Coupling Belousov–Zhabotinsky Oscillations in 3D Reactionware

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WORKSHOP REPORT

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Acknowledgements

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Introduction

Three-dimensional (3D) printing (or Additive Manufacturing), which has been widely used in industrial manufacturing to produce prototypes,[1] has gained increasing momentum and popularity over the past decade in various fields such as medicine,[2] biology,[3-5] millifluidics/microfluidics,[6-8] electronics,[9] and pneumatics.[10-12] The key advantage of 3D printing lies in its ease, speed and price of design and manufacturing compared to commercial alternatives. Recently the concept of 3D-printed bespoke reactors, or "reactionware", for chemical science has been proposed by Cronin *et al.*[13-15] Another advantage of 3D printing is that the designs for a product can be shared online, downloaded, modified and then rapidly reproduced to meet personal demand.

The Belousov-Zhabotinskii (BZ) reaction is one of the most well-known and widely studied oscillating chemical reactions. Although it was first reported in 1958 by B. P. Belousov,[16] it was not widely accepted by the scientific community until the early sixties when more detailed studies had been completed by A. M. Zhabotinskii.[17] The first oscillating reactions of this type were produced by the addition of potassium bromate and citric acid to an acidified solution of cerium IV sulphate. After a short induction period, oscillations in intermediate concentrations are produced by autocatalytic single electron redox reactions involving the metal ion catalyst. The colour of the solution is seen to alternate between clear and pale yellow with a period of a few minutes. This oscillatory behaviour is sustained for several hours during which the substrate, citric acid, is decomposed to produce methanoic acid and carbon dioxide gas. Although all known BZ type oscillating reactions involve potassium bromate as the oxidising agent, the use of several different metal-ion catalysts, organic substrates and acids have been investigated.[18] Oscillations have been successfully obtained using various free metal ions or their complexes, all of which have a single electron couple with a reduction potential of around +1.5 V / SHE.[18]

In order to allow visual observation of the oscillations, $Fe(phen)_2^{2+}$ (ferroin) is often added to the reaction mixture (or even used as the main metal catalyst) as it has a strong red colouration but is bright blue in its oxidised form, ferrin. There is also a large range of organic substrates that give rise to oscillations in this system, although malonic acid (MA) is the most widely used. The mechanistic details of the cerium catalysed BZ reaction of malonic acid were elucidated in 1972 by Field, Kõrõs and Noyes[19] and supplied an intellectual framework for further investigation of this system. The skeleton FKN mechanism (so named after its discoverers) comprises eleven discrete reactions, but for a general mechanistic description, it is often more useful to simplify the FKN mechanism to a three step sequence often referred to as the Simple Oregonator Model (SOM):

{A} $BrO_3^- + 2Br^- + 3CH_2(COOH)_2 + 3H^+ \longrightarrow 3BrCH(COOH)_2 + 3H_2O$

{B} $4M^{(x)} + BrO_3^- + 5H^+ + CH_2(COOH)_2 \longrightarrow 4M^{(x+1)} + 3H_2O + BrCH(COOH)_2$

{C} $BrCH(COOH)_2 + 4M^{(x+1)} + 2H_2O \longrightarrow 5H^+ + Br^- + 4M^{(x)} + 2CO_2 + HCOOH$

The oscillations of the BZ reaction are sensitive to external physical perturbations and may become complex or show chaotic behaviour under some situations. For example, the period of oscillation is known to vary with temperature.[20] The stirring rate is also important with unstirred systems exhibiting chaotic effects while excessive stirring can totally inhibit oscillations.[21-22]

Given the control over the oscillatory behaviour of BZ reactions and the ability of non-oscillating reaction mixture to be excited by such oscillations researchers have theorised its use in basic computation systems,[23-24] and simulated architectures for its use in Boolean logic gates[25] and a binary adder.[26]

A model from which compatibility of a system can be tested is a cellular automaton. It consists of an array of cells which change their state based on a fixed set of rules and the states of their surrounding cells in time steps called generations. From a simple initial pattern of cells complex behaviour can quickly emerge out of such a system.[27] The concept has been extensively studied and a list of Rules exists on which an automaton can rely and reproduce an expected output e.g. Rule 110:

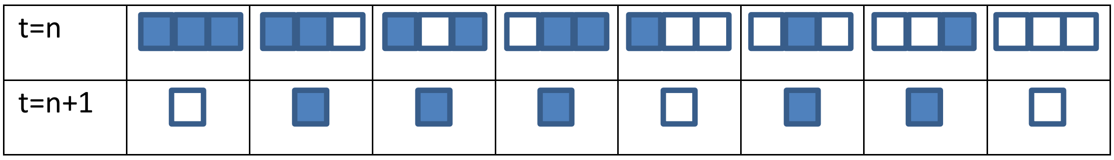


Figure 1: Rule 110 – Every group of three cells at time t = n affects the state of the cell centrally below it in the next generation at t = n+1. Blue cell = 1 state, white cell = 0 state.

Constructing cellular automata to conduct Boolean logic operations was proposed and simulated by Adamatzky.[28] Such a system involved using light sensitive catalyst loaded into a gel to control the reaction over a 2D array. Simulation of a 1D cellular automaton using reaction-diffusion chemistry to reproduce Rule 110 and Rule 60 was reported by Scalise and Schulman.[29] However no evidence could be found of a physical experimental system utilising reaction-diffusion chemistry to embody a cellular automaton.

Building on the previous work on reactionware to facilitate rapid production of different reactor designs, we aimed to investigate whether a series of individual 'cells' containing the BZ reaction could be controlled to emulate a cellular automaton. To do this we arranged patterns of small wells in a 3D-printed block and varied their size, number and fluidic connectivity while monitoring the BZ oscillations optically. Using the knowledge that stirring / not-stirring will influence the oscillatory behaviours of the BZ reaction cells, we positioned magnetic stirring bars in certain cells (in a pre-determined pattern) to see if the pattern of stirred cells was able to influence / drive the oscillations in a) neighbouring cells or b) the whole system.

Results and Discussion

Reactionware Design

The basic starting design for the reactionware comprised a monolithic circular block into which a square arena was inset. This was then divided into equal sized square cells by printing walls at equal spacings. The initial experiment was to determine the maximum sized cell that would continue to produce bulk oscillations over the whole cell, rather than break down into sub-cells. To do this a number of different wall configurations were needed, so the reaction container was configured with a grid of grooves in order to accept several different grids / cells sizes.

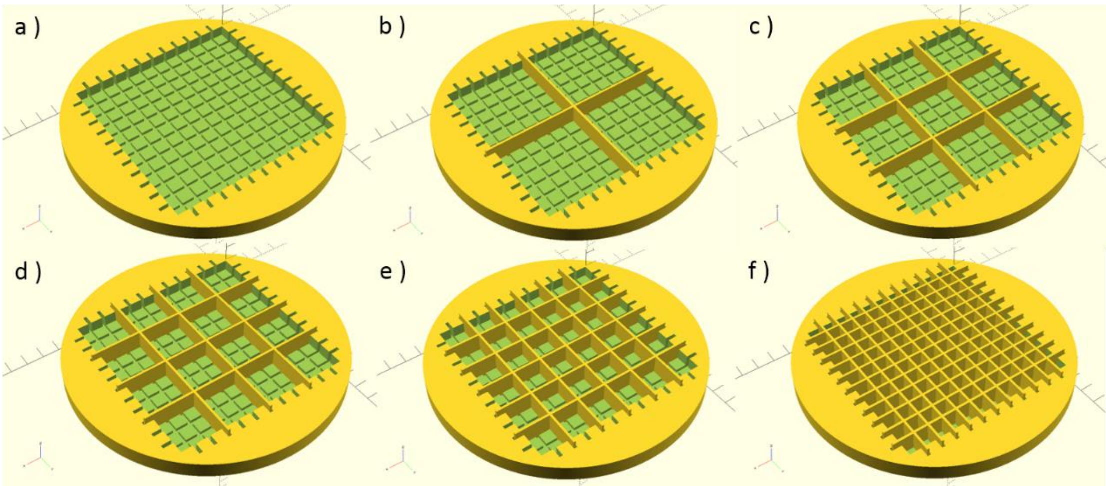


Figure 2: Reactionware of 110x110x5 mm arena divided into an array of 12x12 with grooves to allow for multiple configurations of cells using different wall insets: a) 1x1; b) 2x2; c) 3x3; d) 4x4; e) 6x6; f) 12x12.

A circular base structure was used to minimise the warping seen when printing straight lines/square structures on the 3D-printer. An array of 12x12 cells was used to allow for six different arrays: 1x1, 2x2, 3x3, 4x4, 6x6 & 12x12.

A similar design with a 5x5 array of 8x8x5 mm cells was also printed and used extensively in these experiments.

CAD models were designed using OpenSCAD software. A script was written allowing one to define the number and dimensions of cells and the dimensions of the overall reactor, as well as the number of walls and dimensions of channels between cells, if required.

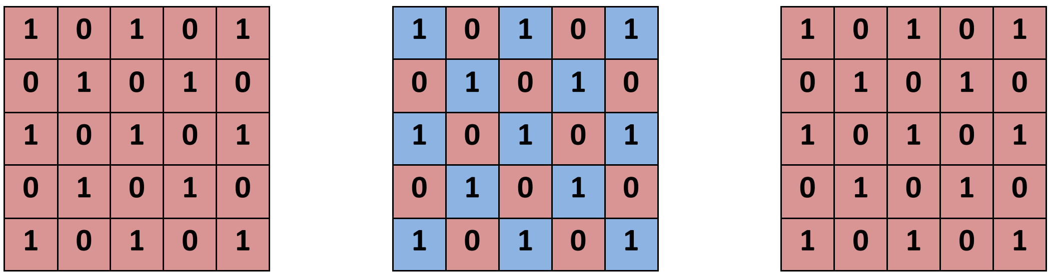
BZ Reaction Parameters and Control

In order to follow the BZ oscillations optically, we chose to use a ferroin catalysed system. This has two advantages: 1) ferroin provides a great blue vs. red colour change that is very easy to distinguish optically; 2) when ferroin is used as an indicator along with another metal salt catalyst, the BZ oscillations often become more complex, as the ferroin driven oscillations can become de-coupled. This de-coupling would make following the oscillations more difficult.

In order to ensure oscillations were visible in the reactionware, the BZ systems were first prepared in bulk and stirred, to allow the oscillations to initiate. Once initiated, the solution was poured into the reactionware and distributed over all the available reaction cells. Where stirring was to be used, the stirrer bars were placed in the cells from the beginning of the reaction but stirring was not started until ca. 5 minutes had elapsed. We found that he BZ oscillations were short-lived / supressed in the absence of stirring in the cells, although some oscillations did still develop. Where oscillations developed in the reactionware, they typically had a recycle time of between 30 seconds and 2 minutes.

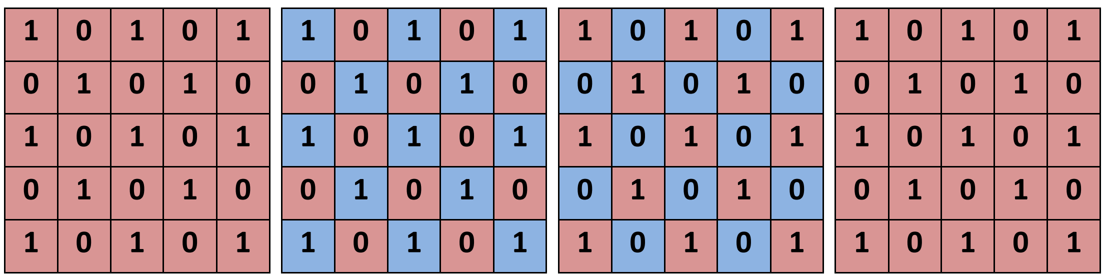
To take advantage of this, the whole reactionware block was placed on a magnetic stirring plate and magnetic stirring beads (3x5mm) were placed in some of the cells. This way the stirring could be turned on and those cells stirred, causing them to begin oscillating. The pattern of beads in the reactionware grid could then impart a pattern on the chemical system, where stirred cells were more likely to oscillate.

The ideal situation for unconnected cells, where pattern enforcement works might look like this (1 denotes a stirred cell while 0 is unstirred):



T=0 **T=n (oscillation)** **… etc**

The ideal situation for connected cells, where pattern enforcement works and drives neighbours might look like this (1 denotes a stirred cell while 0 is unstirred):



T=0 **T=n (oscillation)** **response** **… etc**

Initial Data Analysis and Results

The initial experiments aimed to establish the minimum size of unconnected cell which would support a stable oscillation. Using the two-part design of base and grid, a 85x85x5 mm reactionware arena was printed with a dividing grid that gave a 5x5 pattern of (approx.) 15x15x5 mm cells without any channels connecting them. 45 ml of already oscillating BZ reaction solution was added (recipe in experimental section) and timelapse images were acquired for two hours. Individual oscillations with a period of ~45 seconds were observed in the bulk and mostly the cells remained in phase (there may have been significant leakage and therefore fluidic connection between the cells). The oscillations became weaker and bulk changes were no longer observed after 10 minutes. Instead, the cells were seen to break down into chaotic regions, as might be expected in an unstirred system.

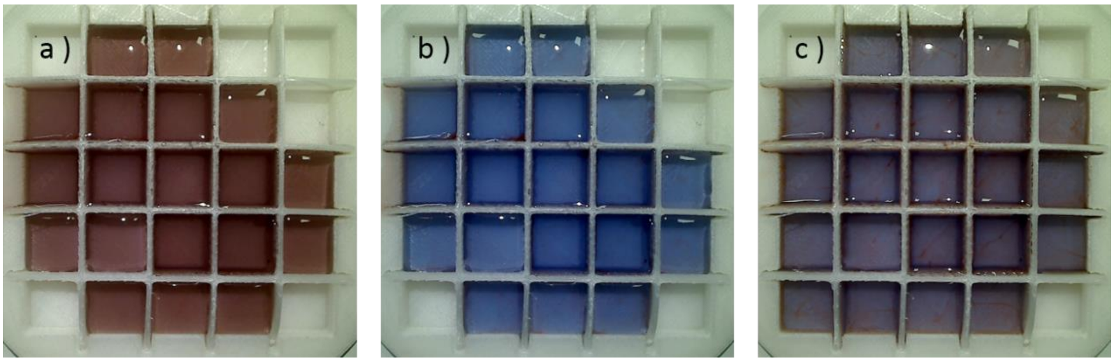


Figure 3: a) & b) Bulk oscillations with all the cells in synch. c) Cells no longer bulk oscillate, instead oscillating with chaotic structures seen as red streaks and spots.

Realising that the individual cells would not oscillate for sufficiently long without stirring, further experiments were carried out on a stirring plate with magnetic stirring beads placed in some wells. Reactionware with a 110x110x5 mm arena with an 11x11 grid of equally spaced grooves allowed different dividers to be used giving either a 12x12 or 6x6 grid of cells. Using a 6x6 grid, cells were (approx.) 12x12x5 mm and the grooved base meant that the fit of the grid was tight – cell to cell leakage was minimised.

A standard BZ mixture was added and stirring bars were added in the pattern: {000000,000000,111111,000000,000000,000000}. Initially bulk oscillations were observed over the whole grid, but after a few cycles, only those cells which were being stirred showed clear oscillations. The pattern was changed to {111111,000000,111111,000000,000000,000000} by the addition of stirring beads and within a few seconds, the newly stirred cells also began to oscillate cleanly. Removal of beads to give the pattern {111000,000000,111111,000000,000000,000000} resulted in the three cells that were no longer stirred oscillating for 3-4 more cycles and then breaking down. This clearly shows that control of individual oscillators (stopping and starting) is possible with stirring.

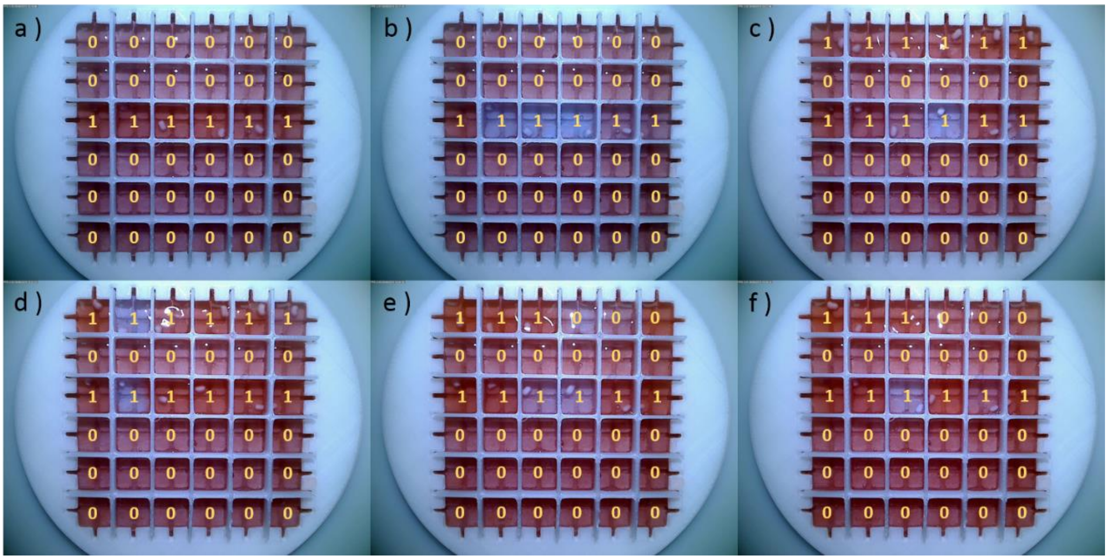


Figure 4: a) Initial uniform bulk oscillation. b) Only stirred cells oscillating. c) Stirrers placed in initially non-oscillating cells. d) Stirrers initiate oscillations in cells. e) Stirrers removed but oscillations continue for a few cycles. f) Only stirred cells continue to oscillate.

Lifting the grid slightly above the bottom of the base in the same reactionware allowed the cells to be fluidically connected. Applying a stirring pattern of {111111,000000,101010,000000,111111,000000}, the stirred cells were seen to oscillate clearly and cells with at least two stirred neighbours, while not oscillating as convincingly, were certainly influenced. This is evident in the timelapse video but is not clearly demonstrable with still images.

In the above experiment, the cells were probably too large to be influenced by just one oscillating neighbour. To solve this, we tested smaller wells. Dividing the same arena into a 12x12 grid resulted in cells that were too small to be imaged easily due to shadowing from the walls.

After some testing, the design that gave the best consistent results was a 55x55x5 mm arena divided into 5x5 grid of square wells (approx. 9x9x5 mm) where the barriers had large channels cut out from the top (to prevent stirrer bars from travelling between cells). Applying the stirring pattern {10101,01010,10101,01010,10101} produced excellent oscillations in the stirred cells which were clearly coupled into the neighbouring unstirred cells.

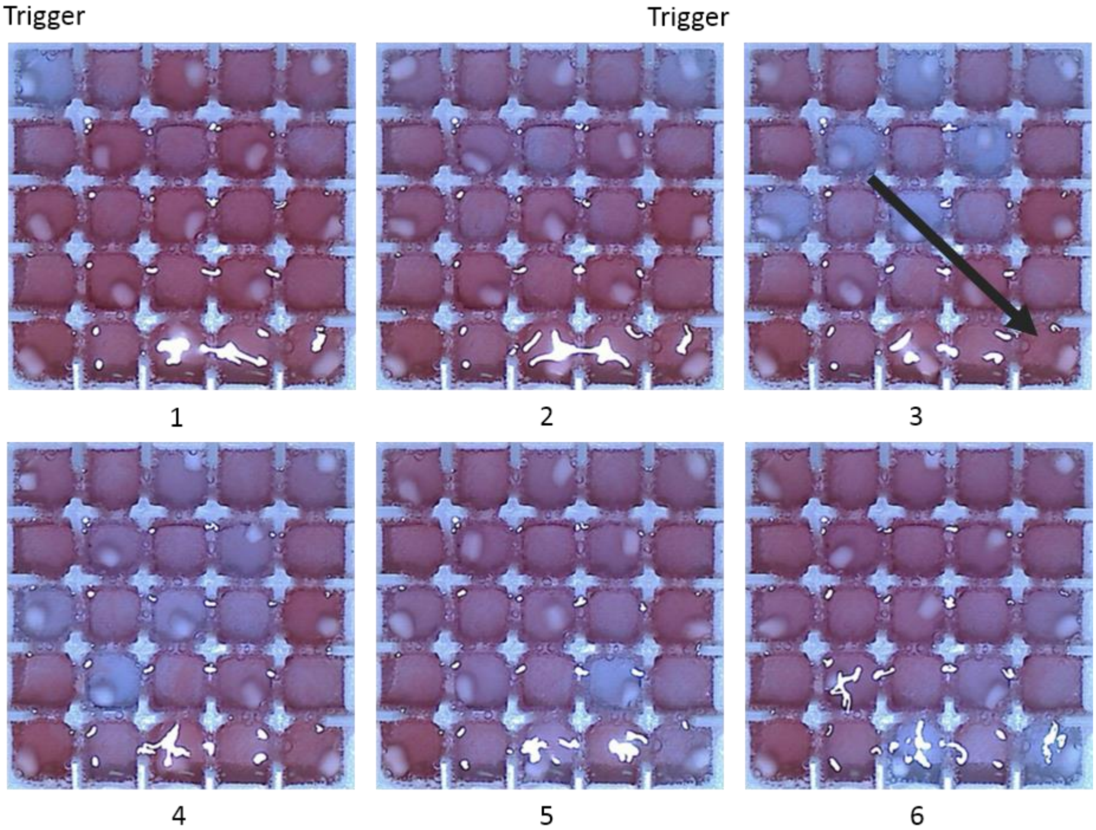


Figure 5: Oscillations eventually coordinated into two triggers in two corners initiating a chemical wave which travelled from one corner to another (in direction of arrow). This behaviour repeated for several cycles until oscillations fell out of phase with each other.

Stirring was stopped and oscillations continued for 3-4 more cycles before dying out. Once stirring was restarted oscillations restarted instantaneously as before.

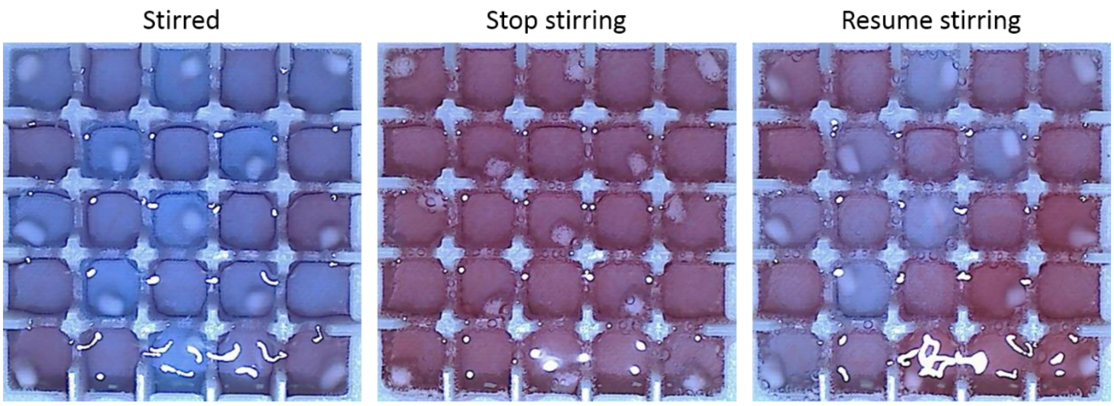


Figure 6: Oscillations could be stopped by switching the stirring off and restarted almost immediately by switching the stirring back on.

Two more 5x5 bases were printed with channels in the walls to allow fluidic connections between the cells. However on this day it appeared that the BZ solution was not oscillating well so no oscillation patterns were observed. For each base an inset with different channel height (from the bottom of the cell) was used. A height of 2mm led to stirrer beads hopping between cells. 3mm proved sufficient to contain the stirrer beads.

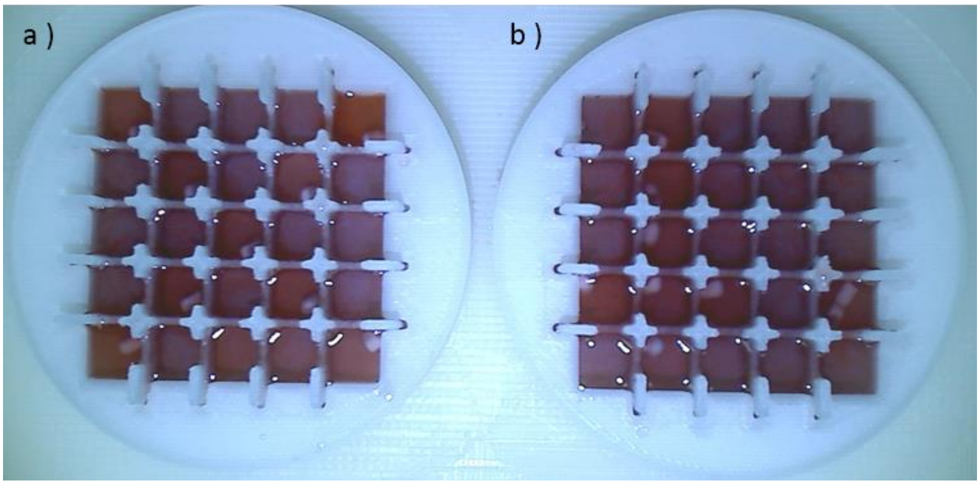


Figure 7: No oscillations observed. a) {10001,01010,00100,01010,10001}. b) {01000,01000,01000,11111,01000}

A monolithic reactor was designed and printed which used circular cells instead of square ones. This guaranteed that the only fluidic connection between cells was through the cutout/printed channels and utilised the printer's accuracy for printing circular structures.

Into a reactor of 6x6 cells of 8 mm diameter and 5 mm depth channels were cut out to allow fluidic connection. Three experiments were carried out with the following configurations:

{101010,010101,101010,010101,101010,010101}

Circular cells produced travelling oscillations as seen in the previous experiment but the cells did not fully bulk oscillate. In addition there were fewer repeated oscillation patterns than the previous experiment. This would imply that the cells were too large to fully bulk oscillate.

{111111,000000,000000,111111,000000,000000}

Apart from the first few whole array oscillations there were no sustained oscillations in any of the cells, stirred or not. There were however flow like patterns in the unstirred cells between the stirred cells. The 'direction' of flow was maintained for the duration of the experiment. It is unclear whether this was physical flow of material or the illusion of flow due to oscillation pattern of the reaction.

{000000,011110,010010,010010,011110,000000}

Less than ten full array oscillations were observed before settling to non-oscillating state. The central four, unstirred cells appeared to approach oscillation after this period but no full oscillations were observed.

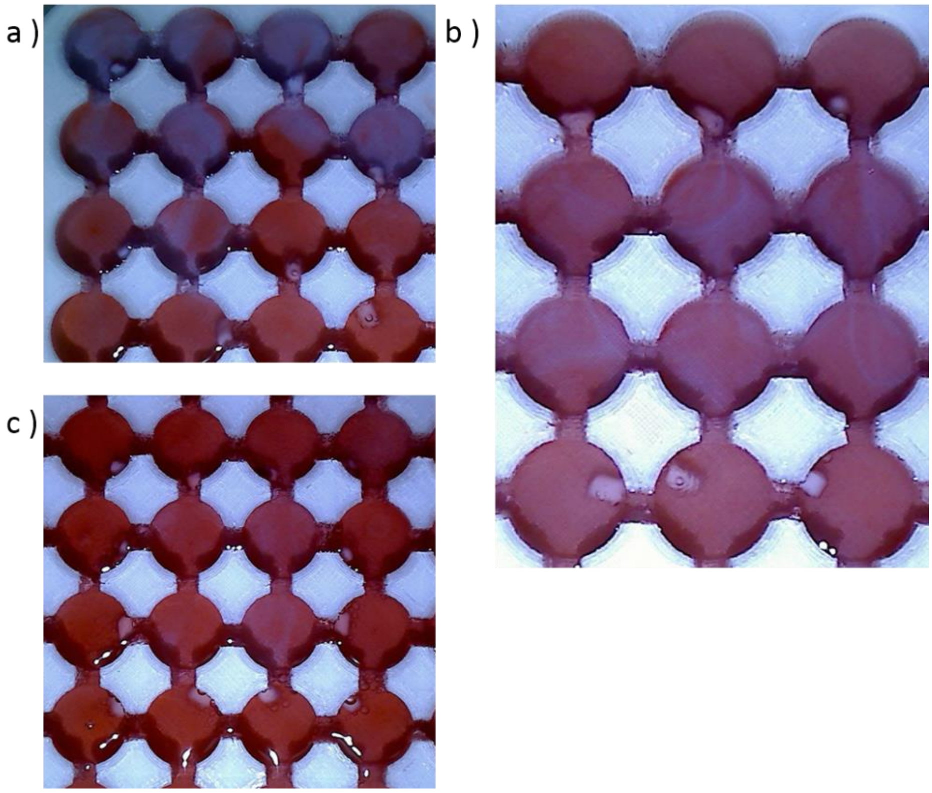


Figure 8: a) {101010,010101,101010,010101,101010,010101} pattern displaying oscillations but remained structured within the cells. b) {111111,00000,000000,111111,000000,000000} pattern displaying no oscillations but flow patterns. c) {000000,011110,010010,010010,011110,000000} showing no sustained oscillations.

The final experiment was used to try the idea of a gravity fed cascade of BZ reaction to see whether oscillations could travel while the reaction mixture was flowing. The following figure depicts the CAD drawing which was 3D printed for use as a cascade:

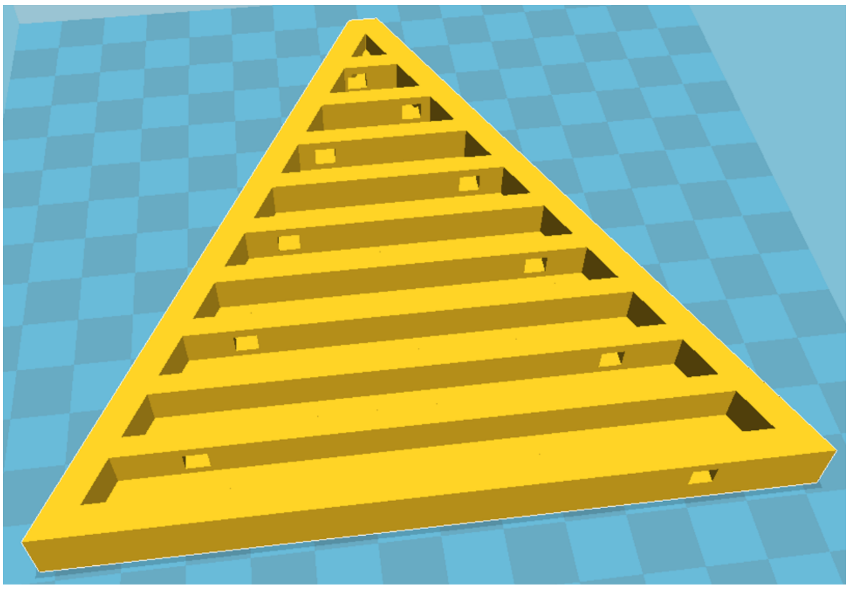


Figure 9: Cascade of ten levels with holes connecting each one to the next. Reactions fed in from pointed top.

It consisted of ten layers within a triangular cascade, where holes in alternate ends of each layer allowed fluid to flow along each layer. Reaction mixture was fed in from the top via a reservoir and collected out of the bottom into a beaker. A glass facing was glued onto the front to allow the colour of the oscillating mixture to be videoed.

Once a reaction mixture was determined to be oscillating, about half was poured into the top reservoir and allowed to flow through and collected in the beaker. The other half was left to stir on a stirrer plate. Before the top reservoir emptied completely the other half of the mixture was added. This was repeated by hand for about 20 minutes.

It was found that oscillations could be observed which travelled from their point of origin in the top reservoir and travelled through the cascade. The rate of flow had two important effects on observing oscillations in the cascade:

1. High flow meant that there was good fluidic connection between the layers of the cascade and any oscillations would travel through all the layers. However this led to shorter periods of time between reservoir refills, which interrupted any oscillation if a refill coincided with an oscillation.

2. Low flow would mean that an oscillation would definitely occur in the time between refills. However this also led to poor fluidic connection between layers and any oscillations which did occur only travelled as far as the first 2-3 layers.

An intermediate flow was found by using a bulldog clip to control the flow through the tubing connecting the top of the cascade and the reservoir. Doing so led to video of footage of several oscillations which travelled through the cascade despite the physical flow of reaction mixture. Improvements would include automation of refilling with pumps and printing with hydrophilic polymer such as polylactic acid to fully fill cascade.

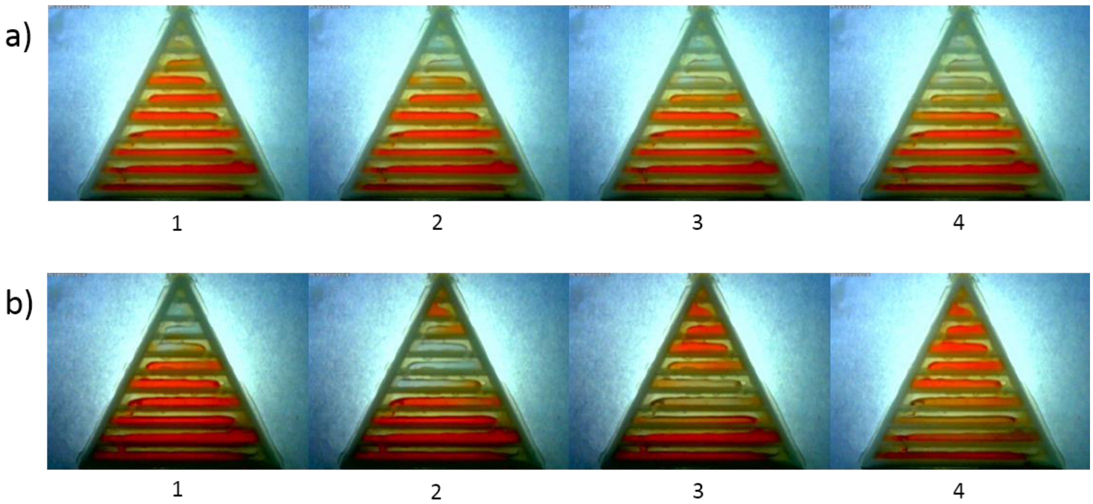


Figure 10: a) Low flowrate led to confinement of oscillation in first three layers. b) Median flowrate led to oscillation travelling through most of the layers of the cascade. High flowrate led to interruption of oscillation before it could be recorded.

Experimental Section

All chemicals were purchased from Alfa Aesar or Beijing Chemical Works, and used as received.

3D Printing: 3D printing was achieved on Airwolf HD2x 3D printer, using Poly-propylene (PP) supplied by Barnes Plastic Welding Equipment Ltd, Blackburn, UK. To prevent corners from lifting due to warping, PP was printed on a 5 mm thick PP sheet

Design Software: 3D-printed reactors used in this work were designed on OpenSCAD computer aided design software (http://www.openscad.org/), and were translated into 3D printer instruction files using open source Cura15 (powered by Ultimaker) software (https://ultimaker.com/en/products/cura-software).

Reactionware: The designs for each type of reactionware were saved in a .scad script to allow for easy changing of reactor parameters. The following files and tables of parameters were used to produce all the reactionware depicted in this report.

GC_BZ-3D_square_in_circular_grooves_with_grid_and_channels.scad

Square	nx/y	cx/y	cz	base	edge	gw	gz	gh	shim	raise	prop
Figure 1	12	9	5	3	10	2	2	6	0.3		0.2
Figure 3	5	16	5	3	10	2	2	6	0.3		0.2
Figure 4	6	18	5	3	10	2	2	6	0.3		0.2
Figure 5	5	11	5	3	10	2	2	6	0.3		0.2
Figure 7 (a)	5	11	5	3	10	2	2	6	0.3	2	0.2
Figure 7 (b)	5	11	5	3	10	2	2	6	0.3	3	0.2

KD_BZ_3d_circle_cell_monolith.scad

Circular	nx/y	cd	ch	gd	gh	gl	edge	base			
Figure 8	6	15	8	3	3	3	3	4			

WZL_BZ-3D_Trapezoid_cascade.scad

Cascade	lay	Hl	h	edge	gap	base	cw	cd	tcw	tcp	in
Figure 9	10	7.5	7.5	5	3.5	3	4	4	2	2	2

BZ Reaction Recipe: We chose to use a ferroin catalysed BZ system as this allows fairly ‘simple’ oscillations which can be easily followed optically. The following recipe was used in the experiments:

- 20ml of 1M Malonic Acid in water
- 20ml of 0.5M $KBrO_3$ in 1M H_2SO_4
- 9ml of 1M H_2SO_4
- 1ml ferroin solution (0.025M, purchased as solution)
- Made up to total 90ml with 39ml water

The experiments were initiated by adding the potassium bromate solution to the other reagents pre-mixed in a beaker. This was stirred for a few minutes to allow bulk oscillations to initiate before being added to the reactionware container.
Initial concentrations of the reagents were as follows:

- Malonic Acid 0.222M
- H_2SO_4 0.322M
- $KBrO_3$ 0.111M
- Ferroin 2.8×10^{-4}M

Imaging: The oscillations were imaged using a Logitech QuickCam Pro 9000 webcam (at 1600x1200px, 24bit) running 1second timelapse with iSpy Connect software. In each experiment, the reactionware was illuminated from above using an LED torch running from a continuous supply to ensure constant output level.

Conclusion

Image analysis and mapping of the oscillations was not within the scope of the workshop and this work is ongoing. Based on the workshop data, the following statements can be made:

Stirring can be used to control individual cells. While cells that were not stirred did sometimes oscillate 3-4 times after initiation, the oscillations were not sustained further, even when the whole arena was used (no divisions). Stirred cells showed sustained oscillation (up to 1.5 hours) and previously unstirred cells could be re-started to oscillate if stirred.

Fluid connectivity played a crucial role in propagation of excitations. Hydrophobicity of PP structures caused poor fluidic connection between cells connected by channels with diameters less than ~3 mm. Even when a fluidic connection could be seen chemical excitations did not appear to propagate for such narrow channels. The hydrophobicity of PP also prevented the channels in the cascade from fully filling. This could be mitigated by using hydrophobic polymers such as polylactic acid (PLA).

Oscillating cells could initiate oscillations in neighbouring cells if fluidically connected. The unstirred cells would remain dormant as an excitable medium and oscillate once initiated by a neighbouring oscillation. As seen in figures 5 & 6 cells over a whole array can couple their oscillations to produce collective oscillations and travelling chemical wavefronts.

Future work will involve the following:

Image analysis of existing and future video data. The videos/image stacks must be analysed to extract colour change data from the cells and assign numerical values to stages of the oscillations. This would allow us to reduce the cells to binary arrays and allow tracking of oscillations to determine relationships, if any, between stirred and unstirred cells, and the arrays as a whole.

From this pattern recognition algorithms could be used. This would be important for determining whether such an array of reaction-diffusion medium could act as a cellular automaton.

An obvious continuation of the work which will be carried out in the Cronin Lab at Glasgow will be to build a reactionware system where each cell can be stirred individually. This will allow the pattern of stirred cells to be stopped / started / modified in real time and the effect of this on the oscillatory behaviour to be followed. It may even be possible to implement some image recognition and feedback, such that stirring can be used to enforce a pattern.

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