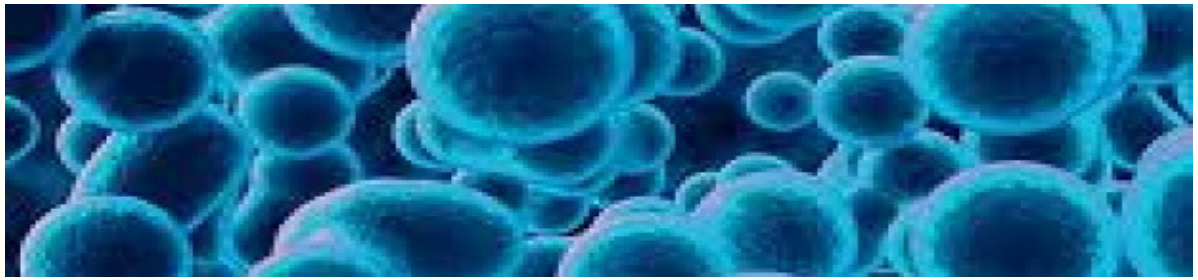

In Vitro study of the growth and interfacial behavior of Vero and HeLa cells

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Aim of the project and overview:

- Our aim is to contribute to the understanding of the interfacial properties of growing colonies of cells.

- For this purpose we growth colonies of Vero and HeLa cells ***in vitro.*** Furthermore, we developed a model for cell colonies growth (Poster 02, in the poster section).

- Growing interfaces are rationalized in terms of the Family-Viseck dynamical scaling theory.

- In both cases, experiments and simulations, we found that growing interfaces belong to the KPZ universality class.

Brief experimental details:

* Vero Cells (African Green Monkey Kidney) are cultured in Petri dishes. These cells continue growing and dividing as long as adequate culture conditions are maintained. Although they are not tumorigenic they show invasive behaviour like that of cancer cells.

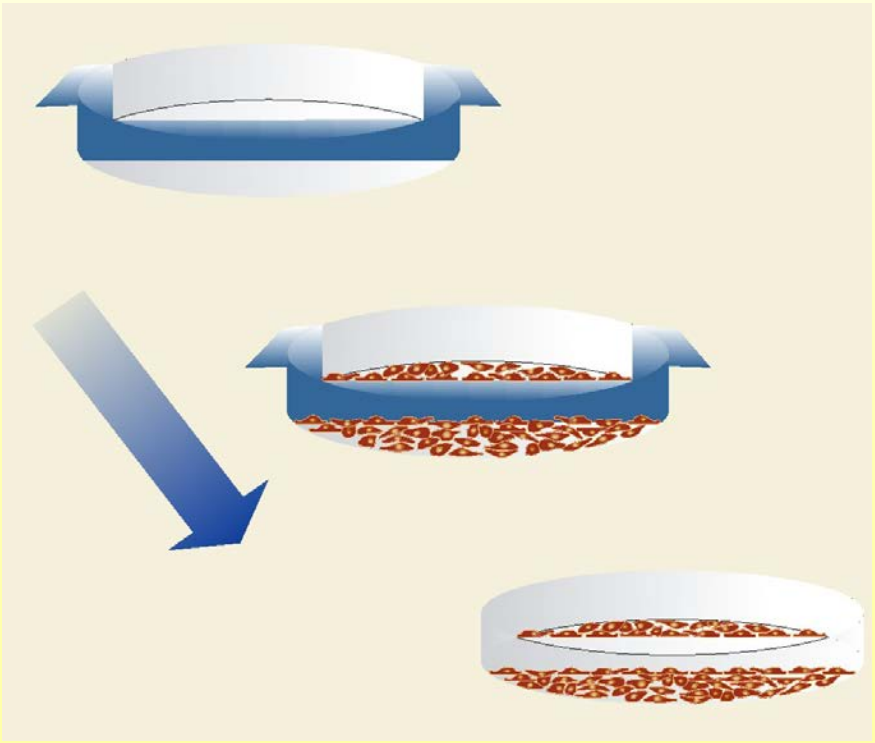
* Colonies are prepared by adding low density (30-40000 cells/ml) Vero cells in a culture medium. In all cases cultures are maintained a 37 ºC, in a (5% CO_2+ 95% air) atmosphere with 95% humidity. Half of the culture medium is changed every 2 days. Full experiments usually take 5-8 days of growth.

Linear and radial experiments:

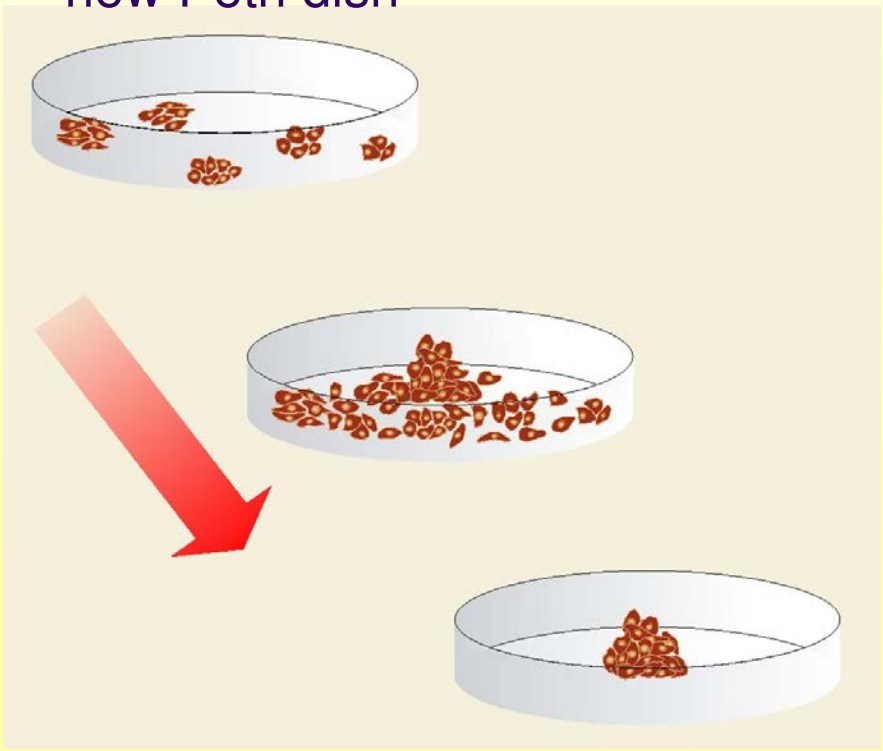
Cell cultures are obtained in Petri dishes of 36 mm diameter, by using standard cell culture medium with antibiotics.

A3

- A Teflon tape is located in the middle of the dish.
- Cell seed and growth.
- Removal of Teflon tape.
- Two linear fronts are obtained.



- Very diluted cell seeds are located in the dish.
- The spontaneous formation of cell colonies is observed.
- The bigger cluster is moved into a new Petri dish



Diapositiva 4

A1 z
Admin, 21/08/2014

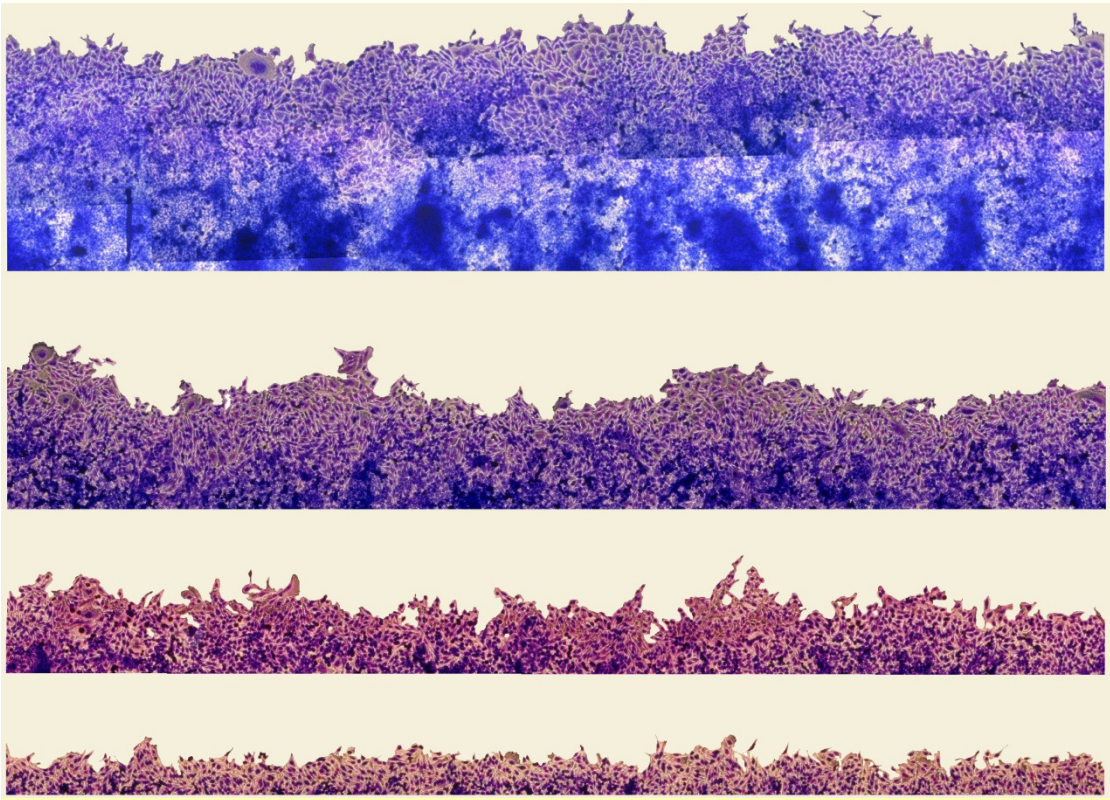
A3 Admin 08/08/2014
Se siembran celulas muy diluidas en una capsula de Petri y se las deja crecer. Al principio las colonias son muy pequeñas y muy irregulares.
Cuando se supera la confluencia del 100% se forman agregados de celulas que se re-siembran en una cápsula de Petri nueva. Crece así una colonia radial pero más grande.
Admin, 08/08/2014

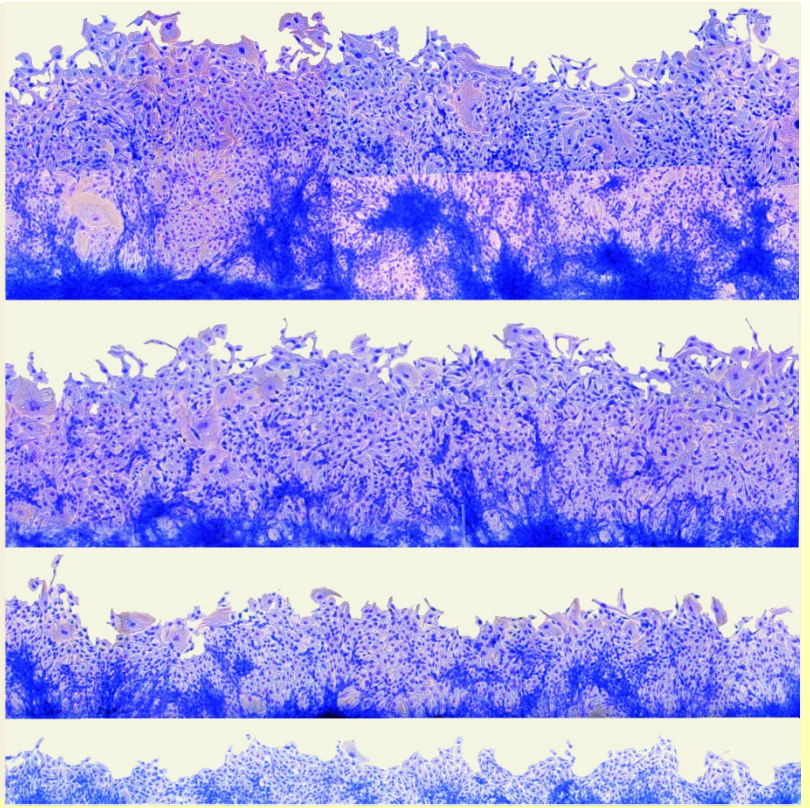
Image composition and binarization.

Colonies are imaged by means of a digital camera coupled to a contrast microscope. By composing different images a resolution of 1 μm = 1.13 pixel is achieved.

Cells are considered as a part of the growing culture when they contact the colony through either cytoplasm or filopodia. Growing profiles are digitalized by using both a digital image processing software and a graphic tablet for hand tracing. Reproducibility of results are obtained within 1% error.

Linear cell cultures

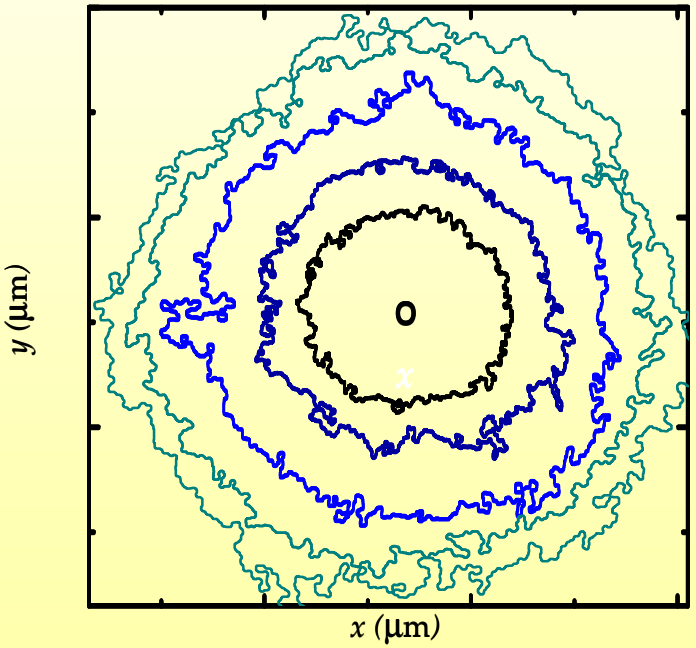


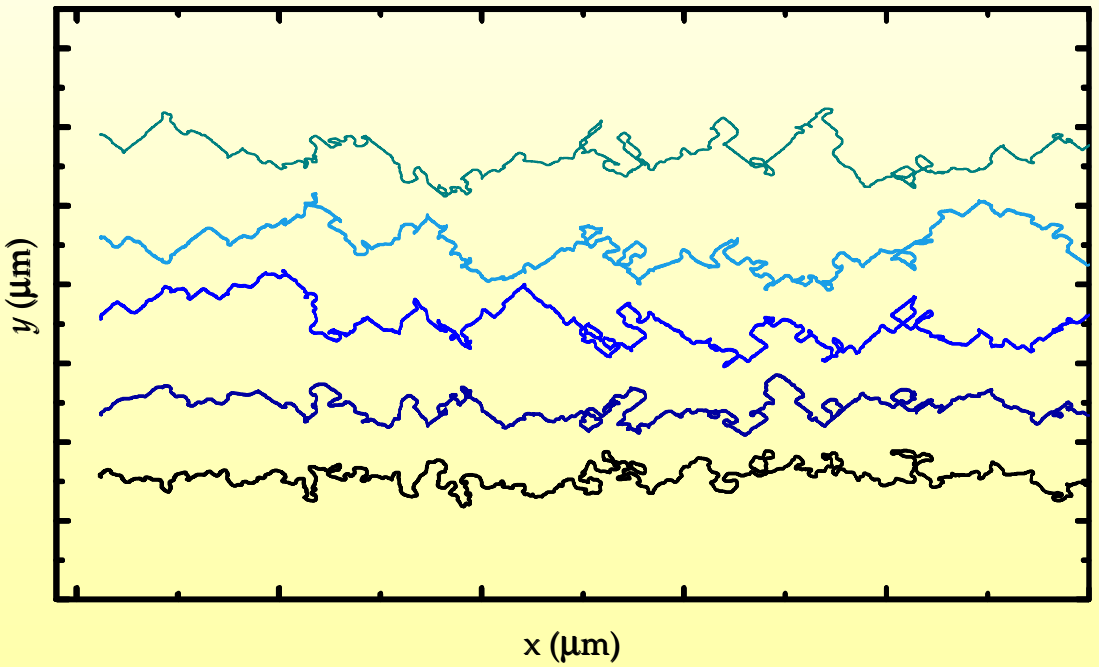


HeLa cell line

Vero cell line

Radial and Linear colony fronts

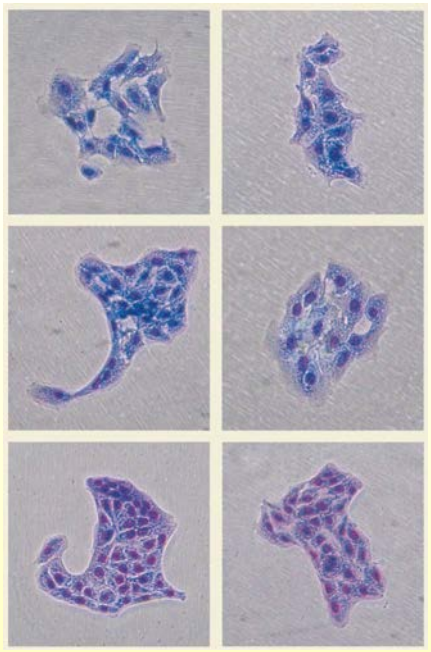


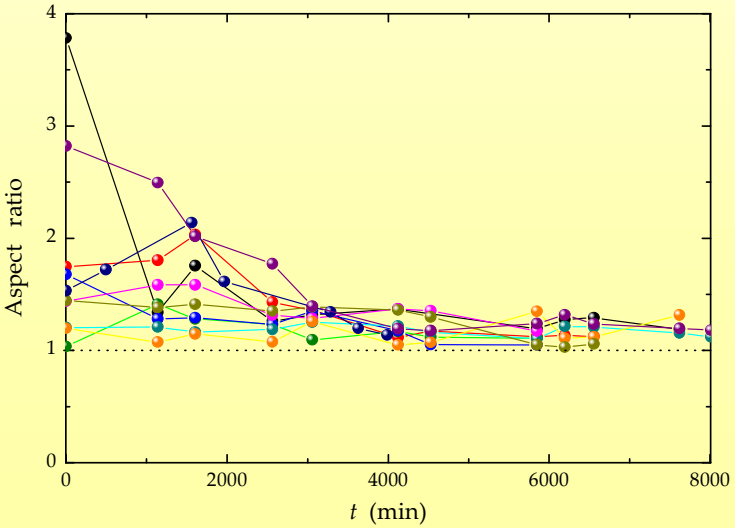


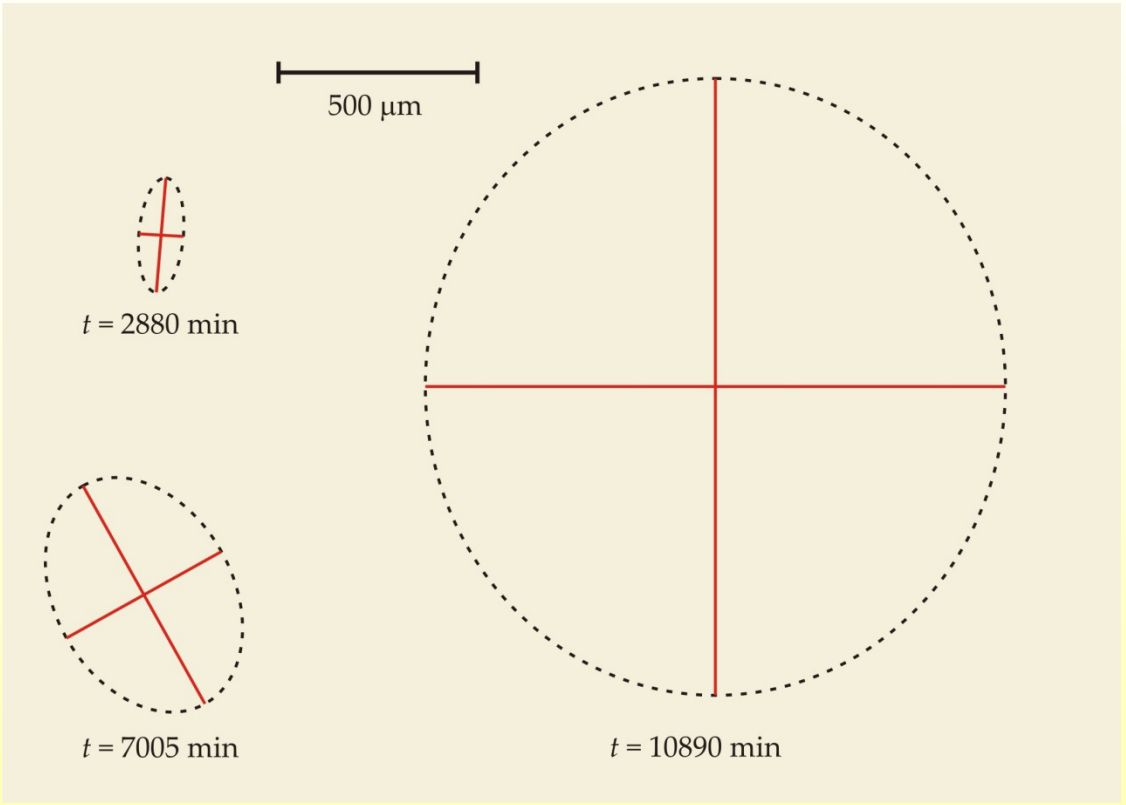
Diapositiva 7

LAH1 Estos son los contornos de frentes reales de crecimiento para que sepan más o menos como son los sistemas
Luis, 19/06/2014

Radial colonies







A4

Diapositiva 8

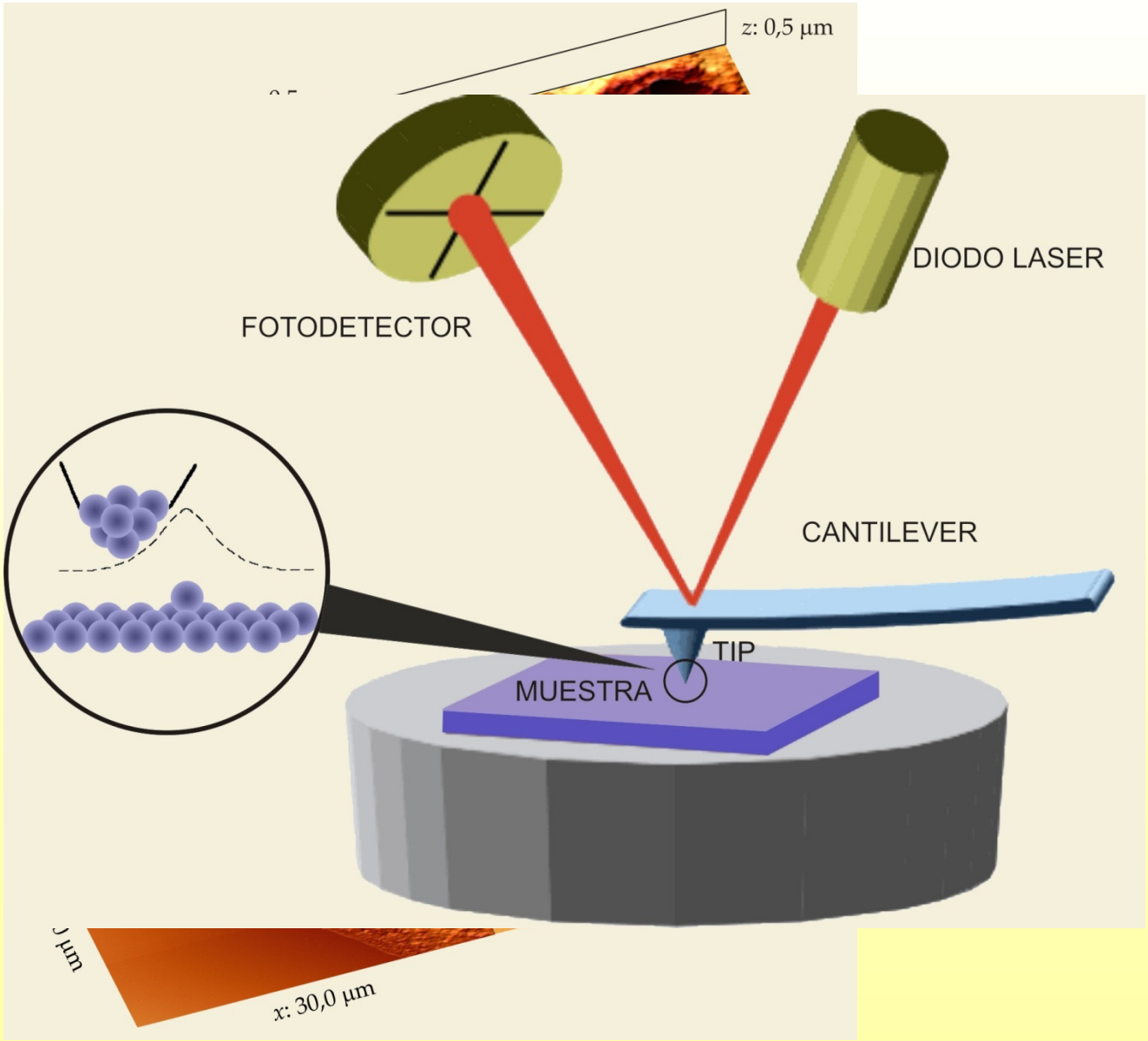
A4 Luis 19/06/2014

Calculo del aspect ratio, un ejemplo.

Evolucion temporal del aspect ratio de todas las colonias

Admin, 11/08/2014

Atomic force microscopy images



Diapositiva 9

A5 Luis 19/06/2014
EL microscopio de fuerzas atomicas tiene una punta de un diametro de unos pocos atomos. Percibe las fuerzas y hace declinaciones del cantilever para mantener la distancia entre la punta y la muestra constante. La altura se mide por el la incidencia del laser.
Admin, 11/08/2014

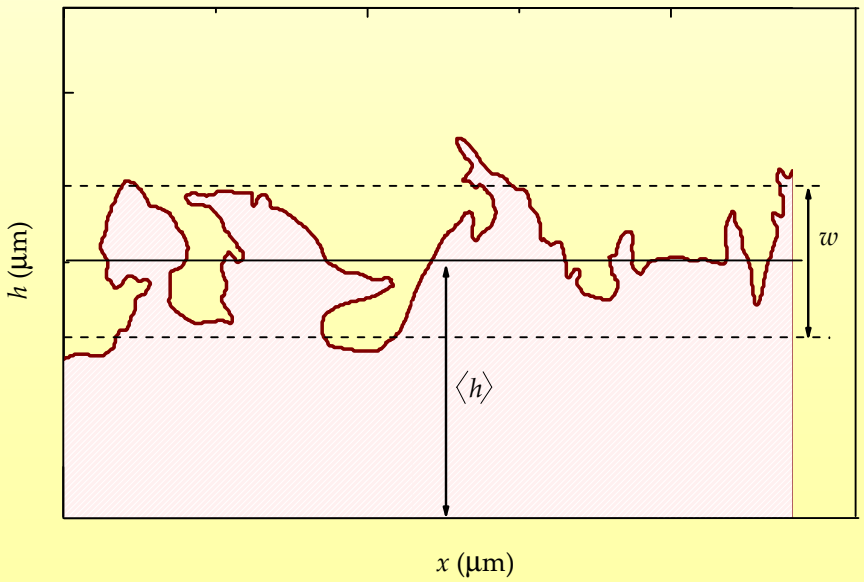
A7 x
Admin, 21/08/2014

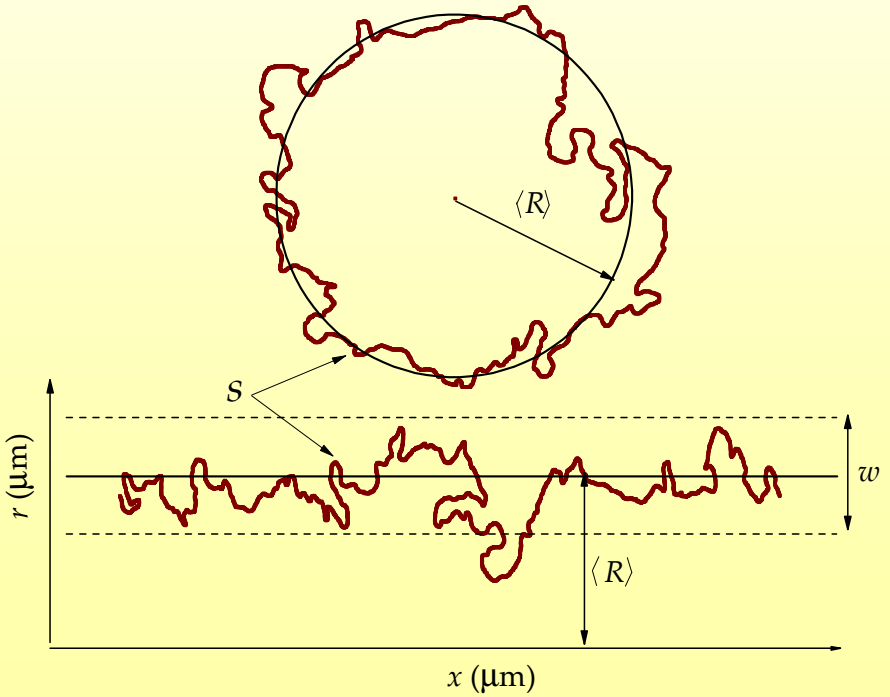
Evaluation of relevant parameters for the characterization of the interface.

$$\langle h(t)\rangle \equiv \frac{1}{L}\sum_{i=1}^{L} h(i,t)$$

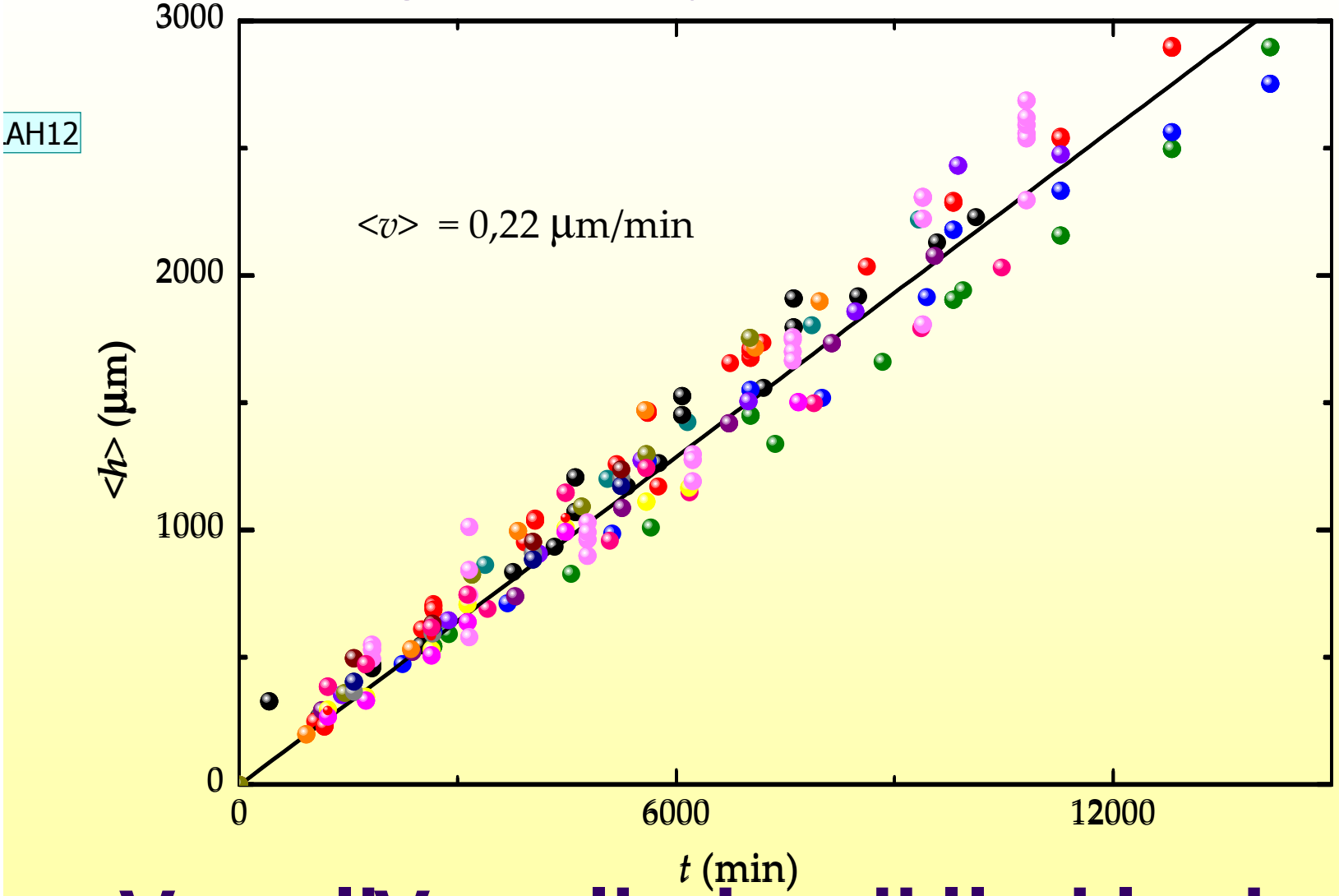
$$w(L,t) \equiv \sqrt{\frac{1}{L}\sum_{i=1}^{L}[h(i,t) - \langle h(t)\rangle]^2}$$

$$S(k,t) = \langle \hat{h}(k,t)\hat{h}(-k,t)\rangle$$





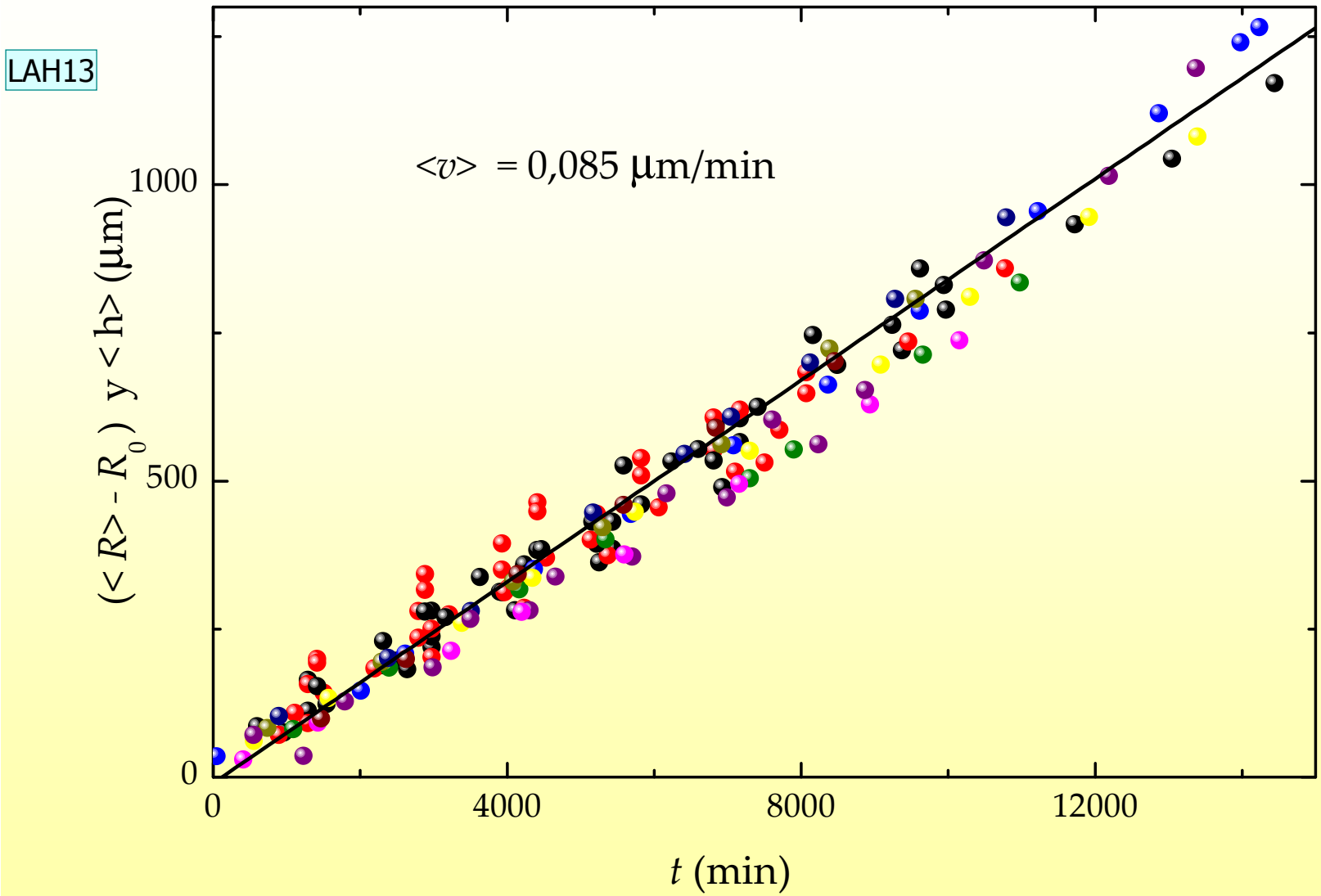
The propagation of the fronts. Calculation of the average velocity.



Vero live and dead cells

Diapositiva 11

LAH12 Velocidad de crecimiento de las colonias lineales y radiales de celulas Vero.
Primero un ejemplo lineal, luego todas las lineales juntas y finalmente se agregan todas las radiales juntas
Luis, 19/06/2014



HeLa linear and radial colonies

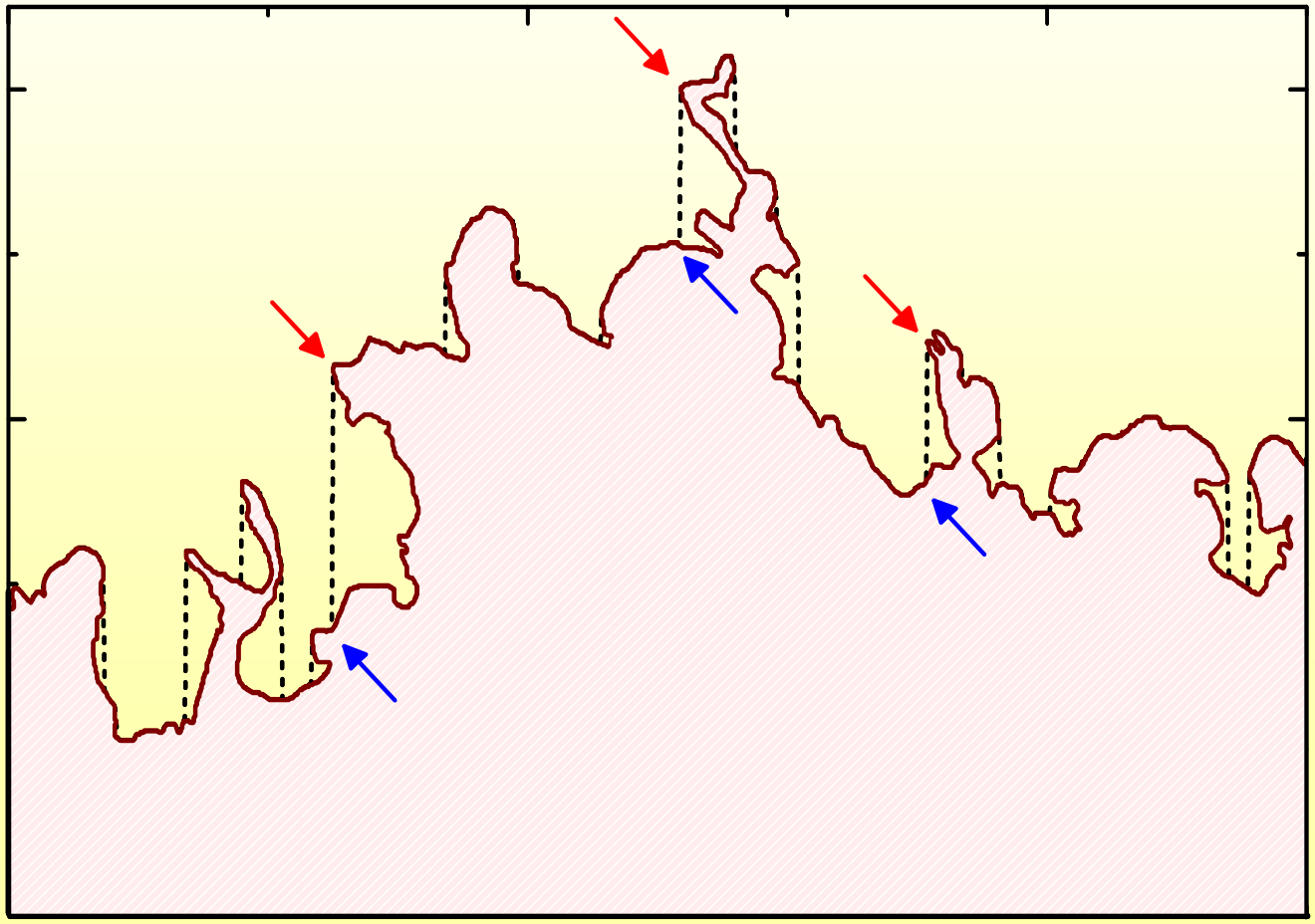
Diapositiva 12

LAH13 Velocidad de crecimiento de la colonias radiales y lineales de celulas HeLa. La velocidad es constante pero diferente. Eso varia en cada tipo celular.

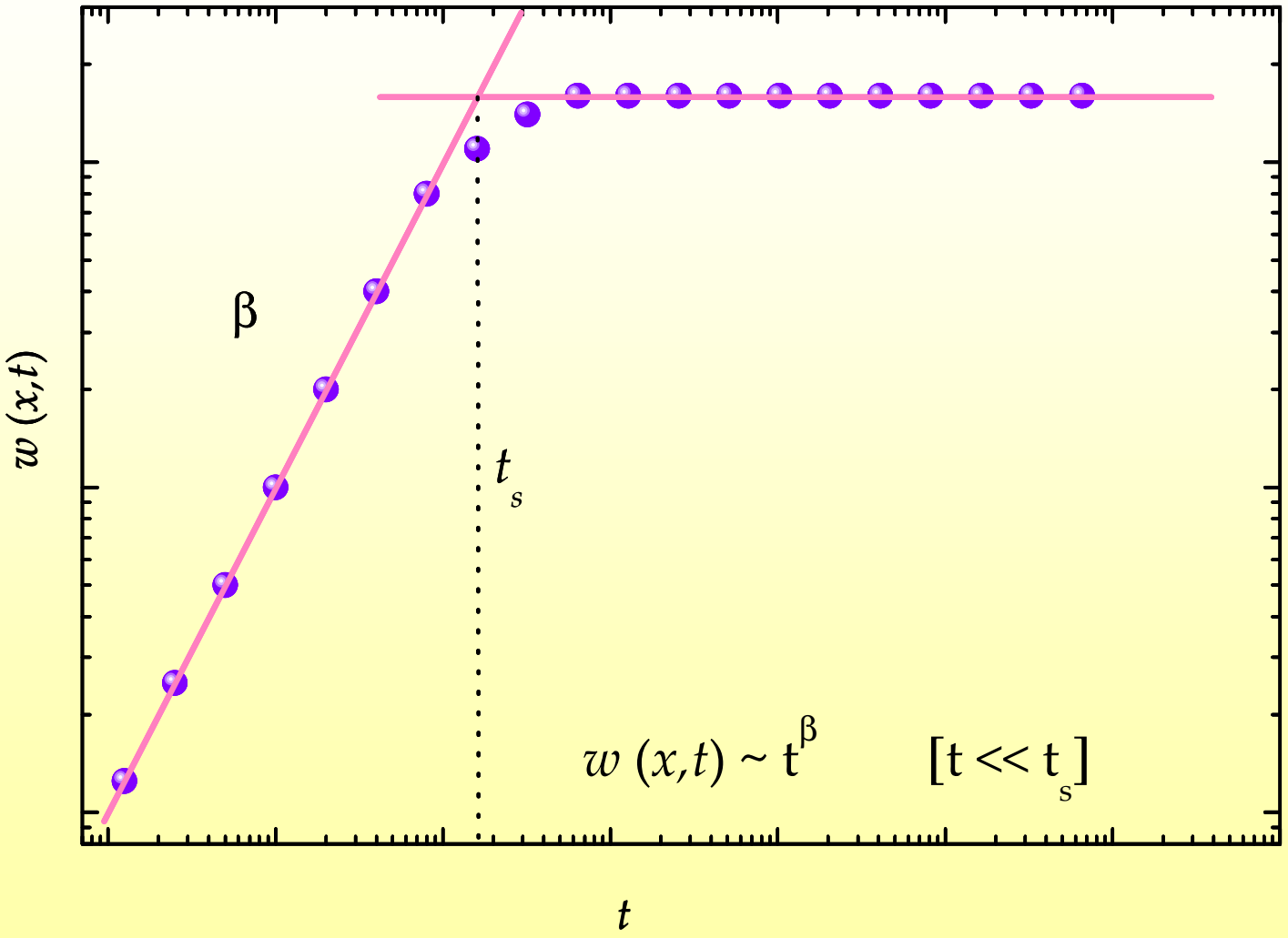
Luis, 19/06/2014

Overhang correction: from multi- to mono-valuated profiles.

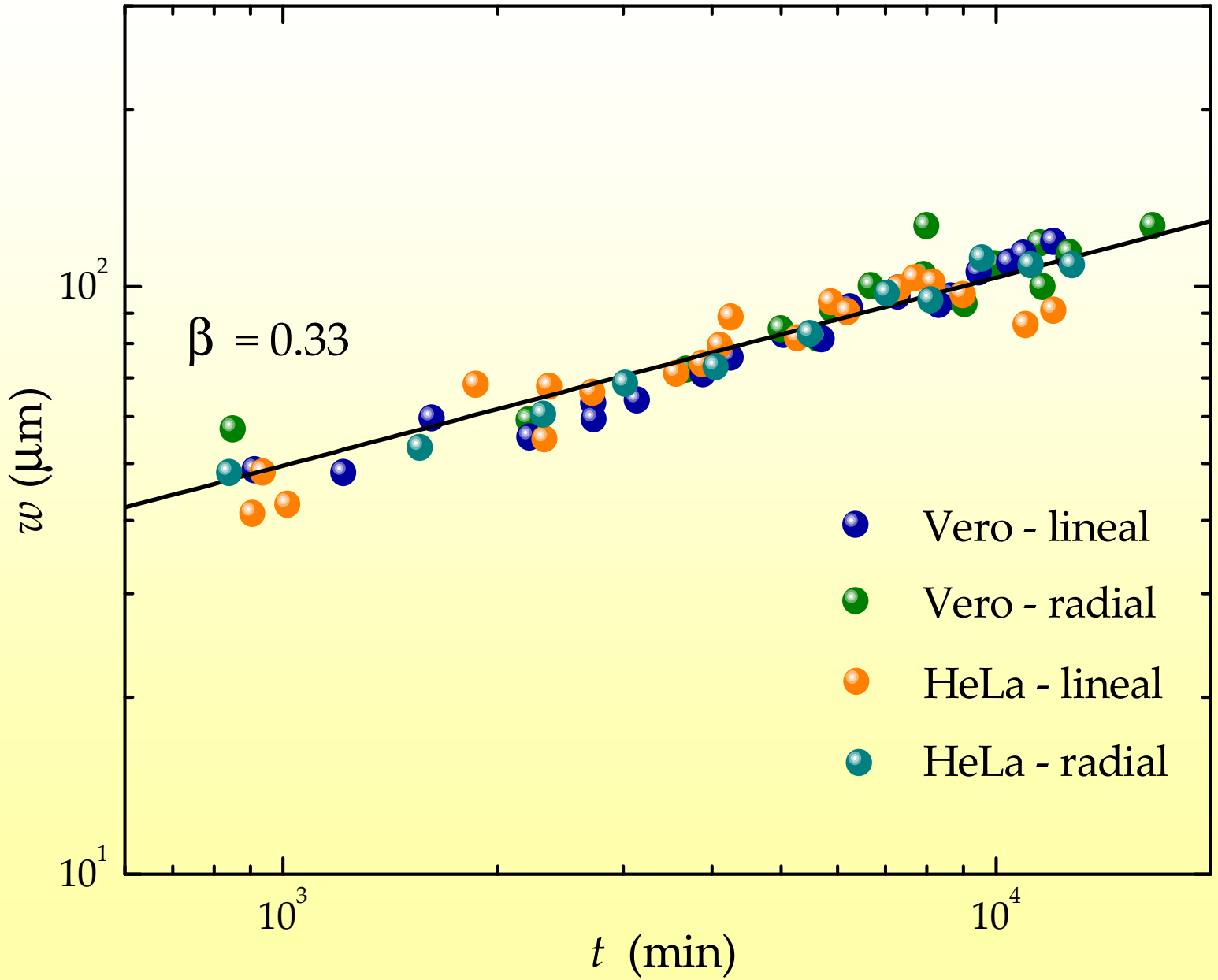
In order to analyze the data, first a single-valued interface is obtained.



Dynamic scaling



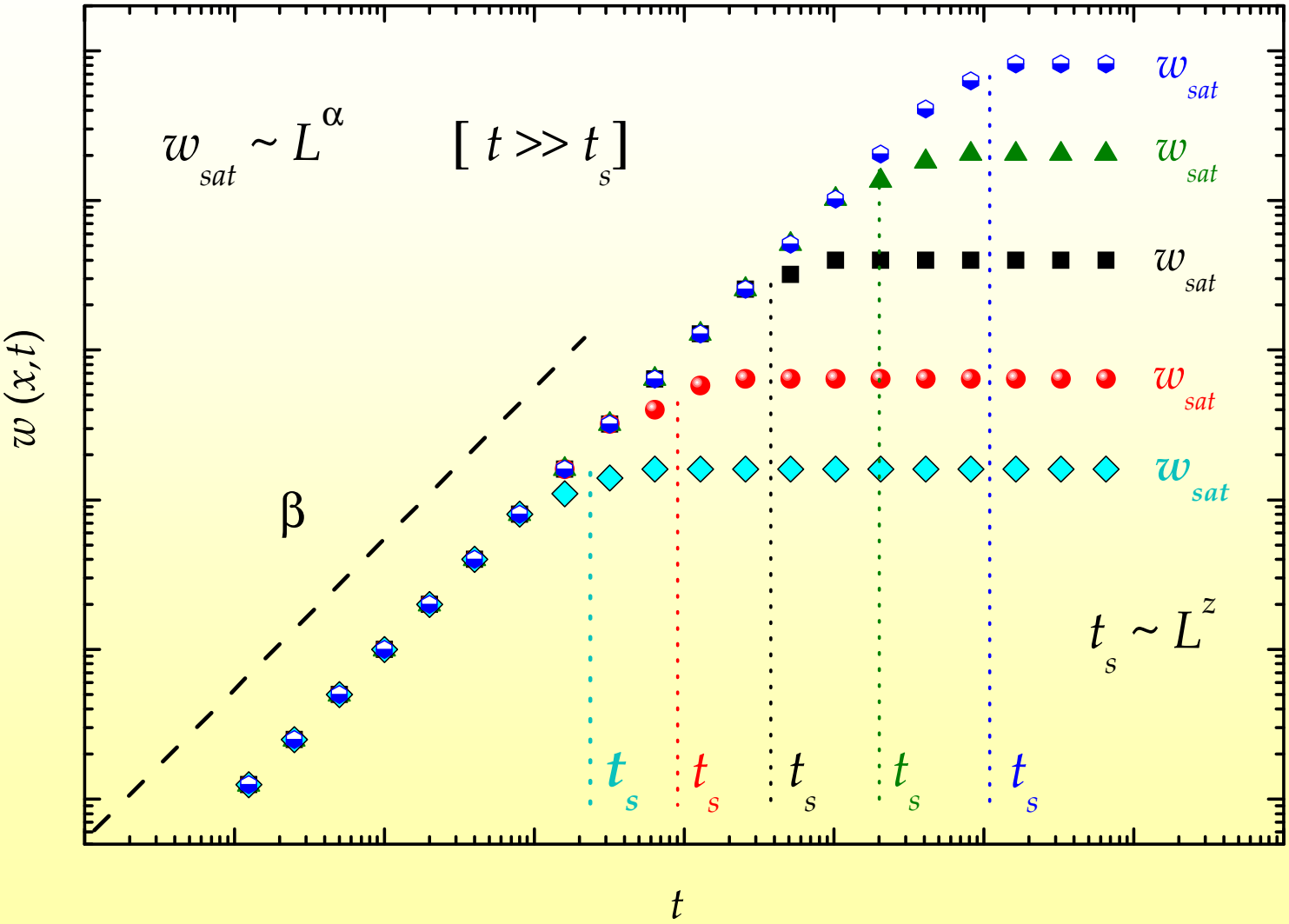
Experimental Growth exponent β



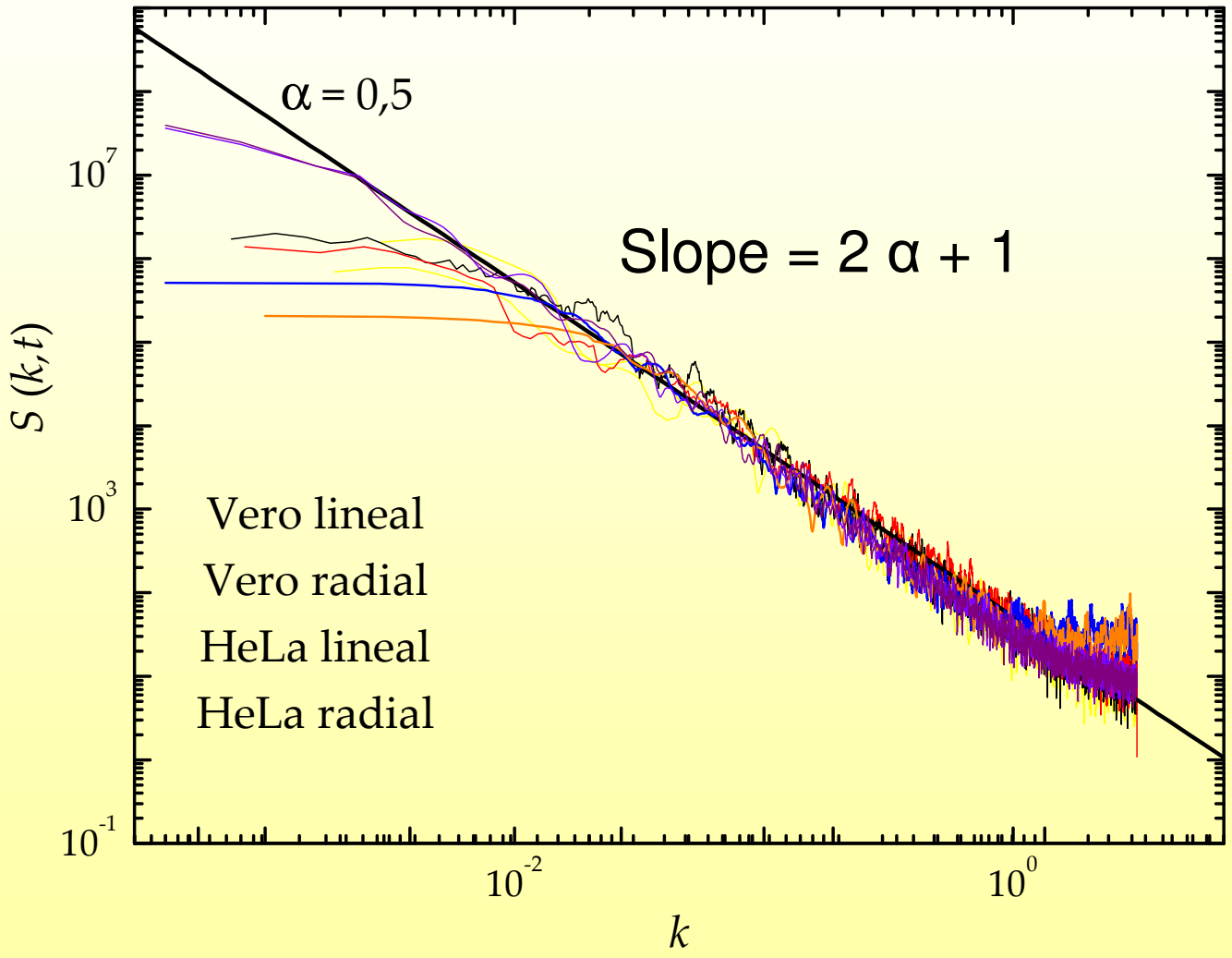
Diapositiva 15

A8 Admin, 21/08/2014

Roughness exponent α.



Experimental structure factor: global roughness exponent α



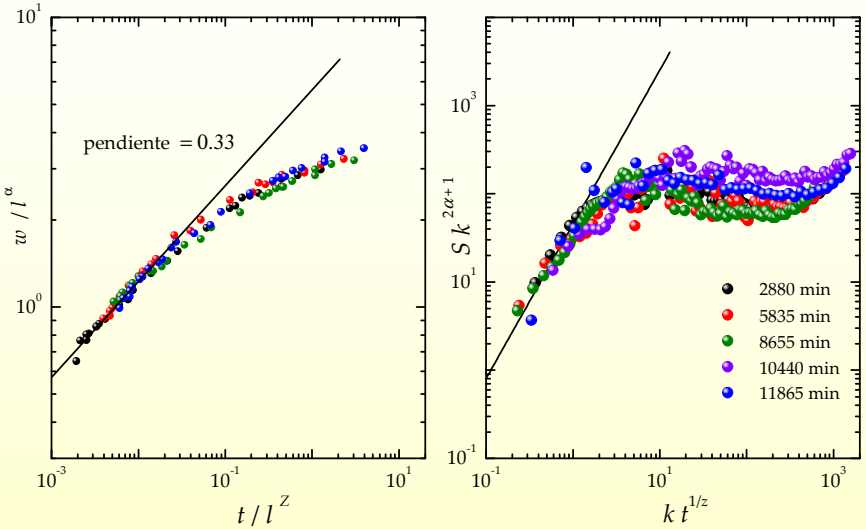
Diapositiva 17

A9 x

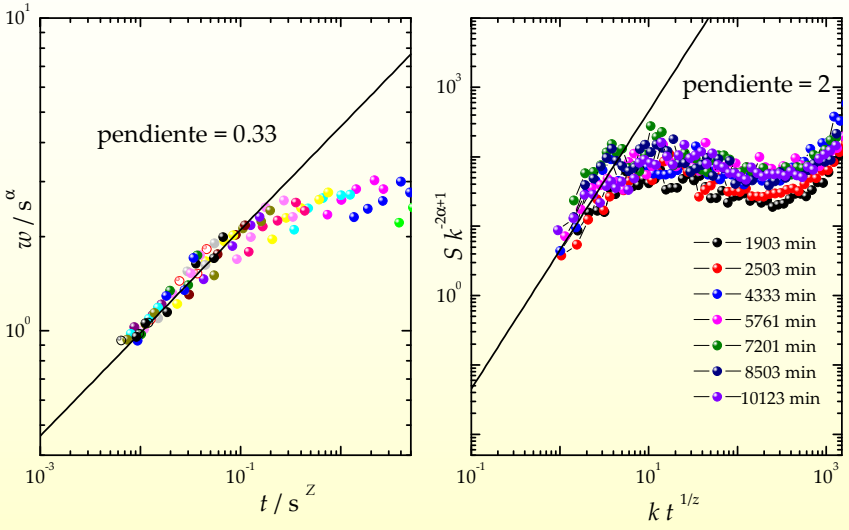
Admin, 21/08/2014

Family-Vicsek and structure factor colapses

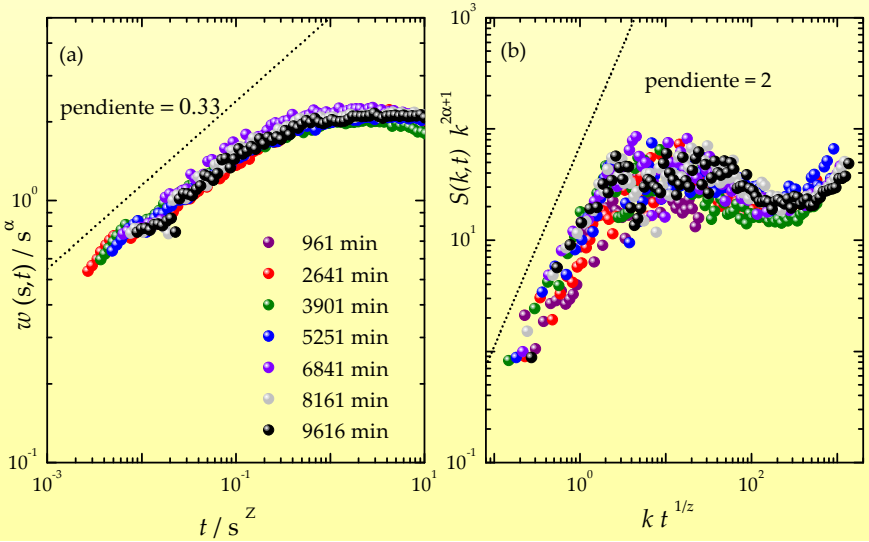
Linear Vero fronts



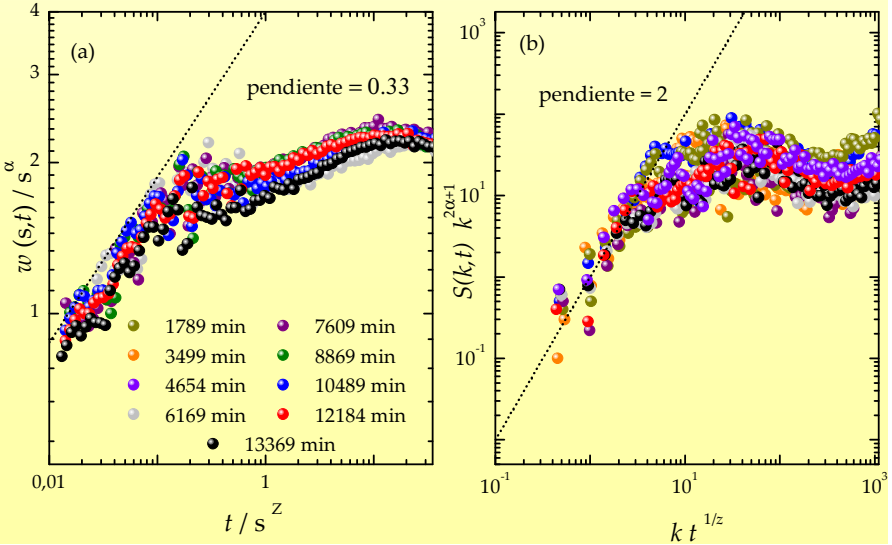
Radial Vero fronts



Linear Hela fronts



Radial HeLa fronts



Conclusions.

- Experimental cultures performed ***in vitro*** exhibit a constant growth velocity. In both cases, radial and linear systems, the growth velocity is the same, within error bars***.***

- At early times the interface roughness of both, radial and linear growing systems, follows a power law in time given by $w \sim t^{\beta}$.

- By measuring the structure we obtained the roughness exponent α.

Conclusions.

- The determined growth and roughness exponents are β =0.33 and α = 0.5, respectively.

- The growing interface of the cultures can then be rationalized in terms of the KPZ universality class.

ACKNOWLEDGMENTS.

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- The project is a collaboration between:

a) A. M. Huergo, A. J. Arvia, M. Pacuale, and A. Bolzán. (Institute of Physical Chemistry, La Plata).

b) B. Moglia, and N. Guisoni. (Institute of Physics of Liquids and Biological Systems, La Plata).

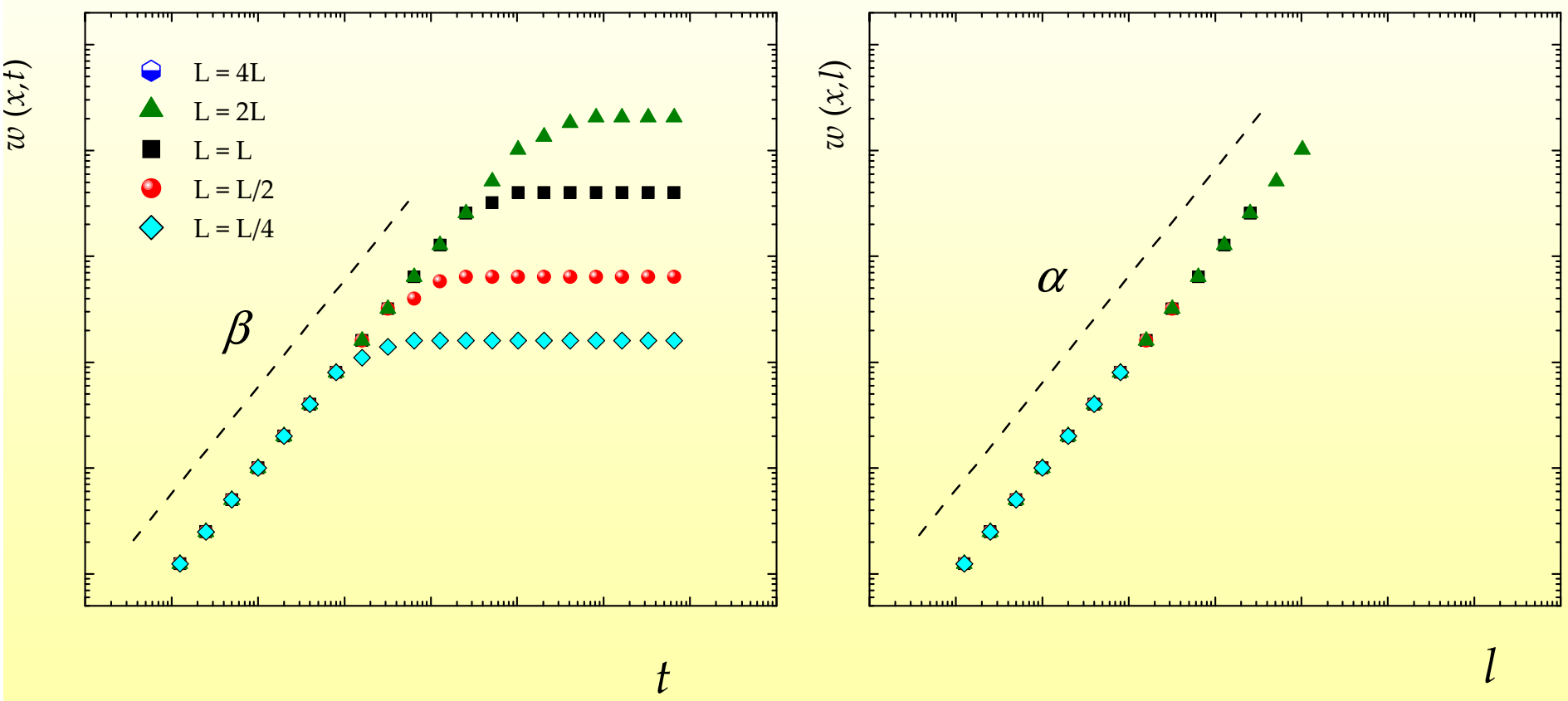
c) P. H. Gonzalez. Faculty of Medicine. La Plata.

Thank you for your attention!!

KPZ equation

$$\frac{\partial h(x,t)}{\partial t} = \nu \nabla^2 h + \frac{\lambda}{2}(\nabla h)^2 + \eta(x,t)$$

Roughness exponent α



Roughness exponent α and structure factor