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Title: Analysis of Multidimensional Microscopy Data Using Cell-ACDC

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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: 23
Number of Shots: 37 (35 SC)

Introduction

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

INTRODUCTION:

1.1. **Francesco Padovani:** We are improving the analysis of multidimensional microscopy data by developing the Analysis of Cell Division Cycle software to overcome the bottlenecks to fast biological discovery.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What are the current experimental challenges?

1.2. **Timon Stegmaier:** Current AI models are not easily accessible. Visualisation and manual correction are essential for achieving high-quality/precise results. But without the right tools, this can be tedious.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.5.1*

CONCLUSION:

What significant findings have you established in your field?

1.3. **Francesco Padovani:** Cell-ACDC is an open-source software framework that enables easy access to AI models for bioimage analysis and ensures high shareability of microscopy data

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.1*

What advantage does your protocol offer compared to other techniques?

1.4. **Kurt Schmoller:** A key aspect of Cell-ACDC is that the community can (easily) integrate new methods into existing workflows with standardized data structure.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.3.1*

What questions will future research focus on?

1.5. **Benedikt Mairhörmann:** Leveraging corrected data from Cell-ACDC for training state-of-the-art methods can lay the foundation for fully automated bioimage analysis.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.1*

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.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.1. **Francesco Padovani, Postdoc at Helmholtz Munich**

and

Kurt M. Schmoller, Principal Investigator at Helmholtz MunichBenedikt: (authors will present their testimonial statements live)

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.2. **Mairhörmann, Postdoc at Helmholtz Munich**

and

Timon Stegmaier, Master Student at Helmholtz Munich,: (authors will present their testimonial statements live)

Beno: reach wider audience and increase the data sharing withing the acdc community

Authors: Could you please also deliver the above statements in German? Two authors can deliver in English and the other two can deliver in German.

Videographer: Please film the testimonials in both English and German

Protocol

NOTE: Videographer shot the screen recordings, but don't use them. Use the lab media files as indicated below

2. Correcting Segmentation and Tracking Errors

Demonstrator: Francesco Padovani

2.1. To begin, click on **Launch GUI…** *(G-U-I)* in the main window of the Visualise and correct module **[1]**.

2.1.1. WIDE: Talent taking a seat at the computer table.

2.2. Click the folder icon in the toolbar of the new window **[1]** and select the folder containing the data, then press **Select Folder** to confirm the selection **[2]**.

2.2.1. SCREEN: "2.2.1_take_1.mp4"

2.2.2. SCREEN: "2.2.2_take_1.mp4"

2.3. Use the dropdown menu to select the channel **phase_contr_preprocessed** *(phase contrast pre- processed)*, then press **Ok** to confirm **[1]**.

2.3.1. SCREEN: 2.3.1_take_2.mp4

2.4. Then, select the segmentation mask name by clicking **Load selected** to load the segmentation file created in the previous step **[1]**.

2.4.1. SCREEN: "2.4.1_take_1.mp4"

2.5. Confirm the image properties by clicking **Ok for loaded Positions [1]**. When prompted, select **No** to prevent loading of additional fluorescence data **[2]**.

2.5.1. SCREEN: "2.5.1_take_1.mp4"

2.5.2. SCREEN: "2.5.2_take_2.mp4"

2.6. Use the mode selector to select **Segmentation and Tracking** mode **[1]**.

2.6.1. SCREEN: "2.6.1_take_1.mp4"

2.7. In the menu bar, navigate to **Tracking** followed by **Select real-time tracking algorithm** and select the desired real-time tracker based on the organism **[1]**.

2.7.1. SCREEN: "2.7.1_and_2.7.2_take_1.mp4"

2.8. Use the left and right arrow keys to navigate between frames **[1]**. Navigate to frame 10 **[2]**.

2.8.1. Talent using keyboard to press left and right arrow keys.

2.8.2. SCREEN: "2.8.2_take_1.mp4"

2.9. Press the key **S** to activate the manual bud separation tool and right-click to automatically split the segmentation mask of cell 1 **[1]**.

2.9.1. SCREEN: "2.9.1_and_2.9.2_take_1"

2.10. Now, navigate to frame 14 **[1]**. Press the key **B** to activate the brush tool and draw the missing segmentation mask for the bud using the left mouse button **[2]**.

2.10.1. SCREEN: "2.10.1_take_1.mp4"

2.10.2. SCREEN: "2.10.2_and_2.10.3_take_1.mp4"

2.11. Continue through the subsequent frames while correcting segmentation and tracking errors using the available tools. Correct at least until frame 42 **[1]**.

2.11.1. SCREEN: "2.11.1_take_1.mp4" 00:30-00:45

3. Cell Cycle Annotations: Asymmetrically Dividing Cells

3.1. Activate **Cell cycle analysis** using the mode selector. When prompted, select **Yes** to go to frame 1 **[1]**.

3.1.1. SCREEN: "3.1.1_and_3.1.2_take_1.mp4"

3.2. Use the left and right arrow keys to navigate between frames **[1]**. Click **Ok** to accept the

initialization of the Cell Cycle Annotation table when prompted **[2]** and navigate to frame 41 **[3]**.

3.2.1. SCREEN: "3.2.1_and_3.2.2_and_3.2.3_take_3.mp4" 00:00-00:03

3.2.2. SCREEN: "3.2.1_and_3.2.2_and_3.2.3_take_3.mp4" 00:04-00:15

3.2.3. SCREEN: "3.2.1_and_3.2.2_and_3.2.3_take_3.mp4" 00:29-00:31 *Video editor: Highlight "Frame n. 41……Last cc annot. Frame n = 41" at the bottom of the image box*

3.3. Right-click on cell 1 or its bud to separate the connection and annotate the cell division event **[1]**.

3.3.1. SCREEN: "3.3.1_take_1.mp4"

3.4. Continue viewing all relevant frames and correct any mistakes in automatic mother-bud assignments using the available tools **[1]**.

3.4.1. SCREEN: "3.4.1_take_1.mp4 00:10-00:20

3.5. To assign a bud to a mother, activate the **Assign Bud to Mother** tool by pressing **A**. Press and hold the right mouse button on the bud, drag to the corresponding mother cell, and release the mouse button **[1]**.

3.5.1. SCREEN: 68954_screenshot_21.mkv"

~~3.6. To annotate unknown cell history, activate the **Annotate Unknown History** tool by pressing **U**. Right-click on the cell to annotate it as having unknown history **[1]**.~~

~~3.6.1. SCREEN: Cursor selecting and using the Annotate Unknown History tool on a cell.~~

3.7. To reinitialize the cell cycle annotation, select the appropriate option from the toolbar **[1]**.

3.7.1. SCREEN: "3.7.1_take_1.mp4"

3.8. To break or rebind a mother-bud association, ensure that no tool is selected. Right-click on an existing mother-bud pair to break the association, or right-click again to re-establish the connection **[1]**.

3.8.1. SCREEN: "3.8.1_take_1.mp4"

4. Cell Cycle Annotations: Symmetrically Dividing Cells

4.1. Activate **Normal division: Lineage tree** *(normal division lineage tree)* using the mode selector **[1]**. When prompted, select **Yes** to go to frame 1 **[2]** and use the left and right arrow keys to navigate between frames **[3]**.

4.1.1. SCREEN: "4.1.1_and_4.1.2_take_2.mp4" 00:00-00:10

4.1.2. SCREEN: "4.1.1_and_4.1.2_take_2.mp4" 00:11-00:16

4.1.3. SCREEN: "4.1.3_take_1.mp4" 00:05-00:16

4.2. Correct errors in the automatic mother-daughter assignments using the tools available in the edit toolbar **[1]**. When prompted, click **Propagate** to apply the changes **[2]**.

4.2.1. SCREEN: "4.2.1_take_1.mp4"

4.2.2. SCREEN: 68954_screenshot_28.mkv 00:10-00:14

4.3. To assign a mother to a new cell ID, activate the **Find Mother for a New Cell ID** tool by pressing **F**. Right-click on the new cell to cycle through candidate mothers **[1-TXT]**.

4.3.1. SCREEN: "68954_screenshot_28.mkv" 00:00-00:08 **TXT: Use Shift + Right-click to cycle backwards through the options**

Results

5. Results

5.1. Nuclear segmentation in tumor spheroids revealed a wide distribution of nucleus volumes, with a substantial number of objects displaying small volumes **[1]**, and a few showing extremely large volumes **[2]**.

5.1.1. LAB MEDIA: Figure 10. *Video editor: Highlight the bars in the graph from 0 to 2000 on the X axis*.

5.1.2. LAB MEDIA: Figure 10. *Video editor: Highlight the sparse bars at the far-right tail from 4000 to 6000 on the X-axis*.

5.2. A 3D view of the tumor organoid displayed numerous segmented nuclei with labeled identifiers **[1]**, and z-slices showed the red segmentation contours applied to each nucleus **[2]**.

5.2.1. LAB MEDIA: Figure 10. *Video editor: Highlight the left panel/ image showing the 3D organoid*.

5.2.2. LAB MEDIA: Figure 10. *Video editor: Highlight the three center z-slice images with red contours outlining individual nuclei*.

5.3. In budding yeast, the H2B protein amount increased sharply at the time of bud emergence **[1]** and plateaued prior to nuclear division **[2]**.

5.3.1. LAB MEDIA: Figure 11A. *Video editor: Highlight the steep upward slope on the blue curve between labels ii and iii on the graph*.

5.3.2. LAB MEDIA: Figure 11A. *Video editor: Highlight the plateau in the blue curve after point iii on the graph*.

5.4. The number of nuclei increased suddenly at the time of nuclear division in the yeast dataset **[1]**.

5.4.1. LAB MEDIA: Figure 11A. *Video editor: Highlight the orange vertical line on the graph at label iii*.

5.5. In mouse embryonic stem cells, the cell area increased steadily until reaching a maximum **[1]**, then decreased during cell division **[2]**, and later began to rise again in the daughter cells **[3]**.

5.5.1. LAB MEDIA: Figure 11B. *Video editor: Highlight the green line labeled "Mother" between to 0 to 400 on X axis*

5.5.2. LAB MEDIA: Figure 11B. *Video editor: Highlight the drop in the green line between point 'i' and point 'ii'*.

5.5.3. LAB MEDIA: Figure 11B. *Video editor: Highlight the two black-grey lines labeled "Daughter 1" and "Daughter 2" after point iii*.

- GUI

Pronunciation link: https://www.merriam-webster.com/dictionary/GUI
IPA: /ˌdʒiːˌjuːˈaɪ/
Phonetic Spelling: jee-you-eye

- segmentation

Pronunciation link: https://www.merriam-webster.com/dictionary/segmentation
IPA: /ˌsɛgmənˈteɪʃən/
Phonetic Spelling: seg-men-tay-shun

- bud (in the sense of "bud emergence")

Pronunciation link: https://www.merriam-webster.com/dictionary/bud
IPA: /bʌd/
Phonetic Spelling: bud

- organoid

Pronunciation link: https://www.merriam-webster.com/dictionary/organoid
IPA: /ɔːrˈgæ.nɔɪd/ or /ɔːrˈgæ.noʊɪd/
Phonetic Spelling: or-ga-noid

- plateaued

Pronunciation link: https://www.merriam-webster.com/dictionary/plateaued
IPA: /plæˈtoʊd/
Phonetic Spelling: pla-tohd

- symmetrically

Pronunciation link: https://www.merriam-webster.com/dictionary/symmetrically
IPA: /sɪˈmɛtrɪkli/
Phonetic Spelling: si-met-ri-cuh-lee