

## Emerging Technologies in Clinical Laboratories as Outlined in Industrial Property Documents

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### Abstract

The purpose of this paper is to present in details, an extended case-study focused on emerging in vitro Diagnostics Technology as reflected on retrieved and evaluated Industrial Property Documents. More specific, this study comprises of the state-of-the-art and the perspectives of automatic Immunoassay Analyzers, it follows the innovation patent-trail from Flow Cytometry to Quantum Dots applications in the in vitro Diagnostics of tomorrow and finally, it provides an overview of the trends in Next Generation Nucleic Acid and Protein Sequencing, all of them as depicted on numerous relevant, retrieved and processed Industrial Property Documents.

**Key words:** IVD-Technology, Clinical Laboratories, Industrial Property Rights, Immunoassay Analyzers, Fluorescence, Quantum Dots, Next Generation Sequencing.

### Introduction

Innovation has become synonymous of competitiveness, however, the increasing importance for Industrial Property (IP) Rights, and more specific for Patent-information, is often disregarded in "academic" Biomedical Technology (BMT) and Medical Informatics (MI) R&D. We synopsise our activities to employ IP-Docs for the optimization of our BMT/MI-students training and participation in R&D activities.

A course on IP-Rights for senior BMT and postgraduate MI-students[1] is being offered since 2003, addressing the origin and the historical development of IP-protection, the essential features and the relevant aspects of the IP-core doctrines, BMT-equipment classification and information retrieval out of patent-documents, by employing the European Patent Office (EPO) [esp@cenet](mailto:esp@cenet) search-engine[25] and their employment in R&D-projects. This "IP-Docs retrieval and evaluation project" is a unique, non-funded, R&D activity in Greece. It has led to about 20 publications, during the last 5 years [2-19] dealing with:

- Development of "semantic" Patent-search techniques.
- Confining the "state of the art" in contemporary Molecular Imaging.
- IP-document based Review of 50 years Biomedical LASER Research.
- IP trends in brain and spine implantable stimulators.
- Manufacturers' R&D-strategy for patients' radiation-protection

outlined in IP-Docs,

The interest for this approach is corroborated by invitations for Lectures and

Tutorials in International Conferences (ITU, IAEA, WHO, IEEE/EMBC etc.) and encourages us to continue our efforts, supported only by our students.

The purpose of this paper is to present in details, an extended case-study focused on emerging in vitro Diagnostics Technology as reflected on retrieved and evaluated Industrial Property Documents [20-23].

More specific, this study comprises of the state-of-the-art and the perspectives of automatic Immunoassay Analyzers, it follows the innovation patent-trail from Flow Cytometry to Quantum Dots applications in the in vitro Diagnostics of tomorrow and finally, it provides an overview of the trends in Next Generation Nucleic Acid and Protein Sequencing, all of them as depicted on numerous relevant, retrieved and processed Industrial Property Documents.

### State-of-the-Art and perspectives of immunoassay analyzers as reflected on IP-documents

Immunoassay is the method used for the detection of a Protein in a specimen through the inherent ability of an antibody to bind to the specific structure. From the pioneers Rosalyn Sussman Yalow and Solomon Berson, who described Radioimmunoassay (RIA) for the first time in 1959<sup>[24]</sup> to present days, various tagging methods have been developed.

It is the purpose of this presentation to contribute a retrospective view, regarding the evolution of immuno-analyzers, as reflected on Industrial Property (IP) documents.

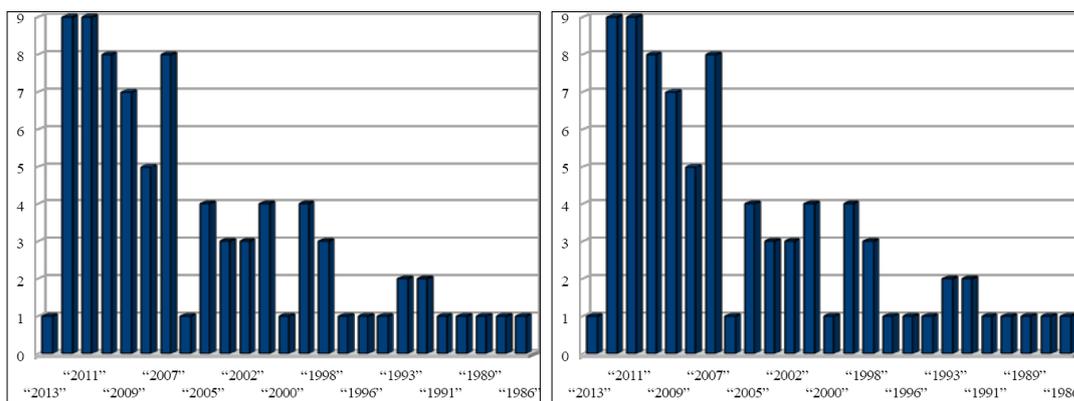
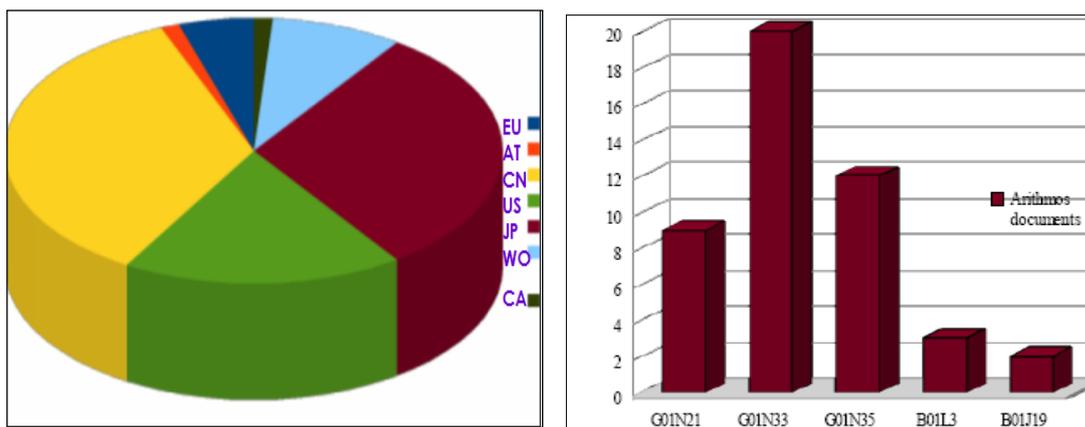


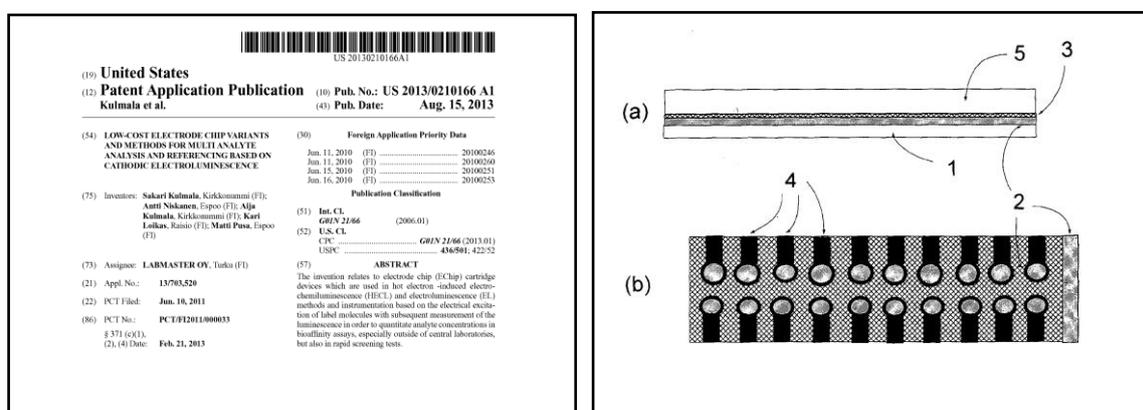
Figure 1. Temporal distribution of Immuno-Assay related IP-Documents (left: title, right: title and abstract search).

Further, by focusing specific on the present trends of the rather dominant Chemiluminescence-Assays (CLIA), as depicted in relevant IP-Docs, to attempt to estimate CLIA's future trends.

The evaluation of relevant IP-Documents, retrieved by employing the on-line esp@cenet search-engine of the European Patent Office (EPO), attempted to create a mapping of the promising techniques and thus, a market-trend prediction, for the next years.



**Figure 2.** Distribution of Immuno-Assay related IP-Documents according to the country of origin (left) and according to the number of IP-Documents per main classification code (right).



**Figure 3.** The US2013210166 (A1) application (left) and a schematic representation of a 20-position E-Chip for Multi Analyte Analysis described in the same document (right).

In total, 82 Immuno-assay and 24 specific Chemiluminescence-assay related IP-Documents have been retrieved and evaluated. A common absolute peak around the year 2011 indicates an increased R&D development in CLIA that is partially due to the introduction of electro-generated CLIA. About 46% of the applications have been filed in the Chinese Patent Office and 37% in the Japanese Patent Office, indicating an important shift towards the Asian markets.

An indicative and promising patent application US2013210166 (A1): «Low-Cost Electrode Chip Variants and Methods for Multi Analyte Analysis and Referencing Based on Cathodic Electroluminescence» is presented in Figure 3. The claimed invention relates to electrode chip (E-Chip) cartridge devices, which are used in hot electron-induced electro-chemiluminescence (HECL) and electrochemiluminescence (EL) methods and instrumentation, based on the electrical excitation of label molecules. The subsequent measurement of the luminescence enables the quantization of analytes' concentrations in bio-affinity assays, especially outside of centralized laboratories, in rapid screening tests.

The present situation of the in vitro Diagnostics (IVD) market shows a dominance trend for high-throughput CLIA-Analyzers. Other methods (RIA, ELISA, PFIA etc.) are still present, however, in smaller scale demands (small or specialized Laboratories).

This emerging picture is fully compatible with the retrospectively outlined trends of CLIA systems, compared to the overall Immuno-Assay systems trends. The physical advantages of Chemiluminescence Assays i.e. the stability of the effect and the high sensitivity due to the prompt “emission” of the total information is fully reflected upon the IP Documents evaluated.

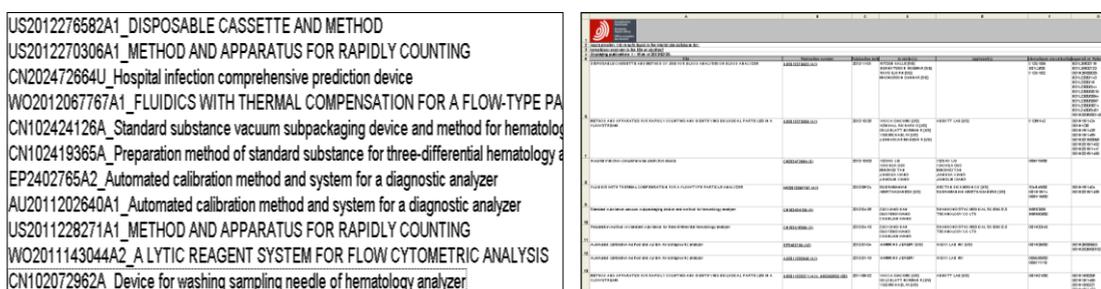
### Following the innovation Patent-Trail from Flow Cytometry to Quantum Dots applications in the In Vitro diagnostics of tomorrow

Fluorescence is being used in the IVD, as a non-destructive way of tracking or analyzing biological molecules, for over forty years. Both usually implementation methods i.e. first, auto-fluorescence (NADH, Tryptophan etc.) and second, “labeling” with extrinsic fluorescent (chemical dye, fluorescent protein or quantum dot), are employed to detect the Biomolecules under investigation.

In this part of our project, the innovation trail has been reviewed first, by focusing mainly on Immuno-fluorescence and Cytometric Assays and second, by attempting to depict the future course of the emerging “Quantum Dots” labeling technology.

The method to accomplish this task was to examine the development of these very important IVD-Techniques, as they are reflected on relevant Industrial Property (IP) Documents.

It was attempted to create a patent-mapping of the promising techniques in these IVD-areas and thus, to reveal probable market-trends, during the next 10 years.

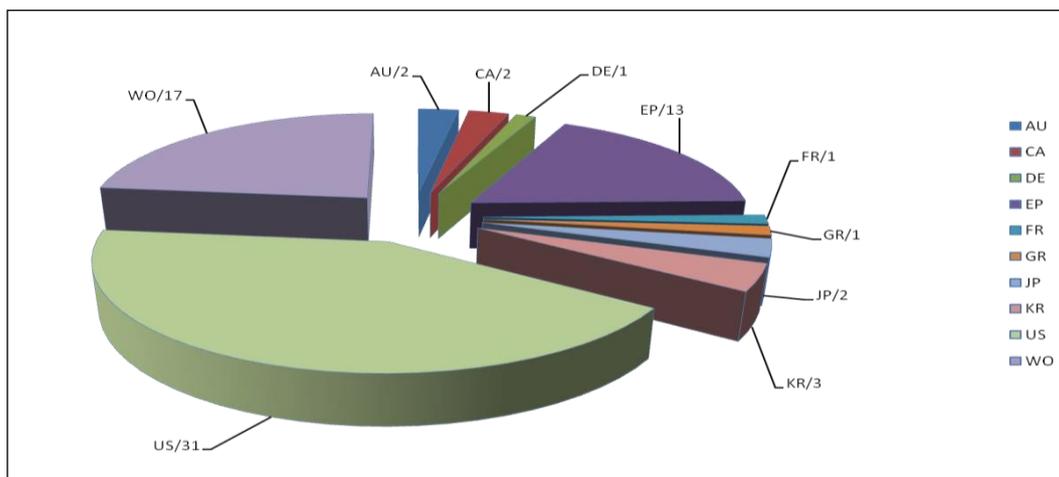


**Figure 4.** An excerpt of the Retrieved IP-Documents (left) and a typical search report of the EPO search-engine esp@cenet (right).

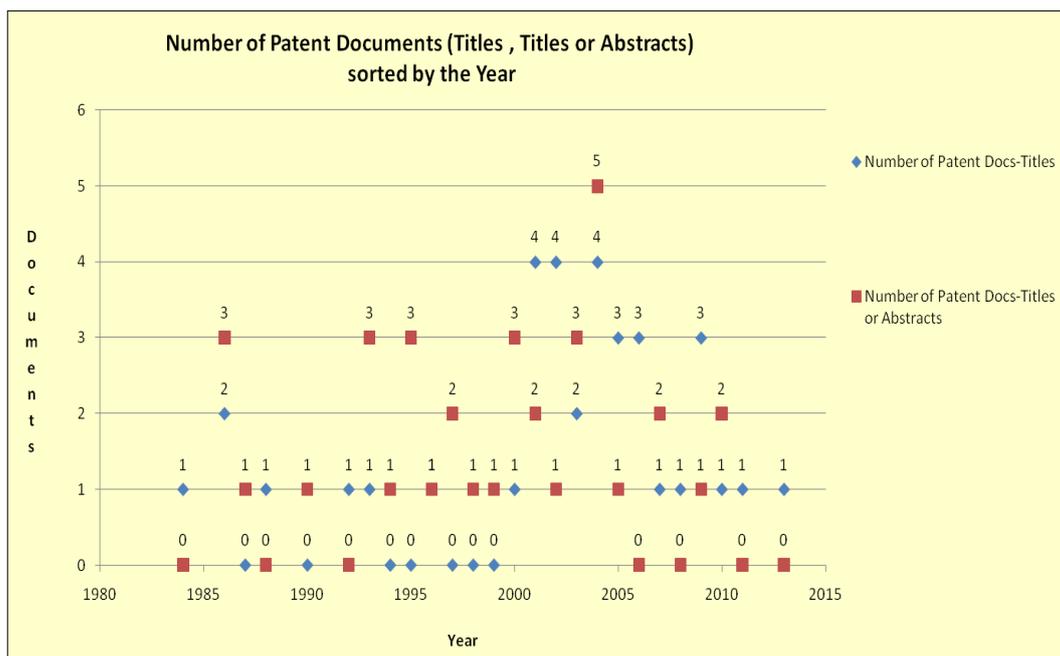
During this IP-search, in total 36 Immunofluorescence, 74 Hybrid Hematology and Flow-Cytometry and 39 Quantum-Dots related IP-Documents have been retrieved and evaluated. The details of the retrieved IP-Documents are presented in Table 1 and in Figures 5-7.

**Table 1.** Details of the retrieved IP-Documents

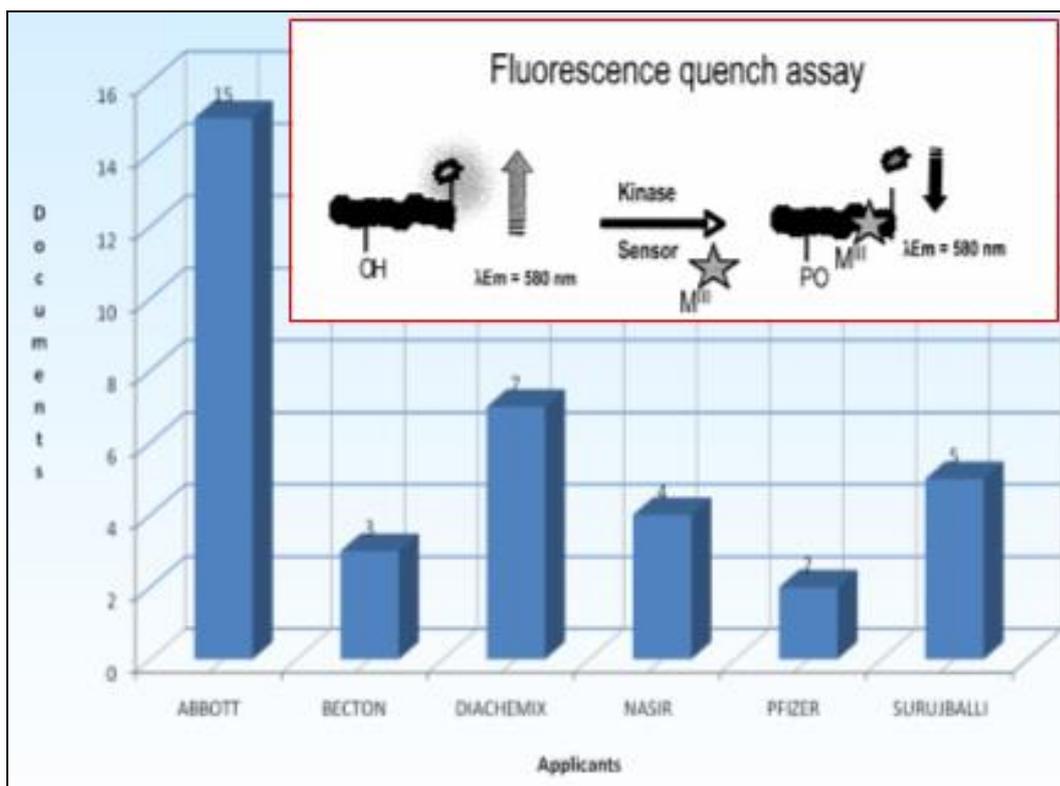
Fluorescence Polarization Assay		Number of Patent Documents	
Data-Base	Keywords	Title	Title & Abstract
WWW	Polarized Fluorescence Assay	0	29
	Fluorescence Polarization Assay	3 5	119
	Immuno-Fluorescence	1 3	60
EP	Polarized Fluorescence Assay	0	8
	Fluorescence Polarization Assay	1 3	40
	Immuno-Fluorescence	0	0
WIPO	Polarized Fluorescence Assay	0	5
	Fluorescence Polarization Assay	1 2	35
	Immuno-Fluorescence	1	2



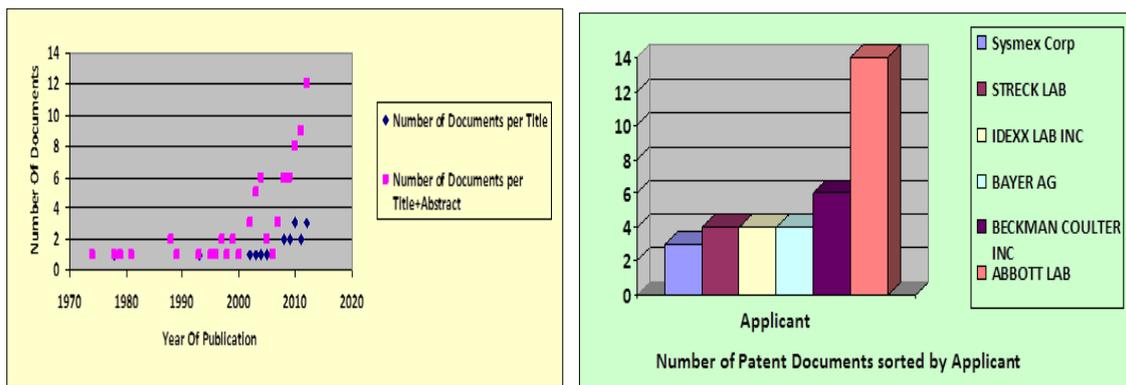
**Figure 5.** Number of relevant IP-Docs sorted by the Patent Office of origin.



**Figure 6.** Fluorescence Polarization Assay (FPA) related IP-Documents (1984-2012).

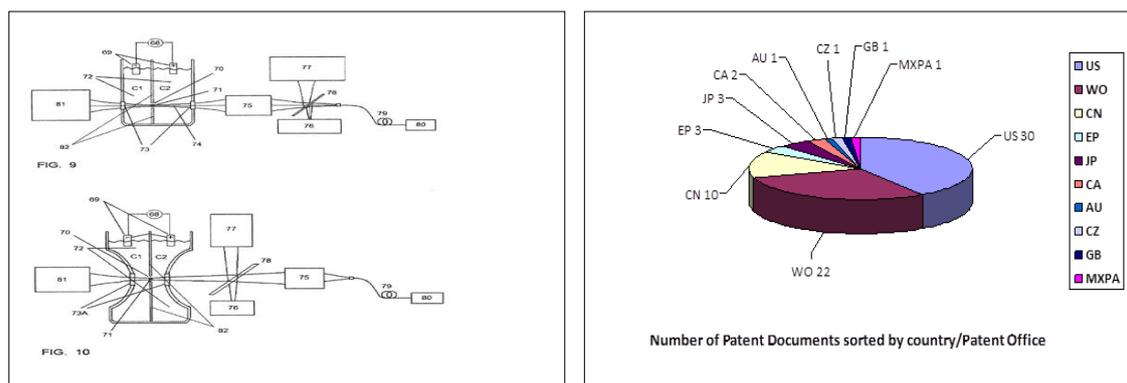


**Figure 7.** Number of IP-Docs sorted by the applicant and as insert a promising Patent Application (US2011/0165603A1) claiming Fluorescence quench assay.

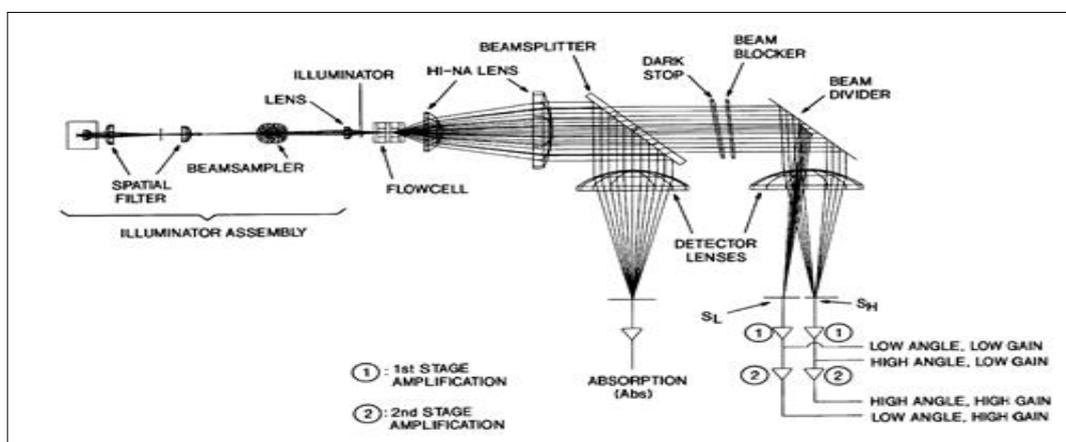


**Figure 8.** IP-Docs per year (left) and the numbers of IP-Docs filed per applicant (right) for Hematology analyzers published during the period 1976-2011.

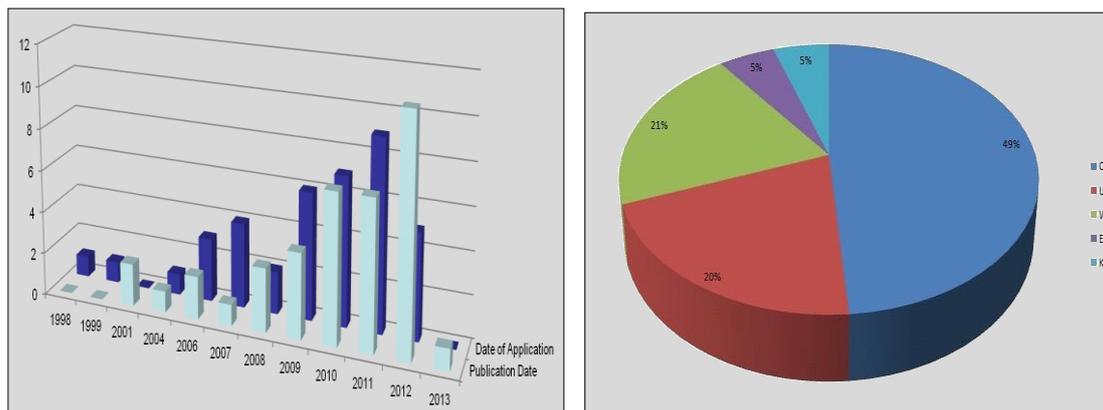
Another important field of applications of Fluorescence in the IVD is the Hybrid Hematology and Flow-Cytometry techniques. We have performed focused searches in the corresponding IPC/CPC subject-matter classes, and the numbers of IP-Docs per year, as well as, the numbers of IP-Docs filed per applicant, for Hematology analyzers published patent applications, for the period 1976-2011, are presented in Figure 8.



**Figure 9.** Number of relevant IP-Docs sorted by the Patent Office of origin (left) and a recent and promising IP-Doc. (US2011254533A1) concerning coaxial illumination of Coulter Aperture (right) in Hematology Analyzers.



**Figure 10.** An important granted Patent US6524858: Single channel single dilution detection method.

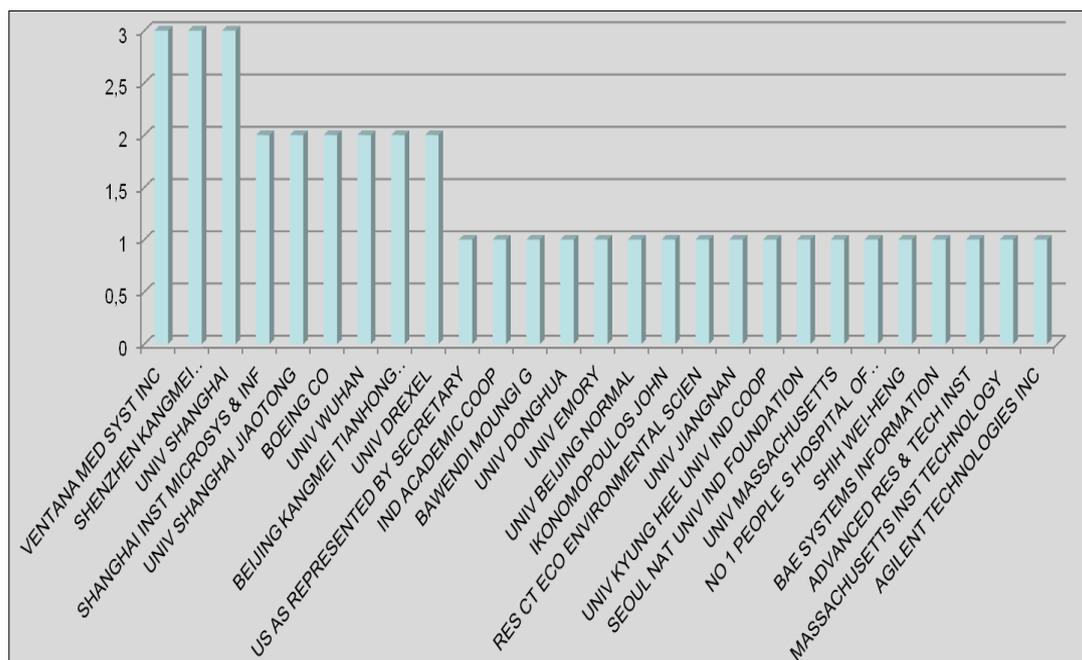


**Figure 11.** Numbers per year of “Quantum Dots” related Number IP-Docs for results concerning the application and the priority dates for the period 1998-2013 (left) and the numbers of relevant IP-Docs sorted by the Patent Office of origin for the same time period (right).

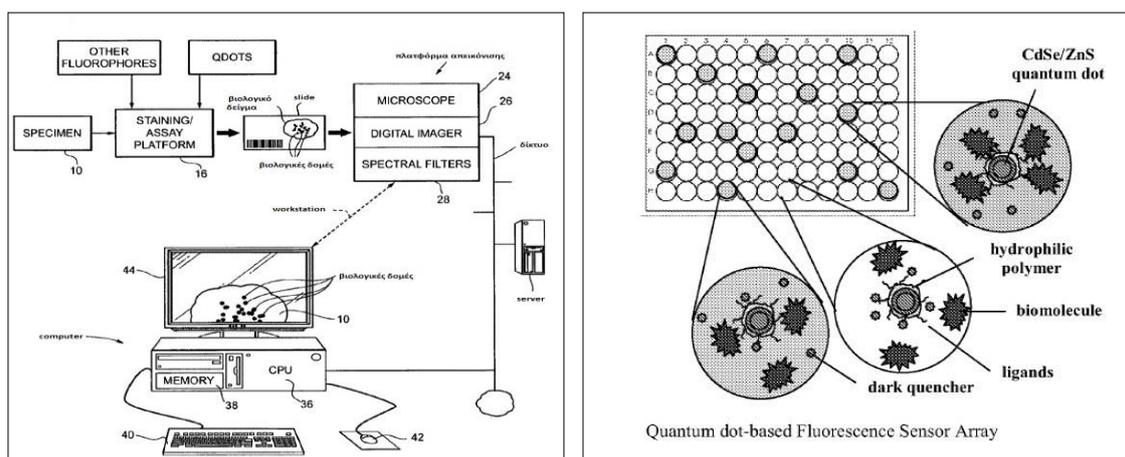
The numbers of relevant IP-Docs, sorted by the Patent Office of origin and a recent Patent Application (US2011/254533A1), claiming a «*Coaxial illumination of Coulter Aperture in full function Hematology Analyzer*» are presented in Figure 9.

Finally, an important granted Patent (US6524858) concerns a single channel single dilution detection method, for the identification and quantification of blood cells and platelets, in a whole blood sample using an automated hematology analyzer and its presented in Figure 10.

Endeavoring to depict the future course of the emerging “Quantum Dots” Fluorescence labeling technology, we have performed a rigorous search of related IP-Docs. Quantum Dots Fluorescence is based on their remarkable physical principles that are resulting in their cardinal features of Photostability and Ligand exchange ability and only their potential Biototoxicity set limits to this emerging «magic» label. The most important results of the patent mapping are presented in Figures 11-13.



**Figure 12.** Number of relevant IP-Docs sorted by Applicant.



**Figure 13.** Two promising IP-Docs: US2011/0235879 A1: Quantitative Multi-spectral Image Analysis of Tissue (left) and WO2010/1411052: Quantum Dot-sensory array for Biological recognition (right).

The filing numbers per year, of “Quantum Dots” related IP-Docs, for the period 1998-2013, for results concerning the application and the priority dates, are presented in Figure 11 (left). The results refer to both, the application and the priority dates of the filed patent documents.

The numbers of relevant IP-Docs sorted by the Patent Office of origin, for the same time period, are also presented in Figure 11 (right). Almost 50% of the IP-Docs have been filed China, about 20% in the USA, 20% in WIPO (World Intellectual Property Organization), 5% in EPO and 5% in Korea, proving the constant displacement of the Industrial Property activities towards Asia, even in emerging Technologies.

Figure 12 displays the number of “Quantum Dots” relevant IP-Docs, sorted by Applicant. The graph corroborates the increasing importance of Asia in «technology stock-exchange» and successfully individualizes the most important Chinese

stakeholders in this scientific-industrial field.

Finally, two promising IP-Docs, the first (US2011/0235879 A1) claiming a «*Quantitative Multi-spectral Image Analysis of Tissue Specimens Stained with Quantum Dots*» and the second (WO2010/1411052) disclosing a «*Quantum Dot-sensory array for Biological recognition*» are presented in Figure 13.

An absolute maximum and plateau is achieved for Immuno-fluorescence related IP-Documents number between the years 2001-2004 and then decreases. For both, Hybrid Hematology/Flow-Cytometry and Quantum-Dots related IP-Documents, after the year 2000, there is a convolutional continuous exponential increase of the filed IP-applications. The results are clearly indicating the increasing involvement trend of Quantum-Dots, especially in modern Flow Cytometry.

### **An overview of the trends in next generation nucleic acid and protein sequencing**

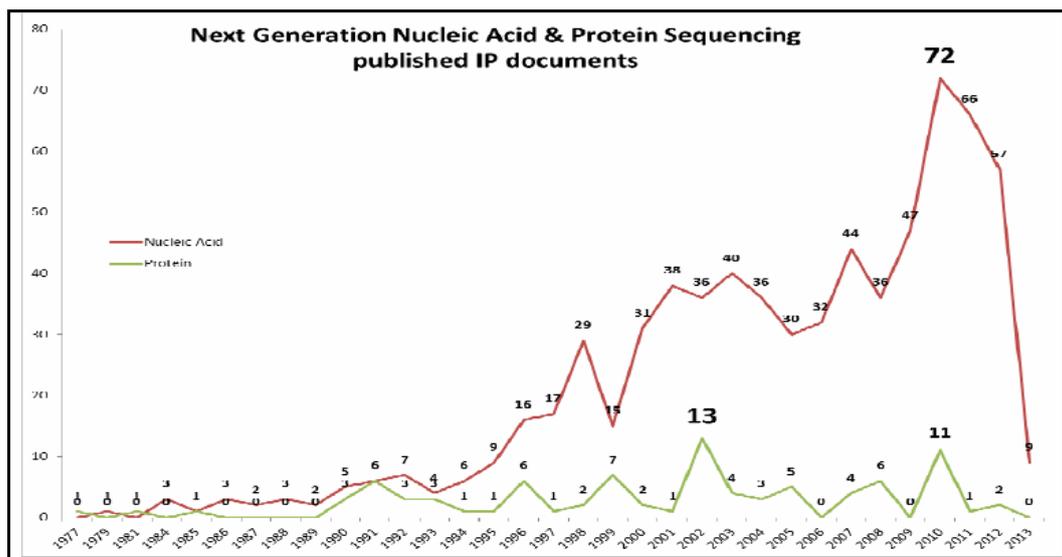
Identifying the functional elements encoded in a genome is one of the principal challenges in modern biology. Sequencing and Decoding Nucleic Acids and Proteins offer knowledge about the cellular functionality and process improving the biomedical aids available. Next Generation techniques provide credible and high throughput genetic information reducing cost and collection time.

Classical sequencing methods have evolved by exploiting the overall evolution of technology in different fields such as imaging microscopy, chemistry and biochemistry, bioinformatics and biotechnology, computing and data storage. Pioneer conventional methods (1st Generation Sequencing) comprised of Auto-radiography, Chain reaction termination, Capillary electrophoresis, Edman degradation and Mass Spectrometry.

**Table 2.** Important Next Generation Sequencing (NGS) methods.

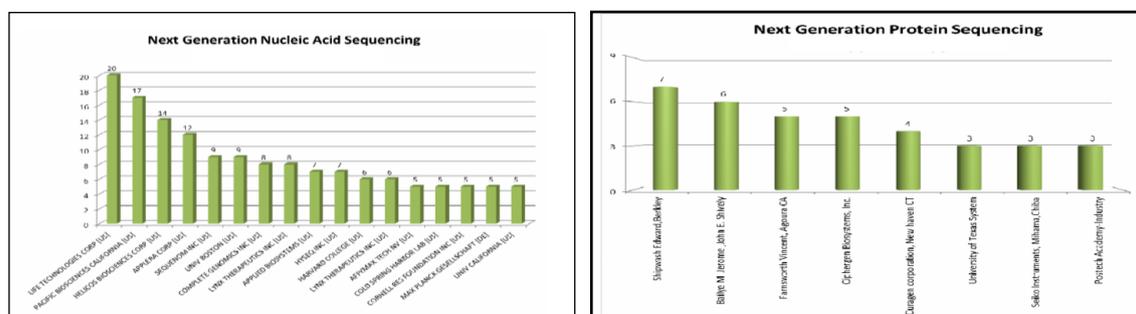
Next Generation Sequencing methods	Next Generation Sequencing methods
Shotgun-PCR	Nanopore and Ion Semiconductor Sequencing (ISFET)
Pyrosequencing	Tandem Mass spectrometry
LASER-linked Polymerase Colony (Polony)	Membranes for solid phase
Real time single molecule sequencing	Chemical degradation in the Gas phase
Reversible terminators	MALDI mass spectrometry
Sequencing by Oleg Ligation Detection	Mass labeling reagents

Next Generation Sequencing (NGS) techniques have evolved taking advantage of the overall evolution of Molecular Biology, ICT and micro/nano Technologies. The most important NGS methods are displayed in Table 2. The numbers of Next Generation Nucleic Acid and Protein sequencing methods related IP-Docs, filed per year during the period 1977-2013, are presented in Figure 14.

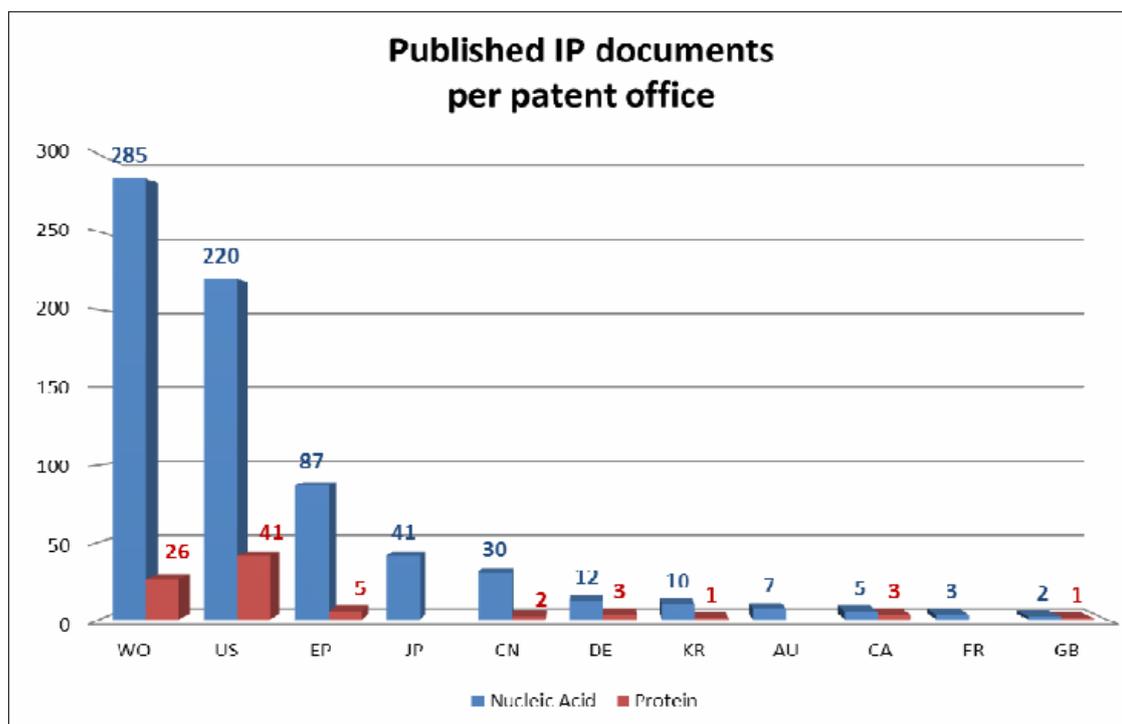


**Figure 14.** Numbers of Next Generation Nucleic Acids and Peptides Sequencing methods related IP-Docs filed per year during the period 1977-2013.

The search concerning Nucleic Acid sequencing reveals a strong trend of IP-Docs related to ion semiconductor sequencing (e.g. US2010137143, WO2010016937, US2006246497), comprising of ISFET array sensors, DNA templates on micro-beads, H<sup>+</sup>-detection and multiple nanopore substrate arrays. Parallel template sequencing provides for rapid base reads and very low cost for equipment. Microscopy-based techniques, non-enzymatic methods, MALDI-TOF MS, Micro-fluidic Sanger sequencing, RNAP sequencing and in vitro virus high-throughput sequencing are considered as methods under development.



**Figure 15.** NGS for Nucleic Acids (blue) and for Proteins (red) IP-Docs per applicant.



**Figure 16.** Numbers of NGS for Nucleic Acids (blue) and for Proteins (red) related IP-Docs per Patent Office of filing.

The main trends in Protein sequencing remains the MALDI-MSD (In Source Decay) and US2010237238 and US8110795 are two interesting examples. Important properties include:

- Peptide backbone fragmentation initiated by side-chain loss at cysteine residue.
- MALDI yields mainly to singly-charged ions without abundant fragmentation.
- Peptide mass finger-printing for the characterization of isolated proteins by the analysis of their

Peptide digest

Finally, the promising nanopore-based protein sequencing are characterized by:

- Single base resolution.
- Unidirectionality.
- Stability of the pore.

I-hemolysin pores with the unfoldase ClpX

## Conclusion

We have presented an extended case-study focused on emerging in vitro Diagnostics Technology methods, as they are reflected on retrieved and evaluated Industrial Property Documents.

More specific, we have presented first, the state-of-the-art and the perspectives of automatic Immunoassay Analyzers, second, we have followed the innovation patent-trail from Flow Cytometry to Quantum Dots applications in the in vitro Diagnostics of tomorrow and finally, we have provided an overview of the trends in

Next Generation Nucleic Acid and Protein Sequencing, all of them as depicted on numerous relevant, retrieved and processed Industrial Property Documents.

We have the feeling that the developed IP-Doc based reviewing approach, offers a useful and convenient methodological tool for researchers, academic teachers and investors, to gain acquaintance and overview in any field of interest, supporting scientific and managerial decision making.

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