European Cooperation in Science and Technology
- COST -
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Secretariat

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COST 4140/10

MEMORANDUM OF UNDERSTANDING
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Subject :	Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action TD1002: European network on applications of Atomic Force Microscopy to NanoMedicine and Life Sciences acronym: AFM4NanoMed&Bio

Delegations will find attached the Memorandum of Understanding for COST Action TD1002 as approved by the COST Committee of Senior Officials (CSO) at its 178th meeting on 25 May 2010.

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

COST Action TD1002
EUROPEAN NETWORK ON APPLICATIONS OF ATOMIC FORCE MICROSCOPY TO NANOMEDICINE AND LIFE SCIENCES ACRONYM: AFM4NANOMED&BIO

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 4159/10 "Rules and Procedures for Implementing COST Actions", or in any new document amending or replacing it, the contents of which the Parties are fully aware of.

2. The aim of the Action is to further develop and transfer the biophysical achievements of Atomic Force Microscopy to NanoMedicine and clinical research.

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 76 million in 2010 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 Chapter V of the document referred to in Point 1 above.

A. ABSTRACT AND KEYWORDS

Atomic Force Microscopy (AFM) has become an enabling platform in nanotechnology. It has provided a great impact in Life Sciences and is becoming indispensable also in NanoMedicine. NanoMedicine is an emerging area, which focuses in imaging, early diagnosis, pathological tissue analysis, and drug delivery. Although significant efforts have been devoted to enhancing the performance of AFM, full exploitation of its capabilities has been hampered by the uncoordinated relationship between researchers active in fundamental sciences, and users in the biomedical field. In addition, due to the swift development of AFM, Life science scientists depend on collaboration with experts in physical sciences to utilize the power of AFM instrumentation. This COST Action aims at bundling the expertise of the most active European AFM laboratories with the biomedical scientific environment into a network to foster further enhancement of AFM instrumental development, and explore and support its extensive applications in Life Sciences and Nanomedicine. Similar to the evolution of the Nuclear Magnetic Resonance technique, which has evolved to enabling Magnetic Resonance Imaging and its widely use in clinical diagnosis, AFM holds the promise to perform the same transition. The result of this COST Action would be to shorten the transition period.

Keywords: AFM Molecular Imaging, Dynamic Force Spectroscopy and Biomolecular Recognition, Cellular Studies on NanoMedicine and Life Sciences, Environmental Nanotoxicology, AFM Instrumentation for Clinical Applications.

B. BACKGROUND

B.1 General background

Inspired by the NIH road map in NanoMedicine, the ESF launched its Scientific Forward Look on NanoMed in 2004. In addition, the EC in 2006 promoted a key initiative, the European Technology Platform (ETP) NanoMed, to unite research with industry partners. The ETP NanoMed, involving 150 member organizations and in its strategic research agenda one of the the topics stressed is nano-diagnostics using imaging. The ESF NanoMed meeting in 2007 recognized Atomic Force

Microscopy as a key technology in the field. Nowadays, the AFM technique is reaching a relative maturity with dramatic improvements made in the last ten years. A strong European AFM community exists, as demonstrated from the AFM BioMed Barcelona meeting in 2007 (200 attendees / 23 states, http://www.afmbiomed.org), reflecting that Europe is at the origin of the AFM invention. Single-molecule AFM studies provide new insights into the supramolecular assembly of synthetic and biological molecules, which is critical for the understanding of biological functions at the nanoscale. While the scientific knowledge in AFM-based research grows exponentially, the technology transfer to NanoMedicine and Life Sciences (NanoMed&Bio) remains challenging. The main hindrance lies in the abrupt research boundary between physicists, who create and develop the technology, and biologists, who apply the technology to solving the problems in life sciences. To bridge the research gap between AFM developments and AFM applications in Nanomedicine and to maintain the European leadership in NanoMed&Bio, this COST Action aims to organize scientific and technological cooperation with experts of different backgrounds. This transdisciplinary Action relies on scientists from different fields such as physics, chemistry, mathematicians, biochemistry, biology and medicine, as well as those in more recently developed and developing fields such as genomics, proteomics, metabolomics and microscope design assembly of synthetic and biological molecules, which is critical for the understanding biological functions at the nanoscale

A Trans-Domain COST Action is believed to provide a key strategic platform and community to bundle these research activities. The COST Action will provide the conditions required for facilitating personal interactions throughout Europe and for increasing collaborations in the AFM community, ranging from physicists to clinicians. Due to the strong interdisciplinary features of the Action, the participants will greatly benefit from the synergy of continuous exchange of experimental skills and research prospects.

This Action will also provide a nurturing environment for participants to apply for European Programmes (within the Seventh Framework Programme), and for individual scientists to apply for Intra-European Fellowships or Initial Training Networks under the Marie Curie Action.

B.2 Current state of knowledge

AFM is widely used in solid-state physics and to a lesser extent in biology. In molecular biology, AFM has been applied to numerous studies due to its extreme force measurement sensitivity, magnification and high signal-to-noise ratio (S/N). Perhaps the most well-known applications is imaging studies of single molecules, but considerable efforts have also been made to quantify inter-molecular binding strengths at the nanometer length scale.

As described above, AFM holds the promise to become a key enabling tool for studying biological materials obtained from patients, as an analytical instrument for detecting single molecules in different specimens such as blood, tissues or cells. Since the AFM tips interact with probed samples, numerous experiments can be designed to identify structures on biological surfaces and to manipulate them. In medical research, a virtually unexplored area is the characterization of the mechanical properties of cells and tissues obtained from patients. Some exciting reports in the literature indeed showed that AFM is able to reveal marked differences in mechanical properties of cells, e.g. the elasticity of cancer cells and normal cells, or cell stiffness changes in response to hormone stimuli. However, this ability of AFM is rarely utilized in medicine. A major reason is the lack of in-depth training for medical scientists in the use and applications of the AFM technique to living matter. This seriously hampers them to start and supervise AFM projects and results in unconscious ignorance of new perspectives evolving from biophysics and bio-nanotechnology. The aim of this Action is to change this situation. So far no EU Framework Programme is directly connected to this COST Action. One of the objectives of the Action is to pave the way for one or several EU Framework Programme proposals.

The Action envisions an establishment of a dynamic network of AFM scientists including the major AFM centres in Europe. These AFM research centres have different focuses or specialization, such as AFM imaging, AFM manipulations, AFM nanomechanics, AFM nanodiagnostics, and so on. These centres will provide a diverse training environment for physician-scientists based on their needs, thus introducing new aspects of technological knowledge for their research. This training strategy will provide the basis for medical scientists to becoming AFM experts. As they return to

their clinical research facilities, they can build up their own AFM laboratories. By interacting with medical scientists, the range of applications of AFM would be significantly enhanced in Nanomedicine and biology. By exploring new research fields of AFM, one may also identify current limitations and propose solutions to overcome them by integrating the network expertise.

B.3 Reasons for the Action

The need for this COST Action emerged after several years of commitments to promoting the AFM technology to life sciences and medicine. Despite tremendous results of AFM applications already obtained from the leading groups during the 90's, it was still very difficult in the early 2000s to find AFM meetings or networks dedicated to fundamental biological problems. Due to fragmented or lack in communication, this Action will address the major research issues on both AFM and Nanomedicine.

This Action aims at bringing together pre-existing informal nationwide or European networks of specialists who are not funded for networking. The Action will gain significant added values from these groups, and reciprocally it will also support the existing links within them as well as create synergies among them.

COST Action objectives:

- Promote the federation and integration of the European community of scientists involved in applying AFM to NanoMed&Bio.
- Reinforce exchanges of scientific experiences specialized on biological applications.
- Gather the knowledge to develop AFM into a tool for clinical diagnosis;
- Explore novel applications of AFM in NanoMed&Bio, pharmaceutical, environmental and food industry.
- Provide rigorous training for students and young scientists to efficiently master AFM operations in NanoMed&Bio.
- Support Short-Term Scientific Missions of young investigators or students among physics and biomedical laboratories.

COST Action expected results:

- The Action will efficiently transfer and continuously update knowledge from AFM researches to medical researchers and clinicians.
- The Action will maintain the leadership of the European AFM groups in AFM technique developments and applications.
- The Action will allow the concerted European standardization of laboratory and clinical methods including the rules and regulations of safety and security related to public health.

Means to reach objectives and expected results:

- Workshops to be held will allow scientists, technologists and professors/teachers to present their study cases and to seek a solution to their problems with gathered experts; seminars will serve to form close relationships among researchers with common interests. This collegial network will provide personal contacts, disseminate AFM knowledge, and help with finding the AFM tools needed to address problems that would be otherwise impossible, or notoriously difficult to solve, in the bio-medical field. A community website will be created to post information of scientist exchanges, job openings and knowledge transfer.
- The Action will support the organization of multidisciplinary European Scientific Conferences on AFM to NanoMed&Bio.
- The Action will standardize AFM operations and data management. Experience feedback will receive particular attention and will be collected by the Action to disseminate information on the developments of AFM technology to the biomedical and diagnostic communities.
- In this Action, emphasis will be given to coordinating the exchange of expertise and Short-Term Scientific Missions such that AFM users will have a chance to gather knowledge from different sources. The Action will also aim to broaden the training of young European AFM scientists from the participating research groups. Participation of women scientists will be encouraged.

B.4 Complementarity with other research programmes

The AFM4NanoMed&Bio COST Action conveys its own specific and innovative objectives. These objectives will be attained by following the strategy that associates scientific accomplishments with technical ones based on biological, chemical and physical principles.

This COST Action will create strong ties with the European Technology Platform (ETP) Nanomedicine whose office will be hosted in the company, VDI/VDE-IT GmbH. The ETP Nanomedicine addresses the issue on applications of nanotechnology to achieve breakthroughs in healthcare. It exploits the improved, often novel, physical, chemical and biological properties of materials at the nanometer scale. Nanomedicine has the potential to enable early detection and prevention of diseases, thereby to improve diagnosis, treatment and follow-up medical care.

Potential members of the Action are involved in several FP6 or FP7 European programmes, mostly in the nanomaterials field. The COST Action will encourage applications to national and European funding agencies or organisations such as:

- National Programmes in Nanosciences in European countries: Nano-Initiative - Action Plan 2010 in Germany, NRP Nanoscience in Switzerland, EPRSC in UK or CNano in France.
- NanoSci-ERA Consortium is a network of public and private bodies that are responsible for financing/managing research programmes in nanoscience. These funding services are established by the countries belonging to the European Research Area (ERA).
- MNT-ERA.NET is a large network of European Micro and Nano Technology (MNT) support programmes from 21 European countries and regions, represented by national and regional ministries or funding agencies. The network was launched in 2004 and its activities are still continuing until 2011 under the 7th EU Framework Program.
- ERA-NET Euronanomed is a joint transnational programme for “Nano from research to patient and industry”.

C. OBJECTIVES AND BENEFITS

C.1 Main/primary objectives

The main objective of the COST Action is to further develop and transfer the biophysical achievements of Atomic Force Microscopy to NanoMedicine and clinical research.

C.2 Secondary objectives

The secondary objectives of the AFM4NanoMed&Bio COST Action are:

- Intensify the dissemination of updated knowledge and know-how among the partners within the Action, and promote joint publications;
- Intensify the rigorous training activities (STSM, Training Schools, Workshops), particularly for Ph. D. students and young researchers;
- Enforce in the key partner community the principal notion embodied in the Action, "a strong and integrated organization", including academic researchers and business-related industries;
- Develop a strategy to build and improve collaborative relationships among the target groups of the Action to interested end-users, particularly focussing on both external and internal communications that disseminate the research results with high potential applicability, and to raise the competitiveness of European industries;
- Create a dedicated website for effectively reporting the COST Action progress as well as scientific and technical information;
- Foster coordinated research activities in AFM on Nanomedicine and competitiveness of scientific proposals in the European Research Area;

Quantitatively the achievement of specific objectives can be evaluated by the following parameters:

- Number of active participants in the COST Action;
- Number of applications for funding by international collaborative research projects;
- Number of presentations and publications of collaborative research projects;
- Number of STSM supported by the Action;

- Number of training activities and young researchers trained;
- Number of academic position promotions and other career advances.

C.3 How will the objectives be achieved?

The objectives of the AFM4NanoMed&Bio COST Action will be achieved by networking the leading European experts in their own field. A powerful combination of expertise and team-work is a key element to success. To facilitate and improve the delivery of AFM technology to Nanomedicine, the COST Action will be organized with five Working Groups (WGs). The WGs will report their activities during the annual Workshop, a meeting that gathers experts from different European countries involved in the COST Action.

The focuses of the five Working Groups will be:

WG 1: High resolution imaging of biological systems of from molecules to cells.
WG 2: Molecular-based force spectroscopy applied to ligand-receptor binding for studying inter-molecular recognition and structural unfolding of molecules.
WG 3: Cellular-based AFM studies on Nanomedicine, Nanodiagnosis and Nanosensing.
WG 4: Research that bridges results from AFM researches with clinical implications and Environmental Nanotoxicology.
WG 5: Instrumentation and device developments for the new generation of AFM equipments and setups aimed at clinical applications.

Each Working Group will decide its priorities, design and define its prior objectives, as well as establish a strategy that splits the work among the WG members. The WG members will present their results at the next Workshop of experts. All the five Working Groups will be responsible for distributing new results, initiatives, guidelines, etc, via the Action website.

The participants of the COST Action provide with manpower, laboratory equipments and facilities; they will also perform experiments and apply for research grants to pursue their scientific objectives.

The number of participating researchers will increase during the course of the Action due to newly joined research groups. Recruitment of new Action participants will be conducted through invitations to workshops, meetings or Training Schools.

To ensure the interaction between COST Action participants and other European researchers, dissemination activities, whenever possible, will be held in conjunction with related International Conferences. The knowledge transfer from academics to industry will be stimulated by including specialists from industries in talk-oriented discussions.

C.4 Benefits of the Action

The major benefit of the AFM4NanoMed&Bio COST Action is described as follows:

- Benefit the AFM society by sharing and exchanging knowledge with experts from different disciplines in the community;
- Benefit NanoMed&Bio sciences directly by identifying specific needs for the relevant researches and gathering everyone's expertise for overcoming the research bottlenecks;
- Accelerate the progress in promoting AFM as a standard nanotechnology for biomedical applications;
- Stimulate European economic growth by starting up related businesses and contributing to innovation in scanning probe microscopy for the European industry;
- Ensure that ESR will be adequately trained in the AFM research subjects that meet the current and future challenges in nanotechnology. In turn, ESR will develop a significant appreciation of the whole intellectual process from basic questions to highly specific investigations in highly specific techniques.

C.5 Target groups/end users

The target groups of the AFM4NanoMed&Bio COST Action will be all the AFM-related research laboratories from European academics, industries and clinics. The experts of these groups will benefit from facilitated communication, which synergizes their researches.

The potential end-users will be government bodies, research consumers, clinics/hospitals and venture capital companies in the biotechnology and pharmaceutical sectors. The end-users benefited from the Action will be Small and Medium Enterprises (SMEs) which will have better access to scientific advances made by European research laboratories. The ultimate end-users will be patients.

The end-users of the COST Action to acquire the disseminated knowledge are:

- Biologists who study structures and functions of a molecular system;
- Environmental scientists who are specialized in bioremediation;
- Biomedical scientists who are involved in validating the AFM application in their fields;
- The computational chemists and biophysicists who analyze AFM data;
- The material scientists who aim to developing novel products for AFM experiment;
- SMEs who links research and industry for introducing latest biotechnology and producing innovative materials;
- The environmental agencies and legislative bodies;
- The regional/national policy makers who set up the priorities for R&D funding and environmental requirements.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

The goal of the Action is to explore the various applications of AFM in NanoMedicine and Life Sciences (NanoMed&Bio), on the molecular, cellular, and tissue basis to decipher the current limitations and open future technologies.

Owing to the exceptionally good signal-to-noise ratio (S/N), AFM is the only technique able to image, at high resolution (HR), single molecules (SM) in physiological conditions. To defy conventional techniques on the study of molecular structures and functions, AFM needs to improve sub-molecular lateral resolution. The first task of the Action is to cope HR imaging with image processing and modelling. The target range of HR imaging will include SM and molecular assemblies, cells, viruses and bacteria.

To study macromolecule-ligand interactions is fundamental to early detection of biomarkers. The excellent S/N allows AFM to study receptor-ligand interactions occurring in a single cell. Molecular interactions and molecular distribution of receptors on the surface of a cell can be characterized by DFS in situ when the reaction happens on a living cell. The challenge in studying the recognition process is to control non-specific event detections. Therefore, the second task of the Action will focus on overcoming the obstacles in detecting specific interactions and handling data automatically.

The concentration limit of biomarker detection in current methods is usually higher than the requisite (<10 -18 M) for early diagnosis. AFM is well suited for detecting specific interactions at very low concentrations leading AFM to as a promising tool for detecting novel biomarkers and those occurred at very early stages of diseases. AFM is perfectly adapted to simple quality evaluation of biomaterials or artificial materials that are going to be introduced into the human body. In brief, the third task of the Action is to develop basic AFM techniques for early biomedical diagnostics, disease developments and biocompatibility assessments in using regenerative medicines. Detection and characterization of toxic substances and their interactions in biological pathways are a multi-scaled challenge ranging from metals to microbes. AFM can be applicable for studying structures and reactions of toxic substances in solid and liquid states. AFM can be used to establish criteria for health risk assessment in nanotechnology and to develop superior protocols for detection and quantitative determination of toxic substances. The fourth task of the Action is to evaluate the competence of AFM to study the toxicological properties of engineered nanoparticles in our environment or in ecologically relevant organisms as required in the emerging fields of nanoecology and nanotoxicology.

To spread the usage of AFM in the NanoMed&Bio community, improvements on operability are required. More robust instrumental equipments and more user-friendly software will be a key in boosting AFM to be widely used. Another very promising application of AFM is to combine AFM with other physical techniques such as Raman spectroscopy and fluorescence detection. The fifth task of the Action is to build a strong cooperation between European AFM manufacturers and researchers, thereby to identify the market needs, and determine priorities in technology developments and open new avenue for both AFM research and European industry.

D.2 Scientific work plan methods and means

Scientific work plan methods and means are presented for each Working Group.

WG 1: High-resolution AFM of biological systems from molecule to cells.

The atomic force microscope (AFM) has become a powerful tool for the structural investigation of biological samples. Compared to other techniques employed in structural biology, the AFM works under native-like conditions: experiments can be performed in physiological buffer at room temperature and under normal pressure. The topography of biomolecules can be acquired at a lateral resolution of ~10Å and a vertical resolution of ~1Å at an extraordinarily low S/N ratio. Imaging techniques in medicine are important for our understanding of pathologies and potential development of cures. It is now clear that much pathology are based on molecular disorders – therefore techniques capable to image at a resolution sufficient to observe single molecules and better must be developed. AFM as a medical nano – imaging tool is able to image individual molecules from healthy or pathological tissues.

Firstly, the Working Group 1 (WG 1) will improve existing sample preparation methods and develop novel strategies. It is of major importance to standardize the starting point of scientific and medical investigation approaches, i.e. the biological sample. This task will be shared with those of WG 2 and WG 3.

Secondly, improved understanding of imaging on the biological objects will also be elaborated, in order to minimize the invasiveness of the investigation. This comprises the evaluation of several technical strategies (such as the development high-sensitivity detectors, non-contact modes, minimal amplitude oscillating imaging modes etc) and adapted conditions (finding the optimal imaging condition and/or the optimal condition for the nativeness of the biological sample, or the most intelligent trade-off between both).

Thirdly, in relation to the prior point, instrumentation will be improved, with focus on pushing towards an "easy-to-handle AFM". This will bring the AFM to a wider use, and aims to bring AFM into the hands of non-physicist users, particularly those active in medical research. This task will be elaborated in tight relation with WG5.

Fourthly, WG 1 will develop instrumentation: More sensitive force control, novel imaging modes, faster scanning for the visualization of dynamic processes (video-speed AFM).

WG 2: Molecule-based force spectroscopy applied to ligand-receptor binding for studying inter-molecular recognition and structural unfolding of molecules.

Due its piconewton force sensitivity, AFM has become a widely used technique for investigating several force-related molecular mechanisms, notably using Dynamic Force Spectroscopy (DFS), such as polymer stretching, cellular membrane elasticity, cell adhesion, cell-receptor recognition, protein folding/unfolding, antibody-antigen biorecognition, (more generally biomolecular interactions) and to detect and manipulate single molecules, or cells, providing new insights into their structure-function relationships, even in physiological and pathological contexts. An understanding of the fundamental mechanisms of molecular recognition is central to understanding processes in living organisms.

Biological systems undergoing biorecognition are studied by pulling apart the two partners involved in a complex, once these have been suitably anchored to the AFM and to a substrate, respectively. One interacting partner is immobilized on the AFM tip and the other on a support, then the functionalized tip is brought into contact with the support and a complex may be formed, provided that the two partners have enough flexibility and orientational freedom. Successively the tip is retracted from the substrate and when the applied external force overcomes the molecular forces, the tip jumps off sharply from contact to a noncontact position and dissociation takes place. Such a jump-off process provides an estimation of the complex unbinding force from which significant kinetics and thermodynamics parameters may be extracted in the framework of suitable phenomenological and molecular models.

Although DFS has demonstrated enormous capabilities to provide detailed information on biological systems, even at the single molecule/cell level, the occurrence of some ambiguous and controversial results has been noted in different experimental and modelling contexts. Therefore, great care should be exercised in both experimental and analysis procedures in order to eliminate possible drawbacks and artefacts which might jeopardize the success of DFS. Careful experiments

will eventually allow one to obtain reliable and reproducible information (as normally required in the biomedical context). Within a broad collaborative milieu involving the related expertise of the most active EU AFM laboratories, the WG 2 will aim at developing well established protocols to be adopted in experiments and applications in life science and nanomedicine.

In particular, special attention will be devoted to the immobilization procedures (binding strength and specificity, active site orientation, flexibility, re-orientational freedom, native configuration, and so on) of the biomolecular partners to the AFM tip and substrate that could single out the specific biorecognition events. It is also important to control the number of interacting partners and to reliably take into account for the unbinding forces. An intense coordinated activity will be devoted to better understand the mechanisms involved in the unbinding processes under the application of an external force to develop alternative theoretical approaches which better describe the experimental results (for instance to derive the equilibrium free energy from the mechanical work performed in non equilibrium measurements). Within such a context, reliable, automatic procedures to analyze the large amount of force curves of DFS experiments will be implemented, by promoting, in this way, DFS as a routine approach in the biomedical field. The WG 2 of the Action will also develop new advanced applications of DFS such as the early detection of biomarkers (especially in a pathological context) where very high detection sensitivity is required. Indeed DFS has demonstrated high potentialities in nano-biodiagnostics, especially in combination with ultrasensitive optical spectroscopies, such as advanced fluorescence.

Finally, the coupling of DFS ability to sense and manipulate single molecules with the detection of mechanical, chemical and electrical effects will be exploited to develop innovative nanodevices such as biosensors, also for application in nanomedicine.

WG 3: Cellular-based AFM studies on Nanomedicine, Nanodiagnosis and Nanosensing.

AFM has become a powerful biomedical research instrument for the visualization, probing and manipulation of biological systems from living cells down to single molecules. Importantly, AFM measurements can be carried out in buffer solution under physiological conditions without labelling procedures, which is fundamental to study the structure and function of biological objects under medical relevance.

One main direction in AFM in Nanomedicine focuses on the mechanical stiffness probing of living cells to elaborate their physiological function state and its dependency from the buffered environment, like the concentrations of Na^+ and K^+. These observations give for the first time insights into functional mechanisms that regulate blood pressure via the stiffness of cells that coat the inner walls of human blood vessels. Another important line of research demonstrated that AFM nanomechanical probing can identify cancerous cells via their stiffness and discriminate between the metastatic cancer and benign cells. AFM studies correlated well with immunohistochemical testing currently used for detecting cancer. It was found that different cancer types displayed a common stiffness.

In recent years the powerful combination of AFM with optical/fluorescence microscopy has opened new avenues for NanoMed&Bio, most importantly the possibility to correlate optical and AFM information. Using the combined AFM-fluorescence technique for the first time simultaneously the electrical plasma membrane potential and mechanical stiffness in a living cell have been monitored. The described method is applicable for any fluorophore, which opens new perspectives in biomedical research. Further important combinations of AFM with other techniques include Raman spectroscopy (TERS, tip enhanced Raman) enabling nanoscale chemical identification promising fundamental breakthroughs for nanosensing and early state diagnosis. Complementary to AFM instrumentation, research on sensing platforms for diagnosis and systems for controlled drug release are of fundamental importance for the development of AFM based Nanomedicine. This includes surface engineering for controlled cell and protein adsorption and the use of vesicles and polymersomes as drug carriers and nanoreactors.

WG 4: Bridging results from AFM technology with clinical implications and Environmental Nanotoxicology.

Nanotoxicology addresses the potentially toxicological interactions between nanostructure materials and living matter and investigates potential risks associated with nanomaterials during their production, use and disposal. Detection and characterization of toxic substances and their interactions with biological pathways is a multi-scale challenge ranging from metals to microbes. AFM, by its operational scale (from the nm to μm) and its versatility, is adapted for studying

structures and reactions of toxic species in solid and liquid states. It offers the capability of three-dimensional visualization and both qualitative and quantitative information on many physical properties including size, morphology, surface texture and roughness. The AFM can characterize nanoparticles in multiple mediums including ambient air, controlled environments, and liquids.

Negative effects of nanotechnology on both public health and the environment are a growing concern. For instance engineered nanoparticles (ENPs) made of single elements like carbon or silver or a mixture of elements/molecules benefit to modern medicine. In several instances, nanoparticles enable analyses and therapies that simply cannot be performed otherwise. However, ENPs also bring with them unique environmental and societal challenges, particularly in regard to toxicity. ENPs have the potential for spreading in the environment, being taken up by organisms. Some of them have already been shown to have noxious effects on organisms.

WG 4 will evaluate the effectiveness of AFM as a tool in the emerging fields of nanoecology and nanoecotoxicology. The application of AFM and achievements in material science and nanotechnology on one side and in biophysics (biology, life science) is now ready to be applied to the environment. AFM will be used for investigating the unique biokinetics and toxicological potential of ENPs, as well as propensities such as their shapes (e.g., spheres, tubes, rods), chemistries (e.g., metals, semiconductors, carbon) and different surface characteristics (coating, charge, porosity). The WG 4 will also focus on applications of AFM ranging from diagnosis of pathological viruses to the study of complex systems such as biofilms and micro/nano gels. Within this Action WG 4 will interact with WG 1 regarding the characterization of nanoparticles at high resolution, with WG 2 concerning force spectroscopy of marine gels and biofilms, with WG 3 on nanodiagnostics and protocols for samples preparation and with WG 5 concerning the selection of the best mode of imaging or characterization of environmental samples.

WG 5: AFM instrumentation and devices for the new generation of AFM equipment aimed at clinical applications.

Several physical imaging techniques, notably, X-ray, nuclear magnetic resonance or ultrasound imaging have evolved as powerful and widely used tools for clinical research and medical diagnosis. With perhaps the exception of X-ray imaging, the development of the medical applications of these techniques was a lengthy process. The ability of the AFM for high resolution imaging of soft materials in physiological conditions makes this instrument a potential candidate for clinical diagnosis. The Working Group 5 intends to shorten the transition period between the development of a highly successful instrument for research in nanotechnology to an instrument with clinical research applications.

The activity of this Working Group is articulated in two different tasks. One is focused on defining the dominant features that should characterized an AFM devoted to clinical applications. The other is devoted to establish the areas of clinical research where the AFM could play a major role. The first task is divided in two sub-tasks. One of these will establish and explore the requirements for achieving a user-friendly instrumental interface. The other sub-task will consider the performance aspects of the instrument in terms of spatial resolution, spectroscopy analysis or fast imaging that are required in clinical research. Some of the technical inputs for this activity will come from the other Working Groups of this Action.

The second task is devoted to establish the most likely areas of clinical research where the AFM is expected have a major impact. From the current research, it can be anticipated that cancer and heart disease diagnosis or immunology would be some of the medical areas where the AFM could readily develop all its potential.

E. ORGANISATION

E.1 Coordination and organisation

The COST Action will be coordinated by a Management Committee (MC) assisted by a Core Group (CG). The CG is composed of the Chair and the Vice-Chair of the Action, 5 nominated Leaders for the Working Groups (WGs) and 2 Task Coordinators (TCs). Each of the 5 WGs will be coordinated by the WG leader with one assisting colleague. Each WG will be composed of at least 5 experts including the Leader and Vice-Leader. Each WG will establish its own strategic plan, priority management and objectives to achieve anticipated results. The CG will be assisted by 2 TCs who include a Dissemination/Editorial Coordinator assisted by one colleague. The 2 TCs will ensure the coordination of the Action and the editorial coordination of the website to be more efficient and flexible.

The MC-CG will be in charge of:

- Receiving, assessing, and approving the bi-annual reports on the activity and progress of the WGs;
- Setting forth the policy and criteria on the inclusion of new members in the network;
- Organising, evaluating and executing the advices suggested by the WGs, the workshops and the annual conferences;
- Spurring frequent communications between research teams with different nationality;
- Organizing the Training Schools;
- Managing the Short-Term Scientific Missions;
- Creating specific items on the Action’s website;
- Appointing the Working Group Leaders during the first meeting;
- Appointing the Task Coordinators during the first meeting;
- Comparing the actual results with the original objectives;
- Ensuring the networking functioning between all participants and WGs;
- Updating the Action’s website;

- Supporting publications for the results from research collaborations within the Action on the special issues of scientific journals;
- Creating and strengthening the links to other relevant EU programmes and industries;
- Promoting the conference grants from ESRs in attending an international conference outside of the COST Action;

The research performed by the network members in the 5 WGs will be financed by local, national, or international funding agencies, independent of the COST Action. This Action will cover all the costs for the activities of the Network, including management, coordination and meeting organisation. The Action shall also initiate international applications for research funding.

Action participants will be free to join more than one WG. Each signatory country will send up to 2 National Representatives, whenever possible one of the two representatives shall be an Early-Stage Researcher (ESR). Participation of female members will be specifically encouraged.

E.2 Working Groups

To maximise the impact of the COST Action, a CG will be constituted with 5 WGs and 2 TCs to foster innovation and knowledge application. The 5 WGs will meet once a year, the same for the CG; the last meeting annually shall be combined with the MC meeting. The WG meetings may include Workshops that focus on specific topics. All participants of the Action will be invited to join at least one of the WGs. Each WG will integrate ESRs and woman scientists from the member states. The joint of members with new competence may take place in a WG during the course of the Action.

The WGs will be in charge of:

- Coordinating and tracking the WG milestones;
- Consulting with the Task Groups to advertise the WG's Action within and outside the COST Action;
- Coordinating the WG meetings and electing local organizers;

- Participating in the MC;
- Processing the written reports required for evaluating the COST Action. To foster cooperation among the 5 WGs, the TCs will be responsible for:
- Coordinating the dissemination of the Action;
- Updating the Action website;
- Inquiring and recruiting new research groups who propose to join the COST Action;

E.3 Liaison and interaction with other research programmes

Interaction with other COST Actions and European as well as international programmes will be maintained throughout the duration of the Action.

Potential members of the Action have already participated in other European programmes or bilateral actions between European countries.

The MC will design suitable strategies of communicating and interacting with the EMRC that is in charge of the Forward Look report on Nanomedicine, the FP7 projects and the Nanotechnology research portal of the European Commission.

Due to the open structure of the Action, attracted research groups can be integrated at any time; and every network member will be invited to recommend competent research groups as potential members.

The world-wide impact of the Action will be emphasized by participating in international conferences.

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.

Among the potential members of the Action, the share of female researchers who are interested to participate in this network is remarkably high. Men and women will have equal opportunities in all the activities (management, communication, research and networking) of the COST Action. An extensive contribution of women scientists will be encouraged.

The MC will encourage the Network Members of the COST Action to include in their research team at least one early-stage researcher (ESR). This Action will promote the involvement, at all the management level, and carrier building of ESRs (MS, MD, Ph. D. and post-doctoral scientists).

During WG meetings and workshops, the WG leaders will favour the invitation of ESRs to present their works and organisations. ESRs will be given the priority for STSM to improve their scientific careers. The WG Leaders will also foster the technological exchange between the participants of this COST Action.

F. TIMETABLE

The AFM4Nanomed&Bio Action will last for 4 years. Milestones and deliverables are defined according to the following schedule.

Year	Date	Milestones	Deliverables
1	Semester 1	Kick-Off Meeting Website Continuous call for STSM Theme-based workshop	MC and Core Group Organization Website set-up Workshop report
	Semester 2	Training School WG-meeting/MC meeting	Training School report WG/MC short reports STSM annual reports Action progress report
2	Semester 3	Continuous call for STSM Theme-based workshop	Website set-up Workshop report
	Semester 4	Training School WG-meeting/MC meeting	Training School report WG/MC short reports STSM annual reports Action progress report
3	Semester 5	Continuous call for STSM Theme-based workshop	Website set-up Workshop report
	Semester 6	Training School WG-meeting/MC meeting	Training School report WG/MC short reports STSM annual reports Action progress report
4	Semester 7	Continuous call for STSM Theme-based workshop	Website set-up Workshop report
	Semester 8	Training School WG-meeting/MC meeting	Training School report WG/MC short reports STSM annual reports Website major update Annual report Action final report

Short Term Scientific Missions and Training Schools are viewed as critical for the Action success. Requests for missions and schools will be proposed to the CG by participants on an ad-hoc basis and will be ensured to include ESRs.

Whenever possible, combined annual MC and WG meetings will be held in conjunction with established European conferences.

Dissemination of the Action will be organised on its dedicated website. According to the annual MC meeting, the Action website will be updated annually during the first semester while a continuous updating of the WGs will be performed by Working Group Leaders.

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: Austria, Belgium, Croatia, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Lituania, Luxembourg, Netherlands, Portugal, Spain, Sweden, Switzerland, United Kingdom. On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at 76 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 developed communication plan, which is to be revised and improved periodically, targets groups which may be interested to the research results and conclusions of the Action. The COST Action will adapt the nature of the message to its target audience.

The target audience for the dissemination is:

- Members of the Action
- ESRs members of the Action
- Other researcher outside the Action
- Academic and Research Institutes
- Other COST Actions, ETP, and Network of Excellence
- Clinicians
- National and European Research Agencies
- National and European Environmental Agencies
- Policy makers
- Opinion makers (at the European and Regional level)
- Nanotechnology-based industries

- Environmental companies
- Media
- General public

H.2 What?

The dissemination will promote the Action's visibility and enable the consortium to obtain feedback and suggestions. The results obtained in this Action will be disseminated in appropriate form according to the description in the following table.

Object of dissemination	Main target audience	Quantity
Website	Academics, Industry, Policy maker, Public	1
Limited-access website	Action participants	1
Electronic discussion forums	Action participants	1
Newsletters	Academics, Industry, Public	1 per WG
WG meetings	Action participants	7
Presentations at scientific conferences	Academics	Several
Workshops	Action participants, Invited speakers, Industries	4
Proceedings	Academics, Industry, Public	1/WG and 1/per conference
Review article	Scientific community	1 per WG
Training Schools	ESRs	4
Final Book	Academics, Industry, Public	1

H.3 How?

To ensure diffusion of results, workshops and conferences will be organized during the 4 years of the COST Action and the Network will publish results of its studies in articles submitted to the best journals. The Dissemination coordinator will support the website by serving as contact person for the external scientific environment. Dissemination will be placed as a standard item on all the MC agenda.

The main dissemination tool will be a website that provides information about the Action:

- Management structure
- Contact points
- List of conferences
- List of workshops
- List of Training schools
- List of publications in peer-reviewed and technical journals
- Proceedings of the meetings
- STSM reports
- Access to annual reports
- Exchange of devices/samples
- Financial reports
- Job opportunity
- Studentships
- Guidelines, manuals, and tutorials

Additional dissemination strategy includes a mailing list at each level of information exchange (MC, WG, TC), teaching at universities, presentation of the Action at major international and country specific meetings and workshops, publications in trade magazines such as for medical and biotechnological industries, and publications of articles in mass media.

People and organization that influence industrial, public and political opinion will be identified and provided with the necessary information as to how society can be benefited from the implementation of solutions generated by the Collaborative Research supported by this COST Action.