FINAL SCRIPT: APPROVED FOR FILMING

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Title: Real-Time Force Measurement Between Emulsion Droplets During Enzymatic Breakdown

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No.**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done.**

3. Filming location: Will the filming need to take place in multiple locations? **No.**

Current Protocol Length

Number of Steps: 7

Number of Shots: 26 (17 SC)

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Huaizhou Jin:** We use optical tweezers to measure real-time forces between individual emulsion droplets during enzymatic digestion to understand the stability during digestion process.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.2.2*

What are the current experimental challenges?

1.2. **Huaizhou Jin:** During the OT experiment, the digestion process is continuous. The optimal concentration of enzymes are difficult to determine, and if we fail to capture droplets in time, we need to restart the digestion all over again.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.3*

What significant findings have you established in your field?

1.3. **Huaizhou Jin:** The slope and maximum force between individual droplets are indeed tied to the oil type (SFA, MUFA, or PUFA based), digestion time, and enzyme type. It appears for digestion with protease oil type does not affect the digestion process.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.1*

What research questions will your laboratory focus on in the future?

1.4. **Huaizhou Jin:** Possibly an experiment that uses both spectroscopic monitoring and force monitoring to see the link between force and changes of chemical structures.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.2*

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Setting up the Optical Tweezers

Demonstrators: Chenhui Song, Huaizhou Jin

2.1. To begin, prepare the sample flowcell **[1]**, and then open the optical tweezers instrument **[2]**. Add approximately 70 microliters of deionized water to the lower objective lens **[3]** and insert the sample chamber into the instrument until a click is heard **[4]**.
Added shot: Talent drops the emulsion sample to a glass slide with tape, then seals the flowcell with a coverslip.

2.1.1. WIDE: Talent opening the optical tweezers instrument.

2.1.2. Talent using a pipette to add 70 microliters of deionized water to the lower objective lens.

2.1.3. Talent inserting the sample chamber.

2.2. Place a drop of immersion oil on the top surface of the sample chamber **[1]**. Then, using the condenser knob, gently lower the condenser lens until it lightly touches the top surface of the sample chamber **[2]**. Close the optical tweezers instrument to complete the setup before activating the laser **[3]**.

2.2.1. Talent applying a drop of immersion oil onto the sample chamber.

2.2.2. Talent carefully lowering the condenser lens until contact is made.

2.2.3. Talent closing the optical tweezers instrument.

2.3. Now, turn on the laser **[1]** and set the power to 100 percent **[2]**. Adjust the lower knob on the optical tweezers system in a clockwise direction to locate the laser spot, moving from bottom to top **[3]**. Observe the laser spots three times **[4]** and set the Z-plane between the second and third sets **[5]**. Then, reduce the laser power to 30 percent **[6]**.

2.3.1. SCREEN: 2.3.1.

2.3.2. SCREEN: 2.3.2.

2.3.3. Talent adjusting the lower knob of the optical tweezers system.

2.3.4. SCREEN: 2.3.4.

2.3.5. SCREEN: 2.3.5.

2.3.6. SCREEN: 2.3.6.

3. **Measurement of the Force-Distance Curve for the Emulsion**

3.1. Use the joystick to capture two droplets of similar size within the channel **[1-TXT]**. Adjust the distance between the two droplets to approximately 10 micrometers **[2]**. Fix the x and y positions of the right optical trap **[3]** and the y position of the left optical trap **[4]**, then mark the droplets with magenta and green boxes, respectively **[5]**.

3.1.1. Talent manipulating the joystick to trap two similar-sized droplets. **TXT: Adjust the flow rates as needed**

3.1.2. SCREEN: 3.1.2.

3.1.3. SCREEN: 3.1.3 .

3.1.4. SCREEN: 3.1.4 .

3.1.5. SCREEN: 3.1.5 00:05-00:16.

3.2. Now, navigate to the **Calibration** menu **[1]** and select Measure to begin calibration **[2]**. If noise levels are excessive, repeat the measurement until satisfactory results are visible **[3]**. Select **Apply** to confirm the calibration **[4]**.

3.2.1. SCREEN: 3.2.1.

3.2.2. SCREEN: 3.2.2.

3.2.3. SCREEN: 3.2.3.

3.2.4. SCREEN: 3.2.4.

3.3. Next, clear the original data using the appropriate software interface **[1]** and measure the force-distance using the calibrated optical trap system **[2]**.

3.3.1. SCREEN: 3.3.1.

3.3.2. SCREEN: 3.3.2 .

3.4. Finally, add the trypsin solution to the system **[1]**. Adjust the settings to measure every 10 minutes, starting 10 minutes after adding the trypsin solution **[2]** to dynamically monitor the emulsions using the optical tweezer system **[3]**.

3.4.1. Talent adding trypsin solution into the sample setup.

3.4.2. SCREEN: 3.4.2. 00:00-00:20

3.4.3. SCREEN: 3.4.3.

Results

4. Results

4.1. Emulsions containing rapeseed oil and whey protein, and milk fat and whey protein, were analyzed using optical tweezers **[1]**. For rapeseed oil emulsions without trypsin, peak interaction force increased with droplet size, reaching over 100 piconewtons at 8 micrometers **[2]**, compared to approximately 10 piconewtons at 3 micrometers **[3]**. A similar trend was observed for milk fat emulsion droplets **[4]**.

4.1.1. LAB MEDIA: Figure 5.

4.1.2. LAB MEDIA: Figure 5C.

4.1.3. LAB MEDIA: Figure 5A.

4.1.4. LAB MEDIA: Figure 5 D E F. *Video editor: Highlight F*

4.2. In both emulsions, the mean peak interaction force increased with droplet diameter, showing a roughly linear relationship **[1]**.

4.2.1. LAB MEDIA: Figure 8A and 8B. *Video editor: Sequentially Highlight the bars from left to right to show the bars become taller. Do this for both A and B*

4.3. Droplets with a diameter of 5 micrometers had the highest capture count among all sizes, indicating optimal trapping efficiency **[1]**, while capture frequencies for 3 micrometer and 10 micrometer droplets were the low **[2]**.

4.3.1. LAB MEDIA: Figure 9. *Video editor: Highlight the tallest bar corresponding to "5" µm on the x-axis*.

4.3.2. LAB MEDIA: Figure 9. *Video editor: Highlight the bars corresponding to 3 µm and 10 µm on the x-axis*.

4.4. After adding trypsin, milk fat emulsion droplets showed a gradual decline in peak interaction force from 31.9 piconewtons at 10 minutes **[1]** to 6.6 piconewtons at 60 minutes **[2]**.

4.4.1. LAB MEDIA: Figure 6 (10min).

4.4.2. LAB MEDIA: Figure 6 (60min).

4.5. In rapeseed oil emulsions, trypsin treatment caused the force-distance curves to decline sharpy, suggesting that the efficiency of enzymatic action is more in the liquid oil phase **[1].**

4.5.1. LAB MEDIA: Figure 7 (40min).

Pronunciation Guide:

1. Microliter

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/microliter
- **IPA**: /ˈmaɪ.krəˌliː.tər/
- **Phonetic Spelling**: MY-kroh-lee-ter(merriam-webster.com)

2. Objective Lens

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/objective%20lens
- **IPA**: /əbˈdʒɛk.tɪv lɛnz/
- **Phonetic Spelling**: ub-JEK-tiv lenz(merriam-webster.com, merriam-webster.com, merriam-webster.com)

3. Immersion Oil

- **Pronunciation link**: https://www.merriam-webster.com/medical/immersion%20oil
- **IPA**: /ɪˈmɜːr.ʒən ɔɪl/
- **Phonetic Spelling**: ih-MUR-zhun oil(merriam-webster.com, merriam-webster.com, merriam-webster.com)

4. Condenser

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/condenser
- **IPA**: /kənˈdɛn.sər/
- **Phonetic Spelling**: kuhn-DEN-ser(merriam-webster.com, merriam-webster.com)

5. Laser

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/laser
- **IPA**: /ˈleɪ.zər/
- **Phonetic Spelling**: LAY-zer(merriam-webster.com, merriam-webster.com)

6. Trypsin

- **Pronunciation link**: https://www.merriam-webster.com/medical/trypsin
- **IPA**: /ˈtrɪp.sɪn/
- **Phonetic Spelling**: TRIP-sin(merriam-webster.com, merriam-webster.com)

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7. Emulsion

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/emulsion
- **IPA**: /ɪˈmʌl.ʃən/
- **Phonetic Spelling**: ih-MUL-shun(merriam-webster.com)

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8. Rapeseed Oil

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/rapeseed
- **IPA**: /ˈreɪp.siːd ɔɪl/
- **Phonetic Spelling**: RAYP-seed oil(merriam-webster.com)

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9. Whey Protein

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/whey
- **IPA**: /weɪ ˈproʊ.tiːn/
- **Phonetic Spelling**: WAY PRO-teen(merriam-webster.com)

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10. Piconewton

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/piconewton
- **IPA**: /ˈpɪk.oʊˌnjuː.tən/
- **Phonetic Spelling**: PIK-oh-new-ton(merriam-webster.com)
