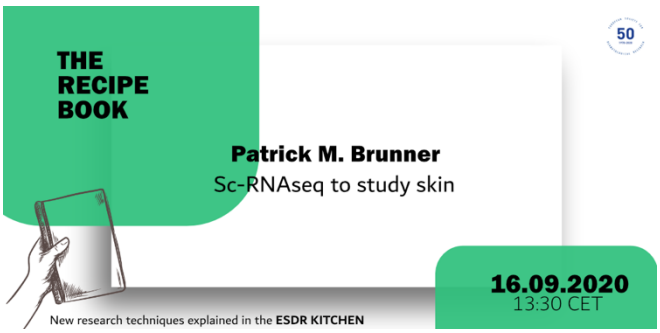

Q & A

The following questions raised during the above session were answered by Patrick Brunner. For further information, he can be contacted at patrick.brunner@meduniwien.ac.at

Q : Roopesh Singh

Different algorithms sometimes produce different results from the same data. How do decide which one to use?

A: It depends on the data and on the research question you have (main interest in large vs. small cell populations, actual samples etc). I think if results are conflicting, they definitely need validation in an independent experiment.

Q : Jeremy Di Domizio

Blister fluids usually contain many neutrophils however they do not seem to be captured in the scRNAseq data, how do you explain such difference?

A: Single-cell analysis of granulocytes in general is a big challenge. They are relatively low in RNA content, with high levels of RNases, and they are very fragile cells that might get damaged during processing.

Q : Jeroen Bremer

I have a practical question regarding tissue storage and dissociation protocols. How should tissue be stored when performing dissociation at a later stage? Is it possible to perform scRNA-seq on cryopreserved (non-fixed) skin?

A: I would advise to store single cell suspensions, if necessary. However, in our hands, fresh material gave us best results. We did not try whole tissue samples so far.

Q : Amanda Nelson

For experiments where before and after treatment effects are being investigated in the same person, how should samples be processed? Does fixing and storing of single cells for sequencing at a later date affect results?

A: We saw that over time, there was a degradation in quality of frozen cells, so I would advise to process the sample freshly each time, trying to be as consistent as possible with processing.

Q : Márta Széll
Do you see significant differences among different cell types of the skin regarding quality control?

A: In our samples (AD, healthy controls) we did not really have large problems in this regard.

Q : Marta Szell
With flow cytometry we study the expression of immune markers on individual cells. As an analogy can we call this method as "flow transcriptometry"? Even the demonstration of the results recall the graphs we produce while demonstrating the flow cytometry results

A: I guess there is an -omics term for everything ;-) but flow cytometry usually uses pre-defined markers that are chosen when planning for the experiment, whereas RNAseq really allows the identification of markers in a less biased, unsupervised fashion.

Q: Sara Brown
Can you comment on the sensitivity threshold of transcriptome analysis in sc data compared to bulk transcriptome analysis?

A: Single cell data are for sure less sensitive, and there is a significant amount of cells that show “technical dopouts” for many markers – So if a marker is missing in your results, I would shoot for a different method and not assume that it is just not there.

Q : Daniel Kaufman
How do you get a single cell isolated out of the skin biopsy or blister?

A: From blister fluid, we get them out by simple centrifugation. For biopsies, we use minced biopsies incubated in collagenase IV in 10% FCS for 40min at 37°C. Epidermal sheets usually only need trypsin incubation for 10 min at 37°C.

Q : Terkild Buus
I found the blistering technique very intriguing. How many viable cells can you get out of a single blister, and how is the distribution of CD45+ vs CD45- cells as compared to cells from biopsies?

A: From blister fluid, we got approximately 5,000 live cells in the fluid phase, with about 5% leukocytes. From epidermal sheets, you get many more, including keratinocytes etc that we did not really count – we aimed at single cell assays that required about 10,000 to 20,000 cells per sample, and this number was usually sufficiently present in each patient. I guess for more extensive FACS analyses, blister samples might have too few cells. However, cell number can be increased when the blister is left on the patient overnight (protected by a plastic cap), but conversely might influence the overall infiltrate, of course. In our experiments, we aimed at FACS sorting the same amount of CD45positive and CD45negative cells in each sample in each patient.

Q : Letian Zhang
Normally how many genes can you get from one cell by 10x?

A: Approximately 500 to 2,000 genes.