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)
James B. Konopka, Stony Brook (Advanced Workshop in Microscopy)
John E. Edwards, Harbor-UCLA Medical Center (Animal models of fungal diseases; clinical mycology)
Joseph Heitman, Duke University (Research talk, career development, student interactions)
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:
Anna Selmecki, Creighton University Medical School
David Boulware, University of Minnesota
Jason Stajich, University of California, Riverside
Jeniel Nett, University of Wisconsin
Julie Djordjevic, University of Sydney
Julian Naglik, King's College London
John Perfect, Duke University Medical Center
Kirsten Nielsen, University of Minnesota
Richard Bennett, Brown University
Sarah Gaffen, University of Pittsburgh
Scott Moye-Rowley, University of Iowa

Teaching Assistants:
Bing Zhai, Memorial Sloan Kettering Cancer Center
Elisa Vesely, Geisel School of Medicine at Dartmouth
Linda Archambault, University of Maine
Robert Zarnowski, University of Wisconsin – Madison
Sarah Beattie, University of Iowa
Yumeng Fan, University of Georgia

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

Our lecture room is Loeb 263 (mornings). Main lab is Loeb 260. Evening seminars will be held at the Starr 209.
Wednesday, July 17
3:00-3:15 pm: MBL facility and lab safety walk through (LB 260).
4:00 pm: TA prepare for Expt. 1. Candida transformation
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: TA prepare strains for Microscope workshop

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

Thursday, July 18
8:00 am: TA training (Loeb 2nd floor lobby)
9:00 am: TA prepare log culture for Expt 1 and Expt 4
8:00 am: Course Introduction (Krysan and Lin)
9:00 am-10:00 am: MBL training (Leob 263)
10:00 am – 11:00 am: Seminar: Jamie Konopka “Candida albicans invasive hyphal growth”
11:00 am : Introduction of Expt 1 and Expt 4
11:30 – 12:30 Lunch

12:30 pm - 4:00 pm:
Start Expt. 1. C. albicans transformation (Krysan)
Start Expt. 4. Cryptococcus transformation (Lin)

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

6:00 – 7:00 pm: continue Expt. 1 and Expt. 4.

7:00 – 9:00 pm: Faculty, TA and student introductions (5 min(!) each)
TA prepare for Expt 6. Candida animal infection (-2 day culture)
TA prepare strains for Expt. 5. Cryptococcus AMT-mediated insertional mutagenesis

Friday, July 19
8:00 am: TA set up the culture (agro and Crypto) for Expt 5.
8:30-9:30 am: Seminar: David Andes “Construction and destruction of the Candida biofilm matrix”
9:45-10:45 am: Seminar: Damian Krysan “Genetic Interactions in Candida albicans”
11:00-12:00 am Introduction to Expt. 6 “Pathogenesis and animal models” (Jack Edward)

1:00-2:00 pm: Introduction to Expt. 5 (Cryptococcus Agro-transformation)
2:00-5:00 pm: Continue Expt. 6 (-1 day culture, walk through the lobster facility)
3:00 – 5:30 pm: microscope training by Dr. Jim McIlvain from Zeiss.
TA: set up fungal/agro culture for **Expt. 5**.

7:00 pm: Genetic manipulation

**Saturday, July 20**

8:30-9:30 am: **Seminar**: Julile Djordjevic “Using *Cryptococcus neoformans* to understand the role of inositolpolyphosphate kinases in cellular function”

9:45-10:45 am: **Seminar**: Jack Edward “Update On Efforts to Develop and Fungal/Candida Vaccine”

10:45-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)

**Microscope workshop** (Jamie Konopka)

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

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

**Sunday, July 21**

8:30-9:30 am: **Seminar**: Robert Cramer “Oxygen and Human Fungal Infection Disease Progression: To Air or Not to Air?”

11:00 am: Introduction to **Expt 2** (David Andes)

1:00 – 7:00 pm:

- Start **Expt. 8** (spheroplasting/ introduce the exp. 8)
- Start **Expt. 2** (inoculation of Candida)
- Introduction to **Expt. 8** (Robb Cramer)
- Continue **Expt. 8** (protoplasting and transformation)
- **Expt. 5. Agrobacterium in Cryptococcus** (collect cells and plate on selective medium)
- **Expt. 6** (continue treatment)
- Continue **Expt. 1, 4** (patch transformants and check tdTomato under microscope, set up overnight culture)

7:00 pm: Mentoring session: career development

**Monday, July 22**

8:30 -9:30 am: **Seminar**: Andy Alspaugh “Signaling, trafficking, and immune evasion for Cryptococcus neoformans”

9:45 -10:45 am: **Seminar**: Sarah Gaffen “The Edge of (IL-) Seventeen”

11:00-noon: mid-term review of experiments

noon -7:00 pm: Continue **Expt. 1** (DNA and PCR, then TA run the gel, students start o/n cultures)
Continue Expt. 2 (set up biofilm plates and treat with fluconazole)
Continue Expt. 6 (Candida infection, treatment)
Continue Expt. 2 (add fluconazole)
O/N culture for Expt 1

7:00 pm: Seminar: Deb Hogan “Fungal diversity and evolution in chronic lung infections”

Tuesday, July 23
8:30-9:30 am: Seminar: Jeniel Nett “Evasion of Neutrophil Defenses by Candida”
9:45 - 10:45 am: Seminar: Julian Naglik “Candidalysin: discovery and function”
11:00 am – noon: Discussion of quantitative data analyses

1:00 - 7:00 pm:
Continue Expt. 1 (Candida transformants: phenotypical testing)
Continue Expt. 2 (Explain XTT assay and perform XTT assay)
Continue Expt. 4 (phenotype and microscope)
Continue Expt. 6 (Candida infection treatment)
Continue Expt. 8 (check plates)
Expt. 7A (spotting assays for stress response)

7:00 -9:00 pm: Seminar: Jason Stajich “Fungal Genomic Diversity at many scales”

Wednesday, July 24
8:30-9:30 am: Seminar: Xiaorong Lin “Cryptococcal development and pathogenesis”
9:45 - 10:45 am: Seminar: Aaron Mitchell “Circuit diversification in the Candida albicans biofilm regulatory network”
11:00 -11:30 am: Introduction to Expt. 7 (Robb Cramer)

1:00 -7:00 pm:
Continue Expt. 1 (check plates)
Continue Expt. 2 (analyze data)
Continue Expt. 4 (examine phenotypes)
Continue Expt. 5 (pick colonies to arrange master plate).
Continue Expt. 6. (Candida infection)
Continue Expt. 8 (check transformants for white ones)
Continue Expt. 7A (watch plates)
TA prepare for Expt. 9

7:00 pm: free time

Thursday, July 25
Day off (with some experimental manipulations required)
TA Start Expt. 7. Mouse model of aspergillosis (day -1, immunosuppression)
Continue treatment for Expt. 6
TA start O/N for Expt. 3.

**Friday, July 26**

8:30 - 9:30 am: Seminar: Ted White “Can’t we all just get along – the effects of media, carbon source and pH on species competition and antifungal susceptibilities.”

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

11:00 am – noon: Introduction to Expt. 3 (Krysan)

1:00 - 7:00 pm:

**Expt. 7: Mouse model of aspergillosis** (infection)
- Continue Expt. 1 (check plates)
- Continue treatment Expt. 6. (Candida infection treatment)
- Continue Expt. 5 (phenotype master plates)
- Expt. 3 (set up MIC plates and set up O/N culture)
- Expt. 4 (check plates)
- Expt. 7 (take pictures)
- TA prepare Expt. 9. (-2)

7:00 pm: Donald Sheppard: Clinical Mycology

**Saturday, July 27**

8:30 - 9:30 am: Seminar: Anna Selmecki “The Impact of Genome Instability on Adaptation and Antifungal Drug Resistance”

11:00 am - noon: Macrophage demonstration (Rob Cramer)

1:00 - 7:00 pm:
- Continue Expt. 3 (MICs, spotting, start o/n culture)
- Continue Expt. 6 (harvest tissues and plate for CFUs)
- Expt. 10 (imaging pre-infected zebra-fish)
- TA prepare Expt. 9 (-1)

7:00 pm: Printing Poster

**Sunday, July 28**

8:30-9:30 am: Seminar: Don Sheppard “A tale of two neutrophils…….”

9:45-10:45 am: Seminar: Rob Wheeler “Innate immune control of Candida albicans dissemination”

9:30 - 9:45 am: Class picture

11:00-noon: Introduction to Zebrafish (Rob Wheeler)

1:00 - 6:00 pm:
- Continue Expt 6. Count colonies
- **Expt. 9 Fungal Leukocyte interactions** (Harvest host cells)
- TA start culture for Expt. 9
Set up Expt. 3 (competition, and O/N for E-test, Group A-B)
Expt. 10 (zebrafish infection, Group C-D)

Expt. 7
TA start culture for Expt. 2
7:00 - 9:00 pm: poster session

Monday, July 29
8:30 - 9:30 am: Seminar: David Boulware “Translational Research in Cryptococcosis”
9:45 - 10:45 am: Seminar: Joe Heitman “Insights into the Cryptococcus Pathogenic Species Complex through Comparative Genomics”
11:00 am – noon: mid-term review of experiments/questions Continue Expt. 9
1:00 – 2:00 pm: Introduction to rabbit model and summarize Expt. 6 (Jack)
2:00 – 7:00 pm: Continue Expt. 3 (E-test, score competition)
   Ted White Nuts/Bolts
   Continue Expt. 9 (harvest and coat ELISA)
   Expt. 2 processing
   Expt. 10 (zebrafish infection, Group A-B)
   Expt. 3 (competition, and O/N for E-test, Group C-D)

7:00 - 9:00 pm: BBQ (in the yard in front of Leob)

Tuesday, July 30
8:30 - 9:30 am: Seminar: John Perfect “Cryptococcus is talking….Are we listening?”
9:45 am – noon: Expt. 10 (zebrafish, Group A-B)
   Expt. 3 (Group C-D scoring)
   Expt. 2 (calcoflour white staining and slide-prep)

1:00 - 7:00 pm:
   Continue Expt. 7 Mouse model of aspergillosis (terminate and lyophilize tissues)
   Expt. 2 (confocal microscopy)
   Finish Expt. 3 (fluorescence-imaging plates)
   Continue Expt. 9 (ELISA)

7:00 pm: Bowling night

Wednesday, July 31
8:30 - 9:30 am: Seminar: Richard Bennett “How Changes in the Genomic Landscape Enable Candida Cells to Adapt to their Host”
9:45 – 10:45 am: Seminar: Kirsten Nielsen “Cryptococcus Cell Wars: When a Bad Yeast Gets Worse”
11:00 am – noon: data analysis of all experiments and prepare presentation
1:00 – 7:00 pm
Continue **Expt. 7. Mouse model of aspergillosis** (DNA extraction and PCR)

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

**Thursday, August 1**

8:30-9:30 am: **Seminar**: Scott Moye-Rowley “Transcriptional control of azole resistance in Aspergillus fumigatus”

11:00-noon: Students gather data, wrap up experiments, and organize their presentation
Finish **Expt. 7 Mouse model of aspergillosis** (data analysis)

12:00-1:30 pm: **Research Ethics and Etiquette Luncheon** (Faculty: Ted White leads discussion)

1:45 -5:00 pm: Faculty and student led group discussion on each experiment

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

**Friday August 2**

Students: Leave Woods Hole
TAs: pack the lab
Experiments:

**Exp. 1: Candida albicans transformation: genetic interaction analysis.**
Objective: (1) To learn to CRISPR/Cas9-mediated genetic manipulation of *C. albicans*. (2) To create a set of *nrg1ΔΔ tfΔΔ* double mutants to identify transcription factors required for the hyperfilamentous phenotype of *nrg1ΔΔ*.
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: David Andes
TAs: Robert Zarnowski

**Exp. 3: Antifungal susceptibility and chemical-genetic interactions.**
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 characterize the potential mode of action of a novel antifungal by assessing chemical genetic interactions using a focused library of *C. neoformans* protein kinase deletion mutants.
Faculty Instructors: Damian Krysan
TAs: Bing Zhai

**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: Elisa Vesely

**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

**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: 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