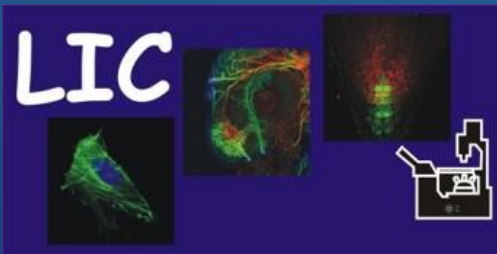




Tutorial - Basic

Huygens Remote Manager

Life Imaging Center 2020



Open - Huygens Remote Manager

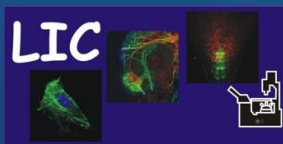
Specification: Huygens Core server (HRM) for deconvolution (256 GB RAM, 2 CPUs, 28 cores, 24 GB GPU VRAM, 6 GB SSD RAID 5, 28 TB storage RAID 5)

Access: <http://rod.lic.zbsa.privat/hrm>

- only accessible within the network of Freiburg University

Licenses available:

- Deconvolution for
 - Confocal
 - Multiphoton
 - Spinning disk
 - STED
 - Light-sheet
 - Widefield
 - Airyscan (ZEISS)
- Colocalization Analysis
- Light-sheet Fusion
- Object Stabilizer
- Time-series



Life Imaging Center

Huygens Remote Manager



Huygens Remote Manager

Help Resources Theme: dark light

User name Password **Log in** Reset

The Huygens Remote Manager is an easy to use interface to the Huygens Software by Scientific Volume Imaging B.V. that allows for multi-user, large-scale deconvolution and analysis.

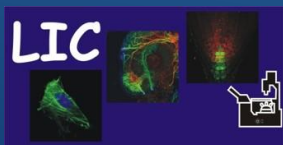
A collaboration of:

- MRI WBI**: Montpellier RIO Imaging National Center for Scientific Research Montpellier
- FMI**: Friedrich Miescher Institute for Biomedical Research
- EPFL**: ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE
- Scientific Volume Imaging Hilversum**: BioImaging and Optics platform EPF Lausanne
- DBSSE**: Single-Cell Facility ETH Zurich
- BIOZENTRUM**: University of Basel The Center for Molecular Life Sciences
- LIN**: Leibniz Institute for Neurobiology Magdeburg
- cni**: Combinatorial Neuroimaging Magdeburg
- UNI FR**: UNIVERSITÉ DE FRIBOURG UNIVERSITÄT FREIBURG
- miap**: Microscopy and Image Analysis Platform University of Freiburg
- MANCHESTER**: The University of Manchester
- UNI FR**: Bioimage | Light Microscopy Facility University of Fribourg
- UNI FR**: BiImaging Facility University of Manchester

Huygens Remote Manager v3.6

Login - Huygens Remote Manager

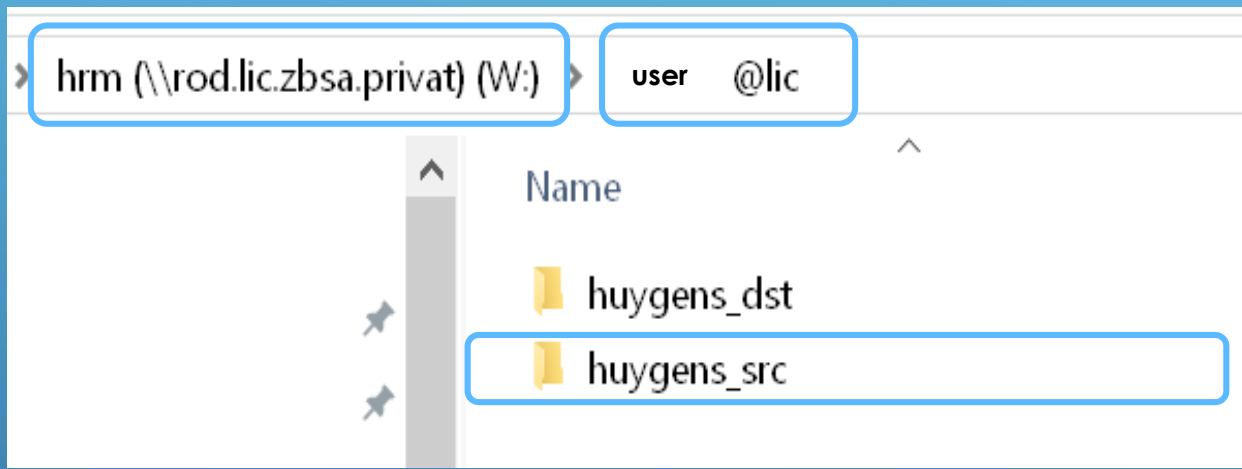
- **Login:** **username@lic** and **password**
- **IMPORTANT:**
 - Please use the same username and password as for your LIC login.
 - Additionally the username has to be supplemented with @ and the logon-domain - all in small letters (i.e. username@lic)



Life Imaging Center

Huygens Remote Manager





Upload Imaging Data to Huygens Remote Server

Down- and Upload image data to a special **network drive W**

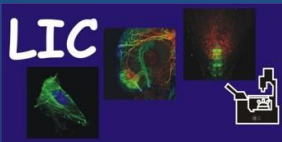
After your first login on the HRM you will find a folder with your username on **drive W**: containing

- (**huygens_dst**) – folder for restored data
- (**huygens_src**) - folder for raw data

Copy your raw image data into the **source folder (scr)**

After completion of your HRM job always clean up the disc space

- Remove raw and processed data
- We have only limited storage disk space!



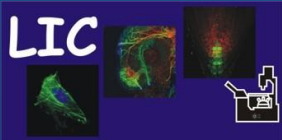
Deconvolution Process in Short !

You have no Parameter and Restoration Templates

- Step A: Launch a Job, then select image files and create and define Image Parameter Settings:
 - Write the specific properties of your images into a template, save this template and select it
- Step B: Restoration settings:
 - Write the preferred deconvolution settings in a template, save this template and select it
- Step C. Start deconvolution – Start JOB

You have Parameter and Restoration Templates

- Step A: Start a Job, then select images files:
 - Select the images and the corresponding templates and go through the process.
- Step B: Start deconvolution - Start JOB



Life Imaging Center

Huygens Remote Manager





Welcome!



Launch jobs

Create and start restoration and analysis jobs.



Raw images

Upload raw images to deconvolve.



Statistics

Summary of your usage statistics.



Queue status

See all jobs.
You have no jobs in the queue.



Results

Inspect and download your restored data and analysis results.

Huygens Remote Manager v3.6

Welcome Page - Huygens Remote Manager

After Login you enter the **Home Panel – Welcome Page**

- To manage here the deconvolution jobs as well as other HRM administrative tasks

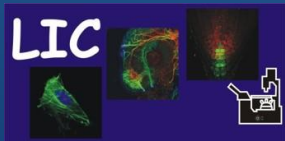
i.e. following shortcuts are available:

Launch a job

Here you create the image parameter and restoration templates

Raw images

Here you could see you data on Drive W, we do not use the integrated upload function



Life Imaging Center

Huygens Remote Manager





Step 1/5 - Select images

? Image file format

Carl Zeiss Image (*.czi)

Images available on server

Fluo-cells_z-stack_for_Deconvolution.czi (Fluo-cells_z-stack_for_Deconvolution:CziUnknow)

When applicable, load file series automatically



Selected images



Quick help

Here you can select the files to be restored from the list of available images. The file names are filtered by the selected file format. Use SHIFT- and CTRL-click to select multiple files.

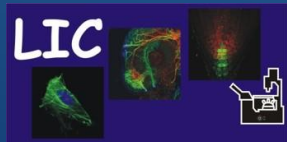
Where applicable, the files belonging to a series can be condensed into one file name by checking the 'autoseries' option. These files will be loaded and deconvolved as one large dataset. Unchecking 'autoseries' causes each file to be deconvolved independently.

Click on a file name in any of the fields to get (or to create) a preview.

Step 1/5 - Select Images

- **Select** the **file format** of the images you want to process
- **Activate automatically loading**

You always get on the right side of the Huygens software a short **help menu**, placing the mouse pointer over one program icon - the specific help is visible on the right side



Life Imaging Center

Huygens Remote Manager





Step 1/5 - Select images

Preview

Image file format

Carl Zeiss Image (*.czi)

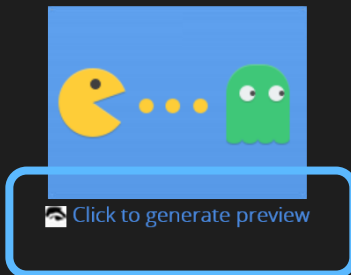
Images available on server

Fluo-cells_z-stack_for_Deconvolution.czi (Fluo-cells_z-stack_for_Deconvolution:CziUnknow

When applicable, load file series automatically

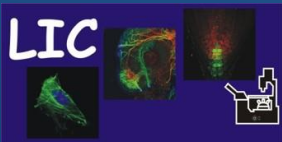


Selected images



Step 1/5 - Select Images

- You can generate a **Preview of your data** by using the link 'click to generate preview'



Life Imaging Center

Huygens Remote Manager





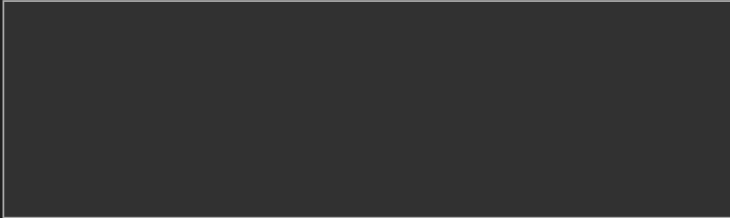
Step 1/5 - Select images

Preview

? Image file format

Carl Zeiss Image (*.czi)

Images available on server



When applicable, load file series automatically



Selected images

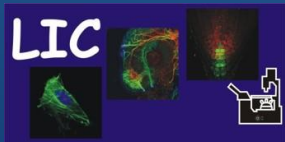
Fluo-cells_z-stack_for_Deconvolution.czi (Fluo-cells_z-stack_for_Deconvolution:CziUnknow



Click to generate preview

Step 1/5 - Select Images

- Move data to the 'selected images' folder
 - Use the Down Arrow and Up Arrow icons to add/remove images to/from the selection
- You can move several files, important image acquisition conditions and parameters have to be the same for all
- Go on



Life Imaging Center

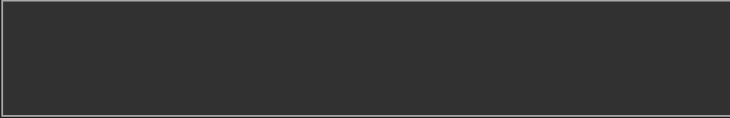
Huygens Remote Manager





Admin image templates

These are the image templates prepared by your administrator.

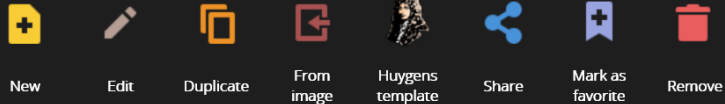


Your image templates

These are your (private) image templates.

Based on Cos7 RFP Mitochondria 63xw Time series of Z stack
 Based on cos7_cells_63xwater_PM_red_golgi_green_nucleus_blue_stack
 Based on LifeAct2cell1_ebene7
 Based on LM1A Red non descanned 512x512 z stack
 Based on Mark and find 005 6 Positionen 6 Channel
 Z1 20x clearing Left
 Z1 20x clearing Right

Template actions:



Placing the mouse pointer over the various icons will display a tooltip with explanations.

For a more detailed explanation on the possible actions, please follow the [? Help](#) link in the navigation bar.

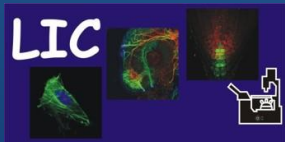
In this step, you are asked to specify all parameters relative to the images you want to restore.

These include: file information (e.g. voxel size); microscopic parameters (such as microscope type, numerical aperture of the objective, fluorophore wavelengths); whether a measured or a theoretical PSF should be used; whether depth-dependent correction on the PSF should be applied.

'Admin image templates' created by your facility manager can be copied to the list of 'Your image templates' and adapted to fit your specific experimental setup.

Step 2/5 - Image Parameters - Create Image Template

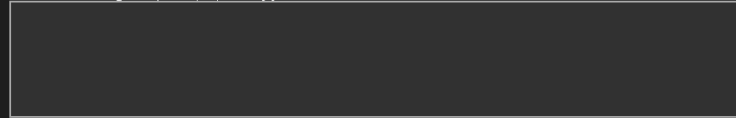
- Specify the **imaging parameters** used during image acquisition
 - These include :
 - File information (geometry, voxel size)
 - Microscope parameters such as Microscope type, numerical aperture of the objective, fluorophore wavelength,....





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These are your (private) image templates.

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 Based on cos7_cells_63xwater_PM_red_golgi_green_nucleus_blue_stack
 Based on LifeAct2cell1_ebene7
 Based on LM1A Red non descanned 512x512 z stack
 Based on Mark and find 005 6 Positionen 6 Channel
 Z1 20x clearing Left
 Z1 20x clearing Right

Placing the mouse pointer over the various icons will display a tooltip with explanations.

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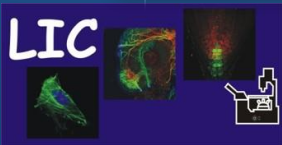
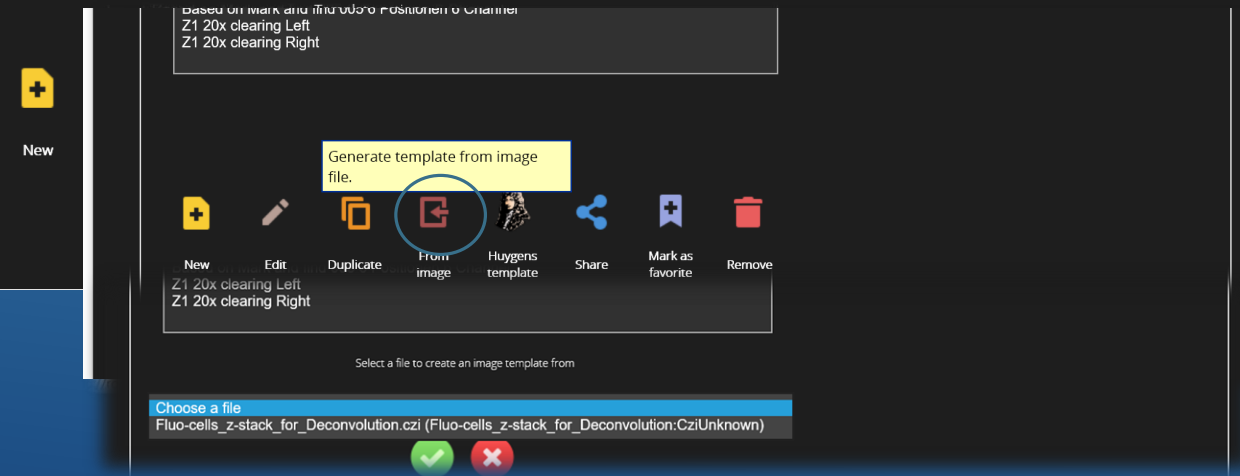
In this step, you are asked to specify all parameters relative to the images you want to restore.

These include: file information (e.g. voxel size); microscopic parameters (such as microscope type, numerical aperture of the objective, fluorophore wavelengths); whether a measured or a theoretical PSF should be used; whether depth-dependent correction on the PSF should be applied.

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Step 2/5 - Image Parameters - Create Image Template

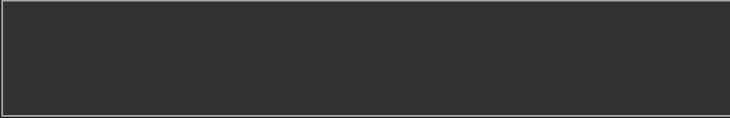
- To create a new Image Parameter Template you have different options:
 - Create a new one
 - Edit and change an old one
 - Duplicate and edit the new duplicate
 - Generate template from image file, will read meta data and use this as presets in new template





Admin image templates

These are the image templates prepared by your administrator.



Your image templates

These are your (private) image templates.

Based on Cos7 RFP Mitochondria 63xw Time series of Z stack
 Based on cos7_cells_63xwater_PM_red_golgi_green_nucleus_blue_stack
 Based on LifeAct2cell1_ebene7
 Based on LM1A Red non descanned 512x512 z stack
 Based on Mark and find 005 6 Positionen 6 Channel
 Z1 20x clearing Left
 Z1 20x clearing Right

Placing the mouse pointer over the various icons will display a tooltip with explanations.

For a more detailed explanation on the possible actions, please follow the [? Help](#) link in the navigation bar.

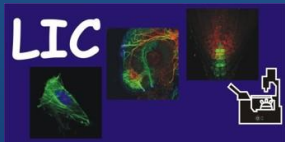
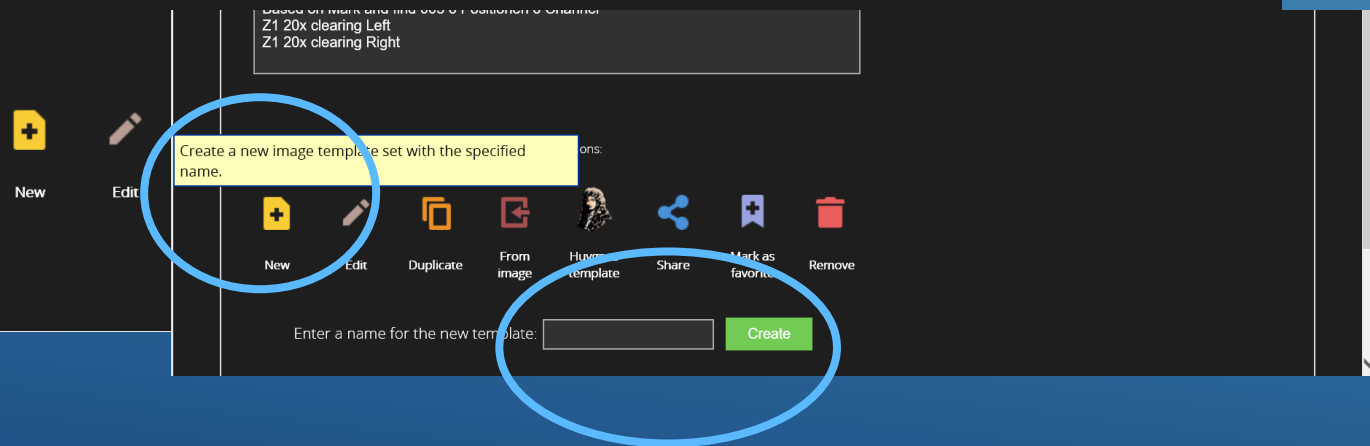
In this step, you are asked to specify all parameters relative to the images you want to restore.

These include: file information (e.g. voxel size); microscopic parameters (such as microscope type, numerical aperture of the objective, fluorophore wavelengths); whether a measured or a theoretical PSF should be used; whether depth-dependent correction on the PSF should be applied.

'Admin image templates' created by your facility manager can be copied to the list of 'Your image templates' and adapted to fit your specific experimental setup.

Step 2/5 - Image Parameters - Create Image Template

- Here we **create** a **NEW One from scratch !!!**
 - Click the button **'New'**.
 - Type a **name** for the new template
 - Click **'Create'**. The template editor opens



Number Of Channels

How many channels (wavelengths) in your datasets?

1 2 3 4 5 6

PSF

Would you like to use an existing measured PSF obtained from bead images or a theoretical PSF generated from explicitly specified parameters?

Theoretical Measured

Here you are asked to define the number of channels in your data and whether you want to use a theoretical PSF or a measured PSF you distilled with the Huygens Software.

Parameter requirements
adapted for czi files



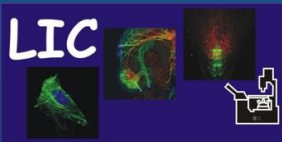
Name	Fluo-cells_z-stack_for_Deconvolution
Description	
Acquisition Date	3/13/2019 16:30:35
Notes	
User	schulung
Scaling X	0.085 µm
Scaling Y	0.085 µm
Scaling Z	0.350 µm
Dimensions	x: 1000, y: 1000, z: 13, channels: 3, 12-bit
Image size	k: 85.02 µm, y: 85.02 µm, z: 4.20 µm
Scan Mode	stack
Zoom	2.5
Objective	Plan-Apochromat 40x/1.4 Oil DIC M27
Position (x,y,z)	Position 1: x: -22806.2 µm, y: -2421.6 µm, z: -30.5 µm
Pixel dwell	0.44 µs
Average	line 2
Master gain	488 CH1: 757 561 CH2: 700 405 CH3: 800
Digital gain	488 CH1: 1.00 561 CH2: 1.00 405 CH3: 1.00
Digital offset	488 CH1: 0.00 561 CH2: 0.00 405 CH3: 0.00
Pinhole	488 CH1: 53 µm 561 CH2: 253 µm 405 CH3: 601 µm
Filters	488 CH1: 508 - 553 561 CH2: 597 - 695 405 CH3: 415 - 480
Beam splitters	MBS : MBS 488/561/633 MBS_inVis : MBS 690+ DBS1 : Mirror
Lasers	488 488 nm : 0.8000 % 561 561 nm : 0.8000 % 405 780 nm : 1.0 %

Step 2/5 - Image parameters - Create Image Template

Specify the imaging conditions used during acquisition

- Select the correct **number of channels**
- **Select PSF type** - because you have not measured a PSF with Beads in your sample use – **theoretical** PSF

If you are not sure how you have acquired your data, open your data file in the Acquisition software to look up the parameter



Microscope Type

How did you set up your microscope?

- array detector confocal
- multipoint confocal (spinning disk)
- single point confocal
- SPIM
- STED
- two photon
- widefield

Numerical Aperture

Cannot be read from file, please fill in.

• NA:

Wavelengths

Cannot be read from file, please fill in.

• excitation (nm):

Ch0: Ch1:

• emission (nm):

Ch0: Ch1:

Objective Type

Cannot be read from file, please fill in.

- air [1.0]
- glycerol [1.4729]
- oil [1.515]
- water [1.3381]
-

Sample Medium

Cannot be read from file, please fill in.

- liquid vectashield / 90-10 (v.v) glycerol - PBS ph 7.4 [1.47]
- water / buffer [1.339]
-

Parameter requirements
adapted for czi files

Step 2/5 - Image Parameters - Create Image Template

- Select the **correct microscope type**
- Specify the **numerical aperture** of the objective
- Specify the **wavelength** of each channel and **emission maximum peak**
- Specify the **Objective type**
- Select **Refractive Index of Embedding Medium**
 - Help about the refractive index of the sample embedding medium can be found in the literature of the mounting or can be measured at LIC

Voxel Size

How were these images captured?

Leave blank to read from file during deconvolution.

XY pixel size (nm) Z-step (nm):

Set to Nyquist rate in Z for 2D datasets.

Calculated from current optical parameters, the (Nyquist) ideal pixel size is 48 nm and the ideal z-step is 130 nm.

+ Online Nyquist rate and PSF calculator

Time Interval

Leave blank to read from file during deconvolution.

• Time interval (s): Set to 0 for a single time point.

Backprojected Pinhole Radius

Cannot be read from file, please fill in.

• backprojected pinhole radius (nm):

Ch0: Ch1:

+ Backprojected pinhole calculator



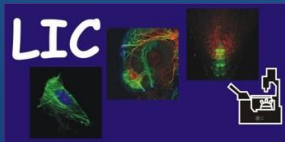
Huygens Remote Manager v3.6

The pinhole of confocal microscopes is a small hole used to get rid of out-of-focus light, thus allowing the recording of real 3D images. Huygens needs this information to properly calculate the PSF of the system. It is important to realize, however, that the Huygens software expects the back-projected pinhole size, and not its physical size. Please use the calculator to obtain the correct size for your microscope.

Parameter requirements
adapted for czi files

Step 2/5 - Image Parameters - Create Image Template

- Remember you can find all asked values when you open your data file in the Acquisition software
- If your raw data are **no time series** use **0** for the interval.
- Open the Backprojected pinhole Calculator** to get the correct values



Step 2/5 - Image Parameters - Create Image Template

Backprojected Pinhole Calculator

- Choose the microscope type from the list
 - To check the used microscope type, go to www.miap.eu
- Follow the instructions of the Quick help
- Write down and go on to transfer the value to the main sheet

Backprojected pinhole calculator

Quick help

Please choose a microscope from the following list:

- Biorad MRC 500, 600 and 1024
- Biorad Radiance
- Leica confocal TCS 4d (parameter P_8)
- Leica confocals TCS 4d, SP1 and NT (Airy disk units)
- Leica confocal SP2
- Leica confocal SP5
- Leica confocal SP8
- Nikon TE2000-E with the C1 scanning head
- Nikon TIE A1R
- Olympus FV300 and FVX

- O
- O
- Yc
- Yc
- Zt
- Zt
- Zt
- Zt
- Zt
- N

Backprojected pinhole calculator

Quick help

Zeiss 780

The following forms will assist you in calculating the (circular-equivalent) backprojected pinhole radius expressed in nanometers, that you can enter directly in the HRM settings.

Please mind that there is one special entry to calculate pinhole distances in spinning disks.

Click Help on the top menu for more details.

Start by selecting your microscope model from the list on the left, or click on cancel at the bottom to go back.

Enter or confirm the requested values and press the calculator button to calculate the **back projected** pinhole radius. Press the back button to go back to the list of microscopes.

Read more about the [Zeiss_780](#) model.

Enter the parameters as reported by your microscope:

Pinhole diameter (microns)

Objective magnification

Backprojected pinhole calculator

Quick help

Backprojected pinhole radius (nm): 392.32

This is the parameter list used in this calculation:

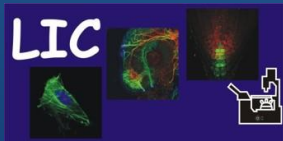
Microscope model: Zeiss_780
Pinhole diameter (microns): 53
Objective magnification: 40
Internal system magnification: 1.9048
Shape factor: 0.564

On the left you can see the result of the calculation and the parameters used for it. Please annotate the calculated value and enter it in the image parameter pages. The value will not be transferred automatically.

You can repeat the calculation with different input values (e.g. for other channels) or proceed to the image parameter pages.



Huygens Remote Manager v3.6



Life Imaging Center

Huygens Remote Manager



Voxel Size

How were these images captured?

Leave blank to read from file during deconvolution.

XY pixel size (nm)

Z-step (nm):

Set to Nyquist rate in Z for 2D datasets.

Calculated from current optical parameters, the (Nyquist) ideal pixel size is 43 nm and the ideal z-step is 130 nm.

[Online Nyquist rate and PSF calculator](#)

Time Interval

Leave blank to read from file during deconvolution.

Time interval (s): Set to 0 for a single time point.

Backprojected Pinhole Radius

Cannot be read from file, please fill in.

backprojected pinhole radius (nm):

Ch0: Ch1:

[Backprojected pinhole calculator](#)



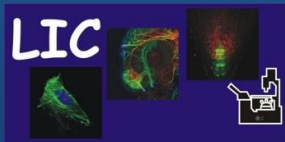
Huygens Remote Manager v3.6

The pinhole of confocal microscopes is a small hole used to get rid of out-of-focus light, thus allowing the recording of real 3D images. Huygens needs this information to properly calculate the PSF of the system. It is important to realize, however, that the Huygens software expects the back-projected pinhole size, and not its physical size. Please use the calculator to obtain the correct size for your microscope.

Parameter requirements
adapted for czi files

Step 2/5 - Image Parameters - Create Image Template

- Insert the **calculated radius** values of the backprojected pinhole
- Go on



The selected objective type and sample medium produce spherical aberration in the image (refractive index mismatch).

Sample Orientation

To remove the aberration please specify the relative position of the coverslip with respect to the first acquired plane of the dataset.

Visualize the image

Plane 0 is closest to the coverslip

Correction Mode

Please note that in certain circumstances, the automatic correction might generate artifacts in the result. If this is the case, please choose the advanced correction mode.

Perform automatic correction



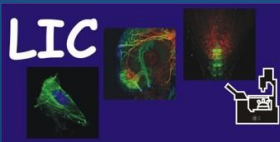
The main cause of spherical aberration is a mismatch between the refractive index of the lens immersion medium and specimen embedding medium and causes the PSF to become asymmetric at depths of already a few μm . SA is especially harmful for widefield microscope deconvolution. The HRM can correct for SA automatically, but in case of very large refractive index mismatches some artifacts can be generated. Advanced parameters allow for fine-tuning of the correction.

Parameter requirements
adapted for czi files

Huygens Remote Manager v3.6

Step 2/5 - Image Parameters - Create Image Template

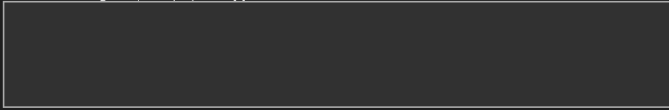
- To remove spherical aberration created by objective type and sample medium **define the orientation of sample plane 0 to the coverslip**
- Perform **automatic correction**
- Finish** Template creation.





Admin image templates

These are the image templates prepared by your administrator.



Your image templates

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Based on Cos7 RFP Mitochondria 63xw Time series of Z stack
Based on cos7_cells_63xwater_PM_red_golgi_green_nucleus_blue_stack
Based on LifeAct2cell4_chan07

Based on LM1A Red non descanned 512x512 z stack
Based on Mark and find 005 6 Positionen 6 Channel
for Manual Template 001
Z1 20x clearing Left
Z1 20x clearing Right

Template actions:



New



Edit



Duplicate



From image



Huygens template



Share



Mark as favorite



Remove



Placing the mouse pointer over the various icons will display a tooltip with explanations.

For a more detailed explanation on the possible actions, please follow the [Help](#) link in the navigation bar.

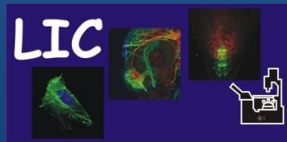
In this step, you are asked to specify all parameters relative to the images you want to restore.

These include: file information (e.g. voxel size), microscopic parameters (such as microscope type, numerical aperture of the objective, fluorophore wavelengths); whether a measured or a theoretical PSF should be used; whether depth-dependent correction on the PSF should be applied.

'Admin image templates' created by your facility manager can be copied to the list of 'Your image templates' and adapted to fit your specific experimental setup.

Step 2/5 - Image Parameters - Create Image Template

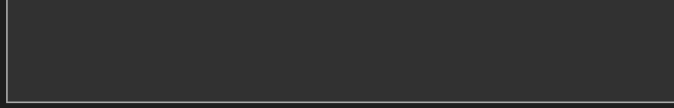
- **Select** your new Image Parameter Template
- Go on...





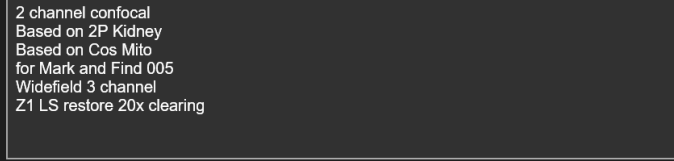
Admin restoration templates

These are the restoration templates prepared by your administrator.

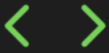


Your restoration templates

These are your (private) restoration templates.

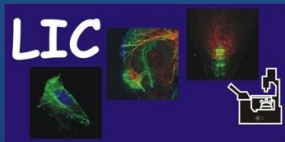


Template actions:



Step 3/5 - Restoration Parameters - Create Restoration Template

- Now create a **Restoration template**
 - specify parameters relative to the restoration of you image (choice of deconvolution algorithm, signal-to-noise ratio, background estimation)
- As before for the Image Parameter Template **you have different options**
- Now we **Create New as before with a new name**



How should your images be restored?

Deconvolution Algorithm

Classic Maximum Likelihood Estimation

Signal/Noise Ratio

SNR:

Ch0: 12 Ch1: 15

Estimate SNR from image

Cropping Mode

Do not crop the image

Background Mode

 automatic background estimation in/near object remove constant absolute value:

Ch0: Ch1:

Stopping Criteria

number of iterations: 40

quality change: 0.05



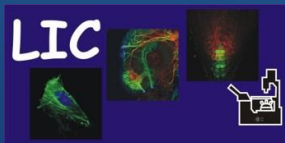
Huygens Remote Manager v3.6

On this page you specify the parameters for restoration.

These parameters comprise the deconvolution algorithm, the signal-to-noise ratio (SNR) of the images, the mode for background estimation, and the stopping criteria.

Step 3/5 - Restoration Parameters - Create Restoration Template

- Define the **Deconvolution Algorithm**
 - The **CMLE** is the most default general deconvolution algorithm available in Huygens, that provides good results for a variety of imaging conditions (**confocal, widefield**).
 - The **GMLE** is a deconvolution algorithm optimized to handle high-noise images, as **STED** or confocal images.
 - The **QMLE** is a deconvolution algorithm that gives good and fast deconvolution results on **low-noise widefield images**.



How should your images be restored?

Deconvolution Algorithm

Classic Maximum Likelihood Estimation

Signal/Noise Ratio

• SNR:

Ch0: Ch1:

+ Estimate SNR from image

Cropping Mode

Do not crop the image

Background Mode

 automatic background estimation in/near object remove constant absolute value:Ch0: Ch1:

Stopping Criteria

number of iterations: quality change: 

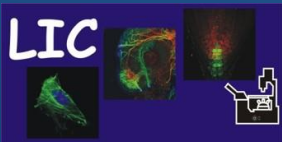
Huygens Remote Manager v3.6

On this page you specify the parameters for restoration.

These parameters comprise the deconvolution algorithm, the signal-to-noise ratio (SNR) of the images, the mode for background estimation, and the stopping criteria.

Step 3/5 - Restoration Parameters - Create Restoration Template

- Estimate the **Signal to Noise Ratio** with the integrated tool - **follow the instructions of the estimator**
 - The Signal-to-Noise ratio (SNR or S/N) controls the sharpness of the restoration result- as higher this value, as sharper the restored image will be.
 - Using a too large SNR value might be risky when restoring noisy originals, because you could be just enhancing the noise.
 - A **noise-free widefield image** usually has SNR values higher than **50**.
 - A **noisy confocal image** can have values lower than **20**
 - A noisy **STED** values below **12**



How should your images be restored?

Deconvolution Algorithm

Classic Maximum Likelihood Estimation

Signal/Noise Ratio

• SNR:

Ch0: Ch1: Estimate SNR from image**Cropping Mode**

Do not crop the image

Background Mode automatic background estimation in/near object remove constant absolute value:Ch0: Ch1: **Stopping Criteria** number of iterations: quality change: 

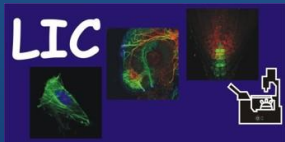
Huygens Remote Manager v3.6

On this page you specify the parameters for restoration.

These parameters comprise the deconvolution algorithm, the signal-to-noise ratio (SNR) of the images, the mode for background estimation, and the stopping criteria.

Step 3/5 - Restoration Parameters - Create Restoration Template

- Set the **background** mode to **automatic**



How should your images be restored?

Deconvolution Algorithm

Classic Maximum Likelihood Estimation

Signal/Noise Ratio

SNR:

Ch0: 12 Ch1: 15

Estimate SNR from image

Cropping Mode

Do not crop the image

Background Mode

 automatic background estimation in/near object remove constant absolute value:

Ch0: Ch1:

Stopping Criteria

number of iterations: 40

quality change: 0.05



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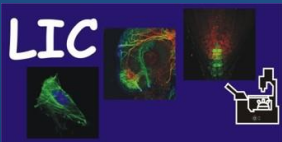
On this page you specify the parameters for restoration.

These parameters comprise the deconvolution algorithm, the signal-to-noise ratio (SNR) of the images, the mode for background estimation, and the stopping criteria.

Step 3/5 - Restoration Parameters - Create Restoration Template

- Define the **stopping criteria** of the algorithm
 - The **Maximum Number of Iterations** is a Restoration Parameter of the Huygens Software that puts a limit to the iterative deconvolution.
 - Another limit is established by the **Quality Change Threshold parameter**.
 - For an initial run you can use 40 iteration and quality change 0.05.**

To achieve best result you can increase or decrease values



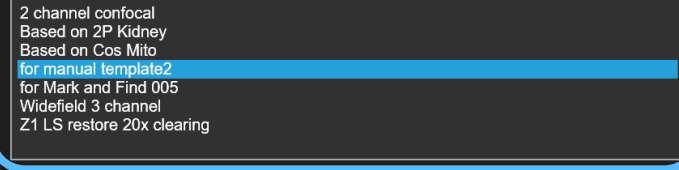
Admin restoration templates

These are the restoration templates prepared by your administrator.



Your restoration templates

These are your (private) restoration templates.



Template actions:



Step 3/5 - Restoration Parameters - Create Restoration Template

- **Select the new Restoration Template**
- Go on

Step 4/5 - Analysis Template

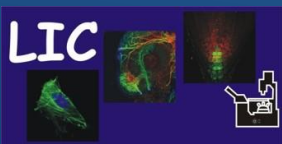
- This step is only active when 2 or more channels have been specified in the previous steps - allows the user to execute Colocalization analysis on the deconvolved images

- Select/create a template or skip**

- Created** with i.e. Click ' **New** ', **type** a name and click ' **Create** '.

- Select whether colocalization should be enabled. For a colocalization run select the channels to be analyzed as well as the colocalization coefficients of interest. Set the threshold to "Automatic estimation" and the Colocalization map to "Pearson". In order to further fine-tune the Colocalization parameters please refer to Advanced deconvolution in HRM.

- Go to next step



Template actions:



New



Edit



Duplicate



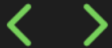
Share



Mark as favorite



Remove



Step 4/5 - Select analysis template

Quick help

Admin analysis templates

These are the analysis templates prepared by your administrator.

A large, empty rectangular box with a thin grey border, intended for displaying administrator-prepared analysis templates.

In this step, you are asked to specify all parameters relative to the analysis of your images.

These are the choices for colocalization analysis, colocalization coefficients and maps.

'Admin analysis templates' created by your facility manager can be copied to the list of 'Your analysis templates' and adapted to fit your analysis needs.

Optional step
Leave selection empty to skip analysis.

Your analysis templates

These are your (private) analysis templates.

A large rectangular box with a thin grey border, containing the text 'coloc no'. The box is highlighted with a blue border in the original image.

New



Edit



Duplicate



Share



Mark as favorite



Remove



Output file format

IMS (Imaris Classic)

Image parameters : for manual template 001

number of channels:	2
microscope type:	single point confocal
numerical aperture:	1.4
objective type:	oil
sample medium:	1.46

Processing parameters : for manual template2

autocrop:	no
signal noise ratio:	12, 15
background estimation:	auto
number of iterations:	40
quality change stopping criterion:	0.05

Analysis parameters :

coloc analysis: no

Selected images

Fluo-cells for manual.czi (Fluo-cells for manual:CziUnknown)



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As a last step, please choose the output file format for your restored images.

Please notice that some output file formats (specifically all TIFF options) are disabled if you set a time interval larger than 0 or you enabled the "When applicable, load file series automatically" option in Step 1 - Select images.


Also, use this as summary to check your parameters. If you spot a mistake, use the links on the left to go back and fix it.


Once you are okay with the parameters, press the launch job button to add the job to the queue and go back to the home page.

Please notice that it is not possible to save multichannel datasets in TIFF-16 bit format.

Step 5/5 – Launch a Job




- As last step **Define Output file format** for the restored images
 - ICS** recommended format - provides good dynamic range and necessary meta data infrastructure to save all the image parameters - creates more than one file for each restored image, all have to be saved and/or copied!
 - IMS** (Imaris classic) – also ok, creates only one file for each restored image, but always only 8bit images
 - Further details: <https://svi.nl/FileFormats>
- Control parameter** summaries as well as the image selection
- Then **start job**

 Queue status


 Yours is the only job in the queue.


Last update: 12:22:29

nr	owner	file(s)	created	status	started	pid	server
You can delete jobs owned by yourself.							
<input type="checkbox"/>	1	an1003@lic	Fluo-cells for manual.czi (Fluo-cells for manual.CziUnknown)	2020-09-28 12:22:27	queued		

 Check All /  Uncheck All *With selected:* 




Huygens Remote Manager v3.6

 Queue status

 Yours is the only job in the queue.

Last update: 12:22:59

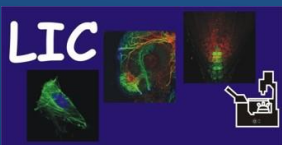
nr	owner	file(s)	created	status	started	pid	server
You can delete jobs owned by yourself.							
<input type="checkbox"/>	1	an1003@lic	Fluo-cells for manual.czi (Fluo-cells for manual.CziUnknown)	2020-09-28 12:22:27	started	2020-09-28 12:22:34	18071 localhost 0

 Check All /  Uncheck All *With selected:* 

Huygens Remote Manager v3.6

Queue Status

- After submitting the job HRM returns to the Home panel.
 - Click **Queue Button** in **Home panel - Welcome Page**
- Deconvolution will run automatically and sequentially if there are jobs of other user too.
 - When a job is finished a notification email is sent to you with a link to the result.



Results
Quick help

Your result files

These are the processed image files currently in your file area.

Fluo-cells_for_manual_czi_5f71b96328ce2_hrm.ims

↓
🗑️
↻

Huygens Remote Manager v3.6

Click on a file name to see a preview.

Click on Detailed View over the file preview to access previews, reports and analysis results.

Select the files you want to download (you can SHIFT- and CTRL-click for multiple selection) and press the download icon to compress the files into an archive and start the download process. (Please mind that large files may take a long time to be packaged before downloading.)

Use the delete icon to delete the selected files instead.

Move your mouse pointer over the action buttons at the bottom to redisplay this help.

Restored Images

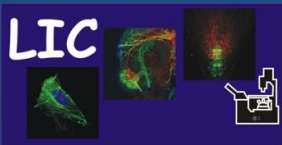
- **Access to the restored images** via HRM home panel - click on – ‘**Result Button**’

or

- Via **File explorer** on **you computer** - File manager: data on drive W/username@lic/: **huygens_dst** (in this case an Imaris file)

zbsa.privat (W:) > an1003@lic > huygens_dst
Search huygens_dst

Name	Date modified	Type	Size
📁 hrm_previews	28.09.2020 12:22	File folder	
📄 Fluo-cells_for_manual_czi_5f71b96328ce2_hrm.hgsb	28.09.2020 12:22	HGSB File	20 KB
📄 Fluo-cells_for_manual_czi_5f71b96328ce2_hrm	28.09.2020 12:22	Imaris Image File	25.392 KB
📄 Fluo-cells_for_manual_czi_5f71b96328ce2_hrm.log	28.09.2020 12:23	Text Document	13 KB
📄 Fluo-cells_for_manual_czi_5f71b96328ce2_hrm.parameters	28.09.2020 12:22	Text Document	7 KB



Life Imaging Center

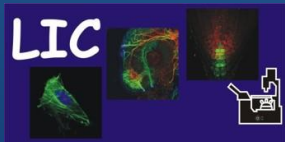
Huygens Remote Manager



Thanks for viewing

For further information

[Detailed - Online HRM - User Manual](#)



Life Imaging Center

Huygens Remote Manager

