Questions	Answers (Written or time location in video)
Did you compare the DIA-SRM with DIA-PRM on same set of peptides in same sample? I am curiously to learn this. Thanks!	live answered 09:07 AM
A key part of my project is to try to detect roughly 100 small proteins (<50 aa) in an organism that remain almost undetected in most LC-MS methods (DDA and DIA), but are known to exist. So I am currently working on predicting peptides for these proteins and generating a spectral library using Prosit. Is it possible to export this data to a PRM method using Skyline without having any previous DDA/DIA data containing these proteins?	If you have protein sequences, you can import them into a Skyline document to come up with a PRM assay. In Skyline you can adjust the peptide settings to mimic what you would expect to see - e.g. if planning on digestion with trypsin, missed cleavages, peptide length, etc, and come up with a list of predicted peptides. You could also import your Prosit predicted peptides as a spectral library into Skyline. You can then use the predicted peptide and precursor information in your Skyline document to build a PRM method. I would suggest an unscheduled PRM method for predicted peptides if they haven't been seen before. If they occur in the samples analyzed you will then be able to analyze them in Skyline and have an idea of the quality of the detections. There is a tutorial and several webinars on PRM in Skyline available: https://skyline.ms/wiki/home/software/Skyline/page.view?name=tutorials. The predicted prosit spectral libraries could also be used as additional evidence or scoring for these peptides.
How much instrument time does it take to do the initial assessment of the %CV in this opitmization approach? How many injections do you do per sample?	3 x 6 = 18 injections
The fragment intensities for triple quad SRMs depends a lot on source parameters and collision energy.. How was this enumerated from DIA runs which are generally done without optimizing source parameters or CE for respective peptides?? was there a second level of SRM optimization involved or went with general DIA params??	live answered 09:16 AM
Is it possible to import a defined set of modifications as a file?	I do not believe there is any way to import modifications from a file. Typically you go to "Settings > Peptide Settings > Modifications" and tell Skyline about all of the modifications that you care about. Alternatively, when you import a peptide search, Skyline will try to guess which UniMod modification corresponds to the modification masses that Skyline saw in the peptide search results.
do you also consider multiple precursor charge states to build out a single set of product ion transitions per peptide?	Skyline can support multiple precursors per peptide. Though, it does change how Skyline scores the best peak. As you can see these tutorial steps are optimized to assume you are going to choose a best precursor rather than treat all precursors equally and assemble a best set of transitions across all detectable precursors.
For inteference free peptides, what threshold/value do you use to determine this	live answered 09:12 AM
how much PRTC peptides you added into each sample to generate the DIA-peptide library?	We typically use either 50 or 150 fmol per injection; somewhat based on the complexity of the sample; if not very complex we use 50 fmol, if more complex maybe up to 150. In the dataset shown I used 150 fmol per injection (3 ul injections of 1 ug peptides each)
How many minimum transtion are required for each peptide?	In this tutorial, the minimum number was set to 3. That is generally considered the minimum at this stage. Though, when isotope labeled standards are added the number can go as low as 1 transition for a fully developed clinical assay.
Is DIA can be done in Orbitraps instruments (PRM)? Which are the main issues/differences to take into account?	live answered 09:10 AM
Why are the same of the peptides eluted at different RTs in LibA, B & C??	These are different runs on a mass spectrometer, potentially on separate days. Retention time will differ across the runs. In most cases, this should be representative of elution time variance in the chromatography.
can you please show again how to give selected "protein names" to keep	You use the Refine > Accept Proteins menu item.
and can you keep certain proteins which does not pass the filrter criteria of 30% CoV	live answered 09:20 AM
Can I import DIA data from any kind of Software, e.g. data searched in Spectronaut?	The menu item to import peptide search results from other software is "File > Import > Peptide Search". The list of types of peptide search results that Skyline can import is on this page: https://skyline.ms/wiki/home/software/BiblioSpec/page.view?name=BlibBuild "Spectronaut" is on that list and it says that you would import a .csv file that you create by exporting a spectral library from Spectronaut.
Can I import DIA data from any kind of Software, e.g. data searched in Spectronaut?	You should be able to import an assay library from Spectronaut, but not yet results in the same way as we did here for EncyclopeDIA. We are working with the development teams most interested in working with us. You can do results import so far for: OpenSWATH, DIA-NN, Paser, and EncyclopeDIA. DIA-NN and Paser are relatively new and only in Skyline-daily.

Is the chromatography that is being used in the final method the same as the individual GPF-DIA injections?	live answered 09:21 AM
Could you filter the peptides by noise to ratio?	This is not currently easily achieved in Skyline.
Could you filter the peptides by noise to ratio?	But you can use any metric to make decisions outside of Skyline and then use Refine > Accept > Peptides to filter your list down to only the peptides you want to take forward to your next round of refinement.
Has this pipeline been used to bring a targeted assay into use in a CLIA lab? If not, are there any groups actively navigating this process?	live answered 09:12 AM
*signal to noise	live answered 09:02 AM
thanks!	live answered 08:58 AM
Can I export the peak areas? Can the total peak area for all transitions be exported?	Yes. Skyline has extensive support for exporting all of the values you see in reports and viewing them during Skyline use in the Document Grid. There is a good tutorial on custom reports in Skyline on the website.
The RT prediction is just based on measured iRT values and not on pep seq is that right?	Yes. That is correct. Skyline also has a built-in implemenation of SSRCalc (Sequence Specific Retention Calculator), but here we are using emprical measurements in DIA to train iRT values that will be used in the SRM method.
A quick follow up on the RT difference query. Do setting a threshold on RT CV is advisable to set more stringent criteria for target selection??	Skyline doesn't make that particular filter easy to implement, but you can use whatever metric you want outside of Skyline and then use Refine > Accept Peptides to narrow your list to just the peptides you want to take forward to your next round of refinement. It would not be hard to export a retention time report, even with retention time standard deviations to develop a list of things you want to keep.
Probably, i missed this part. How was the collision energy predicted based on the DIA data?? Thanks..	live answered 09:17 AM
Is 250 concurrent transitions for all instruments, or just QqQ ?	live answered 09:33 AM
Is there a log to see which peptides are removed and why during your refinement steps?	live answered 09:19 AM
I need to use a core facility to get my DIA data, and take the results from them to make my SRM method. Advice no how to go about giving them instructions on how to acquire the DIA data?	This paper from Pino et al have a nice guide to DIA methods, and have some specific method parameters if you need a starting point! https://pubmed.ncbi.nlm.nih.gov/32312845/
In a single scheduled SRM method, how many transitions at maximum can be taken (to be run in a Triple Quadripole).	live answered 09:30 AM
Thank you for the very informative answer.	live answered 09:16 AM
Great! Thanks!	live answered 09:22 AM
Thank you for the answer!	live answered 09:21 AM
How much variation in abundance do you allow between peptides representing a specific protein?	live answered 09:28 AM
Thanks	live answered 09:21 AM