Estimating Actin Fiber Orientation using Interpolation-Based Grey-Level Co-Occurrence Matrix Computation

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***Abstract*—A novel interpolation-based procedure for the computation of the grey level co-occurrence matrix is defined. Based on this procedure, a method for accurate texture orientation estimation is designed. The robustness of the method is tested against Gaussian noise and blurring. The method is applied to cell microscopy images for the characterization of actin subcellular arrangement.**

***Index Terms*—Image texture, Grey Level Co-occurrence Matrix, Gaussian noise, image blurring, fluorescent microscopy, subcellular analysis, actin.**

I. Introduction

Cell biologists study orientations of actin in a cell from microscopy images that vary spatially and temporally [1]. Actin appears as a set of parallel fibers that change orientation depending on the location in a cell in high resolution (63x) fluorescent microscopy images (see Fig. 1). The problem lies in designing a quantitative measurement of actin orientation that is accurate and robust with respect to microscopy imaging configurations. This is required as understanding the spatial and temporal changes of actin orientation provides insights into sub-cellular processes and cell responses to extracellular matrix mechanics [2]. As of now, in most cases this understanding is limited to visual inspection and qualitative assessment which motivates our work. Past work on the characterization of collagen fibers shows the importance of GLCM derived features, such as homogeneity and energy, which are maximal when computed along the perceived texture directionality [3].

The appearance of actin in a microscopy image can be classified locally as a textured region. One of the computational tools for characterizing texture is the Grey Level Co-occurrence Matrix (GLCM) [4]. GLCM computations are frequently used to capture second order statistics of image textures. They have been used in the past primarily for texture segmentation and classification tasks [5]. In this work, GLCM was used to estimate actin orientation by analyzing features extracted from GLCM, such as homogeneity, contrast, energy, and correlation.

The GLCM is calculated over a selected image region by counting the number of co-occurring intensity pairs. Locations of the co-occurring pixels under consideration are defined by fixed angle and offset (distance) values. In existing software libraries with GLCM implementations such as Matlab, ImageJ, or Pythonxy/scikit-im, the GLCM values are computed over pairs of angles and offsets that are constrained by the image lattice consisting of integer row and column locations of pixels. The lattice constraints on angle and offset values introduce uncertainty in the GLCM estimation since only specific pairs are considered. The novel contribution of this work is to enable the computation of the GLCM for real-valued angle-offset pairs, and to demonstrate the corresponding accuracy improvement of orientation estimation from GLCM derived features.

II. TECHNICAL APPROACH

The actin orientation estimation problem is divided into two steps. The first step focuses on the computation of GLCM derived features over a large set of real-valued angles

and offsets that are not limited to an image lattice. This is achieved by using an interpolation-based GLCM computation that can operate with any real-valued angle-offset pair.

The second step estimates texture orientation; it evaluates accuracy and robustness with respect to image blur and random noise. This was pursued by designing a method for estimating texture orientation from GLCM-derived features, and quantifying the accuracy and noise robustness of texture orientation estimates.

III. Robustness Evaluation and Application

The performance of the developed method was evaluated quantitatively on 258 synthetic images with known texture orientation, before applying the method to images from sub-cellular microscope experiments. The evaluation measured the angular error as the difference between the estimated and the known texture orientation in a given synthetic image. Synthetic images consisted of evenly spaced vertical bars of equal thickness and spacing. The bars were subsequently rotated by various amounts with interpolation, which led to multiple greyscale values. Then, one synthetic data set was created by introducing Gaussian image blur, and another set by adding Gaussian noise.

The experimental evaluation demonstrated minimal effect of blurring on orientation detection as long as the thickness of synthetic lines is larger than the offset used for GLCM computation. The angular error for directionality detection with Gaussian noise did not exceed 1° over the entire synthetic data set. The method was successfully applied to measured fluorescent images of actin as illustrated in Fig. 1. In the future, the actin fiber orientation will be used for spatial and temporal modeling of actin distribution.

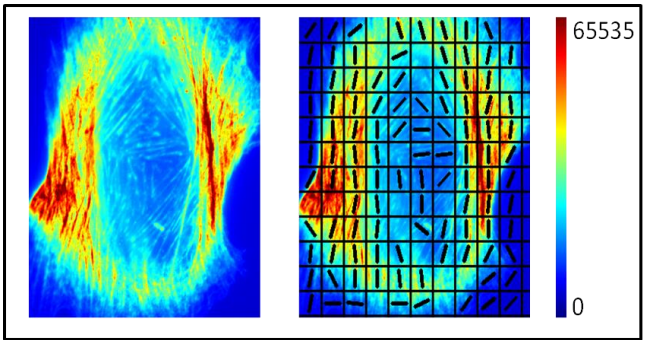

Fig. 1: A fluorescent microscopy image of an actin-stained fibroblast cell, showing actin fibers constituting cellular cytoskeleton (left). Local fiber orientation estimation results (right).

IV. Disclaimer

Commercial products are identified in this document in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the products identified are necessarily the best available for the purpose.

V. References

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