

Automated confocal laser microscopy for high content structure/function analysis of individual neurons in CNS slice preparations

Manoj Kumar Jaiswal¹, Norbert Garbow², Bernhard U. Keller¹

¹Dept. of Neurophysiology, University of Göttingen, 37073 Göttingen, Germany

²Perkin Elmer Cellular Technologies, Hamburg, Germany

UNIVERSITÄTSMEDIZIN : UMG
GÖTTINGEN



ABSTRACT

Previous research in basic and clinical neurosciences has improved the understanding of genetic disturbances associated with human neurodegenerative disease, which has permitted the generation of an increasing number of transgenic mouse models of Alzheimers disease, Parkinson disease and amyotrophic lateral sclerosis (ALS). Although the structural and functional analysis of existing models has generated important insights, the detailed investigation of pathophysiological signal pathways in each mouse model remains a time – consuming challenge.

To address this problem, we set up an automated confocal laser system to perform high content structural and functional analysis of identified neurons in slice preparations of the CNS. Excitation of slices loaded with fluorescent dyes is achieved by multiple laser sources with wavelengths of 405nm, 488nm, 561nm and 640nm respectively. In addition, the system may be equipped with an external pulsed IR laser source for confocal fluorescence lifetime imaging (FLIM). The geometric format of the laser analysis platform is based on 96 – well analysis plates, where computer – controlled water – immersion objectives permit an automated analysis of individual slices on a well-to-well basis. The original laser platform is based on the Perkin Elmer OPERA system and performs automated, high speed point scanning confocal microscopy including a fully automated z-scanning of probes. To simultaneously investigate multiple signal cascades, the system also utilizes simultaneous four – colour imaging and online – analysis for multiplexed assays. Application of solutions and pharmacological compounds is performed by a computer controlled application system with an analysis capacity up to 100.000 image sets per day.

To demonstrate the functionality of the system, we performed confocal laser microscopy on thin slice preparations (150µm) of CNS tissue from young mice. Morphological features of cell soma and dendrites were imaged up to distances of 80 µm into the slice preparation and similar optical parameters were found for images of individual organelles like cell nuclei and mitochondria. In summary, the confocal laser system promises to serve as a valuable tool for a systematic and quantitative analysis of pathophysiological mechanisms in transgenic mouse models at highly effective analysis rates.

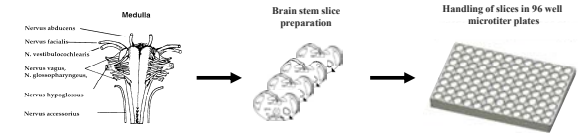
MOTIVATION

Previous research has generated a wealth of information based on the analysis of transgenic animal models of human neurodegenerative disease. While numerous studies have been performed on cell culture models, the detailed characterisation of cellular mechanisms in intact tissue remains an important challenge.

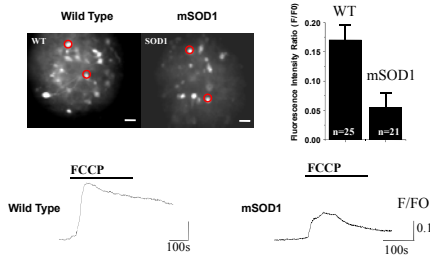
The automated confocal laser scanning system presented here permits structural and functional imaging of molecular / cellular mechanisms in acutely isolated CNS slice preparations. It therefore represents a valuable tool to analyse cellular pathologies in transgenic animal models of neurodegenerative disease with efficient analysis rates.

METHODS

For optical analysis, slice preparations were acutely isolated from CNS tissue and geometrically stabilized on the glass surface of 96 well microtiter plates by light platinum grids. Figures illustrate data from wt and mtSOD1 animal models of human motoneuron disease (ALS, Jaiswal & Keller, 2008).

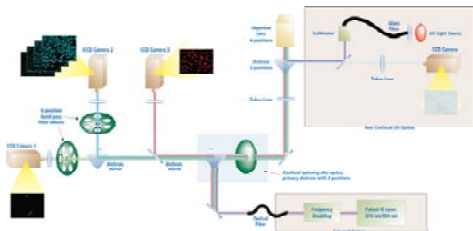


Optimized slice handling and dye loading procedures permit microfluorometric measurements of cytosolic [Ca²⁺] in individual neurons of adult mSOD1 mice (140d) with clear symptoms of motoneuron degeneration (fura-2 AM, hypoglossal motoneurons, rapid CCD Imaging)

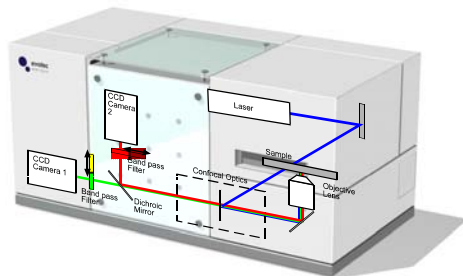


TECHNOLOGICAL DEVELOPMENT

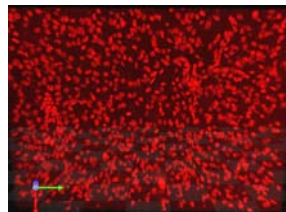
Optical design of confocal laser system:



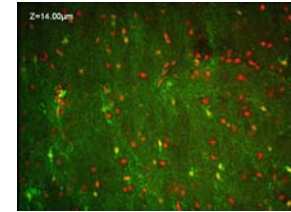
Outside view and optical pathway of confocal laser system:



Imaging of DRAQ5™ / Fluo-4 stained neurons in mouse brain stem slice preparations (see also video)

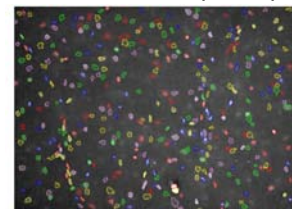


Optical representation of cell nuclei in slice preparation from mouse brain stem (20xW, Draq5, excit. 488nm). Image reflects the superposition of structures with distances 0 < z < 80 µm below the slice surface (see movie).

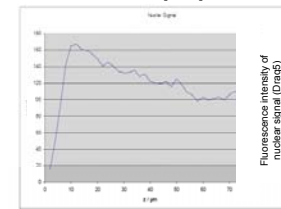


Optical representation of cell nuclei and dendrites in slice preparation from mouse brain stem (20xW, Draq5/Fluo4, excit. 488nm). Distance z = 14µm below slice surface.

Acapella™ imaging analysis including automatic recognition of cell structures (nuclei) in brain stem slice preparations



Automatic recognition of cell nuclei at a depth of 20µm below the surface of the tissue (20x water immersion, Acapella software package).



Average fluorescence intensity of cell nuclei as a function of tissue penetration z.

CONCLUSIONS

- An automated confocal laser scanning system is presented for the analysis of acutely isolated slice preparations from CNS tissue
- The system is based on an automated confocal laser platform with excitation wavelengths of 405, 490 and 780 nm respectively
- A specialized software platform performs an automated recognition of organelles like cell nuclei and mitochondria, which can be monitored up to a distance 80 µm below the slice surface.
- The automated laser scanning system promises to serve as valuable tool to characterize pathophysiological mechanisms in wild type and transgenic animal models of neurodegenerative disease

LITERATURE

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- von Lewinski, F. and Keller, B.U. (2005) Ca²⁺ mitochondria and selective motoneuron vulnerability: implications for ALS. *Trends in Neurosciences* 28 (9), 494 – 500.
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