The following questions were answered by Beatrice Dyring-Andersen during the ESDR Kitchen session above.

Q: What should be the minimum quantity of cells collected by laser capture micro dissection for proteomic analysis?
The mass spectrometry field is in a rapid technological development so the numbers will change accordingly over time. For now, you get reasonable numbers with 500 cells.

Q: Was the difference in skin phototypes also considered in skin atlas project ?
The focus of the Skinatlas was the skin layers, immune cells, endothelial cells and melanocytes. We did not investigate/factor in skin phototypes.

Q: Have you involved the hair follicle proteome in the skin atlas project?
The hair follicle proteome was not included in the Skinatlas and would be a great idea for a project, preferably using LCM combined with MS-based proteomics.

Q : Nice presentation. You talk about data normalization, if you would like to compare two samples for example, what do you use for that?
We normalize all data, filter for valid values (for example 70%) and impute (there are different ways to impute, but the most common one is to add artificial very small numbers to proteins that are not identified in some of the samples so that you don´t have missing values and can continue with statistical analyses). All the parameters depend on the setup of the experiment. Are the samples from the same patients? For 2 groups you could use a T-test. It is important to use false discovery rate correction due to the amount of data.

Q : Hi Beatrice, very interesting talk. I have a question on the peptides (eg. antimicrobial peptides) that are already present on the skin at the time of sampling. Are they always present throughout the sample preparation until acquisition? or they are excluded during fractionization?
Antimicrobial peptides are identified through the method that was presented. They should not be excluded during the sampling processing. In fact, you could quire

some of them on skin.science. There might be exceptions depending on their size, chemical properties and if they are known/predicted.

Q : Was the database you used based on only host proteins? did you also include bacterial/microbial databases?
The data for the Skinatlas was analyzed using human database. All the raw files are available and can be searches against other databases. ☺

Q : Low abundant proteins may be important. How good is your method to detect very low abundant proteins?
Low abundant proteins are indeed very important and your question concerns what is called “the dark corner of the proteome”. We do not know the numbers in this case but I think that we have a deep proteome when we can identify cytokines for example. Or when the proteins are so low-abundant that we can not stain for them properly to validate the findings. The answer is somewhere in the future ESDR meetings.