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TEAM RESULT DOCUMENT

Managing Rheumatic Disease, By Measuring With Ease

TRUSENSE

ZHEJIANG UNIVERSITY

College of Life Science, Zhejiang University

CONTENTS

Summary

BiSheng, named after BiFang, a creature in traditional Chinese mythology who can quickly and accurately target, is an optical biosensor with anti-Adalimumab antibody-based microfluidic chips on porous silicon. When a light beam is applied onto the chips, Adalimumab binding to the adaptors on porous silicon leads to proportional changes in reflection interference, which is used to determine the amount of the antibody. The high reflection performance and adjustable porosity of porous silicon and 3 corresponding algorithms ensure its rapid, sensitive and accurate measurement. Its design concept is user- and environment-friendly, just plugging in the chip and waiting for 5 minutes for the results to be displayed automatically, and the chip will be recycled after use. Portable size, light weight, simple operational procedure and low energy consumption make it possible to be used widely. To further improve users' experience, an online medical platform is being developed, which will enable patients to finish all the processes of diagnosis, testing and treatment without leaving their homes. Team TruSense is always striving to reduce the burden on patients and environment.

Biosensor System and Assay

Molecular Recognition and Assay Reagents

Molecular recognition is based on antigen-antibody specific bonding of adalimumab and anti-adalimumab. In order to detect the binding process, we designed a biosensor chip, transforming chemical signal into detectable light signal for further analysis. The detection method is inspired by the special optical property of porous silicon: Fabry-Perot effect. Since Ghadiri et al. developed interferometry-based porous silicon biosensor in 1997, it has been much improved and optimized , and achieved a current detection limit of interferometric porous silicon biosensors at a picogram level. However, conventional porous silicon biosensors still require a long equilibrium time and tedious labelling procedure. Here, in order to shorten the detection time while maintaining a proper detection precision, we propose to use a special molecular design of the detection layer, and develop a rapid-detection porous silicon chip for adalimumab sensing in blood serum.

The porous silicon detection chip consists of 3 layers. The base layer is the electrochemically etched porous silicon base layer, to which the second layer, i.e. poly(L-lysine) (PLL) layer is linked via (3-Aminopropyl) triethoxysilane (APTES) and glutaraldehyde (GA) bridge. And the abundant amino moieties on PLL layer are modified with another GA bridge to link the third layer or the molecular recognition layer of: the anti-adalimumab antibodies. In order to avoid non-specific binding and adherence, we introduced mPEG as an end-capper, making it possible to use the chip to detect adalimumab in blood serum.

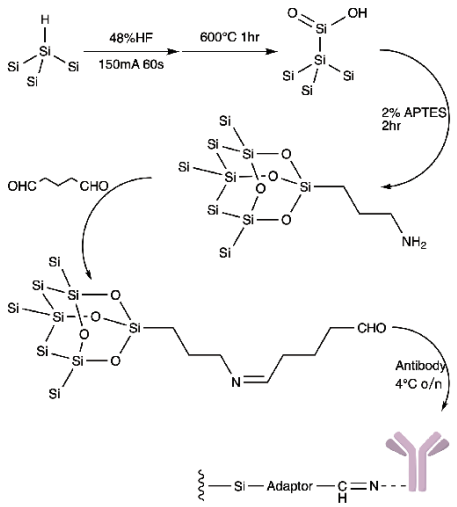


Fig. 1 Preparation of single-layer porous silicon and its chemical and biological modification

Physical Transduction

The porous silicon serves as a FabryPerot resonator, reflecting the incident rays. Lights reflecting off the top and bottom interfaces of the porous silicon film interfere, giving rise to characteristic Fabry–Peŕot fringes in reflection. Constructive interference leading to reflection peaks occurs when the condition indicated in the following Equation 1 is satisfied.

$$\frac{2nL}{\cos\theta} = m\lambda_0 \text{ (Single layer interferometer)} \quad (1)$$

Where n is the refractive index of the porous silicon film, L is the physical thickness of the porous layer, θ is the angle of incident light, m is an integer, and λ_0 is the vacuum wavelength of light. The reflected rays interfere with each other. The data can be obtained using a Reflectometry Interference Spectroscopy (RIS). The signal varies with different chemical condition in the Fabry-Perot resonator. Thus, after antigen-antibody binding, the surface of the porous silicon changes and can be observed by RIS. The interference fringes become closer together as the PSi effective optical thickness (EOT) increases. When molecules infiltrate the single-layer PSi film, the EOT of the film changes, resulting in a shift in the spectral position of the fringes that is proportional to the magnitude of the refractive index change caused by the molecules. For large changes in the EOT of the film that cause the reflection spectrum to shift by more than the width of a single fringe, it becomes challenging to determine how much the spectrum has shifted. In these cases, it is common to analyze the Fourier transform of the reflectance spectrum, which yields a single-peaked curve for which the peak position corresponds to twice the EOT of the PSi film. Details regarding this method, referred to as reflective interferometric Fourier transform spectroscopy (RIFTS), can be found in Pacholski et al.

Cartridge Technology

This instrument can automatically detect adalimumab, its theory is based on characteristics of porous silicon and reflecting and interference theory of ray. The whole detector includes two parts: the detector box and three-tier acrylic clamping piece (TACP).

In the detector box, the main parts include a lead screw stepper motor and peristaltic pump, an optoelectronic switch and some actuators. The lead screw stepper motor is used to move the needle in a vertical direction while the peristaltic pump can inject PBS into TACP at a relatively stable velocity.

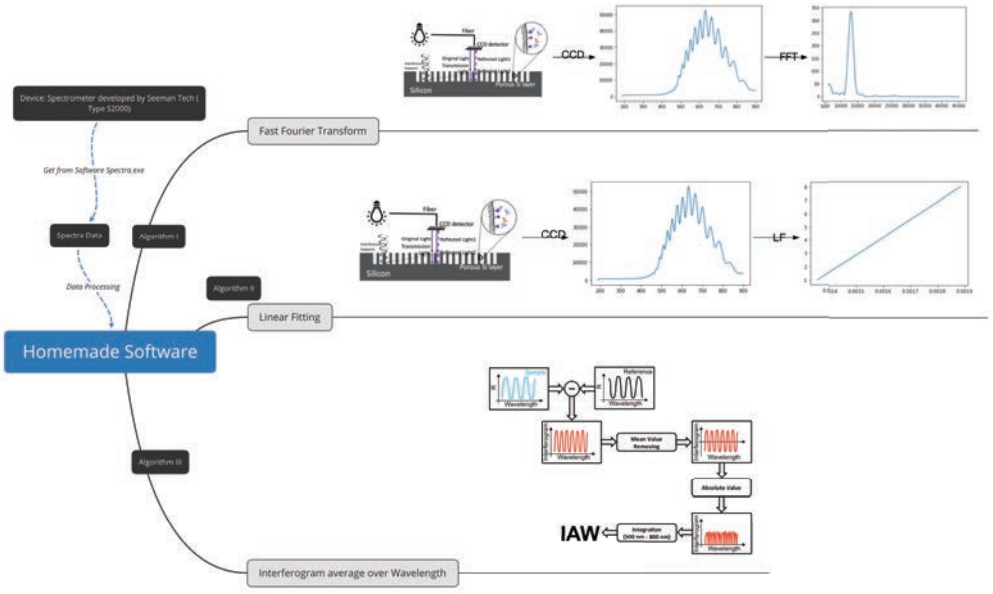


Fig. 2 Software schematic diagram about its principles. The spectrum data gets from the spectrometer developed by Seeman Tech. And there are three algorithms which are explained by flow chart.

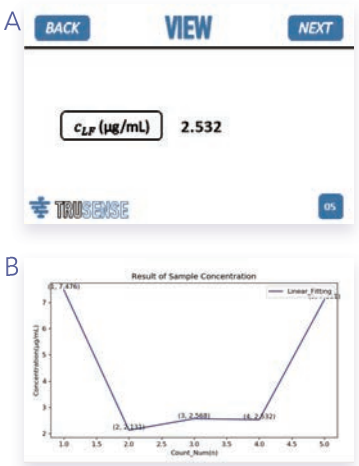


Fig. 2A The result display interface (Note: the result is getting by Linear Fitting Algorithm)
Fig. 2B The history curve about the concentration of Adalimumab (x-axis is sample number, y-axis is the concentration)

When the needle is on top, we can put TACP in a specified position manually. And then what just needs to do is pressing the button, so the needle will go down actuated by stepper motor until it gets to the correct position decided by optoelectronic switch. If the needle has been below the desired position, we can rectify manually by controlling the start-stop with the switch and adjusting the velocity by turning the rotary knob of the peristaltic pump. As for TACP, it is fixed by three acrylic clamping pieces, copping backing, silicone pad and screw-nut. It can also provide a small flow cell for PBS, from where the fiber-optics probe can detect the signals more readily.

Reader Instrument and User Interaction

The reader instrument is a personal computer with a custom-developed software . User can easily use the software to get the concentration of adalimumab. The optical signal, which is reflection spectrum, is transmitted from the spectrometer's light probe, converted into electrical signals by electronic systems, and finally, these simple digital signals are processed by our software to generate the readable spectrum and quantified data. Two algorithms, FFT and IAW, are used in our software in order to minimize the disadvantages caused by the shortcomings of Porous silicon biosensor itself in clinical and diagnostics analysis of nanostructured porous silicon for label-free optical biosensing. Fast Fourier Transform (FFT) reflectance spectroscopy guaranteed reliability while recently researched Interferogram Average over Wavelength (IAW) reflectance spectroscopy has improved its sensitivity. Our Reader software can also be easily migrated to the detection of other protein molecules based on label-free optical biosensing in future development by changing the setting to a universal field.

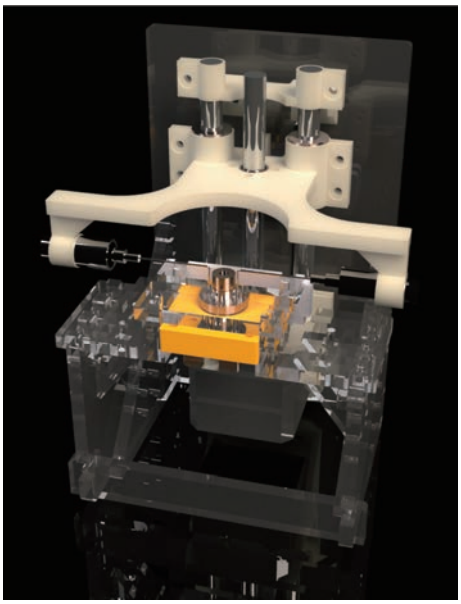


Fig. 3 The detecting part of the biosensor

Novelty & Creativity

Already available

Ordered porous materials is a new type of nanostructured material that first emerged in the 1990s. Due to their unique pore shape and pore size and regular arrangement, they have good selectivity for the size and shape of the analyzed molecules. Biosensors based on porous silicon, one of the well-developed porous materials, have been widely applied from bench to bedside largely owing to its precision and high-throughput. PSi unique photonic properties and wide range of its porosity make it a promising biosensor in both basic and clinical researches. PSi may provide new insights in terms of precision diagnosis, point-of care diagnostics, as well as fast and label-free detection technologies, considering the ab-confined, time-consuming, costly, non-recyclable current detection methods (ELISA, etc.).

New developments

Detection

Rapid detection——with a microfuild system, we are unable to finish one sample detection within 10 minutes and reach a relative error under 5%.

Free-label detection——this kind of label-free technique makes it easy to get a real-time analyzing for it frees us from post hoc processing.

Better LOD performance——Since the degree of precision a spectrometer could be, porous silicon-based biosensor can reach a LOD of ..... Furthermore, with a more complicated biofunctionalization, we managed to reduce LOD by a quarter or one percent if using brand-new analyzing methods.

User Experience

Easy to handle——Our control and motion devices are designed and constructed with modular design theory which makes it easy to handle samples for users.

Reliability and relative low-cost——Optical sensor detection methods are always precise, which give a reliable result. With further development, each test will cost little.

Future Development

Varied porous materials——An increasing number of projects taking advantage of porous materials have shown that other porous materials like AAO could work as well as Psi. That makes cheap testing possible.

Variable samples——According to the method of detecting adalimumab, label-free analysis allows us to detect any other kind of proteins.

Fig 4. (A) Storage (front is for chip storage, back is for icebag); (B) Chip (the harrow Jupiter is sample pool, arrow shows where to add the sample); (C) Sample tank (shown in yellow); (D) User interface (pop-up notices processing is done and press the button indicated with red circle for results); (E) Result viewing; (F) Personal medical records (horizontal axis is sample number, vertical axis is result)

Test Process

Collect Blood

- Collect 15ul blood sample
- Take a chip out of the storage (fig.4A)
- add the sample directly to flow pool (fig. 4B) without dilution

Insert Chips

Push the chip into the sample tank (fig.4C) in the center of the instrument, equipped with sensors to check that it is in the correct position

Automatic Analyze

Just 5 min

Report

- After converting, click the "show" button (fig.4D) to display
- Previous datas are saved to create personal medical records (fig.4E)

After Service (Additional)

- Silicon wafer can be sent back to our company and be recycled
- Instrument is modular and can be partially replaced
- Corresponding algorithms are available for different types of spectrometers

Analytical Performance

Result

We tried three algorithms to fit the standard curve and all showed good linear correlation. Despite this, we were unable to perform more repeated tests to verify our results due to lack of sufficient time and experimental conditions.

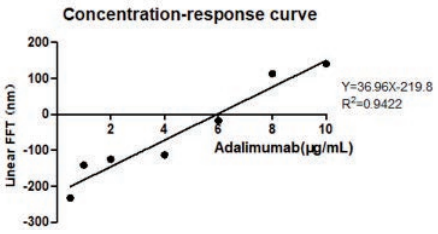


Fig 5. Concentration-Response curve by Linear FFT (Linear fit)

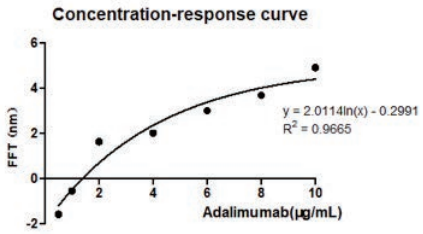


Fig 6. Concentration-Response curve by FFT (Logarithmic fit)

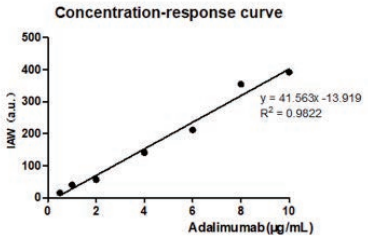


Fig 7. Concentration-Response curve by IAW (Linear fit)

Translation Potential

Stakeholder desirability

According to the timeline from pro-diagnosis to post-diagnosis of a RA patients in China, a customer journey map can be drawn in which there are 5 major stakeholders taking part in, namely the hospital, pharmaceuticals company, patient and family, public institution and insurance company (see in Fig 8.).

Fig 8. 5 Major Stakeholders in user journey map

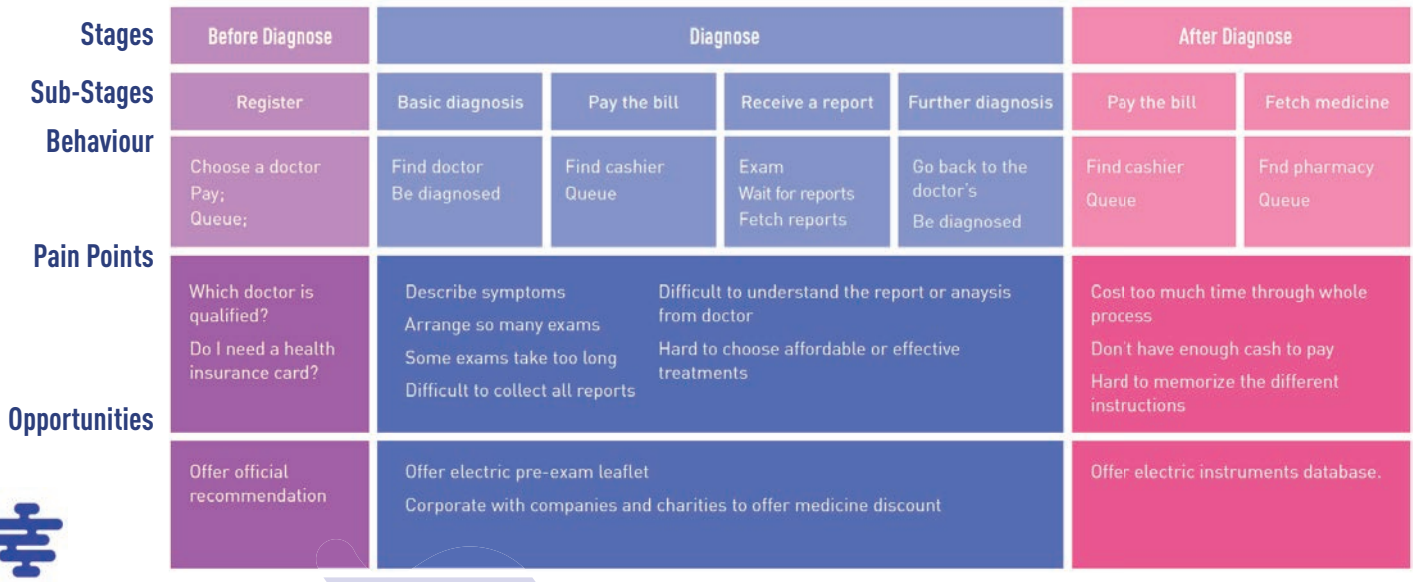
Table 1. The table below shows the behavior and pain of 5 major stakeholders separately, followed by our opportunities of curing their pains.

	Hospital	Pharamaceutical company	Patient and family	Public institution	Insurance company
Behavior	Exam,re-exam and diagnose patients	Provide medicine for patients	Receive treatment and take medicine	Provide humanitarian aid and public aid	Provide insurance to patients
Pain	Overloading workload	low sale	Tardive treatment and unaffordable medicine	Under-employment rate of RA patients and high burden of RA family	Ethical risk, that is to say, people with severer pain are more likely to purchase insurance
Opportunity	Unleash doctors from heavy workload using self-help diagnosis	Co-sale strategy	Accurate medicine tracking	Reduce family burden	Providing a subsidiary applicant situation statement

*focusing on a localized plight, this pharmaceutical company specifically refers to a pharmaceutical company in China.

User Journey Map

To be more specific, we expand the situation of one key stakeholder, the RA patients, by elaborating the User Journey Map of one examination circle.



Need in Market

According to technology and applicable situation, our biosensor belongs to the following market: medical apparatus - in vitro diagnostics (IVD) - immunology diagnosis - monoclonal antibody medicine diagnosis market.

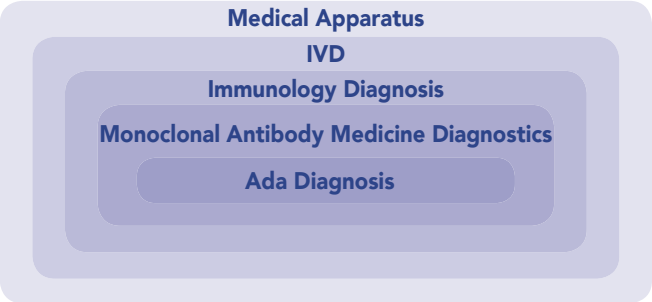


Fig 9. Market segmentation

Medical apparatus: large market, fast growth
With the need for health life increasing, the amount of China medical apparatus market reached 530.4 billion RMB with an year-on-year growth rate of 19.86% in 2018.

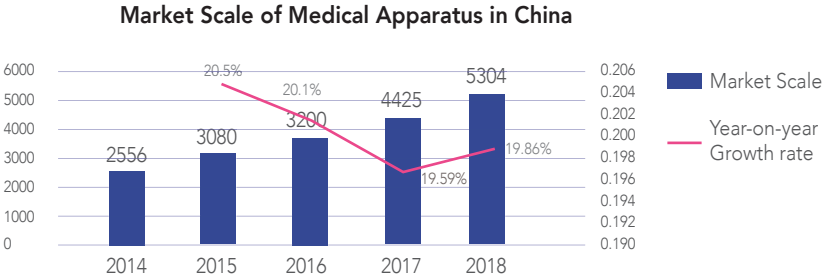


Fig 10. Market Scale of Medical Apparatus in China

IVD Market: great opportunities, promising potential
IVD, refers to tests done on samples such as blood or tissue that have been taken from the human body. To be specific, IVD refers to relative products, including IVD.

The industry chain of IVD is show in Picture.2, in which our prospective corporation covers whole stream.

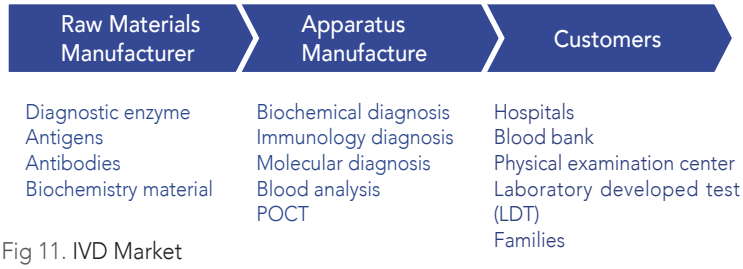


Fig 11. IVD Market

Immunology Diagnosis: import substitution, worth expectation
As the largest and fast segmentation of IVD market, Immunology diagnosis market shows a tendency of import substitution because of the large high-end market share taken by foreign giants.

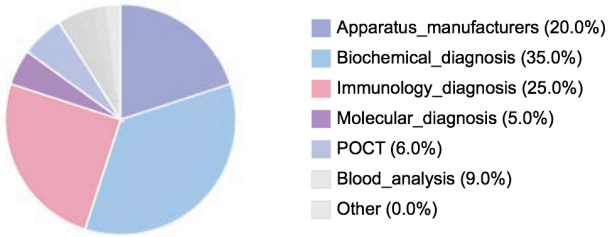


Fig 12. Immunology Diagnosis

Ada diagnosis market: Chinese characteristic, huge growth room
Currently, the RA rate in China is estimated 0.34%-0.36%, means three patients in every 1000 citizens, amounting to 5 million patients, contributing to 16.88% of the global number. To be more specific, the number of female triple that of males. RA can be expect during in age, while middle age females is more like to suffer from it. Remission and disability rate is 8.6% ad 50.3% respectively.
However, Adalimumab has faced a weak sale since its entrance into China in 2010. Etanercept has remained the popular TNF-*α* antibody.

TNF- α	Adalimumab	Etanercept	Infliximab
Global	18.9 billion	8.3 billion	7.8 billion
China	18 million	16 million	167 million

Fig 13. Ada diagnosis market

Value Propersition

Aim to help patients get back to normal life and gain equality and repect from others.

Diagnosis	Blood Test	Treatment
- Go to hospital or get online diagnosis on an App - Pay medical expenses online	- Lend or buy a biosensor from our platform - Do Blood test at home - Get test result on an App	- Consult an expert online - Get treatment - View daily health records

Fig 14. New User Journey Map

Originality of the Solution

We combined online diagnosis with offline diagnosis in the whole process to develop user experience.

Our product line includes device and consumables. Our main consumble is the chip, which is recyclable and has high cost performance.

Device	Consumables	Medicines
1. For sharing 2. For individual use	Chips with different combination	Adamumab with different combination

Fig 15. Product Line

Support

In February 2019, TruSense went to three first-class hospitals in Hangzhou and interviewed representative doctors, nurses and patients.

Fig 16. Interview with doctors, nurses and patients

Business Feasibility

Key activities

First step (1 years)
Speed R&D.
Solicit research subsidy from college and government.

Second step (2-4 years)
Apply for CFDA license.
Apply for patents.
Sell products to LDT*.
Publish paper to advertise academically.

Third step (5-8years)
Open up hospital market.
Open up domestic market through third party like CSO*.

Key partners

Looking along the industry chain of our biosensor, the raw material suppliers in the upstream, government in the middle, and hospitals and third parties in the downstream are three key partners.

Raw material suppliers have a speak to our raw material price. But as there are a bunch of suppliers in the market providing goods with various price, we can strike a balance between the cost and quality by choosing the right supplier.

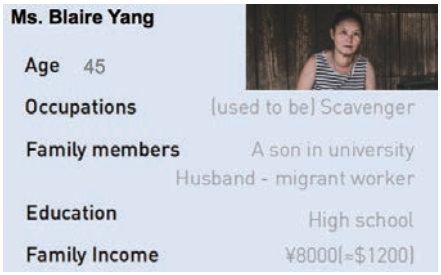
Government has a say in whether license our biosensor or not. As long as a standard producing procedure is achieved, there is no reason for government to block our way.

Hospitals and third parties are predicted to provide us with market channels. For now, our university affiliated hospitals are willing to cooperate, while the optimized CSO depends on further R%D.

First-tier Market Location

Southeast Coast

Persona



Key Channels

Working with the hospital: offline campaign

Medical research magazines, journal-oriented marketing: Nature, Science, etc.

Online medical community platform: Lilac Garden, Ali Health, etc.

Word of mouth / KOL promotion: the patient's community for live broadcast and other content promotion

Distribution: cooperation with pharmaceutical companies

TRUSENSE

Financial Viability

Costs Projection

	Device	Materials	Total
Costs / CNY	8884.6	53.6	8938.2
Costs / EUR	1110.8	6.7	1117.3

Revenue Stream and Business Strategy

A flexible revenue stream will be critic to our R&D especially in the emerging period. In the starting years the research will rely on the subsidy of public department including college lab, university fund, province government project and the like, through which we have succeed in earning our first bucket of gold. Following will the income of selling to LDT of university affiliated hospitals. At last, after building up a connection with hospital market, we will be ready to expand our market channel and scale our profit.

Sales Price

In the third period in which we open up both the hospital and family market, according to the already high price of Ada treatment, it is reasonable to assume that they are affordable a helpful extra treatment.

Team and Support

Team Members

TUTOR

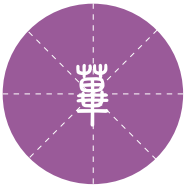
HUANG Liquan

TEAM LEADER

ZHOU Zhenwei

WANG Fanjin

WENG Xin



ZHAO Xiaomin

ZHOU Weihuan

Zou Kexin

CHEN Sheng

Contribute to

Principle of senor, Machanical structure, Product design

FANG Shitao

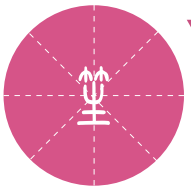
GAO Rujun

Wu Yue

ZHAO Taonan

LOU Biying

XU Hui



Contribute to

Market Research, Marketing Strategy and Communication

XU Yi

ZHANG Linlu

Note: "畢 笙" (Bishen) , a mythical animal from the Classic of Mountains and Rivers, symbolizes precise detection, rapid responce and pain relief.

Therefore, Trusense is divided into "畢" group and "笙" group to create the innovative biosensor system named "畢笙".

Sponsors

SensUs

浙江大学 ZHEJIANG UNIVERSITY 1897

COLLEGE OF LIFE SCIENCES 浙江大學生命科學學院

浙大一院 1947 THE FIRST AFFILIATED HOSPITAL, ZHEJIANG UNIVERSITY

邵逸夫医院 ZUM·LLU 1994

六合华大 BGI·Write

PROSP PRO SPECTRAL TECHNOLOGY 专谱光电

Laurel Venture 朗煜資本

TRUSENSE

Final Remarks

A challenge has been given means there is a lot need to be learned and to be practiced. Our team is hunched over the project and has gained experience, knowledge, friendship during pre-preparation. Proudly speak, all the efforts turned out to be a heady result. Also, we do realize what we have done is not enough.

Look into the future, we'd like to pull out all the stops to improve our biosensor and make it more accurate since there is a compelling evidence that precision medical treatment is a significant driver of new medical technology and equipment. More porous silicon elements will be need, more data will be recorded and operated so that standard curves will be further calibrated. Besides, our team will optimize the transmission system on the basis of better human-machine interaction design to make it ready for commercialization. Furthermore, we firmly believe the competition itself made up of teams worldwide will definitely lead to more attention to healthcare.

We want to thank SensUs for such a golden opportunity sincerely. We can share our ideas with all those creative and intelligent college students from all over the world, which strengthen the bond between colleges, also stretch our minds. Also, we'd like to thank Zhejiang University and the companies mentioned above for supporting us both in finance and conduct propaganda. Hardly could we reach where we are without all those help.

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Appendix

Best-fit values	Linear FFT(nm)	IAW (a.u.)
Slope		
Y-intercept when X=0.0		
X-intercept when Y=0.0	5.947	0.3349
1/slope	0.02706	0.02406
95% Confidence Intervals		
Slope	26.44 to 47.48	35.13 to 48.00
Y-intercept when X=0.0	-279.0 to -160.7	-50.09 to 22.25
X-intercept when Y=0.0	4.978 to 7.171	-0.6129 to 1.078
Goodness of Fit		
r2	0.9422	0.9822
Sy.x	36.49	22.31
Is slope significantly non-zero?		
F	81.57	275.9
DFn, DFd	1.000, 5.000	1.000, 5.000
P Value	0.0003	< 0.0001
Deviation from zero?	Significant	Significant
Data		
Number of X values	7	7
Maximum number of Y replicates	1	1
Total number of values	7	7
Number of missing values	0	0

log(agonist) vs. response -- Variable slope	FFT(nm)
Ambiguous	
Best-fit values	
Bottom	~ -12770
Top	5.023
LogEC50	~ -31.01
HillSlope	~ 0.1051
EC50	~ 0.0
Span	~ 12775
Std. Error	
Bottom	~ 8.878e+007
Top	3.454
LogEC50	~ 28828
HillSlope	~ 0.3264
Span	~ 8.878e+007

95% Confidence Intervals	
Bottom	(Very wide)
Top	-5.969 to 16.02
LogEC50	(Very wide)
HillSlope	(Very wide)
EC50	
Span	(Very wide)
Goodness of Fit	
Degrees of Freedom	3
R2	0.9476
Absolute Sum of Squares	0.7430
Sy.x	7
Number of points	
Analyzed	

Device	Usage	Num	Price per unit / CNY	Price per unit / EUR	Total / CNY	Total / EUR
Spectrometer \ optical fiber \ tungsten lamp	Spectra obtained	1	7000	875	7000	875
Peristaltic pump	Testing process	1	768	96	768	96
Lifting motor	Testing process	1	137	17.125	137	17.125
Flow pool structure	Testing process	1	13	1.625	13	1.625
Instrument shell	Structural components	1	500	62.5	500	62.5
Other structural components	Structural components	1	200	25	200	25
The liquid pool	Structural components	2	20	2.5	40	5
Arduino uno	Control component	1	85	10.625	85	10.625
Power adapter	Control component	1	141.6	18	141.6	18

Consumables	Usage	Num	Price per unit / CNY	Price per unit / EUR	Total / CNY	Total / EUR
Silicon wafer	Chip material	1	20	2.5	20	2.5
HPLC	Silicon wafer etching	1	0.4032	0.504	0.4032	0.504
Ethonol	Silicon wafer etching	1	0.12	0.015	0.12	0.015
1×PBS	Flush and elution	1	2.4	0.3	2.4	0.3
APTES	Chemical modification	1	0.6	0.075	0.6	0.075
Toluene	Chemical modification	1	2	0.25	2	0.25
Glutaraldehyde	Chemical modification	1	0.16	0.02	0.16	0.02
Chitosan	Chemical modification	1	0.0122	0.0015	0.0122	0.0015
Glacial acetic acid	Chemical modification	1	0.0136	0.0017	0.0136	0.0017
PEG derivatives	Serve as end-capper	1	5.364	0.6705	5.364	0.6705
TNF-alpha	specifically combined	1	22.53	2.8163	22.53	2.8163