we follow an alternative approach based on stereology techniques. The fiber process under study in intersected with an independent test fiber process (e.g. random segments): the estimator of the intensity is then derived from the characteristics of the point process of the intersections [1]. In our case, the test process consists in random segments uniform in the window of observation, and the estimator of the intensity is proportional to the number of intersection points [2].

Our approach can be summarized as follows: we first train a detector for finding intersections of fibers with a given straight segment; such task is relatively straightforward and can be accomplished in a general way with a very limited amount of training data. Then, we exploit the probabilistic outputs of such detector, computed on a large amount of random segments, to obtain an estimate of the process intensity \( \hat{L}_A \). We also provide the variance of \( \hat{L}_A \), which quantifies the uncertainty of our estimate.

Compared to segmentation-based methods [3, 4, 5], our approach returns less information; for example, it does not directly provide information about the width of the fibers, or their topology, which may be of interest in some applications. On the other hand, it is general, parameter-free, and can be trained by a non-specialist user on a new scenario with few minutes of user-friendly inputs; on the contrary, designing even simple segmentation algorithms usually requires significant expertise and effort by image processing specialists. Moreover, our approach provides information about the confidence of the resulting measure, and is applicable to difficult

1. The variability due to the fact that we are observing a particular realization of the process; for example, the same angiogenetic mechanism (the process) creates different vascular networks (the realization) in different individuals.

2. The inaccuracy of the method for detecting fibers from images.

In this work, we provide a method for computing the intensity of a fiber process from an image of its realization. Moreover, we also provide an estimate of the uncertainty of the resulting measure, accounting for both aforementioned factors.

Instead of attempting to segment and track fibers, which is the traditional approach for quantifying images of this type, we follow an alternative approach based on stereology techniques. The fiber process under study in intersected with an independent test fiber process (e.g. random segments): the estimator of the intensity is then derived from the characteristics of the point process of the intersections [1]. In our case, the test process consists in random segments uniform in the window of observation, and the estimator of the intensity is proportional to the number of intersection points [2].

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Our approach is useful in different imaging modalities and scenarios; some examples: a) schwann cells in phase contrast microscopy; b, c) allantoid and neural cells under fluorescence microscopy, d) retinal vases from the DRIVE dataset, e) membranes of neural cells in electron microscopy. On each image, a single segment is shown with intersections detected (with \( p > 0.3 \)) after manual training on 10 different segments.

In all our test scenarios where a clear segmentation is not obvious, and human judgment plays an important role (see Figure 5).

The main contributions of the paper (Section 2) consist in: a) a segment-fiber intersection detector which provides probabilistic outputs, easily trained on different scenarios, and competitive with ad-hoc segmentation algorithms; b) a statistical technique which uses such data for the estimation of the intensity of fiber processes along with its uncertainty. Experimental results and examples are provided in Section 3.

2. ESTIMATING THE INTENSITY OF A STATIONARY FIBER PROCESS

As previously mentioned, our approach is based on detecting intersections between fibers in the image and random segments.

Let \( s_i \) be a random segment on the image; the proposed detector is based on a classifier, which for each point \( p_{ij} \) on \( s_i \) considers data from an elongated window \( w_{ij} \) centered on \( p_{ij} \), and aligned with \( s_i \). The classifier maps such data into two classes, intersection/nonintersection, and returns probabilistic outputs. In the current implementation we use a Random Forest Classifier (SVMs, Naive Bayes, TAN and other classifiers reported inferior results) [6]: features are computed by sampling RGB and HSV pixel values in \( w_{ij} \), then using Principal Component Analysis to reduce the dimensionality to a 15-variables feature vector. Improvements on this naive approach, such as using more sophisticated features or classifiers, are definitely possible and would be beneficial in several situations; on the other hand, using raw pixel data allows us to remain completely scenario-neutral and assume nothing about the fiber appearance.

The classifier is trained by user-labeled segments sampled from a set of training images. The first segment is presented to the user, which is asked to click on its intersections with fibers. For each clicked intersection, six positive training examples are generated by considering the point itself and its two neighbors along the segment, along with the corresponding points on the inverted segment. Negative examples are generated from the remaining points on the segment and its inverted version. The detector is then trained, and applied to a second segment. The user is asked to correct detection errors. The process is iterated until the user is satisfied by the detector accuracy.

Intersections with test segments are detected by applying the classifier to each point of the segment; the classifier returns soft information at each point – i.e. the probability of an intersection at \( p_{ij} \). Peaks of the resulting function are considered as candidate intersections, and are assigned the corresponding probability value. Such spatial post-processing of probability values is necessary as the classifier is expected to return nonzero probability values also when not exactly centered on the fiber, due to the fact that user-provided training data necessarily includes positive examples where the intersection point is slightly offset with respect to the point itself. However, due to the training data being symmetric, one can assume that off-center points will be assigned lower probabilities w.r.t. actual intersections, and this assumption is verified in all our test scenarios.

The set of candidate intersection points of a given segment, along with their probabilities, is used to compute a discrete probability distribution on the total number of intersections for segment \( s_i \); such distribution is found as a sum of conditionally independent bernoulli random variables. The resulting number of intersections is the expected value computed from this distribution.

Using such detector, we compute the number of intersections for a large number of random segments generated by a suitable Poisson segment process; the resulting estimator \( \hat{L}_A \) for the fiber process’ intensity \( L_A \) is proportional to the total number of intersections, is unbiased and has good asymptotic properties, such as asymptotic normality. Moreover, an upper bound on the estimator’s variance \( \text{Var}(\hat{L}_A) \) is found by

\[
\text{Var}(\hat{L}_A) \leq \frac{1}{n} \left( \sum_{i=1}^{n} \text{Var}(L_{A_{si}}) \right)
\]
considering the variance due to the particular realization of the fiber process (which depends on the size of the observed area) as well as the variance due to the detector’s inaccuracy, which are computed as per the technique in [2].

3. EXPERIMENTAL RESULTS

We present quantitative experimental results in several datasets with an available ground truth (GT) segmentation (from which we determine the real number of intersections with any segment $s_i$ by counting the connected components of the binary segmentation mask resampled along $s_i$): 

- simulated roots: 49 images [7];
- DRIVE dataset of retina blood vessels [8]: 20 training + 20 testing images;
- Angiogenesis on mouse cornea [9]: 16 images.

Moreover, we show in Figure 1 additional applications in different fields and imaging modalities, including phase contrast, fluorescence, confocal and electron microscopy.

The Angiogenesis dataset represents a real application of the role of intensity estimation. The images show corneas of mice first treated with an angiogenic factor (which induces the creation of a vascular network) and then treated with antibodies (either a placebo or against the angiogenic process). The aim was to decide which antibody was more able to inhibit angiogenesis.

Intensity is instead not an interesting quantity in the DRIVE dataset; we include it in the evaluation because it is a challenging, deeply studied scenario, and allows us to compare our intersection detection results to a number of ad-hoc segmentation algorithms. Note that, to fully address our intent of generality, we use original images in our evaluation and avoid any preprocessing/normalization even if the images have very uneven appearances, leaving to the classifier the burden of becoming insensitive to such artifacts.

A prerequisite for an accurate intensity estimation is that the intersection detector works as expected. Figure 3 reports confusion matrices for the number of detected intersections vs the corresponding ground truth intersections in a set of 3000 random segments. The detector finds a number of intersections well-correlated to the ground truth, save for some underestimation which is to be expected, as tangent fibers may be ignored by the detector but counted in the ground truth.

Figure 4 compares our detector to segmentation algorithms specifically designed for the DRIVE dataset; all algorithms are supervised and trained on the same 20-image DRIVE training set. We consider the absolute error between the computed number of intersections for a segment with respect to the GT, averaged over 2000 random segments on the 20-image DRIVE test set. Note that segmentation is much more information-rich than intensity estimation; on the other hand, such algorithms have been developed ad-hoc for that specific scenario, whereas ours is general (see Figure 1) and just required 5 minutes of training time by a non-specialist.

Other than being well-correlated in number, we also show that the detected intersections are at the expected positions; this is visible in Figure 4 (bottom) and in Figure 5, which shows, on the challenging mouse cornea dataset, the spatial distribution of the intersections detected in many segments (white-background images labeled det. inters.) matches the actual position of the fibers. The same Figure also illustrates a practical application example, in which our method’s outputs are used in order to derive confidence intervals to test the efficacy of an antiangiogenic antibody.

The system is implemented in MATLAB; source code for the intersection detector and intensity estimation is provided in supplementary material and allows to reproduce the presented results. Moreover, intermediate results and full-resolution images are also included.

1The results page for [8] at http://www.isi.uu.nl/Research/Databases/DRIVE/ is kept updated and reports these algorithms’ results.
Fig. 4: Performance comparison between our intersection detector and the intersections computed from the outputs of several segmentation algorithms (including a second manual segmentation) designed specifically for the DRIVE dataset [8]. Box plot of absolute error in the number of intersections per segment, computed on 2000 random segments from the 20-image DRIVE test set.

Fig. 5: Example application on mouse cornea dataset: the intensity of the fiber process modeling vessels in the treated eye is lower than in the control ($p$-value = 0.003). Images in the third column show the spatial distribution of detected intersections, which closely matches the ground truth segmentation.

4. CONCLUSIONS

We presented a general, practical technique for the quantitative analysis of fiber images. Being limited to intensity estimation, the approach provides less data than more traditional, competing approaches based on fiber segmentation/tracking, but has the advantage of being applicable to new scenarios with a simple, quick training. Moreover, the approach works well even when a clear segmentation is hard to define, and returns an estimate of the quality of result, which is a precious output for practical applications.

Experimental results show that the intersection detector on which the estimator is based performs competitively with ad-hoc segmentation algorithms when applied to the DRIVE dataset. Moreover, the resulting intensity estimates and corresponding variances are verified to be correct in synthetic simulations, and matching with expected outputs on real datasets.

5. REFERENCES


