handling, clinical information transfer, and communication technologies.
# Roots of Virtual Microscopy

| Electronic communication phone, FAX, internet | Measurements |
| Digital photography digital camera, laser technology, etc. | Compartments |
| | Stereology |
| | Architecture |
| | Graph theory |
| Telepathology | Image analysis |
| Diagnosis (classic, risk associated) | Therapy (with/without molecular pathology) |
Historical roots of virtual microscopy I

Acoustic electronic communication

1837  Charles Grafton Page, 1837, galvanic music

1860  Antonio Meucci, electronic speech with his (sick) wife

1861  Philipp Reis, (the hors eats no cucumber salad)

1864  Innocenzo Manzetti

1871  David Edward Hughes, Bell Telephone Company, & Alexander Graham Bell

1876  telegraph, telephone, microphone, speaker
Historical roots of virtual microscopy II

Visual electronic communication

- **1843** Alexander Bain, (Black-white Copy telegraph)
- **1925** Rudolf Hell Blitzzerlegeröhre (lightening dismounting valve)
- **1927** first television session
- **1949** image telegraphy
- **1970** CCD Bell Laboratories,
- **1971** Fairchild Imaging, CCD chip, 100*100 pixel) since >2000 digital high resolution cameras (Parasonic, Kodac, ...)
Historical roots of virtual microscopy III
Communication methods

- **1993** Europe, Digital lines (ISDN)
- **1999** LEO (Low Earth Orbit satellites) (S-PCS) First Generation of Personal Communication Systems **Globalstar** (48 Satellites), – **Iridium** (66 Satellites), …
- **Since 2008** large number of satellites, several international satellite companies
- **LEOs** enable world wide mobile telephones, covering of polar regions, no signal delays
What is telepathology?

Telepathology is an electronic, image-related information transfer and classification between 2...n partners either on- or off-line.

• **Conventional technique:** image information is provided by one partner, the other(s) classify.

• **Interactive telepathology:** all partners contribute to classification, either by providing additional information sources (clinical data, experiences, sampling, etc.) or by image transformation procedures (measurement, filters, etc.).
### History of telepathology I

| Year | Event                                                                 |
|------|-----------------------------------------------------------------------|
| 1960 | first trials of NASA (interactive, expert)                           |
| 1976 | skin biopsies in Massachusetts General Hospital (on-line diagnosis) |
| 1986 | bladder biopsies (National Bladder Cancer Group, USA, reference-study, expert) |
| 1988 | breast biopsies (Tromsö, on-line)                                     |
| 1992 | lung biopsies (Heidelberg, reference study)                           |
| 1995 | frozen section analysis (on-line) and expert consultation (off-line, internet) |
History of telepathology II

- **2001** multi-user server (IPATH, UICC-TPCC, AFIP, expert consultation, artificial intelligence)
- **2002** Virtual Pathology Institution (Salomon Islands, Cambodia, Vidi Lung)
- **2004** Virtual slides, virtual microscopy
- **2005** Internet based open automated image measurements (EAMUS™)
- **2006** e-learning via internet (WebMic)
Telepathology expert consultation centers

- **AFIP**: start 1994, 3,500 cases, additional submission of glass slides, 95% concordance
- **iPATH**: start 2001, >8000 cases, remote control microscope, *commercialized 2010*
- **UICC-TPCC**: start 2000, anonymous experts, new concept 2002, virtual microscope, *no longer available.*
- **Campus medicus**: start 2010, replaces iPATH, *commercialized*
- **MECES**: start 2011, integrated measurement and data bank modules, telephone (skype)
The virtual pathology institution
VPI

Experts on duty
The virtual pathology institution VPI

- **Aim:** To maintain a continuous diagnostic service via telecommunication (iPATH)
- **Structure:** Members of the VPI organize themselves for services, i.e., diagnostic duty and reliability (Faculty: Dr. L. Bannach, Dr. G. Haroske, Dr. N. Hurwitz, Dr. K.D. Kunze, Dr. M. Oberholzer, et. al.)
- **Specific institutions without pathologist submit cases via internet (Honaria, Salomon Islands)**
- **In case of diagnostic difficulties additional pathologists can be asked for assistance by the pathologist „on duty“**.
Lectures learned from telepathology & VPI

- Appropriate image quality
- Distributed image judgment
- Long distance image size (< 2 MB)
- Distributed laboratory work possible
- Central administration & supervision mandatory
- Distributed diagnostics possible
- Coordinative case report possible
Interactive and Automated Virtual Microscopy

- **Interactive**: TV screen and interactive software simulating a conventional microscope = pathologist’s work station

- **Automated**: Image software interacts with virtual slide, evaluates (& corrects)
  - Image quality (vignetting, gray value distribution, etc.)
  - Presence of objects & texture (nuclei, fibers, etc.)
  - Region of interest (ROI)
  - Image classification (crude diagnosis)
  - Feedback to LIS (immunohistochemistry, etc.)

- Pathologist’s interaction due to development
Workflow of a conventional pathology institution

Hospital Information System (HIS)
- Acquisition of patient’s data

Laboratory information System (LIS)
- Tissue identification – patient
- Sampling of adequate tissue probes
- Tissue preparation -> glass slide(s)
- Pathologist (Information System)
- Slide examination (image analysis)
- Diagnosis report (preliminary/definitive)
- Further analysis (expert consultation)
- Hospital Information System (HIS)
Workflow of virtual microscopy in a pathology institution

Hospital Information System (HIS)
- Acquisition of patient’s data

Laboratory information System (LIS)
- Tissue identification – patient
- Sampling of adequate tissue probes
- Tissue preparation -> glass slide(s)
- Virtual Microscopy
- Virtual Slide Storage/Retrieval
- Pathologist (Information System)
- Slide examination (image analysis)
- Diagnosis report (preliminary/definitive)
- Further analysis (expert consultation)
- Hospital Information System (HIS)
Interactive features

**Essential:** navigation, magnification, adjustment of brightness, white (color) balance, patient’s identification (bar code)

**Optional:** interactive measurement (tumor size, melanoma), labeling, links to experts, laboratory (additional stains), digital textbooks.
Maximum resolution of the human eye

Maximum point-to-point resolution of the human eye:

35″ (seconds of arc)

1 seconds of arc = \( \frac{1}{3600} \)°

i.e.: \( \frac{2\pi r}{(360° \times 3600)} \)

Field of view: \( \pm 9° \)

Working distance

eye–monitor: 50 – 100 cm

Maximum point-to-point discrimination:

0,085 mm – 0,17 mm

Field of view: 25 – 30 cm

→ 19 inch monitor (4:3) 38,6 cm x 29 cm → 2270 x 1706 pixel (10 cm left)

→ 22 inch monitor (16:9) 48,7 cm x 27,4 cm → 2865 x 1612 pixel (18 cm left)
## Maximum field of view

| Objective (NA) | RES (mm) | field size (mm) | pixel size (cm) | image size (19 inch) |
|---------------|---------|-----------------|-----------------|---------------------|
| 4 (0.12)      | 2.8     | 2.15*1.61       | 1536 * 1150     | 26.1 * 19.5         |
| 10 (0.25)     | 1.3     | 0.86*0.64       | 1323 * 985      | 22.5 * 16.7         |
| 25 (0.50)     | 0.67    | 0.33*0.25       | 985 * 746       | 16.7 * 12.7         |
| 40 (0.70)     | 0.48    | 0.21*0.16       | 875 * 667       | 14.9 * 11.3         |

Minimum pixel size of 19 inch monitor: 2270 * 1706
Minimum pixel size of 22 inch monitor: 2865 * 1612
Required monitor resolution: > 150 pixels/inch
NA= numerical aperture
Rayleigh criterion for microscope resolution (RES):
\[ RES = 0.61 \times \text{wave length} / \text{numerical aperture} \]
Interactive Virtual Microscopy: one or two monitors?

Basics of one monitor display

Maximum field of view of light microscopy (30 * 30 cm)
19 inch (4/3 monitor, 150 pixel/inch, 1536 * 1150 pixels)

Microscopic field of view (30 * 30 cm, 70 – 100 cm working distance):
19 inch screen (x4 – x10): >10 cm free
22 inch screen (x4 – x10): >18 cm free
Interactive Virtual Microscopy: one or two monitors?

Basics of two monitors display

Virtual Microscope

- Microscopic field of view (30 * 30 cm, 70 – 100 cm working distance):
  - 19 inch screen (x4 – x10): >10 cm free
  - 22 inch screen (x4 – x10): >18 cm free

Hospital Information System (DICOM)

- Patient’s data
  - ID: 1234_11
  - Name: Smith  Given name: John
  - Date of birth: *.*.*
  - Previous findings:
    - June, 20, 2011: tbc

- Radiology: 6, 19, 2011
- Ultra-sound: ****
- Clinical diagnosis: tbc & interstitial lung disease
Workflow of advanced virtual microscopy in a pathology institution

Hospital Information System (HIS)
- Acquisition of patient’s data

Laboratory information System (LIS)
- Tissue identification – patient
- Sampling of adequate tissue probes
- Tissue preparation -> glass slide(s)
- Virtual Microscopy & Assistants
- Virtual Slide Storage/Retrieval
- Pathologists Diagnosis System
- Slide examination (image analysis)
- Diagnosis report (preliminary/definite)
- Supervision (Pathologist’s consultation system)
- Hospital Information System (HIS)
Assistant image quality: object measure, hue – saturation – intensity analysis in poor image quality

Split up of intensity

hue - saturation - intensity
analysis
gian_tma
C1_CN_4
Assistant image quality: object measure, hue – saturation – intensity analysis in a “standardized, good quality” image

hue - saturation - intensity analysis

gian_tma
C1_CN_40
Assistant: Selection of ROI I

Original

Selected areas each 20% of original

Selected areas by minimum spanning tree, no relation to original image size
Stomach biopsies, 2.8 µm/pixel, areas
A) selected by graph theory (high gray values)
B) selected by graph theory (low gray values)
C) Area selected by fixed sliding frames
**Assistant: automated magnification**

**Task:** to measure size of nuclei
(required: 1000 pixels, Ram = 1.5 * 10^{-3})

- **Image standardization**
- **Evaluation of segmentation thresholds**
- **Measure potential object size (areas)**
  - too small? -> increase magnification
  - too large? -> decrease magnification

**Results:**
- RAo: 0.15
  - Ram: 1.0 * 10^{-4}
  - Nuclear size: 80 pixels
- RAo: 0.21
  - RAm: 2.1 * 10^{-3}
  - Nuclear size: 800 pixels
Assistant: Tissue Micro Array (TMA)

Breast carcinoma cases of 18 * 8 (144) spots

Glass slide of TMA spots

Individual spot
Tissue Micro Array (TMA)

Algorithm to automated diagnosing spots

Matrix spot identification —— Image acquisition —— Individual spot measurement

Diagnosis algorithm & diagnosis evaluation —— Patient’s image databank —— Final report & quality assurance
Now–a–days in focus: Molecular markers – EGFR and gene analysis (production of proteins involved in DNA reduplication and repair)

Method: (Laser-) Micro dissection

Microdissection of tumor-material out of sections -> DNA Extraction -> PCR -> Sequencing -> Analysis
Mutations in the *EGFR* Gene in EGFR Inhibitor-Responsive Tumors

- Mutations are in *EGFR* gene cluster, within the intracellular tyrosine kinase domain

Adapted from Lynch TJ et al. *New Engl J Med* 4;350:1-11, 2004.
Molecular marker – ERCC1

83 Patients with N2-stage confirmed by mediastinoscopy
Molecular marker – ERCC1, entropy micro- and macrostages

Hwang Cancer 2008 113:1379-1386

320
\{0, 30, 210, 80\}
0.84
1.67 ± 0.3
2.43
Σ No cells
{0, 1+, 2+, 3+}
Entropy
MST Entropy
Σ Entropy macrostages
160
\{148, 12, 0, 0\}
0.28
1.43 ± 0.2
0.92
**Automated virtual microscopy monitor display**

Maximum field of view about 1500 * 1200 pixels

| Patient’s ID: | 1234_11 | VS no: 1 – 2 – 3 – 4 – 5 - ... n |
|--------------|---------|----------------------------------|
| Clinical diagnosis: SNO-MED: | M83503 | ICDO: 8012/3 |
| Stage of performance: | not possible | ready | started | done |
| Image quality | xxx | |
| Area of Interest | xxx | |
| Texture analysis | xxx | |
| Object segmentation | xxx | |
| Structure analysis | xxx | |
| Proposed diagnosis | *** | |
| LIS interaction: | xxx | |

Pathologist’s control at:
- IQ, ROI, OS, PD
- Stage look up (images):
  - ROI, SA
- Confirmed diagnosis:
  - ***
- Expert consultation: on
- Statistics: on
General scheme of development in tissue based diagnosis

Glass slide preparation

Virtual slide preparation

Hospital, Surgery, Endoscopy

Virtual pathology institution

Specific requests: TMA, Predictive diagnosis, Research

Virtual microscopy

Virtual microscopy

Virtual microscopy
Virtual Microscopy, Perspectives

- **Principle constraints:** missing standards for HIS, LIS.
- **Minor constraints:** expensive for simple expert consultation
- **Perspectives:** Ongoing development of pathology specific DICOM standard
- **Integrative processes similar to PACS**
- **Development of virtual microscopy assistants.**
- **Essential for further diagnosis development**
thank you for your attention