



FINAL PUBLISHABLE REPORT 3D-EM

A major goal in the invention of the European “Network of Excellence” (NoE) grant scheme was the integration of outstanding European scientists and laboratories in fragmented fields of research to foster collaboration and progress in a specific research field. The “Network of Excellence” 3D-EM (New Electron Microscopy Approaches for Studying Protein Complexes and Cellular Supramolecular Architecture) was one of the first Networks to be launched in the EC Research Framework Programme 6. At its end after 66 months it now provides an early example to assess the success of this networking approach.

The 3D-EM NoE mainly focussed on three overarching goals:

- (i) Bringing together the experts in the field of structural analysis using electron microscopy to foster close collaboration in research.
- (ii) Establishing a platform of routinely used tools and software, standardisation of methods and data formats, and a joint data resource.
- (iii) Disseminating state of the art technology and best practice methods to the worldwide community via an extensive training programme.

Three “Network Areas” in the 3D-EM NoE covered the scientific approaches in Electron Microscopy (EM) within the Network:

Single Particle Analysis (SPA) deals with the characterisation of protein structures based on the analysis of thousands of purified individual molecules (“single particles”). The 2-dimensional images of single particles are recorded in random steric orientations and provide the basis for the reconstruction of the 3-dimensional model of the particle structure.

A variety of software applications have been developed for this purpose worldwide, and an early task in the NoE lifetime was the side-by-side comparison and evaluation of available software packages for SPA. It resulted in recommendations for use for the different packages in different experimental settings.

The consortium’s SPA experts then moved on to address a major unresolved issue in the field – structural analysis of mixtures of heterogeneous particles. Heterogeneity occurs when flexibility in the single molecule allows for more than one conformation, or when the number or nature of the “building blocks” of a complex may vary. SPA of such heterogeneous samples is exceedingly more complex since it requires that particles in different conformations are assigned to different structural classes. Successful strategies to solve this complexity were laid out by the partner labs of H. Saibil at Birkbeck College, C. Spahn at Charité, S. Fuller at Oxford University, and J.M. Carazo at CSIC Madrid. Thus, the CSIC group devised a comprehensive package for single-particle analysis, “X-Window-based Microscopy Image Processing Package” or XMIPP. It allows to perform the entire 3D-EM image processing work-flow, from the pre-processing of micrographs to the reconstruction and refinement of a 3D model. A graphical user interface makes running XMIPP straightforward even for novice users. The package was made freely available to the public in January 2008, and almost 1.000 downloads since demonstrate its importance for the field.

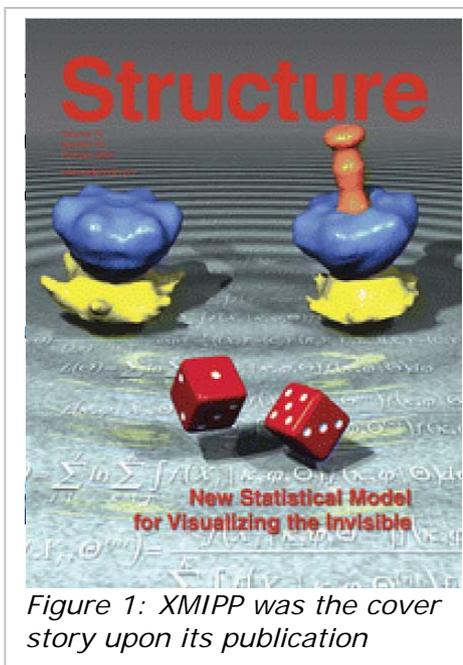


Figure 1: XMIPP was the cover story upon its publication

Cryo-Electron Tomography (CET) is used to reconstruct the 3-dimensional structure of an object from 2-dimensional EM images by recording serial images of the sample from various perspectives or tilt angles. An essential prerequisite for this technology is the availability of an electron microscope with a tiltable object holder. The major European labs in this field successfully concentrated on improving the



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technology and software for tomographic applications with the aim to increase the throughput. In view of the Scientific Advisor Alasdair Steven, "...excellent progress in CET has been stimulated by the NoE".

One important outcome of the efforts is the software package "TOM Toolbox" which was developed for the acquisition and interpretation of CET data by the Baumeister group at MPI of Biochemistry. Since the launch of the first version in 2005, this package has been distributed to over 200 expert laboratories worldwide (see Figure 2). The latest update adapts the software to the novel

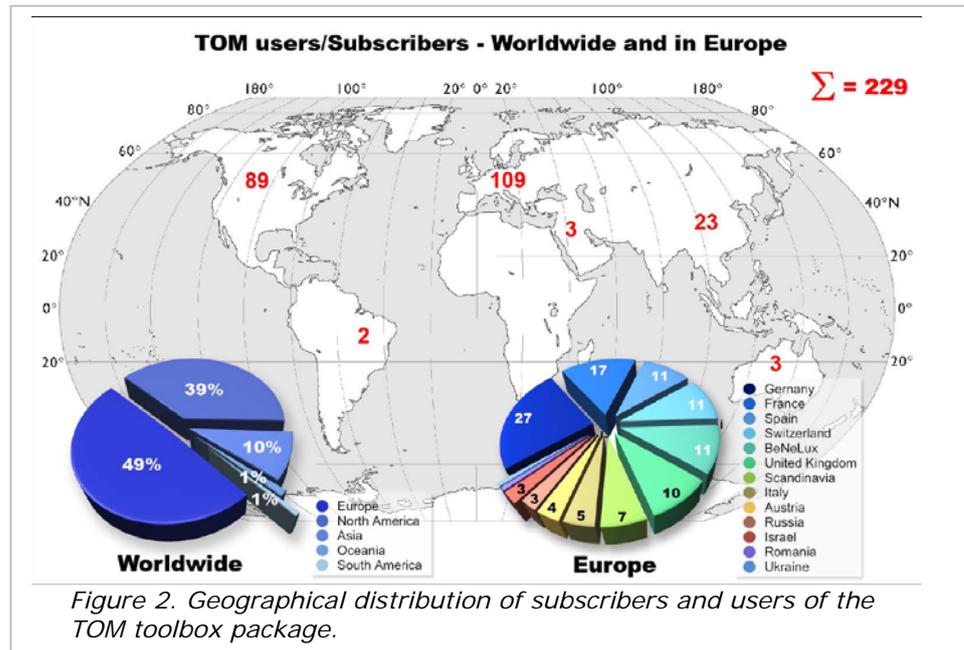
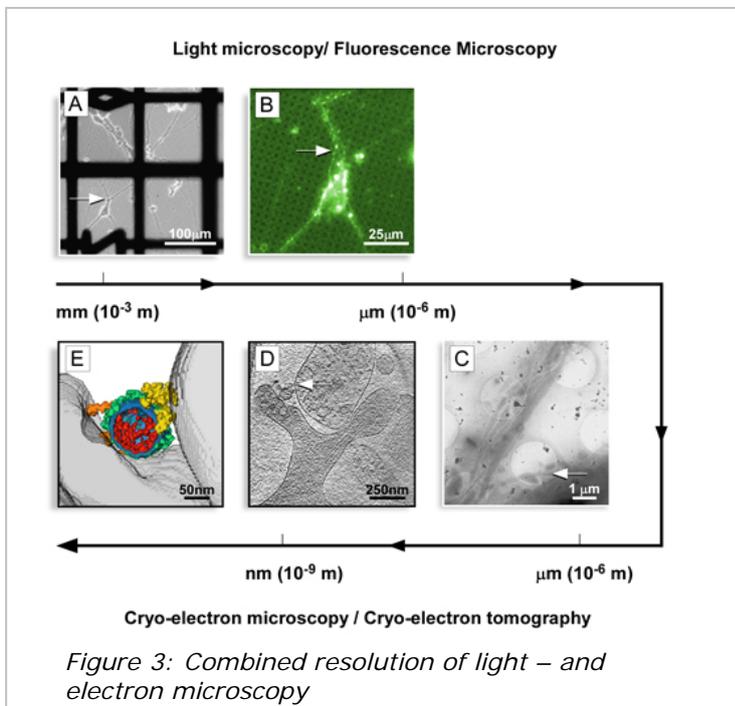


Figure 2. Geographical distribution of subscribers and users of the TOM toolbox package.

high-end electron microscope "Titan Krios" manufactured by 3D-EM commercial partner FEI company. It is the first instrument equipped with both, an automated sample loading system and a specimen stage with dual tilt axis. Together with the software package enabling automatic data acquisition this setup enables for the first time an extensive automatic data sampling of the individual specimen that is required for the analysis of complex biological systems.



Only three years ago, the NoE established a dedicated work package for **Correlative Microscopy**, which strives to achieve a seamless integration of the powerful fluorescence light microscopy with the high resolution obtained only in electron microscopy. The principle is depicted schematically in Figure 3. Obstacles on the road towards this goal include specific but different requirements for cell labelling, sample preparation, and re-identification of exact sample positions in the two different microscopes. Collaborations in this area proved highly effective and, despite the short developmental phase, two alternative setups for the novel hybrid approach to "visual proteomics" were established. Functional prototypes of instrumentation have been built in the partner labs at Leiden Medical

University / University of Utrecht and MPI of Biochemistry, Martinsried, and a first line of instruments is now under construction at FEI company.

2-dimensional Electron Crystallography is an alternative approach for structure determination, especially for membrane proteins. Proteins are directly crystallised on a lipid membrane, their "natural habi-



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tat”, and subsequently analysed by EM. In the 3D-EM network, experts of the Hebert group at Karolinska Institute, Stockholm, and the Carazo laboratory at CSIC Madrid joined forces under the coordination of the Engel lab at the Biocentre Basel in an effort to establish a software platform for the analysis of images obtained (mainly) by 2-dimensional electron crystallography. Their recently finalised *Image Processing Library & Toolbox* (IPLT) has developed into a freely accessible modular platform [www.iplt.org]. It is already in use in a few labs and ready for roll-out into the field.

The **EMDB databank** [www.emdatabank.org] at the **European Informatics Institute** (EBI, Hinxton) is an essential repository for data sets describing the 3-dimensional structure of macromolecules and thus another important asset of the network. The EBI partner has been very active in worldwide efforts to develop and standardise databank formats as a prerequisite for the exchange of data between groups all over the world.

Acknowledging these successful efforts, additional resources have become available for a further improvement of EMDB services. Those were provided in collaboration with the “Research Collaboratory for Structural Bioinformatics” (RCSB), a US based non-profit consortium managing the Protein Data Bank (PDB) for structures of biological macromolecules. The aim is to establish a publicly supported, one-stop deposition and retrieval facility for cryo-EM density maps, atomic models and associated meta-data. This is to be accompanied by a portal for relevant software tools for standardized data format conversion and data integration to facilitate the efficient use of the archived data. Beyond the 3D-EM NoE lifetime, this initiative is supported both by the NIH (for the US partnership at Rutgers) and national UK funding. The EMDB data base is actively involved in the European infrastructure projects ELIXIR [www.elixir-europe.org/] and INSTRUCT [www.instruct-fp7.eu/] to secure EMDB a place in the future of infrastructures in Europe.

Training and Dissemination

Right from the start, the 3D-EM Network had invested tremendous efforts into the establishment and standardisation of **a dedicated training programme in structural biology**. Courses and workshops at basic and advanced level were offered by a variety of partner laboratories, as detailed on the website [www.3dem-noe-training.org]. In summary, over 500 participants were trained in 39 courses to gain state-of-the-art expertise in the relevant technologies. A standardised evaluation of the courses indicated that all organised events were extremely successful and well received. One success story is the introduction of a novel sample preparation method, CEMOVIS, developed at the partner lab of J. Dubochet in Lausanne, to the wider field through the NoE. In the frame of 3D-EM, the Lausanne group organised workshops and training courses to disseminate the new CEMOVIS technology and provided advice on suitable instrumentation. In the course of the project, the partner laboratories at Karolinska Institute, EMBL Heidelberg, and MPI of Biochemistry, Martinsried, acquired expertise in the new method and since the retirement of Prof. Dubochet training courses were held at these partner labs. Meanwhile, the CEMOVIS experts from the Lausanne group have relocated to various EM labs worldwide and further disseminate the method directly. In this showcase example, the power of a Network as a facilitator for the dissemination of best practice was perfectly illustrated.

The training courses were complemented by **a fellowship programme** specifically addressing younger established researchers at Central and Eastern European Universities. It offered individual advanced training and the opportunity for a research “internship” in a network lab with the aim to forge new bonds between partner sites and the home institutions of the fellows. Five researchers used this opportunity and acquired new competencies during their visits in network laboratories. Longer-term effects of this programme will need to be monitored in due course.

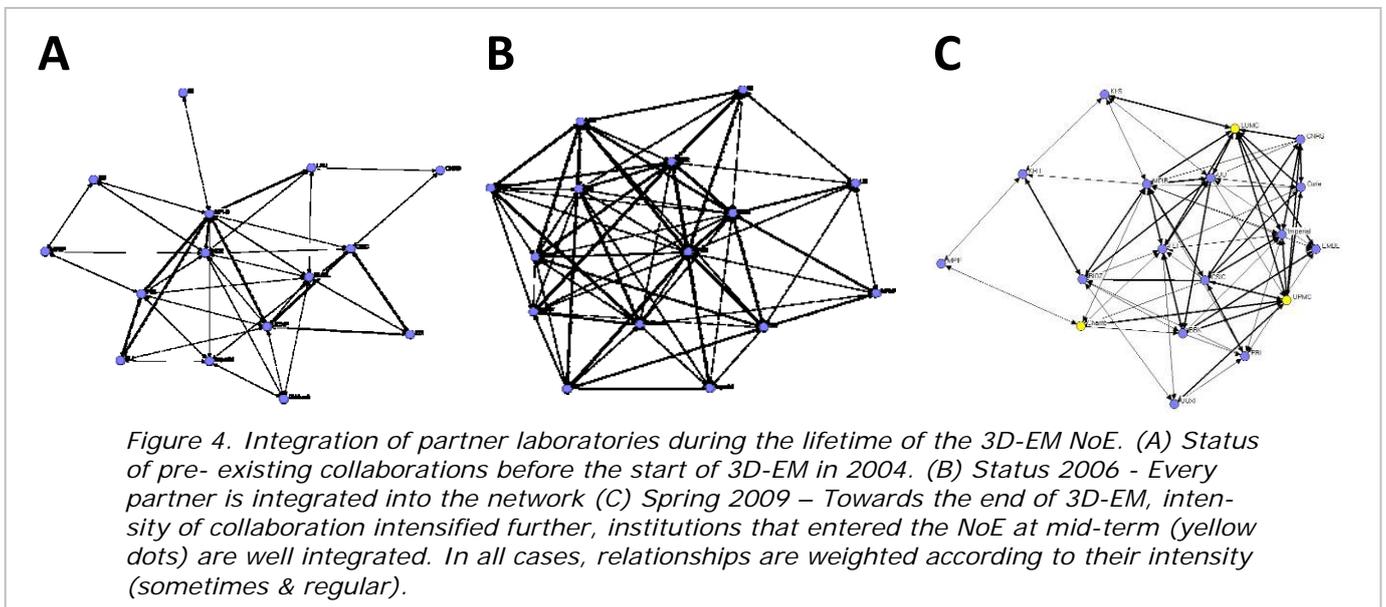
To follow up on the training aspect and broaden its impact, **a teaching DVD “3D-EM in Life Science”** has been produced with contributions from world leading scientists within and beyond the 3D-EM NoE. It covers a wide variety of topics from the history of electron microscopy, the development of different types of instrumentation and approaches to structural analysis to the current use of EM in biomedical research. The DVD will be distributed to training schools and course organisers etc. for free. In their final review, the Scientific Advisors of the NoE praised these efforts and particularly noted “...*much passion from people that make it happen*”.

Integration and Networking

The “defragmentation of the research field” and the creation of synergies and lasting collaborations between leading laboratories was a prime intention for the establishment of the funding instrument of Networks of Excellence. Integrating effects of the activities within the 3D-EM NoE were therefore closely monitored during the course of the project. Standardised questionnaires and individual interviews with the PIs towards project end were used to monitor various parameters for integration, including joint research efforts, joint publications, shared third party funds, and the exchange of personnel.

By these criteria, distinct stages of network formation can be identified:

- (i) A loose pre-existing network of the major labs formed the networking basis for the NoE. The leaders of these core groups launched the NoE initiative and actively participated in its governing bodies throughout the NoE’s lifetime (Fig. 4A).
- (ii) Within the initial two years a consolidated collaborative network (Fig. 4B) evolved and connections were made by all partner labs. Project and Network Area Meetings involving young researchers were instrumental in fostering a direct interaction between the researchers of the partner groups at all levels. Interactions between the young scientists were particularly effective regarding the development of joint research projects interlacing the laboratories.
- (iii) Once the integration of the initial partnership was firmly consolidated, an enlargement of the consortium with three full and three associate groups increased the partnership to 22 laboratories. A final evaluation of integration (including interviews with the group leaders) was performed close to the termination of the NoE in spring 2009. It shows that the network has further expanded and in parallel the intensity of collaborations increased into a tightly knit network, which integrates those partners that joined the NoE during the enlargement process (Fig. 4C). A number of joint publications and exchanges of personnel between partner groups formally confirm the alignment of research interests. Beyond the scope of the 3D-EM NoE, many of the partners are involved in related national initiatives in the field and collaborate in follow-up EU funded projects, thus demonstrating the success of the years with joint activities.



Even more relevant for any long-term structuring effect in this community are the activities that enabled the younger generation to gain interdisciplinary experience and to forge bonds in the field early on in their careers. They are now well prepared to carry on and it is now time for “...the younger generation to pick up the ball”, as the Scientific Advisor stated.

More information on the 3D-EM Network is available on the website: www.3dem-noe.org