CELLPLY

TECHNICAL NOTE

Use of AI to automate the identification and characterization of diverse cells within a co-culture array

HIGHLIGHTS

- VivaCyte Analytical Software powered by deep learning algorithms for image analysis enables fully automated cell detection and data extraction from images
- Application-specific AI models enable detection and analysis of a range of sample types, including cell lines, primary immune and tumor cells, co-cultures and cells mixed with microbeads
- Automated cell detection allows to easily screen co-cultures containing single or multiple effector cells and quantify killing and serial killing activity of individual effector cells

The CellPly VivaCyte platform is designed to streamline operations for multi-parallel functional assays and to deliver actionable data from samples while keeping a near zero end-user hands-on time. The VivaCyte can run application-specific pre-programmed or customized workflows which allow users to run complex assays with robust and reproducible results. For analytical data processing, where many subjective decisions and much hands-on time for data review is normally required, the VivaCyte software enables a hands-free processing to deliver reproducible and robust results for the analytical steps required to transform bio-images to output results. The VivaCyte Analytical Software leverages state-of-the-art deep learning tools and pre-trained models to automatically extract data from thousands of images with minimal end-user processing. This technical note shows examples of live cell detection and automated extraction of single-cell data from images acquired in the CC-Array chips by adopting models suited to a quite diverse set of applications and sample types.

Application-specific AI models for single cell detection

Images and metadata acquired by the VivaCyte instrument are analyzed through a software interface (Figure 1) which displays the images, collects information on sample(s) type, concentration and fluorescent markers and allows to configure the AI models to use. Single cell detection is obtained through a deep learning algorithm exploiting U-net convolutional neural network. The process becomes fully automated once a model is trained. For each application, pre-trained AI models have been tuned to benefit from all available information, i.e. phase contrast images acquired in Z-stacks and/or fluorescent channels for different types of markers. Table 1 lists an example of 3 trained models currently released, while Figure 3 shows examples of images obtained from different applications and processed with these models. It shall be observed that additional models can released for custom applications.

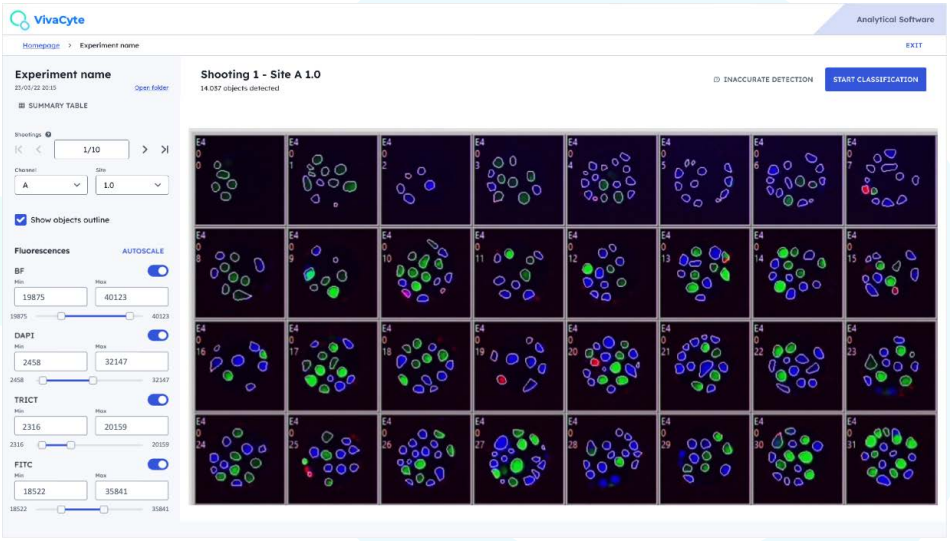

Figure 1. VivaCyte Analytical Software for the visualization of cell detection results.

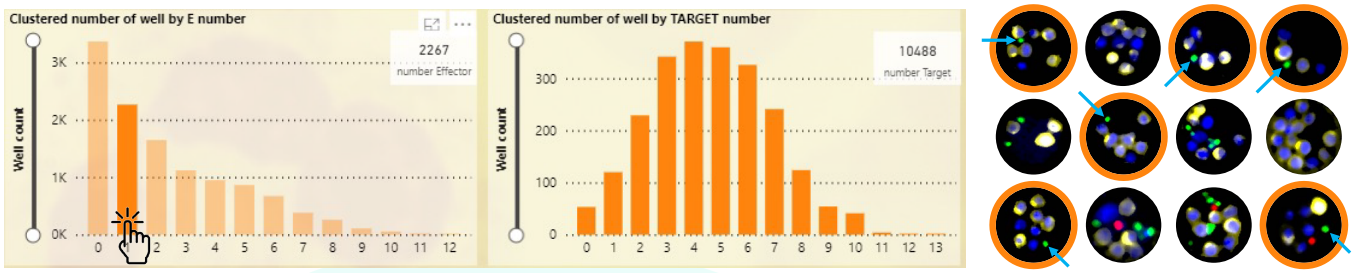

Figure 2. Dashboard functionality of filtering microwells featuring a single effector cells in the co-culture.

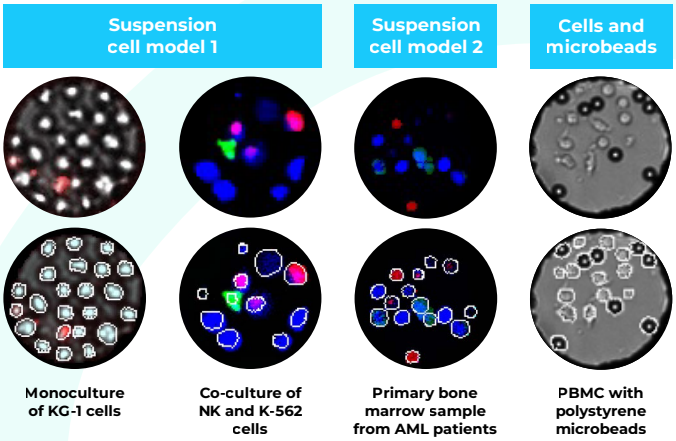

Figure 3. Examples of application-specific AI models for automated cell detection applied to different sample types.

Suspension cells model 1. This model has been trained on phase contrast images enhanced through Z-stacking, together with images expressing the death marker PI, visible in the "orange" fluorescence channel. This combination revealed to be the most effective for both live and dead cell detection. Validation of this model has been carried out on various tumor cell lines, primary immune cells and co-cultures of effector (E) cells and target (T) cell lines. The ability to exploit phase contrast images for cell detection brings the benefit of not requiring the use of a cell tracker marker for cell detection purposes.

Suspension cells model 2. This model is conceived for multi-channel fluorescence images and was trained on image sets featuring 4 different channels (one for live/dead marker, one for cytoplasm staining and two for surface antigen staining). It has been validated on primary bone marrow aspirates. Besides the PI death marker, CMAC was used as cytoplasm marker, which is visible in the "blue" bandwidth, and HLA-DR and CD34 were used as surface markers, in the "green" and "red" channels.

Cells and microbeads model. This model is optimized for applications involving the use of both cells and microbeads, e.g. cytokine release assays based cytokine capturing beads or experiments using CD3/CD28 coated beads for T cell activation. Phase contrast is the only input image needed by the model. The model has been tested on PBMC and microbeads.

Table 1. Example of 3 models trained for different applications

	Suspension cell model 1	Suspension cell model 2	Cells and microbeads
Phase contrast	●	○	●
Z-stack	●	○	○
Live/dead marker	●	●	○
Cytoplasm marker	○	●	○
Surface marker(s)	○	●	○
Validated sample types	Monocultures: KG-1, K562, HL-60, Nalm6, THP-1, NK cells Co-cultures: PBMC, NK cells + target cell lines	Bone marrow aspirates from AML patients (fresh and frozen)	PBMC, T cells + 6um and 8um polystyrene microbeads

Co-culture screening and data visualization

Following cell detection fluorescence intensities, morphological and positional data are extracted for each cell. The software tool suite then features a co-culture screening step where co-cultures are classified according to patterns of E-T cell co-localization, i.e. microwells with the same number of E-T cells are clustered together. An interactive dashboard allows then to select subsets of co-cultures on which output data are calculated, allowing to measure target cell viability at different E:T ratios. As an example, in Figure 2 bar charts show the distribution of co-cultures with respect to number of effector and target cells they contain, which commonly follow a Poisson model. The powerful software interface allows to select in just one click a subset of more than 2,000 microwells featuring an E:T ratio of interest, e.g. in the example one effector cell and any number of target cells. Thanks to this screening step it is possible to attribute the target killing activity in one microwell to the only effector cell it contains, making it possible to define a serial killing profile of the effector cell. Other screening types allow to select a range of E and T cells and evaluate cell killing across many E:T ratios in one experiment. This screening, combined with the upstream AI-based image analysis, provide a unique tool for seamless evaluation of immune cell function with single-cell resolution for diverse range of samples and applications.

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