Genetically encoded biosensors based on fluorescence resonance energy transfer (FRET) have been widely applied to visualize the molecular activity in live cells with high spatiotemporal resolution. The enormous amount of video images and the complex dynamics of signaling events presented tremendous challenges for data analysis and demand the development of intelligent and automated imaging analysis methods specifically designed for the studies of live cell imaging. We have developed advanced and automated computational imaging analysis methods for quantifying and simulating the motion of biosensors, reconstructing the de facto molecular activities, and tracking and analyzing the spatiotemporal molecular interactions in a single live cell with high-throughput power. Based on fluorescence recovery after photobleaching (FRAP) experiments, we have developed a finite element (FE) method to analyze, simulate, and subtract the diffusion effect of mobile biosensors. The results indicate that the Src biosensor located in the cytoplasm moves 48 folds faster ($0.9360.06 \text{mm}^2/\text{sec}$) than those anchored on different compartments in plasma membrane (at lipid raft: $0.1160 \text{mm}^2/\text{sec}$ and outside: $0.1860.02 \text{mm}^2/\text{sec}$). The mobility of biosensor at lipid rafts is slower than that outside of lipid rafts and is dominated by two-dimensional diffusion. Furthermore, we have developed a general correlative FRET imaging method (CFIM) to quantify the subcellular coupling between an enzymatic activity and a phenotypic response in live cells, e.g. at focal adhesions (FAs). CFIM quantitatively evaluated the cause-effect relationship between Src kinase activation and FA dynamics monitored in single cells. CFIM showed that the growth factor-stimulated FA disassembly at cell periphery was linearly dependent on the local Src activation with a time delay. The FA disassembly per unit of Src activation (coupling capacity), as well as the time delay, was regulated by cell-matrix interaction via different integrin receptors. The results revealed a tight enzyme-phenotype coupling in FA populations mediated by integrin $\alpha_v\beta_3$, but not in those by integrin $\alpha_5\beta_1$. Therefore, our computational analysis methods can allow the high-throughput quantification of molecular motions and interactions at subcellular levels in single live cells. The results should advance our systems understanding of the hierarchical interactions of signaling molecular network at subcellular microdomains.