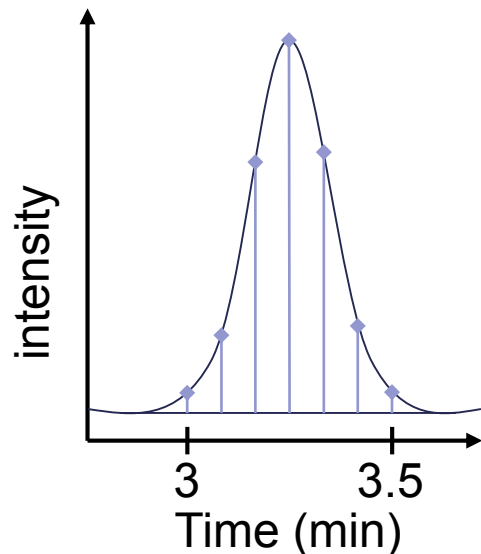
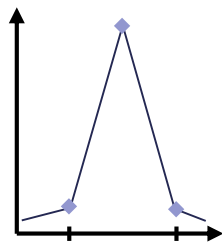


DIA Acquisition

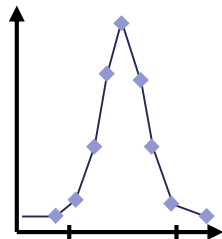


We assumed 30 s peak width at base
3.5 s cycle will collect ~8 DPPP

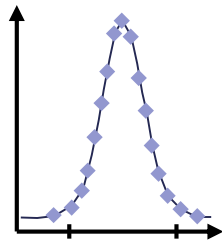


Data Points Per Peak (DPPP)

<7 DPPP = under sampling

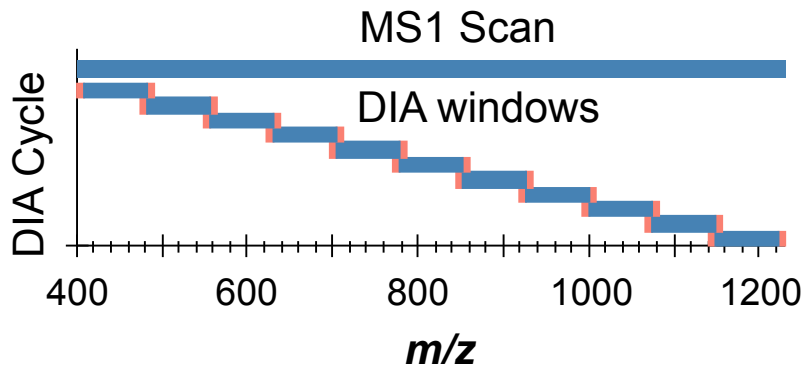
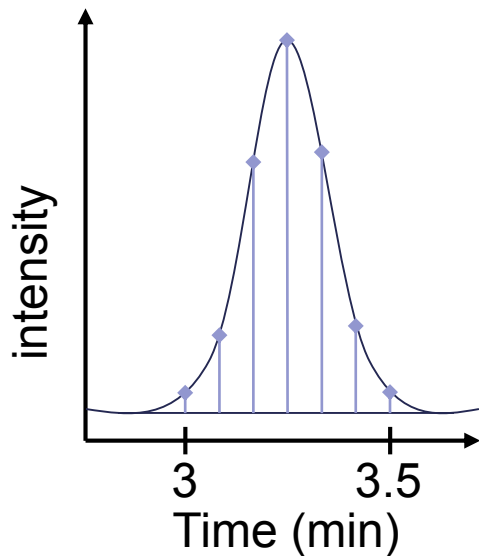


7-10 DPPP = optimal sampling



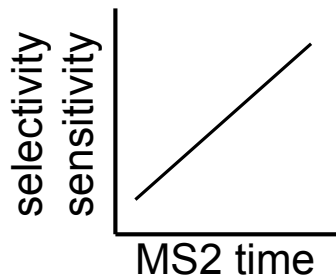
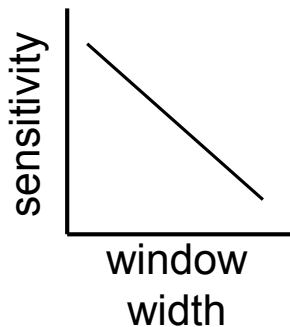
>10 DPPP = over sampling

DIA Acquisition



windows x MS2 acquisition time = cycle time

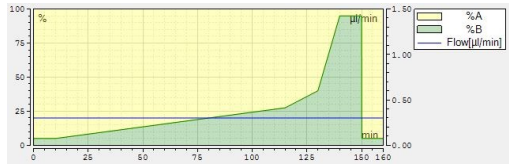
40 (21 m/z width, 400-1200 m/z , 1 Da overlap) x 60 ms = 3.5 s



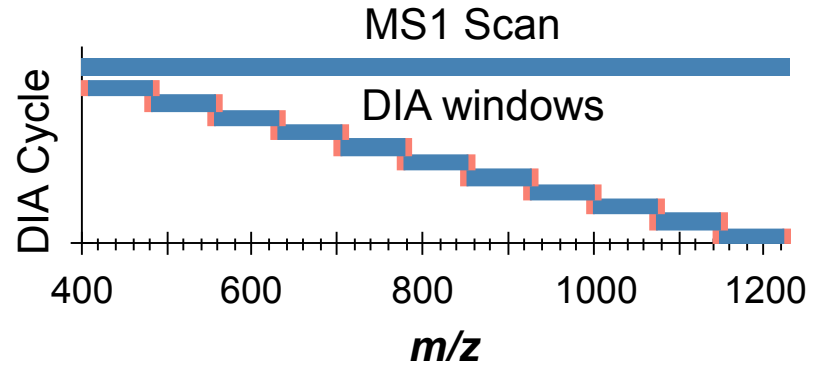
Choices are dependent on chromatography, application and platform.

Making the PRG DIA Method

LC

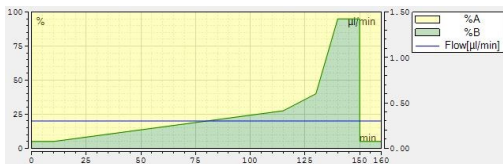


MS



Making the PRG DIA Method

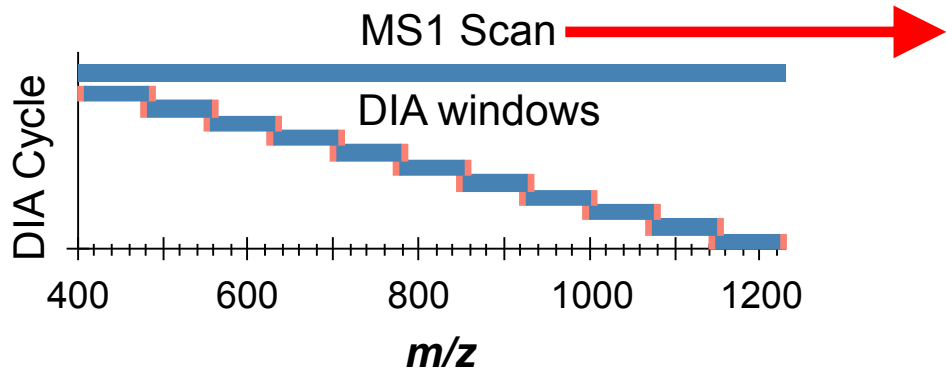
LC



- 130 min two-step gradient that worked well for a tissue lysate
- 1 μg on column recommended

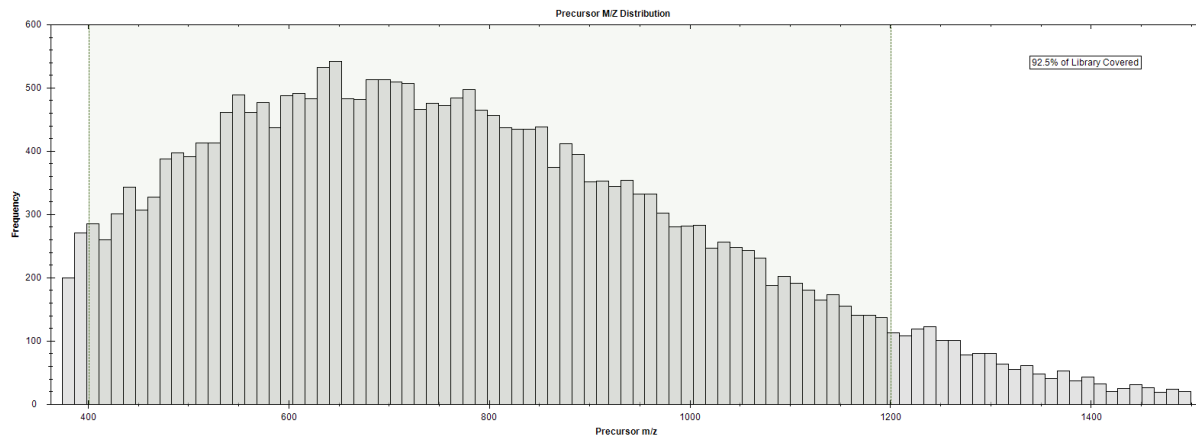
Making the PRG DIA Method

MS



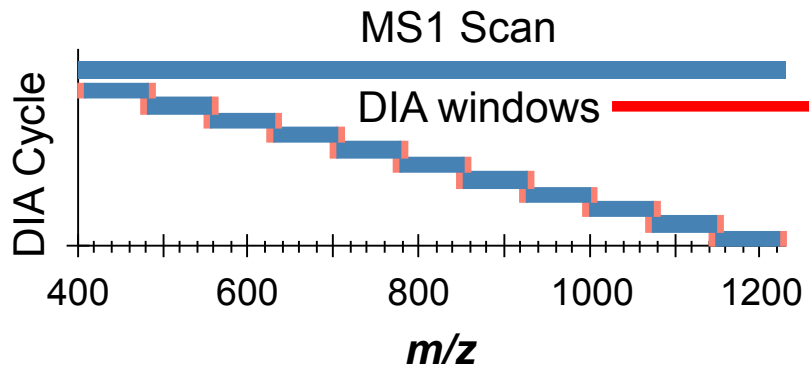
Start at 375 or 400 m/z ?
End at 1000 or 1200 m/z ?

Used 400 to 1200 m/z



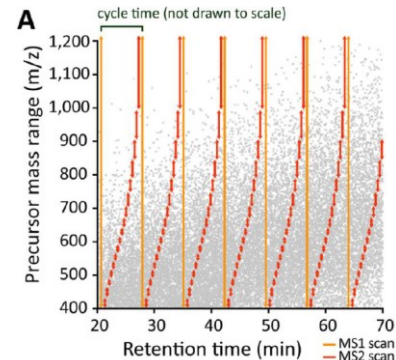
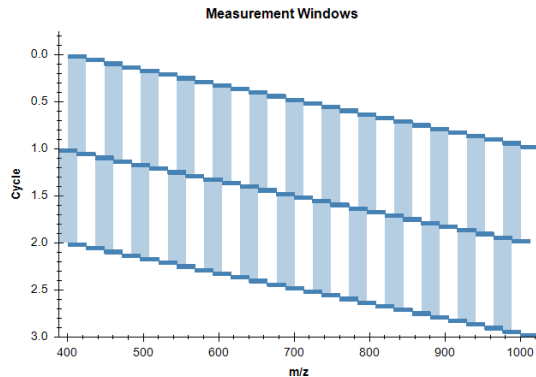
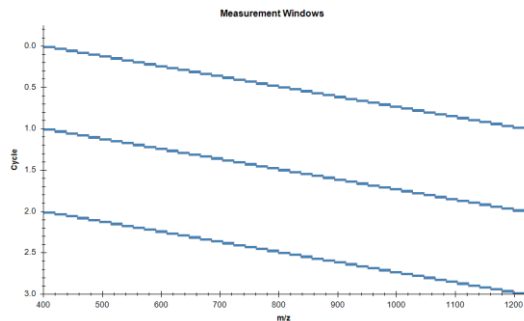
Making the PRG DIA Method

MS

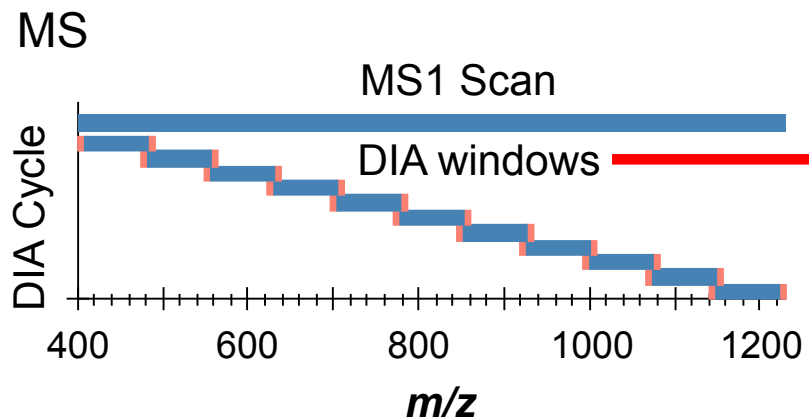


Window Strategies

- sequential segments
 - w/ or w/o overlap
 - static or variable width
- two-cycle overlap
- MSX
- SONAR



Making the PRG DIA Method

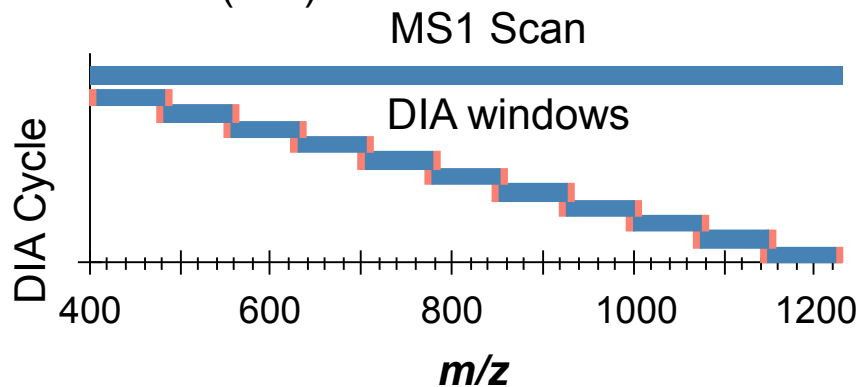
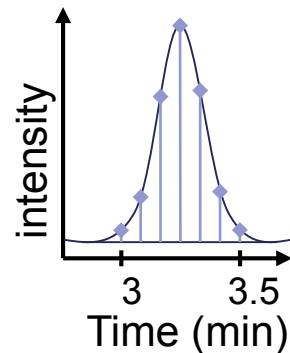


- Used static windows with 1 Da overlap
- Window size based on instrument frequency and DPPP

What we ended up with

Goal: create a base DIA method across platforms.

- Not the best, but standard starting method
- LC: two-step gradient lasting 110-130 minutes
- DIA: try to be at 3.5 sec cycle to be roughly 7-10 dppp if peaks are 30 sec at base
- 1 Da overlapping windows from 400-1200 m/z
- Window width was dependent on instrument scan speed

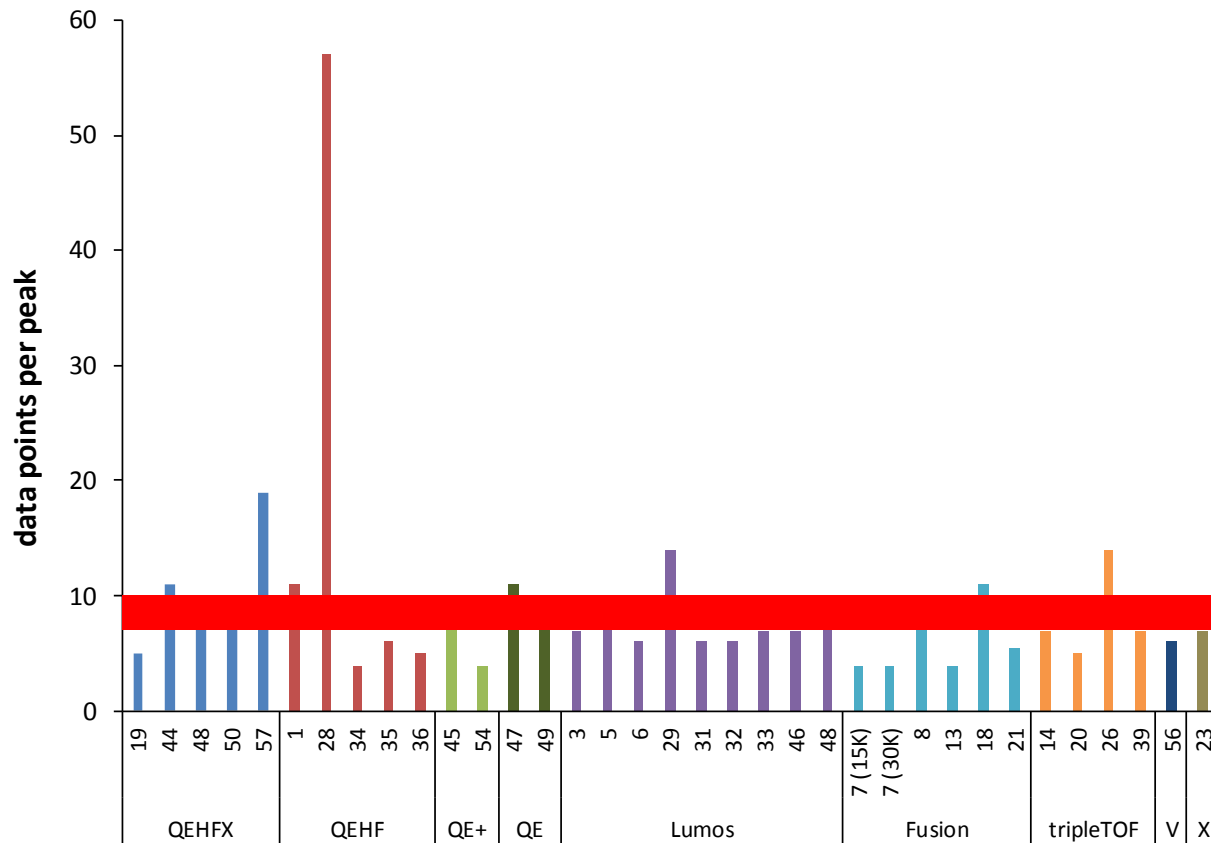


windows x MS2 acquisition time = cycle time
 40 (21 m/z width, 400-1200 m/z, 1 Da overlap) x 60 ms = 3.5 s

But across platforms?

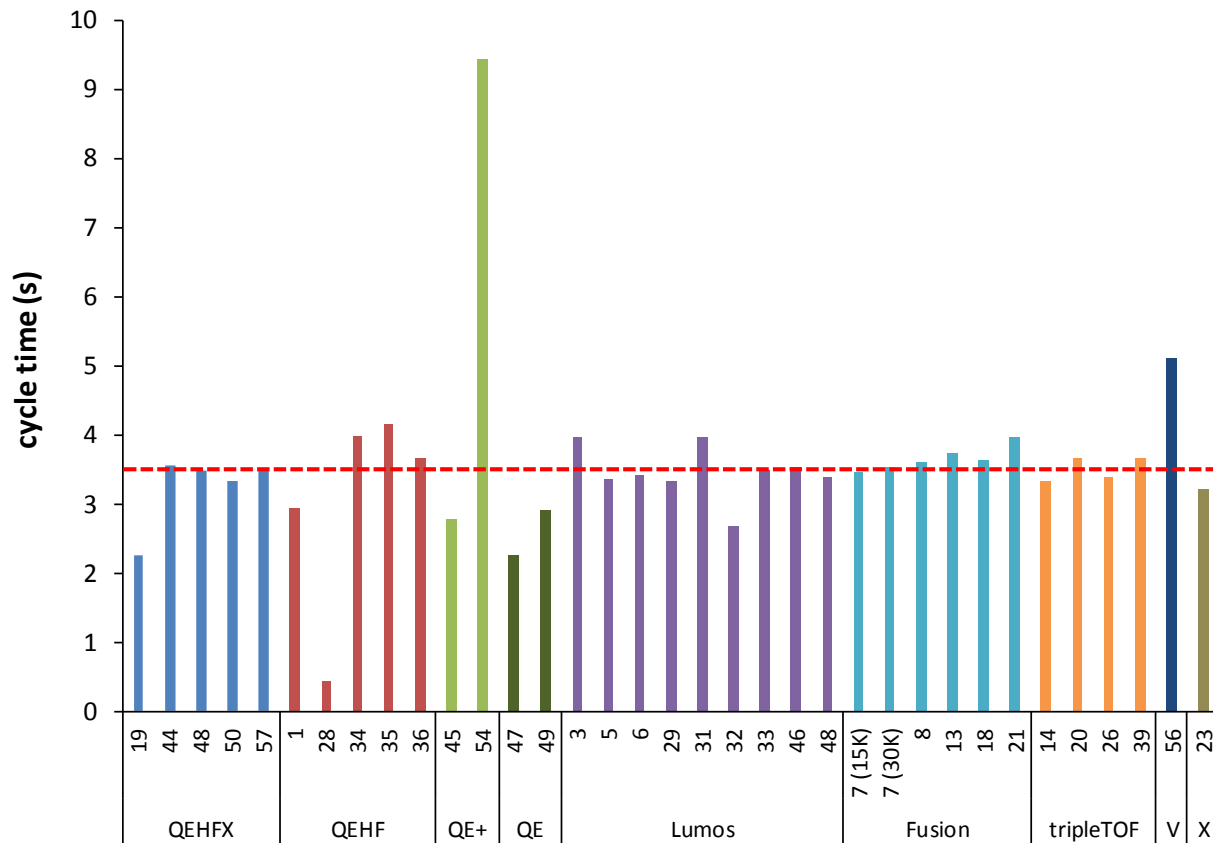
<u>Platform</u>	<u>Lumos 30k MS2</u>	<u>Lumos 15k MS2</u>	<u>QE-HFX</u>		<u>SCIEX</u>
Gradient	130 min	130 min	145 min	Gradient	117 min
Default z	4	4	3	Default z	4
(S-Lens)*/ion funnel RF	(60) 30	(60) 30	40	Scan _____	solution or sensitivity
Resolution FS	120,000	120,000	120,000	AGC FS	1.E+06
AGC FS	1.E+06	1.E+06	3.E+06	mode	profile
mode	profile	profile	profile	Accumulation Time (ms)	250
Max Inj FS	20	20	20	Scan Range	400-1200
Scan Range	399-1200	393-1200	399-1200	Iso Width	11
Iso Width	21	14	21	Number of Segments	80
Number of Segments	40	62	40	window range	399.5-1200.5
window range	399-1200	393-1200	399-1200	CE	auto?
NCE**	HCD30	HCD30	HCD30	MS2 Resolution	30000
Resolution MS2	30000	15000	30000	MS2 Scan _____	solution or sensitivity
Scan Range	200-2000	200-2000	200-2000	MS2 Accumulation Time	100
AGC MS2	1.00E+06	1.00E+06	3.00E+06	mode	profile
Max Inj MS2	60	30	60	cycle time (sec)	3.5
mode	profile	profile	profile		
Parallelization	OFF	OFF	OFF		
cycle time (sec)	3.5	3.5	3.5		

Performance of Participants – DPPP



- Most labs achieved a satisfactory DPPP (7-10)
- After removal of outlier, average DPPP was 7.8
- Considering difficulty of predicting cycle time in trap based instruments, and diversity of platforms, this is surprisingly good.

Performance of Participants – cycle time



- Similar to DPPP, most labs achieved the target cycle time of 3.5 sec
- After removal of three outliers, average cycle time was 3.42 sec

Could be better, yes, but wasn't bad.

All of these choices had consequences but were they significant?

- A two hour two stage gradient?
- MS1 Range?
- Window Strategy?
- Assuming 30 sec peaks at base?
- Tending to have a slower cycle in exchange for tighter windows?