Multi-scale analysis of histopathological images: Experience with follicular lymphoma and neuroblastoma

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Breast Cancer

Lung Cancer


Colon Cancer

Microscopic Slides

Whole-slide Data

- 64990 x 59412 pixels in full resolution
- Original Size: 10.8 Gb; Compressed Size: ≈ 833Mb

8x
40x
Size

50 k x 50 k

5 k x 2 k
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- Enis Cetin, PhD
- Myriam Oger, PhD
- Laura Schmidt, PhD
- Philip Payne, PhD
- Emily Patterson, PhD
- Mike Pennell, PhD
- Kimery Powell, PhD
- Jeff Prescott, PhD
- Nasir Rajpoot, PhD
- Ray Raylman, PhD
- Sidhardt Samsi, MSc
- Olcan Sertel, PhD
- Bulent Yener, PhD

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**Neuroblastoma**

- Neuroblastoma (NB) is a cancer of sympathetic nervous system
- Commonly affects children, especially those under five years old.
Neuroblastoma Classification

Schwannian Development

- Grossly visible Nodule(s) present
- Microscopic Neuroblastic foci present
- Schwannian Nodule(s) absent
- Neuroblastoma (Schwannian stroma-poor)
- Maturing subtype
- Mature subtype
- Ganglioneuroblastoma, Nodular (composite, Schwannian stroma-rich/stroma-dominant and stroma-poor)
- Variant forms*
- Ganglioneuroblastoma, Intermixed (Schwannian stroma-rich)
- Ganglioneuroblastoma, Nodular (composite, Schwannian stroma-dominant)
- FH
- UH

Ganglioneuroma
- (Schwannian stroma-dominant)
- Maturing subtype
- Mature subtype

Variant forms*

FH: favorable histology
UH: unfavorable histology

NB prognosis

Stroma-rich (SR) Differentiating (D) Poorly-differentiated (PD) Undifferentiated (UD)

- Low MKI
  - (<100/5000 cells)
- Intermediate MKI
  - (100-200/5000 cells)
- High MKI
  - (>200/5000 cells)

Detection of mitosis and karyorrhexis (MK) cells
Where is Waldo?

Neuroblastoma Classification

- Stroma Density
- Differentiation
- Mitosis Karyorrhexis Index
Initial Design

NB Digital Slide

Schwannian Stromal Development

Grade of Differentiation

Computer representation

(a) Original image  (b) Partitioned image  (c) Nuclei

(d) Cytoplasm  (e) Neuroni;  (f) Background
Multi-resolution representation and processing

- Gaussian Pyramid
  - Original Image \( I_0 \) zero level of the pyramid

\[
I(i,j) = \sum_{m=-2}^{3} \sum_{n=-2}^{3} w(m,n) I_{l-1}(2i + m, 2j + n)
\]

\[
w(n,m) = w(n)w(m)
\]

\[
w = \begin{bmatrix}
0.25 & -0.5a & 0.25 & a & 0.25 & -0.5a
\end{bmatrix}
\]

Whole-slide Image Analysis

- Whole-slide image
- Classification map
- Parallel Classification
  - Processor 1
  - Processor N
- Assign Classification Labels
  - Stroma-rich
  - Background
  - Stroma-poor
  - Undetermined
**Computerized Classification System**

- Background Identification
- Image Decomposition (Multi-resolution levels)
- Image Segmentation Feature Construction (color, texture, etc.)
- Feature Extraction (PCA, LDA)
- Classification (Bayesian, KNN)
- Multi-resolution Layer Controller (Confidence Region)

**Computer-aided Prognosis**
Neuroblastoma Image Analysis Results

**Grade of Differentiation**

- Undifferentiated Case
- Differentiating Case
- Poorly-differentiated Case

**Schwannian Stromal Development**

- Stroma-rich Case
- Stroma-poor Case

The accuracy of the computer-assisted system is **88.9%** over 45 cases.

The accuracy of the computer-assisted system is **88.4%** over 43 cases.

NB whole-slide classification results
Follicular lymphoma (FL)

- Current FL prognosis is based on manual count of centroblast (CB) cells in ten random high power fields (HPFs).
- Tedious process, time-consuming
- Significant inter- and intra-reader variability
- Sampling bias
- Grade I (<5 CB/HPF)
- Grade II (5-15 CB/HPF)
- Grade III (>15 CB/HPF)
Computer-assisted FL grading

- Two systems for assisted FL grading:
  - CB detection [IEEE TBME'10]
    - Challenging in high grades where the cell morphology is very irregular
  - Perceptual organization [JSPS’09]
    - Spatial distribution of sub-cellular components to capture the overall morphology of cell population

Variability in FL prognosis

- CB detection
  - 10 whole-slide images
  - 10 ROI from each slide
  - CBs marked by 5 pathologists
Variation

CAD-GUI of the grading software

CAD-GUI appearance during the grading

Follicular lymphoma

- CAD for FL prognosis
  - Reduce high variability
  - Avoid sampling bias
  - Reproducible grading

- IHC
- H&E

2X

40X

Grade I

Grade II

Grade III
Follicle Segmentation from H&E Images
Follicle detection from IHC images

Follicle Segmentation from IHC Images
H&E and IHC Registration

- Segmentation of individual cells
  - Variability due to tissue and cell morphology
  - Variability due to slide preparation
  - Separation of clustered cells
- Classification of cells
  - Relevant features
  - Minority class problem due to under representation of CB samples
  - Classification strategy

CB detection

- Segmentation of individual cells
- Classification strategy
- CB detection flowchart
CB detection

An image patch from a whole slide
Segmentation of nuclei
Touching-cell clusters
Non-touching cells
Touching-cell splitting
CB classification

- Initial CB detection based on morphological features
  - Size (area in pixels)
  - nuc / cyt ratio
  - Eccentricity
  - Compactness

ROI image

Likelihood \( \Lambda_{CB} = \prod_i \lambda_i \)

Initial CB detection

CB classification

- Refine the initial CB detection using texture distribution
  - GLRLM
  - Feature extraction (PCA)
  - Classification rule

\[
\text{cell} = \begin{cases} 
CB, & P(f | \mu_{nuc}, \Sigma_{nuc}) = P(\hat{\mu}_{nuc} + 3\sqrt{\Sigma_{nuc}}) \\
\text{notCB}, & \text{otherwise.}
\end{cases}
\]

\[\Sigma_{nuc} = UAU^T\]

- Accuracy of the CB detection system
- 100 ROI images (~2500 cells/ROI)
- Initial detection
  - 90.2% accuracy with 200 FP
- Final refinement
  - 80.7% accuracy with 30 FP

- Current manual system
  - 65.8% accuracy
  - 5.1 FP
FL grading based perceptual organization

- Classification of high-grade FL tissue samples using perceptual organization [JSPS'09]

![](image)

FL grading - MBIR

- Represent connected components of cytological components using ellipses, $\xi_i = \{x_i, y_i, l_1, l_2, s_i, \phi_i\}$
- Morphological features
  - $\mu_i, \sigma_i, \varepsilon_i, f = \{l_1, l_2, s\}$
- Topological features
  - Degree sequence
- Texture of cytological components
  - Nuclei, cytoplasm
  - A color texture approach for images with limited dynamic range [ICASSP'08]
FL grading evaluation

- Evaluated over a dataset consisting of 17 whole-slide images, 10 ROI images extracted from each by 3 different hematopathologists
- Classification results
  - PCA+LDA
  - Bayesian classifier

<table>
<thead>
<tr>
<th></th>
<th>Grade I</th>
<th>Grade II</th>
<th>Grade III</th>
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<tbody>
<tr>
<td>Grade I</td>
<td>74.9%</td>
<td>23.5%</td>
<td>1.6%</td>
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<tr>
<td>Grade II</td>
<td>13.5%</td>
<td>84.6%</td>
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<td>Grade III</td>
<td>0.4%</td>
<td>4.6%</td>
<td>95.0%</td>
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Other Multi-scale Examples

- Incorporation of Mass Spectroscopy with microscopy images
- Cutaneous Lymphoma photographic images and microscopic images
Data Collection : Tissue Image

- H&E image – Regions of interest are Follicle, Mantle and the Intrafollicle regions

Image Registration

- The H&E image and spotted tissue image were registered by manually selecting control points
- Laser spots were overlaid on H&E image for manual classification
Mapping Data Points to H&E Image

- After registration, laser spots were overlaid on H&E image as shown in figure.
- We are interested in the tissue locations corresponding to the mantle, follicle and intra-follicular region.

Generating Ground Truth

- Laser spots were classified into mantle, follicle and intrafollicle regions.
- In the figure red corresponds to mantle, green to follicle and blue to intra-follicular region.
Analysis of MS Data

Note: Red indicates mantle, Green indicates follicle and Blue indicates intra-follicular region

Looking ahead

- Other areas where multi-scale image analysis will be important
Multi-scale analysis is an important part of clinical image analysis

- It is already part of current clinical practice
- Microscopy with initial high resolution images provides an environment to develop many multi-scale algorithms
- Combination of information coming at different scales and from different sources provide critical information to arrive at correct decisions
Acknowledgement

- National Cancer Institute, R01, PI: Gurcan
- National Library of Medicine, R01, PI: Gurcan
- Children’s Neuroblastoma Cancer Foundation (Young Investigator Award: Metin Gurcan)
- CTSA
- Department of Defense
- American Cancer Society
- NFL Charities
- OSU CCC
- Pelotonia Fellowship

CIALAB

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