

Submission ID #: 69531

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21207003>

Title: Whole-Cell Super-Resolution Imaging via DNA-PAINT on a Spinning Disk Confocal with Optical Photon Reassignment

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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**

If **Yes**, we will need you to record using screen recording software.

We recommend using the screen capture program [OBS](https://obsproject.com/). JoVE's tutorial for using OBS Studio is provided at this link: <https://review.jove.com/v/5848/screen-capture-instructions-for-authors?status=a7854k>

As these files are necessary for finalizing your script, please upload all screen-captured video files to your project page as soon as possible:

<https://review.jove.com/account/file-uploader?src=21207003>

- 3. Filming location:** Will the filming need to take place in multiple locations? **Yes**

2 min walk, inside UCL campus

- 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. **No**

Current Protocol Length

Number of Steps: 22

Number of Shots: 40

Introduction

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

INTRODUCTION:

~~What research gap are you addressing with your protocol?~~

- 1.1. **Miruna Tanase**: Our protocol uses DNA-PAINT on a spinning-disk confocal microscope with optical photon reassignment to overcome depth limitations.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What are the most recent developments in your field of research?~~

- 1.2. **Sabrina Simoncelli**: DNA-PAINT now achieves extremely high resolution, with multiplexing and quantitative capabilities, surpassing other SMLM techniques such as PALM and STORM.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What advantage does your protocol offer compared to other techniques?~~

- 1.3. **Cecilia Zaza**: Our protocol achieves deep-cell and tissue DNA-PAINT imaging without sacrificing resolution, using straightforward, commercially available optics.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What are the current experimental challenges?~~

- 1.4. **Sabrina Simoncelli**: High-resolution DNA-PAINT mainly works under TIRF or HILO illumination, restricting imaging depth to just a few microns.
 - 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How will your findings advance research in your field?~~

- 1.5. **Olivia Dalby:** Our findings make DNA-PAINT accessible to many laboratories, enabling nanoscale imaging in cells and tissues at depth.
 - 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.

Protocol

2. Deep Whole-Cell Single-Molecule Localization Microscopy Using Spinning Disk Confocal Systems

Demonstrator: Cecilia Zaza

- 2.1. To begin, use a spinning disk confocal system equipped with an optical photon reassignment unit and an sCMOS (*S-C-M-O-S*) camera [1].
 - 2.1.1. WIDE: Talent showing the spinning disk confocal microscope setup with camera and lasers visible.
- 2.2. Start the system 30 minutes before imaging by turning on the microscope, camera, and lasers [1], then start the computer [2]. Wait for the NIS (*N-I-S*) -Elements software to fully load [3].
 - 2.2.1. Talent switching on the microscope hardware components.
 - 2.2.2. Talent powering on the computer.
 - 2.2.3. SCREEN: 69531_screenshot_1_Bandicam.mp4. 00:10-00:15, 00:42-00:53
- 2.3. Now select the appropriate imaging mode in NIS-Elements by choosing SoRA (*So-Rah*) spinning disk before setting acquisition parameters [1].
 - 2.3.1. SCREEN: 69531_screenshot_1_Bandicam.mp4. 00:54-01:00
- 2.4. Choose the 60X Apo (*A-poh*) numerical aperture 1.49 oil immersion objective to maximize detection of single-molecule events [1].
 - 2.4.1. Talent rotating the objective turret to select the 60X oil objective.
- 2.5. Then select the appropriate magnification based on the objective, camera settings, and sample [1]. Adjust camera binning accordingly to match the selected magnification [2-TXT].
 - 2.5.1. SCREEN: 69531_screenshot_1_Bandicam.mp4. 01:01-01:08
 - 2.5.2. SCREEN: 69531_screenshot_1_Bandicam.mp4. 01:09-01:14
TXT: Binning: Effective pixel sizes :108 nm for 1X (no binning) , 4X (4X binning); 78 nm for 2.8X (2X binning).
- 2.6. Set the exposure time for DNA-PAINT (*D-N-A-Paint*) acquisition and synchronize it with the spinning disk rotation speed [1]. ~~Press the Sync button to automatically match compatible rotation speed [2].~~
 - 2.6.1. SCREEN: 69531_screenshot_1_Bandicam.mp4. 01:14-01:19

~~2.6.2. Talent pressing the Sync button on the spinning disk control pad.~~ **AUTHOR's NOTE: This shot was not filmed. There was an update in the software, and it is no longer required.**

2.7. Now, configure the laser wavelength, laser power, and filter wheel according to the imager dye spectrum [1].

2.7.1. SCREEN: 69531_screenshot_1_Bandicam.mp4. 01:20-01:29

~~2.8. Adjust the laser power to 100 percent using the laser control pad for DNA PAINT imaging [1].~~

~~2.8.1. SCREEN: Talent adjusting the laser power slider to maximum.~~

2.9. To record the laser power after the objective, place a calibrated laser power meter sensor on the microscope stage near the sample plane [1] and record the measured power [2].

2.9.1. Talent positioning the laser power meter sensor on the stage.

2.9.2. Shot of the power meter display showing the recorded value.

2.10. Next, apply a drop of immersion oil to the clean 60X Apo numerical aperture 1.49 oil objective [1]. Secure the prepared immunostained cell sample onto the microscope stage using a stage adapter to minimize drift [2].

2.10.1. Talent dispensing immersion oil onto the objective lens.

2.10.2. Talent placing and securing the sample on the stage.

2.11. Switch to brightfield mode and focus on the glass plane using nanoparticles as a reference [1]. Then activate the **Perfect Focus System** to maintain focus during acquisition [2].

2.11.1. SCREEN: 69531_screenshot_1_Bandicam.mp4. 01:29-01:51

2.11.2. SCREEN: 69531_screenshot_1_Bandicam.mp4. 01:52-01:53

2.12. With Perfect Focus System active, temporarily change the excitation to the green fluorescent protein channel to identify the optimal focal plane [1-TXT]. Use low laser power during this step [2].

2.12.1. SCREEN: 69531_screenshot_1_Bandicam.mp4. 01:53-02:07

2.12.2. SCREEN: 69531_screenshot_1_Bandicam.mp4. 02:04-02:16

TXT: For U2OS mEGFP-Nup96 cells/ HeLa Kyoto mEGFP-Nup107 cells, use the 488 GFP signal

~~2.13. Return to the previously defined SoRA (So-Ran) excitation settings [1].~~ Define the experiment name and saving path [1]. Tick the **Time** box to set the interval, duration and number of loops [2]. Set the interval to **No Delay** and enter the number of loops corresponding to total number of frames [3].

2.13.1. ~~SCREEN: Talent clicking on SoRa Spinning Disk Confocal imaging mode.~~

2.13.2. SCREEN: 69531_screenshot_1_Bandicam.mp4. 02:17-02:25

2.13.3. SCREEN: 69531_screenshot_1_Bandicam.mp4. 02:26-02:27

2.13.4. SCREEN: 69531_screenshot_1_Bandicam.mp4. 02:28-02:32

2.14. Make sure the PFS box at the bottom is selected [1]. Return to the previously defined **SoRA (So-Rah)** excitation settings and press **Run now** [2]. Acquire 15,000 to 20,000 frames for the first protein using a 300-millisecond integration time [3].

2.14.1. SCREEN: 69531_screenshot_1_Bandicam.mp4. 02:33-02:35

2.14.2. SCREEN: 69531_screenshot_1_Bandicam.mp4 02:36-02:47

2.14.3. SCREEN: 69531_screenshot_1_Bandicam.mp4. 02:48-02:54

3. Single-Molecule Localization and Reconstruction Workflow Using Picasso Software

Demonstrator: Miruna Tanase

3.1. For image analysis, load the deconvoluted image stack into the Picasso Localize module [1]. Select **Analyze** and then **Parameters** from the menu bar and input the camera-specific parameters according to the manufacturer specifications [2].

3.1.1. SCREEN: 69531_screenshot_2.mp4 00:00-00:07

3.1.2. SCREEN: 69531_screenshot_2.mp4 00:08-00:25

AND

TEXT ON PLAIN BACKGROUND:

For the sCMOS camera

Parameter Setting:

EM gain: 1

Baseline: 100

Sensitivity: 0.23 (conversion factor, electrons/counts)

Quantum efficiency: 0.95 (depending on the used excitation wavelength)

Pixelsize (nm): 108 (depending on the imaging configuration)

3.2. Next, set the **box side length** to **7** to optimize single-molecule localization precision [1].

3.2.1. SCREEN: 69531_screenshot_2.mp4 00:26-00:29

3.3. Choose a suitable minimum net gradient value and enable **Preview** to visualize detected localizations [1]. Adjust the gradient threshold to minimize background detections [2].

3.3.1. SCREEN: 69531_screenshot_2.mp4 00:30-00:35

3.3.2. SCREEN: 69531_screenshot_2.mp4 00:36-00:40

- 3.4. Now, select **Analyze** and then **Localize** to start spot identification and fitting across all frames [1].

3.4.1. SCREEN: 69531_screenshot_2.mp4 00:41-00:49

- 3.5. Load the resulting .hdf5 (*dot-H-D-F-Five*) into the Picasso Render module to visualize the reconstructed localization map [1].

3.5.1. SCREEN: 69531_screenshot_3.mp4 00:00-00:02

Video Editor: Please freeze screen if necessary

- 3.6. Choose **Postprocess** and then **Undrift by RCC** [1]. Enter an initial segmentation value starting at 1000 to obtain a xy (*X-Y*) drift plot [2-TXT]. Repeat with lower segmentation values to improve drift correction [3].

3.6.1. SCREEN: 69531_screenshot_3.mp4 00:03-00:05

3.6.2. SCREEN: 69531_screenshot_3.mp4 00:06-00:12

TXT: Repeat with lower segmentation values to improve drift correction

- 3.7. Click on **File** and **Save Localization** to save the drift-corrected localization file before closing the Render module [1].

3.7.1. SCREEN: 69531_screenshot_3.mp4 00:13-00:16

- 3.8. Now, load the drift-corrected file into the Picasso Filter module [1] and apply thresholds based on localization precision, point spread function widths, photon counts, and other quality metrics [2].

3.8.1. SCREEN: 69531_screenshot_4.mp4 00:00-00:06

The drift corrected file is being loaded into the Picasso Filter module.

3.8.2. SCREEN: 69531_screenshot_4.mp4 00:07-00:34

- 3.9. Click on **File** and **Save Localization** to save the filtered localization file before closing the Filter module [1].

3.9.1. SCREEN: 69531_screenshot_4.mp4 01:11-01:15

Results

4. Results

4.1. DNA-PAINT imaging of nucleoporin 96 tagged with monomeric enhanced green fluorescent protein in U2OS (*U-Two-O-S*) cells produced a clear super-resolved nuclear pore complex pattern across the field of view [1]. Zoomed-in views revealed paired nucleoporin 96 subunits arranged with the expected 8-fold symmetry of nuclear pores [2].

4.1.1. LAB MEDIA: Figure 1C.

4.1.2. LAB MEDIA: Figure 1D. *Video editor: Highlight the paired bright spots indicated by arrows in individual nuclear pores.*

4.2. Measurement of the Euclidean distance between aligned nucleoporin 96 pairs showed a peak-to-peak separation of 13 plus or minus 2 nanometers [1].

4.2.1. LAB MEDIA: Figure 1E. *Video editor: Show the cross-sectional histogram with the distance marked between the two peaks.*

4.3. Nucleoporin 96, mitochondria, and microtubules were simultaneously visualized in U2OS cells with preserved single-molecule localization precision across all targets [1].

4.3.1. LAB MEDIA: Figure 2B. *Video editor: Show the multicolor reconstruction with nuclear pore complexes in blue, mitochondria in red, and microtubules in green.*

4.4. Large field-of-view imaging of microtubules in HeLa cells demonstrated that localization precision varied with magnification and field size, with reduced precision observed at lower magnification [1].

4.4.1. LAB MEDIA: Figure 3A.

4.5. Higher magnifications produced improved and more uniform localization precision compared with 1X magnification [1].

4.5.1. LAB MEDIA: Figure 3D. *Video editor: Please highlight the dots corresponding to 1x, 2.8x and 4x sequentially*

4.6. Whole-cell imaging was demonstrated by DNA PAINT acquisition of the microtubule network across multiple axial planes spanning the full cell depth [1]. Localization precision gradually decreased with increasing imaging depth [2].

4.6.1. LAB MEDIA: Figure 4A (left images). *Video editor: Sequentially show images from $z = 0\mu m$ to $z = 9\mu m$*

4.6.2. LAB MEDIA: Figure 4A (right plot).

4.7. Double-walled microtubule structures were resolved at multiple axial positions with peak-to-peak distances between 30 nanometers and 40 nanometers [1].

4.7.1. LAB MEDIA: Figure 4C. *Video editor: Sequentially show images 1 – 3 with their corresponding histogram plots*

Pronunciation Guide:

1. Super-resolution
Pronunciation link: <https://www.merriam-webster.com/dictionary/super-resolution>
IPA: /,su:pər,rɛzəˈlu:ʃən/
Phonetic Spelling: soo-per-rez-uh-loo-shuhn
2. DNA-PAINT
Pronunciation link: <https://www.howtopronounce.com/dna-paint>
IPA: /,di:ˌɛnˈeɪ peɪnt/
Phonetic Spelling: dee-en-ay paynt
3. Spinning Disk Confocal
Pronunciation link: <https://www.merriam-webster.com/dictionary/confocal>
IPA: /ˈkɑ:nˌfəʊkəl/
Phonetic Spelling: kon-foh-kuhl
4. Optical Photon Reassignment
Pronunciation link: <https://www.merriam-webster.com/dictionary/photon>
IPA: /ˈfəʊtɑ:n/
Phonetic Spelling: foh-ton
5. Single-Molecule Localization Microscopy
Pronunciation link: <https://www.merriam-webster.com/dictionary/microscopy>
IPA: /maɪˈkrɑːskəpi/
Phonetic Spelling: my-krah-skuh-pee
6. SMLM
Pronunciation link: No confirmed link found
IPA: /,ɛs,ɛm,ɛlˈɛm/
Phonetic Spelling: es-em-el-em
7. PALM
Pronunciation link: <https://www.howtopronounce.com/palm-microscopy>
IPA: /pɑ:m/
Phonetic Spelling: pahm
8. STORM
Pronunciation link: <https://www.howtopronounce.com/storm-microscopy>
IPA: /stɔ:rm/
Phonetic Spelling: storm
9. TIRF
Pronunciation link: <https://www.howtopronounce.com/tirf>
IPA: /tɜ:rf/
Phonetic Spelling: turf
10. HILO
Pronunciation link: <https://www.howtopronounce.com/hilo-microscopy>
IPA: /ˈhaɪləʊ/
Phonetic Spelling: high-loh

11. sCMOS
Pronunciation link: <https://www.howtopronounce.com/scmos>
IPA: /ˌɛsˈsi:mɒs/
Phonetic Spelling: es·see·moss
12. Numerical Aperture
Pronunciation link: <https://www.merriam-webster.com/dictionary/aperture>
IPA: /ˈæpərtʃər/
Phonetic Spelling: ap·er·cher
13. Immersion Objective
Pronunciation link: <https://www.merriam-webster.com/dictionary/immersion>
IPA: /ɪˈmɜːrʒən/
Phonetic Spelling: ih·mur·zhuhn
14. SoRA
Pronunciation link: <https://www.howtopronounce.com/sora-microscopy>
IPA: /ˈsɔːrə/
Phonetic Spelling: saw·ruh
15. NIS-Elements
Pronunciation link: <https://www.howtopronounce.com/nis-elements>
IPA: /ˌɛnˌaɪˈɛs ˈɛləmənts/
Phonetic Spelling: en·eye·es el·uh·ments
16. Immunostained
Pronunciation link: <https://www.merriam-webster.com/dictionary/immunostain>
IPA: /ˌɪmjənoʊˈsteɪnd/
Phonetic Spelling: im·yuh·noh·staynd
17. Perfect Focus System
Pronunciation link: <https://www.merriam-webster.com/dictionary/focus>
IPA: /ˈfoʊkəs/
Phonetic Spelling: foh·kus
18. Green Fluorescent Protein
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescent>
IPA: /ˌflʊəˈresənt/
Phonetic Spelling: floor·eh·suhnt
19. U2OS
Pronunciation link: <https://www.howtopronounce.com/u2os>
IPA: /ˌjuːtuːˈoʊɛs/
Phonetic Spelling: yoo·too·oh·es
20. HeLa
Pronunciation link: <https://www.merriam-webster.com/dictionary/HeLa>
IPA: /ˈhiːlə/
Phonetic Spelling: hee·luh
21. Deconvoluted
Pronunciation link: <https://www.merriam-webster.com/dictionary/deconvolution>

IPA: /ˌdi:kɑːnvəˈluːtɪd/

Phonetic Spelling: dee·kon·vuh·loo·tid

22. Picasso

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

IPA: /pɪˈkɑːsəʊ/

Phonetic Spelling: puh·kah·soh

23. Localization

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

IPA: /ˌləʊkələˈzeɪʃən/

Phonetic Spelling: loh·kuh·luh·zay·shuhn

24. Euclidean

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

IPA: /juːˈkliːdiən/

Phonetic Spelling: yoo·klid·ee·uhn

25. Nucleoporin

Pronunciation link: <https://www.howtopronounce.com/nucleoporin>

IPA: /ˌnuːkliːoʊˈpɔːrɪn/

Phonetic Spelling: noo·klee·oh·por·in

26. Monomeric

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

IPA: /ˌmɒːnəˈmɛrɪk/

Phonetic Spelling: mon·uh·meh·rik

27. Nuclear Pore Complex

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

IPA: /ˈnuːkliər/

Phonetic Spelling: noo·klee·er

28. Mitochondria

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

IPA: /ˌmaɪtoʊˈkɑːndriə/

Phonetic Spelling: my·toh·kon·dree·uh

29. Microtubules

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

IPA: /ˌmaɪkroʊˈtuːbjʊːlz/

Phonetic Spelling: my·kroh·too·byoolz

30. Axial

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

IPA: /ˈæksjəl/

Phonetic Spelling: ak·shuhl

31. Nanometers

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

IPA: /ˈnænəˌmiːtər/

Phonetic Spelling: nan·uh·mee·ter

32. Localization Precision

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

IPA: /prɪ'sɪʒən/

Phonetic Spelling: pruh·si·zhuhn