

Introduction to Analysing Fouling Discs Using ImageJ

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As an introduction to the use of different image-processing techniques in the context of the analysis of VIRTUE discs a series of tutorials will be presented. All of them are based on the freely available image-processing program ImageJ, which has found wide applications in biology, medicine and nanotechnology.

You can download ImageJ in its 'Fiji' distribution from

<https://imagej.net/Fiji/Downloads>

for Windows, MacOS or Linux. Fiji is an open source program and comes bundled with its own Java Runtime Environment and a number of pre-installed plugins, which facilitates installation.

Methods I-IV described here require colour photographs of the whole fouling disc and for methods V and VI, photomicrographs of portions of a disc are analysed. For the individual tutorials, practice images are supplied.

The following six methods can be used to analyse photographs of discs with varying degrees of growth. Deciding which method to use will depend on several factors:

1. Density of growth: Is the disc overgrown with organisms or is the growth sparse?
2. Species present: What species predominates? How big are the individuals?
3. Distribution of the species: Are the organism evenly distributed or are they patchy?
4. Testing hypothesis: Which method is best suited for answering the questions of the experiment?
5. Time availability: How much time is available for analysing the discs?
6. Age of pupils.

A short, tabulated description of the methods can be found below to serve as a guide for teachers and pupils in deciding which of the methods is best suited for their projects.

Most of the tutorials are intended to work in "stand-alone" mode to save the reader from having to reference back and forth between documents. Consequently, there will be some repetition and redundancy among them.

Several points should be considered when using these methods:

1. **Good quality images** are necessary.
2. Consider taking photos of the **top and the underside of the discs**.
3. When taking the photos, the **discs should be immersed in water** (in a basin).
4. Try to avoid as much light reflection as possible. This can be achieved by diffuse or indirect lighting.
5. **Colour photographs** are better suited for this method of analysis.

6. Be sure to have a **reference scale**. Place a ruler or any other object with known dimensions beside the disc while taking the pictures. The diameter of the disc can also be used as a reference scale.
7. As much as possible, before doing the analyses, view the discs under the stereomicroscope or the microscope to identify the organisms growing on the discs.
8. All of these methods have some ambiguity and are never 100% precise! Use with proper judgement!

I. Counting Organisms Growing on a Disc Using ImageJ (Manual)

Characteristic		Remarks
Growth on disc:	sparse to dense	can be used for all kinds of growth; more difficult to use for algae
Level of difficulty:	medium to difficult	
Distribution of species:	patchy to even	
Time needed to analyse one disc:	20 – 30 minutes	depends on the density of growth
Suitable for what ages:	15 years and older	
Skills required:	patience 😊	
Others:		competence in spreadsheet calculations (e.g. Excel) may be helpful; this method can be applied to all kinds of growth; can be applied for photomicrographs

II. Counting Organisms Growing on a Disc Using ImageJ (Automated)

Characteristic		Remarks
Growth on disc:	sparse to not too dense	best suited for photos with good contrast; cannot be used for counting algae
Level of difficulty:	easy to medium	
Distribution of species:	patchy to even	best suited for round organisms like barnacles
Time needed to analyse one disc:	10 – 15 minutes	the required time is not dependent on the density of growth
Suitable for what ages:	14 years and older	
Skills required:	curiosity and willingness to try something new 😊	
Others:		of limited use for photomicrographs

III. Measuring Percentage Cover on the Disc Using ImageJ (Manual)

Characteristic		Remarks
Growth on disc:	sparse to dense	best suited for algal colonies and other colony forming species
Level of difficulty:	easy	
Distribution of species:	patchy	
Time needed to analyse one disc:	10 - 15 minutes	
Suitable for what ages:	13 years and older	
Skills required:	easy maths 😊	
Others:		this method does not require exact identification of species; photo of the entire disc is needed

IV. Measuring Percentage Cover on a Disc using ImageJ (Automated)

Characteristic		Remarks
Growth on disc:	sparse to dense	best suited for algal colonies and colony forming zoobenthos
Level of difficulty:	easy	
Distribution of species:	patchy	
Time needed to analyse one disc:	5 - 10 minutes	
Suitable for what ages:	13 years and older	
Skills required:	easy maths 😊	
Others:		this method does not require exact identification of species; photo of the entire disc is needed

V. Simultaneous Analysis of Multiple Discs Using ImageJ

Characteristic		Remarks
Growth on disc:	sparse	best suited for microalgal colonies (diatoms) and early stages of biofouling; suited for analysing biofilm
Level of difficulty:	easy	
Distribution of species:	patchy	
Time needed to analyse one disc:	5 – 10 minutes	
Suitable for what ages:	13 years and older	
Skills required:	easy maths ☺	
Others:		this method is for a series of photomicrographs taken under the same magnification

VI. Counting Organisms Growing on a Disc Using ImageJ (Manual) - Photomicrographs

Characteristic		Remarks
Growth on disc:	very dense	method is suited for overgrown discs
Level of difficulty:	easy	
Distribution of species:	patchy to even	
Time needed to analyse one disc:	20 minutes	to analyse a single photomicrograph 1-2 minutes are needed
Suitable for what ages:	13 years and older	
Skills required:	easy maths ☺	
Others:		this method is for a series of photomicrographs taken under the same magnification

