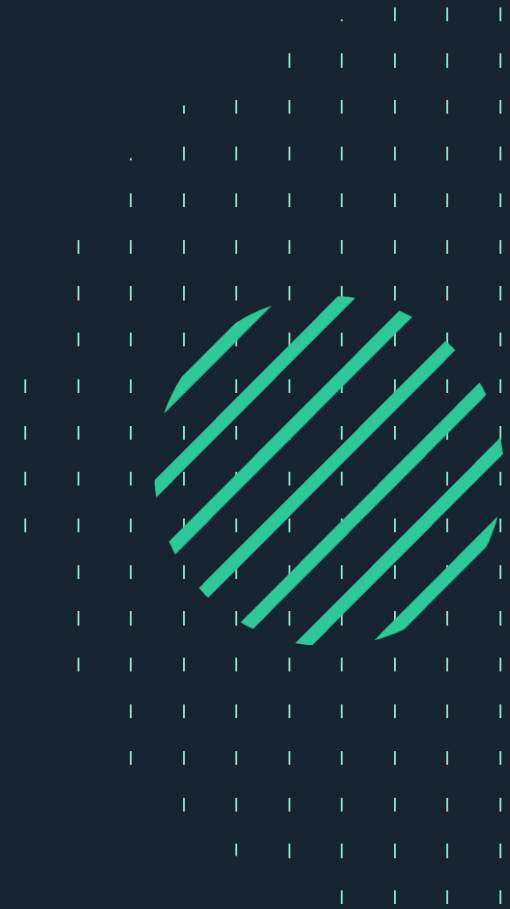
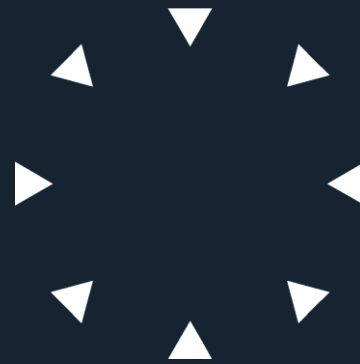


Automating Affinity Selection Mass Spectrometry for Confident Hit Identification



Don D. Nguyen, PhD
Edelris Annual Symposium on AS-MS in Drug Discovery
2026-09-03

Benefits and limitations of Affinity Selection-Mass Spectrometry (AS-MS)

Benefits

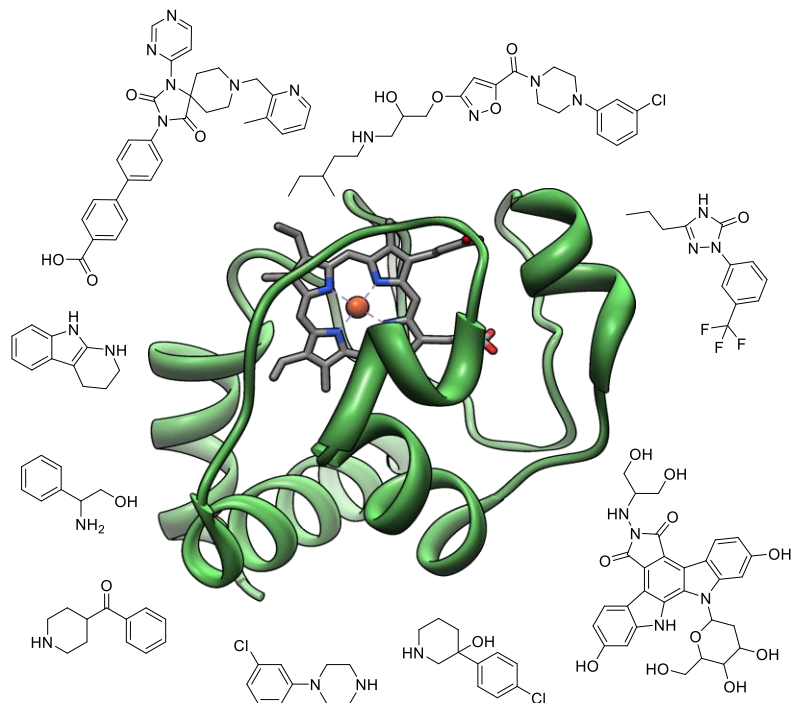
- High throughput binding assay
- Multiplexed screening for hundreds of cpds/well to single cpds (library dependent)
- In solution, label free, straight forward assay development
- Functional effect independent
- Binding site agnostic. AS-MS is able to:
 - ...query multiple binding sites simultaneously, competition assays
 - ...identify unknown binding sites
- Target tractability, 1°-, 2°-screening, orthogonal confirmation, (apparent) K_d

Limitations

- Sufficient affinity required ($10e^{-5}$ M minimally)
- Moderate to slow K_{off}
- Moderate protein consumption
- Separation of target+binders (from non-binders)
 - ...performed at 4°C potentially effecting inducible pockets
 - ...is performed in Ammonium acetate/Aqueous/Organic mixtures (not in buffer)

Affinity Selection-Mass Spectrometry (AS-MS)

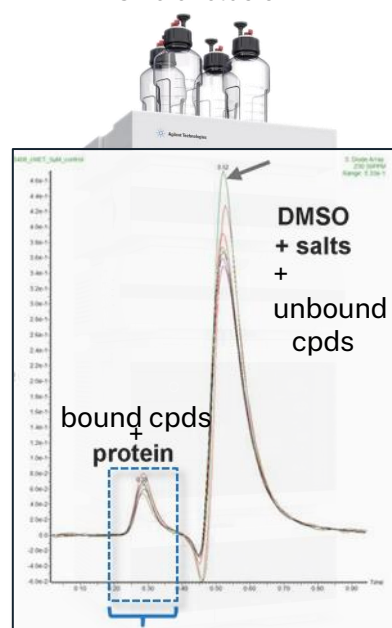
Compound Library Screening



Chromatography

Dimension 1

Size exclusion

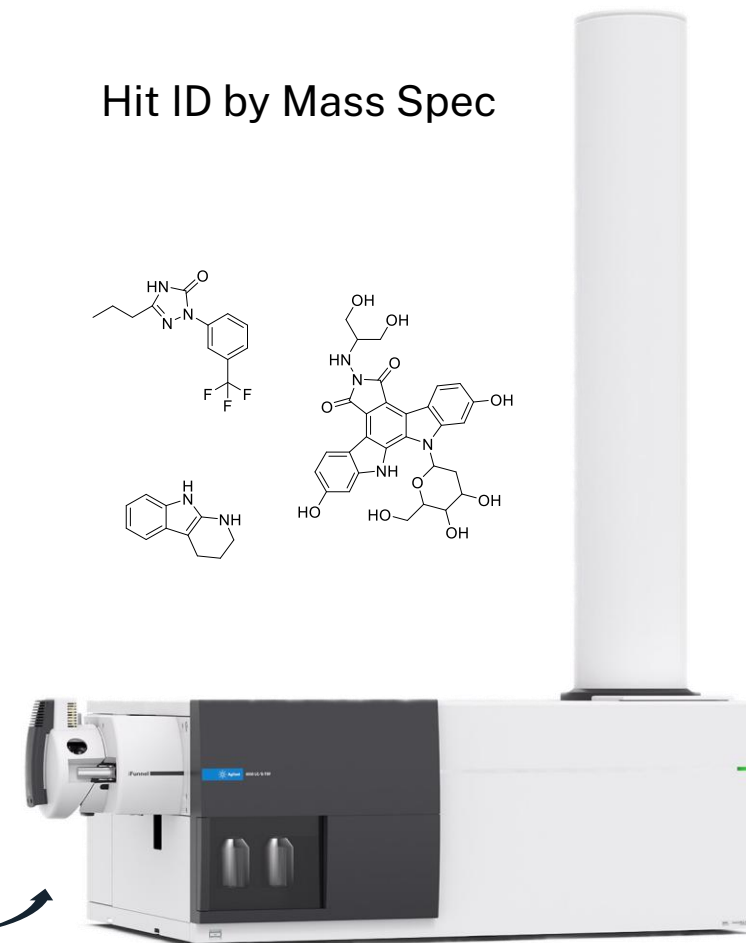
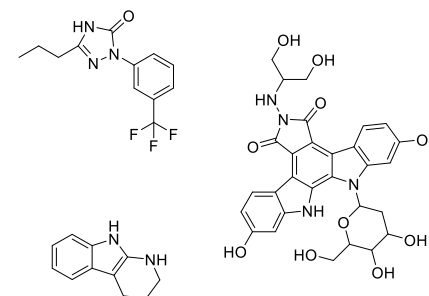


Dimension 2

Reverse phase



Hit ID by Mass Spec

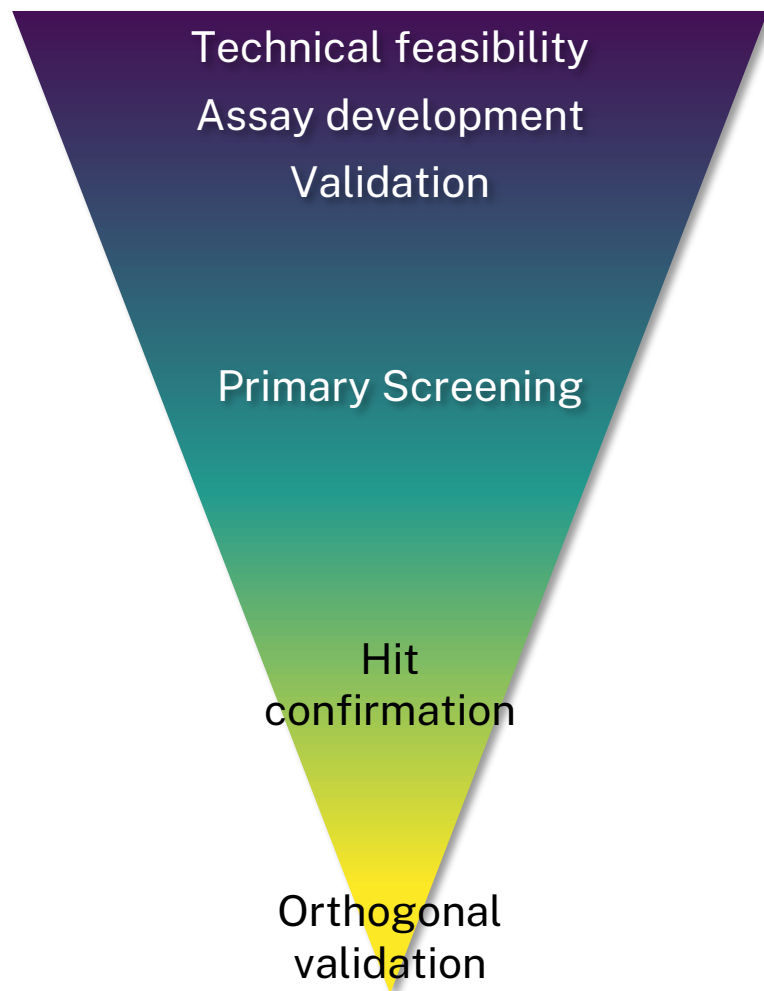
Protein-compound
complex

Protein-compound
complex dissociation
(High temp, low pH, aq/org mixture)

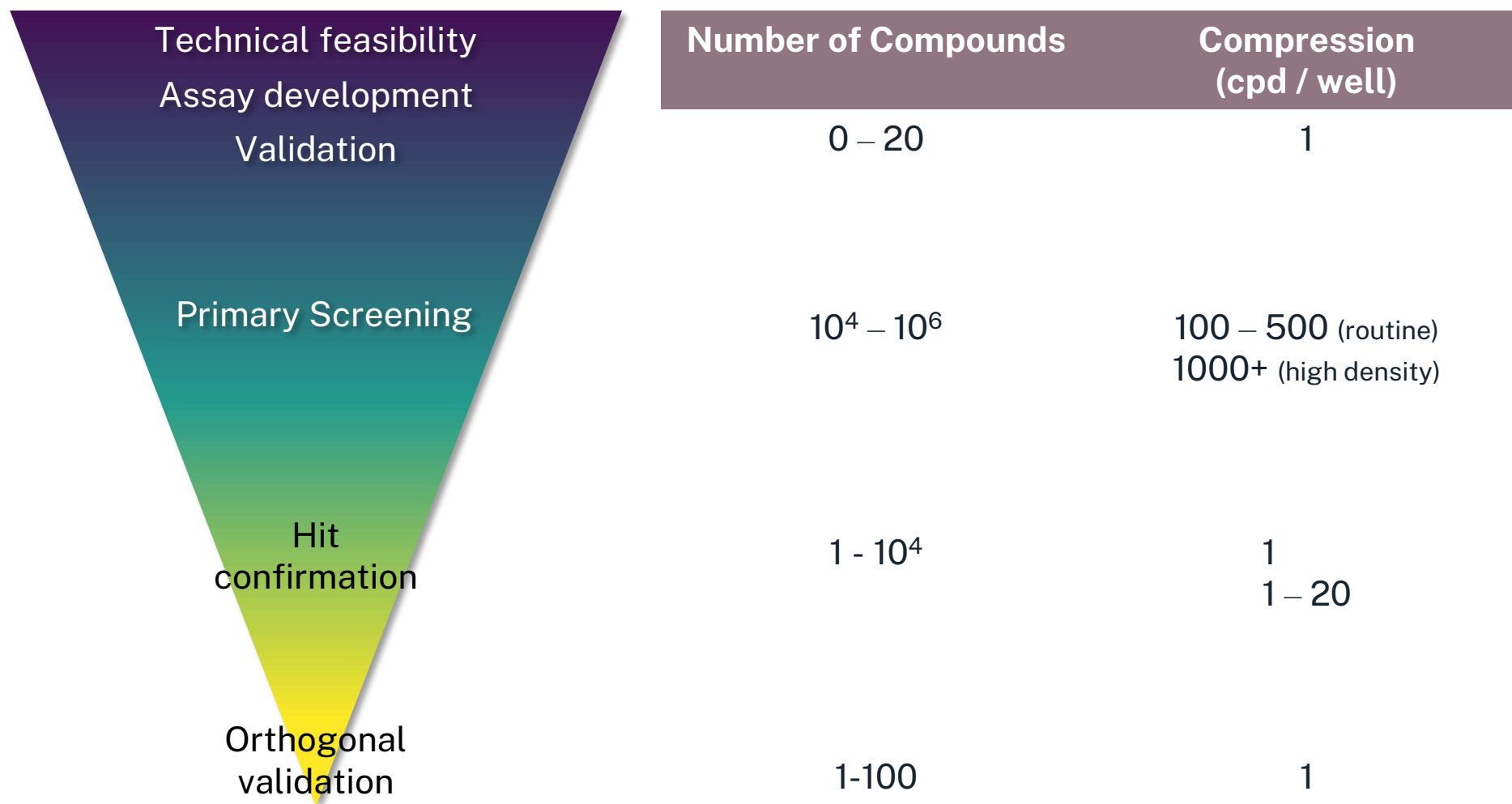
Challenges with AS-MS data analysis



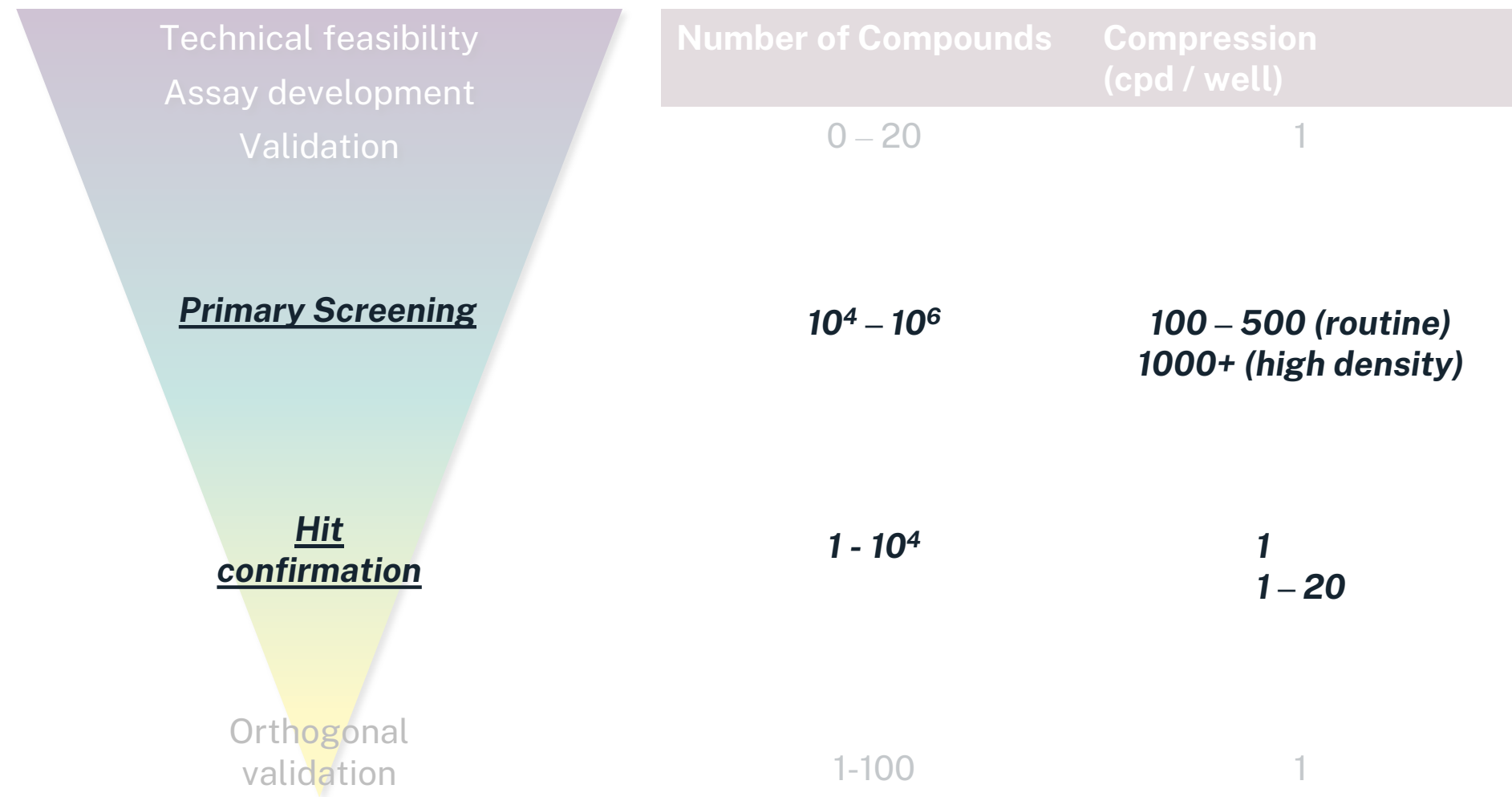
Challenges with AS-MS data analysis



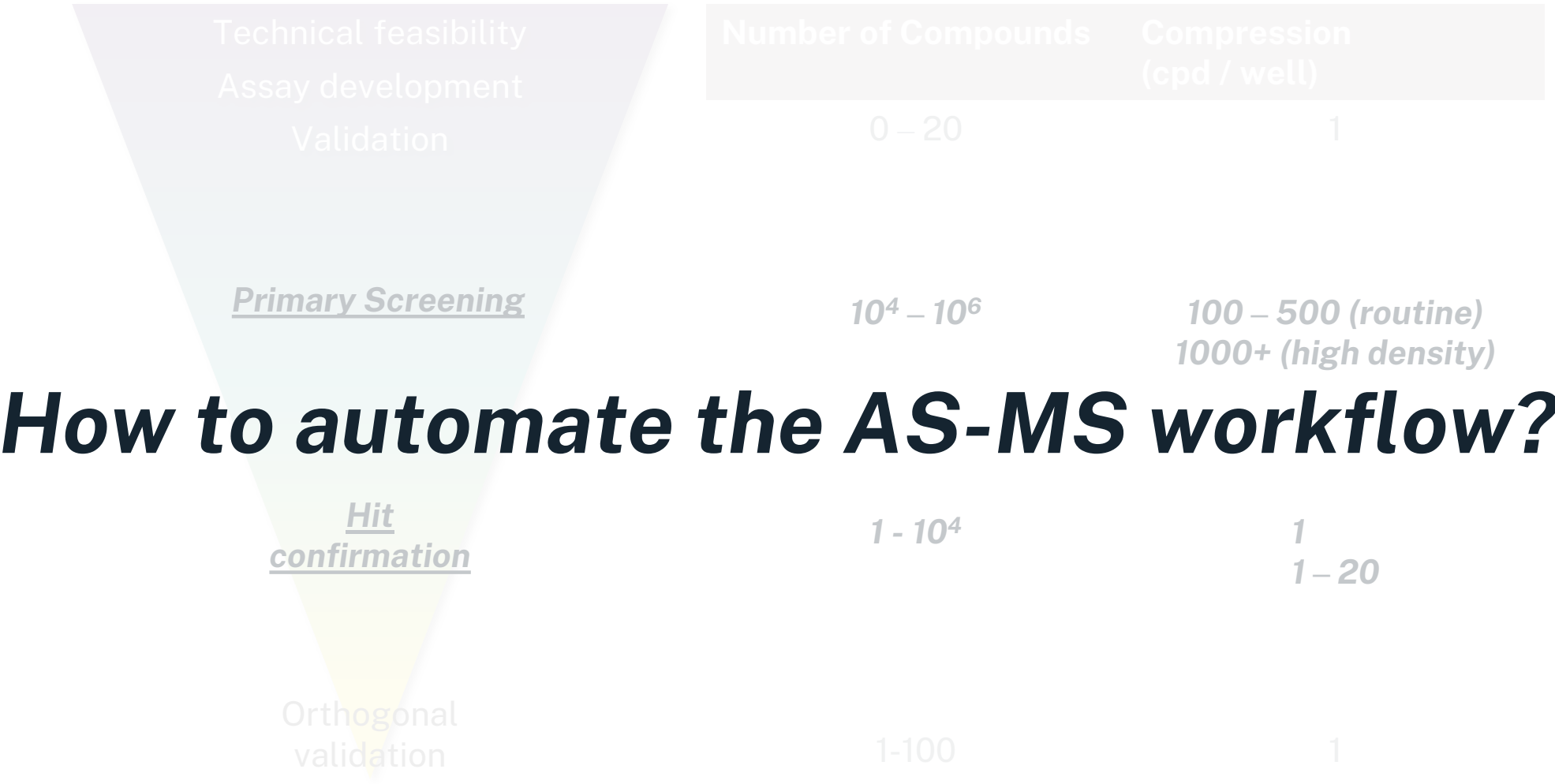
Challenges with AS-MS data analysis



Challenges with AS-MS data analysis



Challenges with AS-MS data analysis

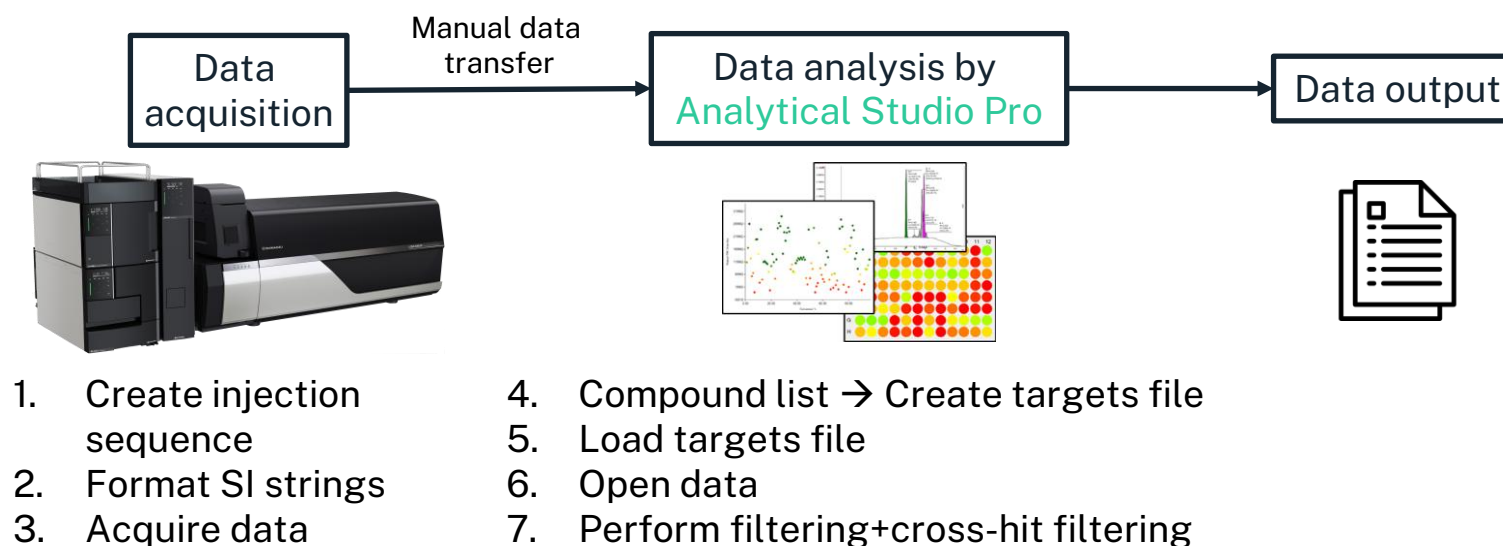


How to automate the AS-MS workflow?

Automating AS-MS:
data flow, processing,
and analysis of
multiplexed and dose
response data

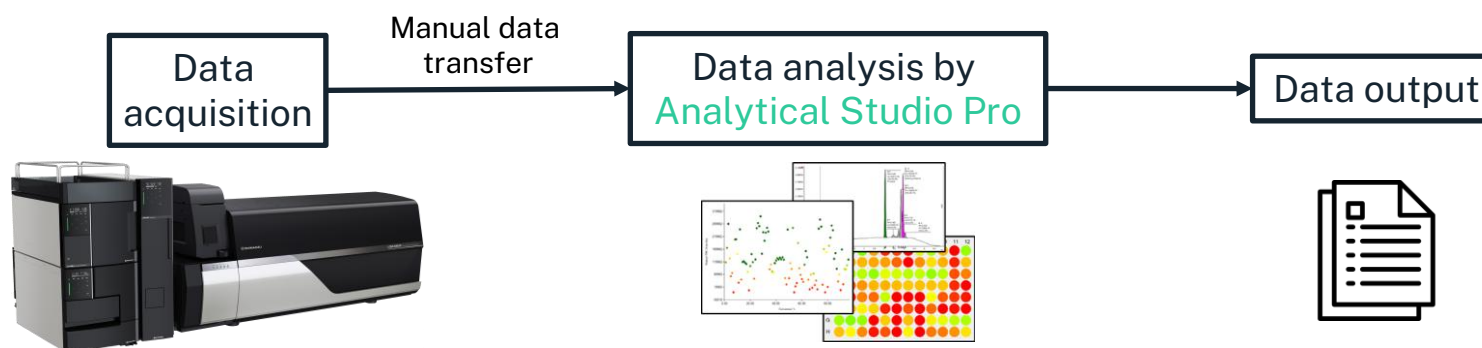


Standalone environment



Standalone environment

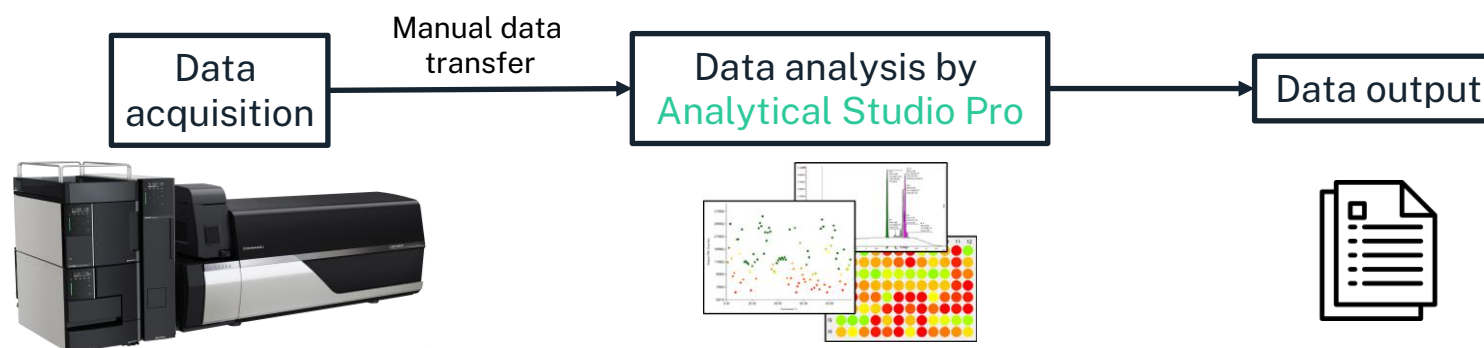
SI=AT!ASMS:CID!Plate123567:PL!01TL;XY;NL;1+8;2+12:F!Formula1_Formula2_Formula..._Formula500:



1. Create injection sequence
2. Format SI strings
3. Acquire data

4. Compound list → Create targets file
5. Load targets file
6. Open data
7. Perform filtering+cross-hit filtering

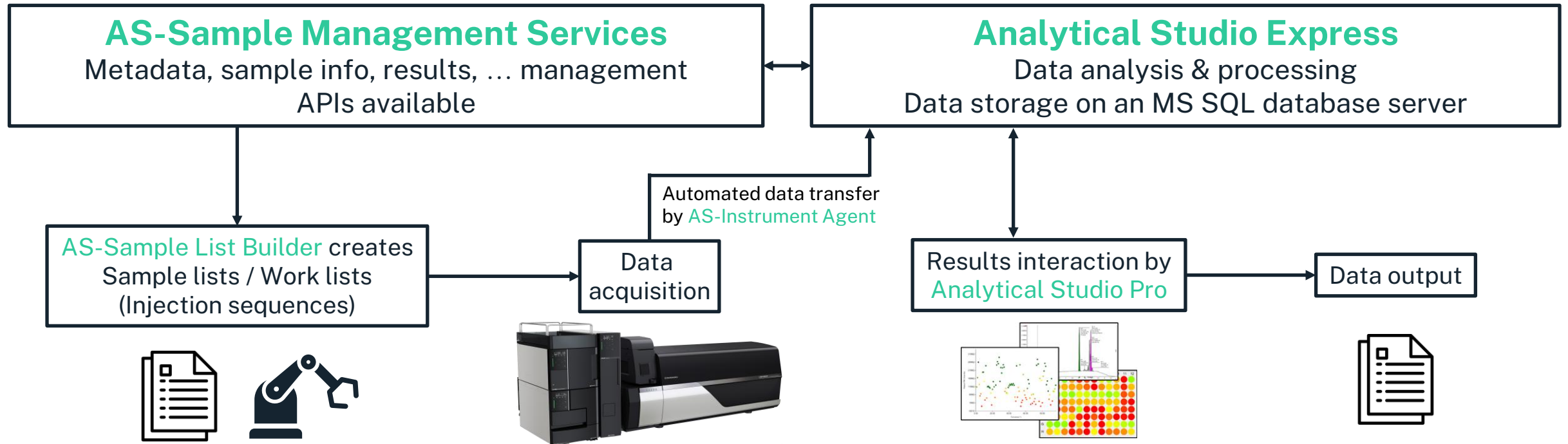
Standalone environment



1. Create injection sequence
2. Format Settings
3. Acquire data
4. Compound list → Create targets file
5. Load targets file
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AS-MS “Automation”

Analytical Studio Sample Management Environment



Automated AS-MS Workflow

Submission and Sample List Generation

A compound list with ID #'s, formulas, and key details for compound identification and processing is submitted to Analytical Studio Sample Management Services (AS-SMS), which generates a sample list.

Initial Analysis

Data is acquired and is analyzed behind the scenes. Peak integration and compound identification based on mass analysis and isotopic pattern matching.

Cross-Hit Filtering

Users review data and remove false positives by identification of background noise across all samples.

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Submitting a compound list to AS-SMS and generating a sample list

A user:

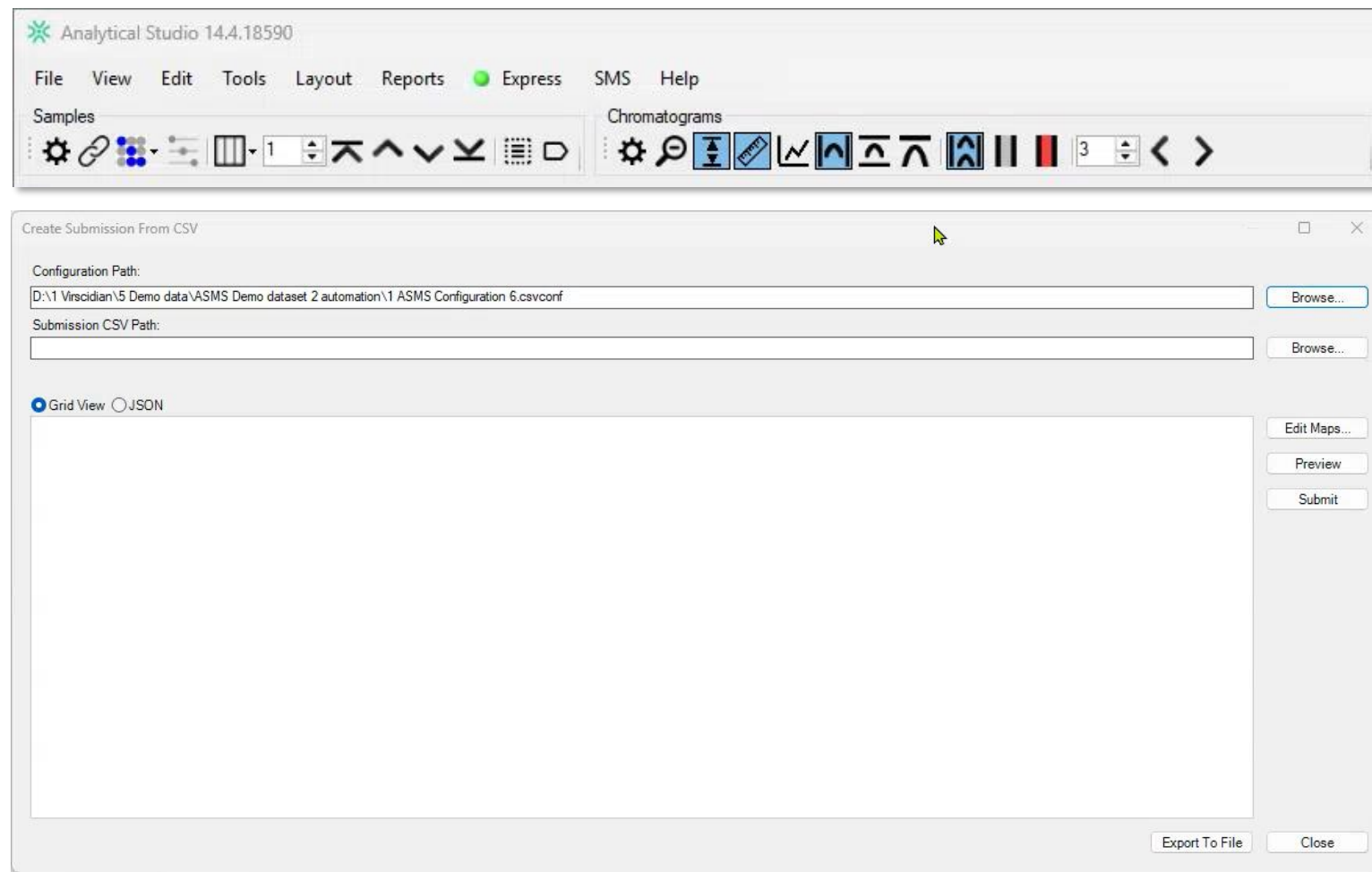
1. Curates a compound list
2. Submits the compound list to AS-SMS
3. Generates a sample list
4. Acquires data

	A	B	C	D	E	F
1	ASMS campaign number	Plate Barcode	PLATE_TYPE	Well	Compound ID	Formula
2	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000001	C17H12N2O4S
3	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000002	C19H29N3O3
4	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000003	C16H11BrN2O3
5	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000004	C17H15NO2
6	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000005	C11H7BrO2
7	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000006	C15H12O6
8	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000007	C21H26FN3O3
9	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000008	C20H24FN3O4
10	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000009	C14H17FN2O6S
11	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000010	C17H15NO5
12	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000011	C15H14N2O4
13	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000012	C16H13NO4S
14	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000013	C20H20N4O5
15	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000014	C18H24N2O2
16	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000015	C17H21N3O3
17	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000016	C10H10F3N3O3
18	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000017	C16H21N3O
19	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000018	C19H17NO4
20	ASMS Automation Demo 1	Plate123567	MTP 384	A03	Cpd0000019	C15H10F2N2O4S

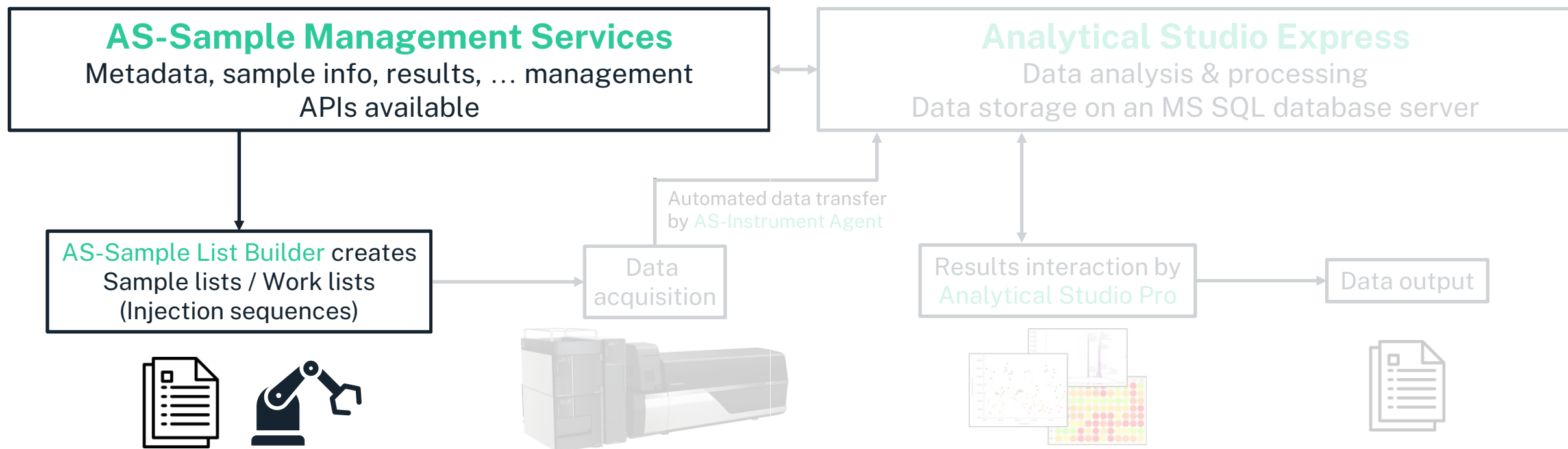
Submitting a compound list to AS-SMS and generating a sample list

A user:

1. Curates a compound list
2. Submits the compound list to AS-SMS
3. Generates a sample list
4. Acquires data



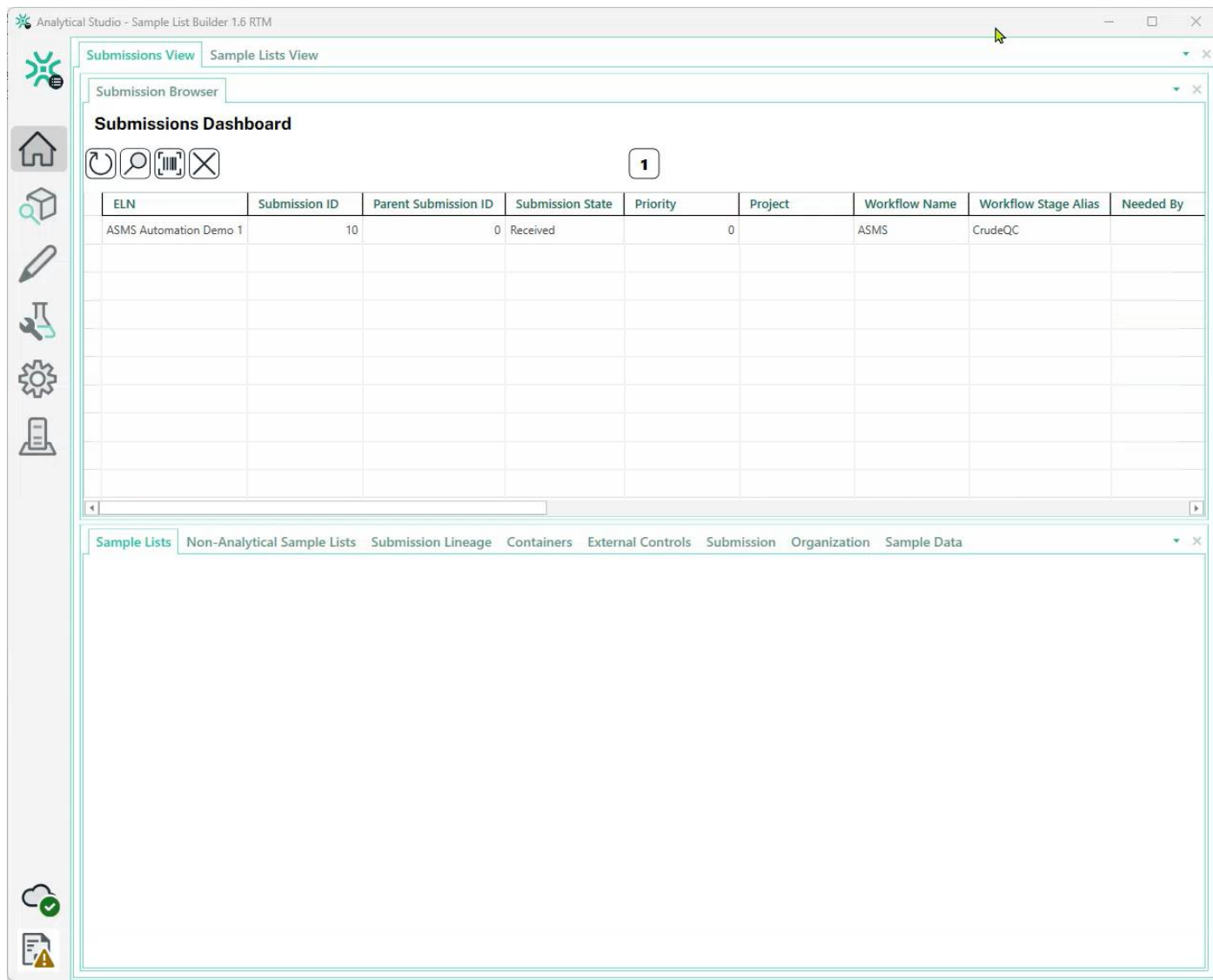
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A user:

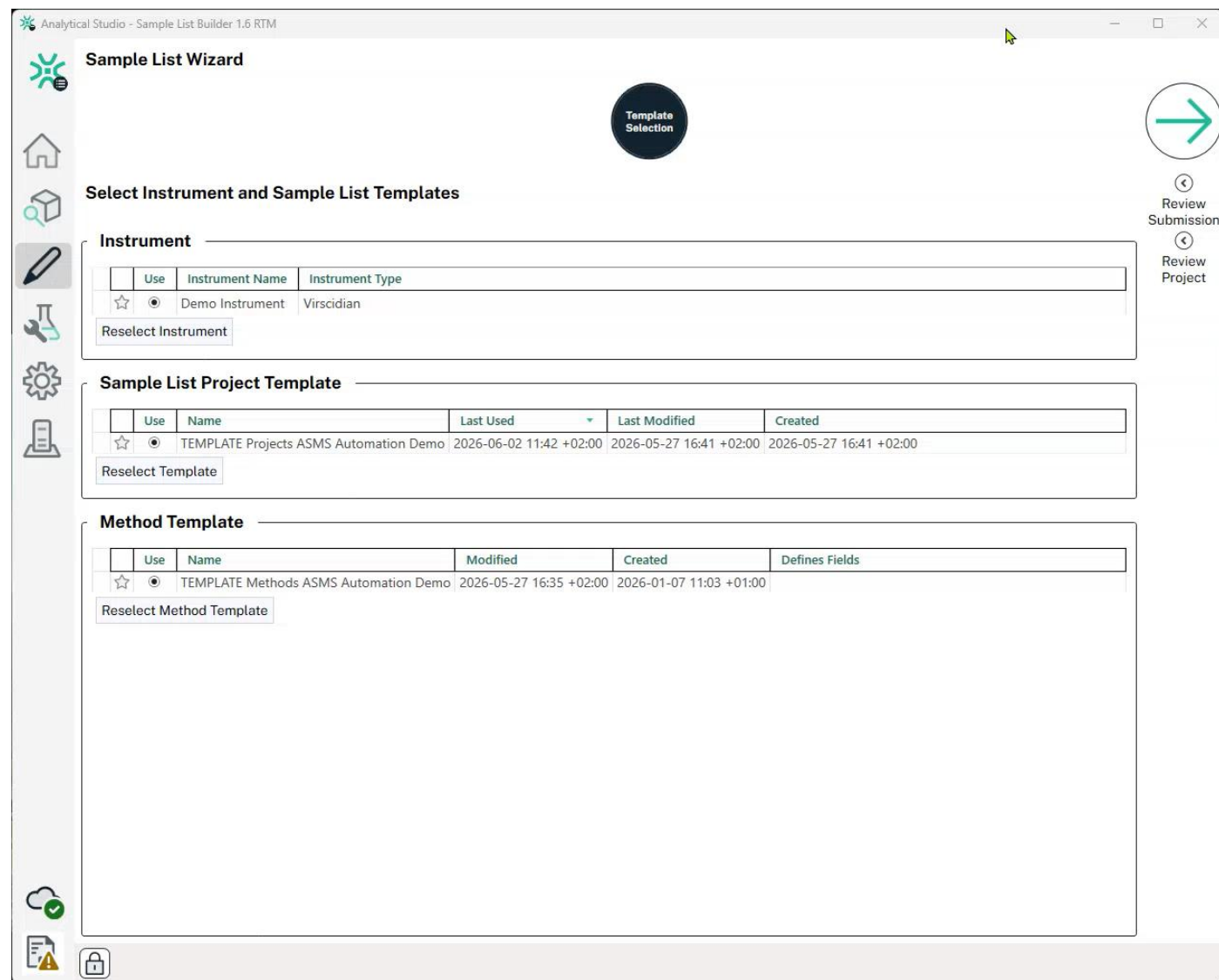
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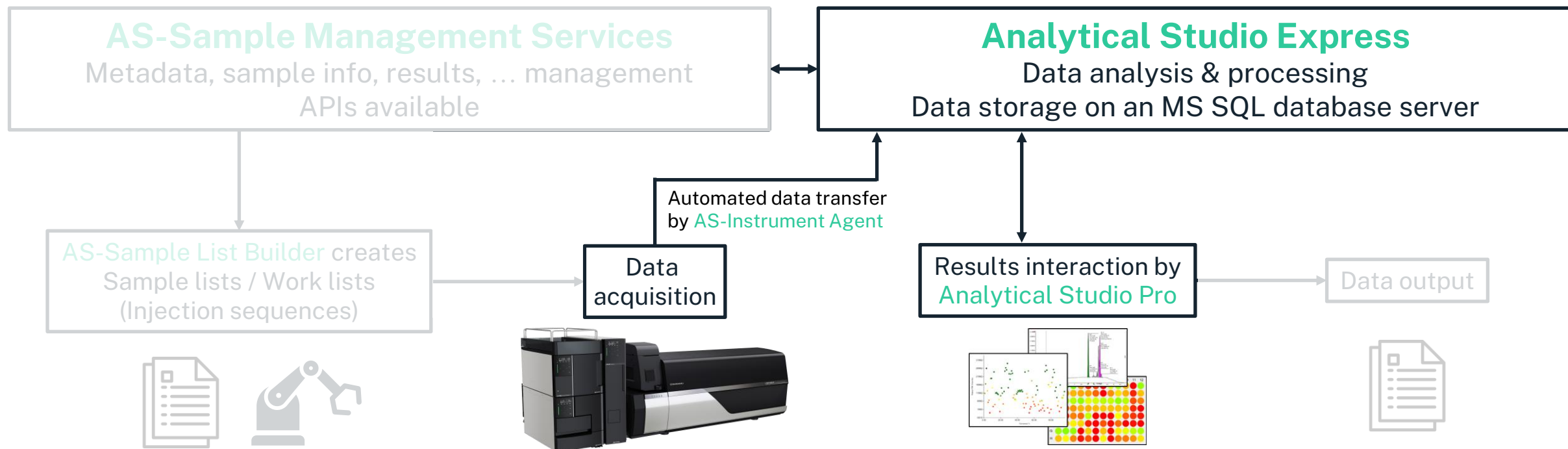
Initial Analysis

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Cross-Hit Filtering

Users review data and remove false positives by identification of background noise across all samples.

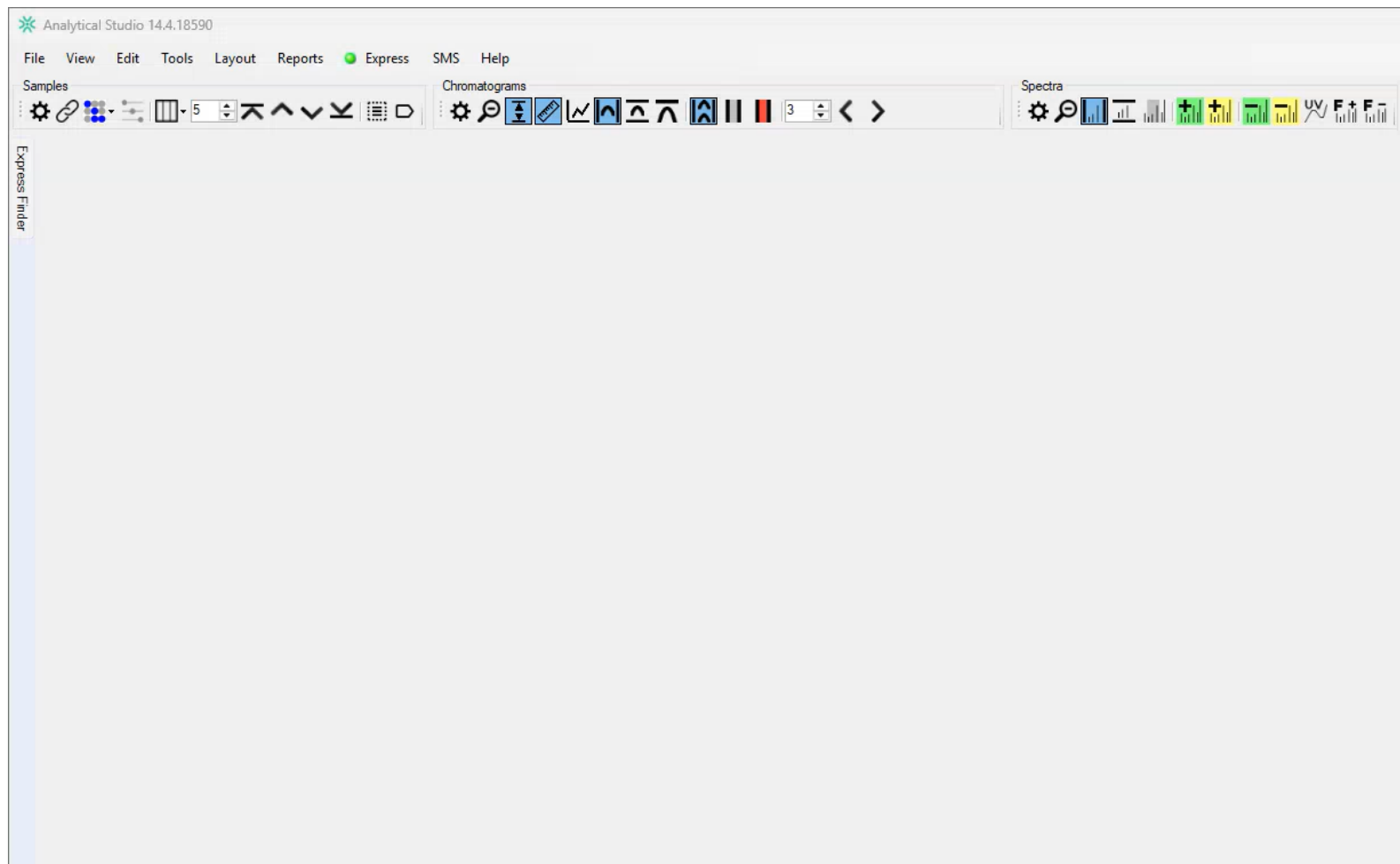
Submitting a compound list to AS-SMS and generating a sample list



Initial analysis: peak integration and compound identification based on mass analysis and isotopic pattern matching

From Analytical Studio -
Express finder:

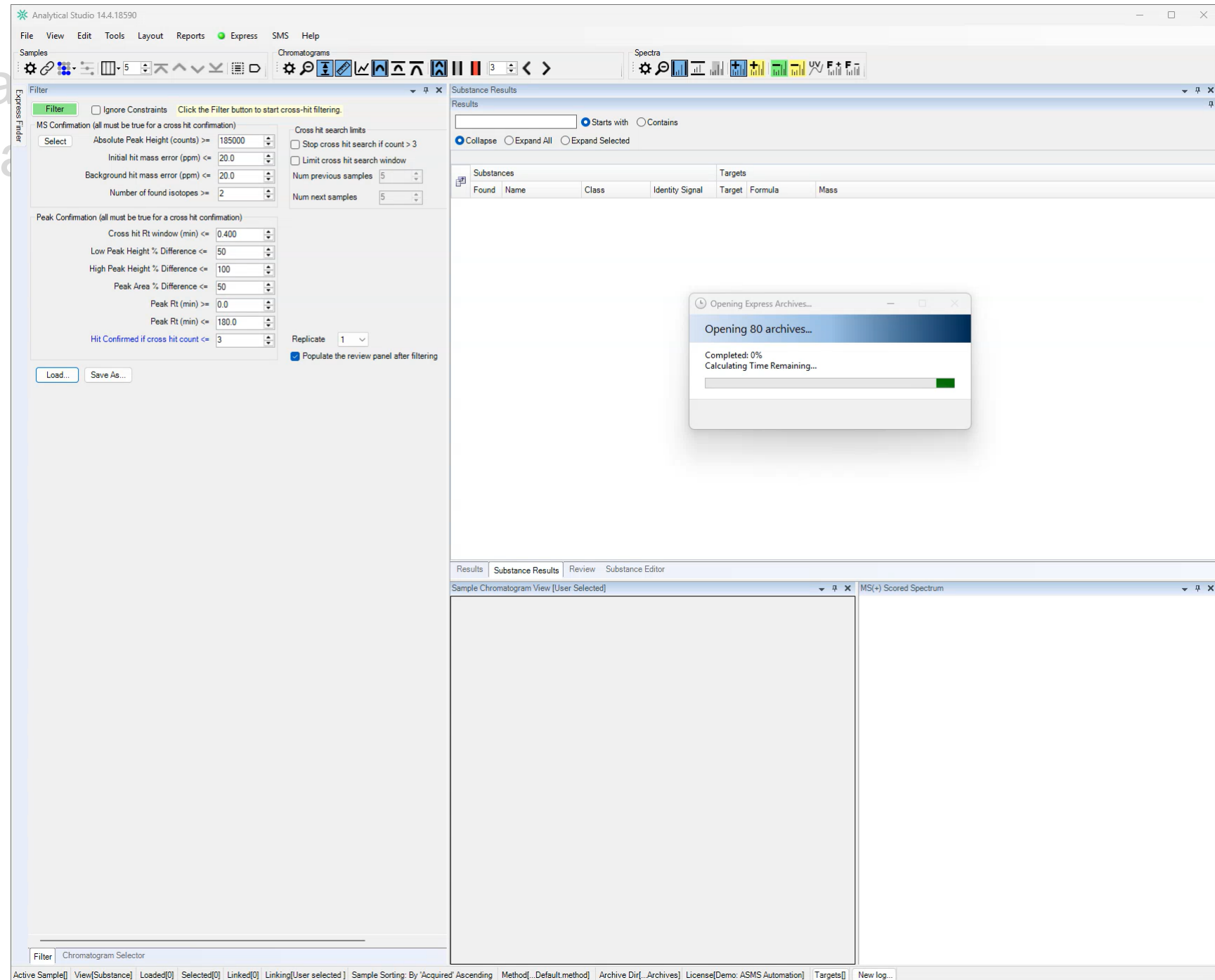
1. Query database
2. Download and (optionally) review results
3. Perform filtering



Initial analysis: peak
based on mass and

From Analytical Studio -
Express finder:

1. Query database
2. Download and (optionally)
review results
3. Perform filtering



Automated AS-MS Workflow

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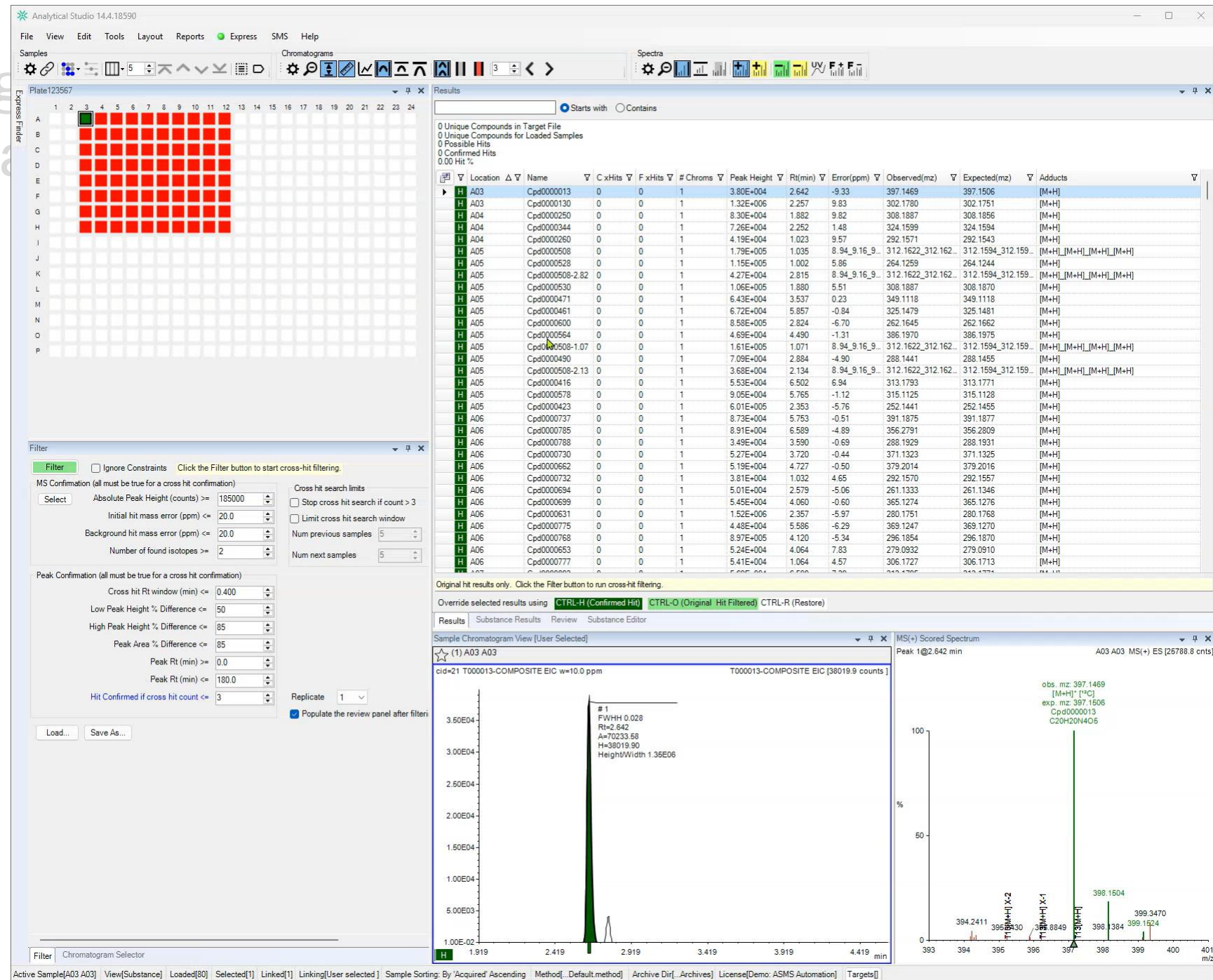
Initial Analysis

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Cross-Hit Filtering

Users review data and remove false positives by identification of background noise across all samples.

2. Interpret results



Cross-Hit Filtering removes false positives by identifying background noise across all samples.

Filter ☐ Ignore Constraints Click the Filter button to start c

MS Confirmation (all must be true for a cross hit confirmation)

Select Absolute Peak Height (counts) $\geq$ 185000

Initial hit mass error (ppm) $\leq$ 20.0

Background hit mass error (ppm) $\leq$ 20.0

Number of found isotopes $\geq$ 2

Peak Confirmation (all must be true for a cross hit confirmation)

Cross hit Rt window (min) $\leq$ 0.400

Low Peak Height % Difference $\leq$ 50

High Peak Height % Difference $\leq$ 85

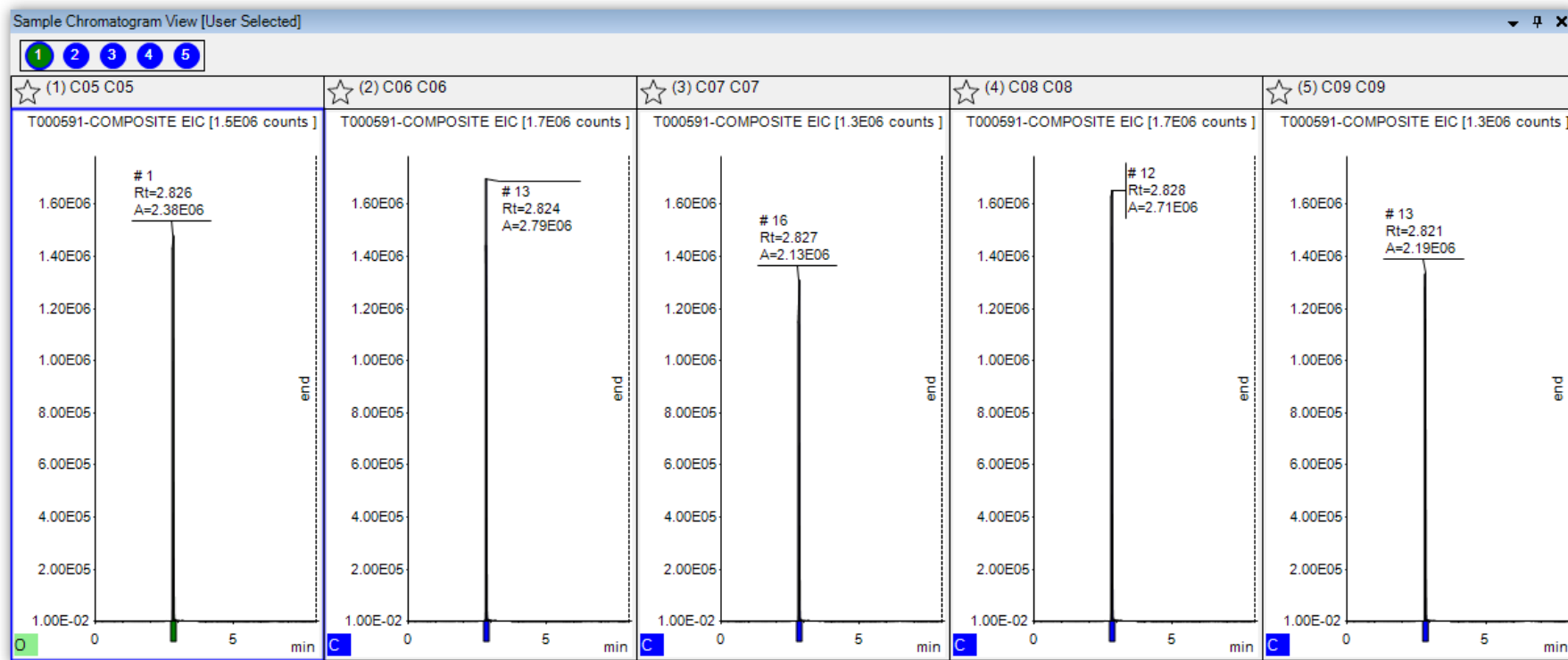
Peak Area % Difference $\leq$ 85

Peak Rt (min) $\geq$ 0.0

Peak Rt (min) $\leq$ 180.0

Hit Confirmed if cross hit count $\leq$ 3

Load... Save As...



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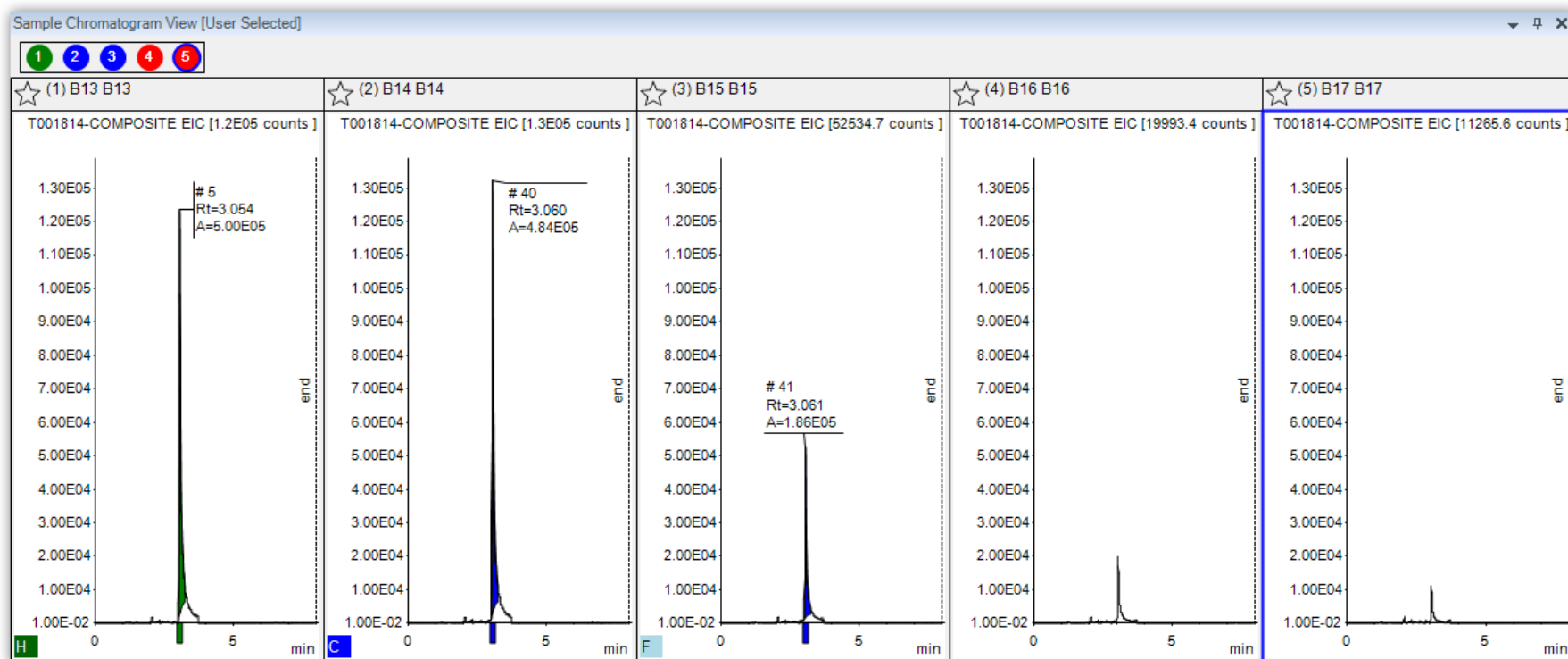
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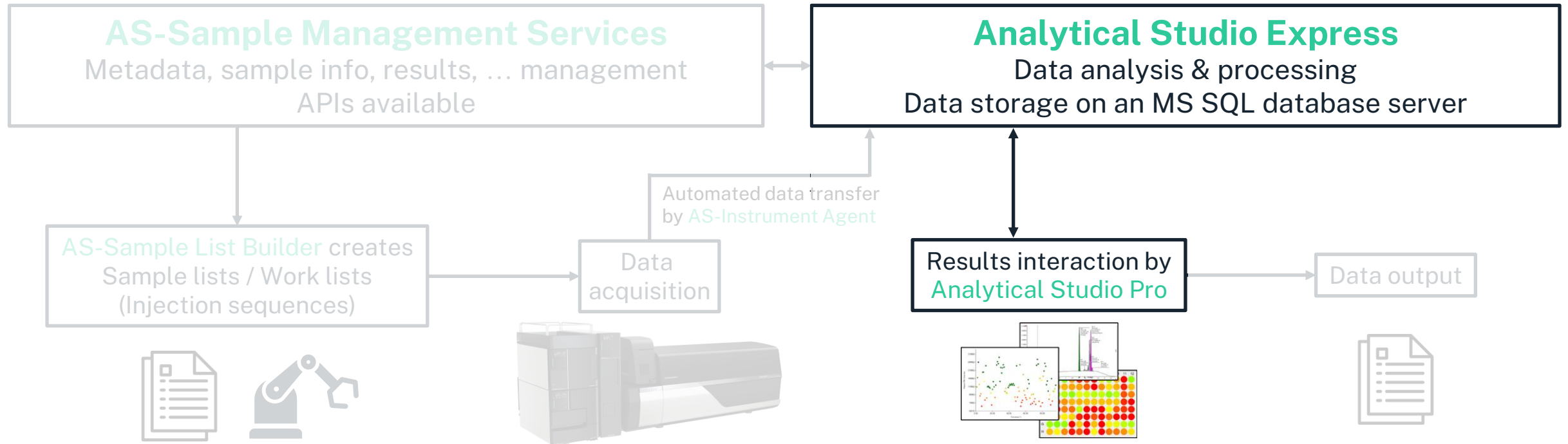
Peak Rt (min) $\leq$ 180.0

Hit Confirmed if cross hit count $\leq$ 3

Load... Save As...



Cross-Hit Filtering removes false positives by identifying background noise across all samples.



AS-MS improvements

	Standalone	AS-SMS+Express
Compound list	Manual	Manual
Sample list	Manually created	Automated via AS-SMS and AS-Sample List Builder
Metadata	Manually curate Sample Info strings	Automated via AS-SMS and AS-Sample List Builder
Initial processing	File > Open > Targets File > Raw data	Download data from AS-Express
Cross-hit filtering	Benchmark	70-80% speed improvement, with the same results
Automation potential	Limited	High potential with API access and integration capabilities

Affinity Selection-Mass Spec

Identifying binders and analyzing dose response data

Automation

Sample list generation, data transfer, and processing

Software improvements

Cross-Hit Filtering and K_d analysis



2026 Dotmatics Summit London

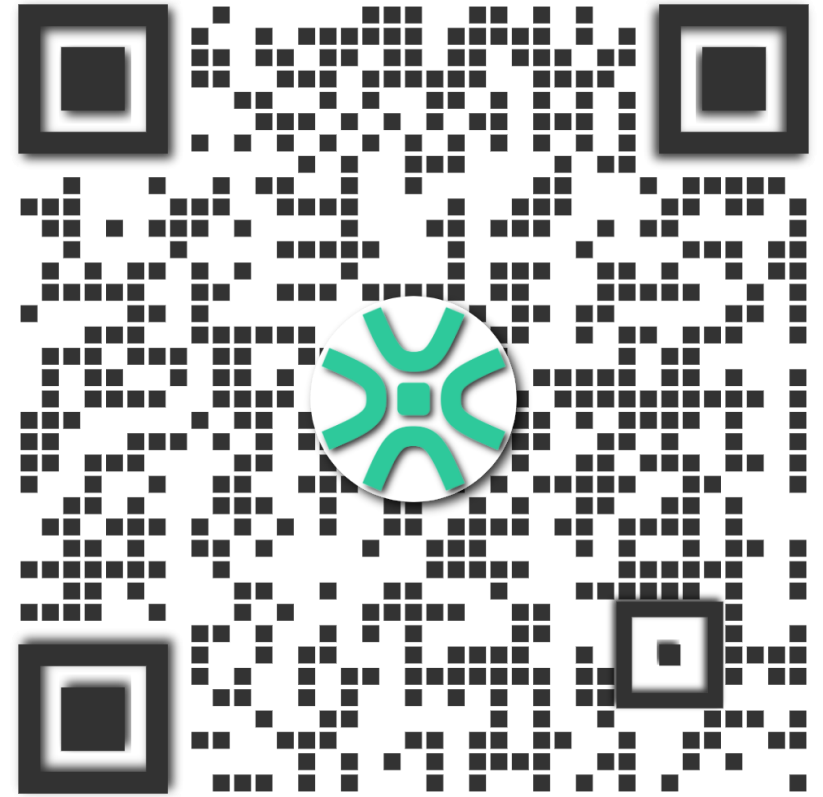
Tuesday, October 20, 2026

St. Pancras Renaissance Hotel, London, UK

8:00 AM - 7:00 PM

From Data to Decisions

See how leading R&D teams are turning everyday lab data into faster, AI-powered decisions



Dotmatics Summit London 2026