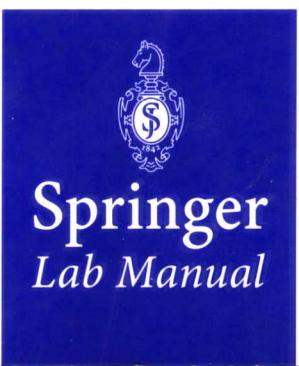


B. Van Duijn  
A. Wiltink  
(Eds.)

# Signal Transduction – Single Cell Techniques



Bert Van Duijn Anneke Wiltink (Eds.)

# Signal Transduction – Single Cell Techniques

With 113 Figures



Springer

BERT VAN DUIJN

Center for Phytotechnology RUL/TNO  
Wassenaarseweg 64  
2333 AL Leiden  
The Netherlands

ANNEKE WILTINK

University of Amsterdam  
Department of Physiology  
Academic Medical Centre  
Meibergdreef 15  
1105 AZ Amsterdam  
The Netherlands

ISBN 3-540-62563-1 Springer-Verlag Berlin Heidelberg New York

**Library of Congress Cataloging-in-Publication Data**

Signal transduction - single cell techniques / Bert Van Duijn, Anneke Wiltink, (eds.). p. cm. - (Springer lab manual) Includes bibliographical references and index.

ISBN 3-540-62563-1 (wire-o-binding : alk. paper)

1. Cellular signal transduction - Laboratory manuals. 2. Cytology - Laboratory manuals. I. Duijn, Bert van, 1961-. II. Wiltink, Anneke, 1961-. III. Series. QP517.C45S556 1997 571.6 - dc21

This work is subject to copyright. All rights are reserved, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other way, and storage in data banks. Duplication of this publication or parts thereof is permitted only under the provisions of the German Copyright Law of September 9, 1965, in its current version, and permission for use must always be obtained from Springer-Verlag. Violations are liable for prosecution under the German Copyright Law.

© Springer-Verlag Berlin Heidelberg 1998  
Printed in Germany

The use of general descriptive names, registered names, trademarks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

Product liability: The publisher cannot guarantee the accuracy of any information about dosage and application thereof contained in this book. In every individual case the user must check such information by consulting the relevant literature.

Cover Design: design & production GmbH, Heidelberg

Typesetting: Mitterweger Werksatz GmbH, Plankstadt

SPIN 10523199 31/3137 5 4 3 2 1 0 ~ Printed on acid free paper -

---

## Preface

*Signal Transduction-Single Cell Techniques* was compiled in response to a rapidly expanding field with an increasing number of possibilities and technical aspects. This laboratory manual provides not only insight into a variety of powerful single cell techniques, but also gives background information and step-by-step protocols for practical applications. In doing so, *Signal Transduction-Single Cell Techniques* is suitable for both single cell specialists and for researchers from outside the single cell field. In addition, the manual contains useful material to be used in teaching related courses.

To study signal transduction in single cells, one has to master many different techniques. Most of these techniques are complicated, contain many pitfalls and require a detailed knowledge of their physical basis. Here, the emphasis is placed on techniques used for handling of cells in experiments and on electrophysiological and fluorescence techniques. The various chapters deal with the theoretical background, the actual recording methods, and analysis of the recorded signals – every chapter being a complete guide. Pitfalls are indicated and useful tips provided.

In Part I, “Handling of Cells in Single Cell Experiments”, there are guidelines for the construction of single cell measurement perfusion chambers, ideas for temperature control and microapplication of drugs, and the application of laser microsurgery. Part II, “Ion Channel and Membrane Potential Measurements Using the Patch-Clamp Technique”, provides multiple examples of the application of different patch-clamp measurement configurations in various cell types from both animal and plant systems. A variety of methods is described in each chapter, enabling the reader to compare and choose the most suitable method for the desired application. In addition, a complete course guiding the reader (experimenter) through the theoretical and practical background of the patch-clamp technique using electrical simulation circuits (without the necessity of having a patch-clamp setup available) is included. Part III, “Fluorescence to Measure Intracellular Ions”, introduces the use

of fluorescent ion sensitive probes, and examples using flow cytometry, microfluorescence, ion imaging and confocal microscopy are given. Emphasis is put on calibration and validation of the different measurement techniques.

With this laboratory manual as a guide, one should be able to perform the experiments described in a well-equipped laboratory without the continuous support of an expert in the field.

Leiden, December 1996

BERT VAN DUIJN  
ANNEKE WILTINK

---

## **Contributors**

**FABIENNE ANDRIS**

Laboratoire de Physiologie Animale  
Université Libre de Bruxelles  
67 rue des Chevaux  
1640 Rhode-St-Genèse  
Belgium

**ERIKA BAUS**

Laboratoire de Physiologie Animale  
Université Libre de Bruxelles  
67 rue des Chevaux  
1640 Rhode-St-Genèse  
Belgium

**LASZLO BENE**

Department of Biophysics  
University Medical School Debrecen  
Nagyerdéi krt 98  
4012 Debrecen  
Hungary

**FEDERICA BERTASO**

Dipartimento di Fisiologia e Biochimica Generali  
Laboratorio di Elettrofisiologia  
Università Statale di Milano  
Via Celoria 26  
I-20133 Milano  
Italy

MARGREET BLOM-ZANDSTRA  
AB-DLO  
P.O. Box 14  
6700 AA Wageningen  
The Netherlands

PETTIE P. BOOIJ  
Institute for Molecular and Biological Sciences  
Faculty of Biology  
Vrije Universiteit Amsterdam  
De Boelelaan 1087  
NL-1081 HV Amsterdam  
The Netherlands

REMKO R. BOSCH  
Department of Biochemistry  
University of Nijmegen  
P.O. Box 9101  
6500 HB Nijmegen  
The Netherlands

SANDOR DAMJANOVICH  
Department of Biophysics  
University Medical School Debrecen  
Nagyerdhei krt 98  
4012 Debrecen  
Hungary

ALBERTUS H. DE BOER  
Institute for Molecular and Biological Sciences  
Faculty of Biology  
Vrije Universiteit Amsterdam  
De Boelelaan 1087  
NL-1081 HV Amsterdam  
The Netherlands

ARIE DE VOS  
Department of Physiology and Physiological Physics  
Leiden University  
P.O. Box 9604  
2300RC Leiden  
The Netherlands

RANDALL L. DUNCAN

Department of Orthopaedic Surgery  
Physiology and Biophysics  
Indiana University Medical Center  
Clinical Building Suite 600  
541 Clinical Drive  
Indianapolis  
Indiana 46202-5111  
USA

MISA DZOLJIC

Department of Anesthesiology  
AMC, University of Amsterdam  
Meibergdreef 9  
1105 AZ Amsterdam  
The Netherlands

RACHEL ERRINGTON

Physiology Department  
Parks Road  
Oxford  
OX1 3PT  
UK

MARCEL T. FLIKWEERT

Kluyver Laboratory of Biotechnology  
Department of Microbiology and Enzymology  
Industrial Microbiology Section  
Delft University of Technology  
Julianalaan 67  
2628 BC Delft  
The Netherlands

MARK FRICKER

Dept. Plant Sciences  
University of Oxford  
South Parks Road  
Oxford  
OX1 3RB  
UK

RESZO GÁSPÁR JR.  
Department of Biophysics  
University Medical School Debrecen  
Nagyerdéi krt 98  
4012 Debrecen  
Hungary

JOCHÉM HERRMANN  
ADIMEC Advanced Image Systems B.V.  
Meerenakkerweg 1  
Postbus 7909  
5652 AR Eindhoven  
The Netherlands

CAN İNCE  
Department of Physiology  
University of Amsterdam  
Academic Medical Centre  
Meibergdreef 15  
1105 AZ Amsterdam  
The Netherlands

KEI INOUYE  
Department of Botany  
Division of Biological Science  
Graduate School of Science  
Kyoto University  
Sakyo-ku  
Kyoto 606-01  
Japan

ATTILA JENEI  
Department of Biophysics  
University Medical School Debrecen  
Nagyerdéi krt 98  
4012 Debrecen  
Hungary

ZOLTAN KRASZNAI

Department of Biophysics  
University Medical School Debrecen  
Nagyerdei krt 98  
4012 Debrecen  
Hungary

JOLANDA LEMMERS

Department of Physiology  
University of Nijmegen  
P.O. Box 9101  
6500 HB Nijmegen  
The Netherlands

OBERDAN LEO

Laboratoire de Physiologie Animale  
Université Libre de Bruxelles  
67 rue des Chevaux  
1640 Rhode-St-Genèse  
Belgium

MIKE MAY

Laboratorium voor Genetica  
Universiteit Gent  
KL Ledeganckstraat 35  
B-9000 Gent  
Belgium

MICHELE MAZZANTI

Dipartimento di Fisiologia e Biochimica Generali  
Laboratorio di Elettrofisiologia  
Università Statale di Milano  
Via Celoria 26  
I-20133 Milano  
Italy

HENK MIEDEMA

Biology Department  
The Pennsylvania State University  
208 Mueller Laboratory  
University Park  
PA 16802  
USA

GYÖRGY PANYI  
Department of Biophysics  
University Medical School Debrecen  
Nagyerdéi krt 98  
4012 Debrecen  
Hungary

CARLO PIERI  
Cytology Center  
Research Department of Gerontology  
Ancona  
Italy

JAN H. RAVESLOOT  
Department of Physiology  
AMC, University of Amsterdam  
Meibergdreef 15  
1105 AZ Amsterdam  
The Netherlands

WIM J.J.M. SCHEENEN  
Department of Biomedical Sciences  
University of Padova  
Via Trieste 75  
35121 Padova  
Italy

ROLF L.L. SMEETS  
Department of Biochemistry  
University of Nijmegen  
P.O. Box 9101  
6500 HB Nijmegen  
The Netherlands

MONIKA TLALKA  
Dept. Plant Sciences  
University of Oxford  
South Parks Road  
Oxford  
OX1 3RB  
UK

**RAFFAELLA TONINI**

Dipartimento di Fisiologia e Biochimica Generali  
Laboratorio di Elettrofisiologia  
Università Statale di Milano  
Via Celoria 26  
I-20133 Milano  
Italy

**JACQUES URBAIN**

Laboratoire de Physiologie Animale  
Université Libre de Bruxelles  
67 rue des Chevaux  
1640 Rhode-St-Genèse  
Belgium

**RUTGERIS J. VAN DEN BERG**

Department of Physiology and Physiological Physics  
Leiden University  
P.O. Box 9604  
2300RC Leiden  
The Netherlands

**BERT VAN DUIJN**

Center for Phytotechnology RUL/TNO  
Wassenaarseweg 64  
2333 AL Leiden  
The Netherlands

**ANTONI VAN GINNEKEN**

Department of Physiology  
AMC, University of Amsterdam  
Meibergdreef 15  
1105 AZ Amsterdam  
The Netherlands

**ZOLTAN VARGA**

Department of Biophysics  
University Medical School Debrecen  
Nagyerdei krt 98  
4012 Debrecen  
Hungary

MARIEKE W. VELDKAMP  
Department of Physiology  
AMC, University of Amsterdam  
Meibergdreef 15  
1105 AZ Amsterdam  
The Netherlands

E. ETIENNE VERHEIJCK  
Department of Physiology  
AMC, University of Amsterdam  
Meibergdreef 15  
1105 AZ Amsterdam  
The Netherlands

Jos A.H. VERHEUGEN  
Neurobiologie Cellulaire  
INSERM U261  
Institut Pasteur  
25 rue de Dr. Roux  
75724 Paris Cedex 15  
France

ARIE O. VERKERK  
Department of Physiology  
AMC, University of Amsterdam  
Meibergdreef 15  
1105 AZ Amsterdam  
The Netherlands

PIET VIS  
Department of Physiology  
University of Nijmegen  
P.O. Box 9101  
6500 HB Nijmegen  
The Netherlands

SAKE A. VOGELZANG  
Vrije Universiteit  
Fac. Biologie  
Vakgroep Molecular and Cellular Biology  
De Boelelaan 1087  
1081 HV Amsterdam  
The Netherlands

WYTSE J. WADMAN

Department of Experimental Zoology  
University of Amsterdam  
Kruislaan 320  
1098 SM Amsterdam  
The Netherlands

MEI WANG

Center for Phytotechnology RUL/TNO  
Department of Plant Biotechnology  
Wassenaarseweg 64  
2333 AL Leiden  
The Netherlands

ZHENG WANG

Department of Physiology and Physiological Physics  
Leiden University  
P.O. Box 9604  
2300 RC Leiden  
The Netherlands

ADAM F. WEIDEMA

Department of Physiology and Physiological Physics  
Leiden University  
P.O. Box 9604  
2300 RC Leiden  
The Netherlands

NICK WHITE

Dept. Plant Sciences  
University of Oxford  
South Parks Road  
Oxford  
OX1 3RB  
UK

PETER H.G.M. WILLEMS

Department of Biochemistry  
University of Nijmegen  
P.O. Box 9101  
6500 HB Nijmegen  
The Netherlands

ANNEKE WILTINK  
Department of Physiology  
University of Amsterdam  
Academic Medical Centre  
Meibergdreef 15  
1105 AZ Amsterdam  
The Netherlands

JULIAN WOOD  
Dept. Plant Sciences  
University of Oxford  
South Parks Road  
Oxford  
OX1 3RB  
UK

DIRK L. YPEY  
Department of Physiology and Physiological Physics  
Leiden University  
P.O. Box 9604  
2300 RC Leiden  
The Netherlands

---

## List of Suppliers

Below the addresses of various companies referred to in various chapters are given.

Altai Nederland B.V.  
Bedrijvenpark Twente 290  
7602 KK Almelo  
The Netherlands  
phone: +31 546 574911  
fax: +31 546 576006

Applied Imaging International Limited  
Hylton Park  
Wessington Way  
Sunderland  
Tyne & Wear  
SR5 3HD  
UK  
phone: +44 (0)191 5160505  
fax: +44 (0)191 5160512  
e-mail: JS @ aii.co.uk

Axon Instruments, Inc.  
1101 Chess Drive  
Foster City, California 94404  
USA  
phone: +1-415-571-9400  
fax: +1-415-571-9500  
e-mail: sales @ axonet.com  
Web: [www.axonet.com](http://www.axonet.com)

Bellco Glass, Inc.  
340 Edrudo Road  
Vineland, New Jersey 08360  
USA  
phone: +1-609 691 1075  
fax: +1-609 691 3247  
e-mail: sales @ bellcoglass.com

BIO-LOGIC Science Instruments  
1 Rue de l'Europe  
ZA de Font Ratel  
F-38640 CLAIX  
France  
phone: +33-76 98 68 31  
fax: +33-76 98 69 09  
e-mail: Bio-Logic @ msn.com  
Web: www.bio-logic.com

BIOSOFT  
P.O. Box 10938  
Ferguson, Missouri 63135  
USA  
phone: +1-314 524 8029  
fax: +1-314 524 8129  
e-mail: info @ biosoft.com  
Web: www.biosoft.com

BIOSOFT  
37 Cambridge Place  
Cambridge CB2 INS  
UK  
phone: +44-1223 368622  
fax: +44-1223 312873

Boehringer Mannheim B.V.  
Postbus 1007  
1300 BA Almere  
The Netherlands  
phone: +31-36 5394911  
fax: +31-36 5394231  
e-mail: biocheminfo.nl @ bmg.boehringer.com  
Web: biochem.boehringer-mannheim.com