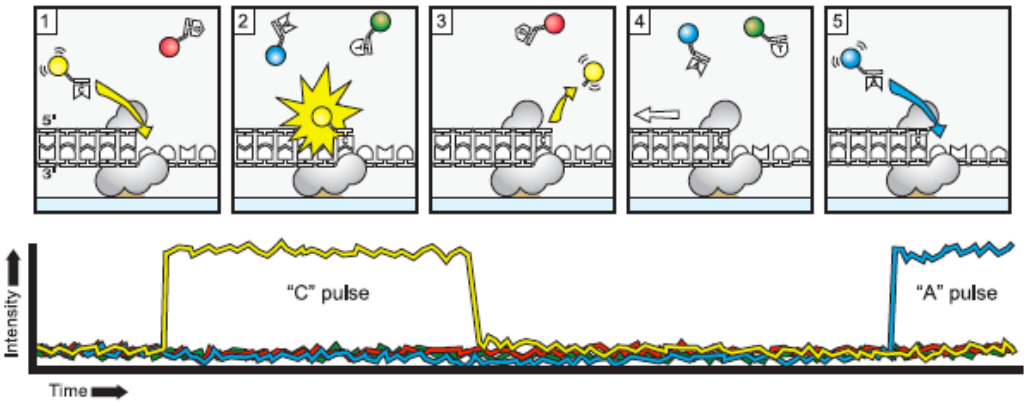
Single Molecule DNA Sequencing

Eid, J., A. Fehr, et al. (2009). "Real-Time DNA Sequencing from Single Polymerase Molecules." *Science* **323** (5910): 133-138.

Levene, M.J., J. Korlach, et al. (2003). "Zero-Mode Waveguides for Single Molecule Analysis at High Concentrations." *Science* **299**: 682-686.

MODEL 1 DNA replication with fluorescent labels



1. Using the diagram above, describe the process of DNA replication highlighting the role of helicase, single stranded binding proteins and DNA polymerase III. Identify where each would be acting in the diagram.

2. Based on the mechanics of DNA replication, explain why the authors chose to link the fluorophore to the terminal phosphate moiety of the dNTP rather than the base. What is a phospholinked dNTP?

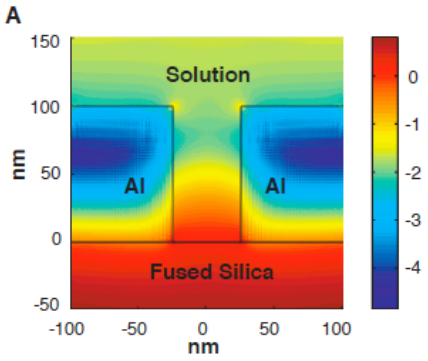
3. Explain how the fluorescent pulse turns 'on' and 'off'.

4. How many fluorescent molecules are detected during each 'read'? How would you estimate the number of photons detected?

5. When DNA polymerase, primer/template, phospholinked dNTPs or metal ions were not present the authors observed <0.01 pulses ZMW^{-1} s^{-1}. Why did they run these experiments and what did this number of pulses indicate?

MODEL 2 Zero Mode Waveguide (ZMW)

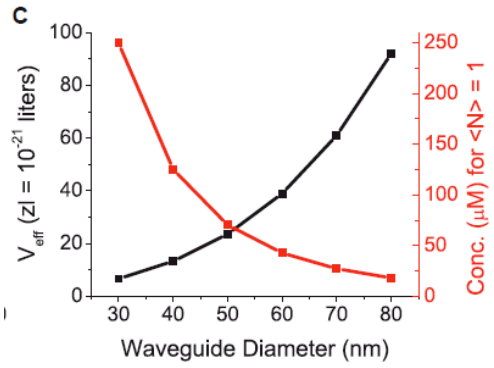
1. Waveguides are structures that direct light. Metal-clad guides exhibit the special characteristic of having a "cut-off" wavelength in which the wave no longer propagates. Based on the figure below, explain where maximum illumination occurs and why this property is important in the experiment.



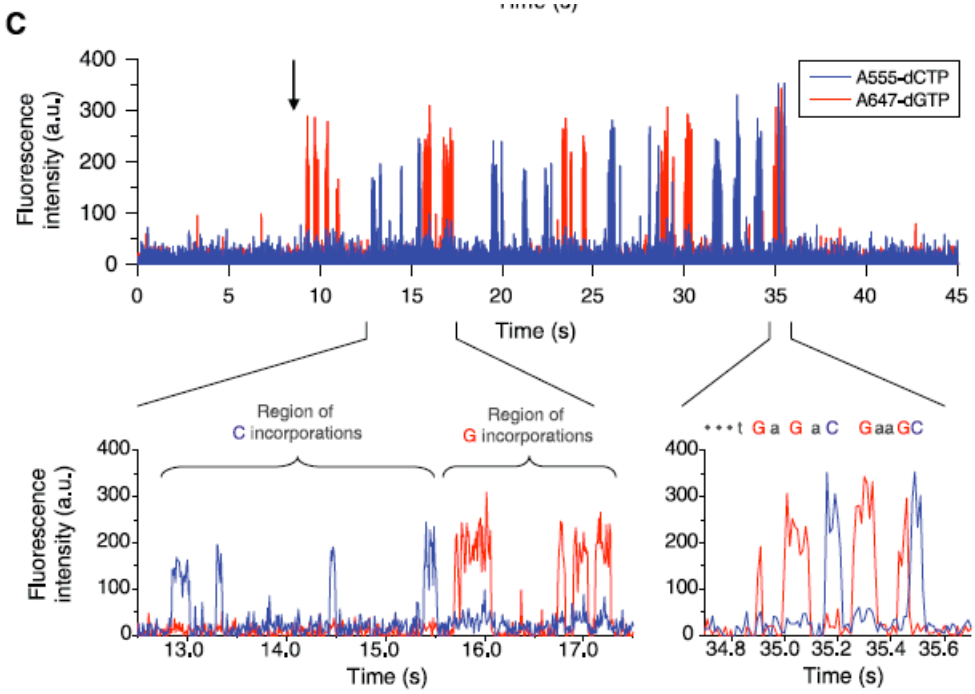
2. For a waveguide with diameter d, the cutoff wavelength is denoted $\lambda_c = 1.7\ d$. Based on the diagram above, what is the approximate value of λ_c? The intensity I in relation to the length of the guide z is given by $I(z) = e^{-z/\Lambda}$ where Λ is a calculated constant for each waveguide based on the equation below. Sketch the relationship between intensity and the length of the guide? One can calculate Λ is using the equation below, where λ_m is the wavelength in the medium composing the core of the waveguide

$$\frac{1}{\Lambda} = 2\sqrt{\frac{1}{\lambda^2} - \frac{1}{\lambda_m^2}}$$

3. The relationship between the effective volume and the diameter of the waveguide is shown on the graph below in black. The corresponding concentration for which there is, on average, one molecule in the volume (<*N*> = 1) is shown in red. Summarize these relationships. What is the significance of these correlations?



MODEL 3 EXPERIMENTAL RESULTS

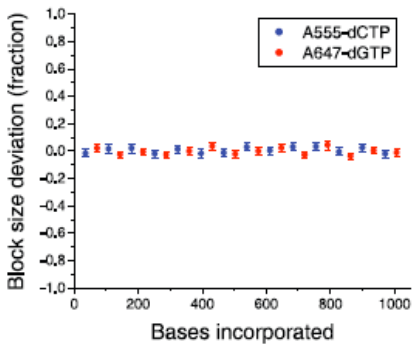


1. Why are only G and C bases detected?

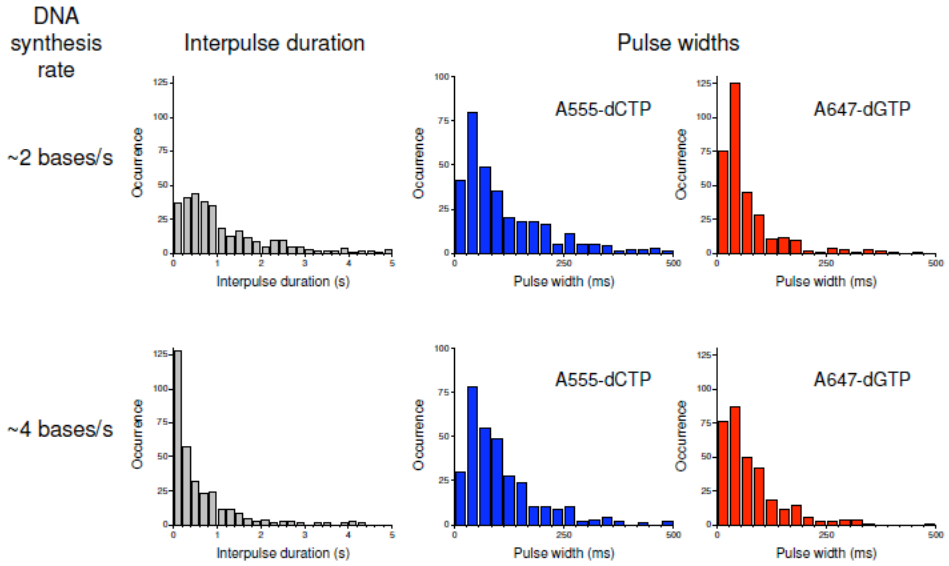
2. The arrow denotes the addition of a metal ion. Why is this necessary?

3. How many C bases are incorporated between 12.5 and 18 seconds? How many G bases are incorporated between 12.5 and 18 seconds? Propose two reasons for gaps between the signals.

4. The authors maintain that the diagram below indicates that "the sequencing approach maintains accuracy irrespective of read length." Explain the meaning of the y-axis and how this supports their claim.



5. Based on the graph below, explain why some polymerase activity demonstrates 2 base/sec incorporation while others demonstrate 4 base/sec incorporation.



6. Propose two challenging questions a student should be able to answer based on the diagram below, which shows the use of four different fluorophores.