ADVANCED SOFTWARE FOR CONFOCAL IMAGE RECORDING
THE ZEN MACRO LIBRARY

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Goals: We have been developing this software since 2009 as an add-on for ZEISS confocal microscopes allowing half-automatic and automatic image recording of multiple 2D or 3D areas over time using different scan settings adapted to the specimen. Recognition of image structures, properties or dynamic changes by the software or outside image analysis tools can be combined with the software to select or exclude, correct or trace the recording areas without or with user interaction. Automated image recording based on guidelines defined by the user for the specific experiment is achieved.

Approach: Most software and hardware functions of ZEISS confocal microscopes can be accessed via an interface to the open-access module VBA (Visual Basics for Applications). We have programmed a library of software tools linked via a common GUI interface to interact, control, extend and enhance the ZEN confocal software. Images recorded via the GUI can be analyzed on-line directly by modules in the library or by external software like ImageJ linked to an open image interface. In a closed loop this allows to adapt the recording scheme during an ongoing experiment, to automatically define scan-areas, -volume, -properties or -protocols based on image properties. A 64bit VBA version will be available.

Achievements: The following features are currently implemented in the software.
1.) Automated large area focus position correction based on cover-glass tilt
2.) Measurement of objective xy-axis shift with position correction after objective change
3.) Multi-resolution Overview image recording with an image based coordinate system
4.) Synchronisation of sample position and overlay of newly recorded confocal images to overview images recorded on any microscope or imaging device (for multimodal imaging with different devices or repetitive, non-continuous recordings of time series or large samples)
5.) Multi-position and/or multi-tile recordings also based on dynamic event recognition with independent or linked recording conditions and time schemes for each tile position. Location and tile definitions manually by drawing on the overview or automated based on sample properties
6.) Heterogeneous spectral (lambda), multi-track recordings over time
7.) Self adjusting 3D-stack position and dimension based on user definable properties
8.) Online multi-object tracing over time in 3D based on image properties
9.) Data processing module for image retrieval, export and further processing

The software is tested and used in a large beta-test at 9 European imaging facilities. Examples are presented for large area recordings of whole chicken embryos, large brain slices; multi-point/multi-tile 2D/3D time-lapse and tracing of Xenopus embryos, cultured cells, and growing Arabidopsis roots; integrating also exact control of external devices (lasers) for photo-manipulation.