**Computer vision profiling of neurite outgrowth morphodynamic phenotypes**

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**Abstract** (150 words)

Neurite outgrowth is a dynamic cell behavior that consists of morphogenetic processes such as neurite initiation, protrusion-retraction cycles, branching and growth cone navigation. Current knowledge of the underlying signaling events stem from molecular perturbations assessed at the steady state, which cannot capture the dynamic nature of this complex process. Here, we present NeuroDynamics an integrated pipeline to study neurite outgrowth dynamics. A microscopy platform allows for high content imaging of neurite outgrowth dynamics. A computer vision approach allows segmentation of cell shape and extraction of features describing neuronal morphodynamics. Statistical analysis then allows to automatically identify features that significantly discriminate between the control and perturbed state. We demonstrate the applicability of our approach by automatically annotating morphodynamic phenotypes in an RNA interference screen targeting a candidate Rho GTPase signaling network identified by a proteomics approach. NeuroDynamics is freely available as open source software to study neuronal outgrowth dynamics.

* You are welcome to add some technical jargon given that we remain in the 150 words limit !
* We can also think of another acronym than Neurodynamics !

**Introduction**

Neurite outgrowth is essential to build the neuronal processes that connect the adult brain. This morphogenetic process is highly dynamic, and consists of a series of stochastic and repetitive events such as neurite initiation, elongation, branching, growth cone motility and collapse. Each of these different morphogenetic behaviors are likely to be regulated by different signaling networks. Furthermore, all these processes occur on length and time scales of microns and minutes to hours, suggesting an exquisite spatio-temporal regulation of the underlying regulating signals. This is not accessible with approaches in which the effects of molecular perturbations are assessed at the steady-state, in which only snapshots of intrinsically dynamic behaviors are captured. Rather, understanding the signaling networks that regulate this complex process might benefit from understanding the effect of molecular perturbations on its dynamics. Advances in automated live cell microscopy now allows for high content image acquisition with high temporal resolution. However, until now, most computer vision approaches have been used to extract image features from cell populations at the steady-state, rather than from dynamic time-lapse datasets. In one study, the temporal context in time-lapse datasets has been taken into account to improve classification of transiently occurring phenotypes, allowing to deconvolve functional states with similar morphology[1](#_ENREF_1).

but no approaches have directly tried to automatically extract information about dynamic cellular features to classify phenotypes.

Different steps to describe in results

1. explain screen and rationale for cell labeling

2. explain microscope acquisition pipeline, explain the need of two resolutions

3. explain computer vision pipeline

4. validation of computer vision pipeline by comparison with ground truth

5. describe feature extraction process: morphological and morphodynamic features

6. describe feature selection algorithms

7. validation of feature selection algorithm by mixing data from two perturb states

8. explain approaches to take into account siRNA noise

9. description of siRNA screen and discussion of biology

**Figures**

**Figure 1.** Global pipeline to analyze neurite outgrowth morphodynamic phenotypes. (olivier and Ludo)

**Figure 2.** Computer vision segmentation of neuronal morphodynamics feature extraction.

(Fethallah and Kevin)

Basically a scheme that describes the different steps in segmenting of the soma, neurites, ...

**Figure 3.** Description of morphodynamic features.

(Fethallah and Kevin)

basically a scheme that summarizes all the different dynamic features that are extracted

**Figure 4.** Morphodynamic phenotype feature selection

(Riwal)

try to make a series of schemes that explain the different steps in feature selection,

vector distance, assessment of interplate and siRNA induced noise.

**Figure 5.** Morphodynamic phenotype description.

(Riwal, Olivier, Ludo)

this will consist of a color-coded map of the different features extracted for each gene perturbation, we will then focus on more specific aspects of what we learned.

**Supplementary Figures.**

**Figure S1.** Experimental controls for lifeact-GFP fluorescent reporter, experimental control for knockdown efficiency by a selected number of siRNAs. (olivier and Ludo)

**Figure S2.** Comparison of ground truth and computer vision segmented data. (Fethallah and Kevin)

**Figure S3.** Feature selection on synthetic data produced by mixing videos from different known sources to test the ability of the algorithm to appropriately identify different morphodynamic signatures (Riwal)

**Supplementary tables.**

**Table S1.** Definition of features. him

1. M. Held, M. H. Schmitz, B. Fischer et al., *Nature methods* **7** (9), 747 (2010).