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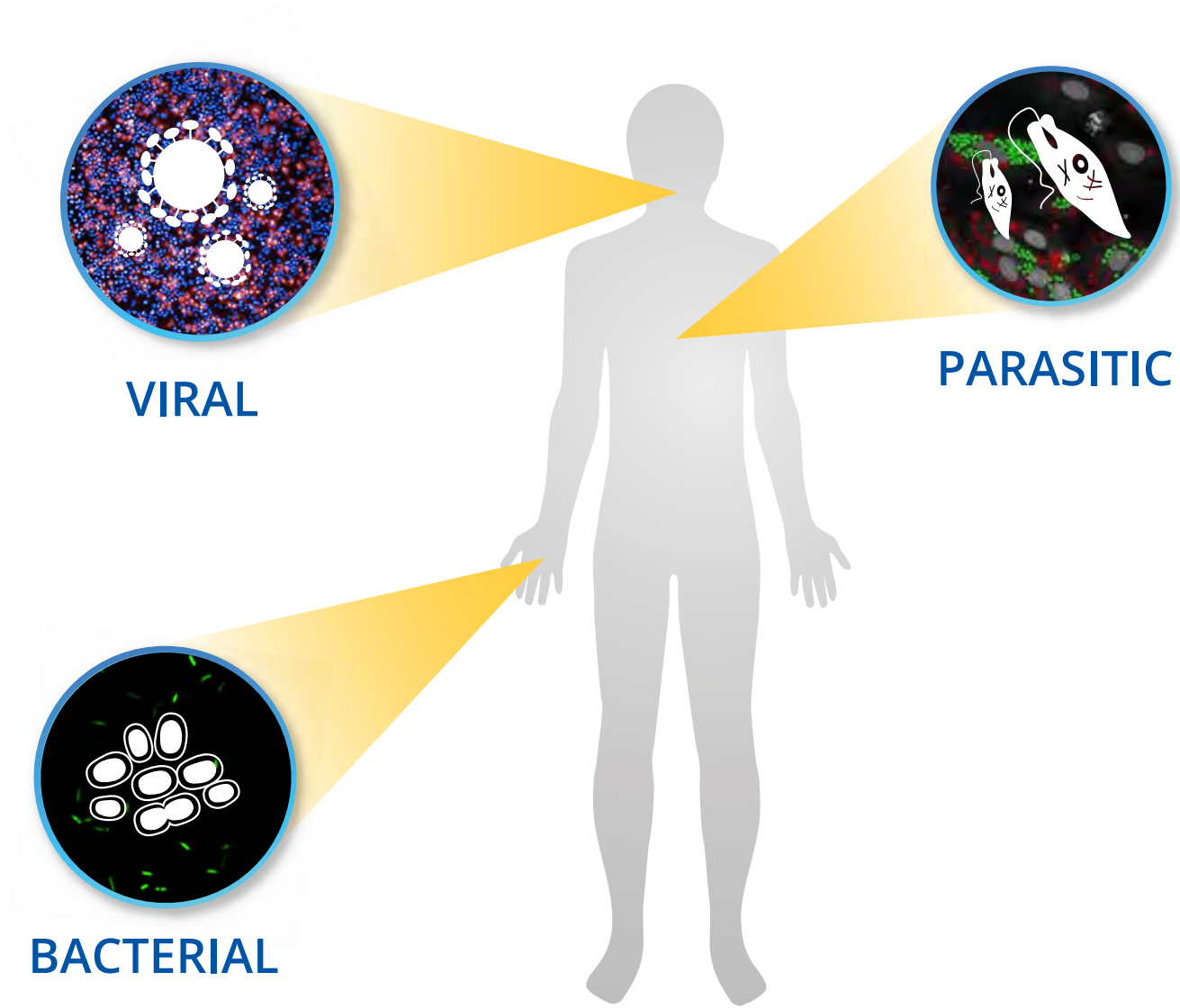
HIGH-CONTENT ANALYSIS ADVANCING YOUR KNOWLEDGE TO HELP COMBAT INFECTIOUS DISEASE



Viral image: Image from Barrows NJ, Campos RK, Powell ST, Prasanth KR, Schott-Lerner G et al. A screen of FDA-approved drugs for inhibitors of Zikavirus infection. Cell Host Microbe 2016;20(2):259–70

Parasitic image: Image from Automated High-Content Assay for Compounds Selectively Toxic to Trypanosoma cruzi in a Myoblastic Cell Line. Alonso-Padilla, J. et al. PLoS Negl Trop Dis. 2015; Jan, 9(1): e0003493.

Bacterial image: Confocal image of Salmonella bacteria labelled with GFP acquired on Opera Phenix with a 63x water objective NA 1.15. Sample courtesy of D. Goulding, Sanger Institute, UK.

Using High-Content Analysis to Study Infectious Disease | Publication Notes and Case Studies | Selected Publications | High-Content Analysis Technologies | PerkinElmer Solutions for High-Content Analysis | SEND ME DETAILS

VIRAL DISEASE

Featured Publication Notes

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SEE FILE

The Role Of TGF-β In Ebola Virus Infection Of Human Hepatocytes As Assessed By Phenotypic Screening

Jason Kindrachuk and colleagues

Ebola virus (EBOV) is a serious public health concern, causing severe hemorrhagic disease in humans with a mortality rate of more than 78%. The authors used a kinome analysis to investigate the host kinome response over time in human hepatocyte cells infected with EBOV. This showed that transforming growth factor β (TGF-β)-mediated pathways are modulated during EBOV infection and replication. Phenotypic screening showed that EBOV infection is inhibited in the presence of TGF-β and related pathway kinase inhibitors.

SEE FILE

A Screen of FDA-Approved Drugs for Inhibitors of Zika Virus Infection

Nicholas Barrows and colleagues

High-content screening and analysis of a panel of 774 FDA-approved drugs identified over 20 molecules that could inhibit Zika virus infection in human cells including cervical, placental, neural stem cells and primary amnion cells – all are potential new therapeutics in the fight against Zika.

SEE FILE

Identification of Proteins Bound to Dengue Viral RNA *In Vivo* Reveals New Host Proteins Important for Virus Replication

Stacia Phillips and colleagues

An *in vivo* approach involving UV crosslinking, antisense-mediated affinity purification and mass spectrometry identified host proteins that physically associate with dengue virus RNA. Small interfering RNA-mediated gene silencing, combined with high-content screening demonstrated that over half of these proteins are likely to be involved in regulating dengue virus replication.

BACTERIAL DISEASE

Featured Publication Notes and Case Study

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Testing Chemical & Genetic Modulators in *Mycobacterium tuberculosis* Infected Cells Using Phenotypic Assays

Vincent Delorme and colleagues

A better understanding of the intracellular stage of *M. tuberculosis* pathogenesis may aid development of new agents in the battle against tuberculosis infection. High-content screening was used to investigate the role of host genes in intracellular replication, identify small molecule inhibitors within the host cell, and examine the role of bacterial genes in intracellular trafficking.

SEE FILE

A Dual Microscopy-Based Assay to Assess *Listeria monocytogenes* Cellular Entry and Vacuolar Escape

J.J. Quereda, J. Pizarro-Cerda and colleagues

Listeriosis-causing *L. monocytogenes* invades mammalian cells and escapes from the vacuole, enabling proliferation in the host cell cytoplasm. A two-step method involving high-content screening was developed to investigate cell invasion and vacuolar rupture in a single experiment, providing a powerful tool which could be used for identifying the factors involved in these processes in bacteria and other pathogens.

SEE FILE

B Cell Selection and Therapeutic Antibody Characterization Using the Operetta High-Content Imaging System

Infection with the intestinal bacterium *Clostridium difficile* is the most common cause of healthcare-associated diarrhoea that can develop in patients after hospitalization and treatment with antibiotics. *C. difficile* is resistant to a wide range of antibiotics and, for this reason, new treatments for severe cases are desperately needed. *C. difficile* infection is mediated by the production of toxins by the bacterium, and treatment with toxin-binding agents, such as antibodies is a promising approach to reducing or inhibiting the clinical manifestations. This case study describes the workflow used by researchers at AIMM to generate antibodies against *C. difficile* ToxB.

PARASITIC DISEASE

Featured Publication Notes

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High-Throughput Assay and Discovery of Small Molecules that Interrupt Malaria Transmission

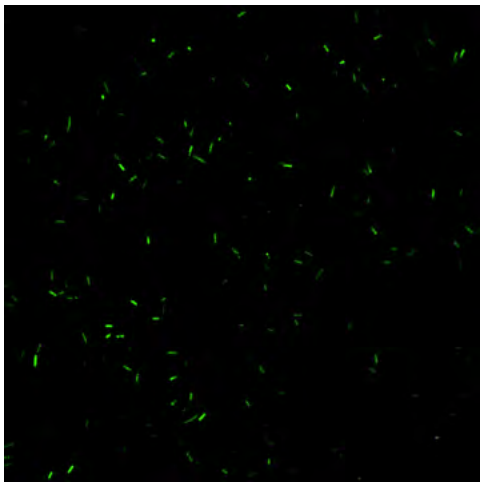
David M Plouffe and colleagues

The Saponin-lysis Sexual Stage Assay (SaLSSA) is a high-throughput, cost-effective assay for identifying small molecules with malaria transmission-blocking activity. A total of 13,983 compounds were analyzed, which included some with consistent low nanomolar transmission-blocking activity. The assay provides a tool for the discovery and development of transmission-blocking drugs.

Using High-Content Analysis To Study Infectious Disease

Infectious diseases caused by bacterial, viral or parasitic pathogens are a major burden to global health. Devastating infections without effective vaccines or anti-microbial treatment still exist, as illustrated by the 2014 West African Ebola virus outbreak that caused more than 11,000 deaths[1] in 10 countries. The increased globalization of modern society, with travel and trade that facilitates the spread of emerging and re-emerging infectious diseases, and phenomena such as anti-microbial resistance, underscore the importance of the development of new preventative and therapeutic approaches.

High-content analysis plays a significant role in infectious disease research **as recently reviewed by Ang and Pethe (2016)**, enabling high-throughput functional and phenotypic assays that can be adapted to a wide range of pathogens. Originally developed as a complementary technology to traditional biochemical high-throughput screening (HTS) in drug discovery, today high-content analysis is established in a far broader area of the life science space as an unbiased imaging method to assess cellular function. Applications include genetic siRNA interference or CRISPR screens for identifying host factors involved in host–pathogen interactions, but also compound screens for drug discovery.



Confocal image of Salmonella bacteria labelled with GFP acquired on Opera Phenix with a 63x water objective NA 1.15, sample courtesy of D. Goulding, Sanger Institute, UK.

More meaningful results

Screening can be performed within the context of a host cell, allowing drugs that target host cell or microbial factors to be discovered. High-content screening allows the analysis of complex processes such as host cell entry, intracellular trafficking or cell-to-cell spread using fixed samples or live cell imaging.

More information from cellular samples

Fully automated imaging and unbiased quantitative image analysis exploits the full potential of microscopy, allowing the characterization of pathogens as well as host cell phenotypes. Infection rates can be determined with high sensitivity, since individual infected cells can be identified. The process of infection can be described with up to hundreds of readouts per cell allowing the linkage of infection rates to other readouts such as host cell morphology or host cell signalling.

More questions answered simultaneously

Data sets are more information-rich compared to non-imaging read-outs, allowing a number of conclusions to be drawn from one experiment, such as drug efficacy against a pathogen as well as drug toxicity on host cells.

More samples analyzed

The automation of imaging tasks allows the analysis of significantly more samples compared to conventional microscopy, enabling genome-wide or kinome-wide siRNA screens or screening of large compound libraries.

[1] Retrieved Dec 12, 2016 from https://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/case-counts.html

Publication Notes and Case Studies

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VIRAL DISEASE

- **The Role Of TGF-β In Ebola Virus Infection Of Human Hepatocytes As Assessed By Phenotypic Screening**
  Jason Kindrachuk and colleagues
- **A Screen of FDA-Approved Drugs for Inhibitors of Zika Virus Infection**
  Nicholas Barrows and colleagues
- **Identification of Proteins Bound to Dengue Viral RNA *In Vivo* Reveals New Host Proteins Important for Virus Replication**
  Stacia Phillips and colleagues

BACTERIAL DISEASE

- **Testing Chemical & Genetic Modulators in *Mycobacterium tuberculosis* Infected Cells Using Phenotypic Assays**
  Vincent Delorme and colleagues
- **A Dual Microscopy-Based Assay to Assess *Listeria monocytogenes* Cellular Entry and Vacuolar Escape**
  J.J. Quereda, J. Pizarro-Cerda and colleagues
- **B Cell Selection and Therapeutic Antibody Characterization Using the Operetta High-Content Imaging System**

PARASITIC DISEASE

- **High-Throughput Assay and Discovery of Small Molecules that Interrupt Malaria Transmission**
  David M Plouffe and colleagues

Selected Publications Featuring High-Content Technologies in the Study of Viral Disease

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VIRAL DISEASE

BACTERIAL DISEASE

PARASITIC DISEASE

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VIRAL DISEASE | BACTERIAL DISEASE | PARASITIC DISEASE

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Selected Publications Featuring High-Content Technologies in the Study of Parasitic Disease

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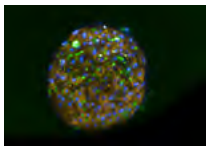
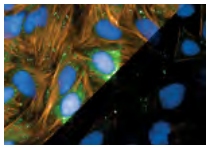
High-Content Analysis And Screening Technologies To Study Infectious Disease

PerkinElmer has more than a decade of experience in developing confocal High-Content Screening systems. The Opera Phenix™ and Operetta CLS™ systems have been fully designed in-house, which allowed control of all design aspects and technologies. Besides combining market leading automated microscopy and image analysis performance, special attention was paid to the requirements of live cell experiments from light management and environmental control, to the ease of cleaning and minimizing of risk of internal contamination of the system.



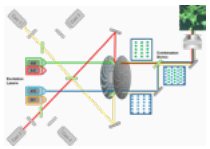
Confocal Imaging

Spinning disk confocality is a fast and gentle technique to reject light from out of focus planes, allowing live cell imaging with minimal photobleaching and phototoxicity. This option gives full flexibility to optimize image quality, depending on the size and morphology of intracellular and extracellular pathogen samples.



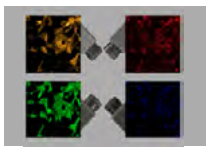
Water Immersion Objectives

Proprietary automated water-immersion objectives with very high numerical aperture deliver and capture more photons allowing imaging of weakly stained samples and provide a higher resolution in XYZ than conventional objectives. This enables good image quality from tiny objects like pathogens inside cells.



Synchrony Optics

The proprietary Synchrony Optics™ technology reduces spectral crosstalk during simultaneous imaging on multicamera Opera Phenix systems. This increases speed up to 4-fold, allowing genome-wide siRNA screens or large compound screens to be done in reasonable time without compromising on sensitivity.



sCMOS cameras

The large format sCMOS cameras – one in the Operetta CLS and up to four in the Opera Phenix – deliver low noise, wide dynamic range and high resolution – perfect for capturing large numbers of cells, e.g. to detect infection rates with high sensitivity.

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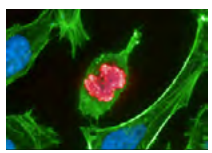
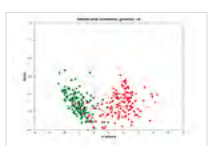


Intelligent Image Acquisition

Intelligent acquisition technology – PreciScan™ – employs a pre-scan at low magnification to identify objects of interest and a re-scan of these objects at higher magnification, limiting the acquisition to just part of the wells or particular wells of interest. This saves time and hard drive space when analyzing rare events like low multiplicities of infection (MOI).

Harmony Software Building Blocks

PerkinElmer's Harmony® software is known for its easy-to-use building blocks, which allow step-by-step image analysis, making it easy for even novice users to generate results, without prior image analysis knowledge. The Operetta CLS and the Opera Phenix are therefore ideally suited for multi-user facilities as new scientists can quickly become familiar with the system and software.



Machine Learning

Machine learning techniques, either supervised or unsupervised, enable the user to distinguish phenotypes using classifiers based on feature combinations rather than on a single parameter or allow image segmentation using a learn-by-example approach.
While other systems may require an image analysis expert to create an algorithm, machine learning makes it easy to do it on your own.

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Solutions For High-Content Analysis And Screening

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Opera Phenix High-Content Screening System

The Opera Phenix™ high-content screening system is the premier confocal solution for today's most demanding applications, such as spheroid imaging or high resolution phenotyping.

Operetta CLS High-Content Analysis System

Uncover deep biological understanding from cell health to bacterial invasion assays with the flexible Operetta CLS™ high-content analysis system.

Harmony High-Content Imaging & Analysis Software

Harmony® provides a complete solution with all the tools you need to set up and automate your experiments, aquire images and analyze data and then store, retrieve and present the results.

Columbus High Volume Data Management & Analysis System

The Columbus™ system is a unique, universal high-volume image data storage and analysis system that brings access to images from a wide range of sources including all major high-content screening instruments via the Internet.

High-Content Profiler

High-Content Profiler™ enables true multiparametric hit selection from high-content screens, from quality control to normalization to machine learning, in one single workflow powered by TIBCO Spotfire®.

cell::explorer Automated High-Content Screening Workstation

cell::explorer™ can automate your entire high-content screening workflow from treatment to readout.

Microplates

We offer microplates designed specifically for high-content screening. For example, our validated CellCarrier™ Ultra plates feature an ultra-low bottom with superior flatness and clarity for optimal image quality day in and day out.

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