Force-Driven Mechano-Typing of B Cells: Multi-Channel Image Analysis of CD40-CD40L Interactions Using AutoEncoder

연세대학교 기계 생물학 연구실
Mechanobiology Laboratory

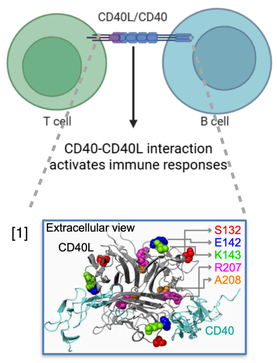
지도교수: 최현규 교수님 담당조교 : 전예림 조교님
2020162029 생화학과 박성현

1. Former Research and Limitation

- Focus on **TCR** mechanics - Previous work has concentrated on T cells. CD40-CD40L mechanics in B cells remain largely uncharacterized.
- **Hand-crafted metrics** only - Analyses have relied on simple features such as spreading area or mean intensity, ignoring rich, non-linear patterns hidden in images.
- **Limited AI prediction** - Although a few studies have linked mutations to force, none have used AI to build predictive models.

2. Objective : Force-Driven Mechano-Typing of B cells

CD40 - CD40L Interaction



- **CD40 - CD40L interaction** plays a critical roles in B cell activation, adhesion, and immune function. Upon binding of CD40L to CD40 on B cells, downstream pathways are activated, leading to proliferation and enhanced cell-substrate adhesion

- **Mutation in CD40L** (e.g. K143A, A208D) disrupt this interaction, reducing both biochemical signaling and mechanical engagement.

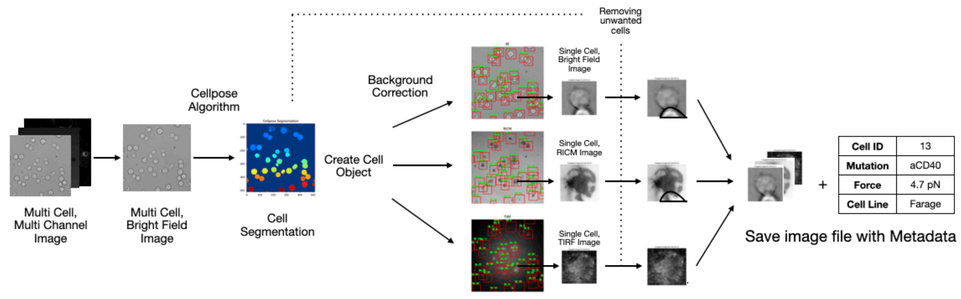
We combine multi-threshold **MTP(Molecular Tension Probe)** sensors, three-channel microscopy (**DIC, RICM, TIRF**), and a deep autoencoder that compresses each cell into a 32-dimensional biophysical features. By aligning these features with the applied force magnitudes and CD40L mutation states, we seek to elucidate how mechanical tension and mutation jointly shape B-cell morphology and mechanics.

[1] Hyun-Kyu Choi et al.,Mechanotransduction governs CD40 function and underlies X-linked hyper-IgM syndrome.Sci. Adv.10, eadl5815(2024)

3. Data Analysis Flow

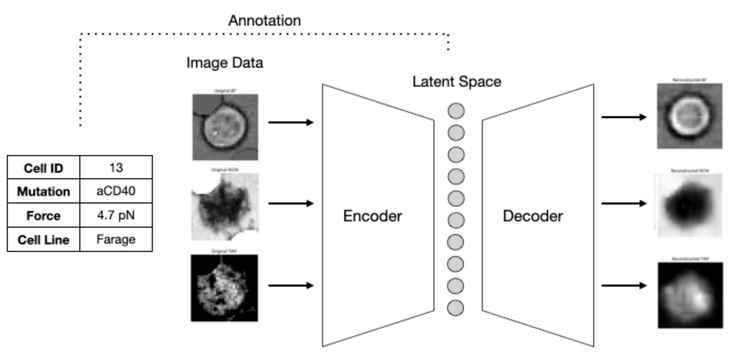
Single-Cell Image Data Extraction

Automatically segment multi-channel microscopy images to extract individual single-cell images.



AutoEncoder

Inferring **Biophysical patterns** from single-cell image through image reconstruction



For every sample data, the intrinsic dimension was computed via Maximum Likelihood Estimation using the log-ratio of distances to its nearest neighbors.

$$\hat{d}(\mathbf{x}) = \left[\frac{1}{k-1}\sum_{i=1}^{k-1}\log\left(\frac{r_k}{r_i}\right)\right]^{-1}$$

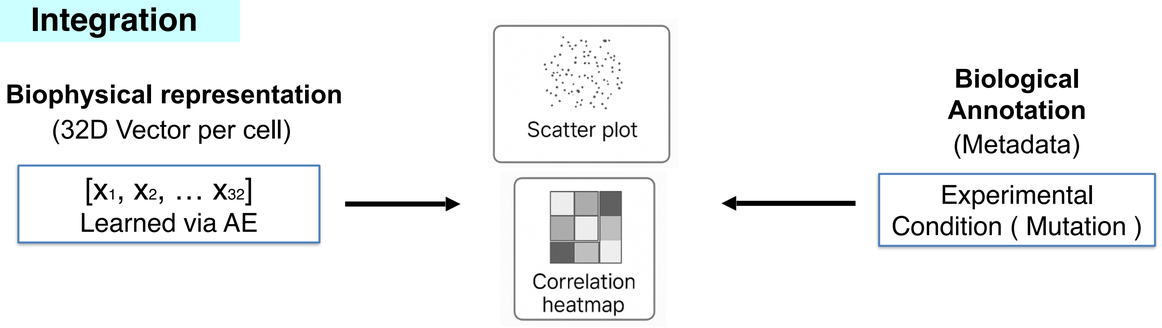
The global intrinsic dimension was then obtained by averaging over all local estimates.

$$\hat{d}_{\text{global}} = \frac{1}{N}\sum_{j=1}^{N}\hat{d}(\mathbf{x}_j)$$

To capture the local biophysical complexity of each cell, we estimated its **intrinsic dimension** based on the spatial distribution of its nearest neighbors in the latent space.

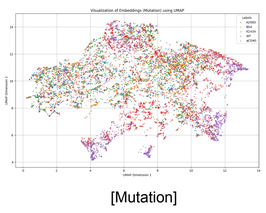
Based on the estimated global intrinsic dimension of 31.3, the latent space was set to 32 dimensions. This allowed the our model to encode each single cell data into a **32-dimensional numerical representation** that reflects its underlying **biophysical patterns**!

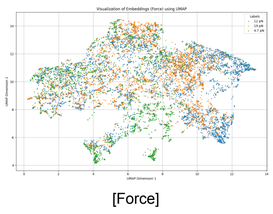
Integration

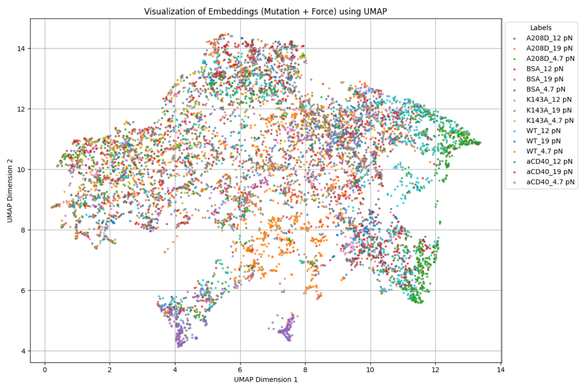


4. Result

Global Biophysical Landscape



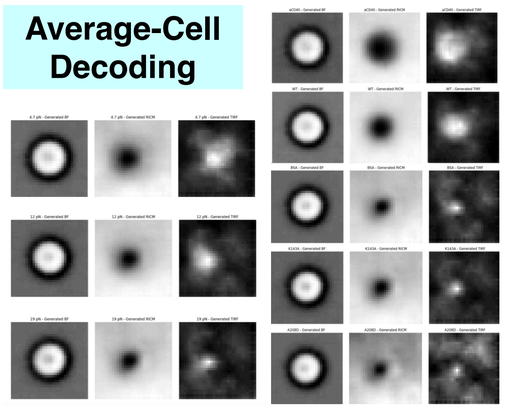




We constructed a analysis platform that learns and clusters **mechano-sensing states** of single cells.

This system enables prediction based on UMAP-embedded biophysical vectors, revealing how B cells adapt to mechanical stimuli.

Average-Cell Decoding

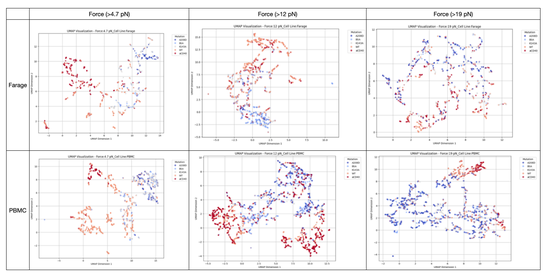


[Force] [Mutation]

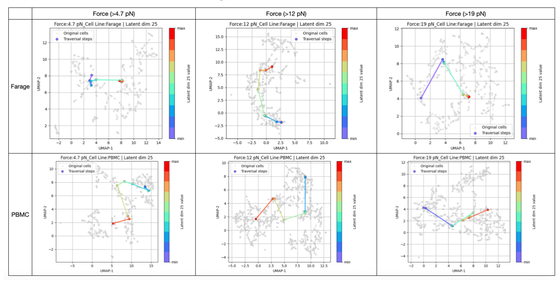
The average 32-D biophysical vector for each condition(Threshold Force, Mutation) was decoded to generate representative **"Average-Cell" images.**

- **Threshold Force** : [>4.7pN] > [>12pN] > [>19pN]
- **Mutation** : [aCD40] > [WT] > [BSA] = [K143A] = [A208D]

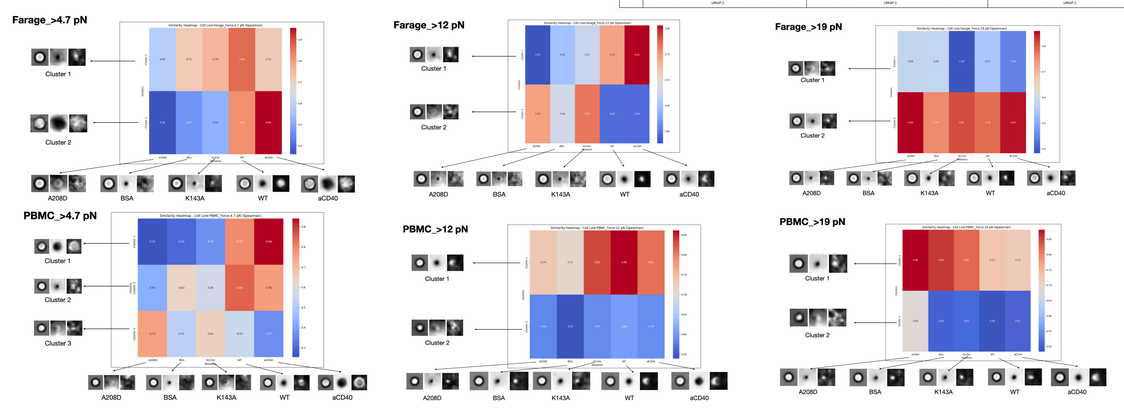
Trajectory Plot (UMAP Visualization)



Changing of biophysical vector dimension 25 induced concurrent modulation of **RICM adhesion and TIRF fluorescence intensity!**



Cell Correlation Heatmap Analysis



(1) Applied **Clustering** method to the 32-D biophysical vectors - no metadata used

(2) Decoded the mean biophysical vector of each cluster to produce **cluster prototypes**, and decoded the mean biophysical vector of each true mutation group to produce **mutation prototypes**

(3) **Pixel-wise difference** between cluster and mutation prototypes (Red = high similarity, Blue = Low Similarity)

5. Further Study

- **Predictive Modeling** for Unknown Mutation : Utilize latent-space embeddings derived from AutoEncoder Analyses to train classifiers, enabling rapid evaluation of newly discovered mutations to determine.
- **Temporal** Dynamics of mechano-types : Expand current static latent-space analysis into longitudinal studies to track how B-Cell morphology, adhesion strength, and receptor-ligand interactions evolve over time under mechanical forces.
- **Generalizable Framework** for Image-Based Mechanobiology : Leverage the established Autoencoder and clustering methodologies to provide a broadly applicable analytical framework for diverse biological systems.

6. Reference

[1] Hyun-Kyu Choi et al.,Mechanotransduction governs CD40 function and underlies X-linked hyper-IgM syndrome.Sci. Adv.10, eadl5815(2024)

[2] Liu B, Chen W, Evavold BD, Zhu C. "Ac-cumulation of dynamic catch bonds between TCR and agonist peptide-MHC triggers T cell signaling". Cell. 2014 Apr 10;157(2):357-368

[3] Carsen Stringer, Tim Wang, Michalismichaelos and Marius Pachitariu "Cell-pose: a generalist algorithm for cellularsegmentation", Nature Methods 18, 100–106(2021)

[4] Y. Liu, L. Blanchfield, V.P. Ma, R. Andar-gachew, K. Galior, Z. Liu, B. Evavold, andK.Salaita,"DNA-basednanoparticletensionsensors reveal that T-cell receptors trans-mit defined pN forces to their antigens for enhanced fidelity", Proc. Natl. Acad. Sci. U.S.A. 113 (20) 5610-5615(2016).