

# Skyline 2023

State of the Project  
15 years after inception

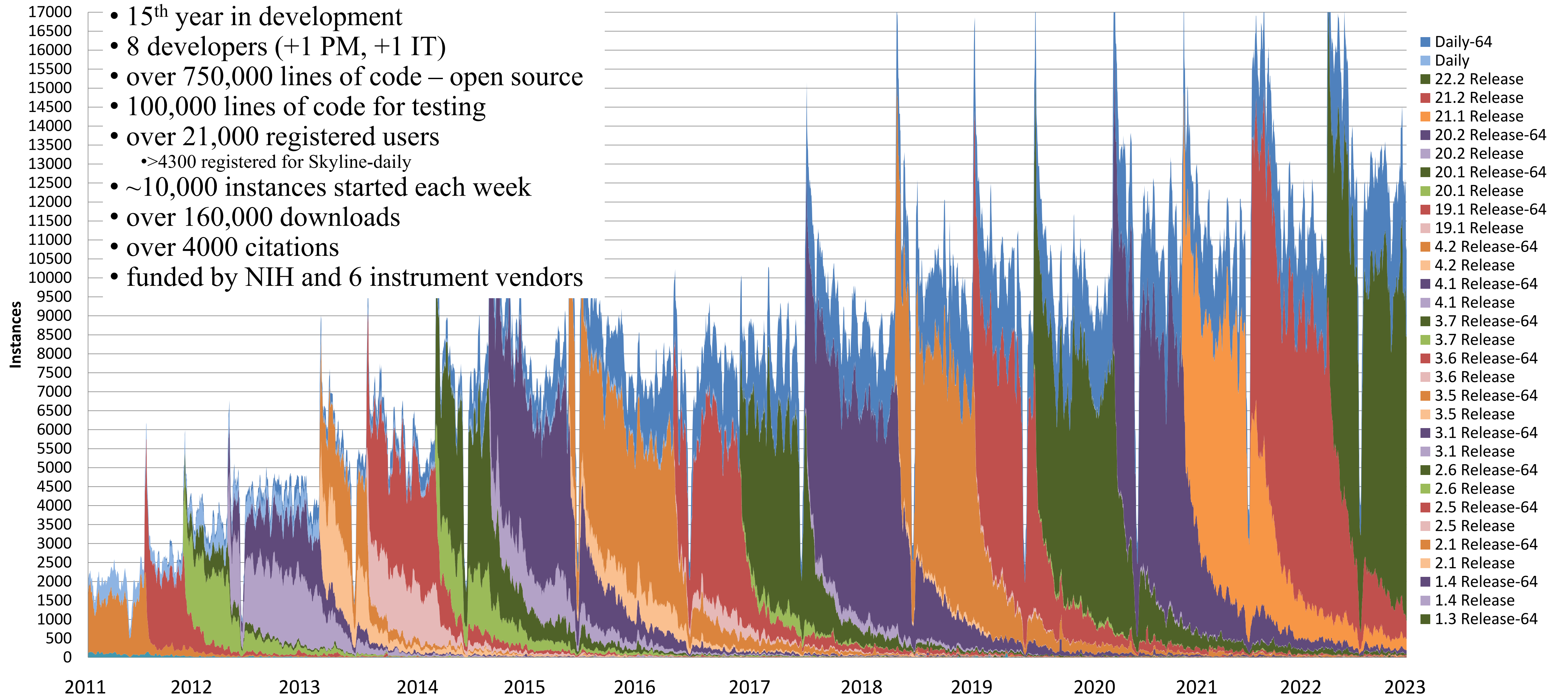
Brendan MacLean



UNIVERSITY *of*  
WASHINGTON

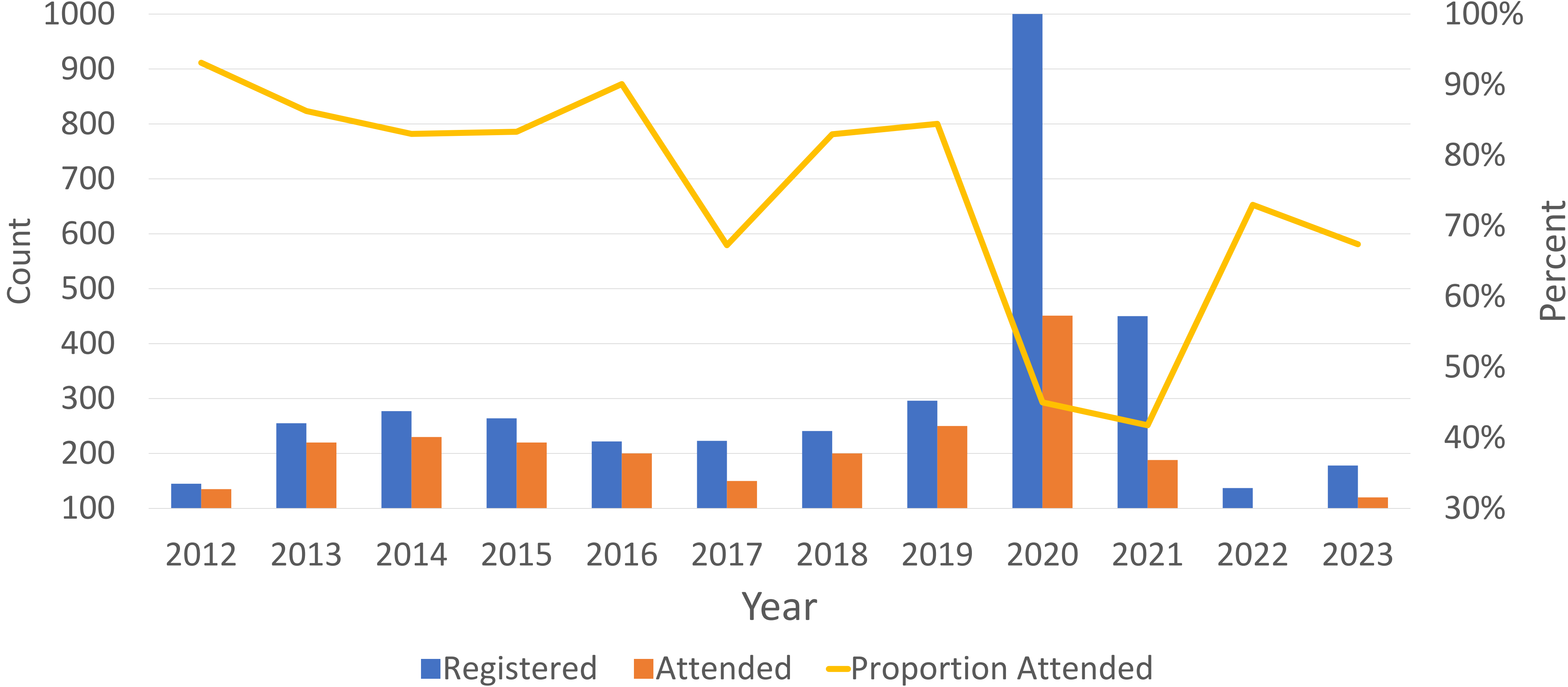


# Skyline Project Overview



# Meeting Attendance by Year

Skyline UGM Attendees by Year



# Teaching Targeted Proteomics In-Person Again

- 2-ASMS, Minneapolis – June 4&5 (25)
- 5-U. of Wa., Seattle – June 27 - July 1 (25)
- 5-PRBB, Barcelona – November 13-18 (25)
- 4-ISAS, Dortmund – March 20-24 (35)
- 2-ASMS, Houston – June 3&4 (43)
- 5-U. of Wa., Seattle – July 10-14 (35)
- 5-PRBB, Bracelona – November 12-17 (25)

## And Online!!

- 2022 UW Skyline Online – October 10-21
- 2023 UW Skyline Online – Fall

# Skyline Achievements

- Protein grouping
- Integration with EncyclopeDIA, DIA-NN, MS Fragger, Paser
- CE Optimization for PRM
- Improved DDA support for MS/MS use
- z+1 and z+2 ion support for E\*D fragmentation
- Improved line plots for dotp/idop/rdotp in Peak Areas graph
- Spectrum property sheets
- Candidate Peaks view for understanding peak scoring
- LOTS of smaller improvements and fixes



# Custom Precursor Filters



Edit Spectrum Filter

Editing spectrum filter on 496.2867++ (heavy)

Property	Operation
Analyzer	Equals
<ul style="list-style-type: none"> <li>MS1 Precursors</li> <li>MS2 Precursors</li> <li>Scan Description</li> <li>Collision Energy</li> <li>Scan Window Width</li> <li>Compensation Voltage</li> <li>Preset Scan Configuration</li> <li>MS Level</li> <li>Analyzer</li> </ul>	

Skyline - OrbitrapVslonTrap.sky

File Edit Refine View Settings Tools Help

Targets

- Pierce standards
  - SSAAPPPPPR
  - GISNEGQNASIK
  - HVLTSIGEK**
  - 496.2867++ (heavy)
  - 496.2867++ (heavy) Analyzer=quadrupole/orbitrap
  - 496.2867++ (heavy) Analyzer=quadrupole/radial ejection linear ion trap
  - ELGQSGVDTYLQTK
  - SFANQPLEVWYSK
  - GLILVGGYGTR
  - GILFVSGVSGGEEGAR
  - LTILEELR
  - ELASGLSFPVGFK
  - LSSEAPALFQFDLK

Ecl\_2021\_0802\_easy\_75um\_50cm\_lit\_orbi\_MS2\_prtcqc\_EH\_16

Intensity (10<sup>6</sup>)

Retention Time

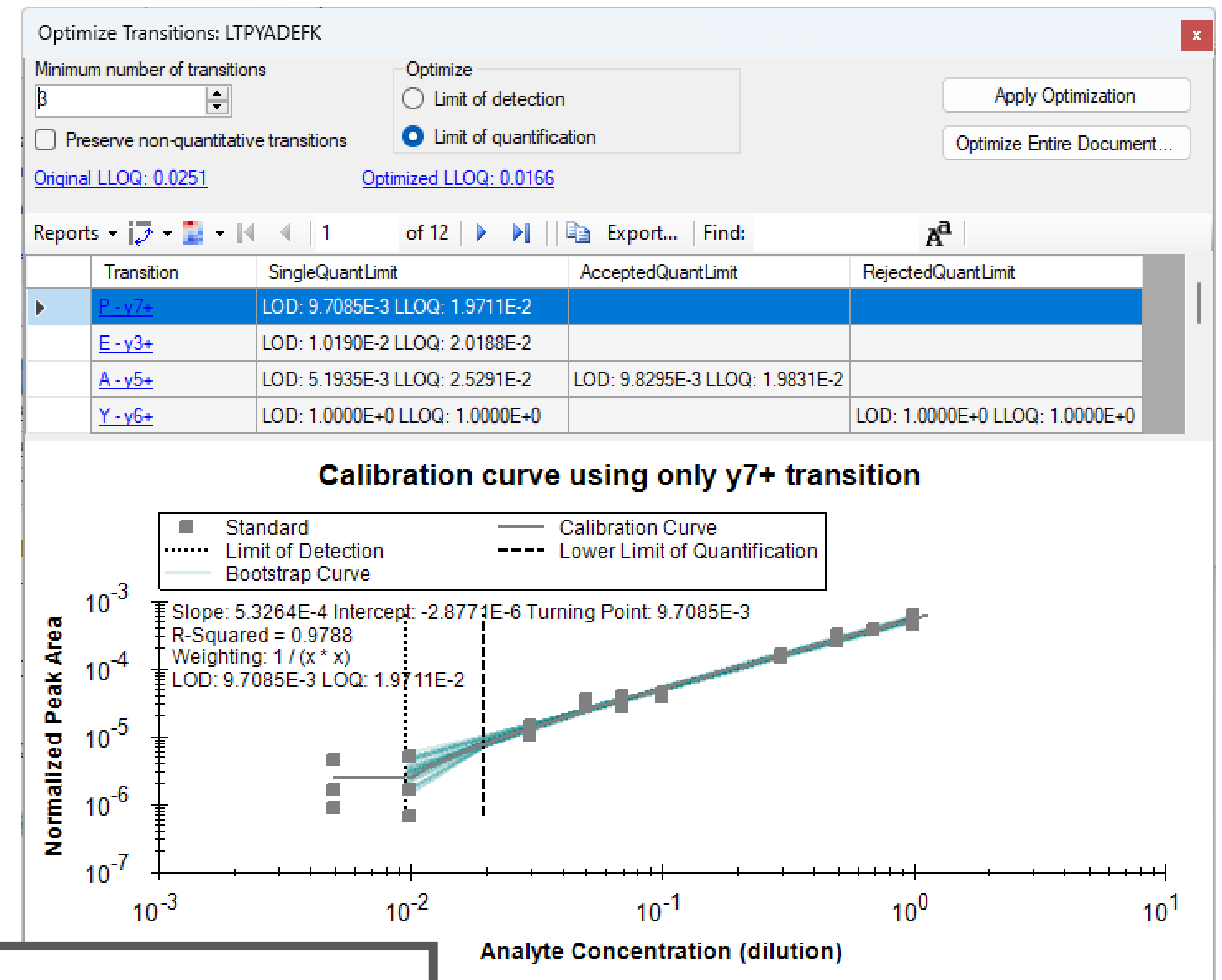
Ready

1/1 prot 3/14 pep 7/42 prec 37/243 tran

# Calibration Curve Transition Optimization

Refine View Settings Tools Help

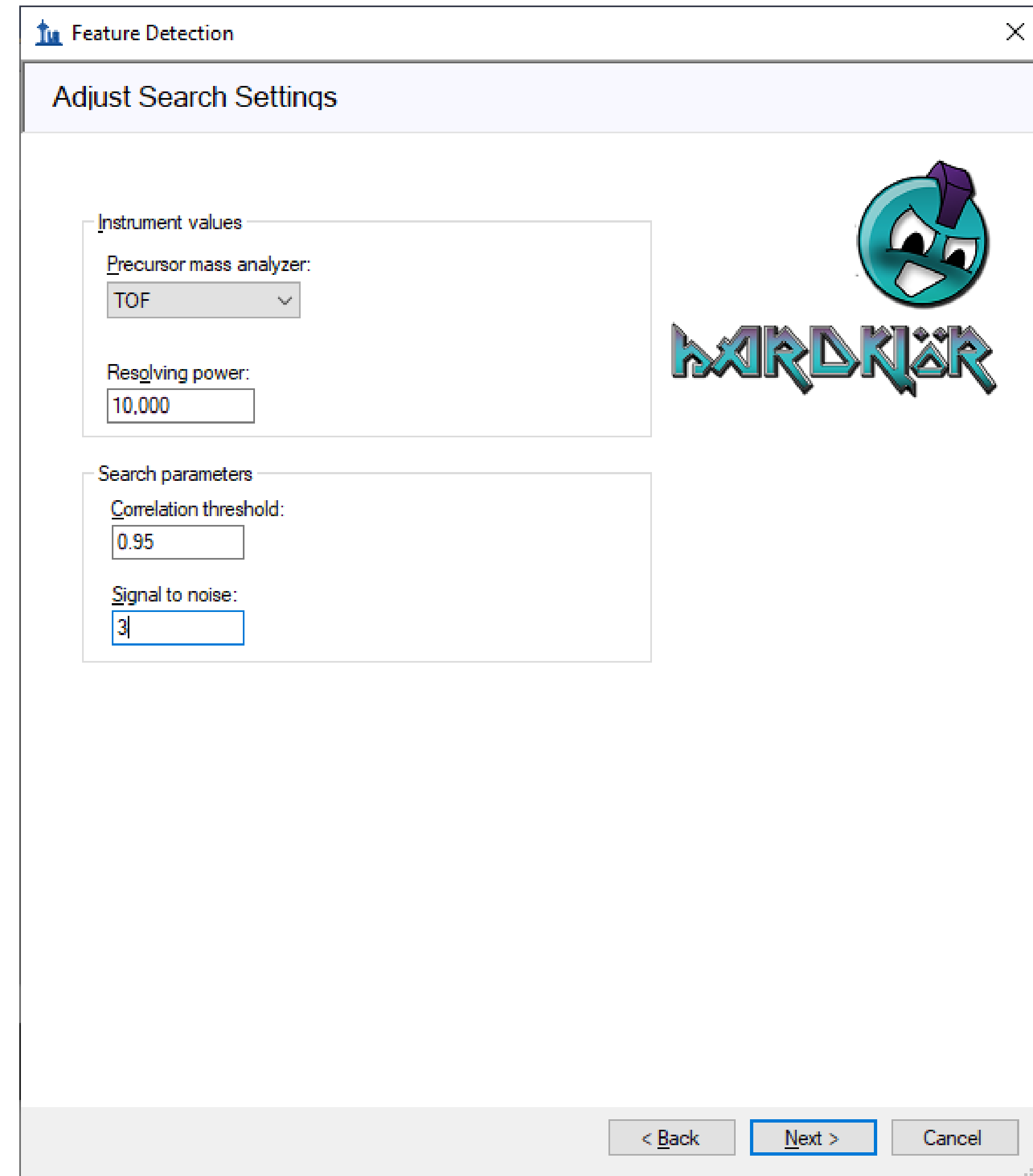
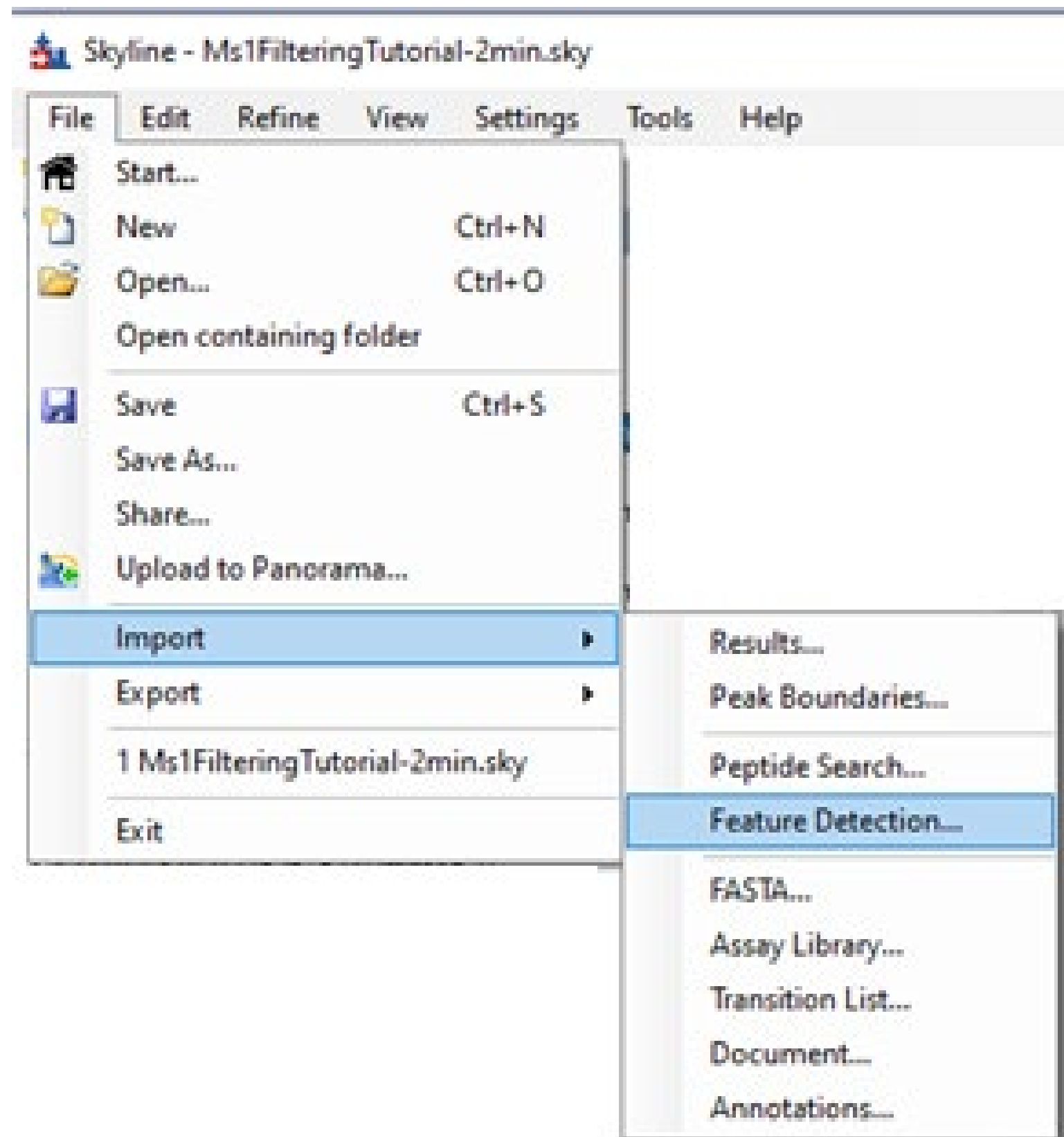
- Reintegrate...
- Add Decoys...
- Compare Peak Scoring...
- Remove Missing Results
- Accept Proteins...
- Remove Empty Proteins
- Associate Proteins...
- Rename Proteins...
- Sort Proteins
- Accept Peptides...
- Remove Empty Peptides
- Remove Duplicate Peptides
- Remove Repeated Peptides
- Optimize Transitions...**
- Permute Isotope Modifications
- Advanced...



Nick Shulman – TP 552

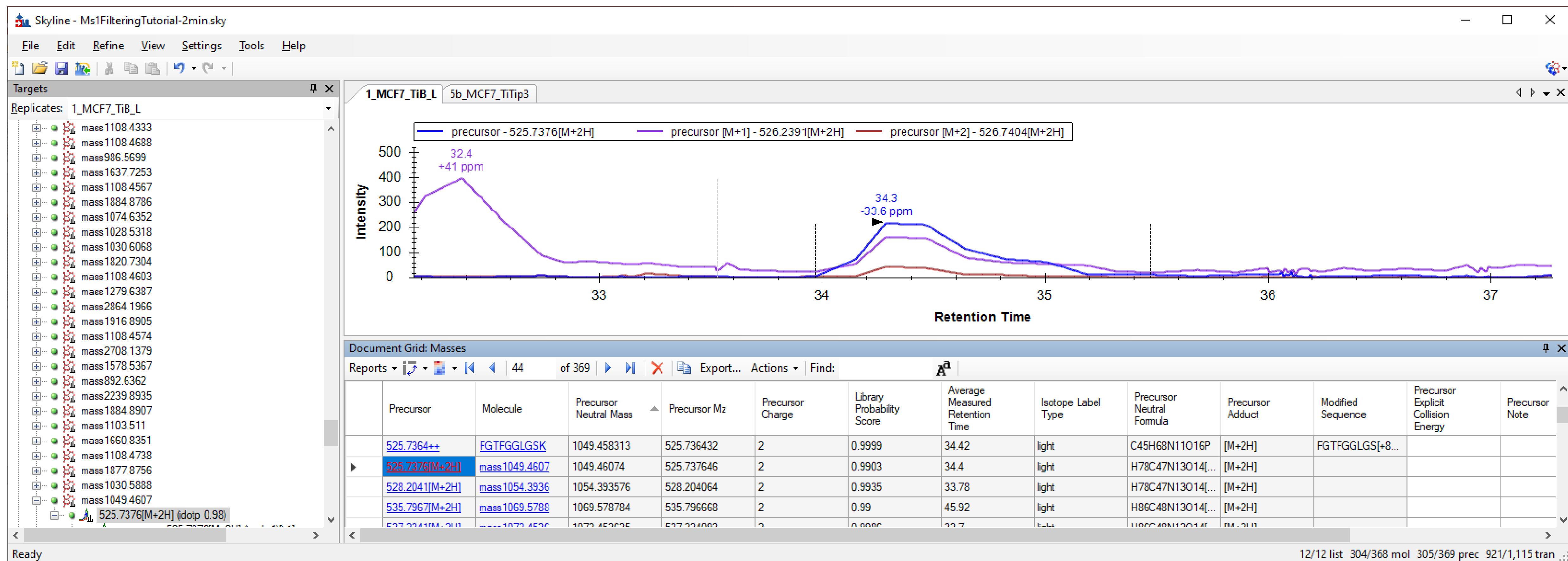
*Optimizing lower limits of quantification and detection by choosing transitions*

# Feature Detection in MS1 Spectra

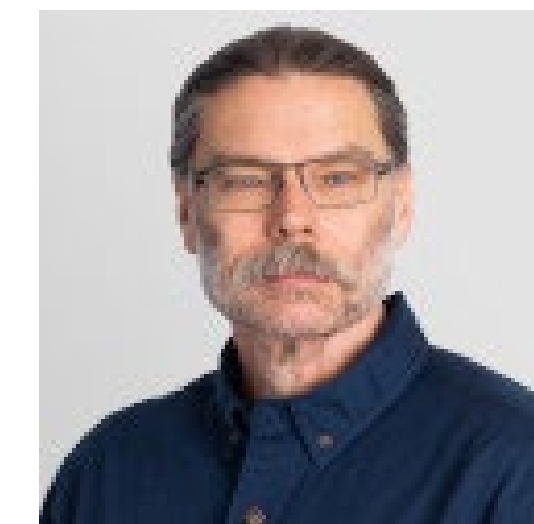




# Feature Detection in MS1 Spectra



# Feature Detection in MS1 Spectra



Skyline - Ms1FilteringTutorial-2min.sky

File Edit Refine View Settings Tools Help

Targets

Replicates: 1\_MCF7\_TiB\_L

- 525.7364++ (idotp 0.97)
  - precursor - 525.7364++ (rank 1)|i 1]
  - precursor [M+1] - 526.2379++ (rank 2)|i 2]
  - precursor [M+2] - 526.7392++ (rank 3)|i 3]
- spIQ9NYF8|BCLF1\_HUMAN
  - R.AEGEWEDQEALDYFSDKESGK.Q [371, 391] (missed 1)
  - K.LKDLFDYSPPLHK.N [505, 517] (missed 1)
  - K.STFREESPLR.I [525, 534] (missed 1)
  - K.STFREESPLR.K [525, 536] (missed 2)
  - R.RIDISPSTLR.K [654, 663] (missed 1)
  - R.RIDISPSTLR.H [654, 664] (missed 2)
  - R.IDISPSTLR.K [655, 663]
  - R.IDISPSTLR.H [655, 664] (missed 1)
- spQ9H6F5|CCD86\_HUMAN
  - R.LGGLRPE\_SPELTSVSR.T [11, 27]
  - R.ALVEFESNPEETREPGSPPSVQR.A [31, 53] (missed 1)
  - R.ALVEFESNPEETREPGSPPSVQR.A [31, 53] (missed 2)
  - R.LQQGAGLESPQGQPEPGAAS\_PQR.Q [72, 94]
- spIP24534|IEF1B\_HUMAN
  - K.YGPADVEDTTGSGATDSKDDDDIDLFGSDDEEESI
- spIP07900|HS90A\_HUMAN
  - K.ESEDKPEIEDVGSDEEEEEKKDGDK.K [251, 274] (missed 1)
  - R.GVVDSEDLPLNISR.E [387, 400]
  - K.DQVANSAFVER.L [500, 510]
- spIP27816|MAP4\_HUMAN
  - K.DMESPTKLDVTLAK.D [277, 290] (missed 1)
  - K.DMESPTKLDVTLAK.D [277, 290] (missed 2)

1\_MCF7\_TiB\_L 5b\_MCF7\_TiTip3

Intensity

Retention Time

precursor - 525.7364++ precursor [M+1] - 526.2379++ precursor [M+2] - 526.7392++

32.4 +43.2 ppm

34.3 -31.3 ppm

ID 34.4

Document Grid: Masses

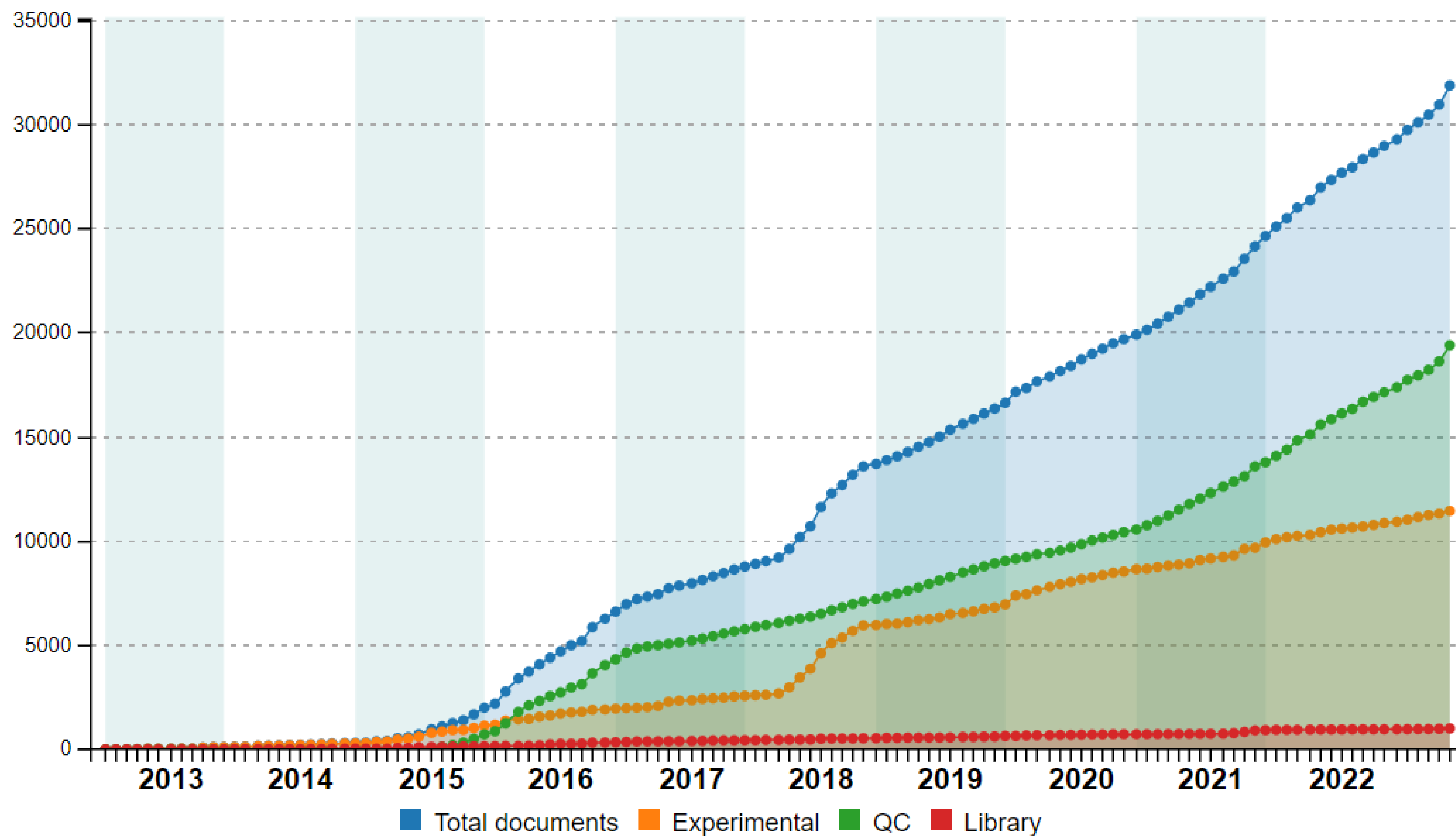
Reports 43 of 369 Export... Actions Find: A<sup>a</sup>

	Precursor	Molecule	Precursor Neutral Mass	Precursor Mz	Precursor Charge	Library Probability Score	Average Measured Retention Time	Isotope Label Type	Precursor Neutral Formula	Precursor Adduct	Modified Sequence	Precursor Explicit Collision Energy	Precursor Note
▶	525.7364++	FGTFGGLGSK	1049.458313	525.736432	2	0.9999	34.42	light	C45H68N11O16P	[M+2H]	FGTFGGLGS[+8...		
	525.7376[M+2H]	mass1049.4607	1049.46074	525.737646	2	0.9903	34.4	light	H78C47N13O14[...	[M+2H]			
	528.2041[M+2H]	mass1054.3936	1054.393576	528.204064	2	0.9935	33.78	light	H78C47N13O14[...	[M+2H]			
	535.7967[M+2H]	mass1069.5788	1069.578784	535.796668	2	0.99	45.92	light	H86C48N13O14[...	[M+2H]			
	537.2341[M+2H]	mass1073.4536	1073.453576	537.234064	2	0.9906	33.7	light	H86C48N13O14[...	[M+2H]			

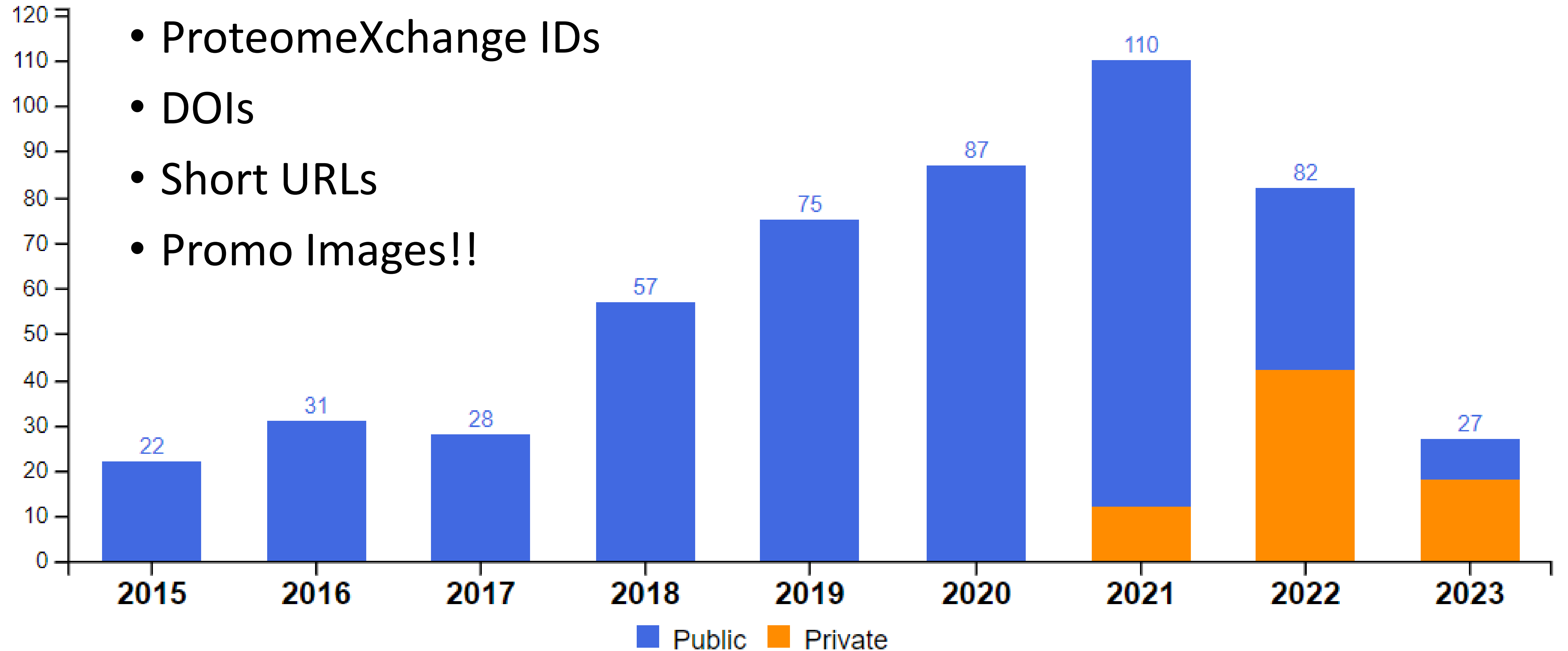
Ready

1/12 list 12/368 mol 12/369 prec 34/1,115 tran ...

# Skyline Documents on



# Panorama Public



# What's Next?

- More great support, instruction, and responding to user feedback
- More workflow integration with free tools: DIA-NN and Encyclopedia
- More precursor filters
  - MS2 -> MS3
  - Fragmentation type: CID vs. HCD vs. ETD vs. EAD
  - Instrument parameters: CE, CoV, Drift Time, 1/K0
- Peptide- and Protein-level detection q values
- Automatic recalibration of RT and mass error

# Instrument Vendor Collaborators

## ▶ Agilent Technologies

- ▶ Marilyn Marx
- ▶ Brian Miller



## ▶ Bruker

- ▶ Sven Brehmer
- ▶ Jens Decker
- ▶ Stephanie Kaspar
- ▶ Gary Kruppa
- ▶ Markus Lubeck
- ▶ Pierre-Olivier Schmit



## ▶ AB Sciex

- ▶ Tim Blacker
- ▶ David Cox
- ▶ Christie Hunter
- ▶ Arianna Jones



## ▶ Shimadzu

- ▶ Tsuyoshi Nakanishi
- ▶ Norio Mukai
- ▶ Toshiya Matsubara
- ▶ Jun Watanabe



## ▶ Thermo-Scientific

- ▶ Philip Remes
- ▶ Sebastien Gallien
- ▶ Bahvin Patel
- ▶ Vlad Zabrouskov



## ▶ Waters

- ▶ Ronan O'Malley
- ▶ Keith Richards
- ▶ Hans Vissers





# Skyline Team



Brendan MacLean



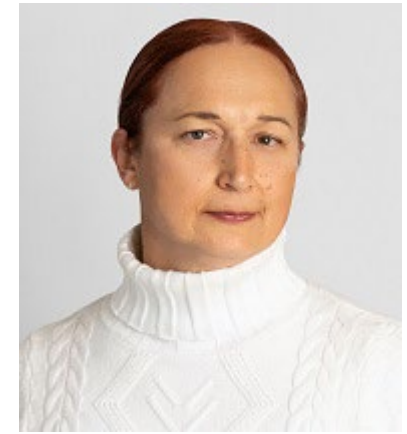
Nick Shulman



Brian Pratt



Vagisha Sharma



Rita Chupalov



Matt Chambers



Brian Connolly



Mark Belanger

