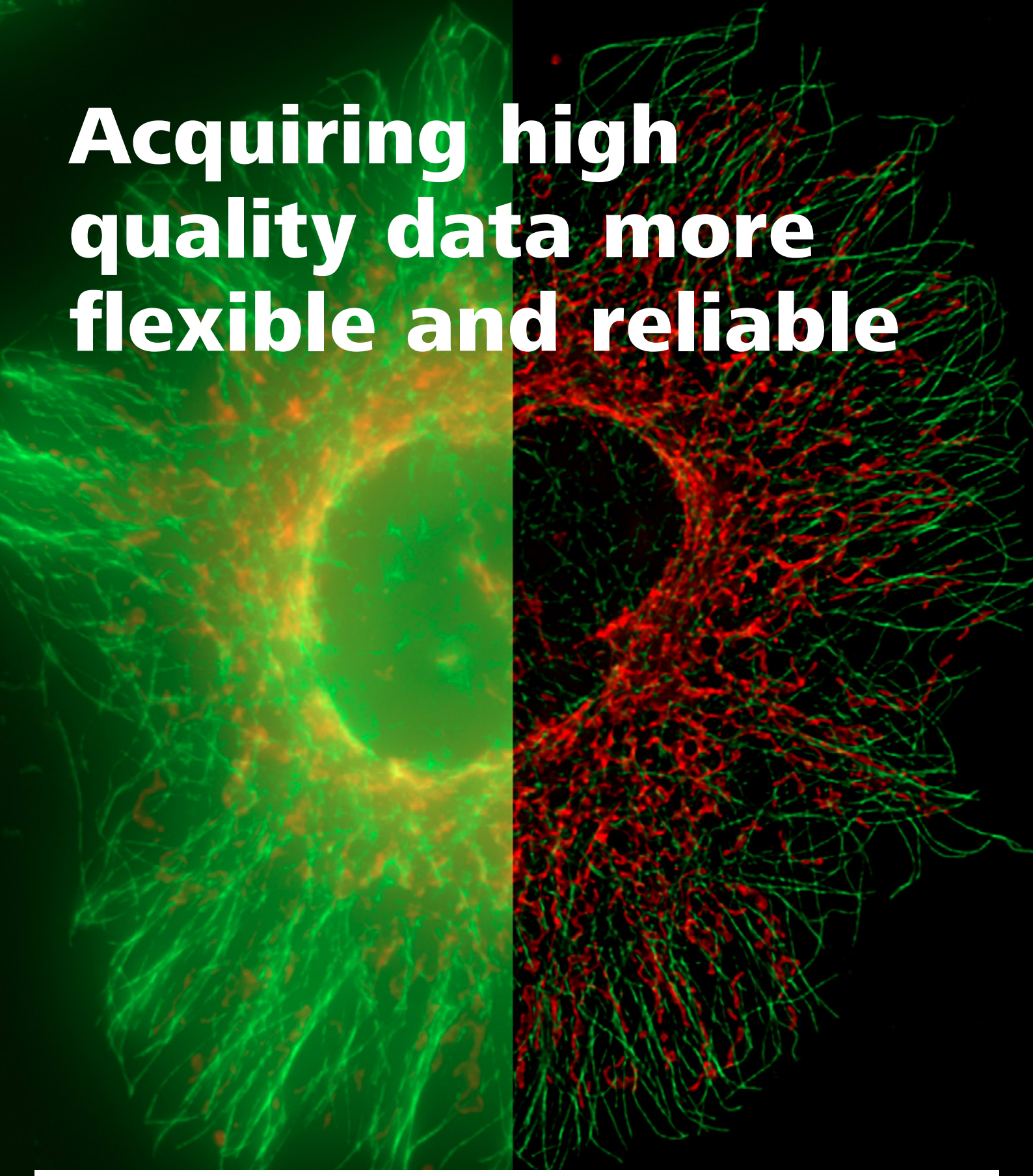

Acquiring high quality data more flexible and reliable

ZEN Deconvolution & Direct Processing for Life Science Application

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ZEN Module Deconvolution

A convenient implementation of proven algorithms

Introduction

Since the introduction to widefield fluorescence microscopy in 1983, deconvolution has witnessed the development of a wide variety of algorithms and has been routinely applied to almost all microscopy techniques. A sharp rise in the popularity of deconvolution is mainly fueled by the rapid improvement of computer hardware, particularly by the parallel processing power of GPUs. The speed of deconvolution, which used to be the bottleneck, has been dramatically improved. Theoretically, widefield fluorescence microscopy benefits the most, since the algorithm reassigns out-of-focus blur, the main source of noise for widefield microscopy, back to the in-focus plane. The result is higher signal-to-noise ratio (SNR) of the in-focus structures and increased resolution. Many 3D microscopy techniques, like laser scanning confocal, have also been regularly processed with deconvolution. Coupled with scanning oversampling and reduced pinhole size, confocal deconvolution can practically increase lateral resolution beyond the theoretical diffraction limit and has been offered by many as the most affordable super-resolution technique.

ZEN Module Deconvolution

ZEN Deconvolution is a collection of scientifically published algorithms grouped based on their inherent principles, processing speed, and quality of improvement:

- Nearest Neighbor
- Regularized Inverse Filter
- Fast Iterative
- Constrained Iterative

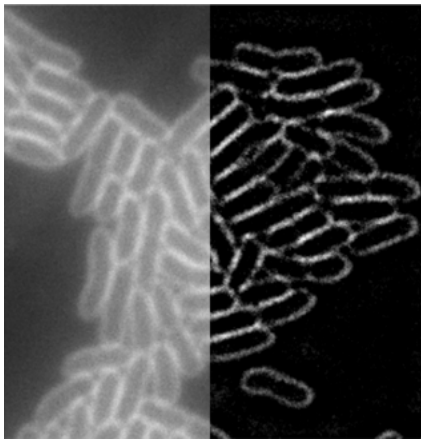
For non-power users, you can simply choose either of the four options, and ZEN will automatically select a set of default parameters to deconvolute your data. Generally, the longer it takes for a deconvolution algorithm, the better the processed image quality. To help you better differentiate the options, they have been renamed for your convenience:

- Simple, very fast
- Better, fast
- Good, medium speed
- Excellent, slow

For users who want to maximize the benefit of deconvolution for their critical data, ZEN offers another set of flexible workflows and advanced options with up to 17 published algorithms. You will find the classical Richardson-Lucy or constrained iterative methods here with advanced regularization and optimization possibilities. One of the major benefits of ZEN Deconvolution is that it reads all the metadata in your image, and it is smartly designed to recommend a suitable set of parameters for that image. All you may need to do is fine-tune the default settings for the best possible results.

Imaging modality	Recommended methods	Recommended parameters	Main improvements	Limitation
Widefield 3D	Constrained iterative	Zero order regularization up to 40 iterations	Axial resolution SNR	Thin sample only need Nyquist Z sampling
Widefield 2D	Deblurring	Default settings	Contrast	Qualitative
Widefield Apotome	Regularized inverse filter	Default settings	SNR	In-focus planes only
Confocal	Constrained iterative	First order regularization up to 10 iterations	Lateral resolution SNR	Need oversampling and small pinhole
Lightsheet	Constrained iterative	Zero order regularization up to 10 iterations	SNR	Long processing time
Spinning disk	Constrained iterative	Zero order regularization up to 20 iterations	SNR	Fixed pinhole and sampling
TIRF	Deblurring	Default settings	Contrast	Qualitative

Recommended methods and parameters with their main improvements and known limitations for different imaging modalities.



2D widefield image of a membrane-stained E. coli sample before and after deblurring.

Starting with ZEN 3.4, one more method is added, deblurring, which is based on a nearest neighbor algorithm. Deblurring can remove out-of-focus blurs more effectively and considerably faster than traditional rolling ball-based background subtraction. Deblurring is thus recommended to process widefield 2D fluorescence images and 3D stacks with non-optimal samplings.

ZEN Module Direct Processing

Parallelizing your image acquisition and processing

Introduction

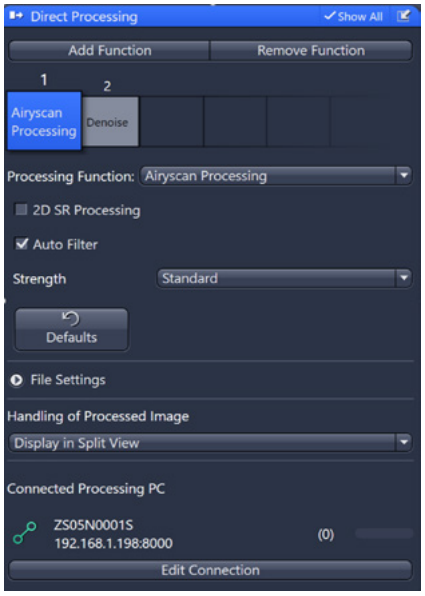
In many cases, the development of modern microscopic techniques makes the data structure more complicated and image processing steps more time-consuming. The raw data cannot provide a visual confirmation of the fine details of the sample and were left untouched after proper processing. Deconvolution, for another example, is a computationally heavy process. The time spent on deconvolution is usually a few times longer than that on the image acquisition. It's thus practically challenging to manage such imaging workflows efficiently and have on-the-fly feedback of the current sample condition for a time-series experiment. ZEN module Direct Processing is designed to address such concerns and improve usability by parallelizing the image acquisition and processing steps. You still enjoy the complete flexibility in designing the experiment while the intuitive user interface will transform your microscope into an automated and versatile platform.

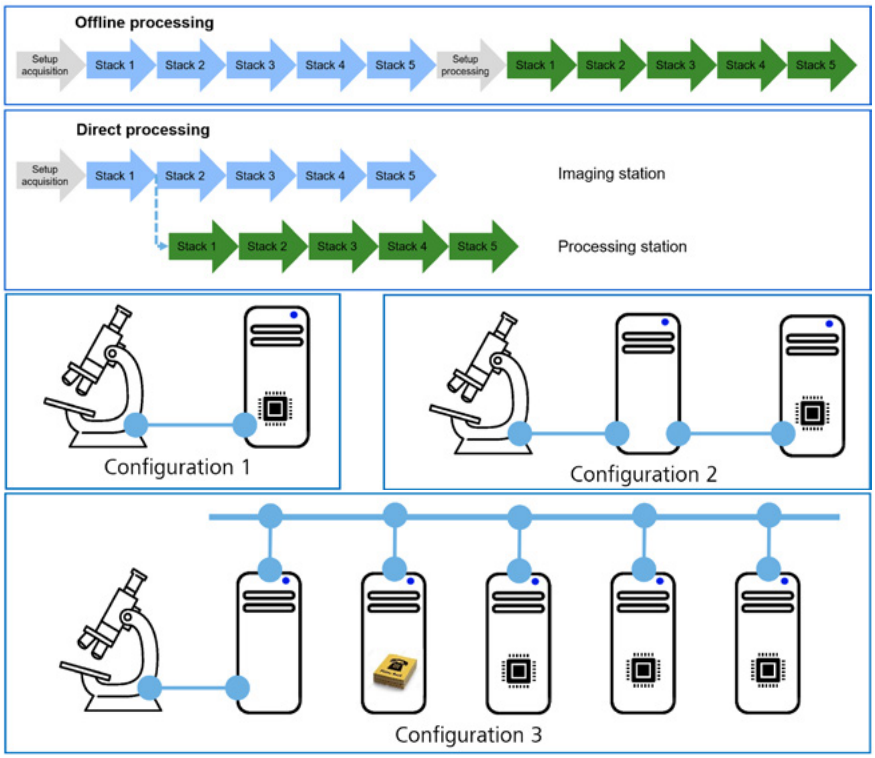
ZEN Module Direct Processing

During image acquisition, ZEN Direct Processing enables you to execute image processing steps on the same PC or another network-connected PC. Seven processing functions are available with ZEN 3.4, and more will be added in the coming versions:

- Airyscan Processing
- Apotome RAW Convert
- Deblurring
- Deconvolution
- Denoise
- Deskew
- Unsharp Mask

Multiple processing steps can be added for a single experiment and the steps will be executed sequentially one after another. To have the earliest possible feedback, ZEN Direct Processing starts to process the smallest processable entity as soon as its acquisition has been completed. In the case of deconvolution, this is typically a z-stack for one channel. You will be able to observe the processed result on the fly.





Direct Processing Configurations

Depends on your microscope setups, there are multiple ways to configure your Direct Processing. The above figure outlines 3 typical configurations:

Configuration 1: The same workstation is used for acquisition and processing.

Configuration 2: A dedicated processing workstation is linked with the acquisition workstation via a network connection. This is the preferred configuration if the image processing is computationally demanding.

Configuration 3: All workstations are connected within a Local Area Network (LAN). You have the option to use a discovery proxy or a simple IP address to flexibly connect either of them.

Deconvolution and Direct Processing is available for multiple platforms

Hardware Requirements

- Axio Observer A1/D1/Z1/3/5/7
- Axio Imager A2/M2/D2/Z2
- Axio Examiner A1/D1/Z1
- Axioscope 5/7
- Axio Zoom.V16
- Celldiscoverer 7 (with LSM 900)
- LSM 800
- LSM 900
- LSM 980
- Lattice Lightsheet 7
- Axiocam mono
- ZEN compatible 3rd party cameras
- CUDA® supported Nvidia graphic card is recommended for GPU Deconvolution

Software Requirements

- ZEN system / pro / Celldiscoverer / Lattice Lightsheet
- ZEN desk is required for processing PC
- ZEN blue 2.6 and above is required for Direct Processing
- ZEN blue 3.4 and above is required for Deblurring processing
- ZEN module Z-stack is required for 3D data acquisition
- ZEN module 3Dxl is highly recommended for visualization and rendering of 3D data

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* Front page image shows the before and after Constrained Iterative Deconvolution of the mammalian U2OS cells labeled for mitochondria (TOM20-mCherry) and microtubules (Tubulin-GFP) structures. Data acquired with Celldiscoverer 7 using a Plan-Apochromat 50×/1.2 water immersion objective and Axiocam 506 mono.