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Efficient Development of High-Content Imaging Assays by Deep Learning

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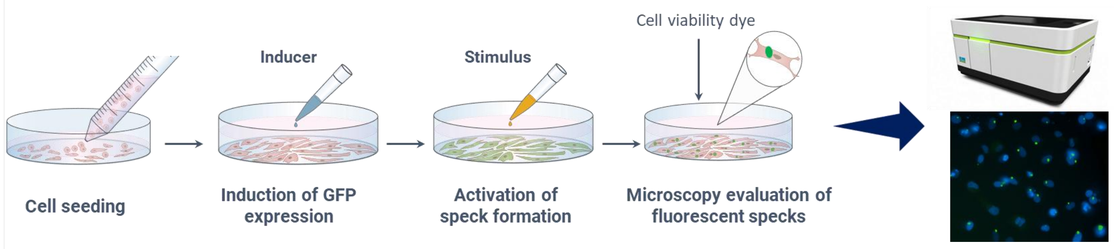
Abstract

Biopharma assay development aims to create robust assays that can run consistently over weeks on automation platforms, and are monitored by well-defined quality control metrics. Usually, such assay development is an iterative process, requiring multiple optimization rounds to determine ideal assay conditions. The development of high content imaging assays typically involves in addition an iterative development of image analysis due to their complex read-out. We report here on an analysis workflow that eliminates this bottleneck.

In a speck formation assay, the best combination of assay window, assay sensitivity, robustness and reproducibility was sought by varying incubation times and the dosing of both inducer and stimulus. For image analysis, a traditional image processing workflow and a deep learning-based workflow were compared. We describe the concepts of both workflows, and assess the benefits of switching from the traditional image analysis workflow to an integrated workflow using a deep learning classifier to quantify the relevant cellular phenotypes. In particular, we highlight the robustness, efficacy and flexibility of the deep learning-based workflow, and its selection as the primary analytical method for this speck formation assay and others at Roche.

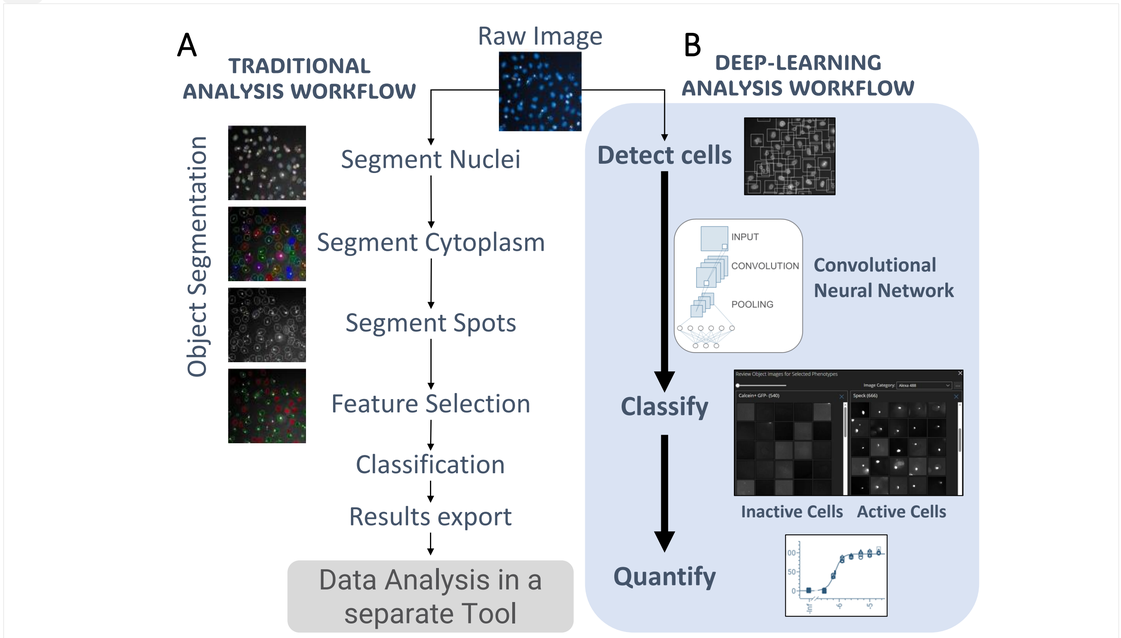
1. Speck Formation Assay



Development of a high-content imaging assay to detect inflammasome activation:

To avoid the high cost of a previously used colorimetric assay for inflammasome activation and to increase physiological relevancy, a high-content imaging assay was developed. In untreated cells, the protein of interest is localized in the cytoplasm and nucleus. Upon inflammasome activation, a large protein speck assembles in the perinuclear area. The final readout is the number of newly formed specks per cell. Assay conditions (incubation time; concentrations of stimulus and inducer) are optimized for the best combination of assay window, assay sensitivity, robustness and reproducibility.

2. Traditional vs Deep-Learning Workflow



(A) Conventional image-analysis workflow typically makes use of the software from the acquisition instrument and requires multiple object segmentations (nucleus, cytoplasm, spots) followed by feature extraction and classification, which all have to be adapted to the assay. Results are manually exported to other software for downstream analysis. This process is repeated for each assay optimization round.

(B) A deep learning (DL) workflow (such as in the Genedata Screener HCS platform) needs nuclear staining only for object detection and automated feature extraction. The scientist defines a training set in the first round for each phenotype; a deep learning model is trained and classifies all images. Class counts are automatically processed further to quantify the effect.

5. Benefits of DL-based Analysis for Assay Development

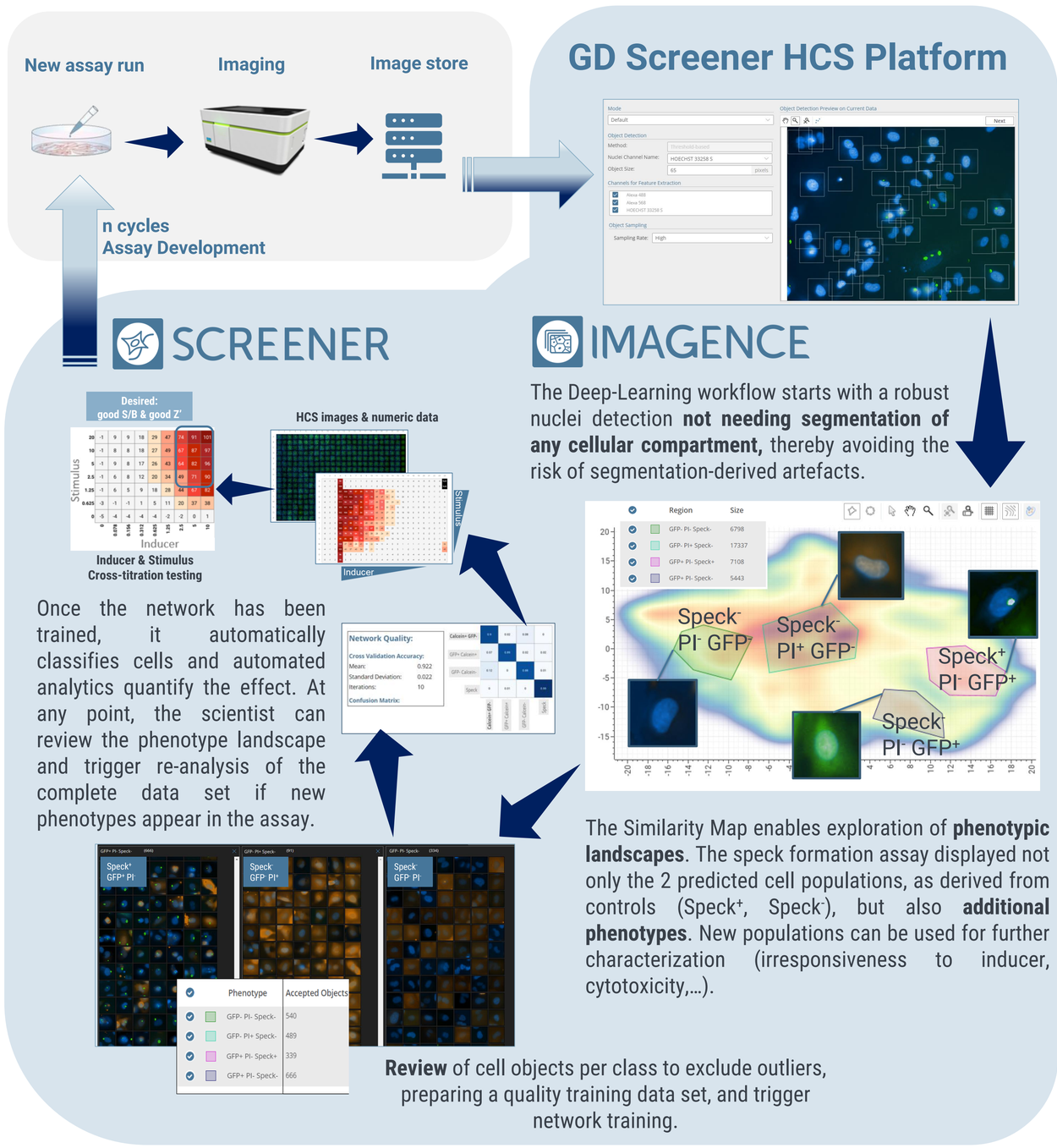
In the development of this speck formation assay, the following benefits were apparent:

- Rapid and straightforward identification of expected phenotype classes
- Easy identification of new phenotypic classes (i.e., for toxicological assessment, or to confirm the mechanism of action), taken into account for further assay optimization
- Streamlined workflow in one integrated solution, which includes a variety of graphical representations that facilitate decision-making
- Faster overall assay development, optimizing the conditions Time, Stimulus & Inducer

For development of high-content assays in general, additional benefits will apply :

1. Rapid set-up across assay formats, no need to optimize segmentation etc. – especially when not running an assay for which a pre-configured conventional workflow exists
2. Segmentation-free workflow gives robustness against assay variations over optimization rounds and time
3. No need for transfer - same analysis platform for assay development and production

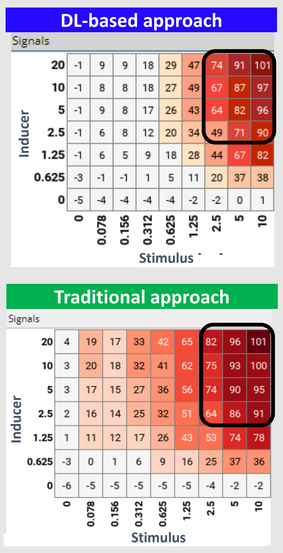
3. Acceleration of Assay Optimization by an Integrated Deep Learning Workflow



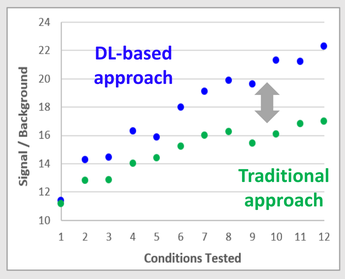
4. Comparing QC Metrics and Assay Results Between Workflows

Integrated HCS Platform SCREENER IMAGENCE

Dose-response matrix of the cross-titration

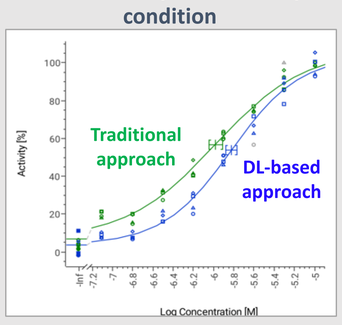


Quality control metrics on tested conditions



Superior assay window with DL method

Overlay of dose-response curves for the finally chosen assay condition



Similar qAC50 values

Overall, both the traditional and the integrated deep learning-based workflow yield comparable results. The DL-integrated workflow, however, presents significant operational benefits for assay optimization in terms of reduced cycle time and versatility across assays.

Conclusion

We present here an integrated analysis workflow using deep learning-based image analysis to accelerate HCS assay development at Roche. It shows a novel benefit of the application of deep-learning to the analysis of high-content imaging data, namely the rapid identification and characterization of new phenotypic classes appearing during assay development and their incorporation into the assay optimized for production. This expands information gain and precision of the assay without additional experimentation. Implemented on the Genedata Screener HCS platform, the workflow has key benefits when compared to workflows relying on traditional image analysis: Faster adaptation to varying assay outcomes as they happen during development, versatile application to different assay formats and designs, increased robustness due to eliminated segmentation steps, and direct translation to production screens. The workflow yields results of comparable, if not better quality than with traditional analysis. Thus, this workflow will be used increasingly for assay development and production screening in a diversity of HCS assays.

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