Summary

Fluorescent nanodiamonds (FNDs) is a kind of advanced material that exhibits various superior properties. They're biocompatible materials and do not interact with the environment. They emit red fluorescence forever and show quantum properties at the room temperature. They show exceptional high sensitivity in diamond relaxometry and are promising as biosensors.

In **Chapter 1**, we had a brief introduction of fluorescent nanodiamonds and their unique properties. We summarized the common ways to deliver FNDs into the cells, the subcellular locations of FNDs, their biocompatibility in different species and quantum applications.

In **Chapter 2**, we investigated the cellular uptake and endosomal escape of FNDs in HeLa cells and HUVECs. First, we studied the endocytosis of FNDs by cells over time on single cell level. Generally, more nanodiamonds can be internalized into cells with the time increasing while a wide range of object numbers per cell has been found even in the same type of cells. Besides, HUVECs can uptake more FNDs than HeLa cells. Next, we evaluated the endosomal escape efficiency with calcein assay. As expected, we found large variations in single cells. However, FNDs in HUVECs can escape from the endosomes more efficiently within 4 hours with less than 25% particles remained. It is important to be aware that cells behave differently. Better understanding of the interactions between FNDs and cells is crucial for sensing and labelling purposes.

In **Chapter 3**, we developed a method to increase the cellular uptake of FNDs in HeLa cells. pHrodo Green Dextran is a pH sensitive green dye and can be coated onto FNDs by simple mixing. With the coating, FNDs can enter the cells efficiently and the surrounding pH can be reflected with the fluorescence intensity of the dye. Overall, coated FNDs are internalized into HeLa cells via endocytosis. The particles were transported from early endosomes, late endosomes to lysosomes and finally excreted by the cells.

In **Chapter 4**, we systematically explored how do the size and concentrations of FNDs affect the sperm cells viability. We chose different size (40 nm, 70 nm and 120 nm) and different concentrations (from 1 μg/mL to 20 μg/mL) for this purpose. We found that sperm cells have better tolerance to large particles with low concentrations. Although FNDs are not general biocompatible to sperm cells, 1 μg/mL 70 nm and 120 nm FNDs are safe to use without interfering metabolic activities or cell viability.

In **Chapter 5**, we measured the free radicals generation in human bronchial epithelial (BEAS-2B) cells and human primary airway epithelial cells (HAECs) with diamond relaxometry. The primary cells were isolated from chronic obstructive pulmonary disease (COPD) patients or healthy donors. After exposed to 15% cigarette smoke extract (CSE), we observed a significant difference in free radicals dynamics between healthy and diseased primary cells. COPD donors are more susceptible to the CSE caused oxidative stress and exhibit lower T1 values. The oxidative cellular response was first successfully captured in 20 min in single cell level. Furthermore, we observed variations in baseline levels and response of free radicals among individual clinical samples.

Finally, in **Chapter 6**, we separately discussed the findings and challenges in each research. We also image the future directions of quantum sensing with fluorescent nanodiamonds.

In this thesis, we have conduct research on various aspects of FNDs including cellular uptake, fate in the cells, biocompatibility and quantum applications. Fluorescent nanodiamonds hold great promise as a labelling and sensing material. We are eagerly anticipating the future applications of nanodiamonds in the biological fields.