UV Photodamage of DNA

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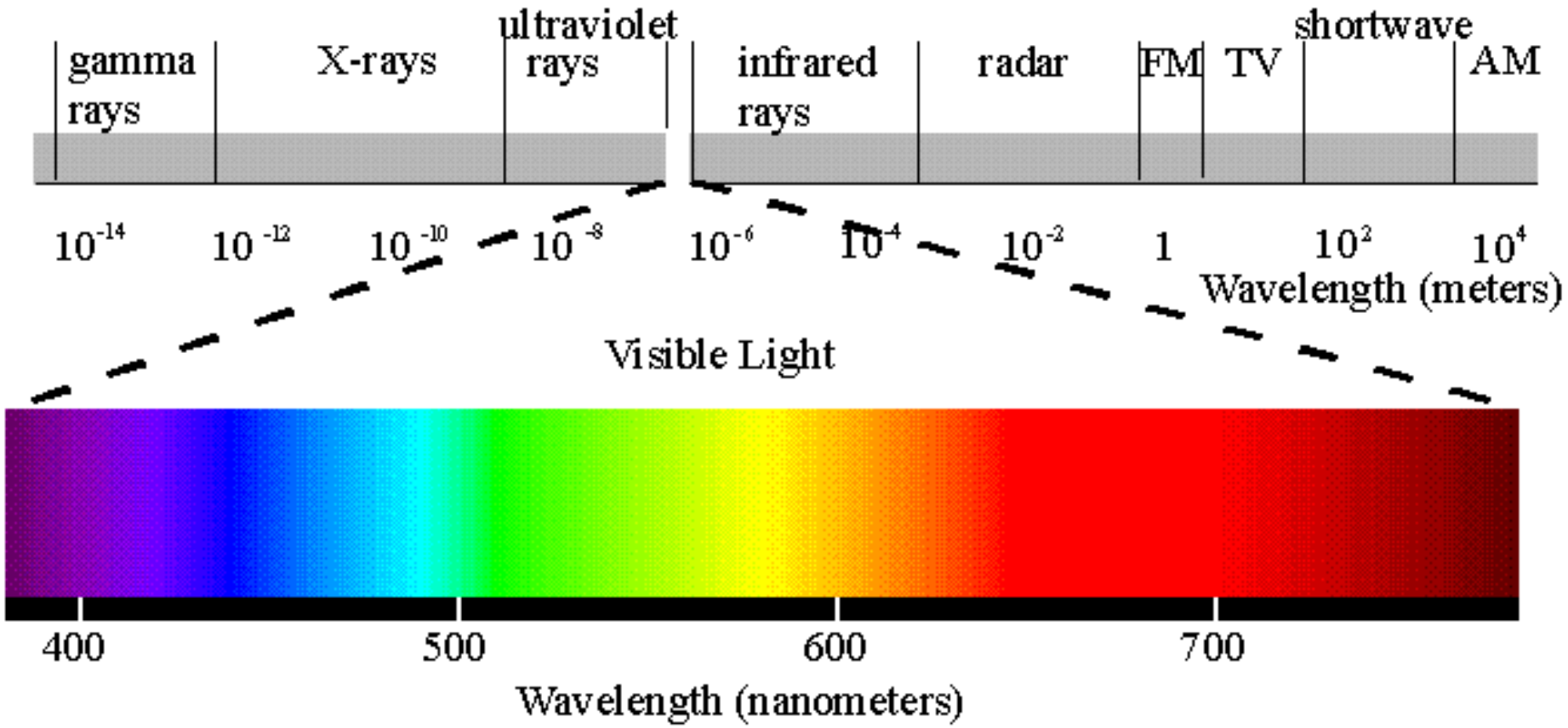
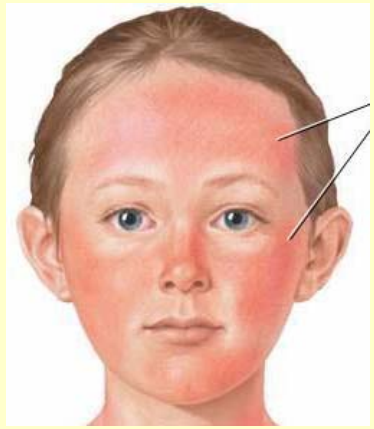
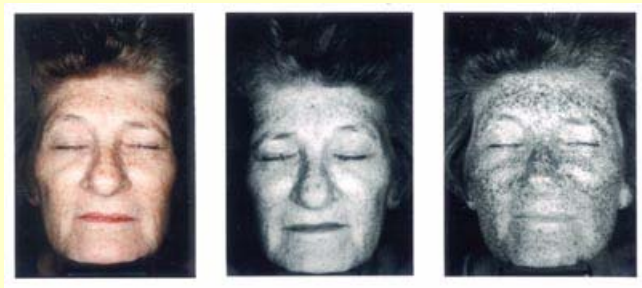
Electromagnetic Spectrum

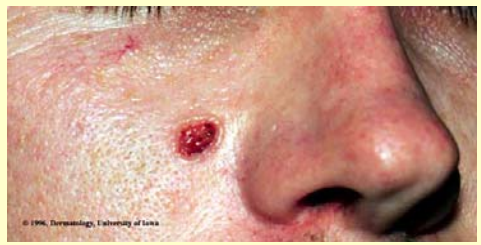


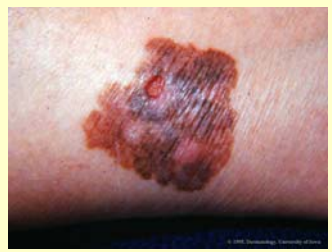
Ultraviolet Light

- Electromagnetic Radiation $\lambda < 380$ nm
  - UVA $320 < \lambda < 380$ nm: high intensity in sunlight, low damage
  - UVB $280 < \lambda < 320$ nm: decreasing intensity, increasing damage
  - UVC $\lambda < 280$ nm: none in sunlight, very damaging
- Good
  - Vitamin D production in skin
  - Disinfecting i.e. “germicidal” = 254 nm UVC
- Bad
  - Erythema “Sunburn”
  - Cosmetic photoaging
  - Immune suppression
  - Cataract induction
  - Skin Cancer induction
    - Basal & squamous carcinomas
    - **Melanoma**

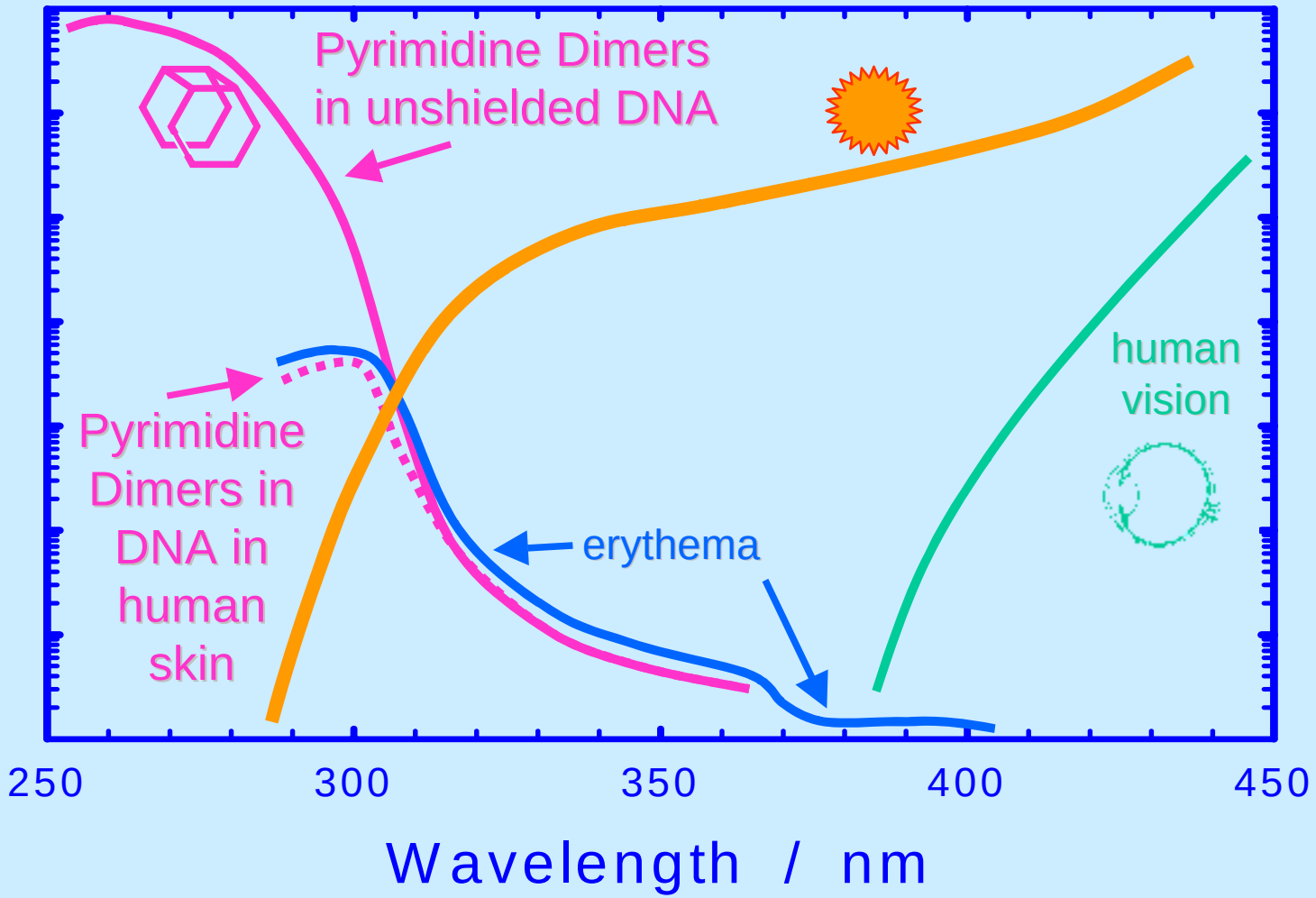








Alphabetical Ultraviolet



Agenda

UV Basics

Ozone Depletion

Human Skin

Plants

Polychromatic Spectra

UV Basics

DNA damage

DNA repair

Quantifying DNA Damage

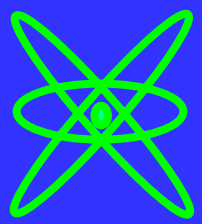
DNA damage

- DNA is (almost) always the most sensitive molecular target in a cell
- The primary photoproducts are
  - Cyclobutyl pyrimidine dimers and
  - 6/4 di-pyrimidines
- The effectiveness of different wavelengths in producing these damages parallel the absorption of DNA (well mostly)

DNA Damages

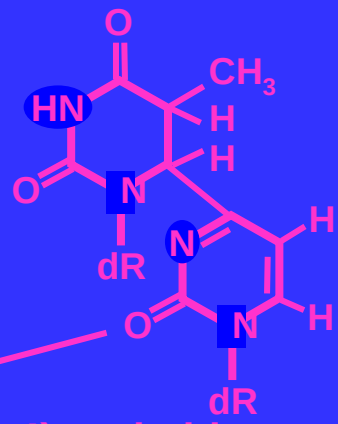
ultraviolet light

chemicals



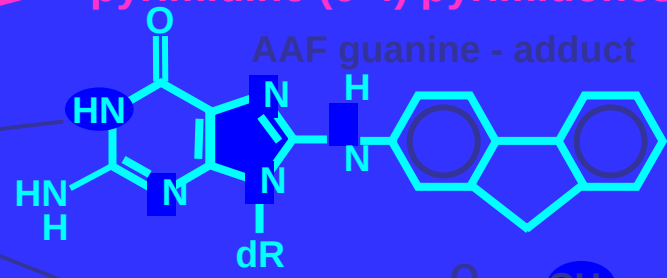
$\alpha,\beta,\gamma,n^o,X \ldots Fe^{+26}$

cyclobutyl pyrimidine dimer

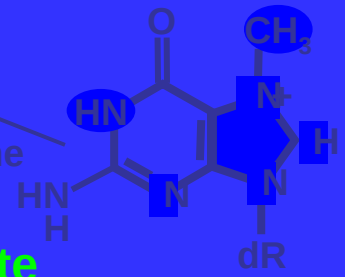


pyrimidine (6-4) pyrimidones

AAF guanine - adduct



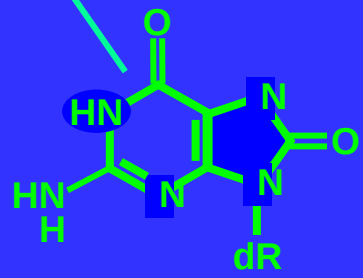
7 Me-guanine



abasic site

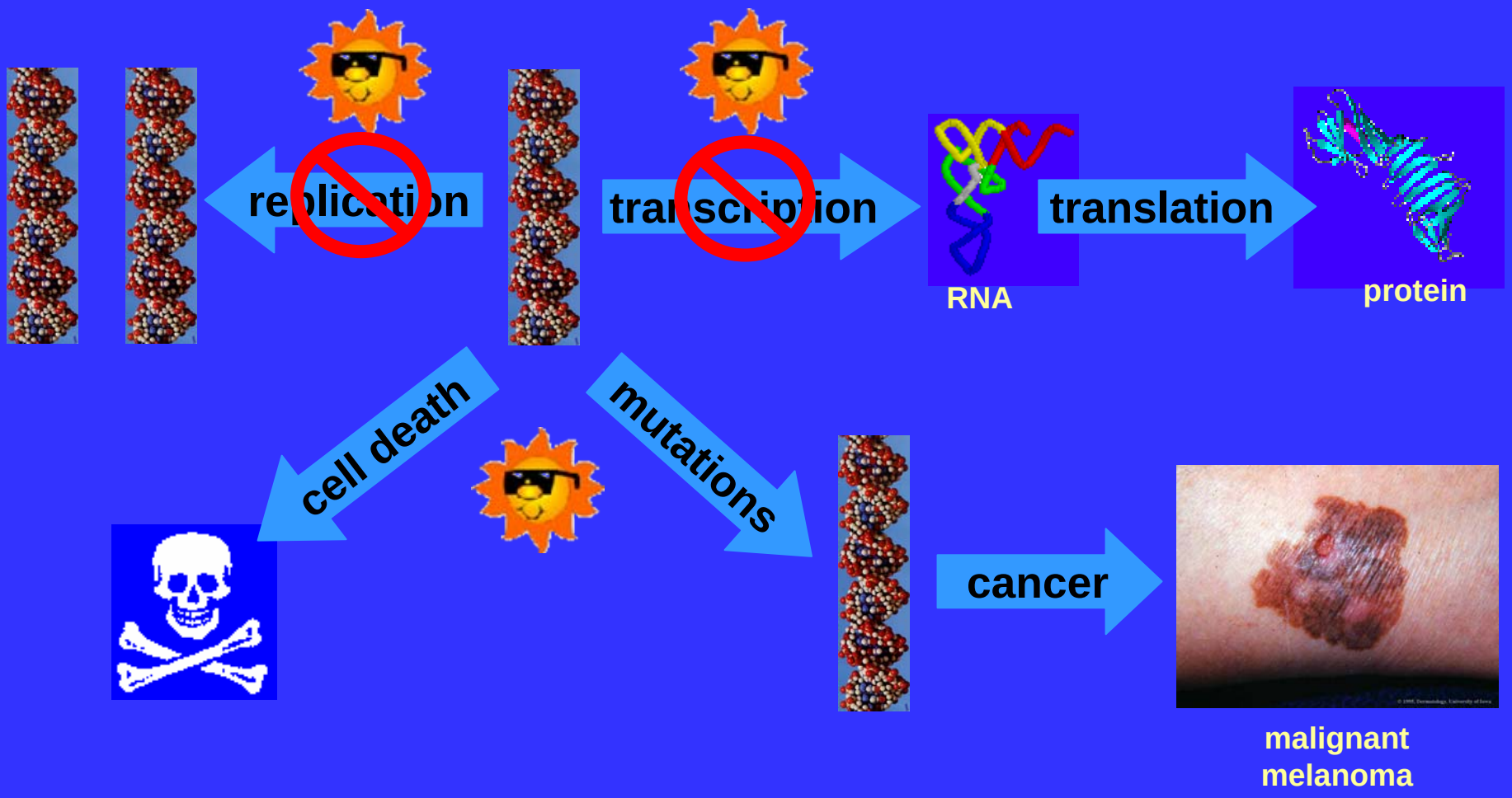
strand break

thymine glycol



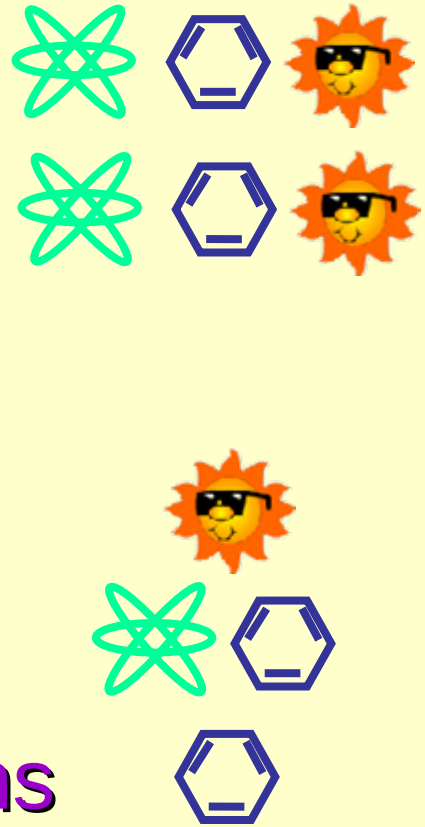
8-oxyGuanine

Is DNA Damage Important?

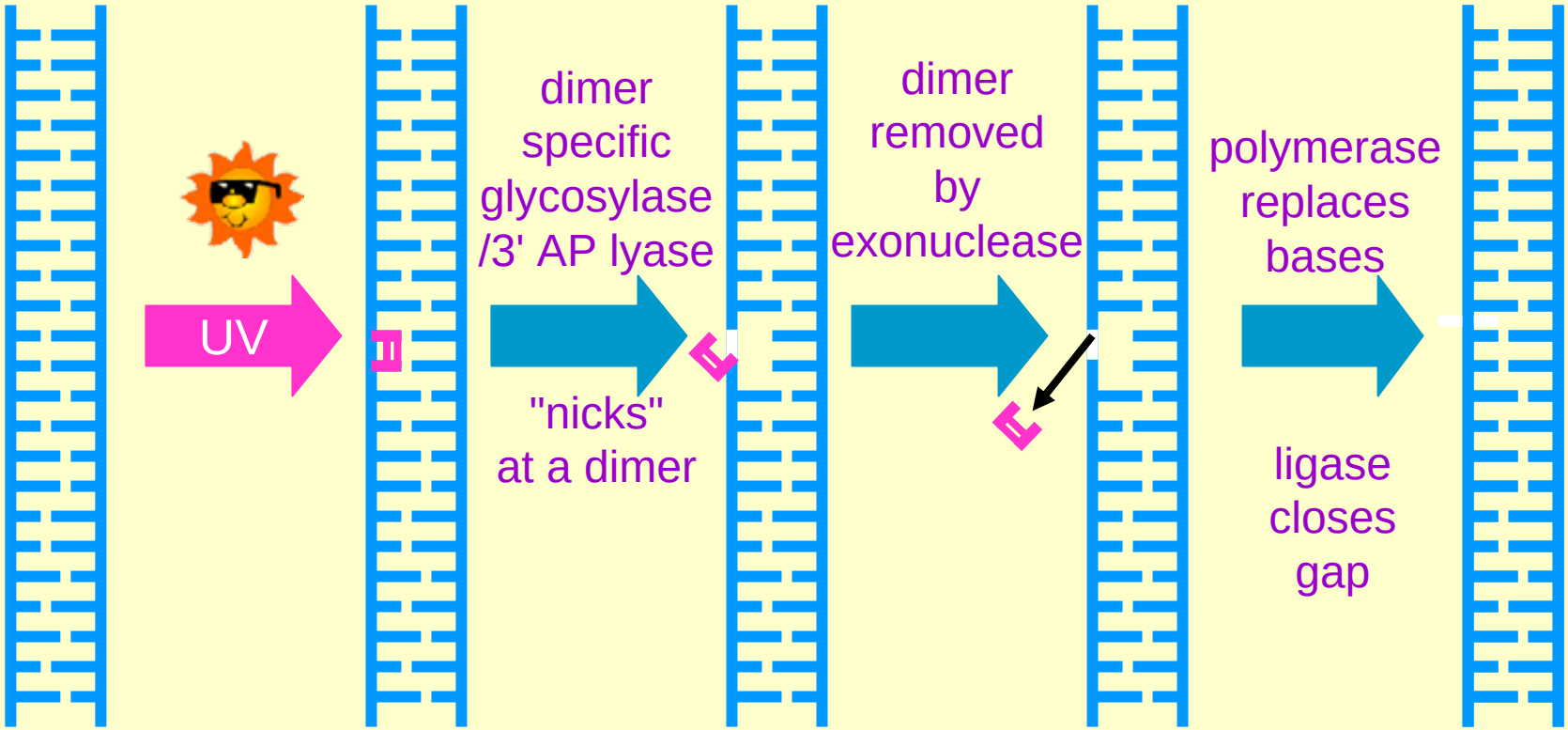


DNA Repair Pathways

- **Base Excision Repair**
- Nucleotide Excision Repair
- Mismatch Repair
- Photoreactivation
- Strand Break Rejoining
- Direct Removal of Alkylations

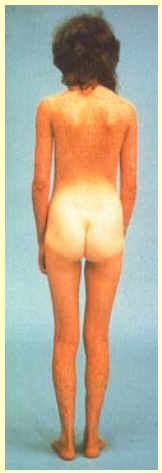


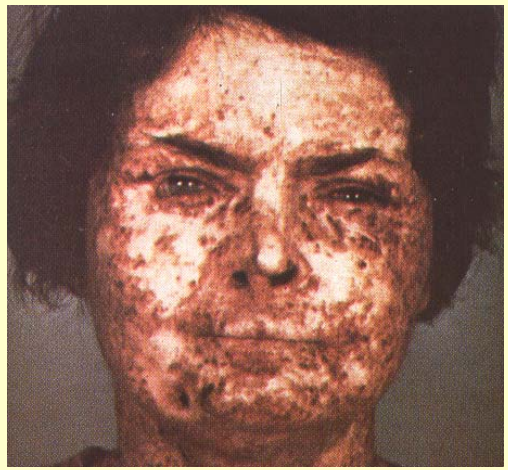
Base Excision Repair by *M. luteus* Pyrimidine Dimer Glycosylase



When DNA Repair Fails

xeroderma pigmentosum

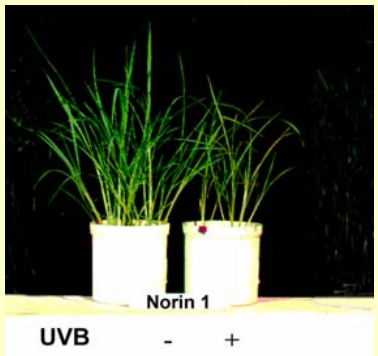


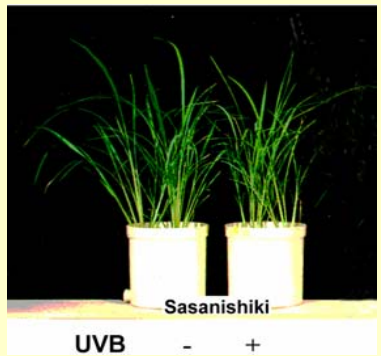


defective nucleotide excision repair

Cleaver, Nature 1968

UV sensitive rice cultivar (Norin 1)





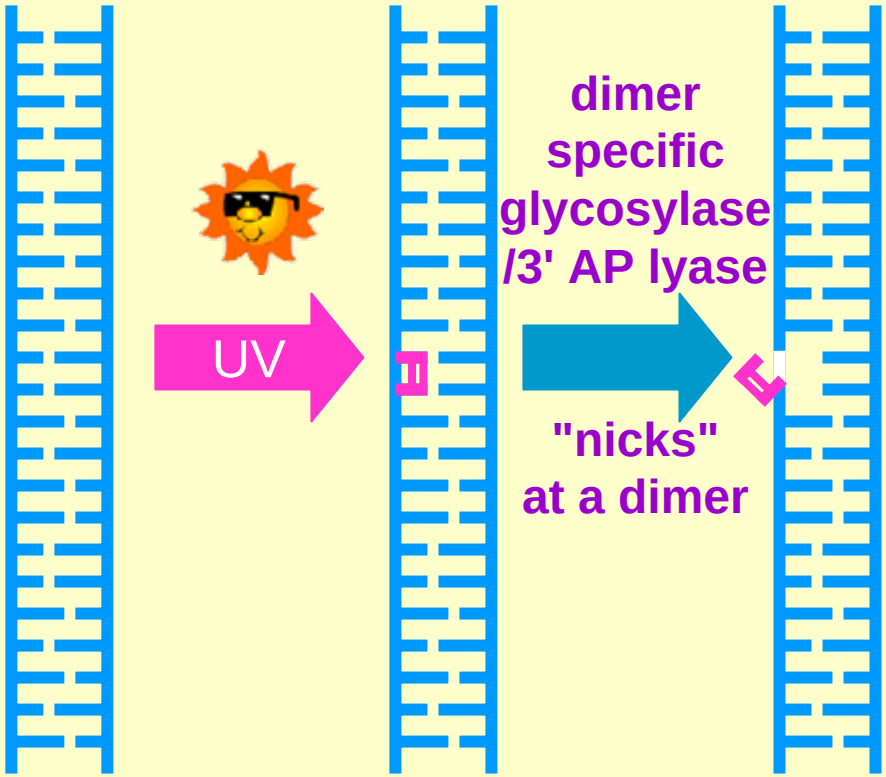
defective photoreactivation

Hidema, Kumagai, & B. Sutherland, The Plant Cell 2000

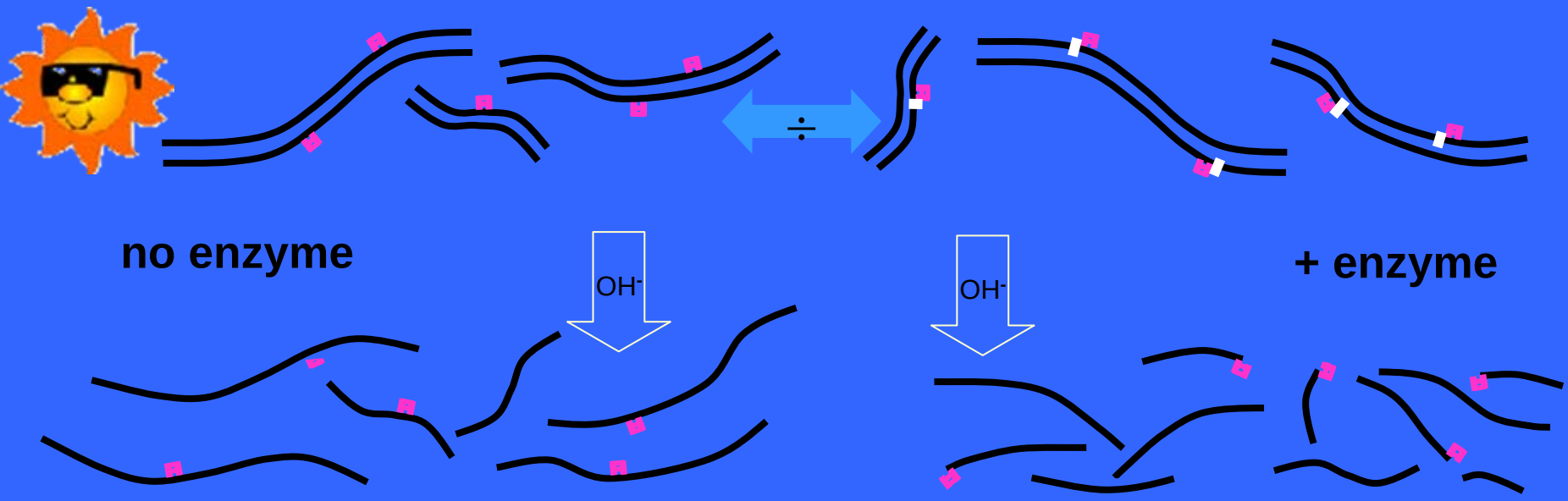
Quantifying DNA Damage

- Acid Hydrolysis & Paper Chromatography
  - Setlow, R.B. and W.L. Carrier, Pyrimidine dimers in ultraviolet-irradiated DNA's. *Journal of Molecular Biology*. **17, 237-251** (1966).
- Alkaline Gradient Centrifugation
  - McGrath, R.A. and R.W. Williams, Reconstruction in vivo of irradiated Escherichia cole deoryribonucleic acid; the rejoining of broken peices. *Nature*. **212, 534-535** (1966).
- Antibodies
  - Mitchell, D.L. and B.S. Rosenstein, The use of specific radioimmunoassays to determine action spectra for the photolysis of (6- 4) photoproducts. *Photochemistry and Photobiology*. **45, 781-786** (1987).
- Comet Assay
  - Olive, P.L., J.P. Banath, and R.E. Durand, Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cells measured using the "comet" assay. *Radiation Research*. **122, 86-94** (1990).
- Gel Electrophoresis & Number Average Length Analysis
  - Freeman, S.E., A.D. Blackett, D.C. Monteleone, R.B. Setlow, B.M. Sutherland, and J.C. Sutherland, Quantitation of Radiation-, Chemical-, or Enzyme-Induced Single Strand Breaks in Nonradioacnzymatictive DNA by Alkaline Gel Electrophoresis: Application to Pyrimidine Dimers. *Analytical Biochemistry*. **158, 119-129** (1986).

Quantifying DNA Damage by Number Average Length Analysis



Quantifying DNA Damage by Average Length Analysis

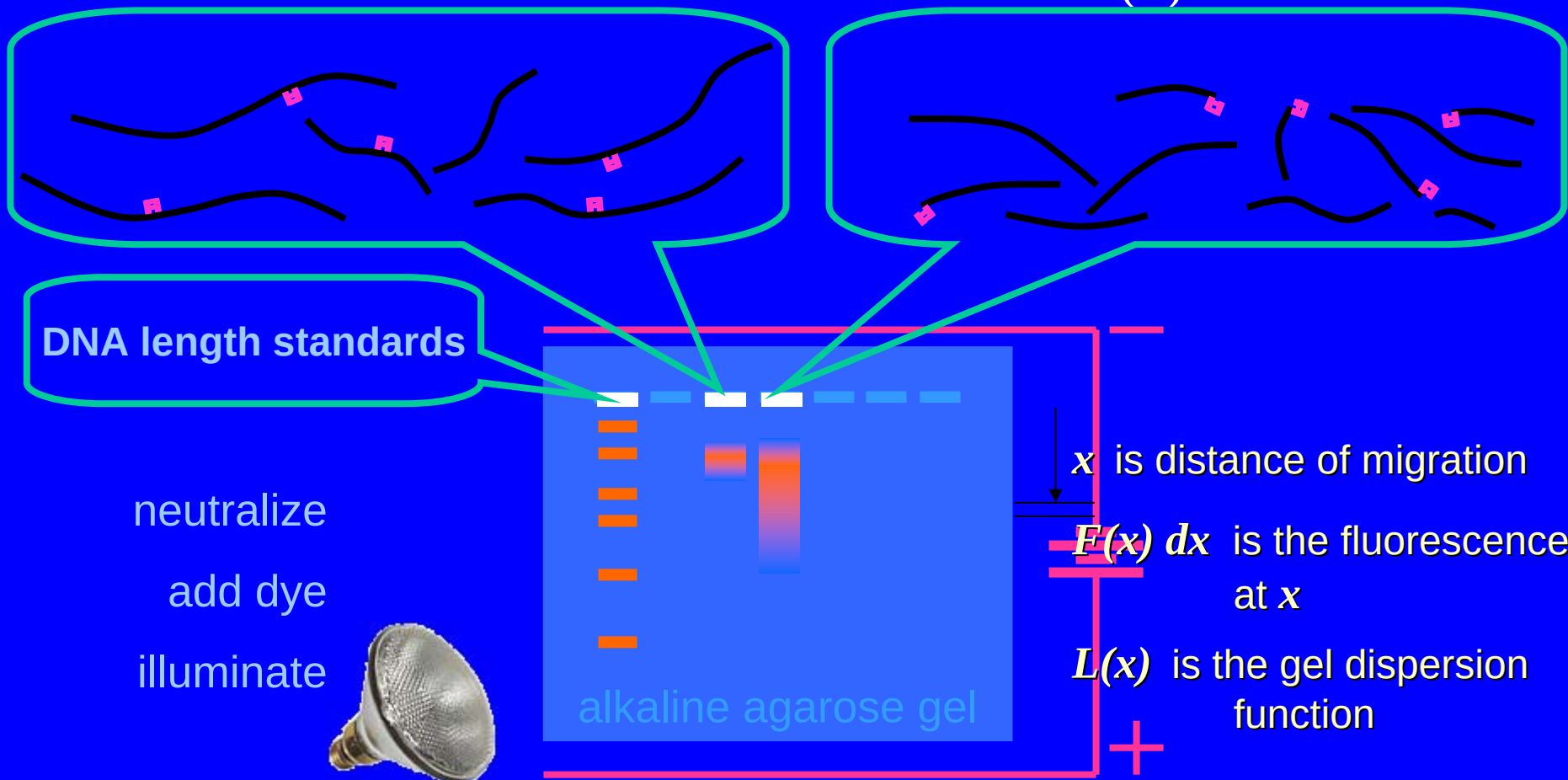


$$\bar{L}_n = \frac{\sum_{i=1}^{N} L_i}{N}, \text{ where } \bar{L}_n \text{ is the number average length of the DNA}$$

$$\Phi = \frac{1}{\bar{L}_n(+enz)} - \frac{1}{\bar{L}_n(-enz)}, \text{ where } \Phi \text{ is the number of lesions per length of DNA}$$

Quantifying DNA Damage by Average Length Analysis and Gel Electrophoresis

$$\overline{L}_n = \frac{\sum_{i=1}^{N} L_i}{N} = \frac{\sum_{L=1}^{\infty} L\,N_L}{\sum_{L=1}^{\infty} N_L} = \frac{\int F(x)\,dx}{\int \frac{F(x)}{L(x)}\,dx}$$



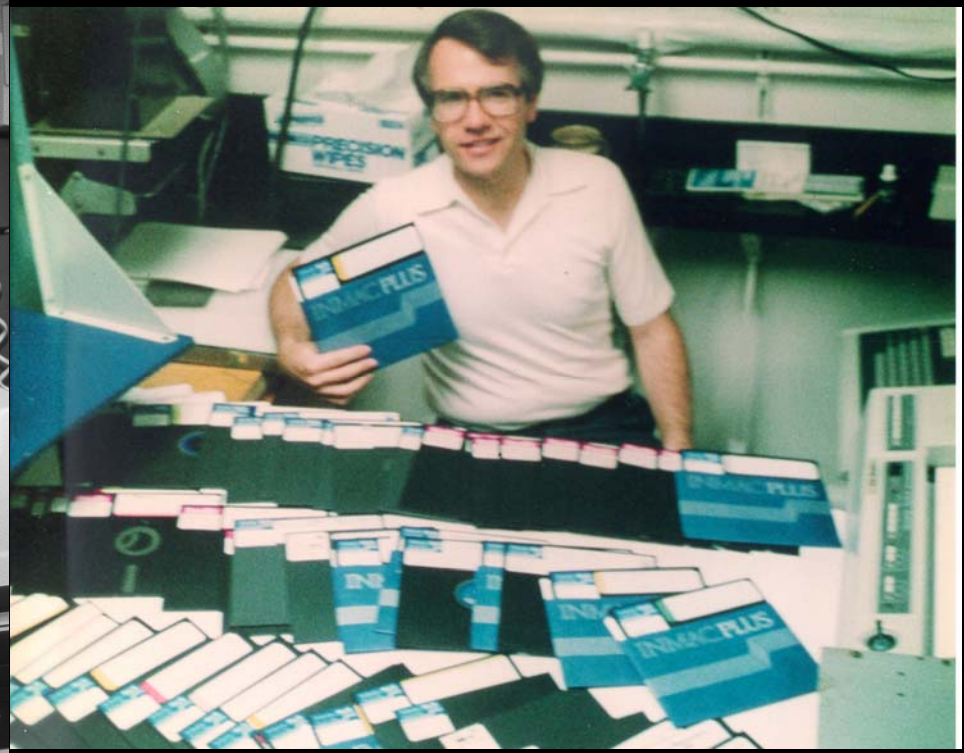
Quantifying DNA Damage by Average Length Analysis and Gel Electrophoresis: **What is Required?**

$F(x)$ → sensitive & linear detection of fluorescence

$L(x)$ → function to relate DNA size and distance of migration

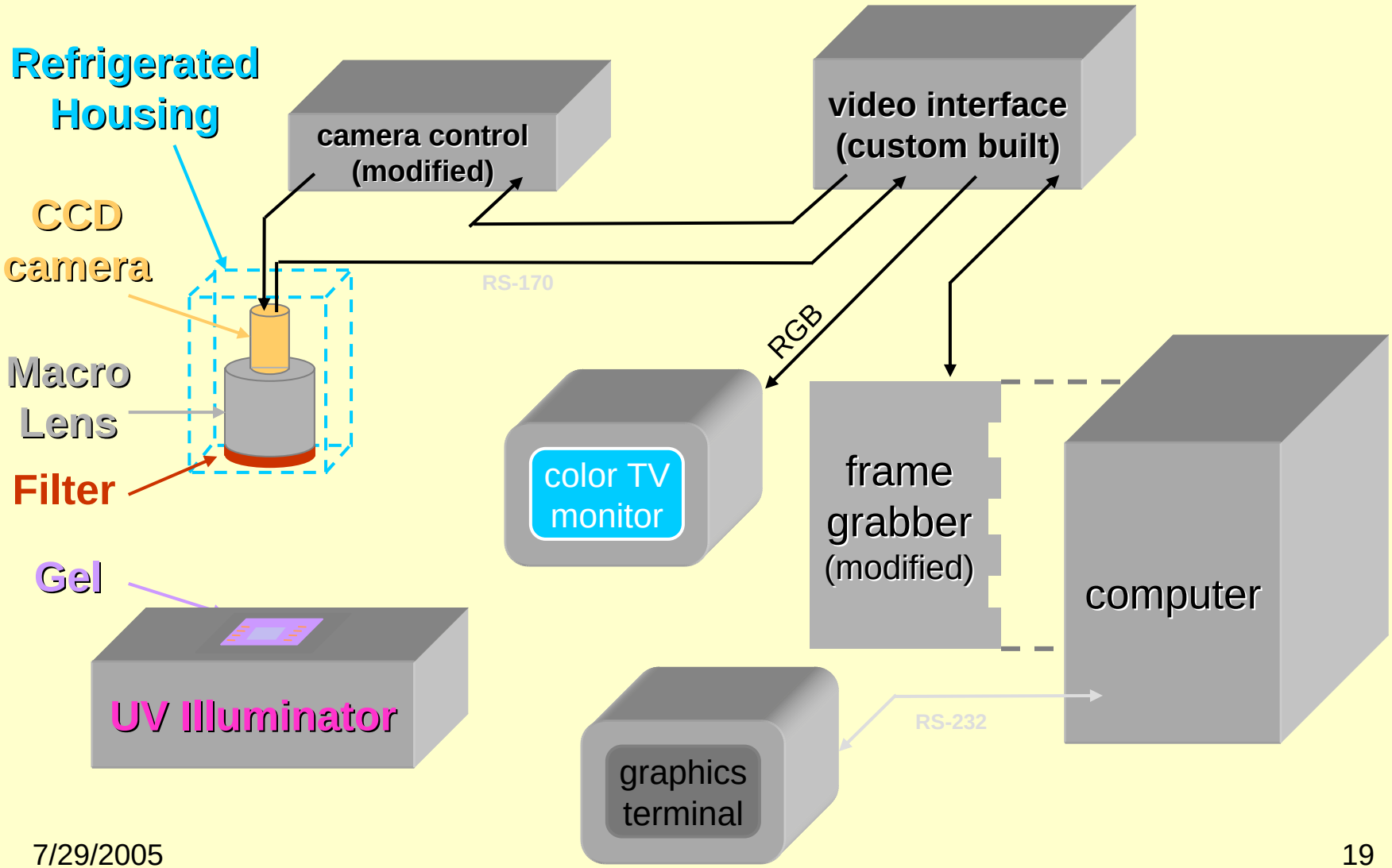
$$\frac{\int F(x)\,dx}{\int \frac{F(x)}{L(x)}\,dx}$$ → transfer data to computer

Record Fluorescence by Photography

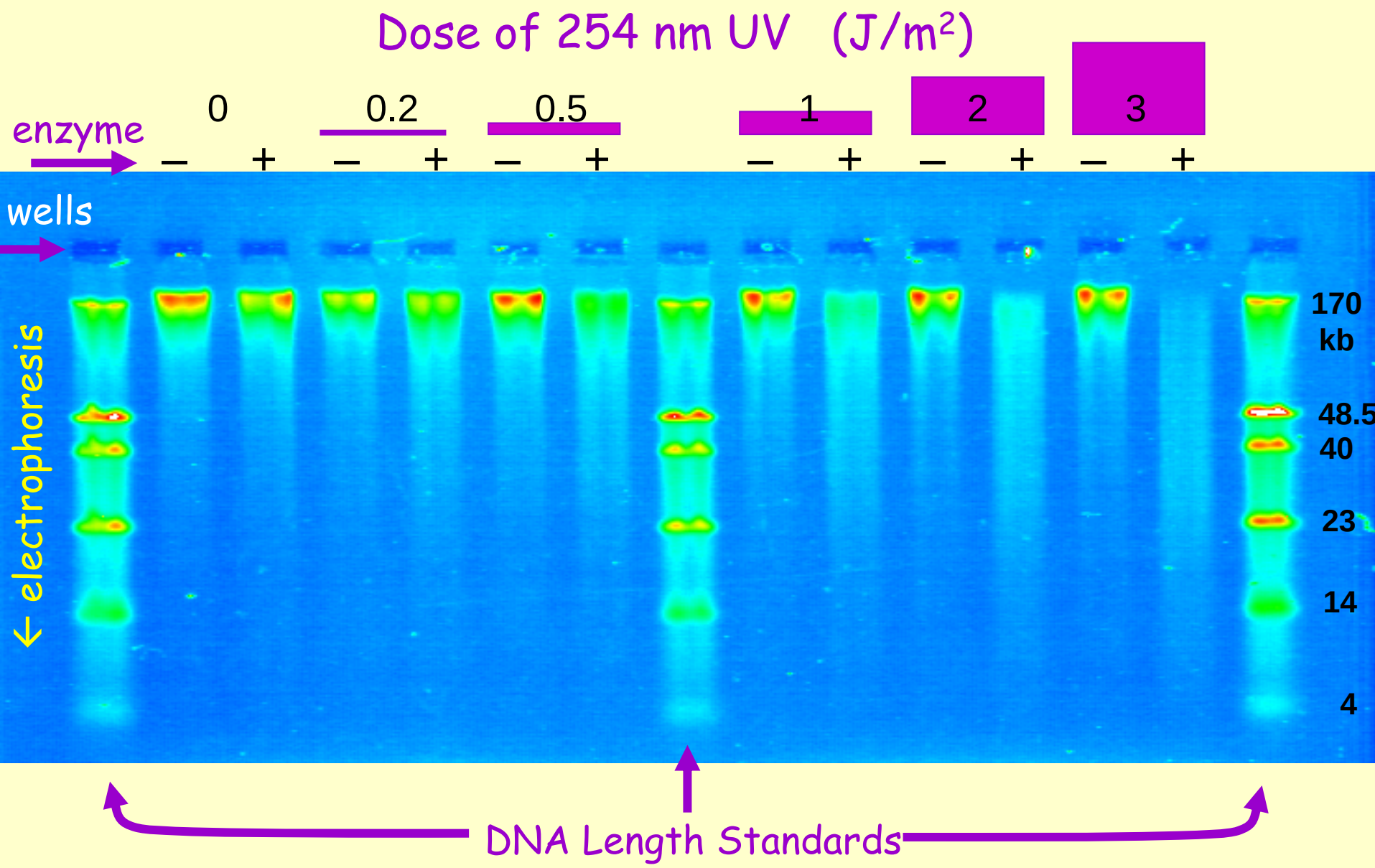


IMAGESystem: First CCD Imager of Gel Fluorescence

Sutherland, J.C., B. Lin, D.C. Monteleone, J. Mugavero, B.M. Sutherland, and J. Trunk, Electronic Imaging System for Direct and Rapid Quantitation of Fluorescence from Electrophoretic Gels: Application to Ethidium Bromide-Stained DNA. *Analytical Biochemistry*. **163, 446-457** (1987).



IMAGESystem: Alkaline Agarose Gel of Human DNA UV Irradiated *In Vitro*

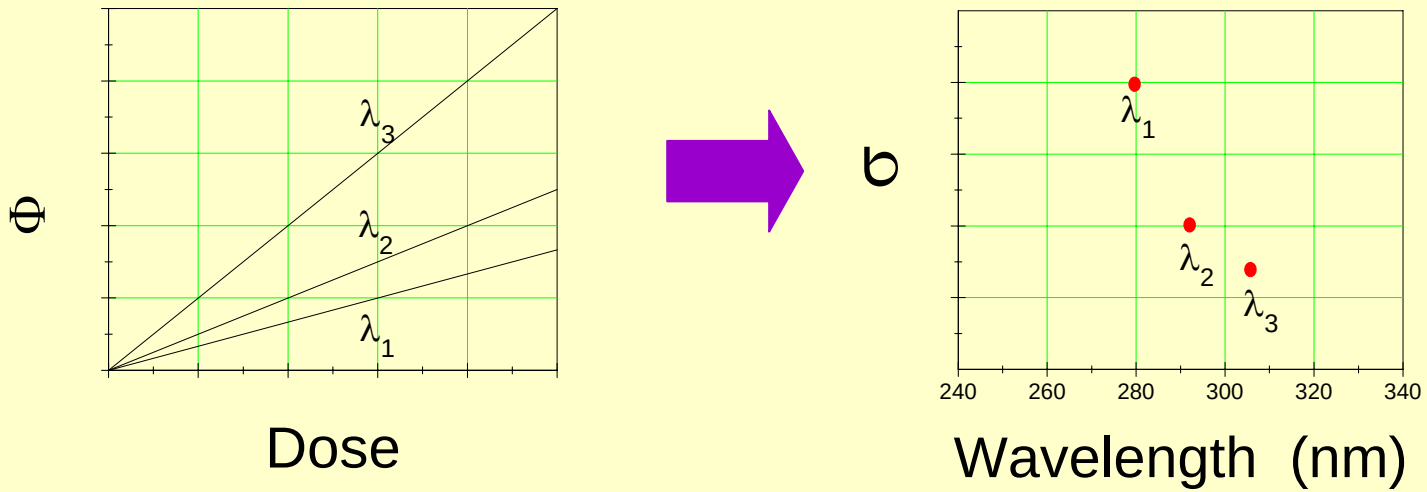


Action Spectra

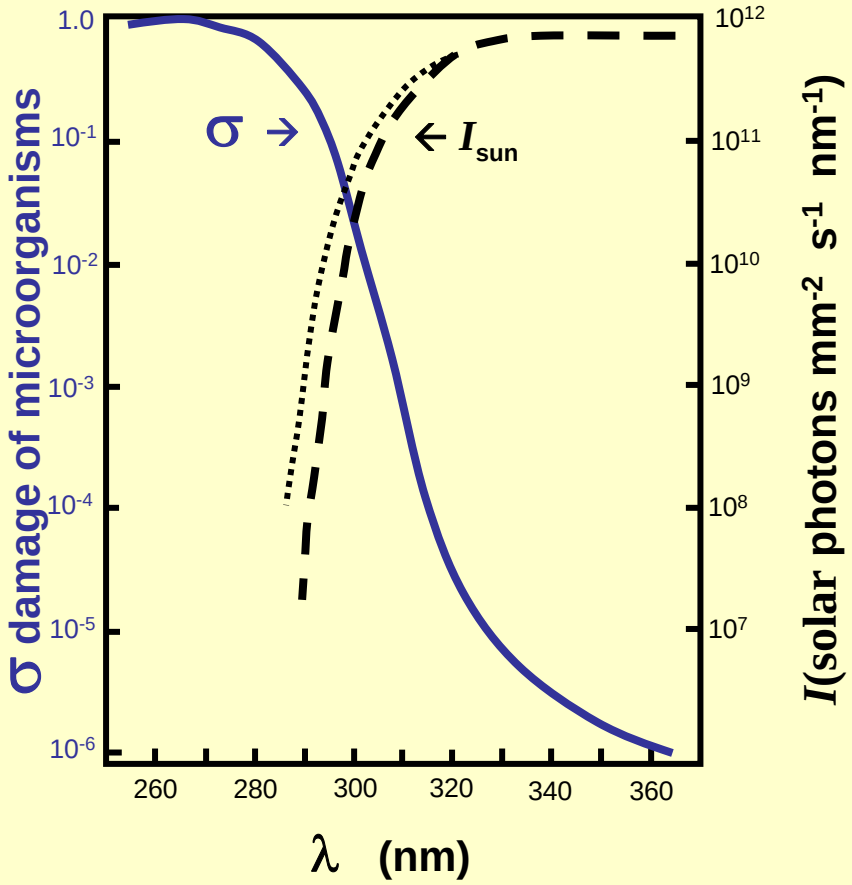
How Effective are Different Wavelengths?

- If effect is a **linear** function of dose for all λ:
  - Photo**chemical** reactions, e.g. dimers, are linear
  - Action spectrum plot of the slopes vs. λ.
  - Physicists would refer to this as cross section, σ

$$\Phi_\lambda = \sigma_\lambda D(\lambda)$$



DNA Action Spectrum and O_3 Depletion



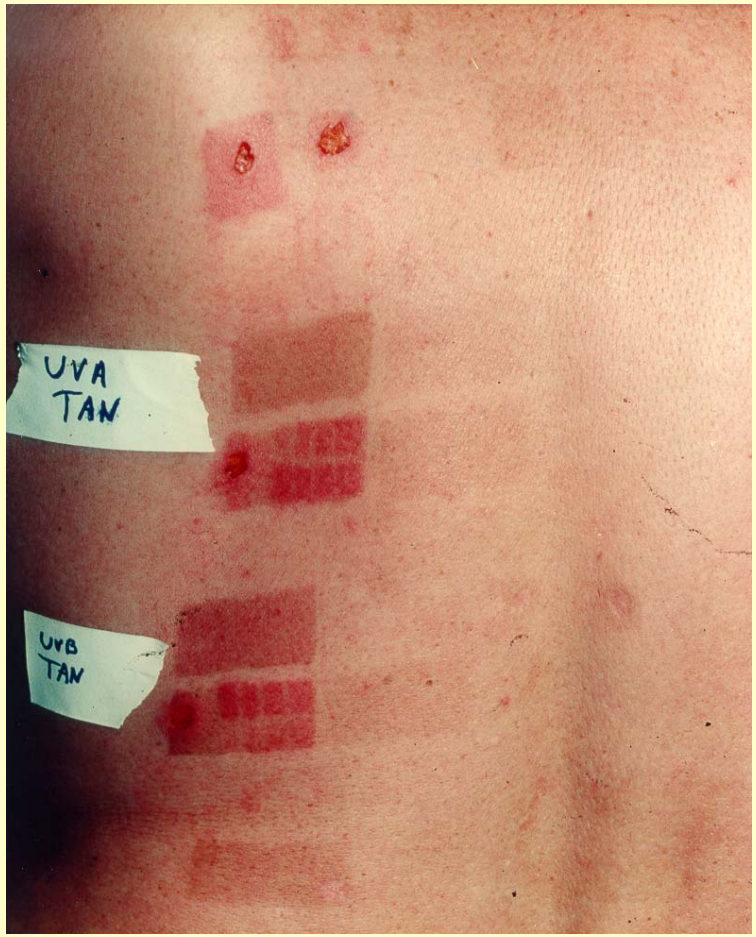
- Action spectrum for biological damage ≈ DNA absorption & rises rapidly for $\lambda < 320$ nm
- Sunlight drops rapidly for $\lambda <$ 320 nm
- Ozone depletion causes shift to shorter wavelengths
- Amplification of effect?

$$< effect > = \int_{\lambda} \sigma(\lambda)\, I(\lambda)\, d\lambda$$

UV DNA Damage in Human Skin

Collaboration with R.W. Gange, Dermatology, Harvard Medical School

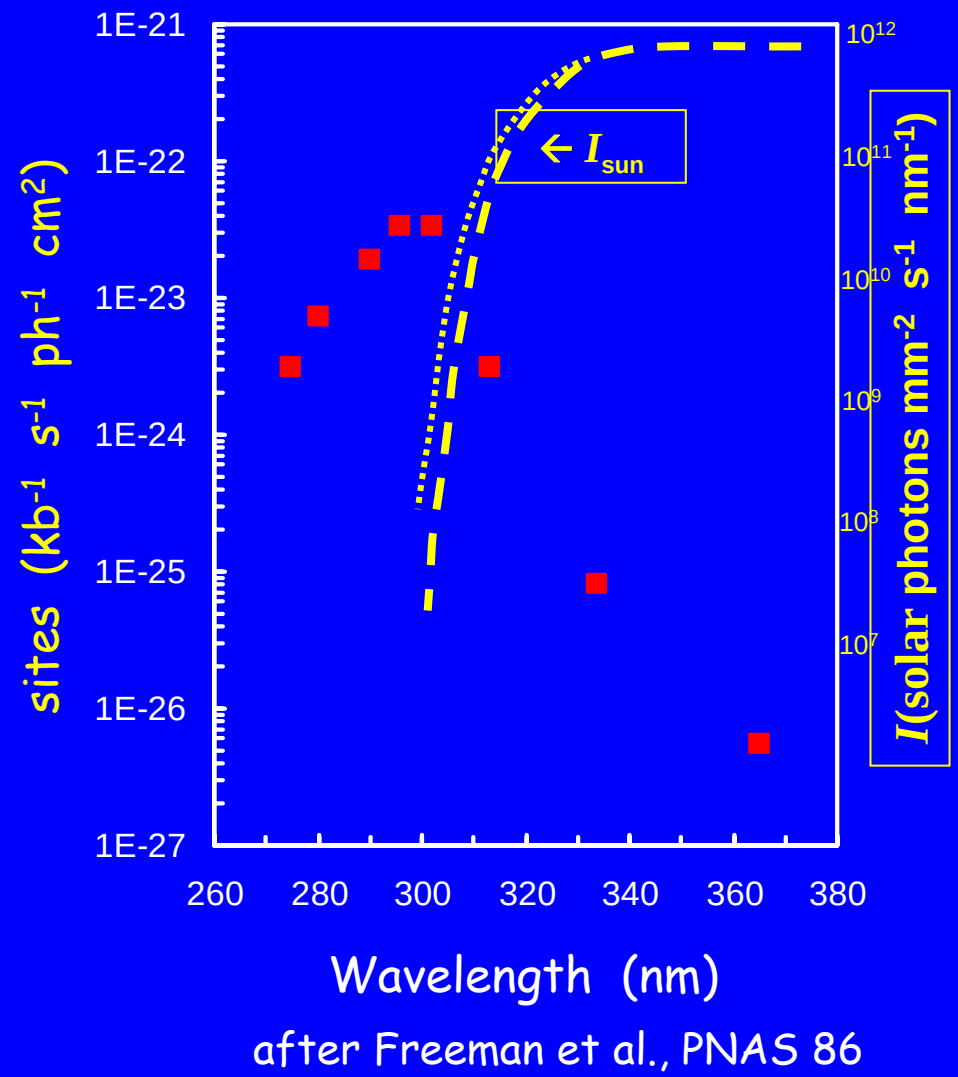
Bill Gange with assistants and volunteer



Back of volunteer showing UV irradiated areas and biopsies

Wavelength Dependence for Dimers in DNA of Human Skin

Collaboration with R.W. Gange, Dermatology, Harvard Medical School



after Freeman et al., PNAS 86

- Data from 35 volunteers
- Each point is average of ≥ 5, normalized to average of all at 302 nm (all volunteers)
- Peak near 300 nm, NOT 260nm
- Dimers formed at 365 nm?

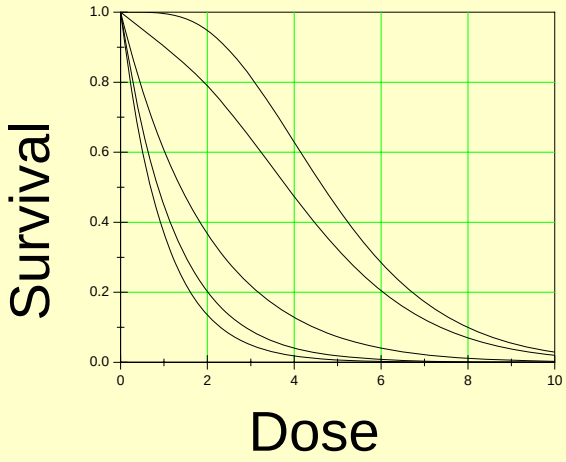
$$< effect > = \int_{\lambda} \sigma(\lambda)\, I(\lambda)\, d\lambda$$

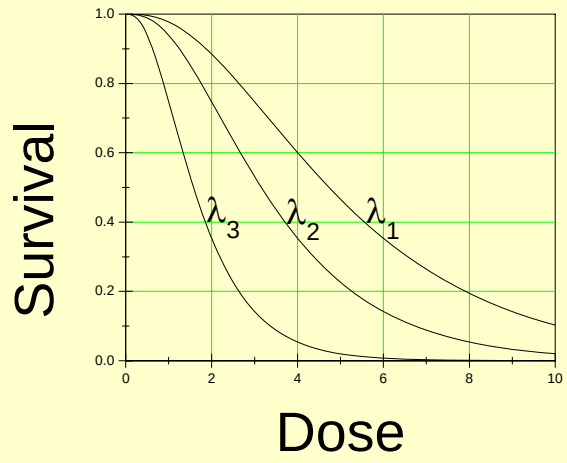
- A 50% reduction in O_3 → 250% ↑ in integrated UV burden at 40° N. latitude

Action Spectra

For Biological Endpoints

- If effect is a **NON**-linear function of dose:
  - **An** Action spectrum is plot of reciprocal of the dose required to produce a given effect vs. λ.
  - Changing end point changes shape of spectrum
  - Many photo-biological reactions are NON-linear:
    - Cell survival
    - Mutation induction
  - **May** be possible to produce an endpoint-independent action spectrum if certain conditions hold (Sutherland, Photochem. Photobiol 2002)



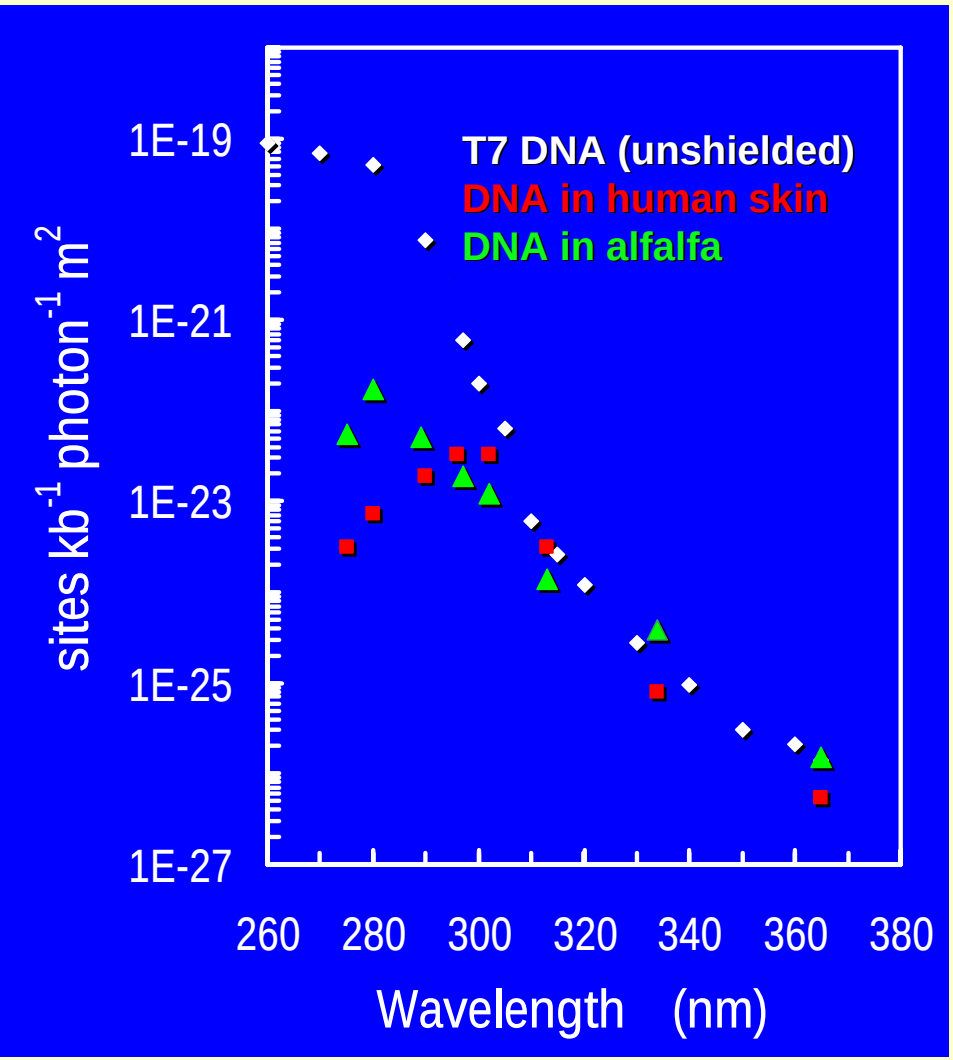


Plants & Ozone Depletion

UV induced DNA Damage in Crop Plants

- Alfalfa, Rice, Soybean, Cotton, Sorghum, .
- DNA Isolation Greatly Different than Human Cells, Bacteria, ...
- Action Spectrum for Dimer Formation in Alfalfa
- Repair: Alfalfa, Rice & Soybean

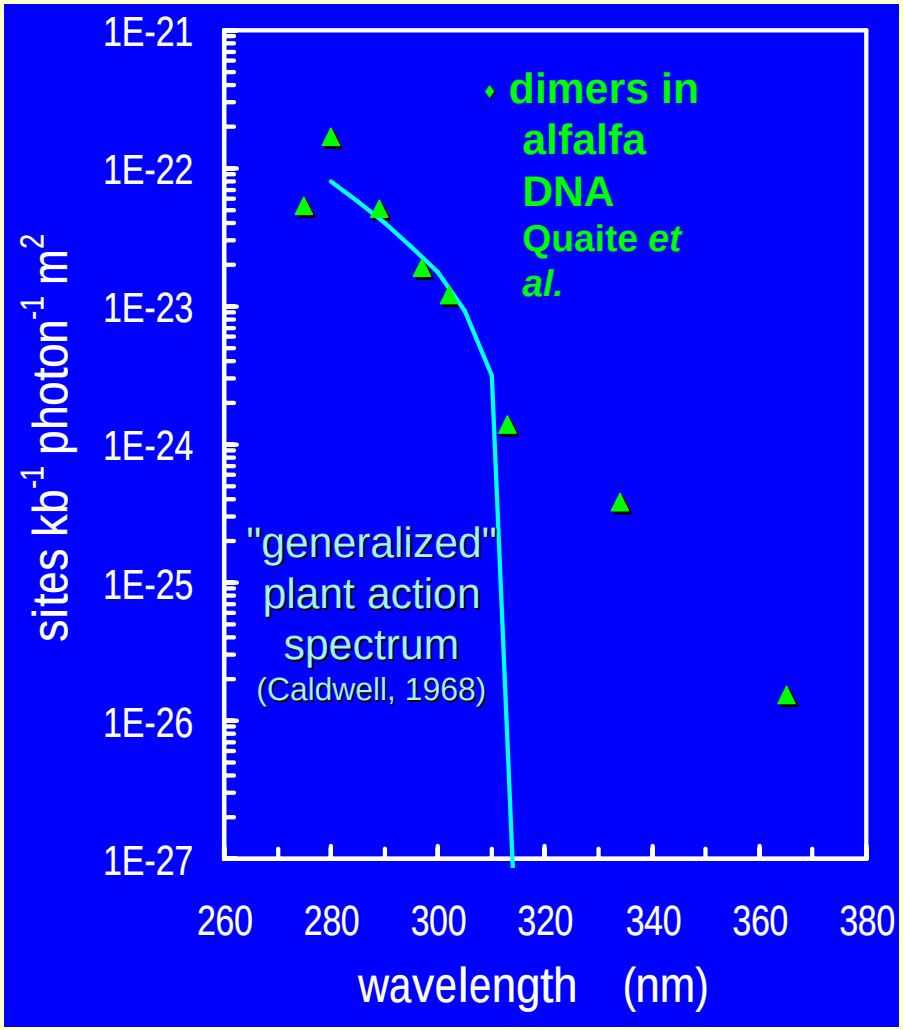
Action Spectrum for Dimers in Alfalfa



- Local absorption ↓ σ for λ < 310 nm in skin and alfalfa.
- Dimers apparently formed to 365 nm in all systems.

- Freeman *et al.* PNAS **86**, 5605-5609
- Quaite *et al.* Nature **358**, 576-578
- Emrick and J. Sutherland, Photochem. Photobiol. 49, 39S

Comparison with "Generalized" Action Spectrum



- good agreement for $\lambda < 310$ nm
- "generalized" action spectrum ignored $\lambda > 315$ nm
- This overestimates effects of O_3 depletion

Quaite, F.E., B.M. Sutherland, and J.C. Sutherland, Action Spectrum for DNA Damage in Alfalfa Lowers Predicted Impact of Ozone Depletion. *Nature*. 358, 576-578 (1992)

- Cross section for damaging DNA low in UVA
- But, intensity UVA >> UVB
- All increase in UV due to O_3 depletion in UVB

$$Fractional\ Increase = \frac{\int_{290}^{380} \sigma I_{post}\, d\lambda - \int_{290}^{380} \sigma I_{pre}\, d\lambda}{\int_{290}^{380} \sigma I_{pre}\, d\lambda}$$

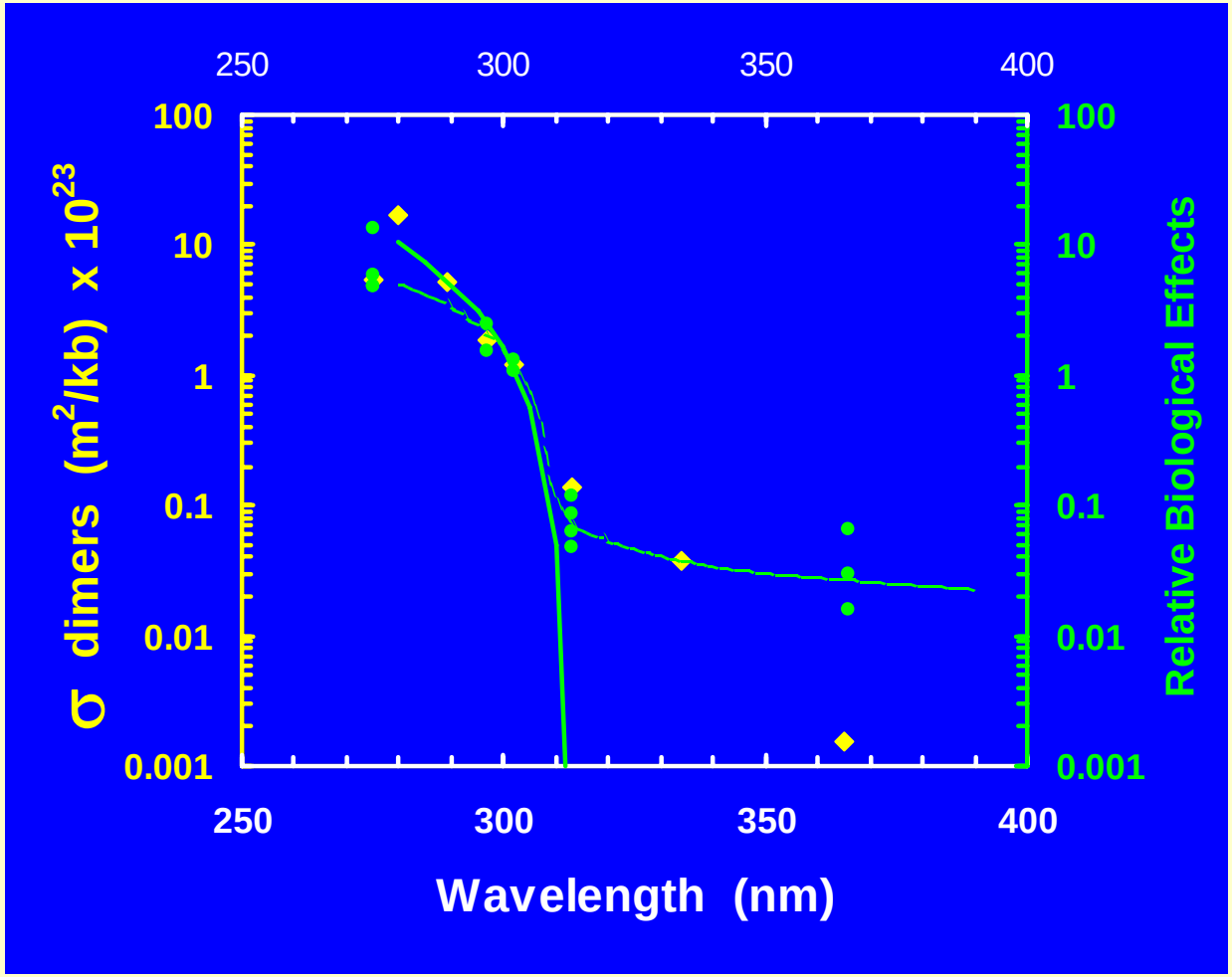
Because UVA does not change

$$Fractional\ Increase = \frac{\int_{290}^{320} \sigma I_{post}\, d\lambda - \int_{290}^{320} \sigma I_{pre}\, d\lambda}{\int_{290}^{320} \sigma I_{pre}\, d\lambda + \int_{320}^{380} \sigma I_{pre}\, d\lambda}$$

Ignoring the UVA makes the denominator too small and thus the ratio too large

Generalized Plant Action Spectrum Revisited

Flint, S.D. and M.M. Caldwell, A biological spectral weighting function for ozone depletion research with higher plants. *Physiologia Plantarum*. 117, 137-144 (2003).



- Old spectrum "incomplete"
- Damage at 365 > dimers
- That's OK: different endpoints
- Is there structure in UVA?
- Are there other absorbers?
- Need more wavelengths!

Depletion of Stratospheric Ozone

- **Is** happening & there will likely by other **anthropogenic** mechanisms.
- **Will** impact the biosphere!
- Action spectra for unshielded DNA overestimate effects for higher organisms.
- Action spectra that ignore $\lambda > 320$ nm overestimate effects for **all** organisms at high latitudes.
- Tunable, high-intensity sources needed for near UV, *e.g.* Jefferson Lab FEL.

Sutherland, J.C.,

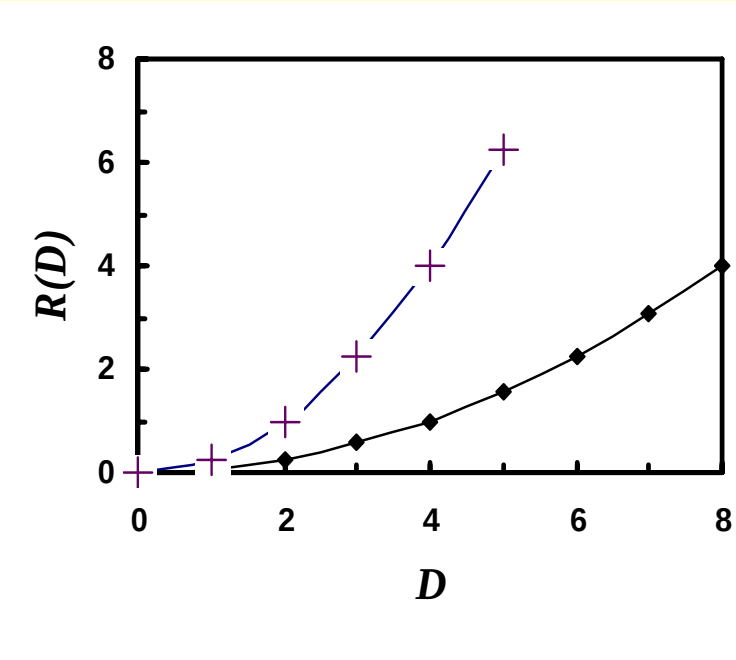
Biological Effects of Polychromatic Light.

Photochemistry and Photobiology. **76, 164-170** (2002)

- Should be possible to predict effects of broad spectrum (e.g. sunlight) from monochromatic dose response data if all have the same shape
- Same shape means that by changing scale of Dose axes, responses can be mapped on top of each other.
- Has not been tested

Spectral Weighting Function

$$R\left(\lambda_{ref}, w(\lambda)D\right) = R\left(\lambda, D\right)$$



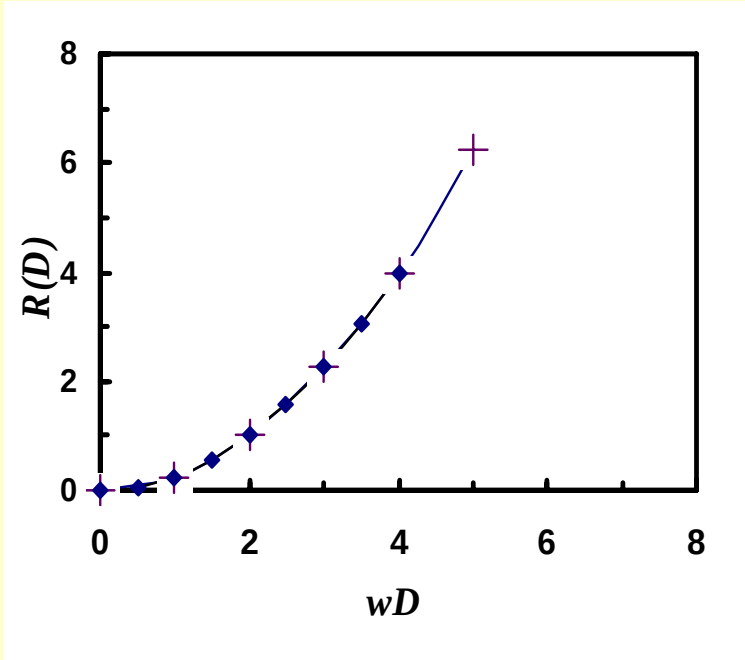


Table 1 Summary of the monochromatic and polychromatic dose response functions.

Type Of Dose Response	Monochromatic Dose Response Function	Polychromatic Dose Response Function
linear	$R(D)=\sigma(\lambda)D(\lambda)$	$R(I(\lambda,t))=\int_{t_1}^{t_2}\int_{\lambda_1}^{\lambda_2}\sigma(\lambda)I(\lambda,t)d\lambda dt$
power law	$R(\lambda,D)=R_O\left(\frac{D}{D_O(\lambda)}\right)^p$	$R(I(\lambda,t))=R_O\left(\iint\frac{I(\lambda,t)}{D_O(\lambda)}d\lambda\,dt\right)^p$
simple exponential survival	$S(D)=e^{-\frac{D}{D_0(\lambda)}}$	$S(I(\lambda,t))=e^{-\iint\frac{I(\lambda,t)}{D_0(\lambda)}d\lambda\,dt}$
shouldered exponential survival	$S(\lambda,D)=1-\left(1-e^{-\frac{D}{D_0(\lambda)}}\right)^n$	$S(I(\lambda,t))=1-\left(1-e^{-\iint\frac{I(\lambda,t)}{D_0(\lambda)}d\lambda\,dt}\right)^n$

Research Opportunities
(Potential Collaborators)

- Can we predict effects of polychromatic spectra?
  - Photoproduct formation (B. Sutherland, BNL)
  - Cell & viral survival and mutation induction? (R. Roper and K. Sullivan, ECU)
  - Photodynamic therapy? (R. Allison, ECU)
  - Wound healing? (K. Sullivan, ECU)
  - Crop productivity?
  - Cancer induction?
- Why are DNA photoproducts formed by 365?
- What other photoproducts are formed by UVA (J Cadet, CNRS Grenoble)

Does UVA Cause Melanoma?