Lattice light-sheet microscopy Multi-Dimensional Analyses (LaMDA) of T-cell receptor dynamics predict T-cell signaling states

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Abstract

The methods of collecting, analyzing, and understanding the dynamics of cell surface receptors with single-cell imaging currently lag behind those for other high-dimensional analyses, such as single-cell omics assays. Here we report the development of lattice light-sheet microscopy multi-dimensional analyses (LaMDA), a pipeline that combines high spatiotemporal-resolution four-dimensional lattice light-sheet microscopy, machine learning, and diffusion maps to analyze T-cell receptor (TCR) dynamics and predict T-cell signaling states without the need for complex biochemical measurements. LaMDA images thousands of TCR microclusters on the surface of live primary cells to collect high-dimensional dynamic data for machine learning, which extracts key dynamic features to build predictive diffusion maps. LaMDA spatiotemporally reveals global changes of TCRs across the 3D cell surface, accurately differentiates stimulated cells from unstimulated cells, precisely predicts attenuated T-cell signaling after CD4 and CD28 receptor blockades, and reliably discriminates between structurally similar TCR ligands. We anticipate broad usage of this approach for other receptors and cells, as well as for guiding the design and development of future immunotherapies for cancer, infection, and autoimmunity.

LaMDA Workflow Overview

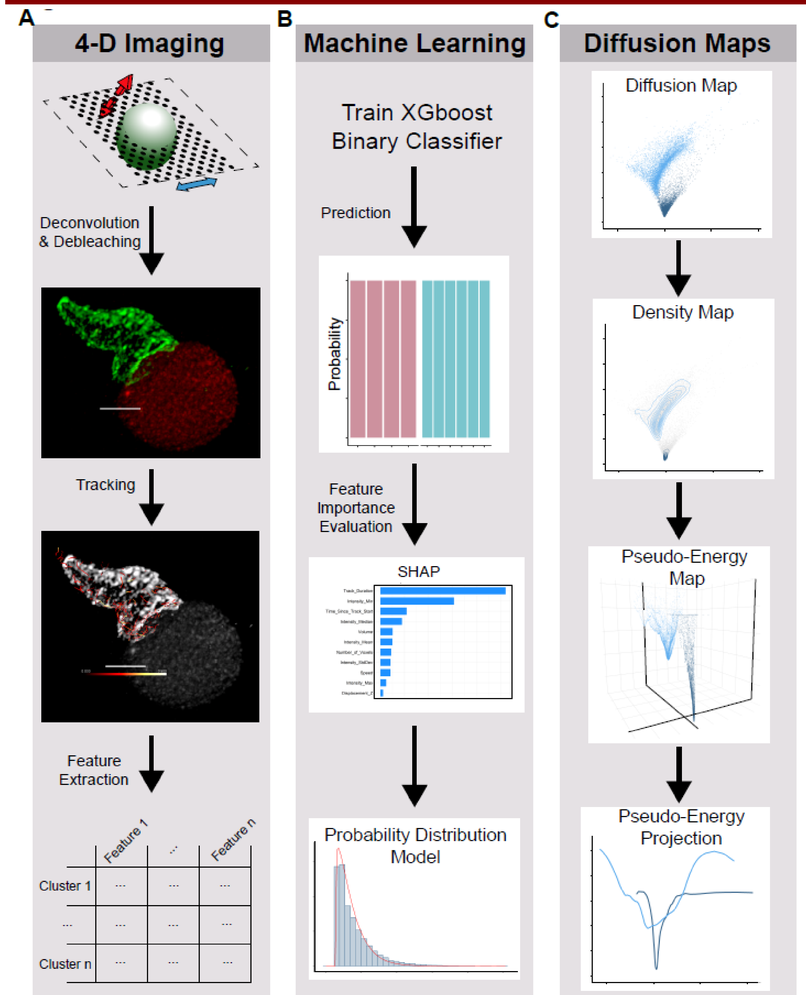
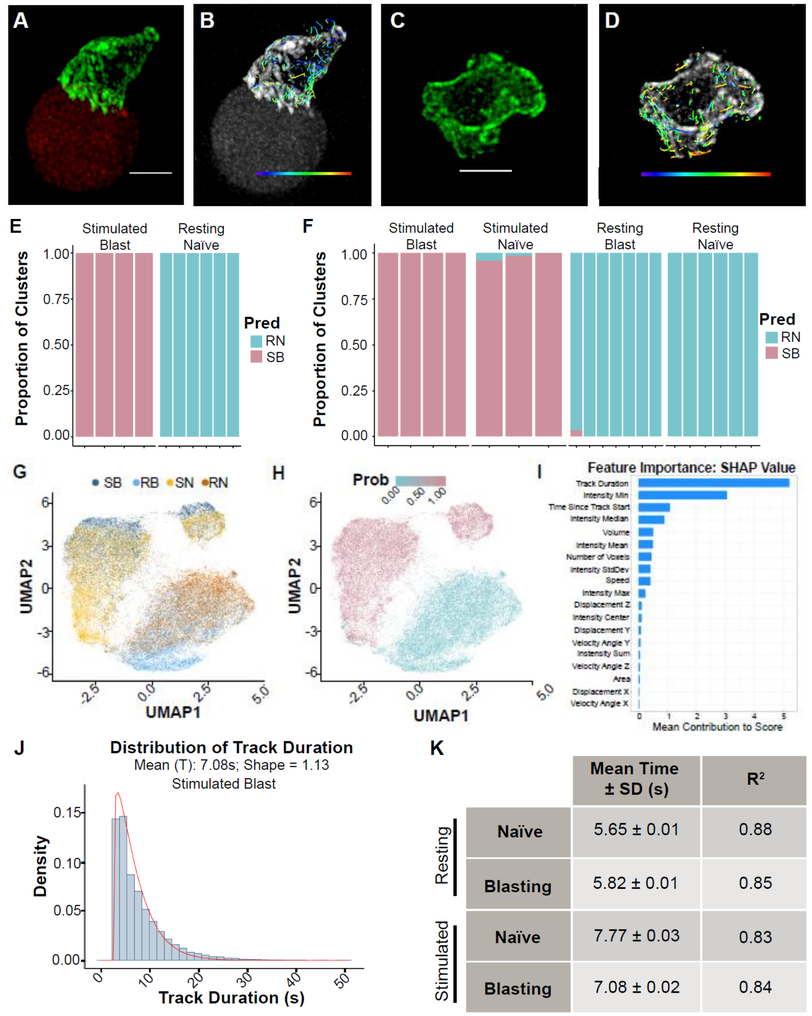


Figure 1:LaMDA Workflow. A. 4-D imaging is conducted with lattice light-sheet microscopy (LLSM). **B.** Machine learning is conducted on extracted features for prediction. **C.** Diffusion maps are used as a dimensionality reduction technique to further quantify variations among cell states.

Machine Learning Differentiate Signaling States



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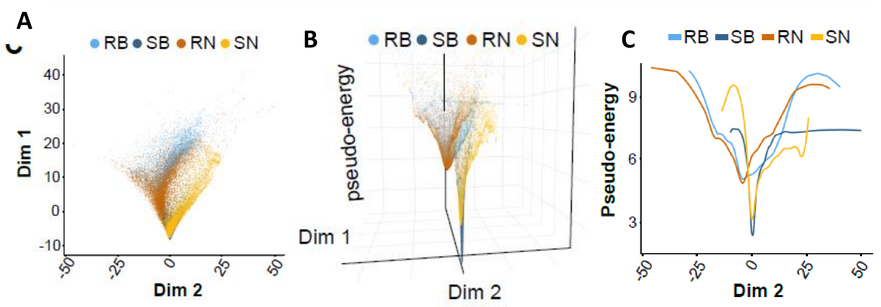
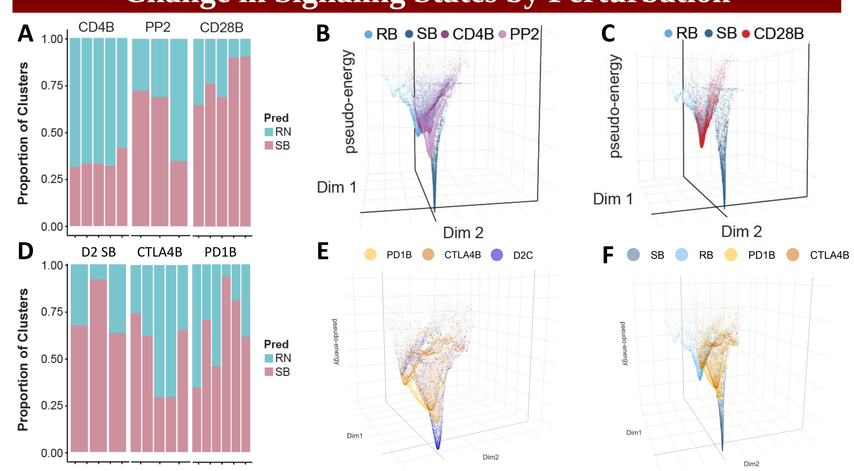
		Mean Time ± SD (s)	R^2
Resting	Naïve	5.65 ± 0.01	0.88
	Blasting	5.82 ± 0.01	0.85
Stimulated	Naïve	7.77 ± 0.03	0.83
	Blasting	7.08 ± 0.02	0.84

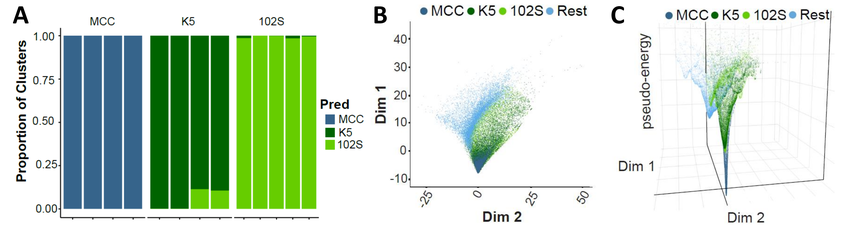
Diffusion Map and Energy Landscape



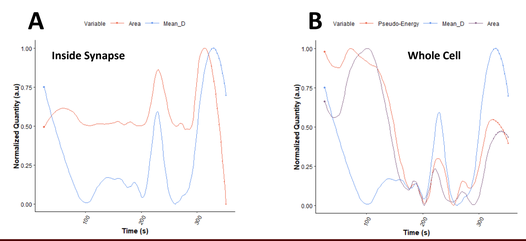
Change in Signaling States by Perturbation



Ligand Discrimination



Dynamic Control of Clusters



Conclusions

- Machine Learning can be used to distinguish between different activation states of T cells
- Resting cells and synapse forming cells have drastically different TCR organization and characterization
- Disrupting TCR signaling shifts the TCR structures towards resting configuration even during synapse formation
- Similar ligands but different affinities have similar signaling states but different cluster stability
- TCR cluster conformations are dynamically regulated by TCR signaling

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