

PREDICTING TRANSVERSE VELOCITY OF EXOSOMES IN A MICROFLUIDIC CHANNEL USING MACHINE LEARNING

Praneetha Pulyala, Xuanhong Cheng, Joshua C. Agar.

Lehigh University, Bethlehem, PA.

1. ABSTRACT:

Exosomes are lipid bilayer vesicles, abundantly found in human physiological fluids such as blood, saliva, urine, breast milk, and semen. Exosomal biochemical composition includes membrane proteins and nucleic acid (DNA, mRNA and miRNA), that play critical role in tissue homeostasis, establishing pathogenic processes such as cancer and neurodegenerative disorders. Obvious physicochemical differences among healthy and diseased exosomes can serve as potential biomarkers for early stage disease diagnosis or even during recuperation. Therefore, efforts are made for exosome isolation that involves centrifugation and size based separation methods. However these methods are expensive and destructive, resulting in poor yield and reduced recovery volumes. To address this challenge, microfluidic based isolation tools are actively considered. COMSOL is used to simulate fluid flow in microfluidic channel. Despite the advantage of lower sample volume requirement for microfluidic technologies, potential disadvantages are tedious device design steps involved in COMSOL and lack of method standardization affecting method variability. Current work is aimed at feasibility evaluation of machine learning to predict critical variables in microfluidic channel design, for efficient isolation of exosomes. Test material flow in microchannel was simulated with COMSOL at varied channel dimensions (height, width and pitch between grooves) to determine transverse velocity of fluid. Data sets (n=57) from COMSOL simulations were fed into Anaconda3 software to map boundary conditions for microchannel dimension vs. transverse velocity. Predicted transverse velocity using Support Vector Machine (SVM) algorithm has 95% conformance to original data sets generated from COMSOL.

2. INTRODUCTION:

Every human cell is capable of secreting multiple types of extracellular vesicles such as exosomes, micro vesicles and apoptotic bodies in support of routine metabolic reactions or tissue homeostasis (Wurding et al, 2010). Among them, the scientific curiosity on exosome formation and their role as messengers has considerably grown in the recent period. Previously exosomes were considered as garbage bags meant for cellular waste disposal, however recent experimental data support their role in intercellular communications as well in disease progression. The late endosomes upon binding to the cellular membrane secrete nano size (30-120nm) spherical vesicles into the extracellular milieu via exocytosis process. They are abundantly observed in physiological fluids such as blood, saliva, urine, breast milk, amniotic fluid, cerebrospinal fluid, pleural effusions, semen and synovial fluids (Taddei et al, 2018).

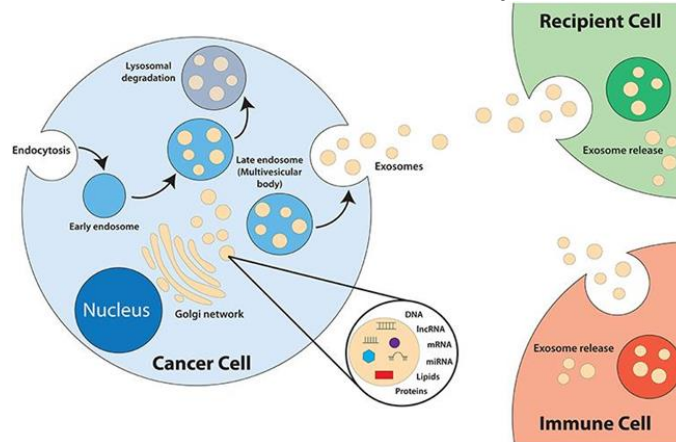
The exosomal membranes are lipid bilayered, and their surface property varies based on cellular origin. Typically these lipid membranes are comprised of phospholipids, cytosolic membrane proteins and encapsulate nucleic acids (DNA, mRNA and miRNA) (Lorena et al, 2015). The specific protein signatures and genetic cargo in exosomes reflect the pathophysiological state of the parental cells they are secreted from. Obvious physicochemical differences among healthy and diseased exosomes are being exploited currently, to identify potential biomarkers (Jin et al, 2015) for early stage disease diagnosis or even during recuperation. Therefore a plethora of research is currently in progress aimed at exosome separation and analysis.

Both in healthy and diseased people contain exosomes in their body fluids, however in certain diseases like cancer excessive levels of exosomes are observed in body fluids. An example is cancer metastasis, where actively secreted exosomes will migrate to the distal sites via circulating vasculature to establish pre-metastatic niche for disease progression (Sangiliyandi et al, 2019). Although exosomes are abundant in body fluids, their isolation pose a significant operational challenge. General evaluation involves exosomal separation, followed by physical and transcriptome analysis. Physical characterization methods include nano-particle tracking and electron microscopy, where the nucleic acid cargo is analyzed by western blot, quantitative polymerase chain reaction (qPCR). Currently differential centrifugation method is considered gold standard for exosomal isolation. However the inherent operations of these methods coupled with the fragile nature of exosomes result in poor recovery and yield. In addition high centrifugation speeds induce colloidal instability, such that exosomes coalesce leading to artifacts in downstream characterization. Therefore alternative approaches for exosome isolation such as immunoaffinity based separation, size exclusion chromatography; precipitation kits and dialysis membrane filtration methods are developed. In brief, the advantages and limitations of these exosome isolation procedures are outlined in [Table 1](#) (Maria et al 2018). The common challenge with above methods is larger sample volume, which is impractical to implement in clinical settings. At this note, microfluidics can offer great advantage, for their miniature size and flexibility to operate at lower sample volumes. Also, the operational times involved in exosome isolation are significantly lower. Although researchers have developed acoustic (Wu et al , 2017) and immunoaffinity based microfluidic technologies, a mechanistic understanding of exosomal isolation based is yet unavailable. Therefore we intend to holistically evaluate exosomal properties, and couple it with fluid dynamics to design a novel microfluidic channel for exosomal isolation and purification. Considering the experimental intricacy in predicting fluid flow, initial experiments were performed with COMSOL to simulate flow of water in microfluidic channel at varied channel geometry (height, width and pitch between grooves) to determine transverse velocity of fluid. Data sets (n=57) from COMSOL simulations was modelled with Anaconda3 software to map boundary conditions for each microchannel dimensions to transverse velocity. Predicted transverse velocity using support vector machine (SVM) algorithm had 95.4% conformance to original data sets generated from COMSOL. Future experiments will be focused on varying the ridge angles and evaluating impact of that on the fluid transverse velocity. The overarching goal of current experiments is to design microfluidic channel to simplify exosome isolation.

Table 1: Techniques for exosomal isolation and their comparison

Isolation method	Principle	Advantage	Limitation
Centrifugation	Density, shape and sized based separation of cell organelles, debris and solutes	Reduced contamination risk and cheap	High cost equipment, tedious and labor intensive processes. Impractical to implement in clinical settings, high speeds can alter exosome structural integrity, impeding downstream characterization techniques.
Size	The size differences among exosomes w.r.t other cell organelles and solutes	Fast and preserves structural integrity. Direct RNA extraction is feasible, and higher reproducibility, moderate sample yield	Exosomes structural integrity could be impacted due to column clogging and vesicle trapped in pores.
Exosome precipitation	Altering physical properties such as solubility of exosomes by incorporating water excluding reagents	Capable of generating large volume exosomes and scalable approach	Specific reagents are needed for exosomal precipitation has to be developed, followed by re-processing of precipitated materials to isolate exosomes.
Immunoaffinity methods	Ligands/receptors are immobilized on column surface to specifically capture exosomes	High purified exosomes with high possibility to subtype exosomes based on surface chemistry	Higher reagent cost, need for development of ligands/receptors. Specificity of exosomes is compromised if cell lysates are loaded into column for separation.
Microfluidics	Depends on the property selected for evaluation (size, affinity, acoustics and density)	Flexible, fast, low cost and portable	Needs sophisticated engineering skills to fabricate microfluidic channels, further validation studies are needed to build robust devices.

Figure 1: Exosomal secretion and fate in human body



3. METHODS AND RESULTS:

3.1. Transverse velocity prediction with COMSOL:

Microfluidic channel (channel) considered in this study design is rectangular shaped with the bottom side being ridged (refer to [Figure 2](#)). The top and lateral sides of the channel are flat surfaces. The channel dimensions are length (C_h) = 30mm, width (C_w) = 0.1mm and depth (C_d) = 0.7mm. Bottom ridges of channel had varied geometrical dimensions. Transverse velocity of water across channel was calculated for each combination of ridge geometry keeping the channel dimensions constant. The ridge dimensions were step by increased between width (r_w) = 0.05-0.25mm, depth (r_d) = 0.01-0.03mm and distance between ridges (displacement, r_{disp}) = 0.1-0.5. A parametric sweep was performed at a step wise increment of 0.05, 0.01, and 0.1mm for length, depth and displacement respectively. Water is the surrogate fluid in this experiment and its physical properties considered for modelling are listed in [Table 2](#). A total of 57 datasets was generated for water transverse velocity with COMSOL (Dede, 2009) (refer to [Figure 4](#)). Each x and y plane had 2000 transverse velocity points, in any given grid combination existing between minimum and maximum points of x and y.

Figure 2: Schematic for microfluidic channel

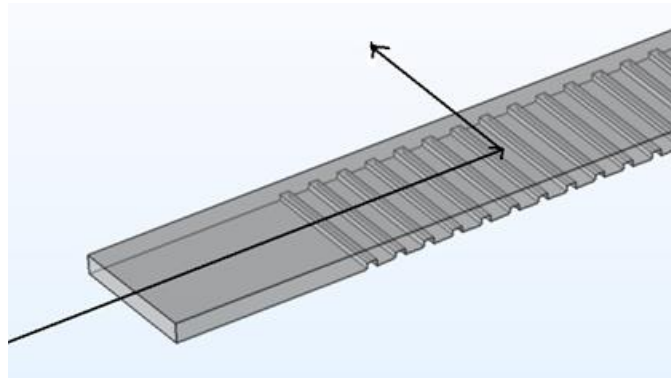
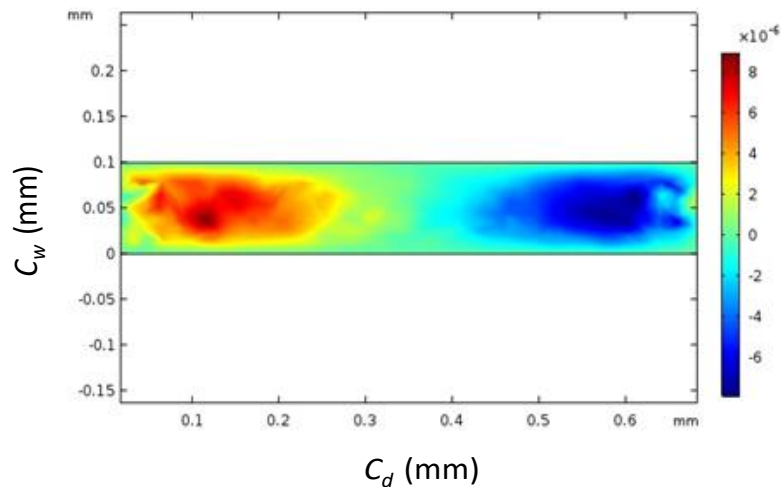


Table 2: Water Properties used in COMSOL software

Properties	Value	Units	Property group
Dynamic viscosity	eta	Pa.s	Basic
Ratio of specific heats	1	1	Basic
Electrical conductivity	5.50E-06	S/m	Basic
Heat capacity at constant pressure	C_p	J/(kg.K)	Basic
Density	rho	kg/m ³	Basic
Thermal conductivity	k	w/(m.K)	Basic
Speed of sound	cs	m/s	Basic

Figure 3: COMSOL heat map for transverse velocity of microfluidic channel



4. DATA ANALYSIS:

4.1. Establish boundary conditions for COMSOL data:

All the 57 data sets from COMSOL were input into Anaconda3 software using excel worksheets (refer to [Figure 4](#)). A good model should have established boundary conditions within which any predictions of either response or variable quantities should be accurate. The boundary conditions were established by incorporating minimum and maximum values for x and y coordinates of each grid, which contain transverse velocity data of water at the given channel geometry.

Figure 4: COMSOL data to train module

rw	rd	disp	x	y	z
0.25	0.03	0.3	0.662837097	0.032219753	-4.57E-06
			0.662751772	0.035441882	-4.88E-06
			0.669715243	0.036033088	-3.80E-06
			0.679007645	0.028251448	-1.24E-06
			0.676898491	0.026928502	-1.25E-06
			0.674418257	0.027861805	-1.85E-06
			0.668235741	0.030188263	-2.69E-06
			0.678781199	0.036802796	-1.71E-06
			0.678931199	0.031138286	-1.45E-06
			0.678670112	0.040997785	-2.09E-06
			0.674257001	0.042658423	-2.62E-06
			0.679064259	0.026113531	-1.18E-06
rw = ridge width			0.659572991	0.033448024	-5.27E-06
rd = ridge depth			0.678554752	0.045354144	-2.05E-06
disp = distance			0.675037748	0.023246616	-2.61E-06
between ridges			0.679299284	0.020700716	-1.30E-06
			0.669498074	0.020737902	-3.43E-06
			0.647757591	0.020616664	-6.00E-06
			0.661304475	0.02069221	-4.36E-06
			0.658230048	0.022970732	-5.38E-06

4.2. Principle Component Analysis:

PCA analysis (Lever, 2017) was performed on COMSOL export data to explain response w.r.t variables and reduce variance in data. The first 5 Eigen values explained ~95.4% of the transverse velocity data generated in COMSOL, enhancing confidence in the model generated.

Figure 5: Schematic for explained variance ratio

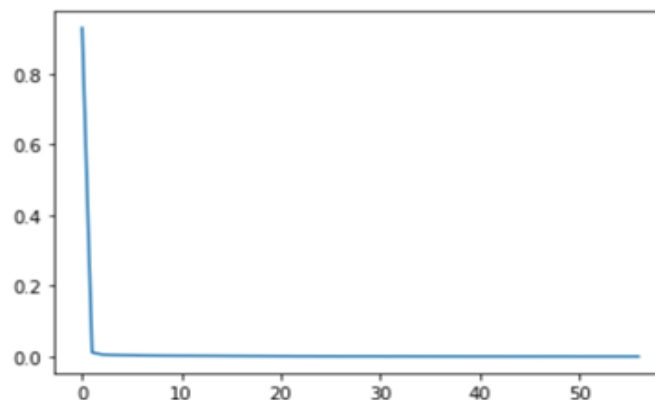
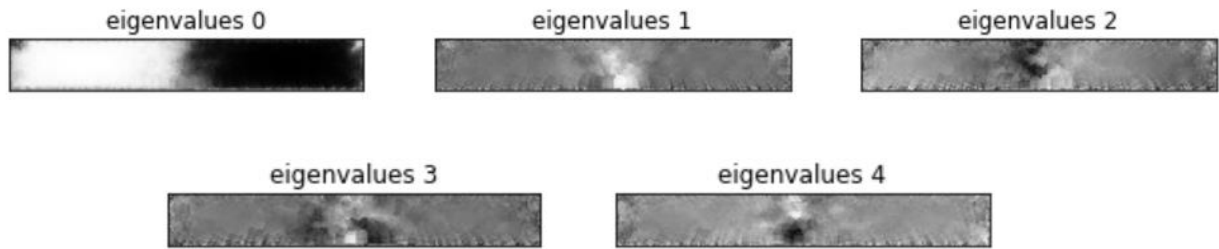


Figure 6: Eigen values generated from PCA



4.3. Support Vector Regression (SVR):

SVR algorithm was used to train the model to compare against trained Eigen values. Data presented in Figure 7 has good conformance between trained and predicted Eigen values. Heat map for the overlay of all 57 curves (refer to Figure 8) is in good agreement with first Eigen value.

Figure 7: A comparison between trained and predicted eigenvalues

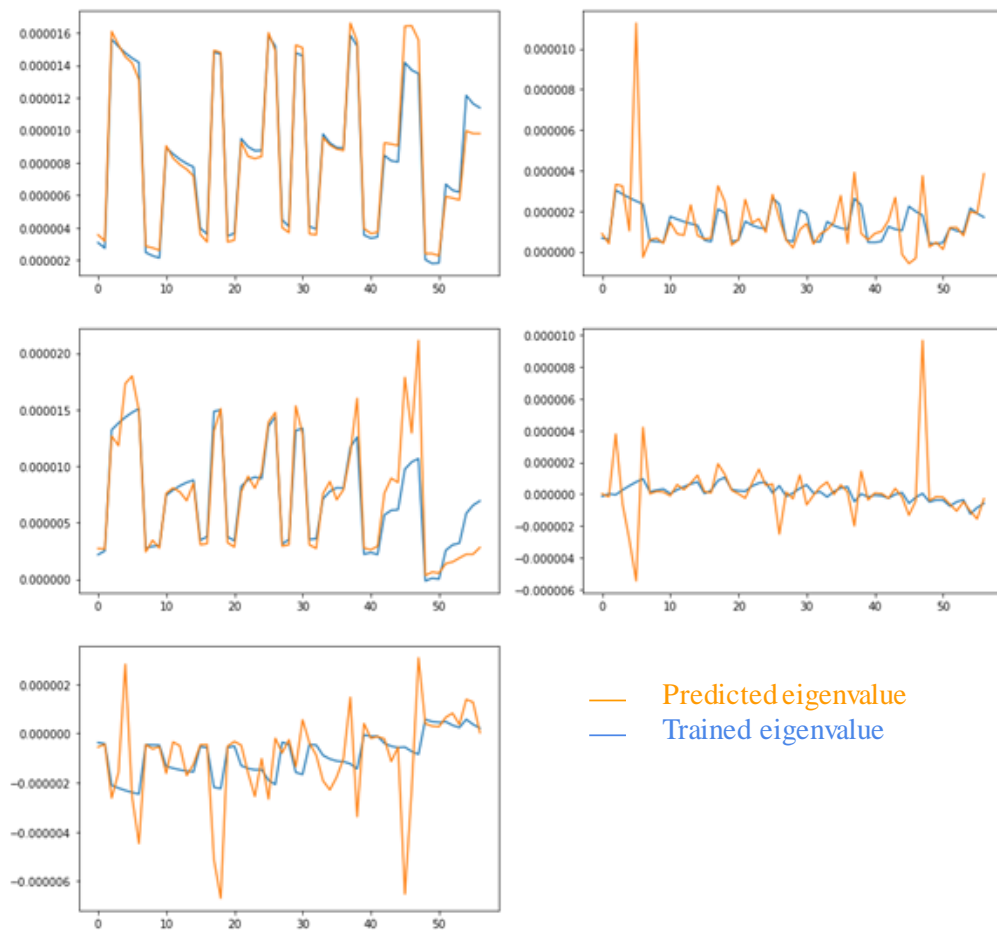
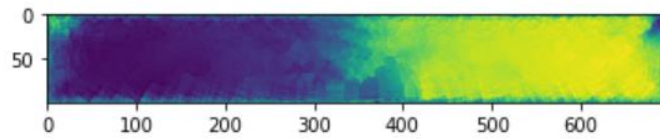


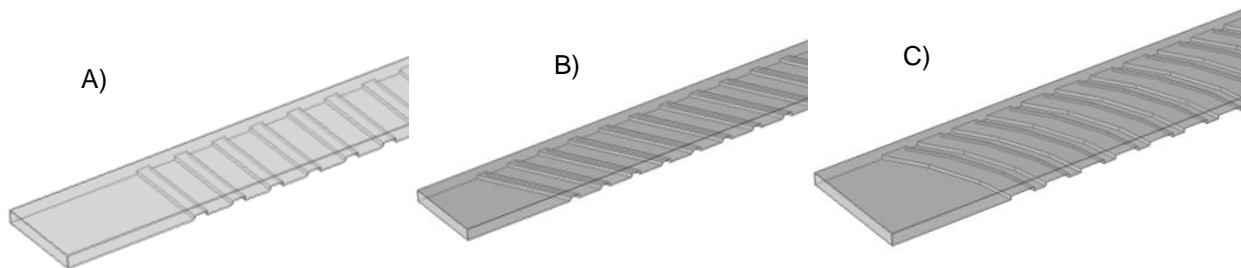
Figure 8: Predicted transverse velocity from machine learning model



5. DISCUSSION:

The fluid flow in a pipe generally follows Poiseuille's equation, with particles in the center layer of pipe possessing highest velocity compared to liquid layers adjacent to walls. The exosomal isolation is considered to be more efficient, when the exosomes are at the center of the microfluidic channel (due to increased transverse velocities). Therefore current experiments are aimed at identifying the right channel design and attributing transverse velocity changes to channel geometry using COMSOL software. The raw data from COMSOL was used to establish boundary conditions using ANACONDA3 application. The general assumption is within the model design space is, prediction should be accurate between responses and variable. To reduce the unexplainable variance, PCA model was further employed to analyze data. This generated a set of 5 Eigen values, which are ~95.4% in agreement with COMSOL data. It indicates robustness of the datasets generated in entire study (n=57 channels). The SVR analysis was employed to validate the model performance against Eigen value. In summary the good agreement of first Eigen value with the overlay of 57 transverse velocity data sets indicate model is very robust. Further experiments are aimed to continue data analysis, to generate additional data, to study flow patterns in curved or angled ridges.

Figure 9: Micro channels with ridges A) parallel to edge of the channel, B) ridges at an angle to the microchannel and C) curved ridges



6. CONCLUSIONS:

Current study is performed to design microchannel with parallel grooves meant for exosomal isolation and characterization, and results indicate successful modelling of the fluid flow (transverse velocity) across the channel. It is well known from literature that, a 45° angle or herring bone model would have a greater impact on transverse velocity and separation of

exosomes. Therefore a step by step optimization of channel parameters, such as size, shape, displacement and angle of the grooves will be studied in very detail (refer to [Figure 9](#)). Followed by channel optimization, surrogate solution (water) suspended with beads is evaluated prior to testing with human physiological fluids. Overarching goal of the current project is to develop a robust microfluidic device for easier isolation of exosomes, and operate at minimal sample volumes.

7. REFERENCES

1. Abraham D. Stroock, e. a., 2002. Chaotic Mixer for Microchannels. *Science*, Volume 295.
2. Dede, E. M., 2009. Multiphysics Topology Optimization of Heat Transfer and Fluid. *COMSOL conference Boston*.
3. Jin Lin, J. L. B. H. J. L. X. C. X.-M. C. Y.-M. X., 2015. Exosomes: Novel Biomarkers for Clinical Diagnosis. *The scientific world journal*.
4. Lever, J., 2017. Principal component analysis. *Nature methods*, 14(7).
5. Lorena Urbanelli, S. B. K. S. G. F. M. L. a., 2015. Exosome-based strategies for Diagnosis and Therapy. *Recent Patents on CNS Drug Discovery*, Volume 10, pp. 10-27.
6. Maria Serena Chiriaco, M. B. A. N. E. P., 2018. Lab-on-Chip for Exosomes and Microvesicles Detection and Characterization. *Sensors*.
7. Sangiliyandi Gurunathan, M.-H. K. ., M. J. M. Q., 2019. Review of the Isolation, Characterization, Biological Function, and Multifarious Therapeutic Approaches of Exosomes. *The Cell*.
8. TaÃnia Soares Martins, J. C. I. M. R. O. A. B. d. C. e. S., 2018. Exosome isolation from distinct biofluids using precipitation and column-based approaches. *PLOS one*.
9. Wu, M., 2017. Isolation of exosomes from whole blood by integrating acoustics and microfluidics. *PNAS*, 114(40), pp. 10584-10589.
10. Wurdinge, C. C. S. J. H. C. L. R. B. L., 2010. Microfluidic isolation and transcriptome analysis of serum microvesicles. *Lab Chip*, Volume 10(4), p. 505–511.