Label-free recognition of non-activated and activated human T cells by Quantative Phase Imaging

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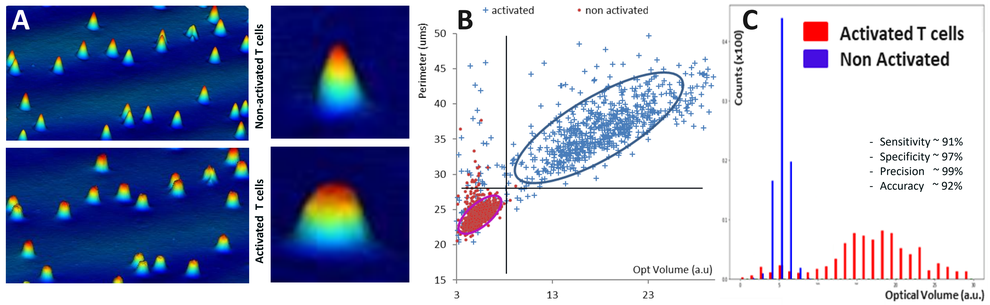
Abstract

There is a gap in current methods for evaluating quality and durability of cells in immunotherapeutic products like CAR-T.
Ability to identify and characterize immune cells on single-cell level without labelling would be highly valuable for biomedical research and clinical trials where unmodified cells are required.
We are developing analytical applications for label-free monitoring of human T cells activation and viral transduction by optical means using image based cell profiling methods.
An application for detection of T cells activation by Quantative Phase Imaging (QPI) was developed. We showed that stimulated cells could be statistically distinguished from non-stimulated by optical morphology fingerprints obtained from their phase images.
Miniaturization of cell bioprocessing platforms and performing cell Quality Control (QC) analysis in a fast and low-cost way would be a benefit. Microfluidic devices were tested and Quantative Phase Imaging was applied for detecting of non-activated and activated T cells in microfluidic chip. This presents a first step towards high throughput analytical platforms for CAR-T cells.

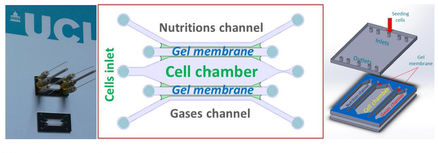
Results

❑ Label free Cell QC method was developed for monitoring of activation T cells by QPI



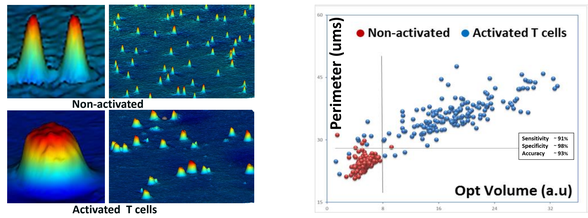
- We are developing analytical methods for label-free monitoring of activation and viral transduction of human T cells by optical means
- Non-activated and activated human T cells were imaged by qMod digital holographic camera (Ovizio Imaging Systems) on slides for 2D flow cytometry (Chemometrics) and shown on Fig. A
- Optical morphology fingerprints were obtained from cell holographic images for each cell being imaged. Then by Principal Component Analysis a few of these fingerprints, which have the most difference for non-activated and activated cells, were identified for further use
- A plot of Phase Optical Volume (POV), which is directly proportional to cell dry mass, versus cell perimeter is shown on a Fig. B above
- It was shown that stimulated T cells could be statistically distinguished from non-stimulated cells by optical morphology fingerprints obtained from their phase images. Standard statistical approach for evaluation of any new method for detection of a disease by biomarkers was used
- Evaluation of this method using Cell Phase Optical Volume as a parameter is shown on the insert to the Fig. C. Evaluation of sensitivity/specificity of detection of activated vs non-activated T cells taken from a second donor showed similar statistical output

❑ Label free recognition of non-activated and activated T cells on a chip by QPI



- The diffusion microfluidic cell culture chip was designed and manufactured
- It is optically transparent and built from Glass an Si
- Melted agar gel is loaded into the chip and guided by hydrophobic Au strips forming vertical gel membranes
- Gel membranes diffuse gases and nutritions in cell chamber

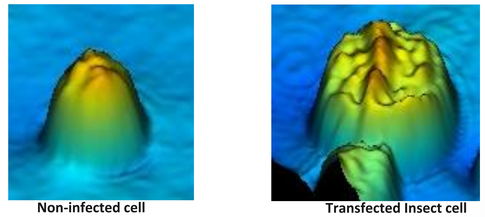
✓ It was shown that activated and non-activated T cells could be distinguished by QPI and the monitoring of activation of immunotherapeutic T cells *on a chip* is feasible



Figures up – schematic and prototype of the cell culture chip used for imaging; Figures down/left – typical phase images of non-activated and activated T cells; Figures down/right – plot of cell morphology fingerprints (cell Phase OV vs Perimeter) obtained from phase images, on the insert – statistics calculated from Phase Optical Volume distributions for non-activated and activated T cells

❑ Can we in the future monitor viral loading in human T cells?

✓Infected cells show changes on cell phase images (shown for infected Insected cells)



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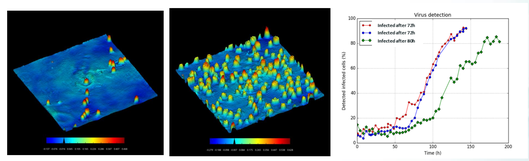
✓ Viral loading monitoring of infected Insected cells



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➢Allowing optimization of viral procedures
➢Can be done for any virus produced in cells

- Changes obtained on phase images of infected Insected cells are believed might be obtained for a viral transduced CAR-T cells allowing monitoring of viral loading in immunotherapeutic T cells

Future work

➢ Developing Critical Analytics applications for in-line QC of therapeutic cells using Quantative PI
➢ Developing relevant methods and techniques of high throughput image/spectra based cell profiling with emphasis on Deep Learning and Big Data
➢ Apply Raman spectroscopy for phenotyping of primary T/CAR-T cells
➢ Developing microfluidic cell culture for therapeutic T cells processing and analysis

Acknowledgements

Funding from the UK Engineering & Physical Sciences Research Council (EPSRC) for the Future Targeted Healthcare Manufacturing Hub hosted at University College London with UK university partners is gratefully acknowledged (Grant Reference: EP/P006485/1)
Financial and in-kind support from the consortium of industrial users and sector organisations is also acknowledged

In collaboration with