Abstract

Cytosolic delivery of foreign nanomaterials into living cells is an important step for cell studies. Delivering such nanomaterials into cells requires overcoming the cell membrane, which is a major biological barrier to macromolecules and nanoparticles. Part I is focused on intracellular delivery by photoporation and relevant applications. we first give a detailed review on photoporation and its main principles. Then we investigate specifically VNB mediated photoporation in comparison with the more traditionally used thermal variant of photoporation. Having found that VNB photoporation is the more efficient mechanism for permeabilizing the cell membrane, we make use of it to deliver of imaging contract agents into cells for improved long-term *in vivo* cell tracking. Next we show that VNB photoporation can be used to deliver extrinsic labels into cells for microscopic visualization of subcellular structures of living cells. Finally, we develop a fully automated VNB photoporation platform, for fast and flexible spatially resolved photoporation of selected cells with several unique applications.

Sizing of nanomaterials in complex biological fluids, which is of importance in a wide range of applications in the life sciences. For instance, nanomedicine formulations may well aggregate after administration into a biological fluid such as blood. In the second part of the thesis we develop a dedicated FRAP method capable of analyzing the distribution of diffusion coefficients of polydisperse systems. These distributions can be converted to size distributions as well since size and diffusion rate are directly linked to one another. After thorough validation we show that our new FRAP method can measure the size distribution of proteins and protein aggregates in undiluted human serum. In addition, we apply the new method to assess intestinal and vascular barrier permeability *in vivo* by measuring the size distribution of probes that permeated through the respective barriers.

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