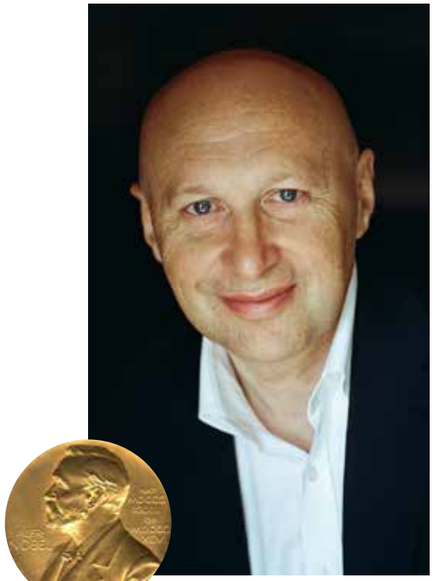
FACILITY LINE

Confocal imaging and superresolution STED at the push of a button



„Confocal scanning microscopy *without* a push-button STED superresolution is no longer state of the art. You easily miss those decisive details that lead to ground-breaking discoveries.“

STEFAN W. HELL - Nobel Laureate in Chemistry 2014
„for the development of superresolution fluorescence microscopy“

Facility Line

Live-Cell

- **Adaptive Illumination**
  DyMIN / RESCue
- **Pulsed gated STED**
- **Fast scanning**
- **Abberior STAR and LIVE dyes**

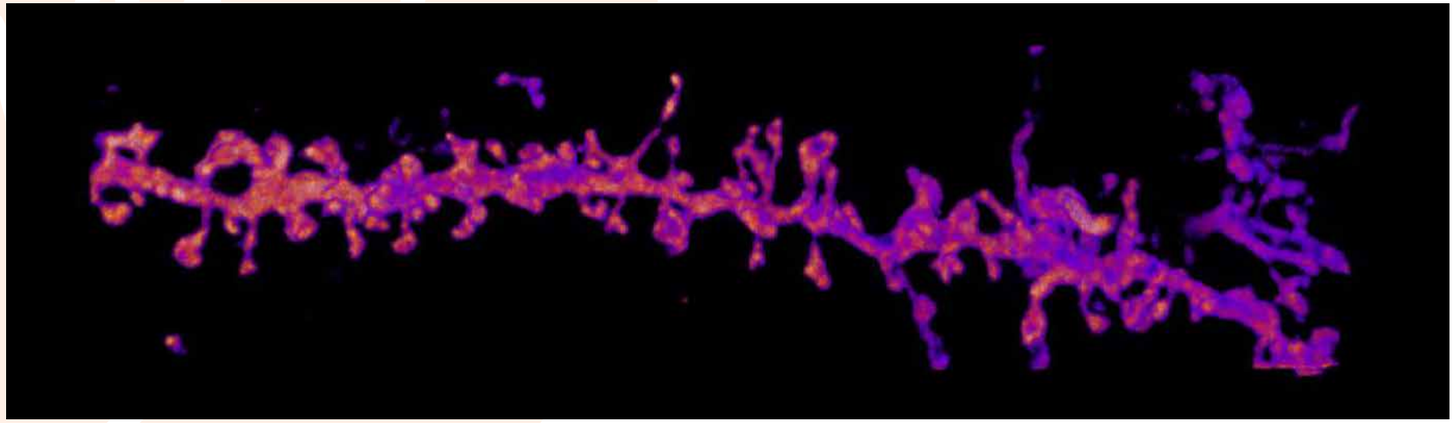
Performance

- **easy3D STED**
  Superior signal and resolution
- **Autoalignment**
  All beams in best shape
- **Rainbow Detection**
  Highly sensitive spectrally
- **Autofocus STED & Confocal**
- **MINFIELD STED**
  Physical resolution below 20 nm
- **Uniquely gentle and sensitive confocal imaging**

Easy-to-use

- **Graphical User Interface (GUI)**
  Easiest User Interface
- **Ergonomic workstation**

Adaptive Illumination (DyMIN, RESCue)

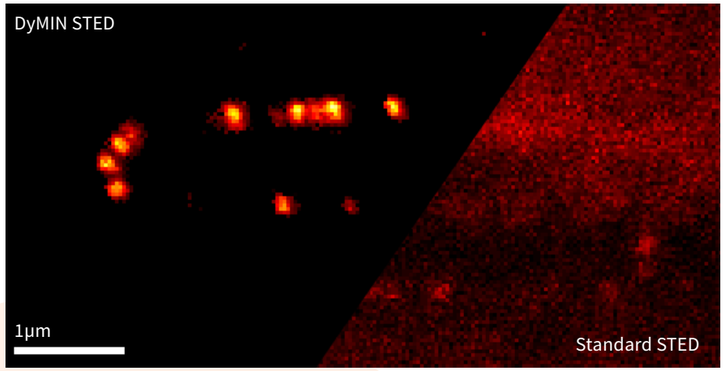


Easy3D DyMIN STED on dendrites in brain slices.
Sample by O. Kaplan & H. Kawabe @ MPI of Experimental Medicine, Göttingen, Germany.

Adaptive illumination schemes in STED microscopy, such as DyMIN and RESCue, maintain the highest superresolution possible while significantly reducing the light dose in the sample. The basic idea is to modulate excitation and STED lasers in a sample-dependent manner to put light only where and when it is needed. For example, lasers are switched off in positions where no fluorescent marker is present.

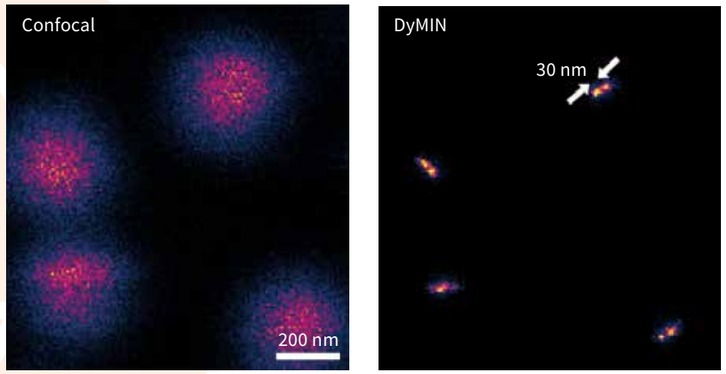
RESCue and DyMIN STED reduce the light dose down to a few percent compared to gated-cw or standard pulsed STED. Thus your living cell experiences orders of magnitudes less light. This saved light exposure can be invested in better resolution, more frames from the same spot, or volume imaging with high 3D-STED resolution. This way, DyMIN and RESCue STED greatly expand the range of STED nanoscopy.

In combination with our easy3D STED, DyMIN records three-dimensional volumes with superior resolution (left).



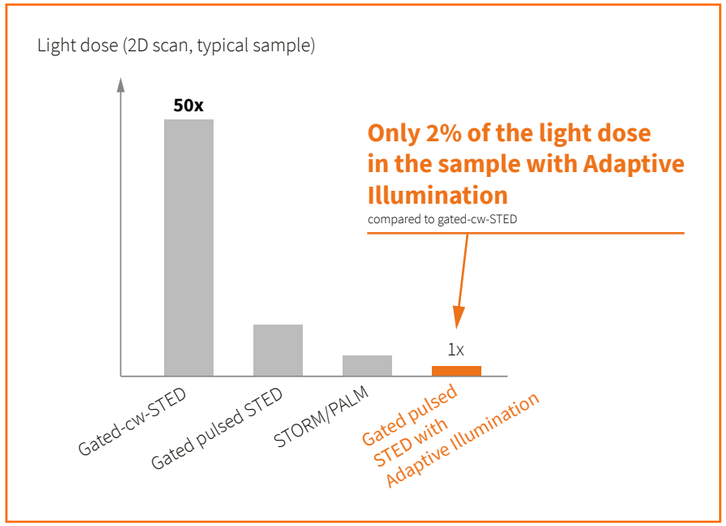
With standard STED imaging, imaging a full volume at high 3D-resolution is not possible (right).

DyMIN STED opens up optical resolutions significantly below 30 nm on the full field of view (right).



DyMIN STED imaging of DNA origami structures at high signal-to-noise ratio clearly separates two fluorescent spots at 30 nm distance (GATTAquant DNA origami sample).

Abberior Instruments - The choice for Live Cell STED



Your advantages:

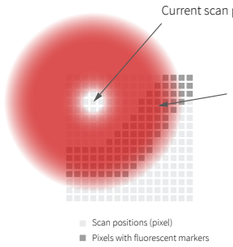
Adaptive illumination STED…

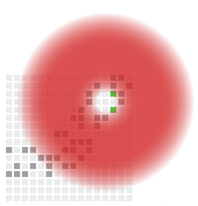
- is the only live-cell superresolution method with resolutions down to 25 nm
- reduces the light dose on your sample by orders of magnitude
- enables time-lapse live-cell superresolution and live-cell superresolution in volumes
- Bonus: RESCue also dramatically reduces the light dose with confocal imaging!

Traditional design

No Adaptive Illumination

Markers are highly exposed to light, before the scan even reaches them.





By the time the scan reaches structure, many fluorophores have already been photobleached.

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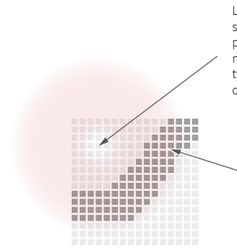
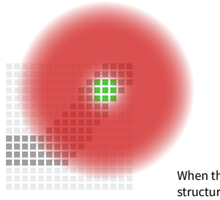
With Adaptive illumination

Light only reaches the sample where it has a positive effect, i.e. where signal can be recorded. Very little pre-bleaching.





When the scan reaches structure, most fluorophores are ready to be imaged. Resolution and signal are greatly improved.

Pulsed gated STED

Excitation light in STED microscopy is always pulsed. Pulsed STED light is the superior concept compared to cw STED, because the same resolution is achieved with 12 times weaker pulsed lasers to reach the same resolution. In case of cw STED, the majority of STED photons arrive outside of the fluorescence time window of the marker used, thus these cw STED photons are useless and potentially harmful to the dye or the sample.

Traditional design

With continous-wave STED approx. 80% of the STED light is at best useless, because it does not arrive during the lifetime of the excited state of the dye. Thus, cw STED requires ~12 times higher average power than pulsed STED.

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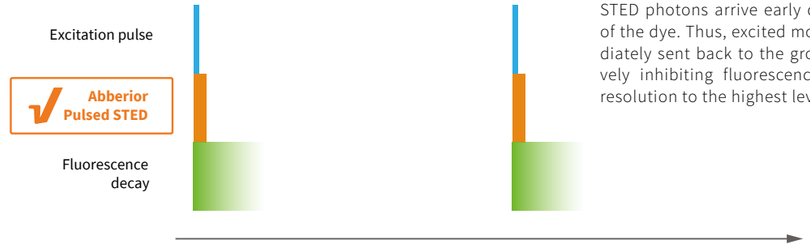
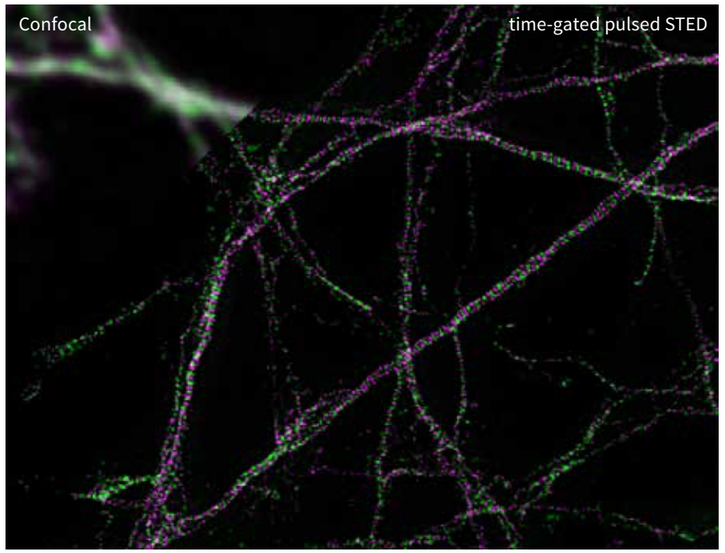
A short-pulsed STED laser ensures that all STED photons arrive early during the lifetime of the dye. Thus, excited molecules are immediately sent back to the ground state, effectively inhibiting fluorescence, and increasing resolution to the highest level.



In synergy with pulsed STED, our avalanche photodiode detectors (APD) are time-gated. Time-gating prevents early fluorescent photons from being counted, which would otherwise blur the image.
Avoiding these photons that arrive too early further enhances the STED resolution.



3-color STED image of primary hippocampal neurons. Note the characteristic ~190 nm beta II spectrin periodicity along distal axons (green) which is only visible in the STED image. Sample by Elisa D'Este @ MPI for Biophysical Chemistry Göttingen, Germany.

Your advantages:
- Orders of magnitude less STED light in pulsed STED mode
- Less bleaching of the dye and significantly less light on your sample!
- Combine time-gating with pulsed STED!

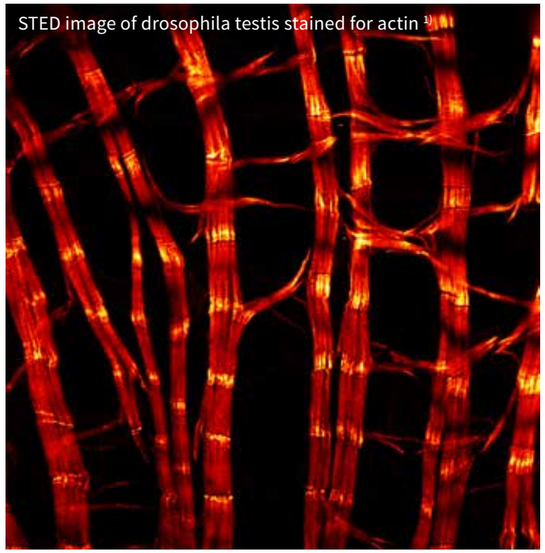
Fast scanning

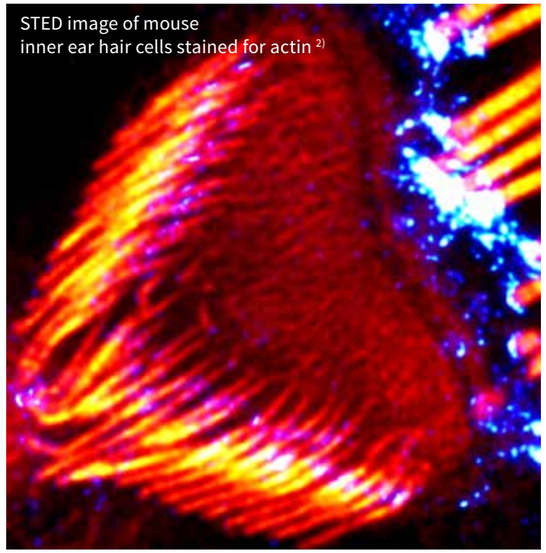
The new Abberior Instruments QUADScanner offers greatly enhanced speed together with the known advantages of the QUADScan-scheme: scanlens-free design with minimal optical losses, compactness and mechanical robustness.

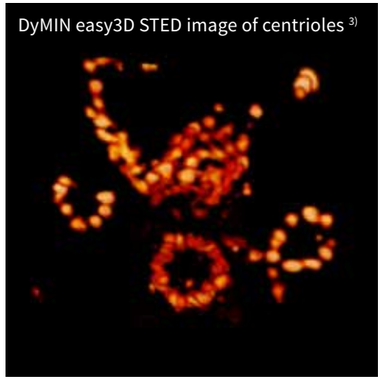
To this end, the QUADScanner is able to operate in two modes: sawtooth scanning for 100%-perfect linearity over the full field of view, and sine scanning for high frame rates up to more than 4 kHz line frequency.

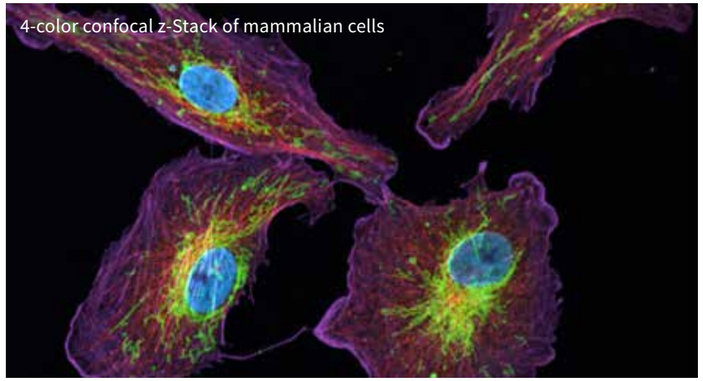
Ultrafast laser switching ensures that the lasers are precisely shut down at the edges of the field to avoid exposure of the sample during the reversal. Not coincidentally, the same ultrafast switching is part of our adaptive illumination (DyMIN, RESCue).

Your advantages:
- Perfectly linear scans with sawtooth mode
- High frame rates with sinus mode
- Low-loss design, no scan lens required
- Fast laser shutdown to avoid unnecessary sample exposure



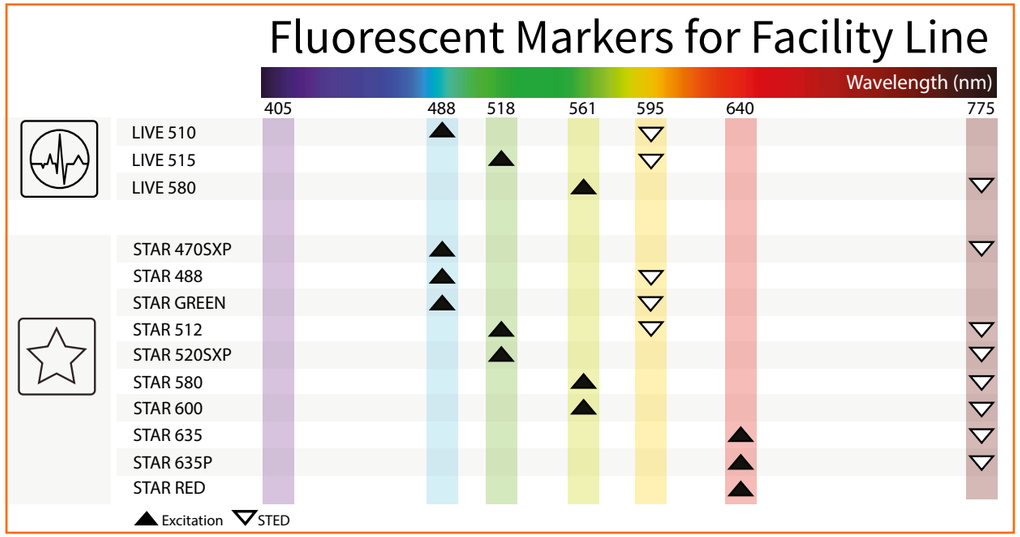






[1] Sample by J. Rehman & Dr. H. R. Shcherbata @ MPI for Biophysical Chemistry Göttingen, Germany
[2] Sample by Dr. Christian Vogl @ InnerEarLab, UMG Göttingen, Germany
[3] Sample by P. Guichard & D. Gambarotto @ University of Geneva, Switzerland

The perfect match - Always use our Abberior STAR and LIVE dyes



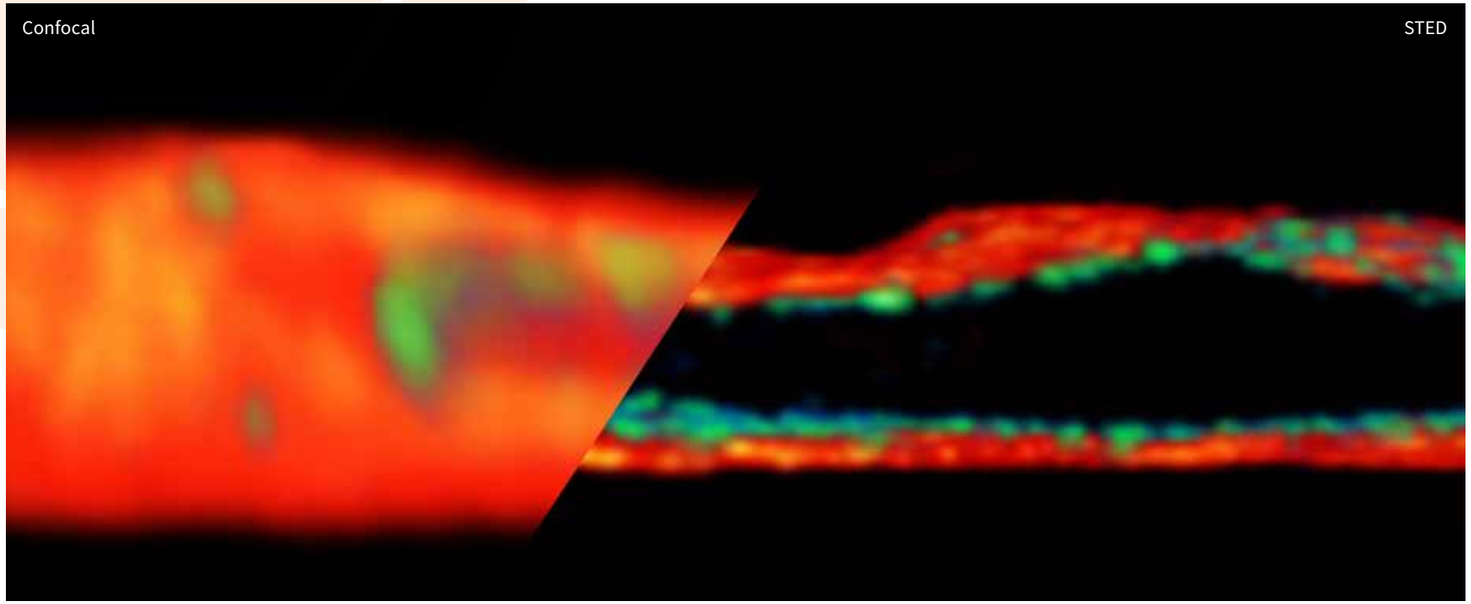
Abberior is a sister company of Abberior Instruments specializing in the development and distribution of fluorescent dyes and labels that are exceptionally well suited for superresolution microscopy (STED, PALM/STORM, MINFLUX, and RESOLFT). Note that, being designed for exceptional brightness and photostability, these markers also yield outstanding results in confocal and epifluorescence microscopy as well as in single-molecule applications.

With more than a decade of research experience in superresolution microscopy and a passion for developing new, functionalized dyes for this new era of microscopy, Abberior is at the forefront of fluorescence labeling for the most demanding, cutting-edge nanoscopy applications. Abberior uses patent-protected technology and continuously develops new labels.

Check our website for the latest additions.

www.abberior.com

easy3D STED



Two-color easy3D STED on lamin and nuclear pore complexes.

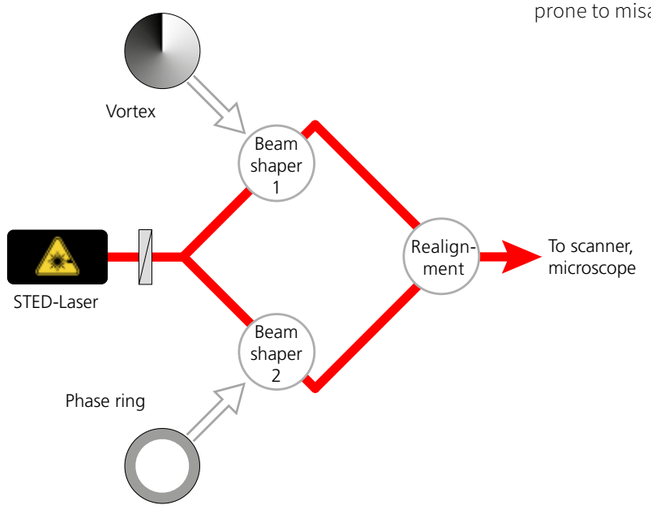
Abberior Instruments offers a unique and proprietary way of generating 2D and 3D STED light patterns, using a fast and freely programmable display (spatial light modulator, SLM). Owing to its versatility, the SLM is also used to correct optical aberrations in our automated alignment procedure.

Our easy3D optical design offers unique advantages that allow us to reach unprecedented 3D STED resolution below 70 nm in all three dimensions, namely:

- One single beam design (instead of two separate beams) allowing ultra stable imaging
- Very short beam length enhancing the stability further
- Correction of sample aberrations for an ideal STED beam zero providing maximal fluorescence yield and best signal-to-noise ratio

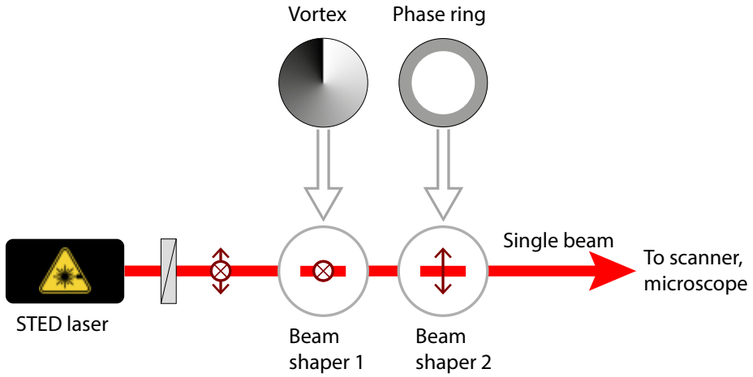
Traditional design

prone to misalignment

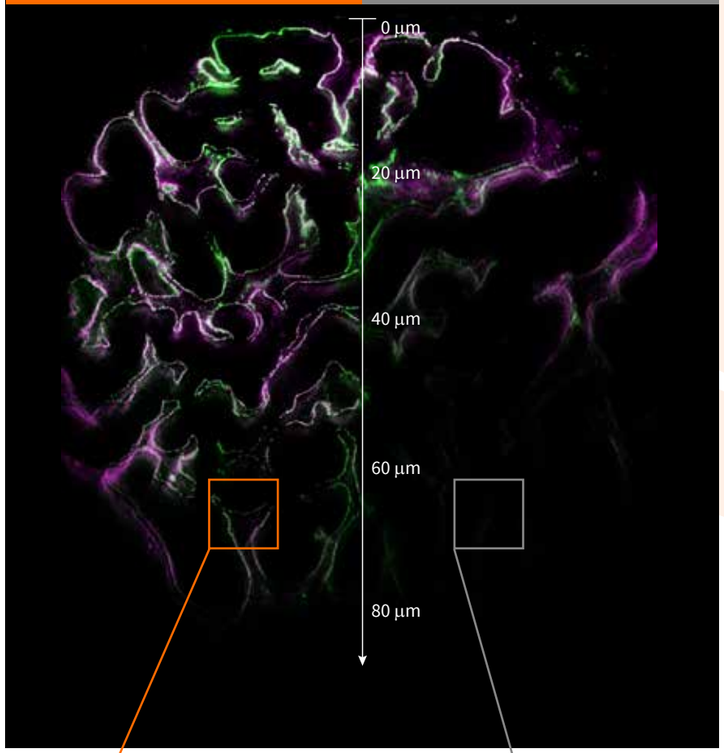


abberior INSTRUMENTS

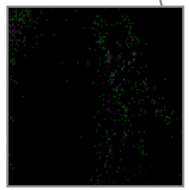
stable by design



abberior INSTRUMENTS — Traditional design



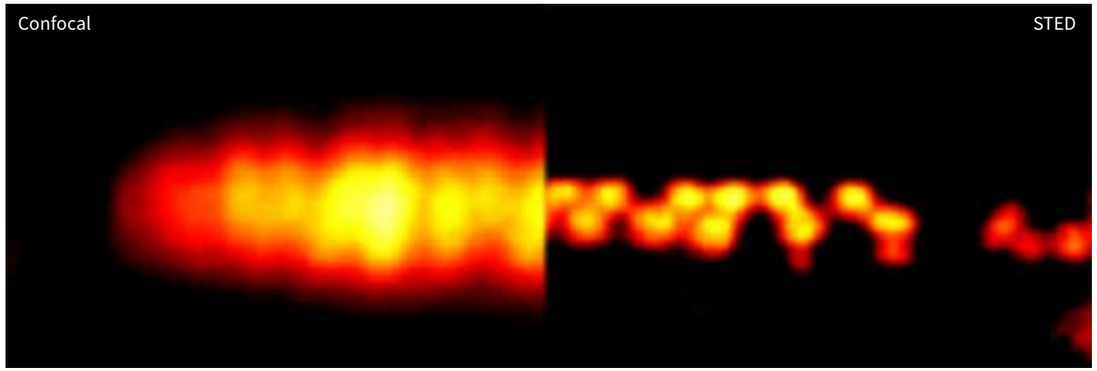
Easy3D STED imaging at 70 µm into rat kidney



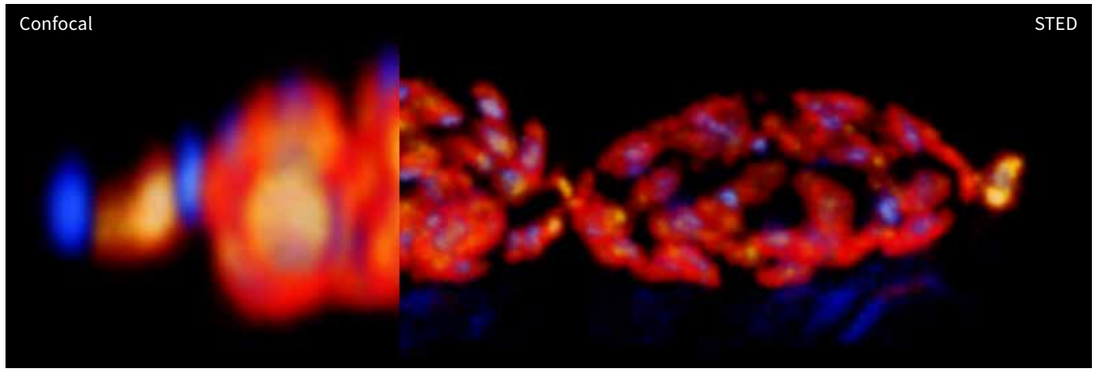
no STED imaging possible

Adaptive optics easy3D STED allows imaging up to 80 µm into cleared rat kidney tissue. Without adaptive optics, the refractive index mismatch between immersion and sample leads to a complete signal loss.

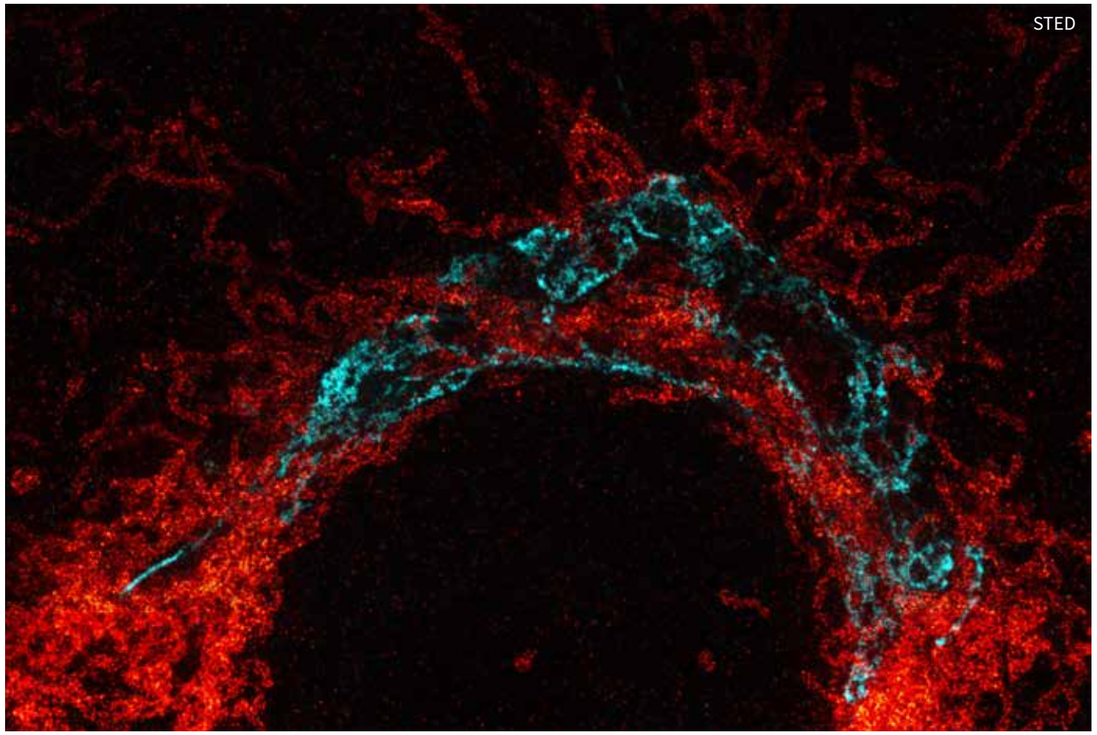
Sample by D. Unnersjö Jess & H. G. Blom @ KTH Stockholm, Sweden.



XZ slice through mouse inner ear hair cells imaged with easy3D RESCue STED. Sample by Dr. Christian Vogl @ InnerEarLab, UMG Göttingen, Germany.

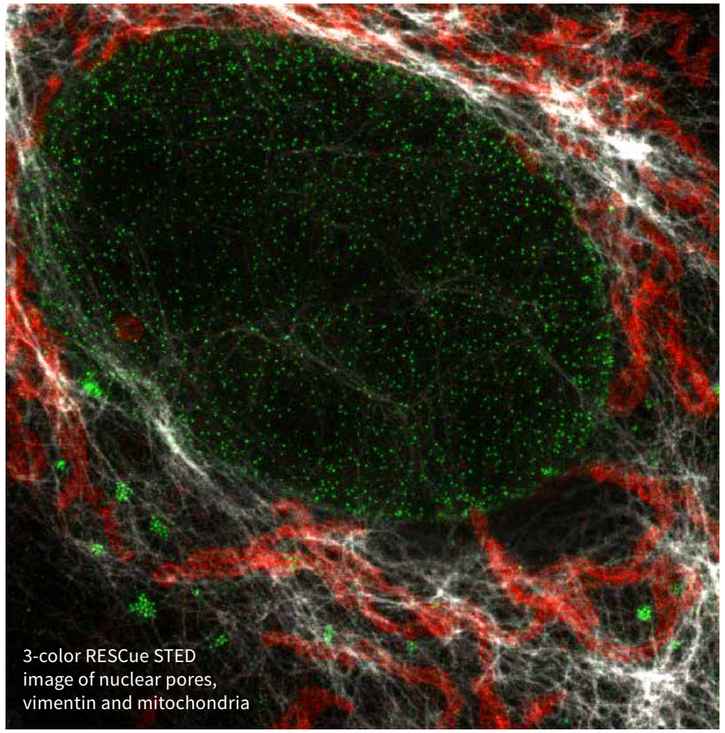


Easy3D STED 3D of active zones at the Drosophila larval neuromuscular junction. Sample by M. Lenz & M. Landgraf @ University of Cambridge, UK.

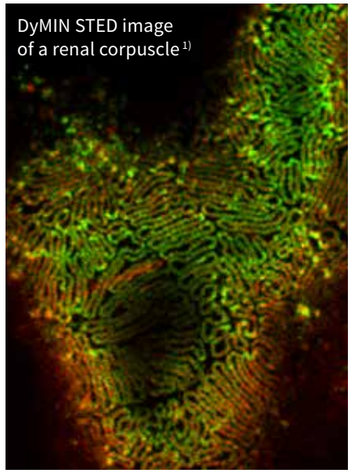


Mitochondrial protein TOM20 and GM130 (Golgi) recorded with DyMIN STED.

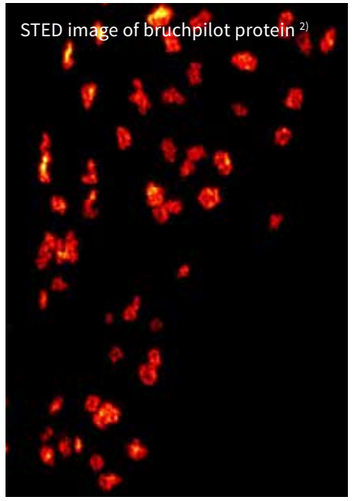
Your advantages:
- Easy3D STED resolution in 80 µm depth
- 70 nm resolution in x, y and z
- One-beam ultra-stable protected easy3D technology



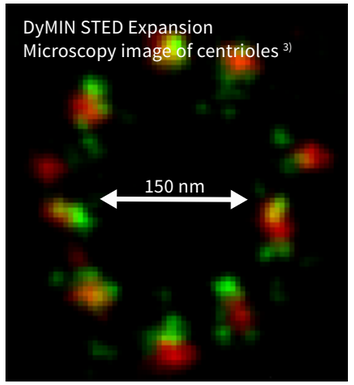
3-color RESCue STED
image of nuclear pores,
vimentin and mitochondria



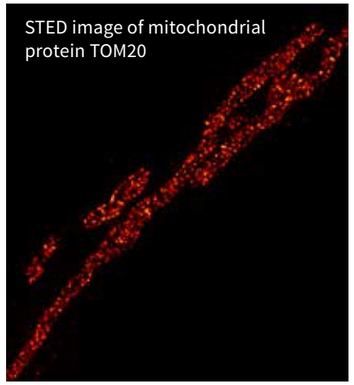
DyMIN STED image
of a renal corpuscle [1)]



STED image of bruchpilot protein [2)]



DyMIN STED Expansion
Microscopy image of centrioles [3)]



STED image of mitochondrial
protein TOM20

[1)] Sample by D. Unnersjö Jess & H. G. Blom @ KTH Stockholm, Sweden
[2)] Sample by Prof. Sigrist @ FU Berlin, Germany
[3)] Sample by P. Guichard & D. Gambarotto @ University of Geneva, Switzerland

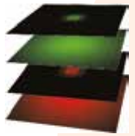
Autoalignment

The Abberior Instruments autoalignment routine harnesses multiple motorized mirrors and fully programmable adaptive optics elements (spatial light modulators, SLM). Abberior's fluorescent adjustment sample assures standardised perfect alignment. Based on two decades of experience in STED microscopy, Abberior Instruments developed the first fully automated alignment procedure in the sample for all beams, STED beam shaping, and the pinhole.

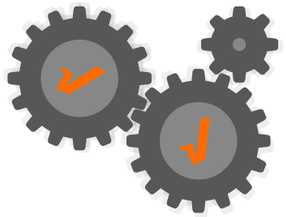
Expect perfect shape and position of all beams as well as reassuring feedback about alignment results!

Be amazed by perfect resolution – always!

First, the system checks if the adjustment sample is sufficient



Then, your Facility Line microscope automatically records multiple images in confocal, 2D-, and 3D-STED mode



Our intelligent autoalignment algorithm processes the data, ...

... and it optimizes the STED-doughnut shapes.

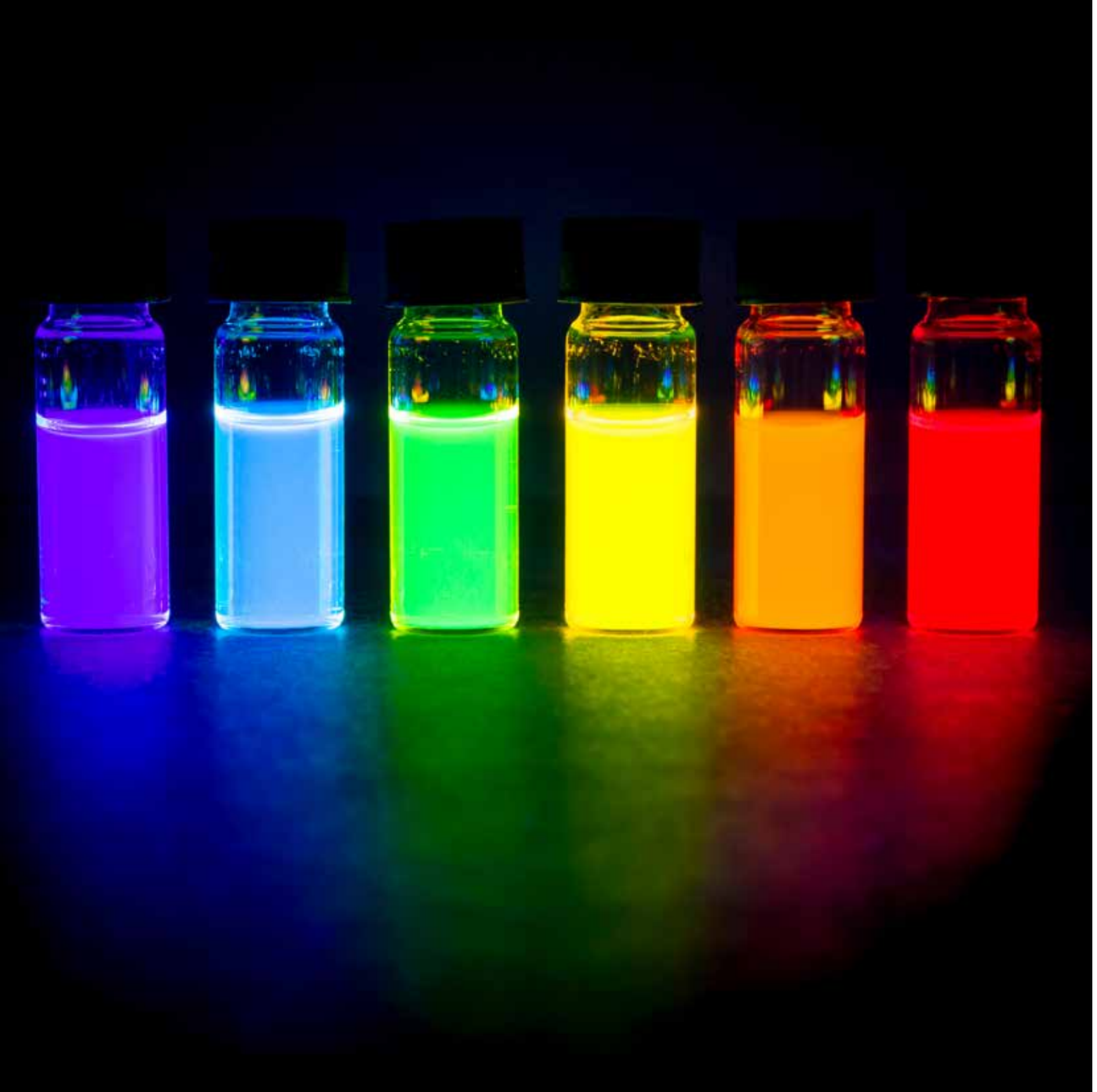
...the position of the pinhole, ...

... automatically aligns the position of all excitation and STED-beams, ...

Done in 90 seconds!

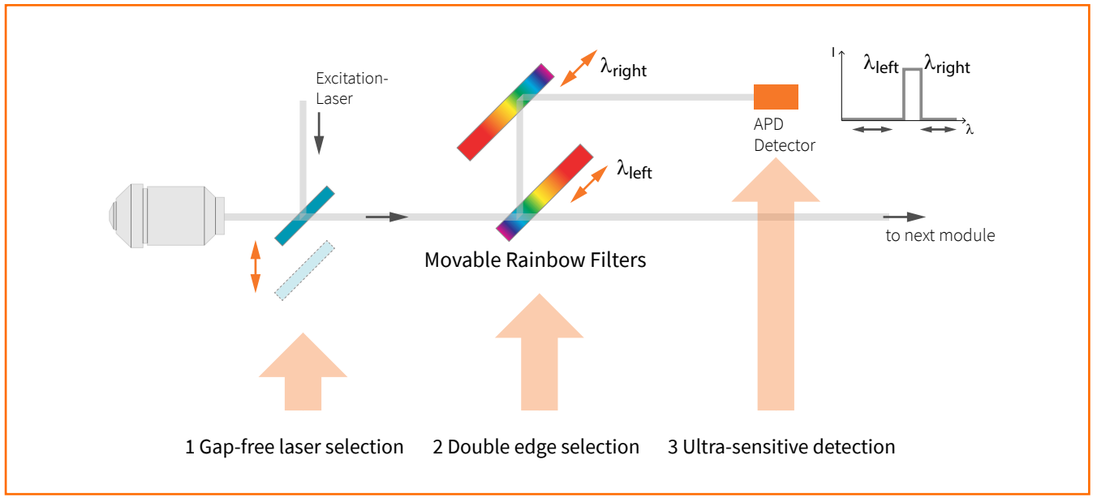
Your advantages:

- Fully automated alignment procedure in the sample!
- 90 seconds for perfect alignment of all beams confocal and STED and the pinhole
- Image with perfect superresolution – always!

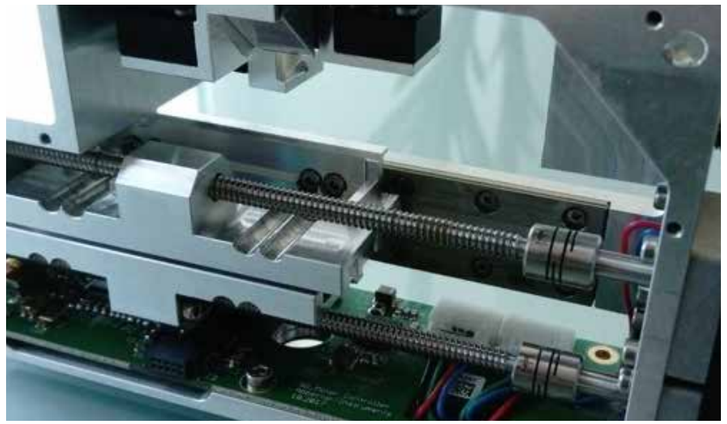


Rainbow Detection
Highly sensitive spectrally

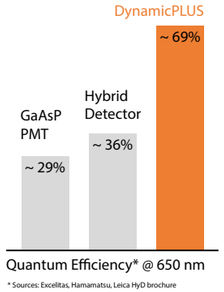
By using flippable filters, any laser combination can be selected by moving individual filters in and out of the beam path. No laser, no filter, no losses. Compared to acousto-optic solutions, images benefit from much better blocking performance of unwanted stray light and higher fluorescence transmission. Enjoy higher flexibility compared to fixed wavelength combinations provided by filters in a wheel.

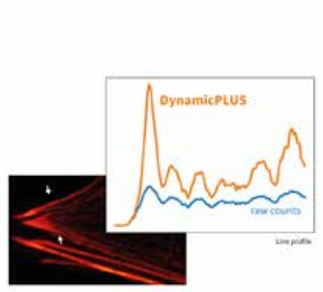


A pair of gradient-coated filters are translated within the Rainbow Detection module in order to freely define the edges of the detection window between 400 and 800 nm. Up to four modules can be installed or upgraded at any time and the fluorescence can be freely distributed between all Rainbow Detection modules.



Capture everything from the faintest details...





...to the brightest confocal images

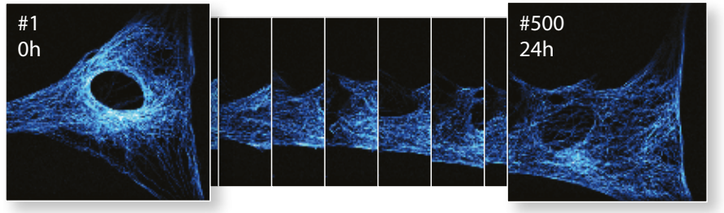
The underlying avalanche photo detectors (APDs) have superior quantum efficiency, up to a factor of two above hybrid detectors. This means that even when the signal levels are low, our APDs still collect plenty of photons for a meaningful image. Typical applications are superresolution STED imaging and experiments with low labeling densities designed to maintain physiological conditions. At the same time, the dead-time compensation of DynamicPLUS yields crisp images of high-signal samples. Thus, also confocal imaging is highly improved. Of course, you can opt between quantitative analysis of raw data or mathematical deconvolution.

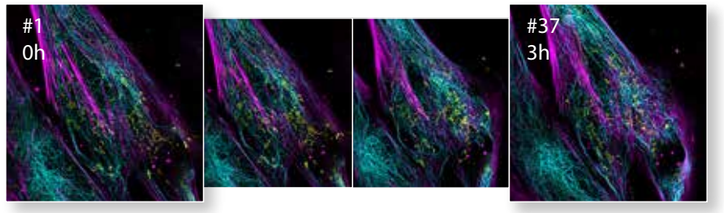
STED image of a growth cone at the tip of the axon of a primary hippocampal neuron at day one in vitro.

Your advantages:

- Free selection of excitation & STED wavelength combinations
- 100% gap-free spectral Rainbow Detection
- Free selection of up to four spectral detection windows
- Avalanche Photo Diodes (APDs) for the highest sensitivity up to 65%

Autofocus STED & Confocal

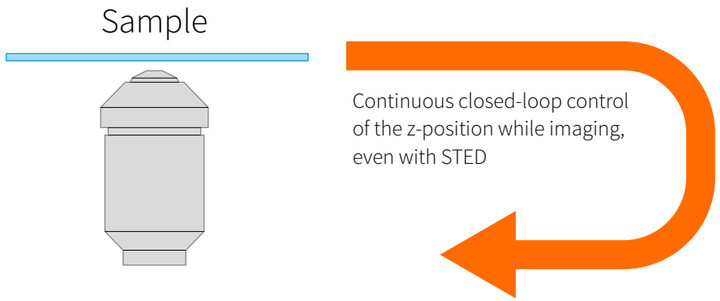




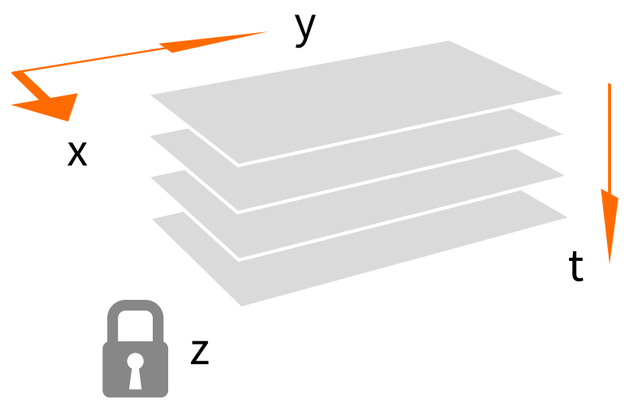
Your advantages:

- Image over hours and days
- Image with constant z plane in xy or even in xyz volume images
- Image with confocal and STED mode without drift in z

The Abberior Instruments autofocus module actively stabilizes the z position of your sample. No focus drift during imaging any more!

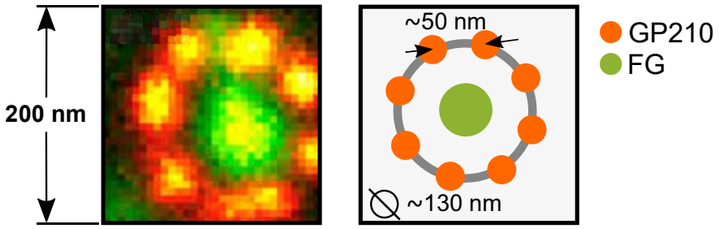


Different imaging modes possible – xy plan and z constant; xz plane over time, xyz stack with z constant in each z section.

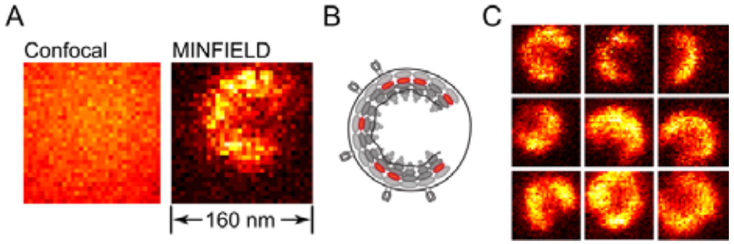


MINFIELD STED

MINFIELD STED confines the field of view to the inner minimum of the STED-doughnut (i.e. imaging fields of e.g. 100 x 100 nm). The dramatically higher signal from the center of the doughnut enables resolutions below 20 nm for many samples.



MINFIELD STED is a co-development between Stefan Hell and coworkers and Aberior Instruments. Read details in: F. Göttfert, T. Pleiner, J. Heine, V. Westphal, D. Görlich, S. J. Sahl, S. W. Hell, „Strong signal increase in STED fluorescence microscopy by imaging regions of subdiffraction extent“, PNAS 1621495114 (2017).



MINFIELD STED presented on human immunodeficiency virus type 1 (HIV-1). Images from J. Hanne et. al. “Stimulated Emission Depletion Nanoscopy Reveals Time-Course of Human Immunodeficiency Virus Proteolytic Maturation“ ACS Nano 10, 8215-8222, 2016.

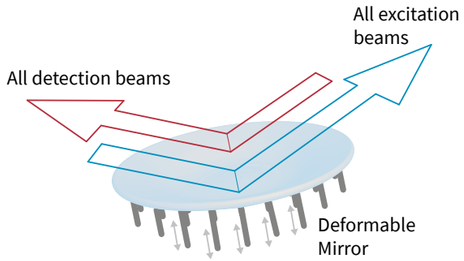
Your advantages:
- Push-button STED superresolution below 20 nm!

Uniquely gentle and sensitive confocal imaging

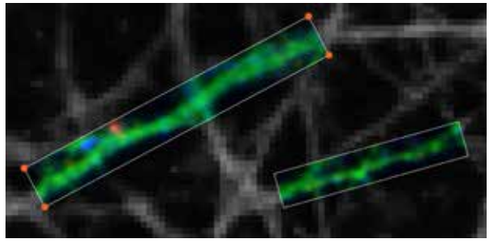
The Facility Line delivers the best confocal imaging money can buy. Why? Simply because extraordinary STED recording requires excellence in all aspects of confocality.

Consequently, our confocal imaging comes with
1. adaptive illumination, which is easily 80% more gentle to your living cells than any other confocal microscope can be;
2. a deformable mirror which, by automatically correcting aberrations, makes correction collars a thing of the past;
3. superior detection efficiency for brilliant signal; and
4. direct counting of fluorescence photons for scientifically rigorous image data.

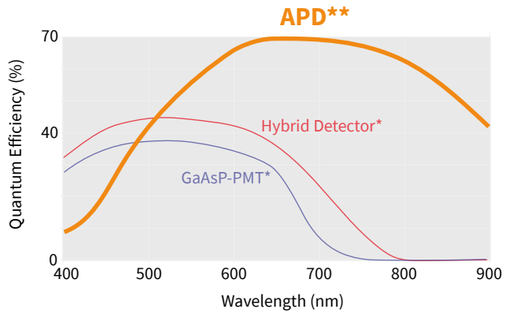
On top, all lasers plus the pinhole are kept in best shape 24/7 by our proprietary autoalignment system, which aligns all beams within 30 seconds.



Our deformable mirror is unique in confocal and superresolution microscopy. It maximizes image quality by eliminating aberrations, optimizing the size and the shape of both the excitation and the detection beam. Thus, both confocal and STED images are brighter and crispier, especially deep within thick samples.



Free rotation of ROIs over the full field of view and around arbitrary axes enables you to image only relevant regions. Moreover, you save time and photons!



Quantum efficiency of the Facility Line's APDs vs typical detectors of other manufacturers. Our images are not disturbed by non-linear photomultiplier gain or image beautification measures. We give you plain photon counts, providing you with the most scientifically accurate data!

Your advantages:
- Confocal imaging with adaptive illumination is 10-100 times more gentle
- Brilliant signal through superior fluorescence detection
- Direct photon counting gives you the most truthful images
- Free rotation of ROIs - image only relevant regions and save time
- Autoalignment of all laser beams and the pinhole
- Superior confocal volume imaging with adaptive optics, particularly in deep tissue

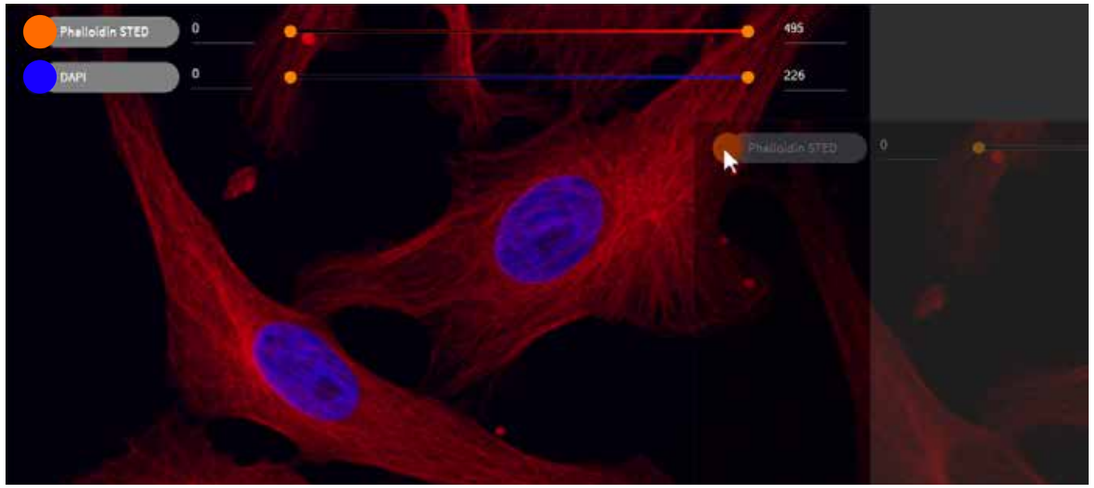
Sources:
* "Leica HyD for Confocal Imaging", Order no.: English 15930030013 - 06/2015/STO, Leica Microsystems
** "SPCM-AQRH Single Photon Counting Module", Rev. 2018-12, Excelitas Technologies Corp.

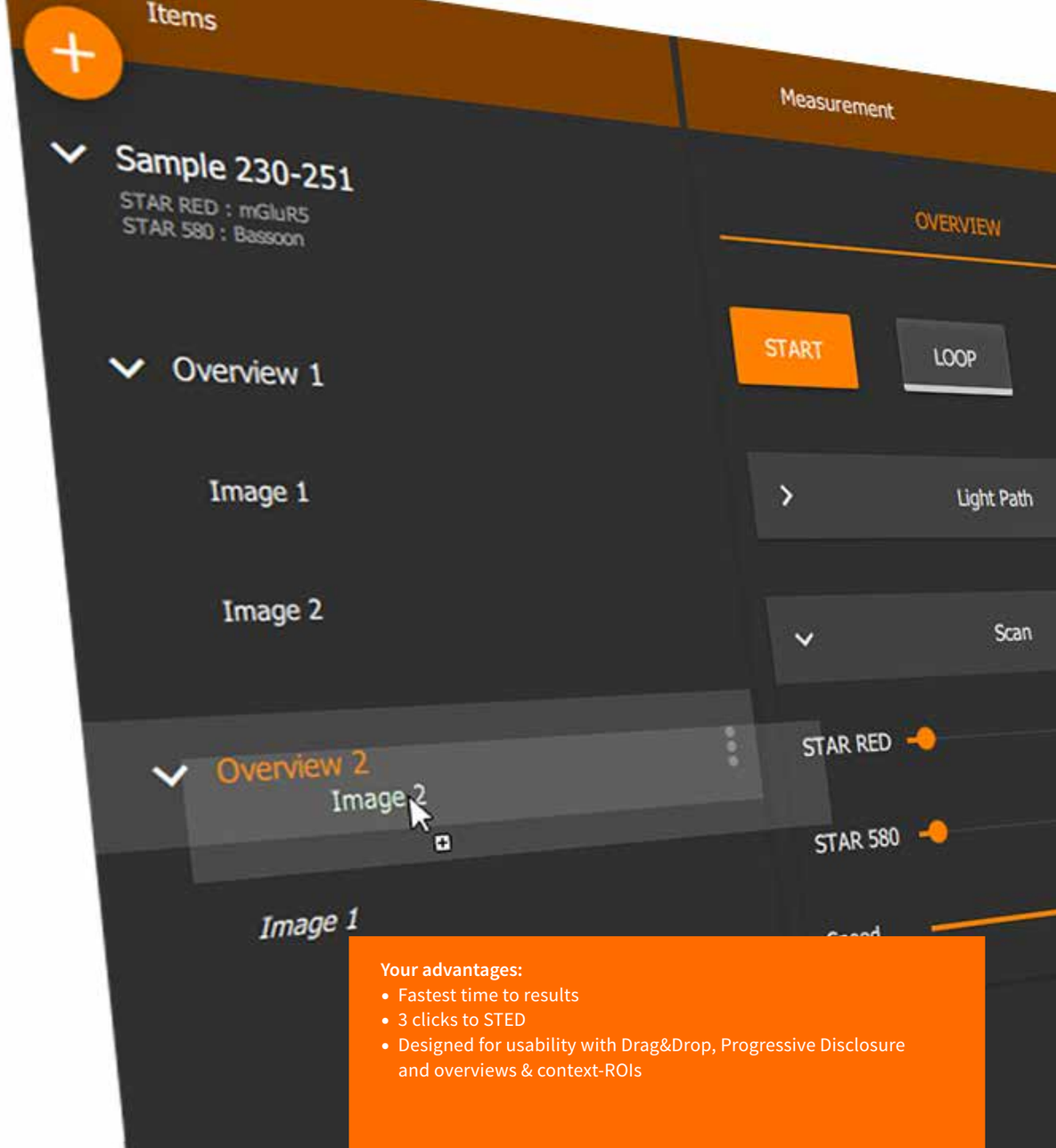
Graphical User Interface (GUI)

The Facility Line-GUI was designed from scratch with easy usability as the prime goal. The user simply enters the dyes that are in the sample. From there, the software applies most settings (e.g. lasers, detectors, pixel size, gating, etc.) automatically. Of course, experienced users can always overwrite the settings.

After selection of the dye, the user performs a quick overview scan and selects one or multiple regions (ROI), which can then be fine-scanned. For these measurements, spatial context with regard to the overview is always kept. Scan settings can ingeniously be transfered via Drag&Drop between overviews or ROIs for a lightning-fast workflow.

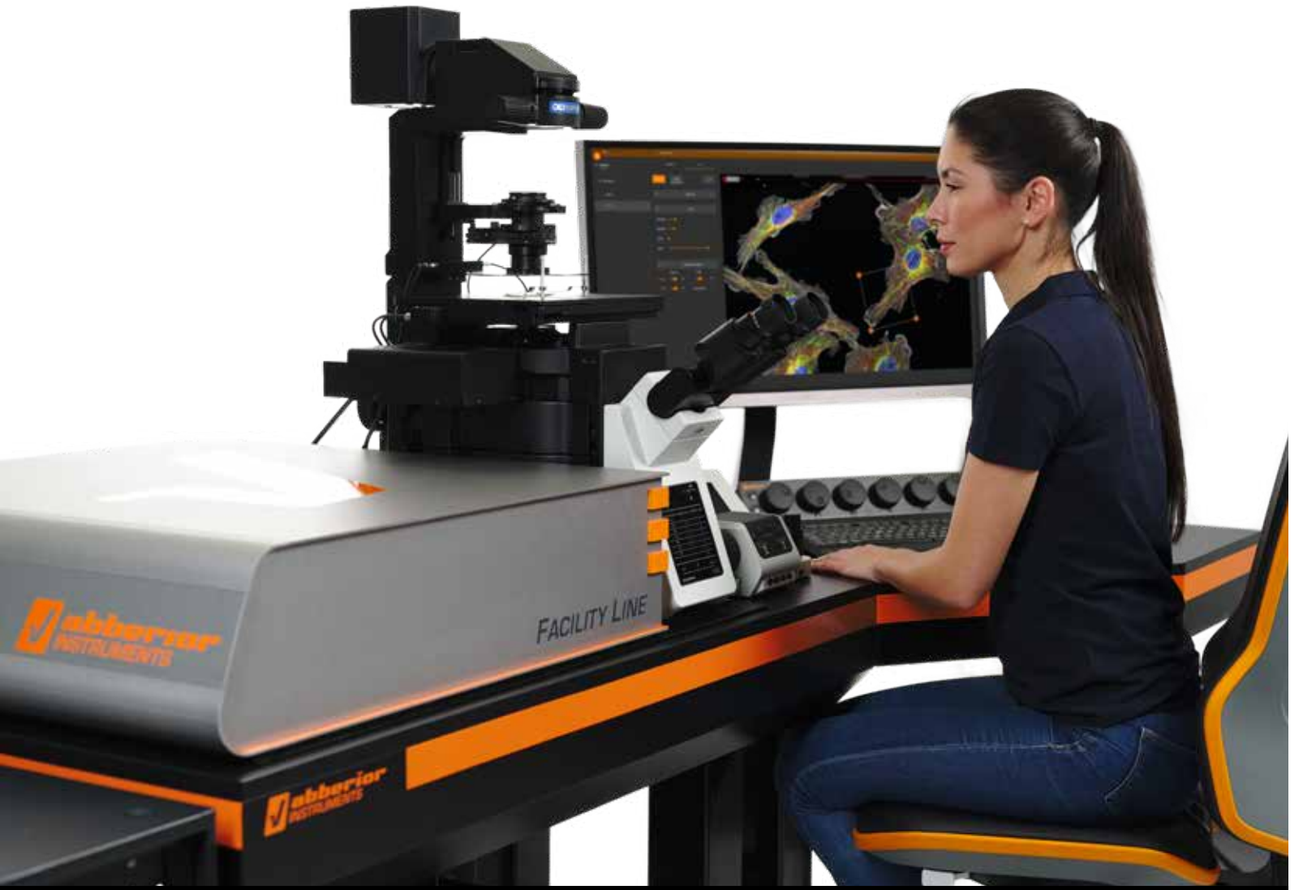
Of course, all our high-end features such as Adaptive Ilumination (DyMIN, RESCue, MINFIELD), Autofocus STED, Adaptive Optics, etc. are at hand, guided by an optimized workflow. Superb confocal imaging and STED comes right at your finger-tips, even for a novice user. As the user gains more experience, progressive disclosure reveals more and more settings for fine-tuning of the results.





Your advantages:

- Fastest time to results
- 3 clicks to STED
- Designed for usability with Drag&Drop, Progressive Disclosure and overviews & context-ROIs



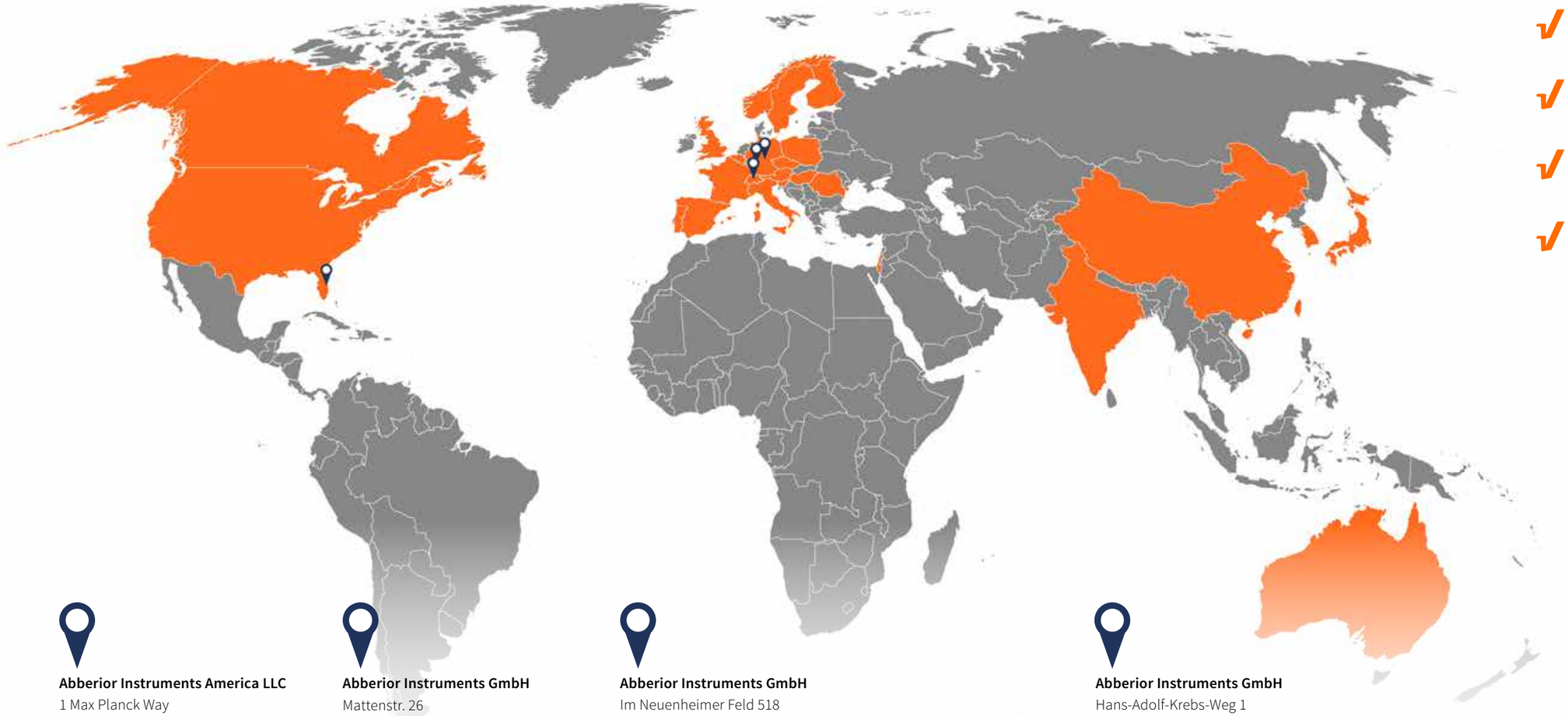
Ergonomic workstation

From day one of designing the Facility Line workstation, we went to great lengths to create a highly ergonomic platform. With a single click of a button in the software, the system automatically fully starts up. The same seating position enables you to look through the eyepieces, work with the PC, and operate the microscope. As a result, you can effortlessly focus on your long-term experiment.

All controls (z-drive, xy-stage, keyboard, mouse, microscope touch panel, widefield illumination, dial panel, etc.) have a well-defined, carefully thought-through location, resulting in an ergonomic and efficient workspace.

No browsing through lists showing how to switch the system on/off in the right order, no readjusting of your seat, no need to stand up in order to look through the eyepieces, no searching for hidden switches or controls.

Hundreds of satisfied users around the world*...



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