Modelling cell and tissue proliferation with applications to therapeutic optimisation in oncology

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http://www-roc.inria.fr/bang/JC/Jean_Clairambault_en.html

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A general framework to optimise cancer therapeutics: designing mathematical methods along 3 axes

1. Modelling the behaviour of growing cell populations on which drugs act: proliferating tumour *and healthy* cell populations in homogeneous tissues, including physiological control by molecular circadian clocks

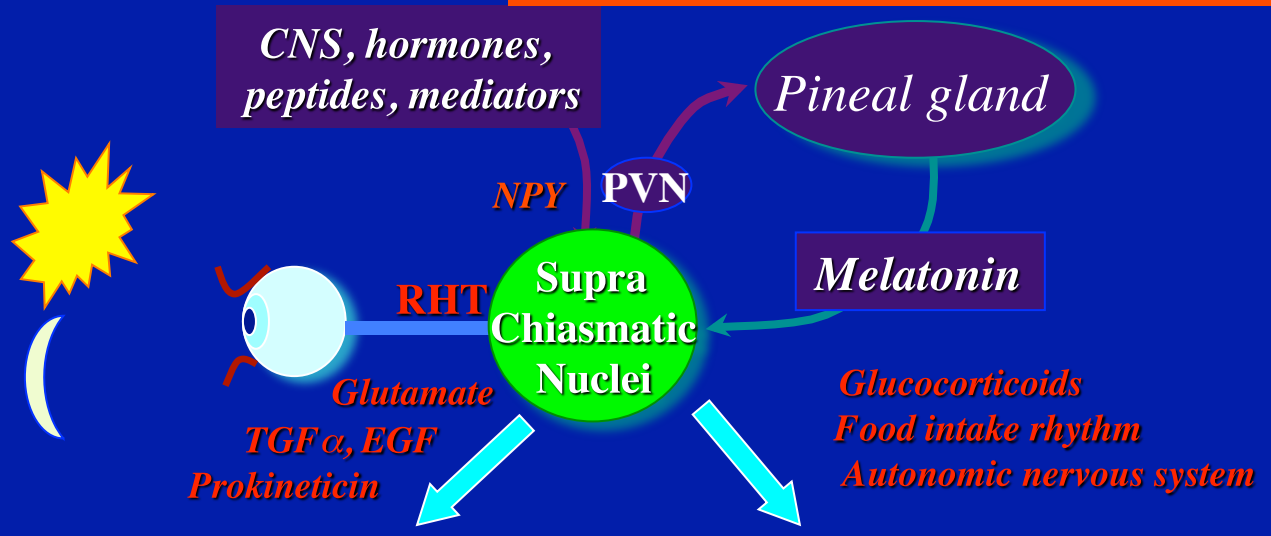
2. Modelling the control system, i.e., fate of drugs in the organism, at the molecular and whole body levels by *molecular pharmacokinetics-pharmacodynamics:* PK-PD, ideally WBPBPKPD (*whole body physiologically based*...)

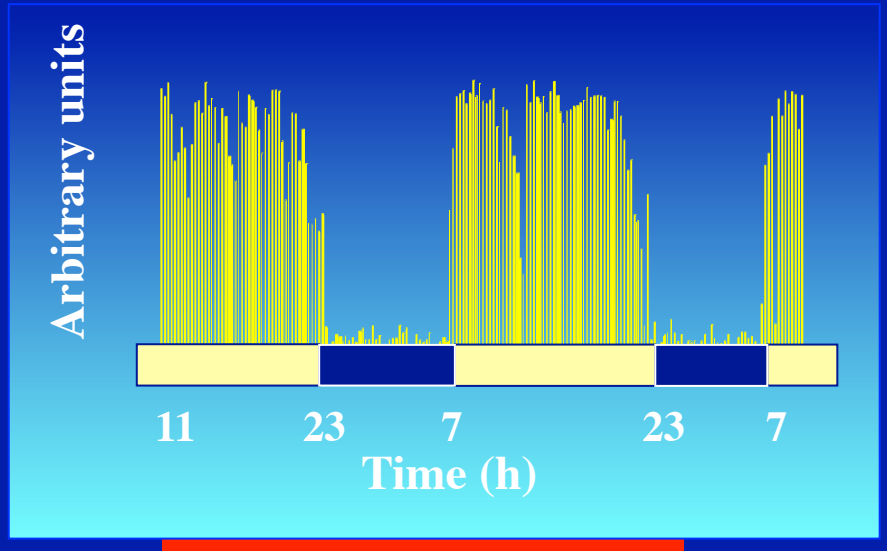
3. Optimising the control: *dynamically* optimised control of drug delivery flows using time-dependent objectives+constraints

(JC Math Mod Nat Phenom 2009; La Recherche 2010; Pers Medicine 2011)

Circadian chronobiology (1): the circadian system

Central coordination





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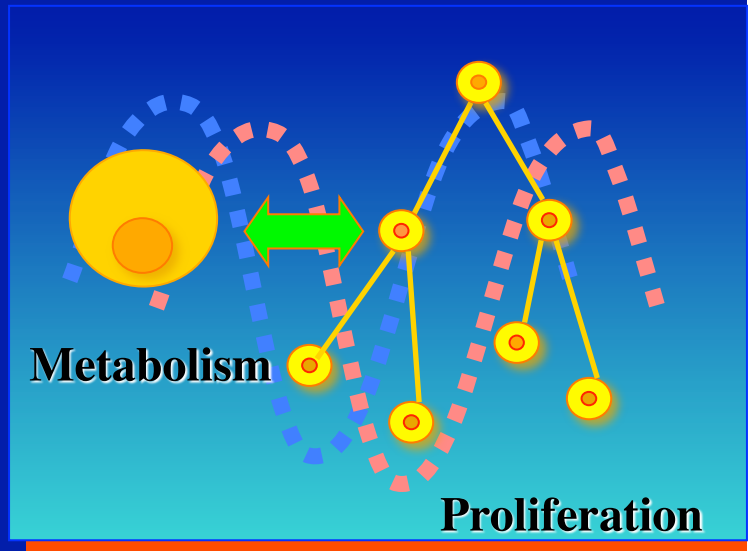


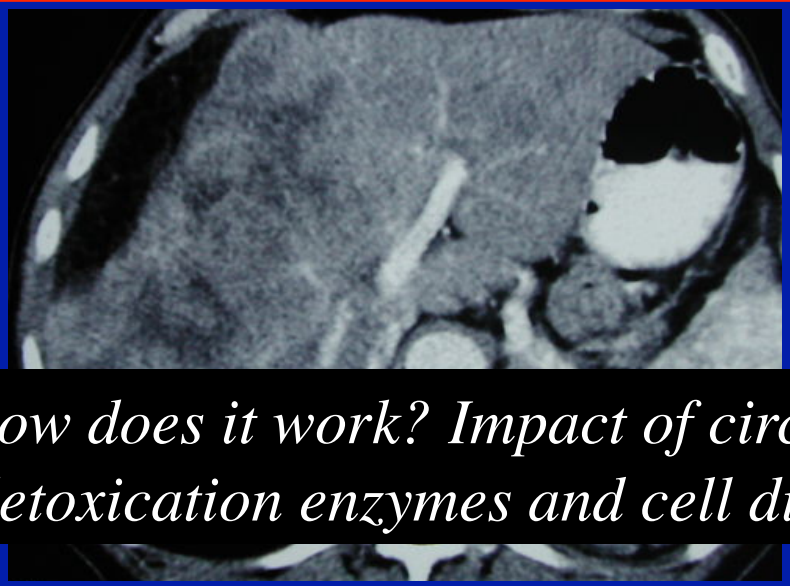
Rest-activity cycle

Peripheral oscillators

Lévi, Lancet Oncol 2001 ; Mormont & Lévi, Cancer 2003

Circadian chronobiology (2): cancer chronotherapy

Metastatic colorectal cancer (Folinic Acid, 5-FU, Oxaliplatin)	Infusion flow		
	Constant	Chrono	
Toxicity			p
Oral mucositis gr 3-4	**74%**	**14%**	$<10^{-4}$
Neuropathy gr 2-3	**31%**	**16%**	$<10^{-2}$
Responding rate	**30%**	**51%**	$<10^{-3}$

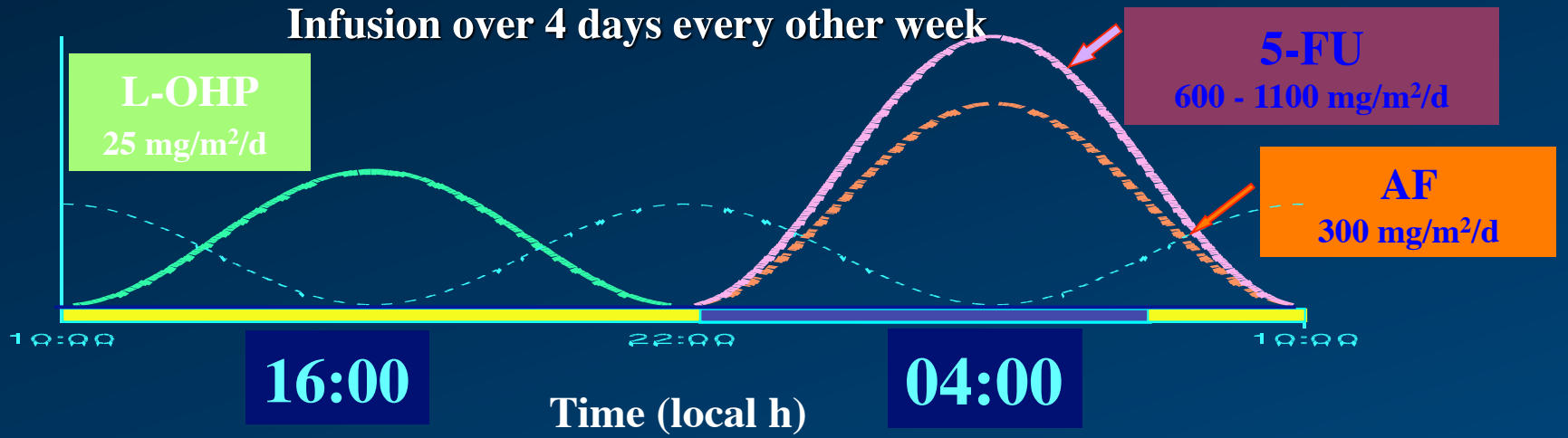


Lévi et al. JNCI 1994 ; Lancet 1997 ; Lancet Onc 2001

How does it work? Impact of circadian clocks on both cell drug detoxication enzymes and cell division cycle determinant proteins

Circadian chronobiology (3): chronotherapy technology

Time-scheduled delivery regimen



Multichannel programmable ambulatory injector for intravenous drug infusion (pompe Mélodie, Aguettant, Lyon, France)

Can such therapeutic schedules be improved?

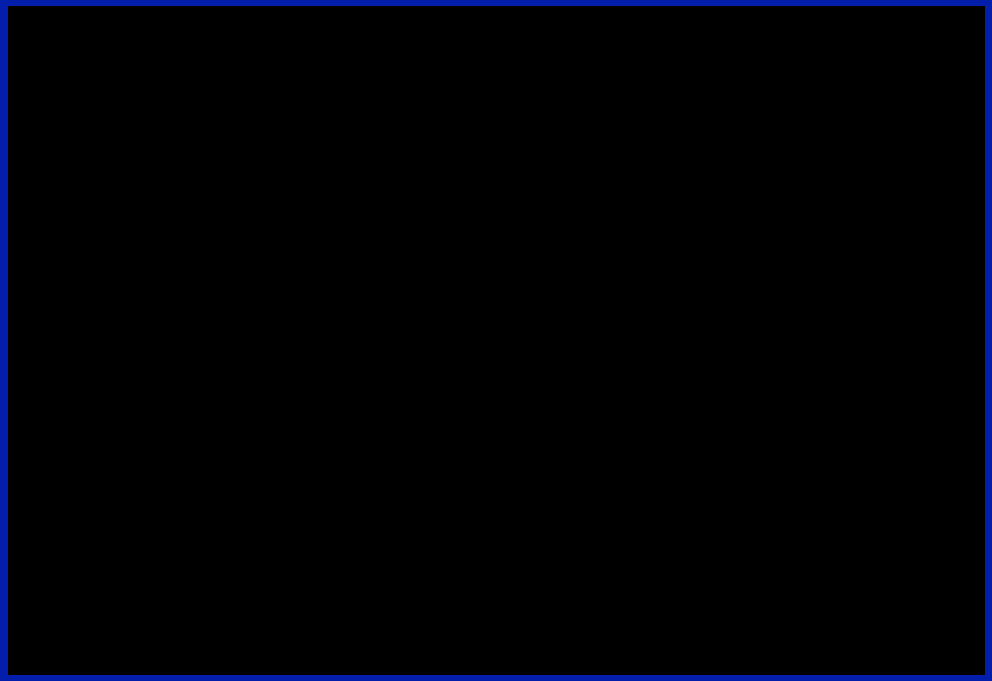
Circadian chronobiology (4): Chronotherapy today in the clinic

Multichannel pump
for chronotherapy

- Centralised programmation
- Any modulation of delivery rate
- 4 reservoirs (100-2000 mL)
- 2 independent channels
- Rates from 1 to 3000 mL/h

Images from the Chronotherapy Unit, Paul-Brousse Hospital, Villejuif, France

Over 2000 cancer patients registered in clinical Phase I, II or III trials



(from Lodish et al., Molecular cell biology, Nov. 2003)

One cell divides in two: a physiologically controlled process at cell and tissue levels in all healthy and fast renewing tissues (gut, bone marrow…) that is *disrupted in cancer*

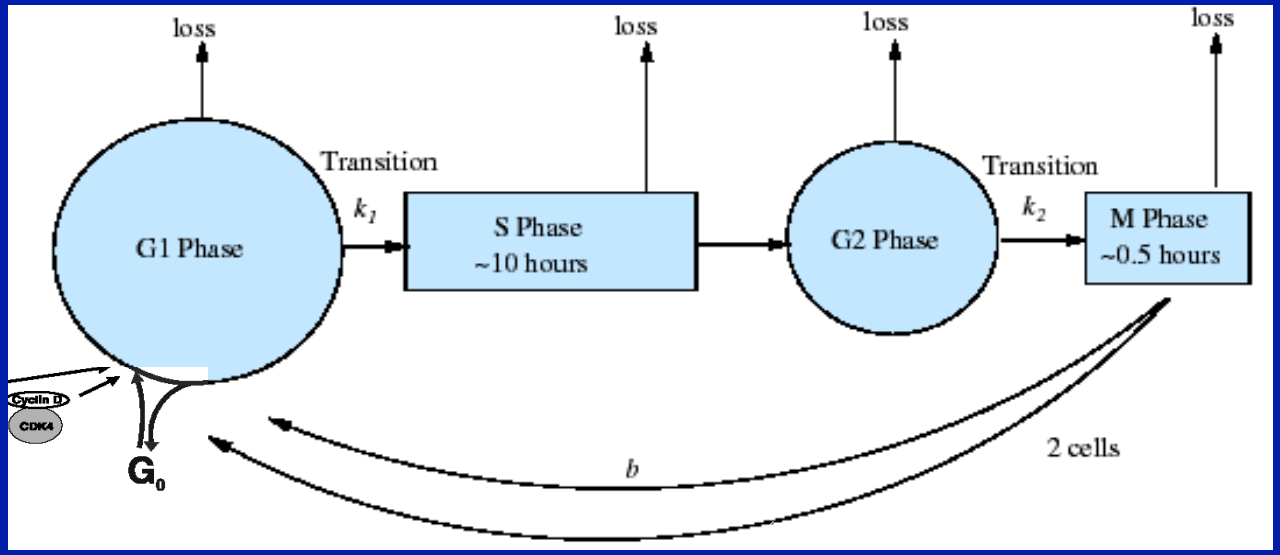
Why model the cell division cycle?

- Need for detailed models of cell proliferation to represent the action of anticancer drugs *in cell populations* with:
  1) Cell cycle phase specificity
  2) Different pharmacological targets on cell cycle control
  3) Action with same targets on tumour cells *and on healthy cells*
     (taking into account *toxic side effects* of anticancer drugs)

- To this aim, even independently of therapeutics, need for models with:
  1) Phase and age-in-phase, possibly cyclin, structure
  2) Transitions between cell division cycle phases (G_1/S, G_2/M)
  3) Exchanges between quiescent and proliferative phases (G_0/G_1)
  4) Targets for control of cell proliferation (physiological / by drugs)

Frame: Age-structured PDE model for the cell division cycle

(here only linear models will be considered, but nonlinear models with feedback are possible)



In each phase i, a McKendrick linear model:

$$\frac{\partial}{\partial t} n_i(t,a) + \frac{\partial}{\partial a}[v_i(a) n_i(t,a)] + d_i(t,a) n_i(t,a) + K_{i\to i+1}(t,a) n_i(t,a) = 0$$

$$v_i(0) n_i(t, a=0) = \int_{\alpha \geq 0} K_{i-1\to i}(t,\alpha)\, n_{i-1}(t,\alpha)\, d\alpha$$

$$K_{i\to i+1}(t,a) = \psi(t) \mathbf{1}_{a \geq a_i}(a)$$

n_i:=cell population density in phase i;
v_i:=progression speed;
d_i:=death rate;

$K_{i-1->i}$:=transition rate (with a factor 2for $i=1$)

d_i, $K_{i->i+1}$ constant or periodic w. r. to time t ($1 \leq i \leq I$, $I+1=1$)

Death rates d_i: ("loss"), "speeds" v_i and phase transitions $K_{i->i+1}$ are model targets for physiological (e.g., circadian) or therapeutic (drug) control $\psi(t)$
[$\psi(t)$: e.g., clock-controlled CDK1 or intracellular output of drug infusion flow]

(Presented in: JC, B. Laroche, S. Mischler, B. Perthame, RR INRIA #4892, 2003; recently: JC, S. Gaubert, T. Lepoutre MMNP, MCM 2009, 2011)

The simplest case: 1-phase model with division

$$\frac{\partial}{\partial t}n(t,a)+\frac{\partial}{\partial a}[n(t,a)]+[d(t)+K(t,a)]\,n(t,a)=0$$

$$n(t,a=0)=2\int_{\alpha\geq 0}K(t,\alpha)\,n(t,\alpha)\,d\alpha$$

$$\text{where } K(t,a)=K_0\psi(t)\mathbb{1}_{[a^*,+\infty[}(a)$$

$$\text{and } \psi(t)=\mathbb{1}_{[0,\tau[}(t),\ 1\text{-periodic}$$

(Here, $v(a)=1$, a^* is the cell cycle duration, and $\tau(<1)$ is the time during which the 1-*periodic control* ψ is actually exerted on cell division)

Then it can be shown that the eigenvalue problem:

$$n(t,a)=e^{\lambda t}N(t,a)$$

$$\int_{\alpha\geq 0}N(t,a)da=1$$

$$\frac{\partial}{\partial t}N(t,a)+\frac{\partial}{\partial a}[N(t,a)]+[\lambda+d(t)+K(t,a)]\,N(t,a)=0$$

$$N(t,a=0)=2\int_{\alpha\geq 0}K(t,\alpha)\,N(t,\alpha)\,d\alpha$$

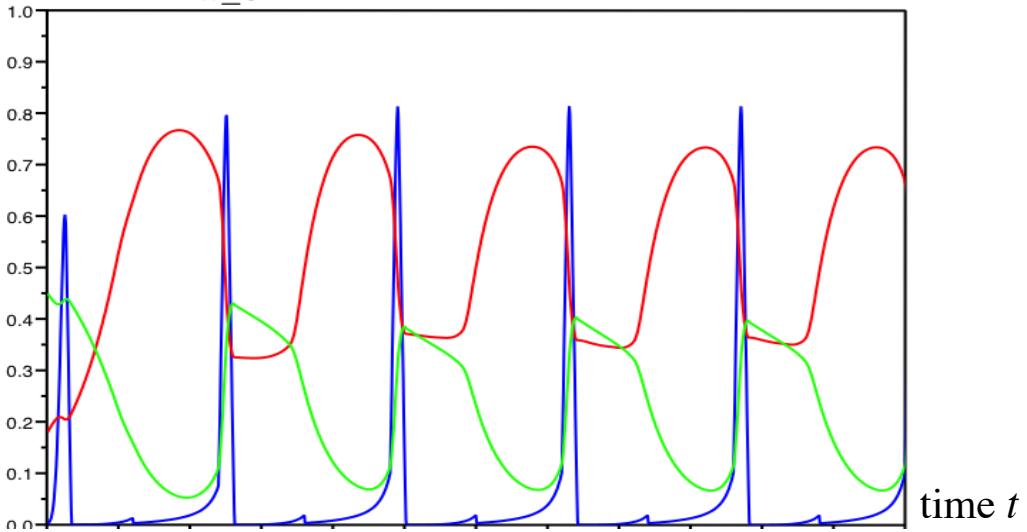
has a unique positive 1-*periodic* eigenvector N, with a positive eigenvalue λ, solution, if $d(t)=d$, $K(t,a)=K(a)$ of Lotka's (=Euler's) equation: $\frac{1}{2}=\int_0^{+\infty}f(x)e^{-\lambda x}dx$, where $f(x)=K(x)e^{-\int_0^x K(y)dy}$ is a p.d.f. if $\int_0^{+\infty}K(x)dx=+\infty$

λ: a growth exponent governing the cell population behaviour

Proof of the existence of a unique growth exponent λ, the same for all phases i, such that the $\widetilde{N}_i(t,a) = e^{-\lambda t} n_i(t,a)$ are bounded, and asymptotically periodic if the control is periodic

Example of control (periodic control case): 2 phases, control on G_2/M transition by 24-h-periodic CDK1-Cyclin B (from A. Goldbeter's minimal mitotic oscillator model)

$$\mathcal{N}_i^{tot}(t) = e^{-\lambda t} \int_{\alpha \geq 0} n_i(t,\alpha) d\alpha, \quad i = 1,2$$



ψ=CDK1 All cells in G1-S-G2 (phase *i*=1) All cells in M (phase *i*=2)

Entrainment of the cell division cycle by ψ= CDK1 at the circadian period

"Surfing on the exponential growth curve"

(= the same as adding an artificial death term +λ to the d_i)

Experimental measurements to identify transition kernels K_{i_i+1}

(and simultaneously experimental evaluation of the first eigenvalue λ)

In the simplest model with d=0 (one phase with division) and assuming $K=K(x)$ (instead of indicator functions $\mathbb{1}_{[a^*,+\infty[}$, experimentally more realistic transitions):

$$\begin{cases} \frac{\partial}{\partial t}n(t,x) + \frac{\partial}{\partial x}n(t,x) + K(x)n(t,x) = 0, \\ n(t,0) = 2\int_0^\infty K(x)n(t,x)dx. \end{cases}$$

Whence (by integration along characteristic lines):

$$n(t+x,x) = n(t,0)e^{-\int_0^x K(y)dy}$$

Interpreted as: if τ is the age in phase at division, or transition:

$$P(\tau > x) = e^{-\int_0^x K(y)dy} \quad \text{with} \quad \int_0^\infty K(x)dx = +\infty$$

With probability density (experimentally identifiable):

$$f(x) = K(x)e^{-\int_0^x K(y)dy} \quad \text{i.e.,} \quad K(x) = \frac{f(x)}{\int_x^\infty f(y)dy}$$

A mathematical result:

The growth exponent λ increases with desynchronisation

where desynchronisation is defined as a measure of phase overlapping at transition

Proliferation, as measured by the Malthus growth exponent, or first eigenvalue, increases with overlapping between cell cycle phases

i.e., the less synchronised phases are, the faster is proliferation

(NB: so far, this has not been extended to the periodic control case, i.e., phase transitions have been assumed to be uncontrolled)

This relies on the Proposition: *(Th. Ouillon's report 2010, see also Billy et al., in revision 2012)*
For a family (f_i) of pdfs with fixed first moment e_i and varying second moment σ_i, λ increases with each σ_i

Proposition 1. *Soit f_i, $1 \leq i \leq I$, une famille de fonctions de densité sur $\mathbb{R}_+$. Les taux de transition associés $K_{i \to i+1}$ sont ainsi donnés par (voir (2)) :*

$$K_{i \to i+1}(x) = \frac{f_i(x)}{\int_x^{+\infty} f_i(x')dx'} = \frac{f_i(x)}{1 - \int_0^x f_i(x')dx'}$$

En supposant $d_i = 0$ $(1 \leq i \leq I)$, la première valeur propre du système (1) $\lambda > 0$ est donnée par (voir [1]) :

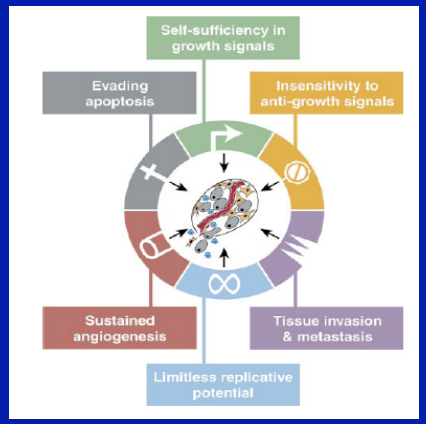
$$\frac{1}{2} = \prod_{i=1}^{I} \int_0^{+\infty} f_i(x) e^{-\lambda x} dx$$

Pour $1 \leq i \leq I$, on pose $e_i = \int_0^{+\infty} x f_i(x)dx$ et $\sigma_i^2 = \int_0^{+\infty} x^2 f_i(x)dx - e_i^2$, et on suppose que les $e_i > 0$ sont constants. Soit $j \in \{1, ..., I\}$. On suppose que les σ_i^2 $(1 \leq i \neq j \leq I)$ sont constants.

Alors λ est croissante avec σ_j^2.

A working hypothesis that could explain differences in responses to drug treatments between healthy and cancer tissues

Healthy tissues, i.e., cell populations, would be well synchronised w. r. to proliferation rhythms and w. r. to circadian clocks, whereas…
...tumour cell populations would be desynchronised w. r. to both, and such proliferation desynchronisation would be a consequence of an escape by tumour cells from central circadian clock control messages, just as they evade most physiological controls, cf. e.g., Hanahan & Weinberg:



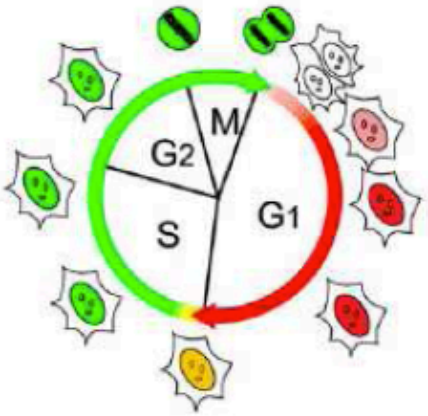
Question:
is cell cycle phase desynchronisation another hallmark of cancer ***in cell populations****?*

Experimental identification of the basic model parameters with *FUCCI reporters* on a 2-phase model G_1 / S-G_2-M
(so far, without circadian control)

Cells:
NIH 3T3 of a common population
(*mouse embryonic fibroblasts*)
without preliminary synchronization

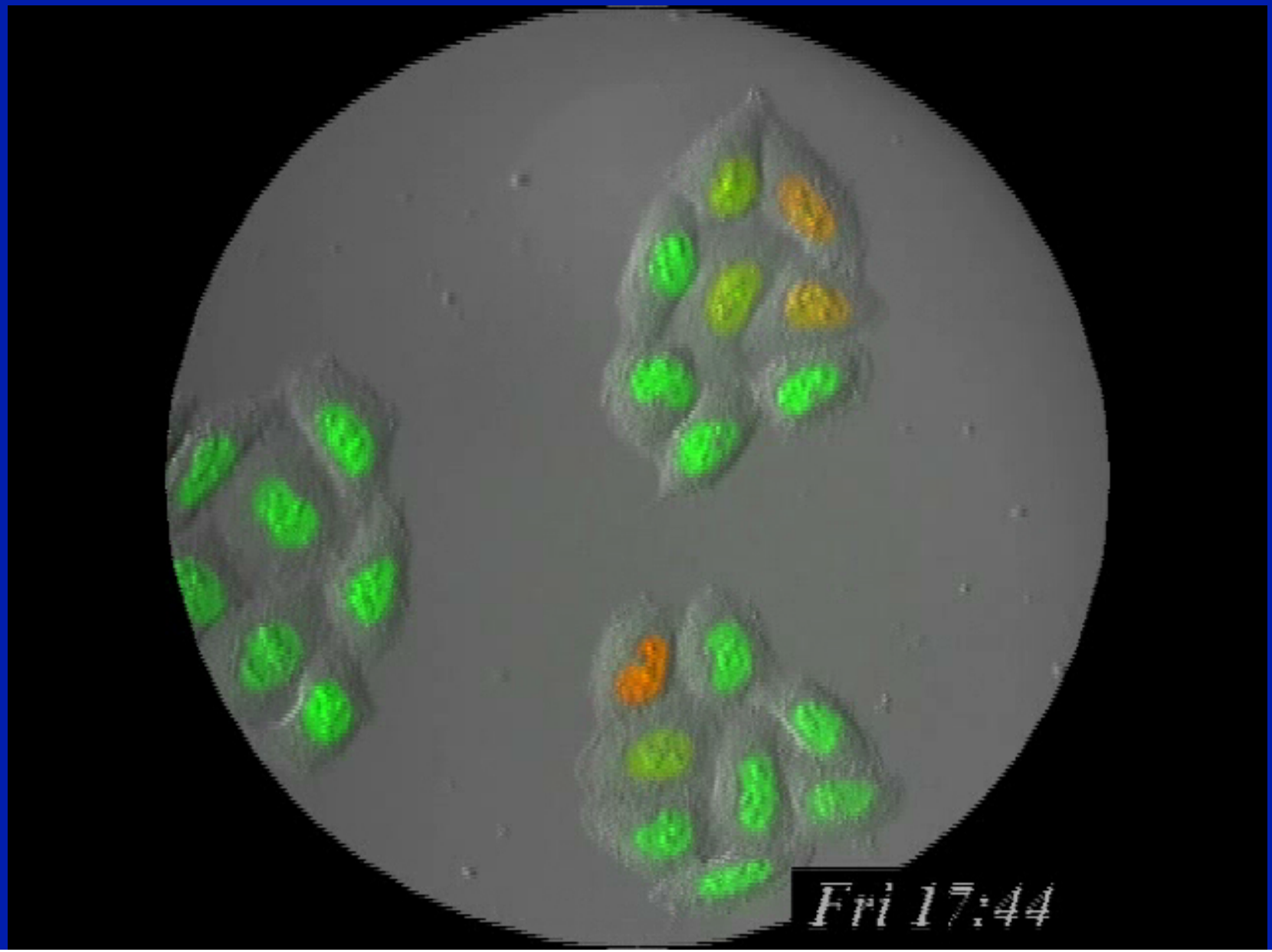
Measures: for each individual cell:
red and green fluorescence time recording

- ⇝ every 15 min
- ⇝ approx. 150 measures for each cell

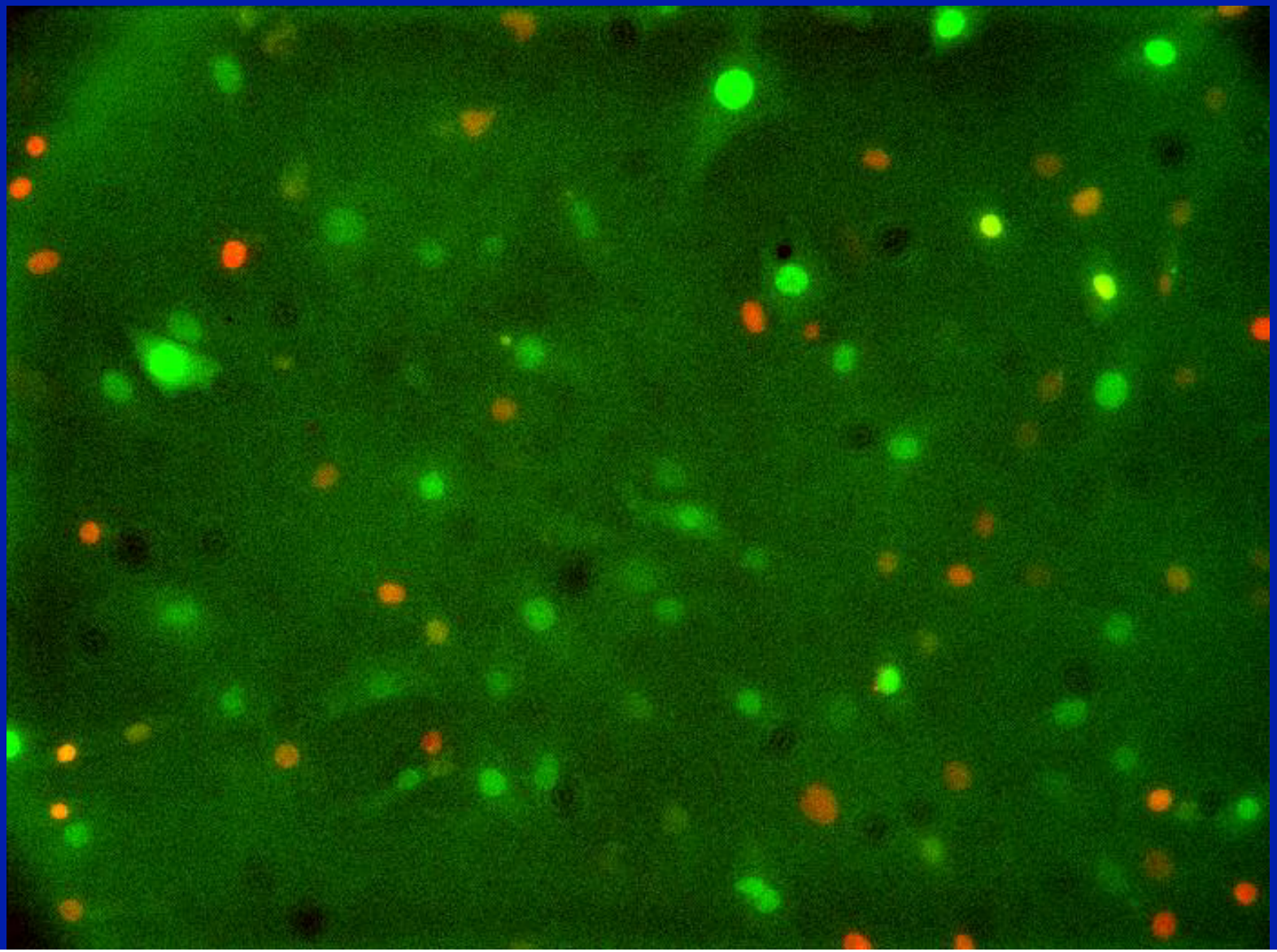


from Sakaue-Sawano et al.
Cell 2008, 132, 487–498

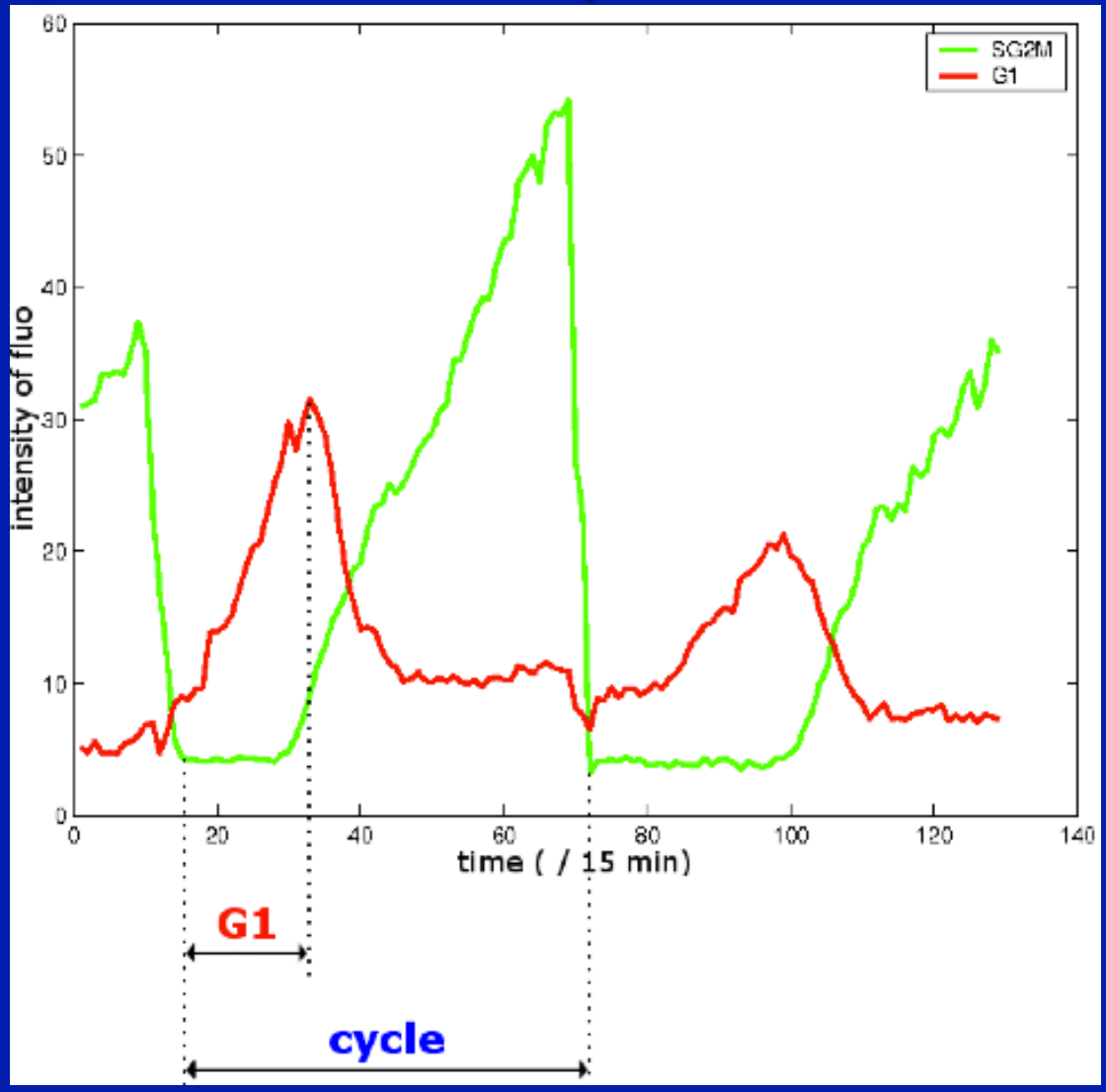
FUCCI: a movie on HeLa cells (Sakaue-Sawano 2008)



Another FUCCI movie on NIH3T3 cells (C. Feillet, F. Delaunay, IBDC Nice)



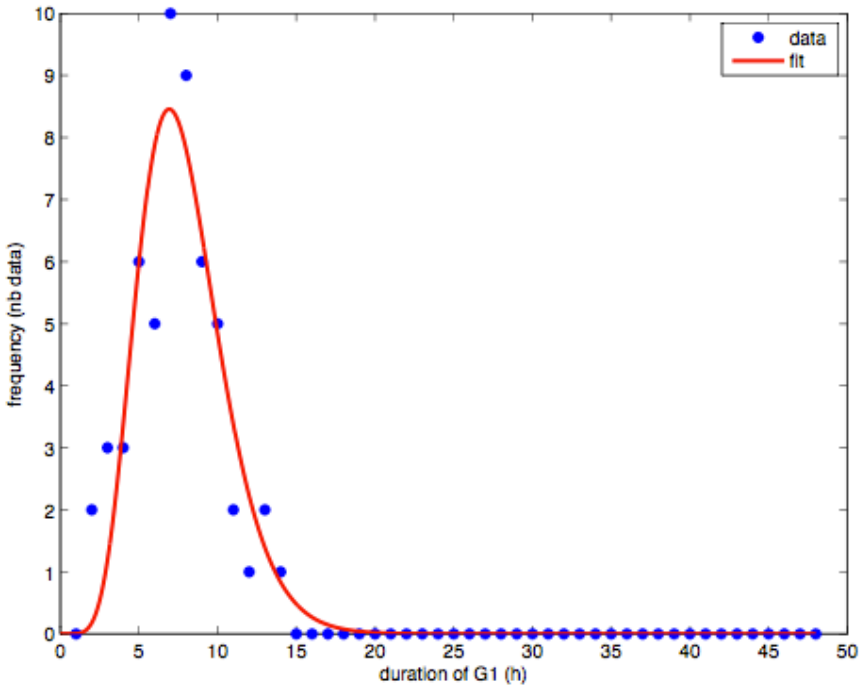
FUCCI reporters +cell tracking (non trivial…):
Measuring time intervals: G_1 and total division cycle durations



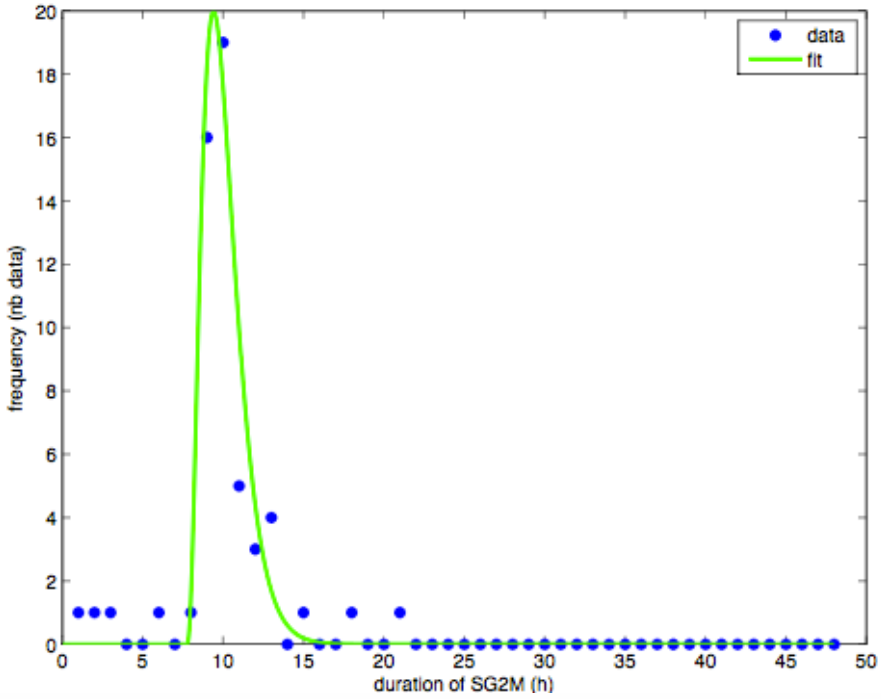
Data from Bert van der Horst's lab, Erasmus University, Rotterdam, processed by Frédérique Billy at INRIA

Phase transitions w.r.t. age x
Pdfs $f(x)$ fitted from data on 50 NIH 3T3 proliferating cells

Density of transition from G1 to SG2M



Density of transition from SG2M to G1



FUCCI data in NIH3T3 cells, that are healthy mouse fibroblasts tracked in liquid medium

Computing the growth exponent, fitting data to p.d.f.s:
Gamma p.d.f.s were best fits and yielded simple computations:

$$f_i(x) = \frac{1}{\Gamma(\alpha_i)}(x-\gamma_i)^{\alpha_i-1}\beta_i^{\alpha_i}e^{-\beta_i(x-\gamma_i)}\mathbb{1}_{[\gamma_i;+\infty[}(x) \qquad i=1,2, \quad \text{where}$$

$$\alpha_1 = 8.28,\ \beta_1 = 1.052h^{-1},\ \gamma_1 = 0h,\ \alpha_2 = 3.42,\ \beta_2 = 1.47h^{-1},\ \gamma_2 = 7.75h$$

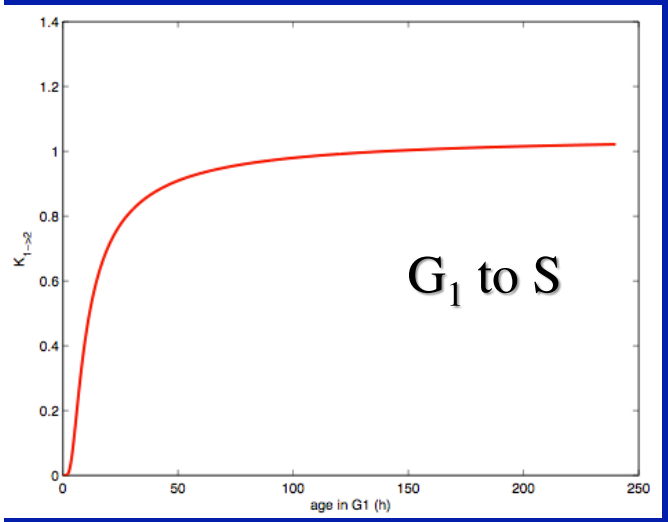
2-phase Lotka's equation simply reads:

$$\left(1+\frac{\lambda}{\beta_1}\right)^{\alpha_1}\left(1+\frac{\lambda}{\beta_2}\right)^{\alpha_2}e^{\lambda(\gamma_1+\gamma_2)} = 2$$

... which yields here $\lambda = 0.039\ h^{-1}$

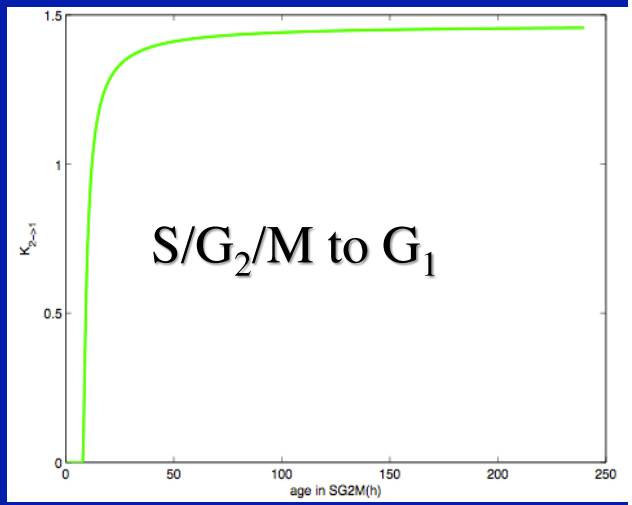
(and yields mean doubling time T_d=17.77 h, with mean cell cycle time T_c =17.95 h)

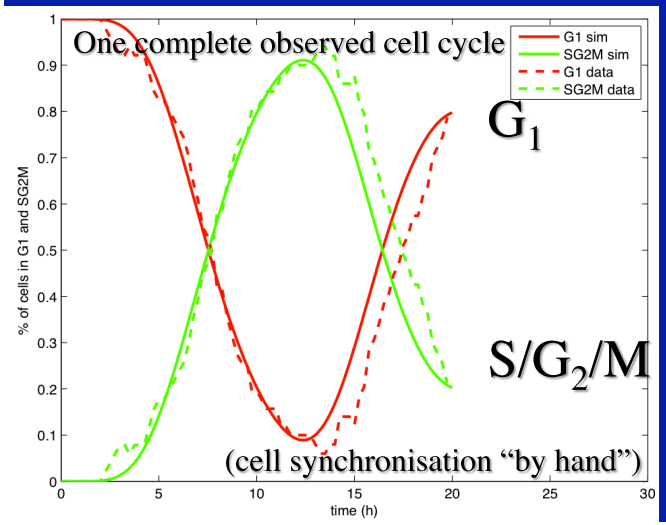
Phase transitions w.r.t. age x
Transition rates $K(x)$ from pdfs $f(x)$ on NIH 3T3 healthy cells and resulting population evolution

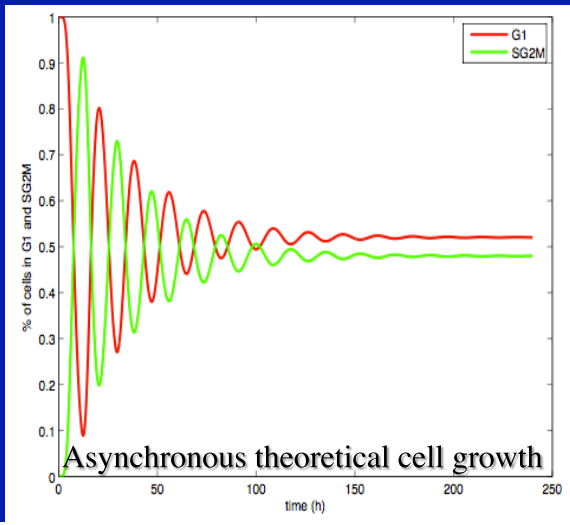


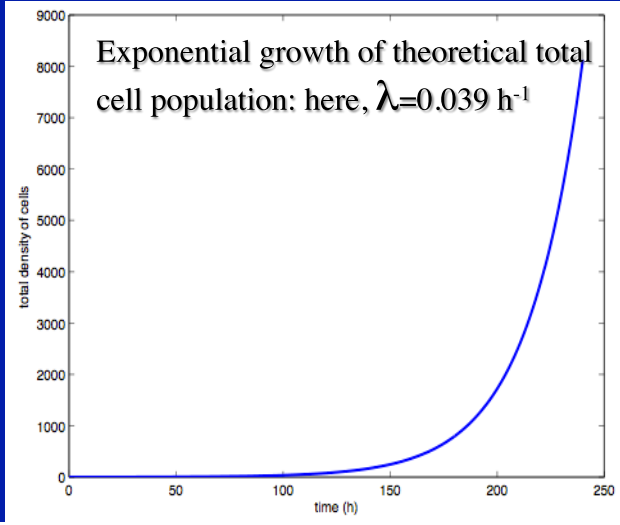
Recalling that in the model
f = p.d.f. of phase duration time
and K = phase transition kernel:

$$K_{i\to i+1}(x) = \frac{f_i(x)}{1 - \int_0^x f_i(\xi)d\xi}$$



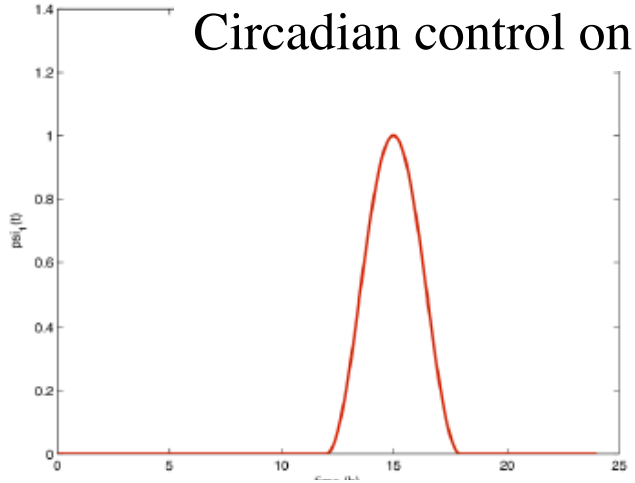


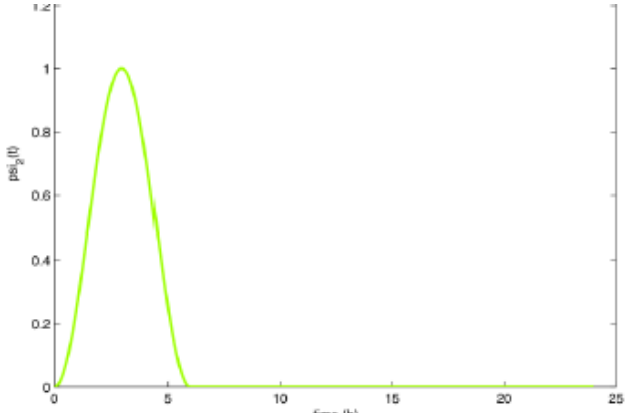




Adding circadian control on phase transitions

Circadian control on transitions: two cosines for ψ_1 and ψ_2

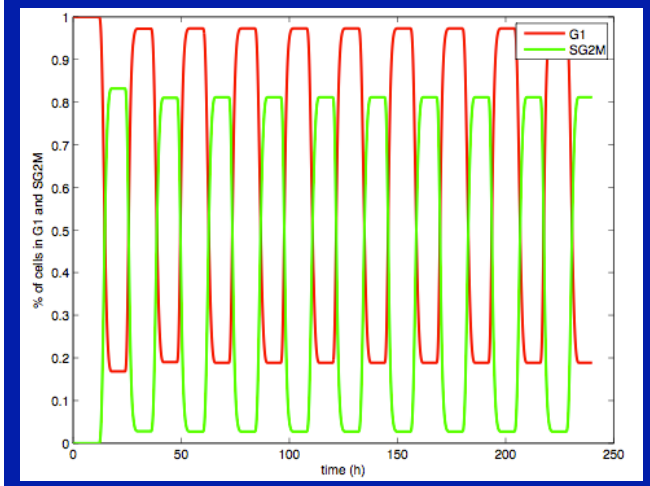




$$\psi_1(t) = \cos^2(2\pi(t-3)/12)\mathbb{1}_{[12;18]}(t)+\varepsilon, \quad \psi_2(t) = \cos^2(2\pi(t-3)/12)\mathbb{1}_{[0;6]}(t)+\varepsilon$$

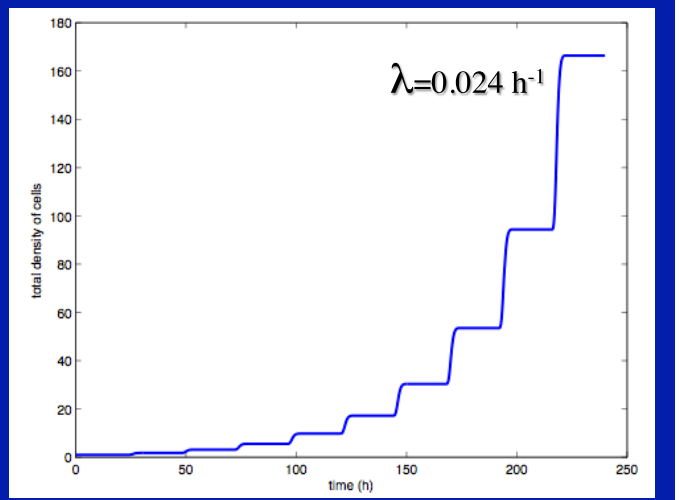
(a 12 h-delay between the two cosines was determined as the one that maximised the λ)

Resulting evolution of the clock-controlled cell population: λ=0.024 h^{-1} (<0.0039 h^{-1})

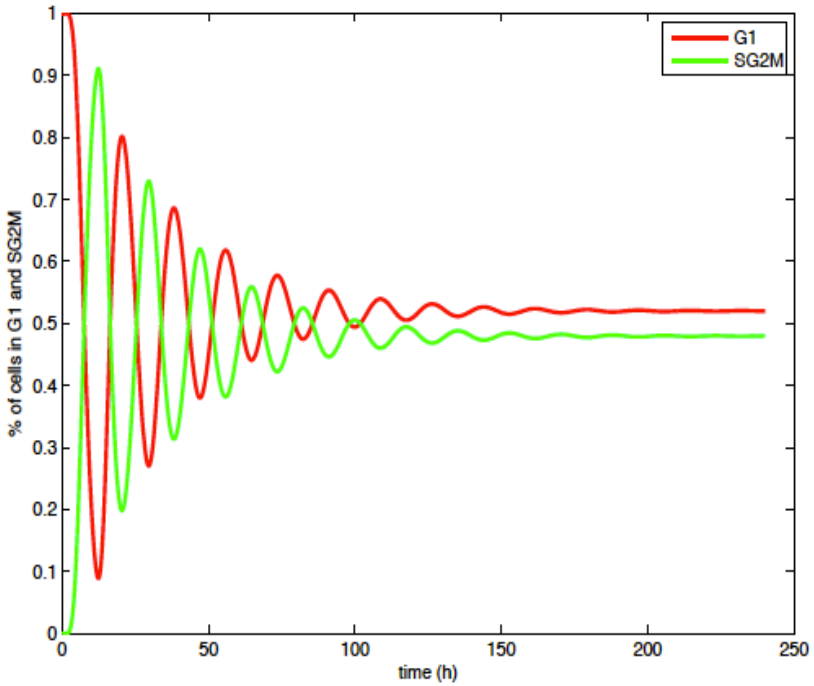


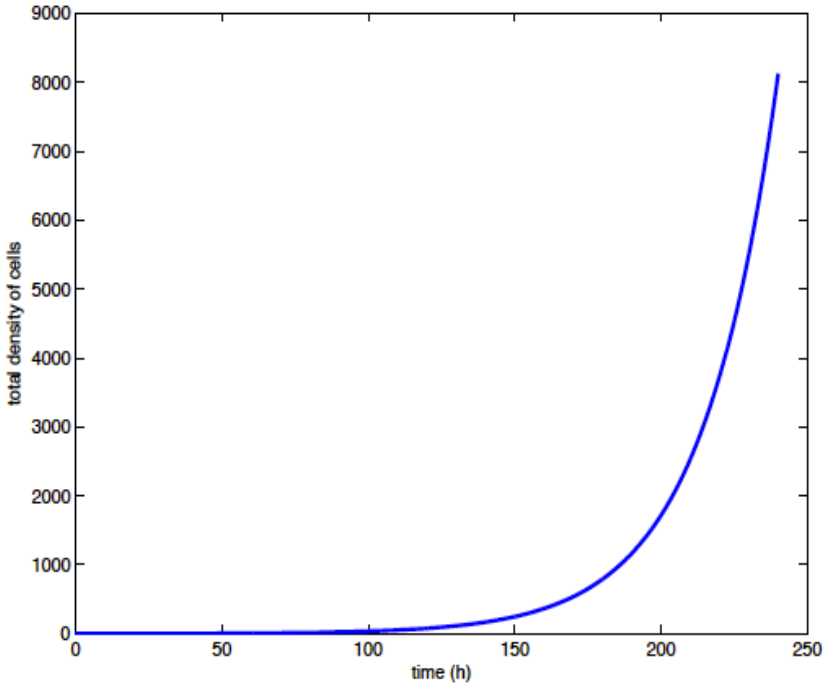
Here we put
$K(x,t) = \kappa(x).\psi(t)$
with κ = FUCCI-identified
and ψ = a cosine

[cosine: in the absence of a better identified clock thus far]



Without time control

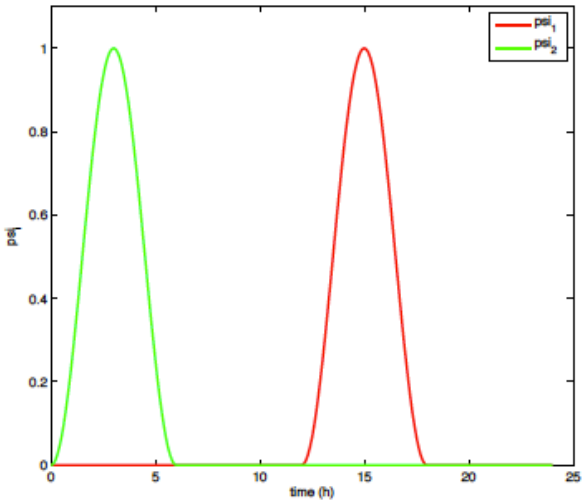


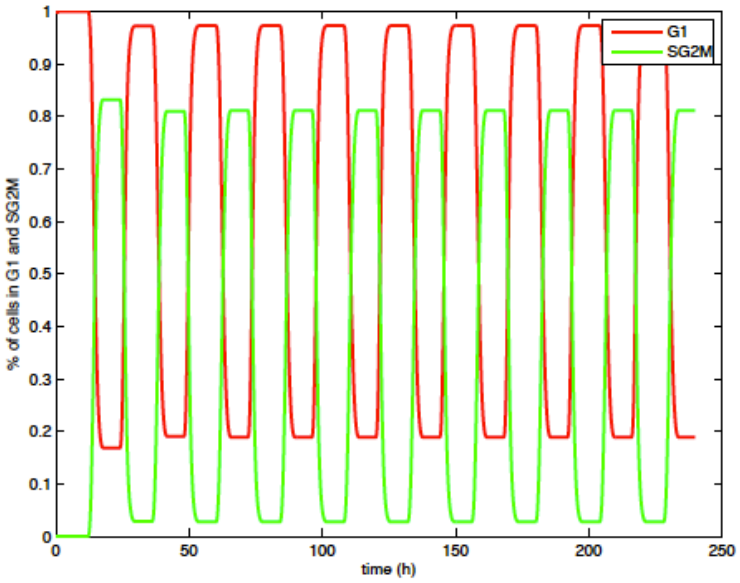


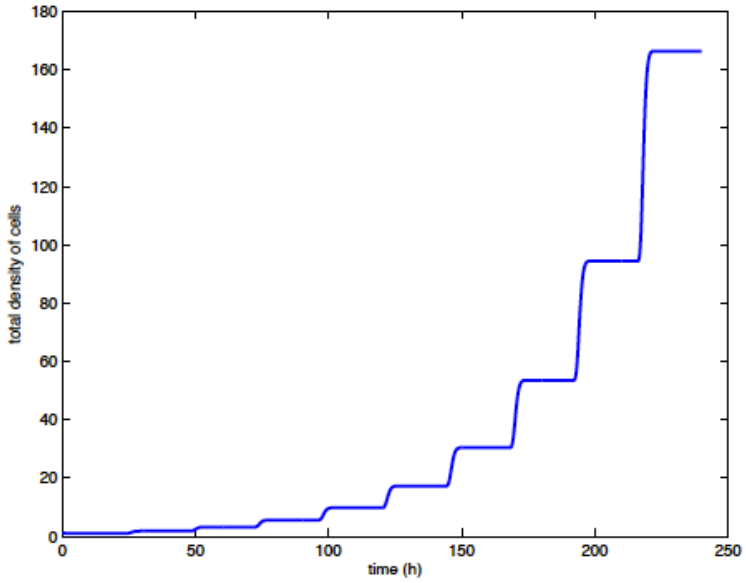
$\lambda = 0.039h^{-1}$ $T_d = 18h$

With time control (1)

$$K_{i\to i+1}(a,t) = \underbrace{\kappa_{i\to i+1}(a)}_{\text{from exp. data}} \times \underbrace{\psi_i(t)}_{\text{circ. clock}} \longrightarrow$$



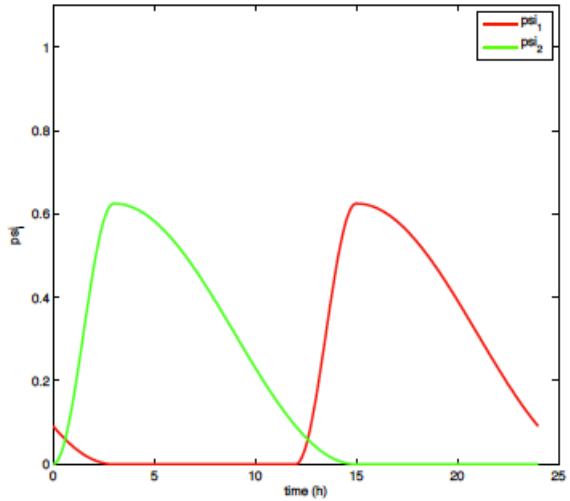


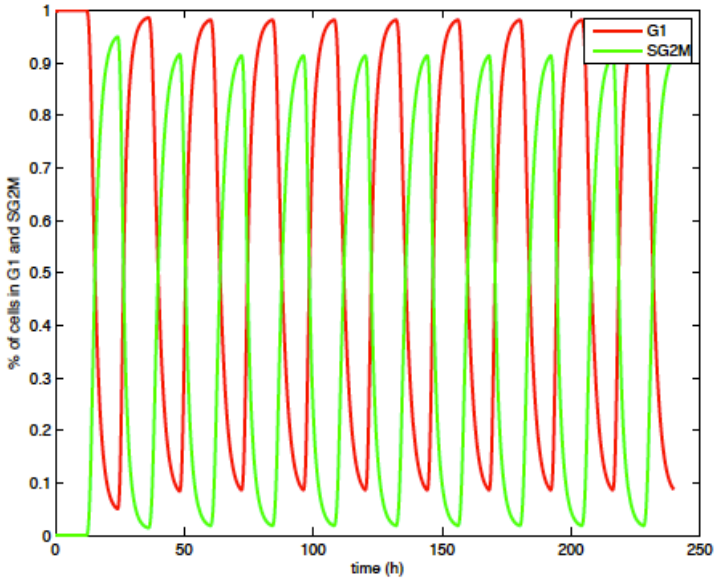


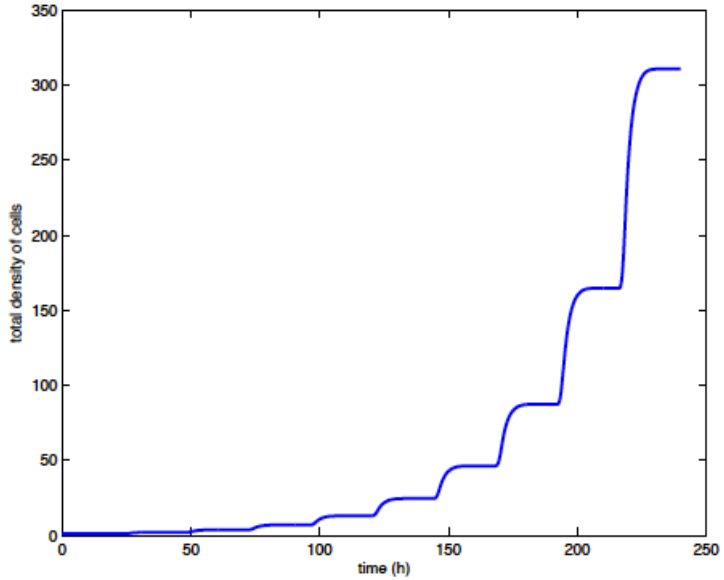
$\lambda = 0.024h^{-1}$ $\quad T_d = 29.4h$

With time control (2)

$$K_{i\to i+1}(a,t) = \underbrace{\kappa_{i\to i+1}(a)}_{\text{from exp. data}} \times \underbrace{\psi_i(t)}_{\text{circ. clock}} \longrightarrow$$

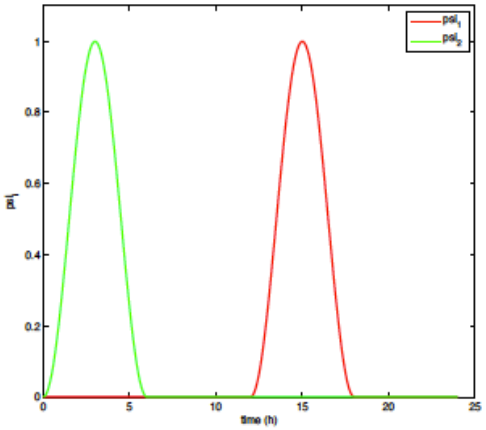


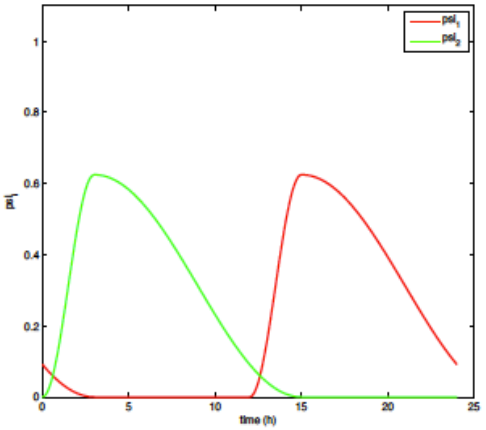


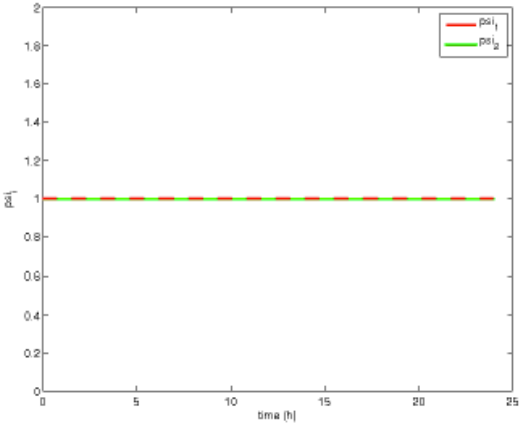


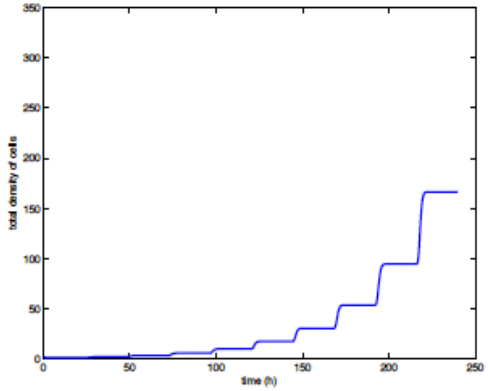
$\lambda = 0.026h^{-1}$ $\quad T_d = 26.3h$

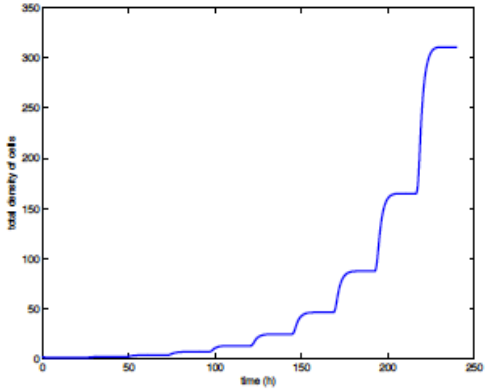
Summary

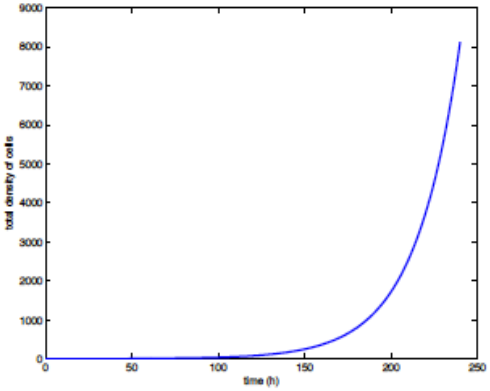












$\lambda = 0.024h^{-1}$
$T_d = 29.4h$

$\lambda = 0.026h^{-1}$
$T_d = 26.3h$

$\lambda = 0.039h^{-1}$
$T_d = 18h$

Theoretical chronotherapeutic optimisation of a 1st eigenvalue (cancer growth) under the constraint of preserving another 1st eigenvalue (healthy tissue growth)

(i.e., what if now we add a drug control, setting $K(x,t) = \kappa(x).\psi(t).[1-g(t)]$?)

- McKendrick's model of cell population proliferation
- Control of proliferation by blocking K_{i_i+1} using theoretic periodic drug delivery:

$K(t,x)=[1-g(t)].\psi(t).\kappa(x)$ *where:* $g(t)$ *is a periodic external control (chronotherapy)*

$\psi(t)$ *is a circadian clock control on the cell cycle*

$\kappa(x)$ *is an [only] age-dependent transition rate*

- Objective function to be minimised: λ_1, 1st eigenvalue of cancer cell population
- Constraint function to be preserved: λ_2 $[\geq\Lambda]$, 1st eigenvalue of healthy cell population
- Design of an augmented Lagrangian by combining λ_1 and $\lambda_2-\Lambda$ (with penalty)
- Arrow-Hurwitz (or Uzawa) algorithm to track saddle points of the Lagrangian
- …thus obtaining only suboptimality (necessary to obtain critical points) conditions

Circadian + pharmacological control on transitions

$K(x,t) = \kappa(x).\psi(t).[1-g(t)]$: κ FUCCI-identified, ψ clock, g optimal drug effect

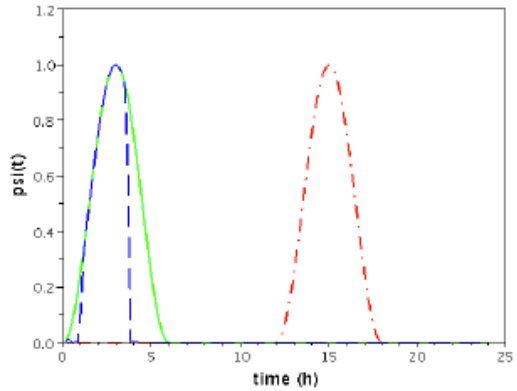


Figure 9: Modelled circadian control for transition G_1 to $S/G_2/M$ (dashdotted line) and transition $S/G_2/M$ to G_1. The natural control for $S/G_2/M$ to G_1 transition is in solid line, the drug induced control is in dashed line.

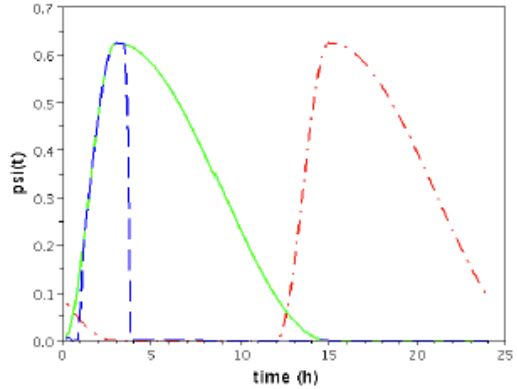


Figure 10: Modelled answer of cancerous cells to circadian control for transition G_1 to $S/G_2/M$ (dash-dotted line) and transition $S/G_2/M$ to G_1. The answer to natural control for $S/G_2/M$ to G_1 transition is in solid line, the drug-induced control is in dashed line.

green and red: ψ

blue: $[1-g].\psi$
(g blocks ψ)

Evolution of the two populations: cancer (blue), healthy (green)

Circadian control, no drug infusion

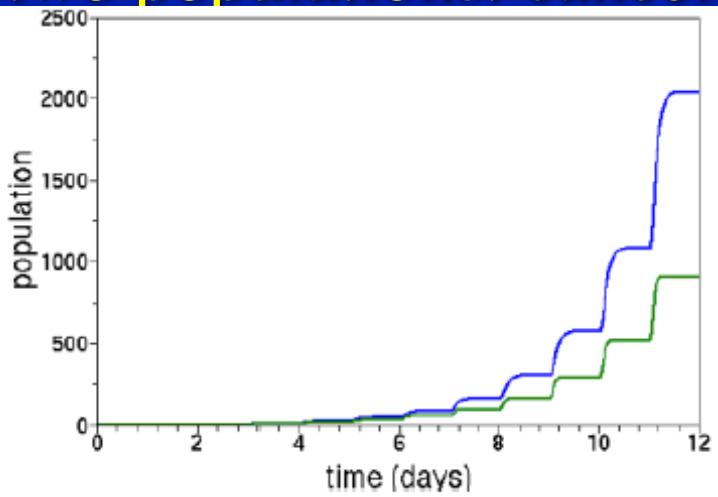


Figure 11: Evolution of the population of cancer (blue, beneath) and healthy (green, above) cells without drug infusion during 12 days. We can see that the populations have different exponential growth rates ($\lambda_{cancer} = 0.026$ and $\lambda_{healthy} = 0.024$). In the beginning, there were as many cancer cells as healthy cells, in the end they represent a much larger part of the total population.

Circadian control, added drug infusion

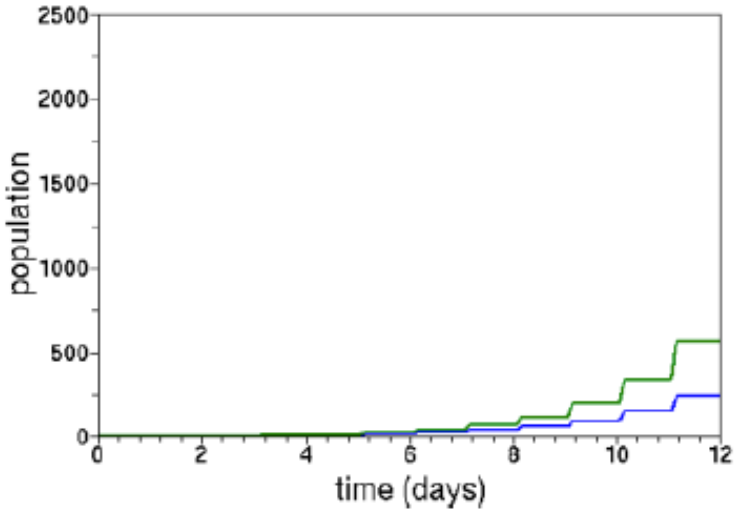


Figure 12: Evolution of the population of cancer (blue, beneath) and healthy (green, above) cells with the drug infusion, starting at time 0, given by the algorithm. Healthy cells keep multiplying ($\lambda_{healthy} = 0.022$) while the cancer cell population is weakened ($\lambda_{cancer} = 0.019$).

(F. Billy et al. 2011, submitted)

Numerical solution to the optimal infusion problem (Uzawa) and effect on eigenvalues, healthy and cancer

Infusion scheme $g(t)$

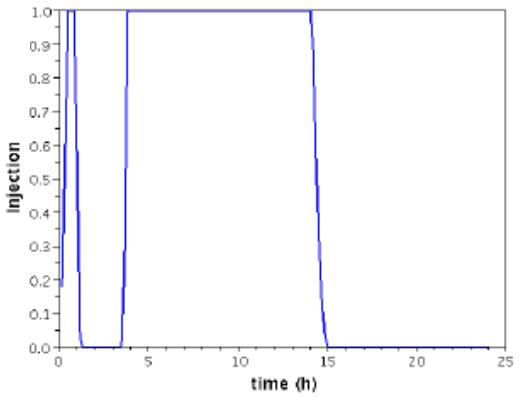


Figure 11: Locally optimal drug injection strategy found by the optimisation algorithm.

Target eigenvalues:
Cancer (blue)
Healthy (green)

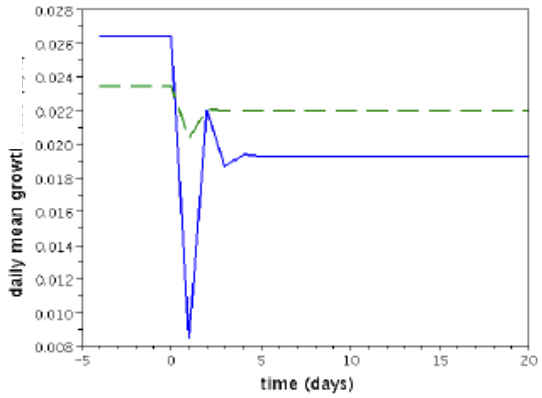


Figure 12: Daily mean growth rates for cancerous (solid line) and healthy cells (dashed line) when starting drug injections at time 0. After a 10 day transitional phase, the biological system stabilises towards the expected asymptotic growth rate

In favour of this approach:
- characterises long-term trends with one number,
- easily accessible target for control
- fits to physiologically structured growth models

Its drawbacks:
- deals with asymptotics, not with transients
- assumes a linear model for proliferation
- assumes periodic control by drugs (but the period can be infinitely long)

What remains to be done to complete the design of this model:

- Identify actual doubling times and compare them with calculated $T_d = \ln 2 / \lambda$

- Replace cosines by identified circadian gating functions

- Identify transition p.d.f.s in a broad variety of cell populations, healthy and cancer

- Assess actual (de)synchronisation in cancer vs. healthy proliferating cell populations

- Relate it with the variance of cell cycle phase duration p.d.f.s (i.e., transition kernels)

- Extend from cell cultures in liquid media to solid tissues (using nonlinear modelling)

Another issue in cancer pharmacotherapeutics: Emergence of drug resistance in cancer cell populations

Instead of controlling drug resistance at the individual cell level (ABC transporters), representing the possible emergence of resistant cell clones due to mutations occurring at mitoses in a *cell Darwinism perspective*.

Assumption: Cancer cell populations, under the pressure of a drug-enriched environment, may develop (costly) mutations yielding resistant cell clones, less fit in a drug-free environment, but better survivors in a hostile environment.

A therapeutic objective, under these circumstances, may be not to eradicate all cancer cells (in fact only all drug-sensitive cells), but instead to let some of them live so as to limit the growth of an emergent resistant cell clone ('adaptive therapy').

A first model with 'resistance gene expression' structure

x ($0 \leq x < +\infty$) is a resistance gene expression level (e.g., activity of an ABC transporter)

The growth dynamics of healthy and tumor cells with a chemotherapy is given by the system

$$\begin{aligned}\frac{\partial}{\partial t}n_H(x,t) = & \Big[\overbrace{\frac{1-\theta_H}{\left(1+\rho(t)\right)^{\beta}}\, r(x)}^{\text{growth with homeostasis}} - \overbrace{d(x)}^{\text{natural apoptosis}} - \overbrace{c(t)\mu_H(x)}^{\text{effect of drug}} \Big] n_H(x,t) \\ & + \frac{\theta_H}{\left(1+\rho(t)\right)^{\beta}} \underbrace{\int r(y) M_{\sigma_H}(y,x) n_H(y,t)\, dy}_{\text{birth with mutation}},\end{aligned} \tag{1}$$

$$\begin{aligned}\frac{\partial}{\partial t}n_C(x,t) = & \Big[(1-\theta_C)\, r(x) - d(x) - c(t)\mu_C(x)\Big] n_C(x,t) \\ & + \theta_C \int r(y) M_{\sigma_C}(y,x) n_C(y,t)\, dy,\end{aligned} \tag{2}$$

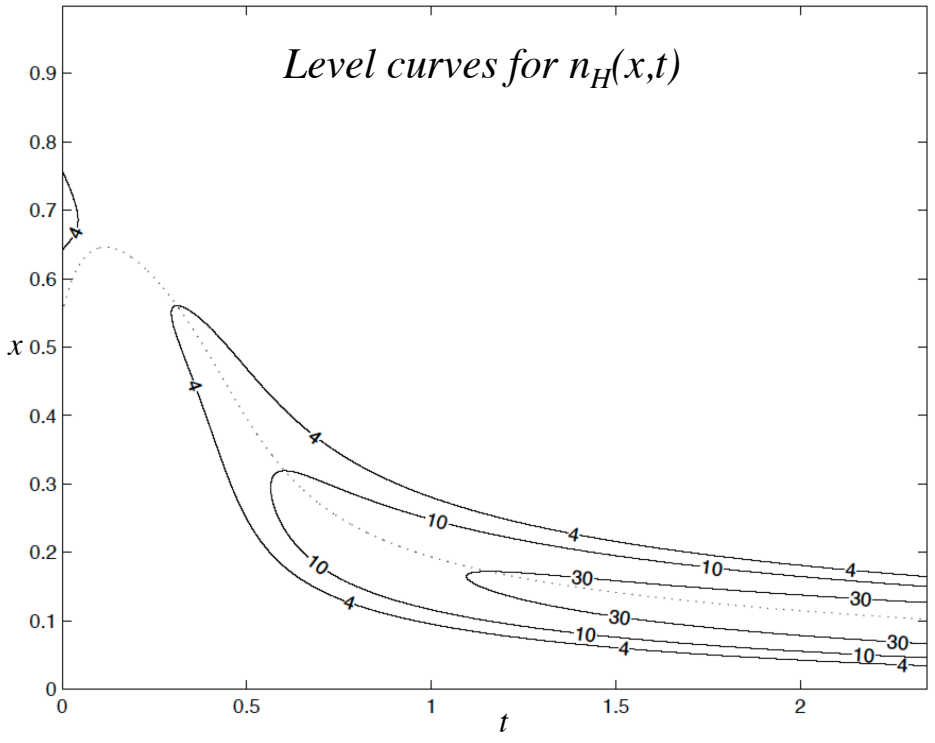
and the total population is defined as

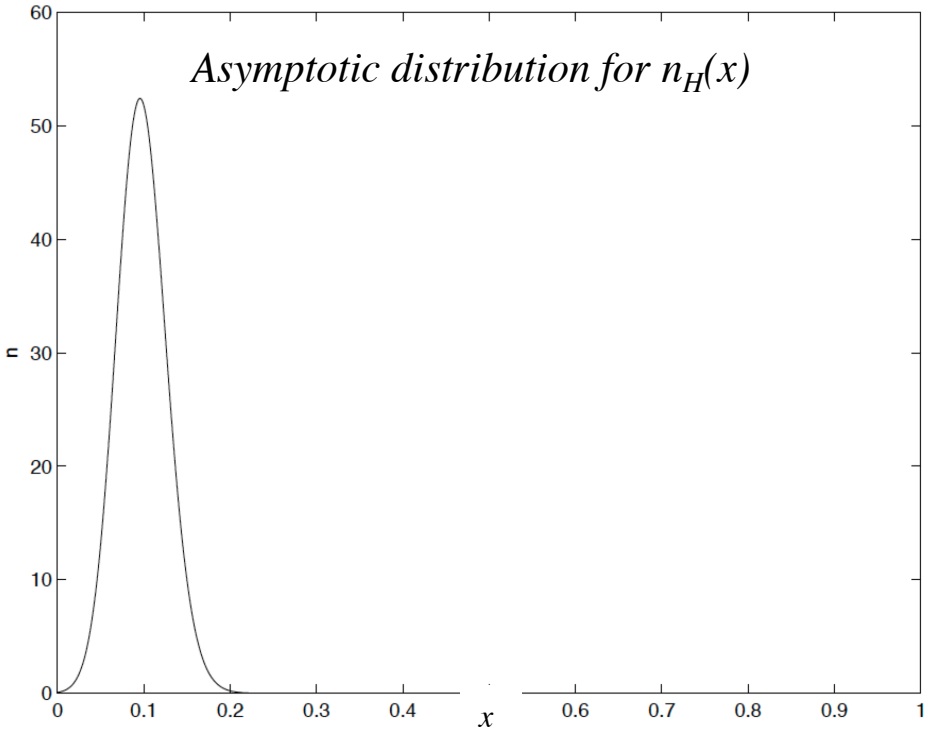
$$\rho(t) = \rho_H(t) + \rho_C(t), \qquad \rho_H(t) = \int_{x=0}^{\infty} n_H(x,t)\, dx, \quad \rho_C(t) = \int_{x=0}^{\infty} n_C(x,t)\, dx. \tag{3}$$

A model that is still not able to yield gene polymorphism in cancer cells...

Probability distribution functions in cell populations for the resistant phenotype under the pressure of a drug

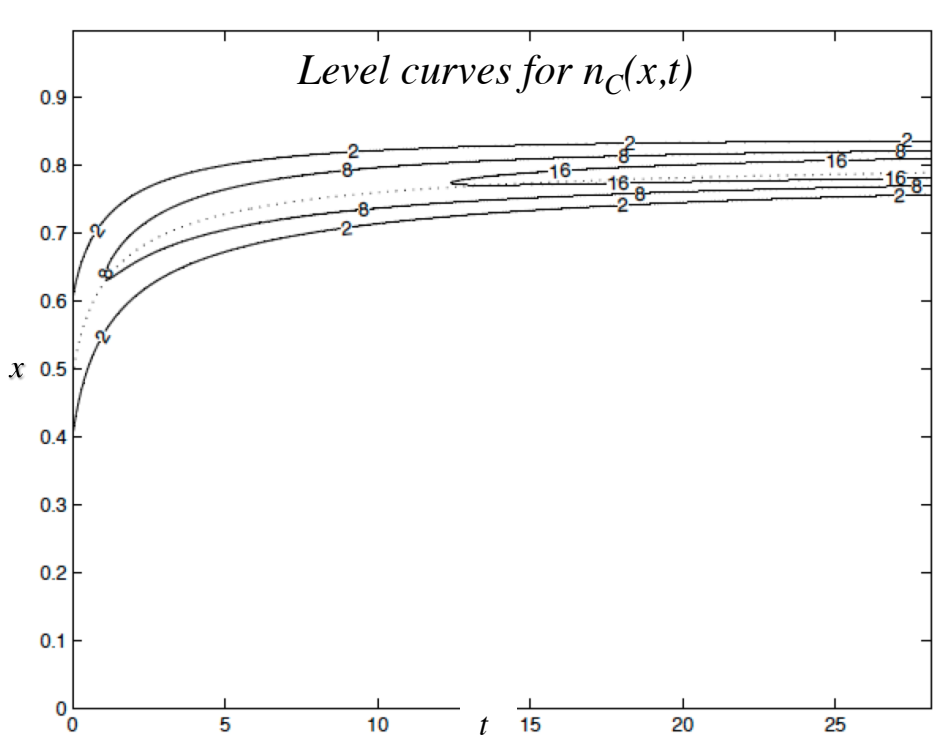
1. No resistance (healthy cells, or sensitive tumour cells)

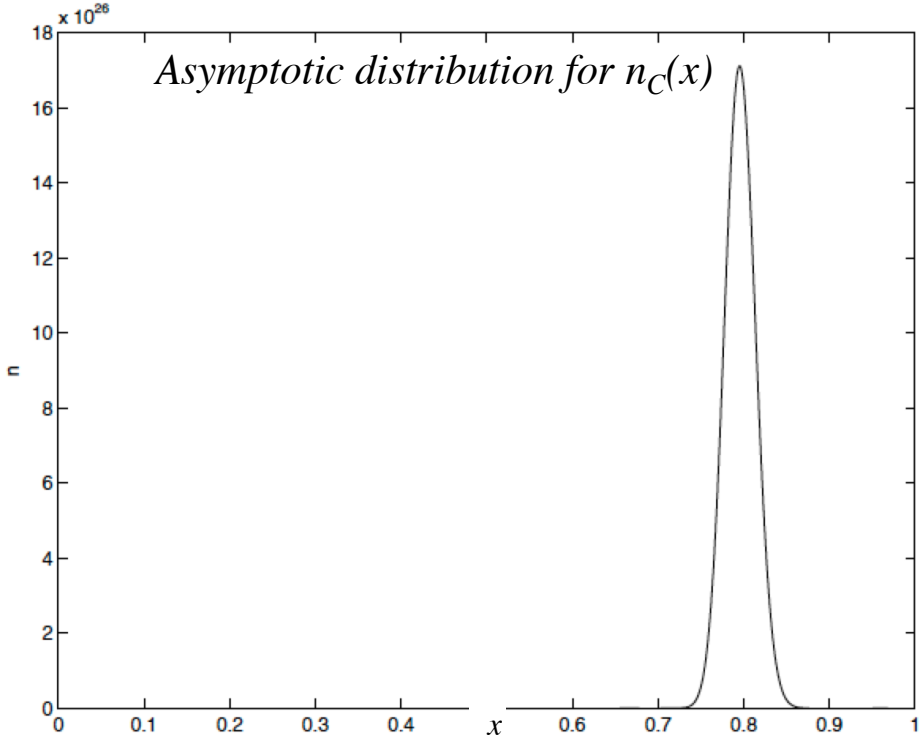




Probability distribution functions in cell populations for the resistant phenotype under the pressure of a drug

2. Resistance (a drug-resistant tumour cell clone)





(JC, A. Lorz, B. Perthame, Submitted, 2011)

Collaborators

INRIA Bang team: *Frédérique Billy, Thomas Lepoutre, Thomas Ouillon, Benoît Perthame*

Other INRIA project-teams: *Stéphane Gaubert, Olivier Fercoq* (Maxplus)

INSERM U 776 "Biological Rhythms and Cancers" (*Francis Lévi*, Villejuif):
Solid tumours, of Mice and Men (particularly colorectal cancer)

EU Network ERASysBio+ C5Sys *Circadian and cell cycle clock systems in cancer*
http://www.erasysbio.net/index.php?index=272
(in particular *Bert van der Horst, Filippo Tamanini* at Erasmus University, Rotterdam)