5 Characterization of the motility features of *Chlamydomonas reinhardtii*

5.1 Purposes

The core of our project is to change the light spectrum sensitivity of *Chlamydomonas*, so the priority is to understand the features of the movement of *Chlamydomonas*, and to determine the standard procedure of characterizing *Chlamydonomas* movement by measuring the motion parameters of wild type *Chlamydomonas* under blue light, in order to characterize the movement features of engineered alga and support further design of experiments at the molecular level.

5.2 Apparatus

5.2.1 Production and Processing of Silica gel Plate

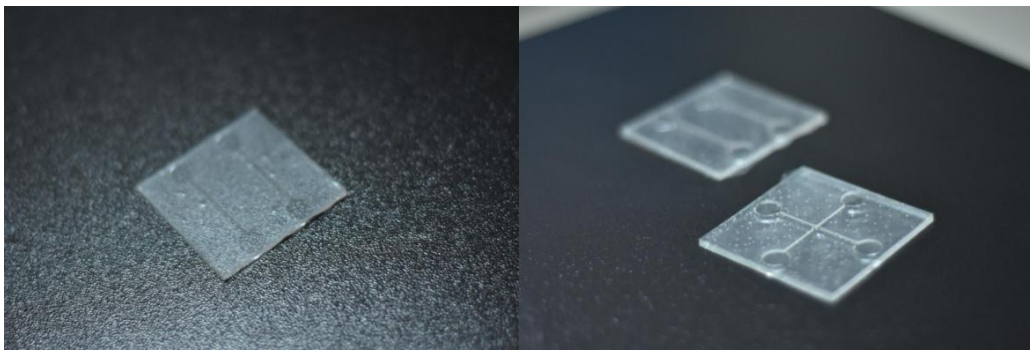

Figure 1. Silica gel plate used for as the swimming lane for microalga

1) Specifications

Overall: Width：50 mm, Length：50 mm;

Channel: Length：40 mm, Depth：0.25 mm, Width：0.25 mm;

Circular pool: Radius：2 mm, Depth：0.25 mm

Specifications of straight silica gel plate and cross silica gel plate specifications are consistent.

2) Production Methods: First, the glass template required for the silica gel plate was carved and poured with PDMS184 silicone rubber. The proportion of the pouring liquid is water: silicone rubber = 10:1; Heating at 80℃ for 4 h, and the plate is ready after cooling the mold.

3) Processing Methods: Before the experiment: Soak the plate in 75% alcohol for 15 min, then wash it with deionized water, after which soak it in 1% BSA solution for 5 min, and then wash again with deionized water.

4) During the experiment： Wash with deionized water after measuring a group of algae. Use qualitative filter paper to absorb residual liquid.

5) After the experiment: Soak in 75% alcohol for 15min and wash with deionized water. Then soak in 1% BSA solution for preservation. For long-term storage, BSA solution should be refrigerated at 4℃ and replaced every 5 days.

5.2.2 Light Source

Figure 2. Light sources and illuminance meter

1) LED lamp (blue light, red light): Used in speed measurement, illumination influence experiment and motion characterization of wild algae; also in motion characterization of engineered algae.

2) UV lamp: Used to validate that UV light can stimulate the exogenous blue fluorescent protein introduced into *Chlamydomonas* to produce endogenous blue light, and the endogenous blue light can induce directional movement of *Chlamydomonas*.

3) Filter plate at 589 nm: Used to validate that the rhodopsin channel protein obtained from volvox can be transformed and expressed in *Chlamydomonas*, so

as to broaden the photosensitive range of *Chlamydomonas* and realize photo-chemo-attractivity at 589 nm.

5.2.3 Construction of the light path

Figure 3. Observation equipment

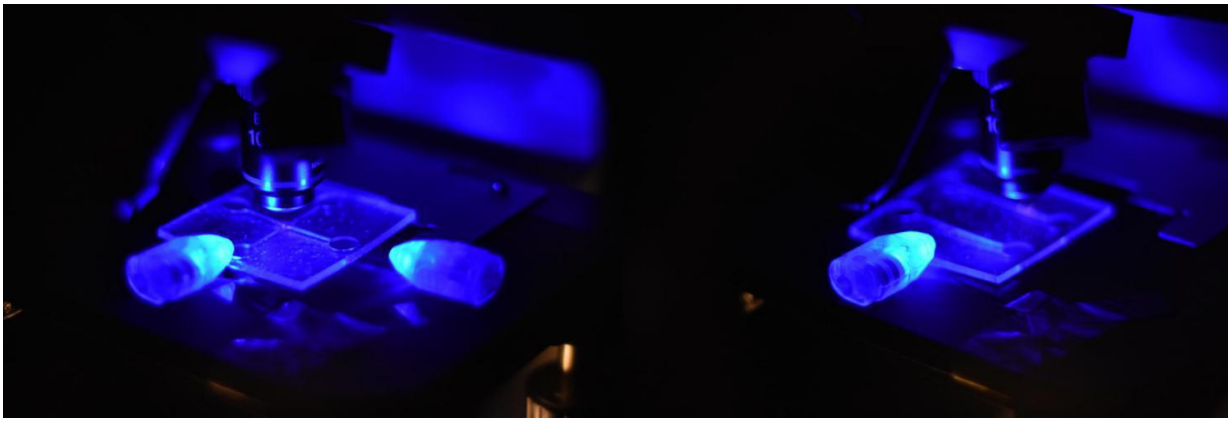

Figure 4. Light Path

1) Paste four signs on the display screen. The left and lower side marks are used to determine the position of the straight silicone plate. The upper and right marks are used to determine the position of the cross-silicone plate; stick a sign on the objective table, each signs spaced 1cm apart to determine the position of the light source.
2) The LED light is placed on one side of the board, sometimes parallel to the track, sometimes perpendicular to the track, thus changing the direction of the light, fixing the distance, and measuring the time for *Chlamydomonas* to move from one side of the board to the opposite side.

3) The illuminance of the light source can be changed by changing the linear distance between the light source and the silicone plate channel to carry out exploratory experiments under different illuminance.

5.3 Measurement of Chlamydomonas motility

5.3.1 Procedures of speed measurement

1) Preparation

Materials: straight silicone plate, 100 μL transfer gun, TAP liquid medium, light source, illuminometer, deionized water, waste liquid tank, 75% ethanol, 1% BSA. First soak the rubber plate with 75% ethanol for 15min, and then soak it with 1% BSA for 5 min. Determine initial concentration of by measuring OD_{750} with microplate reader. Measure the illuminance of the light source at a fixed distance with an illuminometer.

2) Control experiment

a) TAP was used as the channel connecting fluid (about 120 μL) ,add the algal solution to the left side of the channel, and the end point was the right side. In order to reduce disturbance error, the liquid level on the right side should be higher than that on the left. The rubber plate is placed on the loading platform to allow the channel to appear in the field of vision. The shooting field is selected near the end point of the channel to minimize the influence caused by disturbance error. Keep the channel sideline in view parallel to the screen sideline.

b) First, add it to the edge of the circular pool. Gently connect the solution together with a toothpick, or add more TAP solution to one side of the channel plate, so that the liquid level is higher than that of the alga, using the water potential difference to prevent the spontaneous movement of the alga (do not drop the 10 μL algal solution directly into the TAP).

c) In addition to the light of the microscope, the algae movement data were observed and recorded in the absence of external light. Select five groups of parallel data and the average value (blank speed V_1) was calculated.

3) Measurement experiment

The corresponding light source was installed according to the wavelengths of light

that engineered alga responded =. The measurement procedure is the same as the control experiment.

Calculation methods:

a) Measurement of a single *Chlamydomonas*: Measure the length of the channel in the display screen through the scale (or choose the desired length), and wait for Chlamydomonas to completely move from one side of the field of view to the other side, then record the moving time and calculate the speed.

b) Group measurement: Keep the edge lines of the channel the same level as the edge lines of the display screen to measure the distance between the upper and lower sides of the channel. The blue light was placed on the side of the channel for a period of time to make most of the alga gather. Remove the blue light and place a measurement light source on the underside of the channel. When all alga reach the underside, record the time and calculate the speed.

5.3.2 Illumination effect measurement

1) Materials

Straight silicone plate, 100 μL transfer gun, TAP medium, light source, illuminometer, deionized water, 75% ethanol, 1% BSA solution.

First rinse the rubber plate with 75% ethanol for 15 min, and then rinse it with 1% BSA for 5 min. Determine initial concentration of by measuring OD_{750} with microplate reader. Measure the illuminance of the light source at a fixed distance with an illuminometer.

2) Experimental Procedures

See the speed measurement above for slank measurement methods.

3) Illumination effect measurement

a) TAP was used as the channel connecting fluid (about 120 μL) Add the algal solution to the left side of the channel, and the end point was the right side. In order to reduce disturbance error, the liquid level on the right side should be higher than that on the left. The rubber plate is placed on the loading platform to allow the channel to appear in the field of vision. The shooting field is selected near the end point of the channel to minimize the influence caused by disturbance

error. Keep the channel sideline in view parallel to the screen sideline.

b) Do not drop the 10 μL algal solution directly into the TAP. First, add it to the edge of the circular pool. Gently connect the solution together with a toothpick, or add more TAP solution to one side of the channel plate, so that the liquid level is higher than that of the alga, using the water potential difference to prevent the spontaneous movement of the alga.

c) Turn off the external light and measure the illuminance of the source with an illuminometer. The illuminance was changed by changing the distance between the light source and the illuminometer (1cm for each group in this experiment), and the illuminance and distance were marked on the microscope platform.