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MULTIPHOTON MICROSCOPY SUPPORTS SOCIAL SCIENCES TO IMPROVE THE FIGHT AGAINST COVID-19 PANDEMIC

Erwan Ferrandon¹, Petra Pelletier^{2,3*}, Magali Boespflug⁴, Cécile McLaughlin³, Sophie Alain⁵, Claire Lefort¹

¹ University of Limoges, CNRS UMR 7252, XLIM Research Institute, Limoges, France

² University of Paris, Laboratory of Social Psychology, Paris, France

³ University of Limoges, Research Center for Semiotics, Limoges, France

⁴ University of Poitiers, Research Center for Management, Poitiers, France

⁵ University of Limoges, INSERM UMR 1092, Resinfit, CHU Limoges, France

*ORCID ID: 0000-0003-3057-4614, Email: petra.pelletier@gmail.com ; petra.pelletier@unilim.fr

Abstract

We hardly convince people that they are fighting against an invisible enemy with barrier gestures without proper scientific arguments. The aim of this pluri-disciplinary research program is to understand the viral strategy of SARS-CoV-2 virus that is responsible for the Coronavirus disease, and therefore rationalize the perceived biological threat based on socially constructed image of SARS-CoV-2 virus within French population. Indeed, laymen's cognitive processes, emotional reactions and behaviours are driven by rather subjective perception of the reality. Our collaboration with social sciences allows us to seize the SARS-CoV-2 social representations of profane population in order to guide an explicative approach of the current sanitary measures, based on rigorous scientific demonstrations. The first results lead us to a multi-photon microscopy analyses of SARS-CoV-2 virions, devoted to image the structure of the free virion label-free. In that case, the resolution of an optical microscope is a limiting factor. To visualize a target of 120 nm, two main optimizations are necessary: an optimization of the instrumental resolution and of the virion preparation. In the first case, we have chosen a computational strategy resting on a mathematical estimation of the instrumental point-spread-function (PSF), combined with a deconvolution solution in order to reveal details in the image initially lost in blur and noise (Boutet de Monvel et al., 2003). The final aim is to identify the precise shape of the virion in the image by an optical solution and to identify the presence of virions by artificial intelligence tools. Finally, we aim to follow the infectious process in vivo the infected cells in order to reveal its viral strategy in the cells. With such a research strategy, we will be able to follow the efficiency of anti-viral treatments directly within living cells by an optical microscopy technology. Thus, we could demonstrate to laymen SARS-CoV-2 virus specificities that will allow to strengthen the trust between scientists and collectivity and contribute to shape related public policies.

Keywords: COVID-19, SARS-CoV-2, multiphoton microscopy, social sciences, computational image retreatment

1) Introduction

The COVID-19 Coronavirus pandemic has shattered various aspects of laymen's lives. Further, an efficient crisis management of the current sanitary crisis necessitates a multiplicity of daily barrier gestures despite the fact that SARS-CoV-2 virus remains invisible to people. Thus, laymen tend to experience distress while facing the COVID-19 collective trauma (Silver, 2020). Indeed, the biological threat perceived by laymen requires a thorough understanding of the underpinning social construction processes and also a rigorous investigation of proper scientific image of SARS-CoV-2 virus that is responsible for the Coronavirus disease.

Firstly, our mixed-data based research conducted currently in the field of social sciences within French context demonstrated that the COVID-19 Coronavirus is perceived by the prism of profane French population as a dangerous and worldwide spreading virus that represents primarily the impact of COVID-19 sanitary measures, such as social isolation and distancing that are leading to the feeling of stress, depression and anxiety rather than a direct threat to individuals' health (Pelletier, McLaughlin, Valette, Lefort, & Boespflug, 2021).

Additionally, social construction processes that commonly underpin the perception of large-scale societal threats such as contradictory news or advices from mass media experts can lead to laymen's misrepresentations of the reality, exacerbated emotional reactions and illogical behaviours (Pelletier & Drozda-Senkowska, 2019). Also, in the context of sanitary crisis, laymen are torn due to an amalgam between sanitary crisis management decisions and proper scientific explanations that are frequently misunderstood.

Thus, the primary aim of the current research is to demonstrate clear and explicit scientific results, based on rigorous scientific technology that might guide an explicative approach of the current sanitary measures, based on rigorous scientific demonstrations in order to effectively explain the related pandemic challenges to profane population. The scientific technology of Multiphoton microscopy (MPM) allows a rigorous *in vivo* observation of unfixed sample with a high-resolution level to detect viral particles in different condition (Larson, 2011). Viruses used are un-marked because the objective is to detect the signal of autofluorescence generated from the target: SARS-Cov-2 viral proteins studded into the bilayer membrane, including Spike proteins (S proteins).

Firstly, optimization of the two-photon fluorescence microscopy (TPFM) in the observation of the virus alone is needed. Our final aim is to observe a cell *in vivo* during the infection process or the direct effect of anti-viral molecules with live imaging. In the one hand, this strategy would allow to improve the understanding of the viral strategy (Zhang & Kutateladze, 2020). In the other hand and the effects of the antiviral treatments used in a real time would be visualisable. These visuals results represent a concrete argument that might be used by sanitary authorities to convince laymen about the specific dangers of SARS-CoV-2 virus that led to the COVID-19 Coronavirus pandemic outbreak.

2) Methods

Multiphoton microscopy

Excitation conditions with a pulsed beam (central wavelength: 850 nm, pulse duration: 150 fs, repetition rate: 82 MHz, average power: 5mW). Wavelength of emission filter: Magnetic microsphere signal - 575/630nm, expected SARS-CoV-2 viral particle signal - 495/540nm, fluorescent microsphere signal - 430/490 nm. Dichroic wavelength: first – 690nm, second – 570 nm.

Samples

SARS-CoV-2 viral particles were taken from an infected cell culture SP6 after cell lysis in red phenol medium. The supernatant was clarified by centrifugation, filtered by Amicon® Ultra-4 to remove residual remains from cells and diluted in PBS.

In order to concentrate viral particles for microscope observation, some viral particles were also incubated 30 min with fluorescent magnetic microsphere. Supernatant was removed and viral particles coupled to magnetic sulphated cellulose microsphere were rinsed two times in PBS and dilute in PBS. In both cases, fluorescent microspheres of 100 nm diameter were added before deposit 5µL on slide.

Computational image processing

Image processing was led with Fiji and MATLAB software that allow data analysis, exploration and visualisation. Further, deconvolution has been done by fluorescent microsphere signal under MATLAB algorithms (Lee, Wee, & Brown, 2014).

3) Results

Free virions

Our first wide field images from free virions shown some signal agglomerations and various signal intensities (Fig. 1A). These specificities have a heterogeneous repartition. Also, signals obtained have different shapes and sizes.

Computational image processing

Image processing allow us to observe more details due to deconvolution treatment (Doi et al. 2018; Hortholary et al., 2021). By this way, we are able to observe small and clear signals around aggregates (Fig. 1B). Combined to multiphoton 3D acquisitions, image processing software can combine images to a clear representation of the target (Fig. 1C).

Virions attached to magnetic sphere

Particles caught by magnetic microsphere produce signal in the same condition than previously shown (Fig. 2C). By this filtration, we expect to keep only virions stick to magnetic sphere and it seems

that there is less noise or aggregate from unknown biological material. Few green signals are emitted around (A and C) the red zones (B). At least, we can observe a colocalization between the two expected signals.

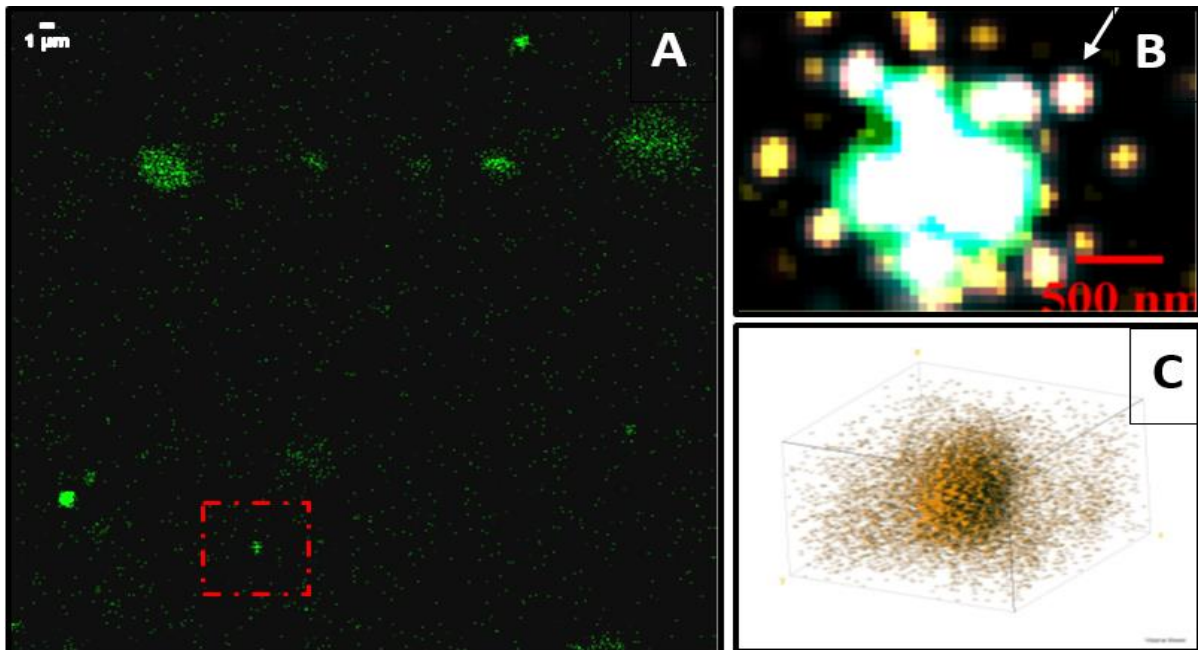


Figure 1: Bi-photon imaging of SARS-CoV-2 lysis infected cells culture. A) Solution from SP6 cell culture infected by $1,7 \times 10^5$ of SARS-CoV-2 viral particles. B) Red frame: Area analyzed under computational processing (B). B) Red frame area after computational processing. White arrow: potential viral particle. C) Aggregate under 3D modelization.

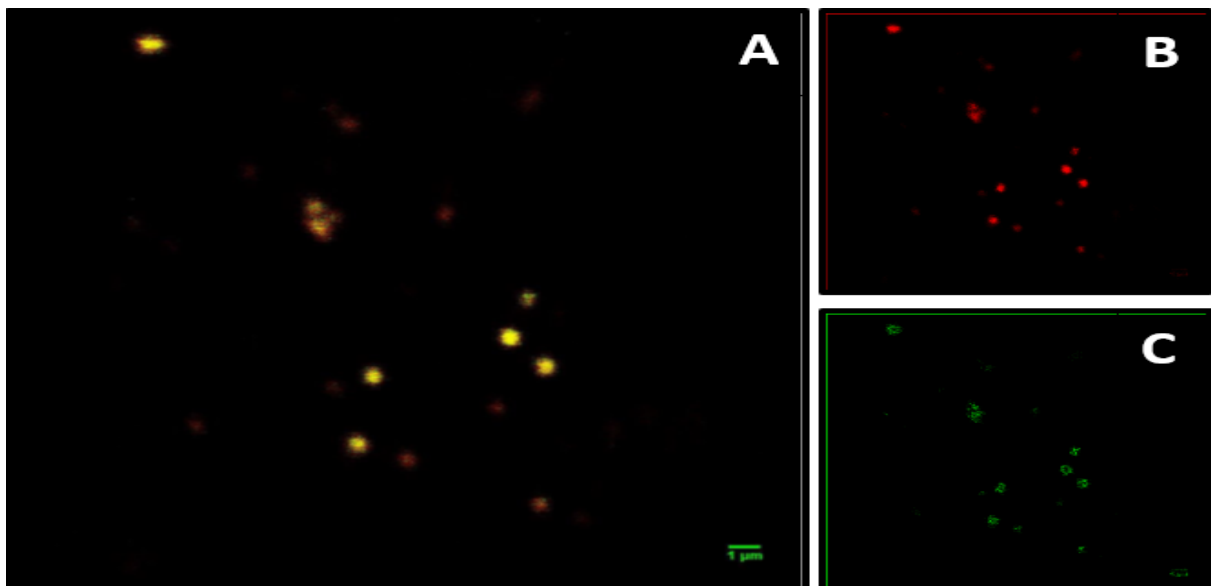


Figure 2: Bi-photon imaging of magnetic microspheres targeting SARS-CoV-2 in lysis infected cells culture. Solution from SP6 cell culture infected by $1,7 \times 10^5$ of SARS-CoV-2 viral particles. Red: magnetic microsphere, Green: Signal from viral particles solution, Yellow: merge. A) Merge of magnetic microsphere and expected viral particle signals. B) Magnetic microsphere signals (Wavelength: 575/630nm). C) Expected SARS-CoV-2 viral particle signals (Wavelength: 495/540 nm)

4) Discussion

We hypothesized that each signal come from unique free virions, probably grouped in the form of aggregates. Aggregates are clearly visible but there is no certitude that we have only viral particles in the free virions condition.

Indeed, it's more probable that we still have some biological materials on the slide diameters that is much greater than diameters of free virions. These could be the rest of cell lysis or culture medium residues. Therefore, we should ensure that there are no other biological objects that can fluoresce in the free virion solution used. According to these results, we have to improve our way to purify the virus solution from cell culture. Moreover, the dimensions at the image of many of these objects do not appear realistic in relation to the diameter expected in the order of 100 nm. Despite the production of 3D images can be particularly useful to analyse SARS-CoV-2 viral strategy, we need preliminarily to be sure that we are imaging exclusively viral particles. The collected wavelength range corresponds to 2-photon fluorescence which can only be endogenous if it comes from free virions, that were unexpected.

The computational strategy used to counter the instrumental physical limit of point-spread-function (PSF) combined with a deconvolution strategy in order to reveal details in the image initially invisible give hopeful result and allow us to show more details under a good resolution (Lefort et al., 2020). However, the reconstruction of the images is currently too much blind to be able to give a reliable conclusion.

Magnetic microspheres are able to concentrate viral particles around their area but it's still weak, that make their discernment almost impossible. It is imperative to verify the origin of these fluorescence signals on the particles and not possible parasites. To be sure that only viral particles are observed, we planned to use electron microscopy as a complemental information.

Obtaining explicit results will help first for explaining clearly the viral strategy of SARS-CoV-2 virus, and allow to the profane population to admit and understand the mechanism of invisible enemy. Such a rigorous scientific proof might allow to increase the overall crisis management effectiveness and therefore rationalize laymen's behaviours in the context of the COVID-19 Coronavirus pandemic (Kreps & Kriner, 2020). These results of real image of SARS-CoV-2 virus will offer a huge support for social sciences and related public policies in order to warn laymen of the concrete dangers of the COVID-19 biological threat.

5) Conclusion

The overall aim of the current research program conducted within French context is to build up a relevant articulation between the hard science and the soft science that will allow to increase the overall crisis management effectiveness in the context of COVID-19 sanitary crisis. Thus, such an innovative research collaboration on SARS-CoV-2 virus that is responsible for the Coronavirus disease would

demonstrate to laymen SARS-CoV-2 virus specificities and contribute to increase the sense of the multiplicity of daily barrier gesture that are imposed to the population as a part of current sanitation policies. In conclusion, such an in-depth investigation of SARS-CoV-2 virus with regard to discrepancies between the biological image and the socially constructed image of the virus will allow to strengthen the trust between scientists and collectivity and contribute to shape related public policies.

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