Biosensors and Bioelectronics 92 (2017) 472–481

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Step-by-step guide to building an inexpensive 3D printed motorized positioning stage for automated high-content screening microscopy

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ARTICLE INFO

Keywords:
3D desktop printing
Arduino
High-content screening
Cell viability
YFPI152L
Glycine receptor
TRPV1
Fluo-4 AM

ABSTRACT

High-content screening microscopy relies on automation infrastructure that is typically proprietary, non-customizable, costly and requires a high level of skill to use and maintain. The increasing availability of rapid prototyping technology makes it possible to quickly engineer alternatives to conventional automation infrastructure that are low-cost and user-friendly. Here, we describe a 3D printed inexpensive open source and scalable motorized positioning stage for automated high-content screening microscopy and provide detailed step-by-step instructions to re-building the device, including a comprehensive parts list, 3D design files in STEP (Standard for the Exchange of Product model data) and STL (Standard Tessellation Language) format, electronic circuits and wiring diagrams as well as software code. System assembly including 3D printing requires approx. 30 h. The fully assembled device is light-weight (1.1 kg), small (33×20×8 cm) and extremely low-cost (approx. EUR 250). We describe positioning characteristics of the stage, including spatial resolution, accuracy and repeatability, compare imaging data generated with our device to data obtained using a commercially available microplate reader, demonstrate its suitability to high-content microscopy in 96-well high-throughput screening format and validate its applicability to automated functional Cl^-- and Ca^{2+}-imaging with recombinant HEK293 cells as a model system. A time-lapse video of the stage during operation and as part of a custom assembled screening robot can be found at https://vimeo.com/158813199.

1. Introduction

Over the past two decades, automated high-content screening (HCS) microscopy has become an indispensable method in cell biology with applications in an almost infinite variety of scientific fields including cancer research and neurosciences.

Conventional HCS systems are typically made up of a fully automated inverted microscope as a central component and a motorized stage mounted on top of the microscope's chassis. The microscope, usually equipped with exchangeable objectives, focusing mechanics, a digital camera and infrastructure allowing for fluorescence or luminescence microscopy, is used for visualization of biological probes at the microscopic level. The motorized stage enables inspection of specimen larger than the field of view of the objective and automatic positioning of biological samples, cultured e.g. in multititer plates, during operation in high-throughput screening mode. Besides these components, conventional HCS devices are equipped with additional parts, such as an incubator, capable of maintaining optimal conditions necessary for culturing cells, liquid-handling systems or robotics for operation in almost autonomous high- or ultrahigh-throughput screening mode.

Commercially available motorized positioning stages are characterized by high-speed scanning capabilities and high positional accuracy in the micro- or sub-micrometer range, achieved through e.g. stable closed-loop control systems. These stages are claimed ideal for manually or automatically positioning a wide range of specimens and samples in many types of microscopy or imaging techniques and applications but are typically proprietary and thus disadvantageous for a number of reasons. First, installation of commercially available positioning devices or integration with existing equipment as well as application and maintenance requires a high level of product-specific skill and hence, is resource-intensive and not user friendly or straightforward. Second, options for interfacing with third-party systems are

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http://dx.doi.org/10.1016/j.bios.2016.10.078
Received 9 August 2016; Received in revised form 21 October 2016; Accepted 25 October 2016
Available online 02 November 2016

often limited at both hard- and software levels, e.g. because available I/O (input/output) ports are not documented and thus, virtually not available to users, software code is closed source or the command set is not provided. Third, customization of conventional positioning stages is hampered for the sake of protection of proprietary hard- and software. For example, hardware modification is typically not permissible and will result in the loss of the warranty and exclusion of liability. Furthermore, proprietary commercial systems are typically cost-intensive and not readily applicable to a broad range of laboratories in low-resource settings or for educational purposes, e.g. in schools or universities.

Despite the fact that there are examples on low-cost 3D printed technologies for cell-based high-content imaging (Campbell et al., 2014; Walzik et al., 2015; Wardyn et al., 2015) and high-throughput screening (Berg et al., 2015; Gregory and Veeman, 2013; Spivey et al., 2014), a positioning stage for automated high-content screening microscopy that is fully open source, scalable and low-cost has not been reported yet. The recent availability of rapid prototyping technology, including 3D desktop printing, open source microcontroller boards and inexpensive consumer electronics, mechanics and robotics parts - also adopted by the 'Maker' movement, a culture of do-it-yourself (DIY) product design (Editorial, 2013; Landrain et al., 2013; Seyfried et al., 2014) - makes it possible to quickly engineer and share user-friendly equipment of reduced cost and complexity.

To overcome the limitations of proprietary technology described above we aimed to develop a motorized stage based on a 'Makers' approach that is fully open source, customizable, robust, affordable to a broad range of research labs and could rapidly and easily be re-built e.g. in so called *fab labs* (fabrication laboratories) and - at the same time - is sufficiently accurate for automated high-content screening microscopy. To this end we aimed to use off-the-shelf electronic components as well as 3D-desktop printing and low-cost open-source microcontroller architecture, both being increasingly used in the development of scientific devices (D'Ausilio, 2012; Frame and Leach, 2014; Leeuw et al., 2013; Pineño, 2014; Schubert et al., 2013; Starosolski et al., 2014; Stokes et al., 2013; Wittbrodt et al., 2014) and proven valuable in the design of technology suitable to educational purposes (Marzullo and Gage, 2012; Shannon et al., 2014; Walzik et al., 2015). We further aimed to evaluate the stage with regard to its positioning characteristics including spatial resolution, accuracy and repeatability and to provide one example each for applications in cancer research and in neurosciences i.e. for high-content imaging in high-throughput screening mode, e.g. for cell fitness screening, and for functional imaging of ion channels, respectively.

In cancer research, high-content imaging in high-throughput screening mode is applied for example in the context of cell viability or fitness screening to identify novel chemotherapeutics. Cellular fitness is indicated by various physiological indicators which are partially and indirectly assessable using fluorescence-based assaying methods and is often measured by quantifying cell number using e.g. fluorescent proteins (Gilbert et al., 2011). In order to demonstrate the applicability of the motorized stage to quantifying cellular fitness, we intended to apply the technology with recombinant human embryo-derived (HEK293^{YFPI152L}) cells, stably expressing YFPI152L, an engineered variant of yellow fluorescent protein (YFP) and to compare data obtained from the custom device with data from a commercial high-end microplate reader.

In neuroscience, high-content imaging, also referred to as functional imaging, has become an integral part for evaluating the biophysical and pharmacological properties of ion channels - pore-forming membrane proteins involved in most physiological and disease processes and considered highly attractive drug targets for therapeutic intervention (Alexander et al., 2015; Braat and Kooy, 2015; Meier et al., 2014; Ortega-Guerrero et al., 2016; Talwar et al., 2013). In functional imaging experiments, the activity of ion channels is mostly assessed indirectly using ion-selective indicators such as loadable or genetically encoded fluorescence dyes that report an ion channel-induced change in intracellular ion concentration via varying fluorescence intensity. For assessing the suitability of the low cost device for functional imaging of ion channels, we aimed to conduct agonist and antagonist concentration-response experiments with glycine receptor (GlyR) chloride channels in combination with YFPI152L as fluorescence probe and with transient receptor vanilloid receptor type 1 (TRPV1) cation channels in combination with Fluo-4 AM as fluorescence indicator.

GlyRs are ligand-gated ionotropic receptors that mediate inhibitory neurotransmission in the central nervous system and conduct an anion current, mainly carried by chloride (Cl^-) upon activation by the amino acid glycine. The channels can be selectively blocked by the high-affinity competitive antagonist strychnine (Lynch et al., 1997). Functional GlyRs are formed from a total of five subunits (α1– α4, β) which assemble either as α homomeric or αβ heteromeric channels (Lynch, 2009). GlyR-activation can be monitored by fluorescence imaging using YFPI152L. YFPI152L, an engineered variant of YFP with greatly enhanced anion sensitivity, is quenched by small anions and is thus suited to reporting anionic influx into cells (Galietta et al., 2001). It has proven useful in screening compounds against many chloride channel types (Balansa et al., 2010, 2013a, 2013b; , 2011, 2009c; , 2011, 2009c; Kruger et al., 2005; Kuenzel et al., 2016).

TRPV1 is a non-selective cation channel that mediates pain perception in nociceptive somatosensory neurons. It is activated by temperatures exceeding 43 °C, by a drop in pH below 6.8 and by capsaicin, the main pungent ingredient in chili peppers (Caterina and Julius, 2001). When activated, the channels conduct a depolarizing cation current, partly carried by Ca^{2+}, leading to an increase in intracellular calcium concentration $[Ca^{2+}]_i$. Capsaicin-induced activation is antagonized by the competitive antagonist capsazepine, a synthetic analogue of capsaicin (Bevan et al., 1992). Activation of TRPV1 and increase of the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ can be assessed using Ca^{2+} selective fluorophores, such as Fluo-4 AM. Fluo-4 AM is a non-ratiometric, high-affinity fluorescent dye for quantifying $[Ca^{2+}]_i$ within a large dynamic range, around a Kd(Ca^{2+}) of 345 nM (Gee et al., 2000). Its fluorescence intensity increases with increasing $[Ca^{2+}]_i$.

Finally, we aimed to create and to provide detailed step-by-step instructions to re-building the stage and to evaluate it with regard to the time required for production and installation.

2. Materials and methods

2.1. Pharmacological reagents

Glycine, strychnine, capsaicin and capsazepine were obtained from Sigma-Aldrich. Glycine was prepared as a 1 M stock in water, strychnine was prepared as 10 mM stock in dimethylsulphoxide (DMSO). Capsaicin and capsazepine were prepared as 100 mM stocks in DMSO. All stocks were frozen at −20 °C. From these stocks, solutions for experiments were prepared on the day of recording.

2.2. Calcium indicator

Fluo-4 AM was obtained from Molecular Probes and was prepared as 10 mM stock in DMSO. Fluo-4 AM stocks were frozen at −20 °C. From these stocks, solutions for calcium imaging experiments were prepared on the day of recording.

2.3. Cell lines

Human embryonal kidney-derived cells (HEK293, CRL-1573) were purchased from *The American Type Culture Collection* (ATCC). The HEK293^{YFPI152L} cell line was generated as described in (Walzik et al., 2015).