Dried Reagent Resuspension For Point Of Care Testing

Application to blood typing

Maxime HUET[1], Arnaud BUHOT[2], Myriam CUBIZOLLES[1] ; maxime.huet@cea.fr

1. Department of Biotechnology, CEA/Université Grenoble-Alpes, 17 avenue des Martyrs, 38054, Grenoble, France;
2. INAC-SPrAM UMR 5819 CEA CNRS/Université Grenoble-Alpes, 17 avenue des Martyrs, 38054, Grenoble, France.

Introduction:

A microfluidic component was designed to collect blood from a finger prick by capillary flow and to perform biological analysis [1,2,3]. It was used to perform ABO blood typing experiments in one step, the blood drop deposit, by agglutination of red blood cells (RBC) using embedded dried reagents. The present study is a first step in the modeling of the whole agglutination assay.

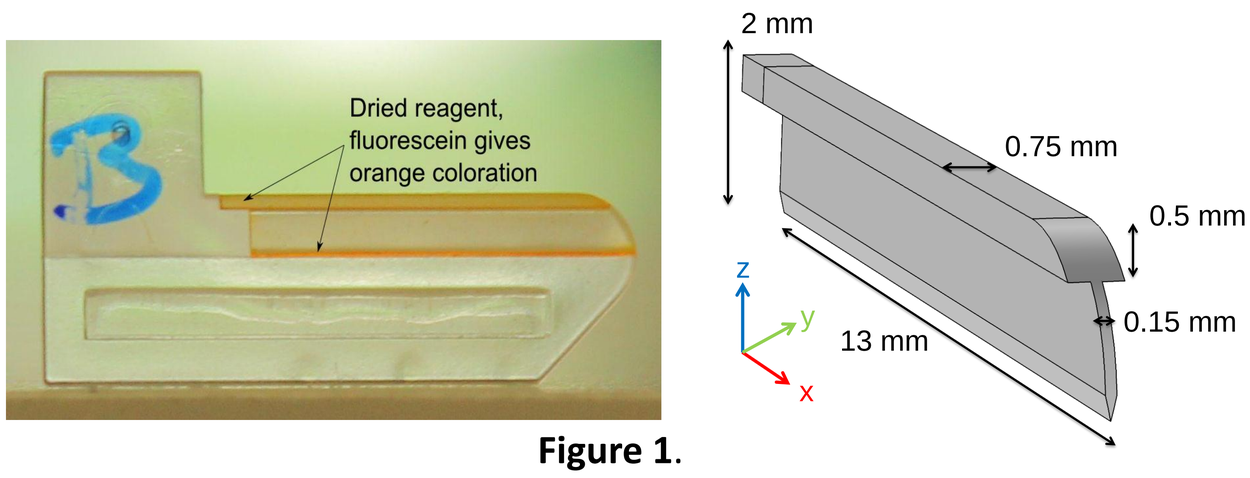


Figure 1.
Left: photo of the microfluidic component with dried reagent.
Right: geometry of the microchannel modeled with COMSOL.

Agglutination, experimental study:

Blood typing experiments were performed with 1:5 diluted blood using a dried reagent that triggers *in vitro* RBC agglutination. Fluorescein was added to the reagent to monitor the resuspension. An image sequence was recorded using a microscope. A video processing algorithm was developed to compute an agglutination indicator that allows successful discrimination between positive and negative agglutination reactions.

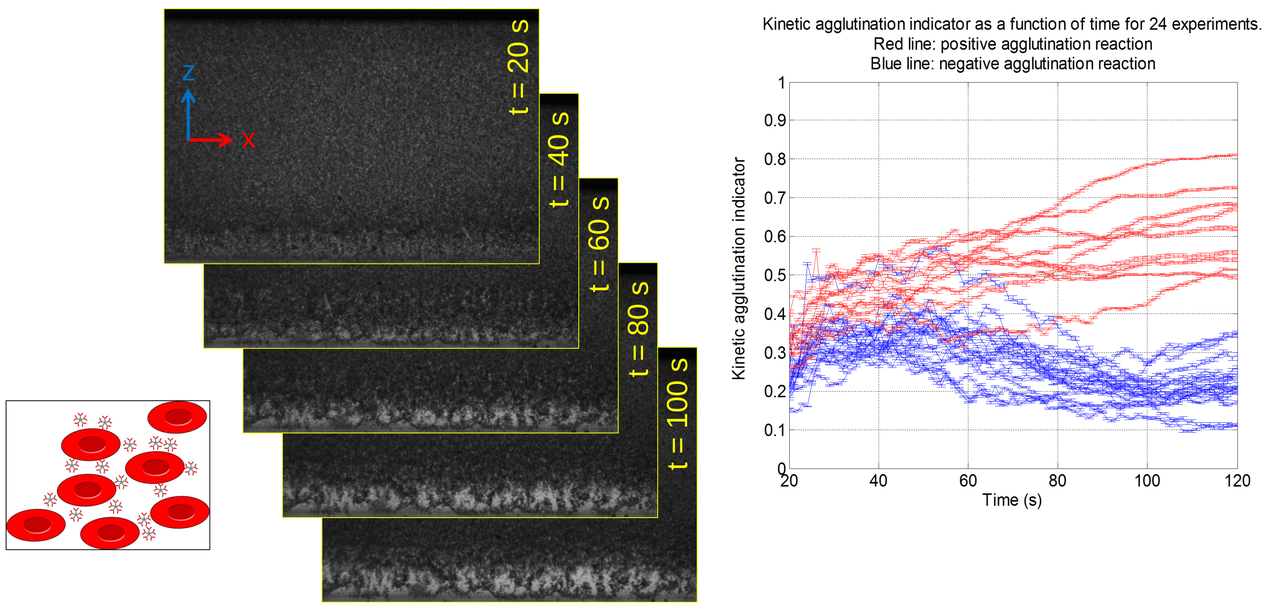
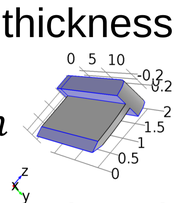
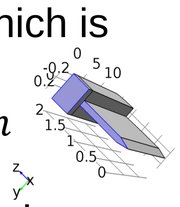


Figure 2. Left: illustration of red blood cells agglutination by antibody.
Middle: some of the images recorded using the microscope (5x objective). Right: agglutination indicator for 24 experiments.

Computational Methods:

Transport of diluted species is used for the diffusion of fluorescein with a (measured) diffusion coefficient of $3.10^{-9}\ m^2/s$. Initial condition is $0\ mol/m^3$ everywhere. The solubilization of dried reagent is modeled by an exponential decay flow : $\frac{\lambda.C_0}{\tau}.e^{\frac{-t}{\tau}}$ with $\tau = 640\ s$, adjusted to correlate the experimental observation, $C_0 = 5{,}3\ mol/m^3$, the concentration of fluorescein in the deposited solution that was dried, λ the thickness of the dry deposit, which is not the same for every surface, here $\lambda = 1\ mm$  and here $\lambda = 0.7\ mm$ 

A no flow condition is applied at the other faces. The temporal solver is run from $t = 0\ s$ to $t = 120\ s$, the typical duration of a biological analysis performed in this microfluidic component.

Results:

For reagent resuspension, human blood plasma was used. The resuspension was recorded with a RGB camera from which the blue channel was subtracted from the green channel. Figures 3 and 4 correspond to the experimental and the simulated results at $t = 100\ s$.

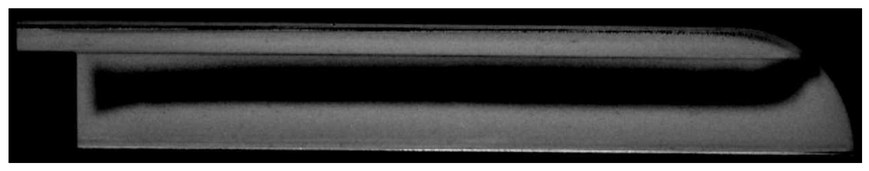


Figure 3. Experimental results at t = 100s

Figure 4. COMSOL results concentration at t = 100s, xz plane

The experimental agglutination indicator was related to the simulated concentration by plotting both as a function of the distance from the V groove, and then plotting one against the other.

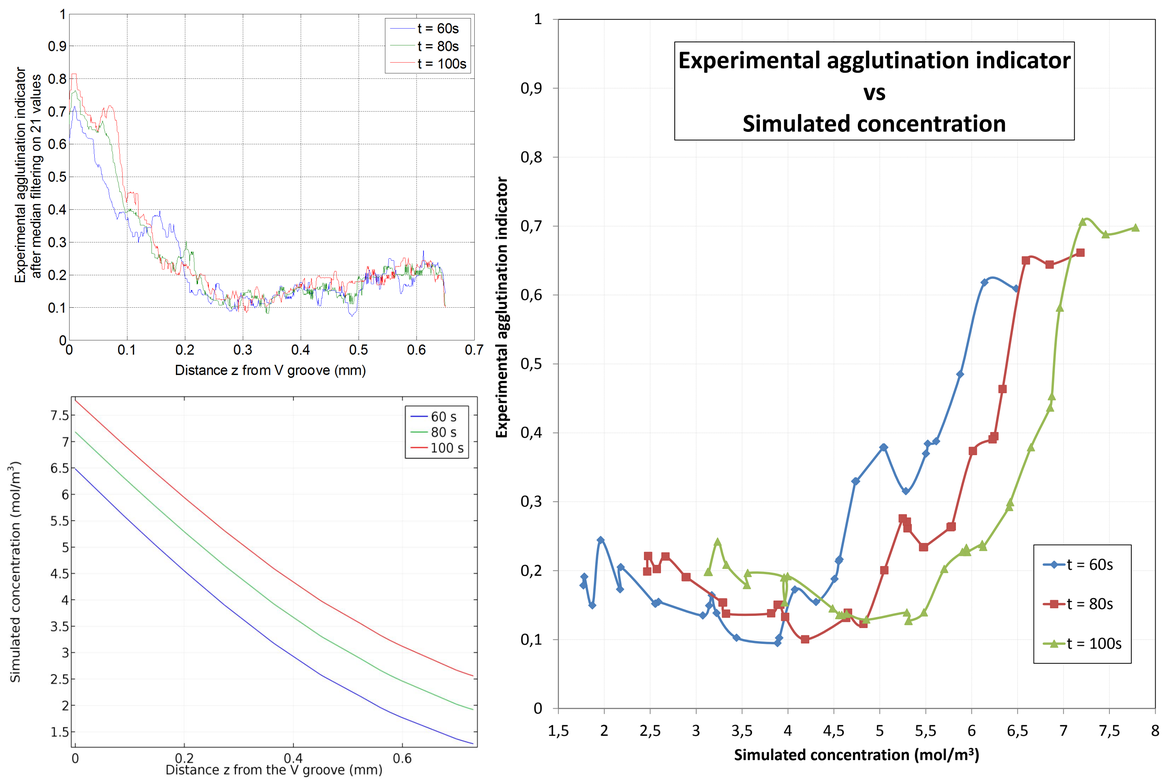


Figure 5. Left: experimental agglutination indicator and simulated concentration as functions of distance z from the V-groove.
Right: correlation between experimental agglutination indicator and the simulated concentration.

Conclusions & perspectives:

Blood typing experiments were successfully performed and kinetics measurements of agglutination were achieved. A 3D model of the reagent resuspension was developed with COMSOL. This model correlates with the experimental resuspension and with the agglutination measurement. Thus it is a satisfying first step to build a more complete model of the agglutination assay.

References:

1. **Whole blood spontaneous capillary flow in narrow V-groove microchannels.** (2015) Sensors and Actuators, B: Chemical, 206, pp. 258-267. *Berthier, J. , Brakke, K.A., Furlani, E.P., Karampelas, I.H., Poher, V., Gosselin, D., Cubizolles, M., Pouteau, P.*

2. **Dispositif de prélèvement d'un échantillon de liquide par capillarite et procédé d'analyse associé.** (2014) WO 2014135652 A1. *P. Pouteau, J. Berthier, V. Poher.*

3. **Development of a Point-Of-Care system to follow up patients under oral anticoagulant therapies.** (2013) GRC Microfluidics, Physics & Chemistry of Challenges, Advances and New Technologies for Diagnostics. Poster. June 9-14, 2013. *M. Cubizolles, J. Spiaczka, J.G. Coutard, A. Douet, J. Berthier, P. Claustre, G. Costa , G. Nonglaton, M.L. Cosnier, P. Pouteau, V. Poher.*