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Revolutionary Application of 3D-Two-Photon Microscopes for Human Therapy and Drug Research

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1. Introduction

Brain diseases are the dominant public health problem, with substantial, and increasing, economic costs.[1] Diagnosis and treatment are severely hampered by the inaccessibility of the living human brain at the cellular level because taking a brain biopsy poses a serious risk to the patient and thus rarely performed. Despite the increasing demands, brain surgery has not changed from the ancient world in one respect: we don't care with the brain at its fundamental granularity, at cellular level. On the other hand, the growth of the CNS drug research is slowed down, due to the absence of the new screening technology, which can measure the neuron activity in situ without significant intervention. Our achievement in the field of 2P uncaging opens the way for fast and accurate in vivo assays, even with ex vivo human brain tissue.

2. Results

Revolutionary 3D-2P-microscope for the diagnose of brain disorder

Our aim is to introduce the Femto-3DforALL microscope for human diagnose and neurosurgical interventions for cellular level functional diagnosis and therapy. Our fast 3D acousto-optical[2,3] (Femto-3DforALL AO) technology is the method of choice (***Figure 1***), which

1) record activity of the brain with over 10 million faster speed as compared to previous methods,
2) allow 3100-fold higher signal-to-nose ratio,
3) provide in vivo motion artifact compensation for the pulsating human brain to preserve the required cellular, subcellular resolution.
4) Furthermore, these parameters can now be provided with our novel mesoscopic objective in large scanning, volumes (FOV = >10 mm).
5) In addition, thanks to the latest achievements, it

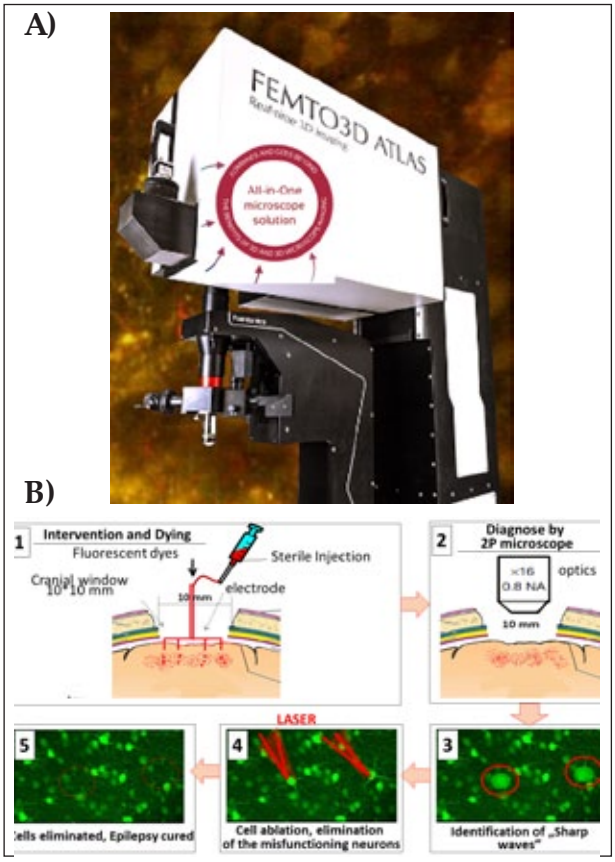

Figure 1 ***A)*** *All-in-ONE solution of our 3DAOforALL microscope extends all currently existing scanning modes into 3D and integrates them in one device. B) Strategy of our novel cellular level diagnosis and therapy (steps 1®5). Red circles indicate hub cells initiating a pathological activity pattern (in step 3 e.g. epilepsy, or major depression). In classic neurosurgery these neurons are eliminated mechanically with a large surrounding block of tissue, loosing many connections, crossing the volume. With cellular level therapy we can achieve the same effect but minimize losses by using cell ablation.*

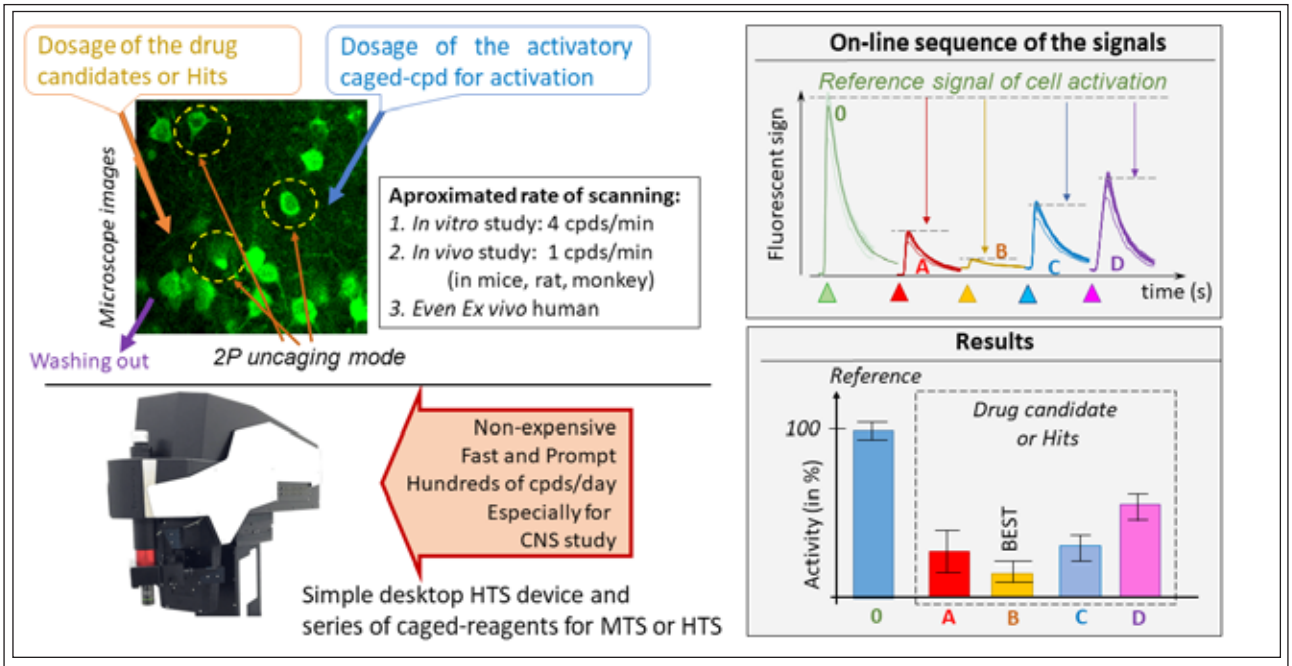

Figure 2 *Schematic representation of the suggested 2P-based biological assay techniques, instruments and the expected results, for the high throughput drug candidates screening.*

can also provide fast and simultaneous 3D photostimulation and 3D photoablation for cellular level therapeutic methods (***Figure 1B***).
6) By means of our novelty, we are able to carry out so called "soft photoablation" in the focal point, which can initiate a spontaneous cell death (apoptosis) at the selected neurons, while not causing damage in the closest surrounding cells.

Revolutionary application of 2P-microscope for the CNS drug research

We develop a new high-throughput-screening technology for pharmaceutical research, which combine the application of our 2P- and uncaging methodology.[3,4] We demonstrated that our caged-compounds (*etc.* DNI-Glu, Q-DNI-GABA *etc.*) can be used as effective reference reagents for the pharmaceutical high-throughput-screening (HTS), even *in vitro* (brain slices) and *in vivo* (living animals) mode to test CNS drug candidates. Noteworthy that this arrangement is applicable for the measurement in human tissues *ex vivo* as well, which offers the possibility to proceed the pharmacological study directly for human treatment, skipping the state of the *in vitro* animal study, explained by ***Figure 2***.

3. Conclusions

In summary, Femtonics' aim is to fill this missing gap with the 3D AO technology and provide the world first cellular level therapeutic brain surgery device, which able to eliminate the misfunctioning neurons, avoiding the elimination of the healthy and functioning tissue. Parallel with that, we demonstrated an effective high-throughput screening assay method for CNS drug research, combining the technical level of 2P uncaging and the caged-neurotransmitters.

4. Acknowledgements

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