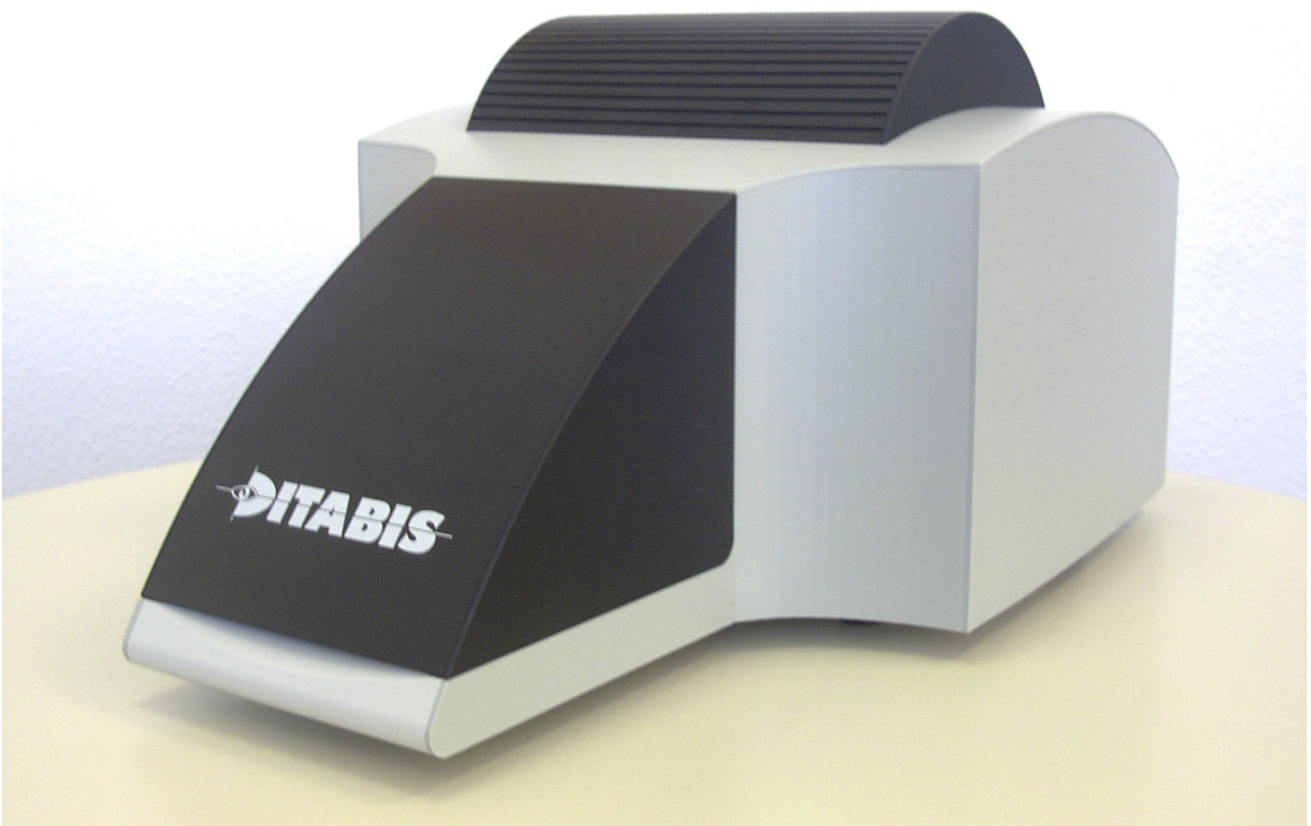


# Microarray Scanner “MArS”

## Preliminary Software Operator Manual



**Disclaimer:**

The present Operator Manual is written in order to support the cooperation partners of DITABIS to test the pre-series instruments of our microarray scanner "MArS". Accordingly it does not replace a complete Operator Manual as delivered with series instruments. The users of "MArS" therefore are encouraged to ask specialists at DITABIS in case of any uncertainty with respect to software or instruments functions, particularly if they might be in conjunction with any risk to health or instrument damages.

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# 1. Software Description

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This chapter describes the operating software of the MARs microarray scanning system. All software dialogs, their functions and relevant safety information are described.

The software is divided into two main function groups. The **Image Viewer** and various **Scanner Control Dialogs**, which are started via the respective buttons on the main screen

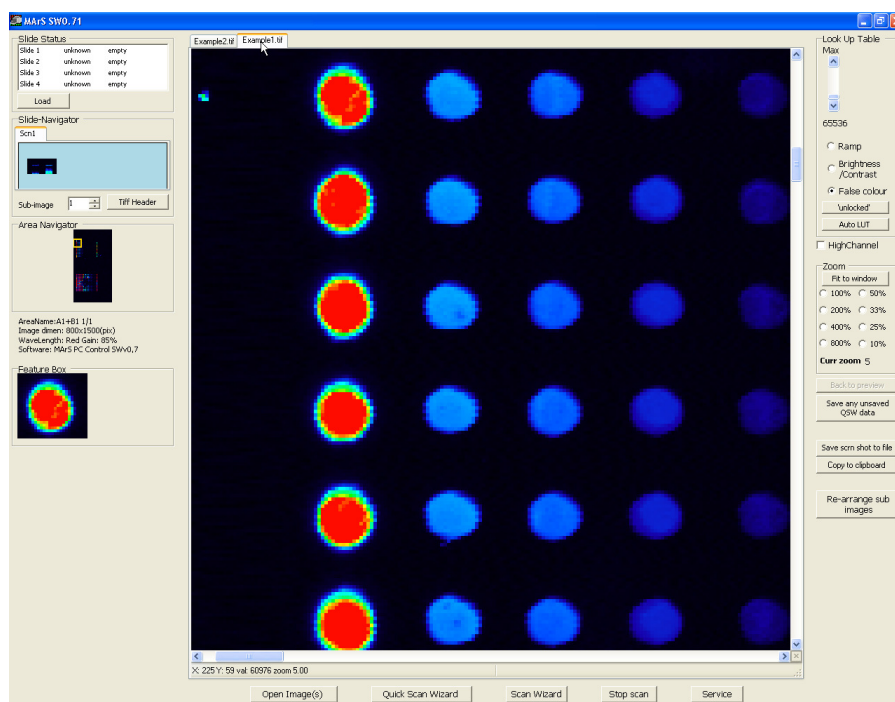
## Image Viewer

The **Image Viewer** acts as main screen of the MARs scanner control software. It displays in several detail views the respective scanned area of a microarray chip. Other functions are to present the status of microarray slides in the instrument, and to provide access to the scanner control dialogs.



Although the MARs instrument allows to scan a microarray biochip with highest flexibility, i.e. many distinct areas on the chip, and each of it at various sizes, gains and wavelength settings, the **Image Viewer** functionality will be limited to view at the same time only one scanned area of the biochip at one wavelength and gain setting. Note: Each scanned image could be a separate TIF image file or a sub-image in a multi TIF file

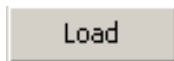
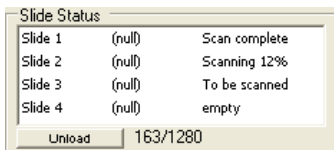
It is not the intention of the **Image Viewer** to provide further microarray image analysis functions but rather to be an easy-to-use viewer for a quick quality control of the obtained scan images. The created TIFF-files can be analysed with virtually any commercial available micro-array analysis software.



### Image Viewer

- |                            |                                      |
|----------------------------|--------------------------------------|
| <b>A</b> Slide Status      | <b>E</b> Feature Box                 |
| <b>B</b> Slide Navigator   | <b>F</b> LUT-Control (Look-Up-Table) |
| <b>C</b> Area Navigator    | <b>G</b> Zoom Factor Box             |
| <b>D</b> Main Display Area | <b>H</b> Control Buttons Section     |

## Slide Status



This box provides a quick overview of the status of each micro-array slide within the instrument. The four loading positions of the scanner are displayed in a table in the same orientation and sequence as they are accessible in the instrument. The messages clearly describe the current status of each slide.

**Load** vs. **Unload** is a toggling button within the Slide Status box being used for loading/unloading micro-array slides into/out of the instrument.

Click **Unload** to move the slide loading table to the front position of the instrument. After opening the lid, the loading table is now accessible by the operator for either unloading scanned slides and/or loading new slides.

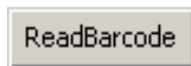
Click **Load** to move the slide loading table to the measuring position in the rear of the instrument. The slides can't be accessed now.




---

*During the loading process, the four loading positions of the slide table are monitored by a reflective sensor for the presence of slides. Loading positions with slides being detected are represented in the **Slide Status** window by a message "loaded". The "loaded" status is only valid, as long the loading table is in the rear position, i.e. not accessible by the user.*

---



Click **ReadBarcode** in order to trigger the instrument to scan for all loaded slides the potential barcode label at the right handed part of the slide.

---

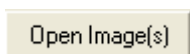
*The geometric slide area to be scanned for the barcode, and which potential barcode types are to be decoded, is specified in a so-called "barcode.slt" file.*

---

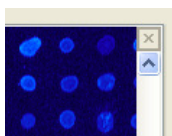
After the barcode reading step the slides remain in the rear position of the instrument, i.e. the slides can't be accessed now. If a barcode was detected and decoded on the slide, its value will be displayed in the second column in the **Slide Status** table.

(The barcode reading/decoding function is not implemented yet.)

### Open Image(s)



For loading previously scanned microarray image(s) press the **Open Image(s)** button within the control button section of the main window. A standard file browsing window will allow you to select one or more 16 bit standard TIF images (including multi paged TIF images).



### Close Image

To close an image simply click on the “X” button located above the vertical scroll bar of the corresponding image



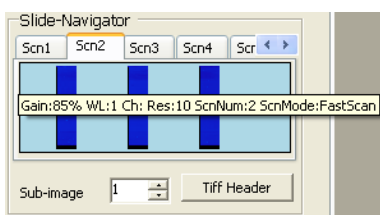
*The filename of the loaded multi-TIFF-image including path information is displayed in the tool tip of the tab page of main display area*

### Slide Navigator

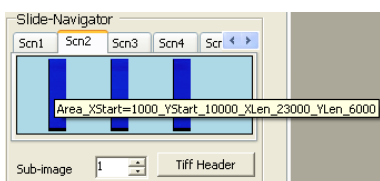
The slide navigator allows you to select and display the desired sub-image of any of the loaded multi-TIF files into the **Main Display Area**.



*When, e.g. Savemode 1 was selected, for each distinct area of a slide - and here for each different gain and/or wavelength setting - a distinct **area sub-image** is stored into the multi-TIF image file. Example: A slide with two geometrical areas, each being scanned at two wavelengths, and using three different gain settings per wavelength will provide a multi-TIF file with 12 sub-images (2 areas x (2 wavelengths x 3 gains each)). \*\* Alternative Savemodes are described in the ScanWizard chapter below.*



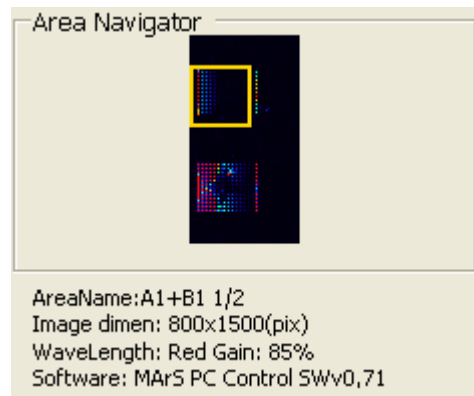
To chose a specific **area sub-image** for displaying, firstly select the desired wavelength/gain settings via the Tabs called **Scn1**, etc. above the schematic slide. The mouse tool tip function will display the corresponding settings values for each **Scn**-tab when the cursor is placed above it.



Secondly select the desired **area sub-image** by clicking onto the corresponding **miniature image** within the schematic slide box. Again the mouse tool tip is displaying information about the underlying area sub-image.

## Area Navigator

The small **Area Navigator** window shows the entire chosen **area sub-image** of the loaded file with a zoom factor that fits the entire area into the image window. Underneath the image window, important information of the TIF-header of the selected **area sub-image** is displayed.



In order to display a selected part of this area at high resolution into the **Main Display Area** draw a rectangle with the pressed mouse button around the desired area. A yellow rectangle will mark the border lines of the data currently displayed in the main display area. Other magnifications or different geometric selections are easily generated by drawing a new yellow rectangle.



---

*The indicating rectangle will also change accordingly, when the zoom factor within the Main Display Area is modified either using the Zoom-Factor box or drawing a rectangle within the Main Display Area – these being two alternative ways to change the magnification.*

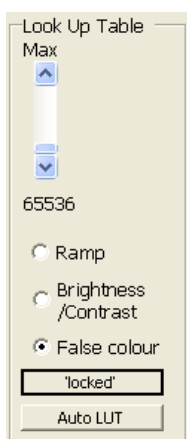
---

## Main Display Area

The **Main Display Area** shows the finally selected part of the current microarray area (= area sub-image) at high resolution. The present image view can be further optimised by using the functions of the **LUT-Control** box (look-up-table) and the **Zoom-Factor** box.

## LUT-Control (Look-Up-Table)

Click a radio button to select one of three general look up table display modes.



**Ramp** or **Brightness/Contrast** are two alternative ways to optimise for best grey values displaying. The two LUT-modes with their corresponding sliders allow two different ways of modifying the gamma curve, i.e. how the real 16-bit fluorescence data are mapped to the displayed 256 grey values of the PC monitor.

The radio button **Color** converts the b/w-image into a false colour LUT display. The corresponding slider modifies the mapping of the grey values to the corresponding false colours.

The **Auto-LUT** button automatically selects well fitting LUT-settings based on highest and lowest grey value of the scanned image.

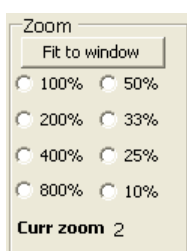
(Auto LUT Function is not implemented yet.)



The toggling button **'locked'** vs. **'unlocked'** allows to compare different loaded microarray images at either identical LUT-settings (= 'locked') or each at its best fitting LUT-settings (= 'unlocked'). Example: If you change in the **'locked'** button state from one image to another (e.g. by clicking another tab of the Main Display Area or even loading a new image) the LUT-settings for the newly loaded image file(s) is taken from the former image. If you click now the button to the **'unlocked'** state the LUT-settings of all the images falls back to their original look up table.

## Zoom Factor Box

Click one of the zoom radio buttons to quickly select a new zoom factor for the image displayed in the **Main Display Area**. The currently selected zoom factor is also displayed in the corresponding info window.

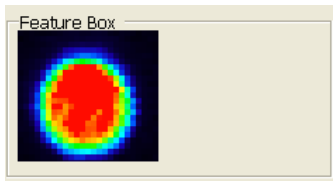


**"Fit to window"** automatically selects an uneven zoom factor which just fits the whole scanned area into the whole Main Display Area. It only should be selected for a first overview, because the following described analysis functions work best at the fixed zoom factors.



*Different zooms alternatively can be selected by drawing a corresponding rectangle either in the **Area Navigator** or the **Main Display Area** – this being an alternative way to change the magnification. Note: The closest fitting "fixed" zoom factor is selected automatically.*

## Feature Box



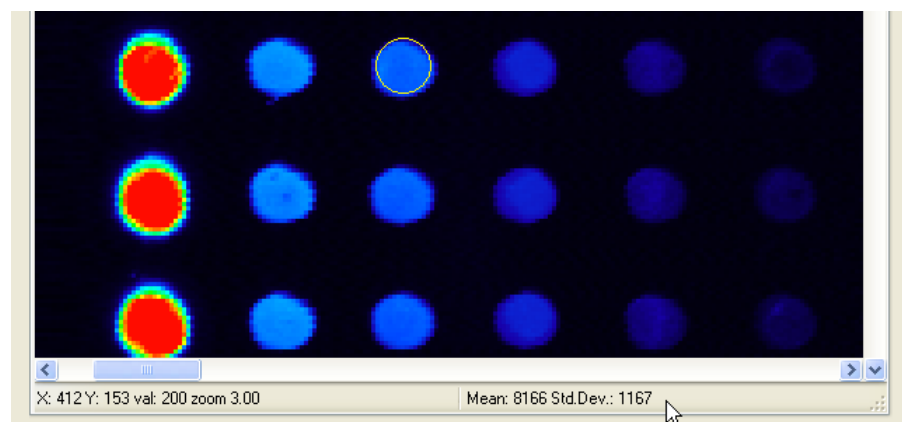
The **Feature Box** in the left lower part of the Image Viewer main window displays a small part of the area sub-image at fixed magnification. Its zoom factor is set to a value so that a typical standard spot of a microarray is viewed in total. Move the mouse cursor above the spot of interest either in the **Main Display Area** or in the **Area Navigator**.

The **X-** and **Y-position** of the centre pixel of the feature box as well as its **gray value** are displayed below main display area.

## Mean and Standard deviation of one spot

Although the MArS-control software is not intended to provide detailed microarray image analysis functions an easy-to-use “single spot analyzer” is integrated to allow a quick quality control of the obtained scan images.

The mean and standard deviation of the gray values of a desired circular region of interest – e.g. a single spot - can be displayed by drawing a circular region with right mouse button onto the image. The values of mean and standard deviation of the pixels within the drawn circle is updated at the completion of drawing the region of interest and are displayed below the main display area on the right hand side.



## QuickScan

**QuickScan** is one of two general ways to start the scan of a microarray. You mainly will use the respective **QuickScan Wizard** to briefly check a new or unknown microarray slide, e.g. for its dynamic range or for the position of the spotted area(s) on the slide.

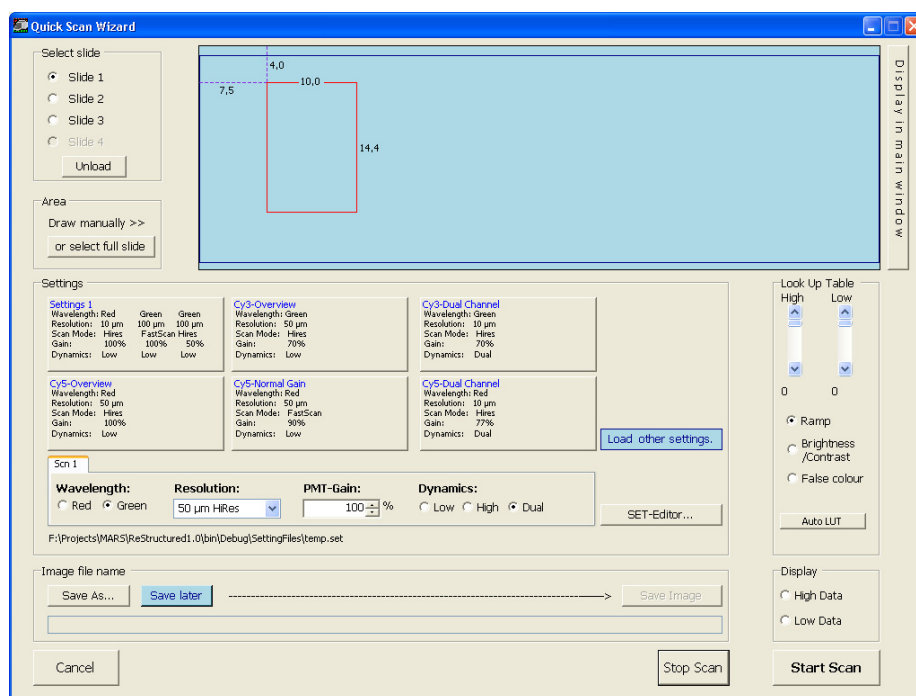


**QuickScan** will allow to scan only one area on the present slide. This area can be programmed to be scanned consecutively with multiple scan settings.

For (routine use) slide scans with several areas, and these e.g. at different wavelength and/or gain settings, use the maximum flexible **ScanWizard**.

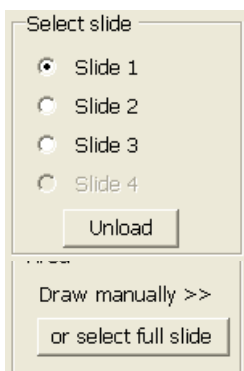
## Defining a Quick Scan

A scan using **Quick Scan** only can be started if minimum one slide is loaded into the rear of the instrument using the load function. Click **Load** either in the Slide Status box of the main window (before starting the QuickScan; see description above) or use the corresponding **Load** button within this Quick Scan Wizard.



### QuickScan Wizard

- |                          |                                 |
|--------------------------|---------------------------------|
| <b>A</b> Select Slide    | <b>E</b> Start Scan             |
| <b>B</b> Area            | <b>F</b> Look Up Table          |
| <b>C</b> Settings        | <b>G</b> Display in main window |
| <b>D</b> Image File Name |                                 |



The **QuickScan Wizard** dialog is divided into five areas that reflect the preparation steps to start a subsequent scan: Select Slide, Area, Settings, Image File Name, and Start. Follow steps 1 to 5 in the dialog to prepare the scan. Any sequence of these steps is allowed.

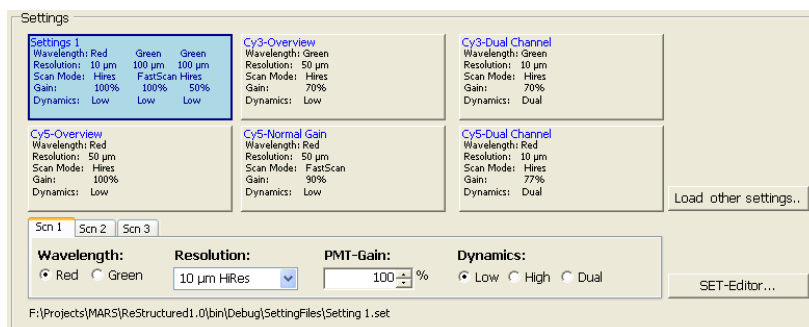
1. Click within the **Select Slide** section the radio button representing the slide you intend to scan. Only radio buttons corresponding to a loaded slide position – i.e. not grayed out – are selectable.
2. The **Area** box prompts you to define the area on the slide you want to scan. You either draw with the pressed mouse button a corresponding rectangle onto the schematic slide on the right; the coordinates and dimensions will be numerically displayed in the schematic slide.



*The maximum scanning area per slide position is restricted to the really visible slide surface, i.e. 23 x 76 mm, because 1mm at each length side of the slide is covered by the slide loading rails. The blue schematic slide shows the real 25 x 76 mm glass slide size, the height restrictions are displayed by the two horizontal blue lines.*

Alternatively you click the **select full slide** button in order to choose the maximum scan-able area of the slide, i.e. the 23 x 76 mm.

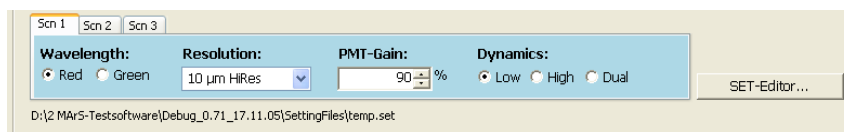
3. In the **Settings** section you have to decide which scan settings (i.e. resolution, wavelength and gain) shall be used for this particular scan. There are three main ways of selecting the scan settings



a) For a quick selection six factory-set settings are prepared as **presets**, each reflecting an own settings file. Click one of the buttons to select your best fitting settings.

b) Another settings file, e.g. with your own optimized settings parameters. can be selected by using **Load other settings...** which opens a standard Windows browser allowing you to load any existing settings file.

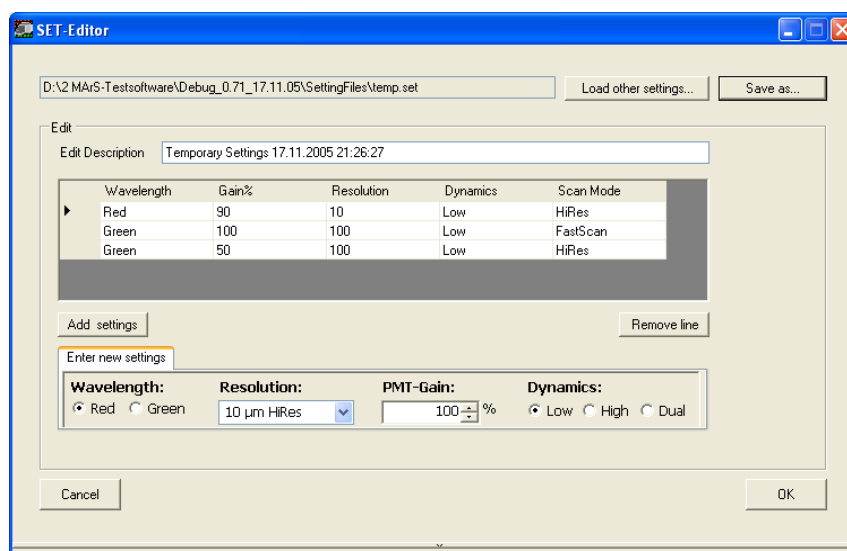
c) A collection of tab pages present below the six **presets** buttons provide a very elegant way to select or quickly modify parameters of the desired scan settings.



The number of tab pages is equal to the number of individual scan settings present in the selected file, i.e. with which the defined area will be subsequently scanned.

Each tab page contains editable fields for each individual scan parameter, that is for wavelength, resolution, scan mode, PMT gain and channel(s). After having selected a stored settings file (.set), the values of these fields are initially identical to the corresponding parameters in the loaded settings file. But now, you can very easily change the value of any parameter of the scan settings to a new desired value. Note, that upon a first value change, the tab page changes to light blue. Also the file name of the previously loaded settings file is change to a (new) default name “**temp.set**”. Pressing “Start Scan” uses these (edited) settings parameters for the subsequent scan.

Click the **SET-editor ...** button to modify and save such a new settings file in a very comfortable way.

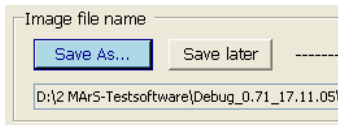


The subsequently applied scan-parameter sets are displayed comprehensively in a table format. A new scan-parameter set can be edited in the below ‘Enter new settings’ tab page and added to the above table by pressing **Add settings**. An existing scan-parameter set can be deleted by selecting the corresponding line in the table and clicking the **Remove line** button. The SET-Editor is explained in more detail in the Appendix chapter.

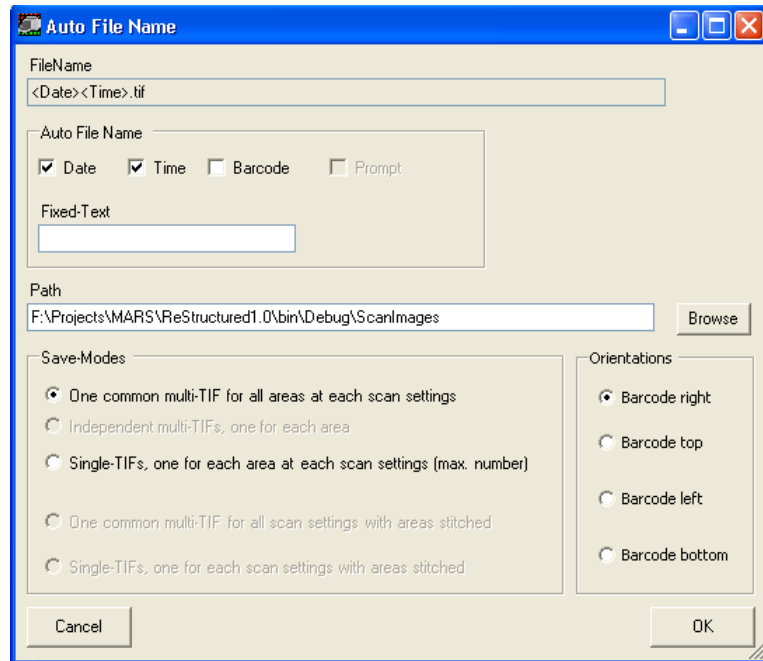


*The SET-Editor and the meaning of the scan parameters will be explained in more detail in a subsequent chapter of the Manual.*

The **Start Scan** button gets enabled once all the required parameters are selected for the scan.



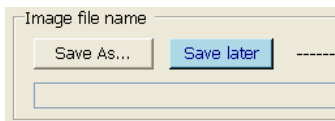
- The **Image file name** group box offers you the possibility to define already before the scan if the subsequent image file shall be immediately saved, and if yes, under which file name. Click **Save As ...** in order to open the following dialog box:



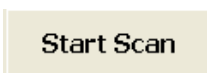
The **Auto File Name** dialog box allows you to assemble the image file-name from an editable fixed text and automatically generated text parts as date, time and the slide barcode. Click the options and/or write the fixed text in the sequence you want to have them shown within the future file name.

In addition you can select a new path where the image file shall be stored, using the **Browse** button.

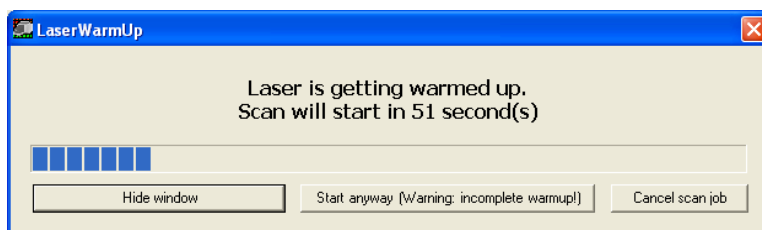
Finally you will decide here for one of the two possible **Savemodes**. For details, please refer to the corresponding explanation in the ScanWizard chapter below.



Alternatively you can click **Save later** in this section. You might use it, if you either don't intend to save the "quick scanned" image, or if you want to decide to save after having viewed it. **Save Image** at the end of the dotted arrow becomes selectable after the corresponding scan is finalized.



- After completing the entries of the above four steps, you can start the scan using the **Start Scan** button. Depending on the status of the instrument, i.e. how long ago the previous scan was done, the following window might appear:



Either doing nothing or pressing the **Hide window** button means, that the scan process will start automatically after the laser warm-up time is finished.

Pressing **Start anyway** will skip the remaining warm-up time, the scan is started immediately. This might be used for a (quick) pre-scan.

**Cancel scan job** cancels the programmed scan and leads back to the Quick Scan dialog box.

### Viewing a Quick Scan in Live Mode

Having pressed **Start Scan** the scanned image will be displayed in live mode into the above schematic slide box, thus allowing you much quicker to potentially start a new pre-scan, e.g. with a slightly modified scanning area. Right handed below the schematic Pre-Scan slide box the same LUT-control features (Look-Up-Table) as already described above for the Image Viewer main window are accessible.

On the right of the schematic Pre-Scan slide box, the button "**Display in main window**" allows you to shift the display of the currently scanned image to the **Main Display Area** - now under high resolution with all the extras features for viewing the image. The corresponding tab of the displayed image is named "scanning".- This action is transiently closing the Quick Scan Wizard window.

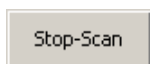
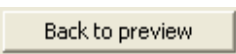
Clicking at any time during the scan the "**Back to preview**" button in the main window shifts the display back to the Quick Scan Wizard window, which is reopened.

As long as the scan is running you can toggle between these two views.

After having started a scan, the **Stop-Scan** button, which is either accessible at the bottom of the ImageViewer main window or of the QuickScan Wizard window allows you to stop the current scan at any time.

The incomplete scan image is lost in this case.

Display in main window



## Scan Wizard

The **Scan Wizard** allows you to program in a very flexible way the scan of up to four micro-array slides. You mainly will use the **Scan Wizard** in your routine research or diagnostics application utilizing stored sets of scanning parameters.

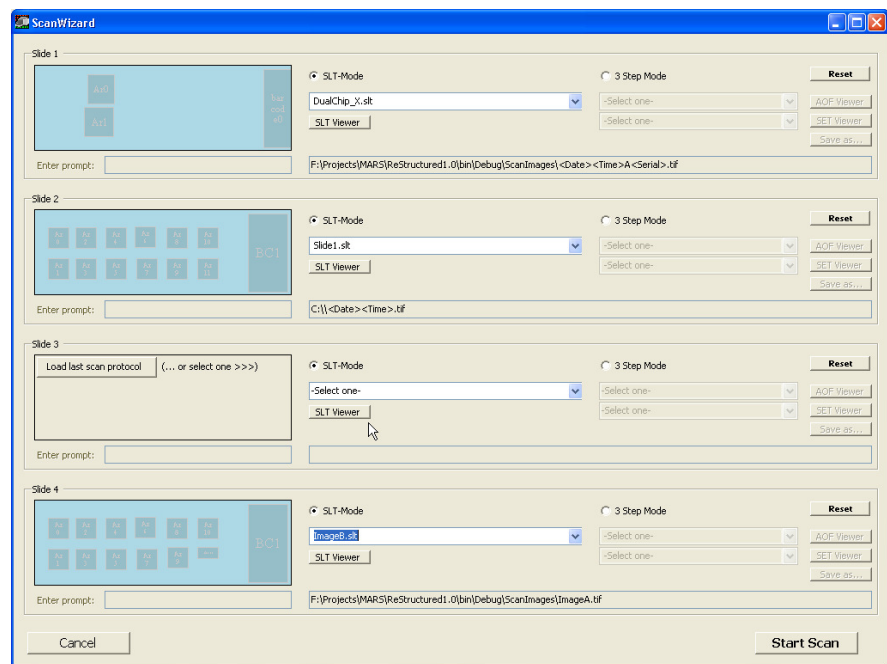


*The use of **Scan Wizard** is the scanning method of choice in order to benefit from the high flexibility and automation of the instrument, i.e. scanning several slides with several areas each, and these at different wavelengths and/or gain settings.*

*If you want to perform a quick scan-check, use the above described **QuickScan** being restricted to only one area on only one slide..*



Click on **Scan Wizard** in the main window to start the corresponding dialog window.



## ScanWizard Dialog steps

The **Scan Wizard** dialog mainly contains four special GUI elements each representing one of the four slides which can be loaded into the scanner. Each of these GUI elements allow to easily program individually the scan job for the slide it represents.

Each GUI element contains:

- a schematic slide box (which is used to display the scan areas selected),
- mode selection radio buttons for selecting the way for programming the scan
- list boxes for slide-type-files (SLT-files), area files(AOF-files), setting files(SET-files), and their corresponding viewer buttons
- and text fields that contain the filename with which the scanned image will be saved.

A scan job for a slide can be programmed either in the so-called **SLT-mode** or in the **3 Step Mode**.

These modes can be selected independently for each slide using the radio buttons located in the top row of this GUI element.

### Defining a scan in the **SLT-mode**

In SLT-Mode a scan job can be programmed selecting only one protocol file i.e., a SLT file.

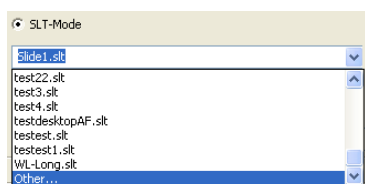
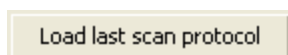


*An SLT file contains all the information that is necessary for a complete scan job*

1. *Scan area information of one or several areas on the slide*
2. *Scan settings information*
3. *SaveMode information including Orientation information and*
4. *File name (rules) with which the image scanned would be saved subsequently*

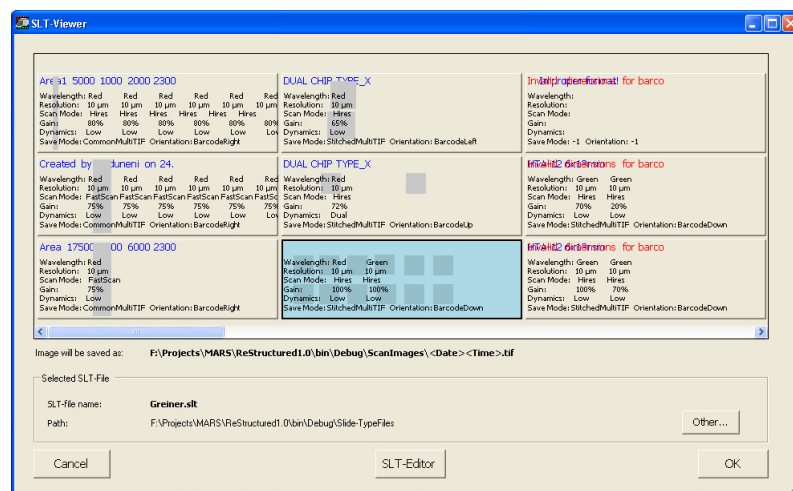
This SLT-file can be selected by the following ways:

1. **Load last scan protocol:** Using this button the software loads the last SLT-file that was used on this slide loading position. This button is present in the schematic slide box
2. **SLT list box:** By this way the desired SLT-file can be selected from the list box. It presents all the SLT-files present in the sub folder "Slide-TypeFiles" of the software's root directory.



In addition to these files another option “**Other...**” is also listed at the end of the list box. Selecting this option opens a standard “Open File dialog box” thus allowing the user to select an SLT-file present in any other folder. The list box now will be filled with the SLT-files present in this new location (i.e. sub-directory).

3. **SLT-Viewer:** Alternatively clicking the “**SLT-Viewer**” button opens a dialog box that contains a graphical representation of the SLT-files present in the list box. Each file is represented with a button depicting the underlying scan parameters: the scan areas present in the corresponding SLT-file are represented with colored rectangles, the scan settings are represented in table format with the Savemode options below. Among these visualized SLT-files, the desired SLT file can be selected by clicking on the corresponding button.



Once the selection for a slide is complete the back ground color of the schematic slide box turns to light blue.

### Defining a scan in the 3 Step Mode

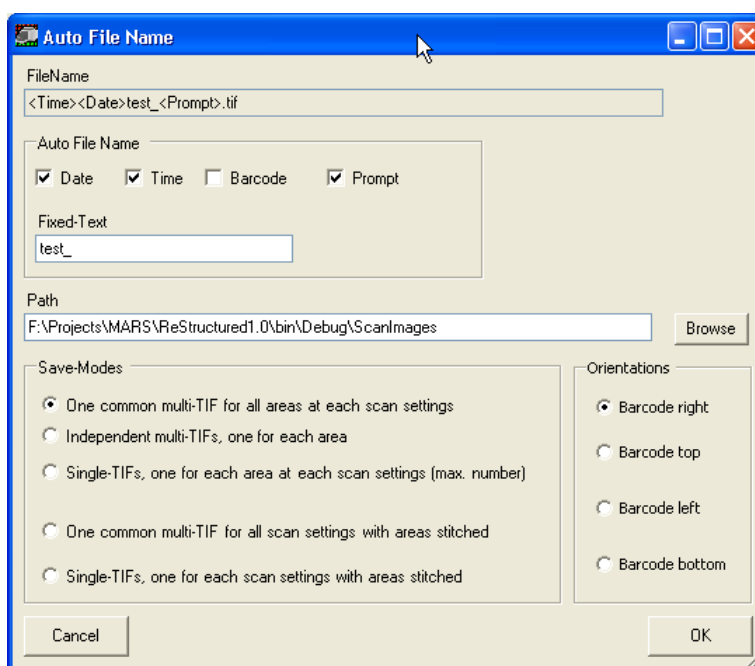
In **3 Step Mode** a scan job can be programmed using an area file(**AOF**-file), a settings file (**SET**-file), and an auto file name being defined with the “**Save as...**” button.

The area files and settings files can be selected from their corresponding list boxes or viewers in the same way as selecting a SLT-file( the procedure for which is described above).

The image file name with which the scanned image will be saved is selected from the “Auto File Name” dialog box that can be opened by “**Save as...**” button. In this dialog box it is also possible to select a different “Savemode” and “Orientation” for the image

#### Save as...

Click on the **Save as ...** button below the SET-viewer button in order to open the following dialog box:



The **Auto File Name** dialog box allows you to assemble the future image file name from an editable fixed text and automatically generated text parts as date, time, prompt and the slide barcode. Click or write the passages in the sequence you want to have them shown within the filename. In addition you can select a new path where the image file shall be stored, using the **Browse** button.

You can decide here with a radio button for one of five possible **Savemodes**. This gives you maximum flexibility for the further image analysis with any commercially available microarray analysis program.



The meaning of the different **Savemodes** are most comprehensively explained by a common example:

Suppose you will scan a slide with four distinct areas. Each area shall be scanned with two wavelengths using for each wavelength three different gain settings.

Multiplying the above mentioned variances (4 areas x 2 wavelength x 3 gains) gives you the total number of physically performed sub-scans, here resulting in 24 sub-images. The different Savemodes now define, how these sub-images are comprised in one or several TIF-files.

**'One common multi-TIF for all areas at each scan settings'** will include all 24 single sub-images in one common multi-TIF file.

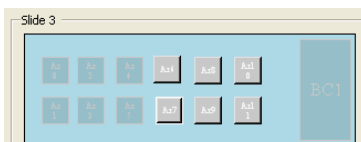
**'Independent multi-TIFs, one for each area'** will create for each of the four areas an independent multi-TIF file. Each of these four multi-TIF files encompasses the corresponding 6 single sub-images (2 wavelengths at 3 gains each).

**'Single-TIFs, one for each area at each scan settings (max. number)'** will save each of the 24 sub-images in a separate single-TIF file.

**'One common multi-TIF for all scan settings with areas stitched'**: The four different areas will be stitched to one common area, i.e. the (non-scanned) pixels in-between the areas are filled up with zero-values, i.e. black pixels. This stitching will be done individually for each wavelength-gain combination, i.e. 6 times. The six stitched multi-area sub-images are saved in one common multi-TIF file.

**'Single-TIFs, one for each each scan settings with areas stitched'** The four different areas will be stitched to one common area, i.e. the (non-scanned) pixels in-between the areas are filled up with zero-values, i.e. black pixels. This stitching will be done individually for each wavelength-gain combination, i.e. 6 times. Each of the six stitched multi-area sub-images is saved in a separate single-TIF file.

Finally you can also select the **Orientation** of the saved scan image with the help four radio buttons present in the corresponding group box. By default 'barcode right' is selected, as this is the orientation in which the slide is loaded and scanned in the scanner.



Each scan area loaded from an area file (in '3 Step Mode') or a SLT-file is represented with a button in the schematic slide box. These buttons act as **toggle buttons** allowing the individual areas to be selected or unselected for this specific scan job!

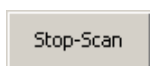
### Start Scan

The start scan button gets enabled once at least one scan area in at least one slide is selected.

Just click on **Start Scan**. The ScanWizard will close. Within the ImageViewer you will observe in live mode the ongoing scan process for

each sub-scan.

During the scan, the Slide Status box of the Image Viewer main window will inform you about the current status of each of the four slides. The image boxes will display in live mode the incoming fluorescence data of the currently scanned area.



After having started a scan, the **Stop-Scan** button, which is accessible at the bottom of the ImageViewer main window allows you to stop the current scan.



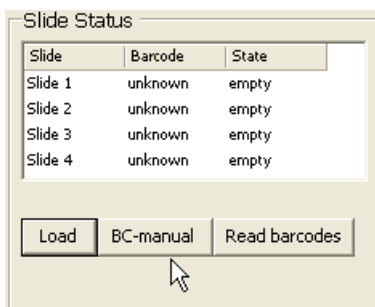

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*The contents of the above mentioned scanning parameter files, i.e. Area File (.aof), Settings File (.set), and Slide-Type file (.slt) and the meaning of the parameters will be explained in more detail in a subsequent chapter of the Manual.*

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## Barcode inclusion to filename and/or the image header

In several cases it is necessary that the filename and/or the header information of an image contains the barcode of the slide to which this image (or images) belongs. In the MARs software two mechanisms are implemented for barcode inclusion, the one based on a manual barcode entry, the other on barcode reading by the scanner.



As shown in the adjacent figure, there are two buttons in the Slide-Status box of the main software-window: “

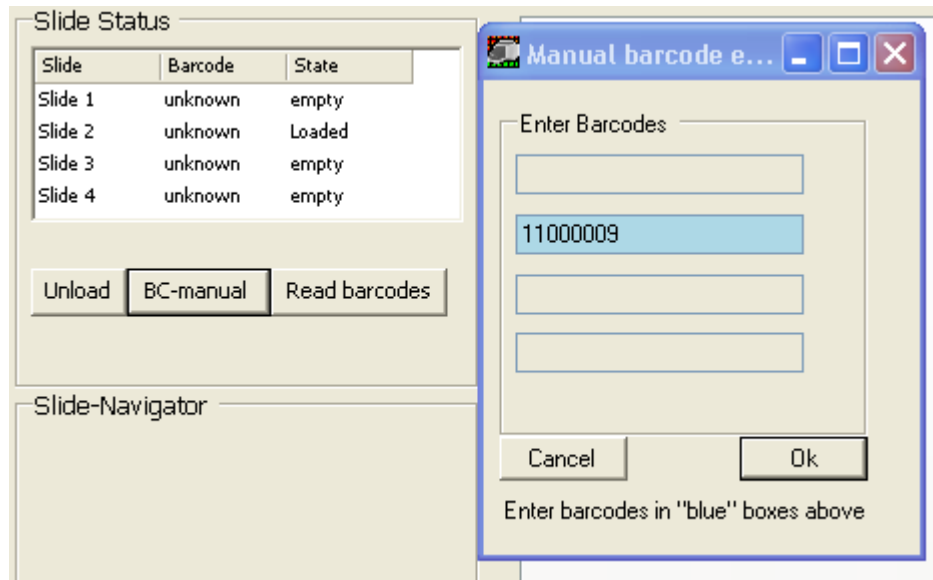
”**BC-manual**” button opens a dialog box to enter the barcode manually, whereas the button “**Read barcodes**” triggers the scanner to decode automatically the barcode from barcode images generated by scanning the barcode region on the slide.

### BC-manual

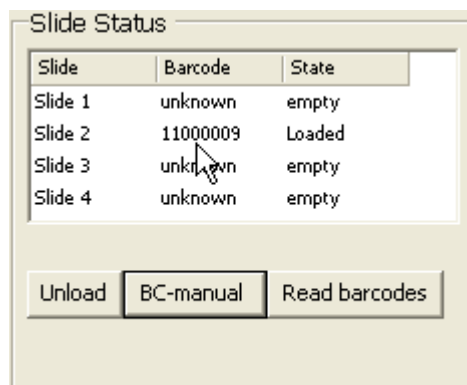
This button allows a user to enter the barcodes manually for the loaded slides. A special sequence minimizes the risk to mix up barcoded slides with respect to their loading positions. Clicking this button performs the following actions

1. If the loading table was outside, the loading plate is moved in first.
2. The loading table then moves outward to a position that allows the user to see the barcode on each slide but will not allow to unload any slide (barcode tracing to loading positions)
3. The following dialog box pops up, prompting the user to enter the barcode for all the loaded slide positions. The loaded positions are marked “blue” in this dialog box. Enter here manually the barcode(s). Clicking **OK** puts the barcode entered in ‘Barcode’-column of the “Slide-

Status” box, whereas clicking the “**Cancel**” button simply forgets the entered barcode(s).



4. The loading table moves back into the internal reference position upon closing this dialog box
5. As shown below in the figure, the entered barcode is now shown in the slide status box.



**Read barcodes**

Pressing this button triggers the scanner to scan an intermediate optical image from a prior defined barcode region on the slide. From this (non-visible) image the software decodes automatically the barcode to a corresponding ASCII string (numbers and/or letters), which are subsequently displayed in the Slide Status box.

Note: The “**Barcode.slt**” file present in the root directory of the software contains all information for scanning the barcode, i.e. the region (area) as well as the settings parameters (PMT-gain, laser wavelength).



## Scan Parameter Files and the Corresponding Editors

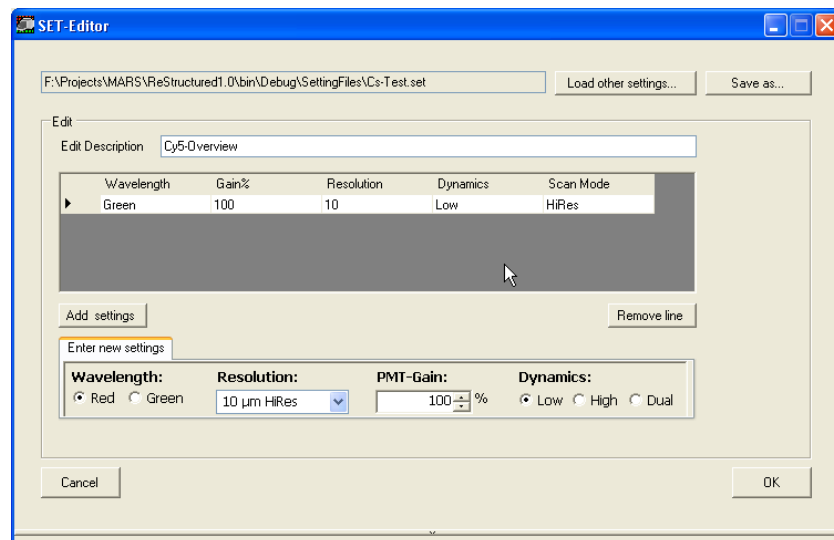
In the Software Description part of this operator manual several times specific **settings files** have been mentioned, which are used to define a dedicated scan job. The following section describes these files and their function in more detail.

### Settings Files (.set) and SET-Editor

Settings Files are either used when programming a scan with the QuickScan function or with the flexible ScanWizard. Their content is defining the scanning parameters for wavelength(s), sensitivity, dynamics and resolution of one or several subsequent scans. These binary files are easily created by the **SET-Editor**. The SET-Editor can be accessed from:

- 'SET-Editor...' -button in the Quick Scan Wizard (QSW),
- In Scan Wizard using the SLT-Mode:  
SLT-Viewer >> SLT-Editor >> SET-Editor
- In Scan Wizard using the 3 Step Mode:  
SET-Viewer >> SET-Editor

A schematic diagram of the common SET-Editor is shown below:



#### Load other settings...

This button allows to load an already existing settings file. Once loading an existing file it is possible to modify the individual fields of the scan settings file.

#### Save as ...

This button allows saving the settings that appear in the table (present below the "Edit description" text box) to a desired file in a desired location. The saved file has the scan settings data in the binary form, which is read by the

MARs software when starting a scan.

#### **Edit Description**

In this text box you can enter text that gives a hint about the settings file that will be created or modified. This reminder text would appear as the top most line in 'quick scan settings'-buttons in the Quick Scan Wizard.

#### **Settings Table**

The table that appears below the edit description text box contains the settings that are configured so far. The number of scan sets configured is equal to the number of lines in this table. Each line has 5 columns representing the five essential settings of a scan set. These columns from 1 to 5 represent the applied Wavelength, Gain%, Resolution, Dynamics, and the Scan Mode respectively. The meaning of these scan parameters is described below.

#### **Remove line**

An already programmed scan set (line) can be removed using this button.

#### **Enter new settings**

Using this tab page it is possible to configure your desired scan set. A scan set is configured by adjusting the individual scan settings with the help of their corresponding fields.

#### **Add settings**

With this button you add a new scan set that is configured in the "Enter new settings" tab. A plausibility check prohibits to repeat a scan set that is already configured in the Settings Table.

#### **OK**

This button closes the SET-Editor dialog box and loads the configured scan sets into the SLT-Editor in case if the SET-Editor was opened from the SLT-Editor or from the QSW.

Note, that (re-)configured settings are ignored by pressing OK, if the SET-Editor was opened from the SET-Viewer. Here you might save your changes to a (new) settings-file using 'Save as ...'.

#### **Cancel**

The SET-Editor dialog box closes and the configured scan sets are ignored.



*There are six pre-defined settings files delivered with the software, three for each wavelength. They will be mainly used in conjunction with the Quick Scan Wizard function. These files are just as a start, you may adapt and use them as a template for creating your own settings files. Their default-names 'Setting 1.set' through 'Setting 6.set' are responsible that they are displayed as quick selection boxes in the Quick Scan Wizard.*

---

## Meaning of the scan parameters in a settings file

Each line in the above mentioned settings table defines one (of possibly several) scan steps using five scan parameters, each. Scans thus defined will be performed according the line sequence in the table.

**Wavelength** specifies which laser and which corresponding fluorescence filter set is used for this scan. '**Red**' laser (CY5); '**Green**' laser (CY3).

**Resolution** (including Scan Mode) specifies the scan resolution in X- and Y-direction. The corresponding list box in the 'Enter new settings'-tab box allows you to define the pixel size ( $\mu\text{m}$ ) you will get in the scanned image. The current software supports either **10**  $\mu\text{m}$ , **20**  $\mu\text{m}$ , **50**  $\mu\text{m}$  or **100**  $\mu\text{m}$  resolution. The alternative abbreviations **HiRes** or **FastScan** following the pixel size value specify whether the slide is scanned in the High Resolution Mode (1:1), or the interpolating FastScan Mode (2:1) – here scanning in Y-direction only every second line but interpolating the skipped line from the two adjacent scanned lines. The FastScan mode strongly reduces the overall scan time. Note, that the selected scan mode is represented in the fifth column in the above settings table.

**PMT-Gain%** is the dominant parameter to define the sensitivity of the instrument for this specific scan step. It controls the gain (amplification) of the photomultiplier (PMT).

**Dynamics** specifies, which preamplifier channel(s) are used for the detector electronics, i.e. the **Low** channel, the **High** channel, or both being called **Dual** mode. Low channel is the recommended standard channel for any selected PMT-gain. The High channel should be used, if even at 100% PMT-gain an increased instrument sensitivity is required. Dual channel is selected, if a broader dynamic range during one(!) scan is desired; this mode will lead to two independent scan images of the same scan area at different (partially overlapping) gains.



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*When combining such a Multi-settings file(.set) with an Area File (.aof) specifying several areas (see below), each of these areas will be scanned with each specified settings combination. After all different areas have been scanned with the settings of the first line, they will be scanned with the settings of the second line, and so on. This is valid either for the SLT-Mode or the 3 Step Mode in the Scan Wizard.*

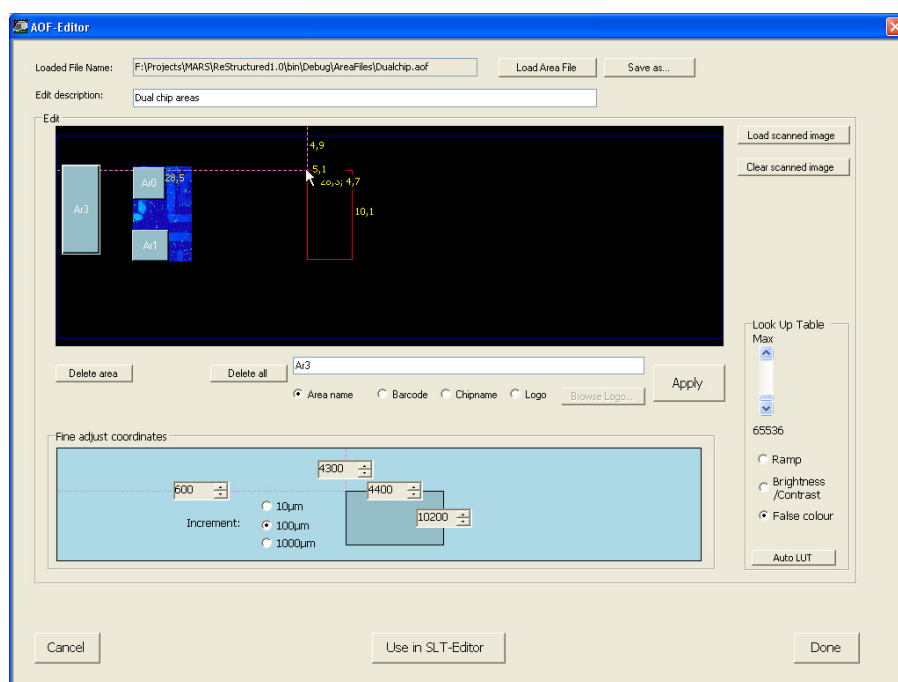
---

## Area File Editor (AOF-Editor)

The AOF-Editor is used for creating the so-called '.aof'-files, i.e. the parameter files specifying the location(s) and dimensions of one or several areas on a slide which are subsequently scanned. Additional areas for barcode scanning, or depicting a slide name/logo are defined here, too.

The AOF-Editor can be accessed from:

- In Scan Wizard using the SLT-Mode:  
SLT-Viewer >> SLT-Editor >> AOF-Editor
- In Scan Wizard using the 3 Step Mode:  
AOF-Viewer >> AOF-Editor



The editor contains a schematic slide box similar to other editors. In this field it is possible to draw the desired scan area(s). The schematic slide box is designed in such a way that its dimensions are proportional to the dimensions of a standard slide. For convenience sake all coordinates and dimensions are displayed lively during the drawing process in 'mm'. The current coordinates of the mouse tip are indicated with respect to the top left corner of the slide box. The length and width of the drawn area and the distance of its top left corner with respect to top left corner of the schematic slide box are also shown.



*One pixel displayed in the schematic slide box on the PC-monitor is equal to 100 µm on the real slide.*

### Load Area File

This button allows to open an already existing area file, thus allowing to modify the individual scan areas in this file. Each scan area in the AOF file is

represented with a corresponding button in the schematic slide box.

**Save as ...**

This button allows to save all the areas that are displayed in the top schematic slide box as an “.aof”-file. Clicking on this button opens a standard “Save file dialog box”, and prompts the user to give a file name and potentially different path.

**Load scanned image**

A button located on the top right corner of the edit group box, allows to load a standard TIF image formerly scanned with the MARs scanner. The respective scan areas will be displayed at the original locations on the slide. The user now can very easily create a scan area by drawing a rectangle over the image where the interested image data is present.

**Look up table control**

This control is useful to adjust the look up table of the loaded image for a better vision.

**Clear scanned image**

The button located below the “Load scanned image” simply clears any image loaded in the schematic slide box if loaded earlier

**Delete Area**

The button located below the slide box on the left side, is used to delete the scan area which is currently selected. A scan area can be selected by clicking on the corresponding scan area button, which then is surrounded by a dotted line.

**Delete all**

This button located below the schematic slide box on the right side of the “Delete Area” button, upon clicking clears all the scan areas present in the slide box.

**Area type radio buttons**

The radio buttons located below the schematic slide box on the right side are used to define the type of a selected area which can be:

- ‘**Area name**’: the normal area(s) to be scanned for fluorescence
- ‘**Barcode**’ area, - the area to be scanned for barcode only  
(not implemented yet, see below)
- ‘**Chipname**’: the area where the entered chip name will be displayed as an ASCII-string in the diverse schematic slide box views of this software
- ‘**Logo**’: the area where a bitmapped logo will be displayed in the diverse schematic slide box views of this software. A separately saved bitmap image is displayed within the specified coordinates. The respective bit-map-file must be located in the “Icons”-sub-directory of the main software

The “Apply” button sets the area type, area name (entered in the corresponding text box), or chipname / logo of the selected scan area.



This **area name** defined here will appear on the scan areas displayed on the schematic slides in the ScanWizard, and later when the image data of a specific scan are displayed in the ImageViewer. Accordingly each area name is saved as part of the TIFF image header for the corresponding fluorescence sub-image.

#### **Fine adjust coordinates:**

The controls in this box allow to fine adjust the start coordinates and dimensions of any selected scan area. This for example is done in order to fine-adjust roughly drawn adjacent scan areas of a compartmented slide for the identical height and area dimensions. The increment radio buttons allow to select the increment steps among one of the values 10 $\mu$ m, 100 $\mu$ m, and 1000 $\mu$ m.

#### **Edit description text box**

The text box located above the slide box is used to add a reminder text that describes the aof file. Its only displayed in this dialog box.

#### **Use in SLT-Editor / Done**

This button located at the bottom in the center of the dialog box is used to load the configured scan areas into the SLT-Editor if this dialog box was opened from the SLT-Editor. The Done button does the same in this case. Note: In case if this AOF-Editor was opened from any other location then the scan areas configured are ignored and the dialog box is closed. Note, that (re-)configured areas are ignored by pressing 'Use in SLT-Editor' or 'Done', if the AOF-Editor was opened from the '3 Step Mode'. Here you might save your changes to a (new) aof-file using 'Save as ...'.

#### **Steps for creating a new AOF file**

1. Irrespective of the case from where you open the AOF editor, click on the "Delete all" button to clear any areas in the schematic slide box
2.
  - a. In case if you already know the size and location of the areas you want to scan, simply draw the areas in the schematic slide box and fine adjust the coordinates and dimensions of the drawn scan areas.
  - b. In case if you want to draw the scan areas based on the image scanned from the QSW, use the "load scanned image" button to load the (pre-scanned) image and then draw the areas possibly followed by a fine adjust.
3. Assigning the area names and area types: By default the software automatically suggests the 'Area names' type, i.e. a normal fluorescence scan area. In case if you want to change these names (also afterwards) simply click on the desired scan area button and change its name in the corresponding text field. Where needed change the area type by clicking on the desired area type radio button followed by a click on the "Apply" button.
4. After configuring all the scan areas simply click on the "Save as..." button and save the aof-file with a desired filename and path. Depending

on the situation you can also use the “Use in SLT-Editor” button.



The **scan area** as defined in either an area-file (or a slidetype-file, see below) is restricted to an area of 23 x 76 mm, because 1mm at each length side of the slide is covered by the slide loading rails.



A “**Barcode**”-area defines the coordinates of a potential barcode label on the slide, which shall be used for an optical scan of the barcode. In this area button the text indicates the (expected) type with which the barcode is decoded. This “function is not yet implemented; it even might be deleted from here in future software versions, e.g. for the case that slide barcodes are only read and decoded as an general scanner function independent from a fluorescence scan; to be decided.)

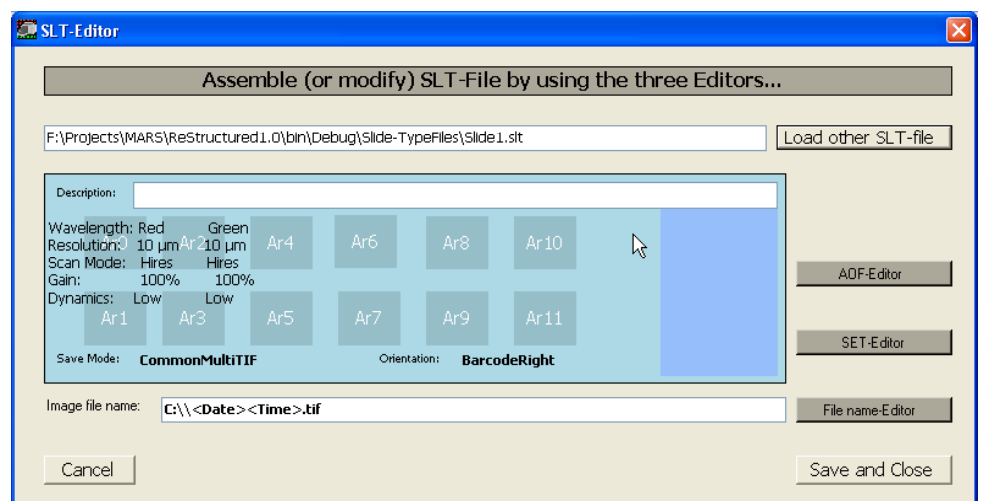
### Slide Type File Editor (SLT-Editor)

The SLT-Editor is used to create the corresponding slide type files (SLT-files). It can be accessed from Scan Wizard using the SLT-Mode (SLT-Viewer >> SLT-Editor).

The SLT file can be understood as a combination of

- an area file (AOF-file),
- a settings file (SET-file),
- the rules for the auto file naming (otherwise entered as step 5 in the ScanWizard)
- including the SAVEMODE and ORIENTATION selection.

In other words, such an SLT-files defines any necessary scan protocol parameter allowing to immediately start a corresponding scan.



In the SLT-Editor a SLT-file is easily assembled from an AOF- file (using the AOF editor), a SET- file (using the SET-Editor) and from the autofilename dialog box (using the File name-Editor) which gives the information about the savemode, orientation and the auto file name.

Similar to the others editors described earlier, a SLT-Editor consists of (refer the figure above):

- A button to load an already existing SLT file for editing
- A schematic slide box to display the individual scan areas, scan settings, Savemode and orientation of the loaded SLT- file
- A text field for depicting the filename with which a slide scanned with this SLT-file will be saved
- Buttons to open AOF-Editor, SET-Editor, and/or the Auto File Name dialog box
- 'Save and Close' button that saves the (modified) SLT-file to a desired file and closes the editor

#### Procedure for creating a SLT-file

1. Use the "AOF-Editor" button to define the scan areas
2. Use the "SET-editor" button to define the scan settings
3. Use the "File name-Editor" button to open the Auto File Name dialog box to specify the
  - Auto file name
  - Savemode
  - Orientation
4. Finally, save the above configuration as a SLT-file using the "Save and Close" button



*The AOF-Editor, the SET-Editor and the File name-Editor have been explained in detail in previous chapters of the Manual.*

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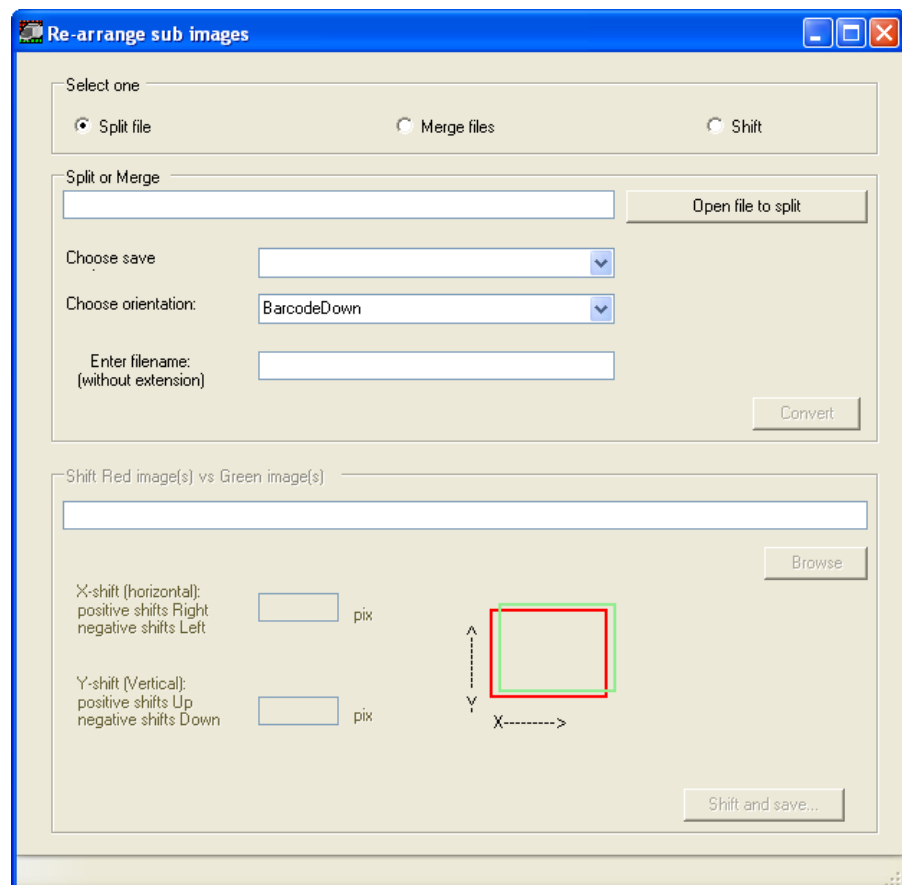
*The SLT-file created using the SLT-Editor is in the binary format, which is readable by the MARs-software*

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## Re-Arranging the TIF Files of Scanned Images

As described earlier in the “Savemode” section, the MARs software can be programmed to generate multi-paged as well as single-paged TIF files. The software also generates images of various scan areas stitched together to form a single TIF image. Up to now all these options were only possible while configuring a scan, i.e. before the scan is started.

With the “Re-arrange sub images” module of the software (which can be accessed from the right-handed “Re-arrange sub images” button in the main window) it is made possible to rearrange already scanned images/files among various Savemodes.



Following is the list of functions handled by this module:

### 1. Splitting a multi paged TIF file

- a. Any multi-TIF file can be split into separate single-TIF files
- b. If the multi TIF file contains images from several scan areas and several scan settings, then it is possible to split these images into one multi paged TIF file per area, i.e., into the file structure of Savemode = 3.

- c. Respectively stitched images can be splitted into the file structure of Savemode 4 or Savemode 5.

It is also possible to change the Orientation of the images during this conversion step.

- 2. **Merging several single paged TIF files** to a desired and valid Savemode format. The main conditions that need to be satisfied here are that all the selected files must belong to same loading slot in the scanner and should be scanned within a time span of 2 hours, i.e. resulting from a former scan of one slide.
  - a. Selected files can be merged to a single multi-paged TIF file
  - b. Selected files can be merged to the file structure Savemode 3
  - c. Selected files can be merged to the file structure Savemode 4
  - d. Selected files can be merged to the file structure Savemode 5
- 3. **Shifting images:** This feature allows to shift (selected) scan image(s) in both directions by simply adding or deleting pixel rows and columns in the image depending on the positive or negative shifts.

This feature could be useful to exactly match the images of an area being scanned with the red and green lasers, where both scans were performed independently by loading the slide twice. Because of unloading and loading this subsequent scanning could produce some shift between the two images .

## Warning



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A proper rearrangement of the images is possible only with images generated by the MARs software and preferably within one scan.

Particularly shifting the images should be careful programmed.

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## Service Functions

The **Service** dialog provides on password protected level several instrument service functions for the service engineer.

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### Warning



**An incompetent use might damage the instrument!**

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## 2. Appendices

### Installation Guide for MARs-Pre-Release-Software Versions

*This chapter describes how to install the pre-release versions of the MARs application software onto your PC. You might get the software either on CD-ROM or via download from the Internet.*

1. You will get a set-up file either on the appended CD-ROM or having it downloaded (e.g. from "StreamLoad") on a directory on your Windows-PC
2. Start the file "MARs-Installation0.X.exe" (e.g. "MARs-Installation0.71.exe") on your PC.
3. You will be asked for a local drive plus destination folder to which the files should be extracted to. Type in e.g. "C:\MARs".
4. If the ".NET framework" is not installed on your computer, i.e. when you install the MARs-software the first time on this particular PC, you firstly have to install it. Start the corresponding setup file **dotnetfx.exe**, which you will find in the appended CD-ROM or having it downloaded (e.g. from "StreamLoad") in a sub folder "MS .net Framework"
5. When running the MARs the first time from you PC, you have to install the corresponding USB-driver:
  - a. First switch **ON** the MARs-Instrument, wait some seconds, and then connect it to your PC via a USB-cable.
  - b. The computer now should recognize a new USB device. Windows will ask you where to search for the driver..Select the directory "usb\_driver" present in the installation CD. The driver now will be automatically installed. At several steps you have to press 'Next' or 'Continue'. Finally Windows shows the information that the installation was successful.
6. To start the software click twice on 'MARs\_Rev0.X.exe' which you will find in your created MARs-SW installation folder (e.g.: "C:\MARs\MARs\_Rev0.71\MARs\_Rev0.71.exe"). Now the instrument can be controlled by the running software.
7. To make starting for routine work easier you might create a shortcut to the program-file 'MARs\_Rev0.X.exe' on your desktop.
8. Make sure that the software directory has the ...

following sub-folders:

Log	Icons
Slide-TypeFiles	ScanImages
SettingFiles	Temp
AreaFiles	

and following files:

parampage.h	MARs.dll
default.set	MARSTick.dll
MARs.ini	MARSTiffs.dll
AN21XX_NET.dll	ScanJobCtrl.dll
MARsComm.dll	Tasman.Bars.dll
MARsEditors.dll	Tasman.Bars.Safe.dll
MARsLutCtrl.dll	noisecorrection_mars.dll
MARsSPB_OldStyle.dll	MARs-Rev0.X.exe
MARs-Slide-Status-Grid.dll	