Immunostaining Analysis Through ImageJ

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Content

• Background Description
• Tool (semi-automatic system)
• Evaluation
• Future Work
• Conclusion
Immunostaining

• Oesophagitis and Colorectal biopsies

• Protein 53 (P53)
  – Tumor suppressor protein
  – Brown (DAB Diaminobenzidine)

• Nuclei
  – Positive nuclei is brown
  – Negative nuclei is blue

• Gland
Digital Image

• Benefits
  efficiency, accuracy
  reproducibility
  (reduce inter-observer-discrepancies)

• Challenges
  objects VS background
  stain
  nuclei
  gland
Color Distribution

- Immunostaining
- Analysis
- ImageJ
What we did

• Semi-automatic tool
• Colour statistical Model
• Positive stain labelling
• Positive slide recognition
• Nuclei segmentation
• Gland segmentation
ImageJ

- Open source
- Ease of use
- Variety functions
- Macros & Plugins
Structure

- Two Stage Process
  - Training
  - Detection

New model

Training

Existed model

Enlarge model

Pre-processing

Save model

Save histogram

Detection

Tool

- Logic Structure
- Statistical Detection Model
- Positive slide recognition
- Nuclei Detection
- Gland Detection

Background

Evaluation

Future
Structure

• Build new model
**Structure**

- Or use pre-build model

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**Tool**
- Logic Structure
- Statistical Detection Model
- Positive slide recognition
- Nuclei Detection
- Gland Detection

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**Training**

New model

Existing model

Enlarge model

Pre-processing

Save model

Save histogram

Detection
Brown Color Collection

- Visualize
- Determine Positive Staining
- Region of Interest

Adjustable Tool

• Logic Structure
• Statistical Detection Model
• Positive slide recognition
• Nuclei Detection
• Gland Detection

Tool

Background

Evaluation

Future
Statistical Detection Model

- Histogram
- Maximum Likelihood Classification
Statistical Detection Model

• Histogram model

\[
\text{Prob}(rgb) = \frac{\#[rgb]}{\# \text{ Total Pixel}}
\]

\[
\text{Prob}(rgb|S) = \frac{\#S[rgb]}{N_S} \quad \text{Prob}(rgb|\bar{S}) = \frac{\#\bar{S}[rgb]}{N_{\bar{S}}}
\]

• Maximum Likelihood ratio

\[
\frac{\text{Prob}(rgb|S)}{\text{Prob}(rgb|\bar{S})} \geq \theta
\]

• RGB, YCbCr, Opponent color model and CbCr
• http://ima.ac.uk/papers/shu2010.pdf
Shades of brown

Y: 0-63

Y: 64-127

Y: 128-191

Y: 192-255
Brown Particle Detection

- Customizable Positive Staining Threshold
- Particle Analyzer (measure size, number)
Particle Density Classification

- Highly recommended area
Nuclei Detection

• Global thresholding & Watershed
  – Isodata
    • *Ridler, TW & Calvard, S (1978)*
  – Otsu
    • *Otsu, N (1979)*
  – IJ_Isodata
    • ImageJ’s original algorithm updated from Isodata
  – Watershed Separation(command of imagej)
    • Works best on smooth convex objects that don’t overlap too much
Comparison of Auto-threshold Method

- Background
- Tool
- Evaluation
- Future

- Logic Structure
- Statistical Detection Model
- Positive slide recognition
- Nuclei Detection
- Gland Detection

- Original
- Otsu
- Isodata
- IJ_Isodata

Large image: 6720*4200 pixel
Segmentation

- Our method
  - Pure brown and blue image
  - Local threshold (IJ_Isodata) on 20*20 square
  - Watershed

- Original  grey  Otsu  IJ_Isodata
Nuclei Detection

- Original
- Our method
- Watershed

Tool
- Logic Structure
- Statistical Detection Model
- Positive slide recognition
- Nuclei Detection
- Gland Detection
Gland Detection

- H&E (Haematoxilin & Eosin)
- DAB (Diaminobenzidine)

**Tool**
- Logic Structure
- Statistical Detection Model
- Positive slide recognition
- Nuclei Detection
- Gland Detection

**Background**

- Stroma (pink)
- A gland unit
- Lumen (white)
- Epithelial nuclei (blue)
- Epithelial cytoplasm (purple)

**Gland Unit**
- Stroma (light blue)
- Lumen (not purely white)
- Cytoplasm (light blue)
- Nuclei (blue)
Gland Detection
Gland Detection
Result Comparison

Correct Detection = \frac{\text{Model Generated Positive Pixels}}{\text{Manually Generated Positive Pixels}}

False Detection = \frac{\text{Model Generated False Positive Pixels}}{\text{Manually Generated Negative Pixels}}

Experimental Results:
- Brown detection
- Positive slide recognition
- Nuclei segmentation
Positive Slide Recognition

- Nuclei within 100 pixel radius $\geq 10$
- 43 Manually Annotated Slides
- Brown Brightness 120-130

**Slide Recognition**

- 100% Correct Detect
- 16% False Positive

**Experimental Result**

- Brown detection
- Positive slide recognition
- Nuclei segmentation

**Evaluation**

- 83% Correct Detect
- 30% False Positive
Nuclei Segmentation

• Positive nuclei (red) \( P_n = \frac{\sum C_b}{\sum C} \) (if \( P_n > \text{threshold} \)
Nuclei Segmentation

- Manually labeled nuclei pixel 61018
- Auto detected nuclei pixel 67525
- The correct detected pixel 49364 (81%)
Future Work

• Refine tool
  – Gland detection on high level recognition
  – Welcome suggestions
Conclusion

• Computerizing slide analysis
• The detail of the existed tool
• The experimental result
• Future plan
Questions?