LIFE-AND-DEATH battles rage in Robert Wheeler’s lab at the University of Maine.

The combatants — zebrafish and Candida albicans — fight to the bitter end in glass-bottom microplates.

Similar perilous battles are being fought inside humans. The C. albicans fungus is a leading cause of hospital-acquired infection that annually kills several thousand patients nationwide.

During the staged scuffles in Wheeler’s lab in Hitchiner Hall, anesthetized zebrafish are injected with Candida and placed in a gelatinous material called agarose.

A laser microscope captures and magnifies the struggles inside the zebrafish blood vessels in real time in high-definition color detail.

The microplate clashes provide the assistant professor of microbiology with the ability to view how immune cells fight the microbe, identify genes involved in virulence, test new drugs and learn how gene perturbations affect host-pathogen interaction.

“We’re using zebrafish to ask really specific questions that cannot be answered another way,” Wheeler says. “These questions have been inaccessible for a long time. We hope to be able to better utilize existing therapies and be able to develop better therapies.”

In March 2012, Wheeler received a three-year, more than $421,600 grant from the National Institutes of Health to ask and answer these questions in the project: “Genetics & Visualization of Innate Host Response to Candida albicans Infection In Vivo.”

The goal is that the resulting answers will save human lives.

The grant is the most-recent funding Wheeler has received during his 13-year quest to unravel the mysteries of Candida.

MILLIONS OF C. albicans live peacefully in digestive tracts of people with healthy immune systems. Despite being the culprit of pesky vaginal infections in adults and oral infections in babies, for the most part, “the organism has evolved to coexist rather than constantly attack,” Wheeler says. “It’s part of our natural microflora.”
Battle lines

But when a person’s immune system is compromised — as occurs with organ transplant patients and people with cancer and human immunodeficiency virus — Candida albicans transforms from peaceful yeast to an invasive, potentially fatal fungus that infects vital organs.

Candida’s Jekyll-to-Hyde conversion proves deadly for about one-third of yeast to an invasive, potentially fatal *Candida albicans* and human immunodeficiency virus — compromised — as occurs with organ infections of the pathogen. It’s clear, says Wheeler, that better diagnostics and therapies are needed.

Wheeler also uses mice to study immunity to *C. albicans*. The research is beneficial, he says, but limited because the live mammals aren’t see-through and don’t fit under a microscope.

But transparent tropical fish larvae measuring a few millimeters fit the bill. And the ability to conduct experiments in vivo — “within the living” zebras — have been and continue to be elucidating.

Zebras also have backbones, share many of the same genes as people, and have the ability to respond to infections and vaccinations in ways similar to humans, Wheeler says.


On Wheeler’s iPad, a battle that lasts for hours is condensed into a time-lapsed movie that can be viewed in minutes. The movie of green zebrafish immune cells gobbling up red *C. albicans* resembles a Pac-Man arcade game.

The Pac-Man reference is one example of how Wheeler explains the fungal host-microbial pathogen interaction in ways that make sense to nonscientists.

He also compares sugar layers of the fungus cell wall using everyday objects and terminology, including M&M candies and GORE-TEX.

Some pharmaceutical drugs make Candida more recognizable. In addition to killing fungi, one antifungal drug has a side effect of uncovering the β-glucan, Wheeler says.

“If we’re able to expose the β-glucan, the immune system goes crazy,” says Remi Gratapac, a postdoctoral research fellow from Grenoble, France. “You see almost a threshold where the immune system is able to cope and if you go just past that, suddenly (β-glucan) can’t cope anymore.”

The UMaine group, says Wheeler, is also trying to better understand how Candida gets from one place to another in the body. Since Candida cannot move independently, Wheeler seeks to discover if the pathogen is carried in immune cells.

Candida, Wheeler says, can change shapes, from bunches of yeast to long filaments. Both shapes serve it well. Candida travels easily in the blood in yeast form and penetrates tissues best as a filament.

Gratapac says that Wheeler, a proponent of re-examining long-standing scientific concepts accepted as true, is “ridiculously clever.”

Researchers in the Wheeler lab include, left to right, Sarah Barker; a postdoctoral research fellow from Yorkshire, England; Xiaojie Ji, first-year biomedical science doctoral student from China; and Remi Gratapac, a postdoctoral research fellow from Grenoble, France.

“‘It’s hard to overstate how instrumental the well-run facility has been,’” Wheeler says. “‘I really don’t know if I could have done this work anywhere else.’”

His research group has already made a significant breakthrough discovery regarding *C. albicans*.

After receiving grants in 2008 and 2009 from the Maine Agricultural and Forest Experiment Station and the National Institutes of Health, he and students Kimberly Brothers and Zachary Newman started viewing interactions between fungi and immune cells. They showed for the first time that NADPH oxidase is required for regulation of *C. albicans* filamentation in vivo.

These observations, first made by Brothers, implied the deadly fungus might spend more time inside zebrafish phagocytes — immune cells that ingest microorganisms, other cells and foreign particles — than researchers had believed.

This, Gratapac says, demonstrates that examining these microscale battles in different hosts can lead to striking insights.

Robert Wheeler