

The study of metabolic flux is at the heart of cancer metabolism research. While genomics reveals what a cell is capable of, transcriptomics gives a view of what it is planning to do, proteomics displays these preparations, metabolomics shows what a cell is actually doing, and finally, **fluxomics** shows the operation of the whole cellular machinery.

Cancer cells have the ability to modulate metabolic pathways to strive even under severe stress. Their lipid supply is provided by several mechanisms for nutrient uptake and removal, increasing *de novo* lipid synthesis, repurposing structural lipids through enzymatic remodeling, or lipid recycling through autophagy. Importantly, most of these lipid acquisition pathways converge in lipid droplets, which combine different **lipid fluxes** and control their usage based on specific cellular needs.

Over the past 70 years, research on lipid metabolism has led to important discoveries in identifying the mechanisms underlying lifestyle diseases, including cancer. Advances in the use of stable isotopes and mass spectrometry have expanded our understanding of the target molecules that contribute to the pathologies and pathways of lipid metabolism. But still challenging is to (1) **quantify lipid flux** to understand the fundamentals of human physiology and pathology and (2) analyze samples from **more than a single time point** to identify lipidomic patterns that predict disease.

Lipid-FLUX offers integrated optical spectroscopy methods with advanced chemometric techniques and machine learning for the analysis of cellular lipid transport. *Lipid-FLUX* allows for high-resolution, **4D spatiotemporal** monitoring of lipid dynamics in living cells. The methodology will be developed on a model system of various cancer cells to localize and quantify lipids in real time. We expect that our results will reveal unique patterns of lipid kinetics in different cell types, which will contribute to a better understanding of cancer development.

The innovation of this multidisciplinary project is three-fold, and concerns three fields of research: (i) **chemistry**: design, and synthesis of new molecular markers to be introduced to cells, of a universal nature, for highly specific and sensitive subcellular detection of lipids, (ii) **bioinformatics**: development of kinetic models and algorithms to quantify metabolic changes in real-time, and (iii) **biomedicine**: development of methodology for studying metabolic pathways in selected cancer models to understand the cellular mechanism of cancer diseases and improve their treatment.

Lipid-FLUX opens new possibilities in cell biology research, offering an innovative, spectroscopy-based tool for studying lipidomics and fluxomics at the level of a single living cell. Further work will focus on optimizing the model and its application in research, which may result in new diagnostic and therapeutic strategies. *Lipid-FLUX* has the potential to revolutionize the approach to studying lipids in cells, providing the tools necessary to discover new aspects of cell biology and develop innovative therapies that in the future may improve the quality of life of patients around the world.