Application Note

LABEL-FREE DETECTION OF LIPID INCLUSIONS

Using the example of lipid inclusions in HepG2 cells, we demonstrate how the VAIDR system can turn a human observation into a quantitative, robust analytical method.

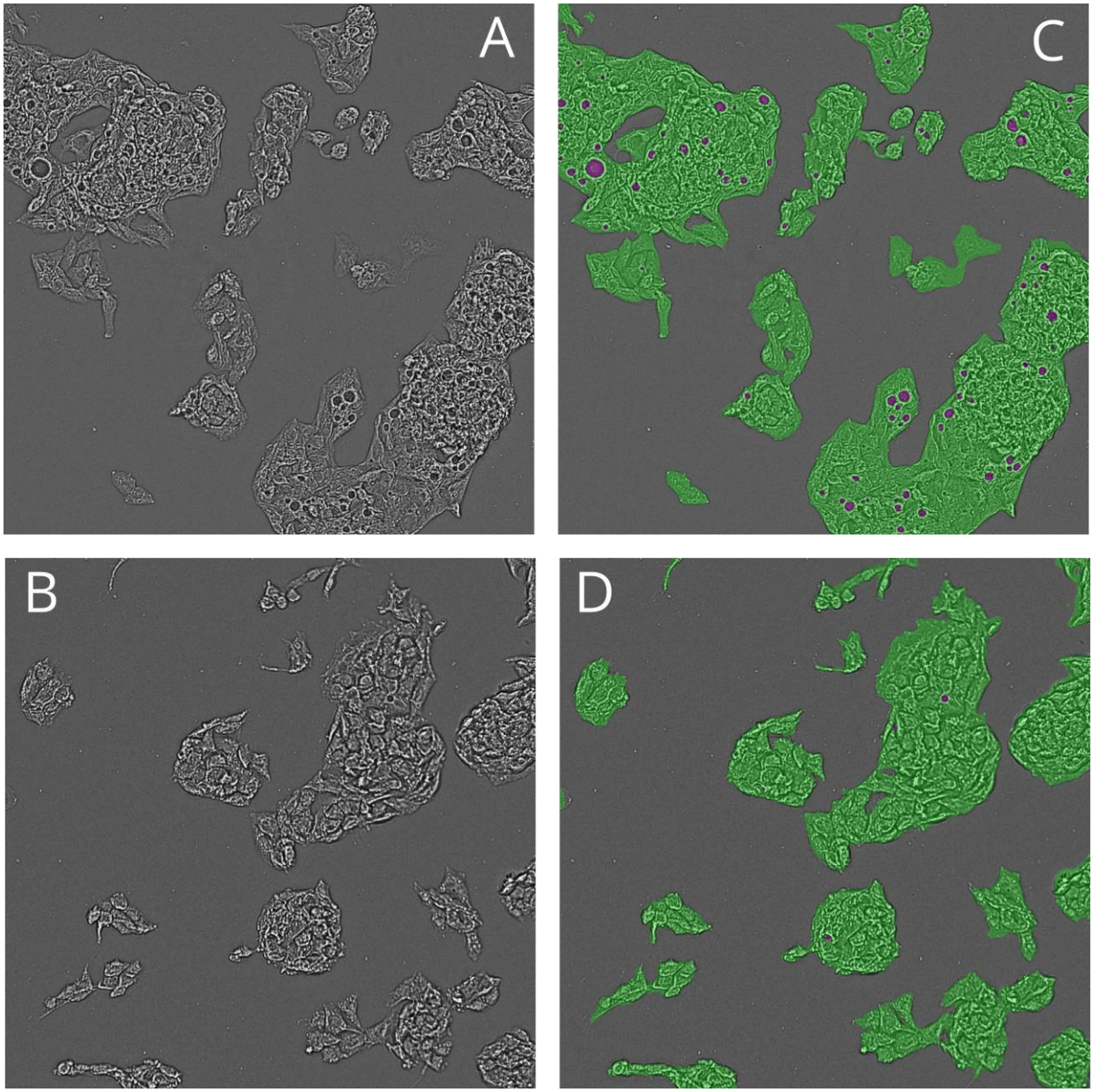

Figure 1: (A) Un-treated HepG2 cells, (B) treated with the antibiotic nitrofurantoin, (C, D) Analysis result with the VAIDR software shows cells in green and lipid inclusions in magenta.

The group of Prof. Stefan Schildknecht of Hochschule Albstadt-Sigmaringen develops sensitive toxicity assays, to increase the safety of novel drug products and to obviate animal testing. One important tool are hepatoxicity assays, e.g. using the liver-carcinoma-derived cell line HepG2.
On coated plates (50 µg/ml poly-L-ornithine and 1 µg/ml fibronectin), HepG2 cells exhibit an abundance of lipid inclusions within the cytosol. As shown in Fig. 1. A, these show up as roughly circular, featureless spaces similar to vacuoli. Following treatment of the cells with nitrofurantoin (30 µM for 24 h), the inclusions disappear (Fig. 1B).
While easily discernible in brightfield- or phase-contrast microscopy, it is not trivial to quantify these structures using label-free microscopy alone. However, the deep-learning-based analysis capabilities of the VAIDR system are perfectly suitable for this kind of task.
To develop an automated method for inclusion quantification, HepG2 cells were plated in 18 wells of a 96 well-plate. 9 wells were left untreated, and the remaining wells were treated with nitrofurantoin (30 µM for 24 h). All wells were imaged (4 images per well) using the VAIDR microscope, which generates digital phase-contrast images. A total of 7 images were then labeled using the labeling interface of the VAIDR system. The labeling process essentially consists of painting the different regions (cells and inclusions, in this case) in specific colors. These labels were then passed to the system to train a classification model.

The model was then used to detect the inclusions in all 18 wells. The automatically analyzed images were used to validate the successful training. The model performs very well at distinguishing the cells from the surrounding background, a task that is valuable, as it allows to quantify cell confluency. Importantly, the model also performs very well at detecting the lipid inclusions.

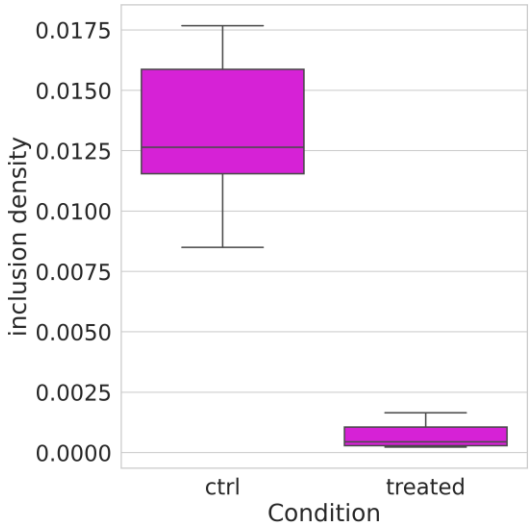

Fig. 2: Lipid inclusion density for control and treated conditions

To evaluate the results quantitatively, inclusion density (the ratio of the areas covered by inclusions and cells, respectively) was computed for each imaged field for all wells. The results are shown in Fig. 2. The untreated wells exhibit a remarkably consistent inclusion density close to 1.5%, while the treated wells consistently have an inclusion density which is about 10 times lower.

In summary, we have shown an example of a successfully developed, non-invasive lipid inclusion quantification method using the VAIDR system. No specific data science knowledge was required to set up the analysis, as all the deep-learning and data-logistics capabilities are easily accessible through the user interface, which can also be controlled remotely from any computer within the organization. Similar analyses can be set up for practically all visually discernible effects using the same methodology.

Reference:

Wijaya LS, Rau C, Braun TS, Marangoz S, Spegg V, Vlasveld M, Albrecht W, Brecklinghaus T, Kamp H, Beltman JB, Hengstler JG, van de Water B, Leist M, Schildknecht S. *Stimulation of de novo glutathione synthesis by nitrofurantoin for enhanced resilience of hepatocytes*. Cell Biol Toxicol. 2021 May 22. doi: 10.1007/s10565-021-09610-3

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