# *Huygens Remote Manager*

**User Guide for version 2.1.x**

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CHAPTER 1

# *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 more details on deconvolution please read "Deconvolution jobs in HRM" on page 12 or other Huygens guides. To run deconvolution jobs, HRM will request a number of microscope and restoration parameters from the user. For more specific information on these parameters, visit the online SVI Wiki [1] or follow the HRM help.

This chapter is an overview of the interface possibilities of HRM. 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, scheduled, batch deconvolution.

Huygens Essential and Huygens Professional have their own integrated scheduler, the 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. Each user has his own account in a web server and can place deconvolution jobs in the queue. HRM runs all jobs listed in the queue, setting priorities across them and alternating over all users. The HRM queue manager uses a simple mechanism to prioritize jobs, which prevents users from monopolizing the queue.

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://www.svi.nl/MicroscopicParameters
   http://www.svi.nl/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, the code can be freely modified and 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 a graphical interface and is designed to work seamlessly with HRM. It includes 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 of the SVI Wiki[4]. Instructions for online testing, downloading and installing HRM are also linked on that page.

Installing HRM on a running regular web server is not very difficult. Apart from the installation instructions that come along with the source code, other practical guidelines can be found in the project's official site[5] and in the SVI Wiki [6].

## *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 users by the system administrator;
- the management of parameter sets that all users can copy or use directly;
- the creation of deconvolution jobs, including image selection, setting microscopic parameters, and setting restoration parameters;
- inspecting the job queue status, and allowing the users to delete their own jobs from it.

HRM is equipped with a simple file uploader/downloader to send 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 a limit for these transactions.

The jobs created via the web interface are dispatched by the HRM queue manager, 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 at http://www.svi.nl/HuCoreMan
3. http://sourceforge.net/projects/hrm
4. http://www.svi.nl/HuygensRemoteManager
5. http://www.huygens-rm.org
6. http://www.svi.nl/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, since 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 Huygens Batch template that

- loads the raw image from a source directory,
- applies the microscopic parameters to it as defined by the user or reads the microscopic parameters from the image metadata,
- optionally loads another image containing the microscope Point Spread Function,
- deconvolves the image using the restoration parameters chosen by the user,
- stores the resulting restored image in a destination directory,
- generates a number of visualizations of the raw and deconvolved images so that the user can see the effect of the restoration,
- and finally writes a tag in the destination directory to inform the HRM queue manager 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.

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 usage*

In this section a summary of the basic HRM workflow is described. HRM shows help links at each stage of the job creation. For more detailed explanations see “Deconvolution jobs in HRM” on page 12.

### Registration and login

HRM offers user management for administrators to handle their managing tasks. User management for other platforms (Active Directory, LDAP) is also supported.

An application form to register as user is available in HRM. The application will reach the administrator who can grant the user further access. A “Request message” field in the registration form allows the applicant to send a message to the administrator along with the application.

An e-mail will inform the user whether the account is activated or rejected. Once activated, the user can login with a user name and password (see Figure 1.1). For security reasons the password is not shown while the user types. Upon mispelling 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

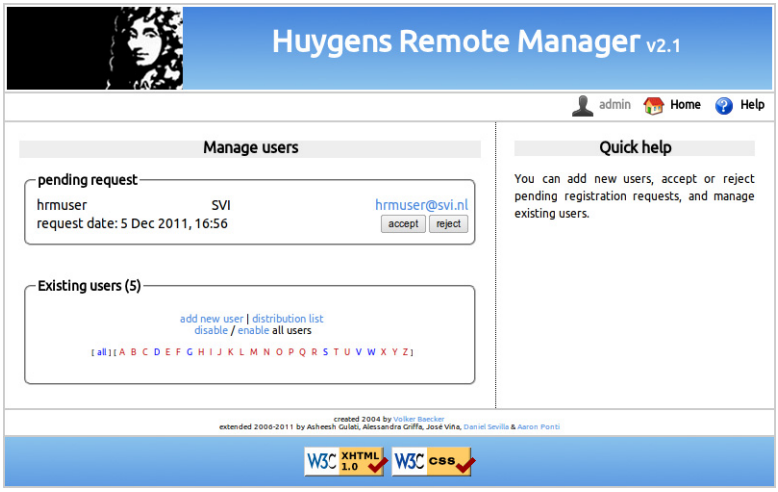


**FIGURE 1.2. The HRM User Management page for the administrator.** Active users and new applications can be easily managed from this panel.

on the user database. It contains, among other things, a “registrations” area that lists the pending account requests to be approved or rejected by the administrator (see Figure 1.2).

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 listed at once.

New user accounts can also be created by the administrator by clicking on “ADD NEW USER”. A user added by the administrator is automatically granted access to HRM.

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 deleted by the administrator by clicking on the corresponding buttons next to the user name.

Additionally, the posibility exists to enable or disable all users at once, which is useful for performing maintenance tasks on the server.

## Job parameters

The HRM parameters are divided into two groups: those describing the image (image or microscopic parameters) and those describing the restoration process (restoration parameters). Both are saved in the HRM database.

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

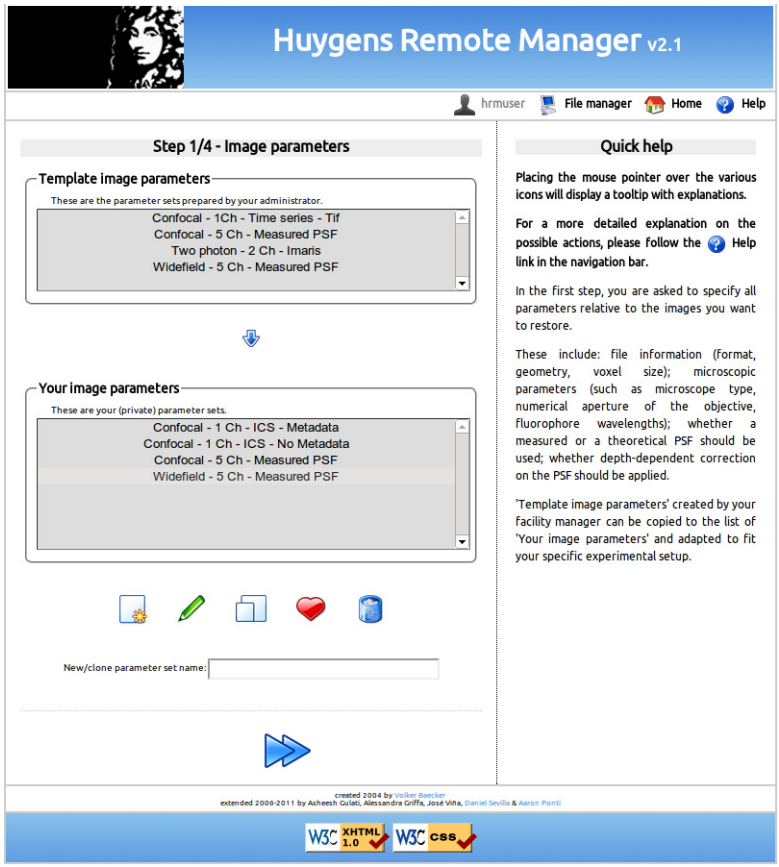


**FIGURE 1.3. Selection of parameter sets in HRM.** The top pane lists sets created by the administrator for all users. The bottom pane lists sets only available for the current user.

Setting image parameters and restoration parameters is similar in HRM. For detailed explanations on the meaning of the parameters and how to determine their values please refer to “Deconvolution jobs in HRM” on page 12 or to the HRM online help for further details.

**HRM includes help links with plenty of further information. There are many image and restoration parameters, and understanding them properly is important to achieve good results.**

### Using an existing parameter set

The page listing parameter sets contains two different panes (see Figure 1.3). The sets created by the administrator (top pane) can be copied to the bottom pane. The sets in the bottom pane can be modified by the user. A parameter set can also be created from scratch by the user.

To copy an administrator parameter set select it at the top pane and transfer it to the bottom pane by clicking on the little down arrow.

The bottom pane will list the sets among which the user can choose. The selected set will be highlighted in the list. Press the forward icon (big rigth arrow) to continue to the next step.

### Creating a new parameter set

- Enter a name for the new parameter set in the "NEW/CLONE" field. The name must be different from the other existing sets. It is best to choose a name that helps remember what the parameter is made for, e.g., it it better to use the microscope name than a family name.
- Press the "CREATE" button.
- Enter the parameters displayed in the subsequent pages. The number of pages is 4 or 5 depending on whether corrections for spherical aberration and measured PSFs are selected.
- Save the parameter set. The save option will show at the last parameter page.

### Copying a parameter set

- Select a parameter set from the bottom pane. The selected set will be highlighted.
- Enter a name in the "NEW/CLONE" field.
- Press the "COPY" button.

The new parameter set will be listed in the bottom pane. The new set can now be edited to modify any of its parameters.

### Editing a parameter set

- Select a parameter set from the bottom pane. The selected set will be highlighted.
- Press the "EDIT" button.
- As in "Creating a new parameter set" enter the parameters displayed in the subsequent pages.
- Save the parameter set. The save optino will show at the last parameter set.

### Making a parameter set default

When using a particular set very frequently it might useful to make it default. The default set will be automatically preselected the next time a set must be chosen.

To make a set default:

- Select a parameter set from the bottom pane. The selected set will be highlighted.
- Press the "DEFAULT" button.

The name of the default set will be highlighted in a different color.

### Deleting a parameter set

- Select a parameter set from the bottom pane. The selected set will be highlighted.
- Press the "DELETE" button.

When a parameter set is deleted its name disappears from the bottom pane. A deleted set can not be restored. If a parameter set was deleted accidentally the only way to get it back is to create a new set and enter all parameter values again. If the set had been used before, the parameter values can be extracted from the summary e-mail sent when the job finished.

### Measured Point Spread Function

To deconvolve the images with a distilled (experimental or measured) Point Spread Function (PSF) the parameter sets must specify it so. In such case, HRM will ask for one PSF file per channel. If an image contains more channels than available PSFs theoretical PSFs will be used for the deconvolution of the remaining channels. Be aware that HRM does not warn about this situation. For more information about the PSF, see the SVI Wiki[7].

It is recommended to provide PSFs in ICS or HDF5 formats, as these formats can provide the most metadata.

### Selecting the images

At this stage the raw datasets can be selected for the restoration. The "Available Images On Server" area lists all the user's images matching the file type of the current parameter set (see Figure 1.4).

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. The images can be removed from the selection by using the up arrow button.

Press the "UPDATE VIEW" button so that HRM can refresh the file list if any images are not displayed.

The Huygens software can read plenty of file formats frequently used in fluorescence microscopy, though HRM may not be fully adapted to use all the formats supported by Huygens. For a full list of file formats supported by Huygens Core please see the most recent online list[8].

7. http://www.svi.nl/PointSpreadFunction
8. http://www.svi.nl/FileFormats

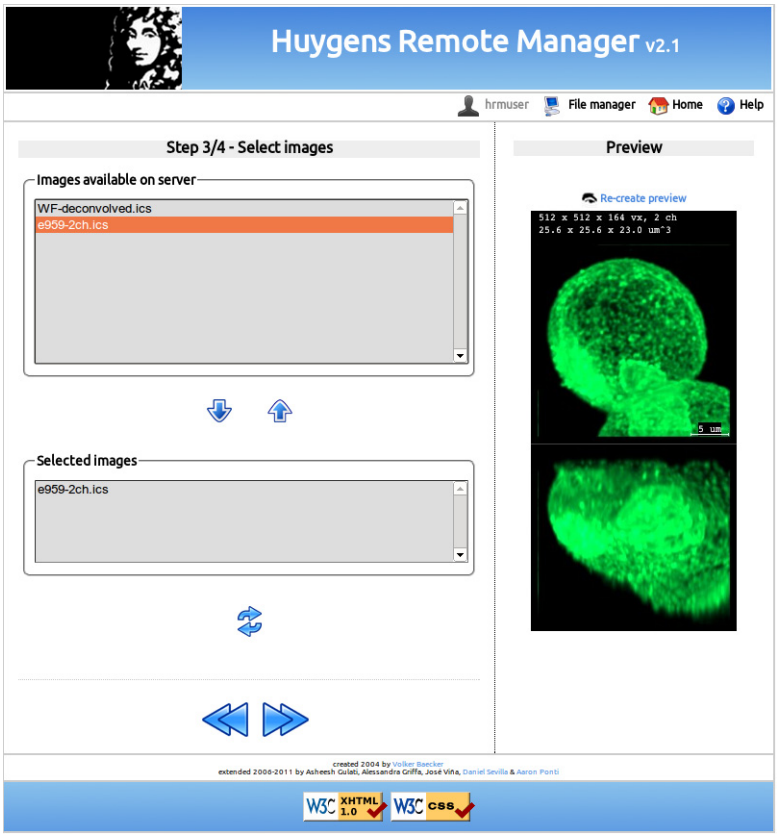


**FIGURE 1.4. Image file selection in HRM.** Only the user's images matching the parameter set can be selected.

### Create the job

In this page an output file format is chosen for the restored images. The job is also finally launched from this page.

To change any of the settings selected so far use the links "Image Parameters" and "Restoration Parameters" displayed on this page. Use the link "Selected Images" to change the image selection.

To create the job simply click on the plug icon (see Figure 1.5). This will launch the job and will go back to the main panel. From the main panel the progress of the queued jobs can be examined by clicking on the "QUEUE" link (see Figure 1.6).

HRM sends the user a notification e-mail when the deconvolution job is finished. The result images are stored in the user's "Deconvolved Images" area.

The deconvolution results can be viewed and downloaded from within the File Manager. Click on the "Deconvolved Images" folder. A list of deconvolved images will be displayed. The images can be individually selected to examine them and download them.

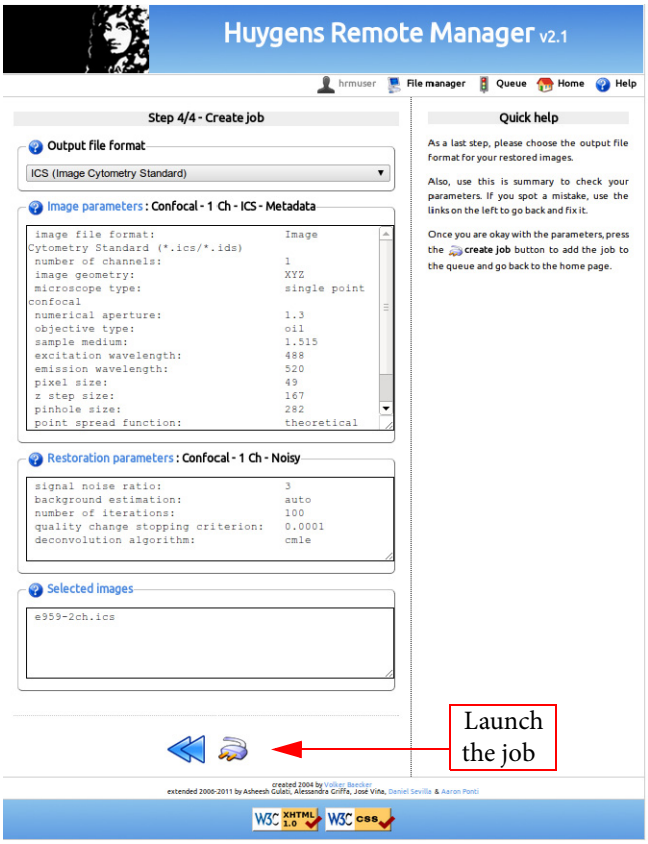


**FIGURE 1.5. Reviewing the parameters and creating an HRM job.** The file type for the deconvolved image must be selected at this point. Cliking on the plug icon launches the deconvolution job.

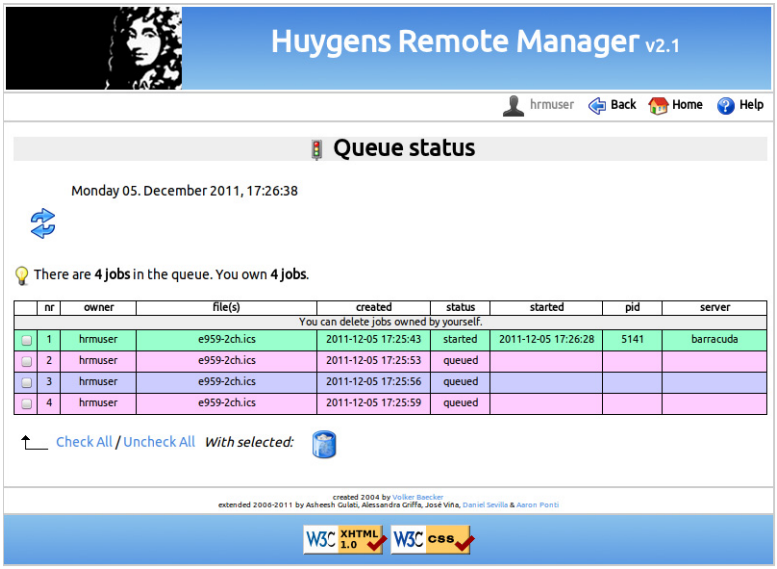


**FIGURE 1.6. The HRM job queue.** All queued jobs are visible to all users, but only owned jobs can be deleted.

CHAPTER 2

# *Deconvolution jobs in HRM*

This chapter explains in more detail how to create and launch deconvolution jobs in HRM. Instructions on how to inspect deconvolution results and how to access statistics in HRM are also included here.

It is recommended to visit an HRM site so that the job launching process can be reproduced. An HRM account at an imaging facility can be requested by sending an application to the corresponding administrator. This task can be performed from within HRM as highlighted in Figure 2.1. Upon cliking on the "REGISTER" link, the user is asked to fill out a short form that will be forwarded to the administrator.

If the imaging facility of interest does not provide access to an HRM installation it is still possible to get familiar with HRM by requesting an account for the HRM demo server[1] at SVI.

With an HRM account deconvolution jobs can be run remotely, in batch mode, with just a few clicks.

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

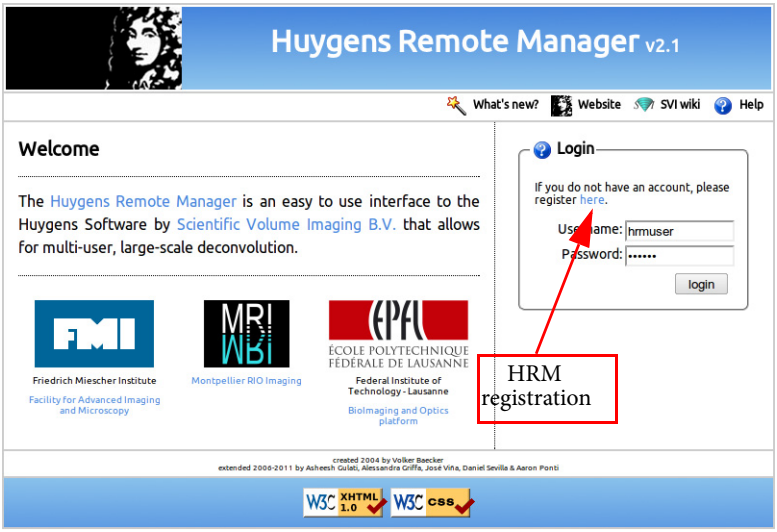


**FIGURE 2.1. The HRM login page.** Hihglighted the link leading to the application form for new accounts.

## *The starting page*

Once logged in HRM, the starting page is displayed, as shown in Figure 2.2. From this page the user can manage the deconvolution jobs as well as other HRM administrative tasks. The following shortcuts are available:

- *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 main panel.

## *Start a Job*

To start a new deconvolution click on the *Start a job* icon. Starting a new deconvolution job is split into 4 main steps:

1. The Image Parameters (parameters of the microscope and the image).
2. The Restoration Parameters (parameters for the deconvolution algorithm).
3. Selection of the raw images.
4. Selection of the output file format.

### The Image Parameters

In *Start a job - Image Parameters* the image parameters can be set and saved. The image parameters are grouped in sets, which can be created, copied, edited, named, deleted, etc. A set of image parameters describes the microscope and the conditions used to acquire the images. A parameter set can be reused in future deconvolution jobs. This will make sense as long as the involved images have been acquired under the conditions specified in the parameter set.

The *Start a job - Image Parameters* page shows 3 main areas (see Figure 2.3):

- *Template Image Parameters:* parameter sets created by the HRM administrator. These can be used as references. They can be copied and changed by the HRM users.
- *Your Image Parameters*: The user's parameter sets . These may be based on administrator templates or created by the user with customized values.
- *New/Clone Parameter Set Name*: Entry field for the name of a new parameter set. Type a name for the new set and click on the "ADD" button. A new set of parameters will be created. It is advised to write clear, easy-to-understand names for the parameter sets.

If the mouse pointer hovers over the icons below the bottom pane an explanation is

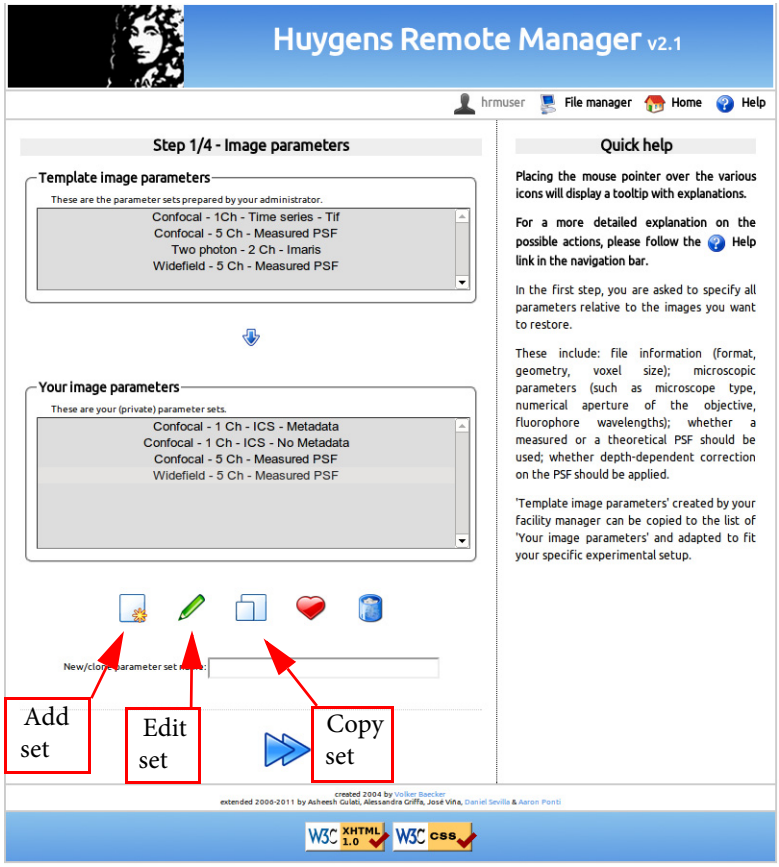


**FIGURE 2.3. The Image Parameter Settings Page.** At the end of this step a microscope setting is selected.

shown for each icon: “Create a new parameter set”, “Edit the selected parameter set”, “Copy the selected parameter set”, “Make a parameter set default”, “Delete the selected parameter set”.

A set of parameters consists of a number of relevant microscopic parameters. The microscopic parameters of a particular set can be seen by editing it. The parameters of a set can be adjusted to meet the needs of the user’s images. After a set has been edited and modified, it can be saved and used with similar images in future deconvolution jobs.

When a set is edited its parameters are displayed with links to the SVI-wiki where explanations are provided. These links are represented by question mark icons throughout HRM.

The set of image parameters provides Huygens Core with information about the images that will be deconvolved. The following image properties are relevant (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 underlying engine, Huygens Core, use **naming standards** such as 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 deconvolution jobs. There are freeware tools available to change file names in batch mode[2].

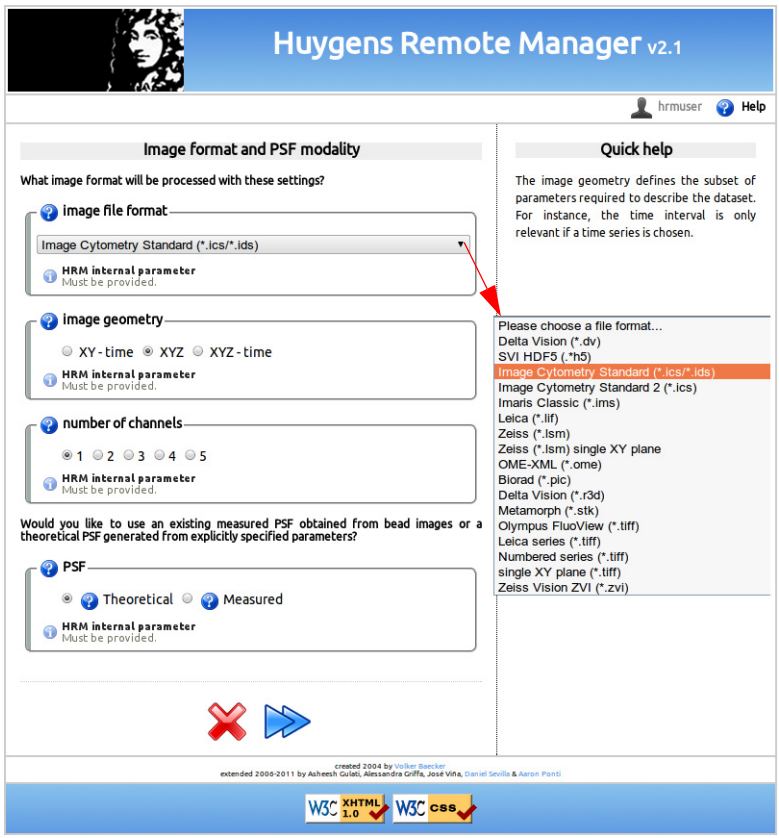


**FIGURE 2.4. Image Parameter Settings.** What kind of images will be deconvolved?

A few examples of different Tif standards include:

- *Olympus FluoView* (*.tiff): This is a particular Tif format (FM multi-layer) that can store multiple 2D planes in a 3D stack (single file).
- *Leica series* (*.tiff): Series of 2D images, with Leica Standard naming. Below, two examples of this standard:
  - XYZ-time, single channel: *name_t00_z000.tif*.
  - XYZ-time, multiple channels: *name_t00_z000_ch00.tif*.
- *Numbered series* (*.tiff): Sequentially numbered series of 2D Tif images. The series is interpreted as a 3D z-stack (no time-lapse, single channel).
- *Single XY plane* (*.tiff): 2D Tif images are considered separately. Use this option when running 2D deconvolutions 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*: The deconvolution needs a Point Spread Function (PSF) to restore the raw data. The Huygens software can compute a theoretical PSF from the parameters of the raw data or it can use a measured PSF. In the latter case, HRM asks for a file containing the measured PSF. In most cases the theoretical PSF works fine.

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

There are a few more questions concerning the optical parameters of the microscope. Figure 2.5 shows a screenshot of these questions in HRM.

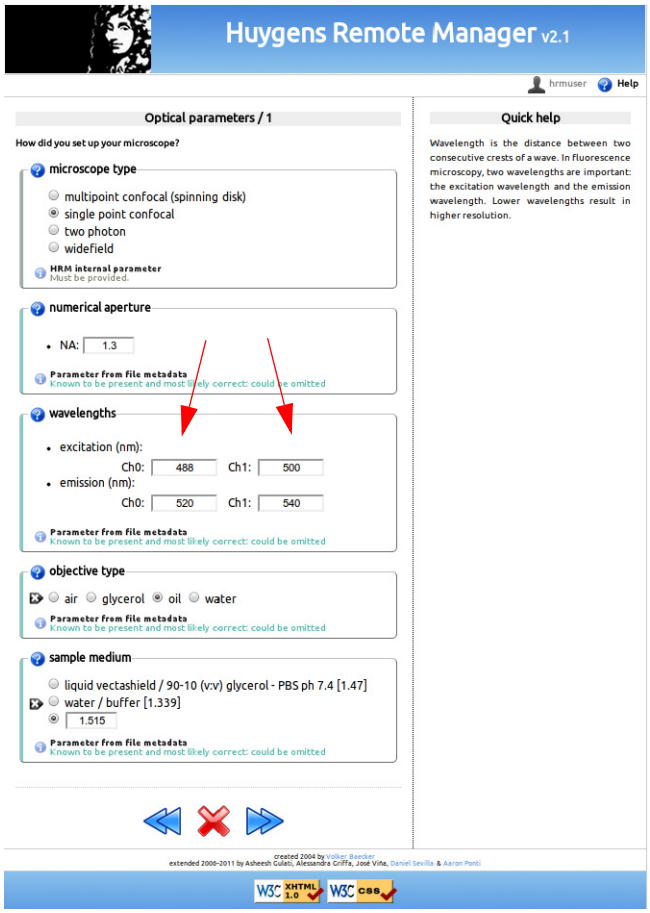


**FIGURE 2.5. Image Parameter Settings.** How was the microscope set up?

- *Microscope type*: whether a Spinning disk confocal, Single point confocal, Widefield or Multiphoton system was used as microscope to take the image.
- *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 displayed 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, air).
- *Sample medium*: The refractive index of the medium in which the sample was embedded (glycerol, polyvinyl alcohol, vectashield or other media).
- *Voxel size*: The voxel size is a very important parameter for the deconvolution of microscopic images. According to the Nyquist criterion its value should not be larger than half the optical resolution of the imaging system. In order to set the voxel size appropriately three different cases can be distinguished depending on the microscope type.

- *Voxel size, widefield and spinning disk 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 possible 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: If a pixel

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

binning is used, it is necessary to take this into account to calculate the pixel size. HRM gives access to a calculator to compute the *xy* pixel size (see "Calculate from CCD pixel size" at the image parameters page). HRM also shows the ideal voxel size for the given optical parameters (numerical aperture, refractive indexes, etc) so that it can be used as a reference (See Figure 2.6). Make sure to set a voxel size

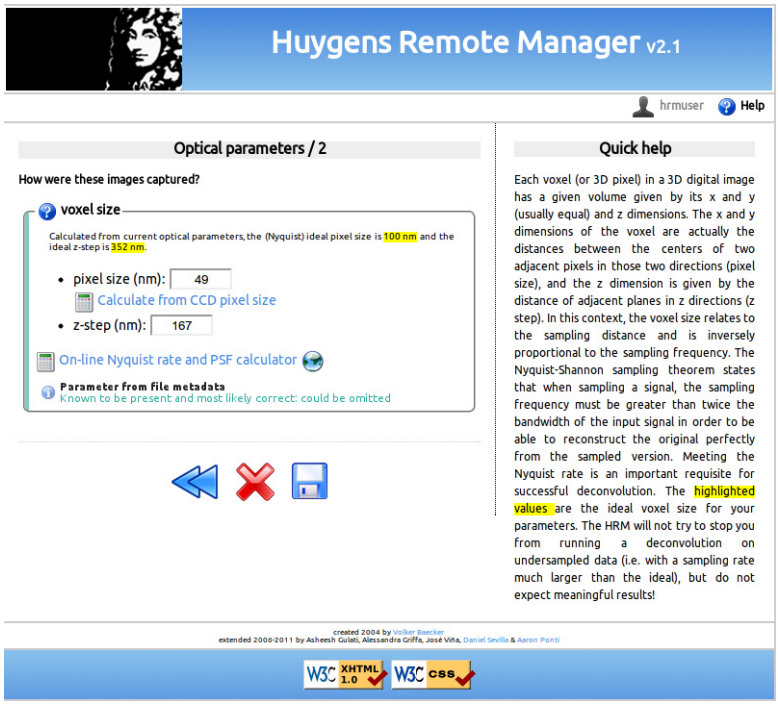


**FIGURE 2.6. Image Parameter Settings.** Widefield microscopy: voxel size. Notice the pixel size calculator.

consistent with the optical resolution of the microscope as undersampled images will often show artifacts after deconvolution. Notice that the z-step value can often be found in the metadata of the image.

- *Voxel size, confocal and 2-photon microscopy*: When doing batch deconvolution it is important that the images be acquired with the same settings. Notice that the sampling density depends on the optics of the imaging system. For confocal imaging this means the same zooming factor and frame size for a given objective.

- *Backprojected pinhole radius, confocal and 2-photon microscopy*: 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. The calculator will ask for the objective magnification and the actual pinhole radius for the computation of the backprojected radius (See Figure 2.7).

- *Backprojected pinhole spacing and radius, Spinning disk microscopy*: (see Figure 2.8).

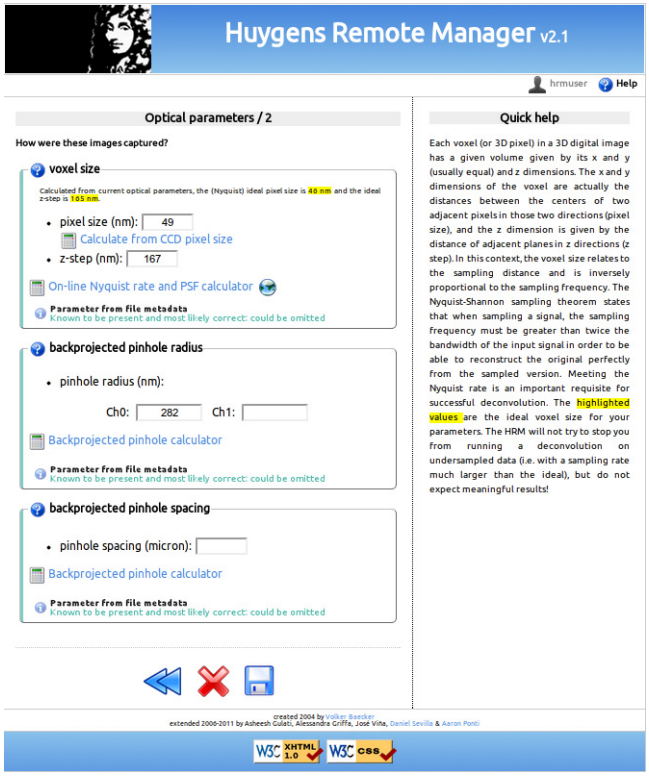


**FIGURE 2.8. Image Parameter Settings.** Voxel size, backprojected pinhole radius and backprojected pinhole spacing for spinnink disk microscopy.

HRM also counts on a calculator to compute the backprojected value of the pinhole spacing for spinning disk microscopy (See Figure 2.9). This calculator lists a number of microscope models to assist the user in the calculation. If, for example, a Yokogawa disk is selected from the list, the pinhole radius is set to 25 µm and the pinhole spacing is set to 253 µm. From these values, HRM computes the backprojected counterparts (as they would be seen on the focal plane).

- *Point Spread Function*: 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 physical scale than that of the PSF, the deconvolution simply cannot be done. In such situation 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 HRM, one can choose to have Huygens Core calculate a theoretical PSF compatible with the raw image or one can upload the measured (distilled) PSF that most resembles the real PSF of the imaging system. In practice the theoretical PSF computed from the image parameters can significantly differ from the experimental (distilled) PSF, because of unavoidable little misalignments and imperfections in the microscope system. Usually a measured PSF is larger and more asymmetric than a theoretical one. The use of a measured PSF can thus improve the deconvolution results.
  - *Distilled PSF file selection*: A measured PSF can be derived 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 with the exact same conditions as the raw images to be

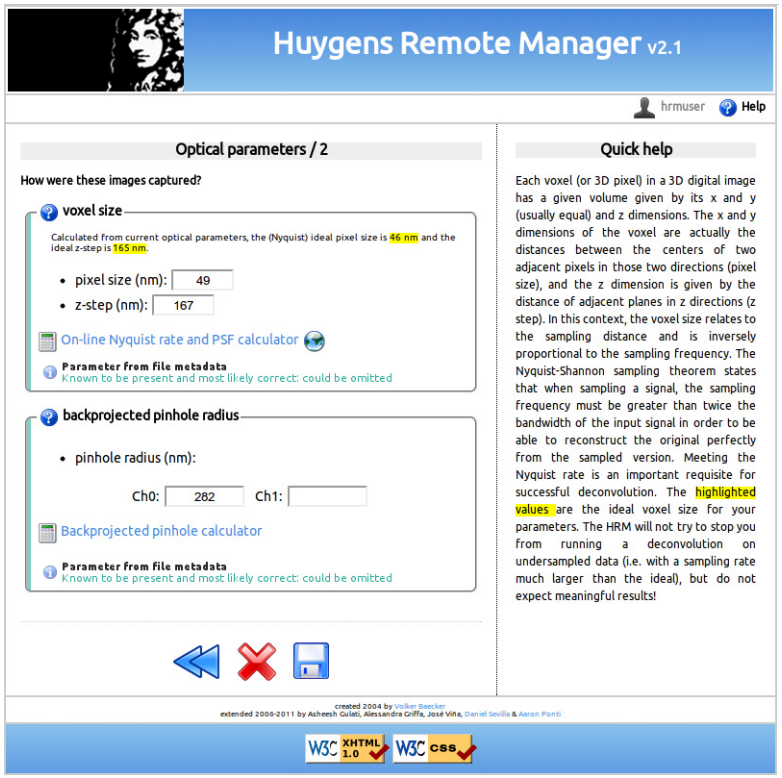


**FIGURE 2.7. Image Parameter Settings.** Voxel size and backprojected pinhole radius for confocal and multiphoton microscopy.


**FIGURE 2.9. Bacprojected pinhole calculator.** Highlighted the calculator for a Yokogawa spinning disk microscope.

deconvolved (See Figure 2.10). HRM will ask to select one PSF file per channel if the measured PSF option is chosen. At the "Distilled PSF file selection" page click on the "BROWSE" button to select a PSF for a specific channel. A list with all-

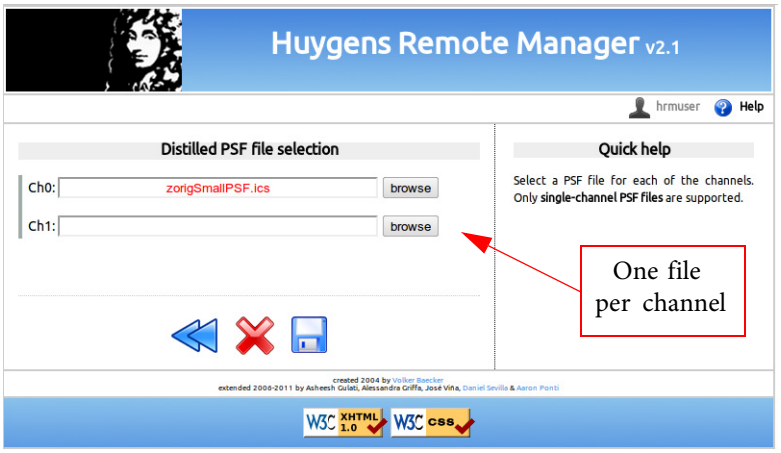


**FIGURE 2.10. Image Parameter Settings.** File selection for measured PSFs.

the user's files compatible with the entered image parameters will be shown. Those files that don't suit the current image paremeters are highlighted in red to stress that they are not good PSF candidates.

- *Theoretical PSF, spherical aberration correction*: if necessary HRM will ask whether to correct the theoretical PSF for spherical aberration (See Figure 2.11). In general, better deconvolution results are achieved if the spherical aberration correction is applied. A few more parameters are necessary for the spherical aberration correction, though. Namely, the position of the coverslip respect to the first slice of the dataset and whether or not the so-called PSF depth-dependent correction (few bricks, slice by slice, or at user-defined depth) must be taken into account. Due to the spherical aberration the PSF size and shape changes with the sample depth. To correct for this effect Huygens Core will generete a "dynamic" PSF adapted to the different z positions (See http://www.svi.nl/MismatchDistortsPsf). Use the Spherical Aberration correction only if there is a significant mismatch between the refractive indexes of the objective and of the sample medium, as the processing is significantly more time-consuming. The following selections play a role in the spherical aberration correction applied to the theoretical PSF:

1. *Specify sample orientation:* Specify the position of the coverslip with respect to the dataset ("Plane 0 is CLOSEST / FARTHEST from the coverslip").
2. *Correction mode*:

   **a.** *Perform automatic correction*:

1. 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 sample 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 case "Perform automatic correction".

   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 spherical aberration.

- *Import metadata*: Most microscopy file formats allow for saving metadata. If the acquisition system works with such file formats it can save parameters such as the sampling sizes, pinhole sizes, numerical aperture, etc, in the raw data. In HRM the user can choose whether this information (image metadata) should be used in the deconvolution job. Some file formats, though, lack the structure to save all the rele-

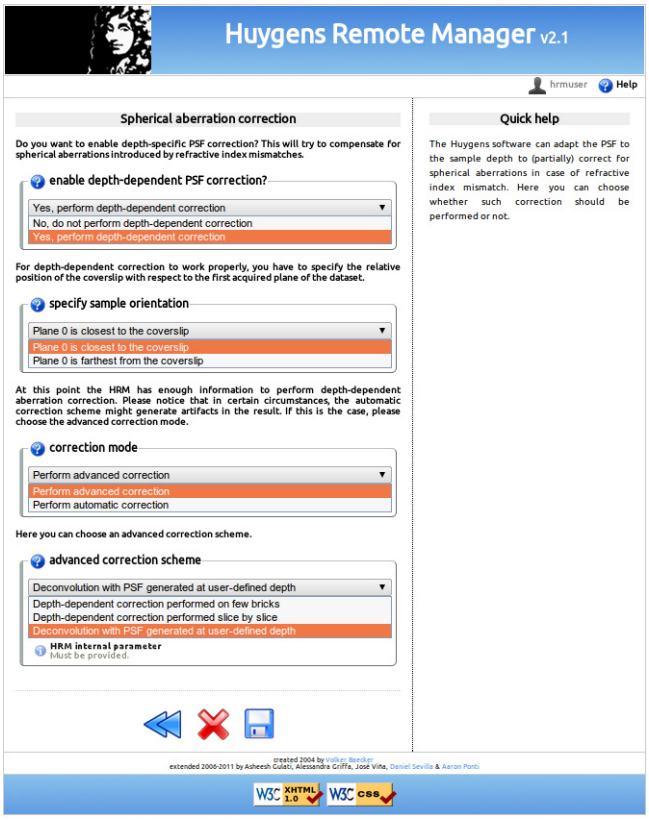


**FIGURE 2.11. Image Parameter Settings.** The spherical aberration correction can be applied when using a theoretical PSF.

vant information of a parameter, e.g. physical units. For this reason, HRM informs, per file type, how reliably the metadata can be used (see Figure 2.12). If a metadata parameter can be trusted the user can skip filling in its value. HRM assists the user in this regard, showing messages on which parameters may be skipped and which must be provided. Notice that entering the image parameters for a deconvolution job in

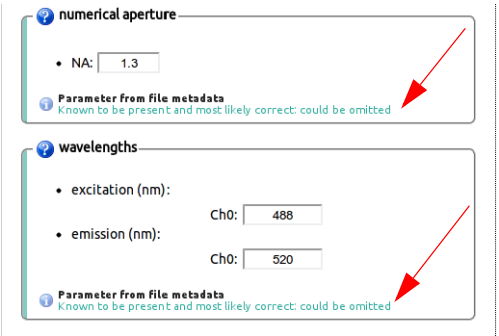


**FIGURE 2.12. Image Parameter Settings.** The confidence level of each metadata parameter is shown below the parameter in HRM.

HRM can be skipped almost entirely if the images contain good, complete, reliable metadata, while the deconvolution results will be optimal. At the end of the deconvolution job, when the user gets a notification email and a link to the restored image

a summary table can be examined listing which parameters were taken from the metadata, as well as their values (see Figure 2.13).

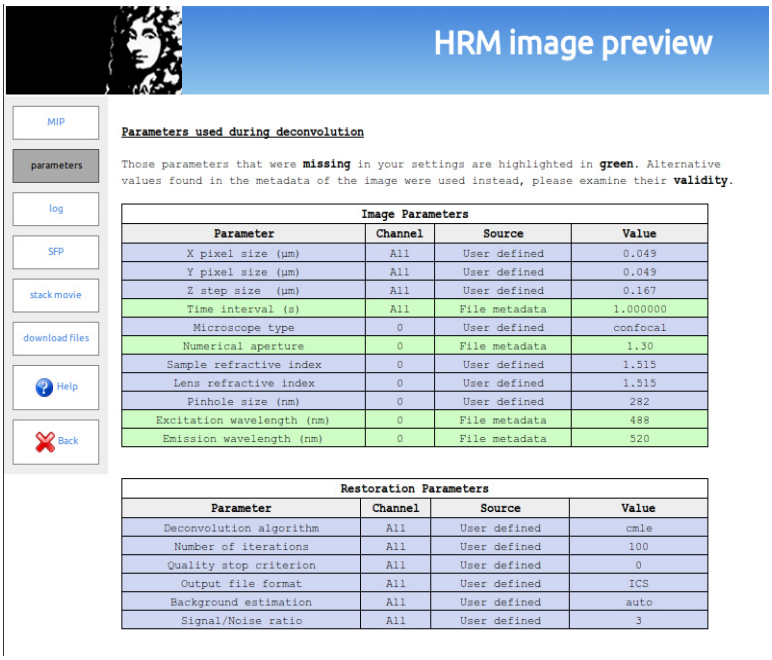


**FIGURE 2.13. Summary table** showing the parameters used in the deconvolution.

At this point, the parameter set is ready and can be saved. The list of all the user's parameter sets will be shown.

Select one parameter set for the deconvolution job and click on the big right arrow to continue (see Figure 2.3).

### The Restoration Parameters

A set of restoration parameters includes options related to the processing of the data. Some of these options refer to pre-deconvolution processing, for instance, the background correction. Most options, though, refer to the deconvolution itself, for example, the Signal to Noise Ratio (SNR), the number of iterations (stopping criterium) or the convergence quality to a solution (stopping criterium).

The background and the SNR are both 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 Parameters (see Figure 2.14) resembles the Image Parameters page (See Figure 2.3). Thus, templates made by the HRM administrator can be selected, copied, and edited for customization. New parameter sets can also be created from scratch.

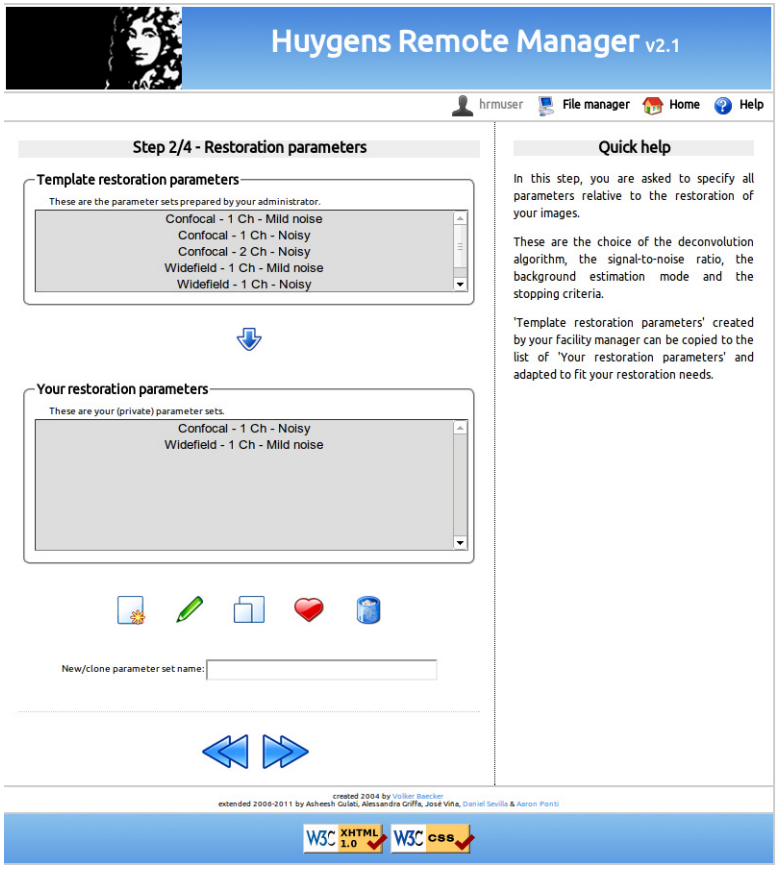


**FIGURE 2.14. The Restoration Parameter Settings.** At the end of this step a restoration parameter set is selected.

A Restoration Parameter set includes the following parameters (See Figure 2.15):

- *Deconvolution algorithm*: Two deconvolution algorithms are available to process the data. The Classic Maximum Likelihood Estimation (CMLE) algorithm and the Quick Maximum Likelihood Estimation (QMLE) (See Figure 2.15).

  The “Classic” algorithm should be used in most circumstances. The “Quick” algorithm is faster, but gives less precise solutions in some cases. One may consider using the “Quick” algorithm in compute-intensive situations, for example, when deconvolving 3D-time series.
- *Background mode*: Three options are available for the backround mode. They return slightly different values so this choice can affect the deconvolution result:
  - *Automatic background estimation:* This estimation usually works fine. A region with a low mean value is found and the background computed there.
  - *In/near object*: Huygens estimates the background 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 the images in the 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 (MLE) algorithm is an iterative method. This means that the algorithm computes sequential solutions which

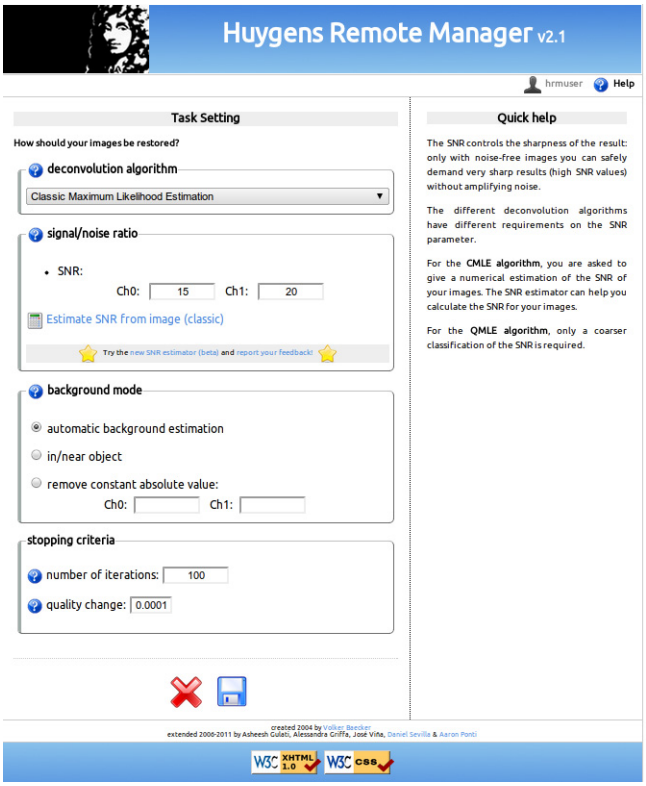


**FIGURE 2.15. The Restoration Parameter Set:** Deconvolution algorithm, SNR estimation, background mode and stopping criteria.

converge to a stable deconvolution result. Whether the convergence is accepted or further improved is controlled by the following parameters:

- *Number of iterations*: sets the maximum number of iterations that Huygens will compute.
- *Quality change*: how much the results of two consecutive iterations differ. If two subsequent results differ less than the Quality Change the convergence has been reached.

- *Signal to Noise ratio estimation*: The deconvolution process may in general increase the noise of the original images, as it restores the high frequencies. For this reason Huygens will correct for noise during the deconvolution process. The SNR parameter defines the degree of noise correction that will be performed and should be a measure of the noise of the original image. The user can assess which SNR value is best or let HRM estimate it automatically.

  For an automatic estimation click on "Estimate SNR from image". Then, select an image (see Figure 2.16) and click on the "CALCULATE" button, the SNR will be estimated for each channel of the selected image.

  The SNR estimation (per channel) will be shown along with four noise simulations with different SNR values. The noise simulations serve to confirm visually the correctness of the automatic SNR estimation. Move the mouse pointer over the different images to see them zoomed in.

  All images deconvolved with the same restoration parameter set should have similar Signal to Noise Ratios. This will be the case if 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 microscope set-

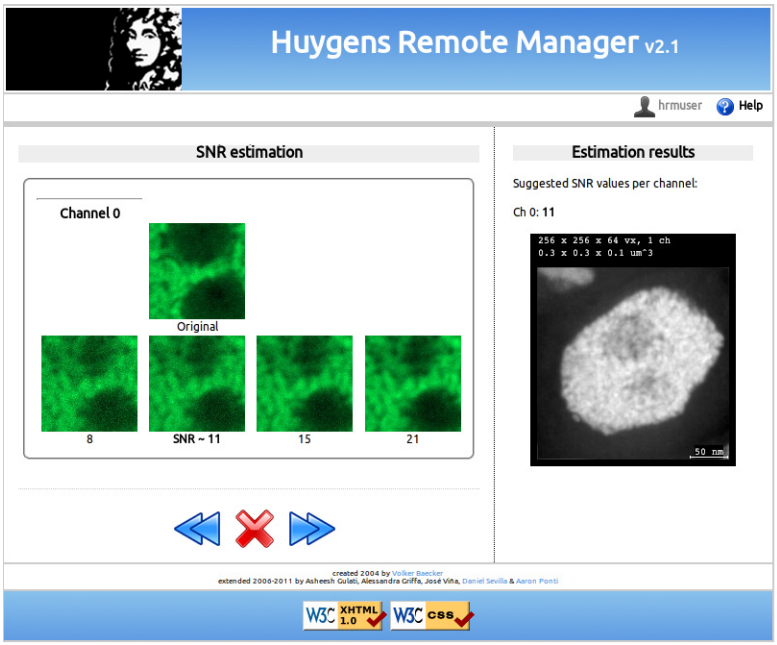


**FIGURE 2.16. The Restoration Parameter Settings.** The SNR estimator.

tings are changed or a different preparation is used the Signal to Noise Ratio should 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 missing, a higher SNR value can be used. On the other hand, if the result looks too grainy one can try to use a lower SNR value.

Since HRM 2.0.0 there exists a new **Beta SNR estimator** that aims at improving the accuracy of the classic SNR estimator. Both tools are currently available to estimate the SNR, though the Beta SNR estimator is being tested and improved. Its estimations in Widefield images and Confocal images free of baseline (black level) may already be accurate. The Beta SNR estimator may not yet find accurate results in confocal images that have a baseline or images that show strong clipping on the lower side of the intensity range.

The Restoration Parameter set is now ready. Upong saving it HRM will show the list of available restoraion parameter sets. Choose one and click on the big right arrow to continue to the next step (Select Images) (see Figure 2.14).

The image and restoration parameter sets can be reused to launch other deconvolution jobs with the same microscopic and processing properties.

### Select images

The selected image and restoration parameter sets can be used to process groups of images (Batch Deconvolution). It is important that these images have SNRs similar to the SNRs of the restoration parameter set. The images should also have similar background levels if the background option “remove constant absolute value” is selected in the restoration parameter set.

In the “Select Images” page the user’s images are filtered by file extension. If the Image Parameter set is specific for TIFF series only TIFF images will be listed.

When an image of the list is selected the right side panel is used for image previews. The user can click on “Click to generate preview” to see a thumbnail of the image.

To carry out the image selection transfer the selected images from the top panel “Images Available on Server” to the bottom panel “Selected images” by clicking on the little down arrow. The images will be added to a stack for batch deconvolution (See Figure 2.17). Then click on the big right arrow to continue to the last step “Create a job”.

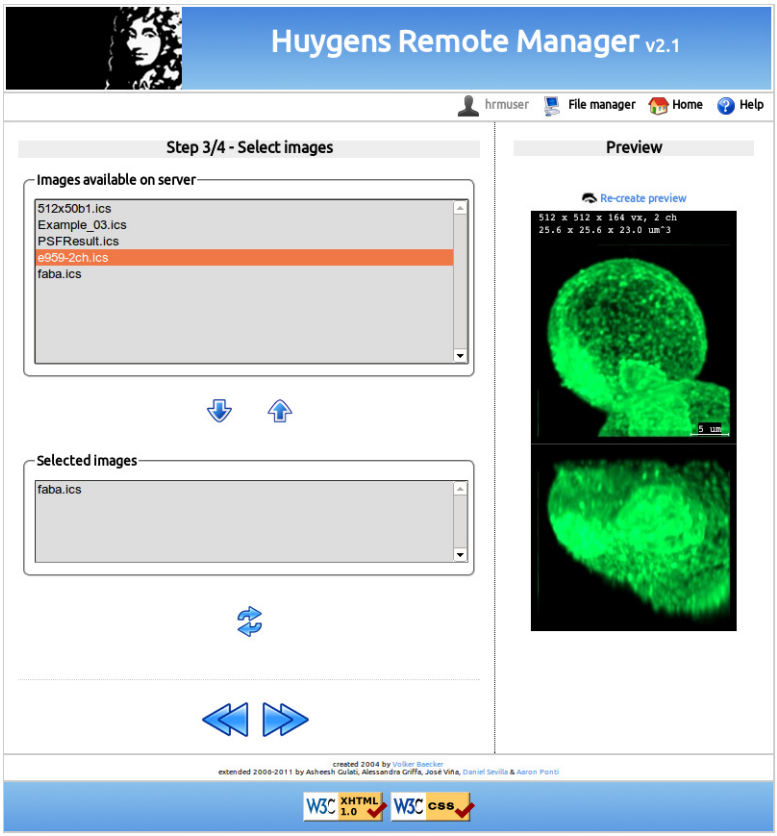


**FIGURE 2.17. Image Selection for batch deconvolution.** A preview of the selected image is displayed on the right side of the page.

### Create a job

In this last step several tables are shown listing the images and settings for the batch deconvolution.

The format of the output images can be selected here. As a guide 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 while it preserves all important details. For 2D imaging, when analysis is required, the TIFF-8bit can be used for output. This format is fine for analysis such as counting or for segmentation, but not for **quantification**. For 2D quantification 16-bit or 32-bit formats are recommended. For 3D vistualization with Huygens ICS, ICS2 or HDF5 are most appropriate.

To change the images or the settings of the Batch Deconvolution click on the corresponding links: “Image Parameters”, “Restoration Parameters”, “Selected Images”.

To launch the deconvolution click on the plug icon (See Figure 2.18). HRM will create one job per image and put it in the job queue.

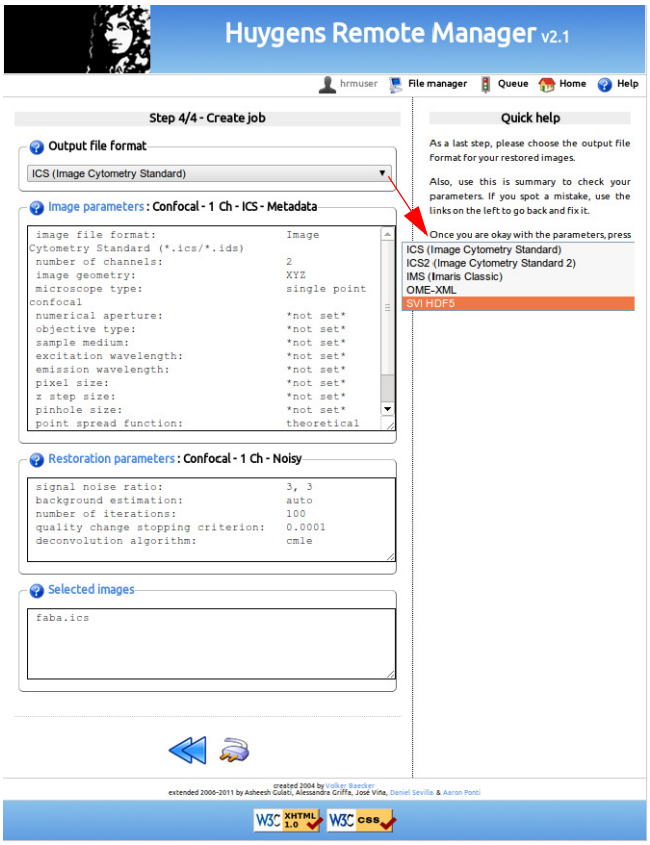


**FIGURE 2.18. Create a Job.** Output file format, summary page and job launch.

After launching the jobs HRM shows the main panel. Click on the Queue Status icon to examine the jobs status or to delete them if they are no longer needed.

## *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 waiting jobs are listed. The job currently processed is marked in green (See Figure 2.19).

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

When the deconvolution is finished, HRM will notify the user by e-mail. If an error occurs the user will also get a notification. In that case please contact the system administrator.

If something seems wrong, try to verify if there is 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.

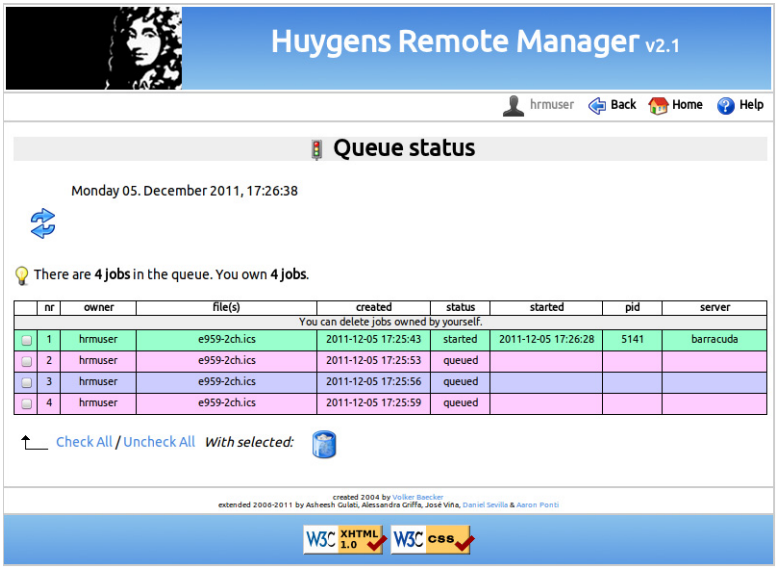


**FIGURE 2.19. The Job Queue.** The job being processed is coloured green. Highlighted in purple are the jobs waiting.

## *The File manager*

If the job has been finished the result can be examined online and also downloaded. For this purpose or to upload new images HRM gives access to a File Manager (main panel) (See Figure 2.2).

Within the File Manager there are two folder icons containing the raw data and the deconvolved images (See Figure 2.20). The raw data folder shows all the files uploaded by the user and available for deconvolution. More files can be uploaded from whithin the folder as well. The deconvolved images folder shows all the restored images. From within this folder the restored images can be examined, downloaded or deleted.

When clicking on the individual restored images their previews are displayed. An option to click for a more detailed view is also shown. The detailed view shows a number of tools to compare the deconvolution result with the original raw data. Namely, depending on the features of the image, a MIP[3] (Maximum Intensity Projection), an SFP[4](Simulated Fluorescence Process), a Slicer and a Stack movie which can all be downloaded. With these tools it can be examined online whether the deconvolution result is satisfactory.

Let us take a closer look at the image comparison tools. Figure 2.21 shows 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 original raw data and deconvolved image can also be compared as SFP (Simulated Fluorescence Process) rendered images (see Figure 2.22).

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

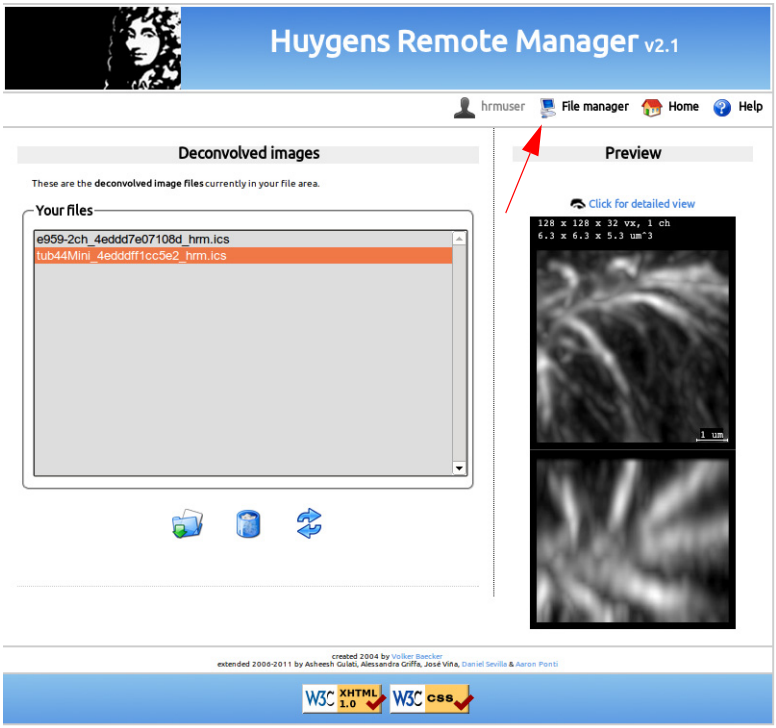


**FIGURE 2.20. The HRM File Manager.** Upload raw data, download deconvolved images, compare originals with results.

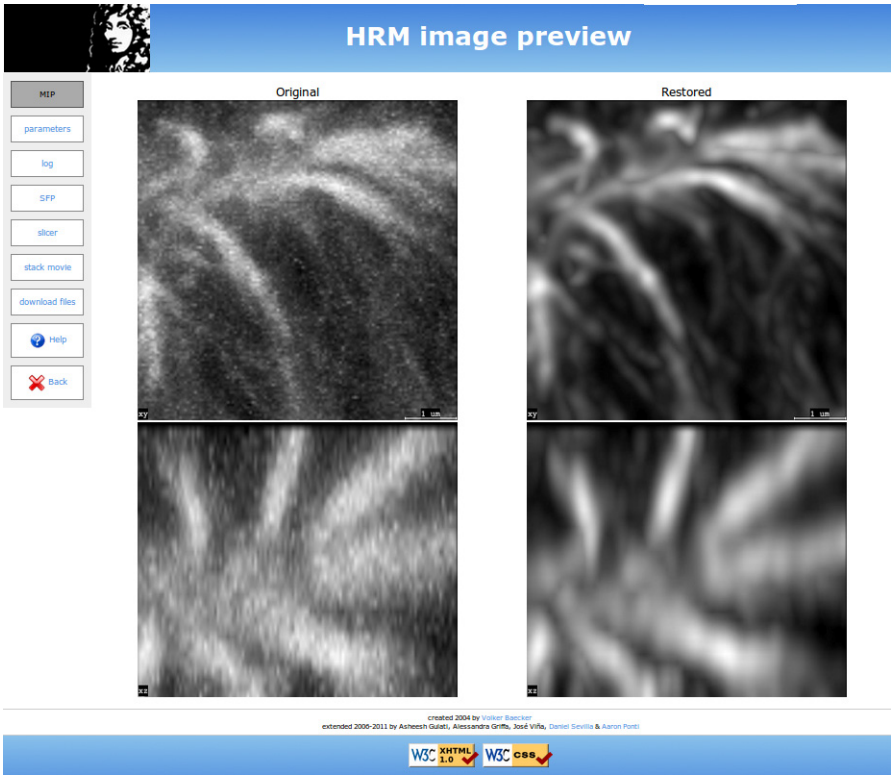


**FIGURE 2.21. The File Manager comparison tools.** A MIP, Maximum Intensity Projection, comparison between the original image and the deconvolution result.

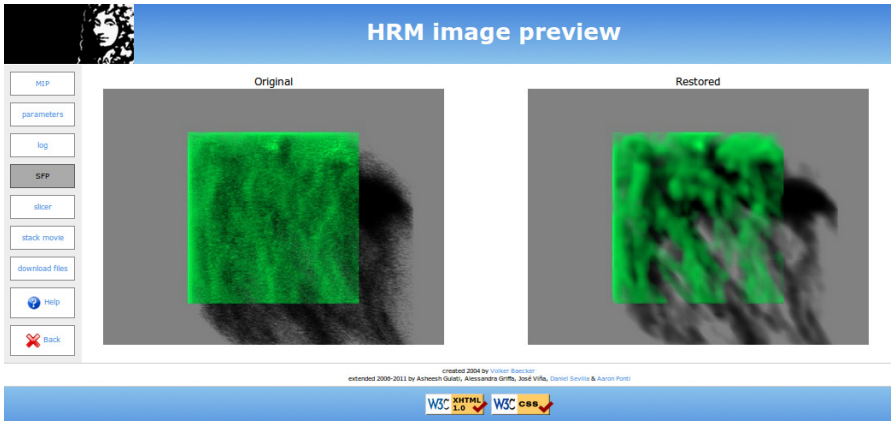


**FIGURE 2.22. The File Manager comparison tools.** An SFP, Simulated Fluorescence Process, comparison between the original image and the deconvolution result.

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 2.23).

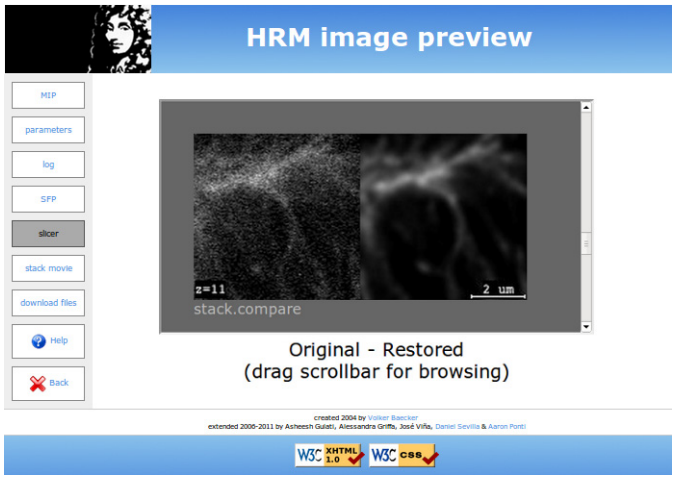


**FIGURE 2.23. The File Manager comparison tools.** The HMR Slicer showing a comparison of raw data and deconvolved data slice by slice.

Clicking on the Stack Movie link downloads a movie with the contents of the z slicer.

## *Deconvolution statistics*

The user statistics can be reached from the HRM starting page (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 used, the percentages of input formats, the type of Point Spread Function, the image geometry, the microscope type, and the time used for the user's deconvolution jobs.

All of this can be split according to the initial and final dates selected by the user to compute the statistics (See Figure 2.24).

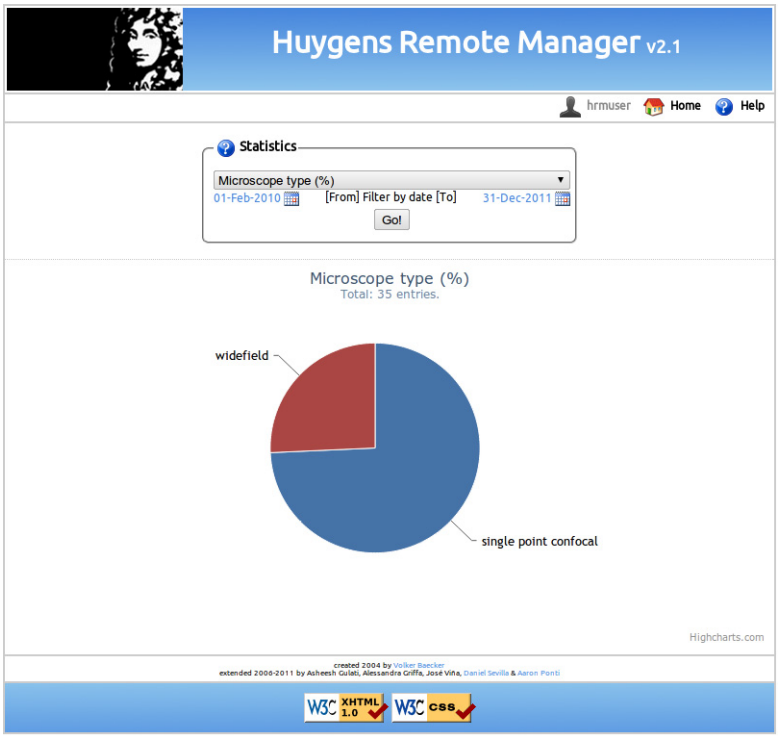


**FIGURE 2.24. Your statistics in HRM.** A straightforward way to check how HRM is being used and what image and restoration trends there are in the deconvolution jobs.