Summary

A fundamental question in cell biology is how proteins interact and get locally self-assembled as macro-molecular structures that execute diverse functions. Cell matrix adhesion sites are macro-molecular assemblies, consisting of more than 150 proteins, involved in various cellular functions such as cell attachment, cell migration, cell morphogenesis, sensing the immediate environment and cell fate determination. A complex network of regulated interactions between adhesion site components generates highly dynamic and spatially heterogeneous adhesion sites that have distinct molecular composition and function. Collectively, these aspects pose fundamental challenges for monitoring how protein networks get assembled and function in adhesion sites. In this thesis novel concepts and approaches are developed to systematically address these challenges: (1) In order to monitor protein networks with high spatiotemporal resolution, sensitive four-color live cell image acquisition, aligning and correction approaches were developed (Chapter 2). (2) To analyze this four-color data I developed live cell compositional imaging approach to derive the spatiotemporal changes in the molecular composition of adhesion sites at a light resolution. Using this approach, the spatial organization of diverse protein-network states and their dynamics at sub-adhesion site resolution were resolved and visualized in spread fibroblasts as well as in fibroblasts responding to mechanical force perturbations (Chapter 3). (3) In order to understand the regulation and function of protein networks it is essential also to understand how the network components influence each other. Therefore, as a complementary data extraction approach, I developed object segmentation and tracking software

that quantifies the relative changes in the levels of the four imaged components in individual adhesion sites. Using such time series and theoretical approaches, potential causal connections between proteins regulating assembling, disassembling and steady-state focal adhesion were inferred. In order to directly derive causal relations within complex biochemical systems it is required to perturb their components. Therefore I developed computational tools to quantify changes in the levels of proteins in focal adhesions in response to acute perturbations of their components and thereby spatially resolving causal relations between them (Chapter 4). (4) Finally, a fundamental problem in studying large and heterogeneous intracellular biochemical systems such as adhesion sites is the inability to co-monitor all the components, which can lead to distinct observed relations between the same subset of observed components. Therefore I was computationally involved in a collaborative development of a statistical method to unmix observations derived from a mixture of protein networks with distinct topologies (Chapter 5). To conclude, in this thesis the above mentioned challenges are addressed, novel tools are developed and thereby structure-function relationships of heterogeneous protein networks of adhesion sites are resolved with high spatial and temporal resolutions.