



Universiteit
Leiden

The Netherlands

Single-molecule microscopy in zebrafish embryos

Góra, R.J.

Citation

Góra, R. J. (2022, November 23). *Single-molecule microscopy in zebrafish embryos*. Retrieved from <https://hdl.handle.net/1887/3487015>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/3487015>

Note: To cite this publication please use the final published version (if applicable).

**SINGLE-MOLECULE MICROSCOPY IN
ZEBRAFISH EMBRYOS**

RADOSŁAW JAKUB GÓRA

Single-Molecule Microscopy in Zebrafish Embryos

Cover and layout by **Radosław Jakub Góra**

ISBN: 978-94-6423-906-5

This publication was funded by the Marie Skłodowska-Curie Interactive Training Network (ITN) *ImageInLife* (Grant Agreement 721537)

Figures on pages 22, 25, 27 and 168 created with BioRender.com

Cover image: an artistic representation of trajectories of single molecules diffusing inside a multicellular organism

Copyright © 2022 by Radosław Jakub Góra. All rights reserved. No parts of this book may be reproduced, stored in retrieval system, or transmitted in any form or by any means without prior permission of the author

SINGLE-MOLECULE MICROSCOPY IN ZEBRAFISH EMBRYOS

Proefschrift

ter verkrijging van

de graad van doctor aan de Universiteit Leiden,
op gezag van rector magnificus prof. dr. ir. H. Bijl,
volgens besluit van het college voor promoties
te verdedigen op woensdag 23 november 2022

klokke 15.00 uur

door

RADOSŁAW JAKUB GÓRA

geboren te Wrocław, Polen

in 1991

Promotores:

Dr. M.J.M. Schaaf

Prof. dr. A.H. Meijer

Promotiecommissie:

Prof. dr. G. van Wezel

Prof. dr. A. Briegel

Prof. Dr. A. Cambi (Radboud University Medical Center, Nijmegen)

Dr. P. Loza-Alvarez (The Institute of Photonic Sciences, Castelldefels)

Dr. J.J. Willemse

Keep the ship outside the spray and surge

Homer, the Odyssey

To everyone who joined me on the ship

TABLE OF CONTENTS

1. INTRODUCTION.....	13
1.1. THE ORIGINS OF SINGLE-MOLECULE STUDIES.....	13
1.2. FLUORESCENCE AND FLUOROPHORES USED IN SMM	14
1.3. THE DEVELOPMENT OF SINGLE-MOLECULE MICROSCOPY (SMM)	16
1.4. MICROSCOPY SETUPS USED FOR SMM.....	19
1.5. SMM TO STUDY PROTEIN DYNAMICS <i>IN VIVO</i>	20
1.6. ANALYSIS OF THE DATA OBTAINED THROUGH SMM IMAGING	22
1.7. H-RAS AND THE GLUCOCORTICOID RECEPTOR (GR): MODEL PROTEINS FOR SINGLE-MOLECULE STUDIES IN THE MEMBRANE AND THE NUCLEUS.....	24
1.8. OUTLINE OF THE DOCTORAL THESIS	26
REFERENCES.....	30
2. ANALYSIS OF THE H-RAS MOBILITY PATTERN IN VIVO SHOWS CELLULAR HETEROGENEITY INSIDE EPIDERMAL TISSUE	37
ABSTRACT	37
2.1. INTRODUCTION	38
2.2. RESULTS.....	41
<i>The mobility pattern of YFP-C10H-Ras and YFP-H-Ras in HEK293T cells.</i>	41
<i>The mobility pattern of YFP-C10H-Ras and YFP-H-Ras in epidermal cells of zebrafish embryos</i>	44
<i>The mobility pattern of YFP-C10H-Ras in zebrafish embryos at different developmental stages.....</i>	47
<i>The mobility pattern of YFP-H-Ras^{V12} and YFP-H-Ras^{N17} in epidermal cells of zebrafish embryos</i>	48
<i>The mobility pattern of YFP-H-Ras and YFP-H-Ras^{V12} in epidermal cells of zebrafish embryos after treatment with Latrunculin B and Methyl-β-cyclodextrin</i>	49
<i>The sources of variability of the results.....</i>	52
2.3. DISCUSSION	55
2.4. MATERIALS AND METHODS.....	60
<i>Zebrafish.....</i>	60
<i>Cell cultures, transfection, and fixation.....</i>	60
<i>Microinjection of DNA in zebrafish embryos.....</i>	61

<i>Treatment of zebrafish embryos with Latrunculin B (LatB) and Methyl-β-cyclodextrin (MBCD)</i>	61
<i>Fluorescence stereomicroscopy</i>	62
<i>Confocal laser-scanning microscopy</i>	62
<i>Total internal reflection fluorescence microscopy (TIRFM)</i>	62
<i>Analysis of protein diffusion patterns</i>	63
<i>Experimental design</i>	65
<i>Statistical Analysis</i>	65
REFERENCES.....	67
SUPPLEMENTARY FIGURE.....	73

3. MULTIFOCAL TWO-PHOTON EXCITATION FLUORESCENCE MICROSCOPY REVEALS HOP DIFFUSION OF H-RAS MEMBRANE ANCHORS IN EPIDERMAL CELLS OF ZEBRAFISH EMBRYOS75

ABSTRACT.....	75
3.1. INTRODUCTION.....	76
3.2. RESULTS.....	79
<i>Imaging of GFP-C10H-Ras molecules in epidermal cells of zebrafish embryos using 2PEFM</i>	79
<i>Analysis of the mobility pattern of GFP-C10H-Ras molecules in epidermal cells of zebrafish embryos using 2PEFM</i>	84
<i>Comparison of the GFP-C10H-Ras dynamics between 2PEFM and TIRFM with different temporal resolutions</i>	86
<i>Analysis of the excitation laser power impact on the GFP-C10H-Ras mobility pattern</i>	90
<i>The mobility pattern of GFP-C10H-Ras in epidermal cells of zebrafish embryos after treatment with Latrunculin B and Methyl-β-cyclodextrin</i>	92
<i>Analysis of the single GFP-C10H-Ras trajectories based on the 2PEFM imaging</i>	95
3.3. DISCUSSION.....	99
3.4. MATERIALS AND METHODS.....	104
<i>Zebrafish</i>	104
<i>Treatment of zebrafish embryos with Latrunculin B (LatB) and Methyl-β-cyclodextrin (MBCD)</i>	104
<i>Sample preparation and mounting</i>	105
<i>Two-Photon Excitation Fluorescence Microscopy (2PEFM)</i>	105
<i>Total internal reflection fluorescence microscopy (TIRFM)</i>	106

<i>Analysis of protein diffusion patterns</i>	106
<i>Analysis of photobleaching</i>	108
<i>Analysis of GFP-C10H-Ras trajectories</i>	109
<i>Experimental design</i>	109
<i>Statistical Analysis</i>	110
REFERENCES.....	112
SUPPLEMENTARY FIGURES.....	118

4. ANALYSIS OF INTRACELLULAR PROTEIN DYNAMICS IN LIVING ZEBRAFISH EMBRYOS USING LIGHT-SHEET FLUORESCENCE SINGLE-MOLECULE MICROSCOPY

.....	121
ABSTRACT	121
4.1. INTRODUCTION	122
4.2. RESULTS.....	126
<i>An LSFM platform for in vivo SMM imaging (LSFSMM)</i>	126
<i>Characterization of detected fluorescence signals from individual YFP-GR molecules</i>	131
<i>Experiments on live fish and data analysis</i>	133
4.3. DISCUSSION	139
<i>LSFSMM setup</i>	139
<i>The glucocorticoid receptor mobility patterns in zebrafish embryos.</i> 140	
<i>The effect of dexamethasone treatment on the mobility patterns of the glucocorticoid receptor subpopulations</i>	142
<i>Variability of the glucocorticoid receptor mobility patterns</i>	144
4.4. MATERIALS AND METHODS.....	144
<i>Zebrafish</i>	144
<i>Microinjection and treatment of embryos</i>	144
<i>LSFSMM setup details</i>	145
<i>Determination of imaging parameters</i>	146
<i>Cultured cells fixation, mounting, and LSFMSM imaging</i>	147
<i>Determination of the fluorescence signals characteristics derived from individual YFP molecules</i>	147
<i>Live zebrafish embryo mounting and LSFMSM imaging</i>	147
<i>Live imaging using the muviSPIM setup</i>	148
<i>Data Analysis</i>	149
<i>Experimental Design</i>	150
REFERENCES.....	153

SUPPLEMENTARY FIGURES.....	158
5. SUMMARY AND DISCUSSION.....	161
5.1. SUMMARY OF THE THESIS.....	161
5.2. THE MOBILITY PATTERNS OF H-RAS AND C10H-RAS IN EPIDERMAL CELLS OF LIVING ZEBRAFISH EMBRYOS (<i>CHAPTERS 2 AND 3</i>).....	163
5.3. THE MOBILITY PATTERN OF THE GLUCOCORTICOID RECEPTOR IN LIVING ZEBRAFISH EMBRYOS (<i>CHAPTER 4</i>).....	169
5.4. THE SOURCES OF VARIABILITY OF THE SMM MEASUREMENTS IN THE ZEBRAFISH EMBRYO MODEL (<i>CHAPTERS 2 AND 4</i>).....	171
5.5. FUTURE PERSPECTIVES.....	172
REFERENCES.....	174
NEDERLANDSE SAMENVATTING	179
CURRICULUM VITAE.....	183
LIST OF PUBLICATIONS.....	185

