Probabilistic Classification of Disease Symptoms caused by *Salmonella* on *Arabidopsis* Plants

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Abstract: Several reports have linked food poisoning with the consumption of raw vegetables and fruits contaminated by *Salmonella*. Most studies suggested an extracellular lifestyle of *Salmonella* on plants. However, more recent studies show that *Salmonella* are also able to colonize the intracellular compartment of various plant tissues causing chlorosis and eventually death of infected organs. The aim of this work is to present a probabilistic classification algorithm for disease symptoms on *Arabidopsis thaliana* plant in order to improve the current biological research. The algorithm itself uses images of *Arabidopsis thaliana* leaves as input and consists of two steps. The first step is the detection of pixels belonging to a leaf. This is done with a globally optimal color segmentation method. The second step is realized with a probabilistic framework to classify each pixel. Finally a morbidity rate is computed based on the classification result.

1 Introduction

In recent time, several reports have linked food poisoning to the consumption of *Salmonella*-contaminated raw vegetables and fruits. Most studies suggested an extracellular lifestyle of *Salmonella* on plants. However, recent results have shown that *Salmonella* bacteria are also able to colonize the intracellular compartment of various plant tissues, causing chlorosis and eventually death of infected organs [SCCH08]. Moreover, similar to other plant pathogens this bacterium triggers complex host defense responses in *Arabidopsis thaliana*. Among other reactions to pathogenic bacteria, plants induce also so-called hypersensitive response (HR). Core of this reaction is the programmed cell death (PCD). PCD is a very tightly controlled process, in which infected areas or organs are sacrificed in order to stop the invaders. On leaves, PCD can be easily visualized since it causes yellowing of plant tissues. This process depends on numerous factors and can

Figure 1: Input images for the proposed classification algorithm. Left image: healthy plant. Right image: sick plant

be prevented by successful pathogens. To investigate how *Arabidopsis thaliana* defends itself and how bacteria interfere with plant immunity, we want to analyze the impact of different bacterial mutants on plant tissues. To solve this task, plants are infected with *Salmonella* and images of infected leaves are taken at different time points after infection. Typical input images can be seen in Figure 1. The task here is to establish an objective measurement for the disease rate in these leafs. This is done in two steps. First, for each pixel in an image the decision has to be drawn if it belongs to the leaf or not. This is done using a convex energy functional whose minimum is the desired segmentation. This topic is presented in the second section. Second, each pixel belonging to a leaf has to be assigned to a class (healthy vs. sick). This classification procedure is described in Section 3.

The workflow of the proposed algorithm is visualized in Figure 2.

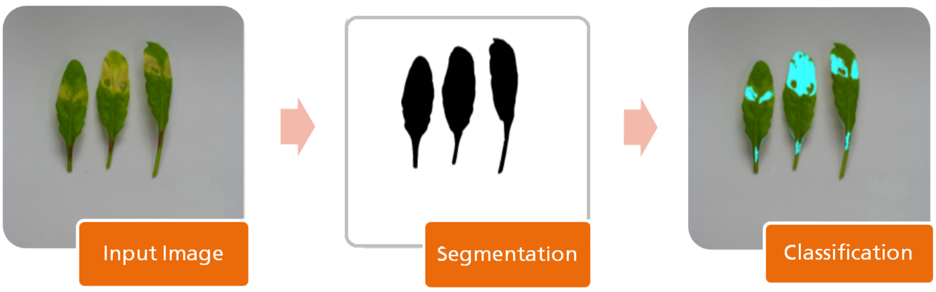

Figure 2: Workflow: First the input image is segmented into foreground (black) and background (white). Then for each foreground pixel a classification is performed. Unhealthy classified pixels are marked cyan.

2 Color Segmentation

The problem of extracting relevant objects from images can be seen as the segmentation of an image into two regions, foreground and background. All pixels labeled as foreground count as part of an object and are interesting candidates for further analysis. Image segmentation is a common task in computer vision, and many solutions have been proposed for this problem. Currently, the best solutions are provided by variational approaches. Three main classes of variational approaches exist for image segmentation, the first one being level sets [OS88, CV01]. The main advantage is that the energy functional being minimized is formulated continuously, so there is no need for discretization. On the other hand, the local optimization of the energy functional does not necessarily lead to a globally optimal solution. The second class are graph cuts [GPS89, BVZ01] with two main advantages: the computation time is generally very short and the solution is approximately globally optimal. The main disadvantage of this approach is the discrete formulation on a graph, leading to discretization errors. A combination of the benefits of those two methods constitutes the third class: total variation (TV) minimization. Chan et al. [CEN04] proposed this method for image segmentation of intensity-based images using a transformed Mumford-Shah model. Additionally in [SHRW09] and [UPCB08], it was shown how this approach can be extended to color images. In this paper, we will rely on [SHRW09] but use a different color space for segmentation.

2.1 TV-Segmentation

The segmentation of an image $I : \Omega \rightarrow [0,1]^3 \subset \mathbb{R}^3$ with $\Omega \subseteq \mathbb{R}^2$ can be seen as separation of the image plane Ω into disjoint regions $\Omega_1, \Omega_2, ..., \Omega_n$, with $\Omega = \Omega_1 \cup, ..., \cup \Omega_n \cup \Gamma$, where Γ denotes the contour of the segmentation. In the case discussed here, there will be only two regions Ω_{obj} and Ω_{bgd}, so we are looking for a binary image $u : \Omega \rightarrow \{0,1\}$.

In [SHRW09] the authors present a convex energy functional based on total variation. In their work they use the HSV color space. In order to be independent of illumination changes this color space is the correct choice. However, since the definition of the hue channel is done in polar coordinates, euclidean distances are not applicable on every channel identically, which is preferable. To obtain an independency of illumination changes and the ability to use the euclidean distance we will use the I1I2I3 color space, proposed by Hafner [Haf99]. The transformation of a RGB pixel value to an I1I2I3 pixel value can be denoted with:

$$I_{123}(\mathbf{x}) = \begin{pmatrix} \frac{1}{3} & \frac{1}{3} & \frac{1}{3} \\ \frac{1}{2} & 0 & -\frac{1}{2} \\ -\frac{1}{4} & \frac{2}{4} & -\frac{1}{4} \end{pmatrix} \cdot I(\mathbf{x}). \tag{1}$$

The first channel contains the illumination information. The second and third channel contain the color information. With this linear transform, we can derive a convex energy functional for color image segmentation in the proposed color space:

$$E(u, \boldsymbol{\mu}_{\text{obj}}, \boldsymbol{\mu}_{\text{bgd}}) = \int\limits_{\Omega} \left(f(I_{123}(\mathbf{x}), \boldsymbol{\mu}_{\text{obj}}) - f(I_{123}(\mathbf{x}), \boldsymbol{\mu}_{\text{bgd}}) \right) u(\mathbf{x}) \mathrm{d}\mathbf{x} + \lambda \int\limits_{\Omega} |\nabla u(\mathbf{x})| \mathrm{d}\mathbf{x}, \tag{2}$$

with

$$f(I_{123}(\mathbf{x}), \boldsymbol{\mu}) = w_1([I_{123}(\mathbf{x})]_{\text{I1}} - \boldsymbol{\mu}_{\text{I1}})^2 + w_2([I_{123}(\mathbf{x})]_{\text{I2}} - \boldsymbol{\mu}_{\text{I2}})^2 + w_3([I_{123}(\mathbf{x})]_{\text{I3}} - \boldsymbol{\mu}_{\text{I3}})^2 \tag{3}$$

denoting a weighted squared sum of the individual channels. For the results presented in this paper we use $w_{\text{I1}} = 0.1$ and $w_{\text{I2}} = w_{\text{I3}} = 0.45$. As additional input we use mean values for the foreground $\boldsymbol{\mu}_{\text{obj}}$ and background $\boldsymbol{\mu}_{\text{bgd}}$ and a smoothing parameter $\lambda \in \mathbb{R}$.

Theorem 2.1. *The proposed energy functional (2) is convex.*

The proof for this theorem is given in Appendix A. Using the Euler-Lagrange equation and a local optimization scheme (e.g successive over-relaxation) we can find the global minimum of (2), which is the desired segmentation. The Euler-Lagrange equation of (2) is

$$\frac{\partial E}{\partial u} = -f(I_{123}(\mathbf{x}), \boldsymbol{\mu}_{\text{obj}}) + f(I_{123}(\mathbf{x}), \boldsymbol{\mu}_b gd) - \lambda \text{div} \left(\frac{\nabla u}{|\nabla u|} \right) \tag{4}$$

3 Probabilistic Classification

The next step in the proposed algorithm is a classification of all pixels that were labeled as part of a leaf by the procedure from the previous section. Each classification algorithm has an offline and online phase. In the offline phase the classification model is learned. The actual classification is then performed in the online phase, where the measurements are checked against the learned model [Bis07].

3.1 Model Learning

In order to learn a non-over fitted model, we take several images from healthy leafs. Then, we perform a segmentation and save all leaf pixel values (several millions). To be independent from illumination changes we only use the second and third channel of the I1I2I3 color space, leading to two dimensional data points. We cluster the data points into M clusters (e.g. $M = 3$) using the k-means algorithm. Finally, for each cluster we compute its mean value $\boldsymbol{\mu}_i$ and covariance matrix Σ_i, with $i = 1, ..., M$. By using this multimodal color distribution we can provide a probabilistic model $\mathcal{M}$ for a healthy leaf. Since this step is quite time consuming, this type of model learning can be done offline before the actual classification task.

3.2 Model Checking

Given a probabilistic model $\mathcal{M}$ representing a healthy plant we can now efficiently check for each labeled pixel $\mathbf{x}$ if it belongs to this model. For this purpose we compute the following likelihood for every labeled pixel.

$$p(\mathbf{x}|\mathcal{M}) = \max_{i=1,\dots,M} \exp\left(-0.5 \cdot ([I_{123}(\mathbf{x})]_{I2,I3} - \boldsymbol{\mu}_i)^T \Sigma_i^{-1} ([I_{123}(\mathbf{x})]_{I2,I3} - \boldsymbol{\mu}_i)\right) \quad (5)$$

Herein, $[I_{123}(\mathbf{x})]_{I2,I3} \in \mathbb{R}^2$ denotes a vector which consists of the I2 and I3 channel information. Given a labeled pixel $\mathbf{x}$, we test the following condition:

$$1.0 - p(\mathbf{x}|\mathcal{M}) \geq \tau. \quad (6)$$

If (6) is true, $\mathbf{x}$ is classified as unhealthy, otherwise as healthy. A typical value for τ is 99.995%.

4 Results

In this section, we present some experimental results achieved with the proposed algorithm. In Figure 3, one can see a screenshot of the graphical user interface (GUI) developed for this task. The big benefit of this GUI is its simplicity and clarity. Users without knowledge about the underlaying algorithms can use them efficiently to classify. Additionally, one can see some classification and segmentation results for a given input image. Some further results are displayed in Figure 4. As it can be easily recognized, the automatic classification results match the visual perception of a human observer. These examples make clear that the proposed algorithm shows reliable results. Unfortunately, we did not have a ground truth for this data to intensively analyze the algorithm, but we can say with fair certainty that this work is a good basis for further development.

5 Conclusion

In this work, we present a probabilistic algorithm for classification of disease symptoms in *Arabidopsis thaliana*, caused by *Salmonella*. First, a detection of leafs in the input image is performed. This is achieved by a globally optimal color segmentation strategy based on total variation. Second, all leaf pixels are classified using a learned multimodal color distribution model and a likelihood function. In practical experiments, we could show a good performance. The presented algorithm can simplify the quantitative evaluation of plant defense reaction to bacterial infection, because he provides an objective rate of disease symptoms.

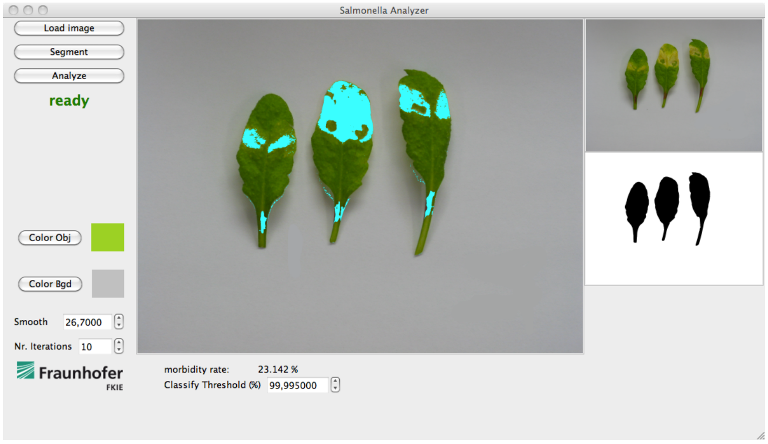

Figure 3: Screenshot of the algorithm GUI. In the center the marked pixels are displayed. On the right the input image and the segmentation result can be seen. On the left and at the bottom control parameters can be set.

A Proof of Theorem 2.1

Proof. For the sake of conciseness, we define

$$D := f(I_{123}(\mathbf{x}), \boldsymbol{\mu}_{\text{obj}}) - f(I_{123}(\mathbf{x}), \boldsymbol{\mu}_{\text{bgd}}). \tag{7}$$

To show that (2) is convex with respect to u we have to demonstrate that

$$\forall u_1, u_2 : E((1-\nu)u_1 + \nu u_2) \leq (1-\nu)E(u_1) + \nu E(u_2) \tag{8}$$

holds for all $\nu \in (0, 1)$. Now we can write:

$$E((1-\nu)u_1 + \nu u_2) = \int_\Omega D((1-\nu)u_1 + \nu u_2)\mathrm{d}\mathbf{x} + \lambda \int_\Omega |\nabla((1-\nu)u_1 + \nu u_2)|\mathrm{d}\mathbf{x} \tag{9}$$

$$= \int_\Omega (1-\nu)Du_1 + \nu Du_2 \,\mathrm{d}\mathbf{x} + \lambda \int_\Omega |(1-\nu)\nabla u_1 + \nu \nabla u_2|\mathrm{d}\mathbf{x} \tag{10}$$

$$\leq (1-\nu)\int_\Omega Du_1 \,\mathrm{d}\mathbf{x} + \nu \int_\Omega Du_2 \,\mathrm{d}\mathbf{x}$$

$$+ \lambda(1-\nu)\int_\Omega |\nabla u_1|\mathrm{d}\mathbf{x} + \lambda\nu \int_\Omega |\nabla u_2|\mathrm{d}\mathbf{x} \tag{11}$$

$$= (1-\nu)E(u_1) + \nu E(u_2) \tag{12}$$

□

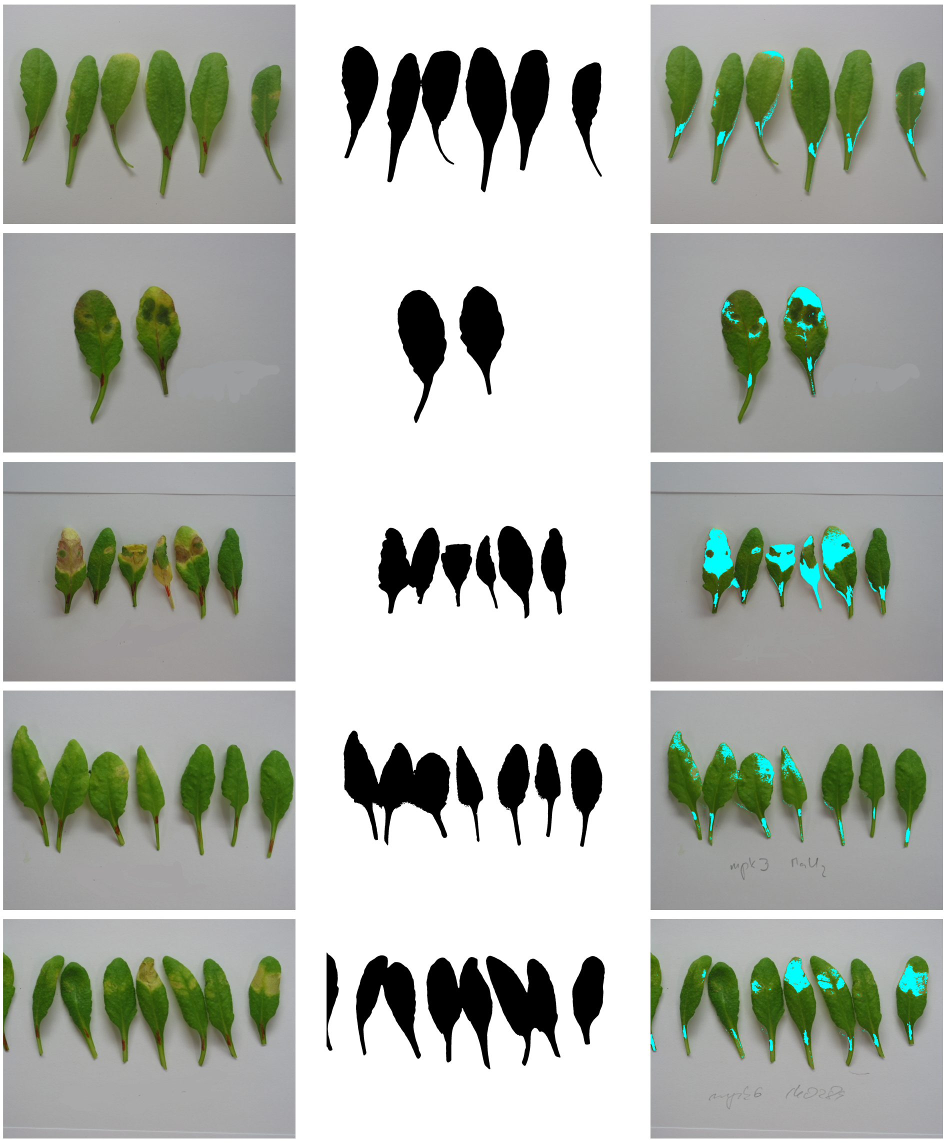

Figure 4: Classification results of the proposed algorithm. Left images: input; middle images: segmentation result; right images: classification visualization, with cyan marking.

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