Project 13: Analysis of host-pathogen interactions through single-cell RNA sequencing
(Supervising Investigator: Alexander Dilthey)

Background: The invasion of a host cell by an intracellular pathogen involves a complex interplay between host defensive mechanisms and the pathogen's attempts to overcome these, e.g. by the manipulation of cell autonomous defense or interferon response pathways. A key question is how these interactions are orchestrated and structured on the transcriptional level, i.e. when and mediated by which transcription factors which genes are activated on the pathogen and the host side. Novel single cell RNA sequencing technologies (scRNA-seq) enable the characterization of host-pathogen interactions at the level of individual cells at unprecedented accuracy and resolution.

Own previous work: The lab has extensive expertise in genomic data science, in the development and application of next-generation sequencing-based assays, and in bioinformatics methods development. Collaborating groups (Pfeffer and Hegemann) in the MOI graduate school have extensive expertise in the functional analysis of the intracellular pathogens *Toxoplasma gondii* und *Chlamydia trachomatis*.

Aim of the project: To characterize host cell invasion and host-pathogen interactions of *Toxoplasma gondii* and *Chlamydia trachomatis* at high resolution using scRNA-seq; to better characterize the kinetics of host defense mechanisms and the strategies employed by the pathogens to overcome these; and to integrate scRNA-seq data with existing functional data on e.g. the GBP effector molecules.

Work program:

- scRNA-seq-based transcriptomic characterization *T. gondii*-infected, *T. gondii*-infected and IFN-γ-stimulated, and *T. gondii* mock infected murine cells at different time points.
- Completion of the genome and complete characterization of the transcriptome of *T. gondii* strain ME49 through long-read sequencing technologies.
- Bioinformatic analysis of the generated scRNA-seq data, in particular with respect to differential gene expression, transcription factors, and expression kinetics.
- Development of algorithms to determine infection status and likely time point of infection of individual cells based on scRNA-seq data.
- Analogous experiments and analyses will be carried out for *C. trachomatis.*