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

Choices are dependent on chromatography, application and platform.
Making the PRG DIA Method

LC

MS

MS1 Scan

DIA Cycle

DIA windows

\[ m/z \]
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

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

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

<table>
<thead>
<tr>
<th>Platform</th>
<th>Lumos 30k MS2</th>
<th>Lumos 15k MS2</th>
<th>QE-HFX</th>
<th>SCIEX</th>
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<td>Gradient</td>
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<td>145 min</td>
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</table>
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?