REUSABLE MODULES FOR HIGH-CONTENT 3D AND 4D IMAGE ANALYSIS

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ABSTRACT

Fully automated high-throughput and high-content experiments need reusable general modules, that can be combined in a flexible way to build the solution. Even though the biological objects or structures may not share any common features, the transformations that act on the structures (like rotations, translations or deformations) are nearly the same in every experiment. In the talk I will show general concepts for the detection, recognition or comparison of complex high-level structures, that we use in our daily work. Invariant features are computed by the integration over the transformation group. Rotation-invariant detection is done using an image representation that encodes the local surrounding of every voxel in spherical harmonics basis functions. A general module for tracking of complex structures can be based on recent high accuracy optical flow methods. The successful applications of these concepts are, e.g., the classification of pollen grains, the rotation-invariant detection of landmarks in zebrafish embryos and the analysis of growth patterns in frog kidney.

Index Terms— Invariant features, group integration, spherical harmonics, optical flow, 3D,4D

1. INTRODUCTION

High content and high throughput microscopic experiments become more and more popular in biomedical research. At the same time we see a shift away from the conventional 2D analysis of single cells towards a 3D and even 4D (3D over time) analysis of cells in a more natural environment up to the analysis of whole organs and organisms. This puts high demands on automated image analysis, because for large amounts of 3D data a manual or even a semi-automated analysis is no longer feasible.

To reduce the time between the design of a new experiment and the availability of the corresponding fully automated image analysis solution it is highly desirable to develop reusable general modules, that can be combined in a flexible way to build the solution. The adaption of such modules to a new task should not be done by tuning dozens of parameters and pre- / post processing steps (that describe the *way* for the computer to reach the goal), but by (interactive) training procedures, such that the user can directly discuss the *goal* with the computer – ideally on the same high level as a discussion with a technical assistant.

1.1. Missing Modules for High Level Structures

General algorithms for detection, segmentation, recognition and comparison of low-level 3D structures (like blobs, tubes, shells, plates, foci, etc.) become more and more available, e.g. from the FARSIGHT project [1]. While these approaches are very useful for a large number of typical image analysis tasks in biomedical research, they may not be applicable when the detection, recognition or comparison of complex high-level structures is required. Example applications in this field are the recognition of pollen grains or parts of them, detection of certain aberrant mitotic patterns in large tissue samples, localization of landmarks in zebrafish embryos, etc. A modelling of such structures using the above primitives, is at least tedious and very problem-specific, but in many cases it will be completely impossible. Here a "learning from examples" approach is much more appropriate.

2. LEARNING FROM EXAMPLES

To build general modules for detection and recognition of such arbitrary complex structures, a thorough analysis of the irrelevant (but unavoidable) intraclass variations of the structures and their images in all applications is necessary.

One very prominent type of variations, especially in fully automated experiments without user interaction, is the arbitrary 3D position and 3D orientation of the object or the structure under consideration. The way to deal with such variations can be roughly categorized into the following categories.

Normalization-based approaches for the compensation of rotation and translation typically only work well for a spe-

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cial subset of structures, or rely on a prior successful segmentation (which contradicts our aim for general reusable modules). For example, the SIFT-like approaches (locate key points by Differential of Gaussians (DoG) and determine their orientation by the prominent gradient direction) have severe problems in 3D biomedical microscopic data, due to the often weak contrasts, the low signal-to-noise ratio and the absorption effects, that dominate the properties of downscaled images.

Model-based approaches are very powerful, but usually require to set up a problem-specific model. Furthermore finding the model parameters for a given data set is usually an ill-posed inverse problem, that needs to be solved by optimizers. The optimization might get stuck in local optima, if the initial parameters are not close enough to the optimal parameters. In high content applications they can usually only be applied, when the initial parameters are found by other approaches.

Purely data driven approaches usually need a large amount of (manually labeled) training data, because the computer has to learn all the possible variations from this data. For rotations a training with the structure of interest under all orientations is feasible in 2D, but hardly possible in 3D. For example, a quantization of the rotation angle in 10 degree steps would increase the size of the training data set in 2D by a factor of 36. In 3D a 10 degree resolution would result in $36^3/\pi \approx 15.000$ orientations, and therefore increase the training data size by 4 orders of magnitude. For other variations such data driven approaches are the appropriate instrument.

Invariance-based approaches can only be applied if an analytical description of the transformations is available, and usually incorporate complex mathematical frameworks. But if such invariants can be found, they provide a direct mapping of the input image to the desired output, without the need for iterative optimizations. Furthermore these approaches can be perfectly combined with data driven approaches and usually help to drastically reduce the required training data.

In the following we will show two closely related concepts for construction of such invariants for the group of rotations and translations (based on group integration and spherical harmonics). Additionally our recent applications of these concepts to several biomedical applications are reported.

3. INVARIANCE VIA GROUP INTEGRATION

The central idea of this framework is not to focus on the structure itself, but to focus on the transformations that may act on the structures without changing their semantic class (e.g. 3D rotations or translations). If there exists an analytical description of these transformations one can construct very general invariant features by integration over the corresponding transformation group (e.g. the group of Euclidean motions). For a given Image $X : \mathbb{R}^d \to \mathbb{R}, \mathbf{x} \mapsto X(\mathbf{x})$ and a transformation $g : \mathcal{F}(\mathbb{R}^d, \mathbb{R}) \to \mathcal{F}(\mathbb{R}^d, \mathbb{R}), X \mapsto gX$ which is an element of a certain transformation group $G \subset \{g \mid g : \mathcal{F}(\mathbb{R}^d, \mathbb{R}) \to \mathcal{F}(\mathbb{R}^d, \mathbb{R})\}$, we can construct an invariant feature by applying a nonlinear kernel function $f : \mathcal{F}(\mathbb{R}^d, \mathbb{R}) \to \mathbb{R}, X \mapsto a$ to the transformed image and integrate over the full transformation group.[2].

$$T[f](X) := \int_G f(gX)dg \tag{1}$$

where dg is the normalized Haar measure. To obtain different features, that describe the given structure, different kernel functions have to be used. The naive computation of the integral (1) would be computationally extremely expensive. So in practice the kernel function will be limited to a class of so-called n-point-kernel functions. I. e., if we can rewrite $f(X)$ as $f\left(X(\mathbf{x}_1), X(\mathbf{x}_2), \ldots, X(\mathbf{x}_k)\right)$ we only need to transform the kernel points $\mathbf{x}_1, \mathbf{x}_2, \ldots, \mathbf{x}_k$ accordingly instead of the whole data set X [2]. This computation is already much cheaper than the naive implementation. The cost can be further reduced by estimating the integral using a Monte-Carlo approach [3], or by using separable n-point-kernel functions, which can be computed by means of the fast Fourier transform [4] or by a spherical harmonics transformation [5, 6]. Further extensions of this framework include the computation of voxel-wise invariants for simultaneous segmentation and classification [5] and the incorporation of local deformations into the transformation group and a probability weighted group integration that make the features robust to arbitrary local deformations [6, 7].

These invariants were very successfully used for the classification of airborne pollen grains. A first data set contains 389 pollen grains from the 26 most prominent German airborne pollen taxa. 896 fully rotation invariant features per pollen grain were extracted using fast parameterizable kernel functions. With these feature set already a simple 1-nearest neighbor classifier was able to classify 386 of 389 (99.2%) correctly [6, 7].

A second data set (that corresponds to a typical biomedical high content experiment) was automatically collected, prepared and recorded over several months by the prototype of a fully automated pollen monitor. The data set contains about 195,000 pollen-like airborne particles recorded in 3D by a transmitted light microscope. About 22,700 of these particles are real pollen, belonging to 33 different pollen taxa, the rest is dust. Training and test data sets were separately recorded. By using a feature vector of about 87,000 invariants and a support vector machine for classification a precision of 97.2% at a recall of 84.6% was be reached (correctly recognized dust not counted) [6, 7]. The prior state of the art (on real air samples) was a precision of about 30% at a recall of 64.9% on a data set with only 8 different taxa.

4. ROTATION INVARIANCE USING SPHERICAL AND TENSORIAL HARMONICS

If the transformations are limited to rotations, we don't need the group integration framework. The data can directly be represented by spherical harmonics (or the spherical Fourier transform) which allows to extract rotation invariant features from the coefficients [8]. There is a close relation between the spherical harmonics approach and the group integration. By using the most simple two-point-kernel function and integration over the group of rotations, the extracted information is even identical. The only difference is that the information is represented similar to an auto-correlation function in the group integration approach and similar to the corresponding power-spectrum in the spherical harmonics approach.

The spherical harmonics (defined on scalar valued images) can be extended to tensorial harmonics [9, 10], which allows the computation of rotation invariant features for tensor valued images. Such images can be generated, e.g. by the voxel-wise computation of the structure tensor, and have significantly outperformed the spherical harmonics computed on the raw data, in an volumetric object classification task [9].

5. 3D OBJECT DETECTION WITH ROTATION INVARIANT FEATURES

A rotation invariant detection of objects and structures can be straight forwardly obtained by using the upper mentioned principles. For this the spherical harmonics transform for the local surrounding of every voxel and a classification of the computed invariant features by Support Vector Machines has to be performed [5]. While the performance of this approach was very good, the computational costs are far beyond the acceptable limits for high content or high throughput experiments.

Recent theoretical insights (based on spherical tensor algebra) have now provided a very fast way to compute voxel-wise spherical harmonic expansions for certain radial functions (Gaussian and Bessel) [10, 11]. The computation only requires convolutions for the radial part of the basis function (e.g. one convolution if a Gaussian is used). After that all expansion coefficients can be directly computed in the real space by so-called spherical derivatives that can be implemented by fast finite differences (like the ordinary derivatives). In a recent application (Detection of certain shaped cell nuclei in a confocal recording of an *Arabidopsis* root tip) rotation invariant voxel-wise features were computed from these coefficients and classified by a Support Vector Machine. The results clearly outperformed Spherical Hough Transform based approaches [11].

6. 3D OBJECT DETECTION WITH TRAINABLE HARMONIC FILTERS

Another very promising approach is to use the spherical tensor calculus for the design of trainable filters [12, 10, 13, 14]. The voxel-wise Gaussian windowed solid harmonic expansions are computed by the same scheme as mentioned in the previous section. But instead of computing invariant features and applying a classifier, the coefficients are non-linearly recombined and scaled by previously learned filter parameters, such that the backward transformation approximates best the desired output. This output is usually a bright dot at the center of the object, that has to be detected. The training is done by creating the desired output image (e.g. containing bright dots at the object centers) for a given training image and by learning the filter parameters with a least square approach. These harmonic filters have been successfully applied e.g., to the detection certain substructures in pollen grains [13]. An extension of this filter to multi-channel images was successfully applied to the detection of complex 3D mitotic structures in cell cultures, and the detection of mitoses in dense human tissue of colorectal cancer [14]. The approach clearly outperformed reference approaches using a steerable filter techniques, or standard morphological approaches.

A recent application of this framework is the rotation invariant landmark detection in confocal recordings of 72hpf zebrafish embryos. These landmarks will allow to bring the 3D dataset into a standard orientation, such that further processing steps (e.g. registration approaches) can start with a well defined parameter set. The first results are already very promising, and we are confident that this could become a central part of a high throughput zebrafish analysis pipeline.

7. ANALYSIS OF 4D DATA

For the analysis of complex 4D structures (3D+t), the same problems occur, like for the static data. Most available approaches are limited to low-level structures like blobs and foci. More complex structures, e.g. tissue with stained cell membranes, where the staining is not perfectly localized, cannot be tracked with these approaches.

The idea for design of general reusable modules is nearly the same as for the invariants. One should not focus on the complex structures itself, but first on the transformations. A very prominent example for that view is the optical flow, which is very common in the low-level computer vision, but seems to be only very rarely used in the biomedical image analysis. Recent optical flow algorithms [15] have reached a sub-pixel-accuracy, that allows to track structures reliably over hundreds of frames. In a recent study we applied a 3D version of this algorithm for tracking of certain anatomical regions in a developing frog kidney over about 240 frames[16]. The data was very noisy, had a strong decrease of intensity in z-direction, and only a very low z-resolution. The results

were surprisingly good. A reliable tracking of all relevant structures was possible, and the accuracy was more than sufficient to perform a quantitative comparison of the different growth patterns.

8. CONCLUSIONS

We have presented concepts that can lead to more general reusable image analysis modules for 3D and 4D data, especially when highly complex structures need to be analyzed. The main idea is to postpone the object or structure specific tasks (like segmentation) to a later step in the image analysis pipeline and to deal with the transformations first. The proposed methods are based on group integration, spherical harmonics analysis or optical flow.

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