

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 2018 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 Iowa*)
Xiaorong Lin, Ph.D. (*University of Georgia*)

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* (Fungal biofilm)
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:

Sven Krappmann, *Friedrich-Alexander University Erlangen-Nürnberg*
Patrick Westfall, *Zymergen*
Tamara Doering, *Washington University at St. Louis*
Jeremy Day, *University of Oxford, Medical Science Division*
Jim Kronstad, *University of British Columbia*
Brendan Cormack, *Johns Hopkins School of Medicine*
Gorge Deepe, *University of Cincinnati Medical Center*
Leah Cowen, *University of Toronto*
Nathan Wiederhold, *UT Health Science Center at San Antonio*

Teaching Assistants:

Yumeng Fan, *University of Georgia*
Virginia Glazier, *Niagara University*
Elisa Vesely, *University of Texas – Houston Medical School*
Norma Solis, *Harbor-UCLA Medical Center*
Sarah Beattie, *University of Iowa*
Linda Archambault, *University of Maine*

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.

2018 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 daily until 6:30 pm). Main lab is Loeb 260. Evening seminars will be held at Speck Auditorium (July 30, August 1, August 3-15) or Starr room 209 (July 31-August 2).

Monday, July 30

9:00 am: TA training by MBL, Loeb 273

3:00-3:10 pm: MBL facility and lab safety walk through.

4:00 pm: TA prepare for **Expt. 1. *Candida* transformation**

4:00 pm: TA prepare for **Expt. 2. *Candida* biofilm**

4:00 pm: TA prepare for **Expt. 4. *Cryptococcus* transformation**

4:00 pm: TA prepare strains for **Expt. 6. *Candida* infection** (-3 day culture)

4:00 pm: Prepare strains for **Microscopy Workshop**

5:30 pm – 8 pm: Opening reception and dinner, Swope Hall (small dining room)

Welcome by Rae Nishi (MBL Director of Education)

Lecture on the history of the MBL (Edwards)

Tuesday, July 31

8:30 am- 9:00 am: Course Introduction (Krysan and Lin)

9:00 am-10:00 am: MBL staff orientation (Lillie Auditorium)

10:00 am – 11:00 am: Seminar: Sven Krappman “**Putting *Aspergillus* virulence in a metabolic context: from nutritional versatility to offspring protection**”

11:00 am - 12:00: Introduction to the lab

1:00 pm - 5:00 pm:

Start **Expt. 1. *C. albicans* transformation** (Krysan)

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

Set up **Expt. 1, 2.**

TA prepare for **Expt 6. *Candida* animal infection** (-2 day culture)

TA prepare strains for **Expt. 5. *Cryptococcus* AMT-mediated insertional mutagenesis**

7:00 pm – 9:00 pm: Faculty, TA and student introductions (5 min(!) each)

Wednesday, August 1

8:30-9:30 am: Seminar: Jeremy Day “**Improving outcomes from cryptococcal meningitis- antifungals, anti-inflammatories and anti-cancer drugs**”

9:45-10:45 pm: Seminar: Jamie Konopka “**Fungal plasma membrane domains that promote resistance to stressful environments in the host**”

Before noon: prepare **Expt. 4** (culture cells on sorbitol).

Continue **Expt. 1** (heat shock and plating)

1:30-3:00 pm: Continue **Expt. 6** (-1 day culture, walk through the lobster facility)

Before 3:00 pm: Whenever the team has 5 min (e.g. during heat shock, then do the

biolistic bombardment for **Expt. 4** leave at room temperature)

3:00 – 5:30 pm: **microscope training** by Dr. Jim McIlvain from Zeiss.

Before 6:30 pm: make sure to recover *Cryptococcus* cells and plate on selective medium for **Expt. 4**.

TA: set up fungal/agro culture for **Expt. 5**.

7:00 pm: Introduction to **Expt. 6** (Jack Edward)

Thursday, August 2

Before 8:30 am: TA: start **Expt. 5. *Agrobacterium* mediated insertional mutagenesis in *Cryptococcus*** (induction medium)

8:30-9:30 am: Seminar: Aaron Mitchell “**Archetype, actuality, and analysis of causal mediation in virulence trait variation**”

10:00-noon: continue **Expt. 6**. (introduction and inoculum calculation)

1:30 pm: continue **Expt. 6**. (Candida infection)

1:30 pm – 7:00 pm:

Continue **Expt. 1, 4** (check plates)

3:00 pm -4:00 pm **Expt. 5. *Agrobacterium* mediated insertional mutagenesis in *Cryptococcus*** (co-culture of crypto/agro on induction medium)

7:30 pm: continue **Expt. 6**. (treatment for Candida infection)

TA prepare for **Expt 2. *Candida* and *Aspergillus fumigatus* biofilm**

9:00 pm: Start culture for **Expt. 8. *Aspergillus fumigatus* transformation**

7:00 pm: **Clinical mycology with Don Sheppard**

Friday August 3

8:30-9:30 am: Seminar: Andy Alspaugh “**How to sense a host: mechanisms of microbial detection of the host environment**”

9:30-10:30 am: Seminar: Don Sheppard “**Microbial exopolysaccharides - virulence factors or drug targets?**”

11:00-noon: Start **Expt. 8** (spheroplasting/ introduce the exp. 8)

1:00 pm – 7:00 pm:

Start **Expt. 2** (inoculation of *Candida*)

Continue **Expt. 8** (protoplasting and transformation)

Expt. 2: 6 hours post inoculation (add Flu)

Continue **Expt. 1** (patch transformants and check tdTomato under microscope, set up overnight culture)

Expt. 2 (inoculation of *Aspergillus fumigatus*)

Saturday August 4

9:00 -10:00 am: Seminar: Robb Cramer “**Aspergillus Colony Morphology, Low Oxygen Fitness and Disease Progression**”

10:00 -11:00 am: Seminar: Damian Krysan “**Antifungal Drug Development**”

11:00-noon Introduction to antifungal drug experiments (Damian)

noon -7:00 pm: Continue **Expt. 1** (DNA and PCR, then TA run the gel, students start o/n cultures)

Continue **Expt. 2** (XTT, crystal violet, and plate reader)

Continue **Expt. 4** (pick transformants)

Continue **Expt. 5** (collect cells from Agro/Crypto plate and plate cells on selective medium)

Continue **Expt. 6** (Candida infection)

TA preparing for **Expt. 9** (i.p. injection)

7:00 pm: Lin and Krysan “Genetic transformation”

Sunday August 5

8:30-9:30 am: Seminar: Xiaorong Lin “**Meiotic machinery in cryptococcal virulence**”

9:45 -10:45 am: Seminar: Jack Edwards “**Fungal Vaccines: Scientific and Developmental Challenges**”

1:00 pm-7:00 pm:

Continue **Expt. 1** (Candida transformants: phenotypical testing)

Continue **Expt. 4** (pick transformants, check fluorescence, phenotypical testing)

Continue **Expt. 5** (incubating)

Continue **Expt. 6** (Candida infection)

Analyze results for **Expt. 2**

4:00 -5:00 pm: print posters

Monday August 6

8:30-9:30 am: Seminar: Patrick Westfall “**Systematic and comprehensive approaches to genetic engineering for the improvement of industrialized microbes**”

10:00 am-7:00 pm:

Continue **Expt. 1** (check plates)

Continue **Expt. 4** (examine mutant phenotype on V8 and fluorescence, select positive transformants from master plates)

Continue **Expt. 5** (pick colonies to arrange master plate).

Continue **Expt. 6.** (Candida infection)
Continue **Expt. 8** (check transformants for white ones)
overnight for the controls)

7:00 pm: Mentoring session: Westfall/Krysan: “**Non-academic career**”

Tuesday August 7

Day off (with some experimental manipulations required)
TA Start **Expt. 7. Mouse model of aspergillosis** (day -1, immunosuppression)
TA start culture for **Expt. 7**
Continue treatment for **Expt. 6**

Wednesday August 8

8:45 -9:45 am: Seminar: Nathan Wiederhold “**Antifungal agents: mechanisms of action & resistance, spectrum of activity, and how to determine these**”

10:00 -11:00 am: Seminar: Rob Wheeler “**Intravital imaging identifies redundant host pathways of Candida dissemination**”

1:00 pm-7:00 pm:

Expt. 7 Mouse model of aspergillosis (infection)
Continue **Expt. 1** (check plates)
Continue treatment **Expt. 6.** (Candida infection model)
Continue **Expt. 5** (phenotype master plates)
TA and students start culture for **Expt. 3** (antifungal).
TA prepare **Expt. 9.** (-2)

7:00 pm: Pizza dinner at breakroom

Thursday August 9

8:30-9:30 am: Seminar: David Andes “**Fungal Biofilm Persistence**”

9:45 -10:45 am: Seminar: George Deepe “**HIF-1a, Glycolysis and and Immune Control of Histoplasma capsulatum**”

11:00 am: Continue **Expt. 3** (E-test, MICs, start o/n culture)

1:00 pm -7:00 pm:

Continue **Expt. 1** (perform double heterozygous phenotypical analyses)
Continue **Expt. 4** (continue checking phenotype)
Continue **Expt. 5** (check phenotypes)
Continue **Expt. 6** (harvest tissues and plate for CFUs)
TA prepare **Expt. 9** (-1)

7:00 pm: Introduction to different drug susceptibility tests (Ted White)

Friday August 10

8:30-9:30 am: Seminar: Brendan Cormack “**NAD⁺ and insights into the regulation of virulence in *Candida glabrata***”

9:30 am: Class picture

10:00 am-6:00 pm:

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

TA start culture for **Expt. 9**

Continue **Expt. 3** (read plates, score growth, plate MICs for fungicidal effect, start competition)

Continue **Expt 6**. Count colonies

7:00 pm-9:00 pm: poster session

Saturday August 11

8:00 am: Continue **Expt. 9** (Stimulate isolated macrophage cells)

8:30-9:30 am: Seminar: Ted White “**Pathways, Pumps and Predominance: The Ins and Outs of Antifungal Drug Resistance**”

9:45-10:45 am: Seminar: Deb Hogan “**Heterogeneity and evolution in *Candida* infections**”

11:00 am – 7:00 pm

Summarize **Expt. 6** (Jack)

Continue **Expt. 9** (inoculate fungal cells, prepare ELISA dilution plate)

Finish **Expt. 5** (check phenotype of agro-transformants on selection media and save them)

Finish **Expt. 4** (check under scope and other phenotypes)

Continue **Expt. 3** (MIC, plate competition, count competition by microscopy, check fungicidal from previous MICs)

7:00 pm: Nuts/Bolts

Sunday August 12

8:30-9:30 am: Seminar: Joe Heitman “**RNAi-dependent epimutations evoke transient antifungal drug resistance**”

11:00 am:

11:00 am – 7:00 pm

1:00 pm: Continue **Expt. 9** (coat ELISA)

Continue **Expt. 7 Mouse model of aspergillosis** (terminate and lyophilize tissues)

Finish **Expt. 3** (fluorescence-imaging plates)

Check other experiments

7:00 pm: Bowling night

Monday August 13

8:30-9:30 am: Seminar: Tamara Doering “**Adventures in cryptococcal biology: biochemistry, gene regulation and pathogenesis**”

9:45 am: Continue **Expt. 9** (ELISA)

Finish **Expt. 3** (FIC determination, score E-test)

Continue **Expt. 7. Mouse model of aspergillosis** (DNA extraction and PCR)

7:00 pm: Mentoring session “Grants” (all faculty members and NIH mycology program officer Dona Love)

Tuesday August 14

8:30-9:30 am: Seminar: Jim Kronstad “**Nutrient sensing and the regulation of virulence in Cryptococcus neoformans**”

9:30-10:30 am: Seminar: Leah Cowen “**Identifying Vulnerabilities in Fungal Pathogens Through Functional and Chemical Genomic Analysis**”

10:30-noon: Students gather data and organize their presentation

Finish **Expt. 9** (data analysis)

Finish **Expt. 7 Mouse model of aspergillosis** (data analysis)

Wrap up other **Expt.**

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

1:45 -5:00 pm: Student led group discussion on each experiment

5:30 -8:00 pm: Course Banquet (Meigs Room)

Wednesday August 15

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 *UPC2* 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* and *Aspergillus fumigatus* 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 Andes

TAs: Virginia Glazier

Exp. 3: Screen for combinatorial interactions between repurposed drugs against *Candida albicans*.

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 transient expression of CRISPR-Cas9 coupled with electroporation to transform *C. neoformans* to delete the *ADE2* gene and integrate different alleles of the *PAS2* constructs. (2) To assess the effects of mutated allele of *Pas2* on its function and subcellular location. (3) To examine the effect of *Pas2* on cryptococcal morphogenesis.

Faculty Instructor: Xiaorong Lin

TAs: Yumeng Fan

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. (3) To screen mutants for filamentation restoration in the *set302Δ* mutant.

Faculty Instructor: Xiaorong Lin

TA: Yumeng Fan

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

TA: 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

TA: 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 instructor: Robert Cramer

TA: 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

TA: Elisa Vesely

Exp. 10: Zebrafish model of Candida infection

Objective: (1) To learn zebrafish care protocols. (2) To microinject zebrafish swimbladders with fungal pathogens. (3) To view and create live images of *Candida albicans*-infected fish to monitor phagocyte responses and fungal-cell/host-cell interactions

Faculty Instructor: Robert Wheeler

TA: Linda Archambault

Microscopy studies

Faculty Instructor: James B. Konopka

TA: Yumeng Fan