Seventh COURSE “MORPHOMETRY AND STEREOMETRY IN NEUROSCIENCES”

Graduate School Neurosciences Amsterdam- Rotterdam

18-22 October 2010
To be held in the colloquium room, MF-H-161, of the Dept. Anatomy & Neurosciences/Pathology, VU University Medical Center (VUmc), Van der Boechorststraat 7, Amsterdam

The aim of this ONWAR Stereology course is to teach how to design, to perform and critically evaluate stereological studies of the nervous system. Stereology is a methodology to achieve quantitative descriptions of the geometry and number of three-dimensional structures from measurements that are made on (nearly) two-dimensional images.

The ONWAR Stereology course covers the following topics:

1) The measurement of volumes, surfaces (area), lengths, and number in 3-dimensional space using ‘2’-dimensional images.
2) The measurement of these structural parameters in homogeneous and non-homogeneous tissues.
3) The design of efficient, unbiased sampling strategies, i.e., "design based" stereology
4) Differential histological shrinkage, how to account for.
5) Confocal microscopy and stereology
6) Morphometry
7) Statistics and stereology

These topics will be dealt with in lectures, practical exercises and demonstrations. Maximum number of participants: 16

Teaching Staff invited

Harry B.M. Uylings, PhD (coordinator) (1)Dept. of Anatomy & Neurosciences, VUmc, Amsterdam
(2) School Mental Health & Neurosci., Div. Brain & Cognition, Univ. Maastricht. hbm.uylings@vumc.nl

Wilma D.J. van de Berg, PhD (coordinator) Dept. of Anatomy & Neurosciences, VUmc, Amsterdam wdj.vandeberg@vumc.nl

Floris G. Wouterlood, PhD Dept. of Anatomy & Neurosciences, VUmc, Amsterdam

Angela Engel, MSc Dept. of Anatomy & Neurosciences, VUmc, Amsterdam

Cathrin Canto, MSc Dept. of Anatomy & Neurosciences, VUmc, Amsterdam

Evelien Timmermans Dept. of Anatomy & Neurosciences, VUmc, Amsterdam

Jaap van Pelt, PhD CNCR, Fac. Earth/Life Sciences, VU University, Amsterdam
PROGRAM

MONDAY 18 OCTOBER 2010

09.00 – 09.30  Introduction.  
Why are the participants interested in this course?  
Introduction to course  
H.B.M. Uylings

09.30 – 10.15  Sampling design (based on lecture notes of A.J. Baddeley, Australia)  
H.B.M. Uylings

10:15 – 10.30  Coffee break

10.30 – 11.15  Unbiased estimation of volume of brain structures  
Theory: Cavalieri’s principle; systematic random sampling; thickness measurement; over- and underprojection  
H.B.M. Uylings

11.15 – 12.00  Practical exercise: estimation of volume of rat neocortex  
H.B.M. Uylings

12.00 – 13.00  Lunch break

13.00 – 13.45  The unbiased estimation of total number of neurons  
Theory: disector method; sampling method; fractionator; delineation of brain region  
W.D.J. van de Berg

13.45 – 14.45  Practical exercise: estimation of total number of neurons; optical disector / fractionator principle  
W.D.J. van de Berg and H.B.M. Uylings

14:45 – 15:00  Tea Break

15.00 – 16.00  Estimation of accuracy  
H.B.M. Uylings
TUESDAY 19 OCTOBER 2010

09.00 – 09.45  Stereology: 3D thinking, 2D measurements leading to 3D estimations
               Based on lecture notes of A.J. Baddeley, Australia (H.B.M. Uylings)

09.45 – 10.15  Anisotropy-Isotropy: how to overcome anisotropy; when necessary; what
               consequences for tissue treatment (orientation; vertical sections)?
               H.B.M. Uylings

10.15 – 10.30  Coffee break

10.30 – 11.00  Estimation of surface and length of 3D fibrillar structures (theory)
               H.B.M. Uylings

11.00 – 12.00  Practical exercise:estimation of surface area of the human cerebellar cortex
               H.B.M. Uylings

12.00 – 13.00  Lunch break

13.00 – 13.45  The nucleator principle: size estimation, spatial distribution (theory). When are
               other approximations preferable?
               H.B.M. Uylings

13.45 – 14.45  Practical exercise: the mean volume of A-cells in rat dorsal root ganglion using
               the nucleator on vertical sections
               H.B.M. Uylings

14.45 – 15.00  Tea break

15.00 – 16.00  Quantitative analysis of synaptic density using design-based stereology
               (Light-microscopy and Electron microscopy)
               W.D.J. van de Berg
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00 – 09:45</td>
<td>Confocal Microscopy, 3D object recognition and counting contacts in 3D</td>
</tr>
<tr>
<td></td>
<td>F.G. Wouterlood</td>
</tr>
<tr>
<td>09:45 – 10:15</td>
<td>Confocal stereology</td>
</tr>
<tr>
<td></td>
<td>W.D.J. van de Berg</td>
</tr>
<tr>
<td>10:15 – 10:30</td>
<td>Coffee break</td>
</tr>
<tr>
<td>10:30 – 11:15</td>
<td>Vascular morphometry</td>
</tr>
<tr>
<td></td>
<td>W.D.J. van de Berg</td>
</tr>
<tr>
<td>11:15 – 12:00</td>
<td>Practical exercise: estimation of capillary loss using Space balls</td>
</tr>
<tr>
<td></td>
<td>W.D.J. van de Berg</td>
</tr>
<tr>
<td>12:00 – 13:00</td>
<td>Lunch break</td>
</tr>
<tr>
<td>13:00 – 17:00</td>
<td>Demonstration Part I: Students will be divided in four groups max. 4 students. Two demonstrations will be followed by the groups today and two on Thursday afternoon.</td>
</tr>
<tr>
<td>13:00-14:30</td>
<td>A) Neurolucida and Stereoinvestigator</td>
</tr>
<tr>
<td></td>
<td>Evelien</td>
</tr>
<tr>
<td></td>
<td>a. Volume estimation</td>
</tr>
<tr>
<td></td>
<td>b. Counting in 3D</td>
</tr>
<tr>
<td></td>
<td>c. Estimation of fiber and capillar length</td>
</tr>
<tr>
<td></td>
<td>d. 3D reconstruction using Neurolucida</td>
</tr>
<tr>
<td></td>
<td>B) Workstations: 1. Neurolucida and Stereoinvestigator</td>
</tr>
<tr>
<td></td>
<td>Harry/Wilma</td>
</tr>
<tr>
<td></td>
<td>14:30-15:00</td>
</tr>
<tr>
<td>15:00-16:30</td>
<td>C) Workstations: 2. Confocal Stereology</td>
</tr>
<tr>
<td></td>
<td>Wilma</td>
</tr>
<tr>
<td>D) Confocal laser scanning microscopy</td>
<td>Floris Wouterlood &amp; Angela Engel</td>
</tr>
<tr>
<td></td>
<td>e. Confocal scanning</td>
</tr>
<tr>
<td></td>
<td>f. Automated object recognition and counting contacts in 3D</td>
</tr>
</tbody>
</table>
THURSDAY 21 OCTOBER 2010

09:00 – 09:45  Methods for 3D reconstruction and analysis of neuronal tree structures  
                Jaap van Pelt

09.45 — 10.15  Computational modeling of neuronal morphology and network formation  
                Jaap van Pelt

10.15 – 10.30  Coffee break

10.30 – 11.15  3-D reconstruction of neurons from multichannel confocal laser scanning image series  
                Floris Wouterlood

10.15 – 12.00  What does neuronal morphology tell us about physiology?  
                Cathrin B Canto

12.00 – 13.00  Lunch break

13:00 – 17.00  Demonstration Part I: Students will be divided in four groups of max. 4 students.

13:00-14:30  A) Neurolucida and Stereoinvestigator  
              Evelien

              g. Volume estimation
              h. Counting in 3D
              i. Estimation of fiber and capillair length
              j. 3 D reconstruction using Neurolucida

              B) Workstations: 1. Neurolucida and Stereoinvestigator  
                 Harry/Wilma

14:30-15:00  Tea break

15:00-16:30  C) Workstations: 2. Confocal Stereology  
              Wilma

              D) Confocal laser scanning microscopy  
                 Floris Wouterlood & Angela Engel

              k. Confocal scanning
              l. Automated object recognition and counting contacts in 3D
FRIDAY 22 OCTOBER 2010

09.00 – 09.45 Neuroanatomical tracing and morphometrics
F.G. Wouterlood

09.45 – 10:15 Practical issues: tissue deformation and shrinkage
H.B.M. Uylings & W.D.J. van de Berg

10.15 – 10.30 Coffee break

10.30 – 11.15 Statistics for Stereology
(based on lecture notes of A.J. Baddeley, Australia)
H.B.M. Uylings

11.15 – 12.00 Describing and testing of relations between variables (regression analysis; when all variables contain a biological and/or non-negligible measuring error; danger of ratios)
H.B.M. Uylings

12.00 – 13.00 Lunch break

13.00 – 13.45 How to deal with biological variability? How many animals do we need to study?
H.B.M. Uylings

13.45 – 14.30 Practical exercise; how many animals do we need for this study?
H.B.M. Uylings

14.30 – 15.00 Tea break

15.00 – 15.30 General discussion

15.30 – 16:00 Course evaluation

16.00 – 17.00 Farewell drink