FINAL SCRIPT: APPROVED FOR FILMING

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Scriptwriter Name: Sulakshana Karkala
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Title: Using LEXY and LINuS Optogenetics Tools and Automated Image Analysis to Quantify Nucleocytoplasmic Transport Dynamics in Live Cells

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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: 24
Number of Shots: 46

Introduction

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

1.1. **Charlène Boumendil** Our lab studies the interplay between chromatin and nuclear pores. We want to understand how nuclear pores affect chromatin organization and how chromatin organization affect nuclear pores functions, including transport.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll: 3.1*

What are the current experimental challenges?

1.2. **Charlène Boumendil:** Nucleocytoplasmic transport is a very fast process. One challenge resides in detecting changes in transport dynamics in live cells.

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

What research gap are you addressing with your protocol?

1.3. **Axelle Donjon:** There are not a lot of tools available to monitor nucleocytoplasmic transport in live cells. By implementing an improved and semi-automated analysis method, we expect to facilitate the usage of these tools.

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

What advantage does your protocol offer compared to other techniques?

1.4. **Axelle Donjon:** Our protocol is based on deep learning. It limits errors and greatly improves the time required to perform the analyses. An analysis that would previously take a day now only lasts one or two hours.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll: 2.17*

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

Protocol

2. Quantitative Analysis of Nuclear Dynamics via FIJI and TrackMate Using mCherry and SiR-DNA Channels

Demonstrator: Axelle Donjon

2.1. To begin, launch the FIJI software on the computer system **[1].**

2.1.1. WIDE: Talent launching the FIJI software.

2.2. Select the **stable phase** as **Image1**, the **illumination phase** as **Image2**, and the **recovery phase** as **Image3 [1]**. Concatenate the 3 images representing three phases to produce a single image **[2]**.

2.2.1. SCREEN: 68585_screenshot_01 00:00-00:06

2.2.2. SCREEN: 68585_screenshot_01 00:07-00:28

2.3. Save the obtained merged image for segmentation by navigating to **File** and selecting **Save As Tiff...** *(Save-As-Tiff)* **[1]**.

2.3.1. SCREEN: 68585_screenshot_01 00:29-00:38

2.4. Next, split the mCherry channel by selecting **Image, Color** and **Split Channels [1]**. Then, save the split image by going to **File, Save As** and **Tiff... [2]**.

2.4.1. SCREEN : 68585_screenshot_02 00:00-00:14

2.4.2. SCREEN: 68585_screenshot_02 00:15-00:25

2.5. To check for bleaching during acquisition, sequentially select **Image, Stacks,** and **Plot Z-axis Profile [1]**. Then click on **Data,** followed by **Copy 1st Data Set,** and paste it into the provided spreadsheet under the Z-axis profile cell **[2]**.

2.5.1. SCREEN : 68585_screenshot_03 00:00-00:06

2.5.2. SCREEN : 68585_screenshot_03. 00:07-00:16

2.6. Now, draw a circular region of interest approximating the average size of a nucleus by clicking on **Analyze,** followed by **Tools,** and clicking on **ROI** *(R-O-I)* **Manager [1].** Add the ROI and save it by navigating to **More** and clicking on **Save... [2]**.

2.6.1. SCREEN: 68585_screenshot_04 00:00-00:15

2.6.2. SCREEN: 68585_screenshot_04 00:19-00:29

2.7. Place the saved ROI in a cell-free region during the time-lapse **[1]**. Ensure **Mean grey value** is selected under **Analyze** and **Set Measurements [2]**. Then click on **More, Multi Measure**, check **Measure all 50 slices** and **one row per slice**, and click **OK** to record the intensity **[3]**.

2.7.1. SCREEN : 68585_screenshot_05 00:00-00:03

2.7.2. SCREEN : 68585_screenshot_05 00:04-00:08

2.7.3. SCREEN : 68585_screenshot_05 00:10-00:16

2.8. Copy and paste all measured intensity values into the provided spreadsheet under the ROI Background cell **[1]**. Then close the image with only the mCherry channel **[2]** and select the saved image with merged channels **[3]**.

2.8.1. SCREEN : 68585_screenshot_05 00:17-00:29

2.8.2. SCREEN : 68585_screenshot_06 00:00-00:05

2.8.3. SCREEN : 68585_screenshot_06 00:06-00:11

2.9. Now open the TrackMate plugin by sequentially clicking on **Plugins, Tracking,** and **TrackMate [1]**. Confirm that time points range from 0 to 49 and click **Next [2]**.

2.9.1. SCREEN : 68585_screenshot_07 00:00-00:08

2.9.2. SCREEN : 68585_screenshot_07 00:09-00:13

2.10. Select the **StarDist** *(Star-disst)* detector and click **Next [1]**. Choose **channel 2 (SiR-DNA)** *(channel-two-Sir-D-N-A)* for nuclei segmentation and wait for the process to complete **[2]**. Leave default parameters in the thresholding window and click **Next [3]**.

2.10.1. SCREEN: 68585_screenshot_07 00:14-00:24

2.10.2. SCREEN: 68585_screenshot_07 00:30-00:45

2.10.3. SCREEN: 68585_screenshot_07 00:46-00:50

2.11. Create filters using **Mean intensity ch1** *(Mean-Intensity-Channel-One)* to exclude nuclei with extreme signal values. Create one filter to select values above the defined threshold and the other to select the values below the threshold **[1-TXT]**. Similarly, add two **Area** filters to remove debris or dying cells and click **Next [2]**.

2.11.1. SCREEN: 68585_screenshot_08 00:00-00:36

TXT: Define the value to only remove the very low or saturated signal

2.11.2. SCREEN: 68585_screenshot_08 00:46-01:00

2.12. Choose the **Simple LAP tracker** and click **Next [1]**.

2.12.1. SCREEN: 68585_screenshot_08 01:01-01:08

2.13. Next, adjust tracking parameters. Set the **Linking max distance** to 30 micrometers, the **Gap-closing** *(gap-closing)* **max distance** to 15 micrometers, and **Gap-closing max frame gap** to 3, then click **Next [1]**.

2.13.1. SCREEN : 68585_screenshot_08 01:08-01:12

Video Editor: Please freeze frame at 01:12

2.14. After calculations, click on **Next** to set filters on tracks **[1]**. To only select nuclei that have been tracked during the entire time course, choose the highest number of spots in the track and click **Next [2]**.

2.14.1. SCREEN : 68585_screenshot_08 01:13-01:15

2.14.2. SCREEN : 68585_screenshot_08 01:16-01:32

2.15. Then tick **Display spot names** to retrace each nucleus in further analyses and customize other display features as preferred **[1]**. At the page bottom, click on **Tracks** and choose **Export to CSV** to save the measurement table **[2]**. Close the table and proceed by clicking **Next [3]**.

2.15.1. SCREEN : 68585_screenshot_09 00:00-00:04

2.15.2. SCREEN : 68585_screenshot_09 00:05-00:12

2.15.3. SCREEN : 68585_screenshot_09 00:13-00:17

2.16. Now, click on **Capture overlay** as action and then press on **Execute** followed by **OK** to generate an image with tracked nuclei **[1]**. Save the image by sequentially clicking on **File, Save As** and choosing **Tiff... [2]**.

2.16.1. SCREEN: 68585_screenshot_09 00:18-00:25

2.16.2. SCREEN: 68585_screenshot_09 00:26-00:34

2.17. To import the TrackMate CSV file into the provided spreadsheet, click on the **Data** tab.

Under the **Get & Transform Data** group, click on **From Text/CSV** *(From-text-or-C-S-V)* and click **Import [1]**.

2.17.1. SCREEN: 68585_screenshot_10 00:00-00:13

2.18. Copy columns **Spot ID (B)** *(Spot-I-D-B)*, **Track ID (C)** *(Track-I-D-C)*, **Frame (I)** *(Frame-I)*, and **Mean intensity ch1 (M)** *(Mean-intensity-channel-1-M)*, and paste into columns A to D of the provided spreadsheet **[1]**.

2.18.1. SCREEN : 68585_screenshot_10 00:14-00:26

2.19. Sort the results alphabetically first by **Track ID (individual nucleus)** *(Track-I-D-Individual-Nucleus)* from cell **B5** and then by **Frame (Time Point)** *(Frame-Time-Point)* from cell **C5 [1]**.

2.19.1. SCREEN : 68585_screenshot_10 00:27-01:00

2.20. Now, open GraphPad prism and create a new sheet and copy-paste the 'time' column from the spreadsheet **[1]**.

2.20.1. SCREEN : 68585_screenshot_11 00:02-00:24

2.21. To generate the relative nuclear intensity graph, navigate to the **Graphs** section and select **XY** as the **graph family** then choose **Points & connecting line with error bars** as the **graph type [1]**. In the Plot settings, choose **Mean**, **Error**, and **SEM**, and click **OK [2]**.

2.21.1. SCREEN : 68585_screenshot_11 00:39-00:42

2.21.2. SCREEN : 68585_screenshot_11 00:43-00:45

2.22. Next, to obtain the export rate, click on **New Data Table** and enter the times in decimals only for stable and illumination phases **[1]**. Paste the values obtained **as relative nuclear intensity** for these phases into the table **[2]**.

2.22.1. SCREEN : 68585_screenshot_12 00:00-00:21

2.22.2. SCREEN : 68585_screenshot_12 00:26-00:34

2.23. In the Results section, click on **XY analyses** followed by **Exponential** then choose **Nonlinear Regression (Curve Fit)** *(Curve-fit)* and click **OK [1]**. Select **Plateau followed by one phase decay as Model** under the parameters. Then choose **a constant equal to 2.25** as **X0**, a **constant equal to 1** as **Y0** and , a **constant equal to 0** for the **plateau [2]**.

Indicate that **K** should be **greater than 0** and press **OK [3].**

2.23.1. SCREEN: 68585_screenshot_13 00:00-00:07

2.23.2. SCREEN : 68585_screenshot_13 00:07-00:21

2.23.3. SCREEN : 68585_screenshot_13 00:22-00:25

2.24. Create a new table and paste-transpose the K values obtained **[1]**. In the **Graphs** section, select **Column** as **graph family,** and choose the **Violin plot** as the **graph type.** Select **Show all points** as the **plot** and click **OK [2]**.

2.24.1. SCREEN : 68585_screenshot_14 00:00-00:18

2.24.2. SCREEN : 68585_screenshot_14 00:24-00:33

Results

3. Results

3.1. Nuclear export with the optogenetic probe LEXY was visualized in live cell imaging **[1].** In the control condition with methanol-treated cells, nuclear mCherry intensity sharply decreased after blue light illumination **[2]**, followed by a gradual recovery during the post-illumination phase **[3]**.

3.1.1. LAB MEDIA: Figure 4.

3.1.2. LAB MEDIA: Figure 4. *Video editor: Highlight the stable and illumination images in the Methanol row*

3.1.3. LAB MEDIA: Figure 4. *Video editor: Highlight the recovery image In the Methanol row*

3.2. Treatment with the export inhibitor Leptomycin B caused a change in nuclear export, as the probe signal remained consistently nuclear throughout all timepoints **[1]**.

3.2.1. LAB MEDIA: Figure 4. *Video editor: Highlight the Leptomycin B 1 nM and 0.5 nm rows*

3.3. Quantitative analysis of relative nuclear intensity showed over time a slow decrease until reaching a plateau at the end of the illumination phase **[1]**. Intermediate Leptomycin B concentrations should display a mild nuclear export **[2].**

3.3.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the Leptomycin B 1 nM curve*

3.3.2. LAB MEDIA: Figure 5A. *Video editor: Highlight the Leptomycin B 0.5 nM curve*

3.4. Export rates calculated from the intensity data confirmed a significantly higher export rate in methanol-treated cells **[1]**, a much lower rate with 0.5 nanomolar Leptomycin B **[2]**, and almost no export at 1 nanomolar Leptomycin B **[3]**.

3.4.1. LAB MEDIA: Figure 5B. *Video editor: Highlight the violin plot for methanol*

3.4.2. LAB MEDIA: Figure 5B. *Video editor: Highlight the violin plot for Leptomycin B 0.5 nM*

3.4.3. LAB MEDIA: Figure 5B. *Video editor: Highlight the violin plot for Leptomycin B 1 nM,*

1. FIJI
Pronunciation link:
https://dictionary.cambridge.org/pronunciation/english/fiji (English Language & Usage Stack Exchange, Cambridge Dictionary)

IPA: /ˈfiː.dʒiː/
Phonetic Spelling: FEE-jee

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2. mCherry
Pronunciation link: No confirmed link found
IPA (American): /ɛmˈtʃɛr.i/
Phonetic Spelling: em-CHER-ee

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3. TrackMate
Pronunciation link: No confirmed link found
IPA (American): /ˈtræk.meɪt/
Phonetic Spelling: TRACK-mate

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4. StarDist
Pronunciation link: No confirmed link found
IPA (American): /ˈstɑr.dɪst/
Phonetic Spelling: STAR-dist

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5. Leptomycin
Pronunciation link: https://www.synonyms.com/pronounce/leptomycin (synonyms.com)
IPA: /ˌlɛp.təˈmaɪ.sɪn/
Phonetic Spelling: lep-tə-MY-sin

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6. optogenetic
Pronunciation link: No confirmed link found
IPA (American): /ˌɑp.toʊ.dʒəˈnɛt.ɪk/
Phonetic Spelling: op-toh-jeh-NET-ik

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7. micrometer
Pronunciation link: No confirmed link found
IPA (American): /maɪˈkrɑː.mɪ.tər/
Phonetic Spelling: my-KRAH-meh-ter

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8. concatenate
Pronunciation link: No confirmed link found
IPA (American): /kənˈkæt̬.əˌneɪt/
Phonetic Spelling: kun-KAT-uh-nate

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9. segmentation
Pronunciation link: No confirmed link found
IPA (American): /ˌsɛg.mənˈteɪ.ʃən/
Phonetic Spelling: SEG-mun-TAY-shun

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10. illumination
Pronunciation link: No confirmed link found
IPA (American): /ɪˌluː.mɪˈneɪ.ʃən/
Phonetic Spelling: ih-loo-mih-NAY-shun

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11. ROI (Region of Interest)
Pronunciation link: No confirmed link found
IPA (American): /ɑrˌoʊˈaɪ/
Phonetic Spelling: R-O-I

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12. bleaching
Pronunciation link: No confirmed link found
IPA (American): /ˈbliː.tʃɪŋ/
Phonetic Spelling: BLEECH-ing

Let me know if you'd like pronunciation help with other terms!