

Quadscanner for High Resolution Scanning Microscopes and Array Reader (P-750)

Key Facts

- Fast, adaptive optical scanning device; calibration with 4 galvanometers
- completely free positioning of the scanning mirrors regarding the conjugated back focal plane
- Application in high resolution microscopes (fluorescence, Raman, STED, localization)
- Application in high resolution array reader

Background

The implantation of this two mirror scanning unit into each of the classical two mirror scanner results into the QuadScanner. This technique decouples mirror rotation and beam shift for both scanning axes. The pupil can also axially be freely positioned and the ratio of each mirror couple is adjusted so that the point of rotation reclines on the pupil plane of the system.

To provide an alignment near the theoretical $L = D$ the mirrors must be positioned in a way, that the conjugated back focal plane is located between both scanner units. In contrast to the three mirror concept the galvanometers no longer need to be precisely positioned at a certain location. A calibration of the mirror ratio allows for complete compensation of mounting tolerances.

Technology

Experimental evaluation of the QuadScanner showed a performance a possible scanning speed with a small ROI.

The first two images were recorded in confocal mode whereas all other images display the STED measurements. Clearly the resolution is greatly increased with STED.

Analysis of the images revealed comparable resolution to fixed sample measurements with much longer dTs. The smallest reasonable dT was 4 μ s. Smaller dTs resulted in a not acceptable SN ratio but could still be performed by the scanner unit. An application of a detection unit consisting out of more than one APD should result in even better performance of the setup.

The presented beam scanning concept is the first beam scanner based on regular galvanometers for a STED microscope. To decouple the rotation and the position of the beam complete for both scanning axes a novel four mirror approach was presented. This concept allows a completely free positioning of the scanning mirrors regarding the conjugated back focal plane. It was shown that the mirror rotation ratio of each two mirror scan unit is important for the later beam position precision. For the implementation of the QuadScanner a calibration routine was developed, which corrects the rotation ratio of each axis for the complete FOV with beam position precision better than 20 μ m in the back focal plane. The STED setup does not work at its optimal performance until this calibration is carried out. This fact was demonstrated at PSF measurements and imaging of Crimson fluorospheres.

This concept uses the unique capability of STED microscopy to tune the resolution by the applied depletion intensity. A confocal pre-scan determines the regions in the FOV with fluorophore information and a successive STED scan re-fines the spatial information only in these regions. This completely new approach to faster beam scanning was demonstrated at technical and biological relevant samples. Depending on the information inside the FOV an image acquisition speed improvement of factor 13 was demonstrated for the technical sample and a factor of 8 for the biological sample. The latter factor reduced the overall image acquisition time to an effective dT well below the reported effective dT of the resonant scanner, resulting in the fastest reported effective dT for a biological relevant STED image. Besides

the impressively enhanced image acquisition speed no drawbacks resulted from the use of the adaptive scan pattern. Instead the image quality was comparable to STED images recorded with 8 times longer dT, thereby implicating a SN ratio, which could not be reached otherwise.

Development Stage

The device is successfully applied in STED microscopes at the DKFZ and the Max-Planck-Institute in Göttingen.

References

“Parallelized STED fluorescence nanoscopy” by Pit Bingen et al. in [Opt Express. 2011 Nov 21;19\(24\):23716-26](#)

Intellectual Property

The priority application was filed December 19, 2008 as EP 08 172 450.2. The PCT application [WO2010069987](#) was granted in Europe as [EP2359178](#) (DE, CH, FR, GB, NL) and granted in USA as [US8520280](#).

Inventors

The investigator is Dr. Johann Engelhardt, Department of Optical Nanoscopy (E190) of DKFZ Heidelberg, Germany.

Commercial Opportunity

This technology is able to improve in particular Fluorescence microscopes, Laserscanners, Raman Systems, STED and Localization Microscopes. Furthermore it can be used for high resolution array readers.

DKFZ Contact:

For further information, including a CDA, please contact:

Dr. Frieder Kern

Deutsches Krebsforschungszentrum

Office of Technology Transfer, T010

Email: F.Kern@dkfz.de

Tel.: +49-(0)6221-42-2952

Fax: +49-(0)6221-42-2956

Figure 2

Adaptive Raster Scanning Procedure

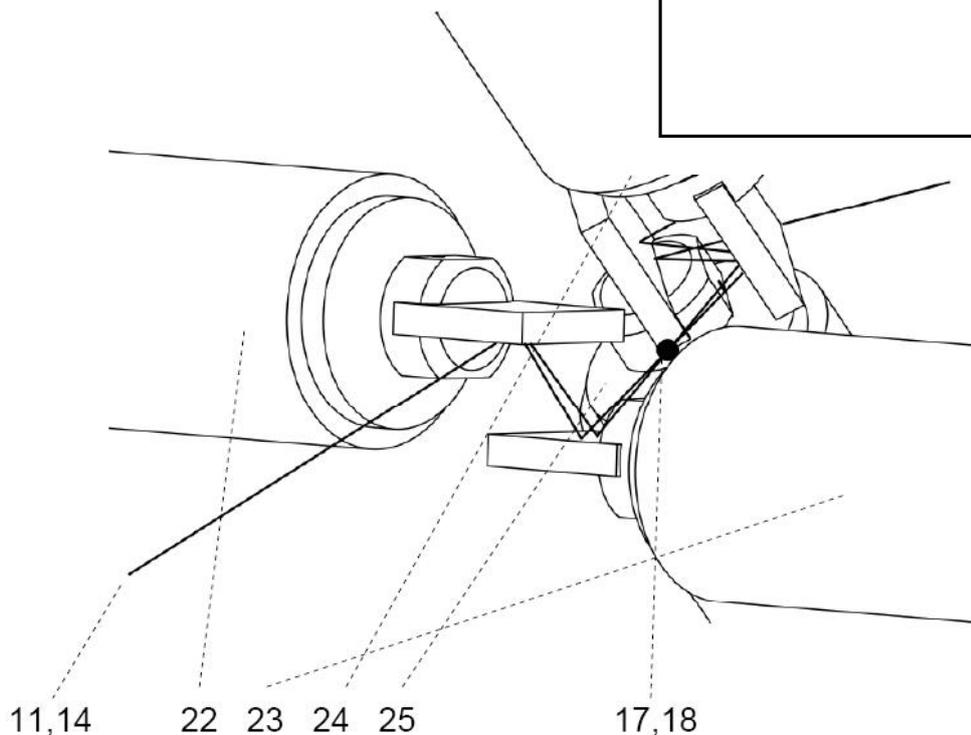
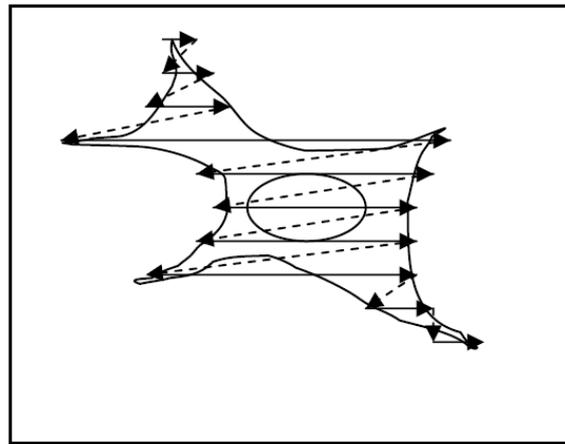


Figure 1

Device using 4 mirrors and 4 galvanometer: Detektion beam (11), STED Laser Beam (14), Pupil Image (17), Intermediate Image (18), Beam Deflector (22, 23, 24, 25)