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Thin-sheet Laser Imaging Microscopy



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Functional principle

Non-destructive optical sectioning in combination with three-dimensional reconstruction and selective visualization are of great help for understanding structure and function of tissues and organisms. With a resolution comparable to wide field fluorescence microscopy, thin sheet laser imaging microscopy is a valuable tool for imaging transparent or fixed and cleared tissues with a size of several mm. Z-stacks are imaged by moving a selectively stained specimen through a laser light sheet, thus causing a plane of fluorescence which can be recorded by a conventional microscope positioned perpendicular to the light sheet (Fig. 1).

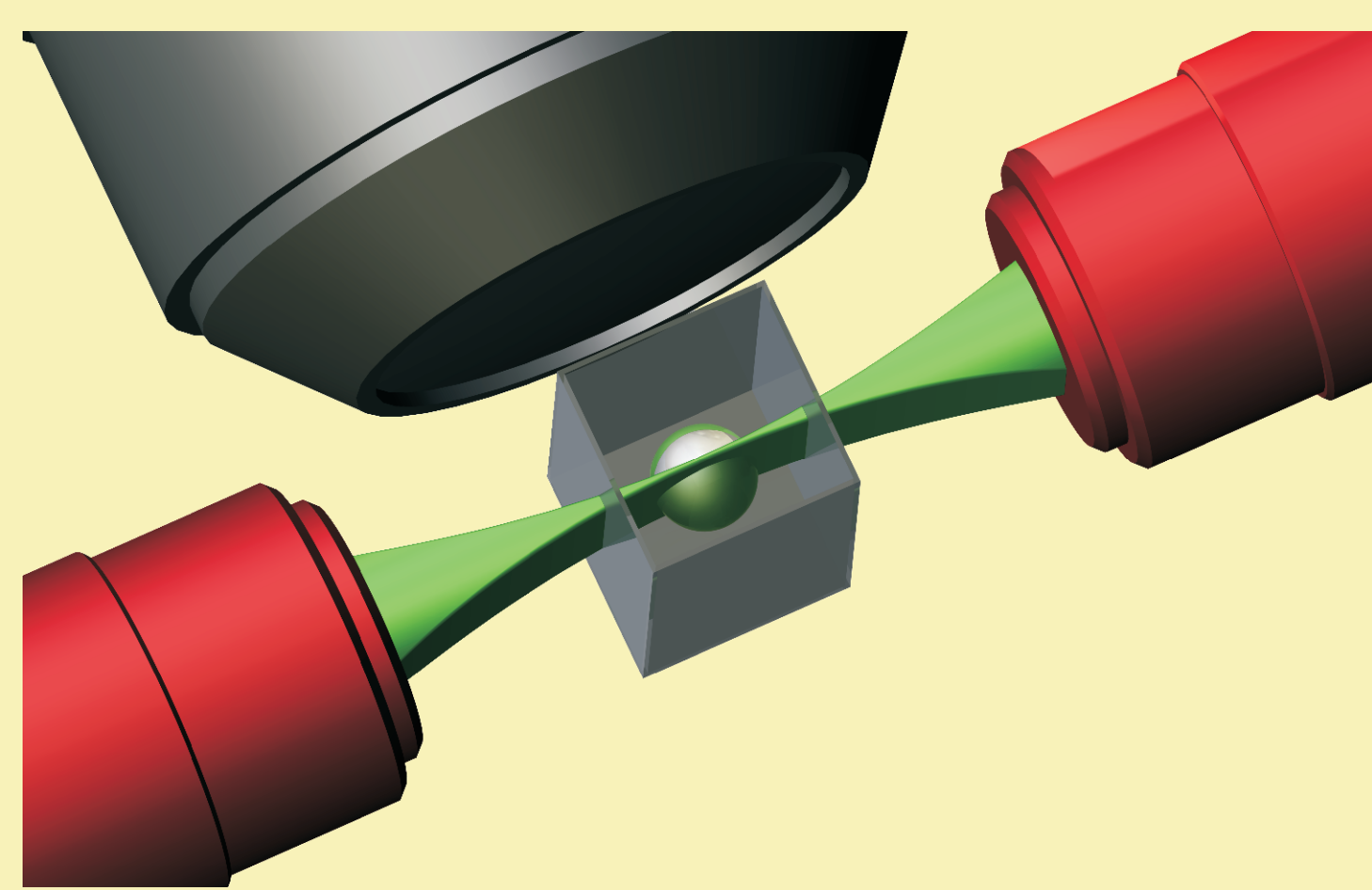


Fig. 1. A cross section of the transparent specimen, symbolized by a sphere, is illuminated by the light sheet. Fluorescent light is emitted by the illuminated region and can be recorded with the microscope.

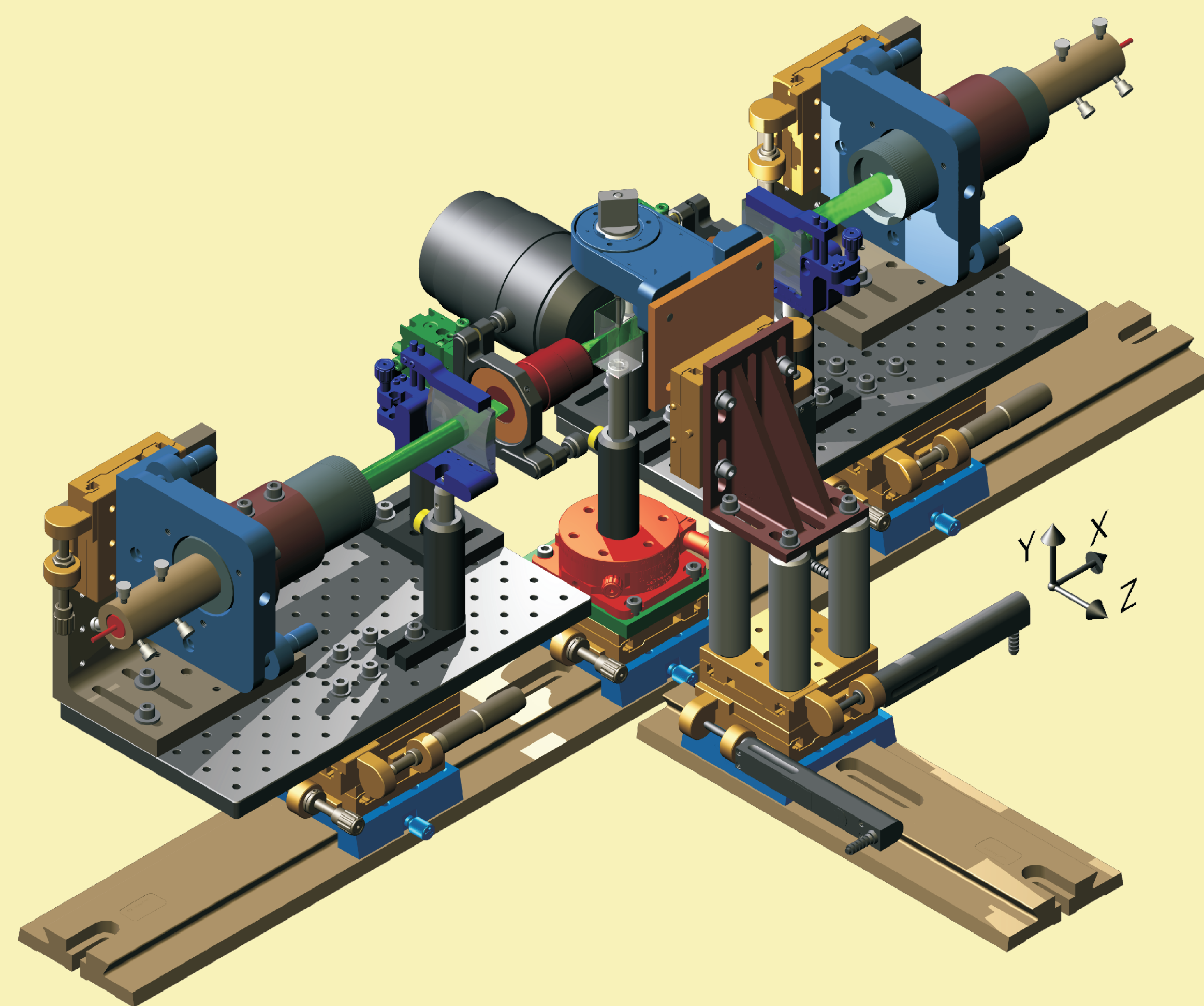


Fig. 2. CAD-diagram of the thin sheet laser imaging microscope installed in the Santi labs at the University of Minnesota. The microscope is symbolized by the objective lens, while the necessary computer and actuator controller are not shown.

General building units

Illuminators for laser light sheet generation:

To generate a light sheet, expanded laser beams have to be focused in one direction while keeping their diameter in the perpendicular direction. As cylindrical lenses [1] suffer from spherical aberration, we use microscope objectives in combination with cylindrical lenses [3,4,6], to ensure a nearly diffraction limited light sheet thickness. Absorption artifacts are reduced by dual side illumination.

Specimen chamber:

For the specimen to be transparent and to avoid light scattering, it has to be placed in a specimen chamber filled with an index matching solution.

Actuator system:

For optimized recording speed and to avoid focussing problems, we move the specimen instead of the illuminators. Translational actuator movement ensures a constant z-sampling across the full width of the specimen as well as image stitching compatibility.

Microscope with digital camera:

Different kinds of microscopes can be used for light sheet based microscopy. In order to allow the actuator system to access the chamber from the top, the microscope has to be positioned horizontally.

Computer with software:

Specimen movement and image acquisition as well as possible shutters and an image stitching process are controlled by a computer via the LabView™ platform.

Shadow artifacts reduction

Shadow artifacts due to light absorption are of major concern in the field of light sheet based microscopy. Dual side illumination [5,6] helps to significantly reduce these artifacts by filling darker lines with light from the second illuminator. The remaining lines can be further reduced by Fourier space filtering.

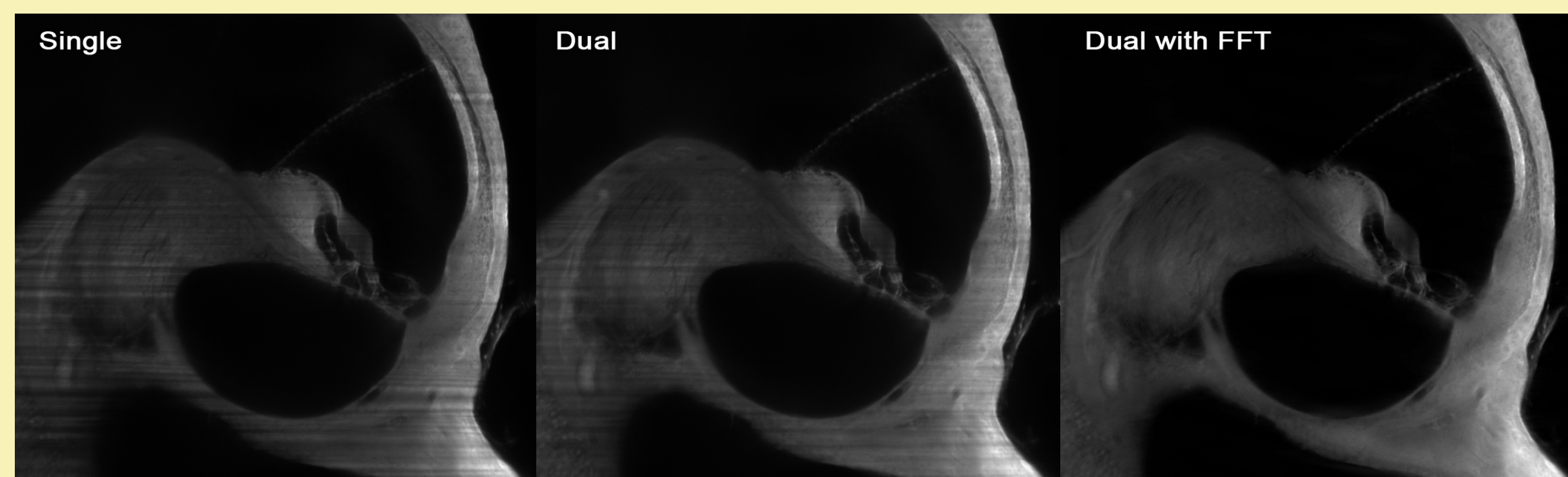


Fig. 3. Comparison of single side illumination with dual side illumination and dual side illumination in combination with Fourier space filtering.

Three-dimensional reconstruction

Three-dimensional reconstructions (Fig. 5) are an excellent tool not only for scientific studies but also for education and communication. They can be obtained from well aligned z-stacks of a specimen by manual (Fig. 6) as well as automatic sectioning using tools like Amira™.

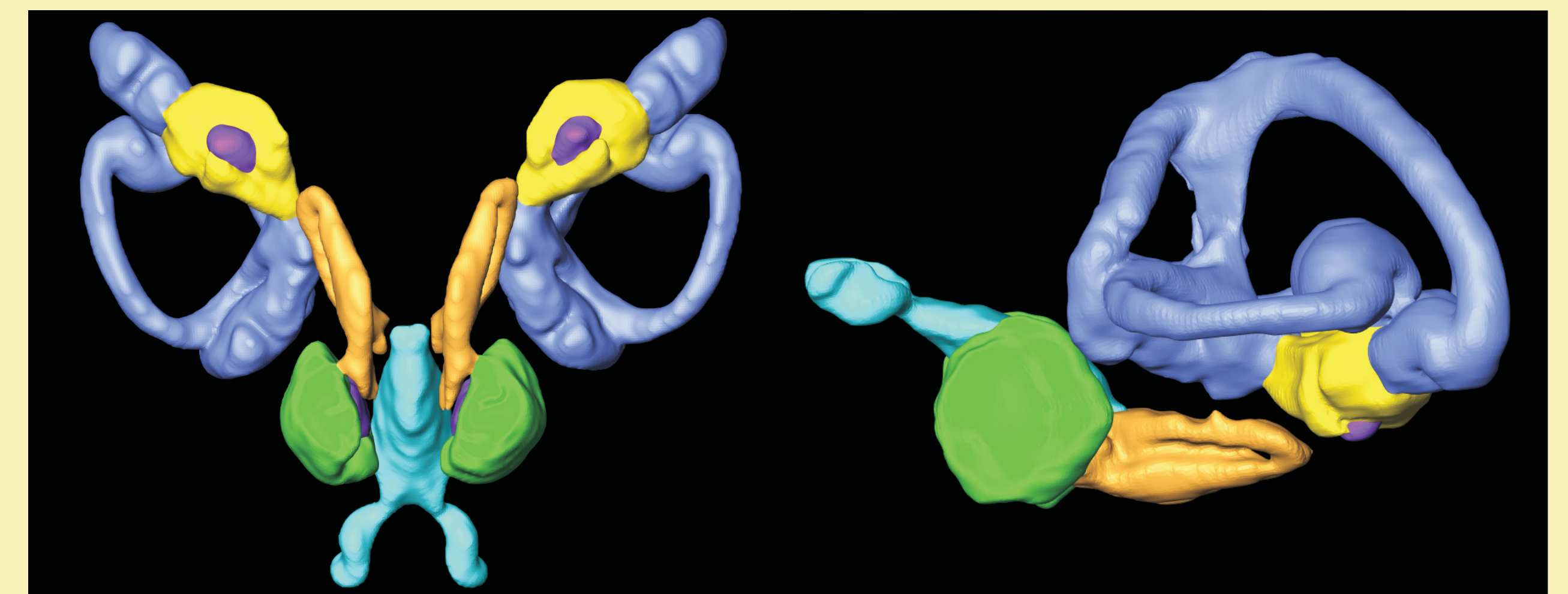


Fig. 5. Two views of the three-dimensional reconstruction of a zebrafish's hearing organs.

Image Stitching

A Gaussian laser beam focused in one direction results in a hyperbolically shaped light sheet of varying thickness across the width of the specimen. By moving the specimen through the smallest region of the light sheet, recording only this region, and stitching these strips together, it is possible to get a well resolved image over the full width of the specimen [2] (Fig. 4).

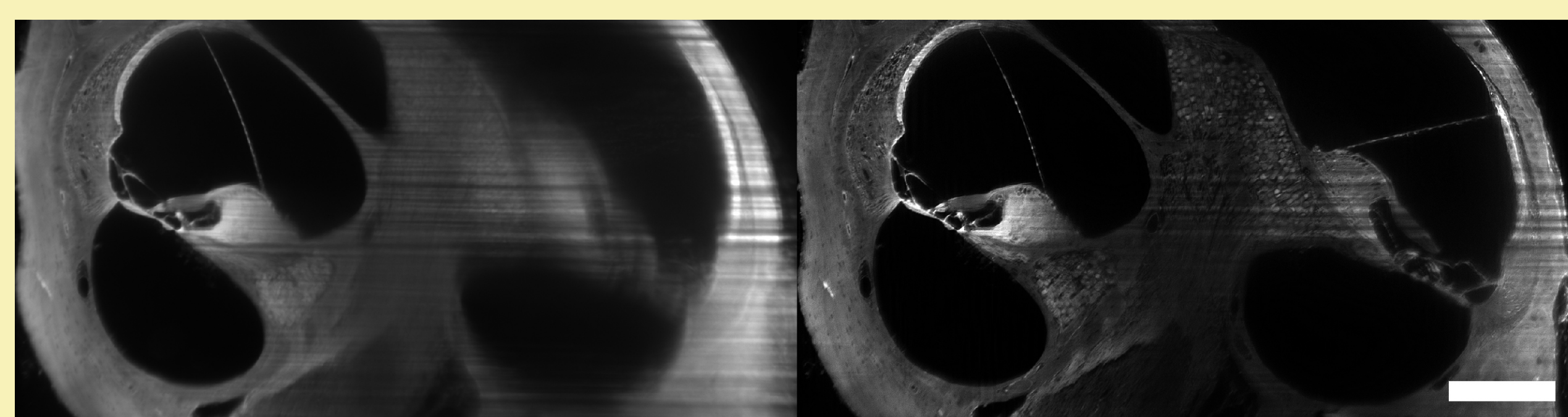


Fig. 4. While the unstitched image on the left shows significantly fewer details in the regions far away from the beam waist, the stitched image on the right benefits from high resolution across the full width of the specimen. Bar = 300µm.

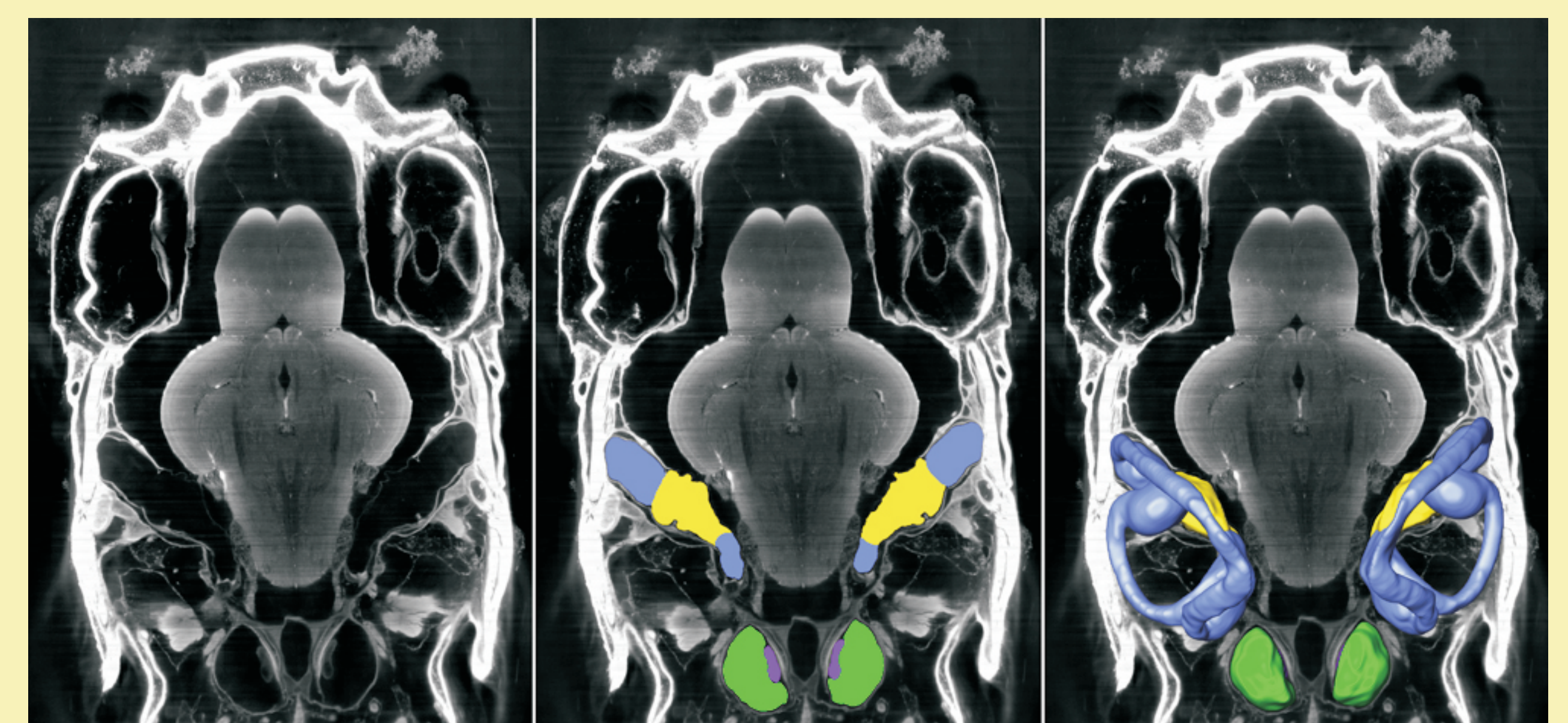


Fig. 6. Steps of a manual 3D reconstruction using Amira™. The relevant regions are manually highlighted using different colors, each color symbolizing a different part of the specimen. After all slices have been processed Amira™ calculates the 3D reconstruction.

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