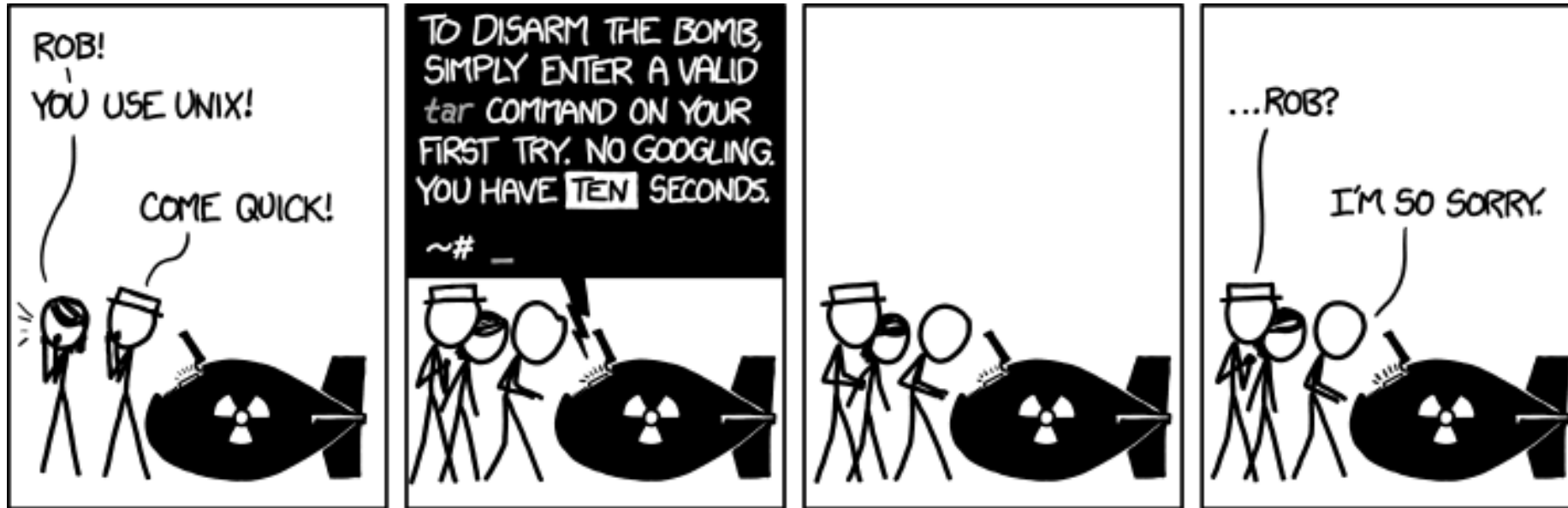


ThermoFisher
SCIENTIFIC

Instrument API and XML Method Modification Interface

ASMS Orbitrap Power User Meeting – June 2nd, 2018, San Diego, CA

Derek Bailey, Ph.D.



1. Instrument Application Programming Interface (IAPI)

- Real-time control and acquisition of Orbitrap Mass Spectrometers using .NET

2. XML Method Modification Interface (XMMI)

- Programmatically modify Fusion-series method files

3. Applications of IAPI

1. Devin Schweppe – Fusion API
2. Christoph Wichmann – Q Exactive API

4. Examples and FAQ for IAPI and XMMI

5. Applications of XMMI

1. Pavel Shliaha – Fusion Method Creation

https://github.com/thermofisherlsms

The screenshot shows the GitHub organization page for Thermo Fisher Scientific - LSMS. The header includes navigation links: Features, Business, Explore, Marketplace, Pricing, This organization, Search, Sign in, and Sign up. The organization's name and logo are displayed, along with the description "Life Science Mass Spectrometry" and a website link. Below this, there are tabs for Repositories (4), People (1), and Projects (0). A search bar and filters for Type and Language are present. The main content area lists four repositories: xcalibur-workbench (Lua, 1 star, MIT license, updated 6 days ago), meth-modifications (C#, 1 star, MIT license, updated on Apr 4), lua-raw-file (C, 1 star, MIT license, updated on Jan 16), and iapi (Instrument Application Programming Interface, 3 stars, MIT license, updated on Nov 30, 2016). A sidebar on the right shows "Top languages" (C, Lua, C#) and "People" (Derek Bailey, dbaileychess).

<https://github.com/thermofisherlsms>

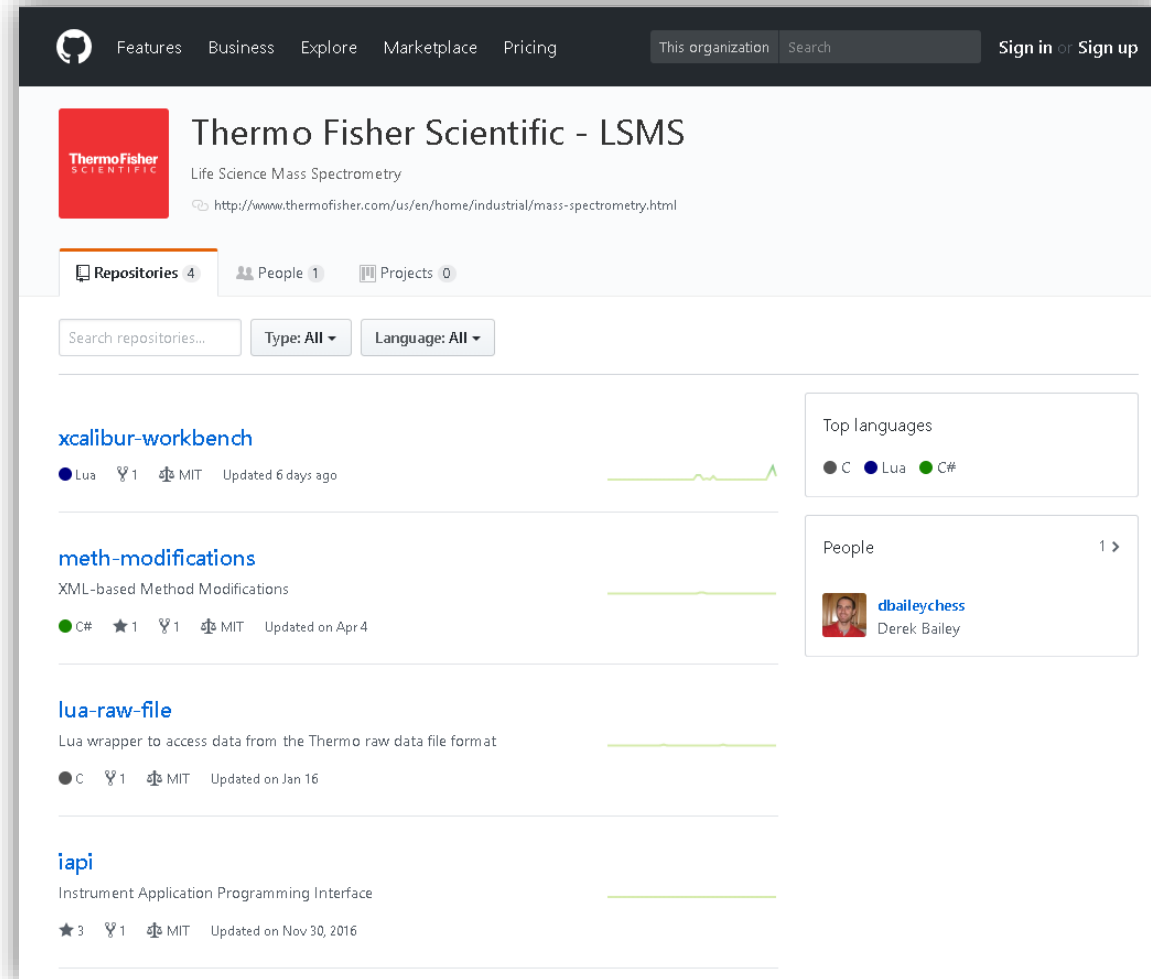
Mike Senko
Tuesday Poster



Covered Today



Covered Today



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Why an API?

“I wish I could do ...”

“Can I do this in method editor?”

“I only want to select peaks that have odd masses ...”

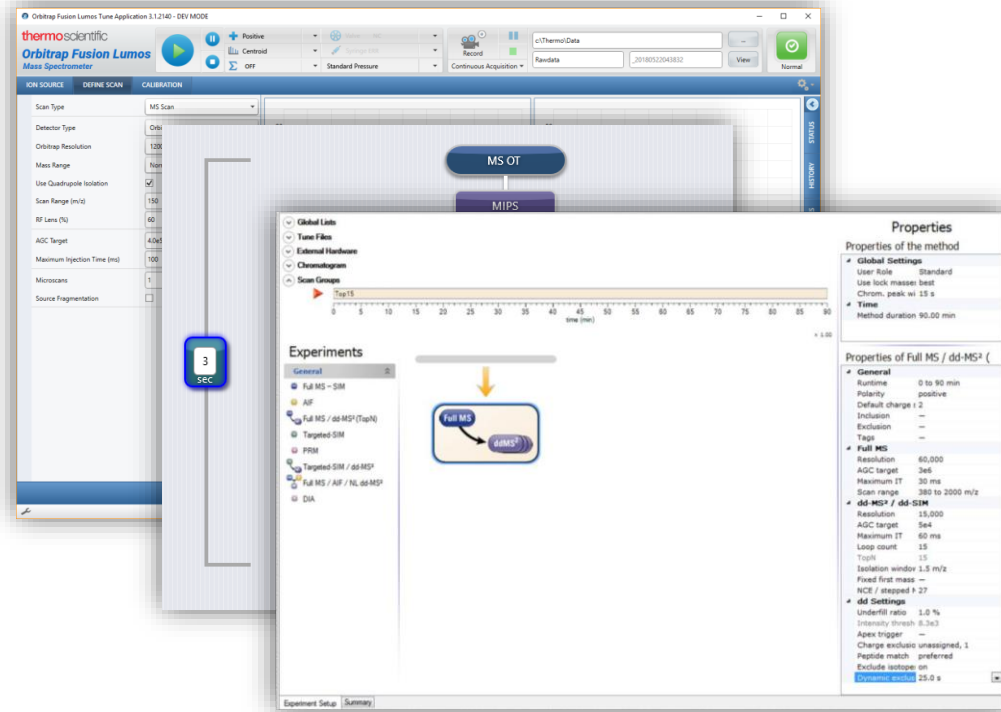
“I have this awesome new experiment ...”

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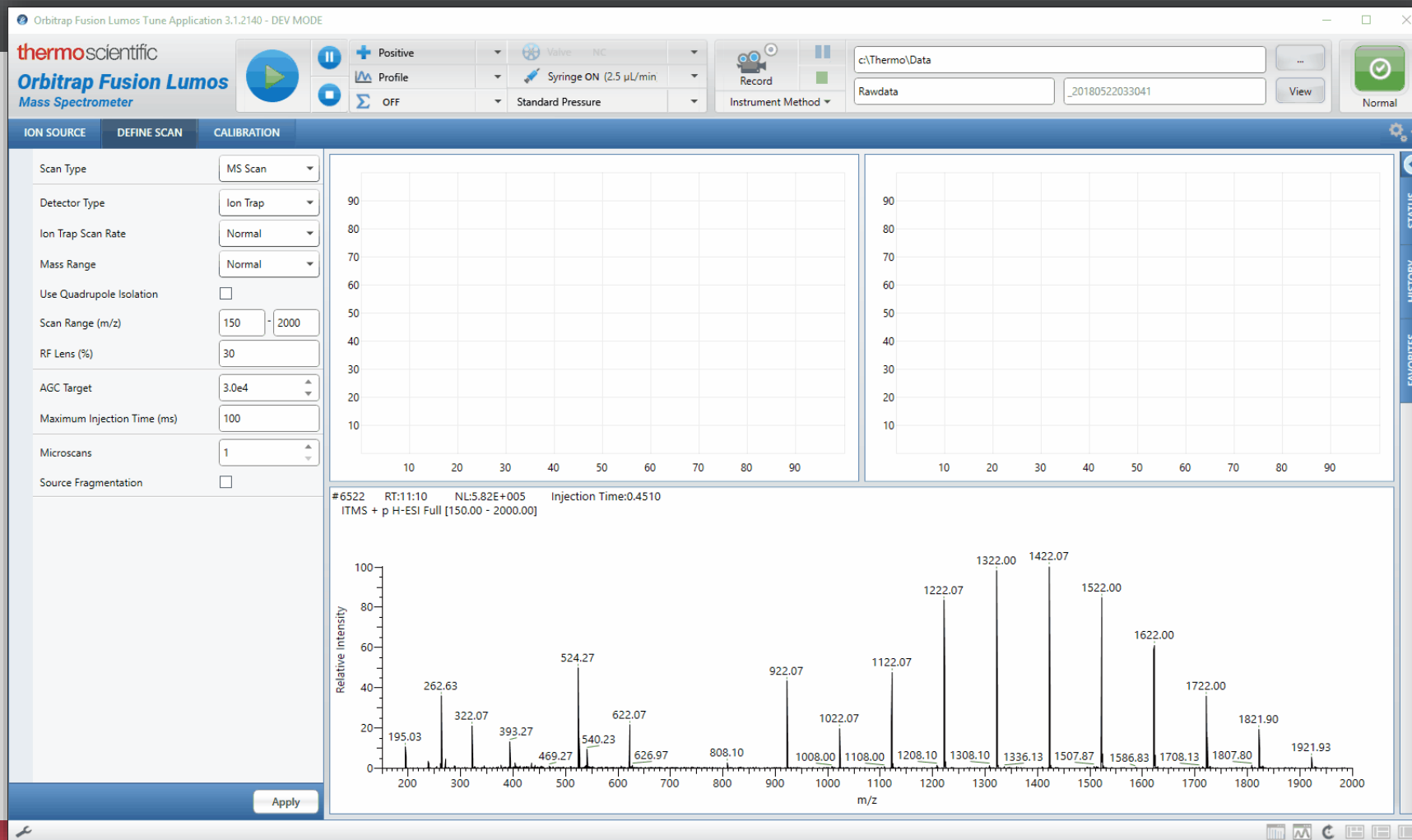
“Can I do this in method editor?”

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- Users can only control the Mass Spectrometer through our **Tune** application and **Method** acquisition.
- Although both provide **great flexibility** in acquisition strategies they don't encompass all possibilities.
- Providing an Application Programming Interface enables users to **extend** the capabilities of the Orbitrap mass spectrometers.
- Allows **real-time** data acquisition and control.



The **IAPI** empowers users to **programmatically** control acquisition of both the **Exactive** and **Tribrid-series** Mass Spectrometers

1. IAPI Architecture

- Interfaces
- Data and Control Flow

2. Receiving Data from the MS

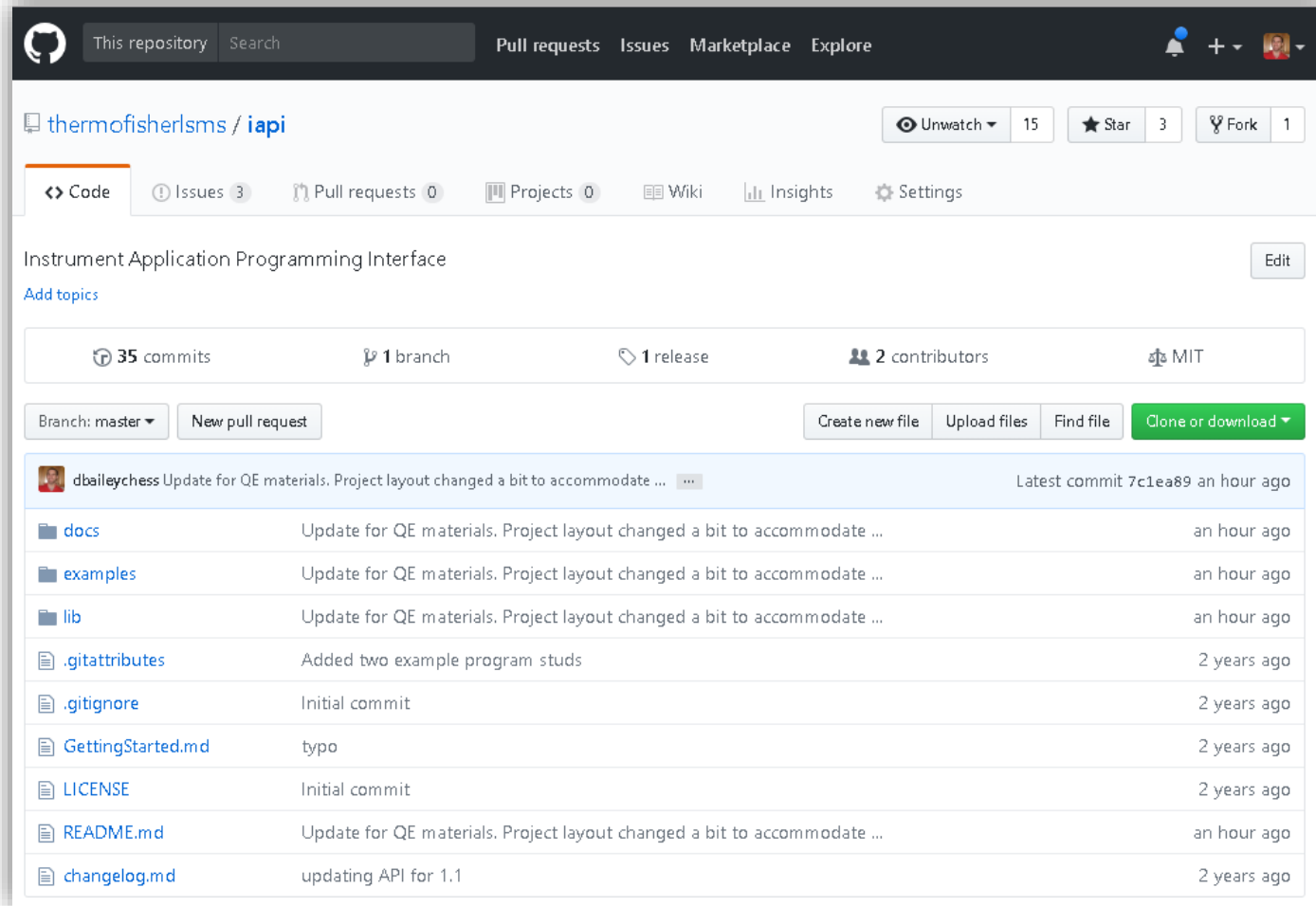
- Interfaces
- Scan Data Stream Subscription

3. Controlling the MS

- Sending Scan Definitions
- Changing other MS parameters

4. Getting Started

<https://github.com/thermofisherlisms/iapi>



- Public interfaces for
 - Tribrid
 - Exactive
- Documentation
 - Previous ASMS Posters
 - Help files
- VS Solution of Example Applications

External Requirements

- Tune Installation
- .NET 4.6.2+
- License Agreement in place
- IAPI License Key Activated

1. IAPI Architecture

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Interfaces

Exactive Series



I-API Version

 API-1.0.dll

 API-1.1.dll

Instrument Extensions

 ESAPI-1.0.dll

 ESAPI-1.1.dll


Misc.

 VI-1.0.dll


Tribrid Series



 API-2.0.dll

 Spectrum-1.0.dll

 Fusion.API-1.0.dll

 Thermo.TNG.Factory.dll

Differences and Similarities between Exactive and Tribrid Series IAPI

- Although IAPI 1.X is **not binary compatible** with IAPI 2.X, they share considerable amount of structure
 - Namespaces and hierarchies are virtually unchanged
 - A few name changes or minor reorganization
 - Removal of some interfaces
 - Spectrum-related interfaces moved into own assembly



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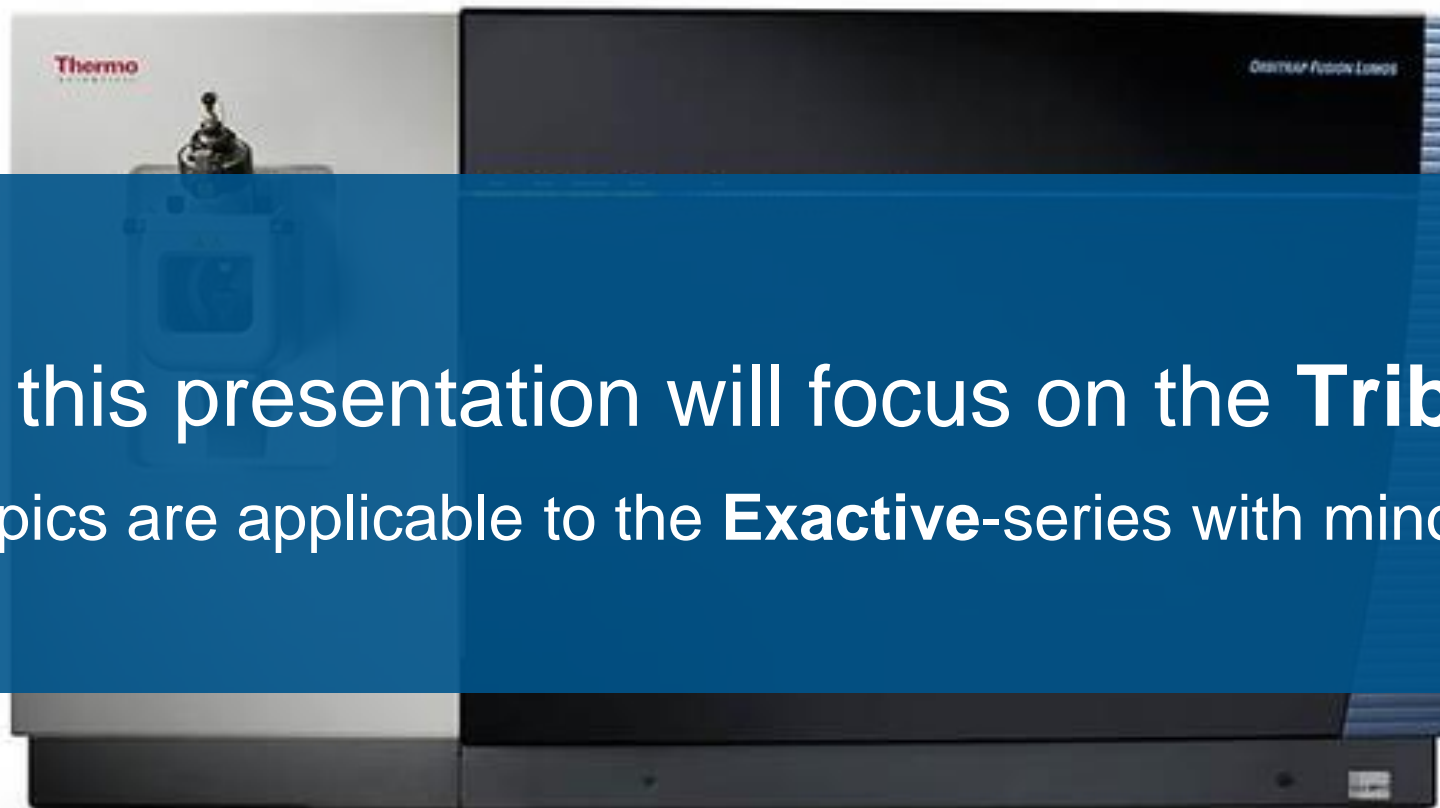
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 - Tribrid-series uses a **Factory** method model with Microsoft's Managed Extensibility Framework (MEF)
 - Exactive-series uses the **System.Reflection.Assembly** API



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 - Exactive-series uses the **System.Reflection.Assembly** API
- Some interfaces are not implemented due to technical reasons or deemed not immediately useful





The rest of this presentation will focus on the **Tribrid-series** IAPI.

Most topics are applicable to the **Exactive-series** with minor differences.

Mass Spectrometer



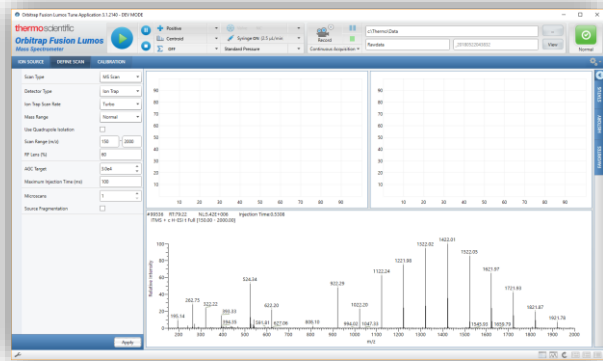
Local Switch



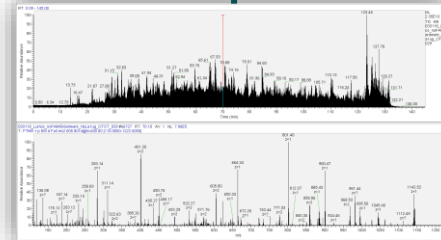
Data System



Tune Application



Instrument Service



Rawfile

I-API Architecture

Mass Spectrometer



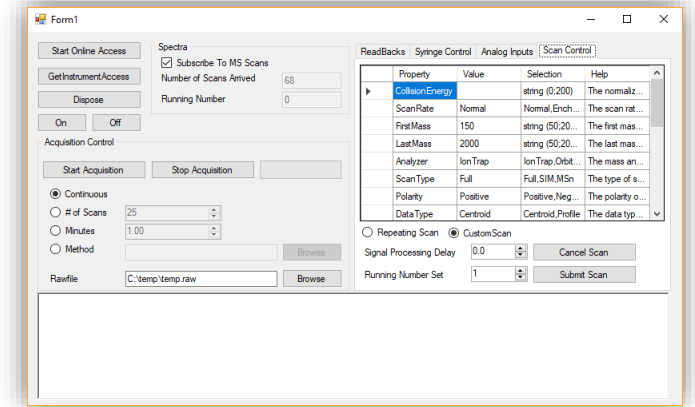
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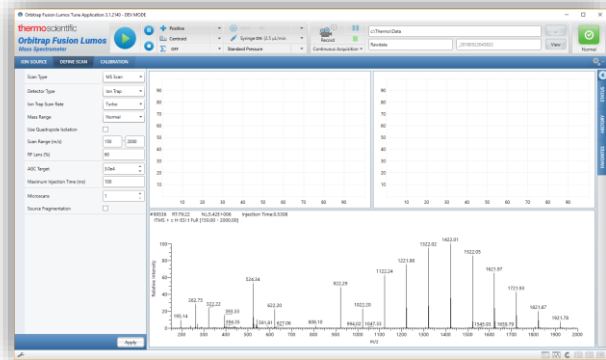
Data System



I-API-Enabled App



Tune Application



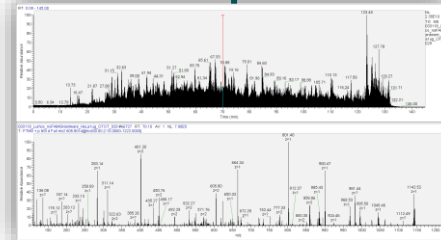
Instrument Service



3rd Party Application



API-2.0.dll



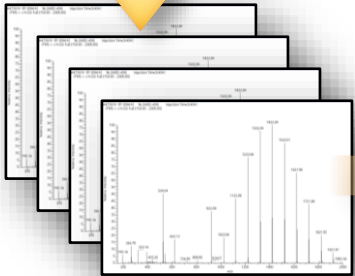
Rawfile

I-API Data and Control Flow

Mass Spectrometer



Scan Data
Stream



Data System



API-2.0.dll



3rd Party
Application

- Displays scan in Tune
- Writes to Rawfile
- Forwards data to API

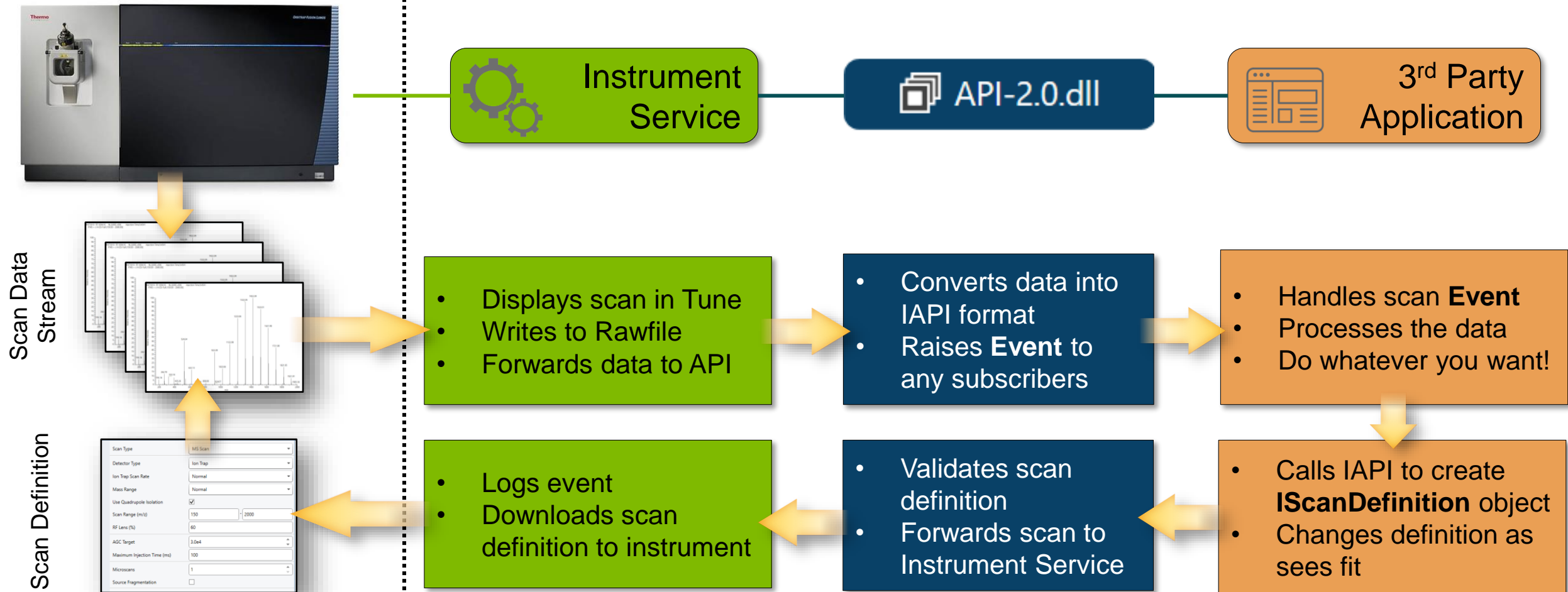
- Converts data into I-API format
- Raises **Event** to any subscribers

- Handles scan **Event**
- Processes the data
- Do whatever you want!

I-API Data and Control Flow

Mass Spectrometer

Data System



1. IAPI Architecture

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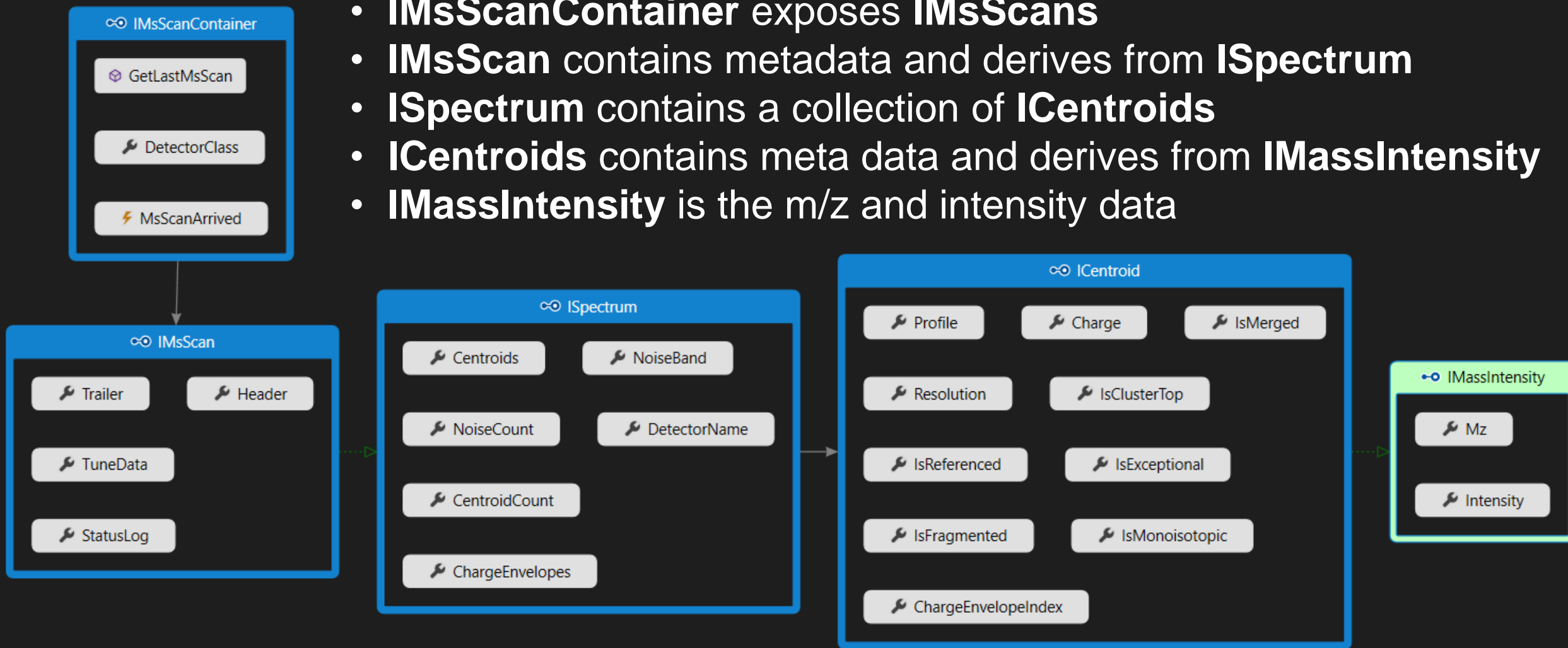
- Interfaces
- Scan Data Stream Subscription

3. Controlling the MS

- Sending Scan Definitions
- Changing other MS parameters

4. Getting Started

- **IMsScanContainer** exposes **IMsScans**
- **IMsScan** contains metadata and derives from **ISpectrum**
- **ISpectrum** contains a collection of **ICentroids**
- **ICentroids** contains meta data and derives from **IMassIntensity**
- **IMassIntensity** is the m/z and intensity data



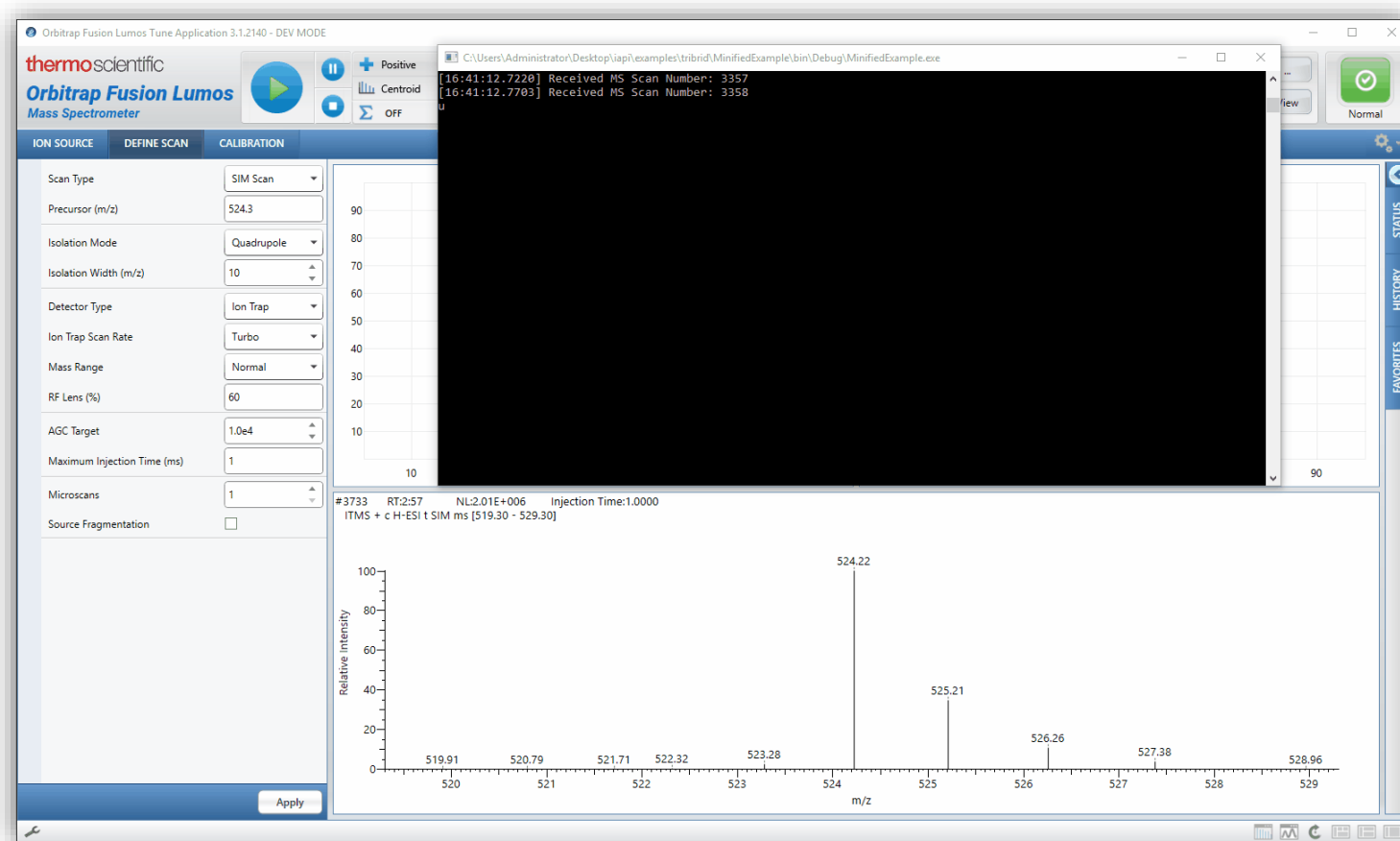
Scan Data Stream

- Receiving the scan data is through the **Event Driven** programming model
- Scans are sent via the .NET **Event** and **EventHandler** mechanism
 - Multiple handlers per scan can be registered
 - Minimal overhead and latency
- Receives Scans from both Tune and Method acquisitions

```
26 .....//Get the MS Scan Container from the fusion
27 .....IFusionMsScanContainer fusionScanContainer = fusionAccess.GetMsScanContainer(0);
28
29 .....//Run forever until the user Escapes
30 .....ConsoleKeyInfo cki;
31 .....while ((cki = Console.ReadKey()).Key != ConsoleKey.Escape)
32 .....{
33 .....    switch(cki.Key)
34 .....    {
35 .....        case ConsoleKey.S:
36 .....            .....//Subscribe to whenever a new MS scan arrives
37 .....            fusionScanContainer.MsScanArrived += FusionScanContainer_MsScanArrived;
38 .....            break;
39 .....        case ConsoleKey.U:
40 .....            .....//Unsubscribe
41 .....            fusionScanContainer.MsScanArrived -= FusionScanContainer_MsScanArrived;
42 .....            break;
43 .....        default:
44 .....            Console.WriteLine("Unsupported Key: {0}", cki.Key);
45 .....            break;
46 .....    }
47 .....}
48 .....}
49
50 .....2 references | Thermo Scientific, 1 hour ago | 2 authors, 2 changes
51 .....private static void FusionScanContainer_MsScanArrived(object sender, MsScanEventArgs e)
52 .....{
53 .....    .....//Print out the scan number of the scan received to console
54 .....    Console.WriteLine("[{0:HH:mm:ss.ffff}] Received MS Scan Number: {1}",
55 .....        DateTime.Now,
56 .....        e.GetScan().Header["Scan"]);
57 .....}
58 }
```

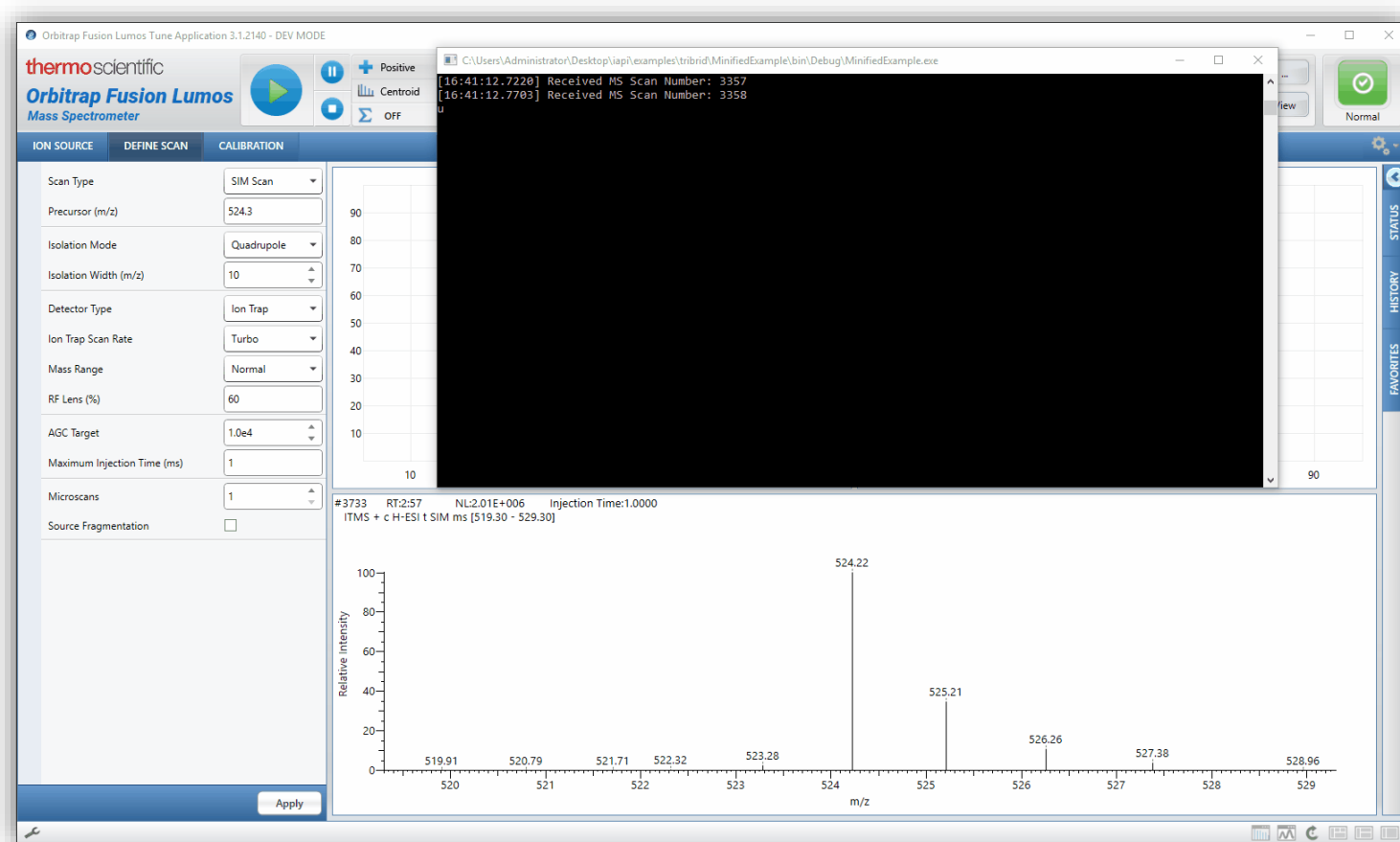
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- tSIM running ~21 Hz (**47.5 ms**) from Tune.
 - Average time of **48.4 ms** between scans received (n = 200)



Scan Data Stream

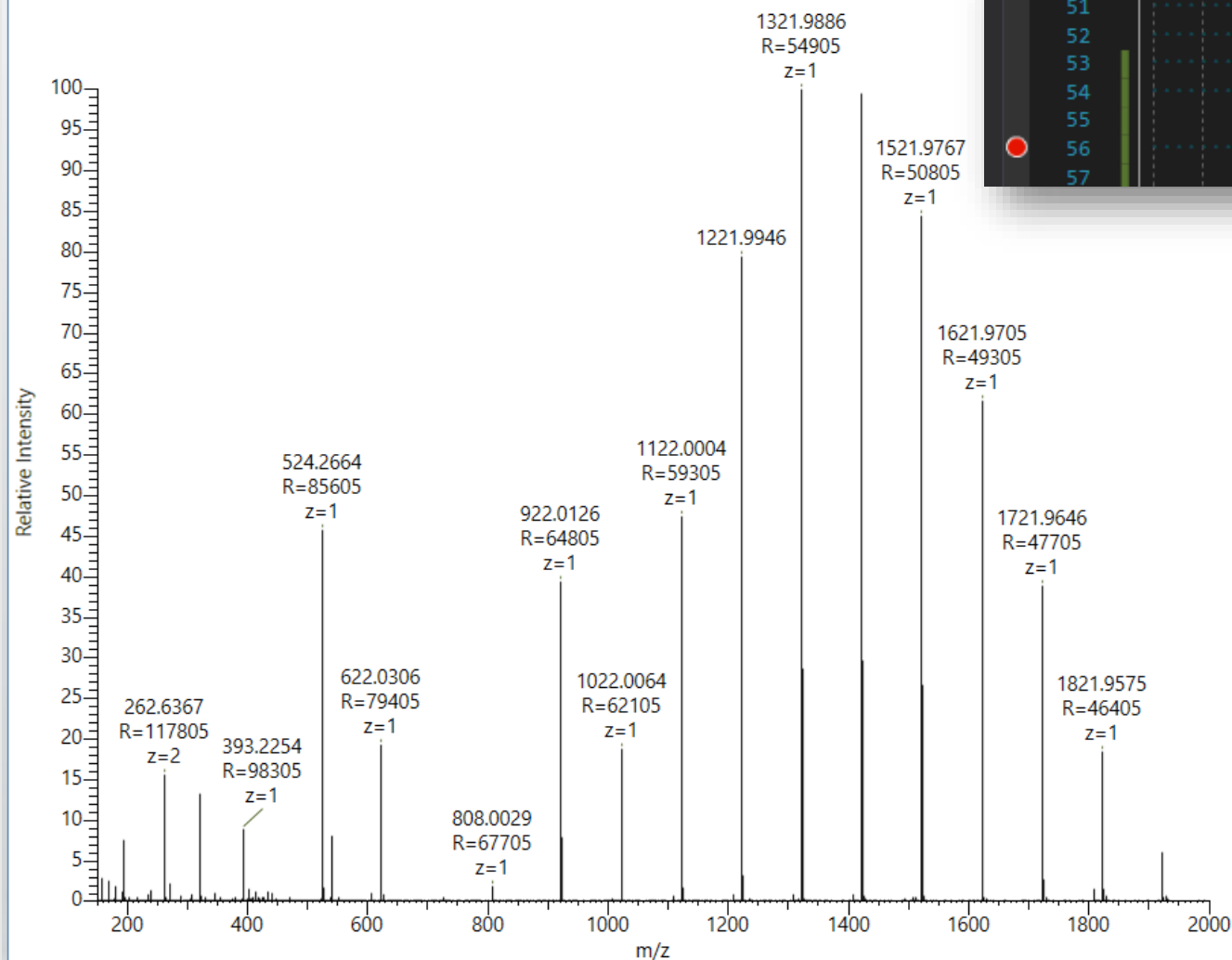
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The IAPI is responsive and has no impact on the MS acquisition

Scan Data Contents

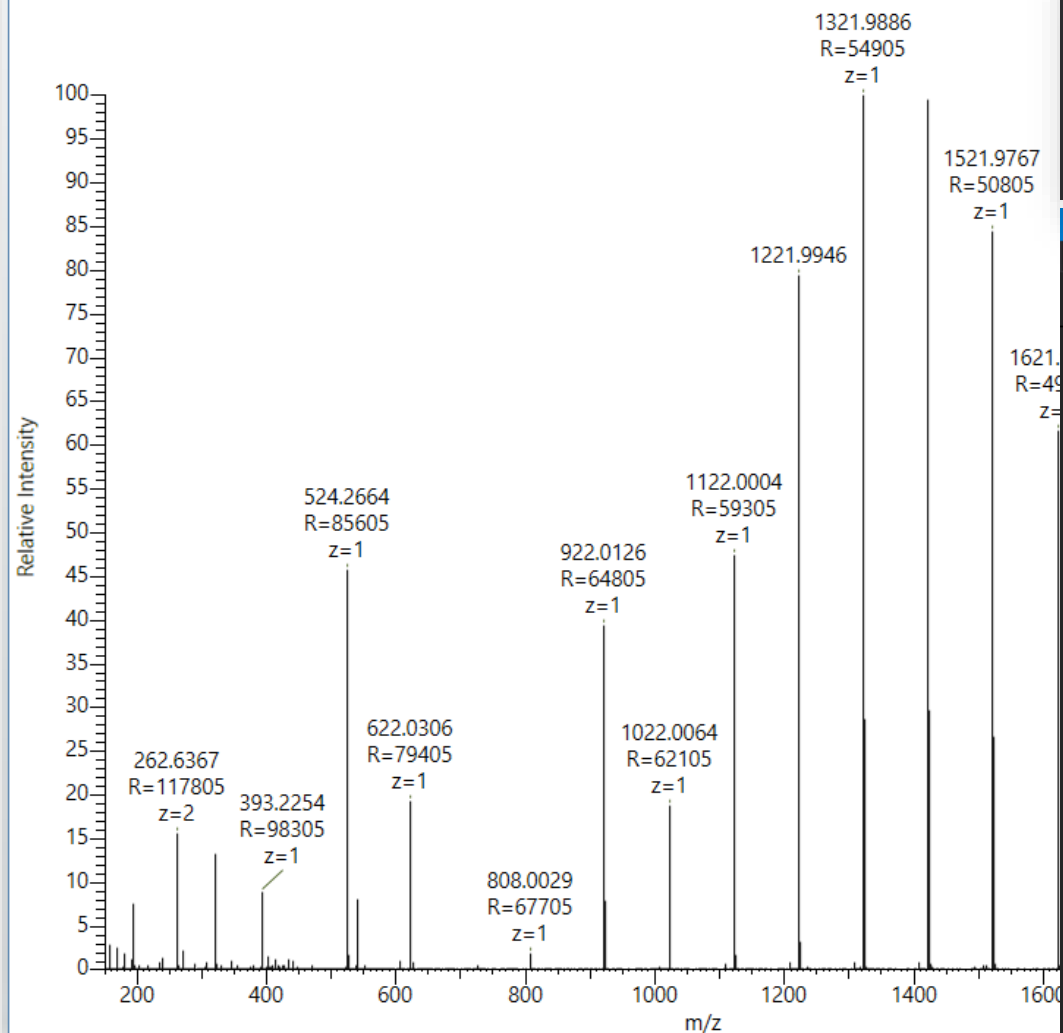
#518053 RT:1234:11 NL:5.89E+007 Injection Time:6.3361
FTMS + c H-ESI Full [150.00 - 2000.00]



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55 .....
56 .....    var scan = e.GetScan();
57 .....}
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57 }
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Watch 1		
Name	Value	Type
scan	{Thermo.TNG.Client.API.MsScanContainer.MsScan}	Thermo.

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2. Receiving Data from the MS

- Interfaces
- Scan Data Stream Subscription

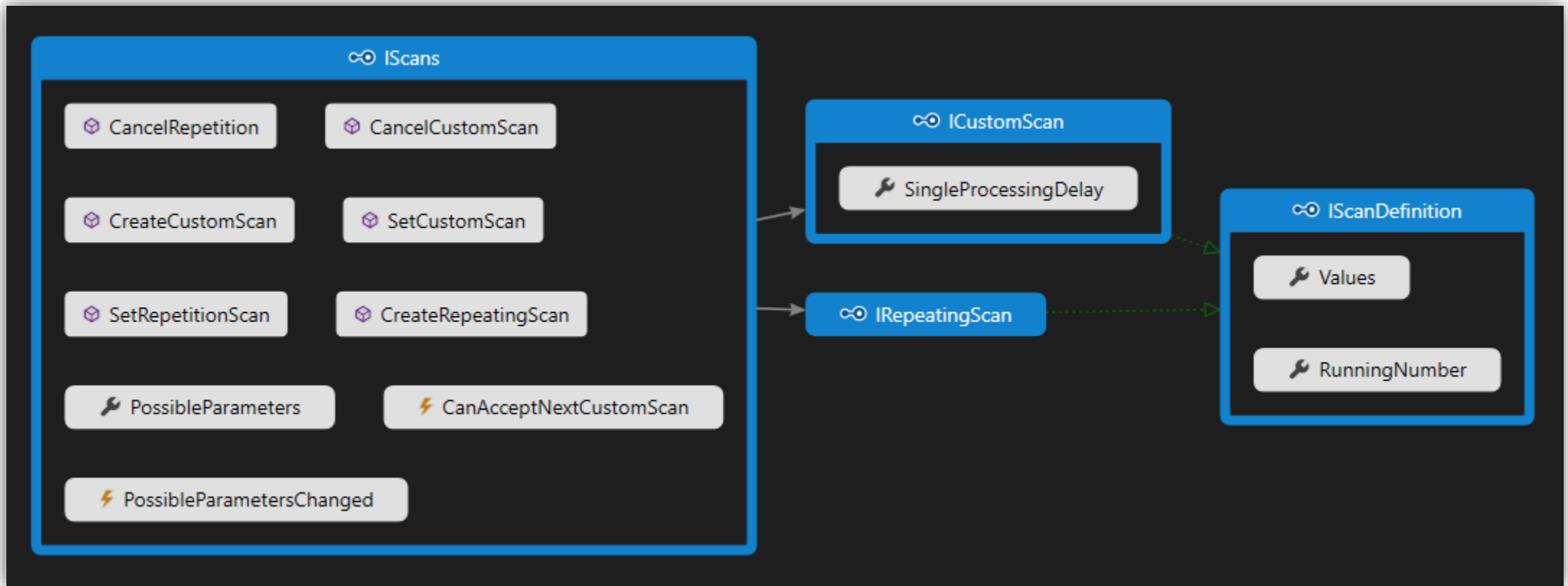
3. Controlling the MS

- Sending Scan Definitions
- Changing other MS parameters

4. Getting Started

IScans Interface

- Sending scans to the instrument is through the **IScans** interface
 - Provides methods for creating different types of **IScanDefinitions**
 - Provides methods for setting and cancelling scans.

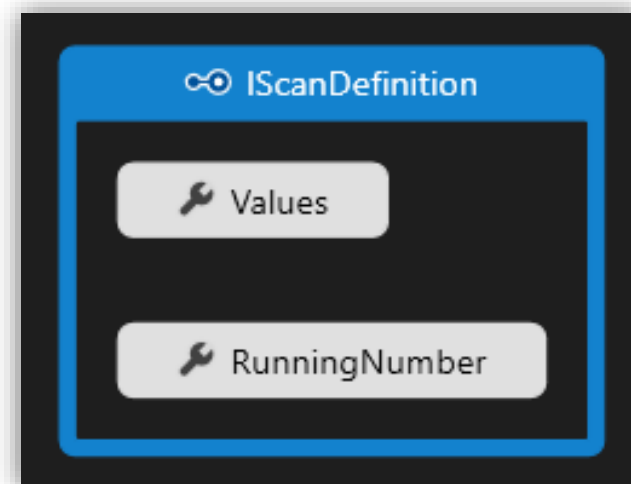


- Scans are defined in the **IScanDefinition** interface
- The **RunningNumber** property is for general purpose use
 - It shows up in the Scan Trailer as the '**Access ID**' field

Scan Header 557 - Rawdata_20180522033041

Name	Value
Dependency Type:	0
Access ID:	1

- The **Values** property is a Dictionary<string,string>
 - Keys are the scan parameters
 - Values are their associated values
 - Values can take different formats based on the parameter
 - Invalid, or nonsense values, are ignored



Example of MS³ of 524@35 191@25
Scan 150 – 550 m/z

```
scan.RunningNumber := 123456789;  
scan.Values["FirstMass"] := "150";  
scan.Values["LastMass"] := "550";  
scan.Values["ScanType"] := "MSn";  
scan.Values["PrecursorMass"] := "524.3;191.2";  
scan.Values["CollisionEnergy"] := "35;25";
```


	Property	Value	Selection	Help
►	ChargeStates		string (0;25)	Charge states for HCD(default 0 is unknown) It is expressed as a string of values, with eac...
	IsolationMode	Quadrupole	None,Quadrupole,Ion Trap	Isolate using the quadrupole or ion trap
	SourceCIDEnergy	0	0-100	Source CID Energy (0 = off).
	ActivationQ	0.25	string (0.05;0.8)	The Activation Q value (0.05-0.8). It is expressed as a string of values, with each value se...
	ActivationType	CID	string (CID;HCD)	The activation type to use at a given MS stage. The available types are (CID,HCD). It is e...
	AGCTarget	3000	3000-100000	The Automatic Gain Control (AGC) target value.
	Data Type	Centroid	Centroid,Profile	The data type to collect the scan in.
	FirstMass	150	string (50;2000)	The first mass of the scan range. It is expressed as a string of values, with each value sep...
	IsolationWidth	0.7	string (0.4;2000)	The isolation width (full-width) for a given MS stage It is expressed as a string of values, wit...
	LastMass	2000	string (50;2000)	The last mass of the scan range. It is expressed as a string of values, with each value sep...
	Analyzer	Ion Trap	Ion Trap,Orbitrap	The mass analyzer.
	MaxIT	100	0.001-8000	The maximum injection time (ms)
	CollisionEnergy		string (0;200)	The normalized collision energy (NCE) It is expressed as a string of values, with each value...
	Microscans	1	1-6000	The number of microscans to collect (1 = don't use microscans)
	OrbitrapResolution	120000	7500,15000,30000,50000,60000,120000,240000,500000	The Orbitrap Resolution
	Polarity	Positive	Positive,Negative	The polarity of the scan.
	PrecursorMass		string (50;2000)	The precursor m/z to isolate at a given MS stage. The first value will be the MS1->MS2 tra...
	ReactionTime	10	string (0.001;100)	The reaction/activation time (ms) for CID activations. It is expressed as a string of values, ...
	SrcRFLens	60	string (0;150)	The RF Lens (%) for the source. It is expressed as a string of values, with each value sepe...
	ScanRate	Normal	Normal,Enhanced,Zoom,Rapid,Turbo	The scan rate of the ion trap
	ScanType	Full	Full,SIM,MSn	The type of scan to perform.

Scan Management

Mass Spectrometer



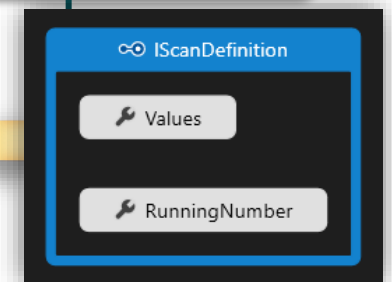
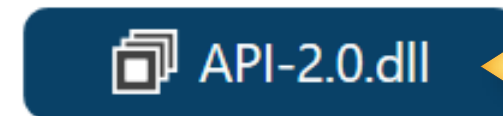
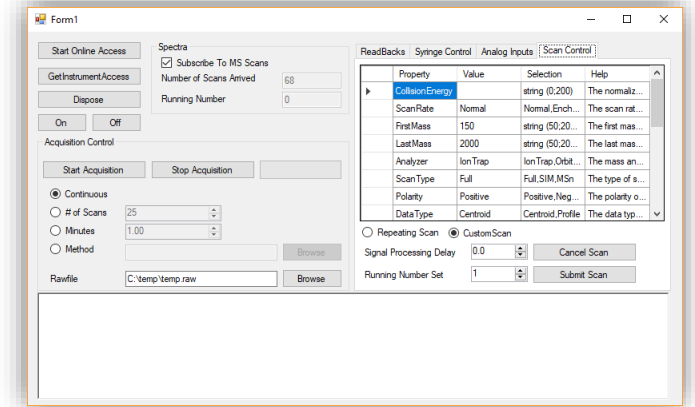
Local Switch



Data System



I-API-Enabled App



Sending Scan to MS

Scan Management

Mass Spectrometer



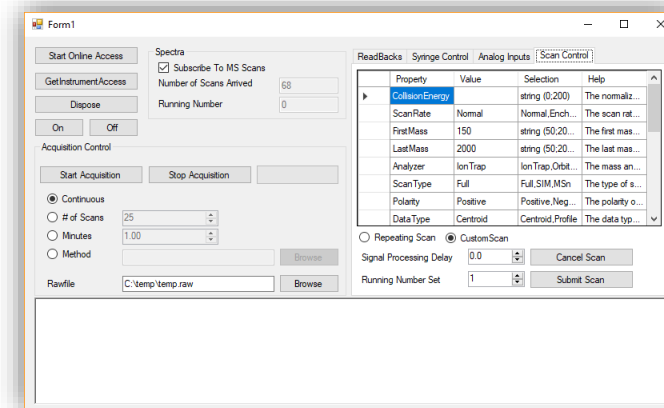
Local Switch



Data System



I-API-Enabled App



Scan Hierarchy

Priority ↑

Custom

Repeating

Method

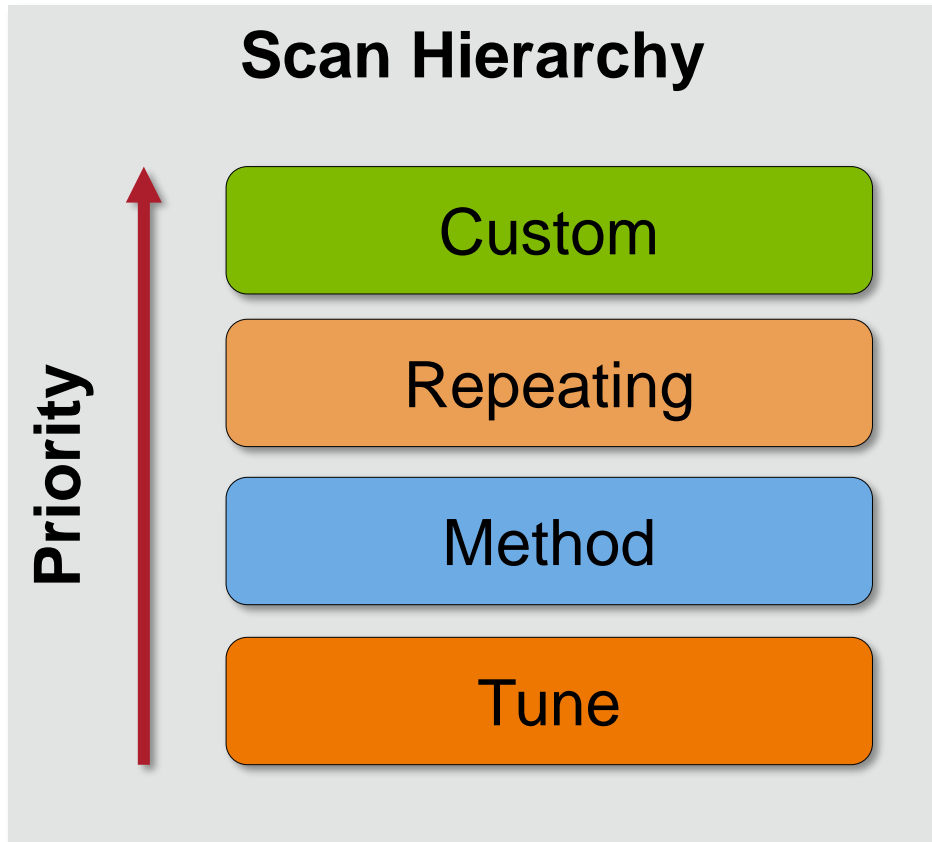
Tune

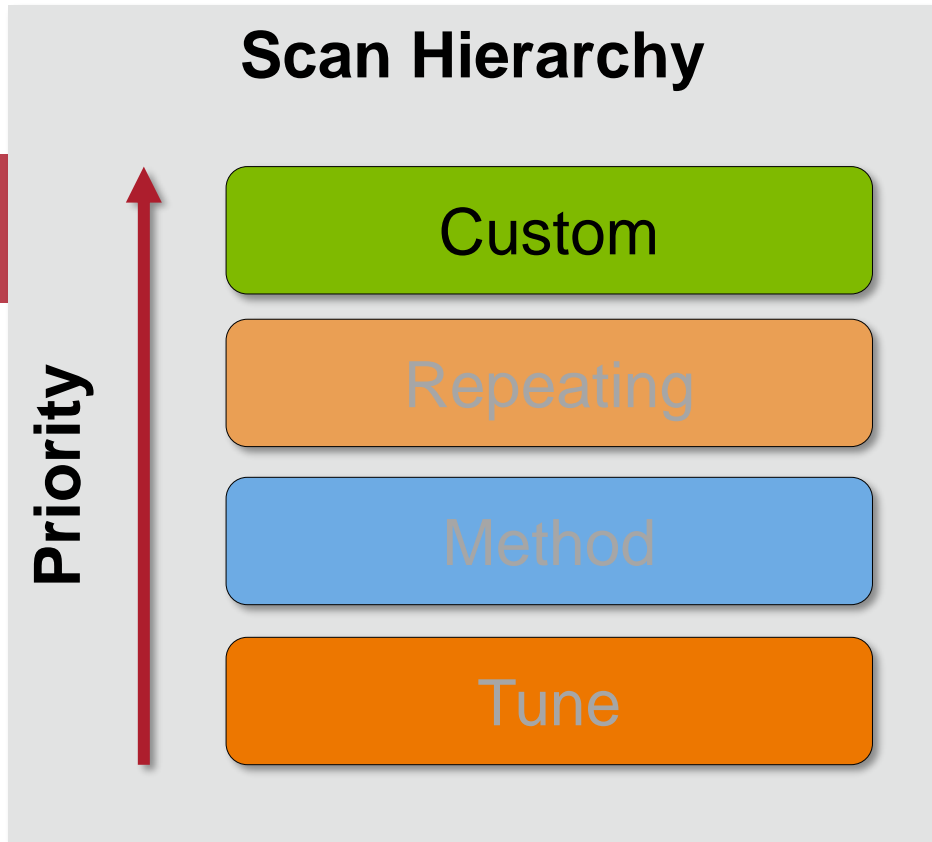
Instrument Service

3rd Party Application

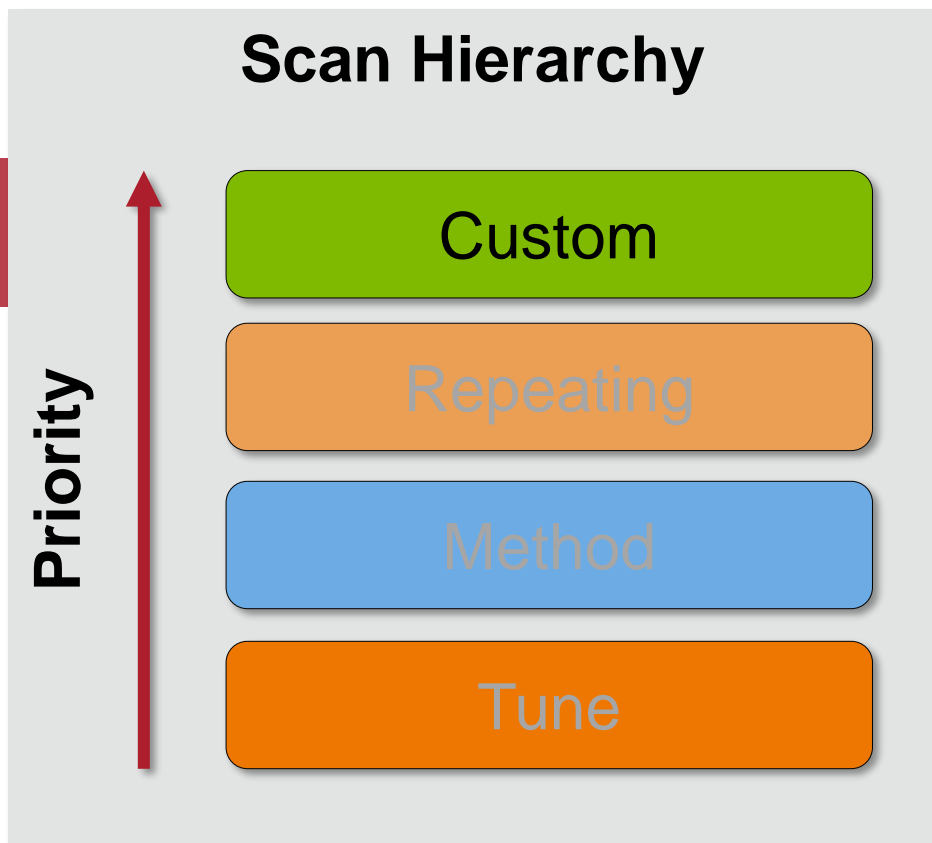
API-2.0.dll

Sending Scan to MS



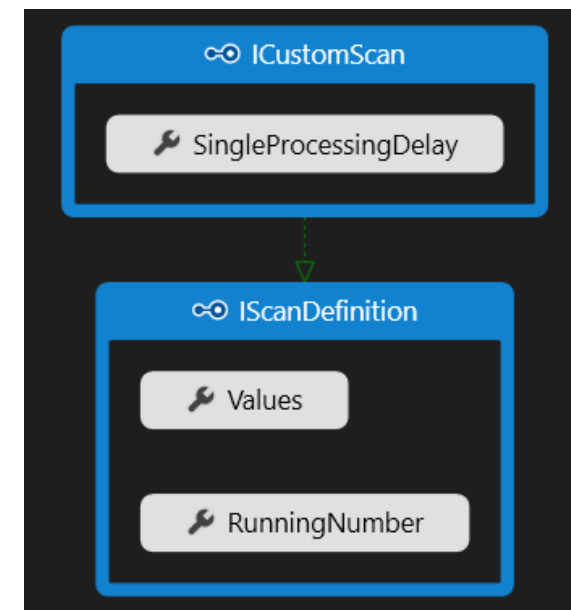


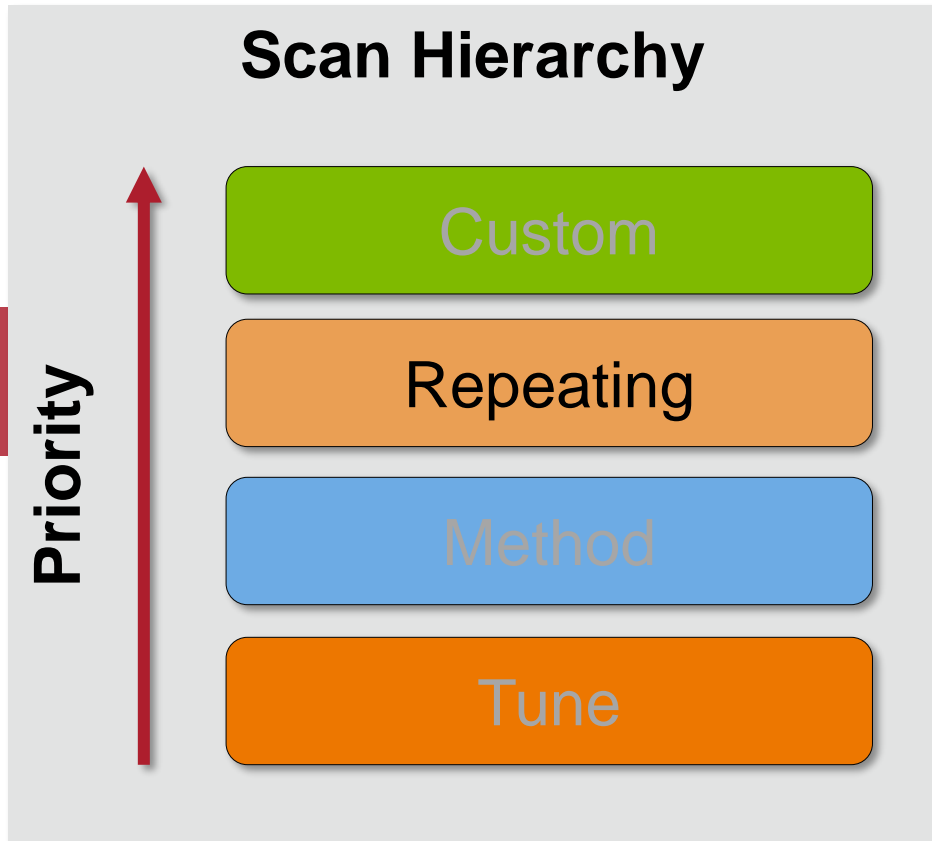
Single scan, drops to lower level after completion



Single scan, drops to lower level after completion

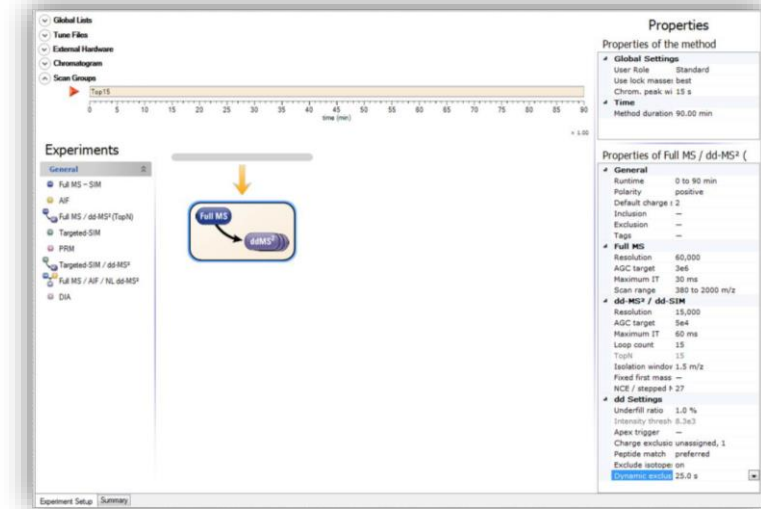
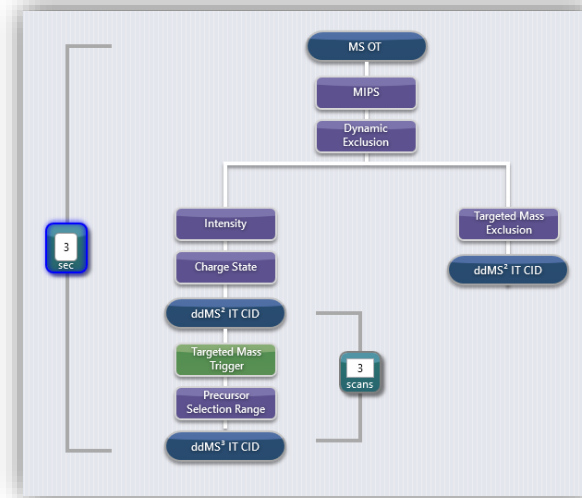
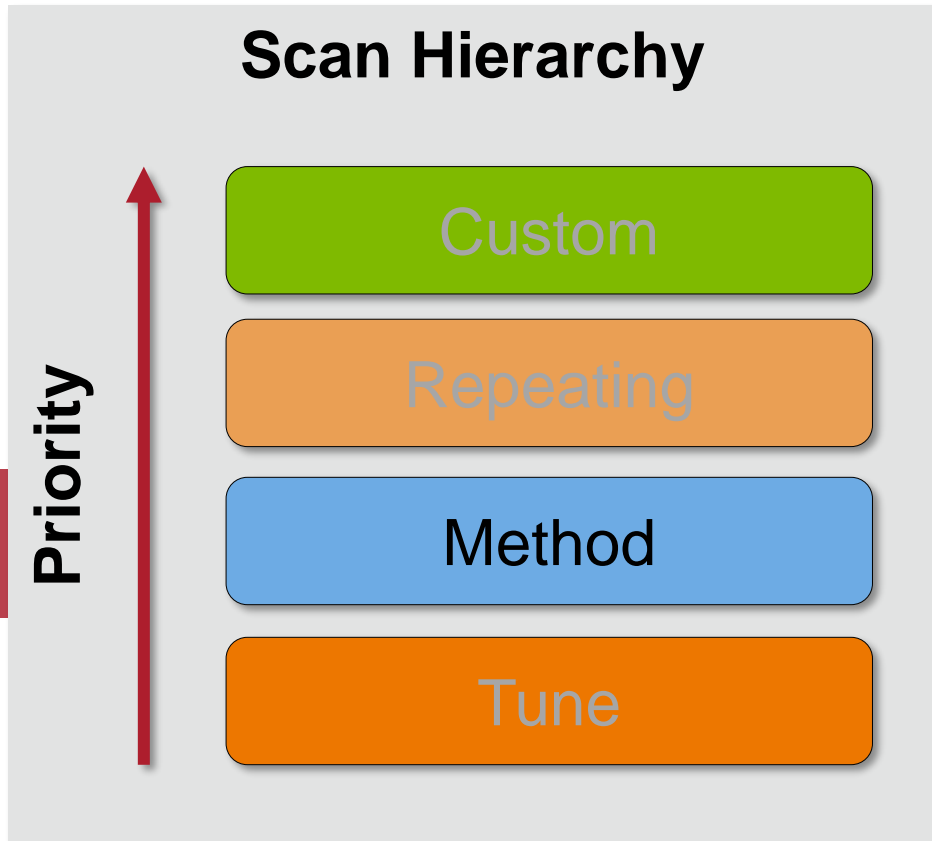
- Once executed, waits the **Single Processing Delay** (s) for a new **Custom** scan to arrive*
- Default delay is 0 seconds (no waiting)
- Immediately ends the wait if a new **Custom** scan arrives or there are pending ones
 - The Tribrid uses a circular buffer (40-capacity) to store them





Repeats scan until explicitly canceled by user

- Continually runs the same scan until:
 1. Cancelled by the user
 2. Replaced by a new Repeating Scan
 3. Preempted by a Custom Scan



Runs Scans and logic defined in the method

- Runs the normal method logic until completion or new IAPI scan arrives

Scan Hierarchy

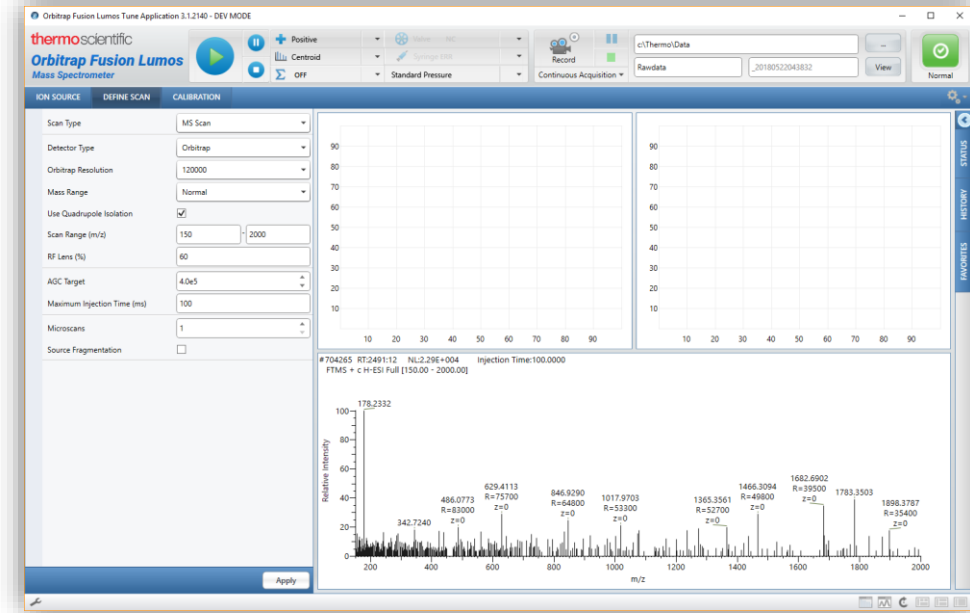
Priority

Custom

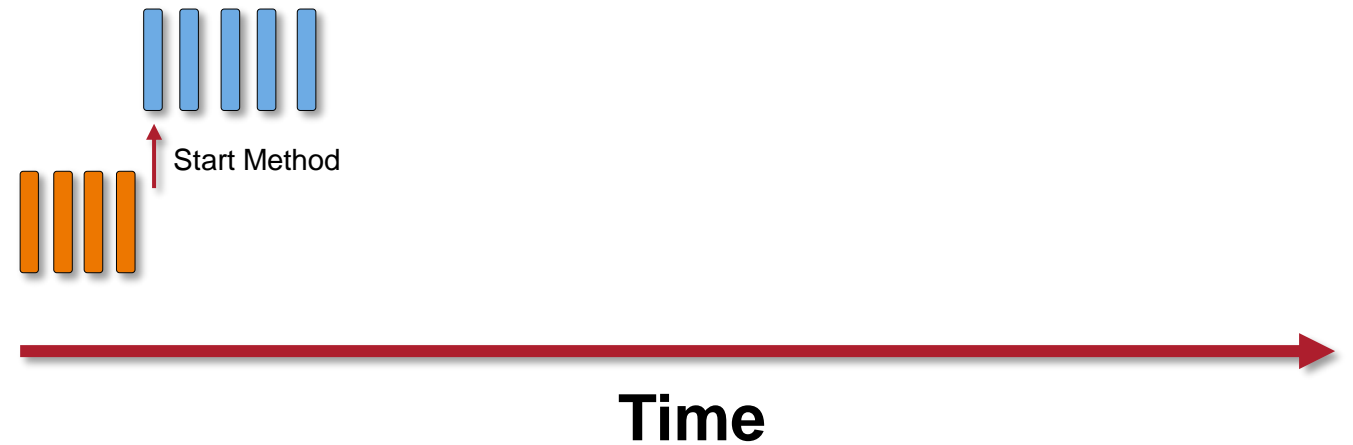
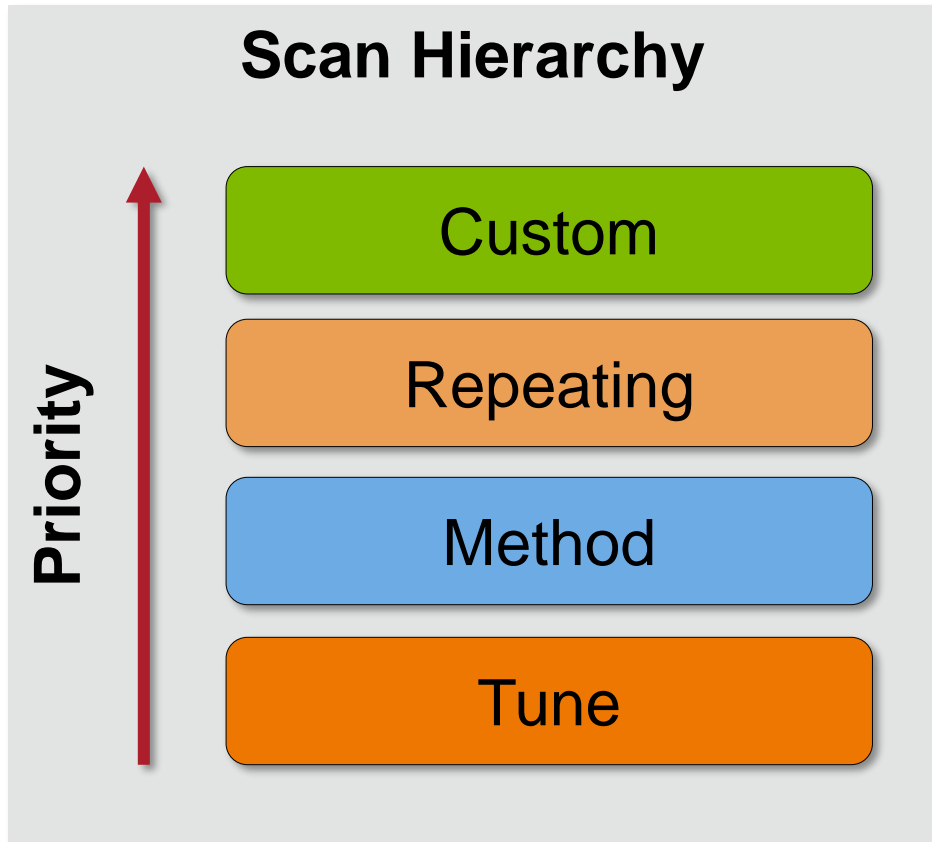
Repeating

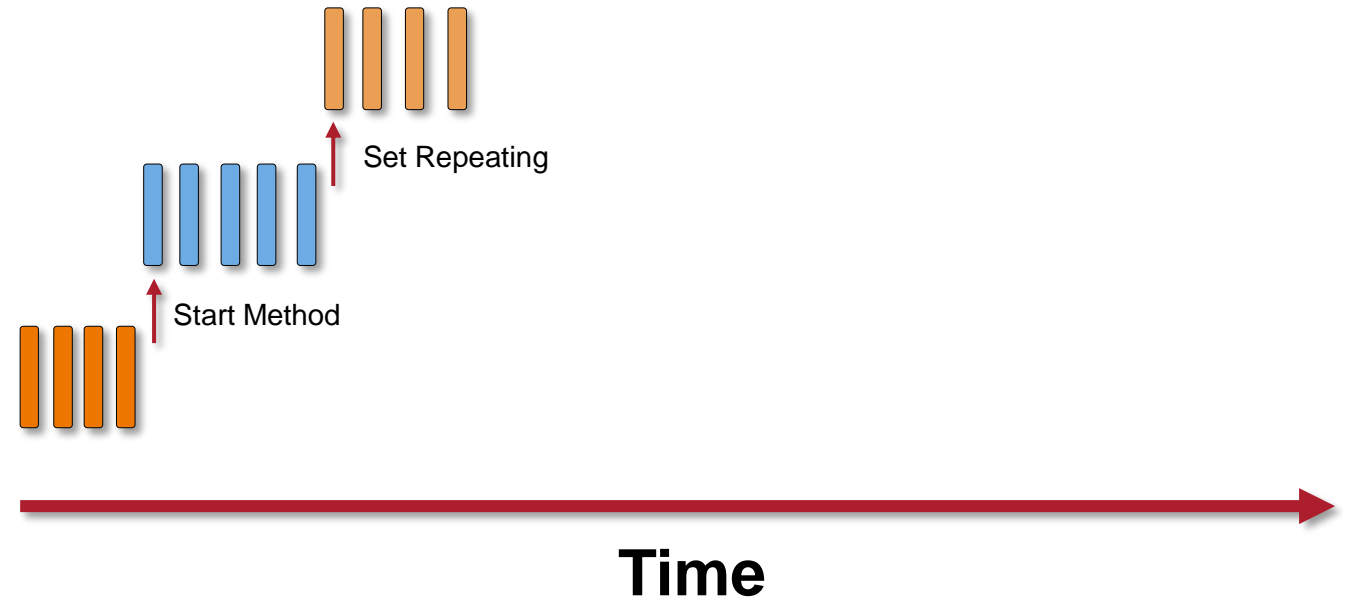
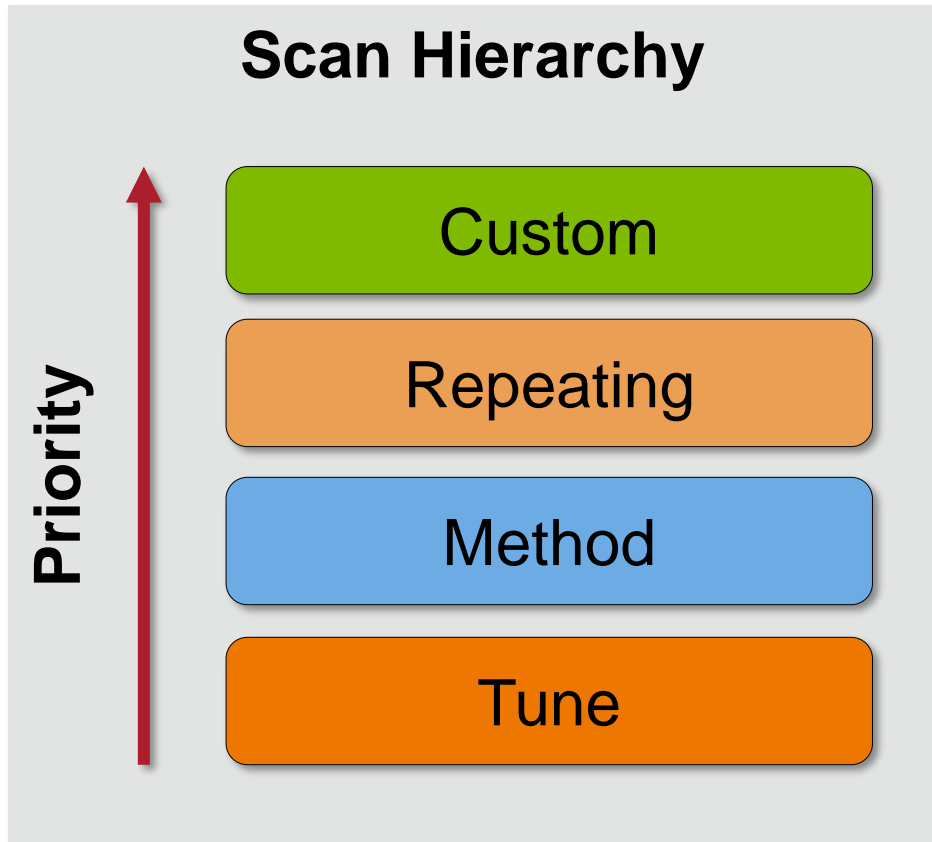
Method

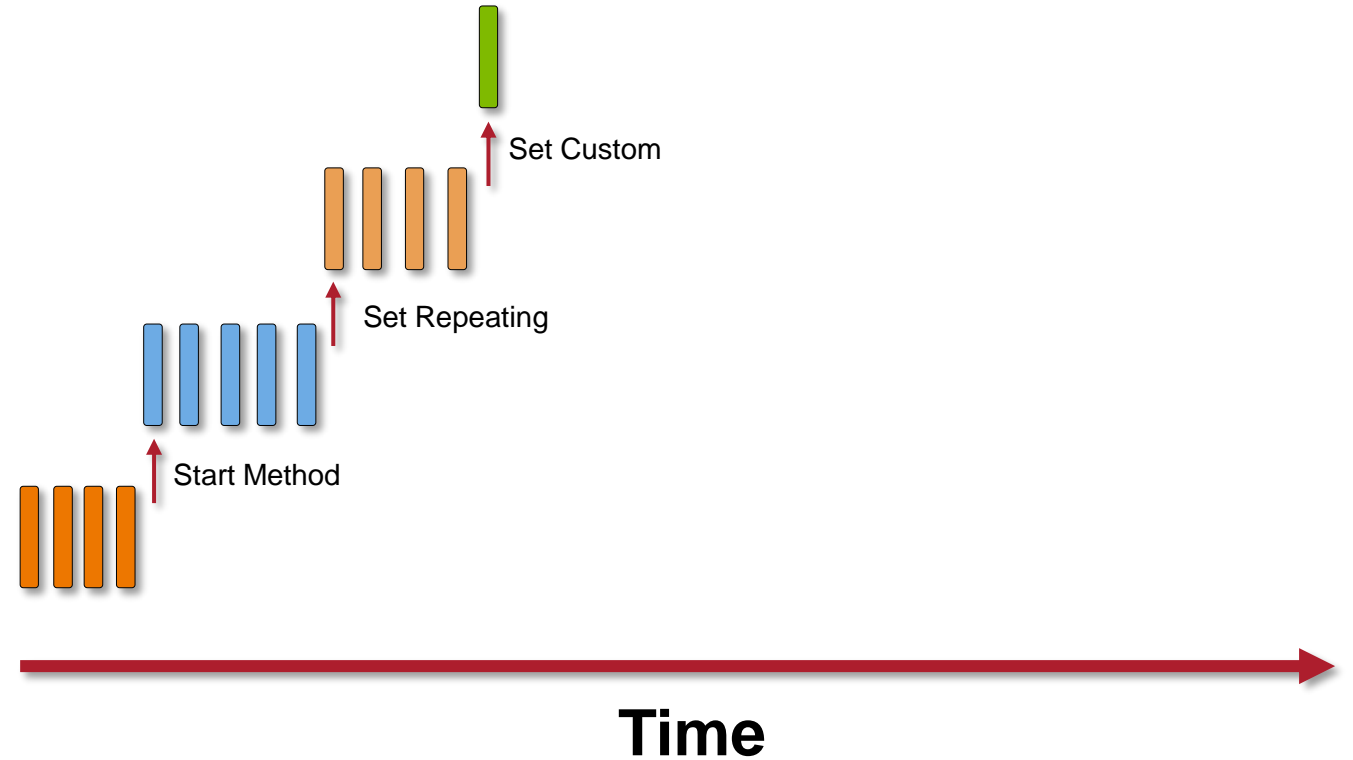
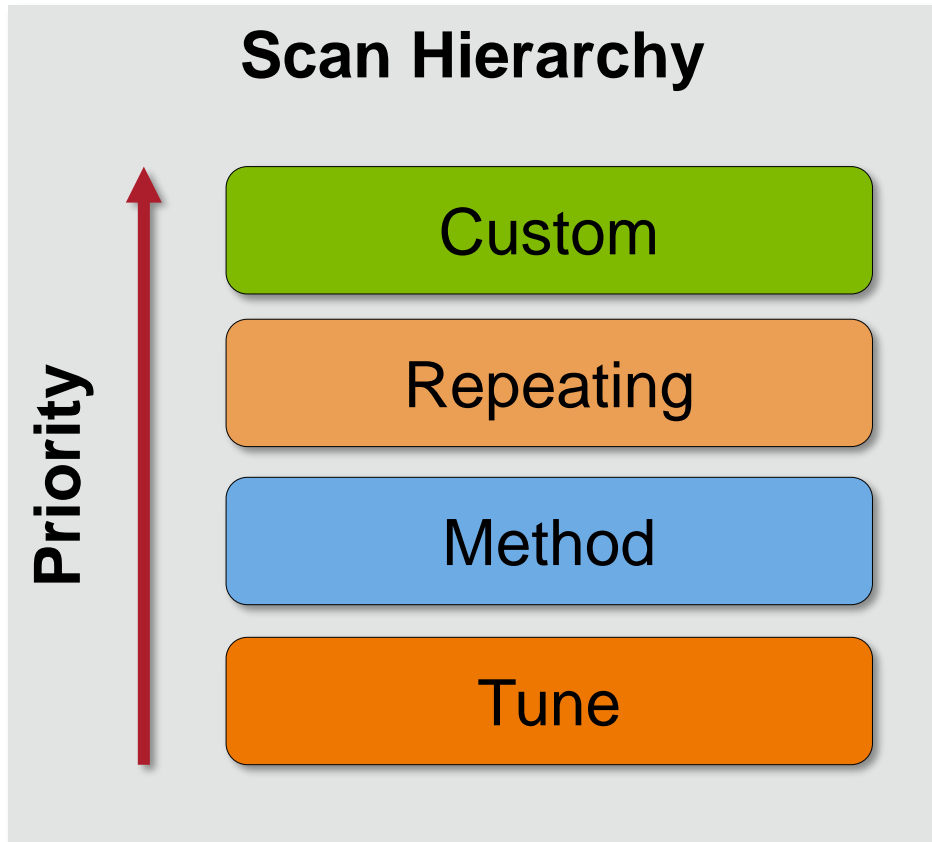
Tune

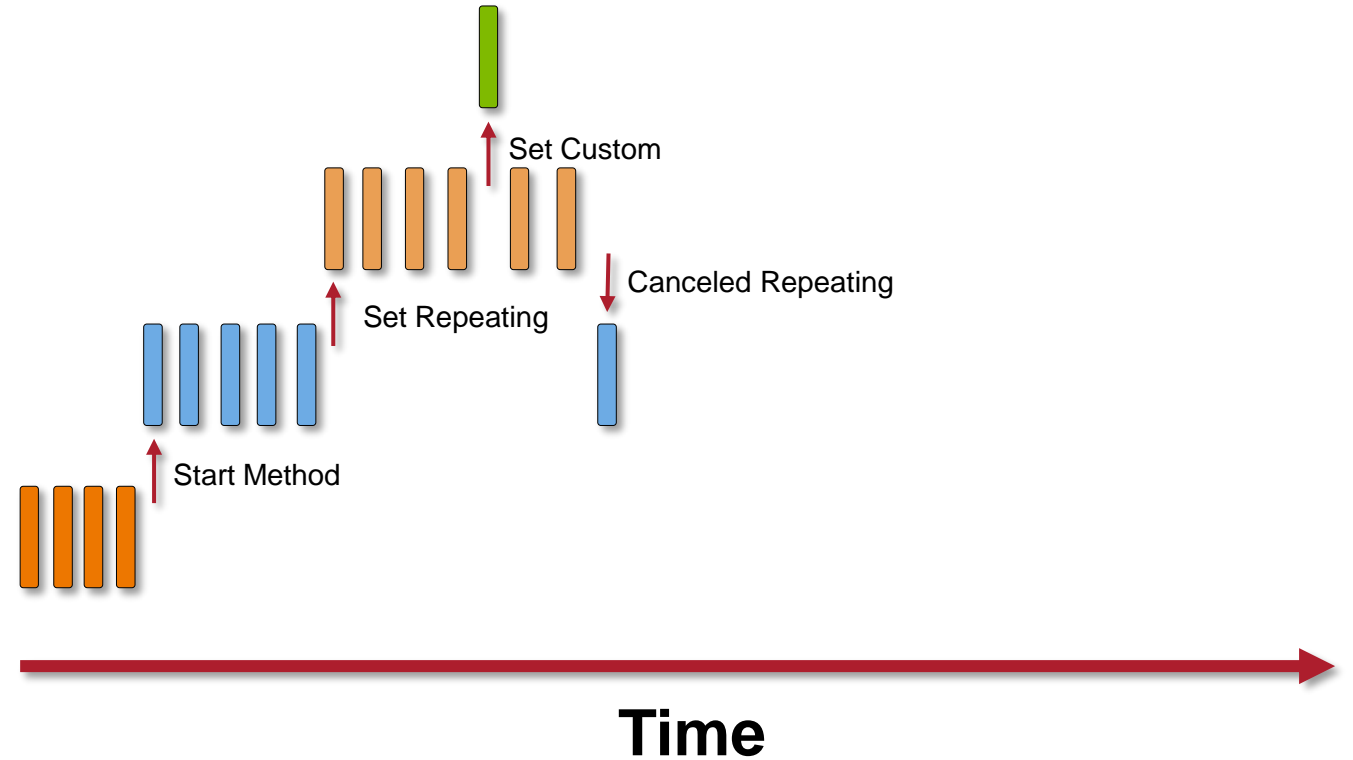
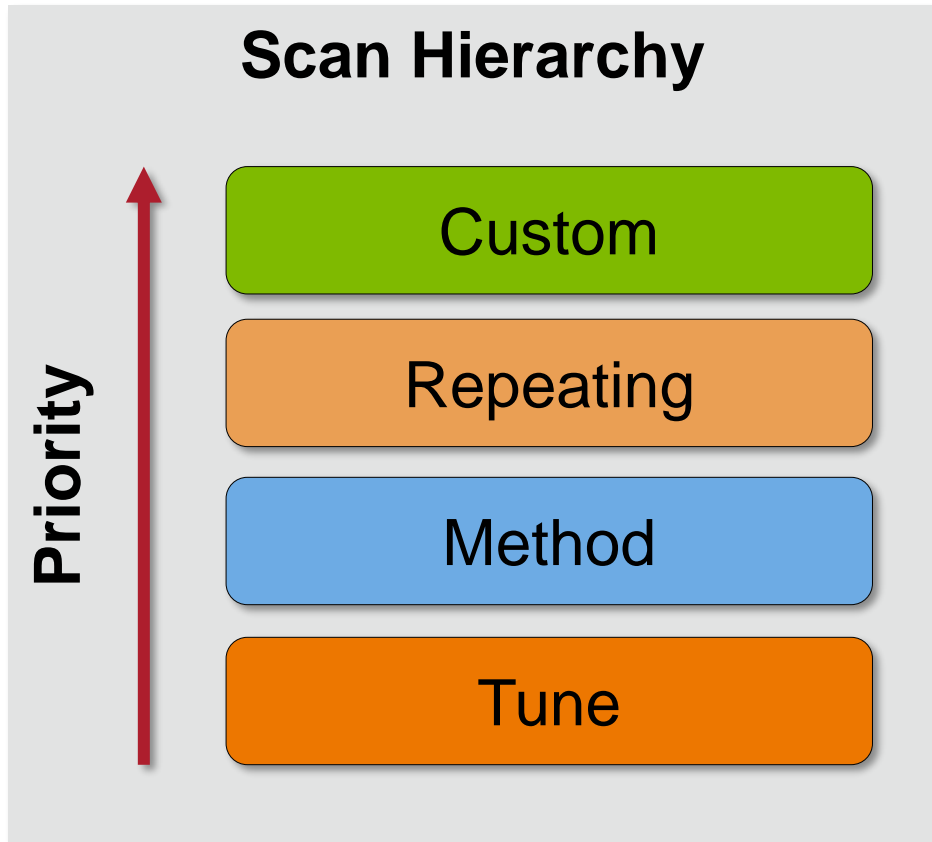


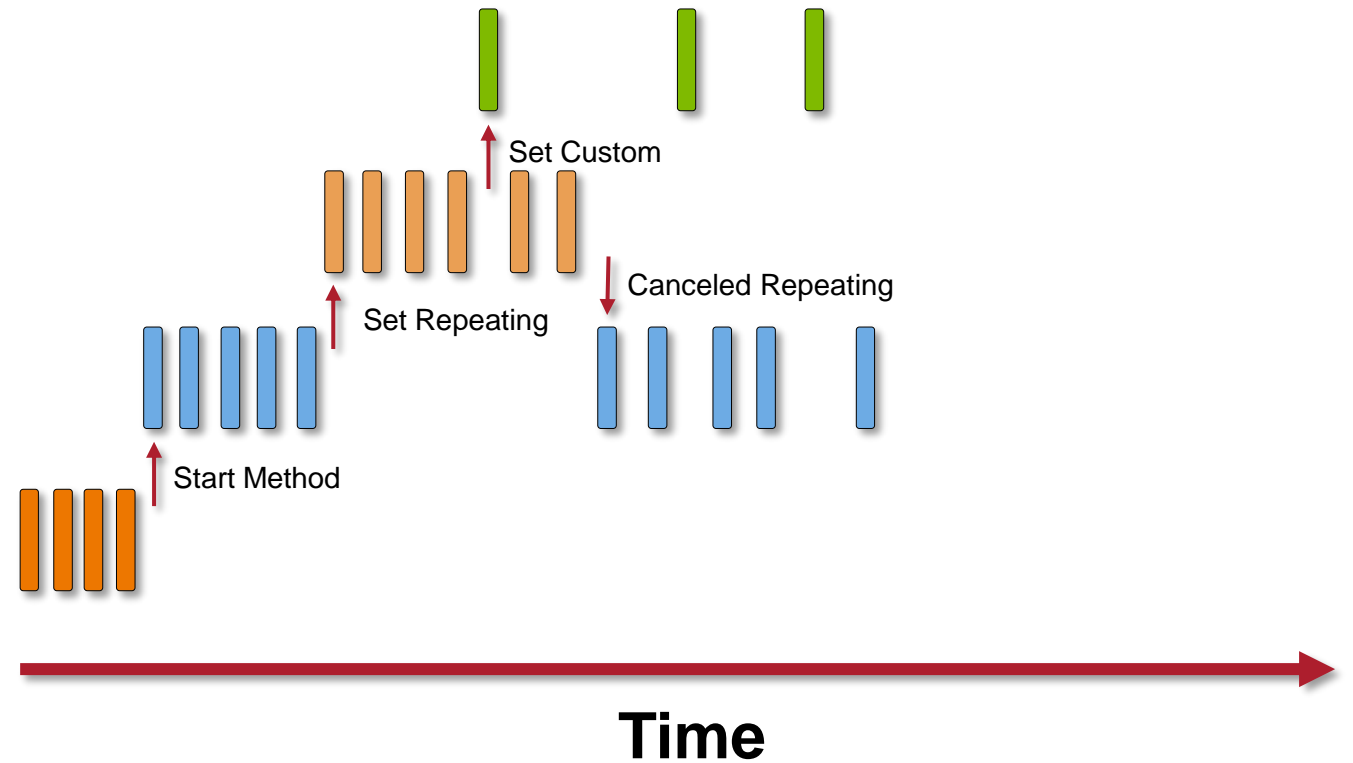
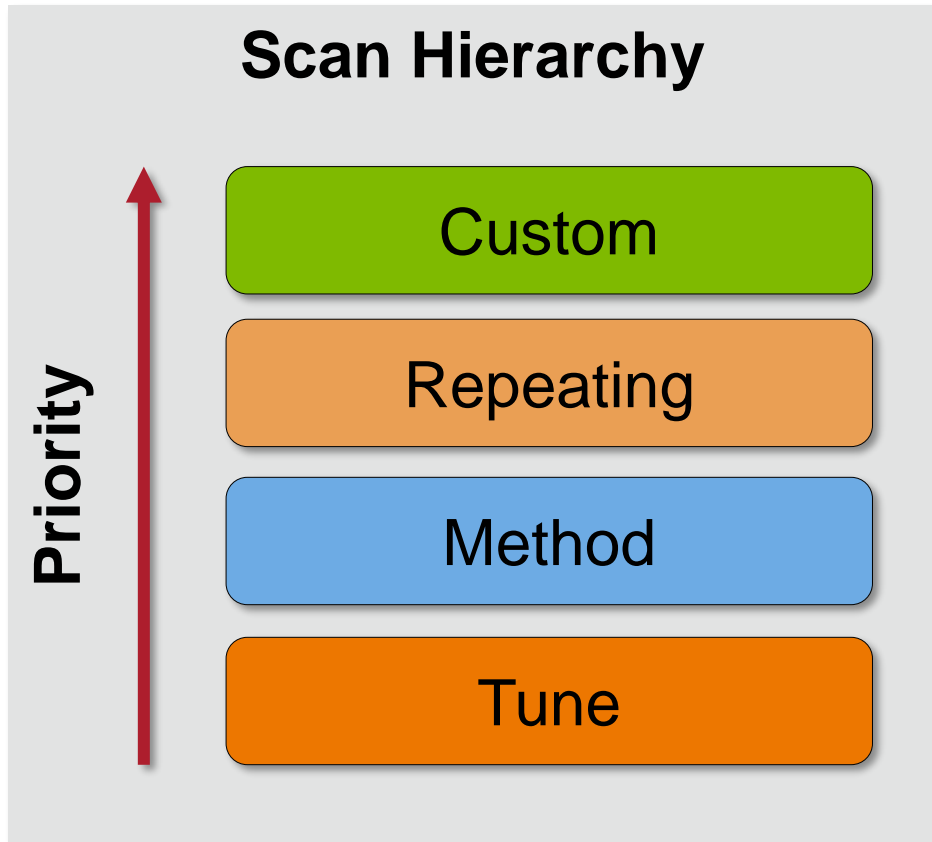
Runs the Scan defined in Tune

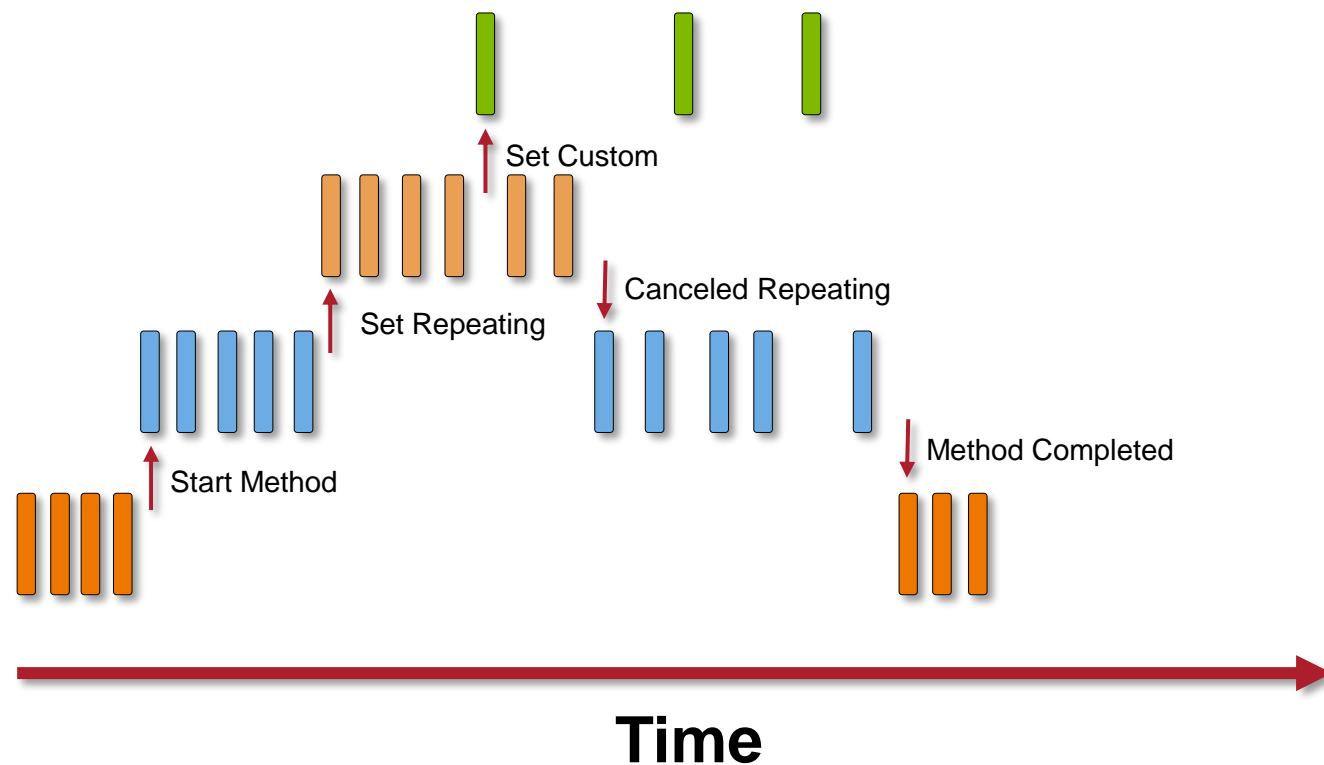
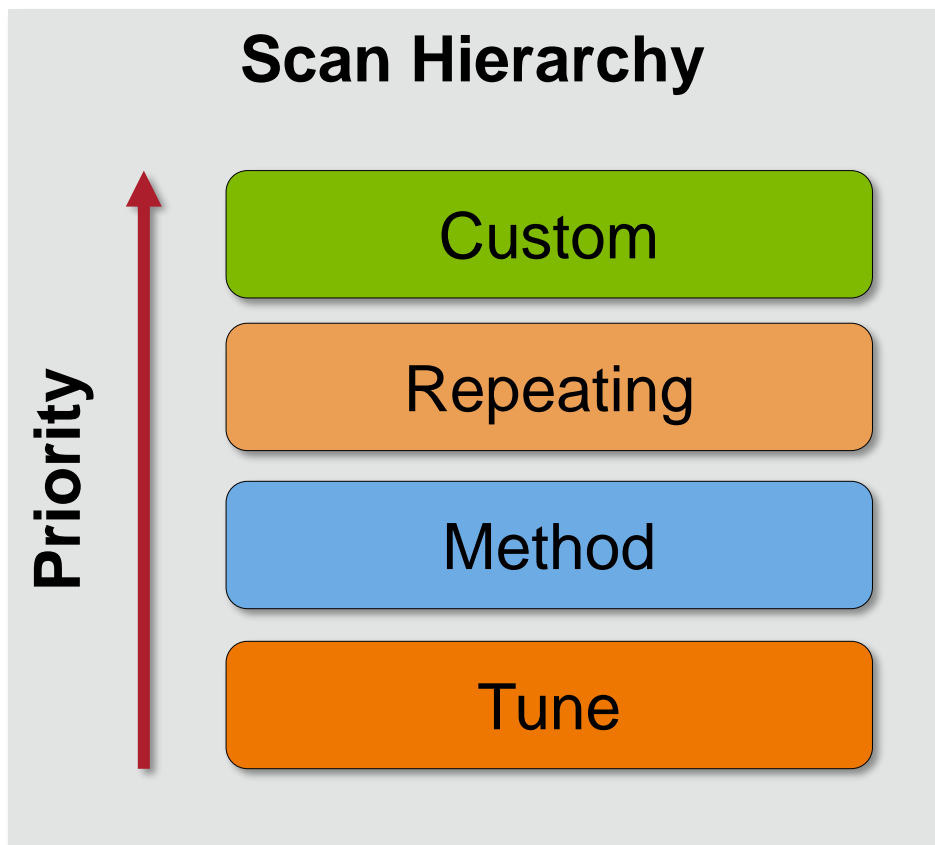


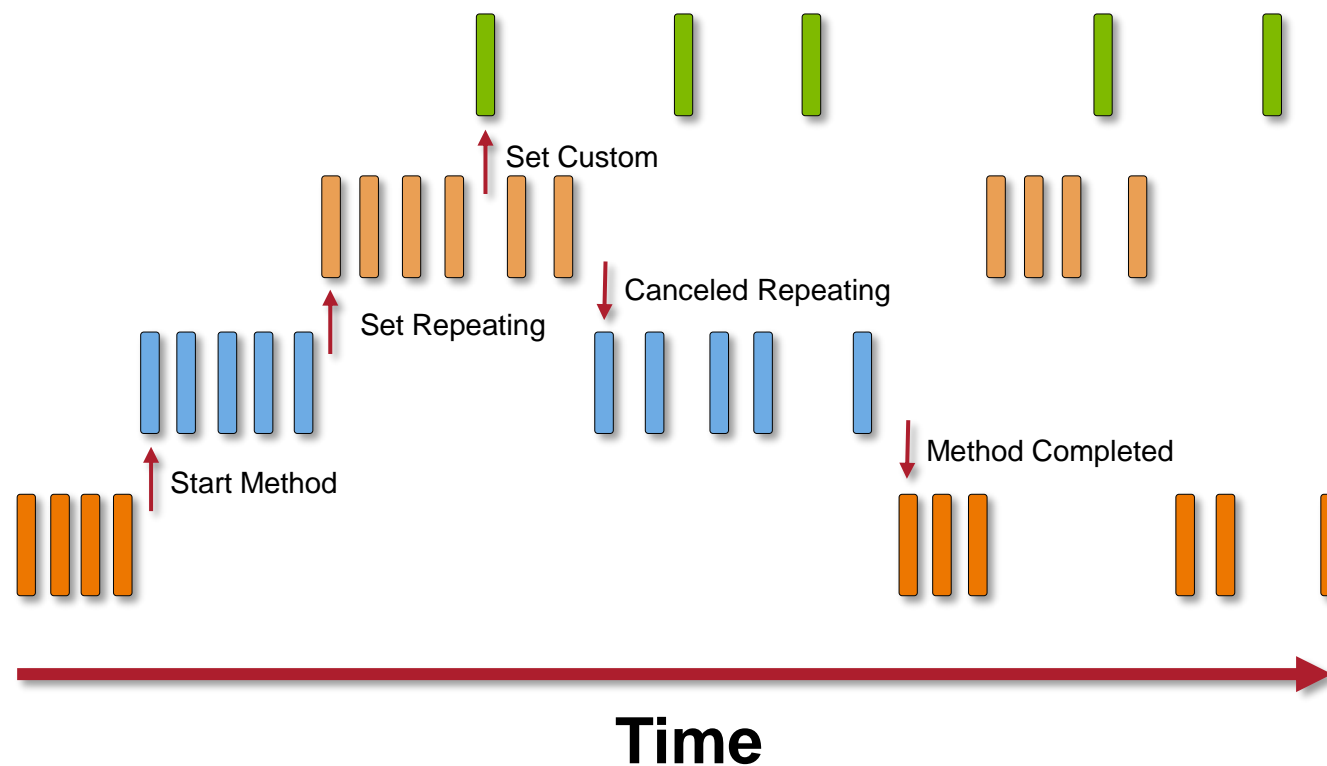
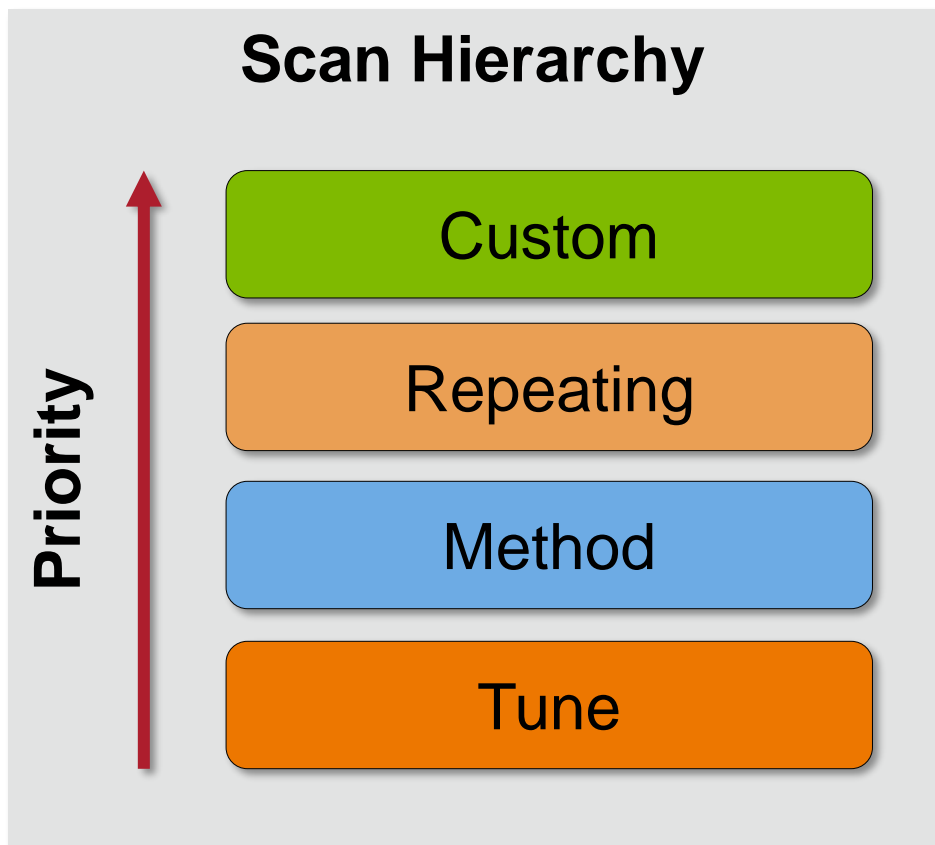












Scan Hierarchy

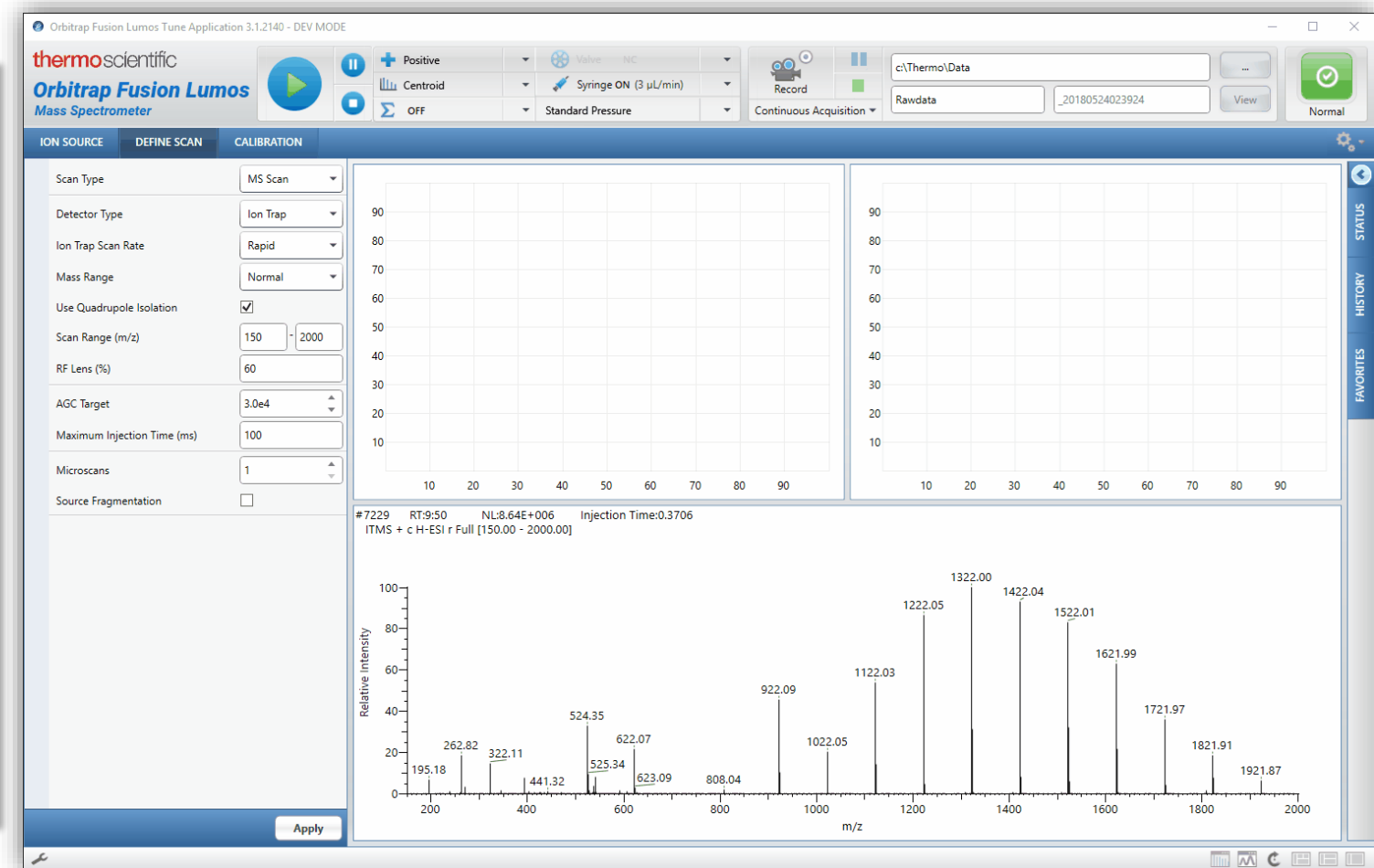
Priority

Custom

Repeating

Method

Tune



Scan Hierarchy

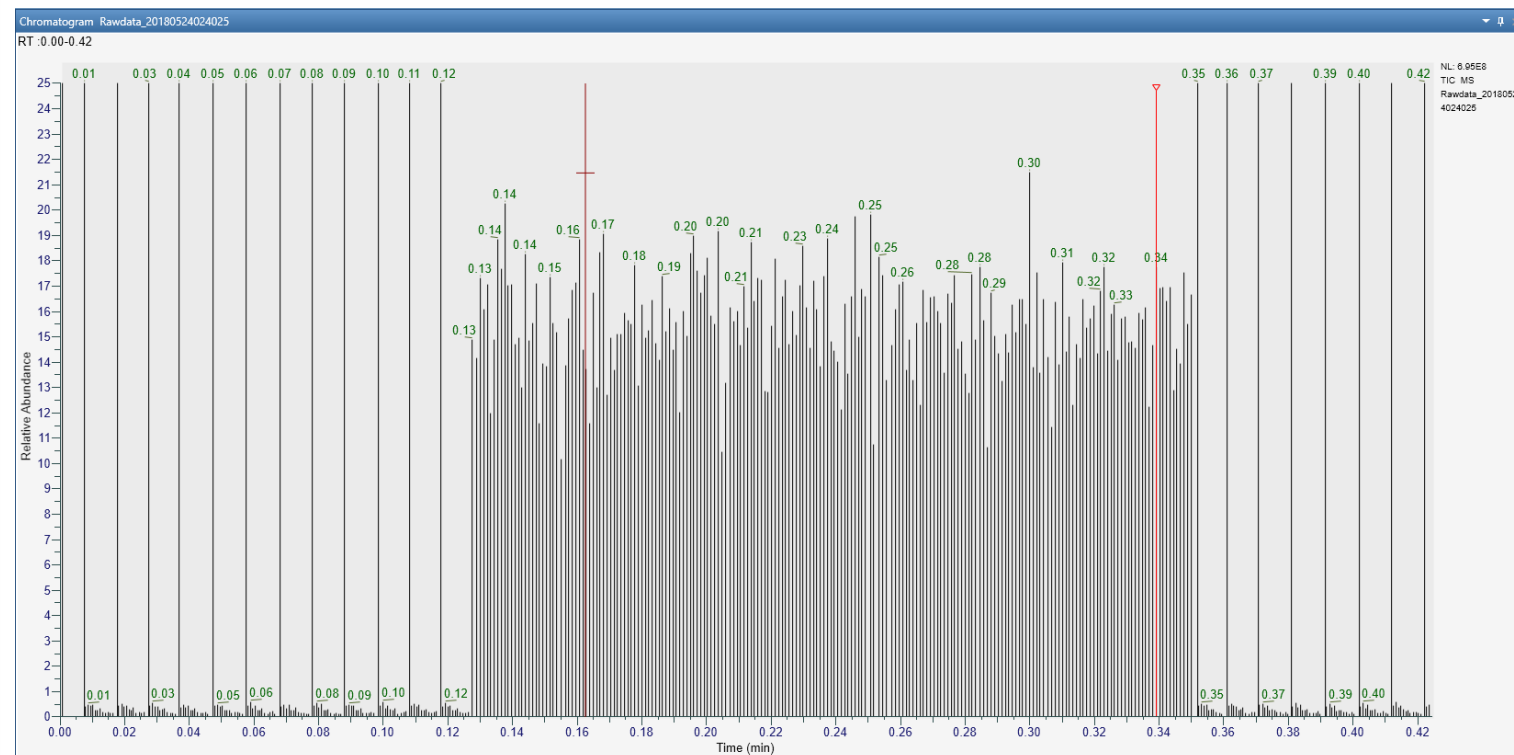
Priority

Custom

Repeating

Method

Tune



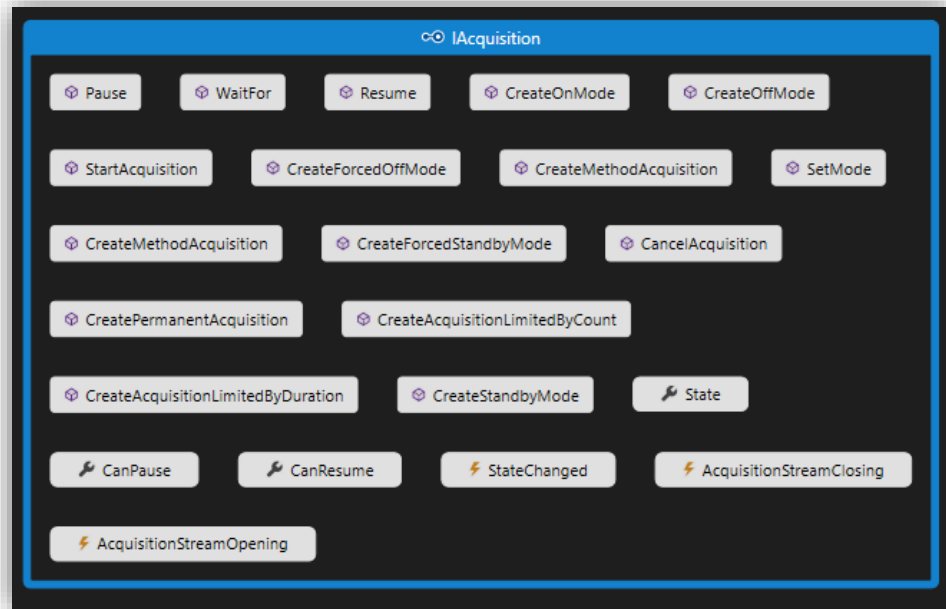
Method
Scans

IAPI
Scans

Method
Scans

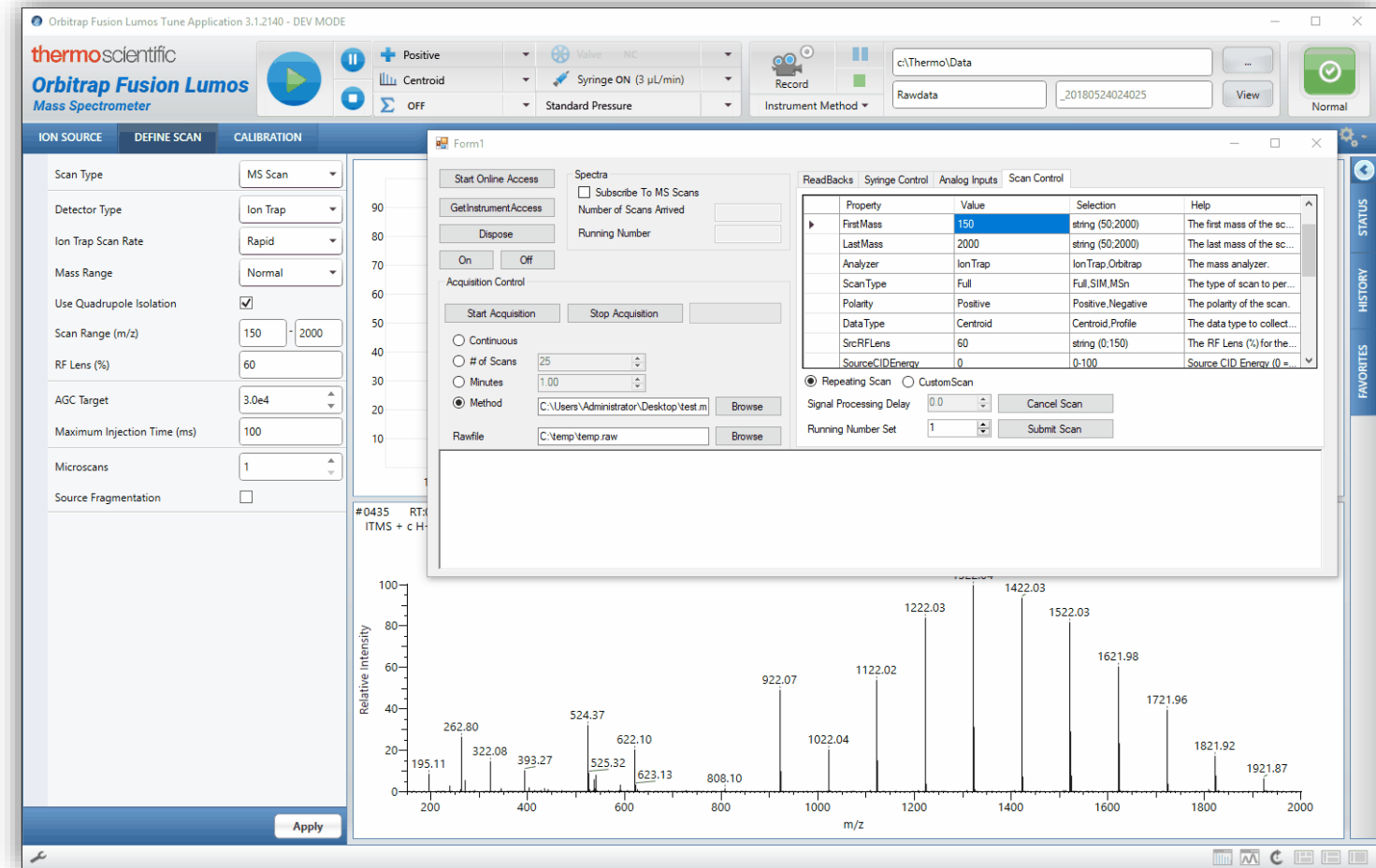
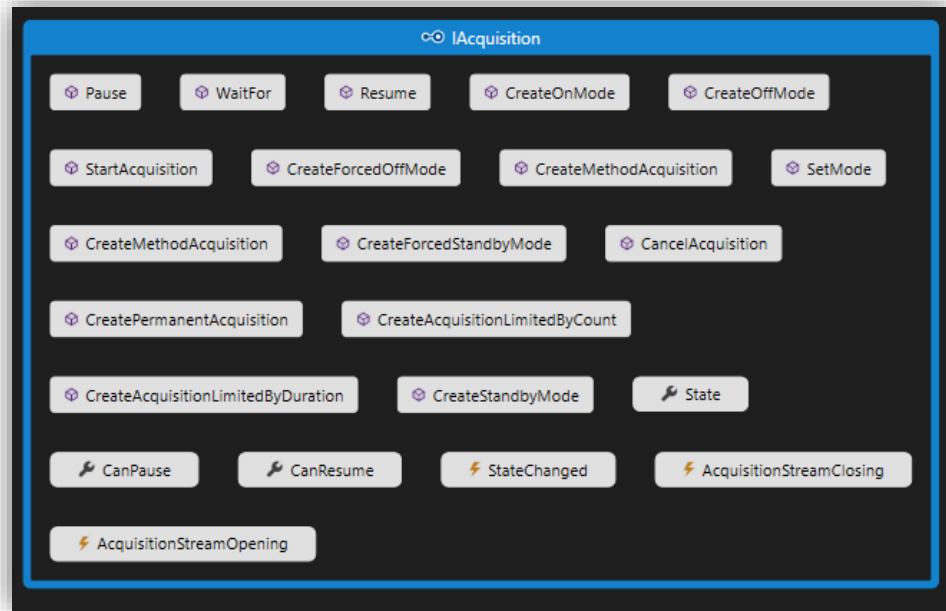
Starting Acquisitions from the IAPI

- The **IAcquisition** interface enables the IAPI to start/stop acquisitions
- It also has **Events** for listening to when an acquisition starts and stops



Starting Acquisitions from the IAPI

- The **IAcquisition** interface enables the IAPI to start/stop acquisitions
- It also has **Events** for listening to when an acquisition starts and stops



1. IAPI Architecture

- Interfaces
- Data and Control Flow

2. Receiving Data from the MS

- Interfaces
- Scan Data Stream Subscription

3. Controlling the MS

- Sending Scan Definitions
- Changing other MS parameters

4. Getting Started

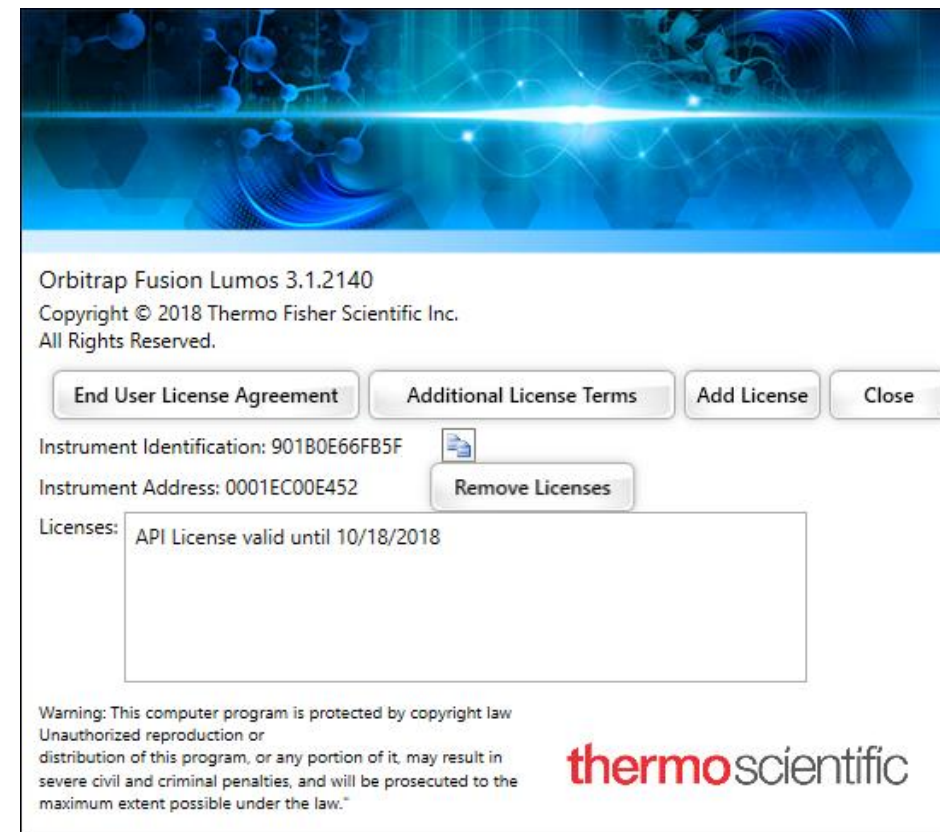
- Access to the IAPI is given after a fully executed IAPI License is in force.
- Contact ThermoMSLicensing@thermo.com to get started
 - Please include which IAPI (**Tribrid** or **Exactive**) you would like access to in your initial correspondence

License Activation

- Access to the IAPI is given after a fully executed IAPI License is in force.
- Contact ThermoMSLicensing@thermo.com to get started
 - Please include which IAPI (**Tribrid** or **Exactive**) you would like access to in your initial correspondence

Activating the License on the Tribrids

- A license key will be provided to you and is entered in Tune's "About Tune" dialog
- Restart both the Instrument and Data system computer and the API will be enabled



1. Instrument Application Programming Interface (IAPI)

- Real-time control and acquisition of Orbitrap Mass Spectrometers using .NET

2. XML Method Modification Interface (XMMI)

- Programmatically modify Fusion-series method files

3. Applications of IAPI

1. Devin Schweppe – Fusion API
2. Christoph Wichmann – Q Exactive API

4. Examples and FAQ for IAPI and XMMI

5. Applications of XMMI

1. Pavel Shliaha – Fusion Method Creation

Method Editor | Global Parameters | Scan Parameters | Summary

METHOD TIMELINE | EXPERIMENT | ACTIONS | SETTINGS

Application Mode: Peptide
Method Duration (min): 60

Experiment # 1 | Time Range (min): 0-60 | CLEAR

Scans

- MS
- MSⁿ

Filters

- Precursor Selection Range
- MIPS
- Intensity
- Purity
- Charge State
- Dynamic Exclusion
- Targeted Inclusion
- Targeted Exclusion
- Apex Detection

Triggers

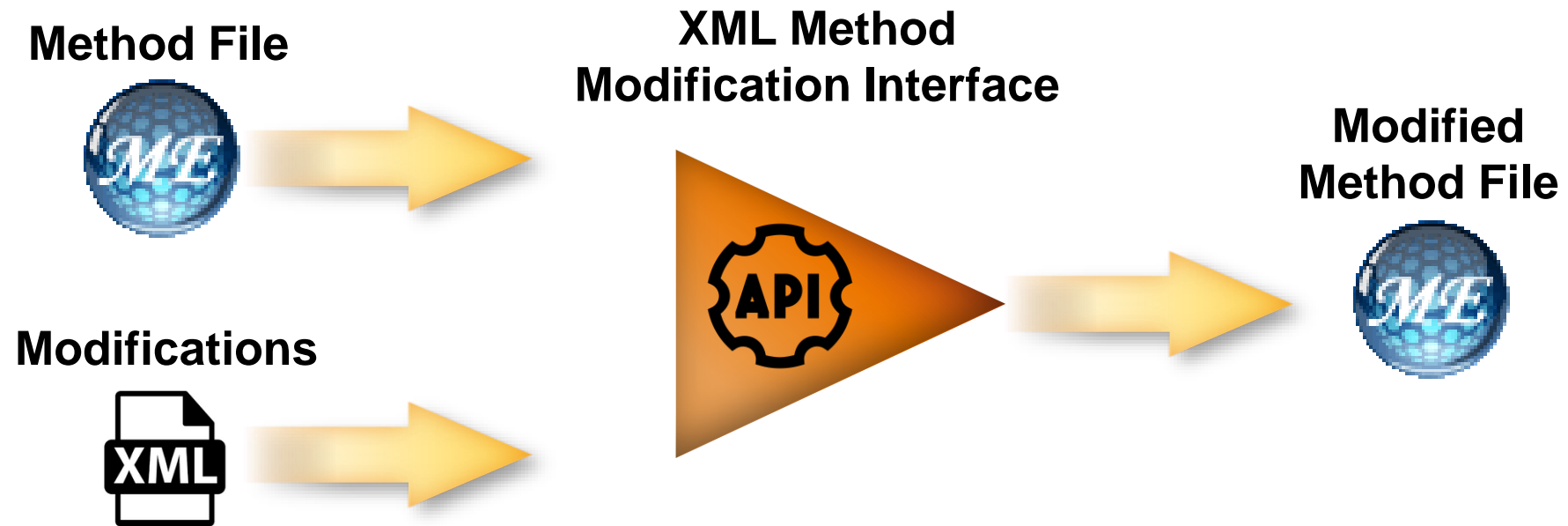
- Targeted Mass Trigger
- Targeted Loss Trigger

MS Scan Properties | Show All

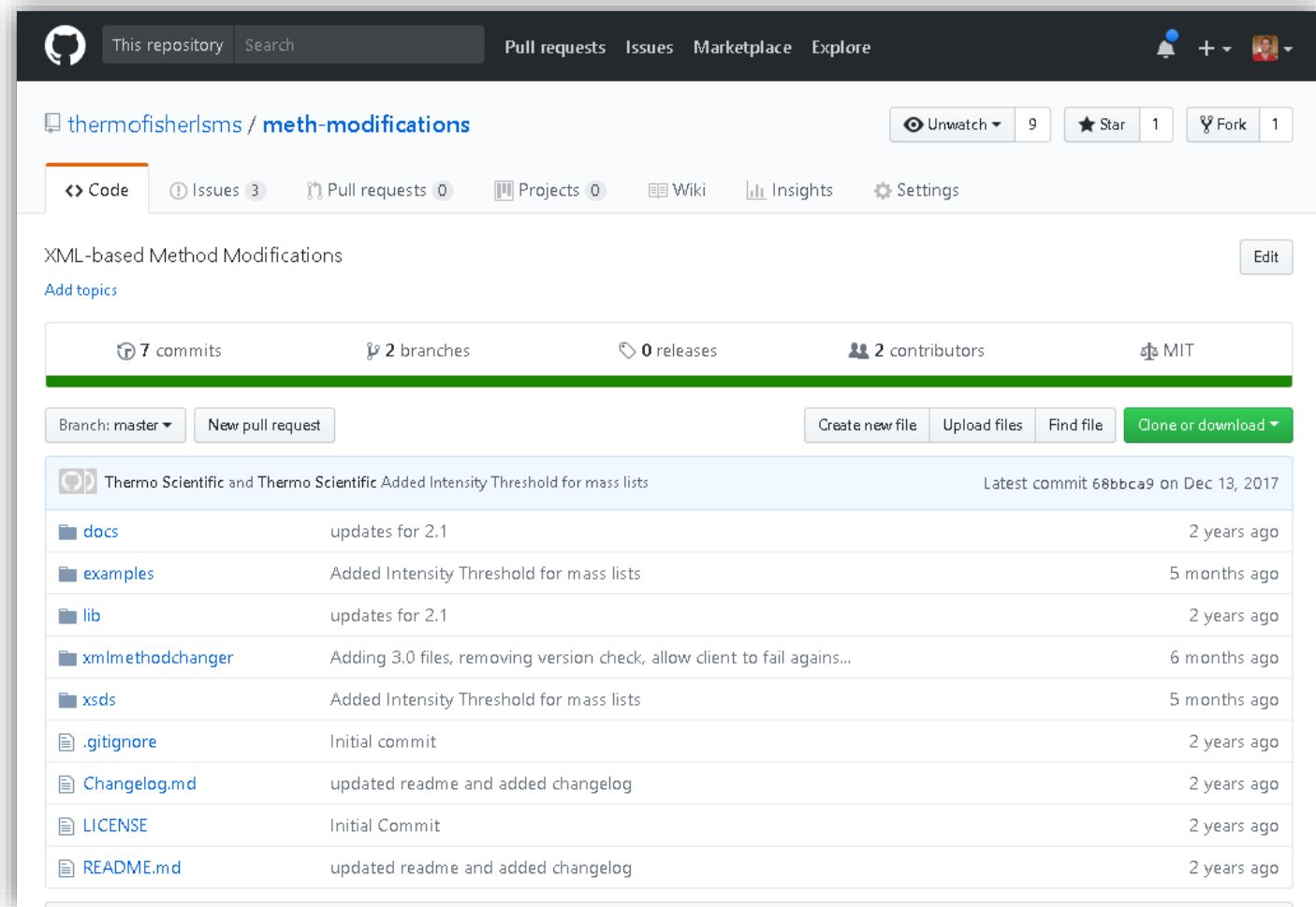
- Orbitrap Resolution: 60000
- Scan Range (m/z): 450-750
- RF Lens (%): 30
- AGC Target: 1.0e6
- Maximum Injection Time (ms): 50

The Method Editor offers tremendous **flexibility** in method creation; sometimes manually editing complex methods is laborious.

Programmatically Modify Fusion Methods



<https://github.com/thermofisherlms/meth-modifications>

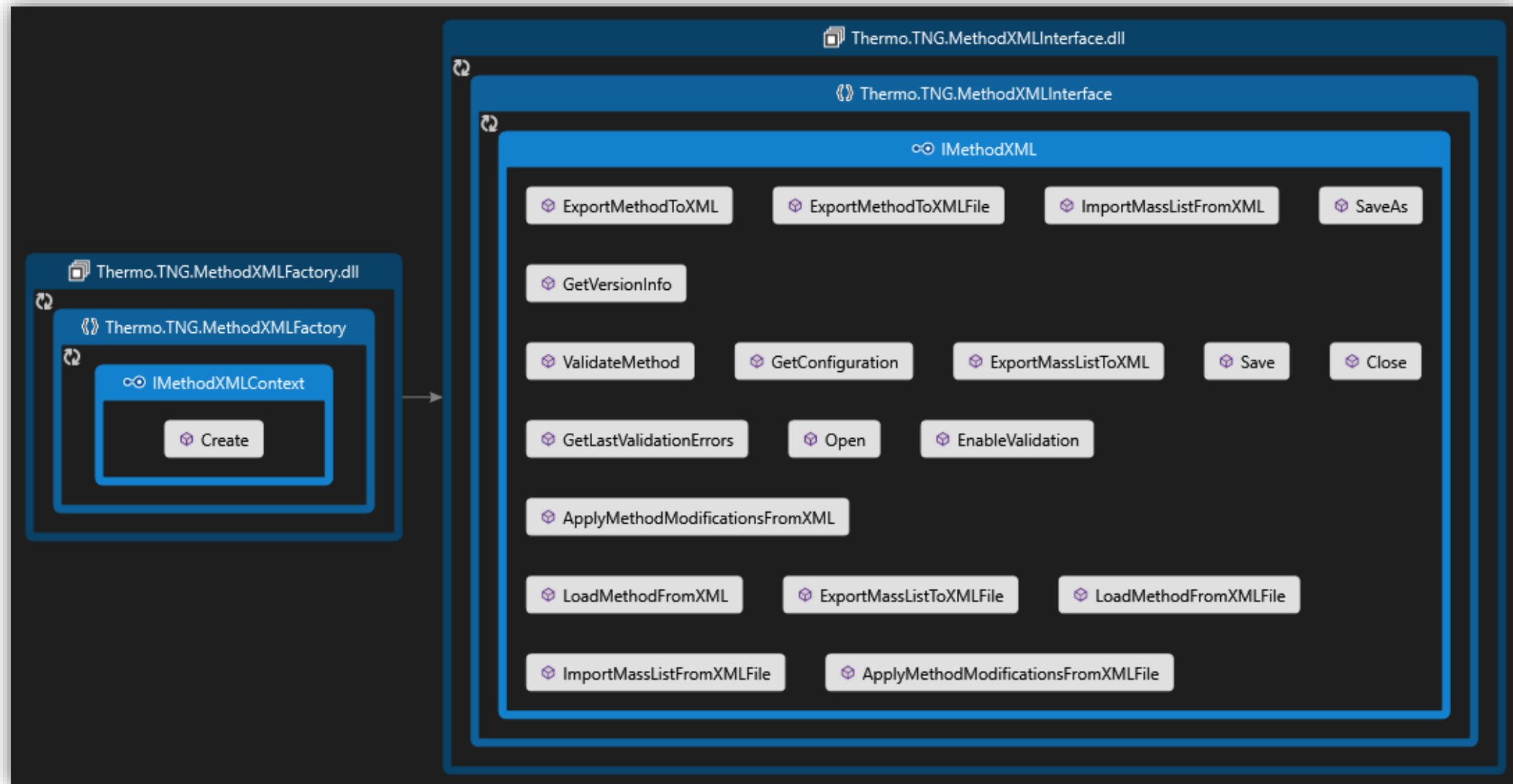


- Supports Fusion and Triple-Quad Methods
 - Versions **1.2, 2.0, 2.1, 3.0** and **3.1**
- Documentation
- Complete Examples
- VS Solution
 - Command Line Program
 - Heavily commented code
- Schemas

External Requirements

- Tune Installation
 - Full or Workstation
- .NET 4.6.2+

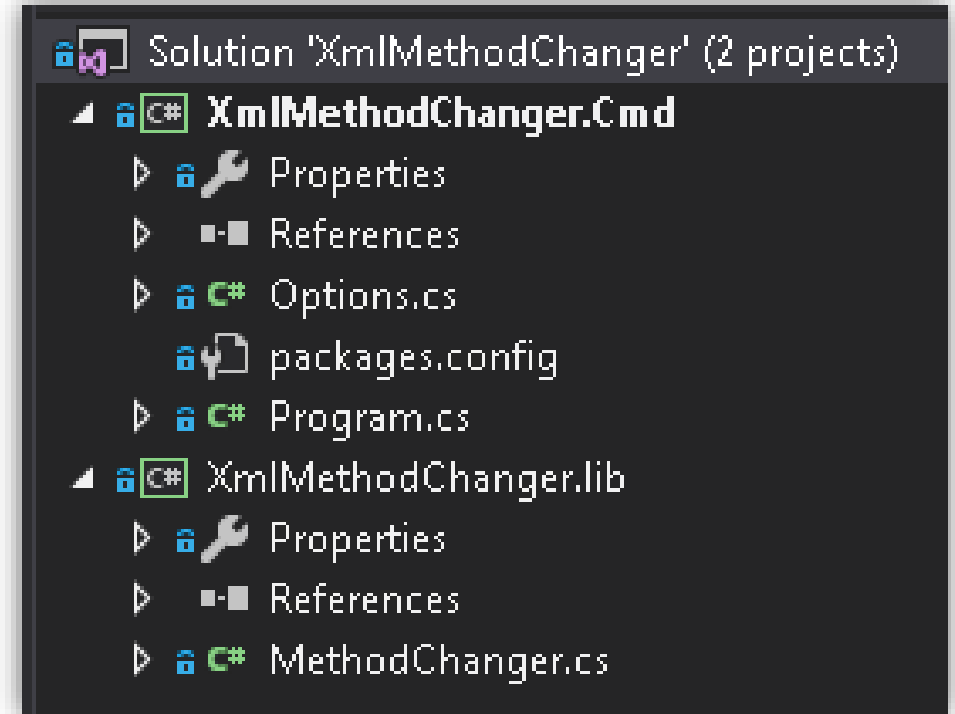
XML Modification Interfaces



Complete Code Example

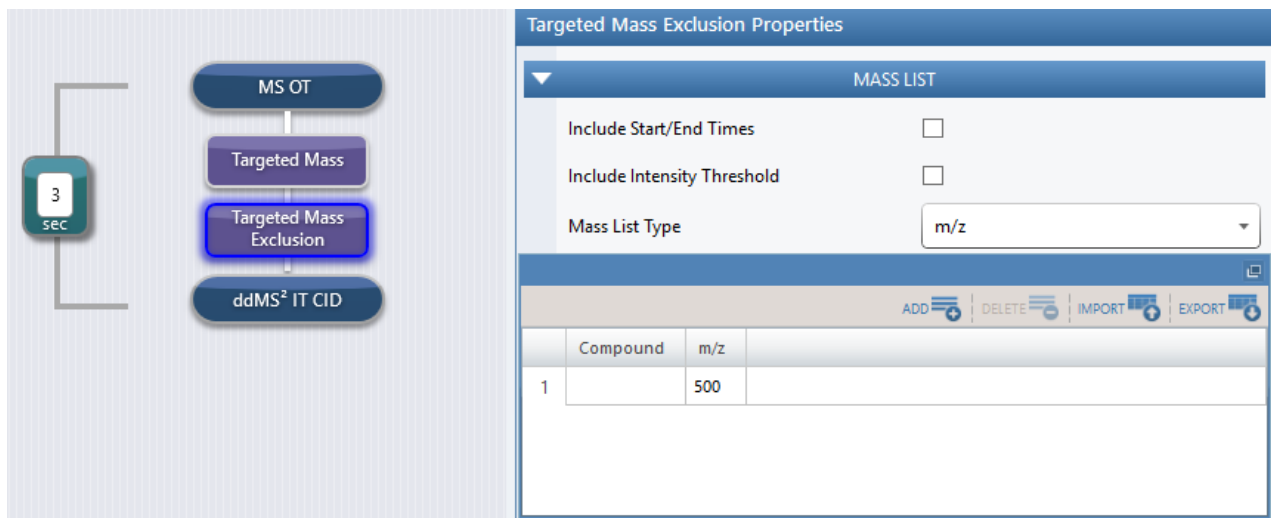
```
1  using Thermo.TNG.MethodXMLFactory;
2  using Thermo.TNG.MethodXMLInterface;
3
4  string templateMethod = @"Files/Fusion/DIA/DIA_Template.meth";
5  string modificationXML = @"Files/Fusion/DIA/DIA.xml";
6  string outputMethod = @"Files/Fusion/DIA/DIA_output.meth";
7
8  string instrumentModel = "OrbitrapFusion"; // alternatively "TSQEndura" or "TSQQuantiva"
9  string instrumentVersion = "1.2"; // just the version number as a string
10
11  using(IMethodXMLContext mxc = MethodXMLFactory.CreateContext(instrumentModel, instrumentVersion))
12  using(IMethodXML mx = mxc.Create()) {
13
14      mx.Open(templateMethod); // Open the template method file
15
16      mx.ApplyMethodModificationsFromXMLFile(modificationXML); // Apply the xml modifications
17
18      mx.SaveAs(outputMethod); // Save the method in memory to disk
19  }
20
```

- The **XmlMethodChanger** Solution contains two projects
 1. XmlMethodChanger.lib
 - A public, static class library (**MethodChanger**)
 - Wraps the two Thermo.TNG.*.dll assemblies
 - Provides public methods for common tasks
 - Serves as an example of the API Usage
 2. XmlMethodChanger.cmd
 - Command-line program that uses XmlMethodChanger.lib
 - Uses third-party NuGet package (Command Line Parser Library)
 - Can be used as a standalone tool for modifying methods



Demonstration

- The “template” method contains the structure of the experiment



The diagram on the left illustrates a 3-second experiment sequence. It features a vertical stack of four components: a green box labeled '3 sec' on the left, and a sequence of four rounded rectangular boxes on the right: 'MS OT' (blue), 'Targeted Mass' (purple), 'Targeted Mass Exclusion' (purple), and 'ddMS² IT CID' (blue). Arrows indicate the flow from 'MS OT' to 'Targeted Mass', then to 'Targeted Mass Exclusion', and finally to 'ddMS² IT CID'. A bracket on the left groups the entire sequence under the '3 sec' label.

The screenshot on the right shows the 'Targeted Mass Exclusion Properties' dialog box. It has a 'MASS LIST' section with the following options:

- Include Start/End Times: ☐
- Include Intensity Threshold: ☐
- Mass List Type:

Below these options is a table with columns 'Compound' and 'm/z'. The table contains one row with the value '1' in the 'Compound' column and '500' in the 'm/z' column. Above the table are buttons for 'ADD', 'DELETE', 'IMPORT', and 'EXPORT'.

Demonstration

- The “template” method contains the structure of the experiment

The diagram on the left shows a 3-second experiment sequence with the following steps: MS OT, Targeted Mass, Targeted Mass Exclusion, and ddMS² IT CID. The screenshot on the right shows the 'Targeted Mass Exclusion Properties' dialog. It includes a 'MASS LIST' section with checkboxes for 'Include Start/End Times' and 'Include Intensity Threshold', and a 'Mass List Type' dropdown set to 'm/z'. Below this is a table with columns 'Compound', 'm/z', and an empty column. The table contains one row with '1' in the 'Compound' column and '500' in the 'm/z' column.

Compound	m/z	
1	500	

- The modification XML specifies a list of transformations to perform on the “template” method file

```
1 <?xml version="1.0" encoding="utf-8" ?>
2 <MethodModifications Version="2" Model="OrbitrapFusionLumos" Family="Calcium" Type="SL">
3   <Modification Order="1">
4     <Experiment ExperimentIndex="0">
5       <MassListFilter MassListType="TargetedMassExclusion">
6         <MassList IntensityThreshold="true">
7           <MassListRecord>
8             <MOverZ>195.12</MOverZ>
9             <Z>1</Z>
10            <IntensityThreshold>5e6</IntensityThreshold>
11          </MassListRecord>
12          <MassListRecord>
13            <MOverZ>262.3</MOverZ>
14            <Z>2</Z>
15            <IntensityThreshold>1e4</IntensityThreshold>
16          </MassListRecord>
17        </MassList>
18      </MassListFilter>
19    </Experiment>
20  </Modification>
21  <Modification Order="2">
22    <Experiment ExperimentIndex="0">
23      <MassListFilter MassListType="TargetedMassInclusion">
24        <MassList IntensityThreshold="true">
25          <MassListRecord>
26            <MOverZ>195.12</MOverZ>
27            <Z>1</Z>
28            <IntensityThreshold>5e7</IntensityThreshold>
29          </MassListRecord>
30          <MassListRecord>
31            <MOverZ>262.3</MOverZ>
32            <Z>2</Z>
33            <IntensityThreshold>1e2</IntensityThreshold>
34          </MassListRecord>
35        </MassList>
36      </MassListFilter>
37    </Experiment>
38  </Modification>
39 </MethodModifications>
```


Demonstration

- The “template” method contains the structure of the experiment

The diagram on the left shows a sequence of three steps: MS OT, Targeted Mass, and Targeted Mass Exclusion, followed by ddMS² IT CID. A bracket indicates the first three steps take 3 seconds. The screenshot on the right shows the 'Targeted Mass Exclusion Properties' dialog. It has a 'MASS LIST' section with checkboxes for 'Include Start/End Times' and 'Include Intensity Threshold', and a 'Mass List Type' dropdown set to 'm/z'. Below is a table with one row: 1 | Compound | m/z | 500.

	Compound	m/z
1		500

- The modification XML specifies a list of transformations to perform on the “template” method file
- The transformed method can then be saved to a separate .meth file

```
1 <?xml version="1.0" encoding="utf-8" ?>
2 <MethodModifications Version="2" Model="OrbitrapFusionLumos" Family="Calcium" Type="SL">
3   <Modification Order="1">
4     <Experiment ExperimentIndex="0">
5       <MassListFilter MassListType="TargetedMassExclusion">
6         <MassList IntensityThreshold="true">
7           <MassListRecord>
8             <MOverZ>195.12</MOverZ>
9             <Z>1</Z>
10            <IntensityThreshold>5e6</IntensityThreshold>
11          </MassListRecord>
12          <MassListRecord>
13            <MOverZ>262.3</MOverZ>
14            <Z>2</Z>
15            <IntensityThreshold>1e4</IntensityThreshold>
16          </MassListRecord>
17        </MassList>
18      </MassListFilter>
19    </Experiment>
20  </Modification>
21  <Modification Order="2">
22    <Experiment ExperimentIndex="0">
23      <MassListFilter MassListType="TargetedMassInclusion">
24        <MassList IntensityThreshold="true">
25          <MassListRecord>
26            <MOverZ>195.12</MOverZ>
27            <Z>1</Z>
28            <IntensityThreshold>5e7</IntensityThreshold>
29          </MassListRecord>
30          <MassListRecord>
31            <MOverZ>262.3</MOverZ>
32            <Z>2</Z>
33            <IntensityThreshold>1e2</IntensityThreshold>
34          </MassListRecord>
35        </MassList>
36      </MassListFilter>
37    </Experiment>
38  </Modification>
39 </MethodModifications>
```

Demonstration

New Volume (J:) > meth-modifications > examples > Fusion > IntensityThreshold

Name	Date modified	Type	Size
Convert.bat	5/16/2018 10:19 AM	Windows Batch File	1 KB
IntensityThreshold.xml	5/16/2018 10:19 AM	XML File	2 KB
Template.meth	5/17/2018 1:58 PM	Xcalibur Instrume...	42 KB

Orbitrap Fusion Lumos Method Editor 3.1.2140 - DEV MODE

File Orbitrap Fusion Lumos

Method Editor

Global Parameters | **Scan Parameters** | Summary

Method Timeline Experiment ACTIONS

Application Mode: Peptide
Method Duration (min): 60

Default Charge State: 1
Internal Mass Calibration: Off

Experiment # 1 Time Range (min) 0-60

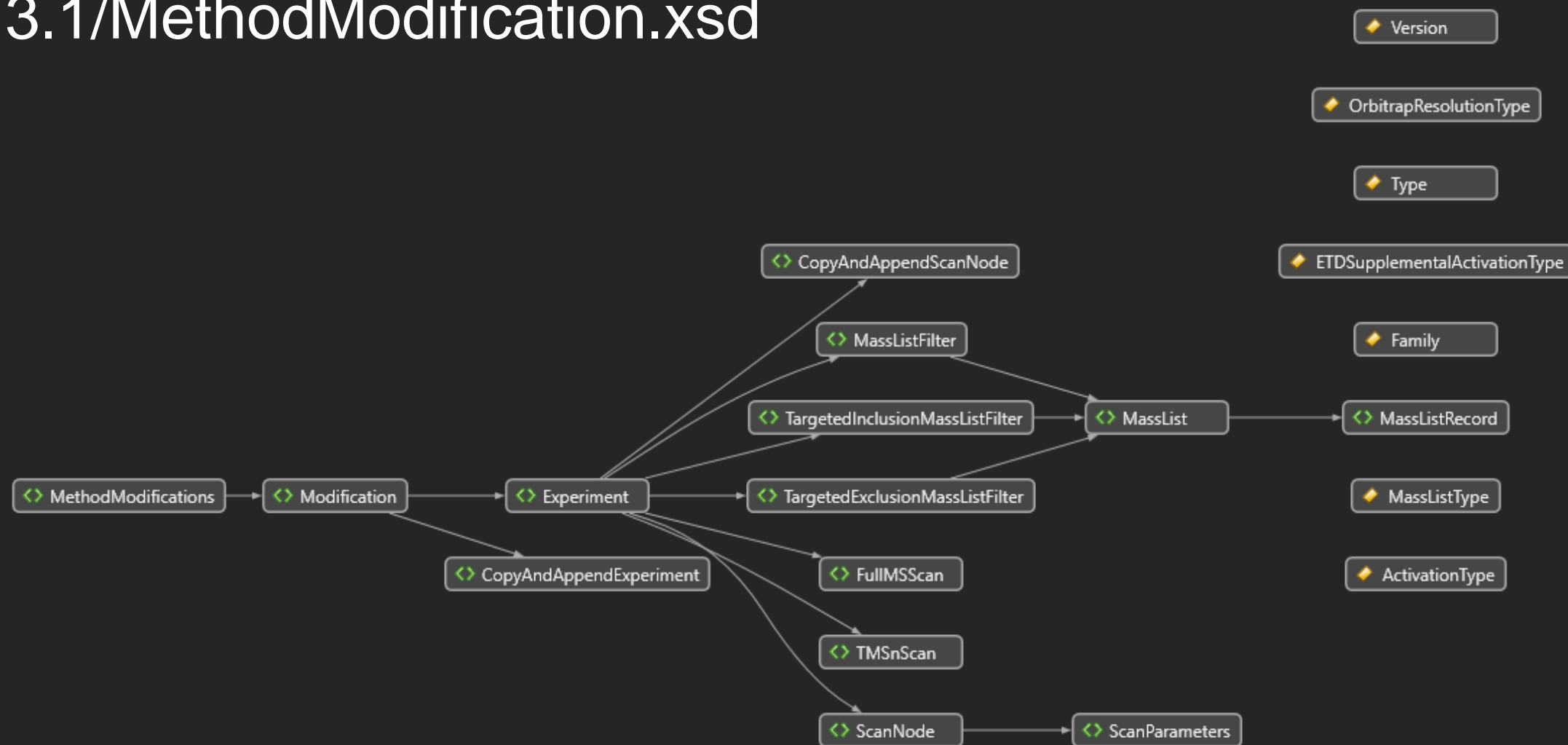
Scans: MS, MSⁿ

Filters: Precursor Selection Range, MIPS, Intensity

Place Scan Here

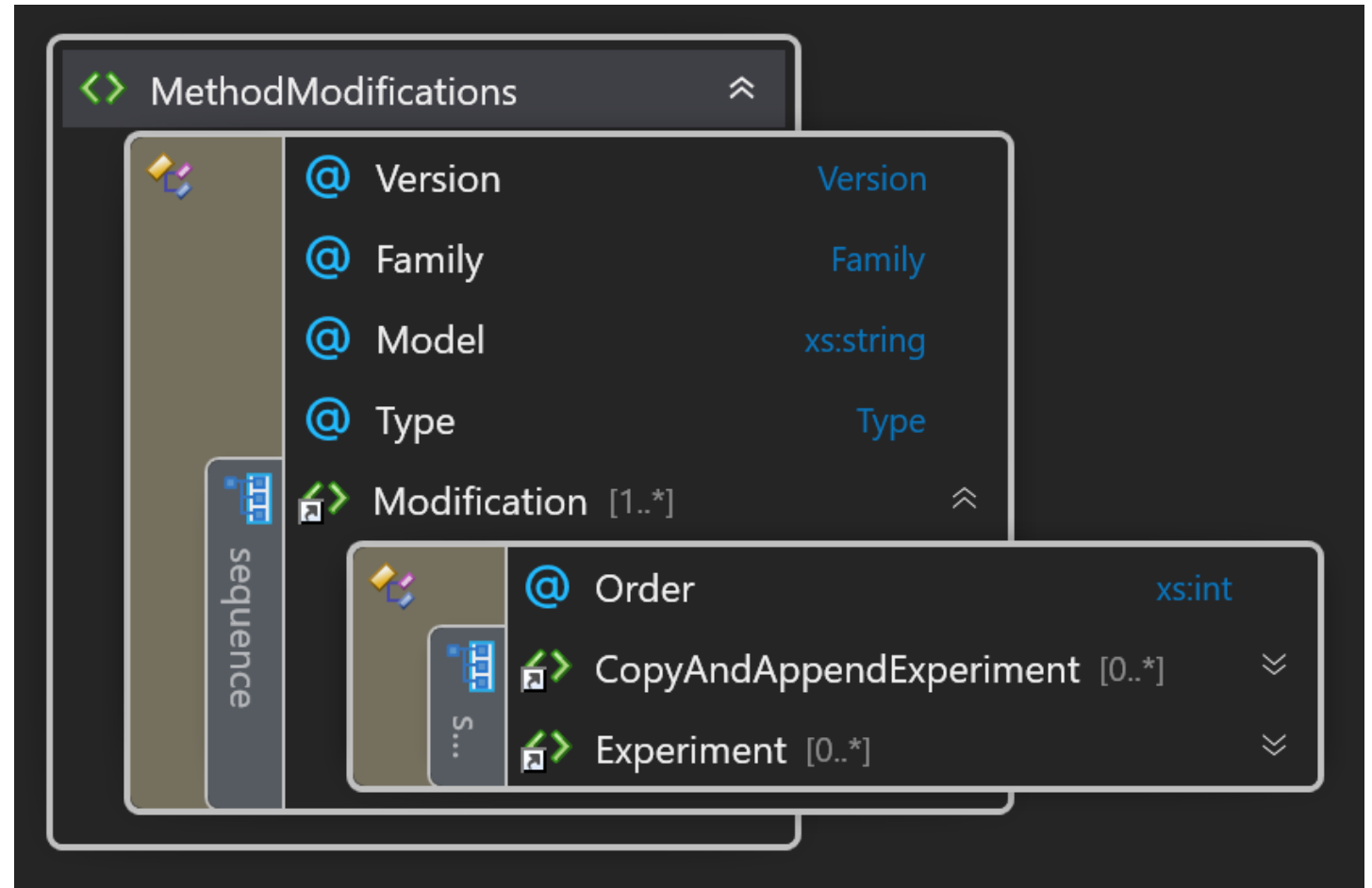
Properties

3.1/MethodModification.xsd



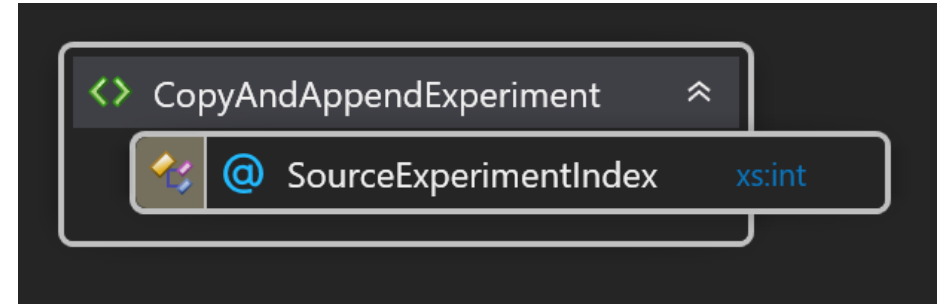
Method Modifications

- The XML contains a collection of **Modifications**
- Each **Modification** contains:
 - An **Order** attribute
 - This is the order that this modification will take effect, from low to high.
 - Collection of **Actions** elements
 - This is what the **Modification** performs on the method
 - **Experiments** are performed first, in the order they are defined in the xml
 - **CopyAndAppendExperiment** are performed second, in the order they are defined in the xml

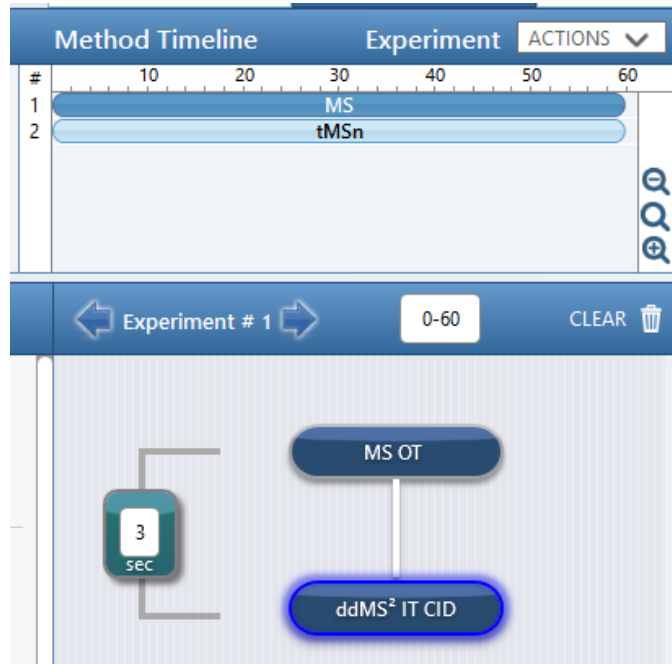


Copy And Append Experiment

- Copies a method experiment and appends it the end of all the experiments
- The attribute **SourceExperimentIndex** (0-based) specifies which experiment to copy

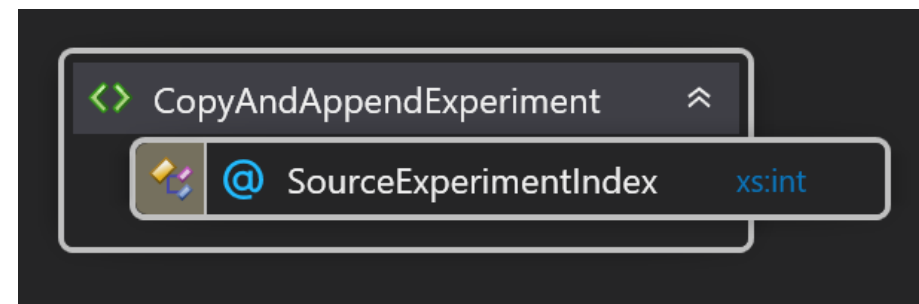


```
<CopyAndAppendExperiment SourceExperimentIndex="0" />
```

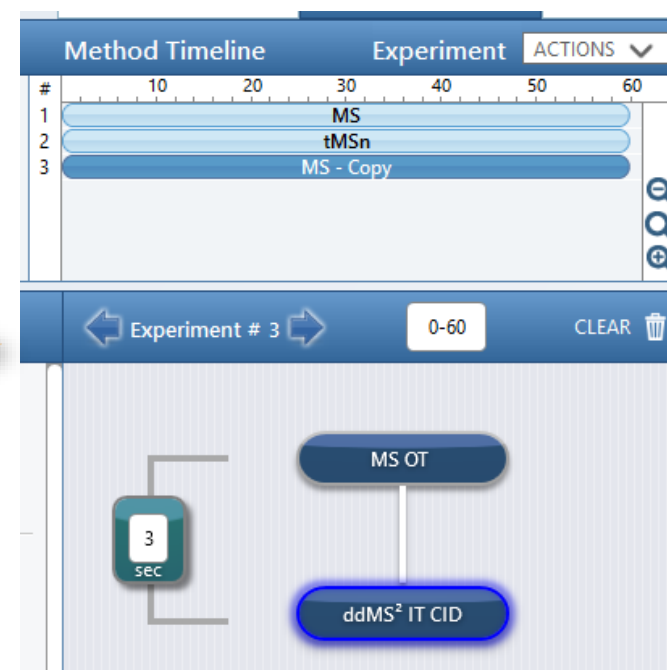
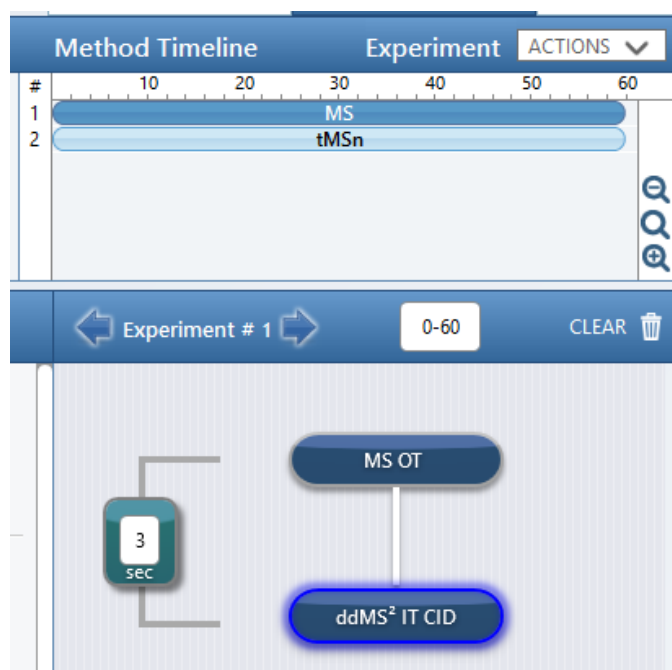


Copy And Append Experiment

- Copies a method experiment and appends it the end of all the experiments
- The attribute **SourceExperimentIndex** (0-based) specifies which experiment to copy

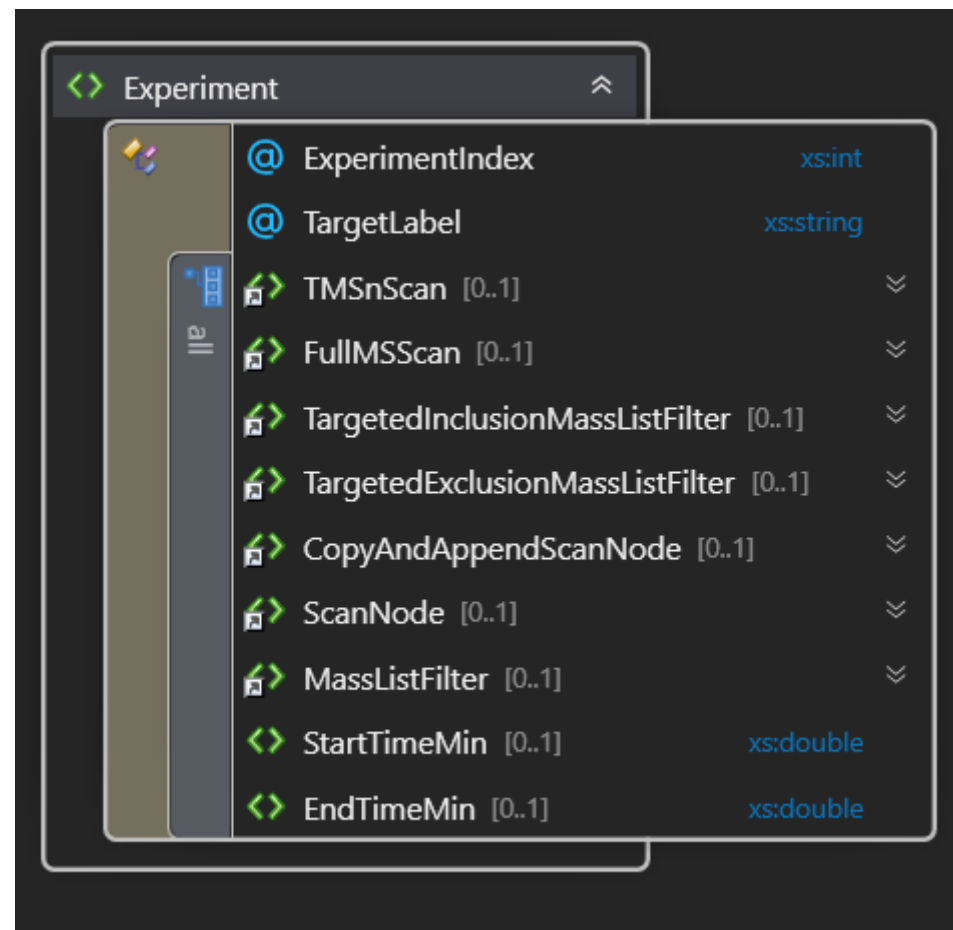


```
<CopyAndAppendExperiment SourceExperimentIndex="0" />
```

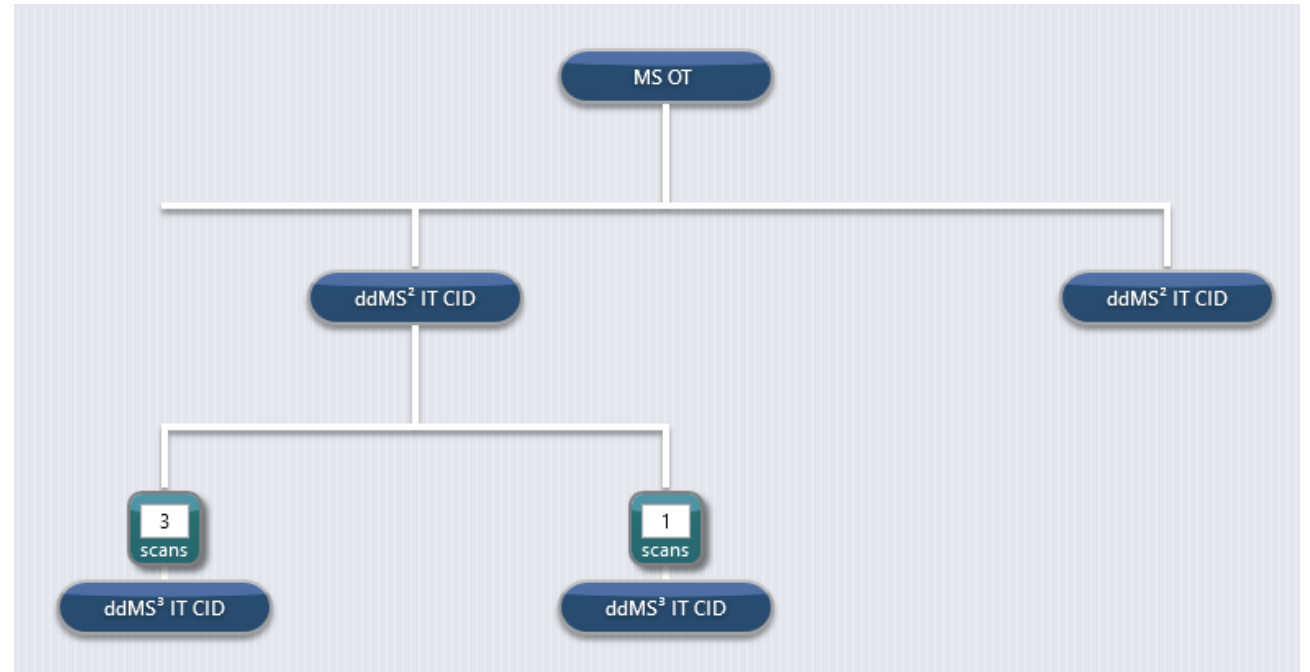


Experiment Modifications

- Modifies an experiment based on the attribute **ExperimentIndex** (0-based)
- Here you can target certain nodes/filters of the selected experiment and modify their contents
- You can also modify the experiment time frame with the **StartTimeMin** and **EndTimeMin** fields.

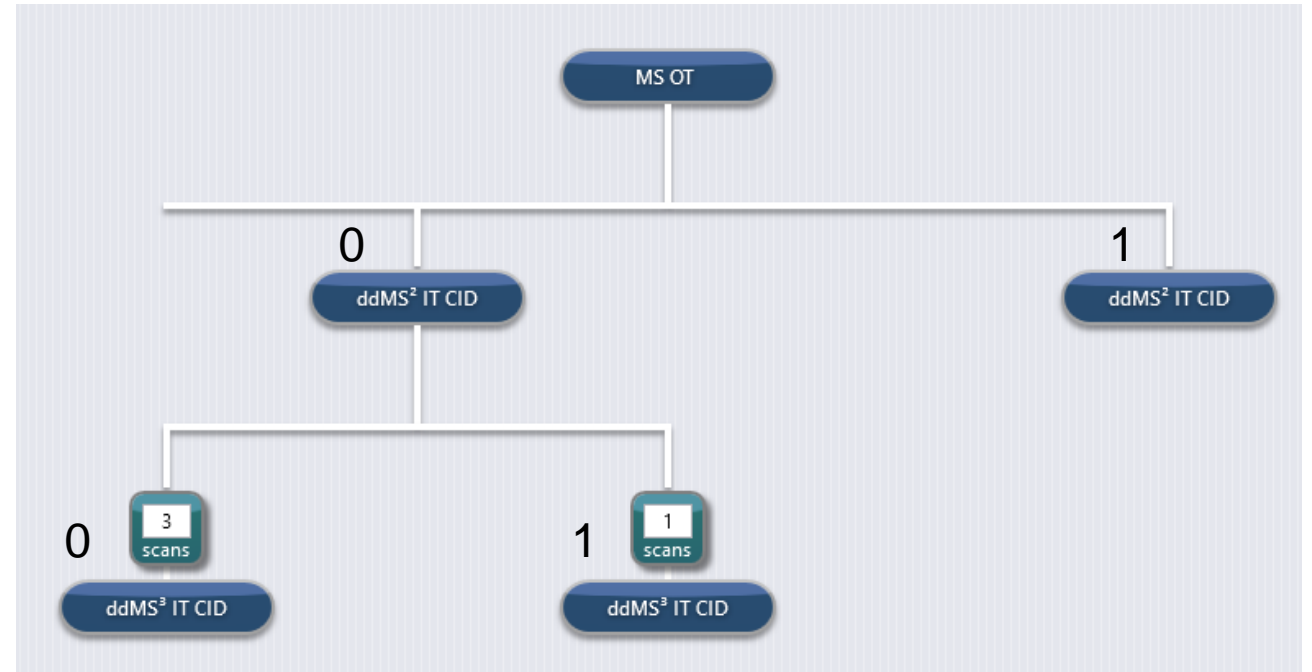


- **SourceNodePosition** is a mechanism to specify a specific node in scan tree



Source Node Position

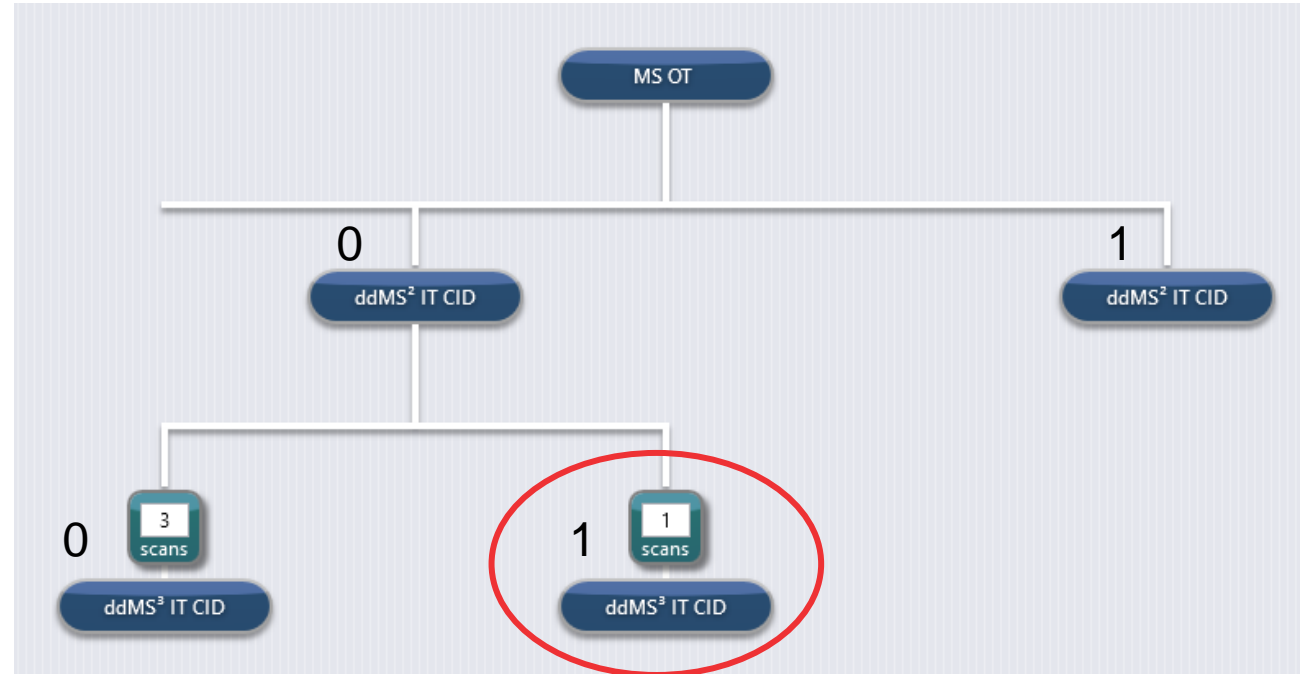
- **SourceNodePosition** is a mechanism to specify a specific node in scan tree
- Each instance of **SourceNodePosition** selects which child node to select; numbered left-to-right and 0-based.



Source Node Position

- **SourceNodePosition** is a mechanism to specify a specific node in scan tree
- Each instance of **SourceNodePosition** selects which child node to select; numbered left-to-right and 0-based.
- To specify the highlighted node, you would need to do:

```
<SourceNodePosition>0</SourceNodePosition>  
<SourceNodePosition>1</SourceNodePosition>
```

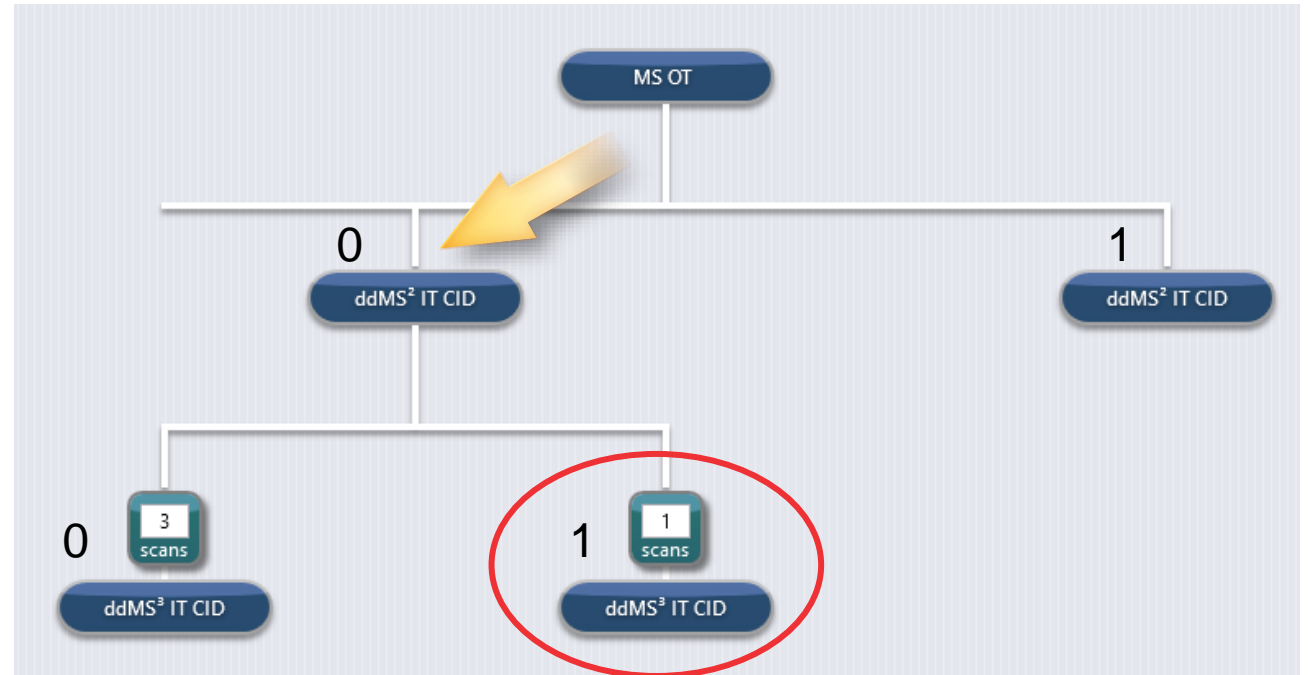


Source Node Position

- **SourceNodePosition** is a mechanism to specify a specific node in scan tree
- Each instance of **SourceNodePosition** selects which child node to select; numbered left-to-right and 0-based.
- To specify the highlighted node, you would need to do:

```
<SourceNodePosition>0</SourceNodePosition>  
<SourceNodePosition>1</SourceNodePosition>
```

- The first instance selects the left branch off the MS1.

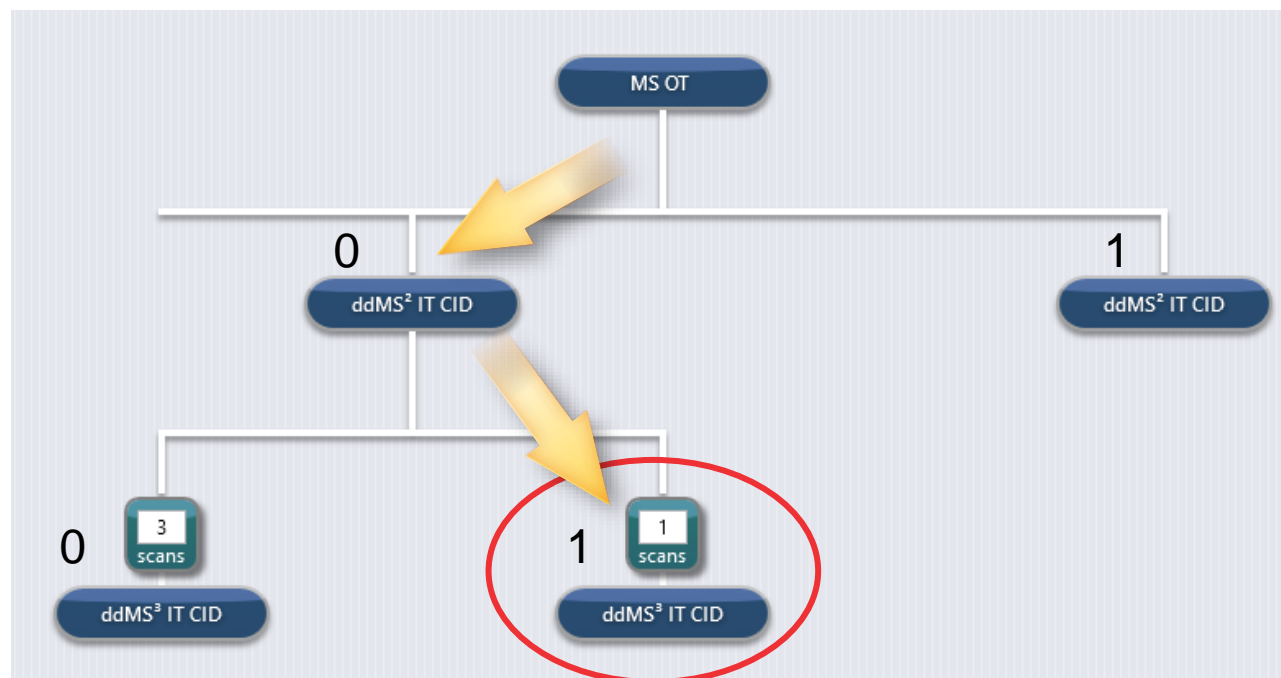


Source Node Position

- **SourceNodePosition** is a mechanism to specify a specific node in scan tree
- Each instance of **SourceNodePosition** selects which child node to select; numbered left-to-right and 0-based.
- To specify the highlighted node, you would need to do:

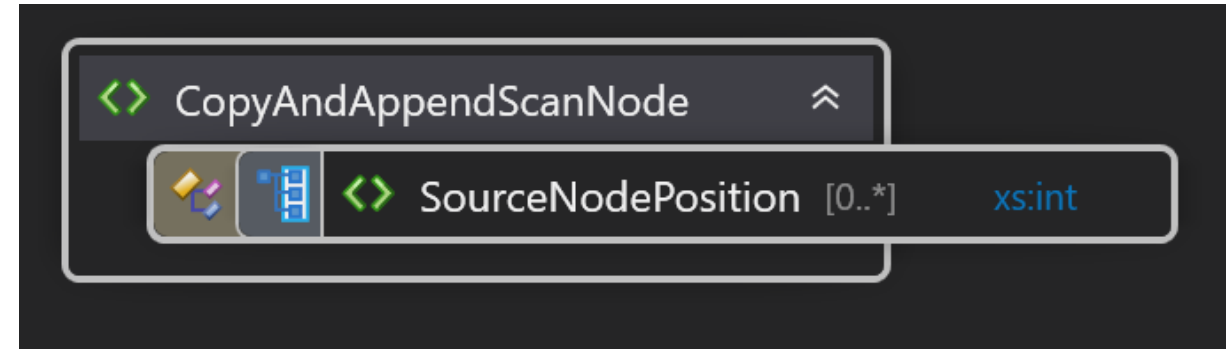
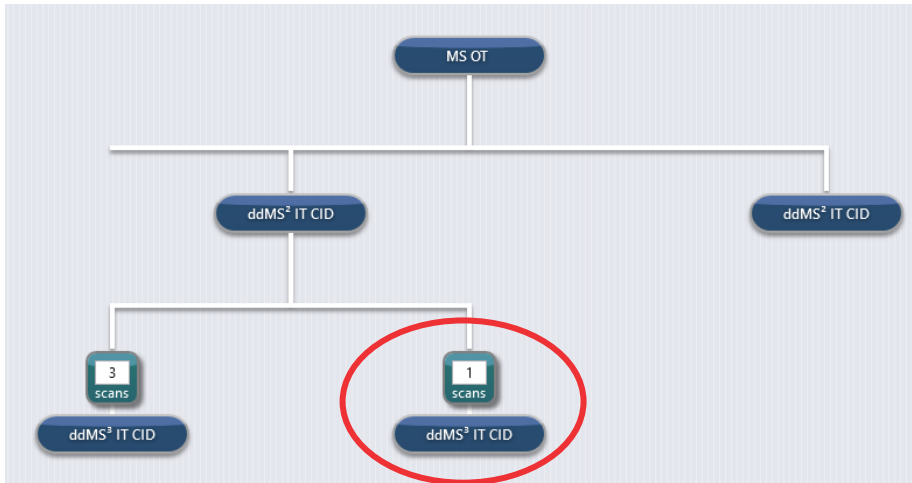
```
<SourceNodePosition>0</SourceNodePosition>  
<SourceNodePosition>1</SourceNodePosition>
```

- The first instance selects the left branch off the MS1.
- The second instance selects the right branch off the ddMS2



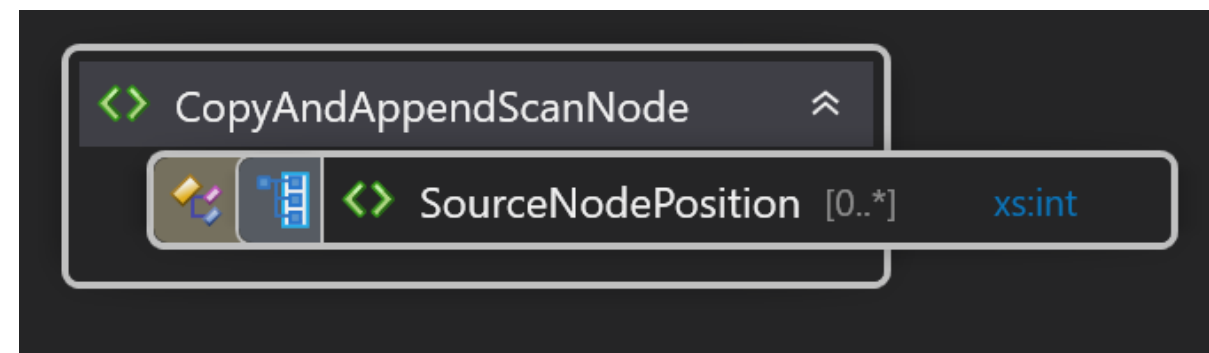
Copy And Append Scan Node

- Copies a scan node in the experiment and appends it to its parent node
- Uses **SourceNodePosition** to specify the specific node in the tree

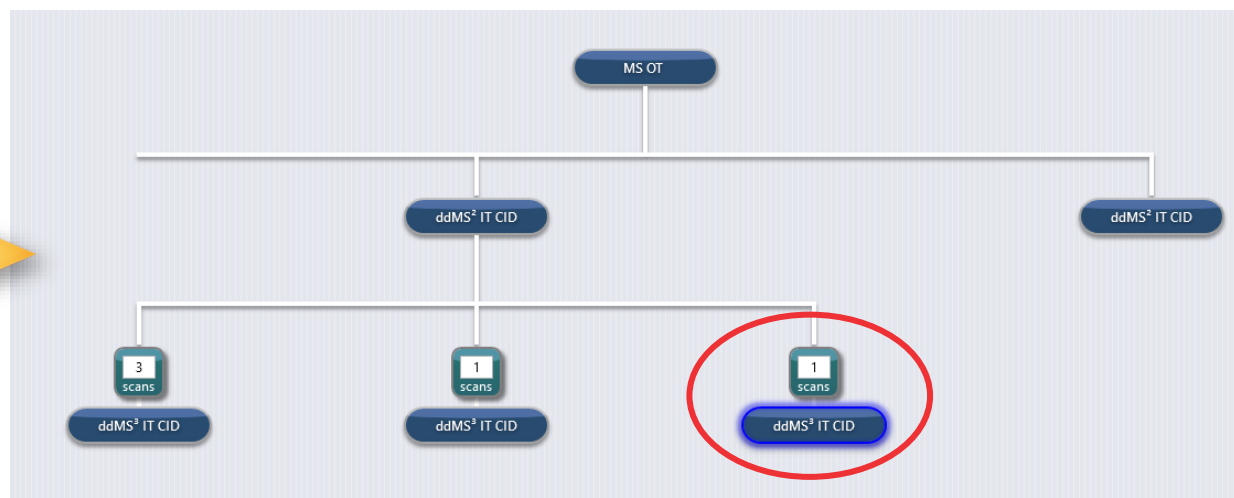
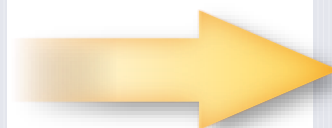
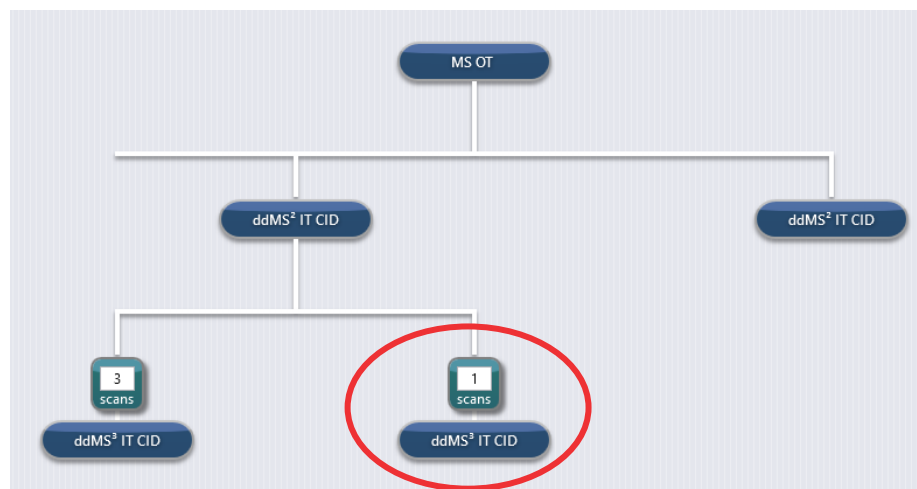


Copy And Append Scan Node

- Copies a scan node in the experiment and appends it to its parent node
- Uses **SourceNodePosition** to specify the specific node in the tree

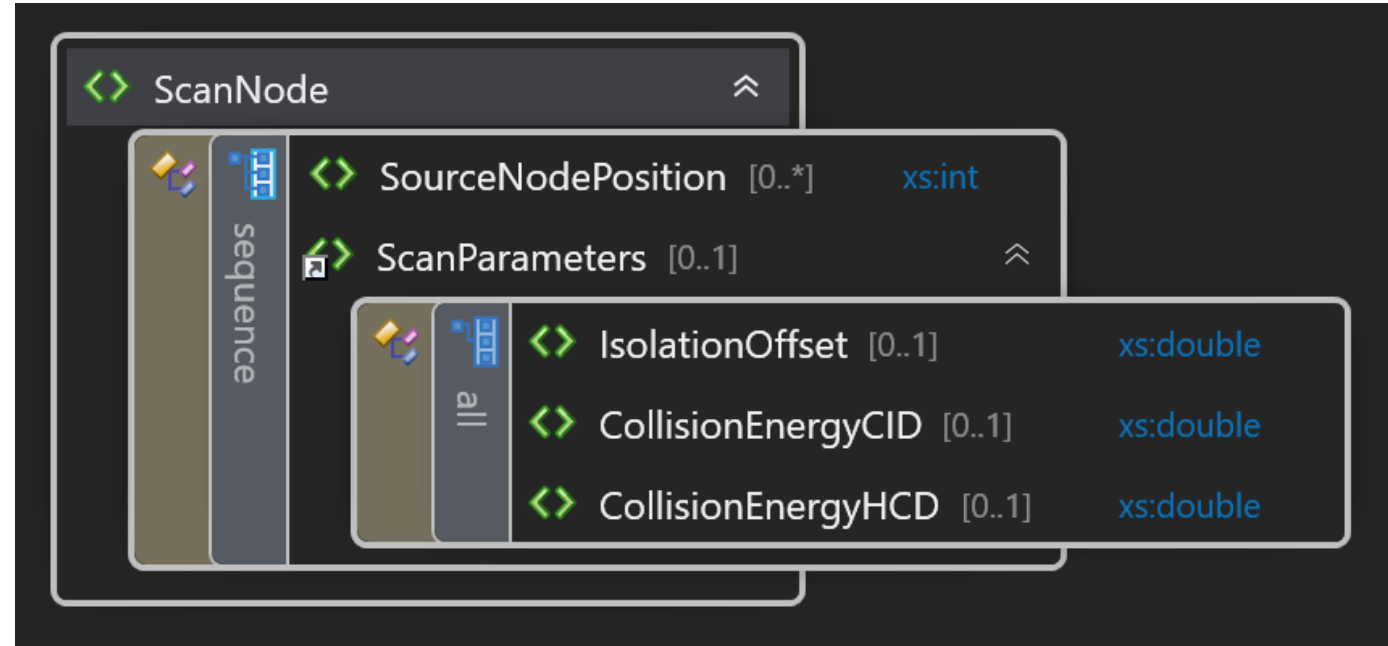


```
<CopyAndAppendScanNode>  
  ..<SourceNodePosition>0</SourceNodePosition>  
  ..<SourceNodePosition>1</SourceNodePosition>  
</CopyAndAppendScanNode>
```



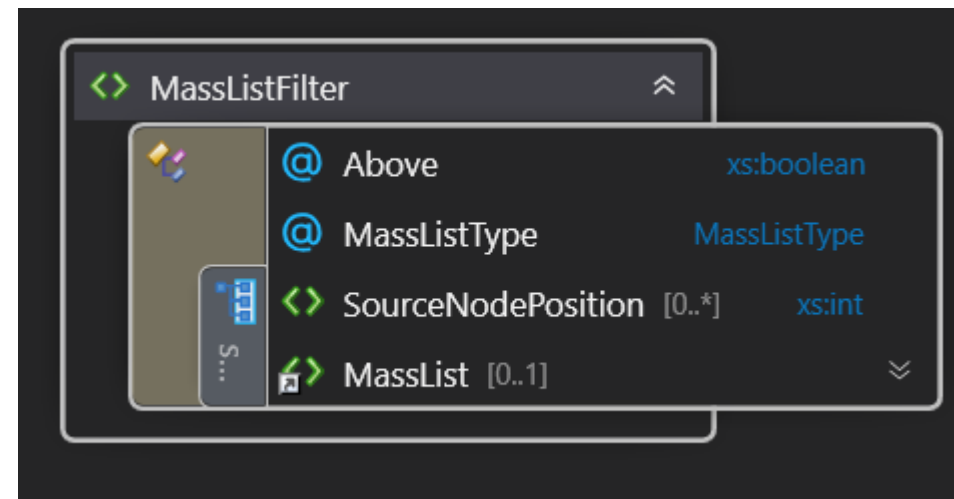
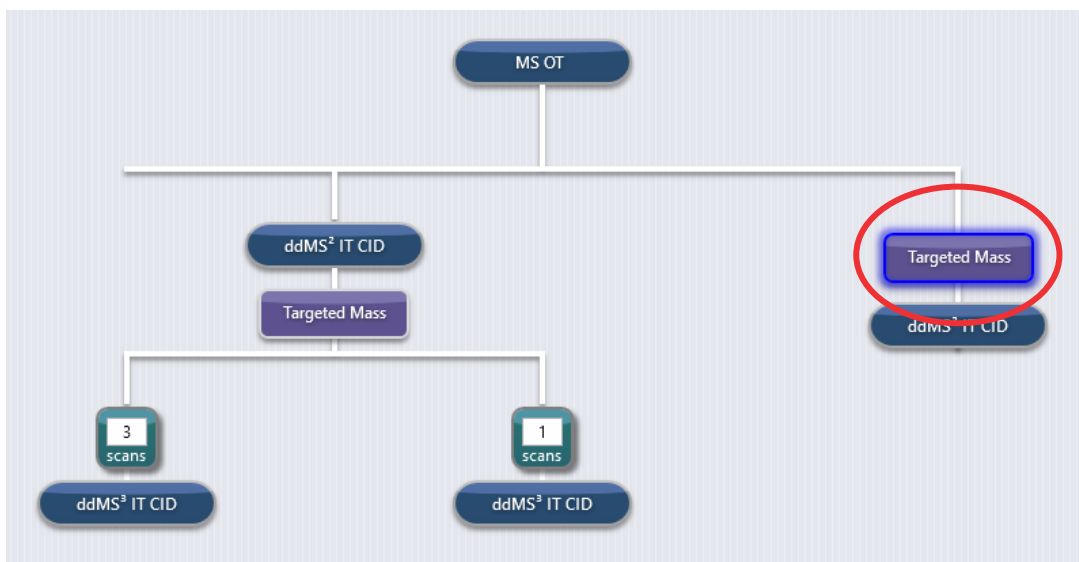
Scan Node Modification

- Modifies a scan based on the specified **SourceNodePosition**.
- You can currently change the
 - Isolation offset
 - CID Collision Energy
 - HCD Collision Energy



Mass List Filter Modification

- Modifies a filter with a mass list based on the specified **SourceNodePosition** and **Above** attribute
- To specify the filter, you first select the node the filter is attached to, and then indicate if the filter is above or below the node.

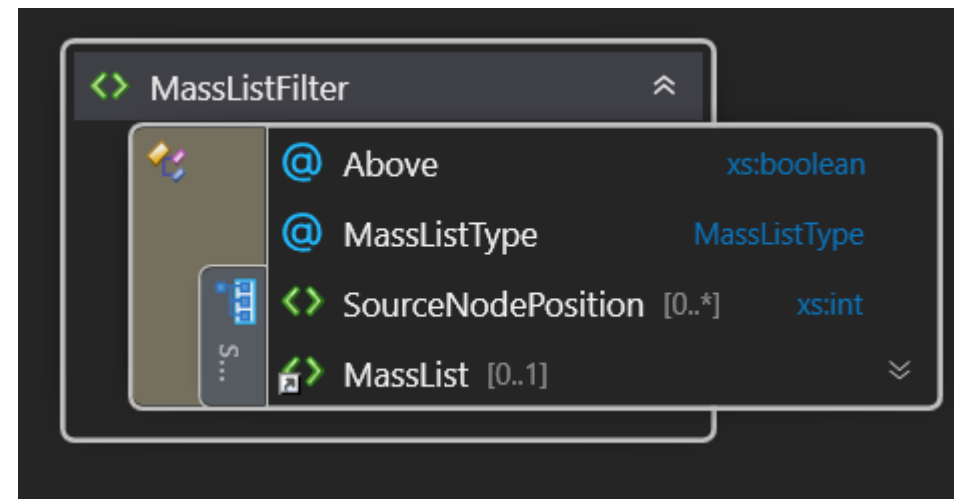
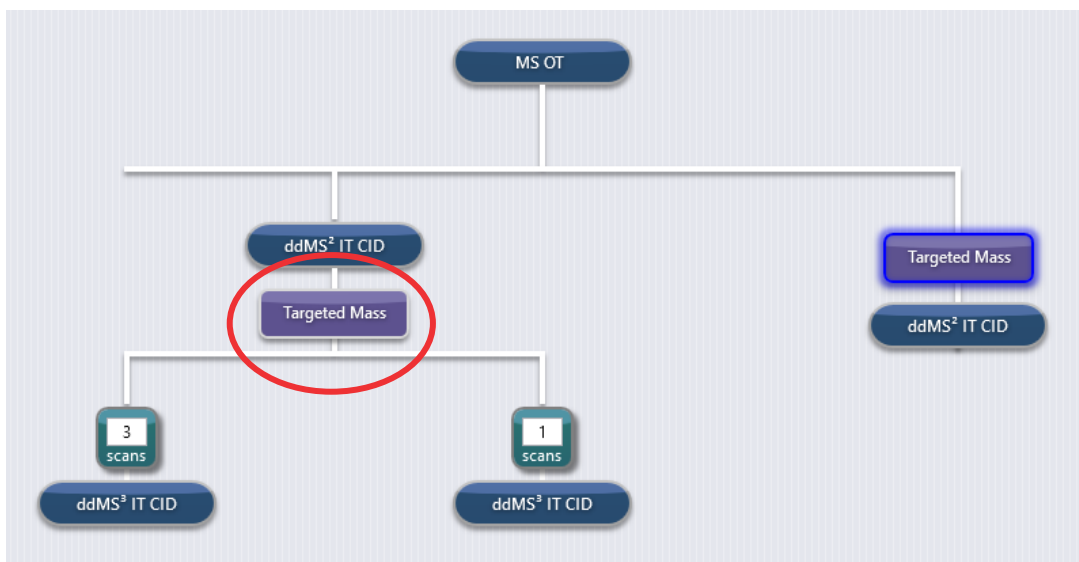


- This filter would be defined as

```
<MassListFilter Above="true" MassListType="TargetedMassInclusion">  
  <SourceNodePosition>1</SourceNodePosition>  
  <MassList />  
</MassListFilter>
```


Mass Filter Modification

- Modifies a filter with a mass list based on the specified **SourceNodePosition** and **Above** attribute
- To specify the filter, you first select the node the filter is attached to, and then indicate if the filter is above or below the node.



- This filter would be defined as

```
<MassListFilter Above="true" MassListType="TargetedMassInclusion">
  · · <SourceNodePosition>1</SourceNodePosition>
  · · <MassList />
</MassListFilter>
```

- And this filter would be defined as

```
<MassListFilter Above="false" MassListType="TargetedMassInclusion">
  · · <SourceNodePosition>0</SourceNodePosition>
  · · <MassList />
</MassListFilter>
```

Mass List Modification

- **MassList** contains a collection of **MassListRecords** which are the entries in the table
- The different Boolean attributes control which columns in the table are used
- The **Append** attribute indicates if the entries are append to the list or are overwritten

Targeted Mass Properties

MASS LIST

Include Start/End Times ☒

Include Intensity Threshold ☐

Mass List Type **m/z & z**

	Compound	Formula	Adduct	m/z	z	t start (min)	t stop (min)
1		MRFA	+H	524.265	1	0	60

ADD DELETE IMPORT EXPORT

Mass Tolerance **m/z**

Low 0.5

High 0.5

Ignore Charge State Requirement for Unassigned Ions ☒

MassList

@ StartEndTime xs:boolean

@ CollisionEnergyCID xs:boolean

@ CollisionEnergyHCD xs:boolean

@ IntensityThreshold xs:boolean

@ ETDReactionTime xs:boolean

@ ScanRange xs:boolean

@ Append xs:boolean

MassListRecord [1..*]

sequence

all

MOverZ [0..1] xs:double

Z [0..1] xs:double

FirstCharge [0..1] xs:double

LastCharge [0..1] xs:double

AdductPositive [0..1] xs:string

Mass [0..1] xs:double

CompoundName [0..1] xs:string

Formula [0..1] xs:string

StartTime [0..1] xs:double

EndTime [0..1] xs:double

CollisionEnergyCID [0..1] xs:double

CollisionEnergyHCD [0..1] xs:double

MSXID [0..1] xs:integer

ETDReactionTime [0..1] xs:double

FirstMass [0..1] xs:double

LastMass [0..1] xs:double

IntensityThreshold [0..1] xs:double

1. Instrument Application Programming Interface (IAPI)

- Real-time control and acquisition of Orbitrap Mass Spectrometers using .NET

2. XML Method Modification Interface (XMMI)

- Programmatically modify Fusion-series method files

3. Applications of IAPI

1. Devin Schweppe – Fusion API
2. Christoph Wichmann – Q Exactive API

4. Examples and FAQ for IAPI and XMMI

5. Applications of XMMI

1. Pavel Shliaha – Fusion Method Creation

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- **Is the I-API Free?**
 - Yes! Though it requires a license agreement with Thermo.
- **Are the Q Exactive and Fusion I-APIs the same?**
 - They are not **binary** compatible, but share the same structure, namespacing, and basic data and control paths.
 - It only requires a minimal amount of work to convert code between the two APIs.
- **How do we obtain the I-API?**
 - Interfaces are shared on <https://github.com/thermofisherlsm/iapi>
 - Contact ThermoMSLicensing@thermo.com to start the process.
- **Can you run the I-API while acquiring an acquisition from a method?**
 - Yes, the I-API works even when in idle, or actively running a method.

- **Are I-API scans parallelized?**
 - When running a method, I-API scans are inserted into the acquisition pipeline that parallelizes the scans with other scans.
 - When running without a method, I-API scans are **not** parallelized.
- **Are the results of the method filters published through the I-API?**
 - The I-API only publishes the raw spectrum, without any of the method filters taking effect.
- **Can we obtain profile data through the I-API?**
 - Currently only the centroided data is returned to the I-API.
- **Is the I-API 64-bit enabled?**
 - No, the main instrument service is a 32-bit service and cannot export a 64-bit interface

- **Can you modify every parameter?**
 - No, only a subset of parameters in a method can be modified using this API.
- **I want to modify parameter X, Y and Z, can you provide it?**
 - Please file an **issue** with the github account describing the desired changes.
 - <https://github.com/thermofisherlsm/meth-modifications/issues>
- **Do you need be attached to an instrument to modify methods?**
 - No, if you have installed the **Workstation** configuration, you can create and modify methods on any computer.
- **Does this work for Exactive methods?**
 - This interface does not currently support Exactive method modification.

I-API Example – Tribrid Series

- Use **Thermo.TNG.Factory.Factory.Create()** to create instrument access container
- Connect to the main service using the **StartOnlineAccess()** call
- Wait until server has connected and get access to a particular instrument

```
1  using Thermo.TNG.Factory;
2  using Thermo.Interfaces.FusionAccess_V1;
3  using Thermo.Interfaces.FusionAccess_V1.MsScanContainer;
4  using Thermo.Interfaces.InstrumentAccess_V1.MsScanContainer;
5  using System;
6
7  namespace MinifiedExample
8  {
9      0 references | Thermo Scientific, 7 minutes ago | 2 authors, 2 changes
10     class Program
11     {
12         0 references | Thermo Scientific, 7 minutes ago | 2 authors, 2 changes
13         static void Main(string[] args)
14         {
15             // Use the Factory creation method to create a Fusion Access Container
16             IFusionInstrumentAccessContainer fusionContainer = Factory<IFusionInstrumentAccessContainer>.Create();
17
18             // Connect to the service by going 'online'
19             fusionContainer.StartOnlineAccess();
20
21             // Wait until the service is connected
22             // (better through the event, but this is nice and simple)
23             while (!fusionContainer.ServiceConnected);
24
25             // From the instrument container, get access to a particular instrument
26             IFusionInstrumentAccess fusionAccess = fusionContainer.Get(1);
27         }
28     }
29 }
```


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