Fast Automatic Recombination of Multiple 3D Datasets for Large Volumes and High Dynamic Range (HDR)

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Motivation

Ongoing biomedical research often requires imaging of large and thick specimen. Such specimens do not fit into the field of view (FOV) of a standard confocal microscope. Moreover the variations in the fluorescence intensities (due to absorption effects) can not be resolved with a single recording. To overcome these limitations a recombination of multiple recordings can be done. While software for such a recombination exists, we have often found it to be limited in applicability. Furthermore recent objectives provide a distortion-free image even at the border of the field of view (FOV). Such datasets allow the use of new fast, efficient and robust techniques.

Approach

Stitching:
Large scale imaging needs to be done at arbitrary positions in the sample to keep recording time and exposure of the probe at a minimum. These arbitrary positions are especially hard to stitch as there is no information to which stacks will correlate.
We use a modified phase-only correlation based on the normalized Fourier spectrum, that allows us to determine pairwise best displacements while being invariant to linear gray value changes. Normalization of the Fourier amplitude is done with the square root, which has shown to be very robust against noise.
The precise pairwise displacement is determined using normalized cross-correlation, to attain a quantitative measure of similarity. Correlation windows are extracted at interest points which are selected based on image contrast. From pairwise displacements we find the globally optimal positions of image stacks using singular value decomposition.
To maintain speed, a multi-scaling approach and a heuristic for choosing likely correlation candidates have been developed.

Bleaching correction:
From the overlapping region, incident image bleaching can be estimated. We have found bleaching to be modelled by a linear process, where the parameters can be gained from the level-wise gray value cooccurrence matrix. Distribution from the overlap into the probe is modelled by a normal distribution where the variance resembles the likeliness of the fluorophore to bleach. This parameter has to be gained from the user by visual inspection.

High Dynamic Range Imaging:
When imaging thick specimen, intensity variations sometimes can not be resolved with a single recording due to the limited dynamic range of the detector. When doing multiple recordings, we propose the use of differently exposed images rather than a multi-averaging, to maintain a high Signal-to-Noise-Ratio (SNR) throughout the dynamic range. Using a noise-model of the confocal microscope, an error normalization can be applied to the high dynamic range recombination of images.

M. Emmenlauer, Feature- und Deformationsmodell-basierte Registrierung von 3D Volumendaten von Zebrafischen. Master Thesis, Universität Freiburg, April 2007.
http://lmb.informatik.uni-freiburg.de/people/emmenlau/diplomarbeit.pdf

Results

We have applied our software toolset to various bio-medical datasets ranging from zebrafish to mouse braincell slices to trichome datasets to single human braincells. With the before mentioned heuristic, very large datasets of up to 68 stacks recorded at arbitrary positions could be stitched in less than 6 minutes on a state of the art single core computer, given a sufficient amount of RAM. For smaller datasets, stitching takes on average less time than needed to load and store the data, typically below three minutes.

Acknowledgements

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Jakob Wolfart, Neurozentrum, University Hospital, Freiburg

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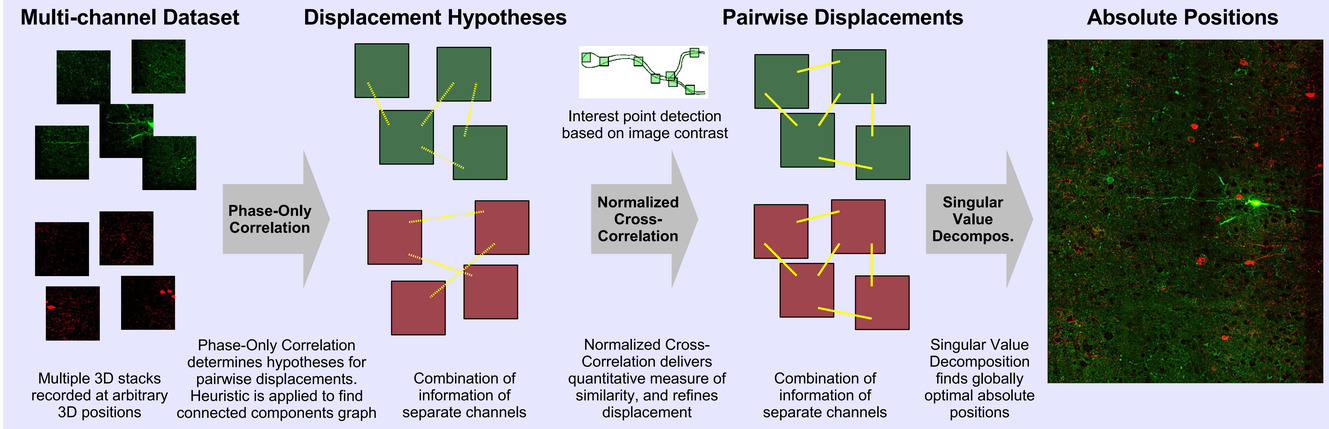
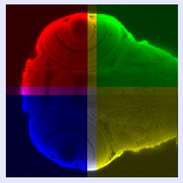
Stitching of large 3D Datasets



Validation

Validation can be done by color overlay or checker-board techniques.
Color overlay shows rainbow artefacts on errors, and homogeneous mix color on perfect overlay.



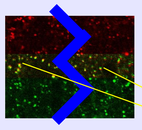


Table rotation: no stitching possible
Red color shines through:
- at the bottom (right)
- and at the top (left)

Benchmarks

All benchmarks have been taken on a 2.6 Ghz Xeon personal computer with 32GB Ram. Shown are times for stitching without bleaching correction, sizes are in voxel.

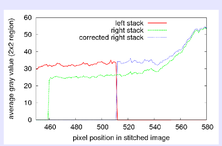
Dataset	Size	Stacks	Time
Mouse, cereb. sec.	9300x4200x77	68	6m 10s
Zebrafish	970x970x480	4	30s
Mouse brain	5000x6300x128	20x2	2m 33s
Single neuron cell	2800x2000x80	6	13s

Bleaching Correction

Level-wise estimation of linear parameters

Incident image bleaching can be estimated from the overlapping parts of two images, where gray values from before and after a recording are available.

The gray value cooccurrence matrix supplies information on the linear parameters of the bleaching function, as well as on its standard deviation.

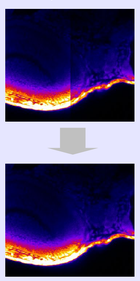


Bleaching visualized:
Average gray values of two overlapping images. Red the first recording, green the second.

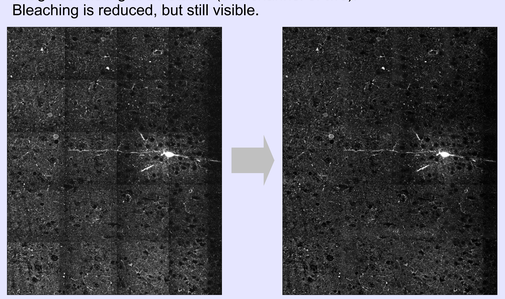
The blue curve depicts the estimated original gray values.

Corrected sample Datasets

Zebrafish
Part of two overlapping Stacks, with color LUT.



Pyramidal neuron, in primary somatosensory cortex of the rat
Image consisting of 20 stacks (one channel of two).
Bleaching is reduced, but still visible.

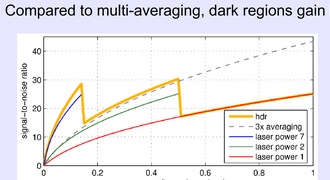


High Dynamic Range Imaging

Signal-to-Noise Ratio (SNR)

Using different exposures (here by varying laser intensities) results in a high SNR over the entire dynamic range while maintaining acquisition time.

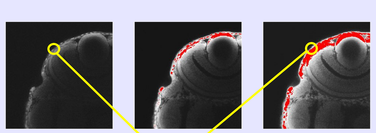
- Compared to multi-averaging, dark regions gain in SNR.



Sample Dataset: 72hpf Zebrafish

Large and thick specimen, average of 500µm in diameter

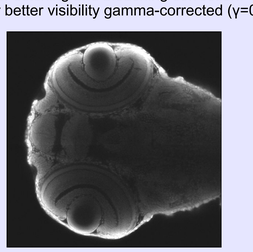
- Recorded at the three laser intensities 1, 5 and 9
- overexposed voxels are colored in red



overexposed voxels can be reconstructed from non-overexposed images

HDR Recombination

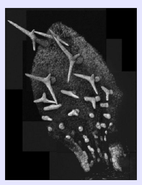
After stitching and bleaching correction.
For better visibility gamma-corrected (γ=0.3)



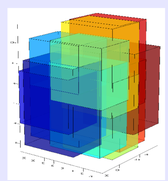
Additional Datasets

Arabidopsis Trichome Dataset

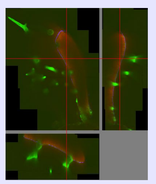
Image consisting of 12 stacks, recorded at arbitrary 3D positions.



Shaded volume rendering



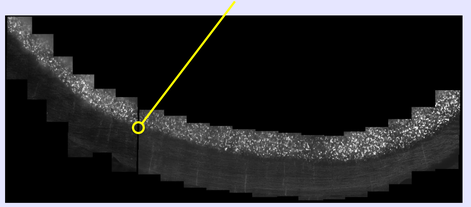
Estimated stack positions in 3D



Orthoview projection

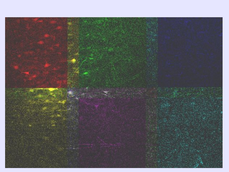
Membrane-GFP transgenic mouse line

Cerebellar section.
Stitching sectioned in 20 + 43 stacks, due to too small overlap of less than 16 pixels (see border).



Rat neuron cell

Color overlay shows good matching of the fine dendrites.



Human brain cell

From the original 12 stacks, only the 7 containing dendrites could be matched.