



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

Secretariat

Brussels, 13 December 2006

COST 338/06

MEMORANDUM OF UNDERSTANDING

Subject : Memorandum of Understanding (MoU) for the implementation of a European
Concerted Research Action designated as **COST Action MP0604: Optical Micro-
Manipulation by Nonlinear Nanophotonics**

Delegations will find attached the Memorandum of Understanding for COST Action MP0604 as approved by the COST Committee of Senior Officials (CSO) at its 166th meeting on 20/21 November 2006.

**MEMORANDUM OF UNDERSTANDING
FOR THE IMPLEMENTATION OF A EUROPEAN CONCERTED RESEARCH ACTION
DESIGNATED AS**

COST ACTION MP0604

OPTICAL MICRO-MANIPULATION BY NONLINEAR NANOPHOTONICS

The Signatories 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 299/06 ‘Rules and Procedures for Implementing COST Actions’, or in any new document amending or replacing it, the contents of which the Signatories are fully aware of.
2. The main objective of the Action is to establish active links between European laboratories working in the field of optical manipulation and related applications and to foster and accelerate long-term development of this field in Europe.
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 20 million EUR in 2006 prices.
4. The Memorandum of Understanding will take effect on being signed by at least five Signatories.
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 the document referred to in Point 1 above.

COST ACTION MP0604

OPTICAL MICRO-MANIPULATION BY NONLINEAR NANOPHOTONICS

A. ABSTRACT AND KEYWORDS

The objective of this Action is to establish active links between European laboratories working in the field of optical manipulation and related applications and to foster and accelerate long-term development of this field in Europe. The goal is to increase knowledge about the basic mechanisms of optical trapping and to develop novel methods of manipulation, micro-patterning and imaging, to be exploited in the future bio-medical technology and in micro-mechanics. The scientific innovation concerns: basic mechanisms of the mechanical light-matter interaction; holographic techniques; nonlinear optical methods in microscopy and trapping. The potential impact on technology concerns the implementation of advanced equipment and devices for: non-destructive and non-invasive manipulation and imaging of micro-objects; patterning and templating of micro-devices; measurements in microfluidics. The scientific exchange resulting from the Action will facilitate the interconnections between these topics to obtain new results in the field of optical manipulation as well as to pave the way to new scientific understanding and technological advancement in the forthcoming era of nanobiotechnology.

Keywords : **Optical Micro-Manipulation, Holographic Optical Tweezers, Micro-Patterning, Microfluidics, Biophotonics**

B. BACKGROUND

At the beginning of the 20th century the concept of light pressure was clarified, however the modern story of optical manipulation starts in the early 1970s with the pioneering work of Arthur Ashkin, which eventually led to the realization of the first “optical tweezers” in 1986, based on a single-beam gradient trap for microscopic particles. In the last two decades a lot of work has been done to improve the ability of optical manipulation and to extend its applications. Laser beams have proven efficient and versatile at manipulating objects ranging in size from tens of nanometres to hundreds of micrometers. These advances enabled precision and sensitivity at the nano-scale and measurement of forces in the femtoNewton range. Applications concerned mainly biology allowing deep investigations on bio-molecular motors and DNA properties. In the recent years the interest in this field grew very quickly, with a growing number of groups in Europe, Northern America and Australasia addressing different scientific issues and technical problems related to it.

The increased rate of achievements in this field is based on some novel concepts and on technological advances concerning: (i) basic foundations of the mechanical light-matter interaction; (ii) holographic techniques; (iii) nonlinear optical methods.

Until a few years ago, virtually all light-manipulation was based on the original optical tweezers technique, in which particles trapped in a single strongly-focused laser beam are moved by translating the laser focus, exploiting the strong electric field gradient in the focal area. In the recent years new approaches stimulated by a wide spectrum of possible applications have been suggested and demonstrated opening both new scientific problems and giving a higher potential technological impact.

While the light induced momentum in the conventional one beam optical tweezers is well understood, the new approaches require a deeper understanding of the mechanisms of the mechanical light-matter interaction, in particular for what concerns: (i) use of complex and multidimensional light patterns (e.g. multi-beam or computer generated holographic light patterns, etc.) ; (ii) use of non-Gaussian beams to transfer angular momentum; (iii) nonlinear optical response of the materials; (iv) dynamics of phototransformation in light-induced folding effects; (v) dynamics of photopolymerisation effect in micropatterning.

In the last few years, researchers realized that much more versatile and general manipulation of molecules and particles was possible using specially-tailored 2D and 3D crystal-like structures of light. This sculpted light has unprecedented potential for manipulating mesoscopic objects. It has already been successfully used to organize small particles, including microbial cells, into patterns and to sort samples of particles according to their size. Three-dimensional light structures can be created by modulating the spatial phase and polarization of laser light and different methods have been recently demonstrated. Some methods are based on computer driven Spatial Light Modulators (SLM) : Generalized Phase Contrast (GPC); Fresnel set-up; holographic. Other approaches use multi-beam interferometry or fast scanning light beams. Some of the participating groups are working on these methods with the aim of reaching a feasible real time optical manipulation of many particles in 3 dimensions. However several problems are still to be solved starting from the limited spatial resolution and low speed of the commercial Spatial Light Modulators presently used to the investigation of other geometries and beam combinations in interferometric techniques.

As an example the promising generalized phase contrast (GPC) method can be mentioned. Based on a combination of programmable spatial light modulators and an advanced graphical user interface, the GPC method enables real-time, interactive, and arbitrary control over the dynamics and geometry of synthesized light patterns. Recent GPC-driven micro-manipulation experiments have shown that the method can provide fully user-guided assembly of many particles in a plane, control of particle stacking along the beam axis, manipulation of multiple hollow beads, and the organization of living cells into 3D colloidal structures. These demonstrations illustrate that the technique can be used not only for the improved synthesis of functional microstructures but also for actuating micro-scale tools: the noncontact method can provide the parallel actuation crucial for sophisticated opto- and micro-fluidic-based lab-on-a-chip systems.

The above mentioned advanced trapping modalities to create specific 2D and 3D arrays can be considered as being akin to a jigsaw. Pockets of excellence have emerged world-wide, to fill in some parts to the 'jigsaw'. However, many key pieces are still completely missing and many more are not quite in place. Thus a coherent, unified approach to tackling the scientific problems using optical trapping has so far remained elusive. In fact this involves not only software and hardware implementation in the current techniques (e.g novel and innovative algorithms for 2D and 3D light field synthesis and development of advanced spatial light modulators), but this is the underpinning and essential key to a number of major fundamental science advances.

Another aspect of innovation in the future of optical manipulation and its applications relies on nonlinear optics. One issue is the role and possibly the exploitation of the nonlinear optical response of materials in light driven nano-motors which are able to move micro-objects or even to fold/unfold macro-object and in light patterning of polymeric and composite materials. This being a field not widely explored yet, where the use of novel photopolymers or anisotropic liquids (liquid crystals) must be investigated.

The other important aspect concerning nonlinear optics is its use in bio-imaging and microscopy. Methods such as Two Photon Fluorescence (TPF), Second and Third Harmonic Generation (SHG, THG) and Coherent Antistokes Raman Scattering (CARS) in Microscopy and their combination have been demonstrated to be very powerful in studying biological systems. The combination of

these methods with optical manipulation techniques will allow collecting previously inaccessible data. In fact the current techniques used in molecular and cell biology to investigate biochemical modifications that take place inside single living cell are not applicable to monitor changes *in situ*. The natural environment of many cells, including blood cells, is in suspension, then, by using optical trapping methods, one can constrain a floating cell in order to collect spectroscopic data in a non-destructive and non-invasive way. Some successful biological applications of optical tweezers combined with Raman spectroscopy have been recently achieved in some groups participating in this COST Action, while the new collaborations born in the frame of this Action will give rise to new approaches in the elaboration of optimal schemes for combining spectroscopic and nonlinear techniques with optical traps in studying single living cells.

In spite of the great potentiality of optical manipulation and of the impressive advancement of the last few years not much exchange has happened between various trendsetter groups in Europe and very few transnational projects are currently running. On the other hand a thematic network to be built in the frame of the forthcoming European VII FP can address only specific aspects of this field, while the mentioned potentiality of optical trapping methods can find higher chances of success by joining in the same Action groups with different expertise and skills. In fact, the field covered by the present Action is very interdisciplinary covering optics, electronics, microlithography, microfluidics and image analysis, just to mention a few. Moreover the methods developed can be applied in almost any field of biology or medicine. Thus a wide network typical of a COST Action will favour the development of novel approaches to optical manipulation as well as the progress of its applications.

Based on the presented state of the art the main objective of the present Action is to establish active links between European laboratories working in the field of optical manipulation and related applications and to foster and accelerate long-term development of this field in Europe. The goal is to increase knowledge about the basic mechanisms of optical trapping and to develop novel methods of manipulation, micro-patterning and imaging, to be exploited in the future bio-medical technology and in micro-mechanics.

In particular the versatility of the mentioned methods opens the door for a range of new applications within biotechnology, materials research in both micro- and nano-scale technology.

One obvious application is to implement an all-optical lab-on-a-chip for non-invasive investigation of growth behaviour in large cell colonies. The technology can be used to maintain natural growth conditions by using sculpted laser light in the near-infrared. For microbiologists, this technology serves as a non-invasive tool for manipulating cells into spatial configurations that can trigger variations in developmental features. For instance, by characterizing biophysical interaction phenomena, including confinement stress, in micro-organisms, microbiologists can better understand microbial cell-growth regulation in bio-films in medical, environmental, or industrial settings.

As already mentioned the optical trapping method coupled to nonlinear microscopy techniques enables non-invasive study of living cells for medical applications (e.g. drug delivery). Therefore applications in advanced optical microscopy/manipulators are envisaged.

Another field of applications concerns micro-mechanics and microfluidics. For what concerns micro-mechanics, a multidimensional optical manipulation method can be used both to fabricate microscopic objects with micrometer dimensions and submicron features (e.g. micro-gear) and to interactively power and actuate them. In this way complex structures can form microfluidic channels, optical waveguide, moving particles getting device for optical sorting of microparticles and micro-pumping. On the other hand these micromechanical devices allow both fundamental

studies of fluids at micro-level (optical peristalsis, non-equilibrium thermodynamics, micro-rehology, etc.) and developing novel technologies for bio-medical diagnostics.

Another area of applications concerns fabrication of new materials by photo-patterning of sub-micron structures to realize photonic devices and bio-sensors.

The COST Action on these topics will strengthen the rapid growth of achievements in this field by establishing wide scale international relations in Europe, stimulating and promoting collaborative research in a multi-disciplinary area. The proposed cooperation, being the fuel necessary to start-up specific research projects among partners of the Action, will favour the development of advanced apparatus for: cell manipulation; micro-patterning and templating; optical imaging for bio-medical applications; control of micro-mechanical light-driven motors.

In the European area, in the frame of VI FP of EU is presently running a NEST- STRP Project "ATOM3D" involving as partners some of the groups who are interested in participating in the present COST Action. This project is complementary to the COST Action, giving a major contribution at the beginning of the Action, since different National and International projects related to the topics of the COST Action will contribute. However it will end in June 2007, thus the Action itself will help in the continuation of its perspective topics. On the other hand a STRP project must be focused on specific objectives which are a part of the more general objectives of a networking activity.

Another European project with some possible interaction with the activities of the Action is the STRP Project "AMPKIN" focussed on studying the dynamic operation of the AMP-activated protein kinase (AMPK) signalling pathway; this project more devoted to investigate biological problems includes one group dealing with optical manipulation.

Two projects running in the frame of the EUROCORE programme "Self-organised Nano Structures" are only partially related to the topics of the COST Action: "Novel Optical Methods for Self-Assembled Nanostructures (NOMSAN)" and "System for Photonic Adjustment of Nano-scale Aggregated Structures" (SPANAS). Again they will be complementary to the Action being specific collaborative projects rather than a networking activity.

On the other hand the Network of Excellence "PHOREMOST" presently running in the frame of the VI FP addressing nanophotonics issues is related to topics very different from the ones addressed in this Action being focused on developing molecular-based optical components.

Therefore no overlapping exists with other initiatives in the European area, and due to the increasing number of groups working in this field it is timely to start a COST Action aimed to establish a wide forum to improve the collaboration among them.

The COST Program offers the best framework for co-ordination of the presented activities. In fact, even if well focused on the development of optical manipulation technology, the activities include several topics requiring very different expertise. Therefore the best way to put together different specific projects is a network and, among the possible network structures, the COST Action is the most suitable one, since:

- (i) it is wider in scope than a thematic network implemented through the Framework Programme, including participation of a large number of countries and different groups for each country and additional partners can join the Action during its life;
- (ii) it does not require a huge amount of money that is needed to support a limited number of national delegates participating in the meetings and to support a program of short scientific missions;
- (iii) the specific research activities of the participating groups are supported in each country by other agencies.

C. OBJECTIVES AND BENEFITS

The main objective of the present Action is to establish active links between European laboratories working in the field of optical manipulation and related applications and to foster and accelerate long-term development of this field in Europe. The goal is to increase knowledge about the basic mechanisms of optical trapping and to develop novel methods of manipulation, micro-patterning and imaging, to be exploited in the future bio-medical technology and in micro-mechanics.

The specific objectives of networking are:

- to establish international relations in Europe within the field of optical manipulation
- to stimulate and promote collaborative research
- to implement an effective and sustained programme of short term scientific missions
- to strengthen linkage with companies interested in technological transfer of the scientific results

From a quantitative point of view the achievement of these specific objectives can be evaluated by the following parameters:

- 1) number of transnational collaborative research proposals prepared including groups participating in the Action;
- 2) number of publications and conference presentations coming out of collaborative researches;
- 3) number of short scientific missions supported by the Action;
- 4) number of participants from industries in the COST meetings;
- 5) number of training schools (open to company members) organised in the dissemination plan

See later in this section the suggested quantitative evaluation criteria.

The main scientific objectives of the research activities to be carried out in the frame of the Action are the following:

- *investigation of novel approaches to optical manipulation* [including use of new beam shapes and light patterns; exploitation of nonlinear optical response of materials for light-driven nano-motors; photo-patterning in polymer and polymer composites, etc.]
- *advancements in the real-time, interactive, and arbitrary control of many micro-particles in 2D and 3D by optical manipulation* [including computer driven methods using SLM (GPC, holographic, Fresnel), interferometric multi-beam techniques, laser scanning, fiber-tweezers, etc.]
- *realization of nano and micro-structures by photo-patterning of polymeric materials* [including fabrication of new materials with tailored physicochemical functionalisation, fabrication of optically activated micro-mechanical devices, realization of ordered periodic structures of nanoparticles, etc.]
- *investigation on microfluidics* [including study of microrheology at nano or micro-scale, optical sorting of micro-particles, optical control of microfluidic components, etc.]
- *implementation of non invasive optical tools for manipulation and study of biological systems* [including lab-on-a-chip for investigation of growth behaviour in large cell colonies, manipulation of cells to trigger variations in the developmental features, etc.]
- *development of new methods coupling optical manipulation tools to nonlinear optical microscopy and spectroscopy for biological systems* [including multi-beam optical traps controlling the living cells without affecting spectroscopic data, single particle trapping and spectroscopic detection, study of time- and space- resolved cell-cell interaction, etc.]

The main objectives concerning the technological impact of these investigations are:

- *implementation of non-invasive tools for manipulating biological systems*

- *implementation of advanced optical microscopy/manipulator apparatus for bio-medical applications*
- *realization of apparatus for real time measurements of physical parameters in microfluids for application in cell biology*
- *realization of advanced apparatus for large area micropatterning and templating*
- *realization of devices based on photo-patterning of polymeric materials (e.g. biosensors, micro-gears)*

The evaluation of the success of the Action can be easily made in relation to the scientific objectives considering the number of peer reviewed publications, conference presentations and invited papers of the participating scientists on these subjects during the Action life.

A similar criterion can be used to evaluate the success in the technological objectives, taking into account patents pending, new apparatus or prototypes realised, new software developed.

The Action will be considered successful with the achievements of the goals listed below.

Networking:

- Year 1 :
 - 1) **5** publications and conference presentations coming out of collaborative researches;
 - 2) **10** short term scientific missions supported by the Action;
 - 3) **1-2** invited speakers from industry at the General Meeting
- Year 2 :
 - 1) **2** transnational collaborative research proposals prepared including groups participating in the Action;
 - 2) **10** publications and conference presentations coming out of collaborative researches;
 - 3) **10** short term scientific missions supported by the Action;
 - 4) **1-2** invited speakers from industry at the General Meeting
 - 5) **1** training school (open to company members) organised in the dissemination plan
- Year 3 :
 - 1) **2** transnational collaborative research proposals prepared including groups participating in the Action;
 - 2) **10** publications and conference presentations coming out of collaborative researches;
 - 3) **10** short term scientific missions supported by the Action;
 - 4) **1-2** invited speakers from industry at the General Meeting
- Year 4 :
 - 1) **2** transnational collaborative research proposals prepared including groups participating in the Action;
 - 2) **10** publications and conference presentations coming out of collaborative researches;
 - 3) **10** short term scientific missions supported by the Action;
 - 4) **1-2** invited speakers from industry at the General Meeting
 - 5) **1** training school (open to company members) organised in the dissemination plan

Scientific and Technical achievements / year:

- 1) **40** publications in peer reviewed journals (total publications on the topics of the Action including both collaborative and non-collaborative research)
- 2) **20** publications in conference proceedings
- 3) **5** new experimental achievements (apparatus/demonstrators/software/patents)

The expected benefits of the proposed COST Action will concern science, technology and, as a consequence - society. The aim is to create an efficient interlink among the European laboratories active in the field of optical micro-manipulation and related applications. For what concerns science the activities to be carried out in the frame of this Action will increase the knowledge in basic physics and possibly biology at micro-scale down to the nano-scale. In particular a deeper insight into the mechanisms of the mechanical light-matter interaction will be achieved by addressing problems linked to amplitude and/or phase modulated complex light patterns, the nonlinear optical response of materials (including new polymer composites) and photopolymerisation processes. Moreover novel results are expected by coupling nonlinear microscopy and trapping.

The technological impact is clear when considering the different devices and instrumentation which will be implemented through the activities of this Action. In particular nanobiotechnology will receive a strong advancement from the successful accomplishment of the objectives, since new tools would be available for non-invasive and nondestructive manipulation of biological micro-objects. On the other hand a number of companies have already expressed the interest in the exploitation of the results of these activities in particular for what concerns advanced optical microscopy and microfluidics devices (LMTB, Zeiss, Micro-Flow Ltd, EuroPhoton, Nikon, Olympus).

The Bi-monthly Optics Report has identified “three additional multi-million and billion dollar industries that laser tweezers will impact. Single molecule manipulation is proving useful in the \$3 billion dollar fertility market, the \$100 million dollar electrophoresis (molecular separation) market, and the micro-machine market expected to reach \$100 billion in the next decade”. The board of National Academy of Sciences, in United States of America, has also viewed that the “optical tweezers is likely to be invaluable in the Human Genome Project”

Finally an appropriate training plan for young academic researcher and company members [realised through a programme of short scientific missions linked to training schools organised in the frame of the Action] will strengthen the academic research and its connections with the technological issues making stronger the linkage between academic and industrial institutions, improving the European position in this field.

D. SCIENTIFIC PROGRAMME

The scientific programme of the proposed Action will be developed along three main lines. One is the “core” activity related to “**Methods of Optical Micro-Manipulation**” while the other two lines shall be considered branches of the first one where the activities have a more applicative character on “**Micropatterning and Microfluidics**” and “**Imaging and Biological Applications**”. A major concern will be in developing techniques and devices representing a breakthrough for the future nanobiotechnology.

Since the scientific programme of a COST Action must provide an open and flexible framework making it possible for any other interested country to join the Action, it will be presented in a schematic way in order to point out the main frames of the activity which include the projects to be carried out by the participating groups.

D.1. Methods of Optical Micro-Manipulation

The main objectives of this activity will be:

- *investigation of novel approaches to optical manipulation*
- *advancement in the real-time, interactive, and arbitrary control of many micro-particles in 2D and 3D by optical manipulation*

These objectives which are linked together will be pursued in a variety of different ways. However, we can distinguish two main frames for the activities to be carried out:

- 1) to work in the “conventional” tweezers scheme of extremely tight focusing (e.g. by 100X microscope objectives) and implementing new geometries, beam configurations and technical methods to manipulate optical traps;
- 2) to exploit other physical mechanisms to overcome the intrinsic limitations of the tweezers scheme and to investigate the optical control of molecular motors able to move meso- and macro-objects

While research in the “conventional” tweezers scheme (a) are expected to get the most advanced achievements in dynamic many particle 2D-3D manipulation, the alternative approaches in (b) are expected to give new solutions to the optical manipulation issues and/or to be promising for optical actuation at the micro-scale.

In the following we give some more details on the different frames:

- 1) The current tweezing geometry uses a microscope objective lens and a standard Gaussian laser beam. This arrangement can only provide a single ellipsoidal trap, elongated along the optic axis. These conventional techniques offer little flexibility for tailoring the optical potential in three-dimensional space and dynamic, multiple trapping can only be realized by time-multiplexing single traps. Multiple beams offer much more than simple multiplexing of the trapping: they are able to create an ‘optical potential energy landscape’. The generation and control of multiple traps will be realised and investigated in several projects. One method is based on the use of acousto-optic deflectors (AODs), which time-share the light beam between each site. Absence from any given site gives the particle an opportunity to diffuse away from its trapped position, which ultimately limits this technique. Then new studies based on holographic or advanced imaging, namely phase-contrast methods, to establish arrays of optical traps will be carried out. Recently, the dynamic projection of holograms and advanced spatial filtering has been demonstrated using a spatial light modulator (SLM). Such devices typically consist of arrays of liquid crystals that can be either optically or electrically addressed. Each pixel can be driven to induce a phase change of $0-2\pi$. A drawback of such systems can be their efficiency: in terms of resolution, we do not yet have sufficient pixels compared with what one finds in static diffractive optical elements. Studies will be performed to overcome the limits of such technology, addressing the issue of the resolution of dynamic holograms and the role of phase-scaling errors.

Among the different methods the General Phase Contrast (GPC) method appears very promising. This method seems reliable to control an arbitrary amount of traps dynamically for real-time interactive multiple-particle manipulation in all three dimensions. The versatility of this method and its application to different physical condition will be tested, by appropriate magnification adjustment or by changing the size of SLM phase patterns.

Other approaches will use SLMs to display the holograms creating the optical potential energy landscape. A new configuration of the optical path (Fresnel set-up) allows one to move, to scale, or to reshape each optical trap individually. This allows one to create more sophisticated optical tools by modular design.

In developing these new methods (GPC, holographic, Fresnel) computer algorithms are needed to determine the final pattern in the image plane. While the application of computer algorithms for the real time calculation of hologram patterns for forming multiple optical traps within the tweezers have been already obtained, further development of the software capabilities will be achieved to obtain a standard interface to optical tweezers driven by the positions of the operators fingertips.

In addition to arrays of traps, holographic technology assists in generating unusual transverse laser modes. The above mentioned algorithms allow one not only to create arrays of traps but also to impose unusual wave fronts of phase on the light, generating non-Gaussian modes. The Laguerre-Gaussian (LG) light beam is one such example of an unusual propagating light mode of interest that can be generated in this way. The peculiar phase distribution of these propagation modes results in an orbital angular momentum of the light field in addition to the spin angular momentum associated with the polarization state of the photon. The mechanical manifestation of such beams in optical traps should be the transfer of both spin angular momentum and orbital angular momentum by a variety of mechanisms, including scattering and absorption. Different projects involving the use of these types of light beams in optical tweezers will be carried out.

- 2) The use of evanescent waves is an important and emergent theme in micromanipulation that exploits the use of near-field optics. It has the potential for creating very small trapping volumes as well as very large area organisation. Evanescent waves penetrate only a small distance into the medium and thus move particles at the surface, leaving the bulk of the sample undisturbed. As near-field optics are not constrained by the diffraction limit, they also offer the potential for creating optical trapping volumes that are significantly smaller than those achieved in conventional optical tweezers. The methodology is also compatible with total internal reflection microscopy. Optical trapping, guiding and even sorting has been demonstrated in such a system using evanescent waves. Many near-field geometries will be under investigation that will be used to enhance the effects of evanescent waves for optical trapping.

Most of the trapping and manipulation experiments using laser tweezers were done in an isotropic fluid environment. The important new features of laser trapping in birefringent media such as liquid crystals have been recently shown: for example, optical trapping allowed researches to study both dipolar and quadrupolar colloidal interactions of particles immersed in a nematic LC. In fact, the presence of inclusions modify locally the average orientation of LC molecules and gives rise to many unique and novel structural forces not observable in ordinary fluids. Being of anisotropic origin, these long-range structural forces are responsible for some fascinating self-assembled colloidal structures, such as 1D chains of particles, 2D hexagonal arrangement of colloids or regular arrays of defects. The unique combination of long-ranged structural forces in liquid crystals and their extraordinary electro-optical properties is therefore a promising direction towards complex liquid crystal self-assembled structures that could provide new means of controlling and manipulating light in photonic devices. Interparticle forces have been measured in nematics and have been exploited to artificially construct multiparticle arrangements. Recently it has been shown that, contrary to intuition, small colloidal particles are efficiently trapped into the laser focus in a birefringent liquid crystal, although their index of refraction is lower than both refractive indices of liquid crystal. These preliminary experiments clearly indicate new aspects of laser trapping in birefringent long-range ordered and soft complex media which will be extensively studied under the frame of this Action. Moreover two features of LC are attractive to be investigated for optical manipulation: collective reorientation and the possible “colossal” nonlinear optical response which could be obtained. These effects could be exploited to reduce the light intensity necessary for optical trapping.

In this way it is also possible to realize molecular-scale motors that can perform work and collectively induce controlled motion of much larger objects. Preliminary experiments have shown that light-driven molecular motors embedded in a liquid-crystal film can rotate objects placed on the film that exceed the size of the motor molecule by a factor of 10,000. On the other hand it is possible the design of polymeric compounds including photo-

isomerisable moieties in order to obtain macroscopic films whose orientation is controlled by linearly polarized light. A more intense investigation of these topics is foreseen and can open new tools for optical manipulation.

Some projects will address the issue of developing apparatus using optical fibers to carry the light to the object to be trapped to overcome the limitations presented by the conventional optical-tweezers set-up consisting of a laser beam entering a suitably modified microscope having a high Numerical Aperture (N.A.) objective. The fibre-optical approach allows increasing the range of situations where trapping of biological micro-particles is possible, because it helps removing the constraints given by the presence of turbid media or thick samples. Moreover, by exploiting the fact that the trapping apparatus is physically decoupled from the imaging one a conventional optical microscope with lower magnification can be used thus allowing a larger field of view, and a greater freedom in viewing and manipulating samples. The demonstrations given recently of trapping by fiber-tweezers had strong limitations due to critical alignment or to a not all-optical approach. New projects using fibers with a multi-core structure and appropriately microstructured end-face will be carried out.

D.2. Micropatterning and Microfluidics

The main objectives of this activity will be:

- *realization of nano and micro-structures by photo-patterning of polymeric materials*
- *investigation on microfluidics*

Several projects will concern light patterning of materials exploiting optical manipulation methods to:

- 1) fabricate new materials with tailored physicochemical functionalisation and to realize new complex three dimensional structures;
- 2) fabricate optically activated micro-mechanical devices and opto-electronic devices;
- 3) study micro-rheology, optical control and measurements in microfluidics, optical sorting of micro-particles

Many different issues will be addressed and are summarized below.

- 1) The irradiation of matter with relatively intense light beams is producing some novel patterning effects in polymer and polymer-composite solutes. Field gradients lead to the formation of relatively solid structures of micron, and potentially submicron, structures owing to some, yet not fully understood, collective behaviours. Higher light intensities result in permanent manipulation and formation of structures by means of photochemical or other effects. In this category micro and nano-patterning of materials will be realised by means of laser-etched or laser-printed structures not yet available by other microfabrication methods. The initial mixtures can also include nanosize dopants undergoing spatial redistribution under illumination and determining the final material properties. Such redistribution takes place in the volume and on the surface of the recording material. On the other hand this optical redistribution can provide an alternative tool for simultaneous manipulation of large numbers of nanoparticles. In this way it is possible to build complex 3D structures by photopolymerisation tailoring both mechanical and optical properties.

Of particular interest in this context will be “optical poling” methods whereby interference of co-resonant multi-photon absorption pathways are made to take place in photo-

isomerizable structures (such as azo-dyes or other candidate structures allowing for some structural modifications that are accessible from an excited state). This approach allows to tailor at will the structure of such media up to higher tensorial order parameters, by playing on the relative phases and polarizations of the incoming beams. In short pulse experiments, this approach belongs to the general realm of quantum coherent control techniques whereby the engineering of short optical pulses allows to steer photochemical reactions or photomechanical behaviour towards desired direction. In spite of its potential, this approach has been so far mainly considered with pure physical properties end-goals and has been poorly exploited at the physics-biology interface, so as to provide new optical tweezing techniques allowing to guide by light functionalized nanoparticles in cell environments.

- 2) These complex structures can form microfluidic channels, optical waveguides, moving particles, even micromechanical devices comprising static and moving components. This gives the possibility of building micromechanical devices for the investigation-manipulation of microscopic objects to be used in biological research as well as in biotechnology. These structures can also be part of high sensitivity interferometric optical sensors or guided-wave opto-electronic devices. Moreover microfluidics systems (pumps, valves, high resolution sorting devices) containing components of all of the above can be realized.
- 3) Microrheology is the study of viscoelastic properties of fluids on the mesoscopic scale, i.e., at an intermediate scale between molecular and macroscopic scale. This field is an emerging area of physics even for the many industrial applications where complex fluids are involved: polymeric solutions, colloids and biological materials. The basic idea of microrheology is to employ microprobes to explore the visco-elastic response of the medium where they are immersed. This local approach offer, with respect to the conventional rheometers, the advantage to investigate heterogeneity of the medium: different sizes of the probe allow to “seeing” the network of the medium at different length scales compared with the mesh size. Therefore optical manipulation of micro-particles is providing the basic tool for these investigations.

Beside visco-elastic measurements many other studies can be performed concerning hydrodynamics, statistical mechanics, non-equilibrium thermodynamics.

D.3. Imaging and Biological Applications

The main objectives of this activity will be:

- *implementation of non invasive optical tools for manipulation and study of biological systems*
- *development of new methods coupling optical manipulation tools to nonlinear optical microscopy and spectroscopy for biological systems*

Optical tweezers have been used to trap several biological materials, including virus, bacteria, cells, and red blood cells. Biological macromolecules have also been studied. However it is now necessary to make final steps to merge optical manipulation with biology, since some nice methods have been developed up to now, but there have been too many latex beads and too few cells in the traps. The variety of possible projects related to biological applications is difficult to include in a restricted number of categories, therefore we will mention some of the envisaged main activities.

- 1) Use of optical manipulation as a non-invasive tool for manipulating cells into spatial configurations that can trigger variations in developmental features. By characterizing biophysical interaction phenomena, including confinement stress, in micro-organisms, microbiologists can better understand microbial cell-growth regulation in bio-films in

medical, environmental, or industrial settings, or, for another example, pro-biotic and pathogenic communities in the gastrointestinal tract. Researchers can investigate the phenomenon of confinement stress in bacteria, yeasts, and molds. Moreover, where confinement stress does play a role in microbial cell-growth regulation, the physiological mechanisms underlying this phenomenon can be studied. A reliable 3D optical micro-manipulation offers a unique tool for studying confinement stress in micro-organisms, because it allows for the arrangement of several particular cells at well-defined 3D distances from each other at well-defined time.

- 2) Structural studies and interactions of biological macromolecules with themselves and with interesting biological targets. Optical tweezers are useful in molecular and cell biology because several important forces are in an accessible piconewton (pN) range: e.g., ligand-receptor binding, DNA stretching, protein unfolding, and molecular motor stall forces. In experiments on the stretching of biological molecules using optical manipulation, one end of a single filament is tethered at the coverslip surface while the other one is attached at a polystyrene bead. If the position sensor is calibrated the force spectrum of the single filament is obtained. One project will extend this technique to the study of conformational changes of synthetic oligonucleotides capable of organizing themselves into secondary structures of DNA with double helix (duplex), triple (triplex) and quadruple (quadruplex).
- 3) The combining of multiple optical tweezers with any micro-spectroscopic technique presents several physical challenges that still need their solutions. In fact, an optical beam used for micro-spectroscopy also exerts radiation forces on a living cells trapped and moved near this analyzing beam by the multi-beam optical tweezers. The optical properties of the trapped cell are not homogeneous and, hence, this additional radiation force perturbs the movement of the studied cell also non-homogeneously. This obviously reduces the spatial resolution of the whole system and makes final images ambiguous. Therefore an ultimate goal is to develop a multi-beam optical trap on the basis of digital holograms that permits to translate a studied living cell in such a way that the presence of the analyzing beam produces minimal effects on acquired spectroscopic data.
- 4) Another step in developing the combination of optical trapping with nonlinear spectroscopic techniques is to obtain 3D images of a single particle (e.g. a living cell in suspension) by nonlinear microscopy techniques such as Second or Third Harmonic Generation (SHG, THG), Two Photon Fluorescence (TPF), Coherent Raman Spectroscopy (CARS), or Raman Spectroscopy. Even though the general requirements for digital holograms that permit one to move an optically trapped micro-object in the 3D space are known, their application to such optically inhomogeneous object as a living cell is not straightforward. This approach is quite different with respect to a standard investigation of an ensemble of particles since it allows observing the response of a single particle. So far, it becomes possible to face many different problems, also related to biological and medical field. For instance, it becomes possible to investigate the interaction of fine particulate produced in combustion processes with single living human cells. Such kind of investigation finds important motivation from the result of epidemiological studies, which have demonstrated the toxic effect of nanoparticles on the human health. Additionally image transport phenomena in living cells can be investigated which allows functional imaging without the need to scan the probe. For instance with this technique one can take snapshots of distributions of targeted Raman-active substances within samples such as lipid-filled vesicles.

E. ORGANISATION

In the **kick-off meeting**, after fulfilling the requirements established in the “Rules and Procedures” (first of all election of Chairperson), the Management Committee (MC) of the Action will address the basic issues for the Action organisation concerning:

- 1) selection of projects to be accepted as activities of the Action
- 2) establishment of Working Groups (WG) and sorting projects accordingly
- 3) election of coordinators of each WG
- 4) introducing collaboration and communication tools
- 5) election of a responsible for Short Term Scientific Missions (STSM) programme

Initially the Action will be organized in three Working Groups (WG):

- WG1 - Methods of Optical Micro-Manipulation
- WG2 - Micro-patterning and Microfluidics
- WG3 - Imaging and Biological Applications

This scheme will be checked after the first year and could be modified according to the actual numerical participation in each WG and the new participants joining the Action. All the participating groups will be invited to provide basic information on their research projects (title, abstract, preference on WG, scientists involved, etc.) and a list of projects for each WG will be prepared.

As basic communication tool among the partners and between the Action and other parties a website will be planned and set-up shortly after the kick-off meeting. The MC will elect a responsible for the website management. It will include a “static” section concerning: (i) general information on the Action programme and objectives; (ii) basic information on participating groups with a link to the group websites; (iii) information and forms to be used for STSM applications; (iv) information for scientists willing to join the Action. A “dynamic” section will provide information on: (i) up-dated timetable of the Action activities (planned workshops, meeting, etc.); (ii) recent scientific and technical achievements and related links; (iii) filed reporting of past activities and achievements. The task to keep the website up to date can be given to PhD students involved in the projects of the Action as a temporarily limited job on a rotating basis.

In the **regular annual meeting of MC** a plan for the co-ordination activity will be approved for the coming year consisting of: organisation of the annual general meeting, proposals for STSM, proposal of WG workshops on specific topics. The organization of workshops and general meetings in conjunction with other international topical meetings will be encouraged. Planning of the activity of the year will be based on the available budget and considering as milestone the organisation of the General Meeting. The committee programme of the General Meeting will include the local organiser, the Action Chair and Vice-Chair and the WG Co-ordinators. According to the available budget, before each meeting the maximum number of participants in charge of the Action will be determined and the National representatives in the MC will communicate to the local organiser and to the Action Chair the names of scientists to be reimbursed.

If necessary, additional extraordinary MC meetings will be organised. However, as far as possible electronic communication will be used among its members. It is foreseen that such a procedure will be necessary for approval of a number of STSM in case of available budget to be used within a restricted time period. Electronic communication will be also used within the MC for preparation of the activity reports which will be delivered at end of each year.

Attention will be paid to improve the collaborations by encouraging the formation of consortia able to prepare proposals under specific calls launched in the frame of the forthcoming VII FP of the European Commission or launched by other national and international agencies. To this aim information on new calls of possible interest for the Action activities will be given through the website and possible invitation to participate in a project proposal will circulate.

A plan will be made for the organisation of training schools, with the aim of organising two schools during the Action life. The schools should be open for company members and provide basic information on optical manipulation methods, devices and applications. For PhD students the schools will be implemented through the STSM programme: a number of missions will be planned as final stages after the school in laboratories participating in the Action.

Liaisons and interactions with other international research programmes as well as with company members will be pursued by inviting international guests to each meeting to present work from other programmes.

In case of other running COST Actions whose objectives would be complementary to the ones of the present Action, effort will be made in agreement with the corresponding MC to organise common workshops able to underline the possible area of multi-disciplinary collaborations.

Provision will be made for gender balance in the election of the Chairpersons (Chair and Vice-Chair), of the coordinators of WG and of any special committees formed to address specific issues (e.g. for standardisation measurements of specific equipment).

The involvement of young people will be a basic characteristic of the Action and will be implemented through the STSM programme essentially devoted to exchange of young PhD students or Post-Doc between the participating laboratories and through the organisation of training schools.

F. TIMETABLE

The Action will run for a total of four years.

The timetable includes the Kick-off meeting at the beginning and one General Meeting of the Action at the end of each year. The General Meeting at the end of the second and of the fourth year can be organised in conjunction with an International Topical Meeting in Optical Manipulation and related subjects. During each year it is foreseen to organize small workshops of each Working Group, as required by the participants. Starting from the second year effort will be made to organize an international school devoted to PhD students and young researchers and open to company members. The School will be also implemented through the STSM programme. At this preliminary stage the organisation of two schools is envisaged.

The Action will start with a Kick-off meeting with presentation of the partners and fixing of the organisation issues. Regular MC Meetings will be organised at the end of each General Meeting. The actual holding of one General Meeting at the end of each year is considered a Milestone of the Action.

A summary of the provisional timetable is presented in the table below.

<i>YEAR</i>	<i>month</i>	<i>Kick-off meeting</i>	
1	3	WP Workshops	Milestone # 1
	6		
	9	General Meeting + MC	
	12		
2	3	WP Workshops Intern. School	Milestone # 2
	6		
	9	General Meeting (* + MC	
	12		
3	3	WP Workshops	Milestone # 3
	6		
	9	General Meeting + MC	
	12		
4	3	WP Workshops Intern. School	Milestone # 4
	6		
	9	General Meeting (* + MC	
	12		

(*) possibly in conjunction with an International Topical Meeting

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: **Austria, Czech Republic, Denmark, France, Germany, Greece, Hungary, Ireland, Italy, Slovenia, Spain, Sweden, 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 **20 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

In general the dissemination will have two aspects: internal and external. The internal dissemination is quite obvious and will be realised through the regular activities of the Action (meetings, workshops, etc.) and the up-dating of the website that will include news, updated timetable of the activities and an area restricted to the Action partners with “work in progress” documents.

However, it is important to underline in this section the external dissemination plan that will address the following main bodies:

- academic or research institutions
- industrial companies
- young researchers

The dissemination plan of the Action will consider the broad target audience interested in its scientific and technical achievements. In fact beside other researchers in the field, the results of the ongoing investigations will be of interest for researchers working in areas related to it both from basic and applicative point of views such as matter physics, photonics, photo-chemistry, biology, biotechnology. The industrial target can be quite broad as well. The main industrial target is related to nano-biotechnology and can include companies involved in developing apparatus such as imaging and manipulation systems, biosensors and microfluidics devices. Micropatterning techniques can also find an interested audience in companies developing photonic devices for different applications such as sensors for environmental monitoring or devices for optical processing and storage.

Due to this broad target the MC will prepare and periodically update a list of research institutions and companies potentially interested in the Action activities keeping them informed by e-mail on those activities, inviting people to participate in the meetings and advertising news appearing on the Action website.

Several “conventional” means of disseminations will be used such as: publications in peer reviewed journals, presentation at international conferences and workshops. Besides that, the publication of special issues of scientific journals as proceedings of Action meetings is foreseen. Possibly special workshops could be devoted to present and discuss with company members recent achievements in order to develop further research activities addressing solutions of specific industrial needs.

An important role will be played by the website that will contain final reports on the activities and achievements of the Action or specific topics addressed by special initiatives. All this material will be available and open to everybody.

Dissemination will be also realised through national and international scientific societies, not only for advertisement of coming events, but possibly to exploit society bulletins or newsletters for reporting the latest achievements with the aim to reach a broader audience.

The participants in this Action will be encouraged, whenever possible, to reach public media (local newspaper, TV , Radio) in order to explain to the public at large the results that can have the strongest impact on everyday life or can be of general interest (in particular concerning biomedical applications).

Finally a very important part of the dissemination activity will be devoted to young researchers. To this aim a plan will be made to organise training schools. At a preliminary stage the organisation of two schools is envisaged during the Action life. They will deal both basic and applicative topics providing information on optical manipulation methods, devices and applications. They will be open to company members and in order to address properly the industries needs, the programme of the school will be discussed and decided in agreement with representatives of companies interested in sending members of their staff as students. On the other hand, for students coming from academic institutions, the programme of the school shall be completed by a short visit to one of the participating laboratories. This visit will be supported through the STSM programme.

As a matter of fact, beside the school, most of this programme will be devoted to support training stages of PhD student or young Post-Docs in the laboratories participating in the Action