

## Customized Protocol Introduction

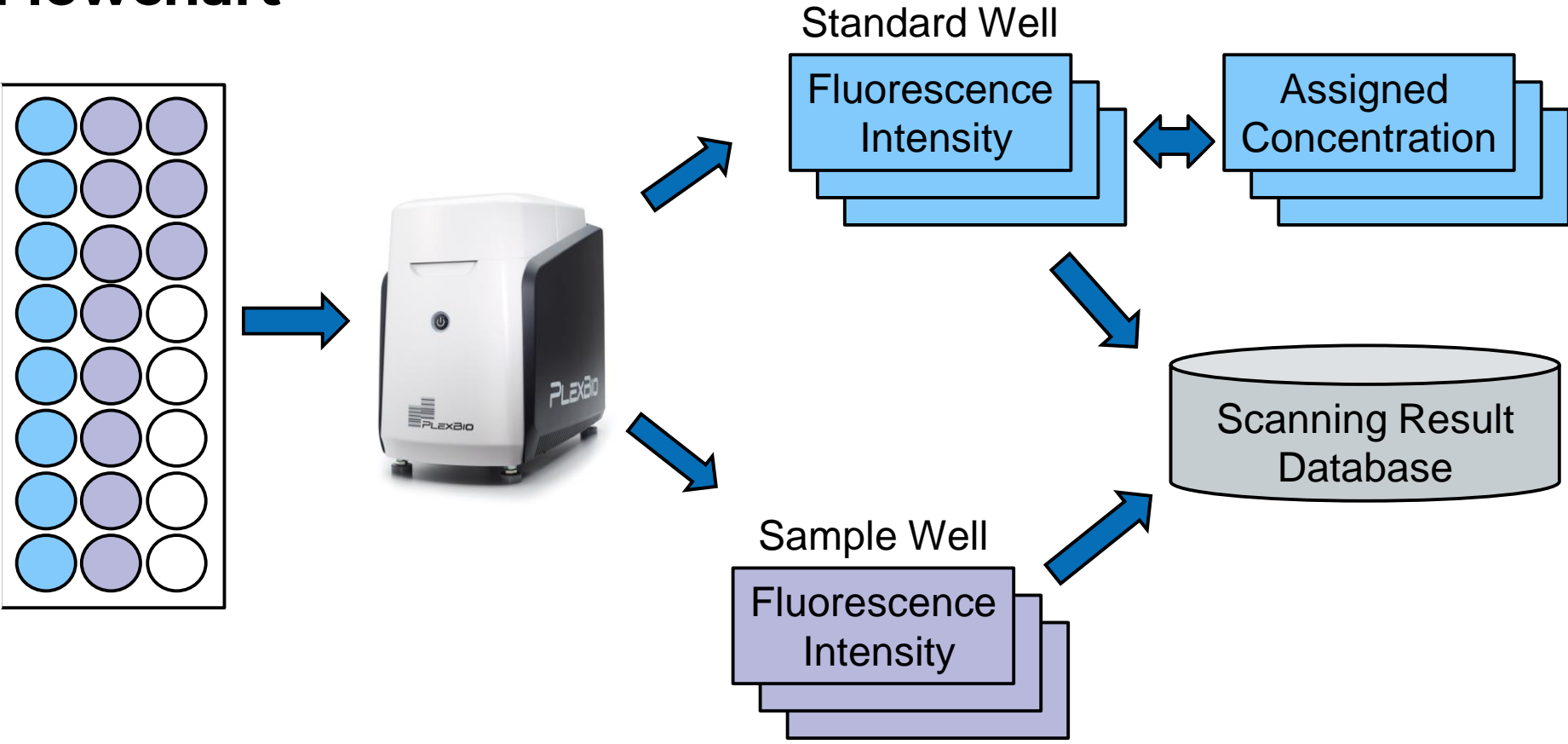
*Software version 1.1.8.1429*

*For RUO license type only*

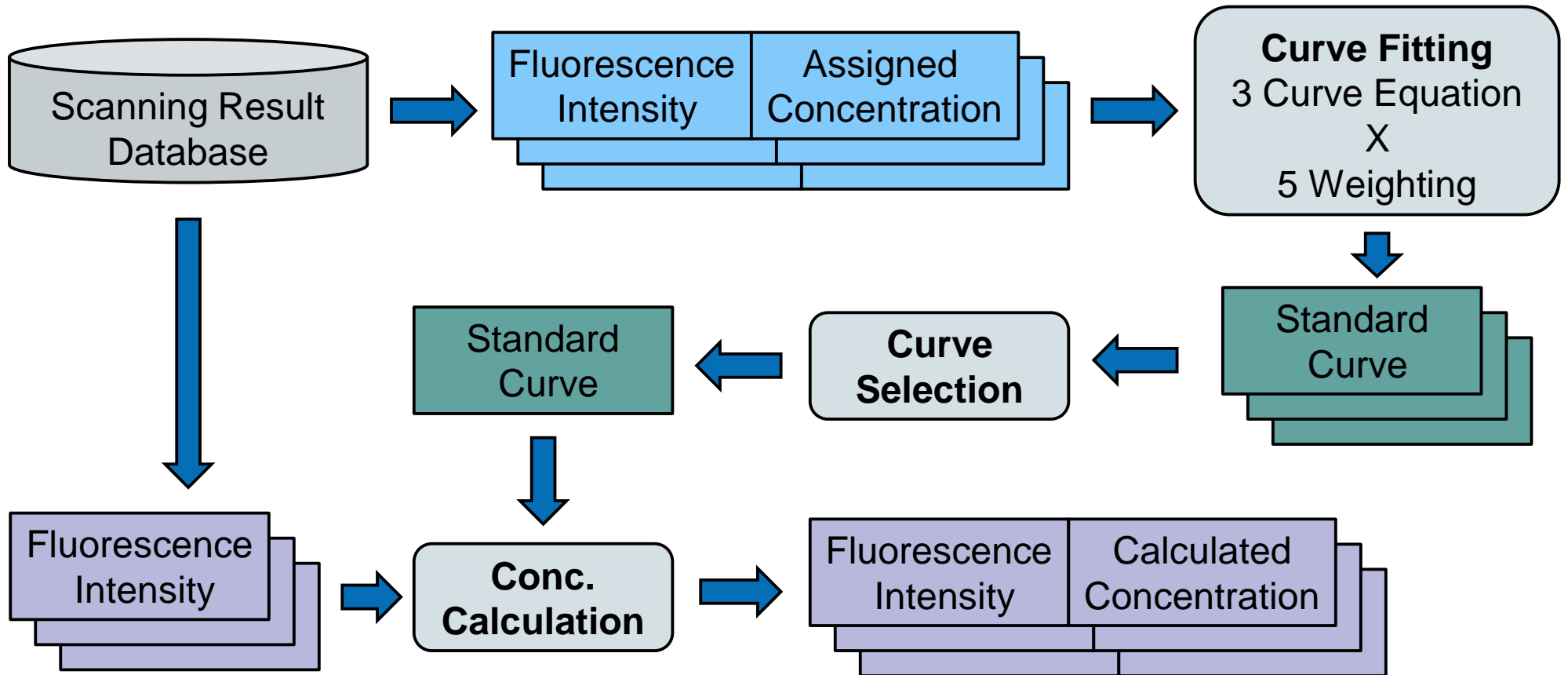
*For quantitative assays only*



## Flowchart



## Flowchart



## Curve Fitting: 3 Curve Equations X 5 Weighting

*Non-linear 4PL*

$$F(x) = b + \frac{a - b}{1 + \left(\frac{x}{c}\right)^d}$$

*Non-linear 5PL*

$$F(x) = b + \frac{a - b}{\left(1 + \left(\frac{x}{c}\right)^d\right)^e}$$

*Non-linear 5PL Ver.2*

$$F(x) = a + \frac{b}{\left(1 + \left(\frac{x}{c}\right)^d\right)^e}$$

**X**

*wSSE*

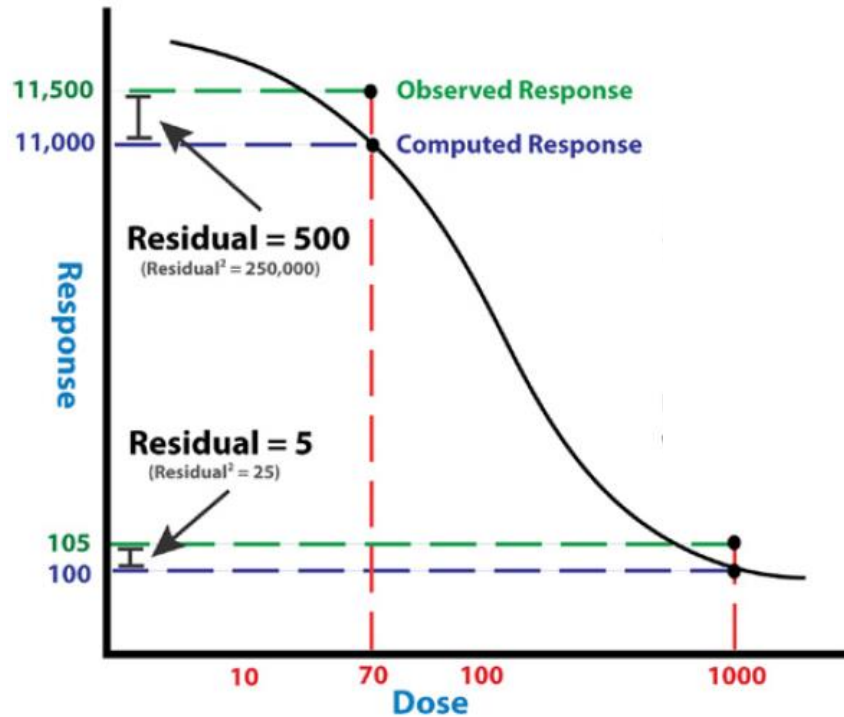
*(weighted sum of squared errors)*

$$wSSE = \sum_{i=1}^N w_i (y_i - \hat{y}_i)^2$$

$$w_i = 1, \frac{1}{y_i}, \frac{1}{y_i^2}, \frac{1}{x_i}, \frac{1}{x_i^2}$$

**Note:** DeXipher software will determine and select the best equation, but it can also be chosen by the user.

## Evaluation of curve fitting: Residue



$$\text{Residual}^2 = (y_i - \hat{y}_i)^2$$

$$SSE = \sum_{i=1}^N (y_i - \hat{y}_i)^2$$

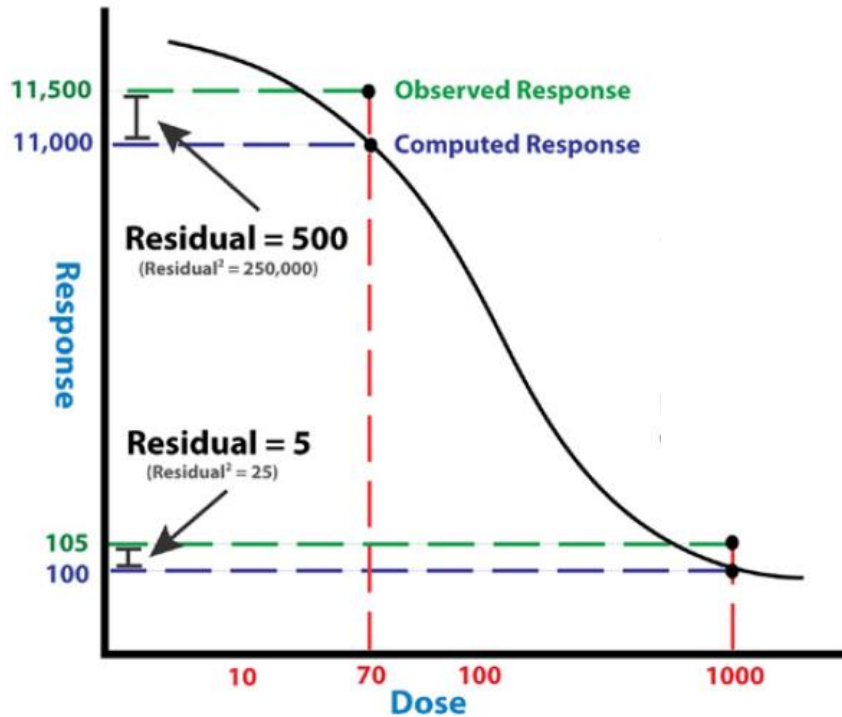
$\hat{y}_i$  = Computed Response at point  $i$

$y_i$  = Observed Response at point  $i$

$N$  = Total Amount of Standard

$SSE$  = sum of squared errors

## Bias of Residue scale



$$Residual^2 = (y_i - \hat{y}_i)^2$$

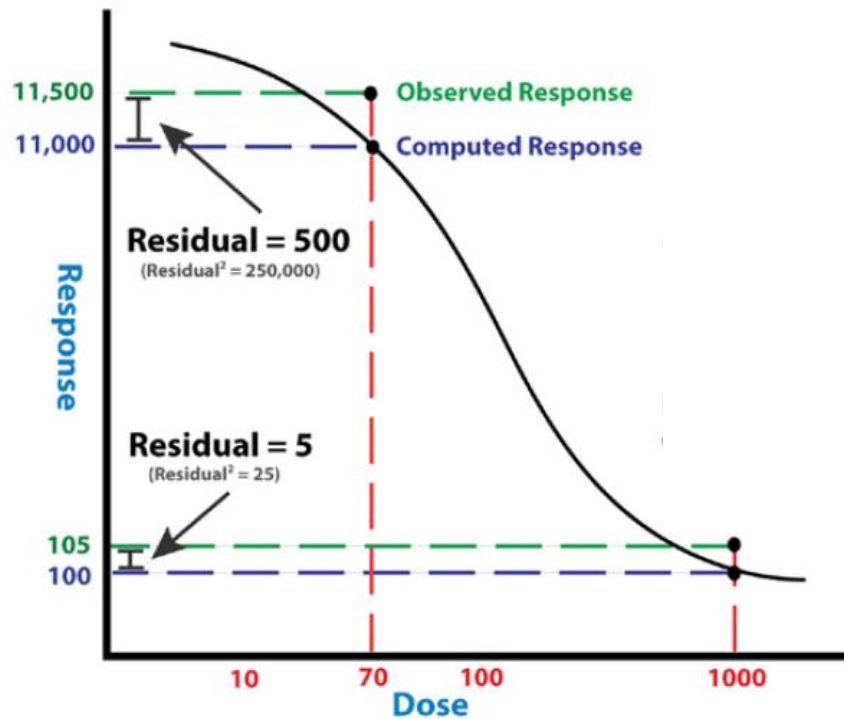
$$Residual_1^2 = (y_1 - \hat{y}_1)^2 \\ = (11500 - 11000)^2 = 250000$$

$$Residual_2^2 = (y_2 - \hat{y}_2)^2 \\ = (105 - 100)^2 = 25$$

10000 X

Larger Y results in larger residue

## Weighted Squared Errors



$$\text{Weighted Residual}^2 = w_i(y_i - \hat{y}_i)^2$$

$$w_i = 1, \frac{1}{y_i}, \frac{1}{y_i^2}, \frac{1}{x_i}, \frac{1}{x_i^2}$$

Here we use  $\frac{1}{y_i^2}$  as  $W_i$ ,

$$\begin{aligned} \text{Weighted Residual}_1^2 &= w_1(y_1 - \hat{y}_1)^2 \\ &= \frac{1}{y_1^2} (y_1 - \hat{y}_1)^2 \\ &= \frac{1}{11500^2} \times (11500 - 11000)^2 = \mathbf{0.0019} \end{aligned}$$

$$\begin{aligned} \text{Weighted Residual}_2^2 &= w_2(y_2 - \hat{y}_2)^2 \\ &= \frac{1}{y_2^2} (y_2 - \hat{y}_2)^2 = \frac{1}{105^2} \times (105 - 100)^2 = \mathbf{0.83 X} \\ &= \mathbf{0.0023} \end{aligned}$$

## Best Curve Selection (Ranking of curves)

$$\begin{aligned} \text{rank}_i &= e_i, & \text{if } e_i \leq c \\ &= e_i + P, & \text{if } e_i > c \\ &= 10P, & \text{if } S_i = \text{NaN (Not a Number)} \end{aligned}$$

$c = 30\%$  (Threshold)  
 $p = 5$  (Penalty)  
 $d_i =$  Experimental MFI at point  $i$   
 $s_i =$  Curve solved MFI at point  $i$   
 $e_i = |(d_i - s_i) / d_i|$

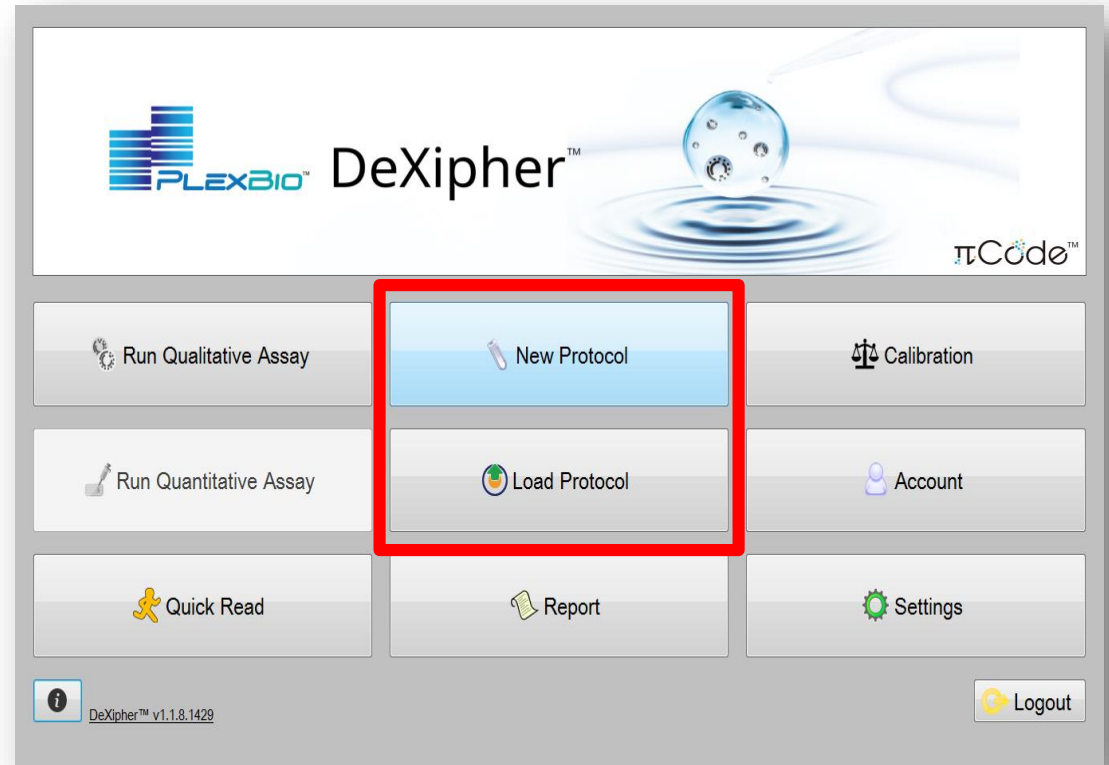
$$\text{Rank} = \sum \text{rank}_i$$

**Best curve: Minimal Rank of 15 curves (3 formula X 5 weighting methods)**



**New Protocol** is for generating and testing new quantitative assays (*Please refer to **A-9\_Creating a New Protocol***)

**Load Protocol** is for use and/or modification of previously created protocols (*Please refer to **A-10\_Loading a Protocol***)



Thank You

**PlexBio**

6F-1, No. 351, Yangguang St.  
Neihu District, Taipei City 11491, Taiwan  
+866-2-2627-5878 | PlexBio.com  
service@plexbio.com