# Detailed tour report on foreign visit:

| 1 | Name & Designation of the Scientist | JAYATI SENGUPTA<br>Senior Scientist (Sct Gr. IV(3)) |
|---|---|---|
| 2 | Name of the Institute/ Centre | Structural Biology and Bioinformatics Division<br>**Indian Institute of Chemical Biology**<br>(Council of Scientific & Industrial Research)<br>4, Raja S.C. Mullick Road,<br>Kolkata-700 032, West Bengal, India |
| 3 | Date of visit | Sept 26th 2011 |
| 4 | Period of visit | 6 Months |
| 5 | Place of visit | National Institute of Health (NIH)<br>Laboratory of:<br>**Dr. Sriram Subramaniam**<br>*Senior Investigator and Chief*,<br>Biophysics Section, Laboratory of Cell Biology,<br>Center for Cancer Research, National Cancer Institute<br>50 South Drive, Room 4306<br>Bethesda, MD 20892-8008, USA |
| 6 | Purpose of the visit | I have got extensive training in single particle 3D reconstruction technique while studying ribosome structure and function during my postdoc in Joachim Frank's lab at Wadsworth Center, NY, where this particular methodology has been developed (the 3D reconstruction software SPIDER).<br><br>However, beyond a certain size range, biological objects are no longer found in identical 'copies,' hence, electron tomography becomes the only resort, and it offers great promises for 3D structural studies of a wide range of specimens.<br><br>My main objective was to learn data collection in high-resolution electron microscope following electron cryotomography (ECT) imaging technique, and the method of 3D reconstruction using the tomography dataset. |
| 7 | Source of sponsorship of the visit | ICMR International Fellowship 2011-2012<br>Letter No. **INDO/FRC/Y-08/2011-1HD**<br>dated 13.07.2011 |

| 8 | What is the scientist's contribution, relevance to ICMR/DHR, India? | Annexure I |
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| 9 | Benefits to the Institute/ Centre from the skills acquired by the scientists during the said visit. | Annexure II |
| 10 | How the skills acquired by the scientist will be utilized? | Annexure III |
| 11 | Whether the Director/OIC satisfied with the tour report? Comments of Director/ OIC on the report | Annexure IV |

## **Details of the achievements**

I) *Technique/expertise acquired*:

My previous postdoctoral training on cryo-EM was focussed on 3D reconstruction of ribosome complexes using 'reference-based single particle method' where I used SPIDER reconstruction software. In this lab I have learned to use many other image processing and image analysis softwares, other approaches for 3D reconstructions (other than reference-based single particle approach), and got chance to use ultra sophisticated electron microscopes.

The training fields are listed below:

*Sample preparation*: High pressure freezing apparatus (demonstration)

*Grid preparation*: Learned a new technique for carbon coating on many grids simultaneously

*Microscopes*: Polara (Hands on), Krios (demo), FIB-SEM (demo)

*Data collection*: Tomography data collection using TOMO software (FEI)

*Data processing*: EMAN/EMAN2, IMOD (tomography data processing) softwares

II) *Research results, including any papers, prepared/submitted for publication*:

Apart from receiving training on different instruments as well as on different methodologies, I have worked with real data by participating in an ongoing project of the host lab.

In eukaryotic genomes, the mobile genome elements are discrete pieces of DNA that can either move from one place to another within a genome or be copied into a new location. The enzymes catalyzing transposition are called transposases. The lab project where I participated is on structural studies of the eukaryotic DNA binding protein Transposase.

**-- *Collected tilt pair images for 'Random Conical Data processing'*.**

Random-conical reconstruction is a method to produce an initial 3D reconstruction when no *a priori* knowledge is available on the structure of a macromolecular assembly.  This approach is well established for studying single particles, as many different 2D projections are obtained with only two exposures of the sample to the electron beam.  For ribosome reconstruction this method is not required now, and 'reference-based' method is used.

I have applied Random-conical reconstruction method on Transposase dataset to create an initial 3D model structure.
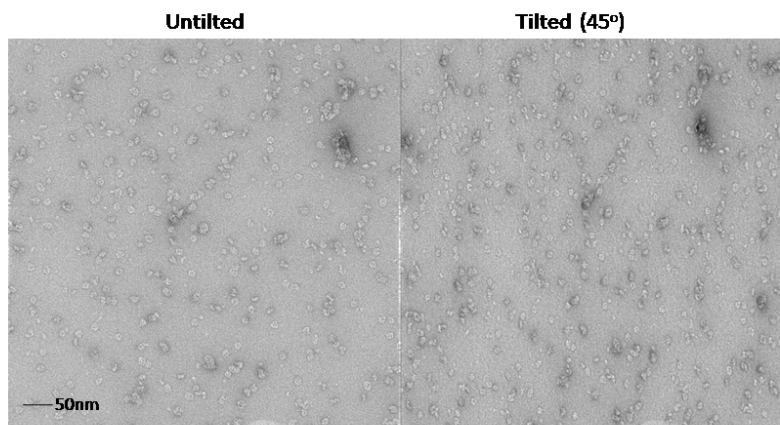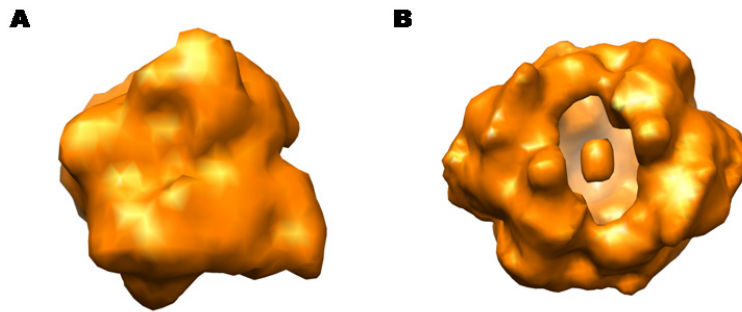


**Untilted**          **Tilted (45º)**

——50nm

**Fig.1:** Tilt pair images of Transposase particles taken for Random Conical tilt reconstruction method

Initial low resolution model structure created from Random Conical method was used to generate the preliminary 3D structure of the protein oligomer using EMAN2 data processing software.

**Fig. 2**: A. Initial low resolution model created using Random conical method of 3D reconstruction.
B. Preliminary 3D structure (using A as reference) of Transposase generated by EMAN2 image reconstruction software

-- *Collected tomography data series on negatively stained sample.*

The most general method for obtaining 3D information by EM is tomography. To a first approximation, images recorded in an electron microscope are "projection" images. "Tomography" refers to an imaging strategy in which projections are recorded of an object from various directions and then a higher dimensional reconstruction is calculated from those projections. In "electron" tomography, a series of two-dimensional projections are recorded in an electron microscope (EM) while incrementally tilting a sample along one or two axes (Tilt series). Each tilt series is aligned and image stack is made (tomogram). A three-dimensional reconstruction is then calculated. This allows the structure of individual macromolecules, organelles, and even whole cells to be imaged in 3D.

The identification and recognition of several molecules in these tomograms are spectacular demonstrations of the potential of cryo-electron tomography to ultimately approach true molecular resolution.
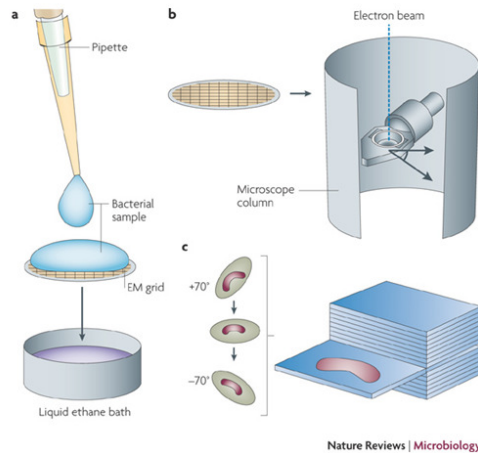
**Fig. 3**: Schematic diagram illustrating the sequence of steps involved in obtaining a three-dimensional image of a plunge-frozen cell using cryo-electron tomography.

Tilt series were collected in Transmission Electron Microscope (T12 and Polara machines) using Tomo software (FEI) for GroEL and Transposase samples.
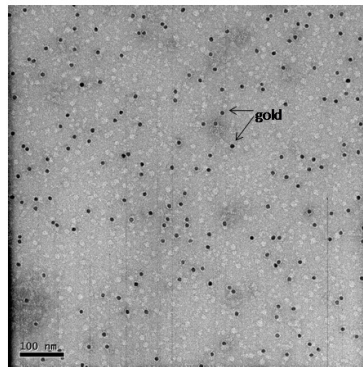


**Fig.4**: Tomographic data collected ($\pm$ 60deg)on this field using gold (10nm) fiducial marker

## -- Tomographic data processing using IMOD (and the in-house data processing pipe line (IMOD-based) of the lab)

IMOD is a set of image processing, modelling and display programs used for tomographic reconstruction and for 3D reconstruction of EM dataset. The package contains tools for assembling and aligning data within multiple types

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and sizes of image stacks, viewing 3-D data from any orientation, and modelling and display of the image files.
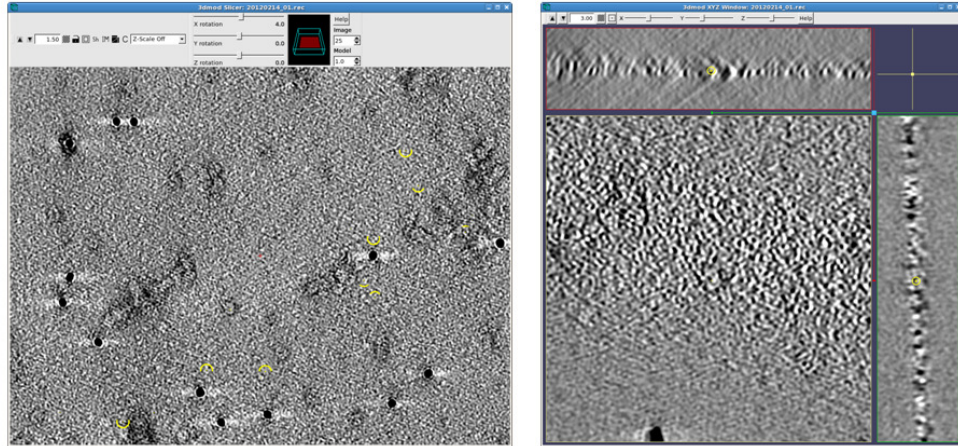
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**Fig 5:** Particle picking form a tomogram using IMOD (3Dmod) data processing software

I have used IMOD data processing software for GroEL (a test dataset) and Transposase datasets.

Further data collection and data processing is still going on in the host lab.

III) *Proposed utilization of the experience in India*:

A state-of-the-art, high-resolution cryo-EM facility is going to be established at our Institute for which order has been placed for 'Polara' (one of the most advanced transmission electron microscope for cryo-electron microscopy of biological samples). With the hands-on experience with this machine, I can jump start my collaborations with scientists of our own Institute as well as from other Institutes to solve 3D structures of different functionally important macromolecular complexes. Furthermore, although single particle approach can be utilized for many of these projects, tomography approach will also be required in some cases, particularly for those large complexes where no previous structural information is available. With the training on tomography data collection and data processing it will be easy for me to adopt this technique wherever required.

**Annexure I**

**The scientist's contribution, relevance to ICMR/DHR, India?**

Structural biology is one of the most important branches in biomedical research. Cryo electron microscopy (cryo-EM) in combination with image analysis tools is increasingly powerful in producing 3D structures of individual molecules and large macromolecular complexes that are unapproachable by other methods. Particularly, this technique has emerged as powerful method for visualizing dynamic, large macromolecular complexes. However, this method is not yet very popular in Indian science. With the postdoctoral experience in single-particle cryo-EM technique, I have joined IICB, Kolkata, and we are going to establish a full-fledged cryo-EM facility at our Institute. ICMR-International Fellowship program enables me to use wide range of cryo-EM techniques to decipher 3D structures of versatile macromolecular complexes which are functionally important in different cellular processes.

**Annexure II**

**Benefits to the Institute/Centre from the skills acquired by the scientists during the said visit.**

A full-fledged, high-resolution cryo-EM facility is going to be established in our Institute under the current five year plan of CSIR, Govt. of India. We are going to purchase one of the most sophisticated cryo-TEM 'Polara'. Several projects have been planned in which, being the only scientist currently with relevant expertise, I will actively participate. Although single particle approach can be utilized for many of these projects, tomography approach (for which I received training) will also be required in some cases. I believe with this training, I can manage to use the cryo-EM facility of our Institute more effectively.

**Annexure III**

**How the skills acquired by the scientist will be utilized?**

Several groups in our Institute deal with cellular organelles and sufficiently large macromolecules. I will be collaborating with scientists of our own Institute as well as from other Institutes. Structural studies using cryo-EM and 3D reconstruction techniques will allow us to interpret biochemical results obtained on these systems in structural terms. With this training I will be able to offer a wide range of cryo-EM applicability for these projects.

**DEPARTMENT OF HEALTH & HUMAN SERVICES**

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892
Laboratory of Cell Biology
Center for Cancer Research
National Cancer Institute
Bldg. 50  Rm. 4306

Tel:    (301) 594-2062
Fax :  (301)480-3834
e-mail: *ss1@nih.gov*

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## REPORT OF HOST INSTITUTE

1.   Name of Professor                        :   Dr. Sriram Subramaniam

2.   Name and address of host institute    :   NIH, Bethesda, MD

3.   Duration of fellowship                  :  6 Months

4.   Brief highlights of the achievements  :

Technique/expertise acquired
- Image processing Softwares: EMAN/EMAN2 (single particle data processing), IMOD (tomography data processing)

- Microscopes:       TEM: Polara, one of the most advanced electron microscopes for cryo-TEM (Hands on), Krios, the most sophisticated electron microscope for cryo-TEM (demonstration), SEM:  FIB-SEM (demonstration)

- Data collection software:  Tomography data collection using TOMO software (FEI)

- Grid preparation:  Learned a new technique for carbon coating on many grids simultaneously

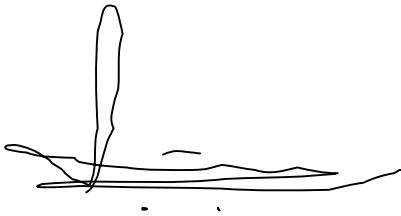- Sample preparation:  High pressure freezing apparatus (demonstration)

Research results
- Worked on an ongoing project of the lab (structural studies of the eukaryotic DNA binding protein Transposase).

- Collected tilt pair images of negatively stained samples for 'Random Conical Data processing

- Collected tomography data series on negatively stained sample

- Tomographic data processing using IMOD (and the in-house data processing pipe line of the lab)

- Further studies on this project are still going on in the lab.

5.  Assessment of the ICMR-IF      :

    Dr. Sengupta has made very rapid progress in the short period that she has been in the lab. She quickly mastered central aspects of various image processing programs that we use in the lab for electron tomography and cryo-electron microscopy.  Her work will very likely be included in a manuscript that we will be submitting in a few months on the structure of the transposase octamer.  More importantly, she has gained critical skills and expertise that will enable her to get off to a running start when she returns to her lab in India and has the opportunity to set up a new, state-of-the-art Polara electron microscope.


6.  Any other comments      : This is an excellent program for young Indian scientists.

    Sriram Subramaniam, Ph. D.
    Senior Investigator and Chief, Biophysics Section
    Laboratory of Cell Biology
    Bldg. 50, Rm. 4306
    National Cancer Institute, NIH
    Bethesda, MD 20892.