SPIRO – the automated Petri plate imaging platform designed by biologists, for biologists

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Abstract

The imaging of plant seedlings, fungal mycelia and bacterial colonies grown on Petri plates is commonly used in phenotyping assays, and is typically done manually despite the procedures being time-consuming and laborious. The main reason for this is the still limited availability of existing automated phenotyping tools and facilities. Additionally, constructing a custom-made automated solution is a daunting task for most research groups specializing in biology.

Here, we describe SPIRO, the Smart Plate Imaging Robot, an automated platform that acquires time-lapse photos of up to four vertically oriented Petri plates in a single experiment. SPIRO was designed for biologists by biologists; thus its assembly does not require experience in engineering or programming and its operation is sufficiently intuitive to be done without training. SPIRO has a small footprint optimal to fit into standard incubators for plants and microbes and is equipped with an LED light source for imaging in the dark, thus allowing acquisition of photos under optimal growth conditions. SPIRO's web-based user interface allows setting up experiments and downloading data remotely, without interfering with samples growth. The robots' 8 MP camera provides excellent image quality suitable for automated image processing, which we demonstrate on the example of two semi-automated assays for analysis of commonly used phenotypic traits: seed germination and root growth.

Moreover, the robot can be easily customized for a specific use, as all information about SPIRO, including the models for 3D-printed structural components, control software, and scripts for image analysis are released under permissive open source licenses.

Keywords: Time-lapse imaging, Phenotyping, Arabidopsis, Roots, Root growth, Seeds, Germination, Automated imaging, Automated image analysis, ImageJ, ImageJ macro, R, Raspberry Pi, 3D printing, Open science hardware, Lab automation

Introduction

Manual imaging of Petri plates using cameras or scanners is a common practice in biology experiments that require phenotyping of plant seedlings or microbial colonies. However, manual imaging necessitates removing the plates from the growth conditions and increases the risk of introducing unwanted variables into the experiment, e.g., changes in temperature, humidity, illumination, vector of gravity, and mechanical stress. Such fluctuations, especially if triggered repeatedly during time-lapse experiments, might significantly impact the phenotypes of interest, including plant seed germination, root growth, or microbial colony growth[1–3]. Furthermore, manual imaging has limited time resolution, causes inconsistencies in the time of imaging and impedes data acquisition during nights.

Automating labor- and time-intensive procedures is crucial to improving research quality and throughput, and open science hardware and software (tools which are freely available and modifiable) can help further this goal[4–6]. Moreover, open source hardware improves the transparency and reproducibility of science while delivering radical cost savings[6], enabling less well-funded labs (including those in low-income countries) to afford high-quality equipment[5].

A plethora of commercial and custom-made automated systems for imaging biological samples on Petri plates are already available[7–13]. However, we struggled to find an affordable platform that would be suitable for imaging Petri plates in standard plant growth incubators. Performing automated imaging under the growth conditions used for other experiments is crucial for direct comparison of the results, therefore we endeavored into developing a custom-made small footprint solution.

The result of our efforts is SPIRO, the compact **S**mart **P**late **I**maging **Ro**bot for time-lapse imaging of vertical Petri plates, which fits into standard plant/microbe incubators. SPIRO was designed for biologists by biologists, introducing end-user insight into its development. We ensured that no prior knowledge of mechanical engineering, electronics, or computer science is necessary for its assembly and operation. SPIRO comprises the absolute minimum of components that warrants robust and reliable high-throughput time-lapse imaging and applicability for a broad range of experimental layouts. Owing to its minimalistic design, building the robot costs less than €200 (as of 2019), and it is not only easy to assemble but also to maintain, making it optimal for every-day use.

To further promote SPIRO's applicability, we have developed two designated assays for high-throughput analysis of images produced by the robot: SPIRO Seed Germination and SPIRO Root Growth Assays. The assays are designed for analysis of phenotypic traits commonly used in plant biology: seed size, germination time, primary root length and growth rate. SPIRO assays encompass complete start-to-finish detailed procedures comprising the preparation of Petri plates, automated imaging under user-defined conditions, semi-automated image processing and statistical analysis of the quantitative data.

SPIRO is powered by the open source computer platform Raspberry Pi[14] and comprises mostly 3D-printed hardware components, making it particularly suitable for customization. For the benefit of the scientific community, we are publishing SPIRO as an open source project with all information about its structural design, electronics, software and designated assays available under permissive licenses allowing unlimited use and modifications in the presence of correct attribution.

Results

General description/overview

SPIRO takes 8 megapixel (MP) time-lapse images of up to four Petri plates positioned on a rotating cube-shaped stage. It is equipped with green LEDs for illuminating plates while imaging in the dark, and is controlled via a web-based user interface (**Fig. 1A-D, Movie S1**). The latter feature enables setting up imaging conditions remotely via an Ethernet cable, Wi-Fi, or a built-in Wi-Fi hotspot, while the robot is inside a growth cabinet. (**Fig. 1A**) SPIRO's dimensions are ca 50 cm × 30 cm × 30 cm (length × width × height), it weighs less than 3 kg, and can easily be moved between locations.

SPIRO performs imaging in cycles, where one cycle comprises: (i) finding the “home” position, at which the first plate holder is facing the camera; (ii) measuring the average light intensity to determine if the ambient light intensity is sufficient for image acquisition without green LED illumination, or otherwise switching the LEDs on; (iii) taking an image and saving the file; (iv) rotating the stage by 90° to place the next plate holder in front of the camera, and repeating steps ii and iii until all plates are imaged. The duration of each cycle is less than two minutes, enabling high temporal resolution time-lapse imaging.

Hardware design

The three main goals of the SPIRO hardware design were that it should be affordable, customizable, and that a person with no previous experience could build it easily. For this reason, we opted to use 3D-printed parts and standard aluminium profiles for the structural components (**Figure 1, Tables S1 and S2, Files S1** and **S2,** and the SPIRO Hardware Repository[15]), and relatively cheap and readily available electronic components. 3D printing is inexpensive, allows for reproducible fabrication, rapid prototyping and modification, and is easily accessible. For example, printing can be done at publicly available facilities such as Makerspaces[16] or ordered from online services (3dhubs[17] or similar). Printed parts can be easily replicated if they get broken or customized for a specific application. We successfully printed and tested four SPIRO prototypes in two independent laboratories using black matte PLA filament (for detailed information about printing, see **Table S2** and the SPIRO Hardware Repository[15]). The hardware proved to be easy to reproduce, robust and durable.

To facilitate use of SPIRO for a broad range of experiments we designed plate holders compatible with the most commonly used plate formats: a 12 cm square (Greiner Bio-One International, Item: 688102) and a 9 cm round Petri plate (Sarstedt, 82.1473.001), and enabled adjusting distance between the camera and the plate holders by moving the camera along the vertical and horizontal aluminum profiles (**Fig. 1A**).

Camera

SPIRO is equipped with a single 8 MP (3280×2464 pixels) color camera, and saves images as RGB PNG files. Image files are stored in a user-defined experiment folder and are automatically sorted into four sub-folders corresponding to each plate holder. Metadata useful for further analysis is included into the file names, i.e., the names contain the plate holder number, date and time of acquisition, and information about day or night mode (for detailed information, please refer to **File S4**). SPIRO acquires excellent quality images regardless of ambient illumination conditions, which is crucial for downstream automated data analysis (**Fig. 1E and F**, **Movie S2**).

We provide assembly instructions for two possible configurations of SPIRO (GitHub SPIRO Hardware[15], **File S2**): the first is based on a Raspberry Pi v2 camera and requires manual adjustment of the focus before starting an experiment; the second one implements an Arducam camera module with motorized focus, which enables remote focusing via the SPIRO software. Both cameras are very compact and allow imaging of complete Petri plates from a short distance without fisheye distortion effects, a feature crucial for quantitative comparison of seed and seedling measurements. In our experience, both configurations deliver the same image quality, and while the first configuration is somewhat cheaper, the second one is more convenient. Notably, the first configuration can be relatively easily upgraded into the second. Furthermore, SPIRO is compatible with a range of MPI CSI (Mobile Industry Processor Interface Camera Serial Interface) cameras, which implementation might require minimal modifications of the camera house. As new cameras are being continuously developed, we strongly recommend checking the SPIRO Hardware repository for potential upgrades.

Computer

SPIRO's electronics layout (**File S2**) is optimized to enable all essential features for robust high-throughput imaging while minimizing costs and assembling complexity. SPIRO is powered by the cheap and readily available Raspberry Pi 3B+ single-board computer[14] that controls the other four components: a camera, stepper motor, positional sensor (mini microswitch) and LED light source. SPIRO's software and acquired images are hosted on a microSD card mounted on the Raspberry Pi computer and can be remotely accessed via Ethernet or Wi-Fi connection. Notably, Raspberry Pi is an open source computer platform designed for development of engineering solutions and is supported by a vivid community. Raspberry Pi is compatible with a multitude of electronics modules, sensors and components, often supplemented with suitable software packages[18]. It is thus optimal for further customizing the current SPIRO layout for specific uses.

Stepper motor and positional sensor

The cube-shaped stage of SPIRO is rotated by a stepper motor (i) during imaging, to position each of the four plate holders in front of the camera and (ii) between the imaging cycles, to ensure the plates are evenly exposed to the ambient conditions and that there is no shading effect from SPIRO's light screen (**Fig. 1**).

The 12 V unipolar stepper motor we recommend provides sufficient force to reproducibly move the weight corresponding to the stage with 4 square Petri plates containing medium and plants and a holding torque that stably holds the stage position during imaging. Importantly, the motor movement is smooth and has no impact on Arabidopsis root growth under normal conditions (**Movie S2**).

The current layout of SPIRO requires two power supply units: a 5 V supply for the Raspberry Pi computer and a 12 V supply for the stepper motor and LED illuminator (**Fig. 1A, Table S1, File S2**). We decided for such configuration, as it drastically simplifies the assembly and maintenance of the robot, in comparison to implementing a single power supply unit.

At the beginning of each imaging cycle, the motor rotates the stage until the pin attached to the bottom of the stage presses the lever of a positional microswitch (**Fig. 1A, Movie S1**). After the signal from the microswitch is detected, the stepper motor makes a predefined number of steps, thus placing the first plate holder in front of the camera. If needed, the number of steps can be adjusted by the user in the "Calibrate motor" settings tab of the SPIRO software. After the image of the first plate holder is taken, the motor rotates the stage by 90° three more times, pausing to acquire images of the other three plate holders of SPIRO.

During prototyping, we considered implementation of either magnetic or infrared (IR) switches. However, a mechanical sensor provides the most robust system that is least susceptible to the presence of magnetic fields or stray light in the environment, and thus is applicable to a broader range of growth cabinets.

LEDs

SPIRO's built-in light source enables imaging of Petri plates in the dark, providing another crucial benefit over manual imaging. The light source comprises a green LED strip mounted on a 3D-printed square frame with a diffuser (**Figure. 1, Movie S1**). The LED frame can slide along the horizontal axis to fine-tune illumination of the Petri plates for individual conditions (**Fig. 1A**).

SPIRO does not require any instructions from the user about the day/night cycles in the growth cabinet. The intensity of ambient illumination is automatically assessed by SPIRO's camera immediately prior to acquiring each image. If sufficient illumination is detected, SPIRO takes an image in the "day" mode (**Fig. 1C, E**), otherwise the robot turns on the LED light source and acquires a "night" image (**Fig. 1D, F**). ISO and shutter speed for image acquisition can be adjusted individually for day and night modes in the web-based interface of the SPIRO software (**Fig. 1B**).

During prototyping, we tested night imaging using IR LEDs and the IR-sensitive Raspberry Pi NoIR camera. However, this increased the cost of the robot, while significantly complicating its electronics layout and focusing procedure, and did not provide satisfactory quality of images suitable for automated image analysis.

Typically, color camera detectors are most sensitive to green light, as they contain double the number of green pixels compared to red or blue. Hence, we speculated that using a green light source for illumination would be most efficient while using the color Raspberry Pi camera for imaging in the dark. Additionally, we took into consideration use of SPIRO for plant imaging. Plants are known to be dependent on the light of blue and red wavelengths for photosynthesis and regulation of the circadian cycle[19,20]. Although a number of studies showed that green light wavelengths also have important regulatory effect during plant growth and development, the reported effects were observed after prolonged irradiation[21]. Thus, we speculated that illuminating Petri plates with green LEDs only during imaging, should have the weakest impact on the growth and circadian cycle of imaged seedlings. To verify whether this was the case, we compared germination rates and root growth of *Arabidopsis thaliana* seeds and seedlings, respectively, imaged using two SPIRO systems, each with and without green LEDs. Germination was assessed using an ANOVA test where germination rate at t=50 h was set as the dependent variable. Germination rate was compared at a single time point (50 h) due to the constraints imposed on germination detection by not having night image data available. For comparing the effect of LED illumination on root growth, plates with identical genotypes and media were imaged with and without LED illumination. Two models were fitted: the first was the standard root growth model as described in the SPIRO Assay Manual (**File S4**), and the second was the same model but with the fixed effect of LED and all of its interaction effects added. Models were then compared using the anova function in R. Our analysis confirmed that green light indeed had no effect on germination and root growth (**Tables 1** and **2**).

Table 1. The results of an ANOVA test with one dependent variable (Germinated before t=50 h), and three independent variables (genotype, system, LED), using four genotypes and 1154 seeds, indicate that SPIRO systems do not differ in performance and that LED illumination does not influence germination rates. The experiment was performed using the protocol provided in **File S4**.

	D.F.	*Sum Sq*	*Mean Sq*	*F-value*	*p-value*
Genotype	3	17.15	5.716	24.723	1.67×10^{-15}
System	1	0.09	0.094	0.407	0.524
LED	1	0.55	0.545	2.357	0.125
Residuals	1148	265.44	0.231		

Table 2. Results of the comparison of root growth models with and without effect of LED, using 316 seedlings on two different SPIRO systems. The better model is the one not including the effects of LED, indicating that LED illumination has no effect on root growth

Model	*Log likelihood*	*Deviance*	χ^2	χ^2 *D.F.*	*p-value*
Without LED	65440	−130880			
With LED	65281	−130563	0	30	1.00

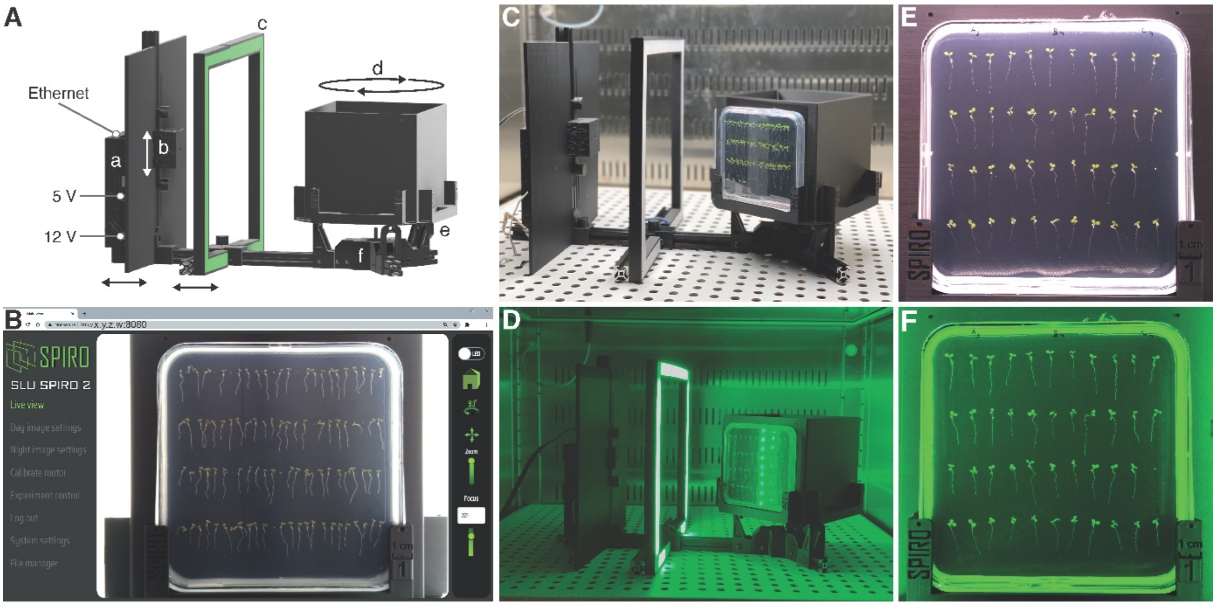

Figure 1. SPIRO, Smart Plate Imaging Robot.

(**A**) 3D rendering image of SPIRO. The robot is controlled by a Raspberry Pi computer placed within the electronics housing (a). The camera house is mounted on a vertical axis with an anti-reflection screen (b). The white arrow indicates possibility of adjusting the camera position along the vertical axis, the black arrows indicate the possibility to tune the distance between the camera and the stage. A green LED frame (c) provides illumination during imaging under night conditions. The cube-shaped stage can accommodate up to four Petri plates (d). At the beginning of each imaging cycle, the home position of the stage (where the first plate holder is facing the camera) is verified with the help of the positional sensor (e). The stage is rotated by a stepper motor (f).

(**B**) SPIRO is controlled by the designated software via a web-based graphical user interface, which allows users to adjust the settings for an experiment and to access imaging data.

(**C**) SPIRO in a plant growth cabinet under day conditions

(**D**) SPIRO in a plant growth cabinet under night conditions. SPIRO automatically detects insufficient illumination and turns on the LED for a fraction of a second while the image is taken.

(**E, F**) Examples of images acquired by SPIRO under day and night conditions, respectively.

SPIRO Accessories

To aid the use of SPIRO we designed a set of essential accessories (**Table S2** and the SPIRO Hardware GitHub Repository[15]).

3D-printable seed plating guides help the user to place Arabidopsis seeds on plates at regular distances from each other and from the edges of the plate. The latter is important to avoid overlaying seed and seedling images with reflections and shadows caused by the Petri plates' rims. The marked regular distance positions for seeds were optimized for the SPIRO Germination and Root Growth Assays described below.

The anti-reflection lids are designed to reduce reflections from seeds and seedlings that are usually visible in the Petri plates' lids. Although such reflections might not be an issue during imaging, their presence is detrimental for automated image processing, as some of them are difficult to automatically differentiate from actual biological samples (for more information see **Fig. 2** in the **File S4**).

Assembling SPIRO

SPIRO was developed specifically to enable its assembly by a person with no expertise or training in engineering, electronics and 3D printing. The complete list of components, step by step instructions, and tutorial videos for assembly are provided in **Tables S1** and **S2,** and **Files S1** and **S2**. However, we highly recommend to check the SPIRO Hardware Repository[15] for potential updates before assembly.

SPIRO hardware includes a set of standard parts, like aluminium profiles, screws and electronic components that need to be purchased. The complete list of these components and links with suggestions on where to purchase them is provided in **Table S1** and the SPIRO Hardware Repository[15]. Our experience of ordering hardware to build SPIRO prototypes in Germany and Sweden reproducibly showed that the most challenging part to acquire are the correct screws. At the moment, we cannot provide a plausible explanation for this peculiar phenomenon and will try to upgrade the SPIRO specifications to reduce the requirements for screws. Notably, approximately one quarter of purchase costs were covering shipping expenses. Furthermore, some parts, such as the LED strips, had to be ordered in excess. Thus, building several SPIROs lowers the price per robot.

The STL, 3MF and F3D files for 3D-printable parts of SPIRO and the printing settings recommendations are provided in **Table S2**, **File S2** and the SPIRO Hardware Repository[15]. SPIRO's hardware was tested and optimized to be printed using PLA (polylactic acid) filament, which is the least expensive and sufficiently robust type of printable plastic. Printing in PETG (Polyethylene terephthalate glycol) and ABS (Acrylonitrile butadiene styrene) plastic is technically possible, but would require adjustment in scaling and printing settings, as the printed parts might shrink or warp significantly.

Printing all SPIRO parts using one 3D printer takes about seven days. The prototyping was done using Prusa i3 MK2/S and MK3S printers. Nevertheless, the pre-arranged components sets we provide (**Table S2,** SPIRO Hardware Repository[15]) can be printed on any type of 3D printer with the printing volume of 25x21x20cm or more. In our experience, the assembly procedure can be completed by a determined untrained person in approximately two full-time work days, while an experienced user can assemble SPIRO in about four hours.

Software and Installation

Since SPIRO was designed to be used within plant growth cabinets, we developed software that allows the remote control of SPIRO via the internet. Besides convenience of use, remote control of the robot is essential to enable setting up imaging parameters under the conditions that will be used during the experiment. SPIRO's software is used for adjusting the camera focus, setting up ISO and shutter speeds for day and night imaging conditions, defining the name and the duration of an experiment and the frequency of imaging, starting and terminating experiments and downloading imaging data (for the detailed information please refer to **File S4** and the SPIRO Software Repository[22]).

SPIRO's software has an intuitive web-based graphical user interface that can be easily accessed from any web browser (**Fig. 1B**). The layout of the software was optimized with the help of several beta-testers to ensure that the interface is sufficiently self-explanatory, does not require training prior to use and contains the complete minimal number of essential features. The program and detailed installation instructions are provided in **File S2** and the SPIRO Software

Repository[22]. The installation procedure requires SPIRO to be connected to the network, which can be done via the Ethernet port of the Raspberry Pi computer (**Fig. 1A**) or by connecting to a Wi-Fi network. For convenience, we recommend assigning SPIRO a static IP number, if this is possible within the user's network. After installation is complete, it is possible to activate a Wi-Fi hotspot from SPIRO's Raspberry Pi computer and use it for future connections to the robot (for instructions see **File S2** and the SPIRO Software Repository[22]).

While setting up and starting a SPIRO experiment is done via an internet connection or Wi-Fi hotspot, running the experiment does not require the robot to be online. However, internet connectivity provides access to images during the experiment run and to the data from the previously completed experiments. The SPIRO software is written in Python 3 and released under an open source 2-clause Berkeley Software Distribution (BSD) license, allowing redistribution and modification of the source code as long as the original license and copyright notice are retained. SPIRO's simple and versatile program for image acquisition includes features making automated imaging possible under conditions that might vary between experiments and laboratories:

- Imaging cycles are carried out at a user-defined frequency and duration. Before each imaging cycle, the stepper motor makes a predefined number of steps after the positional microswitch sensor was detected in order to place the stage in the "home" position.

- Image acquisition is preceded by assessment of illumination intensity. If the average pixel intensity on a sampled image is less than 10 (out of a maximum of 255), the software triggers acquisition under user-defined "night" settings and the LEDs are switched on for the duration of acquisition (less than one second). If the average pixel intensity on the sample image is higher than 10, the image is acquired with user-defined "day" settings.

- Full resolution RGB photos of each of the four plate holders are saved as PNG files on the microSD card mounted on the Raspberry Pi computer, accessible via the web-based user interface of the SPIRO software.

- While idling between imaging cycles, the stepper motor positions the stage at alternating 45° to ensure that all plates are evenly exposed to the conditions in the incubator.

Setting up an experiment

SPIRO was originally designed for imaging *Arabidopsis thaliana* seeds and seedlings. We provide detailed guidelines for casting agar plates, sterilizing and plating seeds, and adjusting imaging settings in **File S2.** For potential updates please refer to the SPIRO Assays Repository[23].

SPIRO assays

To thoroughly assess the data quality acquired using SPIRO and further enhance applicability of the imaging platform for the plant biology community, we developed complete pipelines for two commonly used phenotyping assays that would greatly benefit from automated imaging: seed germination and root growth assays. The assays comprise image processing steps carried out by designated macro scripts in FIJI[24] (distribution of ImageJ) and quantitative data processing steps carried out by custom R scripts. Step by step instructions and scripts are provided in **Files S4** and **S5**. Please note that updates are published in the SPIRO Assays Repository[23].

Each assay starts with pre-processing of SPIRO raw data to combine individual images into time-lapse stack files with a set scale bar. The preprocessed data is then subjected to semi-automated image segmentation with identification of the objects of interest and measurement of their physical parameters, e.g., perimeter, length, and area, at successive time points. The quantitative data is then processed by R scripts to first ensure data quality and then apply custom-designed algorithms that determine seed size, germination time, root length and perform suitable statistical analyses.

The assays were designed to enable applicability for a broad range of experiment layouts and customization for specific uses, thus we introduced several user-guided steps that allow combining seeds or seedlings into groups of interests for analysis, trimming time ranges, renaming samples, removing outliers, etc.

Each assay provides the user with graphical and quantitative outputs and suitable statistical analysis of the data. The assay design ensures quick verification of image segmentation accuracy and identification of potential outliers.

Furthermore, to make combining data from several experiments for the statistical analysis user-friendly, we developed the SPIRO Assay Customizer[25]. Customizer takes the quantitative data from one or several experiments cleaned by the quality control R scripts, and provides the user with an intuitive graphical interface for merging experimental data, regrouping, renaming or removing samples.

The step-wise design of each assay with outputs at intermediate stages allow the user to choose between relying on the provided algorithms or taking over at any point to perform their own analysis of the data.

SPIRO Seed Germination Assay

SPIRO Seed Germination Assay is based on the simple concept that the perimeter of a seed will steadily increase after germination, i.e., the radicle emergence[26], took place. Hence, the image segmentation part of the assay detects individual seeds on the photos (**Fig. 2A, Movie S2**), and tracks changes in the perimeter of each seed within a user-defined time range. The data is then gathered into user-defined groups, e.g., genotypes or treatments, and subjected to a clean-up using a designated R script. After this, for each seed the earliest time point of steady increase in perimeter is detected and identified as the time of germination. The assay is optimized for an imaging frequency of every 30 minutes and thus allows tracking minute differences in germination times. To take into account the effect of imbibition on the seed perimeter and also to compensate for the natural variation in Arabidopsis seed sizes, the germination algorithm normalizes perimeter changes for each seed by comparing it to the same seed perimeter averaged over the first five images in the time-lapse data.

The significance of difference between mean germination times for the user-selected groups of seeds is then assessed by the Kaplan-Meier test (**Fig. 2B**). Furthermore, the assay provides information about the size of individual seeds and the results of t-test comparing seed sizes for user-defined groups (**Fig. 2C**). Additionally, we implemented calculation of other germination parameters that might be valuable for the user, such as rate-of-germination curve, time at maximum germination rate; time required to achieve 50% of total germination efficacy, time required for 50% of total seeds to germinate (for detailed information see **File S4** and the SPIRO Assays repository[23]).

We verified the robustness of the semi-automated SPIRO Seed Germination Assay by comparing its results with germination time points detected manually on the same imaging data. The comparison of germination time points for 172 seeds revealed that the automated assay provides results similar to manual assessment (R^2=0.88, **Fig. 2D**). For this experiments, samples preparation and imaging was done according to the instructions provided in the **File S4**.

While developing the assay we optimized the SPIRO hardware and protocol for seed plating, and introduced seed plating guides that demark positions for placing seeds at optimal distance from each other and plate rims. As a result, when using four 12 cm square Petri plates, it is possible to detect germination for up to 2300 seeds in a single experiment. Additionally, we strongly recommend using SPIRO anti-reflection lids to reduce image segmentation artifacts caused by reflections (**Fig. 2** in the **File S4**).

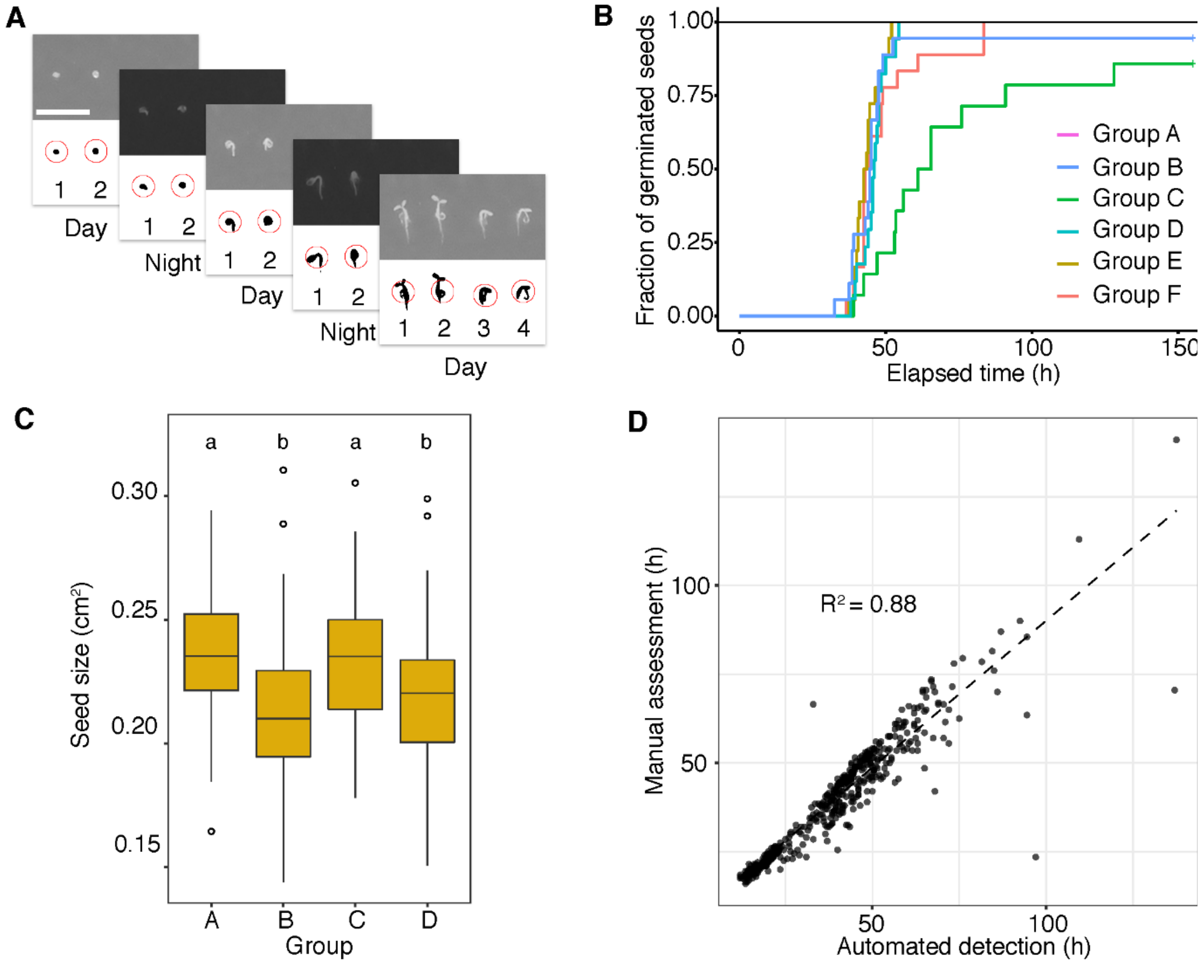

Figure 2. SPIRO Seed Germination Assay.

A. The graphical output of the SPIRO Seed Germination Assay includes a time-lapse stack file, in which each frame contains the original photo and the result of its segmentation, i.e., a mask for each recognized seed annotated by a number and a circle.

B. The assay provides Kaplan-Meier test results for germination of all groups of seeds included into analysis. Additional parameters for germination are calculated using the *germinationmetrics* package for R[27] (for more information see SPIRO Assay manual, **File S3**).

C. The assay also provides a t-test comparison of the mean seed size for the analyzed groups. Boxes represent interquartile range, the horizontal line denotes the median value, circles indicate outliers. Means of the groups that are not significantly different are annotated with the same small case letters, n = 684 seeds.

D. Automatic detection of seed germination using SPIRO Seed Germination Assay provides results very similar to manual assessment of the germination (n=172).

SPIRO Root Growth Assay

Quantifying primary root length of Arabidopsis seedlings is frequently used as a readout for physiological response to mutations or environmental stimuli[13,28,29]. SPIRO is an excellent platform for seedling root phenotyping. We first tested processing of SPIRO images by existing automated image analysis tools for detection of single roots and root systems on time-lapse data[13,30,31]. As these algorithms were optimized for a certain type of imaging data, their applicability for SPIRO-acquired images was limited.

Therefore, we developed the designated SPIRO Root Growth Assay, which uses SPIRO time-lapse data to track primary root length for individual seedlings starting from the germination time-point of the corresponding seed (**Fig. 3A, Movie S2**), builds a root growth rate model for user-defined groups of seedlings (**Fig. 3B**), and then performs statistical analysis comparing root lengths and growth rates for the groups (**Fig. 3C, File S4,** and the SPIRO Assays repository[23]). Similar to the SPIRO Seed Germination Assay, the Root Growth Assay provides the user with a graphical output that show the results of image segmentation for each user-selected group (**Fig. 3A**) and a quantitative output. The latter comprises (i) measurements of the segmented objects performed by the ImageJ macro; (ii) the measurements data cleaned up using a designated R script; (iii) germination time detected for each seed; (iv) curve charts for seedling's root lengths plotted vs absolute time or normalized to individual germination times; (v) curve charts generated by models for root growth rates of user-selected groups of seedlings (**Fig. 3B**); (vi) bar charts showing predicted root lengths for the groups of seedlings at 24-h intervals (**Fig. 3C**) and (vii) results of the statistical analysis comparing growth rates and root lengths between user-defined groups. For more details please refer to the assay manual in **File S4** and the SPIRO Assays repository[23].

Comparison of manual and automated measurements of 141 Arabidopsis roots, revealed that the SPIRO Root Growth Assay provides accuracy comparable with human performance (**Fig. 3D**).

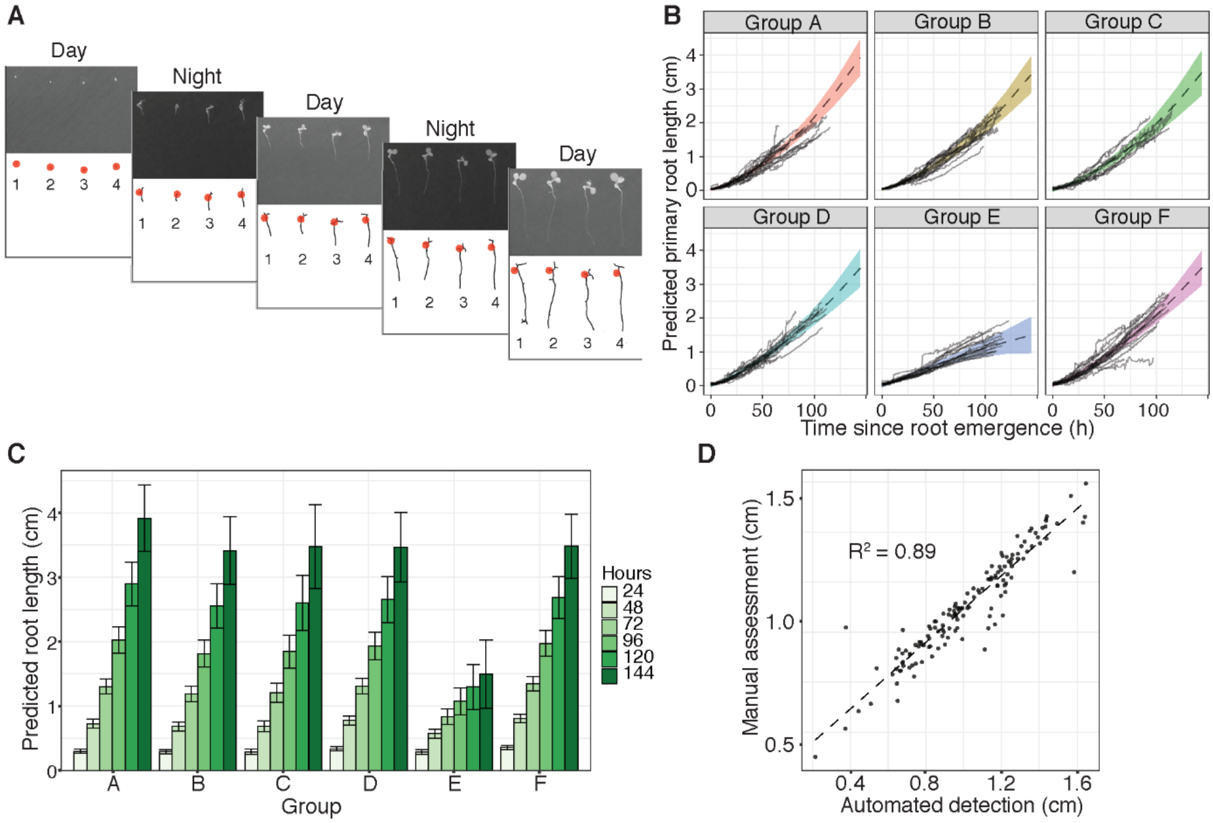

Figure 3. SPIRO Root Growth Assay.

A. Graphical output of the SPIRO Root Growth Assay includes a time-lapse stack file, wherein each frame contains the original photo and the result of its segmentation, i.e., a skeletonized mask for each recognized seedling with denoted root start coordinate (red dot) annotated with a seedling number.

B. The assay builds a model for root growth of each analyzed group (for more information see SPIRO Assay manual, **File S4**). For each root, the elapsed time is calculated from the time of germination for the corresponding seed. Black solid lines indicate root lengths plotted vs time for each seedling, the dotted black line is the predicted root length, and the colored area indicates standard error.

C. Based on the models shown in B, the assay also predicts average root length for each group at 24 h intervals. Error bars show standard error.

D. Automatic detection of root length using SPIRO Root Growth Assay provides results very similar to manual assessment of the root lengths (n=141 seedlings). Manual assessment was done using segmented line tool in ImageJ.

Discussion

In this publication we provide a thorough description of how to build and use the Smart Plate Imaging Robot, SPIRO. SPIRO provides excellent quality of images, acquired under desired growth conditions at regular time intervals. It is an affordable, versatile and robust imaging platform optimal for every-day use in plant biology and microbiology.

Open source engineering solutions can have a great impact on improving the quality, reproducibility, and transparency of research[6] but the benefit of their implementation is habitually undervalued. For instance, the amount of work hours required for manual time-lapse imaging of Petri plates is often underestimated by calculating only the time needed for acquiring images. However, the daily chore of photographing or scanning plates interferes with other work tasks that have to be fitted between imaging sessions and, depending on the desired imaging frequency, can become quite stressful. SPIRO not only frees up user's time by taking over the imaging workload, it also improves the quality of the acquired data and provides remote access to it from anywhere in the world. The latter feature turned out to be surprisingly useful during COVID-19 lockdown regulations.

We strongly believe that reducing the barrier of entry is crucial to the adoption of open science hardware, especially in non-engineering disciplines. In our experience, one of the significant bottlenecks in implementing engineering solutions custom-designed for laboratory use is the requirement of expertise in subjects that are not necessarily popular among biologists, such as mechanical engineering and electronics. SPIRO was developed specifically for biologists, and its design has at its core the concept of being simple and intuitive enough to be assembled and operated with no training in engineering, 3D printing, programming or using the SPIRO per se. Furthermore, we strived to develop an imaging platform that can be built anywhere in the world without requiring access to rare specialized infrastructure and would be affordable also for research groups with limited funding. During preparation of this manuscript, multiple SPIROs have been already constructed in six laboratories located in four different countries using only the provided instructions, thus confirming the need for such platform and validating our approach to making its construction accessible.

The current configuration of SPIRO is optimized for image acquisition under conditions commonly used in plant biology and microbiology. Moreover, the underlying Raspberry Pi platform is ideal for further expanding the system. A large variety of Raspberry Pi-compatible sensors and other input/output modules could be incorporated into a custom-built SPIRO system to accommodate different research needs. For example, cheap sensors for temperature and humidity[18] can be connected to unused general-purpose input/output (GPIO) pins on the computer board. Such upgrades can be valuable when using SPIRO in a growth chamber that does not provide control or logging of these parameters.

We based our design on the use of 3D-printed structural components to further facilitate customization of SPIRO. We provide F3D model files that can be easily modified using Autodesk Fusion 360 software (**File S1**). As of 2021, Autodesk offers a free license tier for academic users, which includes training material. Furthermore, use of 3D-printed parts warrants reproducibility and robustness of the structural components, enables their easy replacement for maintenance and ensures that assembly can be done without access to rare specialized infrastructures. For instance, not only 3D printers are becoming increasingly affordable, with the cheapest models costing less than €200, but prints can also be ordered using commercial services[17] or at public Makerspace facilities[16].

We demonstrate analysis of high-throughput SPIRO-acquired data in the two semi-automated assays for seed germination and seedling root growth. The detailed protocols for the assays comprise the complete procedure from preparing Petri plates to statistical analysis of the data. Both assays provide data analysis accuracy closely matching human performance (**Fig. 2D** and **3D**). Moreover, the SPIRO Seed Germination Assay enables very high temporal resolution enabling the detection of minute differences in germination times. Importantly, despite its small footprint, the platform still provides practical throughput: a single SPIRO can image up to 2300 seeds in a single experiment for the Germination Assay, and 190 seedlings for the Root Growth Assay. In their current versions, the assays are implemented using ImageJ and R, software commonly used in biology labs, which should make their use and customization relatively uncomplicated. We have developed them as "plug-and-play" image analyses that are optimized for SPIRO data and provide outstanding results for the intended purpose. Implementation of the recent advances in machine learning approaches[32–34] will require thorough training of appropriate models for image segmentation, but will eventually enable a more advanced analyses of SPIRO-acquired data such as detangling of crossing roots, measurement of lateral roots, hypocotyls and cotyledons under day and night conditions.

The demand for affordable automated platform for Petri plate imaging is clearly illustrated by the number of publications describing various prototypes of such system[7,9,12,32,33,35]. However, we could not find a solution that would be simultaneously affordable, compatible with standard growth cabinets, provide high-quality data and come with instructions comprehensible for a person not trained in engineering. Hence, we pursued developing SPIRO to establish a platform that would enable universal access to automated high-quality imaging for all research groups, independent on their training or funding situation, and enable easy integration of the automated approach into ongoing research.

For the sake of posterity, the current version of the complete information about SPIRO's construction and use is provided in the supplementary information of this publication. However, SPIRO is under active development, and all updates are made available on the designated GitHub repositories[15,22,23,25]. We release all components of SPIRO as open-source material under licenses allowing unlimited use and modifications as long as proper attribution is present. The assays provided here are only two examples of the broad variety of possible SPIRO applications. The outstanding quality and fidelity of SPIRO's images forms an excellent basis for any Petri plate-based imaging assay. We hope that SPIRO will help alleviate some pains of routine lab work and will also become a stepping stone for advancement of users' interests in developing further solutions. We encourage users to further customize the platform, develop image-analysis pipelines suited for their own research and share optimization with the scientific community.

Competing interests

The authors declare no competing interests.

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List of supplementary material

Supplementary material	File name	Description
Table S1	Table S1. List of components to be purchased.**pdf**	List of components to be purchased and suggestions of vendors
Table S2	Table S2. List of 3D-printable components.**pdf**	List of 3D-printable components and instructions for printing settings
Supplementary File 1	Files for 3D printing.**zip**	Printable (STL, 3MF), and editable (F3D) model files
Supplementary File 2	SPIRO Assembly Instructions.**pdf**	SPIRO Assembly Instructions and links to tutorial videos
Supplementary File 3	spiro-software.**zip**	SPIRO Software GitHub repository archive file
Supplementary File 4	SPIRO Assays Manual.**pdf**	SPIRO Assays Manual
Supplementary File 5	SPIRO Assays.**zip**	SPIRO Assays GitHub repository archive file
Supplementary File 6	SPIRO Assay Customizer.**zip**	SPIRO Assay Customizer archive file
Supplementary Video 1	Supplementary movie 1.**mp4**	SPIRO Overview. A video showing two SPIRO systems placed in a plant growth incubator and the time-lapse video recorded using them
Supplementary Video 2	Supplementary movie 2.**mp4**	Demonstration of the image quality acquired by SPIRO and the capabilities of the two companion SPIRO Assays. The movie shows raw and preprocessed data, as well as illustrates the output of the Germination and Root Growth Assays.

References

1. Quint, M. *et al.* Molecular and genetic control of plant thermomorphogenesis. *Nat. Plants* **2**, 1–9 (2016).
2. Topham, A. T. *et al.* Temperature variability is integrated by a spatially embedded decision-making center to break dormancy in Arabidopsis seeds. *Proc. Natl. Acad. Sci. U. S. A.* **114**, 6629–6634 (2017).
3. De Ligne, L. *et al.* Analysis of spatio-temporal fungal growth dynamics under different environmental conditions. *IMA Fungus* **10**, 1–13 (2019).
4. Pearce, J. M. Return on investment for open source scientific hardware development. *Sci. Public Policy* **43**, 192–195 (2016).
5. Maia Chagas, A. Haves and have nots must find a better way: The case for open scientific hardware. *PLoS Biol.* **16**, e3000014 (2018).
6. Pearce, J. M. Economic savings for scientific free and open source technology: A review. *HardwareX* **8**, e00139 (2020).
7. Yazdanbakhsh, N. & Fisahn, J. High throughput phenotyping of root growth dynamics, lateral root formation, root architecture and root hair development enabled by PlaRoM. *Funct. Plant Biol.* **36**, 938–946 (2009).
8. Subramanian, R., Spalding, E. P. & Ferrier, N. J. A high throughput robot system for machine vision based plant phenotype studies. *Mach. Vis. Appl.* **24**, 619–636 (2013).
9. Nagel, K. A. *et al.* The platform GrowScreen-Agar enables identification of phenotypic diversity in root and shoot growth traits of agar grown plants. *Plant Methods* **16**, 1–17 (2020).
10. https://800ezmicro.com/equipment/anaerobic-microaerobic-systems/32-petrifoto-plate-imaging-system.html.
11. Tovar, J. C. *et al.* Raspberry Pi-powered imaging for plant phenotyping. *Appl. Plant Sci.* **6**, e1031 (2018).
12. Slovak, R. *et al.* A scalable open-source pipeline for large-scale root phenotyping of Arabidopsis. *Plant Cell* **26**, 2390–2403 (2014).
13. Satbhai, S. B., Göschl, C. & Busch, W. Automated high-throughput root phenotyping of Arabidopsis thaliana under nutrient deficiency conditions. *Methods Mol. Biol.* **1610**, 135–153 (2017).
14. Https://www.raspberrypi.org/. No Title.
15. https://github.com/AlyonaMinina/SPIRO.Hardware.
16. https://www.makerspaces.com/what-is-a-makerspace/.
17. https://www.3dhubs.com/.

18. https://tutorials-raspberrypi.com/raspberry-pi-sensors-overview-50-important-components/.

19. Eriksson, M. E. & Millar, A. J. The circadian clock. A plant's best friend in a spinning world. *Plant Physiol.* **132**, 732–738 (2003).

20. Wientjes, E., Philippi, J., Borst, J. W. & van Amerongen, H. Imaging the Photosystem I/Photosystem II chlorophyll ratio inside the leaf. *Biochim. Biophys. Acta - Bioenerg.* **1858**, 259–265 (2017).

21. Folta, K. M. & Maruhnich, S. A. Green light: A signal to slow down or stop. *J. Exp. Bot.* **58**, 3099–3111 (2007).

22. https://github.com/jonasoh/spiro.

23. https://github.com/jiaxuanleong/SPIRO.Assays.

24. Schindelin, J. *et al.* Fiji: An open-source platform for biological-image analysis. *Nat. Methods* **9**, 676–682 (2012).

25. https://github.com/jonasoh/spiro-assay-customizer.

26. Bewley, J. D. Seed Germination and Dormancy. *Plant Cell* **44**, 1055–1066 (1997).

27. Aravind, J., Vimala Devi, S., Radhamani, J., Jacob, S. R. & Kalyani, S. Germinationmetrics: Seed Germination Indices and Curve Fitting. R package version 0.1.3.9000. (2020).

28. Patterson, K. *et al.* Nitrate-regulated glutaredoxins control arabidopsis primary root growth. *Plant Physiol.* **170**, 989–999 (2016).

29. Lucas, M. *et al.* SHORT-ROOT regulates primary, lateral, and adventitious root development in Arabidopsis. *Plant Physiol.* **155**, 384–398 (2011).

30. Betegón-Putze, I., González, A., Sevillano, X., Blasco-Escámez, D. & Caño-Delgado, A. I. MyROOT: a method and software for the semiautomatic measurement of primary root length in Arabidopsis seedlings. *Plant J.* **98**, 1145–1156 (2019).

31. Lobet, G., Draye, X. & Périlleux, C. An online database for plant image analysis software tools. *Plant Methods* **9**, (2013).

32. Gaggion, N. *et al.* ChronoRoot: High-throughput phenotyping by deep segmentation networks reveals novel temporal parameters of plant root system architecture. *bioRxiv* (2020) doi:10.1101/2020.10.27.350553.

33. Yasrab, R. *et al.* RootNav 2.0: Deep learning for automatic navigation of complex plant root architectures. *Gigascience* **8**, 1–16 (2019).

34. Dobos, O., Horvath, P., Nagy, F., Danka, T. & Viczián, A. A deep learning-based approach for high-throughput hypocotyl phenotyping. *Plant Physiol.* **181**, 1415–1424 (2019).

35. Ding, X., Vogel, M., Boschke, E., Bley, T. & Lenk, F. PetriJet Platform Technology: An Automated Platform for Culture Dish Handling and Monitoring of the Contents. *J. Lab. Autom.* **20**, 447–456 (2015).

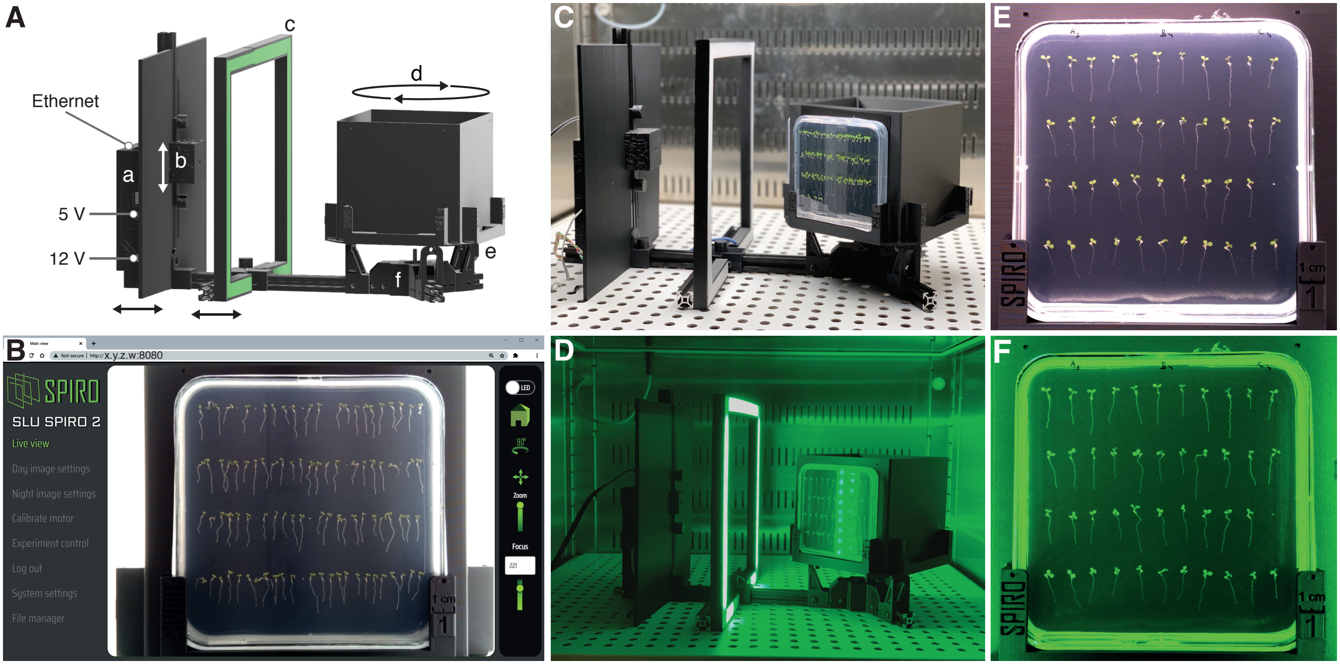

Figure 1. SPIRO, Smart Plate Imaging Robot.

(**A**) 3D rendering image of SPIRO. The robot is controlled by a Raspberry Pi computer placed within the electronics housing (a). The camera house is mounted on a vertical axis with an anti-reflection screen (b). The white arrow indicates possibility of adjusting the camera position along the vertical axis, the black arrows indicate the possibility to tune the distance between the camera and the stage. A green LED frame (c) provides illumination during imaging under night conditions. The cube-shaped stage can accommodate up to four Petri plates (d). At the beginning of each imaging cycle, the home position of the stage (where the first plate holder is facing the camera) is verified with the help of the positional sensor (e). The stage is rotated by a stepper motor (f).

(**B**) SPIRO is controlled by the designated software via a web-based graphical user interface, which allows users to adjust the settings for an experiment and to access imaging data.

(**C**) SPIRO in a plant growth cabinet under day conditions

(**D**) SPIRO in a plant growth cabinet under night conditions. SPIRO automatically detects insufficient illumination and turns on the LED for a fraction of a second while the image is taken.

(**E, F**) Examples of images acquired by SPIRO under day and night conditions, respectively.

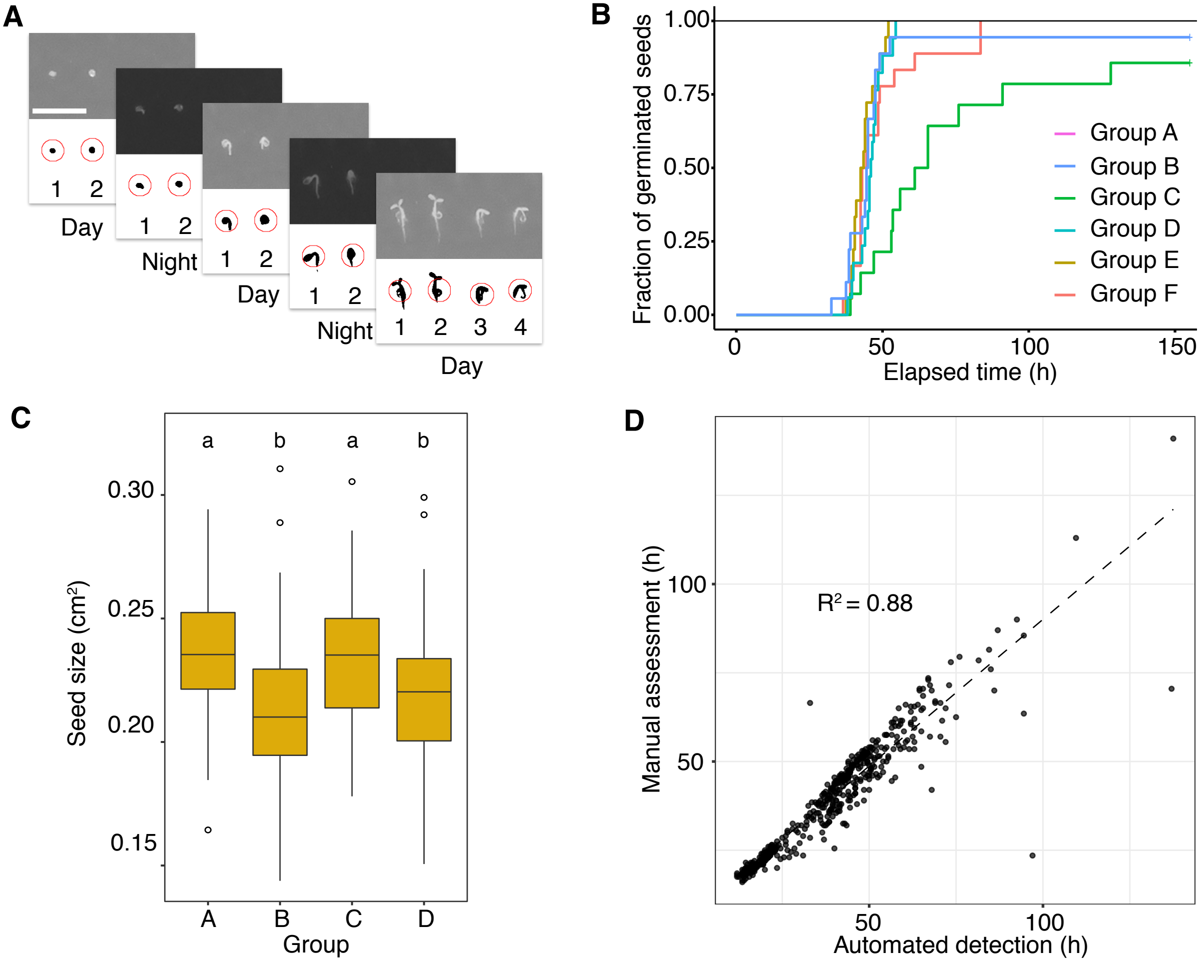

Figure 2. SPIRO Seed Germination Assay.

A. The graphical output of the SPIRO Seed Germination Assay includes a time-lapse stack file, in which each frame contains the original photo and the result of its segmentation, i.e., a mask for each recognized seed annotated by a number and a circle.

B. The assay provides Kaplan-Meier test results for germination of all groups of seeds included into analysis. Additional parameters for germination are calculated using the *germinationmetrics* package for R[27] (for more information see SPIRO Assay manual, **File S3**).

C. The assay also provides a t-test comparison of the mean seed size for the analyzed groups. Boxes represent interquartile range, the horizontal line denotes the median value, circles indicate outliers. Means of the groups that are not significantly different are annotated with the same small case letters, n = 684 seeds.

D. Automatic detection of seed germination using SPIRO Seed Germination Assay provides results very similar to manual assessment of the germination (n=172).

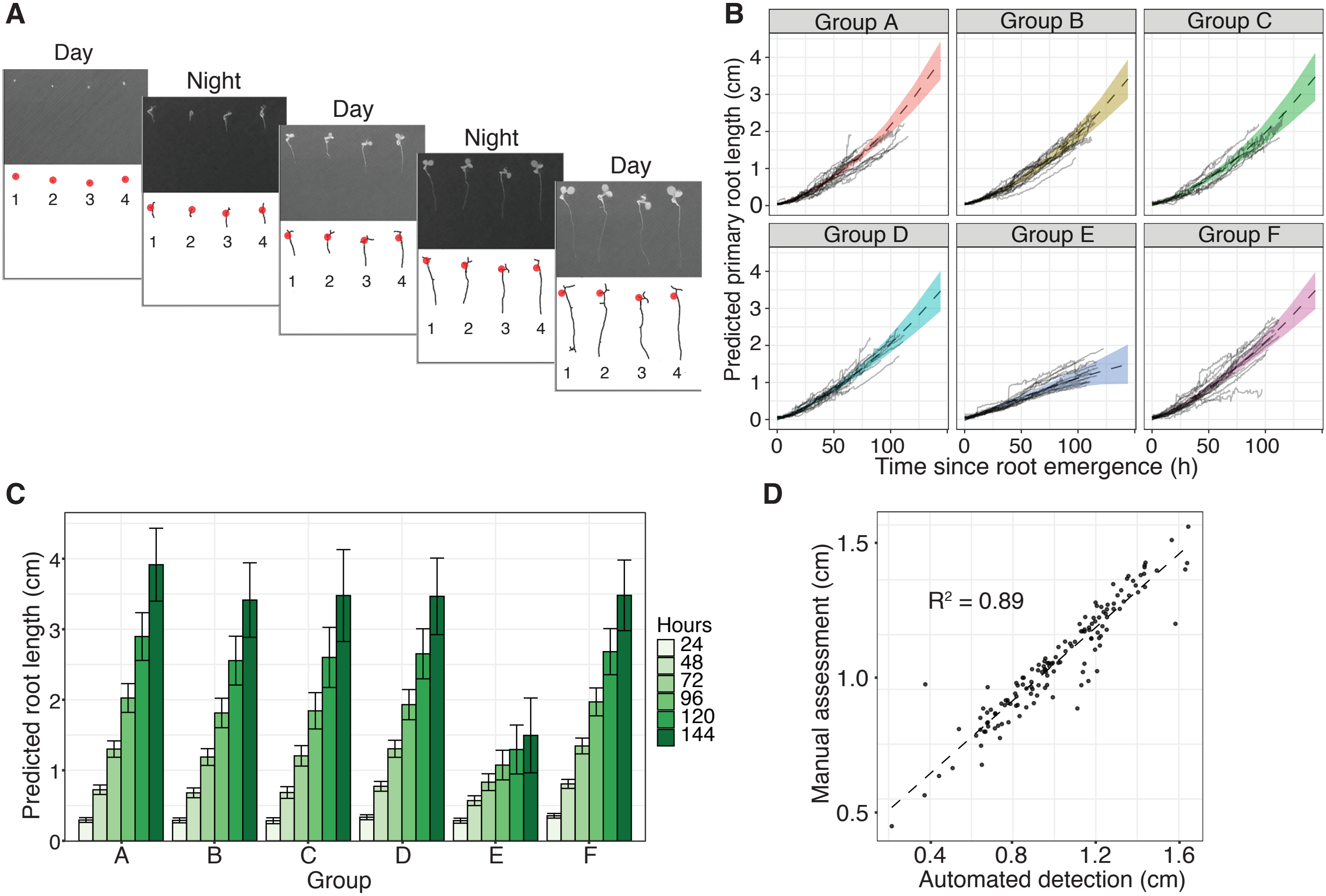

Figure 3. SPIRO Root Growth Assay.

A. Graphical output of the SPIRO Root Growth Assay includes a time-lapse stack file, wherein each frame contains the original photo and the result of its segmentation, i.e., a skeletonized mask for each recognized seedling with denoted root start coordinate (red dot) annotated with a seedling number.

B. The assay builds a model for root growth of each analyzed group (for more information see SPIRO Assay manual, **File S4**). For each root, the elapsed time is calculated from the time of germination for the corresponding seed. Black solid lines indicate root lengths plotted vs time for each seedling, the dotted black line is the predicted root length, and the colored area indicates standard error.

C. Based on the models shown in B, the assay also predicts average root length for each group at 24 h intervals. Error bars show standard error.

D. Automatic detection of root length using SPIRO Root Growth Assay provides results very similar to manual assessment of the root lengths (n=141 seedlings). Manual assessment was done using segmented line tool in ImageJ.