Automated cell tracking and fluorescence analysis to assess individual killer cell efficiency

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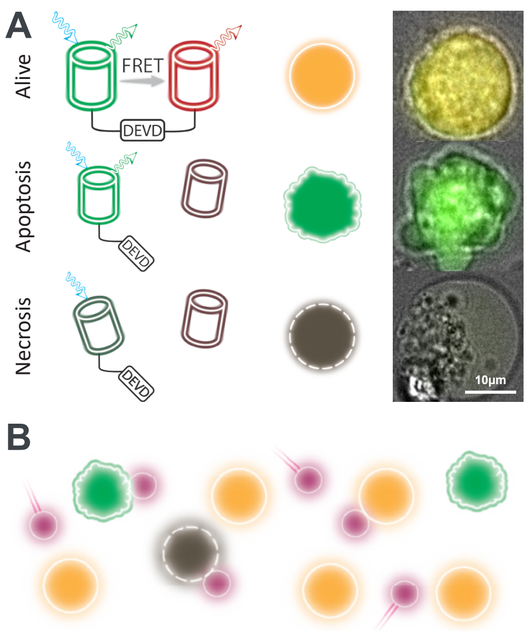
Abstract

Individualized immune therapy of cancer is at the cutting edge of medical advances. To assess the efficacy of new therapeutic treatments, the killing efficiency of human natural killer (NK) cells, specifically targeted at cancer cells needs to be evaluated. Population-based assessment of NK cell killing efficiency is an established method, but inherits several shortcomings. For example, population-level analyses do not provide information about the killing efficiency of individual killer cells. However, this may be a decisive factor for the success of a therapy. Emerging evidence points towards a heterogeneity among individual NK cells, ranging from inefficient killers to "*super killers*".
Based on a novel time-resolved single-cell cytotoxicity assay allowing to assess quality, quantity, and kinetics of target cell death induced by NK cells, we are developing an automated analysis pipeline. This not only allows us to track and analyze individual target cells, but also to assess the killing efficiency of individual NK cells in order to identify potential super killers. A key factor for this analysis is the identification of immunological synapses, which can be achieved using convolutional neural networks. This allows us to determine the fate of each NK and cancer cell, their respective contacts and the time point of cell death induction. The killing history of individual NK cells will give numerous insights into single NK cell cytotoxic efficacies.

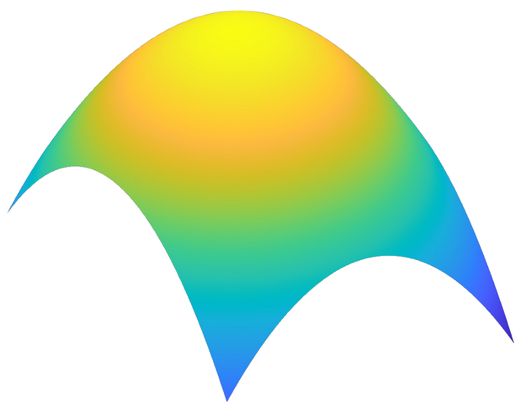


Cell fate determination

Differentiation of cell death types is achieved, using the pCasper-GR construct. NK cells are distinctively visualized, using CellTracker™ DeepRed.

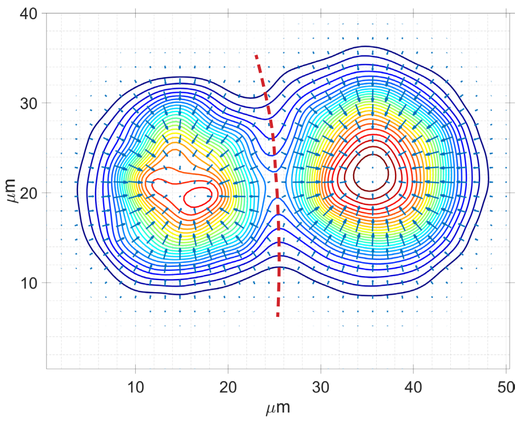
A) Schematic of the pCasper construct with a **GFP** and **RFP** fluorophore. **GFP** is excited by an external light source and in turn causes excitation of **RFP** via Förster resonant energy transfer (FRET). This mechanism is used to deduce not only cell vitality, but also the specific type of cell death.

B) Interaction of **NK cells** with vital **target cells**. Migrating killer cells sequentially induce cell death in the target cells either by **necrosis**, **apoptosis** or a mixed form of cell death, such as secondary necrosis.



Background correction

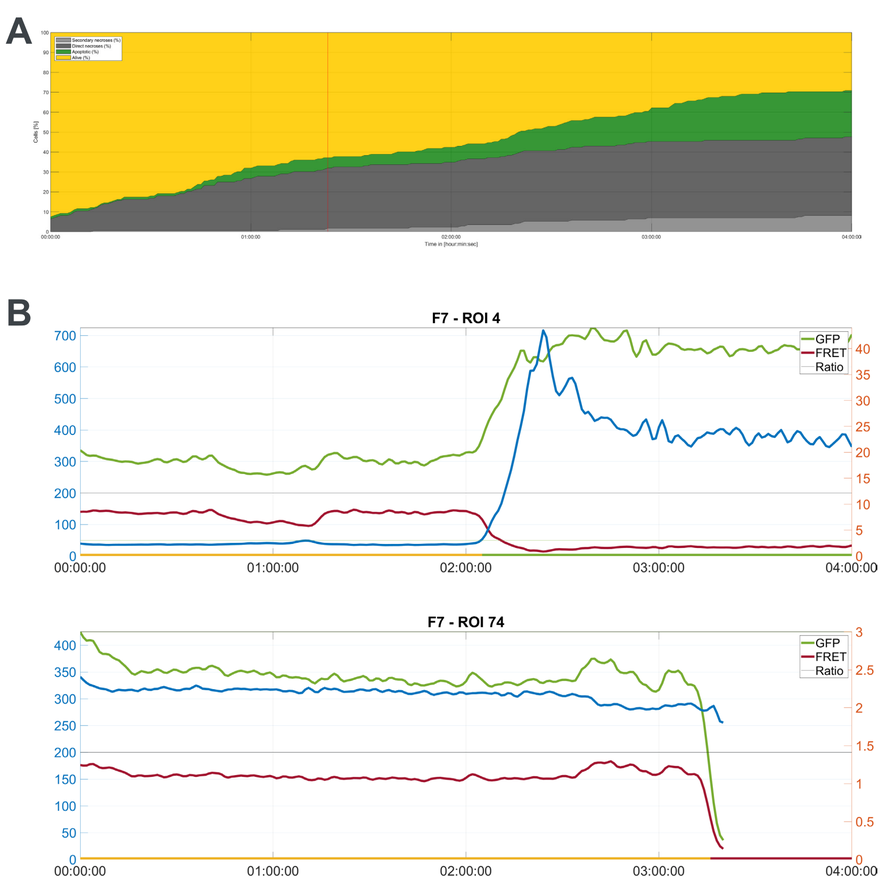
Images often suffer from lens effects such as vignetting, causing a decrease in image brightness towards the edges. This is especially noticable in the image corners. We fit a polynomial surface in a least-squares sense to each frame which is then subtracted in order to compase this effect. In addition, the original image is downsampled prior to the fit to accelerate background estimation.



Automated cell detection & tracking

Our processing pipeline allows to perform segmentation using different custom-made and common segmentation algorithms, such as gradient vector flow. Tracking is automatically performed after segmentation, using a Kalman filter. Frame-to-frame correlations of cells are computed as the L2-norm of the detected centroids and the cell region fingerprint (CRF) of a cell. Batch processing of entire multiwell plates is natively supported.

Cell fate analysis

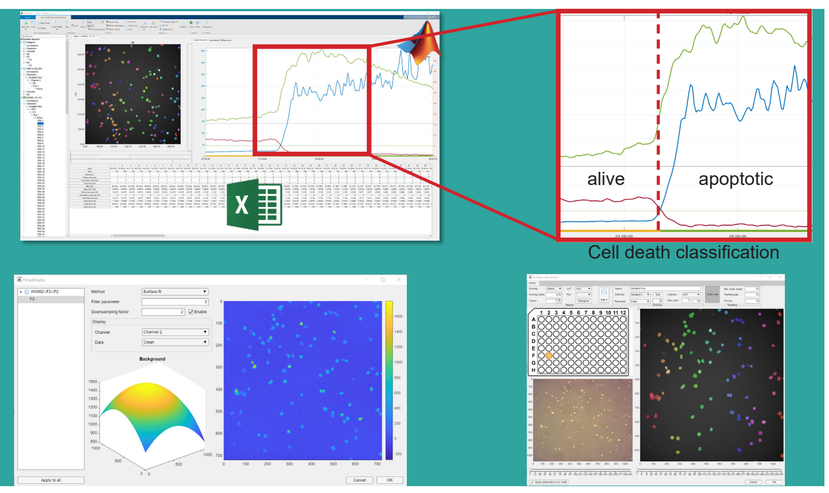


A) Death plots are generated on cell population level, showing the overall killing process over time. The specific type of cell death is color-coded (**green = apoptosis**, **dark gray = nectosis, light gray = secondary necrosis**, **yellow = alive**).

B) Individual, per-cell fluorescence profile plots allow to obtain a detailed analysis of the fate of each cell. This allows to determine the exact type and time point of cell death.

Summary

We are developing a MATLAB-based, easy to use, graphical analysis application to analyze not only individual wells, but entire well plates. This application incorporates different analysis modes, such as cell population analysis and lifetime analysis of individual cells, like target cells and primary killer cells. Current challenges include the robust and reliable analysis of cell contacts including synapse formation and induction of cell death. To achieve this, we are exploring options to train a deep learning nework, based on a U-Net architecture. Reliable synapse detection, will allow us to generate a complete killing history for each individual killer cell in order to identify highly effective *super killer* cells as well as ineffective killer cells.



References

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