European Cooperation in the field of Scientific and Technical Research
- COST -

Brussels, 15 May 2014

COST 028/14

MEMORANDUM OF UNDERSTANDING

Subject :	Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action CM1403: The European upconversion network - from the design of photon-upconverting nanomaterials to biomedical applications

Delegations will find attached the Memorandum of Understanding for COST Action CM1403 as approved by the COST Committee of Senior Officials (CSO) at its 190th meeting on 14 May 2014.

MEMORANDUM OF UNDERSTANDING
For the implementation of a European Concerted Research Action designated as

COST Action CM1403
THE EUROPEAN UPCONVERSION NETWORK - FROM THE DESIGN OF PHOTON-UPCONVERTING NANOMATERIALS TO BIOMEDICAL APPLICATIONS

The Parties to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above and described in the technical Annex to the Memorandum, have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 4114/13 "COST Action Management" and document COST 4112/13 "Rules for Participation in and Implementation of COST Activities" , or in any new document amending or replacing them, the contents of which the Parties are fully aware of.

2. The main objective of the Action is to develop and characterise highly luminescent Photon-upconverting nanomaterials, to design stable and tailor-made surface structures, to establish novel analytical/biomedical applications, and to develop instruments for photon upconversion.

3. The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 44 million in 2014 prices.

4. The Memorandum of Understanding will take effect on being accepted by at least five Parties.

5. The Memorandum of Understanding will remain in force for a period of 4 years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of section *2. Changes to a COST Action* in the document COST 4114/13.

A. ABSTRACT

Photoluminescent upconverting nanomaterials (UCNMs) are lanthanide-doped nanocrystals that emit visible light under near-infrared excitation. The unique anti-Stokes emission enables background-free luminescent detection, which is essential for many diagnostic applications, bioimaging and chemical sensing. UCNMs are highly photostable and display narrow line-like emissions that enable long observation times and multiplexed detection. Research on photon-upconversion is highly interdisciplinary, but currently fragmented without synchronised research actions in Europe. Further progress in the field is severely restricted by the lack of unified methods for the synthesis, functionalisation and characterisation of UCNMs. Missing reference materials and commercial instrumentation make it impossible to compare the results from different groups and precludes the commercialisation of bioanalytical assays, biosensors and diagnostic tools based on these highly promising materials. Consequently, a European network is required to coordinate basic and applied research on UCNMs, standardise procedures, and to make European scientists as well as the high-tech industry aware of this emerging technology. This COST Action is based on a broad range of scientific disciplines to identify and solve numerous research problems such as upconversion enhancement, surface (bio)functionalisation, detection instrumentation, bioanalytical and diagnostic applications, as well as (nano)toxicity.
Keywords: photon-upconversion, rare earths, nanotechnology, point-of-care diagnostic tests, biodetection and bioimaging

B. BACKGROUND

B.1 General background

Fluorescence is an invaluable tool for biomedical applications such as clinical diagnostics and bioimaging, and for optical chemical sensing. To improve the sensitivity and reliability of fluorescence-based technologies, it is of key importance to minimise the background fluorescence originating e.g. from surrounding biological materials. An additional challenge, especially in imaging, is the low photostability of fluorescent dyes. These issues can be solved by using anti-Stokes photoluminescence, i.e. photon upconversion which is a unique form of luminescence of certain upconverting nanomaterials (UCNMs).

Upconverting nanomaterials are composed of an optically inactive host material doped with a combination of rare earth ions (e.g. $NaYF_4$ doped with a combination of Yb^{3+} and Er^{3+} or Yb^{3+} and

Tm^{3+}) that can convert infrared radiation into visible light via sequential absorption of multiple photons (Angew. Chem. Int. Ed. 2011, 50, 5808–5829). The anti-Stokes emission allows to perform background-free detection, and the infrared excitation is barely absorbed and minimally scattered by biological materials enabling measurements in complex sample matrices, e.g. whole blood, and deep tissue imaging. UCNMs have long luminescence lifetimes, and they are extremely photostable, but do not suffer from blinking such as quantum dots. Their surface can be functionalised with ligands or other kinds of molecular shells yielding hybrid UCNMs as highly promising and target-specific optical reporters. Despite many advantages, the UCNMs have not gained yet as broad interest as quantum dots, because both the upconverting reporters and anti-Stokes photoluminescence detection instrumentation have not been commercially available.
The Action members are convinced that the unique features of UCNMs offer simplicity, sensitivity and rapid detection for point-of-care diagnostics as well as for bioimaging. There are, however, many challenges that remain to be solved before UCNMs will live up to their full potential in the biomedical field: (1) The upconversion efficiency of UCNMs is still 10 to 20-fold lower compared to the respective bulk materials (Nanoscale, 2010, 2, 1417-1419). (2) A tailor-made surface functionalisation of UCNMs is critical for biospecific detection, biocompatibility and colloidal stability. (3) There are no reference materials and established protocols available for the synthesis, characterisation and biofunctionalisation UCNMs. (4) The sequential multiphoton excitation renders it difficult to measure the quantum yield of UCNMs. (5) Their detection in general is severely limited by the lack of commercial instrumentation for upconversion luminescence. (6) The chemo- and nano-toxicity of UCNMs and their environmental impact have yet to be studied.
Many fragmented research projects on photon upconversion are carried out simultaneously throughout Europe with local grants, infrastructure and researchers. However, these approaches without coordination will not be very effective in pushing the technology forward, especially in comparison to the great progress in China and North America. The lack of harmonised research actions on both UCNMs and instrumentation has severely hindered the development of the technology and its applications in biomedical sciences. Exploitation of the full potential of UCNMs in biology and medicine requires an exceptional amount of multidisciplinary expertise ranging from materials science and bio/photo-physics over optical engineering to biology and biochemistry. This makes cooperation and synchronised research among European scientists essential. This COST Action shall therefore bring together the extensive and cross-disciplinary expertise of European researchers and academic groups. A coordinated platform of research organisations working on UCNMs is required to promote materials and technology transfer between different research groups and industry and to gain essential access to data and protocols. Furthermore, a transnational and

transdisciplinary approach of the main European research groups in the field will lead to synergistic results and benefit the European research community as a whole.

B.2 Current state of knowledge

Research on rare earth doped UCNMs and photon upconversion has drastically evolved during the past years with a focus on materials science, lighting phosphors, lasers and upconversion lasers. Although rapidly maturing, this technology is currently at a stage where quantum dots were a decade ago. The first reports on stable colloidal UCNMs, which were suitable for bio-applications were published in 2003 (Angew. Chem. Int. Ed. *42*, 3179-3182). UCNMs have been successfully used for the detection of nucleic acids on microarrays, for the diagnosis of various infectious diseases by lateral flow assays and for various homogeneous bioanalytical assays as well as for optical chemical sensors and bioimaging. These applications greatly benefit from the background-free readout under near infrared (NIR) excitation. In diagnostic assays, the use of UCNMs has led to a much lower detection limit compared to conventional fluorophores, especially when analytes are present in complex biological samples such as blood. The use of UCNMs in bioimaging, on the other hand, has extended the range of conventional fluorescence microscopy to deep tissue imaging and photodynamic therapy (ACS nano 2012, 6, 4054-4062). Monodisperse UCNMs down to 10 nm in diameter with the most efficient upconversion host material known to date ($NaYF_4$) can be prepared via different synthetic routes. The reproducibility of syntheses, however, is insufficient and the upconversion efficiency is not optimal for many applications. The same holds for the surface (bio)functionalisation of UCNMs, which is critical for biospecific detection, biocompatibility and colloidal stability. Several proof-of-concept applications ranging from bioanalytical assays to bioimaging have been developed to demonstrate the high potential of UCNMs but their commercial availability is very limited. All instruments have been custom-made using low-cost components, such as infrared laser diodes as excitation light source, standard long-pass and narrow band-pass filters as excitation and emission filters, and solid-state photo detectors which allow relatively simple and inexpensive detection solutions. However, no commercial instrumentation is available for upconversion imaging or bio-assays. Finally, the toxicity of UCNMs and their environmental impact has not been determined, yet.
China has for long invested strongly into lanthanide-doped nanomaterials research and is also the world's largest provider of rare earths. North American interest in the photon upconversion technology is also well represented by top scientific papers and numerous patents. In Europe, there are parallel research efforts aiming at solving the challenges of the UCNM technology, but the lack

of coordination inevitably results in overlapping activities, delaying the predictable scientific, social and economic achievements and leads to a waste of resources. This Action aims at coordinating European research on photon upconversion to develop optimised UCNMs, novel applications, commercial detection instrumentation, and to increase the awareness of the upconversion technology. The Action is innovative and efficient as it comprehensively addresses issues in both basic and applied research with direct commitment of the private sector.

B.3 Reasons for the Action

There is an increasing need for rapid and advanced detection methods for both basic research (e.g. *in vivo* imaging) and different applications (e.g. diagnostics, high-throughput screening). The UCNMs and anti-Stokes photoluminescence detection will generate numerous scientific and technological possibilities, which in turn will have a considerable social and economic impact e.g. on the health care business. However, numerous cross-disciplinary problems have to be solved before UCNMs become a mature technology.

From the companies' perspective, the following issues have been identified before the UCNMs are ready for commercialisation: (1) The design of a tailor-made surface functionalisation is mandatory for obtaining highly stable dispersions of monomeric UCNMs that have a long shelf-life. (2) An optimized surface functionalisation is also required to minimize non-specific binding of UCNMs, which becomes the next limiting factor in assay performance when the luminescence background goes to zero. (3) Standardised reference materials for UCNMs are required to grant batch-to-batch consistency and to scale up the production. (4) Commercial instruments for array readout and *in vivo* imaging must be available before the upconversion technology can be routinely used e.g. for point-of-care diagnostics or clinical applications. Small companies, however, typically do not have the interdisciplinary expertise and facilities to carry out all the optimisation steps in house.

This Action will build a platform for cooperation and interdisciplinary knowledge exchange between scientists from different COST countries and the private sector, and thus enable Europe to take the initiative in developing UCNMs into an established technology and commercial exploitation. Due to their multiple advantages, UCNMs are foreseen to challenge conventional fluorescent reporters used in biomedical applications. The technology is thus expected to lead to earlier diagnoses and also reduce the costs of European and global healthcare. In addition, the environmental load will be decreased compared to e.g. quantum dots, which usually contain toxic heavy metals.

This Action combines the complementary expertise of physicists, chemists, biologists, biochemists,

engineers and the private sector to improve UCNMs, standardise methods for their characterisation, develop new detection instrumentation, evaluate their nanotoxicity and explore their commercialisation. This Action spans from the synthesis and surface chemistry to instrument development, proof-of-principle demonstration of applications and toxicological studies to refine this highly promising reporter technology for presentation to a wider audience.
The future benefits of the COST Action will be the development of new joint research projects and increased national funding for the research on UCNMs. Advances in the innovative photon upconversion technology will also enable the establishment of new spin-off companies leading to new high-tech jobs and strengthening the competitiveness and economical wealth of Europe. This multi-disciplinary collaboration will put Europe at the forefront position in UCNMs research. The methods and interdisciplinary skills related to the photon upconversion technology will be disseminated to young researchers which will strengthen and foster European expertise in the field.

B.4 Complementarity with other research programmes

Existing partly complementary Actions are not sufficient to fulfil the objectives of this Action but collaboration with them will be suggested as joint symposia and workshops. For example, the European F-Element Network (CM1006) is devoted to research on f-elements in general, whereas this Action focuses on the use of certain lanthanides in inorganic materials suitable for generating upconversion luminescence. The Modelling Nanomaterial Toxicity Action (TD1204) uses a computational modelling technique as an effective alternative to experimental testing of nanostructure toxicity. This modelling approach would also be amenable to study UCNMs. The Rational Design of Hybrid Organic-Inorganic Interfaces Action (MP1202) focuses on the interaction of various types of organic and inorganic materials in the molecular level, and thus this Action could benefit from their knowledge.
The photon upconversion luminescence is currently investigated by four FP7 projects: NANOSPEC aims at increasing solar cell efficiencies via upconversion, UCNANOMAT4IPACT studies the applications of UCNMs in photoactivated chemotherapy, CHROMSENSUC aims at increasing the upconversion through chromophores, and LUNAMED (PEOPLE) investigates the use of UCNMs for diagnostics and therapeutic nanomedicine. The Marie Curie ITNs CHEBANA, LUMINET and Mag(net)icFun cover luminescent materials and/or their bioanalytical applications in general. The existing FP7 projects do not significantly overlap with the Action, which aims to coordinate the research on improved UCNMs and their detection methods for the broad use in different applications. The other projects, however, can potentially benefit from this Action, and vice versa.

C. OBJECTIVES AND BENEFITS

C.1 Aim

The main objective of this Action is the development and photophysical characterisation of highly luminescent UCNMs, the design of stable and tailor-made surface structures, establishing novel analytical/biomedical applications and developing instruments for photon upconversion. This Action will enable scientists working on different aspects in this highly multidisciplinary field to exchange their knowledge and coordinate research efforts. The generation of standard materials and standardised methods is a key element in the synchronisation of research and the commercialisation together with industrial partners. Through this Action, Europe can become a global player in the field of photon upconversion, hence creating new jobs in the biomedical industry and decreasing healthcare costs.

C.2 Objectives

The Action aims at coordinating European research to broaden the availability of UCNMs, improve their performance, stimulate new applications and deliver detection instrumentation by implementing the following deliverables:

1. Developing and exchanging standardised protocols and reference materials for the synthesis of highly luminescent UCNMs and their photo-physical characterisation;
2. Developing and exchanging standardised protocols for the surface functionalisation of UCNMs to yield stable suspensions that do not aggregate and can be conjugated to various ligands for binding to target molecules;
3. Establishing safety guidelines by assessing the nanotoxicity of UCNMs;
4. Defining the technical requirements and providing guidelines for developing detection and imaging instruments for UCNMs jointly with industrial partners;
5. Building a network between academia and industry to build prototype instruments for potential commercialisation;
6. Establishing young scientists in the field by joint doctoral theses, Short-Term Scientific Missions (STSMs), workshops and training schools;
7. Interdisciplinary publications on UCNMs and their applications;
8. Promoting UCNMs and the results of the network on the Action website;

This Action will broaden the availability of highly efficient UCNMs, deliver prototype instruments and stimulate new applications, e.g. in point-of-care diagnostics, *in vivo* imaging and single

molecule detection. The deliverables will make researchers and companies aware of the multiple advantages of UCNMs in various applications. At the same time, early-stage researchers (ESRs) will have the opportunity to establish themselves as independent researchers in the field by participating in training schools, symposia and STSMs. The Action will also pursue joint research proposals beyond this Action, thus accelerating the research and strengthening the global research position of Europe.

C.3 How networking within the Action will yield the objectives?

The deliverables will be accomplished by coordinative actions to synchronise the research efforts of individual research groups. Every Action member has its own expertise, research activities and national funding already available, which can be used for the Action. The members have experience in developing, managing and carrying out scientific programmes at the national and international level and the main resources, infrastructure and expertise to achieve the objectives are already available but dispersed within Europe. Hence, this COST Action will bring researchers working on different aspects of the upconversion technology together by taking the following actions:
1. This Action will serve as a hub for establishing and distributing validated reference materials and standard protocols;
2. STSMs are considered as an integral part of knowledge and materials exchange. In particular PhD students and Early-Stage Researchers (ESRs) will be requested to participate;
3. Scientific workshops, research symposia and conferences will be organised as a platform to present and exchange the latest research results and to train graduate students;
4. The Action members, in particular ESRs, will be invited to give seminar talks and lectures at training schools and workshops to promote the Action and to educate students and scientists about the UCNM technology;
5. The Action will be a beacon among individual research activities that will highlight the importance of the upconversion technology for researchers in related fields and industry but also for a wider audience.

C.4 Potential impact of the Action

Scientific impact. The network established by this Action will not only prevent further fragmentation of European research but also lead to synergistic effects, which accelerate the development of tailor-made UCNMs with optimised luminescent properties and surface structure.

The development and distribution of reference materials and standard operating procedures will lead to a higher quality of UCNMs and make the upconversion technique available for researchers that cannot cover every aspect from the design of UCNMs to their applications in their own laboratories. Along the same line, the new instruments for the anti-Stokes luminescence designed together with industrial partners and the evaluation of the nanotoxicity of UCNMs will lower the hurdle for more researchers entering the field and inspire new ideas for research projects and applications. By accelerating the research on photon upconversion, the global research position of Europe will be strengthened.

Economic impact. The availability of optimised and standardised UCNMs will have a strong impact on the commercialisation of the upconversion technology by European companies covering e.g. medical diagnostics, environmental and chemical sensing, but also product authentication and the security business. New business opportunities for start-up companies are created and small and medium enterprises (SMEs) will be able to offer a better product quality, which strengthens the global competiveness of the European hightech industry. *Social impact.* The development of simple point-of-care tests for diagnostics or environment monitoring based on UCNMs is expected to reduce the costs of the global health care system and improve the quality of life, especially in developing countries under low-resource settings. The Action will assist in generating social welfare in Europe through new employment opportunities in industry and research facilities. In addition, the Action will produce highly trained staff and scientists.

Environmental impact. Unlike CdSe quantum dots that are widely used as luminescent reporters, UCNMs do not contain heavy metals, which avoids toxic waste. Moreover, UCNMs can be used for environmental monitoring, where their unique optical properties and excellent photostability can have significant advantages. Photon upconversion has also been employed for improving the efficiency of solar cells, which leads to a more efficient generation of green energy.

C.5 Target groups/end users

The results of the Action will lead to further exploitation of UCNMs by researchers in the fields of materials science, diagnostics, bio/photo-physics, optical engineering, biology and biochemistry. Several SMEs were actively involved in the preparation of this COST Action. The UCNM technology is foreseen to be commercially exploited for simple and rapid diagnostic tests (in particular point-of-care devices), environmental and chemical sensing, microscopy, bioimaging but also for product authentication, security purposes and solar cells. Thus, the end users of this technology are e.g. health care personnel, environmental authorities, security business, and the

general public.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

This Action will coordinate the following research tasks in order to achieve the objectives described in Part C.2:

1. Optimising existing and developing new synthetic procedures to increase the upconversion efficiency of UCNMs, e.g. by adjusting the lanthanide dopant composition and a better control of the dopant distribution, less crystal defects and growing a homogenous protective shell. Plasmonic enhancement of the upconversion luminescence will be investigated. The composition, size and shape of UCNMs will be adjusted with respect to various applications. The rational design of the brightest UCNMs will be supported by theoretical studies;
2. Developing an optimal surface chemistry that leads to stable nanoparticle dispersions, minimizes surface quenching effects and enables the attachment of biomolecules for biomedical applications;
3. The best procedures for the synthesis and surface functionalisation of UCNMs will result in standardised protocols and aid in the preparation of reference materials that will be used to characterise UCNMs under identical conditions among all members of the network;
4. Developing and adjusting instruments for the specific features of upconversion luminescence. These include instruments for the characterisation of UCNMs such as the determination of quantum yields or studies on the power dependency of UCNMs. On the other hand new spectrometers, microwell readers, array scanners, lateral-flow scanners, in vivo imaging systems, and fluorescence microscopes are required for bioanalytical applications of UCNMs. The new instruments will lead to prototypes for potential commercialisation;
5. Implementing low-cost point-of-care diagnostic tests, homogeneous whole blood assays and multiplexed assays, fluorescence imaging and novel optical sensors. The background-free readout of UCNMs in these applications will be compared to conventional fluorophores and quantum dots;
6. Testing of UCNMs for (nano)toxicity.

The execution and completion of these tasks are assigned to five Working Groups (WGs). The research tasks of each WG will be coordinated by a WG Leader, who will guide in achieving the objectives, as well as joint meetings of all WG participants in every year. STSMs of young researchers will foster an efficient collaboration between different groups in order to complete these tasks. New participants are invited to join this Action at the implementation stage and contribute with new research tools and emerging applications beyond the immediate scope of current research.

D.2 Scientific work plan methods and means

WG 1. Materials Research and Photophysical Characterisation

This WG addresses all aspects of materials research of UCNMs to optimise the synthesis, characterise their photo-physical properties, and improve their upconversion luminescence, which is still 10- to 20-fold lower compared respective bulk materials.

1. Enhancement of upconversion efficiency

The upconversion efficiency will be enhanced by systematically synthesizing and evaluating materials of different composition, size, shape and crystallinity. Starting with the best upconversion material known to date ($NaYF_4$ doped with either Er and Yb or Tm and Yb) various synthetic routes will be explored to synthesise UCNMs with an optimal ratio of size and brightness. For example, a more homogeneous distribution of dopant ions and avoiding the formation of sodium vacancies are expected to lead to a higher upconversion efficiency. Growing an undoped shell of host material around the UCNM core will also yield brighter UCNMs because surface quenching effects will be minimised. New dopant compositions (Nd^{3+}) of the shell will be used to shift the excitation wavelength to 800 nm in order to reduce the light absorption of water. Additionally, the plasmonic enhancement of the upconversion efficiency will be investigated and optimised. Standardised protocols for the synthesis of UCNMs will be compiled to provide the optimised UCNMs at an economical scale.

2. Identification of energy loss channels by time-dependent luminescence spectroscopy

The optimization process will be guided by measuring luminescence lifetimes, quantum yields and energy loss channels of UCNMs. After developing dedicated instruments, these measurements will be performed for all core- and core-shell particles to identify how energy transfer and multiphonon relaxation processes inside nanoparticles differ from those of the bulk material and how they are affected by modifications of the synthesis procedures and concentration of sensitizer and acceptor ions.

3. Reference materials

Standardised reference materials will be defined and prepared to establish validated protocols for the qualitative and quantitative characterisation of upconversion luminescence.

WG 2. Surface Functionalisation

Surface functionalisation is required to render UCNMs dispersible in water and for bioconjugation. Different strategies for the reproducible chemical functionalisation of the surface of UCNMs will be assessed to yield tailor-made surface properties.

1. *Dispersibility*

For bioanalytical and biological applications, UCNMs must be colloidally stable in aqueous solutions close to the physiological pH. This can be achieved through surface coating. The coating should also prevent aggregation and be transparent for excitation and emission wavelengths. Furthermore, the surface coating should be as thin as possible for upconversion resonance energy transfer (UC-RET) applications because UC-RET strongly depends on the distance between the UCNM and a quencher unit.

2. *Biocompatibility*

UCNMs can be coated with silica or polyethylene glycol to yield a biocompatible surface, which is required for bioimaging and nanotoxicity research. UCNM-bioconjugates will be characterised in detail to identify the most stable, reproducible and biocompatible UCNMs.

3. *Coupling of biomolecules*

Different methods will be used to introduce functional groups on the surface of UCNMs and to couple them to biomolecules such as proteins or nucleic acids. Standardised protocols for the bioconjugation and purification methods of the UCNMs will be compiled. This will be the basis for the development of different bioanalytical applications.

4. *Long-term stability*

The long-term stability of the bioconjugates e.g. in a dry-reagent system will be studied to enable potential worldwide shipping of the reagents at ambient temperature.

WG 3. Instrument Development

Presently, there are no commercial instruments available for measuring upconversion luminescence and the research is based on in-house built measurement and imaging instruments. This WG focuses on developing dedicated instruments for the photo-physical characterisation or (bio)analytical applications of UCNMs using reference materials from WG 1. The specific demands of users who are involved in assay development or medical imaging (WG 3) will be used to define the technical requirements for developing the instruments.

1. *Instruments for measuring absolute quantum yields and luminescence lifetimes of UCNMs*

The quantum yields and luminescence lifetimes are key photo-physical properties that determine the performance of UCNMs and are important to understand their photo-physical mechanisms. Conventional instruments have to be adjusted for measuring the specific luminescent properties of UCNMs.

2. *Laser development*

The excitation wavelength, power density and operation mode of UCNMs determined by WG1 will

be used to guide the development of diode lasers that are highly efficient, compact and low-cost light sources and have become increasingly attractive for biomedical applications. In collaboration with WG4, the demands for lasers in terms of packaging and interfacing to systems are defined, and lasers are supplied for applications.

3. Development of a single molecule microscope for UCNMs

UCNMs offer an extremely low readout background, do not blink and are extremely photostable. Consequently, UCNMs are well suitable for single molecule applications. This WG will aim to build a dedicated single molecule microscope specifically adapted to the particular photophysical properties of UCNMs. The optimization steps will be done in close relation with WG1 as they are dependent on the underlying photophysics of UCNMs. The developed set-up will be beneficial for WG1 by giving unique information on the photophysics of UCNMs and for WG4 by characterising the interaction between UCNMs and biomolecules.

4. Development and customisation of instruments

Commercial instruments such as (a) fluorescence microscopes, (b) microwell plate readers, (c) flow cytometers, (d) *in vivo* imaging systems, (e) microarray scanners, and (f) lateral-flow scanners will be customised for the research on UCNMs. Prototype instruments will be developed together with industrial partners for the utilisation of UCNMs for (bio)analytical applications.

WG 4. Assays, Sensors and Imaging

Based on the optimised and standardised UCNMs and appropriate detection instruments, new assay formats and applications will be developed. Intrinsically referenced signal detection principles will be investigated, in particular ratiometric dual wavelength measurements that make use of the multiple emission lines of UCNMs, or time-gated measurements to record changes in the luminescence lifetimes.

1. Diagnostic assays

UCNMs are well suited as reporters for point-of-care tests because the upconversion luminescence can be measured free from background even in more complex sample materials such as whole blood. The turnaround time of the assay from sampling to readout is shortened because many sample preparation and purification steps are avoided. Both, heterogeneous and homogeneous immunoassays will be developed based on optimised UCNMs. In addition, UCNMs will be applied in multianalyte bioarray applications.

2. Chemo- and biosensors

UCNMs will be used to develop immunoassays for monitoring bacterial toxins and mycotoxins. The UCNMs will be utilised for the design of nanoprobes for the background-free and ultrasensitive

detection of small molecule chemical analytes such as pH, pO_2, glucose, Ca^{2+} or other ions. Such probes will be designed by combining suitable molecule/ion sensitive fluorophores as energy-transfer acceptors with UCNMs.

3. Encoding schemes for multiplexed analyte detection

The multiple narrow emission bands of UCNMs enable the multiplexed detection of diagnostically relevant analytes. This unique feature of UCNMs will be further exploited by combining the encoding schemes and spectral multiplexing with suspension or solid phase bio-array technologies.

4. Fluorescence imaging and microscopy

The UCNMs produced and characterised in WG 1 and WG 2 will be studied as reporters for both microscopic analysis of cells and *in vivo* imaging. UCNM conjugates will be used to identify and trace membrane-bound or intracellular molecules in living cells. Far-red luminescent UCNMs (e.g. $NaYF_4$:Yb,Tm) will be selected to provide minimal scattering and high contrast. Time-gated measurement will be developed and exploited in particular for Forster Resonant Energy Transfer (FRET)- and sensor applications.

5. Single molecule applications

Single molecule FRET experiments have enabled to characterise the conformations, dynamics and interactions of labelled nucleic acids and proteins. Biomolecules will be labelled with UCNM as the FRET donor and a classical fluorophore as the acceptor. Based on the single molecule instrument developed in WG3, the dynamics of various protein/nucleic acid interactions will be investigated. Additional developments will exploit the signal multiplexing capabilities of UCNM.

WG 5. Toxicity

The dissemination and commercialisation of UCNMs in bioanalytical assays critically depends on the toxicity of UCNMs. Thus, the toxicity of UCNMs will be assessed and safety guidelines will be developed for utilising UCNMs.

1. Interaction with cells

Life-cell imaging (3–7 days) with trend analysis of dividing, necrotic and apoptotic cells combined with manual identification of giant cells will identify the interaction of different UCNMs with cells. The results of these studies will be compared to currently used commercial labels for long-term monitoring of cell such as quantum dots.

2. Cytotoxicity testing

Adverse effects on relevant cell types are identified by basal cytotoxicity testing using screening assays. These assays will be performed on a short-term basis (24 h) in conventional cultures. Cytocompatibility upon longer exposure (4 weeks) is assessed in cells cultured on microbeads in a

bioreactor and cellular effects upon metabolisation and re-uptake of the particles is evaluated.

3. Safety Evaluation

This issue will be approached by experiments and on a theoretical level. Long-term exposures performed in cytotoxicity testing provide the experimental basis for effects of potential metabolisation and re-uptake of the particles. In addition, human risks in the potential application(s) will be considered.

E. ORGANISATION

E.1 Coordination and organisation

The Action will follow the organisational features of all COST Actions described in the documents "COST Action Management" (COST 4114/13) and ""Rules for Participation in and Implementation of COST Activities" (COST 4112/). The main function of the MC is the implementation and overall coordination of the Action and the approval of the budget. During the first MC meeting, the MC will elect the Chair, the Vice Chair, the five WG Leaders, the Short-Term Scientific Mission (STSM) Coordinator, the Website Manager, the Equality Representative and the Industrial Representative. The Industrial Representative will communicate the requirements of industry to the Core Group (CG) and forward the information about commercially interesting results back to industrial partners.

The Chair, the Vice Chair, the five WG leaders, the STSM Coordinator, the Website Manager, and the Equality and Industry Representatives will form the CG. During the 1st MC meeting, the work plan will be established. The MC and the CG will meet once per year and communicate frequently in order to achieve the goals of the Action. The CG will synchronise the research between WGs by collecting the results of each WG and distributing the information to all other WGs. The CG will also be responsible for planning two Training Schools and conferences (see below), and writing the annual report about the progress of the Action in collaboration with the MC.

Five Working Groups (WGs) will be established. The WG Leaders are at the same time members of the CG to ensure an optimal coordination and information transfer. The WG Leaders will coordinate and supervise the research within each WG and communicate recent findings within the CG and MC. The CG in turn will provide feedback on the composition of each WG regarding complementary skills, collaborations, involvement of ESRs, and gender balance. The WG Leaders will organise one WG meeting per year and workshops. The WG Leaders will also be involved in the assignment of STSMs with consent of the STSM Coordinator and MC.

STSMs will be used for transferring practical skills, protocols and standard materials between

different laboratories. Early stage researchers will be the main beneficiaries of the STSMs, allowing them to obtain practical training in various aspects of the upconversion technology. The STSM Coordinator will be responsible for organising and promoting STSMs with assent of the CG and the WG Leaders.

Each WG Leader will organise one workshop. The workshop will take place in one WG laboratory to provide hands-on training on specialised methodologies for selected PhD students and ESRs. Two training schools in the second and fourth year will be organised by the CG for all members of this Action. During these training schools oral presentation on the research progress will be given for all other partners. PhD students will have the opportunity to present their research, too. If possible conferences will be jointly co-organised with other relevant networks. An Action-specific website will be launched and frequently updated by the Website Manager who is also a member of the CG. The website will include (1) comprehensive information about the organisation as well as the past, current, and future activities of this Action, (2) a list of all members and their research activities, (3) recent achievements and publications, (4) a repository of protocols and slides of the training school / workshop presentations, (5) a page to invite new members to this COST Action, and (6) links to other interesting topics for this Action. In addition to this publicly available content, a password protected intranet will be established for the Action members, which includes a repository of protocols, working documents, slides of the training school / workshop presentations, and a discussion forum.

Milestones of the Action are:

M1 - 1st MC meeting and constitution of the Core Group (CC);

M2 - Launch of the Action website in the first 6 months;

M3 - Providing standard protocols and reference materials for all members by the end of the first year;

M4 - Meetings of CG and WGs in every year;

M5 - 5 Workshops for teaching the practical aspects of the upconversion technology in each WG;

M6 - 2 Training Schools on UCNMs by the end of the second and the fourth year;

M7 - 3 conferences;

M8 - Progress reports of each WG and the whole Action.

E.2 Working Groups

The participants of this Action will be invited to join one (or multiple) WGs. The Scientific Programmes of the WGs are described in part D.2. The WG Leaders will coordinate and monitor

the research within each WGs. The CG is a platform for information transfer between the WGs. Members that have joined more than one WG as well as STSMs between the laboratories of the WG will further enable an efficient transfer of knowledge and materials. The members of each WG will meet once per year. Additionally, each WG will be responsible for organising a Workshop.

E.3 Liaison and interaction with other research programmes

This Action will cooperate with multiple on-going European programmes and future programmes of Horizon 2020. For training and integration of early-stage researchers (ESRs), the Action will collaborate with Marie Curie ITNs CHEBANA, LUMINET and Mag(net)icFun, by inviting the ITN-members to give lectures at training schools In addition, the Action will cooperate with other COST Actions, such as TD1204: Modelling Nanomaterial Toxicity.

E.4 Gender balance and involvement of early-stage researchers

This COST Action will respect an appropriate gender balance in all its activities and the Management Committee will place this as a standard item on all its MC agendas. The Action will also be committed to considerably involve early-stage researchers. This item will also be placed as a standard item on all MC agendas.
The participation of women and ESRs is encouraged and gender and age balance are sought, e.g. when the WG Leaders are appointed. In particular, the Equality Representative as a member of the CG will ensure that gender balance and the involvement of early-stage researchers are respected. Early stage researchers will be the main beneficiaries of about 50 STSMs, allowing them to obtain international research experience in other groups. Additionally, ESRs will obtain management and coordination skills and hence increase their visibility, which will have a strong impact on their future careers as well as the capacity building of the Action. The achievement of these objectives, including the research activities within the WGs, will be monitored annually during the meetings of the MC.

F. TIMETABLE

The Action will proceed over four years. A tentative timetable is given below. Due to the nature of collaboration within the COST framework, the specific topics of the work may be shifted with time in order to adjust to specific needs of the Action.

	Year 1	Year 2	Year 3	Year 4
MC/CG Meetings	1*	1	1	1
WG Meetings	1 per WG	1 per WG	1 per WG	1 per WG
Training Schools		1		1
Workshops	1	2	1	1
Conferences		1	1	1
STSMs	9	12	15	12
Annual reports	1	1	1	1

[+] 1st MC meeting / constitution of Core Group (CG)

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: AT, CZ, DE, DK, ES, FI, FR, IT, NL, PL, SE. On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at 44 Million € for the total duration of the Action. This estimate is valid under the assumption that all the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

H. DISSEMINATION PLAN

H.1 Who?

The dissemination plan addresses internal and external target audiences. The internal dissemination is realised through the regular activities of the Action described above. The external dissemination will address the following audiences:

1. External researchers working on UCNMs and related fields in Europe and worldwide;
2. Young researchers, PhD students and post-docs looking for positions in the field;
3. Universities/Research institutions/Academies working on topics related to the Action;
4. National and international research funding agencies;
5. European policy makers and Standard Bodies;
6. Other European projects (Horizon 2020, EUREKA, Marie Curie) and COST Actions;

7. Industry associations;
8. Companies as potential partners for commercialisation of the technology;
9. The general public.

H.2 What?

This Action will use several dissemination methods:
1. An official website to promote the Action to the general public (see E.1) and a password protected intranet for the Action members to strengthen their communication;
2. Establishing e-mailing lists for the Action members;
3. Publication of the results obtained by the Action in peer-reviewed scientific journals and conference presentations. Links to the scientific publications will be added to the website;
4. Publications in popular-scientific non-technical journals, newspapers and media interviews to educate the general public. It is expected that UCNMs in new diagnostic applications will have a strong impact on human health especially in developing countries and hence will meet a broad public interest;
5. Press releases by the respective home institutions of the Action members;
6. Establishing a professional internet network group in LinkedIn to further promote the Action;
7. Joint meetings of all members of the Action.
8. STSMs will lead to a broad dissemination of the knowledge and technologies available in individual members' laboratories.

H.3 How?

Joint research publications generated through the scientific programme of the Action will educate other researchers in the field and in complementary fields. The research methods and the obtained results of the Action can also be included in the laboratory training of undergraduate students in chemistry and biochemistry. The Action will also contribute to the specialised training of young researchers, e.g. by training schools and STSMs. The members of the Action will acknowledge COST by referring to it or by using its logo in peer-reviewed papers, conference posters and presentations, and in websites of the Action.
The Action will pursue commercialisation of new devices, products and applications. The representatives of industry are invited to conference as well as training schools. In addition, the intellectual property rights and their protection are considered within the Action to facilitate the

commercialisation of the technology.