Luciferase-Based Microfluidic Platform for Point of Care Testing and Nanomedicine Applications

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Outlook

- point-of-care testing (POCT) ~ 现场检查设备
- 纳米医学 ≈ **針灸** ~ **Nanomedicine** ≈ Acupuncture
  - (生长) 纳米微观宏观 ↔ (凋亡) 宏微纳米
  - (↑) nano⇐micro⇐macro ⇔ (↓) macro⇒micro⇒nano
  - Growth & apoptosis = 生长 和 凋亡
- Luciferase-based microfluidic platform
  - = 基于荧光素酶的微流体平台
  - ルシフェラーゼベースのマイクロ流体プラットフォーム
- biological measurement = only Biometrics in all Languages
  - Cell by cell = 逐个细胞 = 細胞による細胞 = सेल द्वारा सेल
  - Cell by tissue = 细胞组织 = 組織による細胞 = ऊतक द्वारा सेल
  - Self-organization = 自组织 =自己組織化 = स्व-संगठन = التنظيم الذاتي
- Conclusion

Nanomedicine formula ≈ **Harmony of ☯ = 阴阳 ⇒ 養生學**

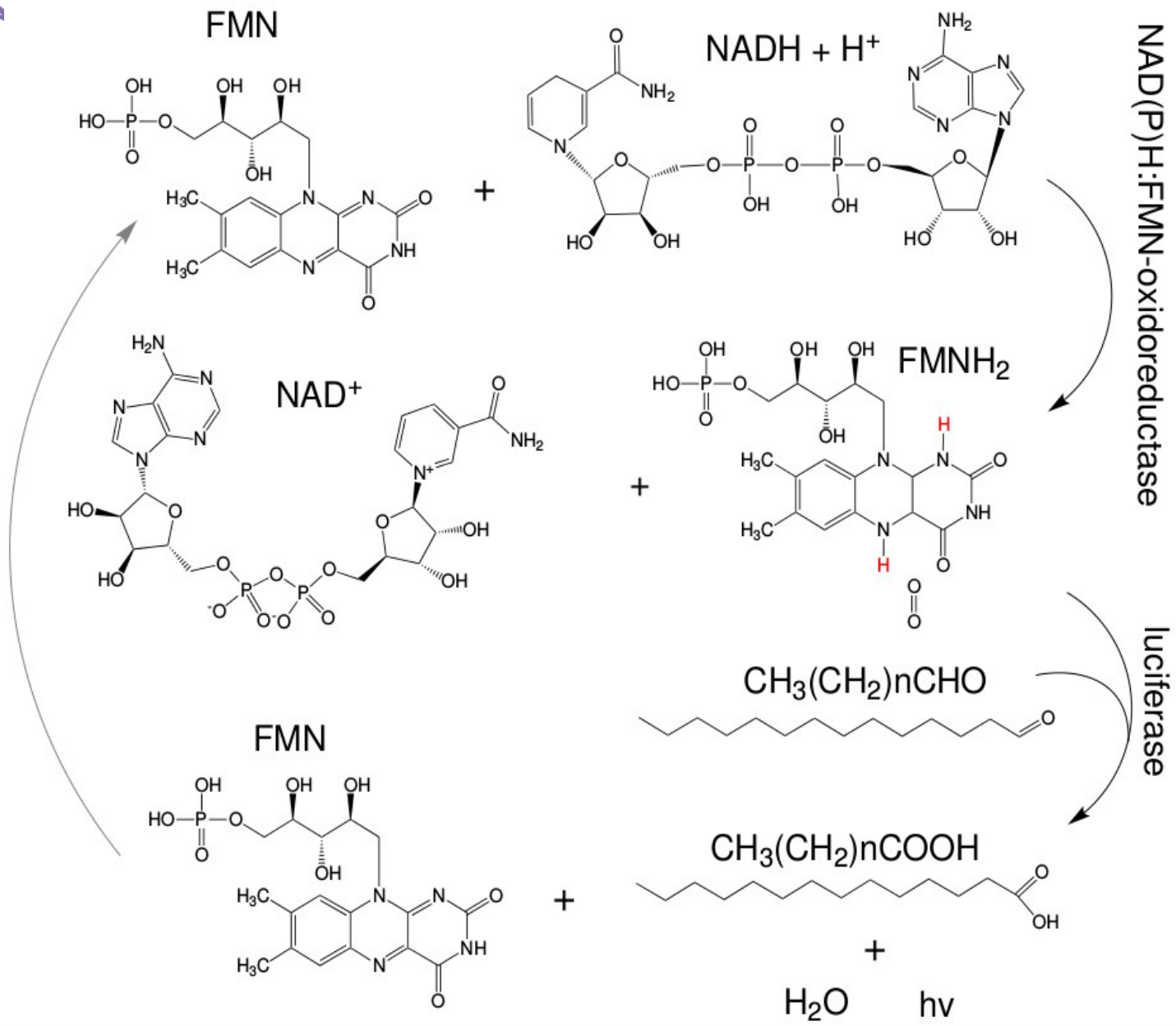
The issues

- The problems of **modern nanomedicine** as the application of nanotechnology are related with problems of microfluidics, because the development of point-of-care testing (POCT) devices for nanomedicine **requires** various types of **disposable microfluidic chips**.
- To address this issue we designed **luciferase-based microfluidic platform** for bioassaying of physiological fluids.
- Design is based on **coupled enzymes of bacterial bioluminescent system**

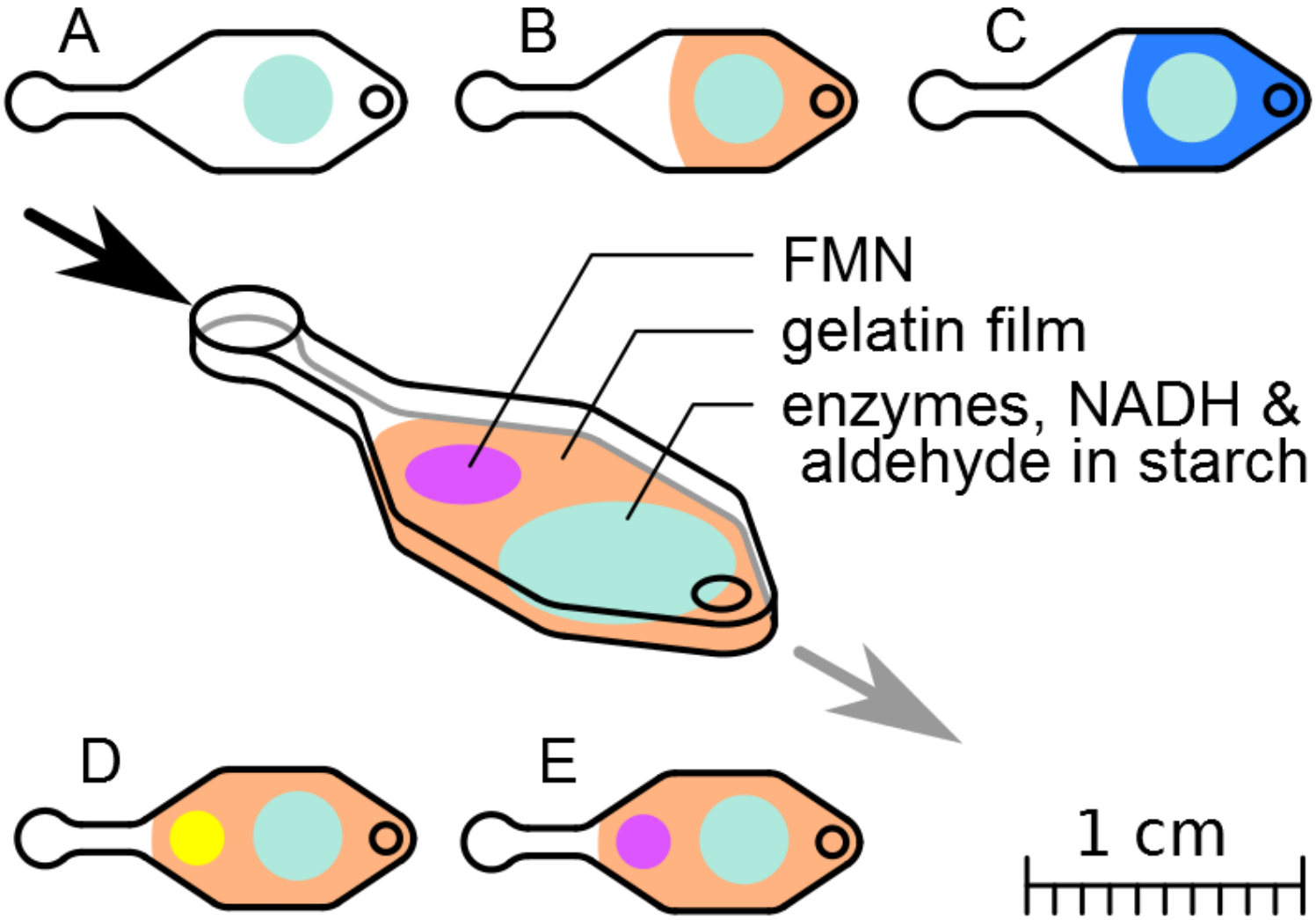
Luciferase-based microfluidic platform

- The platform is based on PMMA chips containing enzymes:
  - NAD(P)H:FMN-oxidoreductase and
  - luciferase with substrates of bacterial bioluminescent system
  - immobilized in dried starch droplets (Enzymolum™).
- After addition of a sample the reagents are releasing from microgel phase and mixing to achieve of bioluminescent signal.
- The signal measurement can be carried out either PMP or MPPC/SiPM, depending on type of POCT device.
- The parameters for sufficient mixing and reagents displacement were studied.

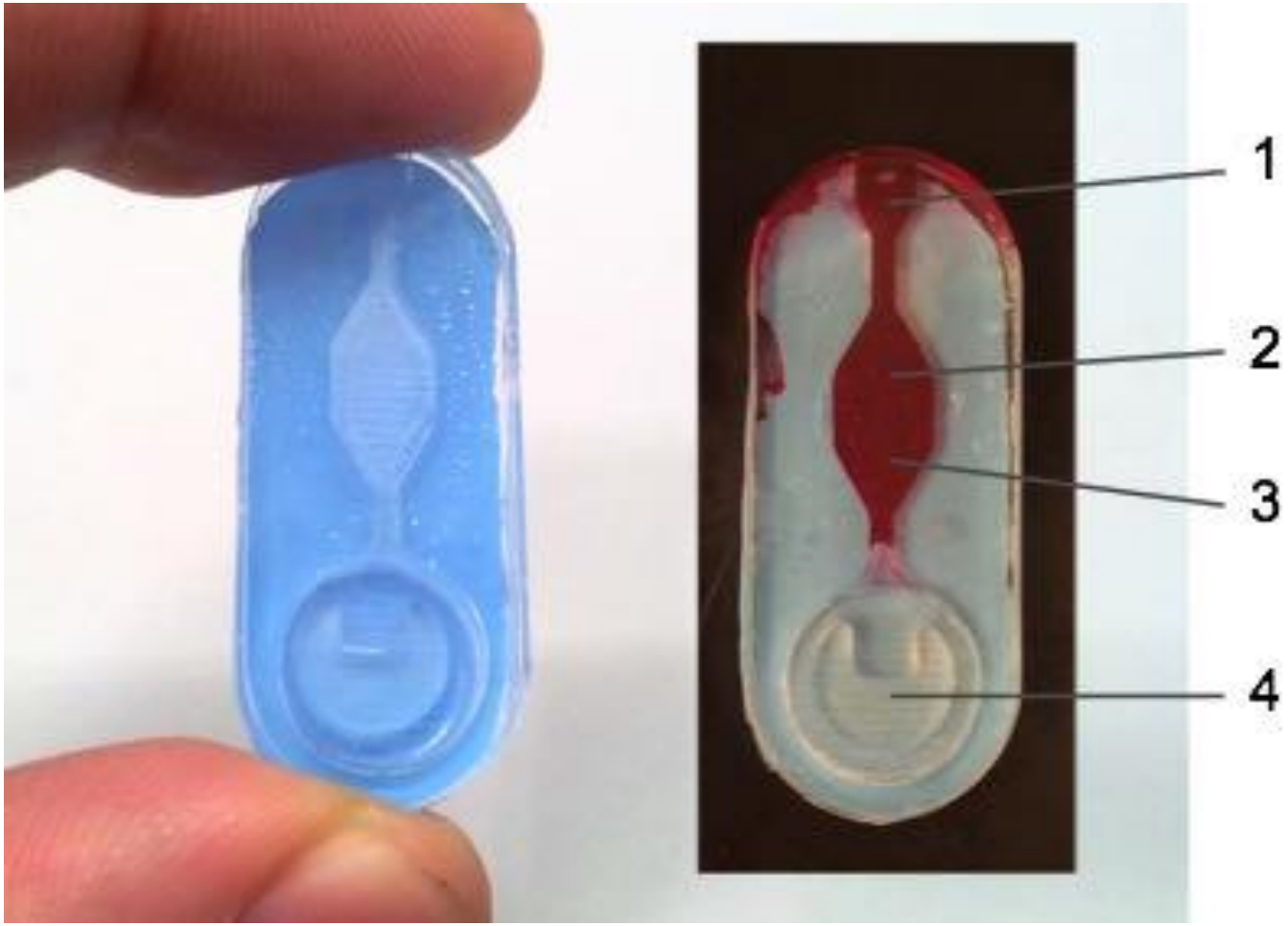
Bioluminescent reaction



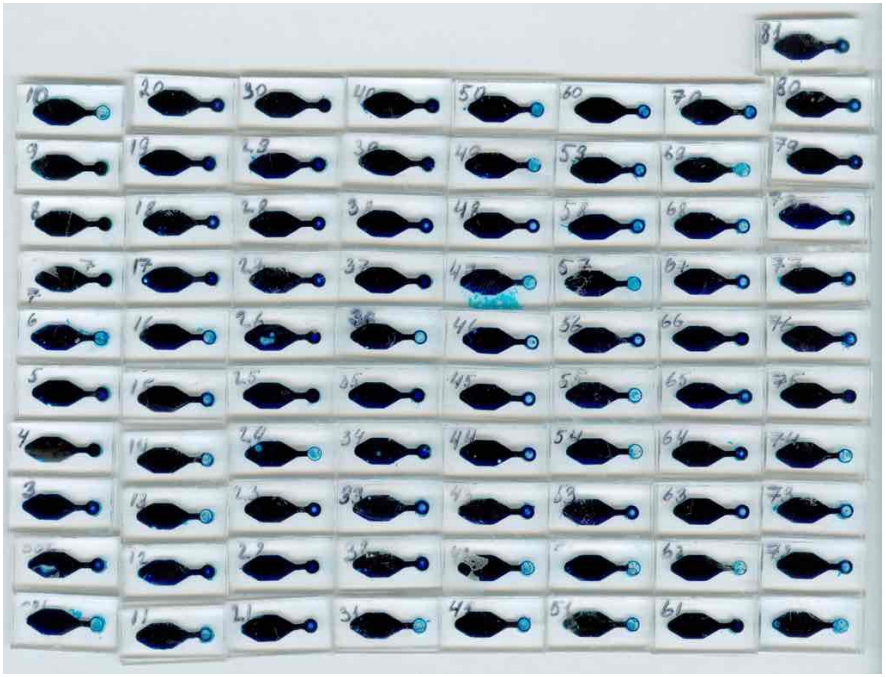
Placement stage reagents into microfluidic chip



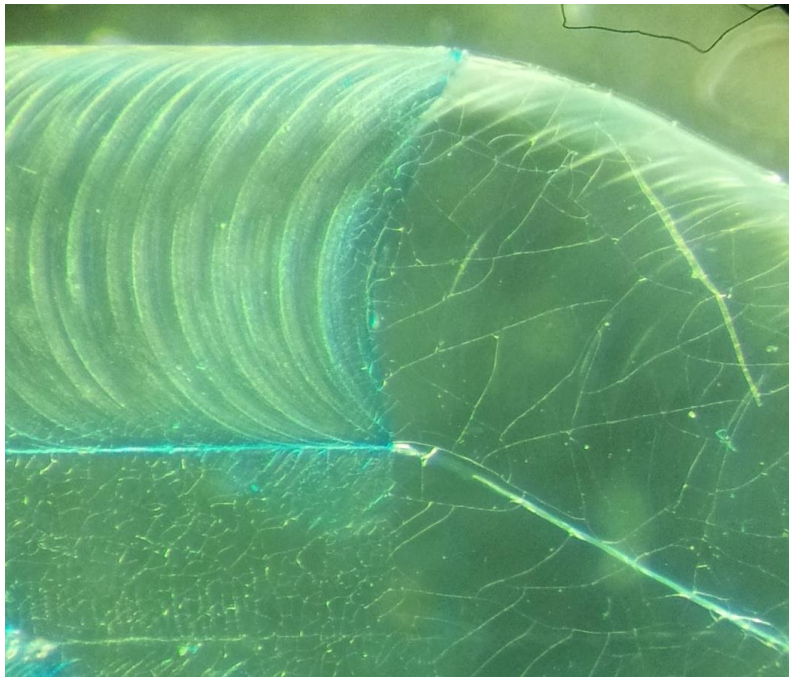
Location topology of all microfluidic chip elements



Prepared the regulations sealing and manufacturing of the chips



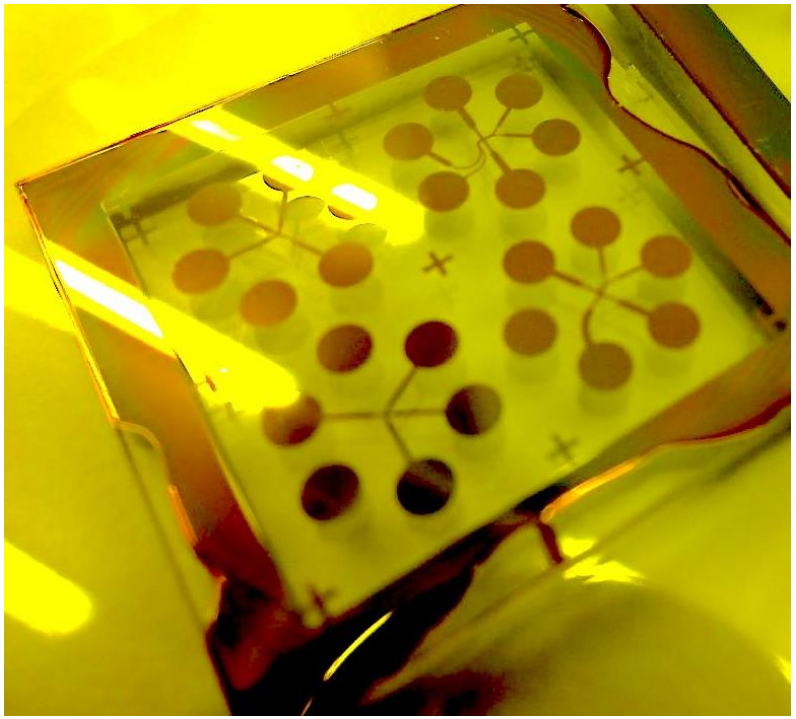
Leak test - quality satisfy



Structures are smoothed up 15 microns; covered by gelatin film (left) & without gelatin (right)

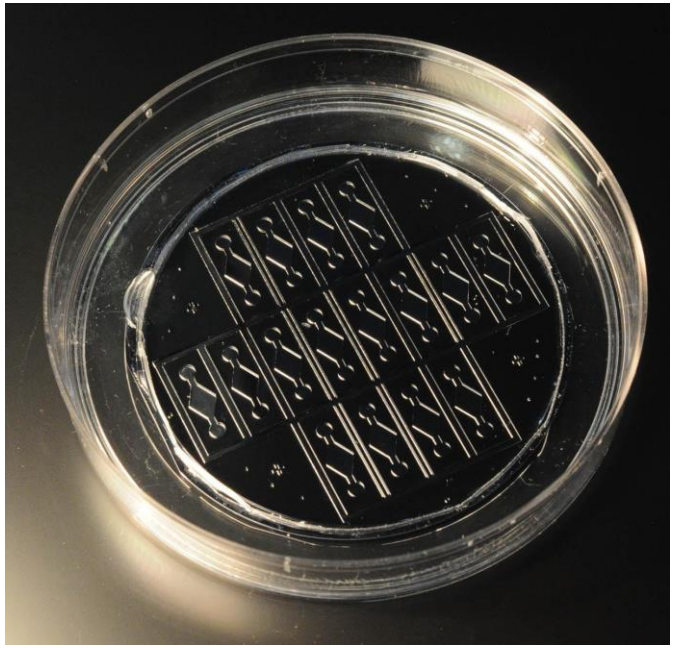
Manufacturing technique of channel surface

- Formation of the surface
  - Photolithography (1 - 300 microns)
  - Milling (> 300 microns)
- Multiple copies
  - Casting
  - Thermal stamping

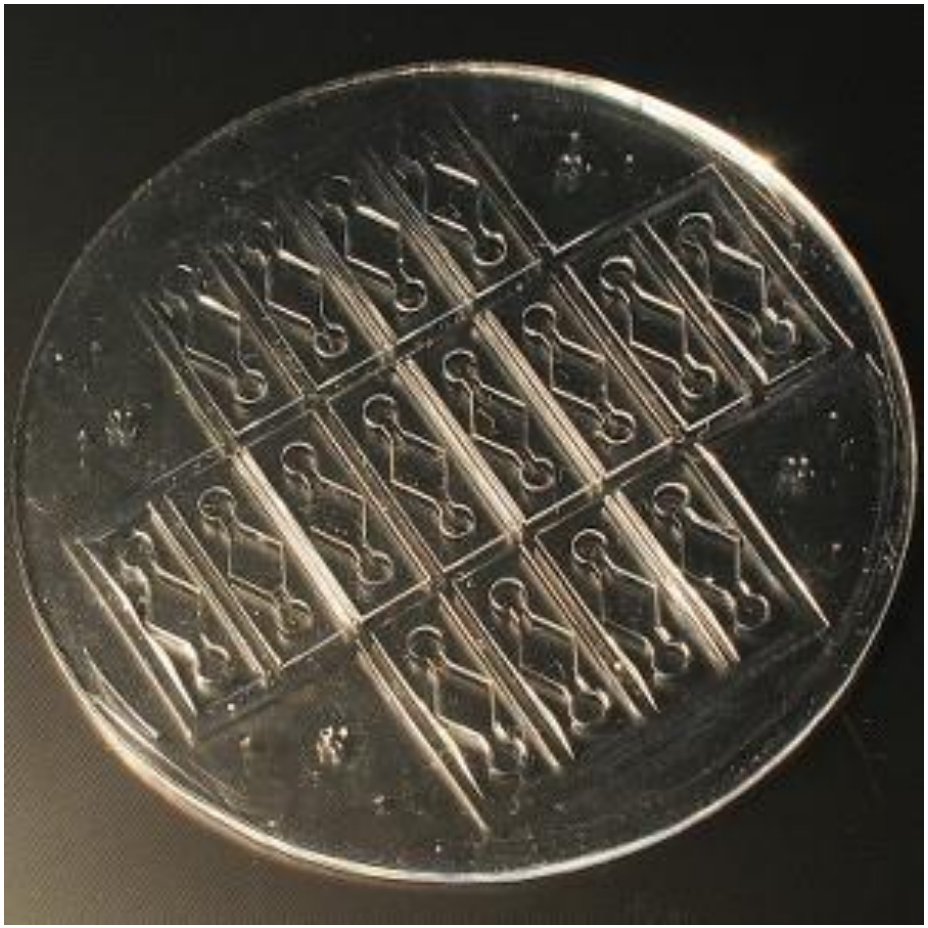


Copying process by thermal stamping

allow to copying the structure from 40 to 150 microns

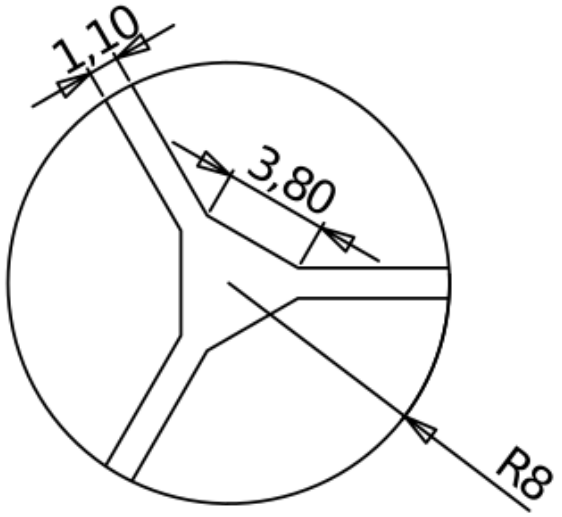


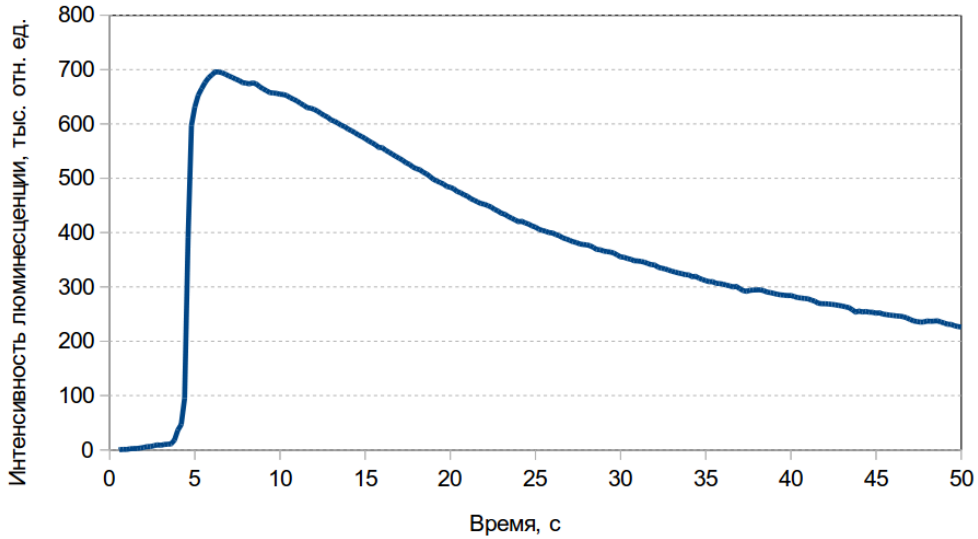
Si/SU-8 master molds for manufacturing microfluidic chips in PMMA with depth of reactors of 450 microns



The stamping result

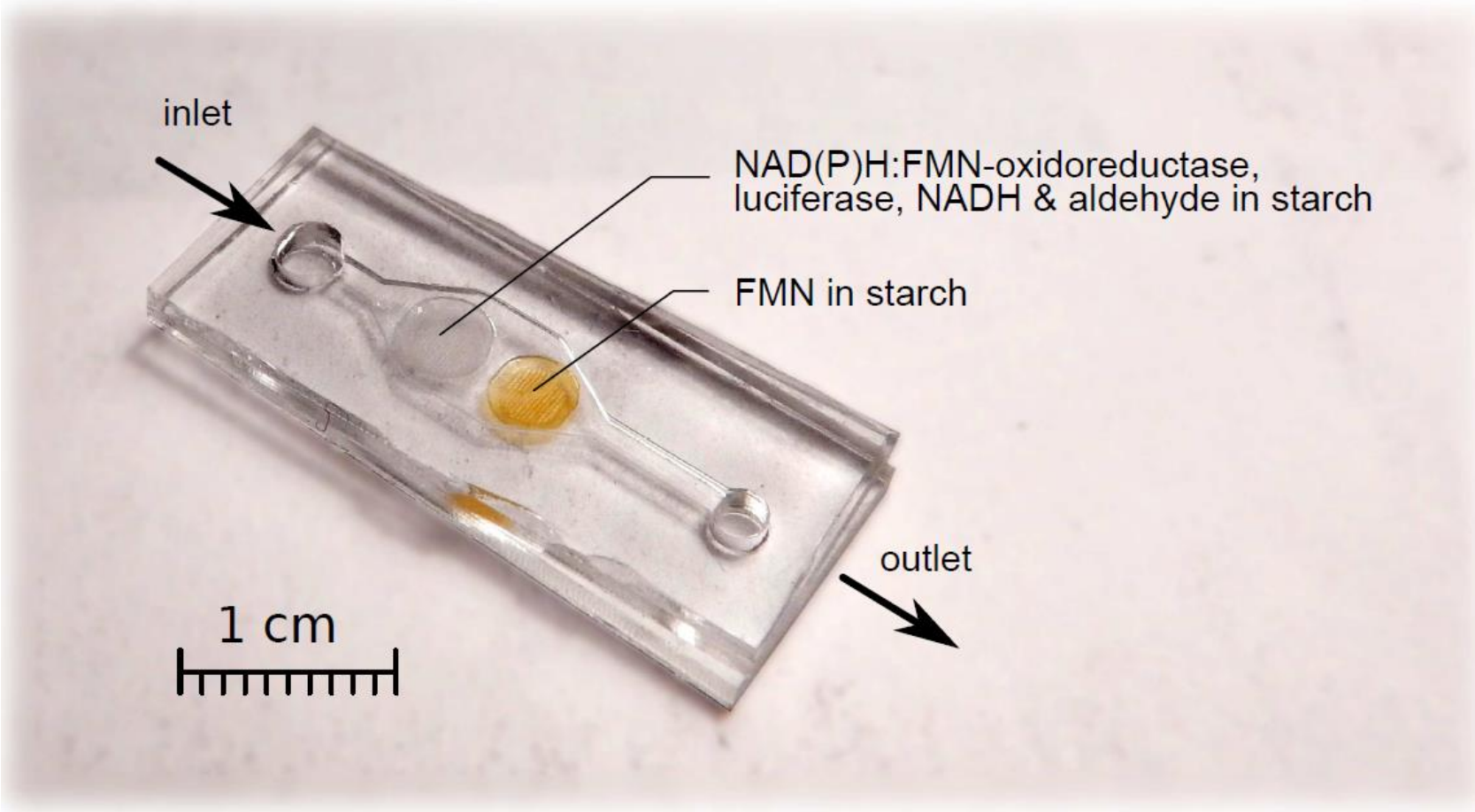
Made chamber is isolated from light for flow detection



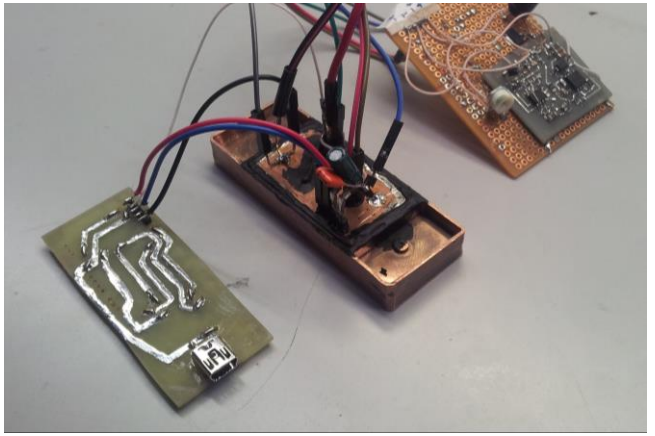


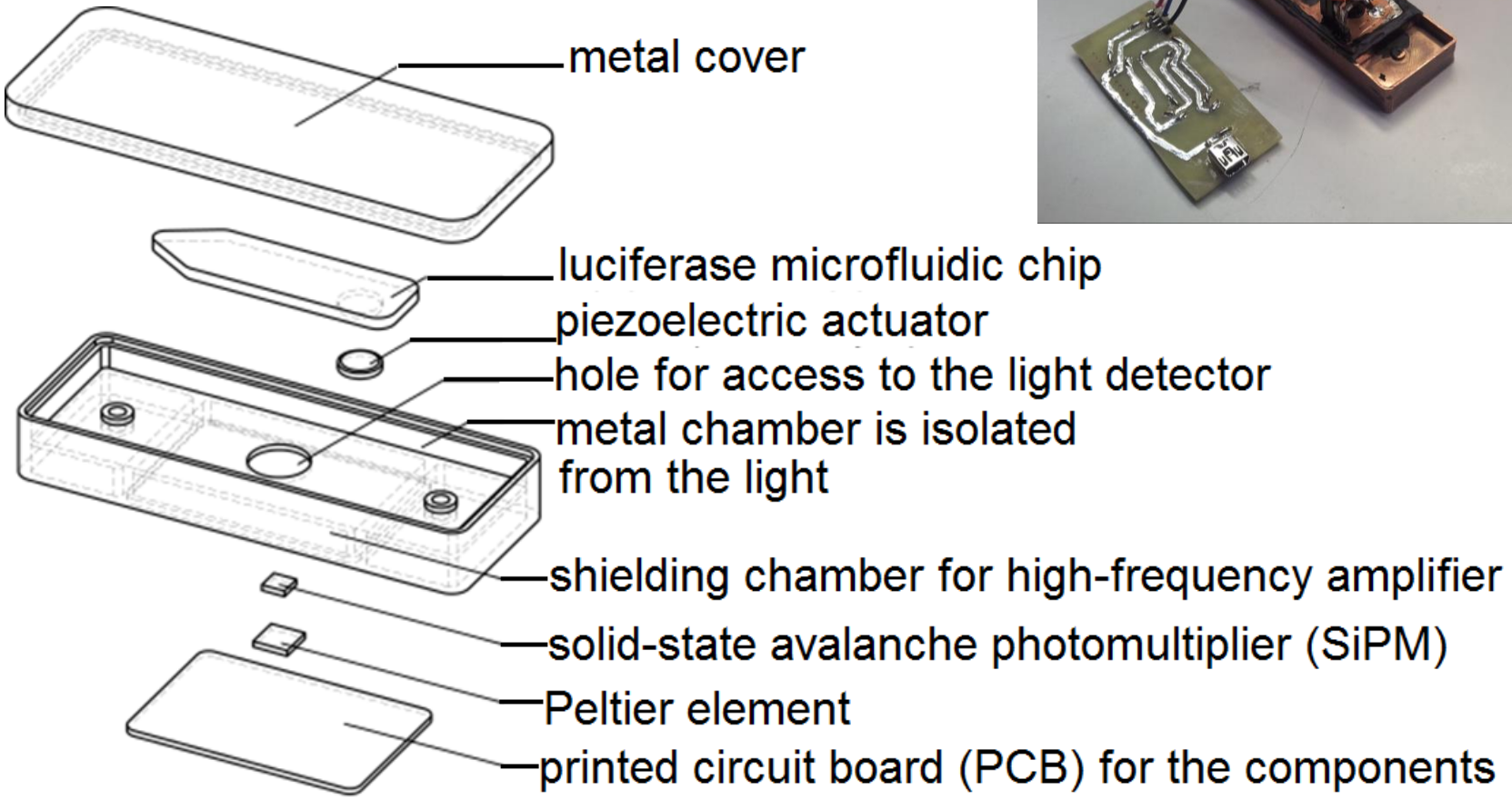
Luminescence kinetics in the microfluidic chip of 20 microliters with 1.6 mM Obelin after calcium adding

Design of disposable chips for luciferase microfluidic platform



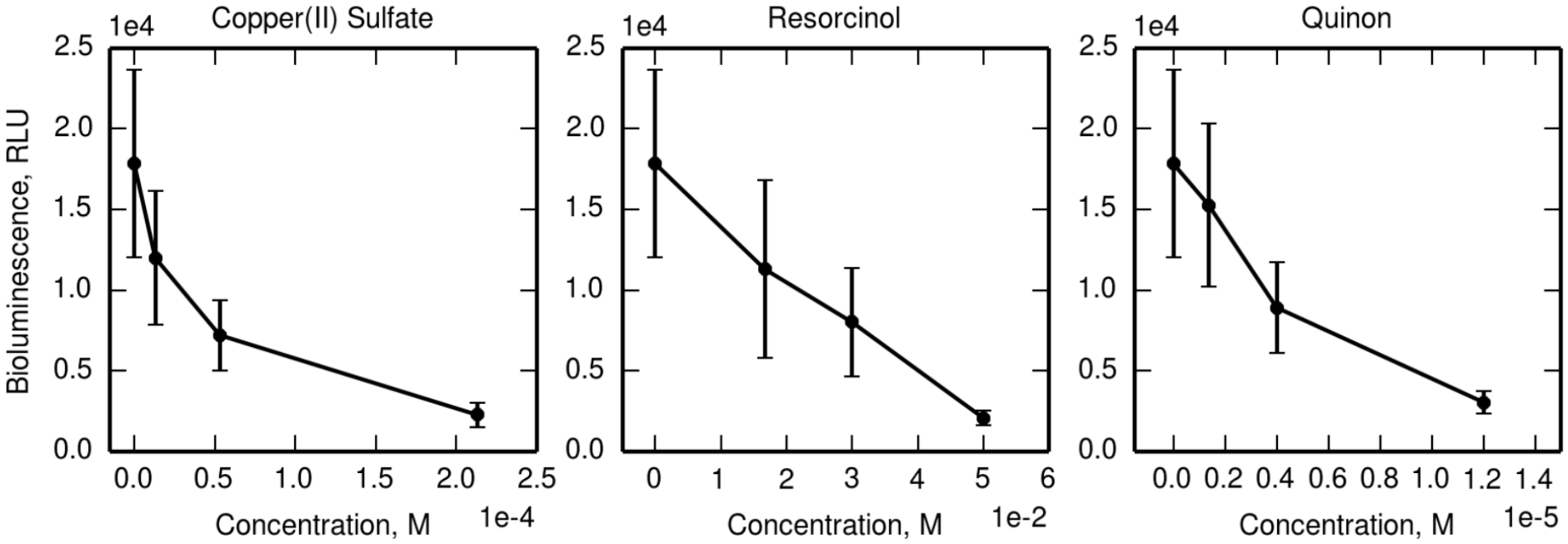
Device for detecting light from luciferase microfluidic chip





Testing of luciferase microfluidic platform

Fabricated devices have checked by model toxins in water, e.g. Copper (II) Sulfate, Resorcinol and Quinone:



Conclusion. **The tested devices have sensitivity at the threshold limit value (TLV) for measured toxicants**

Biological measures 生物学法

- Simplest with depth philosophy
  - 哲学深度的最简单的概念
- The simplest concept of medical depth
  - 医学深度的最简单的概念
- 纳米医学 ≈ 針灸 ~ Nanomedicine ≈ Philosophy
  - 哲 Philosophy
  - 医 Medical
- 針 Needle = action of nanoparticles on cells
  - 灸 = 艾灸 = 纳米颗粒对细胞的作用细胞
  - Cell ⇔ tissue ⇔ organism ≈ 细胞组织生物

生物自然法 **Action is life** 行动是生命

Conclusion: Biological measurement

- Suggested chips do not require laboratory expertise from potential user.
- The prospects for POCT devices on the suggested platform
  - multiplex biological assay,
  - massive parallel measurements, and
  - nanodiamond-based amplification biobarcode assay.
- The research was supported by the grant of the Russian Science Foundation (no.15-19-10041).