DIFFUSION FROM STEADY-STATE PROFILE (DSSP) FOR LOW COST, LOW CONCENTRATION MEASUREMENT OF DIFFUSION

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ABSTRACT

Here we present Diffusion from Steady-State Profile (DSSP), a simple, low-cost technique to measure the diffusivity of labeled proteins in hydrogels that are typically used for 3D cell culture. This is a steady-state technique which allows the use of long-exposure imaging, thereby enabling operation with low protein concentrations without the need for relatively expensive imaging equipment or immunoassays.

INTRODUCTION

We construct a simple microfluidic y-channel with design similar to that of R.F. Ismagilov et al.[1](Fig 1a) However, our devices are prefilled with a cured hydrogel of choice, ensuring plug flow and significantly simplifying calculations. In the y-channel, cell media containing the fluorescently labeled protein of interest is flowed in through one inlet and cell media without analyte through the other. Once the flow in the outlet channel has stabilized (~1 hr) the diffusivity of the protein can be calculated from the steady state intensity profile in the main channel. (Fig 1b)

Diffusion is a typical mode of transport used to create gradients of protein in 3D cell cultures, [2] but the diffusivities of expensive proteins often need to be validated under experimental conditions. Typically, diffusivity is measured using FRAP or by measuring the rate at which an analyte leaches out of a bulk hydrogel via immunoassay.[3][4] Both of these techniques are temporal in nature and require sampling times much shorter than the diffusion time in the system. This poses a direct problem for FRAP, in that less concentration means less expensive reagent wasted, but requires more expensive imaging equipment to catch the lower-intensity signal at the same exposure time. Our technique skirts this problem by operating in the steady state, thus allowing long exposure times; it also has no need for immunoassays.

METHODS

The used hydrogel is fibrin at 3 mg/ml. Flows (~1 micron/sec) are generated via hydro-static pressure in inlets (~1 cm water). After addition of solution, the flow is allowed to develop for ~1 hr before imaging. Imaging is done with a confocal microscope, but could have been done of a simple epifluorescent microscope with sufficiently long exposure. With every sample a cheap, bright, and easily bleachable tracer dye (40 kDa FITC Dextran) was included with the analyte and FRAP of the tracer was used to validate the flowrate in the device and to exclude non-working devices. Analytes were fluorescently labeled before use. In each test, 1 mL of a ~100 ng/mL solution of analyte was used.

RESULTS AND DISCUSSION

Key to the simplicity of this approach is the transformation of the spacial axis in the flow direction to a time axis. This requires two conditions met. First, plug flow is ensured as all flows are through a hydrogel. Second, flow velocities were chosen such that gradients are small in the direction of flow. Under these conditions, profiles perpendicular to the flow direction at different down-stream positions can be thought of as the same profile, simply translated in time. Extraction of the diffusivity was done via analysis of the slope of the profile along the center line (fig 2). For any unique pair of profiles, a unique diffusivity can be extracted.[ref Crank] An average of these values is reported as the final diffusivity.(fig 2c)

In each experiment, diffusivity of an included tracer was validated via FRAP. If the FRAP and DSSP measured diffusivities did not match, the chip was considered defective and discarded. (fig 3a) It is worth noting that the FITC dextran tracer dye does not suffer the same high price that limits the use of FRAP on labeled proteins.

Diffusivities of two cytokines, WNT5a and VEGF165 are shown.(fig 3b) While diffusivity of WNT5a is not well characterized in the literature, VEGF165 results show excellent agreement with the literature values.[6]

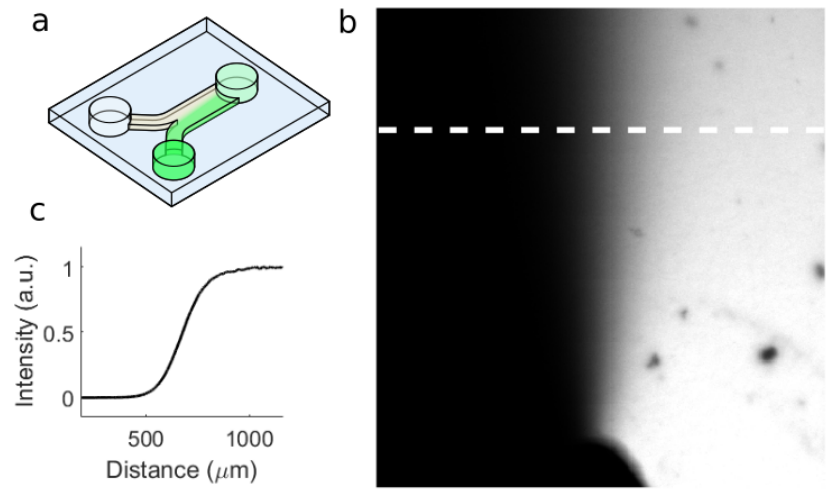

Figure 1: (a) schematic of device showing hydrogel filled y-shaped channel with analyte containing media flowed in through the bottom inlet and analyte free media from the top. (b) Fluorescent image of junction showing the developing profile. Width of the image is 1.25 mm. (c) Intensity profile taken along dotted line.

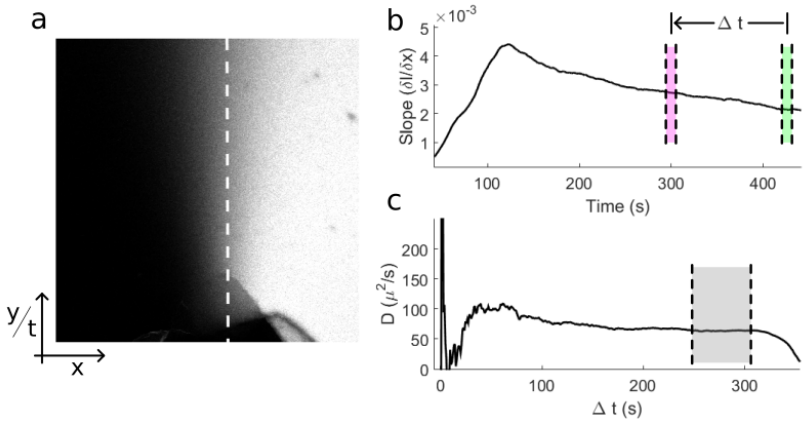

Figure 2: (a) Fluorescent image of junction with center line indicated by the dotted line. Note that under the correct flow conditions the y axis of the image is effectively the time axis for the development of the profile shown in Fig1a. Width of the image is 1.25 mm. (b) Slope of profile perpendicular to the center line as a function of distance along the center line, or time. (c) 2-point diffusivity measurement taken from panel b where the first point is taken at the green slice and the second point (magenta slice) is swept over the profile. The reported value of diffusivity is taken from the plateau shown in gray.

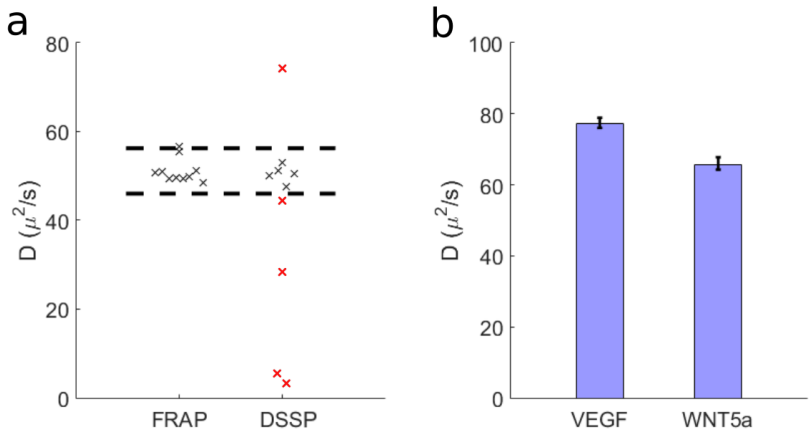

*Figure 3: (a) diffusivity of tracer measured via FRAP compared to diffusivity of tracer measured via DSSP. Devices in which DSSP differed by more than 10% from FRAP were rejected (**red xs**). (b) DSSP values for the diffusivity of VEGF168 and WNT5a. Error bars represent the extents of the data taken from the gray region of Fig2c.*

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