Virtual Microscopy for Hematology

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Digital Images vs. Virtual Images

Digital images make virtual images easier to produce
H. Elizabeth Broome, M.D.

Digital Imaging for Hematology-Outline

- Advantages and limitations of digital imaging
- Available digital imaging systems in US
  - Whole slide imaging such as Aperio
  - Cellavision DM96 and DM1200
  - Medica EasyCell
- FDA issues and validation
- Case Examples

Objectives

At the end of the talk, the participant should be able to:
- describe what digital imaging is currently available for clinical hematology
- compare the performance of digital imaging techniques to manual microscopy
- give specific examples where digital imaging techniques assisted in patient care
Conflict of Interest

• None

Virtual Microscopy; Why Now?

• Advances in technology mean:
  – Quality of digital microscopic image has come close to the human eye
  – Automated slide maker stainiers mean better, more consistent blood smears
  – Robotics create walk away slide imaging capabilities
  – Computer analyses find the best areas of the slide, optimal focus, and “preclassification”
  – Increased storage capacity and band width allow easy processing of large image files
Advantages

- Can view the same image across **time** and **space** for expert review, QC, competency testing and training
  - “Big Brother” is watching you perform your manual differential!
  - Can we trash the slide “micro-locator” yet?
- Easier for viewers with limited expertise on the microscope
- Images do not degrade with time in storage
- Can annotate or further analyze images (computer-aided image analysis)

Disadvantages

- Images are not (yet) as good as with a high-quality microscope
- Optimal viewing requires high-quality equipment and technical expertise
- Does not show all of the smear; may miss cells on edges or platelet clumps in feathered edge
- Users may lose expertise with making smears and using microscope.
“3D” Virtual Slides vs. Multiple 2D Limited Images

- Whole slide imaging operates like a microscope
  - Allows review of the whole slide
  - Some systems focus in 3 dimensions
- Cellavision automatically images optimal viewing areas of the smear plus nucleated cells. The display arranges images in two dimensions.

Cellavision
DI-60
Integrates Cellavision with Sysmex Slide-maker Stainer and Automated Line

Aperio
Approach to Anemia Diagnosis
FDA-approved Hematology
Digital Cell Image Analyzers

<table>
<thead>
<tr>
<th>Capability</th>
<th>Cellavision DM96*</th>
<th>Cellavision DM1200</th>
<th>Medica EasyCell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slide Handling</td>
<td>12 slide per magazine</td>
<td>12 slide per magazine</td>
<td>Carousel for up to 30 slides</td>
</tr>
<tr>
<td>Target Market Slide Volume</td>
<td>50+ Slides per day</td>
<td>25 - 150 slides per day</td>
<td>15-50 slides per day</td>
</tr>
<tr>
<td>On Board Capacity</td>
<td>96 Slides</td>
<td>12 Slides</td>
<td>30 slides</td>
</tr>
<tr>
<td>Slide Scan Time (WBC, RBC, PLT)</td>
<td>~ 2 minutes</td>
<td>~ 2 minutes</td>
<td>~ 4 minutes</td>
</tr>
<tr>
<td>Throughput (WBC,RBC,PLT)</td>
<td>Up to 30 slides/hour</td>
<td>up to 20 slides/hour</td>
<td>up to 15 slides/hour</td>
</tr>
<tr>
<td>STAT Capable</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Power used for imaging</td>
<td>10x, 50x, 100x</td>
<td>10x, 50x, 100x</td>
<td>10x &amp; 100x</td>
</tr>
<tr>
<td>Body Fluid Analysis (Optional)</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Approximate price</td>
<td>$135K</td>
<td>$100K</td>
<td>$65K</td>
</tr>
</tbody>
</table>

*Sysmex DI-60 integrates the DM96 with a slide maker stainer allowing walk away capability for loading all smears onto Cellavision

Aperio vs. Cellavision

<table>
<thead>
<tr>
<th>Bias?</th>
<th>Can do whole slide; usually not</th>
<th>Selection Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>File Size</td>
<td>Large: 100+ megabytes to gigabytes</td>
<td>Smaller: 5 megabytes</td>
</tr>
<tr>
<td>Throughput</td>
<td>25 min+/ slide at 50x oil</td>
<td>35/hr with diff</td>
</tr>
<tr>
<td>LIS interface</td>
<td>No, but can provide links</td>
<td>Yes, can post differentials</td>
</tr>
<tr>
<td>Distribution</td>
<td>Must access through server</td>
<td>Can make jpg’s of images</td>
</tr>
<tr>
<td>Price</td>
<td>$250K for one</td>
<td>$100K each</td>
</tr>
<tr>
<td></td>
<td>$135K each add’l</td>
<td></td>
</tr>
</tbody>
</table>
FDA Issues

- FDA has cleared digital imaging systems for limited uses, e.g. specific IHC staining such as Her2/neu
- FDA has NOT yet approved digital imaging for routine diagnosis
- Cytology: FDA has approved similar systems to Cellavision (e.g. Thin Prep)
- No FDA pre-market notification required for light microscopes

Validating Digital Imaging for Pathology

- >60 routine cases per “application”
- Confirm that all material present on a glass slide is in the digital image.
- For diagnosis: same observer at least 2 weeks apart in random order.
Meta-analysis* of Digital Microscopy for Diagnosis

<table>
<thead>
<tr>
<th>Concordance</th>
<th>86%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discordance</td>
<td>14%</td>
</tr>
<tr>
<td>Concordance and “minor” discordance</td>
<td>98%</td>
</tr>
</tbody>
</table>

23 references for “standard” surgical pathology included. Concordance for each reference ranged from 72% to 98%.

Validation and QC of Cellavision

- Correlation with manual differential using 40+ cases with varied percentages
- Validate quality of images and pre-classification using different stains
- Validate platelet estimation; 40 samples
- Validate RBC morphology and abnormal WBC using truth tables
- Daily QC is just cell localization; >97%
% Neutrophil Correlation Manual, XE5000, DM96

% Lymphs Correlation Manual, XE5000, DM96
% Monocyte Correlation
Manual, XE5000, DM96

% Monocyte Correlation
"Manual" and auto methods
Table 4. Comparison of time taken to complete the 30 differentials on the CellaVision DM96 including reclassification of cells with time taken to perform the same differentials manually

<table>
<thead>
<tr>
<th>Operator</th>
<th>Time for analysis on DM96</th>
<th>Time for manual differential analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 h 5 min</td>
<td>1 h 45 min</td>
</tr>
<tr>
<td>2</td>
<td>1 h 10 min</td>
<td>1 h 40 min</td>
</tr>
<tr>
<td>3</td>
<td>1 h 30 min</td>
<td>3 h 45 min</td>
</tr>
<tr>
<td>4</td>
<td>1 h 40 min</td>
<td>4 h 10 min</td>
</tr>
<tr>
<td>5</td>
<td>1 h 14 min</td>
<td>3 h 10 min</td>
</tr>
</tbody>
</table>


Correct Pre-classification by Cellavision

<table>
<thead>
<tr>
<th>Cell Class</th>
<th>Correct Suggestions by Cellavision (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Segmented neutrophils (n = 3,510)</td>
<td>92.5</td>
</tr>
<tr>
<td>Band neutrophils (n = 868)</td>
<td>57.1</td>
</tr>
<tr>
<td>Lymphocytes (n = 2,585)</td>
<td>96.4</td>
</tr>
<tr>
<td>Monocytes (n = 763)</td>
<td>81.4</td>
</tr>
<tr>
<td>Eosinophils (n = 231)</td>
<td>63.2</td>
</tr>
<tr>
<td>Basophils (n = 50)</td>
<td>80.0</td>
</tr>
<tr>
<td>Blasts (n = 395)</td>
<td>65.1</td>
</tr>
<tr>
<td>Immature myeloid cells (n = 627)</td>
<td>53.2</td>
</tr>
<tr>
<td>Nucleated RBCs (n = 165)</td>
<td>86.7</td>
</tr>
</tbody>
</table>

Kratz et al. AJCP2005 124:770
Case #1

- 5 y.o. girl in ER with lethargy and abdominal pain
- Exam revealed pale membranes and petechiae

Blood Counts
Sysmex XE5000

| WBC: 4.5 x 10^3/ul | Lymphocytes: 90.9%
| RBC: 1.72 x 10^6/ul | Neutrophils: 1.1%
| HGB: 4.7 gm/dL | Monocytes: 6.9%
| MCV: 80.2 fL | Eosinophils: 0.2%
| RDW: 18% | Basophils: 0.7%
| PLT: 18 x 10^3/ul | Imm. Grans: 0.2%

*XE5000 hematology analyzer flags:
“Blasts?” “Immature Gran?”

Case #1 Cont’d
Case #1 Cont’d

Cellavision images were remotely reviewed by a pathologist at her office 15 miles away from the lab.

Case #1 Cont’d

- Diagnosis: probable leukemic blasts
- B-acute lymphoblastic leukemia (B-ALL) confirmed by flow cytometry
- This case illustrates
  - Importance of “blast” flagging by hematology analyzers
  - Importance of manual smear review
  - Use of Cellavision for rapid, remote pathologist review
Case #2

- 17 year old male with no significant medical history presented with bleeding from gums and hematuria x 1 day and cough/malaise/chills x 3 weeks.

- Physical Exam revealed petechiae on extremities and splenomegaly

Case #2 Cont’d

- Blood Counts Sysmex XE5000:
  - WBC: 6.9 x 10³/ul
  - RBC: 4.84 x 10⁶/ul
  - HGB: 14.9 gm/dL
  - MCV: 85.5 fL
  - RDW: 12%
  - PLT: <5 x 10³/ul

- Differential (manual; Cellavision): 13% Segs; 18% Bands; 53% Lymphocytes; 8% Monocytes 8% “other cells”
Case #2 cont’d

“Other cells”

• “Others” are reactive lymphocytes
• Diagnosis: Probable infectious mononucleosis complicated by immune thrombocytopenia
• Positive “monospot” plus IgG and IgM anti-Epstein Barr Virus confirmed recent infection with Epstein Barr Virus
• Platelet count responded to treatment with corticosteroids and IVIg.
Case #3 Digital Image Use in Medical Record

Expurgated screen prints from an electronic medical record:

Case #3 Cont’d

- UCSD Healthcare has Cellavision viewing stations for use by clinicians
- Required moderate initial training and continuing maintenance training
  - Multiple sites requires users to login to different databases
  - Cellavision does not distinguish between “viewer only” vs. active user
- No easy archiving of images into EMR
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Cellavision Advanced RBC (in development)

Spherocytes Highlighted
Summary

- Digital imaging in hematology allows
  - Remote, real-time review of smears
  - Easy designation of “others” for secondary review
  - Competency testing
- Other systems such as Aperio allow
  - “Virtual microscopic” review of entire slides
  - Review of all types of slides, not just blood smears or body fluids
Future

- Improve RBC morphology functions
- Automated screening of blood smears and cytospins for abnormal cells
  - Blasts
  - Increased band neutrophils
  - Increased schistocytes or spherocytes
- Better integration of imaging with hematology automation