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NFFA

Nanoscience Foundries and Fine Analysis

D3.5

Design Study of Nano-Manipulation Facility

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Deliverable D3.6: Design Study of NFFA Nano-Bio labs

1. INTRODUCTION

1.1. Purpose of the document

The purpose of this document is to describe the concept for a NFFA Nano-Bio lab for synthesis and research on combined organic/inorganic nanoscale systems and its integration in a NFFA-RI.

1.2. Application Area

The targets of this document are the members of the NFFA Project, the EC Project Officers, and the general public.

1.3. References

Description of Work (DoW). See at web site:
http://www.nffa.eu/ResearchActivityData.aspx?IdRACT=14&idTypeRACT=1

The design study is based on information shared by NFFA partner facilities and during discussions with representatives from several other institutions in the frame of workshops: 1st NFFA Workshop Jun.09, Co-Nanomet Workshop. Sept.09, SP&AC Workshop. Dec.09). Valuable contributions were also collected during visits at the Molecular Foundry, Berkley and the MPI for Colloids and Interfaces, Potsdam.

1.3.1. Objective of Work Package 3

The Objective of WP3 is the design study of NFFA-RI centres, the technical layout of instrumentation and tools.

1.3.2. Description of work broken down into tasks

The following tasks are defined in WP3:

- T3.1) Design study of the overall infrastructure
- T3.2) Design study of a nanolithography station within the facility
- T3.3) Design study of user-oriented material growth facilities
- T3.4) Design study of user-oriented metrology facilities
- T3.5) Design study of a molecule and nano-particle manipulation lab
- T3.6) Design study of nano-bio labs
- T3.7) Assessment of the possible contribution of existing facilities that could be integrated in NFFA-RI

2. SUMMARY

Nano-manipulation, or positional and/or force control at the nanometer scale, is a key enabling technology for nanotechnology by filling the gap between top-down and bottom-up strategies, and may lead to the appearance of replication-based molecular assemblers [1]. These types of assemblers have been proposed as general purpose manufacturing devices for building a wide range of useful products as well as copies of themselves (self-replication). Presently, nanomanipulation can be applied to the scientific exploration of mesoscopic physical phenomena, biology, and the construction of prototype nanodevices. A growing interest is reserved to bio-materials and bio-nanotechnology to solve these problems specifically for experiments associated to LSFs (Large Scale Facilities). The linking of the macroscopic world to the nano-world of single molecules, nanoparticles and functional nanostructures in devices which match the LSFs beam lines constraints (e.g. microbeam, limited space and time) represents a technological challenge.

As will be outlined in the following, various manipulation techniques have to be made accessible to researchers involved in fine analysis at LSFs. The goal of this task is to shortly review the most significant manipulation techniques and design a manipulation facility distributed into three laboratories, with a special regard to contact-less manipulation techniques. The design is developed starting from the motivation/importance of the MTs for fine analysis at LSFs and from the relevant technical criteria established in section 2. We also consider the synergy with the other Tasks of WP3, especially with Tasks 3.2 (lithography), 3.4 (metrology) and 3.6 (biology).

3. MOTIVATION

The new generation of synchrotron sources can emit X-ray beams with very high brilliance and coherence. On the other hand, also the detectors have evolved toward more and more sensitive detectors (e.g. Pixel detectors such as the PILATUS detector [2]). New fine analysis can thus be carried out on smaller and smaller samples. Therefore, new manipulation techniques, adapted to the micro and nano scale of the samples, will be highly demanded.

Nanomanipulation was enabled by the inventions of the Scanning Tunneling Microscopy (STM) [3], Atomic Force Microscopy AFMs [4], and other types of Scanning Probe Microscopies SPMs. NanoRobotic Manipulators (NRMs), characterized by the capability of 3-D positioning, orientation control, independently actuated multiple end-effectors, and independent real time observation systems, can be integrated with SPMs and largely extend the complexity of nanomanipulation [5,6]. Besides these, Optical Tweezers OT [7], Magnetic Tweezers MTw [8] and electrical methodologies (Electro and DiElectroPhoresis EP & DEP) [9, 10, 11] represent also valuable nanomanipulaton techniques with potential applications for fine analysis experiments in LSFs.

3.1 Sample manipulation for protein micro- and nano- crystallography

Considerable efforts are made by LSFs worldwide to develop high-throughput protein crystallography for structural proteomics. [12] The production of three-dimensional crystallographic structural information of macromolecules can now be thought of as a pipeline which is being streamlined at every stage from protein cloning, expression and purification, through crystallisation to data collection and structure solution. Synchrotron X-ray beamlines are a key section of this pipeline as it is at these that the X-ray diffraction data , that ultimately leads to the elucidation of macromolecular structures are collected. The burgeoning number of macromolecular crystallography (MX) beamlines available worldwide may be enhanced significantly with the automation of both their operation and of the experiments carried out on them. Protein microcrystallography (μPX) [13, 14] represents a complementary approach for studying difficult protein

structures, i.e. proteins which are difficult to crystallize such as membrane proteins. Protein nanocrystallography can be defined as: "*using nanotechnology for the production and characterization of protein crystals at the nano- and subnano-scale*" *(AFM, µPX)* [15]. The extent to which protein crystallography with nanometer-sized beams (nanoPX) will find practical applications will depend not only on the scientific interest in studying ultrasmall crystalline domains but also on systematic studies of radiation damage issues, the availability of advanced sample environments including high precision goniometers, sample characterization and manipulation tools. Pushing the limits to smaller crystals and smaller beam sizes will require the integration of more and more nanotechnology in a µPX beamline and annex laboratories, which justifies the term nanocrystallography.

A µPX goniometer developed by an EMBL/ESRF collaboration incorporates several advanced features (Fig. 1 T3-5(A)). Sample observation and alignment is facilitated by an on-axis zoom-microscope. The minimum beam size is defined by the integrated aperture. [16] A 1*1 μm^2 beam from a KB-mirror system is currently in routine use for µPX at the ESRF-ID13 BL [17]. The optical system is adapted to a scanning µPX goniometer based on a vertical air bearing rotation axis and an x/y/z scanning unit. [17,18] The use of a compact piezo micromanipulator allows keeping the sample in a 1 µm diameter circle of confusion through 360^0 rotation (Fig. 1 T3-5 (B)). This prototype has evolved into a commercial system (Maatel company, France) [19] with an integrated sample changer for automatic loading of pre-frozen samples and is installed at several SR beamlines such as ESRF-ID23-2. An advanced nanogoniometer has been also developed to make use of nanometer sized beams at the ESRF ID13 BL [20] This nanogoniometer serves as a test bed for the development of techniques for transfer, alignment and data collection of ultrasmall protein and biopolymer crystals. It is based on air bearing x/y stages developed for microlithography with laser interferometer position encoders, which are integrated in a marble support structure (Newport/Microcontrole). A vertical air bearing stage (MICOS) provides sub µm eccentricity at the sample position. A microhexapod system is foreseen for sample alignment and x/y/z scanning with sub-10 nm resolution. There are stringent conditions for temperature stability (< ±0.1 ^{0}C) and low vibration level, and therefore a special hutch layout is requested.

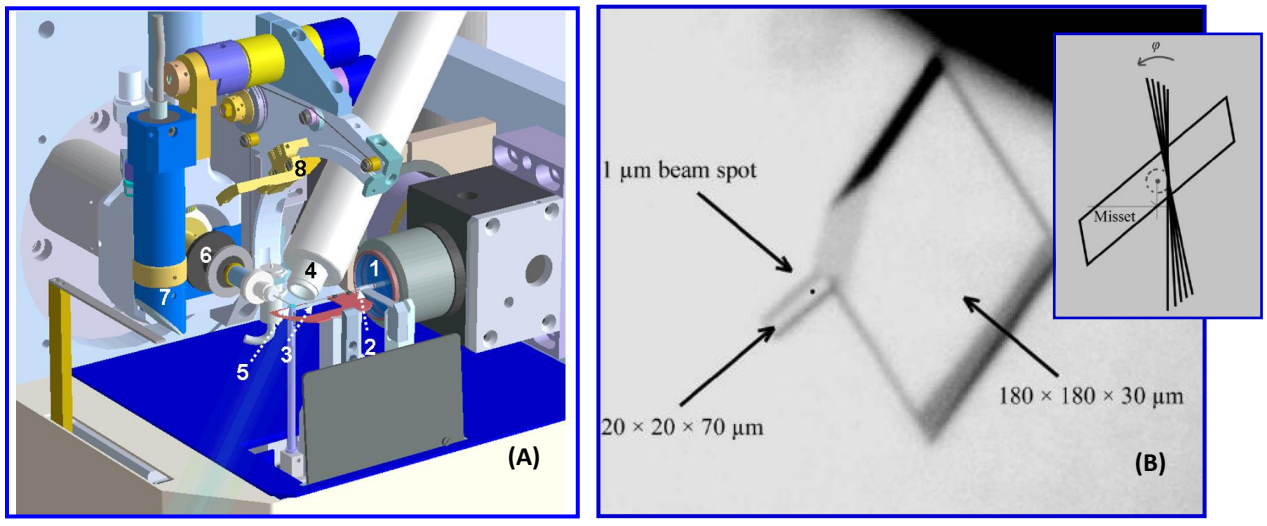


Figure 1 T3-5. (A) Schematic design of µPX goniometer µm beams ; 1: pierced zoom objective; 2: beam defining aperture; 3: guard aperture; 4: cryoflow nozzle; 5: scintillator and photodiode; 6: horizontal rotation axis; 7: illumination; 8: sample transfer system. **(B)** The use of a compact piezo micromanipulator allows keeping the sample in a 1 µm diameter circle of confusion through 360º rotation. Large and small Xylanase II crystals / 1 µm beam spot. Inset: strategy for data acquisition.

3.2 X-ray probing of laser oriented molecules

Ultrafast X-ray imaging will enable to connect the current results, obtained from spectroscopy, on the dynamics of molecular and condensed matter systems with real space motions. [21] Timeresolved X-ray crystallography, which is currently bringing new insights into the function of proteins, will be hugely extended with XFEL (X-ray Free Electron Laser pulses). The extreme intensity of the pulses will allow shrinking crystal sizes down to single molecules, giving three - dimensional movies of conformational dynamics and chemical reactions, and allowing the imaging of macromolecules that cannot be easily crystallized [22]. However, particles such as single macromolecules are very weak X-ray scatterers and therefore a very large number of particles is required to get an useful signal for image reconstruction, even if the number of photons per pulse is very high (10^{12} photons / 100 fs pulse duration / 100 nm spot). Controlling the orientation of the particles with respect to each other during the measurement until enough signal is accumulated and then changing the orientation to get a tomographic series represents a solution to this problem. The orientation of such particles can be controlled using intense laser pulses at [23]. A nonresonant, linearly polarized laser field will align a molecule by interaction with the molecule's anisotropic polarizability; the most polarizable axis within the molecule will align parallel to the laser polarization axis. [24] A manipulation setup to create an interaction volume with a large number of molecules (more than100 bromotrifluoromethane CF_3Br molecules) overlapping, spatially and temporally, with the laser and x-ray beams has been recently demonstrated. [25] (Fig. 2 T3-5)

The X-ray probe of laser-aligned molecules is shown in two experiments. First, the resonant X-ray absorption has been shown to change reversibly in the presence of the laser field by measuring a laser/X-ray cross correlation . Second, the control of X-ray absorption on the Br $1s \rightarrow \sigma^*$ resonance by rotating the alignment of CF_3Br molecules with respect to the X-ray polarization axis has been demonstrated.

Another laser-based method has been proposed recently to control the rotations of asymmetric top molecules in three-dimensional space [26]. The method relies on keeping one axis of a molecule essentially fixed in space along the polarization vector of a nanosecond laser pulse (termed the long pulse) and forcing the molecule to rotate about the aligned axis by an orthogonally polarized, femtosecond laser pulse (termed the short pulse). Strong 3D alignment is observed a few picoseconds after the short pulse and is repeated periodically, reflecting directly the revolution of the molecular plane about the aligned axis.

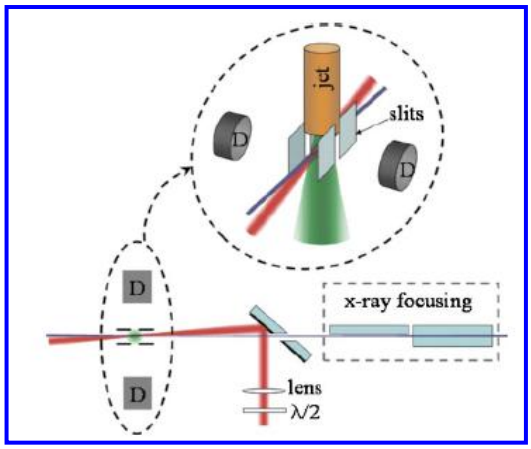


Figure 2 T3-5 (from [25])Schematic of experimental setup, top view. Inset shows the projection of interaction region: jet, collimation slit geometry, and Si drift detectors D. A Ti:sapphire laser (red), 800 nm wavelength, 95 ps pulses at peak intensity of 10^{12} W/cm^2 focused to 40 μm is used for molecule alignment.

3.3 Scanning Probe Microscopy and Synchrotron Radiation

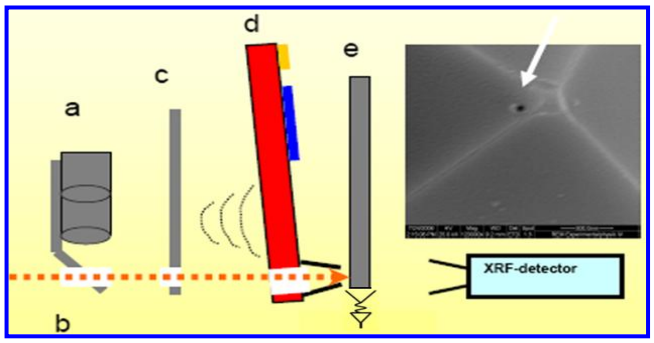
Scanning Probe Microscopes (SPM) are extensively used in nanotechnology to see and manipulate samples of nanometric size, but their application in real time under X-ray beams is just beginning. At the

Photon Factory, KEK, Japan, Dr. T. Okuda (University of Tokyo) and his colleagues have developed a new technique for determining the identity of groups of individual atoms [27]. In order to upgrade the STM by giving it the capability to distinguish chemical species, the research group employed synchrotron X-rays, which excite core-level electrons in the sample's atoms. In this way, secondary electrons can be detected by the STM as they tunnel across the gap. The important point here is that the tunneling current depends on the chemical species. Accordingly, the technique provides chemical imaging. The current spatial resolution is around 10 nm. In the present research, Fe and Ni L absorption edges were chosen to control the core-level excitation. [28].

Another recent achievement is the in situ AFM (Atomic Force Microscopy) development at the ESRF Grenoble (ID01) [29] . The main goal of using an AFM for in-situ measurements is the ability to interact with the sample while probing it with the X-ray beam. One possible interaction is the application of a pressure onto the sample. This can be done employing the AFM tip as an indentation device. A hollow-tip AFM has been implemented recently at the Synchrotron Soleil [30] for simultaneous study of the topological and chemical information on a nanoscale (Fig. 3 T3-5).

Figure 3 T3-5
Concept of hollow tip AFM combined with XRF

The SR beam enters from the left (red dot line),
a: microscope, b: pierced mirror
c: moveable aperture (xy), d: HOT-AFM cantilever
e: sample, f: electron or photon-detector;
inset: image of pierced tip with 50-nm hole, see arrow.
(adapted from ref [30])



4. RELEVANT TECHNICAL CRITERIA

In order to identify the optimum set of infrastructure for manipulation in context of the proposed NFFA Centers we identify the following technical criteria to judge the relevance of the various manipulation methods and tools.

4.1. Materials and environment

The type of material and the environment mainly dictate the manipulation technique/strategy. Dielectric, metallic, magnetic, biological samples are considered in different environmental conditions: fluid, gas, or vacuum. Contact and contact-less manipulation techniques should be considered according to a series of criteria as: sample fragility, required resolution, freedom degrees of the manipulation, robustness. Since the sample are very small and delicate (at least the biological samples), contact-less manipulation is more preferred.

4.2. Spatial resolution

The spatial resolution of the sample manipulation is given by the resolution of the imaging / observation system. The sample can be micro or nano sized but nanometric and sub-nanometric spatial resolution is considerd for its manipulation. This might be achieved using imaging components already used for manipulation or additional specialized components. For instance, the resolution of a normal optical tweezers system is limited to the resolution of optical microscopy but it can be increased to nanometric resolution using interferometric techniques.

4.3. Freedom degrees

Different manipulation techniques can provide complete or limited freedom degrees. Usually, more freedom degrees means also lower spatial resolution. A trade-off should be found for each application but systems which allow high spatial resolution for more freedom degrees are desired for the NFFA centers.

A map of the criteria introduce above and their relationship is shown in Fig. 4 T3-5.

4.4. Flexibility

The large variety of nanomanipulation applications require techniques, that allow for precise and complex sample manipulation for a large variety of materials and environments. Contact-less manipulation methods are more flexible than contact techniques, allowing usually complementary investigations as force measurements, which highly increase the information about the sample and its interaction with the environment.

4.5. Robustness

The success rate of a process step in nanomanipulation will be crucial for users of NFFA manipulation stations, as they need a high degree of reliability to get samples prepared in time for experiments at LSFs.

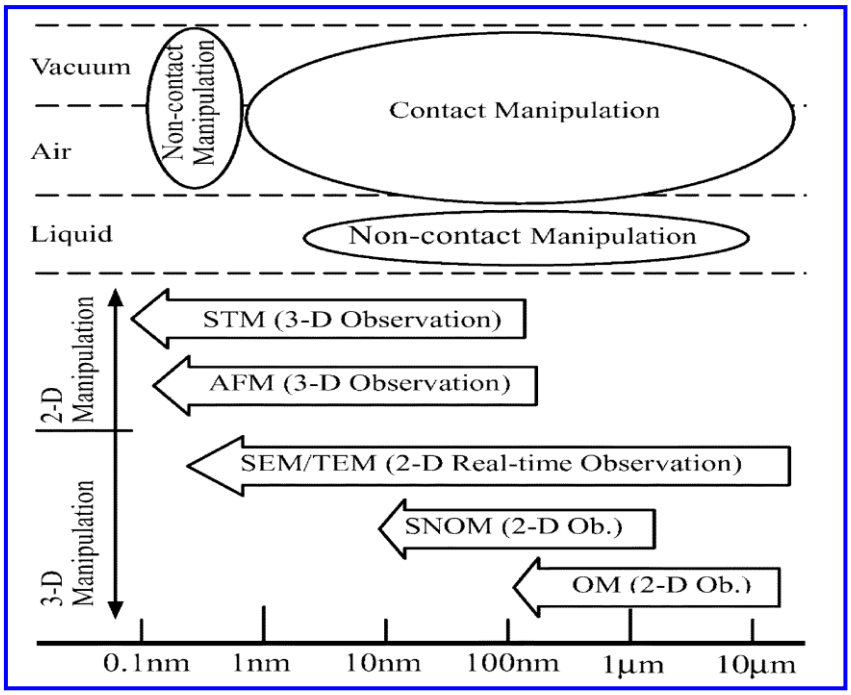


Figure 4 T3-5 Imaging/Observation and Manipulation.

Strategies for nanomanipulation can be broadly classified into three types: 1) lateral noncontact; 2) lateral contact; and 3) vertical manipulations. Generally, lateral noncontact nanomanipulation is mainly applied for atoms and molecules in UHV with an STM or bioobjects in liquid with optical tweezers or magnetic tweezers, whereas contact nanomanipulation can be used in almost any environment mainly with an AFM but hardly for atomic manipulations, and vertical process is adopted by NRMs.

4.6. Manipulation speed

Manipulation speed depends on the complexity of the manipulation (number of freedom degrees, complementary force measurements) required by the experiment. For instance, very high manipulation speed is required and can be achieved for molecule alignment in an XFEL diffraction experiment, while a low speed is required to get the rotation of the sample in a protein crystallography experiment. However, an optimization of the manipulation speed is desired for each technique. Sample to be manipulated is usually not bigger than the beam size at a LSF (i.e. up to a few square millimeters).

4.7. Protocols and data-standards

In order to guarantee a high degree of reproducibility and compatibility between the manipulation stations of different NFFA Centers, it is essential to establish a set of standards, especially regarding the manipulation, imaging, force measurements protocols, and CAD data. This will enable users to transfer their results between manipulation stations, to take advantage of the data repository, and to add experiences into the data repository (see WP-4).

4.8. Mode of operation and access

Manipulation techniques are diverse and rather complex equipment is involved. A high level of cautiousness, training, and experience is a prerequisite to reproducibly obtain good results, in reasonable time, and without damage to the equipment. Therefore, the access for users of LSFs to nanomanipulation equipment should be cascaded in three main modes, depending on the degree of cost and robustness: *Open access* to nanomanipulation equipment implies, that the LSF users are enabled to operate the equipment themselves. Expert staff will still be required for proper maintenance of the equipment and training of the users. In the case of non-standard, advanced tools it may be advantageous to have dedicated scientific staff doing research on the technique to keep the facility at the cutting edge of technology.

Limited access to the nanomanipulation station equipment implies, that only users with frequent use of the tools over a long time (e.g. PhD students or researchers permanently located at the site) are allowed to operate the equipment.

Remote access is the mode of operation for equipment that, due to its high degree of sensitivity and extreme risk of damage by improper use, is only operated by dedicated staff. Users can be involved in the use by providing e.g. samples and exposure data files or masks.

Training of users can be programmed through virtual sessions connected via internet with the manipulation experiments developed in the NFFA centers.

5. DISTRIBUTED MANIPULATION LABORATORY AT NFFA CENTERS: EQUIPMENT AND TECHNIQUES

Based on the technical criteria introduced above, the main manipulation techniques (MTs), classified in contact and contact-less, are discussed in this section. A special regard will be given to contact-less techniques.(5.1- 5.3) which provide more flexibility than the contact techniques (5.4-5.5) in terms of the manipulation freedom degrees and the environment in which they can be implemented. A summary including cost and recommended number of tools in the manipulation stations distributed over the NFFA centers is given in Table 1 T3-5.

5.1 Optical Manipulation

Optical manipulation implies the use of the radiation pressure of light on small samples immersed in liquid, gas or vacuum. Accordingly the technique is called optical tweezers (liquid) or optical levitation (gas or vacuum).

Optical tweezers are arguably the most versatile manipulation technique. It can be used to exert forces in excess of 100 pN on particles ranging in size from nanometers to micrometers while simultaneously measuring the three-dimensional (3D) displacement of the trapped particle with sub-nanometer accuracy and sub-millisecond time resolution. These properties make optical tweezers extremely well suited also for the measurement of force and motion.

An optical trap is created by focusing a laser to a diffraction-limited spot with a high numerical aperture (NA) microscope objective Fig. 5 T3-5 (A) [7].

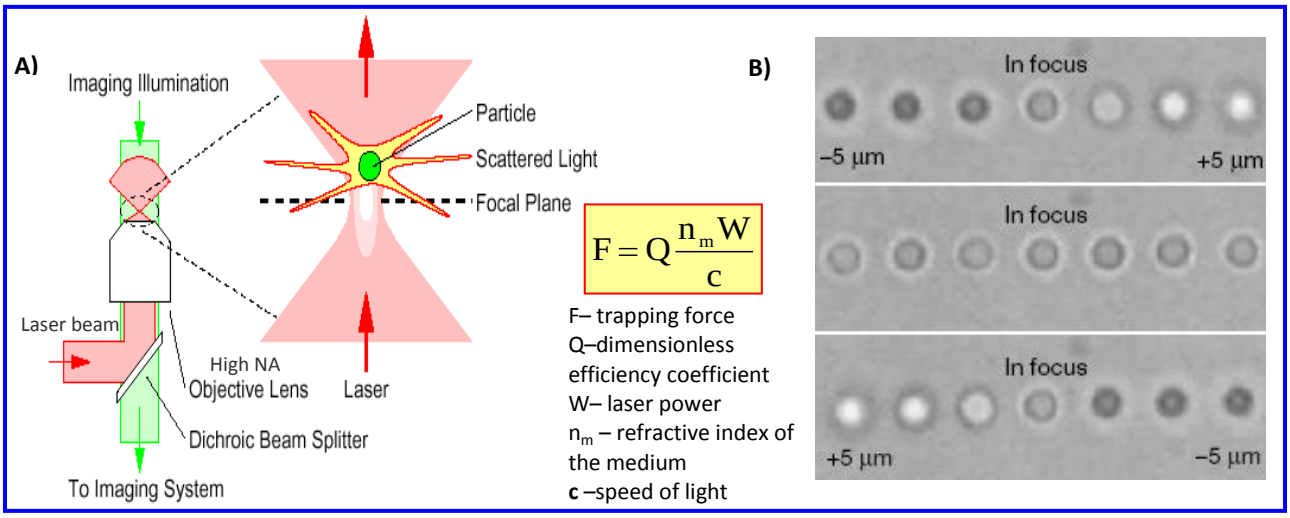


Figure 5 T3-5 (A) Optical tweezers setup: a strongly focused laser beam is directed on the sample cell. Intensity gradients in the converging beam draw small colloidal particles toward the focus, where the gradient force dominates and the particles can be trapped in three dimensions. **(B)** Multiple trapping and manipulation in 3D demonstrated by seven 1 µm diameter silica spheres being moved up and down through seven different planes (from ref [37]).

Dielectric particles in the vicinity of the focus experience a 3D restoring force directed toward the focus. The dielectric particle is polarized by the optical field, and the interaction of this optically induced dipole with the steep gradient near the focus of the laser results in a force directed along the gradient. In addition to the gradient force, there is what has been termed a scattering force directed along the beam propagation direction, which results in a shift of the equilibrium trapping position slightly past the focus. To form a stable trap with optical tweezers, the gradient force along the optical axis must overcome this scattering force, which necessitates the very steep gradient obtained with a high NA objective. For small displacements (~150 nm) of the trapped object from its equilibrium position the force is linearly proportional to the displacement, and the optical trap can be well approximated as a linear spring. The spring constant, or stiffness, depends on the steepness of the optical gradient (how tightly the laser is focused), the laser power and the polarizability of the trapped object. Particles ranging in size from ~20 nm to several micrometers can be stably trapped . These include single cells [32], organelles within cells [33], lipid vesicles [34] and polystyrene or silica microspheres used alone or as probes linked to a molecule of interest [35].

Using different techniques to split the trapping laser beam [36] multiple particles can be trapped and manipulated independently (Fig. W3-5-5 (B)). This could be used for sample delivery and positioning or to trigger chemical reactions between two or more different particles in SR experiments.

A special sample cell, allowing the access of the X-ray beam to the investigated particle, should be considered. A custom optical tweezers microscope using a capillary as sample cell has been recently tested for liposome investigation with SAXS [38] (Fig. W3-5-6 (A)). The concept of multiple trapping is illustrated creating two traps in which liposomes are attracted forming two clusters (Fig. 6 T3-5 (B)).

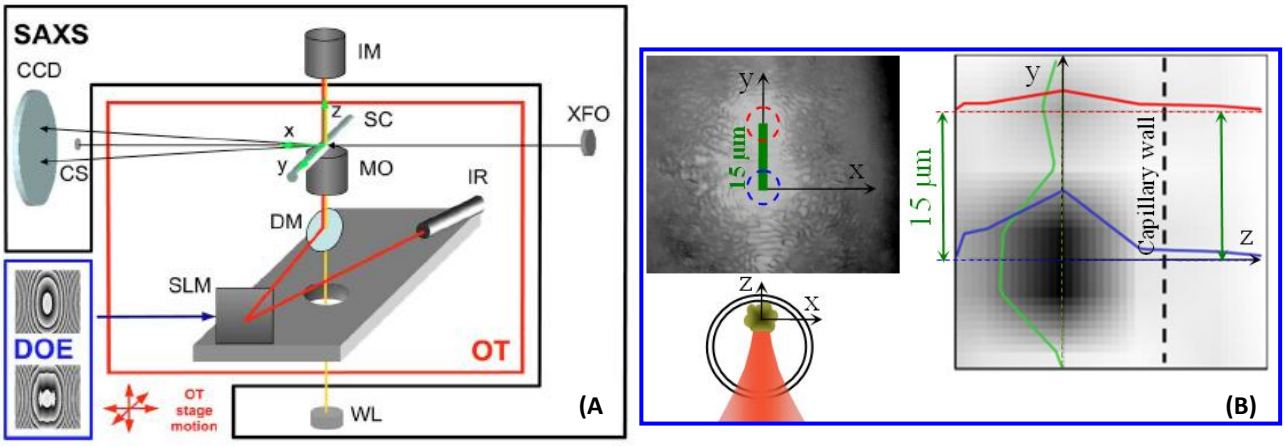


Figure 6 T3-5 (A) Custom OT setup combined with SAXS: the capillary SC, attached to the OT stage (red box), is illuminated by the white light (WL) source and observed by the imaging microscope (IM) lens, which are attached to the SAXS stage (black box). The x-ray beam is microfocused by x-ray focusing optics (XFO) inside the SC and the scattered light is recorded by the CCD. The IR laser beam is directed onto the spatial light modulator (SLM) and its wavefront shaped by diffractive optical elements (DOEs) (blue inset) to control the focus and the splitting of the beam which is then reflected to the microscope objective (MO) by the dichroic mirror (DM). The laser beam is focused by the MO into the SC to form the trap. The OT stage can be moved along the three axes, as indicated by the red arrows. **(B)** Two clusters of liposomes trapped at separate positions, indicated by red respectively blue lines: a microscope image of the traps (infrared laser on) is shown at the top-left corner and the X-ray scanning diffraction image (azimuthaly integrated intensities, mesh 3x5 µm x µm) from the two clusters, with the corresponding intensity profiles, is shown on the right.

Optical levitation allows trapping and manipulation of particles in a medium less viscous than water. Levitation is obtained by balancing the downward force of gravity with the upward force generated by the radiation pressure of a mildly convergent laser beam on a microparticle [39]. Levitation of dielectric solid and hollow particles and of liquid drops in air and levitation of dielectric microparticles in high vaccum have been demonstrated by Ashkin and collaborators [40,41]. Since the laser beam is moderately focused, the working distance for levitation is much higher than that of the optical tweezers. This implies an important advantage: the possibility to monitor easily the particles from a lateral view (Fig. 7 T3-5) or access the sample with another investigation beam, which can be an X-ray beam. To reach a stable levitation stability in high vacuum, a feedback system is required to control the levitating light power. This provides also another important capability, namely automatic force measurement. Even if optical levitation offers a series of considerable advantages, it has been applied just in a few applications. However it can be reconsidered for sample manipulation in experiments with SR since it is very flexible and quite simple to be implemented. The levitated particles could be functionalized to carry different reagents in the spot of the SR beam. Liquid drops could be at their turn used as envelops of molecules to reduce damaging by the X-ray beams.

Optical alignment of molecules is another application of the optical fields to sample manipulation, already mentioned in the first section. In terms of freedom degrees of manipulation it allows less but faster control. Molecules provided by a spray like device at high flow rate can be oriented by using the polarization of the laser beam.

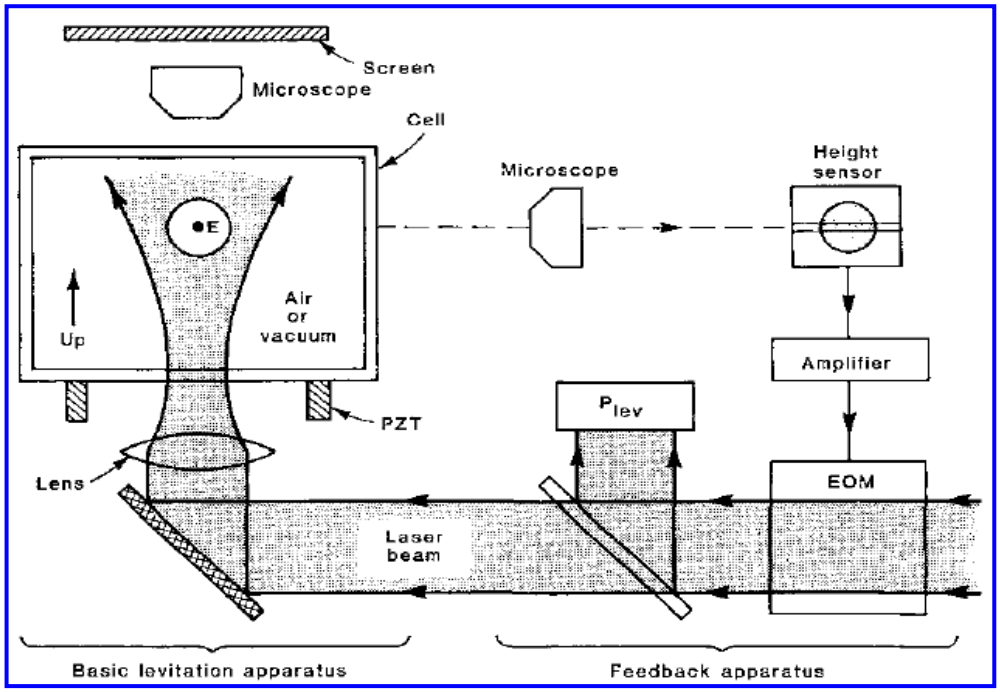


Figure 7 T3-5. Basic apparatus for optically levitating dielectric spheres and feedback stabilization apparatus for levitating in vacuum and measuring forces. Abbreviations: *PZT,* piezoelectric ceramic shaker; *EOM,* electrooptic modulator

5.2 Magnetic Tweezers

Magnetic tweezers, in their basic form, consist of a pair of permanent magnets placed above the sample holder of an inverted microscope outfitted with a charge-coupled device (CCD) camera linked to a frame grabber [8]. The concept of magnetic tweezers is similar to that of optical tweezers: a magnetic particle in an external magnetic field experiences a force proportional to the gradient of the square of the magnetic field. High forces can be achieved with relatively small magnetic field strengths, provided that a very steep field gradient can be generated. The fields generated by sharp electromagnetic tips (Fig. W3-5-8 (A)) have been used to apply forces higher than 200 pN on micrometer-sized magnetic particles.[42] Electromagnetic tweezers are capable of exerting forces in excess of 1 nN and can be used to manipulate, and importantly rotate, magnetic particles ranging from 0.2 to 5 µm. Magnetic tweezers afford a passive infinite-bandwidth force clamp over large displacements. These characteristics are ideally suited to the study of nucleic acid enzymes, particularly DNA topoisomerases [43,44] (Fig. 8 T3-5 (B)) and the rotary motors. Owing to the steep gradient, however, the force falls off rapidly with displacement away from the magnet. Consequently, appreciable force can only be applied on a particle in close proximity to the magnet, and the force is not constant for small displacements of the magnetic particle in the vicinity of the magnet. Larger magnets provide a higher magnetic field strength and a shallower field gradient, resulting in forces that vary more slowly over a larger area. A single magnet can be used to supply an attractive pulling force on a magnetic particle, but a minimum of two magnets are required to generate torque and apply force.

Magnetic tweezers offer some advantages over other non-contact manipulation techniques and are particularly well-suited for certain measurements. They do not suffer from the problems of sample heating and photodamage that plague optical tweezers. Moreover, magnetic manipulation is exquisitely selective

for the magnetic beads used as probes, and is generally insensitive to the sample and microscope chamber preparation. These features permit noninvasive force and displacement measurements in complex, heterogeneous environments. Because of the properties of the magnetic field used to impose force, magnetic tweezers offer the prospect of highly parallel single-molecule measurements, which would be difficult or impossible to achieve with other single-molecule force spectroscopy techniques.

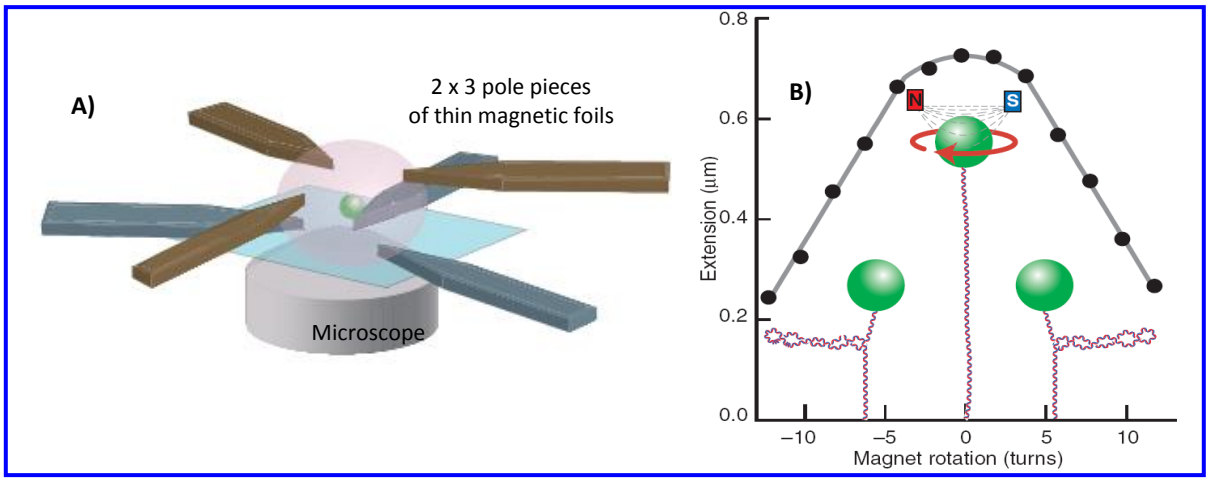


Figure 8 T3-5 Magnetic Tweezers (A) Schematic representation of an electromagnetic tweezers pole configuration permitting full three dimensional control over the position of the bead (adapted from ref. [42]. The pole pieces are sandwiched between electromagnetic coils in an assembly that mounts on an inverted microscope (not shown). **(B)** Measuring DNA topology with magnetic tweezers: the extension is measured as a function of rotation for a 1 µm super-paramagnetic bead tethered to a surface by a 3-kb molecule of DNA under 0.4 pN of pulling force. As the DNA is over- or under-wound (supercoiled) there is a slight decrease in extension near zero turns, which is due to the accumulation of twist in the DNA molecule. At ± 4 turns the DNA buckles, forming a plectoneme loop. Each subsequent turn increases the plectoneme by another loop, leading to a linear decrease in extension from 4 to 12 turns. Removal of the plectonemes by the activity of a topoisomerase can be directly observed in real time by monitoring the extension of a supercoiled DNA molecule. Schematics are not drawn to scale. From ref [43].

5.3 Dielectrophoretic trapping and manipulation

Dielectrophoresis is the movement of a dielectric object due to forces generated by a non-uniform electric field, while electrophoresis relates to the movement of a charged particle in an electric field. When a dielectric particle is suspended in an electric field, polarised charges are induced on its surface, which establishes electric dipoles. The magnitude and direction of these induced dipoles depend on the frequency and magnitude of the applied electric field, the morphology of the particles, and the dielectric properties of particle and medium [45]. The interaction between the induced dipoles and the electric field can generate a dielectrophoretic (DEP) force affecting the particle (Fig. W3-5-9). Since the direction of the DEP force is determined by the spatial variation of the field, the particle always moves toward/against the direction of the electric field maxima.

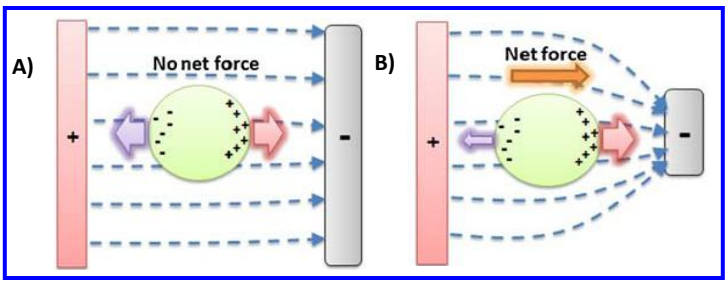


Figure 9 T3-5 Dielectrophoretic force
(A) No net force is produced in an uniform electric field **(B)** A net force is produced in a non uniform field because of the magnitude of the gradient of the electric field.

The strength of the DEP force strongly depends on the medium and particles' properties (such as their dimensions and morphologies) and on the frequency, phase and magnitude of the electric field [46]. The DEP force can be positive or negative. Positive DEP force traps particles in regions with strong electric field gradients, while negative DEP force repels them from such regions. Dielectrophoresis applied within microfluidic systems is well suited for applications such as separation and sorting, trapping and assembling, patterning, purification, and characterization in a wide range of environmental, biological, and clinical applications. [46] A DEP system can be configured in different versions, the most populars being probably the "parallel finger paired electrodes" and the "micro tip electrodes" (Fig. W3-5-10). In the second case, the electrodes generate the maximum electric field gradient at the tips, and hence generate a large trapping DEP force. As the triangular micro tips produce a relatively low effective trapping area, multiple pairs of electrode tips can be patterned to enhance the trapping efficiency.

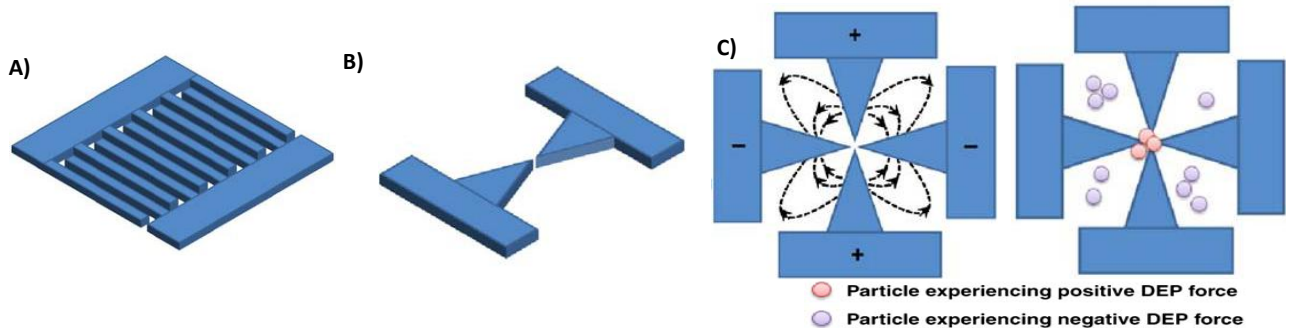


Figure 10 T3-5 DEP systems (A) Parallel finger paired electrodes configuration: the electrodes generate the greatest electric field gradient directly above their surface. **(B)** Single paired micro tips electrode: the electrodes generate the maximum electric field gradient at the tips generating a large DEP force. **(C)** Crossed-electrode paired micro tips configuration increases the effective trapping area. This design is efficiently used for capturing single nano particles [47].

5.4 Scanning probe microscopy (SPM) manipulation

Scanning probe microscopes: Scanning Tunneling Microscope (STM) and Atomic Force Microscope (AFM) allow the highest imaging resolution and can be applied to particles as small as atoms with atomic resolution (see T3-4). The possibility to move the probe makes possible to use SPM for nanomanipulation or/and high resolution lithography (in fact, it is difficult to separate for this case manipulation from lithography, see also T3-2). However, due to their positioning limitations and environment requirements (mainly for STM) , standard SPMs can hardly be used for complex manipulations and in 3D space.

Scanning Tunneling Microscopy (STM) Manipulation

The use of STM for lithography/nanomanipulation is specified already in T3-2. However, one aspect interesting to be mentioned for manipulation is the test of more advanced tips such as nanotube brushes or similarly functionalized microstructures [48,49].

Atomic Force Microscopy (AFM) Manipulation

AFM is more robust and flexible than STM for manipulation/lithography, as specified in T3-2. Beside its applications in manipulation AFM can be used for force spectroscopy as a complementary technique in fine analysis. An important feature of AFM is the ability to conduct measurements of biological samples under near-physiological conditions 108,109. AFM allows measurement of inter- and intramolecular interaction

forces with piconewton resolution. When used for one-dimensional force measurements this specialized version of an atomic force microscope is called a molecular force probe (MFP) and its use is fundamentally distinct from imaging AFM. When used in an imaging mode, the AFM cantilever scans the surface of the specimen line after line, whereas in an MFP the cantilever is moved only in the vertical direction, perpendicular to the specimen plane 25,110. The vertical motion of the cantilever is controlled by piezoelectric actuators affording sub-nanometer resolution. The displacement of the cantilever is monitored directly with either a capacitor or a linear voltage differential transformer. As a result, high-resolution force-versus-extension curves of single molecules can be recorded using an MFP. The most important characteristics of AFM/MFP are presented in Table 11 T3 -5 in comparison with the optical tweezers and magnetic tweezers [50]

Table 11 T3-5

Comparison of single-molecule manipualtion and force spectroscopy techniques
from ref [50]

	Optical tweezers	Magnetic (electromagnetic) tweezers	AFM
Spatial resolution (nm)	0.1–2	5–10 (2–10)	0.5–1
Temporal resolution (s)	10^{-4}	10^{-1}–10^{-2} (10^{-4})	10^{-3}
Stiffness (pN nm^{-1})	0.005–1	10^{-3}–10^{-6} (10^{-4})	10–10^{5}
Force range (pN)	0.1–100	10^{-3}–10^{2} (0.01–10^{4})	10–10^{4}
Displacement range (nm)	0.1–10^{5}	5 –10^{4} (5–10^{5})	0.5–10^{4}
Probe size (μm)	0.25–5	0.5–5	100–250
Typical applications	3D manipulation Tethered assay Interaction assay	Tethered assay DNA topology (3D manipulation)	High-force pulling and interaction assays
Features	Low-noise and low-drift dumbbell geometry	Force clamp Bead rotation Specific interactions	High-resolution imaging
Limitations	Photodamage Sample heating Nonspecific	No manipulation (Force hysteresis)	Large high-stiffness probe Large minimal force Nonspecific

5.5 Microgrippers

Compared to manipulation using ‘single ended’ tools such as SPM tips, a microfabricated gripper could provide better control over the applied forces, as well as a more well-defined mechanical grip on the object since rotation will be limited by the gripper geometry. It is also possible to make direct electrical and mechanical measurements on the grabbed object if the gripper arms are made of conducting material or can provide a force feedback signal.

Over the last few years, several research groups have been developing grippers for micromanipulation and they have also become commercially available from companies such as Nascatec and Zyvex [51]. Many different gripper actuation principles and designs have already been explored, but often only to the point of proving the actuation capability of the device, rather than actual testing of the device on real samples.

At the nanoscale gripper structures have been demonstrated to manipulate nanoparticle samples both under ambient conditions [49], *in situ* SEM [52], and in liquids: DNA [53] and cells [54]. It appears that grippers could find a wide variety of applications provided there is further development and specialization of the gripper functionality for the various environmental conditions. [55] With grippers, one could imagine the development of a nanoscale workshop where individual nanocomponents could be picked up, characterized and tested, in a way not unlike how millimetre sized components are normally handled with tweezers in an electronic workshop. Common tasks would then be to transport an individual nanostructure from the original substrate onto a transmission electron microscope (TEM) grid for initial examination, and then to mount it in a prototype electronic or mechanical nanoscale device, or to mount it in ways that cannot easily be achieved by direct growth or deposition.

6. REQUIRED NUMBER AND LOCATION OF MANIPULATION STATIONS

In the table below, the various manipulation tools for NFFA centers are listed summarizing the criteria defined in section 4. In the lower part of the table, the required number of tools and dedicated manpower is given. The numbers are based on the assumption, that three NFFA centers are created.

	Contact-less manipulation				***Contact manipulation***		
	Optical Manipulation		***Magnetic Tweezers***	***Dielectrophoretic Tweezers***	***Scanning Probe Microscopy Manipulation***		***Microgripper***
	Optical Tweezers	***Optical Leviation***			***STM***	***AFM***	
Resolution (*)	*Medium/high*	*Medium/high*	*Medium /high*	*Medium*	*Very high*	*Very High*	*High/VeryHigh*
Environment	*Liquid*	*Air/Vacuum*	*Liquid*	*Liquid*	*Vacuum*	*Air/Liquid/ Vacuum*	*Air/Liquid/Vacuum*
Freedom Degrees	*3D+force measurement*	*3D*	*3D+force measurement*	*2D*	*2D*	*2D+force measurement*	*3D*
Flexibility	*Very high*	*High*	*Medium*	*Medium*	*Low*	*Medium*	*High*
Robustness	*High*	*High*	*High*	*Medium*	*Low*	*Medium*	*Medium*
Access mode	*Open*	*Limited*	*Open*	*Limited*	*Limited*	*Limited*	*Limited*
Required Environment	*Lab or clean room*	*Lab or clean room*	*Lab or clean room*	*Lab or clean room*	*Lab or clean room*	*Lab or clean room*	*Lab or clean room*
Level of standardization	*High*	*Medium*	*Low*	*Low*	*Medium*	*High*	
Required units	*3*	*3*	*3*	*3*	*2*	*2*	*3*
Cost per unit (M€)	*0.4*	*0.2*	*0.2*	*0.2*	*0.5*	*0.5*	*0.25*
Total investment (M€)	*1.2*	*0.6*	*0.6*	*0.6*	*1*	*1*	*0.75*
Dedicated staff per unit	*1*	*0.5*	*0.5*	*0.5*	*1*	*1*	*1*
Total staff	*3*	*1.5*	*1.5*	*1.5*	*2*	*2*	*3*

(*) Resolution ranges: medium 0.2 – 10 μm, high 1 – 300 nm, very high 0.1-10 nm.

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