Segmentation-Free Quantification of Spots on a Homogeneous Background

Max Hermann* University of Bonn, Germany
Reinhard Klein* University of Bonn, Germany
Thomas Schultz* MPI for Intelligent Systems, Tübingen, Germany; University of Bonn, Germany

ABSTRACT

A recurrent problem in biological image analysis is to quantify the number and size of spots on a homogeneous background. Most automated approaches rely on segmenting the individual spots, which becomes unreliable when the image contains artifacts, noise, or confounding objects. Therefore, practitioners often resort to tedious and time-consuming manual counting and measurements. As an alternative, we propose a visual analytics approach to this problem. It is based on Total Variation Flow, a partial differential equation that changes the intensities of image regions at a rate inverse to their scale. From this, we derive novel quantitative per-pixel measures of scale and density, and we show how the results can be combined with tools for visualization and selection to achieve a fast summary of median size and spot density in an image. Given a set of images, our framework places them on a 2D map that makes it easy to quickly compare them with respect to spot sizes and density. Our system is applied to real-world data from Stimulated Emission Depletion (STED) microscopy.

1 INTRODUCTION

Many biological microscopy images, such as those of membrane protein clusters [5] in Fig. 1, show spots on a homogeneous background. Analysis of such images requires counting and measuring the sizes of those spots, and even though sophisticated automated algorithms have been proposed for their segmentation [2], practitioners frequently resort to manual analysis because automated segmentation is not reliable enough in many real-world scenarios. In this work, we propose a novel approach to this problem that avoids having to segment the spots, and combines automated analysis with a small amount of user interaction to provide increased robustness.

2 RELATED WORK

We exploit a relationship between the size of image regions and the amount by which minimizing total variation, a popular method for noise removal [4], changes their intensity. Even though this relationship has been discovered early on [6], to our knowledge, it was used in a practical application only once, for texture segmentation [3]. Unlike this previous use case, we require *quantitative* scale estimates: While it is sufficient for texture segmentation to have a measure that distinguishes large from small scales [3], we require a measure with physical units that can be interpreted by a biologist. To increase accuracy, we also have to be more careful when setting several parameters. Human guidance can provide valuable help with this; therefore, we develop a visual analytics system that helps the domain expert understand and effectively use our total variation based scale measure.

3 A NOVEL SCALE MEASURE FROM TOTAL VARIATION

We now introduce total variation (TV) regularization and flow, explain its dependence on the size of image structures, and derive novel quantitative measures of scale and spot count in an image.

*e-mail:{hermann,rk,schultz}@cs.uni-bonn.de

IEEE Symposium on Visual Analytics Science and Technology 2014
November 9-14, Paris, France
978-1-4799-6227-3/14/$31.00 ©2014 IEEE

3.1 TV Regularization and TV Flow

In total variation (TV) based image regularization, the original grayscale image is modeled as a function $f : D \to \mathbb{R}$ with $D \subset \mathbb{R}^2$, and a regularized version u is obtained as the differentiable function $u : D \to \mathbb{R}$ that minimizes the energy functional

$$E(u;\alpha,f) := \iint_D \left(1/2\,(u-f)^2 + \alpha\|\nabla u\| \right) dx\,dy, \qquad (1)$$

where the parameter α controls the degree of smoothness of u.

In Total Variation flow (TV flow) [1], the original image f is embedded into a family of increasingly smoothed images $u(\mathbf{x},t)$, where $\mathbf{x} \in D$ and $t \in [0,\infty)$ is an artificial "time" parameter that specifies the degree of smoothness. TV flow assumes the initial value $u(\mathbf{x},0) = f$ and finds $u(\mathbf{x},t)$ for $t > 0$ by solving the partial differential equation $\partial u/\partial t = \operatorname{div}\left(\nabla_{\mathbf{x}} u/\|\nabla_{\mathbf{x}} u\|\right)$.

In general, the result of TV flow $u(\mathbf{x},t)$ at time $t = \alpha$ approximates TV regularization with parameter α [3].

3.2 Total Variation and Scale

In piecewise constant images, the absolute difference δ between the image intensity of a pixel in the original image, compared to the TV regularized image with parameter α, can be written as [6]

$$\delta = \alpha/s \quad \text{with} \quad s = |\Omega|/|\partial\Omega|, \qquad (2)$$

where the scale s of a pixel is defined as the ratio of the area $|\Omega|$ over the boundary length $|\partial\Omega|$ of the image patch the pixel belongs to.

The scale measure that was previously used for texture segmentation [3] is based on TV flow rather than TV regularization. It computes an average scale $\bar{m}$ over a time window $t \in [0,T]$:

$$\bar{m} = 4\frac{T - \int_0^T 1_{\partial_t u = 0}\,dt}{\int_0^T |\partial_t u|\,dt} \qquad (3)$$

Compared to the scale measure s, $\bar{m}$ replaces δ, the overall intensity change, by $\int_0^T |\partial_t u|\,dt$, the integrated amount of absolute change during $t \in [0,T]$. Given the approximate equivalence of TV flow after time T and TV regularization with parameter $\alpha = T$, $\int_0^T |\partial_t u|\,dt \approx \delta$ if the sign of $\partial_t u$ is the same throughout the interval $[0,T]$. Second, $\bar{m}$ replaces the regularization parameter α with $T - \int_0^T 1_{\partial_t u = 0}\,dt$, i.e., it subtracts periods during which pixel intensities did not change. Finally, $\bar{m}$ contains an additional factor of 4.

3.3 Proposed Scale Measure

In this work, we propose the following new scale measure:

$$\sigma = \gamma\frac{\int_{T_{\text{start}}}^{T_{\text{stop}}} 1_{|\partial_t u| > \Theta}\,dt}{\int_{T_{\text{start}}}^{T_{\text{stop}}} |\partial_t u| 1_{|\partial_t u| > \Theta}\,dt} \qquad (4)$$

Even though it is also based on TV flow, σ differs from $\bar{m}$ in three ways: First, it allows for a "burn-in" interval $[0, T_{\text{start}}]$ before we start the measurement. Originally, each individual pixel acts as a small region [3]. $T_{\text{start}} > 0$ allows for formation of more meaningful regions, and elimination of noise. Second, we introduce a

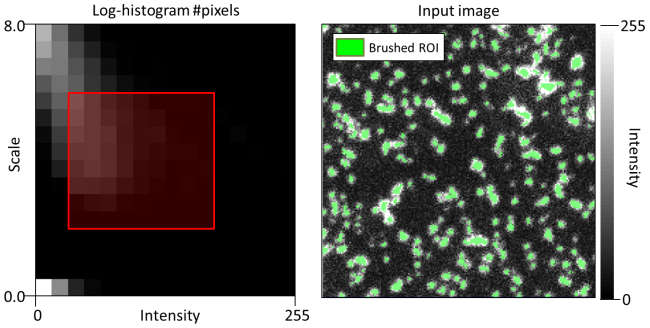

Figure 1: Brushing a joint histogram of scale and intensity makes it easy to select foreground pixels, shown here on a STED image.

threshold $\partial_t u < \Theta$ to reduce the impact of higher-order structures, which result when spots in the original image merge, on the scale measurements. Another way to reduce their impact would be to select a shorter T_{stop}; however, since low-contrast spots vanish more quickly than high-contrast ones, it is difficult to find a single T_{stop} that works well for the entire image.

Finally, we replace the fixed factor 4 by a variable shape factor γ, which can be used, depending on the physical size of a pixel and the shape of the expected spots, to calibrate σ to the physical units of length, specifying the radius of the spot. According to Eq. (2), for circular spots with constant intensity and pixel edge length l, $\gamma = 2l$.

3.4 Proposed Density Measure

A second parameter of interest is the number of spots per area. The area covered by a circular spot of radius σ is $A = \pi\sigma^2$. Thus, a pixel of area l^2 and scale σ accounts for the fractional part $\nu = l^2/\pi\sigma^2$ of a spot. The sum $\Sigma\nu$ of fractional spot counts of all foreground pixels in an image region provides a segmentation-free estimate of the contained number of circular spots. Normalizing by the overall region size A_R provides a spot density measure $\rho = \Sigma\nu/A_R$.

4 A Semiautomated Framework For Spot Analysis

We have designed and implemented a visual analytics framework to compute the scale and density measures defined in Sec. 3 for a given set of images, and to place them on a 2D map, allowing for a fast overview and navigation. A screenshot of our system is part of the supplementary material.

4.1 Selecting the Parameters

The main parameters of our scale measure σ are start time T_{start} and threshold Θ; the stopping time T_{stop} has little impact, since at later times, only large structures persist, which are filtered out by Θ.

To help select a suitable value of T_{start}, we provide a slider that allows the user to explore different values of diffusion time t to identify a point at which image noise has been removed, but the desired cluster structures are still well-preserved. Examples are shown in the supplementary material.

The derivative threshold Θ can be set based on Eq. (2): Since $\partial_t u \approx s^{-1}$, an upper bound $r_{\max}$ of relevant spot sizes leads to $\Theta = 2l/r_{\max}$.

4.2 Fast Spot Selection Using Brushing and Linking

Computing the density measure ρ from Sec. 3.4 requires to distinguish foreground pixels (belonging to spots) from the background. Our framework allows the user to quickly select these pixels by brushing a joint histogram of intensity and scale, excluding pixels that are too dark to be part of a spot, or outside the range of relevant scales. A mask highlighting the selected pixels is overlaid on the images, making it easy to confirm the selection (cf. Fig. 1).

We emphasize that, even though this step amounts to segmenting the image into foreground and background, it is fundamentally different from approaches that segment *individual spots* in order to count and measure them. The latter task is much harder, and is affected much more severely by noise or artifacts. For example, very few pixels could cause individual spots to merge or split, abruptly changing their size and number in the traditional approach; in our case, adding or removing pixels has a continuous and limited impact on the result.

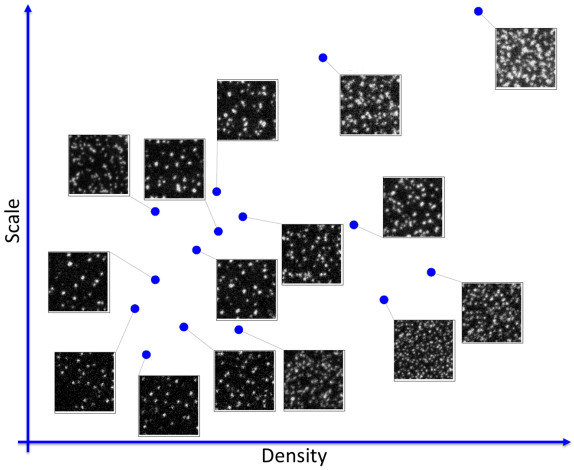

Figure 2: A set of microscopy images laid out according to their median scale and spot density, represented by image patches.

4.3 Mapping Images For Fast Navigation

Frequently, biologists acquire a large number of images, and would like to obtain a quick overview of how they differ with respect to spot density or scale. To this end, we place images on a 2D map, whose axes are given by the overall density (horizontal) and median scale (vertical) of the pixels selected in Sec. 4.2.

An example of such a map is shown in Fig. 2 (large version in the supplementary). Our system links this map to all other views, enabling the user to identify specific images (e.g., outliers), and to examine them in detail, along with their joint histograms.

5 Conclusion

We derive a novel scale measure from TV flow that allows for quantitative analysis of spots in biological images without requiring their segmentation. We present the theoretical foundations, as well as a visual analytics framework that is more robust to noise and image artifacts than fully automated segmentation-based methods, but requires far less interaction than manual counting and measuring.

Acknowledgements

The authors wish to thank Elisa Merklinger and Thorsten Lang (LIMES, University of Bonn, Germany) for co-designing the study and for providing preanalyzed raw data.

References

[1] F. Andreu et al. Minimizing total variation flow. *Differential and integral equations*, 14(3):321–360, 2001.

[2] E. Bernardis et al. Pop out many small structures from a very large microscopic image. *Medical Image Analysis*, 15(5):690–707, 2011.

[3] T. Brox and J. Weickert. A TV flow based local scale estimate and its application to texture discrimination. *Journal of Visual Communication and Image Representation*, 17(5):1053–1073, 2006.

[4] L. I. Rudin, S. Osher, and E. Fatemi. Nonlinear total variation based noise removal algorithms. *Physica D*, 60(1):259–268, 1992.

[5] J. J. Sieber et al. Anatomy and dynamics of a supramolecular membrane protein cluster. *Science*, 317(5841):1072–1076, 2007.

[6] D. M. Strong. *Adaptive Total Variation Minimizing Image Restoration*. PhD thesis, University of California at Los Angeles, 1997.