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Software for Extraction of Cell Properties from Dielectrophoretic Data using Well Electrode

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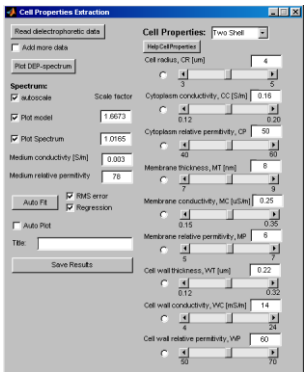
Graphical abstract



Abstract

Dielectrophoresis (DEP) method offers a lot of advantages such as cell characterization and separation on mixtures of cells in modern laboratories. By knowing the cells characteristic, separation of any interested cells from blood or other biological fluids could be performed for medical diagnostic research. Additionally, the effect of drugs to cell could be investigated further. In this study, a user friendly software has been developed using GUI Matlab to extract the properties of cell from dielectrophoretic data. The data contains changes of light intensity of the moving cells under the influenced of DEP force in the DEP well electrode. The intensity is measured from a series of images captured by a microscope. By fitting the model curve to the dielectrophoretic data with two friendly functions namely Auto Plot and Auto Fit, user is able to extract the cell properties faster. In this study, the results of live and dead yeast cell properties were successfully determined based on a total of six dielectrophoretic data.

Keywords: Dielectrophoresis; well electrode; cell properties

Abstrak

Kaedah dielektoporesis (DEP) menawarkan banyak kelebihan seperti pencirian sel dan pengasingan campuran sel-sel di dalam makmal moden. Dengan mengetahui sifat-sifat sel, pengasingan sel-sel daripada darah atau cecair biologi boleh dilakukan untuk penyelidikan diagnostik perubatan. Selain itu, kesan dadah terhadap sel boleh disiasat lanjut. Dalam projek ini, perisian yang mesra pengguna telah dibangunkan dengan menggunakan GUI Matlab untuk mengekstrak sifat-sifat sel daripada data dielectrophoretic. Data ini mengandungi perubahan keamatan cahaya untul sel-sel yang bergerak di bawah dipengaruhi daya DEP dalam elektrod telaga DEP. Keamatan cahaya adalah diukur daripada imej-imej yang ditangkap oleh mikroskop. Dengan menggunakan dua fungsi mesra iaitu *Autoplot* dan *AutoFit* untuk memuatkan lengkung model terhadap data dielektoporetik, pengguna dapat mengekstrak sifat-sifat sel dengan lebih cepat. Dalam kajian ini, berdasarkan jumlahnya enam data dielektoporetik, keputusan sifat-sifat sel yis yang hidup dan mati telah berjaya ditentukan.

Kata kunci: Dielectophoresis; elektrod telaga; sifat-sifat sel

1.0 INTRODUCTION

The term dielectrophoresis (DEP), first defined by Pohl as motion and precipitation of suspensoids in divergent electric fields.[1] The rapid development of dielectrophoresis technology was proved by about 2000 publications over the past 10 years.[2]

Dielectrophoresis technology has been widely used for a lot of applications such as separation of particles[3], characterization of different types of cells[4], differentiation between viable and nonviable cells[5], isolation and detection of cells[6]. For separation and characterization of cells, several patterns of electrodes have been introduced to conduct dielectrophoretic experiments. Kumar *et al*.[7] was proposed nanometers gap of the electrodes to trap nanoparticles. Jang *et al*.[8] was used quadrupole electrodes to trap and manipulate HeLa cells. Lewpiriyawong *et al*.[9] had designed conducting Polydimethylsiloxane (PDMS) electrode to characterize and separate yeast and bacteria. By using dielectric affinity column containing a microelectrode array, Becker *et al*.[10] had demonstrated separation and characterization of breast cancer cell. Moreover, DEP well electrode that characterizes and separates yeast has been investigated experimentally by Hoettges *et al*.[4]

Every cell has its own unique properties such as permittivity and conductivity. Due to that, the DEP well electrode developed by Hughes group is utilized to determine cell properties from dielectrophoretic data. The data contains changes of light intensity of the moving cells under the influenced of DEP force in the well. The data is then used by the developed user friendly GUI software

to determine the properties of cell. Two features namely Auto Plot and Auto Fit are added to the software to facilitate user to extract the cell properties easy and much faster.

Dielectrophoresis (DEP) is the motion of suspensoid particles relative to that of the solvent resulting from polarization forces produced by an inhomogeneous electric field.[2] When particles are in the non-uniform electric fields, interface charges are induced, electrical polarization occurs along the direction of the electric fields.[11] In contrast, if the particles are in uniform electric fields, the equal electrostatic forces will act on both opposite ends of the dipole and cause no net movement, unless particles have a net charge and zero value frequency of electric field. However, if the fields are spatially non-uniform, then the forces on either side of particles will be difference, then the DEP force can induce motion of particles.

By referring to Figure 1(a), when the electrical polarizability of particles exceeds the suspending medium, then DEP force has the same direction with the gradient of electric fields. Hence, the particles move to the strong electric field region (positive dielectrophoresis, pDEP). In contrast, if the electrical polarizability of particles is less than medium, DEP force will has opposite direction to the gradient of electric fields which is shown in Figure 1(b) and the particles will move to the weak electric field region (negative dielectrophoresis, nDEP). The polarizability of particles depends strongly on their composition, morphology, phenotype and the electric field frequency region.[12]

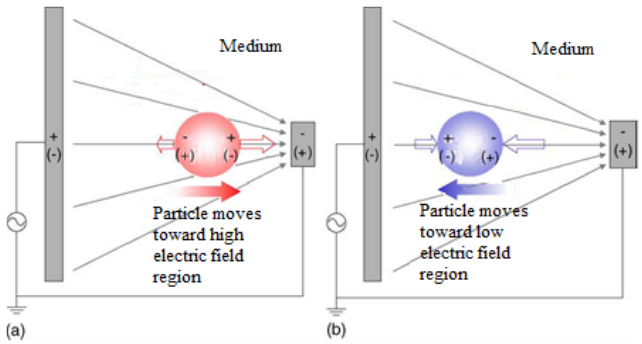


Figure 1 Direction of particle movement depending on DEP response: (a) positive DEP and (b) negative DEP

For a spherical particle which possesses no permanent dipole moment, the time-average DEP force acting on the particle in a medium of absolute permittivity, is [2]

$$F_{DEP} = 2\pi\varepsilon_m r^3 \,\mathrm{Re}\left[CM(\omega)\right]\nabla\left|E_{rms}\right|^2 \tag{1}$$

where ε_m is permittivity of surrounding medium, r is radius of the particle, Re represents real part, CM is Clausius–Mossotti factor, ∇ represents gradient operator and E is amplitude root mean square (rms) of the electric field.

Clausius–Mossotti factor (CM) is the relationship between the frequency (ω) dependent dielectric permittivity of the medium and the particle and defined by[2]

$$CM(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \tag{2}$$

where ε^*_p is effective permittivity of the particle and ε^*_m is effective permittivity of the medium. From equation 2, if effective permittivity of the particle is less than effective permittivity of the medium, then CM will be less than zero, so that nDEP occurs and the particles will be repelled from high field gradient. In contrast, if the effective permittivity of the particles is greater than effective permittivity of the medium, then CM will be greater than zero, pDEP is happened to pull the particle to high field gradient.

For a spherical particle with homogeneous structure as shown in Figure 2, the effective permittivity, ε* can be calculated by using formula

$$\varepsilon^* = \varepsilon - \frac{j\sigma}{\omega} \tag{3}$$

where ε is permittivity, j is the square root of -1, σ is the conductivity and ω is the angular frequency (2πf) of the applied ac electric field.

However, most biological particles are having complex internal structures with multiple shells. The particles contain cytoplasm, membrane and wall structures. Hence, the dominant permittivity of each shell (multi-shell model) can be reduced to one effective permittivity (homogenous model) by using smeared-out sphere approach.[13] Figure 2 shows the reduction of heterogeneous spherical model (multi-shell model) to a homogeneous model.

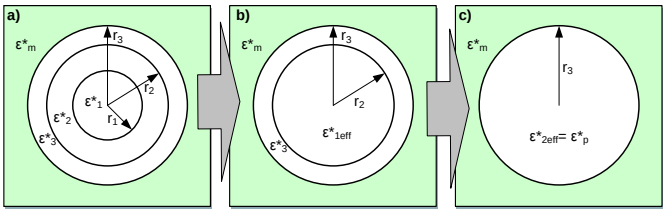


Figure 2 Reduction of heterogeneous spherical particle to a homogeneous particle

The permittivity of the innermost sphere and first shell (Figure 2a) are simplified to an effective complex permittivity (Figure 2b) by equation

$$\varepsilon^*_{1\mathrm{eff}} = \varepsilon^*_2\left[\frac{\left[\left(\frac{r_2}{r_1}\right)^3 + 2\left(\frac{\varepsilon^*_1 - \varepsilon^*_2}{\varepsilon^*_1 + 2\varepsilon^*_2}\right)\right]}{\left[\left(\frac{r_2}{r_1}\right)^3 - \left(\frac{\varepsilon^*_1 - \varepsilon^*_2}{\varepsilon^*_1 + 2\varepsilon^*_2}\right)\right]}\right] \tag{4}$$

where r_1 is the radius of the innermost sphere , r_2 is the radius to the outside of the first shell, ε^*_1 is the complex permittivity of innermost sphere and ε^*_2 is the complex permittivity of the first shell.

For a double-shell sphere particle like yeast, the double-shell sphere model (Figure 2a) can be simplified to homogenous sphere model (Figure 2c) by using formula

$$\varepsilon^*_p = \varepsilon^*_{2\mathrm{eff}} = \varepsilon^*_3\left[\frac{\left[\left(\frac{r_3}{r_2}\right)^3 + 2\left(\frac{\varepsilon^*_{1\mathrm{eff}} - \varepsilon^*_3}{\varepsilon^*_{1\mathrm{eff}} + 2\varepsilon^*_3}\right)\right]}{\left[\left(\frac{r_3}{r_2}\right)^3 - \left(\frac{\varepsilon^*_{1\mathrm{eff}} - \varepsilon^*_3}{\varepsilon^*_{1\mathrm{eff}} + 2\varepsilon^*_3}\right)\right]}\right] \tag{5}$$

where r_3 is the radius from the centre of the sphere to the outside of the cell wall, ε^*_3 is the complex permittivity of the second shell

and ε^*_{1eff} is the first effective complex permittivity which is obtained from equation 4.

2.0 EXPERIMENTAL

There are some procedures to get dielectrophoretic data from well electrode (Figure 3) before the properties of cell could be extracted by the developed software. The well with diameter of 700 µm was constructed by drilling through 7 layers of electrodes (copper) and 8 layers of insulators (fiberglass reinforced, FR4). The thickness of each of the electrode layer is 70 µm and insulation layer is around 120 µm. A glass slide is glued at the bottom of the well.

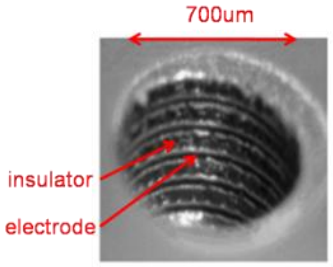


Figure 3 Structure of well electrode

Yeast cells (Saccharomyces cervisiae) are grow overnight in a simple mixture of medium, made from 2g of yeast extract-peptone-glucose (YPD) broth mixed with 40ml of deionised (DI) water. The grown cells are pipetted in an Eppendorf tube and centrifuged at 180g (1500 r.p.m.) for 3 minutes. The supernatant is then removed and the cell pellet is washed by resuspending it with 1ml of iso-osmotic medium. The medium conductivity is adjusted to 3mS/m by adding a few drops of phosphate buffered saline (PBS). The reading is verified using conductivity meter (HI8733, HANNA instruments). The centrifugation and washing process are repeated three times before the final concentration of the cells is obtained. To prepare non-viable yeast cells, the grown cells are heated to 90°C for 20 minutes.[14]

To start with the experiment, the well electrode is placed on the stage of a microscope. The live yeast cells with a concentration of 10^8 cells/ml are loaded into the well. A light source is used to light up the well to observe the behaviour of cells. A signal of 20Vppis applied to the well and verified using the digital oscilloscope. When the electric field is applied, cells are either pushed from the well edge to the centre which leads to intensity of light at the centre and edge decreases and increases respectively, or pulled towards the well edge which results in light intensity of the centre well increases. Typically, a total of 25 images of the energised well are captured over a period of 120 seconds. The captured images will then be compared to the initial image to measure the changes of light intensity. The measurement is carried out at 14 frequency points from 1 kHz to 20 MHz and is repeated 3 times. To obtain the dielectrophoretic data for the dead yeast cells, similar steps are repeated. The data gathered is then plotted and fitted with the double shell model using the developed software. The conductivity, permittivity and the thickness of each layer that forms the internal cellular components can then be extracted.

Figure 4 shows the flow of the process to extract the cell properties while Figure 5 shows the developed GUI software.

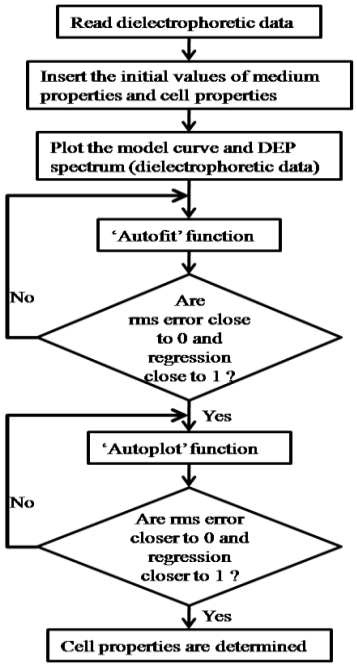


Figure 4 Flow of process to extract cell properties

The user is needed to insert the dielectrophoretic data that obtained from the experiment using the well electrode. Initially, user is required to enter an assumption value for each parameter of cell properties in the edit box provided. Those values are substituted in the shell model equation e.g. single shell, double shell to plot DEP model spectrum. This curve is fitted to the dielectrophoretic data by adjusting each value of the cell properties parameters. To determine the best fit between those curves, the regression and root-mean-square error (RMSE) values are calculated to be close to 1 and 0 respectively.15 The closer the regression to 1, the stronger is the correlation between the model prediction and the actual parameters value. Meanwhile, a smaller RMSE value indicates better model performance.

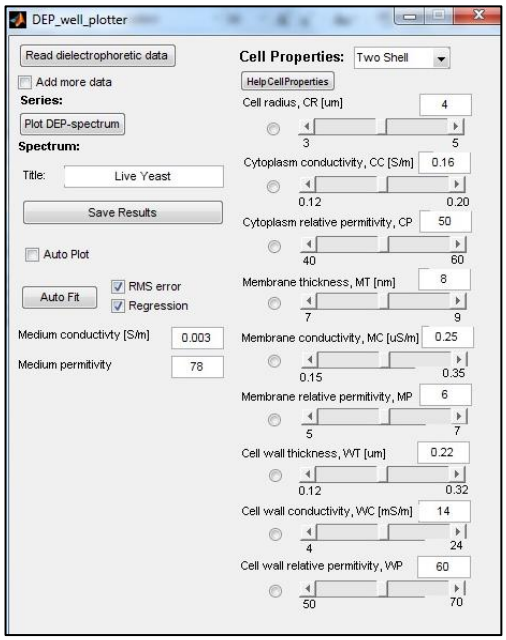


Figure 5 GUI software

By using the developed software, a user can determine any unknown cell properties easy and fast. The 'HelpCellProperties' function will illustrate the structure of cells with parameters labelling for user guidelines. The Auto Fit function is created to minimize the time of try and error in attaining the best fitted curve between the model and the data. User can define the minimum and maximum values of the cell parameters by clicking the radio button (Figure 6).

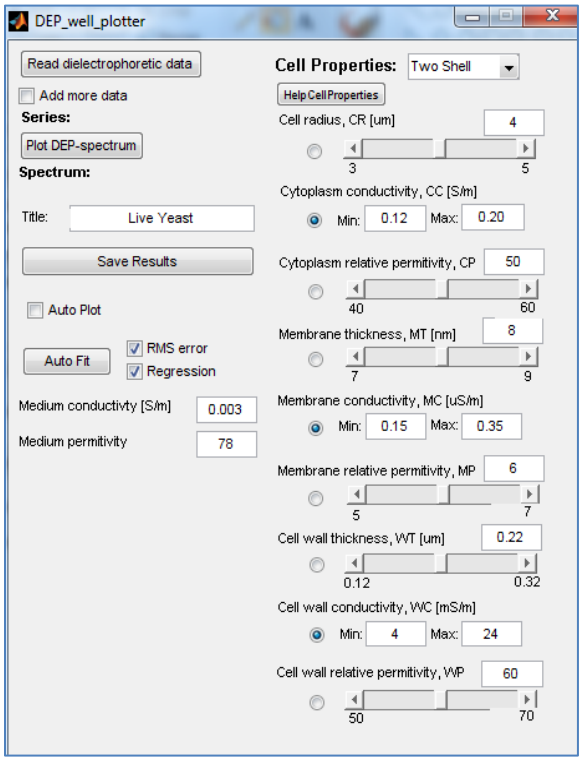


Figure 6 The GUI can determine the cell properties based on the minimum and maximum values defined by a user

With the Auto Fit function, the values of each parameter are determined when the model curve is fitted. In order to make model curve fit better, another function called Auto Plot is created. This function will plot the model curve in real time when any value of parameters is slightly changed using slider or edit box. User can observe the parameters that contribute to the changes of model curve in getting the best fitted curve. The best fitted model curve is achieved when the regression and RMSE values are much closer to 1 and 0 respectively. Both Auto Fit and Auto Plot functions provide friendliness of determining parameters of cell properties. Moreover, the results of cell properties can be saved by the 'Save Results' button.

■3.0 RESULTS AND DISCUSSION

The analysis of this study was done by using 6 dielectrophoretic data (3 live yeast data and 3 dead yeast data). These data were plotted and fitted by using the developed GUI software. The Auto Fit was clicked to fit the model curve with the dielectrophoretic data based on the assumption values of the parameters entered. To attain better fitted curve, the Auto Plot was selected. User can easily alter any parameters and observe the changes of each parameter to the model curve in real time. The final parameters of the cell properties were determined once the value of RMSE and regression were closed to 0 and 1 respectively. Figure 7 shows the best fitted DEP spectrum of live yeast cell for data 2. Table 1 summarizes the properties of live yeast cell based on three dielectrophoretic data and compared with the properties published by Huang *et al*.[16]

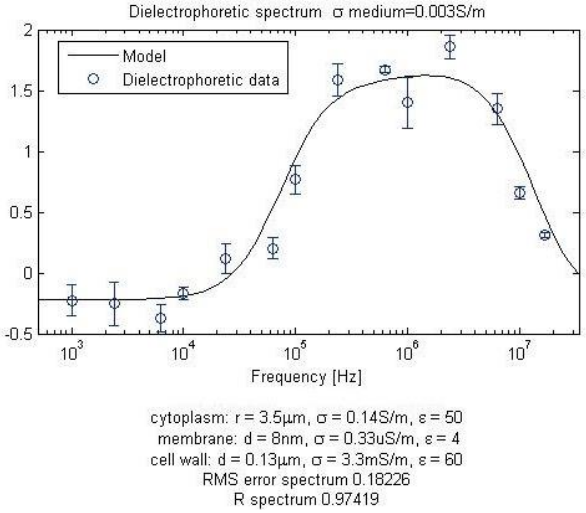


Figure 7 DEP spectrum for live yeast

Table 1 Comparison of live yeast cell properties between Data Published[13] and three dielectrophoretic data

Cell Properties	Data Published	Data 1	Data 2	Data 3
Cell radius (μm)	4.0	3.5	3.5	3.5
Cytoplasm conductivity (S/m)	0.16	0.18	0.14	0.18
Cytoplasm relative permittivity	50	50	50	50
Membrane thickness (nm)	8	8	8	8
Membrane conductivity (μS/m)	0.25	0.33	0.33	0.33
Membrane relative permittivity	6	4	4	4
Cell wall thickness (μm)	0.22	0.13	0.13	0.13
Cell wall conductivity (mS/m)	14.0	4.5	3.8	4.4
Cell wall relative permittivity	60	60	60	60
RMS error		0.228	0.182	0.248
Regression		0.958	0.973	0.946

It can be found that the live yeast properties were slightly different with the data published in parameters such as cell radius, cell wall thickness and conductivity of cytoplasm and membrane. However, the conductivity of cell wall of the experimented yeast had less 70% from the published value. Based on three dielectrophoretic data (data 1, data 2 and data 3), it can be concluded that the live yeast cell used for the experiment had radius of 3.5μm, the cytoplasm conductivity was between 0.14 and 0.18S/m, the cytoplasm relative permittivity was 50, the membrane thickness was 8nm, the membrane conductivity was 0.33μS/m, the membrane relative permittivity was 4, the cell wall thickness was 0.13 μm, the cell wall conductivity was between 3.8 and 4.5 mS/m, the cell wall relative permittivity was 60. The dielectrophoretic data was best fitted to the model with the average values of the RMSE and regression about 0.22 and 0.96 respectively.

Similarly to the dead yeast, three dielectrophoretic data were plotted and fitted. Figure 8 shows the best fitted DEP spectrum of dead yeast cell for data 6. Meanwhile, Table 2 summarizes the properties of dead yeast cell based on three dielectrophoretic data and compared with the properties published by Huang *et al*.[16]

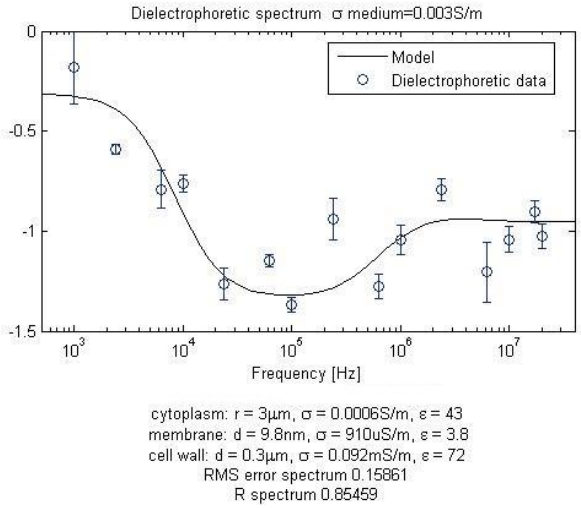


Figure 8 DEP spectrum for dead yeast

Table 2 Comparison dead yeast cell properties between Data Published[13] and three dielectrophoretic data

Cell properties	Data Published	Data 4	Data 5	Data 6
Cell radius (μm)	3.50	2.80	2.98	3.00
Cytoplasm conductivity (mS/m)	7.00	0.45	0.58	0.60
Cytoplasm relative permittivity	50.0	43.2	54.3	43.0
Membrane thickness (nm)	8.0	9.5	7.9	9.8
Membrane conductivity (μS/m)	160	980	963	910
Membrane relative permittivity	6.0	3.8	4.5	3.8
Cell wall thickness (μm)	0.25	0.30	0.31	0.30
Cell wall conductivity (mS/m)	1.500	0.081	0.088	0.092
Cell wall relative permittivity	60.0	75.0	70.6	72.0
RMS error		0.260	0.256	0.159
Regression		0.762	0.858	0.855

Most of the parameters of dead yeast cells have large difference with data published especially the conductivity of cytoplasm, membrane and cell wall. The discrepancies may be caused by the differences in yeast strain and the growth conditions used in the experiments. Referring to three dielectrophoretic data (data 4, data 5 and data 6), a conclusion can be made that the cell radius was between 2.8 and 3 μm, the cytoplasm conductivity was between 0.45 and 0.6 mS/m, the cytoplasm relative permittivity was between 43 and 54.3, the membrane thickness was between 7.9 and 9.8 nm, the membrane conductivity was between 910 and 980 μS/m, the membrane relative permittivity was between 3.8 and 4.5, the cell wall thickness was about 0.3 μm, the cell wall conductivity was between 0.081 and 0.092 mS/m, the cell wall

relative permittivity was between 70.6 and 75. The dielectrophoretic data was best fitted to the model with the average values of the RMSE and regression about 0.23 and 0.83 respectively

■4.0 CONCLUSION

A user-friendly GUI software has been developed to be more pleasant and easy for user to characterize any type of cell. In addition, user could save much time when determining the properties of the cell. Once the properties are known, DEP spectrum of the cell could be plotted. Based on the DEP spectrum, user can do many applications such as cell manipulation, separation, concentration and sorting.

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