

## How to use the SR-DIP software module

### Introduction to differential intensity processing (SR-DIP)

SR-DIP is an image processing method which uses the intensity differences of consecutive images to obtain position information of the fluorescence labels in the sample. These position information can be used to create one super resolution image. Prerequisite is a series of images (time series) of a static sample that shows sufficient differences in intensity in the image sequence (moderate bleaching). Hence SR-DIP is best used with fixed samples, avoiding motion artefacts of the sample itself. The required time series does not need to be obtained on a confocal system, however, the good signal to noise properties of such systems help to obtain good results with SR-DIP.

The method was developed by researchers from the VIB in Leuven, Belgium, and was published in 2012. The implementation used in the SR-DIP software is license protected in order to cover the license royalties of the respective patent.

The full pdf is open access, [click here](#) to read it.

## Sub-diffraction imaging on standard microscopes through photobleaching microscopy with non-linear processing

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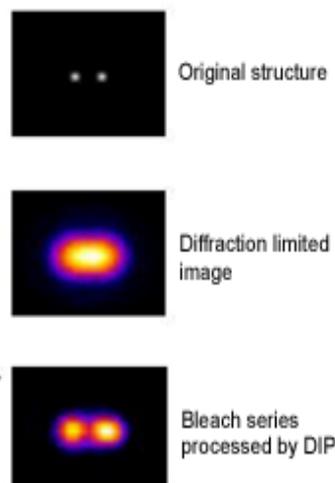
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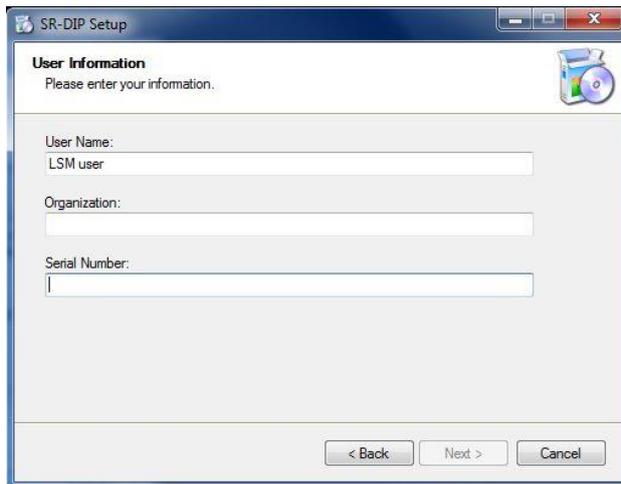
The example images on the left illustrate the effect of SR-DIP. A sub-resolution structure of two points will not be resolved sufficiently by normal (diffraction limited) confocal imaging, but with SR-DIP the achievable resolution is almost doubled.

## Installation of SR-DIP and required license key

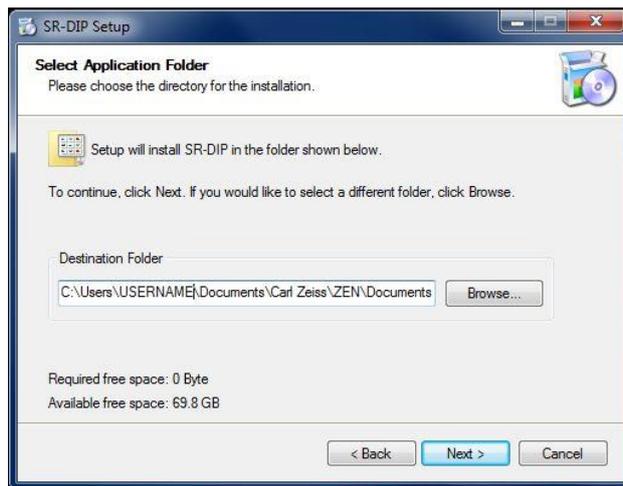
There is a setup procedure which guides you through the installation of SR-DIP for your ZEN 2012 blue or ZEN 2.0/2.1/2.3 blue software.



After accepting the license agreement, fill in your user name (default is LSM User) and the serial number which you received from ZEISS after ordering your SR-DIP license (ZEISS # 000000-2177-835) from your ZEISS sales contact.



In case the application folder is not found, you can also browse the Carl Zeiss\ZEN\Documents folder.



## Image Acquisition for SR-DIP

One can acquire the necessary images with any ZEISS imaging system that is able to acquire time series data of about 50 time points or more, with ideally 5% difference of fluorescence intensity by bleaching. Good results have been obtained with ZEISS systems LSM 7 LIVE, LSM 700, LSM 710, LSM 780, LSM 880 and LSM 800. As well camera based 3D sectioning systems like ELYRA, Cell Observer SD, VivaTome or Apotome.2 can be used with SR-DIP in case sufficient bleaching is present in the data.

Sample: static sample (ideally fixed and embedded), labelled with common fluorescent labels. Any movement in between images of the time series will lead to artefacts in the resulting SR-DIP image.

Sampling (x,y): ideally 4x oversampling. This corresponds to twice the amount of pixels that are set after pressing the Optimal button of LSM systems (2x Optimal).

SR-DIP works only for single z-slices, z-stacks cannot be processed.

Experimental setup:

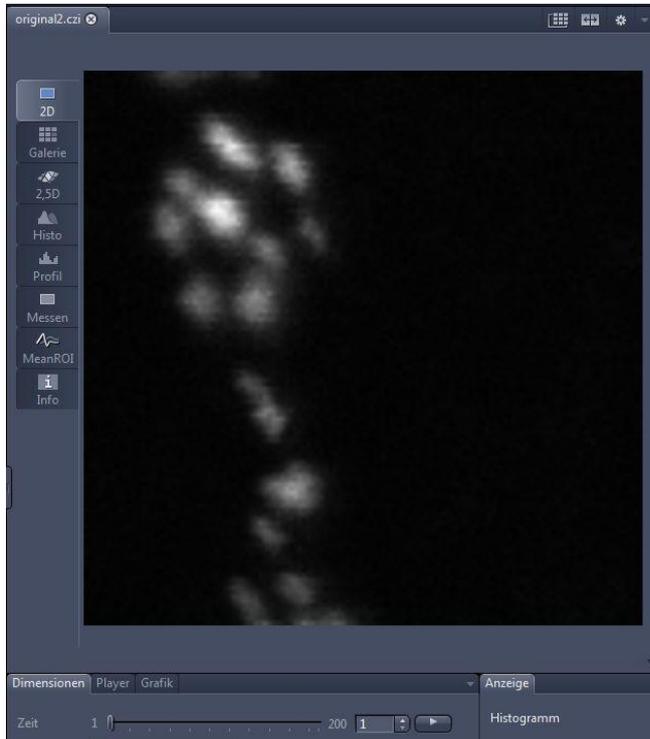
- a) Time series with at least 50 images. Laser power for imaging set to a value that results in a 5% reduction of fluorescent intensity in each successive image. The reduction of intensity can be checked with the Histogram or Mean of ROI View Tabs in ZEN software.
- b) Time series with Bleaching after each image (Repeat bleach after # of scans set to 1 in Bleaching tool window in ZEN) with at least 50 images. Instead of a region of interest (ROI) the complete image is bleached.

Each bleach event should reduce the fluorescent intensity by 5%. The reduction of intensity can be checked with the Histogram or Mean of ROI View Tabs in ZEN software. The Iterations or bleaching laser intensity can be set accordingly.

The resulting time series can be processed with the SR-DIP software module and will produce one image with enhanced resolution.

## Using SR-DIP

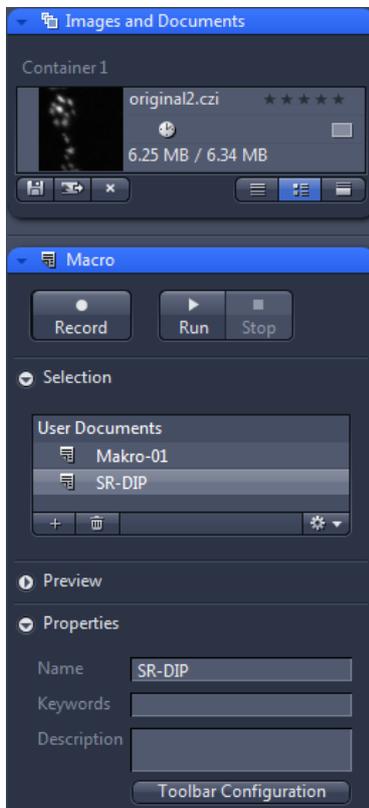
First open an image series which shows a moderate bleaching effect in ZEN blue software. Avoid empty images at the end of the series, or series without noticeable differences between consecutive images.



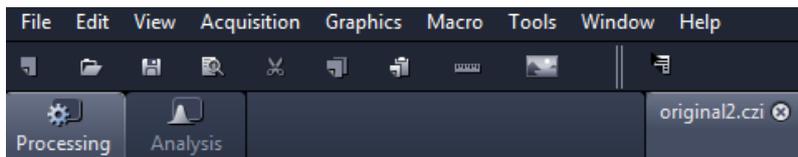
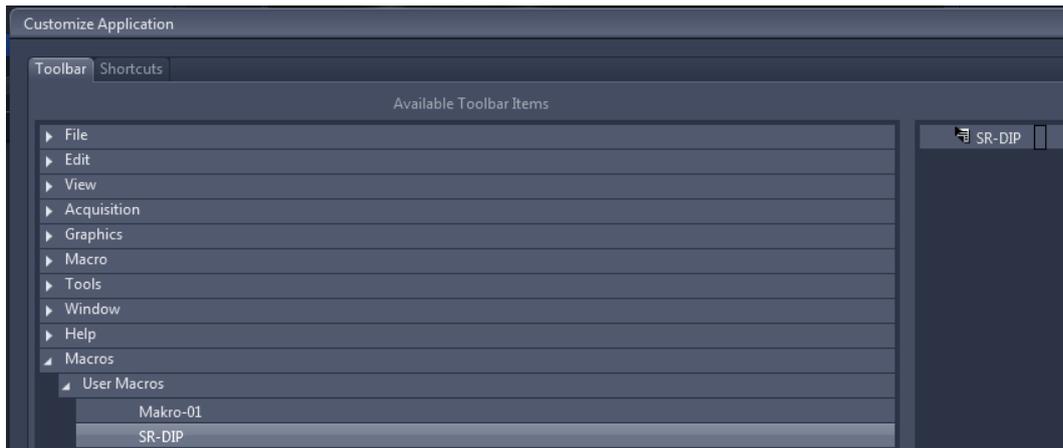
The gallery view is a good tool to judge the time series. Create a subset of a longer time series if necessary.



Next start SR-DIP as an OAD macro in the right tool area of ZEN. This is available in all ZEN blue configurations. It is not needed to start the macro editor to run SR-DIP. Click on SR-DIP and run.

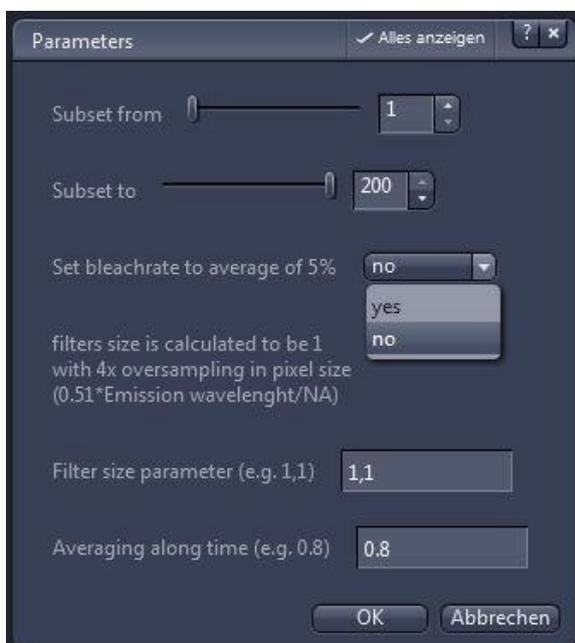


It is possible to create a shortcut at the top toolbar of ZEN by using the toolbar configuration and choosing SR-DIP from the list. This will create a new button in ZEN which starts SR-DIP.



After starting SR-DIP, you can choose a number of parameters in order to adjust the default settings to the properties of your time series. The defaults might work, but it is recommended to try and adjust the parameters and check the result with your individual data set.

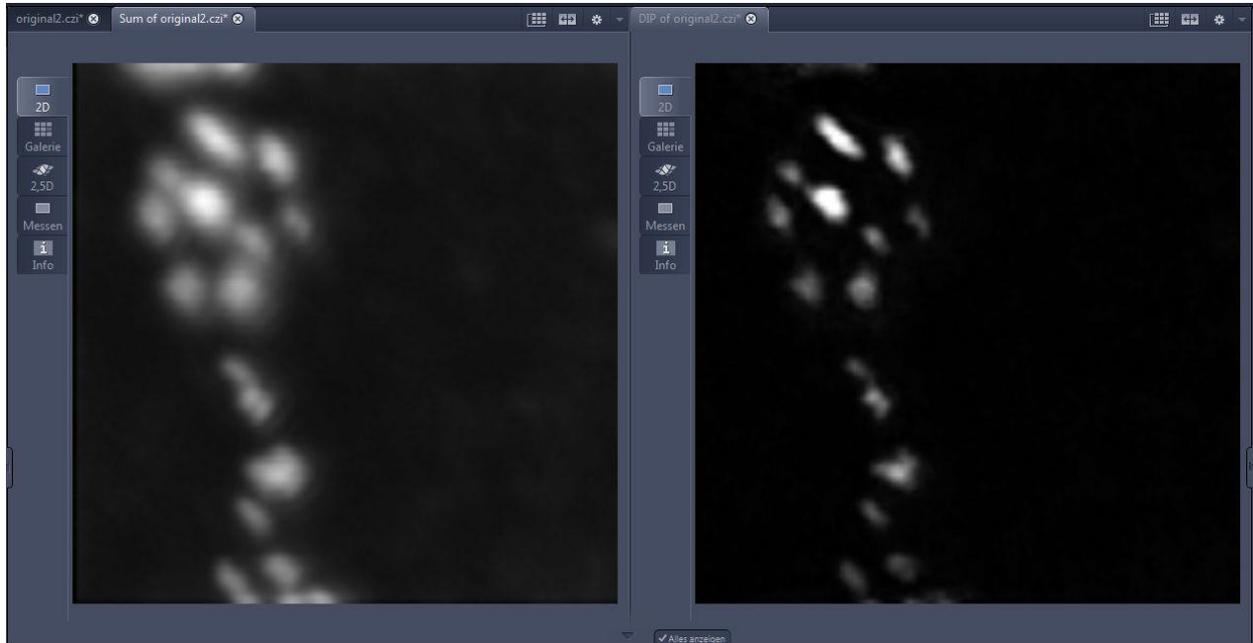
At the top of the SR-DIP parameter menu, you can select a subset and adjust the bleach behavior of your data. SR-DIP works well with e.g. 50 up to a few hundred images. Bleach rates of 5% average are ideal, in this case choose yes. For bleach rates other than 5% choose no. This value depends on your sample, especially how linear your sample bleaches, and allows to adjust for best structure fidelity.



Depending of the oversampling factor (x,y dimensions) during the acquisition of the data, you might want to adjust the filter size. On a LSM system, the Optimal button for the pixel format will give you about 2x oversampling. In order to obtain higher resolution, you need to increase the sampling further, e.g. to 2x optimal, resulting in 4x oversampling. In this case the filter size parameter\* is 1,1.

Depending on the noise in your time series, you will need an averaging. The strength\* can be adjusted with 0.8 as a good default value to start with.

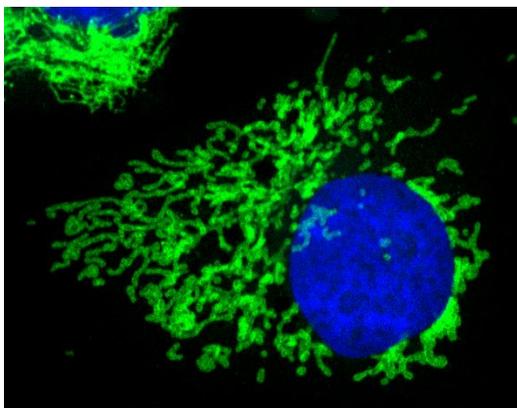
After clicking OK, the processing starts and calculates first a sum image of the series and then the DIP superresolution result.



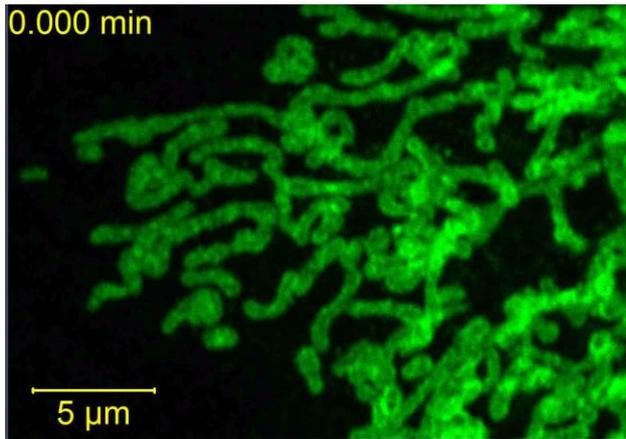
\*Filter size and strength are values needed for the processing function, which includes also a deconvolution step. While these values are not self-explaining, you might imagine the filter size as your lever to set resolution and averaging as your lever to set image smoothness. Both parameters can influence your result and need some trial and error optimization, since they depend vastly on your sample and acquisition conditions.

#### Example:

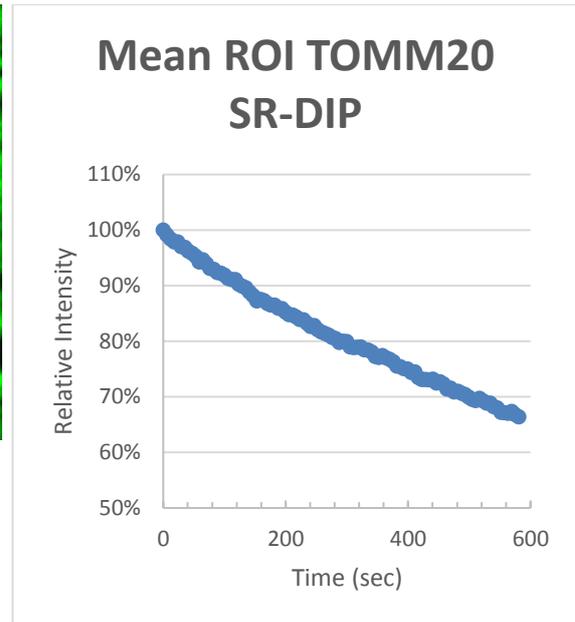
TOMM20-Alexa 568



1) Acquisition of Time Series experiment, bleaching the fluorescent signal



The movie is an attachment of this pdf file. You can find it on the left hand side menu in the section marked with a paper clip.



2) Result:

