ADVANCED SOFTWARE FOR MULTI-PURPOSE, INTELLIGENT CONFOCAL IMAGE RECORDING, THE ZEN MACRO LIBRARY

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Goals: The goal of this development is a software allowing half-automatic and automatic image recording of multiple 2D or 3D areas over time on a confocal microscope using different scan settings adopted to the specimen. Recognition of image structures, properties or events by the software or by 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. In the final version fully automated image recording based on guidelines defined by the user for the specific experiment is achieved.

Approach: We have 7 Zeiss confocal microscopes that are controlled by the ZEN 2010 software. Most software and hardware functions can be accessed via an interface to the open-access module VBA (Visual Basics for Applications). We have programmed a set of software tools consisting of different macros and connected via a common GUI interface, to tightly interact with and control the Zen software. Confocal 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, when images change during an ongoing experiment, to automatically define scan-areas, -volume, -properties or -protocols based on image properties.

Achievements: The following modules are currently implemented in the software.
1.) Automated large area \textbf{focus position correction} based on cover-glass tilt
2.) Manual or automatic \textbf{overview, multi-resolution image} recording combined with position coordinate storage
3.) \textbf{Multi-position and/or multi-tile recordings} with independent or linked recording conditions and time schemes for each position. Location and tile definitions can be done manually by drawing on the overview or automated by sample based properties.
4.) \textbf{Self adjusting 3D-stack position} based on user definable sample properties
5.) \textbf{Online multi object tracing} based on image properties using the motorized stage
6.) \textbf{Optimal gain estimation} for each confocal PMT detector.
7.) \textbf{Data processing} module for image retrieval, export and further processing with external software. Image coordinates and table movements can be im- and exported from the ZEN software or written to ASCII text files for usage by external software packages and to measure growth or migration speed of objects.

The software was used for large area recordings of whole chicken embryos, multi-point/multi-tile 3D time-lapse of Xenopus embryos and cultured cells, tracking of growing Arabidopsis roots, and of fixed brain slices.