**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 !

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, from the analysis side, most computer vision approaches have until now only been used to automatically extract image features from cell populations at the steady-state[1](#_ENREF_1),[2](#_ENREF_2), rather than from dynamic time-lapse datasets. In one study about mitosis, the temporal context in time-lapse datasets has been taken into account to improve classification of functional cellular states by deconvolving ambiguous, transiently occurring phenotypes with similar morphology[3](#_ENREF_3). In another perturbation screen about cell scattering, global dynamic features such as cell migration speed have been measured[4](#_ENREF_4). However, no approaches have directly tried to automatically extract information about multiple dynamic cellular features to classify phenotypes.

Here, we present Neurodynamics, an integrated pipeline that allows to study dynamic neurite outgrowth phenotypes in response to molecular perturbations. Neurodynamics consists of three entities: 1. a high content imaging platform for neurite outgrowth dynamics, 2. an automated neuronal shape segmentation algorithm that extracts morphological and morphodynamics features, 3. a feature selection algorithm that allows automatic inference of the neurite outgrowth morphodynamic phenotype. A scheme depicting the global approach is shown in Fig.1. We demonstrate the efficiency of our approach to study a variety of molecular perturbations.

**Results**

**High content imaging of neurite outgrowth dynamics**

To study neurite outgrowth dynamics, we used the mouse N1E-115 cell neuroblastoma cell line, that can be easily triggered to extend neurites by simple serum starvation and plating on the extracellular matrix protein laminin. To visualize soma and neurite morphology, we used a fusion of green fluorescent protein (GFP) with the F-actin binding peptide lifeact[5](#_ENREF_5" \o "Riedl, 2008 #5). This provided high contrast on neurites and soma without excessive accumulation of fluorescence in the thick somata of differentiated N1E-115 cells, allowing for adequate imaging in N1E-115 cells by wide field microscopy (Figure 2a). For unambiguous cell identification, we also simultaneously labeled the nucleus with a mCherry fusion with a nucleus localization sequence (NLS), expressed from the same expression vector than Lifeact-GFP (Supplementary Fig.1a). We observed that transient expression of this construct did not affect neurite outgrowth in N1E-115 cells (Supplementary Fig.1b). To perturb different signaling pathways, we optimized a method to co-transfect the fluorescent marker and siRNAs simultaneously in cells, and observed efficient knockdown in response to knockdown of a panels of mRNAs (Supplementary note 1). To perform high content live cell imaging, we optimized our microscope for fast acquisition of multiple wells of a 24 well plate (Supplementary note 2). This allowed to acquire 240 fields of view in two channels across a 24-well plate with 12 minute time resolution for 20 hours. We performed different sets of experiments with 10 and 20x air objectives. Using 10 x objectives allowed for aquisition of a large field of view with typically 20 objects that could be consistently be observed for long time periods. The 20x objective allowed high resolution imaging of morphological features such as filopodia, but the moving cells often migrated out of the field of view resulting in loss of these objects. Typical 10x and 20x movies are shown (Supplementary movie 1 and 2). In these experiments, we tracked neuronal morphodynamics of differentiated cells that were replated on laminin, allowing to observe almost the whole neurite outgrowth process. We observed that illumination of neurons in the early phase of neurite outgrowth was toxic to the cells, leading to their death. We found that starting the observation process 3 hours post-plating, a time at which cells also had extended 3-4 neurites, allowed the cells to survive until the end of the imaging process.

Describe verbally the neurite outgrowth process here ?

**Automated computer vision analysis of neurite ougrowth dynamics**

To capture the dynamic neurite outgrowth trajectories of neurons in the native and perturbed state, we developed a computer vision pipeline that allowed to automatically segment the soma and neurites in each frame of the timelapse datasets.

Fethallah and Kevin can you please write a 10 line long, accessible paragraph that explains the segmentation procedure and the quantification of its efficiency in comparison with the manually annotated ground truth. We can add a supplementary note (Supplementary note 3) that describes the process in depth.

Can you please also sketch figure 2 that is dedicated to the segmentation process. This figure will show:

1. raw green and red images (it is the 1st figure in which we will show this).

2. schematics of successive steps in the image segmentation.

3. A fully segmented time series of neurons as an example.

4. Some kind of efficiency evaluation in comparison with the manually annotated ground truth.

Please then write another paragraph that explains the different static and dynamic features that are extracted.

Please sketch figure 3, which explains graphically how some static and dynamic features are extracted. You can represent some of the trajectories of one or two features over time. For this, you can use one of the presentation that Kevin once made. I would try to show a graph of a feature trajectory that is stochastic like the protrusion/retraction cycles of neurite outgrowth. This shows that we look at highly dynamic events, and thus illustrates the benefit of our approach. We can add a number of supplementary movies with different morphological features that are analyzed (soma, each new neurites). Please prepare also supplementary tables about the definition of features (supp tables 1 and 2). Then we also need a supplementary note that explain the format in which the morphodynamic history of each cell is explained.

1. describe feature selection algorithms

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

3. explain approaches to take into account siRNA noise

**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)

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

(Fethallah and Kevin)

**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 lifeact-GFP/NLS-mCherry reporter and siRNA transfection.

(**a**) Structure of the Lifeact-GFP IRES NLS-mCherry expression vector. (**b**) Effect of Lifeact-GFP expression on neurite outgrowth.

(ludo and olivier)

**Figure S2.** 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 static features.

**Table S2.** Definition of dynamic features.

**Supplementary notes**

**Supplementary note 1.** Description of RNA interference experiments.

Olivier and ludo

**Supplementary note 2.** Description of microscope setup used for high content acquisition.

Olivier and ludo

**Supplementary note 3.** In depth description of computer vision segmentation and evaluation of the method by comparison with ground truth.

Fethallah and Kevin

**Supplementary note 4.** Description of html format in which the cell trajectories are described

Fethallah and Kevin

**Supplementary movies.**

**Supplementary movie 1.** Representative timelapse movie of N1E-115 cells expressing the GFP-lifeact-IRES-NLS-mcherry construct with the 10x PlanApo objective.

**Supplementary movie 2.** Representative timelapse movie of N1E-115 cells expressing the GFP-lifeact-IRES-NLS-mcherry construct with the 20x PlanApo objective.

**Supplementary movie 3.**

**References**

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2. C. Collinet, M. Stoter, C. R. Bradshaw et al., *Nature* **464** (7286), 243 (2010).

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

4. D. Loerke, Q. le Duc, I. Blonk et al., *Science signaling* **5** (231), rs5 (2012).

5. J. Riedl, A. H. Crevenna, K. Kessenbrock et al., *Nature methods* **5** (7), 605 (2008).