The project aims to use microscopy and spectroscopy with mid-infrared light to quantify the metabolic activity of cancer cells, its relationship to the cellular microenvironment, and its response to treatment with drug candidates. IR microscopy allows quantitative monitoring of cellular transformations with single cell resolution, in real time, and without the use of exogenous trackers or markers. It is non-invasive and requires minimal or no sample pre-treatment. Most importantly, it allows simultaneous detection and identification of multiple molecular species, in an untargeted approach. The spatial resolution of the measurement will allow investigating individual cells, the intercellular space, and the surrounding microenvironment within a complex cellular sample. The format of the spectroscopic measurement will be akin to the one used for both fast and steady state kinetic measurements of macroscopic samples of individual enzymes, and will provide corresponding information, including the possibility of implementing isotopic labeling and inhibition studies at the level of the individual cell. With the present project, we will build onto the state-of-the-art to develop the capability to quantitatively discriminate between different metabolic states, using as a benchmark the comparative metabolic profiling of cancer and non-cancer cell lines. We will implement this competence by addressing the interaction between metabolism, cellular morphology, and the cellular microenvironment. Finally, we will quantitatively test the activity of specific molecules of potential pharmacological interest for the regulation of cellular metabolism.