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Cover Letter

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Dear Editors,

This letter accompanies the revised manuscript (initially submitted 15 March 2019) entitled **"Specific labelling of mitochondrial nucleoids with SYBR Gold dye and their time-lapse super-resolution imaging by structured illumination microscopy"** which we would like to be considered for publication as a video protocol in JoVE. The present manuscript is based on our original research article: *Jevtic, V., Kindle, P. & Avilov, S. V. SYBR Gold dye enables preferential labelling of mitochondrial nucleoids and their time-lapse imaging by structured illumination microscopy. PLoS One. 13 (9), e0203956, (2018).*

We addressed all comments of JoVE reviewers and editors (as described in details in the attached file), except we kept rare use of commercial names of substances.

Yours sincerely,

Sergiy Avilov, on behalf of all co-authors

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TITLE:

Specific Labeling of Mitochondrial Nucleoids for Time-Lapse Structured Illumination Microscopy

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KEYWORDS:

mitochondrial nucleoid, mitochondrial DNA, asymmetric cyanine, DNA fluorescent stain, structured illumination microscopy, live cell microscopy

SUMMARY:

The protocol describes specific labeling of mitochondrial nucleoids with a commercially available DNA gel stain, acquisition of time lapse series of live labeled cells by super-resolution structured illumination microscopy (SR-SIM), and automatic tracking of nucleoid motion.

ABSTRACT:

Mitochondrial nucleoids are compact particles formed by mitochondrial DNA molecules coated with proteins. Mitochondrial DNA encodes tRNAs, rRNAs, and several essential mitochondrial polypeptides. Mitochondrial nucleoids divide and distribute within the dynamic mitochondrial network that undergoes fission/fusion and other morphological changes. High resolution live fluorescence microscopy is a straightforward technique to characterize a nucleoid's position and motion. For this technique, nucleoids are commonly labeled through fluorescent tags of their protein components, namely transcription factor A (TFAM). However, this strategy needs overexpression of a fluorescent protein-tagged construct, which may cause artifacts (reported for TFAM), and is not feasible in many cases. Organic DNA-binding dyes do not have these disadvantages. However, they always show staining of both nuclear and mitochondrial DNAs, thus lacking specificity to mitochondrial nucleoids. By taking into account the physico-chemical properties of such dyes, we selected a nucleic acid gel stain (e.g., SYBR Gold) and achieved preferential labeling of mitochondrial nucleoids in live cells. Properties of the dye, particularly its high brightness upon binding to DNA, permit subsequent quantification of mitochondrial nucleoid motion using time series of super-resolution structured illumination images.

INTRODUCTION:

Circular 16.5 kbp DNA molecules constitute the genetic material of mitochondria, encoding 22 tRNAs, 2 rRNAs, and 13 polypeptides needed for mitochondrial oxidative phosphorylation complexes. Mitochondrial DNA bound to mitochondrial transcription factor A (TFAM) and several other proteins form the mitochondrial nucleoids[1–4]. Mitochondrial nucleoids move and redistribute between the components of the mitochondrial network[5,6] during its morphological remodeling, fission or fusion depending on cell cycle phase, stress, and other factors (reviewed in Pernas et al.[7]). In addition, the motion of mitochondrial nucleoids, is implicated in systemic lupus erythematosus disease[8] and may play a role in other diseases. Fluorescence microscopy is a straightforward technique for live-cell studies of organelles, but the technique has a resolution of >200 nm, which is larger than the size of mitochondrial nucleoids (~100 nm[9–12]). This limit has been circumvented by so called "super-resolution" techniques, such as stimulated emission depletion (STED) and single molecule localization microscopy (SMLM)[13,14]. So far, mitochondrial nucleoids and other DNAs were imaged in live cells by direct stochastic optical reconstruction microscopy (dSTORM)[15]. Fine sub-mitochondrial structures with positions correlating with mtDNA were observed by STED in live cells[16]. However, these super-resolution techniques require high illumination intensity, which causes phototoxic effects on living cells[17]. Therefore, time lapse imaging of mitochondrial nucleoids with resolution beyond diffraction limit is challenging. To address this, we used super-resolution structured illumination microscopy (SR-SIM[18]. SIM requires a much lower illumination power dose than STED and SMLM[19]. Furthermore, in contrast to STED and SMLM techniques, SIM permits straightforward multicolor three-dimensional (3D) imaging, and it does not require particular photophysical properties of the fluorophores or imaging buffer composition[19].

The conventional strategy for labeling mitochondrial nucleoids in live cells is fluorescent tagging of a mitochondrial nucleoid protein, such as TFAM[20]. However, in many cases, this strategy is not suitable. Moreover, overexpression of fluorescent protein-tagged TFAM produces a serious artifact[21]. Labeling of DNA with organic dyes has advantages over a fluorescent protein (FP)-based strategy. Organic dyes are free of constrains related to FP tagging: they can be used for any type of cells or tissue and can be applied at any time point of an experiment. Live cell imaging of mitochondrial nucleoids has been reported with several DNA-binding dyes: DAPI[22], SYBR Green[23], Vybrant DyeCycle[24], and picoGreen[15,25,26]. A substantial drawback of most DNA-binding dyes for nucleoid labeling is that they stain all DNA within the cell. Targeting a dye solely to mitochondrial DNA is highly desirable. To achieve that, careful selection of a dye possessing suitable physico-chemical properties is necessary. Lipophilic dyes possessing delocalized positive charge, such as rhodamine 123, are known to accumulate in live mitochondria, which preserve their negative membrane potential. In addition, an ideal dye for specific labeling of mitochondrial nucleoids should bind DNA with high affinity and emit bright fluorescence upon DNA binding. Considering these requirements, certain cyanines are promising (e.g., picoGreen), but nuclear DNA is abundantly stained by these dyes simultaneously with mitochondrial DNA[15,25,26]. The present protocol describes specific labeling of mitochondrial nucleoids in live cells with another cyanine dye, SYBR Gold (SG), and tracking of the nucleoids in time lapse super-resolution SIM videos. Moreover, SG-stained live cells can be imaged by any type of inverted fluorescent microscope (confocal, spinning disk, epifluorescence, etc.) suitable for living cells and equipped with a 488 nm light source.

PROTOCOL:

NOTE: All the cell lines mentioned here were cultured in high glucose Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), glutamine, penicillin/streptomycin, and pyruvate. Equilibrate all media and supplements to be used on the day of labeling and imaging by warming them up to 37 °C in an incubator set to 5% CO_2. All cell culture work including labeling takes place in sterile conditions under a laminar flow hood.

1. Live cell labeling

1.1. One day before the labeling procedure, culture A549 (human lung carcinoma) cells on an 8 well chambered slide or 35 mm Petri dish with #1.5 glass bottom. Dilute the cell suspension to have approximately 50,000 cells/mL and seed 250 µL of the cell suspension to each well of the 8 well chambered slide.

1.2. Prepare the following dilutions of SYBR Gold (SG) commercial stock solution: 1:500, 1:1,000, and 1:5,000. Prepare Mitotracker Deep Red (far red stain) or Mitotracker CMXRos Red (red stain) (**Table of Materials**; commercial stock diluted 1:2,000) in phenol red-free culture medium. Prepare the same set dilutions of picoGreen as for SG.

NOTE: The solutions described in step 1.2 are the 2x labeling solutions. Protect all the solutions containing fluorescent dyes from light as much as possible.

1.3. Wash the adherent cells with PBS once. Add phenol red-free culture medium to each well. Use a volume equal to 1/2 of the total well capacity (e.g., add 125 µL if the total well capacity is 250 µL).

1.4. Add the 2x labeling solution prepared in step 1.2 to each well. Use a volume equal to 1/2 of the total well capacity. Incubate the cells for 30 min at 37 °C under 5% CO_2.

Note: Do not incubate the cells with SG labeling solution for longer than 1 h, because longer incubation will cause labeling of nuclear DNA.

1.5. Carefully aspirate the medium containing the fluorescent dyes and wash the cells once with PBS.

1.6. Add phenol red-free cell culture medium and keep the cells in the dark in a CO_2 incubator at 37°C until imaging.

1.7. Image living cells on an SR-SIM microscope equipped with an incubation unit (see below).

2. SR-SIM image acquisition

2.1. Install a stage-top incubator on the microscope stage. Set the desired temperature and CO_2 concentration (e.g., 37 °C and 5% for mammalian cells) and keep warm for at least 1 h before starting image acquisition.

2.2. Switch on all the components of the SR-SIM microscope, including the lasers, and leave them to warm up for at least 1 h.

2.3. Choose a high magnification, high numerical aperture (NA) immersion objective (e.g., 100x 1.46 NA oil) recommended for SR-SIM by the microscope manufacturer.

2.4. Install a chambered slide or 35 mm dish with labeled cells (from step 1.6 on the incubated microscope stage.

2.5. Locate the area of interest on the sample, preferably with oculars. To achieve the best SR-SIM imaging quality, choose the cells that are well attached to the glass.

2.6. Use a back-tinned high-end electron multiplying charge-coupled device (EM-CCD) camera to acquire SR-SIM images.

2.7. Using the image acquisition software, set a high EM gain recommended for the camera used (e.g., 300).

2.8. Before acquiring the time lapse series for nucleoid tracking, acquire a two-color SR-SIM image of the same field of view: one channel for mitochondrial staining and another one for SG. Set the first color channel appropriate to the mitochondrial stain used (e.g., for a far red mitochondrial stain, set the excitation at 633 nm or longer and a 650 nm longpass emission filter). For the SG signal, set the excitation at 488 nm and a 500–550 nm bandpass emission filter.

2.9. Using the software, set the lowest laser power possible for both 488 nm and far red stain lines.

NOTE: A typical setting is 1% laser output power adjusted by an acousto-optical tunable filter (AOTF).

2.10. If the SIM microscope acquires channels only sequentially (single-camera setup), then switch off the far-red stain detection channel.

2.11. Set the acquisition of a single focal plane by switching off the Z-stack acquisition by unticking the **Z-stack** box in the software.

2.12. Set the shortest possible EM-CCD camera exposure time.

2.13. Set three rotations of the grid rather than five rotations.

2.14. To increase the frame rate, in the **Acquisition** tab of the software, set the camera to read the data only from the central area of camera sensor instead of "**Full Chip**".

NOTE: For example, switching from reading an area of 1,000 x 1,000 pixels full sensor to reading only 256 x 256 pixels allows a reduction in camera exposure time from 50 ms to 13.4 ms and the total frame time from 1.8 s to 1.2 s. If only a central part of the camera sensor is read, then the field of view will be very small for the 100x objective typically used for SR-SIM. A shorter exposure time will reduce the signal-to-noise ratio of the images.

2.15. Optimize laser power and camera exposure time: acquire SR-SIM two-dimensional (2D) images of labeled cells at several laser power values (e.g., 0.5%, 1%, 1.5%, 2%) and several exposure times (e.g., 13.4 ms, 25 ms, 50 ms).

2.16. Process the raw SIM datasets (see step 3.1).

2.17. Choose laser power and camera exposure times that yield SIM images with bright spots in the mitochondria (i.e., the nucleoids) with little or no artifacts generated by SIM processing. Inspect the areas outside of the mitochondria (e.g., 1% power of the 488 nm solid-state laser and 25 ms exposure of EM-CCD camera).

2.18. Start acquisition of the time lapse series using the settings optimized in steps 2.15–2.17.

3. Data processing and analysis

3.1. Process raw SIM datasets with the structured illumination module of a suitable software (**Table of Materials**): choose the **Auto** checkbox for SIM processing parameters. For automatic processing of multiple files, use the batch processing tool, deselect **Use Current for Batch**, click **Run Batch** and select multiple files for SIM processing.

3.2. Convert SIM-processed time series datasets to *ims* format with Imaris file converter software (i.e., the version corresponding to the version of Imaris software).

3.3. Open a converted file in the software (version 8.4.1 or later) that has a license for the Lineage (or Track) module.

3.4. Start the "**Spots**" creation wizard by clicking **Add New Spots** icon. A wizard for creation will be launched.

3.5. Choose the **Create** tab of the wizard.

3.6. On the first step of the wizard, click **Track Spots (over time)** and proceed to the second step of the wizard.

3.7. Set **Estimated XY Diameter** to 0.1–0.15 µm, click **Background Subtraction** and proceed to the third step.

3.8. Adjust the threshold in the filter "**Quality**" by dragging the vertical line in the histogram so that the majority of the nucleoids are detected as "spots", while the artifacts are not detected (i.e., the detected spots are marked as balls overlaid on the image). Check if this is the case on each frame and readjust the threshold if necessary. Proceed to the fourth and then to the fifth step of the wizard.

3.9. Choose the **Autoregressive Motion** algorithm. Set **Max Distance** to 0.5 µm. Set **Max Gap Size** to 0.

3.10. Look at each frame in the time series and check if any false tracks are drawn and if any gaps between tracks are introduced (tracks built by the wizard with current settings are instantly marked as lines overlaid on the image). Adjust "**Max Distance**" if necessary. Proceed to the sixth step of the wizard. Choose the **Track Duration** filter and set a threshold of 3–5 s.

3.11. If the currently detected spots or tracks are not optimal, go back to the preceding steps of the wizard using navigation buttons (in the bottom of wizard window) needed to fine-tune any parameter for spots or tracks creation.

3.12. When the fine-tuned parameters allow the software to detect all the spots and build tracks correctly, click the green arrow navigation button in the wizard to confirm creation of the tracks.

3.13. Extract the statistics of the tracks by clicking the **Statistics** icon window. Choose the needed statistics parameters (**Detailed** and **Average Values** tabs under **Statistics**) and export the values as csv files by clicking the **Floppy Drive** icon for quantification and visualization.

NOTE: Maximum track speed, mean track speed, track length, and track displacement are important to characterize nucleoid motion.

3.14. If the image dataset needs to be presented or published, then create snapshots and/or video files representing the time lapse series with optionally overlaid tracks using the **Snapshot** or **Animation** tools.

4. Confocal image acquisition

4.1. Install a stage-top incubator on the microscope stage, set desired temperature and CO_2 concentration (i.e., 37 °C and 5% for mammalian cells) and keep warm for at least 1 h before starting image acquisition.

4.2. Switch on all the components of a confocal laser scanning microscope, including the lasers, and leave them to warm up for at least 1 h.

4.3. Choose a desired middle to high magnification objective and spatial sampling according to Nyquist criteria.

NOTE: In this protocol, a 100x 1.46 NA oil objective yielded a spatial sampling of 50 nm/pixel. Most software that controls microscopes can automatically calculate this value. As a rule of thumb, spatial sampling should ensure 2–3 pixels per the theoretical optical resolution calculated by the Abbe equation (e.g., 80–120 nm for a theoretical resolution of 240 nm).

4.4. For the SG channel, set the excitation at 488 nm and a detection range of 500–550 nm. For the mitochondrial stain channel, set to 561 nm and 570–630 nm emission for the red channel dye, or 633 nm excitation and >650 nm emission for the far red channel dye.

4.5. Set the lowest possible laser power (typically 1% or less) that permits acquisition of the signals with PMT detector gains of 700 mV.

4.6. Acquire two-color confocal images of the cells, where the mitochondrial nucleoids are detected in the "SG channel" and mitochondria are detected in the "red" or "far red" channel.

REPRESENTATIVE RESULTS:

Characterization of live cell labeling with SG

First, the distribution of SG in the cells upon incubation with the dye at various dilutions was characterized by confocal microscopy. After incubation with high concentrations of SG or picoGreen, both dyes mostly labeled the nuclei and showed a punctate staining in the cytoplasm (**Figure 1**), similarly to published data for another positively charged cyanine dye (i.e., picoGreen)[15]. However, upon incubation with SG at 1:10,000 dilution, faint staining appeared in the nuclei, while in the cytoplasm, we observed a pattern of bright spots (**Figure 1**). On the other hand, incubation with picoGreen dye diluted 1:10,000 yielded mostly nuclear staining. The SG signal was much brighter than that of picoGreen at the same concentration. The data showed that SG is more suitable for imaging mitochondrial DNA than other similar DNA-binding dyes.

To confirm that the bright dots are localized in the mitochondria, we stained living cells simultaneously with SG and far red mitochondrial stain. The latter is a positively charged cell permeable organic dye that accumulates in the mitochondria of living cells. Upon incubation with SG at 1:10,000 and 1:50,000 dilutions, nearly all SG staining occurred in mitochondria, while upon labeling at 1:500 and 1:1,000 dilutions, significant staining of the nuclei and cytoplasm occurred (**Figure 2**).

Further, we characterized the time course of staining live cells with SG by time lapse microscopy (**Figure 3**). The plots of SG fluorescence intensity in mitochondria vs. time (**Figure 3B**) suggested that after 45 min, nucleoid staining was close to saturation. Thus, we recommend incubation times of ~30–60 min.

We tested how SG intracellular distribution changes upon fixation and/or permeabilization of the cells. Fixation (2% paraformaldehyde [PFA]) of the live-stained cells caused a slight redistribution

of the dye to the nucleus (**Figure 4A,B**). Permeabilization of the fixed cells (0.1% Triton X100) eliminated the SG dotted pattern in mitochondria, and staining of the nuclei was dominant (**Figure 4C**). If the SG was added to the cells after fixation and permeabilization, it distributed uniformly across the cytoplasm and nuclei (**Figure 4D**). Thus, SG labeled cells can be fixed if necessary, but the dye is not suitable for protocols that require permeabilization.

Live cell SR-SIM and mitochondrial nucleoids tracking

Live cells costained with SG and a far red mitochondrial stain (**Table of Materials**) were 3D imaged by a super-resolution SIM technique. As in the confocal images (**Figure 2**), mitochondrial nucleoids appeared as bright spots within the mitochondria (**Figure 5A**). Further, we acquired a time series of 2D SIM images and tracked the positions of the nucleoids at a resolution beyond the diffraction limit. Immediately before starting the time series we acquired SIM 3D stacks in the SG and the mitochondrial stain channels. Then we acquired a time series in the SG channel only and tracked the mitochondrial nucleoids in order to quantify their motions. The track mean speed was 0.042 µm/s, and the maximal instant speed of tracking was 0.078 ± 0.012. The majority of nucleoids did not displace far from their original positions but showed short-distance random motions that were probably confined to the mitochondrial network, as an overlay of tracks on the mitochondrial images suggests (**Figure 5B**). Few rapid directional displacements occurred during a typical time series (**Movie 1**).

FIGURE LEGENDS:

Figure 1: Comparison of picoGreen and SG live cell labeling. A549 cells were incubated with picoGreen or SG at indicated dilutions and imaged. Representative fields of view of the labeled cells in the green channel. LSM880 Airyscan Fast, 20x/Air objective, scale bar 50 = µm. Single optical sections. White squares mark the areas shown at a higher magnification to the right of the entire 423 x 423 µm fields of view. For 1:1,000 and 1:2,000 dilutions, the same images are shown at two brightness settings: the "default" brightness settings optimized for 1:10,000 dilution, and the "reduced brightness" settings adjusted to avoid detector saturation. This figure has been modified from Jevtic et al.[27].

Figure 2: SG localization in live cells upon labeling at different concentrations. HeLa cells were incubated for 30 min with a mixture of 0.25 µM Mitotracker CMXRos Red and the indicated SG dilution. The solution was replaced with DMEM, and the images were acquired on a LSM880 Airyscan microscope, 63x 1.4 oil objective, with a sequential acquisition of color channels. Single optical slices are shown (scale bar = 10 µm). This figure has been modified from Jevtic et al.[27].

Figure 3: HeLa cells during labeling with SYBR Gold (SG). First, live HeLa cells were labeled with Mitotracker CMXRos Red and washed. SG (final dilution 1:10,000 in DMEM) was added to the cells and a time lapse acquisition was obtained. LSM880 microscope, 63x 1.4 Oil objective, sequential acquisition. Z-stacks were acquired at each time point. The maximum intensity projections are shown. (**A**) Mean intensities of SG fluorescence over time in several regions of interest. (**B**) Representative fields of view showing the regions of interest where SG fluorescence was measured (colored rectangles). (**C**) A field of view at several time points during incubation

with SG. A square region marked with white line is shown at a higher magnification in the right column. This figure has been modified from Jevtic et al.[27].

Figure 4: Effect of fixation and permeabilization on SG localization in the cells. HeLa cells were stained with SG (stock diluted 1:10,000) and Mitotracker CMXRos Red (0.25 µM) for 30 min. Single optical slices were acquired on a spinning disk microscope; sequential acquisition; scale bar = 10 µm. (**A**) Live HeLa cells stained with SG. (**B**) Live HeLa cells stained with SG and then fixed with 2% PFA in PBS for 30 min. (**C**) Live HeLa cells stained with SG, then fixed with 2% PFA in PBS for 30 min and permeabilized with 0.1% Triton X100 for 15 min. On the lower panel, the same image is shown, but the brightness in the green channel is set higher. (**D**) HeLa cells fixed with PFA, permeabilized with 0.1% Triton X100 for 15 min, and then stained with SG and Mitotracker CMXRos Red. To acquire the images shown in (D), the EMCCD gain of the camera for the green channel was reduced by a factor of 12 in comparison to **A**–**C** to avoid overexposure. This figure has been modified from Jevtic et al.[27].

Figure 5: Nucleoid tracking on live SIM images. Representative images of a field of view of DMSO-treated cells. Top: the two-color SIM image taken before the acquisition of the time lapse series. Green = SG channel; magenta = mitotracker channel. Bottom: nucleoid tracks from a 50-frame SIM time series in the SG channel (**Movie 1**); frame time = 1.8 s; tracking by Imaris 8.4.1 software. Tracks are color-coded by maximal track speed (µm/s); PS1 Elyra, SIM mode, 100x/1.46 Oil objective; scale bar = 10 µm. This figure has been modified from Jevtic et al.[27].

Movie 1: SIM time lapse series showing representative SG-stained cells. Scale bar = 5 µm. Detected mitochondrial nucleoids are marked as white spheres. Tracks are visualized as "Dragon tails" (8 frames length) and color-coded according to their maximal instant speeds (color bar in the bottom right shows speeds in µm/s). Mitochondrial nucleoids tracking and visualization by Imaris 8.4.1 software. This video has been published in Jevtic et al.[27].

DISCUSSION:

There are several critical components to the protocol: To achieve preferential labeling of mitochondrial DNA, the concentration of the DNA binding dye during incubation should be kept very low (e.g., a 1:10,000 dilution of a typical commercial stock), and the incubation time should be 30 min. The incubation time should never exceed 1 h. SYBR Gold dye should be used; other DNA-binding dyes are not bright enough to generate a strong signal upon labeling at a low concentration.

The limitation of our protocol is that the dye is washed out from the mitochondrial nucleoids during a permeabilization step. Therefore, the described procedure is not suitable for conventional immunofluorescence protocols. In this case, nucleoids in fixed cells can be efficiently labeled with other techniques, such as antibodies against DNA or mitochondrial transcription factors (TFAM).

Direct labeling of mitochondrial nucleoids with the DNA binding organic dye has advantages over fluorescent protein labeling: any type of cell can be labeled within <1 h, without temporal or

other constrains of transient or stable expression of fluorescent protein-tagged constructs. Also, in current protocols, conventional fluorescent protein-tagging of TFAM was reported to cause artifacts. Moreover, SG efficiently stains nucleoids only if the mitochondrial membrane potential is intact. This prevents image acquisition of biologically irrelevant "sick" and dead cells, which cannot be avoided with FP-based staining. Finally, previously published protocols based on DNA binding dyes did not achieve preferential staining of mitochondrial DNA.

The proposed protocol is fast and simple, thus we assume it will be widely used for live imaging of mitochondrial nucleoids by various fluorescence techniques, both diffraction limited (e.g., laser scanning confocal, TIRF, etc.) as well as super-resolution SIM.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure

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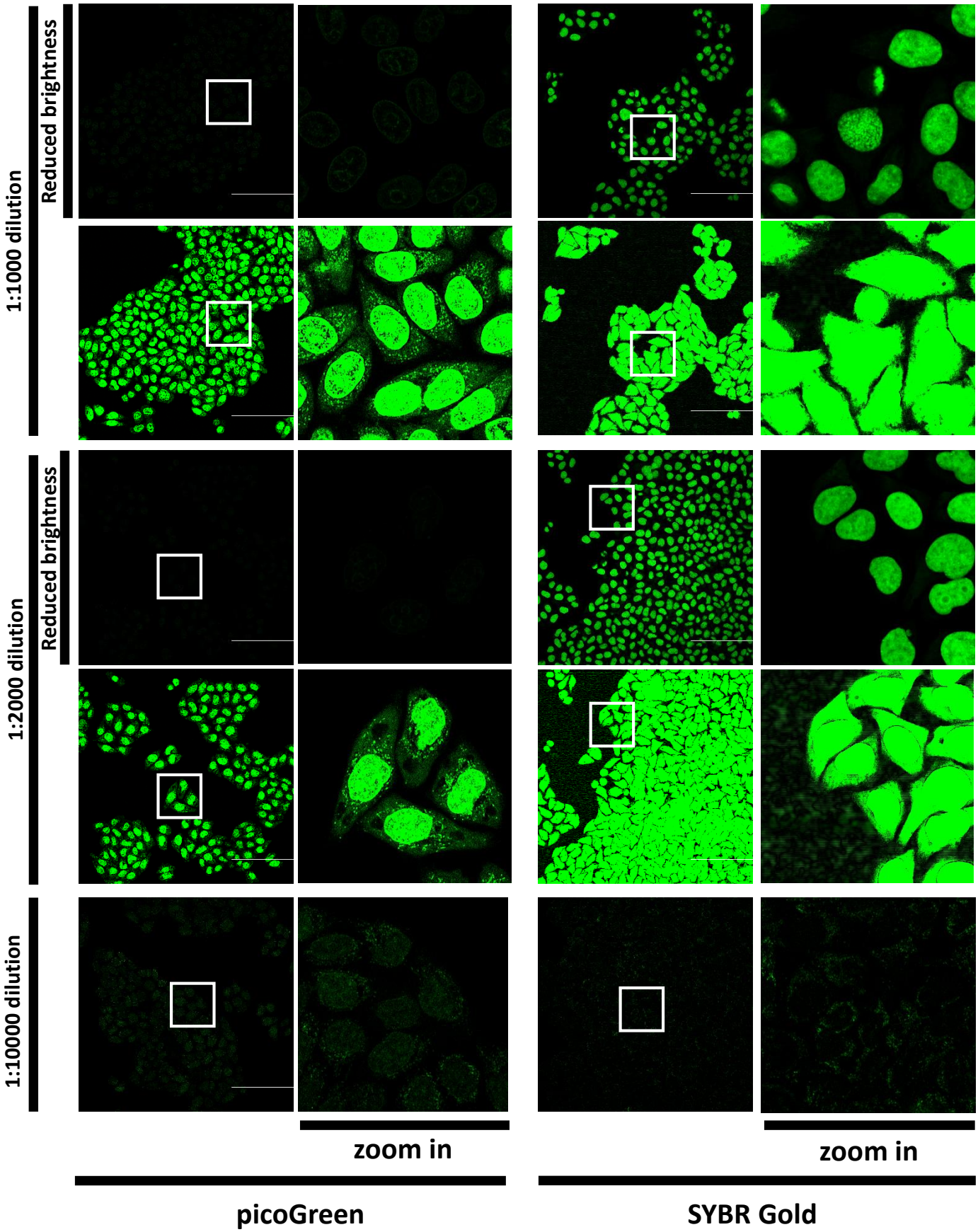

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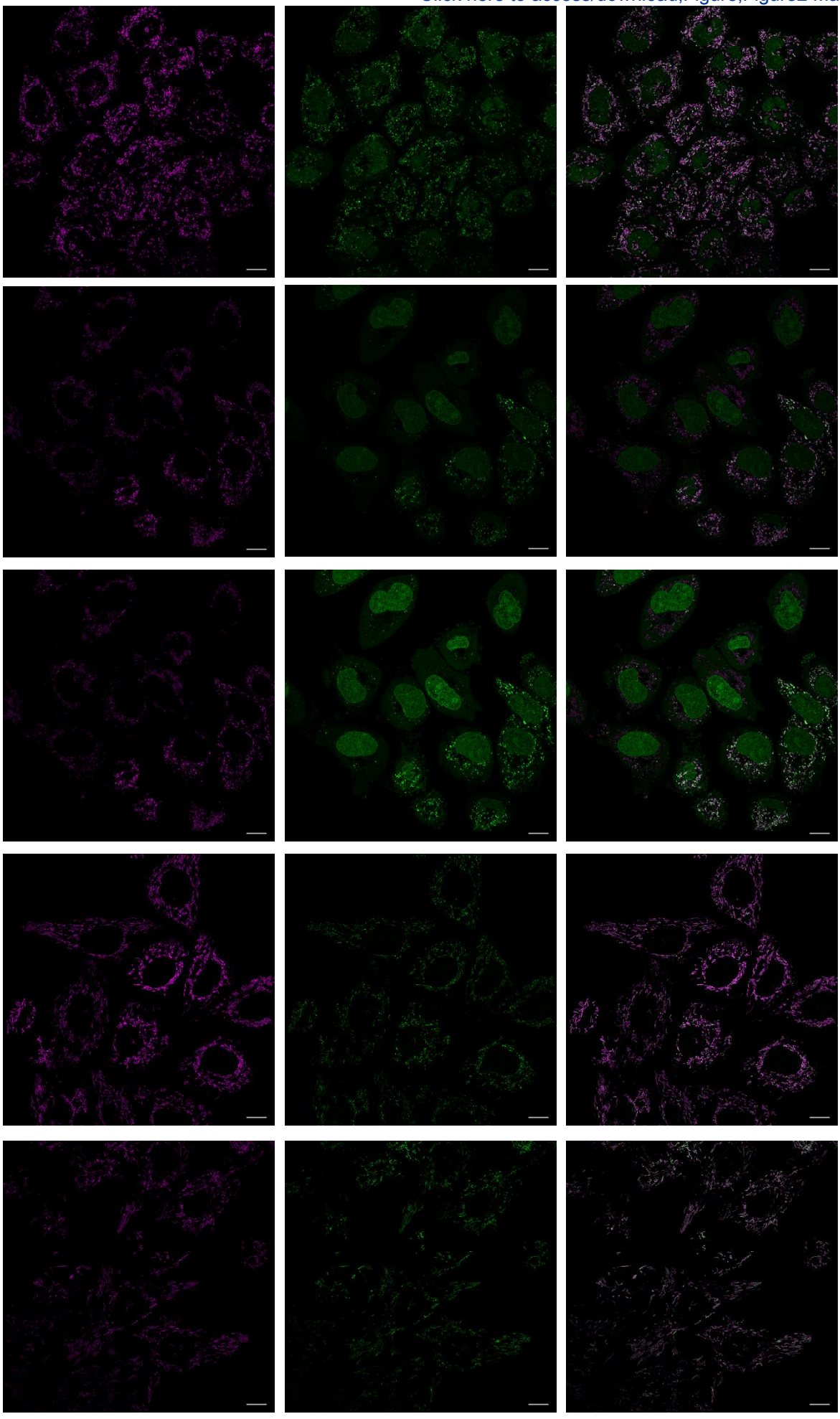

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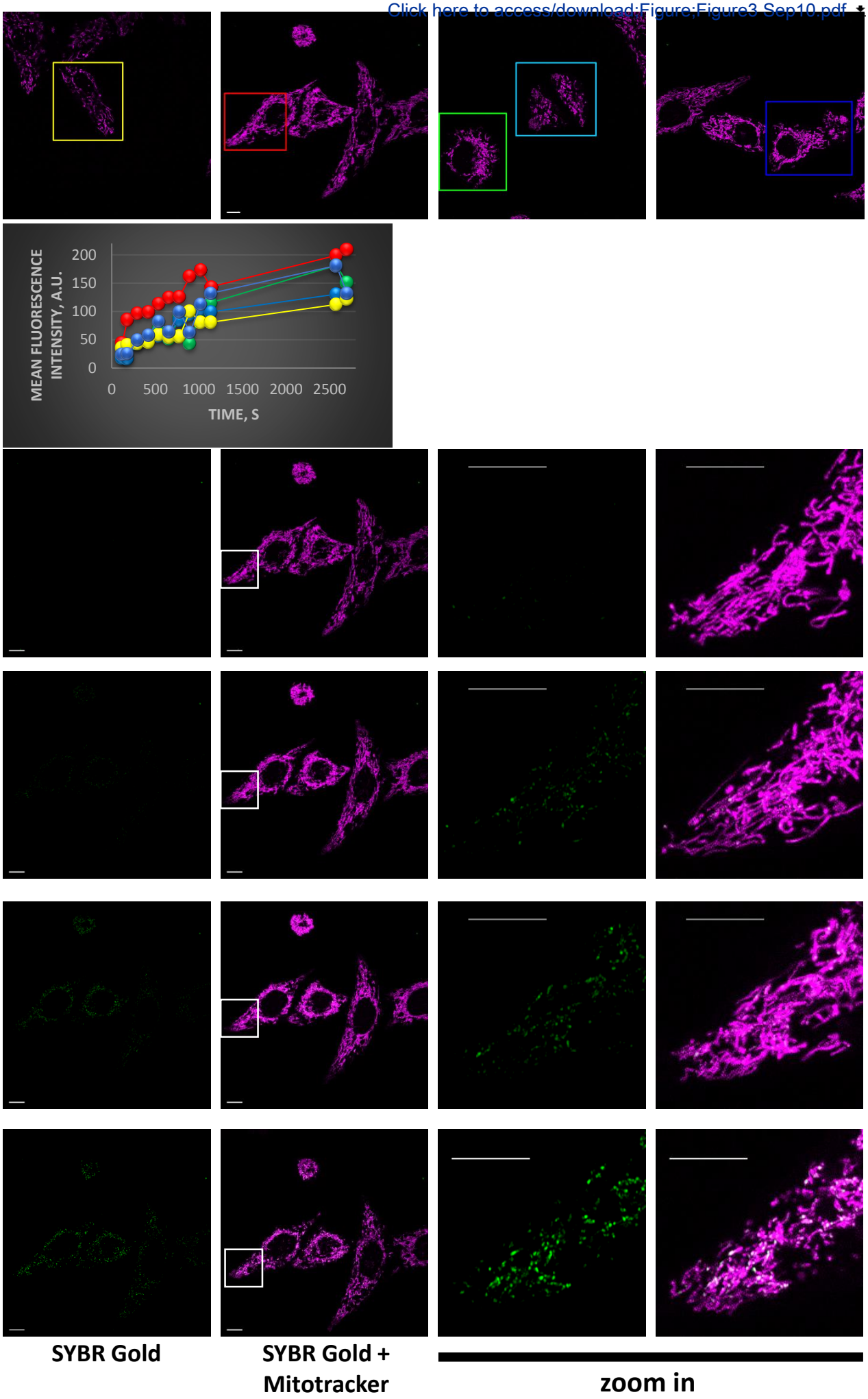

Figure

Figure

Video 1

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Video or Animated Figure

SA-007 c14 488SIM-50ms-1-0per_Nov15-rt.avi

Table of Materials

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Name of Material/Equipment	Company	Catalog Number
Elyra PS1	Carl Zeiss	
high glucose DMEM	GIBCO/ThermoFisher	31966021
ibidi 35-mm dish, glass bottom	Ibidi Gmbh	81158
ibidi 8-well microSlide, glass bottom	Ibidi Gmbh	80827
Imaris 8.4.1	Bitplane/Oxford Instruments	
iXon 885	Andor Technologies	
LSM880 Airyscan	Carl Zeiss	
Mitotracker CMXRos Red	ThermoFischer	M7512
Mitotracker Deep Red FM	ThermoFischer	M22426
picoGreen	ThermoFischer	P7581
Plan Apochromat 100x/1.46 Oil objective	Carl Zeiss	
SYBR Gold	ThermoFischer	S11494
Zen Black 2012 software	Carl Zeiss	

Comments/Description

multi-modal super-resolution microscope containing module for super-resolution structured illumination microscopy (SR-SIM)

image porcessing and visualisation software package
EMCCD camera with back-illuminated sensor
laser scanning confocal microscope with array detector
red live cell mitochondrial stain
far red live cell mitochondrial stain
cell permeant DNA stain

cell permeant DNA stain
image acquisition and processing software

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Dear Editors,

The following corrections are made to address the inquiries:

Line 142 – “step 1.5” replaced with “step 1.6”

Line 222 – corrected

Line 367 - corrected

screenshot 1

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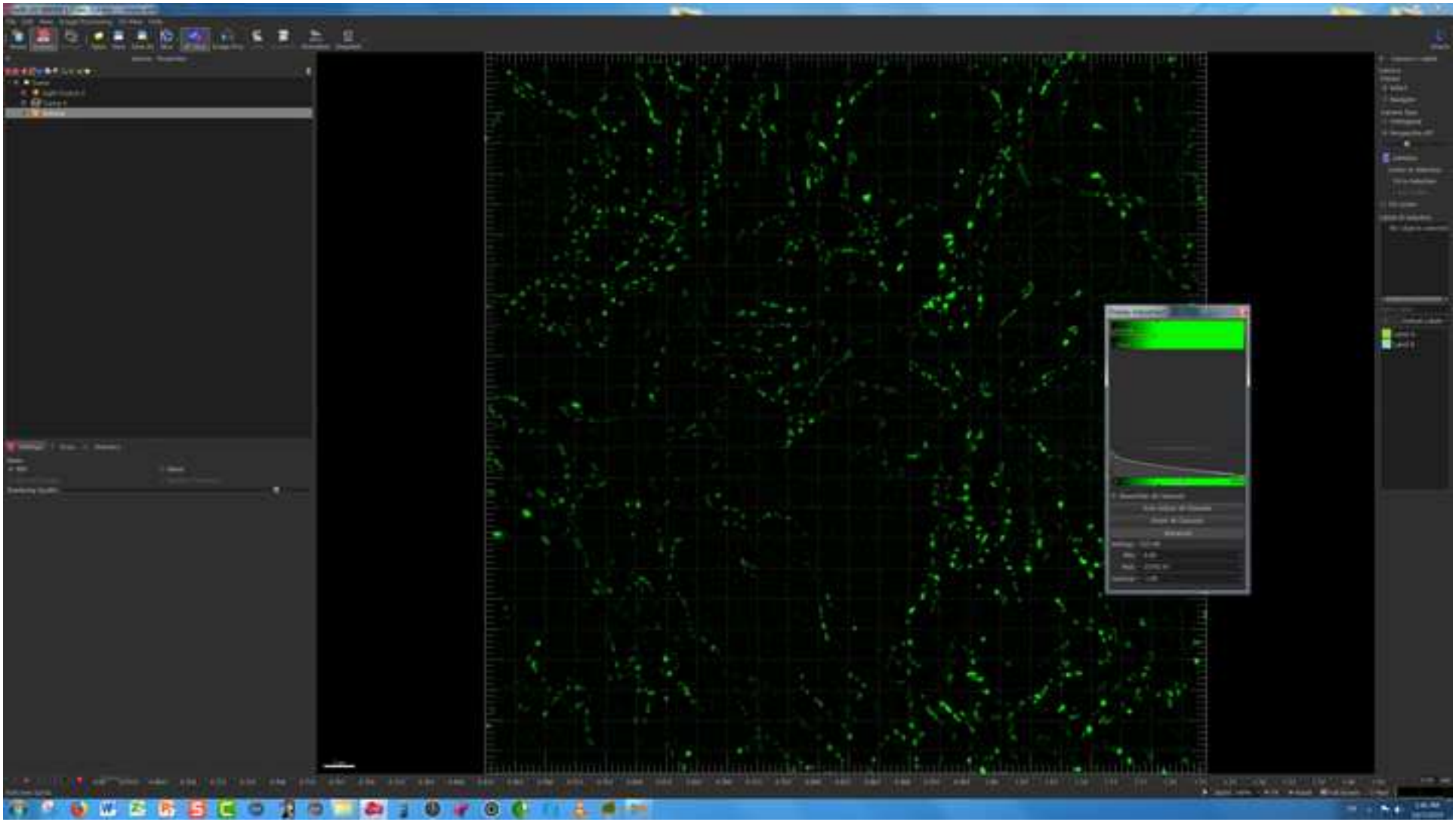

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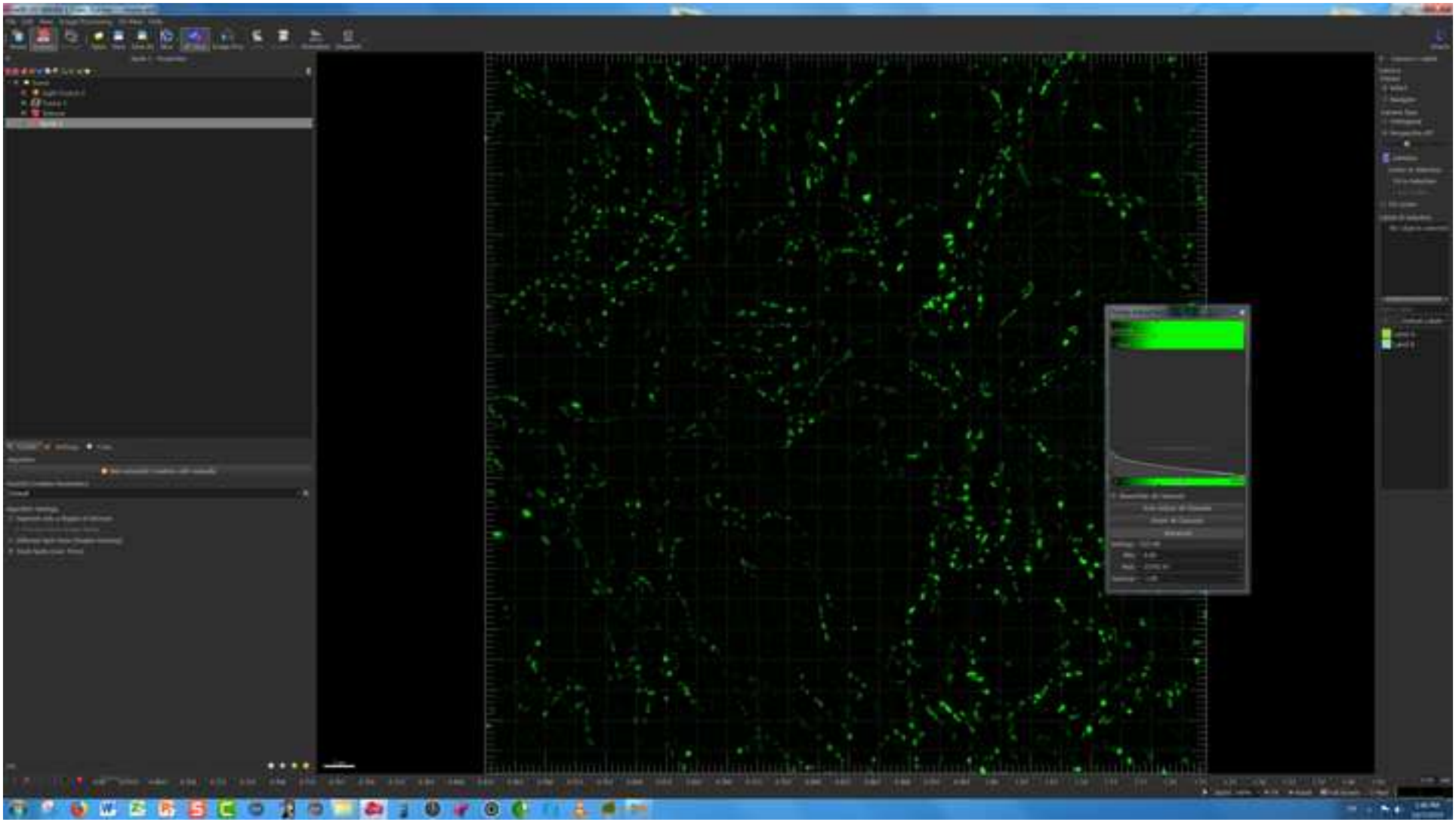

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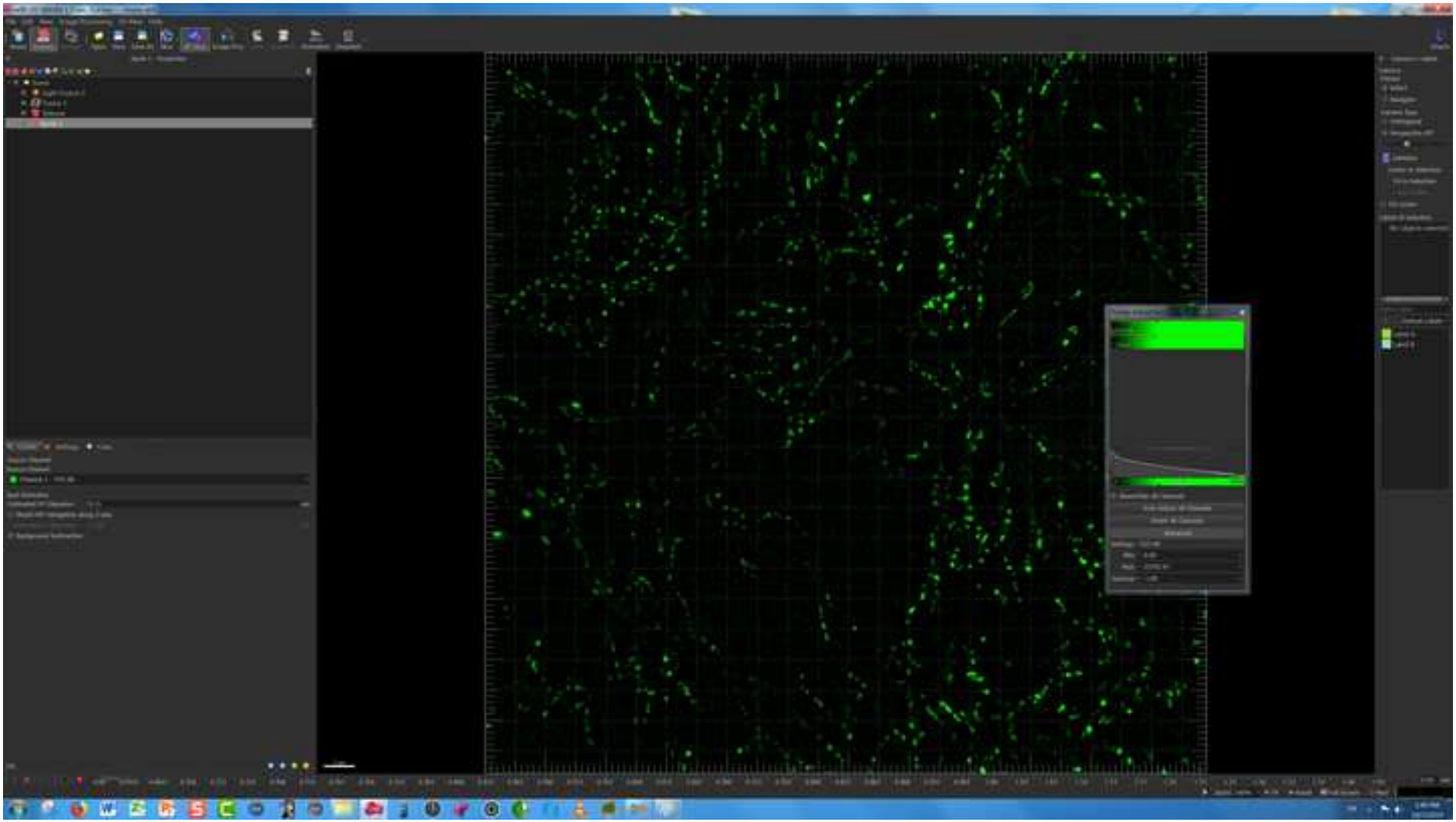

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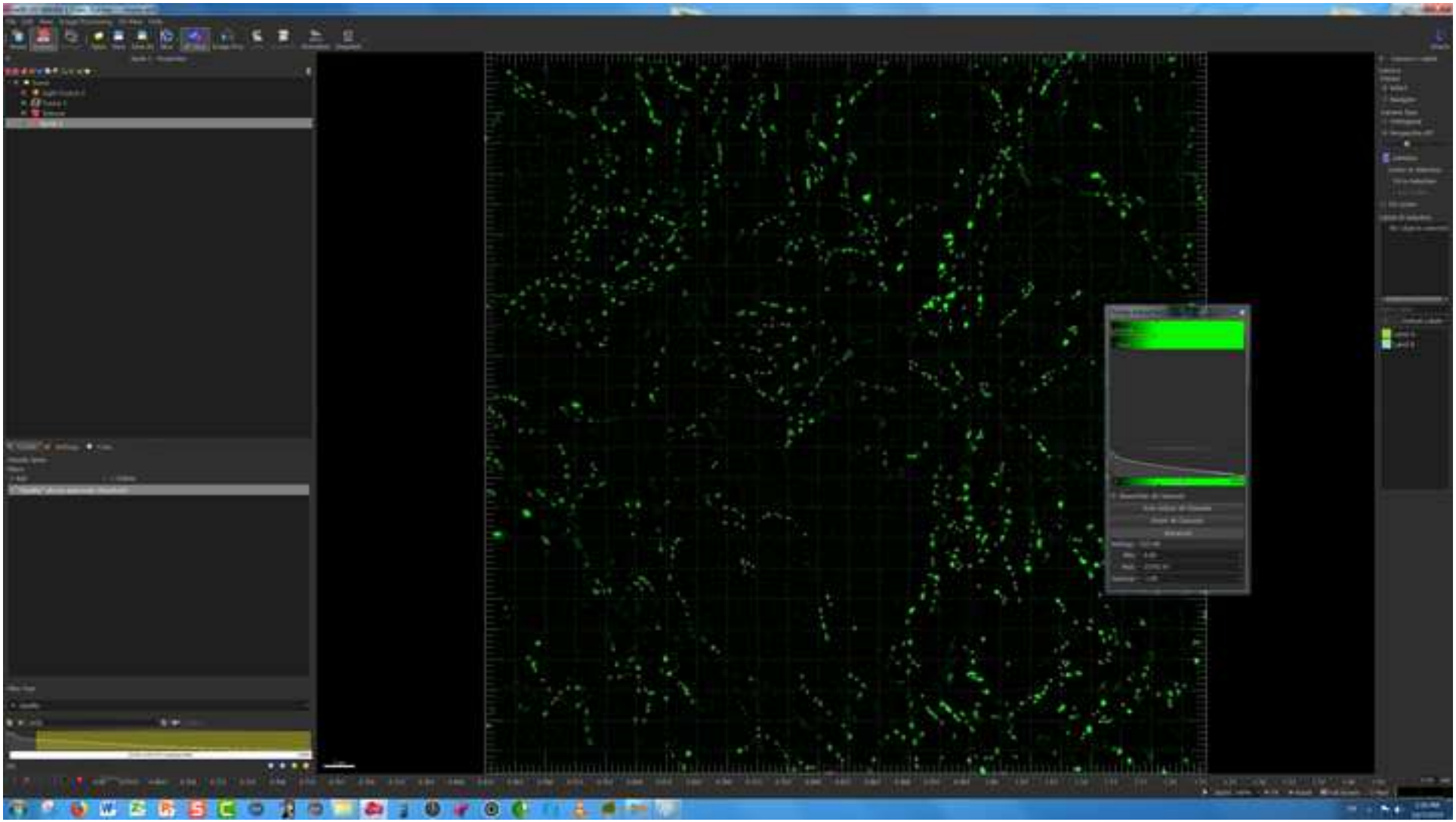

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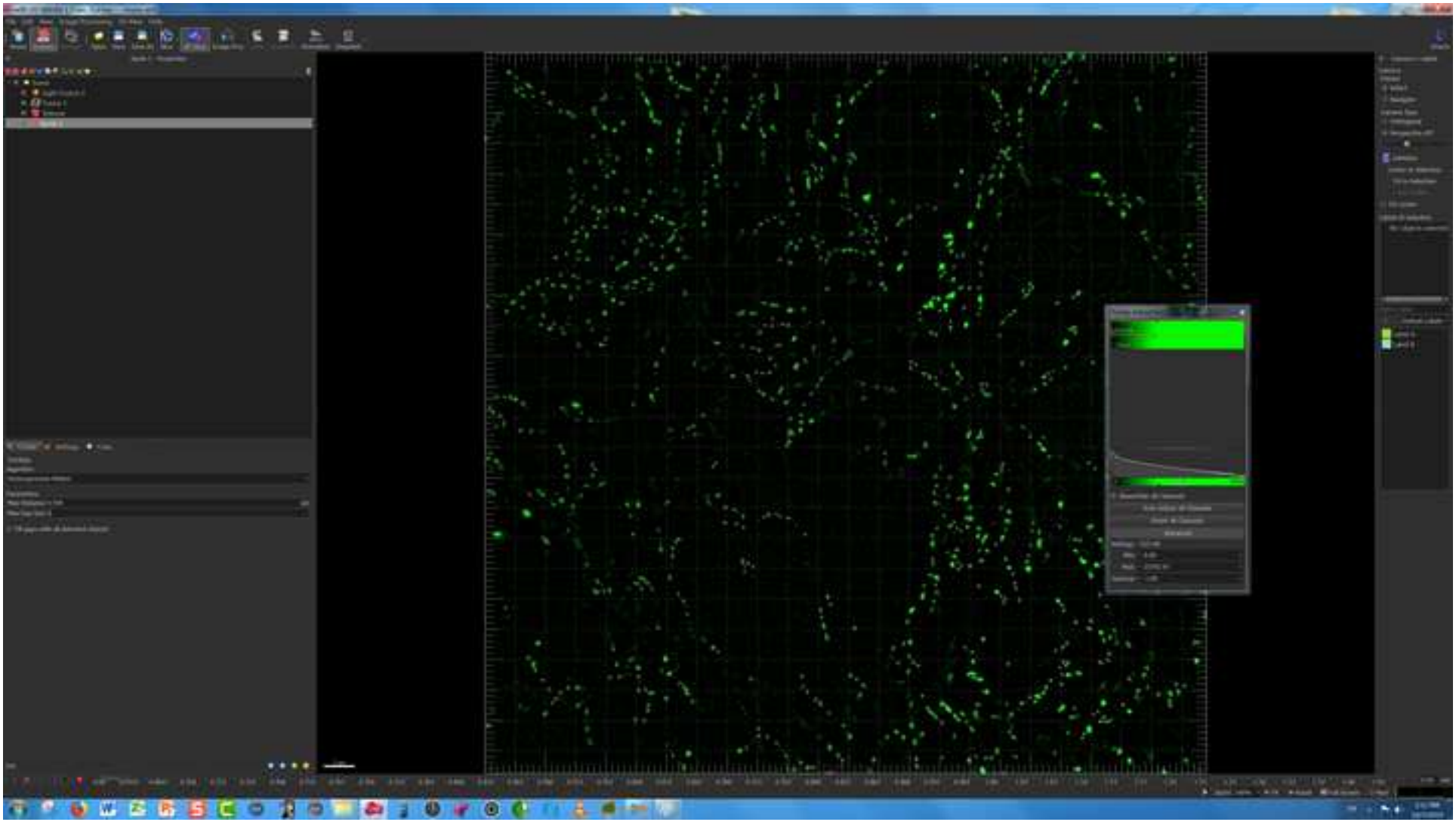

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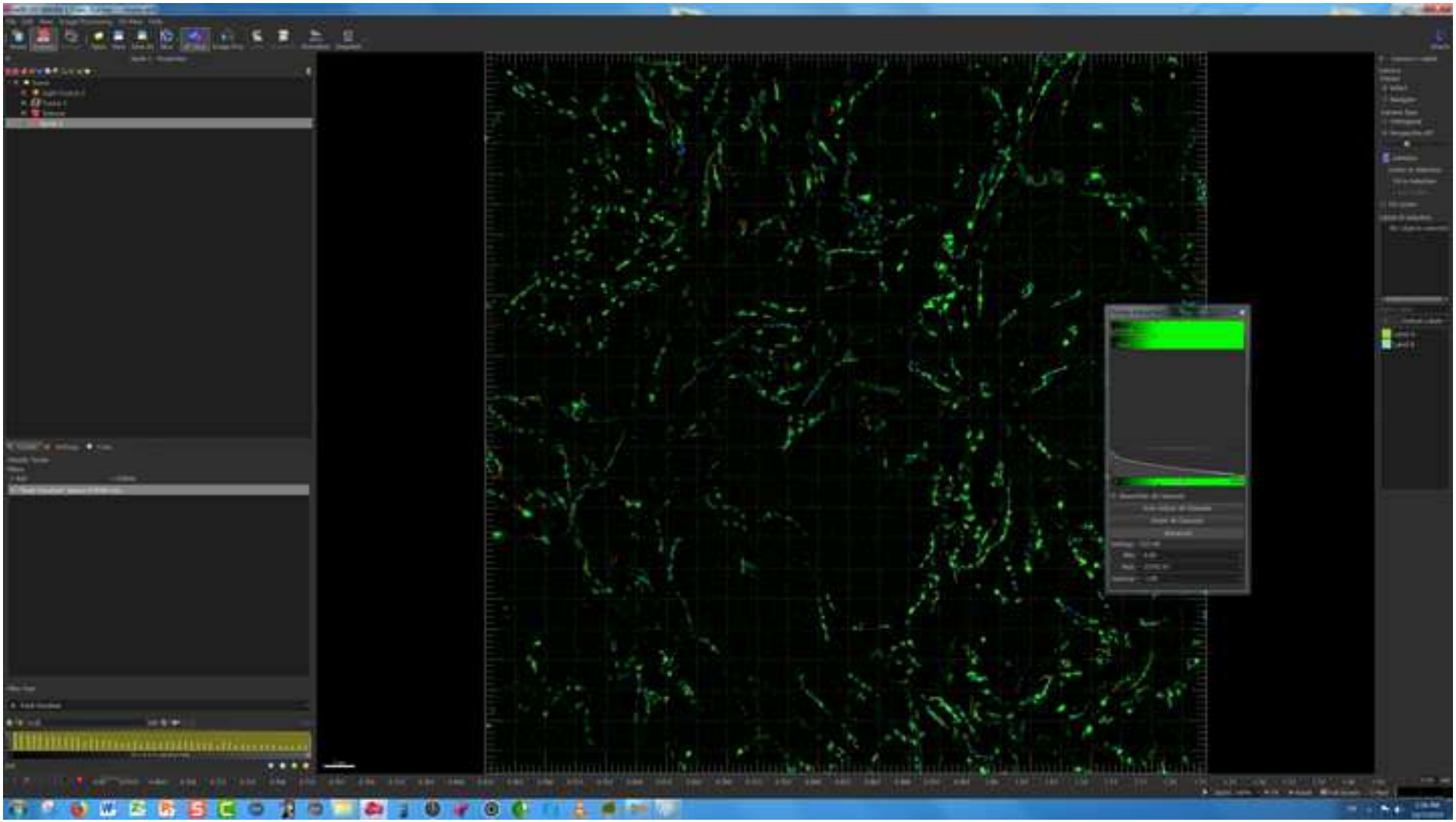

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