

Setting up BoxCar-Like methods on a Fusion

BEN ORSBURN

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Many BoxCar-like methods can be set up on the Tribrids. I group them as 3 things

1) BoxCar method that most resembles the original study (BoxFahrt)

2) BoxCar Assisted MS2 Fragmentation (BAMF) – the use of BoxCars to actively select the ions used for MS/MS. At present, I believe this can only be performed on the Fusion and Lumos devices

3) BoxCarDIA – BoxCar in conjunction with Data Independent Acquisition – this is a test method that does appear to work, but I can't find anything that can process the data.

How to set up any BoxCar like method on a Tribrid (Fusion Tune 3.0 shown)

Tribrid instruments can have multiple “Experiments”. Each MS1 or MS2 acquisition type requires it’s own “Experiment”

In the Example below (BAMF), I have 3 Experiments. Each Experiment can be configured independently.

The screenshot displays the 'Method Editor' interface for a Tribrid instrument, specifically the 'Scan Parameters' tab. The interface is divided into several sections:

- Method Timeline:** A horizontal bar at the top showing the method duration (137 min) and a timeline with markers at 22.8, 45.7, 68.5, 91.3, 114.2, and 137 minutes. Below the timeline, three horizontal bars represent different experiments.
- Experiment 3 Configuration:** The main area shows the configuration for 'Experiment 3' with a time range of 0-137 min. A central flowchart illustrates the scan sequence: a 1.5 sec delay, followed by tSIM, MIPS, Charge State, Dynamic Exclusion, and ddMS² IT HCD.
- Data-Dependent MSⁿ Scan Properties:** A panel on the right lists various parameters for the scan, including Isolation Window (m/z), Activation Type, HCD Collision Energy (%), Detector Type, Ion Trap Scan Rate, and First Mass (m/z).

Data-Dependent MS ⁿ Scan Properties	
Isolation Window (m/z)	2.5
Activation Type	HCD
HCD Collision Energy (%)	25
Detector Type	Ion Trap
Ion Trap Scan Rate	Rapid
First Mass (m/z)	100

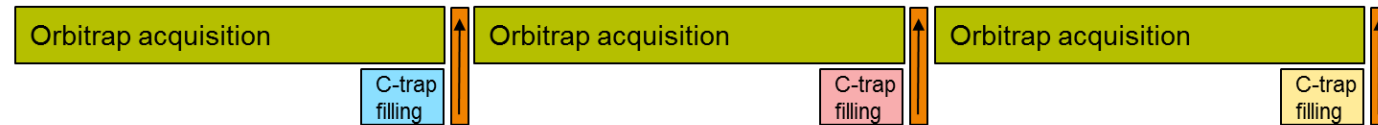
BoxCar is essentially Multiplex Targeted-SIM (msx-TSIM)

-msx allows isolation and collection prior to a scan

-factory instrument parameters limit you to 10 msx per scan

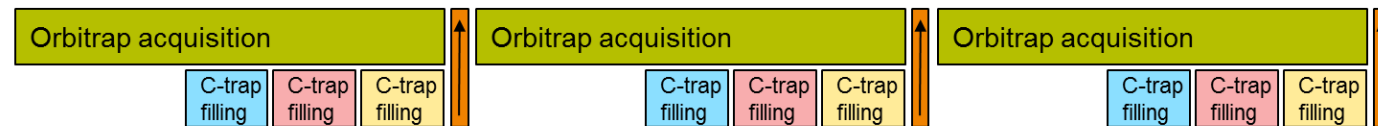
-BoxCar in MaxQuant.Live does not have this limitation. In the original BoxCar study 12 windows are utilized

SIM of 3 ions



- Overall cycletime can be too long for a series of SIMs

Multiplexed SIM of 3 ions



Each filling can have it's own target!!

- Filling of multiple ions into C-Trap within one scan cycle
- Upto 10 ions can be used for multiplexing

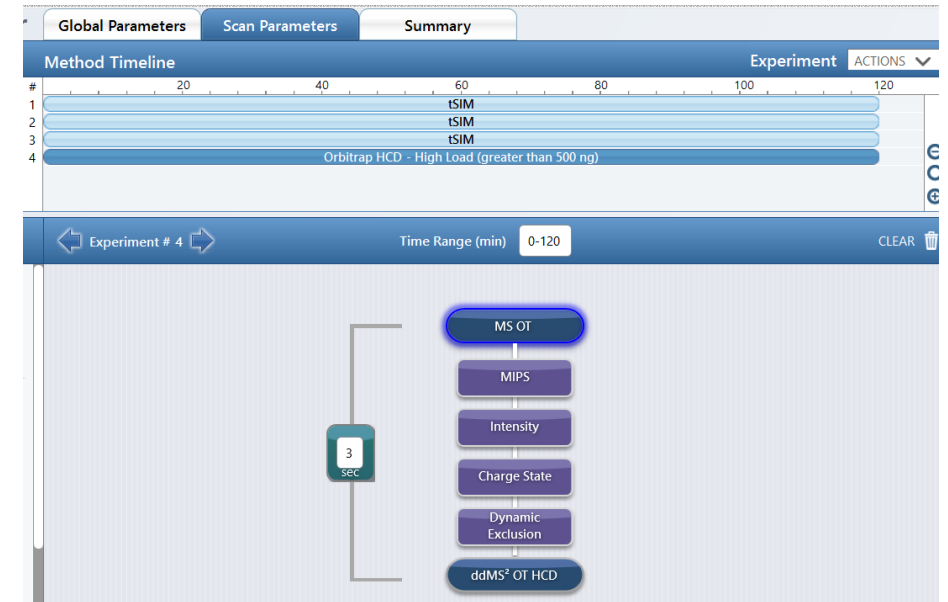
BoxFahrt methods

BoxFahrt methods come close to replicating the original BoxCar method.

This method requires 4 separate “Experiments”

3 BoxCars are used to acquire more democratic precursor distributions.

A normal MS1 with ddMS2 triggered off of the MS1 is the final 4th experiment

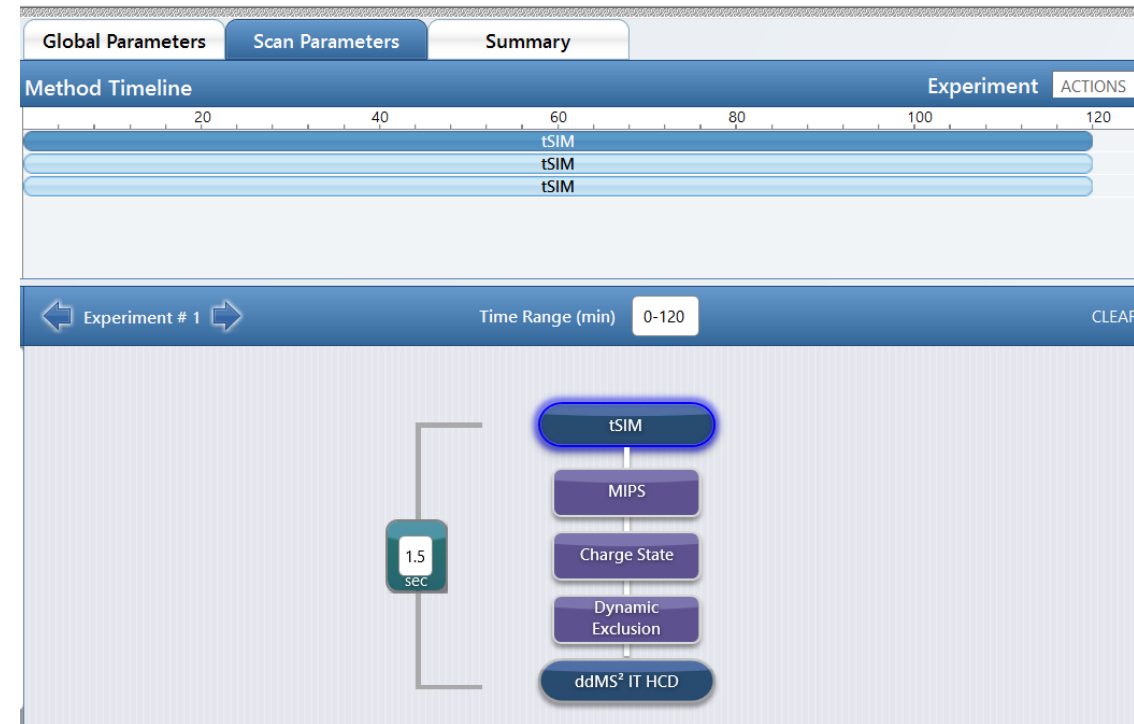


BoxCar Assisted MS/MS Fragmentation (BAMF)

BAMF utilizes only 3 experiments.

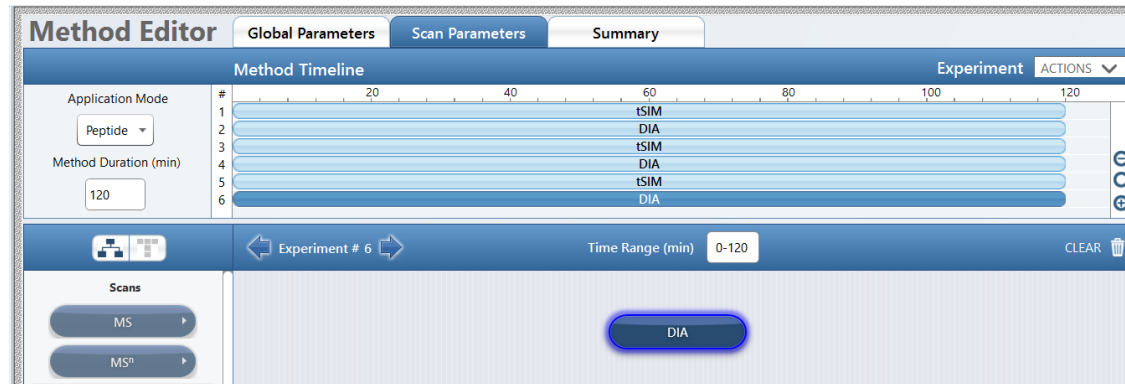
Ions selected for fragmentation are chosen directly from the BoxCar scans rather than the MS1

As such, ions are selected for fragmentation democratically, allowing MS/MS fragmentation and sequencing of ions that can not be seen in a “regular” full MS1 scan



BoxCarDIA

This is an experimental method that does appear to work, however I have had no luck as of ASMS 2019 finding anything that can process the data. Please contact me if you would like to have a RAW file.



The method utilizes 6 experiments alternating between 3 BoxCar (msx-TSIM) scans and 3 small window DIA scans.

Using the ion trap in parallel with the Orbitrap effectively allows DIAs to be acquired with no increase in cycle time