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Prep Sheet

Creative Microbiology

Authored by Tianyue Zhang, M.Sc and Yuan Chen, M.Sc

Getting Started

Before teaching the lesson plan, complete the following steps prior to class.

- ☐ Setup an OpentronsAI account by going to opentrons.ai from Chrome browser
- ☐ Run through the process of prompting OpentronsAI to write a protocol and ensure you are comfortable with the process

Need Additional Support?

For technical support, please check our Opentrons Help Center for relevant articles. If you need further support, please contact support@opentrons.com.

If you have questions related to the lesson plan, please reach out to the authors, Kennedy Bae, at kennedy@opentrons.com.

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Educator Guide

Creative Microbiology

Authored by Tianyue Zhang, M.Sc and Yuan Chen, M.Sc

Purpose

This lab serves to immerse students in an interdisciplinary journey into biology and automation. Beginning with the collection and culturing of bacteria from their hands, students progress to automating colony isolation through computer vision and eventually design their unique drawings using the bacteria as colors. Students will experience cutting-edge lab equipment through the Opentrons OT2 Robot, and work through a series of pipelines of lab automation. This lab will equip students with the knowledge to streamline experiments and seek innovative solutions across different fields.

Student Audience

This lab caters to high school and undergraduate levels. The duration of each run spans approximately 15-20 minutes, offering flexibility to accommodate a broad spectrum of class sizes.

Background Knowledge

Students should have a conceptual understanding of liquid pipetting, bacteria culture, and programming in Python.

Core Competencies

Gaining Laboratory Experience:

Students will learn how to use lab equipment including Opentrons OT2 Robot; pipetting and plating microbiomes as well as standard handling, cleaning, and lab safety procedures.

Experience the Efficiency of Automation:

By utilizing automation as a tool, students will gain insight into streamlining experiments and artwork design, reducing repetitive labor in execution, and paving the way for future applications across various domains.

Integration of Biology and Design

Gain insights into the interdisciplinary nature of lab automation in biology, computer vision, and arts. Combine knowledge from different disciplines to challenge a complex task collaboratively.

Introduction to Computer Vision for Advanced Classes:

This course includes an optional intro-level image analysis pipeline. Students should understand basic computer vision concepts such as watershed, thresholding, and edge detection to conduct parameter tuning for better detection accuracies.

Supplies

Opentrons Equipment

- ☐ Opentrons OT-2 Automated Liquid Handling Robot
- ☐ Opentrons p300 Single-channel GEN2

Non-Opentrons Equipment

- ☐ USB Camera with auto-focus
- ☐ Incubator
- ☐ 3D printed LB plate tray in black
- ☐ A computer connected to Opentrons OT-2

Labware

- ☐ Opentrons OT-2 96 FIlter Tip Rack 200 ul
- ☐ Opentrons OT-2 96 Filter Tip 2 for each colony
- ☐ Opentrons 15 Tub Rack with Falcon 15 ml conical Tubes

- [ ] 100mm round LB plate
- [ ] LB Omni Tray
- [ ] Swaps

Reagents

- [ ] Water
- [ ] LB

Experimental Duration

Required Class Sessions

2

Lab Run Time

Estimated total time: 2.5-3 hrs

Pre-session:

- 5 minutes to collect samples from hands (1 week before sessions)

First session:

- Taking pictures for the plates: 5 minutes for each group
- Design and run protocol for colony picking: 10-15 minutes for each group to calibrate and run the protocol.

Second session:

- Design a drawing protocol and 10-20 minutes for each student to run the pipeline
- The final plates should be ready in 1-2 days

Lab Preparations:

1. **Experimental Setups**
    a. Prepare LB agar in both a 100mm plate and an Omnitray with a depth of 0.8-1 cm and wettable swabs for bacteria collection
    b. Incubator:
        i. Set to 37 ℃
        ii. Plastic wrap (cling film)
        iii. labels
2. **Camera Setup:**
    a. Adjust focus of the camera to make sure max resolution
    b. To ensure the LB tray's center aligns with the center of the image for easy processing:
        i. Open the 2x2 grid feature in the photo application.
        ii. Align the marker located at the center of each of the four edges of the black holder with the grid lines.
    c. To optimize image quality:
        i. Securely fix the camera position
        ii. Close off any direct light sources that illuminate the plate surface.
3. **Software Setup**
    a. Download our code from github:
    b. Install Anaconda on a laptop/desktop using the

following procedure:

i. Visit the Anaconda page, and install the Anaconda following the instructions
ii. Open the Anaconda terminal and change the working directory to the folder with the downloaded github repository (colony_picking)
iii. create a environment and install the required packages with the following command:

conda env create -f environment.yaml

And it will create a new anaconda environment named “opentrons”. This environment will be used to run pick colonies as well.

If you're anticipating the inclusion of an advanced computer vision analysis pipeline, particularly involving deep learning, students can incorporate their own Python file containing their model. This model can then be loaded in main.py for the purpose of object detection and segmentation. The expected input is an image, and the desired output should provide the coordinates of the centers of the largest four colonies detected (in cases where there are more than four colonies present).

Basic Troubleshooting

1. A video demonstration of a complete process can be accessed via the link.
2. Do a trial run and labware check with empty labware before class; this way any unexpected occurrences can be resolved before students arrive.
3. If you need to reach out to Opentrons Support, kindly mention that you are participating in our Opentrons for Education program and provide the date of your upcoming lab class. For queries regarding Opentrons protocol library and protocol designer, please refer to Opentrons FAQs at https://support.opentrons.com/s/.

Required Pre-Lab Activities

Prior to starting this lab, students should have the following technical abilities and theoretical knowledge:

- Standardized lab safety requirements and how to prepare and clean up biological samples.
- Execute manual and automated liquid handling such as multi-dispense; mixing reagents; and acknowledging the potential deviations of using a robot and manual when the volumes are small.

- Understanding of how to interpret images as an input for computers, which factors will affect the image quality, and how to avoid such influences.

Procedure Guide

Session 1

Growing bacteria sampled from hands

1. zPrepare a 100mm agar plate.
2. Swipe the wetted cotton swab across the surface of your hands to collect a generous sample and transfer it to the plate
3. Incubate the plate at 37 ℃ for 2-7 days until at least 4 colonies are approximately 1 cm in diameter

Colony Identification Using Computer Vision

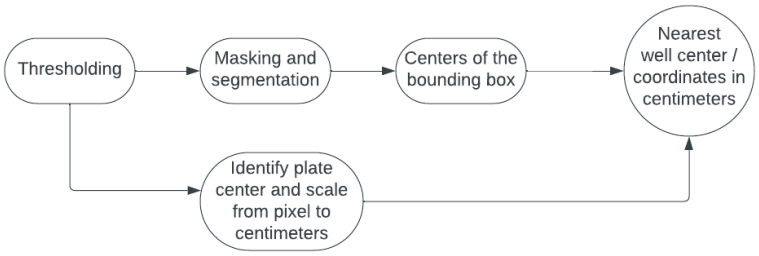
a. Open the Anaconda terminal and navigate to the folder containing our code (colony_picking)
b. Activate the Anaconda environment named opentrons by running:
    conda activate opentrons
c. Calibrate the camera such the center of the plate and image are aligned
d. Take two pictures:
    i. One picture without the LB plate.
    ii. Another picture with the LB plate.
    iii. Move these pictures into the colony_picking folder

e. Rename the picture without the LB plate as pure_black, and the picture with the LB plate as p1
f. Run CV pipeline by calling (current working directory is colony_picking)

```
python main.py
```

This will generate a csv file with the real location of the bacteria colonies named “points.csv”

And here is our general workflow diagram:



g. Go to the Opentrons Protocol Library (https://library.opentrons.com/p/cmu_colony_picking) and upload the “points.csv” at corresponding entry place, and input the information of OT-2 setup corresponding to the real setup, and download a protocol in py format.
h. Upload the py file in the Opentrons APP, and the bacterial picking will be initiated.

Automated plate drawing with bacteria: Picking Colonies ~ 5 minutes

In this step, students will use Opentrons OT2 to pick up the bacteria in the 100mm plate drop in a 15 ml tube to prepare for drawing in the next session.

4. Launch the Opentron Protocol Designer software on your computer.
5. Select Instruments and Consumables:
    a. Select the P300 Single-Channel GEN2 pipette.
    b. Choose the Opentrons OT-2 96 Filter Tip Rack 200 µL tips.
6. Define Layout:
    a. Choose a dummy 960-well plate layout.
    b. Define the positions of colonies or samples based on colors and numbers.
    c. Determine the positions of the tubes where the dispensed liquid will go.
    d. Add 3 ml LB to each tube to dispense bacteria
7. Move the pipette tip to the nearest well based on the colony color and number.
8. Aspirate 1 µL of liquid from the well.
9. Dispense the aspirated liquid into the corresponding tube.
10. Mix the contents in the tube 20-50 times.
11. Clearly label and save the tubes in an incubator.

Trouble shooting:

- Calibrate every plate tube rack before running the protocol:
    a. Ensure the tip will slightly touch the surface of the agar when picking bacteria
    b. Ensure the tip will dip into the agar 1-2 mm when

drawing
    c. No crushing with tubes, tube racks, or the edges of the wells
    d. No direct light source right on top of the OT-2 machine. This will interfere with the cv result.
- Ensure that multi-dispensing without blowout is enabled.
- Set the dispensing volume to 1 µL.
- If you find that the generated mask is incorrect, check to see if there are any other objects besides the LB tray in the camera's view while calibrating. If there are additional objects, remove them so that the camera is only capturing the LB tray.

Session 2

1. **Automated plate drawing with bacteria: Drawing ~ 20 minutes per student**

Allow students to design their own drawings on the dummy 960 plates.

Drawing tips:

Ensure the tip is calibrated to sit slightly below the agar surface. In cases of uneven agar surfaces, calibrate to the shallowest point and dispense 0.5-1ul at each spot.

2. **Clean up**

Students are required to bleach used plates and tubes before

leaving the lab. Used tips should be disposed of properly.

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Student Guide

Creative Microbiology

Authored by Tianyue Zhang, M.Sc and Yuan Chen, M.Sc

Purpose

This lab immerses you in an interdisciplinary journey into biology and automation. Beginning with the collection and culturing of bacteria from hands, you will progress to automating colony isolation through computer vision and eventually design your unique drawings using the bacteria as colors.

You will gain hands-on experience with cutting-edge lab equipment by using an Opentrons liquid handling robot (OT-2), and work through a series of pipelines of lab automation. You will be equipped with the knowledge to streamline experiments and seek innovative solutions across different fields.

Equipment

Opentrons Equipment

- ☐ Opentrons OT-2 Automated Liquid Handling Robot
- ☐ Opentrons p300 Single-channel GEN2

Non-Opentrons Equipment

- ☐ USB Camera with auto-focus
- ☐ Incubator
- ☐ 3D printed LB plate tray
- ☐ A computer connected to Opentrons OT-2

Labware

- ☐ Opentrons OT-2 96 FIlter Tip Rack 200 ul
- ☐ Opentrons OT-2 96 Filter Tip 2 for each colony
- ☐ Opentrons 15 Tub Rack with Falcon 15 ml conical Tubes 1 for each colony
- ☐ Black plate holder
- ☐ 100mm round LB plate
- ☐ LB Omni Tray
- ☐ Cotton-tipped swabs

Reagents

- ☐ diH2O
- ☐ Lysogeny broth (LB)

Experimental Procedure

Session One: Collecting and culturing bacteria sampled from hands

This will be formed up to one week before Session Two.

1. Prepare a 100mm agar plate.
2. Pre-moisten the cotton swab with water.
3. Swipe the moistened cotton swab across the surface of your hands to collect a generous sample.
4. Transfer the sample to the plate by gently wiping the swab over the surface of the agar.
5. Incubate the plate at 37 ℃ for 2-7 days until at least 4 colonies are approximately 1 cm in diameter.

Session Two: Colony identification and colony picking

At the start of this lab session, you will review the lab by watching this brief video:

Creative Microbiology: A Course Designs for Automation a...

Colony Identification Using Computer Vision

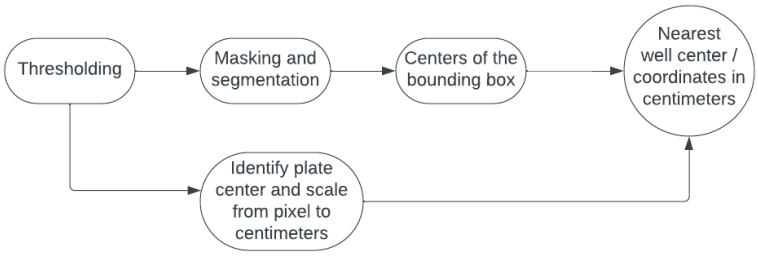
1. Open the Anaconda terminal and navigate to the folder containing our code (colony_picking)
2. Activate the Anaconda environment named opentrons by running:

   conda activate opentrons
3. Calibrate the camera such the center of the plate and image are aligned

4. Take two pictures:
    a. One picture without the LB plate.
    b. A second picture with the LB plate.
    c. Move these pictures into the colony_picking folder
5. Rename the picture without the LB plate as pure_black, and the picture with the LB plate as p1
6. Run CV pipeline by calling (current working directory is colony_picking)

```
python main.py
```

    i. This will generate a csv file with the real location of the bacteria colonies named “points.csv”
    ii. And here is our general workflow diagram:



7. Go to the protocol in the Protocol Library (https://library.opentrons.com/p/cmu_colony_picking) and upload the “points.csv” at corresponding entry place, and input the information of OT-2 setup corresponding to the real setup, and download a protocol in py format.
8. Upload the py file in the Opentrons APP, and the bacterial picking will be initiated.

Automated plate drawing with bacteria: Picking Colonies ~

5 minutes per plate

You will use Opentrons OT2 to pick up the bacteria from the 100mm plate and deposit into a 15 ml tube to prepare for drawing in the next session.

1. Launch the Opentron Protocol Designer software on your computer.
2. Select Instruments and Consumables:
    a. Select the P300 Single-Channel GEN2 pipette.
    b. Choose the Opentrons OT-2 96 Filter Tip Rack 200 µL tips.
3. Define Layout:
    a. Choose a dummy 960-well plate layout.
    b. Define the positions of colonies or samples based on colors and numbers.
    c. Determine the positions of the tubes where the dispensed liquid will go.
    d. Add 3 ml LB to each tube to dispense bacteria
4. Move the pipette tip to the nearest well based on the colony color and number.
5. Aspirate 1 µL of liquid from the well.
6. Dispense the aspirated liquid into the corresponding tube.
7. Mix the contents in the tube 20-50 times.
8. Clearly label and save the tubes in an incubator.

Troubleshooting:

- Calibrate every plate tube rack before running the

protocol:

a. Ensure the tip will slightly touch the surface of the agar when picking bacteria
b. Ensure the tip will penetrate 1-2 mm into the agar when drawing
c. No crashing with tubes, tube racks, or the edges of the wells

- Ensure that multi-dispensing without blowout is enabled.
- Set the dispensing volume to 1 µL.
- If you find that the generated mask is incorrect, check to see if there are any other objects besides the LB tray in the camera's view while calibrating. If there are additional objects, remove them so that the camera is only capturing the LB tray.

Session Three

Automated plate drawing with bacteria: Drawing ~ 20 minutes per plate

Design individual drawings on the dummy 960 plates.

Drawing tips: Ensure the tip is calibrated to sit slightly below the agar surface. In cases of uneven agar surfaces, calibrate to the shallowest point and dispense 0.5-1ul at each spot.

1. **Properly wrap and store your drawing plates in an incubator for 1-2 days.**

2. Clean up

Bleach used plates and tubes before leaving the lab. Used tips should be disposed of properly.

Post-Lab

After 1-2 days of incubation, you will have the opportunity to view and photograph your final plates.

After viewing, ensure you bleach and properly dispose of your plates before leaving the lab.