Amplification Efficiency:

linking baseline and bias in qPCR data analysis

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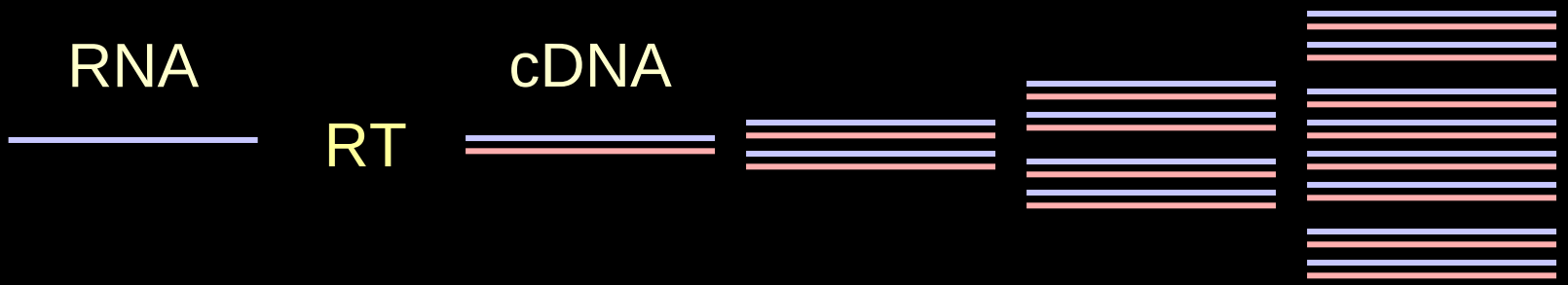
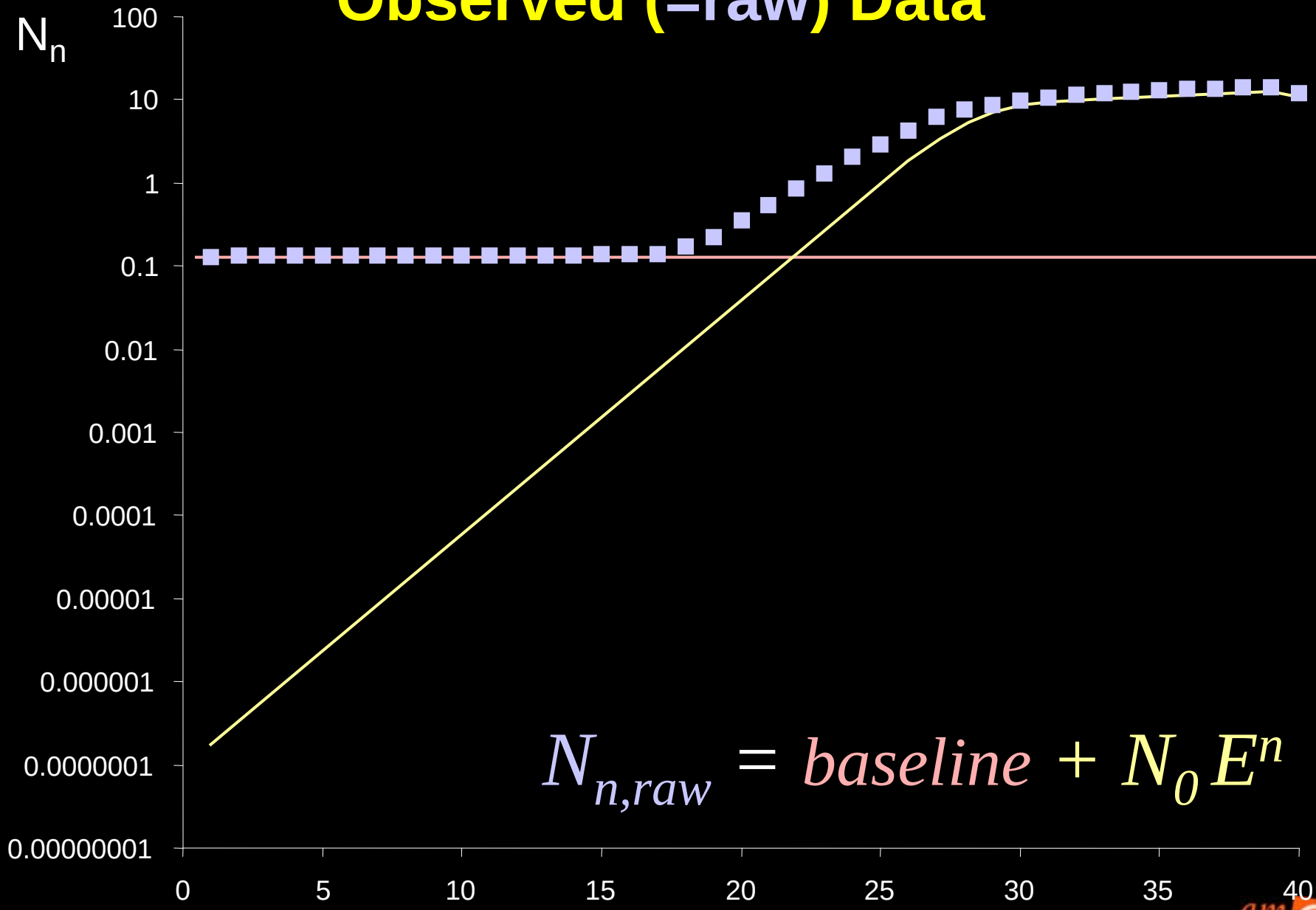
qPCR Data



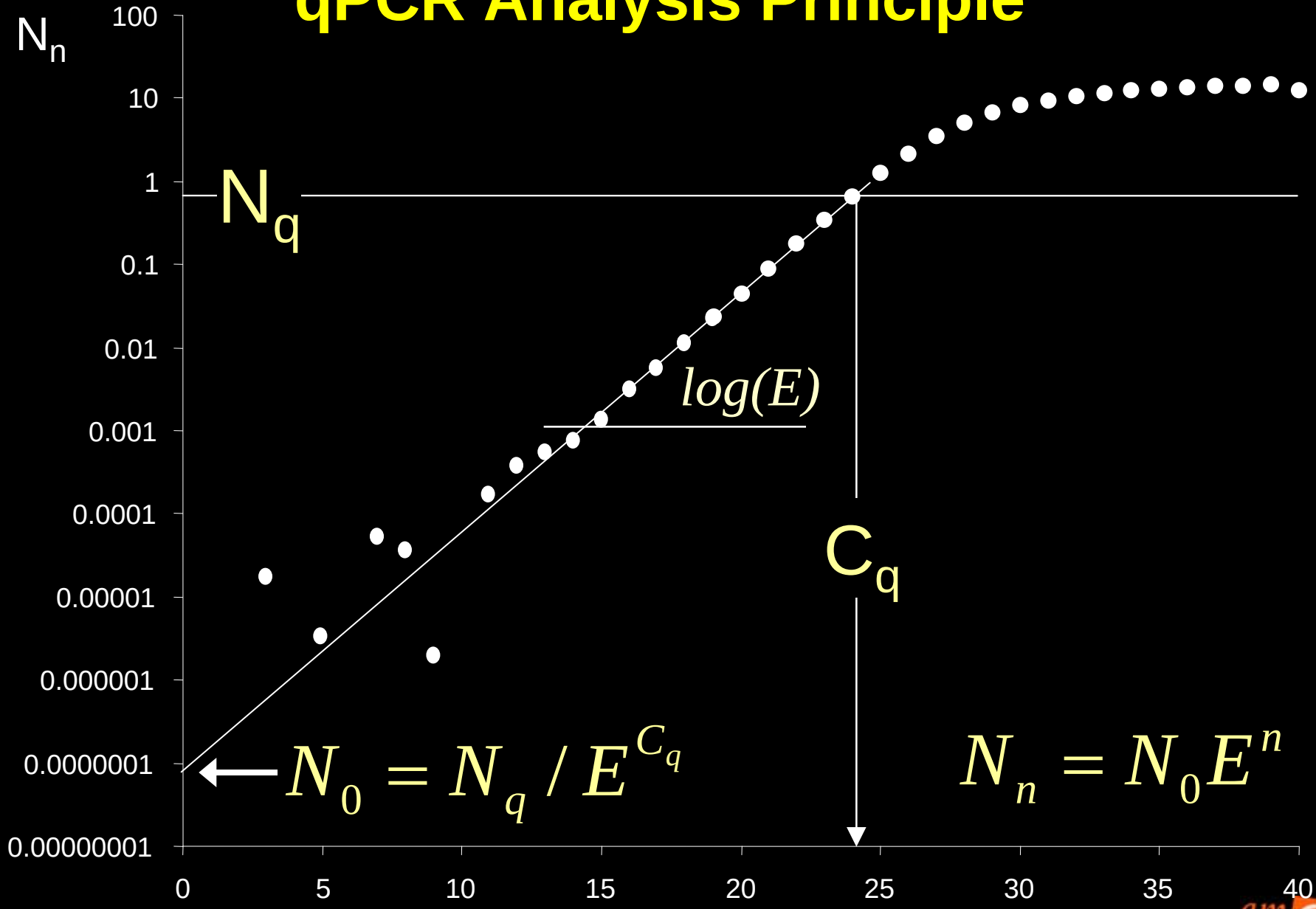
$$N_n = N_0 E^n$$

- N_n: PCR product after n cycles
- n: number of PCR cycles
- E: Efficiency (1-2; 2=100%)
- N_0: start concentration

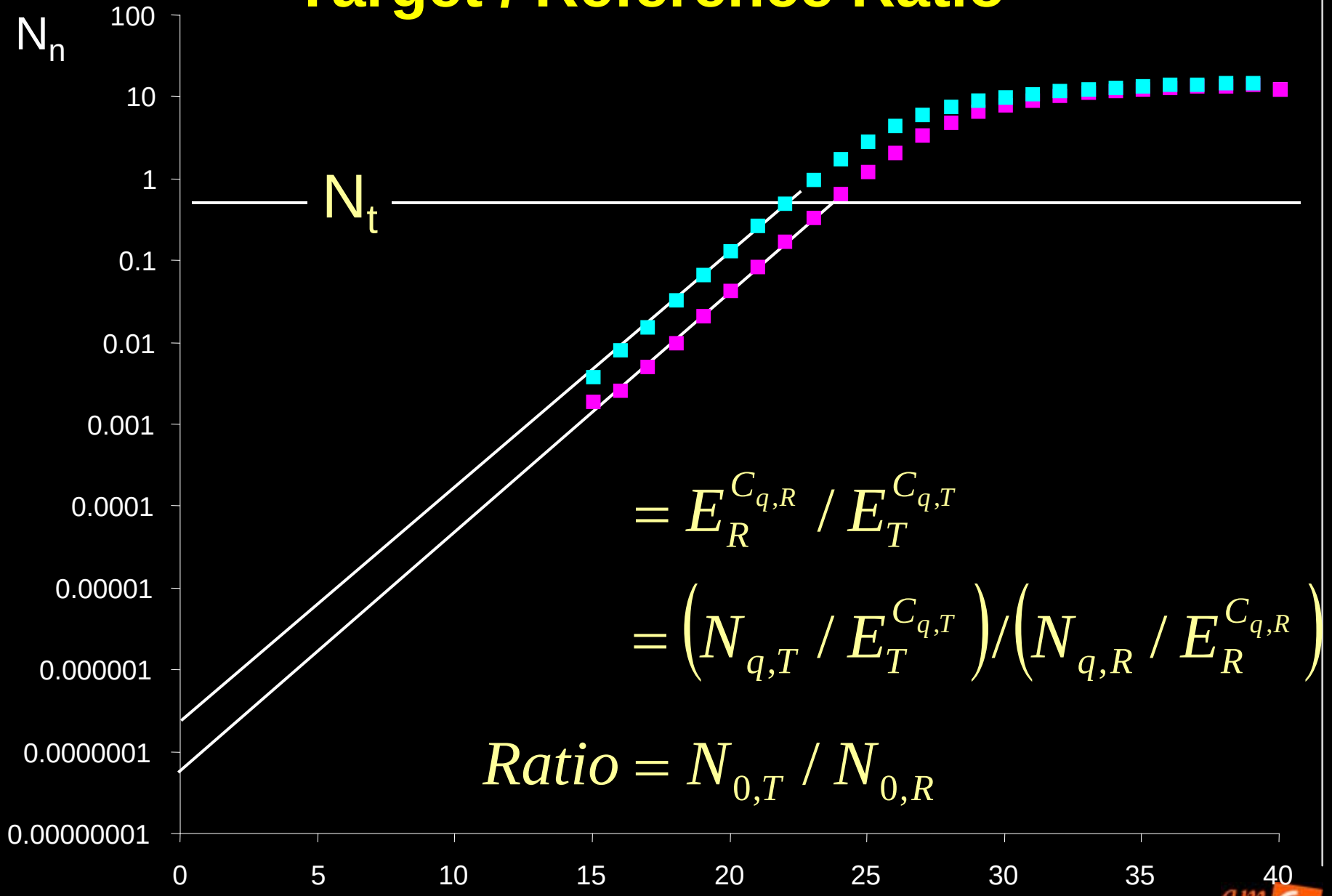
Observed (=raw) Data



qPCR Analysis Principle



Target / Reference Ratio

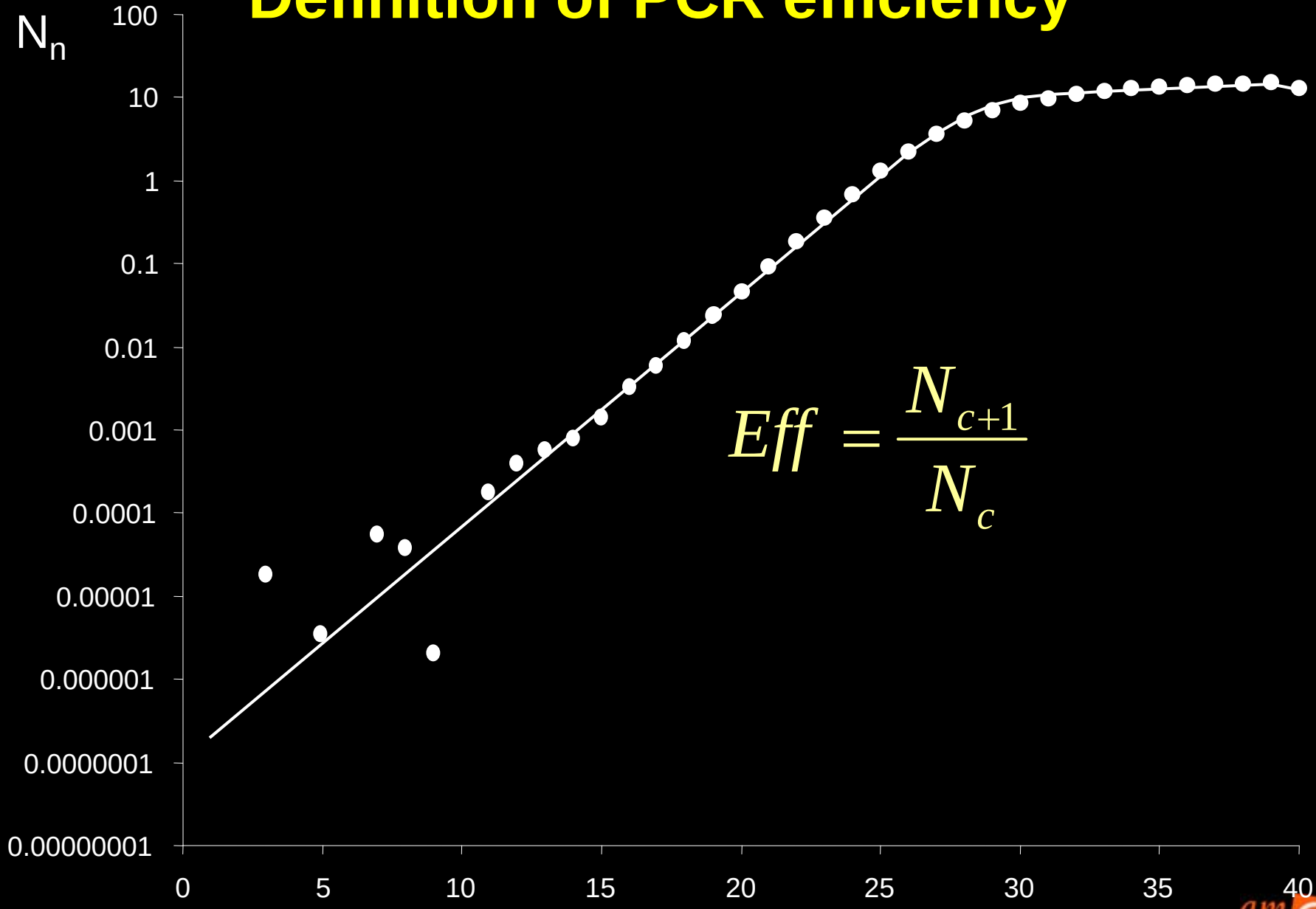


$$= E_R^{C_{q,R}} / E_T^{C_{q,T}}$$

$$= \left(N_{q,T} / E_T^{C_{q,T}}\right) / \left(N_{q,R} / E_R^{C_{q,R}}\right)$$

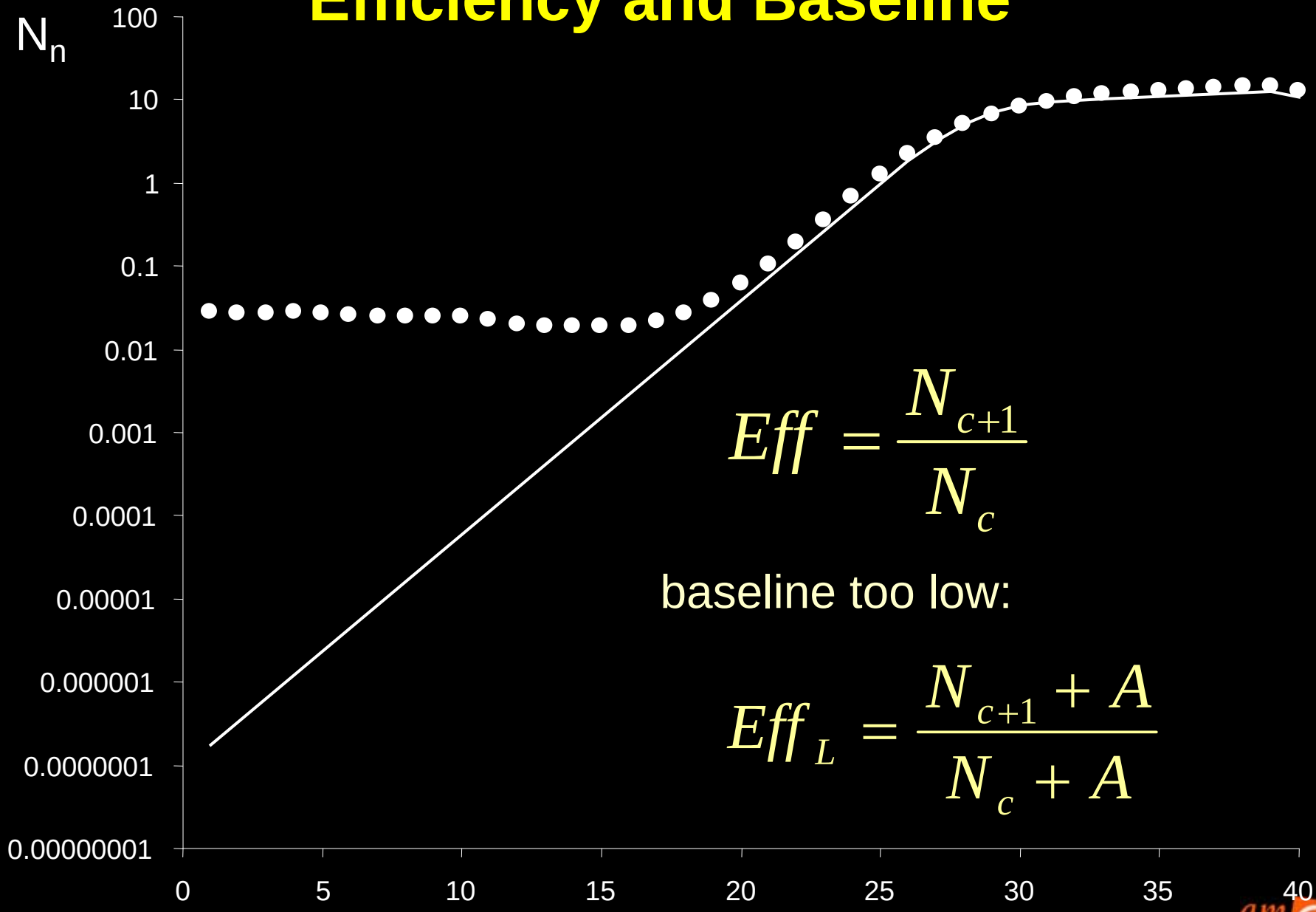
$$Ratio = N_{0,T} / N_{0,R}$$

Definition of PCR efficiency



$$Eff = \frac{N_{c+1}}{N_c}$$

Efficiency and Baseline

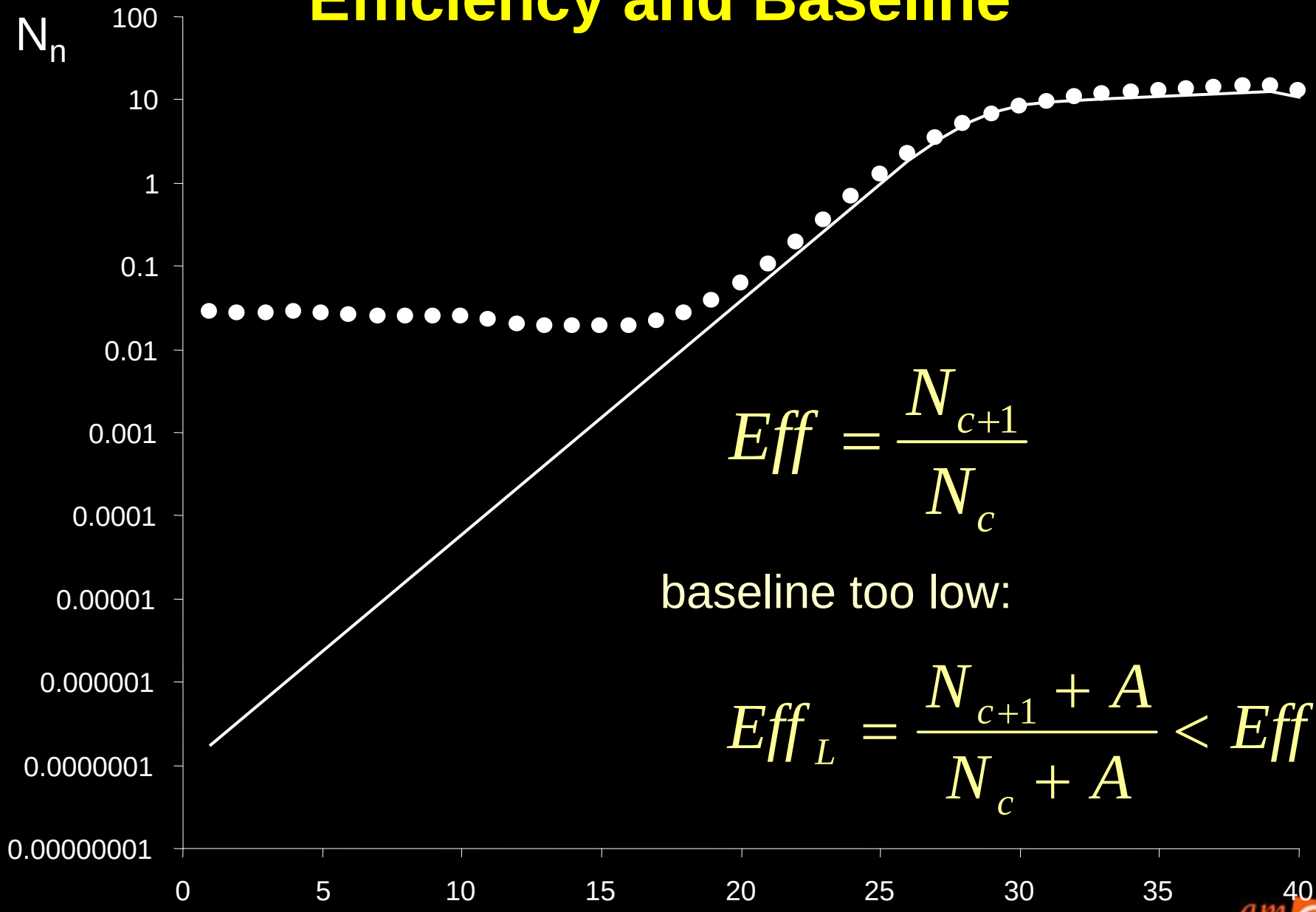


$$Eff = \frac{N_{c+1}}{N_c}$$

baseline too low:

$$Eff_L = \frac{N_{c+1} + A}{N_c + A}$$

Efficiency and Baseline

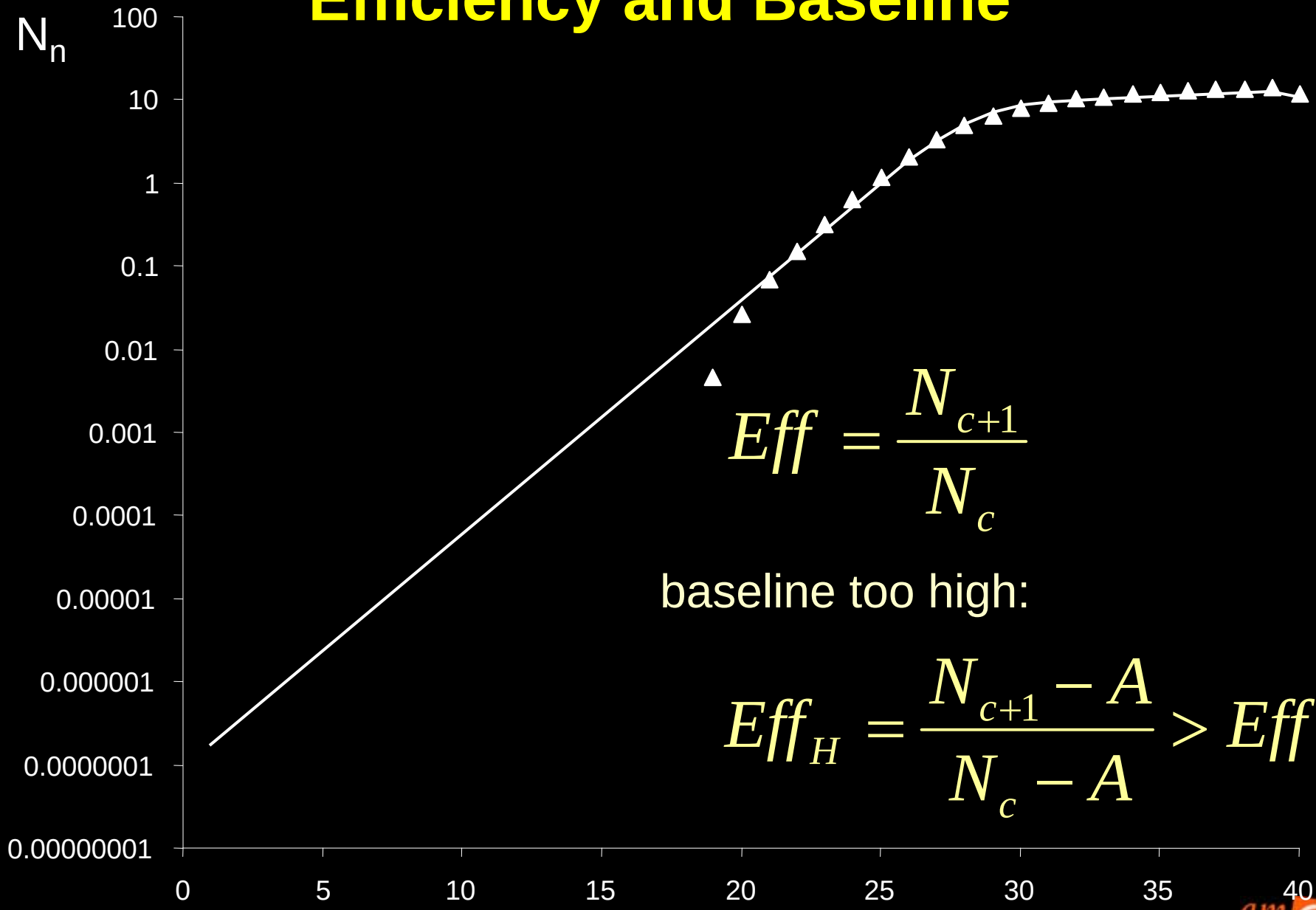


$$Eff = \frac{N_{c+1}}{N_c}$$

baseline too low:

$$Eff_L = \frac{N_{c+1} + A}{N_c + A} < Eff$$

Efficiency and Baseline

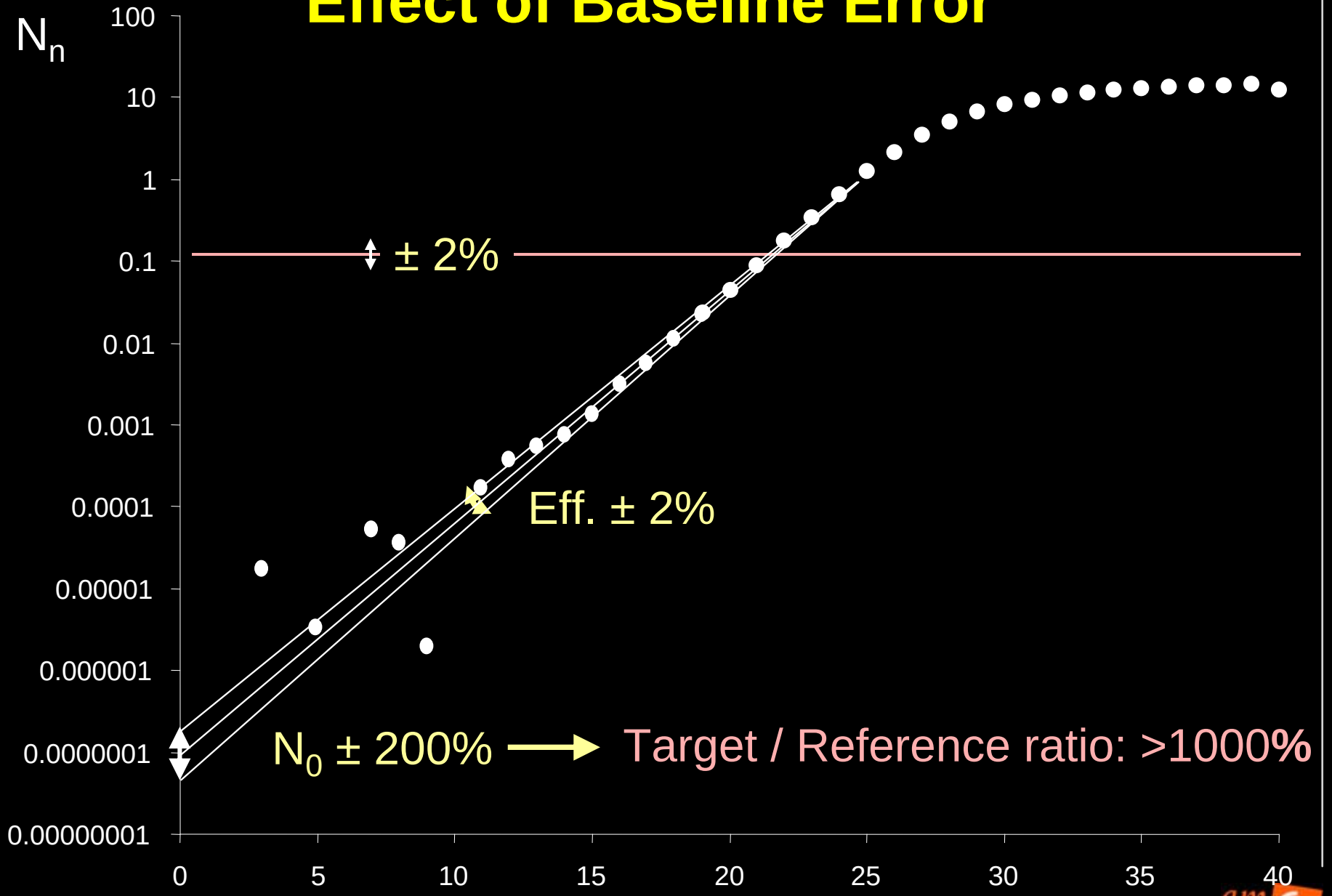


$$Eff = \frac{N_{c+1}}{N_c}$$

baseline too high:

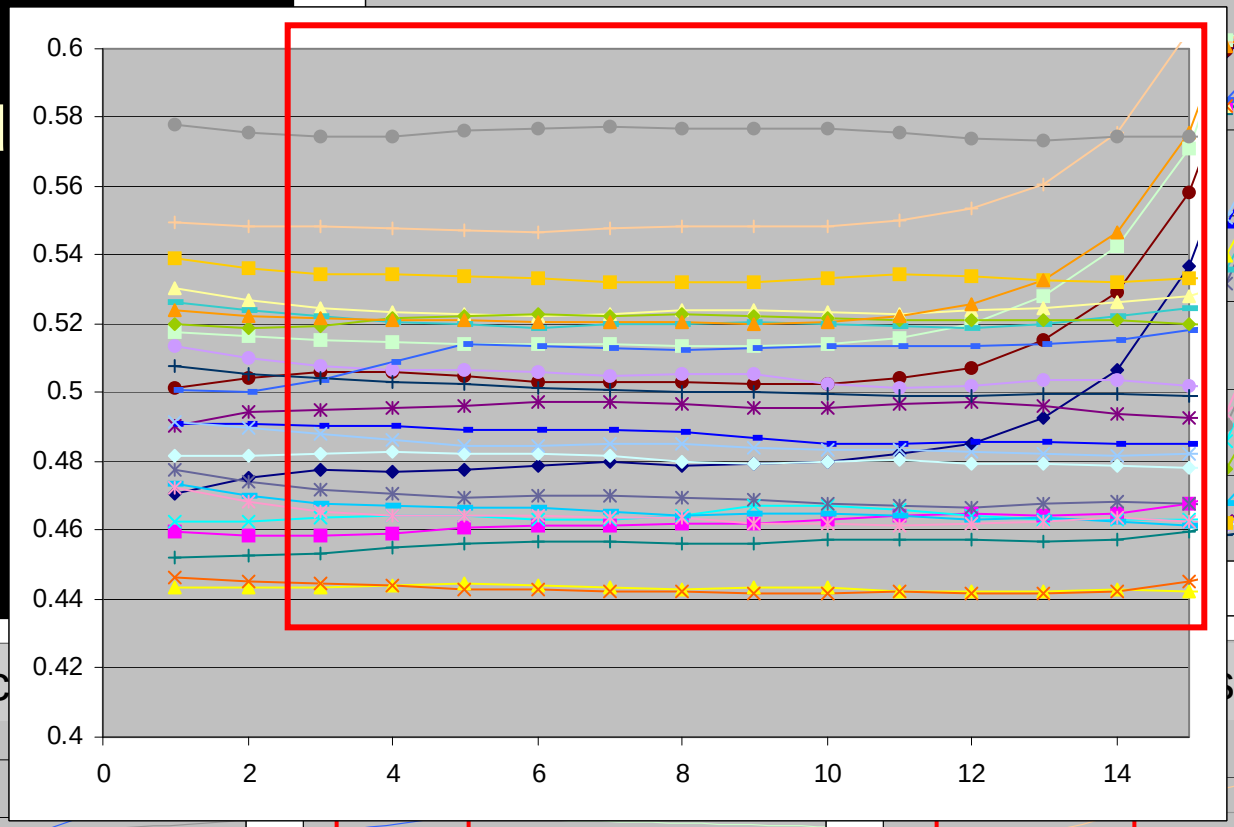
$$Eff_H = \frac{N_{c+1} - A}{N_c - A} > Eff$$

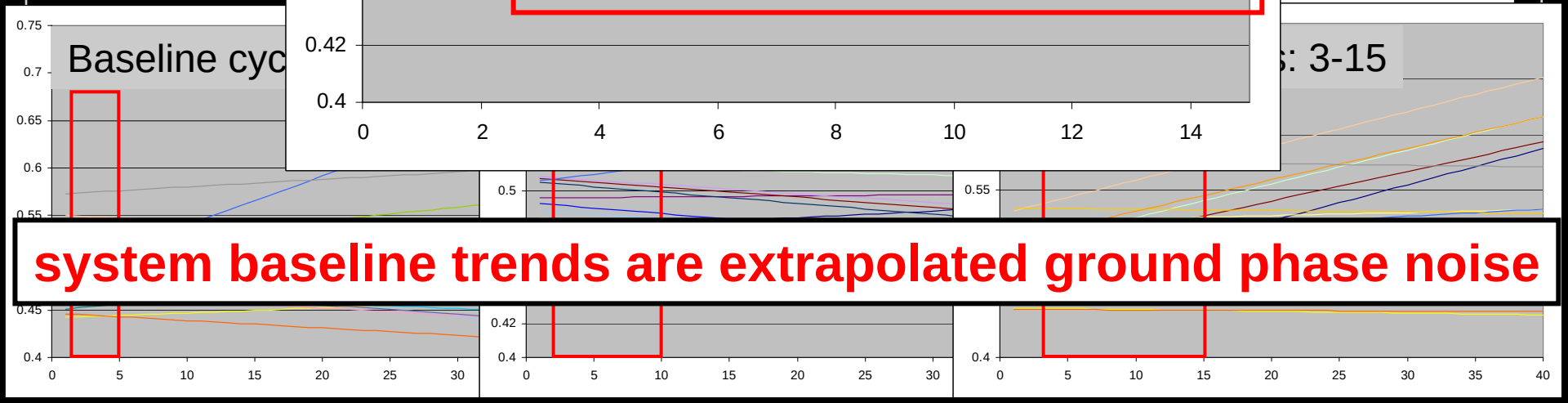
Effect of Baseline Error



System Baseline Correction

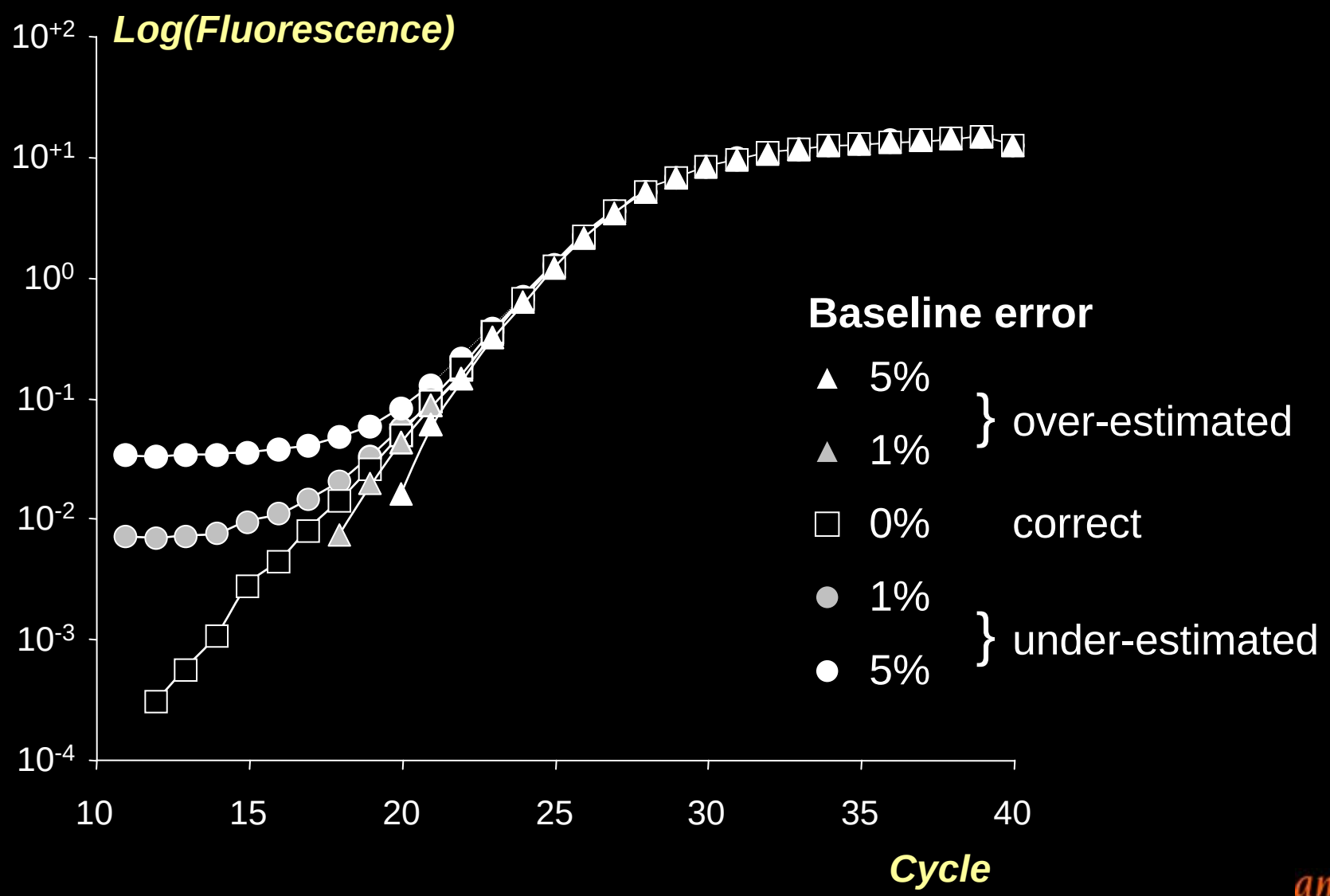
baseline = regression line through X ground cycles



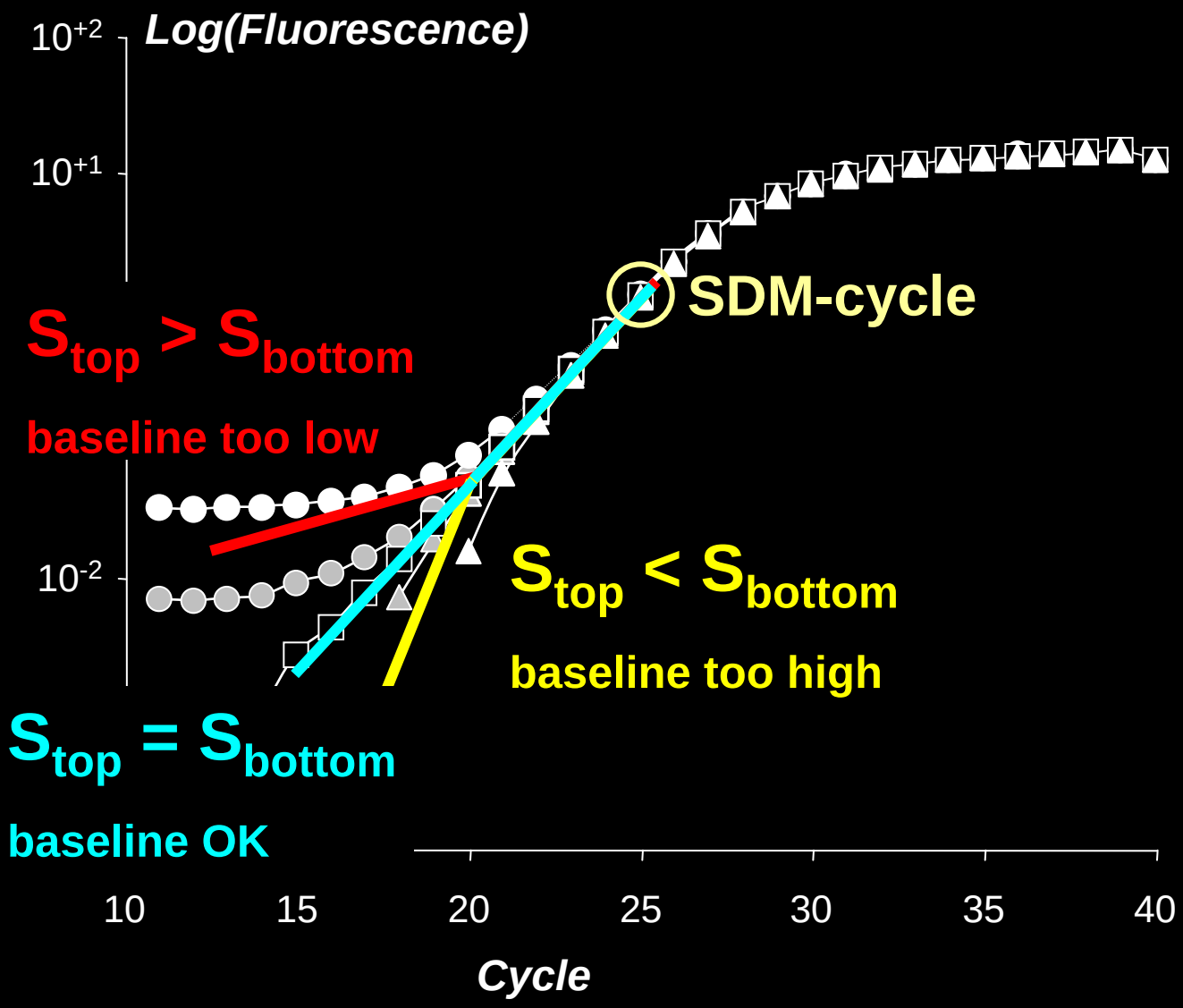


system baseline trends are extrapolated ground phase noise

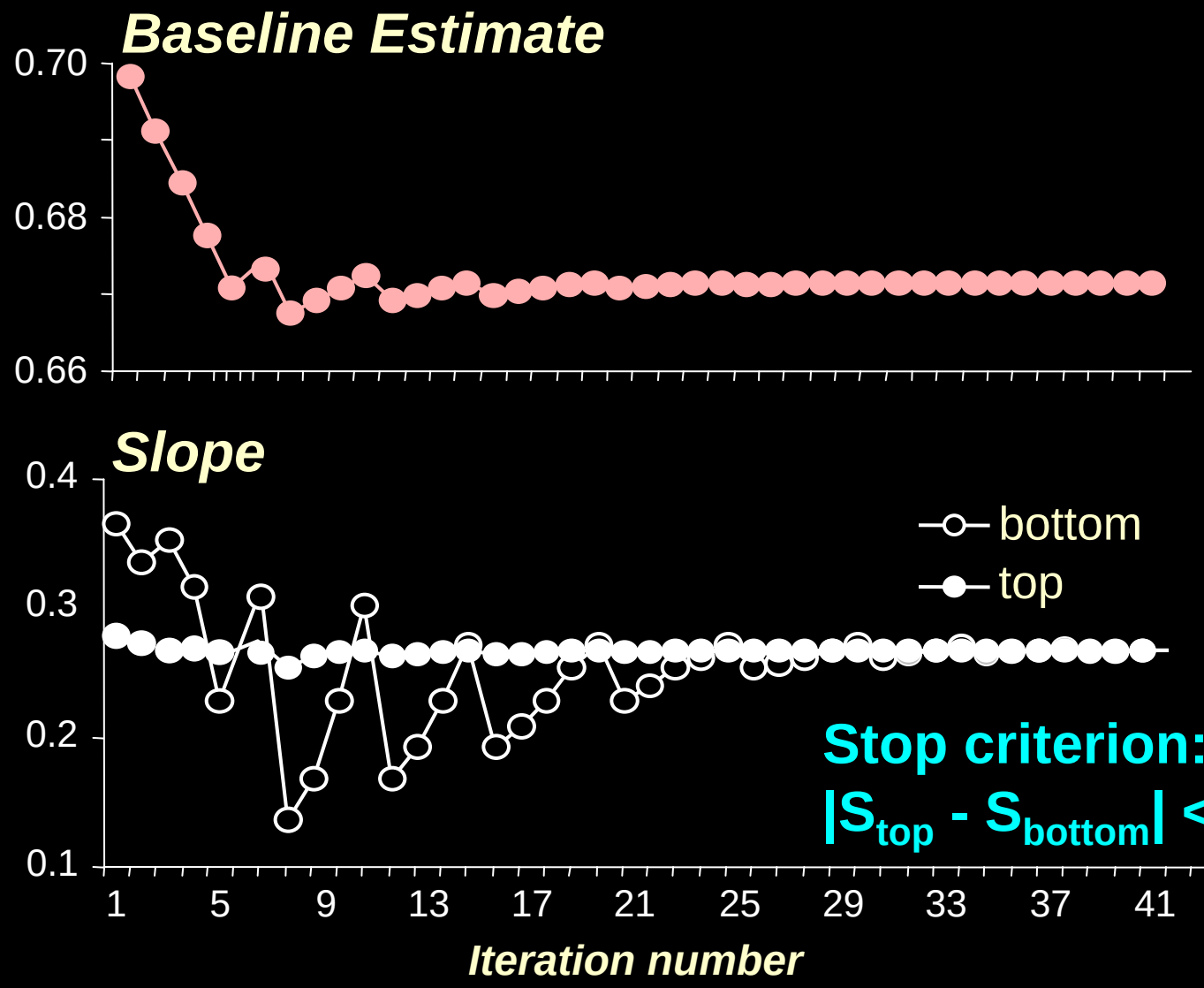
Recognizing Baseline Error



Baseline Estimation Principle



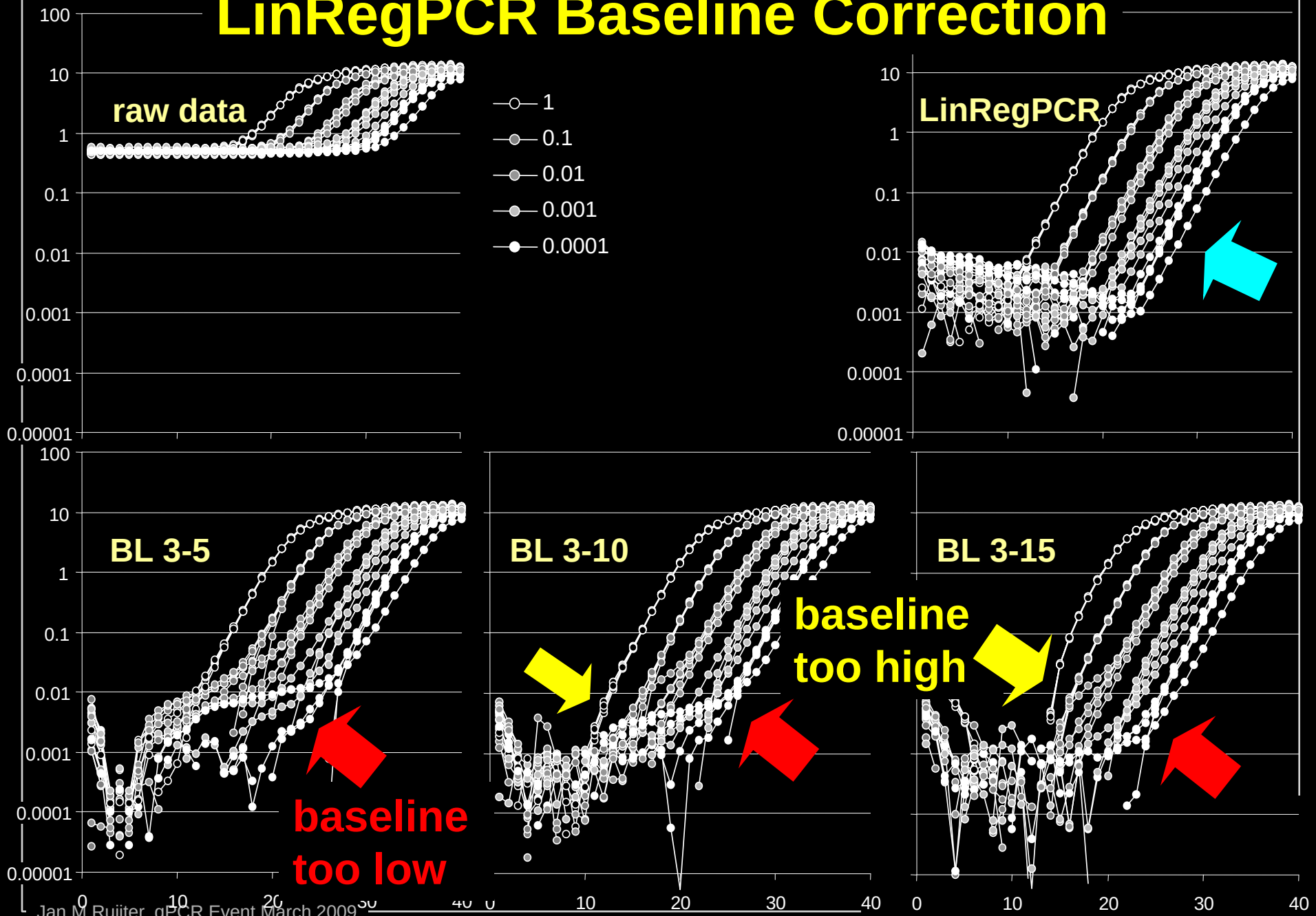
Baseline Estimation Algorithm



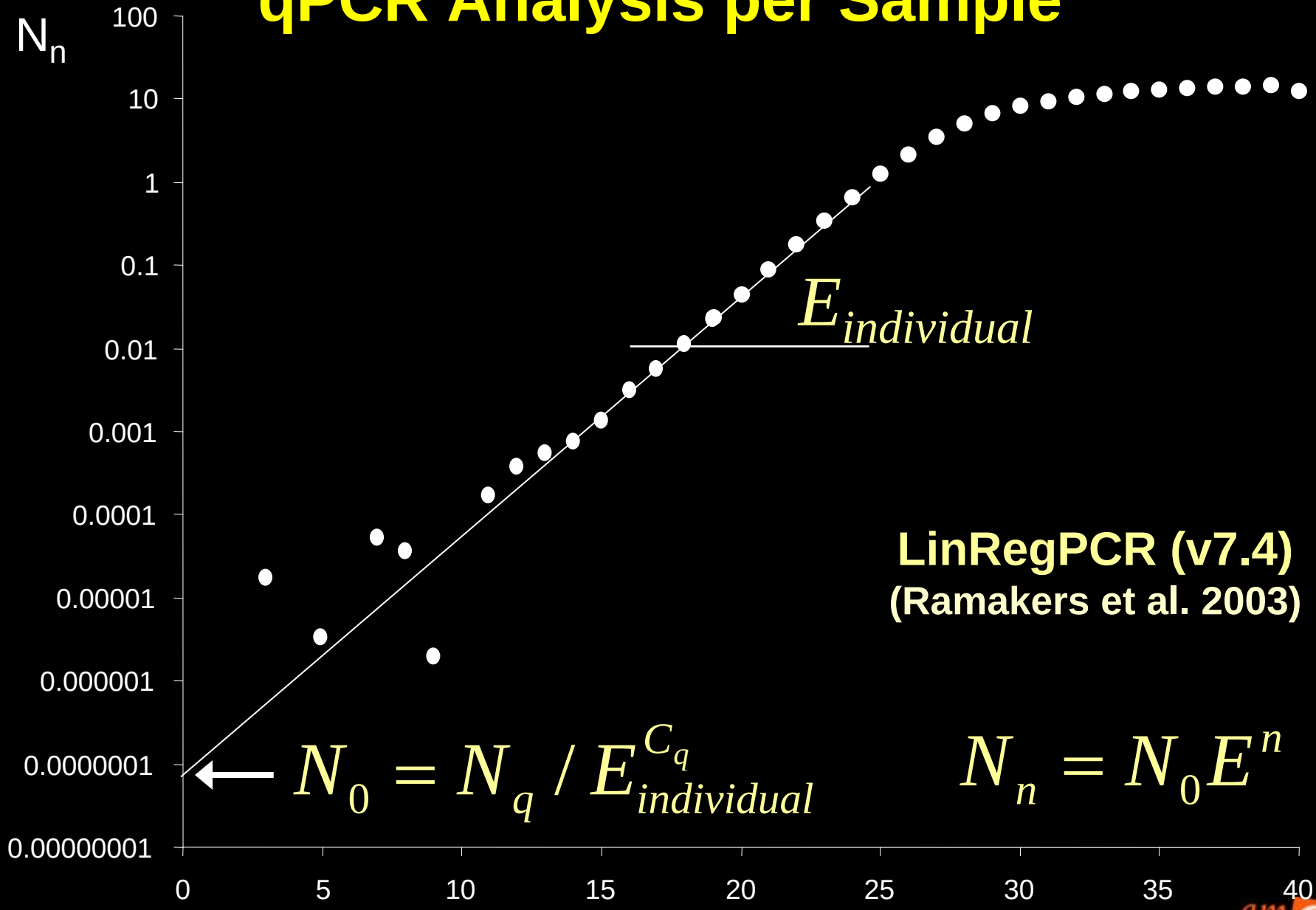
Stop criterion:

$$|S_{top} - S_{bottom}| < 0.00001$$

LinRegPCR Baseline Correction

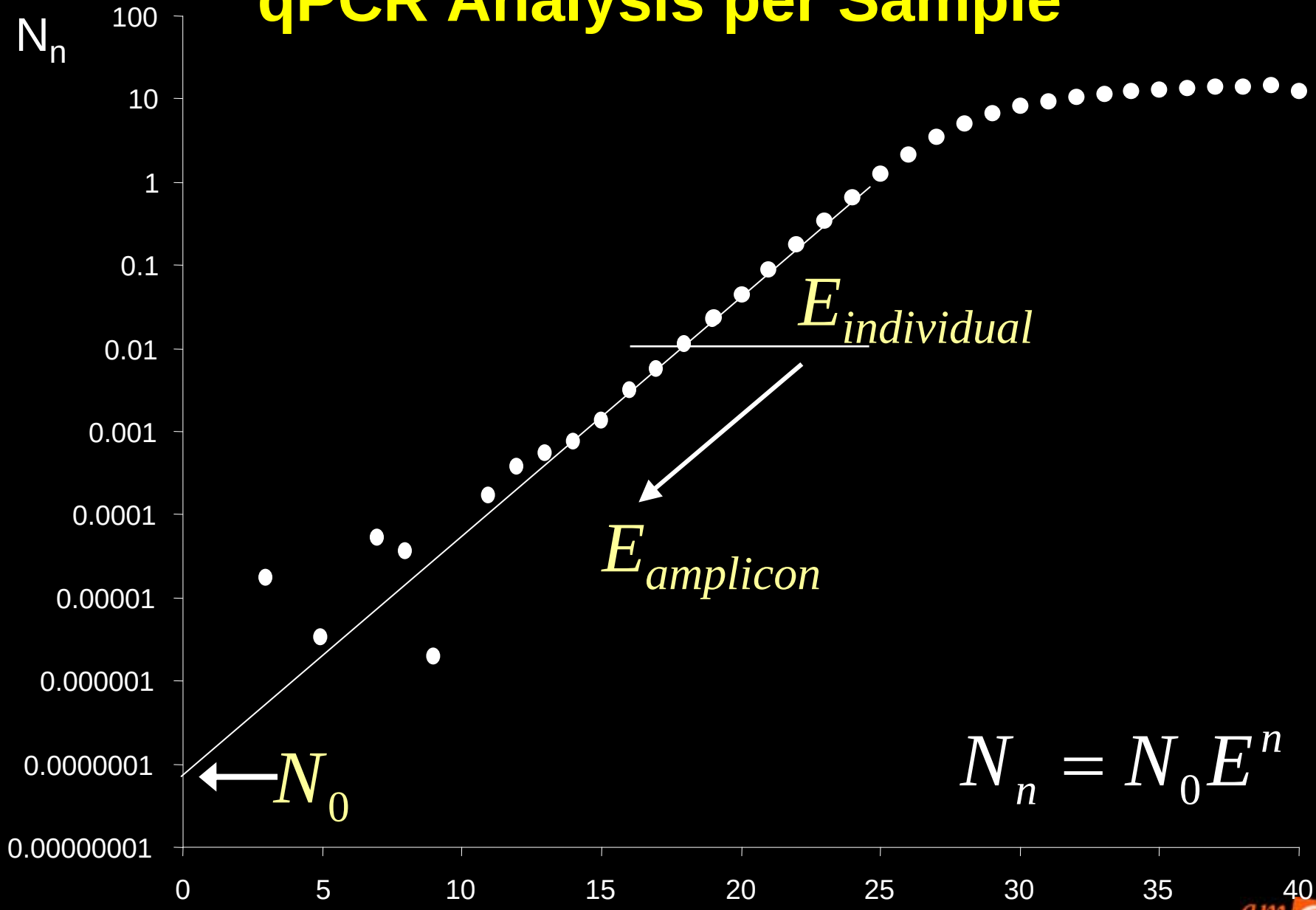


qPCR Analysis per Sample

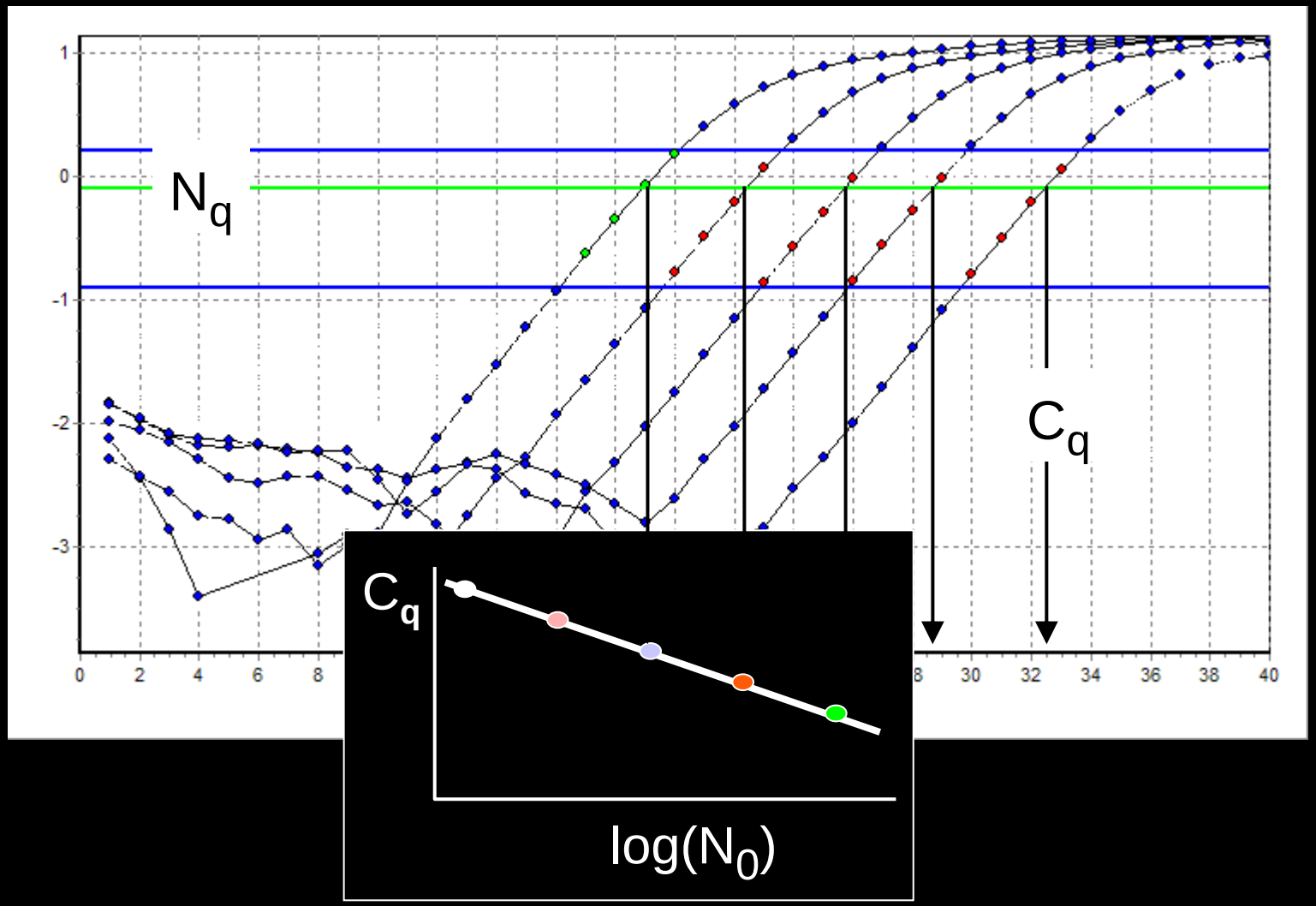


LinRegPCR (v7.4)
(Ramakers et al. 2003)

qPCR Analysis per Sample



Efficiency derived from Standard Curve



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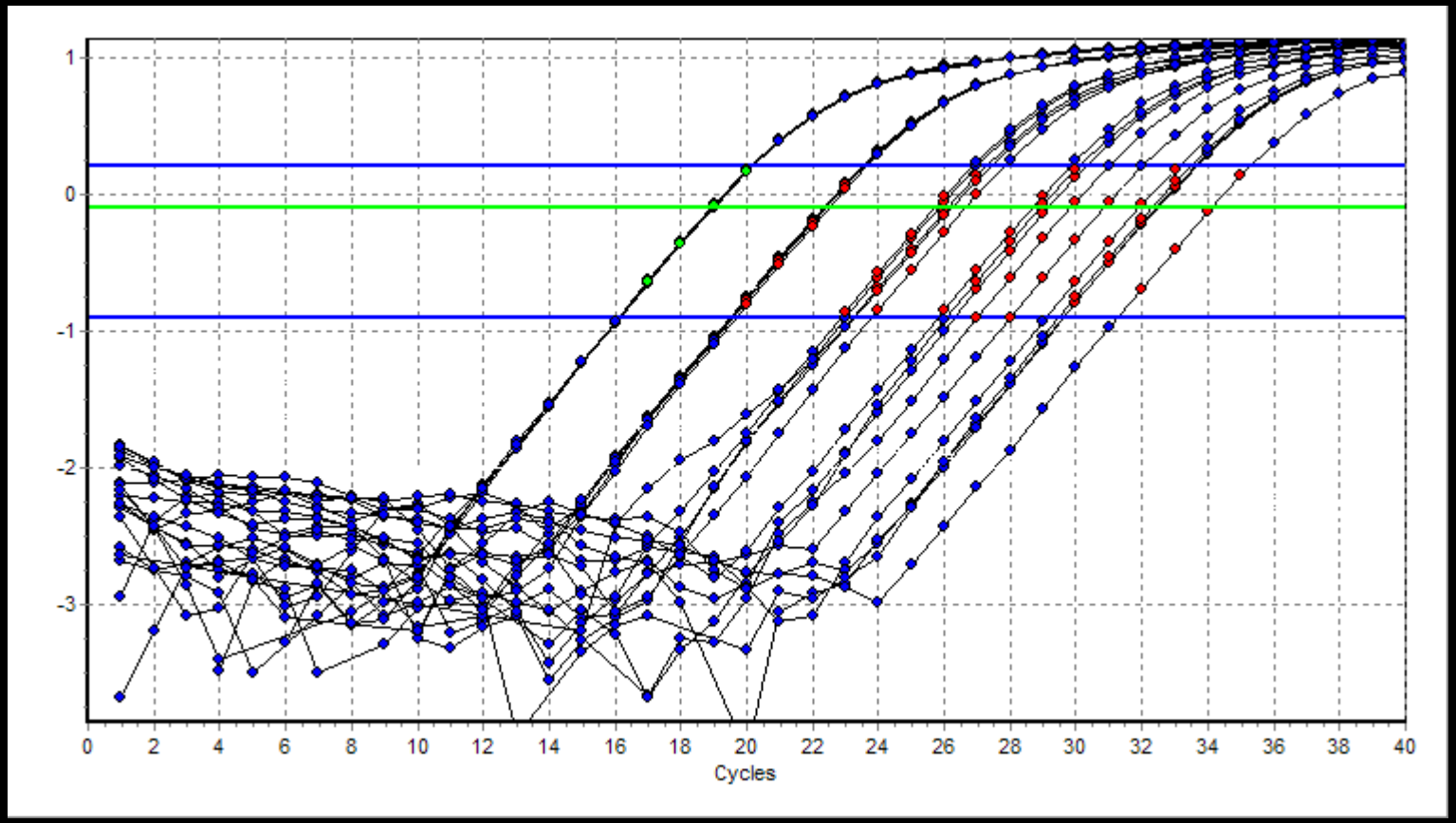
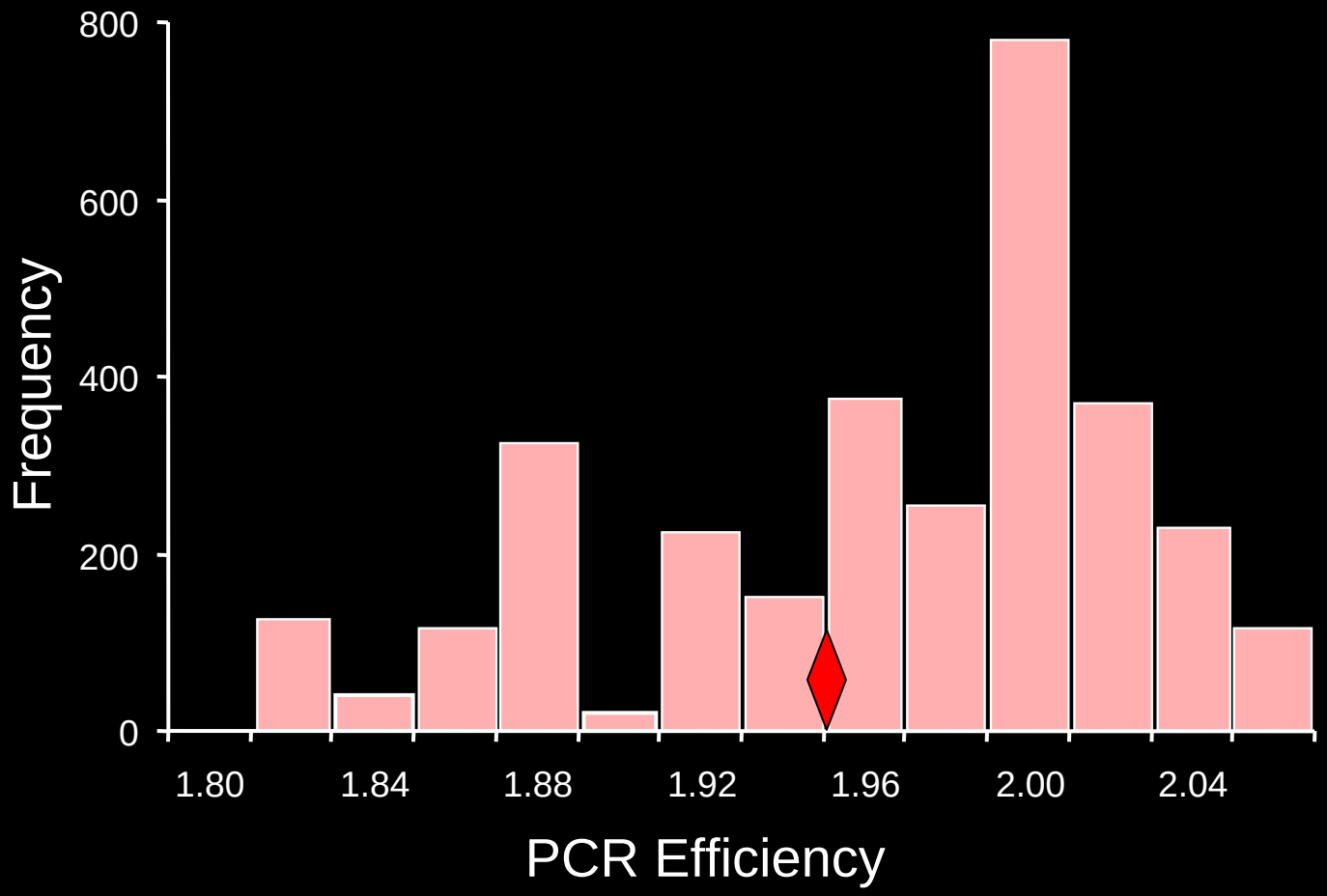


5 dilutions
5 replicates per dilution } 3125 standard curves

Efficiencies derived from Standard Curve



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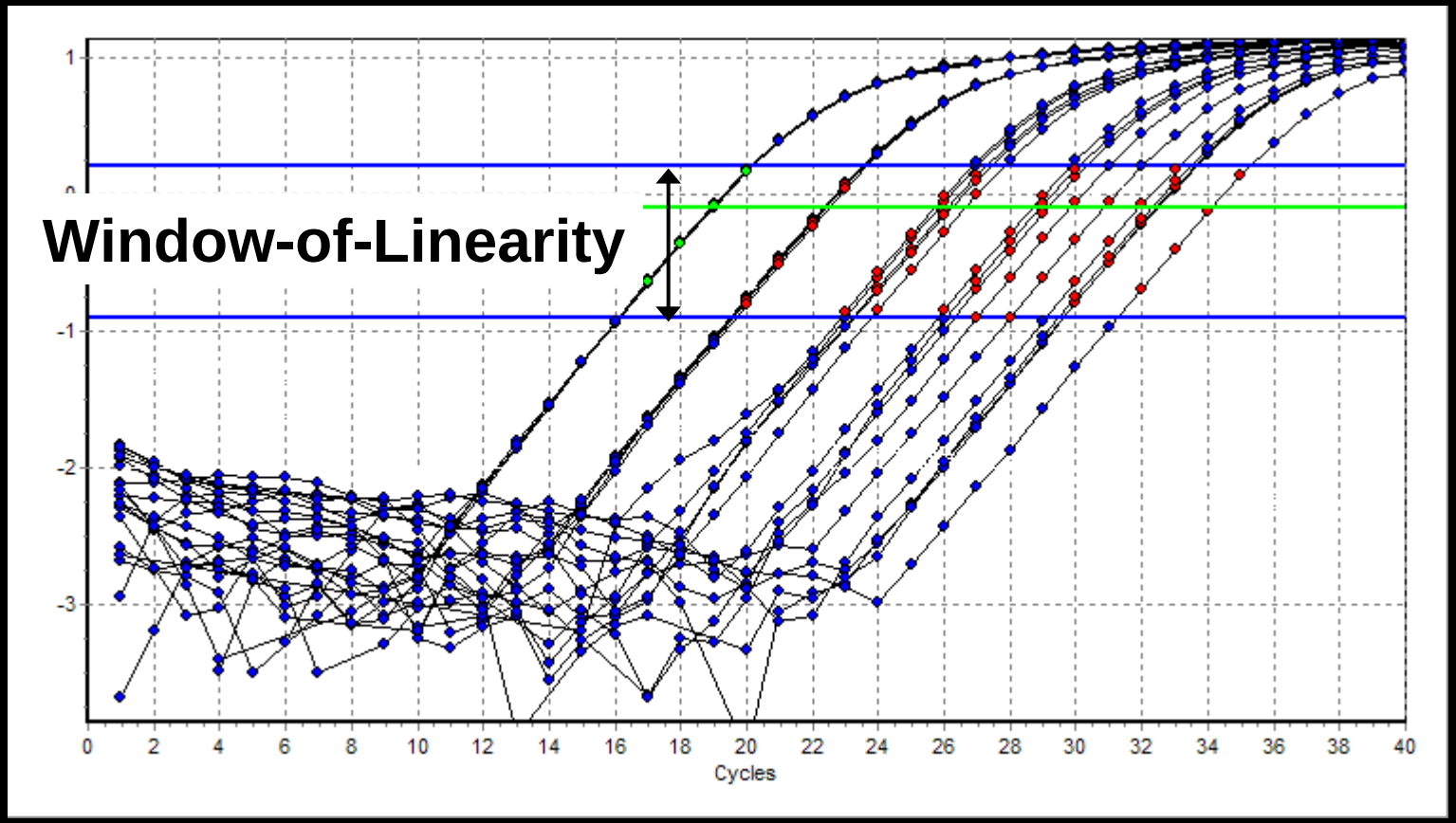
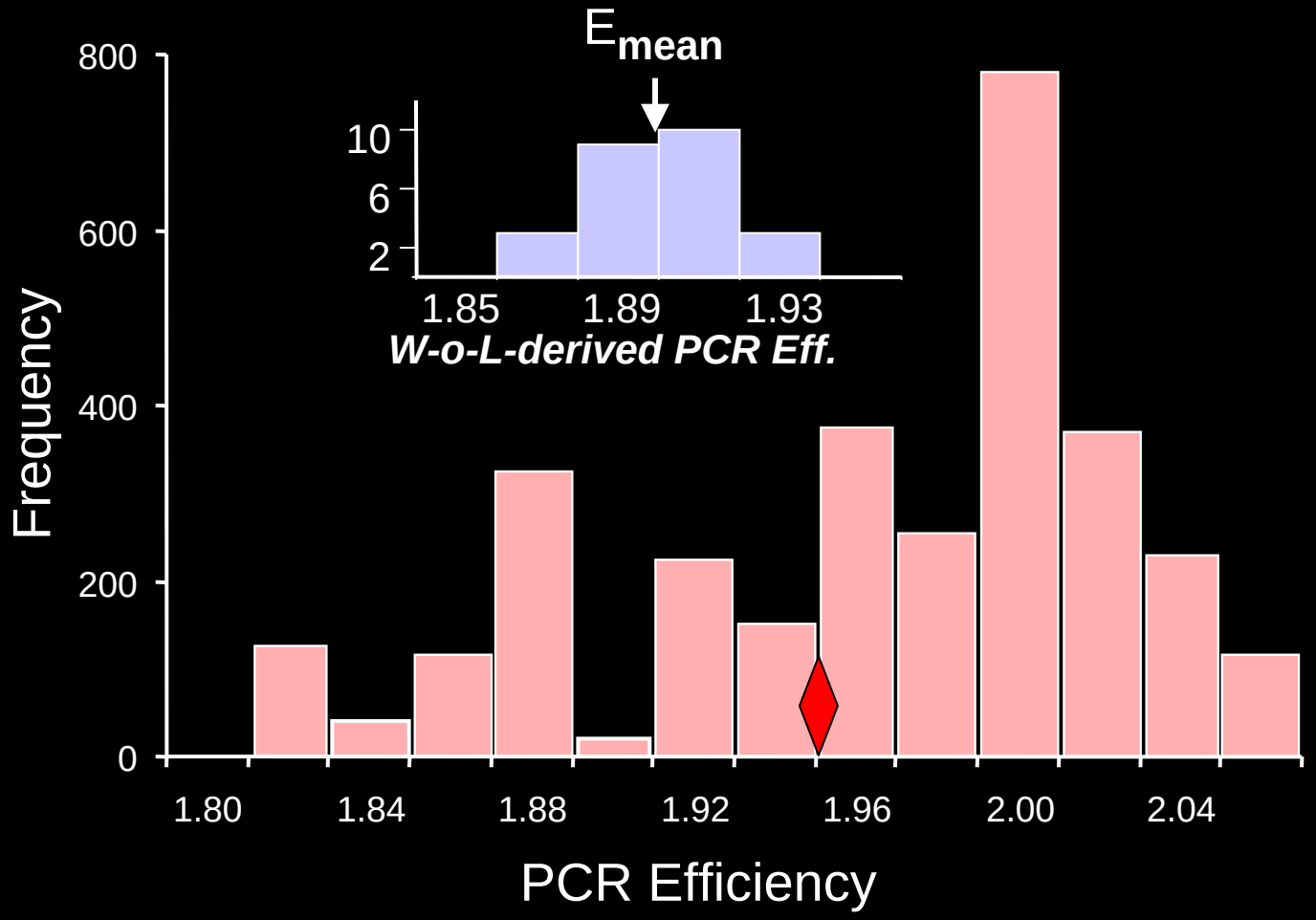
Efficiency derived from Standard Curve



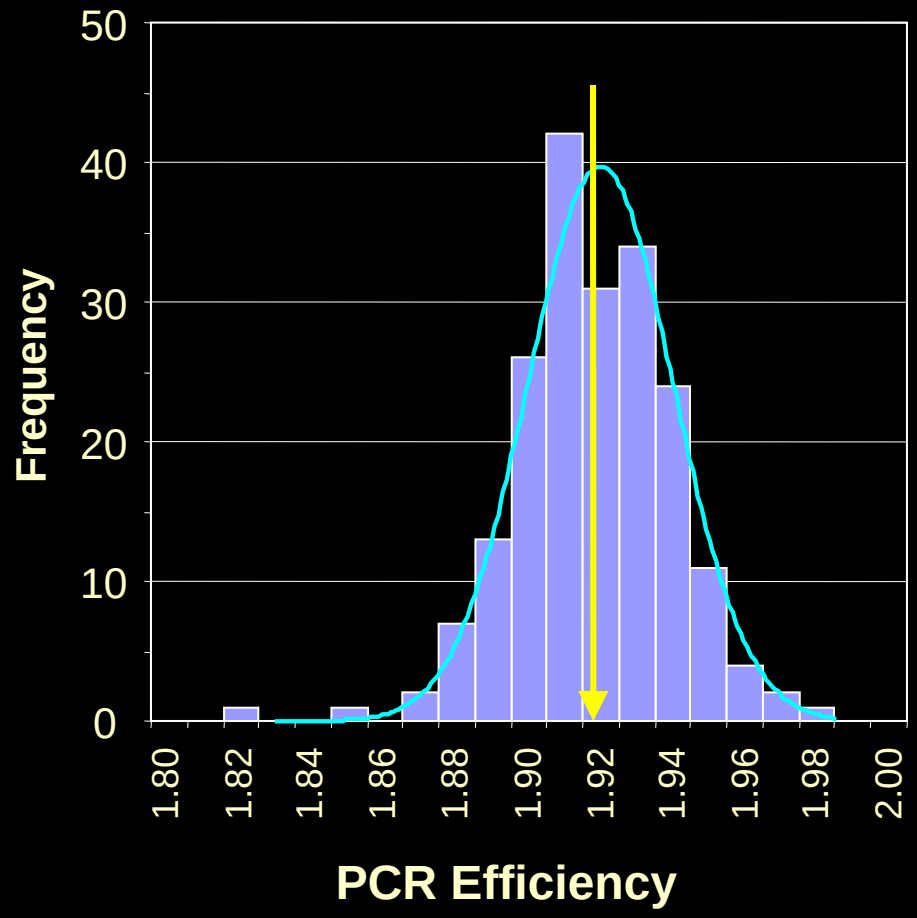
5 dilutions
5 replicates per dilution } 25 individual efficiency values

Efficiencies derived from Standard Curve

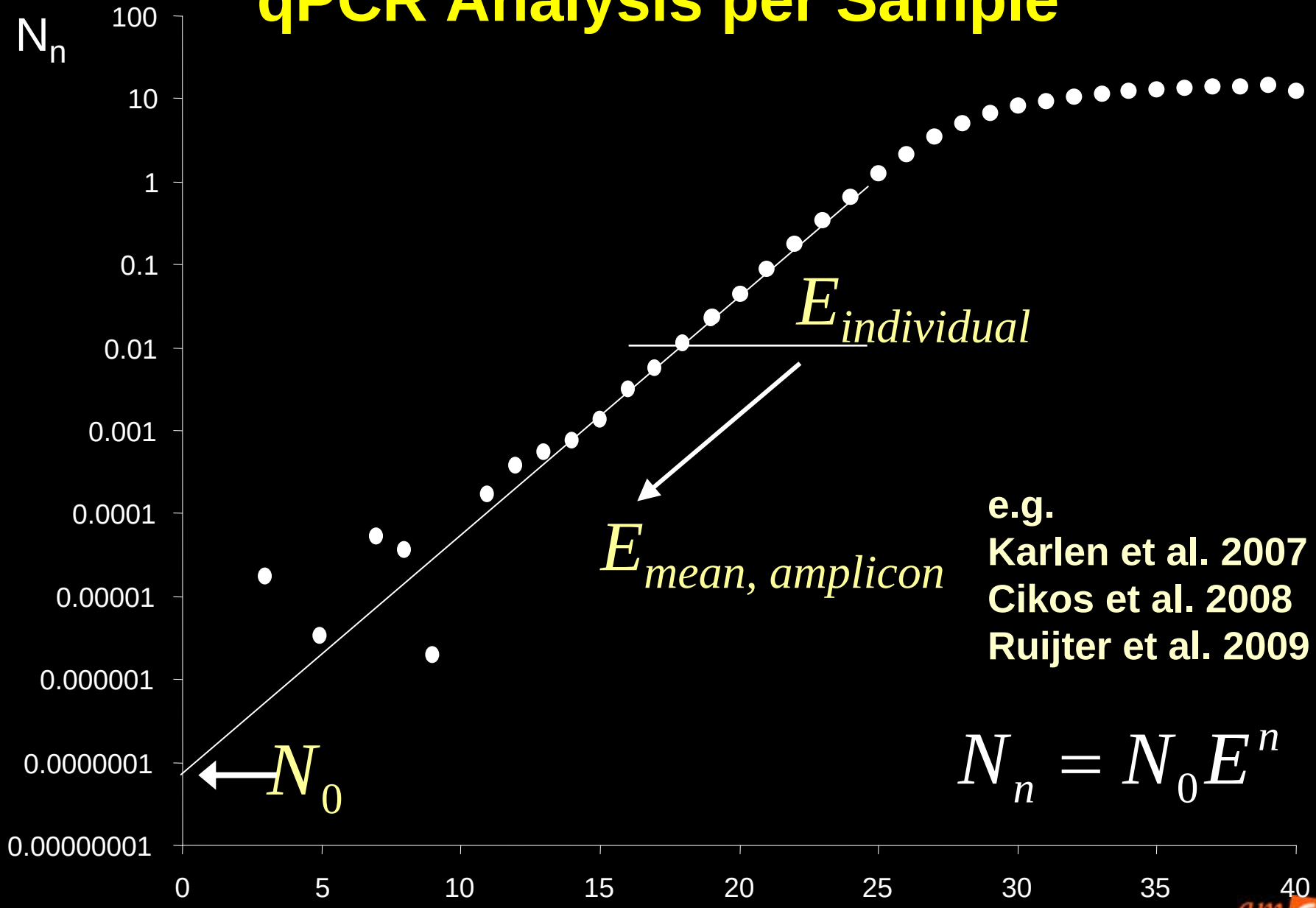


Variation of individual PCR Efficiencies

GAPDH: 20 biological samples, 10 replicates/sample



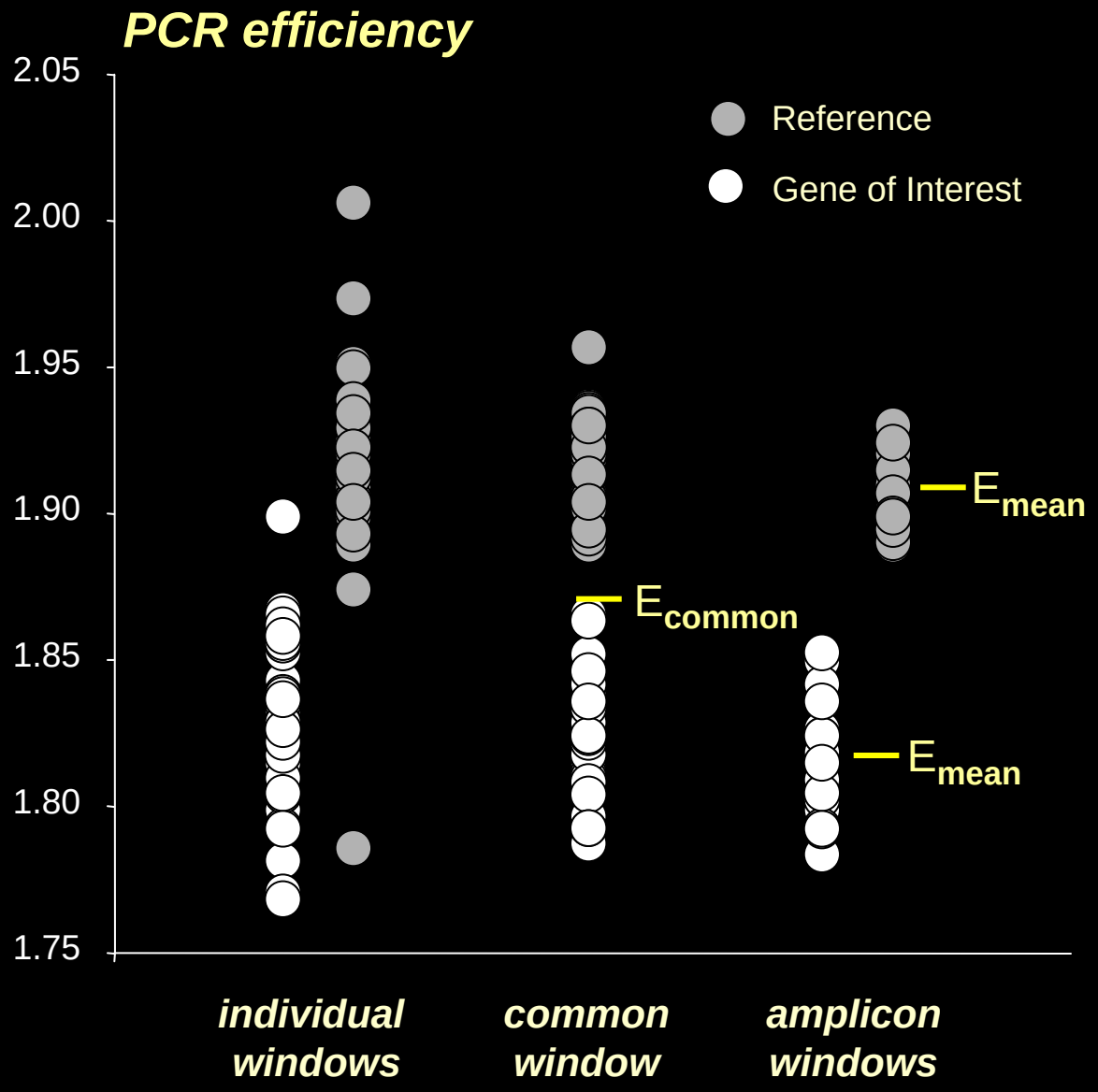
qPCR Analysis per Sample



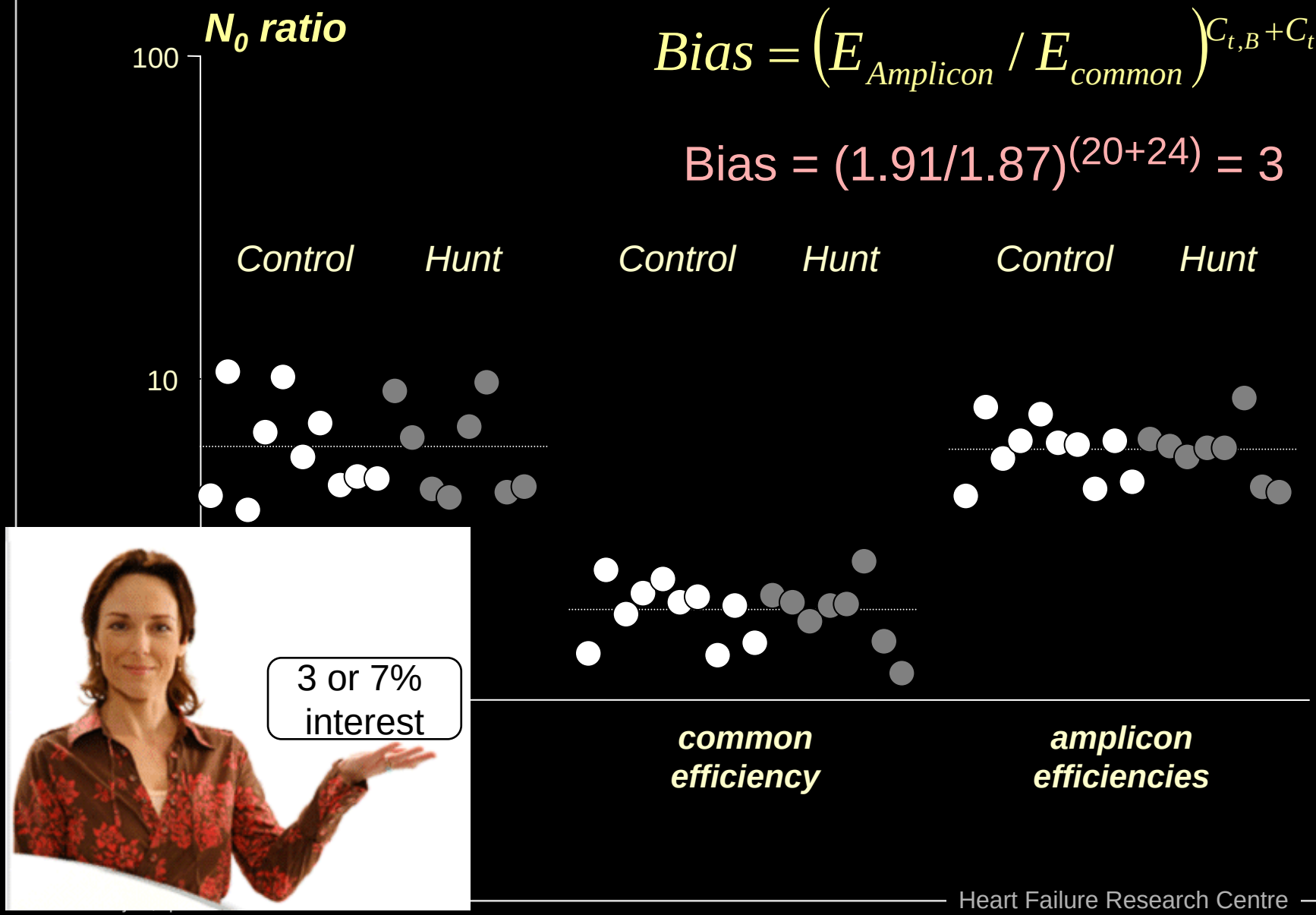
e.g.
Karlen et al. 2007
Cikos et al. 2008
Ruijter et al. 2009

$$N_n = N_0 E^n$$

Efficiency and Bias



Expression Ratio



$$Bias = \left(E_{Amplicon} / E_{common}\right)^{C_{t,B}+C_{t,A}}$$

Bias = $(1.91/1.87)^{(20+24)} = 3$

Paper and Program

Nucleic Acids Research Advance Access published February 22, 2009

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doi:10.1093/nar/gkp045

Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data

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program (latest version) and manual:
http://LinRegPCR.HFRC.nl **(direct download)**

Frequently Asked Questions:
http://LinRegPCR.nl

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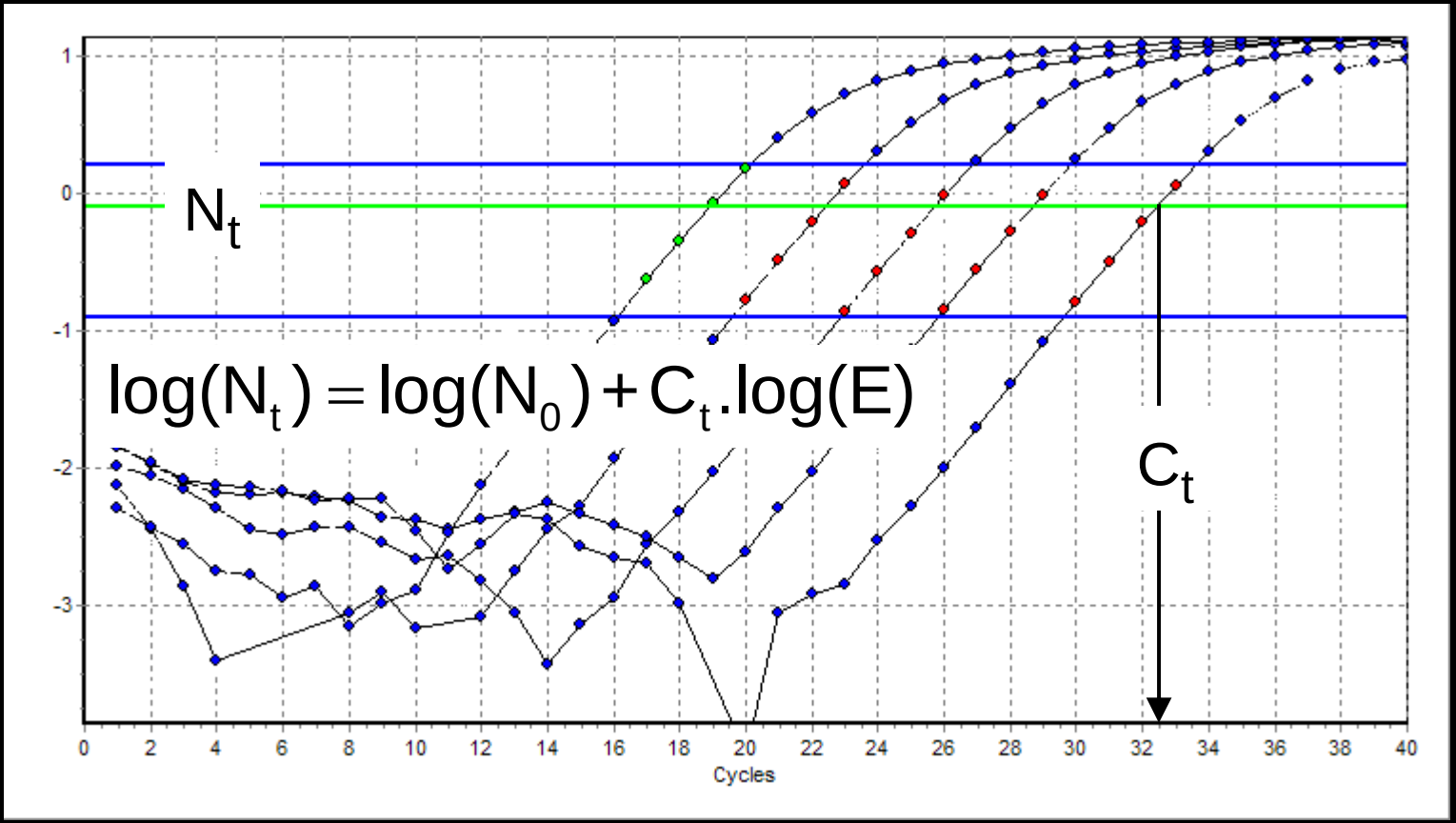
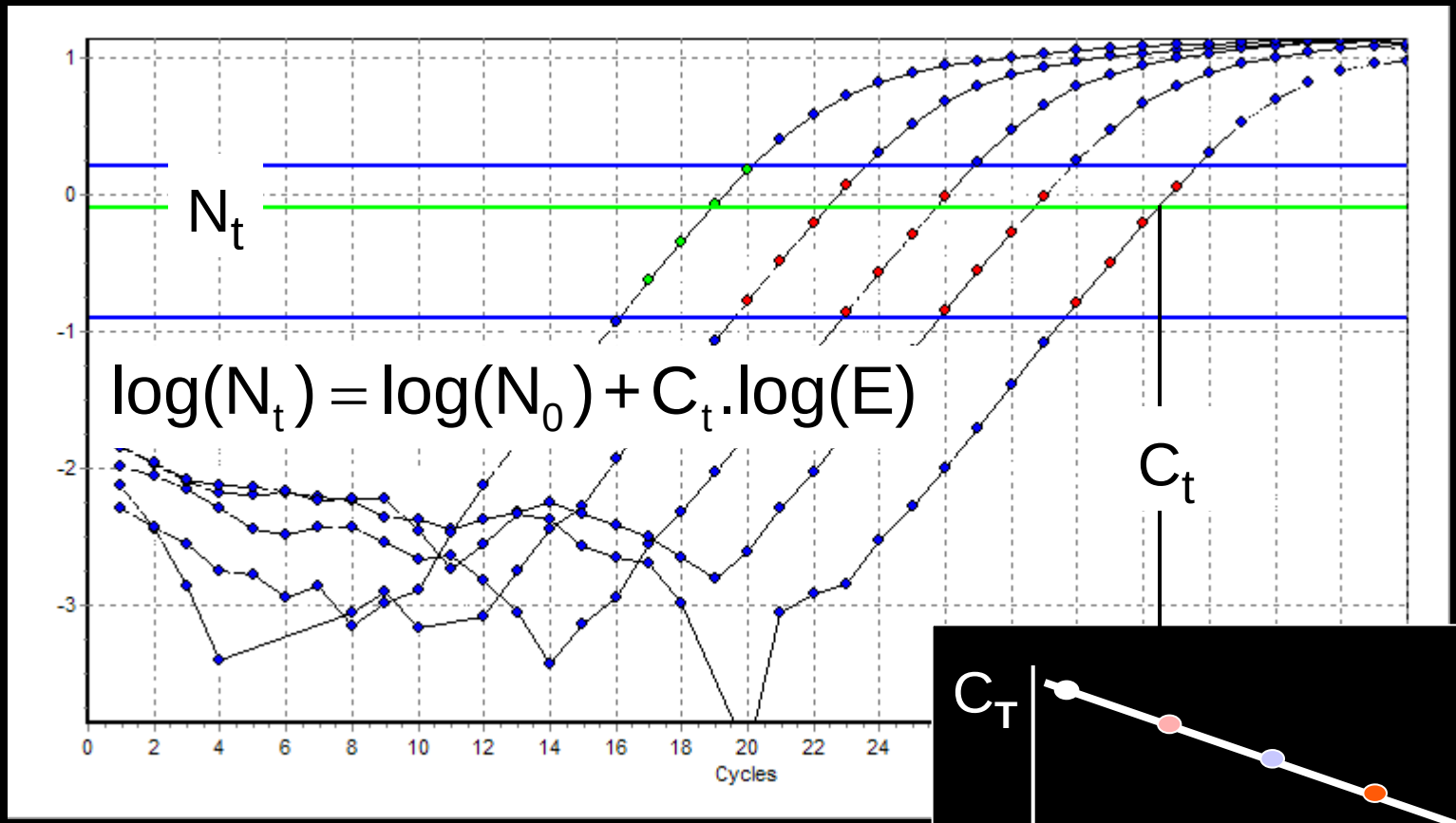
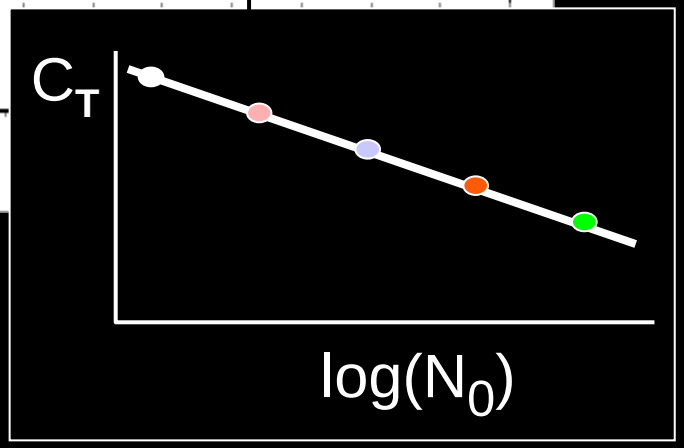


$$C_t = \frac{\log(N_t)}{\log(E)} - \frac{1}{\log(E)}\log(N_0)$$

Efficiency derived from Standard Curve





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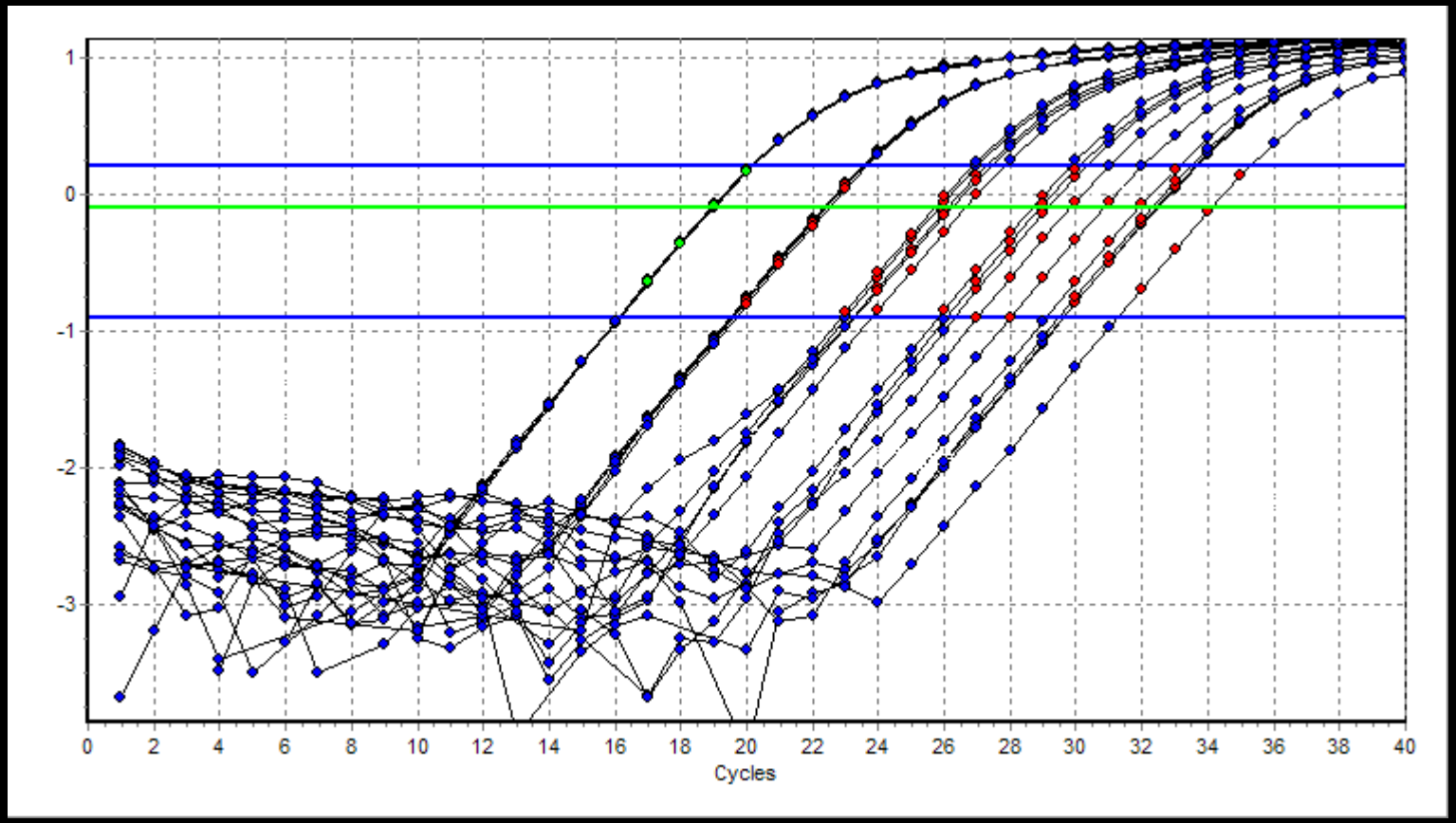

5 dilutions
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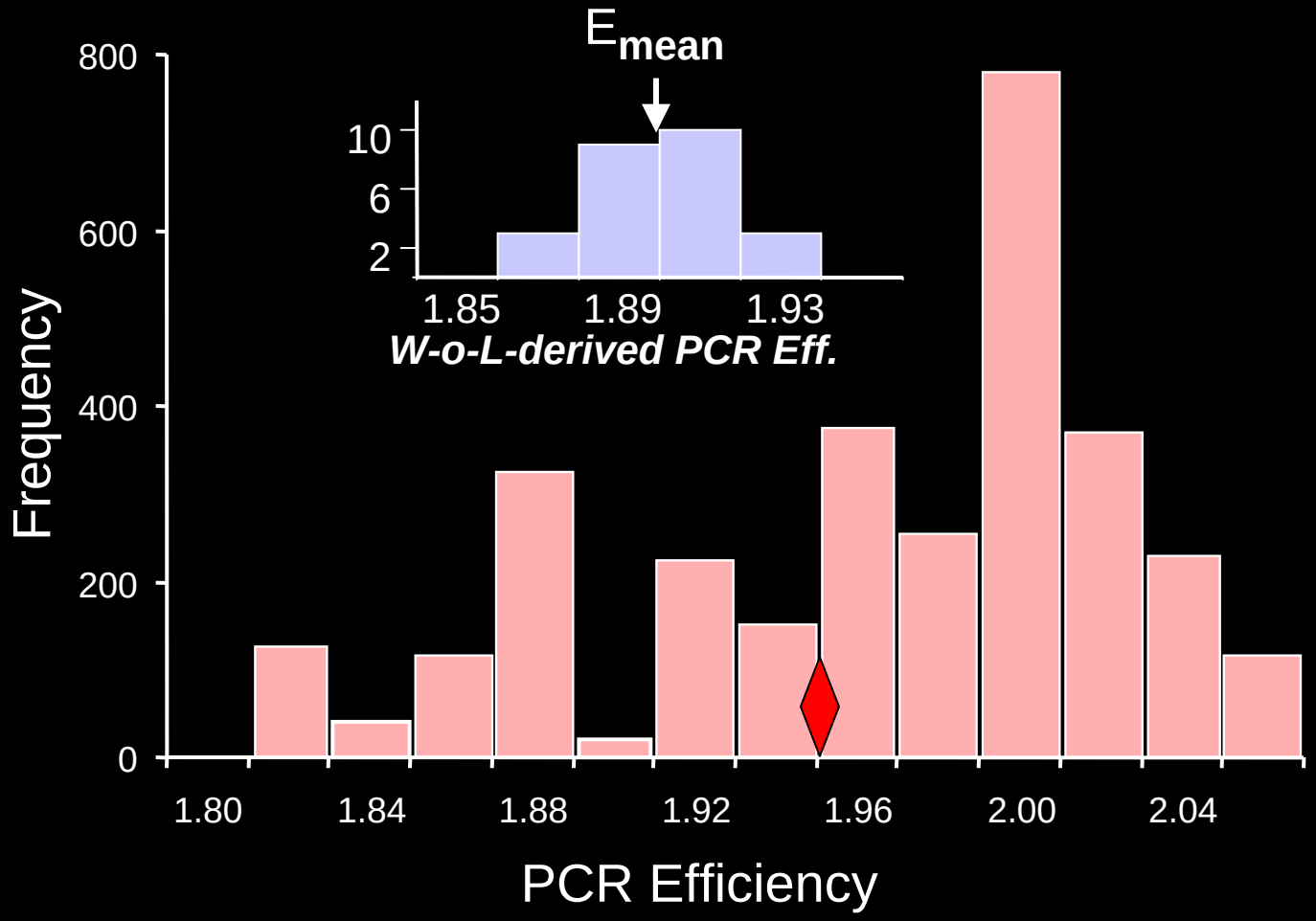
Efficiencies derived from Standard Curve



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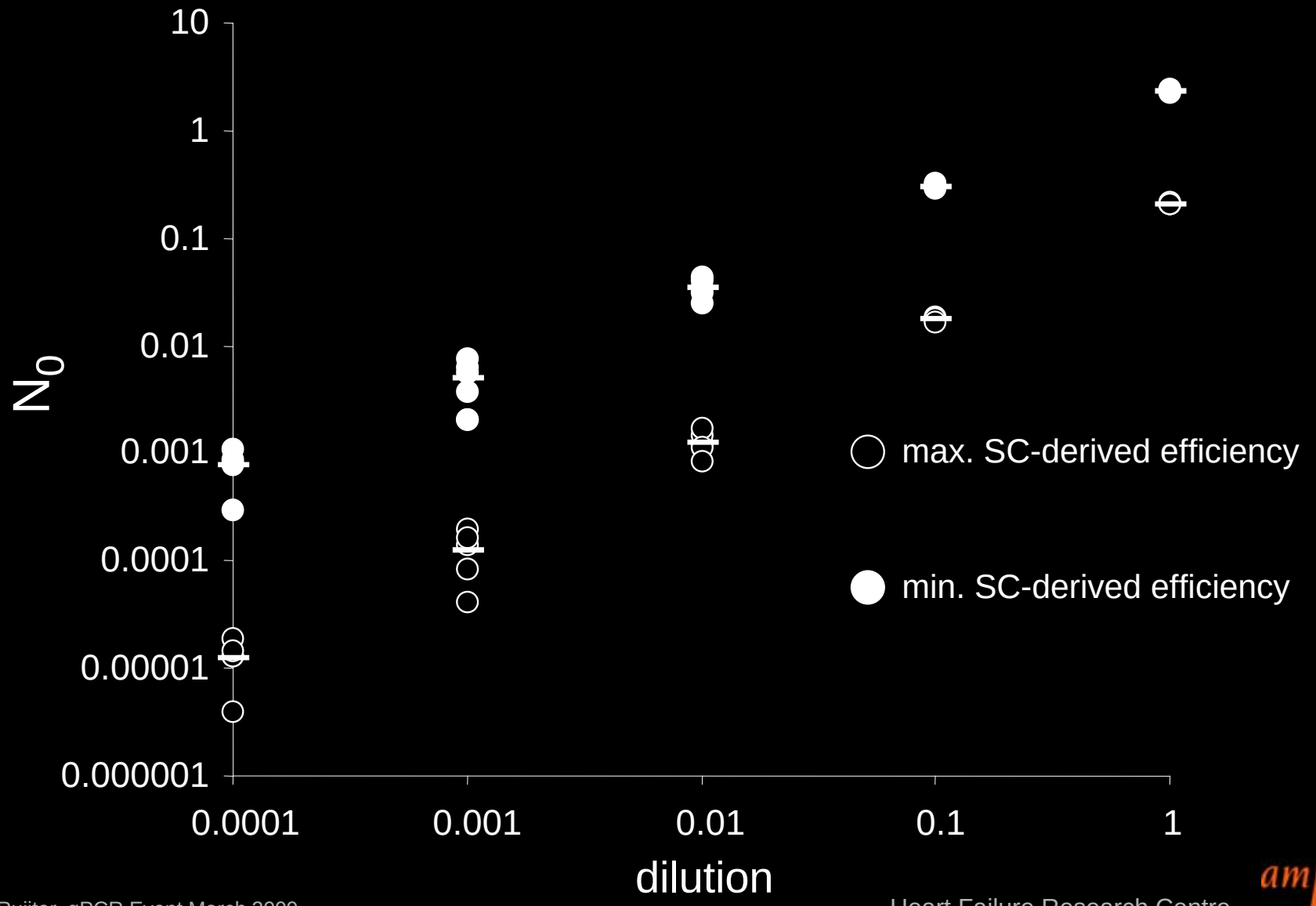
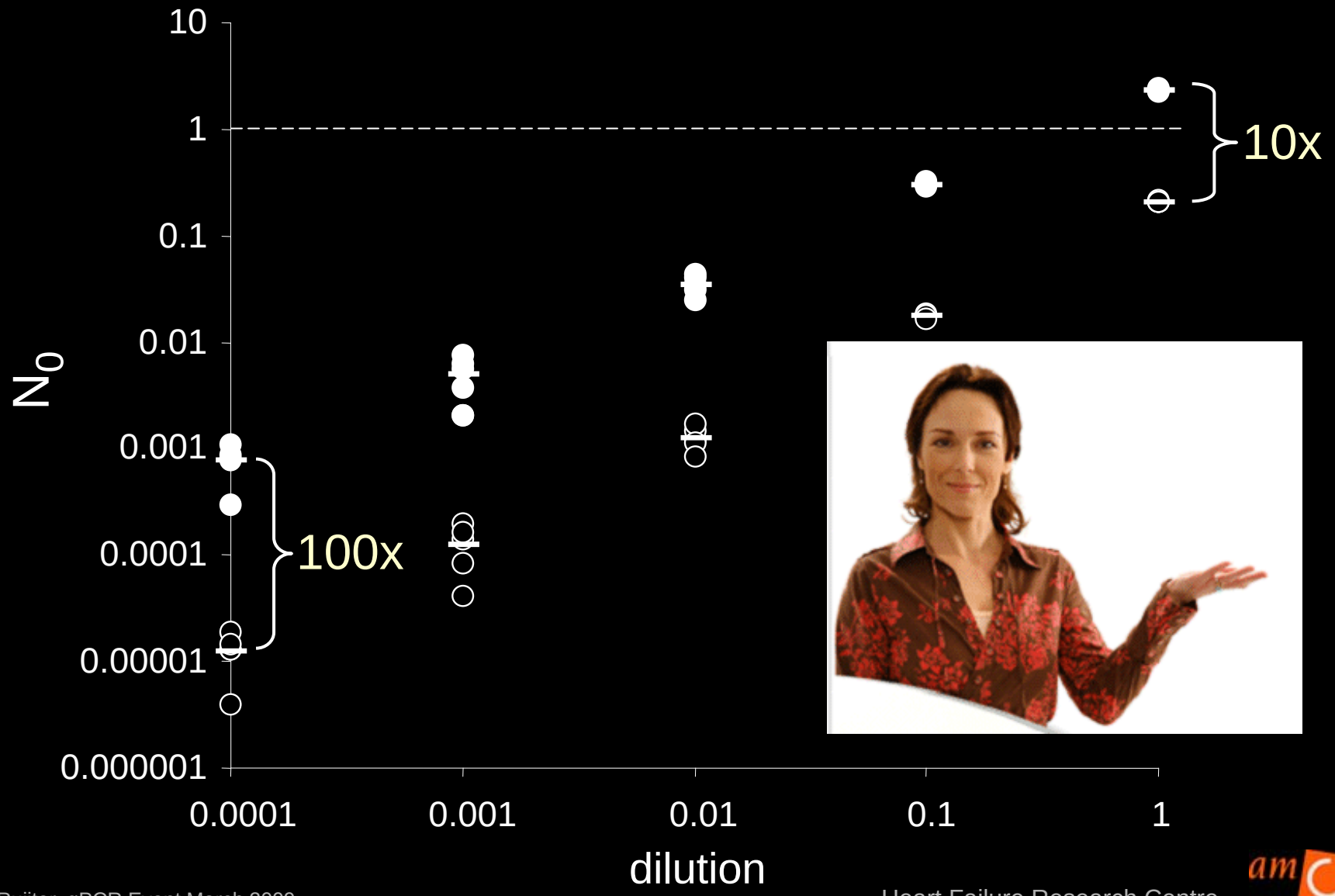
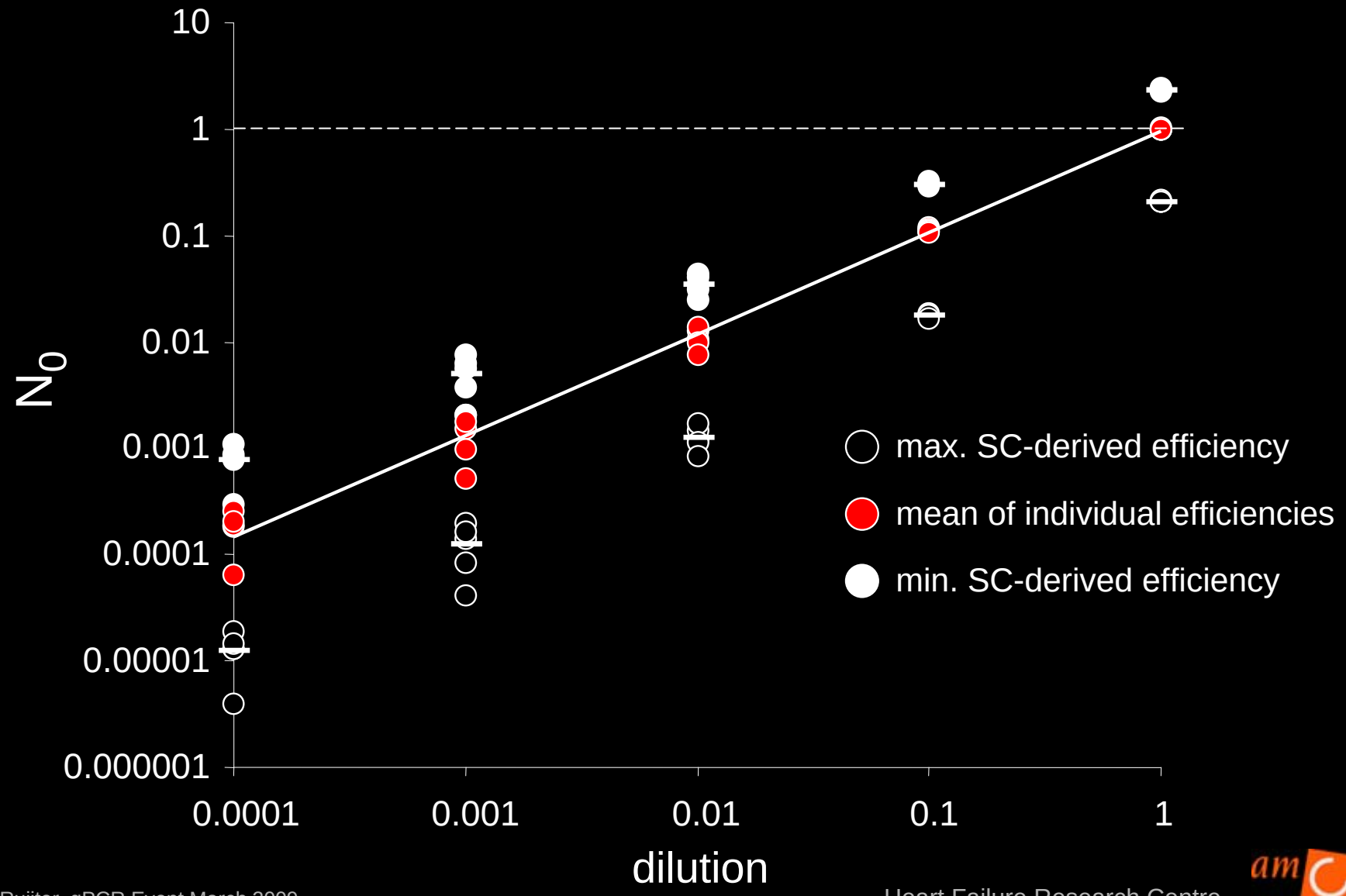
Bias from using the Wrong Efficiency



Bias from using the Wrong Efficiency



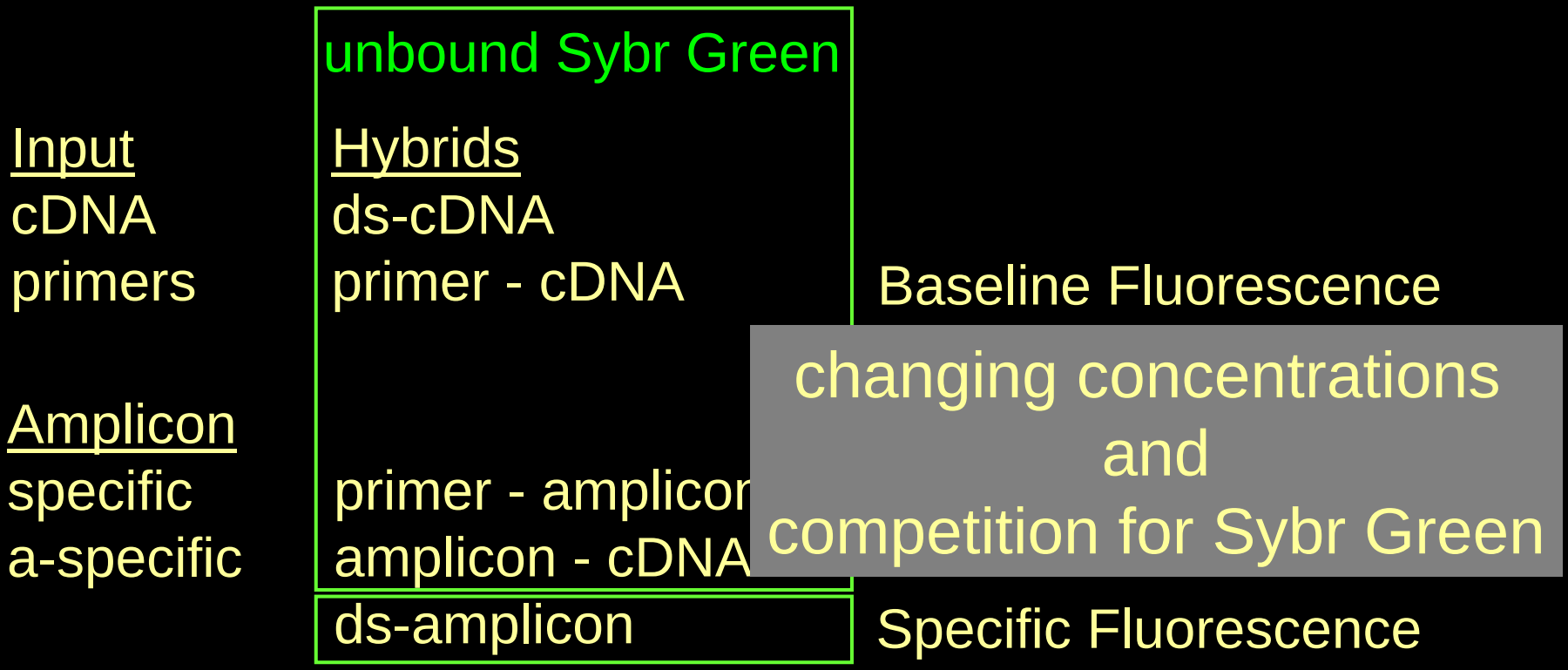
Unbiased N_0 with Mean Efficiency



Baseline Model ?

Input	Hybridisation	Amplification	
cDNA	ds-cDNA		
primers	primer - cDNA	specific amplicon a-specific	
Amplicon			
specific	primer - amplicon	specific amplicon	(expon)
a-specific	amplicon - cDNA ds-amplicon	a-specific	(linear)

Baseline Model ?



Fluorescence values in first cycles

cannot

be used to estimate the baseline