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Title: Live-Cell Imaging of Single-Cell Arrays (LISCA) – A Versatile Technique to Quantify Cellular Kinetics

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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 similar? **N**

2. **Software:** Does the part of your protocol being filmed demonstrate software usage? **Y**

3. **Interview statements:** Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **42**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Anita Reiser**: Live-Cell Imaging on Single-Cell Arrays, called LISCA, allows an automated readout of the fluorescence time courses of hundreds of individual cells **[1]**.

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

REQUIRED:

1.2. **Daniel Woschée**: The advantage of the approach is that individual cell responses are recorded from spatially isolated cells in identical microenvironments, allowing an efficient image analysis with great statistics **[1]**.

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

Protocol

2. Microstructured Single Cell Array Fabrication

2.1. To fabricate a single-cell array, use a scalpel to cut a single PDMS (P-D-M-S) master piece containing six micropattern stripes out of the PDMS layer **[1]** and place the master piece on the lab bench with the micropattern facing up **[2]**.

2.1.1. WIDE: Talent cutting PDMS

2.1.2. Talent placing stamp onto bench

2.2. Use a razorblade to cut each of the six micropattern stripes into a PDMS stamp, cutting off some of the patterned areas to ensure that the edges of the stamps are open **[1]**.

2.2.1. Stamp being cut *Videographer: Important step*

2.3. Next, carefully scratch the protective foil of the coverslip of a six-chamber slide to mark the channel positions of the slide **[1]** and place the coverslip on the bench with the protective foil facing down **[2]**.

2.3.1. Foil being scratched

2.3.2. Talent placing coverslip onto bench

2.4. Use tweezers to place the stamps on the coverslip at the marked channel positions with the micropatterns facing down **[1]** and check the attachment of the stamps under a microscope **[2]**. The squares in contact with the slide will appear darker than the interspace **[3]**.

2.4.1. Stamp(s) being placed

2.4.2. Talent at microscope, checking attachment

2.4.3. LAB MEDIA: 2.4.3. *Video Editor: please highlight some squares*

2.5. The pattern quality is decreased by squares that are not properly attached to the slide **[1]**.

2.5.1. LAB MEDIA: 2.4.4. *Video Editor: please highlight squares on left of image with bright portions* The authors add information about the effects of dirt particles of the pattern quality that I deleted, since dirt particles cannot be viewed in this example. But fyi in case they ask why their edit was not included

2.6. If the stamps have securely attached, treat the coverslip and the stamps with oxygen plasma at 0.2 millibars of pressure and approximately 40 Watts for 3 minutes to make the surfaces between the PDMS stamps and the coverslip hydrophilic **[1]**.

2.6.1. Talent placing coverslip/stamps into plasma cleaner

2.7. At the end of the plasma cleaning, place the coverslip into a biosafety cabinet **[1]** and add 15 microliters of pegylated polylysine solution to each stamp so that the solution is absorbed into the hydrophilic pattern of the stamp **[2]**.

2.7.1. Talent placing coverslip into BSC

2.7.2. Talent adding PLL-PEG to stamp(s) *Videographer: Important step*

2.8. After 20 minutes, rinse the stamps with 1 milliliter of ultrapure water **[1]**, use tweezers to remove the stamps from the slide **[2]**, and rinse the coverslip a second time with a second milliliter of ultrapure water **[3]**.

2.8.1. Talent rinsing stamps

2.8.2. Talent removing stamps *Videographer: Important step*

2.8.3. Talent rinsing coverslip

2.9. When the coverslip has dried completely, attach a six-channel sticky slide to the coverslip, taking care that the micropatterned areas align with the bottom of the channels **[1]**, and add 40 microliters of PBS and 40 microliters of fluorescently labeled fibronectin solution to each channel **[2]**.

2.9.1. Slide being attached to coverslip

2.9.2. Talent adding PBS or FN to channel(s), with PBS and FN containers visible in frame

2.10. To mix the two solutions, remove 40 microliters from one reservoir and add it to the opposite reservoir of the same channel three times to generate a homogenous solution **[1]**.

2.10.1. Solutions being mixed

2.11. When all of the channels have been mixed, incubate the slide for 45 minutes at room temperature **[1]** before washing each channel three times with 120 microliters of PBS per wash **[2]**.

2.11.1. Talent setting timer, with slide visible in frame

2.11.2. Talent washing channel(s), with PBS container visible in frame

3. Cell Seeding

3.1. After the last wash, add 40 microliters of a 400,000 cells/milliliter cell suspension of interest and 40 microliters of cell growth medium to each channel **[1-TXT]** and mix the cell solution with the medium as demonstrated **[2]**.

3.1.1. WIDE: Talent adding cells or medium to channel(s), with medium and cell containers visible in frame **TEXT: See text for cell and medium preparation details**

3.1.2. Solutions being mixed

3.2. After mixing, remove 40 microliters of suspension from each channel so that only the channels are filled with cell suspension **[1]** and place the slide into a cell culture incubator **[2]**.

3.2.1. Suspension being removed

3.2.2. Talent placing slide into incubator

3.3. After 1 hour, check for cell adhesion by phase-contrast microscopy **[1]** and add 120 microliters of 37-degree Celsius cell growth medium to each channel **[2]**.

3.3.1. Talent at microscope, checking slide

3.3.2. Talent adding medium to channel(s), with medium container visible in frame

3.4. Then return the slide to the cell culture incubator for 3 more hours **[1]**.

3.4.1. Talent placing slide into incubator

4. Perfusion System

4.1. To set up a perfusion system, connect a 1-millilter syringe filled with 37-degree Celsius cell growth medium to the inlet tube of the system **[1]** and use the valve to fill the tube with medium **[2]**.

4.1.1. WIDE: Talent connecting syringe to system

4.1.2. Talent filling tube with medium *Videographer: Important step*

4.2. Connect the inlet tube to the reservoir of one channel, taking care that no air bubbles are trapped **[1]**, and connect a serial connector to the reservoir opposite to the inlet tube of the current channel **[2]**.

4.2.1. Tube being connected to reservoir

4.2.2. Connector being connected to reservoir

4.3. Connect the rest of the channels as demonstrated until the required number of channels are connected in series **[1]** and connect the outlet tube directly to the free reservoir of the final channel **[2]**.

4.3.1. Channels being connected

4.3.2. Talent connecting outlet tube to reservoir

4.4. Then fill the connected tube with medium to confirm that the perfusion system does not leak **[1]**.

4.4.1. Talent filling tube with medium

5. Time-Lapse Microscopy

5.1. For time-lapse imaging of the cells, to set up a time-lapse protocol for recording a phase-contrast image and a fluorescence image **[1]**, set the optical configuration for phase-contrast imaging and fluorescence imaging **[2-TXT]**.

5.1.1. WIDE: Talent setting imaging parameters, with monitor visible in frame

5.1.2. Added shot: SCREEN: screenshot_5.2.1_5.2.3_5.4.3: 00:06-00:16 Moved from 5.2.1.

5.2. Use a 10x objective **[1]**, the appropriate fluorescence filters **[2]**, and an automated focus correction system to ensure a better image quality for the long-term measurements **[3]**. Select a 10-millisecond exposure time for the phase-contrast imaging and a 750-millisecond exposure time for recording the eGFP (E-G-F-P) fluorescence intensity **[4]**. Set a time schedule with a 10-minute time interval between the consecutive loops through the position list and an observation time of 30 hours **[5]**. For 5.2.1.-5.2.4., the authors have suggested showing both files and to emphasize each parameter in each image as mentioned. The have also provided a "Screenshot Suggestion" file that explains their request in further detail. Please feel free to ask Anastasia to reach out to the authors and explain why a screen capture video would be better.

5.2.1. Added shot: LAB MEDIA: screenshot_5.2.2._PhC and screenshot_5.2.2._GFP *objective*

5.2.2. Added shot: LAB MEDIA: screenshot_5.2.2._PhC and screenshot_5.2.2._GFP *filters*

5.2.3. Added shot: LAB MEDIA: screenshot_5.2.2._PhC and screenshot_5.2.2._GFP *automated focus correction system*

5.2.4. Added shot: LAB MEDIA: screenshot_5.2.2._PhC and screenshot_5.2.2._GFP *exposure time*

5.2.5. SCREEN: screenshot_5.2.1_5.2.3_5.4.3: 00:17-00:30

5.3. Next, place the six-channel slide on the single-cell arrays in the sample holder of the 37-degree Celsius heating chamber of the microscope **[1]** and tape the perfusion system tubing to the stage **[2]**.

5.3.1. Talent placing slide onto stage

5.3.2. Tubing being taped

5.4. Insert the free ends of the outlet tubes into a 15-milliliter conical tube to collect the liquid waste **[1]** and set the position list for the scanning time-lapse measurement **[2]**, taking care that the number of positions will be able to be scanned within the defined time interval between consecutive loops through the position list **[3]**.

5.4.1. Talent placing tubing into tube

5.4.2. Talent setting position list

5.4.3. SCREEN: screenshot_5.2.1_5.2.3_5.4.3: 00:33 - 01:07 *Video Editor: please speed up*

5.5. Then start the time-lapse measurement **[1]**.

5.5.1. SCREEN: screenshot_5.5.1: 00:05 - 00:19 *Video Editor: please speed up*

6. Fluorescent Marker-mRNA Transfection

6.1. For transfection of the cells with a fluorescent marker, use a syringe to flush the tubing system with 1 milliliter of 37-degree Celsius PBS, taking care that the microscope stage doesn't move **[1-TXT]**.

6.1.1. WIDE: Talent flushing system *Videographer: Important step* **TEXT: Pause time-lapse as necessary**

6.2. After flushing, fill the system with 300 microliters of serum-reduced medium supplemented with the mRNA of interest to a final concentration of 0.5 nanograms of mRNA/microliter **[1]** and allow the lipoplexes to incubate for 1 hour **[2]**.

6.2.1. Medium/mRNA being added to slide

6.2.2. Talent setting timer, with setup visible in frame

6.3. At the end of the incubation, flush the system with 1 milliliter of 37-degree Celsius complete cell growth medium to remove any unbound mRNA **[1]**.

6.3.1. System being flushed

7. Image Analysis and Fluorescence Readout

7.1. At the end of the analysis **[1]**, view the channels listed in the channel menu and scroll through the time frames to inspect the pre-selected cells and their integrated fluorescence signal **[2-TXT]**. Click on a cell of interest to highlight its fluorescence time course **[3]** or click on a time course to find the corresponding cell **[4]**.

7.1.1. WIDE: Talent scrolling through time frames, with monitor visible in frame

7.1.2. SCREEN: screenshot_7.1.2_t1: 00:17-01:02 *Video Editor: please speed up* **TEXT: Press Home to jump to first timeframe**

7.1.3. SCREEN: screenshot_7.1.3_t2: 00:18-00:24

7.1.4. SCREEN: screenshot_7.1.3_t2: 00:27-00:32

7.2. Press **Shift** while clicking on a cell to select or de-select it. De-select any cells that are not viable or not confined to an adhesion spot or that are attached to another cell to exclude them from further analysis **[1-TXT]**.

7.2.1. SCREEN: screenshot_7.2.1_t1: 00:11-00:19 **TEXT: Optional: Highlight cell and press Enter**

7.3. When all of the cells are selected or de-selected appropriately, save the single-cell time courses for the cell area and the integrated fluorescence in an appropriate directory **[1]**.

7.3.1. SCREEN: screenshot_7.3.1_t1: 00:08-00:22 *Video Editor: please speed up*

Results

8. Results: Representative Image Analysis and Fluorescence Readout

8.1. To quantify the translation onset time after the transfection and the strength of the cellular eGFP (E-G-F-P) expression, a three-stage translation model can be employed **[1]**.

8.1.1. LAB MEDIA: Figure 6A *Video Editor: please emphasize equation*

8.2. The model solution for eGFP can be fitted to single-cell time courses as illustrated **[1]**.

8.2.1. LAB MEDIA: Figure 6B *Video Editor: please emphasize green data lines*

8.3. Here histograms of the translation onset time **[1]** and the expression rate of lipoplex transfected cells can be observed **[2]**.

8.3.1. LAB MEDIA: Figure 6C *Video Editor: please emphasize onset histogram*
8.3.2. LAB MEDIA: Figure 6C *Video Editor: please emphasize right histogram*

8.4. As both parameters are estimated for each cell **[1]**, the correlation of these parameters can be analyzed as demonstrated in the scatterplot **[2]** and compared to cells transfected with lipid nanoparticles **[3]**.

8.4.1. LAB MEDIA: Figure 6C
8.4.2. LAB MEDIA: Figures 6C and 6D *Video Editor: please emphasize blue data points*
8.4.3. LAB MEDIA: Figures 6C and 6D *Video Editor: please emphasize red data points*

8.5. As illustrated, cells transfected with lipid nanoparticles exhibit less cell-to-cell variability compared to cells transfected with lipoplexes **[1]** and the population average demonstrates a faster onset of translation as well as a higher expression rate **[2]**.

8.5.1. LAB MEDIA: Figures 6C and 6D *Video Editor: please keep red dots emphasized*
8.5.2. LAB MEDIA: Figures 6C and 6D *Video Editor: please emphasize outlined red dot*

Conclusion

9. Conclusion Interview Statements

9.1. **Anita Reiser**: Our approach can also be adapted to alternative micropatterning methods. Most important for LISCA is a good cell confinement and a combination of automated image analysis with a convenient user supervision **[1]**.

9.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

9.2. **Joachim O. Rädler**: As you have seen, cell behavior is heterogeneous and single-cell time-lapse movies provide access to the unbiased dynamics of the inherent biochemical networks, for example in gene expression, apoptosis, or cell killing assays **[1]**.

9.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera