Quantitative Analysis of CD4+ and CD8+T Cells Structures and Morphology Based Classification

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Hypothesis and Specific Aims

Hypothesis

The diffraction imaging flow cytometry method allows label-free classification of B and T cell subtypes

Aims

1. Quantitative study of lymphocyte morphology through confocal imaging
2. Acquisition of Cross-Polarized Diffraction Images
3. Cell Classification by SVM with confocal and diffraction image data

Motivation of Research

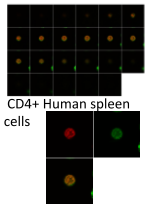
- To investigate the correlation between diffraction images of lymphocytes and their 3D morphology.
- To develop an innovative label-free method for rapid and accurate assay of leukocytes.

Background and Significance: The Immune system

- First line of defense against a microbial invasion or abnormal cells
- Adaptive and Innate responses
  - Adaptive responses
    - Requires prior exposure to certain pathogens.
    - Body produces antibodies against pathogens.
    - Lifelong protective immunity to reinfection of the same pathogen.
  - Innate responses
    - Does not require prior exposure to particular pathogens.
    - Macrophages engulf pathogens.
    - Does not provide lifelong immunity to reinfection of the same pathogen.

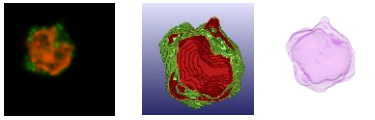
Quantitative study of lymphocyte Morphology : 3D reconstruction analysis

- Cell Imaging and Morphology Analysis (CIMA) software to reconstruct the 3D images.
- Import z-stacks
- CIMA software
  - Segmentation
  - Interpolation
  - 3D morphological feature parameters data
  - 3D reconstructed image of cell



CD4+ Human spleen cells

3D reconstruction of CD4+ T cell



Confocal slice of a CD4 + T lymphocyte obtained from a peripheral blood sample

3D image reconstructed of a CD4+ T lymphocyte obtained from a peripheral blood sample (by slice with 3D intracellular features)

3D image reconstructed of a CD4+ T lymphocyte obtained from a peripheral blood sample (whole cell with intracellular features)

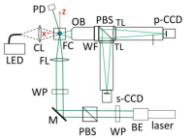
3D morphological feature parameters data of CD4+ and CD8+ T cells from human spleen sample

Mean ± Standard deviation

Parameter	Symbol	Unit	CD4+ T (n =42)*	CD8+ T (n =77)*	p*
Cell grid perimeter	GP_c	μ m	2454 ± 590	2142 ± 677	0.014
Cell surface area[b]	S_c	μ m²	165.7 ± 30.3	180.0 ± 25.2	0.007
Nuclear grid perimeter	GP_n	μ m	2220 ± 455	1874 ± 617	0.002
Nuclear surface area[b]	S_n	μ m²	150.5 ± 24.6	157.1 ± 23.0	0.151
Nuclear volume[c]	V_n	μ m³	97.29 ± 21.9	104.2 ± 20.1	0.085
Nuclear surface to volume ratio	SV_{rn}	μ m⁻¹	1.582 ± 0.256	1.528 ± 0.157	0.157
Mitochondrial grid perimeter	GP_m	μ m	196.9 ± 173	124.7 ± 119	0.009
Mitochondrial surface area[b]	S_m	μ m²	12.24 ± 10.5	9.953 ± 11.0	0.274
Mitochondrial volume[c]	V_m	μ m³	0.881 ± 1.06	0.818 ± 1.40	0.801
Mitochondrial surface to volume ratio distance ratio	SV_{rm}	μ m⁻¹	2.965x10⁸ ± 3.30x10⁸	3.660x10⁸ ± 3.27x10⁸	0.272
Mitochondrion-to cell volume ratio	Vr_{mc}	-	0.0080 ± 0.009	0.0061 ±0.010	0.288

*n = number of imaged cells, p-values were obtained by a two-sample t-test method where equal variances are assumed
[b]S = N_s so with N_s as the number of voxels on the membrane of the organelle and so as the diagonal plane area of the voxel.
[c]V = N_v v_0 with N_v as the number of voxels inside the organelle of interest and v_0 as voxel volume.

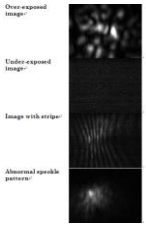
Acquisition of Cross-Polarized Diffraction Images : The p-DIFC System



Schematic diagram of an experimental p-DIFC system for acquisition of s- and p-polarized diffraction images. BE: beam expander; WP: half-wave plate; PBS: polarizing beam splitter; M: mirror; FL: focusing lens; FC: flow chamber; CL: condenser lens; PD: photodiode; OB: objective; WF: 532 nm wavelength filter; TL: tube lenses; CCD: camera. The x-axis and z-axis are labeled by red lines.

Acquisition of Cross-Polarized Diffraction Images: Preprocessing

- Images pairs will be flited using an in-house developed preprocessing software.
- Software removes
  - Overexposed image pairs
  - Underexposed image pairs
  - Large speckled image pairs
  - Strip image pairs



Examples of diffraction image (provided by W. Jiang).

Support Vector Machine Cell

- 3D morphological feature parameters and the p-DI feature parameters data is imported into an in-house developed software(LIBSVM) based upon the SWM algorithm.
- The software will use the SVM algorithm to recognize patterns within the feature parameters of both 3D morphological feature parameters and the p-DI feature parameters data and perform a binary classification of the different biological cell types
- Software will be used to obtain optimized SVM models for classification.

Support Vector Machine Cell Classification

- Feature parameter vector, represents the ith cell for each cell type in a parameter space

$$c_i = \sum_{m=1}^{N_{tra}} g_{im} u_m$$

- Kernel function
  a) Linear: $K(x_i, x_j) = x_i^T x_j$
  b) Polynomial : $K(x_i, x_j) = (\gamma x_i^T x_j + r)^d, \gamma > 0$
  c) Radial basis function(RBF): $K(x_i, x_j) = \exp\left(-\gamma \|x_i - x_j\|^2\right), \gamma > 0$
  d) Sigmoid: $K(x_i, x_j) = \tanh(\gamma x_i^T x_j + r)$
- Matrix Q of rank N_{tra} for mapping into a feature space
  - Elements are identified as *Qij =titj K(ci , cj)* where the cell type identifier of *ti* and *tj*(=1 or -1 for the two types of cells) and the index *i or j* ranging from 1 to N_{tra}.
- Five-fold cross validation with single parameters on the training data set.

Support Vector Machine Cell Classification

- Model is saved and evaluated with a decision function

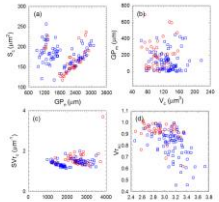
$$F(x) = \sum_{i=1}^{N_{tra}} t_i a_i K(c_i, c_j) + b$$

- SVM will perform a binary classification of the data based upon the outcome of the decision function, whether *F>0* or *F<0*.
- Classification Accuracy

$$A = \frac{TP + TN}{TP + TN + FP + FN}$$

The outcomes are labeled as TP, TN, FP, and FN where TP or TN represent the number of correctly identified cells for each cell type and FP or FN represent the number of incorrectly identified cells for each cell type.

3D Morphological Feature Parameters data of CD4+ and CD8+ T cells from human spleen sample



Scatter plot of 42 CD4 cells and 77 CD8 cells with p < 0.05.

Support Vector Machine Cell Classification

Table 2. SVM Classification results CD4+ and CD8+ (single parameter)

Epoch	A_{tra} and A (%) for each kernel					
	Linear		RBF		Sigmoid	
	Training	Test	Training	Test	Training	Test
1	96.67	37.29	95	32.20	93.33	37.29
2	66.67	79.66	65	30.51	65	49.15
3	73.33	59.32	73.33	61.02	70	50.85

Number CD4+ training data = 30, Number of CD4+ testing data = 12, Total number of CD4+ = 42 Number CD8+ training data = 30, Number of CD8+ testing data = 47, Total number of CD8+ = 77

Summary

- Research plan
  - Quantitative study of lymphocyte morphology through confocal imaging and 3D reconstruction
  - Acquisition of Cross-Polarized Diffraction Images to investigate classification
  - Apply SVM algorithm for Cell Classification with image data
- Goal
  - to develop label-free “fingerprint” for rapid and morphology based assay of human lymphocytes.

References

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