A Synthetic Genetic Edge Detection Program

Tabor JJ, Salis HM, Simpson ZB, Chevalier AA,
Levskaya A, Marcotte EM, Voigt CA, Ellington AD
Cell. 2009 Jun 26;137(7):1272-81

Presented by Helen Chen
Journal Club 11/05/2009

> *What I cannot create I do not understand.*
> ~Richard Feynman

- Limitations of analysis in understanding complex systems
- Value of design in facilitating discovery
- Design requires recognizing the realistic trade-offs and compromises required for collective system function

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Genetic Circuits

- Standardized, well-characterized components to test and apply understanding of natural systems
- Program living cells with genetic parts e.g. promoters, transcription factors
- Parts combined to construct genetic versions of electronic circuits e.g. switches, memory
- Current challenge: assemble multiple genetic circuits into larger programs for engineering more sophisticated behaviors

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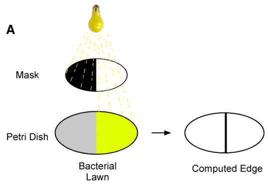
Edge Detection

- Well-studied computational problem used to determine boundaries of objects
- Scan for white pixel, compare intensity to eight neighboring pixels
- If any of neighbors is black, pixel = edge

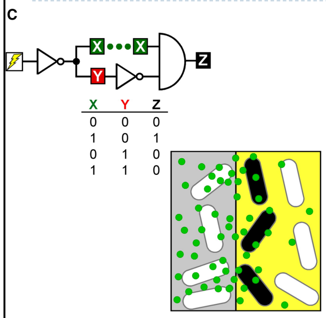
Goal

- Implement a parallel edge detection algorithm wherein each bacterium within a population functions as an independent signal processor, and the population cooperates to find the edges.
- Mathematically Model



A synthetic genetic edge detection program. Tabor JJ, Salis HM, Simpson ZB, Chevalier AA, Levskaya A, Marcotte EM, Voigt CA, Ellington AD. **Cell.** 2009 Jun 26;137(7):1273. Figure 1A

Genetic Edge Detection Algorithm



X	Y	Z
0	0	0
1	0	1
0	1	0
1	1	0

- Pseudocode: IF NOT light, produce signal. IF signal AND NOT (NOT light), produce pigment.
- Produce signal –generate diffusible communication signal
- Produce pigment – produce black pigment

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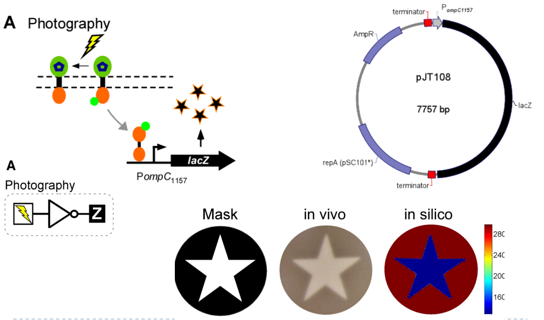
Genetic Edge Detection Algorithm

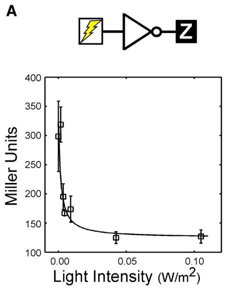
- I. Photographic Bacteria using Dark Sensor
- II. Cell-Cell Communication
- III. Inverter
- IV. Full Logic Function

I. Photographic Bacteria using Dark Sensor



A synthetic genetic edge detection program. Tabor JJ, Salis HM, Simpson ZB, Chevalier AA, Levskaya A, Marcotte EM, Voigt CA, Ellington AD. **Cell.** 2009 Jun 26;137(7):1275. Figure 3A, S2

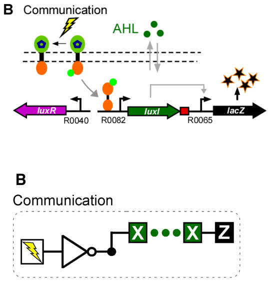
Transfer Function of Dark Sensor



$$f_{\text{light}} = \frac{K}{K+L}(\beta_{\max} - \beta_{\min}) + \beta_{\min}$$

β_{max} = 298 and β_{min} = 125 (outputs in Miller units), L = intensity of light (W/m^2), fit parameter is K = 0.0017 W/m^2 (R^2 = 0.75)

II. Cell-cell Communication



- Communicating using *V. fischeri* quorum sensing system
- Dark → transcription of *luxI* →membrane diffusible compound 3-oxohexanoyl-homoserine lactone (AHL) (Engebrecht , 1984).
- AHL binds to constitutively expressed LuxR → *β* - galactosidase
- Additional blurring component

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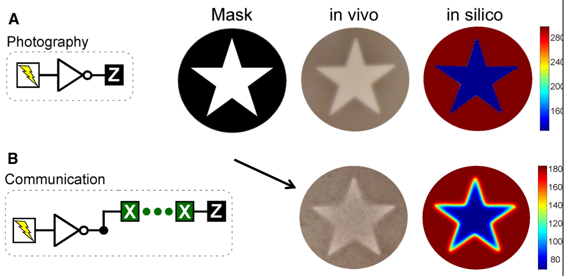
II. Cell-cell Communication



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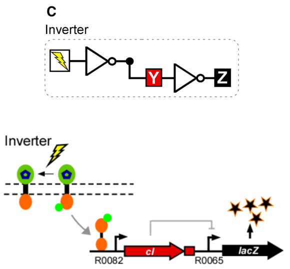
III. Inverter



- B-gal expressed only where AHL AND light, or NOT (NOT light), are present
- NOT function = genetic inverter, (Yokobayashi et al., 2002)
- *cI* gene from phage *λ* → dimeric transcriptional repressor turns OFF the output promoter when the input promoter is ON
- Negative bacterial photograph

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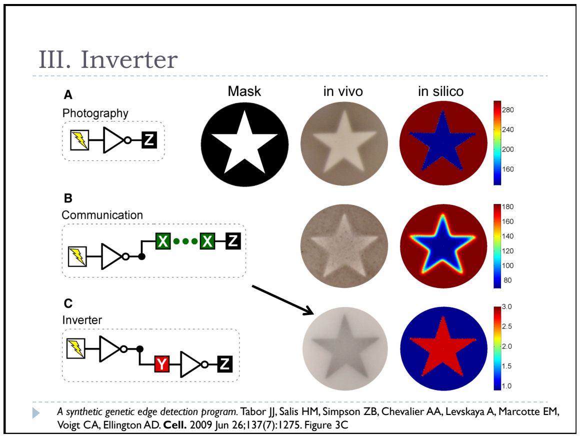

III. Inverter

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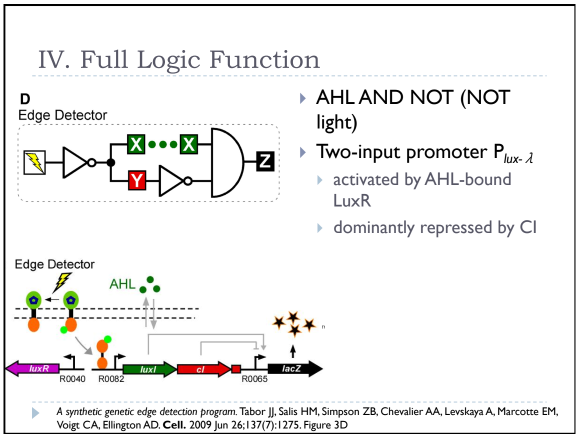

IV. Full Logic Function

- AHL AND NOT (NOT light)
- Two-input promoter $P_{lux\text{-}\lambda}$
  - activated by AHL-bound LuxR
  - dominantly repressed by CI

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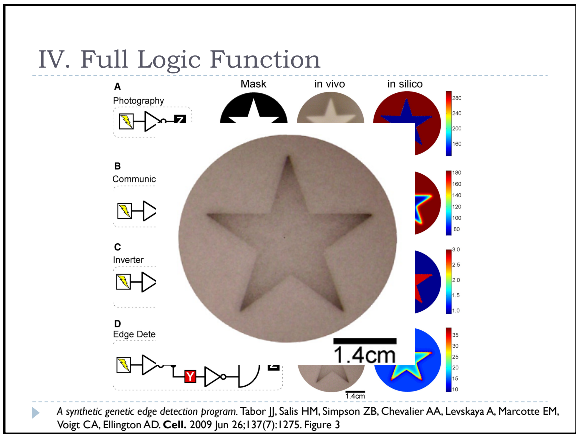

IV. Full Logic Function

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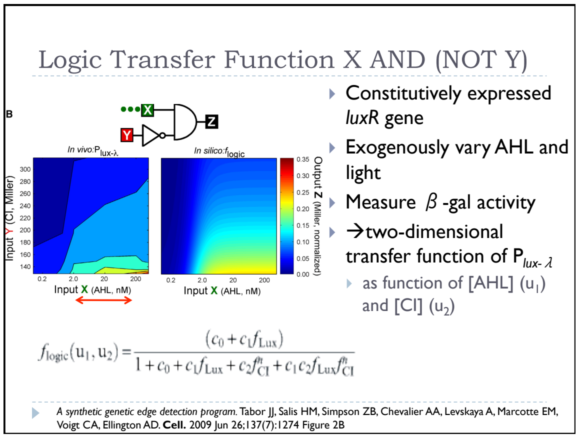

Logic Transfer Function X AND (NOT Y)

- Constitutively expressed *luxR* gene
- Exogenously vary AHL and light
- Measure β -gal activity
- →two-dimensional transfer function of $P_{lux\text{-}\lambda}$
  - as function of [AHL] (u_1) and [CI] (u_2)

$$f_{\text{logic}}(u_1,u_2)=\frac{(c_0+c_1 f_{\text{Lux}})}{1+c_0+c_1 f_{\text{Lux}}+c_2 f_{\text{CI}}^n+c_1 c_2 f_{\text{Lux}} f_{\text{CI}}^n}$$

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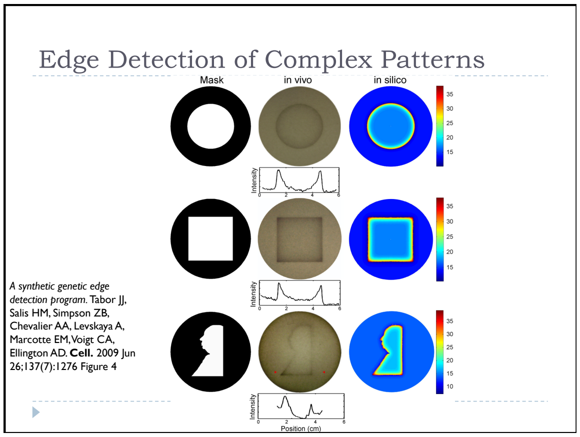

Edge Detection of Complex Patterns

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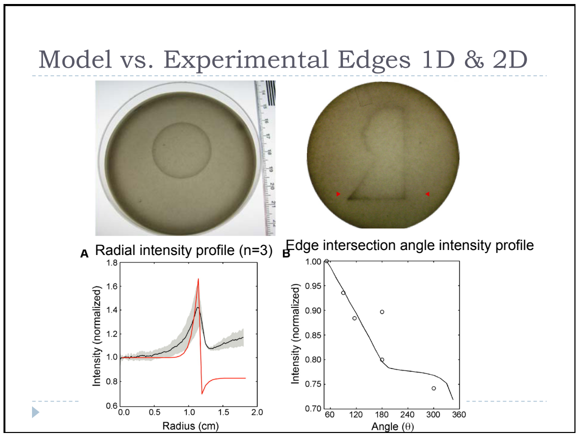

Model vs. Experimental Edges 1D & 2D

Summary – Predictive Models

- Different circuit combinations produced expected behaviors that can be predicted by a mathematical model parameterized with data from the characterization of the individual circuits
  - Will not be true for all circuit combinations

Next: More Complex Genetic Programs

- How to combine functions to create more complex genetic programs
- Well-characterized parts, perform reliably when linked to others without unforeseen higher-order effects (Kim and Tidor, 2003)
- Interactions between synthetic circuits and host systems→ regulatory and metabolic cross-talk, how engineered circuits impose burdens on host cells

Bottom-up Analysis

Synthetic systems as working models for natural counterparts

- Simplicity and tractability → amenable to mathematical analysis
  - generate testable hypotheses regarding contribution of parameters to overall function
- Thorough characterization of simple genetic parts → development of predictive mathematical tools → program cells for functions which approach the sophistication of natural systems

Acknowledgments

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- 20.109 Staff

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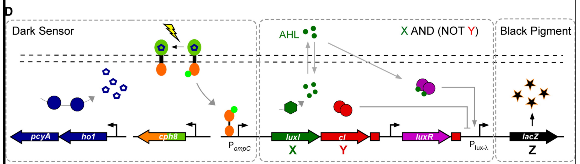
Questions?

Conversion of pseudocode to molecular genetic system



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Reaction-Diffusion Model

- $\frac{\partial u_1}{\partial t} = D\nabla^2 u_1 + \kappa_1 f_{\text{light}} - \kappa_2 u_1$
- $u_2 = \kappa_3 f_{\text{light}}$
- $u_3 = \kappa_4 f_{\text{logic}}(u_1, u_2)$
  - u_1 = [AHL] , u_2 [CI dimers], u_3 = [β -galactosidase]
- A model of the complete edge detector system is constructed based on the individually measured f_{light} and f_{logic}
- Quantifies dynamics of light-dependent production of AHL and CI, AHL diffusion, production of the β -galactosidase reporter and degradation of all products

Independent Info Processing

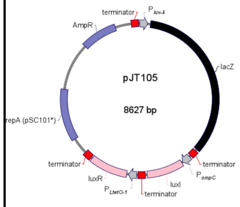
- Logic and cell-cell communication drive natural processes e.g. pattern formation, development
- Cells respond to local signal without information regarding position – reduce info processing
- Competing efforts also use cell-cell communication to program multicellular behaviors
  - e.g. turbidostat, cell density-dependent transcription regulators, synthetic ecosystems, pattern forming
  - BUT use genetically distinct populations (AHL senders and receivers)

Predictive Models

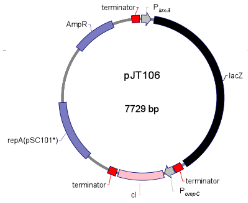
- Predicting behavior of genetic programs = limiting step in programming cellular behavior
- Transfer function – quantitative relationship between circuit inputs and outputs
- Examine in silico (by computer) prior to physical construction
- Drive applications in biotechnology and bottom-up studies of natural regulatory systems

Plasmids

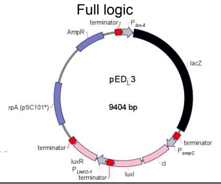
communication



inverter



Full logic



Quantifying the Effect of Angle of Intersection on Edge Intensity (5B)

- Create a series of unit circle *in silico* masks where θ degrees of the circle are in the light with 360- θ degrees in the dark and where θ is varied from 50 to 345 degrees
- For each mask, the solution of the reaction-diffusion model is computed, which predicts the maximum edge intensity. The maximum edge intensity is the β -galactosidase concentration at the edge location.
- The model predictions compare favorably with the experimentally observed edge intensities of the asymmetrical silhouette mask at the selected angle intersections

Edge Intensity versus Angle

- The raw grayscale pixel intensities from the asymmetrical silhouette mask are extracted at selected angle intersections (ImageJ, 1.40 g, Wayne Rasband, NIH) and background corrected. The background corrected intensities are then normalized by dividing by the maximum value in the data set (x = 51°, y = 1.0 in Figure 5B). The experimental values are compared to Miller Unit predictions from varying the angle of intersection in the reaction diffusion model as described below.

Determination of the Logic Transfer Function, f_{logic}

- The steady-state concentration of β -gal is determined by the transcription rate of the LuxR-activated, CI-repressed $P_{lux\text{-}\lambda}$ promoter, which is quantified by the f_{logic} function. The Shea-Ackers formalism is used to enumerate the binding states of LuxR and CI bound to the promoter ([Ackers et al., 1982] and Bintu et al., 2005b)
- The steady-state concentration of β -galactosidase is proportional to the probability of RNA polymerase initiating transcription