Mass cytometry uniquely enables high-dimensional single-cell proteomic analysis for system-level discovery and comprehensive functional profiling applications. The large 40-plus marker proteomic panels routinely analyzed using mass cytometry provide simultaneous measurement of the breadth of cell types and the depths of their functions in a single tube. We will describe the basic principles and workflow of the technology, including recent advancements incorporated into the newest CyTOF® system, Helios™. Using data from published reports, we will discuss the scientific questions that are being addressed with mass cytometry, the analysis methods used to explore the high-content data that is generated, and the unique insights and discoveries gained by using this technology. We will also discuss the suite of reagents and tools that simplify development and execution of large-panel experiments, enabling researchers to unravel the complex heterogeneity inherent to biological systems.