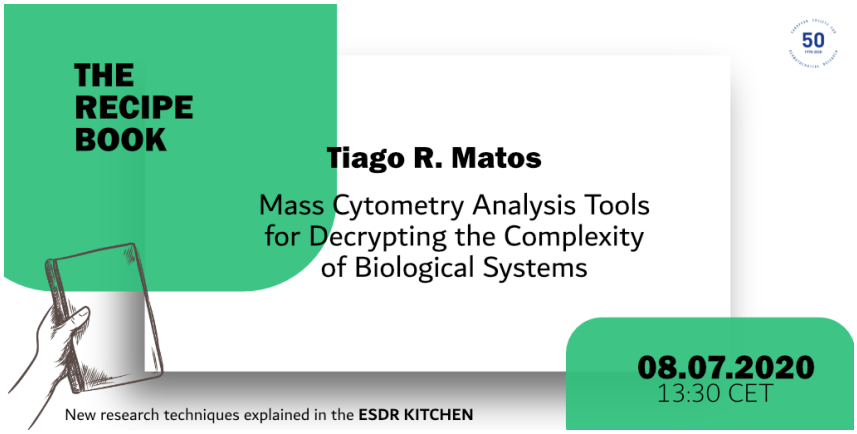

Q & A

The following questions raised during the above session were answered by Tiago Matos. For further information, he can be contacted at tiagoreismatos@gmail.com

Q. For the skin samples you have used - do they need to be fresh or is frozen ok?

A. The samples can be both fresh or frozen. However, it is crucial that the sample processing is identical for all samples.

Q. Any examples of application of mass cytometry in wound healing?

A. I know that certain researchers are using mass cytometry in wound healing, such as Frank Niessen and Sue Gibbs from the Amsterdam University Medical Centers, Location VUMC. However, I haven't come across any publication on this topic yet. On the website of Fluidigm, you can find most publications that have used mass cytometry.

Q. How would you imagine to increase the number of targets to be detected?

A. It is possible by combining panels.

See: Bendall SC, Simonds EF, Qiu P, Amir ED, Krutzik PO, Finck R, *et al*. Single cell mass cytometry of differential immune and drug responses across the human hematopoietic continuum. *Science* 2011;332(6030):687e96

or Sen N, Mukherjee G, Arvin AM. Single cell mass cytometry reveals remodeling of human T cell phenotypes by varicella zoster virus. *Methods*. 2015;90:85-94.

Furthermore, Fluidigm is adding more isotopes, allowing in the future to analyse more parameters simultaneously.

Q. Is there a lower limit to the sensitivity - i.e. what is the lowest percentage of cells one could detect?

A. It is advised to run a minimum of 5 million cells. Have in consideration that only +/-50% of the cells introduced are analysed. For the analysis tools it has been suggested to have at least 300 events per subset of cells. I have run sometimes a few thousands of cells, which allowed to see expression of some transcription markers. However, I wasn't able to use trajectory detection or cluster analysis tools.