

LIFE SCIENCE MASS SPECTROMETRY

SCiLS™ Lab 2024b – What's New?



SCiLS

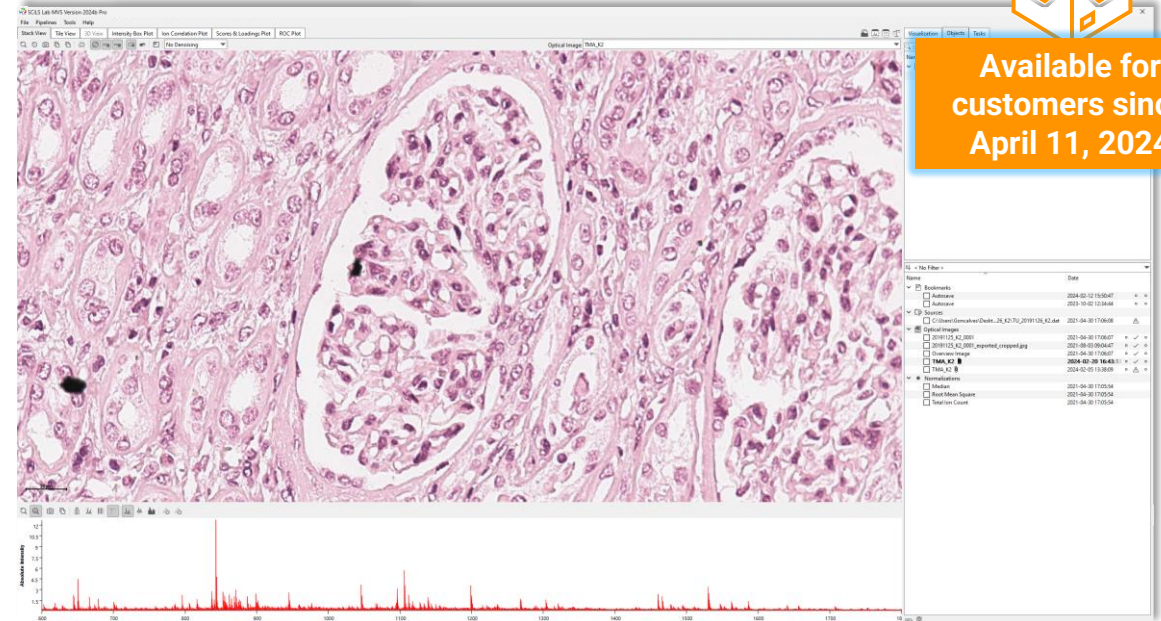


SCiLS™ Lab 2024b – What's New?



Available for
customers since
April 11, 2024

- 01 *High-resolution optical images:*
Microscope images at full resolution
- 02 *Optical image co-registration:*
Improved image co-registration workflow
- 03 *T-ReX Feature finding:*
Feature finding during manual data import
- 04 *Multiple other feature enhancements:*
Various other improvements and changes

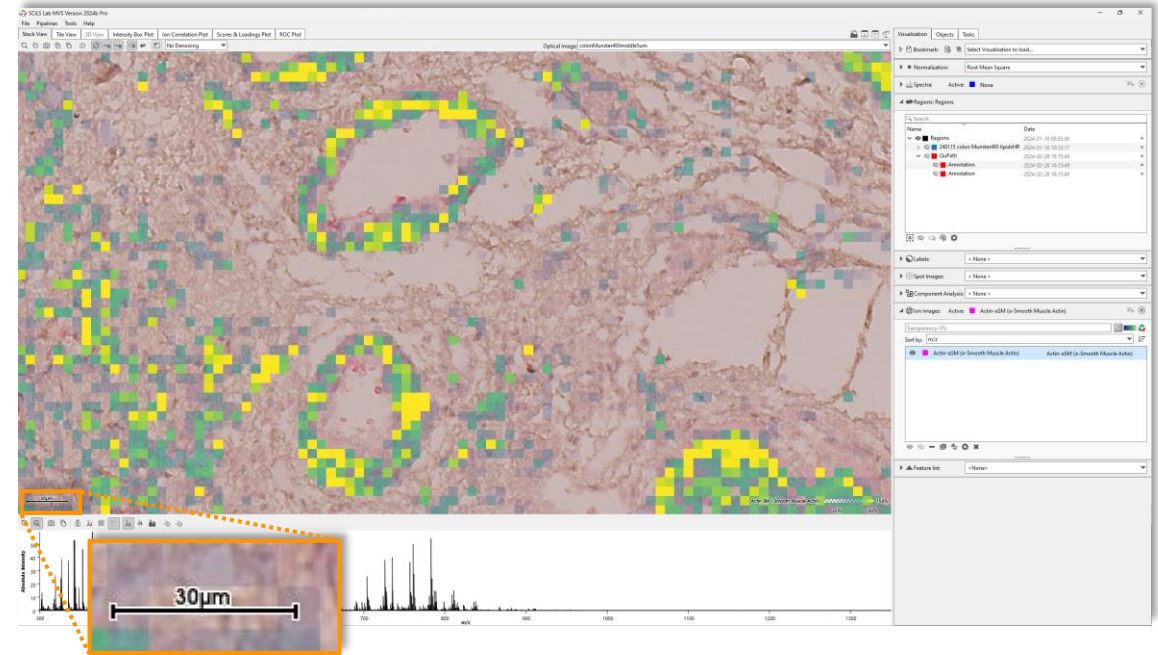


01

High-resolution optical images

High-resolution optical images: High-resolution optical images in SCiLS™ Lab Stack View

- SCiLS Lab supports rendering optical images in Stack View at **full resolution**
- **Import** high-resolution optical images using
 - SCiLS Lab optical image import
 - QuPath-to-SCiLS export plugin
 - SCiLS Exchange Format (SEF) files
 - SCiLS API
- Whole-slide *brightfield* imaging data in file formats supported by the **OpenSlide** library are compatible
 - **NOTE:** *whole-slide fluorescence data is not supported*



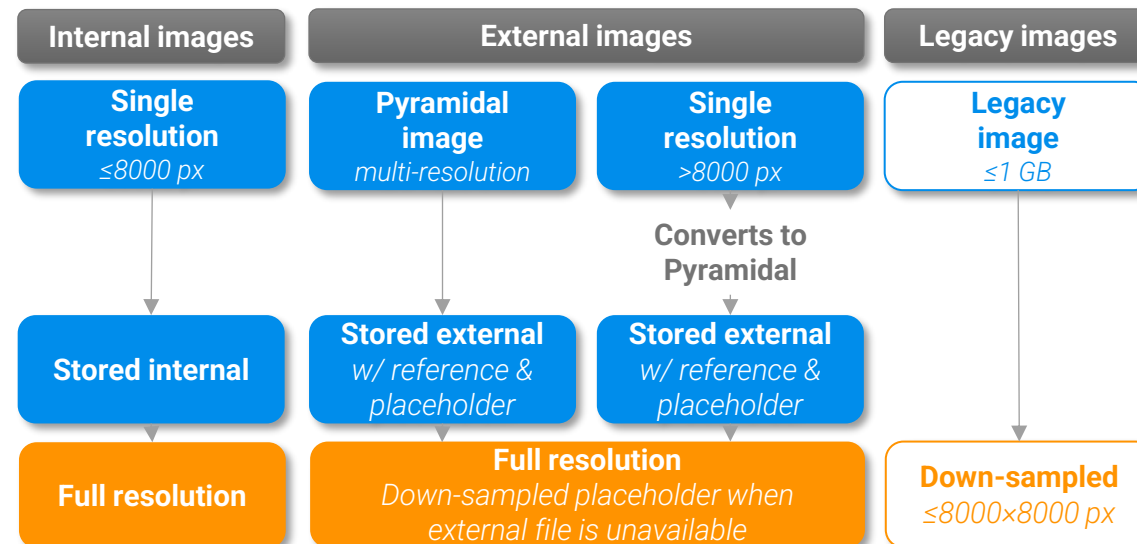
Data formats supported by the OpenSlide library

Data format / vendor	Extensions	Data format / vendor	Extensions
Aperio	.svs, .tif	Philips	.tiff
DICOM	.dcm	Sakura	.svslide
Hamamatsu	.vms, .vmu, .ndpi	Trestle	.tif
Leica	.scn	Ventana	.bif, .tif
MIRAX	.mrxs	Generic tiled TIFF	.tif



High-resolution optical images: Optical image storage in SCiLS™ Lab

- Optical image handling has fundamentally changed in [SCiLS Lab 2024b](#)
- Previously, optical images were
 - Stored in the .slx file
 - Maximum file size of 1 GB
 - Down-sampled to max. 8000×8000 pixels
 - Rendered as down-sampled image in Stack View
- Now, data sets can contain
 - Legacy images
 - Internal images
 - External images

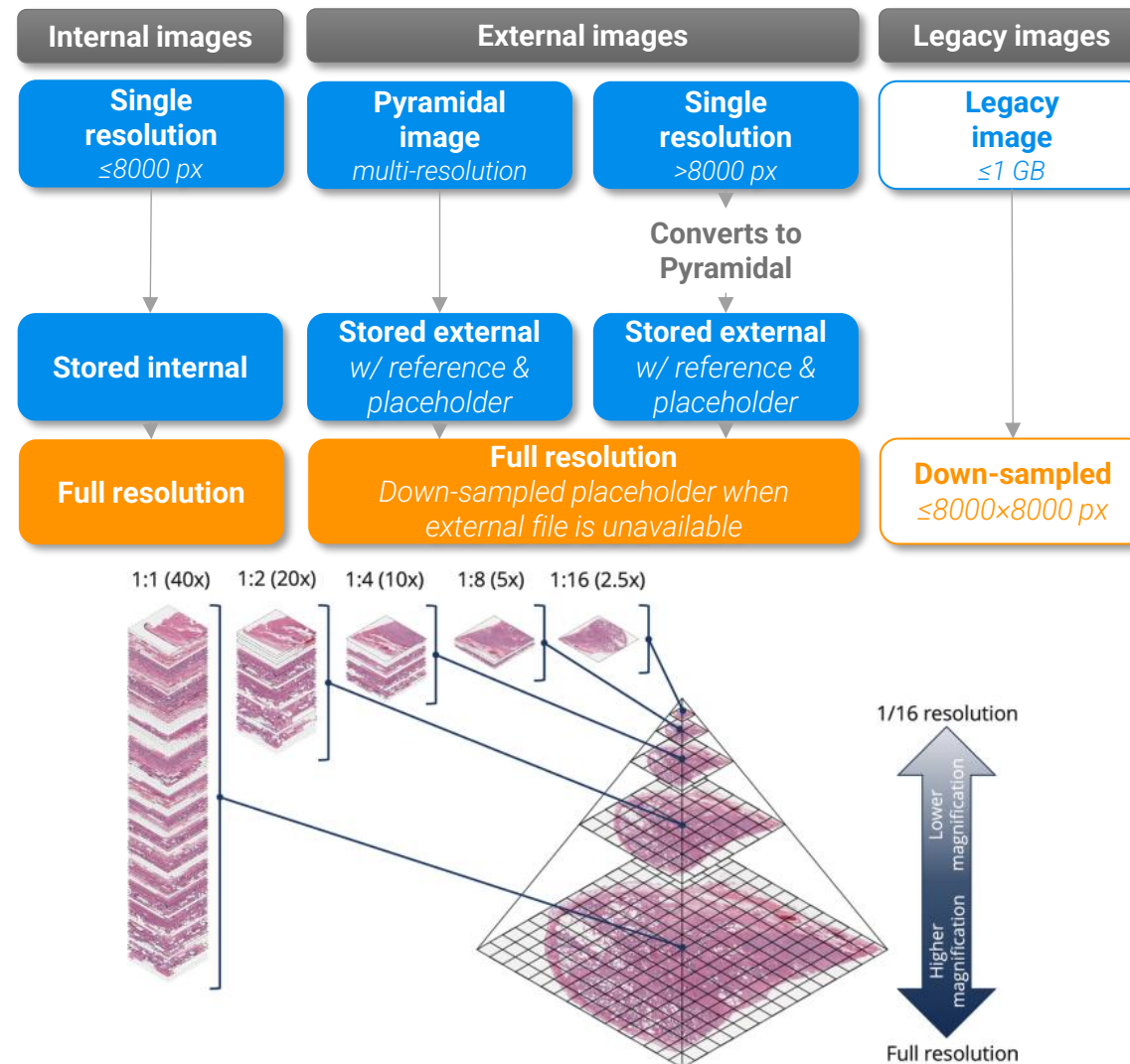


Visualization			Objects	Tasks
Search				
Name	Date			
Regions	2021-04-30 17:04:16			o o
TMA-dataset	2021-04-30 17:05:54			o o o
No Filter				
Name	Date			
Bookmarks				
Autosave	2023-10-02 12:34:44			o o
Autosave	2024-02-05 13:55:10			o o
Sources				
C:\Users\Goncalves\Desktop\TU_20191126_K2\TU_20191126_K2.dat	2021-04-30 17:06:08			▲
Optical Images				
20191125_K2_0001	2021-04-30 17:06:07			o ✓ o
20191125_K2_0001_exported_cropped.jpg	2021-08-03 09:04:47			o ✓ o
Overview Image	2021-04-30 17:06:07			o ✓ o
TMA_K2	2024-02-05 13:38:09			o ✓ o
Normalizations				



High-resolution optical images: Optical image storage in SCiLS™ Lab

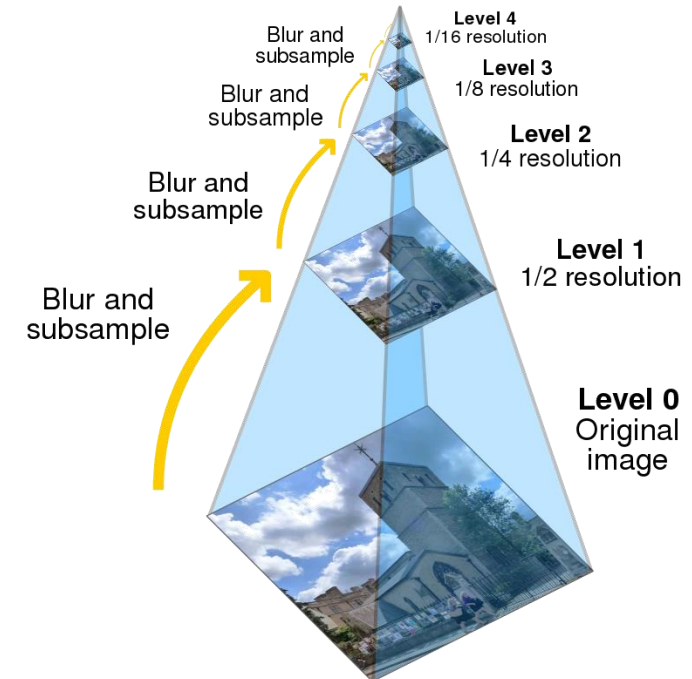
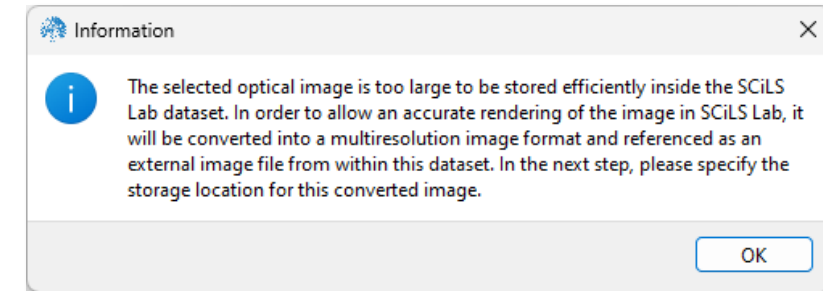
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 - Stored in the .slx file
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 - Rendered as down-sampled image in Stack View
- Now, data sets can contain
 - Legacy images
 - Internal images
 - External images
- Objects tab** displays optical image information
 - Internal/Legacy or matching image found at path: ✓
 - Path inaccessible or non-matching image found: ⚠





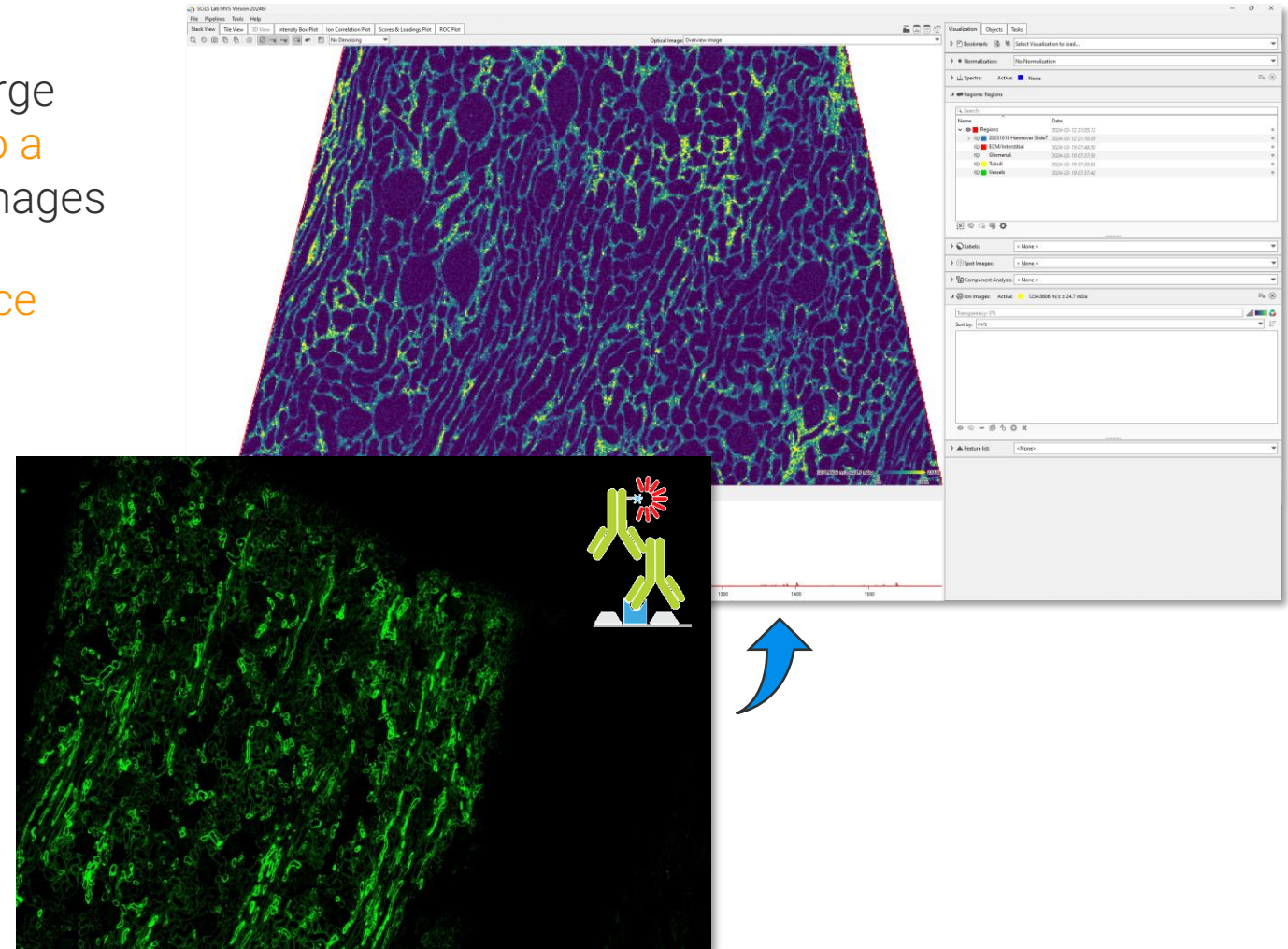
High-resolution optical images: Optical image storage in SCiLS™ Lab

- To facilitate high-resolution rendering for large “flat” images, these images are converted to a **pyramidal format** and treated as External images



High-resolution optical images: Optical image storage in SCiLS™ Lab

- To facilitate high-resolution rendering for large “flat” images, these images are **converted to a pyramidal format** and treated as External images
- Allows importing pre-processed **fluorescence microscopy** images into SCiLS Lab



High-resolution optical images: Updating external image locations in the Objects tab

- In case external image files are **re-located** the external file references will need updating
 - Warning** upon opening SCiLS Lab data set
 - High-resolution rendering unavailable for affected external images, **placeholder** image is shown
- Objects tab** displays optical image information
 - Internal/Legacy or matching image found at path: ✓
 - Path inaccessible or non-matching image found: ⚠
- The “**Edit**” properties dialog can be used to:
 - Update** path to external image
 - Remove** the external file reference
 - External image files unaffected
 - Placeholder image unaffected
 - Cannot be undone

The screenshot displays the SCiLS Lab software interface. At the top, a red warning banner reads: "High resolution visualization not available, please check and update the path in the Objects tab." Below this, a "Warning" dialog box is open, stating: "1 optical image(s) referenced by this dataset cannot be found at their expected external location(s). Check the Optical Images list in the Objects tab to update the external reference(s). Don't show again for this dataset". In the background, the "Objects" tab is visible, showing a table of optical images with columns for Name and Date. The "Edit" dialog box is also open, showing the "Name" field set to "TMA_K2" and the "Path" field set to "D:/Datasets/Nightly/Train-t...TMA_K2/TMA_K2-multires.tiff". The "Information" section of the dialog shows: "Produced by: SCiLS Lab Nightly Version 12.01.15659.1" and "Date: 2024-02-05 13:38:09".

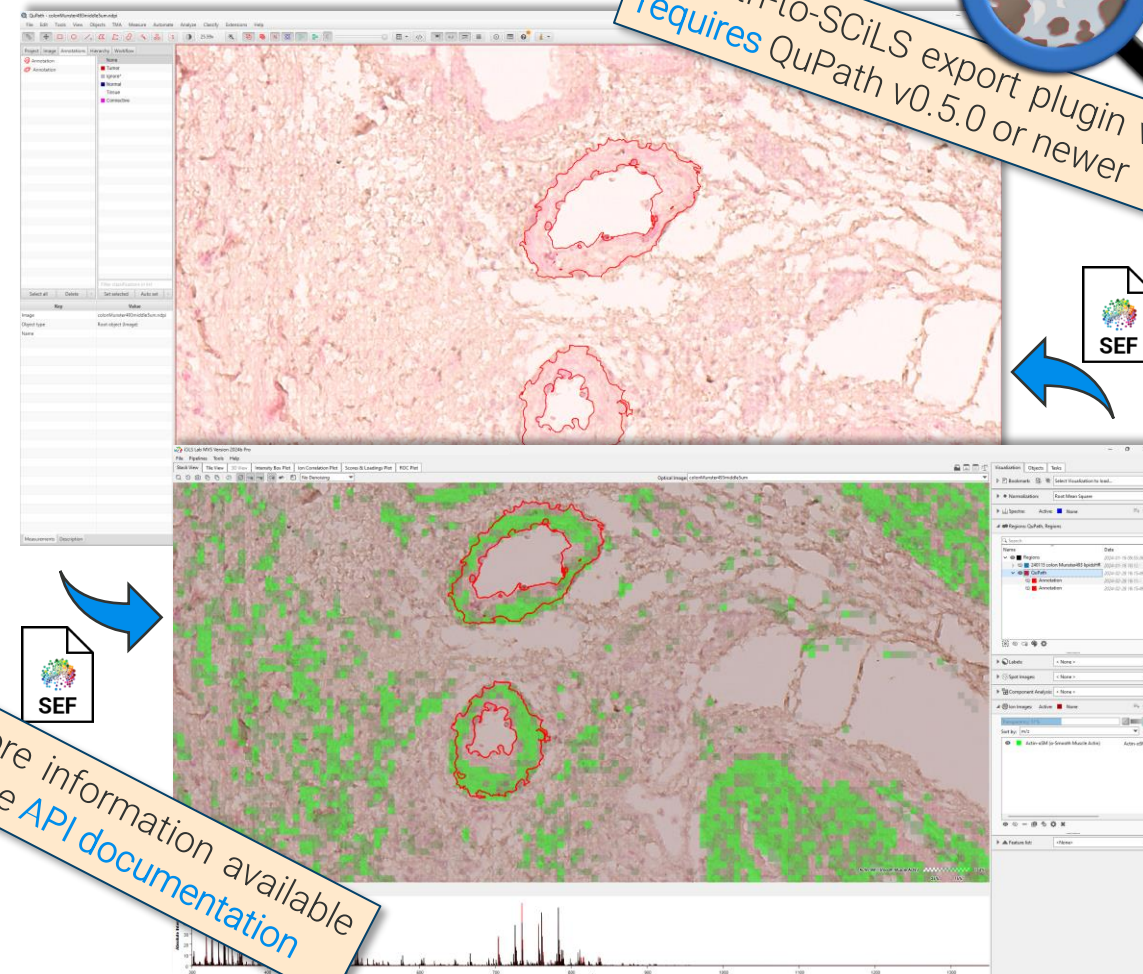


SCiLS



High-resolution optical images: Improved integration between SCiLS™ Lab and QuPath

- The **QuPath-to-SCiLS export plugin** facilitates the transport of region annotations between SCiLS Lab and QuPath
- Plugin v1.4 transports **external optical image references** between SCiLS Lab and QuPath
- SCiLS Lab and QuPath access the same external optical image to **avoid data duplication**
- A reference to an external optical image can be added to the **updated SCiLS Exchange Format**

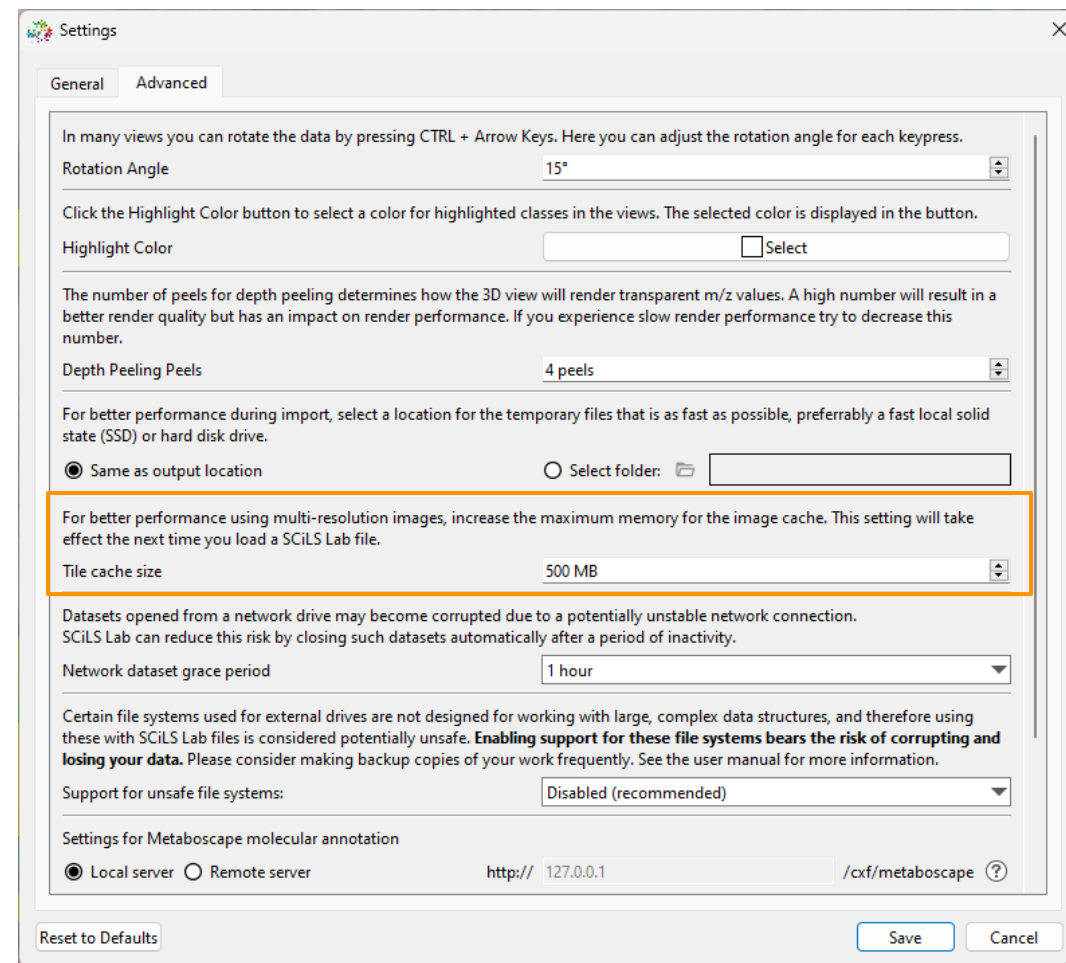


QuPath-to-SCiLS export plugin v1.4
requires QuPath v0.5.0 or newer

More information available
in the **API documentation**

High-resolution optical images:: SCI LS™ Lab performance high-resolution optical image rendering

- Whole-slide imaging data is read from disk and is not fully loaded into memory. When navigating through an image, the image tiles that were “visited” are temporarily stored, or cached, in memory to revisit them more efficiently later
- To enhance the user experience and improve the performance of viewing high resolution optical images in SCI LS Lab, the **Tile Cache Size** can be increased on the **Advanced** tab of **File>SCI LS Lab Settings**

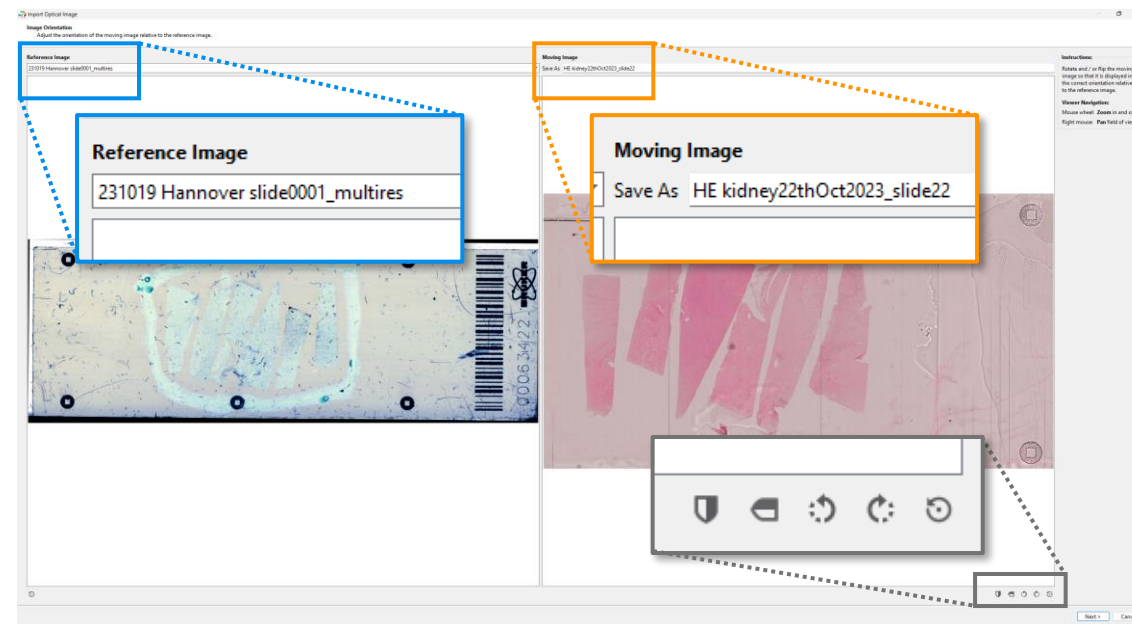


02

Optical image co-registration

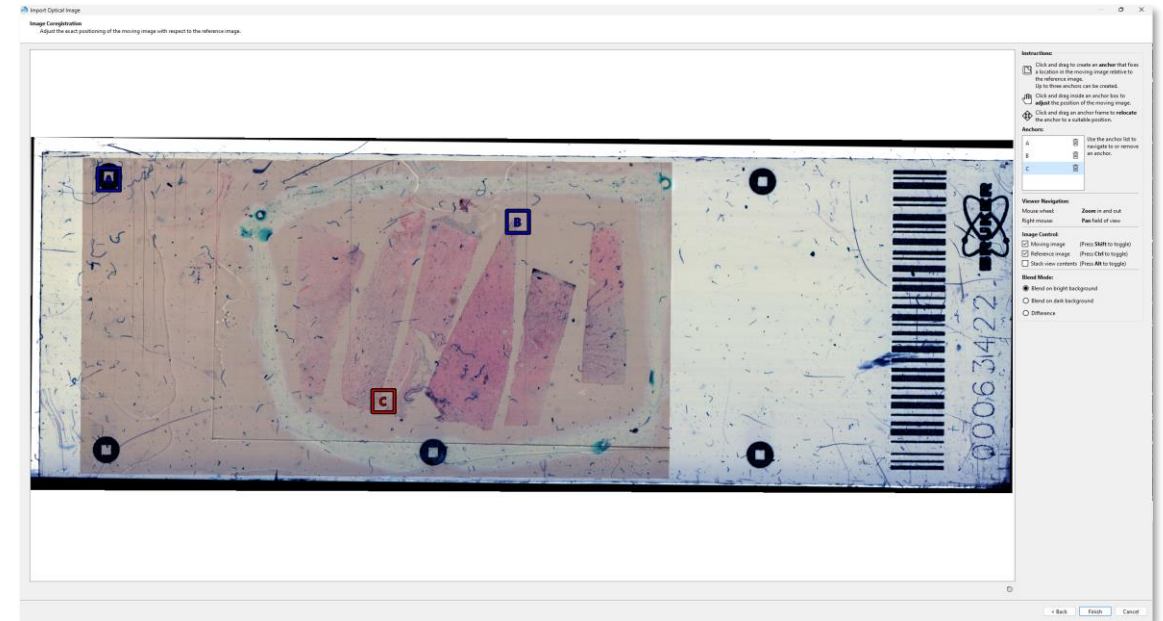
Optical image co-registration: Improved image co-registration workflow

- To accommodate working with high-resolution optical images, SCiLS™ Lab has a new **two-step** and **interactive** image **co-registration** module
- **Access** the new image registration module via **File > Import > Optical Image (Co-Register new image)**
- **Select** the image you want to co-register to the MALDI Imaging data, this is the **Moving image**
- In the registration wizard, select the **Reference image**, which is already present in the data set
- Use the **Image Orientation** step to match the orientation of the moving image to the reference image



Optical image co-registration: Improved image co-registration workflow

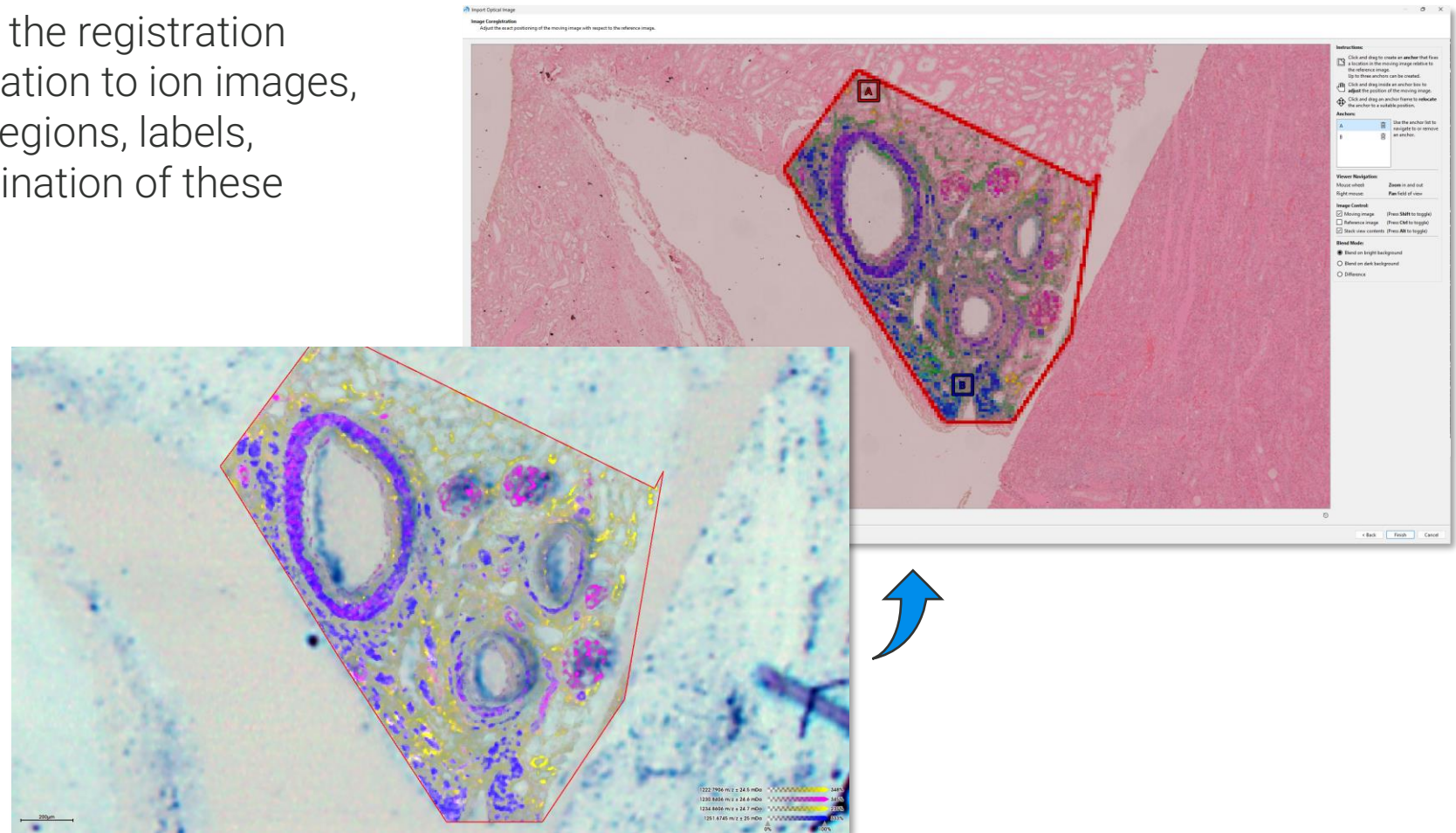
- Following Image Orientation, continue to the **Image Co-registration**, which is based on **anchors** to adjust the moving image
 - 1 anchor – adjust position
 - 2 anchors – adjust rotation and scaling
 - 3 anchors – compensate for shearing artefacts
- The detailed registration is **interactive** and immediately displays the result of the input actions
- Anchor operations
 - Create by clicking in the registration window
 - Move to adjust the moving image position
 - Relocate (click on the teachbox border and drag)
 - Delete to undo image transformation





Optical image co-registration: Improved image co-registration workflow

- **Display Stack View content** in the registration window to allow direct registration to ion images, component analysis results, regions, labels, external images, or any combination of these

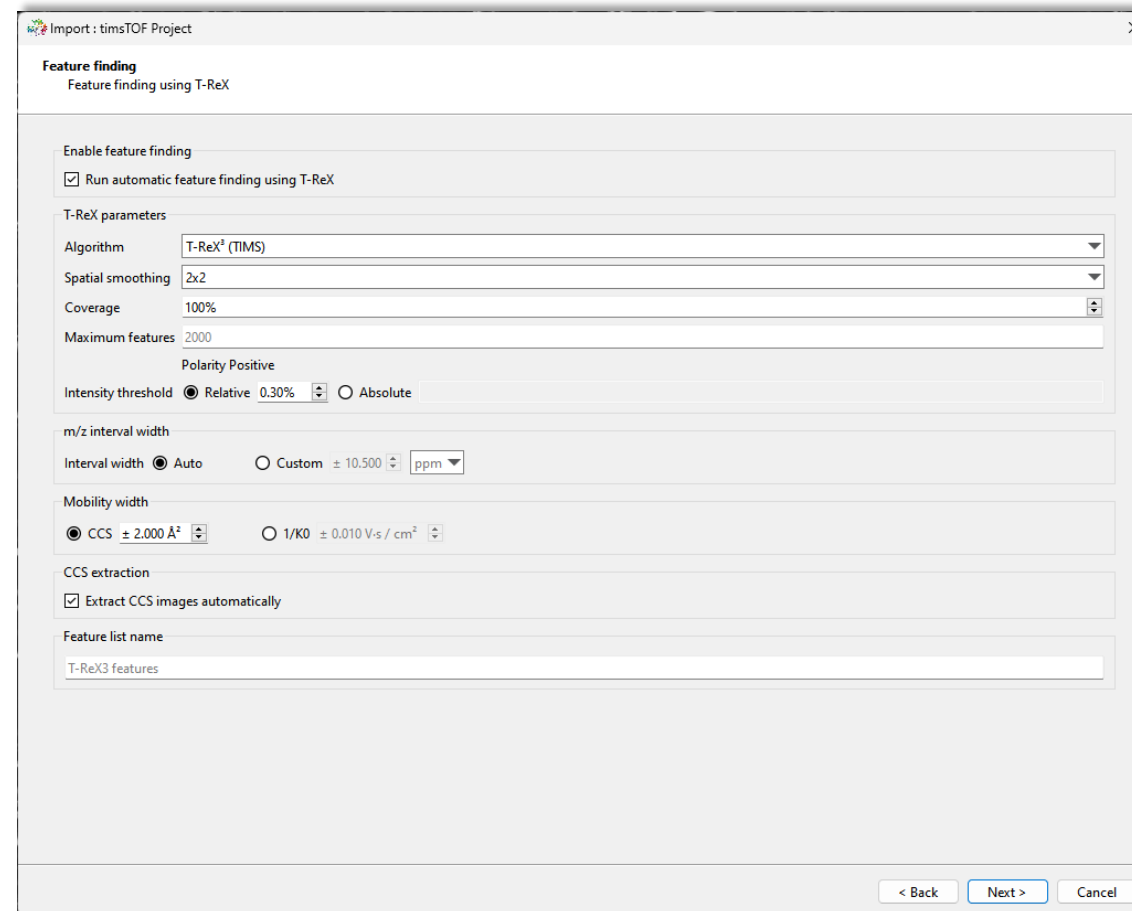


03

T-ReX[®] Feature Finding

T-ReX[®] Feature Finding: Feature Finding upon manual import of data

- During the **manual import** of timsTOF fleX and/or centroided MRMS data into SCiLS[™] Lab, **T-ReX Feature Finding** can be enabled to run during the import procedure
 - T-ReX² for MRMS and QTOF data
 - T-ReX³ for TIMS data
- For CCS-enabled timsTOF fleX data, the **CCS extraction** can also be enabled to run during data import
- Parameters and results are identical to operating the Feature Finding tool in SCiLS Lab




The screenshot shows a software dialog box titled 'Import: timsTOF Project'. Inside, there is a section for 'Feature finding' with the subtitle 'Feature finding using T-ReX'. The settings are as follows: 'Enable feature finding' is checked with 'Run automatic feature finding using T-ReX'. Under 'T-ReX parameters', the 'Algorithm' is set to 'T-ReX³ (TIMS)', 'Spatial smoothing' is '2x2', 'Coverage' is '100%', and 'Maximum features' is '2000'. The 'Polarity' is set to 'Positive', and the 'Intensity threshold' is 'Relative 0.30%'. For 'm/z interval width', 'Interval width' is 'Auto'. For 'Mobility width', 'CCS ± 2.000 Å²' is selected. Under 'CCS extraction', 'Extract CCS images automatically' is checked. The 'Feature list name' is 'T-ReX3 features'. At the bottom right, there are buttons for '< Back', 'Next >', and 'Cancel'.

04

Multiple feature enhancements

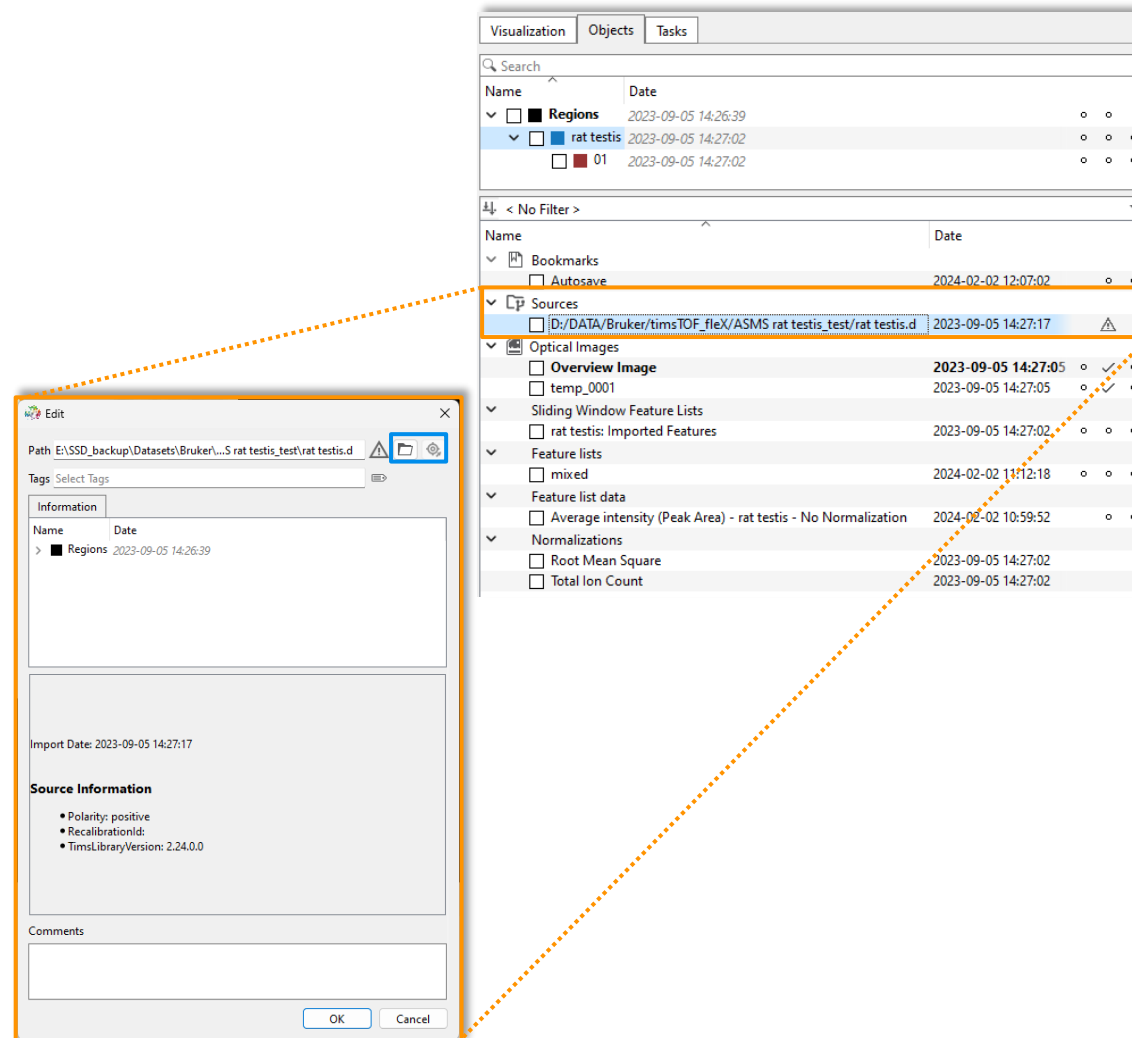
Multiple feature enhancements: Changed mouse & keyboard interactions in Stack View

- The **mouse and keyboard interactions** to navigate in Stack View have been updated

Move mouse	 Left button	 Right button	 Middle/3 rd button
No key	Select regions	Pan the image	Pan the image
SHIFT	Select regions	Rotate the image (Stack View)	Pan the image
CTRL	Toggle region selection	Pan the image	Pan the image
ALT	Intersect selected regions	Pan the image	Pan the image

Multiple feature enhancements: Improvements of the Objects tab functionality

- Display of **data sources** has been moved from File Properties to **Objects tab**
- Accessibility of source data is shown in Objects tab
 - Accessible: ✓
 - Inaccessible: ⚠
- **Update path** to the source data set via the “Edit” properties
- For timsTOF fleX data, a change in the active calibration of the source data is shown: ⓘ
- The **calibration file** active at the time of importing the source data into SCiLS Lab can be **exported**

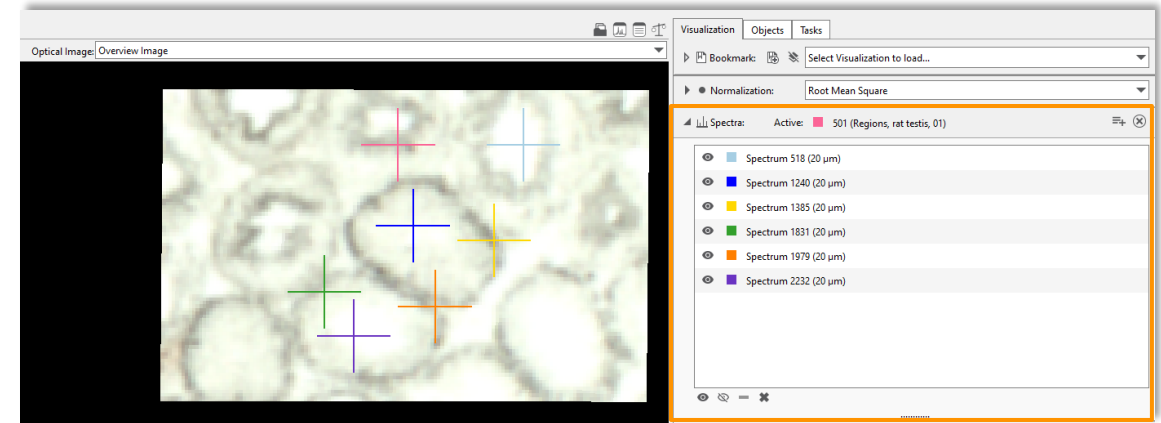


The screenshot displays the SCiLS Lab interface with the 'Objects' tab selected. The main window shows a list of objects with columns for Name and Date. A specific object, 'rat testis', is expanded to show its 'Sources' section, which includes a file path: 'I:\D:\DATA\Bruker\timsTOF_fleX\ASMS rat testis_test\rat testis.d'. An orange box highlights this path. Below the main window, an 'Edit' dialog box is open, showing the same file path in the 'Path' field. The dialog also displays 'Information' (Name, Date), 'Import Date' (2023-09-05 14:27:17), and 'Source Information' (Polarity: positive, RecalibrationId, TimsLibraryVersion: 2.24.0.0). An orange box highlights the 'Edit' dialog, and a dotted orange line connects the highlighted path in the dialog to the highlighted path in the main window's 'Sources' list.





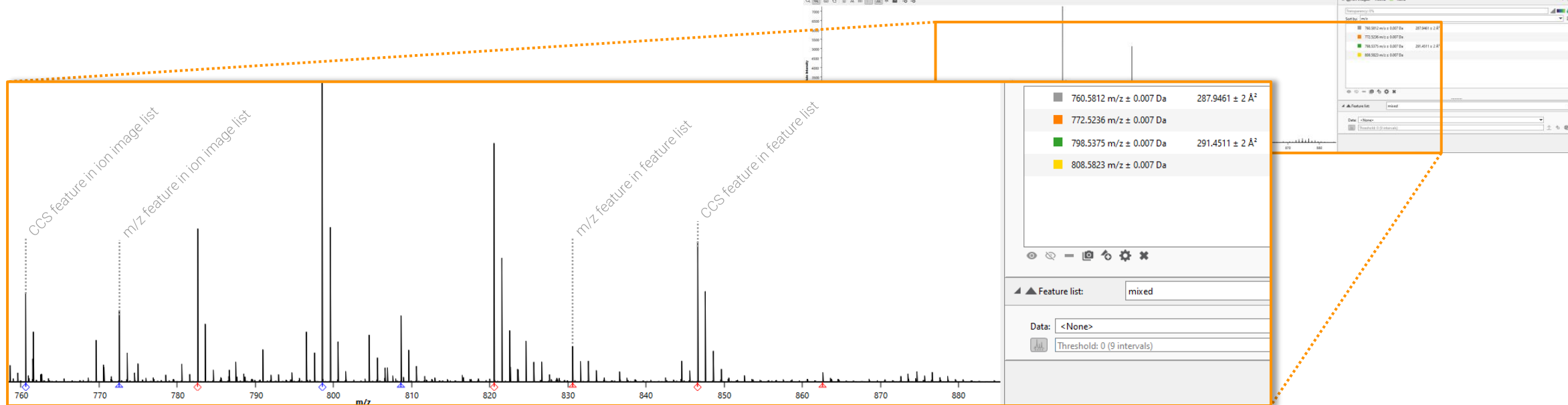
Multiple feature enhancements: Improved single spectrum viewing

- Displaying **single spectra** has been improved to work like displaying ion images
- Select a single spectrum with a **single click**
- Add single spectra to the Spectra list by using **Alt + click**



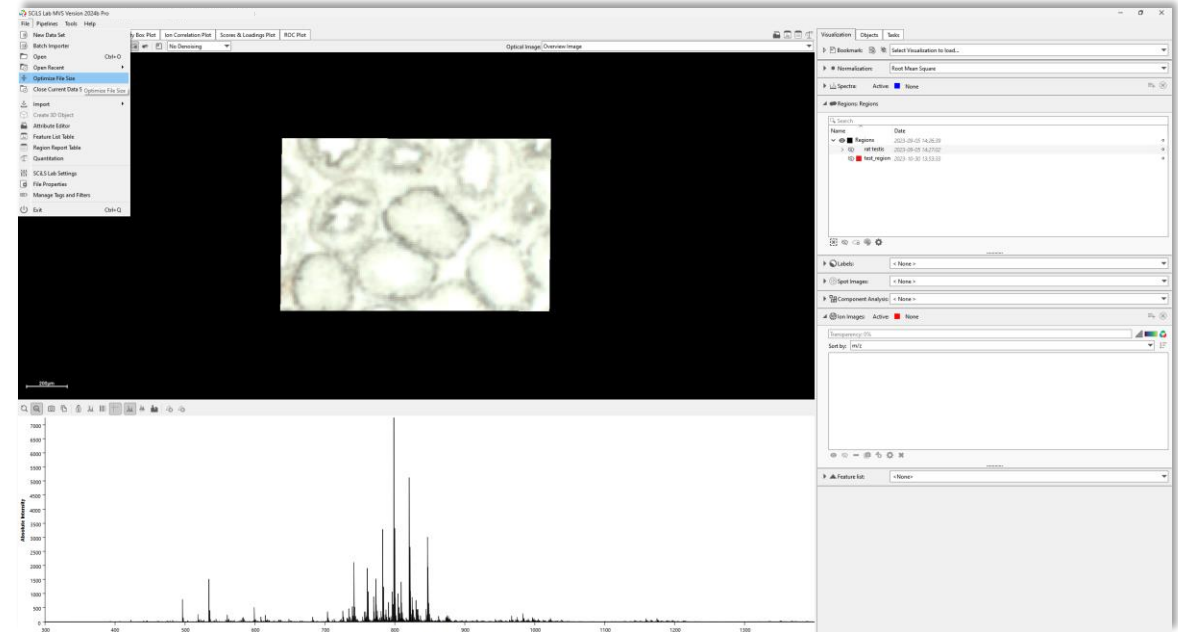
Multiple feature enhancements: Spectrum plotter differentiates m/z and CCS features

- m/z and CCS features have different icons in the Spectrum panel
 - m/z feature 
 - CCS feature 
- Colors differentiate features in the *ion image list* from features in the active *feature list*



Multiple feature enhancements: Optimize SCiLS™ Lab file size

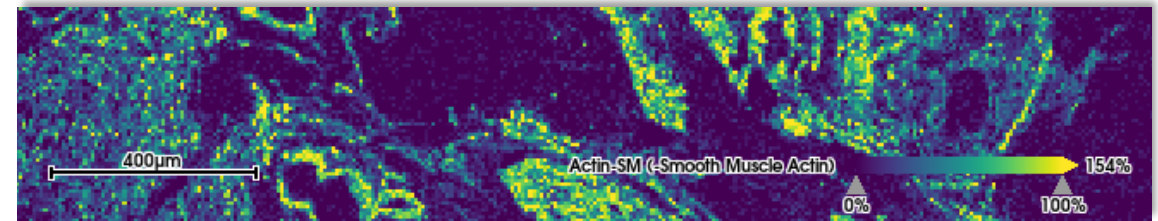
- Over time, the .slx file of a SCiLS Lab data set can accumulate redundant information, putting a burden on storage space
 - For example, unused extracted CCS images
- File > Optimize file size** will clean up the SCiLS Lab data set and remove redundant information, saving disk space



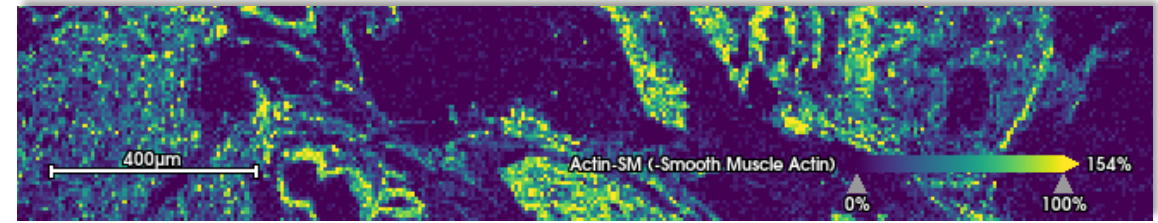
	Data set 1	Data set 2
Number of pixels	66925	66925
.slx size after import	319 MB	94 MB
# CCS features after import	873	0
# CCS features after feature filtering	10	10
.slx size after feature filtering	319 MB	100 MB
.slx size after "optimize file size"	98 MB	100 MB

Multiple feature enhancements: Improved Stack View readability

- To **enhance the readability** of the ion information, scale bar, color bar and error messages in Stack View, a thin text outline has been introduced
- The color and outline are dependent on the **Background Color** setting that can be adjusted via **File > SCiLS Lab settings**



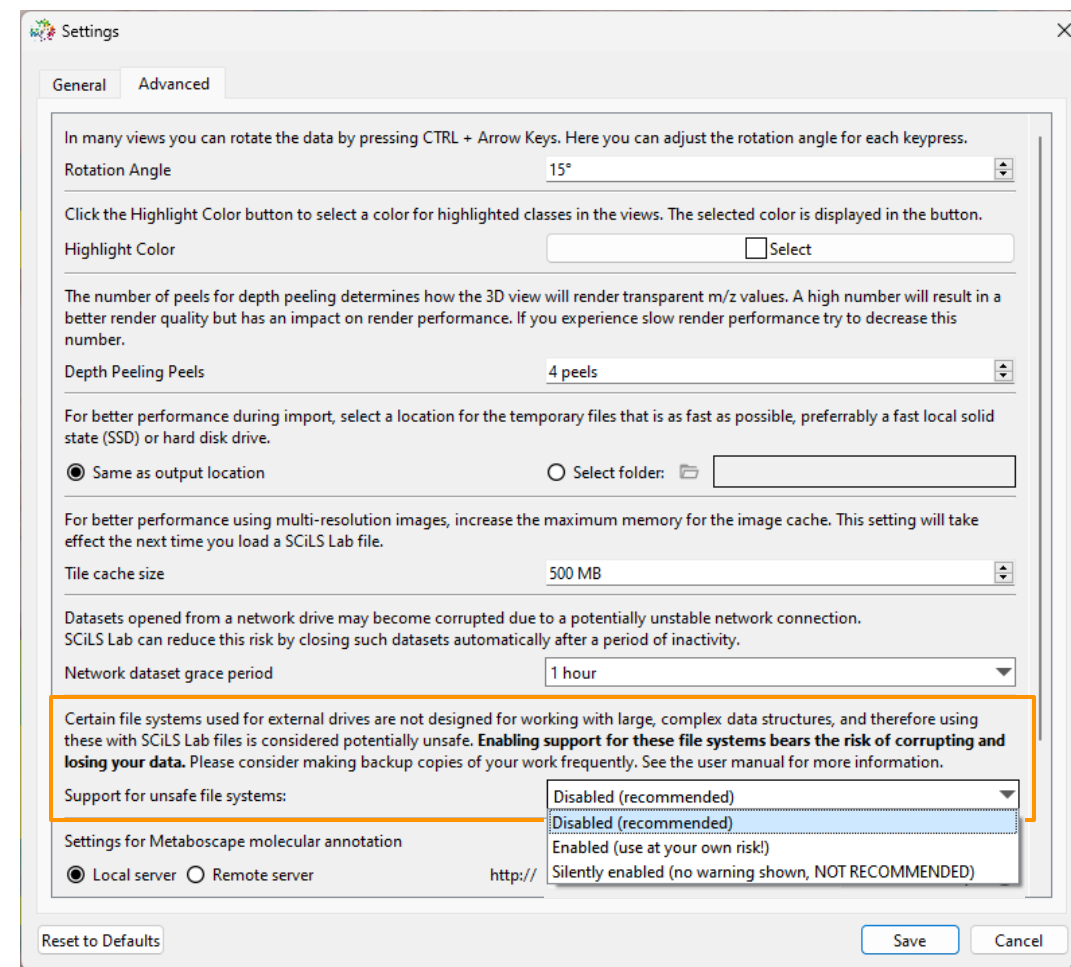
Background Color: White



Background Color: Black/Gradient

Multiple feature enhancements: Prevent loading data from unsafe file systems

- After several customer cases involving irreparable **.slx file corruption** on external drives, the use of certain file systems (incl. exFAT) has been deemed unsafe
- Opening SCiLS™ Lab files from unsafe file systems, or storing new SCiLS Lab files on unsafe file systems using the Importer is therefore **disabled** by default
- Through **File > Settings** users can enable reading and writing to unsafe file systems, at the risk of causing irreparable damage to their data





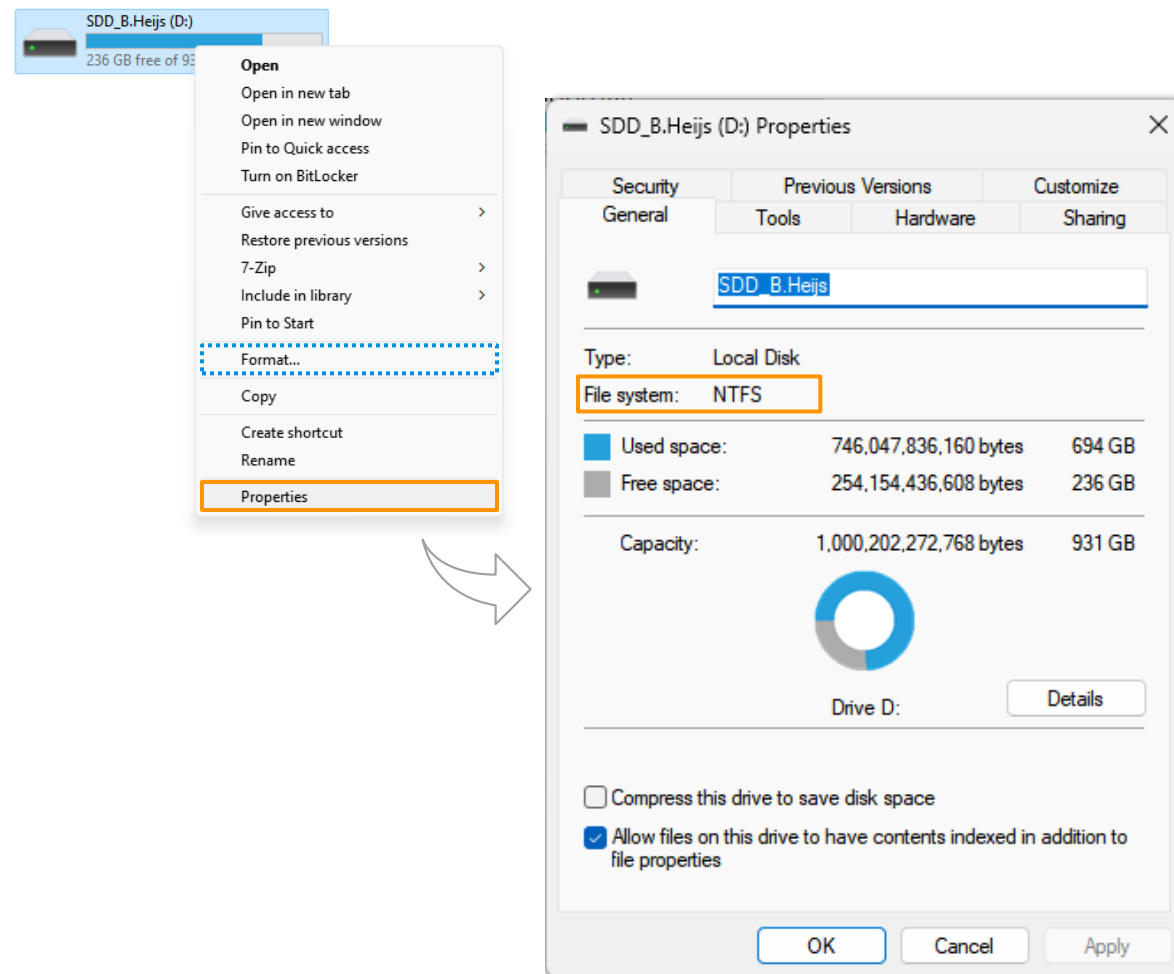
Multiple feature enhancements:

Prevent loading data from unsafe file systems using the API

- Similar to SCiLS™ Lab, the **SCiLS API**, by default, can no longer access files that are stored on unsafe file systems.
- Although not recommended, users can override this restriction when creating a new API session.
 - **R client:**
 - **SCiLSLabOpenLocalSession()**
 - Set “**overrideFilesystemCheck**” to **TRUE**
 - **Python client:**
 - **LocalSession()**
 - Set “**override_filesystem_check**” to **TRUE**

Multiple feature enhancements: Prevent loading data from unsafe file systems

- To check the current file system of a storage device, **This computer > right-click drive > Properties**
- To change an unsafe file system, select **“Format...”** and choose a file system considered safe
 - For example, NTFS
 - **NOTE:** Formatting a drive erases all its content. Create a backup before taking this action!



05

SCiLS API improvements



Multiple feature enhancements: Multi-modal imaging integration using the SCiLS™ API

- The introduction of **External Features** in SCiLS 2024a creates new possibilities for the analysis of **multi-modal imaging** data in SCiLS Lab
- From SCiLS 2024b onwards, the **SCiLS API** can write External Features into a data set, which allows users to **write any image** into a data set
- For joint analysis, images should be **co-registered and re-sampled** to the SCiLS Lab coordinate system
- **Example scripts for R and Python**, as well as detailed explanations on re-sampling strategies are provided in the **SCiLS API documentation**

[SCiLS Lab API Documentation](#)

[View page source](#)

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[UMAP with CCS images - R](#)

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Multimodal Analysis - R

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[Implementation of helper functions and resampling code](#)

[Creating a Multimodal Dataset with MALDI, IR, \$\mu\$ XRF and ICP-MS data](#)

[The \$\mu\$ XRF measurement](#)

[The ICP-MS measurement](#)

[Analyzing Multimodal Data in SCiLS Lab](#)

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Multimodal Analyses with SCiLS Lab - R

In this example, the SCiLS Lab API is used to merge a multimodal dataset obtained using matrix-assisted laser desorption/ionization (MALDI) Imaging (20 μ m pixel size), infrared microscopy (IR, 5 μ m pixel size), laser ablation inductively-coupled plasma mass spectrometry (LA-ICP-MS, 25 μ m pixel size) and micro-X-ray fluorescence (μ XRF, 30 μ m pixel size). Merging these datasets will allow their joint analysis and the calculation of correlations between the different datasets.

The data is described in the preprint: Kronenberg K, Werner J, Seeba M, Rave H, Linsen L, Steiger K, et al. *A multimodal view at cancerous liver tissue by chemical bioimaging and image segmentation strategies*. ChemRxiv. Cambridge: Cambridge Open Engage; 2023 and was kindly provided by the authors.

The μ XRF and IR data were acquired from the same section, MALDI and ICP-MS were acquired from different adjacent sections. The MALDI Imaging data were acquired on a TIMS-TOF fleX instrument (Bruker Daltonics, Bremen, Germany), the IR measurement was acquired on a Hyperion II-ILIM (Bruker Optics, Ettlingen, Germany), the μ XRF was acquired on an M4 TORNADO (Bruker Nano, Berlin, Germany) and the LA-ICP-MS was acquired on an iCAP TQ ICP-MS (Thermo Fisher Scientific, Bremen, Germany) coupled to an LSX 213 G2+ laser system (CETAC Technologies, Omaha, NE, USA).

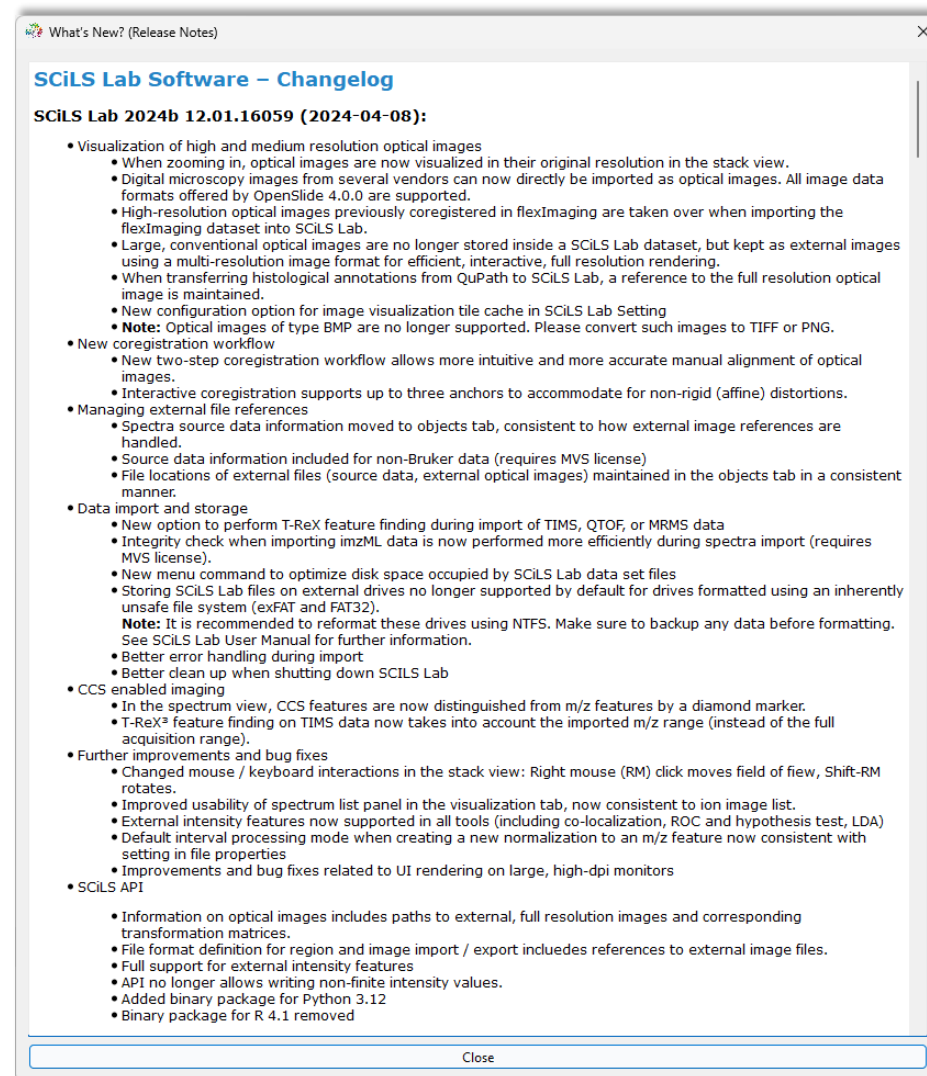
SCiLS Lab supports an 'external feature' type. External features are intensities that are associated with measured spots in a SCiLS Lab dataset that are not derived from the source measurement of the SCiLS Lab dataset. Since they are not conceptually related to the measurement, they don't have an m/z (and ion mobility) value and are identified only by their name. External features can be imported from other SCiLS Lab datasets using the SCiLS Ion Image Mapper or written by the API. In this example, we use external features to combine the different modalities.

This document is showing both of how to 'just do it' and a more detailed explanation of how the techniques work.

SCiLS™ Lab 2024b Changelog

Open changelog in SCiLS Lab:

- **Help > What's New**, or
- **Ctrl + B**



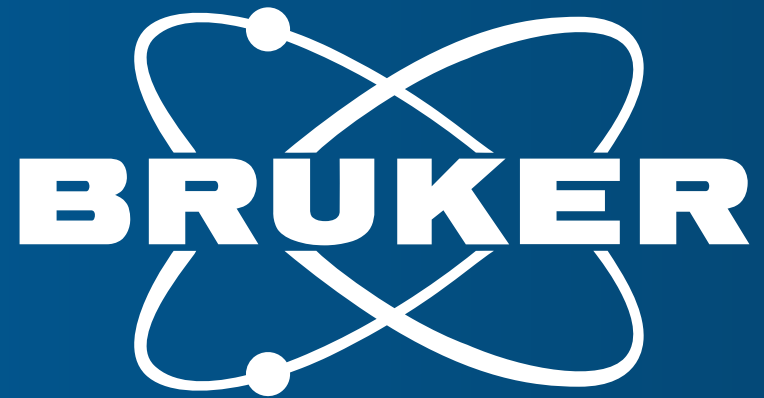
What's New? (Release Notes)

SCiLS Lab Software – Changelog

SCiLS Lab 2024b 12.01.16059 (2024-04-08):

- Visualization of high and medium resolution optical images
 - When zooming in, optical images are now visualized in their original resolution in the stack view.
 - Digital microscopy images from several vendors can now directly be imported as optical images. All image data formats offered by OpenSlide 4.0.0 are supported.
 - High-resolution optical images previously coregistered in flexImaging are taken over when importing the flexImaging dataset into SCiLS Lab.
 - Large, conventional optical images are no longer stored inside a SCiLS Lab dataset, but kept as external images using a multi-resolution image format for efficient, interactive, full resolution rendering.
 - When transferring histological annotations from QuPath to SCiLS Lab, a reference to the full resolution optical image is maintained.
 - New configuration option for image visualization tile cache in SCiLS Lab Setting
 - **Note:** Optical images of type BMP are no longer supported. Please convert such images to TIFF or PNG.
- New coregistration workflow
 - New two-step coregistration workflow allows more intuitive and more accurate manual alignment of optical images.
 - Interactive coregistration supports up to three anchors to accommodate for non-rigid (affine) distortions.
- Managing external file references
 - Spectra source data information moved to objects tab, consistent to how external image references are handled.
 - Source data information included for non-Bruker data (requires MVS license)
 - File locations of external files (source data, external optical images) maintained in the objects tab in a consistent manner.
- Data import and storage
 - New option to perform T-ReX feature finding during import of TIMS, QTOF, or MRMS data
 - Integrity check when importing imzML data is now performed more efficiently during spectra import (requires MVS license).
 - New menu command to optimize disk space occupied by SCiLS Lab data set files
 - Storing SCiLS Lab files on external drives no longer supported by default for drives formatted using an inherently unsafe file system (exFAT and FAT32).
 - **Note:** It is recommended to reformat these drives using NTFS. Make sure to backup any data before formatting. See SCiLS Lab User Manual for further information.
 - Better error handling during import
 - Better clean up when shutting down SCiLS Lab
- CCS enabled imaging
 - In the spectrum view, CCS features are now distinguished from m/z features by a diamond marker.
 - T-ReX³ feature finding on TIMS data now takes into account the imported m/z range (instead of the full acquisition range).
- Further improvements and bug fixes
 - Changed mouse / keyboard interactions in the stack view: Right mouse (RM) click moves field of view, Shift-RM rotates.
 - Improved usability of spectrum list panel in the visualization tab, now consistent to ion image list.
 - External intensity features now supported in all tools (including co-localization, ROC and hypothesis test, LDA)
 - Default interval processing mode when creating a new normalization to an m/z feature now consistent with setting in file properties
 - Improvements and bug fixes related to UI rendering on large, high-dpi monitors
- SCiLS API
 - Information on optical images includes paths to external, full resolution images and corresponding transformation matrices.
 - File format definition for region and image import / export includes references to external image files.
 - Full support for external intensity features
 - API no longer allows writing non-finite intensity values.
 - Added binary package for Python 3.12
 - Binary package for R 4.1 removed

Close



Innovation with Integrity