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MPI-INF

DISSERTATION

# Physarum loves OATS!

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supervised by  
Prof. Kurt MEHLHORN

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# 1. NEFI

## 1.1. abstract

Networks are amongst the central building blocks of many systems. Given a graph of a network, methods from graph theory enable a precise investigation of its properties. Software for the analysis of graphs is widely available and has been applied to study various types of networks. In some applications, graph acquisition is relatively simple. However, for many networks data collection relies on images where graph extraction requires domain-specific solutions. Here we introduce **NEFI**, a tool that extracts graphs from images of networks originating in various domains. Regarding previous work on graph extraction, theoretical results are fully accessible only to an expert audience and ready-to-use implementations for non-experts are rarely available or insufficiently documented. **NEFI** provides a novel platform allowing practitioners to easily extract graphs from images by combining basic tools from image processing, computer vision and graph theory. Thus, **NEFI** constitutes an alternative to tedious manual graph extraction and special purpose tools. We anticipate **NEFI** to enable time-efficient collection of large datasets. The analysis of these novel datasets may open up the possibility to gain new insights into the structure and function of various networks. **NEFI** is open source and available at <http://nefi.mpi-inf.mpg.de>.

## 1.2. introduction

The study of complex network-like objects is of increasing importance for many scientific domains. The mathematical study of networks, Graph Theory, formalizes a network's structure by modeling the constituents of a network as *vertices* and the pairwise relations between them as *edges*. Some communities traditionally refer to vertices as nodes or sites and to edges as arcs or links. Networks are ubiquitous in everyday life. Examples are as diverse as the Internet, social networks, transportation networks, metabolic networks, blood vessels or the vein networks of leaves. For a comprehensive review see [19].

In situations where the extraction of a mathematical graph from a physical network is easy, the size of graphs that can be analyzed quickly increased from hundreds to millions of vertices. At the same time it became feasible to build large

databases of various types of networks. This enabled the application of software incorporating methods from statistics and graph theory to obtain many results that changed our understanding of large scale network structures. However, digitization remains difficult for many types of networks, e.g. leaf venations, blood vessels or food webs, and therefore ready-to-analyze datasets are often not available. In these cases, investigation on a larger scale requires tedious and sometimes error prone data acquisition.

In many experimental settings networks are initially available as high quality images obtained under laboratory control. Before any analysis can take place, it is necessary to extract the associated graphs from these images. This requires the identification of vertices and edges within the depicted structure. This process can quickly become very work-intensive even for smaller networks, which makes automated solutions indispensable.

Leveraging advances in computer vision, several authors have proposed and successfully implemented solutions for domain specific graph extraction applications. The authors of [20], [21] consider the mycelial networks of *P. impudicus*. They use watershed segmentation in combination with a novel enhancement step designed to highlight curvilinear features in the input networks. Based on the segmented image a skeleton is computed and used to extract the graph representing the input network. The resulting method is designed to be brightness and contrast invariant in order to correctly extract the networks grown by *P. impudicus* from challenging noisy or low contrast images.

Baumgarten et al. [4], [5] investigate the vein networks of *P. polycephalum*. For segmenting the input image they rely on careful constant thresholding followed by a sequence of restoration algorithms that try to repair the network in the segmented image. Next, the restored segmented image is used to compute a skeleton. After applying another sequence of correction steps, the skeleton is scanned to extract the graph of the input network.

In [7] a more general algorithm applicable to a variety of problems is proposed. Based on an original stochastic model, the authors use Monte Carlo sampling to obtain junction-points in the input image. This technically involved solution guarantees structural coherence for the resulting graph representation. Further examples include the extraction of road networks [18], retinal blood vessel analysis [14] and the extraction of plane graphs [25].

The three above mentioned algorithmic solutions for the network extraction problem exhibit one or more of the following limitations:

- They do not build on top of well-established computer vision methods and tend to rely on ad-hoc algorithms. As a result the quality of the method and its implementation could likely be improved. In addition, a lot of time is spent on reimplementing algorithms that are already available.

- They are not implemented or only available as pseudo-code.
- They are implemented but not designed for easy of use, distribution and extendability.

We are aware that the primary objective of the work cited above is not the production of reusable software, but of algorithms and tools for solving a concrete research question. As a result, the above authors have limited time for researching advances in computer vision, following best software engineering practice or writing documentation respectively.

From experience we know that when producing an easy-to-use software, a large part of the required work consists of specifying and improving the user-interface as well as working out minor bugs and annoyances. This type of work, while time consuming, is essential for any software aiming to reach a non-negligible audience. However, efforts like these are hardly attractive to researchers whose focus is on obtaining the next result. While we understand that under these circumstances the aforementioned limitations arise naturally, we strongly believe that it is necessary to overcome those limitations in order to increase the value and the impact of scientific software in general and network extraction software in particular.

To this end, we introduce **NEFI**, a lightweight piece of ready-to-go software intended to enable the non-expert to automatically extract networks from images. **NEFI** constitutes an extensible framework of interchangeable algorithms accessible through an intuitive graphical user interface.

We emphasize at this point that we do not claim to introduce novel techniques for image processing or computer vision. Instead, our contribution consists of a reusable, flexible and easily extendable toolbox combining well-known methods, which have become standard in their respective fields of origin, in a meaningful way. By introducing **NEFI**, we hope to make these methods more widely accessible to practitioners in other fields.

**NEFI**'s segmentation is based on a combination of standard routines available in **OpenCV**, (2015), [6]. These algorithms are known to perform well on clean and uncluttered images obtained under controlled laboratory conditions. However, on more challenging inputs of low contrast, strong gradients or similar irregularities, their performance is severely reduced. Nevertheless, in these cases more involved algorithms, currently not implemented as part of a reliable library and thus not integrated into **NEFI**, may still be able to process these images. To help meet this situation, **NEFI** was designed with extendability in mind. As a result users will find it easy to build on-top of **NEFI**'s code in order to add their own implementations of more sophisticated methods.

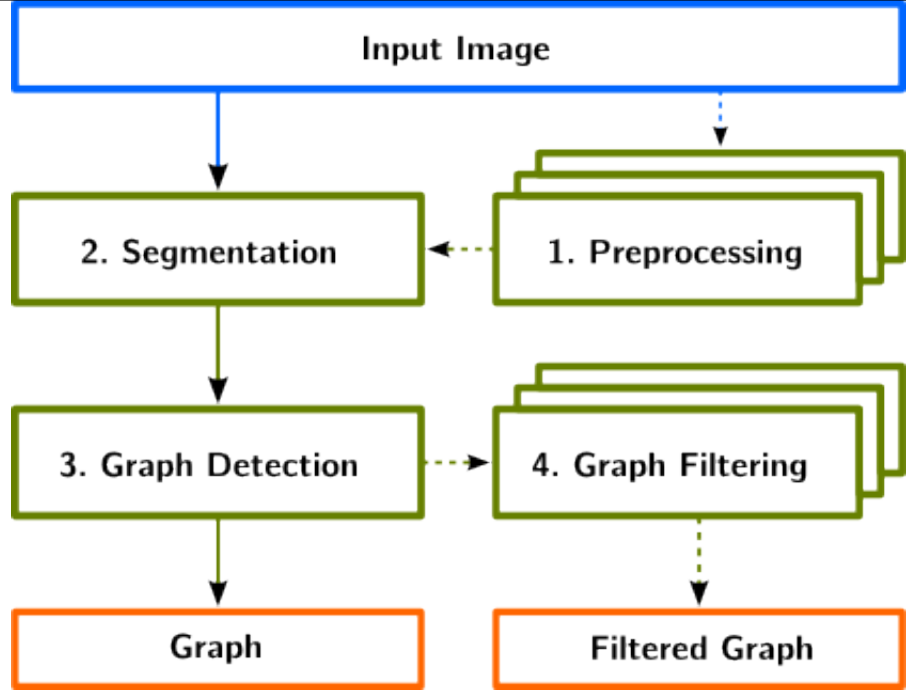


Figure 1.1.: A flow chart illustrating **NEFI**'s pipeline components in green boxes. Dashed arrows depict optional sections of the pipeline. Blue and orange boxes denote **NEFI**'s input and possible outputs respectively.

### 1.3. Network Extraction From Images

**NEFI** features a collection of image processing routines, segmentation methods and graph algorithms designed to process 2D digital images of various networks and network-like structures. Its main function is executing a so-called extraction pipeline, designed to analyze the structures depicted in the input image. An extraction pipeline, for short pipeline, denotes an ordered sequence of algorithms. A successful execution will return a representation of the network in terms of an edge-weighted undirected planar graph. Computed weights include edge lengths and edge widths. Once the graph is obtained, available graph analysis software [2], [3], [11], [15], [16], [27] or custom written scripts can be deployed to investigate its properties.

A typical pipeline combines algorithms from up to four different classes: preprocessing, segmentation, graph detection and graph filtering, see Figure 1.1. A more detailed description follows below.

For each pipeline section, **NEFI** typically offers several interchangeable algorithms to choose from. After executing preprocessing routines, a segmentation algorithm separates foreground from background. Then the foreground is thinned

to a skeleton from which the vertices and edges of the graph are determined. In the process various edge weights are computed. Finally, the graph can be subjected to a variety of useful graph filters. Figure 1.2 illustrates the intermediate results of **NEFI**'s pipeline steps listed in the order of their execution. When a pipeline is executed, **NEFI** makes all intermediate results available via its clean and intuitive GUI, see Figure 1.3.

Using the GUI all basic functions of **NEFI** can be accessed in an intuitive fashion. To facilitate ease-of-use, most of **NEFI**'s algorithms come with default parameters based on the settings in **OpenCV** [6], which were found to perform well on our test sets as well as on many other images.

There are various predefined pipelines to get started immediately. Alternatively, users may freely combine the various methods to build custom pipelines. Both approaches allow the user to experiment with the available methods in order to close in on the optimal settings for the data. Once a pipeline is constructed, it can be saved and reused. **NEFI**'s simple pipeline concept together with a self-explanatory graphical user interface make working with **NEFI** intuitive and straightforward. **NEFI** also offers a command-line mode, which is suited for batch processing.

**NEFI** comes with a number of example images from different domains which we use to produce the figures in this work. Figure 1.4 and Figure 1.5 show **NEFI**'s output on two images using predefined pipelines. Blue squares denote the vertices and red lines the edges of the detected graph. The thickness of the detected edges corresponds to thickness of the depicted structures. For comparison the extracted graph is drawn on top of the input image. We present a detailed quantitative evaluation in a later section.

We stress that **NEFI** can deal with a range of inputs from various domains as long as they are of sufficient quality. In addition to the examples shown above, it has been successfully used to process images of natural (e.g. leaf venation, patterns of mud cracks) as well as man-made structures (tilings). It is also straightforward to add custom extensions. We provide a well documented platform which allows programmers to include more specialized segmentation algorithms or additional graph filters. For an overview of alternative graph extraction approaches see for example [9].

Next, we discuss the purpose and design of each major stage of the pipeline and highlight some of **NEFI**'s strong points.

### 1.3.1. Preprocessing Collection

The preprocessing section of the pipeline offers various standard image processing algorithms intended to be used prior to the segmentation step. Preprocessing methods may be exploited to affect the output of the segmentation step. For

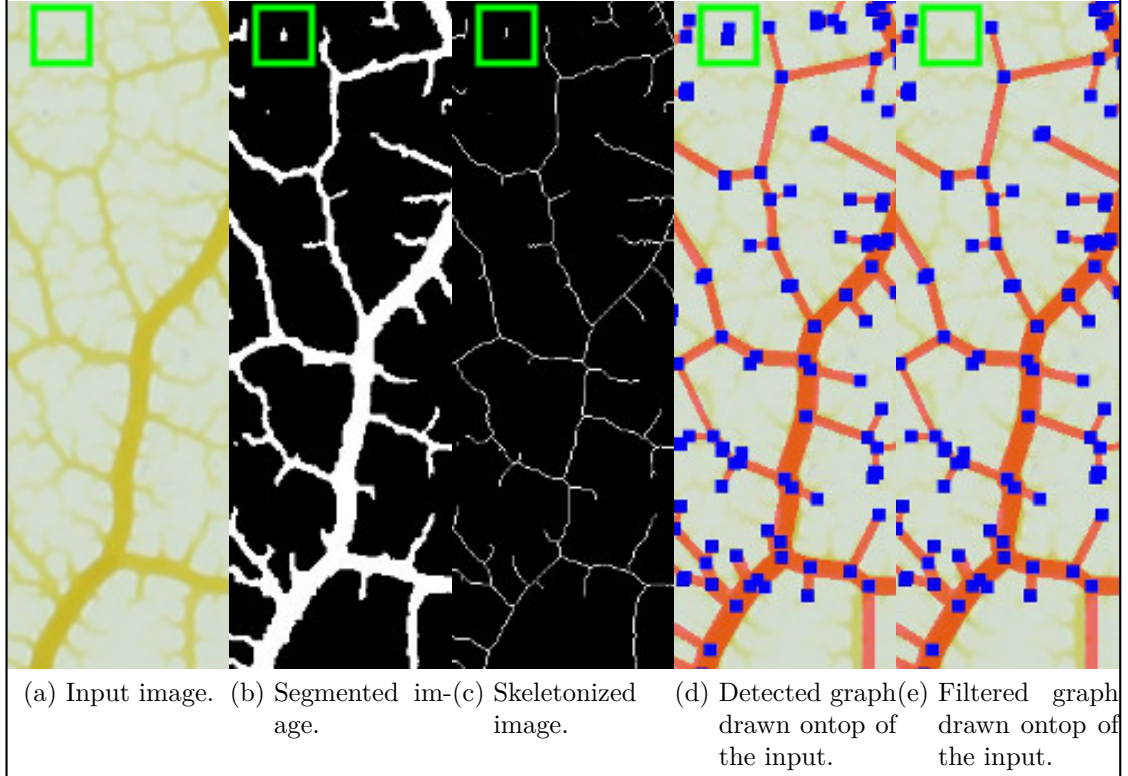


Figure 1.2.: Direct comparison of **NEFI**'s pipeline steps given a slice of an image of a slime mold (*P. polycephalum*). From left to right: input image, segmented image, skeletonized image, detected graph and filtered graph. The green square contains a very faint vein which the segmentation did not pick up fully. Thus, the skeleton becomes fragmented which leads to spurious vertices in the detected graph. By applying a graph filter we remove stray vertices without manipulation of the segmented or the skeletonized image. Similar filtering can remove “dead-ends”, i.e. vertices that do not belong to any cycle in the graph.



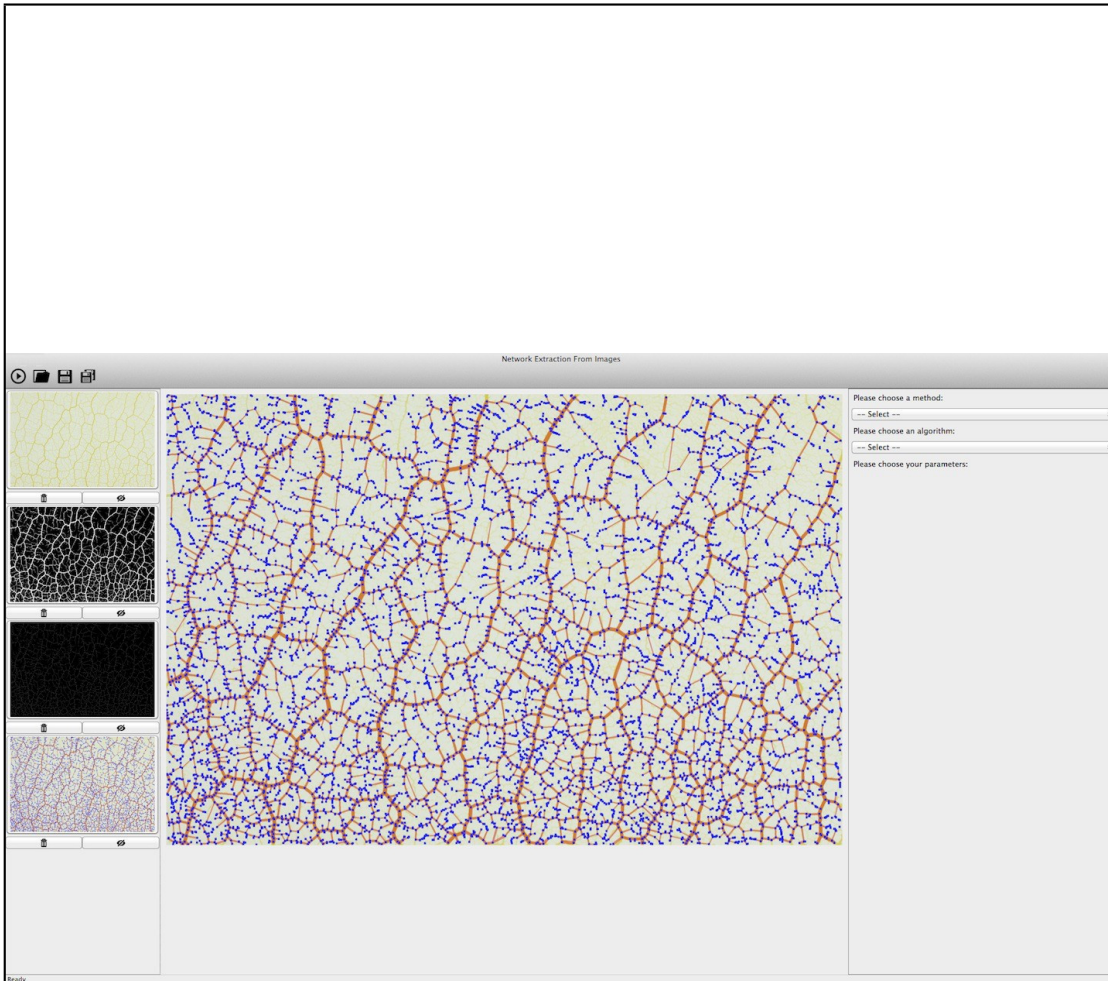


Figure 1.3.: A screenshot of **NEFI**'s GUI running on Mac OS. On the left hand side **NEFI** lists intermediate results as thumbnails. Bringing the final result to the center workspace allows for direct visual assessment of the quality of the extracted graph. On the right hand side **NEFI**'s pipeline elements can be accessed via drop-down menus. The image was produced in a collaboration with the KIST Europe.

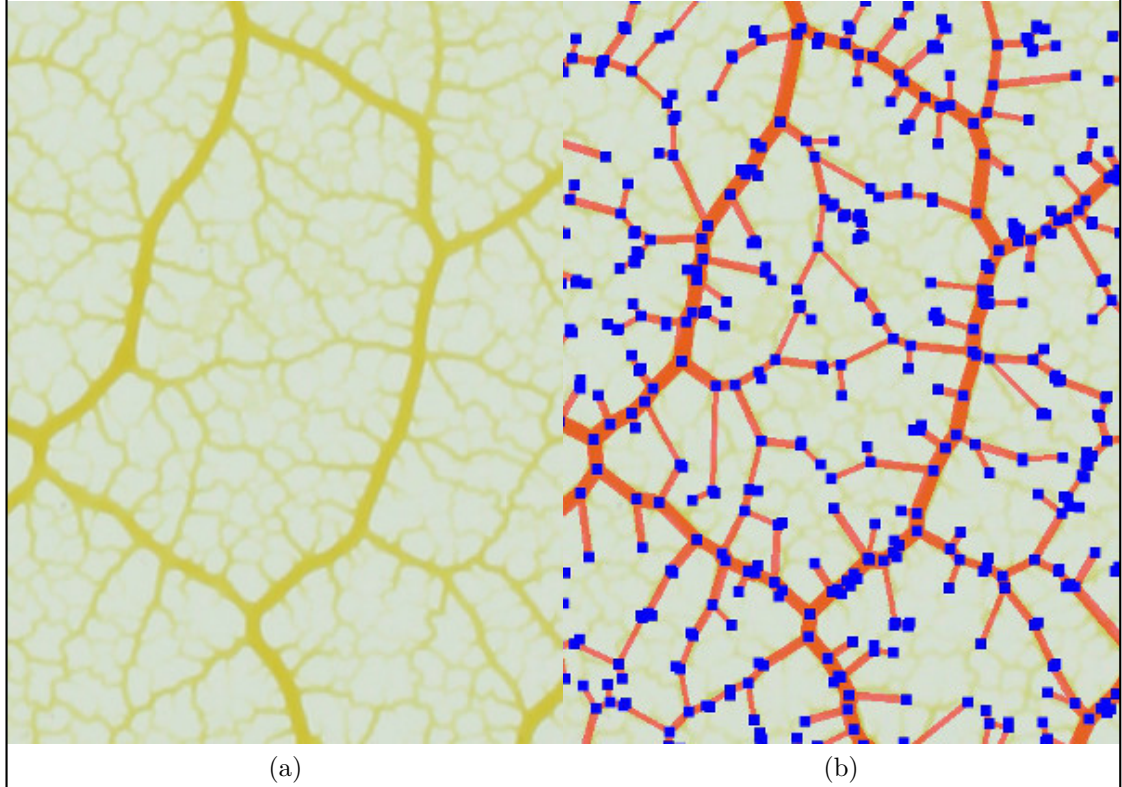


Figure 1.4.: Extracted graph of the network formed by a slime mold (*P. polycephalum*). The left hand side shows the input image depicting the network. The right hand side shows the extracted graph overlayed on top off the same image for direct comparison. Note, that no filters have been applied. The image was produced in a collaboration with the KIST Europe.



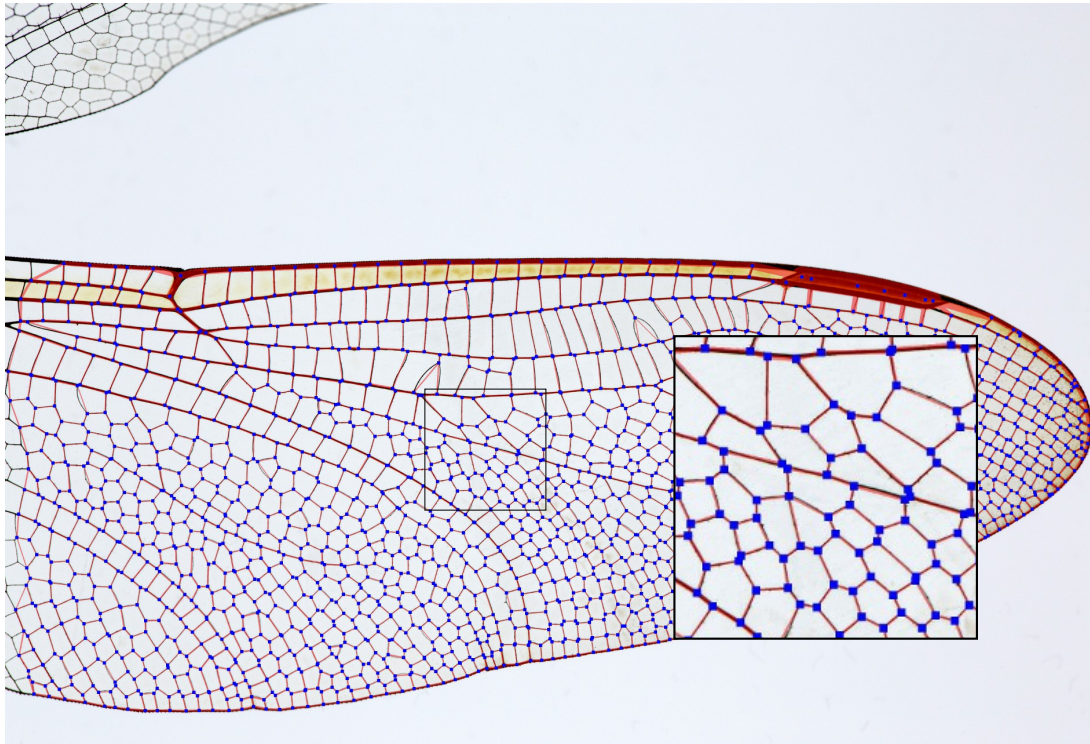


Figure 1.5.: Extracted graph of the vein network exhibited by a wing of a dragonfly (*A. junius*). Note, that after the use of various filters a very clean-looking graph is obtained. Image courtesy of Pam and Richard Winegar.

example, adding a slight blur to an input image may benefit the overall result by reducing the amount of spurious white pixels in the segmented image. However, blurring too much will remove fine detail and reduce accuracy in determining the thickness of depicted edges. As a result, we recommend to experiment with different approaches and parameter settings in order to decide how to use preprocessing. For images of sufficient quality we found that excellent results can be obtained without preprocessing.

NEFI relies on **OpenCV** [6] for preprocessing and offers Gaussian and Median Blurring, Denoising as well as Bilateral Filtering.

### 1.3.2. Segmentation Collection

The goal of the segmentation step is separating the image foreground, i.e. the structures of interest, from the remaining image. **NEFI** builds on top of **OpenCV** [6] combining different segmentation algorithms. The general-purpose algorithms shipped with **NEFI** have become standard in image processing and perform reliably well if input images are clean, devoid of strong gradients and have a good contrast between fore- and background. Conversely, if the input becomes more challenging, the effectiveness of **NEFI**'s segmentation degrades quickly and more complex or domain-specific algorithms become necessary. We defer a quantitative study of how the properties of the input image affect **NEFI**'s performance to the Section *Evaluation*.

**NEFI**'s segmentation is designed such that several algorithms can be used interchangeably. We included basic thresholding algorithms like Otsu's method [1] or adaptive thresholding as well as more involved segmentation routines such as guided watershed [17] and the GrabCut algorithm [24]. The last two methods receive as an additional input a so-called marker. The better the markers approximate the foreground, the better these algorithms work. **NEFI** offers several marker strategies which can be used interchangeably together with the respective marker based segmentation routines.

Interchangeability of the algorithms is a core design principle of all pipeline steps.

This design facilitates easy experimentation with different methods. Our own experience shows that often it is not clear a priori which methods work for a given input image. This decision usually also depends on the desired degree of detail in the final output, where less sensitive methods might produce fewer false positives. This ease of experimentation with quick visual feedback from the GUI is one of the major strong points of **NEFI**.

The flexibility is not limited to the algorithms we provide. Instead, **NEFI**'s software design makes it easy to integrate additional methods. We expect that in practice challenging inputs will be encountered for which the algorithms currently

offered by **NEFI** will be insufficient. In these cases a potential user may choose to implement additional, perhaps domain-specific, methods. By extending **NEFI**, the user can rely on existing modules and thus save a lot of time. The authors are convinced that improved extendability is another strong point of our work.

### 1.3.3. Graph Detection Collection

The graph detection collection consists of algorithms that take a segmented image as input and detect the nodes and the edges of the graph. We offer a colloquial description of the actual algorithm because we do not rely on well-documented library code for this section of the pipeline.

The first step for graph detection is called thinning. Here we reduce the segmented foreground such that every line is only one pixel thick, while preserving the connectivity properties of both the foreground and the background pixels. The result of this process is called the skeleton of the segmented image. To do so we implemented the algorithm by Guo and Hall [10]. It always produces thin results and preserves 8-connectivity of the foreground pixels. A pure Python implementation proved to be fairly slow, hence we chose to implement this function as a C extension.

For fairly thin foreground features this method is nearly flawless and finds a skeleton where the lines lie in the center of the foreground areas. However, large foreground sections lead to artifacts in the skeleton whose exact shape depends on the noise present at their borders.

On the skeleton we then detect the positions of nodes. For this purpose we adapt criteria from thinning algorithm by Zhang and Suen [28]. A white pixel becomes a node if its removal creates exactly one or at least three 4-connected white components in its 1-neighborhood. In the former case the pixel forms the end of a path, otherwise it is the meeting point of at least three edges.

Note that due to this step, the maximum degree of the graphs we detect is limited to four. This is inevitable if nodes are detected at single-pixel locations. For higher degree nodes we will create several nodes of limited degree that are very close to each other and can be merged by a later post-processing step.

Given the node positions, it is very simple to find the edges. We perform a variant of breadth first search on the white pixels in the skeleton, starting from each node simultaneously. Each white pixel around a node gets a unique number and a queue. In each step we iterate over all queues and take out the first pixel. If it is unmarked, we mark it with the unique number of this queue and enqueue all its white neighbors. Otherwise, we have detected an edge, i.e. there is a path along white pixels that connects two nodes.

While walking along the pixels we record the length of the edge. Horizontal and vertical steps count as one unit, diagonal steps count as  $\sqrt{2} \approx 1.41$  units.

The diameter of an edge calculated by computing a distance transform on the segmented image. This assumes that the thinned edge lies in the middle of the actual edge. Computing the diameters is now a simple lookup of each edge-pixel from the skeleton in the distance transformed image. As we have the diameters along the whole edge on hand by this procedure, we then compute a median and a variance.

For handling the graph we rely on **NetworkX** [12].

#### 1.3.4. Graph Filter Collection

The graph filter collection offers the possibility to add powerful processing steps that directly apply to the graph obtained after graph detection.

Often it is possible to improve the result by removing unwanted artifacts in the segmented image or during later processing stages. A common strategy, used for example in [4], [5], consists of “reparing” the errors in the skeleton using heuristics or user assisted methods. However, these methods carry the potential danger of introducing additional errors.

NEFI pursues a novel approach by exploiting the structure of the extracted graph. First, we retain a maximum of structural information by not altering the segmented image or the skeleton at all, i.e. we establish the graph including all artifacts. Then we use dedicated graph filters to remove said artifacts. For example, if the network in the input image is reasonably large, it will result in a large connected component in the graph. Small components resulting from noise can thus be removed effectively and safely. Since the effects of filtering the graph can immediately be evaluated by visual inspection, we prefer graph filtering over less transparent approaches that take place before the graph was established. Figure 1.2 illustrates the use of filtering.

We have used filtering with sensitive segmentation to obtain surprisingly good results. Overly sensitive segmentation picks up fine detail but also introduces artifacts. However, almost all of the artifacts result in very small components that can easily be removed by filtering. The desired detail will remain mostly unaffected because it is part of the largest component. The graph depicted in Figure 1.5 was obtained using this technique.

Filtering may also be used to remove parts of the graph which are not of interest. The following filters are predefined in **NEFI**. A filter removing everything not in the largest connected component, one smoothing vertices of degree two except if this introduces parallel edges and finally, one which removes all vertices and edges that are not contained in a cycle. Filters may be freely combined in any order. Naturally, the filter collection is designed for extendability.

Graph filtering and its various applications delivers excellent results. To our knowledge, no other software offers such tools as part of its core workflow. For

this reason, we consider this one of **NEFI**'s strong points.

## 1.4. Evaluation

To assess **NEFI**'s performance we investigate the output quality given input images of varying quality. Additionally we report on **NEFI**'s speed.

### 1.4.1. Defining a Graph Similarity Measure

As a quality measure, we need to quantify the degree of congruence between the graph depicted in the original input image  $i$  and the graph computed by **NEFI**.

Let  $A = (V_A, E_A)$  be the *true* graph correctly describing the structure depicted in  $i$ , with  $V_A$  and  $E_A$  denoting its vertex and edge set respectively. We call  $A$  the *ground truth*, which is of course not known in general. Furthermore, let  $B$  denote the graph obtained by executing one of **NEFI**'s pipelines. Note that,  $A, B \in \mathcal{G}$ , where  $\mathcal{G}$  denotes the set of undirected edge-weighted planar graphs where vertices are labeled with their respective euclidean coordinates in the plane. With these definitions we propose a similarity measure  $s$  mapping any pair of graphs  $A, B \in \mathcal{G}$  onto a number  $s \in [0, 1]$ .

We compute a correspondence of vertices in  $A$  to vertices in  $B$ . Two edges  $e \in E_A$  and  $f \in E_B$  then correspond if their endpoints correspond. We choose the correspondence such that the following intuitive notion of similarity are optimized.

1. Positions of vertices in  $V_A$  are similar to positions of corresponding vertices in  $V_B$ .
2. Edges in  $E_A$  including their weights are similar corresponding edges in  $E_B$ .

For an exact definition of  $s$  and the notions of *similarity* and *correspondence*, we refer the reader to the supplementary material.

We require that the measure is maximal if any graph  $A$  is compared with itself, that is  $s(A, A) = 1$ . Consequently, if  $A$  is completely different from  $B$  we have  $s(A, B) = 0$ . This minimum value is assumed if no viable correspondence between  $V_A$  and  $V_B$  can be found. Naturally, the value of  $s(A, B)$  increases (decreases) if the similarity between  $A$  and  $B$  increases (decreases).

### 1.4.2. Similarity Measure for Evaluation

In order to determine the overall quality of **NEFI**'s output we need to quantify the degree of similarity between two graphs  $A = (V_A, E_A)$  and  $B = (V_B, E_B)$  using a graph similarity measure. Let  $A, B \in \mathcal{G}$ , where  $\mathcal{G}$  is a set of undirected

Connect this part to the next section instead ...

edges-weighted planar graphs where the nodes are labeled with their respective coordinates in the euclidian plane. Then we may define a similarity measure  $s$  as follows:

$$s : \mathcal{G} \times \mathcal{G} \rightarrow [0, 1] \quad (1.1)$$

$$\forall A, B \in \mathcal{G} : s(A, B) = \text{matching}(A, B) \quad (1.2)$$

Where  $\text{matching}(A, B)$  denotes the normalized cost of a minimum cost graph matching problem which we will define shortly. Given this definition the degree of similarity between  $A$  and  $B$  is quantified by the value of  $s(A, B)$ . The similarity measure we propose has the following desirable properties:

- $A$  compared to itself yields a maximum similarity score of  $s(A, A) = 1$ .
- If there are no vertices in  $V_A$  with corresponding similar nodes in  $V_B$  and no edges  $E_A$  with corresponding similar edges in  $E_B$  the minimum similarity score of  $s(A, B) = 0$  is obtained.
- The similarity score  $s(A, B)$  increases (decreases) if the similarity between  $A$  and  $B$  increases (decreases).

We proceed with making the notions of *similarity* and *correspondence* more precise by constructing a minimum cost graph matching problem. Given the two graphs  $A, B \in \mathcal{G}$ , let  $n_A, n_B$  denote the number of nodes and  $m_A, m_B$  denote the number edges of the respective graphs. Note that  $n_A$  and  $n_B$  can differ, as can  $m_A$  and  $m_B$ .

We can now define a matching between  $A$  and  $B$  consisting of three parts. First we define a matching between the node sets  $V_A, V_B$ , then we define a matching between the edges sets  $E_A, E_B$  and finally we introduce a coupling between the two. To obtain a solution to the problem we rephrase the combined matching problem as an integer linear program and solve it using IBM CPLEX. For ease of exposition, however, we discuss the problem using the language of matchings.

For the node matching, we match each node  $i \in V_A$  with at most one node  $j \in V_B$ . We define a variable  $x_{ij}$  that is 1 if we match  $i$  with  $j$  and 0 otherwise. With a match we associate a cost of  $\Delta_V(i, j)$ . The function  $\Delta_V(i, j)$  returns the euclidean distance between node  $i$  and node  $j$ . If any node  $u$  remains unmatched, we assign a penalty of  $p_V(u)$  to it. A node can either be penalized or matched. We thus compute a minimum cost node matching, in which it is favorable for nodes in  $A$  to be matched with similar nodes in  $B$ . We call such a matching pair of similar nodes *corresponding*.

The edge matching proceeds analogously to the node matching. Each edge  $e \in E_A$  is matched with at most one edge  $f \in E_B$ . We denote this match with

a decision variable  $y_{ef}$  and associate a cost of  $\Delta_E(e, f)$  with it. The function  $\Delta_E(e, f)$  returns the sum of the differences in edge weights between edge  $e$  and edge  $f$ . If any edge  $d$  remains unmatched, we assign a penalty of  $p_E(d)$  to it. An edge can either be penalized or matched. We thus compute a minimum cost edge matching, in which it is favorable for edges in  $E_A$  to find a match with a similar edge in  $E_B$ . We call such a matching pair of similar edges *corresponding*.

As of yet both matchings are independent of each other. In particular this allows an edge  $e$  to be matched with an edge  $f$  independently from their respective positions in the plane as long as they have similar weights. A more meaningful matching favors pairing similar edges where the endpoints of both edges are similar to each other, i.e. geographically close. This additional constraint introduces a coupling between node and edge matching. To enforce coupling we add the constraint that an edge  $e = (a, b) \in E_A$  can only be matched to an edge  $f = (a', b')$  if the respective nodes participate in a matching as well. That is, we require  $a$  to be matched to  $a'$  and  $b$  to be matched to  $b'$  or alternatively  $a$  to be matched to  $b'$  and  $b$  to be matched to  $a'$ .

By combining node matching, edge matching and the additional coupling constraint we obtain the final minimum cost matching problem. We rephrase the uncoupled matching problem as an integer linear program (ILP) as follows:

$$\text{minimize : } f = \sum_{\substack{i \in V_A \\ j \in V_B}} \Delta_V(i, j) x_{ij} + \sum_{i \in V_A} p_V(i) \bar{x}_i + \sum_{j \in V_B} p_V(j) \bar{x}_j + \quad (1.3)$$

$$\sum_{\substack{e \in E_A \\ f \in E_B}} \Delta_E(e, f) y_{ef} + \sum_{e \in E_A} p_E(e) \bar{y}_e + \sum_{f \in E_B} p_E(f) \bar{y}_f \quad (1.4)$$

$$\text{s.t. : } x_{ij} \in \{0, 1\} \quad i \in V_A \quad j \in V_B \quad (1.5)$$

$$y_{ef} \in \{0, 1\} \quad e \in E_A \quad f \in E_B \quad (1.6)$$

$$\forall i \sum_j x_{ij} \leq 1 \quad \forall j \sum_i x_{ij} \leq 1 \quad (1.7)$$

$$\forall e \sum_f y_{ef} \leq 1 \quad \forall f \sum_e y_{ef} \leq 1 \quad (1.8)$$

$$\bar{x}_i = 1 - \sum_{j \in V_B} x_{ij} \quad \bar{x}_j = 1 - \sum_{i \in V_A} x_{ij} \quad (1.9)$$

$$\bar{y}_e = 1 - \sum_{f \in E_B} y_{ef} \quad \bar{y}_f = 1 - \sum_{e \in E_A} y_{ef} \quad (1.10)$$

To introduce the coupling between the vertex and the edge matching, we add an additional constraint as described above:

$$\forall e = (a, b), f = (a', b'): \quad 2y_{ef} \leq x_{aa'} + x_{ab'} + x_{ba'} + x_{bb'} \quad (1.11)$$



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Given the optimal solution to the ILP the optimal matching for nodes and edges can be recovered. Thus, we know what matching pairs have been formed and which nodes and edges remained unmatched. We use this information to define true positives, false positives and false negatives as described in the main manuscript.

In addition, we obtain the optimal value of the objective function  $OPT$ , i.e. the cost associated with the selected optimal matching. The value of  $OPT$  includes both the costs incurred by node and edge matches as well as the penalty terms arising from nodes and edges that cannot be matched. As a result it reflects the similarity between the two graphs  $A$  and  $B$ .

We point out that  $OPT = 0$  if a graph  $G$  is matched with itself. Every single node and edge finds its exact copy as a match of cost 0 for itself and thus no penalties arise. The other extreme is realized when there are no vertices in  $V_A$  with corresponding similar nodes in  $V_B$  and no edges  $E_A$  with corresponding similar edges in  $E_B$ . In this case the value of  $OPT$  attains its maximum  $\overline{OPT}$  because all nodes and edges of both graphs are penalized. Naturally, the value of  $OPT$  increases (decreases) with decreasing (increasing) graph similarity. Given these observations it is natural to use  $OPT$  and  $\overline{OPT}$  to define a similarity measure between two graphs  $A, B \in \mathcal{G}$  as follows

$$s(A, B) = \text{matching}(A, B) = 1 - \frac{OPT(A, B)}{\overline{OPT}(A, B)} \quad (1.12)$$

with

$$\overline{OPT}(A, B) = \sum_{i \in V_A} p_V(i) + \sum_{j \in V_B} p_V(j) + \sum_{e \in E_A} p_E(e) + \sum_{f \in E_B} p_E(f) \quad (1.13)$$

As a practical remark, we note that the number of variables entering the integer linear program grows like  $\mathcal{O}(n_A n_B + m_A m_B)$ . As a result, the linear program may become prohibitively large even for graphs of moderate size.

To meet this issue, it is convenient to define a circle with search radius  $r$  centered around each node  $i$ . Our goal is to exclusively consider nodes  $j$  within this search radius to consider as possible matches for node  $i$ . In other words, we label all nodes  $j$  outside the search circle as too expensive. To correctly enforce this idea in the integer linear program, we require  $p_V(u) > r$  for all nodes  $u$  in both graphs.

With this definition in place, none of the nodes  $j$  outside of the search circle will be selected as an optimal match for node  $i$  because two nodes  $i$  and  $j$  are matched if and only if the cost of forming the pair is smaller than the penalty due



for payment in case the match is not established. Thus, the respective variables  $x_{ij}$  may safely be omitted from the linear program.

Choosing the search radius and adjusting the penalties accordingly in order to control the size of the linear program seems more natural to the authors than the other way around. This approach has the additional advantage that a sensible value for the search radius can often be inferred by the looking at the length scales of the structures of interest in the original input image.

The number of variables  $y_{ef}$  referring to edge pairs can be reduced by separately applying the previous argument to each of the nodes involved. We enumerate the set of all possible edges  $f = (a', b')$  to be matched with edge  $e = (a, b)$ . The edges  $f = (a', b')$  are constructed by connecting all nodes  $a'$  within a radius  $r$  around the node  $a$  with all nodes  $b'$  within a radius  $r$  around node  $b$ . This process can create self-loops  $f = (a', a')$  which we exclude as candidates. If there are no nodes within a distance  $r$  from  $a$  or  $b$ , the associated edge variables  $y_{ef}$  can be omitted from the linear program. To correctly enforce this idea in the ILP, we normalize the cost of the edges associated with the variables  $y_{ef}$  and choose the edge penalties as  $p_E(d) > r$  for all edges  $d$  in both graphs. There is considerable freedom in the choice of the edge penalties as long as they are large enough that sensible matches  $y_{ef}$  are not ignored in favor of paying cheaper penalties.

It is advised to experiment with different values or to simply choose them larger than the largest edge match cost. The latter choice has the effect that whenever a match is available it will be selected, even if the cost is extremely large. As a result this choice is justified in particular when the edge costs are known to be comparable in magnitude. As a concluding remark, we stress that the choice of penalties for nodes and edges affects the final value of the similarity measure  $s(A, B)$  via the normalization. As a result, when using this measure to compare several graphs with each other, the penalties and tracking radius  $r$  must be chosen consistently in order to guarantee the viability of the comparison.

### 1.4.3. Evaluation of NEFI's Output

We proceed with the evaluation of **NEFI's** output using the above similarity measure. To do so we create a set  $\mathcal{A}$  of ground truth graphs such that  $\mathcal{A} \subset \mathcal{G}$ . We start by processing a real-life set  $I_0$  of images of the slime mold *P. polycephalum* with **NEFI**. Thus we obtain a set of graphs  $\mathcal{B}_0 \subset \mathcal{G}$ . Given those graphs we obtain the set  $\mathcal{A}$  by distorting the graphs  $B_i \in \mathcal{B}_0$  using different graph filters. At this point we will not use the images in  $I_0$  or the graphs in  $\mathcal{B}_0$  anymore.

Next, we turn the graphs in  $\mathcal{A}$  into a test set  $I_1$  of 2D images by simply drawing them. The drawing preserves the euclidean positions of the nodes, the edge lengths and the thickness of the edges. As a result, the image  $i \in I_1$  depicts the graph  $A_i \in \mathcal{A}$ . In other words, we know the *ground truth*  $A_i$  for every image  $i$  in the test

set  $I_1$ .

To compare different segmentation methods, we prepare a set  $\mathcal{P}$  of pipelines differing only in the segmentation algorithms used. The parameters of the pipeline were chosen manually for each test set using experimentation and visual inspection.

Given the sets  $I_1$  and  $\mathcal{A}$  as well as our similarity measure we can now evaluate **NEFI**'s output. We take an image  $i \in I_1$  and process it with a given  $p \in \mathcal{P}$  to obtain a graph  $B_i \in \mathcal{B}_1$ , i.e. the graph **NEFI** extracted from the input image. Then we compute the similarity  $s(A_i, B_i)$ . To obtain statistical statements, we repeat this procedure for all images and all pipelines.

During the computation of  $s(A_i, B_i)$ , we record features **NEFI** failed to detect in  $i$ , namely the number of vertices (edges) in  $A_i$  which remain without corresponding vertices (edges) in  $B_i$ . This is the number of false negatives (FN). Furthermore, we record the number of vertices (edges) in  $B$  for which no corresponding vertices (edges) exist in  $A_i$ . These are features which **NEFI** detects in  $i$  but which are in fact not present. These are false positives (FP). Finally we record the number of vertices (edges) in  $B_i$  that have corresponding elements in  $A_i$ . That is, features correctly extracted from the image  $i$ , which we count as true positives (TP). Unfortunately, we cannot determine the number of true negatives (TN) in a similar fashion. For this reason we restrict ourselves to computing sensitivity ( $\frac{TP}{TP+FN}$ ) and precision ( $\frac{TP}{TP+FP}$ ) in the results of the evaluation. Sensitivity and precision reported in the following Tables combine the respective values for vertices and edges.

Table 1.1 summarizes the results of processing the set  $I_1$ . The images in  $I_1$  are ideal inputs for **NEFI** for which all our segmentation routines produce very good results. Otsu's method and adaptive thresholding yield perfect segmentations. Hence, any difference between **NEFI**'s output and the ground truth cannot originate in the segmentation part of the pipeline but must be attributed to thinning and graph detection. The excellent correspondence between **NEFI**'s output and the ground truth confirms that thinning and graph detection are very reliable.

One might question the validity of using graphs which were detected by **NEFI** in the first place as the input set. However, the approach is valid because the origin of the images in  $I_1$  has no significance regarding **NEFI**'s performance. In other words, they are just as hard or as easy to process as images obtained in any other comparable way.

The perfect images in  $I_1$  do not represent real life input very well. Therefore we produced three more test sets and evaluate them as described above.

For the set  $I_2$  we take the images in  $I_1$  and change the brightness of the edge drawings randomly. As a result the local contrast between foreground and background varies widely across the image. To create set  $I_3$  we take the images in

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Method	Similarity score $s$	Sensitivity	Precision
Otsu's method	$0.984 \pm 0.005$	$0.970 \pm 0.011$	$0.998 \pm 0.001$
Adaptive threshold	$0.984 \pm 0.005$	$0.970 \pm 0.011$	$0.997 \pm 0.001$
Watershed (deletion/erosion)	$0.980 \pm 0.006$	$0.959 \pm 0.013$	$0.988 \pm 0.001$
Watershed (distance transform)	$0.906 \pm 0.121$	$0.837 \pm 0.160$	$0.998 \pm 0.001$
Watershed (adaptive)	$0.977 \pm 0.008$	$0.956 \pm 0.016$	$0.998 \pm 0.001$
Grabcut (deletion/erosion)	$0.984 \pm 0.005$	$0.970 \pm 0.011$	$0.998 \pm 0.001$
Grabcut (distance transform)	$0.983 \pm 0.005$	$0.967 \pm 0.011$	$0.998 \pm 0.001$

Table 1.1.: Summary of the evaluation of 250 ideal test images  $I_1$ .

Method	Similarity score $s$	Sensitivity	Precision
Otsu's method	$0.868 \pm 0.018$	$0.704 \pm 0.028$	$0.987 \pm 0.005$
Adaptive threshold	$0.941 \pm 0.010$	$0.853 \pm 0.034$	$0.976 \pm 0.025$
Watershed (deletion/erosion)	$0.859 \pm 0.018$	$0.693 \pm 0.028$	$0.984 \pm 0.006$
Watershed (distance transform)	$0.408 \pm 0.176$	$0.239 \pm 0.154$	$0.987 \pm 0.007$
Watershed (adaptive)	$0.966 \pm 0.008$	$0.936 \pm 0.016$	$0.984 \pm 0.017$
Grabcut (deletion/erosion)	$0.864 \pm 0.019$	$0.696 \pm 0.029$	$0.986 \pm 0.005$
Grabcut (distance transform)	$0.858 \pm 0.020$	$0.688 \pm 0.030$	$0.986 \pm 0.005$

Table 1.2.: Summary of the evaluation of 250 test images  $I_2$  with edges drawn with random brightness.

$I_1$  and insert a color gradient into the background while leaving the foreground unchanged. Set  $I_4$  is obtained by taking the images in  $I_1$  and subjecting them to a global blur.

Table 1.2 summarizes the results of processing the set  $I_2$ . We observe that both similarity score as well sensitivity are deteriorating for almost all methods except for adaptive thresholding and watershed based on adaptive thresholding. Adaptive thresholding is still able to compensate the local changes in brightness present in the test images and returns segmented images of high quality. We stress that for images showing more severe irregularities the performance of these methods is expected to suffer.

Note that the precision remains comparably high, which indicates that the vertices and edges detected by **NEFI** are indeed part of the ground truth.

Table 1.3 summarizes the results of processing the set  $I_3$ . We observe that almost all methods, with the exception of adaptive thresholding and watershed based on adaptive thresholding perform very poorly. In particular watershed based on a distance transform marker is completely unable to handle the input images.

Method	Similarity score $s$	Sensitivity	Precision
Otsu's method	$0.737 \pm 0.060$	$0.602 \pm 0.065$	$0.911 \pm 0.065$
Adaptive threshold	$0.984 \pm 0.005$	$0.970 \pm 0.011$	$0.998 \pm 0.001$
Watershed (deletion/erosion)	$0.752 \pm 0.047$	$0.588 \pm 0.061$	$0.977 \pm 0.009$
Watershed (distance transform)	$0.334 \pm 0.303$	$0.240 \pm 0.261$	$0.943 \pm 0.043$
Watershed (adaptive)	$0.982 \pm 0.005$	$0.967 \pm 0.012$	$0.997 \pm 0.001$
Grabcut (deletion/erosion)	$0.733 \pm 0.053$	$0.573 \pm 0.066$	$0.987 \pm 0.005$
Grabcut (distance transform)	$0.742 \pm 0.052$	$0.582 \pm 0.065$	$0.983 \pm 0.008$

Table 1.3.: Summary of the evaluation of 250 test images  $I_3$  with a color gradient in the background.

Method	Similarity score $s$	Sensitivity	Precision
Otsu's method	$0.953 \pm 0.011$	$0.909 \pm 0.022$	$0.993 \pm 0.003$
Adaptive threshold	$0.947 \pm 0.010$	$0.863 \pm 0.028$	$0.989 \pm 0.005$
Watershed (deletion/erosion)	$0.950 \pm 0.010$	$0.915 \pm 0.022$	$0.981 \pm 0.010$
Watershed (distance transform)	$0.738 \pm 0.127$	$0.575 \pm 0.147$	$0.915 \pm 0.059$
Watershed (adaptive)	$0.954 \pm 0.010$	$0.909 \pm 0.329$	$0.975 \pm 0.020$
Grabcut (deletion/erosion)	$0.950 \pm 0.012$	$0.903 \pm 0.024$	$0.993 \pm 0.003$
Grabcut (distance transform)	$0.918 \pm 0.024$	$0.838 \pm 0.046$	$0.990 \pm 0.004$

Table 1.4.: Summary of the evaluation of 250 blurred test images  $I_4$ .

Table 1.4 summarizes the results of processing the set  $I_4$ . We observe that almost all methods, with the exception of watershed with distance transform, perform reasonably well. Sensitivity and similarity scores are slightly smaller than the results obtained for the optimal test images  $I_1$ . This is due to the fact that blurring an image  $i$  causes the depicted edges to appear slightly wider. This increase in width is detected in the edges of the graphs  $B_i$ . As a result the similarity score  $s(A_i, B_i)$  decreases accordingly.

Summarizing the results we conclude that the quality of a graph detected by **NEFI** depends on the input image and the selected pipeline. We have established that the major factor determining the quality of the extracted graph is indeed the segmentation step. Errors introduced by thinning and graph detection appear negligible in comparison.

Consequently, **NEFI**'s major limitations arises from the limited applicability of its the segmentation algorithms. As a result, **NEFI** operates best on clean and uncluttered images such as images produced under controlled laboratory conditions. More difficult input may still be processed, possibly at the cost of reduced

Pipeline element	Image small ( $1152 \times 864$ )	Image large ( $5760 \times 3840$ )
Watershed	< 1	2
GrabCut	6	160
Adaptive threshold	< 1	7
Guo-Hall thinning	< 1	12
Vertex detection	< 1	5
Edge detection	< 1	6
Computing edge weights	< 1	5

Table 1.5.: Timings of some of **NEFI**'s pipeline elements on images of different size. All values are in seconds. The timings were obtained on a MacBook Pro notebook equipped with a 2.4 GHz Intel i5 processor and 8 GB RAM.

quality. For these inputs, domain specific algorithms might be necessary and can be implemented as extensions for **NEFI**. Alternatively, the segmentation step can be entirely outsourced to more specialized third-party software. Given the externally segmented image as an input, **NEFI**'s pipeline may proceed directly with graph detection.

We refer the reader to the Supplementary Material for a short guide that summarizes our experience when dealing with more challenging input.

#### 1.4.4. Evaluation of Speed Performance

**NEFI** was designed to efficiently process large quantities of images. Thus it outsources computationally intensive tasks to highly optimized and reliable libraries such as Itseez, **OpenCV** [6] and NetworkX Developer Team, **NetworkX** [12]. Table 1.5 illustrates the effectiveness of some of **NEFI**'s algorithms.

### 1.5. Limitations of NEFI

In the last section we have seen how the quality of the extracted graph changes depending on the input image and the selected segmentation algorithms. We have established that the major factor determining the quality of the extracted graph is indeed the segmentation step. As a result **NEFI**'s major limitation naturally arises from the limited domain of effectiveness of **NEFI**'s general purpose segmentation collection. It's methods do not work very well if the input contains irregular background or color/brightness gradients, has low contrast or insufficient resolution to detect structures that are either too dense or too fine. For these inputs, domain

specific algorithms are necessary and should be implemented as extensions for **NEFI**. Alternatively, the segmentation step can be entirely outsourced to more specialized third-party software. Given the externally segmented image as an input **NEFI**'s pipeline may proceed directly with graph detection.

In general, however, **NEFI**'s domain of effectiveness is limited to sufficiently clean and uncluttered images such as images produced under controlled laboratory conditions. We refer the reader to the supplementary material for a short troubleshooting guide that summarizes our experience when dealing with more challenging input.

Another limitation arises due to the nature of **NEFI**'s vertex detection. Since we walk the skeleton in search junctions, no vertices of degree two are detected. Furthermore, vertices of high degree (4 or more) are split into several degree three vertices. The latter can be undone by merging vertices using a suitable graph filter.

As a concluding remark, we stress that **NEFI** tries to compensate some of its limitations by offering the possibility to work with segmented images from other sources or to integrate additional algorithms with comparably little effort. **NEFI** should be regarded as a flexible platform suitable for further development rather than a universal solution to the network extraction problem.

## 1.6. Synergies With Other Software

### 1.6.1. Analysis of Graphs

NEFI is a tool that facilitates data acquisition, which is a necessary precursor to data analysis. To analyze **NEFI**'s output one can either rely on open source graph analysis software [2], [3], [11], [15], [16], [27] or write custom programs. **NEFI** can output many common graph formats, readable by most popular graph libraries. To get the user started immediately, we provide a minimal Python program that illustrates the basic steps required to perform graph analysis. It shows how to read **NEFI**'s output from disk and how to compute a histogram of a given edge attribute. The code can be downloaded from **NEFI**'s project page at the Max Planck Institute for Informatics, <http://nefi.mpi-inf.mpg.de>.

### 1.6.2. Third-party Segmentation Software

**NEFI**'s graph detection takes a segmented image as an input. Such an image need not be produced by using **NEFI** but can be obtained by relying on arbitrary third-party segmentation algorithms or tools.

In this context an interesting tool called *ilastik* [26] was brought to our attention. *ilastik* offers a so-called *classification workflow* in which a pixel classifier is trained

by interactive user inputs. The trained classifier can then be used to automatically segment previously unseen images. The segmented images obtained in this way can then directly be turned into graphs using **NEFI**.

By using **NEFI** in conjunction with third-party software the benefits of both can be realized.

## 1.7. Discussion

We anticipate **NEFI** to become a valuable tool that allows scientists from any domain to automate graph extraction from images in an intuitive fashion requiring no expert knowledge. We hope that researchers will be able to spend more time on analyzing their data and less time on processing it. By providing a flexible platform for graph extraction, we invite experts to extend and improve **NEFI** in order to introduce their contributions to a wider interdisciplinary audience. In the long run we would like **NEFI** to further the field of network science by promoting the creation of new network databases.

## 1.8. Acknowledgments

We thank Prof. M. Hauser for sparking our interest in this topic. We thank Prof. K. Mehlhorn for supporting this project with valuable comments and encouragement. We thank P. and R. Winegar as well as the Korea Institute of Science and Technology Europe for contributing sample data. This work was supported by the Max Planck Institute for Informatics and the IMPRS Saarbrücken.

### 1.8.1. General Information about NEFI

**NEFI** is an open source Python application and available at its project page at Max Planck Institute for Informatics, <http://nefi.mpi-inf.mpg.de>. **NEFI**'s homepage includes a gallery of various use-cases and a comprehensive guide containing instructions on how to download, install and use **NEFI** on Windows, Mac and Linux. Additionally, a supplementary datasets is available for download there, allowing for a quick evaluation of **NEFI**'s main features. This dataset can be used to reproduce the figures and evaluation results shown in this manuscript.

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# A. Guide on how to use NEFI

## A.1. Properties of ideal and non-ideal images

In order to obtain good graph extraction results, **NEFI** relies on input of certain quality. In this section we give a description of desirable properties of ideal input images.

Since, the determining factor of the quality of **NEFI**'s graph extraction is the segmentation step, ideal images should enable a nearly perfect separation of foreground and background. Such images should have a high contrast between the depicted structures of interest and the background. At the same time it is very important that images are free of strong reflections or shadows because such areas are likely to show an even higher contrast to the background than the actual structures of interest. As a result, the segmentation algorithms are prone to identify these regions as foreground causing the actual structures of interest to be ignored. The presence of strong color or brightness gradients can have similar detrimental effects and should thus be prevented if possible. See Figure A.1 and Figure A.2 for examples of challenging images which **NEFI** will have difficulties working with.

Another factor that influences segmentation, and by extension graph detection, are contaminations of different origin. Parts of the image might not belong to the object of interest at all. Such regions should be removed before loading the image into **NEFI**, see Figure A.3.

Figure A.4 depicts an image lacking in a similar way. The image contains objects that are technically part of the network one would be interested to extract, however, they are not suited very well to be represented as a graph. In particular, they will be picked up correctly in the segmentation step yielding four large areas of white pixels. Subsequently, thinning will try to reduce these to a lines resulting in more or less unpredictable results. While the remaining parts of the structure will be processed correctly, such images do not constitute ideal candidates for processing with **NEFI**.

Summarizing this information we can collect the following desirable properties for an ideal image:

- High contrast between foreground and background.

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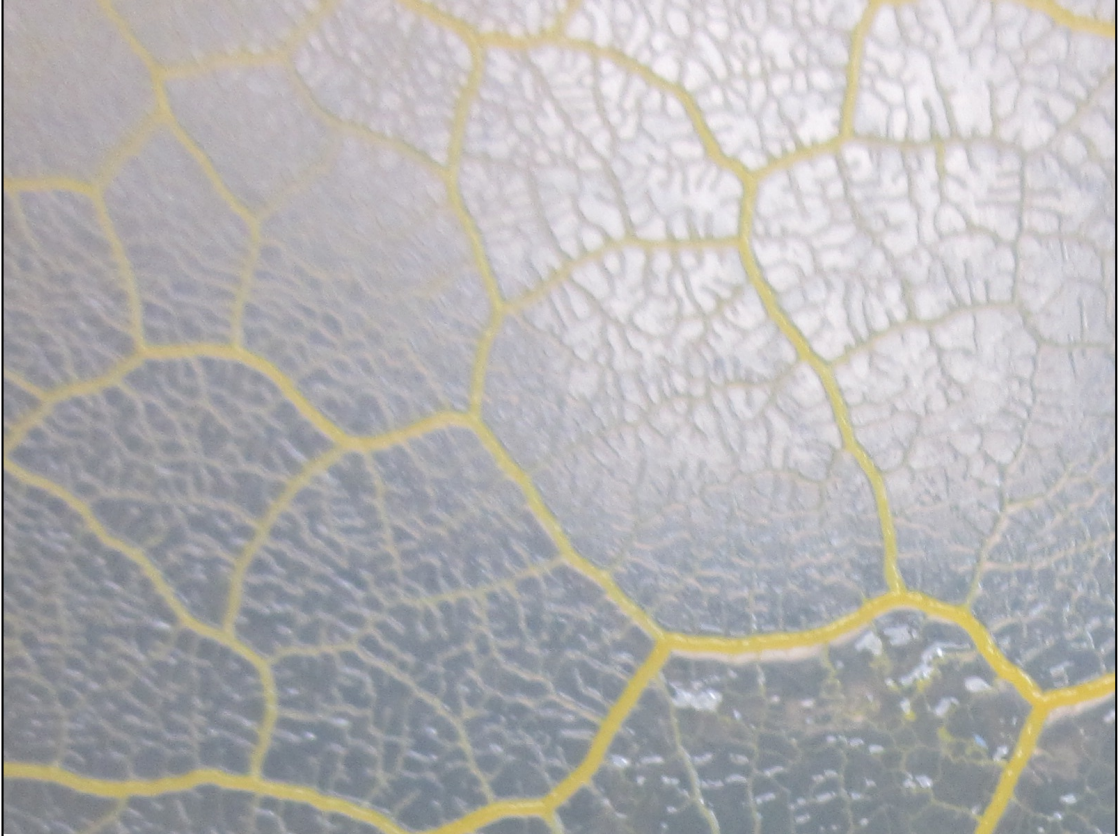


Figure A.1.: This image of *P. polycephalum* contains strong light reflections in the upper right quadrant which will cause **NEFI**'s segmentation algorithms to be lead astray. The fact that the image is not properly focused is less of a problem in comparison.



Figure A.2.: This image of *P. polycephalum* was not illuminated evenly from below. As a result it contains a brightness gradient which is detrimental for many of the segmentation algorithms currently implemented in **NEFI**. The fact that the image contains a lot of visible noise makes it a bad candidate for processing with **NEFI**.



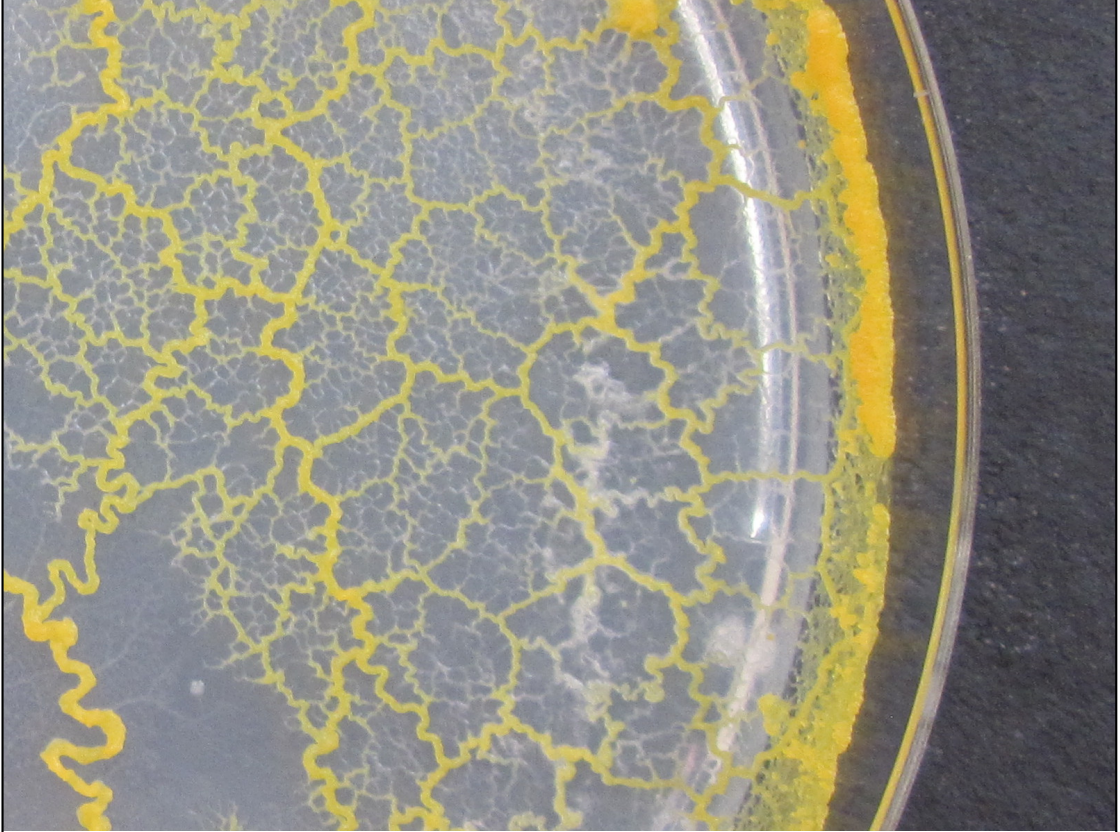


Figure A.3.: In addition to the network of *P. polycephalum* the image contains the edge of a petri dish and pieces of the background on the right hand side (as well as light reflections). Prior to any attempt of processing the image, petri dish and background should be removed.

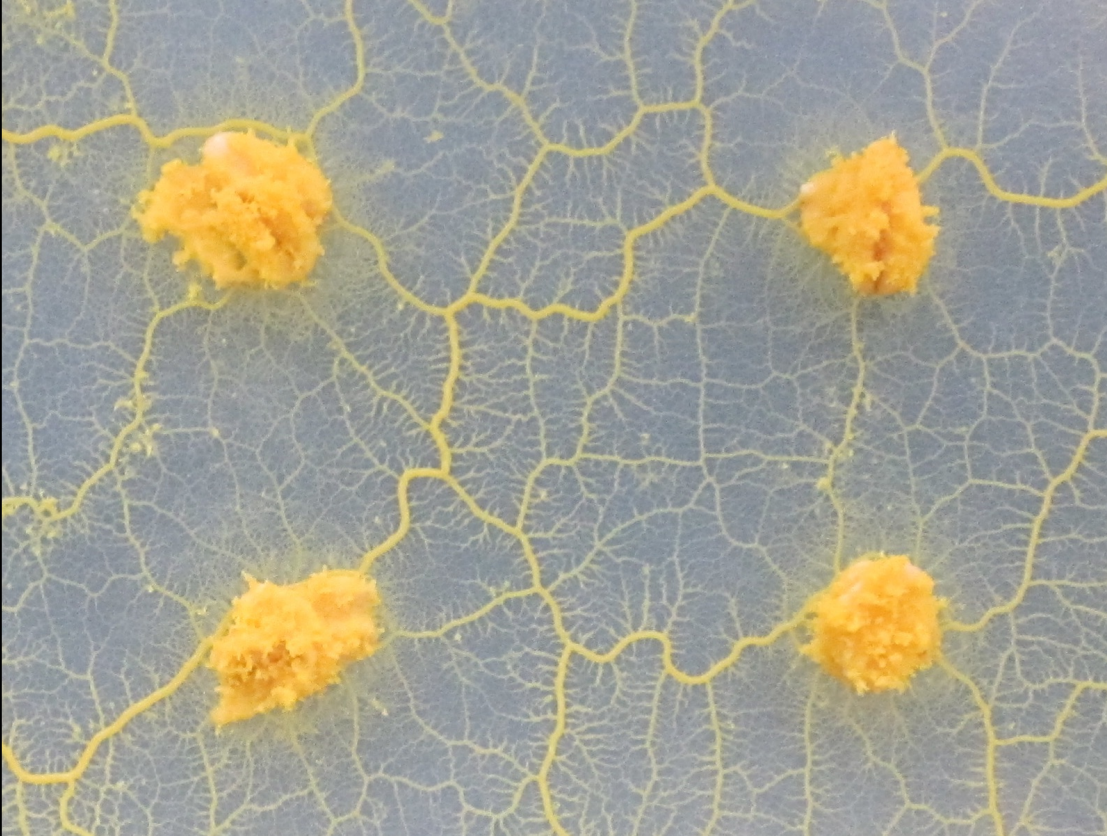


Figure A.4.: The network of *P. polycephalum* depicted in the image contains 4 massive, non-network-like regions (oat flakes completely covered by the mold). After segmentation, thinning will try to reduce these regions to lines leading to unpredictable results. The remaining parts of the network, however, will be extracted correctly.



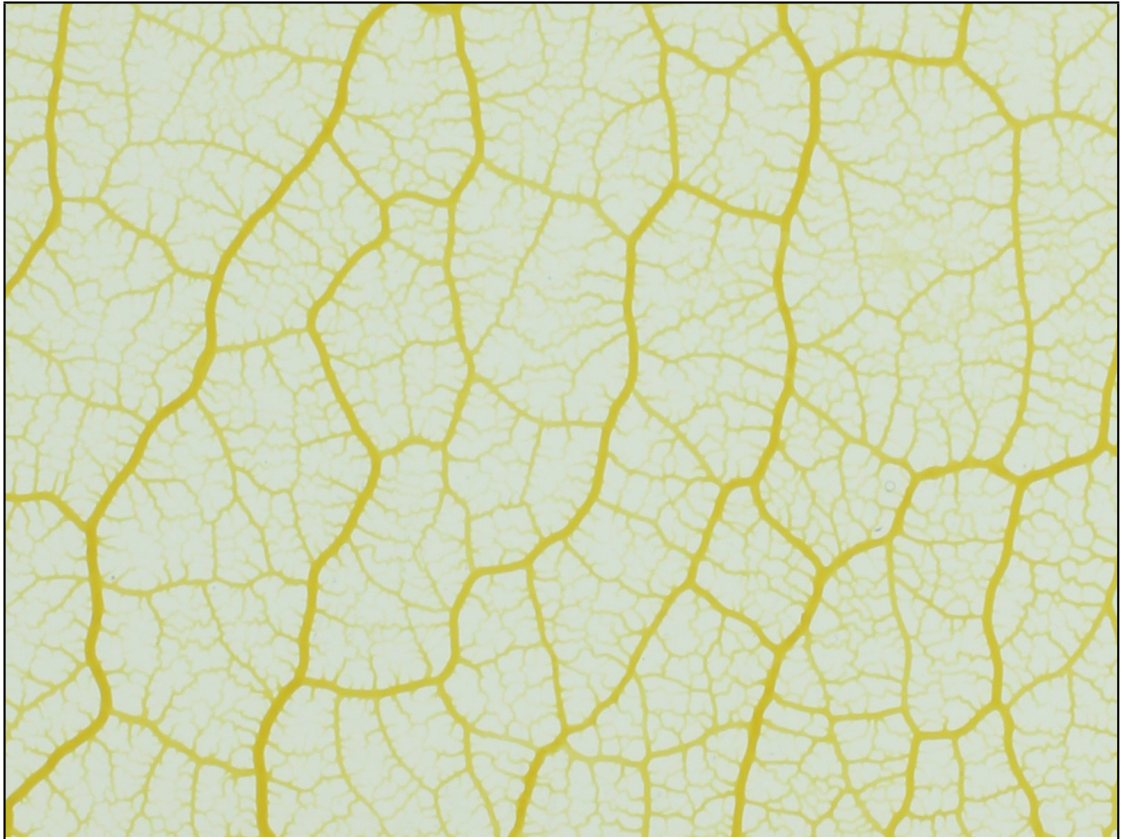


Figure A.5.: An ideal image of *P. polycephalum*. The image has been obtained using bright field illumination and was produced in a collaboration with the KIST Europe.

- Uniform background void of reflections, shadows as well as color or brightness gradients.
- No contaminations that might disrupt the segmentation process.

When producing images to be processed with **NEFI** one should strive to fulfill these properties whenever possible. See Figure A.5 and Figure A.6 for examples of very good input images.

## A.2. How to deal with challenging images

**NEFI** operates best on images produced under controlled laboratory conditions that fulfill the properties described in the last section. However, more difficult

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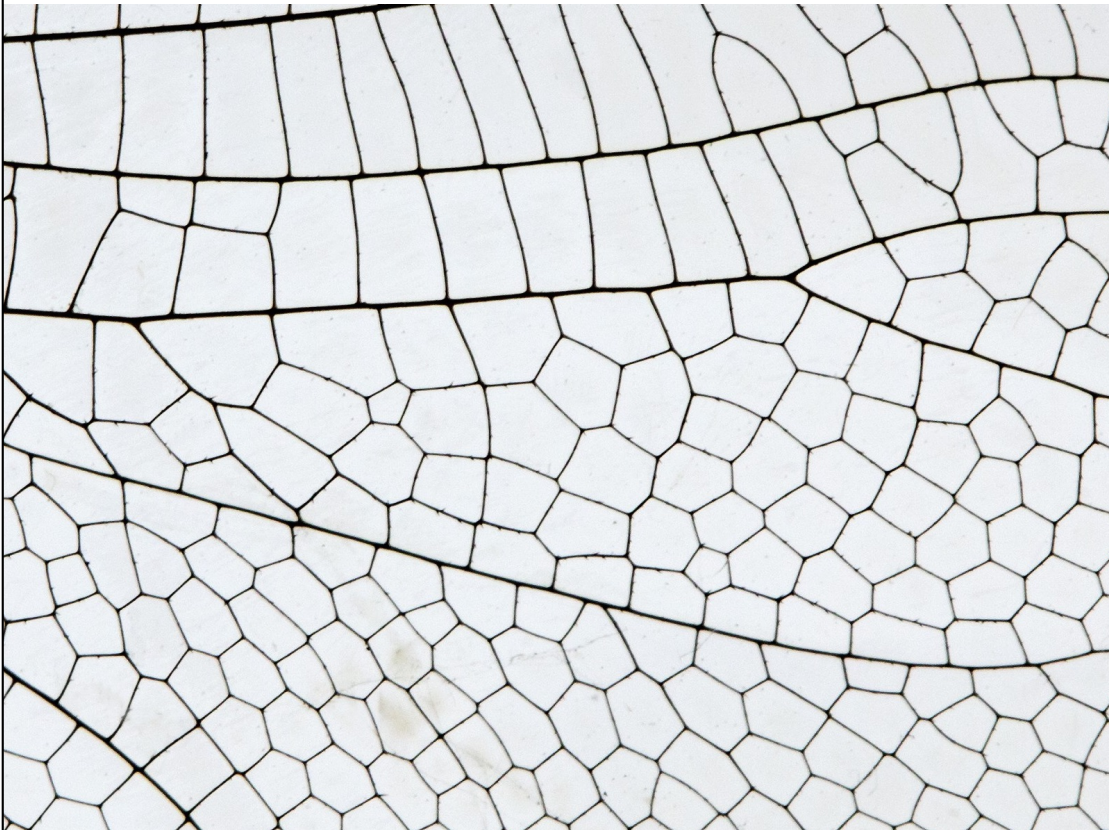


Figure A.6.: An ideal image depicting a detail of the wing of *A. junius*. Image courtesy of Pam and Richard Winegar.

inputs may still be processed, but most likely at the cost of reduced quality. Based on our experience when dealing with more challenging input and the results of our evaluation, we are able to formulate the following recommendations for the usage of NEFI:

**Otsu's method** may be used on noisy or blurred images. It will do reasonably well as long as if the image has a high contrast between network and background. Adaptive threshold, watershed based on adaptive threshold, and GrabCut with deletion and erosion may even perform slightly better under these conditions. Otsu's has the advantage that no parameters have to be set in order to get good results. The choice should be based on the desired degree of resolution of the extracted graph. We would recommend these methods to process Figure A.3 after clipping the image accordingly.

**Adaptive threshold and watershed based on adaptive threshold** may be used if differences in contrast between foreground and background are local and not too strong. If this is the case good results may still be obtained. Both methods allow to analyze images that contain a color gradient in the background or that contain edges which have differing levels in brightness. One might try to process images like Figure A.1 and Figure A.2 with these methods while experimenting with different parameter settings. However, such images remain challenging. NEFI's current algorithms may not deliver sufficient results.

**Preprocessing methods** like Gaussian and Median Blurring, Denoising as well as Bilateral Filtering can be used to remove small artifacts, contaminations or irregularities from the image. Although these methods can reduce the amount of artifacts produced during segmentation and thinning, improvements come at a price. For example, imposing a strong blur causes the depicted edges to appear slightly wider. This effect will propagate through the pipeline causing the edge widths of the final graph to overestimate the true widths depicted in the image. We recommend to use preprocessing with care especially if a high accuracy regarding edge weights is required.

**Graph filtering** enables the removal of unwanted artifacts and spurious vertices caused by irregularities in the input which propagate through the pipeline. However, filtering can only repair the result up to a given point. While the ability to add custom filters is powerful, it appears pointless to filter a graph established on the basis of a failed segmentation. We encourage users to visually verify the integrity of the established graph and only then to proceed with filtering.



We anticipate users to encounter images **NEFI**'s algorithms will be unable to handle properly. In this situation the user will have to rely on different segmentation solutions.

As a first suggestion, we recommend to user to look for available software specializing in segmentation. In this regard we like to point out the Kitware, **ITK Project** [13]. It's *C++* code base has been developed by the medical image processing community and serves as the basis for many other specialized tools that deal with image processing as well as segmentation.

After a segmented image has been obtain using specialized third party segmentation software, **NEFI** can take this image and proceed with graph detection and filtering directly.

If no proper software is available, the user has the option to extend **NEFI**'s segmentation capabilities by adding more sophisticated code. When doing so, one can built on top of existing features already implemented in **NEFI**. The literature offers a wealth of different approaches to segmentation leading to algorithms of varying complexity. Before diving into any implementation efforts, we strongly recommend to survey existing methods and their domains of effectiveness by consulting [8], [22], [23].

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