Studies on the kinetics of biomolecular transport through nuclear membrane are of interest from a fundamental physics perspective as well as from the point of view of biological applications. The mechanism of this transport and the parameters that control the transport phenomena are not well understood. A proper understanding of the transport phenomena will help in developing methodologies which will be of interest to biomedical research, especially related to gene therapy. Confocal fluorescence microscopy is one of the most widely used imaging techniques employed to study biomolecular systems. However, because of the high cost, commercial confocal microscopes are not affordable to many laboratories. Also most commercial designs are not adaptable to different individual laboratory requirements. Here we report on the design and development of a cost effective and versatile confocal laser scanning microscope that can play a vital role in studies on biomolecular transport through membranes. Results on the nuclear transport of different sizes of FITC labelled dextran which is a model drug molecule are also reported. Studies were carried out in digitonin permeabilized cells using an in-house constructed confocal laser scanning microscopy in the time lapse imaging scheme. We specifically examined the kinetics of transport of dextrans of 4, 10, 20, 40 and 70 kDa through the nuclear membrane of live A549 cells and determined the diffusion rate constants. The intake and intracellular distribution of dextran were found to be dependent on the molecular weight of the dextrans. This analysis can provide us an estimate of the effective pore size available for nuclear transport of these biomolecules. The model can be used to study the transport of proteins and DNA molecules through nuclear membranes. The method described in this work is not restricted to nuclear transport processes alone. It may be used, for instance, to analyse translocation of different biomolecules like proteins and nucleic acids across different bio-membranes.

Acknowledgement: Authors gratefully acknowledge the financial support from DBT, DRDO and DST, Govt. of India. S. Arunkarthick would like to acknowledge the CSIR, Govt. of India for SR Fellowship.
Fig. 1: Experimental Setup of our Home Built Confocal Laser Scanning Microscope. SL- Scan Lens, X and Y- Scanning mirrors, DM- Dichroic Mirror, S- Shutter, FC-Fiber Coupler, F-Optical Fiber, BPF- Band Pass Filter, PMT- Photomultiplier Tube, M1-M4 – Mirrors and DAQ- Data Acquisition Card.

Fig. 2: Pollen Grain Serial Optical Sections by Confocal Microscope. (A) Figures A-J shows Ten confocal cross-sections of dye labeled pollen grain. 3D reconstruction of the pollen grain serial optical sections using ImageJ.

Fig. 3: Preliminary results on nuclear transport study. Time course study of nuclear accumulation of 4 kDa FITC-Dextran.