

# Semi-supervised learning for phenotypic profiling of high-content screens (DRAFT)

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## Abstract

Semi-supervised machine learning techniques are particularly useful in experiments where data annotation and classification is time- and resource-consuming or error-prone. In biological experiments this is often the case. Here, we apply a graph-based machine learning method to classify cells in different stages of infection with the Semliki Forest Virus (SFV), which features have been extracted from image analysis of fluorescence microscopy results, obtained in turn from a genome-wide high-content screening experiment. The aim of this project is to investigate whether and to which extent intelligent control experiment design combined with semi-supervised learning can reach the accuracy of a human annotator and/or in certain cases substitute it.

## Introduction

Recent advancements in high-throughput microscopy and data analysis made possible to perform large scale biological experiments and automatically evaluate them. For the detection of sub-cellular changes caused by different perturbations in the cell (RNAi or drugs), often supervised machine learning (SML) is used. Reliable training of an SML method, however, requires significant effort from a field expert.

As an alternative, semi-supervised machine learning (SSL) methods make use of information intrinsically found in the entire data, both annotated and unannotated, thus allowing to make use of a larger amount of information by exploiting, alongside with the annotated data, the relative distribution of unannotated data on the feature space<sup>[1]</sup>. Furthermore, common sources of error in manually-annotated data (subjective data interpretation by the annotator, instrument capabilities and calibration, noise, ...) can affect the quality of the annotations, and in some cases SSL techniques can account for and correct such misannotations<sup>[2]</sup>.

The semi-supervised learning paradigm, under a few assumptions<sup>1</sup>, has proven valuable in exploring and classifying biological data in fields as diverse as drug-protein interactions<sup>[3]</sup>, gene expression<sup>[4]</sup>, and medical diagnosis<sup>[5]</sup>.

## Materials and Methods

### High-content screening

A human genome-wide siRNA library was used to produce human cell cultures with knocked-out genes, stored in a collection of 55 384-well plates spanning the entire genome. These cell cultures were exposed to a genetically engineered fluorescent SFV strand as a transduction vehicle for green fluorescent protein (GFP) genes, and the corresponding GFP production on all of the cultures was tracked over time. 3 repetitions of this experiment were carried out.

The protein expression was stopped at  $t$  after culture infection with SFV, for all siRNA-mediated phenotypes, and microscopic pictures of the sample were obtained under a light microscope. Wild-type cultures were also exposed to SFV, and protein expression was stopped at 4, 5, 6, and 7 hours after exposure and analyzed as negative control experiments, in the exact same manner as for the silenced samples. A control sample set for non-exposure to the virus was also collected and analyzed in the same way as described above.

<sup>1</sup>Smoothness, Cluster, and Manifold assumptions, see <sup>[1]</sup> p. 4-6

## Image acquisition and analysis

For every sample at each infection stage, 9 tiled images were captured via a light microscope, by composing the green fluorescent signal of the produced protein, and a blue-colored image of the nuclei.

Image segmentation of all microscopic pictures to identify individual cells, and the subsequent feature extraction per identified cell, was done with CellProfiler<sup>[6]</sup>. A total of 93 features were retrieved and used in this experiment, corresponding to color intensity, area, shape, and texture descriptors. (For the complete list of features extracted, see Appendix A)

## Unannotated data

The above process was performed automatically on the 380.160 images (55 plates  $\times$  384 wells  $\times$  9 sites  $\times$  2 channels) retrieved from the siRNA-mediated phenotypes. All 93 features<sup>2</sup> were extracted as floating point values, and stored in text files, one line per cell. Unlabeled information for around 100.000.000 cells was collected by this process.

## Annotated data

From the genome-wide information, a small subset of the data was manually annotated by an expert on SFV infection, by visually identifying cell phenotypes directly from the segmented microscopic images and cross-checking with the time annotation on the respective source plate, and classifying them into the different stages of infection. This manual point-and-click process yielded 3098 annotated cells.<sup>3</sup>

software  
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for this?

## Control data

As control cultures, 5 plates treated with a control siRNA that has no effect on the expressed phenotype of the cells were used. Control plates not exposed to SFV, as well as infected plates analyzed at 4, 5, 6, and 7 hours after infection, were used to retrieve control information during the time course. The exact same image analysis and feature extraction procedure described for the unannotated cultures was performed on these plates.

## Semi-supervised learning implementation

A graph-based label propagation (label spreading<sup>[7]</sup>) approach was followed. In this kind of approach, an undirected graph is built using the data points (cells) as vertices, and edges are created for all pairs of vertices that satisfy a neighboring condition, with weights proportional to some measurement of association between the pair of vertices. This degree of association is often assumed as related to the distance between the data points in the  $n$ -dimensional feature space, in a linear, exponential, or Gaussian fashion, among others, and can be either limited in space ( $k$ -nearest neighbors, cutoff distance, ...), or consist of a complete graph that considers all possible pairwise relationships.

In the original formulation, a vector of initial labels  $\hat{Y}^{(0)}$  is created by assigning the actual labels to the vertices corresponding to annotated data, and neutral labels to the unannotated data; then, in an iterative fashion, described in equation 1, all vertices *learn* the labels from their neighbors at a rate  $\alpha$  (and preserve their initial labeling at a rate  $1 - \alpha$ ). This process is repeated until convergence to a  $\hat{Y}^{(\infty)}$ .

$$\hat{Y}^{(t+1)} \leftarrow \alpha \mathcal{L} \hat{Y}^{(t)} + (1 - \alpha) \hat{Y}^{(0)} \quad (1)$$

The *laplacian* term controlling the particular strength of label spreading between any two data points is given by  $\mathcal{L} \leftarrow \mathbf{D}^{-1/2} \mathbf{W} \mathbf{D}^{-1/2}$ , with  $\mathbf{W}$  the weight or affinity (distance-related) matrix between nodes in the graph (and  $\mathbf{W}_{ii} = 0$ ), and  $\mathbf{D}$  the diagonal degree matrix  $\mathbf{D}_{ii} = \sum_j \mathbf{W}_{ij}$ .

In the present implementation, the availability of *experimentally supervised data* from the experimental control samples was exploited. For each of these samples, the experimental conditions were known (in particular, the time after exposure to the virus), and a specific phenotype (label) could therefore be expected. To make use of this information, initial labels were assigned not only to the manually-annotated data points, but also to these experimentally supervised data points, by using the labels corresponding to their expected infection phase.

<sup>2</sup>In total, 95 features were extracted from the images. Spatial coordinates, however, were regarded as of little, if any value for data analysis in this work.

<sup>3</sup>A small number of imaging artifacts were also identified manually. However, accounting for such information was out of the scope of this work.

The  $n$  data points are classified in four types: labeled data, experimentally-supervised data with high labeling confidence (data points taken from the uninfected control samples), experimentally-supervised data with standard labeling confidence (data points taken from the infected control samples), and unlabeled data, with cardinalities  $n_l, n_h, n_s, n_u$  respectively. Different learning rates per each type ( $\alpha_l, \alpha_h, \alpha_s$ , and  $\alpha_u$ , respectively) were used to construct a diagonal  $n \times n$  matrix  $\mathcal{A}$ , such that  $\mathcal{A}_{ii} \in \{\alpha_l, \alpha_h, \alpha_s, \alpha_u\}$ , according to the class of the data point  $i$ . The actual values are found by hyperparameter optimization (detailed in next sections).

The variant of the label spreading algorithm presented here, uses then the slightly modified (*experimentally supervised data-aware*) iterative formula:

$$\hat{Y}^{(t+1)} \leftarrow \mathcal{L}\mathcal{A}\hat{Y}^{(t)} + (\mathbf{I} - \mathcal{A})\hat{Y}^{(0)} \quad (2)$$

The label spreading algorithm: 1. Read the  $n \times f$  feature matrix  $\mathbf{M}$  containing the  $f$  selected feature values for all  $n = n_l + n_h + n_s + n_u$  data points. 2. Compute the affinity matrix  $\mathbf{W}$  from the pairwise distances between all data points in  $M$ . 3. Create the initial label vector  $\hat{Y}^{(0)}$  from all  $n$  points, according to their type. 4.

### Development and runtime environment

To read and analyze the data, a script was coded in Python 2.7.4. Extensive use of the open source libraries `numpy` and `scipy` were used for matrix and numerical manipulation, as well as `matplotlib` for data visualization and graphical user interface.

Many options for this script are customizable via command line parameters. Appendix B includes a description of all the possible parameters and a quick user guide.

### Feature selection

The values for all the features from the annotated cells were analyzed with Weka<sup>[8]</sup>. The InfoGain attribute evaluator was used to determine the information gain ratio for each feature, and the top 4 features were chosen as the selected dimensions to represent the data for further analysis.

ad-hoc

Due to the heterogeneity on the range of values between the selected features, spanning several orders of magnitude, normalization of the data was required. A standard z-score normalization was applied on all the dimensions. This normalization, however, does not account for the fact that some data dimensions are more important than others in terms of information gain. The relative information gain score among the group of selected features was used to construct a scaling factor or weight for feature normalization, so that pairwise distances over more important dimensions are smaller than distances over less important ones.

$$w_d = \frac{\max F}{F_d} \quad (3)$$

The selected features were:

ID	Description	Score	Weight
4	Standard deviation of green intensities (whole cell)	1.3275	1
3	Mean of green intensities (whole cell)	1.1739	0.8843
2	Standard deviation of green intensities (nuclei)	1.0605	0.7989
1	Mean of green intensities (nuclei)	0.9868	0.7434
92	Information measure of correlation (scale 5, whole cell)?	0.9288	0.6997
53	Correlation (scale 3, whole cell)?	0.9241	0.6961
93	Maximal correlation coefficient (scale 5, whole cell)	0.9081	0.6841

### Data pre-processing

Text files in both `arff` and `txt` formats containing feature information for labeled, soft-labeled, and unlabeled data were read into a feature matrix  $\mathbf{M}$  ( $n$  data points  $\times m$  features).

The `arff` files containing *labeled data* were read and loaded into the feature matrix, by filtering the read fields to include only the features obtained in the feature selection phase. The possible classes or labels are loaded from the `arff` file by parsing the line starting with `@attribute class` (this prefix can be overridden via the `--label-line-prefix` parameter in the command line). As a parameter to the program, a list of ignored labels can be also passed with the option `--ignored-labels`. Data points annotated with any of these label identifiers

will be left out of the feature matrix. No further sampling was performed over the labeled data, i.e. all remaining (non-ignored) data points were kept.

The txt files containing *soft-labeled data* were read a similar way, except there was no need for parsing any formatting of the files. Each line in these txt files corresponds to a cell, and contains the values for the features, space-separated, in the same ordering as the labeled files. To assign actual soft labels, the relative file location in the file system was used as follows: the user indicates a root directory with all the soft-labeled data, and the files are expected in different directories within, which are internally mapped (via a python dictionary) to the actual labels.

The txt files containing *unlabeled data* were read exactly as described above for the soft-labeled data. A default neutral label was assigned to all entries read from this files.

Due to the massive amount of information, sampling parameters over the soft-labeled and unlabeled data were implemented. The command-line parameter `--num-samples N` controls how many data points to use from both soft-labeled and unlabeled data together ( $N/2$  each). An additional flag parameter `--class-sampling` indicates that the script must sample the soft-labeled data uniformly over classes, to avoid sampling bias due to large differences between the number of data points on each class.

As an outcome of this pre-processing step, the feature matrix containing the values of the selected features for the labeled, soft-labeled, and unlabeled cells (after sampling, when specified) was returned, along with the initial label matrix.

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### Data normalization

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### Graph construction

The graph was internally represented by its weight matrix  $\mathbf{W}$  ( $W_{ij} > 0$  if there exists an edge between the vertices  $x_i$  and  $x_j$ , zero otherwise), plus a  $n \times m$  label matrix  $Y$  ( $n$  cells,  $m$  possible labels or classes), with valid values ranging from 0 to 1. A value of  $Y_{i,j} = 1$  represents complete confidence that the  $i$ -th cell in the data set, belongs to the  $j$ -th phenotypic class of cells. Likewise, a value of 0 indicates absolute disbelief that a cell corresponds to a class, and values of 0.5 indicate complete uncertainty about class membership.

### Label propagation

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### Hyperparameter optimization

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## Results

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## Discussion

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## Conclusions

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## Appendix A

### Features analyzed

Position and intensity		Nuclear shape features	
1	nuclei location $x$	11	Area
2	nuclei location $y$	12	Eccentricity
3	green mean intensity nuclei	13	Solidity
4	green std intensity nuclei	14	Extent
5	green mean intensity cells	15	Euler number
6	green std intensity cells	16	Perimeter
7	blue mean intensity nuclei	17	Form factor
8	blue std intensity nuclei	18	Major axis length
9	blue mean intensity cells	19	Minor axis length
10	blue std intensity cells	20	Orientation

[Haralick features here]

## Appendix B

# Script parameters and help

up to date?

```
$ python hcs.py -h
usage: hcs.py [-h] [-t] [-l LABELED_FILE [LABELED_FILE ...]]
              [-u UNLABELED_FILE [UNLABELED_FILE ...]] [-s SOFT_LABELED_PATH]
              [-L NUM_LABELED_POINTS] [-n NUM_SAMPLES] [-c]
              [--max-iterations MAX_ITERATIONS] [-d WIDTH]
              [-nf {exp,knn3,knn4,knn5,knn6}]
              [-dm {euclidean,cityblock,cosine,sqeclidean,hamming,chebyshev}]
              [-f FEATURE_INDEX [FEATURE_INDEX ...]] [-q]
```

### Label propagation

#### optional arguments:

```
-h, --help            show this help message and exit
-t, --test            Performs a test run.
-l LABELED_FILE [LABELED_FILE ...], --labeled LABELED_FILE [LABELED_FILE ...]
                        Labeled files.
-u UNLABELED_FILE [UNLABELED_FILE ...], --unlabeled UNLABELED_FILE [UNLABELED_FILE ...]
                        Unlabeled files.
-s SOFT_LABELED_PATH, --soft-labeled SOFT_LABELED_PATH
                        Path to soft labeled files. One directory per label
                        expected.
-L NUM_LABELED_POINTS, --num-labeled NUM_LABELED_POINTS
                        Number of labeled data points to use. Default: use all
                        available
-n NUM_SAMPLES, --num-samples NUM_SAMPLES
                        Number of samples. Default: 3000
-c, --class-sampling  Distributes the number of samples given by
                        [NUM_SAMPLES] uniformly over all soft classes
--max-iterations MAX_ITERATIONS
                        Maximum number of iterations. Default: 1000
-d WIDTH, --display-columns WIDTH
                        Max width used for matrix display on console
-nf {exp,knn3,knn4,knn5,knn6}, --neighborhood-function {exp,knn3,knn4,knn5,knn6}
                        Neighborhood function to use. Default: exp
-dm {...}, --distance-metric {euclidean,cityblock,cosine,sqeclidean,hamming,chebyshev}
                        Metric for calculating pairwise distances. Default:
                        euclidean
-f FEATURE_INDEX [FEATURE_INDEX ...], --features FEATURE_INDEX [FEATURE_INDEX ...]
                        Selected feature indices (as given by the labeled
                        data).
-q, --quiet           Displays progress and messages.
```