

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 2016 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

Course directors:

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

Faculty-In-Residence:

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

Lecturers:

Karl Kuchler Vienna, *Medical University of Vienna*
YongSun Bahn, *Yonsei University*
David Ande, *University of Wisconsin – Madison*
Tobias Hohl, *Memorial Sloan Kettering Cancer Center*
Anita Sil, *UCSF*
Li-Jun Ma, *University of Massachusetts – Amherst*
William Steinbach, *Duke University*
Christina Hull, *University of Wisconsin – Madison*
Richard Bennett, *Brown University*
Terry Roemer, *Merck*

Teaching Assistants:

Virginia Glazier, *University of Rochester School of Medicine*
Pedro Miramon-Martinez, *University of Texas – Houston Medical School*
Norma Solis, *Harbor-UCLA Medical Center*
Srijana Upadhyay, *Texas A&M University*
Alex Hopke, *University Of Maine*
Sourabh Dhingra, *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.

2016 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).
Main lab is Loeb 207.

Sunday, June 12

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 strains for microscopy

5 pm – 8 pm: Opening reception and dinner, Swope Hall. Welcome by ??? (MBL Director of Education)

Lecture on the history of the MBL (Edwards)

Monday, June 13

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

9:00 am-10:00 am: MBL staff orientation to MBL Loeb 263

10:00 am – 11:00 am: Seminar: Li-Jun Ma. “Genome dynamics & fungal pathogenicity”

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

1:00 pm: Nuts and bolts: Li-Jun Ma “Design & Analysis of RNA-seq Experiments”

2:00 pm - 5:00 pm:

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

Start **Expt. 2. *C. albicans* biofilm** (set up plates) (Krysan)

TA Start **Expt. 4. *Crypto* biolistic** (start culture H99 and *cln1Δ*) (Lin)

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

TA prepare for **Expt 6. *Candida* animal infection**

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

Tuesday, June 14

8:30-9:30 am: Seminar: David Andes “**Fungal and Host Participation in *Candida* Biofilm Matrix Assembly and Function**”

9:30-11:00 am: Lin and Krysan “Genetic transformation”

11:00 am - 12:00: Jamie Konopka: Nuts and Bolts on microscopy.

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

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

2:00 – 4:00 pm: **microscope training** by Dr. Jim McIlvain from Zeiss.

Before 4:30 pm: Whenever the team has 5 min (e.g. during heat shock, then do the biolistic bombardment for **Expt. 4** leave at room temperature)

Before 7:00 pm: make sure to recover *Cryptococcus* cells and plate on selective medium for **Expt. 4.**

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

continue **Expt. 6** (-2 day culture)

7:00-8:00 pm: Seminar: Bill Steinbach "Fifteen years of killing *Aspergillus fumigatus*: Newer molecular targets and approaches"

Wednesday, June 15

Before 9:00 am: TA: start **Expt. 5. *Agrobacterium* mediated insertional mutagenesis in *Cryptococcus*** (induction medium)

9:00-10:00 am: Seminar: Karl Kuchler Vienna "**Invasive candida infections - put a brake on inflammation and survive**"

10:00-11:00 am: Seminar: Jamie Konopka "Multiple roles of the fungal plasma membrane in virulence: seeing is believing"

11:00 am – 7:00 pm:

Continue **Expt. 1, 4** (check plates) Expt 6 (TAs: passage culture)

Continue **Expt. 2** (harvest biofilm and RNA extraction, store RNA at -20°C)

12:30 pm: Continue **Expt. 6** (-1 day culture, walk through the lobster facility)

Late afternoon **Expt. 5. *Agrobacterium* mediated insertional mutagenesis in *Cryptococcus*** (co-culture of crypto/agro on induction medium)

7:00 pm: Seminar: Richard Bennett "Catching Lightning in a Bottle: *Candida* the Chameleon"

8:00 -9:00 pm: Karl Kuchler Vienna: Academic career development mentoring session.

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

Thursday, June 16

8:30 -9:00 am: Introduction to *Aspergillus* transformation (Robb Cramer)

9:00-10:00 am: Seminar: Tobias Hohl "Visualizing and Dissecting Antifungal Immunity in the Lung"

10:00-10:15 am: Start **Expt. 8** (spheroplasting)

10:00-11:00 am: Introduction to **Expt. 6** (Jack Edward)

11:00-noon: continue **Expt. 6.** (introduction and inoculum calculation)

1:00 pm – 7:00 pm:

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

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

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

7:00 pm: continue **Expt. 6.** (treatment for *Candida* infection)

Friday, June 17

8:30 – 9:00 am: start competition *Candida* culture (Damian)
9:00 -10:00 am: Seminar: YongSun Bahn “**Systematic Functional Analysis of Pathobiological Networks in *Cryptococcus neoformans***”
10:00 -11: 00 am: Robert Cramer on **animal models for fungal infections**

11:00 -7:00 pm: Continue **Expt. 1** (DNA and PCR)
Start **Expt 7. Mouse model of aspergillosis** (day -1, immunosuppression)
TA starts culture for **Expt. 3** (H99 cells).
Continue **Expt. 2** (cDNA and RT-PCR)

7: 00 pm: Seminar: Robert Cramer “**Environmental Sampling and Experimental Evolution Reveal New Insights Into *Aspergillus fumigatus* virulence**”

Saturday, June 18

9:00-10:00 am: Seminar: Anita Sil “**Regulation of cell shape and virulence by temperature in *Histoplasma capsulatum***”
10:00 -11: 00 am: Introduction to antifungal drug experiments (Damian)
11:00 -noon: Microscopy (competition assay)

1:00 pm-7:00 pm:
Continue **Expt. 7 Mouse model of aspergillosis** (infection)
Continue **Expt. 1** (*Candida* transformants: run gel)
Finish **Expt. 2** (RT-PCR data analysis)
Continue **Expt. 3** (setup drug testing)
Continue **Expt. 4** (pick colonies)
Continue **Expt. 5** (collect cells from Agro/Crypto plate and plate cells on selection medium)
Continue **Expt. 6.** (*Candida* infection)

7: 00 pm: Seminar: Don Sheppard “***Aspergillus* exopolysaccharide - a sticky situation**”

8: 00-9:00 pm: Career development “**women in science**”

Sunday, June 19

Day off (with some experimental manipulations required)

Monday, June 20

8:30-9:30 am: Seminar: Terry Roemer “**Leveraging conditional essentiality in anti-microbial lead finding**”

9:30 -10:30 am: Seminar: Damian Krysan “**Antifungal drug discovery**”

11:00 am-7:00 pm:
Start **Expt. 9a,b. Fungal Leukocyte interactions** (Harvest host cells)
Continue **Expt. 3** (read plates, score growth, start overnight for FIC)
Continue **Expt. 7. Mouse model of aspergillosis**

Continue **Expt. 5** (pick colonies to arrange master plate).
Continue **Expt. 4** (examine cell morphology compared to H99, get positive cells from master plates, spot on YPD at two temps.)
Finish **Expt. 8** (check phenotypes on plate)
Continue **Expt. 1** (students: overnight culture for positive clones, TA overnight for the controls)
Continue **Expt. 6.** (Candida infection)

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

Tuesday June 21

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

8:30-9:30 am: Seminar: Aaron Mitchell “**Ironing out Candida albicans biofilms**”

9:45 -10:45 am: Seminar: Xiaorong Lin “**Cryptococcus morphogenesis and virulence**”

10:45 am:

Continue **Expt. 9a,b** (inoculate Aspergillus and Candida)

1:00 pm-7:00 pm:

Continue **Expt. 7.** Mouse model of aspergillosis

Continue **Expt. 3** (E-test, choose FIC drugs for Expt. 3)

Continue **Expt. 6.** (Candida infection, harvest tissues and plate for CFUs)

3:45 pm: Continue **Expt. 9a,b** (prepare for Elisa and RT-PCR)

Continue **Expt. 6.** (Candida infection, harvest tissues and plate for CFUs)

7:00 pm: Clinical mycology with Don Sheppard

Wednesday, June 22

8:30 -9:30 am: Seminar: Jack Edward “**Immunotherapy for Candida infections: scientific and developmental challenges**”.

9:30 -10:30 am: Introduction to different drug susceptibility tests (Ted White)

11:00 am: Continue **Expt. 3** (E-test, choose FIC drugs for Expt. 3)

Lunch

1:00 pm -7:00 pm:

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

Continue **Expt. 1** (perform double heterozygous phenotypical analyses)

Continue **Expt. 9a,b** (RNA, cDNA and RT-PCR, coat the Elisa plates)

Continue **Expt. 4** (Do serial dilution and spotting assays to check thermo-tolerance)

Continue **Expt. 5** (check melanization defect from transformants)
Continue **Expt. 6.** (Candida infection)

7 pm: Seminar: Aaron Mitchell on “**CRISPR-Cas9 system**”

Thursday, June 23

8:30-9:30 am: Seminar: Joe Heitman “**Sexual reproduction and the evolution of eukaryotic microbial pathogens**”

9:30 am: Class picture

10:00 am: continue **Expt. 9** (wash and block)

10:15-11:00 am: Seminar: Ted White “**The central role of ergosterol biosynthesis in fungal biology**”

11:00 am-7:00 pm:

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

1:00 pm: Finish **Expt. 9a,b** (Elisa and data analysis)

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

6:30 pm: Pizza dinner at Damian’s cabin

Friday, June 24

8:30-9:30 am: Seminar: Deborah Hogan “Fungal microbiome in CF patients”

9:45 -11:00 am: Seminar: Melanie Wellington “Come on Candida, light my fire!”

11:00 am – noon: Finish **Expt. 7 Mouse model of aspergillosis** (PCR and data analysis)

11:00 am – 7:00 pm

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

Finish **Expt. 4** (check thermo-tolerance & cell morphology/size under scope)

Finish **Expt. 5** (check melanization/capsule/ or other phenotypes)

7:00 pm: Bowling night

Saturday, June 25

8:30-9:30 am: Seminar: Christina Hull “TBA”

9:45-11:00 am: Summarize Expt. 6 (Jack)

11:00 am: Finish **Expt. 1** (competition assay)

Gather data, summarize, analyze, and wrap up most experiments

Lunch

1:00 -2:00 pm: Seminar: Arturo Casadevall “The origin of microbial virulence”

2:00 -4:00 pm: group discussion with Arturo Casadevall

4:00 -5:00 pm: print posters

7:00 pm: Mentoring session “Grants and Papers” (all faculty members)

Sunday, June 26 (mini-symposium)

4:00 pm-6:00 pm poster
7:00 pm: Course Banquet

Monday June 27

8:30-10:00 am: Students gather data and organize their presentation
10:00 -11:00 am: Discussion about **Expt. 8 and 9** (Cramer and Wellington)
11:00-noon: Wrap up **Expt. 1, 2, 3, 5.**

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

1:30-2:30 am: Seminar: Andy Alspaugh “**Hidden in plain sight: immune avoidance by fungal pathogens**”

2:30 -5:00 pm: Student led group discussion on each experiment

7:00 pm: Barbecue

Tuesday, June 28

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: Pedro Miramon-Martinez and Virginia Glazier

Exp. 2: Transcriptional profiling of *Candida albicans* biofilms.

Objective: (1) To provide instruction on manipulations and technical considerations pertaining to transcript analysis in fungi. (2) To examine the difference in gene expression of *Candida* biofilm cultured under different conditions by performing RT-PCR.

Faculty instructor: Damian Krysan

TAs: Virginia Glazier and Pedro Miramon-Martinez

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 and Pedro Miramon-Martinez

Exp. 4: Genetic manipulation of *Cryptococcus neoformans*

Objectives: (1) To use a biolistic transformation apparatus to transform *C. neoformans*, establishing stable genomic integration of *CLN1* overexpression constructs. (2) To assess the effects of overexpression of *CLN1* on cell cycle/morphology. (3) To generate the effect of overexpression of *CLN1* on thermo-tolerance.

Faculty Instructor: Xiaorong Lin

TAs: Srijana Upadhyay and Virginia Glazier

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 melanization defects.

Faculty Instructor: Xiaorong Lin

TAs: Srijana Upadhyay and Virginia Glazier

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 and Pedro Miramon-Martinez

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: Sourabh Dhingra 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 and Xiaorong Lin

TAs: Sourabh Dhingra and Srijana Upadhyay

Exp. 9a, b: Fungal interactions with leukocyte (*A. fumigatus* and *C. albicans*)

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. (3) To compare the response of primary murine peritoneal macrophages to the fungal pathogens *C. albicans* and *A. fumigatus*

Faculty Instructor: Robert Cramer and Melanie Wellington

TAs: Alex Hopke and Sourabh Dhingra

Microscopy studies

Faculty Instructor: James B. Konopka

TAs: Srijana Upadhyay and Pedro Miramon-Martinez