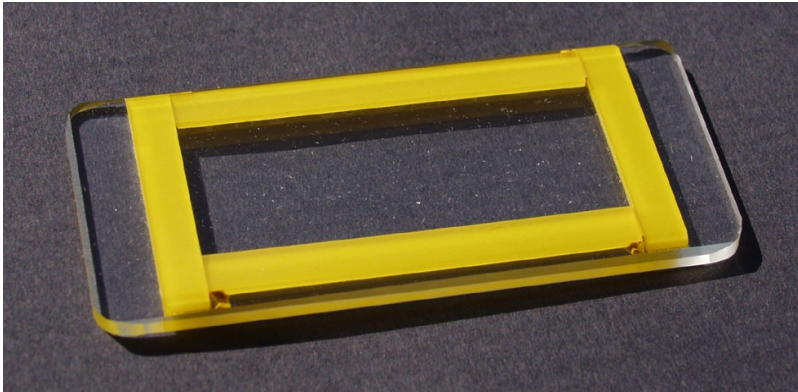
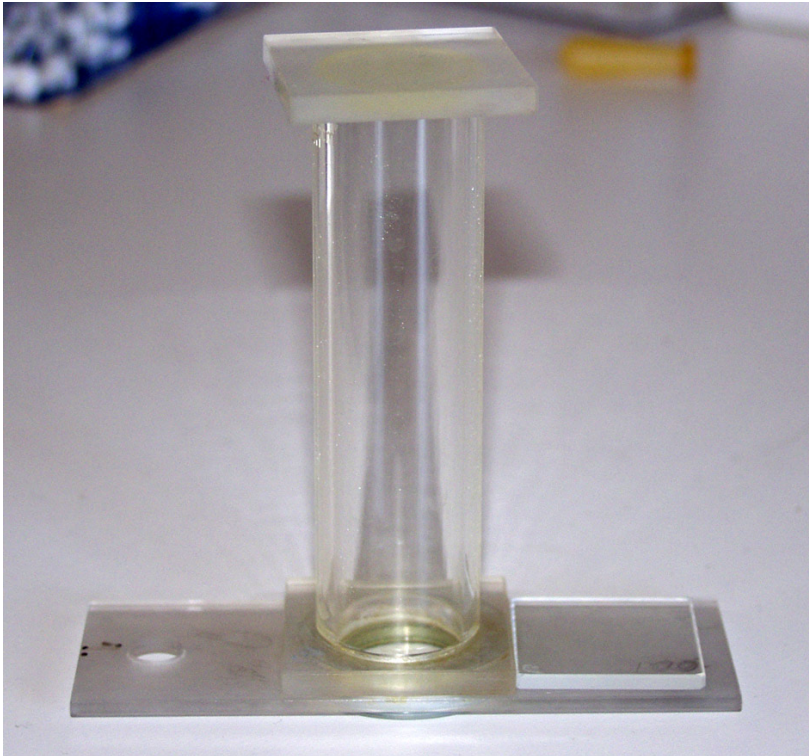


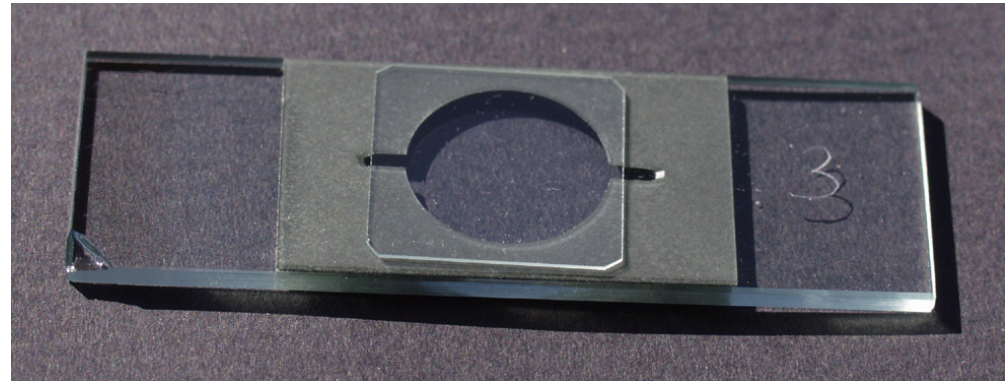
# Determinação da concentração de microalgas em cultura por microscopia óptica



**Sedgewick-Rafter**



**Sedimentation chamber**



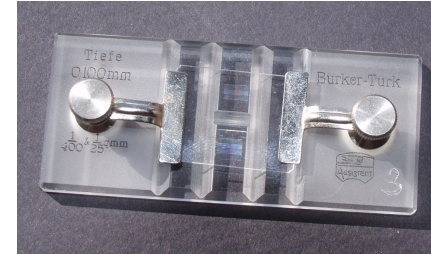
**Palmer Maloney**



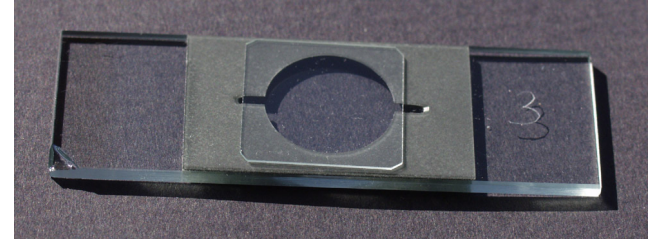
**Neubauer**

# Câmaras de contagem

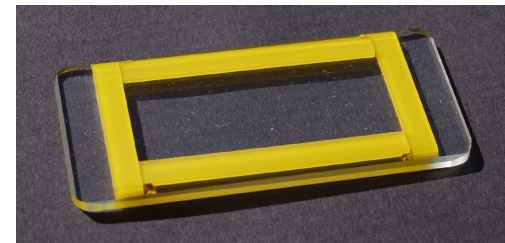
- Hemacitômetro (0,1 mm profundidade)
  - Dimensões das células: 2-30 $\mu$ m



- Palmer Maloney – volume 0,1ml
  - Dimensões das células: 5-150 $\mu$ m



- Sedgwick-rafter – volume 1ml
  - Dimensões das células: 50-500 $\mu$ m





# Câmaras de contagem

Table 4.2. Characteristic dimensions of different types of counting chambers.

Chamber type	depth mm	area mm <sup>2</sup>	volume μl	detection limit cells/l	reliable counts cells/l	diameter or size of ruling
Improved Neubauer	0.1	9	0.9	10 <sup>6-7</sup>	10 <sup>8-9</sup>	3x3 mm square
Fuchs-Rosenthal	0.2	16	3.2	10 <sup>6</sup>	10 <sup>8</sup>	4x4 mm square
Palmer- Maloney	0.4	250	100	10 <sup>4</sup>	10 <sup>6</sup>	17.9 mm diameter
Workshop type*	1	200	200	10 <sup>4</sup>	10 <sup>5-6</sup>	16 mm diameter
	2	200	400	10 <sup>4</sup>	10 <sup>5-6</sup>	
Sedgewick-Rafter	1	1000	1000	10 <sup>4</sup>	10 <sup>5</sup>	50x20mm rectangle
2 ml sedimentation cylinder**	10	200	2000	10 <sup>3</sup>	10 <sup>4</sup>	16 mm diameter

\* May be used on standard or inverted microscopes

\*\* To be used on inverted microscopes

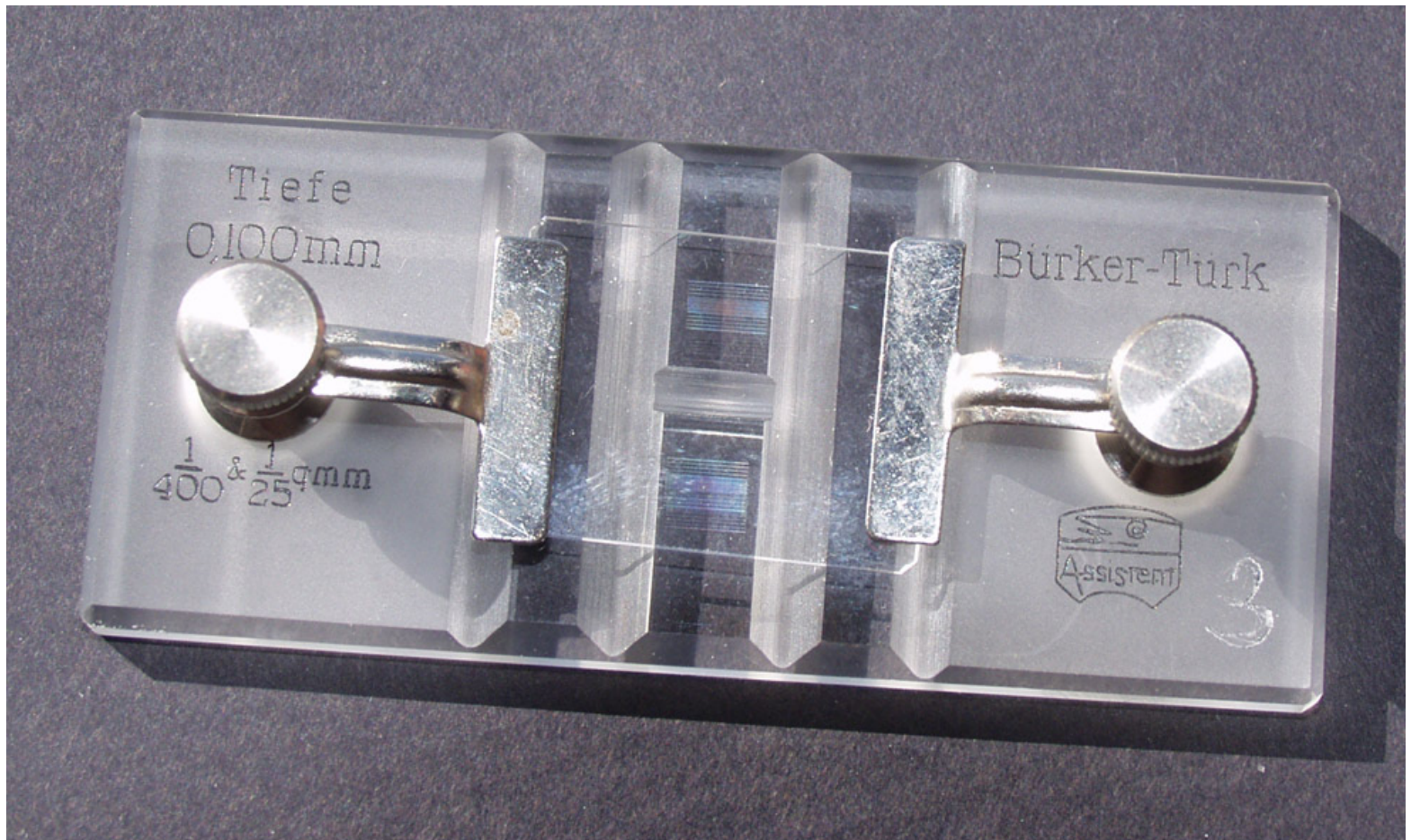
Tempo de sedimentação:

- Profundidade da câmara
- Velocidade de sedimentação (depende do fixador usado, e.g. Lugol>Formol)

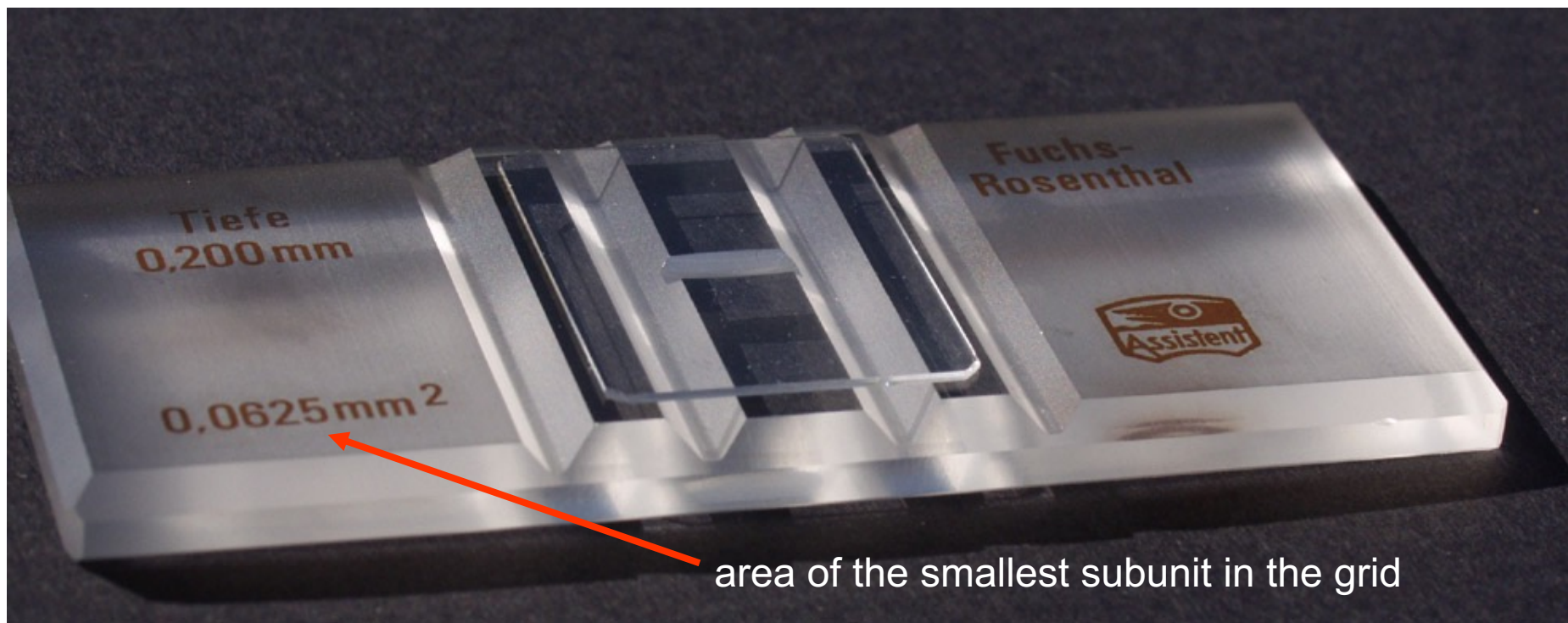
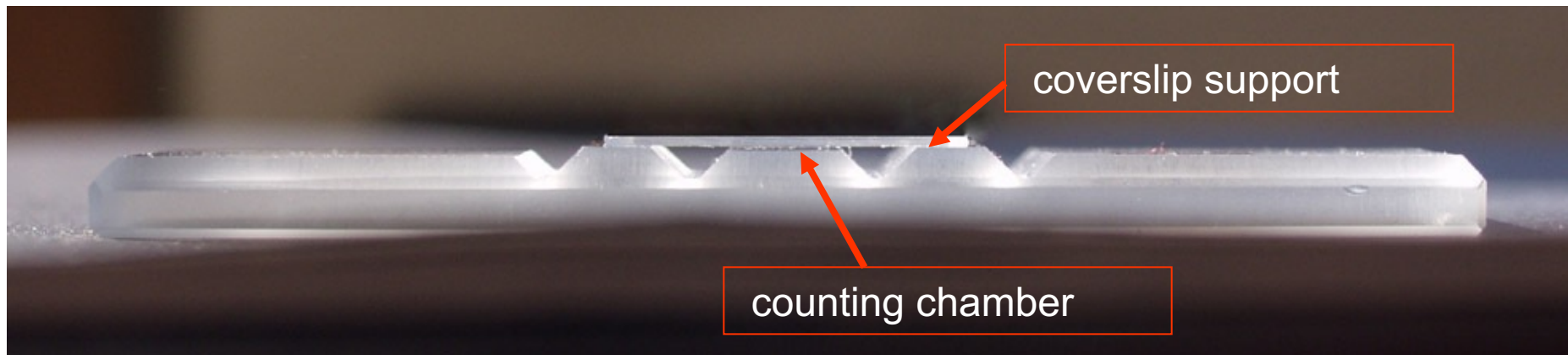
Tempo de sedimentação Improved Neubauer,  
0,1 mm profundidade = 2 minutes

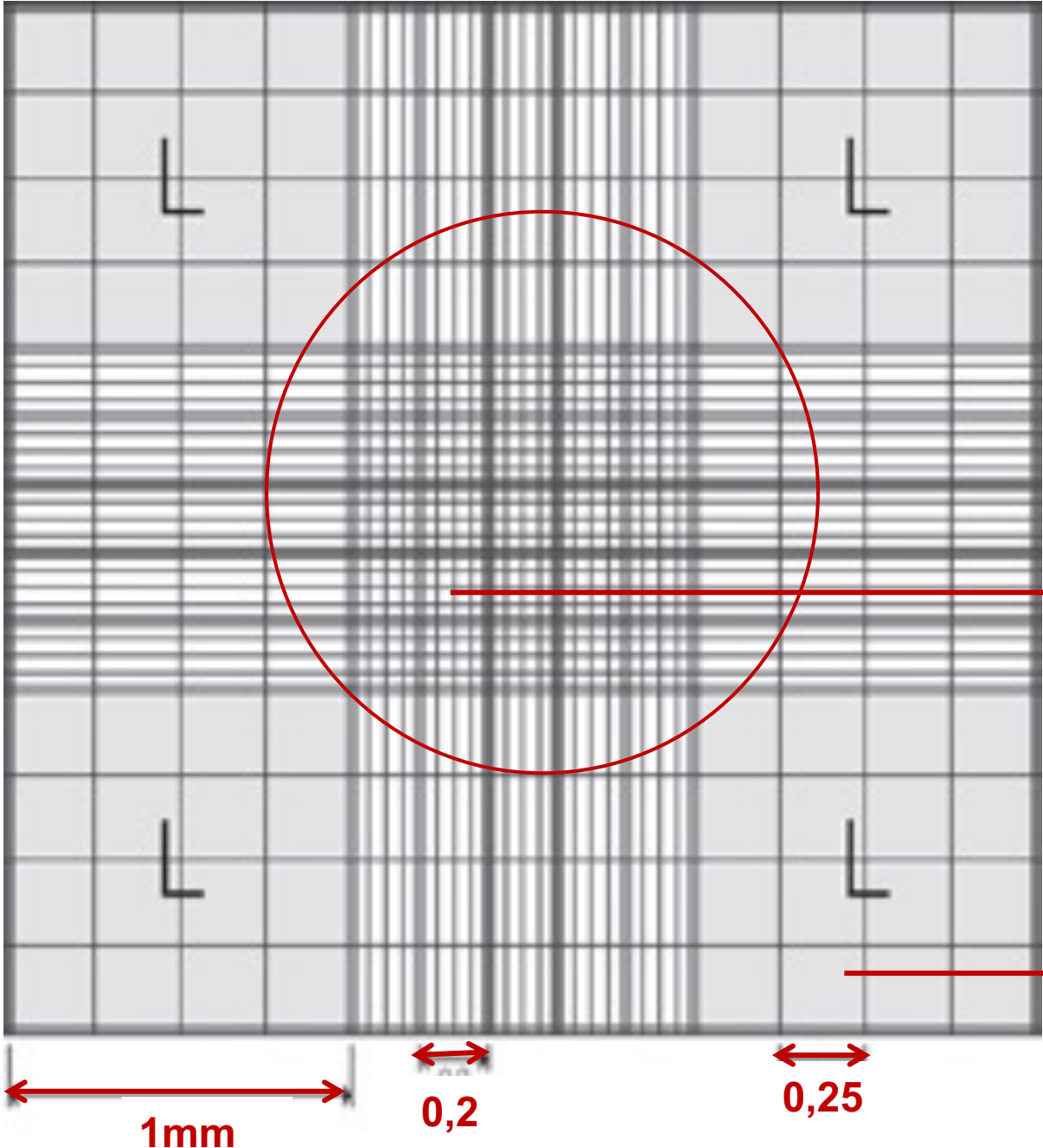
# Câmaras de contagem com retículo

- Fuchs-Rosenthal, Neubauer, Bürker-Türk









Profundidade= 0,1mm  
 Área total= 9 quadrados  
 $1\text{mm}^2 = 9\text{mm}^2$   
 Volume total=  $9\text{mm}^2 * 0,1\text{mm}$   
 $= 0,9\mu\text{l}$

Volume Quadrado "L" =  
 $1\text{mm}^2 * 0,1\text{mm} = 0,1\mu\text{l}$

Volume =  
 $0,04\text{mm}^2 * 0,1\text{mm} = 0,004\mu\text{l}$

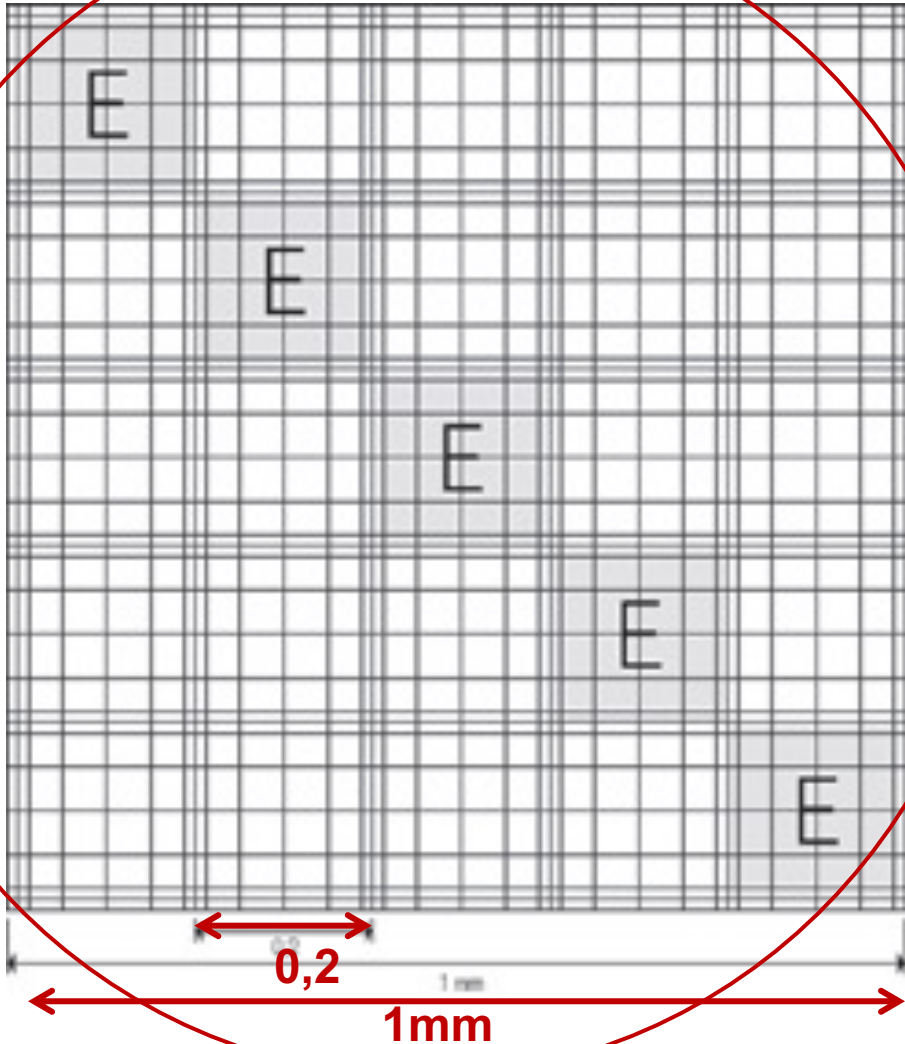
Volume =  $0,0625\text{mm}^2 * 0,1 = 0,00625\mu\text{l}$

Neubauer improved

Fórmula de cálculo (válida universalmente):

$$\text{Partículas } \mu\text{l}^{-1} = \frac{\text{partículas contadas (n)}}{\text{superfície contada (mm}^2\text{)} * \text{profundidade da câmara (mm)} * \text{fator diluição}}$$

Quadrado central



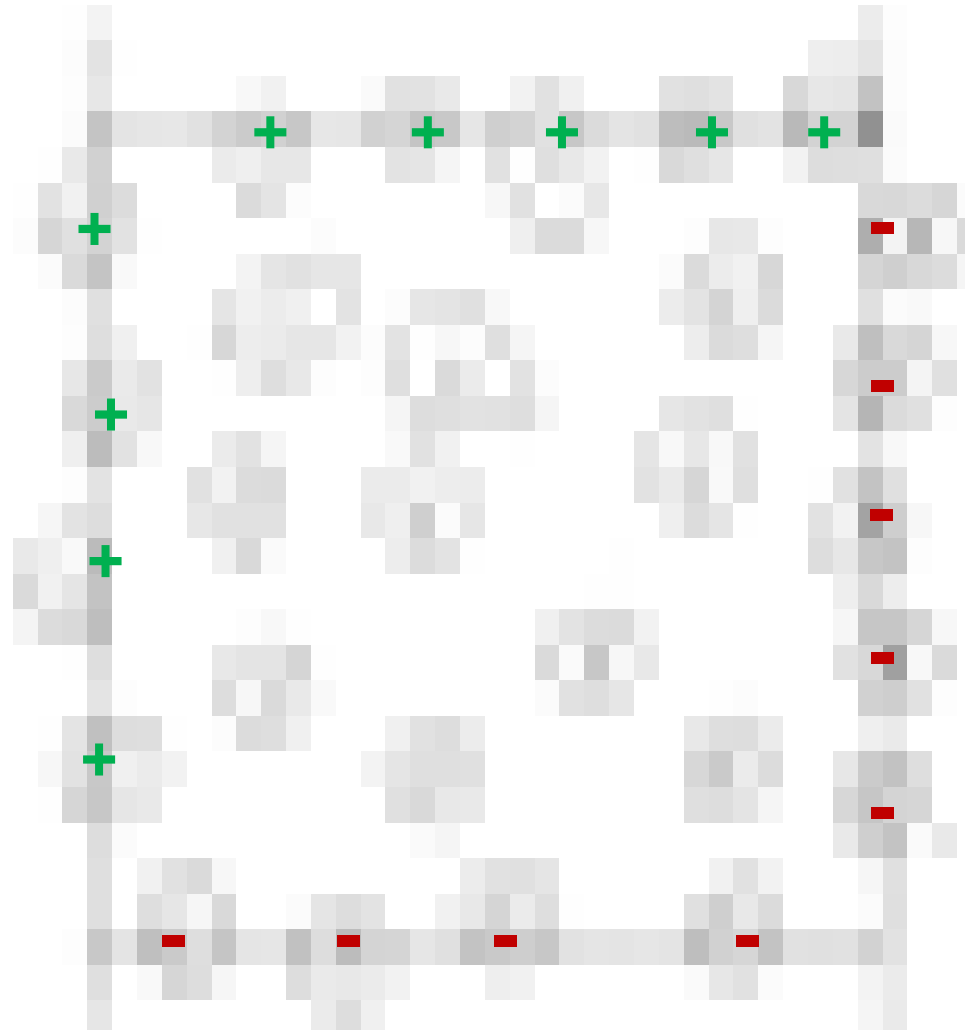
Exemplo (eritrocitos sangue) câmara Neubauer improved

1. Células contadas: 528 eritrocitos
2. Superfície contada: 5 quadrados médios, correspondem a 0,2 mm<sup>2</sup>
3. Profundidade da câmara: 0,1 mm
4. Fator diluição: 1 : 200

$$\text{Eritrócitos } (\mu\text{l}^{-1}) = (528 * 200) / 0,2 * 0,1 * 1 = 5,28 * 10^6 \text{ eri } \mu\text{l}^{-1} \text{ sangue}$$



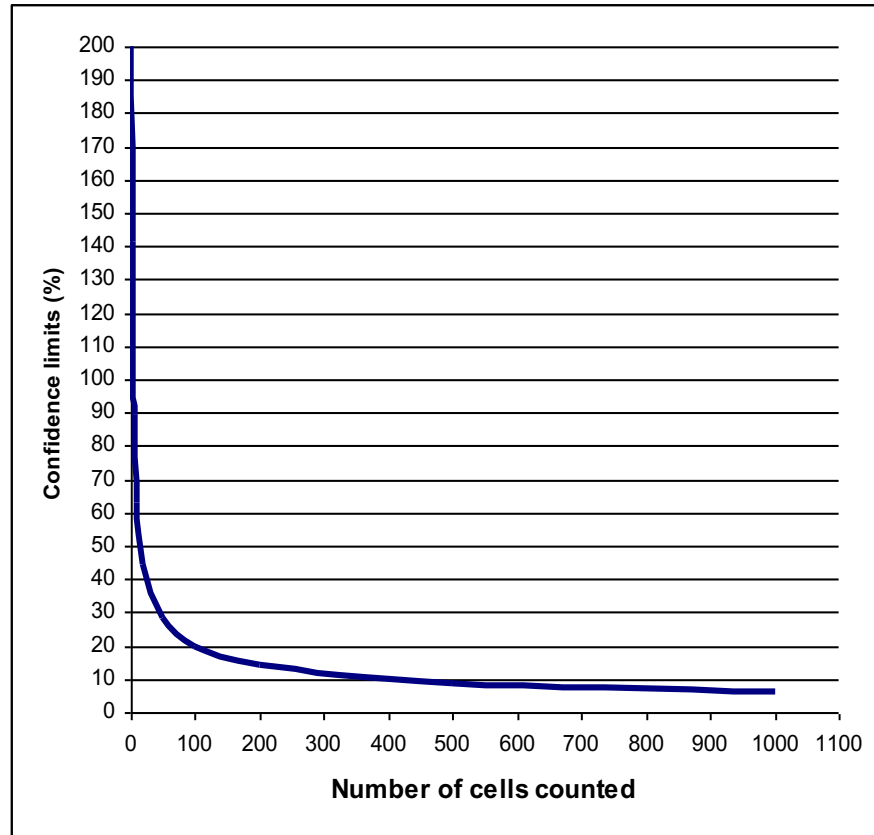
# Que células contar?



# Variabilidade morfológica nas células de *H. pluvialis*?



# Quantas células contar?



$$\text{Limites de confiança (95\%)} = \frac{\pm(2 \cdot \sqrt{n} \cdot 100\%)}{n} = \pm \frac{200\%}{\sqrt{n}}$$