**EMRP Call 2012 – Open Excellence, Industry and SI Broader Scope**

EURAMET
European Association of National Metrology Institutes

Selected Research Topic number: **SRT-x08**
Version: 1.0

# Title: Advanced microscopy for multiparametric molecular imaging


## Abstract

Advances in biophysics have initiated the development of a range of novel quantitative microscopy techniques which overcome the limitations of traditional light microscopy. This presents an opportunity to apply these novel techniques to address some of the most significant challenges in biomedical research, such as understanding the dynamic molecular interactions that occur within living cells and how these relate to cell function and disease. Through collaboration with leading innovators in the field of microscopy the JRP should exploit, refine and validate emerging advanced imaging techniques to support the development of a novel approach to measuring complex molecular events and understanding their interactions.


## Conformity with the Work Programme

This Call for JRPs conforms to the EMRP Outline 2008, section on “Grand Challenges” related to Industry & Fundamental Metrology on pages 8, 13 and 14.


## Keywords

Super-resolution microscopy, label-free microscopy, time-resolved microscopy, fluorescence microscopy.


## Background to the Metrological Challenges

Super resolution fluorescent imaging techniques, which overcome the diffraction barrier that hinders traditional light microscopy, are being developed with spatial resolution improved from 250 nm to 20 nm. Such techniques include stimulated emission depletion (STED), structured illumination (SIM), photoactivated localisation microscopy (PALM), fluorescence PALM (FPALM), stochastic optical reconstruction microscopy (STORM), and direct STORM (dSTORM). These new approaches are beginning to be used to address some of the most significant challenges in biology, such as understanding the molecular interactions that occur within living cells and tissues. Use of these imaging approaches will lead to an improved understanding of cell biology as well as of diseases such as cancer, neurodegeneration and diabetes.

Previously, electron microscopy (EM) and atomic force microscopy (AFM) were used to study events such as protein aggregation in fixed excised tissue or *in vitro*. However, super resolution fluorescent imaging techniques are essential if biological interactions are to be measured *in situ,* in a cellular context, at the molecular level. Highly specific and versatile labelling tools are also available for functional imaging using immunocytochemistry or fluorescent protein technology. These methods have the potential to allow the spatial distribution of virtually all of the cellular substructure, or proteins, to be analysed in living cellular samples. A measurement approach for a ‘single cell system biology’ needs to be developed that can allow the dynamic analysis of multiple cellular events to be made as well as an understanding of how these events interact and are related in disease states. Examples of super resolution fluorescent imaging techniques and their current state-of-the-art applications are given below:

Total Internal Reflection (TIRF) microscopy allows the selective visualisation of events at the interface of the cell basal plasma membrane and a substrate, probing the living cell surface and its microenvironment to a depth of around 60 nm. TIRF can also be used to measure the fluorescence of a single molecule.

Förster (or Fluorescence) Resonance Energy Transfer (FRET) is a valuable tool to quantify molecular dynamics in biophysics and biochemistry, such as protein-protein interactions, protein–DNA interactions, and


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protein conformational changes. FRET is scalable from single molecule detection (smFRET) up to millimetre scale.

Fluorescence-Lifetime Imaging Microscopy (FLIM) is generally applied to the study of receptor signalling and intracellular trafficking. It can further be used as in conjunction with confocal microscopy, two-photon excitation microscopy, and multiphoton tomography, enabling the reconstruction of 3D structures from micrometre to millimetre scales. Two photon excitation has also recently been demonstrated to open up options for deep 3D imaging with super-resolution.

## Scientific and Technological Objectives

Proposers should address the objectives stated below, which are based on the PRT submissions. Proposers may identify amendments to the objectives or choose to address a subset of them in order to maximise the overall impact, or address budgetary or scientific / technical constraints, but the reasons for this should be clearly stated in the JRP-Protocol.

The JRP shall focus on the development of advanced quantitative imaging approaches for the multiparametric microscopy measurements of dynamic cellular processes. A system should be developed to allow the interdependencies of cellular events such as receptor signalling, protein-protein interactions, protein trafficking, mRNA transcription and translation and cell structural changes to be correlated using the microscopy techniques described below.

The specific objectives are

1. To develop quantitative microscopic techniques with super-resolution and/or time-resolution and to develop advanced nonlinear-optics imaging approaches including two-photon excitation fluorescence, CARS (Coherent Anti-Stokes Raman Spectroscopy), SRS (Stimulated Raman Spectroscopy), and SHG (Second Harmonic Generation).
2. To perform multiparametric analysis combining label-free microscopies including CARS, SRS, SHG and Terahertz SNOM (Scanning Near Field Optical Microscopy).
3. To examine algorithms and software for image reconstruction in order to combine these different microscopies for integrated quantitative analysis
4. To apply multiple complementary advanced imaging approaches to a common biological questions (such as a neurodegenerative disease cell model) and to compare the type and quality of the data obtained.

Proposers shall give priority to work that enables the traceable measurements and characterisation required in the future for understanding of dynamic cellular processes.

Proposers shall give priority to work that enables new metrological methods and techniques in the future through excellent science. The project need not address metrology directly.

Proposers should establish the current state of the art, and explain how their proposed project goes beyond this.

The total eligible cost of any proposal received for this SRT is expected to be around the 1.8 M€ guideline for proposals in this call. The available budget for integral Research Excellence Grants is 84 months of effort.

## Potential Impact

The project should be designed to bring together the best scientists in Europe and beyond whilst exploiting the unique capabilities of the National Metrology Institutes and Designated Institutes. Significant non-NMI/DI and international participation in the projects is expected and proposers should make full use of the larger budget for Research Excellence Grants available for this SRT.

You should detail other impacts of your proposed JRP as detailed in the document “Guide 4: Writing a Joint Research Project”

You should also detail how your approach to realising the objectives will further the aim of the EMRP to develop a coherent approach at the European level in the field of metrology and includes the best available contributions from across the metrology community. Specifically the opportunities for:

- improvement of the efficiency of use of available resources to better meet metrological needs and to assure the traceability of national standards
- the metrology capacity of Member States and countries associated with the Seventh Framework Programme whose metrology programmes are at an early stage of development to be increased

- outside researchers & research organisations other than NMIs and DIs to be involved in the work

## Time-scale

The project should be of up to 3 years duration.