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## Polarity-JaM: An image analysis toolbox for cell polarity, junction and morphology quantification

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Cell polarity involves the asymmetric distribution of cellular components such as signaling molecules and organelles within a cell, asymmetries of a cell's shape as well as contacts with neighbouring cells. Gradients and mechanical forces often act as global cues that bias cell polarity and orientation, and polarity is coordinated by communication between adjacent cells.

Advances in fluorescence microscopy combined with deep learning algorithms for image segmentation open up a wealth of possibilities to understand cell polarity behaviour in health and disease. We have therefore developed the open-source package Polarity-JaM, which offers versatile methods for performing reproducible exploratory image analysis. Multi-channel single cell segmentation is performed using a flexible and user-friendly interface to state-of-the-art deep learning algorithms. Interpretable single-cell features are automatically extracted, including cell and organelle orientation, cell-cell contact morphology, signaling molecule gradients, as well as collective orientation, tissue-wide size and shape variation. Circular statistics of cell polarity, including polarity indices, confidence intervals, and circular correlation analysis, can be computed using our web application. We have developed data graphs for comprehensive visualisation of key statistical measures and suggest the use of an adapted polarity index when the expected polarisation direction or the direction of a global cue is known a priori.

The focus of our analysis is on fluorescence image data from endothelial cells (ECs) and their polarisation behaviour. ECs line the inside of blood vessels and are essential for vessel formation and repair, as well as for various cardiovascular diseases, cancer, and inflammation. However, the general architecture of the software will allow it to be applied to other cell types and image modalities. The Polarity-JaM package integrates these analyses in a reproducible manner with a level of documentation that allows the user to analyse image data accurately and efficiently, see <https://polarityjam.readthedocs.io>. In addition to a Python-API, we provide a Napari plugin with a graphical user interface and a Web-App for statistical analysis [www.polarityjam.com](http://www.polarityjam.com).

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