Chapter 6

Summary and outlook

This thesis was divided into two parts. In the first part I developed novel tools to generate precise geometric reconstructions of neuronal shapes from confocal image stacks. These can be done semi-automatically on complex branching neurons and can achieve a degree of precision not previously available at the corresponding respective optical resolution. The semi-automatic reconstruction methods are described in chapter 2.
I further established techniques for full automation of the reconstruction process. These can be applied on 'simple' neurons (such as cerebellar Purkinje cells) and still yield maximum precision, however they are restricted to clearly-stained neuronal branches. Furthermore, I developed methods to quantitatively investigate the distribution of labeled molecules (i.e. synaptic proteins, cell surface molecules etc.) along neuronal surfaces. Together, these new methods open up new frontiers in functional neuro-anatomy. One example is that synapse distributions through dendritic trees can be now estimated from light microscopy work. This work is presented in chapter 3.
In the second part of the thesis I used these novel methods to analyze the role of dendritic filopodia for postembryonic dendritic growth and for synaptogenesis of motorneurons during the metamorphosis of *Manduca sexta*. The results are described in chapter 4 and demonstrate that two different types of filopodia, namely shaft and tip filopodia, exist within one dendritic tree. Both undergo a different morphogenesis during ongoing dendritic growth and synaptogenesis. The data further indicate that shaft filopodia may mainly be important for guiding in axon terminals which have just made contact with dendritic filopodia, whereas the tip filopodia seem to have little to do with synaptogenesis, but are, on the other hand, important for steering growing dendrites through developing neuropils.
On the basis of the new methods and the results on dendrite development and synaptogenesis within developing dendritic trees, several future challenges can now be faced:

First, one could employ the techniques to localize the sites of input synapses not only on filopodia but throughout entire dendritic trees. This might help to figure out the rules as to where and when synapses are formed during postembryonic remodelling. Second, such synapses localization analysis can be broken down according to the different transmitter types by combining transmitter and synaptic protein immuno-cytochemistry with precise neuronal surface reconstruction. And finally, the role of specific synapse distribution rules through dendritic trees for adult behavior can be addressed by theoretical computational analysis with multi-compartment modelling. Geometric reconstructions can be equipped with the sites of various different types of input synapses and exported into modelling software like NEURON. This allows us to compare random synapse distributions with those found in the tissue, and to compare rules for synapse distribution found at different developmental stages on different dendritic geometries. Since the function of the Motorneuron 5 in *Manduca sexta* is well understood at the larval and the adult stages, this yields the unique possibility to investigate the effects on dendritic shape and synapse distribution through this complex geometry in the light of neuronal function, and even in a behavioral context.