

Course Mission:

Fungal diseases are significant causes of mortality and morbidity in both the developed and the developing world. The recent increases in the incidence and severity of invasive fungal infections are directly attributable to new susceptible patient populations. Examples of these large, at-risk populations include patients with AIDS; hospitalized patients being treated for cancer and autoimmune disorders; and those receiving organ transplants. Despite this increasing threat, our understanding of the basic pathophysiology of fungal disease lags far behind our understanding of bacterial, parasitic, or viral diseases. Furthermore, the number of antifungal therapies in clinical use is limited, and there is a paucity of novel antifungal strategies in the current drug pipeline. To address the need for more research in the area of fungal diseases, this course aims to:

- a) increase students' breadth of knowledge in fungal pathogenesis research
- b) introduce and explore both standard and cutting edge systems for the analysis of fungal virulence
- c) create an environment that fosters interactions and idea-exchange among students, faculty, and the greater mycology research community.

Course material is suitable for advanced graduate students, post-doctoral fellows, research faculty, and clinician-scientists.

The specific objectives of the 2017 Molecular Mycology course:

- To present the current conceptual models for the pathogenesis of medically important fungi, with a focus on the most frequently encountered pathogens – *Candida*, *Cryptococcus*, and *Aspergillus*
- To train students in molecular manipulation of *Candida*, *Cryptococcus*, and *Aspergillus*
- To provide hands on experience with mammalian and cell culture models to assess virulence and analyze different types of host-pathogen interactions
- To present a broad perspective on experimental issues pertinent to pathogenic fungi, such as the definition and determination of virulence, the determination of host responses relevant to infection, and the quantification of antifungal susceptibility
- To instruct students in techniques relevant to the analysis of the function of fungal gene products such as determination of essentiality, microscopic analysis of morphology and fluorescent protein fusions, comparison of RNA expression profiles of wild-type and mutant strains, assessment of chromosome content
- To provide insight into the clinical aspects of fungal diseases from the perspective of the host and the pathogen
- To provide an introduction to tools for comparative genome and transcriptional analysis
- To discuss with students about academic careers and alternative careers, and also about career development issues.

Course directors:

Damian Krysan, M.D., Ph.D. (*University of Rochester School of Medicine*)
Xiaorong Lin, Ph.D. (*University of Georgia/Texas A&M University*)

Faculty-In-Residence:

Aaron Mitchell, *Carnegie Mellon University* (Genetics tools to study fungal pathogenesis)
Andrew Alspaugh, *Duke University School of Medicine* (Signal transduction, pH regulation, cryptococcal pathogenesis)
David Andes, *University of Wisconsin – Madison*
Deborah Hogan, *Geisel School of Medicine at Dartmouth* (Inter-kingdom interaction)
Donald C. Sheppard, *McGill University* (*Aspergillus fumigatus*: Molecular Manipulation and Host-pathogen Interaction)
John E. Edwards, *Harbor-UCLA Medical Center* (Animal models of fungal diseases; clinical mycology)
Joseph Heitman, *Duke University* (Research talk, career development, student interactions)
James B. Konopka, *Stony Brook* (Advanced Workshop in Microscopy)
Robert A. Cramer, *Geisel School of Medicine at Dartmouth* (Fungal Immunology, *Aspergillus* pathogenesis)
Robert Wheeler, *University of Maine* (Fungal mammalian cell interaction)
Theodore C. White, *University Of Missouri – Kansas City* (Antifungal susceptibility and resistance mechanisms)

Lecturers:

Amariliz Rivera, *Rutgers University*
Hitendra Madhani, *University of California-San Francisco*
Axel Brakhage, *Leibniz Institute for Natural Product Research and Infection*
Floyd Wormley, *University of Texas-San Antonio*
Carol Munro, *University of Aberdeen*
Gerard Wright, *McMaster University*
Cheryl Quinn, *InnovaTID Pharmaceuticals*
Bridget Barker, *Northern Arizona University*
Mairi Noverr, *LSU Health Sciences Center*

Teaching Assistants:

Virginia Glazier, *University of Rochester School of Medicine*
Elisa Vesely, *University of Texas – Houston Medical School*
Norma Solis, *Harbor-UCLA Medical Center*
Youbao Zhao, *University of Georgia/Texas A&M University*
Sarah Beattie, *Geisel School of Medicine at Dartmouth*

Course coordinator:

Carol Edwards, *Harbor-UCLA Medical Center*

Course Overview: Training is provided by laboratory exercises, seminars/lectures, demonstration exercises, nuts and bolts discussions, and informal panel discussions.

Lectures/seminars by faculty and visiting lecturers provide additional insight into clinical issues, and current conceptual models describing the pathogenesis of *Candida*, *Aspergillus*, and *Cryptococcus* and other fungi such as *Histoplasma*, *Coccidioides*, *Penicilliosis*, and *Pneumocystis*. Speakers from academia and industry lecture on more specialized topics including drug targets, molecular diagnostic techniques, genome structure, evolution, vaccine strategies, interactions between fungi and other host-associated microbes, and host defenses and immune responses.

Laboratory exercises led by course directors and instructors, focus on *Candida*, *Aspergillus*, and *Cryptococcus*. Exercises provide experience with genetic manipulation of these pathogenic fungi, in vitro and in vivo pathogenicity assays, host responses relevant to fungal diseases, advanced antifungal susceptibility testing, RNA expression analysis, comparative genome analysis, and microscopic analysis of fungi. Experimental introduction and interpretation sessions provide more information regarding issues relating to experimental design and analysis.

Demonstrations from visiting faculty provide students with the opportunity to have hands-on experience with additional techniques and strategies and to interact with lecturers and faculty in smaller groups. They include comparative genomic analysis tools, analysis of antifungal susceptibilities in filamentous fungi, interaction of mammalian immune cells and fungi, and microscopic analysis of fungal cells.

Nuts and bolts discussions from faculty provide the students the knowledge/experience that is not easily obtained by reading literature/books. These discussions include factors that need to be considered during planning experiments such as high throughput screen, using fluorescence proteins, transcription analyses, animal models, or the history of genetics in fungal pathogen research.

Panel discussions on medical mycology, research ethics, and professional development are also part of the course curriculum.

2017 Molecular Mycology Classroom and Workshop schedule:

(Please review the protocol for each experiment ahead of time. Your exact schedule is subject to change, depending on the progress of your experiments.)

The experimental plan of the syllabus is subject to change depending on student progress.

Our lecture room is Loeb 263 (reserved from 8:30-11:00am and from 7-9pm) from July 31 to August 8. Then our lecture room will be switched to Lillie Auditorium for August 9-16. Main lab is Loeb 260.

Monday, July 31

5:30 pm – 8 pm: Opening reception and dinner, Swope Hall (small dining room)
Jack Edwards: Lecture on the history of the MBL and MOMY

Tuesday, August 1

9:00 am-10:00 am: MBL staff orientation to MBL Loeb 263
10:00 am – 11:00 am: Seminar: Mairi Noverr. “Promoting protective immunity to polymicrobial fungal-bacterial sepsis”

4:00 pm - 5:00 pm: Jamie Konopka: Nuts and Bolts on microscopy.

Wednesday, August 2

8:30-9:30 am: Seminar: Aaron Mitchell “**Genetic strategies for insight into fungal pathogenesis**”
9:45-10:45 pm: Seminar: Jamie Konopka “**Stress Management: plasma membrane roles in resisting stressful environments in the host**”

Thursday, August 3

8:30-9:30 am: Seminar: Don Sheppard “**Aspergillus exopolysaccharide - a sticky situation**”
9:45-10:45 am: Seminar: David Andes “**Interrogation of the Candida Biofilm Matrix**”

Friday August 4

9:00-10:00 am: Seminar: Andy Alspaugh “**Hidden in plain sight: immune evasion by fungal pathogens**”

7:00 pm: Clinical mycology with Don Sheppard

Saturday August 5

9:00 -10:00 am: Seminar: Xiaorong Lin “**Impact of *Cryptococcus* life cycle on virulence**”

Sunday August 6

8:30-9:30 am: Seminar: Robb Cramer “**Disease Progression Factors: How “Fungus” Reduce Stress During Infection**”
9:45 -10:45 am: Seminar: Jack Edwards “**Progress on the development of a fungal vaccine**”

6:00 pm-8:00 pm Student Poster Session

Monday August 7

8:30-9:30 am: Seminar: Cheryl Quinn “**Antifungal drug discovery in the**

pharmaceutical and biotechnology industries”

7:30 pm: Seminar: Amariliz Rivera “**Type III interferon is a critical regulator of innate antifungal immunity**”

Tuesday August 8

Day off (with some experimental manipulations required)

Wednesday August 9

9:45 -10:45 am: Seminar: Damian Krysan “**Antifungal drug discovery**”

10:45 -11:45 am: Seminar: Deb Hogan “**Heterogeneity in chronic lung infections**”

Thursday August 10

8:30-9:30 am: Seminar: Carol Munro “**The fungal cell wall as an antifungal target**”

9:45 -10:45 am: Seminar: Axel Brakhage “**Cross talk of *Aspergillus fumigatus* with macrophages and neutrophils**”

Friday August 11

8:30-9:30 am: Seminar: Floyd Wormley “**Induction of Long-Lived Immunity by Innate Cells Against Cryptococcosis**”

11:00 am-7:00 pm:

Start **Expt. 9. Fungal Leukocyte interactions** (Harvest host cells)

Continue **Expt. 3** (read plates, score growth, start overnight for FIC)

Saturday August 12

8:30-9:30 am: Seminar: Hiten Madhani “**Epigenetic memory over geological timescales**”

9:45-10:45 am: Seminar: Rob Wheeler “**In vivo imaging of Candida-innate immune interactions in a transparent vertebrate**”

Sunday August 13

8:30-9:30 am: Seminar: Gerry Wright “**Expanding antifungal drug targets through compound combinations**”

9:45-10:45 am: Seminar: Ted White “**Analysis of azole resistance mechanisms using overexpression in *Saccharomyces cerevisiae***”

Monday August 14

8:30-9:30 am: Seminar: Joe Heitman “**Calcineurin as a globally conserved virulence factor: Structure guided design of inhibitors as anti-fungal agents**”

Tuesday August 15

8:30-9:30 am: Seminar: Bridget Barker “**Population genomic analysis and assessment of recombination in 2 species of Coccidioides**”

12:00-1:30 pm: Research Ethics and Etiquette Luncheon (Faculty: Heitman, White, Wheeler, Lin and Krysan)

Wednesday August 16

Students: Leave Woods Hole

TAs: pack the lab

Experiments:

Exp. 1: *Candida albicans* transformation and complex haploinsufficiency for the study of genetic interactions in a diploid fungus.

Objective: (1) To learn to transform *C. albicans*. (2) To create transcription factor *EFG1* mutants in a set of heterozygous transcription factor deletion mutants. The final mutants will be heterozygous mutants for two different transcription factors. Assay these mutants' phenotypes for genetic interaction studies.

Faculty instructor: Damian Krysan

TAs: Elisa Vesely

Exp. 2: *Candida albicans* biofilms.

Objective: (1) To assess the quantity of biofilm cells during the formation process. (2) To understand the pros and cons of each quantification method. (3) To estimate viable cell burden and biomass.

Faculty instructor: Don Sheppard and David Ande

TAs: Virginia Glazier

Exp. 3: Screen for combinatorial interactions between repurposed drugs against *Cryptococcus neoformans*.

Objectives: (1) To learn to perform different techniques for assessing drug susceptibility in clinically relevant fungi. (2) To assess fungicidal vs. fungistatic activity for antifungal agents. (3) To assess drug interactions (additive, synergistic, or antagonistic).

Faculty Instructors: Damian Krysan, Ted White, and Don Sheppard

TAs: Virginia Glazier

Exp. 4: Genetic manipulation of *Cryptococcus neoformans*

Objectives: (1) To use a biolistic transformation apparatus to transform *C. neoformans*, establishing stable genomic integration of different PAS3 constructs. (2) To assess the effects of mutated allele of Pas3 on its function and subcellular location. (3) To examine the effect of Pas3 on cryptococcal morphogenesis.

Faculty Instructor: Xiaorong Lin

TAs: Youbao Zhao

Exp. 5: Insertional mutagenesis of *Cryptococcus neoformans* through *Agrobacterium*-mediated transformation and genetic screen design

Objective: (1) To familiarize students with a broadly applicable DNA transfection method. (2) To create novel strains for genetic screens for various defects.

Faculty Instructor: Xiaorong Lin

TAs: Youbao Zhao

Exp. 6: Determination of the efficacy of antifungal compounds using an immunocompetent murine model of hematogenously disseminated candidiasis

Objective: (1) To obtain experience with and assess utility of the mouse model for disseminated candidiasis. (2) To assess the efficacy of antifungal drugs against candidiasis in the mouse model. (3) To learn the pros and cons of the mouse model for *Candida*

infections.

Faculty instructor: Jack Edwards

TAs: Norma Solis

Exp. 7: Mouse model of aspergillosis

Objective: (1) To obtain experience with and assess usefulness of the mouse model for aspergillosis. (2) To analyze fungal burden based on quantitative analysis of fungal DNA in tissues.

Faculty instructor: Robert Cramer

TAs: Sarah Beattie and Norma Solis

Exp. 8: Genetic Engineering of *Aspergillus fumigatus* (transformation)

Objective: To illustrate basic molecular methods for *Aspergillus* transformation and mutant phenotypic analysis.

Faculty instructors: Robert Cramer Lin

TAs: Sarah Beattie

Exp. 9: Fungal interactions with leukocyte

Objective: (1) To harvest primary macrophages from an animal host. (2) To use an in vitro co-culture system to assess interactions between mammalian phagocytes and pathogenic fungi.

Faculty Instructor: Robert Cramer and Robert Wheeler

TAs: Elisa Vesely and Virginia Glazier

Microscopy studies

Faculty Instructor: James B. Konopka

TAs: Elisa Vesely