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Title: Real-Time Label-Free Imaging and Quantitative Analysis of Macrophage Morphodynamics Using Optical Diffraction Tomography

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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**

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

- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **YES**

Current Protocol Length

Number of Steps: 11

Number of Shots: 35

Interviews

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

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Chen Sun:** We developed a label-free ODT-based workflow to monitor dynamic macrophage behavior at the single-cell level.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Chen Sun:** Traditional endpoint assays cannot continuously track live-cell dynamics, while ODT enables long-term label-free observation of living cells.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.3. **Ke-bin Shi:** The most important consideration while performing this protocol is maintaining stable focus and appropriate cell density for reliable long-term single-cell tracking.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Ke-bin Shi:** This workflow can be combined with multimodal imaging, 3D reconstruction, and additional quantitative morphological analyses.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Ke-bin Shi:** Future studies may further improve automated analysis in complex cellular environments and expand this workflow to other live-cell systems.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

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

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

What problem in your field of research does your JoVE article address?

- 1.6. **Ke-bin Shi, Professor, Peking University**: (authors will present their testimonial statements live)
 - 1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What value do you expect this video article to have for other researchers working in your field?

- 1.7. **Ke-bin Shi, Professor, Peking University**: (authors will present their testimonial statements live)
 - 1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Please film the testimonials in both English and Chinese

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Optical Diffraction Tomography (ODT) Acquisition

Demonstrators: Chen Sun and Jie-jie Zhu

- 2.1. To begin, take cultured RAW264.7 (*Raw-Two-Sixty-Four-Point-Seven*) macrophage cells with 80 to 90 percent confluency and treat them with lipopolysaccharide and Baicalin [1]. Then, turn on the optical diffraction tomography imaging system [2] and start the environmental control module [3].
 - 2.1.1. WIDE: Talent at the working area, adding LPS and BAI+LPS to the culture dish.
***Videographer:** Please obtain CU shot as well for this shot.*
 - 2.1.2. Talent turning on the optical diffraction tomography imaging system.
 - 2.1.3. Talent starting the environmental control module. **NOTE: It has been filmed by the photographer**
- 2.2. Set the stage temperature to 37 degrees Celsius [1] and the carbon dioxide concentration to 5 percent [2]. Then, allow the system to equilibrate for at least 20 minutes [3].
 - 2.2.1. Talent setting the stage temperature to 37 degrees Celsius.
 - 2.2.2. Talent setting the carbon dioxide concentration to 5 percent.
 - 2.2.3. Shot of the system equilibrating.
- 2.3. After opening the acquisition software, place the culture dish on the imaging stage [1]. Use a 60X (*Sixty-X*) objective with a numerical aperture of 1.42 to locate cells [2] and select isolated cells with clear boundaries for imaging [3]. **NOTE: The VO is edited for the deleted shot**
 - ~~2.3.1. SCREEN: Show the acquisition software opening. **NOTE: SC quality not good**~~
 - 2.3.2. Talent placing the culture dish on the imaging stage.
 - 2.3.3. SCREEN: 7.12_JOVE_71209_2.3.3.mp4
 - 2.3.4. SCREEN: 7.12_JOVE_71209_2.3.4.mp4
- 2.4. Set the imaging interval to 8 minutes and the total number of frames to 181 [1].
 - 2.4.1. SCREEN: 7.12_JOVE_71209_2.4.1.mp4
- 2.5. Enable the autofocus function to maintain focus during time-lapse imaging and start time-lapse acquisition [1]. Monitor system stability during acquisition [2].
 - 2.5.1. SCREEN: 7.12_JOVE_71209_2.5.1.mp4

2.5.2. Talent monitoring system stability during acquisition.

3. Quantitative Analysis of Macrophage Morphodynamics

3.1. After image acquisition, the software automatically performs image reconstruction using the built-in standard algorithm. Wait until the progress bar is complete [1].

3.1.1. SCREEN: 7.12_JOVE_71209_3.1.1+3.1.2.mp4

3.2. In the analysis software, import the raw dataset by selecting **Import Raw Data**, browsing to the target folder, and choosing the file [1].

3.2.1. SCREEN: 7.12_JOVE_71209_3.2.1.mp4

3.3. Then, click “**Cell**” to perform automatic segmentation using consistent settings across datasets [1].

3.3.1. SCREEN: 7.12_JOVE_71209_3.3.1.mp4

3.4. Select cells that do not overlap with neighboring cells [1], are not in contact with image boundaries [2], and can be continuously tracked throughout the time series [3].

3.4.1. SCREEN: 7.12_JOVE_71209_3.4.1.mp4

3.4.2. SCREEN: 7.12_JOVE_71209_3.4.2.mp4

3.4.3. SCREEN: 7.12_JOVE_71209_3.4.3.mp4: 00:02-end

3.5. Select a fixed number of cells per field of view for consistent analysis across datasets [1]. Go to the **Long-term Tracking** module [2].

3.5.1. SCREEN: 7.12_JOVE_71209_3.5.1.mp4: 00:03-end

3.5.2. SCREEN: 7.12_JOVE_71209_3.5.2.mp4

3.6. Then, enter the start and end frame [2]. Click **Track** to initiate tracking based on the consistency of cell positions between consecutive frames [1].

3.6.1. SCREEN: 7.12_JOVE_71209_3.6.1.mp4

3.6.2. SCREEN: 7.12_JOVE_71209_3.6.2.mp4

3.7. Click on **Export**, then select **Chart System** followed by **Track Chart**. Choose **Cell** as the segmentation category and specify the tracking frame range [1].

3.7.1. SCREEN: 7.12_JOVE_71209_3.7.1.mp4

3.8. Select the output parameters, such as **Area** and **Perimeter**. Click **Export Image or Excel** and save the file [1].

3.8.1. SCREEN: 7.12_JOVE_71209_3.9.1.mp4: 00:15-00:35

3.9. Calculate the average migration speed in micrometers per hour for each cell based on the centroid displacement across the entire time series [1].

3.9.1. SCREEN: 7.12_JOVE_71209_3.10.1 00:05-00:24

Results

4. Results

4.1. Time-lapse optical diffraction tomography imaging enabled continuous recording of single-cell morphological changes over time [1].

4.1.1. LAB MEDIA: Figure 2.

4.2. At early timepoints, cells in both lipopolysaccharide and lipopolysaccharide with Baicalin groups exhibited relatively round morphology with clear boundaries and minimal variation [1].

4.2.1. LAB MEDIA: *Figure 2. Video editor: Highlight the panels labeled 6 h in both rows, showing round cells with smooth edges*

4.3. After 8 hours, cells showed increased morphological diversity, including spreading, irregular edges, and protrusions [1]. At later stages, these changes intensified, with greater contour complexity and heterogeneity.[2].

4.3.1. LAB MEDIA: Figure 2. *Video editor: Highlight the panels labeled 12 h and 18 h showing cells with uneven shapes and extensions at the same location as shown in the 24 h image.*

4.3.2. LAB MEDIA: Figure 2. *Video editor: Highlight the 24 h panels and the inset showing irregular and complex cell shapes*

4.4. Representative single-cell tracking trajectories were visualized over time for both treatment groups [1].

4.4.1. LAB MEDIA: Figure 3A.

4.5. Projected cell area remained relatively stable during the early phase in both groups, fluctuating around 140 to 170 square micrometers [1]. After approximately 12 hours, a subset of cells exhibited increased projected area, with more pronounced changes during 12–24 hours [1].

4.5.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the area plot under LPS and BAI+LPS and emphasize the lines cluster around 140–170 (y axis) between 0 and 8*

4.5.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the area plot under LPS and BAI+LPS and emphasize the lines between 12-24 h*

4.6. Under lipopolysaccharide treatment, some cells showed rapid expansion in projected

area exceeding 400 to 500 square micrometers [1]. However, under lipopolysaccharide treatment with Baicalin, most cells maintained a projected area of 250 to 300 square micrometers during the later stage [2].

4.6.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the lines in the LPS area plot between 400-500 (y-axis) and between 12-24 h*

4.6.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the lines in the BAI+LPS area plot, between 250-300 (y-axis) at 12-24 h*

4.7. Cell perimeter remained around 40 to 50 micrometers during the early phase in both groups [1].

4.7.1. LAB MEDIA: Figure 3B. *Video editor: Highlight perimeter plot and emphasize the lines in the early time region (0-12 h) where values cluster near 40-50*

4.8. In the mid-to-late phase, some cells treated with lipopolysaccharide exceeded 100 micrometers in perimeter [1], while most cells treated with lipopolysaccharide and Baicalin remained around 70 micrometers at later stages [2].

4.8.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the lines staying above 100 in the LPS perimeter plot between 12-24 h*

4.8.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the lines between 40-80 (y-axis) in the BAI+LPS perimeter at 12-24 h*

1. RAW264.7

Pronunciation link: No confirmed link found

IPA: No verified IPA available

Phonetic Spelling: raw two·sixty·four point seven

2. Macrophage

Pronunciation link: <https://www.merriam-webster.com/dictionary/macrophage>

- IPA: /'mæk.rəˌfeɪdʒ/
Phonetic Spelling: mak·ruh·fayj
3. Confluency
Pronunciation link: <https://www.merriam-webster.com/dictionary/confluence>
IPA: /kən'fluː.ən.si/
Phonetic Spelling: kun·floo·uhn·see
 4. Lipopolysaccharide
Pronunciation link: <https://www.merriam-webster.com/medical/lipopolysaccharide>
IPA: /ˌlɪp.oʊ.pɑː.li'sæk.ə.raɪd/
Phonetic Spelling: lip·oh·pol·ee·sak·uh·ryde
 5. Baicalin
Pronunciation link: <https://www.howtopronounce.com/baicalin>
IPA: /'baɪ.kəlɪn/
Phonetic Spelling: bye·kuh·lin
 6. Diffraction
Pronunciation link: <https://www.merriam-webster.com/dictionary/diffraction>
IPA: /dɪ'fræk.ʃən/
Phonetic Spelling: dih·frak·shuhn
 7. Tomography
Pronunciation link: <https://www.merriam-webster.com/dictionary/tomography>
IPA: /tə'mɑː.grə.fi/
Phonetic Spelling: tuh·mog·ruh·fee
 8. Equilibrate
Pronunciation link: <https://www.merriam-webster.com/dictionary/equilibrate>
IPA: /ˌiː.kwə'laɪ.breɪt/
Phonetic Spelling: ee·kwuh·ly·brayt
 9. Numerical aperture
Pronunciation link: <https://www.merriam-webster.com/dictionary/aperture>
IPA: /nuː'mer.i.kəl'æp.ə.tʃər/
Phonetic Spelling: noo·mer·ih·kuhl ap·er·cher
 10. Autofocus
Pronunciation link: <https://www.merriam-webster.com/dictionary/autofocus>
IPA: /'ɔː.touˌfoʊ.kəs/
Phonetic Spelling: aw·toh·foh·kuhs
 11. Segmentation
Pronunciation link: <https://www.merriam-webster.com/dictionary/segmentation>
IPA: /ˌseg.mən'teɪ.ʃən/
Phonetic Spelling: seg·muhn·tay·shuhn
 12. Morphodynamics
Pronunciation link: <https://www.howtopronounce.com/morphodynamics>
IPA: /ˌmɔːr.foʊ.dai'næm.ɪks/
Phonetic Spelling: mor·foh·dye·nam·iks
 13. Reconstruction
Pronunciation link: <https://www.merriam-webster.com/dictionary/reconstruction>

IPA: /ˌriː.kənˈstrʌk.fən/

Phonetic Spelling: ree·kun·struk·shuhn

14. Trajectories

Pronunciation link: <https://www.merriam-webster.com/dictionary/trajectory>

IPA: /trəˈdʒek.tə.ɪz/

Phonetic Spelling: truh·jek·tuh·reez

15. Centroid

Pronunciation link: <https://www.merriam-webster.com/dictionary/centroid>

IPA: /ˈsen.trɔɪd/

Phonetic Spelling: sen·troyd

16. Displacement

Pronunciation link: <https://www.merriam-webster.com/dictionary/displacement>

IPA: /dɪsˈpleɪs.mənt/

Phonetic Spelling: dis·plays·muhnt

17. Morphological

Pronunciation link: <https://www.merriam-webster.com/dictionary/morphological>

IPA: /ˌmɔːr.fəˈlɑː.dʒɪ.kəl/

Phonetic Spelling: mor·fuh·loj·i·kuhl

18. Protrusions

Pronunciation link: <https://www.merriam-webster.com/dictionary/protrusion>

IPA: /proʊˈtruː.ʒən/

Phonetic Spelling: proh·troo·zhuhn

19. Heterogeneity

Pronunciation link: <https://www.merriam-webster.com/dictionary/heterogeneity>

IPA: /ˌhet.ə.oo.dʒəˈniː.ə.ti/

Phonetic Spelling: het·uh·roh·juh·nee·uh·tee

20. Micrometers

Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometer>

IPA: /maɪˈkraː.mə.tə/

Phonetic Spelling: my·krah·muh·ter