Lyndon Zimmermann BE(Mech)

ASIS SCIENTIFIC
PH: 08 8340 8444
EMAIL: SALES@ASISSCIENTIFIC.COM.AU
WEB: WWW.ASISSCIENTIFIC.COM.AU

ASIS SCIENTIFIC + Dolls House Artisans

RevC 12OCT2025

Guide to the use of the SMART#42069 7" LCD Biological Digital Microscope in conjunction with StrangeView software using prescribed accoutrements

1. Introduction

Naa marni? Marni'ai! [Greeting and response from the Kaurna Pangkarra, in the Kaurna Warra]

The objective of this Appendix is to guide citizen scientists in the use of this digital microscope for phytoplankton counting, particularly of the Karenia spp genus. In the South Australian context Karenia mikimotoi is the most infamous of this genus, but has cousins! Also of interest are Karenia's rivals, and species that can co-exist.

Users armed with this Guide with at least moderate scientific orderliness, manual dexterity and a tolerance for confusion arising from Hanzi (Chinese pictograph) menus should find this microscope/ software/ accoutrement combination convenient and rewarding to use, producing useful images within an hour.

This Appendix makes no recommendations on interpreting hazard levels from the identifications and counts.

The author would graciously consider improvements and corrections to this document!

2. Setting up the digital microscope system

2.1 General layout – wet lab/ dry lab

I have my microscope on my desk plugged into my regular computer with a nice big screen. I view the task directly on the computer, and captured mages are loaded directly onto the computer, immediately available for upload to the Facebook Group, iNaturalist, or for emailing.

Dual screen, one dedicated to the task and the other to the file management, would probably improve the system.

2.2 PC connection: Load StrangeView software

[Where did I get the Source code?]

Don't plug the microscope in, just install the StrangeView software onto your PC and start it up. It should come up with a screen title 'StrangeView' with a top menu and a Source menu to the left. "Please connect USB microscope" should appear on the main screen.

It will probably come up default English. If not, the far left 'Globe' icon is the popdown to change language.

The 'gear wheel' icon can be used to change the target directory for images.

Familiarise yourself with the other menu icons, but they may not make sense, yet.

The StrangeView Users Manual has one page – it says ‘Users Manual’. ‘About’ says it is Version 1.1, no more.

2.3 Television connection: use HDMI cable

Connect via the ‘HDTV’ port on the objective gantry. [Not trialled]

2.4 Standalone mode: use TF card and battery

No memory card specifications provided. [Not trialled]

No battery provided. Size 18650 [Not trialled]

2.5 Tablet or smartphone connection

Use TOX-APP to connect via WiFi. Press and hold WiFi button on microscope 2-3 seconds to initiate connection. Password 12345678 [Not trialled]

2.6 Set SMART-42069 microscope parameters

The microscope used was (presumably Software Version) Version TNZH-0-V40 TIENIU

There may be a better trick, but a simple step is to power the microscope with a dumb USB power supply to set language.

There are two blue knobs under the stage, left one is called ‘Light Source’ in the Instruction Booklet and the other is called ‘WiFi’. In this iteration of this Guide we won’t use WiFi, but still call this knob the ‘WiFi knob’.

First step is to set or confirm language, most likely either English or Hanzi (Chinese). Connect the microscope to the dumb power supply using the USB cord and press the power button (at the back) briefly to power up. It will take a couple of seconds. Press the menu button (three horizontal lines) below the stage and a menu in either English or Hanzi should appear.

If Hanzi then rotate the WiFi knob counterclockwise until some Hanzi with ‘(language)’ following appears. Select this and press the WiFi knob. Rotate clockwise again until ‘English’ (or your preferred language) appears. Press the WiFi knob again to select.

Repeat the process to select 50Hz Power Frequency. [Is it important?]

Either a 4X, 10X or 40X scaleplate can be selected [relevance?] Most taxa identification will be done using the 40X objective lens (yielding 550x total magnification), so perhaps select that one.

Scroll through the other options for familiarity.

Press the Menu button again to return to the objective. Note the calibration.
Using the 10X option mine said Measure size x 7.37 = Cell size (um)

Turn the microscope off with a long press of the power button to Save and power down. Disconnect the USB cable from the dumb power supply.

2.7 Connecting the microscope to the software

With the StrangeView software running [is this necessary?] connect the microscope USB cable to the computer. Use the power button at the rear to start the microscope.

Rotate the WiFi knob to the middle menu item, either in Hanzi or English ‘PC Camera’. Press the WiFi knob to select. The objective view will be replaced by ‘PC Camera’ on the microscope screen and the objective view will appear on StrageView.

If the microscope has been turned off (using the power button at the rear) it may not start up again. Unplug and reinsert the USB cable and select 'PC camera' again.

3. Dry familiarisation with the microscope and StrangeView software

3.1 Dry inspection of the haemocytometer

With the stage fully down rotate the objectives turret so the 4/0.1 objective lens is in position providing 55x magnification. It is the shortest one. Attempt to position the etched portion of the haemocytometer directly under the objective using the X-Y knobs under the stage.

Whilst watching the screen, raise the stage until the haemocytometer etching becomes visible. The total counting area is 3mmx3mm, but only about 2.5mm wide by 1.5mm tall will be visible at this stage. Exploring with the X-Y knobs will allow you to see nine 'quads'.

There is a slider on the bottom right of the StrangeView screen which will provide some digital zoom. In standalone mode the 'WiFi' knob can be rotated to provide a further 2x zoom, in 20 steps. Neither zoom appears to be of much use – image capture ignores them.

The Specifications table of the SMART-42069 reports Optical magnification 55x to 550x suggesting:

SMART-42069 Objective	Overall optical magnification	Dimension of smallest graticule
4/0.1 160/-	55x	Small graticule ~0.015mm 5 graticules ~0.075mm
10/0.25 100/0.17	~140x	Small graticule ~0.006mm 5 graticules ~0.03mm
40/0.65 160/0.17	550x	Small graticule ~0.0015mm 5 graticules ~0.0075mm

The nine quads contain a total of 0.0001ml under a 'heavy cover slip'.

Each corner quad consist of 16 squares each 0.25 x 0.25mm (250 x 250µm).

The central quad consists of 25 squares 0.2 x 0.2mm (200 x 200µm), each square with 15 smaller squares 0.05x0.05mm.

The edges have even finer engravings, reported to be 0.005mm (5µm) apart.

Rotate the turret to engage the 10/0.25 objective lens and refocus. Counting area is now 4-1/2 x 2-1/2 of those 0.2 x 0.2mm squares.

Rotate the turret to 40/0.65. Counting area of the central portion is now about half of one of those 0.2 x 0.2mm squares, or eight of those 0.05 x 0.05mm small squares.

3.2 Digital zoom using StrageView

Unfortunately this doesn't work for image capture. The captured image will be back at the 1x.

Position the subject 1/5 x 1/5 from the top left-hand corner of the screen. Use the slider bottom left to zoom in, the subject will slide to the middle of the image. Maximum zoom is 2x.

3.3 Calibration of the crosshairs using StrangeView

[Not trialled]

3.4 Calibration of the crosshairs in standalone mode

[Not trialled]

4. Wet work with the microscope and StrageView

4.1 Summary of steps

(from the spreadsheet)

1 Have a look at a drop of the live sample on the counting slide as soon as you get it under the microscope. If it is already quite dense, either count it live, or stain it with Lugol's but do not let it settle and count it unconcentrated - Usually concentration is required.

2 Take lots of photos of all the things you can in the live sample, taking photos from the same organism from different angles if possible, then wash off the slide. Save the photos and label them Organism A, B, C etc.

3 Put 50 mL of sample into a settling tube and pipette 0.25mL of Lugol's media into the tube, screw cap on, then tip up and down a few times.

4 Allow to settle in a cool park room for 18-24 hours, then use a pipette or syringe to carefully remove the top 45mL of sample, leaving the bottom 5mL undisturbed.

5 Cap and tip the tube up and down a few times.

6 Take a small amount out with a pipette and place a drop on each half of the [haematometer] counting cell.

7 Count how many of each organism are in the counting cells (See figure page for more details of what area is a cell). Count all cells on the slide, unless there is lot of organisms on the slide, at which point you count as many as reasonable.

8 Calculate the cells per mL for each organism using the raw data page of this spreadsheet.

9 Upload the photos for each organism, along with their count in the comments field, any observations around fish kills etc and sample location details to iNaturalist. If you have an idea of general identification (diatom, dinoflagellate etc) add it as a starting identification.

4.2 Get wet #1.1 presence or absence in live sample

Don’t waste time! Drop a sample onto your haemocytometer slide, pop on the cover slide and have a look. You’ll soon have at least an intuitive idea of the presence of Karenia spp and other plankton. The scene will likely be quite comic with phytoplankton scooting around like ‘The Jetsons’ (if you are old enough to know who or what they were) and impossible to capture images. Maybe you’ll find a docile one, grab an image of it!

Live samples can be concentrated by drawing them to light directed onto a section of the sample jar.

Note sample location and date, magnification, image capture time and date on your worksheet.

The visible features of a live, or freshly dead, subject can be different to the treated and thoroughly dead ones. Capture images of both, if possible. Organisms can be euthanised with methylated spirits, Lugols Solution, sodium hypochlorite solution (pool chlorine). Place a drop of your poison of choice at one edge of the cover slide and touch some paper fleetingly to the opposite edge, to wick the poison through.

Different taxa will respond in it’s own way to a given

4.3 Get wet #1.2 capture some video (if you can!)

Or just watch. Watching the subject in motion provides a proxy 3D view. Karenia is flattened, evident when in motion.

4.4 Get wet #2.0 Kill the blighters!

Syringe precisely 50ml of well shaken samples into suitably labelled 50ml centrifuge tubes then syringe 0.25ml of Lugols media in, close the lid and agitate gently. Make sure you read the Lugols Media MSDS (Material Safety Data Sheet) and wear safety gloves (especially if you don't want iodine stains on your skin).

4.5 Get wet #2.1 further genus (or maybe species) identification

Before we start the arduous count process we need to be confident about what we are counting. Search the haemocytometer quads, and further afield, for interesting organisms. There will be plant fragments and probably pieces of sand from your well-agitated sample. Scoot around using the 140x magnification then zoom in to 550x for a better look. Perhaps a better microscope will be more accurate, but with a budget microscope the subject may need to be offset at 140x to be in the field of view at 550x, and then refocussed after the objective change. Sharper eyes (and grey matter, probably after the count) may distinguish further taxa. We're not just looking for Karenia spp, we're seeing what else is there, or not there.

Don't bother with the 2x digital zoom in StrangeView, captured images revert to the 1x.

Taxa identification can be done on samples half an hour after dosing with Lugols media. They will be dead but not settled out.

4.6 Get wet #3 bloom count

Just like our initial taxa identification we can do a bloom count on freshly (half an hour) Lugols-euthanised samples. Multiplier is one. Count protocol to follow.

4.7 Get wet #4 Lugols media + 18 hours prep

Lugols media is going to euthanise and stain the phytoplankton within half an hour. It will also make them heavier, helping them sink during the remaining undisturbed 17.5 hours, to gently concentrate into the cone in the bottom of the centrifuge tube.

So why not centrifuge them? Good question but centrifuging these fragile euthanised phytoplankton is going to blitz them, they'll be soup!

After 18 **UNDISTURBED** hours syringe the supernatant (top liquid) off, always drawing from just under the surface, leaving 5ml of concentrated phytoplankton at the bottom. Cap the centrifuge tube. Give the syringe a good freshwater rinse afterwards. Syringe the next sample.

Now we have prepped samples.

4.8 Counting prepped samples

Yay! We've mucked around with tubes of seemingly perfectly clear water, adding chemicals, treating them like eggs, what's the fuss?

Drop the stage, power up the microscope and start the StrangeView software.

Put the haemocytometer on a 'wet table'. Give the concentrated phytoplankton sample a bit of a gentle shake and pipette droplets onto the two grids of the haemocytometer. Squirt a bit of the sample down the drain before applying the second drip.

Place a 'heavy' (0.4mm) slide cover over the sample to squeeze the viewing area down to 9 x 0.0001ml.

Following the pre-described process there should be very limited possibility of sand getting between the haemocytometer and the 'heavy' slide cover and mucking things up. 'Limited possibility' means it is possible.

The haemocytometer has drainage channels to allow excess sample to flow away when the slide cover is applied. Use some paper towel to wipe excess away from the edges and underside of the haemocytometer.

Set the objective to 10/0.25 (ie 140x) with the stage down. At this magnification only the two or maybe three rows of the one-sixteenth-quad squares will appear, when focussed, using the SMART-42069 microscope.

Do a quick scout around, zoom in and capture images of anything interesting or concerning, pay attention to noting time and magnification when images are captured. These images will be important in validation of your count, by others later. If the target taxa are absent from the counting area have a scout around outside the counting area. If you find them outside the count area consider doing another slide.

Traverse the X-Y to the top two rows of Quad 1 (ie top left) and count the identified phytoplankton. Note. Count the lower two rows of Quad 1. Note. Repeat for all the Quads, noting Quads 4, 5 and 6 have five rows, so record the top two then the bottom three.

My record sheet goes 1, 2, 3, 6, 5, 4, 7, 8, 9 to make counting and recording easier.

5. Reporting

5.1 Sort images into folders

Fresh images will have the collection date in the metadata but concentrated samples will probably be the day after. Sort into folders (probably by sample date) and assign meaningful prefixes (probably sample site and treatment) to the auto-generated filenames.

5.2 Georeference images

Use GeoSetter or similar software to bulk georeference images, rather than one at a time in iNaturalist.

5.3 Identify taxa

Where possible identify the taxa of the images and enter as the 'Title' in the metadata.

5.4 Process the count

Add up the nine individual Quad counts for each of the two grids counted. Assuming a concentration factor of 10:

$$\text{Plankton Count} = \frac{n^{o} \text{ of plankton}}{n^{o} \text{ of cells (squares) counted}} \times 10^{3}$$

Do this for all the identified taxa and create a summary table for that Location/ depth/ time/ treatment sample.

A more general equation is:

$$\text{Plankton Count} = \frac{1}{\text{how many times concentrated}} \times \frac{n^{o} \text{ of plankton}}{10^{-4}} \times \frac{1}{n^{o} \text{ of cells (squares) counted}}$$

5.5 Tabulate count for 'Luke's HAB display app'

Download the spreadsheet template from the Phytoplankton of South Australia Facebook Group.

Add additional columns to the right, if desired, for your own records. These may include sample time, sample depth, total depth, Secchi depth, turbidity NTUs, pH, salinity EC, weather conditions, dolphin sightings, fish mortality.

Submit records to Peri Coleman for amalgamation, in accordance with instructions on the Phytoplankton of South Australia Facebook Group Page. Only submit data in accordance with the official template.

5.6 Upload to iNaturalist

A core rule for iNaturalist is each observation is to be of a single individual. Single individual, not a collection of images from a single sample or bloom. In the case of phytoplankton this will generally mean one image per iNaturalist observation.

Accepted practice amongst the Phytoplankton of South Australia FB Group at time of writing was to create a Summary Count Table and attach it to each individual observation from that sample. If you capture images of three different taxa then submit three observations, with identical location, time and Summary Count Table in the 'notes'.

Your Summary Count Table will probably be based on your 'Luke's HAB display app' spreadsheet. To convert the 'Luke's HAB display app' entry (with titles along the top row) to a format suitable for iNaturalist (with titles in the left column) in Excel:

- copy and paste the titles row to another location in a spreadsheet
- copy and paste the desired sample data to the row below that title row
- Copy <CTRL C> the two rows, click on the spreadsheet cell you want to be the top left and Paste – Transpose.

Your entry will be copied and transposed.

Assign your best suggestion of species, genus or higher. 'Identifiers' (people) will hopefully be attracted to your observation based on the initial suggestion and perhaps your reputation and make further suggestions.

iNaturalist will attempt a suggestion based on image recognition if you are slow to enter your own, and these will vary widely in accuracy. Best to do a bit of a search before accepting the suggestion. I had the situation with an image of a sea cucumber where I accepted the iNaturalist suggested of a type of cucumber I frequently eat pickled! I'm sure Identifiers had a laugh at that one.

Appendix A. Sample Phytoplankton counting sheet

Sample date/seq & time:	Sample location & sampler	Depth:	Foam	200mm	3000mm
		Jar#	Surface	500mm	

Live sample image time/ magnifications/ cover slip 55x/140x/550x x2

Date and time Lugols Solution added:

Fresh dead sample image time/ magnifications

Counter/ Date and time sample count:

Total counts: cells/ml

Quad	1	2	3	6	5	4	7	8	9
Upper 2									
Lower 2/3									

Images:

Sample date/seq & time:	Sample location & sampler	Depth:	Foam	200mm	3000mm
		Jar#	Surface	500mm	

Live sample image time/ magnifications/ cover slip 55x/140x/550x x

Date and time Lugols Solution added:

Fresh dead sample image time/ magnifications

Counter/ Date and time sample count:

Total count: cells/ml

Quad	1	2	3	6	5	4	7	8	9
Upper 2									
Lower 2/3									

Images: