TISSUEFAXS SPECTRA

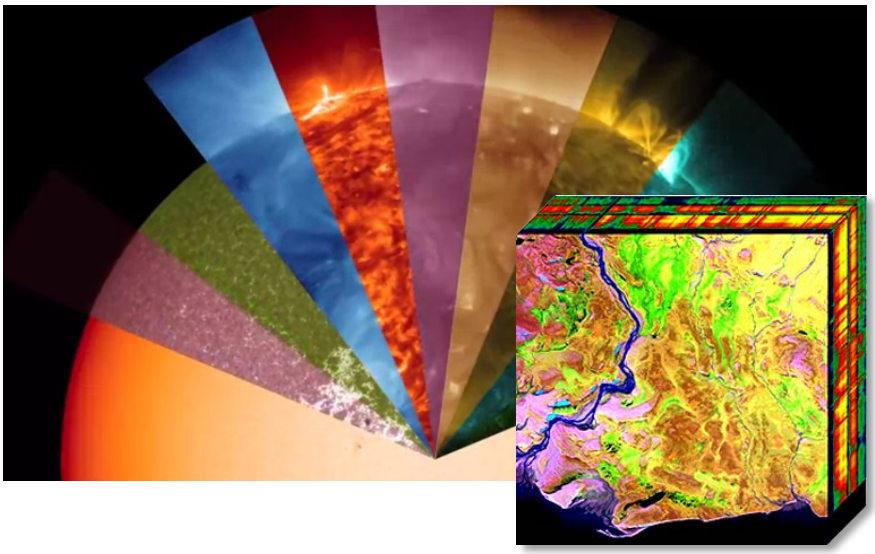
MULTISPECTRAL IMAGING SYSTEMS

TISSUEGNOSTICS
IMAGING SOLUTIONS

TissueGnostics
Spectral Imaging

Multispectral imaging captures data within specific wavelength ranges across the visible and NIR spectrum using various technologies.

The data obtained through multispectral imaging is used to view or reveal information about the sample which may not be revealed by traditional color imaging. The applications for this imaging approach are broad in scope, and include biological imaging and analysis.



Multispectral microscopy significantly improves separation of concurrent immunological labeling enabling the multiplexing of colorometric or fluorescent information.

The term ‘lambda stack‘ or ‘lambda cube‘ describes the data structure created by imaging a field of view across a range of wavelengths. Each layer of the stack represents one image of the field taken at a specific wavelength.

TissueGnostics uses tunable liquid crystal filter technology with rapid switching to scan lambda cubes.

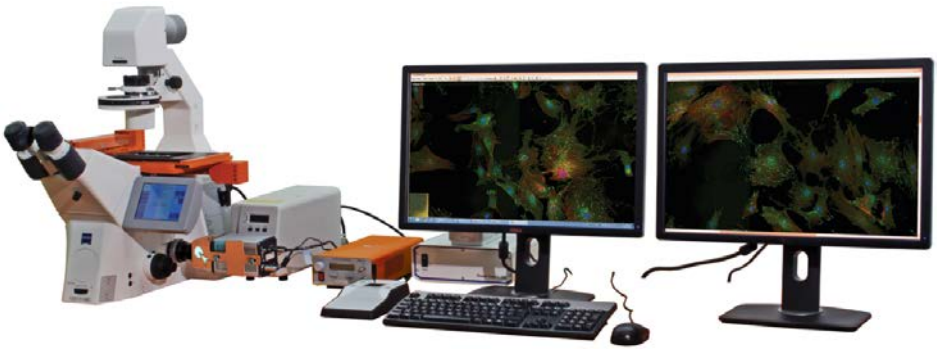
TISSUEFAXS SPECTRA SYSTEMS

TissueFAXS SPECTRA systems are available in upright and inverted configurations .

They are based on TissueGnostics TissueFAXS Tissue Cytometry systems. For details and information about TissueFAX please see www.tissuegnostics.com.

TissueFAXS SPECTRA PLUS upright system

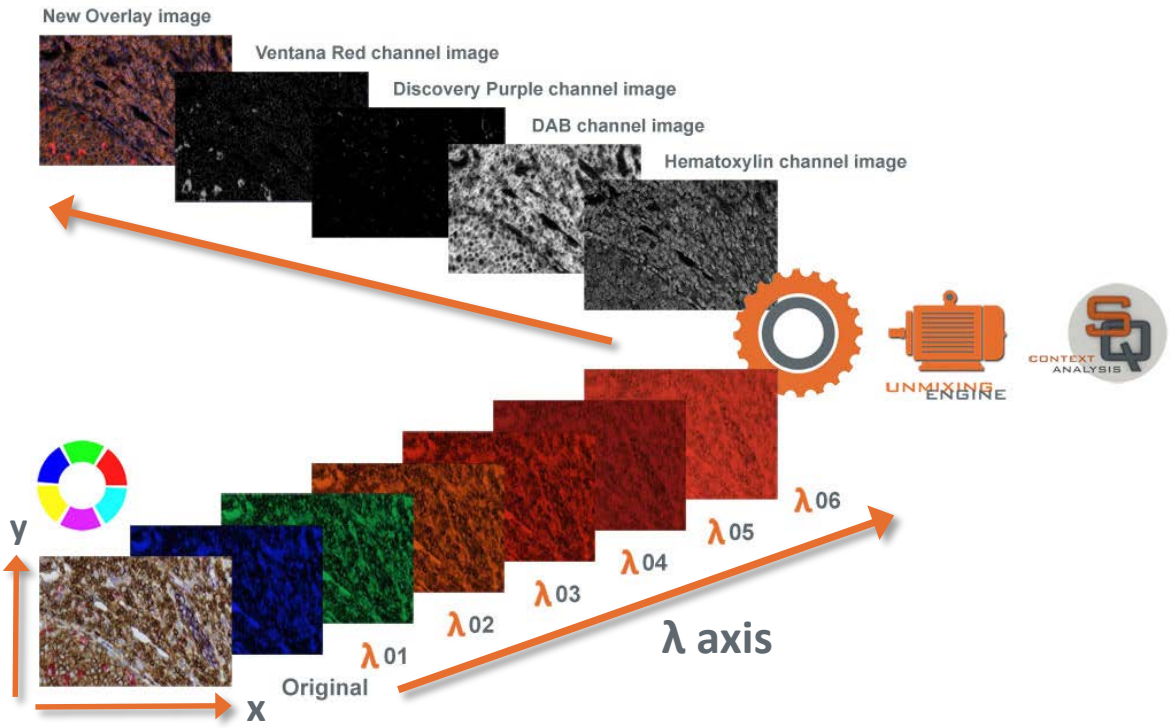
TissueFAXS SPECTRA i PLUS inverted system



The SPECTRA unit and its associated software can also be added to existing TissueFAXS systems as well as compatible other automated microscopy systems as an upgrade.

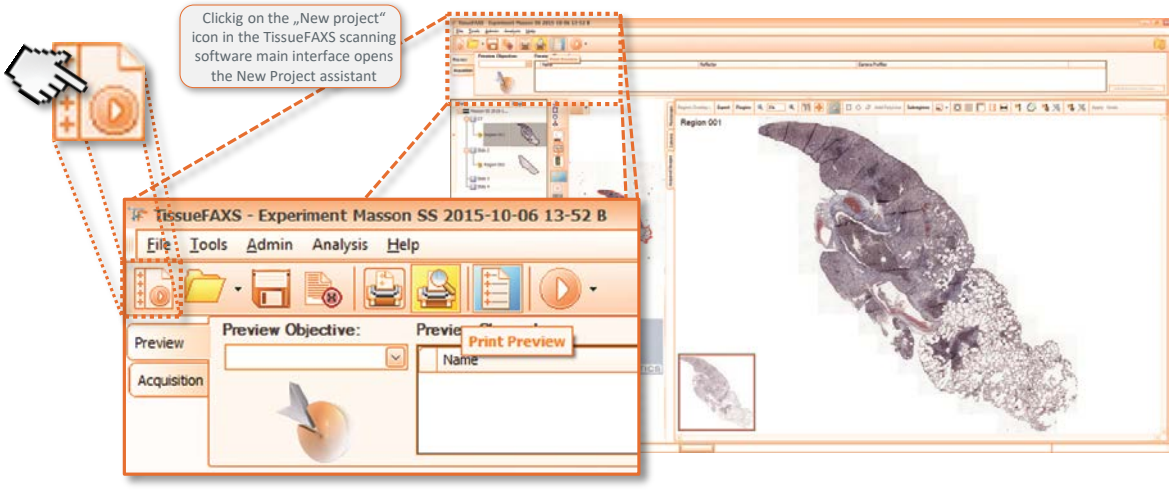
THE TISSUEGNOSTICS MULTISPECTRAL WORKFLOW

The schematic below shows the TissueFAXS SPECTRA workflow on a brightfield sample. Images and digital slides scanned on the TissueFAXS tissue cytometer are spectrally unmixed in StrataQuest analysis software or with a preconfigured StrataQuest application.

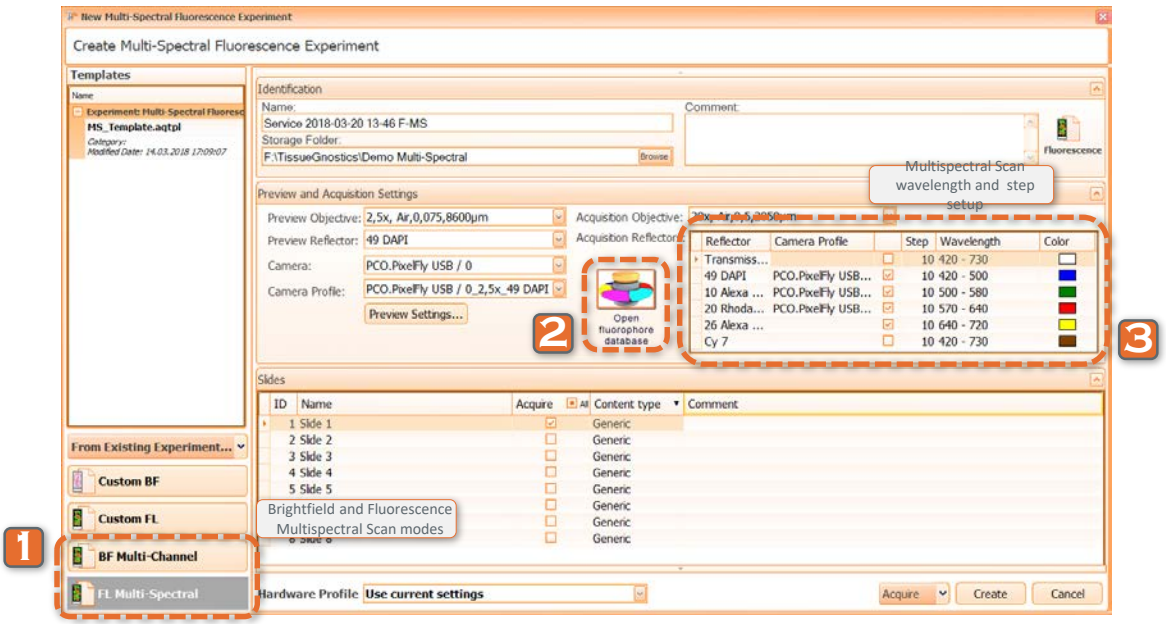


TissueFAXS multispectral imaging of both brightfield and fluorescense samples produces a complete spectrum of the sample along the λ axis at every image pixel.

The screenshots below show the acquisition software workflow.



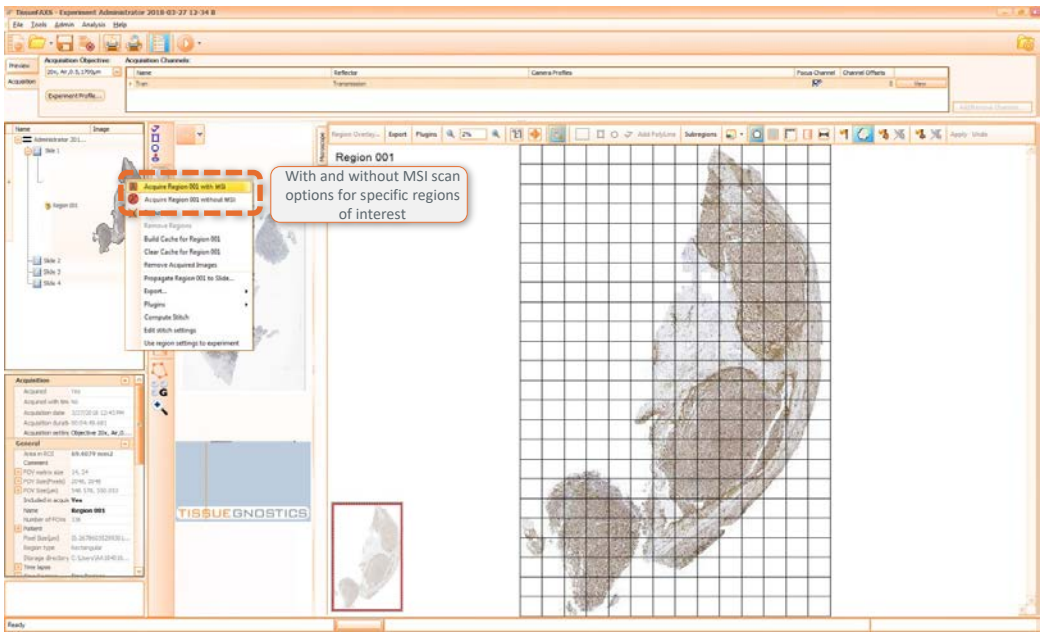
MULSTISPECTRAL IMAGING



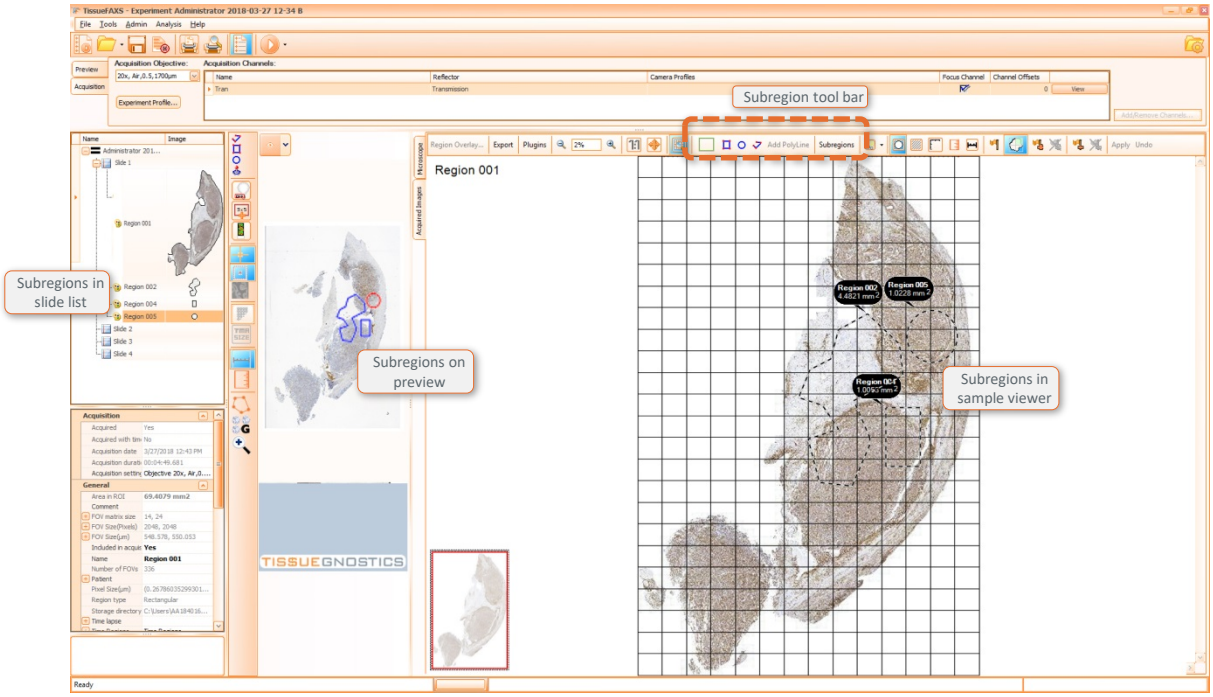
Start a multispectral scan by selecting ‘FL Multi-Spectral‘ as shown in (1) above.

A user editable fluorophore database (2) will provide basic wavelength ranges and step sizes for acquisition. All multispectral imaging parameters can be manually controlled (3).

After establishing imaging parameters, a low magnification preview scan at 2.5X (shown below) completes the configuration for tissue detection and automated multi-spectral scanning.

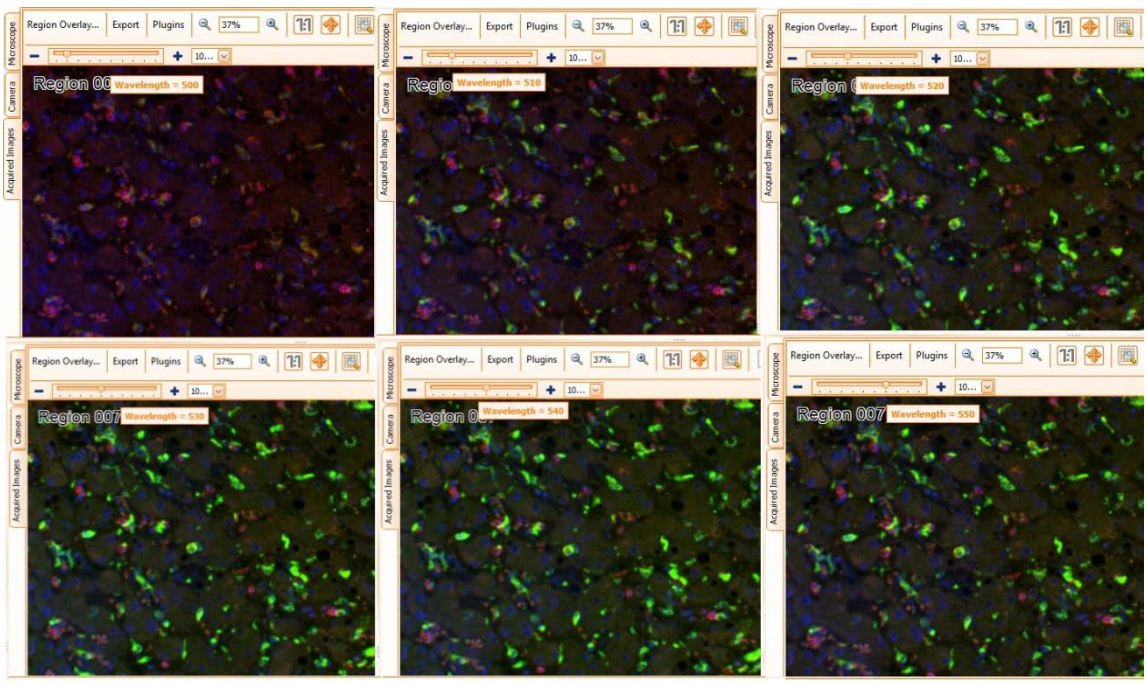


MULSTISPECTRAL IMAGING



Some samples require a high magnification image upon to properly select areas for multi-spectral imaging. In this case the definition of the area for multi-spectral imaging can be performed upon the high resolution scan, as shown in the screenshot above.

Multispectral imaging is viewed using a spectral slider control to select the discrete wavelength band for display. The screenshot below shows the response of the data to slider selection from 500nm to 580nm with a 10nm step.



SPECTRAL UNMIXING

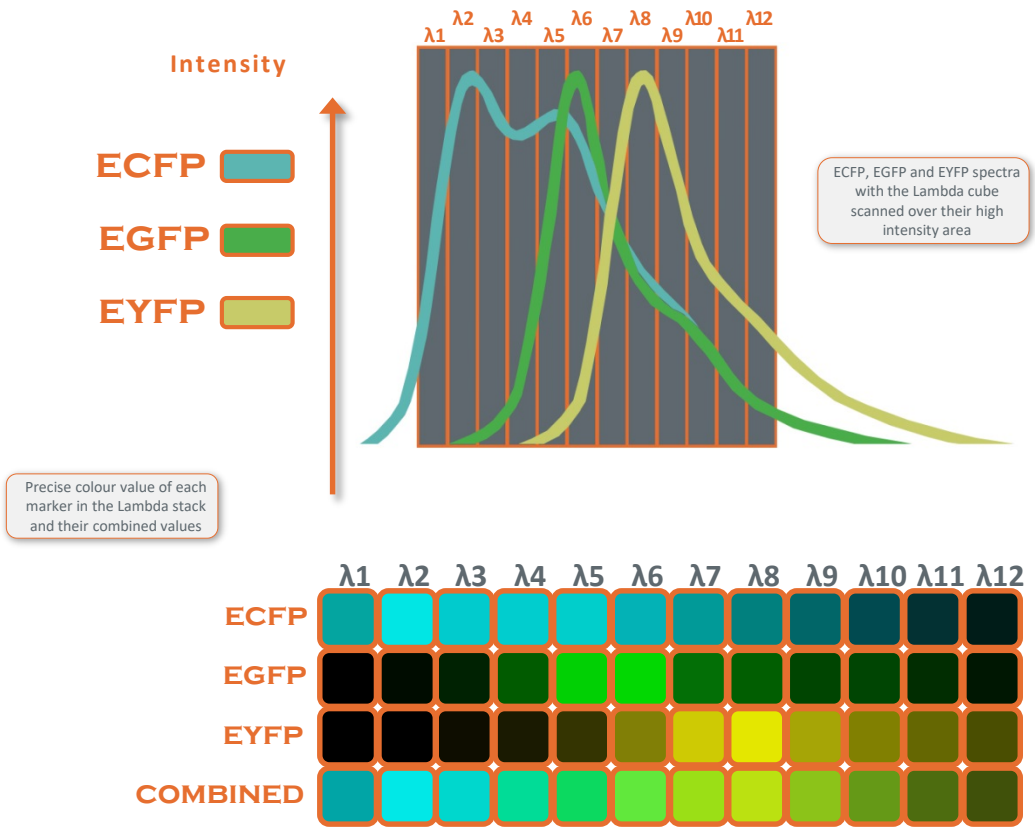
Spectral unmixing determines the relative contribution of each fluorophore or stain in every pixel of the image and conveys the following benefits:

- Separation of multiplexed immunohistochemical staining
- Separation of multiplexed immunofluorescence
- Removal of background and autofluorescence
- Increases number of concurrent markers/stains
- Augments morphological and colormetric information content

Spectral unmixing requires the acquisition of reference spectra for each label used in the sample as well as for background and intrinsic fluorescence.

Reference spectra can be from picked spots in the sample with pure staining or by scanning a separate reference Lambda cube for each marker or from a reference database. Background and autofluorescence are treated as supplementary markers.

The following example shows the principle of spectral unmixing using overlapping spectra of fluorescent labels ECFP, EGFP and EYFP.

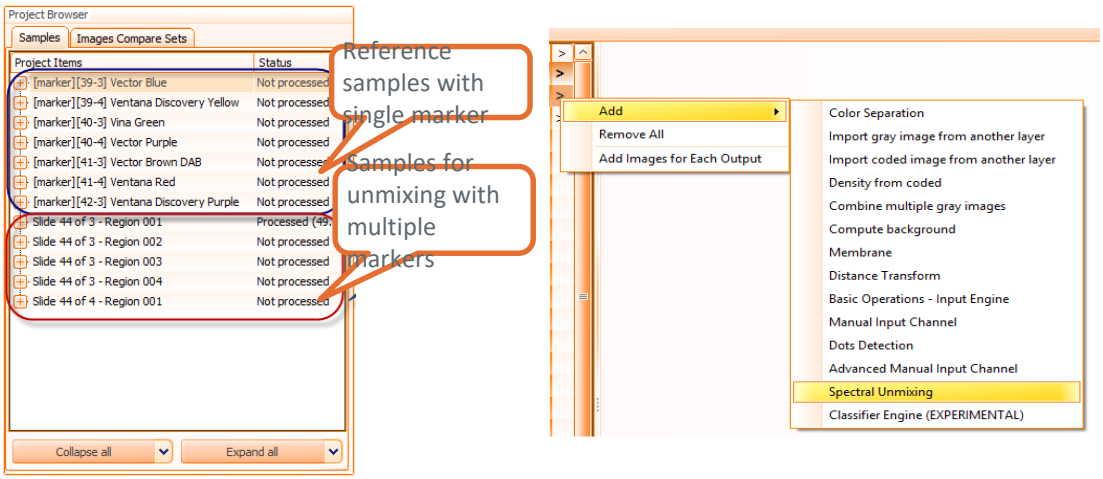


Reference spectra for ECFP, EGFP and EYFP are required to unmix the combined signal using StrataQuest. Background and autofluorescense spectra can also be obtained to improve the signal to noise ratio of the overall image.

SPECTRAL UNMIXING

Data acquired using TissueFAXS SPECTRA can either unmixed during acquisition from within TissueFAXS SPECTRA S scanning software or processed post acquisition using either the Spectral Unmixing Engine included in the StrataQuest whole slide analysis software or the available StrataQuest Unmixing Application.

Fluorophore spectra are input into the Spectral Unmixing Engine using any combination of manual selection from within the image, numerical input, or import from a database of previously acquired spectra*.



* Note: When manually selecting colors take care to ensure that the fluorescense contained within the selected pixel(s) represents a pure fluorophore. Spectral unmixing quality is dependent upon identification of the multiple linearly independed spectral bases to the total fluorescense produced by the sample.

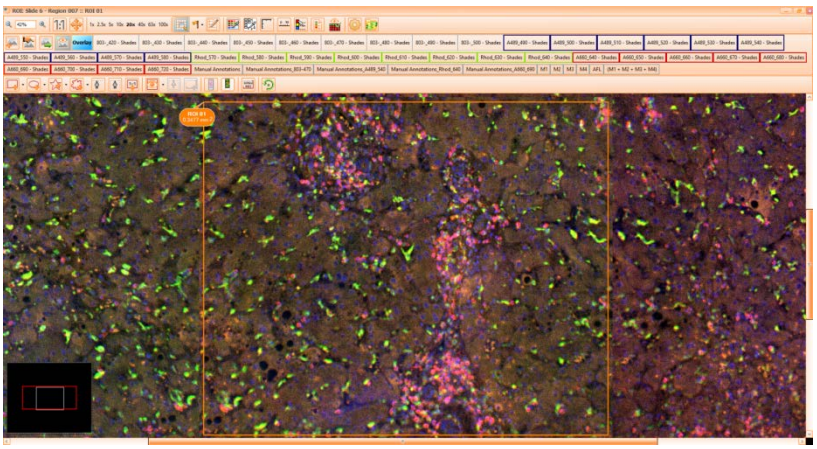
The StrataQuest Spectral Unmixing APP

The StrataQuest Spectral Unmixing APP is accessed as an input extraction from within StrataQuest Plus. The unmixing app simplifies the process of adding the necessary reference spectra to the unmixing modeal, and executing the unmixing step.

SPECTRAL UNMIXING

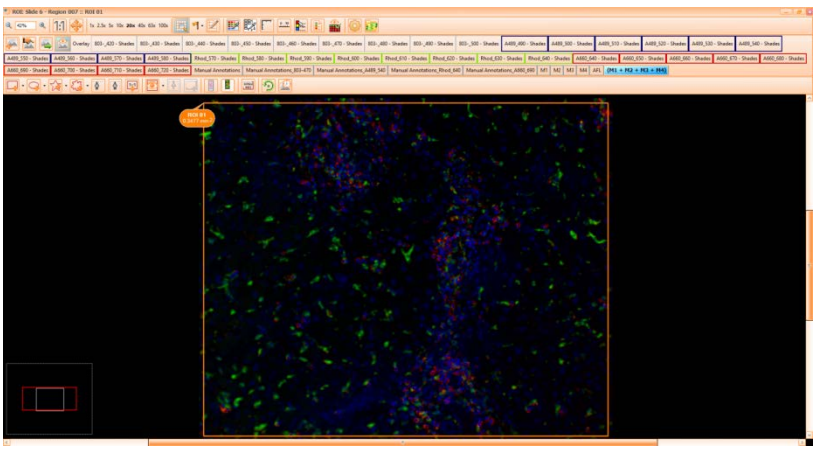
The results of the unmixing process can are viewed either within StrataQuest or the StrataQuest APP spectral viewer.

The two image below show the dramatic contrast and signal improvement obtained using spectral unmixing to remove background and autofluorescence from a spectrally scanned sample.



BEFORE

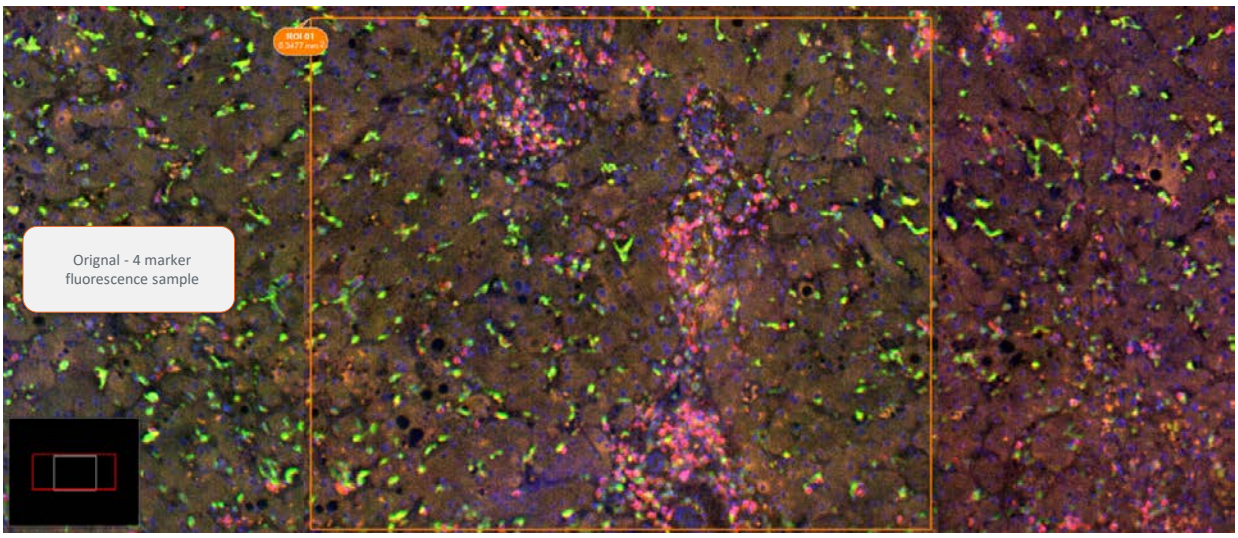
Commparison the Overlay of the Orignal of a 4 marker fluorescence sample and the unmixed result

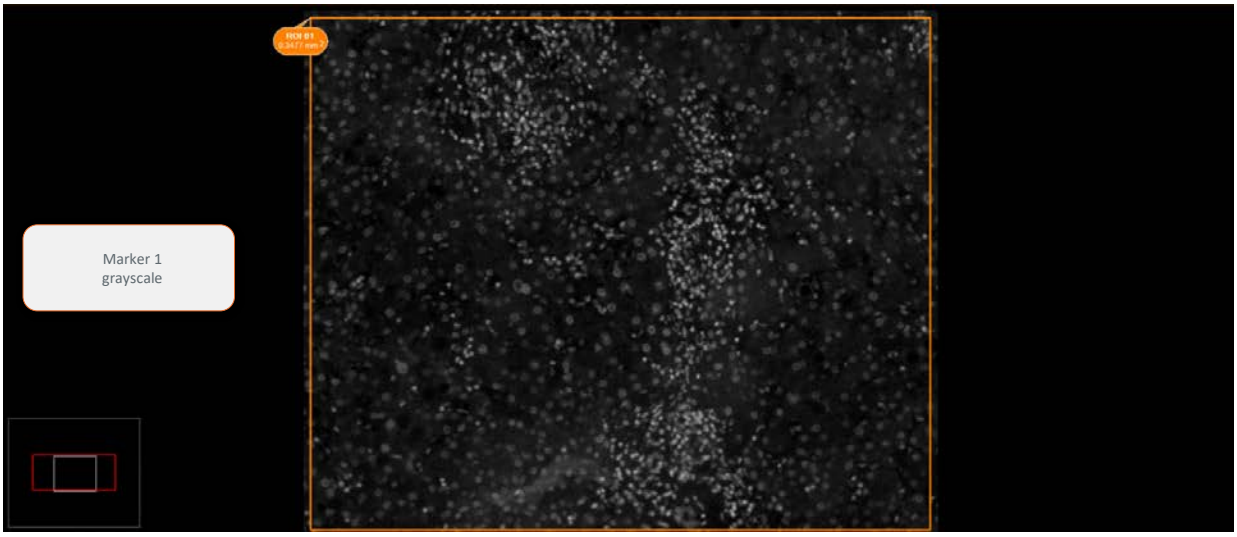


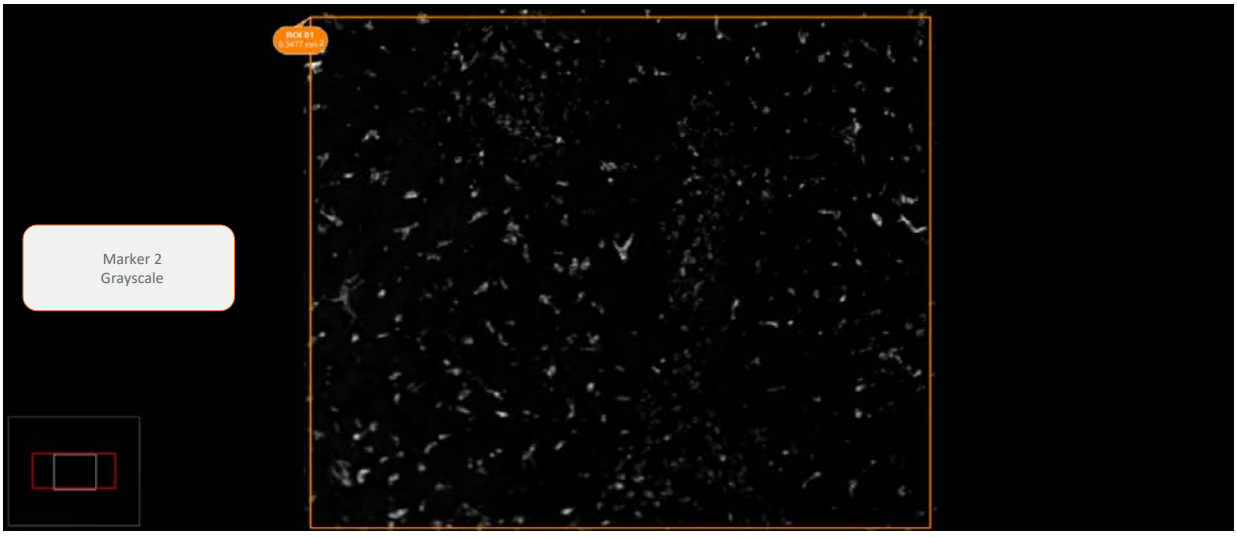
AFTER

SPECTRAL UNMIXING

Example imaging: Original image at top, unmixed channels 1 and 2 below

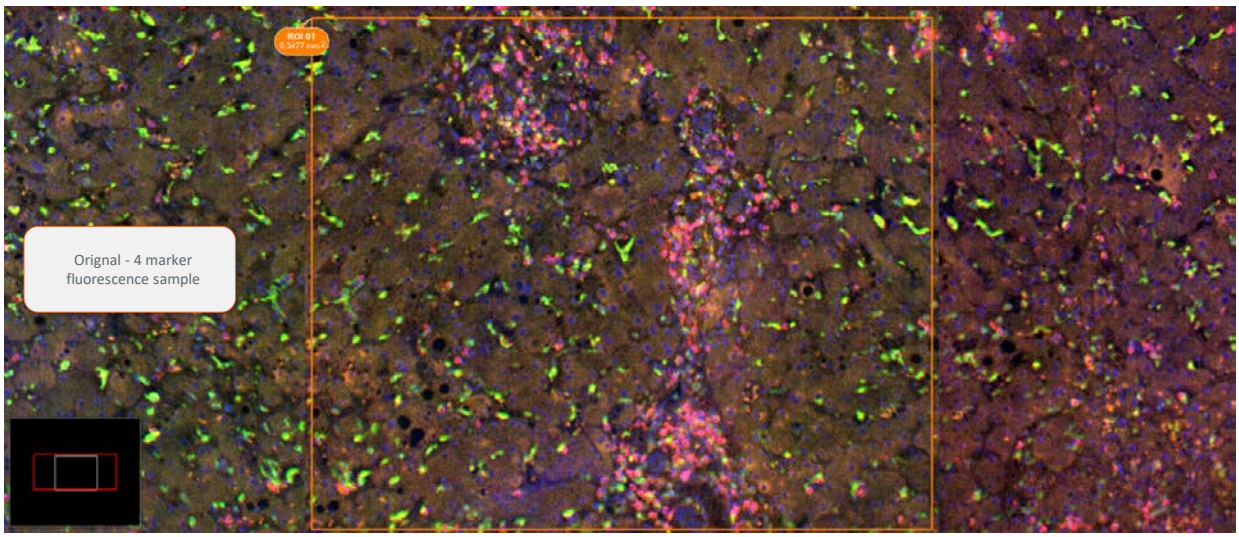


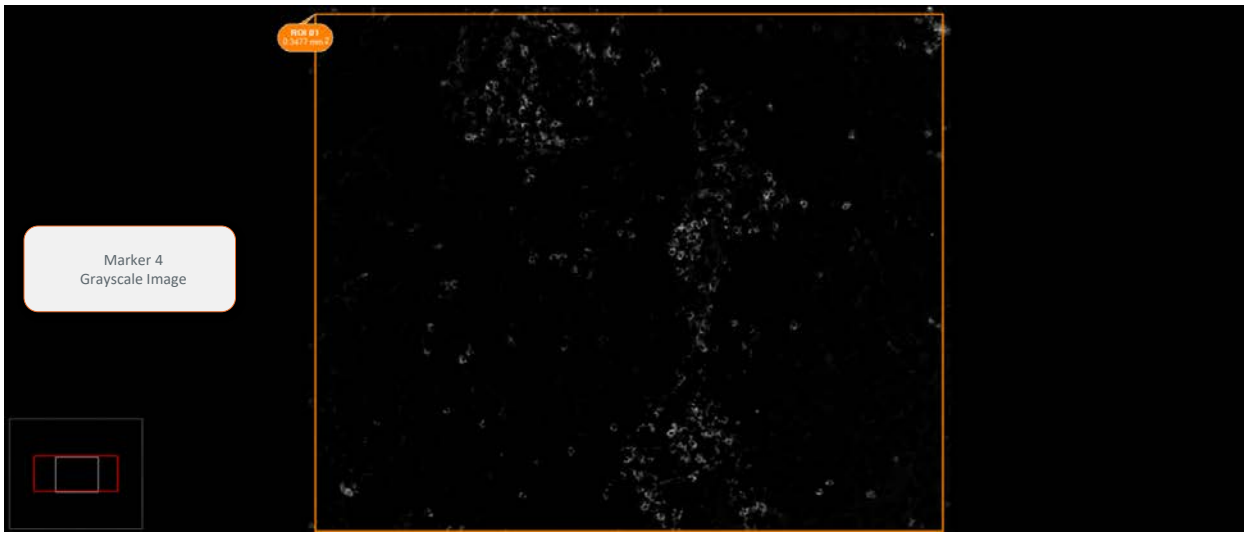




SPECTRAL UNMIXING

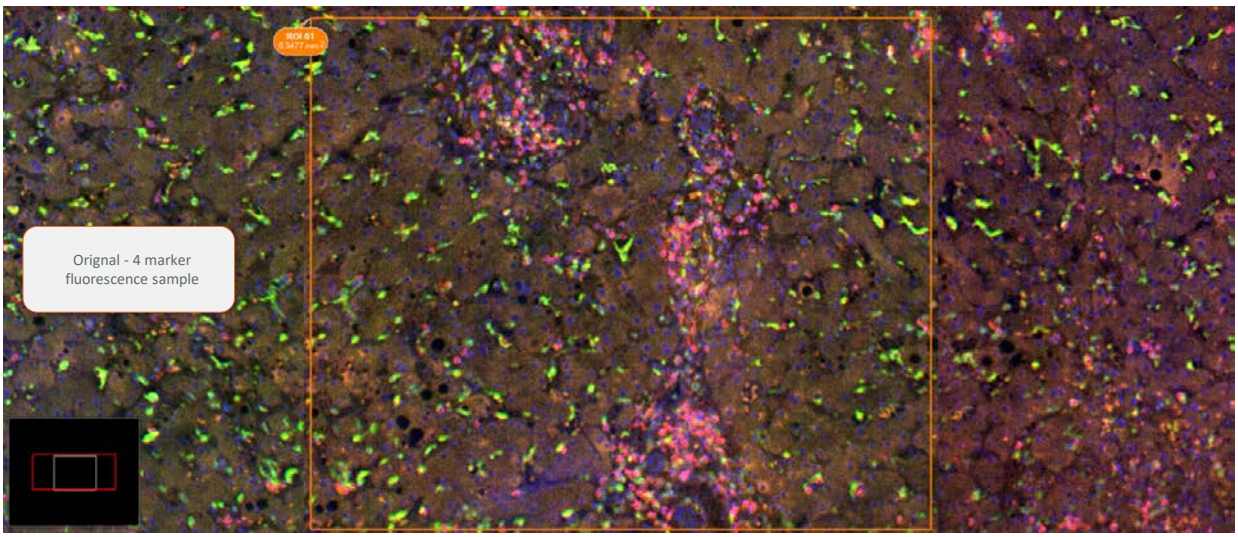
Example imaging: Original image at top, unmixed channels 3 and 4 below

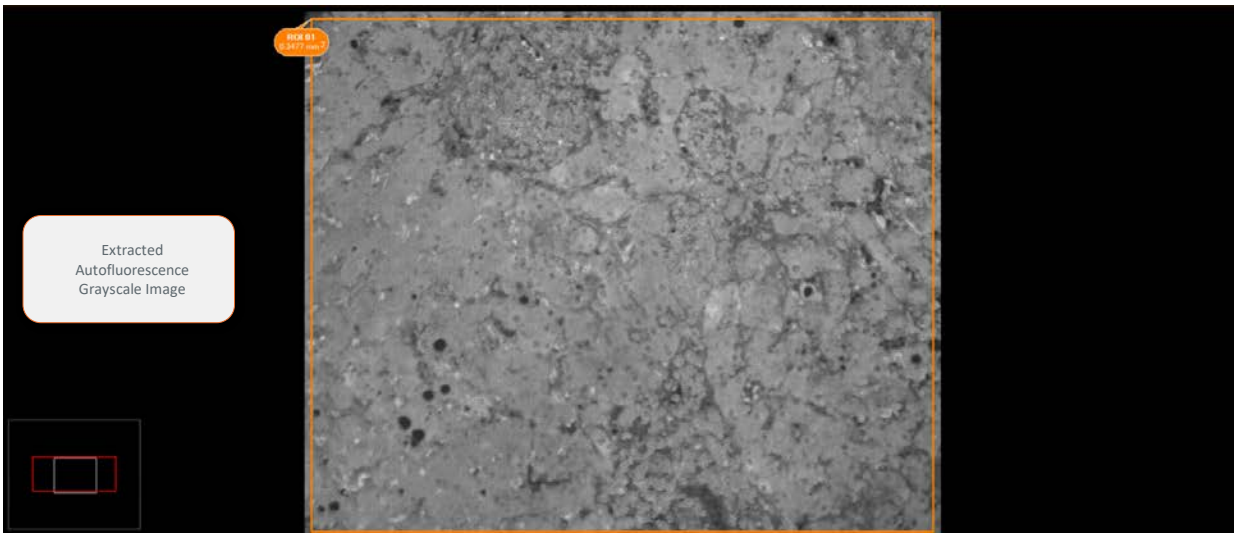


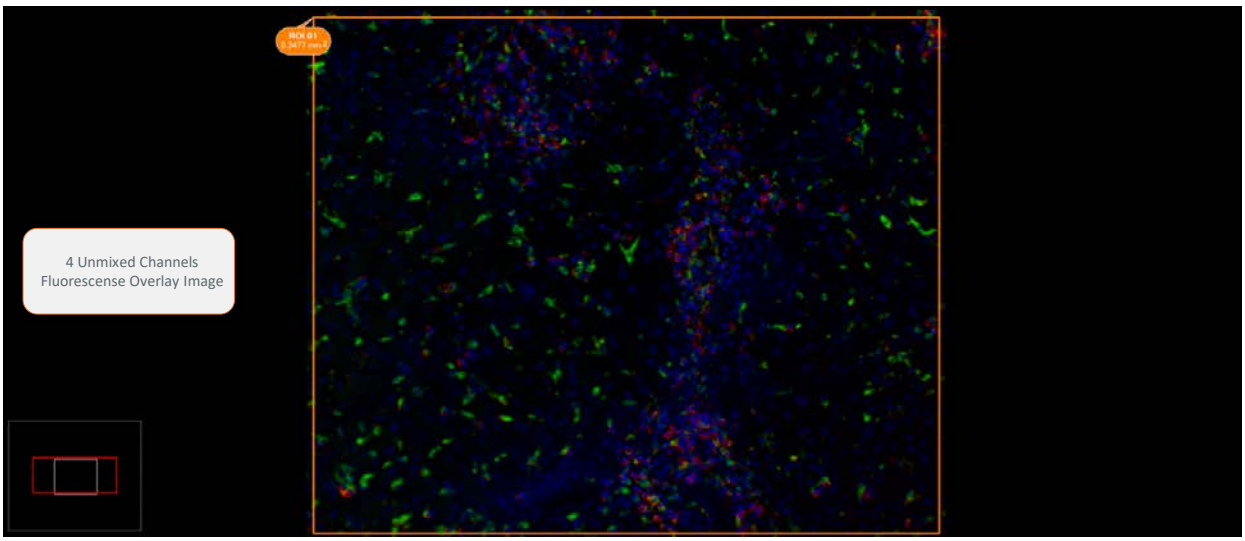


SPECTRAL UNMIXING

Example imaging: Original image at top, background/autofluorescense and unmixed result shown below







TISSUEGNOSTICS

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