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Development of High Content Imaging Assays for Lethal Viral Pathogens

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Filoviruses Ebola and Marburg are among the most pathogenic agents that cause hemorrhage fevers in humans and non-human primates. Filovirus infection causes significant morbidity and mortality, and to date, there exist no FDA approved vaccines or antiviral drugs to treat this deadly infection. The development of effective therapeutic and preventative measures to Ebola and related viruses has been limited due to lack of suitable high throughput assays for screening chemical libraries. Traditional assays to monitor viral replication and study the effect of antiviral compounds include qualitative visual observation of cytopathic effects and quantitation of infectious virus particles using plaque assays or of viral RNA transcripts by real time RT-PCR. However, these assays are time consuming, tedious and not amenable to high-throughput screening for drug discovery. Assays based on non-infectious virus-like particles or pseudotype viruses carrying the filovirus glycoprotein are currently being used to study and identify inhibitors of viral entry. However, these systems do not mimic the characteristics of authentic virions.

High content imaging is a state-of-the-art technology that is increasingly being applied for primary drug screening. Although these screens provide cell population data, more detailed information from individual cells can be extracted during image analysis. Image-based screens to monitor viral infection following gene targeted knock-down strategies or treatment with chemical compounds serve to identify critical host targets and therapeutic lead molecules. In this study, we describe the development and optimization of bioassays based on image acquisition and analysis for the highly pathogenic filoviruses Ebola and Marburg. Further, we demonstrate the benefits of statistical analysis of single-cell data for assay optimization and to account for heterogeneity in the viral antigen staining pattern. In conclusion, we demonstrate the potential application of image-based screening assays for highly pathogenic viruses.