

Batch QCPA: Worked examples v1.1

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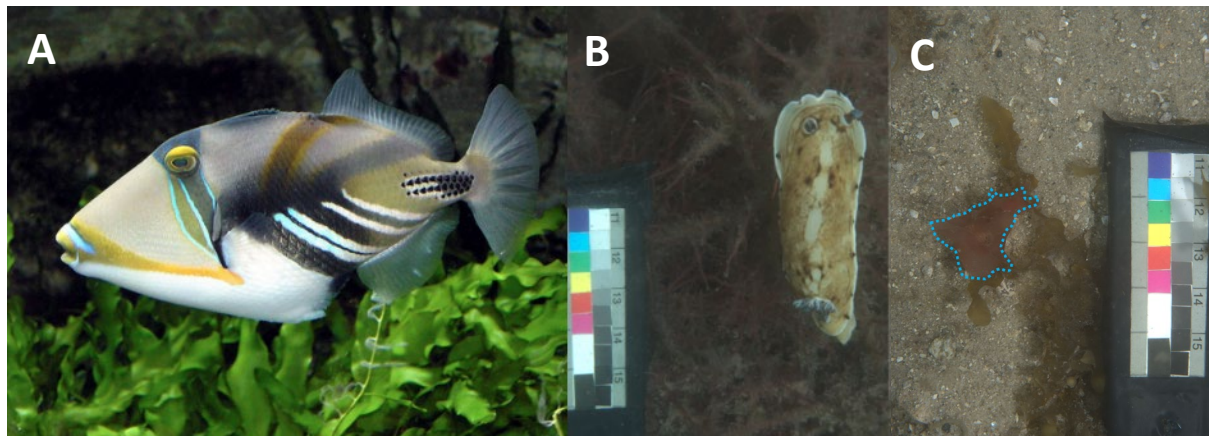
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Example 1: A trichromatic observer without UV sensitivity

This example uses data from nudibranch molluscs photographed underwater. Nudibranchs are incredibly diverse, colourful marine animals known for their defensive colouration. We will use the visual system of a triggerfish (*Rhinecanthus aculeatus*) to analyse the images with all available analyses. This example highlights using two different visual models, as we pretend some images in the test data were taken with a different light source. We will base our modelling on the cone responses under a slightly green underwater illumination at 5m depth.



A) *Rhinecanthus aculeatus* (A. Pingstone, public domain). Calibrated images from the test dataset of *Aphelodoris varia* (B) and *Aplysia* sp. highlighted with blue dashed line (C) by CvdB.

To run this example, you will need:

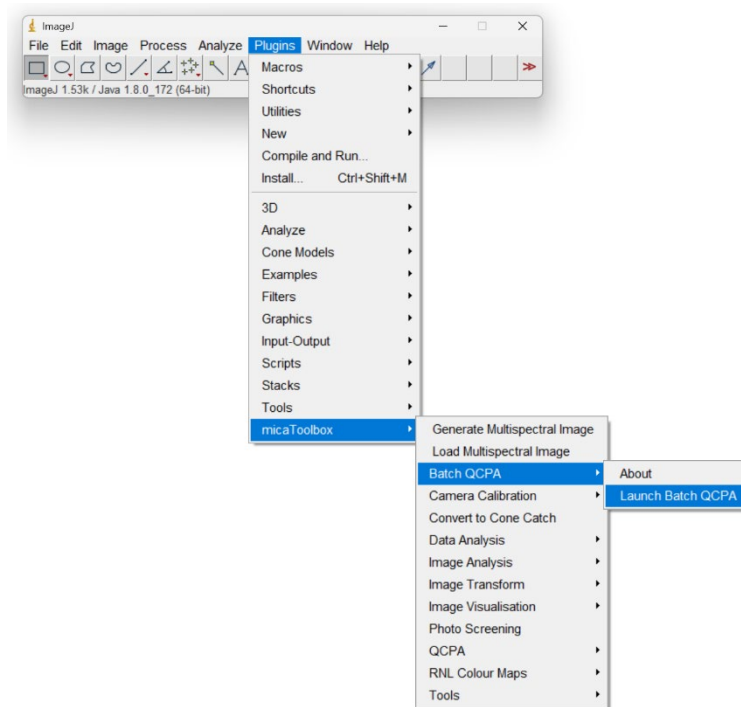
1. The test data provided here: <https://doi.org/10.48610/3cdcc1f>
2. The following visual models (provided with the test data):
 - a. Olympus_PEN_E_PL5_Olympus_60mm_f2_8_PV62white_VK6R_Combined_to_Triggerfish_5m_green_water
 - b. Olympus_PEN_E_PL5_Olympus_60mm_f2_8_VK6R_to_Triggerfish_5mGreenWater

Copy the JAVA and CLASS files in the 'cone mapping models' folder provided in the test data folder into your local directory of imageJ into 'plugins' -> 'Cone Models'.

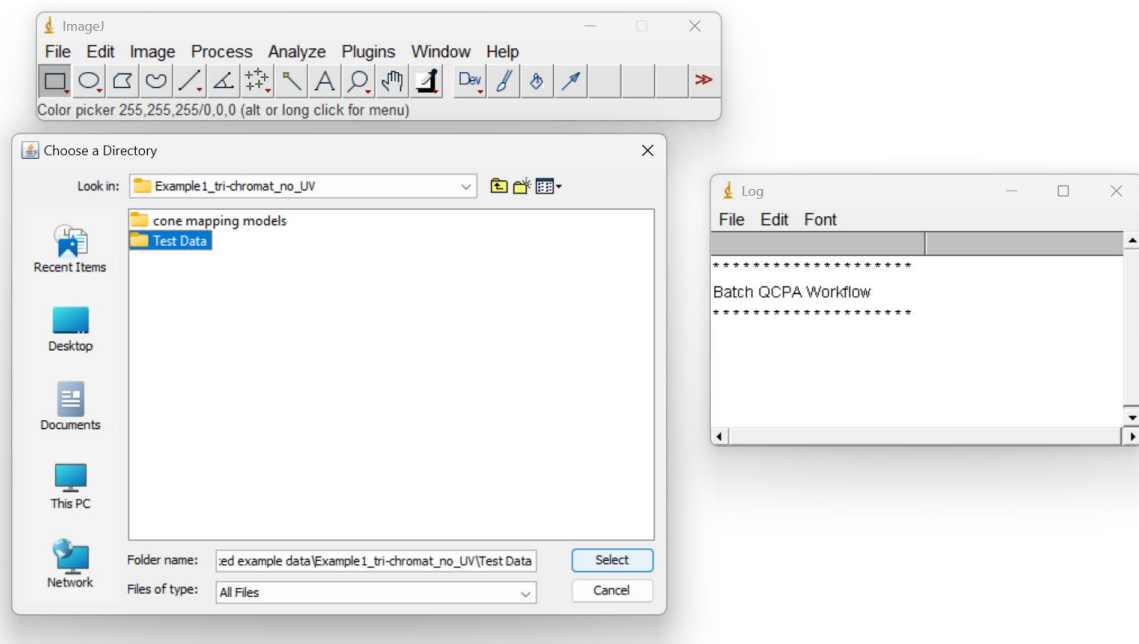
3. The latest version of the MICA toolbox & ImageJ available [here](#)
4. The correctly installed QCPA batch script, available here: <https://github.com/cedricvandenbergh/QCPA-batch-script>

See the batch script manual for detailed instructions on installing the toolbox and the batch script.

Step 1: Launch the batch script (Plugins -> micaToolbox -> Batch QCPA -> Launch Batch QCPA)



Step 2: Select the 'Test Data' folder in the 'Tri-chromat no UV' example folder.



Step 3: Provide the general input setting for the analysis.

- We will work with '.ORF' images, the RAW file format for Olympus cameras.
- We have not renamed our .mspec images. Thus, the script will look for files called '.mspec'.

- c. We will simulate viewing distances of 30 cm and 50 cm. This will make processing comparably fast. Note that the larger the images, the higher the spatial acuity of the modelled visual system, and the closer the viewing distance, the longer processing will take, as the resulting images will remain large.
- d. Triggerfish (*Rhinacanthus aculeatus*) have 3 known cone types and, thus, spectral sensitivities involved in bright-light colour vision.
- e. As the images were taken with variable animal rotations, each animal folder contains a .txt file called 'rotation.txt' specifying the number of degrees the image needs to be rotated to be aligned with the anterior-posterior axis of the animal. Therefore, 'Enable rotation' should be ticked.
- f. We will choose a [lower chromaticity limit](#) of 0.03. This means that, below 3% luminance, the observer should not be able to perceive any colour.
- g. We will [replace negative cone catch values](#) in our images with values of 0.001.
- h. We will analyse all three ROIs available in the dataset: The animal, the animal and its background and the background by itself.
- i. We will apply all modules available in the QCPA batch script.

Main QCPA Input Parameters

Choose your file Extension

Enter your mspec filename

Add viewing distances in same unit as the scale bar in each image

If using more than one distance ensure values are separated by a comma

Number of spectral sensitivities (excl. luminance channel) required?

☒ Enable rotation?

Choose your lower chromaticity limit

Replace negative cone catch values with:

Use 'IGNORE' above to ignore these settings and use a value of 0

Select the ROIs you would like to measure below:

☒ Animal ROI

☒ Animal + Background

☒ Background ROI

Select the modules you would like to use below:

Note that Colour maps will not work with less than two colour channels (dichromacy)

☒ CAA,BSA,VCA & Particle Analysis

☒ LEIA

☒ GabRat

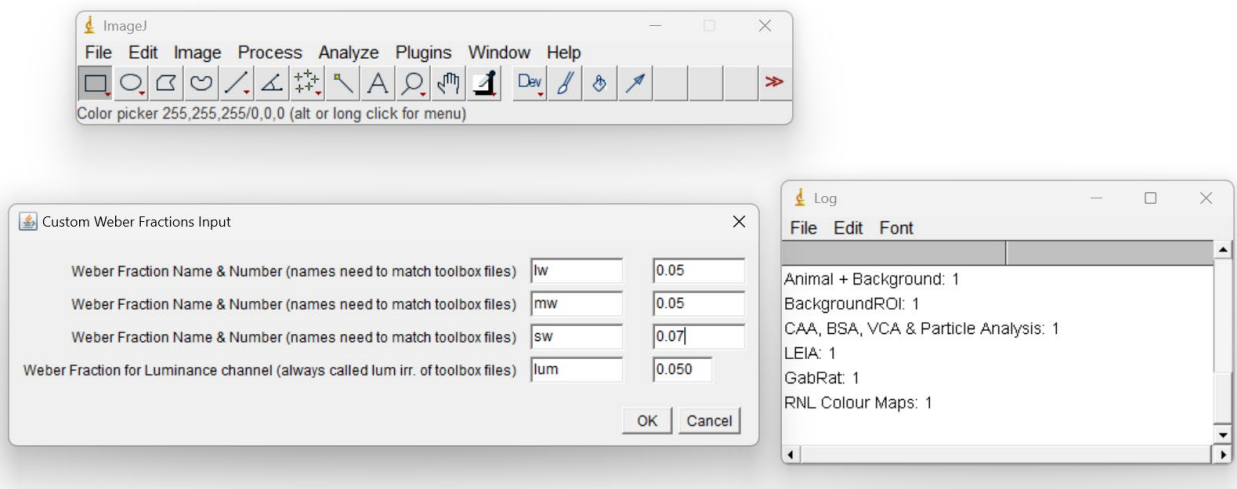
☒ RNL Colour Maps

OK Cancel

Step 4: Provide Weber fractions

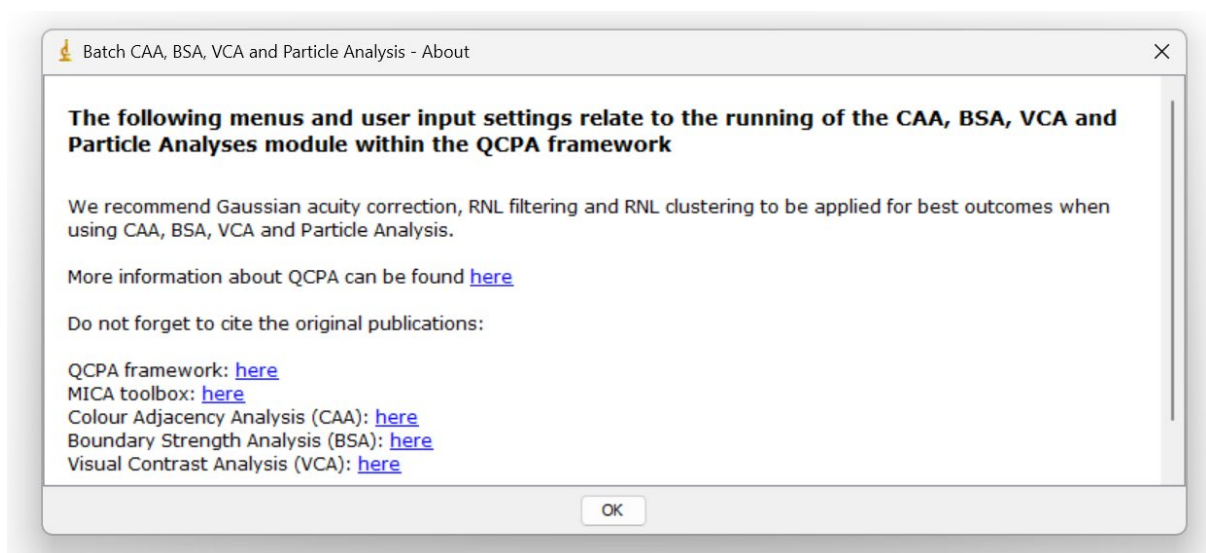
- Studies using *R. aculeatus* as a modelled observer typically assume a very conservative noise level of 0.05 in every type of photoreceptor. Luckily, we have a good understanding of the relative abundance of each type of photoreceptor and we can [calculate the corresponding channel-specific Weber fractions](#): lw: 0.05, mw: 0.05, sw: 0.07.
- R. aculeatus* uses its double cone (the fused 'unit' of the mw & lw receptors) for luminance contrast detection. Thus, in the csv file in the toolbox that was used to train the mapping function, the luminance channel is called 'dbl'. Therefore, the luminance channel in the mapping function is also called 'dbl'. However, the batch script automatically assumes the last channel in that file to be the luminance channel and will refer to it as 'lum'. Thus, no

need to call it anything else and, importantly, make sure your sensitivity file in the toolbox, used to train your mapping function, has a luminance channel.

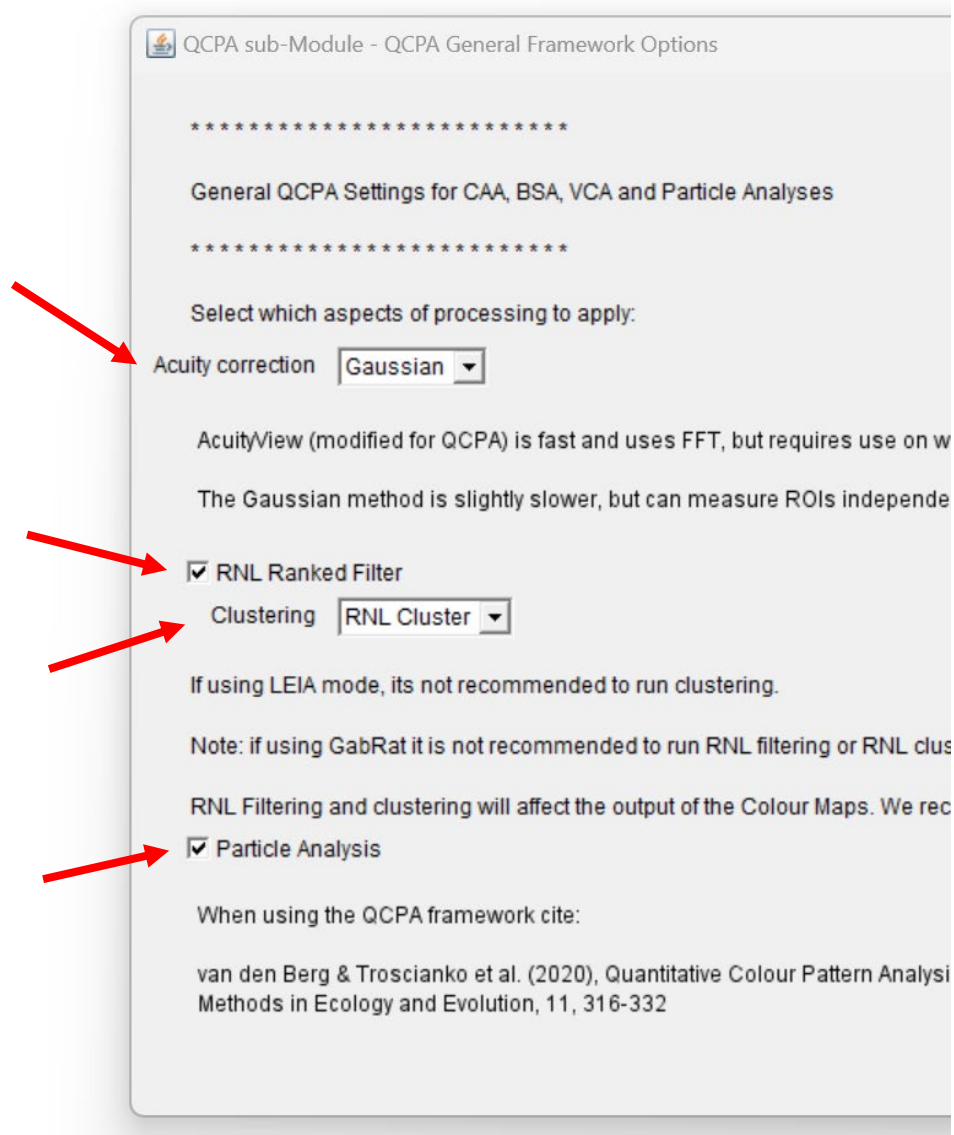


Step 5: Provide settings relating to module 1 – CAA, BSA, VCA and Particle Analysis

- a. The script will prompt an intro panel with useful links and recommendations for settings and reminders to cite the correct corresponding literature for use in publications.



- b. We will be using the Gaussian acuity modelling (not AcuityView) implemented in the QCPA as the outlines of our animals and the backgrounds are not rectangular, and we are interested in analysing ROIs individually rather than the whole image.
- c. To use module 1, clustering is mandatory. We, therefore, use the RNL clustering, as the naïve Bayes clustering is not available because it would require manual input for each image.
- d. We will enable particle analysis
- e. RNL filtering is enabled, as recommended, following spatial acuity modelling.



- f. We will use acuity provided in cycles per degree (cpd). Behavioural experiments and histological data suggest a maximum acuity of about 3-5 cpd in triggerfish. We'll be conservative and use 3 cpd.
- g. We will be using the 'viewing distance' method, which is the only option available in the batch script.
- h. We will rescale our images to a pixel/MRA ratio of 5 to maximise processing speed while minimising the loss of relevant spatial information.

Acuity correction

QCPA acuity correction (CAA, BSA, VCA & Particle Analysis)

Acuity Settings

Acuity units: Cycles per degree

Acuity value: 3

Distance/Angle Settings

Method: Viewing distance

If using 'Viewing distance' the image must contain a scale-bar, and the must match the scale bar units (e.g. mm). All angles are in degrees

Rescale to px per MRA: 5

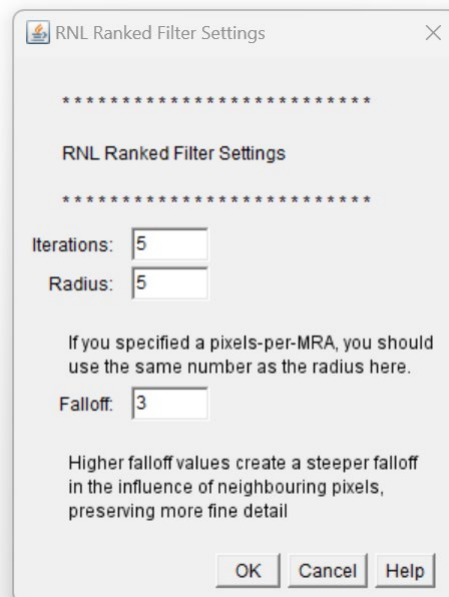
Automatically re-scale the image to a set number of pixels per MRA, 0=0

Background Info

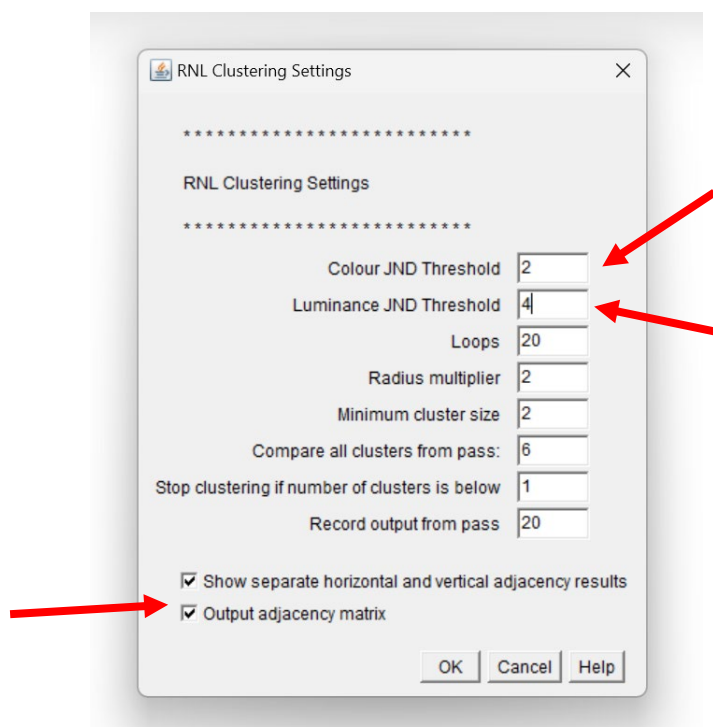
AcuityView in QCPA is based on a 2016 Matlab script by S. Johnsen. Bo However, a host of other/additional processing steps may go into truly a

If you use AcuityView rather than Gaussian, please also consider citing: Caves, E. M. & Johnsen, S. AcuityView: An r package for portraying the e Methods in Ecology and Evolution 9, 793-797 (2018).

- i. We will use the RNL filter with 5 iterations, a radius of 5 and a falloff of 3.



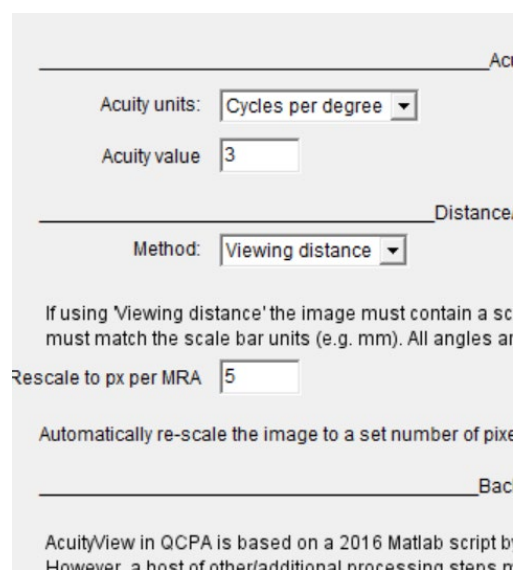
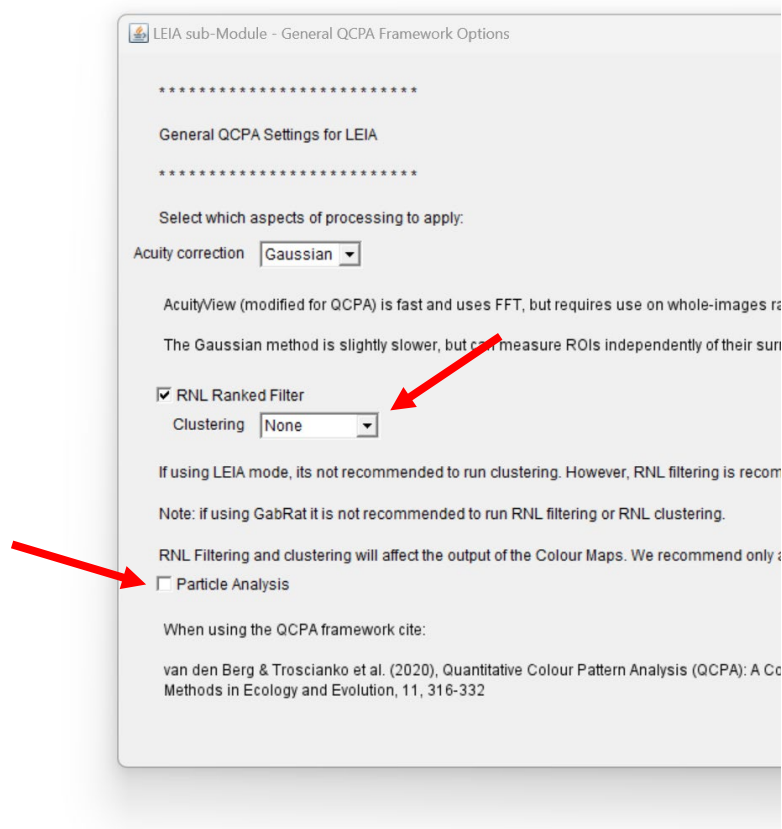
- j. We will run the analysis with a chromatic JND threshold of $2 \Delta S$ and an achromatic threshold of $4 \Delta S$. These values align with recent findings from behavioural experiments.
- k. The rest of the settings we will leave at default. However, we will tick the options for all the output.



Step 6: Provide input settings relating to module 2 - LEIA

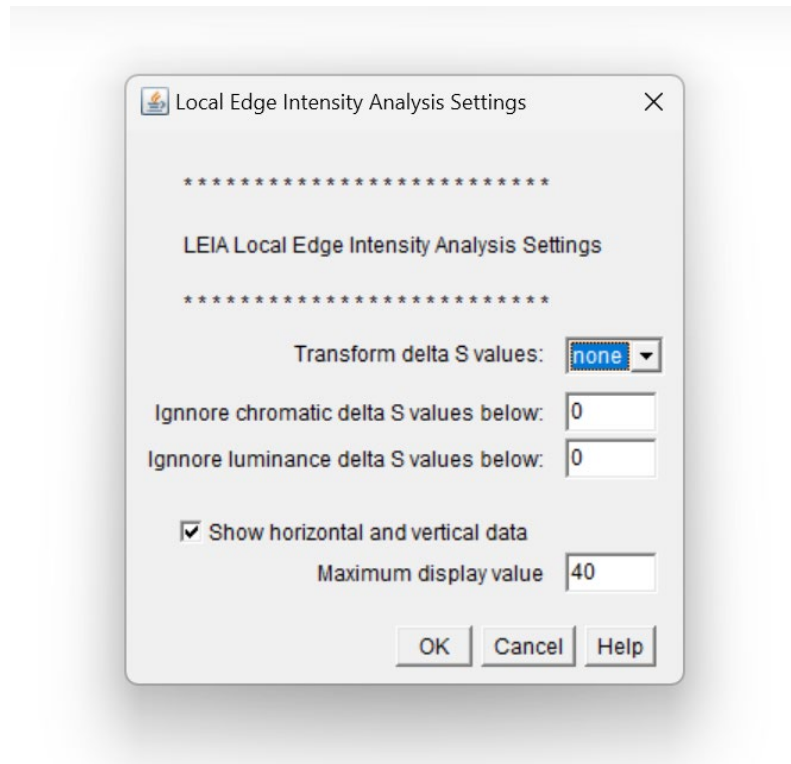
For LEIA, we will:

- NOT cluster the images, as we want to keep as many informative edges as possible.
- NOT run particle analysis, as our images for LEIA will not be clustered.



LEIA requires a set of unique input choices at the end:

- a. We will not transform the ΔS values of the edges detected by LEIA.
- b. We will ignore chromatic and achromatic edges with negative values.
- c. We will have the horizontal and vertical edges reported in addition to the overall edge contrast.
- d. We will choose a maximum display contrast of 40 (this does not impact the data).



Step 7: Provide input settings relating to module 3 - GabRat

For GabRat we will:

- a. NOT cluster the images
- b. NOT run particle analysis, as our images for GabRat will not be clustered
- c. NOT run RNL clustering, as we want edges in our images left as unprocessed as possible following acuity modelling.
- d. Run our Gaussian acuity correction with a value of 1cpd, the assumed λ_{\max} of the CSF in a triggerfish (this is unique to GabRat).

GabRat sub-Module - General QCPA Framework Options

General QCPA Settings for GabRat

Select which aspects of processing to apply:

Acuity Correction Gaussian

AcuityView (modified for QCPA) is fast and uses FFT, but requires use on whole
The Gaussian method is slower, but can measure ROIs independently
of their surroundings

☐ RNL Ranked Filter

Clustering None

If using LEIA mode, its not recommended to run clustering. However, RNL filtering

Note: if using GabRat it is not recommended to run RNL filtering or RNL clusterin

RNL Filtering and clustering will affect the output of the Colour Maps. We recomr

☐ Particle Analysis

When using the QCPA framework cite:

van den Berg & Troscianko et al. (2020), Quantitative Colour Pattern Analysis (Q
Methods in Ecology and Evolution, 11, 316-332

Acuity correction

QCPA acuity correction (GabRat)

Acuity Set

Acuity units: Cycles per degree

Acuity value 1

Note: Unlike other QCPA modules, GabRat should be used with

Distance/Angle :

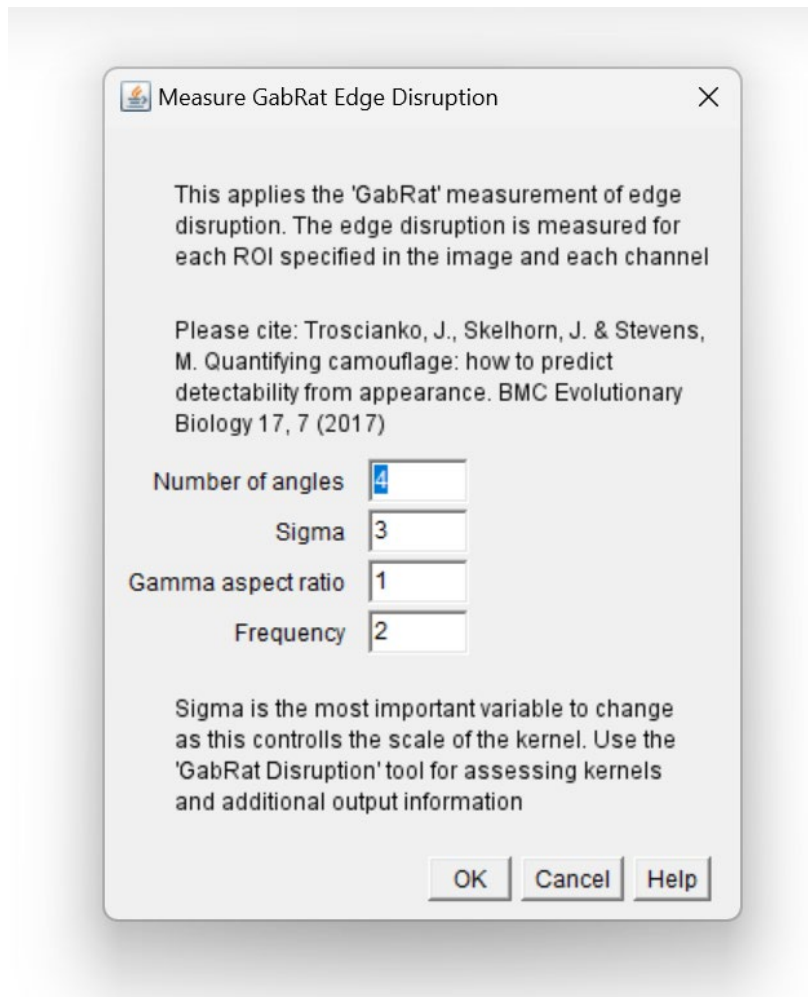
Method: Viewing distance

If using 'Viewing distance' the image must contain a scale-bar
must match the scale bar units (e.g. mm). All angles are in de

Rescale to px per MRA 5

GabRat requires a set of specific inputs at the end:

We will leave these settings at default. In a nutshell, The acuity modelling applied to the image means we won't need to adjust the properties of the Gabor filter (leave default settings) → It's all happening at the scale of a receptive field already due to our image rescaling.



Step 8: Provide input settings relating to module 4 – Colour Maps

For Colour maps, we will:

- NOT cluster our images
- Use the RNL filter, as we want to restrict the range of colours in our image (try with and without to see the difference).
- NOT enable particle analysis, as our images won't be clustered.

Colour Maps require the user to set the resolution of the Colour Map files. We recommend using the default resolution of 4 pixels per JND.

Colour Maps sub-Module - General QCPA Framework Options

General QCPA Settings for Colour Maps

Select which aspects of processing to apply:

Acuity Correction Gaussian

AcuityView (modified for QCPA) is fast and uses FFT, but requires a large ROI. The Gaussian method is slower, but can measure ROIs independent of their surroundings

☒ RNL Ranked Filter

Clustering None

If using LEIA mode, it's not recommended to run clustering. However, if using GabRat it is not recommended to run RNL filtering.

RNL Filtering and clustering will affect the output of the Colour Maps.

☐ Particle Analysis

When using the QCPA framework cite:

van den Berg & Troscianko et al. (2020), Quantitative Colour Perception Methods in Ecology and Evolution, 11, 316-332

Acuity

Acuity units: Cycles per degree

Acuity value 3

Distance

Method: Viewing distance

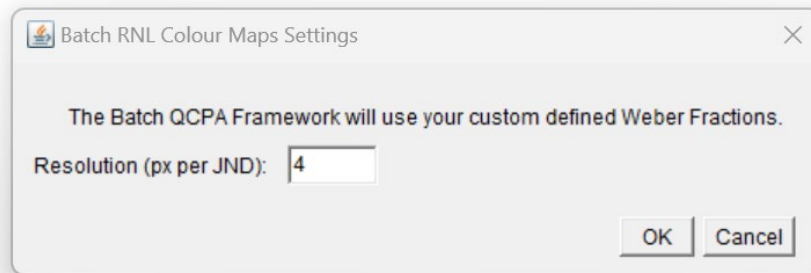
If using 'Viewing distance' the image must contain a scale bar. The scale bar must match the scale bar units (e.g. mm). All angles are in degrees.

Rescale to px per MRA 5

Automatically re-scale the image to a set number of pixels per MRA.

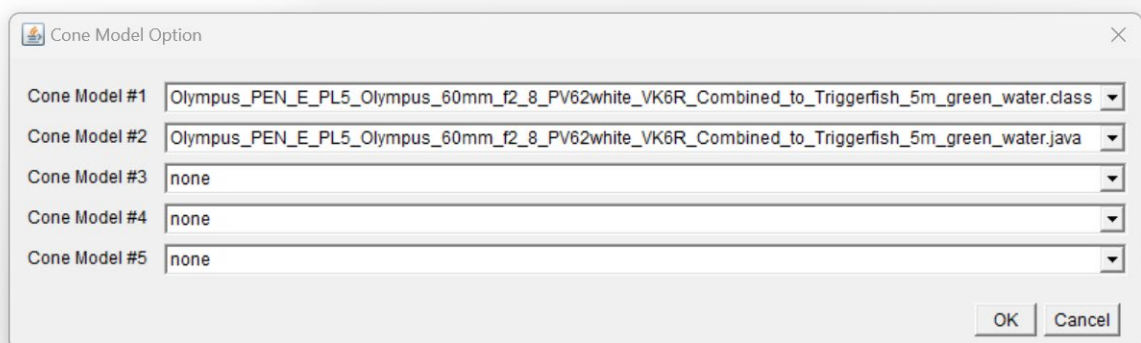
Back

AcuityView in QCPA is based on a 2016 Matlab script by [citation]. However, a host of other/additional processing steps have been added.

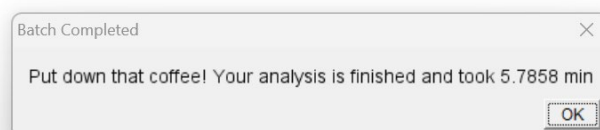


Step 9: Define which cone mapping functions ('visual models') to use

- a. We will be using:
 Olympus_PEN_E_PL5_Olympus_60mm_f2_8_PV62white_VK6R_Combined_to_Triggerfish_5m_green_water as our cone model number 1 and
 Olympus_PEN_E_PL5_Olympus_60mm_f2_8_VK6R_to_Triggerfish_5mGreenWater as our model number 2.
- b. You might notice that, in fact, all images in the dataset have model 1 specified in their corresponding .txt file, so the analysis would run even if we had just specified one model. We're doing this here to demonstrate how.



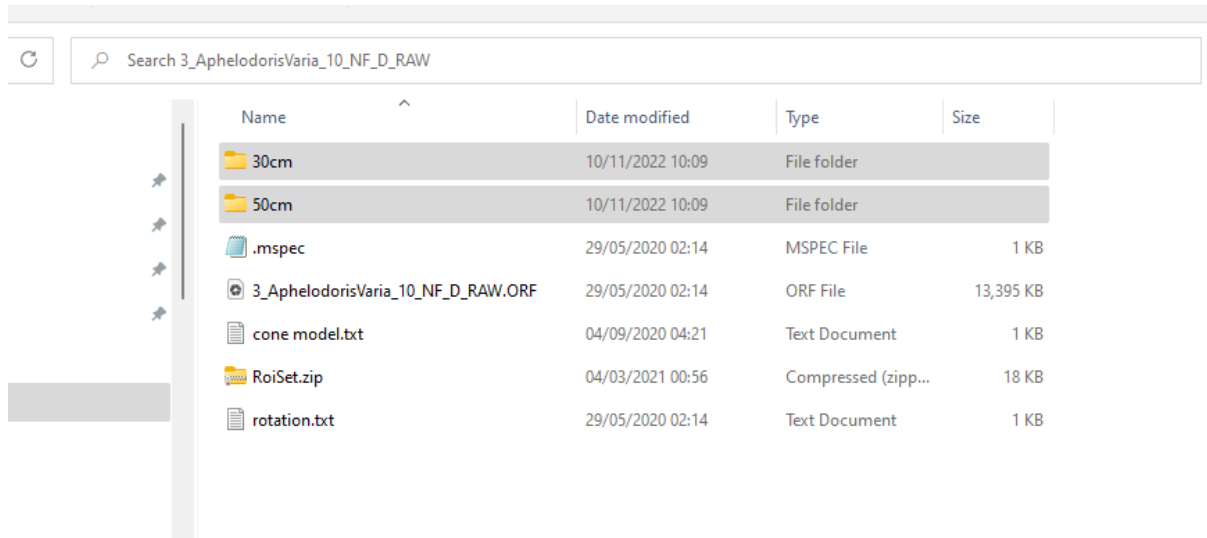
The analysis should take a couple of minutes to run.



Example 1: Data output

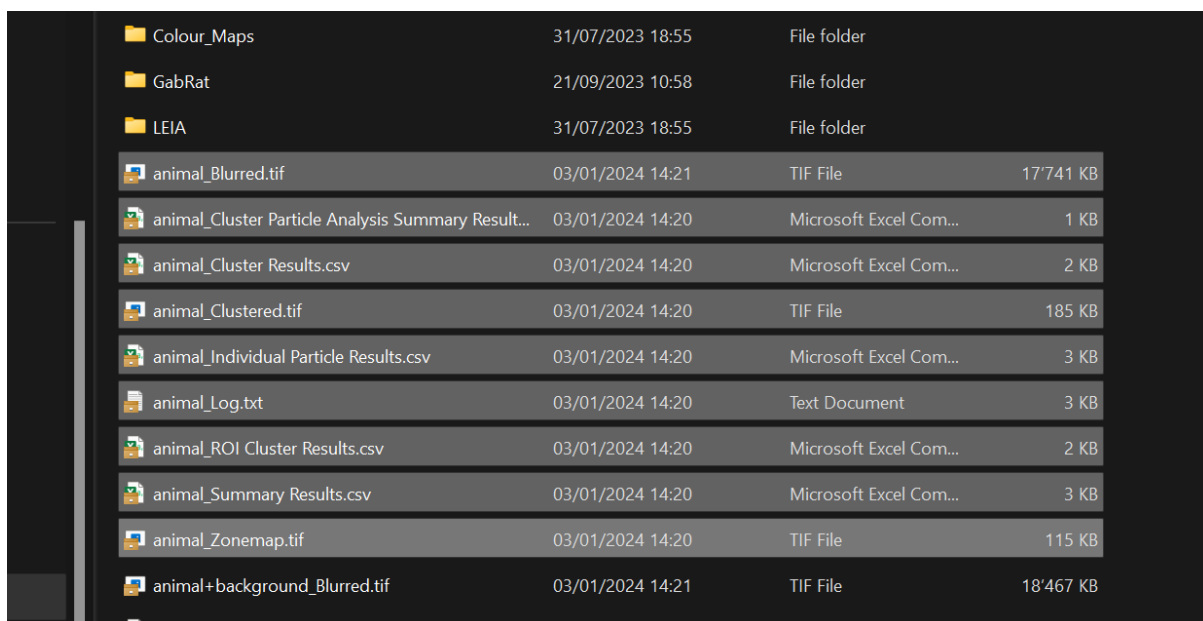
The batch script will provide a detailed log for the settings used at the top level. Please upload this log with any published data and modelling files to promote reproducibility.

All data output will be stored in a distance-specific folder. In our case 30cm and 50cm.



Name	Date modified	Type	Size
30cm	10/11/2022 10:09	File folder	
50cm	10/11/2022 10:09	File folder	
.mspec	29/05/2020 02:14	MSPEC File	1 KB
3_AphelodorisVaria_10_NF_D_RAW.ORF	29/05/2020 02:14	ORF File	13,395 KB
cone model.txt	04/09/2020 04:21	Text Document	1 KB
RoiSet.zip	04/03/2021 00:56	Compressed (zip) File	18 KB
rotation.txt	29/05/2020 02:14	Text Document	1 KB

Within each distance folder, a log file for each ROI and module can be found, together with all generated output **for module 1**:



Colour_Maps	31/07/2023 18:55	File folder	
GabRat	21/09/2023 10:58	File folder	
LEIA	31/07/2023 18:55	File folder	
animal_Blurred.tif	03/01/2024 14:21	TIF File	17'741 KB
animal_Cluster Particle Analysis Summary Result...	03/01/2024 14:20	Microsoft Excel Com...	1 KB
animal_Cluster Results.csv	03/01/2024 14:20	Microsoft Excel Com...	2 KB
animal_Clustered.tif	03/01/2024 14:20	TIF File	185 KB
animal_Individual Particle Results.csv	03/01/2024 14:20	Microsoft Excel Com...	3 KB
animal_Log.txt	03/01/2024 14:20	Text Document	3 KB
animal_ROI Cluster Results.csv	03/01/2024 14:20	Microsoft Excel Com...	2 KB
animal_Summary Results.csv	03/01/2024 14:20	Microsoft Excel Com...	3 KB
animal_Zonemap.tif	03/01/2024 14:20	TIF File	115 KB
animal+background_Blurred.tif	03/01/2024 14:21	TIF File	18'467 KB

Specifically, for each ROI you will find:

'ROI NAME' blurred.tiff → a multispectral .tiff of your ROI after acuity modelling & RNL filtering (or without filtering if you don't use the RNL filter). This is great for visualisations (i.e. false colour presentation images).

'ROI NAME' Cluster Particle Analysis Summary Results.csv → A summary of all individual clusters (summarised across individual sub-particles) in the segmented image. This is a great way to get a suite of descriptors summarising each colour pattern element, such as its average orientation, total size, solidity, etc. This is a base ImageJ function.

'ROI NAME' Cluster Results.csv → A range of basic summary metrics for each cluster in the ROI, such as different mean chromaticity metrics, mean receptor channel stimulation etc.

'ROI NAME' Clustered.tiff → A multispectral .tiff of your ROI after RNL clustering. This is great for visualisations (i.e. false colour presentation images).

'ROI NAME' Individual Particle Results.csv → Particle descriptors for all sub-particles. A good source of information on shape of individual colour pattern elements.

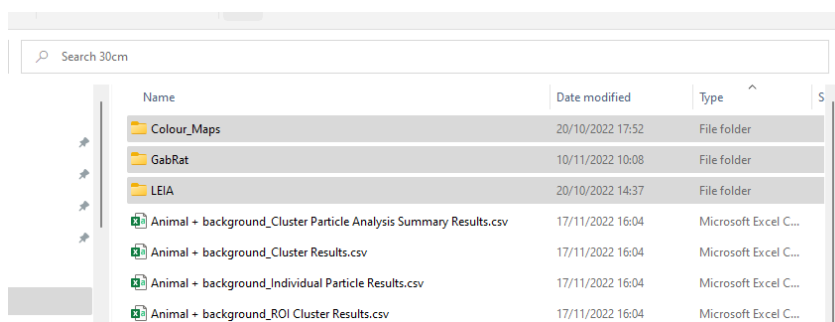
'ROI' Log.txt → A log file of all processing steps and their respective settings applied in module 1.

'ROI NAME' ROI Cluster results.csv → Summary of the spectral properties of the colour pattern elements. This is where you find the transition matrices. This file is the basis for all computations for the CAA, BSA, VCA.

'ROI Name' Summary Results.csv → All CAA, VCA, BSA parameters.

'ROI NAME' Zonemap.tiff → A .tiff file of the clustered ROI as a zone map, i.e., with each cluster numbered 1 to k.

For modules 2 – 4, all data output is stored in corresponding subfolders:



Name	Date modified	Type
Colour_Maps	20/10/2022 17:52	File folder
GabRat	10/11/2022 10:08	File folder
LEIA	20/10/2022 14:37	File folder
Animal + background_Cluster Particle Analysis Summary Results.csv	17/11/2022 16:04	Microsoft Excel C...
Animal + background_Cluster Results.csv	17/11/2022 16:04	Microsoft Excel C...
Animal + background_Individual Particle Results.csv	17/11/2022 16:04	Microsoft Excel C...
Animal + background_ROI Cluster Results.csv	17/11/2022 16:04	Microsoft Excel C...

Colour Maps:

Each ROI is saved as a colour map cloud .tiff file that can be used to visualise colour space and/or calculate colour space overlap using the colour map functions in QCPA (see [here](#)).

Additionally, a detailed log can be found in the main folder.

LEIA:

For each ROI, the user will have the numerical output of LEIA in a .csv file and the visualised chromatic and achromatic LEIA contrast saved as a .tiff. The latter are fantastic for visualisation purposes.

Additionally, a detailed log can be found in the main folder.

GabRat:

The user will find a .csv file with the GabRat values for all ROIs. Only the animal ROI is of significance, as the kernel runs along the outside of the animal ROI.

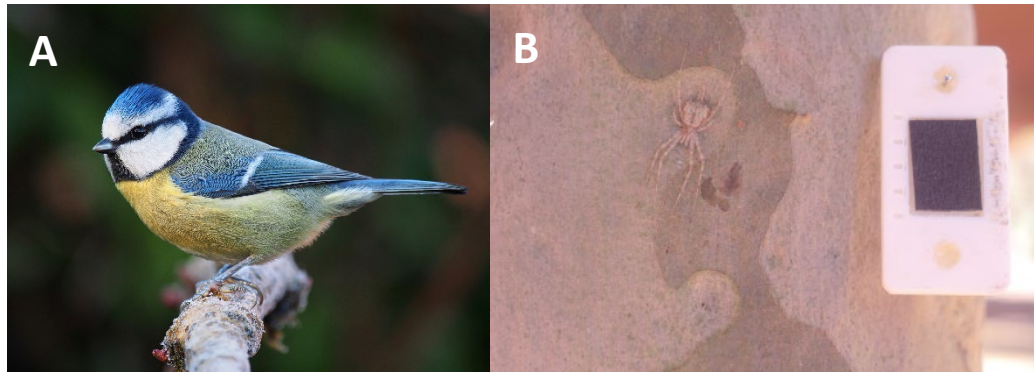
Additionally, a detailed log can be found in the main folder.

To assist with compiling data out of these folders & files, please see the complementary R-script library provided here:

<https://github.com/CaraConradsen/QCPA-r-script>.

Example 2: A tetrachromatic observer with UV sensitivity

For this example, we will investigate a bird predator (European Bluetit, *Parus major*) looking at spiders (*Tamopsis brisbanensis*) on a tree trunk. These image data and calibration files have been kindly provided by Alfonso Aceves.



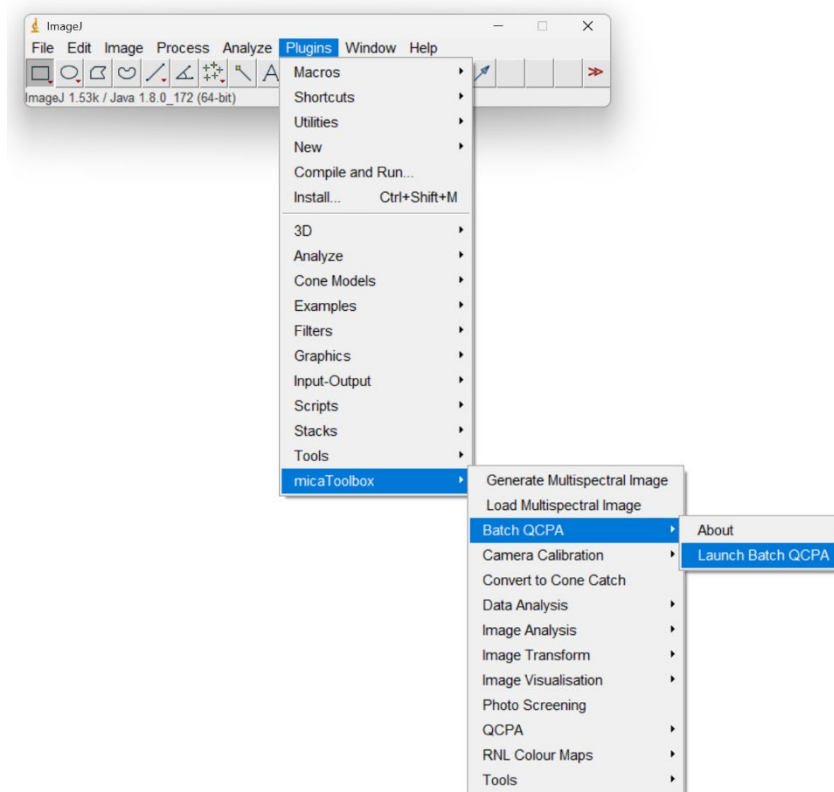
A) *Parus major* (© Francis C. Franklin / CC-BY-SA-3.0); B) Calibrated image of *Tamopsis brisbanensis*, taken by A. Aceves (with permission).

To run this example, you will need:

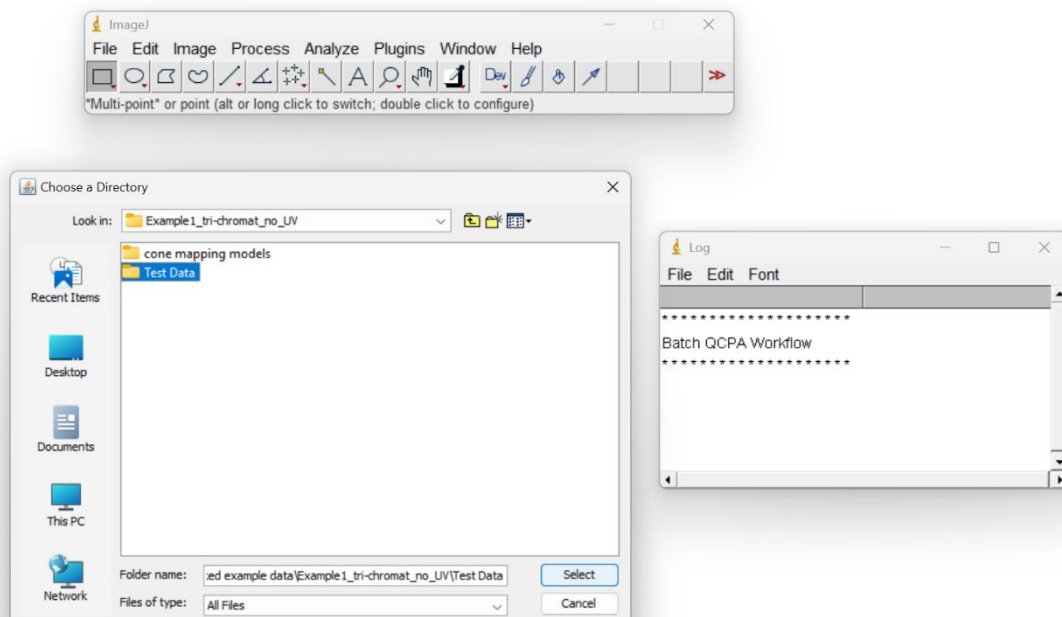
1. The test data provided here: <https://doi.org/10.48610/3cdcc1f>
2. The following visual model (provided with the test data):
 - a. Nikon_D7000_Novoflex_35mm_D65_to_Bluetit_D65
Copy the JAVA and CLASS files in the 'cone mapping models' folder provided in the test data folder into your local directory of imageJ into 'plugins' -> 'Cone Models'.
3. The latest version of the MICA toolbox & ImageJ available [here](#)
4. The correctly installed QCPA batch script, available here:
<https://github.com/cedricvandenbergh/QCPA-batch-script>

See the batch script manual for detailed instructions on installing the toolbox and the batch script.

Step 1: Launch the batch script (Plugins -> micaToolbox -> Batch QCPA -> Launch Batch QCPA)



Step 2: Select the 'Test Data' folder in the 'tetra-chromat with UV' example folder.



Step 3: Provide the general input setting for the analysis.

- a. We will work with '.NEF' images, the RAW file format for Nikon cameras.
- b. We have not renamed our .mspec images. Thus, the script will look for files simply called '.mspec'.
- c. We will simulate viewing distances of 30 cm and 50 cm. This will make processing comparably fast. Note that the larger the images, the higher the spatial acuity of the modelled visual system, and the closer the viewing distance, the longer processing will take as the resulting images will remain large.
- d. Bluetits (*Parus major*) have 4 known cone types and, thus, spectral sensitivities involved in bright-light colour vision, plus a double cone responsible for luminance contrast perception.
- e. As the images were taken with variable animal rotations, each animal folder contains a .txt file called 'rotation.txt' specifying the number of degrees the image needs to be rotated to be aligned with the anterior-posterior axis of the animal. Therefore, 'Enable rotation' should be ticked.
- f. We will choose a [lower chromaticity limit](#) of 0.03. This means that, below 3% luminance, the observer should not be able to perceive any colour.
- g. We will [replace negative cone catch values](#) in our images with values of 0.001.
- h. We will analyse all three ROIs available in the dataset: The animal, the animal and its background and the background by itself.
- i. We will apply all modules available in the QCPA batch script.

Main QCPA Input Parameters

Choose your file Extension

Enter your mspec filename

Add viewing distances in same unit as the scale bar in each image

If using more than one distance ensure values are separated by a comma

Number of spectral sensitivities (excl. luminance channel) required?

☒ Enable rotation?

Choose your lower chromaticity limit

Replace negative cone catch values with:

Use 'IGNORE' above to ignore these settings and use a value of 0

Select the ROIs you would like to measure below:

☒ Animal ROI

☒ Animal + Background

☒ Background ROI

Select the modules you would like to use below:

Note that Colour maps will not work with less than two colour channels (dichromacy)

☒ CAA,BSA,VCA & Particle Analysis

☒ LEIA

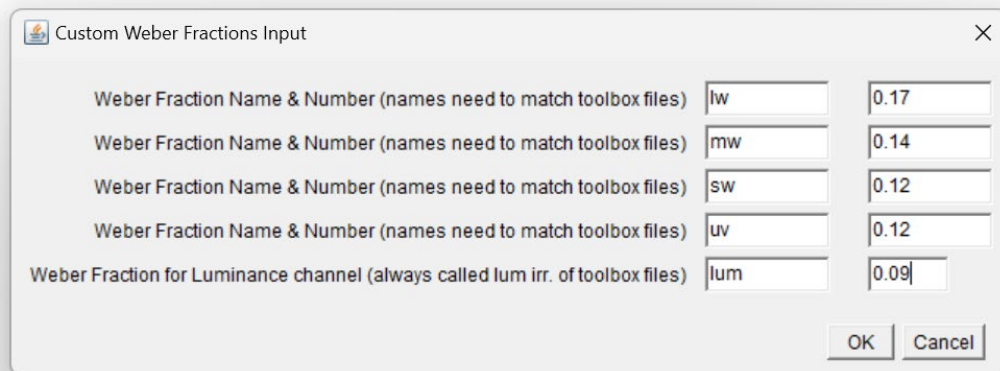
☒ GabRat

☒ RNL Colour Maps

Step 4: Provide Weber fractions

- a. Hart et al., 2000 showed that photoreceptors are distributed at the following ratios (uv:sw:mw:lw:dbl): 1:1.92:2.68:2.7 with the (double) dbl cone relative abundance of 4.86. When normalised to the most abundant class of receptors, we get: 0.21:0.4:0.55:0.55:1. Silvesti et al., (2021) have determined that a Weber fraction of 0.2 reflects a 1 ΔS JND for the lw cone and thus, following the equations outlined [here](#), we get a receptor-specific noise of 0.09, this then let's us calculate the appropriate Weber fractions as follows: 0.17:0.14:0.12:0.12:0.09 (uv:w:mw:lw:dbl). Here, dbl, is the luminance channel.

- b. *P. major* uses its double cone for luminance contrast detection. Thus, in the csv file in the toolbox that was used to train the mapping function, the luminance channel is called 'dbl'. Therefore, the luminance channel in the mapping function is also called 'dbl'. However, the batch script automatically assumes the last channel in that file to be the luminance channel and will refer to it as 'lum'. Thus, no need to call it anything else and, importantly, make sure your sensitivity file in the toolbox, used to train your mapping function, has a luminance channel.



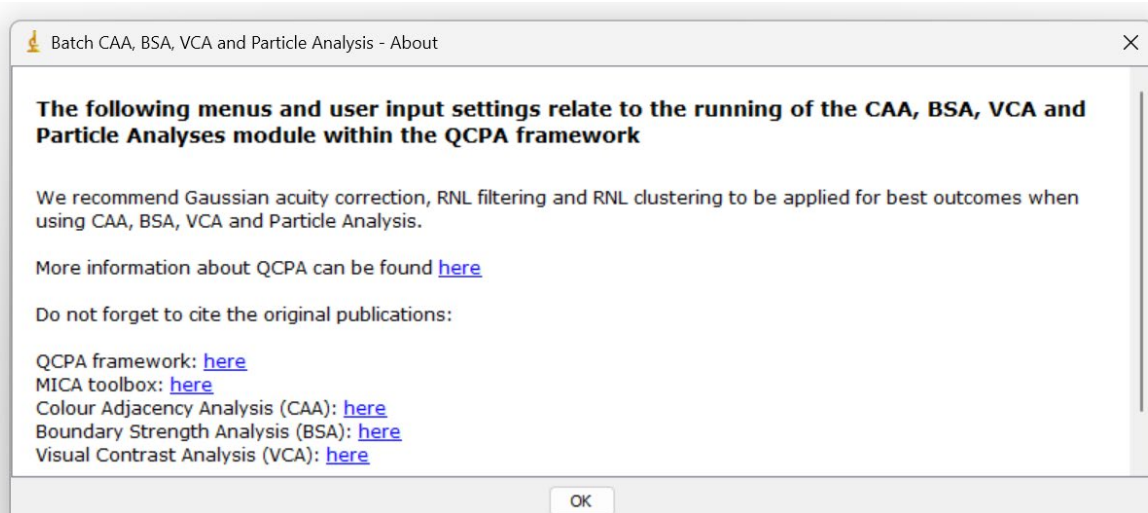
Custom Weber Fractions Input

Weber Fraction Name & Number (names need to match toolbox files)	lw	0.17
Weber Fraction Name & Number (names need to match toolbox files)	mw	0.14
Weber Fraction Name & Number (names need to match toolbox files)	sw	0.12
Weber Fraction Name & Number (names need to match toolbox files)	uv	0.12
Weber Fraction for Luminance channel (always called lum irr. of toolbox files)	lum	0.09

OK Cancel

Step 5: Provide settings relating to module 1 – CAA, BSA, VCA and Particle Analysis

- a. The script will prompt an intro panel with useful links and recommendations for settings and reminders to cite the correct corresponding literature for use in publications.



Batch CAA, BSA, VCA and Particle Analysis - About

The following menus and user input settings relate to the running of the CAA, BSA, VCA and Particle Analyses module within the QCPA framework

We recommend Gaussian acuity correction, RNL filtering and RNL clustering to be applied for best outcomes when using CAA, BSA, VCA and Particle Analysis.

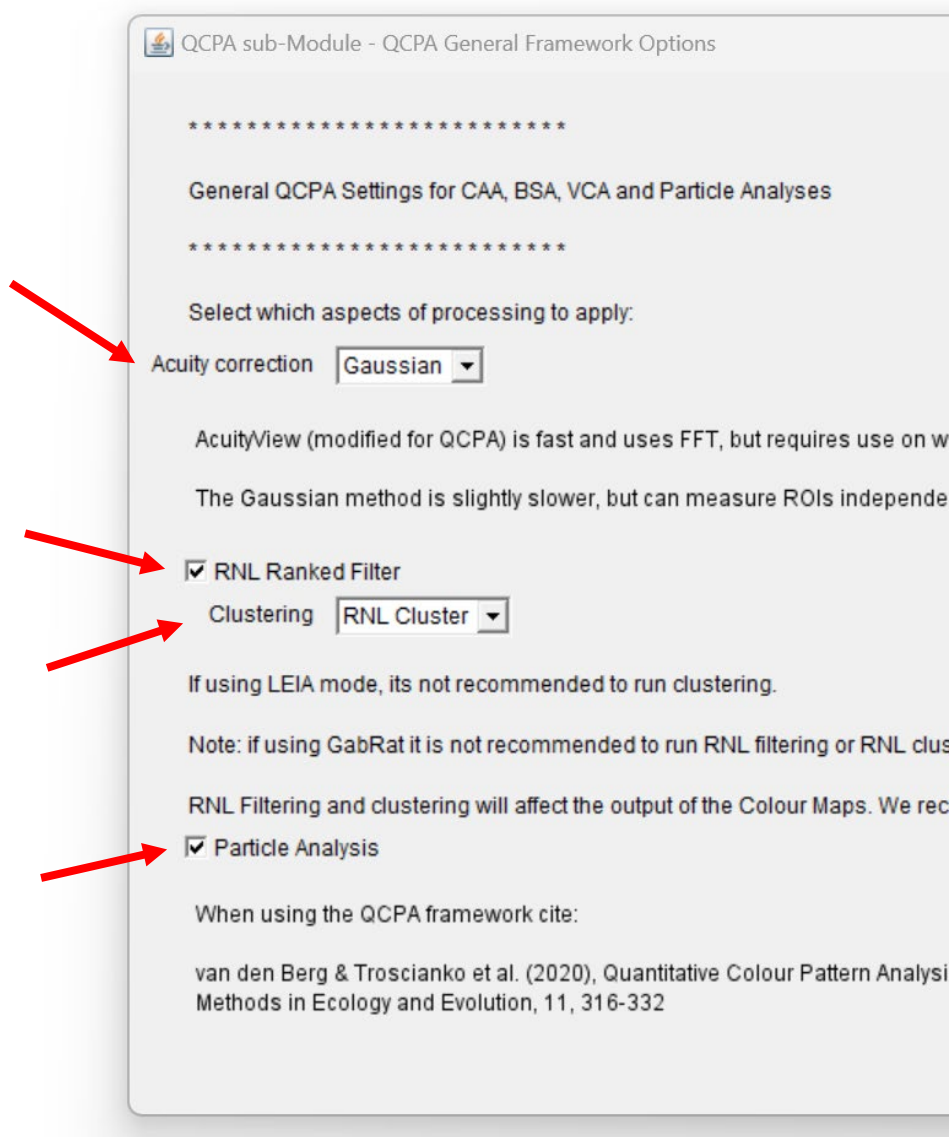
More information about QCPA can be found [here](#)

Do not forget to cite the original publications:

QCPA framework: [here](#)
 MICA toolbox: [here](#)
 Colour Adjacency Analysis (CAA): [here](#)
 Boundary Strength Analysis (BSA): [here](#)
 Visual Contrast Analysis (VCA): [here](#)

OK

- b. We will be using the Gaussian acuity modelling (not AcuityView) implemented in the QCPA as the outlines of our animals and the backgrounds are not rectangular, and we are interested in analysing ROIs individually rather than the whole image.
- c. To use module 1, clustering is mandatory. Therefore, we use the RNL clustering, as the naïve Bayes clustering is unavailable because it would require manual input for each image.
- d. We will enable particle analysis.
- e. RNL filtering is enabled, as recommended, following spatial acuity modelling.



- f.* We will use acuity provided in cycles per degree (cpd). Behavioural experiments and histological data suggest a maximum acuity of about 6 cpd in tits (Martin, 2018) which we will use here.
- g.* We will use the 'viewing distance' method, the only option available in the batch script.
- h.* We will rescale our images to a pixel/MRA ratio of 5 to maximise processing speed while minimising the loss of relevant spatial information.

Acuity correction

QCPA acuity correction (CAA, BSA, VCA & Particle Analysis)

Acuity Settings

Acuity units:

Acuity value:

Distance/Angle Settings

Method:

If using 'Viewing distance' the image must contain a scale-bar, and the units entered must match the scale bar units (e.g. mm). All angles are in degrees

Rescale to px per MRA:

Automatically re-scale the image to a set number of pixels per MRA, 0=off. A value of 5

Background Info

AcuityView in QCPA is based on a 2016 Matlab script by S. Johnsen. Both, Gaussian, However, a host of other/additional processing steps may go into truly approximating

If you use AcuityView rather than Gaussian, please also consider citing:
Caves, E. M. & Johnsen, S. AcuityView: An r package for portraying the effects of visual
Methods in Ecology and Evolution 9, 793-797 (2018).

- i. We will use the RNL filter with 5 iterations, a radius of 5 and a falloff of 3.

RNL Ranked Filter Settings

RNL Ranked Filter Settings

Iterations:

Radius:

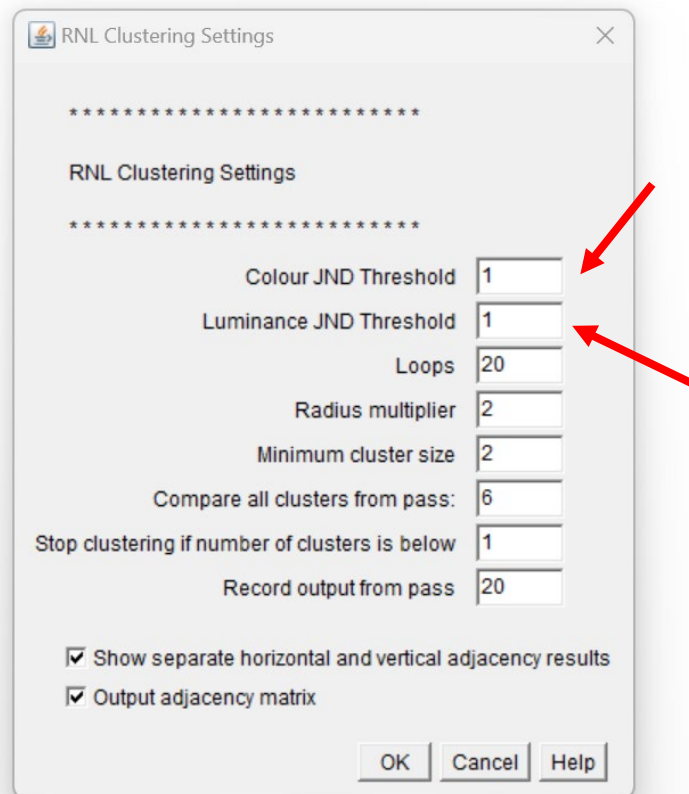
If you specified a pixels-per-MRA, you should use the same number as the radius here.

Falloff:

Higher falloff values create a steeper falloff in the influence of neighbouring pixels, preserving more fine detail

OK Cancel Help

- j. We will run the analysis with a chromatic JND threshold of 1 ΔS and an achromatic threshold of 1 ΔS . These values are much less conservative as we have calibrated our Weber fractions using findings from behavioural experiments.



- k. The rest of the settings we will leave at default. However, we will tick the options for all the output.

Step 6: Provide input settings relating to module 2 - LEIA

For LEIA, we will:

- NOT cluster the images, as we want to keep as many informative edges as possible.
- NOT run particle analysis, as our images for LEIA will not be clustered.

LEIA sub-Module - General QCPA Framework Options

General QCPA Settings for LEIA

Select which aspects of processing to apply:

Acuity correction Gaussian

AcuityView (modified for QCPA) is fast and uses FFT, but requires use on whole-images rather than ROIs.

The Gaussian method is slightly slower, but can measure ROIs independently of their surroundings.

☒ RNL Ranked Filter

Clustering None

If using LEIA mode, it is not recommended to run clustering. However, RNL filtering is recommended.

Note: if using GabRat it is not recommended to run RNL filtering or RNL clustering.

RNL Filtering and clustering will affect the output of the Colour Maps. We recommend only using one of these options.

☐ Particle Analysis

When using the QCPA framework cite:

van den Berg & Troscianko et al. (2020), Quantitative Colour Pattern Analysis (QCPA): A Comprehensive Framework for Analysing Visual Information in Ecology and Evolution, 11, 316-332

Acuity correction

QCPA acuity correction (CAA, BSA, VCA & Particle Analysis)

Acuity Settings

Acuity units: Cycles per degree

Acuity value 6

Distance/Angle Settings

Method: Viewing distance

If using 'Viewing distance' the image must contain a scale-bar, and the units entered must match the scale bar units (e.g. mm). All angles are in degrees.

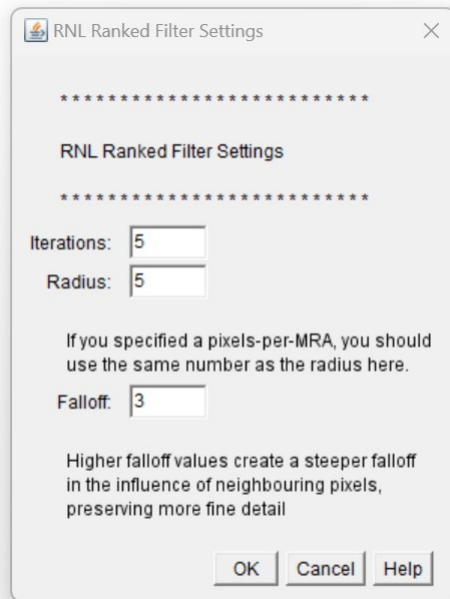
Rescale to px per MRA 5

Automatically re-scale the image to a set number of pixels per MRA, 0=off. A value of 5 will re-scale the image to 5 pixels per MRA.

Background Info

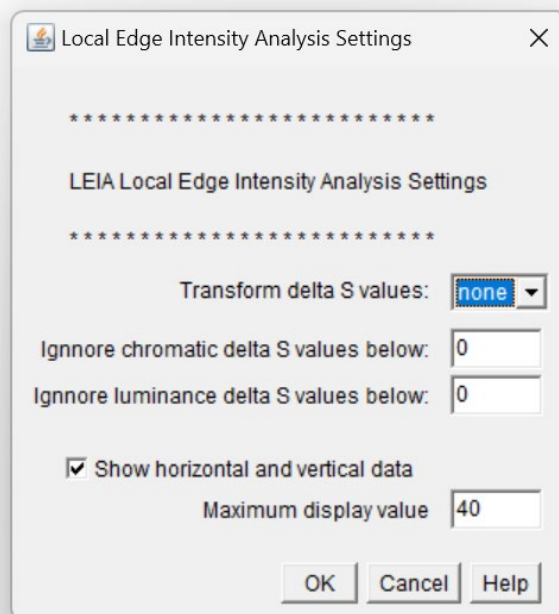
AcuityView in QCPA is based on a 2016 Matlab script by S. Johnsen. Both, Gaussian and AcuityView methods can be used to approximate visual acuity. However, a host of other/additional processing steps may go into truly approximating visual acuity.

If you use AcuityView rather than Gaussian, please also consider citing:
Caves, E. M. & Johnsen, S. AcuityView: An R package for portraying the effects of visual acuity on colour pattern analysis. Methods in Ecology and Evolution 9, 793-797 (2018).



LEIA requires a set of unique input choices at the end:

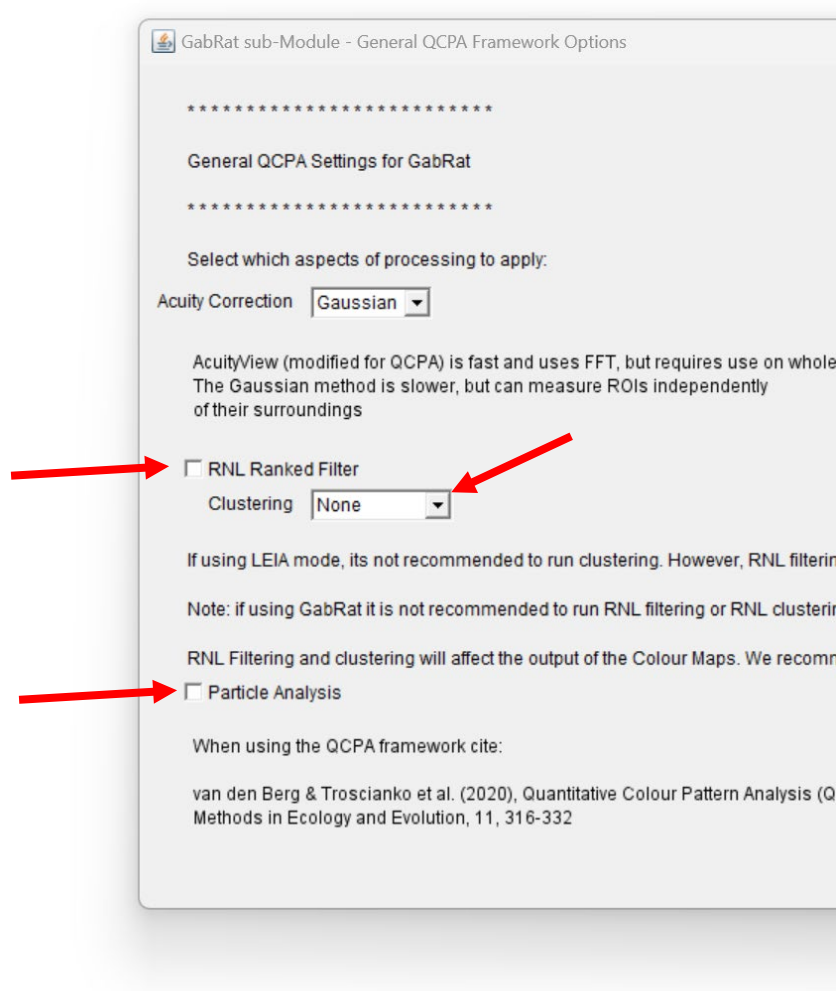
- a. We will not transform the ΔS values of the edges detected by LEIA.
- b. We will ignore chromatic and achromatic edges with negative values
- c. We will have the horizontal and vertical edges reported in addition to the overall edge contrast.
- d. We will choose a maximum display contrast of 40 (this does not impact the data).

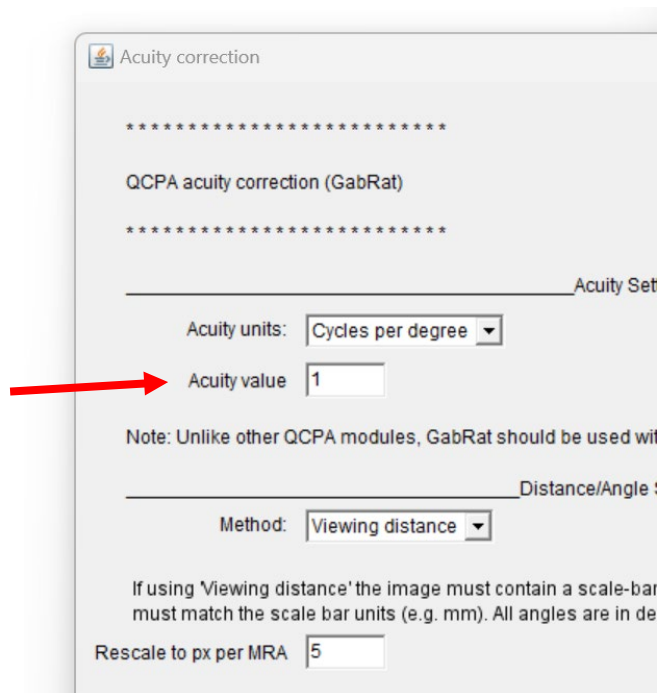


Step 7: Provide input settings relating to module 3 - GabRat

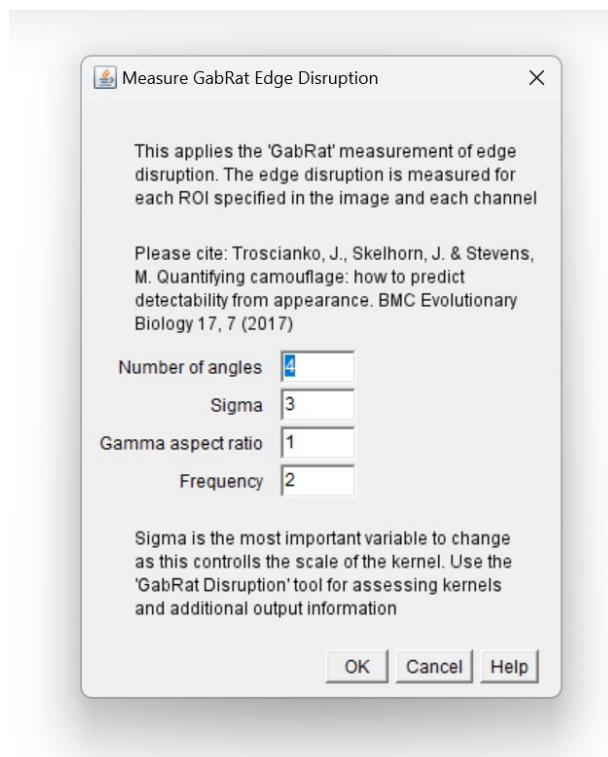
For GabRat we will:

- a. NOT cluster the images
- b. NOT run particle analysis, as our images for GabRat will not be clustered
- c. NOT run RNL clustering, as we want edges in our images left as unprocessed as possible following acuity modelling.
- d. Run our Gaussian acuity correction with a value of 1cpd, the assumed λ_{\max} of the CSF in a songbird (Harmening & Wagner, 2011).





GabRat requires a set of specific inputs at the end:

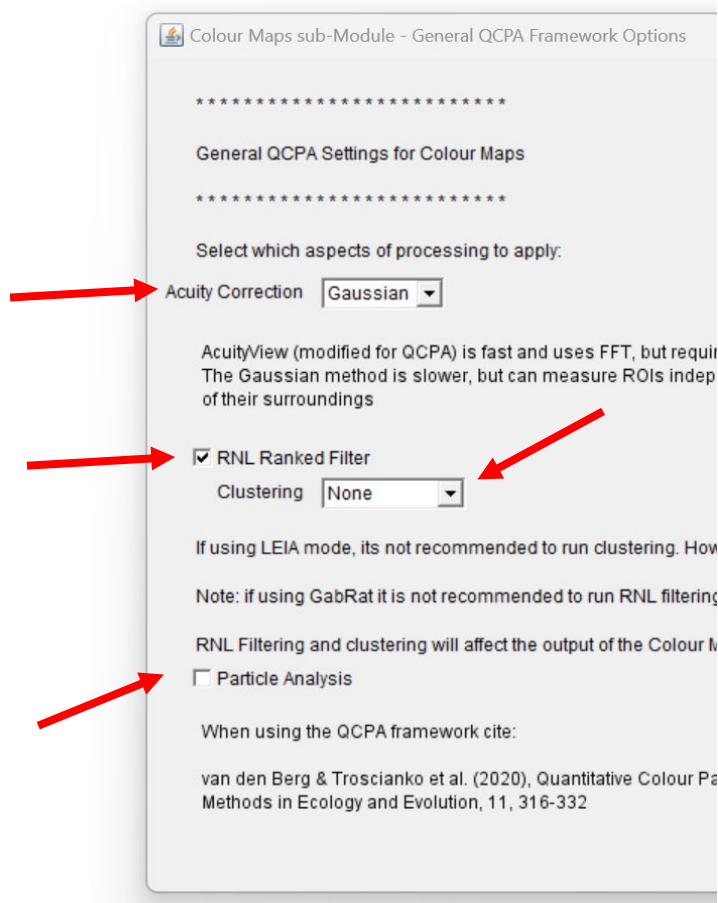


We will leave these settings at default. In a nutshell: The acuity modelling applied to the image means we won't need to adjust the properties of the Gabor filter (leave default settings) → It's all happening at the scale of a receptive field already due to our image rescaling.

Step 8: Provide input settings relating to module 4 – Colour Maps

For Colour maps, we will:

- NOT cluster our images
- Use the RNL filter, as we want to restrict the range of colours in our image (try with and without to see the difference).
- Not enable particle analysis, as our images won't be clustered.



Colour Maps sub-Module - General QCPA Framework Options

General QCPA Settings for Colour Maps

Select which aspects of processing to apply:

Acuity Correction Gaussian

AcuityView (modified for QCPA) is fast and uses FFT, but requires a scale bar. The Gaussian method is slower, but can measure ROIs independent of their surroundings.

☒ RNL Ranked Filter

Clustering None

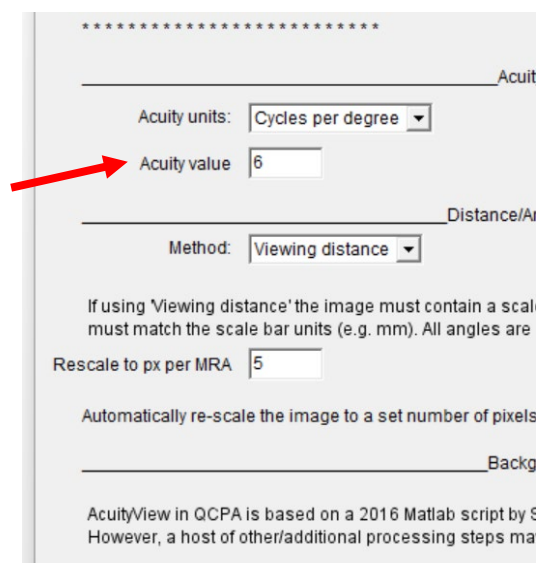
If using LEIA mode, it is not recommended to run clustering. However, if using GabRat it is not recommended to run RNL filtering.

RNL Filtering and clustering will affect the output of the Colour Map.

☐ Particle Analysis

When using the QCPA framework cite:

van den Berg & Troscianko et al. (2020), Quantitative Colour Perception Methods in Ecology and Evolution, 11, 316-332



Acuity

Acuity units: Cycles per degree

Acuity value 6

Distance/Acuity

Method: Viewing distance

If using 'Viewing distance' the image must contain a scale bar. The scale must match the scale bar units (e.g. mm). All angles are in degrees.

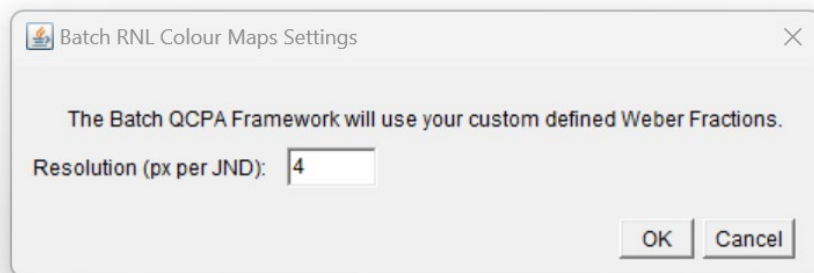
Rescale to px per MRA 5

Automatically re-scale the image to a set number of pixels

Backg

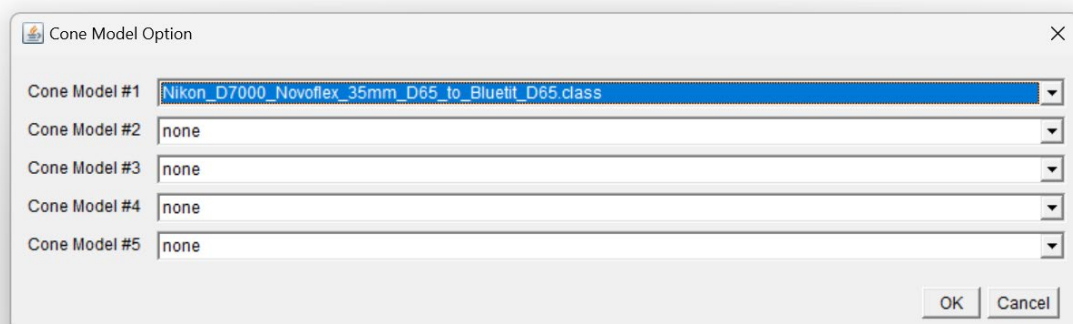
AcuityView in QCPA is based on a 2016 Matlab script by S. Troscianko. However, a host of other/additional processing steps may be applied.

Colour Maps require the user to set the resolution of the Colour Map files. We recommend using the default resolution of 4 pixels per JND.

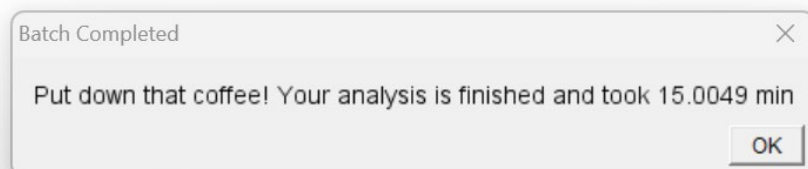


Step 9: Define which cone mapping functions ('visual models') to use

- a. We will be using: Nikon_D7000_Novoflex_35mm_D65_to_Bluetit_D65.

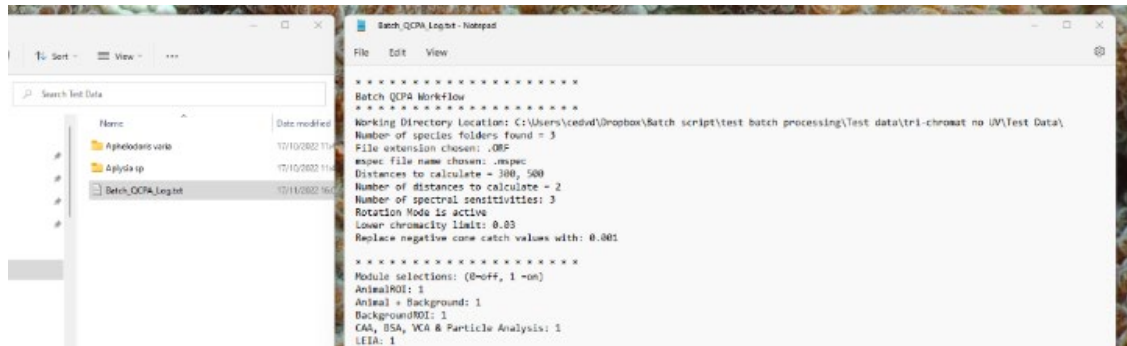


The analysis should take a couple of minutes to run. Note that this analysis will take longer than example 1 due to the significantly increased spatial acuity of the viewer.



Example 2: Data output

The batch script will provide a detailed log for the settings used at the top level. Please upload this log with any published data and modelling files to promote reproducibility.

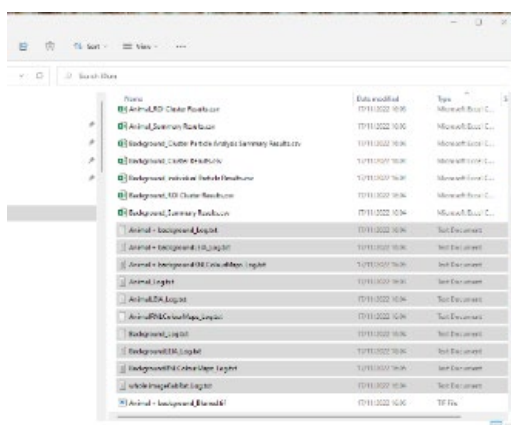


All data output will be stored in a distance-specific folder. In our case 30cm and 50cm.

The screenshot shows a file explorer window with the search bar containing 'Search 3_AphelodorisVaria_10_NF_D_RAW'. The file list is as follows:

Name	Date modified	Type	Size
30cm	10/11/2022 10:09	File folder	
50cm	10/11/2022 10:09	File folder	
.mspec	29/05/2020 02:14	MSPEC File	1 KB
3_AphelodorisVaria_10_NF_D_RAW.ORF	29/05/2020 02:14	ORF File	13,395 KB
cone model.txt	04/09/2020 04:21	Text Document	1 KB
RoiSet.zip	04/03/2021 00:56	Compressed (zipp...	18 KB
rotation.txt	29/05/2020 02:14	Text Document	1 KB

Within each distance folder, a log file for each ROI and module can be found, together with all generated output **for module 1**:



Specifically, for each ROI you will find:

'ROI NAME' blurred.tiff → a multispectral .tiff of your ROI after acuity modelling & RNL filtering (or without filtering if you don't use the RNL filter). This is great for visualisations (i.e. false colour presentation images).

'ROI NAME' Cluster Particle Analysis Summary Results.csv → A summary of all individual clusters (summarised across individual sub particles) in the segmented image. This is a great way to get a suite of descriptors summarising each colour pattern element, such as its average orientation, total size, solidity, etc. This is a base ImageJ function documented [here](#).

'ROI NAME' Cluster Results.csv → A range of basic summary metrics for each cluster in the ROI, such as different mean chromaticity metrics, mean receptor channel stimulation etc.

'ROI NAME' Clustered.tiff → A multispectral .tiff of your ROI after RNL clustering. This is great for visualisations (i.e. false colour presentation images).

'ROI NAME' Individual Particle Results.csv → Particle descriptors for all sub-particles. A good source of information on shape of individual colour pattern elements.

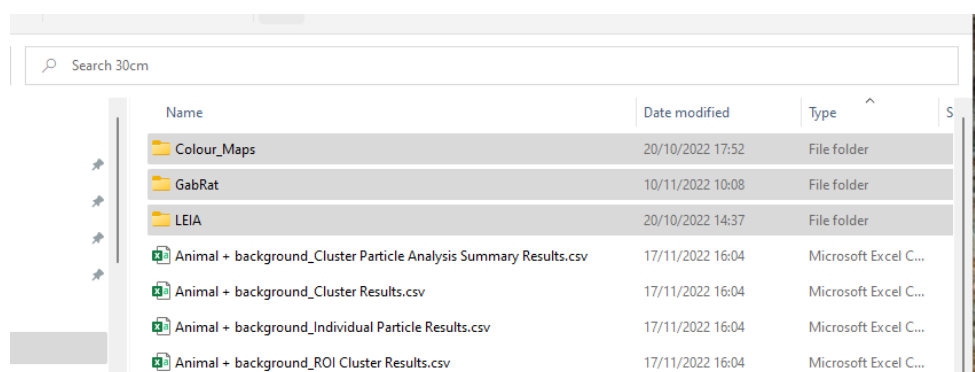
'ROI' Log.txt → A log file of all processing steps and their respective settings applied in module 1.

'ROI NAME' ROI Cluster results.csv → Summary of the spectral properties of the colour pattern elements. This is where you find the transition matrices. This file is the basis for all computations for the CAA, BSA, VCA.

'ROI Name' Summary Results.csv → All CAA, VCA, BSA parameters.

'ROI NAME' Zonemap.tiff → A .tiff file of the clustered ROI as a zone map, i.e. with each cluster numbered 1 to k.

For modules 2 – 4, all data output is stored in corresponding subfolders:



Name	Date modified	Type
Colour_Maps	20/10/2022 17:52	File folder
GabRat	10/11/2022 10:08	File folder
LEIA	20/10/2022 14:37	File folder
Animal + background_Cluster Particle Analysis Summary Results.csv	17/11/2022 16:04	Microsoft Excel C...
Animal + background_Cluster Results.csv	17/11/2022 16:04	Microsoft Excel C...
Animal + background_Individual Particle Results.csv	17/11/2022 16:04	Microsoft Excel C...
Animal + background_ROI Cluster Results.csv	17/11/2022 16:04	Microsoft Excel C...

Colour Maps:

Each ROI is saved as a colour map cloud .tiff file that can be used to visualise colour space and/or calculate colour space overlap using the colour map functions in QCPA (see [here](#)).

Additionally, a detailed log can be found in the main folder.

LEIA:

For each ROI, the user will have the numerical output of LEIA in a .csv file and the visualised chromatic and achromatic LEIA contrast saved as a .tiff. The latter are fantastic for visualisation purposes.

Additionally, a detailed log can be found in the main folder.

GabRat:

Here, the user will find a .csv file with the GabRat values for all ROIs. Only the animal ROI is of significance, as the kernel runs along the outside of the animal ROI.

Additionally, a detailed log can be found in the main folder.

To assist with compiling data out of these folders and files, please see the supplementary R-script library provided here:

<https://github.com/CaraConradsen/QCPA-r-script>