

Titre: Title:	3D multiplex immunoplasmonics microscopy
Auteurs: Authors:	Éric Bergeron, Sergiy Patskovsky, David Rioux, & Michel Meunier
Date:	2016
Type:	Communication de conférence / Conference or Workshop Item
Référence: Citation:	Bergeron, É., Patskovsky, S., Rioux, D., & Meunier, M. (mai 2016). 3D multiplex immunoplasmonics microscopy [Résumé]. 10th World Biomaterials Congress, Montréal, Québec (3 pages). Publié dans Frontiers in Bioengineering and Biotechnology, 4. https://doi.org/10.3389/conf.fbioe.2016.01.02114

 **Document en libre accès dans PolyPublie**
Open Access document in PolyPublie

URL de PolyPublie: PolyPublie URL:	https://publications.polymtl.ca/4856/
Version:	Version officielle de l'éditeur / Published version Révisé par les pairs / Refereed
Conditions d'utilisation: Terms of Use:	CC BY

 **Document publié chez l'éditeur officiel**
Document issued by the official publisher

Nom de la conférence: Conference Name:	10th World Biomaterials Congress
Date et lieu: Date and Location:	2016-05-17 - 2016-05-22, Montréal, Québec
Maison d'édition: Publisher:	Frontiers
URL officiel: Official URL:	https://doi.org/10.3389/conf.fbioe.2016.01.02114
Mention légale: Legal notice:	

3D multiplex immunoplasmonics microscopy

Eric Bergeron^{1*}, Sergiy Patskovsky^{1*}, David Rioux^{1*} and Michel Meunier^{1*}

¹ Polytechnique Montréal, Department of Engineering Physics, Canada

Introduction: Selective labeling and identification of receptors on cells can provide important clinical information, such as distinction between healthy and diseased cells, early detection and evolution of a disease, patient-specific drug selection and monitoring of the therapeutic response^[1]. Immunofluorescence is the gold standard for efficient detection of antigens expressed by cells. Antibodies (Abs) conjugated to fluorescent dyes are mainly developed in the visible wavelengths and remain limited by photobleaching, high sensitivity to the environment, low light intensity, and wide absorption and emission spectra^[2]. Tunable plasmonic nanoparticles (NPs) should provide higher multiplexing capacity than immunofluorescence since NPs are photostable over time, emit high light scattering at a specific wavelength (plasmon peak) and can be synthesized and functionalized with Abs^{[3]-[6]}. The scattering peaks of silver (Ag) and gold (Au) nanospheres (NSs) are around 450 and 550 nm, respectively, and the ones from Au nanorods (AuNRs) can be extended from 600 to 2200 nm^[7]. Microscopy at various wavelengths allows low illumination and fast integration times for spectral characterization of NPs in cellular environment^{[8]-[12]}. We aim to use reflected light microscopy (RLM) for three-dimensional (3D) wide-field imaging of Abs-functionalized NPs (immunoplasmonics fNPs) targeting cell surface receptors as an alternative to immunofluorescence.

Materials: Abs anti-CD44 and anti-EGFR from abcam and Abs anti-K_V1.1 from Alomone Labs. Orthopyridyl-disulfide-poly(ethylene glycol) (5kDa)-*N*-hydroxysuccinimide (OPSS-PEG-NHS) and HS-PEG (5kDa) from Nanocs. 80 nm AgNSs from Ted Pella. 100 nm AuNSs and 40 nm x 92 nm AuNRs from Nanopartz. PBS and DAPI from Sigma-Aldrich. Secondary Abs (Alexa 488 and Cy3) from Life Technologies.

Methods: Abs were conjugated to OPSS-PEG-NHS (OPSS-PEG-Ab)^{[10],[13]}. Citrate-capped NPs were functionalized with 0.01 OPSS-PEG-Ab/nm² and 5 μM HS-PEG (5kDa): CD44-AgNSs, EGFR-AuNSs and K_V1.1-AuNRs. The stability of fNPs was confirmed by UV-vis spectroscopy. The expression of CD44, EGFR and K_V1.1 receptors was detected by immunofluorescence and RLM on MDA-MB-231 breast cancer cells and on 661W photoreceptors. Cells were incubated for 3 h with 8 μg/mL fNPs, washed with PBS and fixed. RLM system for 3D wide-field imaging was built on an inverted Eclipse Ti microscope (Nikon) with spectral filters (500, 580 and 700 nm, Thorlabs) for fast z-scanning, optimal spectral separation and optical contrast of the fNPs in cellular environment (Fig. 1).

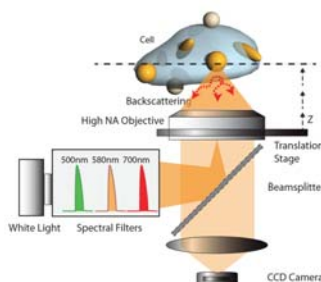


Fig. 1. RLM set-up for 3D wide-field imaging. Spectral filters are centered around the average plasmon peak of each type of NPs in cellular environment: 500 nm for AgNSs, 580 nm for AuNSs and 700 nm for AuNRs.

Results and discussion: Deconvolution was applied to each image and treated with ImageJ to generate an image taking into account the average plasmon peak of each type of NP (Fig. 2).

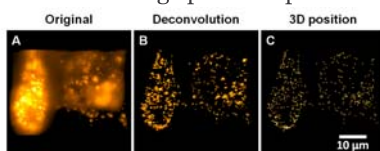


Fig. 2. Image treatment. (A) 3D image of EGFR-AuNSs on MDA-MB-231 cells obtained with 100 nm step z-scan by RLM set-up. (B) Deconvolution of the image using experimental point-spread function. (C) 3D position of NPs obtained by local maximum filter and 3D Object Counter (ImageJ).

Immunofluorescence demonstrated the expression levels of targeted receptors (Fig. 3A-F): CD44⁺ EGFR⁺ K_v1.1⁺ MDA-MB-231 and CD44⁻ EGFR⁻ K_v1.1⁺ 661W. The exposure time to detect CD44 was 80 ms while it was longer for EGFR and K_v1.1 (500 ms). By increasing the exposure time, the background fluorescence and photobleaching become more important. This problem is solved with improved 3D identification of stable fNPs selectively labeling targeted cells by RLM (Fig. 3G-L).

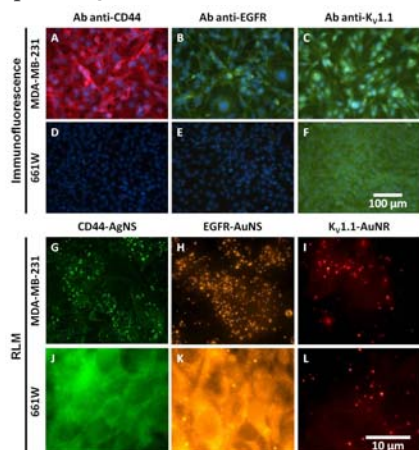


Fig. 3. Identification of CD44, EGFR and K_v1.1 cell surface receptors on CD44⁺ EGFR⁺ K_v1.1⁺ MDA-MB-231 and CD44⁻ EGFR⁻ K_v1.1⁺ 661W cells by immunofluorescence and by RLM.
 (A-F) Immunofluorescence with primary Abs detected with Cy3 or Alexa Fluor 488 conjugated to IgG Abs. Cell nuclei were stained with DAPI.
 (G-L) RLM with CD44-AgNSs, EGFR-AuNSs and K_v1.1-AuNRs.

Conclusion: The developed technology is simple and compatible with standard fluorescence microscopy set-up. This technology with optical analysis of biomarkers is ready for clinical applications as an alternative to immunofluorescence.

This work was supported by Le Fonds de recherche du Québec and the Natural Science and Engineering Research Council of Canada. EB received funding from Fonds de recherche du Québec – Santé. Dr. Laudine Desreumaux-Communal and Prof. Anne-Marie Mes-Masson from CRCHUM are acknowledged for fruitful discussions.

References:

- [1] J. Aaron, N. Nitin, K. Travis, S. Kumar, T. Collier, S. Y. Park, M. José-Yacamán, L. Coghlan, M. Follen, R. Richards-Kortum and K. Sokolov (2007) Plasmon resonance coupling of metal nanoparticles for molecular imaging of carcinogenesis in vivo, *Journal of Biomedical Optics* 12(3):034007.
- [2] P. Zhang, S. Lee, H. Yu, N. Fang and S. H. Kang (2015) Super-resolution of fluorescence-free plasmonic nanoparticles using enhanced dark-field illumination based on wavelength-modulation, *Scientific Reports* 5:11447.
- [3] X. Huang, P. K. Jain, I. H. El-Sayed and M. A. El-Sayed (2007) Gold nanoparticles: Interesting optical properties and recent applications in cancer diagnostics and therapy, *Nanomedicine* 2(5):681–693.
- [4] K. Weintraub (2013) The new gold standard, *Nature* 495(7440):S14–S16.
- [5] D. Rioux and M. Meunier (2014) Alloy nanoparticles, process for their preparation and use thereof, USPTO61945276.
- [6] S. Patskovsky, E. Bergeron, D. Rioux, M. Simard and M. Meunier (2014) Hyperspectral reflected light microscopy of plasmonic Au/Ag alloy nanoparticles incubated as multiplex chromatic biomarkers with cancer cells, *Analyst* 139(20):5247–5253.
- [7] K. Seekell, M. J. Crow, S. Marinakos, J. Ostrander, A. Chilkoti and A. Wax (2011) Hyperspectral molecular imaging of multiple receptors using immunolabeled plasmonic nanoparticles, *Journal of Biomedical Optics* 16(11):116003.
- [8] N. Fairbairn, A. Christofidou, A. G. Kanaras, T. A. Newman and O. L. Muskens (2013) Hyperspectral darkfield microscopy of single hollow gold nanoparticles for biomedical applications, *Physical Chemistry Chemical Physics* 15(12):4163–4168.
- [9] S. Patskovsky, E. Bergeron and M. Meunier (2015) Hyperspectral darkfield microscopy of PEGylated gold nanoparticles targeting CD44-expressing cancer cells, *Journal of Biophotonics* 8(1–2):162–167.
- [10] S. Patskovsky, E. Bergeron, D. Rioux and M. Meunier (2015) Wide-field hyperspectral 3D imaging of functionalized gold nanoparticles targeting cancer cells by reflected light microscopy, *Journal of Biophotonics* 8(5):401–407.
- [11] S. Patskovsky, D. Rioux, M. Meunier and E. Bergeron (2014) A method for imaging a sample incorporating plasmonic nanoparticles and device therefore, USPTO61987274.
- [12] H. Wang, G. Rong, B. Yan, L. Yang and B. M. Reinhard (2011) Optical sizing of immunolabel clusters through multispectral plasmon coupling microscopy, *Nano Letters* 11(2):498–504.
- [13] E. Bergeron, C. Boutopoulos, R. Martel, A. Torres, C. Rodriguez, J. Niskanen, J.-J. Lebrun, F. M. Winnik, P. Sapieha and M. Meunier (2015) Cell-specific optoporation with near-infrared ultrafast laser and functionalized gold nanoparticles, *Nanoscale* (in revision).

Citation: Bergeron E, Patskovsky S, Rioux D and Meunier M (2016). 3D multiplex immunoplasmonics microscopy. *Front. Bioeng. Biotechnol. Conference Abstract: 10th World Biomaterials Congress*. doi: 10.3389/conf.FBIOE.2016.01.02114

Copyright: The abstracts in this collection have not been subject to any Frontiers peer review or checks, and are not endorsed by Frontiers. They are made available through the Frontiers publishing platform as a service to conference organizers and presenters.

The copyright in the individual abstracts is owned by the author of each abstract or his/her employer unless otherwise stated.

Each abstract, as well as the collection of abstracts, are published under a Creative Commons CC-BY 4.0 (attribution) licence (<https://creativecommons.org/licenses/by/4.0/>) and may thus be reproduced, translated, adapted and be the subject of derivative works provided the authors and Frontiers are attributed.

For Frontiers' terms and conditions please see <https://www.frontiersin.org/legal/terms-and-conditions>. Received: 27 Mar 2016; Published Online: 30 Mar 2016.

*** Correspondence:**

Dr. Eric Bergeron, Polytechnique Montréal, Department of Engineering Physics, Montréal, QC, Canada, Email1

Dr. Sergiy Patskovsky, Polytechnique Montréal, Department of Engineering Physics, Montréal, QC, Canada, sergiy.patskovsky@polymtl.ca

Dr. David Rioux, Polytechnique Montréal, Department of Engineering Physics, Montréal, QC, Canada, david.rioux@polymtl.ca

Dr. Michel Meunier, Polytechnique Montréal, Department of Engineering Physics, Montréal, QC, Canada, michel.meunier@polymtl.ca