SMARTNANO

NEWSLETTER

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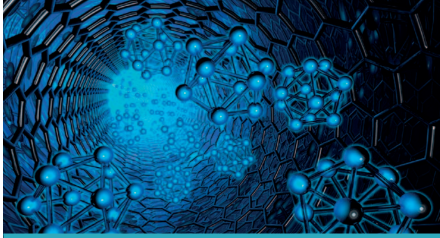
only for project dissemination activities



Who is Abich?
An interview to Stefano Todeschi

Dr. Stefano Todeschi

Co-founder and CEO at Abich srl

Could you give us a brief overview of your scientific services and their applications?

The main target of our company is providing testing and analysis services, mainly to cosmetic and biomedical companies. We offer a wide range of different assays, from chemical analyses to in vivo, in vitro or microbiological tests. Specifically, we focus on in vivo cosmetic tests performed on human volunteers and in in vitro tests, thus we work a lot with cell cultures and re-constructed tissues. We are also continuously developing new in vitro meth-ods as an alternative to the use of animals: we actually were the first compa-ny, in Italy, to do this. For this reason, we received an award from the Italian Hall of Commerce in 2004 as young innovative company.
We also perform regulatory consulting and R&D services, like formulation of topical products or investigation on the composition and properties of new natural or synthetic ingredients. Some years ago we had to screen a wide numbers of unknown herbal extracts from the boreal forest, to look for phar-maceutically or cosmetically active components. This led us to discover new properties through in vitro-testing like for example depigmenting or collagen synthesis inducing activity, and to test these extract for safety allowing their use in vivo on humans.

You recently opened a subsidiary laboratory in Canada, what is the reason for this choice?

We think Montreal is a good location to create a bridge between the European and FDA regulation. This allow us to test cosmetics and sunscreens according to different rules and to ease the import-export activity of European and North-American

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Analysis and research at Abich srl

Abich srl is a Contract Research Organization (C.R.O.) providing research and analysis for the industry, mainly manufacturers of cosmetics, medical devices and other consumer products. It was established in 2002 in Tecnoparco del Lago Maggiore as a start-up initiative and soon awarded with the National Price for most innovative companies in Italy. In 2007 the company moved to found its own facility, which was designed to optimize the operation and management of an advanced chemical and biological laboratory.

Since then **Abich** opened new facilities in Milan and in Montreal for the clinical testing of cosmetics, medical devices and drugs, and collaborated with the Molecular and Cellular Immunology Laboratory of the San Raffaele Hospital (Milan) to develop innovative testing methods and research projects about allergies to topical products.

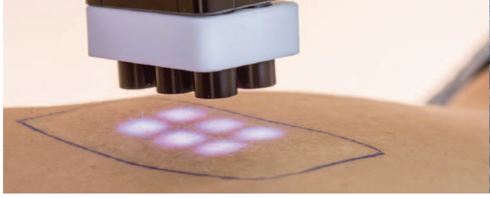
The cosmetological and clinical facility performs both instrumentals and observational tests on human volunteers in order to investigate the performance and tolerability of topical products like cosmetics, sunscreens and medical devices.
A network of specialized physicians (including pecialist in dermatology, allergology, pediatrics, dentistry, ginaecology, ophthalmology and so on) and researchers design and supervise the testing protocols and activities

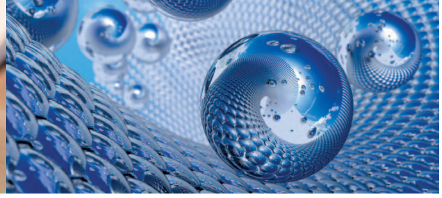
Abich offers a wide range of services in the field of biological and chemical analysis to be applied on cosmetics, biomedicals, textiles, drugs, environmental samples, as well as for industrial hygiene, analytical chemistry and biological R&D. The research activities focus on the development of new biotechnology-based methods to assess the safety and effectiveness of industrial and consumer goods such as chemicals, raw materials, botanicals, cosmetics, medical devices, detergents and textiles. The company has developed and implemented a large number of alternative in vitro assays to test the biological properties and safety of products and ingredients for human use. In the field of nanotechnologies, **Abich** collaborates to the Smartnano project to develop innovative analytical techniques (IFFFF) to detect nanoparticles in complex matrixes like cosmetics and foods.

Is the use of titanium dioxide nanoparticles in sunscreens safe?

The increasing use of chemicals in the form of nanoparticles, the so-called nanomaterials, which are used as ingredients in a wide range of consumer products, has led the Scientific Committee for Consumer Safety (SCCS) to carry out a new assessment about the safety of a set of substances.

One of them is titanium dioxide (TiO_2), a mineral UV-filter commonly used in the nanoscale (particles smaller than 100 nm in diameter) in sunscreen products. Some





minerals, if used in this scale, become almost invisible, but still absorb UV radiation, with the added advantage of giving an increased protection and maintaining a far better texture and compliance in product application. Since the biological and toxicological behavior of nanoscale TiO_2 showed to be different from the larger sized particles, there were concerns about the safety of this widely employed sunscreen and it use as UV filter was under discussion until 2008, when a new set of data was submitted to the SCCS.
The last revision of SCCS work (SCCS/1516/13 Revision of 22 April 2014) confirmed the safety of this component in his nano form to be used as an UV-filter, up to a maximum concentration of 25%. The revision however also established some limitations. The ingredient must not be used in formulations that could lead to inhalation, such as sprays or powder products, and the purity of the ingredient has to be ≥ 99%. Alternatively, in case of a lower purity percentage, the impurities must be demonstrated to be safe. Titanium Dioxide is currently regulated - irrespectively of its form - as a UV-filter in a concentration up to 25% for cosmetic products in Annex VI, entry 27 of the Cosmetics EU regulation 1223/09.

The extended version of the opinion of SCCS is available here. However, it should be noted that the development of new assessment methods is an ongoing process, and we have to consider that the impact of titanium dioxide NPs released in the environment has not been evaluated completely yet.

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companies. In addition, in Canada and in the USA the ban on animal testing is being enforced in these years, and we wish to use our ex-perience in In vitro models to propose alternative testing methods in the North-American market. This seems very promising. Canadian regulation supports financing companies to perform R&D activities locally, and this also is a favoring element for selling our services there.

Who is your main target customer?

We mainly work with cosmetic, medical devices, drugs and chemical ingredi-ents companies, in Italy, all over Europe, in USA, Canada, Australia and New Zealand. In addition, a part of our turnover comes from financed research projects (like Smartnano) and from local authorities.

In what particularly do you feel innovative?

Surely, one of the most innovative aspects of our company is the continuous development of practices that allow us to test the safety and the efficacy of products for human employment without using animals for research. These tools also allow us to look into aspects that we could not investigate in vivo, and with very high precision. For example, these methods allow us to investi-gate the performance of an enzyme or the percutaneous absorption of an in-gredient.

What kind of service you see more promising in your field?

In the past 15 years, we have acquired a very deep understanding of Sun-screen products testing, both in vivo and in vitro. This kind of test is required more and more often as a consequence of the increasing concerns related to irreversible damage caused by sun rays.
We have 3 solar simulators and we can perform SPF and Water resistance testing according to ISO, Colipa, FDA and Australian standards and regula-tions.
We also developed protocols for testing sea-water or chlorinated water re-sistance and for permanent SPF.
In addition, we internally developed a software to enable our customer to evaluate the resulting SPF of a theoretical formulation in silico. The simulator is freely accessible through our website www.abich.it following registration.

What kind of R&D technology programs are you developing?

*We are involved in the **Smartnano** project, which is focused on the devel-opment of strategies for the detection and analysis of nanomaterials in con-sumer products (such as cosmetics) and environmental matrices. However, we also develop analytical methods and in-house assay techniques for specific testing purposes. As an example, we recently developed an anti-pollution test performed on in vitro reconstructed epidermis and a mucoadhesion assay for medical devices.*

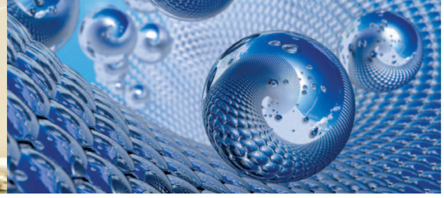
What about the driving force that brought Abich into Smartnano?

*As part of the 7th Framework Program, **Smartnano** gave us a unique op-portunity to interact and collaborate with a large group of European industri-als and academics, thus helping us to expand our field of interaction and our knowledge in the intriguing field of nanomaterials. Our role in the project is that of leader of the dissemination and communication of the results and we also play a key role in the development and validation of the analytical methods.*

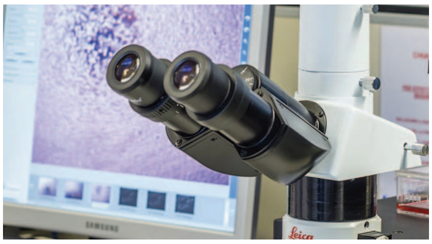
SmartNano Objectives

Nanotechnology is having an increasing impact in many industrial sectors. Paints, food, consumer products such as cosmetic sunscreens and anti-odorants: the use of nanoparticles is now widespread in an over Nanotechnology is having an increasing impact in many industrial sectors. Paints, food, consumer products such as cosmetic sunscreens and anti-odorants: the use of nanoparticles is now widespread in an overwhelming number of goods. It is estimated that 3 to 4 new products containing nanomaterials enter the market every week and that in 2014 1.6 trillion Euros of manufactured goods were based on nanotechnology. Engineered nanoparticles (ENPs) are used in these products to provide specific properties, and can be traced in environmental and biological samples.

In 2013, in response to our increasing need for detection and analysis of nanoparticles, European industrials and academics launched the EU-funded **Smartnano** project. The consortium's leading purpose of developing a technology platform for the measurement of engineered nanoparticles could be a key tool in assessing the fate and potential risks for the population of this substances. The purpose of the project is to develop an innovative, ecologically friendly and cost-effective technology platform that provides a complete solution "from sample-to-result" for the detection, identification, and measurement of ENPs in a wide range of complex matrices.



Samples and matrices



The **Smartnano** platform has to be versatile enough to sample preparation procedures for complex matrices, ENP separation, nanoparticles detection and characterization.

The consortium developed several protocols for the pre-treatment of ENP-containing samples prior to AF4 separation. Several methods for matrix simplification have been tested and bench-top experimental protocols were developed and optimized. Working sample preparation protocols have been completed for challenging samples, such as TiO_2 nanoparticles (NPs) in sunscreen lotions, SiO_2 NPs in milk, and Ag NPs in environmental samples. For SiO_2 in biota samples, a protocol was developed which allows the AF4 separation of the supernatant cell lysed component of bacterial and diatoms cell cultures. These sample preparation methods thus far have proved absolutely essential to the successful measurement of particle size distribution in complex samples.

Supercritical CO_2 extraction techniques were used to simplify complex matrices containing ENPs. The techniques have been evaluated for four different model systems. A simple extraction method was found to effectively remove water and CO_2-soluble compounds from each material, resulting in a residue that contained the ENPs. Under optimal extraction conditions, the residues were dry films or foams that could readily be dissolved or suspended in a suitable solvent for subsequent analysis.

In order to allow an easier manipulation and handling of the materials containing the ENP, a cartridge system was developed. A stainless steel holder with either a stainless steel or Teflon insert allowed for accurate filling of the cartridge cavity with the sample and easy collection of the resultant dried ENP residue prior to further processing.

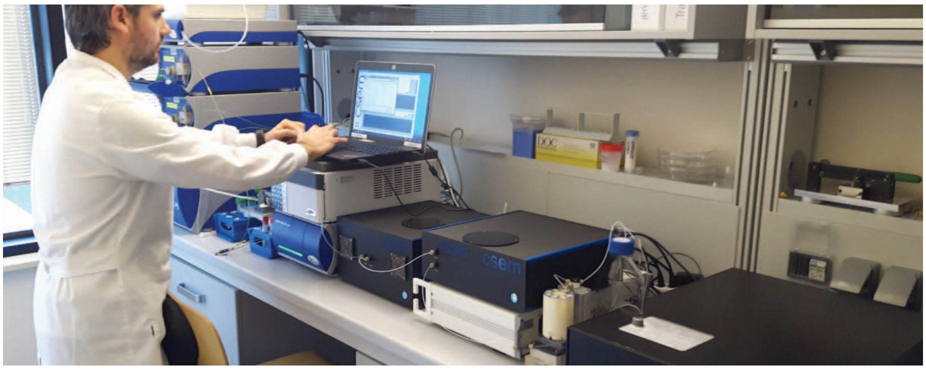
Separation of engineered nanoparticles in complex matrices

The **Smartnano** project focused its efforts in the development of an innovative AF4 macroscopic separation module. The **Smartnano** consortium made significant progress towards an upgraded, cost effective AF4 separation system based on a miniaturized FFF cartridge. Prototypes of the miniaturized separation cartridge module (including a novel Split technology) and clamping system were developed and tested. All prototypes were designed following the principles of obtaining an ecological (lower volumes), cost-effective (cheap, disposable separation cartridges) and easy to use (easily replaceable cartridges using a snap-on clamping system) platform. The test measurements were based on the aforementioned 4 model system samples. The measurements showed that the microfluidic based separation cartridge module increases the sensitivity of the system by a factor of four and more.

Detection of engineered nanoparticles in the samples

For the detection of nanoparticles, a sensitive Multi-Angle Light Scattering (MALS) module was developed. The system was tested with in-line light scattering measurements of water samples containing titanium dioxide, silver and gold nanoparticles. The advantages of the system are the compact optical design, the highly sensitive measurement system, the cost-effective design and easily replaceable flow-through cartridge.

The **Smart-Nano** consortium also developed a sensitive UV absorption module for the detection of nanoparticles. The system was tested on the same samples used for the MALS detector. Again, a compact optical design, cost-effective and easily replaceable flow-through cartridge model, with a wavelength tunability between 200 and 470 nm, fulfilled the project objectives.



Further achievements

The **Smartnano** consortium also successfully demonstrated the use of ICP-MS for the hypersensitive identification and partial quantification of ENPs in two configurations (off-line and on-line). The direct on-line coupling of the **Smartnano** AF4 system to commercial ICP-MS is a major accomplishment. This capability will allow the use of ICP-MS as a hypersensitive quantitative detector for several metal-based ENPs. Specifically, the detection of titanium at ppb levels has been demonstrated for titanium dioxide nanoparticles in a model sunscreen matrix, both in the on-line and off-line configuration. The particle size distribution of a polydispersed silver nanoparticles mixture has also been demonstrated in the on-line configuration at ppb levels. In addition, single particle detection via ICP-MS has been achieved in the case of silver nanoparticles at ppt sensitivity.

In order to facilitate the modular compatibility of the **Smartnano** platform with other existing characterization and detection technologies, a collection cartridge module has also been designed for easy and safe transfer of AF4-separated fractions to other analytical facilities.

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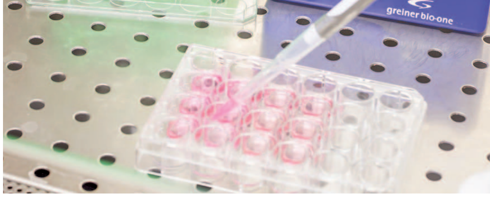
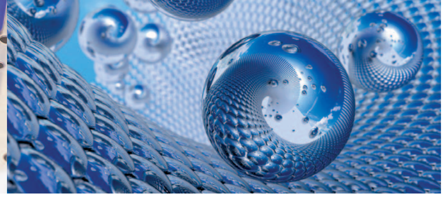
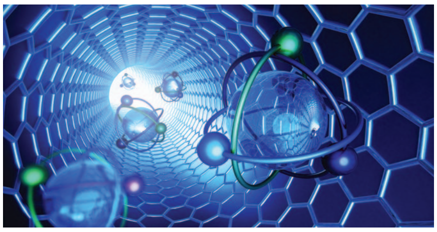




System integration



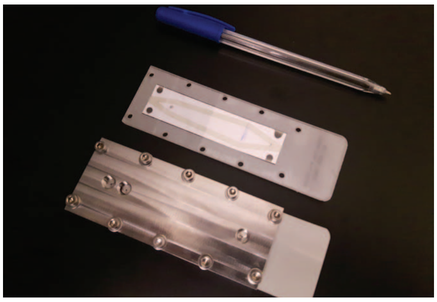
In the last month, the modules described above were integrated and optimized for the separation, size measurement and quantification (SSQ) system. Two different designs for the miniaturized disposable cartridges have been successfully built and used to achieve separation of standard samples containing mixtures of NP of different sizes. All the different parts (separation, size, quantification) have been successfully integrated at the hardware and software level and the protocol for installation, calibration and verification for the integrated SSQ module has been developed. The workflow for the complete size, separation and quantification module has been developed and tested and it was possible to separate complex mixtures of NP of different materials such as proteins, latex, titanium dioxide, silver, and gold. In conclusion, the main objectives of this task have been achieved and the availability of well performing SSQ module, together with the protocols for instrument installation and for the separation of standard ENP mixtures set the stage for the successful continuation of the integration and development work on the complete measurement platform. Optimized workflow procedures have been successfully developed for complex samples, such as sunscreen lotions, food matrices and environmental samples containing ENPs. The results show that the **Smartnano** platform, based on miniaturized, disposable cartridges, is able to correctly detect nanoparticles in very complex and challenging samples. In most cases, it can also be used to accurately determine particle size and distribution. These results set the stage for the successful implementation of the application specific platform validation of the subsequent work package.

Nanoparticle characterization

An homodyne dynamic light scattering (hDLS) detector module was also developed. The performance of the system was tested with a variety of samples including ENPs (TiO_2, Au), proteins, biomolecules and micelles in the size range of 0.2-200 nm.

The performance was benchmarked against other commercially available conventional DLS systems. The prototype DLS system was designed to work online with the UV and MALS detector in an integrated platform.

According to our current estimates, both systems are fully compatible with the existing EU and US regulation. No complications or limitations related to these specific systems were identified. Environmental concerns and required safety measures were addressed and observed to be minimal, according to the defined ecologically-sound objectives.



Completing SmartNano project

Currently, the complete **Smartnano** platform prototype, including the miniaturized separation system and online UV, MALS and hDLS detector, is undergoing the final validation on four standard complex samples treated through supercritical CO2 extraction. The results of the ongoing tests are encouraging and the final conclusions of this validation work package will be available within the next quarter, completing the project objectives.

Closing notes

The Smart-Nano project is approaching completion.
The validation of the project's research and the resulting prototype is in progress, including complete tests on the device and the related samples and standards that were created for nanoparticles in complex matrices. The results are promising.
The next issue of the Smartnano newsletter will be the closing number and will include an interview to external stakeholders.
The consortium is also starting to presents its results through international conferences and publications.
Please visit the Smartnano official website to check our latest publications and our presence in oncoming events, or to register for the project's newsletter.

Agenda | *Next events*

April 12-14, 2016
In cosmetics.
Paris Expo Porte de Versailles,
75015 Paris, France
www.in-cosmetics.com/

Featuring: **Smartnano project innovation seminar**
Thursday 14th April at 11.50 hr | Hall 1
www.in-cosmetics.com/en/Sessions/18299/Detecting-and-measuring-nanoparticles-in-consumer-products-the-SMART-NANO-platform

Smartnano Partners that will attend the event:
Abich Srl | **Stand R111**
Feyecon carbon dioxide technologies | **Stand C41**
First Time Exhibitors section

May 22-26 2016.
18th International Symposium on Field Flow-Based Separations
Dresden, Germany
www.ipfdd.de/de/veranstaltungen/conferences-and-workshops/fff2016/
Smartnano Partners that will attend the conference:
CSEM - Centre Suisse d'Electronique et de microtechnique
Postnova Analytics Gmbh

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