# Intrapatient and Interpatient Heterogeneity Assessment of Adhesion Dynamics of RBCs in Patients with Sickle Cell Disease


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## Abstract


Sickle Cell Disease (SCD) is an inherited blood disorder affecting more than 14 million genetically predisposed individuals worldwide. One of the particularly painful aspects of SCD is the blockage of microvasculature caused by the aggregation of misshapen sickle red blood cells (RBCs), which have heightened adhesive interactions with inflamed endothelium. Hence, the key to tackling SCD lies in understanding adhesive dynamics on endothelial walls.

In this work, we have developed a methodology for studying the biophysics of cell adhesion using high-throughput detachment experiments integrated with kinetic modeling. This methodology is then applied to analyze SCD adhesion dynamics heterogeneity at intrapatient and interpatient scales using Artificial Intelligence-based image analysis algorithms. Assessment of kinetic parameter values for different SCD patient samples is shown to collapse into a universal curve that is unique to the protein-ligand pair and has not been previously observed. The methodology developed in this study for analyzing adhesion characteristics is a novel approach and can easily be transferred for studying other protein-ligand interaction systems at a coarser single-cell scale.


## Experimental Setup

A microfluidic RBC adhesion assay was designed that recreated the vascular bed environment in vitro. Blood samples of 23 SCD patients were flowed through micro-channels functionalized with laminin (Ln) and real-time videos of cell detachment at a set of ramped flow conditions were obtained that captured the dynamics of protein receptor-ligand interactions at a single-cell scale. A schematic of experimental setup is shown in Figure 1. Shear velocities began from 5 μL.min$^{-1}$ and were ramped at 2.5, 3.0, 3.5, 4.5 μL.min$^{-2}$ for each sample trial. These velocities were then converted to dynamic force values using velocity profile given by the Navier-Stokes equation solution given in [1].

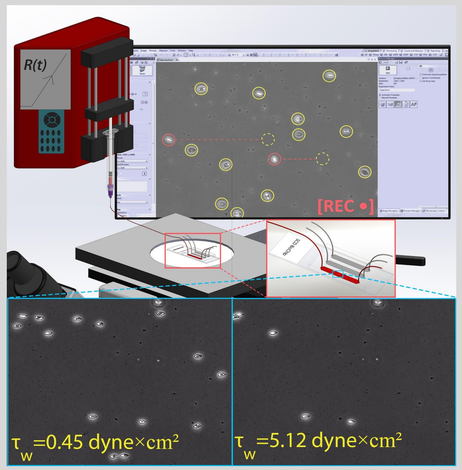


Figure 1: Schematic of the experimental setup with real video frames as recorded by an optical microscope camera. Enlarged view of the microfluidic channel is shown at two different time stamps.

## Kinetic Model

Mean adhesion lifetime of a single-molecule protein-ligand system with a single-dominant minimum energy state under a constant force is given by Bell Model (Equation 1). [2]

To account for single-cell level detachment of sickle RBCs, an extension of the Bell Model is developed. A schematic chain-reaction diagram of the model is shown in Figure 2. By solving the master equation, a Mean First Passage Time (MFTP) of reaching N=0 is analytically obtained (Equation 2), which is equivalent to mean adhesion lifetime of the cell.

**TABLE 1: Definitions of Symbols Appearing in the Model**

| | |
|---|---|
| Spontaneous dissociation rate | $k_s$ |
| Stress free bond length | $x_0$ |
| Constant Force | F |
| Thermodynamic beta | β |
| Average lifetime for a single molecule bond | $\tau_s$ |
| Spontaneous rebinding rate | $k^s_{on}$ |
| Number of protein-ligand clusters | N |
| Average lifetime for N molecule bond | $\tau^N_s$ |

$$\tau_s = \frac{1}{k_s} e^{-\beta F x_0} \quad (1)$$

$$\tau_s^N = \frac{1}{k_s} \times \frac{(N-1)!}{N} \times \left(\frac{k_{on}^s}{k_s}\right)^{N-1} \times \mathrm{Exp}\left[-\beta F x_0 \sum_{i=1}^{N} \frac{1}{i}\right] \quad (2)$$

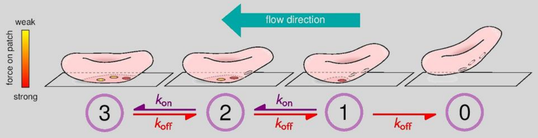


Figure 2: Schematic diagram of multiple-bond kinetic model with N=3.

## Interpatient Heterogeneity

### Cell Identification and Detachment Tracking

Ln-adhered sickle cells are identified and segmented using a background-dependent thresholding of the intensity gradient magnitude. Identified cells are then 'tracked' until all initial bonds are broken using an algorithm shown in Figure 3. This gives the adhesion lifetime of each individual sickle RBC.

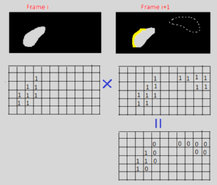


Figure 3: Visualization of our detachment tracking algorithm

### Model Parameter Estimation using Maximum Likelihood Analysis

To estimate model parameter values for each of the 23 patients, we performed an Maximum Likelihood Estimate (MLE) analysis with four free parameters: $x_0$, $k_s$, $k^{on}_s$, and N. Two different distributions, Gaussian and Exponential, were tested using Akaike information criterion (AIC) analysis and it was found that Exponential was preferred over Gaussian. Finally, model fit estimates were compared among patients to analyze the 'severity' of different patients.

## Deep Learning Implementation

Previous studies have found a relation between cell adhesion and dynamic deformability of cells. To study the impact of finer intrapatient deformability variation on adhesion characteristics, we implemented a complementary pair of deep-learning algorithms

### Improved Cell Segmentation using Pixel-wise Classification

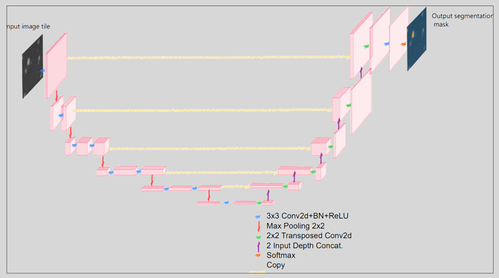


Figure 4: Network architecture for segmenting Ln-adhered sickle cells. Training set consists of pixel-labeled cell templates. The network follows an encoder-decoder structure to reconstruct the 32X32 segmented image

### Classifying Cell Deformability using Transfer Learning

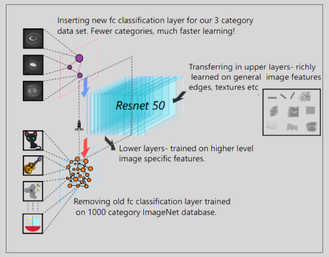


Figure 5: Network architecture for classifying cell templates into three classes - deformable cell, non-deformable cell, and non-adhered object. Network parameters are directly transferred from pretrained Resnet50,

## Intrapatient Heterogeneity

### Combined Network Performance and Separate Fit Estimates

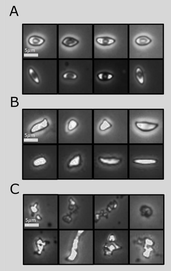


Figure 6: Instances of cells belonging to each of our 3 classes.

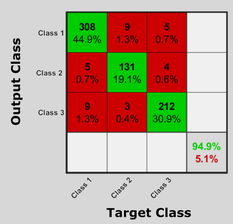


Figure 7: Confusion Matrix representing accuracy performance on the testing set of classification network

The trained deep learning networks when implemented together resulted in testing accuracy of 94.9%. Using the network, identified cells were divided into two classes – deformable and non-deformable. MLE analysis is then separately performed to obtain and compare the model fit values of patients with differences in cell deformability characteristic.

## Results and Discussion

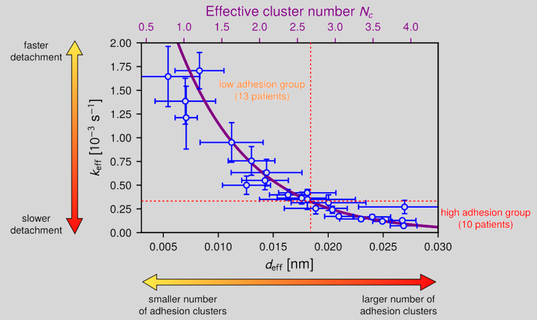


Figure 8. Sample wise Detachment Index Curve. The parameter values of each of the 23 samples are shown with error bars resulting from average of the 4 ramp values. Heat maps predict heightened 'stickiness' of sickle RBCs.

The result of model parameter value estimates from the MLE analysis is shown in Figure 8. As it can be seen from the figure, assessment of kinetic parameter values for different patient samples collapses onto a single effective Bell Model-like curve. This is a result that has not been previously observed. It is independent of the intrapatient deformability variation, as those values are averaged within the error bars of Figure 8, without affecting the curve behavior. However, interpatient heterogeneity still exists, and our model is able to predict patients with heightened adhesive dynamics or 'stickiness' of RBCs. We predict that these patients might be at relatively higher risk for Vaso-occlusive crisis events. The heterogeneity assessment techniques we have developed as part of this work are easily transferrable for studying other protein-ligand systems at a coarser single-cell scale. In future work, we want to test our kinetic map of single-molecule to single-cell adhesion dynamics in systems where range of model parameters are already known.

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