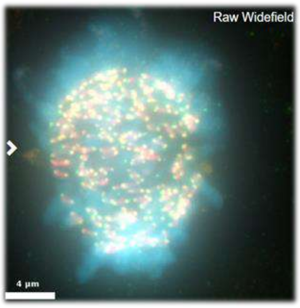
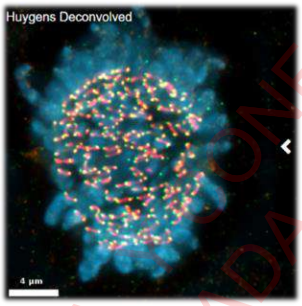
Huygens Professional Deconvolution Guide

- ### What is Deconvolution?

Deconvolution is a computational technique applied to images to compensate for the optical limitations of the system used in their acquisition by reducing the out-of.-focus blurring. The use of different Deconvolution algorithms is particularly effective when processing data obtained in Widefield Microscopy, Laser Scanning Confocal Microscopy (LSM), Spinning Disk Confocal Microscopy, and Super-resolution Microscopy…

"A Practical Guide to Deconvolution of Fluorescence Microscope Imagery". David S.C. Biggs





- ### Key Aspects when acquiring images to deconvolve

1. The size of each pixel must satisfy the Nyquist criterion

2. Avoid saturated pixels

3. Optimize the Signal to Noise (S/N) ratio

4. Acquire a minimum of 3 Z planes

System	Image saving format
Zeiss LSM510 y LSM710 Confocal	**.lsm format**
Zeiss LSM800 y LSM900 Confocal	**.czi format**
NikonA1R in vivo Confocal	**.nd2 format**; and the **.xml file** containing the acquisition information
Olympus SpinSR10 Confocal	**.vsi format**; and save the **folder containing the ".ets" file** (metadata)
Leica Stellaris 8 STED+FLIM Confocal	**.lif format**
Zeiss Widefield Microscopes (sCMOS y F.R.E.T.)	**.stk format**

- ### For more information:

You can find detailed information about the deconvolution program "Huygens" on the following website:

https://svi.nl/Huygens-Deconvolution

- More information about the possibilities of this software could be found through the following link:

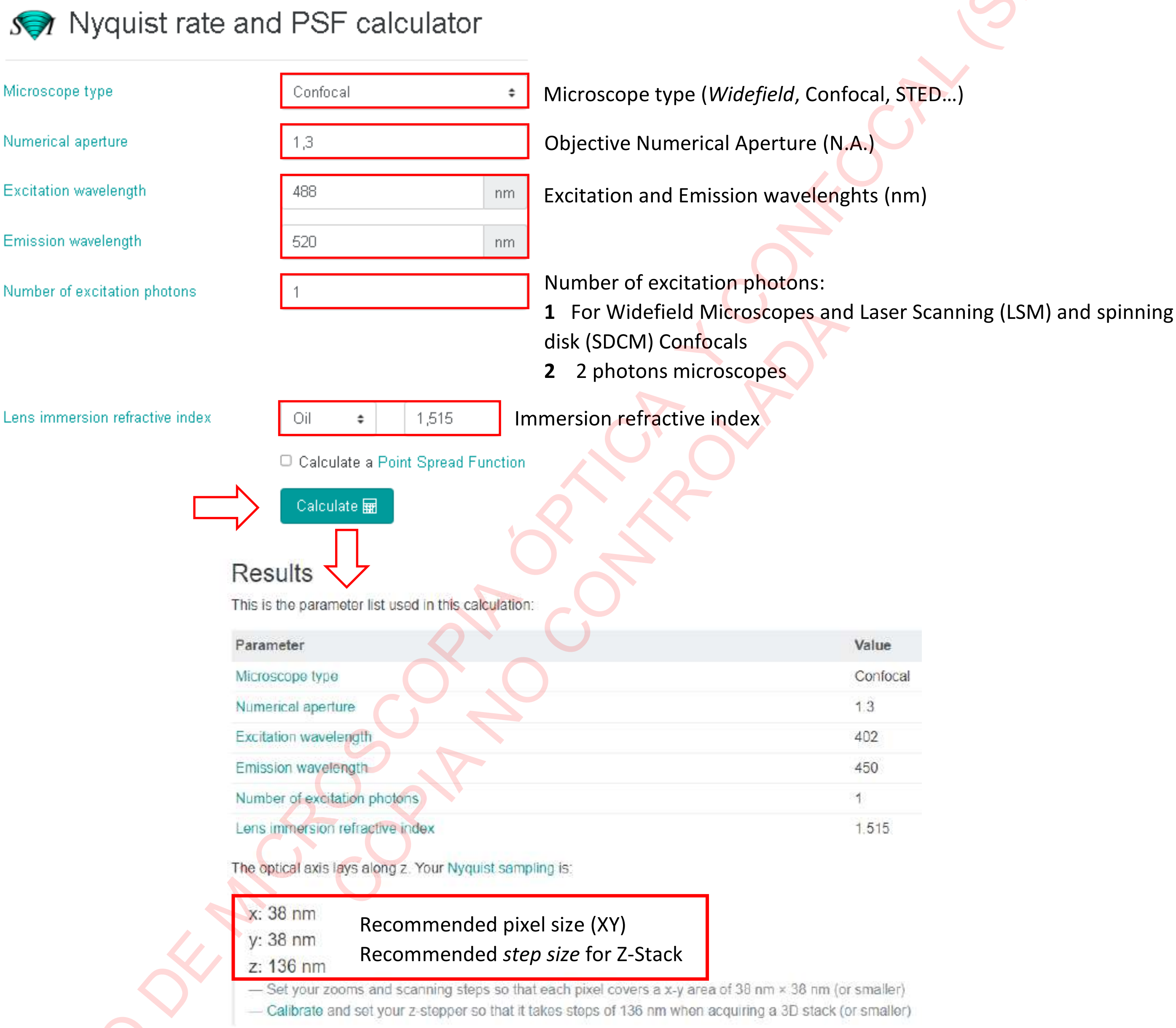
https://svi.nl/FAQ

How to Calculate pixel size of images to deconvolve

When acquiring images using a microscope and saving them, the ideal is to achieve a sampling density that meets the Nyquist criterion.

Huygens (*Scientific Volume Imaging*) has an online calculator where you will have to select:

https://svi.nl/NyquistCalculator



Depending on the values obtained (X, Y, Z); the different parameters (zoom, z-stack step size…) must be adjusted to satisfy these requirements.

IMPORTANT

When images are acquired in confocal microscopes using a **pinhole value of 1 Airy Unit (AU)**, the lateral sizes (XY) obtained in the calculator, and those listed in the following tables, can be **increased up to 1.6 times** without detriment to the quality of the Deconvolution.

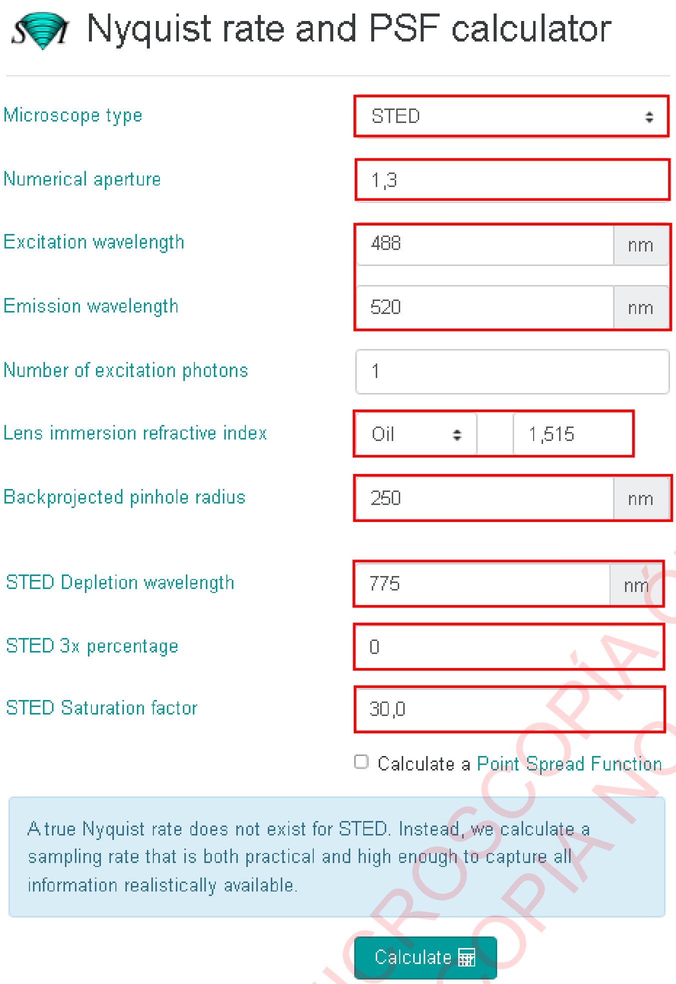
In cases where **small pinhole values (< 0.5 AU)** are used, these sizes can be **increased up to 1.3 times**; and in the case of using **large pinholes (> 4 AU)**, these sizes can be **increased up to 2 times**.

STED IMAGE DECONVOLUTION

When acquiring Super-resolution images (STED), 86X (1.2) and/or 100X (1.4) objectives will be used.

To perform a correct Deconvolution of the acquired images, we will use the SVI Huygens online calculator to get the recommended pixel size (XY) and Z-step needed in our image acquisition.

https://svi.nl/NyquistCalculator



Objective numerical aperture: **1.2** (86X obj.) or **1.4** (100X obj.)

Excitation and emission peak wavelengths of the fluorophore

Immersion medium **Water** (86X) or **Oil** (100X)

Backprojected pinhole value of the selected objective. See below.

Depletion laser wavelength (**775 nm**)

STED Depletion Laser Power Percentage

Percentage used in the z (axial) depletion beam (**STED 3D**)

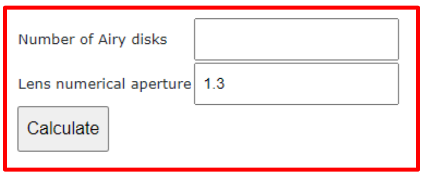
How to calculate the Backprojected pinhole?

To calculate the Backprojected Pinhole we will use the online calculator available at the following link:

https://svi.nl/LeicaConfocal_TCS_SP8

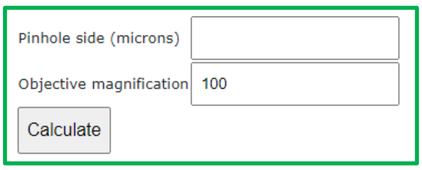
(1) In case of adjusting the Pinhole in terms of Airy Units (AU)

In this case, we have to take into account that Leica presents the Pinhole value in reference to the 580 nm excitation line; so that when we adjust the AU to our reference channel, we will have to see what this value is for the wavelength 580 nm; along with the numerical aperture of the objective used.



(2) In case of adjusting the Pinhole in terms of microns (µm)

In this case, we will set the Pinhole to be displayed as microns (µm) in the LAS X program, noting that value and the objective magnification to perform the calculations.



Last modification: 12/07/2022

Objectives and Numerical Apertures (N.A.) available at the SMOC

- **ZEISS CONFOCALS**

LSM510 vertical & LSM710 (Inverted and Vertical)

Objective (Immersion Media)	Numerical Aperture (N.A)
25X (Oil)	0.8
40X (Oil)	1.3
63X (Oil)	1.4
100X (Oil)	1.4
63X (Water) *LSM710 Inverted only*	1.2
100X (Oil) *LSM510 vertical only*	1.3

LSM800 Inverted and LSM900 Vertical

Objective (Immersion Media)	Numerical Aperture (N.A)
20X (Air)	0.8
25X (Oil)	0.8
40X (Oil)	1.3
63X (Oil)	1.4
100X (Oil)	1.4
63X (Water) *LSM800 Inverted only*	1.2

- **OLYMPUS SPINSR10 CONFOCAL**

Objective (Immersion Media)	Numerical Aperture (N.A)
10X (Air)	0.4
20X (Air)	0.8
30X (Silicone)	1.05
40X (Air)	0.95
40X (Silicone)	1.25
60X (Silicone)	1.3
100X (Oil)	1.45

	50 µm Disk + Lens 1X		SoRa Disk + Lens 1X		SoRa Disk + Lens 3.2X	
Objective	*Backprojected Pinhole (nm)*	*Pinhole Spacing (µm)*	*Backprojected Pinhole (nm)*	*Pinhole Spacing (µm)*	*Backprojected Pinhole (nm)*	*Pinhole Spacing (µm)*
10X	2500	25.3	1250	12.7	390.63	3.95
20X	1250	12.7	625	6.4	195.31	1.98
40X	625	6.33	313	3.17	97.66	0.99
60X	416.7	4.22	208.4	2.11	65.11	0.66
100X	250	2.53	125	1.27	39.06	0.40

IMPORTANT WHEN DECONVOLVING SPINNING DISK IMAGES

When deconvolving spinning disk images, we have to take into account which **Disk (50 µm or SoRa)** and **magnification lens (1X or 3.2X)** combination was used in the image acquisition, since the Backprojected pinhole and the pinhole spacing values vary depending on the combination selected.

Likewise, we must check if the excitation wavelengths coincide with the channels that we want to deconvolve (**405:** BFP, DAPI, Hoechst; **488:** Alexa 488, FITC, GFP; **561:** Alexa 555, Alexa 594, Rhodamine, TexasRed, TRITC; **640:** Alexa647, Cy5, To-Pro3).

In the Microscopic Parameters window, we will have to check if the values shown agrees with the combination that was used during the image acquisition.

Last modification: 12/07/2022

- **NIKON A1R IN VIVO CONFOCAL**

Objective (Immersion Media)	Numerical Aperture (N.A)
20X (Aire / *Air*)	0.75
20X (Aceite / *Oil*)	0.75
40X (Aceite / *Oil*)	1.3
60X (Aceite / *Oil*)	1.4
60X (Agua / *Water*)	1.2

- **LEICA STELLARIS 8 STED+FLIM CONFOCAL**

Objective (Immersion Media)	Numerical Aperture (N.A)
20X (Aire / *Air*)	0.75
40X (Aceite / *Oil*)	1.3
63X (Aceite / *Oil*)	1.4
86X (Agua / *Water*)	1.2
100X (Aceite / *Oil*)	1.4

- **WIDEFIELD MICROSCOPES: F.R.E.T. and SCMOS (ZEISS)**

FRET		sCMOS	
Objective (Immersion Media)	**Numerical Aperture (N.A)**	**Objective (Immersion Media)**	**Numerical Aperture (N.A)**
10X (Air)	0.45	10X (Air)	0.3
20X (Air)	0.8	20X (Oil)	0.5
25X (Oil)	0.8	25X (Oil)	0.8
40X (Oil)	1.3	40X (Oil)	1.3
63X (Oil)	1.4	63X (Oil)	1.4
100X (Oil)	1.3	100X (Oil)	1.3
40X LD (Air)	0.6	40X (Air)	0.6