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Title: Development of an Innovative LED-Based Illumination Device for In Vitro Application of Photodynamic Therapy with Rose Bengal

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 22
Number of Shots: 43

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

NOTE: Use the last ones

1.1. **Guillaume Grolez:** The scope of our research is to develop fundamental and translational projects aiming to characterize the effects of Photodynamic Therapy in the treatment of cancers with no effective therapeutic options.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What are the current experimental challenges?

1.2. **Anne-Sophie Dewalle:** The current experimental challenges are to develop the technologies necessary for the implementation of Photodynamic Therapy from *in vitro* to clinic applications, including new photosensitizers and new illumination devices.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.1*

What advantage does your protocol offer compared to other techniques?

1.3. **Anne-Sophie Dewalle:** Compared to other techniques, our protocol involves a low-cost and homemade device enabling homogeneous illumination of a 96-well plate under physiological conditions inside a cell culture incubator**.**

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.1*

What research questions will your laboratory focus on in the future?

1.4. **Guillaume Grolez:** In the future, one of our main objectives is to develop new PDT packages each comprising both a photosensitive compound enabling specific cancer cells targeting and an associated illumination device.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.2.1*

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Seeding the Cells before Treatment

Demonstrator: Clément Bouchez

2.1. To begin, remove the cultured HepG2 *(hep-G-2)* cells from the incubator **[1]** and place the flask under the pre-cleaned microbiological safety station **[2]**.
- 2.1.1. WIDE: Talent opening the incubator and retrieving the culture flasks.
- 2.1.2. Talent placing the culture flask in the microbiological safety station. NOTE:: Use last take

2.2. Using a pipette, remove the culture medium from the culture flask **[1]**. To eliminate residual medium, rinse with phosphate-buffered solution **[2]** while gently mixing, then carefully remove the buffer **[3]**. NOTE: The VO is adjusted for the additional shot
- 2.2.1. Talent aspirating the medium from the culture flask inside the safety cabinet.
- 2.2.2. Talent pipetting phosphate buffer solution into the flask (take 2)

Added shot: Gently swirling before aspirating it out.

2.3. Then, apply 3 milliliters of trypsin solution containing 0.25 percent trypsin and 0.53 millimolar EDTA to the cells **[1]** and place the culture back in the incubator at 37 degrees Celsius for 5 minutes to detach the cells **[2]**.
- 2.3.1. Talent adding trypsin to the culture flask under the safety station.
- 2.3.2. Talent placing the treated flask inside the incubator and closing the door.

2.4. After 5 minutes, add 7 milliliters of culture medium to the flask to neutralize the trypsin and suspend the cells **[1]**. Now, transfer the trypsin, medium, and detached cells into a 15-milliliter centrifuge tube **[2]**.
- 2.4.1. Talent pipetting medium into the flask and mixing gently. NOTE: Use take 2
- 2.4.2. Talent transferring the cell suspension into labeled 15 milliliter tubes.

2.5. To count the cells, pipette 20 microliters of the cell solution into a clean tube **[1]** and add 20 microliters of trypan blue solution **[2]**. After mixing, load the stained mixture onto the counting slide **[3]** and insert it into the cell counter **[4]**.
- 2.5.1. Talent dispensing 20 microliters of cell suspension into a microtube.
- 2.5.2. Talent adding 20 microliters of trypan blue and mixing with a pipette.
- 2.5.3. Talent loading the stained sample onto the slide.
- 2.5.4. Talent inserting the slide into the cell counter.

2.6. Next, add culture medium to a tube **[1],** then introduce cells to reach a final concentration of 150,000 cells per milliliter **[2]**. Seed 1.5×10^4 cells per well into a white 96-well plate with a clear flat bottom **[3]**. Prepare two plates for each viability

reading time **[4]**. NOTE: The VO is edited for the additional shot.

2.6.1. Talent pipetting culture medium into a tube containing cells to dilute them to the target concentration. NOTE: Use take 2

Added shot: 2.6.1B EXTRA add cell into medium

2.6.2. Talent dispensing 1.5 × 10^4 cells into each well of the 96-well plate.

2.6.3. Talent labeling and setting aside two plates for each time point.

2.7. Incubate the plates for 24 hours before treatment to let the cells attach **[1]**.

2.7.1. Talent placing the 96-well plates into the incubator and close the door. NOTE: Use 2nd part

3. Rose Bengal Treatment of the Cells

NOTE: Color grade this part in order to create the illusion of darkness

3.1. To prepare a stock solution of Rose Bengal, dissolve it in 10 percent saline solution **[1]**.

3.1.1. WIDE: Talent adding Rose Bengal powder to a tube containing 10 percent saline solution. NOTE: CU + MED is filmed

3.2. Dilute the Rose Bengal treatment solutions at concentrations ranging from 0 to 100 micromolar in cell culture medium **[1-TXT]**.

3.2.1. Talent pipetting stock solution into multiple tubes arranged on the bench. **TXT: Concentrations: 0, 5, 10, 25, 50, 75, and 100 µM**

3.3. Now, remove the culture medium from the cell plates **[1]** and add 100 microliters of the prepared Rose Bengal treatment solutions to each well **[2]**.

3.3.1. Talent aspirating the medium from each well of the 96-well plate.

3.3.2. Talent pipetting 100 microliters of each Rose Bengal concentration into the corresponding wells.

3.4. Incubate the cells with Rose Bengal for 2 hours to allow internalization **[1]**.

3.4.1. Talent placing the treated microplates into the incubator and closing the door.

3.5. After 2 hours, remove the Rose Bengal solution from each well **[1]**, wash the cells two times with PBS **[2]**. Aspirate the PBS **[3]** and add 100 microliters per well of Rose Bengal-free culture medium **[4]**. Cover the microplates with aluminum foil to protect them from light **[5]** and set aside half of the plates for illumination during the photodynamic therapy assay **[6]**.

3.5.1. Talent aspirating Rose Bengal solution from the well.

3.5.2. Talent adding phosphate-buffered saline to a well.

3.5.3. Talent aspirating phosphate-buffered saline from the well.

3.5.4. Talent adding fresh Rose Bengal-free culture medium to the wells.

3.5.5. Talent covering microplates with aluminum foil.

3.5.6. Talent labeling and grouping plates for photodynamic therapy and dark conditions. NOTE: Use the last take

4. Operating Procedure for the CELL-LED Device

NOTE: Color grade this part in order to create the illusion of darkness

4.1. Connect the male connector on the light distributor to the female connector of the light source **[1]**.

4.1.1. Talent aligning and connecting the light distributor cable to the LED light source.

4.2. Remove the photodynamic therapy and dark condition microplates from the incubator **[1]**. Unwrap the photodynamic therapy plate **[2]**. Then, set the dimmer on the LED driver to the maximum level to deliver an average irradiance of 0.62 milliwatt per square centimeter across the 96 wells **[3]**. After that, place the plate on the light distributor **[4]**. NOTE: The VO is edited for the moved shot

4.2.1. Talent retrieving the plates from the incubator.

4.2.2. Talent unwrapping the plate that was covered with foil. NOTE: Use take 2

4.3.1 Show the LED driver control panel with settings being adjusted. NOTE: The authors suggested moving this shot before 4.2.3

4.2.3. Talent placing the photodynamic therapy plate onto the aligned light distributor panel. NOTE: 4.2.2 and 4.2.3 are filmed in a single shot

4.3. ~~Then, set the dimmer on the LED driver to the maximum level to deliver an average irradiance of 0.62 milliwatt per square centimeter across the 96 wells **[1].**~~ Illuminate the microplate until the desired light dose on the cells is achieved **[1]**. NOTE: The VO is replaced from 4.3 to 4.1 for the moved shot

4.3.1. ~~Show the LED driver control panel with settings being adjusted~~. NOTE: This step is moved before 4.2.3

4.3.2. Shot of the plate being illuminated.

4.4. Once the desired light dose is reached, turn off the device **[1]**. Rewrap the photodynamic therapy microplate in aluminum foil **[2]** and return it along with the control plate to the incubator until the viability test is performed **[3]**.

4.4.1. Talent switching off the LED system.

4.4.2. Talent covering the photodynamic therapy plate again with foil.

4.4.3. Talent placing both plates back into the incubator.

5. Cell Viability Assay After Treatment

5.1. After completing the photodynamic therapy assay, place the illuminated plate in the incubator for 24 hours to allow for post-treatment cellular response **[1]**.
   5.1.1. Shot of the plate lying inside the incubator.

5.2. After the incubation period, retrieve one illuminated and one non-illuminated plate **[1]**. Add 100 microliters of reagent from the cell viability assay kit into each well to measure mitochondrial metabolism and ATP production **[2]**.
   5.2.1. Talent removing both microplates from the incubator.
   5.2.2. Talent using a multichannel pipette to dispense 100 microliters of reagent into every well of both plates.

5.3. Incubate the plates in the dark for 10 minutes before proceeding with luminescence measurement **[1]**.
   5.3.1. Talent placing the foil-covered plates into a dark incubation chamber.

5.4. After 10 minutes, read the luminescence in each well using a multimodal reader **[1]**.
   5.4.1. Talent loading the plate into the luminescence reader.

5.5. Consider the luminescence from the untreated control wells as representing 100 percent viability **[1]**. Normalize the luminescence values from the treated wells to this control to calculate the percentage of viability for each treatment condition **[2]**.
   5.5.1. Talent working at a computer entering values.
   5.5.2. Shot of the computer screen displaying the resulting percentage viability values, grouped by treatment concentration and condition.

5.6. Repeat the same protocol for cell viability measurement at additional post-treatment time points such as 24 hours, 48 hours, and 72 hours **[1]**.
   5.6.1. Talent removing the plate from the reader and placing another one.

Results

6. Results

6.1. Illumination alone, without Rose Bengal, did not alter HepG2 cell viability at any of the tested light doses **[1]**, and Rose Bengal alone, without light, also caused no significant change across all concentrations **[2]**.

6.1.1. LAB MEDIA: Figure 5. *Video editor: Highlight the three blue BARS under "Light*".

6.1.2. LAB MEDIA: Figure 5. *Video editor: Highlight the seven grouped bars under the "RB" label* .

6.2. Rose Bengal-mediated photodynamic therapy using CELL-LED-550/3 *(cell-L-E-D-five-fifty-bar-3)* significantly reduced HepG2 cell viability at all light doses, showing a strong cytotoxic effect **[1]**.

6.2.1. LAB MEDIA: Figure 5. *Video editor: Highlight the bars corresponding to 25,50, 75 and 100 µM under the label "PDT" for 0.30 and 0.60 J/cm2 and highlight all bars for 1.22 J/cm2 (These bars have ** on them*).

6.3. The overlap between the LED emission profile and the absorption spectrum of Rose Bengal confirmed the spectral compatibility of the CELL-LED-550/3 device with the photosensitizer **[1]**.

6.3.1. LAB MEDIA: Figure 6. *Video editor: Highlight the peak of the green line (LED spectrum) overlapping with the peak of the pink line (Rose Bengal spectrum) in the 550 nanometer region*.

1. **heterogeneity**
   Pronunciation link:
   https://dictionary.cambridge.org/pronunciation/english/heterogeneity (Cambridge Dictionary)
   IPA: /ˌhɛt̬.ə.roʊ.dʒəˈneɪ.ə.t̬i/ (Cambridge Dictionary)
   Phonetic Spelling: *het-uh-roh-jeh-NAY-uh-tee*

2. **chemotherapy**
   Pronunciation link:
   https://dictionary.cambridge.org/pronunciation/english/chemotherapy (Cambridge

Dictionary)
IPA: /ˌkiː.moʊˈθer.ə.pi/ (Cambridge Dictionary)
Phonetic Spelling: *kee-moh-THER-uh-pee*

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3. **cytotoxic**
   Pronunciation link: https://dictionary.cambridge.org/pronunciation/english/cytotoxic (Cambridge Dictionary)
   IPA: /ˌsaɪ.ʈoʊˈtɑːk.sɪk/ (Cambridge Dictionary)
   Phonetic Spelling: *sahy-toh-TAHK-sik*

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4. **illuminance**
   Pronunciation link: https://www.oxfordlearnersdictionaries.com/definition/english/illuminance (Oxford Learner's Dictionaries)
   IPA: /ɪˈluːmɪnəns/ (Oxford Learner's Dictionaries)
   Phonetic Spelling: *ih-LOO-mih-nens*

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5. **oncotherapy**
   Pronunciation link: *No confirmed link found in major dictionaries* (it's a less common combo word "onco-therapy").
   IPA (constructed): /ˌɒnkoʊˈθerəpi/
   Phonetic Spelling: *on-koh-THER-uh-pee*

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6. **photodynamic**
   Pronunciation link: https://www.merriam-webster.com/dictionary/photodynamic (howjsay.com) *(the medical/technical dictionary)*
   IPA: /ˌfoʊ.toʊ.daɪˈnæm.ɪk/ (howjsay.com)
   Phonetic Spelling: *foh-toh-dye-NAM-ik*

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7. **Rose Bengal**
   Pronunciation link: https://www.merriam-webster.com/medical/rose%20bengal (howjsay.com)
   IPA: /ˈroʊz ˈbɛngəl/ (howjsay.com)
   Phonetic Spelling: *ROHZ BEN-guhl*

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8. **cytotoxicity**
   Pronunciation link: https://dictionary.cambridge.org/pronunciation/english/cytotoxic (Cambridge Dictionary) *(you take "cytotoxic" and add the "-ity")*
   IPA: /ˌsaɪ.ʈoʊˌtɑːkˈsɪr.i/ *(approximate, where the stress shifts slightly)*
   Phonetic Spelling: *sahy-toh-tahk-SIH-tee*

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9. **viability**
   Pronunciation link: https://www.merriam-webster.com/dictionary/viability

(howjsay.com)
IPA: /ˌvaɪəˈbɪlɪti/ (howjsay.com)
Phonetic Spelling: *vy-uh-BIL-ih-tee*

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10. **incubator**
    Pronunciation link: https://www.merriam-webster.com/dictionary/incubator
    (howjsay.com)
    IPA: /ˈɪŋ.kjuː.beɪ.tər/ (howjsay.com)
    Phonetic Spelling: *ING-kyoo-bay-ter*
