6th International Symposium on Systems Biology of Microbial Infections

HKI Center for Systems Biology of Infection, Jena, Germany

SBMI 2021 Program

Please note that all presentations are directed to CET.

Thursday 11 November

10.00 Opening

Session I: Virus

Chair: Thilo Figge (Jena)

10.10-10.50 **Stefanie Deinhardt-Emmer** (Jena)

The crosstalk between respiratory pathogens and the host: the alveolar homeostasis in the face of the enemy

10.50-11.20 **Paul Rudolph** (Jena) **& Johannes Forster** (Wuerzburg)

A state based model and feasibility study to design an optimal COVID-19 surveillance protocol for child care facilities

11.20-11.40 **Gabriel Krause** (Berlin)

Virtual infection model and cell generator: an open source Godot-based framework for immune reaction studies

11.40-12.00 **Julian Kauk** (Jena)

'Flatten the curve' of misinformation: Epidemic models as a promising new framework for studying the spread and containment of low-credibility information in social networks

12.00-13.00 Break

Session II: Fungi, Part I

Chair: Christoph Saffer (Jena)

13.00-13.40 **Paul Bowyer** (Manchester/UK)

Aspergillus - the Evolution will be Televised

13.40-14.10 **Amelia Barber** (Jena) **& Tongta Sae-Ong** (Jena)

Aspergillus fumigatus pan-genome analysis identifies genetic variants associated with human infection

14.10-14.30 **Katarina Jojić** (Jena)

Dual localization and role of the aminotransferase of the sphingofungin biosynthetic cluster in the pathogenic fungus Aspergillus fumigatus

14.30-14.50 Break

Session III: Bacteria, Part I

Chair: Sandra Timme (Jena)

14.50-15.30 **Denise Kirschner** (Ann Arbor, MI/USA)

A systems biology approach to understanding the immunobiology of tuberculosis infection and treatment

15.30-15.50 **Nicolas Personnic** (Lyon/FR)

Bacterial eco-pathology: how the individuals shape the community

15.50-16.10 Break

Session IV: Fungi, Part II
Chair: Carl-Magnus Svensson

16.10-16.50 **Réka Albert** (University Park, PA/USA)
Mathematical modeling of the Candida albicans yeast to hyphal transition reveals key decision points and predicts control strategies

16.50-17.10 **Nadja Thielemann** (Wuerzburg)

Intestinal mycobiome shifts are associated with liver fibrosis in NAFLD patients

17.10-17.30 **Daniel Wüstner** (Odense/DK)

Label-free imaging of polyene macrolides and their ergosterol-dependent interaction with yeast cell membranes

Friday 12 November

Session V: Inflammation and Immune Response
Chair: Philipp Praetorius

10.00-10.40 **Matthias Gunzer** (Essen/Duisburg)

Whole organ quantitative biology with light sheet microscopy to uncover the impact of sterile and infectious inflammation on tissue integrity

10.40-11.00 **Jan Dudeck** (Magdeburg)

Directional mast cell degranulation of tumor necrosis factor into blood vessels primes neutrophil extravasation

11.00-11.20 **Stefan Schuster** (Jena)

Optimizing defence, counter-defence and counter-counter defence in host-pathogen interactions – A modelling study

11.20-11.40 **Rustam Guliev** (Jena)

Discrimination between macrophages phenotypes using Raman spectroscopic imaging

11.40-12.00 **Artur Yakimovich** (London/UK, Dresden)

Novel Machine Learning Approaches in Image-based Host-pathogen Interactions Analysis

12.00-13.00 Break

Session VI: Bacteria, Part II
Chair: Carl-Magnus Svensson

13.00-13.30 **Effie Bastounis** (Tübingen) **& Raúl Aparicio** (Zaragoza/SP)

Borrelia burgdorferi induces alterations in the biomechanics and gene expression of host endothelial cells at early but not late in vitro infection

13.30-14.00 **Claudia Vilhena** (Jena) **& Zoltan Cseresnyes** (Jena)

Super-resolution microscopy reveals the dynamic subcellular localization of pneumococcal proteins during an immunological challenge

14.00-14.20 **Natalia O. Dranenko** (Moscow/RU)

Evolution of ipaH family proteins in human and non-human hosts Escherichia

14.20-14.40 Break

Session VII: Fungi, Part III
Chair: Sandra Timme (Jena)

14.40-15.00 **Dalia Sheta** (Wuerzburg)

M-CSF fosters tissue resident alveolar macrophages to protect from lethal invasive aspergillosis early after hematopoietic cell transplantation

15.00-15.20 **Christoph Saffer** (Jena)

Comparative simulations of fungal infection dynamics in the human and murine lung

15.20-15.40 **Susann Hartung** (Jena)

Quantitative characterisation of Aspergillus fumigatus hyphal growth in an "Invasive aspergillosis-on-chip" disease model of the human lung

Session VIII: Co-Infection
Chair: Paul Rudolph (Jena)

15.40-16.20 **Sherli Koshy-Chenthittayil** (Farmington, CT/USA)

Streptococcus oralis and Lactobacillus paracasei interactions through the lens of an agent-based model

16.20-16.50 **Raquel Alonso-Román** (Jena) **& Sascha Schäuble** (Jena)

Lactobacillus rhamnosus colonisation antagonizes Candida albicans by forcing metabolic adaptations that compromise pathogenicity

16.50-17.00 Closing words

Keynote Speakers

Mathematical modeling of the *Candida albicans* yeast to hyphal transition reveals key decision points and predicts control strategies

Réka Albert[1], DJ Wooten[1], JGT Zañudo[2], D. Murrugarra[3], AM. Perry[4], A. Dongari-Bagtzoglou[5], Reinhard Laubenbacher[6], Clarissa J Nobile[7]

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[3] University of Kentucky, Lexington, KY, USA
[4] University of California Merced, Merced, CA, USA
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[6] University of Florida, Gainesville, FL, USA

Phenotypic plasticity between two morphological phenotypes, yeast and hyphae, is a key mechanism by which *Candida albicans* can thrive in many microenvironments and cause disease in a host. Understanding the decision points and key driver genes controlling this important transition and how these genes respond to different environmental signals is critical to understanding how *C. albicans* causes infections. My group built a mathematical model of the *C. albicans* yeast to hyphal transition, integrating multiple environmental factors and regulatory mechanisms. We validated the model by a systematic comparison to prior experiments. The discrepancies motivated alternative hypotheses that are testable by follow-up experiments. Analysis of this model (by methods initiated by my group) revealed two time-constrained windows of opportunity that must be met for the complete transition from the yeast to hyphal phenotype, as well as control strategies that can robustly prevent this transition. Our collaborators experimentally validated two of these control predictions in *C. albicans* strains lacking the transcription factor UME6 and the histone deacetylase HDA1, respectively. We expect this model will serve as a strong base from which to develop a systems biology understanding of *C. albicans* morphogenesis.

Reference:
Mathematical modeling of the Candida albicans yeast to hyphal transition reveals novel control strategies, DJ Wooten, JGT Zañudo, D Murrugarra, AM Perry, A Dongari-Bagtzoglou, Reinhard Laubenbacher, Clarissa J Nobile, Réka Albert, PLoS computational biology 17 (3), e1008690 (2021)

Aspergillus - the Evolution will be Televised

Paul Bowyer[1,2], Sara Gago[1,2], Mike Bromley[1,2], Aiah Khateb[1,2], Marcin Fraczek[1,2]

[1] Manchester Fungal Infection Group, Manchester, United Kingdom

[2] Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom

Aspergillus fumigatus is the most important mould pathogen of man. This fungus affects the health of over 20 million people worldwide and is responsible for >350,000 deaths annually. The majority of mortality due to *Aspergillus* infection is caused by chronic infections that follow tuberculosis, COPD, sarcoidosis or other lung damaging conditions. These infections typically last a period of several years and carry a cumulative year on year mortality of about 8%.

We recently observed that fungi resident in the lung for >5 years had significant changes in their genomes. This adaptation is the end product of an adaptive process that begins with first contact of the fungal spores with the lung epithelium. Specific genome types of *A. fumigatus* preferentially infect in cases of chronic disease. Phagocytosis and killing of spores are the first steps in compatibility. This is followed by signalling from epithelium to specialised immune cells. Finally, genetic factors in the host play a strong role in receptivity of the host to fungal infection and persistence of the infection.

Fungi that survive this initial interaction, which typically lasts a few hours to days, become resident and begin to establish infections. In CPA these lead to lung cavitation and formation of fungal balls with devastating consequences for the host. Almost all chronic infections become drug resistant – either before or after antifungal treatment as adaptation to the stresses in the lung induces drug resistant phenotypes. Adaptation appears to be driven by large scale genome rearrangements more frequently than by single nucleotide changes.

Understanding features of the *A. fumigatus* genome that allow survival of early interactions at the lung epithelium and that occur during growth in the lung is critical to our understanding pf pathogenicity and may allow targeted diagnostic approaches to identifying at-risk infections.

The crosstalk between respiratory pathogens and the host: the *alveolar homeostasis* in the face of the enemy

Stefanie Deinhardt-Emmer[1,2]

[1] Jena University Hospital, Jena, Germany
[2] The Buck Institute for Research on Aging, Novato, CA USA

The process of breathing is realized in a highly specialized system, the *alveolus*. The fragile *alveolar homeostasis* is challenged during life by numerous respiratory pathogens resulting in epithelial and endothelial damage, barrier disruption, and robust inflammatory response. Nevertheless, various host defense strategies preserve this balance by immune cell migration and the expression of specific substances. However, these processes can be strongly influenced by important comorbidity factors, e.g., obesity and aging.

The host-pathogen crosstalk can be investigated by using different model systems. Considerable progress has been made in this area in recent years; organoids, the alveolus-on-a-chip, and humanized mouse models are available to figure out the pathomechanisms behind the infection.

Own data demonstrate that especially bacterial co-infections in the context of viral disease lead to severe barrier damage and that inhibitors of intracellular signaling processes can result in a sufficient inhibition of virus replication. The current COVID-19 pandemic demonstrates clearly that host factors such as senescence can lead to an increased susceptibility, which, due to the broad organ tropism of SARS-COV-2, can also result in damage outside the lung.

Using sophisticated technologies, system biology can improve the understanding of the interplay between pathogens and the host to establish new solutions against our enemies.

Whole organ quantitative biology with light sheet microscopy to uncover the impact of sterile and infectious inflammation on tissue integrity

Matthias Gunzer[1,2]

[1] Institute for Experimental Immunology and Imaging, University Hospital Essen, Essen, Germany
[2] University of Duisburg-Essen, Duisburg, Germany

Organs are delicate 3-D structures with high degrees of functional organization. These structures are affected on the microscopical but also meso- and macroscopical level by pathological conditions. We have been using light sheet fluorescence microscopy (LSFM) to investigate the fine details of organs in unprecedented detail with a focus on blood vessels systems and infiltrating immune cells. In this talk I will briefly introduce the principal method and then demonstrate on several examples how inflammation massively affects a delicately structured environment.

A systems biology approach to understanding the immunobiology of tuberculosis infection and treatment

Denise Kirschner[1,2]

[1] Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI USA
[2] Center for Systems Biology, University of Michigan Medical School, Ann Arbor, MI USA

Tuberculosis (TB) is one of the world's deadliest infectious diseases. Caused by the pathogen *Mycobacterium tuberculosis* (Mtb), the standard regimen for treating *TB* consists of treatment with multiple antibiotics for at least six months. There are a number of complicating factors that contribute to the need for this long treatment duration and increase the risk of treatment failure. The structure of granulomas, lesions forming in lungs in response to *Mtb* infection, create heterogeneous antibiotic distributions that limit antibiotic exposure to *Mtb*. We can use a systems biology approach pairing experimental data from non-human primates with computational modeling to represent and predict how factors impact antibiotic regimen efficacy and granuloma bacterial sterilization. We utilize an agent-based, computational model that simulates granuloma formation, function and treatment, called GranSim. A goal in improving antibiotic treatment for TB is to find regimens that can shorten the time it takes to sterilize granulomas while minimizing the amount of antibiotic required. We also created a whole host model to study *Mtb* dynamics within a human host. Overall, we use these models to help better understand *TB* treatment and strengthen our ability to predict regimens that can improve clinical treatment of *TB*.

Streptococcus oralis and *Lactobacillus paracasei* interactions through the lens of an agent-based model

Sherli Koshy-Chenthittayil[1], Linda Archambault[1,2], Angela Thompson[2], Anna Dongari-Bagtzoglou[2], Reinhard Laubenbacher[3], Pedro Mendes[1,4]

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[3] Department of Medicine, University of Florida, Gainesville, FL USA
[4] Center for Cell Analysis and Modeling, University of Connecticut School of Medicine, Farmington, CT USA

The oral commensal *Streptococcus oralis* is known to increase the virulence of *Candida albicans* infections in murine oral candidiasis and epithelial cell models through mechanisms which promote the formation of tissue-damaging biofilms. Many probiotic *Lactobacillus* species are known to inhibit the growth of *Streptococcus* species. We used an iterative collaborative process between experimentation and modeling to create and test a three-dimensional agent-based model containing *L. paracasei* and *S. oralis* in a biofilm. The model included growth, division, decay, and mechanical interactions of each individual bacterium in the biofilm. Through this process, the mostly unexplored relationship between *S. oralis* and *L. paracasei* in biofilm growth has been investigated to reveal the inhibitory nature of *L. paracasei* on *S. oralis* biofilms.

Talks

Lactobacillus rhamnosus colonisation antagonizes *Candida albicans* by forcing metabolic adaptations that compromise pathogenicity

Raquel Alonso-Román[1], Sascha Schäuble[2], Mohammad H. Mirhakkak[2], Antonia Last[1], Lars Möller[1], Jakob L. Sprague[1], Peter Großmann[2], Katja Graf[1], Rena Gratz[1], Selene Mogavero[1], Slavena Vylkova[3], Gianni Panagiotou[2], Bernhard Hube[1], Mark Gresnigt[4]

[1] Department of Microbial Pathogenicity Mechanisms – Hans Knöll Institute, Jena, Germany
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Dysbiosis of the intestinal bacterial microbiota caused by antibiotic treatment can initiate overgrowth of commensal *Candida* species – a predisposing factor for disseminated candidiasis. The gut colonizing bacterium and probiotic *Lactobacillus rhamnosus* can antagonize *C. albicans*, the most frequent cause of disseminated candidiasis. To uncover the molecular mechanisms of bacterial antagonism, we investigated the interplay between *C. albicans*, *L. rhamnosus*, and intestinal epithelial cells (IECs) by integrating transcriptional andSDF metabolic profiling, and reverse genetics. Untargeted metabolomics combined with *in silico* modelling of *L. rhamnosus* metabolism not only identified antivirulence and antifungal metabolites, but also suggested that IECs foster bacterial growth by releasing specific metabolites such as citric acid, gamma-glutamylalanine and carnitine, which was confirmed experimentally. Bacterial growth on IECs modifies the metabolic environment, including the removal of carbon sources energetically favoured by *C. albicans.* Consequently, *C. albicans* is forced to transcriptionally rewire its metabolism and cell biology, which is associated with the reprogramming of specific genes that play a role in virulence. Deletion mutants of these genes exhibit a reduced damage potential, providing a causal explanation for the antagonistic effects driven by metabolic alterations in our model. Altogether, our research suggests that intestinal colonization with bacteria can antagonize *C. albicans* by reshaping the metabolic environment, forcing metabolic adaptations and consequently reducing fungal pathogenicity.

Aspergillus fumigatus pan-genome analysis identifies genetic variants associated with human infection

Amelia Barber[1,8], Tongta Sae-Ong[2], Kang Kang[2], Bastian Seelbinder[2], Jun Li[3,4], Grit Walther[5], Gianni Panagiotou[2,6], Oliver Kurzai[1,5,7]

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[3] Department of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong, China
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[7] Institute for Hygiene and Microbiology. University of Würzburg. Würzburg, Germany
[8] Junior research group Fungal Informatics, Leibniz Institute of Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany

Aspergillus fumigatus is an environmental saprobe and opportunistic human fungal pathogen. Despite the more than 300,000 cases of invasive disease globally each year, a comprehensive survey of the genomic diversity present, including the relationship between clinical and environmental isolates, and how this genetic diversity contributes to virulence and antifungal drug-resistance, has been lacking. In this study, we define the pangenome of *A. fumigatus* using a collection of 300 environmental and clinical genomes from a global distribution, 188 of which were sequenced in this study. We find that of the 10,907 unique orthogroups, 7,563 (69%) are core and found in all isolates, while 3,344 show presence/absence variation, representing 16-22% of each isolate's genome. Using this large genomic dataset of both environmental and clinical samples, we found an enrichment for clinical isolates in a genetic cluster whose genomes also contain more accessory genes, including more transmembrane transporters, proteins with iron-binding activity, and genes involved in both carbohydrate and amino acid metabolism. Finally, we leverage the power of genome-wide association to identify genomic variation associated with clinical isolates and triazole resistance as well as characterize genetic variation in known virulence factors. This characterization of the genomic diversity of *A. fumigatus* allows us to move away from a single reference genome that does not necessarily represent the species as a whole and better understand its pathogenic versatility, ultimately leading to better management of these infections.

Borrelia *burgdorferi* induces alterations in the biomechanics and gene expression of host endothelial cells at early but not late *in vitro* infection

Effie Bastounis[1], Raúl Aparicio Yuste[2], Annalena Reuss[1], Grace Blacker[3], Michal Caspi Tal[3], María J. Gómez Benito[2]

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[3] Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA USA

Bacterial pathogens can alter host cell mechanics to promote their own dissemination through tissues. Borrelia burgdorferi (Bb), the causative agent of Lyme Disease, is an extracellular bacterial pathogen that can spread to distant tissues by travelling in and through the vasculature, lined by a single continuous endothelial cell monolayer. To examine whether Bb infection induces changes in host endothelial cell biomechanics, we used video-microscopy to monitor endothelial cell responses to prolonged exposure to Bb. We found that initially Bb cells homogeneously spread over the endothelial cell monolayers maintaining their spirochete morphology and increased motility, but over time less motile Bb aggregates formed, some of which were intracellular, viable and persisted overtime. We hypothesized that the different forms that Bb attained over the course of infection (spirochetal versus aggregate form) could impact distinctly host cell biomechanics and response to infection. Consistently, we found that host cell motility, cell-extracellular matrix and cell-cell forces in response to infection were transiently reduced during the early stages of infection but reverted to levels similar to those of cells not exposed to infection at later stages when bacterial aggregates formed. To examine how biochemical signaling might regulate the time-dependent biomechanical responses to infection, we performed RNA sequencing and discovered upregulation of multiple host cell innate immune signaling pathways during early but not late infection where differences were minimal compared to cells originating from uninfected wells. Altogether our findings suggest that the changes in host cell innate immune signaling, and biomechanics are tightly regulated and linked to the different morphological forms that Bb cells attain. Our studies also shed light into the processes that may contribute in rendering Bb infection chronic and might facilitate efforts on better understanding Lyme disease.

Evolution of ipaH family proteins in human and non-human hosts *Escherichia*

Natalia O. Dranenko[1], Maria Tutukina[1,2,3], Mikhail Gelfand[1,2], Olga O. Bochkareva[4]

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[4] Institute of Science and Technology (IST Austria), Klosterneuburg, Austria

Shigella and enteroinvasive *Escherichia coli*, human-restricted pathogens, can enter epithelial cells of the colon, multiplicate within them, and move between adjacent cells using the type 3 secretion system (T3SS) encoded by the pINV virulence plasmid. A large family of T3SS effectors, E3 ubiquitin-ligases encoded by the ipaH genes, plays a key role in the Shigella pathogenicity via modulation of the cellular ubiquitination leading to the degradation of host proteins. Nevertheless, ipaH gene repertoire in the genus and its impact on strain virulence is still unclear. Recently, the presence of T3SS and ipaH genes in Escherichia marmotae, a potential marmot pathogen, was declared. We performed comparative genomic analysis of the ipaH genes in Shigella and enteroinvasive Escherichia from human and non-human hosts. Shigella ipaH genes as well as their regulatory elements are highly conserved but less than half of Shigella genomes held a complete set of ipaH genes; gene losses and duplications are not consistent to species tree and nomenclature. In contrast to Shigella, non-human-host IpaH proteins reveal two different types of NEL C-terminal domain and a diverse set of subtract-binding domains; only ipaH9.8 gene was found both in human-host and non-human-host Escherichia. These results provide a framework for understanding of host-pathogens interactions and the role of effectors' composition.

Directional mast cell degranulation of tumor necrosis factor into blood vessels primes neutrophil extravasation

Anne Dudeck[1], Jan Dudeck[1]

[1] Otto-von-Guericke Universität Magdeburg, Medical Faculty, Institute for Molecular and Clinical Immunology, Magdeburg, Germany

Tissue resident mast cells (MCs) rapidly initiate neutrophil infiltration upon inflammatory insult, yet the molecular mechanism is still unknown. Here, we demonstrated that MC-derived tumor necrosis factor (TNF) was crucial for neutrophil extravasation to sites of contact hypersensitivity-induced skin inflammation by promoting intraluminal crawling. MC-derived TNF directly primed circulating neutrophils via TNF receptor-1 (TNFR1) while being dispensable for endothelial cell activation. The MC-derived TNF was infused into the bloodstream by directional degranulation of perivascular MCs that were part of the vascular unit with access to the vessel lumen. Consistently, intravenous administration of MC granules boosted neutrophil extravasation. Pronounced and rapid intravascular MC degranulation was also observed upon IgE crosslinking or LPS challenge indicating a universal MC potential. Consequently, the directional MC degranulation of pro-inflammatory mediators into the bloodstream may represent an important target for therapeutic approaches aimed at dampening cytokine storm syndromes or shock symptoms, or intentionally pushing immune defense.

Discrimination between macrophages phenotypes using Raman spectroscopic imaging

Rustam Guliev[1], Ute Neugebauer[1], Natalie Arend[1], Max Naumann[1], Christian Kretzer[2], Oliver Werz[2], Jürgen Popp[1]

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Macrophages are important cells of the innate immune system. They play many roles in host defense which is reflected by their different activated subtypes. Depending on their function and phenotype, macrophages are classified into classically activated macrophages (pro-inflammatory M1 cells), alternatively activated macrophages (anti-inflammatory M2 cells), and non-activated (resting M0 cells). Fast characterization of macrophage phenotypes would be important for studying the contribution of the various subtypes to the various stages of the inflammation process.

In this work, the ability of label-free Raman spectroscopic imaging to discriminate between different macrophage phenotypes was investigated. Macrophages were derived from peripheral blood monocytes of three different human healthy donors. 67 Raman images of the three macrophage phenotypes (M0, M1, and M2) were analyzed. The spectra of the images were processed using chemometric methods of unmixing (N-FINDR), correction (Extended Multiplicative Scatter Correction, EMSC), and discrimination (principal component analysis and linear discriminant analysis, PCA-LDA). The discrimination models were validated using leave-one-donor-out cross-validation. The results show that Raman imaging is able to discriminate between pro- and anti-inflammatory phenotypes with good quality. The detected differences also correspond to the biochemical feature of the various phenotypes.

Quantitative characterisation of *Aspergillus fumigatus* hyphal growth in an "Invasive aspergillosis-on-chip" disease model of the human lung

Mai T.N. Hoang[1,2], Zoltan Cseresnyes[3], Susann Hartung[1,2], Marco Blickensdorf[3], Christoph Saffer[3], Knut Rennert[4,5], Alexander S. Mosig[4,5], Marie von Lilienfeld-Toal[1,2], Marc Thilo Figge[3,6]

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Invasive pulmonary aspergillosis is a great threat to immunocompromised patients as treatment options are limited and only successful upon early diagnosis which leads to high mortality rates. Infectious agents are conidia of the mold *Aspergillus fumigatus* that enter the lung alveoli but, in immunocompetent humans, are cleared by innate immune cells. In immunocompromised patients, however, conidia can germinate and grow into filamentous bodies (hyphae) leading to tissue destruction and invasion of blood vessels. To date, complex human cell-based models to investigate invasive aspergillosis are rare and often lack the quantification of growth parameters of individual hyphae. Our novel "invasive aspergillosis-on-chip" model is composed of human lung epithelial cells at an air-liquid-interface and medium-perfused human endothelial cells separated by a porous membrane. Models were infected by FITC-labelled *A. fumigatus* conidia on the epithelial side and fungal growth was detected by confocal microscopy. Three-dimensional (3D) data were analysed using automated and semi-automated systems biology methods. The structure of the models was reconstructed by the fluorescence of Calcofluor White-labelled hyphae, the reflected light image identifying the membrane pores and the FITC fluorescence identifying the conidia. Reconstructed hyphae allowed us to compute the morphometric measures of the individual hyphae (length, number of branches and branching levels) in 3D. Additionally, computational data analysis of the reconstructed membrane pores and hyphae revealed an interesting behaviour of invading hyphal branches under physiologic and diseased conditions as well as under influence of antifungal drugs. The development of this versatile "invasive aspergillosis-on-chip" system is very promising in respect to its potential applications in understanding the pathophysiology in invasive aspergillosis and providing a much-needed tool for animal-free drug screening.

Dual localization and role of the aminotransferase of the *sphingofungin* biosynthetic cluster in the pathogenic fungus *Aspergillus fumigatus*

Katarina Jojić[1,2], Sandra Hoefgen[1], Stefan Hoffmann[3], Alexander Bissell[1,2], Zoltán Cseresnyés1[3], Marc Thillo Figge[3,4], Vito Valiante[1]

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Filamentous fungi are potent producers of structurally diverse natural products and are a subject of extensive research for the development of novel drugs. *Aspergillus fumigatus*, a relevant human pathogenic fungus, produces a class of sphingolipid inhibitors, named as *sphingofungins*. They inhibit the serine palmitoyl transferases (SPTs) that catalyze the first step in the biosynthesis of sphingolipids in eukaryotes and are known for the important structural and signaling role in the cell. These compounds have a potential to be used as therapeutics against human neurodegenerative diseases connected to the deregulation of sphingolipid metabolism. Our localization studies showed that the biosynthesis of *sphingofungins* is partially compartmentalized in the perinuclear endoplasmatic reticulum (ER) and in independent ER-derived vesicles together with the SPTs. Additionally, we developed high-throughput screening of the fungal hyphae using confocal microscopy for image acquisition and subsequent image processing and analysis in order to confirm the colocalization of these enzymes. The obtained results posed a question what are the mechanisms by which the fungus reduces the poisonous effects of its own product. We identified that the aminotransferase of the cluster plays a dual role - promotes the first crucial step during the biosynthesis of *sphingofungin* in the cytosol, and in parallel helps in the sphingolipid biosynthesis when localized in the vesicles, thus reducing self-poisoning effects. This work revealed the presence of enzymes in fungi that have different roles based on their cellular localization.

Virtual infection model and cell generator: an open source Godot-based framework for immune reaction studies

Gabriel Krause[1,2], Zoltán Cseresnyés[1,2], Fabian Kriegel[3]

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[2] GfOP Neumann & Partner mbH, Michendorf, Germany
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The availability of high-performance computers, the increasing utilization of Machine Learning, and the ability to observe microbiological objects in three dimensions and over time, open up a wide range of applications for image-based computer simulations. These applications reproduce experimental images or synthetic models to predict biological events based on measured microscopy data. We developed a Godot—based simulation framework, where 3-dimensional datasets serve as the basis for accurate simulations of the behaviour of epithelial and endothelial cells, macrophages and viruses. The physiological response of these components simulate phagocytosis and viral infection in the tissue model under various conditions. Smaller-scale simulations are able to run on standard home computers or even laptops, whereas larger projects will naturally require scaled-up resources.

Our goal was to create a versatile program which finds its use in various fields for testing and understanding the details of infection processes that are behind various diseases. In addition, the tool can be used to examine the effects of possible drugs and treatments designed to fight these simulated infections and ailments. By using an open-source engine, the framework is freely accessible, thus ensuring easy acceptance by the community. The software is easy to handle, the script is written inside Godot using a Python-like language, thus providing an easy learning curve for the large community of Python programmers. By easily modifying and rearranging the simulated environment, as well as by being able to utilize real-life cell shapes via 3D surface file imports from e.g. Imaris-based 3D surface reconstructions, our tool provides a versatile solution for a wide range of cell biology—related problems.

'Flatten the curve' of misinformation: Epidemic models as a promising new framework for studying the spread and containment of low-credibility information in social networks

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Misinformation in social networks are considered to have manifold adverse effects on the individuals using them, e.g., erosion of trust in politicians and traditional newspapers or reduced adherence to pro-environmental behavior and public health measures in a pandemic situation. Acquiring a better understanding of how such misinformation diffuses through a social network and what can be effective countermeasures therefore seems reasonable. Pioneering works on the diffusion of misinformation have revealed that epidemic models constitute a useful framework to characterize the spread of misinformation in social networks. However, relatively little is known about the effects which specific countermeasures social media platforms can implement (e.g., fact-checking or post-deletion) can have on the spread of misinformation. In a recently published work (see https://doi.org/10.1371/journal.pone.0256179), colleagues and I have yielded first evidence about the effectiveness and sufficiency of such countermeasures in containing the spread of misinformation in social networks. We used a simple epidemic SIR (Susceptible-Infected-Removed) model to characterize the spread of a specific conspiracy theory diffusing on Twitter in 2020, and we also created representations of both fact-checking and tweet-deletion in the epidemic model. We found that the spread of the conspiracy theory can be modeled adequately within an epidemiological framework, indicating parallels between misinformation diffusion and the spread of infectious diseases. Furthermore, our subsequent simulations indicate that fact-checking is an effective mechanism in an early stage of misinformation diffusion, while tweet-deletion shows only moderate efficacy but is less time-sensitive. In general, an early response seems critical to gain control over the spread of misinformation through social networks. The further potential and perspectives of epidemic models for characterizing the dynamics and containment of misinformation in social networks will be discussed.

Bacterial eco-pathology: how the individuals shape the community

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Bacterial clonal growth ensure the efficient colonization of the ecological niches as well as the hosts. To ensure their survival, even a small population of genetically identical bacteria must engage in elaborated social behaviors. This is rendered possible by the reversible expression of alternative phenotypes among isogenic individuals, within the micrometer ranges, a phenomenon termed phenotypic heterogeneity. Pathogens are often acquired from their ecological reservoir. A prime example is the waterborne Gram-negative bacterium and re-emerging pathogen, *Legionella pneumophila* that causes a severe pneumoniae: the Legionnaires' disease (LD). *L. pneumophila* colonizes and persist in virtually all water systems where the bacterium has evolved as facultative intracellular parasite of free living protozoa. True cellular playgrounds, those natural interactions have largely shaped the bacterium behavior and the virulence program that is involved during the infection of lung associated phagocytes. Fostered by an innovative combination of single-cell and Omics technologies, our research unveiled *L. phenotypic* heterogeneity as a major feature in the eco-pathological life-cycle of this bacterium. Here, I will discuss (i) the community behavior of *L. pneumophila*, (ii) the mechanisms driving this bacterium cell-to-cell variations and (iii) the contribution of *L. pneumophila* phenotypic heterogeneity to the infection process and the therapeutic failures.

Selected article:

Striednig B,..., Personnic N. Quorum sensing governs a transmissive Legionella subpopulation at the pathogen vacuole periphery. *EMBO Rep*. 2021 Sep 6;22(9): e52972. doi: 10.15252/embr.202152972.

Personnic N, et al. Quorum sensing controls persistence, resuscitation, and virulence of Legionella subpopulations in biofilms. ISME J. 2021 Sep 19. doi: 10.1038/s41396-020-00774-0.

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A state based model and feasibility study to design an optimal COVID-19 surveillance protocol for child care facilities

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During the global COVID-19 pandemic day care centers (DCCs) were closed to avert transmission. However, children are only mildly affected by the disease and closure of DCC imposes negative effects on children's health. Therefore, (re)opening DCCs while simultaneously providing continuous surveillance testing might be a feasible alternative. The objective of the study is to design an optimal COVID-19 surveillance protocol by state-based modeling and proof its feasibility.

To evaluate various strategies we developed a virtual infection spread model for DCCs as state-based model (SBM), which simulates the infection spread in a DCC after the introduction of one primary case. For each individual, viral load kinetics is modeled and infection transmission is modeled using an aerosol transmission and infection risk calculator. Using the SBM we evaluate an optimal DCC setting with respect to (i) how many children have to participate in regular testing, (ii) how frequent testing has to be performed, (iii) and can DCCs remain open with continuous surveillance.

To investigate feasibility, we conducted a 12-week longitudinal study starting in October 2020. Nine pre-selected DCCs in Wuerzburg (Germany) were assigned to four different surveillance approaches (modules) for the detection of SARS-CoV-2 by RT-PCR. Asymptomatic children and childcare workers (CCWs) were screened by mid-turbinate nasal swabs twice weekly (module 1, one DCC), once weekly (module 2, one DCC) or by home-sampled saliva twice weekly (module 3, two DCCs). In module 4, symptomatic children, CCWs and the respective household contacts of five DCCs were offered tests on demand.

Consent to surveillance (71%) and successful study participation (68%) was highest in non-invasive home-sampled saliva testing. During the 12-week study period, no SARS-CoV-2 infection was detected in asymptomatic individuals. Simulation results of the SBM show that the expected number of an additional infection is less than 1 provided that twice-weekly testing and children participation of over 50% is realized. Furthermore, it supports the importance of testing on Mondays to minimize the risk of outbreaks.

In conclusion, surveillance of SARS-CoV-2 in DCC by continuous non-invasive sampling is feasible and the SBM provides evidence of a low risk of outbreaks by twice weekly testing.

Comparative simulations of fungal infection dynamics in the human and murine lung

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Alveolar macrophages (AM) constitute the first line of immune cells in the lung and are responsible for clearing all types of microbial invaders, such as the pathogenic fungus *Aspergillus fumigatus.* If not efficiently cleared, intruding conidia can form hyphae within hours leading to life-threatening infections like invasive aspergillosis. However, the absolute number of AM, *i.e.* in human and mice, is still controversially discussed. In the human lung, a range of four up to 46 AM per alveolus is covered [1, 2, 3, 4]. Similarly, AM number for the murine lung varies [3, 5].

In previous studies, we developed a hybrid spatio-temporal agent-based-model (hABM) [6, 7], which allowed to simulate virtual infection scenarios of *A. fumigatus* in a single alveolus. This model considers a realistic to-scale representation of the alveolus, consisting of a ¾ sphere, with alveolar epithelial cells (AEC) of type 1 and 2 as well as pores of Kohn (PoK). In the model, the AEC, on which a conidium is located, secretes chemokines that diffuse on the inner surface of the alveolus. AM are able to sense the chemokine gradient, which directs their migration towards the conidium. Previously, this model was applied to compare infection dynamics in human and mice [8] and to investigate the role of PoK [9]. This allows to accurately simulate host-pathogen interactions in the human and murine lung, to compare these hosts and to gain quantitative insight.

In this study, we use the hABM to investigate the impact of the number of AM on infection clearance during *A. fumigatus* lung infections for various chemokine parameters for the human and murine system. As a measure of infection clearance, we compute the infection score, which is the fraction of simulations in which the fungus was not detected before onset of germination, *i.e.* before six hours. Fitting joined analytical functions to the *in silicio* results enables us to derive a surrogate model that aggregates quantitative infection probabilities for given AM, chemokine parameters and the number of conidia per alveolus for the human and murine system, respectively. Furthermore, associated AM domains of the human and murine system can be normalized making them comparable such that systematic characteristics can be explained with the aid of its analytical model parameters. This allows to draw conclusions about differences between the two host environments. Depending on these parameters we gain quantitative understanding about the transferability of experimental observations in mice to the human system.

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Optimizing defence, counter-defence and counter-counter defence in host-pathogen interactions – A modelling study

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In host-pathogen interactions, often the host (attacked organism) defends itself by some toxic compound and the parasite, in turn, responds by producing an enzyme that inactivates that compound. In some cases, the host can respond by producing an inhibitor of that enzyme, which can be considered as a counter-counter defence. An example is provided by cephalosporins, β-lactamases and clavulanic acid (an inhibitor of β-lactamases). Here, we tackle the question under which conditions it pays, during evolution, to establish a counter-counter defence rather than to intensify or widen the defence mechanisms. We established a mathematical model describing this phenomenon, based on enzyme kinetics for competitive inhibition. We use an objective function based on the area under the curve. The optimal allocation of defence and counter-counter defence can be calculated in an analytical way. The calculation provides a threshold value for the dissociation constant of the inhibitor. Only in the case of strong binding of the inhibitor, it pays to have a counter-counter defence. This theoretical prediction accounts for the observation that not for all defence mechanisms, a counter-counter defence exists. Our results should be of interest for computing optimal mixtures of β-lactam antibiotics and β-lactamase inhibitors such as sulbactam, as well as for other molecular-ecological interactions and to fight antibiotic resistance in general.

In this interplay, a paradox occurs: If the inactivating enzyme is very efficient, the toxin becomes useless. If that is no longer produced, the enzyme becomes useless, so that production of the toxin becomes useful again. The question arises whether this leads to an oscillatory change in strategies or whether a steady state is attained as a sort of compromise, in which both species produce an optimal amount of defence chemical and enzyme, respectively? We tackle that question by using methods from game theory.

M-CSF fosters tissue resident alveolar macrophages to protect from lethal invasive *aspergillosis* early after hematopoietic cell transplantation

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Opportunistic infections, such as invasive aspergillosis, pose life-threatening risks for patients undergoing hematopoietic cell transplantation (HCT). Therefore, we investigate first-line host defense mechanisms to protect from pulmonary *A. fumigatus* infection. Here we addressed whether cytokines, including M-CSF and IL-34, affect alveolar macrophages (AMs) function after allogeneic HCT to improve protection from invasive aspergillosis.

To this end we employed dynamic confocal and light sheet fluorescence microscopy, flow cytometry and functional in vitro assays in combination with in vivo mouse models of allo-HCT (8 Gy TBI, C57BL/6> Balb/c) and invasive *A. fumigatus* lung infection.

HCT recipients survived *A. fumigatus* infection when infected 6 days but not 4 days after HCT. AMs showed the highest frequency, proliferation and phagocytic activity in the lung, when compared to neutrophils and monocytes, suggesting that AMs were responsible for protecting mice from *A. fumigatus* infection. This observation was confirmed by selective AM depletion, which rendered mice vulnerable to infection. Subsequently, we investigated whether cytokines could boost AMs to achieve earlier protection. In vitro, M-CSF but not IL-34, increased AMs migration speed (0.6, 0.3 µm/min respectively) and versatility (diffusion co-efficient of 0.78, 0.52 µm2/min, respectively). Next, we treated HCT-recipients with M-CSF and subsequently infected them intratracheally with *A. fumigatus* 4 days after HCT. M-CSF boosted myelopoiesis by 2-fold and, importantly expanded tissue-resident AMs by 1.5-fold. Additionally, M-CSF improved AMs killing capacity and protected 90% of HCT recipients from lethal *A. fumigatus* infection on day 4 after HCT. Notably, M-CSF treatment did not protect from lethal aspergillosis upon local depletion of AMs in HCT recipients. Thus, M-CSF holds great potential for clinical application by fostering AMs responsiveness to protect from early *A. fumigatus* infections in HCT recipients.

Label-free imaging of polyene macrolides and their ergosterol-dependent interaction with yeast cell membranes

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The increasing resistance of several pathogenic yeast species against known drugs is alarming and demands not only development of novel *antifungals* but also a thorough mechanistic understanding of drug action. *Polyene macrolides* (short polyenes) are naturally occurring amphiphilic antifungals, synthesized in various Streptomyces bacteria. Polyenes, such as amphotericin B or nystatin are often used as last line of defense, since resistance is relatively low, while other polyenes, like natamycin are used primarily as food preservatives. Polyene macrolides are supposed to target ergosterol in the plasma and vacuole membrane, but despite decades of research, their detailed mechanism of action has not been unequivocally determined. Large polyenes, such as amphotericin B or nystatin can form ion channels in lipid bilayers, while natamycin, a smaller polyene, does not and likely directly interferes with membrane protein function. We have developed an ultraviolet (UV)-sensitive imaging platform to detect the weak intrinsic fluorescence of polyenes and thereby directly observe their interaction with model and cell membranes. For nystatin, we observe that micron-sized aggregates form, which can interact with the plasma membrane (PM) of mammalian cells. Both, nystatin and natamycin bind extensively to the PM of baker's yeast, Saccharomyces cerevisiae, as inferred from a strong UV signal in this membrane. Loading a yeast mutant, unable to synthesize ergosterol with cholesterol gave much less binding of both polyenes to the yeast PM. Sterol import into these yeast cells and consequently polyene binding required the ABC transporters Aus1/Pdr11, suggesting these transporters as novel drug targets. Binding of natamycin to the yeast PM caused vacuolar targeting of the proton pump Pma1 but did not alter the lateral distribution of the eisosome marker Sur7. Molecular dynamics (MD) simulations, electron paramagnetic resonance spectroscopy (EPR) and nuclear magnetic resonance spectroscopy (NMR) show that natamycin interferes preferentially with ergosterol-dependent acyl chain ordering of membrane phospholipids. Together, these results explain the selective fungicidal effect of the polyenes nystatin and natamycin by their differential interaction with yeast membranes containing ergosterol compared to the mammalian cholesterol. Our label-free imaging platform is currently combined with X-ray microscopy of yeast ultrastructure and deep-learning assisted phenotype classification of vacuole function upon polyene treatment. This multimodal imaging approach will provide a useful tool set for characterizing polyene action in biofilms in the future.

Intestinal mycobiome shifts are associated with liver fibrosis in NAFLD patients

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Background

Non-alcoholic fatty liver disease (NAFLD) belongs to the most common chronic liver diseases and gained an estimated prevalence of around 25-30% in Europe. Its pathogenesis is characterized by excessive accumulation of fat in hepatocytes leading to a non-alcoholic fatty liver (NAFL). With ongoing fat accumulation, inflammatory processes could set in leading to a non-alcoholic steatohepatitis (NASH). This stage also involves fibrosis, which could lead to complete scarring of liver tissue. As the liver is closely connected to the gut via portal vein blood supply, it is thereby exposed to high-fat diet related implications like intestinal microbiome compositional shifts. As significant bacteriome compositional shifts were identified before in patients with advanced NAFLD in comparison to healthy controls, this may indicate a similar compositional shift in the human intestinal *mycobiota*.

Materials & Methods

To investigate the human intestinal mycobiome diversity in NAFLD we isolated microbial DNA from stool samples of 188 NAFLD patients as well as 27 healthy controls and used it for ITS1 sequencing. The mycobiome data was then correlated to multiple disease parameters.

Results

A wide variety of 224 fungal species were identified in the stool samples, including *Saccharomyces cerevisiae, Candida albicans* and *Debaryomyces hansenii*. First results of our ongoing bioinformatical analysis revealed a significant increase of *Candida* species as well as *Debaryomyces hansenii* abundance in samples of patients with advanced liver fibrosis in comparison to patients with early liver fibrosis.

Conclusion

Our data showed that the abundance of various fungal species correlates with liver fibrosis in NAFLD patients. The future analysis will focus on the correlation of mycobiome and bacteriome data, which are also available for all samples of this study. This might reveal possible interaction mechanisms between intestinal fungi and bacteria which influence the progress of NAFLD.

Super-resolution microscopy reveals the dynamic subcellular localization of pneumococcal proteins during an immunological challenge

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Streptococcus pneumoniae is a gram-positive human pathogen that colonizes the upper respiratory tract and acts as the causal agent of otitis media, bronchitis and community-acquired pneumonia.

On the bacterial surface, *S. pneumoniae* harbours several proteins with diverse functions (transport channels, carbohydrate metabolic enzymes, iron receptors) that protrude to the extracellular space. A subset of these proteins is the so-called Choline-Binding Protein (CBP) group.

To allow a precise subcellular localization, single-cell super-resolution Structured Illumination Microscopy (SIM) coupled with batch processing image analysis was used under various conditions.

The super-resolution 3D image stacks were processed using HuygensPro and Imaris. The 2D images were processed using custom-developed workflows written in JIPipe (www.jipipe.org). The protein-specific signals were reconstructed by fitting uniformly small spheres over the bacterial volume. This analysis indicated that the protein distribution is not homogenous along the bacterial surface, but rather concentrated along an equatorial plane of the bacterium. Average distances to the five nearest neighbours showed a similar, equatorial distribution, and revealed a linear relationship between the fluorescence intensity of the clusters and their average distance to their 5-nearest neighbours. The redistribution of peptide clusters was similarly characterized during cell growth using various strains and growth conditions. End-point experiments of peptide distributions were examined using template matching algorithms to reveal the various stages of development during cell division.

The localization of the analysed CBP was not homogeneous along the cell wall which contrasts with the current knowledge presented in the literature. This finding is suggestive of a protective effect exerted by this CBP on designated positions on the cell wall that might correlate with immune attack. The linear relationship between protein cluster intensity and neighbour distance suggests a dynamic and fine-tuned allocation of proteins to the cell wall to avoid areas with simultaneously high protein density and intensity.

Our work presents a novel view and an optimized analytical tool to provide insights into the dynamic spatial positioning of pneumococcal proteins, thus opening new ways to reassess how these pathogens exploit their protein machineries to evade the immune system.

Novel Machine Learning Approaches in Image-based Host-pathogen Interactions Analysis

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The research of interactions between the pathogens and their hosts is key for understanding the biology of infection. Commencing on the level of individual molecules, these interactions define the behavior of infectious agents and the outcomes they elicit. Discovery of host-pathogen interactions (HPIs) conventionally involves a stepwise laborious research process. However novel computational approaches including machine learning and deep learning allow to significantly accelerate the discovery process, particularly for rich information sources like microscopy. One example of such approaches includes an algorithm we have devised to detect interactions between intracellular *Toxoplasma gondii parasites* and the host cell innate immune response molecules with high accuracy from micrographs obtained in high-content fashion. In another example, it was possible to detect intracellular and extracellular poxvirus virions in 3D superresolution micrographs without specific immunohistochemical labelling. This was possible through transfer-learning-enabled deep learning model inference from seemingly irrelevant fluorescence channels, allowing to distinguish minute changes in virus particle signal upon internalization. Finally, bringing temporal dimension as a source of information for deep learning algorithms allows predicting infection outcomes in a population of infected and uninfected host cells employing time-lapse microscopy data. Altogether, these examples suggest a great potential for HPI analysis using novel machine learning.

Additional comments

This talk is a collective overview of the following peer reviewed publications:

1) Fisch, D., Yakimovich, A., Clough, B., Wright, J., Bunyan, M., Howell, M., ... & Frickel, E. (2019). Defining host–pathogen interactions employing an artificial intelligence workflow. Elife, 8, e40560.

2) Andriasyan, V., Yakimovich, A., Petkidis, A., Georgi, F., Witte, R., Puntener, D., & Greber, U. F. (2021). Microscopy deep learning predicts virus infections and reveals mechanics of lytic-infected cells. Iscience, 24(6), 102543.

3) Yakimovich, A., Huttunen, M., Samolej, J., Clough, B., Yoshida, N., Mostowy, S., ... & Mercer, J. (2020). Mimicry Embedding Facilitates Advanced Neural Network Training for Image-Based Pathogen Detection. Msphere, 5(5), e00836-20. Furthermore it will outline the future directions for the group I am about to establish at CASUS HZDR.

Organization

This symposium is organized by the research group Applied Systems Biology (ASB) at the Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany.

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