



Tips & Tricks :Axioscan, Slide Scanner

The Axioscan is a fully automated epi-fluorescence slide scanner, designed for high-throughput digitization of a large number of samples. With a capacity of up to 100 slides, it supports image acquisition in brightfield, polarized light (useful for observing collagen stained with Sirius Red), and fluorescence with excitation wavelengths of 385, 430, 475, 555, 590, 630, and 735 nm, allowing up to 7 simultaneous colors. It is equipped with two cameras (712 mono and 705 color), a Colibri 7 LED light source, and a filter system. This Zeiss microscope, available on the GIGA Cell Imaging platform, operates based on "profiles" that define the actions to be applied to each slide. It features four objectives (5x, 10x, 20x, 40x) for brightfield and fluorescence microscopy, and a dedicated 20x objective for polarized light.

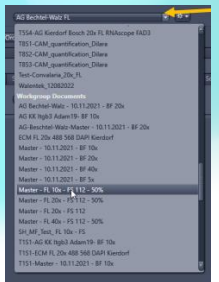
Using the Axioscan

During training, it is recommended to bring your own positive and negative slides to create a customized profile with assistance from a platform member. Before starting a new experiment, make sure to validate your settings with the platform staff to ensure optimal results. Due to high demand, the Axioscan should be booked in advance, especially for urgent projects. Data are stored on the system for four months, organized into cycles from January to April, May to August, and September to December, after which they will be automatically deleted. For many applications, the 20x objective is sufficient, particularly in brightfield, which saves time and reduces file size compared to using the 40x objective. If a platform member performs the acquisition, it is mandatory to provide a CSV file listing the slide names and their imaging order.

Profile Naming

For efficient organization, name your profiles using the following format: microscopy type, your initials, the objective used, and the number of colors or wavelengths for fluorescence.

- Fl_jm_40x_2couleurs ou Fl_jm_40x_488_594
- Bf_jm_20x
- Pol_jm_20x



Active/Inactive Folders

Two folders on the desktop streamline profile management: "Active Profiles" for profiles currently in use and visible in the software menu, and "Inactive Profiles" for archived profiles that can be reactivated if needed.

Pre-scan

The pre-scan provides an overview of the slide, allowing for automatic or manual delineation of the tissue and focus point placement. Optimize speed settings to save time and ensure a clear contrast between the tissue and the slide background.

- Settings for brightfield and polarized light pre-scan:
 - Exposure time
 - Light intensity (LED power)
- Settings for fluorescence pre-scan:
 - Exposure time
 - Light intensity (LED power)
 - Binning: combines multiple adjacent pixels into one, reducing resolution and acquisition time.

Tissue Detection Zone

Automatic: Adjust display parameters to clearly differentiate the tissue from the background. For highly transparent or low-contrast samples, manual delineation may be necessary.

Manual: Use drawing tools (rectangle, circle, freehand, polygon) to precisely define the tissue area.

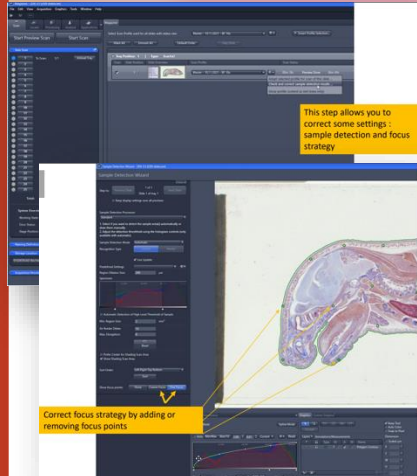
Setting the Focus

- Coarse Focus:

A preliminary focus at low magnification (5x) to define the area for final focus. Place between 3 to 10 points depending on the tissue area to optimize time.
- Fine Focus:

The final focus is performed using the acquisition objective (10x, 20x, 40x), with a higher number of points to ensure optimal sharpness (5 to 10 times more points than Coarse Focus).

For fluorescence, select the most intense fluorochrome to facilitate automatic focusing.

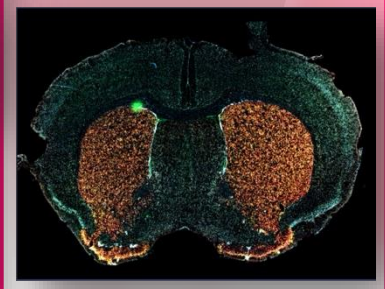


Acquisition Parameters and Data Analysis

As with other microscopes, it is essential to preserve the sample by limiting LED intensity (recommended range is between 5% and 15%, while the default is set at 50%). Select the microscopy mode (brightfield, polarized, fluorescence), and for fluorescence, adjust the LED power and exposure time for each fluorochrome, using the appropriate filters.

➢ **Stitching:** To speed up the acquisition process, it is advisable to perform stitching offline.

➢ **Opening and analyzing images:** Use QuPath, a free software compatible with Windows, Linux, and macOS, for data analysis.



ALWAYS KEEP IN MIND :
You are the only one responsible for your data
Never use the platform's computers as a back-up for your data !