# Project 4: Learning 3D Morphological Programmes of T Cell Activation

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**Project summary:** T cell activation is accompanied by extensive reorganisation of cell architecture, including changes in nuclear morphology, cytoskeletal organisation, organelle positioning, and cell-cell interactions. While transcriptomic programmes underlying activation have been studied in great detail, the corresponding three-dimensional morphological changes remain poorly characterised. Most high-content imaging analyses rely on two-dimensional projections, discarding volumetric information that may contain important signatures of immune cell function. Consequently, we currently lack computational methods capable of extracting, interpreting, and organising 3D cellular phenotypes at scale.

To address this challenge, the Trynka Group has developed Tglow, a high-throughput imaging platform that captures rich volumetric images of primary human T cells throughout activation. By combining brightfield imaging with multi-channel 3D fluorescence microscopy, Tglow provides an unprecedented view of the spatial organisation of immune cells over time. In parallel, ongoing CRISPR perturbation experiments generate genetically perturbed activation trajectories, enabling direct investigation of the molecular regulators that drive specific morphological programmes.

This project will develop computational approaches to discover and interpret three-dimensional morphological phenotypes from these unique datasets. Rather than relying on conventional 2D projections, we will analyse complete volumetric image stacks to quantify features that are only visible in three dimensions, including nuclear architecture, cytoskeletal organisation, organelle positioning, and spatial relationships between intracellular structures. We will compare classical image-derived measurements with modern deep learning approaches that learn representations directly from volumetric data, determining which best capture biologically meaningful variation.

The resulting 3D representations will be used to identify activation trajectories, quantify the effects of genetic perturbations, and determine whether volumetric morphology provides improved sensitivity and interpretability compared with conventional 2D analyses. By linking morphological programmes to activation state and genetic perturbations, the project will establish a scalable framework for studying immune cell behaviour through high-dimensional imaging.

The Trynka lab contributes world-leading expertise in human immunology, functional genomics, CRISPR screening, and large-scale imaging of primary T cells, together with access to unique longitudinal and perturbation datasets. The Ewald lab contributes expertise in machine learning for biological imaging, including high-dimensional image representations, deep learning, and interpretable computational analysis of cellular morphology. Together, this new collaboration provides an exceptional environment for developing computational methods at the interface of artificial intelligence and immunology.

**Scientific & professional training:** At the Sanger Institute, the Fellow will receive training in primary human T cell biology, high-content volumetric microscopy, CRISPR-based perturbation experiments, and large-scale image generation and quality control. At EMBL-EBI, they will develop expertise in computational image analysis, three-dimensional image processing, machine learning, representation learning, and scalable analysis of high-dimensional imaging datasets. The Fellow will participate in joint supervision, lab meetings, seminars, and conferences across both institutes, contributing to collaborative publications, open-source software, and interdisciplinary research at the interface of computational biology and immunology.

**Potential for new research directions:** The computational framework developed during this fellowship will establish a foundation for analysing volumetric cellular phenotypes across many biological systems beyond T cells. The methods could be applied to other immune cell types, organoids, developmental systems, and high-content drug screening datasets where three-dimensional morphology is increasingly being captured but remains underutilised. More broadly, the project will help establish 3D phenotyping as a new computational modality for linking cellular architecture to gene function, disease mechanisms, and therapeutic response.