A deep learning and novelty detection framework for rapid phenotyping in high-content screening

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ABSTRACT Supervised machine learning is a powerful and widely used method for analyzing high-content screening data. Despite its accuracy, efficiency, and versatility, supervised machine learning has drawbacks, most notably its dependence on a priori knowledge of expected phenotypes and time-consuming classifier training. We provide a solution to these limitations with CellCognition Explorer, a generic novelty detection and deep learning framework. Application to several large-scale screening data sets on nuclear and mitotic cell morphologies demonstrates that CellCognition Explorer enables discovery of rare phenotypes without user training, which has broad implications for improved assay development in high-content screening.

INTRODUCTION Advances in microscope automation have facilitated the systematic study of cellular phenotypes resulting from genetic or chemical perturbations. Prevailing image analysis pipelines rely on supervised machine learning to classify cellular phenotypes based on user-defined collections of statistical image features (Boland and Murphy, 2001; Carpenter et al., 2006; Neumann et al., 2006; Bakal et al., 2007; Jones et al., 2009; Ramo et al., 2009; Held et al., 2010; Messelwitz et al., 2010; Sommer and Gerlich, 2013; Boutros et al., 2015; Mattiazi Usaj et al., 2016). This approach has revealed new gene functions in various RNA interference (RNAi)-based screens (e.g., Goshima et al., 2007; Neumann et al., 2010; Schmitz et al., 2010; Gudjonsson et al., 2012; Sommer and Gerlich, 2013; Liberali et al., 2014; Boutros et al., 2015; Cuylen et al., 2016). While supervised machine learning is in principle broadly applicable, it requires extensive user interaction for the development of new biological assays. A major limitation of supervised machine learning is the requirement for classifier training for each new assay or variation in the experimental conditions. The classifier training requires representative images for all expected phenotype classes. As possible phenotype morphologies are not always completely known a priori, screening assay development often involves extensive and time-consuming pilot screens that are inspected visually for manual annotation of phenotype classes (Conrad and Gerlich, 2010). This process can be facilitated by interactive learning (Jones et al., 2009), yet rare phenotype classes might not be represented in pilot screens. Moreover, current high-content screening analysis software relies on user-curated collections of feature extraction algorithms, which often require specific software adaptations when establishing new cell biological assays. Developing classifiers for new biological assays has hence remained a major bottleneck in high-content screening.

These limitations might be overcome by unsupervised learning methods, which estimate phenotypic content based on intrinsic data structure. However, high cell-to-cell variability and experimental noise often preclude sensitive and reliable detection of low-penetrance phenotypes. The accuracy of unsupervised phenotype detection can be improved by object features beyond the single-cell level, such as the temporal context (Zhong et al., 2012; Failmezger et al., 2013) or the cell population context (Rajaram et al., 2012; Liberali et al., 2014), but such information is not always applicable for a given biological assay. The detection of unknown phenotypes might be facilitated by novelty detection methods (Yin et al., 2008, 2013; Manning and Shamir, 2014), yet the performance of such methods in genome-wide screening has not yet been tested.
The dependence of machine learning approaches on user-curated image features might be overcome by feature self-learning from the data. This can be achieved by deep learning methodology (LeCun et al., 2015), which has demonstrated impressive performance in various domains, such as face recognition (Taigman et al., 2014) and speech recognition (Sainath et al., 2013). Recent analyses of protein localization in budding yeast by deep learning indicates a high potential in bioimaging (Kraus et al., 2017; Parnamaa and Parts, 2017), but applicability to genome-scale human cell screening data has not yet been explored and an integration of novelty detection with deep learning is not available in community-standard software for high-content screening.

We here present the novelty detection and deep learning software CellCognition Explorer. The software yields phenotype scores based on deviation of cell morphologies from negative control images. We demonstrate that CellCognition Explorer enables sensitive and accurate cellular phenotype detection in genome-scale screening data without the need for extensive user interaction for data annotation. In addition, deep learning of image-derived features overcomes the dependence on user-curated feature analysis collections and accurate cell segmentation outlines. Hence, CellCognition Explorer greatly facilitates rapid screening assay development even when cellular phenotypes are not known a priori.

**RESULTS**

CellCognition Explorer software

We have developed CellCognition Explorer, a machine learning framework for the detection of abnormal cell morphologies without prior user training. Two core technologies enable the unsupervised classification task. Deep learning methods infer numerical descriptors of individual cell objects directly from the raw image pixel data, thus circumventing feature engineering and software adaptations for new biological assays. Novelty detection methods then learn a statistical model of the natural phenotype variation within the negative control cell population. This yields an accurate classification boundary for subsequent detection of any morphological deviations in large-scale screening data—even for phenotypes that are not known a priori.

CellCognition Explorer provides a pipeline for integrated data analysis from raw images to phenotype scores (Figure 1). The software package consists of two programs: The main CellCognition Explorer program provides interactive data visualization tools and the possibility of performing versatile analysis workflows using novelty detection methodology, as well as conventional supervised learning methods. It is controlled by a simple graphical user interface (Supplemental Figure S1) that works on all major computer operating systems. CellCognition Deep Learning Module is a separate program for graphics processing unit (GPU)-accelerated high-performance computing of deep learning features (Supplemental Figure S2). The implementation as two separate programs provides optimal flexibility for installation of the interactive data exploration and workflow design tool, while enabling the efficient computation of deep learning features by dedicated GPU hardware. Both programs are controlled by graphical user interfaces and distributed as open source software embedded within the CellCognition platform (Held et al., 2010; http://software.cellcognition-project.org/explorer/). Interoperability with widely used bioimage software packages, including ImageJ/Fiji (Abramoff et al., 2004; Schindelin et al., 2012), CellProfiler (Carpenter et al., 2006), and Bioconductor (Gentleman et al., 2004), is enabled via the standardized file format cellH5 (Somer et al., 2013).

Cell phenotyping by novelty detection

The novelty detection algorithms implemented in CellCognition Explorer are designed to learn intrinsic cell-to-cell variability in an untreated negative control cell population autonomously, which sensitizes the classifier to perturbation-induced phenotypes. Abnormal cell phenotypes are then scored either based on the weighted cell object distance in feature space relative to the mean and covariance of a control cell population (Mahalanobis distance,
MD; Pimentel et al., 2014), based on their likelihood after the multivariate probability density of control conditions is estimated (kernel density estimation, KDE; Pimentel et al., 2014), or by fitting a nonlinear hyperplane to control cell objects (one-class support vector machine; Scholkopf et al., 2001) (see Materials and Methods). These methods can score and classify cell objects based on conventional precomputed numerical features (e.g., Boland and Murphy, 2001; Murphy et al., 2003; Carpenter et al., 2006; Held et al., 2010) or based on learned representations from the original image pixel data when combined with deep learning (Vincent et al., 2010; Durr and Sick, 2016; Kraus et al., 2016).

To test the performance of the novelty detection methods, we generated a data set representing the full spectrum of morphological phenotypes for a chromatin marker. HeLa cells stably expressing fluorescent histone 2B fused to mCherry (H2B–mCherry) were subjected to RNAi depletion of 1214 genes previously identified as important for mitosis from a genomewide screen (Neumann et al., 2010), using individual transfection of two different small interfering RNAs (siRNAs) per target gene, followed by live-cell imaging on an automated epifluorescence microscope. To establish a reference annotation, we detected cell objects based on local adaptive thresholding and watershed segmentation and calculated 239 conventional numerical features describing texture and shape (Held et al., 2010). We then randomly subsampled 10,000 cell objects and manually annotated their “ground truth” phenotypes by classification into nine different morphology classes: normal interphase nuclei and various different outlier morphologies, representing mitotic stages, dead cells, and abnormal nuclear shapes (Figure 2a, b).

![FIGURE 2](image-url)

**FIGURE 2:** Novelty detection with CellCognition Explorer. (a) Phenotype distribution in principal component subspace of 10,000 manually annotated HeLa cells stably expressing H2B-mCherry. The cell images were randomly selected from 2428 RNAi experiments to establish a representative collection of all cellular phenotypes. The nine phenotype classes illustrate the most common nuclear and mitotic chromatin morphologies. Colors indicate phenotype groups as illustrated by examples. (b) Performance of different novelty detection methods. True positive and false positive rates (receiver operating characteristic curve) were calculated for the indicated methods based on the data shown in a. (c) Comparison of phenotype scoring by conventional supervised learning (Held et al., 2010) and novelty detection. Each dot represents one of 2428 different siRNAs; negative controls: no-targeting siRNAs; positive controls: siRNAs causing strong mitotic phenotypes. Phenotype scores are defined as the fraction of HeLa-H2B-mCherry cells deviating from normal interphase morphology. For detailed analysis results, see Supplemental Table S2. (d) Phenotype scoring of 2428 siRNAs as in a by novelty detection using CellCognition Explorer. Red bars indicate the distribution of the top 100 ranked siRNA hits identified by conventional supervised learning; see c.
Supplemental Table S1, full data set available at http://software.cellcognition-project.org/explorer/). In the principal component subfeature space (Pimentel et al., 2014), interphase nuclei appeared as a single compact cluster, whereas the different outlier phenotype groups scattered broadly in different regions.

All three novelty detection methods implemented in CellCognition Explorer accurately classified normal interphase nuclei as inlier objects and other morphologies as outliers, consistent with phenotype scoring with supervised analysis (Figure 2b). To extend the performance tests to the full data set, we next quantified the abundance of abnormal cell phenotypes in each of the 2428 RNAi conditions. The fraction of cells with outlier morphologies calculated with novelty detection methods consistently matched the reference state-of-the-art analysis using supervised learning by support vector machines (Held et al., 2010) (SVMs; Figure 2, c and d, and Supplemental Figure S3). Thus, novelty detection with CellCognition Explorer accurately identifies abnormal cell morphology phenotypes without the need for extensive data annotation.

Deep learning of cell features
The CellCognition Deep Learning Module automatically extracts numerical feature sets that adjust to specific cell morphology markers used in an assay. This is achieved by a convolutional autoencoder, a multilayered artificial neural network (Hinton and Salakhutdinov, 2006) that learns a representation (encoding) for a collection of images. This method requires only center coordinates of cell objects as an input and is thus independent of the accurate object segmentation contours that are normally necessary for conventional user-curated feature sets to calculate shape features. The features derived by deep learning serve as an input for the novelty detection method (see above) and can also be used for fully automated phenotype scoring by novelty detection, thereby overcoming dependence on manually curated feature sets.

Application to genomewide screening
To test the applicability of CellCognition Explorer to high-throughput screening, we aimed to recompute phenotype scores for published primary image data of a genomewide RNAi screen for mitotic damage repair regulators (Neumann et al., 2010). The original phenotype scoring of these screening data was based on classification using supervised learning (Neumann et al., 2010), in which the development of a reliable classifier required a multimonth phase of iterative pilot screening and visual inspection for classifier training (Neumann et al., 2006, 2010). The analysis of millions of images during pilot screening had ultimately led to the discovery of many previously unknown phenotypes (Figure 4a). Identifying all abnormal cell morphologies prior to classifier training would abrogate the need to visually inspect the full data set, thereby solving a major bottleneck in data analysis.

We used the CellCognition Deep Learning Module to extract self-learned features for phenotype scoring based on novelty detection. The top-ranked RNAi phenotypes according to phenotype penetrance were very similar to the originally published hit list (Figure 4b), even though information on abnormal phenotype morphologies from pilot screening was not taken into account. Hit lists generated by our new methods are hence highly enriched for abnormal cell morphologies, which greatly facilitates further phenotype analysis by visual inspection, supervised classification, or unsupervised clustering.

To probe the versatility of our methods, we next reanalyzed primary image data from another large-scale RNAi screen for DNA damage repair regulators (Gudjonsson et al., 2012). The primary assay probed the regulation of DNA damage repair protein...
DISCUSSION

CellCognition Explorer bypasses the need for extensive pilot screening to capture all possible phenotype morphologies and thereby solves a major bottleneck of conventional supervised approaches. CellCognition Explorer yields a list of top-ranked screening targets directly from large-scale screening data, which can be used for mechanistic follow-up studies or further subclassified using conventional supervised methods.

By avoiding human bias arising from supervised classifier training (Zhong et al., 2012), novelty detection with CellCognition Explorer helps to improve the consistency between different screens. Furthermore, the independence from manually curated feature sets facilitates the development of new cell biological assays. The inference of image features by deep learning does not take the image segmentation contours into account and therefore has the potential to facilitate the analysis of cellular markers that are difficult to segment.

We here demonstrate how CellCognition Explorer can be used to detect novel phenotypes in nuclear morphology screening data. CellCognition Explorer supports processing of multichannel images and we are currently extending the software for segmentation of other cell compartments and cell tracking for kinetic readouts (Hoefler, unpublished observations).

Overall, the simple graphical user interface of CellCognition Explorer and the independence of centralized large-scale computing infrastructure is well suited to applying deep learning and novelty detection to diverse questions in cell biology. Hence, CellCognition Explorer provides new opportunities for high-content screening and for exploratory research in diverse biological fields.

MATERIALS AND METHODS

Cell culture

A HeLa cell line stably expressed histone H2B fused to mCherry and lamin B1 fused to EGFP (Daigle et al., 2001) was generated from a HeLa Kyoto cell line as previously described (Schmitz and Gerlich, 2009). HeLa cells were cultured in DMEM (Life Technologies) supplemented with 10% (vol/vol) fetal bovine serum (FBS; Life Technologies), 1% (vol/vol) penicillin-streptomycin (Sigma-Aldrich), 500 μg ml⁻¹ G418 (Life Technologies), and 0.5 μg ml⁻¹ puromycin (Calbiochem). Homogeneous expression levels of the two transgenes were ensured by fluorescence-activated cell sorting. The cell line tested negatively for mycoplasma contamination in our quarterly testing routine. The parental HeLa cell line (“Kyoto strain”) was obtained from S. Narumiya (Kyoto University, Japan) and validated by a Multiplex human Cell line Authentication test (MCA).

siRNA library transfection

A total of 1114 genes of the MitoCheck genome-wide RNAi screen validation data set (Neumann et al., 2010) were targeted by two
siRNAs. To test target specificity, all siRNAs were mapped against the 2013 human genome (ENSEMBL V70), resulting in a unique match. siRNAs were delivered in 384-well imaging plates (Falcon) using solid-phase reverse transfection. Cells were seeded on the imaging plates using a Multidrop Reagent Dispenser (Thermo Scientific).

Automated microscopy
At 40 h after cell seeding, plates were imaged on a Molecular Devices ImageXpressMicro XL screening microscope using a magnification 20×, 0.75 NA, S Fluor dry objective (Nikon). Four positions with two Z-sections each (4 μm offset) were acquired in each well. Images were flat-field-corrected with the Metamorph software (Molecular Devices) using images acquired in empty wells to compensate for inhomogeneous illumination.

Image preprocessing for reference annotation with conventional supervised learning
To detect individual cells, we used local adaptive thresholding followed by a watershed split-and-merge segmentation as provided in the CellCognition framework (Held et al., 2010). For each cell, the segmentation outline, its center of mass, and its bounding box were computed and saved to the cellH5 format (Sommer et al., 2013). Then, 239 cellular morphology features describing the texture, shape, and intensity distribution of each single cell object were computed as in Held et al. (2010). As a reference annotation in the chromatin morphology screen, we trained a multiclass SVM on nine phenotype classes with 592 training examples, achieving a cross-validation accuracy of 85.1%.

Outlier detection
An outlier is an observation that deviates so much from other observations as to arouse suspicions that it was generated by a different process (Hawkins, 1980). Novelty detection methods aim to identify outliers by inference based on intrinsic properties of the data without any further annotations. We implemented three state-of-the-art novelty detection methods: Mahalanobis distance (MD; Mahalanobis, 1936), kernel density estimation (Pimentel et al., 2014), and one-class SVM (OC-SVM; Scholkopf et al., 2001). All methods find novelty based on preextracted morphology features or on data-adaptive learned feature representations by deep learning autoencoders.

Mahalanobis distance
MD is based on the parametric assumption that the extracted features follow a multivariate Gaussian distribution (Mahalanobis, 1936). The Mahalanobis distance (Eq. 1) incorporates the mean μ and the covariance matrix S (multidimensional spread) of the data:

$$MD(x) = (x - \mu)^T S^{-1} (x - \mu)$$ (1)

For estimation of the mean and covariance, data samples were drawn from negative control conditions. Negative control conditions will also contain aberrant phenotypes (e.g., polypoid cells). To reduce the effect of strong outliers on the estimation process, some instances were excluded based on their univariate feature distributions (i.e., cells with the most extreme single feature values) prior to the estimation of the multivariate mean and covariance.

The mean feature vector represents the ideal normal cell, whereas the covariance estimates its multivariate spread. Once the mean and the covariance of the multivariate Gaussian distributions are estimated, an empirical cutoff distance is estimated (relative to negative control data) in such a way that 15% of the data points have a greater Mahalanobis distance than the chosen cutoff and therefore are considered abnormal. Cells from other experimental conditions are then assigned to the normal category (inliers) when their Mahalanobis distance is smaller than the calculated cutoff, or to the abnormal category (outliers) if the distance is greater. For all experiments shown in the article, >50,000 cells were sampled from negative control conditions for estimation of the parameters for outlier detection. For the chromatin morphology screen (Figure 2, c and d), a 10% cutoff was used. Negative control conditions in the screen for damage repair regulators (Figure 4, c and d) contained only very few outliers; a 5% cutoff value was hence chosen. The varying thresholds were empirically determined based on the expected number of outliers in the data sets.

Kernel density estimation
KDE is nonparametric estimation of a probability distribution (Pimentel et al., 2014). Conceptually, KDE is related to histograms. Instead of quantizing a discrete probability density by counting how many data points fall into each bin, KDE does not require binning and outputs a continuous function. KDE places a zero-centered kernel function K of a bandwidth h on each data point followed by summing all these kernels:

$$KDE_h(x) = \frac{1}{n} \sum_{i=1}^{n} K_h(x - x_i)$$ (2)

A multivariate Gaussian density distribution was used as the kernel K. The bandwidth h is given by the variance of the Gaussian distribution. In places where many data points are located, the estimated density will be high, while at places where no or few data points lie, the density will approach zero. Similarly to the cutoff parameter of Mahalanobis distance, a separation into abnormal (outlier) and normal (inlier) locations was achieved by thresholding so that the resulting estimated density had 15% of the data points classified as novel and 85% classified normal. The choice of an appropriate bandwidth is the key to KDE, because it determines the smoothness of the resulting density (e.g., too small bandwidths lead to spiky nonoverlapping densities). Therefore, a cross-validation strategy was used to estimate appropriate bandwidth from holdout data.

One-class support vector machine
Another nonparametric and nonlinear way to estimate densities in high-dimensional spaces is the OC-SVM (Scholkopf et al., 2001). Similarly to the standard two-class SVM (Vapnik and Lerner, 1963) the OC-SVM yields a separating hyperplane with a maximal margin to distinguish the majority of the data from the origin. When a Gaussian kernel is used in the SVM optimization framework, the hyperplane implicitly corresponds to nonlinear decision boundaries in input space (Boser et al., 1992). With this, the OC-SVM models arbitrary densities in high dimensions as opposed to more simplified ellipsoidal distributions when MD is used. Similarly to KDE, a parameter g (inverse SD of the Gaussian kernel) accounts for the smoothness of the resulting enclosing hyperplane. In the experiments shown in the article, g was optimized empirically by a grid search on a logarithmic scale. The parameter n in OC-SVM–based novelty detection is the maximal fraction of data points in the training set that can be assigned being an outlier. In accord with the methods described previously, we set n = 0.15.

Self-learned feature representation with autoencoders
The novelty detection algorithms described above can be trained on a manually curated feature set (e.g., as in Held et al., 2010). Such
features are defined a priori and do not depend on the actual data at hand. Hence, information for distinguishing novel phenotypes from normal morphologies might not be sufficiently expressed by general feature sets.

To overcome this limitation, we implemented a class of artificial neural networks (ANNs) termed autoencoders (AE) to learn a data-dependent features representation directly from pixel data from individual cropped-out cells in an unsupervised manner. The bounding box size for cropping can be chosen freely by the user. An AE is an ANN that adjusts its internal weights to reconstruct its input as closely as possible (subject to a given objective function) by first learning how to encode its inputs effectively in the encoding layer and then decoding this back to the original domain with an inverse decoding layer (Hinton and Salakhutdinov, 2006). Once an AE has learned how to encode cell images into a code, this process is iterated to stack AEs in a hierarchical manner to generate deeper networks called stacked AEs (Hinton and Salakhutdinov, 2006). To ensure that an AE is not learning the simple identity function, the internal parameters have fewer degrees of freedom than its input/output (contractive autoencoding).

Recent progress in designing deep learning networks for various tasks in computer vision has led to the incorporation of convolutional and pooling layers (e.g., Krizhevsky et al., 2012). Convolutional layers refer to learnable image filters usually of size 3 × 3 or 5 × 5 applied to the input images. Like other network weights in an ANN, these filters are simultaneously adapted during optimization to yield feature maps, which improve the overall network performance. To reduce the overall spatial dimensionality, convolutional layers are combined with max-pooling layers, which effectively downsamples the input image by selecting only the maximum pixel value for a defined neighborhood region (Krizhevsky et al., 2012)—in our experiments, 2 × 2. We combined the stacked-contrastive-AE model with convolutional and pooling layers to achieve more expressive codes in the AE paradigm. For faster AE training, dropout layers have been proposed (Vincent et al., 2010), which randomly set a fixed percentage of inputs in each layer to zero. This compels the network to learn to reconstruct from corrupted inputs, which leads to less overfitting and faster training convergence.

To analyze the data shown in the article, we used AEs with a variable sequence of one or more convolutional layers (Supplemental Tables S5–S7), followed by max-pooling layers and a central stacked AE. The convolutional and pooling layers are mirrored in the decoding part of the AE with their corresponding inverse deconvolutional and unpooling layers. Between each pair of layers in the encoding part of the AE, optional dropout layers are inserted with a dropout probability ranging from 10 to 90%. The convolutional and fully connected layers require a nonlinear activation function. In our implementation, sigmoid (s) and linear-rectifier (r) activation functions are supported.

Autoencoder layout
To analyze the screening data—chromatin morphology (Figure 3, b and c), MitoCheck RNAi screen (Figure 4, a and b), and DNA damage repair RNAi screen (Figure 4, c and d)—we designed AEs such that an input image of a typical cropped single cell (40 × 40 pixels) is encoded through a series of convolutional layers, pooling layers, and fully connected layers, which successively reduce the input dimensions from 40 × 40 = 1600 image pixels to a code length of 144 or 64. Note that the decoding part in each AE is composed of layer-wise inversions of the encoding part. The layout of all AEs with precise information about the convolutional and dense layers can be found in Supplemental Tables S5–S7.

Autoencoder training
AEs were trained with stochastic gradient descent (SGD) based on the sum of squared residuals of input and output as objective function (Vincent et al., 2010). The SGD method resembles a stochastic approximation of the gradient descent optimization method, where the true gradient of all the data is approximated by the gradients of so-called mini-batches, randomly selected subsets of the training data of a certain size m. All weights in the network are updated according to the back-propagated gradients of the objective function. This process is repeated and each round is referred to as one training epoch. Empirically, we found that alternating two variants of SGD—Nesterov momentum (Nesterov, 1983) and adaptive gradient descent (AdaGrad; Duchi et al., 2011)—leads to fast convergence. Nesterov momentum adds a momentum term to the update rule to stabilize the learning process and to avoid local minima (Bengio et al., 2013). AdaGrad modifies SGD by introducing a per-parameter learning rate leading to faster convergence (Duchi et al., 2011).

For the AE used in chromatin morphology screening, we used first Nesterov momentum SGD updated for 128 epochs with a mini-batch size of 128, a learning rate of 0.02, and a momentum of 0.8 followed by AdaGrad updates for 128 epochs with a mini-batch size of 128 and a base learning rate of 0.1.

For the AE depicted in MitoCheck RNAi screening, we used a mini-batch size of 128. Nesterov momentum updates with a learning rate of 0.1 and a momentum of 0.5 were followed by AdaGrad updates with a learning rate of 0.1, both for 64 epochs. Then we reiterated with slightly adjusted values: a learning rate of 0.02 l and a momentum of 0.9 for the Nesterov updates and a learning rate of 0.05 for AdaGrad.

For the AE shown in DNA damage repair RNAi screening, we used first Nesterov momentum SGD with a learning rate of 0.02, a momentum of 0.9, and a mini-batch size of 128 followed by AdaGrad with a learning rate of 0.05 and a mini-batch size of 64.

CellCognition Explorer software package. The CellCognition Explorer software package consists of two programs: 1) the CellCognition Explorer main program, from which the novelty detection methods (e.g., MD) and supervised learning algorithms (multiclass SVM) can be executed and visualized; and 2) a program for the generation of self-learned feature representations (codes).

CellCognition Explorer main program. The software imports images of cell populations and converts them into galleries of individual cells. Original multichannel raw images are preprocessed in advance: Thumbnails, features, and outlines are calculated and saved to cellH5/hdf5. The software stores experimental conditions along with cell objects to enable efficient data export and visualization of results by statistical plots, such as object class counts per condition. The software provides functions for cell segmentation and conventional feature extraction as in Held et al. (2010) for multiple image channels.

The graphical user interface is designed to handle a few thousand cells smoothly on a standard desktop PC. Larger data sets can be loaded in batches. The software is available as a binary stand-alone version for Mac OSX and Windows 7.

CellCognition Explorer Deep Learning Module. With this program, the user can train a deep learning AE from negative controls. The main input is a cellH5 file (Sommer et al., 2013) containing the image data and center positions of all detected cell objects, together with a position mapping (text-file) indicating negative control positions used for randomly sampling cells for the training process.
After training, the resulting AE model can be used in the encoding step. The output file of the encoding step can be loaded directly into the CellCognition Explorer main program for novelty detection and/or supervised learning. CellCognition Explorer Deep Learning Module provides a graphical user interface to specify all processing parameters (Supplemental Figure S2). The software is provided as source code (LGPL) and as a binary installer using the docker framework. For installation in MacOS X, a docker execution script is provided.

**Implementation details**

CellCognition Explorer is implemented in Python 2.7, and the deep learning module makes use of the Theano (ver. 0.7.0) and lasagna (ver. 0.2) library for deep learning and GPU computing interfacing with NVIDIA CUDA (ver. 5.5). For the graphical user interfaces, PyQt5 (ver. 5.3.2) binding to the C++ library Qt (ver. 5.3.1) was used. Novelty detection methods are based on the sklearn library (ver. 0.16.0). Image-based screening data are segmented and processed using CellCognition Analyzer (ver. 1.6.0) and analysis results were saved in the cellH5 format (ver. 1.3.1).

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