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## Research Article

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# **New pesticide detection method: Combining microfluidics with GC-MS coupling technique**

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## **ABSTRACT**

Pesticide residues entering the human body through the food chain can induce many chronic diseases and pose a serious threat to human life and health. To break away from the dependence of traditional detection methods on equipment and time, a 3D printed chip with high mixing performance was designed based on microfluidic technology. Its excellent mixing effect further improves the efficiency and accuracy of gas chromatography-mass spectrometry (GC-MS) detection. The overall structure of the chip channel is in an "S" shape, and circular flow guide columns, pressure-driven convection structures, rectangular turning structures, and stratified rotation structures are set in the channel path. A total of 8 sets of simulation experiments were conducted to study the mixing performance of the overall structure of the chip. Under the conditions of a diffusion coefficient of  $1\text{e-}11\text{ m}^2/\text{s}$  and a Reynolds number (Re) of

0.5-200, the mixing index was as high as 96.67-99.53%, indicating that the chip has extremely excellent mixing performance. At the same time, colorimetric experiments and GC-MS detection experiments were carried out. In the colorimetric experiment, the mixing effect of the chip was in good agreement with the simulation cloud map. In the detection experiment, the detection value of the pesticide solution (DDV, Dichlorvos) mixed by the microfluidic chip was highly consistent with the concentration of the diluted pesticide solution, verifying the scientific rationality of the microfluidic chip structure. This provides a certain theoretical and practical basis for promoting the integrated interaction of microfluidic technology and GC-MS detection technology.

**Keywords:** 3D printing , pesticide residue detection , microfluidic chip , micro-mixing

## Introduction

In modern agricultural production, the use of pesticides is a crucial means to ensure crop yields. However, the extensive and long-term use of pesticides has brought about significant and overlooked potential hazards to the environment, animals, plants, and human health [1-3], forming a full-chain risk from the field to the table. Therefore, pesticide residue testing is urgently needed [4-8].

The current mainstream pesticide detection methods include chromatographic detection, mass spectrometry detection, surface-enhanced Raman spectroscopy detection, etc. [9-13]. Although these methods have advantages such as high accuracy and high selectivity, they have shortcomings such as complex operation, time-consuming and labor-intensive, and can only be limited to laboratory scenarios, making it difficult to achieve real-time and rapid detection. With the continuous progress of micro-technology, microfluidic detection technology has emerged. This technology, with core advantages such as integration, high throughput, high mixing efficiency and high sensitivity, has gradually gained prominence in pesticide residue detection [14-16] and biomedical fields, effectively complementing the limitations of

traditional detection methods. These technologies that combine microfluidics with pesticide detection include dual-mode fluorescence colorimetric sensors [17], surface-enhanced Raman spectroscopy (SERS) aptamer sensors [18], colorimetric sensors [19], and methods based on fluorescence resonance energy transfer (FRET) [20]. In the process of detecting trace pesticides, efficient mixing is a key step to ensure the complete reaction and improve the sensitivity and accuracy of detection. Especially in microfluidic chips or portable detection devices, since the fluid is in a low Reynolds number laminar flow state, natural diffusion mixing is slow, and active mixing needs to be achieved through targeted design [21]. Dibya Jyoti Behera et al. [22] summarized the method of paper-based microfluidic devices ( $\mu$ PADs) through the design of labyrinth-shaped hydrophilic channels to extend the diffusion path driven by capillary force, achieving sufficient mixing of pesticides and reagents, adapting to various detection methods, and being widely used in on-site analysis of organophosphorus and pyrethroid pesticides. Although this method can adapt to the qualitative detection of most pesticides, it has a defect in quantitative detection accuracy. Liu et al. [23] developed a double-layer fluorescent paper-based sensor, promoting the mixture of samples and GO-Cy3-labeled probes through capillary action, detecting oxidol and combining a smartphone platform and LeNet model to complete the detection within 10 minutes. Sarida Naorungroj's [24] review systematically summarized the evolution of microfluidic electrochemical sensing platforms, pointing out that through fluid control strategies (such as accelerated flow, sequential flow, etc.) it is possible to effectively improve mixing and reaction efficiency, and has achieved high-sensitivity detection of paraquat and organophosphorus pesticides on various substrates, providing an important reference for the large-scale application of microfluidic technology in pesticide residue monitoring.

The aforementioned method cannot achieve a balance between "speed and accuracy" in pesticide detection. Moreover, the pesticide residue limit standards in international trade are becoming increasingly strict, which has become a key barrier for agricultural product exports. There is an urgent need for precise and efficient

pesticide residue detection technologies to support food safety supervision and trade clearance. Traditional detection methods are no longer able to meet the dual requirements of "rapid screening+precise confirmation".

In the research on the mixing performance of microfluidic chips, the mixing index, energy consumption (characterized by pressure drop), and operational adaptability (Reynolds number, diffusion coefficient) are the core evaluation dimensions. The current designs still have significant limitations in their overall performance under harsh conditions. At low Reynolds numbers and low diffusion coefficients, the mixing index of Najafpour et al. [25]'s 3D electroosmotic structure was only 92.88%, while Jin et al. [26]'s fan-shaped barrier structure, although using the same low diffusion coefficient ( $1\text{e-}11\text{m}^2/\text{s}$ ) as this study, had a mixing index of 87%-92% and a pressure drop of 1541Pa. Wu and Li et al. [27-28]'s electro-osmotic piezoelectric vibration scheme, although driven by eddy currents or external fields, relied on high diffusion coefficients and even so, the mixing index was still below 97%, and the pressure drop was as high as 35000Pa, with extremely high energy consumption costs. Some designs were also limited by passive diffusion-dominated mixing modes, such as Bie, Wang, and Yazdani et al. [29-31]'s sudden expansion and contraction configurations, which overly relied on molecular diffusion at low Reynolds numbers and lacked active flow field disturbances. The mixing index was significantly lagging behind, and the Zaitri and Chen et al. [32-33]'s multi-branch complex channels, although optimizing the flow field through diversion recombination, had weak fluid disturbances at low Reynolds numbers and still could not meet the requirements for mixing degree. Meanwhile, the flow channel structure design also led to an increase in energy consumption for some schemes, such as Sun and Pourghasemian et al. [34-35]'s fishbone and snake-shaped flow channels, affected by the compression effect of the flow channel space or the increase in flow channel resistance, the pressure drop increased significantly during operation. Farahani and Bayareh et al. [36-37]'s helical electroosmotic composite design also needed additional barrier structures or high voltage input, resulting in limited

improvement in the mixing index and a sharp increase in pressure drop, with limited practicality and economy. In conclusion, even though the existing designs were in more favorable diffusion conditions (some schemes had diffusion coefficients up to 230 times that of this study's chip), their performance still had significant shortcomings. Either the upper limit of the mixing index was only 96%, or the pressure drop was as high as 35000Pa, or both did not meet the requirements. However, the chip designed in this study could still achieve high mixing index (96.67%-99.53%) and low energy consumption (pressure drop of only 2.4356-1153.969Pa) in a wide Reynolds number range ( $Re=0.5-200$ ) under harsh conditions (diffusion coefficient as low as  $1e-11m^2/s$ ), and its comprehensive performance was significantly superior to the existing schemes.

This research combines microfluidics with the GC-MS combined method and uses the 3D printing processing method to fabricate microfluidic chips with high precision and smooth flow channels. Thanks to the unique advantages brought by the "microchannel scale (micrometer level)" of microfluidics technology, it becomes a key breakthrough to solve the pain points of traditional detection. It not only improves "speed" but also retains "accuracy", and reduces the consumption of chemical reagents and the impact on the environment.

## **1. Microfluidic chip structure design**

As shown in Figure 1, the length of the microfluidic chip is 15.3 mm, the width is 12 mm, and the thickness is 2 mm. The main structure of the microfluidic chip consists of multiple complex-sectional flow channels. The chip's inlet and outlet are circular channels with a diameter of 0.6 mm, the inlet length is 2.5 mm, and the outlet length is 2.4 mm. At the inlet, the channel extends laterally for 2.5 mm, bends inward by a quarter of a circle with a radius of 1 mm, then extends vertically by 1 mm, and subsequently intersects with another inlet through a quarter circle with a radius of 1 mm. At the beginning of S1 stage, the radius of the quarter circle is 1 mm, the cross-sectional channel is rectangular, with a length of 0.4 mm and a width of 0.2 mm,

and it contains three circular guide columns with a diameter of 0.2 mm. In the second half of S1 stage, the channel is divided into two layers, with a length of 2.5 mm, the inner surface distance of the two layers is 0.4 mm, each layer of the channel has a rectangular cross-section with a length of 0.4 mm and a width of 0.1 mm, and the angle between the two channels is  $77.32^{\circ}$ . The matrix circular guide columns are embedded in the divided channels, with a diameter of 0.12 mm. The main function of the structure at the beginning of S1 stage is to change the direction of the fluid, and the circular guide columns play the role of compressing and expanding the fluid. The structure in the second half of S1 stage mainly performs liquid diversion, the diversion effect can promote the mixing of the liquid, and at the same time, in combination with the matrix circular guide columns, the flow within the channels realizes efficient convection and diffusion, thereby achieving the purpose of preliminary mixing. In the S2 stage, a "diversion-convection" structure is adopted. The total length of the channels in the S2 stage is 2.3 mm, the thickness of the diversion channels at the diversion point is 0.1 mm, the lengths of the three diversion channels are 0.75, 0.5, and 0.75 mm respectively, the width is 0.2 mm, the thickness of the confluence channels at the confluence point is 0.15 mm, and the lengths of the two confluence channels are 0.55 and 0.55 mm respectively, the width is 0.2 mm. To reduce the retention of bubbles during the experiment, the structure at the right-angle section is rounded, with a radius of 0.05 mm. At the junction of S1 stage and S2 stage, and also at the junction of S2 stage and S3 stage, the same rounding treatment is carried out, with a radius of 0.1 mm. In the S2 stage, the liquid continuously performs "diversion", "convection", "compression", and "diffusion" behaviors, greatly improving the degree of liquid mixing. By utilizing the Dean Flow effect caused by the bending of the microchannel, the fluids in the left and right channels achieve efficient convection and diffusion, thereby achieving the purpose of thorough mixing. The S3 stage can be divided into three parts, from top to bottom, it can be called the rectangular turning channel, the diversion spiral channel, and the matrix guide column channel. The length of the first part structure is 2.9 mm, the thickness is 0.2 mm, and the width is 0.4 mm. The first part structure contains 6 rectangular baffles, which run

through the entire channel, the length and width of each baffle are 0.2 mm, and the distance between each baffle is 0.2 mm. The distance between the first baffle and the end of the S2 stage is 0.25 mm, the first part structure is connected to the second part structure by a diversion rotation structure, the last baffle in the first part structure is 0.45 mm away from the connection structure. The length of the diversion rotation structure is 0.6 mm, the diversion structure can change the originally layered left and right fluids into layered up and down, allowing the fluids to flow into the second part structure in a more easily mixed state. The length of the second part structure is 2.5 mm, each side of the structure is formed by rotating a rectangular section with a length of 0.4 mm and a width of 0.1 mm. The rotation direction is as shown in the figure. The length of the third part is 1.1 mm, the width is 0.4 mm, and the thickness is 0.2 mm. It contains a matrix-shaped circular deflector column with a diameter of 0.12 mm. In the S3 stage, the liquid undergoes "compression" and "expansion" behaviors through a rectangular turning channel, and the continuous change of the fluid flow direction and effective collision promotes the mixing of the fluid. The second part structure performs the function of diversion and rotation on the fluid. The circular deflector column in the third part structure can increase the stretching and folding of the liquid interface. The combined action of the three parts structures promotes further mixing of the liquid. The structure in the S4 stage is exactly the same as that in the S2 stage. The structure in the S5 stage consists of layered rectangular turning channels and turning spiral channels, and its function is the same as that of the aforementioned similar structures. The structure in the S6 stage is to further roll the already fully mixed liquid to avoid the accumulation of local concentration gradients.

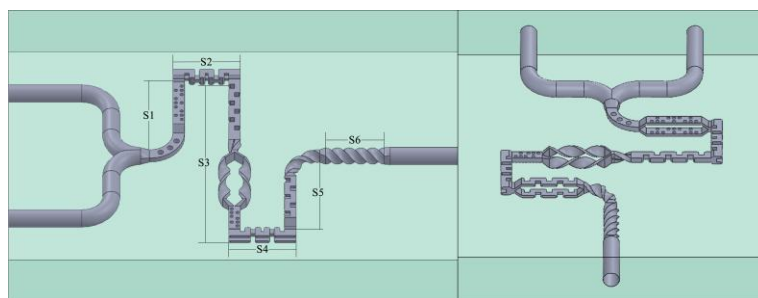


**Figure 1.** Schematic diagram of the overall structure of the microfluidic chip

The microfluidic chip structure designed in this study integrates the core reinforcement mechanism of mixing. Each structure collaboratively enhances the mixing efficiency from three dimensions: interface optimization, flow field disturbance, and flow state reconstruction. Layered structure: It shortens the diffusion-dominated mixing path. The fluid in the microfluidic channels is mostly in laminar flow, and the mixing mainly relies on molecular diffusion. The diffusion rate is inversely proportional to the square of the diffusion distance. The layered structure enables two inlet liquids to form parallel thin liquid layers, reducing the "lateral diffusion distance" from the overall width of the channel to the thickness of a single liquid layer, significantly reducing the diffusion mass transfer resistance and laying the foundation for subsequent enhanced mixing. At the same time, the parallel arrangement of the layered interface can ensure the uniformity of liquid contact and avoid local mixing dead zones. Circular guide column structure: Local vortices and velocity gradients are introduced. The circular guide column is equivalent to the "flow disturbance unit" in the microchannel. When the fluid flows around, a low-pressure vortex zone (tail flow effect) is formed behind the column. On the one hand, the vortices will draw different liquids from both sides, breaking the parallel flow state of laminar flow and increasing the stretching and folding of the liquid interface; on the other hand, the velocity difference around the guide column (lower velocity near the column wall and higher velocity in the inter-channel passage) forms a shear force, tearing the liquid layer into finer liquid filaments, increasing the contact specific surface area, and shifting from "passive diffusion" to "active convection disturbance". "Compression-diffusion" structure: It uses the inertial effect to enhance shear and stretching. This structure is similar to a variant of the Venturi effect, with the core being the regulation of the flow field through changes in the channel cross-sectional area. In the compression section: The reduced cross-sectional area leads to an increase in flow velocity, causing the fluid to be compressed and generating strong shear force, further refining the liquid filament size; in the diffusion section: The increased cross-sectional area causes a sudden drop in flow velocity, converting kinetic energy

into pressure energy, forming a radial expansion flow, further "tearing" the refined liquid filaments and distributing them uniformly, while the velocity gradient triggers the inertial effect, breaking the radial concentration homogeneity and promoting convection mixing. This structure is particularly suitable for enhancing the mixing efficiency of high-viscosity liquids, overcoming viscous resistance through kinetic input. "Diversion-convection" structure: It realizes "separation-recombination" of multi-scale mixing. The diversion structure splits the mixed flow after layering into multiple parallel branches, further reducing the thickness of the liquid layer in each branch; in the recombination stage, the multiple branches intersect or oppose each other, forming a convection effect. The liquids in different branches undergo lateral exchange when they converge, equivalent to completing a "multiple separation-recombination" cycle, significantly improving the mixing uniformity. This structure can effectively avoid the problem of "local mixing being sufficient but overall mixing being uneven" in a single channel, and is particularly suitable for scenarios with fluctuating flow rates. Rectangular turning channel structure: It induces Dean flow to promote radial mixing. At the rectangular turning point of the microchannel, the fluid is subjected to centrifugal force, generating a secondary flow (Dean flow) perpendicular to the main flow direction: the fluid on the outside of the channel is pushed outward by centrifugal force, while the fluid in the inner part replenishes to the center, forming a closed circulation. The Dean flow forces the two liquids to exchange positions radially, breaking the limitation of "axial parallel flow and radial slow diffusion" in laminar flow, stretching the interface to the entire channel cross-section, upgrading the mixing from "one-dimensional diffusion" to "two-dimensional convection+diffusion", significantly shortening the mixing length. Rotational structure: It introduces three-dimensional circulation and winding effect. The rotational structure causes the fluid to generate angular momentum, forming a three-dimensional rotating flow field: The shear force generated by the circumferential rotation rolls the liquid layer into a spiral shape, increasing the interface area; the coupling of axial flow and circumferential rotation forms "spiral convection", with the liquid continuously rolling during the forward movement,

avoiding the accumulation of local concentration gradients; for liquids with significant differences in density or viscosity, the rotational flow field can counteract the stratification effect caused by gravity or buoyancy, ensuring the mixing uniformity.

Each structure forms a complete synergy chain of "pre-mixing (layering), primary disturbance (diversion column), enhanced stretching (compression-diffusion), multi-scale segmentation (diversion-convection), radial mixing (turning channel), and three-dimensional homogenization (rotation)", which enhances the mixing efficiency from three core elements: diffusion path, contact area, and flow field dimension.

## 2. Analogue simulation

### 2.1 Basic governing equations of fluids

The fluid flow pattern in the microfluidic chip channel differs fundamentally from that at the macroscopic scale. The mixing process is the result of the combined action of fluid dynamics and mass transfer. To systematically describe this process and guide chip design, this paper constructs a complete theoretical framework covering the flow basis, transport mechanism, pressure drop characteristics, and effect evaluation. Since the accuracy of the simulation results is predicated on the establishment of correct governing equations [38, 39], the following equations are introduced to ensure the accuracy of the simulation results.

The continuity equation states that during the process of solution mixing, the total amount of substances remains unchanged. The continuity equation is the manifestation of the law of conservation of mass in fluid mechanics, and its expression is:

$$\nabla \cdot u = 0 \quad (1)$$

In the formula:

$u$  represents the velocity vector of the center of mass of the mixture.

The Navier-Stokes equations, together with the continuity equation, form the fundamental closed set of equations that describe the motion of incompressible fluids. Their vector expression is:

$$\rho \mathbf{v} \cdot \nabla \mathbf{v} = -\nabla p + \nabla \cdot \mu (\nabla \mathbf{v} + (\nabla \mathbf{v})^T) \quad (2)$$

In the formula:

$\rho$  is the density of the fluid ( $\text{kg/m}^3$ ),

$\mathbf{v}$  is the velocity of the fluid ( $\text{m/s}$ ),

$\mu$  is the dynamic viscosity of the fluid ( $\text{kg/(m*s)}$ ),

$p$  is the pressure (Pa),

$T$  is the transpose symbol.

According to the convection-diffusion equation in the theory of fluid mechanics [29]:

$$\frac{\partial C}{\partial t} + \mathbf{V} \cdot \nabla C = D \nabla^2 C \quad (3)$$

In the formula:

$\mathbf{V}$  represents the velocity vector field of the fluid,

$D$  is the diffusion coefficient,

$C$  represents the concentration of the solute.

From Equation (3), it can be seen that only by increasing the diffusion time and enhancing the convective intensity can micro-mixing be promoted.

The Re equation. The fluid flow within the microfluidic chip follows the principle of mass conservation. By using the calculation formula of the Re, the fluid characteristics within the chip can be inferred [30, 31]. The expression is as follows:

$$Re = \frac{\rho v d}{\mu} \quad (4)$$

In the formula:

$d$  represents the characteristic length of the pipeline.

$\Delta P$  refers to the pressure drop between the inlet and outlet sections of the microchannel, where the pressure is the average of the static pressure of the section.  $\Delta P$  can be defined as:

$$\Delta P = P_{\text{in}} - P_{\text{out}} \quad (5)$$

In the formula:

$P_{\text{in}}$  represents the inlet static pressure (Pa),

$P_{\text{out}}$  represents the outlet static pressure (Pa).

Regarding diffusive mixing, the Einstein equation provides [32]:

$$\delta^2 = 2Dt \quad (6)$$

In the formula:

$t$  is the diffusion time,

$\delta$  is the transverse diffusion length.

Equation (6) shows that both diffusion time and convective intensity are positively correlated with micromixing efficiency, while the diffusion coefficient is generally constant. For a given microfluidic chip design, reducing the flow velocity to prolong diffusion time, and incorporating structures such as guiding pillars and baffles in the channels, can effectively enhance convective effects and thereby improve mixing efficiency.

In the research of fluid mixing in microfluidic chips, the mass mixing index (M) is an indicator used to quantify the mixing effect, and its core formula is usually based on the uniformity of the concentration distribution of the target component (such as a tracer or solute) in the fluid.

$$M = 1 - \frac{\sqrt{\frac{1}{N} \sum_{i=1}^N (Y_i - \bar{Y})^2}}{\bar{Y}} \quad (7)$$

$Y_i$  is the mass fraction of a certain component in the  $i$ -th grid cell.  $\bar{Y}$  is the average mass fraction of this component at the outlet.  $N$  is the total number of grid cells at the outlet. A value of  $M$  closer to 1, the more uniform the mixing; the closer to 0, the less uniform the mixing.

The above equations can systematically guide the design, simulation and performance optimization of micro-channel mixing structures. Especially in passive mixers, controlling the flow and mass transfer processes through the geometric structure is a key breakthrough point. Finally, the calculation of the mass fraction

mixing index is used to preliminarily determine the mixing effect [44].

## 2.2 Simulation parameter settings

The three-dimensional model of the microfluidic chip was drawn using the SolidWorks software, and the model was imported into the Fluent software for simulation. The parameters of the solution were set as follows: density of  $1000 \text{ kg/m}^3$ , viscosity of  $0.001003 \text{ kg/(m}\cdot\text{s)}$ , molar mass of  $18.0152 \text{ g/mol}$ , and mass diffusion rate of  $1\text{e-}11 \text{ m}^2/\text{s}$ . The two inlet fluid input speeds were the same. The inlet was a velocity inlet, and the outlet was a pressure outlet. The channel walls were set as no-slip boundaries. Eight sets of simulations under different Re values were set up to verify the mixing effect of the microfluidic chip under different fluid states.

## 2.3 Analysis of simulation result

To study the mixing performance of microfluidic chips with three-dimensional complex cross-section channels, the component transport model in Fluent software was used to simulate the flow velocity field of the chips. A total of 8 sets of simulations were conducted with Reynolds numbers (Re) set at 0.5, 1, 5, 10, 20, 50, 100, and 200 to verify the mixing effect of the chips under different conditions. The performance indicators selected were the common mass fraction mixing index (M) and pressure drop.

As shown in Figure 2, the two-phase fluid is in a clearly laminar state during the S1 stage. Through the compression and expansion effects of the circular guide column on the liquid, as well as the turbulence effect, the initial mixing of the two-phase fluid is achieved. In the S2 stage, the "compression-expansion" effect of the fluid and the "diversion-convection" effect are used to further mix the two-phase fluid. When the Re is low ( $\text{Re} = 0.5\text{-}20$ ), the effect of fluid mixing in the S2 stage is more obvious, and the further mixed fluid can be sent to the S3 stage. At medium Re ( $\text{Re} = 50$ ,  $\text{Re} = 100$ ), the effect of fluid mixing in the S2 stage slightly weakens because the fluid velocity increases and the laminar flow phenomenon becomes more obvious. However, through subsequent structures, the weakened mixing effect can be compensated. At  $\text{Re} = 200$ , the laminar effect is broken, and the fluid molecules become more disordered, completing the basic mixing of the fluid. The S3 stage

structure fully mixes the fluid that has basically completed the mixing, prolonging the fluid contact time, and enabling the fluid to be fully mixed through diffusion and centrifugal force effects. The structures in S4, S5, and S6 stages collide and tumble the fully mixed fluid to avoid local concentration accumulation, achieving a mixing index of over 96% to 99.53%. As shown in Figure 3, thanks to the interaction of various structures, the mixing efficiency is improved through three core elements: diffusion path, contact area, and flow field dimension, resulting in a final mixing index of 96.67% - 99.53%.

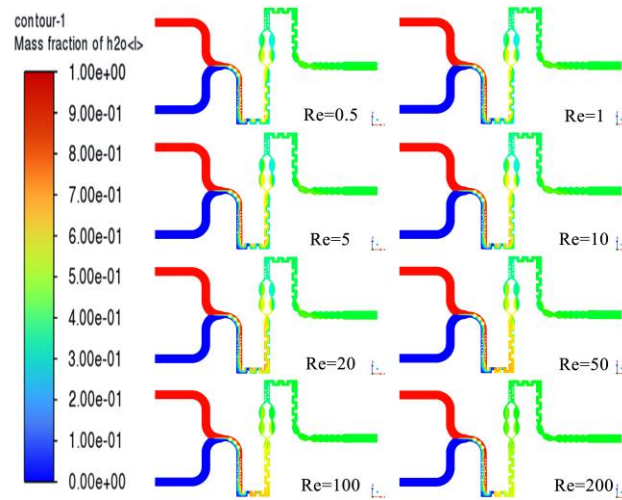


**Figure 2.** Overall mixed cloud map

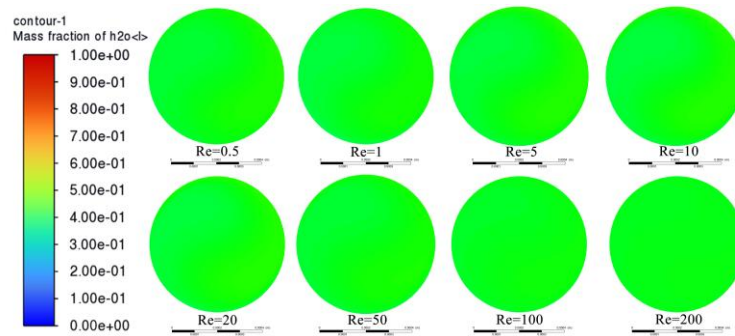


**Figure 3.** Exported Cloud Map

By analyzing Table 1 and Figure 4 comprehensively, the mixed index shows a three-stage characteristic of "low Re stable  $\rightarrow$  medium Re gradual recovery  $\rightarrow$  high Re increase" as the Re number changes. Moreover, the mixed index remains above 96.67% throughout the entire Re range, indicating that the designed multi-structure collaborative chip has excellent mixed performance. Low Re range (Re = 0.5-10): Molecular diffusion dominates, with high and stable mixed efficiency. When Re is

less than or equal to 10, the fluid is in a typical laminar state (inertial force is much smaller than viscous force), and the mass transfer process mainly relies on molecular diffusion. The built-in layered structure divides the two liquids into parallel thin liquid layers, reducing the lateral diffusion distance from the overall width of the channel to the micrometer level, significantly reducing the diffusion and mass transfer resistance. Therefore, even at  $Re = 0.5$ , the mixed index reaches 97.37%. As  $Re$  increases from 0.5 to 10, the residence time of the fluid in the channel linearly shortens, while the inertial effect in the low  $Re$  range is weak. The circular guide columns, compression diffusion, and other disturbance structures are difficult to form effective convective disturbances, and the mixing still relies mainly on diffusion. Thus, the mixed index shows a slight decrease from 97.37% to 96.67%, but overall it remains at a high mixed level above 96%. This verifies the core role of the layered structure in low  $Re$  diffusion mixing.

Medium  $Re$  range ( $Re = 20-50$ ): Inertial diffusion effect cooperates, with mixed efficiency gradually recovering. After  $Re$  is greater than 10, the inertial effect begins to appear (inertial force and viscous force ratios gradually become balanced), and the fluid kinetic energy is sufficient to activate the active convective structures of the chip: the wake vortex formed behind the circular guide columns sucks the liquids from both sides, increasing the stretching and folding degree of the "liquid-liquid" interface; the "compression-diffusion" structure further refines the liquid size through the strong shear force generated by the sudden change in cross-sectional area; the "split-flow-convection" structure realizes "separation-recombination" multi-scale mixing. These structures generate convective disturbances that effectively supplement the deficiency of molecular diffusion and partially counteract the effect of shortened residence time caused by the increase in flow velocity, causing the mixed index to rise from 96.67% at  $Re = 10$  to 97.65% at  $Re = 50$ , indicating that the mass transfer dominant mechanism has shifted from "pure diffusion" to "inertial convection-diffusion synergy". The complex structure design of the chip has officially enabled the convective function to take effect.

High  $Re$  range ( $Re = 100-200$ ): Inertial convection dominates, and mixed efficiency significantly increases. Circular guide columns, rectangular bends, and rotational structures

successfully induce strong secondary flows and vortices, achieving chaotic convection mixing dominated by inertial force, thus breaking through the bottleneck of pure diffusion mixing. When  $Re$  is greater than 100, the inertial effect dominates the flow field, which upgrades from two-dimensional laminar flow to three-dimensional complex flow state: the Dean flow intensity generated by the rectangular bend channel is proportional to the square of  $Re$ , forcing the liquid to exchange positions radially, upgrading the mixing from "one-dimensional diffusion" to "two-dimensional convection+diffusion"; the spiral circulation formed by the rotational structure wraps the liquid layer into a spiral shape, increasing the contact area of the "liquid-liquid" interface, while the axial flow and circumferential rotation coupling forms a three-dimensional rolling flow field, avoiding the accumulation of local concentration gradients. The strong convective disturbances generated by the multi-structure collaboration effectively eliminate the limitation of mass transfer efficiency due to molecular diffusion rate, causing the mixed index to rise from 98.72% at  $Re = 100$  to 99.53% at  $Re = 200$ , approaching the fully mixed state, fully verifying the effectiveness of the chip's "layered pre-mixing-inertial disturbance enhancement-three-dimensional flow field homogenization" collaborative design, successfully breaking through the mass transfer bottleneck of laminar mixing. Within the range of  $Re = 0.5-200$ , the standard deviation of the mass fraction mixing index is 0.0097, which is less than 1%. This indicates that within the wide range of Reynolds numbers, this microfluidic chip has stable and excellent mixing performance.

**Table 1.** Mixing index and pressure drop of the chip under different Reynolds numbers

| Sequence | Re  | Quality fraction mixing index (M) | Standard deviation( $\sigma$ ) | Pressure drop (Pa) |
|----------|-----|-----------------------------------|--------------------------------|--------------------|
| 1        | 0.5 | 0.9737                            | 0.0097                         | 2.4356             |
| 2        | 1   | 0.9707                            |                                | 4.8721             |
| 3        | 5   | 0.9679                            |                                | 24.4544            |
| 4        | 10  | 0.9667                            |                                | 49.1283            |
| 5        | 20  | 0.9679                            |                                | 99.0336            |

|   |     |        |          |
|---|-----|--------|----------|
| 6 | 50  | 0.9765 | 254.7452 |
| 7 | 100 | 0.9872 | 532.5854 |
| 8 | 200 | 0.9953 | 1153.969 |

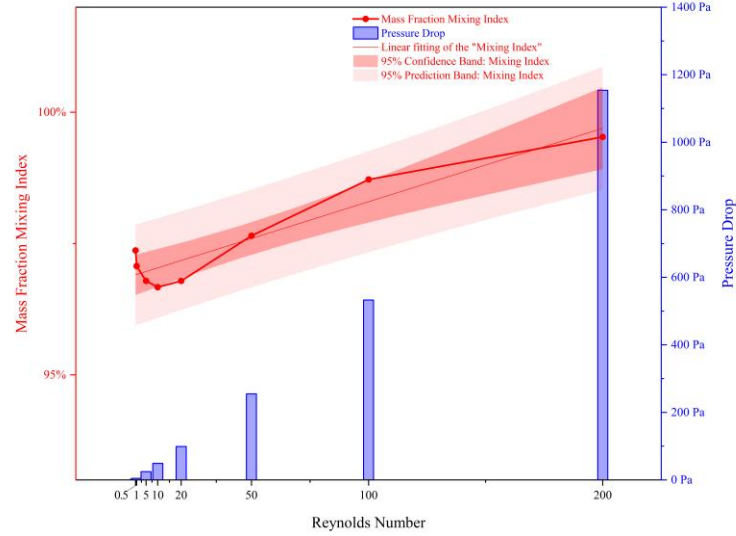


**Figure 4.** The mixing index and pressure drop of the chip under different Reynolds numbers

As shown in Figure 5, it is the simulation flowchart and the cross-sectional mixed cloud chart of each stage. Due to the excellent fluid mixing effect and moderate energy loss under the condition of  $Re = 20$ , in order to balance the mixing efficiency and pressure drop, this  $Re$  flow condition was selected for analysis and subsequent experiments. If the flow rate is too fast, it is not conducive to controlling the volume of the required mixed solution. The detection liquid required for trace detection only needs a few milliliters, and if the flow rate is too fast, it will cause waste of the relevant solution. Choosing an appropriate flow rate can reduce the consumption of chemical reagents and reduce the impact on the environment. If the flow rate is too slow, the required mixing time will increase significantly, which is contrary to the research concept and does not reflect the efficiency of the microfluidic chip. In the simulation flowchart of the microfluidic chip, the cross-sectional mixed cloud charts of the starting point of S1 stage, the starting point of S2 stage, the end point of S2 stage, the starting point of S4 stage, the end point of S4 stage, the end point of S5

stage and the outlet were selected respectively, and the quality fractions were derived for calculation. The obtained mixing indices were 0.0583, 0.2075, 0.4764, 0.8134, 0.9415, 0.9489, and 0.9679 respectively. From the mixing index, it can be seen that when the two-phase fluid first contacts, the mixing index is only 0.0583. After the initial mixing by the structure of S1 stage, the mixing index reaches 0.2075, increasing by 0.1492. At the end point of S2 stage, the mixing index is 0.4764. After the fluid undergoes further mixing in the structure of S2 stage, the mixing index increases by 0.2689. Thanks to the longer length of the S3 stage structure, the time for fluid diffusion and mixing is increased, and the mixing index is raised by 0.3370. The S4 stage structure raises the mixing index by 0.1281. In the later stage of mixing, since the fluid has reached a basically uniform mixed state, the increase in the mixing index becomes more difficult. The S5 stage structure raises the mixing index by 0.0074, and the S6 stage structure raises the mixing index by 0.0190. The mixing index at the outlet is 0.9679, and the fluid is in a basically uniform state, meeting the requirements of the subsequent detection experiments.

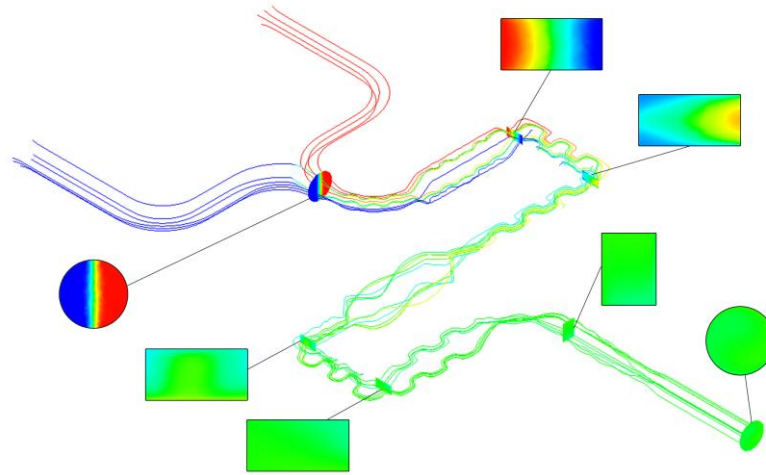


**Figure 5.** Simulation streamline diagram and cross-sectional cloud diagrams at each stage ( $Re = 20$ )

### 3. Experimental section

#### 3.1 Introduction to experimental equipment

As shown in Figure 6(a), the chip printing equipment uses a resin 3D printer purchased from BREUCK Company, model BL3D-50-P2. The horizontal resolution is 25  $\mu\text{m}$ , the size accuracy is 0.02 mm, the printing layer thickness is 10-100  $\mu\text{m}$ , and

the construction speed is 10-30 mm/h. The printing material is selected as the high-precision resin of model BPHP-01, with a color of transparent yellow, hardness of 86-88HB, bending strength of 95-100 MPa, tensile strength of 55-65 MPa, and elongation at break of 5-8%.

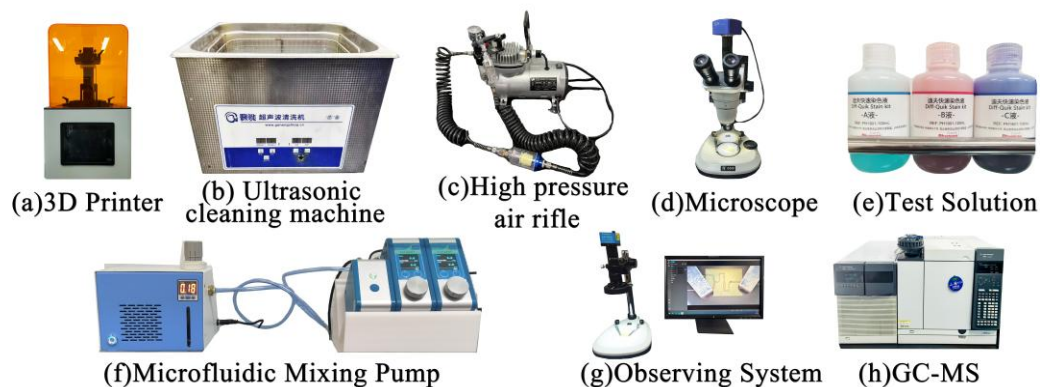


**Figure 6.** Main experimental equipment and instruments

Figure 6(b) shows the Songneng brand ultrasonic cleaning machine. Figure 6(c) shows the 750-30L model silent air compressor.

As shown in Figure 6(d), it is the COIC 1200CJS computer-type upright metallographic microscope produced by Chongqing Aotu Optical Instrument Co., Ltd. The microscope is connected to the computer screen and observed through the Uop View software.

As shown in Figure 6(e), the colorimetric mixed experimental reagent is the Diffe rapid staining solution purchased from Fuzhou Feijin Biotechnology Co., Ltd. As shown in Figure 6(f), the colorimetric mixed experimental equipment adopts the OxyGEN system purchased from Fluigent Company. This system has a modular interface suitable for developing and running time-based microfluidic chip detection, and is equipped with a protocol that is easy to edit and complete the entire automated experiment.

As shown in Figure 6(g), the observation equipment uses the GP-660V electronic microscope purchased from Kunshan Gaopin Precision Instrument Co., Ltd. This microscope is used with high-definition CCD and display, featuring a long working distance, wide field of view, and high-definition imaging quality.

As shown in Figure 6(h), the detection equipment uses the triple quadrupole gas

chromatography-mass spectrometry (GC/MS) instrument purchased from Agilent Technologies, model: 7000C GC/MS Triple Quad. This equipment has powerful separation and detection capabilities and is widely used for qualitative and quantitative analysis of organic compounds in complex mixtures.

### **3.2 3D Printing experiment section**

This experimental process uses the equipment shown in Figure 6(a) to fabricate the microfluidic chip, employs the equipment in Figures 6(b) and 6(c) for chip cleaning, and uses the equipment in Figure 6(d) for microscopic scanning of the chip to observe the printing quality of the chip. It consists of five steps: model establishment, model processing, chip printing, chip cleaning, and characterization observation.

Compared with traditional microfluidic chip fabrication techniques (such as lithography-chemical etching [45], soft lithography [46], CNC processing [47], etc.), the 3D printing technology [48] adopted in this experiment demonstrates many core advantages for the complex structure and precise fabrication requirements of microfluidic chips. The microfluidic chip designed in this paper integrates complex internal flow channels and functional units such as layered structure, circular flow guide structure, "compression-diffusion" structure, "diversion-convection" structure, rectangular turning structure, and rotational structure. Each structure is closely arranged in space and has three-dimensional cross-correlation. Traditional lithography technology is difficult to achieve the integrated fabrication of complex three-dimensional flow channels and usually requires multiple steps of lithography and bonding processes, which can easily lead to flow channel alignment deviations, loose bonding, and high costs of lithography equipment and complex operation. However, 3D printing technology, based on the "layer-by-layer stacking" forming principle, can directly form the constructed three-dimensional model at one time without the need for molds and multiple assembly steps, ensuring the integrity and connectivity of complex flow channel structures, while avoiding production errors caused by multiple process connections. In addition, 3D printing technology is fast in forming, low in cost, and simplifies the fabrication process. If the flow channel

structure needs to be adjusted, it only requires modifying the three-dimensional model and reprinting, without the need to re-make molds, significantly shortening the design iteration cycle and reducing the experimental cost.

After completing the modeling with SolidWorks 2023 software, export the STL format file, and then import it into Materialise Magics slicing software for model preprocessing. The model preprocessing process includes the following steps: Model positioning: Set the thickness direction of the chip as the printing height direction. This direction can minimize the use of support structures and ensure that the flow channel structure is completely formed along the printing direction, which is conducive to maintaining the geometric shape of the channel; Slicing layering: Set the printing layer thickness to 40  $\mu\text{m}$  (taking into account the material properties and equipment accuracy to balance the forming accuracy and printing efficiency), and the slicing software automatically divides the three-dimensional model into continuous two-dimensional slice layers; Adding support structures: Add lightweight column-shaped supports at the edges of the chip inlet and outlet to prevent the chip from deforming or detaching during printing. The connection surface of the support structure and the chip is set as an inclined plane. Carefully inspect and manually optimize the support structure to ensure that it is easy to remove and does not damage the main structure of the chip.

Equipment calibration process: Check the cleanliness of the resin tank, use a lint-free cloth dipped in anhydrous ethanol to wipe the transparent film on the bottom of the resin tank, removing residual impurities and fingerprints; Calibrate the levelness of the printing platform, complete the platform calibration through the automatic leveling function of the equipment, ensuring that the distance between the printing nozzle and the platform is uniform (the initial distance is set at 0.1 mm, matching a 40  $\mu\text{m}$  layer thickness); Adjust the intensity of the printing light source and the exposure time, based on the parameters of the printing material (BPHP-01 resin), set the exposure time for each layer and the bottom layer (the bottom layer needs to enhance adhesion to prevent the model from falling off).

3D printing process: Pour the BPHP-01 high-precision transparent yellow resin

into the resin tank of the printer. The resin level should cover the printing area in the tank to ensure that the first layer slice can fully contact the resin. Import the pre-processed slice file into the printer control system and confirm the printing parameters: layer thickness, exposure time, construction speed are 20 mm/h (between the equipment's rated range of 10-30 mm/h, balancing efficiency and accuracy). Close the equipment protection door, start the printing program. During the printing process, observe the operation status of the equipment in real time, confirm that the resin level is stable, the nozzle movement is without deviation, and the model has no obvious deformation or warping. Keep the equipment working environment clean and dry throughout the printing process (temperature 23 °C, humidity 40%-60%, avoiding the resin performance being affected by the environment).

Post-printing processing procedure: Put on nitrile gloves, remove the chip model from the printing platform, avoiding direct contact with the inner wall of the flow channel; Use fine pointed pliers to slowly peel off the supporting structure along the connection surface between the support and the chip body; Place the chip without support into the device in Figure 6(b) for ultrasonic cleaning (for 5 minutes) to remove the unsolidified residual resin on the surface, then rinse three times with deionized water, and finally dry the remaining moisture using the device in Figure 6(c) (the pressure of the tank of the high-pressure cleaning equipment is 0.7 MPa, and the duration of high-pressure air cleaning is 4 minutes).

Characterization and observation of microfluidic chips: Using the microscope equipment shown in Figure 6(d), the actual printed channel cross-section, surface roughness and dimensional accuracy of the chip were evaluated. The chip was placed flat on the stage, and then observed at low magnification ( $5\times$ ) to determine the area to be observed, and then switched to  $50\times$  for observation.

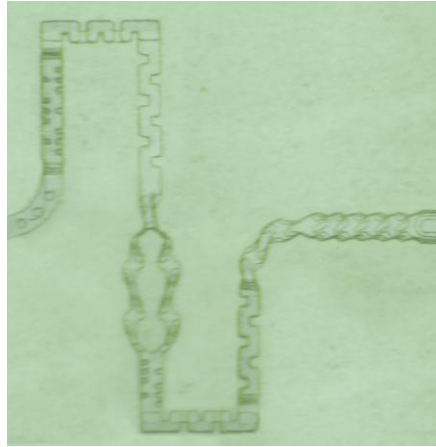


**Figure 7.** Microscope observation image

From Figure 7, it can be clearly seen the layer thickness during the printing process, the surface roughness of the channels, and the dimensional accuracy of the structure. In the S1 stage structure, the micro-channel printing structure is clear and distinct, the circular guide columns have no obvious defects, the transitional layer structure is smooth, and there is no obvious deformation; in the S2 stage structure, the micro-channel is clearly visible locally, the channel has no deformation, and the printing accuracy is in good consistency with the three-dimensional model; in the S3 and S4 stage structures, the right-angle turning channel structure is clear, the rectangular baffles are not missing, the layer rotation structure is consistent with the three-dimensional model, the layer thickness is obvious, the channel has no obvious deformation, and the circular guide columns are not missing; in the S5 stage structure, the layer transition structure is clear and smooth, the rectangular baffles have no missing parts, and the turning corners are distinct; in the S6 stage structure, the rotation structure has obvious layer thickness, and the rotation shape is clearly visible. In conclusion, the chip has basically met the expected precision requirements and the structure is complete, meeting the requirements of subsequent experiments.

### **3.3 Colorimetric mixture experiment section**

In this experimental process, solution in Figure 6(e) was used as the staining solution. The device in Figure 6(f) was employed as a micro-injector pump to inject the staining solution into the inlet of the microfluidic chip. The mixing effect of the microfluidic chip was evaluated through the observation system in Figure 6(g).

This experiment thoroughly examined the fluid mixing performance of the microfluidic chip under a specific  $Re$  ( $Re = 20$ ). The specific experimental steps were as follows: Firstly, appropriate amounts of Diffusion Rapid Staining Solution A and B were taken as the staining agents and diluted at two gradients of 1:10 and 1:40 to obtain the solution for the colorimetric mixing experiment. Subsequently, using the OxyGEN fluid control system, the prepared diluted solutions were precisely introduced into the inlet of the microfluidic chip. The experiment was strictly carried out in accordance with the initial flow rate conditions set in the previous simulation. During the flow of the fluid through the chip channels, the mixed area was continuously and dynamically observed using the GP-660V electron microscope to capture the real-time changes at the fluid interface. To systematically evaluate the mixing effect under the same  $Re$  condition, different local images at different positions were selected for swapping or magnification processing in stages S1, S3, and S5.

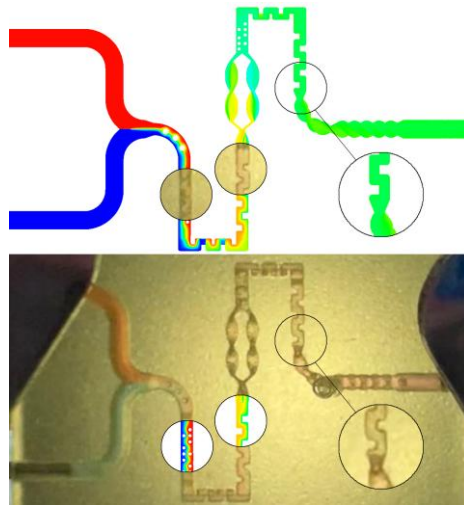


**Figure 8.** Local Comparison Diagram of Simulation and Hybrid Experiment

As shown in Figure 8, by conducting a detailed comparative analysis of the microscopic images collected from the above three positions, the spatial evolution of the mixing process is clearly presented: In the two local images, during the S1 stage, the two colored fluids exhibit relatively obvious laminar flow characteristics, with distinct interfaces and a low degree of mixing. In the S3 stage, although there are still some distinguishable color differences in the edge areas on both sides of the flow

channel in the simulation model cloud image, the main area of the channel has shown significant improvement in mixing, with the color of the color-mixed experiment becoming lighter and no obvious color difference being observed, indicating that the overall mixing effect of both is approaching a good state. In the S5 stage, the fluid color is in a uniform state, indicating that the mixing has been quite sufficient. In conclusion, by comparing the actual mixing state observed in the mixing experiment with the mixed cloud images obtained from the previous simulation, the two show good consistency. This result strongly validates that the designed microfluidic chip has stable and efficient fluid mixing performance under  $Re = 20$  conditions.

### **3.4 GC-MS Detection experiment section**

#### **3.4.1 Experimental principle**

This experiment aims to evaluate the mixing performance of the microfluidic chip in the solution of DDV and methanol. The core design principle of the experiment is to strictly control variables and use the same initial concentration of solution samples. By setting the target experimental group and two control experimental groups for parallel comparison, the final quantitative evaluation of the structural rationality and micro-mixing efficiency of the microfluidic chip is conducted based on the dataized concentration detection results.

Target experimental group (chip experimental group): The sample solution is processed in a microfluidic chip to simulate the flow and mixing process of the solution within the chip in actual applications.

Magnetic stirring group: The solution is in a continuous and intense rotational motion state, ensuring the stability of temperature and guaranteeing that the DDV molecules are distributed highly uniformly in the solution.

Static control group: The solution undergoes natural static treatment without any external force intervention. The purpose of this group is to verify and quantify the potential interfering factors (such as the sedimentation and stratification of DDV particles or molecules, the local concentration inhomogeneity caused by non-uniform mixing, and the possible physical property changes due to natural cooling, etc.) on the final DDV concentration detection results. The data of this group is expected to

deviate from the benchmark value due to insufficient mixing or separation effects.

It is predicted that the solution introduced into the microfluidic chip will achieve a good mixing effect, and the concentration of DDV should be uniformly distributed in the solution. That is to say, the experimental data of the chip experimental group is closer to the diluted DDV solution concentration, and is closer to the experimental data of the magnetic stirring group, while showing a significant difference from the experimental data of the static control group, indicating that the mixing effect of the microfluidic chip is more excellent.

### 3.4.2 Experimental pre-treatment

Before the formal test, a standard solution needs to be prepared to draw the DDV detection standard curve. Take 0.05, 0.1, 0.2, 0.5, and 1  $\mu\text{g/mL}$  as the test sample points for the curve concentration, as shown in Figure 9(b). After systematic analysis, the  $R^2$  of the standard curve is 0.9867, indicating that the linear correlation between the detection solution concentration and the standard curve is high, which meets the detection requirements of the subsequent experiments.

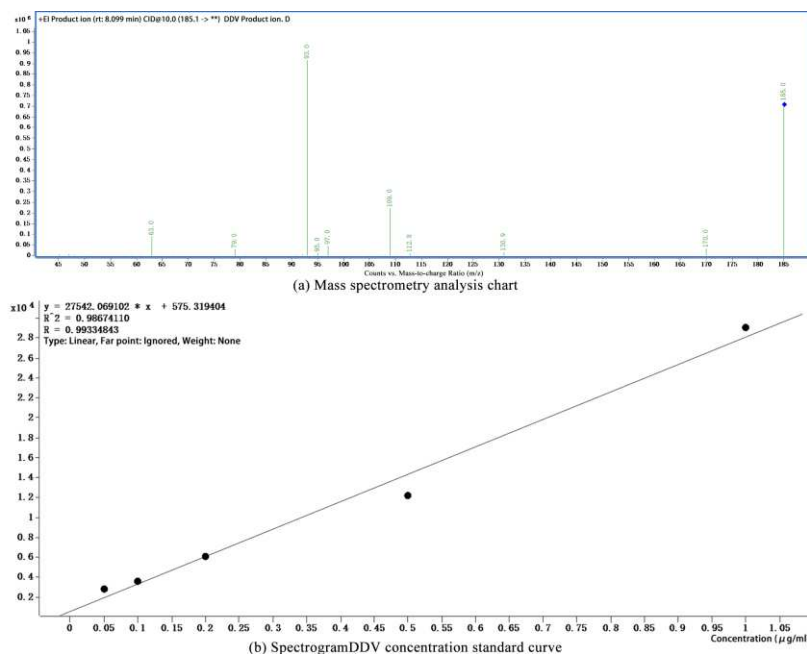


**Figure 9.** Image of GC-MS pre-treatment. (a) is the mass spectrum, (b) is the standard curve

Since the gas chromatography-mass spectrometry method has strict requirements for the analytical solution, high-purity methanol was selected as the matrix solvent for the experiment. Three sets of experiments were set up (the theoretical concentration of

the solution configured in each group of experiments was 0.1 ug/mL), as follows:

Chip Experiment Group: Using a pipette, take 0.02 mL of the standard substance of the dichlorvos solution in methanol (with a concentration of 100 ug/mL) and place it in a beaker. Pour high-purity methanol into the beaker to a total volume of 10 mL. Shake the beaker for five minutes to ensure the solution is evenly mixed. Pour the diluted solution into a storage bottle, and in another storage bottle, pour 10 mL of high-purity methanol. Introduce the solutions from both storage bottles into the OxyGEN system for the microfluidic chip mixing step.

Magnetic Stirring Group: Using a pipette, take 0.1 mL of the dichlorvos solution standard substance in methanol (with a concentration of 100 ug/mL) and place it in a beaker. Pour high-purity methanol into the beaker to a total volume of 100 mL, and use a magnetic stirrer to stir for 15 minutes.

Static Control Group: Using a pipette, take 0.1 mL of the dichlorvos solution standard substance in methanol (with a concentration of 100 ug/mL) and place it in a beaker. Pour high-purity methanol into the beaker to a total volume of 100 mL, and then let it stand for 15 minutes.

After preparing three samples, the temperature of the solution was measured. It should be noted that the pre-treatment stage of the experiment was strictly conducted under standard room temperature conditions (25°C). Through experimental testing, the solution temperatures of the magnetic stirring group, the chip experiment group, and the static control group were 25.7°C, 25.2°C, and 24.6°C respectively. There were minor differences in the temperatures of the three groups of solutions. This might be due to the continuous friction and collision between the magnetic rotor and the liquid molecules during its rotation. At the same time, the rotation of the magnetic rotor would drive the entire liquid to form a vortex, and the liquid molecules would also collide with each other, which led to an increase in the internal energy of the liquid. The direct manifestation of this increase in internal energy is the rise in temperature.

#### **3.4.3 Testing experiments and data analysis**

The concentration of the prepared three samples was immediately detected using the GC/MS Triple Quad instrument. To ensure the accuracy and reliability of the

experimental data and to evaluate the stability of the method, three independent parallel samples were tested for each sample. Finally, the DDV concentration value of each sample was taken as the arithmetic mean of the three parallel detection results. The initial concentration value of the prepared solution (0.1  $\mu\text{g/mL}$ ) was selected as the reference concentration for the experiment, which was used as the target reference for evaluating the mixing effect of the microfluidic chip in the subsequent evaluation.

**Table 2.** Average concentration data of three DDV sample solutions

| Group             | Average concentration<br>( $\mu\text{g/mL}$ ) | The difference                                                       |                                |
|-------------------|-----------------------------------------------|----------------------------------------------------------------------|--------------------------------|
|                   |                                               | from the reference<br>concentration<br>value (0.1 $\mu\text{g/mL}$ ) | Ratio of the difference<br>(%) |
| Chip Integration  | 0.0946                                        | 0.0054                                                               | 5.4                            |
| Magnetic Stirring | 0.0925                                        | 0.0075                                                               | 7.5                            |
| Static Treatment  | 0.0776                                        | 0.0224                                                               | 22.4                           |

As shown in Table 2, the comparative analysis of the three sets of experimental data indicates that the mixing method has a significant impact on the uniformity of the prepared DDV solution and the subsequent GC-MS detection results. The dilution ratios of the three experimental groups (chip experiment group, magnetic stirring group, and static control group) are consistent, ensuring that the differences in the detection results are due to the different mixing methods and mixing degrees (assuming the dilution steps are precise and ignoring actual operational errors). The detection results show that the ratio of the difference between the chip experiment group and the theoretical concentration of the prepared solution is 5.4%, the ratio for the magnetic stirring group is 7.5%, and the ratio for the static control group is 22.4%. Among them, the static control group only relies on molecular diffusion for mixing, which has the lowest efficiency, and the distribution of dichlorvos in the solution is uneven, resulting in a significant deviation of the detection value from the theoretical diluted concentration (0.1  $\mu\text{g/mL}$ ); the magnetic stirring group achieves active mixing through mechanical stirring, but it takes 15 minutes and there may be a possibility of insufficient local mixing during the stirring process, and the detection value is slightly lower than that of the chip experiment group; while the microfluidic chip utilizes the

efficient mixing effect at the micrometer scale to uniformly disperse DDV pesticides and methanol in a shorter time, reducing the detection deviation caused by local concentration differences. Its detection results are closer to the theoretical value, fully demonstrating the efficiency and stability of the microfluidic mixing technology. Based on the above detection results and experimental data analysis, the microfluidic chip mixing technology is superior to traditional magnetic stirring and static mixing, as it can significantly shorten the mixing time and improve the uniformity of the solution mixture, providing a more efficient and reliable mixing scheme for GC-MS sample preparation, and helping to improve the accuracy and reproducibility of target substance detection. The temperature fluctuation range of the three solutions in the experiment was small ( $24.6^{\circ}\text{C}$ - $25.7^{\circ}\text{C}$ ), which can exclude the significant interference of temperature changes on the volatilization of the target substance or the detection signal, ensuring the validity of the data comparison.

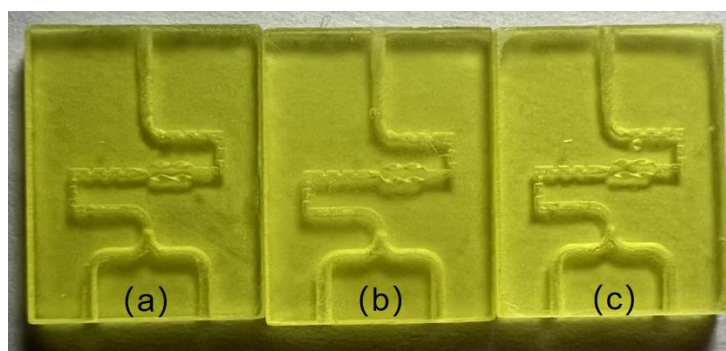


**Figure 10.** Physical image of microfluidic chip

To further verify the stability of the repeated use of the same microfluidic chip and the variability of the detection results between chips from different printing batches, this study selected three chips with exactly the same printing parameters and identical printing environment conditions (such as temperature, humidity, and printing accuracy) (the physical pictures of the chips are shown in Figure 10). The verification was carried out through GC-MS detection experiments, and the specific process and results are as follows:

The detection solution was prepared according to the steps described above to ensure the consistency of the experimental samples. Firstly, the reusability of the same chip was verified: taking chip (a) as the research object, three independent detection

experiments were conducted successively following the experimental steps described above; after each experiment, the standardized cleaning process of "clear water rinsing → alcohol rinsing → clear water rinsing twice → ultrasonic cleaning → high-pressure gas drying" was strictly followed to eliminate the interference of residual samples on the subsequent detection. The GC-MS detection results of the three times were 0.0945  $\mu\text{g/mL}$ , 0.0927  $\mu\text{g/mL}$ , and 0.0939  $\mu\text{g/mL}$  respectively. The maximum difference among the three sets of data was only 0.0018  $\mu\text{g/mL}$ , indicating extremely small data fluctuations. Subsequently, the variability of the detection results for different chips was examined: taking the same batch of diluted detection solution as before, the same batch was introduced into chips (b) and (c) respectively, and the GC-MS detection was completed following the same experimental steps, resulting in detection results of 0.0941  $\mu\text{g/mL}$  and 0.0934  $\mu\text{g/mL}$  respectively. Compared with the initial detection result of chip (a) (0.0945  $\mu\text{g/mL}$ ), the maximum difference in the detection results of the three chips was only 0.0011  $\mu\text{g/mL}$ .

Based on the comprehensive experimental data, it can be concluded that this microfluidic chip has reliable reusability after standardized cleaning ( $n=3$ ), with minimal fluctuations in the results of three consecutive tests. Moreover, even for chips from different production batches (as the printing parameters and environment are consistent, they can be regarded as equivalent chips from the same batch), the variability of the test results is extremely small, further verifying the stability and reliability of this chip in the detection application.

### **3.5 Results and discussion**

This experimental system successfully verified the technical feasibility of microfluidic chips in achieving high-performance and low-energy consumption mixing. This characteristic makes it particularly suitable for application scenarios with strict requirements for mixing speed and sample loss, such as rapid and accurate detection of trace pesticide residues. Due to the unique advantages brought by the "microchannel scale (micron level)", microfluidic chips have become a key breakthrough in addressing the pain points of traditional detection methods. Their core advantages lie in achieving efficient and rapid mixing of fluids within a

micro-space through effects such as laminar flow diffusion and chaotic mixing, with features of low reagent usage, miniaturization, and easy integration. Current detection technologies face a contradiction between "speed and accuracy": rapid screening methods cannot meet the need for precise quantitative analysis, while traditional GC-MS can achieve accurate qualitative and quantitative analysis, but the pre-treatment is cumbersome and time-consuming, making it difficult to adapt to on-site emergency detection and large-scale sample screening scenarios. This paper optimizes the mixing process of pesticides and extractants to break through the barrier of "pre-treatment-detection" connection, achieving simultaneous improvement in detection efficiency and accuracy. In traditional pesticide detection, the extensive use of extractants (such as methanol and acetonitrile) not only increases detection costs but also generates volatile organic pollutants, causing environmental burdens. The core goal of green analytical chemistry is "reducing chemical reagent consumption and lowering environmental impact", and the low reagent usage characteristic of microfluidic technology is highly consistent with this orientation. Applying microfluidics to the mixing process of pesticides and methanol can significantly reduce the consumption of organic solvents, which not only complies with environmental protection policy requirements but also provides a new path for the sustainable development of detection technology.

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submission of the article for publication.

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