Basics of Optical Systems in Real-Time PCR Instruments for Virus Detection

(INVITED TALK)

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Organized by Indian Institute of Technology Delhi

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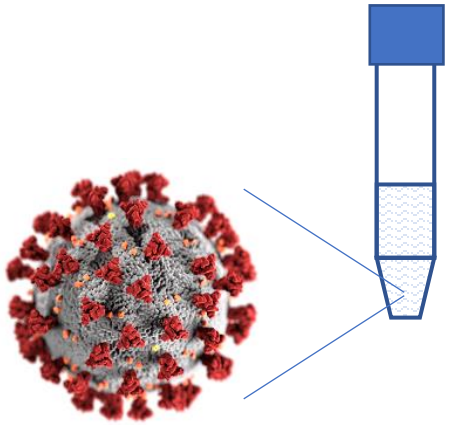
- B.A. Physics, B.S. & M.S. Optics, University of Rochester (Class '97)
- Associate Editor, SPIE Spotlight Series – Optical Design & Engineering
- Author of three books and some papers on optical system design

Typical SARS-CoV-2 PCR Detection Workflow

Sample Collection



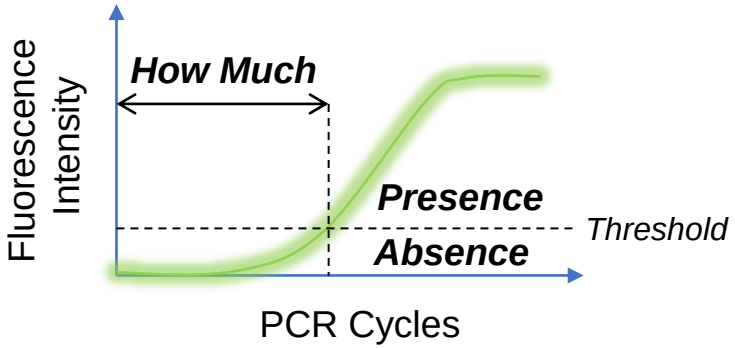
1 e.g., Take a swab from saliva, sputum…

Nucleic Acid Isolation

RNA Purification or Isolation Kit/System

2 If the virus is present, its RNA (single-stranded DNA) is obtained here

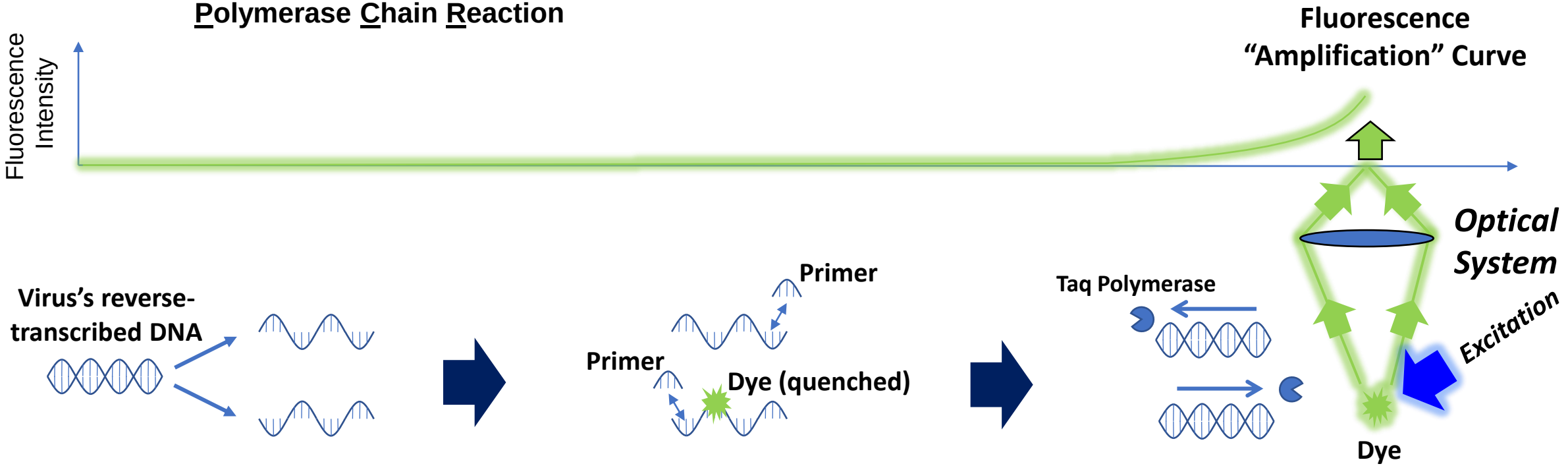
Reverse Transcription + Real-Time PCR



3 Convert its RNA to DNA, then use PCR to test if it is "cloneable"

Real-Time PCR using Applied Biosystems™ TaqMan® "Assay"

Polymerase Chain Reaction

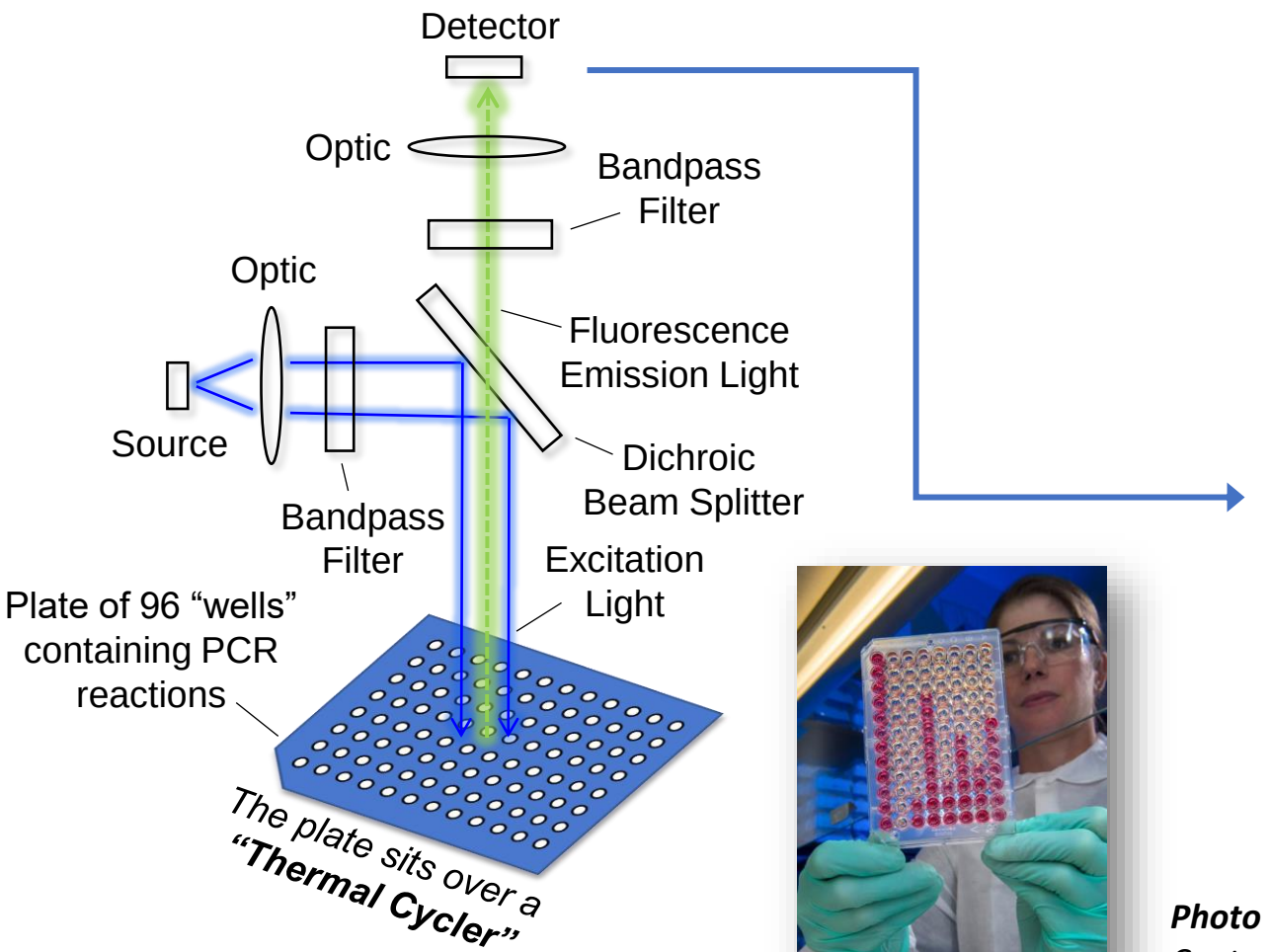


1 Raise temperature to separate the virus's reverse-transcribed DNA (assuming it's present)

2 Test if the primer's genetic sequence matches the sequence on the separated strands

3 If match is successful, then as temperature is lowered, dye is "freed" as two new DNAs are "stitched" from the primers

Basic Components of a Real-Time PCR Optical System



To design the optics, we have to think about what makes this curve and how it is useful to people

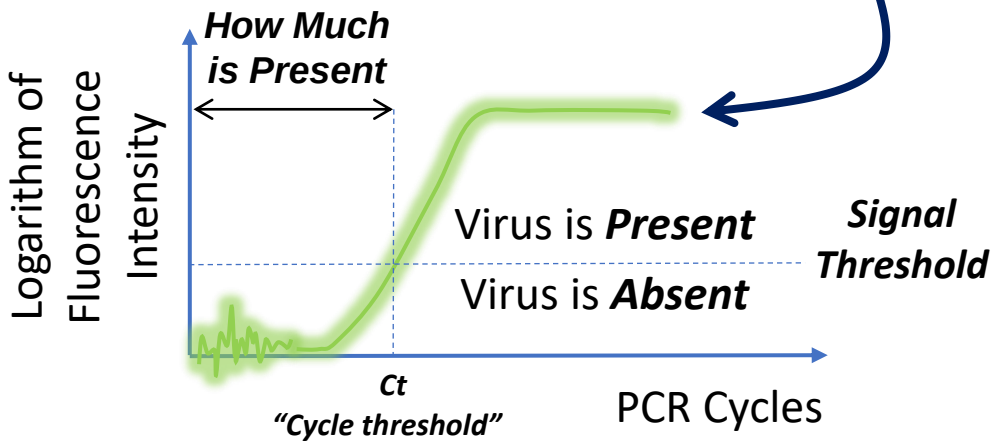


***Photo Credit**: Centers for Diseases Control and Prevention, PHIL ID: #23214*

Fundamentals of Real-Time PCR Detection (TaqMan® Assays)

Initial concentration of target DNA (halved, because in TaqMan® assays, dyes attach to only ***one*** of the strands of a DNA molecule they seek)

Dye's quenching efficiency $0 \ll q \leq 1$

Total concentration of quenched dyes depletes as concentration of fluorescent dyes increases

Background can arise from plate autofluorescence, sensor dark currents, etc.

We are ignoring this part of the curve for now

Total signal $F_{tot,j}$

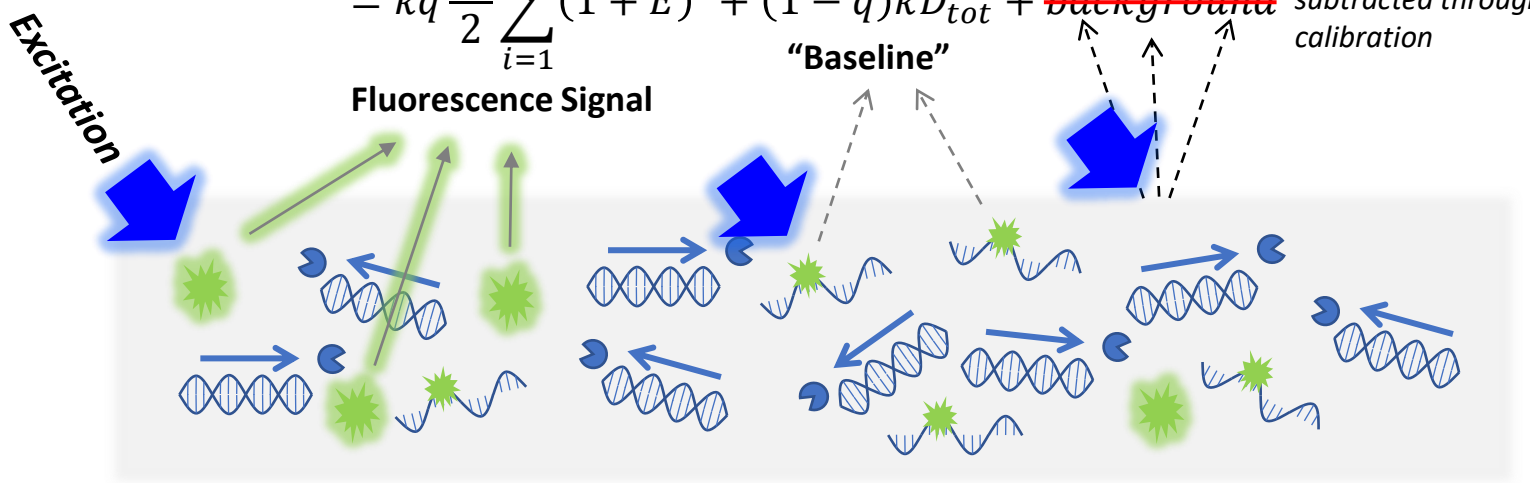
Optical factor

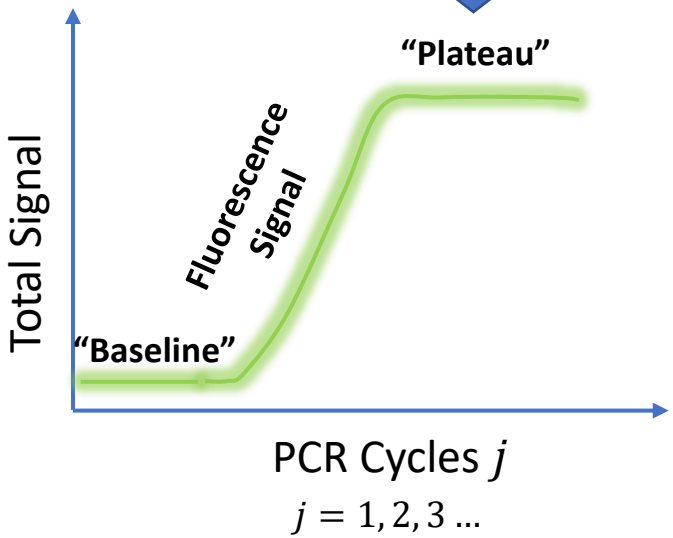
PCR "efficiency"

$$F_{tot,j} \approx k\frac{C_o}{2}\sum_{i=1}^{j}(1+E)^i + (1-q)k\left[D_{tot} - \frac{C_o}{2}\sum_{i=1}^{j}(1+E)^i\right] + background$$

$$= kq\frac{C_o}{2}\sum_{i=1}^{j}(1+E)^i + (1-q)kD_{tot} + \cancel{background}$$

Background can be subtracted through calibration





The Log-Linear Curve of Real-Time PCR

Fluorescence intensity after subtracting baseline

Geometric series

Like the future value of an annuity

Like compound interest

$$F_j \approx kq\frac{C_o}{2}\sum_{i=1}^{j}(1+E)^i = \frac{kq}{2}\frac{C_o(1+E)}{E}\left[(1+E)^j - 1\right] \approx kC_o(1+E)^j$$

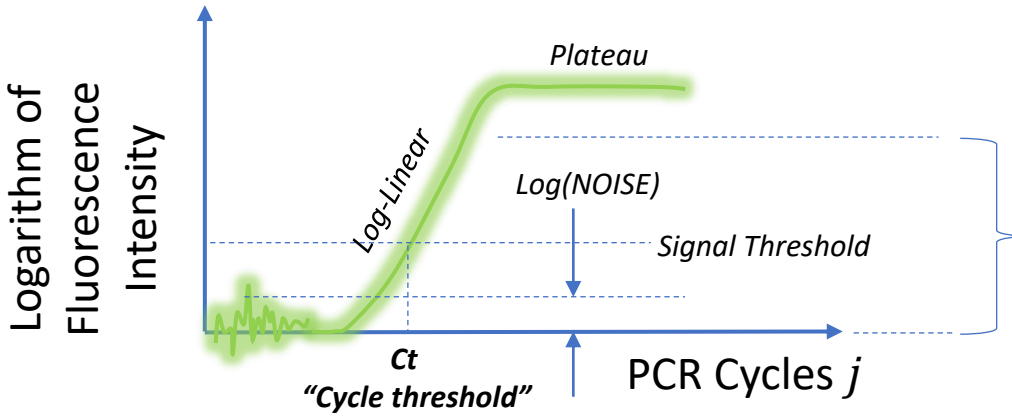
Approximation for large j and for $0 \ll E \leq 1$, and $q \approx 1$

Add noise from background

$$F_j \pm Noise \approx kC_o(1+E)^j\left(1 \pm \frac{1}{\sqrt{F_j}} \pm \frac{\mathrm{LOD}}{C_o(1+E)^j}\right)$$

Signal shot noise

This is saying that the amplifying DNA concentration must reach a "limit of detection" (LOD), beyond which the signal becomes limited only by its own shot noise (and that's what we want!)



$$\log(F_j \pm Noise) \approx \log(1+E)\,j + \log(kC_o) \pm \log(Noise)$$

$$y \approx mx + b \pm Noise$$

y: TOTAL SIGNAL

mx: AMPLIFYING SIGNAL

b: INITIAL SIGNAL

$Noise$: BACKGROUND NOISE

Goals of Optical System Design for Real-Time PCR

1 Minimize the **LOD** (this is like a "**noise equivalent concentration**", which is the minimum concentration of DNA needed to reach a fluorescent signal "at the maximum noise level")

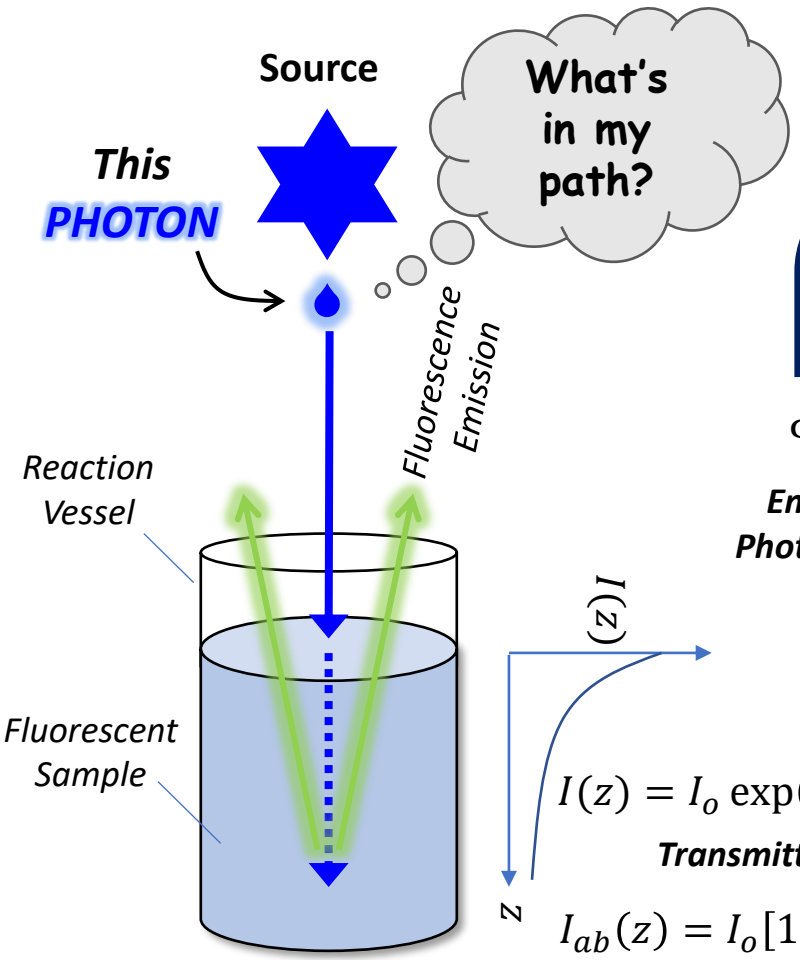
$$F_j \pm Noise \approx kC_o(1+E)^j\left(1 \pm \frac{1}{\sqrt{F_j}} \pm \frac{\mathrm{LOD}}{C_o(1+E)^j}\right)$$

2 Maximize $\boldsymbol{k}$

This is a quantity that involves all of the factors and variables associated with the optical system (and sometimes, also involves factors from the chemistry of the reaction)

Where does k come from?

HINT: You have to "ride" a ***PHOTON***



$$k$$

Detected Fluorescence

$$F \approx C_o \int_{-\infty}^{+\infty} \frac{I_o}{h\nu} \frac{Q f_{em}(\lambda)\lambda}{\int_{-\infty}^{+\infty} f_{em}(\lambda)\lambda d\lambda} a\varepsilon A z T(\lambda)\, \eta(\lambda) d\lambda$$

Transmittance of filters, lenses, etc.

Sensor's quantum efficiency

Quantum Yield

$$\Phi(\lambda)_{em} \approx \frac{I_o}{h\nu} \frac{Q f_{em}(\lambda)\lambda}{\int_{-\infty}^{+\infty} f_{em}(\lambda)\lambda d\lambda} a\varepsilon C_o A z$$

Emitted Photon Flux

Note: we are ignoring reflections, scatter, numerical apertures, and probably many other factors (but you get the idea)

Absorbed Photon Flux $\Phi_{ab} \approx \frac{I_o}{h\nu} a\varepsilon C_o A z$

Illuminated sample volume

$$I(z) = I_o \exp(-a\varepsilon C_o z)$$

Transmitted Irradiance

$$I_{ab}(z) = I_o[1 - \exp(-a\varepsilon C_o z)] \approx I_o a\varepsilon C_o z$$

Low-order term in a Taylor expansion

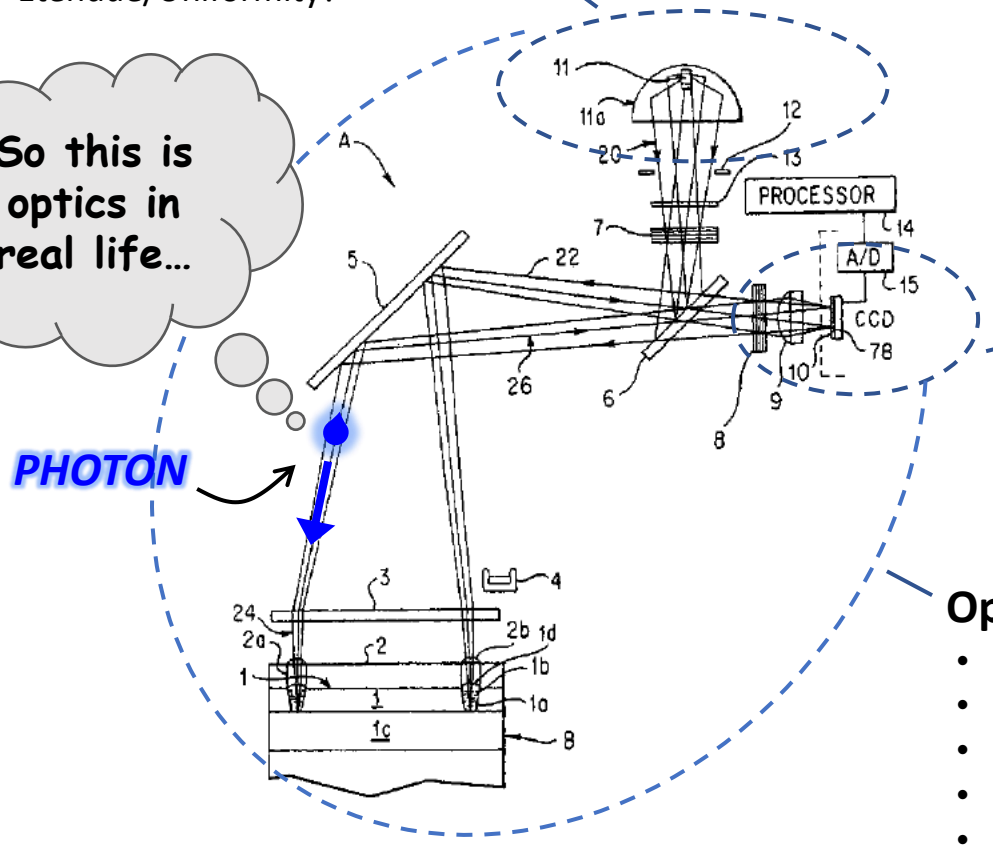
Absorbed Irradiance

Example: An imaging-based real-time PCR optical system

- Reflectors or lenses?
- Source spectrum?
- Étendue/Uniformity?

Illumination Design

So this is optics in real life…



(12) **United States Patent**
Oldham et al.

(10) **Patent No.: US 7,498,164 B2**
(45) **Date of Patent: Mar. 3, 2009**

(54) **INSTRUMENT FOR MONITORING NUCLEIC ACID SEQUENCE AMPLIFICATION REACTION**

(75) Inventors: **Mark F. Oldham**, Los Gatos, CA (US); **Eugene F. Young**, Marietta, GA (US)

(73) Assignee: **Applied Biosystems, LLC**, Carlsbad, CA (US)

(58) **Field of Classification Search** 435/808, 435/288.7, 809, 303.1, 288.4; 378/42, 45; 422/82.08; 250/483.1, 459.1, 461.2, 461.1; 356/73
See application file for complete search history.

(56) **References Cited**
U.S. PATENT DOCUMENTS

Lens Design

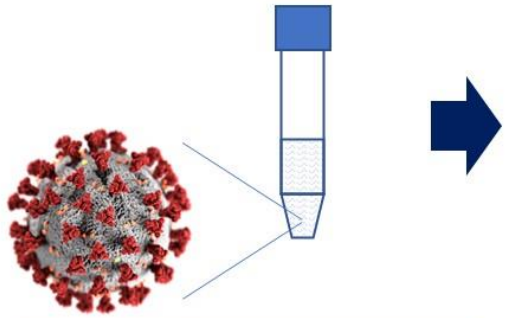
- What's the most suitable lens design form?
- Does lens relative illumination play a role?
- What ray angles are incident on the filters? (the %T bandpass of thin-film filters shifts towards shorter wavelengths at high incident angles)

Optical System Design

- Have we accounted for all factors in k? – "What" does the photon "feel"?
- Is this the most suitable optical architecture to meet system requirements?
- Talk to everyone: engineers, software developers, biologists, managers, marketing…
- Perform Monte Carlo simulation for system tolerancing analysis
- Explore and identify new technologies that may be applied to solve problems

Digital PCR: absolute quantification of DNA

Take Very *Dilute* Samples



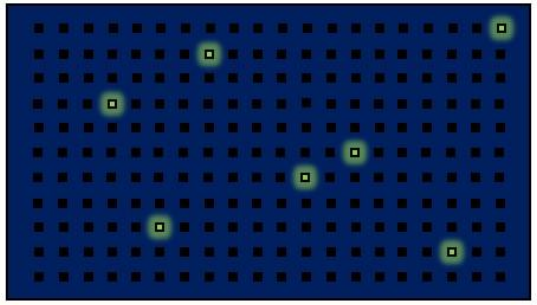
1 e.g., Take a swab from saliva (or, for liquid biopsies, from blood)

Nucleic Acid Isolation + Sample Partitioning

RNA isolation, then insert sample into ~ 20k microarray partitions

2 If the virus is present, its RNA (single-stranded DNA) is obtained here

Reverse Transcription + PCR + Count Individual DNA Molecules



3 Count bright partitions (each has ~ single to a few DNA molecules inside!)

Probability of having x counts of DNA per partition ➡ $P(x) \approx \frac{e^{-\mu}\mu^{x}}{x!}$ ➡ $P(0) \approx e^{-\mu} \approx (N-B)/N$

Solve for μ ➡ $\mu \approx -\ln\left(\frac{N-B}{N}\right) \pm uncertainty$

N = *Total number of partitions*

B = *Number of bright partitions*

μ = *Mean number of DNA molecules per partition*

From the recent Thermo Fisher newsroom (Sep 20, 2021)

ThermoFisher SCIENTIFIC

Thermo Fisher Scientific Adds Digital PCR to Genetic Analysis Capabilities

Applied Biosystems QuantStudio Absolute Q Digital PCR System*, the first integrated digital PCR solution, is ideal for oncology, cell and gene therapy development and other research applications

> dPCR has quickly become the standard for nucleic acid quantification in oncology, cell and gene therapy development and other research applications because its absolute quantification enables higher accuracy and precision. Thermo Fisher recently acquired Combinati and its cutting-edge dPCR technology to rapidly develop and commercialize it alongside an expanding portfolio of assays.

(19) **United States**
(12) **Patent Application Publication**
Siew et al.

(10) **Pub. No.: US 2021/0140885 A1**
(43) **Pub. Date: May 13, 2021**

(54) **OPTICAL SYSTEM, AND METHOD OF ILLUMINATING A SAMPLE PLANE**

(71) Applicants: **Advanced Instrument Pte. Ltd.**, Singapore (SG); **Combinati Incorporated**, Palo Alto, CA (US)

(72) Inventors: **Ronian Siew**, Vancouver (CA); **Sheau Yeng Wei**, Singapore (SG); **Ju-Sung Hung**, Palo Alto, CA (US)

(73) Assignees: **Advanced Instrument Pte. Ltd.**, Singapore (SG); **Combinati Incorporated**, Palo Alto, CA (US)

Publication Classification

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G01N 21/64 (2006.01)

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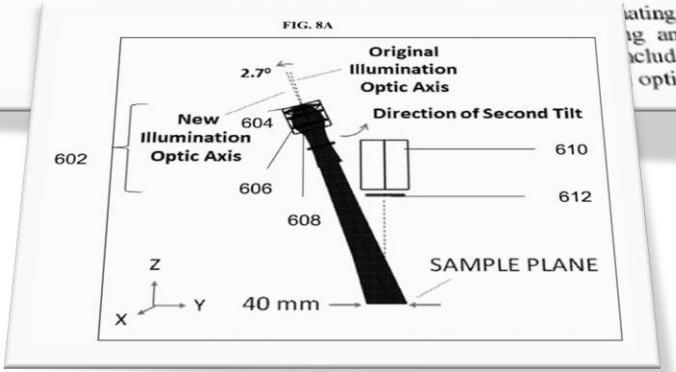
CPC ***G01N 21/6428*** (2013.01); ***G01N 21/6452*** (2013.01); *G01N 2201/062* (2013.01); *G01N 2021/6439* (2013.01); *G01N 2021/6478* (2013.01); ***G01N 21/6456*** (2013.01)

(57) **ABSTRACT**



Some Novel PCR Detection-Related Developments

- J. H. Son, B. Cho, S. Hong, S. H. Lee, O. Hoxha, A. J. Haack, and L. P. Lee, "***Ultrafast photonic PCR***," Light Sci. Appl. **4**, e280 (2015)

Uses light to heat and cool for PCR!

- V. J. Gadkar, D. M. Goldfarb, S. Gantt, and P. A. G. Tilley, "***Real-time Detection and Monitoring of Loop Mediated Amplification (LAMP) Reaction Using Self-quenching and De-quenching Fluorogenic Probes***," Scientific Reports **8**, 5548 (2018)

Real-time DNA detection without thermal cycling!

- J. Nurmi, H. Lilja, and A. Ylikoski, "***Time-resolved fluorometry in end-point and real-time PCR quantification of nucleic acids***," Luminescence **15**(6), 381-388 (2000)

Eliminates background autofluorescence!

- X. Fan and S-H Yun, "***The potential of optofluidic biolasers***," Nature Methods **11**, 141-147 (2014)

Essentially converts bio-samples into lasers!

- P. Mohammadyousef, M. Paliouras, M. Trifiro, and A. G. Kirk, "***A Novel Portable Fluorophore-free Photonic qPCR for Point-of-Care Applications***," in Biophotonics Congress: Biomedical Optics 2020 (Translational, Microscopy, OCT, OTS, BRAIN), OSA Technical Digest (Optical Society of America, 2020), paper TTh4B.6

Uses ultra-violet absorption by DNA!

Acknowledgements

I thank Professors Anurag Sharma, Joby Joseph, and Kedar Khare of the Indian Institute of Technology Delhi for their kind invitation and support for this talk

Questions?

PS: I didn't talk about "multiplexing"
(so, you can ask me about it here ☺)