OptoCS – Optogenetic Cell Sorting

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ABSTRACT

The research field of optogenetics uses genetic engineering to precisely control cellular activities with light. What might sound like science fiction, has the potential to solve many problems of mankind ranging from biofuel-production, the generation of nutritious food to individualized medicine for various diseases. Many optogenetic researchers are limited in their experimental repertoire due to a general lack of suitable illumination devices specifically made for optogenetic research. This consortium developed a specialized illumination device which enables the combination of optogenetics with fluorescence activated cell sorting (FACS) for the first time. Together with researchers across the globe, this consortium aims at making optogenetic research easier and more efficient to help solve actual problems with this fantastic technology.

Keywords: Optogenetics; Flow Cytometry; Fluorescence activated cell sorting (FACS); Immuno-cancer-therapy

1. INTRODUCTION

The field of optogenetics is a relatively young discipline in the research field of biology investigating possibilities to remotely control cells with visible light. Optogenetics is a technique that uses genetic engineering to precisely control cellular activities with light. The optogenetic toolbox consists of a variety of photoactivatable proteins, which are often of bacterial or plant origin. These so-called photoreceptors have the property to change their conformation and affinity upon illumination with light of a specific wavelength. This conformational change can e.g. lead to the opening of an ion channel or oligomerization of signaling proteins triggering signal transduction. Genetic fusion of optogenetic proteins to signaling components allows for the reversible optical control of virtually any signaling pathway[1]. Among the cellular mechanisms already targeted by optogenetics are e.g. apoptosis, cell cycle progression, cell proliferation and differentiation, cell migration and many more. While the dynamic evolution of this field of study does not allow for a complete table, these data represent a helpful collection and starting point for any research.

Optogenetics will help deciphering signaling pathways and provide therapeutic approaches for diseases such as mood disorders, Parkinson's, addiction, diabetes, blindness and cancer.

Despite the revolutionary potential of optogenetics, the analysis spectrum for scientists is very limited. The light stimulus can be compared to the dosing of a stimulating agent. While researchers are used to pipetting stimulating agents at exact molar concentrations, the possibilities for light stimulation with the same accuracy barely exist. opto biolabs from this consortium has recently launched its pxONE illumination adapter: It enables high-throughput analysis of optogenetic samples using flow cytometry for the first time[2].

The goal of the OptoCS consortium was to expand the experimental repertoire even further by adapting the pxONE to fluorescence-activated cell sorting (FACS). During the Attract Phase 1 funding period, we were able to build a prototype that was successfully connected to a cell sorting machine. We are estimating the development time to market to be another 20-26 months. Cell sorting is a standard method in e.g. individualized immuno-cancer therapy, but not yet feasible for the sorting of optogenetic cell samples. Optogenetic Cell Sorting will bridge the gap between basic research and medical applications and will be pivotal for the development of optogenetic therapies.

2. STATE OF THE ART

The most commonly used analysis method for optogenetic research is fluorescence microscopy where the in-built lasers serve as the light source for optical stimulation and analysis. The availability of fluorescence microscopes in most research institutes makes this an attractive method for optogenetic

research labs. Despite the many advantages of fluorescence microscopy like e.g. high spatial resolution, the analysis of dynamic processes is very slow and limited to relatively small numbers of cells. Flow cytometry offers the standardized analysis of thousands of cells per second recording over 30 parameters at single cell resolution. Fluorescence activated cell sorting (FACS) is a special form of flow cytometry where cells can be separated into individual sample tubes according to their fluorescent properties (= parameters). Depending on the FACS machine used, cells can be batch-sorted, meaning all cells with certain properties are pooled into one sample tube, or single-cell-sorted, meaning that individual cells with defined properties are each sorted in to a separate sample tube. Cell sorting is a powerful tool for the generation of cell lines, subsequent single cell analysis (RNA-Sequencing, Proteomics etc.) and purification of genetically engineered cell samples for therapeutic approaches. Despite the many advantages of FACS, it is not possible to combine this technique with optogenetics, yet. An illumination adapter recently launched by opto biolabs enables the combination of flow cytometry and optogenetics, but is not yet suitable for FACS machines. The goal of this project is to build an illumination adapter that will enable the FACS-mediated sorting of optogenetic cell samples.

3. BREAKTHROUGH CHARACTER OF OPTO-CS

Optogenetic research is already revolutionizing basic research and more importantly, some optogenetic tools have already reached clinical studies to conquer human disease.

This consortium believes that optogenetic research will significantly change the way we do basic and applied research, treat patients, synthesize products and the way we produce nutritious food for society.

Despite the potential of this technology, researchers still struggle with a very limited repertoire of suitable illumination devices. Many researchers build their own illumination devices or improvise with flashlight devices. This does not only restrain the individual research projects but more importantly renders many experiments impossible to reproduce. Hence, it is pivotal for optogenetic researchers to be able to rely on standardized illumination procedures that can be controlled and reproduced within research labs but also across different institutions.

The sorting of cells based upon their reactivity to distinct light pulses was not possible before. Using the OptoCS prototype, this project enables to transform optogenetics from a mostly basic research application to a more clinical and therapeutic focus.

4. PROJECT RESULTS

4.1 Building of OptoCS prototype

Different approaches where tested to integrate the pxONE illumination device into the FACS Aria cell sorter, including the use of an external pumping system to inject the cell sample into the cell sorter.
The final prototype however uses the internal pressure system of the FACS Aria and is depicted in Fig. 1.
The OptoCS prototype is a pressure chamber that can encapsulate the pxONE and seal it air tight. The pressure line applies the same pressure to the OptoCS prototype as it would have in the in-built cell sample chamber. The cell sample outlet delivers the cell sample into the cell sorter. The injection lines allows the addition of small volumes to the cell sample during cell sorting experiments for e.g. stimulating agents.

4.2 Integration of OptoCS prototype into the FACS Aria cell sorting system

The pxONE is placed into the OptoCS prototype and screwed tied to apply pressure and air-seal the system (Fig. 2). The sample line and the pressure line are connected to the cell sorter. This elegant solution allows to control the pressure and hence the travelling speed of the cell sample with the software of the cell sorter, meaning that the usual work flow of cell sorting stays exactly the same and will increase the acceptance of this new technology among researchers. It also means, that the OptoCS device will be compatible with many different cytometers which significantly expands the number of researchers profiting from this technology.

Fig. 1. Nr.1: pxONE illumination device with inserted sample tube; Nr.2: OptoCS prototype with connection tubes: (a) sample line (b) injection line (c) pressure line

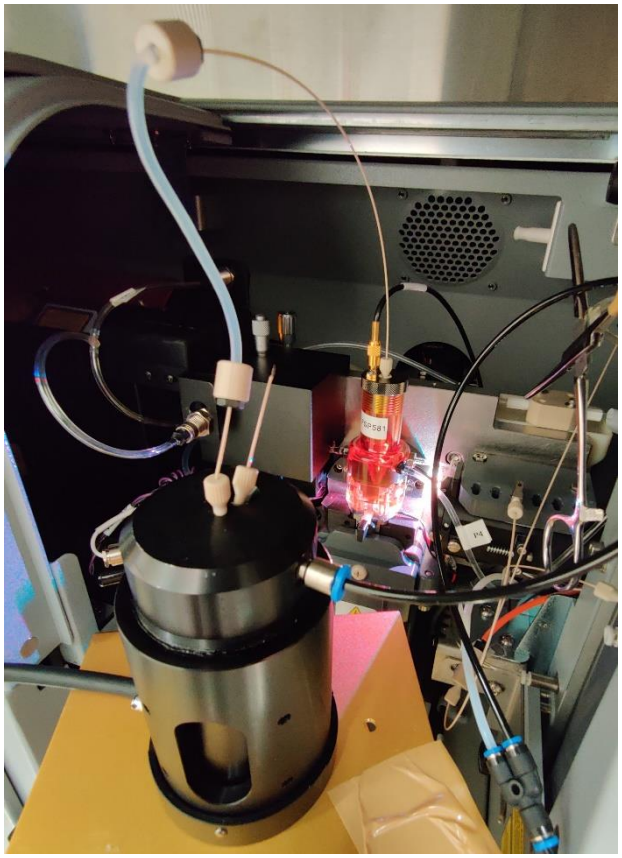

Fig. 2. The pxONE inserted in the OptoCS prototype and connected to a FACS Aria analysis chamber. The connection of the OptoCS prototype to the FACS Aria enables light stimulation and cell sorting at the same time.

4.3 Control of illumination using the OptoControl Software

The cell sample illumination happens inside the integrated pxONE and is controlled via an external software. The amount of light used to treat and stimulate the cells is pivotal for the experimental outcome. The in-built LEDs can be controlled in the ms.-min. range to enable a maximum variation of light stimulation. Importantly, it is possible to save illumination protocols for later experiments or to share these protocols with other research institutions, increasing reproducibility of optogenetic research.
This software is compatible with Windows 10 since most cytometers and cell sorting machines also run exclusively with Windows systems.

5. FUTURE PROJECT VISION

During the next years, this consortium envisions to develop further illumination devices combining the culturing, analysis and application of optogenetic systems. We envision an automated culturing system illuminating optogenetic cell samples, taking automated samples for flow cytometric analyses, intelligently adjusting the illumination protocol accordingly and sorting cells whenever required. Such a fully automated optogenetic system would be highly useful for optogenetically controlled product synthesis as well as optogenetically induced cell differentiation and isolation. Our biggest passion however lies in optogenetics-based personalized medicine. The OptoCS platform offers a versatile tool to isolate cells based upon their responsiveness to light and use these for the treatment of cancer, diabetes, neurological diseases and many more.
All the above research areas will benefit from OptoCS and similar smart illumination solutions.

5.1. Technology Scaling

The first and most challenging technical hurdle was solved during the Attract Phase 1: Installing an external cell sample unit to a cell sorter which can illuminate and chill the cell sample during flow cytometric sorting experiments. This experimental proof of concept translates into a TRL 3 for the OptoCS prototype. During the next developmental steps, the following components need to be added to the current OptoCS prototype:

1. Stirring module for the cell sample reservoir

One essential component will be a magnetic stirring module preventing the cell sample to settle to the bottom of the tube and allowing the addition of stimulating agents to the cell sample during cell sorting experiments. A first 3D printed prototype already exists and will be implemented into the OptoCS device.

2. Sample illumination along the sample line

The final OptoCS device will allow for a 2-step illumination procedure prior to the cell sample injection into the cell sorter. Firstly, sample illumination inside the pxONE (already achieved) and secondly, sample illumination along the way to the cell sorter. This also allows for the analysis and sorting of very fast and kinetic reactions and will be the most challenging yet to solve requirement for the OptoCS device. The speed of the cell sample is controlled by the cell sorter, allowing for easy implementation and compatibility. But the illumination duration needs to be adjusted to the cell sample speed and

yet allow for a great variety of illumination protocols. Some researchers require milliseconds of illumination followed by quick analysis. Other researchers require seconds of illumination and some delay prior to the analysis. The OptoCS device needs to fulfil all those different requirements. A realization concept for this already exists and could be implemented during Attract Phase 2.

3. Temperature control of the sample line

Illumination of the sample line can cause significant heating especially considering the very small volume being illuminated. Hence, a cooling system will be implemented in the sample line to prevent excessive heating and cell damage.

4. Combining all components into one device

The final step will be to combine and assemble all single components into one functional device, that can easily be attached and detached to and from flow cytometers and sorters from different brands to make this a universally applicable device for optogenetic research.

5.2. Project Synergies and Outreach

The OptoCS consortium already comprises key experts from different fields. Prof. Wilfried Weber is an internationally renowned optogenetics researcher from the University of Freiburg and guides the here developed device towards researchers needs. Dr. Malte Paulsen and Dr. Diana Ordonez from the EMBL in Heidelberg lead the core facility for flow cytometry and are essential for the smooth implementation of these devices into different cytometers. The start-up company opto biolabs from the University of Freiburg has already successfully launched their first illumination device for flow cytometry and is the link between research and commercialization. The two founders Dr. Kathrin Brenker and Luis Köbele are the driving force behind this project. They are responsible for the product development and certification process.
This consortium is always eager to collaborate and is already in close contact to the glassomer team (EU-Attract Phase 1 funded: OptoGlass3D) at the University of Freiburg to produce glass components for the OptoCS device.
More importantly, there is a waiting list of over 10 flow cytometry core facilities and research labs in Europe, Canada and the US who are eager to test the OptoCS prototype. This consortium aims at close collaboration with these research labs for beta-testing of the OptoCS device prior to product launch. At the same time, this technology will be available to highly motivated research groups even prior to the product launch.
Furthermore, there is an increasing interest in this technology from flow cytometer companies. These collaborations range from co-marketing strategies to the co-development of illumination devices. These collaborations will help to make this technology available to more researchers much faster and facilitate scaling once the OptoCS device has reached the market. opto biolabs from within this consortium has recently launched a white paper series to inform about novel research possibilities by combining flow cytometry with optogenetics. This format will be used in the future to inform about the possibilities of optogenetic cell sorting.

5.3. Technology application and demonstration cases

Optogenetic cell sorting will revolutionize optogenetic research in many ways. The OptoCS platform will offer many possibilities to aid basic and applied research. Together with our partnering flow cytometry facilities, we will be able to offer this technology to many researchers within Europe and across the globe. The close collaboration of this consortium with the EMBL in Heidelberg is key, as the EMBL is a teaching institute and very experienced in the training of novel techniques to their partnering institutions.

5.3.1. Development of novel optogenetic tools.

The development of novel optogenetic proteins or tools is a very time consuming procedure and involves the stepwise mutation of existing optogenetic proteins, followed by the analysis of each generated mutant to find successful candidates. A successful candidate can e.g. be a protein, that is excited by far-red wavelengths rather than blue light to enable in vivo applications. Using optogenetic single cell sorting, scientists will be able to analyse thousands of mutants at the same time and simply sort single responding or non-responding cells into 96-well plates. The fast, efficient and reliable development and characterization of optogenetic tools is essential for the development of optogenetic applications.

5.3.2. Single-cell-analysis of optogenetic experiments

Optogenetic tools allow for the remote, reversible control of distinct signaling proteins of complicated signaling pathways. Currently, there is a huge interest in retrieving single-cell resolved proteomics, RNA- and DNA-sequencing data. Using the OptoCS platform, it will be easy to disperse single, sorted cells in multi-well plates for further analysis and compare, e.g. responding from non-responding cells or make more complicated comparisons. This will drive biological discovery fast forward in the field of optogenetics.

5.3.3. Opto-CS platform for individualized therapies

Moreover, optogenetic cell sorting will be essential for optogenetics-based individualized cancer therapy. Here, patient immune cells are harvested, genetically altered to

become photo-activatable, sorted (with optogenetic cell sorting!) and injected back into the patient as treatment regimen. The first mouse melanoma has already been treated with individualized, optogenetic cancer therapy[4]. Hence, this consortium believes that optogenetic cell sorting will not only have a major impact on basic research today, but shape the possibilities for individualized cancer therapy tomorrow[3,5].

5.4. Technology commercialization

The commercialization of the OptoCS project is already fully planned and on its way. The start-up opto biolabs was recently incorporated and will finalize its first investment round within the next 1-2 months. Opto biolabs has recently launched its first product, an illumination device for flow cytometry. Hence, the team around opto biolabs is experienced in all the necessary steps to convert a lab prototype into a certified product. This consortium is very aware that the OptoCS device contains signficantly more challenging components with some yet unsolved problems. Hence, the estimated duration to launch will be around 2 years under the assumption that two engineers, one flow cytometry expert and one optogenetic scientist will work on this project full time. The main cost drivers are personnel as well as parts and components for the device iterations. The personnel costs will be around €300K per year and the costs for parts and components will be around €65K per year.

5.5. Envisioned risks

The major risk of this project is the co-dependence on the optogenetics market. All measurable indicators point towards optogenetics becoming a major success with an ever increasing number of optogenetic research labs, optogenetic proteins, publications and clinical applications. A great development to simplify optogenetics is the development of optogenetic nanobodies and other reagents which do not require the tedious work of genetic engineering anymore[6]. Hence, it is very likely that optogenetic cell sorting will become an essential part of this development.

A smaller risk is the possible lack of awareness for this technology. Historically, optogenetics is closely linked to microscopic analysis techniques and many optogenetic researchers are unaware of flow cytometry in general. Hence, we aim at an aggressive information strategy, including conference visits, webinars, white papers, workshops and social media marketing to increase the awareness of optogenetic flow cytometry.

Minor risks include the product certification process and IP-strategy. The OptoCS device is not a medical device and will hence only need simple certification for electrical devices. The team of opto biolabs is experienced in this process. Concerning the IP-strategy, the illumination devices developed here are partly already patented and partly in the patenting process. Although patents cannot guarantee safety from being copied, this consortium is following a stringent patenting strategy to maximize IP-protection.

5.6. Liaison with Student Teams and Socio-Economic Study

Should this project get selected for ATRACT Phase 2 funding, this consortium will happily share all necessary information for a collaboration with MSc. Level student teams. This consortium will be happy to host a student team for an exchange meeting on site if the pandemic situation allows for such a meeting. The responsible person for this collaboration will be Dr. Kathrin Brenker.

This consortium will be happy to participate in socio-economic studies through interviews, technology impact references or similar suitable ways to support the ATTRACT initiative.

6. ACKNOWLEDGEMENTS

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