Leica HCS A

High Content Screening Automation

User Manual

LAS AF Matrix M3

English Version V01

- 15.12.2009 -

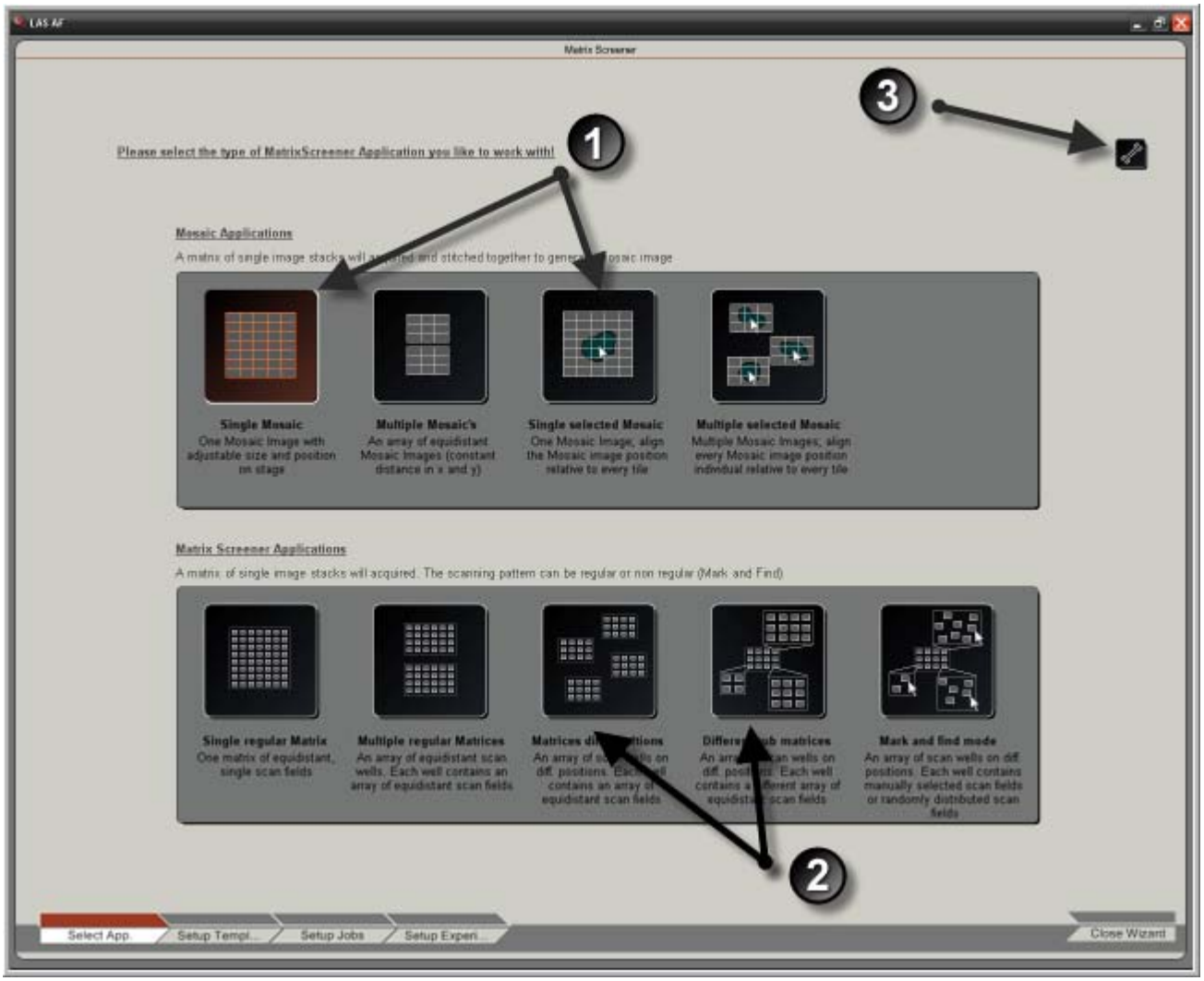
Calling up MatrixScreener Wizard

Note: A system with motorized specimen stage is required when using MatrixScreener Wizard. The motorized specimen stage must be initialized when LAS AF is started.

Start LAS AF and select MatrixScreener Wizard from the top left menu bar under **Leica Microsystems LAS AF** .

The MatrixScreener Wizard starts with the **Select App.** operating step, in which you can do the following:

- Select the suitable application from two types of MatrixScreener applications.
- Configure global settings that are valid for all MatrixScreener applications.



Mosaic Applications

1 This includes all applications related to joining many individual images together to create an image mosaic. Depending on the requirements of your experiment, you can select the suitable application here.

Matrix Screener Applications

2 This includes all applications related to carrying out experiments that run at different positions. Depending on the requirements of your experiment, you can select the suitable application here.

3 In addition to the individual settings for the respective MatrixScreener application, there are global settings that apply for all MatrixScreener applications. You can use the button with the tool symbol to configure or modify these global settings.

See also:
Structure of the MatrixScreener Wizard
Applications of the MatrixScreener Wizard
MatrixScreener Wizard: Global settings
MatrixScreener Wizard: Scan field functions

MatrixScreener Wizard: Setup Template
MatrixScreener Wizard: Setup Jobs
MatrixScreener Wizard: Setup Experiment

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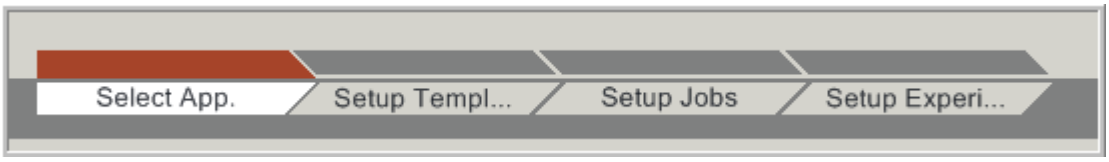
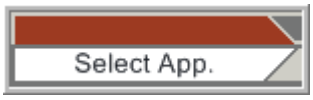
Structure of the MatrixScreener Wizard

The MatrixScreener Wizard is divided into logically sequenced operating steps that are displayed as arrow symbols in a bar at the bottom of the screen. By clicking the mouse on an arrow symbol, you can move from one operating step to another.





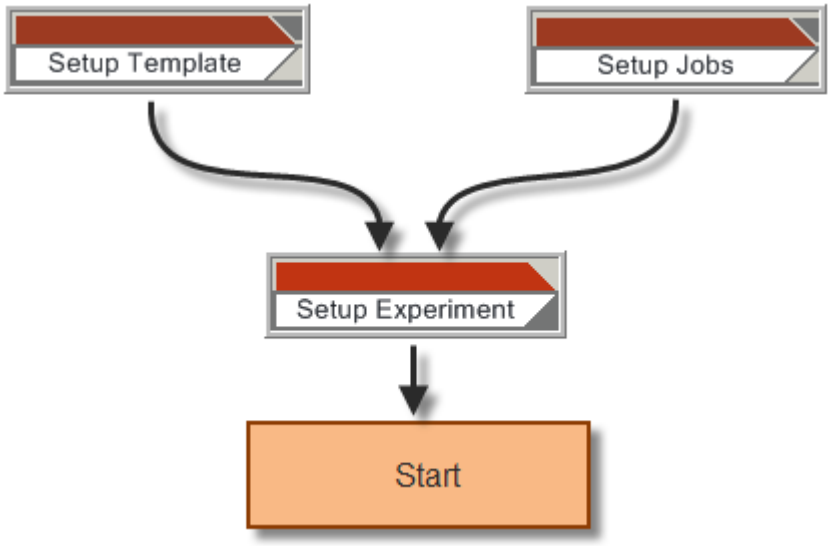
The MatrixScreener Wizard starts with the **Select App.** operating step, in which you can select the desired application.

In the **Setup Template** operating step, you can configure the basic settings for your experiment by creating a Scanning Template or selecting an existing one. This defines the spatial structure of your experiment.

In the **Setup Jobs** operating step, you can configure the settings for image acquisition and define and organize jobs and experiments (**Pattern**). Experiments can consist of multiple items (jobs, pauses and other **Pattern**s) that are processed in succession.

In the **Setup Experiment** operating step, you can merge the settings from the **Setup Template** and **Setup Jobs** operating steps, i.e. you can assign jobs and experiments to the Scanning Template.

The basic principle of the MatrixScreener applications arises from this structure:



Note: You can save the experiments of the MatrixScreener applications and reload them later. If you have defined and saved an experiment, then you can begin directly with the **Setup Experiment** operating step: load the saved experiment, put on the specimen slide, and start the experiment.

Tip: If the experiments of the MatrixScreener applications are saved on a network drive, all connected MatrixScreener systems can access them. This way an experiment can be used directly by a connected system without the need for other settings, if this system has the same hardware configuration as the system on which the experiment was defined.

See also:
Applications of the MatrixScreener Wizard
MatrixScreener Wizard: Global settings
MatrixScreener Wizard: Scan field functions
MatrixScreener Wizard: Setup Template
MatrixScreener Wizard: Setup Jobs
MatrixScreener Wizard: Setup Experiment

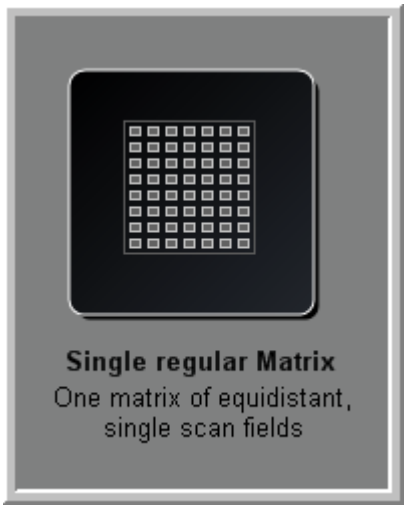
Applications of the MatrixScreener Wizard

Mosaic Applications

This includes all applications related to joining many individual images together to create an image mosaic. Depending on the requirements of your experiment, you can select the suitable application here.

Matrix Screener Applications

This includes all applications related to carrying out experiments that run at different positions. Depending on the requirements of your experiment, you can select the suitable application here.

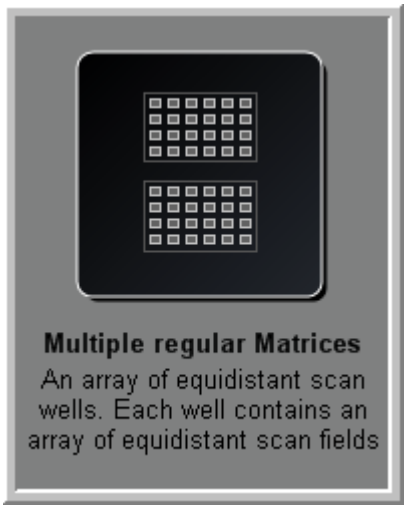


Single regular Matrix

The **Single regular Matrix** application is the simplest of the **Matrix Screener Applications**. With this application you can define only one matrix, which can consist of multiple scan fields. For that reason, this application is primarily suited for chambered coverglasses with one chamber and for fixed specimens on a regular specimen slide.

To get a quick start using this application, you can use the following Quick Tutorial:

MatrixScreener Wizard Quick Tutorial: Single regular Matrix



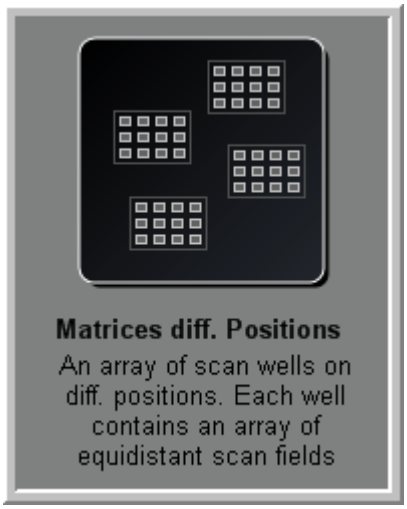
Multiple regular Matrices

With the **Multiple regular Matrices** application you can define a series of matrices, and each matrix can consist of multiple scan fields. The number of scan fields is the same in each well or chamber of the specimen slide. For that reason, this application is primarily suited for well plates and chambered coverglasses.

To get a quick start using this application, you can use the following Quick Tutorial:

MatrixScreener Wizard Quick Tutorial: Multiple regular Matrices

Matrices diff. Positions



With the **Matrices diff. Positions** application you can define multiple matrices in various positions, and each matrix can consist of multiple scan fields. The number of scan fields is the same in each well or chamber of the specimen slide. The starting point of a matrix can be individually adjusted. For that reason, this application is primarily suited for well plates and Tissue Micro Arrays (TMA).

To get a quick start using this application, you can use the following Quick Tutorial:

MatrixScreener Wizard Quick Tutorial: Matrices diff. Positions

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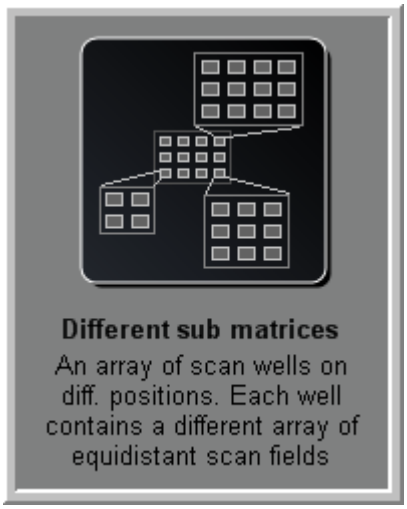
Different sub matrices

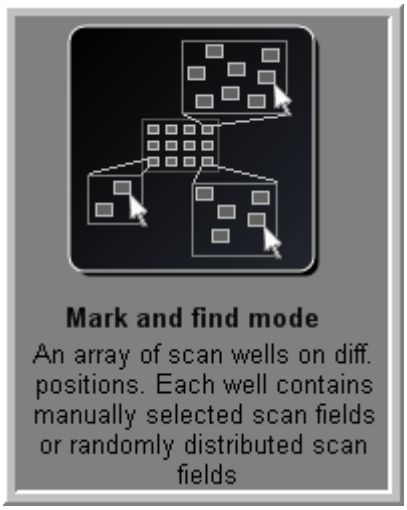


With the **Different sub matrices** application you can define multiple matrices in various positions, and each matrix can consist of multiple scan fields. Each well or chamber of the specimen slide can include an individual number of scan fields. The starting point of a matrix can be individually adjusted. For that reason, this application is primarily suited for mixed specimens and for tissue sections of different sizes, which are applied to the specimen slide at various places.

To get a quick start using this application, you can use the following Quick Tutorial:

MatrixScreener Wizard Quick Tutorial: Different sub matrices

Mark and find mode



The **Mark and find mode** application is intended for implementing multi-position experiments, i.e. certain positions can be marked in the specimen and be approached with the specimen stage in sequence during the experiment. You can define multiple matrices in various positions, and each matrix can consist of multiple scan fields. The starting points in the matrices can be defined in advance. You can (manually or randomly) assign the Mark & Find positions to the scan fields in the wells or chambers of the specimen slide. Scan fields without assigned Mark & Find positions are automatically deactivated and thus excluded from the image acquisition. For that reason, this application is primarily suited for experiments in which individual cells of the specimen are manually selected in certain wells or chambers of the specimen slide.

To get a quick start using this application, you can use the following Quick Tutorial:

See also:
Structure of the MatrixScreener Wizard
MatrixScreener Wizard: Global settings
MatrixScreener Wizard: Scan field functions
MatrixScreener Wizard: Setup Template
MatrixScreener Wizard: Setup Jobs
MatrixScreener Wizard: Setup Experiment

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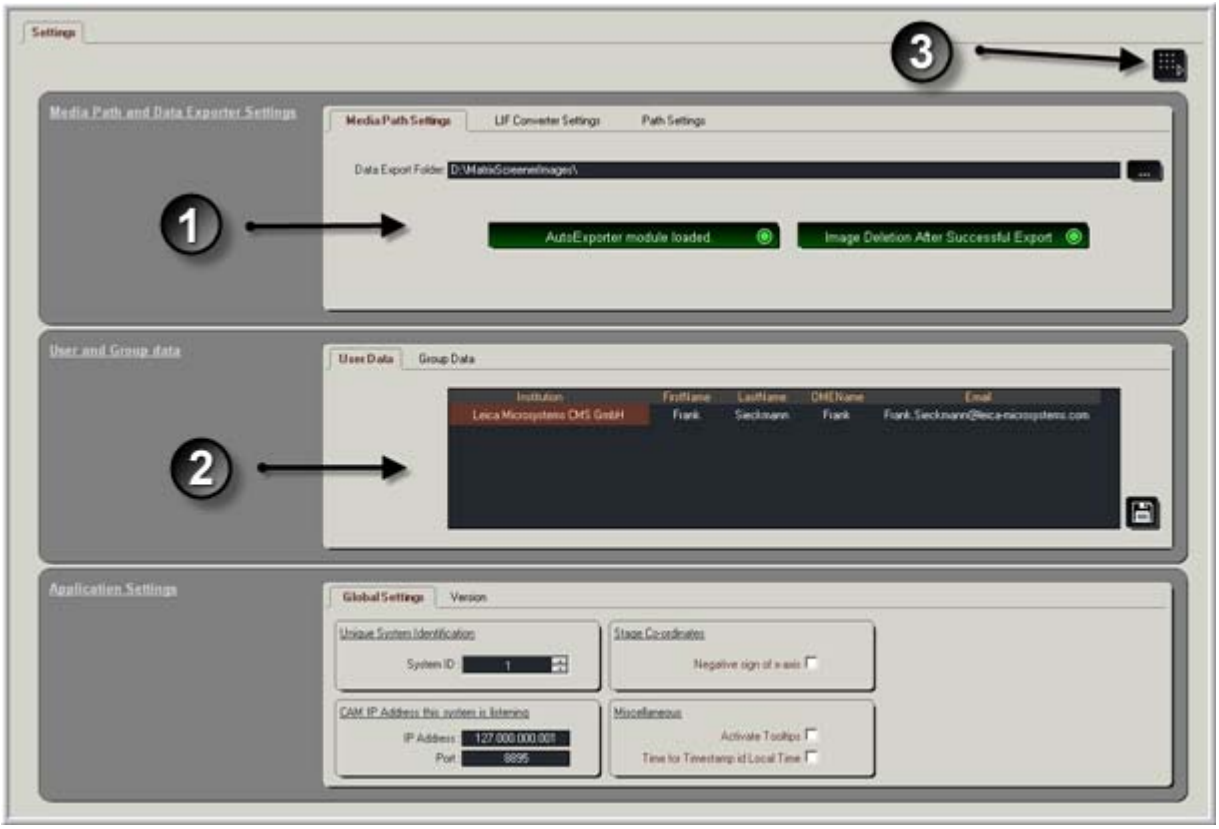
MatrixScreener Wizard: Global settings

In addition to the individual settings for the respective MatrixScreener application, there are global settings that apply for all MatrixScreener applications. To access the dialogs in which can you can configure or modify these global settings, click the button with the tool symbol on the start page of MatrixScreener Wizard (**Select App.** operating step).

The following settings are globally valid:

- Storage location and file structure for the MatrixScreener images (including Metadata)
- Storage location for Scanning Templates
- Information about the users



Media Path and Data Exporter Settings

Define the storage locations and file structures here, for example for the MatrixScreener images (including Metadata) and for Scanning Templates.

User and Group Data

Here, enter information about the users (Experimenter and Group according to OME).

Click this button to return to the MatrixScreener Wizard start page (**Select App.** operating step).

See also:

MatrixScreener Wizard: Media Path Settings
MatrixScreener Wizard: LIF Converter Settings
MatrixScreener Wizard: Path Settings
MatrixScreener Wizard: User Data
MatrixScreener Wizard: Group Data

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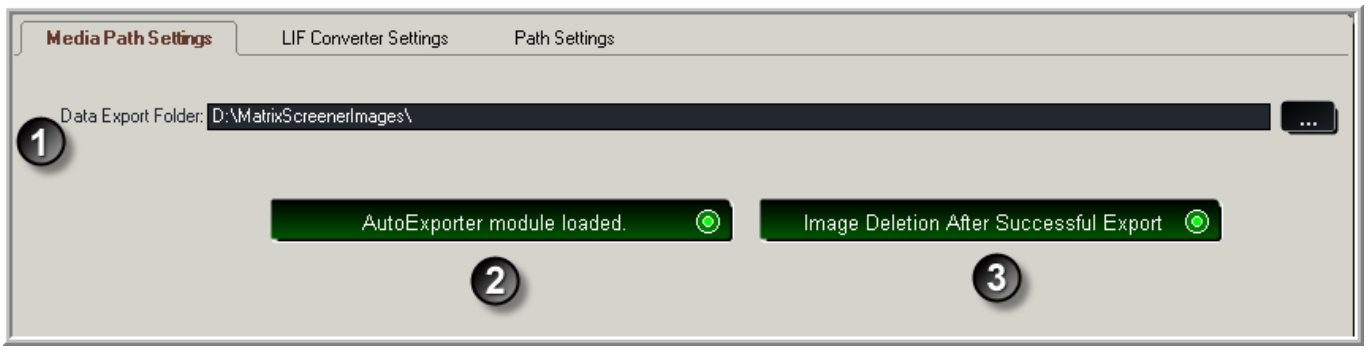
MatrixScreener Wizard: Media Path Settings



Data Export Folder

1

Here, you can define the path to the storage location for your experiment data. This path is called the **Data Export Folder** and is part of the total path, i.e. it specifies the location of the file structure in which the MatrixScreener images and the corresponding Metadata are stored. The **Data Export Folder** can link to any destination drive, such as the local hard disk drive of the workstation (default setting), a network drive or a USB drive.

IMPORTANT: MatrixScreener Wizard uses the **Data Export Folder** only if the **AutoExporter module** is loaded.

AutoExporter module

2

Here, you can load the **AutoExporter module**, which ensures that the MatrixScreener images and the corresponding Metadata are stored in the background, i.e. without impairing the actual scanning operation.

Note: You can adapt the file structure for the data to be stored to the requirements of your experiment in the **LIF Converter Settings** tab.

Image Deletion After Successful Export

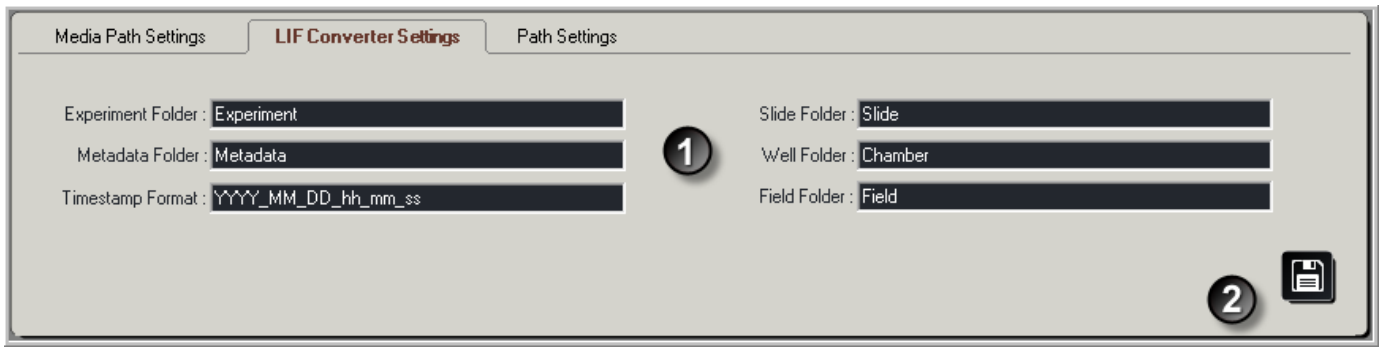
3

You can enable the **Image Deletion After Successful Export** function here. This ensures that the MatrixScreener images already stored in the file structure are deleted from the **Experiments** tab in LAS AF. This is particularly recommended during long experiments with a large number of images, as otherwise the system memory (RAM) of the workstation available for an experiment will gradually be used up.

Note: If you have loaded the **AutoExporter module**, you should also always enable the **Image Deletion After Successful Export** function.

See also:
MatrixScreener Wizard: Global settings
MatrixScreener Wizard: LIF Converter Settings
MatrixScreener Wizard: Path Settings
MatrixScreener Wizard: User Data
MatrixScreener Wizard: Group Data

MatrixScreener Wizard: LIF Converter Settings



1 Here, you can adapt the file structure in which the MatrixScreener images and the corresponding Metadata are stored according to the requirements of your experiment. The file structure itself is predefined, but you can name the main directory (**Experiment Folder**), subdirectories (**Slide Folder**, **Well Folder**, **Field Folder**) and the directory for the Metadata (**Metadata Folder**) as desired. In addition, under **Timestamp Format**, you can define the format in which a real time stamp is appended to the name of the main directory.

IMPORTANT: MatrixScreener Wizard uses the settings configured here only if the **AutoExporter module** is loaded. You can load the **AutoExporter module** in the **Media Path Settings** tab.

2 This button saves the settings you have configured.

Note: When saving modified settings, LAS AF automatically creates a backup copy of the original settings.

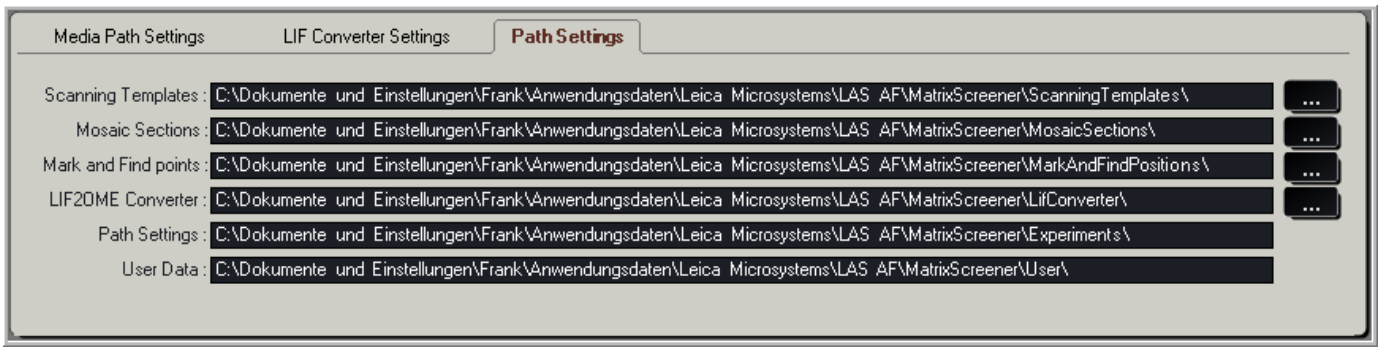
Important details

As the name **LIF Converter Settings** implies, the LIF data generated by LAS AF are converted when stored to the file structure, to the OME-compatible file formats OME.TIFF (images with embedded Metadata) and OME.XML (Metadata).

The **LIF Converter Settings** include an XML file (LIFCONVERTER.XML) that controls the manner in which the MatrixScreener images and the corresponding Metadata are stored in the file structure. You edit this file by naming the main directory and subdirectories and defining the format for the real time stamp. The LIFCONVERTER.XML file is stored in every Windows user directory; therefore, each user can configure his or her own settings under **LIF Converter Settings** and store his or her experiment data individually.

See also:
MatrixScreener Wizard: Global settings
MatrixScreener Wizard: Media Path Settings
MatrixScreener Wizard: Path Settings
MatrixScreener Wizard: User Data
MatrixScreener Wizard: Group Data

MatrixScreener Wizard: Path Settings



Here, enter the paths for working with the MatrixScreener Wizard:

- **Scanning Templates**: Storage location of the Scanning Templates
- **Mosaic Sections**: Storage location of the manually selected mosaic sections
- **Mark and Find Points**: Storage location of the manually selected mark & find positions
- **LIF2OME Converter**: Storage location of the **LIF Converter Settings**
- **Path Settings**: Storage location of the paths for working with the MatrixScreener Wizard
- **User Data**: Storage location of the information about the users

The paths can refer to any destination drive, e.g. to the local hard disk drive of the workstation (default setting: Windows user directory), a network drive or a USB drive.

Tip: If multiple users work jointly on experiments on different MatrixScreener systems at the same time, we recommend saving the Scanning Templates on a network drive. This creates a joint platform that can be used by the connected MatrixScreener systems for experiments.

Important details

Your settings and information about experiments, users etc. are saved primarily in XML format. The XML files are self-descriptive in that before each file name, a meta tag appears in the form of a defined keyword in curly brackets (human-readable design). The keywords can help you identify a XML file at first glance and assign it to an experiment. You can also search for a certain keyword using Windows search. The following table provides some examples.

File type	Meta tag	Description / file content
{Experimenter}UserData.xml	{Experimenter}	Information about the user (Experimenter according to OME)
{Group}GroupData.xml	{Group}	Information about the user group (Group according to OME)
{MatrixScreenerData}ApplicationPaths.xml	{MatrixScreenerData}	Paths for working with the MatrixScreener Wizard
{ScanningTemplate}MatrixApp0.xml {ScanningTemplate}MatrixApp0.lrp	{ScanningTemplate}	Scanning Template Job settings
{MosaicSections}MosaicSections.xml	{MosaicSections}	Manually selected mosaic sections
{MarkAndFind}MarkandFind.xml	{MarkAndFind}	Manually selected mark & find positions

See also:
MatrixScreener Wizard: Global settings
MatrixScreener Wizard: Media Path Settings
MatrixScreener Wizard: LIF Converter Settings
MatrixScreener Wizard: User Data
MatrixScreener Wizard: Group Data

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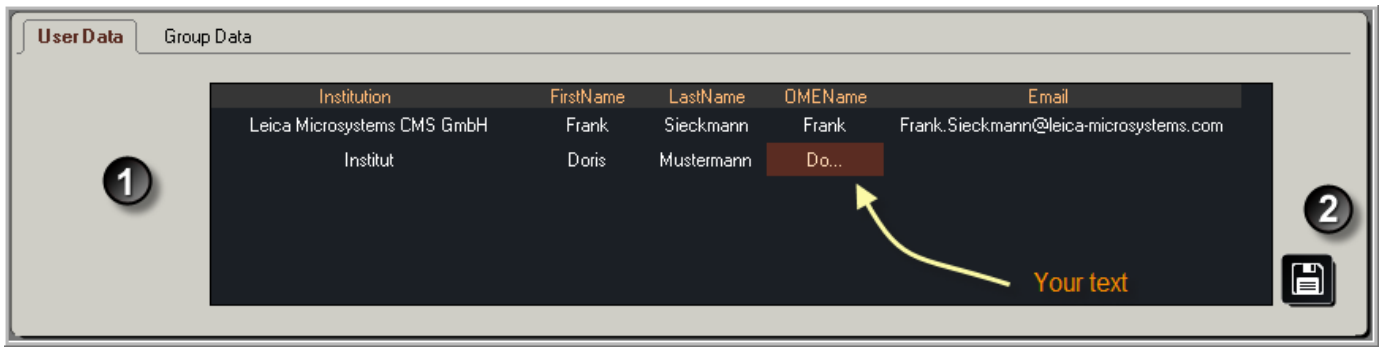
MatrixScreener Wizard: User Data



1 Here, you can create the user (Experimenter according to OME) for an experiment and enter all required information about the user. In case more than one user is working on one experiment, you can add users to the list: double-clicking the blank line under an existing user creates another user.

2 You can use this button to save your settings.

See also:

MatrixScreener Wizard: Global settings
MatrixScreener Wizard: Media Path Settings
MatrixScreener Wizard: LIF Converter Settings
MatrixScreener Wizard: Path Settings
MatrixScreener Wizard: Group Data

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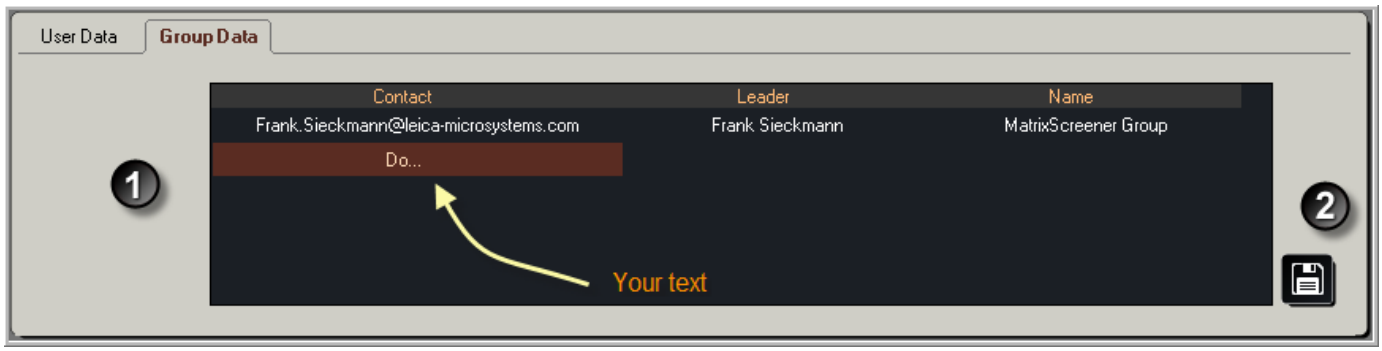
MatrixScreener Wizard: Group Data



1 Here, you can create a user group (Group according to OME) for an experiment and enter all required information about the user group. By default, only one user group is defined for each experiment; however, you can add groups to the list if necessary: double-clicking the blank line under an existing user group creates another user group.

2 You can use this button to save your settings.

See also:
MatrixScreener Wizard: Global settings
MatrixScreener Wizard: Media Path Settings
MatrixScreener Wizard: LIF Converter Settings
MatrixScreener Wizard: Path Settings
MatrixScreener Wizard: User Data

MatrixScreener Wizard: Scan field functions

Each scan field in a well can have functions that were assigned to it by the user as part of an experiment. Note that one scan field can have multiple different functions, but any given function can only be assigned to each scan field once. If a scan field has been assigned a function, this scan field appears with a colored outline in the **Scanning Template** view. The following functions are available with the corresponding color coding:

Pink outline: **Autofocus** scan field (automatic focus search)

Blue outline: **Drift Compensation** scan field (compensation for any focus drift that may occur)

Yellow outline: **Single Object Tracking** scan field (scan field for object tracking)

Green outline: **Pump** scan field (water replenishment when using water as the immersion medium)

Red outline: Scan field that is disabled and thus excluded from the image acquisition

If multiple functions have been assigned to one scan field, LAS AF automatically defines a logical sequence for executing them. For example, compensation of focus drift is executed before object tracking, and water replenishment is executed before the automatic focus search.

Note: The **Single Object Tracking** function is available in the **MatrixScreener Applications** only (not in the **Mosaic Applications**).

Note: The Water Immersion Micro Dispenser is required for the **Pump** function. When using a water immersion objective, the Water Immersion Micro Dispenser provides for an automatic supply of water immersion during long-term experiments and live cell experiments.

Note: In the **Mosaic Applications**, only entire mosaic areas can be disabled (not individual scan fields).

See also:
MatrixScreener Wizard: Assigning scan field functions
MatrixScreener Wizard: Assigning the Autofocus function
MatrixScreener Wizard: Assigning the Drift Compensation function

MatrixScreener Wizard: Assigning the Single Object Tracking function
MatrixScreener Wizard: Assigning the Pump function
MatrixScreener Wizard: Disabling and re-enabling scan fields

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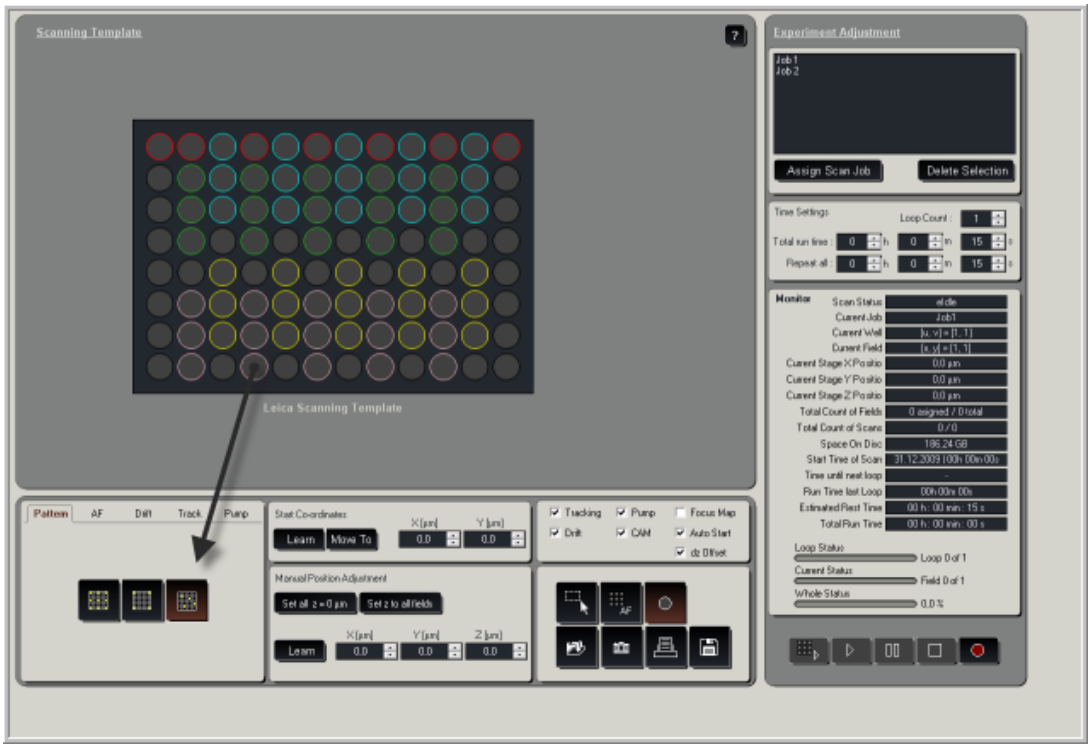
MatrixScreener Wizard: Assigning scan field functions

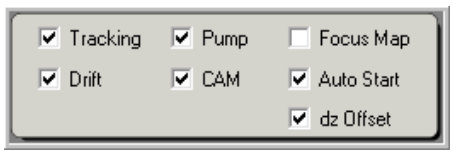
Brief description: Each scan field in a well can have functions that were assigned to it by the user as part of an experiment. You can assign the functions using either the LAS AF graphical user interface or using the keyboard.

Assigning scan field functions using the LAS AF graphical user interface

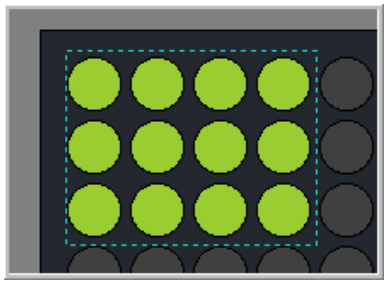
For both **Mosaic Applications** and **MatrixScreener Applications**, the dialogs in which you can assign certain functions to scan fields are located on the bottom left in the last operating step **Setup Experiment**:



Before you can assign a function to one or more scan fields, both of the following requirements must be in place:

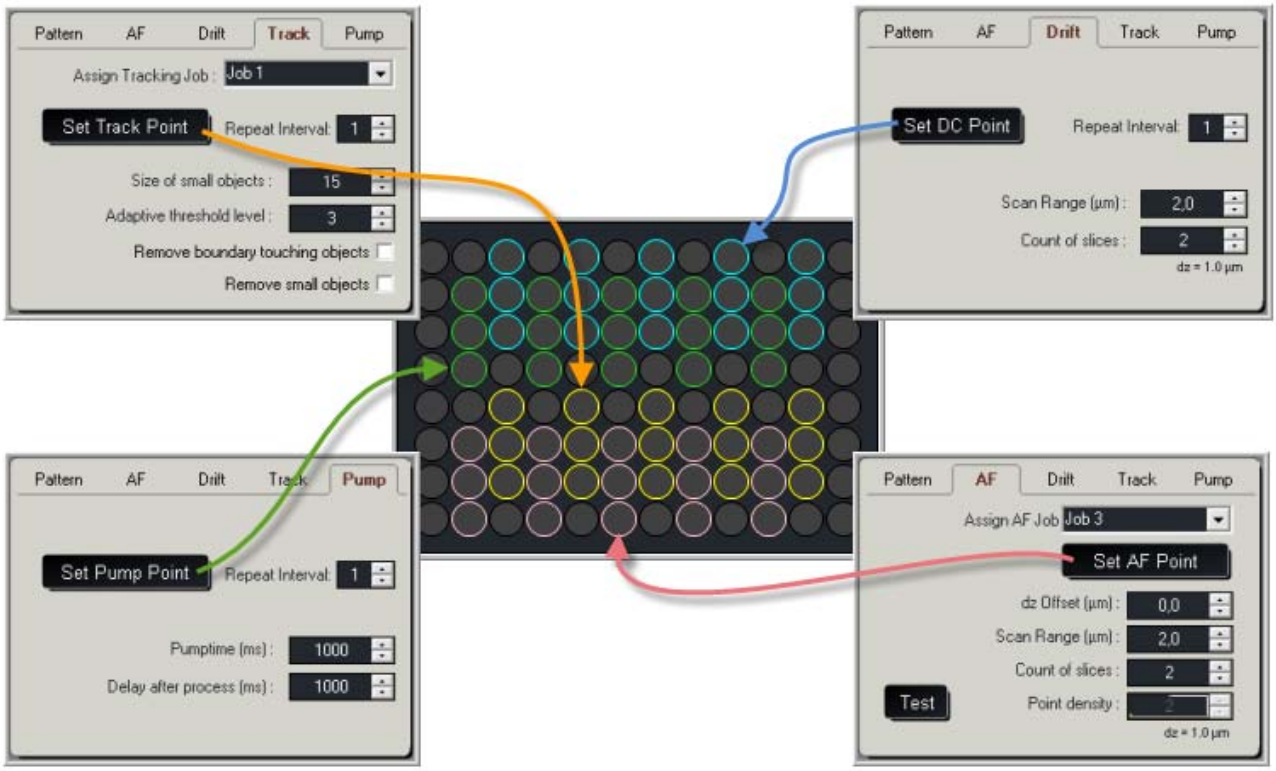


The desired scan field functions must be enabled. You can likewise enable the scan field functions in the last operating step **Setup Experiment**.



The desired scan fields must be selected. You can select an individual scan field by clicking it in the **Scanning Template** view. To select multiple scan fields, you can either hold down the Shift key while clicking all desired scan fields in succession in the **Scanning Template** view or hold down the left mouse button while drawing a rectangle over the scan fields to be selected. Selected scan fields are shown in light green.

If both of these requirements are met, you can assign the desired scan field functions to the selected scan fields.



IMPORTANT: The settings for a certain scan field function apply to all scan fields to which this scan field function is assigned. If, for example, 10 scan fields have the **Pump** scan field function (water replenishment when using water as the immersion medium) and you change the duration for water replenishment for a water immersion objective under **Pumptime**, the modified setting applies to all 10 scan fields. This applies correspondingly for the other scan field functions.

Note: If a certain scan field function is assigned to a scan field, you can remove the assignment by reassigning the respective scan field function to the scan field. In this way, you can toggle back and forth between two states, i.e. scan field function enabled and disabled (so-called toggling: assigning a scan field function inverts the current state).

Assigning scan field functions using the keyboard

You can also assign scan field functions using the keyboard, which makes assignment easier and faster, particularly for a large number of scan fields (if you assign scan field functions using the keyboard, the motorized specimen stage is not moved). You can assign scan field functions using the following keys:

To assign the **Autofocus** scan field function (automatic focus search) to a scan field or remove this assignment, hold down the **A** key and click the desired scan field in the **Scanning Template** view.

To assign the **Drift Compensation** scan field function (compensation for any focus drift that may occur) to a scan field or remove this assignment, hold down the **D** key and click the desired scan field in the **Scanning Template** view.

To assign the **Single Object Tracking** scan field function (object tracking) to a scan field or remove this assignment, hold down the **T** key and click the desired scan field in the **Scanning Template** view.

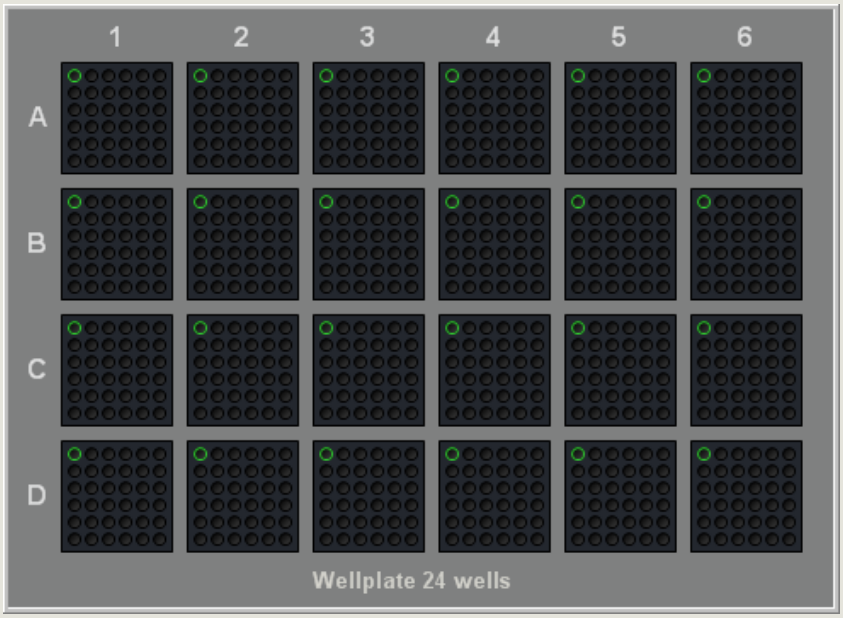
To assign the **Pump** scan field function (water replenishment when using water as the

immersion medium) to a scan field or remove this assignment, hold down the **P** key and click the desired scan field in the **Scanning Template** view.

To disable a scan field and thus exclude it from the image acquisition or re-enable a disabled scan field, hold down the **E** key and click the desired scan field in the **Scanning Template** view.

Example

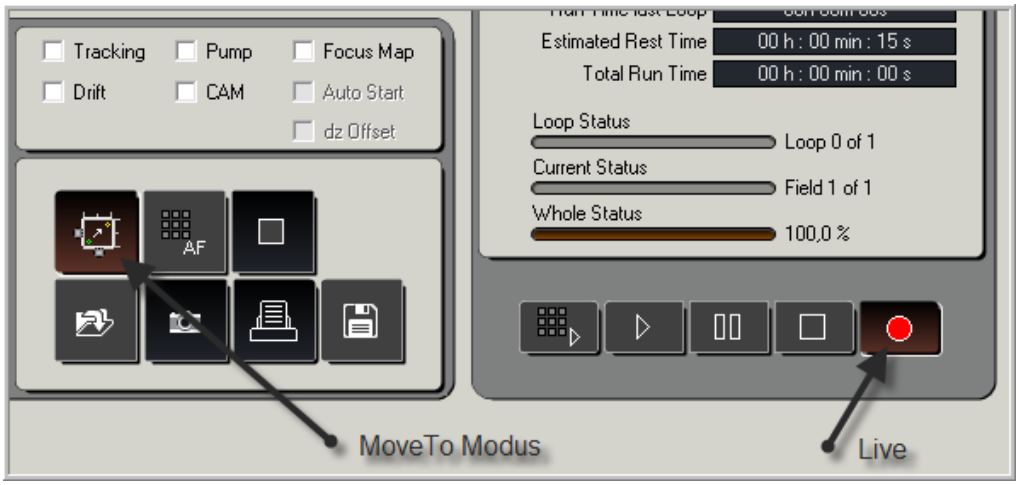
You are using a well plate with 24 wells and have positioned 6 × 6 scan fields in each well. The first scan field in each well has the **Pump** scan field function (water replenishment when using water as the immersion medium), i.e. whenever moving to the first scan field of a well, a little water is to be replenished on the water immersion objective first before the actual scan operation in this well begins. The resulting pattern in the **Scanning Template** view then looks something like this:



Reviewing assigned scan field functions

You can review assigned scan field functions using the **Live** image acquisition, i.e. you can review whether a selected scan field contains information that is relevant to the experiment. For example, it is not logical to assign a scan field the **Autofocus** scan field function (automatic focus search) or the **Drift Compensation** scan field function (compensation for any focus drift that may occur) if no information that is relevant to the experiment is present there and thus acquired images do not contain any information that can be used for the automatic focus search.

To review an assigned scan field function, enable the **Move To** mode (if the **Move To** mode is enabled, the current position of the motorized specimen stage is marked by a scan field that is flashing green in the **Scanning Template** view). Clicking the desired scan field moves the specimen stage to the corresponding position. There, you can use the **Live** image acquisition to check whether the scan field contains information relevant to the experiment.



See also:

[MatrixScreener Wizard: Scan field functions]

[MatrixScreener Wizard: Assigning the Autofocus function]

[MatrixScreener Wizard: Assigning the Drift Compensation function]

[MatrixScreener Wizard: Assigning the Single Object Tracking function]

[MatrixScreener Wizard: Assigning the Pump function]

[MatrixScreener Wizard: Disabling and re-enabling scan fields]

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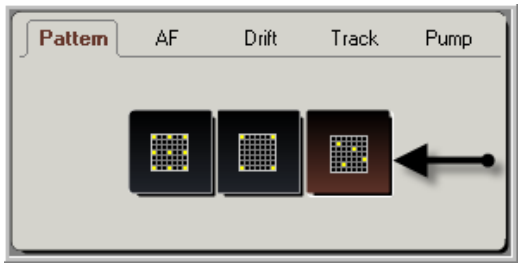
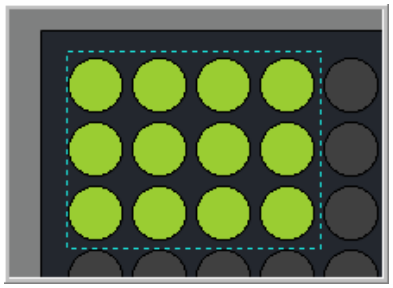
MatrixScreener Wizard: Assigning the Autofocus function

Before you can assign the **Autofocus** scan field function (automatic focus search) to one or more scan fields, both of the following requirements must be in place:

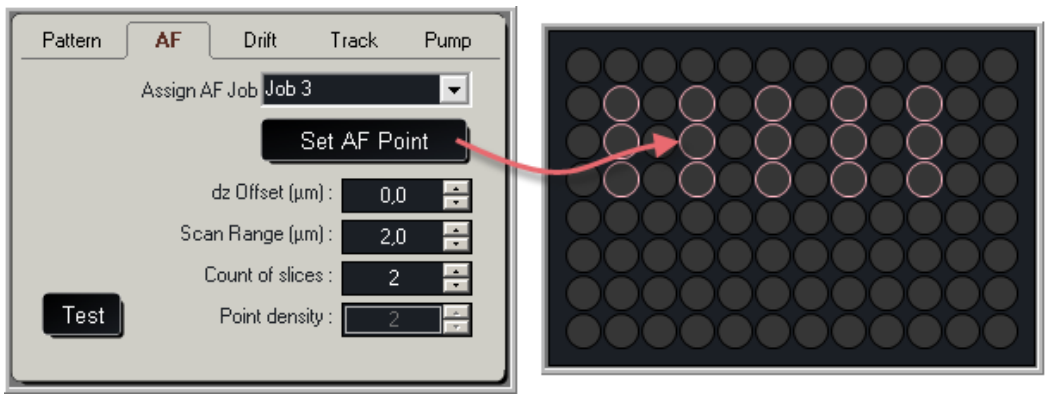


The **Individual Autofocus Measurement Points** function must be enabled in the **Pattern** tab. You enable this function in the last operating step **Setup Experiment**.



The desired scan fields must be selected. You can select an individual scan field by clicking it in the **Scanning Template** view. To select multiple scan fields, you can either hold down the Shift key while clicking all desired scan fields in succession in the **Scanning Template** view or hold down the left mouse button while drawing a rectangle over the scan fields to be selected. Selected scan fields are shown in light green.

If both of these requirements are met, you can assign the **Autofocus** scan field function to the selected scan fields. To do so, in the last operating step **Setup Experiment**, go to the **AF** tab at the bottom left and click the **Set AF Point** button.



Autofocus scan fields appear outlined in pink in the **Scanning Template** view.

Note: You can remove the **Autofocus** scan field function from a scan field by assigning it this function again (so-called toggling: assigning a scan field function inverts the current state).

You can also assign the **Autofocus** scan field function to a scan field, or remove this assignment, using the keyboard by holding down the **A** key and clicking the desired scan field in the **Scanning Template** view.

See also:
MatrixScreener Wizard: Autofocus settings
MatrixScreener Wizard: Scan field functions

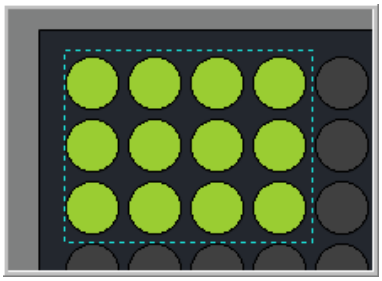
MatrixScreener Wizard: Assigning scan field functions
MatrixScreener Wizard: Assigning the Drift Compensation function
MatrixScreener Wizard: Assigning the Single Object Tracking function
MatrixScreener Wizard: Assigning the Pump function
MatrixScreener Wizard: Disabling and re-enabling scan fields

MatrixScreener Wizard: Assigning the Drift Compensation function

Note: The **Drift Compensation** function is, in principle, an automatic focus search that corrects any focus drift that may occur in the z-direction. A focus drift can occur due to temperature fluctuations, for example.

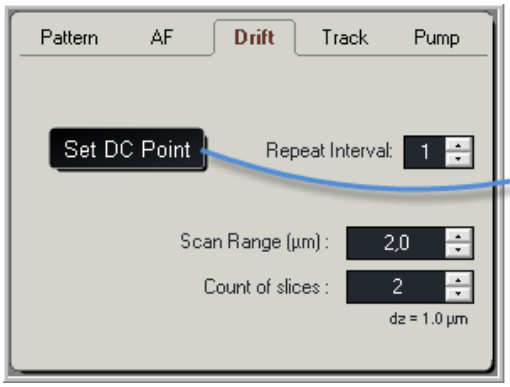
Before you can assign the **Drift Compensation** scan field function (compensation for any focus drift that may occur) to one or more scan fields, both of the following requirements must be in place:

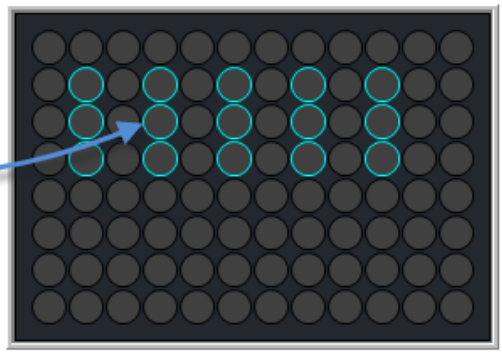
The **Drift Compensation** scan field function must be enabled. You enable this function in the last operating step **Setup Experiment**.



The desired scan fields must be selected. You can select an individual scan field by clicking it in the **Scanning Template** view. To select multiple scan fields, you can either hold down the Shift key while clicking all desired scan fields in succession in the **Scanning Template** view or hold down the left mouse button while drawing a rectangle over the scan fields to be selected. Selected scan fields are shown in light green.

If both of these requirements are met, you can assign the **Drift Compensation** scan field function to the selected scan fields. To do so, in the last operating step **Setup Experiment**, go to the **Drift** tab at the bottom left and click the **Set DC Point** button.





Drift Compensation scan fields appear outlined in blue in the **Scanning Template** view.

Note: You can remove the **Drift Compensation** scan field function from a scan field by assigning it this function again (so-called toggling: assigning a scan field function inverts the current state).

You can also assign the **Drift Compensation** scan field function to a scan field, or remove this assignment, using the keyboard by holding down the **D** key and clicking the desired scan field in the **Scanning Template** view.

See also:

MatrixScreener Wizard: Drift Compensation settings
MatrixScreener Wizard: Scan field functions
MatrixScreener Wizard: Assigning scan field functions
MatrixScreener Wizard: Assigning the Autofocus function

MatrixScreener Wizard: Assigning the Single Object Tracking function
MatrixScreener Wizard: Assigning the Pump function
MatrixScreener Wizard: Disabling and re-enabling scan fields

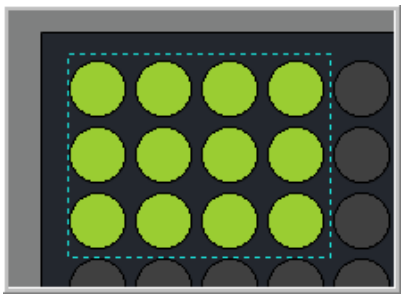
MatrixScreener Wizard: Assigning the Single Object Tracking function

Note: The **Single Object Tracking** function is available in the **MatrixScreener Applications** only (not in the **Mosaic Applications**).

Note: The **Single Object Tracking** always follows only one object in the image, preferably the largest and brightest.

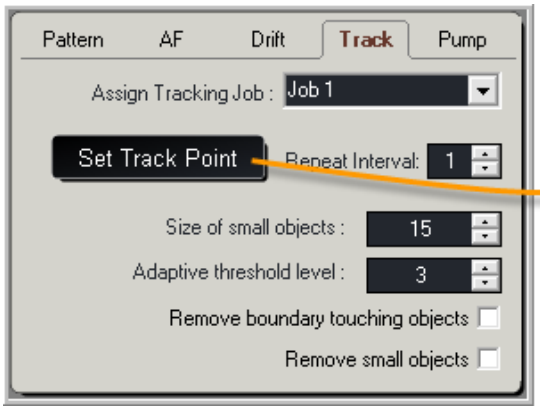
Before you can assign the **Single Object Tracking** scan field function (object tracking) to one or more scan fields, both of the following requirements must be in place:

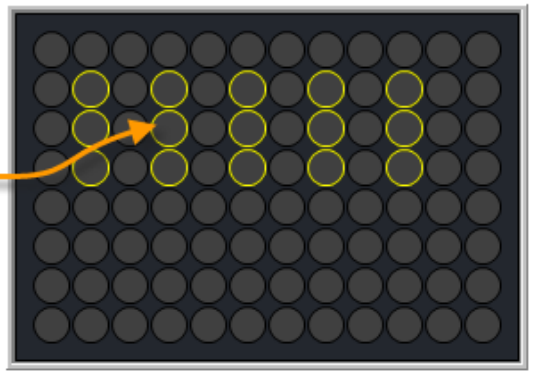
The **Single Object Tracking** scan field function must be enabled. You enable this function in the last operating step **Setup Experiment**.



The desired scan fields must be selected. You can select an individual scan field by clicking it in the **Scanning Template** view. To select multiple scan fields, you can either hold down the Shift key while clicking all desired scan fields in succession in the **Scanning Template** view or hold down the left mouse button while drawing a rectangle over the scan fields to be selected. Selected scan fields are shown in light green.

If both of these requirements are met, you can assign the **Single Object Tracking** scan field function to the selected scan fields. To do so, in the last operating step **Setup Experiment**, go to the **Track** tab at the bottom left and click the **Set Track Point** button.





Single Object Tracking scan fields appear outlined in yellow in the **Scanning Template** view.

Note: You can remove the **Single Object Tracking** scan field function from a scan field by assigning it this function again (so-called toggling: assigning a scan field function inverts the current state).

You can also assign the **Single Object Tracking** scan field function to a scan field, or remove this assignment, using the keyboard by holding down the **T** key and clicking the desired scan field in the **Scanning Template** view.

See also:

[MatrixScreener Wizard: Single Object Tracking settings]
[MatrixScreener Wizard: Scan field functions]
[MatrixScreener Wizard: Assigning scan field functions]
[MatrixScreener Wizard: Assigning the Autofocus function]
[MatrixScreener Wizard: Assigning the Drift Compensation function]
[MatrixScreener Wizard: Assigning the Pump function]
[MatrixScreener Wizard: Disabling and re-enabling scan fields]

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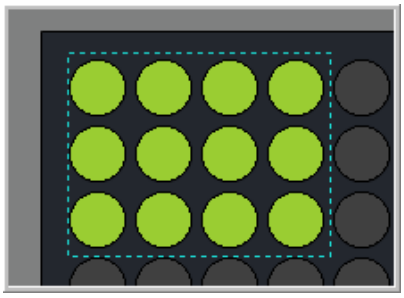
MatrixScreener Wizard: Assigning the Pump function

Note: The Water Immersion Micro Dispenser is required for the **Pump** function. When using a water immersion objective, the Water Immersion Micro Dispenser provides for an automatic supply of water immersion during long-term experiments and live cell experiments.

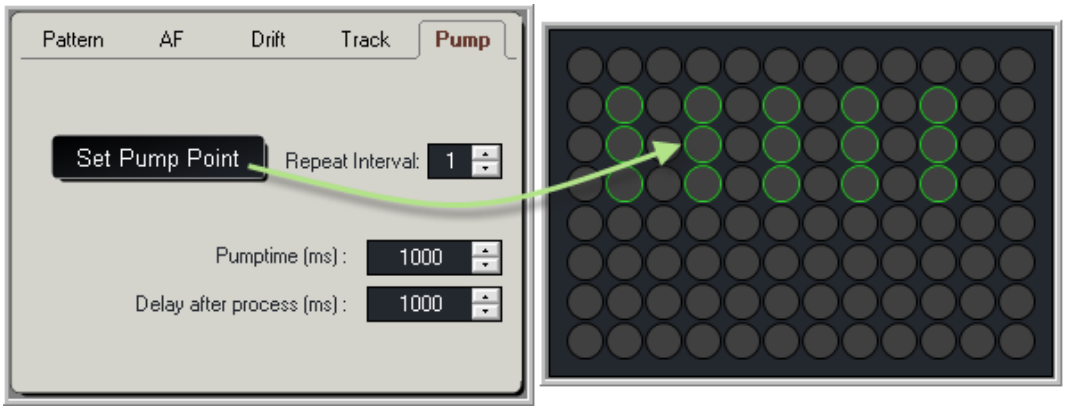
Before you can assign the **Pump** scan field function (water replenishment when using water as the immersion medium) to one or more scan fields, both of the following requirements must be in place:

The **Pump** scan field function must be enabled. You enable this function in the last operating step **Setup Experiment**.



The desired scan fields must be selected. You can select an individual scan field by clicking it in the **Scanning Template** view. To select multiple scan fields, you can either hold down the Shift key while clicking all desired scan fields in succession in the **Scanning Template** view or hold down the left mouse button while drawing a rectangle over the scan fields to be selected. Selected scan fields are shown in light green.

If both of these requirements are met, you can assign the **Pump** scan field function to the selected scan fields. To do so, in the last opeating step **Setup Experiment**, go to the **Pump** tab at the bottom left and click the **Set Pump Point** button.



Pump scan fields appear outlined in green in the **Scanning Template** view.

Note: Under **Delay after process**, you can set a delay in order to allow time for the specimen slide to set before the actual scanning operation begins.

Note: You can remove the **Pump** scan field function from a scan field by assigning it this function again (so-called toggling: assigning a scan field function inverts the current state).

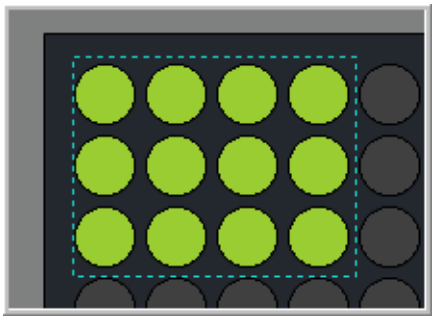
You can also assign the **Pump** scan field function to a scan field, or remove this assignment, using the keyboard by holding down the **P** key and clicking the desired scan field in the **Scanning Template** view.

See also:
MatrixScreener Wizard: Pump settings
MatrixScreener Wizard: Scan field functions
MatrixScreener Wizard: Assigning scan field functions
MatrixScreener Wizard: Assigning the Autofocus function
MatrixScreener Wizard: Assigning the Drift Compensation function
MatrixScreener Wizard: Assigning the Single Object Tracking function
MatrixScreener Wizard: Disabling and re-enabling scan fields

MatrixScreener Wizard: Disabling and re-enabling scan fields

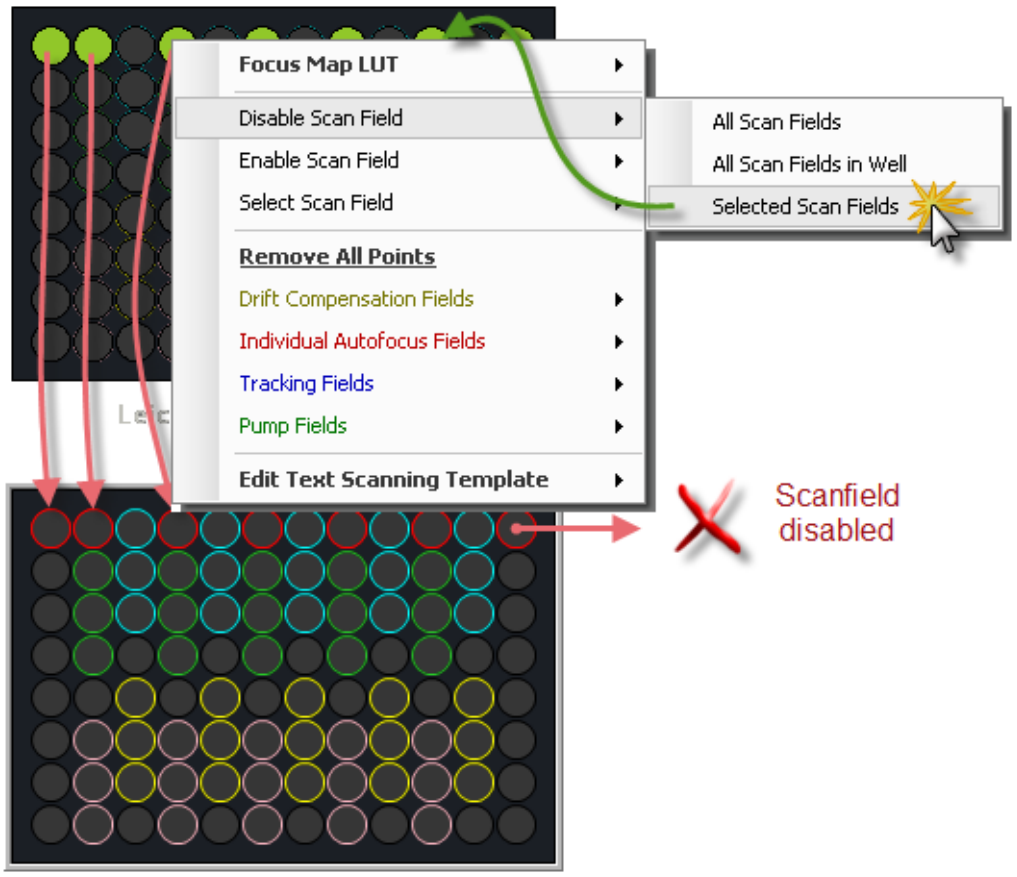
Note: In the **Mosaic Applications**, only entire mosaic areas can be disabled (not individual scan fields).

Before you can disable or re-enable one or more scan fields, the following requirement must be met:



The desired scan fields must be selected. You can select an individual scan field by clicking it in the **Scanning Template** view. To select multiple scan fields, you can either hold down the Shift key while clicking all desired scan fields in succession in the **Scanning Template** view or hold down the left mouse button while drawing a rectangle over the scan fields to be selected. Selected scan fields are shown in light green.

If this requirement is met, you can disable the selected scan fields. To do so, call up the context menu by right-clicking anywhere in the **Scanning Template** view and select **Disable Scan Field** > **Selected Scan Fields**.



Disabled scan fields appear outlined in red in the **Scanning Template** view.

To re-enable disabled scan fields, call up the context menu again by right-clicking anywhere in the **Scanning Template** view and select **Enable Scan Field** > **Selected Scan Fields**.

Note: Disabled scan fields are only temporarily excluded from the image acquisition. You can re-enable a scan field at any time - even while an experiment is in progress - in order to include it in the image acquisition.

You can also disable a scan field or re-enable one that is disabled by holding down the **E** key and clicking the desired scan field in the **Scanning Template** view.

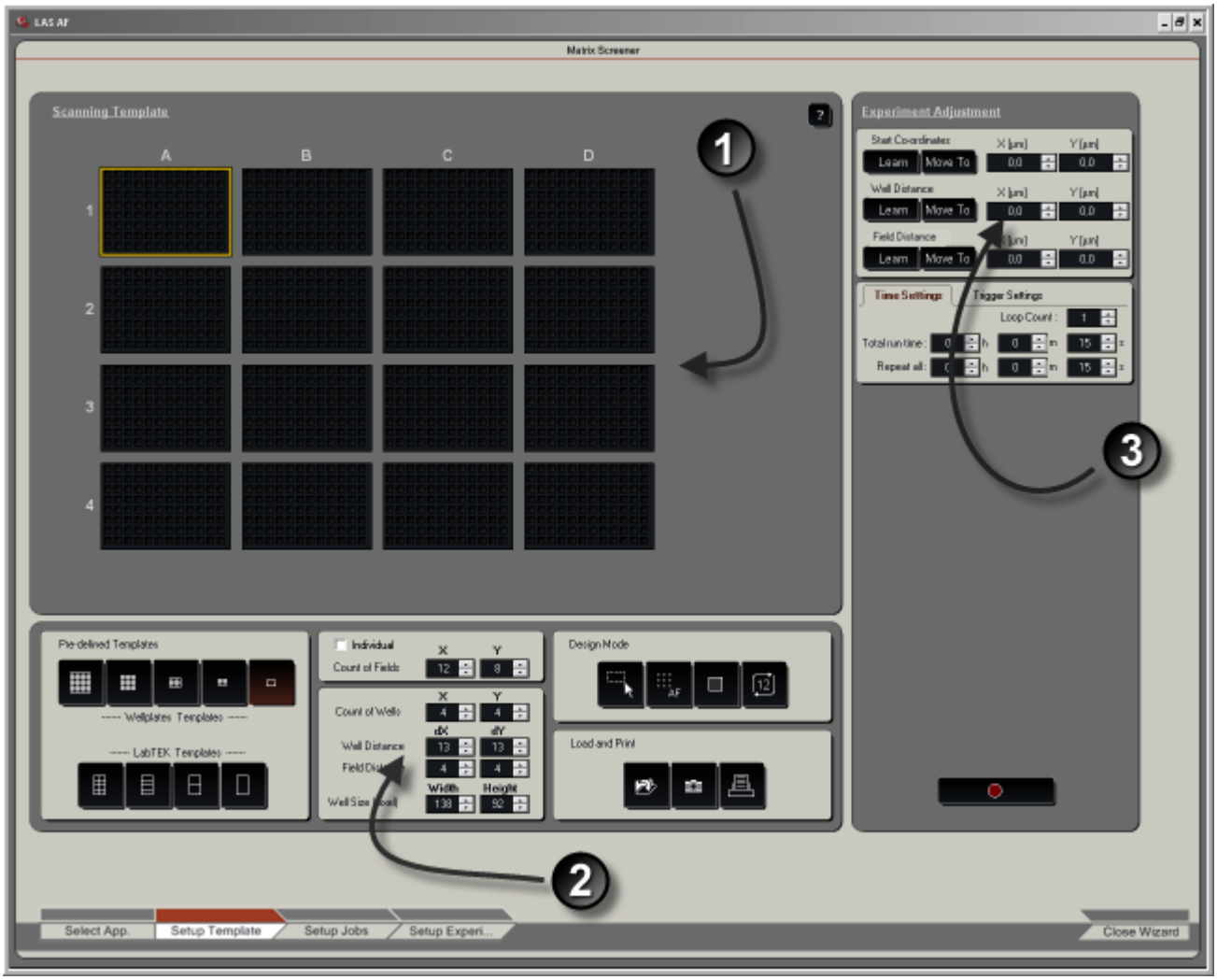
See also:
MatrixScreener Wizard: Scan field functions
MatrixScreener Wizard: Assigning scan field functions
MatrixScreener Wizard: Assigning the Autofocus function
MatrixScreener Wizard: Assigning the Drift Compensation function
MatrixScreener Wizard: Assigning the Single Object Tracking function
MatrixScreener Wizard: Assigning the Pump function

MatrixScreener Wizard: Setup Template

Note: First, select the suitable application for your experiment on the MatrixScreener Wizard start page (**Select App.** operating step) before switching to the **Setup Template** operating step.

In the **Setup Template** operating step, you can configure the basic settings for your experiment by creating a Scanning Template or selecting an existing one. This defines the spatial structure of your experiment.

- Type of specimen slide used (well plate or chambered coverglass)
- Number of wells or chambers and distances between the wells or chambers
- Number of scan fields, distances between the scan fields and positions of the scan fields (xy-coordinates)



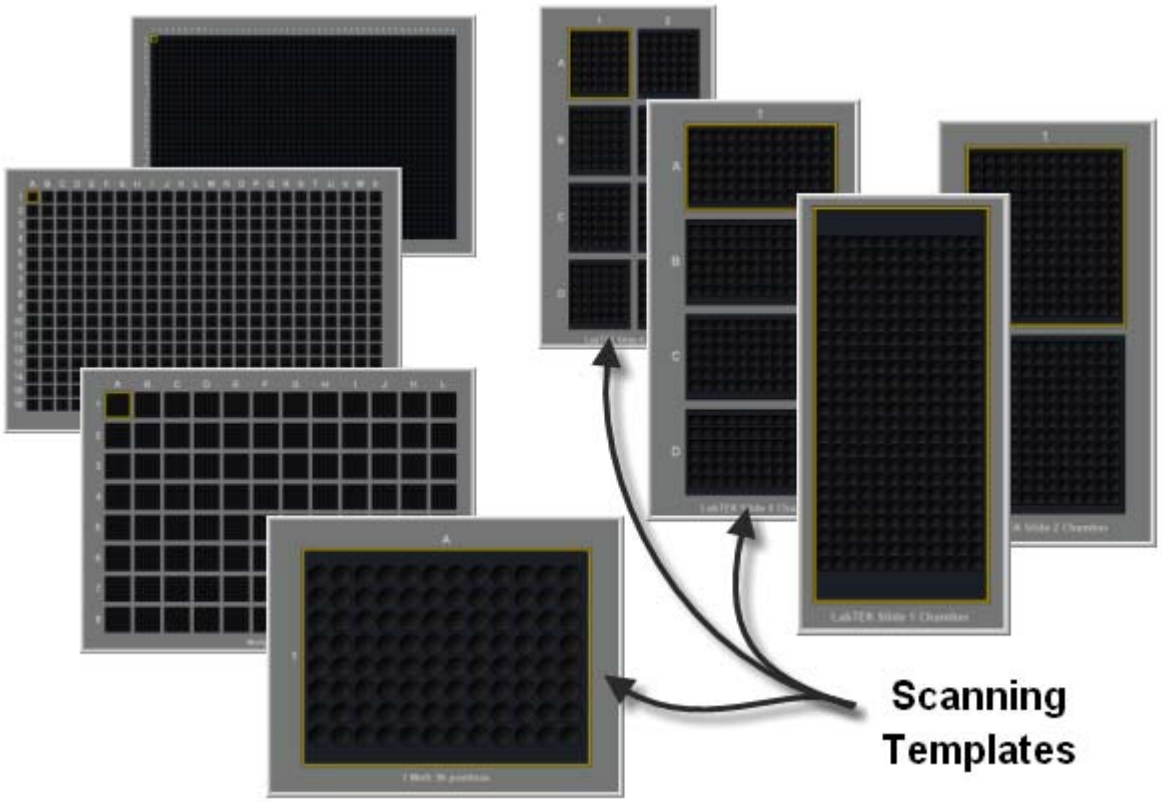
Note: The settings are configured here interactively, i.e. adjustments or changes to a Scanning Template are shown immediately in the **Scanning Template** view.

1	The **Scanning Template** view; the current Scanning Template is displayed here. You have several options for finding a suitable Scanning Template for your experiment: you can create one yourself, load an existing one or select one that is predefined; in the **Setup Template** operating step, depending on the application and type of specimen slide used (well plate or chambered coverglass), predefined Scanning Templates are available for selection at the bottom left.
2	Here, you can create a Scanning Template or adapt an existing or predefined Scanning Template to the requirements of your experiment. You can define the number of wells or chambers, the distances between the wells or chambers and the number of scan fields in a well or chamber.
3	Here, you can configure the distances between the scan fields and the positions of the scan fields (xy-coordinates).

See also:
MatrixScreener Wizard: Scanning Templates
MatrixScreener Wizard: Creating or adapting a Scanning Template
MatrixScreener Wizard: Assigning coordinates to scan fields
MatrixScreener Wizard: Setup Jobs
MatrixScreener Wizard: Setup Experiment

MatrixScreener Wizard: Scanning Templates

The MatrixScreener Wizard can map all common specimen slides for screening experiments (well plates, chambered coverglasses) in the form of Scanning Templates. You have several options for finding a suitable Scanning Template for your experiment: you can create one yourself, load an existing one or select one that is predefined; in the **Setup Template** operating step, depending on the application and type of specimen slide used (well plate or chambered coverglass), predefined Scanning Templates are available for selection at the bottom left.



Note: Scanning Templates are created or adapted to the requirements of an experiment in the **Setup Template** operating step. The allocation of jobs and experiments takes place only after the jobs and experiments have been defined in the (next) operating step **Setup Jobs**.

Note: You can save Scanning Templates that you have created yourself and reload (and modify) them later. This enables you to exchange Scanning Templates with other users.

Tip: If multiple users work jointly on experiments on different MatrixScreener systems at the same time, we recommend saving the Scanning Templates on a network drive. This creates a joint platform that can be used by the connected MatrixScreener systems for experiments. Specify the storage location of the Scanning Templates in the **Path Settings** tab, which you can reach by clicking the button with the tool symbol on the start page of MatrixScreener Wizard (**Select App.** operating step).

See also:
MatrixScreener Wizard: Setup Template
MatrixScreener Wizard: Creating or adapting a Scanning Template
MatrixScreener Wizard: Assigning coordinates to scan fields

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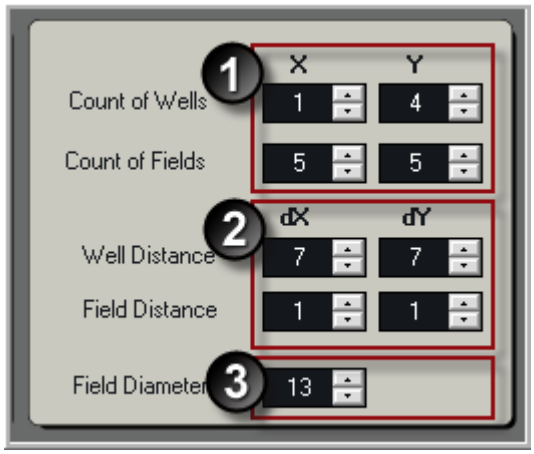
MatrixScreener Wizard: Creating or adapting a Scanning Template

You have several options for finding a suitable Scanning Template for your experiment: you can create one yourself, load an existing one or select one that is predefined; in the **Setup Template** operating step, depending on the application and type of specimen slide used (well plate or chambered coverglass), predefined Scanning Templates are available for selection at the bottom left.

Following is a simple example of how to create a Scanning Template yourself in the **Setup Template** operating step (the corresponding applies if you want to adapt an existing or predefined Scanning Template to the requirements of your experiment). The objective is to create a Scanning Template for an experiment for which a chambered coverglass with 4 chambers is used as the specimen slide. 5 × 5 scan fields are to be positioned in each chamber. Configure the required settings in the following dialog in the **Setup Template** operating step:



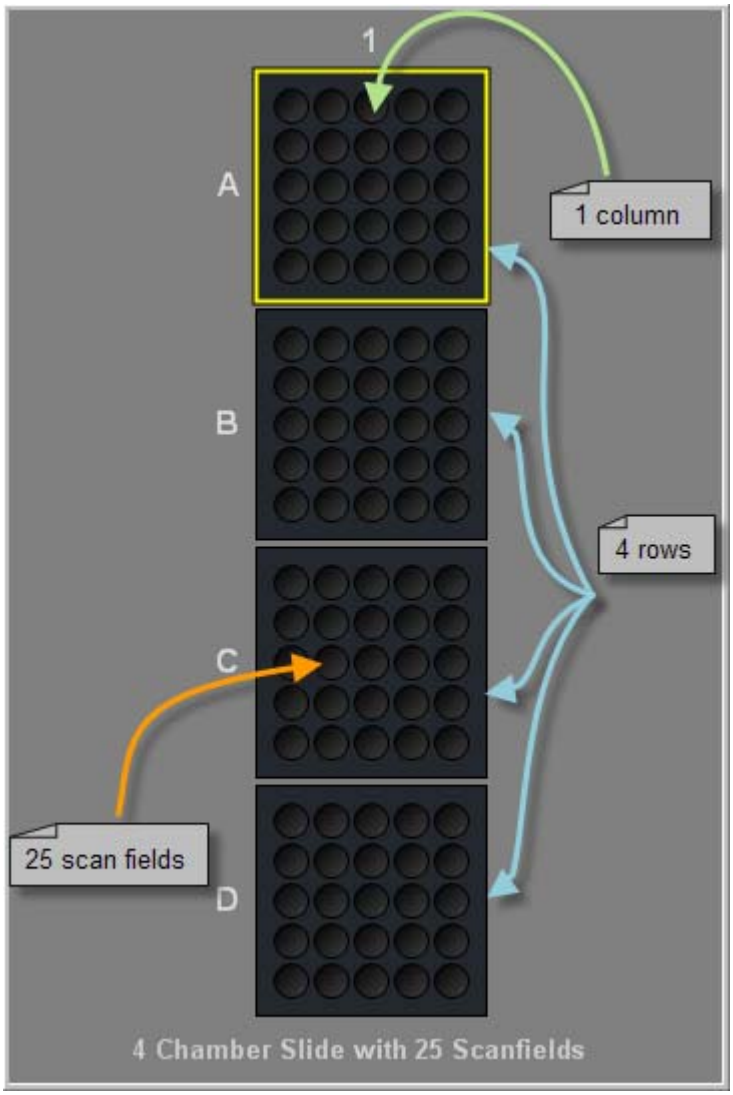
Note: The settings are configured here interactively, i.e. adjustments or changes to a Scanning Template are shown immediately in the **Scanning Template** view.

1. Under **Count of Wells**, set the number of chambers; under **Count of Fields**, set the number of scan fields in each chamber. In this example, therefore, it is 1 column (**X**) with 4 chambers (**Y**) and 5 × 5 scan fields per chamber.

2. You can adjust the distances between the chambers (**Well Distance**) and between the scan fields (**Field Distance**) in order to control the display of the Scanning Template in the **Scanning Template** view. This can be necessary if the number of scan fields is very large, in which case all may not fit in the **Scanning Template** view.

3. The corresponding applies for the diameter of the scan fields (**Field Diameter**), which you can adjust here if necessary so that all scan fields are displayed in the **Scanning Template** view.

Thus the Scanning Template is created for a chambered coverglass with 4 chambers and 5 × 5 scan fields per chamber and looks something like this in the **Scanning Template** view:



Note: You can save Scanning Templates that you have created yourself and reload (and modify) them later. This enables you to exchange Scanning Templates with other users.

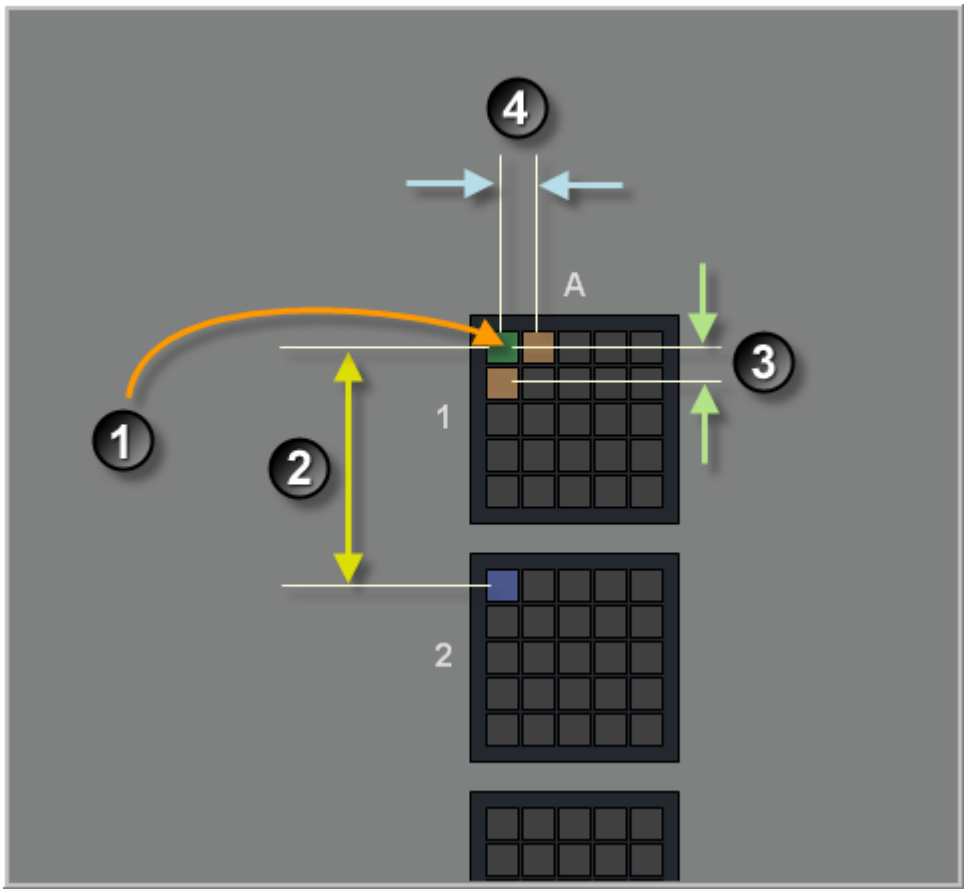
Tip: If multiple users work jointly on experiments on different MatrixScreener systems at the same time, we recommend saving the Scanning Templates on a network drive. This creates a joint platform that can be used by the connected MatrixScreener systems for experiments. Specify the storage location of the Scanning Templates in the **Path Settings** tab, which you can reach by clicking the button with the tool symbol on the start page of MatrixScreener Wizard (**Select App.** operating step).

See also:
MatrixScreener Wizard: Setup Template
MatrixScreener Wizard: Scanning Templates
MatrixScreener Wizard: Assigning coordinates to scan fields

MatrixScreener Wizard: Assigning coordinates to scan fields

Following is a simple example of how to assign xy-coordinates to the scan fields in a Scanning Template in the **Setup Template** operating step, i.e. how to assign the scan fields their position on the specimen stage. For this example, a Scanning Template is used, which maps a simple chambered coverglass as the specimen slide: 1 column with 4 chambers and 5 × 5 scan fields per chamber (for how to create this Scanning Template, refer to MatrixScreener Wizard: Creating or adapting a Scanning Template).

In a newly created Scanning Template, the xy-coordinates of all scan fields are initially set to 0. In this simple example, the distances between the chambers and between the scan fields are always equal, so that only 4 values have to be configured to assign the scan fields their position on the specimen stage:

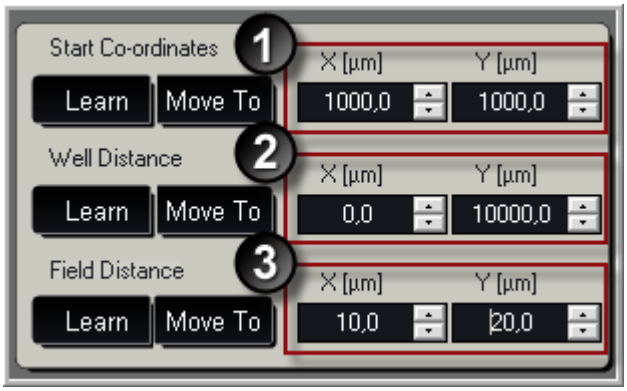


1	Starting point (always the upper left scan field in the first well or chamber)
2	Vertical distance between two chambers in the y-direction (as the distances between the chambers are always equal, you only have to specify one value)
3	Vertical distance between two scan fields in the y-direction (because the distances between the scan fields are always equal, you only have to specify one value)

4 Horizontal distance between two scan fields in the x-direction (because the distances between the scan fields are always equal, you only have to specify one value)

Note: If the Scanning Template maps a well plate or chambered coverglass on which the wells or chambers are arranged not only in one column, but in multiple columns, the horizontal clearance between two wells or chambers in the x-direction must also be configured as the fifth value.

In this example, the starting point is to be assigned the position x = 1000 µm and y = 1000 µm on the specimen stage. The vertical distance between two chambers is to be 10000 µm, the vertical distance between two scan fields 20 µm and the horizontal distance between two scan fields 10 µm. Configure the required settings in the following dialog in the **Setup Template** operating step:



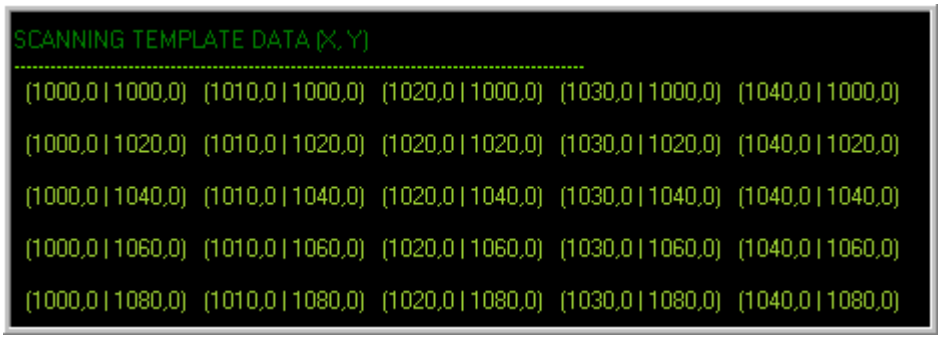
1 Under **Start Co-ordinates**, set the position of the starting point on the specimen stage: **X = 1000 µm** and **Y = 1000 µm**.

2 Under **Well Distance**, configure the distance between two chambers: **X = 0 µm** (horizontal distance, not required in this example) and **Y = 10000 µm** (vertical distance).

3 Under **Field Distance**, configure the distance between two scan fields: **X = 10 µm** (horizontal distance) and **Y = 20 µm** (vertical distance).

You can enter these values manually or have them entered by LAS AF by moving the specimen stage to the respective position and clicking the **Learn** button; for this method, you first have to move to the starting point, then move to the other positions to determine the distances to the starting point. By clicking the **Move To** button, you can move the specimen stage to the respective position, where you can use the **Live** image acquisition to check whether the entered coordinates are correct.

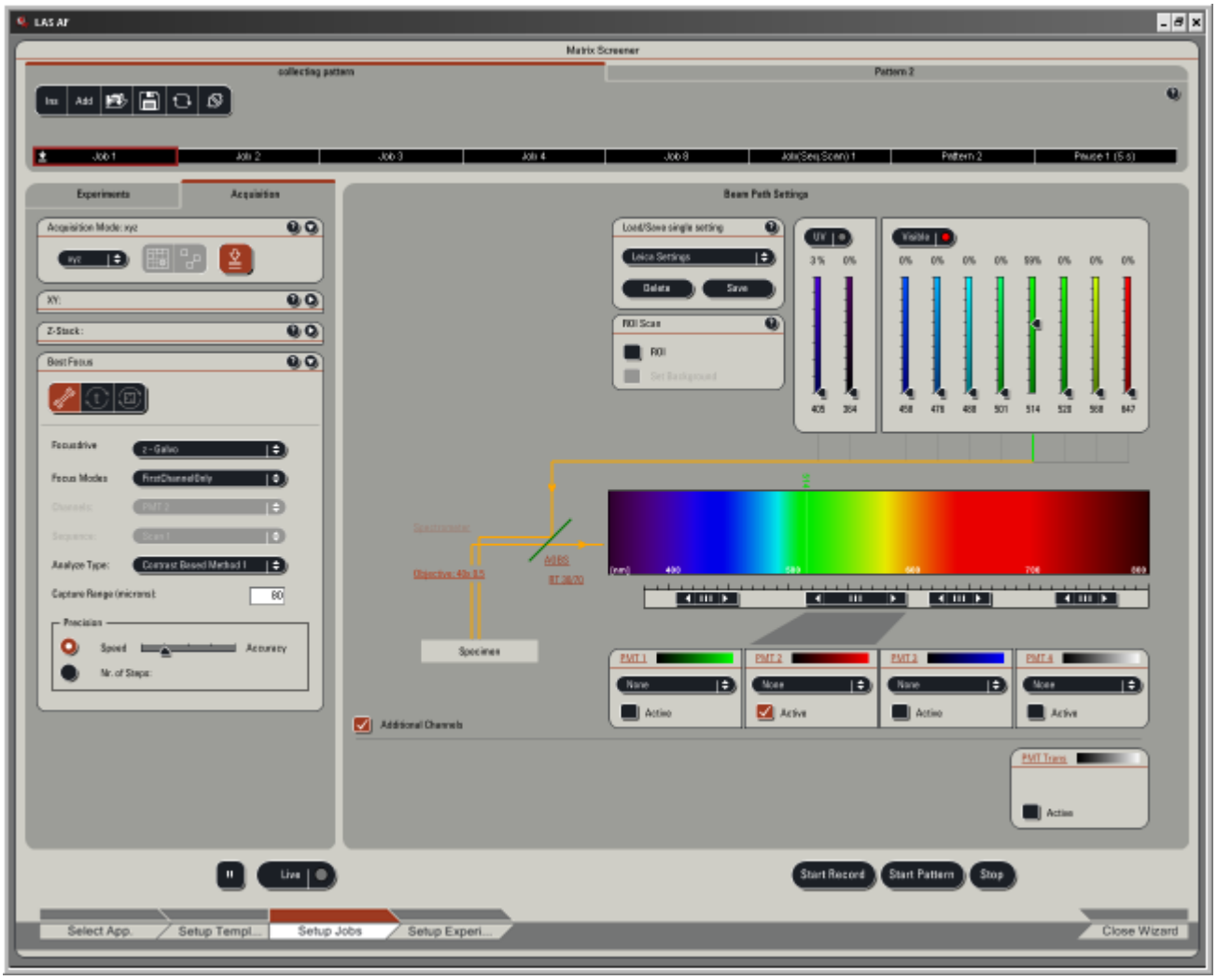
Once all required values are entered, you can view the coordinates of all scan fields in the log window of the MatrixScreener Wizard. To do so, press the **Crtl + P** key combination to call up the log window; the log window is initially empty. Then, move the mouse pointer in the **Scanning Template** view over the Scanning Template and press the **F6** button; this updates the content of the log window and displays the coordinates of all scan fields. The following illustration shows an excerpt from the log window with the xy-coordinates of the 25 scan fields that are positioned in the first chamber of the chambered coverglass:



See also:
MatrixScreener Wizard: Setup Template
MatrixScreener Wizard: Scanning Templates
MatrixScreener Wizard: Creating or adapting a Scanning Template

MatrixScreener Wizard: Setup Jobs

Brief description: In the **Setup Jobs** operating step, you can configure the settings for image acquisition and define and organize jobs and experiments (**Pattern**). Experiments can consist of multiple items (jobs, pauses and other **Pattern**s) that are processed in succession.



All regular LAS AF functions for image acquisition are available here. Which functions are available in particular depends on the configuration of your system.

Once all jobs and experiments (**Pattern**) have been defined, they can be allocated to the Scanning Template in the **Setup Experiment** operating step.

See also:
Pattern
Best Focus
MatrixScreener Wizard: Setup Template
MatrixScreener Wizard: Setup Experiment

MatrixScreener Wizard: Setup Experiment

Brief description: In the **Setup Experiment** operating step, you can merge the settings from the **Setup Template** and **Setup Jobs** operating steps, i.e. you can assign jobs and experiments to the Scanning Template.

Mosaic Applications

1 Here, you can configure the settings for the scan field functions: **Autofocus**, **Drift Compensation** and **Pump**.

Note: The **Single Object Tracking** function is not available in the **Mosaic Applications**.

Here, you can toggle back and forth between different displays of the Scanning Template in the **Scanning Template** view, save, load and print a Scanning Template

2 as well as create a screenshot of the Scanning Template.

3 Listed here are the jobs and experiments that you defined in the **Setup Jobs** operating step. You can assign these jobs and experiments to the Scanning Template.

> **Note:** In **Mosaic Applications**, you can always only assign one job or one experiment to one Scanning Template.

4 Here, you can define loops to process an experiment repeatedly.

5 Here, the status of an ongoing experiment is displayed.

6 Here, you can enable the scan field functions: **Autofocus**, **Drift Compensation** and **Pump**.

> **Note:** The **Single Object Tracking** function is not available in the **Mosaic Applications**.

7 With these buttons you can control the flow of an experiment.

Matrix Screener Applications

1 Here, you can configure the settings for the scan field functions: **Autofocus**, **Drift Compensation**, **Single Object Tracking**, and **Pump**.

2 Here, you can toggle back and forth between different displays of the Scanning Template in the **Scanning Template** view, save, load and print a Scanning Template as well as create a screenshot of the Scanning Template.

3 Listed here are the jobs and experiments that you defined in the **Setup Jobs** operating step. You can assign these jobs and experiments to the Scanning Template.

Note: In the **MatrixScreener Applications**, you can assign multiple jobs and/or experiments to one Scanning Template, i.e. certain scan fields can be selected in the Scanning Template for one job or experiment.

4 Here, you can define loops to process an experiment repeatedly.

5 Here, the status of an ongoing experiment is displayed.

Here, you can enable the scan field functions: **Autofocus**, **Drift Compensation**,

6 **Single Object Tracking**, and **Pump**.

7 With these buttons you can control the flow of an experiment.

See also:
MatrixScreener Wizard: Autofocus settings
MatrixScreener Wizard: Drift Compensation settings
MatrixScreener Wizard: Single Object Tracking settings
MatrixScreener Wizard: Pump settings
MatrixScreener Wizard: Setup Template
MatrixScreener Wizard: Setup Jobs
MatrixScreener Wizard: Scan field functions

Leica MICROSYSTEMS Living up to Life

MatrixScreener Wizard: Autofocus settings

Important details

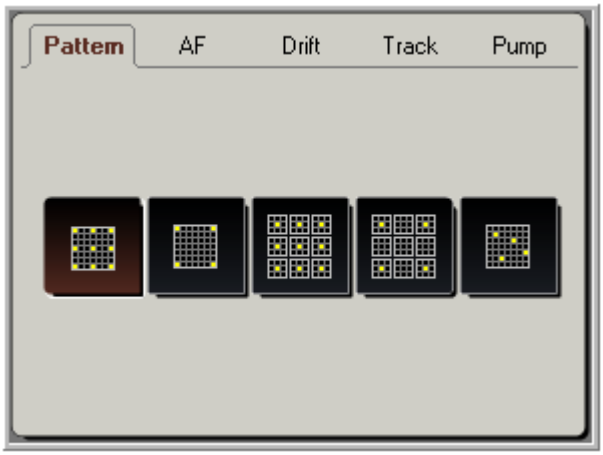
The prerequisite for automatic focus search is an **Autofocus** job, which can be defined in the **Setup Jobs** operating step. An **Autofocus** job is used to focus in the scan fields dedicated for that purpose and thus is configured in such a way that the structures of the specimen that are to be brought into focus are displayed with the highest possible contrast.

The selection of the excitation wavelengths for an **Autofocus** job always depends on the conditions of the experiment. In order to avoid photobleaching of the specimen, you should select a long wavelength excitation and a low laser intensity when defining an **Autofocus** job.

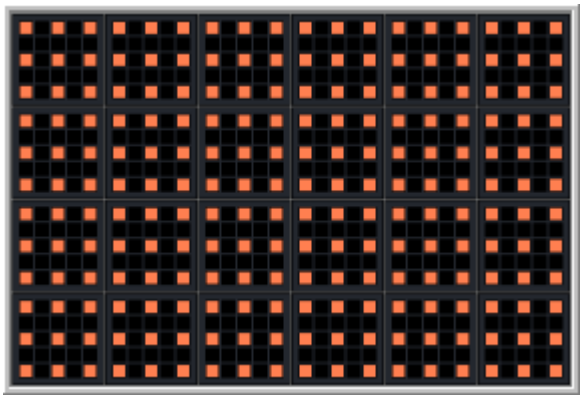
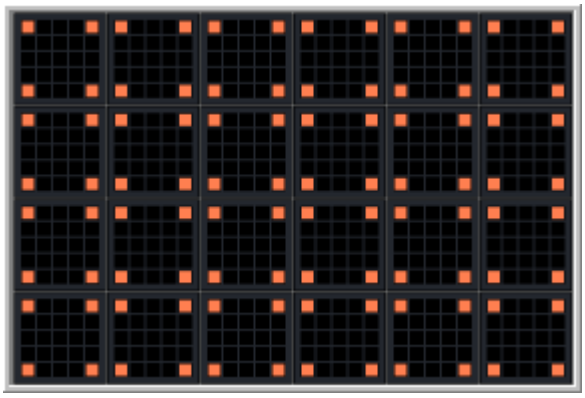
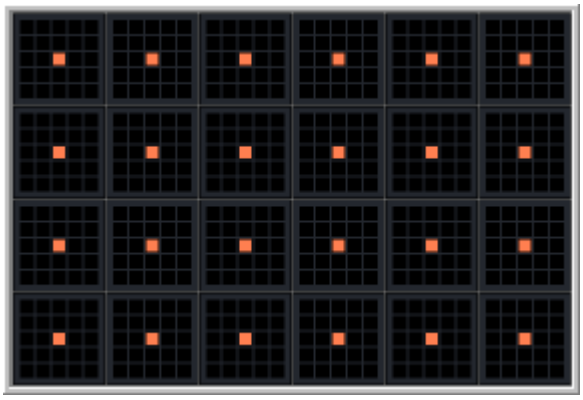
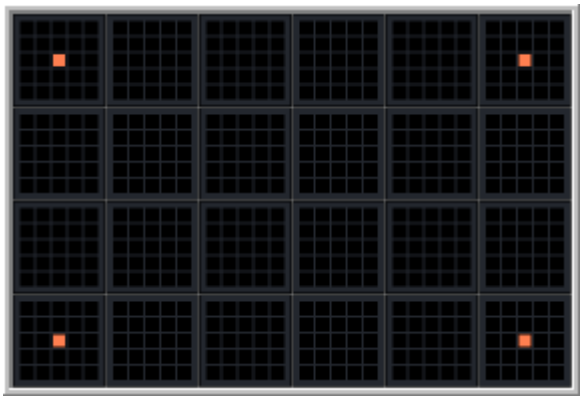
In order to achieve a good focus quickly when defining an **Autofocus** job, select a small scan format (e.g. 64 × 64), a high scan speed (e.g. 800 Hz) and bidirectional image acquisition in the **XY** dialog (**Setup Jobs** operating step).

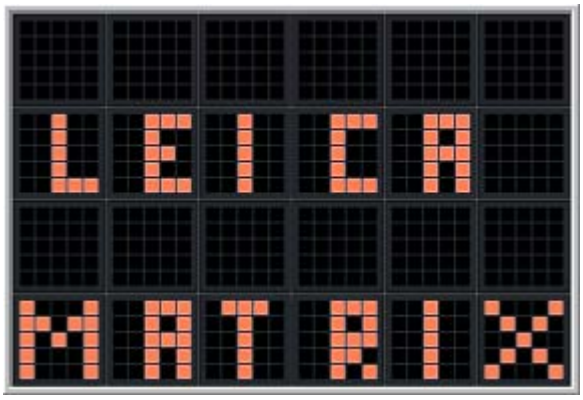
Autofocus scan fields

The **Autofocus** function requires scan fields in which the automatic focus search is carried out, and which are used as the basis for creating a Focus Map. The automatic focus search determines the position (z-coordinate) of the best focal plane in each **Autofocus** scan field; the system uses interpolation to automatically calculate the positions of the best focal plane lying between the **Autofocus** scan fields. The spatial pattern of the **Autofocus** scan fields is determined in the **Setup Experiment** operating step, at the bottom left in the **Pattern** tab:



Depending on the requirements of the experiment, different **Pattern**s with **Autofocus** scan fields are available, which can be placed over the specimen slide (well plate or chambered coverglass). Once you select a pattern, the corresponding **Autofocus** scan fields are displayed in the **Scanning Template** view:

Pattern	Scanning template view	Description
		For this pattern, the **Autofocus** scan fields are distributed uniformly across all wells or chambers. This way, one Focus Map is created for the entire specimen slide. In order to adapt the automatic focus search to the experiment conditions, the density of the **Autofocus** scan fields can be adjusted in the **AF** tab under **Point density** (see below).
		For this pattern, the **Autofocus** scan fields are placed in the corners of the wells or chambers. This way, a separate Focus Map for each well or chamber is created on the basis of 4 **Autofocus** scan fields.
		For this pattern, the **Autofocus** scan fields are placed in the center of the wells or chambers and the determined position of the best focal plane is applied across the entire well or chamber. This way, a separate Focus Map with a consistent focal plane is created for each well or chamber.
		For this pattern, the **Autofocus** scan fields are placed in the center of those wells or chambers that are located in the outer corners of the specimen slide. This way, one Focus Map for the entire specimen slide is created based on 4 **Autofocus** scan fields. **Note:** This pattern should be used for very flat specimens only.
		Here you can define the

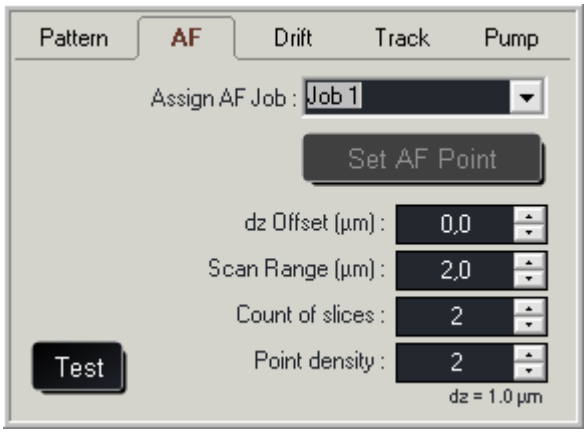


Autofocus scan fields yourself by assigning the **Autofocus** scan field function to the scan fields manually. When doing so, use the scan fields in which you already have determined good focus (e.g. using **Live** image acquisition). This way, the Focus Map is not created based on a rigid pattern, but flexibly based on focus ranges already known to have good focus.

How to assign the **Autofocus** scan field function to the selected scan fields manually is described here: MatrixScreener Wizard: Assigning the Autofocus function.

Settings for automatic focus search

When you have determined the **Autofocus** scan fields, you can switch from the **Pattern** tab to the **AF** tab to configure the settings for automatic focus search:



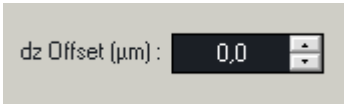
Here, you can select an **Autofocus** job for automatic focus search which you have defined in the **Setup Jobs** operating step.

Note: The **Autofocus** job selected here is also used for the **Drift Compensation** function.

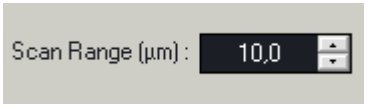
Using this button, you can assign the **Autofocus** scan field function to the selected scan fields. The prerequisite is that you have selected the **Individual Autofocus Measurement Points** function in the **Pattern** tab to determine the **Autofocus** scan fields yourself.

Note: **Autofocus** scan fields appear outlined in pink in the **Scanning Template** view.

For **Autofocus** jobs, there are different methods to determine the best focal plane in the specimen. When defining an **Autofocus** job, you can select a method in the **Best Focus** dialog under **Analyze Type** (**Setup Jobs** operating step).



You will need the **dz Offset** setting if you selected the **Reflection Based Method** for an **Autofocus** job. This method uses the strongest reflection signal of the cover slip to determine the best focal plane; this way, the Focus Map is created at the height of the cover slip surface. However, because the specimen is not located directly on the cover slip plane, you can enter the distance to the cover slip surface here to move the Focus Map into the actual focal plane of the specimen.



Enter the range in z-direction, in which automatic focus search is carried out in each **Autofocus** scan field here.

Here, set the number of individual images (horizontal xy-sections) that the system acquires during automatic focus search in an **Autofocus** scan field (within the range determined in **Scan Range**). Note that the more individual images are acquired, the more precise the automatic focus search; the fewer individual images are acquired, the faster the automatic focus search (at the expense of precision).

Tip: An appropriate number of individual images for automatic focus search can be determined using the following rule of thumb: the distance between two individual images should correspond to approximately half the focus depth of the objective used.

If you have selected the **Multiple AF points** pattern in the **Pattern** tab, the **Autofocus** scan fields are distributed uniformly across all wells or chambers of the specimen slide. This allows you to adjust the density of the **Autofocus** scan fields. Note that the greater the "waviness" of the surface used for focusing (specimen or cover slip surface, depending on the automatic focus search method), the higher the density of the **Autofocus** scan fields should be, and thus the smaller the value configured here should be.

Note: Value 1 is used for automatic focus search in each

	scan field, value 2 for every second scan field, value 3 for every third scan field, etc.
	By clicking on this button, you can test your settings for the automatic focus search.

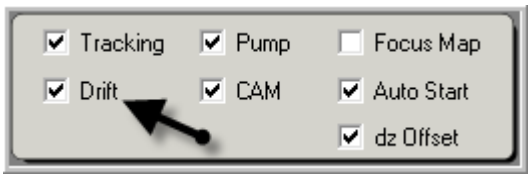
See also:
MatrixScreener Wizard: Assigning the Autofocus function
MatrixScreener Wizard: Setup Jobs
Best Focus
MatrixScreener Wizard: Setup Experiment
MatrixScreener Wizard: Drift Compensation settings
MatrixScreener Wizard: Single Object Tracking settings
MatrixScreener Wizard: Pump settings

MatrixScreener Wizard: Drift Compensation settings

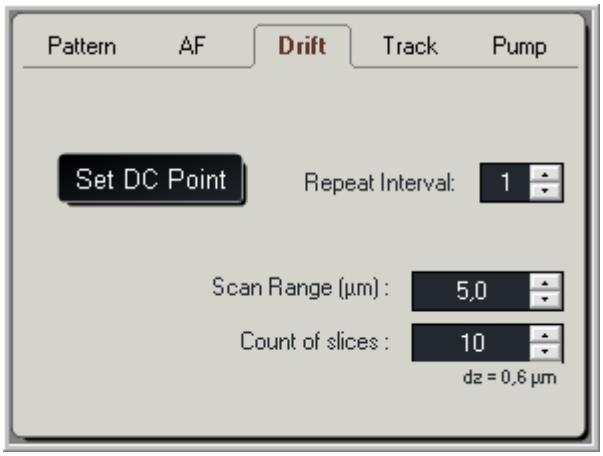
Note: The **Drift Compensation** function is, in principle, an automatic focus search that corrects any focus drift that may occur in the z-direction. A focus drift can occur due to temperature fluctuations, for example.

IMPORTANT: The **Autofocus** job selected in the **AF** tab is automatically used for the **Drift Compensation** function.

The **Drift Compensation** function (compensation for focus drift that may occur) must first be activated before it can be used. You enable this function in the last operating step **Setup Experiment**.

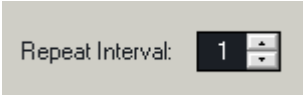


The settings for the **Drift Compensation** function can be configured in the **Setup Experiment** operating step, at the bottom left in the **Drift** tab:



Using this button, you can assign the **Drift Compensation** scan field function to the selected scan fields.

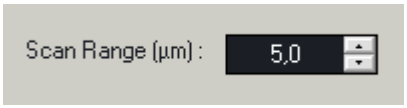
Note: **Drift Compensation** scan fields appear outlined in blue in the **Scanning Template** view.



Here, you can define the interval for carrying out the compensation of focus drift.

Note: Value 1 is used for compensating focus drifts during

each scan cycle, value 2 for every second scan cycle, value 3 for every third scan cycle, etc.



Enter the range in z-direction in which focus drift compensation is carried out in each **Drift Compensation** scan field here.

Set the number of individual images (horizontal xy-sections) that the system acquires during focus drift compensation in a **Drift Compensation** scan field (within the range determined in **Scan Range**) here. Note that the more individual images are acquired, the more precise the focus drift compensation; the fewer individual images are acquired, the faster the focus drift compensation (at the expense of precision).

Important details

The setting under **Repeat Interval** is used to define the interval in which focus drift compensation is repeated. The interval in this case is not a fixed given value, but depends on the duration of a complete scan cycle, i.e. on the time required to completely acquire all scan fields on the specimen slide once. For that reason, the interval can vary depending on the experiment and specimen slide. If, for example, a complete scan cycle takes 1 hour and the value you configured in **Repeat Interval** is 5, then focus drift compensation is carried out every 5 hours.

See also:

MatrixScreener Wizard: Assigning the Drift Compensation function
MatrixScreener Wizard: Setup Jobs
MatrixScreener Wizard: Setup Experiment
MatrixScreener Wizard: Autofocus settings
MatrixScreener Wizard: Single Object Tracking settings
MatrixScreener Wizard: Pump settings

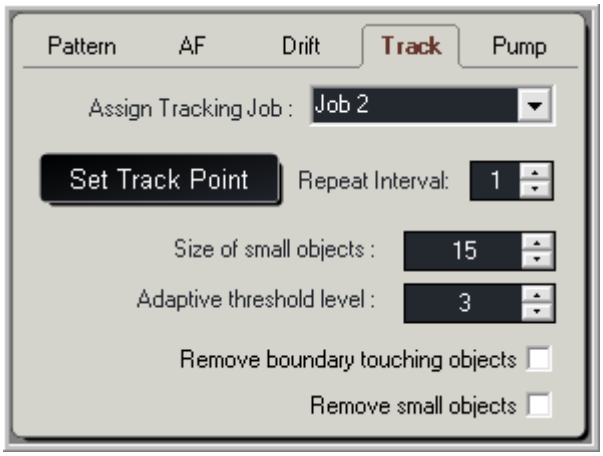
MatrixScreener Wizard: Single Object Tracking settings

Note: The **Single Object Tracking** function is available in the **MatrixScreener Applications** only (not in the **Mosaic Applications**).

IMPORTANT: The **Single Object Tracking** always follows only one object in the image, preferably the largest and brightest. The total number of objects in the image should be small (1 to 6). If the object in question moves out of the center of the image over time (e.g. after cell division), it is centered in the image prior to image acquisition.

The **Single Object Tracking** function must first be activated before it can be used. You enable this function in the last operating step **Setup Experiment**.

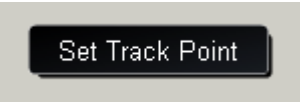
The settings for the **Single Object Tracking** function can be configured in the **Setup Experiment** operating step, at the bottom left in the **Track** tab:



Here, you select a job that you defined for object tracking in the **Setup Jobs** operating step (if you did not define a specific **Single Object Tracking** job, you can also use the main job of your experiment). The job settings must be selected in such a way that the object to be tracked is clearly highlighted in the image, i.e. it is displayed as intensively as possible and with the highest contrast and lowest noise.

Note: Images that are acquired using the job selected here as part of the **Single Object Tracking** function

are only used for object tracking; they are not stored.



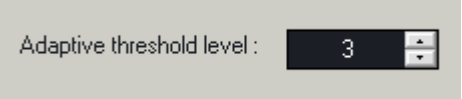
Using this button, you can assign the **Single Object Tracking** scan field function to the selected scan fields.

Note: **Single Object Tracking** scan fields appear outlined in yellow in the **Scanning Template** view.

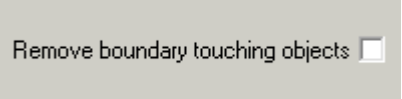
Here, you can define the interval for carrying out the object tracking.

Note: Value 1 is used for object tracking for each scan cycle, value 2 for every second scan cycle, value 3 for every third scan cycle, etc.

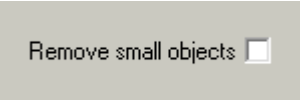
Here, you can set the diameter (in pixels) to a specific size from which the objects should be ignored during object tracking (in the example shown here, all objects with a diameter less than 15 pixels).



Here, you can adjust the detection stage for object tracking. This is done using a threshold value which adjusts flexibly to the respective image conditions and reacts dynamically to variations in contrast or local gray level variations (e.g. due to shading). The higher the value configured here, the more sensitive the detection will be.



Here, you can define that all objects that protrude over the edge of the image are ignored during object tracking.



Here, you can define that all objects that have a diameter (in pixels) smaller than the value configured in **Size of small objects** will be ignored during object tracking.

Note: The settings for the **Single Object Tracking** function can also be modified during an ongoing experiment. You can already see the effect of the modified settings in the next **Single Object Tracking** scan field that is scanned after the change was made.

Tip: To carry out object tracking in three dimensions, you can combine the **Single Object Tracking** function (xy-correction) with the **Drift Compensation** function (z-correction).

Important details

The setting under **Repeat Interval** is used to define the interval in which object tracking is repeated. The interval in this case is not a fixed given value, but depends on the duration of a complete scan cycle, i.e. on the time required to completely acquire all scan fields on the specimen slide once. For that reason, the interval can vary depending on the experiment and specimen slide. If, for example, a complete scan cycle takes 1 hour and the value you configured in **Repeat Interval** is 2, then object tracking is carried out every 2 hours.

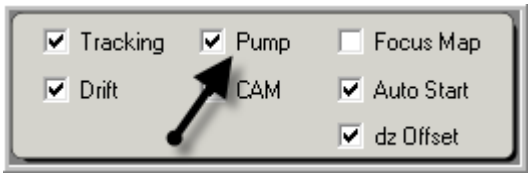
See also:

MatrixScreener Wizard: Assigning the Single Object Tracking function
MatrixScreener Wizard: Setup Jobs
MatrixScreener Wizard: Setup Experiment
MatrixScreener Wizard: Autofocus settings
MatrixScreener Wizard: Drift Compensation settings
MatrixScreener Wizard: Pump settings

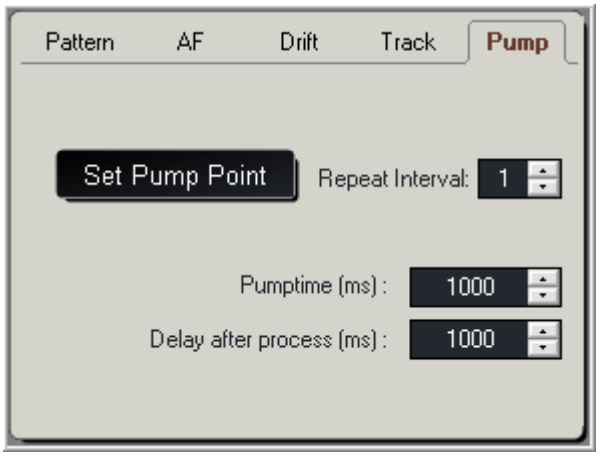
MatrixScreener Wizard: Pump settings

Note: The Water Immersion Micro Dispenser is required for the **Pump** function. When using a water immersion objective, the Water Immersion Micro Dispenser provides for an automatic supply of water immersion during long-term experiments and live cell experiments.

The **Pump** function (water replenishment if water is the immersion medium) must first be activated before it can be used. You enable this function in the last operating step **Setup Experiment**.



The settings for the **Pump** function can be configured in the **Setup Experiment** operating step, at the bottom left in the **Pump** tab:



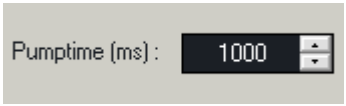
Using this button, you can assign the **Pump** scan field function to the selected scan fields.

Note: **Pump** scan fields appear outlined in green in the **Scanning Template** view.

Define the interval for water replenishment here.

Note: Value 1 is used for water replenishment during each scan cycle, value 2 for every second scan cycle, value 3 for every third scan cycle, etc.

Specify the duration of the water replenishment here (in **ms**). The duration of the water replenishment should be



selected based on the ambient temperature and the configured interval for water replenishment in order to compensate for water loss due to evaporation during an ongoing experiment.

Note: If there is a lot of water loss in a short time, you should not only increase the duration of water replenishment, but also reduce the water replenishment interval.

As a result of water replenishment, it is possible that the best focal plane that was configured will be moved temporarily. This effect occurs when the specimen slide is lifted slightly due to the water replenishment and, as a result, the specimen is moved out of focus.

Under **Delay after process**, you can set a delay in order to allow time for the specimen slide to set before the actual scanning operation begins. This way, you can make sure that images are not acquired until the best previously configured focal plane is recovered.

Important details

If there is a lot of water loss during an ongoing experiment, there is a danger that the water reservoir of the Water Immersion Micro Dispenser is completely emptied due to the continuously required water replenishment. In this case, we recommend that you position the water reservoir outside of the objective nosepiece so that it is possible to fill it with water for water replenishment during an ongoing experiment if necessary.

When working with a climate chamber, also position the water reservoir in the climate chamber. Doing so will ensure that the temperature of the water in the water reservoir corresponds to the interior temperature of the climate chamber and that optical artifacts that may occur due to the temperature dependence of the refractive index are avoided.

See also:
MatrixScreener Wizard: Assigning the Pump function
Water Immersion Micro Dispenser
MatrixScreener Wizard: Setup Jobs
MatrixScreener Wizard: Setup Experiment
MatrixScreener Wizard: Autofocus settings
MatrixScreener Wizard: Drift Compensation settings
MatrixScreener Wizard: Single Object Tracking settings

MatrixScreener Wizard Quick Tutorial: Single Mosaic

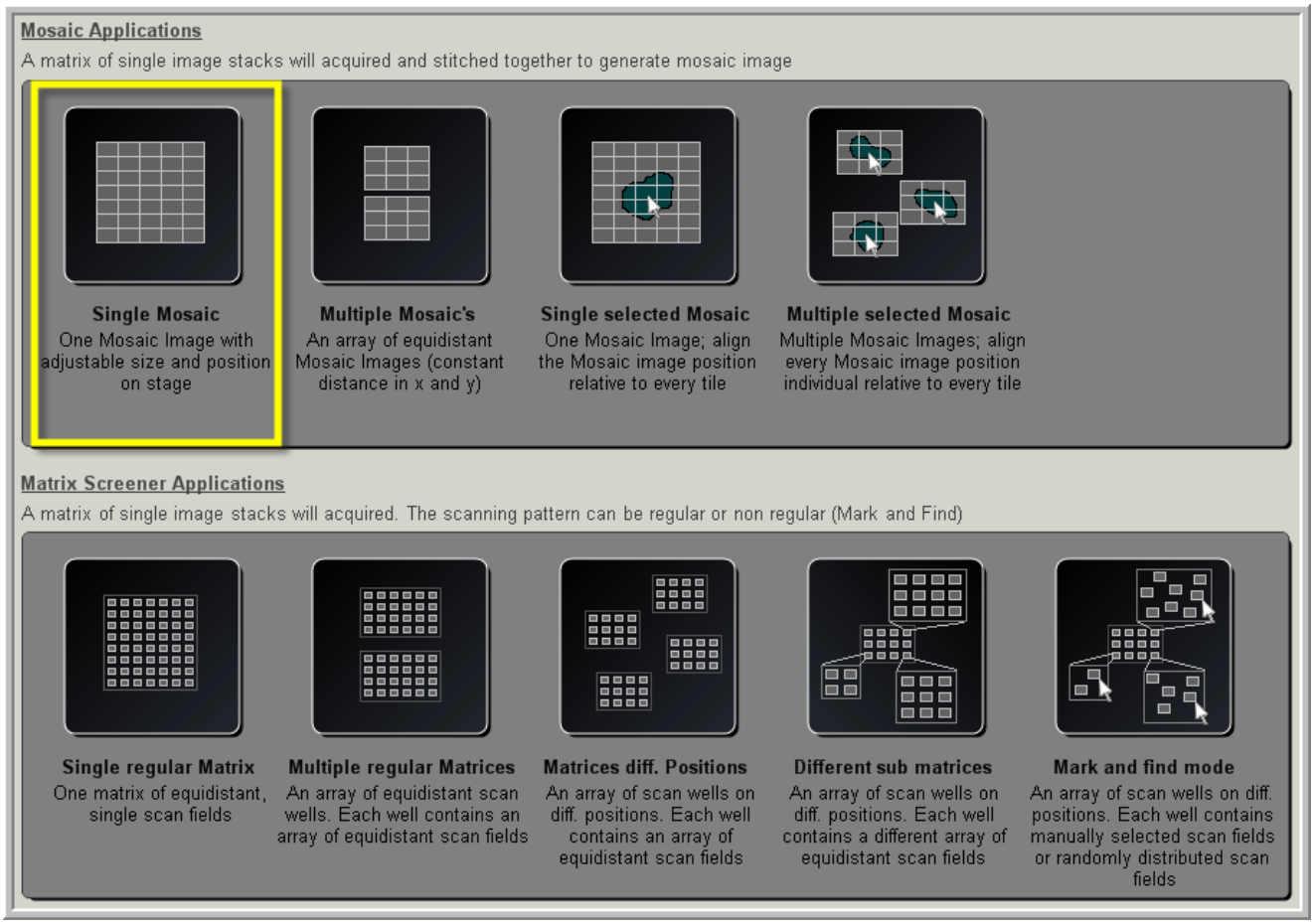
Brief description: This Quick Tutorial helps you get a quick start using the **Single Mosaic** application.

Start application

1. Start LAS AF.
2. Select MatrixScreener Wizard from the top left menu bar under **Leica Microsystems LAS AF**.

The MatrixScreener Wizard starts with the **Select App.** operating step, in which you can select the desired application.

3. Click **Mosaic Applications** > **Single Mosaic**.



The **Single Mosaic** application is started now.

Switch to the **Setup Template** operating step to begin with the settings for your experiment.

Setup Template

In the **Setup Template** operating step, you can configure the basic settings for your experiment by creating a Scanning Template or selecting an existing one. This defines the spatial structure of your experiment.

After configuring all required settings, you can switch to the **Setup Jobs** operating step.

Setup Jobs

In the **Setup Jobs** operating step, you can configure the settings for image acquisition and define and organize jobs and experiments (**Pattern**). Experiments can consist of multiple items (jobs, pauses and other **Pattern**s) that are processed in succession.

After configuring all required settings, you can switch to the **Calibration** operating step.

Calibration

In the **Calibration** operating step, you can configure the settings for acquiring an image mosaic.

After configuring all required settings, you can switch to the **Setup Experiment** operating step.

Setup Experiment

In the **Setup Experiment** operating step, you can merge the settings from the **Setup Template** and **Setup Jobs** operating steps, i.e. you can assign jobs and experiments to the Scanning Template.

After configuring all required settings, you can start the experiment.

The experiment is running now and you can track the progress of the experiment under **Experiment Adjustment** > **Monitor**.

MatrixScreener Wizard Quick Tutorial: Multiple Mosaics

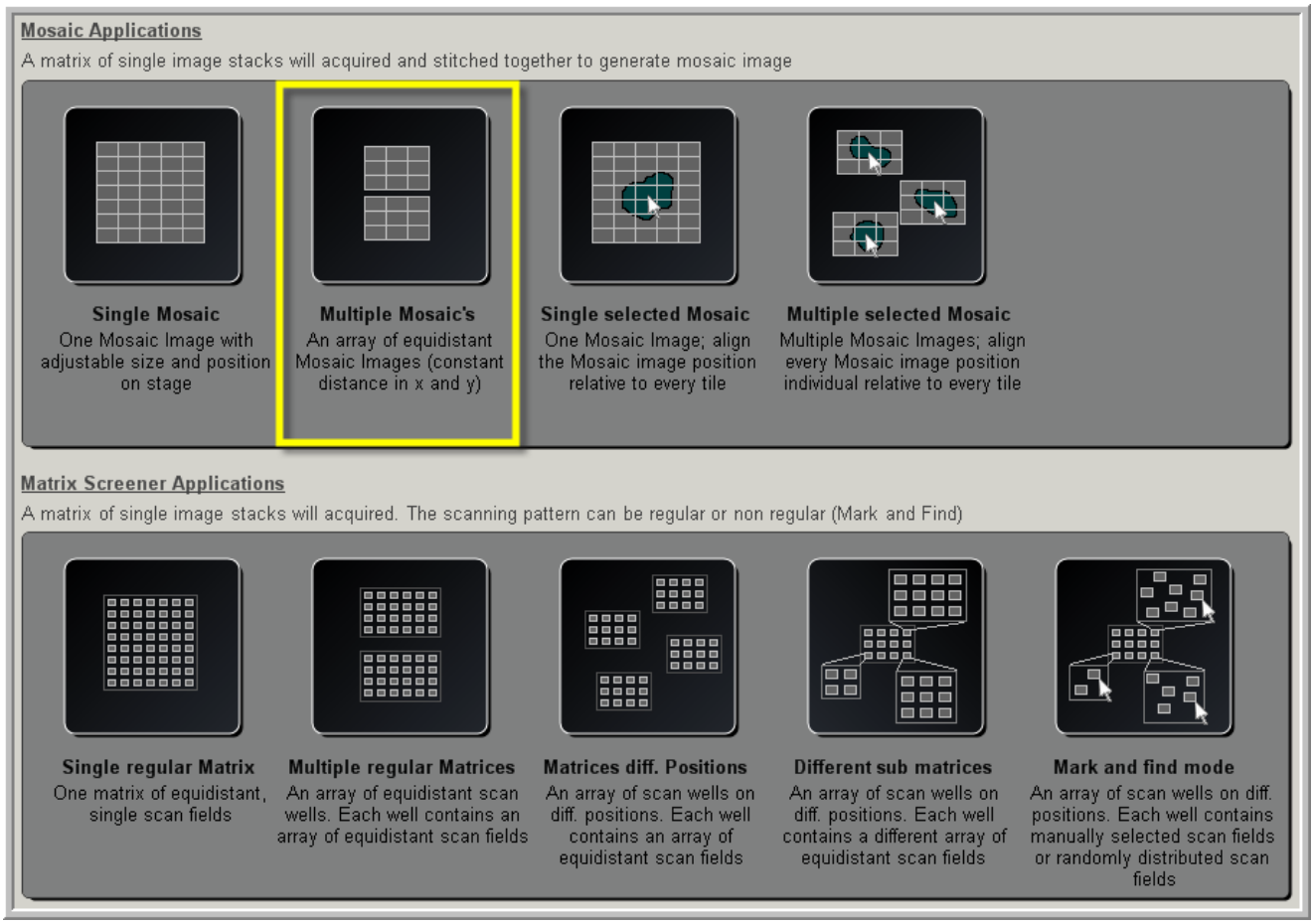
Brief description: This Quick Tutorial helps you get a quick start using the **Multiple Mosaics** application.

Start application

1. Start LAS AF.
2. Select MatrixScreener Wizard from the top left menu bar under **Leica Microsystems LAS AF**.

The MatrixScreener Wizard starts with the **Select App.** operating step, in which you can select the desired application.

3. Click **Mosaic Applications** > **Multiple Mosaics**.



The **Multiple Mosaics** application is started now.

Switch to the **Setup Template** operating step to begin with the settings for your experiment.

Setup Template

In the **Setup Template** operating step, you can configure the basic settings for your experiment by creating a Scanning Template or selecting an existing one. This defines the spatial structure of your experiment.

After configuring all required settings, you can switch to the **Setup Jobs** operating step.

Setup Jobs

In the **Setup Jobs** operating step, you can configure the settings for image acquisition and define and organize jobs and experiments (**Pattern**). Experiments can consist of multiple items (jobs, pauses and other **Pattern**s) that are processed in succession.

After configuring all required settings, you can switch to the **Calibration** operating step.

Calibration

In the **Calibration** operating step, you can configure the settings for acquiring an image mosaic.

After configuring all required settings, you can switch to the **Setup Experiment** operating step.

Setup Experiment

In the **Setup Experiment** operating step, you can merge the settings from the **Setup Template** and **Setup Jobs** operating steps, i.e. you can assign jobs and experiments to the Scanning Template.

After configuring all required settings, you can start the experiment.

The experiment is running now and you can track the progress of the experiment under **Experiment Adjustment** > **Monitor**.

MatrixScreener Wizard Quick Tutorial: Single selected Mosaic

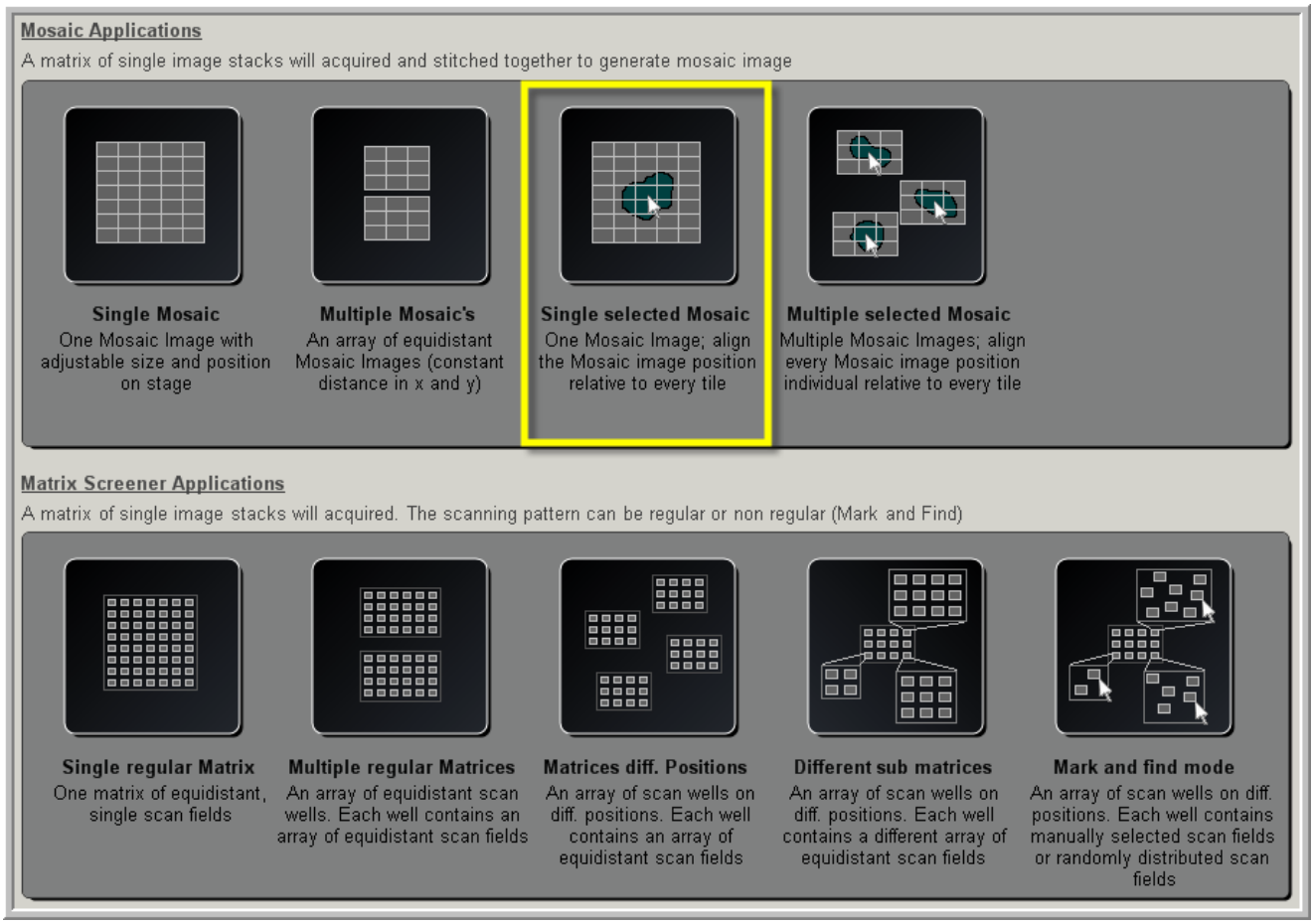
Brief description: This Quick Tutorial helps you get a quick start using the **Single selected Mosaic** application.

Start application

1. Start LAS AF.
2. Select MatrixScreener Wizard from the top left menu bar under **Leica Microsystems LAS AF**.

The MatrixScreener Wizard starts with the **Select App.** operating step, in which you can select the desired application.

3. Click **Mosaic Applications** > **Single selected Mosaic**.



The **Single selected Mosaic** application is started now.

Switch to the **Setup Template** operating step to begin with the settings for your experiment.

Setup Template

In the **Setup Template** operating step, you can configure the basic settings for your experiment by creating a Scanning Template or selecting an existing one. This defines the spatial structure of your experiment.

After configuring all required settings, you can switch to the **Setup Jobs** operating step.

Setup Jobs

In the **Setup Jobs** operating step, you can configure the settings for image acquisition and define and organize jobs and experiments (**Pattern**). Experiments can consist of multiple items (jobs, pauses and other **Pattern**s) that are processed in succession.

After configuring all required settings, you can switch to the **Calibration** operating step.

Calibration

In the **Calibration** operating step, you can configure the settings for acquiring an image mosaic.

After configuring all required settings, you can switch to the **Setup Experiment** operating step.

Setup Experiment

In the **Setup Experiment** operating step, you can merge the settings from the **Setup Template** and **Setup Jobs** operating steps, i.e. you can assign jobs and experiments to the Scanning Template.

After configuring all required settings, you can start the experiment.

The experiment is running now and you can track the progress of the experiment under **Experiment Adjustment** > **Monitor**.

MatrixScreener Wizard Quick Tutorial: Multiple selected Mosaic

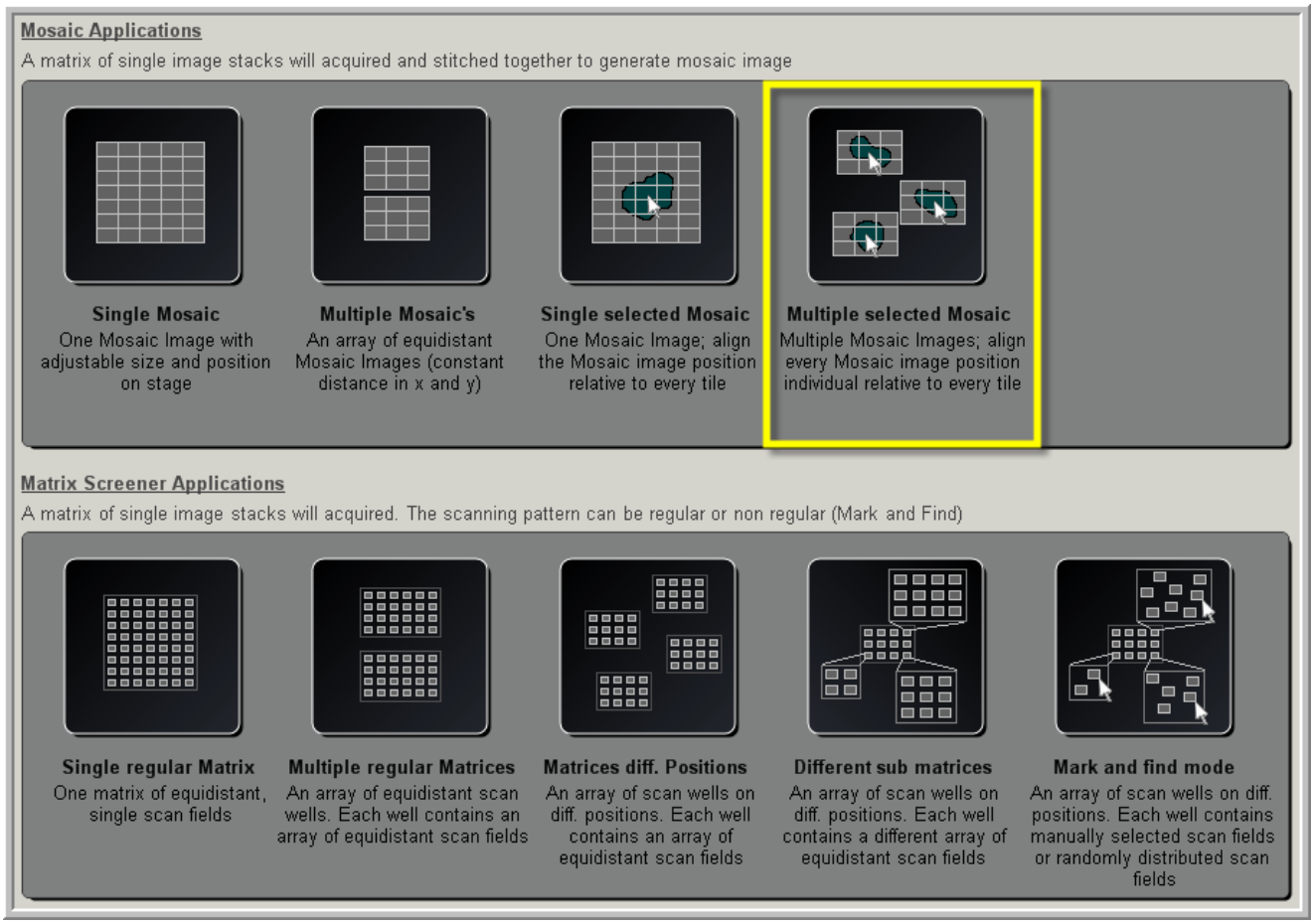
Brief description: This Quick Tutorial helps you get a quick start using the **Multiple selected Mosaic** application.

Start application

1. Start LAS AF.
2. Select MatrixScreener Wizard from the top left menu bar under **Leica Microsystems LAS AF**.

The MatrixScreener Wizard starts with the **Select App.** operating step, in which you can select the desired application.

3. Click **Mosaic Applications** > **Multiple selected Mosaic**.



The **Multiple selected Mosaic** application is started now.

Switch to the **Setup Template** operating step to begin with the settings for your experiment.

Setup Template

In the **Setup Template** operating step, you can configure the basic settings for your experiment by creating a Scanning Template or selecting an existing one. This defines the spatial structure of your experiment.

After configuring all required settings, you can switch to the **Setup Jobs** operating step.

Setup Jobs

In the **Setup Jobs** operating step, you can configure the settings for image acquisition and define and organize jobs and experiments (**Pattern**). Experiments can consist of multiple items (jobs, pauses and other **Pattern**s) that are processed in succession.

After configuring all required settings, you can switch to the **Calibration** operating step.

Calibration

In the **Calibration** operating step, you can configure the settings for acquiring an image mosaic.

After configuring all required settings, you can switch to the **Setup Experiment** operating step.

Setup Experiment

In the **Setup Experiment** operating step, you can merge the settings from the **Setup Template** and **Setup Jobs** operating steps, i.e. you can assign jobs and experiments to the Scanning Template.

After configuring all required settings, you can start the experiment.

The experiment is running now and you can track the progress of the experiment under **Experiment Adjustment** > **Monitor**.

MatrixScreener Wizard Quick Tutorial: Single regular Matrix

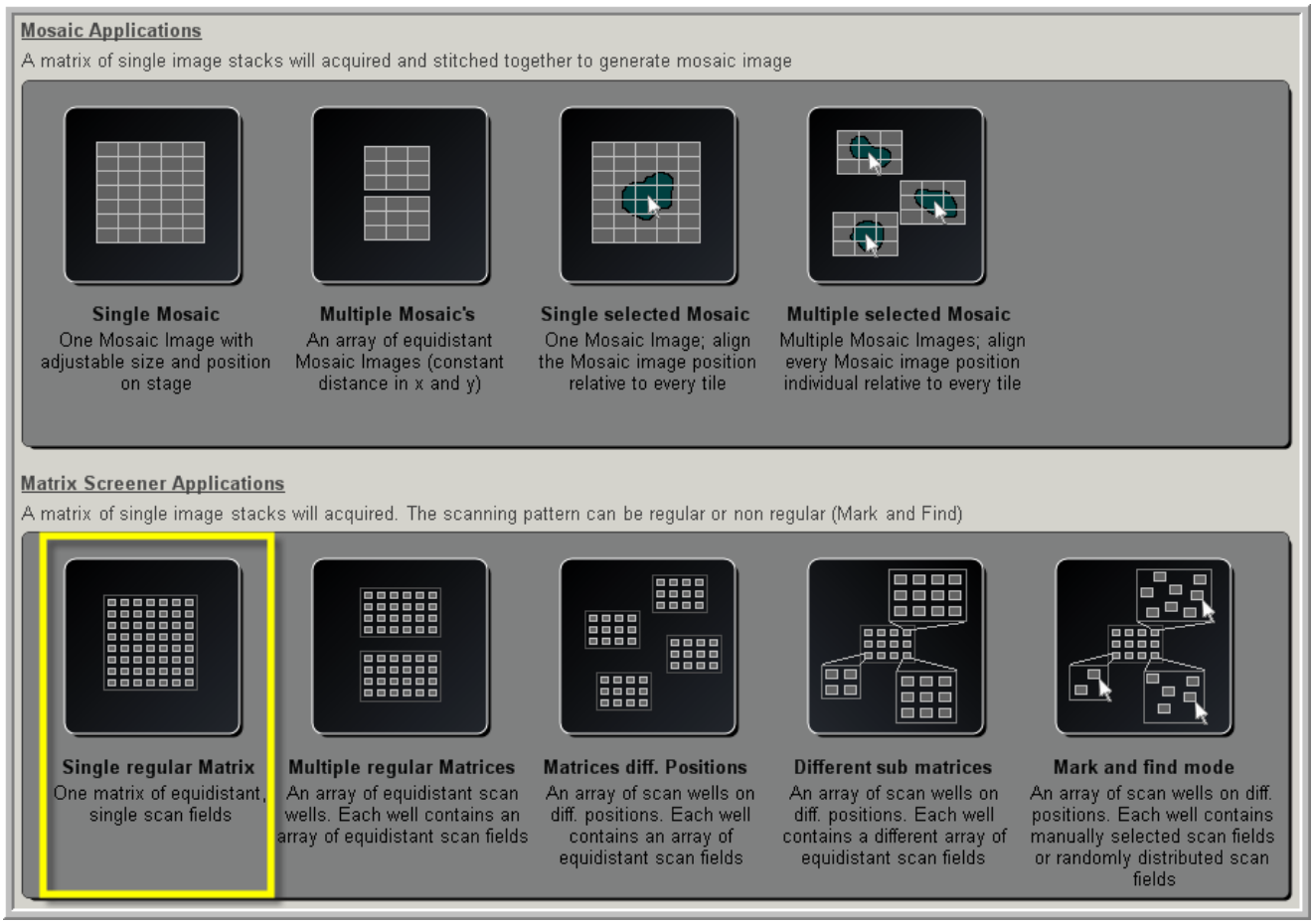
Brief description: This Quick Tutorial helps you get a quick start using the **Single regular Matrix** application. The **Single regular Matrix** application is the simplest of the **Matrix Screener Applications**. With this application you can define only one matrix, which can consist of multiple scan fields. For that reason, this application is primarily suited for chambered coverglasses with one chamber and for fixed specimens on a regular specimen slide.

Start application

1. Start LAS AF.
2. Select MatrixScreener Wizard from the top left menu bar under **Leica Microsystems LAS AF**.

The MatrixScreener Wizard starts with the **Select App.** operating step, in which you can select the desired application.

3. Click **Matrix Screener Applications** > **Single regular Matrix**.

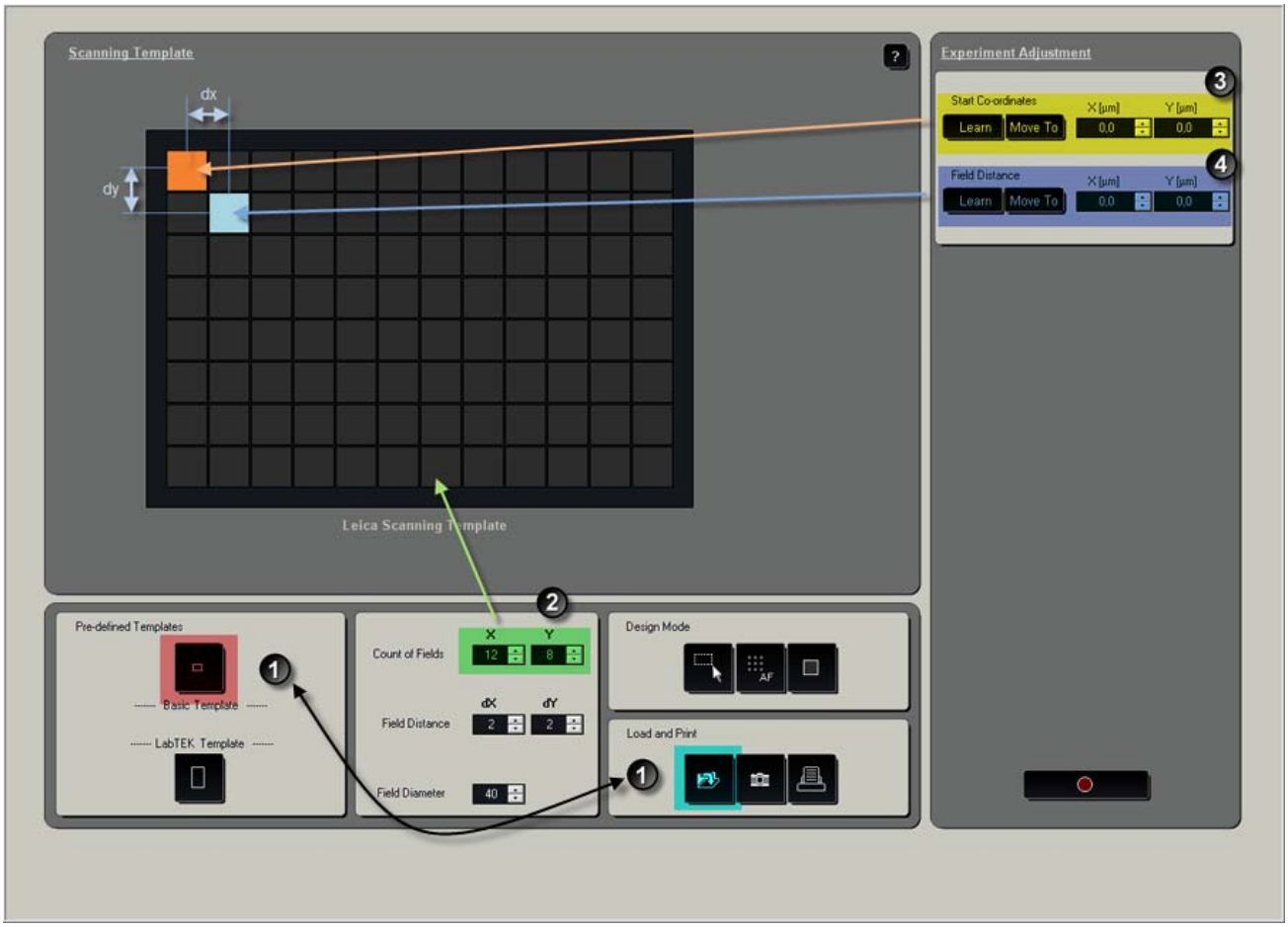


The **Single regular Matrix** application is started now.

Switch to the **Setup Template** operating step to begin with the settings for your experiment.

Setup Template

In the **Setup Template** operating step, you can configure the basic settings for your experiment by creating a Scanning Template or selecting an existing one. This defines the spatial structure of your experiment.



The following operating steps refer to the numbers in the screenshot shown here.

1. Select a Scanning Template. You have two options:
   - Either select a predefined Scanning Template under **Pre-defined Templates**.
   - Or load an existing Scanning Template under **Load and Print**.
2. Adjust the Scanning Template to the requirements of your experiment by adjusting the number of scan fields under **Count of Fields**.
3. Define the starting point of your experiment by moving the specimen stage to the respective position and then click the **Learn** button under **Experiment Adjustment** > **Start Co-ordinates**.

   The starting point is always the upper left scan field in the Scanning Template; it is marked orange in the screenshot shown here.
4. Adjust the horizontal and vertical distance between the scan fields by moving the specimen stage to the scan field lying to the right under the starting point and then click the **Learn** button under **Experiment Adjustment** > **Field Distance**.

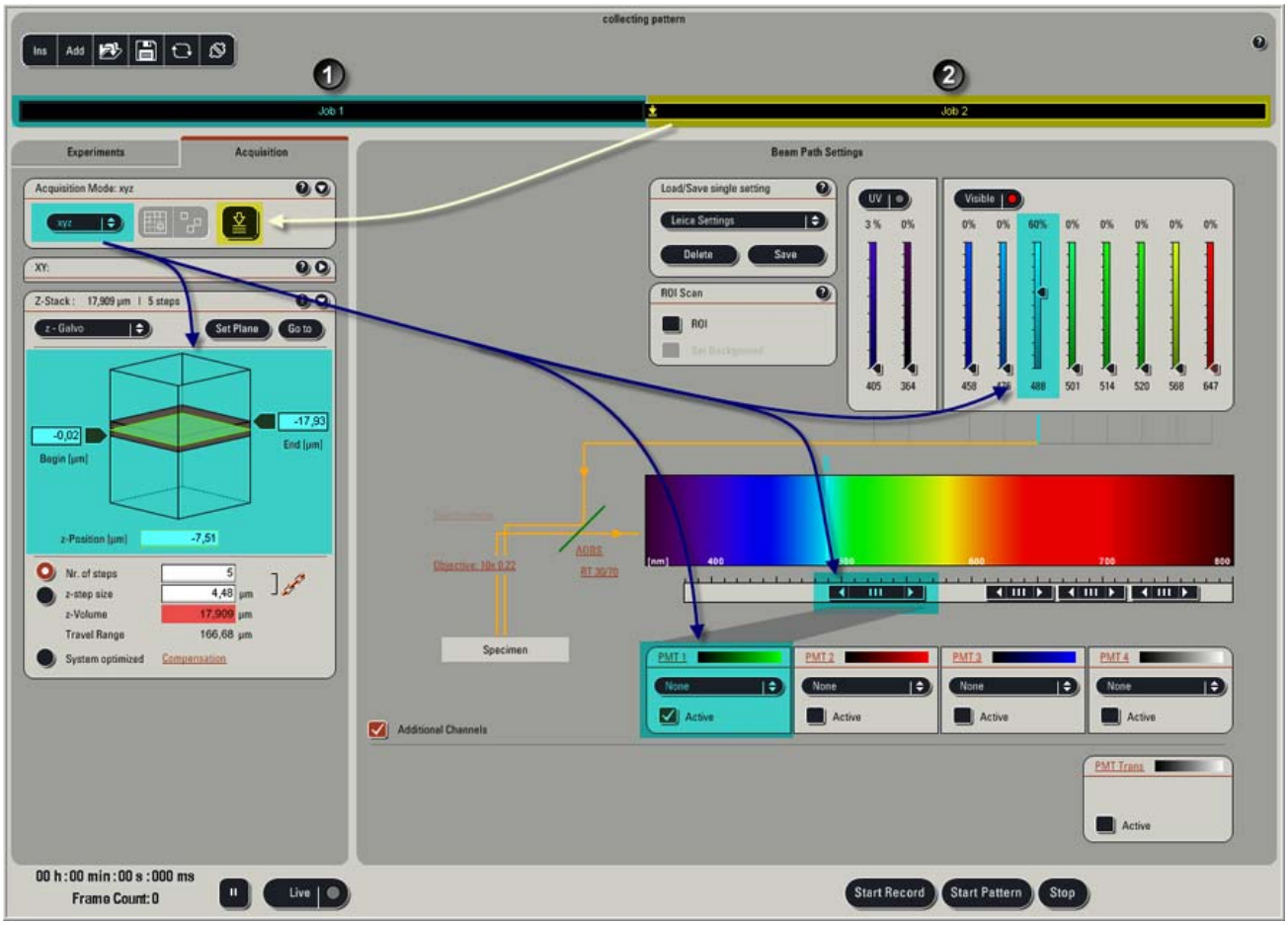
   The horizontal and vertical distance between the scan fields is marked in the screenshot shown here with dx and dy; the scan field lying to the right under the starting point is marked light blue.

The Scanning Template is now prepared for the experiment.

Switch to the **Setup Jobs** operating step.

Setup Jobs

In the **Setup Jobs** operating step, you can configure the settings for image acquisition and define and organize jobs and experiments (**Pattern**). Experiments can consist of multiple items (jobs, pauses and other **Pattern**s) that are processed in succession.



The following operating steps refer to the numbers in the screenshot shown here.

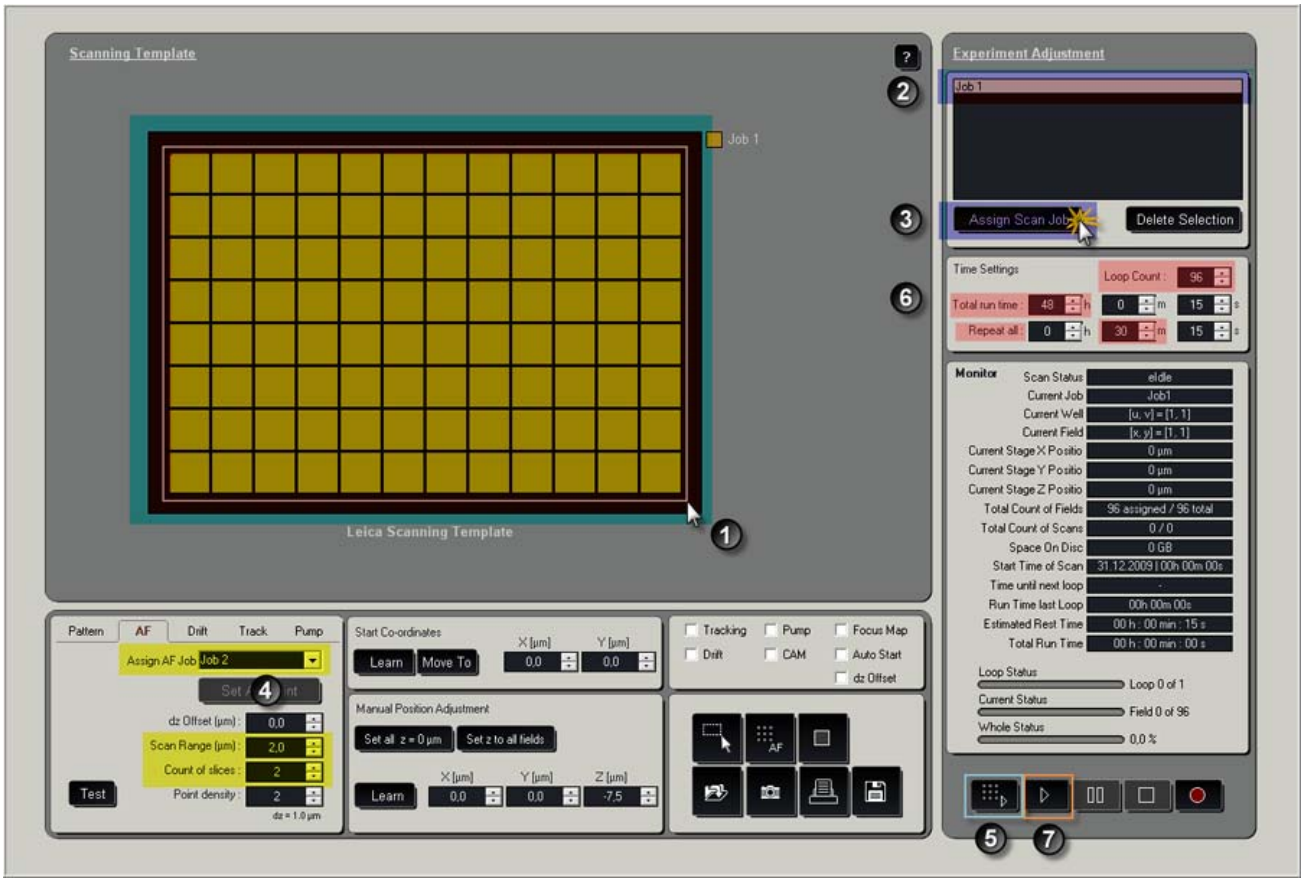
1. Define at least one job with all required settings for the image acquisition.
2. Define a **Autofocus** job for the automatic focus search.

The jobs for performing the experiment are now defined.

Switch to the **Setup Experiment** operating step.

Setup Experiment

In the **Setup Experiment** operating step, you can merge the settings from the **Setup Template** and **Setup Jobs** operating steps, i.e. you can assign jobs and experiments to the Scanning Template.



The following operating steps refer to the numbers in the screenshot shown here.

1. Select scan fields in the **Scanning Template** view by holding down the left mouse button and dragging a rectangle over the respective area of the Scanning Template.

   In general, for experiments with the **Single regular Matrix** application, select all scan fields.

2. Select a job from the list box under **Experiment Adjustment** via mouse click.

3. Assign the selected job to the selected scan fields by clicking the **Assign Scan Job** button under the list box.

4. At the bottom left in the **AF** tab, select the **Autofocus** job for the automatic focus search and then adjust the following parameters: the range in z-direction in which the automatic focus search is carried out (**Scan Range**) and the number of individual images (horizontal xy-sections) that the system acquires during the automatic focus search (**Count of slices**).

   The individual images (horizontal xy-sections) are acquired within the range defined under **Scan Range**.

5. Make sure that the starting point of the experiment (upper left scan field) lies near the focus range and then start the automatic focus search by clicking the corresponding button to the bottom right.

6. Optional: Under **Experiment Adjustment** > **Time Settings**, you can define loops to process an experiment repeatedly.

   If no loops are defined, an experiment is run once. If an experiment is supposed to run 2 days, for example, and during this time it is to be repeated every half hour, configure the following settings: set 30 minutes under **Repeat all** and 48 hours under **Total run time**. The number of loops resulting from this (in this case, 96) is automatically calculated by the system and displayed under **Loop Count**.

7. Start the experiment by clicking the corresponding button to the bottom right.

The experiment is running now and you can track the progress of the experiment under **Experiment Adjustment** > **Monitor**.

MatrixScreener Wizard Quick Tutorial: Multiple regular Matrices

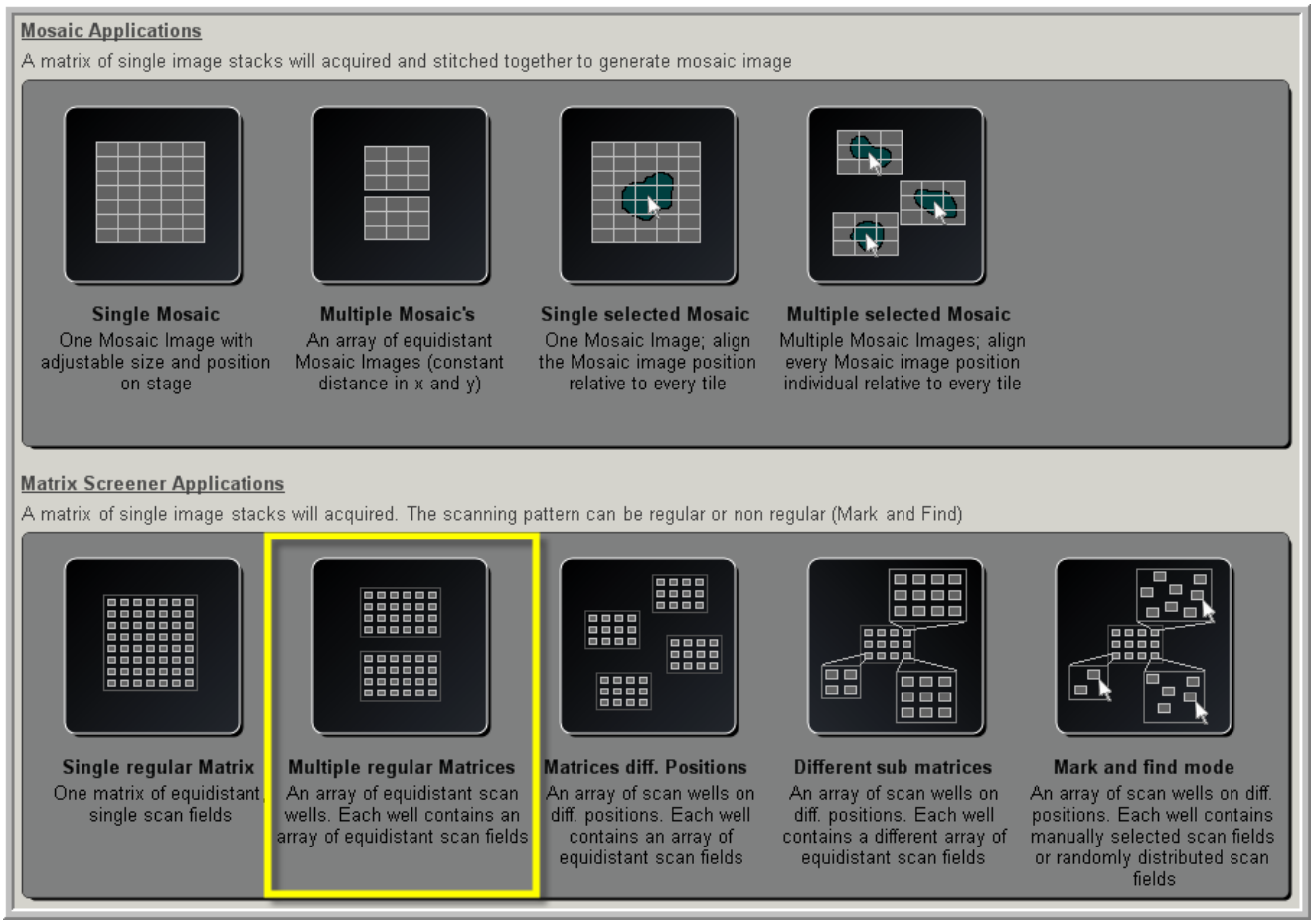
Brief description: This Quick Tutorial helps you get a quick start using the **Multiple regular Matrices** application. With the **Multiple regular Matrices** application you can define a series of matrices, and each matrix can consist of multiple scan fields. The number of scan fields is the same in each well or chamber of the specimen slide. For that reason, this application is primarily suited for well plates and chambered coverglasses.

Start application

1. Start LAS AF.
2. Select MatrixScreener Wizard from the top left menu bar under **Leica Microsystems LAS AF**.

The MatrixScreener Wizard starts with the **Select App.** operating step, in which you can select the desired application.

3. Click **Matrix Screener Applications** > **Multiple regular Matrices**.

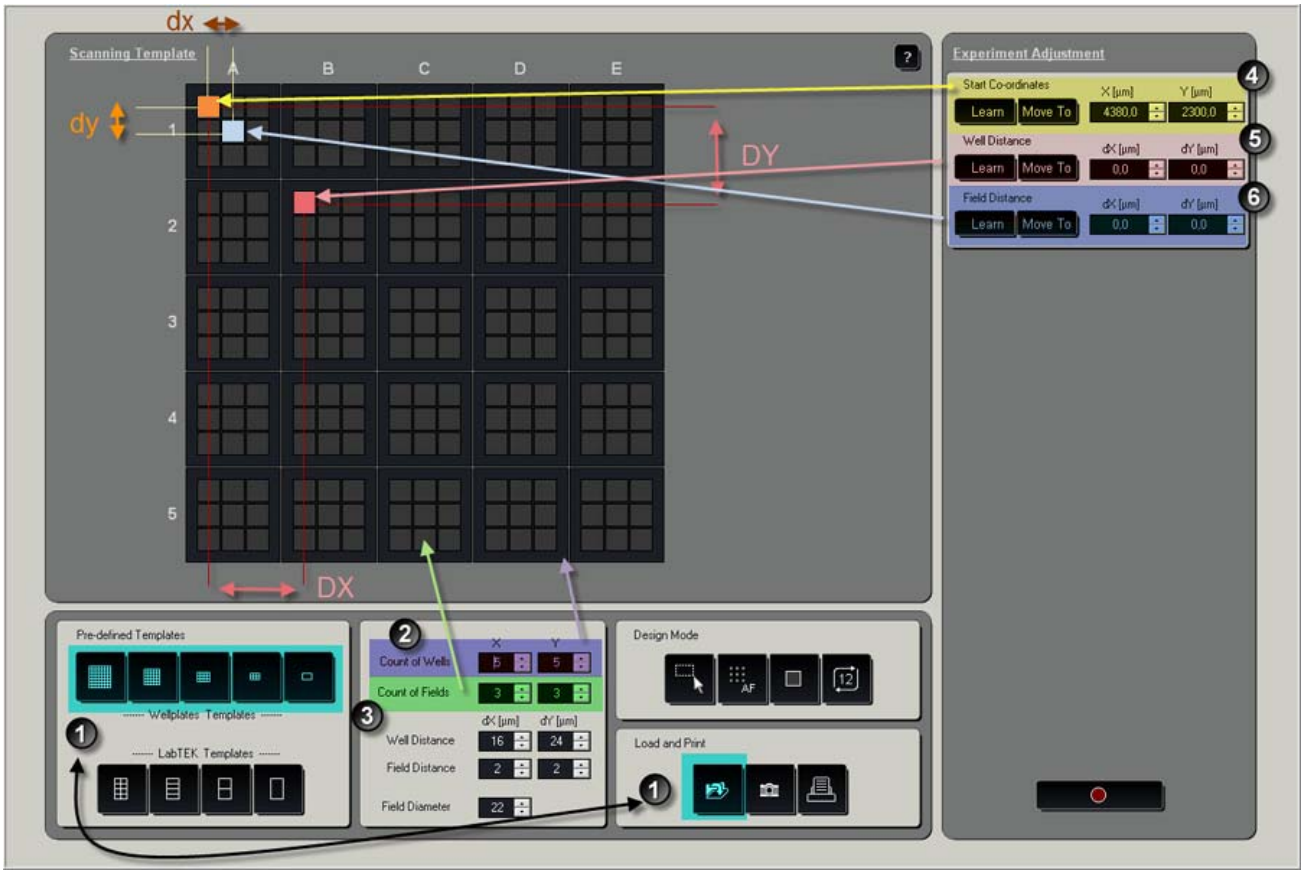


The **Multiple regular Matrices** application is started now.

Switch to the **Setup Template** operating step to begin with the settings for your experiment.

Setup Template

In the **Setup Template** operating step, you can configure the basic settings for your experiment by creating a Scanning Template or selecting an existing one. This defines the spatial structure of your experiment.



The following operating steps refer to the numbers in the screenshot shown here.

1. Select a Scanning Template. You have two options:
   - Either select a predefined Scanning Template under **Pre-defined Templates**.
   - Or load an existing Scanning Template under **Load and Print**.
2. Adjust the Scanning Template to the requirements of your experiment by adjusting the number of wells or chambers of the specimen slide under **Count of Wells**.
3. Adjust the Scanning Template to the requirements of your experiment by adjusting the number of scan fields under **Count of Fields**.

   This adjustment applies for the entire specimen slide, i.e., the number of scan fields is the same in all wells or chambers.
4. Define the starting point of your experiment by moving the specimen stage to the respective position and then click the **Learn** button under **Experiment Adjustment** > **Start Co-ordinates**.

   The starting point is always the upper left scan field in the Scanning Template; it is marked orange in the screenshot shown here.
5. Adjust the horizontal and vertical distance between the wells or chambers by moving the specimen stage to the initial scan field of the well or chamber lying to the right under the starting point and then click the **Learn** button under **Experiment Adjustment** > **Well Distance**.

   The horizontal and vertical distance between the wells or chambers is marked in the screenshot shown here with DX and DY; the first scan field of the well or chamber lying to the right under the starting point is marked pink.
6. Adjust the horizontal and vertical distance between the scan fields by moving the specimen stage to the scan field lying to the right under the starting point and then click the **Learn** button under **Experiment Adjustment** > **Field Distance**.

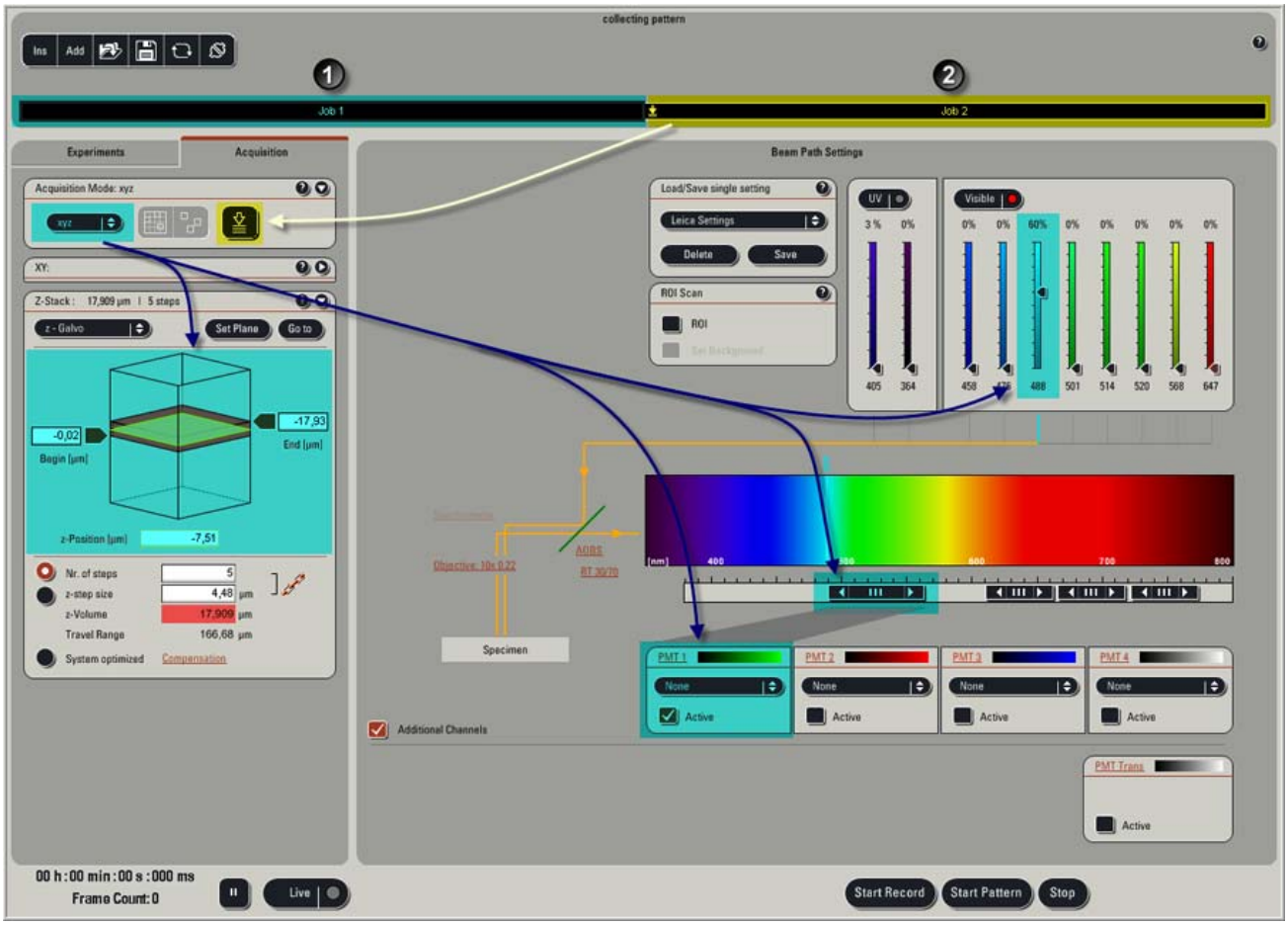
   The horizontal and vertical distance between the scan fields is marked in the screenshot shown here with dx and dy; the scan field lying to the right under the starting point is marked light blue.

The Scanning Template is now prepared for the experiment.

Switch to the **Setup Jobs** operating step.

Setup Jobs

In the **Setup Jobs** operating step, you can configure the settings for image acquisition and define and organize jobs and experiments (**Pattern**). Experiments can consist of multiple items (jobs, pauses and other **Pattern**s) that are processed in succession.



The following operating steps refer to the numbers in the screenshot shown here.

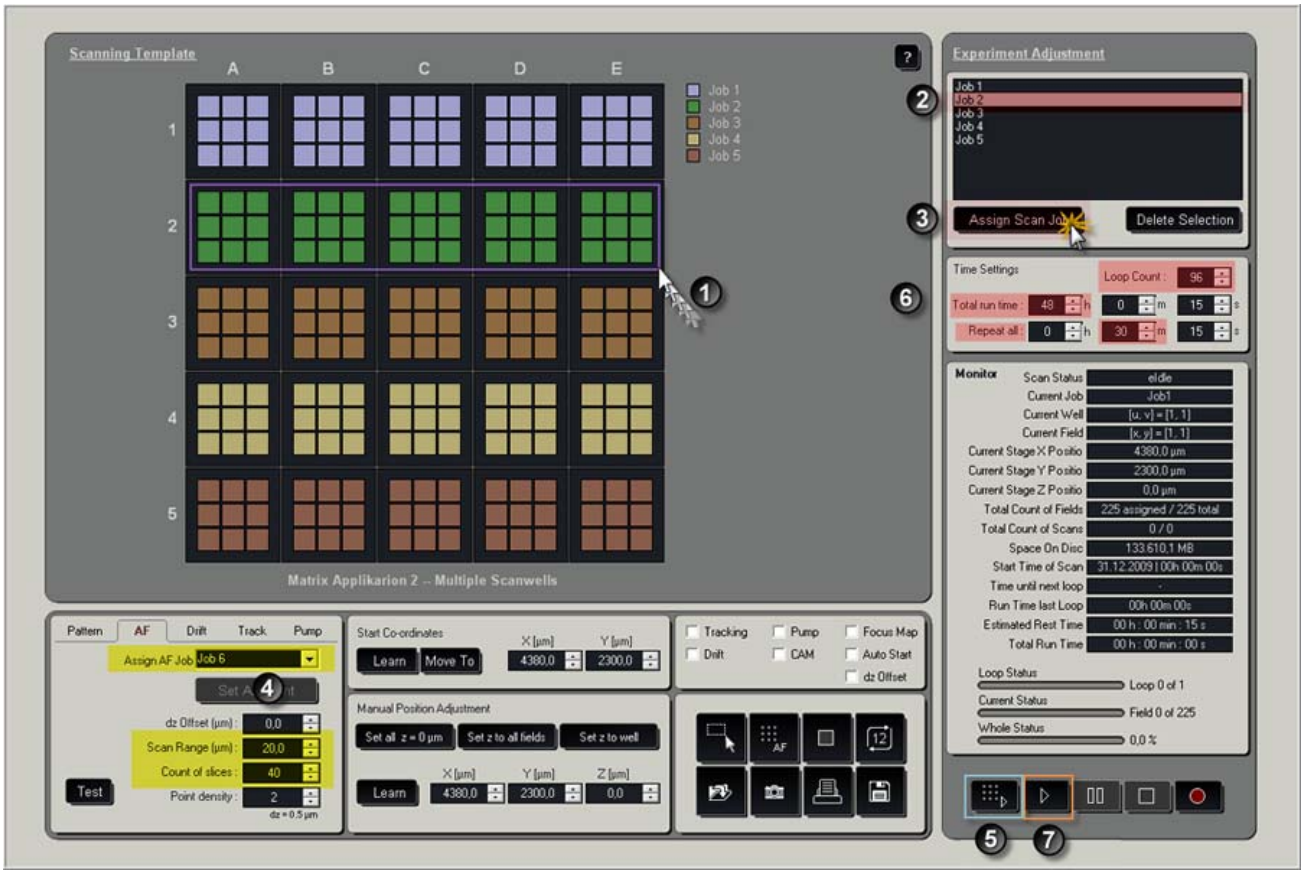
1. Define at least one job with all required settings for the image acquisition.
2. Define a **Autofocus** job for the automatic focus search.

The jobs for performing the experiment are now defined.

Switch to the **Setup Experiment** operating step.

Setup Experiment

In the **Setup Experiment** operating step, you can merge the settings from the **Setup Template** and **Setup Jobs** operating steps, i.e. you can assign jobs and experiments to the Scanning Template.



The following operating steps refer to the numbers in the screenshot shown here.

1. Select scan fields in the **Scanning Template** view by holding down the left mouse button and dragging a rectangle over the respective area of the Scanning Template.
2. Select a job from the list box under **Experiment Adjustment** via mouse click.
3. Assign the selected job to the selected scan fields by clicking the **Assign Scan Job** button under the list box.
4. At the bottom left in the **AF** tab, select the **Autofocus** job for the automatic focus search and then adjust the following parameters: the range in z-direction in which the automatic focus search is carried out (**Scan Range**) and the number of individual images (horizontal xy-sections) that the system acquires during the automatic focus search (**Count of slices**).

   The individual images (horizontal xy-sections) are acquired within the range defined under **Scan Range**.
5. Make sure that the starting point of the experiment (upper left scan field) lies near the focus range and then start the automatic focus search by clicking the corresponding button to the bottom right.
6. Optional: Under **Experiment Adjustment** > **Time Settings**, you can define loops to process an experiment repeatedly.

   If no loops are defined, an experiment is run once. If an experiment is supposed to run 2 days, for example, and during this time it is to be repeated every half hour, configure the following settings: set 30 minutes under **Repeat all** and 48 hours under **Total run time**. The number of loops resulting from this (in this case, 96) is automatically calculated by the system and displayed under **Loop Count**.
7. Start the experiment by clicking the corresponding button to the bottom right.

The experiment is running now and you can track the progress of the experiment under **Experiment Adjustment** > **Monitor**.

MatrixScreener Wizard Quick Tutorial: Matrices diff. Positions

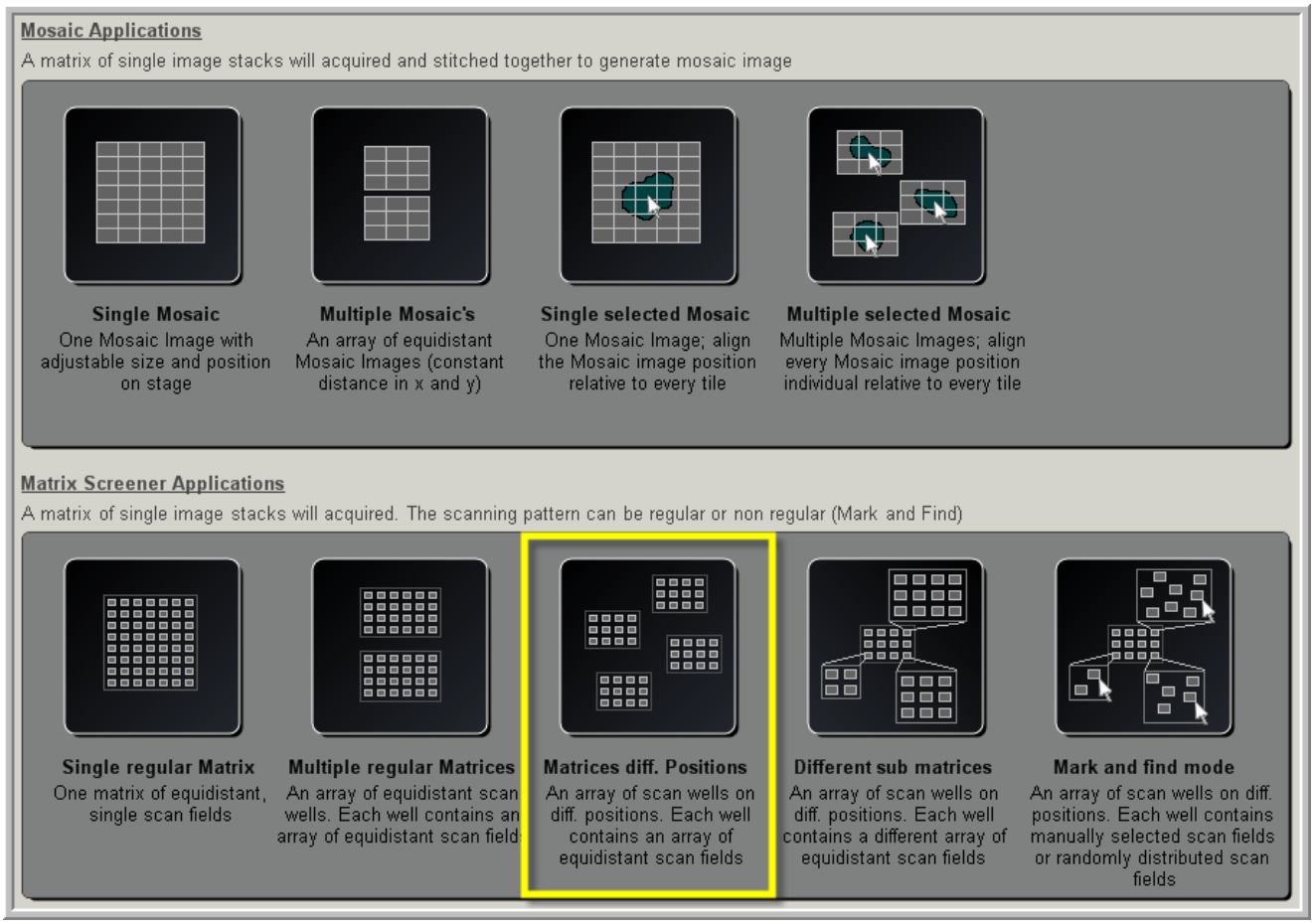
Brief description: This Quick Tutorial helps you get a quick start using the **Matrices diff. Positions** application. With the **Matrices diff. Positions** application you can define multiple matrices in various positions, and each matrix can consist of multiple scan fields. The number of scan fields is the same in each well or chamber of the specimen slide. The starting point of a matrix can be individually adjusted. For that reason, this application is primarily suited for well plates and Tissue Micro Arrays (TMA).

Start application

1. Start LAS AF.
2. Select MatrixScreener Wizard from the top left menu bar under **Leica Microsystems LAS AF**.

The MatrixScreener Wizard starts with the **Select App.** operating step, in which you can select the desired application.

3. Click **Matrix Screener Applications** > **Matrices diff. Positions**.

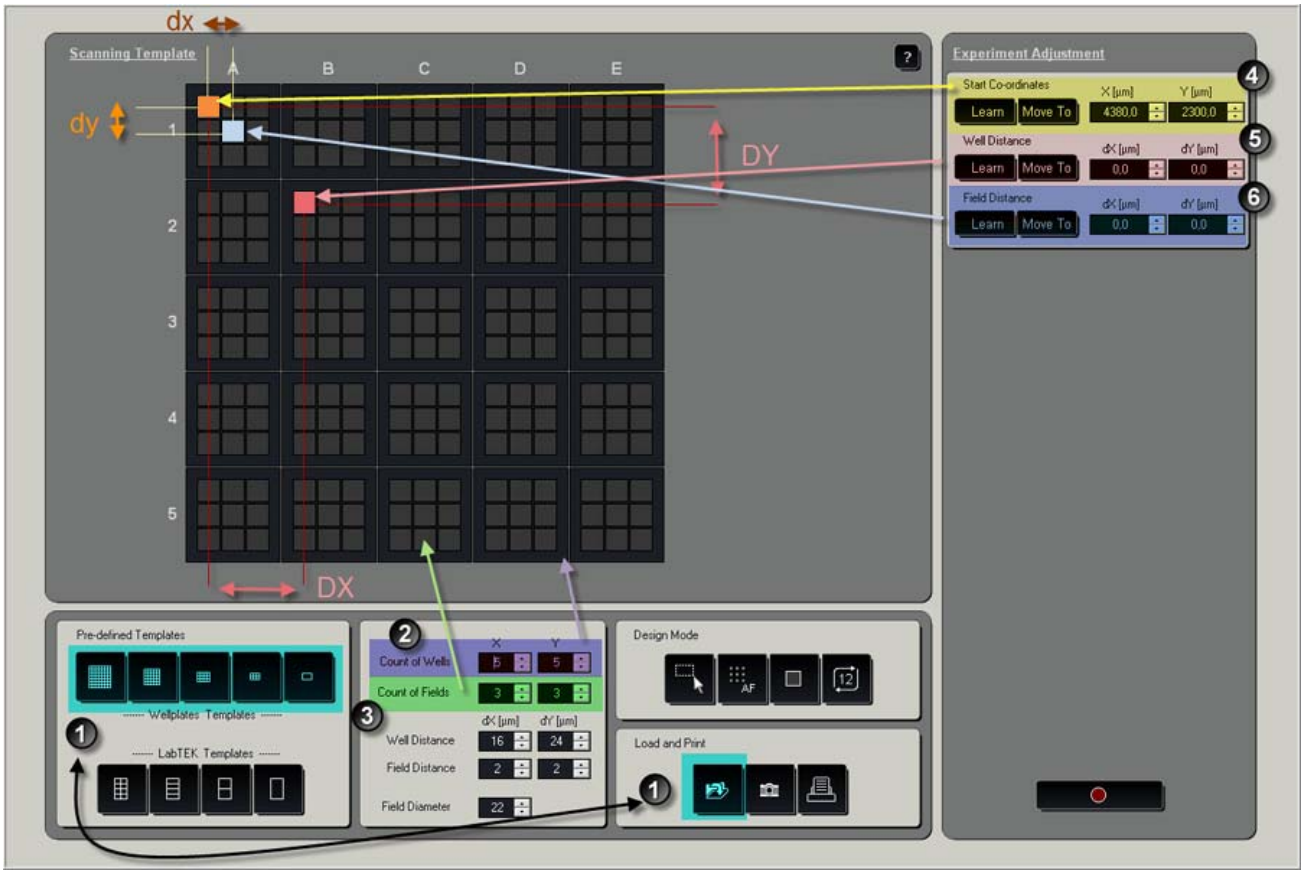


The **Matrices diff. Positions** application is started now.

Switch to the **Setup Template** operating step to begin with the settings for your experiment.

Setup Template

In the **Setup Template** operating step, you can configure the basic settings for your experiment by creating a Scanning Template or selecting an existing one. This defines the spatial structure of your experiment.



The following operating steps refer to the numbers in the screenshot shown here.

1. Select a Scanning Template. You have two options:
   - Either select a predefined Scanning Template under **Pre-defined Templates**.
   - Or load an existing Scanning Template under **Load and Print**.
2. Adjust the Scanning Template to the requirements of your experiment by adjusting the number of wells or chambers of the specimen slide under **Count of Wells**.
3. Adjust the Scanning Template to the requirements of your experiment by adjusting the number of scan fields under **Count of Fields**.

   This adjustment applies for the entire specimen slide, i.e., the number of scan fields is the same in all wells or chambers.
4. Define the starting point of your experiment by moving the specimen stage to the respective position and then click the **Learn** button under **Experiment Adjustment** > **Start Co-ordinates**.

   The starting point is always the upper left scan field in the Scanning Template; it is marked orange in the screenshot shown here.
5. Adjust the horizontal and vertical distance between the wells or chambers by moving the specimen stage to the initial scan field of the well or chamber lying to the right under the starting point and then click the **Learn** button under **Experiment Adjustment** > **Well Distance**.

   The horizontal and vertical distance between the wells or chambers is marked in the screenshot shown here with DX and DY; the first scan field of the well or chamber lying to the right under the starting point is marked pink.
6. Adjust the horizontal and vertical distance between the scan fields by moving the specimen stage to the scan field lying to the right under the starting point and then click the **Learn** button under **Experiment Adjustment** > **Field Distance**.

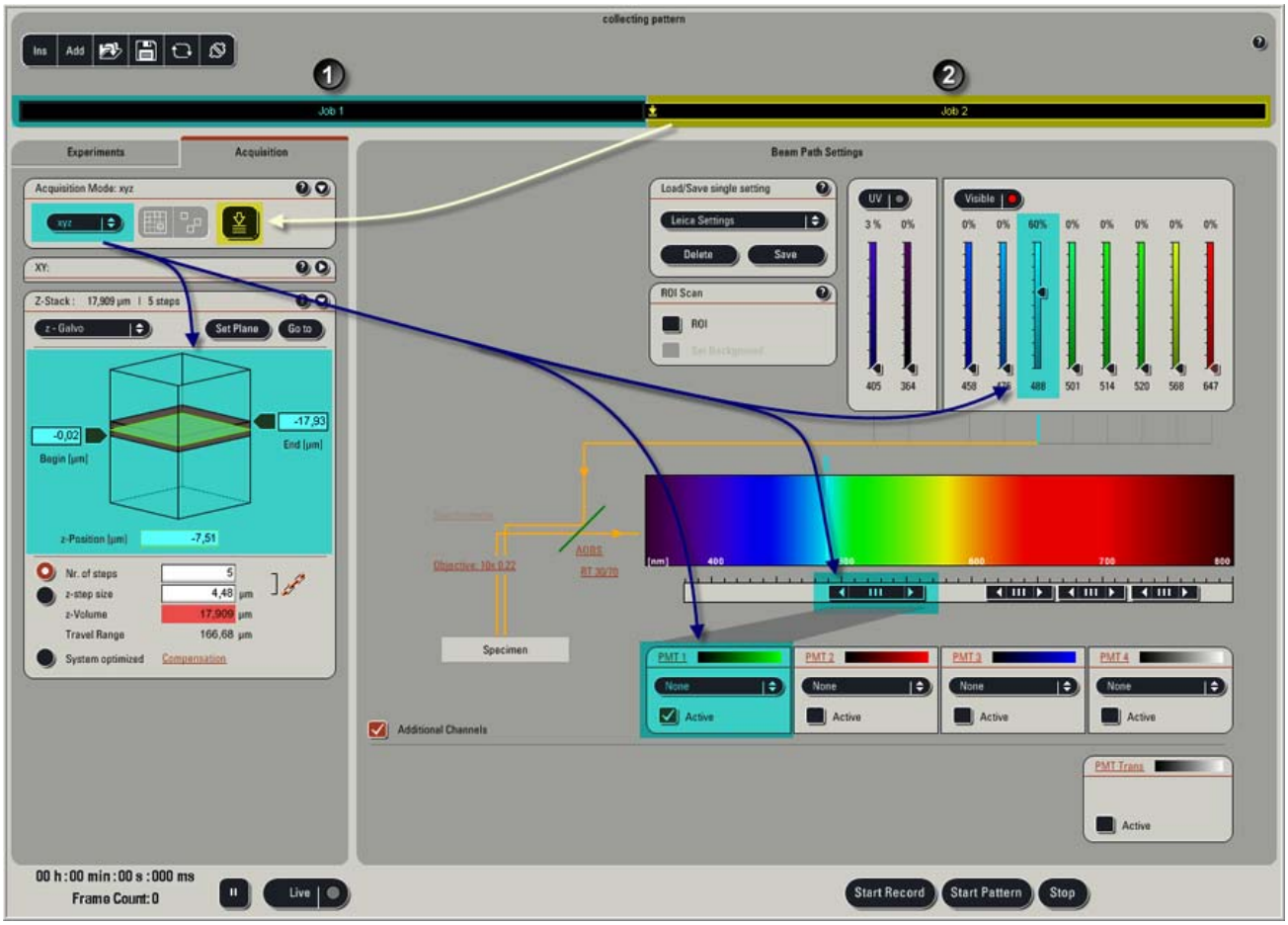
   The horizontal and vertical distance between the scan fields is marked in the screenshot shown here with dx and dy; the scan field lying to the right under the starting point is marked light blue.

The Scanning Template is now prepared for the experiment.

Switch to the **Setup Jobs** operating step.

Setup Jobs

In the **Setup Jobs** operating step, you can configure the settings for image acquisition and define and organize jobs and experiments (**Pattern**). Experiments can consist of multiple items (jobs, pauses and other **Pattern**s) that are processed in succession.



The following operating steps refer to the numbers in the screenshot shown here.

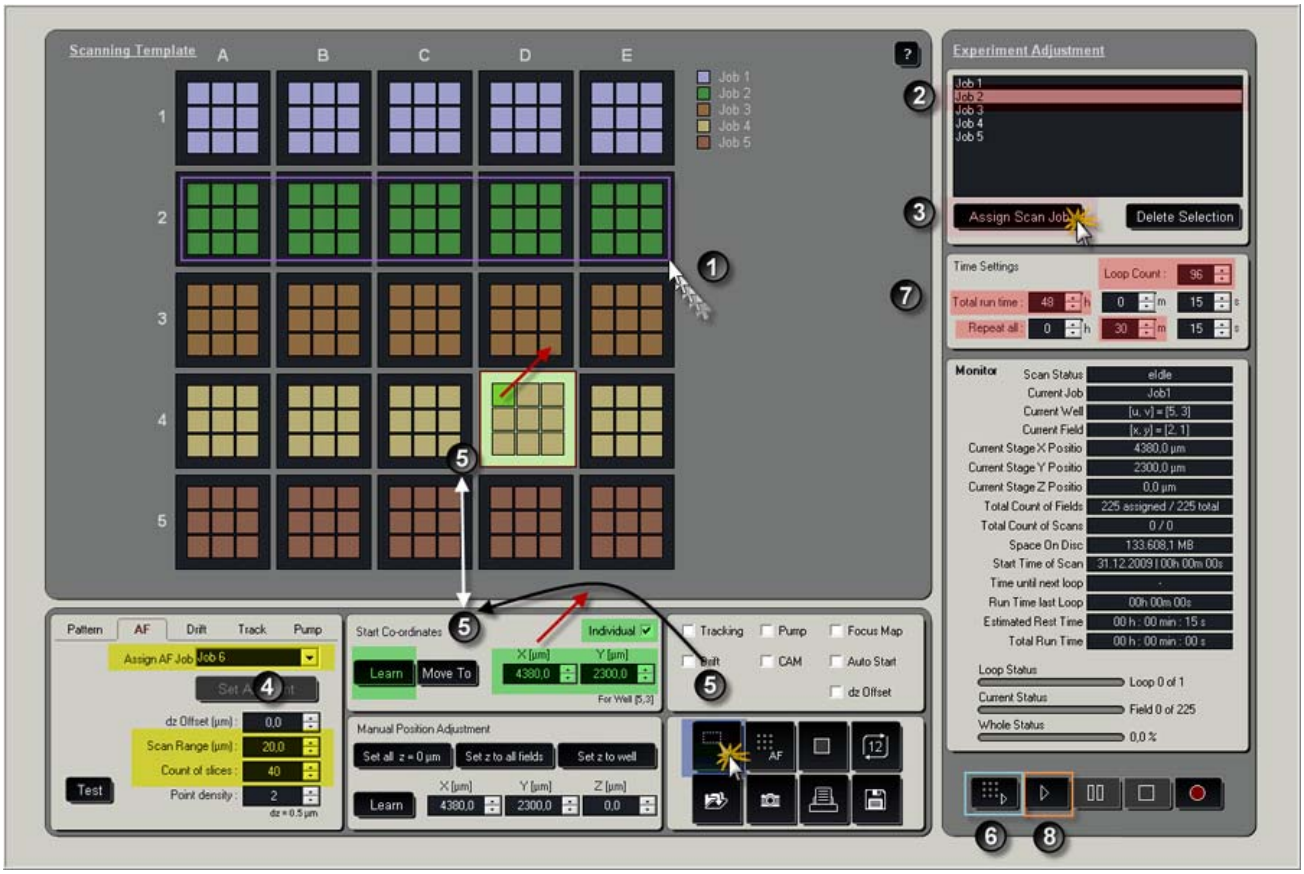
1. Define at least one job with all required settings for the image acquisition.
2. Define a **Autofocus** job for the automatic focus search.

The jobs for performing the experiment are now defined.

Switch to the **Setup Experiment** operating step.

Setup Experiment

In the **Setup Experiment** operating step, you can merge the settings from the **Setup Template** and **Setup Jobs** operating steps, i.e. you can assign jobs and experiments to the Scanning Template.



The following operating steps refer to the numbers in the screenshot shown here.

1. Select scan fields in the **Scanning Template** view by holding down the left mouse button and dragging a rectangle over the respective area of the Scanning Template.
2. Select a job from the list box under **Experiment Adjustment** via mouse click.
3. Assign the selected job to the selected scan fields by clicking the **Assign Scan Job** button under the list box.
4. At the bottom left in the **AF** tab, select the **Autofocus** job for the automatic focus search and then adjust the following parameters: the range in z-direction in which the automatic focus search is carried out (**Scan Range**) and the number of individual images (horizontal xy-sections) that the system acquires during the automatic focus search (**Count of slices**).

   The individual images (horizontal xy-sections) are acquired within the range defined under **Scan Range**.
5. Optional: Move a certain well or chamber in the Scanning Template by defining a new starting point.

   To define a new starting point for a certain well or chamber, follow these steps: enable the **Individual** option under **Start Co-ordinates** and select all scan fields of the well or chamber; then enable the **Move To** mode and move the specimen stage to the desired new starting point (upper left scan field of the well or chamber); then click the **Learn** button under **Start Co-ordinates**.
6. Make sure that the starting point of the experiment (upper left scan field) lies near the focus range and then start the automatic focus search by clicking the corresponding button to the bottom right.
7. Optional: Under **Experiment Adjustment** > **Time Settings**, you can define loops to process an experiment repeatedly.

   If no loops are defined, an experiment is run once. If an experiment is supposed to run 2 days, for example, and during this time it is to be repeated every half hour, configure the following settings: set 30 minutes under **Repeat all** and 48 hours under **Total run time**. The number of loops resulting from this (in this case, 96) is automatically calculated by the system and displayed under **Loop Count**.
8. Start the experiment by clicking the corresponding button to the bottom right.

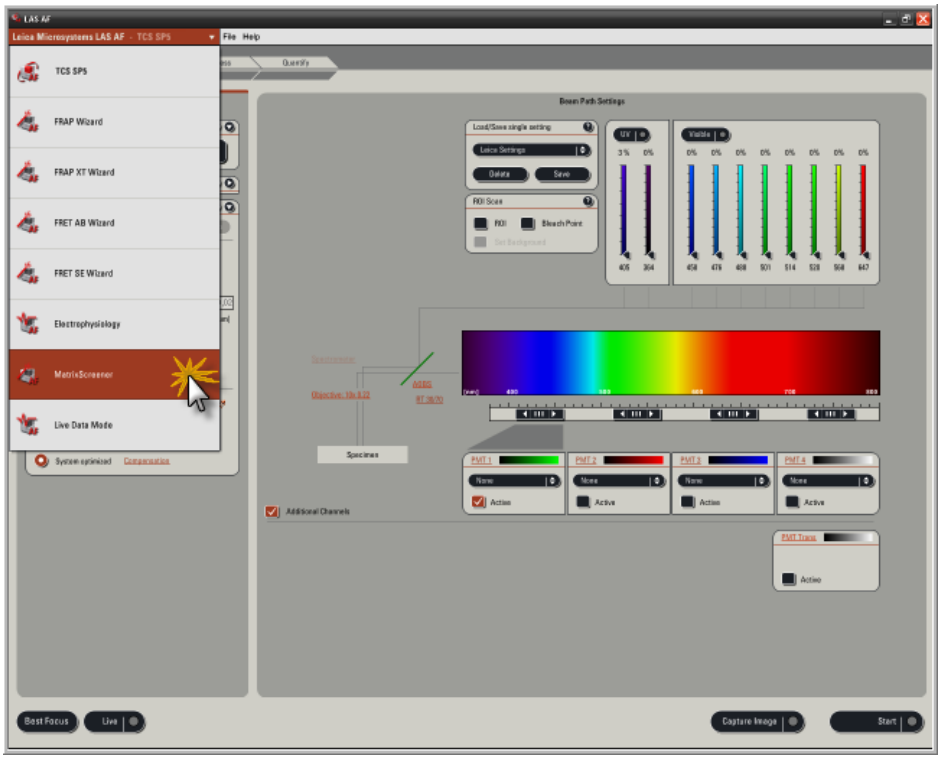
The experiment is running now and you can track the progress of the experiment under **Experiment Adjustment** > **Monitor**.

MatrixScreener Wizard Quick Tutorial: Different sub matrices

Brief description: This Quick Tutorial helps you get a quick start using the **Different sub matrices** application. With the **Different sub matrices** application you can define multiple matrices in various positions, and each matrix can consist of multiple scan fields. Each well or chamber of the specimen slide can include an individual number of scan fields. The starting point of a matrix can be individually adjusted. For that reason, this application is primarily suited for mixed specimens and for tissue sections of different sizes, which are applied to the specimen slide at various places.

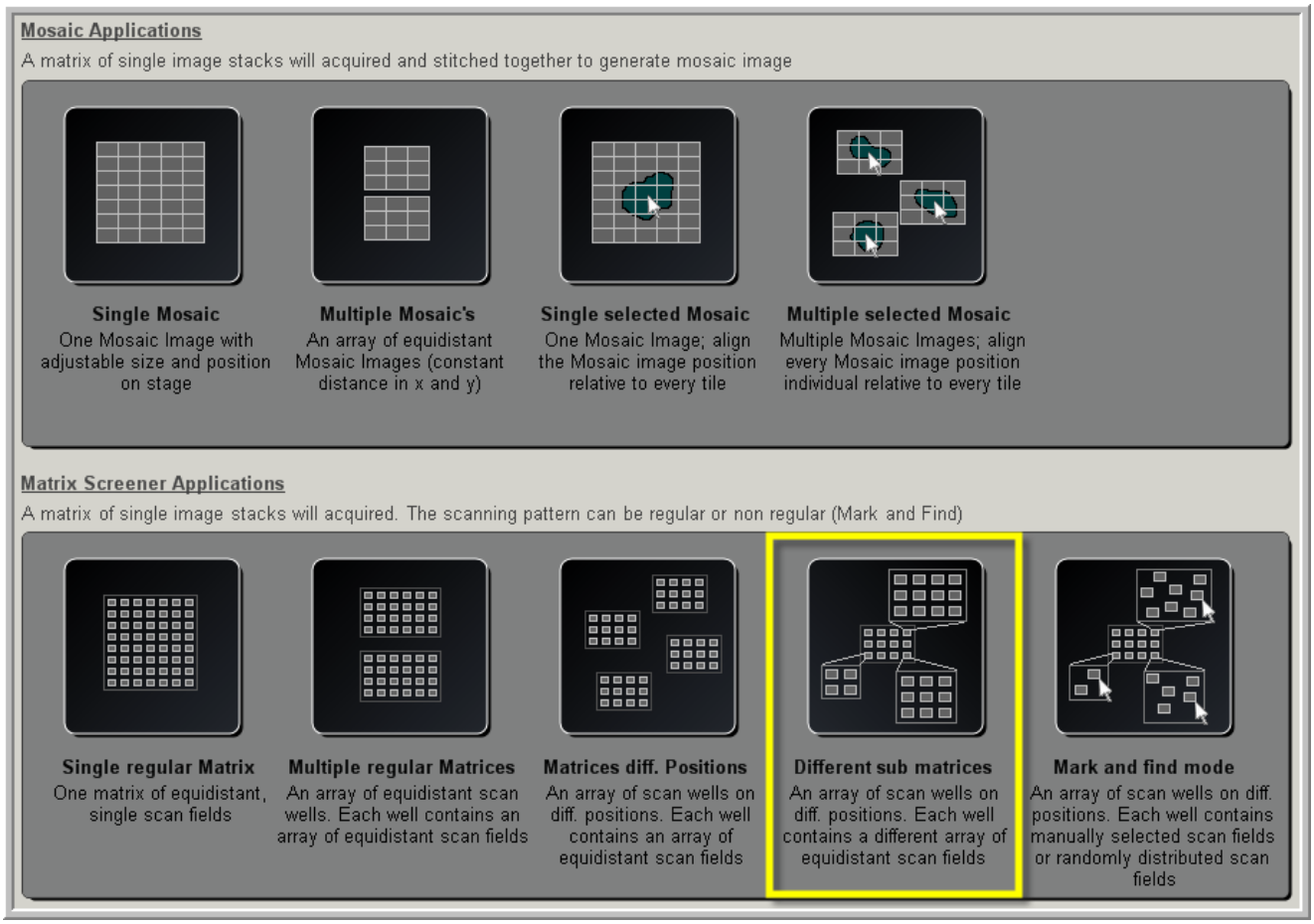
Start application

1. Start LAS AF.
2. Select MatrixScreener Wizard from the top left menu bar under **Leica Microsystems LAS AF**.



The MatrixScreener Wizard starts with the **Select App.** operating step, in which you can select the desired application.

3. Click **Matrix Screener Applications** > **Different sub matrices**.

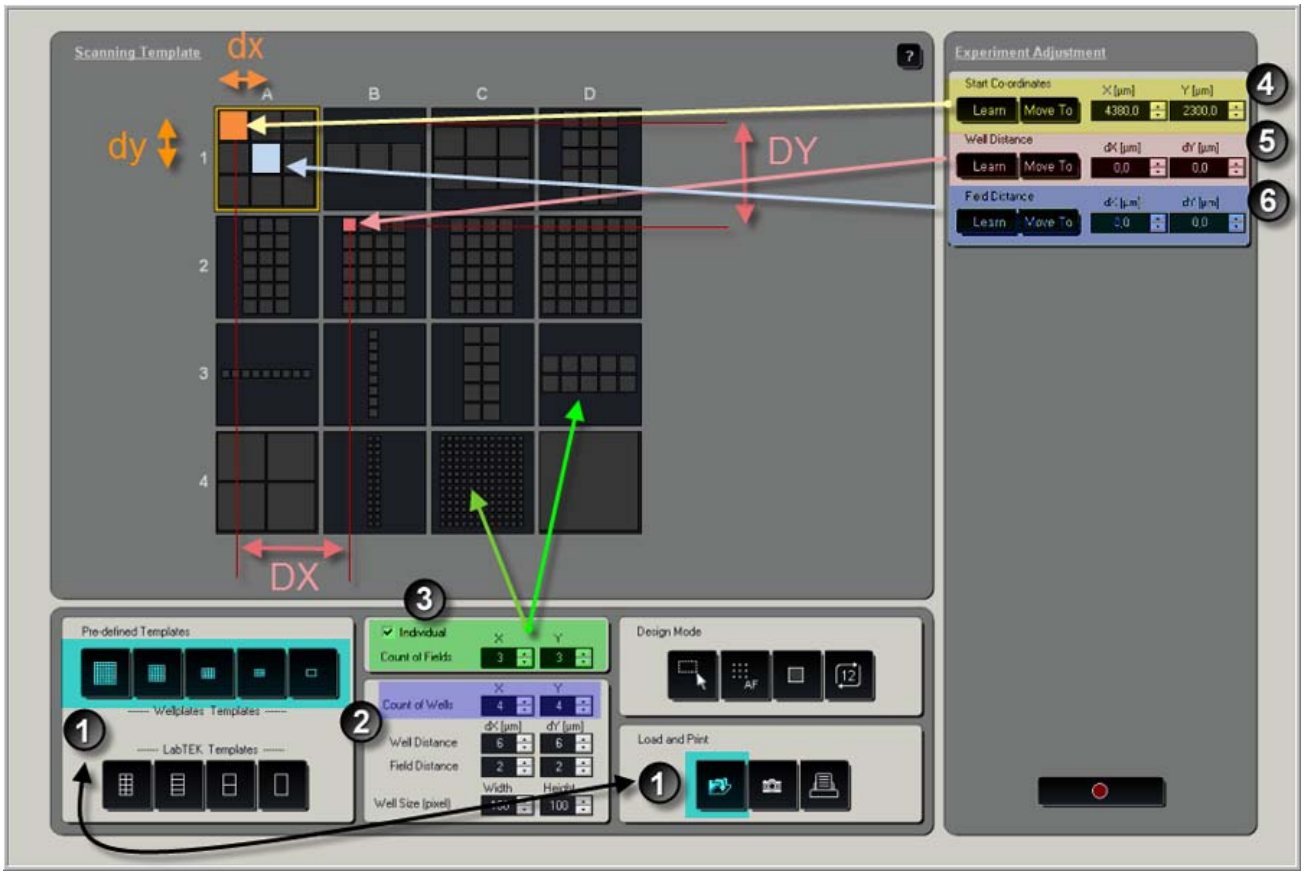


The **Different sub matrices** application is started now.

Switch to the **Setup Template** operating step to begin with the settings for your experiment.

Setup Template

In the **Setup Template** operating step, you can configure the basic settings for your experiment by creating a Scanning Template or selecting an existing one. This defines the spatial structure of your experiment.



The following operating steps refer to the numbers in the screenshot shown here.

1. Select a Scanning Template. You have two options:
   - Either select a predefined Scanning Template under **Pre-defined Templates**.
   - Or load an existing Scanning Template under **Load and Print**.
2. Adjust the Scanning Template to the requirements of your experiment by adjusting the number of wells or chambers of the specimen slide under **Count of Wells**.
3. Adjust the Scanning Template to the requirements of your experiment by adjusting the number of scan fields under **Count of Fields**.

   This adjustment applies for the entire specimen slide, i.e., the number of scan fields is the same in all wells or chambers. If the **Individual** option is enabled, you can set a separate number of scan fields for each well or chamber.
4. Define the starting point of your experiment by moving the specimen stage to the respective position and then click the **Learn** button under **Experiment Adjustment** > **Start Co-ordinates**.

   The starting point is always the upper left scan field in the Scanning Template; it is marked orange in the screenshot shown here.
5. Adjust the horizontal and vertical distance between the wells or chambers by moving the specimen stage to the initial scan field of the well or chamber lying to the right under the starting point and then click the **Learn** button under **Experiment Adjustment** > **Well Distance**.

   The horizontal and vertical distance between the wells or chambers is marked in the screenshot shown here with DX and DY; the first scan field of the well or chamber lying to the right under the starting point is marked pink.
6. Adjust the horizontal and vertical distance between the scan fields by moving the specimen stage to the scan field lying to the right under the starting point and then click the **Learn** button under **Experiment Adjustment** > **Field Distance**.

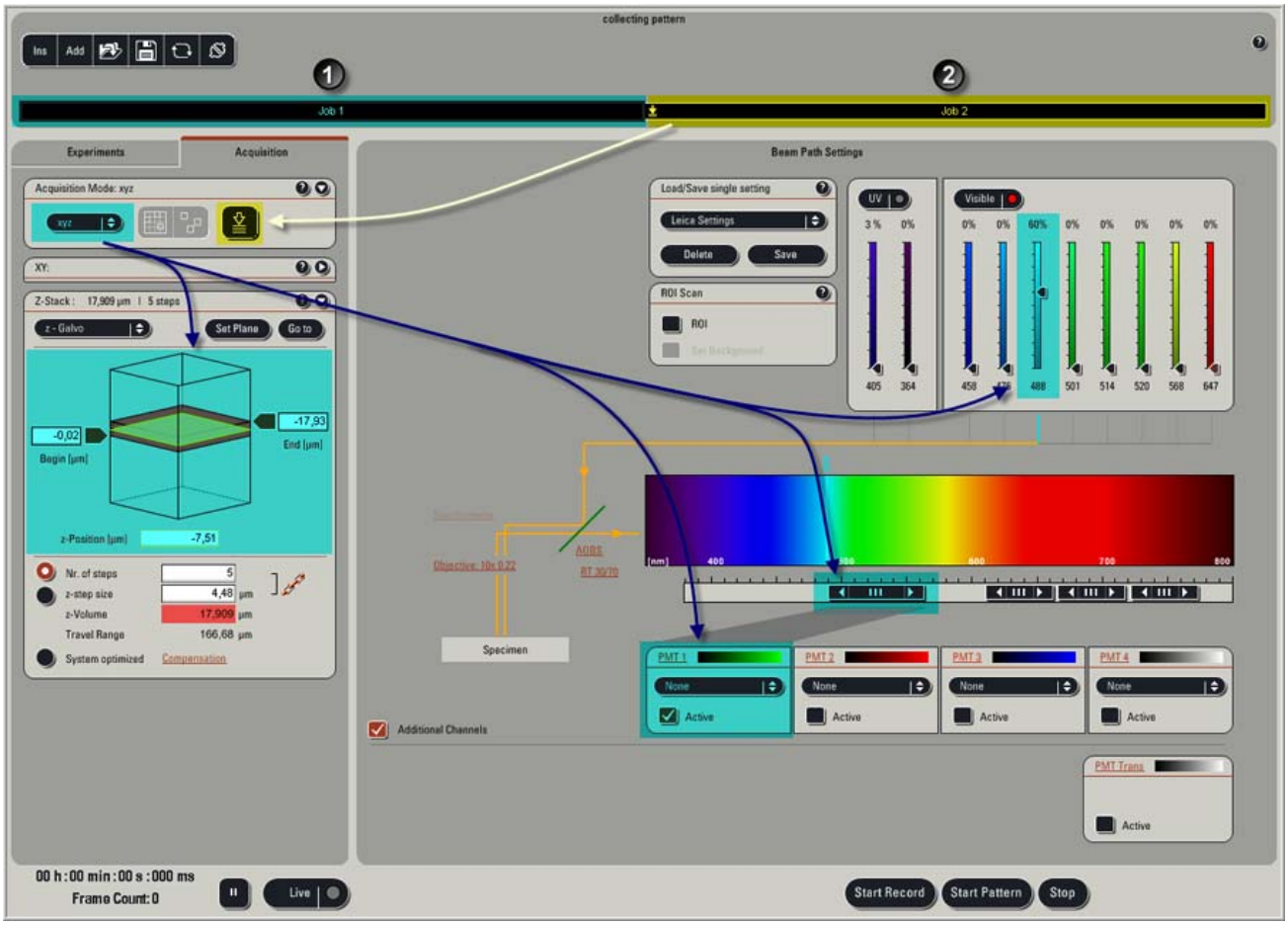
   The horizontal and vertical distance between the scan fields is marked in the screenshot shown here with dx and dy; the scan field lying to the right under the starting point is marked light blue.

The Scanning Template is now prepared for the experiment.

Switch to the **Setup Jobs** operating step.

Setup Jobs

In the **Setup Jobs** operating step, you can configure the settings for image acquisition and define and organize jobs and experiments (**Pattern**). Experiments can consist of multiple items (jobs, pauses and other **Pattern**s) that are processed in succession.



The following operating steps refer to the numbers in the screenshot shown here.

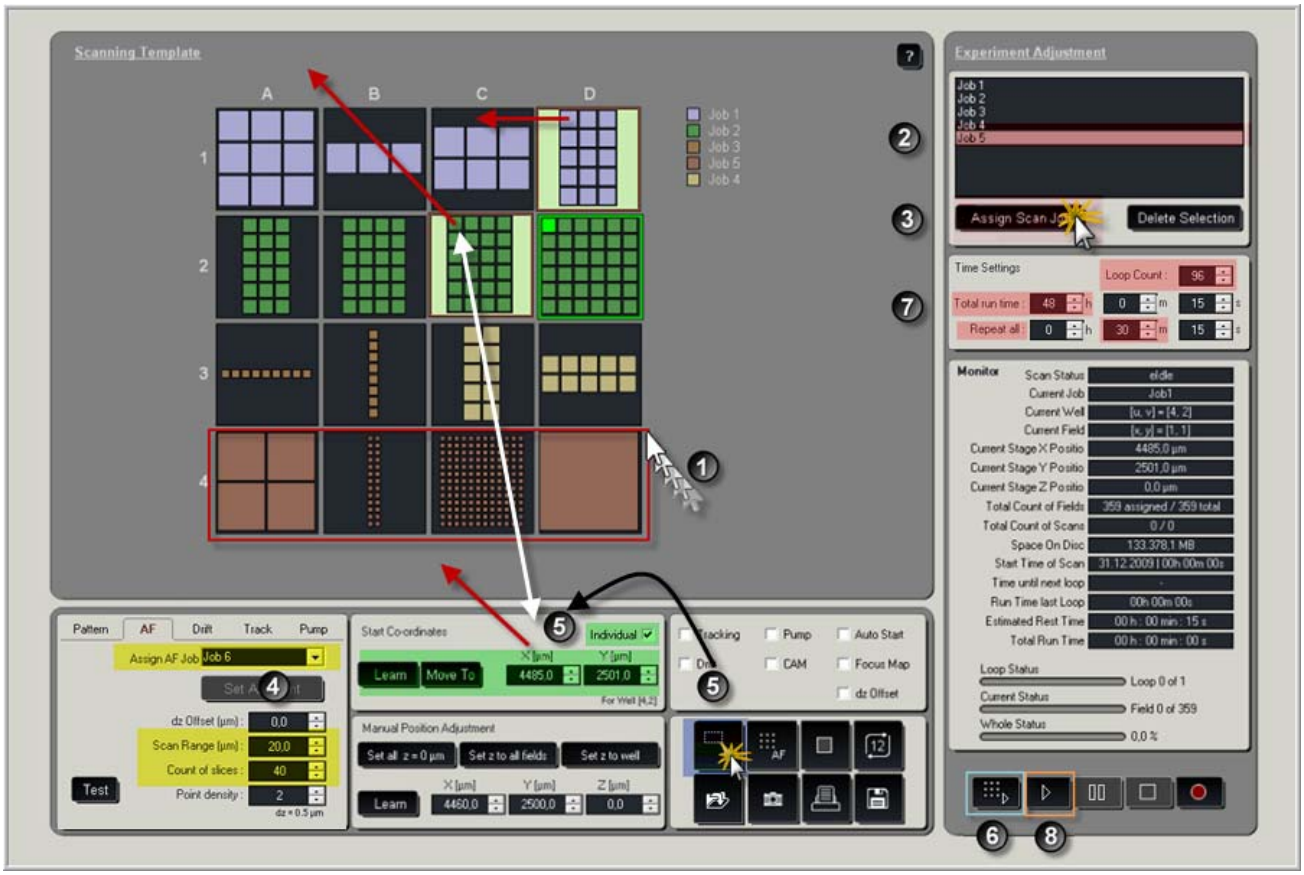
1. Define at least one job with all required settings for the image acquisition.
2. Define a **Autofocus** job for the automatic focus search.

The jobs for performing the experiment are now defined.

Switch to the **Setup Experiment** operating step.

Setup Experiment

In the **Setup Experiment** operating step, you can merge the settings from the **Setup Template** and **Setup Jobs** operating steps, i.e. you can assign jobs and experiments to the Scanning Template.



The following operating steps refer to the numbers in the screenshot shown here.

1. Select scan fields in the **Scanning Template** view by holding down the left mouse button and dragging a rectangle over the respective area of the Scanning Template.
2. Select a job from the list box under **Experiment Adjustment** via mouse click.
3. Assign the selected job to the selected scan fields by clicking the **Assign Scan Job** button under the list box.
4. At the bottom left in the **AF** tab, select the **Autofocus** job for the automatic focus search and then adjust the following parameters: the range in z-direction in which the automatic focus search is carried out (**Scan Range**) and the number of individual images (horizontal xy-sections) that the system acquires during the automatic focus search (**Count of slices**).

   The individual images (horizontal xy-sections) are acquired within the range defined under **Scan Range**.
5. Optional: Move a certain well or chamber in the Scanning Template by defining a new starting point.

   To define a new starting point for a certain well or chamber, follow these steps: enable the **Individual** option under **Start Co-ordinates** and select all scan fields of the well or chamber; then enable the **Move To** mode and move the specimen stage to the desired new starting point (upper left scan field of the well or chamber); then click the **Learn** button under **Start Co-ordinates**.
6. Make sure that the starting point of the experiment (upper left scan field) lies near the focus range and then start the automatic focus search by clicking the corresponding button to the bottom right.
7. Optional: Under **Experiment Adjustment** > **Time Settings**, you can define loops to process an experiment repeatedly.

   If no loops are defined, an experiment is run once. If an experiment is supposed to run 2 days, for example, and during this time it is to be repeated every half hour, configure the following settings: set 30 minutes under **Repeat all** and 48 hours under **Total run time**. The number of loops resulting from this (in this case, 96) is automatically calculated by the system and displayed under **Loop Count**.
8. Start the experiment by clicking the corresponding button to the bottom right.

The experiment is running now and you can track the progress of the experiment under **Experiment Adjustment** > **Monitor**.

MatrixScreener Wizard Quick Tutorial: Mark and find mode

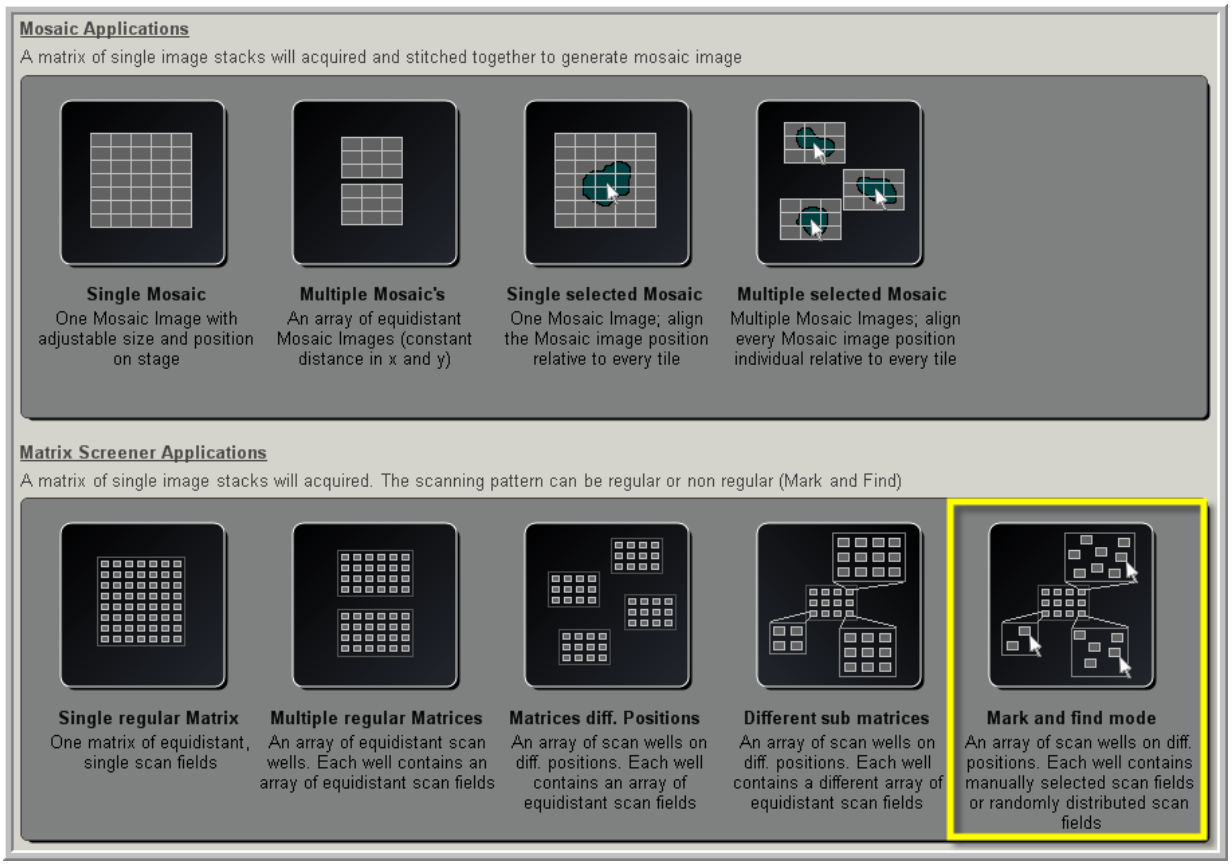
Brief description: This Quick Tutorial helps you get a quick start using the **Mark and find mode** application. The **Mark and find mode** application is intended for implementing multi-position experiments, i.e. certain positions can be marked in the specimen and be approached with the specimen stage in sequence during the experiment. You can define multiple matrices in various positions, and each matrix can consist of multiple scan fields. The starting points in the matrices can be defined in advance. You can (manually or randomly) assign the Mark & Find positions to the scan fields in the wells or chambers of the specimen slide. Scan fields without assigned Mark & Find positions are automatically deactivated and thus excluded from the image acquisition. For that reason, this application is primarily suited for experiments in which individual cells of the specimen are manually selected in certain wells or chambers of the specimen slide.

Start application

1. Start LAS AF.
2. Select MatrixScreener Wizard from the top left menu bar under **Leica Microsystems LAS AF**.

The MatrixScreener Wizard starts with the **Select App.** operating step, in which you can select the desired application.

3. Click **Matrix Screener Applications** > **Mark and find mode**.

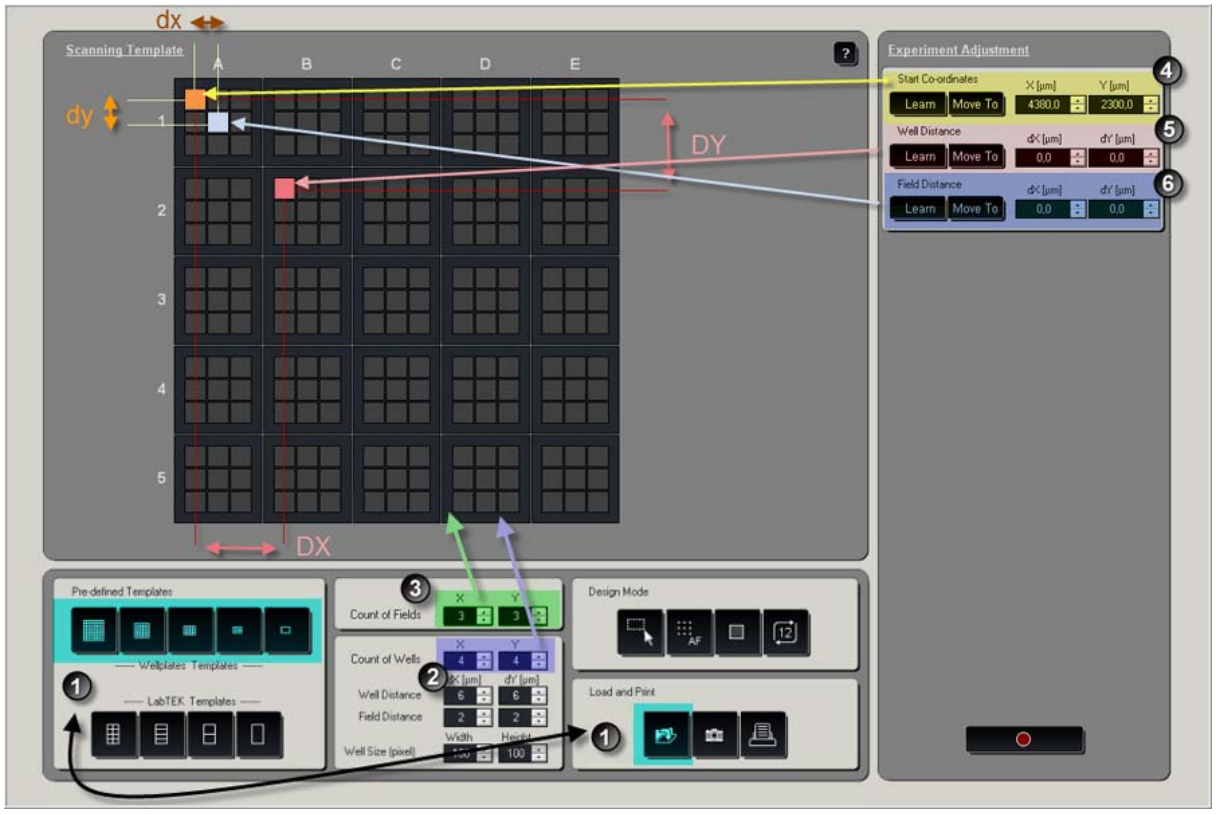


The **Mark and find mode** application is started now.

Switch to the **Setup Template** operating step to begin with the settings for your experiment.

Setup Template

In the **Setup Template** operating step, you can configure the basic settings for your experiment by creating a Scanning Template or selecting an existing one. This defines the spatial structure of your experiment.



The following operating steps refer to the numbers in the screenshot shown here.

1. Select a Scanning Template. You have two options:
   - Either select a predefined Scanning Template under **Pre-defined Templates**.
   - Or load an existing Scanning Template under **Load and Print**.
2. Adjust the Scanning Template to the requirements of your experiment by adjusting the number of wells or chambers of the specimen slide under **Count of Wells**.
3. Adjust the Scanning Template to the requirements of your experiment by adjusting the number of scan fields under **Count of Fields**.

   This adjustment applies for the entire specimen slide, i.e., the number of scan fields is the same in all wells or chambers.
4. Define the starting point of your experiment by moving the specimen stage to the respective position and then click the **Learn** button under **Experiment Adjustment** > **Start Co-ordinates**.

   The starting point is always the upper left scan field in the Scanning Template; it is marked orange in the screenshot shown here.
5. Adjust the horizontal and vertical distance between the wells or chambers by moving the specimen stage to the initial scan field of the well or chamber lying to the right under the starting point and then click the **Learn** button under **Experiment Adjustment** > **Well Distance**.

   The horizontal and vertical distance between the wells or chambers is marked in the screenshot shown here with DX and DY; the first scan field of the well or chamber lying to the right under the starting point is marked pink.
6. Adjust the horizontal and vertical distance between the scan fields by moving the specimen stage to the scan field lying to the right under the starting point and then click the **Learn** button under **Experiment Adjustment** > **Field Distance**.

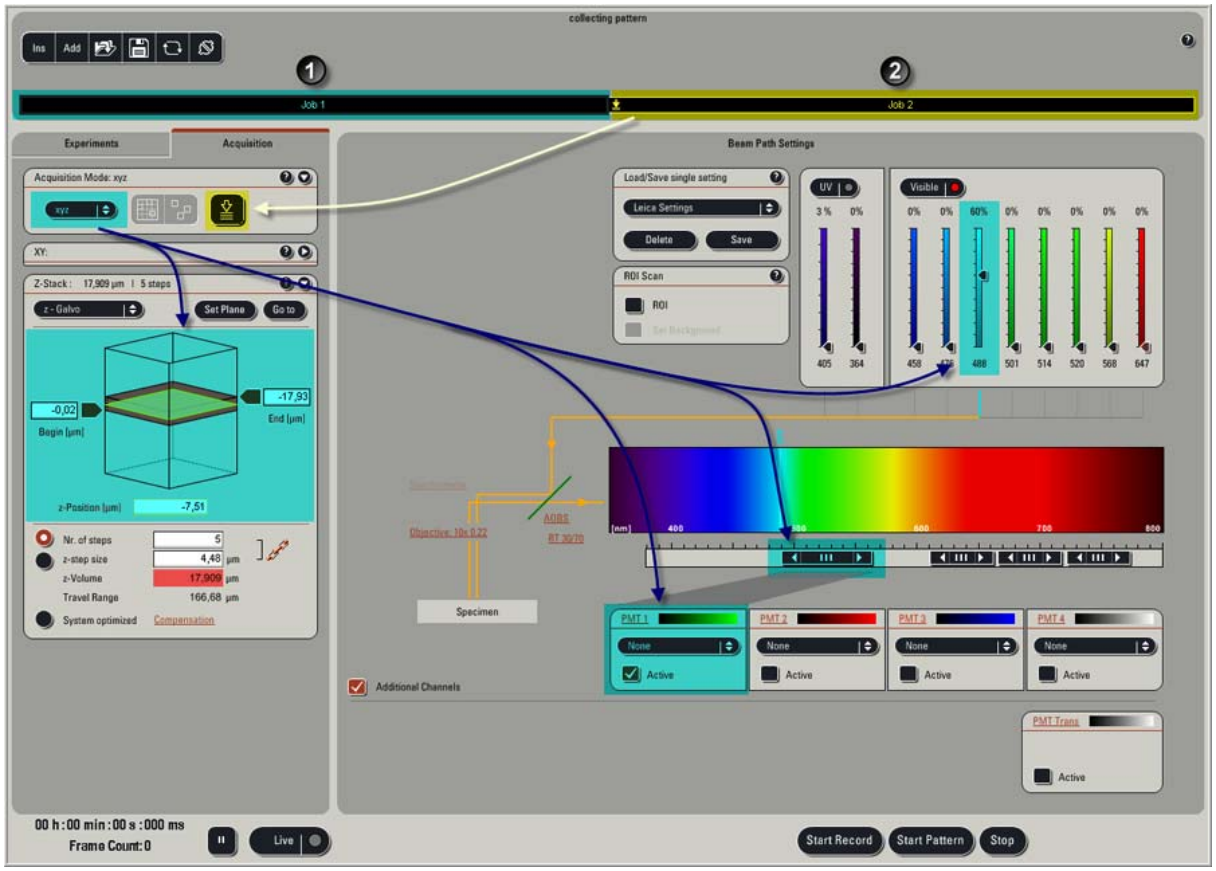
   The horizontal and vertical distance between the scan fields is marked in the screenshot shown here with dx and dy; the scan field lying to the right under the starting point is marked light blue.

The Scanning Template is now prepared for the experiment.

Switch to the **Setup Jobs** operating step.

Setup Jobs

In the **Setup Jobs** operating step, you can configure the settings for image acquisition and define and organize jobs and experiments (**Pattern**). Experiments can consist of multiple items (jobs, pauses and other **Pattern**s) that are processed in succession.



The following operating steps refer to the numbers in the screenshot shown here.

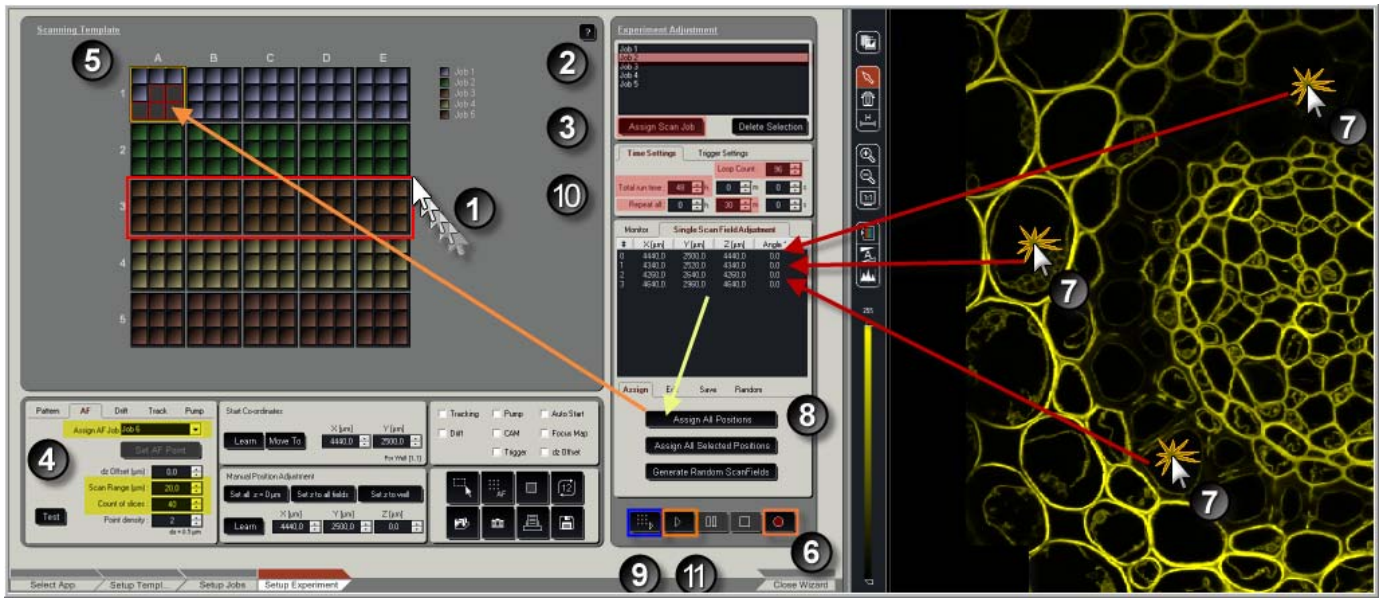
1. Define at least one job with all required settings for the image acquisition.
2. Define a **Autofocus** job for the automatic focus search.

The jobs for performing the experiment are now defined.

Switch to the **Setup Experiment** operating step.

Setup Experiment

In the **Setup Experiment** operating step, you can merge the settings from the **Setup Template** and **Setup Jobs** operating steps, i.e. you can assign jobs and experiments to the Scanning Template. Additionally, in this operating step you can assign the Mark & Find positions to the scan fields in the wells or chambers of the specimen slide.



The following operating steps refer to the numbers in the screenshot shown here.

1. Select scan fields in the **Scanning Template** view by holding down the left mouse button and dragging a rectangle over the respective area of the Scanning Template.
2. Select a job from the list box under **Experiment Adjustment** via mouse click.
3. Assign the selected job to the selected scan fields by clicking the **Assign Scan Job** button under the list box.
4. At the bottom left in the **AF** tab, select the **Autofocus** job for the automatic focus search and then adjust the following parameters: the range in z-direction in which the automatic focus search is carried out (**Scan Range**) and the number of individual images (horizontal xy-sections) that the system acquires during the automatic focus search (**Count of slices**).

   The individual images (horizontal xy-sections) are acquired within the range defined under **Scan Range**.
5. In the **Scanning Template** view, select a well or chamber of the specimen slide to assign your Mark & Find positions.
6. Move the specimen stage to the position of the selected well or chamber and start the **Live** image acquisition with the corresponding button on the bottom right; focus on the specimen cells that are relevant for the experiment.
7. Mark the desired cells in the display window in sequence by holding down the Ctrl key and double-clicking.

   The coordinates of the cell are taken over as Mark & Find positions and listed under **Experiment Adjustment** > **Single Scan Field Adjustment**.
8. Assign the Mark & Find positions listed under **Experiment Adjustment** > **Single Scan Field Adjustment** to the selected well or chamber. Three options can be selected under **Experiment Adjustment** > **Assign**:
   - Use the **Assign All Positions** button to assign all listed Mark & Find positions.
   - Select certain Mark & Find positions and assign them using the **Assign All Selected Positions** button.
   - Use the **Generate Random ScanFields** button to assign the Mark & Find positions randomly.

   Each well or chamber can be assigned only as many Mark & Find positions as there are scan fields available (e.g. a maximum of 9 in the screenshot shown here). Excess scan fields to which no Mark & Find positions were assigned are automatically deactivated and thus excluded from the image acquisition. Disabled scan fields appear outlined in red in the **Scanning Template** view.

   Double-clicking on a well or chamber in the **Scanning Template** view transfers the Mark & Find positions included there into the list under **Experiment Adjustment** > **Single Scan Field Adjustment**. To delete certain Mark & Find positions assigned to a well or chamber, select them in the list and click the **Delete Position** button under **Experiment Adjustment** > **Edit**; then reassign the remaining Mark & Find positions to the well or chamber.
9. Make sure that the starting point of the experiment (upper left scan field) lies near the focus range and then start the automatic focus search by clicking the corresponding button to the bottom right.
10. Optional: Under **Experiment Adjustment** > **Time Settings**, you can define loops to process an experiment repeatedly.

    If no loops are defined, an experiment is run once. If an experiment is supposed to run 2 days, for example, and during this time it is to be repeated every half hour, configure the following settings: set 30 minutes under **Repeat all** and 48 hours under **Total run time**. The number of loops resulting from this (in this case, 96) is automatically calculated by the system and displayed under **Loop Count**.
11. Start the experiment by clicking the corresponding button to the bottom right.

The experiment is running now and you can track the progress of the experiment under **Experiment Adjustment** > **Monitor**.

MatrixScreener Wizard: Glossary

A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X | Y | Z

F

Focus Map	A **Focus Map** is a two-dimensional map that maps the topological course of the best focal plane either in the specimen or on the cover slip surface (depending on the method that is used for automatic focus search) and thus traces the "waviness" of the specimen or cover slip surface. The basis of the focus map is provided by the **Autofocus** scan fields that are displayed in the **Scanning Template** view. The automatic focus search determines the position (z-coordinate) of the best focal plane in each **Autofocus** scan field; the system uses interpolation to automatically calculate the positions of the best focal plane lying between the **Autofocus** scan fields.

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L

LIF	Leica Image File (LAS AF-specific file format); LIF files contain all data of an experiment, such as the acquired images and the corresponding Metadata as well as the instrument parameters and hardware settings used.

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M

Metadata	Metadata ("data about data") are structured data that describe the acquired images themselves as well as their structure and content relationships.

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O

OME	Open Microscopy Environment (open standard for microscope data); the objective of OME is to create a manufacturer-independent and thus nonproprietary standard for easier exchange and advanced processing of microscope data. For this purpose, open file formats such as OME-TIFF (images with embedded Metadata) and OME-XML (Metadata) have been developed.

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S

Scanning Template	The actual experiment file that completely describes an experiment.

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