Comments to the Editor

Comments on the Article ''The Universal Dynamics of Tumor Growth'' by A. Brú et al.

We read with great interest the article published by Brú et al. titled ''The Universal Dynamics of Tumor Growth'' (*Biophysical Journal* 85:2948–2961). Therein, a number of in vitro growing cell line colonies and developing in vivo developing tumors are analyzed. Brú et al. infer that their results indicate a common growth dynamics that is compatible with the molecular beam epitaxy (MBE) process. The authors achieved this conclusion based on the following reasoning. First, a fractal analysis of the contours was performed. Once the universality class was identified by means of that analysis, a stochastic equation sharing the same universality class was introduced: the linear MBE model. Then, experimental assays to test the features imposed by that model were carried out. Herein, we demonstrate that the scaling analysis performed by the authors is inconsistent and consequently misleading. Moreover, we also show that the experimental evidence provided to check the characteristics imposed by the linear MBE equation do not point exclusively to that model. Finally, we indicate the source of such inconsistent analysis, namely, an effect due to the geometry of tumors, and how to fix it.

First, we briefly review some scaling concepts for fractal super-rough growth processes. As indicated by Brú and coworkers, the scaling exponents and the scaling functions that define the universality class of a fractal growth process can be obtained by measuring the local width function and the power spectrum. Both the exponents and the scaling functions are usually found by means of a collapse procedure (Barabási and Stanley, 1995). This technique allows the local width and/or the power spectrum at different times to collapse into a single universal curve (the scaling function) when the right exponents and scaling hypothesis are used. In the particular case of the local width function for a super-rough growth process such as MBE, it has been established that the collapse technique should give a scaling function with a crossover between two well differentiated regimes, each of them with characteristic power-law decays (e.g., Ramasco et al., 2000, and references therein). In a double logarithmic plot, the behavior is the following. For $l/t^{(1/z)} < 1$, the slope of the scaling function, $W(l,t)/l^{\alpha_{\text{global}}}$, must be $(\alpha_{\text{local}} - \alpha_{\text{global}})$, and for $l/t^{(1/z)} > 1$ the slope should be $-\alpha_{\text{global}}$. A reliable collapse procedure must be consistent with these scaling exponents.

In Figs. 3 and 4 of the article by Brú and co-authors, the scaling analysis for the colon adenocarcinoma cell line is presented as a representative case. The inset in Fig. 3 shows a collapse of the local width curves when the super-rough scaling hypothesis is assumed and the following values of the exponents are used: $z = 4, \alpha_{\text{global}} = 1.5$, and $\alpha_{\text{local}} = 0.91$. In accordance with the above discussion, the slope of the scaling function for $l/t^{(1/z)} > 1$ should be $-\alpha_{\text{global}} = -1.5$. It is easy to check that this is not the case. In fact, the slope is approximately half of that value. Therefore, the collapse is not *self-consistent* with the initial scaling hypothesis and consequently is incorrect. It is worth noting that the behavior of the local width curves shown in Fig. 3 does not fit within any of the known scaling hypotheses (Ramasco et al., 2000) (and clearly does not fit into the super-rough class) since no saturation of the local width is reached. Moreover, the same kind of criticism applies to the collapse and functional form of the power spectra shown in Fig. 4. These facts pose the interesting question of whether or not tumor growth processes define a new scaling behavior. We comment on this point below. Notice the crucial importance of such incorrect scaling analysis since the universality class and a consequent model satisfying it depend on the exponents that are obtained.

Once the universality class of the process has been identified, with the unfortunate shortcoming indicated above, the authors introduce in their article a stochastic equation that shares that same class: the linear MBE model. Their subsequent experimental assays are intended to show that the developing tumors and colonies satisfy that equation. Three features are examined: cell surface diffusion, cell proliferation restricted to the periphery, and linear growth rate. Concerning surface diffusion, only preliminary results for a particular kind of cell are presented in Fig. 5. These results show a single cell migrating along the colony border toward a site with a larger coordination number. It has recently been shown that a number of biological processes present anomalous diffusion properties (Palmer et al., 1999; Kues et al., 2001; Caspi et al., 2002; Suh et al., 2003; Wong et al., 2004). In fact, a fractional transport approach leading to subdiffusive behavior has been recently considered in the context of tumor development (Iomin et al., 2004). Obviously, surface diffusion, i.e., the subdiffusive process that may lead to MBE behavior, is not the only way of diffusing anomalously. In the studies referred to above, the subdiffusive transport properties are typically observed and quantified by measuring the mean square displacement (MSD) as a function of time. The moving attributes of the migrating cell shown in Fig. 5 may *qualitatively* indicate an anomalous diffusive process compatible with the MBE equation. However, only by measuring the MSD, or an equivalent quantity, may one

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quantitatively elucidate what kind of subdiffusive process drives cell migration. As a matter of fact, a multitude of subdiffusive processes favoring sites with high coordination number can be envisioned.

On the other hand, by conveniently labeling cells and monitoring the evolution of the tumor radii as a function of time, the authors indeed demonstrate that the proliferation activity is mainly located at the periphery and that the system increases its size linearly. (There must be a typo in the vertical axis of Fig. 9: log$r$ should read r. Otherwise the growth would be exponential and not linear as stated. The inset is correctly labeled.) These important results rule out the widespread belief of Gompertzian growth in tumor development. However, we must point out that *none* of these results provide unequivocal evidence of MBE dynamics. To see this, consider the following general form of a stochastic equation,

$$\frac{\partial h(x,t)}{\partial t} = F + G[h(x,t)] + \eta(x,t),$$

where $G[h(x,t)]$is in general a nonlinear functional of $h(x,t)$ and/or its derivatives, F is the growth rate, and $\eta(x,t)$ is a white noise, uncorrelated in space and time, with zero mean value. Within this general framework, in the particular context of tumor growth, $h(x,t)$ would represent the tumor interface, and the linear MBE model corresponds to the case $G[h(x,t)] = -K(\partial^4 h(x,t)/\partial x^4)$. Then, it is trivial to prove using symmetry considerations that *any* functional satisfying the symmetry $G[h] = -G[-h]$ induces a linear growth rate for the average value of $h(x,t)$ (Barabási and Stanley, 1995). (We do not consider herein systems that may undergo phase transitions, i.e., spontaneous symmetry breakings.) Hence, the measured linear growth rate may indicate a certain general mathematical structure for the evolution equation, but does not point to a particular functional form. Finally, cell proliferation on the periphery is not a distinctive characteristic whatsoever of the linear MBE equation. For example, cells in the so-called Eden model grow at the periphery, and this model shows neither a super-rough scaling hypothesis nor MBE exponents (Barabási and Stanley, 1995). Therefore, *none* of the experimental assays carried out to test the features imposed by the MBE model *quantitatively* link the observed phenomenology with that equation. The assays help to discard models that are inappropriate to describe the observed growth but do not pinpoint a particular equation.

As the authors state, there are two important differences between this and other growth problems: the geometry, and a system size that is changing in time. Still, the literature provides scaling tools to overcome the difficulties in the analysis under such constraints. For instance, these tools were developed and used to analyze a similar problem in the biological context (Galeano et al., 2003). Brú and co-authors assert correctly that the dynamical exponent, z, is a measure of how information is transmitted along the interface, and that when that information acknowledges the finiteness of the system, then saturation of the different statistical functions (local width, power spectrum, etc.) is reached. However, when the system size is changing in time, one must consider the dilation effect. Note that a growing system size alters the *effective* velocity at which information is transmitted. By neglecting this fact, as the authors do, the dynamical exponent is masked by this effect and may lead to wrong conclusions about the scaling properties. Following this argument, one can easily envision that if the system size grows faster than the velocity at which the information is transmitted, then no saturation can be reached. Such a scenario is certainly plausible considering the data shown in Figs. 3 and 4, where saturation is not obtained. Notice that in this case, tumor growth would not define a new scaling behavior. In any case, an analysis in terms of the dilation effect would still be required to deduce the correct value of the exponents and obtain a self-consistent collapse. One can account for the experimental data by rescaling space as $x \rightarrow x \cdot f(t)$, where $f(t) \propto t^a$ is a dilation factor that takes into account the increase of size in time. As a matter of fact, since a linear growth of the average radii of the tumors was observed and their geometry is circular, a value $a = 1$would be expected. Thus, we suggest that the authors reanalyze the data in terms of a corrected scaling hypothesis (see Galeano et al., 2003, for details) to check this statement and shed light on this important topic. Such a reanalysis is required to obtain the correct scaling hypothesis, critical exponents, and universality class, which in turn could lead to an appropriate model. Notice that we are not excluding the applicability of the MBE model after a reanalysis. However, as has been demonstrated herein, the study carried out by Brú and co-authors does not provide, in its present form, unequivocal evidence of tumor growth following the linear MBE model.

We end with some concluding remarks. Statistical mechanics is a discipline that tries to understand how collective behavior arises from mutual interactions between individual units. A particularly suitable process to study within this formalism is the ontogeny of tumors and colonies since cellular activity drives the growth and evolution of the system as an ensemble. More specifically, fractal analysis techniques are certainly a powerful tool that may establish a connection between the different spatiotemporal scales in tumor growth. The essence underlying fractal analysis is scale invariance. Scale invariant properties lead to universality classes and thus to the identification of the universal mechanism responsible for such growth (Nunes Amaral and Barthélémy, 2004). We have shown that the article ''The Universal Dynamics of Tumor Growth'' by A. Brú et al. disregards that essence when the increase in system size is ignored in the scaling analysis. As a consequence, the proposed connection with a mechanism inducing those properties makes no sense. In fact, we have also shown that the subsequent experimental assays that were carried out do not pinpoint a specific model and hence a mechanism. In summary, the underlying universal mechanism

governing the ontogeny in tumors, if any, is still an open problem to be tackled.

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REFERENCES

Barabási, A. L., and H. E. Stanley. 1995. Fractal Concepts in Surface Growth. Cambridge University Press, Cambridge.

Caspi, A., R. Granek, and M. Elbaum. 2002. Diffusion and directed motion in cellular transport. *Phys. Rev. E.* 66:011916.

Galeano, J., J. Buceta, K. Juarez, B. Pumariño, J. de la Torre, and J. M. Iriondo. 2003. Dynamical scaling analysis of plant callus growth. *Europhys. Lett.* 63:83–89.

Iomin, A., S. Dorfman, and L. Dorfman. 2004. On tumor development: fractional transport approach. ArXiv:q-bio.TO/0406001.

Kues, T., R. Peters, and U. Kubitscheck. 2001. Visualization and tracking of single protein molecules in the cell nucleus. 2001. *Biophys. J.* 80: 2954–2967.

Nunes Amaral, L. A., and M. Barthélémy. 2004. Complex systems. *In* Advances in Condensed Matter and Statistical Physics. E. Korutcheva and R. Cuerno, editors. Nova Science Publishers, New York.

Palmer, A., T. G. Mason, J. Xu, S. C. Kuo, and D. Wirtz. 1999. Diffusing wave spectroscopy microrheology of actin filament networks. *Biophys. J.* 76:1063–1071.

Ramasco, J. J., J. M. López, and M. A. Rodríguez. 2000. Generic dynamic scaling in kinetic roughening. *Phys. Rev. Lett.* 84:2199–2202.

Suh, J., D. Wirtz, and J. Hanes. 2003. Efficient active transport of gene nanocarriers to the cell nucleus. *Proc. Natl. Acad. Sci. USA.* 100:3878–3882.

Wong, I. Y., M. L. Gardel, D. R. Reichman, E. R. Weeks, M. T. Valentine, A. R. Bausch, and D. A. Weitz. 2004. Anomalous diffusion probes microstructure dynamics of entangled F-actin networks. *Phys. Rev. Lett.* 92:178101.

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