Real-time fluorescence lifetime acquisition system

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ABSTRACT

We have developed a novel method for measuring the fluorescence lifetime instead of or in addition to its intensity. We demonstrated an acquisition system that is extremely fast, compact and significantly less expensive than current approaches. We are seeking for partners among optical instrumentation manufacturers for licensing and technical cooperation agreements.

This article covers the analysis of technology transfer processes in correlation with expectations of newly developing biotech market. It concludes that technologies scaling project synergies and outreach are of crucial importance for the development of the technology for market purposes.

Keywords

Fluorescence lifetime; silicon photomultiplier; waveform sampling; knowledge transfer; innovation; patent search; role of market analysis, technology commercialization.

1. INTRODUCTION

This paper describes the application of high-energy physics technology for real-life applications. This is an area, which has always been considered to have a large potential, but too little has been realized. In particular in the detector area estimates show that there could be numerous unused technologies.

Based on the mature technology developed for high-energy physics, we developed a technology that targets primarily at medical, biomedical, biotechnology and pharmaceutical fields, all of which experience significant market growth in the current time period, in particular in the past ten years. The application areas include a detection of the presence of certain organic compounds, measurements of the properties of samples or tissue through the concentration of certain organic compounds, non-invasive determination of the chemical environment in the sample and non-invasive medical diagnostics and guided surgery.

In this article we will first touch upon the promising market capitalization. We will describe the technology at hand into more details, including the benefits, arising from it, the state-of-the-art and the technology scaling. Secondly, we will touch upon the patent databases searches which assisted us in estimating the technology potential, commercialization and IP protection strategy. Lastly, we will touch upon further technology development and market development plans.

1.1 Market evaluation

In the last several years, global market of fluorescence lifetime imaging microscopy developed smoothly, with an average growth rate of 4%. In 2016, global revenue of fluorescence lifetime (FL) imaging microscopy was nearly 155 MUSD; the actual production was about 545 units.

The major players in global Fluorescence Lifetime Imaging Microscopy market include Leica, Olympus, Zeiss, Becker & Hickl, HORIBA, PicoQuant, Bruker, Nikon, Lambert and Jenlab.

We expect this technology to enter the biotech market, which alone (but not limited to the FL imaging microscopy) is expected to hit 727b USD in 2025 [5], providing tangible benefits for society. Many emerging applications require sensors with a wide field of view, good spatial resolution and very fast acquisition times - a parameter envelope not yet reached by present research.

Our goals are to develop a device that uses a wide field illuminator (diffused laser) and a wide field detector, using a single laser pulse, capable of continuous sub millisecond frame rates. Global Fluorescence-Lifetime Imaging Microscopy market is projected to reach \$ 1.8 Billion by 2020, with a GAGR of 4% from 2016, and Asia will have a big dynamic momentum on the market growth.

2. TECHNOLOGY DESCRIPTION

2.1 Background

Fluorescence is the emission of light by certain substances (fluorophores) after they are illuminated with light of specific excitation wavelengths. Measurements of the fluorescent light emitted by various samples are used in a very wide range of applications, such as imaging of cell structures, tracking of antibodies and DNA sequencing in biology, detection of cancer cells in medicine and quality control in pharmacy. Besides intensity, the fluorescence lifetime (FL) can also be measured, as pioneered in application of fluorescence lifetime imaging microscopy (FLIM). This has many advantages over the base method, such as independence from fluorophore concentration, reduced damage to the sample (photobleaching) and ability to measure properties of the microenvironment in which the fluorophore is located (pH, oxygenation…).

Currently, FL measurements require sophisticated and expensive instrumentation. Typically, the fluorescence lifetime is determined with time correlated single photon counting (TCSPC) method, which is intrinsically slow. Mature technological developments in the field of high energy physics (HEP) enable direct waveform sampling technology as important and a very cost-effective tool for fast FL applications. By measuring the photodetector signal resulting from complete fluorescence response, FL can be estimated even from a single excitation pulse.

Real-time Fluorescence Lifetime Acquisition System (RfLAS) was assembled from low cost, commercially available components in order to demonstrate the feasibility of such approach. Calibrated FL standards with lifetimes in the range of 2 ns – 9 ns were used to test RfLAS accuracy and performance for different levels of available fluorescence light intensity and photodetector configurations. Using our prototype, we show that FL of all three fluorescence standards could be measured with an accuracy better than 10% from only a single pulse of excitation light, which improves below 1% level by averaging over only a few tens of pulses. Therefore, RfLAS demonstrates that FL can be acquired practically in real-time for a much lower price point than current state of the art.

The three critical components – the photodetector, waveform sampler and data processing algorithms – lend themselves perfectly for implementation in a single chip. These are also areas of expertise of the authors, and the institutes they are affiliated with. The envisioned integrated detector would push the performance and robustness beyond the present state, and more importantly, using CMOS technology at scale, would collapse the price per unit, opening possibilities to use FL obtained information in much wider areas as currently available.

2.2 State of the art

In TCSPC method, FL is determined from a histogram of measured time delays between excitation pulses and individual fluorescence photons, resulting from said excitations. If more than one photon is detected per pulse, the accuracy is degraded (pile-up effect), so the fluorescence signal has to be at a single photon level. The excitation pulse has to be repeated many times in order to obtain sufficient time delay histogram statistics, leading to long acquisition times and possible photo bleaching of the sample.

The acquisition times are even longer if imaging is required. In this case, laser excitation is scanned over the sample, and sufficient TCSPC statistics have to be accumulated for each scan position (image pixel). Alternative imaging approach is possible with single photon avalanche diode (SPAD) arrays, recently developed specifically for FL application with time-to-digital converters (TDC) implemented on a single chip. These devices have an intrinsic limiting factor, the sensitive area is somewhere between 1% and 20 % [1] as most of the space is used for electronics, and prototypes have a relatively small pixel count.

FL is also measured using frequency-domain technique, where it is derived from phase shift between modulated excitation illumination and resulting modulation in fluorescence signal, and gated detection, where FL is estimated from ratios of fluorescence signal at specific time gates.

Currently, FL measurements require sophisticated and expensive setups, and certain time to reconstruct the FL. In case of imaging, a few frames per second can be achieved at best for sufficient image resolutions [2].

2.3 Technology scaling

In our development plan, we will first build from the selected off the shelf components, a highly integrated multi-channel version of the device. It will be fully decoupled from laboratory equipment; therefore, it can be lent or sold to early adopters. These are crucial for us, we need early feedback, dissemination, and to validate and demonstrate the device in a real operational environment. An extremely important aspect is also presence on the market. Having a community of users, and a device that can be demonstrated in real operating environments will create the foundation for the third step.

Secondly, having built up the necessary experience, and deep understanding of the system, we will make an integrated scalable sensor, the real breakthrough in FL high speed imaging. The sensor will integrate efficiency optimized SiPMs, bump bonded to the electronics wafer, which could be produced in different technologies, with different performances, for different applications.

Taking in consideration mass production, these sensors can be made at a very competitive price. CMOS technology is also very affordable at scale, has a known roadmap and is very well supported. These factors provide a secure path to aggregate scalable solutions.

2.4 Project synergies and outreach

During the initial phases of the technology development, we were searching for cooperation with potential users and partners, focused on fast FL acquisition. We will be able to quickly form a consortium capable of advancing RfLAS. Laying the foundation in the dissemination program, we should build quickly a community of users to provide application test cases and feedback, and most importantly increment to TRL 5-7.

For additional dissemination, we intend to leverage one of the strong points of our technology, its simplicity. We will take an abundant amount of knowledge gained and develop an open source, open hardware, single channel FL acquisition toolkit, composed of hardware solutions based on off the shelf components, data acquisition software and library of end-user experience. The feedback and exposure will directly benefit the project, and increase the speed of development.

2.5 Technology application and demonstration cases

Measurement of FL is a still growing field of research with many applications not realized. A technique, improved in acquisition speed, and even more importantly, lower entry cost, has the potential to advance many fields of science and open new industrial applications. We have discussed concrete applications with potential users, including a pharmaceutical production company, high tech company developing monitoring and metrology technology for food industry and national health institute.

With just this batch of early adopters, RfLAS would improve development and monitoring of biopharmaceutical production. This includes an increase of the quality of food available to consumer and reduction of wasted food by measuring the ripeness of fruits and detecting presence of bacteria on food products; advance the accuracy and speed of diagnostics of histological samples; and contribute to a wide range of material science research.

3. ANALYSIS OF MARKET OPTIONS

3.1 Technology assessment

Supported by a group of specialists we performed a state of the art examination for the mentioned technology.

We found that technology has a significant advantage over the current state of the art. Some technologies touch on similar measurement methods and use language and definitions in patent claims to cover a very wide range of almost all measurement options, but do not cover the details of photodetector implementation. This is one of the significant improvements of our technology: we use silicon photomultipliers (for photodetectors), in connection with the digitization of the signal from the photodetector using a chip and the principle of fast waveform detection.

There are also related patent applications and patents, which describe significantly slower, more complex devices or use alternative technology (TCSPC), which requires higher laser energy input to operate. The higher laser energy also results in photobleaching, which is in our technology avoided due to single photon regime of acquisition. The most related patent application, which also uses a silicon photomultiplier in conjunction with the use of a digitization chip and direct waveform sampling, does not describe a significant improvement in technology. These are namely the simultaneous capture of several sensors simultaneously, capturing the spectrum, which is an important analytical contribution in the submitted patent application of the presented device in the analysis and processing of fluorescent times.

3.2 Benefits and market placement

A silicon photomultiplier is a very fast photodetector, whose response to a single photon is faster than the fluorescence lifetime. Therefore, the shape of the electronic signal, i.e., the waveform, output by the silicon photomultiplier will follow the exponential decay of the fluorescence light resulting from a single pulse of excitation. If the resulting waveform is sampled with sufficient accuracy, the need for long accumulation of single-photon arrival times and large excitation light intensities can thus be avoided. Excitation light with low intensities reduces the risk of photo bleaching. Silicon photomultiplier photodetectors and waveform sampling chips developed for the needs of high-energy physics experiments have become low-cost, off-the-shelf components. Thus, the method allows a cost-effective way to measure the fluorescence lifetime and, at the same time, avoids lengthy data acquisition and photo bleaching of the sample.

The main advantages of the method proposed over TCCSP are cost-effective compared to common TCCSP technology, long accumulation of single-photon arrival times and large excitation light intensities of TCCSP are improved and excitation light with low intensities reduces the risk of photo bleaching.

The technology is in late early stage of development and is fully available for demonstration. It has been developed with the core funding of Slovenian Research Agency and also supported in part by ATTRACT Phase I. Due to the situation in the technology and market field, it was determined, that it is high relevance that its IPR status is arranged.

3.3 Database searches

We have prepared an overview of the state of the art with the help of the commercial patent database Derwent Innovation. In the review, we considered patent applications and patents filed anywhere in the world, and searched using the following key phrases: fluorescence lifetime, silicon photomultiplier, waveform sampling, and a specific content keyword that the authors of this contribution consider as a part of their secret knowhow and is not going to be revealed.

We tested different combinations of words and compared the obtained results with each other. We reviewed the results of the following search strings in more detail:

1.Fluorescence AND lifetime AND silicon AND photomultiplier AND waveform AND sampling AND specific content keyword (No records)

2.Fluorescence AND lifetime AND photomultiplier AND waveform AND sampling (6 records)

3.Fluorescence AND waveform AND photodetector (51 records)

4.Fluorescence AND waveform AND sampling AND photomultiplier (14 records)

The results obtained with the second, third and fourth search sets contain 71 results. Upon examination, 6 of the 51 hits turned out to be highly relevant and 2 relevant. Of the other 14, 4 were highly relevant and two relevant, and of the last six, 3 were highly relevant and 2 relevant. It turned out that the most relevant search was under point 4, where the largest share of relevant hits was. However, the search under point 3 is also important for finding market orientation.

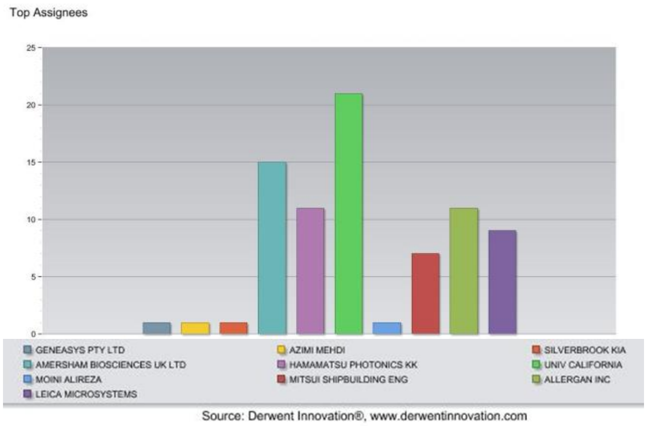

Figure 1: Top Assignees in the World in the field of fluorescence lifetime measurements

Even though we kept in mind that a proper Freedom-To-Operate analysis (FTO) can only be performed when the product is defined, the patent analysis also has given the authors insight into which companies have shown an interest in this type of technology. Top Assignees in the World can be found in Figure 1. Derwent Innovation overview of the field shows a current prevalence of US Universities and Japanese companies in the closest technology searches.

Due to the results of the review of the state of the art, we decided to prepare documentation for the disclosure of the official invention. it makes sense that intellectual property is properly registered with the JSI (it can also be used as a technical improvement / hidden knowledge) - also in terms of the possibility of rewarding inventors for inventions created during working hours.

3.4 Market assessment

The size of the market for measuring fluorescence time, according to data from companies engaged in market research, is currently estimated at over 250 mil. EUR at an estimated average annual growth, since 2016, somewhere around 4%. The advantage over the existing offer is mainly in the relatively favorable design / price of the technology (silicon photomultiplier), speed of data capture and processing, prevention of photobleaching and especially in the possibility of simultaneous capture of multiple wavelengths of light, obtaining important additional information for further processing.

Of course, the market analysis would be more significant and in particular more reliable, if we could identify the first specific application(s) to be addressed by the technology, and then count the potential end users and multiply by the assumed price of the equipment to arrive at an accessible first market. This is an ongoing process which we hope to continue in the next steps.

We have already established contacts with companies, but without adequate protection of intellectual property, contacts cannot grow into more serious forms of conversations and exchange of technical information.

Following proper registration of intellectual property with the JSI, the marketing plan is expected to include: (i) the preparation and publication of a technology offering in commercial databases; (ii) contacting the main players from the list we created as part of internal market research; (iii) depending on the response from the main players, active marketing to other potential partners (through direct contact of potential partners, participation in international partnership events and active marketing within sectoral groups, a project of the European Commission).

3.5 Continuation of intellectual property protection

Given the high technological potential (according to the state of the art) and the high market potential, it makes sense to apply to the Office for the Protection of Intellectual Property, which conducts a full test, UK-IPO, which we also propose to find out within 6 months the invention is new and on an inventive level. Namely, we will receive an international opinion on the patentability of the technology (ISR - International Search Report) from a certified ISA (International Search Authority), on the basis of which we will be convinced of the novelty and inventive step of the proposed technology.

The selection of an office that performs a full test is also a precondition for co-financing the work of patent attorneys within the Technology Transfer project financed by Slovenian Ministry of Science and Sports.

Technology and market assessments proved the relevance and the need for patent application protection.

3.6 Technology commercialization

We are in the process of obtaining IP protection for the core aspects of our development, with patent applications currently filed in UK and European offices. We are in talks with two companies interested in technology, with one we are in the process of signing NDA. Other private entities expressed interest for the development of front ends and data display software. The multichannel instrument will support our commitment to advance as quickly as possible to step three of our development program, to enable the community and users to have on disposal a price competitive and robust instrument for their application.

4. FURTHER TECHNOLOGY, IP AND MARKET DEVELOPMENT PLANS

4.1 Envisioned risks

Our main target is the development program of highly integrated sensors, potentially having some degree of data processing on chip. Modelling, design, production, assembly and testing of such devices are, in a vast majority, also areas of expertise of the authors [6, 7], and the institutes they are affiliated with. We intend to prepare a simulation of such a device, to predict its performance and share the performance envelope with early adopters to shape its final form.

The physical aspect requires multiple R&D cycles which is slow and costly. To mitigate the failure in this task, we will start by assembling some of the ideas we already have on low cost CMOS fabs and unveil potential issues toward high integration. At each iteration, interested users shall be able to test our devices in their respective environments.

4.2 Liaison with student teams and socio-economic study

Our group are open for collaborations, and look forward to establish reliable partnership with users, partners and stakeholders. Our plan envisages their presence from the very beginning and will provide support in their future endeavors, by providing them with better and more advanced instruments. Of special interest are Master students, the next generation of STEM engineers, which will, one hopes, adopt our technology. It is very rewarding having the possibility to empower the younger generation, and give them tools to cover the fear of missing out new opportunities in such an early stage, searching for other possible applications of the developed chip, that may include PET, encrypted LIDAR, and other machine vision applications

5. CONCLUSIONS

The researchers come from a Slovenian public research organization. Their research involves experimental particle physics on large particle accelerators and development of complex detectors. They have analyzed their options with transferring the technology in question, performed market and technology assessment and decided upon an IP and market strategy. Future steps involve in particular wider interaction with potential customers and further development towards a product for the market.

Even though the process of the transfer is described in a historically relevant manner, the authors also acknowledge, that there have been many setbacks within the process itself. It is not that every step has been performed flawlessly, without mistake, setback, delay or disappointment. For example, it took a long time to arrange the internal take up of the technology at the public research organization, even longer to arrange for the dual ownership between the two primary owners, both public research organizations. We need to point out these facts, although we are, for non-disclosure issues not entitled to discuss the details here.

The partners are sought among optical instrumentation manufacturers. As a public research organization, the researchers are available for different sorts of collaboration: Potential partners are offered a license to the granted patent under licensing agreement. Technical cooperation for the development of a complete instrumentation device for measuring the fluorescence lifetime by this method is also considered a viable option.

The timing of technology development is suitable for inclusion in technological processes in the market. With the analysis of the market and patent saturation, we gained an overview of the state of the art and the possibilities for further market orientation. In our opinion, with the timely protection of intellectual property, we have achieved an optimal position for further marketing activities.

6. ACKNOWLEDGMENTS

This project has received funding from the ATTRACT project funded by the EC under Grant Agreement 777222.

Technology marketing support has been obtained through CTT, JSI.

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