T cell-mediated recognition of peptide-major histocompatibility complex (pMHC) class I and II molecules is crucial for the control of intracellular pathogens and cancer, as well as for stimulation and maintenance of efficient cytotoxic responses. Such interactions may also play a role in the development of autoimmune diseases. Novel insights into this mechanism are crucial to understanding disease development and establishing new treatment strategies. MHC multimers have been used for detection of antigen-responsive T cells since the first report by Altman et al. showed that tetramerization of pMHC class I molecules provided sufficient stability to T cell receptor (TCR)-pMHC interactions, allowing detection of MHC multimer-binding T cells using flow cytometry. Since this breakthrough the scientific community has aimed for expanding the capacity of MHC multimer-based detection technologies to facilitate large-scale epitope discovery and immune monitoring in limited biological material. Screening of T cell specificity using large libraries of pMHC molecules is suitable for analyses of T cell recognition potentially at genome-wide levels rather than analyses restricted to a selection of model antigens. Such strategies provide novel insights into the immune specificities involved in disease development and response to immunotherapy, and extend fundamental knowledge related to T cell recognition patterns and cross-recognition by TCRs. MHC multimer-based technologies have now evolved from detection of 1-2 different T cell specificities per cell sample, to include more than 1000 evaluable pMHC molecules using novel technologies. Here, we provide an overview of MHC multimer-based detection technologies developed over two decades, focusing primarily on MHC class I interactions.