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### Title

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### Permalink

<https://escholarship.org/uc/item/9w68d3xq>

### Journal

Cell Biology International, 38(8)

### ISSN

1065-6995

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### Publication Date

2014-08-01

### DOI

10.1002/cbin.10324

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Peer reviewed

## METHODOLOGY

# 3D Arrays for high throughput assay of cell migration and electrotaxis

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## Abstract

Cell behaviour in 3D environments can be significantly different from those in 2D cultures. With many different 3D matrices being developed and many experimental modalities used to modulate cell behaviour in 3D, it is necessary to develop high throughput techniques to study behaviour in 3D. We report on a 3D array on slide and have adapted this to our electrotaxis chamber, thereby offering a novel approach to quantify cellular responses to electric fields (EFs) in 3D conditions, in different matrices, with different strains of cells, under various field strengths. These developments used *Dictyostelium* cells to illustrate possible applications and limitations.

**Keywords:** 3D matrices; array; assay; cell locomotion; electrotaxis; morphology

## Introduction

Cell migration is fundamental to many important biological processes including development, regeneration, wound healing and immune responses (Locascio and Nieto, 2001; Luster et al., 2005). Defects in cell behaviour have serious consequences, including delayed wound healing, ineffective immune responses to infection and mental retardation (Wang et al., 2005; Etienne-Manneville, 2008). Cell culture in 2D planar dishes has provided an important enhancement to our understanding of both the phenomena and mechanisms underlying cell behaviour such as spreading, migration and division.

Cell migration and other behaviour in 3D, however, can be significantly different from those seen in 2D cultures (Knight et al., 2000). For example, in a 3D matrix, non-malignant mammary cells form polarized, growth-arrested acinus-like colonies, whereas this phenotype is lost when cells are cultured *ex vivo* on 2D plastic substrata. Significantly, malignant mammary cells form disorganised, proliferative and nonpolar colonies (Lee et al., 2007). In 2D models, no significant difference was observed between the malignant and non-malignant cell lines, while the 3D migration kinetics

of the non-invasive cell line was lower than the migration kinetics of the invasive cell line (Hazgui et al., 2005). Overall cellular signalling pathways and cell morphology are dramatically influenced by 3D culture as opposed to traditional 2D monolayers (Weigelt et al., 2010). Many other cell types behave differently in 3D matrices of different materials (Even-Ram and Yamada, 2005; Zaman et al., 2006; Mandal and Kundu, 2009; Klemke et al., 2010; Tayalia et al., 2011). Because 3D systems more closely mimic the *in vivo* situation, it is critical to verify important results from 2D cultures in 3D systems.

Many different types of 3D matrices have been produced that require efficient experimental techniques to determine their effects on cell behaviour. For example, modification of the matrix composition, electrical charge, density, etc. yields hundreds of thousands of different 3D matrix environments for culturing and transplanting cells (Tibbitt and Anseth, 2009; Bott et al., 2010; Tai et al., 2010; Ehrbar et al., 2011; Galie et al., 2011). Different treatments of cells in 3D with various modalities, such as growth factors, toxic agents and different mechanical and physical properties further increase the experimental conditions and pose a challenge for efficient determination of cell behaviour in a large number of situations.

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Applied electric fields induce directional migration of many types of cells in culture dish. This phenomenon is known as galvanotaxis/electrotaxis (Robinson, 1985). The significance of electrotaxis in wound healing and regeneration is gradually being recognised (McCaig et al., 2005, 2009; Zhao, 2009; Zhao et al., 2012). Most of the experimental evidences are obtained from a single chamber experimental system. Several new experimental systems were reported recently. Those include multiple-chamber with different electrical gradients, or fluidics chambers that combine electric potential gradients with shear flow or chemical gradients (Li and Lin, 2011; Li et al., 2012; Liu et al., 2013). In an attempt to develop a 3D electrotaxis with capacity to test multiple 3D matrix at the same time, we developed and tested a 3D electrotaxis array system.

We aimed to develop a high throughput technique for screening of cell behaviour. A 3D array technique was developed in combination with multi-focal plane field time-lapse microscopy as an efficient screening tool for high throughput quantification of cell behaviour, emphasising the need for screening of electric field (EF)-guided cell migration (electrotaxis/galvanotaxis) in 3D. Direct current (dc) EFs provide a directional signal that guides migration (Zhao et al., 1997, 2006; Yao et al., 2008; Zhao, 2009; Guo et al., 2010). 3D culture systems for galvanotaxis have been reported before (Song et al., 2007; Sun et al., 2012). Here we report a different system with 3D arrays that allows simultaneous testing of multiple extracellular matrices. This high throughput 3D array technique on slides offers a novel approach to the quantification of cellular responses to EFs with a high efficiency that could not otherwise be achieved.

## Materials and methods

### Cell cultures and 3D matrix preparation

*Dictyostelium* cells (AX2) of  $1.0 \times 10^7$  cells were starved for 8 h. Low density cell suspensions were mixed in 500  $\mu$ L (w/v) of low melting point agarose (Sigma–Aldrich) of different final concentrations (0.2, 0.3 and 0.5%) in DB: 5 mM  $\text{Na}_2\text{HPO}_4$ , 5 mM  $\text{KH}_2\text{PO}_4$ , 1 mM  $\text{CaCl}_2$ , 2 mM  $\text{MgCl}_2$ , pH 6.5).

The agarose gel mixed with cells was loaded onto the 3D matrix array region spot by spot on a slide or the bottom of a Petri dish. 3D matrix arrays of various sizes can be designed and fabricated as required. An array of  $4 \times 5$  spots is shown (Figure 1B). More spots can be made for greater numbers of tests.

### 3D array for high throughput electrotaxis assay

We developed a 3D array in an electrotaxis chamber, as previously described (see Figure 1, and Zhao et al., 1996; Song

et al., 2007). The electrotaxis chamber was mounted onto an imaging system with a motorised stage. EFs were applied as previously described (Zhao et al., 1996; Song et al., 2007). Two agar salt bridges (1.5% agar dissolved in DB) were connected to the DB reservoirs at the ends of the chamber.

### Time-lapse microscopy of cell migration in 3D

Cells were visualised with a microscope (Zeiss Axiovert) and time-lapse images were collected with a cooled CCD camera (Hamamatsu C4742-95). To track cell migration along the Z axis, seven sequential planes were acquired at Z axial spacing of 2  $\mu$ m by the SimplePCI 5.3 imaging system, with a motorised X, Y, Z stage (BioPoint 2, Ludl Electronic Products Ltd.). Motorised control allowed imaging of cell behaviour in multi-field, and multi-Z (focal plane) positions. The apparatus enabled observation and recording of cell behaviour in various 3D matrices under many different conditions within one experiment. An array to 100 spots of 3D gel in an electrotaxis chamber was produced, thus generating high throughput. Cell migration was recorded at various planes along the Z axis. The migration of cells along the Z axis were confirmed and quantified by analysing images in different focal planes.

### Analysis and quantification of cell behaviour

Movement of individual cells was tracked using ImageJ (v.1.44n) software. Trajectory speed and displacement speed were measured by tracing the positions of cells. The tracking data were exported to Excel and Origin (v.7.5) for analysis. The angle that each cell moved with respect to the EF vector was measured and its cosine value was calculated to give a directedness value (Guo et al., 2010). The mean values and standard errors from three independent experiments were calculated and used for statistical analysis.

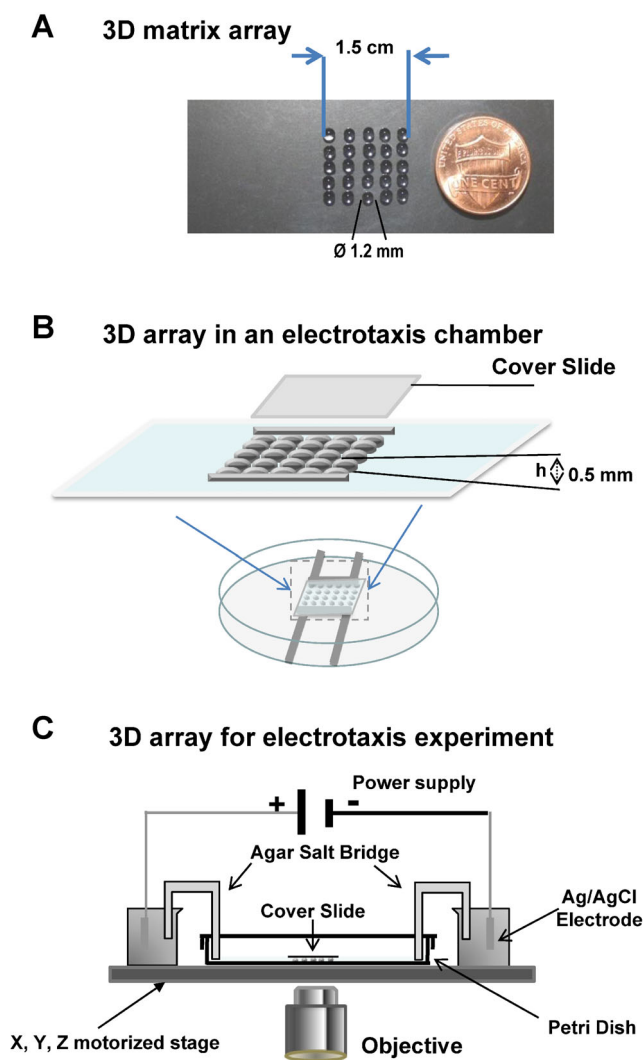
### Statistical analysis

Data are expressed as mean  $\pm$  SEM. Statistical differences between groups were assessed with unpaired, two-tailed Student's *t*-test, with statistical significance set at  $P < 0.05$ . Statistical analysis was performed using the software of SPSS 10.0.

## Results and discussion

### Implementation of the design

An example array of 3D matrix spots of  $4 \times 5$  allows 20 conditions be recorded in one run (Figure 1). Such an array can be easily laid on a microscope slide with agarose matrix mixed with cells and loaded in an area of 1.5 cm  $\times$  1.5 cm.



**Figure 1** 3D matrix array for high throughput cell migration and electrotaxis assay. (A) 3D matrix array on a slide that allows 25 tests at the same time. Increasing the array size could increase numbers of test over 100 different matrices on one slide for cell morphology, and cell migration assay. (B and C) The 3D matrix array provides a unique high throughput migration assay for electrotaxis in 3D. Schematic illustration of the high throughput microchip in study of cell migration in 3D matrix. The 3D matrix array can be incorporated in an electrotaxis chamber, thus allows for high throughput 3D electrotaxis assay.

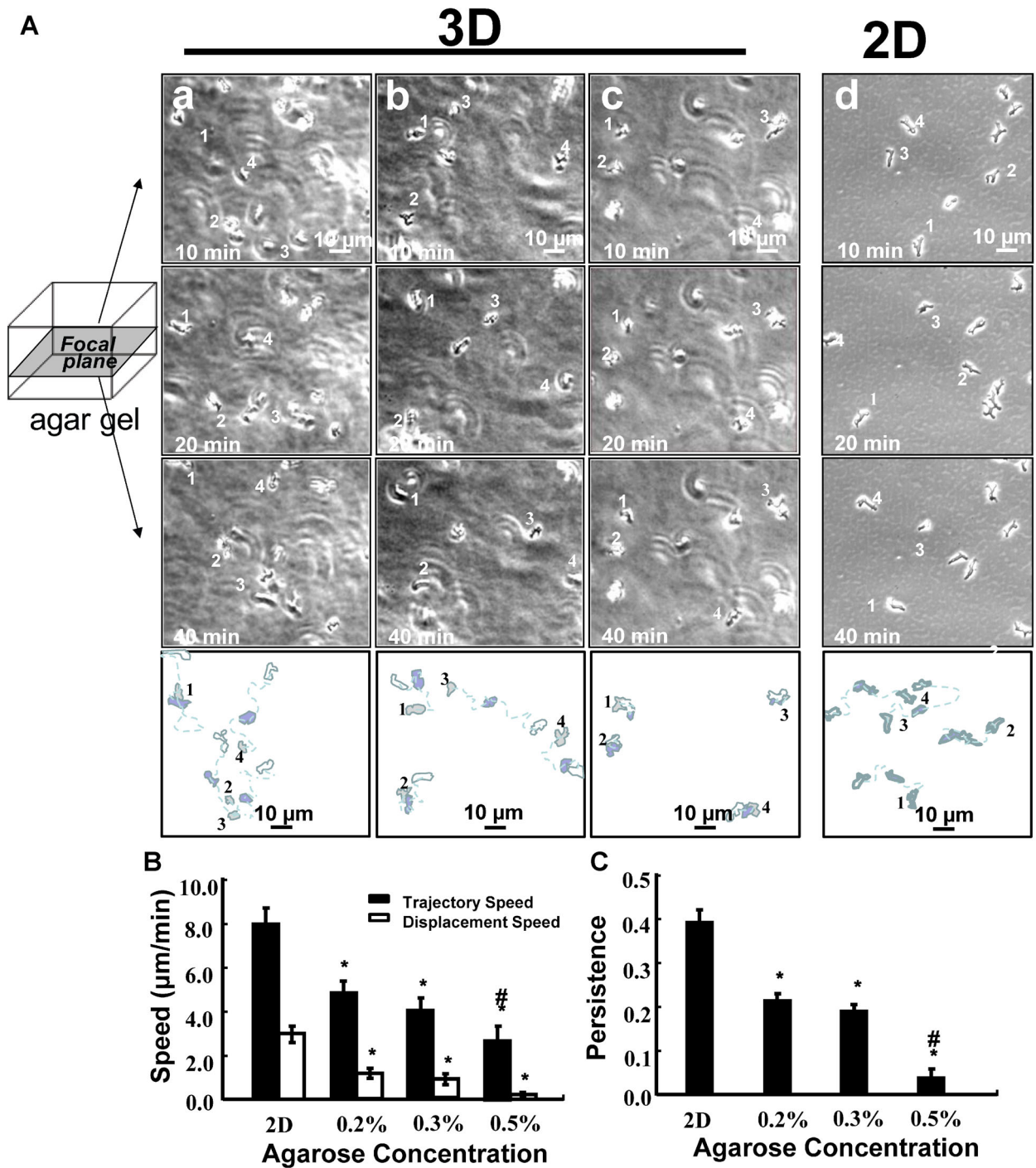
Each spot was of diameter of 1.2 mm or less. With photolithography (Chen et al., 2011) or laser ablation technique, much smaller spots with cells in 3D could be fabricated.

The advantage of this technique allows many 3D matrices to be tested in a single experiment. When combined with multi-field time-lapse microscopy, this method offers a high throughput assay of cell morphology and behaviour in 3D environments. In the case shown in Figure 1, at least 20 samples were tested in a single experiment. Recently 3D arrays have been developed for high throughput assays (Lee et al., 2008; Fernandes et al., 2010; Derda et al., 2011). The focus is on toxicological assays, and the distribution of

populations of cells in the different compartments. No assay of individual cell migration has yet been reported, and our method offers the first high throughput analysis of electrotaxis/galvanotaxis in 3D matrices.

#### *Dictyostelium discoideum* cells in different 3D matrices migrated at different rates

*Dictyostelium discoideum* cells, a popular model system for chemotaxis, were used (Zhao et al., 2002; Willard and Devreotes, 2006; Hoeller and Kay, 2007) to compare cell migration in the 3D array to that in 2D culture. To define motility in different concentrations of agarose, cells



**Figure 2** 3D agarose matrix array show basal motility of cells in 3D gel depends on the matrix density. (A) *Dictyostelium* cells migrate in 3D matrixes of agarose of different concentrations (a, 0.2%; b, 0.3%; c, 0.5%; d, in 2D). The initial position of each cell was marked with a number. (B) *Dictyostelium* cells in 3D culture migrated slower than that in 2D culture. Cell motility in 3D matrix is significantly affected by density of the matrix. (C) migration persistence in 3D is significantly lower than that of those in 2D culture, more significant in higher density of the agarose. Scale bar = 10 μm. Average of 30–45 cells from three independent experiments. (\* $P < 0.01$  when compared to that on 2D; # $P < 0.01$  when compared to that in 0.2 and 0.3% agarose).

developed for 6–8 h were suspended in low melting point, 0.2, 0.3 and 0.5% agarose. For simplicity, we compared migration of single cells on the *X–Y* plane. The *D. discoideum* cells showed obvious migration in 0.2 and 0.3% agarose. In 0.5% agarose, *D. discoideum* migration was almost abolished in the gel with only occasional protrusion of pseudopodia being observed (Figure 2A, see also Supporting Information Movie S1). The cells had significantly lower displacement and trajectory speeds in 3D agarose matrix (Figure 2B). The data indicate that the motility of *D. discoideum* was significantly lower in higher concentrations of agarose.

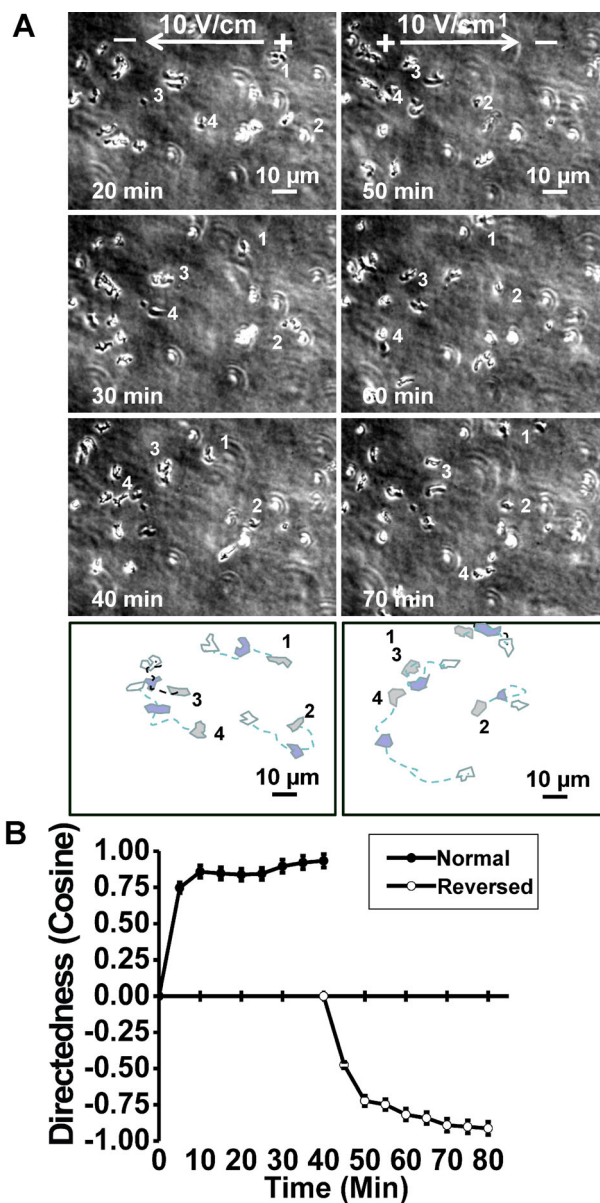
Persistence index quantifies how often cells make turns; the results indicate that the cells in 3D culture made significantly more turns than those in 2D culture (Figure 2C).

We also analysed cell migration in 3D with *Z* information by taking time-lapse images with *Z* steps of 2  $\mu\text{m}$ . Extracting *X, Y, Z* coordinates from those images and plotting the 3D positions showed the path of a selected cell (Figure S1). Many *D. discoideum* cells moved in and out of the focal plane.

Migration of many types of cells in 3D matrices is significantly different from that in traditional 2D tissue culture system (Knight et al., 2000). Proliferation and differentiation of cells, biochemical signalling, DNA uptake, transcription and cell–cell interaction are also dependent on property of ECM (including, e.g. chemical composition, adhesiveness, compliance and electrical charge) (Gray-Schopfer et al., 2007). More research has been focusing on developing engineering materials to create 3D environment that mimicking physiological and pathological conditions (Sontheimer, 2008; Fiorio Pla et al., 2012; Valero et al., 2012). For example, recent advance in chemistry allows production of 3D materials with controlled property of electrical charge (Xiao et al., 2011), which gives rise to large number of different types of 3D materials. Our method provides a powerful high throughput assay for evaluation of those materials on cell behaviour in 3D.

#### *Dictyostelium discoideum* cells migrated towards the cathode in 3D agarose in EFs

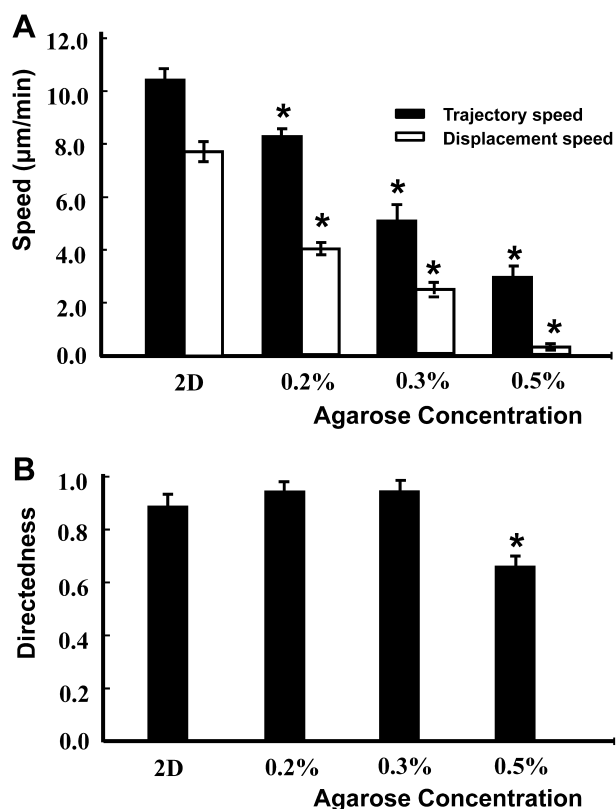
Dc EF has been used to guide cell migration, but the mechanisms are very poorly understood. To study electrotaxis in 3D, the 3D array on slide technique was adapted to our electrotaxis chamber (Figure 1C). This 3D array not only provides a model of electrotaxis in 3D, but also offers a unique high throughput method to study a vast array of conditions simultaneously. To test electrotaxis, we exposed these cells in the 3D (0.3%, final concentration) agarose array to an EF. Cells migrated evidently towards the cathode in 3D. When the EF field polarity was reversed, the cells reversed their direction of migration towards the new cathode (Figure 3, see Movie S2). This result is consistent



**Figure 3** Electrotaxis of cells in 3D matrices. (A) Time-lapse images show EF-guide cell migration. When the field polarity was reversed, the cells changed the migration direction. (B) quantification of the migration directedness shows the reversal of migration direction. In 0.3% agarose, EF = 10 V/cm. Data in (B) are mean  $\pm$  SEM from 20 to 45 cells. Scale bar = 10  $\mu\text{m}$ .

with observations of cell migration in 2D (Shanley et al., 2006).

*Dictyostelium discoideum* cell migration decreased with increasing agarose concentration. In a 0.5% agarose matrix, the *D. discoideum* cells migrated towards the cathode, but the migration was highly restrained by the agarose matrix compared to that seen in 0.2 and 0.3% agarose (Figure 4).



**Figure 4** Quantification of the directional migration of *D. discoideum* AX2 cells in 2D and 3D culture in an applied EF of 10 V/cm. (A) Trajectory speed and displacement speed and (B) directedness. Average of 30–45 cells from three independent experiments ( $P < 0.01$ ).

We have initiated new experiments to use this 3D array system to study molecular mechanisms of electrotaxis in 3D matrix, testing the effects of manipulation of adhesion molecules, inhibition and mutation of adhesion molecules on cell migration in 3D and in electric fields.

## Conclusion

We have developed a 3D array method which allows high throughput screening of cell behaviour in different matrices, under different conditions. The practicability of this technique has been shown with *D. discoideum* cells. Combination of this technique with the electrotaxis chamber offers a unique approach for screening cell behaviour in response to EFs in 3D.

## Acknowledgements and funding

This work was supported by a grant from NSF [MCB-0951199], California Institute of Regenerative Medicine [RB1-01417], NIH [1R01EY019101]. This study was sup-

ported in part by an Unrestricted Grant from Research to Prevent Blindness, UC Davis Ophthalmology; Yunnan Province Talented Recruiting Program [2009CI127] and National Science Foundation of China [U1132603]. We thank Wellcome Trust for continuous support [WT082887MA].

## Author contribution

M.Z., S.Z., P.N.D., A.M. designed research; S.Z. and R.G. performed research; M.Z., S.Z. analysed data and wrote the paper.

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### Supporting Information

Additional supporting information may be found in the online version of this article.

**Movie S1. *Dictyostelium* cell migration in 3D in 0.3% of agarose.**

**Movie S2. *Dictyostelium* cell migration in 3D in 0.3% of agarose in EF.**

**Figure S1. Migration trajectory of a *Dictyostelium* cell in 3D matrix.** A *Dictyostelium* cell in 0.3% agarose matrix migration for 60 min.

Received 19 October 2012; accepted 2 April 2013.  
Final version published online 7 May 2013.