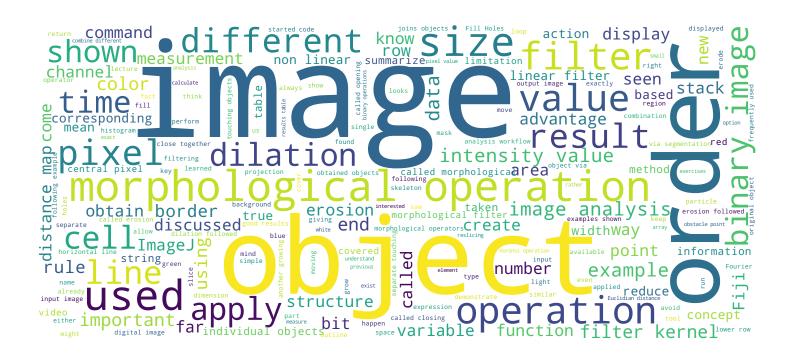


# **Morphological operations**

#### **Image Processing & Analysis for Life Scientists**

Olivier Burri, Romain Guiet & Arne Seitz









#### Introduction





- Morphological Operations
- Usage in Image Analysis

Welcome to this lesson covering morphological operations. In the previous lecture you have learned how to define an object via segmentation. Just as a reminder, segmentation is a protocol to distinguish between an object and its background. In this lecture, I want to explain what we can do with the obtained objects. These so called morphological operations are needed in order to adjust the obtained objects to the structure we are interested in. These operators can also be used to create completely new objects. So let's get started.

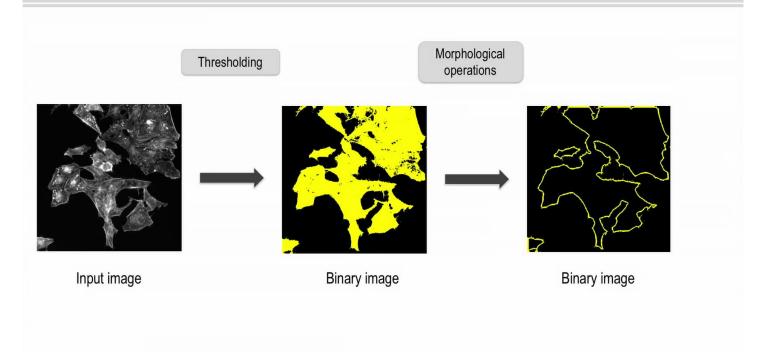
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#### Motivation





You might remember how to use thresholding in order to come from an image to an object. In the example shown here you see Hela cells which were stained with Phalloidin in order to visualize actin. From that image, we can obtain the object corresponding to the area covered by the cells by simple thresholding.

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### Introduction





- Morphological Operations
- Usage in Image Analysis

Today I will demonstrate how we can use so called morphological operations in order to come from that object to an object which is corresponding to the outline of the object by using these so called morphological operators.

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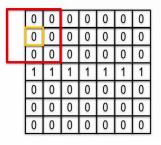
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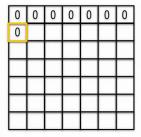
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Filter

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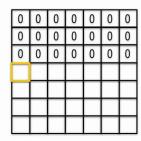
So what are morphological operators? They are indeed a subclass of non-linear filters. And now we will just apply them to binary images, which have only two intensity values. We calculate the new image as we move the filter over an image. Now we define rules like the following: "If the center pixel is distinct from 0 we are making the surrounding pixels white too." What happens If we apply that rule to the image shown here? The central pixel —surrounded by the yellow square- has the value 0. So no action had to be taken. Thus the intensity value of the new pixel is also 0. And this is true for the entire first row.

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**Filter** 

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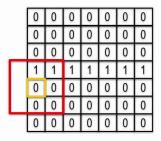
**Output** 

And also, in the 2nd and 3rd row no action has to be taken if we apply our rule. So we end up with a binary image only consisting of the intensity value 0. So until now, it looks like a pretty boring or even useless non-linear filter. In the fourth row, it is the first time that the central pixel of our filter kernel is deviating from 0. So here we have to apply our rule and set all pixel intensity values to 1.

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**Filter** 

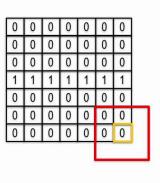
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**Output** 

Please be aware of the fact that we are even overwriting pixel which had been previously set to 0 By moving the kernel towards the last pixel of the row we only encounter intensity values of 1. So we have to apply our rule for each and every pixel of that row. If we move to the next row, the central pixel value is again 0. So no action has to be taken.

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**Filter** 

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**Output** 

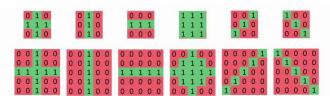
And this holds also true for that row. And the two following ones. So let's have a look on the outcome. By applying this morphological filter, we have converted an image with a horizontal line of a width of one pixel to an image with a horizontal line of 3 pixel. So we extended the width of the line. The underlying operation of this filter is called a "Dilation".

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- A type of non-linear filtering
  - · Kernels of different shapes



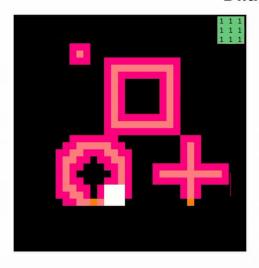
- Where neighbors are affected by simple rules
  - Eg. "If the center pixel is white, make all pixels surrounding it white too" (or according to the kernel's shape)

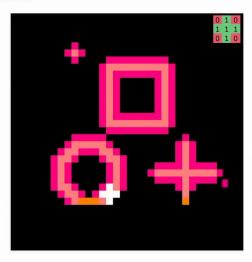
We have used a quadratic filter kernel where all the elements had the value 1. However, it is also possible to use more sophisticated filter kernels as shown in the diagram here. As you are already used to the concept of filtering you shall be able to say which of the shown filter kernels are giving the exact same result as the one used before. Just a small hint. You should find two filters. And as with all filters, we can also vary the size of the filter kernel. The line of the width 1 will then be transferred to a line with a size corresponding to the size of the kernel.

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#### **Dilation**





In the following videos, you can see how morphological filters work, and how the output depends on the geometry of the filter.

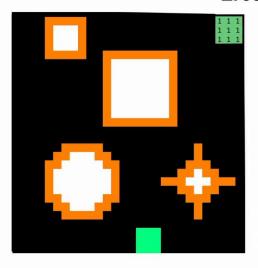
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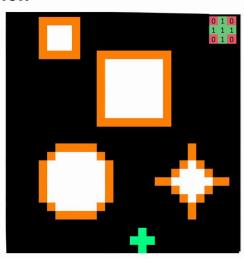
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#### **Erosion**





The morphological operation, which is doing the opposite of a dilation is called erosion. The shown video summarizes again the working principle of that filter and how the 2 different ilter kernels work.

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- Erosion
- Dilation

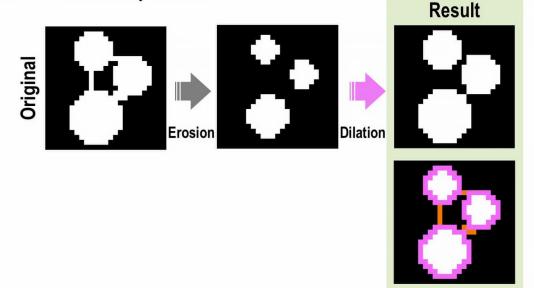
So far we have looked at single morphological operations namely erosion and dilation. Now we want to explore what happens if we combine them. Interestingly, combing 2 opposite morphological operations is giving a result which may differ from the input image. And the order of the operation matters. This is demonstrated in the two following examples.

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#### **Opening**: Erosion followed by Dilation



The combination of a dilation followed by an erosion is called closing. This operation joins objects which are close together to a single object. Therefore this operation can be used to decrease the number of objects in a given image. The advantage of a dilation is that the size of the objects is similar to the size of the original object. An erosion followed by a dilation is called opening. This is a very useful operation in order to obtain individual objects. The advantage over a single erosion is – similar the case of the closing- that the area of the object is closer to the area of the original objects.

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- Erosion
- Dilation
- Closing
  - Dilation+Erosion
- Opening

Summary

Erosion+Dilation

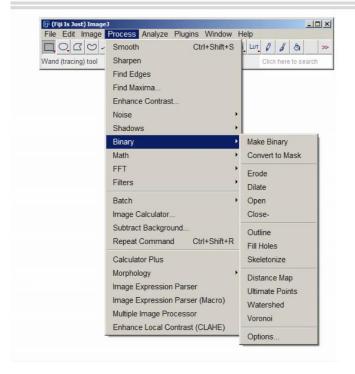
So to summarize what had been covered so far: we have discussed the morphological operations of dilation and erosion. The first one is making binary objects larger and the latter one is shrinking them. The size and shape of the kernel is important for the output image as morphological operations are non-linear filters. The combination of these two operations is often resulting in an image which is different from the input image. A dilation followed by an erosion is called closing and joins objects which are close together. An erosion followed by a dilation is called opening and separates objects which are close together. Combining these opposite operations has the advantage that the size of the objects is close to the size of the input objects. Morphological operations are powerful tools in order to create so called masks, which are frequently used in image analysis workflows. It is important to keep in mind that these operations require a binary image as input.

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### **Binary-Based Operations**







Morphological operations can be also found in ImageJ. All the operations, we have discussed so far are implemented and can be found under the Bianary submenu in the Process menu. In the exercises, you will learn that each and every command is influenced by the options which can be accessed at the end of that submenu.

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## **Binary-Based Operations**





- Watershed
- Fill Holes
- Skeletonization
- Voronoi
- Distance Map

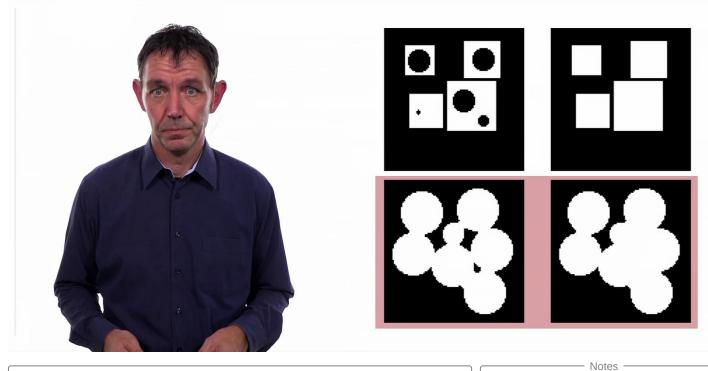
After the basic morphological operations we will now have a look at the more complicated ones. Here, it is less important to know the mathematical procedure behind the operation but rather know in which situations such a filter can be applied as well as know the limitations of these filters.

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#### **Fill Holes**





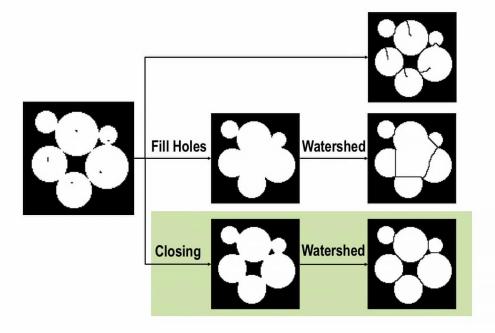
A very prominent and often used filter is the so called whatershed algorithm. It is used in order to separate touching objects. In essence, it first erodes the object to find the ultimate eroded points. So each object is shrunk to a single point only. Then these points are dilated until they either reach the border of the object or the border of another growing object. This operation is giving good results for objects which are not overlapping too much and which are homogeneous. As you can see in the lower row, it gives less good results if the objects are not homogeneous. The "Fill Holes" operation is more intuitive than the previous one. It creates homogeneous objects by filling the holes. It is an operator which is frequently used. However it also has limitations as it tends to fill up the area between objects as can be seen in the examples shown in the lower row.

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### **Combined Operations**





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Watershed Alone

The following example illustrates that it is important to combine different morphological operators in order to separate touching objects. For a human interpreter, it is easy to identify six different circles as individual objects. If we want to avoid to draw these objects by hand during an analysis workflow we need to combine different operations. Here we use the closing operation in order to fill the holes. This is working well as the holes are quite small. After doing so, we can apply the watershed algorithm in order to separate the objects.

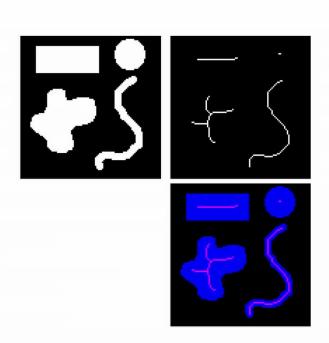
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#### **Skeletonization**







Sometimes it is advantageous to reduce a structure to its skeleton. Not too surprisingly the command skeletonize is performing exactly this task. We can think of skeletonize as a clever way of erosion. This can be seen in the examples shown here. A structure is reduced to a line which, in the ideal case, is a representation of the structure. Especially when tracing filamentous structures like, for example, neurites this can be a very powerful approach.

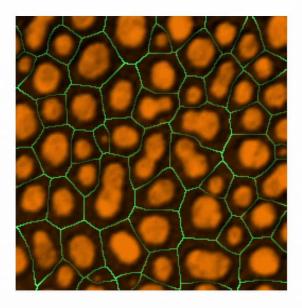
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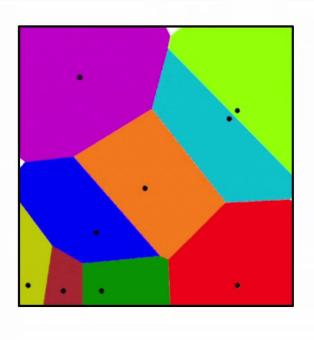


Skeletonization was a way to reduce a structure. Now we want to have a look at the Voronoi operation which is doing the opposite. It is growing a region. Various scientists had proposed it at the same time, therefore it is also known as Dirichlet teselation. It is used in order to partition the space between individual objects as shown in the image here. The objects were given the borders between them – displayed in green- were calculated used a voronoi operator.

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For me the most intuitive way to understand the operation is a region growing mechanism as shown in the short movie.

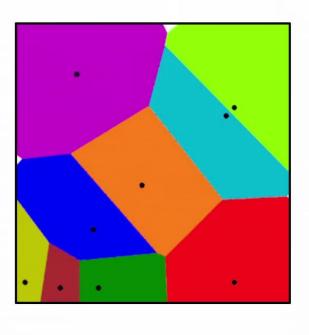
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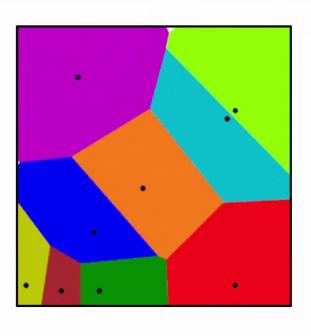


We reduce the objects to seed point and grow a region around these points.

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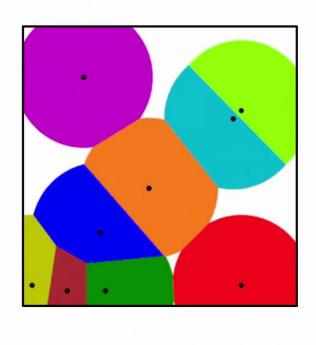
The growing stops locally once it hits another growing region or the border of the image.

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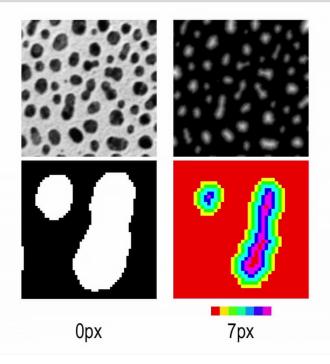
At the end, every point is part of exactly one region.

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#### **Distance Map**





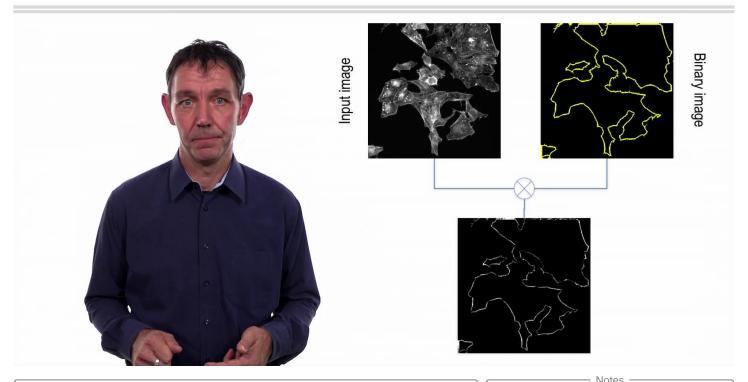


Finally, yet importantly, I want to introduce distance maps. Here we are calculating the distance from each pixel to its nearest obstacle point. An obstacle point is either the border of an image or the border of the object itself. There are different variants of distance calculation available. In image analysis the mostly used on is the Euclidian distance map. You might recognize the similarity of the distance map and the skeleton of the object. In fact the calculation of the skeletion, the ultimate points and the voronoi operation are based on the Euclidian distance map.

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#### **Summary**





Summary -

With the binary operations you have learned so far it shall be not too complicated to produce a binary image which is defining the rim of an object, in this case the outline of a cell. All we have to do is to create the object via segmentation. With fill holes, we make the object homogeneous. Then we create a copy of this object and erode it. Depending on the size of the filter kernel, we obtain an object which is smaller. By subtracting this smaller object from the original one, we obtain a binary image just outlining the cells. This binary image can be used in order to measure the amount of a protein localizing to the plasma membrane. This demonstrates the strength of binary operations in order to come to masks or objects. They are key in order to perform state of the art image analysis.

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#### **Summary**





- Erosion
- Dilation
- Closing
  - Erosion+Dilation
- Opening
  - Dilation+Erosion
- Watershed
- Fill Holes
- Skeletonization
- Voronoi
- Distance Map

So it is time to summarize. We have discussed a variety of morphological operations. Starting with the simple ones called erosion and dilation. They are used in order to grow or shrink objects. Combining these operations lead to new operations called Closing and Opening. Furthermore we have discussed more complicated operations like Watershed and Voronoi. Morphological operations are key in order to create masks which are essential for measurements in image analysis. Up to now, we have only discussed morphological operations which had been applied to binary images. However, it shall be mentioned that there are also morphological filters available working with grey scale images. With this slide, we are at the end of the lecture. Hope to see you next time and take care.

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