# Surface Coating and Cytoplasm-to-Nucleus Translocation

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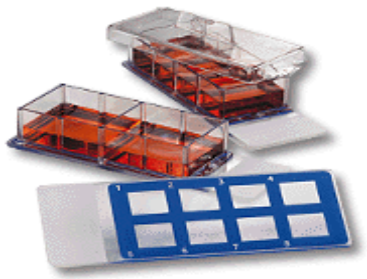
Goal: To understand how different surface treatments affect measures of Cytoplasm-to-Nucleus Translocation assay (CNT).

The study was performed on untreated MCF7 cells grown in chamber slides:

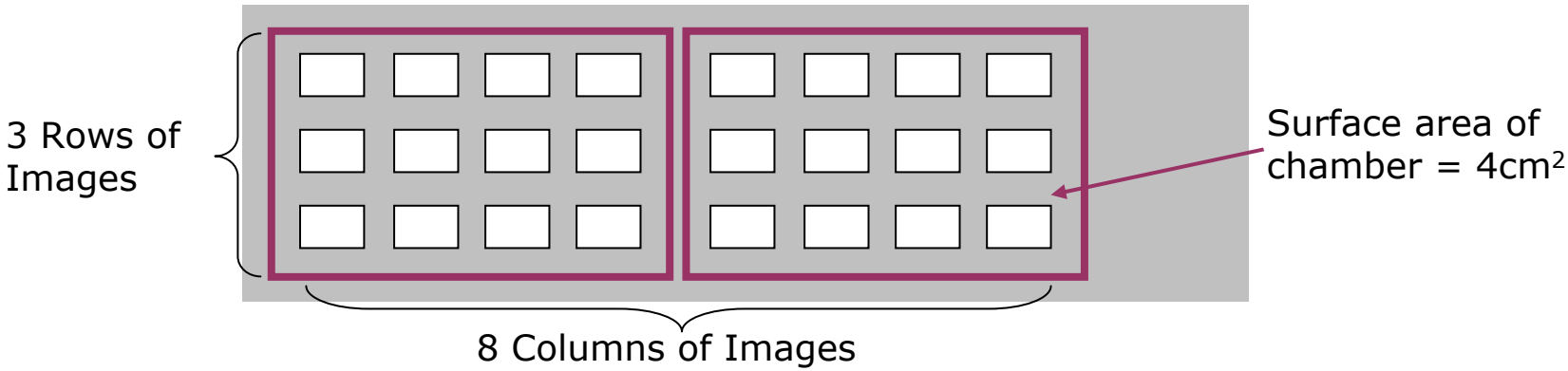
www.bdbiosciences.com/ptProduct.jsp?backLink=ptProductList.jsp&backName=Product%20List&prodId=364840.

Chamber slides were chosen because they provide a choice of surface treatments on a flat surface, eliminating possible effects of non-flat bottom of microplates on CNT measures.



Coating: Collagen I, Fibronectin, Poly-D-Lysine

# Scan Fields and Surface Treatments. Analysis of Variance.




Images were acquired on the CellCard reader with Nikon 4X PlanApochromat 0.2NA objective and Retiga EX camera (resolution 1360*1024 pixels). Pixel size in image plane = 1.62µm; frame size = 2.20mm*1.66mm. Number of images = 24.

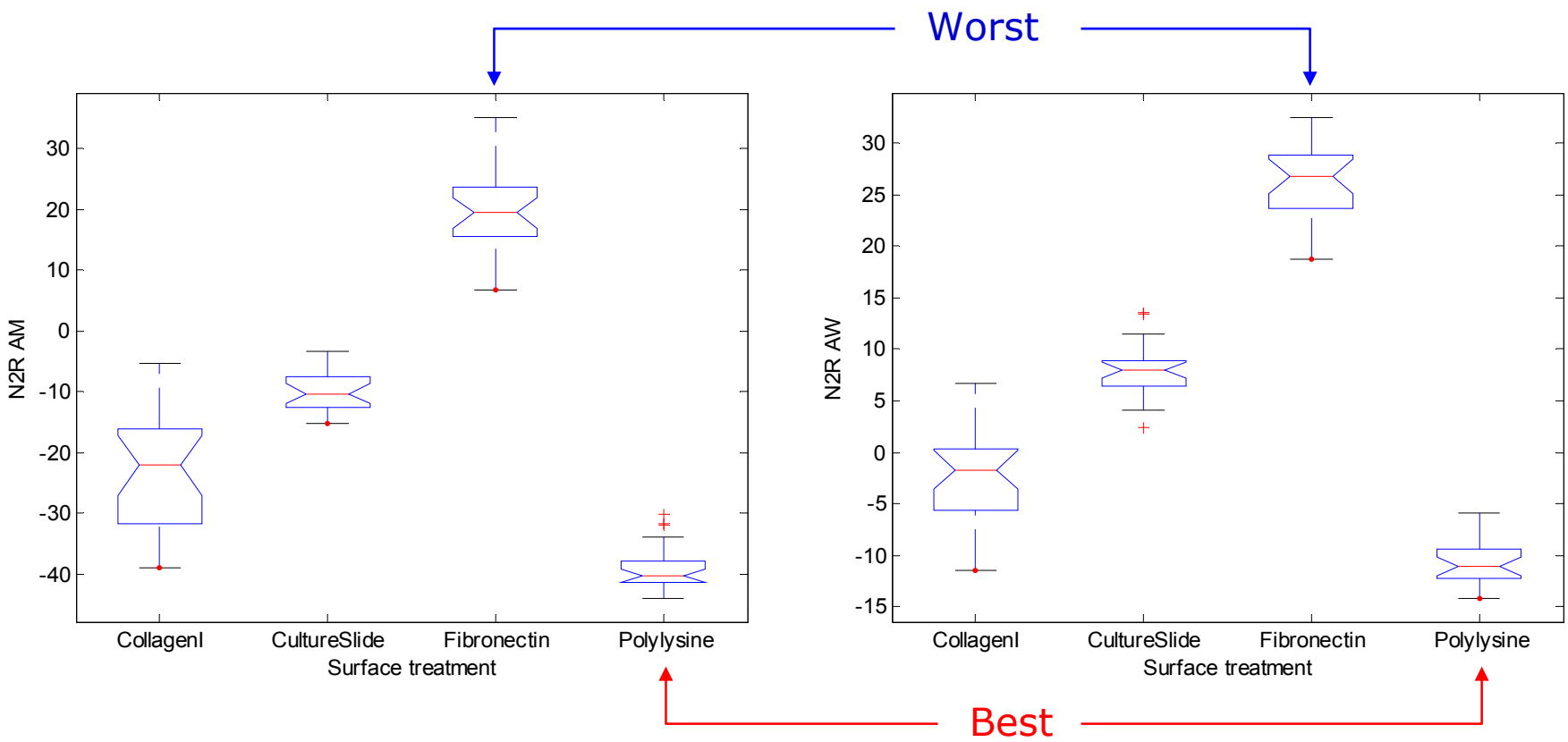
ANOVA

| | | | Slope1 | | | Slope3 | | | N2R_AW | | | N2R_AM | | | N_AM | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DF | Mean square | Fisher's F | Pr > F | Mean square | Fisher's F | Pr > F | Mean square | Fisher's F | Pr > F | Mean square | Fisher's F | Pr > F | Mean square | Fisher's F | Pr > F |
| Source of variation | Surface treatment | 3 | 3602.0 | 117.4 | < 0.0001 | 6184.1 | 115.7 | < 0.0001 | 6028.1 | 493.0 | < 0.0001 | 14961.4 | 378.3 | < 0.0001 | 5044.0 | 276.6 | < 0.0001 |
| | Row | 2 | 59.7 | 1.9 | 0.149 | 93.1 | 1.7 | 0.182 | 8.3 | 0.7 | 0.511 | 81.4 | 2.1 | 0.134 | 28.2 | 1.5 | 0.219 |
| | Column | 7 | 51.6 | 1.7 | 0.124 | 87.2 | 1.6 | 0.138 | 14.3 | 1.2 | 0.330 | 58.3 | 1.5 | 0.188 | 14.4 | 0.8 | 0.596 |

Analysis of variance was performed for five CNT measures: Slope1, Slope3, N2R_AW, N2R_AM, and N_AM using surface treatment, image row and image column as factors. For all measures surface treatment is a significant factor and image position is not.

# CNT at Different Surface Treatments. Ring-based Measures.

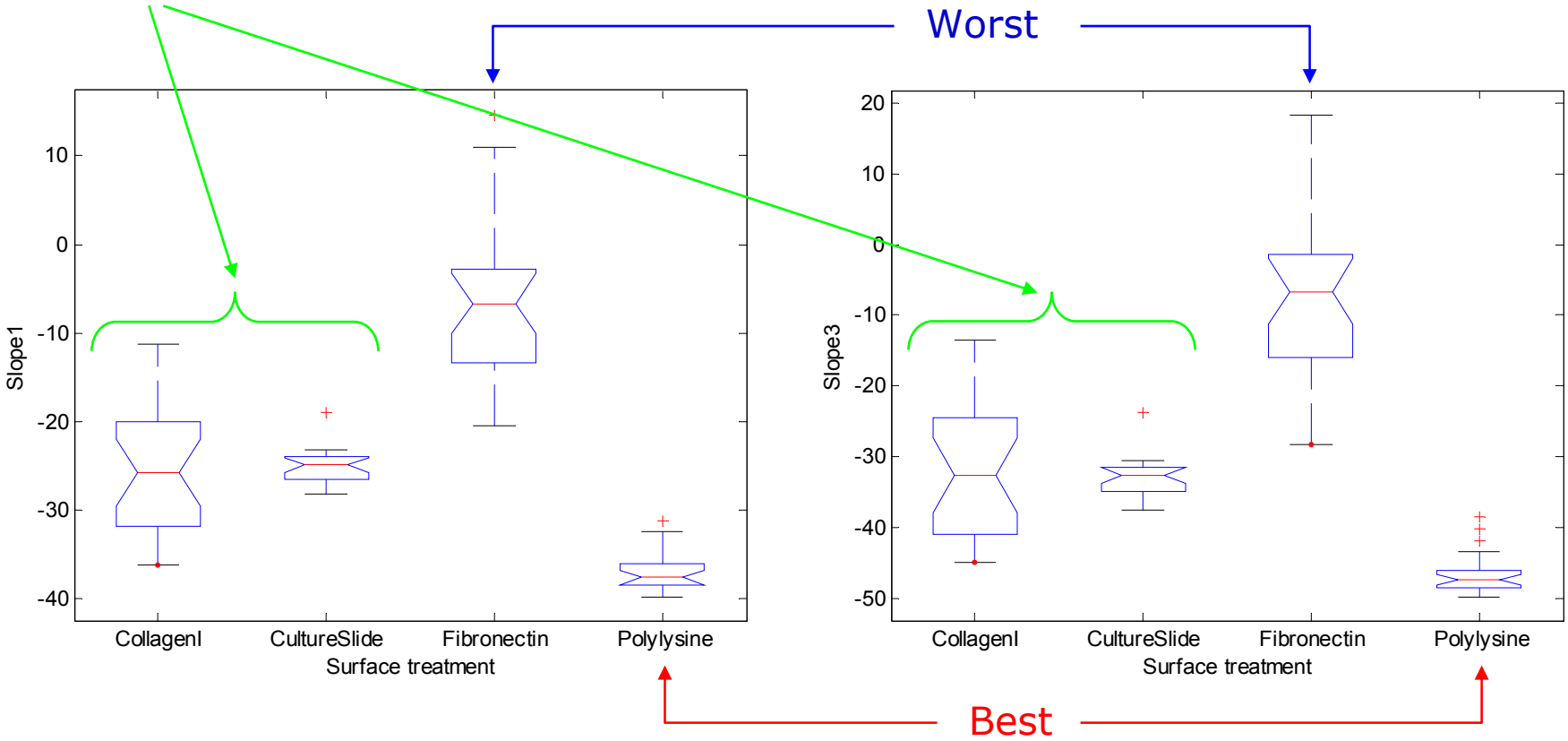
The desired result for nontreated cells is to obtain a measure as negative as possible and with as low variation as possible.



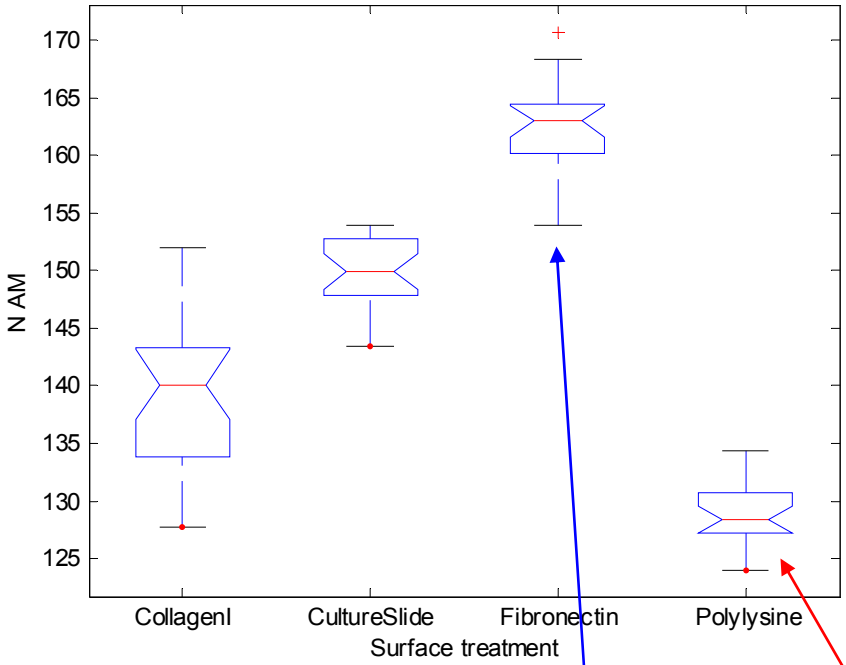

Each sample consists of 24 values corresponding to the 24 images acquired on the chamber slide.

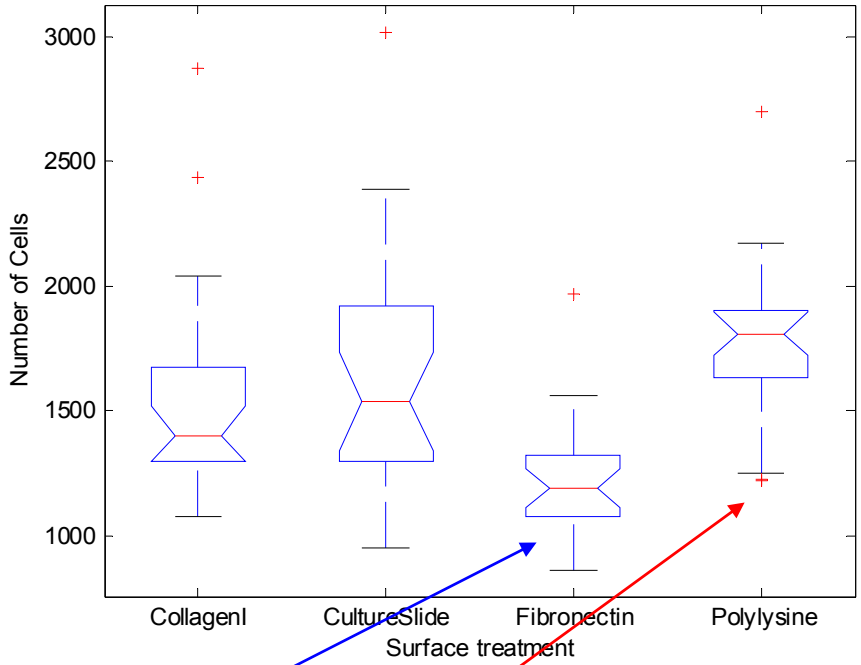
# CNT at Different Surface Treatments. Slope-based Measures.

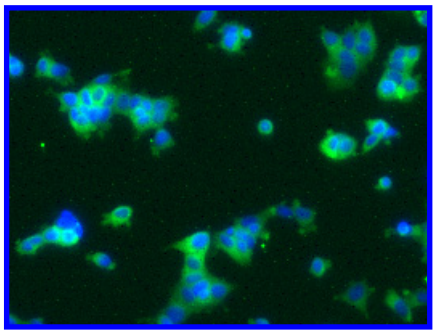
For slope-based measures the untreated CultureSlide gives the same average values as CollagenI, but has much less variation among scan fields.



# CNT at Different Surface Treatments. Signal Stain in the Nucleus and Number of Cells.



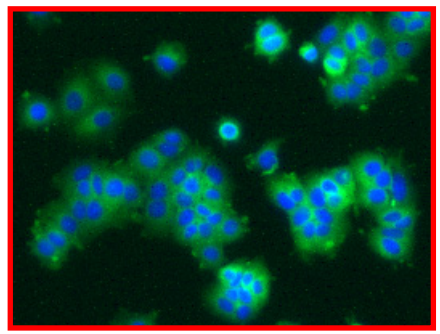
Fibronectin

Greatest amount of signal in the nucleus points to thicker cells

Smallest number of cells points to the worst attachment

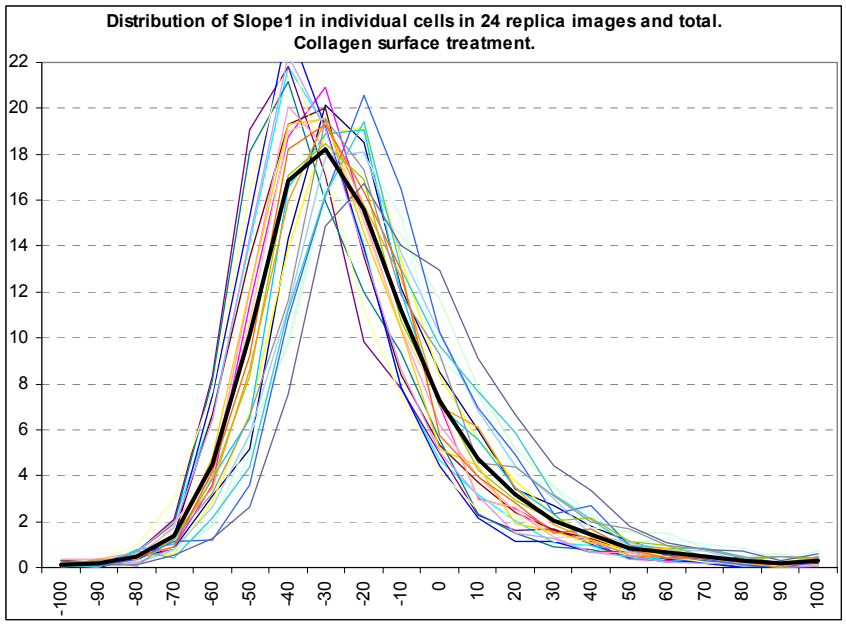
Greatest number of cells points to the best attachment

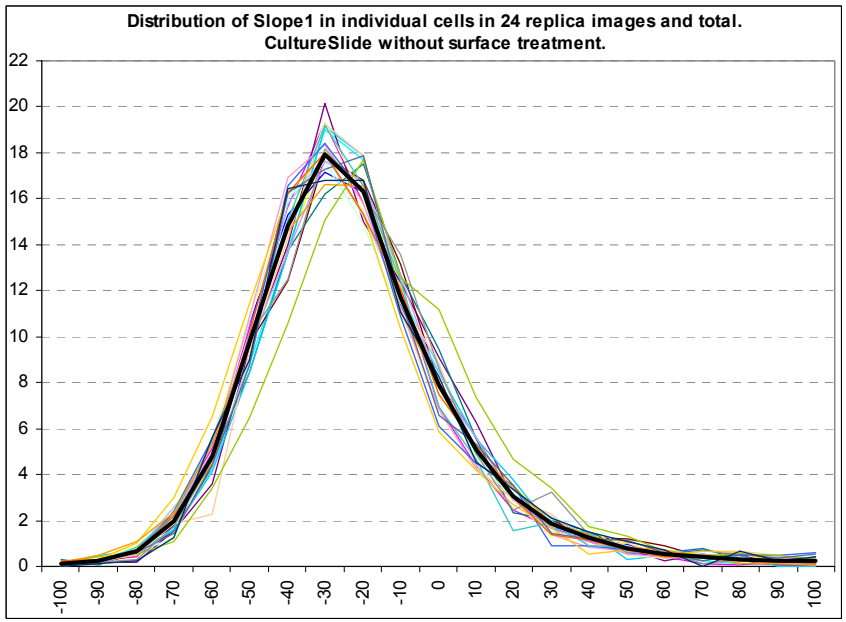
Smallest amount of signal in the nucleus points to flatter cells

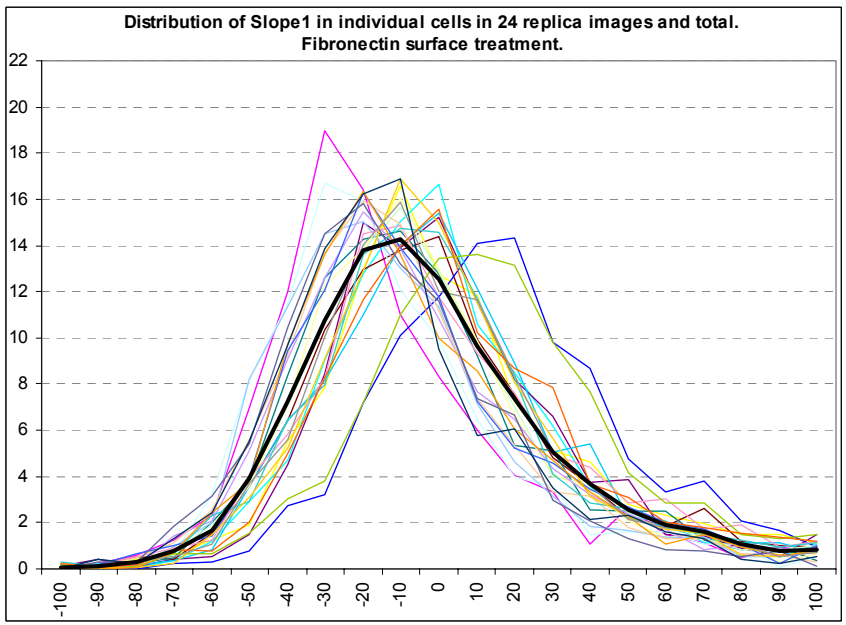


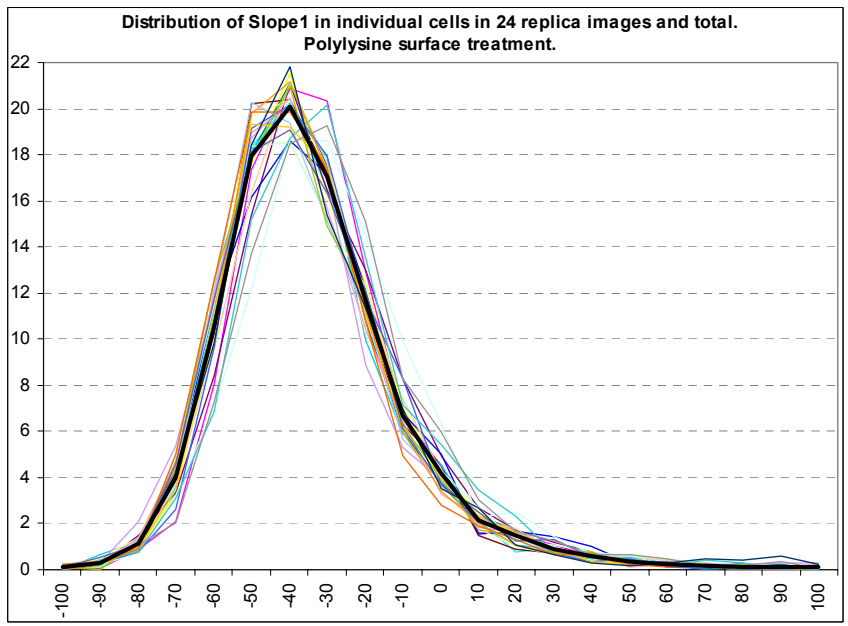
Polylysine

# Distributions of Cells by Slope Value in Individual Fields

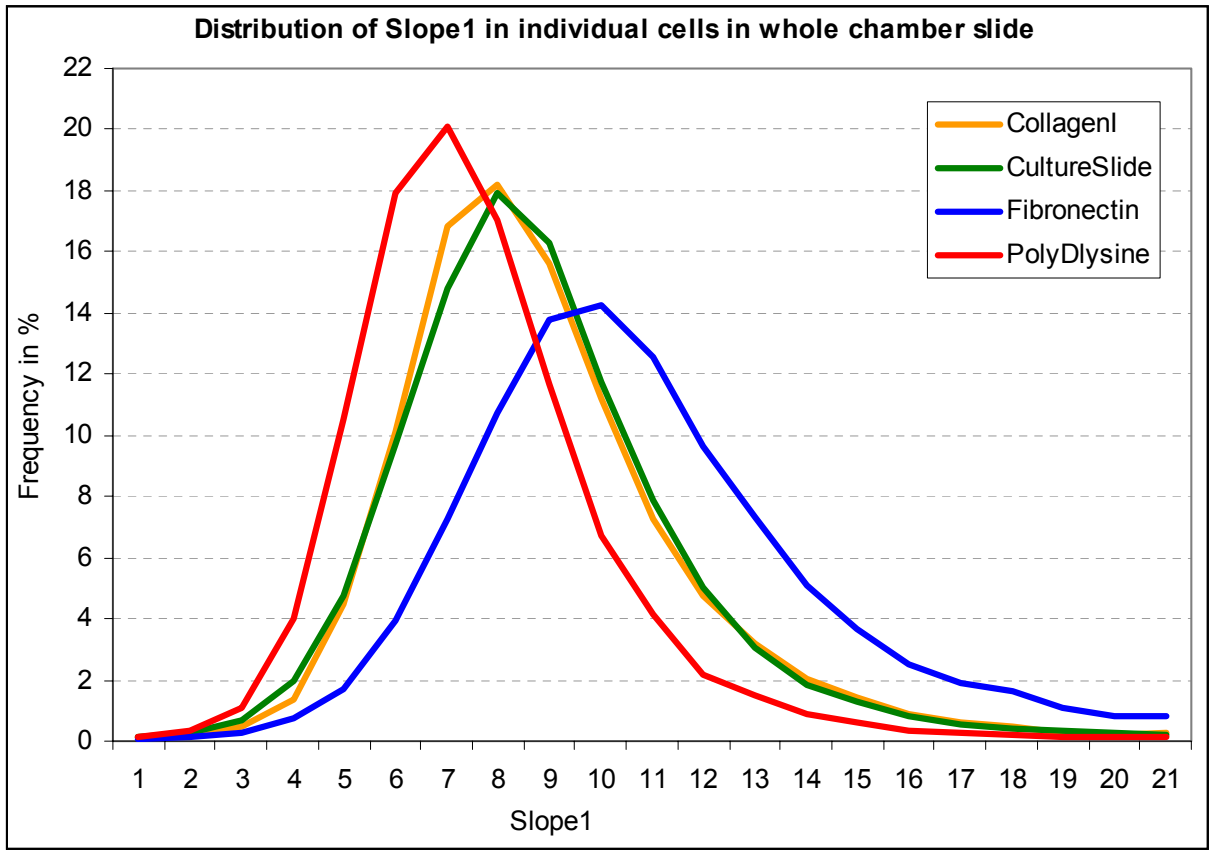


# Distributions of Cells by Slope Value in the whole Chamber Slide. Conclusions on Surface Treatment.




1. Polylysine gives the tightest histograms within fields and the closest histograms among fields
2. Fibronectin gives the widest histograms within fields and the greatest variation among fields
3. Individual histograms are tighter for Collagen, but fields are more similar for CultureSlide

# General Conclusions

1. Surface treatments significantly affect CNT measures. Some treatments may produce the same average effect, but have different variation.
2. Histograms in all images are unimodal and similar in shape but may shift along X-coordinate with change of image position on the slide. This indicates a slow change in surface properties along the slide.
3. Increasing area may not improve the assay estimate (measured e.g. by standard error of mean) if there is a drift in surface conditions among imaged areas.
4. Ranking of surface treatments depends on the algorithm used to analyze the images (e.g., for ring measures Collagen is better than CultureSlide, but for slope measures CultureSlide is better than Collagen)
5. Fibronectin is uniformly the worst treatment for CNT, but it is the best treatment for cell count, as it gives the smallest variation.

Qualification: Results may be different for different cell types.