APMC13 BRISBANE 2025
13th Asia Pacific Microscopy Congress
Brisbane, Australia, February 2-7, 2025

Leveraging the Rich Spatiotemporal Features of Lattice Light-sheet Microscopy with Machine Learning and AI

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Live-cell imaging allows scientists to observe the dynamics of living cells across time. Lattice Light-sheet (LLS) microscopy is one such method that captures these processes at high spatiotemporal detail in 4D. LLS enables us to observe previously unknown events, however, the large data size, specialized processing needs, and the complexity of the feature rich datasets pose significant challenges for maximizing the utility of this technology.

To this end, we developed napari-lattice, a python plugin within napari, an n-dimensional viewer that streamlines LLS analysis. It enables users to extract specific regions of interest within LLS data without processing the entire volume. Furthermore, napari-lattice integrates seamlessly with standard image analysis pipelines, enabling segmentation and feature extraction in a single end to end workflow.

We applied the napari-lattice workflow to live-cell imaging of Neutrophil extracellular trap (NET) formation, a form of programmed cell death exhibiting dynamic changes in cell shape, topology and nuclear DNA conformation, as multilobular nuclei decondense and DNA is extruded extracellularly. Using primary human neutrophils, we study how cells from different donors behave under various NET-inducing stimuli. To enable this, we developed an end-to-end workflow that extracts morphological information in 2D and 3D for live cells over time, which is modular and scalable. Traditionally, time series data is summarized using basic statistics such as mean, maximum, number of peaks and area under the curve. However, this approach fails to capture the full complexity and dynamics of the temporal changes. We address this limitation by using tsfresh, a python package that computes multiple statistical properties to summarize temporal changes.

By combining these rich features with supervised machine learning and explainable AI we can identify specific morphological feature changes and their combinations that distinguish NETosis induced by different stimuli. This approach enables data-driven hypothesis generation, allowing users to infer the distinguishing spatiotemporal events underlying distinct biological processes.

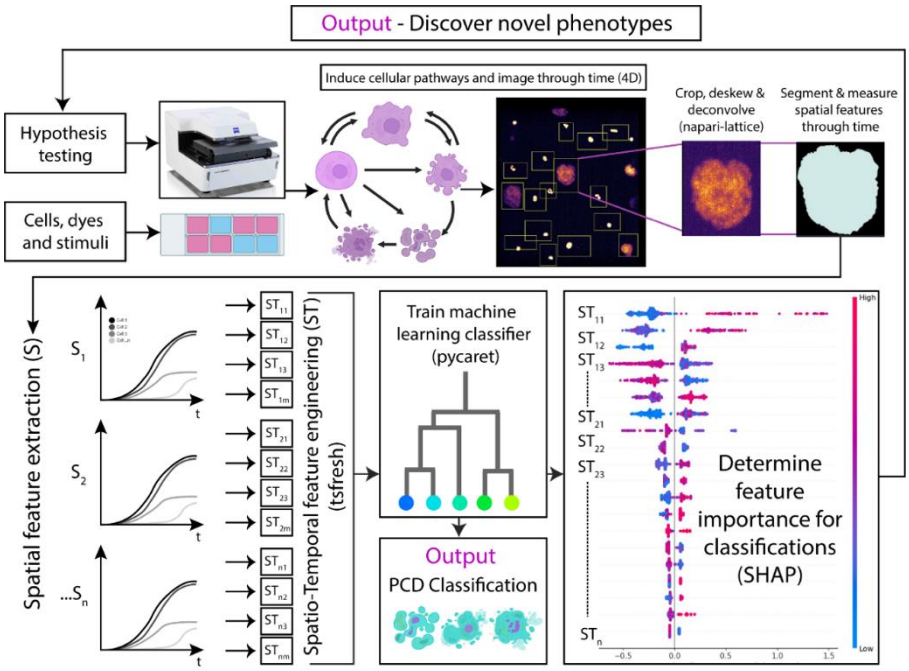

Fig. 1. Live cell imaging is performed on cells in parallel under multiple conditions in 4D using LLS microscopy. Using napari-lattice, LLS specific processing is applied to extract cells. Using python packages such as, cellprofiler and scikit-image, an exhaustive list of morphological, topological and textural image features are extracted through time. These timeseries data are processed using a python package, tsfresh to create multiple temporal features for each extracted image features. The spatiotemporal features are subsequently used to train and evaluate a machine learning classifier (tree-based) for each condition. Using feature importance and shapely values, the top features are identified, which can be used to identify biologically relevant phenotypes under different conditions