Advancing fragmentation strategies development in LC-MS metabolomics

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ViMMS VIRTUAL MS
https://github.com/sdrogers/vimms

1. Introduction

In untargeted metabolomics, tandem mass spectrometry is used to identify compounds by matching fragmentation spectra to a database. The more MS features for which high-quality spectra are acquired, the more molecules will be identified. The number of spectra acquired depends upon the choice of **fragmentation strategy**.

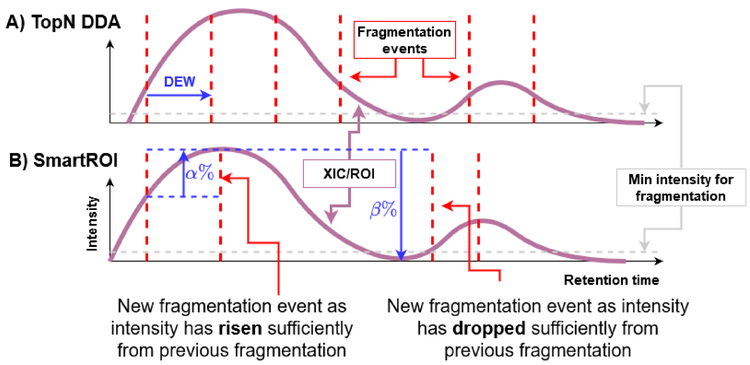
Common fragmentation strategies (e.g. DDA) are suboptimal. For example, TopN strategies often fragment large peaks many times (even with dynamic exclusion windows), and waste MS2 scans fragmenting noise. Developing new strategies is hard due to expensive instrument time and the lack of software to support the process.

2. SmartROI: a novel fragmentation strategy

We present a new fragmentation strategy, **SmartROI**, in which fragment spectra acquisition is informed by regions of interests (ROIs), **constructed in real-time from MS1 scans**. Fragmentation events are triggered only for precursor ions within ROIs thus reducing fragmentation of unreliable signals.

SmartROI is based on a traditional Top-N DDA duty cycle, but with additional rules to prevent unnecessary repeated fragmentations of a chromatographic peak whilst still allowing multiple peaks within an ROI to be fragmented.

In particular, SmartROI keeps track of the intensity of the ROI and only allows new fragmentation events under particular conditions.

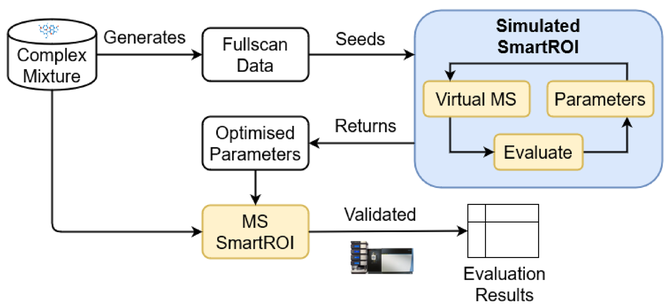


SmartROI compared with a Top-N strategy. Keeping track of an ROI in real-time allows for better targeting of MS2 events.

3. Developing SmartROI in ViMMS

Virtual **M**etabolomics **M**ass **S**pectrometer (**ViMMS**) is an LC-MS/MS simulator framework built in Python that allows for scan-level control of the MS2 acquisition process in-silico [1] and therefore **rapid prototyping** of new acquisition methods. SmartROI, as well as other standard fragmentation strategies were implemented as controllers within ViMMS.

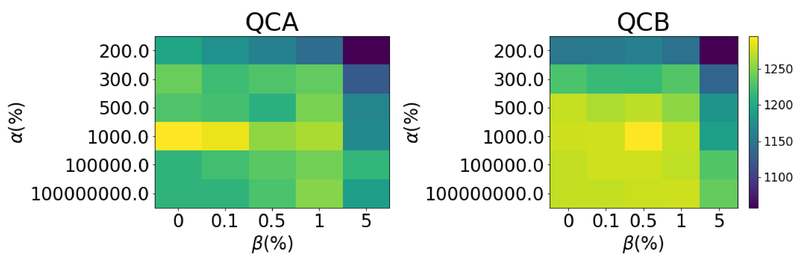
New fragmentation strategies like SmartROI can be optimised and tested within ViMMS to determine quickly if they give improved results without using an actual mass spectrometer.



Flow diagram demonstrating the process of developing and optimizing a new fragmentation strategy. Yellow boxes were performed on top of ViMMS.

Optimisation

SmartROI was **optimised in simulation** and compared to a standard Top-N strategy. Two complex samples (serum 'QCA', and beer 'QCB') were used to seed the simulations. Performance was evaluated using **coverage**: the number of picked peaks that include one or more fragmentation events.



Simulated coverage results for the α and β parameters of SmartROI on the QCA and QCB samples.

4. Evaluation

From simulation, $\alpha = 1000\%$ (how much peak intensity has to increase to allow re-fragmentation) and $\beta = 0.1\%$ (how much intensity has to drop to allow re-fragmentation) were chosen. These were used for validation on the actual mass spectrometer.

A translational layer was added to allow controllers within ViMMS, including SmartROI, to run on Thermo Fusion instruments. To evaluate performance, peaks were picked using MZmine2, with **coverage** calculated for:

- Picked: based on the picked peaks of each method.
- Aligned: based on the picked peaks that are common to both methods when aligned.

SmartROI improves upon the Top-N DDA controller:

- **It improves coverage**: 20% improvement in coverage means substantially more picked peaks are fragmented.
- **It is more efficient**: SmartROI improves coverage using fewer MS2 scans and therefore more MS1 scans . This results in higher quality chromatographic peaks for intensity quantitation.

Data	Top-N				SmartROI			
	No. picked peaks	Cove-rage	No. MS1 Scans	No. MS2 Scans	No. picked peaks	Cove-rage	No. MS1 Scans	No. MS2 Scans
QCA (picked)	5186	1083	643	6359	3016	**1182**	1218	3790
QCA (aligned)	1481	590	643	6359	1481	**709**	1218	3790
QCB (picked)	5667	1225	644	6401	3387	**1508**	1217	4054
QCB (aligned)	2094	890	644	6401	2094	**1107**	1217	4054

Experimental results for SmartROI on the QCA and QCB samples. The bold column highlights the higher coverage of SmartROI compared to Top-N across all settings.

5. Conclusions

A novel strategy SmartROI was introduced that detects ROIs in real-time and triggers fragmentation events only for ROIs. SmartROI was developed and optimised on top of ViMMS. It was validated on a real instrument and demonstrated substantial improvement in acquisition coverage over a traditional Top-N scheme.

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