Universidad Veracruzana

Secretaría Académica
Dirección General de Investigaciones
Instituto de Ciencias de la Salud
ACTA
Consejo Técnico

En la ciudad de Xalapa-Enríquez, Ver., siendo las diez horas del día veintitrés de mayo del dos mil diecinueve, con fundamento en los artículos 20 fracción XI, 75, 76 y 77 de la Ley Orgánica; 303, 304 y 305 del Estatuto General, ambos de la Universidad Veracruzana, reunidos los Cc. Gaudencio Gutiérrez Alba, Mónica Flores Muñoz, investigadores, Betzaida Salas García, en su carácter de Consejero-Investigador y María Gabriela Nachón García, Directora, todos miembros del Consejo Técnico del Instituto de Ciencias de la Salud de la Universidad Veracruzana, reunidos en el espacio que ocupa la Sala de Juntas de la citada entidad académica, con el objeto de tratar los asuntos mencionados en la convocatoria de fecha veintidos de mayo del dos mil diecinueve, suscrita por la Dra. María Gabriela Nachón García, Directora del ICS, y que para mayor conocimiento se transcriben a continuación los puntos a tratar:----------

1.- Lista de asistencia y declaración de quórum
2.- Lectura y aprobación de acta de la sesión anterior
3.- Analizar y aprobar en su caso, la petición de la Dra. Elisa Hortensia Tamariz Domínguez, para prolongar su estancia académica que actualmente está llevando a cabo en el Instituto de Ciencias Fotónicas de Barcelona, España. El periodo será del veintisiete de mayo al seis de junio del año en curso, para desarrollar el proyecto denominado: "Live-imaginig of cytoskeleton and cell adhesión proteins dynamics during optical quidance of fibroblasts and neurons", el cual será en colaboración con el ICFO, número de proyecto HRA0124-00, con vigencia del 01 de mayo al 31 de julio de 2019. Este proyecto fue aprobado por parte de la Unión Europea que consiste en financiar la estancia de un grupo de investigación por 15 días en alguno de los Institutos europeos de investigación que utiliza láseres. ----------
4.- Asuntos Generales

En el marco de lo anterior y con fundamento en el artículo 78 de la Ley antes citada, los miembros del Consejo Técnico hemos llegado a los siguientes:

ACUERDOS:

PRIMERO. Se contó con la asistencia de cuatro de los integrantes del H. Consejo Técnico.----------

SEGUNDO. Se aprueba acta de la sesión anterior. ----------

TERCERO. Se analiza la petición de la Dra. Elisa Hortensia Tamariz Domínguez, para prolongar su estancia académica que actualmente está llevando a cabo en el Instituto de Ciencias Fotónicas de Barcelona, España. El periodo será del veintisiete de mayo al seis de junio del año en curso, para desarrollar el proyecto denominado: "Live-imaginig of cytoskeleton and cell adhesión proteins dynamics during optical quidance of fibroblasts and neurons", el cual será en colaboración con el ICFO, número de proyecto HRA0124-00, con vigencia del primero de mayo

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al treinta y uno de julio de dos mil diecinueve. Este proyecto fue aprobado por parte de la Unión Europea, que consiste en financiar la estancia de un grupo de investigación por quince días en alguno de los Institutos europeos de investigación que utiliza láseres. También se considera que este proyecto está enfocado a continuar con los experimentos que venían realizando alumnos del Doctorado en Ciencias de la Salud, además, el obtener recursos para la adquisición de reactivos y así continuar con los experimentos. ESTE CUERPO COLEGIADO, ACUERDA OTORGAR EL AVAL CORRESPONDIENTE PARA QUE SE PROLONGUE LA ESTANCIA ACADÉMICA DE LA DRA. ELISA HORTENSIA TAMARIZ DOMINGUEZ, EN EL INSTITUTO DE CIENCIAS FOTÓNICAS DE BARCELONA ESPAÑA, DURANTE EL PERIODO DEL VEINTISIETE DE MAYO AL SEIS DE JUNIO DEL AÑO EN CURSO, CONSIDERANDO LA PERTINENCIA ACADÉMICA DEL CITADO PROYECTO, YA QUE FAVORECERÁ EL IMPULSO A LA INVESTIGACIÓN Y A LOS POSGRADOS, DE ACUERDO AL PLADEA DE NUESTRO INSTITUTO. ----------

CUARTO. No hubo asuntos generales que tratar. ----------

No habiendo nada más que agregar, se cierra la presente acta, siendo las once horas del mismo día de su fecha, firmando al margen y calce los que en ella intervenimos. ----

BETZAIDA SALAS GARCÍA
CONSEJERO-INVESTIGADOR

MÓNICA FLORES MUÑOZ
INVESTIGADOR

GAUDENCIO GUTIERREZ ALBA
INVESTIGADOR

UNIVERSIDAD VERACRUZANA
INSTITUTO DE CIENCIAS DE LA SALUD XALAPA, VER.

MARÍA GABRIELA NACHÓN GARCÍA,
DIRECTORA

Responder a todos | Eliminar | No deseado | Bloquear | ···

noticia y petición carta

ET

Elisa Tamariz <elisatammx@gmail.com>
Mié 22/05/2019 03:55 AM
Nachon Garcia Maria Gabriela

Buenos días Gaby

Espero todo vaya bien por allá. Te quiero comentar una buena noticia:
Me han aprobado un proyecto por parte de la Unión Europea que consiste en financiar la estancia de un grupo de investigación por 15 días en alguno de los institutos europeos de investigación que utiliza láseres (el programa se llama LaserLAb)., en este caso para el ICFO, en lugar donde me encuentro ahora.
El proyecto que sometimos esta enfocado a continuar con los experimentos que realizó Norma y que necesitamos concluir para completar su publicación, dentro de este proyecto esta incluida ella y mi colaborador el Dr. Remy, De manera que tendrán el financiamiento para poder regresar y realizar más experimentos.
Además a través de este proyecto el laboratorio del Dr. Pablo Loza, con quien estoy colaborando, puede recibir algo de dinero para comprarnos los reactivos que necesitamos.

Debido a esta aprobación puedo extender mi estancia por una semana más y completar el dinero que me hace falta para pagar los viáticos y hospedaje.
Por lo anterior te quiero pedir permiso para cambiar mi fecha de regreso al dia 07 de junio en vez del 30 de mayo como estaba previsto.
Si no hay inconveniente requiero de una carta de autorización de tu parte para hacer esta estancia.

Te anexo un borrado que he preparado conteniendo los puntos que me indican debe contener la carta. He incluido la versión en ingles y en español pues no estoy segura de en que idioma debe ser. Te pediría por favor si pudieras checarlas y si estas de acuerdo enviármelas membretadas y firmadas por ti.

Anexo a este correo la respuesta de aceptación, la copia de los requisitos que me piden en el cual me solicitan tu carta, y el proyecto que envie a LaserLab.

Muchos saludos y nuevamente gracias por todo tu apoyo
Elisa

Final decision for proposal ICFO002590

LASERLAB-ACCESS@laserlab-europe.eu<laserlab-access@laserlab-europe.eu>

sáb 11/05/2019 5:45

Para Tamaríz Domínguez Elisa Hortensia <etamariz@uv.mx>;

Dear Elisa Tamariz,

We wish to inform you that your proposal ICFO002590, Live-imaging of cytoskeleton and cell adhesion proteins dynamics during optical guidance of fibroblasts and neurons
has been accepted on the basis of scientific quality by the LASERLAB-EUROPE Selection Panel. The final comment of the Selection Panel is as follows:

The subject of this proposal is certainly interesting. In order to complete its goals the host team has to be helpful . Both referees recommend to asccept this proposal under the condition that the host agrees with the planned experiments and gives sufficient support. The referees write:

"The study of cellular protrusions during and after exposure in a laser tweezer or optical guidance system is an interesting scientific topic with some clinical relevance in neurology. The host institution has the required super-resolution light microscopy and nanoscopy facilities as well as competent laser scientists. What I am missing is some information about the biolab in Barelona, which should be quite specialized, e.g. in viral cell transfection. The researchers are part of an interdisciplinary (physical/biological) group and appear well qualified for this project. The experimental approach, procedure and method are appropriate, but the description could sometimes be more concrete. What I do not understand is that for the fluorescence measurements the excitation wavelength is larger than the detection wavelength. Possibility this is an error which could be easily explained by the applicants.

The proposal aims at live-imaging of cytoskeleton and cell adhesion protein dynamics in laser induced ? cell protrusions?, using so called laser-induced ?optical guidance? techniques. The proposal is somewhat confusingly argued: While the goals of the project are sufficiently stated and the suggested experimental approach appears to be applicable in this regard, the proposal is apparently hastily compiled. Redundant/unstructured information is provided, incl. the same Fig. twice. The main applicant, Dr. Eliza Tamariz apparently has had previous collaboration with the host lab, ICFO. The proposal may be successful if the ICFO team strongly agrees/is committed to carry out the project. A successful completion of the project, including publication of the results in a decent journal, may be likely only, if the host team is fully convinced and committed. Hence, this reviewer recommends funding of the proposal by Laserlab EUROPE only if the host team is fully convinced"
The proposal is accepted, but the applicants should be informed about the remarks of the referees.

The host institute will contact you shortly to clarify all necessary details regarding the implementation of the research project.

Please, be aware that, due to the continuous acceptance of proposals by our host institutes, occasional oversubscriptions may occur.
In these cases LASERLAB-EUROPE will make every effort to accommodate your research at another suitable host institute, in compliance with the Access Policy (see http://www.laserlab-europe.eu/transnational-access/how-to-apply-for-access/how-to-apply-for-access#access).
In addition, please note the information for users at http://www.laserlab-europe.eu/transnational-access/information-for-users

Sincerely,

Daniela Stozno

Coordinator's office
laserlab-access@laserlab-europe.eu

FULL PROPOSAL

Live-imaging of cytoskeleton and cell adhesion proteins dynamics during optical guidance of fibroblasts and neurons

Keywords
Optical tweezers, confocal microscopy, cells, cytoskeleton, adhesion

Abstract
Highly focused near-infrared (NIR) laser has been used to induce and attract cells protrusions in a technique called optical guidance (OG); however there are limited experimental evidences of the cellular events behind the laser effects and optimal stimulation protocols have not been reported. Our group has been studying OG in neurons (PC12 cells) and fibroblasts (3T3 cells), finding that orientation and advance of PC12 and 3T3 protrusions remain after the laser is turned off, but the observed increase in velocity stops when radiation ceases. We also find that cell leading edge status is determinant for laser effects on the velocity of protrusion. We are very interested in characterizing the role of actin cytoskeleton and cell-substrate adhesion proteins, as biological events involved in the cells response to NIR laser irradiation. We intend to use the expression of fluorescent proteins actin-GFP, tubulin-RFP and talin-RFP in 3T3 and PC12 cells, to study the *in vivo* dynamics of these proteins during OG. To perform such experiments we require a confocal microscopy adapted to an in vivo cell imaging setup, coupled to optical tweezers. These experiments will describe for the first time the role of some of the main proteins involved in cell motility and protrusion, and how the previous status of those proteins could influence the NIR laser effects over the cells.

Objective
To study the role of actin and tubulin cytoskeleton and the cell adhesion protein talin, in cell protrusion induced by highly focused near infrared laser, by performing in vivo time lapse experiments in 3T3 and PC12 cell lines.

Introduction
Highly focused NIR lasers induce an attraction of cell protrusions through a technique called optical guidance (OG). This has been described for fibroblasts[1-3] and neurons[4-10] using several laser regimes, wavelengths and powers. Although different mechanisms have been suggested to take part in the cellular response, limited experimental evidences have been reported. It has been suggested that optical gradient of the NIR radiation induces cytoskeleton polymerization at the site of stimulation, enhancing actin filaments formation and the regulation of filopodia and lamellipodia at the leading edge of the stimulated neurons[6,8]; however only fixed cell samples have been shown as partial evidences and no conclusive results have been obtained[5]. On the other hand, there is no evidence of the role of cell adhesion during NIR induced cell protrusion.
OG is an interesting approach as a non-invasive method to guide cell projection for clinical applications, as for example neuro-regenerative procedures. However, a more thorough study for deeper comprehension of the cellular mechanisms involved is needed.
During regular cell projection, plasma membrane protrusions are formed at the leading edge as lamellipodia and filopodia. The former are wider protrusions rich in actin filaments organized as a branched filaments network, while the latter are rich in parallel actin filaments[11]. In both cases complex interactions of actin filaments and cell adhesion proteins influence elongation or retraction of cells´ leading edge [12]. Retrograde actin filaments flow is fundamental for cell advance when it is coupled by cell-substrate contacts mediated by adhesion proteins that provide mechanical stabilization of cell protrusions, therefore supporting forward projection [13,14]. It has not been reported whether some of those events are enhanced or inhibited during OG. The variability and complexity of cell protrusion dynamics and the specialized infrastructure and equipment to perform such experiments make this goal a tough one.
We intend to use a confocal microscope coupled to optical tweezers for in vivo time lapse recording of fluorescent proteins under NIR laser stimulation, to observe the dynamics of cytoskeleton proteins as tubulin and actin, and the protein of cell adhesion talin. Our aim is to characterize if optical guidance has an impact on these proteins and what kind of modifications could be induced under laser

irradiation to produce the optical guidance effect. The comprehension of cytoskeleton and cell adhesion dynamics will help to understand the molecular condition under which cells under OG have a better performance. This is necessary to establish and improve the conditions for reaching in the future a possible clinical application for neuroregeneration.

This experimental approach using fibroblast and PC12 cells with OG has been part of a multidisciplinary project to understand the biological mechanisms behind OG, and has been performed by researchers in neurobiology (Elisa Tamariz from the University of Veracruz, Mexico), and physics (Remy Avila from the National Autonomous University of Mexico). This research was part of the Ph.D. thesis of Norma Medina-Villalobos who obtained her degree last September. There has been a previous fruitful collaboration with Dr. Pablo Loza-Álvarez coordinator of the Super-Resolution Light Microcopy & Nanoscopy facility (SLN) at Institute of Photonic Science in Barcelona Spain (ICFO) (see Avila et al 2018). To further analyze protein dynamics under OG we expect to use the facilities at ICFO such as the biolab and the SLN that provide the ideal conditions to perform the experiments and obtain relevant results.

Experimental methods

a) Cell culture and fluorescent protein expression

Cell line of fibroblasts (3T3) and neurons (PC12) will be cultured over coverslips previously covered with collagen type I. In the case of PC12 cells will be differentiated to neurons by adding neural growth factor (NGF).

Cells will be single or double infected with baculovirus vectors containing the sequence of cytoskeleton proteins actin and tubulin coupled to the green fluorescent protein (GFP) or red fluorescent protein (RFP), and the cell adhesion protein talin coupled to RFP.

b) Laser stimulation and fluorescent protein signal recording

Coverslips with infected cells will be mounted in a chamber with growth cell medium, and adapted to a Tokai Hit stage top incubator coupled to a Nikon Confocal C1-Si inverted microscope to control temperature at 37°C and 5% of CO_2. Actin-GFP and/or Tubulin-RFP or Talin-RFP proteins will be excited at 488 nm and 561 nm respectively and emitted fluorescence will be collected through 450/35 nm and 515/30 nm band passer emission filter respectively. For optical guidance a Ti:Sapphire (Ti:sap) laser, operating in CW mode at 810-nm wavelength positioned by using a pair of galvanometric mirrors, and entering to the microscope through the rear port will be use. The laser will be located about 5 µm away from the cell leading edge and will be displaced while the cells advance. In vivo time lapse recording of confocal optical sections and transmission images will be obtained every 30 seconds with or without NIR laser stimulation for several time periods. Transmission images and z sections will be processed by FIJI imageJ software to analyze the results.

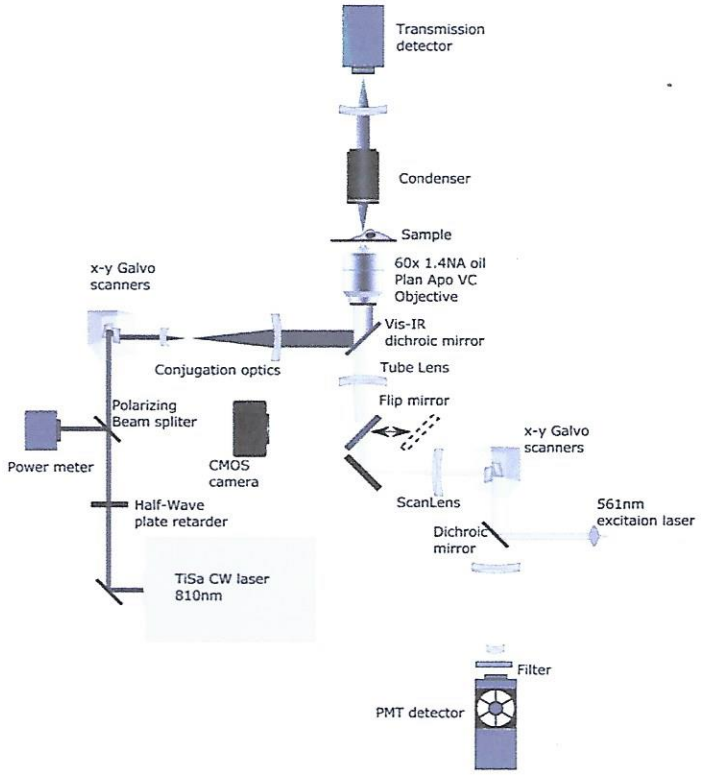
Working plan

Activity	Week 1	Week 2	Week 3	Week 4	Description
Culture of cell lines					Defrost 3T3 and PC12 cell and *in vitro* culture to obtain cells stocks to perform all the experiments
Viral vector titration for single and double expression of fluorescent proteins					Optimal number of viral particles per cell (ppc) will be stablish to obtain the suitable protein expression to observe fluorescent signal for confocal microscopy recording
Standardization of optical guidance protocol					NIR laser irradiation conditions and laser displacement will be set up. Control experiments without fluorescent proteins
Optical guidance plus confocal microscopy experiments					Record of fluorescent proteins dynamics under several optical guidance time schedules
Data analysis					Image processing and data analysis

Description of experimental arrangement

The instrument is based on a Nikon Eclipse Ti inverted microscope equipped with a C1si confocal system. The CW Ti:Sa laser is positioned using a galvanometric mirrors pair entering through the rear port. The NIR beam is imaged in the back focal plane of the objective by means of the conjugation optics and reflected by the short-pass (FF720-SDi01-25x36) dichroic mirror. The power of the NIR laser is controlled by measuring the power reflected by the power meter after a polarizing BS cube when rotating a half-wave retarder. Transmission images can be acquired either using the transmission detector when using the confocal unit or using a CMOS camera (DCC1545M-Thorlabs) when illuminating with the standard halogen lamp. During the experiments the sample is mounted inside a chamber with temperature held at 37 °C using a Tokai Hit stage top incubator.

Sketch of experimental setup



Time schedule including expected duration of access

Time Schedule for biolab

Activity	hr	Total
Culture of cell lines A viral vector expression	4hr/3 days a week/4 weeks	48 hr

Time Schedule for super-resolution light microcopy & nanoscope facility (SLN)

Activity	hr/day/week	Total hr
Standardization of optical guidance protocol	4hr/3 days /1 week	12 hr
Optical guidance plus confocal microscopy experiments	6hr/3days/3 Weeks	54 hr

Estimated difficulty of the experiments

Moderate to high

Expertise of the group

The multidisciplinary group working in the project of optical guidance of cell projections is formed by researchers of the biomedical and optics field, with expertise in neurobiology and biophotonics. We have been investigating for several years the biological mechanisms behind optical guidance, as an approach to better understand the phenomena to stablish and improve the conditions for reach in the future a possible clinical application. The group is formed by:

1) Dr. Elisa Tamariz, Ph.D. in Cell Biology, senior researcher at Biomedical Department of Health Science Institute of University of Veracruz, México, head of the Axonal Projection and Neuroregeneration laboratory. Dr. Tamariz is an expert in *in vitro* culture of neurons and fibroblasts, expression of fluorescent proteins, confocal microscopy, in vivo time lapse recording of cells, cell adhesion and cytoskeleton dynamics, and imagen analysis.
2) Dr. Remy Avila , Ph. D. in Physics, senior researcher at the Center of Applied Physics and Advanced Technology, at National University of México, expert in optics, biophotonics, optical tweezers, optical guidance, fluorescent microscopy and confocal microscopy.
3) Dr. Norma Medina-Villalobos recently obtained her Ph.D. in Heath Science (September 2018), under the supervision of Drs. Elisa Tamariz-Domínguez and Remy Avila, with a thesis about biological mechanisms of optical guidance. Expert in cell culture, optical guidance and statistical analysis of data.

It is worth mentioning that this group is already familiar with ICFO, SLN infrastructure and Dr. Pablo Loza´s group from a previous collaboration that lead to a publication in a prestigious journal (Avila et al 2018).

References

1. Albrecht-Buehler, G. Surface extensions of 3T3 cells towards distant infrared light sources. *J Cell Biol* **114**, 493-502 (1991).
2. Albrecht-Buehler, G. A long-range attraction between aggregating 3T3 cells mediated by near-infrared light scattering. *Proc Natl Acad Sci U S A* **102**, 5050-5055 (2005).
3. Avila, R., *et al.* Optical tweezers experiments for fibroblast cell growth stimulation. *Proc. of SPIE* **9129**, 91291U-91291-91297 (2014).
4. Carnegie, D.J., Cizmar, T., Baumgartl, J., Gunn-Moore, F.J. & Dholakia, K. Automated laser guidance of neuronal growth cones using a spatial light modulator. *J Biophotonics* **2**, 682-692 (2009).
5. Carnegie, D.J., Stevenson, D.J., Mazilu, M., Gunn-Moore, F. & Dholakia, K. Guided neuronal growth using optical line traps. *Opt Express* **16**, 10507-10517 (2008).
6. Ehrlicher, A., *et al.* Guiding neuronal growth with light. *Proc Natl Acad Sci U S A* **99**, 16024-16028 (2002).
7. Graves, C.E., McAllister, R.G., Rosoff, W.J. & Urbach, J.S. Optical neuronal guidance in three-dimensional matrices. *J Neurosci Methods* **179**, 278-283 (2009).
8. Mohanty, S.K., Sharma, M., Panicker, M.M. & Gupta, P.K. Controlled induction, enhancement, and guidance of neuronal growth cones by use of line optical tweezers. *Opt Lett* **30**, 2596-2598 (2005).
9. Stevenson, D.J., *et al.* Optically guided neuronal growth at near infrared wavelengths. *Opt Express* **14**, 9786-9793 (2006).
10. Mathew, M., *et al.* Signalling effect of NIR pulsed lasers on axonal growth. *J Neurosci Methods* **186**, 196-201 (2010).
11. Ridley, A.J. Life at the leading edge. *Cell* **145**, 1012-1022 (2011).
12. Giannone, G., *et al.* Periodic lamellipodial contractions correlate with rearward actin waves. *Cell* **116**, 431-443 (2004).
13. Galbraith, C.G., Yamada, K.M. & Sheetz, M.P. The relationship between force and focal complex development. *J Cell Biol* **159**, 695-705 (2002).
14. Gallo, G., Yee, H.F., Jr. & Letourneau, P.C. Actin turnover is required to prevent axon retraction driven by endogenous actomyosin contractility. *J Cell Biol* **158**, 1219-1228 (2002).
15. Avila R, Tamariz E, Medina-Villalobos N, Andilla J, Marsal M, Loza-Alvarez P. Effects of near infrared focused laser on the fluorescence of labelled cell membrane. *Sci Rep*. **8**, 17674 (2018)

LASERLAB EUROPE: Application for experimental time

Description of the project (to be provided in pdf format)
Please note that for CLF, FELIX, FERMI, LULI and PALS specific forms should be used for this part!

Please make sure that in your proposal

- the main goals and novel scientific aspects of the planned work become clear,
- the choice of host infrastructure is properly motivated (in Part 3),
- key references are included (in Part 4 if they are from the participating scientists).

PART 2: Detailed scientific description of the project

List the main objectives of the proposed research *(half a page maximum)*:

To study the role of actin and tubulin cytoskeleton and the cell adhesion protein talin in cell protrusion induced by highly focused near infrared laser by performing in vivo time lapse experiments in 3T3 and PC12 cell lines

Give a brief description of the scientific background and rationale of your project *(half a page maximum)*:

Highly focused NIR lasers induce an attract cell protrusions through a technique called optical guidance (OG). This has been described for fibroblasts and neurons using several laser regimes, wavelengths and powers. Although different mechanisms have been suggested to take part in the cellular response, limited experimental evidences have been reported. It has been suggested that optical gradient of the NIR radiation induces cytoskeleton polymerization at the site of stimulation, enhancing actin filaments formation and the regulation of filopodia and lamellipodia at the leading edge of the stimulated neurons; however only fixed cell samples has been shown as partial evidences and no conclusive results have been obtained. In the other hand there is no evidence of the role of cell adhesion during NIR induced cell protrusion.
OG is an interesting approach as a non- invasive method to guide cell projection for clinical applications, as for example neuro-regenerative procedures; however, a more thorough study for deeper comprehension of the cellular mechanisms involved is needed. During regular cell projection, plasma membrane protrusions are formed at the leading edge as lamellipodia and filopodia. The former are rich in actin filaments organized as a branched filaments network, while the last are wider protrusions with parallel actin filaments. In both cases complex interactions of actin filaments and cell adhesion proteins influence elongation or retraction of cells´ leading edge. Retrograde actin filaments flow is fundamental for cell advance when it is coupled by cell-substrate contacts mediated by adhesion proteins that provide mechanical stabilization of cell protrusions, therefore supporting forward projection. If some of this events are enhanced or inhibited during OG has not been reported yet, the variability and complexity of cell protrusion dynamics, and the specialized infrastructure and equipment to perform such experiments make this goal a though one.
We intend to use a confocal microscope coupled to optical tweezers for in vivo time lapse of fluorescent proteins under NIR laser stimulation. The comprehension of cytoskeleton and cell adhesion dynamics will help to understand the molecular condition under which cells with OG have a better performance; as an approach to understand the phenomena, and to stablish and improve the conditions for reach in the future a possible clinical application for neuroregeneration.
The multidisciplinary group working in the project of optical guidance of cell projections is formed by researchers of the biomedical and optics field, with expertise in neurobiology and biophotonics. We are recently beginning to explore biological mechanisms behind optical guidance,
The equipment and facilities such as biolab and the laser lab present at the Institute of Photonic Science in Barcelona Spain, besides the previous fruitfull collaboration with Dr. Pablo Loza, coordinator of the Super-Resolution Light Microcopy & Nanoscope facility, certainly will be the better option to perform experiments and obtain relevant results.

Present the proposed experimental method and working plan *(half a page maximum)*:

Experimental methods

Cell culture and fluorescent protein expression
Cell lines of fibroblasts (3T3) and neurons (PC12) will be cultured over coverslips previously covered with

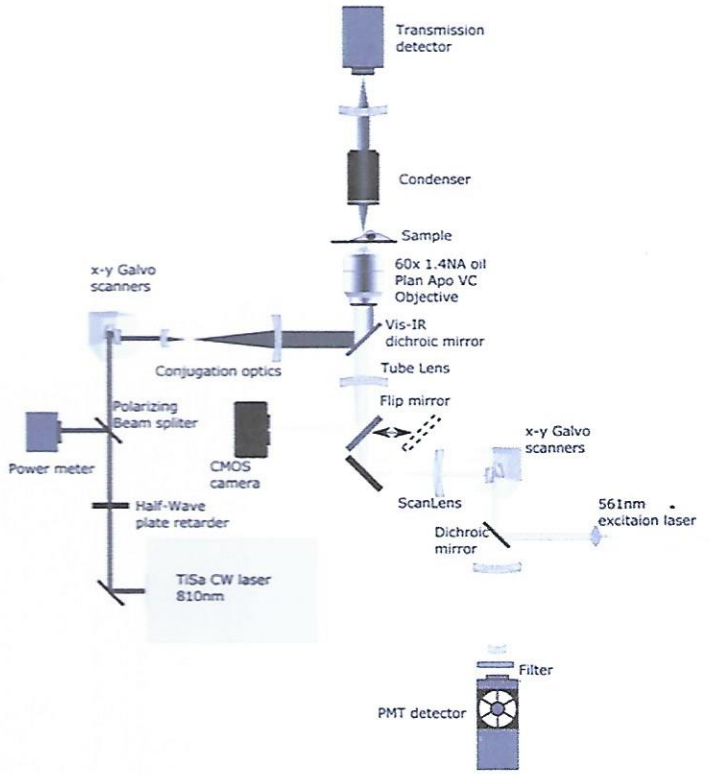
collagen type I. In the case of PC12 cells will be differentiate to neurons by adding neural growth factor (NGF). Cells will be single or double infected with baculovirus vectors containing the sequence of cytoskeleton protein actin or talin coupled to the fluorescent proteins green or red fluorescent protein (Actin-GFP, Tubulin-RFP), or the cell adhesion protein talin coupled to red fluorescent protein (Talin-RFP).

Laser stimulation and fluorescent protein signal recording
Coverslips with infected cells will be mounted in a chamber with growth cell medium, and adapted to a stage top incubator coupled to a Confocal inverted microscope to control temperature at 37°C and 5% of CO_2. Actin-GFP and Tubulin-RFP and or Talin-RFP proteins will be excited at 488 nm and 561 nm respectively and emitted fluorescence will be collected through 450/35 nm and 515/30 nm band passer emission filter respectively. For optical guidance a Ti:Sapphire (Ti:sap) laser, operating in CW mode at 810-nm wavelength positioned by using a pair of galvanometric mirrors, and entering to the microscope through the rear port will be use. The laser will be located about 5μm of the cell leading edge and will be displaced while the cells advance. In vivo time lapse recording of confocal optical sections and transmission images will be obtained every 30 seconds with our without NIR laser stimulation for several time periods. Transmission images and z sections will be processed by FIJI imageJ software to analyze the results.

Working Plan:
Week 1- Cultures of 3T3 and PC12 cells to obtain cells stocks to perform all the experiments
-Viral vector titration for single and double expression of fluorescent proteins
Week2- Standardization of optical guidance protocol
Week3- Optical guidance plus confocal microscopy experiments
Week4- Optical guidance plus confocal microscopy experiments, data analysis

Give a brief description of the experimental arrangement including main optics and diagnostics required *(half a page maximum)*:
The instrument is based on a Nikon Eclipse Ti inverted microscope equipped with a C1si confocal system. The CW Ti:Sa laser is positioned using a galvanometric mirrors pair entering through the rear port. The NIR beam is imaged in the back focal plane of the objective by means of the conjugation optics and reflected by the short-pass (FF720-SDi01-25x36) dichroic mirror. The power of the NIR laser is controlled by measuring the power reflected by the power meter after a polarizing BS cube when rotating a half-wave retarder. Transmission images can be acquired either using the transmission detector when using the confocal unit or using a CMOS camera (DCC1545M-Thorlabs) when illuminating with the standard halogen lamp. During the experiments the sample is mounted inside a chamber with temperature held at 37 °C using a Tokai Hit stage top incubator.

Please provide a sketch of the experimental set-up.



Indicate the proposed time schedule including expected duration of access time *(half a page maximum)*:

Time Schedule for biolab

Activity	hr	Total
Culture of cell lines A viral vector expression	4hr/3 days a week/4 weeks	48hr

Time Schedule for super-resolution light microcopy & nanoscope facility (SLN)

Activity	hr/day/week	Total hr
Standardization of optical guidance protocol	4hr/ 3 days /1 week	12hr
Optical guidance plus confocal microscopy experiments	6hr/3days/3 Weeks	54hr

Please estimate the difficulty of the experiment (high, medium, moderate).
High

Host infrastructure	
Indicate your preferred LASERLAB-EUROPE host infrastructure:	The Super resolution light microscopy and nanoscopy (SLN) facility at ICFO
Explain briefly why your project will be best carried out at this specific host infrastructure: The SLN facility have an inverted confocal microscopy with stage top incubator coupled to optical tweezers, in the same place there is a biolab with all the equipment to perform cell culture.	
Please indicate the name of the local correspondent, if known:	Pablo Loza-Alvarez
If possible, list other LASERLAB-EUROPE facility(ies) where your experiment could alternatively be carried out:	

Additional information	
Have you already submitted an access proposal to any of the participating facilities under this or previous EU programmes?	No
If yes, please indicate the name of the institution, submission date and reference number for each such proposal:	
Is this a resubmission of a previous proposal (see guideline#1)?	No
If yes, please give the exact reference number and submission date. Please describe briefly the changes made in comparison to the rejected version.	
Is this proposal intended to complete an earlier project funded under the EU access programme at the same facility (see guideline#2)?	No
If yes, please give the exact reference number and submission date. Please indicate also what has been achieved in the previous experiment and the reasons why the objectives have not been fully obtained.	
Is this project part of a Ph.D. thesis?	Yes
If yes, please give the name of the Ph.D. student and the supervisor. Norma Medina-Villalobos Ph. D.(obtained her degree in September 2018 with part of this project, we intended to perform the experiments at SLN to complete a research paper about her thesis) Elisa Tamariz supervisor Remy Avila supervisor	
Has the subject of your planned experiments already been published by other groups?	No
If yes: In what aspect is your planned research different from that of the other groups? What is the goal of your project and why is it important?	

Guidelines

1. Select "yes" if this application is a revised version of a proposal submitted to LASERLAB-EUROPE before and rejected by the selection panel.
2. Select "yes" if this project would be a continuation of a project with identical objectives already carried out at the same infrastructure.

PART 3: Technical information
When possible, please specify your requests regarding the laser characteristics (wavelengths, pulse energy, power, line width, pulse length, repetition rate, focusing optics, etc.), auxiliary equipment and diagnostics to be provided or any other specific requirements (including specific support such as target lab, scientific computing, etc.): Ti:Sapphire (Ti:sap) laser, operating in CW mode at 810-nm wavelength positioned by using a pair of galvanometric mirrors, and entering to an inverted confocal microscope through the rear port, coupled to a stage top incubator. The power at the exit of the microscope objectives ranges between 40-60 mW.
List all samples and chemicals to be brought to the LASERLAB-EUROPE facility: Fibroblast 3T3 cell Neurons PC12 cells DMEM Fetal Bovine Serum Penicillin/streptomycin Phosphate Buffered Saline Tripsin/EDTA Collagen Type I Cell Ligh vectors: -Actin-GFP -Talin-RFP -Tubulin-RFP o GFP

Information on any safety issues concerning the experiment. Please tick the appropriate boxes and give detailed information for each potential risk present during the experiment (except for laser risk). ☐ Chemical risk: ☐x Biological risk: ☐ Radiological risk: ☐ Other risk(s):	
Does your proposal involve ethical issues, e.g. biological samples, genetic information, dual use (civilian and military or terrorist)? If yes, the proposal and the potentially necessary ethical approvals have to be discussed with the host lab prior to starting the experiments.	No

PART 4: Additional information about the applicant's (and group's) expertise

Expertise of the group in the domain of the application (including theoretical support):

Elisa Tamariz-Domínguez: Ph.D. in Cell Biology, neurobiologist, expert in *in vitro* culture of neurons and fibroblasts, expression of fluorescent proteins, *in vivo* time lapse recording under confocal microscopy in 2D and 3D culture systems, analysis of cell adhesion and cell protrusion dynamics. Research experience in axon guidance, molecular mechanisms of axon projection, neuroregeneration and biophotonics.

Remy Avila-Foucat: Ph.D. in Physic, expert in optics, advanced microscopy, optical tweezers, optical guidance, fluorescent microscopy, confocal microscopy. Research experience in biophotonics and optical guidance.

Norma Medina-Villalobos, Ph.D in Health Science, expert in optical guidance, cell culture, image analysis, confocal microscopy, statistical analysis. Research experience in optical guidance and biophotonics.

The group is familiar with the SLN infrastructure and Dr. Pablo Loza´s group from a previous collaboration that lead to very interesting results and a publication in a prestigious journal

Short CV of the applicant:

Elisa Tamariz-Domínguez
Author name: Elisa Tamariz
2000 PhD. Cell Biology, Center of Research and Advanced Studies (CINVESTAV), Mexico.
1996 MSc. Cell Biology, Center of Research and Advanced Studies (CINVESTAV), México.
1994 Bachelor in Biology, Universidad Nacional Autónoma de México.
Professional Experience
2011- Senior Researcher, Department of Biomedicine, Health Science Institute, Veracruzana University. Head of the Axonal Projection and Neuroregeneration Laboratory. México
2005-2010 Associated Researcher, Neurobiology Institute, National University of Mexico (UNAM)
2004-2005 Postdoctoral Researcher, Neurobiology Institute, National University of México (UNAM)
2001-2002 Postdoctoral Researcher, Cell Biology Department, Southwestern Medical Center, University of Texas, EUA.
Grants and Awards
2005- National Researcher Level I distinction, National Council of Science and Technology (CONACYT), National System of Researchers (SNI).
2007 First Place to best MSc Project presented by the student under my supervision Claudia García-Peña Acosta. International Cajal Club Annual Meeting
2008 First Place to best PhD Project. Project presented by the student under my supervision Claudia García-Peña Acosta. XV Meeting of the Neurobiology Institute, National University of Mexico, (UNAM)
2008-Young Researcher Grant. National Council of Science and Technology (CONACYT)
2012 First Place to best MSc. Project presented by student under my supervision Ariadna Ríos, XIX Meeting of Neurobiology Institute, National University of Mexico (UNAM)
2012 Full Time Professor Grant, Public Education Council (SEP)
2014 Outstanding Professor and Mentoring Performance. Universidad Veracruzana and Public Education Council (SEP)
2015 First Place, Young Talent Award, State Science and Technology Council, to Joaquin A. Rodriguez student under my supervision with the project: Optical guidance of cell projection

2018 "Arte Ciencia y Luz Award" to best MSc. thesis at Universidad Veracruzana, to the student under my supervision Ana Vela-Alcántara

Research Interests

Mechanism of Axonal Projection and Neuroregeneration
Biological mechanisms in optical guidance of cells
Stem Cells differentiation and mechanotransduction

Scientific Society Member

Society for Neuroscience
Mexican Society for Developmental Biology
Mexican Biophotonic Network

Remy Fernand Avila Foucat
Author name: Remy Avila

Education

1998 PhD. Physics, Université de Nice Sophia-Antipolis, France.
1994 MSc. Images in Universe Sciences, Université de Nice Sophia-Antipolis, France.
1993 Bachelor in Physics, Universidad Nacional Autónoma de México, Mexico.

Professional Experience

2010- Senior Researcher, Centre for Applied Physics and Advanced Technology, Universidad Nacional Autónoma de México, México
2017-2018 Sabbatical stage at Instituto de Ciencias Fotónicas (ICFO), Barcelona, Spain.
2003-2010 Senior Researcher, Centre for Radioastronomy and Astrophysics, Universidad Nacional Autónoma de México, México
1999-2003 Associate Researcher, Centre for Radioastronomy and Astrophysics, Universidad Nacional Autónoma de México, México
2014 - 2017 Coordinator of the Bachelor´s program in Technology, Universidad Nacional Autónoma de México, México
2018 - Coordinator of Graduate Studies at Centre for Applied Physics and Advanced Technology, Universidad Nacional Autónoma de México, México

Grants and Awards

2005 - National Researcher Level II distinction, Council of Science and Technology (CONACYT), National System of Researchers (SNI).
1999-2005 National Researcher Level I distinction, Council of Science and Technology (CONACYT), National System of Researchers (SNI).
2004 National University Award for Young Academicians in the field of Technological Innovation.
2000 Allen prize of the Optical Society of America for the best thesis in remote sensing.

Research Interests

Biophotonics
Optical Tweezers
Advances Microscopy
Optical Turbulence

Scientific Society Member

Optical Society of America
Mexican Optical Academy

Principal Journals reviewer

Monthly Notices of the Royal Astronomical Society
Optics express
Optics letters
Journal of Optics

Norma Lyssette Medina Villalobos

Education

2018 PhD. Health Science. Health Science Institute, Veracruzana University. Mexico
2014 MSc. Science and Technology. Guadalajara University. Mexico

Professional Experience

2009- Laboratory technician. Department of Integral Institutional Formation (DFII). Cell culture Laborator Instituto Politécnico Nacional (IPN).
2011-2013 Teacher for the Propaedeutic course. Instituto Politécnico Nacional (IPN). León Guanajuato, México.
2016 Teacher National University of Mexico (UNAM), Querétaro México
2017 Teacher Polytechnic of Valle of Mexico University. Estado de México, México.
2019 Teacher Iberoamerican University (Ibero-Leon). León Guanajuato, México.

Research Interests

Analysis of images.
Statistical evaluation of the cellular projection
Mechanism of Axonal Projection and Neuroregeneration
Biological mechanisms in optical guidance of cells
Adhesion proteins involved in the cell protrusion.
Scientific Society Member
Mexican Biophotonic Network

A list of 5 recent, relevant publications of the participating scientists in the field of the project:

Elisa Tamariz-Domínguez:

-Tamariz E., Grinnell F. *Modulation of fibroblast morphology and adhesion during collagen matrix remodeling.* Mol. Biol Cell (2002) 13:3915-3929.
-Tamariz E, Díaz-Martínez N. E, Díaz N. F, García-Peña C. M, Velasco I, Varela-Echavarría A. *Axon responses of stem cell-derived dopaminergic neurons to semaphorin 3A and 3C.* J. Neuroscience Res. (2010) 88:971-980.
-Díaz-Martínez NE, Tamariz E, Díaz NF, García-Peña CM, Varela-Echavarría A, Velasco I. *Recovery From Experimental Parkinsonism by Semaphorin-guided Axonal Growth of Grafted Dopamine Neurons.* Mol Ther. (2013) Jun 4. doi: 10.1038/mt.2013.78.
-Tamariz E., Varela-Echavarría A. *The discovery of the growth cone and its influence on the study of axon guidance.* Front in Neuroanat (2015) 9:1-9.
-Avila R, Tamariz E, Medina-Villalobos N, Andilla J, Marsal M, Loza-Alvarez P. *Effects of near infrared focused laser on the fluorescence of labelled cell membrane.* Sci Rep. (2018) Dec 5;8(1):17674. doi: 10.1038/s41598-018-36010-1.

Remy Ávila-Foucat

-Avila, R., Tamariz, E., Medina-Villalobos, N., Andilla, J., Marsal, M. and Loza-Alvarez, P., *Effects of near infrared focused laser on the fluorescence of labelled cell membrane.* Sci. Rep. 8(1), 17674 (2018). https://doi.org/10.1038/s41598-018-36010-1
-Remy Avila, Norma Medina-Villalobos, Elisa Tamariz, Roger Chiu, Luz María Lopez-Marín, Angélica Acosta, and Victor Castaño. *Optical tweezers experiments for fibroblast cell growth stimulation.* Proc. SPIE 9129, Biophotonics: Photonic Solutions for Better Health Care IV, 91291U (8 May 2014); https://doi.org/10.1117/12.2064939.
-R. Avila, O. Rodríguez-Herrera, A. González-Suárez, and J. Ascencio-Rodríguez. *Optical chaining of tens of silica beads with single trap.* in Optics in the Life Sciences, OSA Technical Digest (online) (Optical Society of America, 2015), paper OtM2E.3. https://doi.org/10.1364/OTA.2015.OtM2E.3.
-Remy Avila, Joaquín Ascencio-Rodríguez, Daniel Tapia-Merino, Oscar G. Rodríguez-Herrera, and Arturo González-Suárez. *Optical concatenation of a large number of beads with a single-beam optical tweezer.* Opt. Lett. 42, 1393-1396 (2017) https://doi.org/10.1364/OL.42.001393.
-Remy Avila, Elisa Tamariz, Norma Medina-Villalobos, Jordi Andilla, Maria Marsal, and Pablo Loza-Alvarez. *Cell membrane molecular dynamics under a NIR focused laser.* Proc. SPIE 10876, Optical Interactions with Tissue and Cells XXX, 108760L (1 March 2019); doi: 10.1117/12.2507904; https://doi.org/10.1117/12.2507904

Norma Medina-Villalobos

- Avila R., Medina-Villalobos N, Tamariz E. Chiu R., Lopez-Marín L., Acosta A., Castaño V. *Optic tweezers experiments for fibroblast cell growth stimulation.* Proc. SPIE 9129, Biophotonics: Photon Solutions for Better Health Care IV, 91291U (8 May 2014); doi: 10.1117/12.2064939
- Avila R, Tamariz E, Medina-Villalobos N, Andilla J, Marsal M, Loza-Alvarez P. *Effects of near infrared focused laser on the fluorescence of labelled cell membrane.* Sci Rep. (2018) Dec 5;8(1):17674. doi: 10.1038/s41598-018-36010-1.
-Avila R, Tamariz E, Medina-Villalobos N, Andilla J, Marsal M, and Loza-Alvarez P. *Cell membrane molecular dynamics under a NIR focused laser.* Proc. SPIE 10876, Optical Interactions with Tissue and Cells XXX, 108760L (1 March 2019); doi: 10.1117/12.2507904; https://doi.org/10.1117/12.2507904

Instituto de Ciencias de la Salud

Universidad Veracruzana

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Calle 21 de agosto No. 9 Bis, esq. Calle Ruiz Cortínez
Col. Hidalgo, Xalapa, Ver.

Teléfono
(228) 3 52 46 08,

Xalapa, Veracruz a 22 de mayo del 2019

A quien corresponda

Por medio de la presente hago constar que la Dra. Elisa Tamariz Domínguez está adscrita al Instituto de Ciencias de la Salud de la Universidad Veracruzana, como Investigadora Titular de tiempo completo con contrato permanente. Cuenta con seguro de gastos médicos proporcionado por la Universidad, además de un seguro internacional de viaje adquirido por la investigadora.

La Dra. Tamariz cuenta con la autorización para realizar una estancia en el Instituto de Ciencias Fotónicas del Barcelona (ICFO), del 27 de mayo al 06 de junio, para llevar a cabo el proyecto denominado ¨Live-imaginig of cytoskeleton and cell adhesión proteins dynamics during optical guidance of fibroblasts and neurons¨ proyecto conjunto que se lleva a cabo en colaboración con el ICFO, número de proyecto HRA0124-00, con vigencia del 01 de mayo al 31 de julio de 2019, y que se llevará a cabo en colaboración con el Dr. Pablo Loza-Alvarez

Sin más por el momento quedo a sus apreciables órdenes, saludos cordiales.

UNIVERSIDAD VERACRUZANA
INSTITUTO DE CIENCIAS DE LA SALUD XALAPA, VER.

ATENTAMENTE

Dra. María Gabriela Nachón García

Directora

75 ANIVERSARIO Universidad Veracruzana 1944-2019

Instituto de Ciencias de la Salud

Universidad Veracruzana

Instituto de Ciencias de la Salud

May 22, 2019. Xalapa, Veracruz.

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(228) 3 52 46 08,

To whom it might concern

I hereby certify that Ph.D. Elisa Tamariz Domínguez has a tenure track position as a Principal Researcher at Instituto de Ciencias de la Salud de la Universidad Veracruzana. She has health insurance from the Universidad Veracruzana and also individual international Travel insurance.

Dr. Tamariz has the authorization to complete a research stay at the Institute of Fotonique Sciences (ICFO) from May 27 to June 07 of 2019, to work in the collaboration project entitled. ¨Live-imaging of cytoskeleton and cell adhesion proteins dynamics during optical guidance of fibroblasts and neurons¨ project number HRA0124-00, from May 1 to July 31 of 2019, at Dr. Pablo Loza-Alvarez research group.

I remain to your appreciable orders, cordial greetings.

UNIVERSIDAD VERACRUZANA
INSTITUTO DE CIENCIAS DE LA SALUD
XALAPA, VER

Sincerely,

Dra. María Gabriela Nachón García

Director

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