WIDEFIELD APPLICATION LETTER

reSOLUTION

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Well Plate Acquisition Wizard

Leica
MICROSYSTEMS

Well Plate Acquisition Wizard

The Well Plate Acquisition Wizard is an optional module within LAS AF and is available for AF7000, AF6500 and AF6000. It allows for an easy setup of time-lapse experiments based on micro plates including the definition of Z-stacks, tile scans and sub arrays within the wells. Patterns for 96 well plates, 24, 12 and 6 well plates are predefined. Differing patterns can be user defined.

A wizard guides the user through all steps from pattern selection to well activation, acquisition setup and post-processing. A best focus algorithm searches for the focus in each well before the experiment starts.

Hardware requirements:

Microscope:
Leica DMI 6000 B

Stages:
11 522 068 Motorized 3-plate stage (SuperZ optional)
11 522 100 Scanning stage (SuperZ optional)
11 532 536 Scanning stage H117N1DM

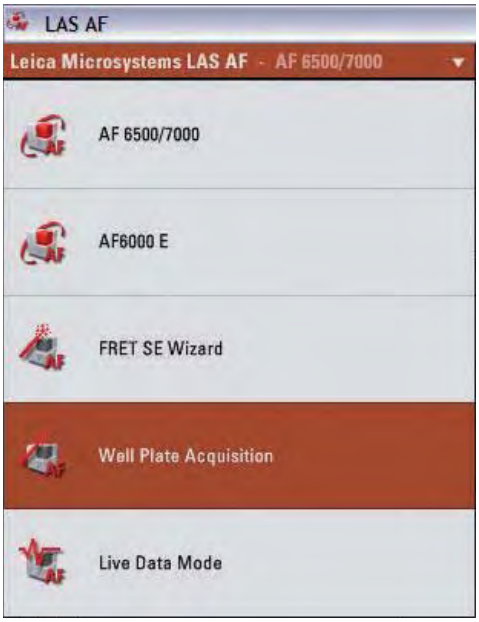
Inserts and CO_2-covers:
11 532 338 Stage insert for micro plates
11 521 735 CO_2 cover for micro plates
11 640 416 Insert SuperZ for micro plates
11 640 406 Incubator SuperZ for micro plates

Micro plates:
Glass bottom micro plates with 0.17mm glass thickness. Flat bottom plastic micro plates might be working when using objective magnifications of 20x and below.

Note: DIC contrast does not work with plastic bottom micro plates or micro plates covered by a plastic cover.

Starting the Well Plate Acquisition Wizard:

Select the Well Plate Acquisition Wizard in the pull down menu of the LAS AF main menu. The Well Plate Acquisition Wizard is an optional module within LAS AF and is dongle protected. The application is greyed out if the Well Plate Acquisition wizard is not licensed or if the stage is not initialized by LAS AF.
Since the Well Plate Acquisition Wizard requires two monitors this application also is greyed out if a second monitor is missing.



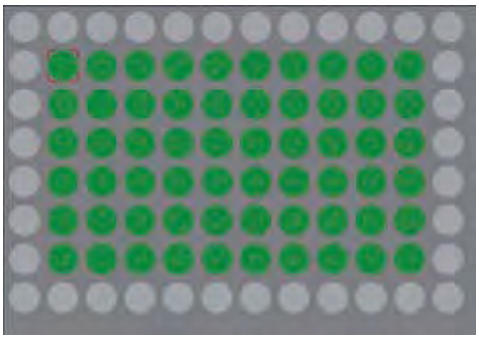
The wizard consists of 5 steps and an overview of the experimental workflow.

- Step 1: selection of micro plate pattern, user defined patterns, activation and deactivation of wells
- Step 2: alignment of micro plate, channel definition for best focus
- Step 3: best focus routine searches for focus level in each well
- Step 4: setup of acquisition parameters, start experiment
- Step 5: merging tile scan images

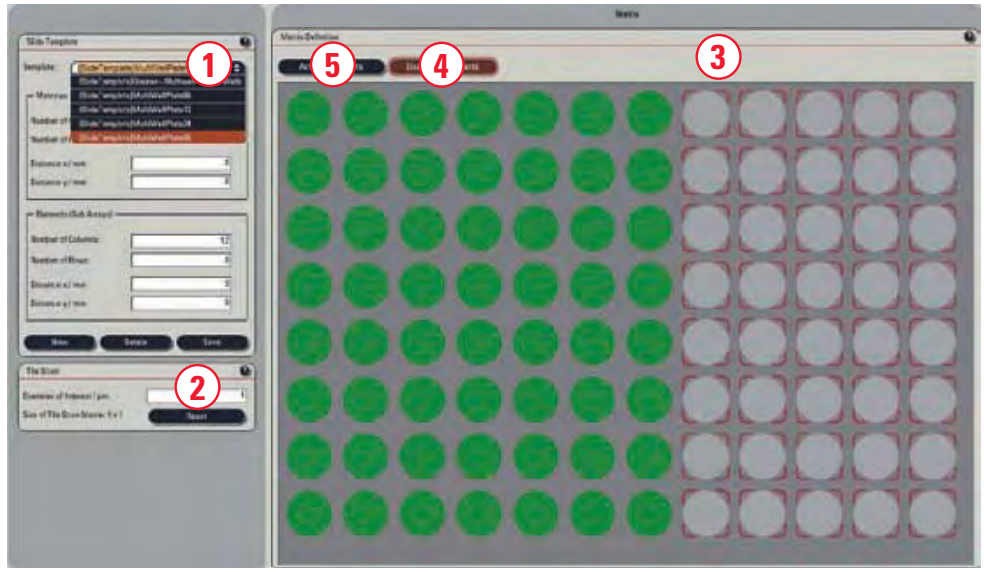
Step 1: Define Pattern

In Step 1 the type of micro plate is selected and the wells to be visited during the experiment are activated. A predefined pattern for 96 well plates, for 24, 12, and 6 well plates can be selected in the template pull down menu.

The predefined **template 96 – 60 wells** is a 96 well plate pattern with deactivated outer wells. This pattern is required when screening with high NA objectives with a short working distance. Due to the short working distance the objective can-touch the micro plate frame and make the outer wells inaccessible. Alternatively the outer wells of the 96 well plate template can be deactivated in the matrix definition window.



96 – 60 wells template



Well Plate Acquisition Wizard Step 1: Selection of micro plate pattern

Workflow:

1. Select a predefined micro plate **template (1)** or define your own template.

2. Type in a diameter of interest if a **tile scan matrix (2)** needs to be acquired in each well. The diameter determines a tile scan matrix covering the area describe by the diameter. If no tile scan matrix is required just press the reset button or type in the number "1".

3. Deactivate wells which are of no interest for the experiment. Wells can be deactivated in the **matrix definition window (3)** by keeping the left mouse button pressed while moving over the wells. The selected wells are then each marked with a red frame. Press **Deactivate Elements (4)** to deactivate the wells. Selected wells can be activated again by pressing **Activate Elements (5)**.

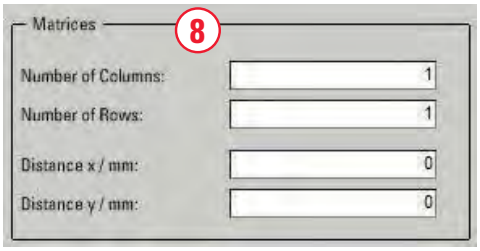
Attention! If a merged tile scan image exceeds a size of approx. 200 – 400MB, the images cannot be exported anymore from LAS AF. Reducing bit depth in the camera interface or increasing binning in the acquisition interface will reduce image size. Alternatively a lower magnification can be selected to obtain a larger tile scan area.

Note: You can go back to step 1 from any other step of the wizard to change the tile scan size or to activate and deactivate wells.

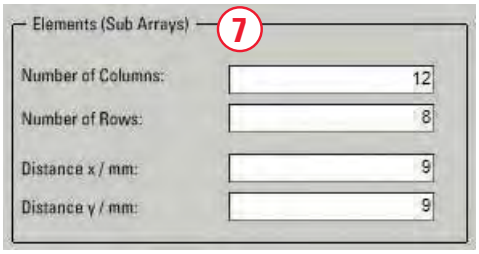
Defining your own micro plate pattern:

1. Press **New (6)** in the **Slide Template** window.

2. **For templates with no sub arrays**: Type in the numbers in the matrices section as displayed:



Define the number of columns and rows including the distances in X and Y of the micro plate in the **elements (sub arrays) (7)** section. The distances are defined from center to center of adjacent wells.

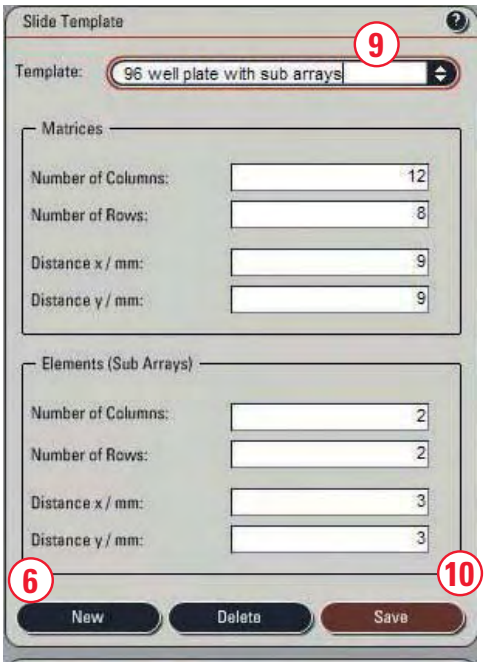


3. **For templates with sub arrays**: Define the number of columns and rows including the distances in X and Y in the **matrices (8)** section. The distances are defined from center to center of adjacent wells.

Sub arrays are patterns within each well. They can be defined accordingly in the **elements (sub arrays) (7)** section.

4. Define a **template name (9)** and **Save (10)**. Once the save button is pressed, the newly generated template will appear in the matrix definition window.

Note: Each position of a sub array still can be combined with a tile scan matrix. Each single element of a sub array can be deactivated separately.

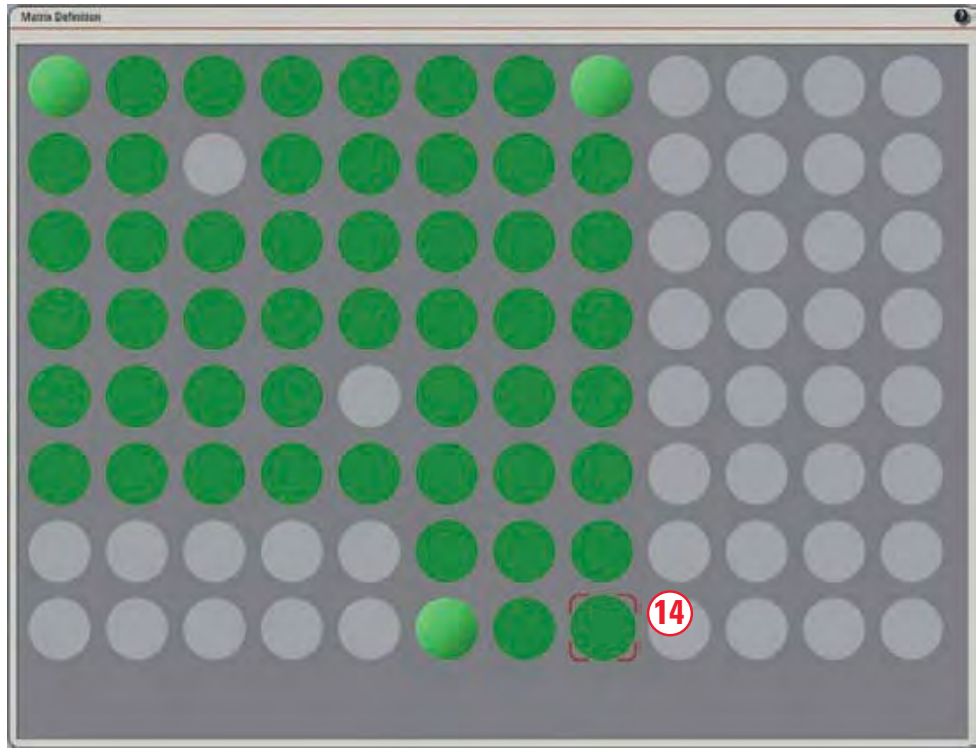


Definition of a new template with sub arrays

Step 2: Align

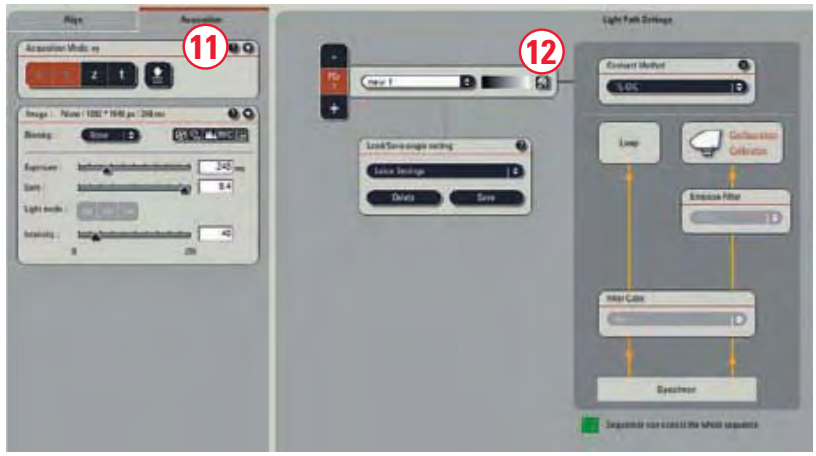
In step 2 the micro plate is aligned and the acquisition parameters for the best focus function are defined. The best focus function will be used in the next step to search for the focus level in the wells automatically and generate a focus map of the micro plate. For micro plate alignment four active wells in the corners of the screening area are necessary.

Note: The XY position of the first corner is already sufficient to identify the XY-position of the micro plate. The XY position of all other corners is not necessary to correct for, if the stage moves correctly to the wells in the remaining corners. In each corner it is important that the focus level is read in correctly. These focus levels will be used to generate a first approximation of focus level distribution of the micro plate and is required to generate the focus map in the next step of the Wizard.



Workflow:

1. Go to the **Acquisition (11)** tab and define a channel **(12)** used for the best focus function. Additional channels can be defined, but best focus will use the first channel only for focus search. Best focus works with both, brightfield and fluorescence channels. Please make sure the images are not saturated. Otherwise the best focus algorithm might not find a focus level.



2. Go to the **Align (13)** tab and move the micro plate to the position marked with a red frame **(14)**.

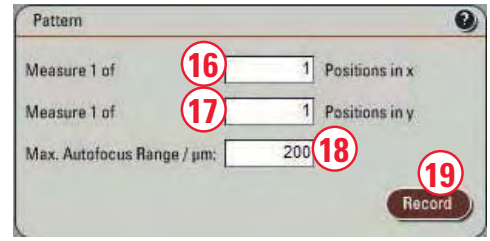
3. Find the focus within this well manually and press **Read Position (15)**.

4. The stage now will move automatically to the next corner of the matrix. Find the focus within the well manually and press **Read Position**. This procedure will be repeated for the next two corners of the matrix. Once all four corners are aligned, a message will indicate that alignment is completed. The user now can navigate within the micro plate by a left mouse click on the wells in the matrix definition window. The user cannot proceed to step 3 if alignment is not completed.

Note: If any of the positions used for alignment are deactivated or if there is no specimen to focus at, please mark an adjacent active position by left mouse click and use this position for alignment.

Step 3: Focus Map

In this step a focus map of the micro plate is generated. A best focus function automatically searches for the focus in each specified well. The user also can navigate within the micro plate by a mouse click on the desired well. Focus levels can be corrected manually, if necessary.



Workflow:

1. Type in every nth number of wells in x- **(16)** and in y- **(17)** direction the best focus function should automatically search the focus level for. Focus levels of non-selected wells are estimated by interpolation.

Note: It is recommended that every well in X- and in Y-direction is selected for automatic focus search to increase the accuracy of the focus map. The best focus function always uses the motorized z-drive of the microscope to generate the focus map.

2. Type in the max. autofocus range **(18)**. This number defines the capture range of the autofocus and depends on the objective´s depth of field and the evenness of the micro plate.

Note: 200µm max autofocus rangeis a good number to start with. Using a larger number can result in focusing on the cover slip in brightfield mode. This problem does not occur in fluorescence mode.

3. **Press Record (19)**. The best focus function now searches for the focus level in each of the selected wells. If the focus capture range is not sufficient or the measurement points in X- and Y-direction need to be changed, the focus map generation can be stopped by pressing **Stop**.

Note: The best focus algorithm searches for best contrast images by moving in coarse steps through the capture range and reducing step size the closer it comes to the best contrast image.

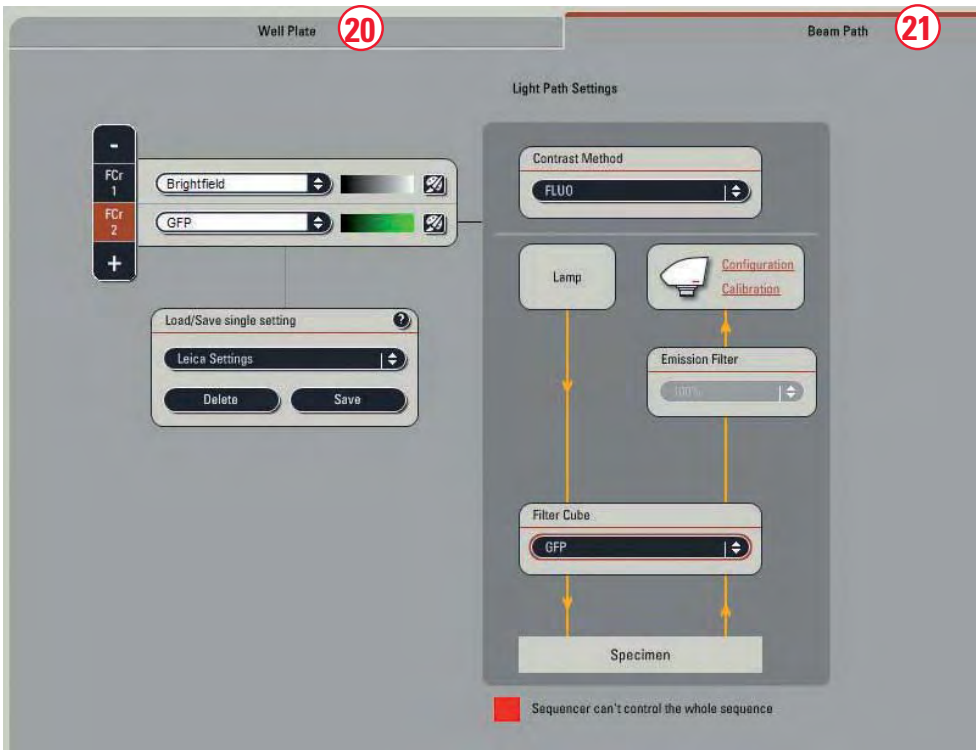
4. The focus level in each well can be reviewed by a left mouse click on the desired well. Once the stage arrived at the well a live image can be activated.

5. The focus level of each well can be corrected for by focusing manually and pressing **Read z-level**.

Step 4: Acquire

Step 4 defines the acquisition parameters of the well plate experiment. The time-lapse experiments can be defined with Z stacks, different channels and different contrasting methods.

Note: You can go back to step 1 of the wizard to change the tile scan size or to activate or deactivate wells. Even wells used for the well plate alignment can be deactivated.



Workflow:

1. Define the acquisition parameters in the acquisition interface.

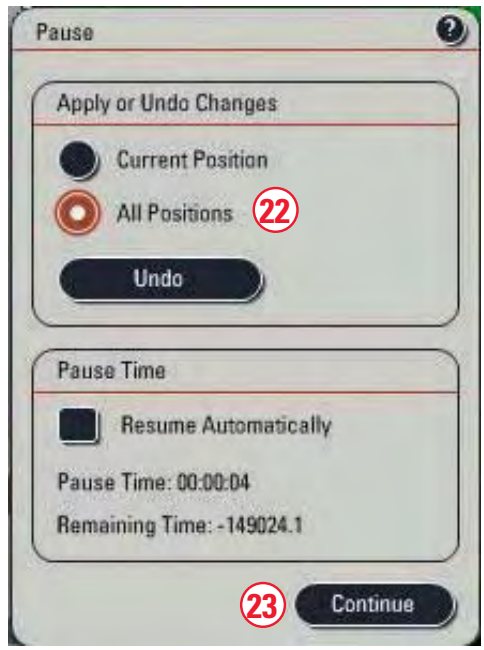
Note: Use the **well plate** (20) tab to navigate within the micro plate. Z stacks can be defined with Z-wide or with the fine focus, if available. The Z-stack in each well will be centered to the z-values stored in the focus map.

2. Use the **beam path** (21) tab to define channels.

Note: Relative focus correction cannot be used in the Well Plate Acquisition Wizard.

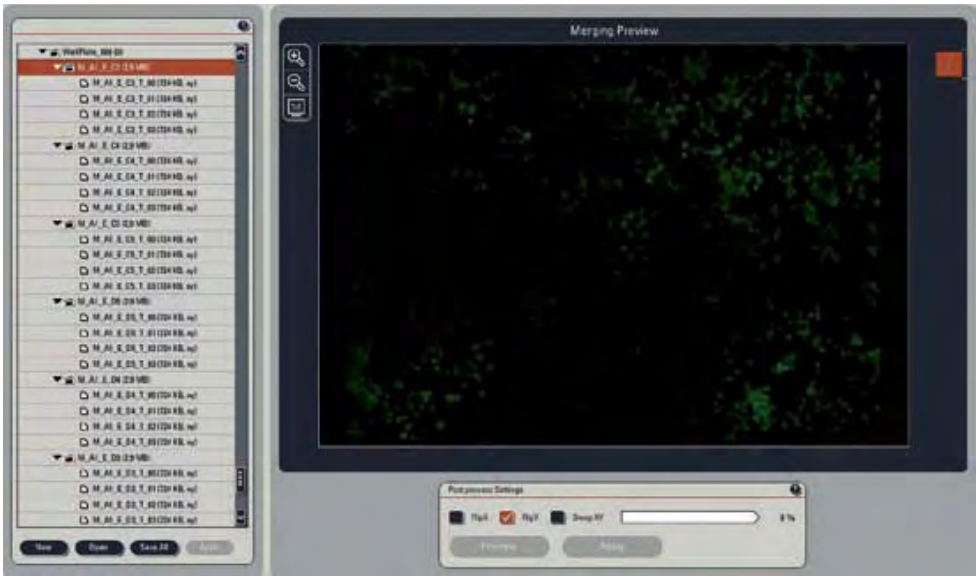
3. Start the well plate experiment by pressing **Start.**

Note: During the experiment the best focus function is deactivated. Focus corrections can be done with the **Pause and Relocate** button, if necessary. Press **Pause and Relocate** after a cycle is finished and apply an offset to all micro plate positions by selecting **All Positions** (22) and pressing **Continue** (23).



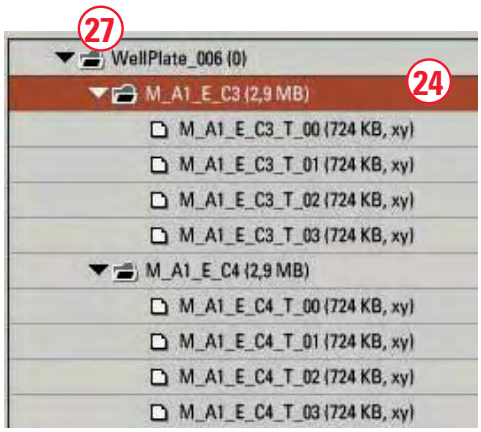
Step 5: Post-Process

This step is used to merge tile scan images. It only is needed if a tile scan area was defined in step 1.



Workflow:

1. Go to a well position **(24)** within the experiment tree. The last two digits of the folder´s name define the position of the well within the micro plate. C3 for example is row C, column 3.



2. Use **FlipX**, **FlipY** or **SwapXY** **(25)**, if the tile scan images in the merging preview are not displayed in the right order.

3. Once the merging preview shows a correct tile scan image, this image can be merged by pressing **Apply** **(26)**.

4. To merge all images of the experiment, go to the experiment header **(27)** and press **Apply**.

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