nature portfolio

Peer Review File

Large-scale-integration and collective oscillations of 2D artificial cells

Corresponding Author: Dr Joshua Ricouvier

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors describe a new microfluidic chip design which allows them to diffusively couple an array of 30x30 “artificial cells”. The artificial cells constitute compartments which harbor DNA brushes and a cell free expression system capable of expressing “programmed” gene circuits such as homeostatic protein synthesis and oscillations. The major advance of the system over previous designs is that the compartments are laid out in a 2D grid rather than in a 1D array. In principle, this allows for a wider range of spatio-temporal patterns to be implemented and studied that explicitly require two spatial dimensions.

Unfortunately, the authors do provide concrete examples of such use cases. The phenomena they demonstrate (oscillator synchronization and phase waves) do not require a 2D system and can equally be observed in 1D. The manuscript therefore presents a solution that is looking for a problem and it is not clear what advantage one gains by the significantly increased engineering effort.

Taken together, I cannot recommend the manuscript for publication in Nature Communications and instead suggest that the authors submit to a more specialized journal.

A significant fraction of the main text deals with characterizing the diffusion of proteins through the channels. While this is obviously important to validate the experimental setup, most of it is simply confirming what one would expect from elementary considerations about diffusion in quasi-1D channels. More of this could be delegated to the Methods section.

On the other hand, important quantifications regarding the oscillations are missing: How many oscillation cycles can the system sustain? What is the distribution of the single oscillator frequencies? Are the oscillation frequencies uniform across the chip when the oscillators are not coupled?

I actually suspect that the phase waves that propagate from the boundary towards the center are simply an artifact of non-uniform oscillator frequencies where the oscillators near the boundary are slightly faster than those in the interior. This would explain why the authors observe an apparent decrease in the phase wave velocity over time: the frequency gradient would lead to a growing phase gradient which corresponds to a decreasing apparent velocity of the phase waves.

In fact, phase waves are not really a “collective mode” in the first place, since they do, by definition, not require spatial coupling. A phase gradient set up through the initial condition or non-uniform parameters is sufficient to produce phase waves. Trigger waves on the other hand require spatial coupling and are therefore a genuine collective phenomenon. Observing trigger waves would therefore significantly strengthen the manuscript. In 2D trigger waves can form spirals. Such spiral waves are a genuine 2D phenomenon. Their observation would therefore justify the extra engineering effort required to build a 2D array and make the manuscript interesting to a broader audience.

Minor issues

The abstract contains jargon like “collective modes” without proper explanation. The last sentence of the abstract is a bit of a buzzword collection that has little to do with what was actually done in the manuscript.

In the introduction and discussion, previous literature on synthetic coupled oscillators should be cited and addressed: Tompkins et al, Chaos (2015), Litschel et al., Lab Chip (2018). What advantages does the system implemented by the authors have over these previous experiments?

Line 49: I don't think the word "discovered" is appropriate here. Change to "observed".

Line 145: I don't understand the meaning of the phrase "we immobilized the genetic oscillator circuit on 30x30 cells"

Lines 176-178: What do "softer" and "rigid" mean here? I suggest the authors use more precise language.

I find the use of the word "geometry" throughout the manuscript a bit confusing. The authors refer to the small-scale geometric parameters, i.e. the width and lengths of channels etc. However in the context of reaction-diffusion systems, geometry usually refers to the "large-scale" geometry of the domain, i.e. its overall shape, whether it is flat or curved etc. Reaction-diffusion patterns can be quite sensitive to such features, similarly to how one can "hear the shape of a drum". The authors should consider using a different word than "geometry" or at least explain clearly what they mean by it.

Most of the captions are very terse and hard to understand. It would help if the authors could provide some explanation rather than just stating technical details. The description of Fig. 1B "Allocation of compartments according to gene constructs spotted" is unclear. Similarly, the description of Fig. 3D "Space-time pattern for a line of ten compartment orthogonal to the wave" is not clear.

Section V in the SI appears to describe only a single oscillator. It is not clear how the simulation results shown in Fig. 4e,f were obtained. It would be useful if the authors could include snapshots or movies from these simulations.

Typos

Line 82: (ref)

SI line 218: concentratTapez une équation ici.ion

Reviewer #3

(Remarks to the Author)
Intercellular communication and collective behaviors are ubiquitous in biology but difficult to study due to the complexity of the interactions and viability requirements. It is therefore very attractive to characterize the formation of collective patterns in simplified and well-controlled environments. Ricouvier and colleagues developed a microfluidic biochip that provides a well-controlled environment to observe reaction diffusion patterns arising from an array of 900 coupled cell-free gene expression reactions in a 2D space. In this system, they characterize the emergence of waves that propagate through an array of coupled genetic oscillators. The system the authors developed will be exciting to a diverse readership in physics, synthetic biology and related fields.

The transition from coupled compartments arranged in 1D chains, which the group reported previously, to a large 2D system, is a non-trivial advance. Ricouvier et al. overcame this challenge with a complex microfluidic 3D layout. What makes the design especially notable, in my opinion, is the ability to maintain gene expression at steady state levels for extended periods of time and the ability to precisely control diffusion rates between compartments through the architecture. The analyses and figures are of a high quality.

I have a few suggestions to improve the readability of the manuscript, in particular for scientists with different backgrounds, and to allow the community to build on the results.

Details provided in the Methods section

It would be nice to move the Methods section into the main text and to expand the description of the methods.

What are the sequences of the individual regulatory sequences and the entire linear DNA constructs that you use in the manuscript? You developed a highly robust genetic oscillator that will be interesting for other researchers to use in other systems. Providing annotated DNA sequences in the supplementary information pdf or in genbank format will help other researchers build on the results of the paper. I am aware that the oscillator has been described before (Tayar et al. 2017, PNAS) but it is difficult to piece together the circuit's DNA sequences from the referenced publications.

What concentrations do the individual DNA templates of the circuits have in the compartments on chip (or in the DNA spotting solution)?

The section about the preparation of TXTL reagents lacks detail. e.g. how is linear DNA stabilized? Source of the T7 RNA polymerase for some of the DNA templates that have T7 promoters? What was the composition of the reaction mixture?

Main manuscript

Line 82: A reference is missing.

Line 93: Fig. S3 doesn't seem to be the correct figure to reference here for the oscillator.

Lines 116-119: Can you clarify what result you are describing? I do not understand the sentence about the dynamics of isolated versus coupled compartments and the reference to Fig. S5. Are you referring to Fig. 2d?

In Fig. 2d, why don't coupled compartments reach a steady state? Would steady states be expected later on in time? Does your model capture and explain the peaks in the protein concentration dynamics?

Fig. 3d: Could you indicate in the overview images which 10 compartments are shown?

Line 164: It seems the figure reference should refer to Fig. 4a(inset) instead of Fig. 4b.

Lines 149ff, 167ff: Do you have an explanation why the phase waves propagate from the edges into the center? The waves in movie 2 are beautiful. Some more discussion of the result would be interesting.

Can you comment on how repeatable the results from the biochip are? For example, do the phase waves always look the same or do they differ from chip to chip? Similarly, for the same chip architecture, do you get the same steady states and oscillation periods for the calculated lifetimes of a particular compartment without coupling? Do the results in Fig. 1i originate from multiple independent chips?

Lines 174 – 187: I find the comparison between the weakly coupled oscillators (beta = 0.3, shown in Fig. S7 a&c) and the strongly coupled oscillators (beta = 1.1, shown in Fig. 3) very interesting but the description and the discussion of the results (e.g. the speeds of the phase waves) is very brief and mathematical. Could you expand the section, make it more accessible for non-physicists and add a supplementary video to compare the results from the two coupling modes?

In general, it would be helpful to refer to specific subpanels of the supplementary figures throughout the text, so it is clearer which specific results you are describing. My comments above mention some of the instances, where I was particularly confused but I noticed it in general.

Supplementary information

Page 6, line 143: reference to Fig. S2, did you mean S3?

Fig. S1 a/b: Not clear what the images show and what the design of the channels is. Can you add a drawing of the design and labels?

Fig. S2c: With what device were the droplets generated? In the methods section you mention two different devices for DNA deposition.

Fig. S3a: What does the red arrow mean?

Fig. S5b: Could you provide more details what the graph shows and how it was generated?

Fig. S5e: In my pdf, the graph is empty.

(Henrike Niederholtmeyer)

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have convincingly addressed all my concerns, improved the manuscript significantly and performed additional experiments and quantifications (presented in Figs. 4 and 5) that answer my previous questions. Overall, the quality of the manuscript has greatly improved and I think it will be of interest to a broad readership. I therefore recommend it for publication in Nature Communications.

I have only a few minor remarks:

In the introduction (line 50), the authors switch quite abruptly from describing what's done in the paper back to describing previous studies. I think it would be clearer if they moved the latter up and finished the introduction with a summary of their

own findings. In particular, they could emphasize that the 2D architecture allowed them to investigate the transversal profiles of the oscillation fronts for different coupling strength. This is impossible in 1D.

The grammar of the sentence in line 230 after the comma is broken.

Finally, the authors have no completely removed the term "phase wave" from the manuscript. I don't think this is a good idea, since this is the established term for the phenomenon they observe. I would therefore recommend that they briefly mention this, for instance in the section entitled "Front propagation in 2D coupled oscillator lattices" where they discuss the uncoupled lattice experiments (beta = 0). These experiments demonstrate that coupling is not crucial for the formation of the phase gradient and apparent front propagation, but is needed to smoothen the front and setting a lower bound for the propagation velocity.

Reviewer #3

(Remarks to the Author)
In their revised manuscript, Ricouvier et al have further extended and improved their analyses and descriptions. I would like to repeat my opinion that the development of a 2D array of such a large number of interconnected cells with controllable coupling is an impressive feat. It is exciting to have an experimental platform that allows the analysis of reaction diffusion patterns that are produced by cell-free genetic circuits in 2D. This setup offers tight control over all the important parameters and will be of interest to scientists from many different fields. The authors demonstrate the capabilities of their platform with a nice analysis of coupled oscillators. I found it very interesting how the degree of coupling influences the waves the oscillating network generates. I was very happy to see the additional explanations, analyses and results and look forward to seeing this paper published.

Minor edits:
- Line 145: Figure reference should be to Fig. 3d, I think.
- Thanks for adding details to the methods section and the sequences of the oscillator. Please also state concentrations or ratios of the different linear templates as this is important for network function and dynamics.

Reviewer #1 (Remarks to the Author):

Comments

The authors describe a new microfluidic chip design which allows them to diffusively couple an array of 30x30 "artificial cells". The artificial cells constitute compartments which harbor DNA brushes and a cell free expression system capable of expressing "programmed" gene circuits such as homeostatic protein synthesis and oscillations. The major advance of the system over previous designs is that the compartments are laid out in a 2D grid rather than in a 1D array. In principle, this allows for a wider range of spatio-temporal patterns to be implemented and studied that explicitly require two spatial dimensions.

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A significant fraction of the main text deals with characterizing the diffusion of proteins through the channels. While this is obviously important to validate the experimental setup, most of it is simply confirming what one would expect from elementary considerations about diffusion in quasi-1D channels. More of this could be delegated to the Methods section.

> Reply
>
> We thank the reviewer for the thoughtful comments on emphasizing the unique aspects of 2D, and the suggestion to delegate the considerations to the Methods. While the steady-state expression and diffusion model of the individual compartment seems a direct extension of work published 10 years ago, it was not an elementary step to work out and validate. Therefore, we felt that a summary of the physical principles and considerations would benefit the general readership. Following the reviewer's advice, we have now shorted the main text to a more concise and lucid summary of the physical principles, while expanding on the rigorous considerations in the Methods and Supp Info. With respect to innovation in 2D, we now believe the extended new data and analysis describe better the propagating 2D fronts in the coupled oscillating 2D array.

Comments

On the other hand, important quantifications regarding the oscillations are missing: How many oscillation cycles can the system sustain? What is the distribution of the single oscillator frequencies? Are the oscillation frequencies uniform across the chip when the oscillators are not coupled?

> Reply
>
> We thank the reviewer for these clarifying questions. The system can sustain oscillations as long as there is efficient TXTL reaction flowing in. Typically, we run the experiments between 12 and 20 hours. This led to observe between 6 to 10 oscillations with a period of 120 minutes in average. When the oscillators are not

coupled, their period exhibits a dispersity of 5%. These data are now presented in the main text and Supplementary Informations figures.

Comments

I actually suspect that the phase waves that propagate from the boundary towards the center are simply an artifact of non-uniform oscillator frequencies where the oscillators near the boundary are slightly faster than those in the interior. This would explain why the authors observe an apparent decrease in the phase wave velocity over time: the frequency gradient would lead to a growing phase gradient which corresponds to a decreasing apparent velocity of the phase waves.

Reply

We thank the reviewer for this comment. The oscillators are based on the activation and inhibition of transcription of mRNA from the DNA brush. Activator and inhibitor proteins are diluted in the medium and diffuse out from the compartment to its environment. At the lattice boundaries, compartments have less neighbors and their mirror compartments are perfectly synchronized. This change of coordination induces a slightly shorter period and higher amplitude, which generates fronts travelling toward the center of the lattice, persisting on distances of over fifteen compartments for strongly coupled lattices ($\beta = 1$). Weakly coupled lattices are less susceptible to the boundaries, as initial conditions, small variations in the underlying flow and inhomogeneities, dominate in creating propagating fronts with a reduced speed and shorter spatial extent of a few compartments. We added new experimental data in the SI, and new supplementary movies to emphasize the difference between coupled, weakly coupled and not coupled arrays. To strengthen this point, we added the additional data and extensive analysis presented in the new Figure 4 and 5.

Comments

In fact, phase waves are not really a “collective mode” in the first place, since they do, by definition, not require spatial coupling. A phase gradient set up through the initial condition or non-uniform parameters is sufficient to produce phase waves. Trigger waves on the other hand require spatial coupling and are therefore a genuine collective phenomenon. Observing trigger waves would therefore significantly strengthen the manuscript. In 2D trigger waves can form spirals. Such spiral waves are a genuine 2D phenomenon. Their observation would therefore justify the extra engineering effort required to build a 2D array and make the manuscript interesting to a broader audience.

Reply

We thank the reviewer for these insightful comments. We have removed the confusing term of “phase waves”, as they are indeed pseudo-waves that may be seen in an uncoupled lattice of oscillators due to initial conditions and phase/period inhomogeneities having nothing to do with coupled oscillations. We have now explicitly identified 2D fronts in coupled lattices as continuously deformed contours in 2D, with shape, smoothness, speed, and spatial extent determined by the coupling strength in the lattice. We characterized their dynamics using the phase gradient, angular autocorrelation function and power spectrum. We find long-range ordering of the phase variations, and an hourglass shaped Fourier transform that reveals a minimal velocity. We find these new 2D structures very

exciting. With respect to spiral waves, this is indeed a great idea, yet is a challenge that is well beyond the scope of this paper. Our system is currently a self-oscillating medium, and further genetic programming is required to achieve an excitable medium. While this is left for future work, the present manuscript lays the basic foundations of 2D systems.
New extended data and new Figures 4 and 5 as well as new Figures in the SI are supporting the description of the 2D fronts.

Minor issues

Comments
The abstract contains jargon like "collective modes" without proper explanation. The last sentence of the abstract is a bit of a buzzword collection that has little to do with what was actually done in the manuscript.

In the introduction and discussion, previous literature on synthetic coupled oscillators should be cited and addressed: Tompkins et al, Chaos (2015), Litschel et al., Lab Chip (2018). What advantages does the system implemented by the authors have over these previous experiments?"

Reply
The use of "collective modes" has been removed and abstract revised accordingly.

Previous literature on synthetic coupled oscillators: Tompkins et al, Chaos (2015), Litschel et al., Lab Chip (2018): have now been cited and addressed in the main text.

Our system differs in two main aspects. Firstly, it is encoded by genes that are expressed in a transcription-translation reaction. As such, there are degrees of freedom of design and programming not possible with chemicals. The present manuscript focuses on the case study of oscillators encoded by a set genes with control knobs difficult to achieve with chemicals.

Secondly, our system is inherently discrete with the heart of the oscillator in the DNA brush, where the newly made activator and repressor proteins carry out their function inside the DNA brush in binding DNA sequences to regulate their mutual expression and implement the feedback circuit. These new proteins diffuse from one cell to another through the capillaries, but they do not function outside the brush.

Comments
Line 49: I don't think the word "discovered" is appropriate here. Change to "observed".

Line 145: I don't understand the meaning of the phrase "we immobilized the genetic oscillator circuit on 30x30 cells"

Lines 176-178: What do "softer" and "rigid" mean here? I suggest the authors use more precise language.

Reply
We addressed all the comments above.

Comments
I find the use of the word “geometry” throughout the manuscript a bit confusing. The authors refer to the small-scale geometric parameters, i.e. the width and lengths of channels etc. However in the context of reaction-diffusion systems, geometry usually refers to the “large-scale” geometry of the domain, i.e. its overall shape, whether it is flat or curved etc. Reaction-diffusion patterns can be quite sensitive to such features, similarly to how one can “hear the shape of a drum”. The authors should consider using a different word than “geometry” or at least explain clearly what they mean by it.

Reply
We thank the reviewer for the comment. We removed the word “geometry” and use exact terminology related to width and lengths of channels, as well as shape and domains, as suggested.

Comments
Most of the captions are very terse and hard to understand. It would help if the authors could provide some explanation rather than just stating technical details. The description of Fig. 1B “Allocation of compartments according to gene constructs spotted” is unclear. Similarly, the description of Fig. 3D “Space-time pattern for a line of ten compartment orthogonal to the wave” is not clear. Section V in the SI appears to describe only a single oscillator. It is not clear how the simulation results shown in Fig. 4e,f were obtained. It would be useful if the authors could include snapshots or movies from these simulations.

Reply
The captions have been fixed and movies added.

Reviewer #3 (Remarks to the Author):

Intercellular communication and collective behaviors are ubiquitous in biology but difficult to study due to the complexity of the interactions and viability requirements. It is therefore very attractive to characterize the formation of collective patterns in simplified and well-controlled environments. Ricouvier and colleagues developed a microfluidic biochip that provides a well-controlled environment to observe reaction diffusion patterns arising from an array of 900 coupled cell-free gene expression reactions in a 2D space. In this system, they characterize the emergence of waves that propagate through an array of coupled genetic oscillators. The system the authors developed will be exciting to a diverse readership in physics, synthetic biology and related fields.

The transition from coupled compartments arranged in 1D chains, which the group reported previously, to a large 2D system, is a non-trivial advance. Ricouvier et al. overcame this challenge with a complex microfluidic 3D layout. What makes the design especially notable, in my opinion, is the ability to maintain gene expression at steady state levels for extended periods of time and the ability to precisely control diffusion rates between compartments through the architecture. The analyses and figures are of a high quality.

I have a few suggestions to improve the readability of the manuscript, in particular for scientists with different backgrounds, and to allow the community to build on the results.

The authors thank Prof Henrike Niederholtmeyer for her thoughtful and positive review.

Details provided in the Methods section

Comments
It would be nice to move the Methods section into the main text and to expand the description of the methods.

Reply
Thanks to this suggestion we modified the main text accordingly.

Comments
What are the sequences of the individual regulatory sequences and the entire linear DNA constructs that you use in the manuscript? You developed a highly robust genetic oscillator that will be interesting for other researchers to use in other systems. Providing annotated DNA sequences in the supplementary information pdf or in genbank format will help other researchers build on the results of the paper. I am aware that the oscillator has been described before (Tayar et al. 2017, PNAS) but it is difficult to piece together the circuit's DNA sequences from the referenced publications.

Reply
The genetic sequences have been added to the Supplementary Materials section of the paper. Additional informations are available upon request.

Comments
What concentrations do the individual DNA templates of the circuits have in the compartments on chip (or in the DNA spotting solution)?

Reply
The DNA spotting solution contains DNA concentrated 150-300nM. It allows DNA surface density at 500-1000 strands/μm^2. These informations have been added to the main text accordingly.

Comments
The section about the preparation of TXTL reagents lacks detail. e.g. how is linear DNA stabilized? Source of the T7 RNA polymerase for some of the DNA templates that have T7 promoters? What was the composition of the reaction mixture?

Reply
Linear DNA is either stabilized by adding Lambda GamS in the TXTL mixture or by using TXTL from a modified strain which is delta-recBCD. This statement has been added to the methods section of the main text. Therefore expression is

sustained as long as there is efficient cell extract flowing in the chip, approximately 10-20 hours depending on the experiments.

Main manuscript

Comments
Line 82: A reference is missing.

Reply
Change has been made accordingly.

Comments
Line 93: Fig. S3 doesn't seem to be the correct figure to reference here for the oscillator.

Reply
Change has been made accordingly.

Comments
Lines 116-119: Can you clarify what result you are describing? I do not understand the sentence about the dynamics of isolated versus coupled compartments and the reference to Fig. S5. Are you referring to Fig. 2d?

Reply
Overall the text was fixed and clarified.

Comments
In Fig. 2d, why don't coupled compartments reach a steady state? Would steady states be expected later on in time? Does your model capture and explain the peaks in the protein concentration dynamics?

Reply
Fig 2d shows the dynamics of a single source diluting out into empty neighboring compartments, as well as to the well, and this occurs recurrently at every step as the distance from the sources increases. The dynamics is therefore set by the compartment lifetime and the dilution time scales, yielding profiles that have a peak and decay. We did not observed steady state during the typical 8h experiments. For comparison, we added the dynamics of non-coupled compartments expressed on the same chip, which do reach steady-state during the same experiment.

Comments
Fig. 3d: Could you indicate in the overview images which 10 compartments are shown?
The figure has been changed.
Line 164: It seems the figure reference should refer to Fig. 4a(inset) instead of Fig. 4b.

Reply
Fig 3d and Fig 4 have been changed.

Comments

Lines 149ff, 167ff: Do you have an explanation why the phase waves propagate from the edges into the center? The waves in movie 2 are beautiful. Some more discussion of the result would be interesting.

Reply
We now provide a clear account of the effect of the boundaries.

Comments
Can you comment on how repeatable the results from the biochip are? For example, do the phase waves always look the same or do they differ from chip to chip? Similarly, for the same chip architecture, do you get the same steady states and oscillation periods for the calculated lifetimes of a particular compartment without coupling? Do the results in Fig. 1i originate from multiple independent chips?

Reply
We now present replica experiment of strongly and weakly coupled lattices as well as uncoupled ones. The data shows that general features are in repeatable with qualitatively the same dynamic. Specific features come about a signature of every chip related to initial conditions and impurities. Weakly coupled lattices are more sensitive to defects inside the lattice than to the natural boundaries. For unregulated expression of GFP, uncoupled compartment and coupled lattices exhibit qualitatively the same dynamic. For the oscillator, coupled oscillator tend to be slightly slower. This is now discussed in the text.

Comments
Lines 174 – 187: I find the comparison between the weakly coupled oscillators (beta = 0.3, shown in Fig. S7 a&c) and the strongly coupled oscillators (beta = 1.1, shown in Fig. 3) very interesting but the description and the discussion of the results (e.g. the speeds of the phase waves) is very brief and mathematical. Could you expand the section, make it more accessible for non-physicists and add a supplementary video to compare the results from the two coupling modes?

Comments
In general, it would be helpful to refer to specific subpanels of the supplementary figures throughout the text, so it is clearer which specific results you are describing. My comments above mention some of the instances, where I was particularly confused but I noticed it in general.

Reply
Thank you. The description and discussion have been extended and clarified.

Supplementary information

Comments
Page 6, line 143: reference to Fig. S2, did you mean S3?

Fig. S1 a/b: Not clear what the images show and what the design of the channels is. Can you add a drawing of the design and labels?

Reply
Yes, a drawing of the design has been added to the figure.

Comments
Fig. S2c: With what device were the droplets generated? In the methods section you mention two different devices for DNA deposition.

Reply
These images were taken after spotting using the Sciflexarray S3 (Scienion). The information has been added to the SI.

Comments
Fig. S3a: What does the red arrow mean?

Reply
The red arrow represents the direction of the underlying flow. The information has been added in the caption.

Comments
Fig. S5b: Could you provide more details what the graph shows and how it was generated?

Reply
Information has been added in the caption.

Comments
Fig. S5e: In my pdf, the graph is empty.

Reply
We were careful to add the graph in the revised version.

(Henrike Niederholtmeyer)

Response to referees

Referee 1

In the introduction (line 50), the authors switch quite abruptly from describing what's done in the paper back to describing previous studies. I think it would be clearer if they moved the latter up and finished the introduction with a summary of their own findings. In particular, they could emphasize that the 2D architecture allowed them to investigate the transversal profiles of the oscillation fronts for different coupling strength. This is impossible in 1D.

Changes have been made accordingly l.43 and l.54. The modified text is written in red.

The grammar of the sentence in line 230 after the comma is broken.

The addition is in red l.242

Finally, the authors have no completely removed the term "phase wave" from the manuscript. I don't think this is a good idea, since this is the established term for the phenomenon they observe. I would therefore recommend that they briefly mention this, for instance in the section entitled "Front propagation in 2D coupled oscillator lattices" where they discuss the uncoupled lattice experiments (beta = 0). These experiments demonstrate that coupling is not crucial for the formation of the phase gradient and apparent front propagation, but is needed to smoothen the front and setting a lower bound for the propagation velocity.

We now discuss phase waves l.221

Referee 2

- Line 145: Figure reference should be to Fig. 3d, I think.

Change has been made on Figure 3.

- Thanks for adding details to the methods section and the sequences of the oscillator. Please also state concentrations or ratios of the different linear templates as this is important for network function and dynamics.

We added the ratios in the Supplementary Information, section VI.b.