ESTP Pathology 2.0 group:

Digital Image Analysis and Multiplexing of Biomarkers – Part 1

April 28th 2015
Disclaimer

I work for Flagship Biosciences Inc.:
- Assay Development and Digital Image Analysis company
- CLIA-certified histology laboratory
- Support big pharma and small biotech companies

For more information, please visit www.flagshipbio.com
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I work for Flagship Biosciences Inc.:

- Assay Development and Digital Image Analysis company
- CLIA-certified histology laboratory
- Support big pharma and small biotech companies
- Analysis of animal & human tissues
  - Drug trials (retrospective & prospective)
  - Clinical Diagnostics
  - Medical Devices
  - Infectious & inflammatory disease research

All algorithm examples shown in this presentation are proprietary to Flagship Biosciences.

For more information, please visit www.flagshipbio.com
Goals of this presentation

1. Reduce the fear that image analysis may replace pathologists
2. Share my excitement for this novel technology
3. Leave you with the assurance that you already know most of what you need to be part of an image analysis team
Agenda

Digital Image Analysis – the Basics

The Image Analysis Team
- The Pathologist’s Role in an Image Analysis Team
  - Slide/tissue and scanning quality control
  - Annotation review
  - Post-analysis review

Closing Remarks
Digital Image Analysis – the Basics

Tools available:
- Free/open source (e.g. ImageJ)
- Commercially available (software and toolkits)
- Service providers

Three main categories:
- Area-based measurements
- Cell-based measurements
- Object-based counting/measurements

Goal: Fit-for-purpose/application-targeted algorithm tools
Digital Image Analysis – the Basics

Area-based measurements:

- Color of each pixel quantified → measure area covered by defined stain
  - Classification based on intensity possible
- Example:
  - Quantification of liver fibrosis in a mouse model
Digital Image Analysis – the Basics

Area-based measurements:
Digital Image Analysis – the Basics

Area-based measurements:

- Mouse liver
- Stained with Picrosirius Red (PSR) - collagen
Digital Image Analysis – the Basics

Area-based measurements:

- Mark-up image: PSR stained areas in blue
Digital Image Analysis – the Basics

Area-based measurements:

- Total liver area = black
Digital Image Analysis – the Basics

Cell-based measurements:

- Morphometry-based assessment
  - Pixels grouped based on similarity to define structures (e.g. cells) → data collection per structure

- Combination solutions
  - Assessment of individual pixels + assessment of cell populations → automated identification complex tissue structures (e.g., glomeruli, tumor in normal tissue) → data collection
Digital Image Analysis – the Basics

Cell-based measurements:
- Morphometry-based assessment
- Tumor/stroma separation based on cell morphology
- No IHC stain needed to identify tumor
Digital Image Analysis – the Basics

Cell-based measurements:

- Combination: pixel information (cell) + relationship of cells to each other

- Detection of Ki67+ cells in murine PanIN
Digital Image Analysis – the Basics

Cell-based measurements:

- Combination: pixel information (cell) + relationship of cells to each other

- Detection of Ki67+ cells in murine PanIN
Digital Image Analysis – the Basics

Cell-based measurements:
- Combination: pixel information (cell) + relationship of cells to each other

![Images of cell-based measurements](image-url)
Digital Image Analysis – the Basics

Object-based measurements:

- A certain structure is being detected and quantified
  - Fat vacuole, vessel, muscle fiber
  - Data is collected for the entire population and individual events counted
Digital Image Analysis – the Basics

Object-based measurements:
The Image Analysis Team
The Image Analysis Team

Variable number of members

Key competencies:
- Computer programmers – coding of algorithms
  - Not needed/not allowed with some commercial systems
- TIA analysts – algorithm tuning
  - For some commercial systems, an image analysis expert is needed
- Biological expertise of the disease process investigated
- Pathology expertise – central at all steps!
- Technicians
The Pathologist’s Role in an IA Team

TIA solutions cannot be validated for a wide variety of applications / different slides, therefore approval of each image analysis result by a pathologist is currently only possible way to go!

The Pathologist’s Role in an Image Analysis Team

- Slide/tissue and scanning quality control
- Annotation review
- Post-analysis review
The Pathologist’s Role in an IA Team

The Paradox of Image Analysis

Gold Standard: Pathologist’s assessment

Algorithm expected to be better than human scores

Validation by comparison to

IA solution development

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The Pathologist’s Role in an IA Team

Why it is so hard to be the gold standard:

- Eyes can’t be trusted
- A.k.a. the Craik–O’Brien–Cornsweet illusion

*The Journal of Neuroscience, 1 October 1999, 19(19): 8542-8551*
The Pathologist’s Role in an IA Team

Why it is so hard to be the gold standard:

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The Pathologist’s Role in an IA Team

Blind orion searching for the rising sun by nicolas poussin, 1658; metropolitan museum of art
# The Pathologist’s Role in an IA Team

<table>
<thead>
<tr>
<th>PATHOLOGIST</th>
<th>Image Analysis</th>
<th>Annotations</th>
<th>Scanning</th>
<th>Histology</th>
</tr>
</thead>
</table>
|             | • Appropriate algorithm development | • Correct annotation  
• Target tissue present | • Damage during transport  
• Scanning artifacts | • Cutting and staining  
• Fixation  
• Tissue collection |
The Pathologist’s Role in an Image Analysis Team

- Slide/tissue and scanning quality control
- Annotation review
- Post-analysis review
The Pathologist’s Role in an IA Team

Slide and scanning quality control

- Slide/tissue quality
  - Effects of tissue harvesting, handling, fixation type and time, processing time, slide preparation, staining etc.
  - Staining quality!
    - Optimized stain, dynamic range, appropriate staining etc.

- Scanning quality
  - Stitching issues/compensation lines
  - Out-of-focus scans; focal blurs
  - Fixable vs. non-fixable artifacts

- Target tissue present?
The Pathologist’s Role in an IA Team

Slide/tissue and scanning quality control

- Slide quality – histology artifacts

Tissue Folds

Air bubbles
The Pathologist’s Role in an IA Team

Slide/tissue and scanning quality control
- Staining quality

Algorithm can identify “blue” round areas as nuclei

What the algorithm “sees”:
- Hematoxylin
- DAB

What the pathologist sees:
- DAB with hematoxylin counterstain
The Pathologist’s Role in an IA Team

Slide/tissue and scanning quality control
- Staining quality

No identifiable “blue round structures” present to allow for nuclear detection by algorithm

What the pathologist “should see”:
DAB with hematoxylin counterstain

What the algorithm “sees”:

Hematoxylin

DAB
The Pathologist’s Role in an IA Team

Slide/tissue and scanning quality control

- Slide quality – histology artifacts

A void in DAB staining where the nucleus is ≠ nuclear hematoxylin staining

No identifiable “blue round structures” present to allow for nuclear detection by algorithm

What the algorithm “sees”:

- Hematoxylin
- DAB

What the pathologist “should see”:

DAB with hematoxylin counterstain
The Pathologist’s Role in an IA Team

Slide/tissue and scanning quality control

- Slide quality – scanning artifacts
The Pathologist’s Role in an IA Team

Slide/tissue and scanning quality control

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The Pathologist’s Role in an IA Team

Slide/tissue and scanning quality control

- Slide quality – scanning artifacts
The Pathologist’s Role in an IA Team

Slide/tissue and scanning quality control
- Slide quality – scanning artifacts

Compensation lines
The Pathologist’s Role in an IA Team

Annotation review

- Manual image masking
- Aim for including 80-100% of target tissue

Reasons for manual annotations
- Focus analysis on true target tissue
- Decrease surface area to cover by algorithm
- Decrease run time for algorithm
- Decrease overall amount of data gathered
- Aid in algorithm development
The Pathologist’s Role in an IA Team

Annotation review

- Manual image masking
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The Pathologist’s Role in an IA Team

Annotation review

- Manual image masking
- Aim for including 80-100% of target tissue
The Pathologist’s Role in an IA Team

Post-analysis review

- Is the algorithm performing well?
- Is it defining cells appropriately?
- Is it detecting the right cells/cell population?
- Is it binning/scoring staining intensities appropriately?
The Pathologist’s Role in an IA Team

Post-analysis review

- Area-based measurements

**Top:** drug-treated cornea

**Left to right:** decreasing values selected as threshold to detect staining intensities above background

**Bottom:** vehicle-treated cornea

NHP eye
The Pathologist’s Role in an IA Team

Post-analysis review
  - Nuclear detection – split/merge

Rat eye
The Pathologist’s Role in an IA Team

Post-analysis review
- Nuclear detection – split/merge

Too few nuclei are detected overall and the size of individual nuclei shown in the mark-up is too large.
The Pathologist’s Role in an IA Team

Post-analysis review

- Nuclear detection – split/merge

Rat eye

FAIL

PASS
The Pathologist’s Role in an IA Team

Post-analysis review
- Region of interest (ROI)

Mouse pancreas
The Pathologist’s Role in an IA Team

Post-analysis review

- Region of interest (ROI)

Mouse pancreas
The Pathologist’s Role in an IA Team

Post-analysis review
- Region of interest (ROI)

Mouse pancreas

FAIL

PASS
The Pathologist’s Role in an IA Team

Post-analysis review

- Vessel detection

Rabbit tissue
The Pathologist’s Role in an IA Team

Post-analysis review
- Vessel detection

Rabbit tissue
The Pathologist’s Role in an IA Team

Post-analysis review
- Vessel detection

Rabbit tissue
The Pathologist’s Role in an IA Team

Post-analysis review

- Tumor/stroma separation

Human tissue
The Pathologist’s Role in an IA Team

Post-analysis review
- Tumor/stroma separation

Human tissue

Not all epithelial neoplastic cells are detected by the algorithm
The Pathologist’s Role in an IA Team

Post-analysis review
- Tumor/stroma separation

Human tissue

FAIL

PASS
The Pathologist’s Role in an IA Team

Post-analysis review

- Scoring – gates/thresholds (cytoplasm)

Human tissue

0 = blue
1+ = yellow
2+ = orange
3+ = red
The Pathologist’s Role in an IA Team

Post-analysis review

- Scoring – gates/thresholds (cytoplasm)

Human tissue

![Image of human tissue with scoring criteria]

0 = blue
1+ = yellow
2+ = orange
3+ = red

FAIL

PASS
The Pathologist’s Role in an IA Team

Post-analysis review

- Scoring – gates/thresholds (dots/cell)

Human tissue

0  = blue
1+  = yellow
2+  = orange
3+  = red
The Pathologist’s Role in an IA Team

Post-analysis review

- Scoring – gates/thresholds (dots/cell)

Human tissue

0 = blue
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FAIL

PASS
Closing Remarks

Goals of this talk:

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Closing Remarks

Currently no business case for wide clinical adoption of IA

Game changer:
- Complex Image Analysis Companion Diagnostics

It’s time for our profession to embrace these new technologies and shape the way we will work with them in the future
Questions?

Don’t miss the next webinar:

**Digital Image Analysis and Multiplexing of Biomarkers – Part 2**

Helen Angell, BSc, PhD
Senior Scientist, Molecular Pathology, Translational Science, Oncology
iMed, AstraZeneca

Thank you to ESTP, IFSTP and IATP for sponsoring this webinar!