Cortical neural networks:

light-microscopy-based anatomical reconstruction,

numerical simulation and analysis

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Collaborators

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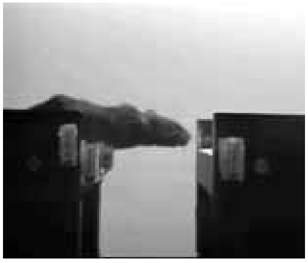
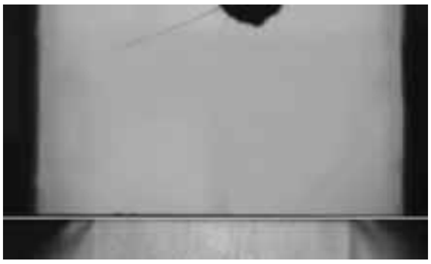
Motivation

- Fundamental question in neuroscience:
  How does the brain translate sensory input into behavior?
  - ➔ Describe and understand at cellular level, complete circuits that can drive behavior.
- Widely used model system: somatosensory whisker system in rodents

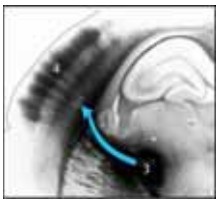




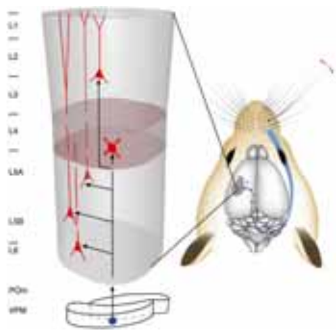
- Decision making; example: gap crossing
  - ➔ Reveal relation between structure and function (anatomy ↔ physiology)

Biological background: cortical column

- Somatosensory cortex processes information from whisker
- Information pathway:
  whisker → brain stem → thalamus (VPM) → cortical column
- Basic anatomical unit: cortical column
- 1-to-1 whisker-column correspondence
- Column divided into layers 1-6



Modified from V.C. Wimmer et al (2010): *Dimensions of a projection column and architecture of VPM and POm axons in rat vibrissal cortex*. Cereb Cortex, 20(10), 2265–2276



Modified from Helmstaedter et al. (2007): *Reconstruction of an average cortical column in silico*. Brain Res. Rev. 55(2): 193-203

Approach

Reconstruct cortical column

↓

Numerically simulate its behavior

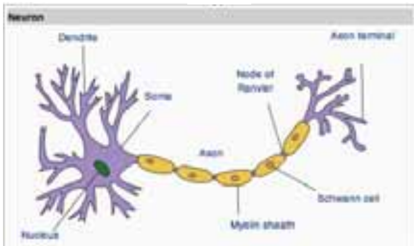
↓

Analyze simulation results

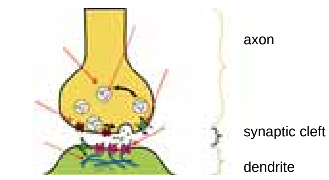
Reconstruction

Ideal:

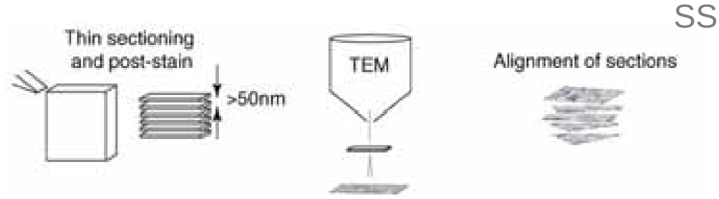
Dense reconstruction of all *neurons* and their *synaptic connections* within a cortical column of 1 individual.



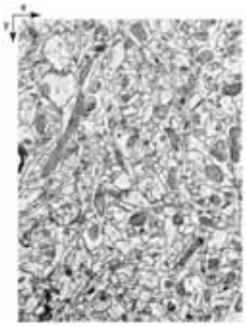
From: wikipedia



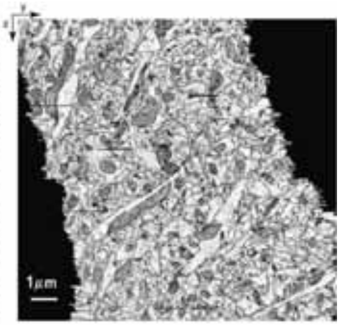
SS TEM



From: *K. Briggman and W. Denk.* Towards neural circuit reconstruction with volume electron microscopy techniques. Curr. Opin. Neurobiol. 16:562-70 (2006).

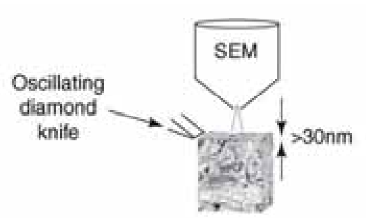


50 nm section of rat neocortex at 7 nm lateral resol. (xy plane)



Registered (aligned) stack of 239 sections resectioned in silico (yz plane).

SBF SEM



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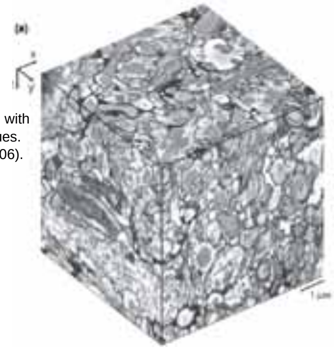
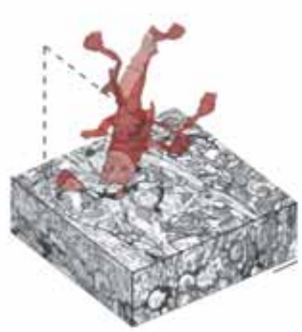


350 mm³ from adult rat barrel cortex 13.2nm/pixel, 253 sections each 30 nm thick

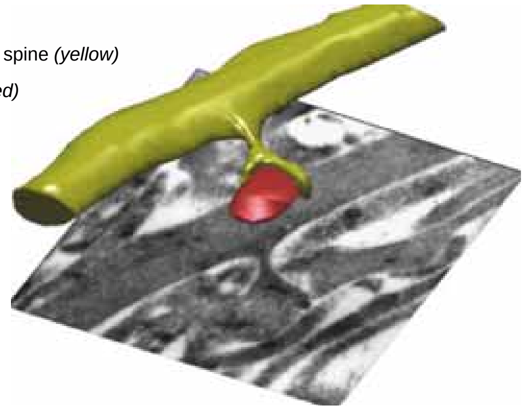


Manually traced spiny dendrite

Synaptic connection

Reconstruction of

- dendrite segment with spine *(yellow)*
- pre-synaptic bouton *(red)*



From: *K. Braun,* Univ. Magdeburg

Reconstruction approach

Ideal:

Using electron tomographic technique perform dense reconstruction of all *neurons* and their *synaptic connections* within a cortical column of 1 individual

technically not yet feasible

Instead “Reverse engineering”:

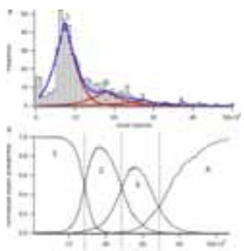
Using light microscopy, collect *somata and neurites* from different sources and combine them in a single model and establish rule-based connection

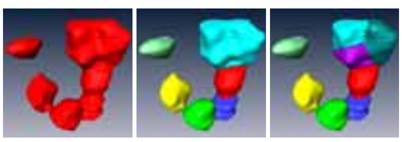
What anatomical data do we need?

- 3D neuron distributions
- 3D VPM axon reconstructions
- 3D dendrite reconstruction
  for a representative sample of neurons of all occurring cell types
- Spatial frequencies of all cell types
- Spine- and bouton densities
  (number of pre-/post-synaptic connection sites per µm axon/dendrite)

3D neuron distribution

- Input: 3D confocal images
  containing stained somata (cell bodies)
- Problem: detect somata, resolve overlap
- Method:
  1) Binary segmentation
  2) Morphology-based splitting of clusters
  3) Volume model-based splitting of clusters

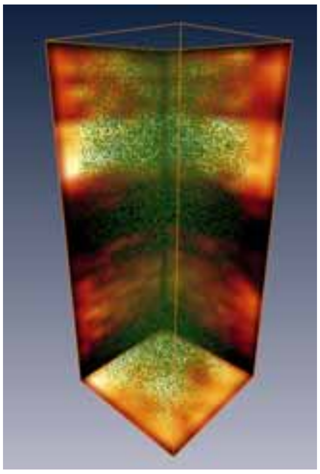




Oberlaender et al. (2009): *Automated three-dimensional detection and counting of neuron somata*. J Neurosci Methods, 180(1):147–160

3D neuron distribution (2)

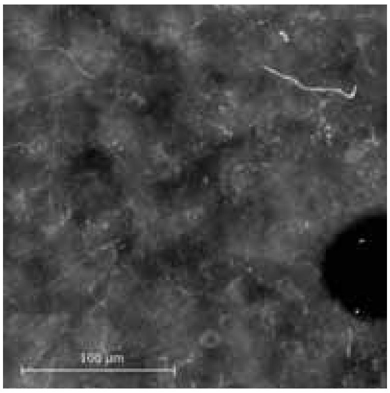
- Volume containing cortical column
- 2 x 2 x 40 confocal sections
- ~18k neurons in cortical column
- Density varies w.r.t. cortical depth



H.S. Meyer et al (2010): *Number and laminar distribution of neurons in a thalamocortical projection column of rat vibrissal cortex*. Cereb Cortex. 20:2277-2286

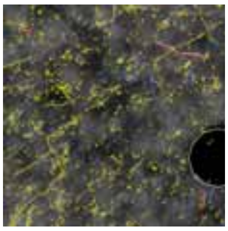
Morphology reconstruction

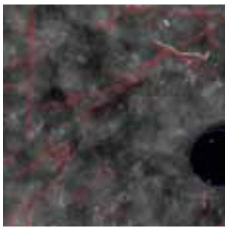
- Input: stack of transmitted light brightfield images (~35 sections)
- Problem: trace axons and dendrites, align stack, combine sections
- Complications: thin foreground structures, uneven dye penetration, stained background, large data (~20 GB/section)
- Approach:
  1) Automatic section tracing
  2) Interactive intra-section post-processing
  3) Automatic alignment
  4) Interactive inter-section post-processing (final quality control)
  5) Optional semantic labeling



Morphology reconstruction: “inverse tracing”

- Staining problems and sectioning may cause skipping of branches
- Ensure completeness
- Very conservative automatic tracing, accepting over-segmentation
- Manually remove false positives using dedicated tool (Amira filament editor)

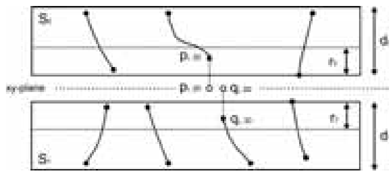


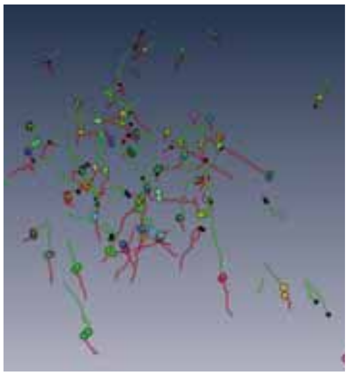


V.J. Dercksen et al (2012): *Interactive visualization—a key prerequisite for reconstruction of anatomically realistic neural networks*. In: Visualization in Medicine and Life Sciences II. Springer-Verlag. In press.

Morphology reconstruction: section alignment

- Input: thin sections containing traced fragments
- Problem: alignment
- Point matching of near-boundary points
- Detection of maximal clique in distance compatibility graph
- Scaling and rigid alignment

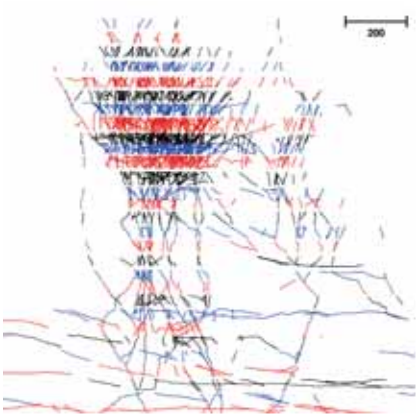




V.J. Dercksen et al (2009): *Automatic alignment of stacks of filament data*. Proc IEEE International Symposium on Biomedical Imaging: From Nano to Macro (ISBI), 971-974.

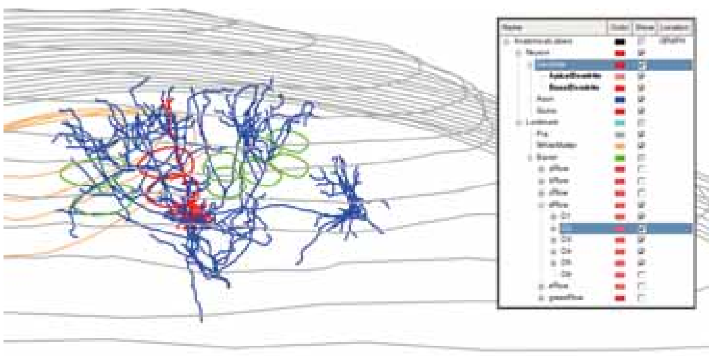
Morphology reconstruction: alignment

- Time: ~10s / slice pair
- Integrated in Filament Editor, allowing interactive control
- Interactive inter-slice connecting of fragments
- Removal of false positives



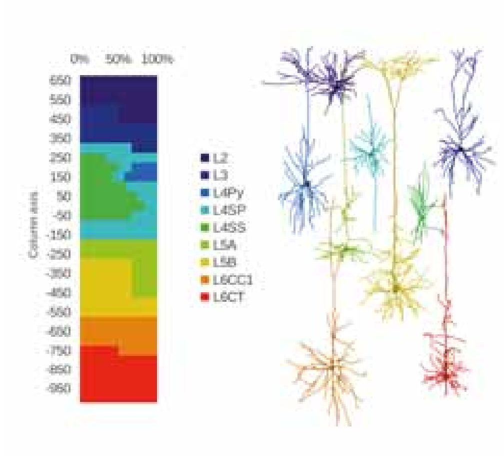
Morphology reconstruction: semantic labeling

- Semantic labeling of sub-structures and landmarks for visualization and analysis



Morphology reconstruction: dendritic cell types

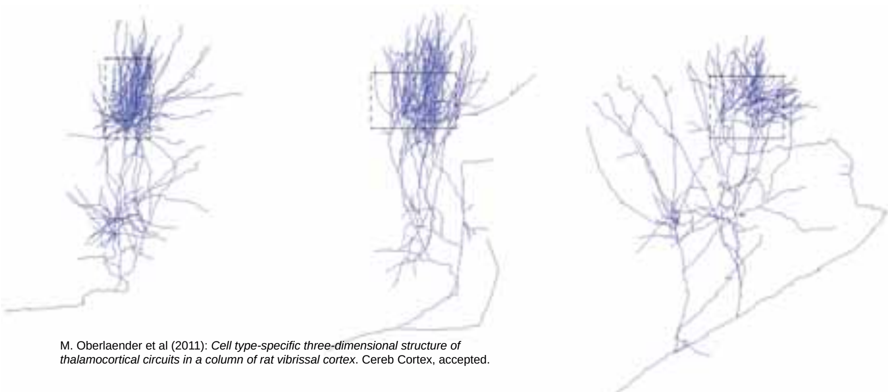
- ~100 dendritic reconstructions
- Classification:
  9 excitatory cell types
- Relative frequency
  w.r.t cortical depth
  (Cortical depth recorded
  during dye injection)



M. Oberlaender et al (2011): *Cell type-specific three-dimensional structure of thalamocortical circuits in a column of rat vibrissal cortex*. Cereb Cortex, accepted.

Morphology reconstruction: axons

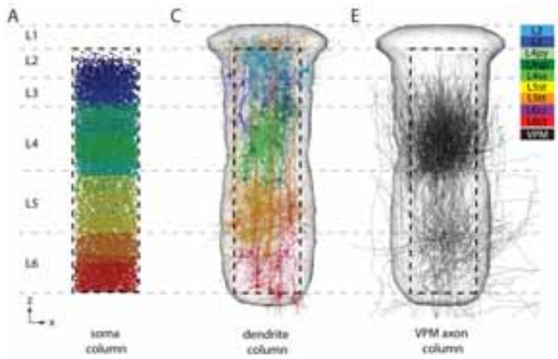
- Axons: Long-ranging, thin, complex
- VPM axons project into column
- Convey input from whiskers



M. Oberlaender et al (2011): *Cell type-specific three-dimensional structure of thalamocortical circuits in a column of rat vibrissal cortex*. Cereb Cortex, accepted.

Cortical column assembly

- Place somata according to given neuron density
- Place dendrites by duplicating morphologies, satisfying cell type frequency



M. Oberlaender et al (2011): *Cell type-specific three-dimensional structure of thalamocortical circuits in a column of rat vibrissal cortex*. Cereb Cortex, accepted.

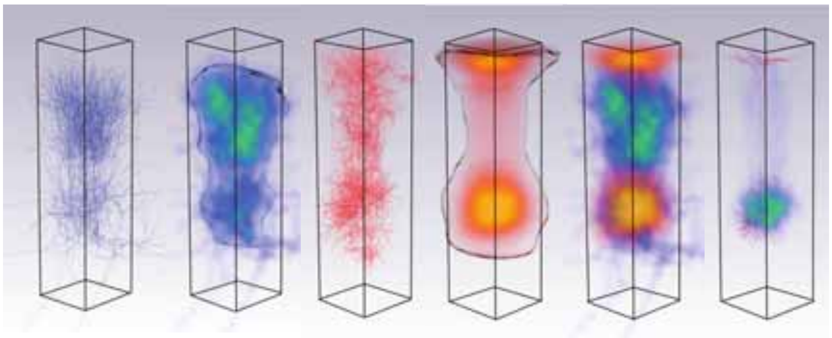
Synaptic connectivity

- Number of synapses estimated from structural overlap (Peters' rule)
- Synapse estimate in 50^3 μm^3 grid cells
  - # boutons = axon length * bouton density
  - # spines = dendrite length * spine density
  - # synapses: $c_{i,j}(x,y,z) = f(x,y,z) * s_i(x,y,z) * \frac{b_j(x,y,z)}{S(x,y,z)}$

  $c_{i,j}$ = # synapses of post-synaptic neuron i with pre-synaptic type j
  b_j = # boutons of pre-synaptic type j
  s_i = # spines of cell i
  S = total # spines
  f = optional factor for inhibitory cell compensation

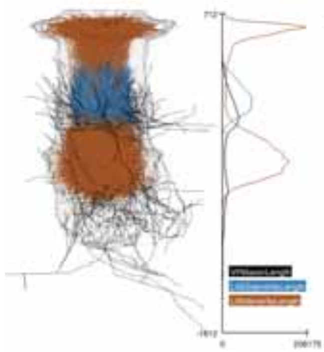
- Synapse position input for simulation
- Realization of pre-synaptic cell for each synapse is generated before simulation (depending on convergence/divergence parameters)

Synaptic connectivity: VPM-L5B example



Visual analysis of anatomical network properties

- Current work: Interactive exploration of neural network properties
- Collect evidence to validate model, support hypotheses
- Produce images for publication
- Tool: query-based selection and evaluation for visual and quantitative analysis



Example:

```
selection VPMaxon = axon where CellTypeIs('VPMaxon')
selection L5Bdendrites = dendrite where CellTypeIs('L5B')
selection L4SSdendrites = dendrite where CellTypeIs('L4SS')
profile VPMaxonLength = length(VPMaxon, Z, 25)
profile L4SSdendriteLength = length(L4SSdendrites, Z, 25)
profile L5BdendriteLength = length(L5Bdendrites, Z, 25)

selection allDendrites = dendrite
profile L5Bsynapses =
      synapses(VPMaxon, L5Bdendrites, allDendrites, XYZ, 50
profile L4SSsynapses =
      synapses(VPMaxon, L4SSdendrites, allDendrites, XYZ,
50)
```

Simulation of signal flow
(Stefan Lang)

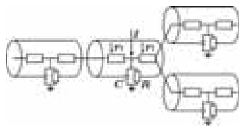
- Membrane potential (V)
- Hodgkin-Huxley equations describe V in response to input/output current through membrane channels and synapses

$$C\frac{dV}{dt} = -(\sum_i I_i + I_{leak}), \quad I_i = \frac{V-E_i}{R_{i(V,t)}}$$

- Cable equations describe V change through compartments

$$\frac{r_m}{r_l}\frac{\partial^2 V}{\partial x^2} = c_m r_m \frac{\partial V}{\partial t} + V$$

- Coupled PDEs of reaction-diffusion type
- Finite Volume scheme
- t-dependent eqs implicitly discretized by Crank–Nicholson or Backward–Euler schemes
- Simulation software NeuroDUNE www.neurodune.org



Simulation parameters

- Anatomical parameters
  - Number of neurons
  - Morphology
  - Spine, bouton densities
  - Number of synapses
  - Synapse positions on dendrites
  - ...
- Physiological parameters
  - Electrical parameters
    Ion channel conductances, reversal potentials, resting potential, etc.
  - Convergence/divergence
  - Pre-/post-synaptic neuron pairing
  - Input signal (#spikes)
  - Input synchrony
  - Synaptic efficacy
  - ...

Simulation parameters

- Anatomical parameters
  - Number of neurons → Anatomical data
  - Morphology → Anatomical data
  - Spine, bouton densities → Values reported in literature
  - Number of synapses → Anatomical data
  - Synapse positions on dendrites → Parameter study

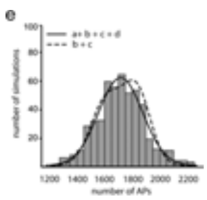
  ... ...

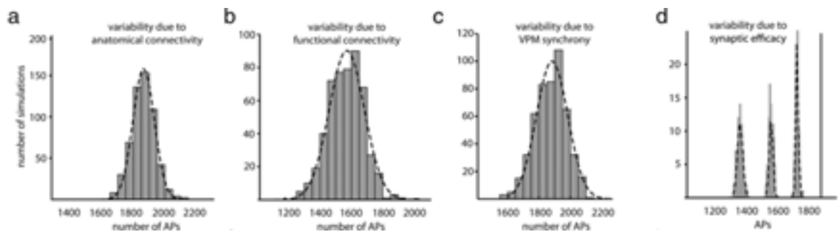
- Physiological parameters
  - Electrical parameters → Values reported in literature
    Ion channel conductances, reversal potentials, resting potential, etc.
  - Convergence/divergence → Values reported in literature
  - Pre-/post-synaptic neuron pairing → Parameter study
  - Input signal (# spikes) → Measured in vivo
  - Input synchrony → Parameter study
  - Synaptic efficacy → Parameter study
  - ... ...

S. Lang et al (2011): *Simulation of signal flow in 3D reconstructions of an anatomically realistic neural network in rat vibrissal cortex*. Neural Netw. 24:998-1011.

Simulation: parameter sensitivity study

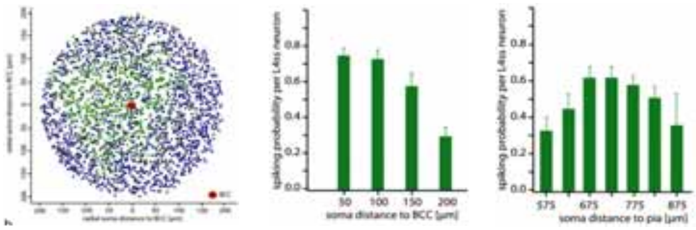
- Model: VPM (285) → L4 spiny stellates (2770)
- Monte Carlo: 507 runs per trial
- Dependent parameter: number of L4SS spikes
- 4 independent parameters were varied:
  - Anatomical connectivity (synapse positions)
  - Functional connectivity (pre- and post-synaptic neuron pairs)
  - VPM input spike timing (synchrony)
  - Synaptic efficacy (% spikes that elicit post-synaptic signal)
- Functional connectivity and input synchrony explain most variance





Simulation: example result

- Location-specific spiking response
- Spiking response decreases
  - with distance to barrel column center (BCC)
  - towards layer boundaries (L3, L5)
- This behavior has been reported for L2/3 cells in literature



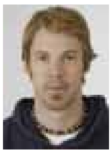
Future challenges

- Tools for explorative analysis of simulation results
- Anatomic modeling of larger brain volumes
  (currently: barrel field, ± 25 columns)
- Utilization (also) of electon-tomographic images:
  particularly automatic segmentation of TB sized data

→ Gradual, step-wise improvement of understanding:
  anatomy – physiology – function

Acknowledgement to collaborators

Vincent J Dercksen
ZIB, Berlin



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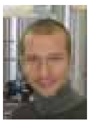
Further acknowledgements



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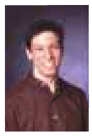

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