LIFE SCIENCE MASS SPECTROMETRY

SCiLS™ Lab 2025a – What's New?

01

Multiple feature enhancements

SCiLS™ Lab 2025a – What's New?

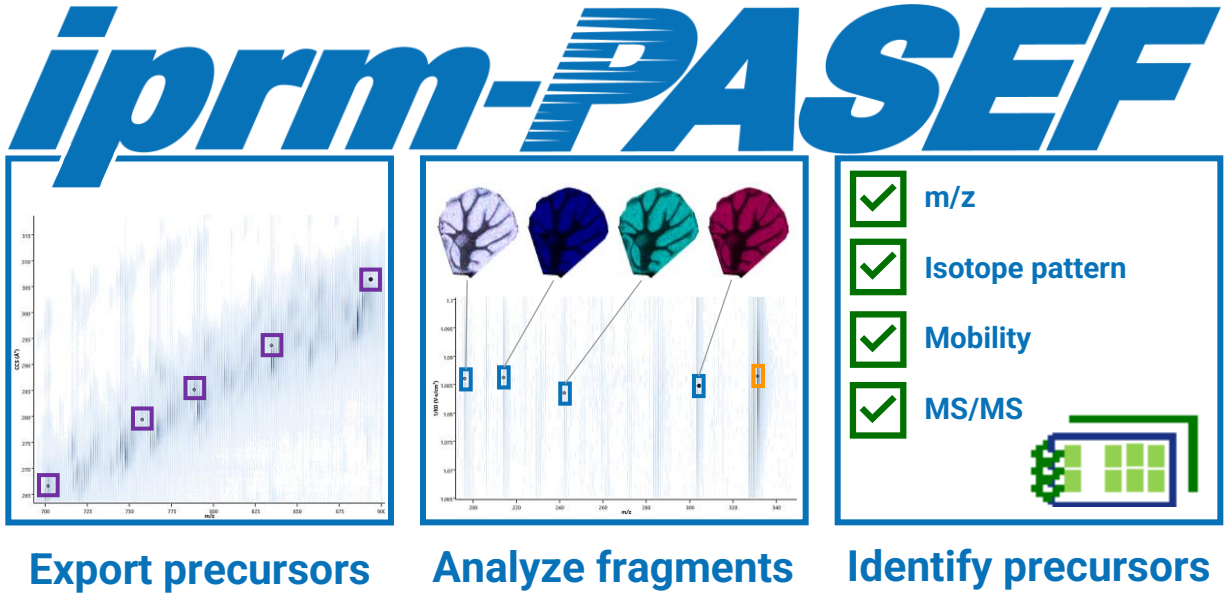
01 *Multiple feature enhancements:*
Various improvements and changes

02 *iprm-PASEF:*
Fragmentation-based molecular annotation

03 *T-ReX® feature finding:*
Visualize T-ReX deisotoping results

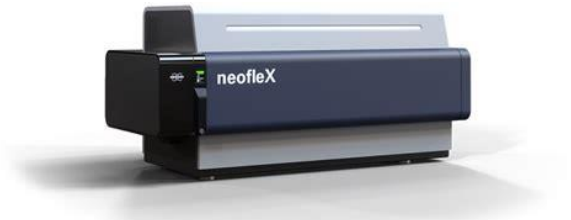
04 *SCiLS API improvements:*

05 *neofleX support:*

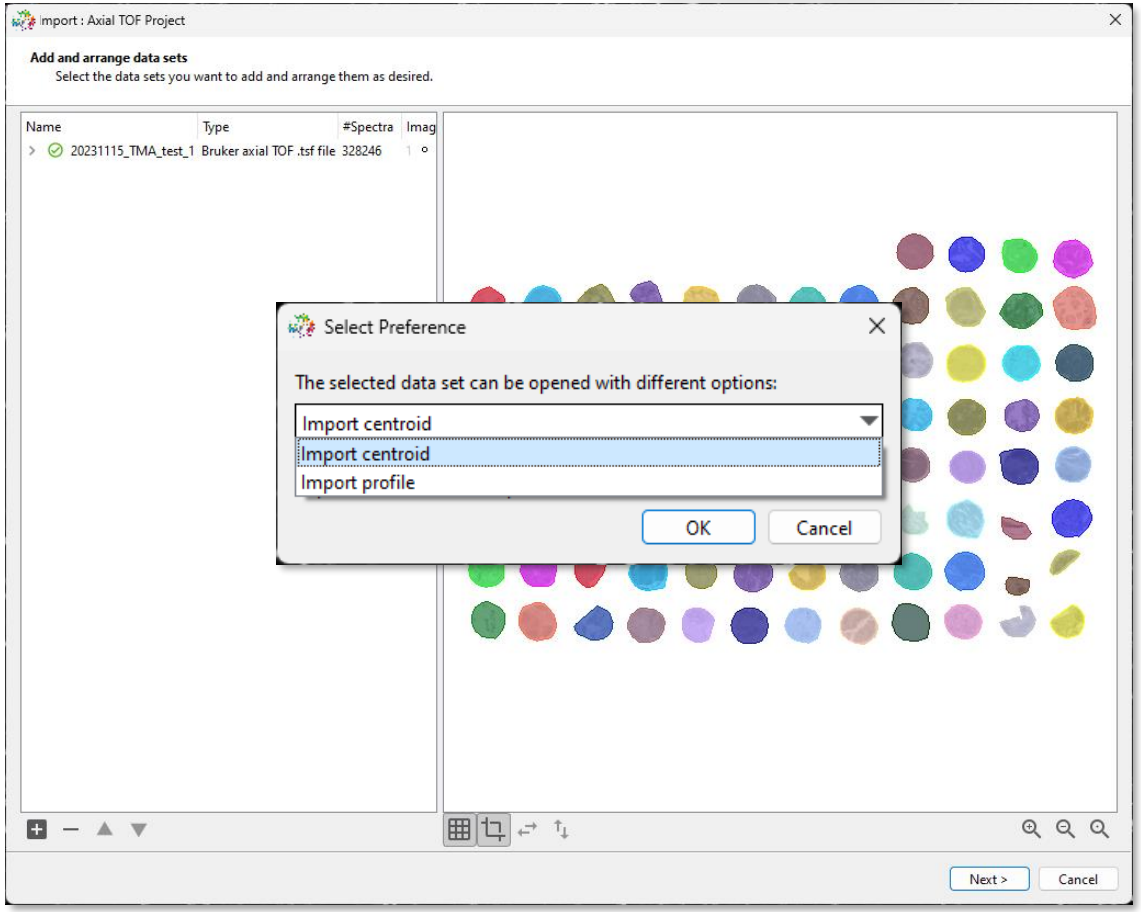


Available for Customers since October 1st, 2024

Multiple feature enhancements
Importing neofleX data into SCiLS™ Lab



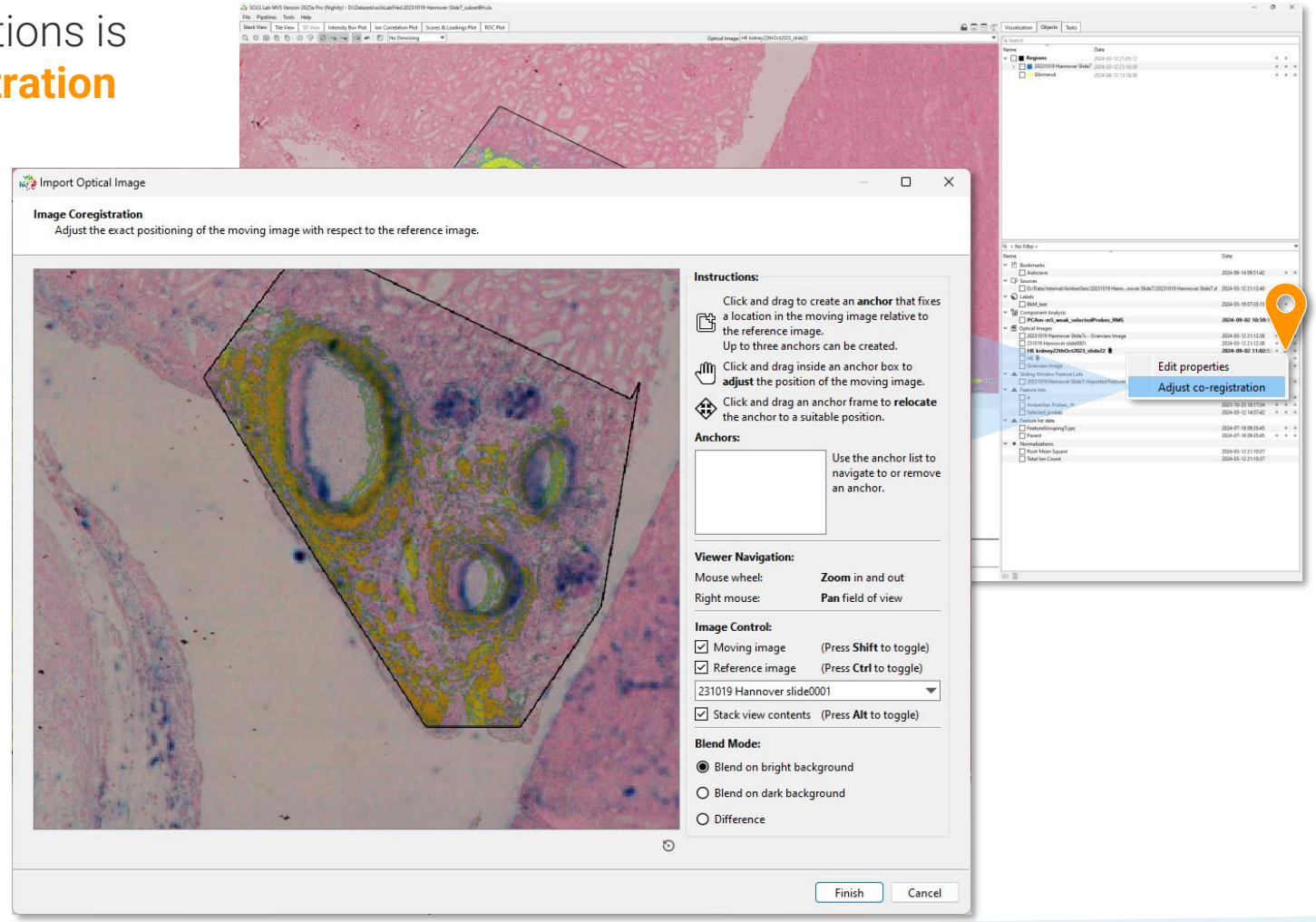
- SCiLS Lab 2025a can read .d/tsf data acquired by the all-new **neofleX** axial TOF instrument
- Import **centroid** or **profile** neofleX data into a SCiLS Lab “Axial TOF project”
- neofleX profile data can be combined with rapifleX profile data (.d container format)
- Combining centroid and profile axial TOF data is not supported
- T-ReX® Feature Finding is not enabled for neofleX data sets



Multiple feature enhancements

Adjust registration for previously imported optical images

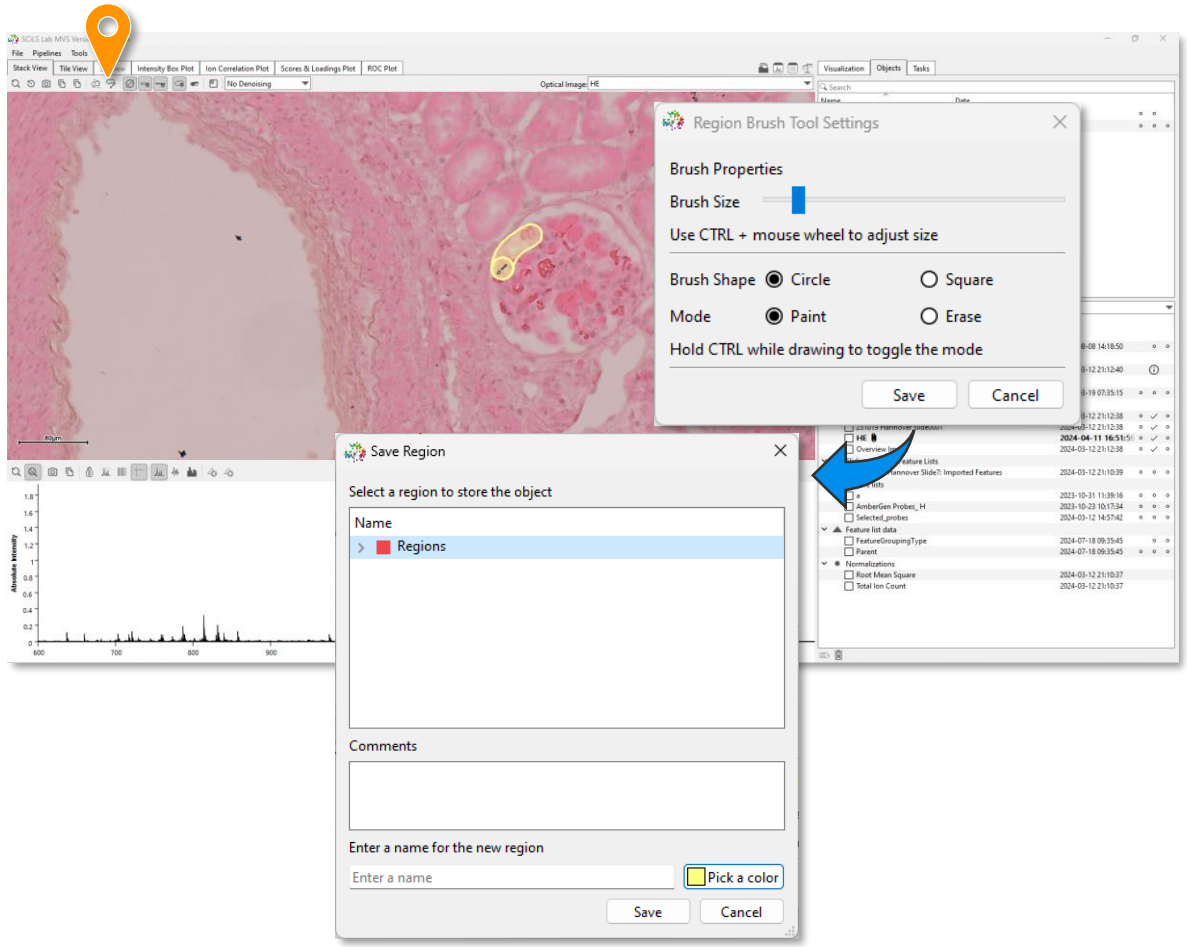
- In case any of the optical image registrations is unsatisfactory, one can **Adjust co-registration**
  - No image orientation required
  - Detailed registration identical to optical image import
  - Allows changing the reference image
  - New registration overwrites previous
  - Cancelling restores original registration



Multiple feature enhancements

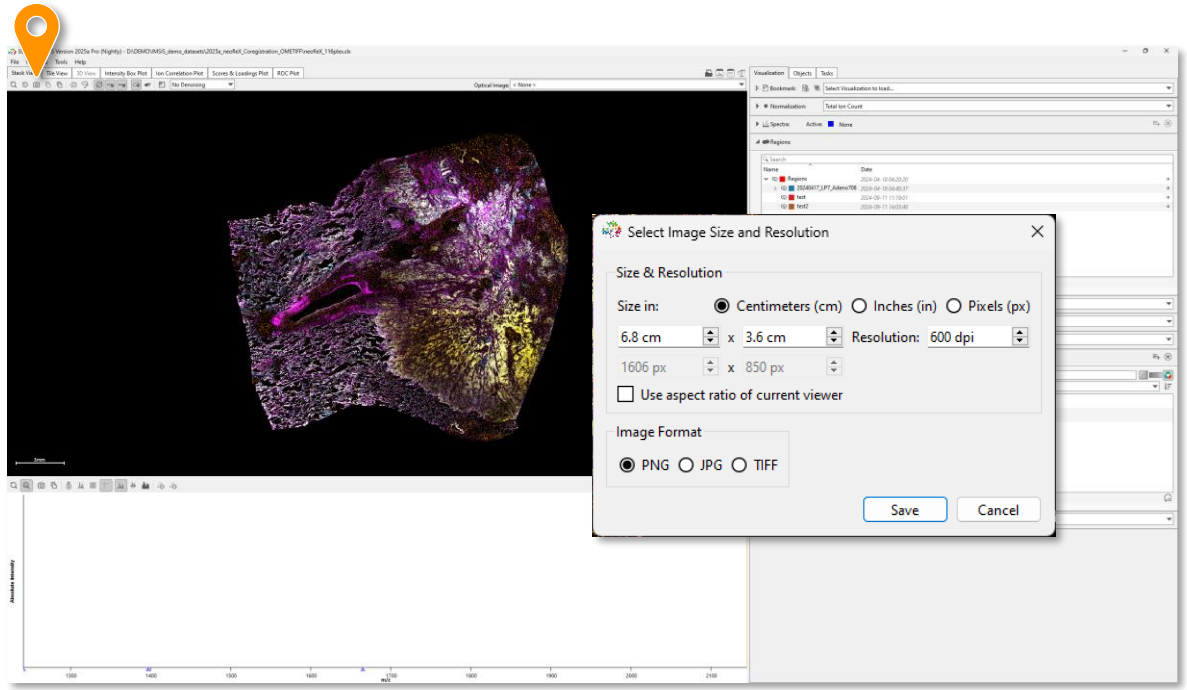
Brush tool for region of interest definition

- SCiLS™ Lab 2025a now includes a **brush tool** to define regions of interest:
- Switch between **Painting** and **Erasing**
- Switch between **Circular** and **Square** brush shape
- Fluently **change brush size** while painting
- Allows to **create disjoined regions**



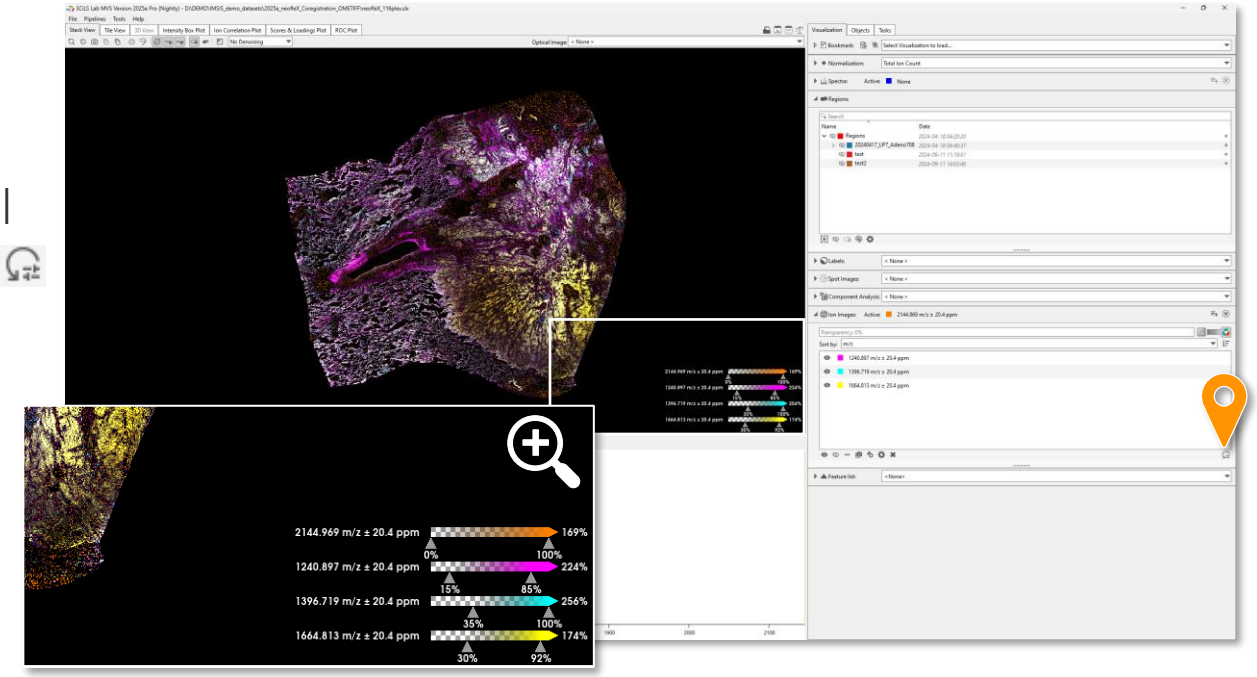
Multiple feature enhancements
Improved screenshot export

- Different usage of images often come with specific **size and image resolution requirements**.
- Set the dimensions and image resolution when using **Save screenshot**:



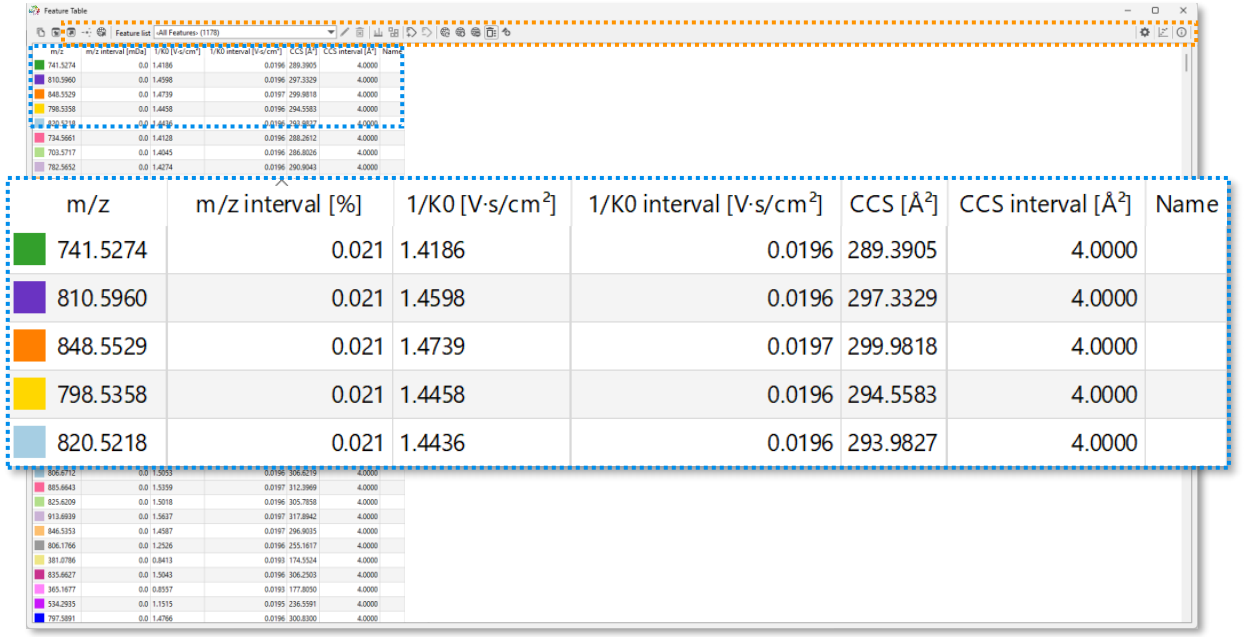
Multiple feature enhancements
Individual colorbar scaling in Stack View

- Set color bars for individual images independently when in **image blend mode**:
- Reinstate the default 0% and 100% positions for all images simultaneously using **Reset all color bars**:



Multiple feature enhancements
Feature Table update

- SCiLS™ **Feature Table** has been updated
- **Functionality-based grouping** of tools
- **m/z and mobility intervals** now have their own Feature Table columns *(hidden by default)*

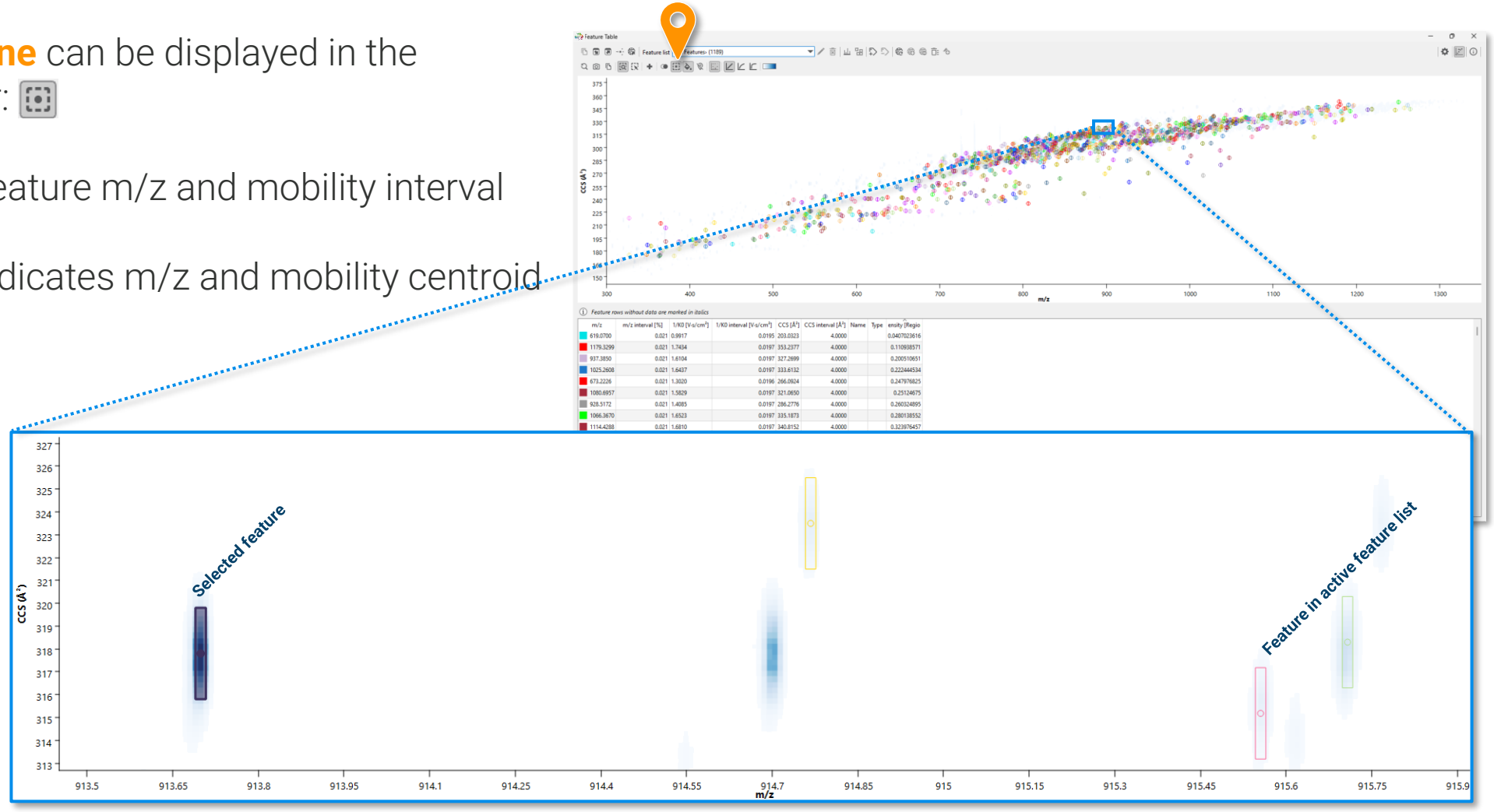


m/z	m/z interval [%]	1/K0 [V·s/cm²]	1/K0 interval [V·s/cm²]	CCS [Å²]	CCS interval [Å²]	Name
741.5274	0.021	1.4186	0.0196	289.3905	4.0000	
810.5960	0.021	1.4598	0.0196	297.3329	4.0000	
848.5529	0.021	1.4739	0.0197	299.9818	4.0000	
798.5358	0.021	1.4458	0.0196	294.5583	4.0000	
820.5218	0.021	1.4436	0.0196	293.9827	4.0000	

Copy-to-clipboard
Import feature list
Export feature list
Export iprm-PASEF parameters
Export feature images (OME-TIFF)
Select feature list
Edit name
Delete current feature list
Add intensity column(s)
Add "Component Analysis loadings" column
Create molecular annotations
Delete annotations for selected features
Extract missing CCS images
Create ion images from selection
Remove selected ion images
Remove selected rows from feature table
Create new feature list from selected rows
Manage columns
Show feature navigator
Show detailed feature information
Copy-to-clipboard (detailed feature info)

Multiple feature enhancements
Feature Navigator improvement

- **CCS feature outline** can be displayed in the Feature Navigator:
- Indicates actual feature m/z and mobility interval
- Original marker indicates m/z and mobility centroid

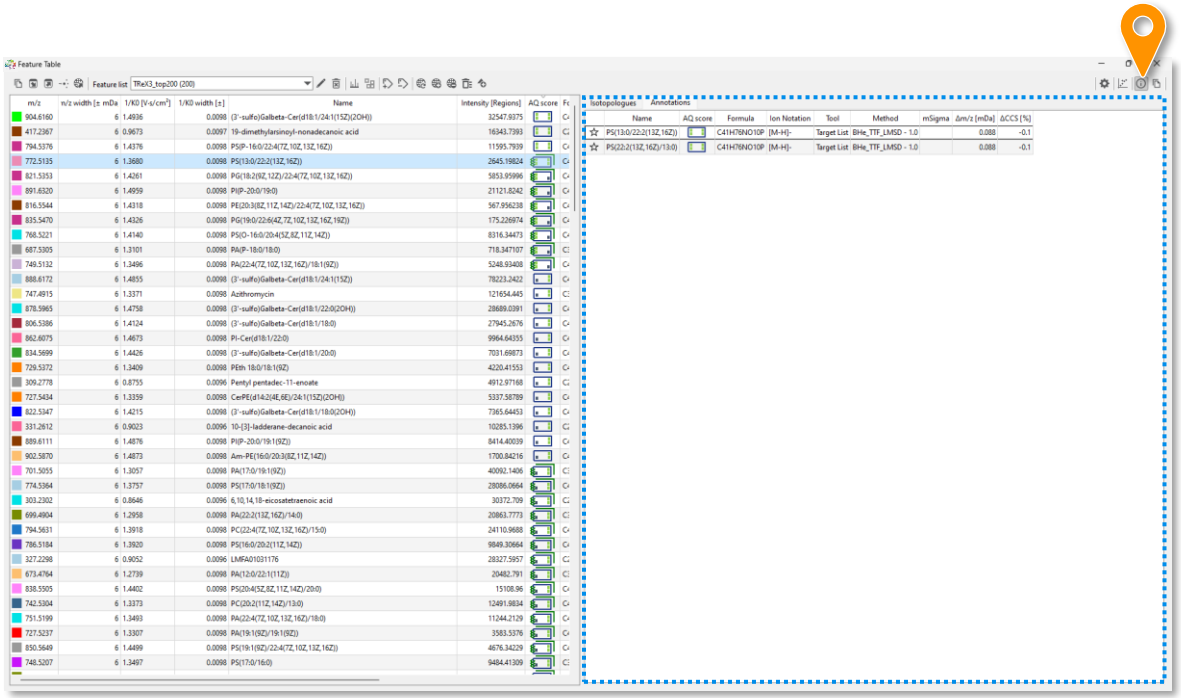


Multiple feature enhancements
Feature Table detailed information tabs

- Specific feature details are now shown in the **Detailed Information** panel of the Feature Table

- Sub-panels for different sets of information:
  - **Isotopologues**
    - Only for data sets compatible with T-ReX® feature finding
  - **Annotations**
    - Available for all data sets, only used when performing MetaboScape-powered Molecular Annotation
  - **Fragments**
    - Only available in iprm-PASEF data sets

- Contents of the Detailed Information sub-panels can be exported using the dedicated **copy-to-clipboard** button



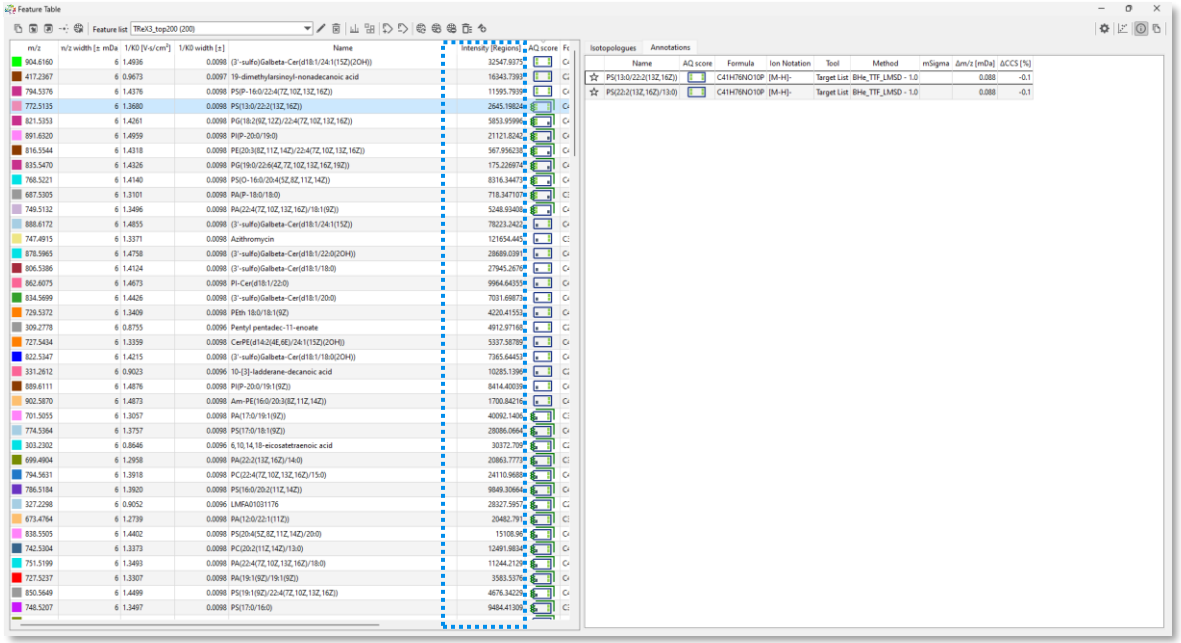
Multiple feature enhancements
Feature Table – Intensity [Regions] column

- Previously, the Feature Table did not contain a default intensity column, and the user had to manually create one if they wanted to sort a feature list based on intensities.

- Now, the Feature Table includes the default **"Intensity [Regions]"** column which always displays the **non-normalized average root region ("Regions") intensity**

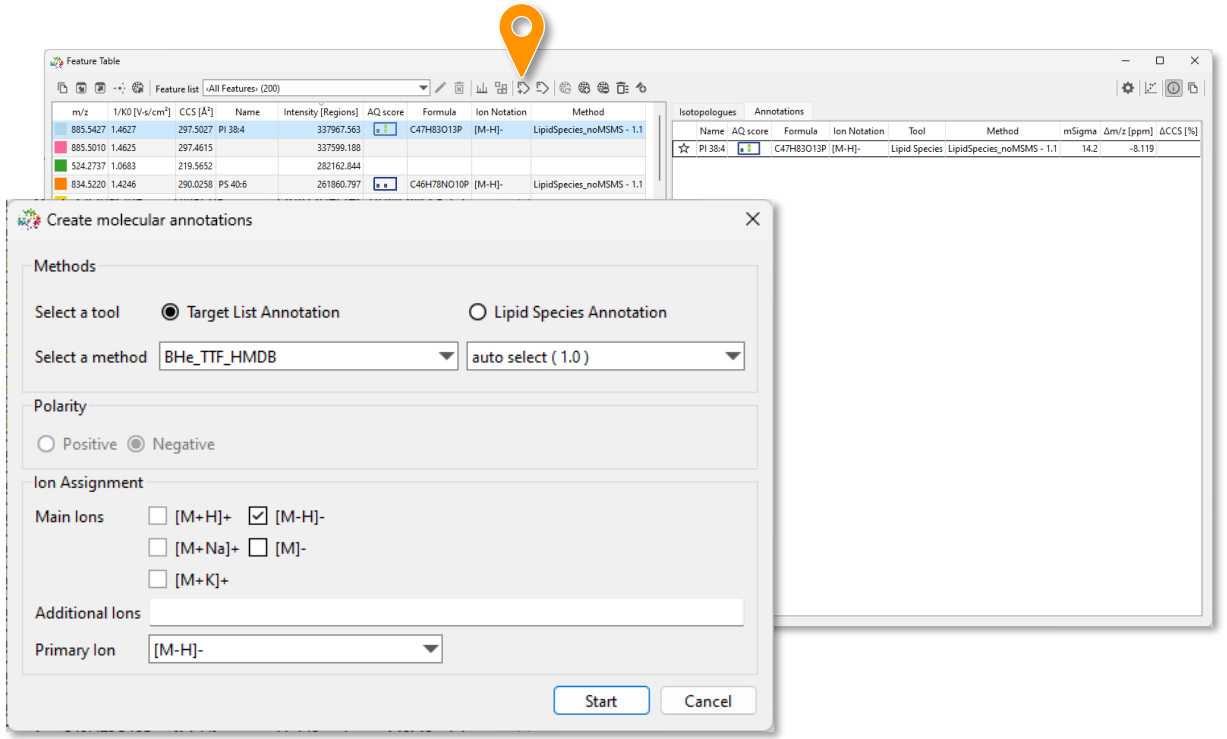
- For mass-mobility features these intensities are only available after extracting the CCS ion images

- The "Intensity [Regions]" column cannot be deleted, but can be hidden from the Feature Table



Multiple feature enhancements
MetaboScape®-powered rule-based Lipid Species annotation

- An additional tool for MetaboScape–powered Molecular Annotation available
- Methods list filtered on active tool selection
- Rule-based **Lipid Species** annotation
  - m/z accuracy
  - Isotope pattern
  - Mobility (if available)
  - MS/MS (can be disabled in MetaboScape)
- In case of MS1 data, only returns species level annotations, which avoids over-interpretation of the lipid identity
  - **NOTE:** *by default, Lipid Species annotation methods require MS/MS information. This parameter can be turned off in MetaboScape.*



MetaboScape annotation in SCiLS Lab

Requires MetaboScape® 2025a or newer

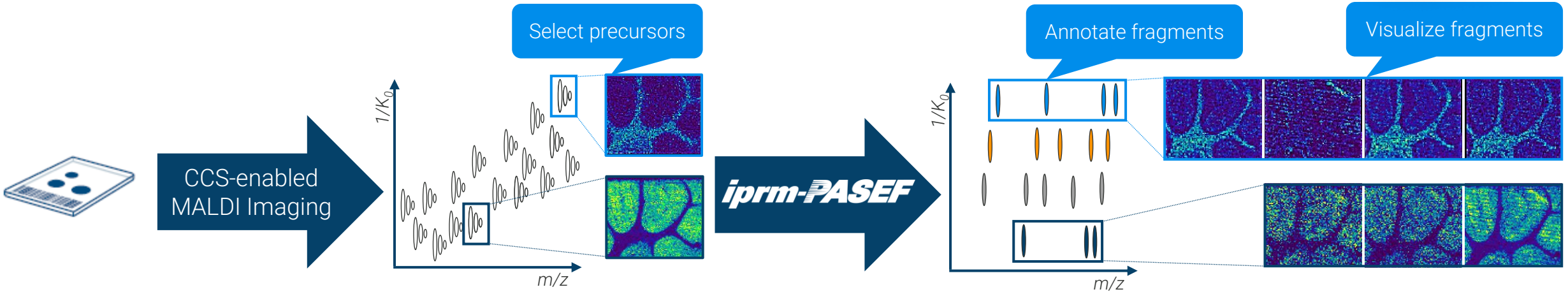
02

iprm-PASEF

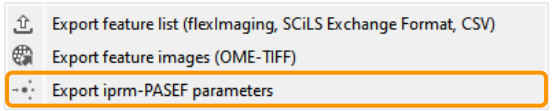
iprm-PASEF
A workflow to identify your images with confidence

timsTOF fleX-based targeted fragmentation with spatial fidelity

- Targeted and CCS-enabled precursor isolation and fragmentation with spatial fidelity
- The first **fully integrated** commercial imaging solution for fragmentation-based Molecular Annotation – **Acquire, Analyze, Annotate**

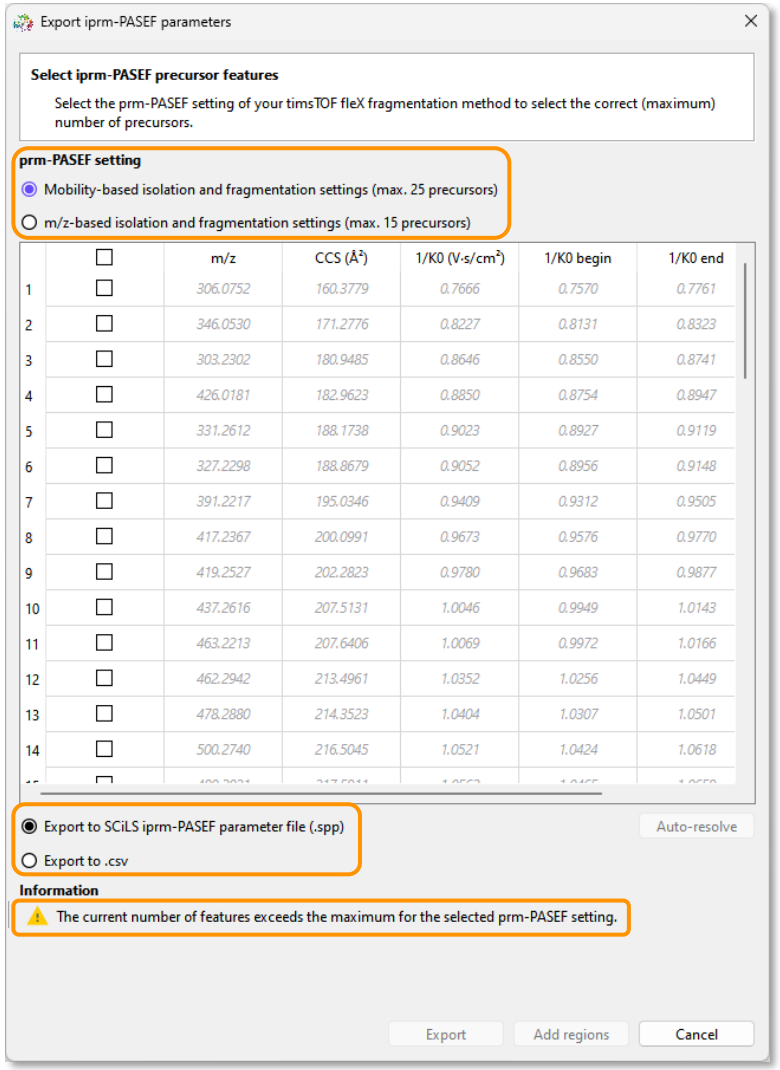


iprm-PASEF
Create target precursor list in SCiLS™ Lab

- New "**Export iprm-PASEF parameters**" option in CCS-enabled MALDI Imaging datasets
  - Feature table: 
  - Objects tab: 

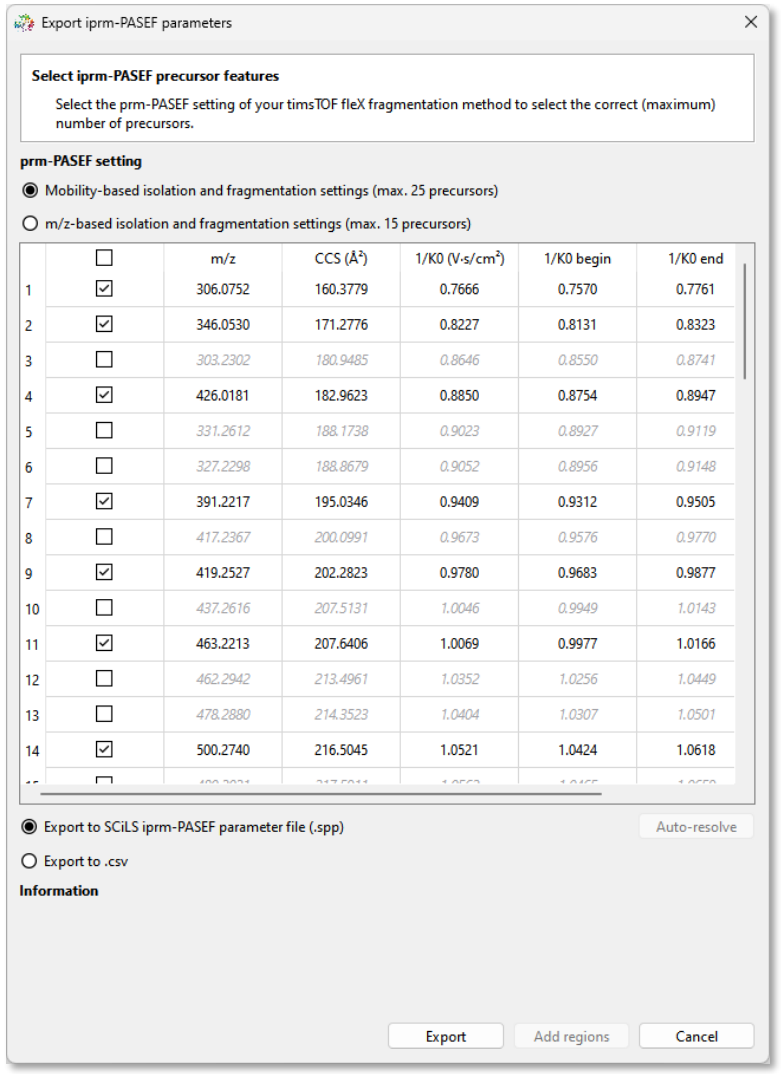
- **prm-PASEF setting** in timsControl determines maximum Precursor list length
  - **NOTE:** *does not alter setting in timsControl*
  - **Warning** shown when maximum feature list length is exceeded, this disables export

- Define **export format**
  - SCiLS iprm-PASEF parameter file (.spp)
    - Can includes feature AND regions
    - Read by timsControl & flexImaging
  - Comma-separated values (.csv)
    - Only includes features
    - Read by timsControl



iprm-PASEF
Create target precursor list in SCiLS™ Lab

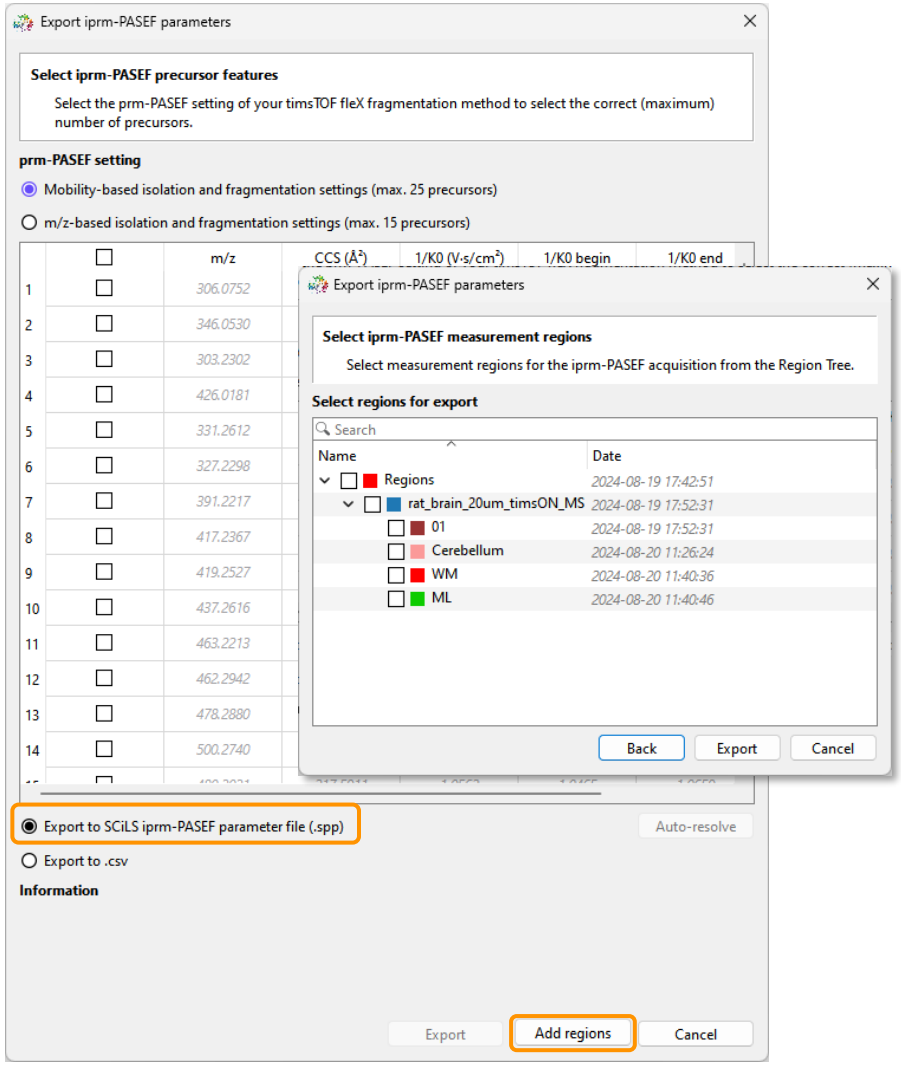
- Table contains features in **current feature list**
- Precursor list **restrictions**
  - Precursor list length
    - determined by prm-PASEF setting
  - Only non-overlapping 1/K0 windows allowed
    - $1/K0_{end \rightarrow begin} \geq 0.01$
- **Resolve overlapping $1/K_0$ windows** by:
  - Manual adjust of 1/K0 begin and/or 1/K0 end values
    - Double-click on value and change
  - Uncheck conflicting features
  - Auto-resolve conflicting 1/K0 windows
    - Only enabled when number of features does not exceed maximum precursor list length
    - Adjusts 1/K0 begin and 1/K0 end values of features with overlapping 1/K0 windows
    - Always includes 1/K0 centroids of both original features
  - Export disabled until all overlapping 1/K0 are resolved



		m/z	CCS (Å²)	1/K0 (V·s/cm²)	1/K0 begin	1/K0 end
1	☑	306.0752	160.3779	0.7666	0.7570	0.7761
2	☑	346.0530	171.2776	0.8227	0.8131	0.8323
3	☐	303.2302	180.9485	0.8646	0.8550	0.8741
4	☑	426.0181	182.9623	0.8850	0.8754	0.8947
5	☐	331.2612	188.1738	0.9023	0.8927	0.9119
6	☐	327.2298	188.8679	0.9052	0.8956	0.9148
7	☑	391.2217	195.0346	0.9409	0.9312	0.9505
8	☐	417.2367	200.0991	0.9673	0.9576	0.9770
9	☑	419.2527	202.2823	0.9780	0.9683	0.9877
10	☐	437.2616	207.5131	1.0046	0.9949	1.0143
11	☑	463.2213	207.6406	1.0069	0.9977	1.0166
12	☐	462.2942	213.4961	1.0352	1.0256	1.0449
13	☐	478.2880	214.3523	1.0404	1.0307	1.0501
14	☑	500.2740	216.5045	1.0521	1.0424	1.0618

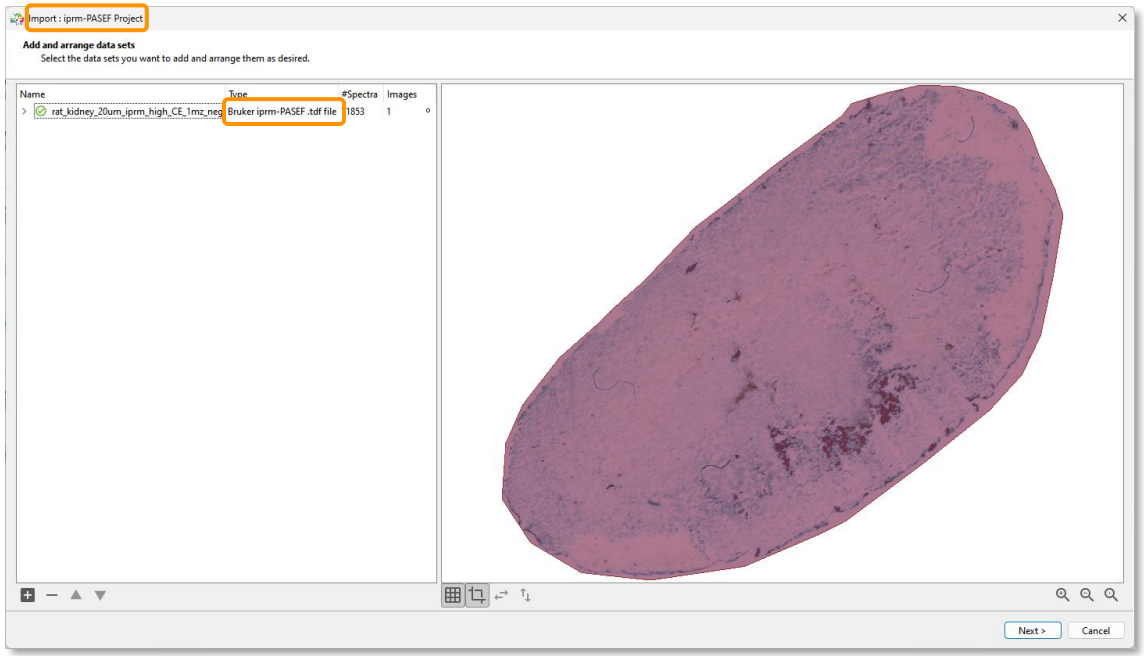
iprm-PASEF
Create target precursor list in SCiLS™ Lab

- **Add regions** to import and define measurement regions in flexImaging
  - Regions cannot overlap as flexImaging does not support overlapping measurement regions
  - Disjoined regions appear as individual measurement regions in flexImaging



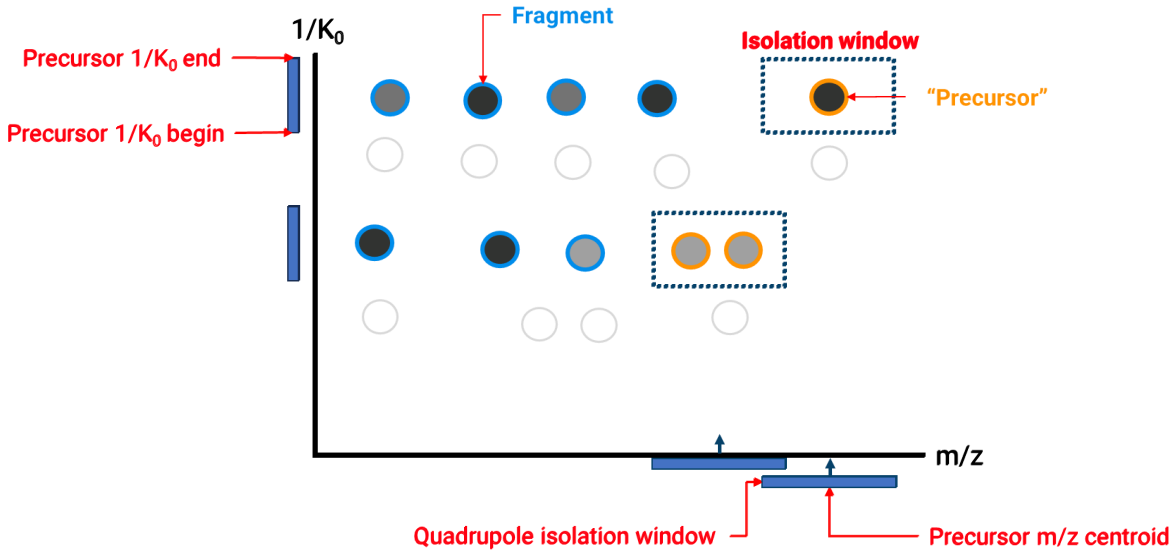
iprm-PASEF
Import iprm-PASEF data into SCiLS™ Lab

- iprm-PASEF source data sets will be imported into SCiLS Lab as an **"iprm-PASEF project"**
- iprm-PASEF data cannot be mixed with other data types (e.g. "regular" .tdf timsTOF fleX data)
- T-ReX® feature finding can be performed upon manual import of iprm-PASEF projects
  - Only returns features found within the mobility boundaries of the precursor isolation windows
  - Default settings and internal parameters have been optimized for iprm-PASEF data



iprm-PASEF
Feature relations in iprm-PASEF data sets

- Features are grouped by the **Isolation Windows**
- Features are split into three different types:
  - **Precursors**
    - All features within quadrupole isolation window and mobility window
  - **Fragments**
    - All other features within mobility windows
  - **None**
    - All feature outside the isolation window
    - Are not detected by T-ReX® feature finding
- Features are automatically grouped and characterized in the Feature Table



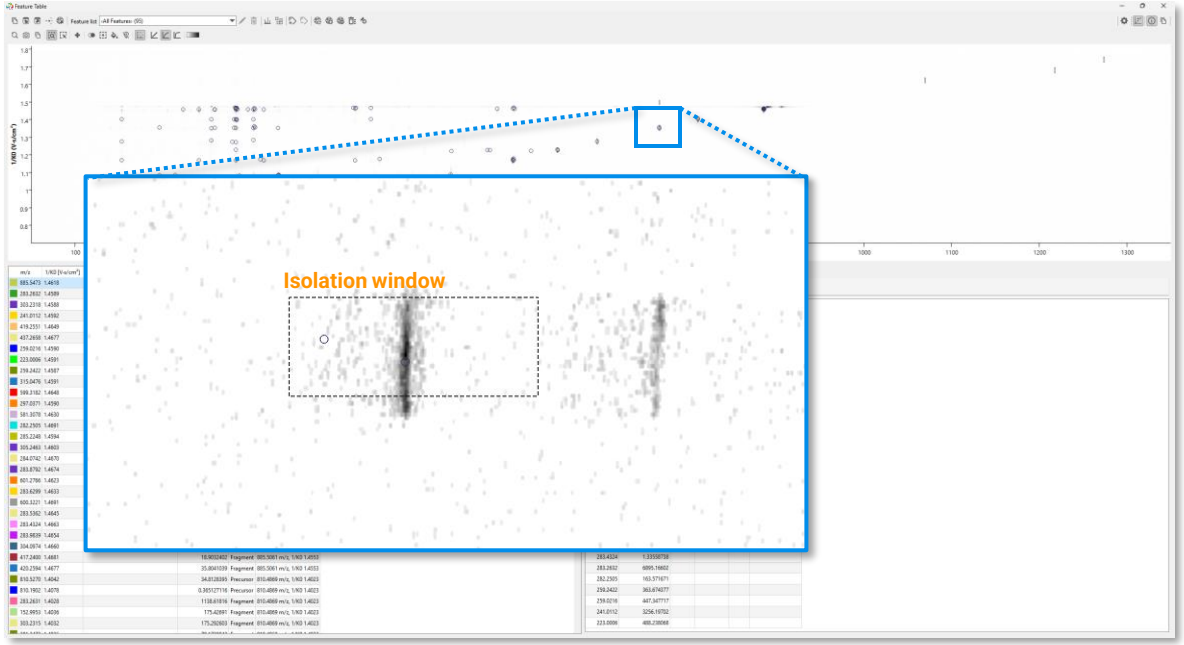
iprm-PASEF

Feature relations in iprm-PASEF data sets

- Features are detected within and can be grouped by the **Isolation Windows**

- Features can be split into two different types:
  - **Precursors**
    - All features within quadrupole isolation window and mobility window
  - **Fragments**
    - All other features within mobility windows
  - **None**
    - All feature outside the isolation window
    - Are not detected by T-ReX® feature finding

- Features are automatically grouped and characterized in the Feature Table



iprm-PASEF
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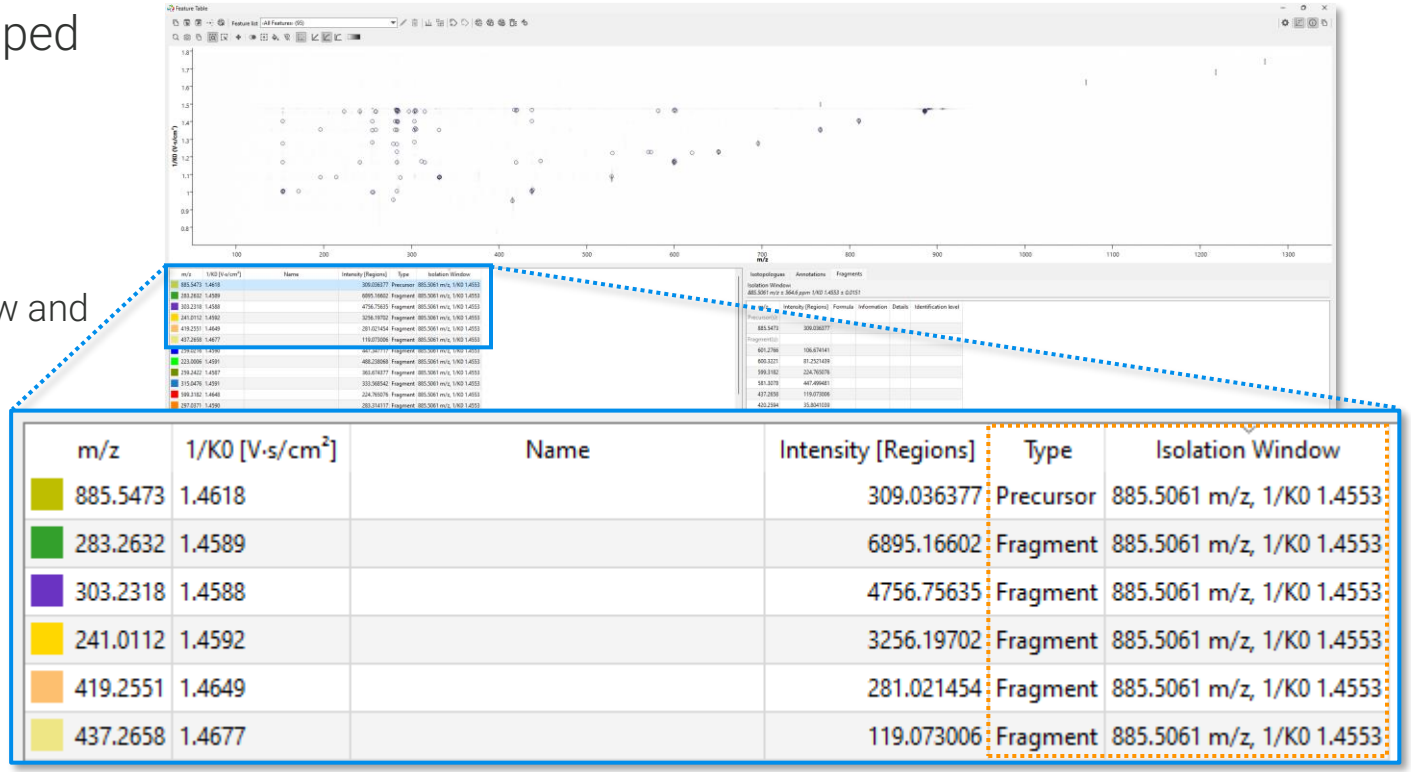
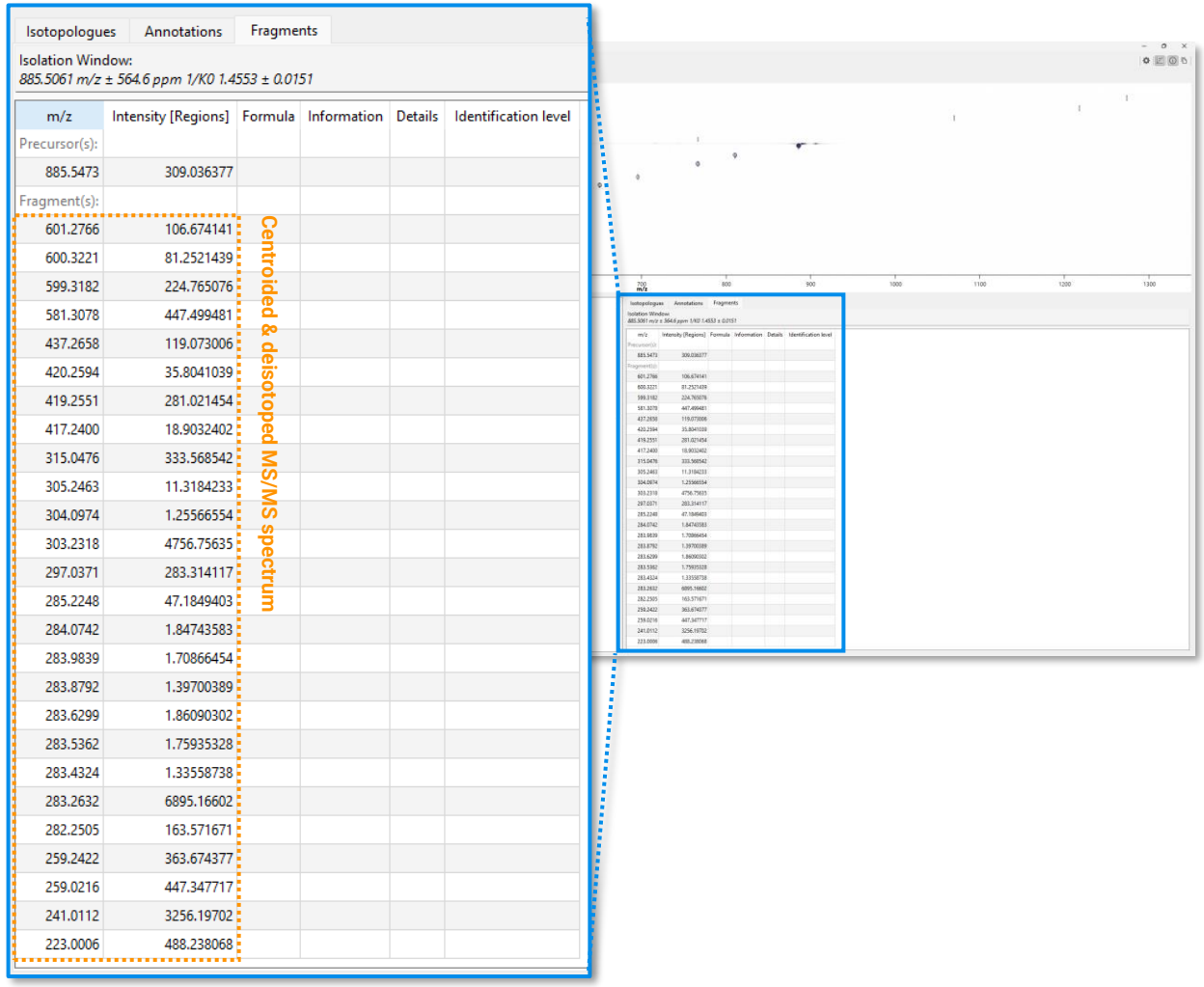


m/z	1/K0 [V·s/cm²]	Name	Intensity [Regions]	Type	Isolation Window
885.5473	1.4618		309.036377	Precursor	885.5061 m/z, 1/K0 1.4553
283.2632	1.4589		6895.16602	Fragment	885.5061 m/z, 1/K0 1.4553
303.2318	1.4588		4756.75635	Fragment	885.5061 m/z, 1/K0 1.4553
241.0112	1.4592		3256.19702	Fragment	885.5061 m/z, 1/K0 1.4553
419.2551	1.4649		281.021454	Fragment	885.5061 m/z, 1/K0 1.4553
437.2658	1.4677		119.073006	Fragment	885.5061 m/z, 1/K0 1.4553

iprm-PASEF
Feature relations in iprm-PASEF data sets

- Fragments sub-panel in the detailed feature information panel shows all precursors and fragments associated to the active feature and present in the active feature list

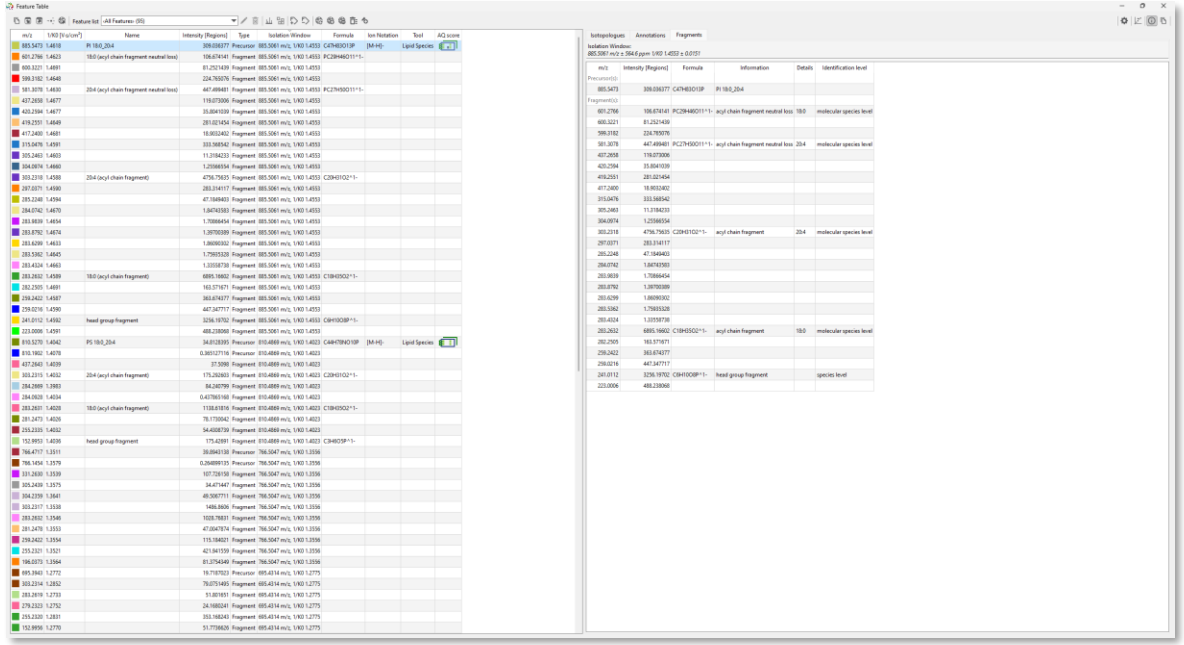


iprm-PASEF

MetaboScape-powered molecular annotation for iprm-PASEF

- Following MetaboScape®-powered Molecular Annotation, **precursor features are annotated** based on:
  - Precursor mass error
  - Precursor isotope pattern quality
  - Precursor mobility error
  - MS/MS spectrum

- Annotation options including MS/MS information:
  - **Rule-based Lipid Species annotation**
    - Requires MetaboScape 2025a or newer
    - Provides fragment annotations
  - **Target list w/ associated Spectral Library**
    - Requires MetaboScape 2023b or newer
    - Identifications restricted to Target list entries



Acquire Bruker Spectral Libraries here:

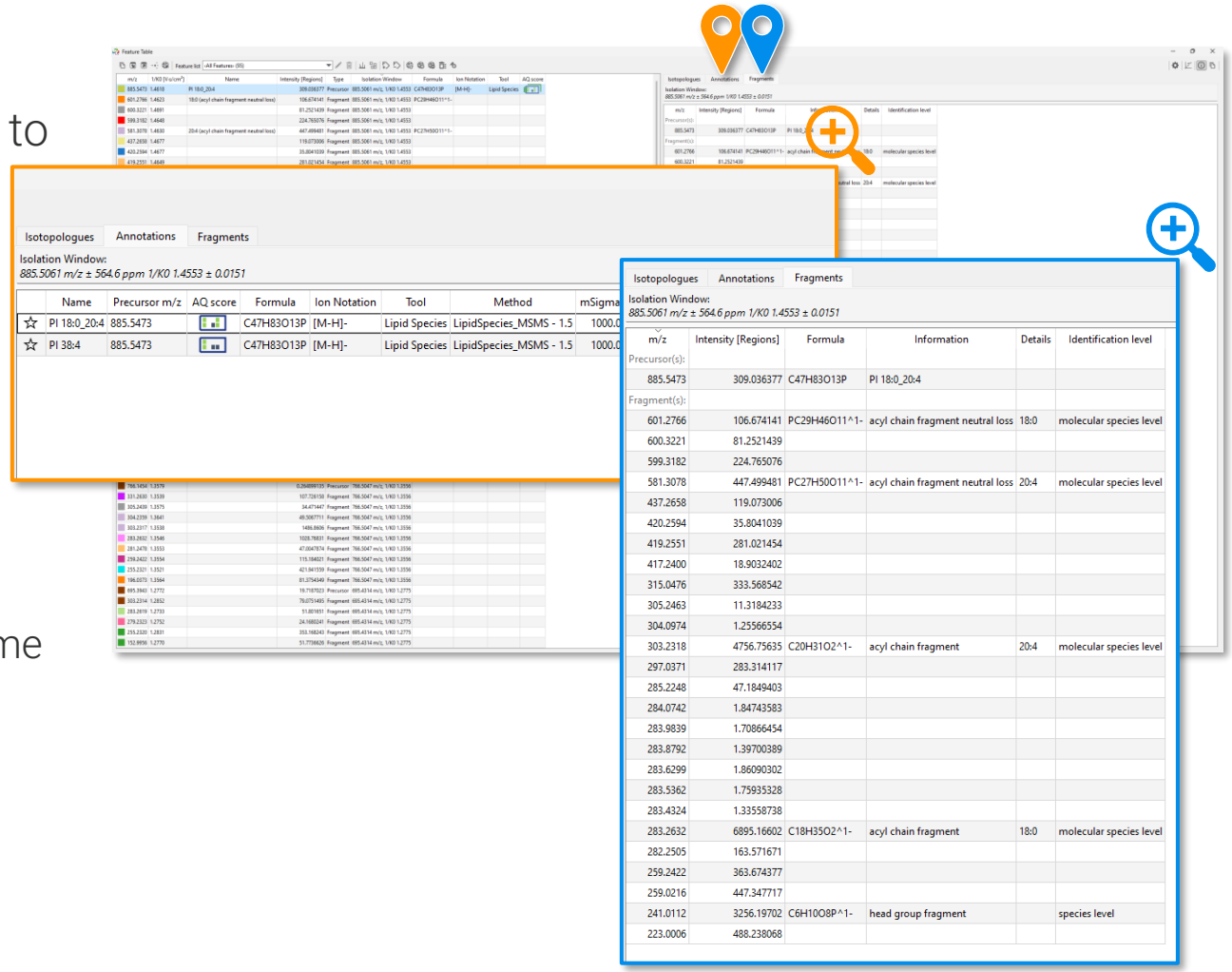
MetaboScape annotation in SCiLS Lab

iprm-PASEF

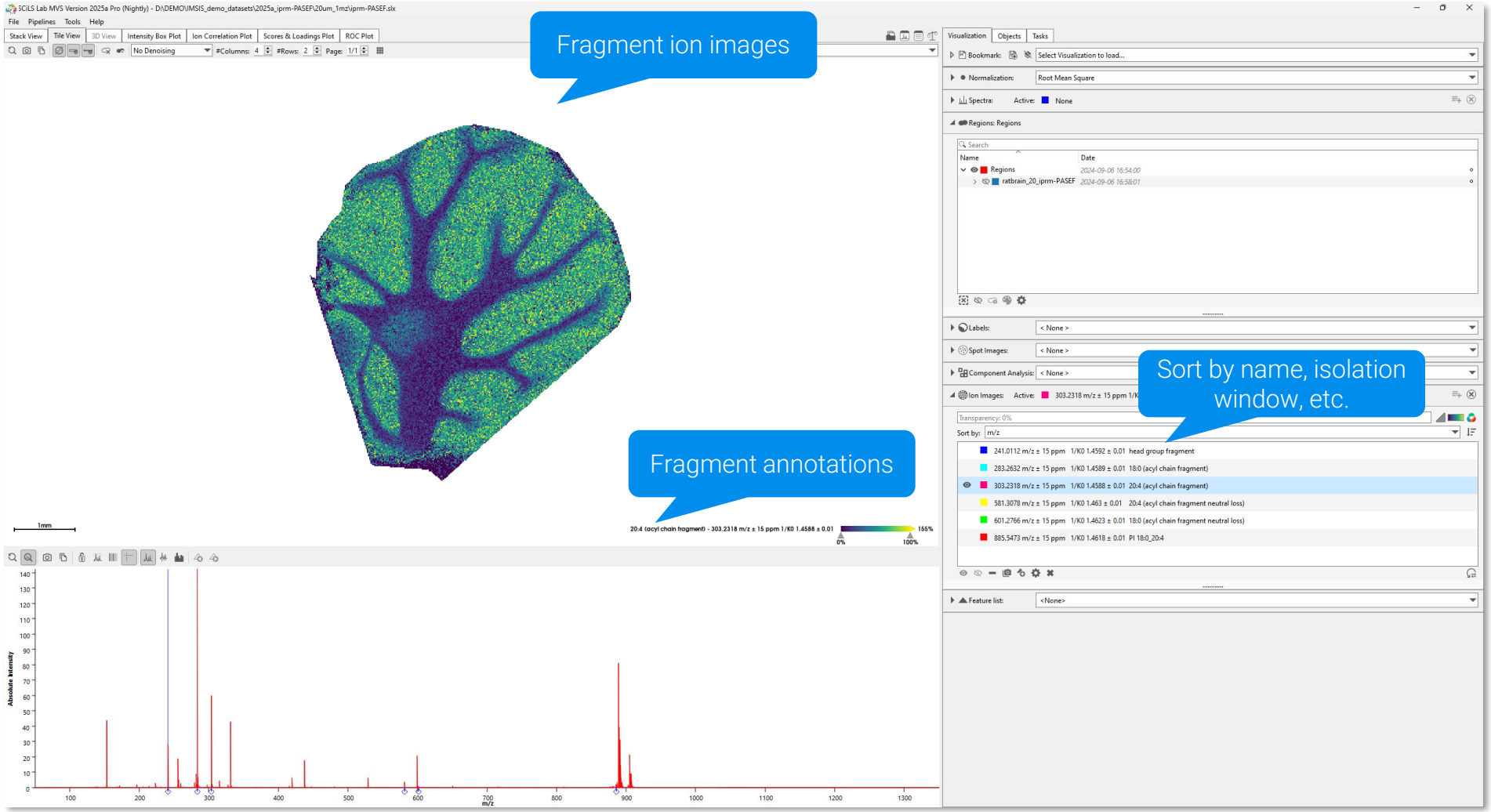
MetaboScape-powered molecular annotation for iprm-PASEF

- **Annotations sub-panel** of the detailed feature information displays all annotations associated to the Isolation Window of the active feature.
  - Annotations are sorted by AQ score
  - The highest-ranking annotation is active by default
  - Only one active annotation per isolation window

- Fragments sub-panel of the detailed feature information shows the annotations of the fragments that "explain" the active annotation
  - Fragment annotations are also added to the Name column of the Feature Table



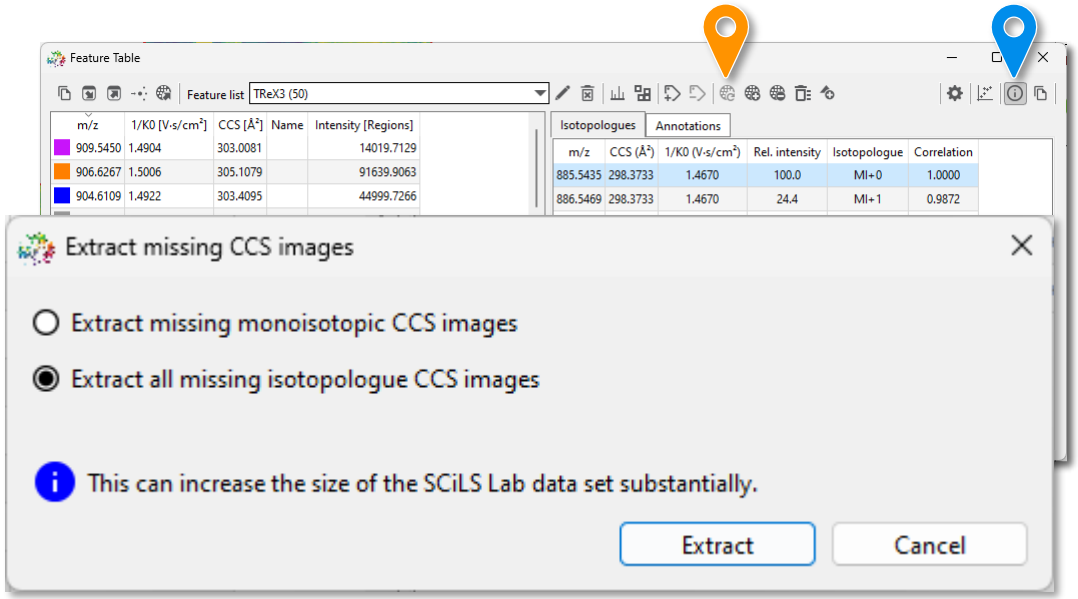
iprm-PASEF
Display fragment ion images



03
T-ReX® feature finding

T-ReX feature finding
Display T-ReX® isotopologues in SCiLS™ Lab

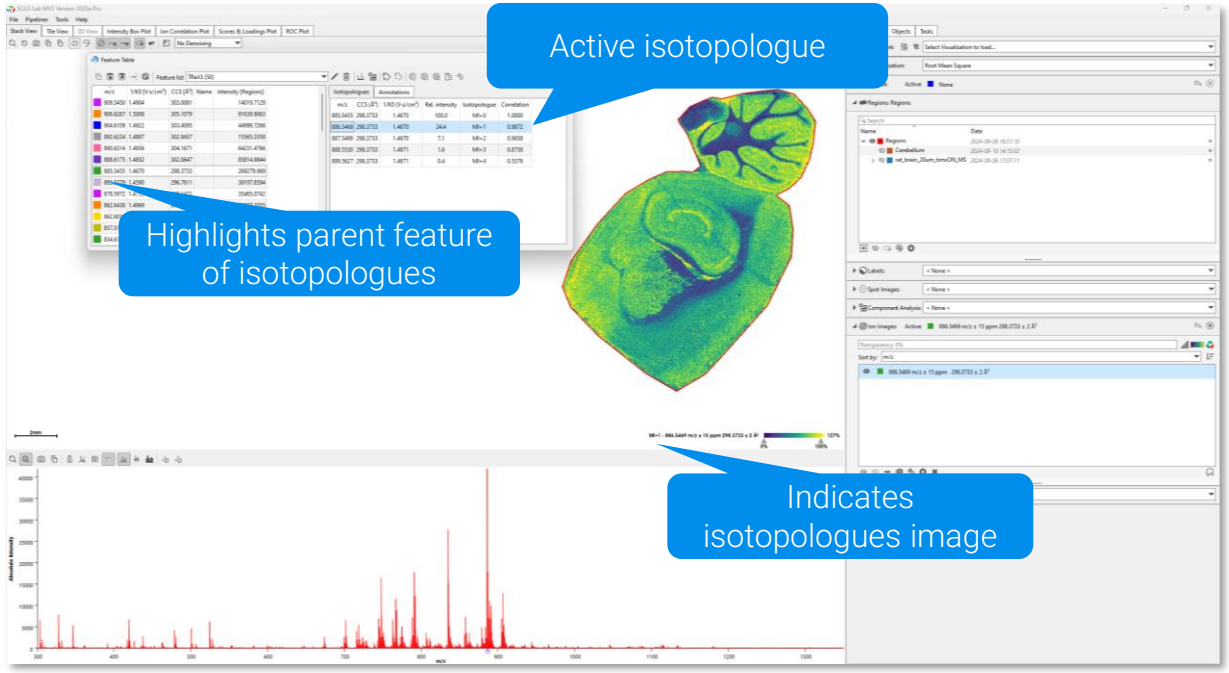
- T-ReX feature finding algorithm performs **deisotoping**, and stores the isotopologue details with its features
- The **isotopologues** sub-panel of the detailed feature information shows the isotopologues detected by T-ReX feature finding
- For CCS-enabled images, isotopologues ion images can be extracted separately
- Relative isotopologue intensities are extracted by the T-ReX feature finding algorithm.
- Correlation column shows Pearson correlation between the isotopologue and the monoisotope



T-ReX feature finding

Display T-ReX® isotopologues in SCiLS™ Lab

- Isotopologue features can be added to the Ion Image list and shown in various plots:
  - Correlation plots
  - Correlation table
  - Box plots
  - ROC plots

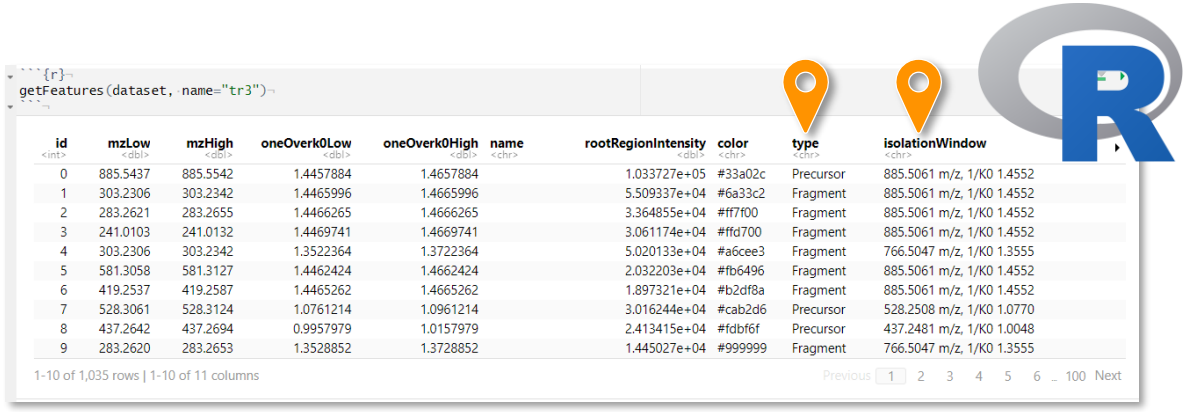


04

SCiLS API improvements

SCiLS API improvements

- Added support for R 4.4, binary package for R 4.2 removed
- **Normalizations** can now be called via **Name,** rather than their uuid's
- For **iprm-PASEF** data sets, the Fragment and Precursor types, as well as their relation to the Isolation Windows is maintained, and shown in the Feature table.
  - This allows **easy reconstruction of MS/MS spectra** for auxiliary database searches.



```{r}
getFeatures(dataset, name="tr3")
```

id <int>	mzLow <dbl>	mzHigh <dbl>	oneOverk0Low <dbl>	oneOverk0High <dbl>	name <chr>	rootRegionIntensity <dbl>	color <chr>	type <chr>	isolationWindow <chr>
0	885.5437	885.5542	1.4457884	1.4657884		1.033727e+05	#33a02c	Precursor	885.5061 m/z, 1/K0 1.4552
1	303.2306	303.2342	1.4465996	1.4665996		5.509337e+04	#6a33c2	Fragment	885.5061 m/z, 1/K0 1.4552
2	283.2621	283.2655	1.4466265	1.4666265		3.364855e+04	#ff7f00	Fragment	885.5061 m/z, 1/K0 1.4552
3	241.0103	241.0132	1.4469741	1.4669741		3.061174e+04	#ffd700	Fragment	885.5061 m/z, 1/K0 1.4552
4	303.2306	303.2342	1.3522364	1.3722364		5.020133e+04	#a6cee3	Fragment	766.5047 m/z, 1/K0 1.3555
5	581.3058	581.3127	1.4462424	1.4662424		2.032203e+04	#fb6496	Fragment	885.5061 m/z, 1/K0 1.4552
6	419.2537	419.2587	1.4465262	1.4665262		1.897321e+04	#b2df8a	Fragment	885.5061 m/z, 1/K0 1.4552
7	528.3061	528.3124	1.0761214	1.0961214		3.016244e+04	#cab2d6	Precursor	528.2508 m/z, 1/K0 1.0770
8	437.2642	437.2694	0.9957979	1.0157979		2.413415e+04	#fdbf6f	Precursor	437.2481 m/z, 1/K0 1.0048
9	283.2620	283.2653	1.3528852	1.3728852		1.445027e+04	#999999	Fragment	766.5047 m/z, 1/K0 1.3555

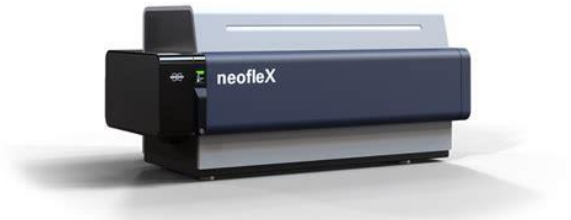
1-10 of 1,035 rows | 1-10 of 11 columns

Previous 1 2 3 4 5 6 … 100 Next

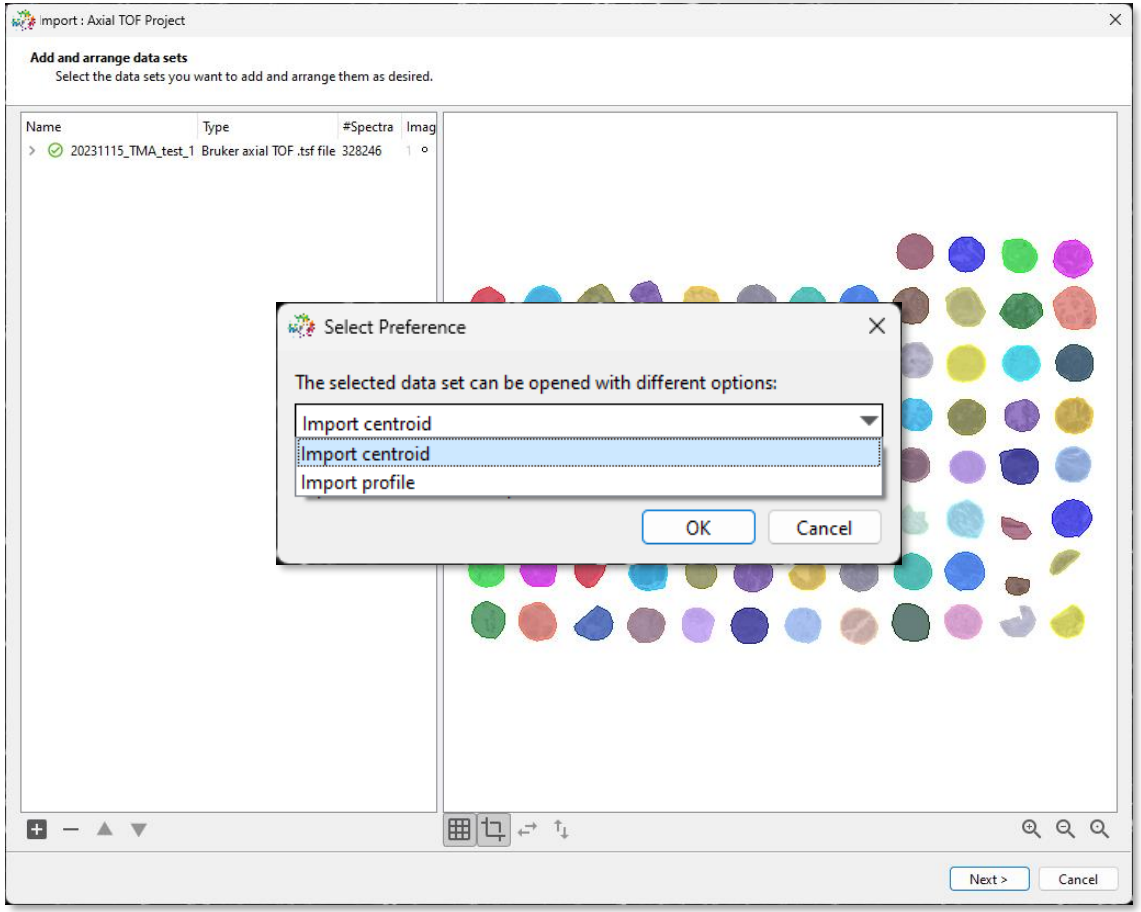
05

neofleX support

neofleX support
Importing neofleX data into SCiLS™ Lab



- SCiLS Lab 2025a can read .d/tsf data acquired by the all-new **neofleX** axial TOF instrument
- Import **centroid** or **profile** neofleX data into a SCiLS Lab “Axial TOF project”
- neofleX profile data can be combined with rapifleX profile data (.d container format)
- Combining centroid and profile axial TOF data is not supported
- T-ReX® Feature Finding is not enabled for neofleX data sets

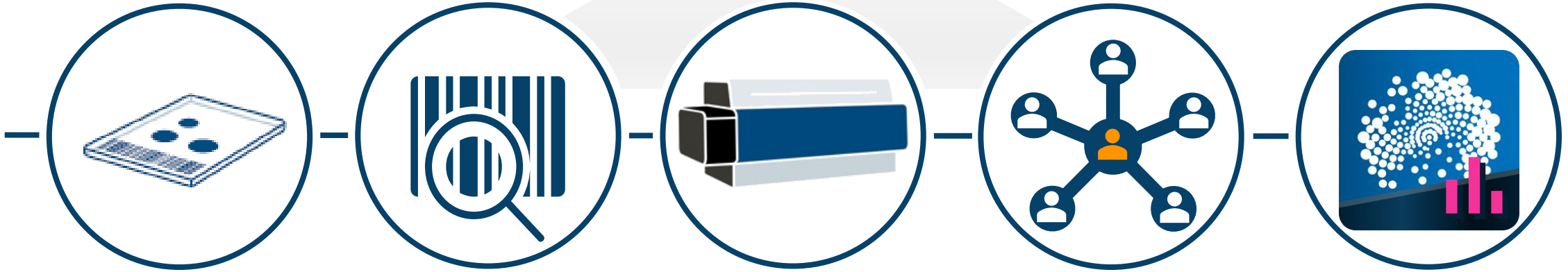


neofleX support

SCiLS™ autopilot – smoothly from sample to images

Powered by

SCiLS **autopilot**



Automated acquisition setup for neofleX, timsTOF fleX & rapifleX

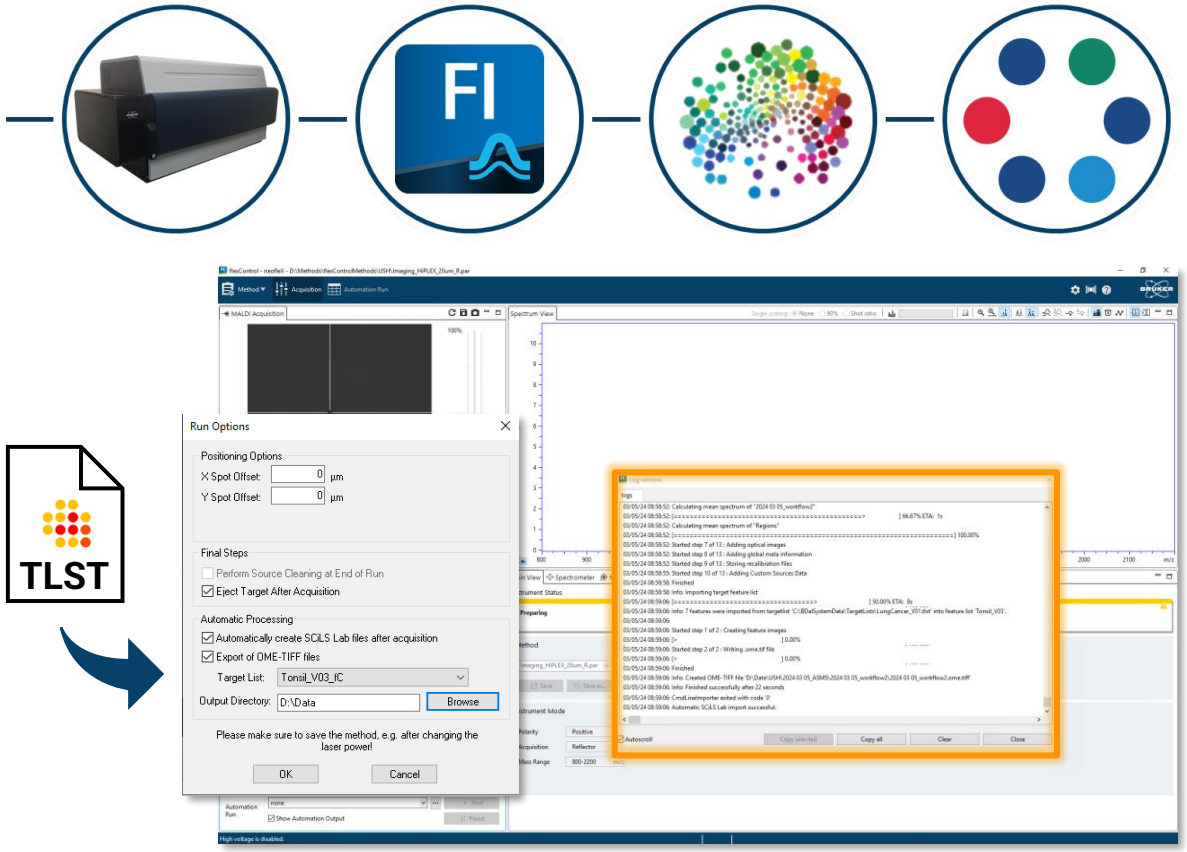
Automated SCiLS™ Lab data import and pre-processing

Automated image extraction and OME-TIFF export

OME-TIFF

Multiple feature enhancements
Auto-convert to OME-TIFF

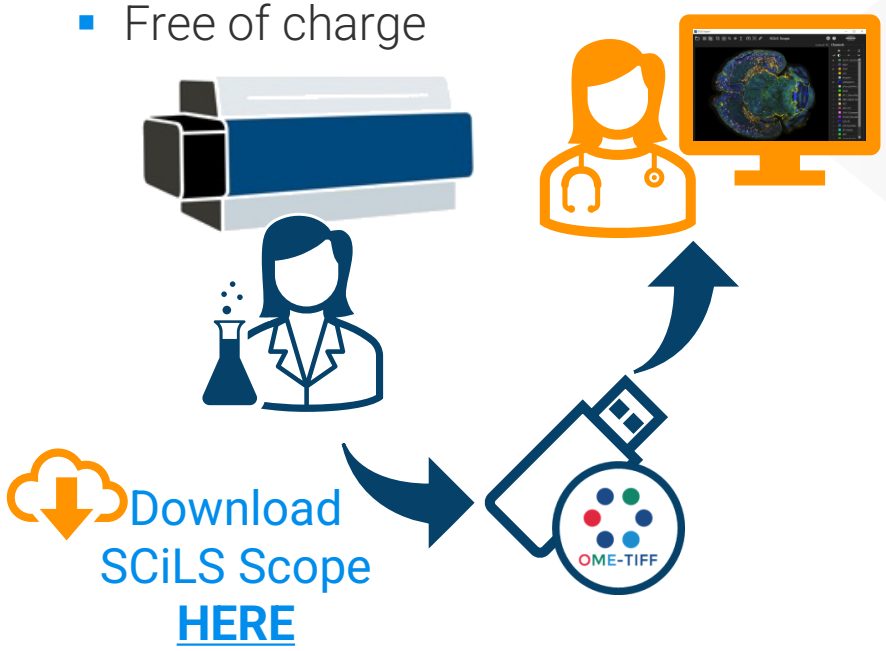
- SCiLS autopilot can now automatically generate a SCiLS Lab file, and export the features specified in a Target List (.tlst) file to OME-TIFF format
- Acquisition PC needs access to an active SCiLS Lab license (no seat consumed), or the SCiLS autopilot converter license
- Requires the following software versions:
  - flexControl 5.0
  - flexImaging 7.5
  - SCiLS Lab 2024b

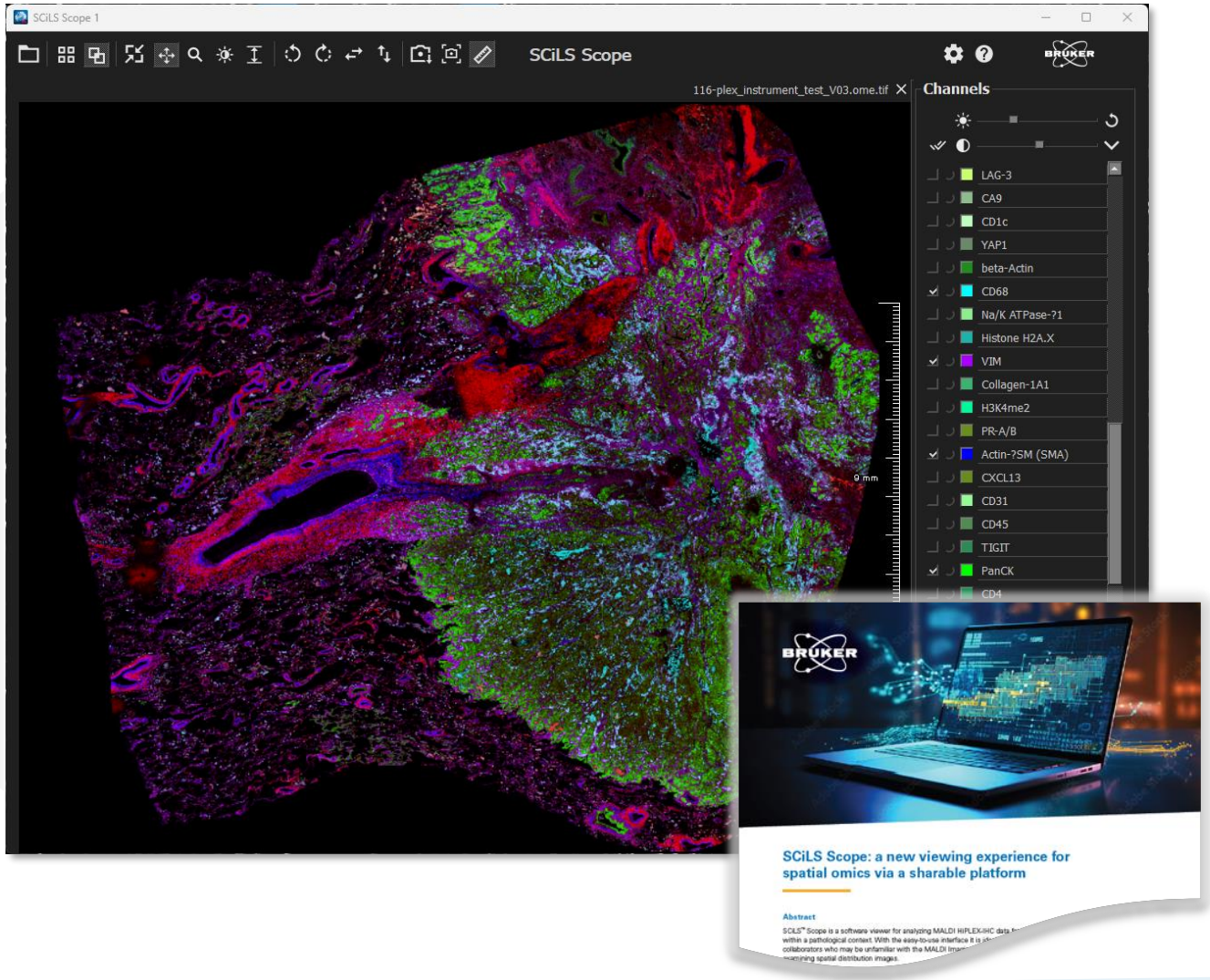


SCiLS™ Scope – access images in an instant

Easy-to-use viewer for MALDI HiPLEX-IHC images

- Share results efficiently in OME-TIFF format
- Select and adjust your images of interest
- Measure distances between relevant structures
- Free of charge

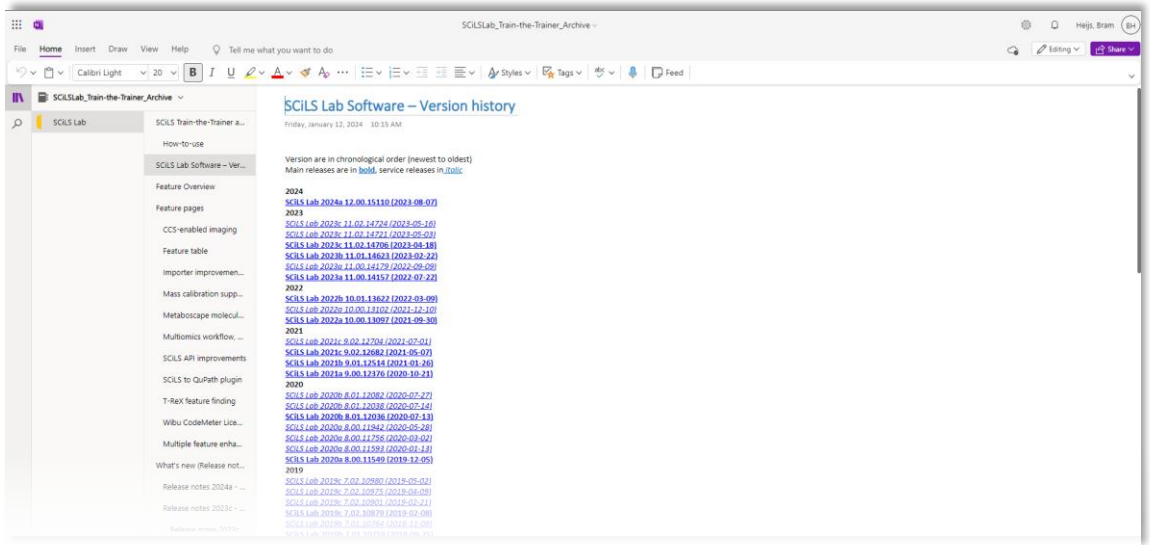




SCiLS™ Lab Train-the-trainer Archive

SCiLS™ Lab Train-the-trainer Archive

- What does it contain?
  - SCiLS Lab change log
  - Feature development history
  - Links to available Train-the-Trainer slides
- What's it for?
  - Knowledge transfer
  - Consolidating SCiLS Lab training material
  - Easy access to SCiLS Lab training material
- Where to find it?
  - MS TEAMS > TEAMS > IMM > SCiLSLab
    - Files > Release Notes & What's New
      - SCiLSLab_Train-the-Trainer_Archive



Thank you!

Bram Heijs, PhD
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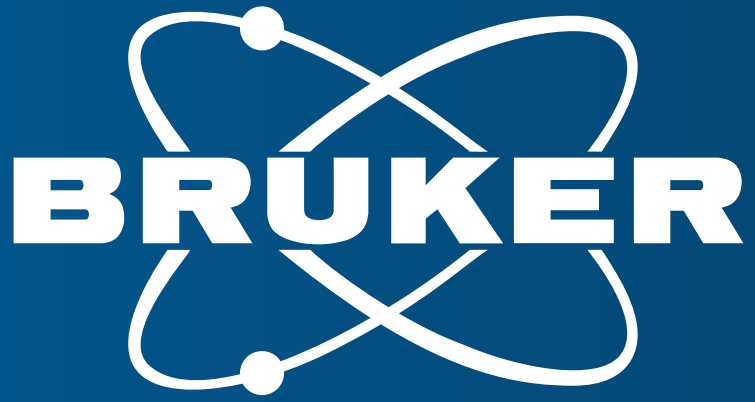
bram.heijs@bruker.com



Innovation with Integrity