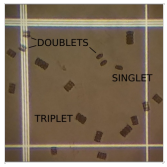


Version 2

May 29, 2020

Counting Microalgae Culture Density V.2

In 1 collection

Jakub Nedbal[1]

[1]King's College London

1 *Works for me* dx.doi.org/10.17504/protocols.io.bd8ni9ve

Jakub Nedbal
King's College London

ABSTRACT

Counting culture cell density offers a reliable way of consistend seeding density for freshly inoculated cultures and allows tracking the growth of the cultures. Cell densiry counting, as described in this protocol, is performed manually under the microscope in a Neubauer hemocytometer.

.

EXTERNAL LINK

https://app.labstep.com/sharelink/1bfe1dae-317f-460d-a934-6d23c1696ac2

GUIDELINES

Neubauer hemocytometer is used for cell counting to assess the cell culture density.

The cell counting involves several steps:

- Dissociating clumped cells
- Sampling the cell culture
- Loading the hemocytometer
- Counting cells
- Calculating cell culture density

MATERIALS TEXT

Neubauer Improved Haemocytometer
Counting Chamber
Counting Chamber

Hawksley AC1000

Void depth: 0.1 mm
Counting Area: 1mm²

CK2
Inverted phase contrast microscope

Nikon CK2

Objective: MVC-10X (Newport Corporation)

Citation: Jakub Nedbal (05/29/2020). Counting Microalgae Culture Density. https://dx.doi.org/10.17504/protocols.io.bd8ni9ve

P20 pipette and pipette tips

Sterile flow hood

70 % dentarured ethanol

Lab cleaning tissue wipes

Kimwipes™ or similar cleaning wipes

Hemocytometer Sidekick app

SAFETY WARNINGS

Counting cells requires logging their numbers using tally counters or their electronical equivalents. The repeated tapping can cause fatigue and repetitive strain injury. Make sure to take breaks between counting cells.

70 % denatured ethanol is a hazardous substance and must be handled accordingly.

BEFORE STARTING

Make sure you have a Neubauer hemocytometer or other counting chamber, tally counter or a phone app recording counts, a tissue culture microscope with 10× objective, and P20 pipette with tips.

A soft tissue and 70 % denatured ethanol are required for wiping the hemocytometer and the coverslip clean.

Handle the cultures in a sterile laminar flow cabinet and work aseptically to avoid their contamination.

Loading Microalgae into the Hemocytometer

1 Wipe down the sterile laminar flow cabinet with 70 % denatured ethanol solution.

Prepare the hemocytometer, P20 pipette and pipette tips in the sterile cabinet.

2 Mark down the temperature on the shaker.

Thermometer on the illuminated orbital shaker platform.

Dissociate any cell clumps by thorough vortexing.

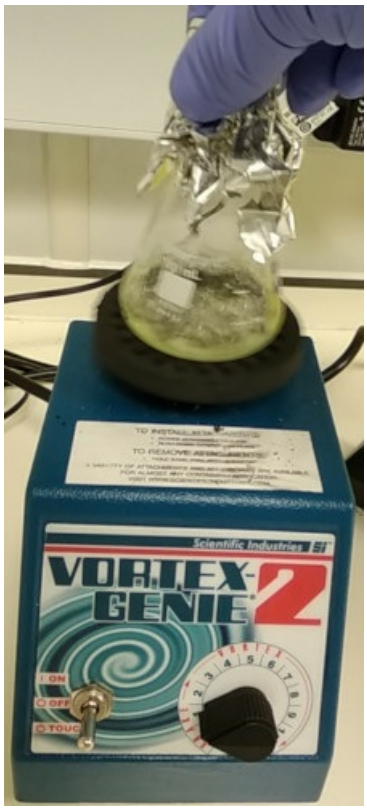
Citation: Jakub Nedbal (05/29/2020). Counting Microalgae Culture Density. https://dx.doi.org/10.17504/protocols.io.bd8ni9ve

3

Do not vortex too vigorously to avoid the cotton wool plug getting splashed on.

Vortex *D. quadricauda* for at least 5 seconds.

Vortex *C. vulgaris* for at least 30 seconds.



Vortex cells thoroughly. Avoid soaking the cotton wool plug with the cell suspension.

3.1 When *C. vulgaris* culture is left growing for a long time, the cells start clumping, forming a green rim of cell on the sides of the flasks near the surface of the medium. The cell clumps are difficult to dissociate and make assessing the cell culture density difficult if not impossible. In which case, the cells need to be dissociated by trituration in the sterile flow cabinet. This is done using a 20 ml syringe with a 21G syringe. The culture is aseptically drawn through the needle out of the culture flask into syringe. It is then expelled back into the flask, with the expelled jet aimed at the coagulated cells stuck to the walls of the flask. Repeating the sucking and expelling the culture suspension through the needle about 10 times dissociated the clumps and allows the cells to be counted in the hemocytometer.

4 Bring a clean hemocytometer with a coverslip on into the laminar flow hood.

Transfer the culture flask into the laminar flow hood.

Remove the aluminium foil from the culture flask.

Spray the tweezers with 70 % denatured ethanol. Let dry.

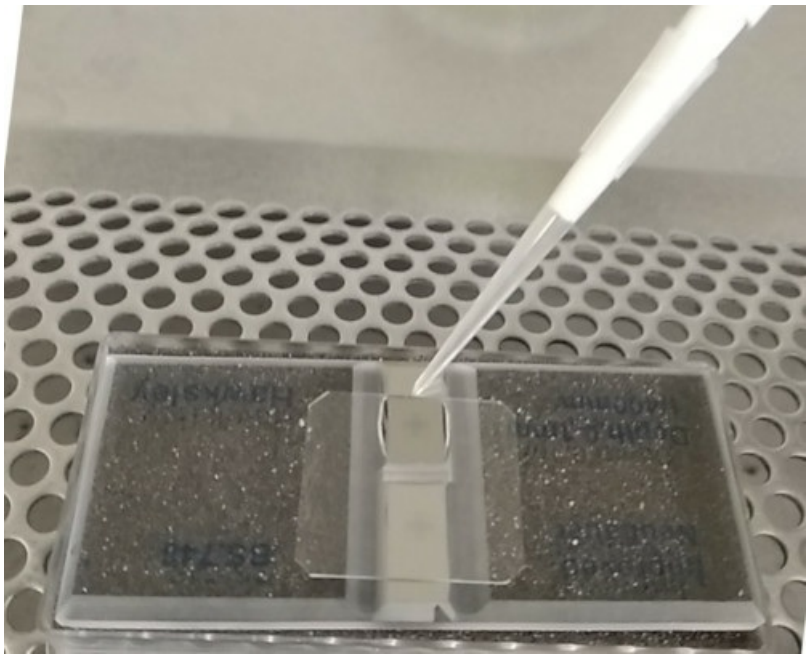
Lift the cotton with pair of sterilized tweezers. Do not lay the wool on any surface. Keep it held in the tweezers until it is plugged back into the flask opening.

Citation: Jakub Nedbal (05/29/2020). Counting Microalgae Culture Density. https://dx.doi.org/10.17504/protocols.io.bd8ni9ve

Remove the cotton wool plug from the flask opening using tweezers and hold it in the tweezers until it can be plugged back in.

5 Pipette out **10 µl** sample of the algal culture.

Load it into one chamber of the hemocytometer.



Microalgae cell suspension is being loaded into one chamber of the hemocytometer.

Citation: Jakub Nedbal (05/29/2020). Counting Microalgae Culture Density. https://dx.doi.org/10.17504/protocols.io.bd8ni9ve

6 Shake the flask to resuspend any cells that started settling.

Pipette out another **10 µl** sample of the microalgae culture **using a new tip**.

Load the second chamber of the hemocytometer, following the steps described above.

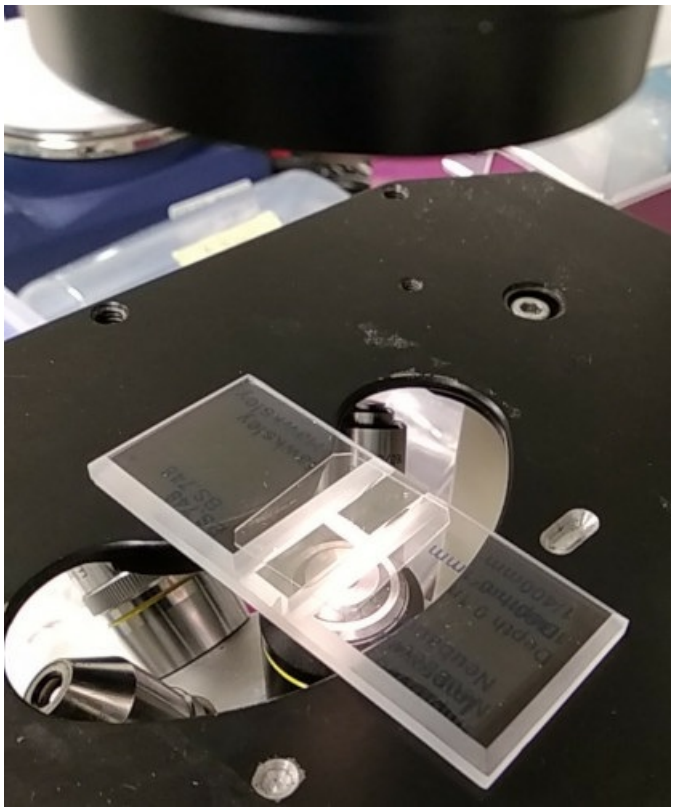
7

Reseal the culture flask with the cotton wool plug and aluminium foil cap.

Return flask onto the orbital shaker.

Counting Cell Culture Density

8 Bring the hemocytometer to the microscope with a 10× phase contrast objective and sufficient working distance.



Loaded hemocytometer on a microscope stage is ready for counting.

9 ***Desmodesmus quadricauda*** - counting coenobia

D. quadricauda cells form coenobia. Count the number of coenobia, not individual cells.

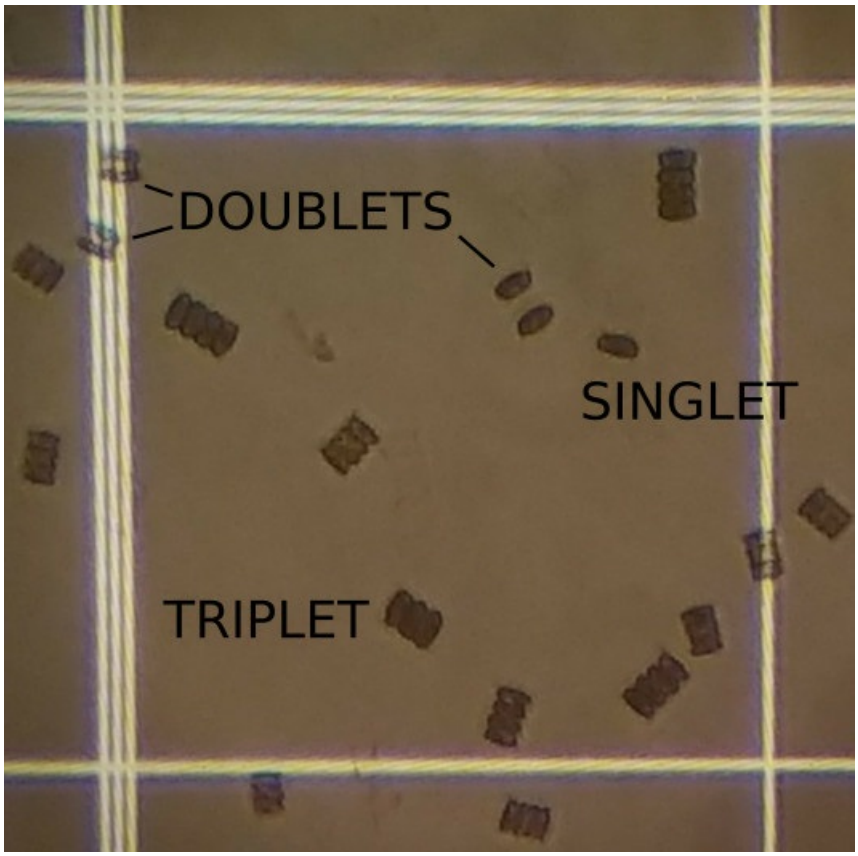
Repeat the count three times - one for coenobia with two cells, one for coenobia with 4 cells, and one for coenobia with 8 cells.

The counting in the hemocytometer app allows counting two cell types at the same time. This makes it possible to count coenobia with 4 and 2 cells at the same time, for instance.

Count cells in both chambers and the 8 outer squares in each chamber.

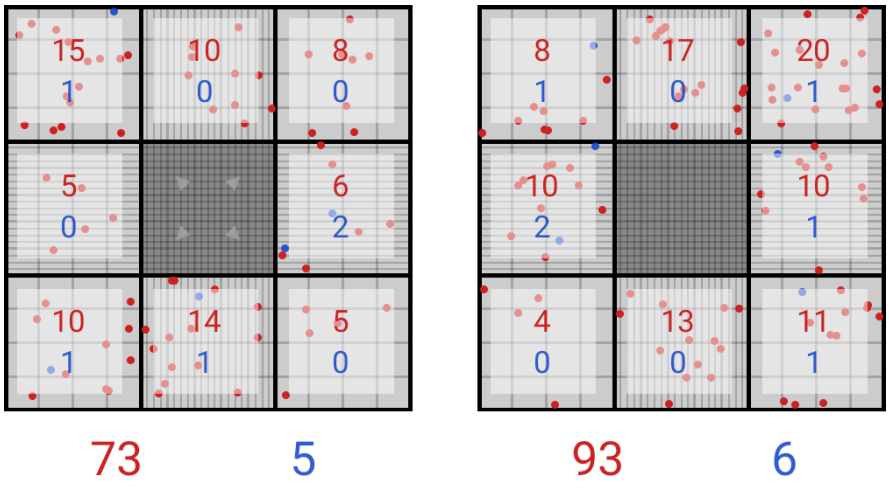
Citation: Jakub Nedbal (05/29/2020). Counting Microalgae Culture Density. https://dx.doi.org/10.17504/protocols.io.bd8ni9ve

Sometimes, coenobia contain non-canonical number of cells, such as singlets, triplets, etc., as in the image below. To account for them, use a system that counts the approximate number of cells: For example, count two singlets count as a single doublet, two triplets count as one quadruple and one double, or a triplet and a singlet counts as one quadruple.



Most D. quadricauda coenobia contain two, four or eight cells. Some, however, contain a non-canonical number of cells.

Use the Hemocytometer Sidekick App to count the coenobia in each segment of the hemocytometer. The snapshot below shows the count of 8 squares, withe coenobiea with 4 cells in red and coenobia with 2 cells in blue.



Screengrab from the Hemocytometer Sidekick App shows the counts of coenobia with four cells (red) and coenobia with two cells (blue). The two sets of nine squares represent each of the two counting chambers of the hemocytometer, with counting done in the outer eight segments.

Square	Doublets	Quadruples	Octuples

Citation: Jakub Nedbal (05/29/2020). Counting Microalgae Culture Density. https://dx.doi.org/10.17504/protocols.io.bd8ni9ve

LT1			
CT1			
RT1			
LM1			
RM1			
RB1			
CB1			
RB1			
LT2			
CT2			
RT2			
LM2			
RM2			
LB2			
CB2			
RB2			

Table to record the number of coenobia with two, four and eight cells. The squares in the rows are abbreviations of two letters and one number. The first letter (L, C, T) stand for "left", "center", and "right". The second letter (T, M, B) stand for "top", "middle", "bottom", the last number (1, 2) stand for counting chamber one or two.

10 ***Desmodesmus quadricauda*** - calculating cell culture density.

Multiply the number of coenobia with two cells in each square by 2. Multiply the number of coenobia with four cells by 4. Multiply the number of coenobia with eight cells by 8. The sum of the three resultant numbers gives the total number of cells (not coenobia) in each square of the hemocytometer.

The average of the number of cells in each of the 20 squares multiplied by 10000 ml^{-1} gives the cell culture density in cells per milliliter.

11 Clean the hemocytometer and the coverslit with 70 % denatured alcohol using Kimwipes or equivalent tissue paper.

Load the *C. vulgaris* in the same way described above.

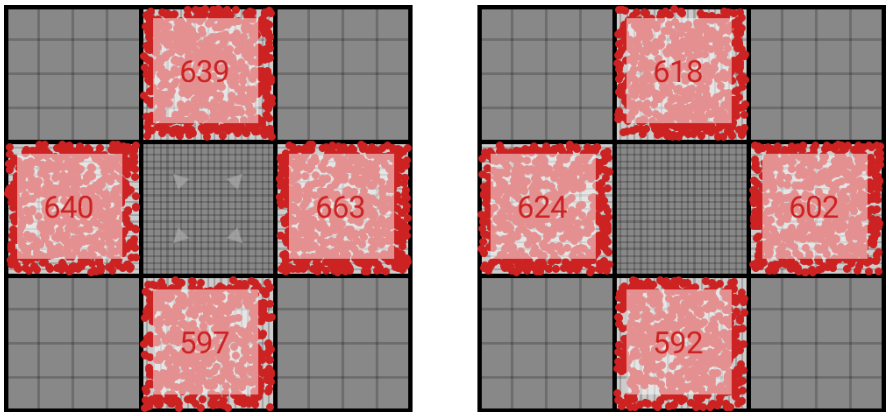
12 ***Chlorella vulgaris*** - counting cells

C. vulgaris cells form dense cultures. This means too many cells are in each square of the hemocytometer to count. On the other hand, the high number of cells means that the relative spread of counts in different squares of the chamber is relatively small.

Count cells in both chambers of the hemocytometer, but only in the four squares forming a plus sign to simplify the process, as in the image below.

When the cell density is very high (>5×10^{6} ml^{-1}), do not count cells in all 20 sub-rectangles of the hemocytometer. Instead count only a subset of the sub-rectangles in each square and scale the obtained number appropriately, to obtain the total in the entire square.

Citation: Jakub Nedbal (05/29/2020). Counting Microalgae Culture Density. https://dx.doi.org/10.17504/protocols.io.bd8ni9ve



Screengrab from the Hemocytometer Sidekick App shows the counts of C. vulgaris cells in four segments in two chambers of the hemocytometer.

Square	Count	Sub-rectangles
CT1	0	20
LM1	0	20
RM1	0	20
CB1	0	20
CT2	0	20
LM2	0	20
RM2	0	20
CB2	0	20

Table to record the number of cells. the squares in the rows are abbreviations of two letters and one number. The first letter (L, C, T) stand for "left", "center", and "right". The second letter (T, M, B) stand for "top", "middle", "bottom", the last number (1, 2) stand for counting chamber one or two. When the cell desnity is high, it is impossible and makes little sense to count cells in the entire segment of the hemocytometer. Each segment is divided into 20 sub-rectangles. Depending on the density, choose a number of sub-rectagles to count in. Count all 20 sub-rectangles for low–density cultures, or just one sub-rectangle for high density cultures. Note the number of sub-rectangles that counting was done it in the table above.

13 ***Chlorella vulgaris*** - calculating cell culture density

As described in the above step. Counting cells in all sub-rectangles of each square for very dense cultures would be long and tedious, without adding much value. When only four (out of the total of 20) sub-rectangles were counted, multiply the obtained number by five to get the total of cells in each square.

The multiplication can be done manually, or directly in the Hemocytometer Sidekick app by setting a dilution factor of 8 after the count is finished, as in the screenshot below.

The average of the number of cells in each of the 8 squares multiplied by 10000 ml^{-1} gives the cell culture density in cells per milliliter.

14 Clean the hemocytometer and the coverslit with 70 % denatured alcohol using Kimwipes or equivalent tissue paper.

Clean the sterile flow hood and tidy the spaces used.

Turn off the lamp on the microscope.

15 References

- Hemocytometer Sidekick app
- Manual Cell Counting protocol

Citation: Jakub Nedbal (05/29/2020). Counting Microalgae Culture Density. https://dx.doi.org/10.17504/protocols.io.bd8ni9ve

- growthCurves: Matlab file to analyse and graphically represent cell counting results.

Citation: Jakub Nedbal (05/29/2020). Counting Microalgae Culture Density. https://dx.doi.org/10.17504/protocols.io.bd8ni9ve