

# Towards Automatic Segmentation of Serial High-Resolution Images

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**Abstract.** Developing barley grains are to be visualised by a 4-D model, in which spatiotemporal experimental data can be integrated. The most crucial task lies in the automation of the extensive *segmentation* procedure. Because of constraints like incomplete a-priori expert knowledge and the complexity of this specific segmentation task, learning techniques like *Artificial Neural Networks (ANN)* yield promising solutions. In this work we present our first good *segmentation results*. Two different supervised trained ANN classifiers were applied, on one hand, the well-established borderline-learning *Multiple-Layer Perceptron (MLP)* and on the other hand, the prototype-based *Supervised Relevance Neural Gas (SRNG)*. While so far segmentation was mainly achieved using manual tools, now almost automatic segmentation becomes more feasible.

## 1 Introduction

The geometric reconstruction of biological structures in 3-D models establishes new facilities for the analysis of structural and functional interrelationships as well as developmental processes of these biological objects in a spatial or even spatiotemporal (4-D) context. The intended purpose of such models often lies in the generation of standardised anatomical atlases in which various spatial experimental data can be integrated. We are working on the generation of a 4-D atlas model of a barley grain with ideally about 25 different time steps, that allows the integration of experimental data, e.g. gene-expression data. The research of spatiotemporal processes – which yield the accumulation of nutrients like starch and proteins in the grain – will be significantly promoted. So far the emphasis lies on the generation of high-resolution, stand-alone models that ensure the recognisability of even minor components like small sized tissues [1]. Experience gained from a predominantly manual modelling of highly resolved grain models clearly showed the need for automation and facilitation of many repetitive, time-consuming and work-intensive steps. This is even more important as many geometric models at different time steps have to be created in order to establish a time-dependent geometric model that represents complex biological development processes. The creation of 3-D models comprises a sequence of nontrivial steps. In contrast to relatively small 3-D models based on direct 3-D image generating methods, such as *Nuclear Magnetic Resonance (NMR)* or

confocal microscopy, the creation of high-resolution seed models based on many hundred up to several thousand of serial cross-sections – as in our case – requires the solution of additional problems. These are e.g. the handling of an enormous amount of data and the regeneration of the spatial integrity of the 2-D images of the cross-sections in the 3-D model according to the original object [2, 3].

However, the most crucial task lies in the automation of the segmentation procedure. In this step the biologically relevant materials or tissues are identified or labelled within the raw image material. Strictly speaking, each pixel has to be assigned to one of about 20 model-specific materials. Since the segmentation is to be done over all images of a grain it is by far the most work-intensive step in the workflow. In this work in progress we focus on the automation of the segmentation procedure. We deal with specifically generated, bluish dyed high-resolution images of cross-sections of barley grains [1]. Due to the high degree of complexity available automatic standard methods failed and therefore the segmentation had to be manually done by drawing the borderlines of each of the materials over all images. A flexible and easily adaptable segmentation solution is required since a couple of constraints are to be dealt with:

- Incomplete, partly inconsistent and (even in the modelling process itself) developing expert knowledge of the histological background,
- Varying appearance of cross-sections in different grain-parts and at different developmental stages,
- Subjectiveness and hence lack of (exact) reproducibility as well as
- Varying requirements (e.g. the question which materials are relevant?).

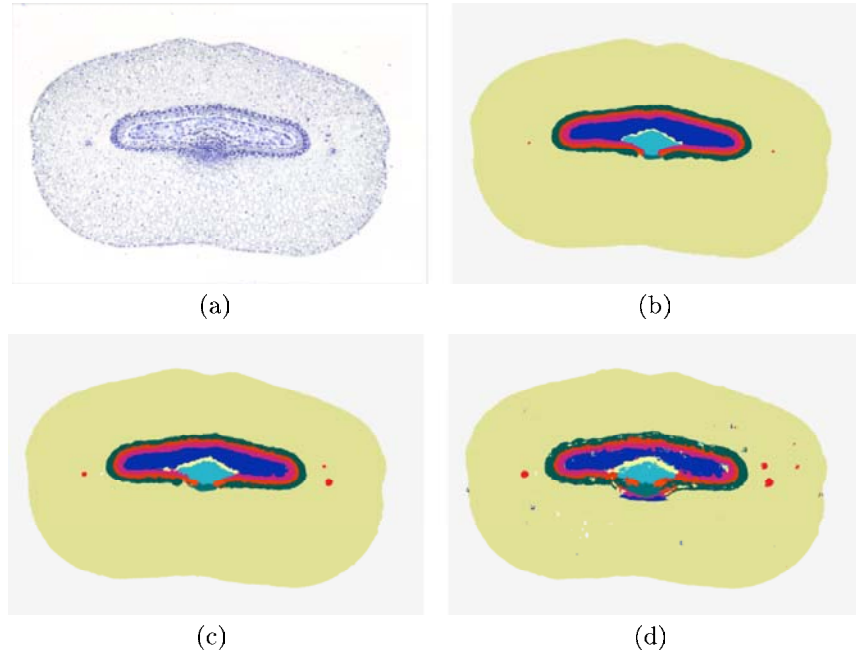
The segmentation is also characterised by a very high level of complexity, since the discrimination of two neighbouring materials often turned out to be Gordian.

## 2 Methods

Due to the degree of difficulty we decided in favour of a segmentation based on a prior pixel-wise classification. A very promising attempt to combine the pros of human-like adaptive and intelligent processing on the one hand and computer-aided high-throughput processing on the other hand is to utilize soft computing techniques like *Artificial Neural Networks (ANN)* [4, 5] which generally offer intelligent and trainable data processing, particularly within an image processing framework.

So far two different supervised trained ANN classifiers were applied to exemplary image stacks, on the one hand the well-established borderline-learning *Multiple Layer Perceptron (MLP)* [6, 7, 8] and on the other hand the prototype-based *Supervised Relevance Neural Gas (SRNG)*, which is an initialisation-tolerant version of Kohonen's well-established *Learning Vector Quantization (LVQ)* classifier [9]. Our first good segmentation results are based on an underlying feature vector with 170 properties concerning colour, geometry and symmetry (such as Cartesian and polar coordinates, distance to centroid, absolute angle to symmetry axis) and particularly texture according to varying neighbourhoods (such as

**Fig. 1.** Automatic segmentation of an exemplary cross-section of a grain: a) original colour image of a cross-section, b) according manual segmentation by a biologist, c) results of automatic segmentation based on MLP, d) results of automatic segmentation by SRNG.



Gaussian filters, histogram based features). For simplification reasons we used for the proof of concept the same feature vector for both classifiers although we are aware of the fact, that different classifiers may require slightly different feature sets to perform optimally. All features were z-score-transformed for the normalisation of the attributes.

We used 2 stacks of respectively 10 arbitrarily chosen consecutive images from different grain parts. Respectively 3 typical images were selected to randomly extract training examples – for the MLP a natural material representation (density of training samples equals density of data samples) was chosen whereas for the SRNG a uniform presentation was preferred. In the recall phase both classifiers were applied to the whole image stack. In each image stack 11 respectively 12 materials were to be recognised.

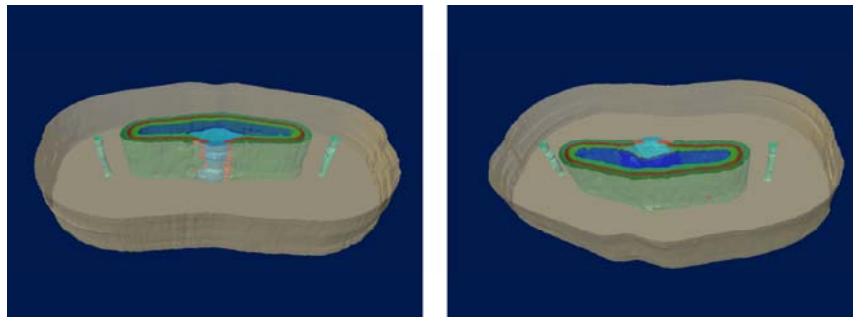
### 3 Results

We introduce our first good results based on an underlying initial feature vector. By comparison with formerly completely manually segmented images we achieved with both methods classification accuracies of about 90% per image. In Tab. 1 the confusion matrix according to classification results over a full stack of

**Table 1.** Confusion matrix of results with MLP / natural distribution of pixels for 10 consecutive images (model 7 *Days After Flowering (DAF)*, style). All model-specific materials are assigned one-to-one to a specific label index in ascending order. Gaps in the label numbering are referred to materials being not present in the considered part of the grain. The entries in the main diagonal are in most cases close to 100%. Higher numbers at other matrix positions indicate not yet satisfactorily characterised materials.

Label-No.	0	2	6	8	9	10	11	12	13	14	16
0	98.65	1.32	0	0.01	0	0	0.01	0	0	0	0.01
2	0.66	98.66	0.02	0.39	0.02	0.02	0	0	0	0.08	0.15
6	0	0.21	73.86	9.42	0.06	2.51	11.18	0.01	1.25	1.49	0
8	0	5.97	10.36	81.48	0.05	1.33	0.18	0.05	0.06	0.52	0.01
9	0	0	0	0	90.43	0	4.15	5.10	0.32	0	0
10	0	0.08	3.60	5.02	0.19	60.12	0.58	0.42	0.93	29.07	0
11	0	0.02	6.86	0.11	6.86	0.23	81.37	1.12	3.05	0.39	0
12	0	0	0.02	0	7.88	0	2.00	75.08	15.02	0	0
13	0	0.01	0.30	0	1.72	0.19	1.77	3.15	88.52	4.33	0
14	0	1.04	0.09	0	0	2.21	0	0	0.09	96.57	0
16	0	20.44	0	0	0	0	0	0	0	0	79.57

10 images in relation to the manually segmented reference images is to be seen. In principle with MLP very reliable borderlines were achieved, smaller materials sometimes were omitted. With SRNG the single positions of even tiny materials were detected, but the borders look rather jagged and many misclassified islands occur (Fig. 1). Based on the strengths of both automatic segmentation methods and a simple postprocessing procedure mainly using structural knowledge, first model-segments were created with the visualisation software Amira<sup>TM</sup> (Fig. 2).



**Fig. 2.** Two perspective views of one 3-D model based on 10 automatically segmented images of cross-sections of a barley seed (7DAF, style, 20 fold stretched in height).

## 4 Discussion

Our results show that a nearly automatic segmentation of cross-sections of developing barley seeds is feasible now. The expert can focus on essential modelling decisions. Only a tiny but representative subset of the images will have to be manually segmented for the training of the ANN classifiers. Adaptations can be achieved in the modelling process itself or in response to changed requirements without much effort. Furthermore an automation of the segmentation step is an inevitable precondition for the generation of high-resolution 4-D atlas models based on a considerable number of individual grains at the different developmental stages. Further research concerns the application and comparison of further classification methods, the development of a reduced but nevertheless robust feature vector, and the design of an adequate postprocessing procedure.

We are convinced that our automatic segmentation method does not only work within different grain parts and different developmental stages of barley but can be applied to similar species such as wheat as well. From the conceptual point of view this method is also generally extensible to medical applications.

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