Across the world, millions of people contract invasive infections that are difficult to diagnose and treat, and can be life-threatening. The frequency of invasive fungal infections has increased significantly over the past two decades and continues to do so. Fungal infections have a major impact on human health (Pfaller et al., 2006), causing high levels of morbidity and mortality, and resulting in increased healthcare costs (Spellberg et al., 2008). Around 1.5 million people die each year as a result of invasive pathogenic fungal infections, and more than 90% of these deaths result from species belonging to one of four genera: Cryptococcus, Candida, Aspergillus and Pneumocystis (Brown et al., 2012).

In this article, we determine the challenges associated with infection persistence and antifungal therapeutics. Additionally, we will explore how increased sensitivity in the latest pre-clinical imaging instruments is giving researchers new insight into fungal infections in order to shape the future development of more effective treatments.

Although anybody can contract an invasive fungal infection, patients most at risk are those that are hospitalised or with compromised immune systems, as these patient groups struggle to fight infection. In the US, from 1980 to 1997, the annual number of deaths in which invasive mycosis was listed on the death certificate increased by 320% (Low and Rostein, 2011). This was due in part to the increasing number of organ transplants and the use of new and more potent chemotherapeutics and regimens, which increased the pool of immunocompromised patients dramatically (Low and Rostein, 2011).

**Challenges Associated with Antifungal Therapeutics**

Despite medical advances, the impact of invasive fungal infections on the global healthcare system continues to grow. The *Candida* fungus is the most common cause of healthcare-associated bloodstream infections in the US. Each case is estimated to result in an additional three to 13 days of hospitalisation and increased healthcare costs of $6000 to $29,000 per patient (Magill et al., 2014; Morgan et al., 2005).

Although there is a significant market for antifungal therapeutics, treating invasive fungal infections remains challenging due to their increased resistance to first- and second-line antifungal drugs, such as fluconazole and echinocandins, including anidulafungin, caspofungin, and micafungin (CDC, 2015). Statistics show that approximately 7% of all *Candida* bloodstream isolates tested at CDC are resistant to fluconazole, whilst approximately 1% of all *Candida* tested at CDC showed echinocandin resistance (Cleveland et al., 2012; Lockhart et al., 2012; Hajjeh et al., 2004; Kao et al., 1999).

The causes of antifungal resistance are numerous. Some species of fungi are naturally resistant to specific types of antifungal medication, whereas others may develop resistance over time due to improper antifungal use. Studies indicate that antibacterial medications can also contribute to antifungal resistance, for example by creating favourable conditions for *Candida* growth (Ben-Ami et al., 2012). Common measures to prevent and reduce the incidence of antifungal infections include the growing role of infection control staff, education and co-operation of healthcare professionals and patients, and also improving diagnostic techniques on offer.

**Disease Dynamics and Persistence**

Overcoming the persistence of fungal infections is a key driver for the development of effective antifungal treatments. Even when a therapy is considered to have been successful, infection may still be present in the tissue at levels too low for detection by common imaging and analytical techniques; consequently, if a treatment is withdrawn, relapse of the infection can occur (Delarze and Sanglard, 2015). Therefore, gaining a deeper understanding of the dynamics of infection as well as developing new, more effective treatment options remains a major goal for researchers around the globe.

Preclinical-imaging is the visualisation of living animals for research purposes. The technique involves using multiple imaging modalities and bioluminescence, which detect the luciferase light emission from engineered cells to monitor tumour cells, infections, gene expression and monitoring response to therapy in real-time. Pre-clinical in vivo imaging is widely regarded as a key tool within the drug discovery and development pipeline, giving researchers clear visibility of cellular changes at a molecular level and the ability to visualise where fungal cells congregate and persist in the body. The result is a deeper understanding of disease progression, and the mode of action and pharmacokinetics of potential antifungal therapeutics.

Technological advances are increasingly aiding researchers to optimise in vivo testing in the pre-clinical setting and translate their work from in vivo models into the clinical situation. The development of multi-modal pre-clinical imaging systems is transforming research practice, facilitating use of complementary imaging techniques to improve understanding of disease progression and drug efficacy. One such multi-modal imaging system* enables five imaging modalities to be conducted consecutively, with simple, fast and automatic transfer between different imaging techniques and accurate coregistration of the images produced.

**Enhanced camera functionality now brings a new level of sensitivity, speed and versatility to non-invasive procedures, providing unprecedented performance levels during extremely low-light applications and thereby enabling researchers to discover important biological mechanisms in disease to inform treatment and monitoring.**
The Candida species, in particular C. albicans, have specific virulence traits and can easily develop resistance to antifungal therapeutics. Dr Sanglard and his research team are currently working to gain an improved understanding of the dynamics and progression of C. albicans infection, and to identify novel antifungal agents and establish their activities in vivo using animal models of infection.

Sanglard’s researchers are using pre-clinical imaging to study how infection moves around the body and ascertain where infected cells might collect. The multi-modal system allows them to detect fungal infections at extremely low levels in vivo in the kidney tissue, which is notoriously difficult to detect. It is typical to see signs of disease at $10^5 - 10^6$ cells per kidney; however, fungal infection can persist at $10^2 - 10^3$ cells per kidney. The enhanced camera sensitivity has enabled the researchers to detect infection at unprecedented low levels of $10^2 - 10^3$ cells per kidney (Figure 1).

Dr Sanglard said: ‘The sensitivity of the Xtreme II is unparalleled, enabling us to detect very low levels of C. albicans infected cells within the kidney and other tissues. In fact, in our hands we see the Xtreme II being twice as sensitive as other instruments we have used, allowing us to observe disease progression earlier, and to explore where any infection persists after therapy.’

**BOX OUT:** Achieve new levels of sensitivity, speed and versatility in pre-clinical research. Advances in pre-clinical imaging technology are providing researchers with enhanced capabilities to characterise and measure biological processes in vivo, thereby paving the way for a better understanding of physiological and disease mechanisms in the pre-clinical setting.

The Bruker Xtreme II combines five imaging modalities as standard, with access to Bioiminescence, Multispectral VIS-NIR Fluorescence, Direct Radioisotopic Imaging and Cerenkov radiation. A high-speed digital X-ray scanner adds to the functional images with morphological features. The latest software allows simple, fast and automatic transfer between different imaging techniques and accurate co-registration of the images produced.

Enhanced camera capabilities now bring a new level of sensitivity, speed and versatility to non-invasive procedures, enabling researchers to discover important biological mechanisms in disease to inform treatment and monitoring. By cooling the camera to less than -90°C and using exceptionally low-read noise electronics, unprecedented performance levels during extremely low-light applications – such as bioluminescence and Cerenkov imaging – are achieved.

The use of multi-modal animal beds, which are compatible with a number of instruments, removes any requirement to disturb the animal during a cross-platform study and ensures accurate positioning to facilitate the layering of images to improve insight during subsequent study.

**Conclusion**

As the global demand for antifungal therapeutics continues to rise – driven largely by the high mortality rates and increased healthcare costs associated with severe fungal infections – researchers are focusing their studies on the disease dynamics and the effects of treatment in greater detail. Imaging is at the forefront of the revolution in pre-clinical information, providing increasingly valuable insights into disease mechanisms and progression. A range of imaging modalities are already being used to inform therapeutics in a number of clinical areas, most significantly in infectious disease research fields, such as antifungal studies.

Technological advances are aiding the translation of pre-clinical research work from in vivo models into the clinical situation. The development of highly sensitive, multi-modal systems is bringing new cross-platform capabilities, facilitating enhanced insight, more accurate analyses and improved access to useful data and enabling higher test throughput. Looking forward, these innovations will surely put more powerful data into the hands of researchers, further accelerating the development of antifungal drugs and informing clinical practice.

*Bruker Xtreme II*
References


