

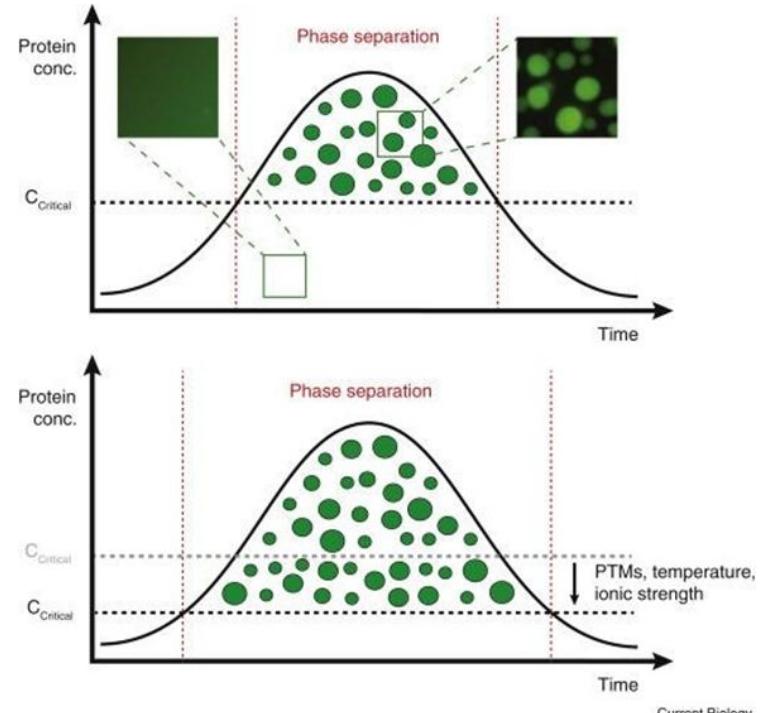
# Liquid-liquid phase separation: Continuum modelling

PHYS/BIOE 230 Project Presentation

Arnab Roy

# Biological Motivation of phase separation

- Cellular organization is essential for living organisms to regulate their metabolism and responsiveness and liquid protein droplets phase separate from rest of cytoplasm to help in this organization.
- Past experiments have already shown that membraneless organelles can assemble through phase separation.
- Liquid-liquid phase separation (LLPS) can be particularly helpful for quantifying replication of viruses.



Alberti, S (2017).

DOI:<https://doi.org/10.1016/j.cub.2017.08.069>

Current Biology

# Biological motivation Contd...

- Scientists have recognized the importance of phase separation during viral infections. LLPS provides a powerful framework for understanding the viral life cycle from viral replication to evasion of host immune surveillance. As a result, this review focuses on the progress of LLPS research in viral infection and immune regulation to provide clues for antiviral therapeutic strategies.

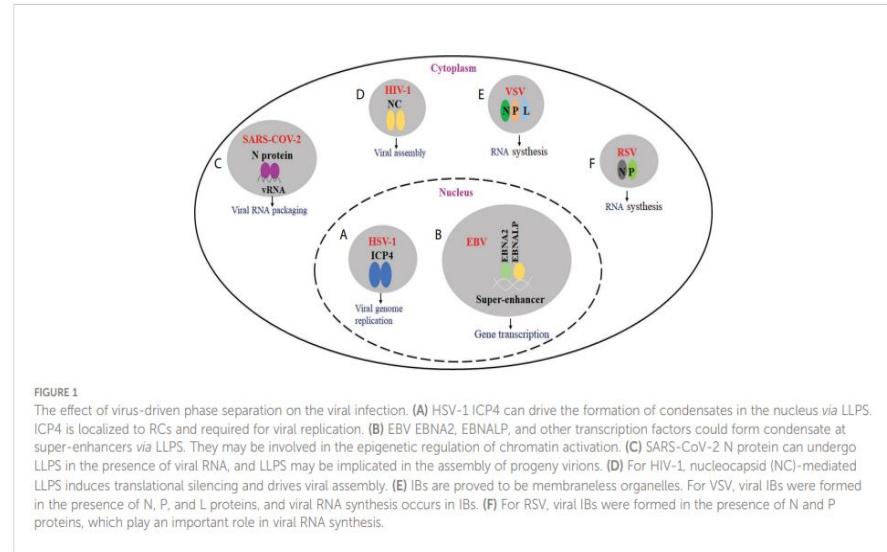


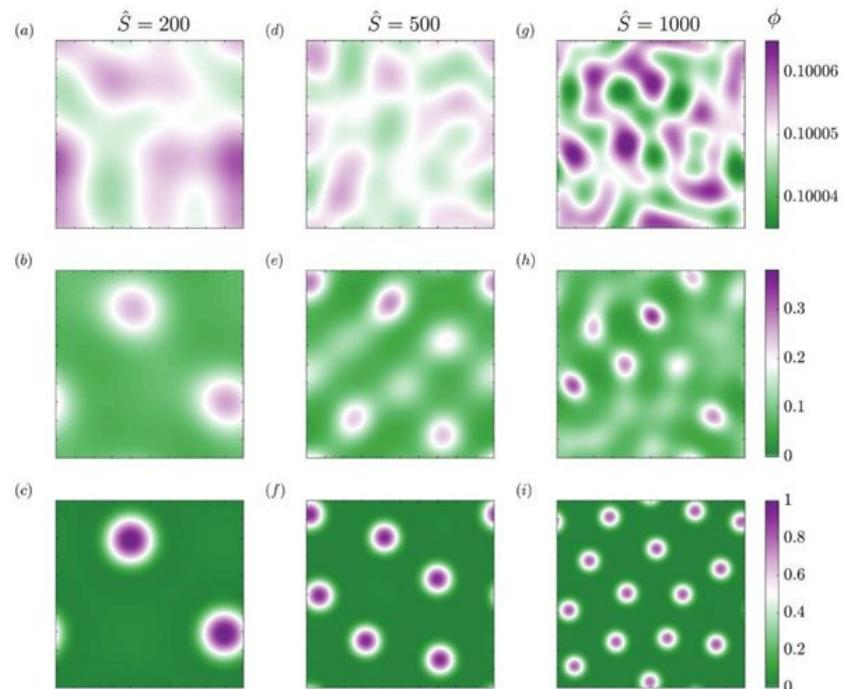
FIGURE 1

The effect of virus-driven phase separation on the viral infection. (A) HSV-1 ICP4 can drive the formation of condensates in the nucleus via LLPS. ICP4 is localized to RCs and required for viral replication. (B) EBV EBNA2, EBNALP, and other transcription factors could form condensates at super-enhancers via LLPS. They may be involved in the epigenetic regulation of chromatin activation. (C) SARS-CoV-2 N protein can undergo LLPS in the presence of viral RNA, and LLPS may be implicated in the assembly of progeny virions. (D) For HIV-1, nucleocapsid (NC)-mediated LLPS induces translational silencing and drives viral assembly. (E) IBs are proved to be membraneless organelles. For VSV, viral IBs were formed in the presence of N, P, and L proteins, and viral RNA synthesis occurs in IBs. (F) For RSV, viral IBs were formed in the presence of N and P proteins, which play an important role in viral RNA synthesis.

# Two component phase separation model

Idea:

- Start with defining a free energy density for the system based on some average density field  $u$
- Think like two different phases are mixed and coexisting together on a flat surface, we have to separate them.
- Define some interface between the two phases with thickness  $\delta$  and to form this interface, there should be some energy cost  $k$  (i.e. you can relate surface tension here)
- Spontaneous phase separation occurs as  $u$  evolves with time.



Mahapatra, A. (2021).  
<https://doi.org/10.1039/d1sm00502b>

# Model Formulation

- The main idea behind ‘continuum modelling’ is to take a density or averaged field quantity instead of tracking every individual particles (particle-based modelling)
- We consider a normalized density field  $u$  described by the free energy density( $f$ ):

$$f = \frac{k}{2} \left[ |\nabla u|^2 + \frac{1}{2\delta^2} (u^2 - 1)^2 \right]$$

Where  $k$  is the interfacial energy cost (surface tension) and  $\delta$  denotes thickness of the interface.

- Dynamical Equation (kind of a continuity equation):

$$\frac{\partial u}{\partial t} = -\frac{k}{\tau} \nabla^2 \left[ \nabla^2 u + \frac{1}{\delta^2} (u - u^3) \right]$$

# Numerical Methods

- Initialization of  $u$ : We used gaussian distribution with slight negative bias to initialize the 2D array for  $u$  on the square lattice.
- Simulation box size: square box of domain  $100 \times 100$ . It is divided into 100 cells, so size of each cell is 1.
- Spatial discretization: Wrote down the discrete laplacian (2nd order spatial derivative) on the 2D grid using `np.roll` command (as we used in class)
- Time update: Next task is to solve the time evolution equation of  $u$ . We solved that using two methods - (1) Forward Euler time-stepping (2) Runge-Kutta 4th order.
- **Yet to do** : Try to create animation or movie of the whole time evolution dynamics starting from random initial density all over the box to finally forming droplets due to phase separation.

## Runge-Kutta (4th order)

- The need for RK4 comes because Forward Euler time-stepping can only handle very small  $dt$ .
- RK method is kind of a higher order expansion of Euler method.
- The idea of this method is to find time derivatives at multiple points in between  $t$  and  $t+dt$  and effectively averaging them to find the total time derivative.

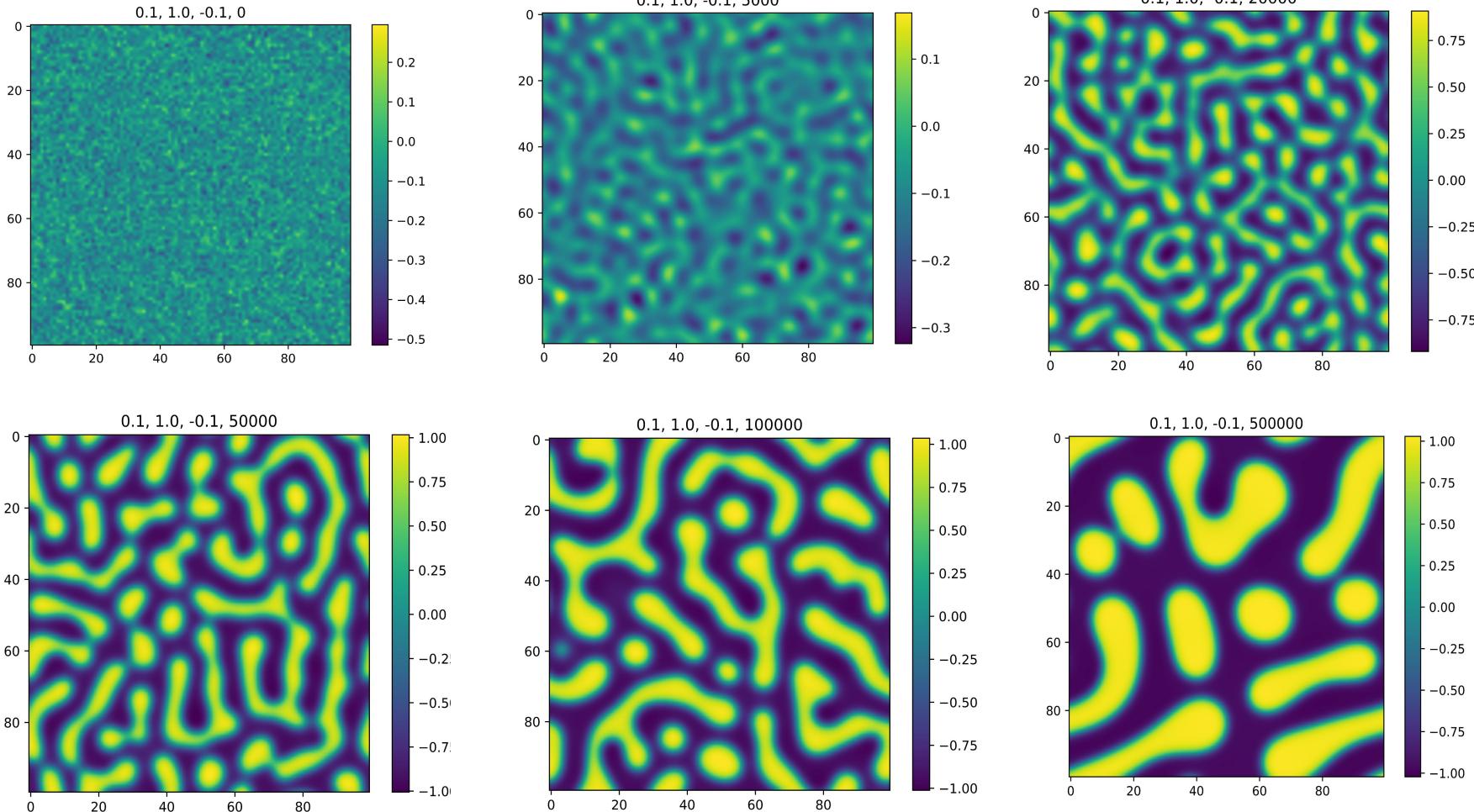
$$k_1 = f(t_n, y_n),$$

$$k_2 = f\left(t_n + \frac{h}{2}, y_n + h \frac{k_1}{2}\right), \quad y_{n+1} = y_n + \frac{1}{6}h(k_1 + 2k_2 + 2k_3 + k_4)$$

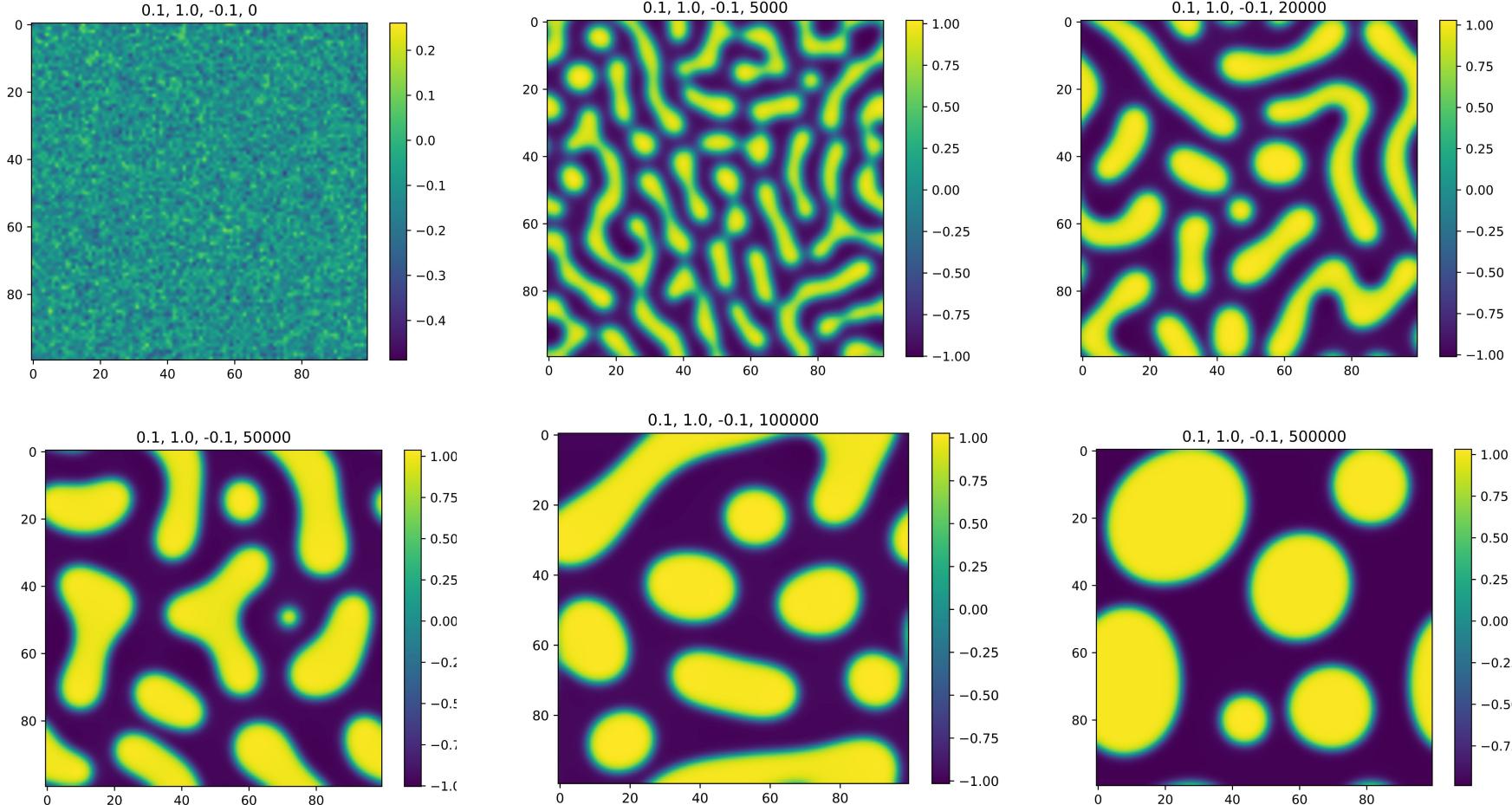
$$k_3 = f\left(t_n + \frac{h}{2}, y_n + h \frac{k_2}{2}\right),$$

$$k_4 = f(t_n + h, y_n + hk_3).$$

# Forward Euler Method : $dt = 0.01$ (sim\_t=1000 ~ real time 1s)

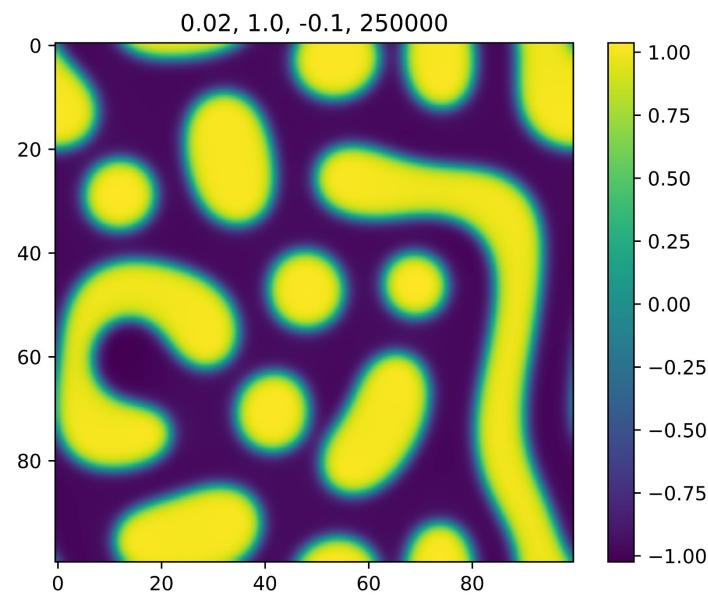
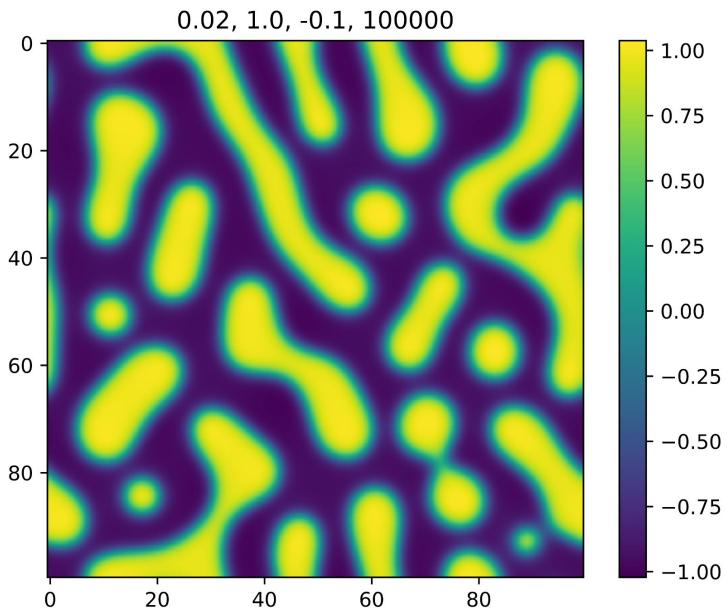


# Runge-Kutta 4th order : dt =0.1 (sim\_t=1000 ~ real time 4s)



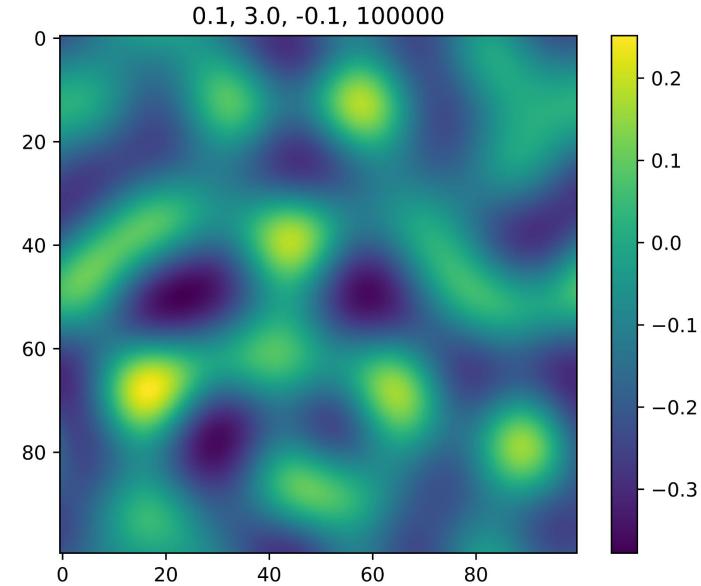
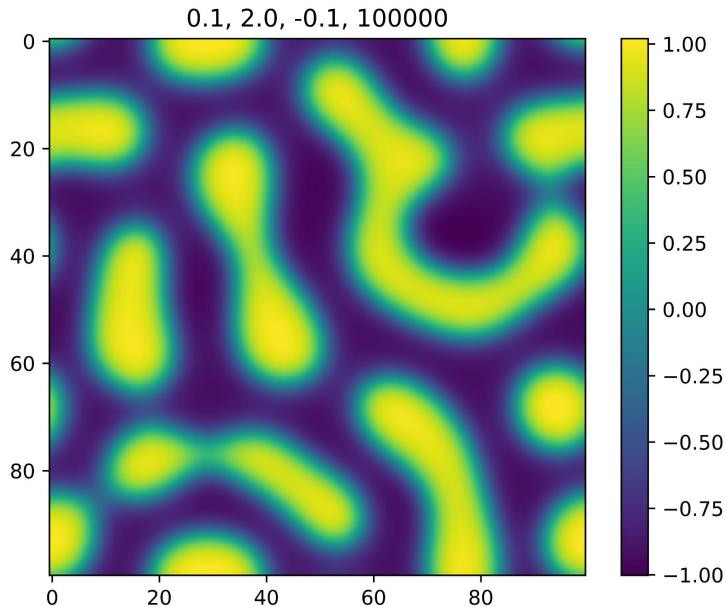
## Effect of parameters on time scale of phase separation(RK4)

- Effect of tau : Increasing tau means low D, hence it makes the phase separation much more slower



# Effect of parameters on time scale of phase separation

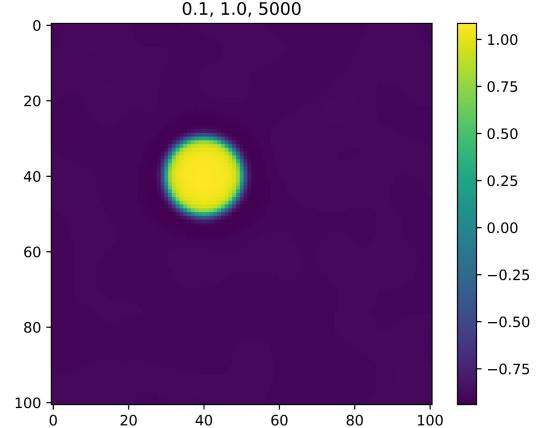
- Effect of  $\delta$  : Increasing  $\delta$  makes the phase separation much more difficult



# Play with initialization of the system

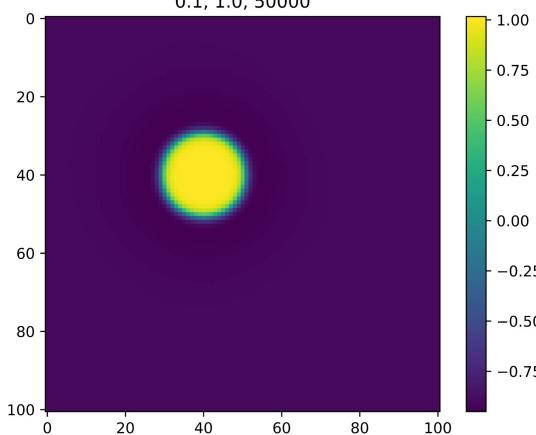
- Instead of initializing system with a random initial average density  $u$  being in the range of -1 to 1 with a slight negative bias towards -1, here I am initializing the system as a single initial droplet where inside the droplet  $u=1$  and outside the droplet,  $u=-1$
- This initialization is done by using interpolation function “hyperbolic tangent ( $\tanh$ )” function which interpolates from a region of +1 to -1 within small thickness with adding some random small noise. Noise helps in equilibration of the system.
- Defined a circular region of radius  $R$  and then defined initial density value:  
$$u(r) = \tanh((R-r)/d)$$
 [Took help from Kinjal in this..]

0.1, 1.0, 5000

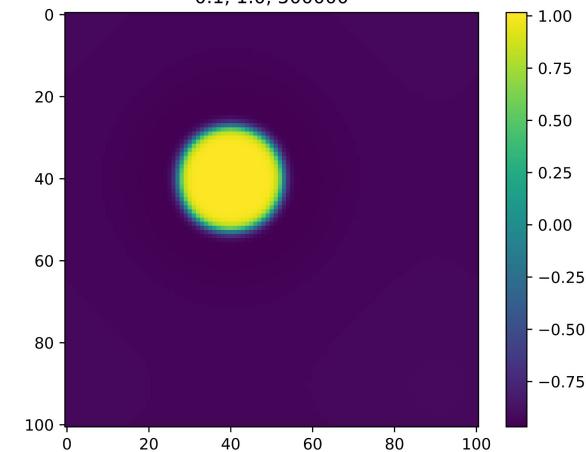


Initialization: Single droplet(Euler method)

0.1, 1.0, 50000

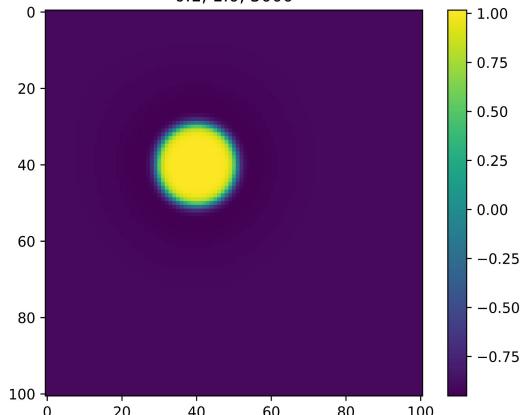


0.1, 1.0, 500000



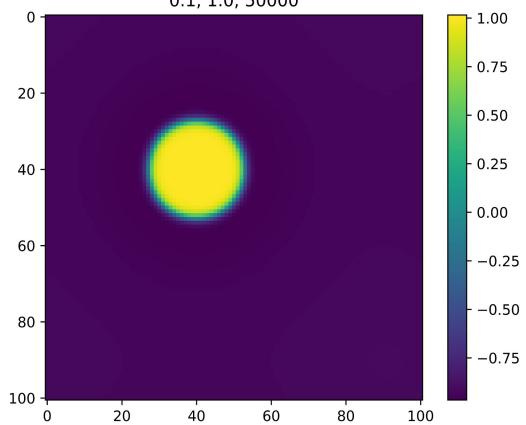
Ostwald ripening

0.1, 1.0, 5000

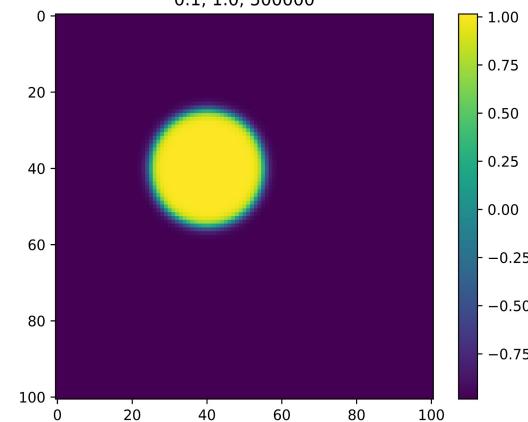


## Initialization: Single droplet(RK4 method)

0.1, 1.0, 50000



0.1, 1.0, 500000



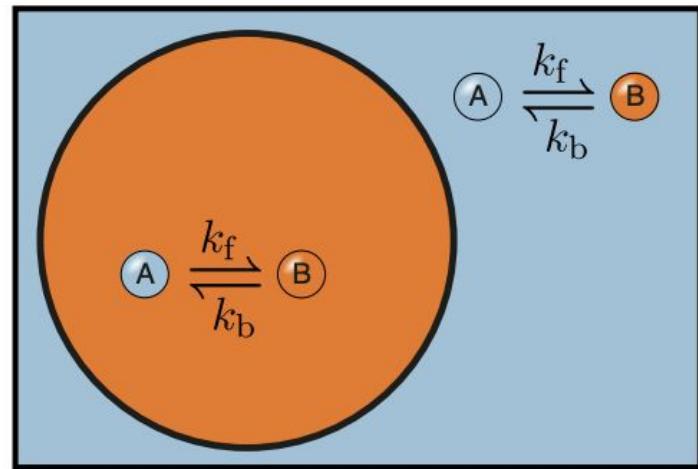
Ostwald ripening

# Droplet dynamics with reactions (reaction-diffusion system)

- Here, we are considering 1st order chemical reaction is happening between two components: A(solute present in liquid outside,blue) and B (droplet forming material,orange)
- Hence, the dynamical equation will contain an additional source term (s) contributing the dependence on  $u$  from the kinetics of the reaction:

$$\frac{\partial u}{\partial t} = -\frac{k}{\tau} \nabla^2 [\nabla^2 u + \frac{1}{\delta^2} u(1 - u^2)] + s$$

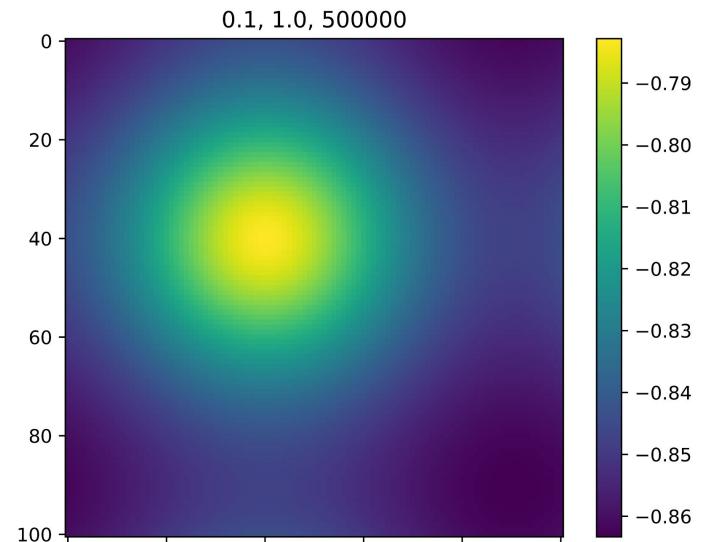
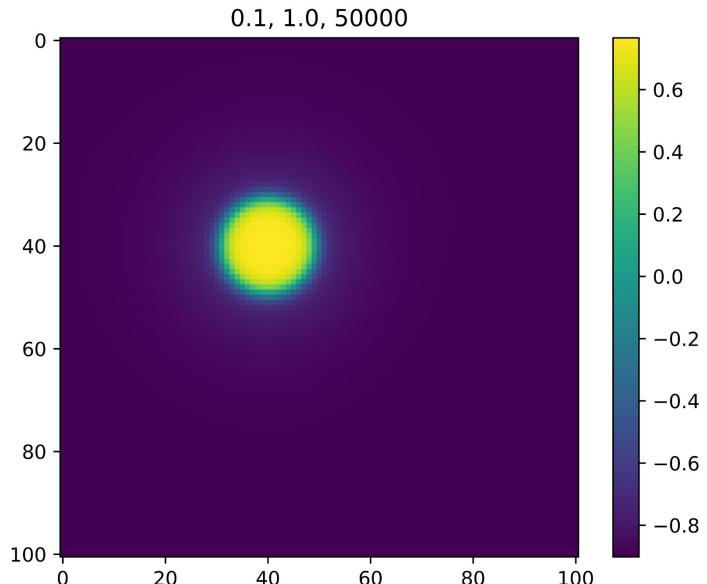
$$s = k_f \frac{(1 - u)}{2} - k_b \frac{(1 + u)}{2}$$



Zwicker, D., Hyman, A.A., Julicher, F. (2015). Suppression of Ostwald ripening in active emulsions. DOI: <https://doi.org/10.1103/PhysRevE.92.012317>

# Initialization: Single droplet with chemical reaction

Radius( $R$ ) = 10



Forward Euler time update

# Things I tried beyond these....

- Wrote a generalized code for this droplet formation or phase separation model combining RK4 method, Forward Euler method and also using “numba” package of python to speed up the computation.
- I am using `@jit` (just-in-time) decorator (as discussed in last class) to speed up the coding. Computation time reduces by 3 times from using RK4 (using same  $dt = 0.1$ )
- I tried varying the magnitude of random noise while initializing single droplet. I saw that if noise amplitude is higher, then it dominates and after some time lot of small droplet patches emerge which is just like ‘random initialization’.

# Next Steps (Future Plan)

- ❖ Initial phase separation model (with random initialisation):
  - Plan to write a code for calculating size distribution of droplets (how average radius of droplet changes with time)
  - Try to find out critical 'interface thickness' beyond which phase separation doesn't occur.
  - Try the simulation for larger box size and different initial densities.
  - Numerically compare speeds of Forward Euler & RK4 methods (although already having a qualitative idea after doing few simulations, still it would be better to quantitatively show the speed difference with varying  $dt$ )
- ❖ Single & two droplet initialization model: (with & without reaction)
  - Have to understand the role of chemical reaction better. The idea/concept is: if there is no chemical reaction (passive case), one single droplet will continue growing with time (Ostwald Ripening) but involving reaction will suppress this and shrink the droplet.
  - Literature says there is a critical radius  $R_{crit}$  above which droplet grows and below which droplet shrinks, our task is to find such  $R_{crit}$

# Conclusion

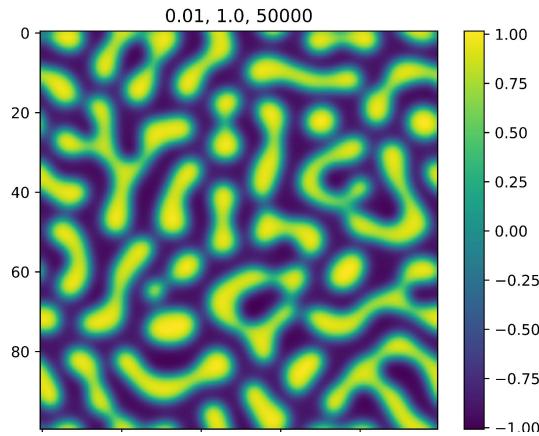
- In the initial 2 component phase separation model, we showed how starting from a random initial configuration spontaneously phase separation occurs and leads to formation of droplet of one component(say, oil) with high density (+1) in a sea of another liquid component(say,water) with low density (-1).
- Determined how modifying parameters like  $\tau$  (related to Diffusion constant),  $\delta$  (interface thickness) affects phase separation process.
- Tried to simulate single and multiple initial droplets to see how their size evolves with time (grows or shrinks) in presence and absence of chemical reaction.

Thank You

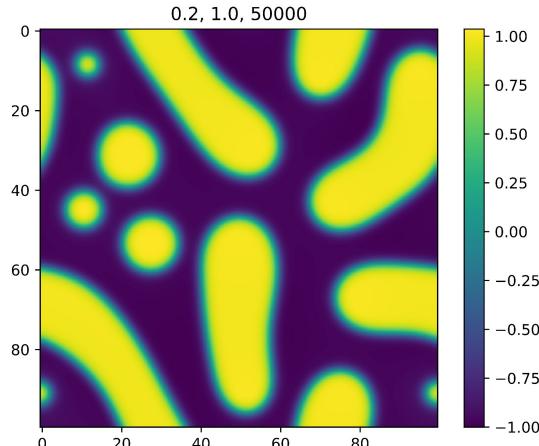
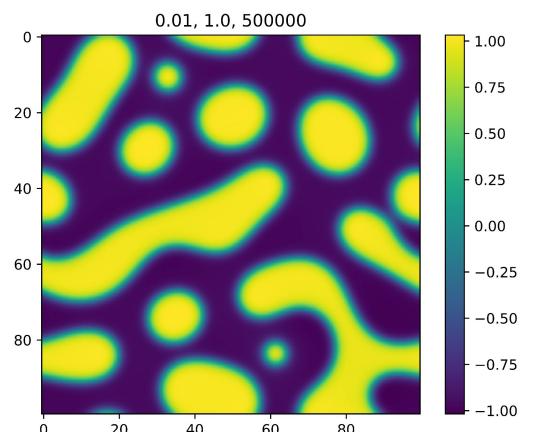
# Final Modifications and updated results

Phys 230 Final Project  
Arnab Roy  
12 May 2023

# Variation of Surface tension( $k$ ): random initialization, No reaction

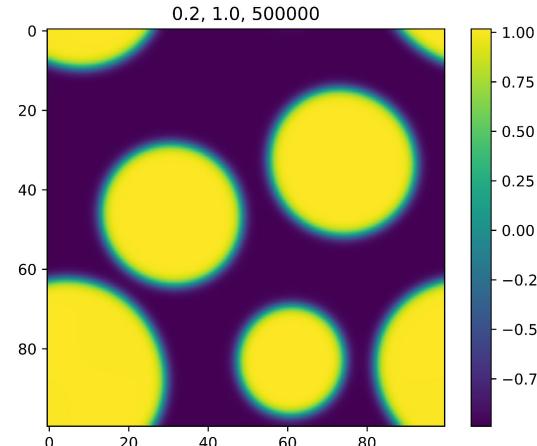


$k = 0.01$   
(10 fold reduction  
than before)



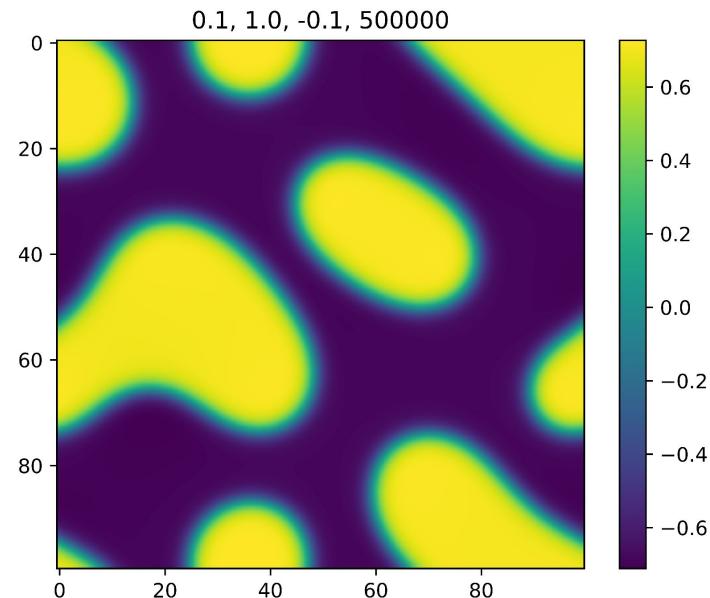
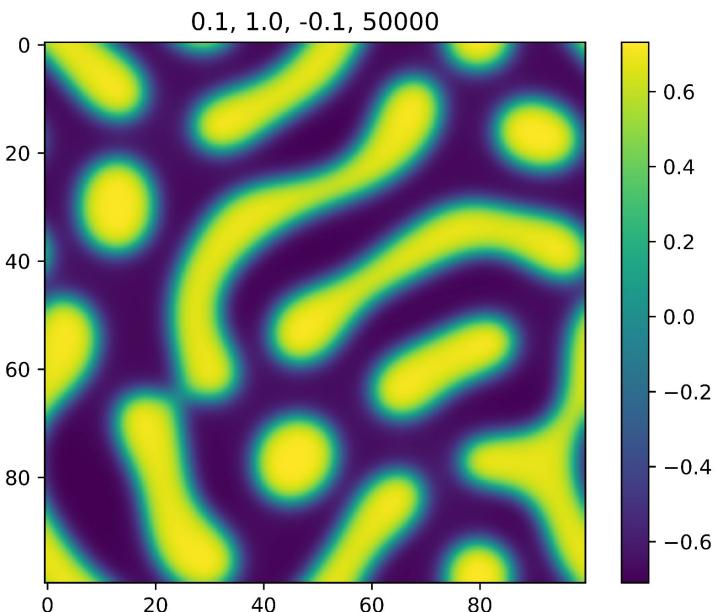
$k = 0.2$   
(2 fold increase  
than before)

Surface tension( $k$ ) has a maximum threshold for this simulation and I found it to be 0.2 here.



## Random initialization with chemical reaction

$$k_f = 0.8, k_b = 0.2$$

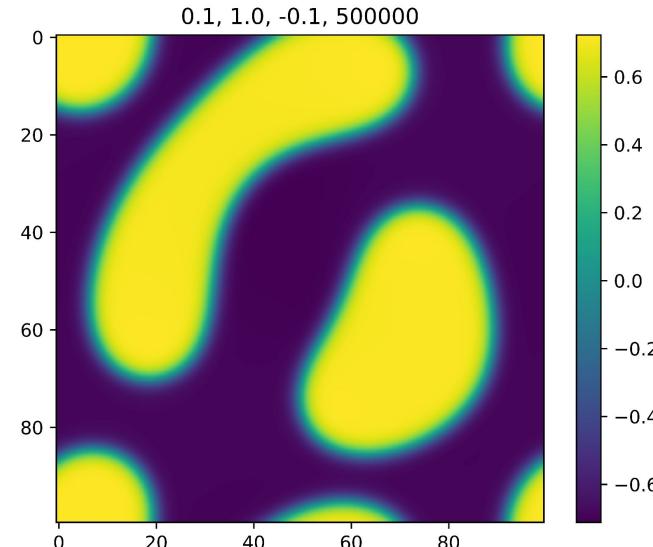
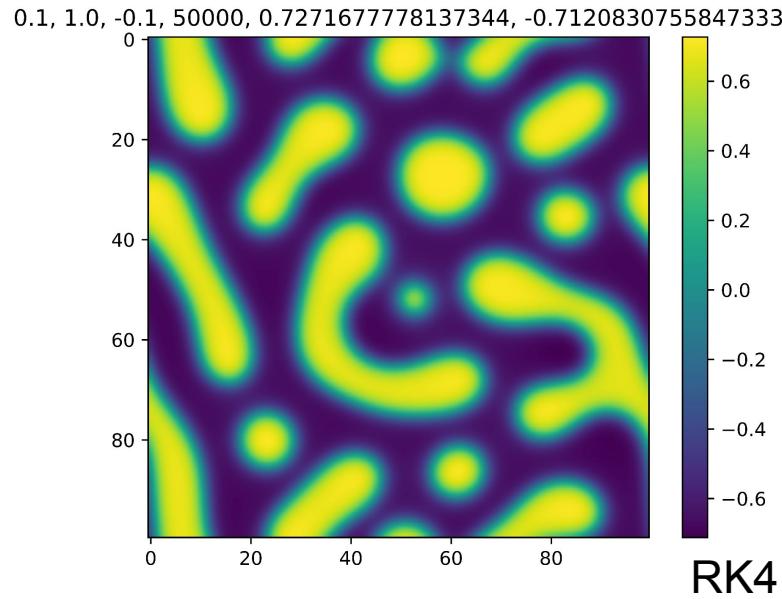


## RK4 time update

# Random initialization with chemical reaction

Another trial rates:  $k_f = 0.55$ ,  $k_b = 0.45$

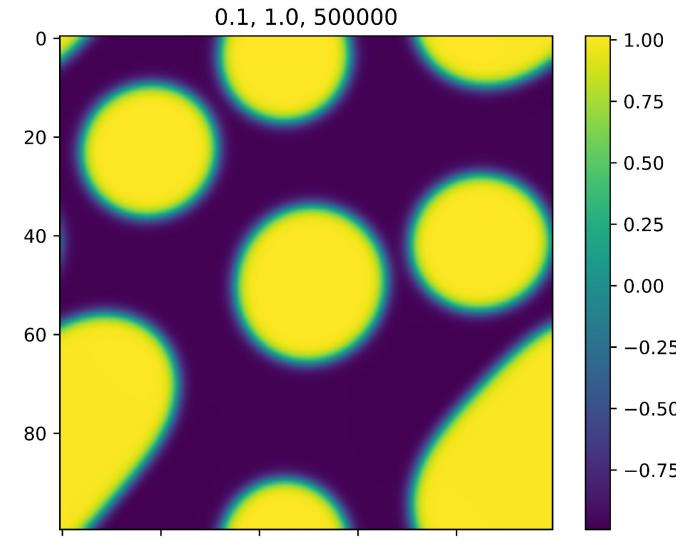
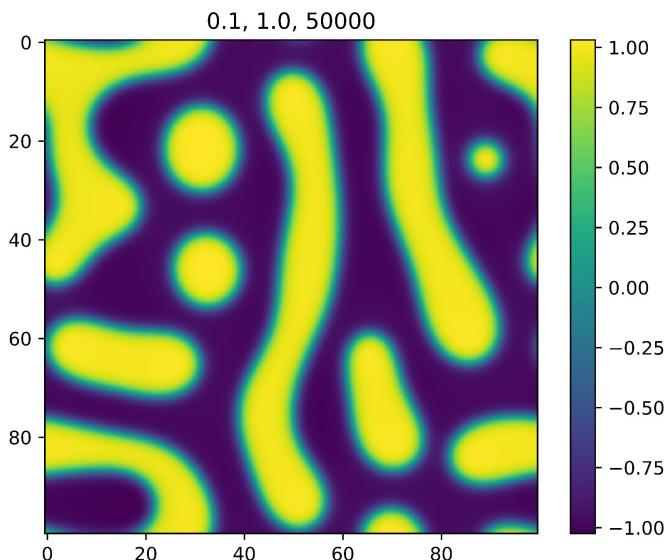
(Comparing with the last slide, Individual values of  $k_f, k_b$  are not affecting the dynamics significantly as long as the overall reaction rates are large)



Next intuition: Try much smaller reaction rates (also the Zwicker et.al paper talks about very small  $k_f, k_b$ )

## Random initialization with chemical reaction

Much much smaller reaction rates:  $k_f = 0.0005$ ,  $k_b = 0.005$

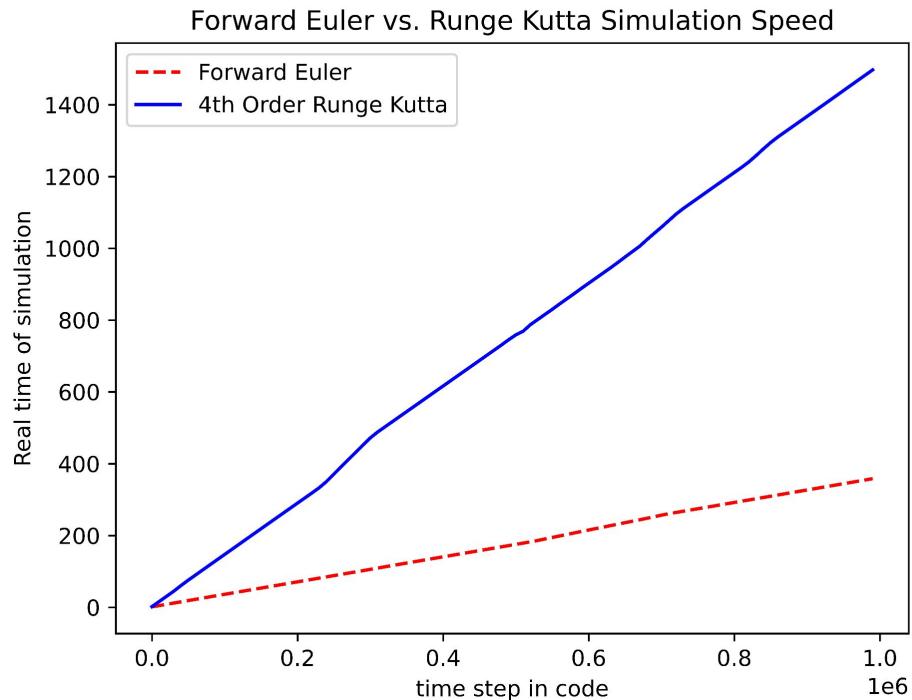


## RK4 time update (with numba)

Observation: Growth rate of droplet is faster now as reaction rate is very small and also the final steady state  $u$  inside doesn't deplete upto  $\sim \pm 0.7$  like the previous two slides.

# Speed Comparison: Forward Euler vs Runge-Kutta 4th order

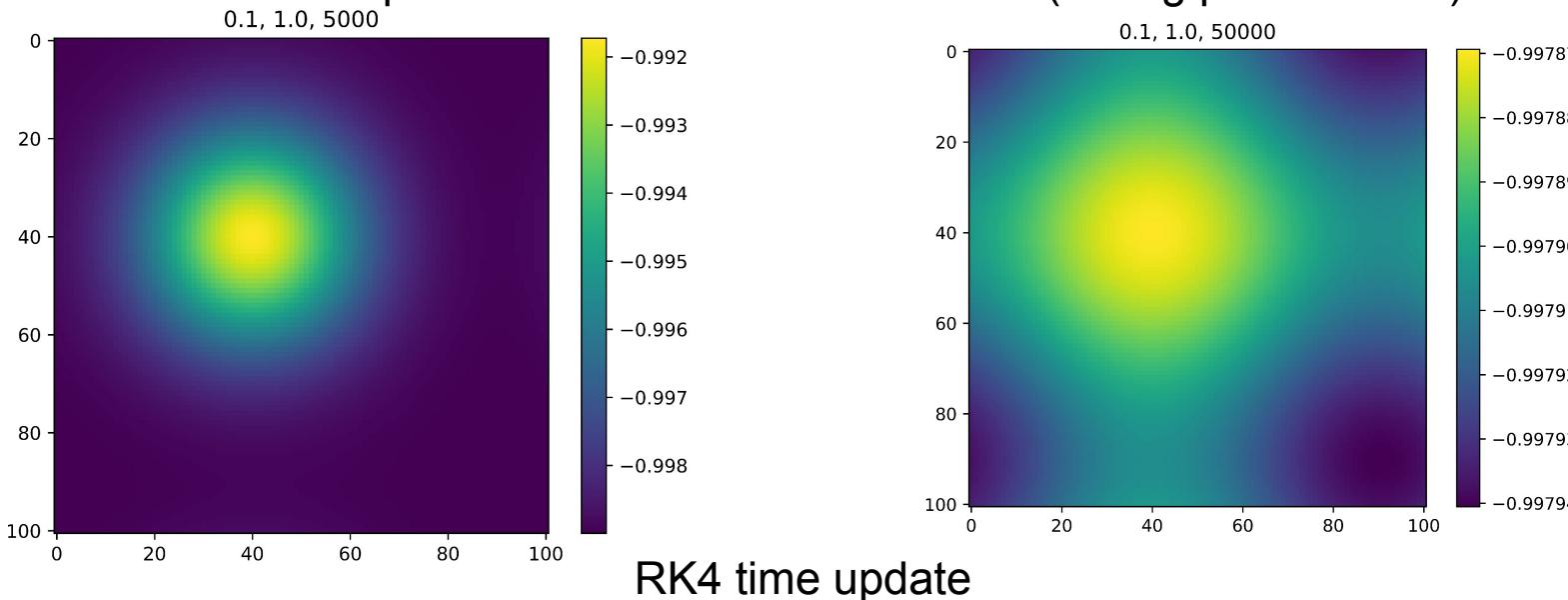
- Compared two different time stepping algorithm used in the project.
- It clearly shows the intuitive argument that RK4 method takes  $\sim 4$  times more real time to complete the simulation for a particular `simulation_end_time` or `time_step` (xaxis is in  $10^6$ ).
- RK4 can take higher interval `dt` but Euler method is not numerically suitable for high `dt`.



## Initialization: Single droplet with reaction (smaller radius)

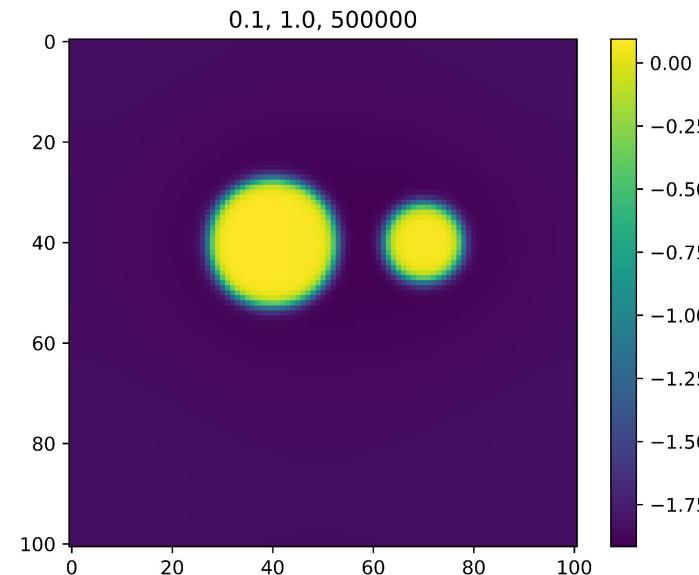
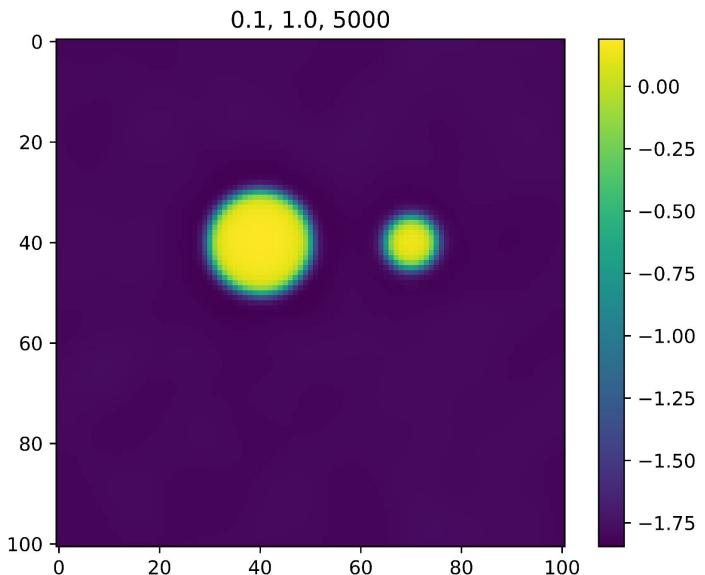
$$\text{Radius}(R) = 1$$

Compare with  $R=10$  as shown before (during presentation)



Droplet is not at all stabilised here. The colorbar here is somewhat misleading. Actually everything should be completely violet i.e. low in density which suggests fast reaction rates with smaller droplet vanishes quickly and whole system trying to read state with very low  $u$

Initialization: Two droplets with reaction( $R_1=10, R_2=5$ )



Here the observation says that both the droplets are increasing in size with time even in presence of the chemical reaction(ignore the colorbar, it reaches 0 at maximum and -2 as minimum because for 1 droplet,outside everything is -1 and so,at the spot of 2nd droplet it becomes 0 and so on). I feel my initialization is not correct here. I'll try to delve into it later.

# Conclusion and possible further steps

- ❖ Succeeded in showing liquid-liquid phase separation using two component binary fluid model.
  - Were able to show effects of parameter variation ( $k, \tau, \delta$ ) in phase separation time scale.
- ❖ Were able to show different initialization of the system
  - Successfully analyzed full time scale of phase separation starting from random initialization with both Forward Euler and Runge-Kutta(4th order) method and compared their speed in computational time.
  - Were able to initialize the system with a single droplet at a particular place on the grid using interpolating hyperbolic tangent function and make it evolve in time to finally show it grow in time - which is the phenomena known as Ostwald ripening.
- ❖ Tried to include first order chemical reaction scheme in the system and investigated how it affects the system for different initial condition - a) random initialization with slight negative density, b) single droplet.
  - For random initial state, the chemical reactions couldn't suppress growth of the droplets, although very fast reaction rates reduce the steady state density to  $\sim \pm 0.7$ , rather than reaching to  $\pm 1$
  - For single droplet initial state, very fast chemical reaction shrink the droplet and diffuse everything to reach a state with  $u$  close to -1
- ❖ Next step would be to understand the two droplet dynamics with effect of reaction to see if Ostwald ripening is suppressed and how.