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Omnisphero: A novel computational approach for high content image analyses (HCA) of organoid neurosphere cultures *in vitro*.

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Introducing Omnisphero

Recently new approaches were introduced for safety and efficacy testing *in vitro* to enhance a so far poor predictivity. These approaches move away from high throughput screenings of classical tumour-derived cell systems towards a medium throughput/high content analysis using more complex primary human cell systems, such as neurospheres. Therefore we developed Omnisphero, a user friendly analysis tool for neuronal identification, morphological and 3D specific endpoint evaluation in 3D high density heterogeneous cell cultures. Omnisphero outperforms frequently used commercially available software with high accuracy and precision algorithms.

Establishing HCA for neurosphere endpoint evaluation

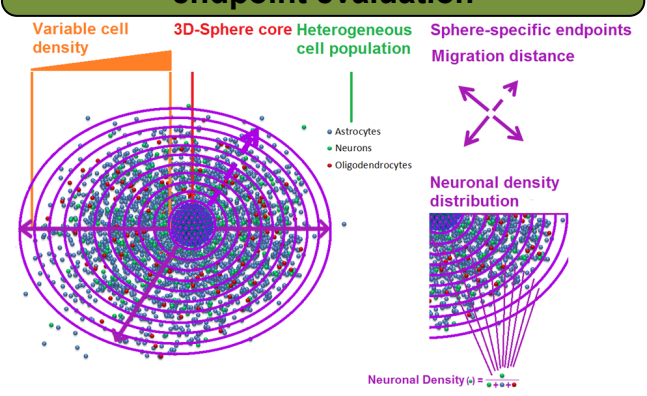

Fig. 1: Establishing HCA in the 'Neurosphere Assay'. Neurodevelopmental toxicants have shown to be a serious risk for society, making regulatory testing of chemicals, including petroleum products and petrochemicals, indispensable. Since testing according to the current regulatory guidelines employing animal experiments is time- and cost intensive we established the human 'Neurosphere Assay' which is able to mimic basic processes of brain development: Proliferation, differentiation, migration and apoptosis. However, automated high content image analyses (HCA) of such complex systems remains challenging due to (i) a variable cell density within the migration area, (ii) a 3D sphere core, leading to unfocused images, (iii) heterogeneous cell population of neurons and glia cells, (iv) sphere-specific endpoints.

Neuronal identification

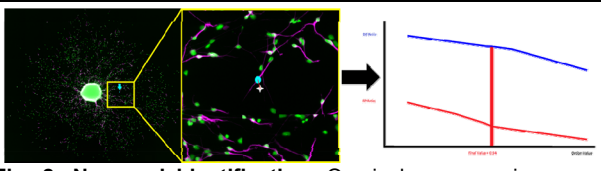

Fig. 2: Neuronal identification. Omnisphero comprises a self-developed algorithm called 'Neuron Tracer' designed to quantify neurons in complex heterogeneous 3D cell systems. This algorithm can be used without any previous knowledge due to a new concept implemented in Omnisphero: The user defines a neuron by manual assignment with a counting tool instead of defining an object over parameter settings. The position which is marked by the user (white cross) is mapped on the closest surrounding nucleus (blue dot). Obtained positions are consequently used to iterate all parameters of the 'Neuron Tracer' algorithm until the best ratio between detection power (DP) and false positive (FP)-rate is achieved[2].

Visualization of identified neurons

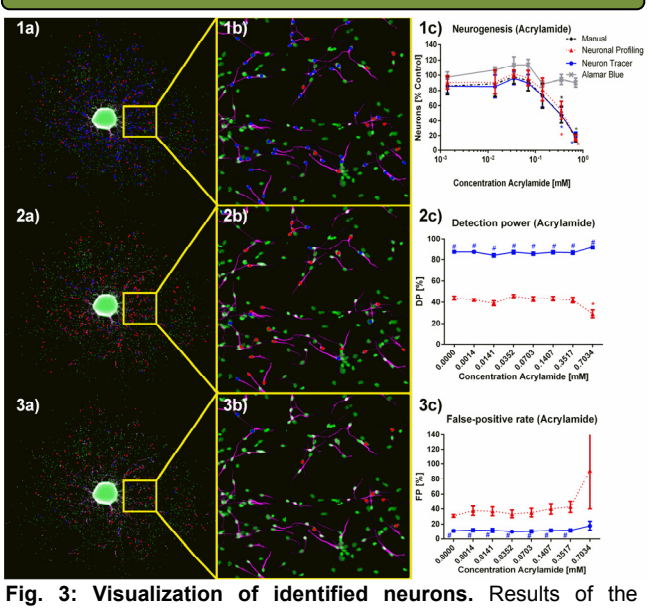

Fig. 3: Visualization of identified neurons. Results of the neuronal identification obtained with the 'Neuron Tracer' algorithm or with other software like the Neuronal Profiling bio-application (Thermo-Fischer) can be visualized within Omnisphero. It is possible to either display all neuronal positions of one or two algorithms on the overview image (1a) or in case a gold standard is available only false negatives (2a) and false positives (3a). The user can also zoom into regions of interest (1b,2b,3b) to identify subpopulations of neurons not identified by an algorithm or to spot systematic errors of an algorithm. The data can additionally be visualized as concentration response curves (1c) and DP (2c)- or FP (3c)- rate- graphs for different algorithms [2].

Literature

[1] Baumann, Barenys…Fritsche et al., *Current Protocols in Toxicology*, **2014**, 59, 12.21.1-12.21.24

[2] Schmuck, Temme, Mosig… Fritsche et al., (*in preparation*)

[3] Schmuck, Temme, Barenys… Fritsche et al., *Neurotoxicology*, **2014**, 43, 127-133

Neuronal morphology

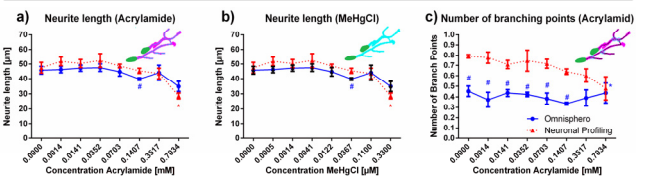

Fig. 4: Neuronal morphology. Identified neuronal coordinates (Omnisphero) were used to re-analyze neuronal morphology with the Neuronal Profiling software and compared to the original evaluation of the Neuronal Profiling software. While neurite length (a) and neurite area (b) show comparable results, the number of branching points (c) is significantly lower using the results of Omnisphero compared to the original evaluation. This might be explained by the high FP-Rate of the Neuronal Profiling software which leads to an introduction of artificial branching points [2].

Radial migration distance

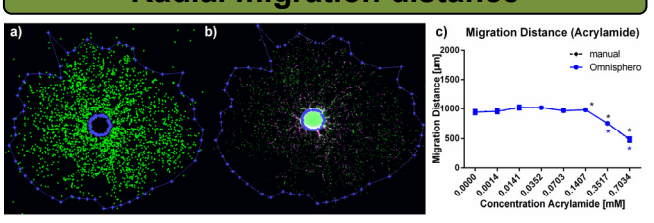

Fig. 5: Migration distance. Migration distance is assessed by using the density of coordinates of identified cell nuclei (a) and the intensity information of the original image (b). The migration distance is calculated as the mean distance between the sphere core and the furthest migrated cells. Results obtained by Omnisphero and manual evaluation are plotted against the concentration (c) [2].

Neuronal density distribution

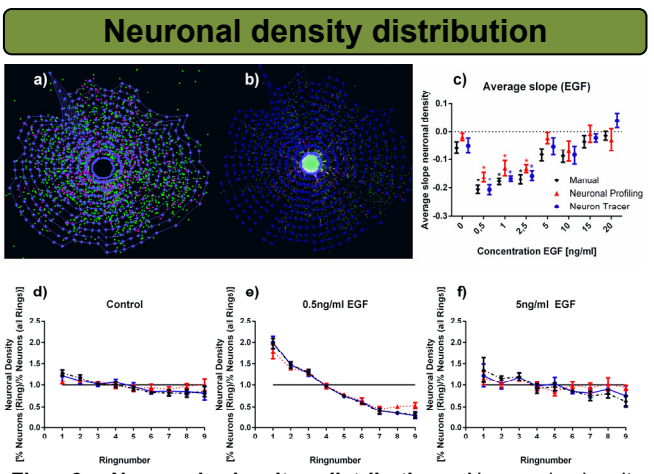

Fig. 6: Neuronal density distribution. Neuronal density distributions are assessed by dividing the evaluated migration area (Fig. 5) into ten rings (a+b) with equal widths. In each ring the ratio between the number of neurons and the number of cell nuclei is normalized to the ratio within the entire migration area. This results in a distance-dependent function of neuronal density (d-f). Since only the images, the coordinates of nuclei and neurons are required, evaluations of different origins can be used to assess this endpoint. Density distribution graphs can be condensed by plotting the average slope of each function for all concentrations (c) [2].

In vivo migration analyses: BrdeLuxe

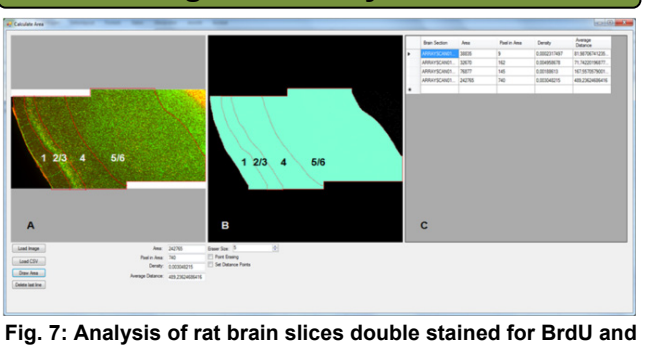

Fig. 7: Analysis of rat brain slices double stained for BrdU and Nissl. Brain slices were double stained for BrdU incorporation (red) and Nissl (green) for identification of brain layers. The red channel was analyzed using the Spot Detector algorithm (Thermo Fischer) to obtain positions of BrdU-positive cells. The derived data were analyzed using the self-written program BrdeLuxe. This program enables an automated evaluation of cortical layers for total number of $BrdU^{+-}$-cells and $BrdU^{+-}$-cells density in each cortical layer. This opens the opportunity to compare *in vitro* with *in vivo* migration [3].

Summary & Conclusion

Omnisphero is the preferred program for neuronal identification in high-density mixed neuronal and glial cultures:

- **High accuracy and precision algorithms for neuronal identification and morphological evaluation in neurospheres**
- **A visualization tool to compare neuronal identification evaluations of different algorithms**
- **Evaluation of neurosphere-specific endpoints: Radial migration and neuronal density distributions**

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