Application Note

LABEL-FREE QUANTIFICATION OF NEURITES

Quantitative assessment of neurite networks in recent years emerged as sensitive readout for the identification of both adverse and protective properties of pharmacological interventions. We developed a label-free method for the quantification of neurite mass, employing state-of-the art machine learning techniques. Our instrument allows its application by laboratory staff without the necessity of intensive training of the operators in data science.

Neurites: A sensitive indicator of toxicity

The group of Prof. Stefan Schildknecht (Albstadt-Sigmaringen University, Germany) focuses on the development of cellular in vitro models for basic research and for their application in pharmacological and toxicological investigations. For such investigations, the human neuronal line LUHMES is regularly applied and allows the differentiation of neuronal cells with a dopaminergic phenotype in less than a week. Based on their architecture and limited capacity for renewal, neurons represent a particularly sensitive cell type of the human body and are therefore an attractive model both for the identification of harmful conditions, and for the discovery of novel intervention strategies to prevent damage. The loss of correct neuronal function in vivo is not necessarily dependent on cell death but can already be initiated by rather modest harmful input. Hence, experimental detection of moderate alterations in the integrity of the neurite network emerged as reliable and sensitive readout for the identification of adverse compounds or conditions.

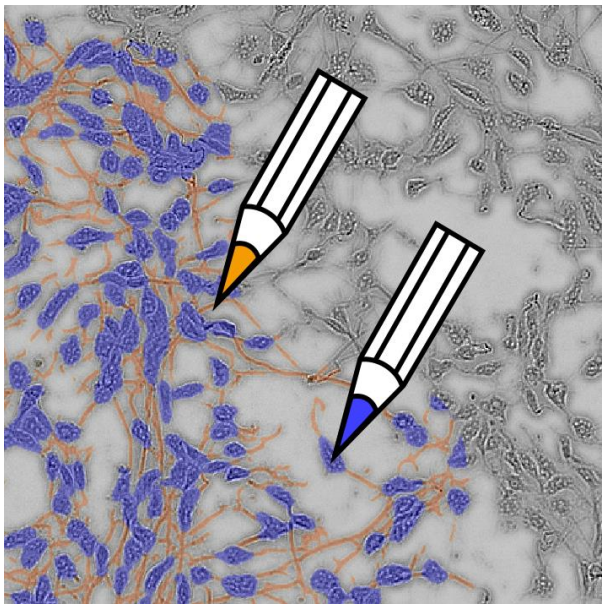

Fig. 1: Visually labeling somata and neurites

Robust quantification is challenging.

To implement a neurite quantification assay, the method developer needs to choose between fluorescently staining the cells or following a label-free approach. The former option has the advantage of substantially simplifying the image analysis. However, the downside is that reagents and lab-effort are expensive, fixation may alter the fragile morphology and the cells can only be observed once.

The label-free approach is much more cost-effective, sensitive, suffers less from processing-induced variability and enables multiple time points as well as a continued cultivation of the cells for other purposes. However, the image analysis of bright-field or phase-contrast images is not trivial and requires special skills and tools.

VAIDRs artificial intelligence can be trained without data science skills.

Using VAIDRs simple drawing interface, a few images are labeled by hand. For the current purpose, two classes are defined: soma and neurite. As shown in Fig. 1, the labeling process consists of painting the somata blue and the neurites orange. Clicking a button starts the training of the AI.
When the first training round is finished, the results can be checked visually. Errors can be corrected by painting over the pre-painted images and re-starting the training.

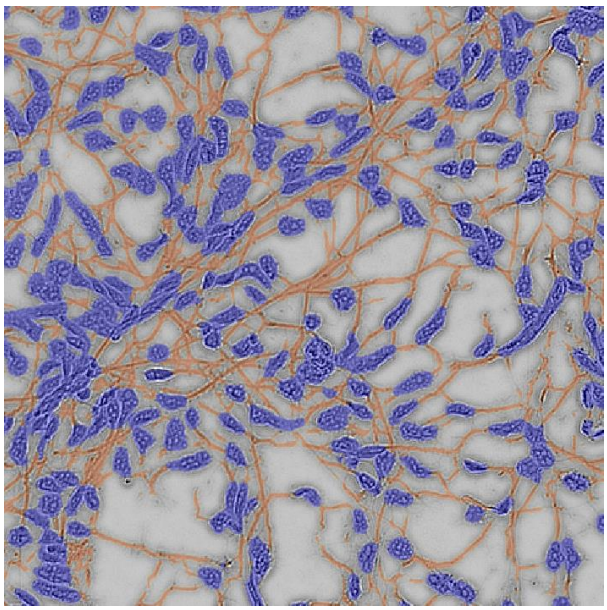

Fig. 2: Automatically detected neurites (orange) and somata (blue)

Quantification and data handling are automated.

Once neurites and somata are detected correctly (Fig. 2), the trained AI can be applied to quantify the images of interest. The data are selected by filtering the data overview table to the desired measurements and marking them for analysis with the neurite quantification. Results are generated and automatically linked to the entries in the data overview table. They are available for future review and can always be found by filtering to the desired measurements.
Several automated modes of visualizing the results are available. However, to offer maximum flexibility, all results and metadata can also be exported in common file formats. As an example, we exported the quantification results to a CSV file and used a generic external tool (e.g., Excel) to compute quantities of interest: (1) The confluency, which is the total cell area (somata plus neurites) divided by the image area. (2) The neurite density, which is the area covered by neurites divided by the total cell area. When plotting neurite density vs confluency for several wells cultured under identical conditions (Fig. 3), it becomes clear that the neurite density is limited by the available area: More confluent wells exhibit a smaller neurite density, as more area is taken up by the somata. This is important to consider when using neurite density as a quantitative readout.

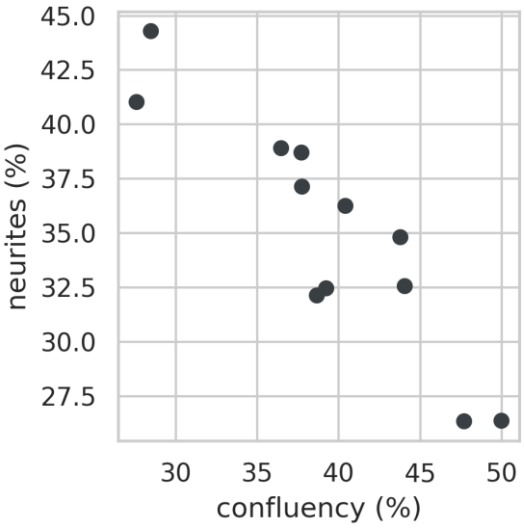

Fig. 3: Evaluation of neurite density vs confluency for 12 wells

In summary, we have described how the VAIDR system can be used to develop and employ a method that quantifies the neurite network in 2D cell culture without the need for staining. Training the machine learning algorithm and evaluating the results can all be achieved without the need for specialized training in data science.

References:

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For more information about the group of Stefan Schildknecht, visit www.hs-albsig.de/personendetailseite/stefan-schildknecht

To learn about VAIDR, visit www.vaidr.de