

MycoProof Basidio User Manual

In combination with Mycotype® **Basidio**^{QS} Microarray Detection Kit
Software Version: 1.0



MycoProof **Basidio**

Bio **type**®

powered by Qualitytype AG 2009

Table of contents

1. Introduction	3
2. Setup	3
2.1 System requirements	3
2.2 Starting the installation.....	4
2.3 Licensing.....	4
2.4 Increasing or decreasing the utilized RAM.....	5
3. The user interface and how to work with it.....	6
3.1 Menu and tool bar.....	7
3.2 Windows	7
3.3 Minimizing and maximizing windows	7
3.4 Adjusting width and height of a window.....	8
3.5 Arranging windows.....	8
3.6 How to work with tabs	9
3.7 Direct printing	10
3.8 Shortcuts.....	10
3.9 Language settings.....	10
4. User Administration	11
4.1 The user view	11
4.2 Create new user	12
4.3 Edit a user	12
4.4 Reset password	13
4.5 Delete user	13
5. Import and manage samples.....	13
5.1 Sample View.....	13
5.2 Import samples	16
5.3 Open Samples	17
5.4 Analysis.....	18
5.5 Quality.....	18
5.6 Results of analyzed features	19
5.7 Show image.....	20
5.8 Edit sample details	22
5.9 Export samples	23
6. Import calibration slide	25
7. Glossary	26
8. Supplement	27
8.1 Calculation of spot signal intensity.....	27
8.2 Calculation of quality sensors.....	27
8.3 Calculation of fungi features	27

1. Introduction

MycoProof **Basidio** was designed for the evaluation of microarray experiments carried out with the MycoType[®] **Basidio**^{QS} test kit. The application features the import, analysis and management of image data generated by a fluorescence scanner. All data are stored in a secured database and can be queried by means of user defined filters. Relevant data can be exported as PDF as well as CSV documents. The graphical user interface (GUI) was developed for the special needs of wood surveyors, enabling a fast and clear evaluation of chip experiments.

2. Setup

MycoProof **Basidio** is offered as a single-position system and all data is stored in a local database. We offer a free demo license, which is only valid within a restricted time span, as well as an unlimited license for unrestricted use of the application.

2.1 System requirements

MycoProof **Basidio** is executable with the following Windows systems:

Windows 32 Bit

- Windows 2000 Professional/Server
- Windows Server 2003
- Windows XP Home/Professional
- Windows Vista
- Windows 7

Windows 64 Bit

- Windows Server 2003
- Windows XP
- Windows Vista
- Windows 7

Minimum configurations recommended:

- PC running Microsoft Windows 2000, XP, Vista or 7
- 512 MB RAM available (however, we recommend to use more than 521 MB RAM)
- approximately 300 MB free hard disc space (without database)
- screen resolution of 1024 x 768 pixel

2.2 Starting the installation

- Boot your operating system and close all active applications.
- Insert the CD-ROM into the optical drive.
- Launch the setup program called MycoProofBasidioSetup.exe.
- The setup program will propose a directory as destination folder. It is possible to change the directory. However, never install more than one product in the same directory.
- After successful installation, MycoProof **Basidio** can be started using the shortcut in the Windows Start menu that has been created during the installation.

2.3 Licensing

In order to use MycoProof **Basidio**, you need to provide a license key. The license key can be purchased from Biotype Diagnostic GmbH. However, there is also the possibility to request a free demo license key, which enables you to test the application for a limited period.

You can request the license key via email (mycoproof@biotype.de). Please note that we can only send a license key if you send us your System Identification. The System Identification does not contain data from your system.

To obtain the System Identification, please observe the following steps:

- Log in as user „Admin“ (password „admin“). To this end, select the menu item **File/Login....**
- You have to designate a directory to store your data in.
- Once you are logged in, select the menu item **?/Licenses.**

Your System Identification is shown in the **License Request** section.

Clicking on the link **Send license request** will start your mail application will. If there is no default mail application configured, you have to manually copy the system identification into the clipboard (**Copy button**), open your mail application, create a new e-mail, paste the system identification into the body and send it to the address cited above.

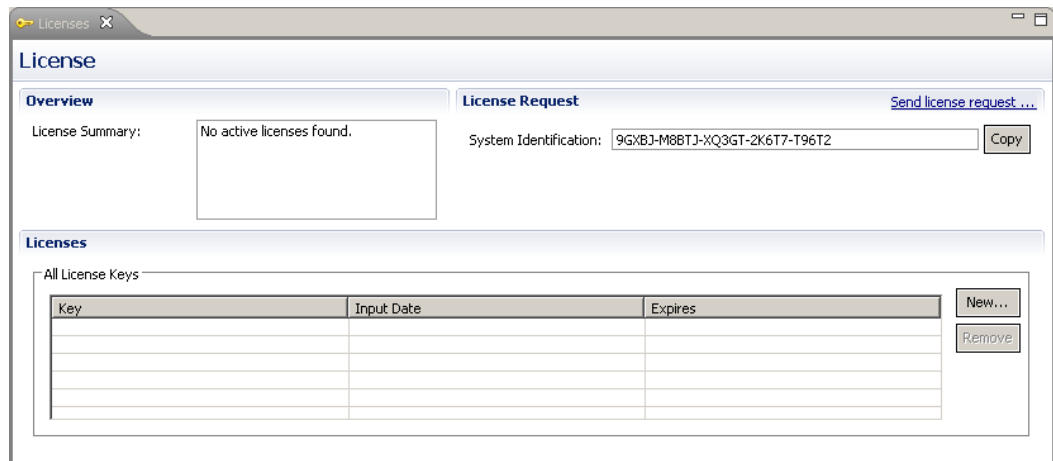


Fig. 1 License Editor

When we have received your license request we will contact you. Add your new license key to the software by following these steps:

- Login as administrator (User “Admin”, default password “admin”)
- Open the License Editor by the menu **?/Licenses**.
- Choose the **New** button in section **All License Keys**
- Copy your license key into the appearing dialog and press the **Finish** button

The populated key will now appear at the table in section **Licenses**. Please note that changes to the licensing system will only take effect after restarting the application.

Please keep your license key safe!

2.4 Increasing or decreasing the utilized RAM

You can determine how much RAM MycoProof **Basidio** is allowed to utilize. By default, the program occupies 256 MB upon launch and may increase it to up to 512 MB in case of need.

The RAM to utilize can be adjusted in the initialization file (INI file). The INI file is located in the same directory like the executable file of MycoProof **Basidio**. The default path is: **C:\Programme\Biotype\MycoProof**. The initialization file has the same name like the executable file, but another file extension (MycoProof.**ini**).

Open the INI file. The RAM occupied automatically can be adjusted in the line **-Xms??m**, where '???' represents the desired RAM in megabytes. The RAM occupied at maximum can be adjusted in the line **-Xmx??m**, where '???' represents the desired RAM in megabytes. Save the file, when you made the desired changes.

Note: Newer operating systems such as Microsoft Windows Vista do not permit common users to access the program directory. In this case, ask your administrator to make the changes, please.

3. The user interface and how to work with it

The user interface of MycoProof **Basidio** is highly interactive.

The screenshot displays the MycoProof Basidio software interface. The main window is titled "sampleOneCustomerTwo_2" and shows an "Analysis" section. Under "Quality", a message states: "This section provides control spots of image calculation. Analysis is only successful, if every result is valid. Background noise of image: 1,071 RFU. A valid layout of the spot grid was found." Below this is a table with columns "Feature", "Signal (RFU)", and "Result".

Feature	Signal (RFU)	Result
<input checked="" type="checkbox"/> ALR Negative Control		VALID
<input checked="" type="checkbox"/> ALR Positive Control		VALID
<input checked="" type="checkbox"/> Fluorescence Positive Control		VALID
<input checked="" type="checkbox"/> PCR Positive Control		VALID

Below the quality table is the "Features" section, which states: "The following features were analyzed. For each spot signal is a symbol icon shown, which represents whether the spot was found or not. The feature is marked valid, if at least one spot and its related reference spot was found and the calculated result (RFU) is greater than the threshold. Encountered irregularities are shown in the Plausibility-Column." This is followed by a table with columns "Feature", "Signal (RFU)", "Reference Signal (...)", "Result", and "Plausibility".

Feature	Signal (RFU)	Reference Signal (...)	Result	Plausibility
<input checked="" type="checkbox"/> Antrodia sinuosa			negative	
<input checked="" type="checkbox"/> Antrodia vaillantii			negative	
<input checked="" type="checkbox"/> Antrodia xantha			negative	
<input checked="" type="checkbox"/> Bjerkanthera adusta			negative	
<input checked="" type="checkbox"/> Coniophora arida			negative	
<input checked="" type="checkbox"/> Coniophora marmorata			negative	
<input checked="" type="checkbox"/> Coniophora olivacea			negative	
<input checked="" type="checkbox"/> Coniophora puteana			negative	
<input checked="" type="checkbox"/> Daedalea quercina			POSITIVE	

At the bottom of the interface, there is a "Sample View" section showing a table of matches:

Sample Name	Remark	Image	Created By	Created At
sampleOneCustomerTwo_4		C:\samples\sampleOneCustomerT...	John Smith	01/06/2010
sampleOneCustomerTwo_3		C:\samples\sampleOneCustomerT...	John Smith	01/06/2010
sampleOneCustomerTwo_2		C:\samples\sampleOneCustomerT...	John Smith	01/06/2010
sampleOneCustomerTwo		C:\samples\sampleOneCustomerT...	John Smith	01/06/2010
firstSampleCustomerOne		C:\samples\firstSampleCustomer...	John Smith	01/06/2010

The bottom status bar shows "User: john" and "14M of 127M".

Fig. 2 The user interface of MycoProof **Basidio**

The subsequent sections will explain the general design of the user interface and its general functions.

3.1 Menu and tool bar

All features of the application can be accessed by the menu bar. Some important features are also available by the tool bar.

3.2 Windows

There is a window showing all stored samples (Sample View). It can be found in the lower part of the main window by default. The upper part of the main window is used for the so called editors. Double clicking on an item in the Sample View will open a specific editor containing all information about that specific sample. You can open several editors at the same time. If you want to switch to another window, simply click on the tab of the editor you want to bring to front. As an alternative, you can use the shortcut **Strg+F6**.

The user interface of MycoProof **Basidio** offers extensive opportunities for rearrangement, allowing a user its adaptation to his personal preferences. These are the principles operations of the user interface are based on:

- Calling of (additional) data opens (additional) windows, which are arranged in tabs
- Open windows do not close as long closing is not directly induced by the user.
- The size of the windows can be modified at will.
- Also the arrangement of the window is adjustable. Every window can be shifted to other positions (e.g. to a special edge of the main window).

The subsequent sections explain the general handling of windows in MycoProof **Basidio** and are intended to help making your work with the program as convenient and effective as possible.

3.3 Minimizing and maximizing windows

By means of the **Minimize** and the **Maximize** buttons situated in the top right of every window you can minimize and maximize windows.



The 'Minimize' and the 'Maximize' button

Minimize

Pressing the **Minimize** button will minimize the complete window area with all its tabs. A field with two buttons will appear at one edge of the main window.



A minimized window (with one 'Restore' button)

Each field contains a **Restore** button. Pressing this button will bring back the window in its original size and position while at the same time the two buttons disappear.

Note: You can shift the field into other positions, if you like. To do so, bring your mouse pointer onto the dashed line. The mouse pointer will turn into an arrowed cross. Now press the left mouse button and keep it pressed while shifting the two buttons until they are in the desired position.

Maximize

If you press **Maximize**, the window will enlarge until it occupies the whole main window, while other areas are minimized. You can reverse this by clicking on the **Restore** button that has now replaced the **Maximize** button.

Alternatively, you may maximize a window by double clicking on the corresponding tab. To restore the window in its original size and position, simply double click on the tab for another time.

3.4 Adjusting width and height of a window

To adjust the window size, place the mouse pointer directly onto the border of the window. The mouse pointer will turn into a double-headed arrow. Press the left mouse button and keep it pressed while moving the mouse pointer until it is in the desired position.

3.5 Arranging windows

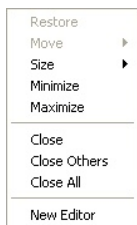
A further advantage of the technology used in MycoProof **Basidio** is the possibility to freely arrange windows. This way you can, for instance, display the results table of two samples directly next to each other.

To arrange windows, click on the corresponding tab and move the mouse into the desired direction while keeping the left mouse button pressed. A black arrow and a grey frame indicate the position of the window if you would let go the mouse button in the current position. Stop pressing the mouse button when you have found the desired arrangement.

Windows can also be rearranged using the pop-up menu. Use the right mouse button to click on a tab and select **Move**. Now you have two possibilities:

– **Editor:** Moves the chosen tab within the editor area

– **Tab group:** Moves whole tab group



Pop-up menu of an editor window

Views such as the **Sample View** can also be detached from the main window and the general user interface. To detach a view, open the pop-up menu and select the item **Detached**. To reverse the procedure, simply perform the same steps again.



Pop-up menu of a Sample View window

You can open more than one sample by simply double clicking on another sample in the **Sample View** window.

3.6 How to work with tabs

Windows are not closed when you open new windows. Instead, the new window is opened in the same area like the old one. Tabs allow switching between the windows.

The order of the tabs can be changed at will. Simply click on a tab and move it forwards or backwards into the desired position while keeping the left mouse button pressed.

Individual tabs can be closed by pressing the **X** button (**Close**) next to their tab title. However, you can also close several editor tabs at the same time. To this end, open the pop-up menu and select one of the following menu items:



Pop-up menu of an editor tab

- **Close:** Closes the selected tab
- **Close Others:** Closes all tabs in the editor area but the one selected
- **Close All:** Closes all tabs in the editor area

These functions allow for the handling of a large number of tabs (windows) open at the same time.

3.7 Direct printing

The contents of some windows can be printed directly. To this end, click the **Print** button on the main window toolbar. Alternatively, use the menu **File/Print** or the shortcut **Strg+P**.

There is a preview shown. Use the corresponding buttons to browse between pages or zoom in and out.

If you want to print the document as shown, use the **Finish** button. Use **Abort** to leave the preview without printing.

3.8 Shortcuts

The majority of the applications features are also accessible by keyboard shortcuts. All shortcuts are displayed beside the menu item. For example, use **Strg+S** for saving the contents of an open sample.

3.9 Language settings

By default, MycoProof **Basidio** is loaded with the language settings set in the operating system for standards and formats. However, you can determine another language that is used independently from the operating system settings.

This has to be set in the initialization file (INI file). The INI file is located in the same directory like the executable file MycoProof **Basidio**. The default path is: **C:\Programme\Biotype\MycoProof**. The initialization file has the same name like the executable file, but another file extension (MycoProof.**ini**).

Open the INI file and add the following line: **-Duser.language=XX** and replace 'XX' by the two-place lower-case ISO-code for the referring language. The line has to be situated after the line **-vmargs**. You can additionally set a country format. To this end, add the line **-Duser.country=XX** and replace 'XX' by the two-place upper-case ISO-code for the referring country. The line has to be situated after the line **-vmargs**, too. Then save the file.

At the moment, you can choose between the following languages:

German (ISO-Code: DE) and **English** (ISO-Code: EN).

Note: Newer operating systems such as Microsoft Windows Vista do not permit common users to access the program directory. In this case, ask your administrator to make the changes, please.

4. User Administration

MycoProof **Basidio** distinguishes between three types of users:

Administrator

Administrators can create new users and manage licenses. They are not authorized to create or edit samples. One administrator account is automatically created during installation. It cannot be deleted. You can create as many administrator accounts as you like without needing further licenses.

Laboratory Head

The user type is not allowed to do administration tasks, but Laboratory Heads can create new samples, and read and edit all samples stored in the database.

Assistant

The user type is not allowed to do administration tasks and Assistants are only allowed to edit the samples they have created by their own. However, Assistants can read all samples that are stored in the database.

User accounts can only be edited by administrators. For further information, please read the following sections.

4.1 The user view

User accounts can be managed in the user view by the administrator, i.e. they can be created, edited, locked, unlocked and deleted.

To open the user view, login as administrator. By default, the user view is shown at the lower part of the application (otherwise open the user view by the menu **Window/User View**).

The user view shows all users in the database with their login name and display name. All required features in order to manage users are accessible by the user view toolbar.



User view toolbar

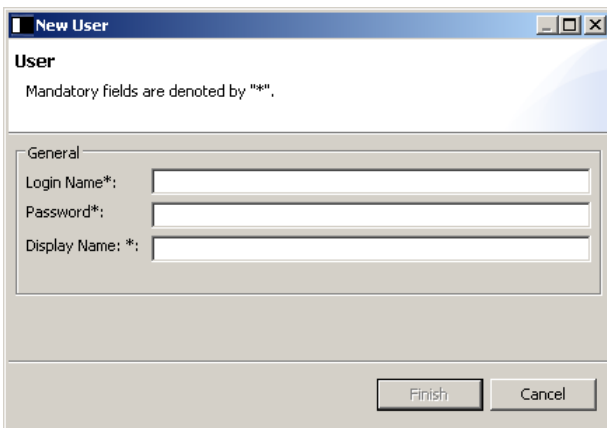
- **Create new user (icon plus sign):** Creates a new user
- **Open user (icon document):** Opens a user for editing
- **Delete (icon red cross):** Deletes a user account

- **Reset password (icon key):** Resets the users password

All features are described in the subsequent sections.

4.2 Create new user

Press the button **Create new user** from the user view toolbar in order to create a new user account. An input form is shown:



The image shows a 'New User' dialog box with the following fields and buttons:

- General section:
 - Login Name* (text input)
 - Password* (password input)
 - Display Name: * (text input)
- Buttons: Finish, Cancel

Creating a new user

Fill the input fields:

- **Login Name:** This name has to be used by login in combination with the password
- **Password:** The user's password
- **Display Name:** This name will be used by the application for showing the full name of the user

Finish the creation process by pressing the corresponding button. Please note that the **Finish** button will only be toggled active, when all fields are filled.

4.3 Edit a user

In order to edit the user's details, double click on its corresponding item in the user view.

The screenshot shows a web application window titled "User" with a user profile for "John Smith". The form is divided into four sections:

- User:** Active (checked), Login Name (john), Display Name (John Smith).
- Contact:** E-Mail (john@thesmiths.com), Phone Number (0123/45678910), Fax Number (0123/45678911).
- User Group:** Assistant (selected from a dropdown menu).
- Organization:** Name (The Smiths Labs), Street (Schmidtstraße), House Number (1), Postal Code (12345), City (Schmidthausen).

Fig. 3 User editor

You are not allowed to edit the Login Name. This name is unique within the database and is used to match samples and surveyors safely.

All the other fields are enabled for editing.

Please take care not to change the User Group of the last administrative user to **Assistant** or **Head**. There will be no possibility to manage users than.

In order to lock a user, deactivate the checkbox **Active**. This user is not allowed to login than. Please take care not to toggle the last administrator as inactive.

4.4 Reset password

The administrator can reset the password of any user without knowing the old one. Select the desired user from the user view and press the **Reset Password** – Icon (the key icon). Enter and confirm the new password in the appearing dialog box.

4.5 Delete user

Select the user to delete in the user view and press the icon **Delete User**. Confirm the deletion by clicking **OK** at the appearing dialog box.

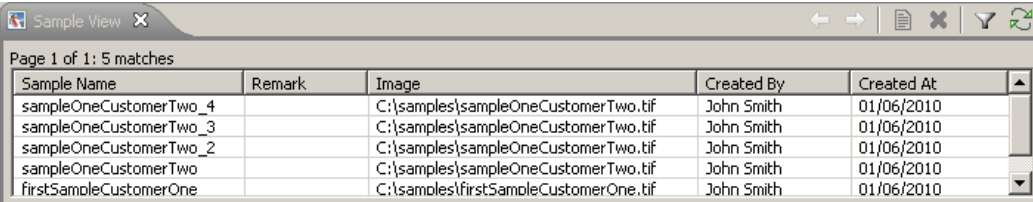
Attention: The deletion of users cannot be undone.

5. Import and manage samples

5.1 Sample View

When logged in as **assistant** or laboratory **head**, the sample view will be shown at the lower part of the application by default. You can also manually open the sample view by the menu item **Window / Sample View**.

In this window all stored samples are listed.



Page 1 of 1: 5 matches

Sample Name	Remark	Image	Created By	Created At
sampleOneCustomerTwo_4		C:\samples\sampleOneCustomerTwo.tif	John Smith	01/06/2010
sampleOneCustomerTwo_3		C:\samples\sampleOneCustomerTwo.tif	John Smith	01/06/2010
sampleOneCustomerTwo_2		C:\samples\sampleOneCustomerTwo.tif	John Smith	01/06/2010
sampleOneCustomerTwo		C:\samples\sampleOneCustomerTwo.tif	John Smith	01/06/2010
firstSampleCustomerOne		C:\samples\firstSampleCustomerOne.tif	John Smith	01/06/2010

Fig. 4 Sample View

By default, the last 100 samples are listed (see filter feature below). For each sample, the following information is displayed:

- **Name:** The unique sample name
- **Remark:** A comment belonging to the sample
- **Image:** The path to the image the sample was created from
- **Created By:** The display name (full name) of the user who created the sample
- **Created At:** The creation date of the sample

All features can be accessed by using the sample view toolbar.



Sample view toolbar

The following actions can be executed:

- **Open Sample (document icon):** Opens the selected sample to show the analysis results or edit sample details
- **Delete sample (cross icon):** Deletes the selected samples
- **Refresh (green arrows icon):** Refreshes the view from the database
- **Sample Filter (funnel icon):** Search samples

You can select one or more samples and open them by clicking the document icon.

In order to show only certain samples in the view, open the filter dialog box (click the toolbar's funnel icon).

Fig. 5 Sample filter

Now you can filter the stored samples by the shown criteria. Use a so called wildcard in the field Sample Name and Sample Number to find samples with entries like the given value. If you look for all samples having the string “Sample” in their name, use the search string “*Sample*”.

Pressing **OK** will activate the filter. If there are no matching samples, the sample list will remain empty. To reset the filter, open the filter dialog box once more, clear all filter criteria fields and press **OK**.

5.2 Import samples

First of all, a sample is represented by an image file, which was created by a fluorescence scanner. This file is now imported to MycoProof **Basidio** and analyzed. The result of the analyzing process will be stored in the database, along with the original image and some meta information.

MycoProof **Basidio** is able to recognize up to four sub arrays on one image. If the image is rotated at 90°, 180° or 270°, it will be automatically rotated back to the right position during the import process.

You can reach the import feature by the menu **File / Import Images** (Shortcut **Strg-I**), or simply use this icon from the toolbar:



Sample Import Icon

Now the import dialog is shown. Select one or more images in TIF-format by pressing the **Browse** button. Then you have to define the resolution of the image data, given as micrometer per pixel. By default, a value of 10 is suggested. This has been proven to be a sufficient resolution and is also supported by the majority of the fluorescence scanners.

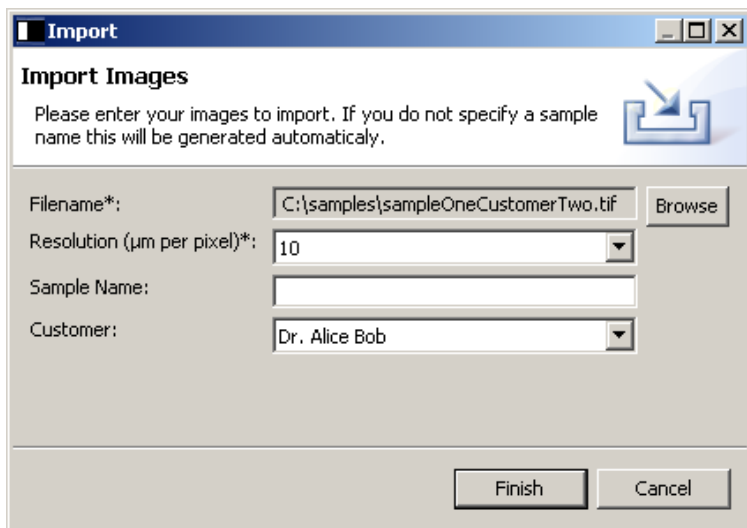


Fig. 6 Import of images

By leaving the field Sample Name blank, the sample name will be generated from the file name of the imported image file, otherwise the given string is taken as the sample base name.

Sample names are unique within the system and cannot be assigned more than once. If there is a collision detected during the import, all subsequent samples will obtain a sample name consisting of the name and a concatenated underscore followed by a

consecutive number (beginning with 2). This strategy is also used if more than one sub arrays are found within one image.

Furthermore, you can select a customer from a list of all customers that were assigned so far. The selected customer is assigned to all imported samples than. Please note that you are also able to assign a customer afterwards.

The imported samples are added to the Sample View during the process of import.

5.3 Open Samples

Use the Sample View to open samples (menu **Window / Sample View**). Select the desired sample from the list and open it by double clicking the sample or by clicking the document icon from the icon bar. Now the so called Sample Editor is opened in the upper part of the application.

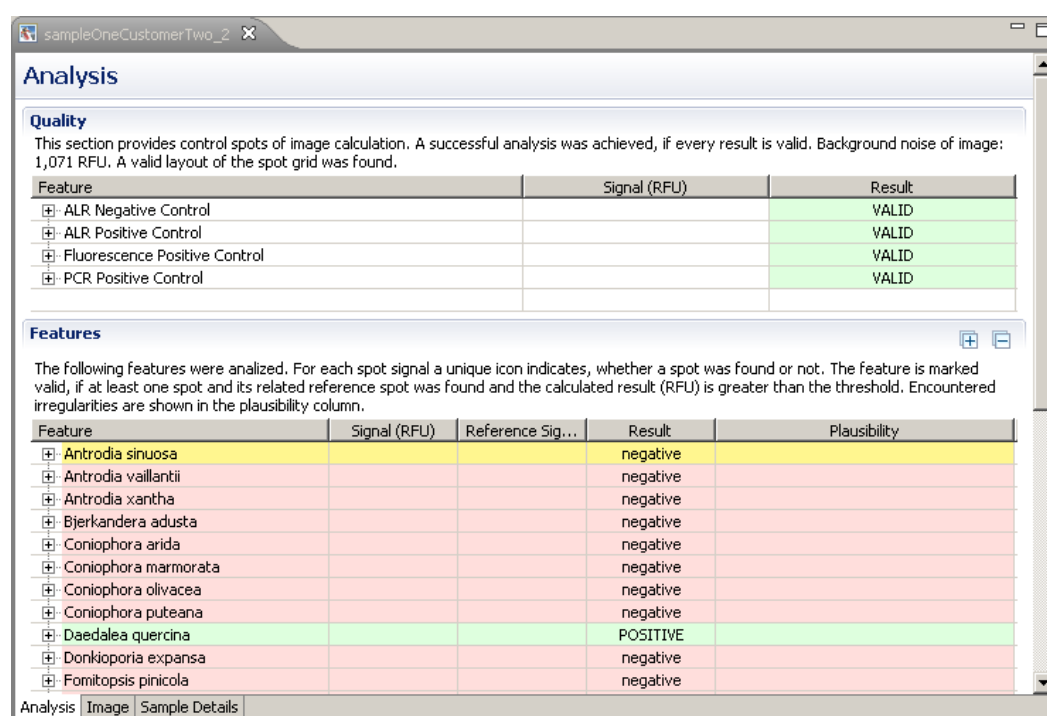


Fig. 7 Sample Editor / Analysis

The editor has three tabs, which are shown in the lower tab bar;

Analysis Image Sample Details

Tabs of the Sample Editor

- **Tab Analysis:** Find all analysis results within this page
- **Tab Image:** The original image of the sample
- **Tab Sample Details:** All meta data belonging to the sample, like sample number, customer or remarks

Click on the tab title to bring the tab page to the foreground.

5.4 Analysis

The analysis page within the Sample Editor is divided into two areas: Results of quality analysis are shown in the upper part, while the results of feature measurements can be found in the lower table.

The details of the analyzed features are structured as a tree, where the parent node displays the feature result summary as VALID resp. invalid. The child nodes represent the results of the specific probes dedicated to the feature.

Child nodes can be expanded resp. collapsed by clicking the plus- resp. minus icon.

5.5 Quality

The quality section shows all quality criteria of Mycotype® **Basidio**^{QS}. Here you can find helpful information covering the quality of the DNA microarray and the efficiency of PCR and ALR as well as the presence of reaction inhibitors.

Quality		
This section provides control spots of image calculation. A successful analysis was achieved, if every result is valid. Background noise of image: 1,071 RFU. A valid layout of the spot grid was found.		
Feature	Signal (RFU)	Result
⊕ ALR Negative Control		VALID
⊕ ALR Positive Control		VALID
⊕ Fluorescence Positive Control		VALID
⊕ PCR Positive Control		VALID

Fig. 8 Quality section

The text at the top of the section offers a description of the background noise of the image in RFU as well as a statement whether a valid spot layout was found or not. In this context valid means that all positive control spots were found and their measured values are greater than the threshold and all negative control spots were not found.

The default view shows the quality features as collapsed tree items, which you can expand by clicking the plus icon beside the feature name. The table features the following columns:

- **Feature:** The name of the quality feature (parent node), resp. the name of the quality probe (child node)
- **Signal (RFU):** The signal intensity of the probes in RFU, which is calculated from the actual probe area (foreground) and the background noise of the intermediate surrounding of the probe (background). The median of the foreground minus the median of the background is displayed in box brackets.
- **Result:** The result is shown as an interpreted statement (VALID / invalid) for the whole feature as well as for the single quality probes. A positive control is valid, if the spot has been found and the signal is greater than 1500 RFU. A negative control is only marked valid, if the spot has not been found. If you are interested in the calculation methods in detail, please have a look at the appendix.

Furthermore, the background colours of the table rows refer to the result:

- **Green:** The feature / the spot is valid
- **Red:** The feature / the spot is invalid

5.6 Results of analyzed features

You can find all analyzed features in the lower part of the Analyze Tab.

Features

The following features were analyzed. For each spot signal a unique icon indicates, whether a spot was found or not. The feature is marked valid, if at least one spot and its related reference spot was found and the calculated result (RFU) is greater than the threshold. Encountered irregularities are shown in the plausibility column.

Feature	Signal (RFU)	Reference Si...	Result	Plausibility
Antrodia sinuosa			negative	
Antrodia vaillantii			negative	
Antrodia xantha			negative	
Bjerkandera adusta			negative	
Coniophora arida			negative	
Coniophora marmorata			negative	
Coniophora olivacea			negative	
Coniophora puteana			negative	
Daedalea quercina			POSITIVE	
DaeQue1a	✓ 1,158 [1,4...	✓ 2,229 [2,5...		1,693
DaeQue2a	✗ 306 [559 - ...	✗ 558 [850 - ...		428
Donkioporia expansa			negative	
Fomitopsis pinicola			negative	
Gloeophyllum abietinum			negative	
Gloeophyllum sepiarium			negative	
Gloeophyllum trabeum			negative	
Laetiporus spp.			negative	
Leucogyrophana mollusca			negative	
Leucogyrophana pinastri			negative	
Neo/Lentinus lepideus			negative	
Oligoporus placenta			negative	
Phellinus ferruginosa			negative	
Pleurotus spp.			negative	
Schizophyllum commune			negative	
Serpula lacrymans			negative	
Serpula himantioides			POSITIVE	
Stereum spp.			negative	
Tapinella panuoides			negative	

Fig. 9 Features section

The text above the section describes the table structure and gives some notes about the result interpretation. By default, all features are shown as collapsed tree nodes, giving an overview about the result of all features (Result column). You can either expand a single feature node by clicking the plus icon, or by the use of the Expand All function, provided by the plus icon in the section title bar. There is a corresponding Collapse All function (minus icon) as well.



Icons providing the actions **Expand / Collapse all**

The feature table provides the following columns:

- **Feature:** Name of the fungi at the parent node, name of the spot at the child node
- **Signal (RFU):** Intensity of signal in RFU and the spot found / spot not found icon. Signal intensity is calculated as the difference between foreground and background. Beside the signal you can find the specific signals of the foreground and background

in box brackets. The symbol icon indicating a spot detection shows a green check mark, the one indicating that no spot was found a red cross.

- **Reference Signal (RFU):** Shows the same information like the column Signal (RFU), but concerning the Reference Spot, which means the duplicate of the spot.
- **Result:** The overall result, given as interpreted statement for the feature (VALID, invalid), and as numerical value for the single spots. Please refer to the appendix for further information about the calculation methods.
- **Plausibility:** If there was one spot found, but no appropriate reference spot, there is a note displayed in this column.

Furthermore, the background of the features is colourized in dependence of their results, which was inspired by the colours of traffic lights:

- **Green:** The feature was found.
- **Yellow:** The feature was found, but the calculated result of at least one involved spot was only within the range from 600 and 1500 RFU. Also, if there is a plausibility issue, the feature is coloured yellow.
- **Red:** The feature was not found.

The particular spots are also colourized:

- **Green:** The spot as well as the reference spot was found, and the calculated result is greater than 1500 RFU.
- **Yellow:** Spot and reference spot were found, but the calculated result is just in the range of 600 and 1500 RFU.
- **Red:** At least one spot was not found, resp. the signal is lower than 600 RFU.

Feature	Signal (RFU)	Reference Signal (RFU)	Result	Plausibility
[-] Antrodia sinuosa			negative	
AntSin1a	✓ 804 [1,1...]	✗ 937 [1,519 - 582]	870	Spot and control spot differ
AntSin2a	✗ 185 [454 ...]	✗ 382 [811 - 429]	283	
[-] Antrodia vaillantii			negative	
AntVai1a	✗ 257 [492 ...]	✗ 481 [827 - 346]	369	
AntVai2a	✗ 87 [361 -...]	✗ 96 [383 - 287]	91	
[+] Antrodia xantha			negative	
[+] Bjerkandera adusta			negative	
[+] Coniophora arida			negative	
[+] Coniophora marmorata			negative	
[+] Coniophora olivacea			negative	
[+] Coniophora puteana			negative	
[-] Daedalea quercina			POSITIVE	
DaeQue1a	✓ 1,158 [1,1...]	✓ 2,229 [2,574 - 345]	1,693	
DaeQue2a	✗ 306 [559 ...]	✗ 558 [850 - 292]	428	
[+] Donkioporia expansa			negative	

Fig. 10 Feature details

5.7 Show image

For displaying the image, on which the analysis is based on, switch to the Image tab of the Sample Editor. The shown image was perhaps cropped and rotated before storing. Cropping means all superfluous margins around the found array were removed. Additionally, the image is shown by the use of a so called lookup table. Thus, an image with original greyscale colours is displayed with rainbow colours and so you can identify spots with lower signal intensity as well.

The title section also states the path on the file system of the source image at the time of import.

Furthermore, the image tab provides a feature for hiding resp. showing the spot raster. Use this icon:



Icon symbolizing Toggle **Spots**

The spot mask draws colourized borders around the found spots. The colourization is again based on the traffic lights principle:

- **Green circle:** The spot was found and the signal is greater than 1500 RFU
- **Yellow circle:** The spot was found, but the signal is just in the range between 600 and 1500 RFU
- **Red circle:** The spot was not found resp. the signal is lower than 600 RFU

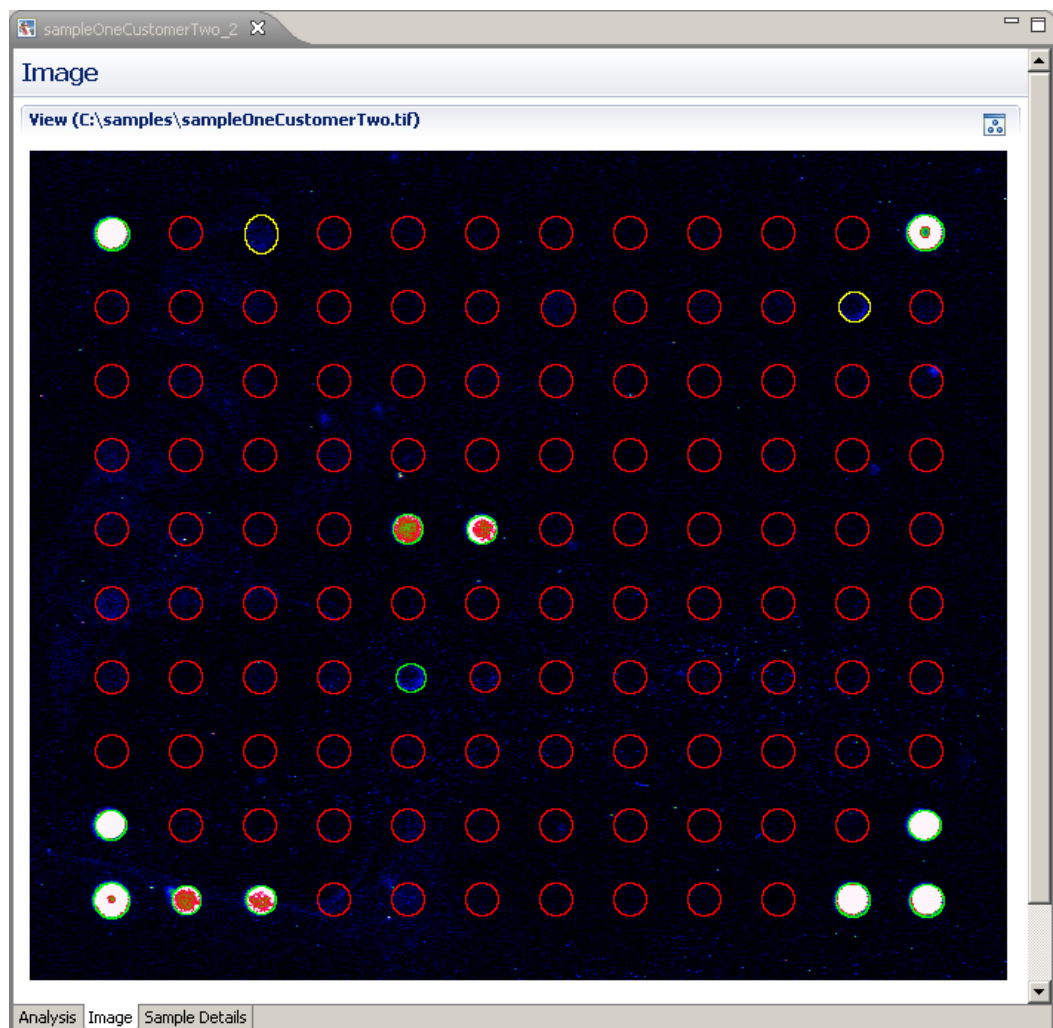


Fig. 11 Sample Editor / Image tab

5.8 Edit sample details

Further information about the sample can be edited at the **Sample Details Tab** of the Sample Editor.

Fig. 12 Sample Details Tab

You can edit all data within the page, except the following with an informative nature:

Section **General**:

- **Sample Name**: This name is unique within the database and cannot be changed due to reasons of auditability
- **Created By, Created At**: The user who created the sample and the time of creation cannot be changed.

Section **Array Design**:

- All information in this section cannot be changed, because it is predefined by the chip manufacturer Biotype Diagnostic GmbH

Section **Client**:

- Either you choose to create a new client and allocate it to the sample (green plus sign icon) , or to edit the assigned client (document icon), or you look for a existing client from the database (magnifier icon)
- Please note that changes of the client will affect all samples which use the same client. Consider to create a new client if you do not want that.
- Changes have to be saved by using the menu item **File / Save** (shortcut **Strg+S**), or by clicking on the floppy icon at the toolbar of the application

Please note that you are only allowed to edit samples which you have created by your own. Other samples cannot be changed due to reasons of data security, unless you belong to the user group of laboratory heads.

5.9 Export samples

The export features are only available with an opened Sample Editor:



Icons providing exports to PDF and CSV

Clicking the left PDF icon will start the export routine, which will generate a PDF document containing all relevant information belonging to the sample. As an alternative, choose menu item **File/Create PDF report** (shortcut Strg+R). The left icon symbolizes the CSV export (menu **File/Export sample to CSV**).

In order to generate export files, you have to select a target file on the file system. After creating a PDF file, MycoProof **Basidio** will try to open your system PDF viewer application and load the exported file.

The CSV file contains all numerical data and its interpretation. It enables you to process the calculated data in any application that can read CSV files, e.g. Microsoft Excel.

Mycotype Basidio^{QS}

Sample:	sampleOneCustomerTwo_2		
Sample ID:	S42-23		
Created at:	01/06/2010		
Created by:	John Smith The Smiths Labs Schmidtstraße 1 12345 Schmidhausen	Phone:	012345678910
		Fax:	012345678911
		E-Mail:	john@thesmiths.com
Created for:	Dr. Alice Bob	Company/Institute:	Sample Labs GmbH
Software:	Mycoproof Basidio 1.0		
Positive Findings:	Daedalea quercina, Serpula himantoides		

Quality Report	Signal (RFU)	Result
ALR Positive Control	85184, 85238, 85251	VALID
ALR Negative Control	164(Spot Not Found), 172(Spot Not Found)	VALID
PCR Positive Control	85237, 85129	VALID
Fluorescence Control	85112, 84909	VALID
Noise Level	1071	

Image

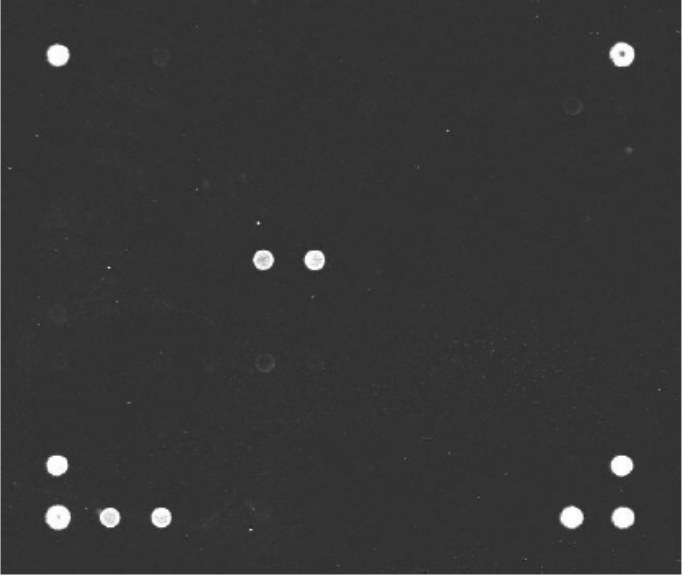


Fig. 13 Cover sheet of an exported PDF file

6. Import calibration slide

Biotype Diagnostic GmbH provides a calibration slide. By the help of that slide, the fluorescence scanners of various manufacturers can be calibrated to a defined sensitivity. To this end, scan the slide, and select the menu item **File / Show signal intensity of calibration result** (shortcut Strg+K). Now you can select your scanned image file by clicking the button Browse.

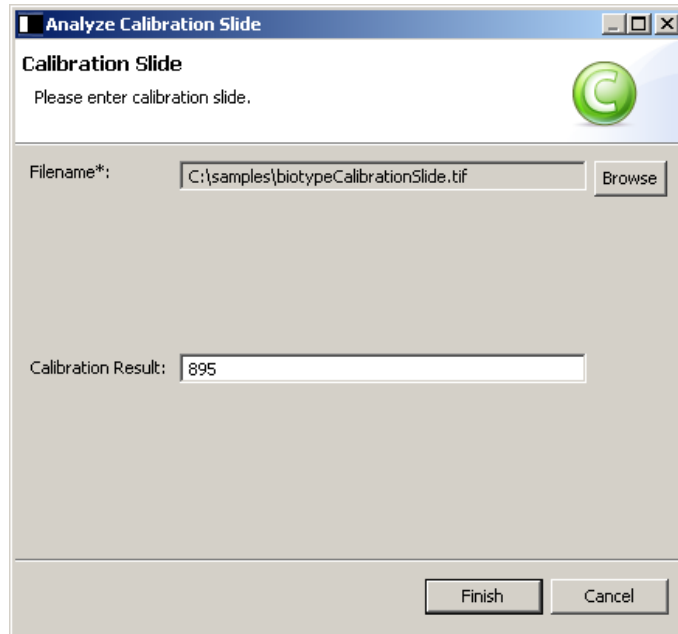


Fig. 14 Analyze Calibration Slide

Use the numerical value shown in the field **Calibration Result** to calibrate your fluorescence scanner. Close the dialog box by the Button **Finish**.

7. Glossary

ALR:

The Arrayed Ligation Reaction is a specific microarray detection technology. A fluorescence labeled ligation oligonucleotide is coupled to an immobilized DNA probe via a thermophilic ligase, if both the ligation oligonucleotide and the DNA probe are complementary to the PCR amplicon.

Button:

Clicking on a button will perform a particular operation.

CSV:

Abbreviation for 'comma-separated values'. A data format saving data of simple structure in a text file (.txt). The advantage of CSV files is their small size.

Drop-down list:

A control element offering several options, of which one can choose one. Usually, the list displays only one element (the one currently selected). Clicking on the drop-down menu extends the field to a list showing further elements. The desired element can then be chosen with the mouse pointer or the arrow keys.

Editor:

A window providing detailed information about an item, e.g. editing windows (sample windows, image windows, windows presenting the results of calculation).

Menu Bar:

A bar in the upper part of the main window. It provides access to different menus. The menus in turn contain functions. Typical menus of a menu bar are, for instance, 'File' and 'Edit'.

PDF:

A data format allowing to display documents in a fixed layout (font type, page breaks, etc.) regardless of the software applied.

PCR:

The Polymerase Chain Reaction is an enzyme driven amplification of a well defined DNA fragment.

RFU:

The Relative Fluorescence Unit is the scale unit of the fluorescence intensity.

Spot:

A spot is a circular area on a planar microarray surface that comprises a multitude of DNA probe molecules. It is possible to immobilize multiple spots of a certain probe type on one microarray.

Toolbar:

A bar comprising several buttons. Toolbars are part of many windows and view. They provide functions for the referring context. Most toolbars can be shifted at will, however, when you open a new window they are, by default, located in the top part.

View:

Specialized windows of MycoProof **Basidio** providing overviews for specific element types including some basic information about them as well as functions to work with the elements.

8. Supplement

8.1 Calculation of spot signal intensity

The signal intensity of a spot is calculated from the restrained spot area and the background noise of the surrounding area of the spot. The surrounding area is defined as a square of 500 x 500 μm , which is located at the center of the ideal position of the spot. The spot signal intensity is derived from the median signal intensity of all foreground pixels minus the median signal intensity of all background pixels. The signal intensity is given as RFU value.

A spot is found when an approximately circular area of 17 to 33 square pixels (at a resolution of 10 μm /pixel) was found in the spot grid square and the signal of this spot (foreground - background) is higher than 600 RFU. The foreground is the median value of all pixels in this approximately circular spot area. The background is the median of all pixels in the spot grid square minus the pixels of the foreground.

8.2 Calculation of quality sensors

The analysis results of both the feature and the single spots belonging to a certain quality sensor are shown as a deduced declaration (VALID/ invalid). A spot of a positive control is valid if the probe was found and the spot signal is above 1500 RFU. A spot of the negative control is valid, if the spot has not been found. A quality feature is valid, if all associated spots are valid. Otherwise, the feature is invalid.

8.3 Calculation of fungi features

In MycoProof **Basidio** the features represent the dry rot fungi that are detectable with Mycotype **Basidio**^{QS}. The result of each feature is shown as a deduced declaration (POSITIVE/negative). The calculation of each feature (e. g. *Antrodia sinuosa*) is depended on two different DNA probes (e. g. AntSin1a, AntSin2a) that are present as duplicates (spot/reference spot). Thus, four spots are included in the calculation of a single feature.

The result of a feature is positive, if at least one DNA probe (e. g. AntSin1a) has been found completely (spot and reference spot) and the final fluorescence value is above 1500 RFU. Otherwise, the feature is negative. The final value of a DNA probe is the average fluorescence intensity of the two corresponding spots (signal/reference signal). In some cases a fixed signal percentage of an associated feature is subtracted from another feature signal to ensure a clear specificity. The following probes are offset:

- **AntVai1a** minus 1 % of the average value from SerLac2a and SerLac2b, if ConMar6a > 1000 RFU and SerLac > 2000 RFU
- **ConOli7a** minus 6 % of the average value from ConMar4a and ConMar6a
- **DaeQue2a** minus 6 % of the average value from AntXan1a and AntXan2a
- **GloSep1a** minus 10 % of the average value from AntSin1a and AntSin2a
- **LeuPin2a** minus 3 % of the average value from PleSpp1a and PleSpp2a
- **PleSpp1a** minus 2 % of the average value from AntXan1a and AntXan2a
- **SteSpp1a** minus 4 % of the average value from PleSpp1a and PleSpp2a
- **SteSpp1b** minus 5 % of the average value from FomPin4a and FomPin5a
- **TapPan2b** minus 4 % of the average value from LaeSpp1a and LaeSpp2a minus 2.5 % of the average value from PleSpp1a and PleSpp2a

MycoProof Basidio User Manual

Software Version: 1.0
Powered by: Qualitype AG
Date: 2009-12

Notes

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