
Huygens Remote Manager



User Guide for HRM version 1.2.x



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Huygens Remote Manager

In this manual the Huygens Remote Manager (HRM) is presented. HRM is a collaborative open-source interface to Huygens Core that allows scheduled multiuser deconvolution through a web server.

For deconvolution details please refer to “Deconvolution jobs in HRM” on page 13 or to other Huygens Guides. In order to run deconvolution jobs, HRM will request from the user a number of microscope and restoration parameters. For more specific information on these parameters, visit the online SVI-wiki¹ or follow the HRM help.

This chapter is an overview of the interface possibilities of HRM, included in this guide as a quick reference. Being a collaborative project, the HRM capabilities may change and expand very quickly. It is advisable to follow the HRM online help.

What is HRM

The Huygens Remote Manager (HRM) is a web task manager for servers that acts as an interface to Huygens Core to do multiuser batch deconvolution.

Huygens Essential and Huygens Professional have their own integrated Batch Processor which is more intended for single-user deconvolution. Multiple users may run simultaneous sessions of the Batch Processor but the multiple processors will compete for the same hardware resources, likely resulting in a slowdown.

HRM, however, has a queuing system intended for multiple users. Different users have their own accounts in a web server and place deconvolution jobs in the queue. HRM runs all jobs listed in the queue setting priorities across them and alternating over all users. A quota system for improved queue management can easily be implemented, and will probably be included in future versions of HRM.

HRM is flexible enough to control different computation servers and split the jobs among them, allowing centralized administration of the deconvolution jobs in a cluster. The HRM queue manager runs in background in Unix-like systems (Linux and Mac OS

1. <http://support.svi.nl/wiki/MicroscopicParameters>
<http://support.svi.nl/wiki/RestorationParameters>

X). HRM is not the only way to use Huygens Core, which is available for Linux, Mac OS X and Windows. Because HRM is an open source project, it can be freely modified and the code of HRM reused to adapt it to any particular needs. Other completely different interfaces that communicate with Huygens Core to use the Huygens restoration, visualization and analysis algorithms could be developed using the Huygens Core Programmer Guide ².

Huygens Core works by default without graphical interface and is designed to work seamlessly with HRM. It will include more and more features especially intended for web interfaces such as HRM, or any other alternative interface.

Where to find HRM

The open source HRM is developed by Huygens users at the Montpellier Rio Imaging facility, the Facility for Advanced Imaging and Microscopy at the Friedrich Miescher Institute (FMI, Basel), and the BioImaging and Optics Platform at the Ecole Polytechnique Fédérale de Lausanne. Scientific Volume Imaging participates in this project by contributing its experience in deconvolution and software engineering. HRM is a free and open source project, and can be found in SourceForge ³.

More information about HRM and links to other HRM resources can be found in the HRM online article in the SVI-wiki⁴. Instructions for online testing, downloading and installing the HRM code are also linked on that page.

On a running regular web server installing HRM is not very difficult. Apart from the installation instructions that come along with the source code, other practical guidelines based on other users' experiences can be found in the SVI-wiki ⁵.

HRM requirements and technical features

HRM consists of two main components: a web based interface and a queue manager. The web interface allows:

- the management of different users by the system administrator;
- the management of template settings that all users can copy or use directly;
- the creation of deconvolution jobs by the users, including image selection, setting of microscope parameters, and setting of restoration parameters;
- inspecting the job queue status, and deleting the user's own jobs from it.

HRM is equipped with a simple file uploader/downloader to send the raw images from the user's local machine to the HRM server, as well as to retrieve the deconvolution results from the server. The server administrator can set up the largest file size allowed in these transactions.

The jobs created by the users via the web interface are dispatched by the queue manager of HRM, which runs in the background, to any of the dedicated servers running Huygens Core. When the job is finished, an e-mail informs the user that the restored datasets are available.

2. An on-line version is available <http://www.svi.nl/HuCoreMan>

3. <http://sourceforge.net/hrm>

4. <http://support.svi.nl/wiki/HuygensRemoteManager>

5. <http://support.svi.nl/wiki/HrmInstallation>

More specifically, HRM requires a web server with PHP and e-mail capabilities, a database backend to store deconvolution parameters, job descriptions and, optionally, user accounts, a file server to temporarily store input and restored datasets, and one or more processing servers running Huygens Core.

The setup is highly configurable the file server, the processing servers and the queue manager can either be all hosted by the same machine or be distributed across two, three or more computers.

How HRM communicates with Huygens Core

For each deconvolution task in the job queue the HRM queue manager automatically generates a Tcl script that

- loads the raw image from a source directory,
- applies the microscopic parameters settings to it as defined by the user in a template,
- optionally loads another image containing the microscope Point Spread Function,
- deconvolves the image using the restoration settings chosen by the user,
- stores the resulting restored image in a destination directory,
- and finally writes a tag in that destination directory to let the HRM queue manager know that the job is finished.

When the job is finished the queue manager optionally sends the user an e-mail announcing the end of the job and its status. This is handled by HRM itself and not by the above mentioned Tcl script.

Multiple jobs can be processed in parallel depending on how HRM is configured, the multiprocessing capabilities of the server and the number of available computation servers.

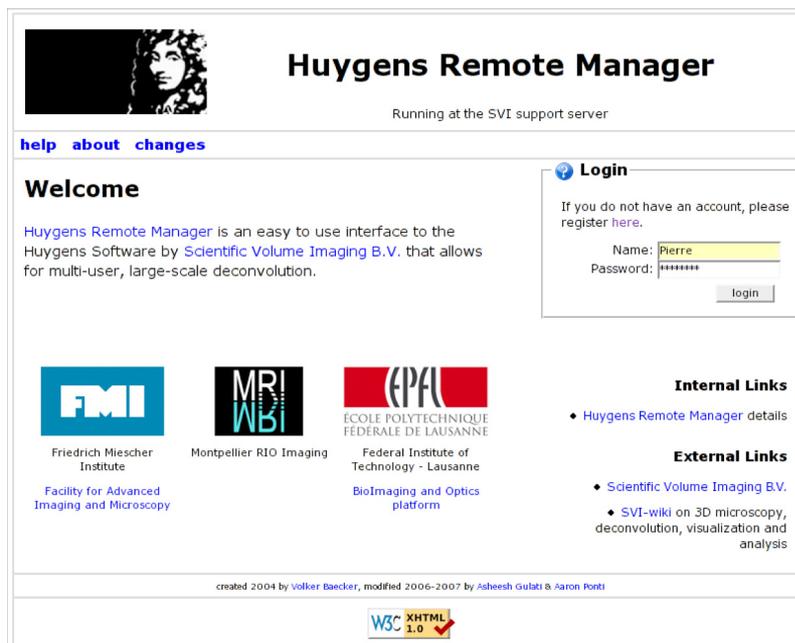


FIGURE 1.1. The login page of the Huygens Remote Manager. Users with an active account can login, new users can apply for an account.

Basic HRM usage

In this section a summary of the basic HRM work flow is described. Help links are accessible in HRM at each stage of the creation of deconvolution jobs. For more detailed explanations see “Deconvolution jobs in HRM” on page 13.

Registration and login

To become an HRM user one needs to apply for an account in the system. This application can be sent directly via a registration form available in HRM. The application will reach the HRM administrator who will likely grant the user further access to HRM. When filling out the registration form, all entries but the “Request message” field are mandatory. The “Request message” field in the registration form lets the applicant send the HRM administrator a message along with the application.

An e-mail will inform the user as soon as the account has been activated or rejected. Upon account activation, the user can login with the chosen user name and password. For security reasons the password is not shown while the user types. Upon misspelling a name or a password a message will appear stating that the account does not exist. Be aware that the name and the password are case sensitive, i.e. “pierre” and “Pierre” are different names.

User management

This area is enabled for the HRM administrator only. It allows to perform maintenance on the user database. It contains, for instance, a “registrations” area that lists the pending account requests to be either approved or rejected by the administrator.

The existing users are grouped by the initial letter of their user name. This allows the administrator to filter users for further administrative processing, such as edition, deletion, rejection, etc. Alternatively all users can be shown at once.



FIGURE 1.2. The HRM User Management Page for the administrator. Active users and new applications can be easily managed from this panel.

New users can be created by the administrator by clicking on “add new user”. A user added by the administrator automatically gets access to HRM granted.

Clicking on the “distribution list” link allows the administrator to send an e-mail to all registered users.

Each user can be edited, enabled/disabled, or even deleted by the administrator by clicking on the corresponding buttons next to the user name.

Additionally, the possibility exists to enable or disable all users at once, when seeing the unfiltered list of all users.

Furthermore, the User Management is optional in HRM. In case the administrator chooses other authentication methods the User Management is handled outside HRM.

Job settings

The HRM parameter settings are divided into two groups: those describing the image (settings of the microscope) and those describing the restoration process (deconvolution settings). Both are saved in the HRM database.

The HRM settings can be created by the administrator so that they are available across the system, to be used by all users, or can be created individually by each user, either from scratch or based on the settings created by the administrator. The settings created by users are visible only in their account and are not visible across the system by other users. A setting is saved with a name and can be reused later.

Setting both image parameters and restoration parameters is carried out similarly in HRM. For detailed explanations on the meaning of these parameters and how to determine their values please refer to “Deconvolution jobs in HRM” on page 13 or to the HRM online help for further details.

Notice again that every stage in HRM comes along with help links with plenty of further information. There are many image and restoration parameters, and understanding them properly is important to achieve good results.

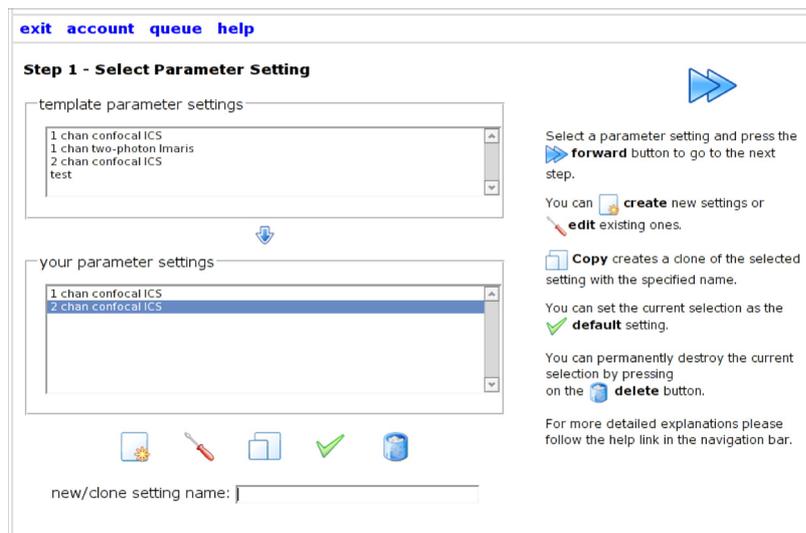


FIGURE 1.3. Setting selection in HRM. The top pane lists the setting templates created by the administrator for all users. The bottom pane lists settings only available for the current user.

Using an existing setting

Notice that there are two different setting panes on the setting screens. The settings in the bottom pane “your parameter settings” can be modified by the user. The settings template created by the administrator (top pane) can be copied into the bottom pane. Once at the bottom pane the settings can be further worked out to meet the needs of the user. Settings can also be created from scratch if that is more suitable to a particular user.

To copy any of the administrator setting templates from the top pane the template has to be selected and it then has to be transferred to “your parameter settings” (bottom pane) by clicking on the blue down arrow.

The bottom pane “your parameter settings” will list the settings among which the user can choose the most suitable for his or her deconvolution jobs. The selected setting will be highlighted in the list. Press the forward icon to continue to the next step.

Creating a new setting

First enter the name of the new setting. The name must be different from the names of already existing settings. Besides this restriction any name can be used. It is best to choose a name that helps remember what the setting is made for, e.g. it is better to use the name of the used microscope than to use a family name.

Press the CREATE button. Several pages where to enter different sets of parameters will be presented. The number of pages where to set these parameters is 3 or 4, depending on whether corrections for spherical aberration and selection of a measured PSF are chosen. The option to save the setting will eventually show at the last page.

Copying a setting

In order to create a setting that is similar to an already existing setting:

- Select from the bottom pane the setting to be copied. The selected setting will be highlighted.
- Enter a name in the field "new/clone setting name".
- Press the COPY button.

The new setting will be shown in the bottom pane list. It will contain the same parameter values of the copied setting. The new setting can now be edited to modify any of its parameters.

Editing a setting

Select the setting to be edited from the bottom pane list. The selected setting will be highlighted. Then press the EDIT button.

When editing a setting the user is led through the same steps as when creating a new setting. If there are steps where no parameters need to be changed one can press the forward button to continue to the next step. After saving the setting the user will be redirected again to the "select parameter setting" page where all the available settings are listed.

Making a setting default

When using a particular setting very frequently the user might want to set it as default. The default setting will be automatically preselected the next time the user logs in HRM.

To make a setting default, select the setting from the bottom pane list. The selected setting will be highlighted. Press the DEFAULT button.

The name of the default setting will be highlighted in a different color when it is not selected.

Deleting a setting

To delete a setting select the setting from the bottom pane list. The selected setting will be highlighted. Press the DELETE button.

Be careful: the setting will be deleted immediately without any further questions. Once it is deleted its name will disappear from the list. A deleted setting can not be restored any more. If a setting was deleted accidentally the only way to get it back is to create a new setting and enter all values again. If this setting had been used before, the user can look for the parameter values in the summary e-mail that HRM sent when the job was finished.

Selecting the images

At this stage the images to be processed can be selected. The "available images on server" area lists all images on the user account that match the file type of the current parameter settings. If no images are listed on this area it is quite likely that there are no images of the selected type in the user account.

SHIFT+CLICK and CONTROL+CLICK can be used to select multiple images. Press the down arrow button to add the images to the "selected images" area. Images can be removed from the selection in the same way using the up arrow button.

If more images are added to the file server after reaching the image selection stage, they will not be shown until the UPDATE VIEW button is pressed so that HRM can rescan the user account and refresh the file list.

If the image setting involved in a particular deconvolution job states that an experimental Point Spread Function (PSF) is to be used in the computation, HRM will ask for the corresponding PSF file per channel. Thus, keep in mind that multichannel raw images require a PSF file per channel for deconvolution. For more information about the PSF, see the SVI-wiki⁶.

If an image template is selected for the deconvolution of an image with more channels than what the template specifies, HRM will use theoretical PSFs for the remaining channels, up to the number of channels of the raw image to be deconvolved. Be aware that HRM does not warn about this situation.

The PSFs must be always saved in ICS format to store all the metadata necessary for Huygens Core to do the deconvolution.

The Huygens software can read plenty of file formats frequently used in fluorescence microscopy, but HRM may not be fully adapted to make use of some of these formats.

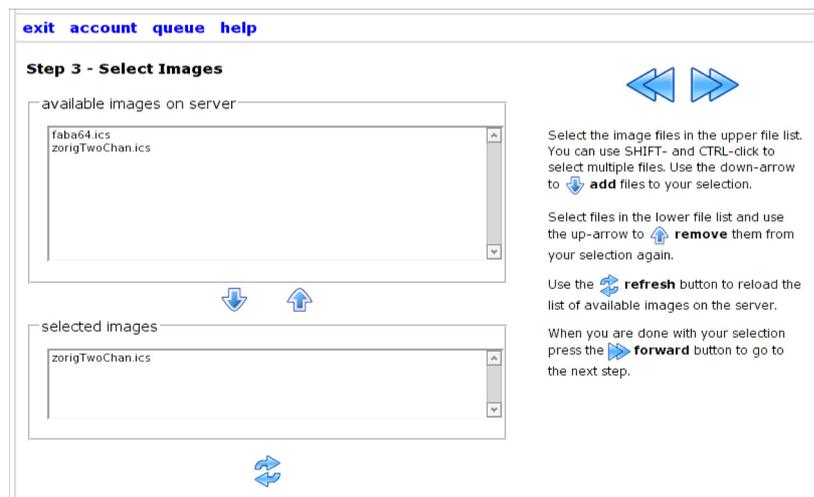


FIGURE 1.4. Image file selection in HRM. Only images matching the currently selected settings stored in the user account are shown for selection.

For a full list of file formats supported by Huygens Core please see the most recent on-line list⁷.

Create the job

This is the last “Start a job” page, where HRM needs to be told which output file format to use for the deconvolved images and where the deconvolution job is launched.

If the user would like to change any settings before creating the deconvolution job it is recommended to use the links “image parameters” or “restoration parameters” displayed

6. <http://support.svi.nl/wiki/PointSpreadFunction>

7. <http://support.svi.nl/wiki/FileFormats>

on this page. They will take the user back to the corresponding setting selection page, where different settings can be selected or the current one be again edited.

Likewise, in order to change the images on which to run the deconvolution job one can click on the “selected images” link. This shows back the “Select images” page where the image selection can be changed.

To create the job at this point simply click on the plug icon. This will launch the job and will show again the HRM home page. The progress of the queued jobs can be checked by clicking on the “queue” link at the top of the home page.

HRM sends the user a confirmation e-mail when the deconvolution job is finished. The result images are stored in the “Results” area of the user’s HRM account.

The deconvolution results can be checked out and downloaded using HRM. From the HRM home page, click on the File Manager button and then on the “Results” link on the top menu. There a list of deconvolved images is displayed. The images can be selected to check them out, if requested, to download them to a local machine. Upon clicking on the blue down arrow the selected images will be packed in a single tar file and then downloaded by an Internet browser.

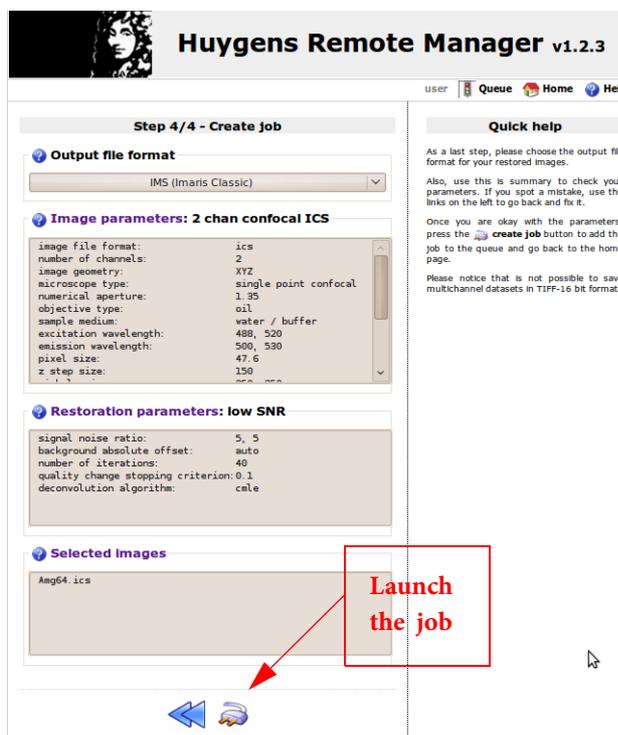


FIGURE 1.5. Reviewing the settings and creating an HRM job. The file type for the deconvolved image must be selected at this point. Clicking on the plug icon launches the deconvolution job.



Huygens Remote Manager

Running at the SVI support server

[exit](#) [results](#) [help](#)

Job Queue Status

You can delete queued jobs owned by yourself. 

 Tuesday 12 February 2008, 08:31:21

nr	owner	file(s)	created	status	started	pid	server
1	guest	zorigTwoChan.ics	2008-02-12 08:30:21.730618	started	2008-02-12 08:30:44.585136	8802	localhost
<input type="checkbox"/>	2	guest	faba64.ics	2008-02-12 08:31:11.004877	queued		
<input type="checkbox"/>	3	guest	faba64.ics	2008-02-12 08:31:12.637514	queued		
<input type="checkbox"/>	4	guest	zorigTwoChan.ics	2008-02-12 08:31:12.640824	queued		
<input type="checkbox"/>	5	guest	zorigTwoChan.ics	2008-02-12 08:31:12.73464	queued		

Check All / Uncheck All With selected: 

created 2004 by Volker Baecker, modified 2006-2007 by Asheesh Gulati & Aaron Ponti



FIGURE 1.6. The HRM job queue. All the queued jobs are visible to all users, but only the jobs owned by the current user can be deleted.

A more detailed explanation on how to create and launch deconvolution jobs in HRM is included in this chapter. Other related tasks such as how to check out deconvolution results and how to access statistics with HRM are also explained here.

A number of HRM screenshots are shown explaining the job launching process step by step. In order to reproduce these steps it is useful to visit an HRM site, such as that of an imaging facility that offers HRM usage.

If one does not yet have an HRM account at an imaging facility, one can still request an HRM account by sending an application to the corresponding administrator. This task can be performed from HRM as highlighted in Figure 2.1. Upon clicking on the register link, the user is asked to fill out a short form that will be forwarded to the administrator, who can then grant further access to HRM.

If the imaging facility of interest does not provide access to an HRM installation one can still get familiar with HRM by requesting an account for the HRM demo server¹.

With an HRM account deconvolution jobs can be run in batch mode, with just a few clicks and, typically from any computer within the intranet of the facility that hosts HRM.

1. <http://www.svi.nl/hrm>



FIGURE 2.1. The HRM login page. Hihglighted the link leading to the application form for new accounts.

The starting page

Once logged in HRM, its starting page is displayed, as shown in Figure 2.2. In the starting page, the user has access to several shortcuts that manage deconvolution jobs as well as other HRM administrative tasks. These shortcuts are:

- *Start a job*: Start a new deconvolution or a batch of deconvolutions.
- *File Manager*: Upload, download and view raw and deconvolved data.
- *Your account*: View and change logging data.
- *Queue status*: See all jobs, manage owned jobs.
- *Your statistics*: Summary of job statistics.

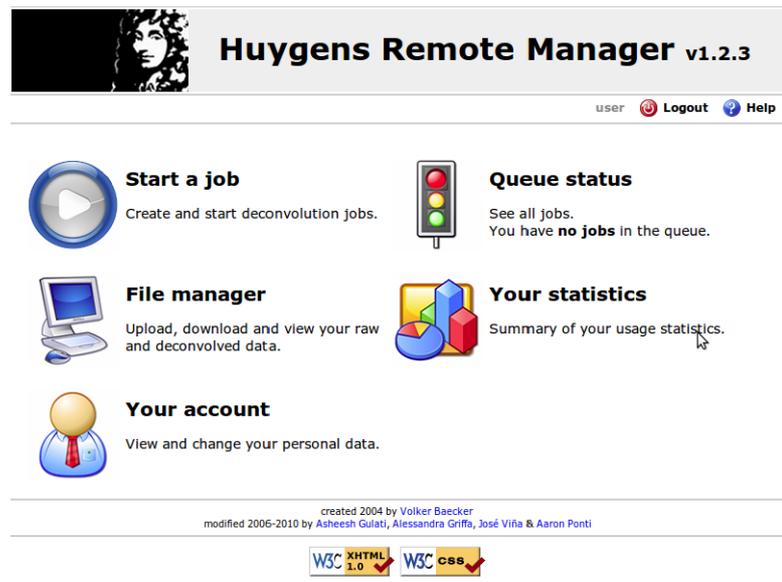


FIGURE 2.2. The HRM starting page. All HRM processes can be handled from this panel.

Start a Job

To start a new deconvolution one has to click on the *Start a job* icon and complete a number of preliminary steps. As will be explained later in more detail, this procedure is split into 4 main steps:

1. The Image Parameter Settings (settings of the microscope).
2. The Restoration Parameter Settings (settings of the deconvolution algorithm).
3. Selection of images to deconvolve.
4. Selection of the output file format and job launching.

The Image Parameter Settings

In *Start a job - Image Parameter Settings* the image settings can be created and saved, which allows the user to run very straightforward deconvolutions on images that have been taken under the conditions stated therein.

The *Start a job - Image Parameter Settings* page looks like Figure 2.3. On this page there are 3 main areas:

- *Template image parameters:* Pre-settings set up by the HRM administrator upon which more complete settings can be built. The HRM users can then work out those templates to create templates that fully meet their needs.
- *Your image parameters:* The settings to be really used for deconvolutions. These may be based on any of the templates existing in the pre-settings or may be specifically made by the user in order to create customized settings.
- *New/clone setting name:* Field to write the name of a new setting. It is advised to write clear, easy-to-understand names for these settings. By clicking later on the “add” button (first button) a new setting with the specified name is created.

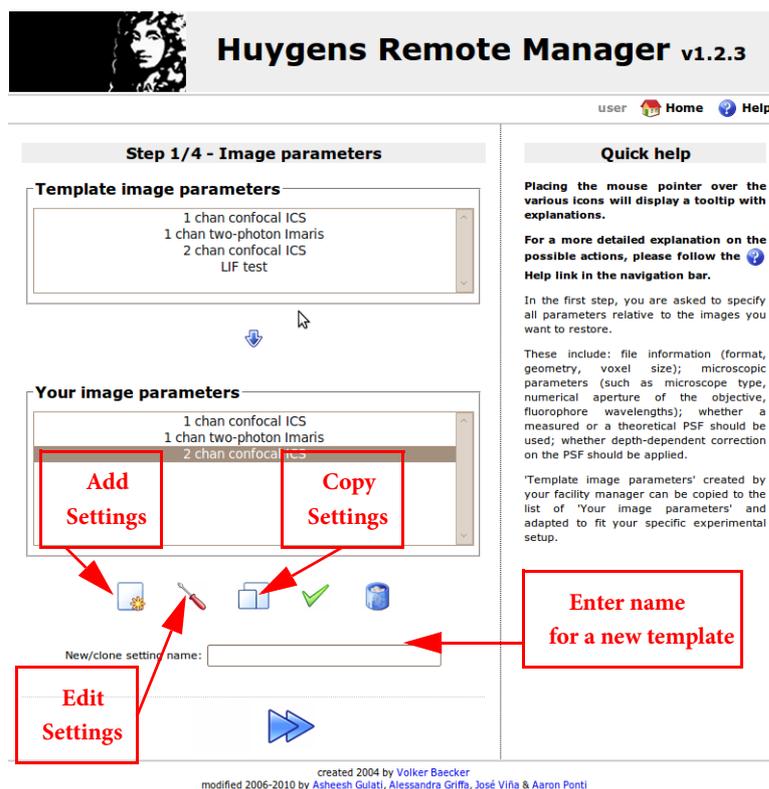


FIGURE 2.3. The Image Parameter Settings Page. At the end of this step a microscope setting is selected.

Notice that when the mouse hovers over any of the icons under “Your image parameters” a short explanation shows up informing about what can be done by clicking on these icons: add a new setting, edit a setting, copy a setting, make a setting default, delete a setting.

An image parameter setting consists of a number of relevant microscope parameters. We can see the microscope parameters in a particular setting by, for example, editing one of the settings listed in the pane “Your image parameters”. Some of these parameters might need a bit of fine-tuning to fully meet the needs of the user’s images. After a setting has been edited and modified, it can be saved and used with similar images in future deconvolution jobs.

Note that all the parameters of a setting are displayed along with links to the SVI-wiki where detailed explanations on the parameters and how to estimate them are provided. These can be reached by clicking on any of the question marks shown throughout HRM.

The image or microscope setting tries to answer the question of what kind of images are to be deconvolved. To get an accurate answer to this question the user will be asked about the following information (See Figure 2.4).

- *Image format:* The format of the image file is directly linked to the acquisition system of the microscope (LEICA, ZEISS, Tiff series, etc).

Both HRM and the engine on which it relies, Huygens Core, use **naming standards**, for instance, the LEICA standard. When handling exotic **Tif** file names, such as the Tif exports from PerkinElmer UltraView, it is recommended to rename the files

before proceeding with the deconvolutions jobs. There are freeware tools available to change file names in batch mode².

A few examples of different Tif standards include:

- *Olympus FluoView* (*.tif): This is a particular Tif format (FM multi-layer) that can store multiple 2D planes in a 3D stack (single file).
- *Leica series* (*.tif): Series of 2D images, with Leica Standard naming. Below, two examples of this standard:
 - ExampleXYZ-time, single channel: name_t00_z000.tif.
 - ExampleXYZ-time, multiple channels: name_t00_z000_ch00.tif.
- *Numbered series* (*.tif): Sequentially numbered series of 2D Tif images. The series is interpreted as a 3D z-stack (no time-lapse, single channel).
- *Single XY plane* (*.tif): 2D Tif images are considered separately. Use this option when running 2D deconvolution on single Tif images.
- *Image geometry*: Whether the image consists of 3D stacks, time-series or 4D stacks (3D plus time).
- *Number of channels*: Number of “fluorescent” channels in the image. Note that no transmission channels can be deconvolved!
- *PSF*: In order to calculate deconvolutions the Huygens software can previously compute a theoretical Point Spread Function, PSF, from the image parameters or it can use a measured PSF instead. In the latter case, HRM asks for a file containing the measured PSF. In most cases the theoretical PSF works fine.

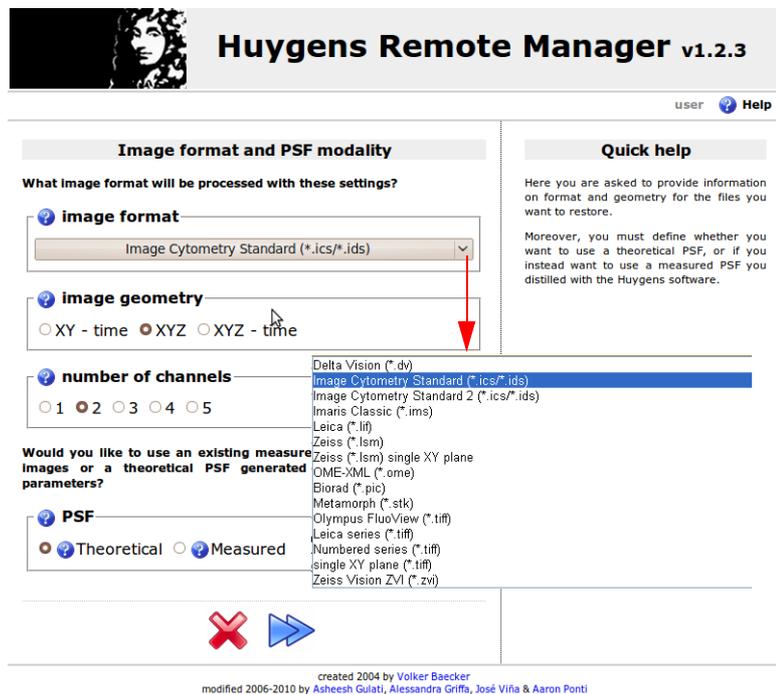


FIGURE 2.4. Image Parameter Settings. What kind of images will be deconvolved?

2. <http://www.snapfiles.com/get/denrenamer.html>

There are a few more questions for HRM to figure how the microscope was set up. Figure 2.5 shows a screenshot with some questions on optical parameters as they are presented in HRM.

The screenshot shows the 'Optical parameters / 1' section of the Huygens Remote Manager v1.2.3. The interface includes a 'Quick help' sidebar on the right. The main content area is titled 'How did you set up your microscope?' and contains several sections with radio buttons and input fields:

- microscope type:** Radio buttons for 'multipoint confocal (spinning disk)', 'single point confocal' (selected), 'two photon', and 'widefield'.
- numerical aperture:** A text input field for 'NA' with the value '1.35'.
- wavelengths:** Two sections: 'excitation (nm)' with 'Ch0' (488) and 'Ch1' (520) input fields; and 'emission (nm)' with 'Ch0' (500) and 'Ch1' (530) input fields. Two red arrows point to the 'Ch0' and 'Ch1' input fields in the excitation section.
- objective type:** Radio buttons for 'air', 'glycerol', 'oil' (selected), and 'water'.
- sample medium:** Radio buttons for 'liquid vectashield / 90-10 (v:v) glycerol - PBS ph 7.4 [1.47]', 'water / buffer [1.339]' (selected), and an empty input field.

At the bottom of the form are navigation arrows: a blue left arrow, a red 'X' button, and a blue right arrow. A footer note reads 'created 2004 by Volker Bescher'.

FIGURE 2.5. Image Parameter Settings. How was the microscope set up?

- *Microscope type:* In the microscope type section HRM asks whether a Confocal, Widefield or Multiphoton microscope was used to take the images. If a confocal microscope was used it is necessary to report which type, whether Spinning disk or Single point confocal.
- *Numerical aperture:* The numerical aperture describes the amount of light coming from the focus that the objective can collect. It depends on the half angle of the maximum cone of light that can enter or exit the lens. It is directly linked to the resolution of the objective. The numerical aperture is recorded on the objective, right next to the magnification.
- *Wavelengths:* Excitation and emission wavelengths of each channel. For the emission wavelength the central value of the emission spectrum of the fluorophore can be considered. Make sure to insert these values in the same order as they were acquired.
- *Objective type:* Dry or immersion objective (oil, water, glycerol).
- *Sample medium:* The refractive index is a property of the medium. Therefore the refractive indexes of glycerol, polyvinyl alcohol, vectashield and other media are different.

The voxel size is also an important parameter for the deconvolution of microscopic images. In order to set it appropriately there are three different cases to distinguish, depending on the type of microscope.

- *Voxel size:* Widefield microscopy

On widefield images, the xy pixel size depends on the physical size of the CCD camera element, the objective magnification, the binning, and the eventual magnification factors introduced by the microscope tube and the c-mount.

In the frequent case in which the tube factor and the c-mount factor are equal to 1, the xy pixel size is given by:

$$xy_{pixel} = \frac{CCD_{pixel}}{Obj_{magnification}}$$

If a pixel binning was used, it is necessary to take this into account when calculating the pixel size. To compute the XY pixel size one can use the “Calculate from CCD pixel size” link available at the image parameter settings page. Notice that the z-step value can be found in the metadata of the image.

Make sure to set a voxel size consistent with the optical resolution of the system with which the images were taken. The ideal voxel size can be found for the current acquisition settings highlighted on the “Quick help” column (See Figure 2.6) which is presented on the right side of the page while setting these image parameters.

Note that if the images are undersampled the results of deconvolutions can be unsatisfactory and show artifacts.

Huygens Remote Manager v1.2.3

user ? Help

Optical parameters / 2

How were these images captured?

• **voxel size**

- xy pixel size (nm):
- size of the z-step (nm):

[On-line Nyquist rate and PSF calculator](#)

• **backprojected pinhole radius**

- pinhole radius (nm):

Ch0 Ch1

[Backprojected pinhole calculator](#)

Quick help

Here you have to enter the voxel size as it was set during the image acquisition. Remember that the closer the acquisition sampling is to the Nyquist **ideal sampling rate**, the better both the input and the deconvolved images will be!

With current optical parameters, the ideal pixel size is **45 nm** and the ideal z-step is **147 nm**.

The Huygens Remote Manager will not try to stop you from running a deconvolution on undersampled data (i.e. with a sampling rate much larger than the ideal), but do not expect meaningful results!

created 2004 by Volker Baecker
modified 2006-2010 by Asheesh Gulati, Alessandra Griffa, José Viña & Aaron Ponti

W3C HTML 1.0 W3C CSS 2.1

FIGURE 2.6. Image Parameter Settings. Widefield microscopy: voxel size and pinhole radius. Notice the pixel size calculator.

- *Voxel size and backprojected pinhole radius: Confocal and 2-photon microscopy.*
As stressed in the explanation above the pixel size and the z-step are two really important data. These parameters can be found in the metadata of the images. The voxel size should not be larger than half the optical resolution of the imaging system, according to the Nyquist criterion.

In the “Quick help” column, highlighted in yellow, the ideal voxel size for the current image parameters can be found.

When doing batch deconvolution, make sure to always have the same pixel size and Z sampling. For confocal imaging this means that the same zooming factor and frame size should be used for a series of images taken with a given objective.

The ideal sampling density depends on the optics of the imaging system. As the Point Spread Function (PSF) is the basic “brick” of which the images are “made”, one should record details at least on the scale of the PSF to gather all the available information. Failing at that may spoil any attempt to do deconvolution, because deconvolution works on the PSF scale.

If the digital sampling occurs at a much larger of a physical scale than that of the PSF, deconvolution simply cannot be done. Many PSFs would be recorded inside each image voxel, and it would not be possible to improve and enhance the image by deconvolving it.

In confocal images the “backprojected pinhole radius” is the radius of the pinhole as it would be seen on the focal plane. This number can be calculated in HRM by clicking on the “Backprojected pinhole calculator” link.

For the computation of the backprojected pinhole radius the user will be asked for the pinhole radius for each channel that was used to take the image, and for the objective magnification (See Figure 2.7).

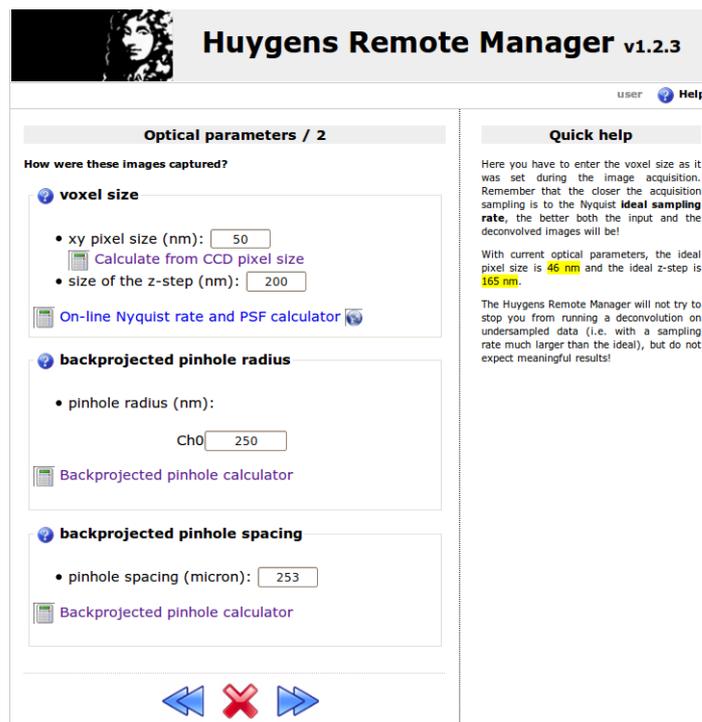


FIGURE 2.7. Image Parameter Settings. Voxel size, backprojected pinhole radius and spacing for multiphoton microscopy.

- *Voxel size, backprojected pinhole spacing and radius:* Spinning disk microscopy. Where the voxel size is concerned, the same principles explained in the section above apply to Spinning disk microscopy as well.

To explain the case of spinning disk microscopy a system containing a Yokogawa disk will be taken as an example. In that case, the physical size of the pinhole radius is typically $25\ \mu\text{m}$, while the physical spacing between pinholes is $253\ \mu\text{m}$. In HRM both the backprojected pinhole radius and the spacing (as they would be seen on the focal plane) can be derived by dividing these values by the objective magnification. To compute the backprojected pinhole radius and spacing click on the “Backprojected pinhole calculator” link and select “Yokogawa spinning disk (pinhole radius/distance)” from the list (See Figure 2.8). As can be seen in Figure 2.8 there are many more microscopes from which one can choose a backprojected pinhole calculation.

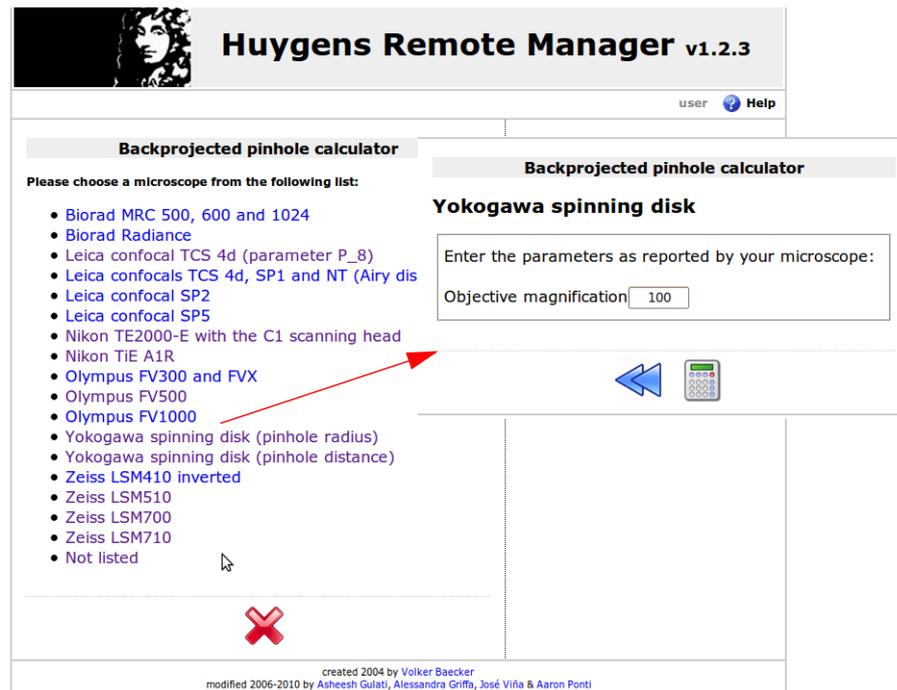


FIGURE 2.8. Backprojected pinhole calculator. Highlighted the calculator for a Yokogawa spinning disk.

- *Point Spread Function:* In HRM one can choose to have a theoretical PSF calculated by Huygens Core for the image or one can upload the measured (distilled) PSF that most resembles the real PSF of the imaging system.
- 1. *Distilled PSF file selection:* In practice the theoretical PSF computed from the image parameters can significantly differ from the effective PSF, because of unavoidable little misalignments and imperfections in the microscope system.

Usually a measured PSF is larger and more asymmetric respect to a theoretical one. The use of a measured PSF can thus improve the deconvolution results.

A measured PSF can be retrieved from images of fluorescent beads, for example using the SVI Huygens PsfDistiller. However a good PSF is relatively complicated to measure, as one needs to acquire multiple images, for each wavelength, and exactly with the same conditions with which the images will be acquired later on (See Figure 2.9).

If one chooses to use a measured PSF, HRM asks to select one PSF file for each channel of the image.

To see the PSF file selector the user has to click on the “BROWSE” button. A list with all the existing image files in the HRM user account that are compatible with the selec-

tion will be shown. Files with parameters very different from those stated in the user's current setting are shown in red to highlight that they are not good PSF candidates for the current setting.

2. *Theoretical PSF, spherical aberration correction*: If one chooses to work with a theoret-

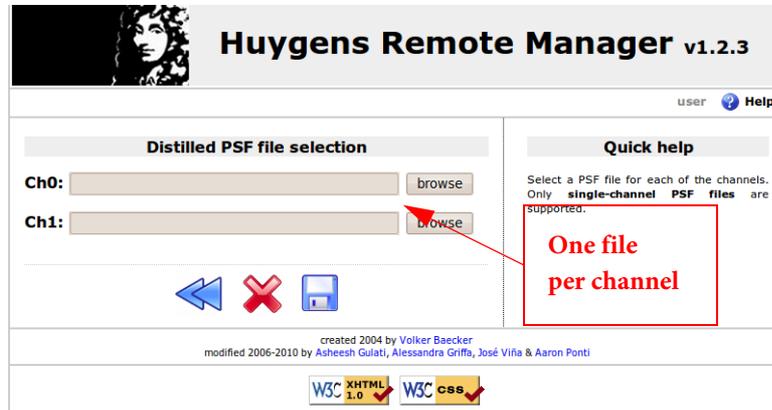


FIGURE 2.9. Image Parameter Settings. File selection for measured PSFs.

ical PSF the PSF file selector will be skipped and the user will be taken to a different page where to choose whether or not to perform a Spherical Aberration correction (SA) when deconvolving the dataset.

In general, better deconvolution results are achieved if an SA correction is applied. In order to do that one needs to supply some additional information. Namely, the position of the coverslip respect to the first slice of the dataset, and eventually whether or not the user wants to take into account PSF depth-dependent correction on a few bricks, PSF depth-dependent correction slice by slice, or PSF at user-defined depth.

The spherical aberration implies a variation of the PSF size and shape with the sample depth. To correct for SA Huygens Core will generate a “dynamic” PSF, adapted to the different z positions (See <http://www.svi.nl/MismatchDistortsPsf>).

Use the SA correction only if a significant mismatch between the refractive indexes of the objective and of the mounting medium is present, as the processing is significantly more time-consuming.

Notice that if the depth-dependent PSF correction is enabled in the Spherical Aberration Correction page, HRM will show the following extra fields for selection (See Figure 2.10):

1. *Specify sample orientation*_z: Specify the position of the coverslip respect to the dataset (“Plane 0 is CLOSEST / FARTHEST from the coverslip”).

2. *Correction mode*:

- a. *Perform automatic correction*:

In this case the stack will be divided into a certain number of bricks. Each brick will be deconvolved with a PSF adapted to the depth, considering the mismatch of refractive indexes between the mounting medium and the objective medium.

- b. *Perform advanced correction*:

1. *Depth-dependent correction on few bricks*: The number of bricks into which the stack will be divided for the deconvolution is limited. The deconvolution will be faster than in the above case “Perform automatic correction”.

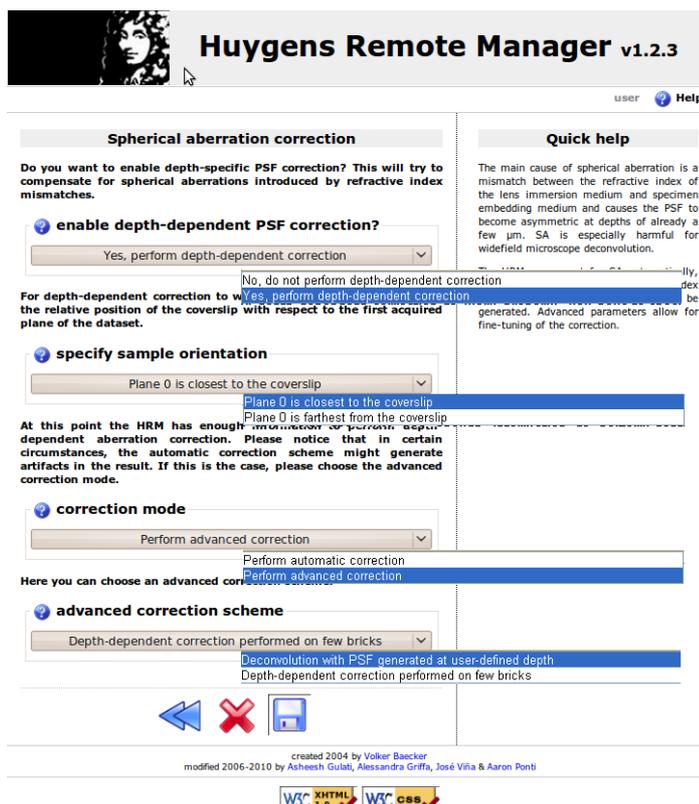


FIGURE 2.10. Image Parameter Settings. The spherical aberration correction can be applied when using a theoretical PSF.

2. *Deconvolution with PSF generated at user-defined depth:* A unique PSF will be used, but calculated at a depth in the sample defined by the user. The main idea is to use a “mean” PSF to partially correct for the SA.

HRM has at this point enough information about the type of images to be deconvolved. The Image Parameter Setting is ready. Save the setting after the selections at the Spherical Aberration Correction page and HRM will show again the page where to choose among all the Image Parameter Settings.

Notice that one of the nice advantages of working with settings is that if one would like to deconvolve more images taken under similar conditions (similar wavelengths, pinholes, voxel sizes, etc) one would be able to reuse this very setting with no further editing, allowing to launch deconvolution jobs very quickly.

Once back at the Image Parameter Setting page the user might want to choose the setting that was just edited. After this selection click on the big blue right arrow to continue with other selections.

The Restoration Parameter Settings

At this point, in the “Restoration Parameter Settings”, the user has to set up a few options related to the processing of the data. Some of these options tell HRM about what kind of pre-deconvolution processing will be performed on the images. For instance, how much to correct for background. The most options, though, establish how the deconvolution will be run. For example, what Signal to Noise Ratio (SNR) the images have, how many

iterations the deconvolution algorithm will try, or how well the deconvolution algorithm converges to a solution before stopping.

Notice that two restoration parameters such as the background value and the SNR are directly linked to different important acquisition parameters. Namely:

- Gain / offset
- Time exposure / scanning velocity
- Summing / averaging
- Laser power
- Spectral detection range

The initial page of the Restoration Parameter Settings looks very much like the Image Parameter Settings page (See Figure 2.11). This means that one can select among existing templates made by the HRM administrator, copy them, edit them for customized settings, add new settings from scratch and so forth.

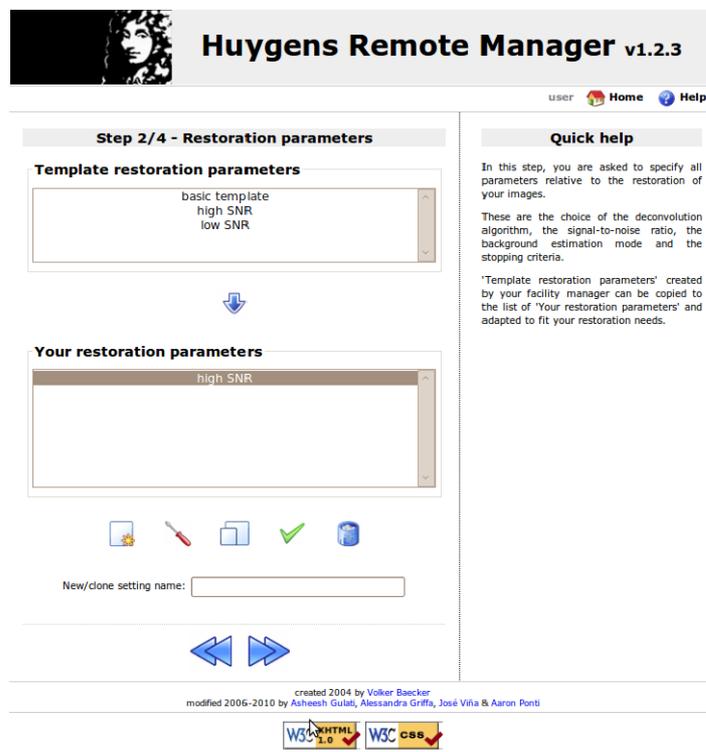


FIGURE 2.11. The Restoration Parameter Settings. At the end of this step a restoration template is selected.

A Restoration Setting can be edited to access the following parameters that need to be set up (See Figure 2.12):

- *Deconvolution algorithm*: There are two deconvolution algorithms available to process the data. The Classic Maximum Likelihood Estimation (CMLE) algorithm and a quicker version of it (See Figure 2.11).

The “Classic” algorithm should be used in most circumstances. The “Quick” version of the algorithm is faster but in some cases gives less precise solutions. One may con-

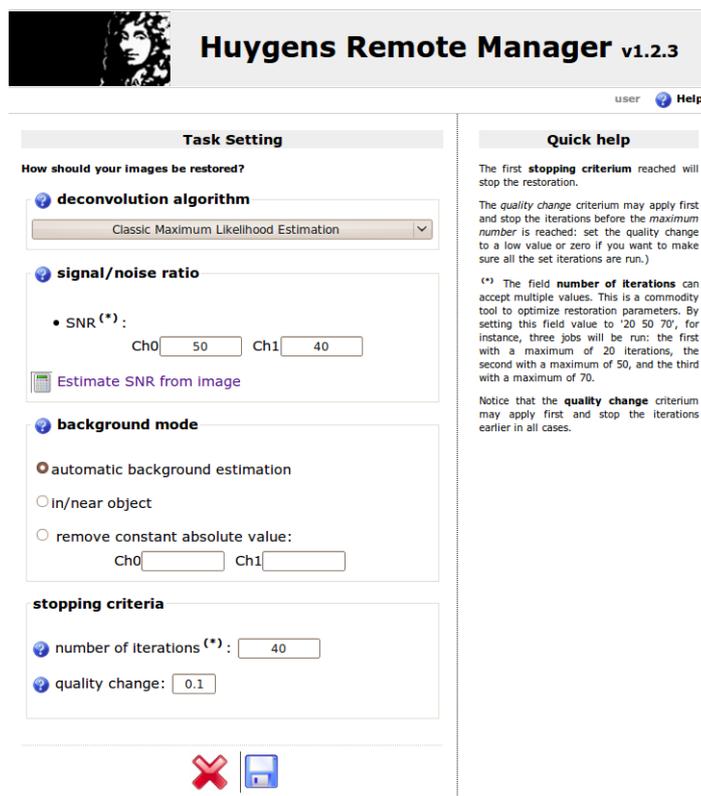


FIGURE 2.12. The Restoration Parameter Settings: Deconvolution algorithm, SNR estimation, background mode and stopping criteria.

sider using the “Quick” implementation in compute-intensive situations, for example, when deconvolving 3D-time series.

- **Background mode:** The following three options return slightly different background values and can therefore affect the deconvolution result:
 - *Automatic background estimation:* A region with a low mean value is found. The background is computed on that region. This estimation usually works fine.
 - *In/near object:* The Huygens software estimates the background value around intensity peaks. This option can be interesting for example when having bright little objects in a cell with a strong cytoplasmic background.
 - *Remove constant absolute value:* To make sure that the same background level is removed from all images in batch, insert manually a measured mean background for each channel. This option is typically useful for those interested in doing fluorescence quantification or stitching.
- **Stopping criteria:** The maximum likelihood estimation algorithm for deconvolution is an iterative method. This means that the algorithm computes sequential solutions which converge to a stable deconvolution result.

Although the default stopping criteria usually behave well they can be changed to match particular needs:

- *Number of iterations:* It is the maximum number of iterations that Huygens Core will compute.
- *Quality change:* represents how much the results of two consecutive iterations differ. If the two results are really similar it means that convergence has been reached.

- *Signal to Noise ratio estimation*: In this section the HRM SNR estimator can be run or a specific SNR estimation can be typed instead.

The deconvolution process may in general increase the noise of the original images, as it restores the high frequencies. For this reason Huygens Core will correct for noise during the deconvolution process.

The SNR parameter defines the degree of noise correction that will be performed. One can use the “Estimate SNR from image” link to estimate the SNR value on one of the images. As a first step, an image that is representative for the stack of images that will be deconvolved with this setting has to be selected, as shown in Figure 2.13. Then

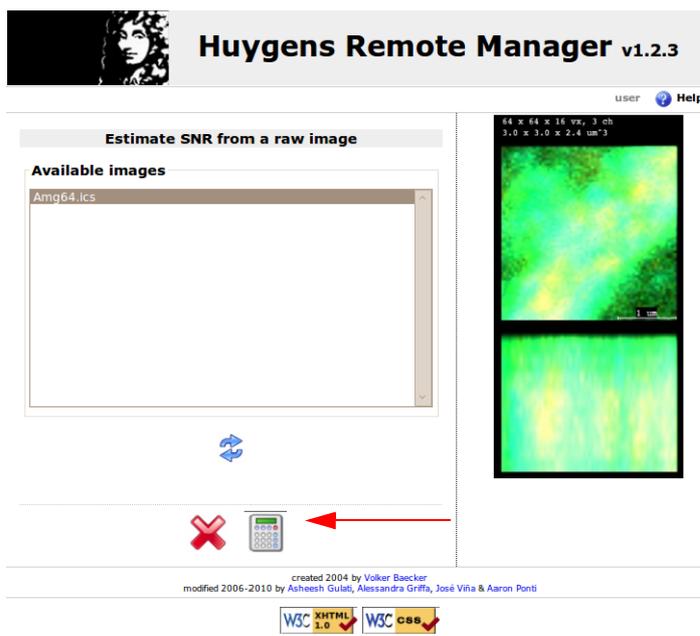


FIGURE 2.13. The Restoration Parameter Settings. The HRM SNR estimator.

by clicking on the “CALCULATE” button the SNR will be estimated on each channel of the selected image.

Besides the SNR estimation, for each channel, four deconvolution simulations with different SNR values around the estimated one will be displayed.

Comparing the different deconvolution simulations, one can visually confirm the correctness of the automatic SNR estimation. Move the mouse over the different images to see a zoom.

Again, when doing batch-processing, make sure that all the images in the stack have similar SNRs.

This will be the case as long as the images have been taken with the same Gain, Offset, laser power and image averaging for confocal imaging and the same Gain and time exposure for widefield imaging. Each time these settings are changed on the microscope or a different preparation is used the Signal to Noise Ratio will have to be re-estimated.

The SNR is a delicate parameter as it can highly influence the deconvolution result. On the one hand, if the deconvolution result looks too smooth and details are miss-

ing, a higher SNR value can be used. On the other hand, if the result looks too noisy one can try to use a lower SNR value.

The deconvolution can be run with more than one SNR value: simply type multiple values, separated by a space, in the SNR fields. HRM will create one deconvolution job per SNR value. When running deconvolutions on multichannel images make sure to type SNR values for all channels.

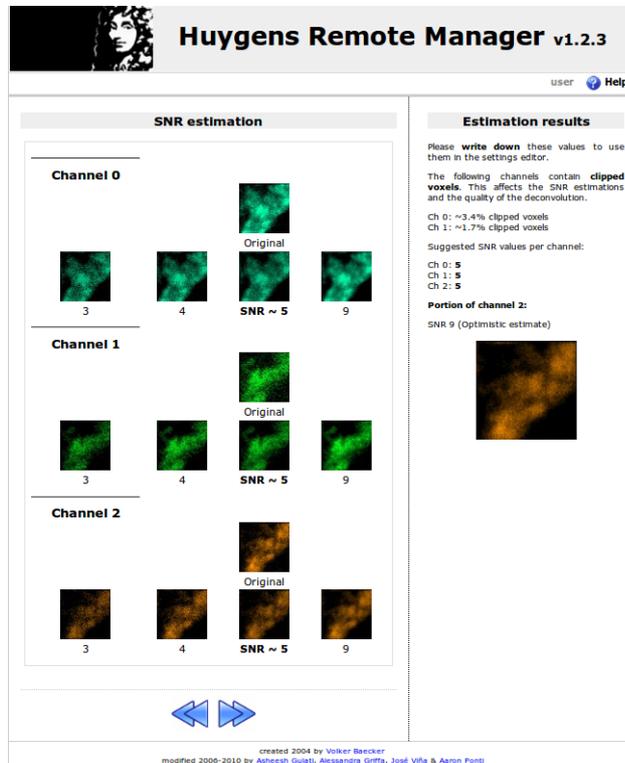


FIGURE 2.14. The Restoration Parameter Settings. The HRM SNR estimation output page.

If the Restoration Parameter Setting is saved at its last page HRM will show again the Restoration Parameter Setting page where one can choose among all the available Settings. The user might want to choose the one setting that was just edited. After this selection click on the big blue right arrow to move on to the next step of the job creation procedure.

If one would like to run deconvolutions with the same restoration settings in the future, that is, with the same deconvolution algorithm, background mode, stopping criteria and SNR, one could use the same Restoration Parameter Setting with no further editing. This will, as pointed out throughout this manual, allow to set other deconvolution jobs very straightforwardly.

Select images

At this point HRM knows all about the type of restoration that the user would like to use in the deconvolution, as well as the image parameters with which the images were taken.

If there are several images matching the selected settings then a Batch Deconvolution can be run on them effectively. Make sure that the same SNR values for all the images

involved in the Batch Deconvolution can be used. If the background option “remove constant absolute value” is selected make sure as well that all images for the Batch Deconvolution have similar background values.

In order to select images for a Batch Deconvolution one needs to be at step 3/4 of the “Start a job” process: “Select Images”. This means that both an Image Parameter Setting and a Restoration Parameter Setting have already been selected.

In the “Select Images” page the user can see all the images in the user account that are compatible with the selected settings.

If the Image Parameter Setting is specific for TIFF series or the LEICA format, only images with TIFF or LEICA formats will be shown for selection.

When clicking on the images for selection a preview of each image is shown, when possible. This preview is displayed on a panel on the right side of the page.

To carry out the final selection select and convey the images from the top panel “Images Available on Server” to the bottom panel “Selected images” by clicking on the little blue down arrow. The selected images will be added to a stack for batch deconvolution (See Figure 2.15). Then click on the big blue right arrow to move on to the last step of “Start a job”: Create a job.

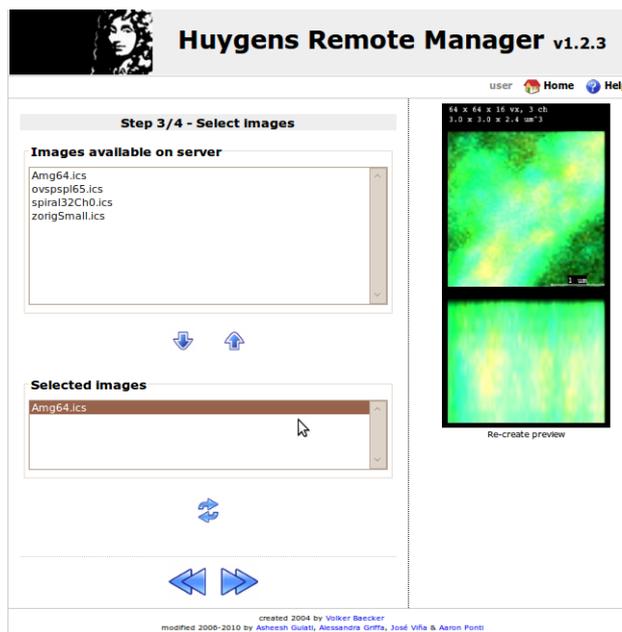


FIGURE 2.15. Image Selection for batch deconvolution. A preview of the selected image is displayed on the right side of the page.

Create a job

At this point the deconvolution job launching is almost ready. In this last step different summaries are presented stating the options with which the bath deconvolution is about to be run, as well as the specific images in the image stack. There is one last option for the user to select: the format of the output image.

As a guide for which format to select one can stick to the following rules. For 3D analysis the “ICS format” is appropriate, or even the most recent “ICS2” which is a multichannel, 32 bit format that stores all the deconvolution information, preserving all important details.

For 2D imaging, when analysis is required, the TIFF-8bit for output can be used. This format is fine for analysis such as counting or for segmentations, but not for **quantification!**

For visualization with IMARIS, the IMS format is fine. This is an 8 bit format (2 to 4 times lighter than 32bit).

If one would like to change a specific setting one has to click on the setting name, at the Create a Job page. This will take the user back to the setting selection. Likewise, one could click on the Selected Images link if other images had to be added or removed.

To proceed with the deconvolution with the current settings, click on the plug icon to launch the job (See Figure 2.16). HRM will automatically create one job per image and send it to the job queue where all the jobs are dispatched for deconvolution.

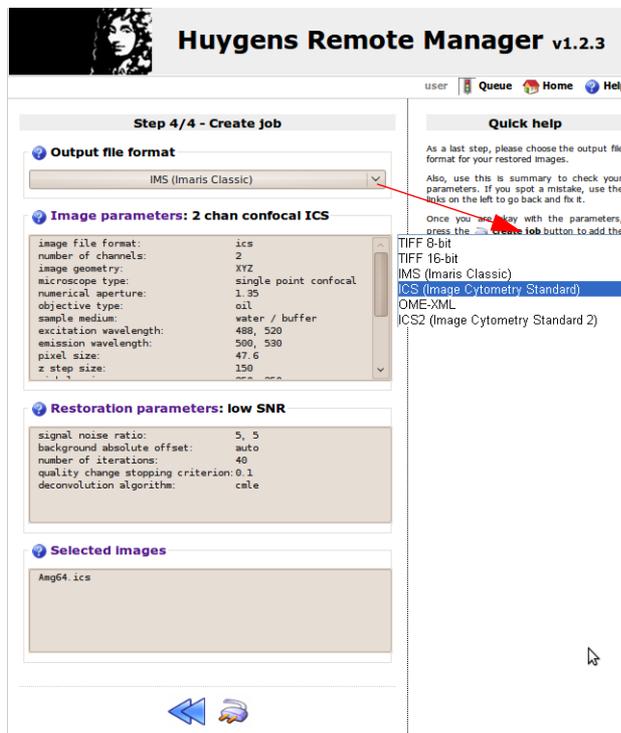


FIGURE 2.16. Create a Job. Output file format, summary page and job launching.

After launching the jobs HRM will show again the starting page where one can click on the Queue Status icon to check out what the status of the jobs is or to delete them if one is no longer interested in deconvolving them.

The Queue status

HRM manages the deconvolution of multiple jobs owned by different users through a queue. When clicking on the “QUEUE STATUS” button all the jobs in the queue that wait

to be executed are listed. The job that is currently being processed is marked in green colour (See Figure 2.17).

Huygens Remote Manager v1.2.3

user Home Help

Job queue

Friday 16. July 2010, 14:53:22

- There are **4 jobs** in the queue.
- You own **4 jobs**.

Quick help

You can delete queued jobs owned by yourself.

nr	owner	file(s)	created	status	started	pid	server
<input checked="" type="checkbox"/> 1	user	Amg64.ics	2010-07-16 14:53:11	started	2010-07-16 14:53:18	5401	localhost
<input type="checkbox"/> 2	user	ovspspl65.ics	2010-07-16 14:53:11	queued			
<input type="checkbox"/> 3	user	spiral32Ch0.ics	2010-07-16 14:53:11	queued			
<input type="checkbox"/> 4	user	zorigSmall.ics	2010-07-16 14:53:11	queued			

Check All / Uncheck All With selected:

created 2004 by Volker Baecker
modified 2006-2010 by Asheesh Gulati, Alessandra Griffo, José Vilia & Aaron Ponti

FIGURE 2.17. The Job Queue. In green colour the jobs being processed, highlighted in purple the jobs waiting.

To monitor the owned jobs in the queue and optionally delete them, select the corresponding lines and click on the “TRASH BIN” button.

A notification e-mail for each of the jobs is sent to the user when the deconvolution is terminated. If something went wrong HRM will notify that the job terminated with an error. In this case, or if no deconvolution report is received after a reasonable time, please contact the system administrator.

If something seems wrong, try to verify if there was a mistake in the settings. Try to contact the system administrator otherwise.

Notice that because HRM can be installed on a combination of dedicated servers the deconvolution process is usually performed with a good computation speed.

The File manager

If the job has been finished one might want to check out the result and perhaps also download it to a local machine. One might also want to upload new images for further processing. All these tasks are easily handled from the File Manager, which can be reached from the starting page of HRM (See Figure 2.2).

Once at the File Manager there are two little folder icons on the top menu with the words “Originals” and “Results” (See Figure 2.18). By clicking on the little “Originals” icon all raw images in the user account available for processing are listed. Notice that the File Manager in Figure 2.18 shows a list of deconvolved images and a preview of the selected one.

Notice that more raw data can be uploaded from the “Originals” page, and existing raw data can be deleted. From the “Results” page deconvolved images can be downloaded or deleted. All the images that are not removed will still be available when the user logs in next time.

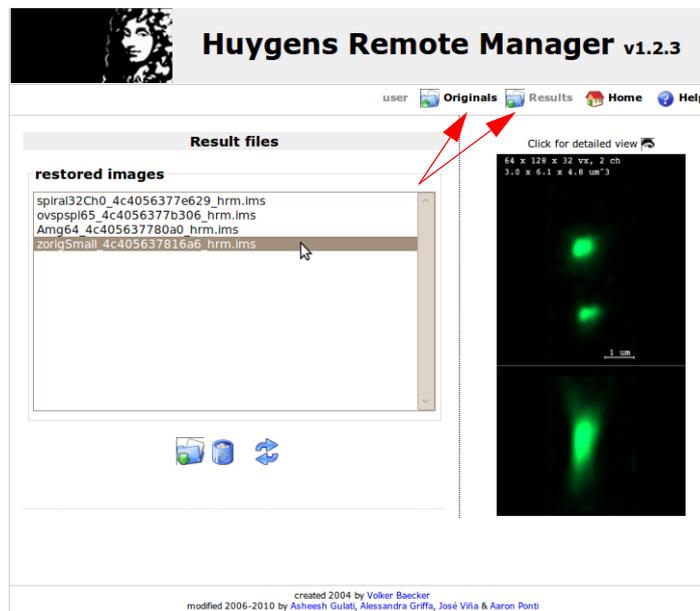


FIGURE 2.18. The HRM File Manager. Upload raw data, download deconvolved images, compare originals with results.

When going over the list of results, previews of the images are displayed and an option to click for a more detailed view presented. In this more detailed view there are a number of tools to compare the deconvolution results with the original raw data. Namely, depending on the features of the image, an MIP³ (Maximum Intensity Projection), an SFP⁴ (Simulated Fluorescence Process), a Slicer and a Stack movie ready for download.

With these tools one can check online how the deconvolution improved the image, prior to downloading any files.

Let us take a closer look at the image comparison tools. See in Figure 2.19 a typical MIP result, comparing the original image with the deconvolved data set. On the left side of this page, there are links to the other comparing tools so that the user can quickly switch between tools.

The Simulated Fluorescence Process, SFP, is shown in Figure 2.20 also comparing the two data sets: original raw data and deconvolved image.

The Slicer allows the user to compare the original image and the deconvolved data set slice by slice along the z coordinate at any depth (See Figure 2.21).

Clicking on the Stack Movie link raises a pop-up (See Figure 2.22) asking if a movie player should be opened where to see the Stack Movie immediately or if the movie should rather be downloaded to a local machine.

3. <http://www.svi.nl/MaximumIntensityProjection>
4. <http://www.svi.nl/SFP>

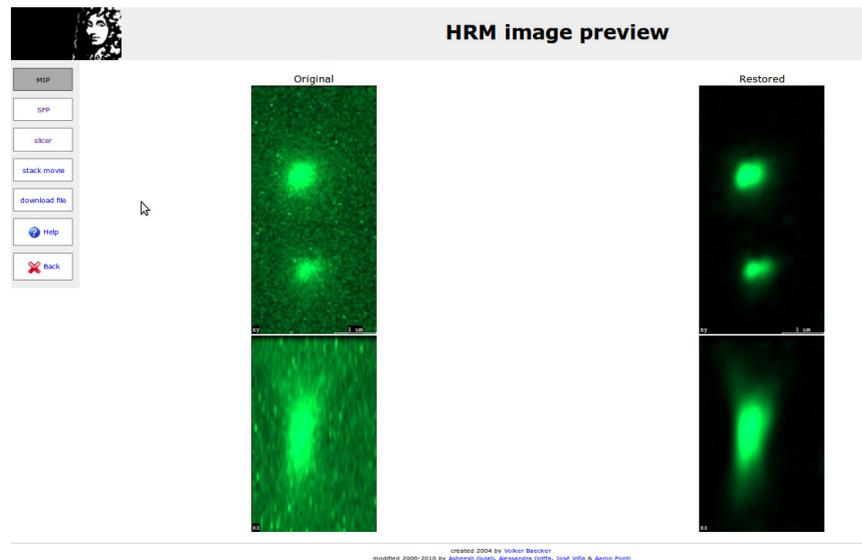


FIGURE 2.19. The File Manager comparison tools. A MIP, Maximum Intensity Projection, comparison between an original image and the deconvolution result.

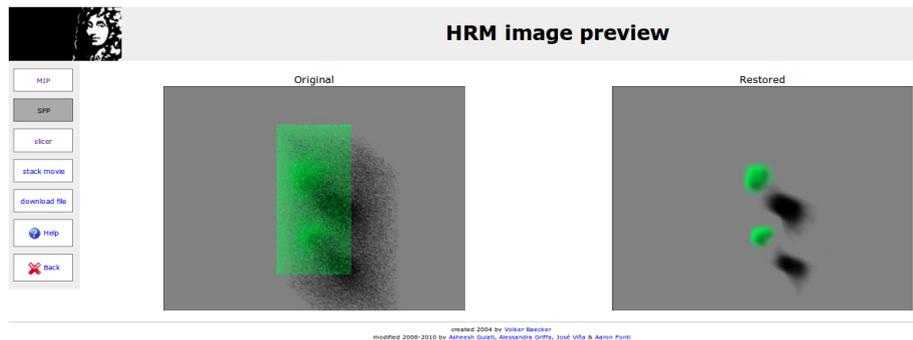


FIGURE 2.20. The File Manager comparison tools. An SFP, Simulated Fluorescence Process, comparison between the original image and the deconvolution result.

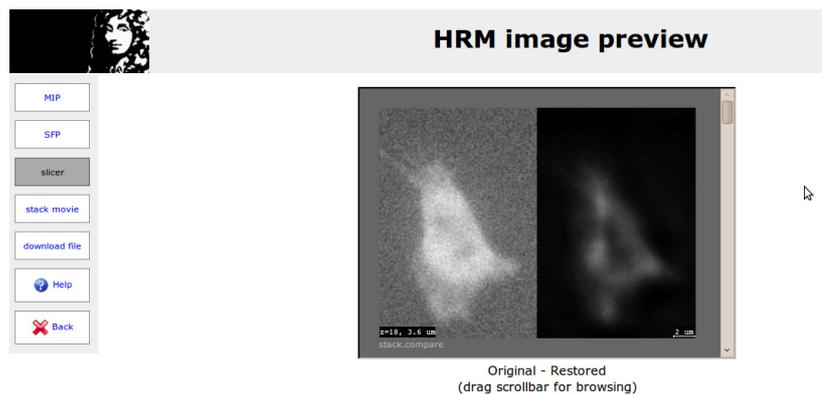


FIGURE 2.21. HMR Slicer: Comparison of raw data and deconvolved image slice by slice.

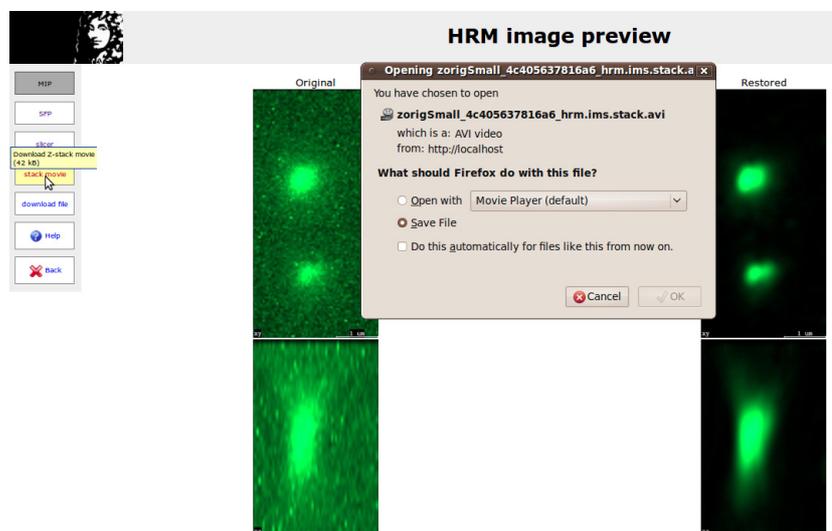


FIGURE 2.22. The File Manager comparison tools. Open or download a stack movie comparing the original image and the deconvolution result.

Your deconvolution statistics

The user statistics can be seen from the the starting page of HRM (See Figure 2.2). This turns out to be a straightforward and useful way for anyone to quickly see how he or she is using HRM and what the image and restoration trends are.

Clicking on *Your Statistics* brings the user to a page that summarizes and shows statistical information about the deconvolution jobs. This page collects and displays data about the percentages of output formats that were used, also the percentages of input formats, the type of Point Spread Function, the image geometry, the microscope type, and also how much time HRM used for the deconvolution jobs of the user.

All of this can be split according to the initial and final dates selected by the user to compute the statistics (See Figure 2.23).

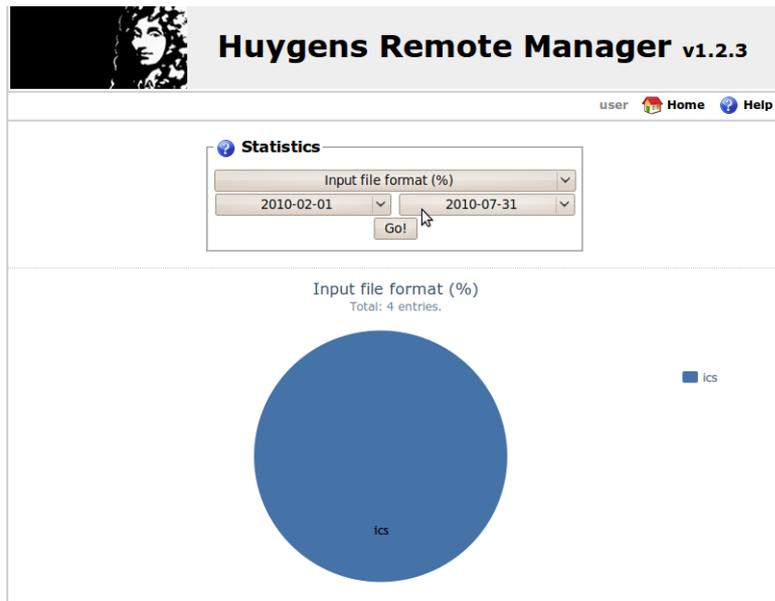


FIGURE 2.23. Your statistics in HRM. A straightforward way to check how HRM is being used and what image and restoration trends there are in the deconvolution jobs.