

OPTICAL FILTERING FOR FLUORESCENCE DETECTION – DIFFRACTION GRATING APPROACH

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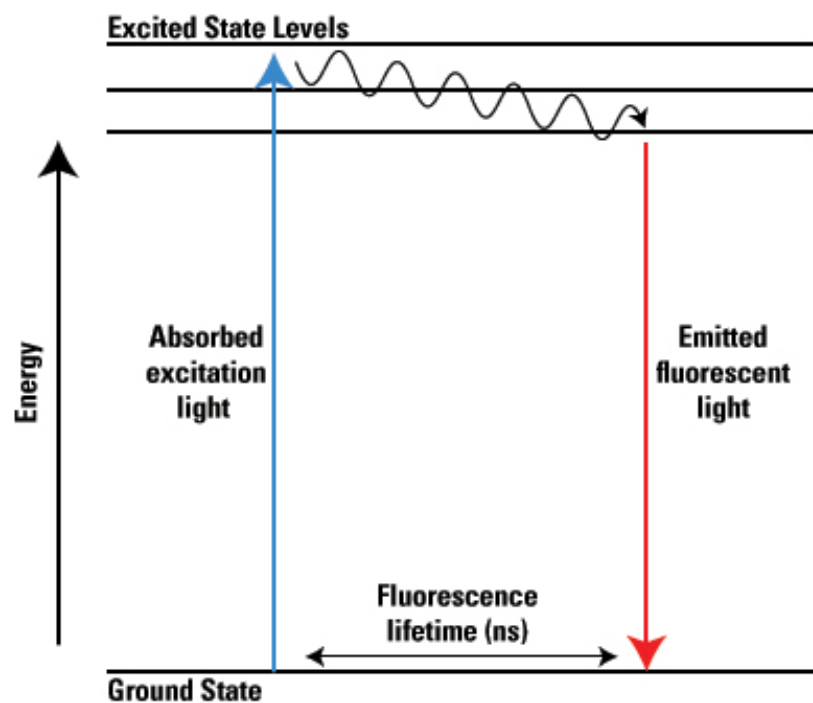
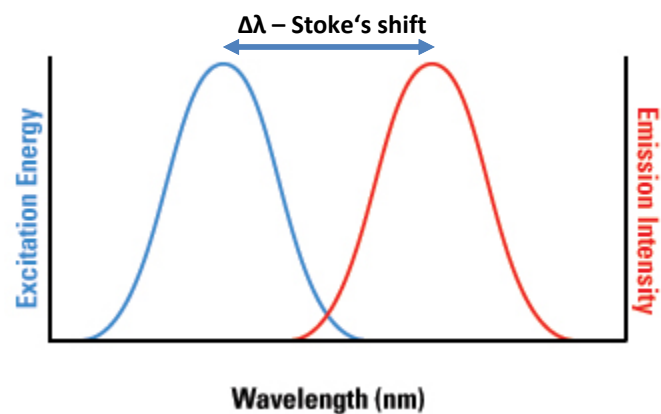


Outline

- Fluorescence detection
 - › Why the need for optical filters in fluorescence detection?
 - › Fluorescence detector structure
- Filtering structures
 - › Diffraction grating based filters
- Results
 - › Period & height dependence
 - › Grating material dependence
 - › Incident angle dependence
- Realistic conditions
- Conclusions

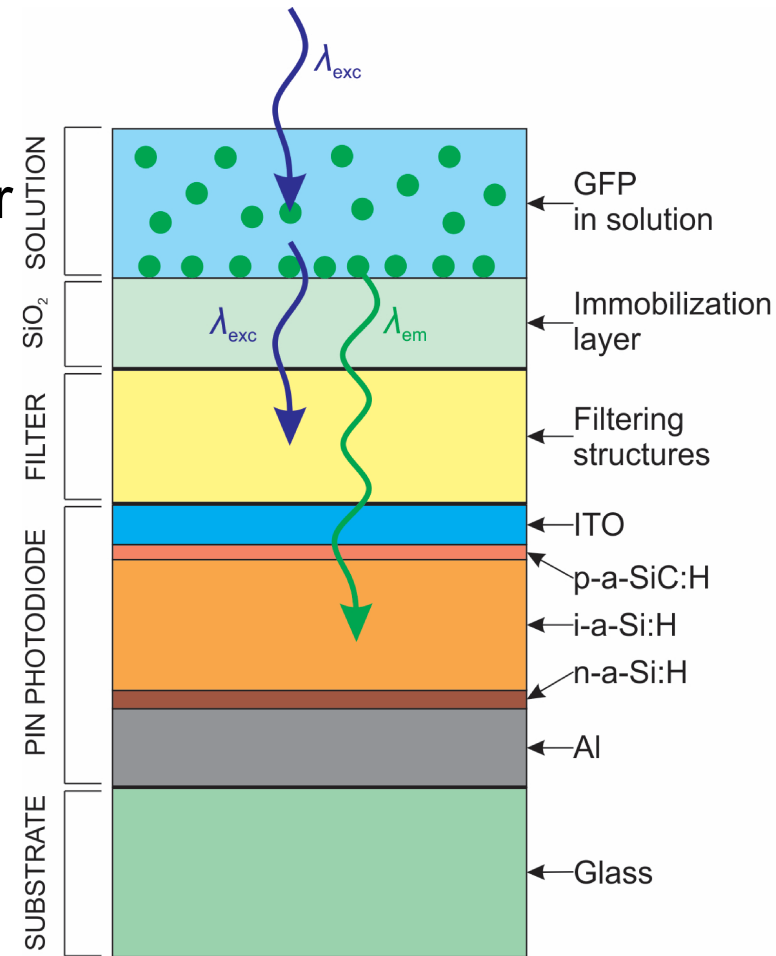
Fluorescence

Basic principles:



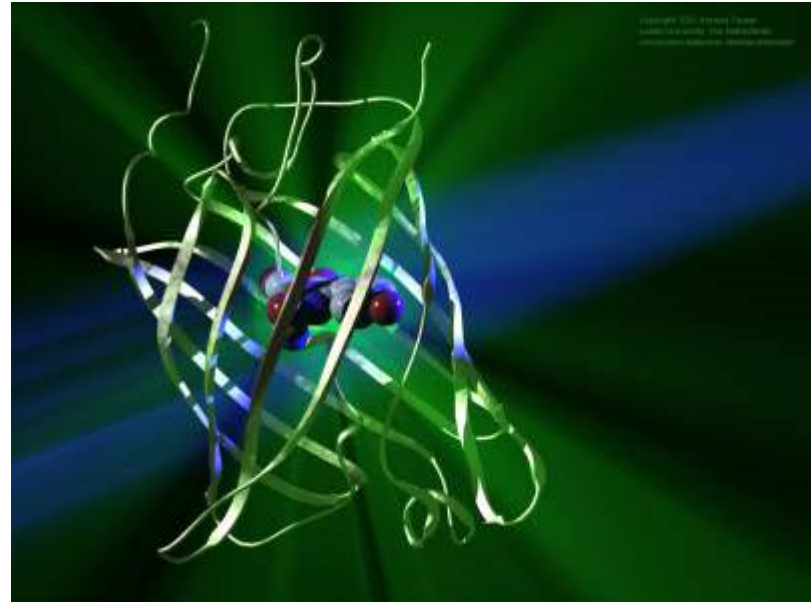
Fluorescence detector structure

- Glass substrate
- Broadband thin film a-Si photo-detector
- Filtering structures
 - › Absorbing filter
 - › Diffraction grating as filter
 - › Combination of both
- SiO₂ immobilization layer
- Fluorophores in solution



Fluorescence & fluorescence detection

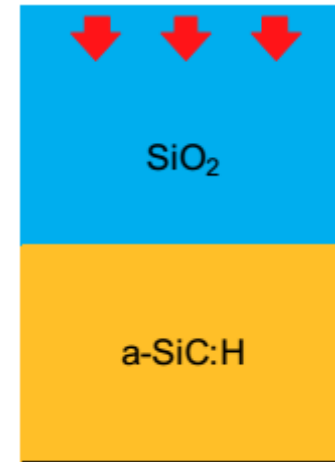
- Focus on a green fluorescence protein (GFP)
- Quantification of biomolecules, drug discovery, etc.
- GFP is **non-toxic!**
- Excitation light (480 nm)
- Emission light (510 nm)
- Small Stokes' shift of only **30 nm !**
- Broadband photo-detectors
- **Selective** optical filtering is essential !



Optical filters - solutions

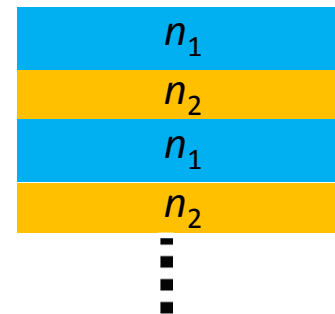
■ Absorbing filters

- › Simple solutions
- › Easy to fabricate
- › Non-efficient for small Stokes' shift fluorophores



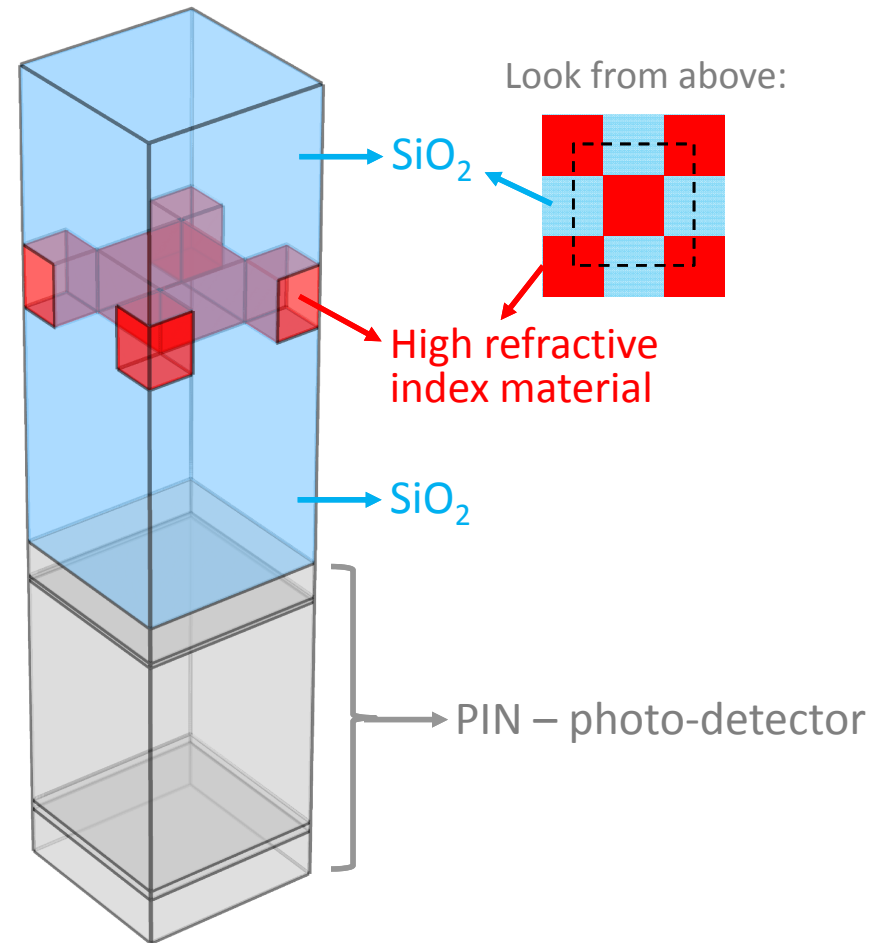
■ Multilayer interference filters

- › Efficient
- › A lot of fabrication steps



Diffraction grating based optical filters

- Grating embedded in low refractive index material
- $n_{\text{grating}} > n_{\text{SiO}_2}$
 - › Grating: TiO_2 and p-a-SiC:H
- Checkerboard like grating
 - › Possible other configurations & shapes

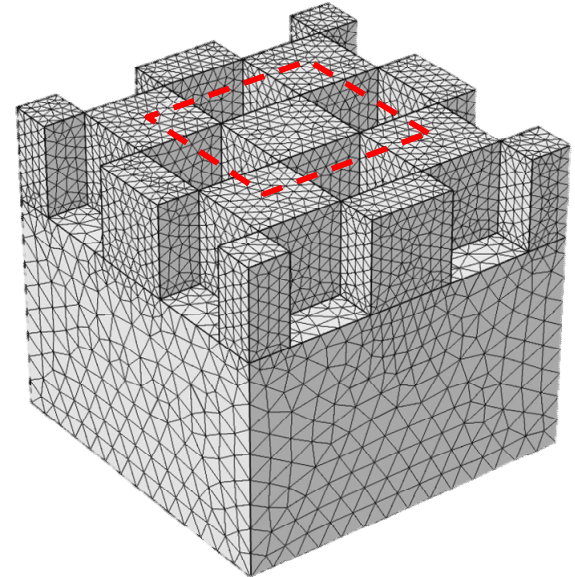
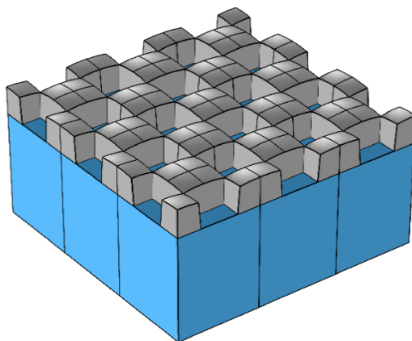
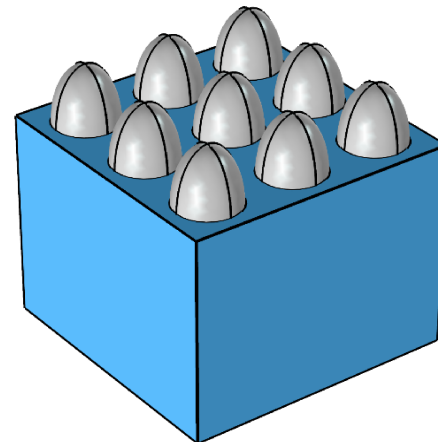


Simulations

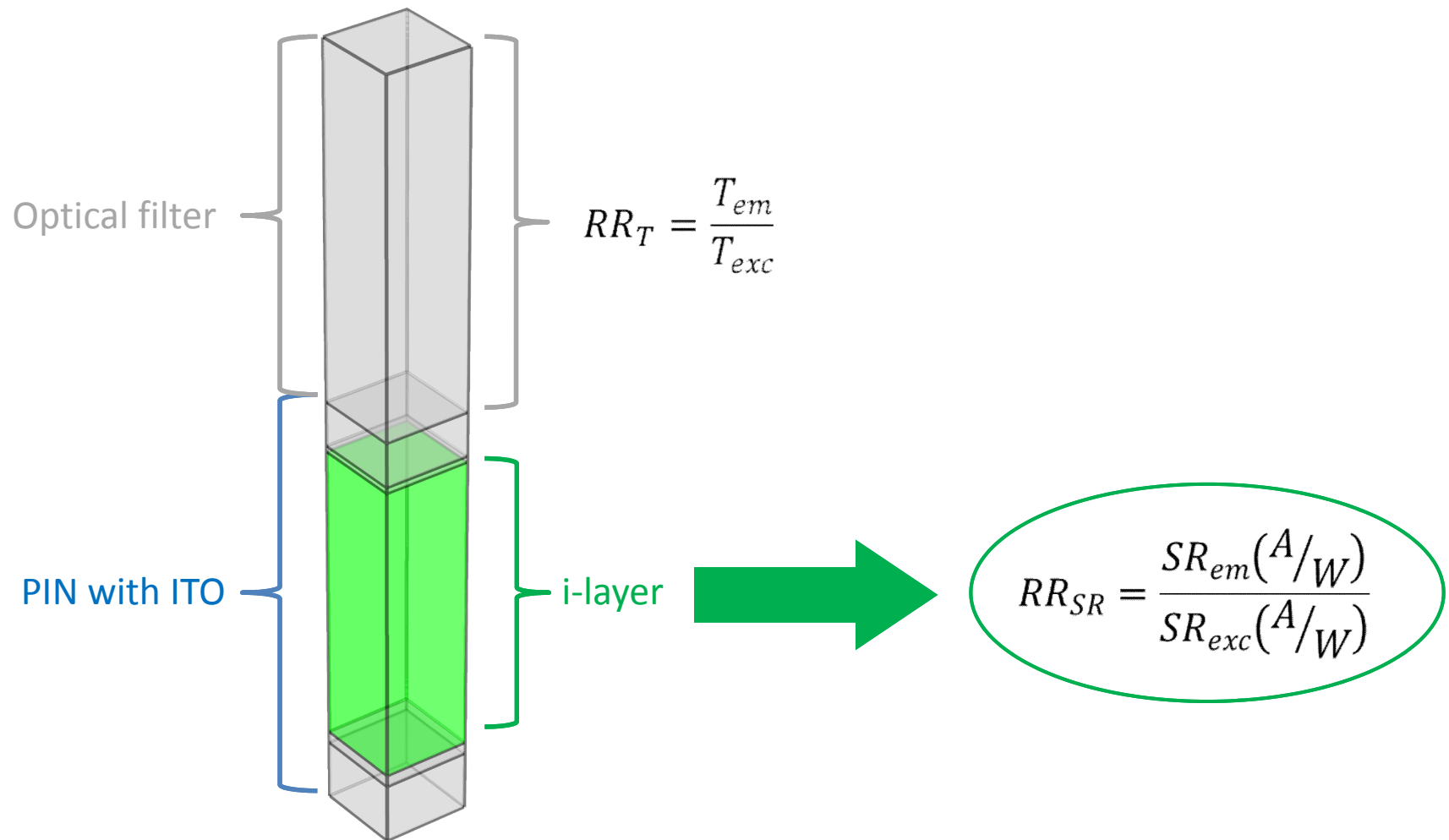
- Simulations using Comsol Multiphysics



- 3-D simulation
- Finite element method – FEM
 - Various shapes & materials

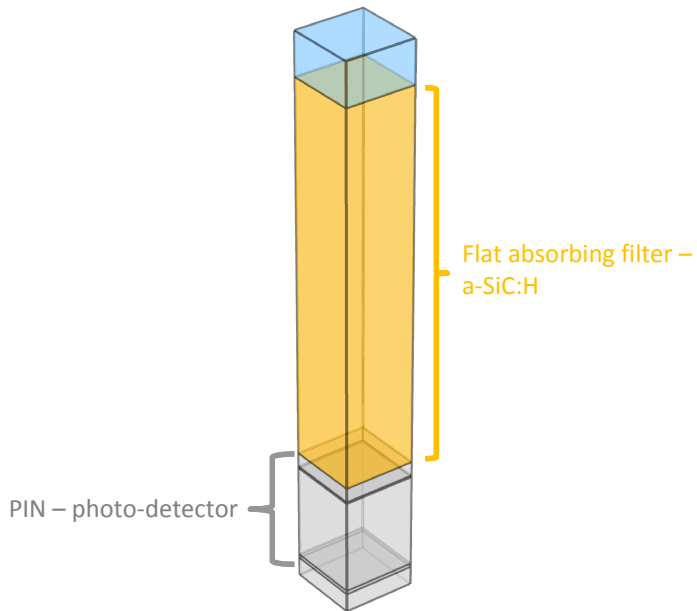
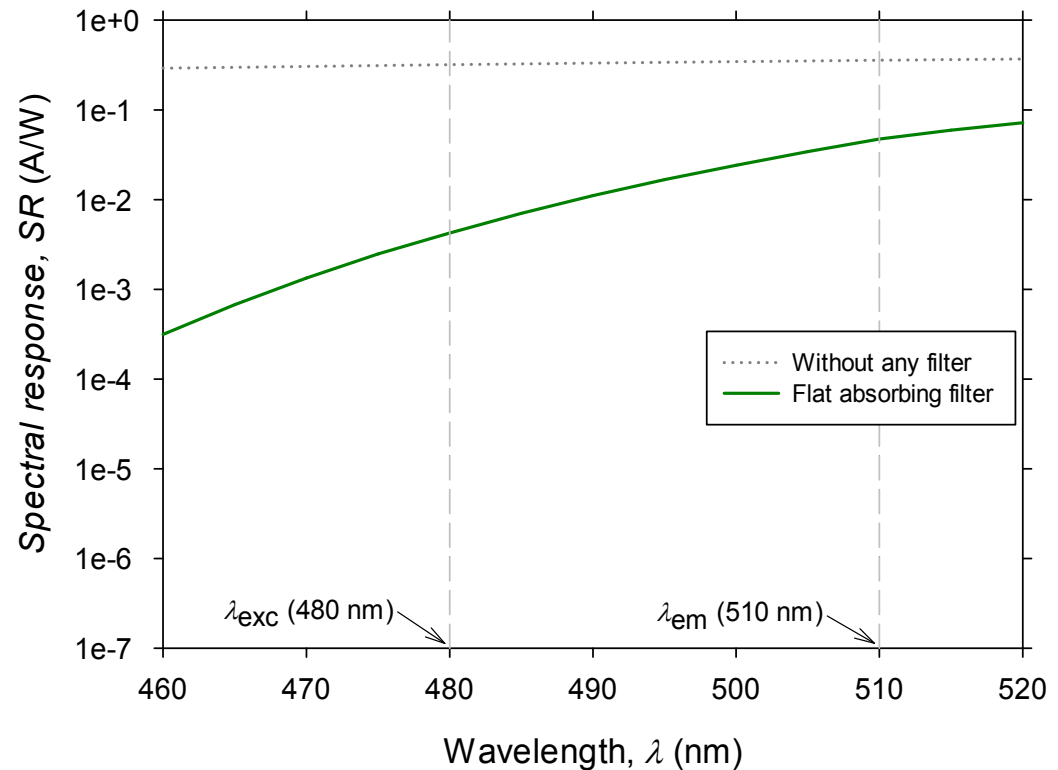


Simulations & evaluating results



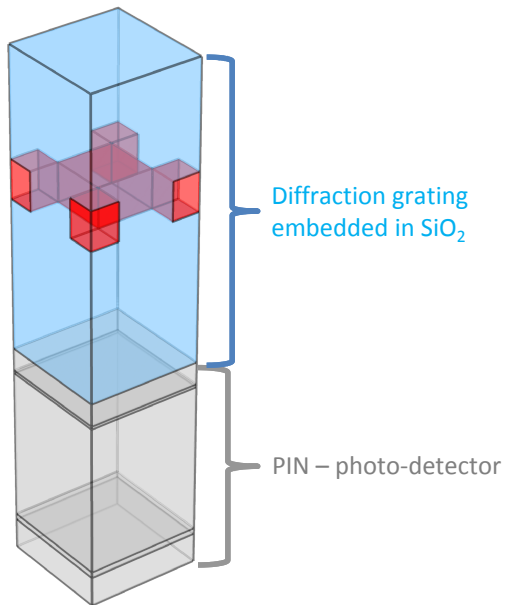
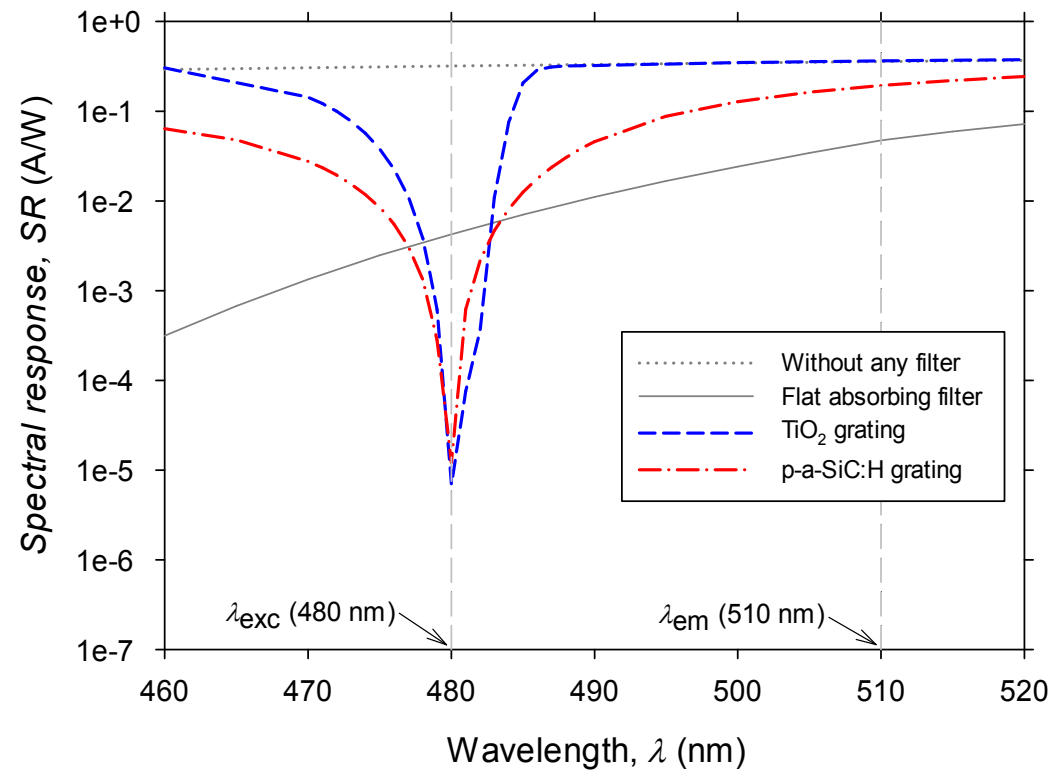
Results – absorbing filters

- Absorbing a-SiC:H filter
 - › reference
- Best ***RRs*** ≈ 11.1



Results – embedded diffraction grating

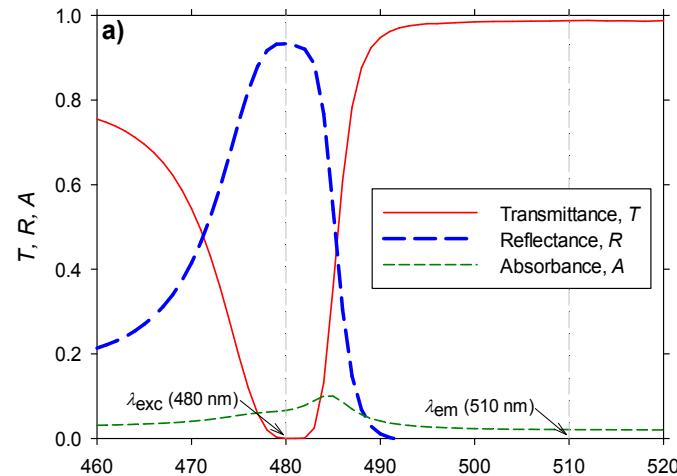
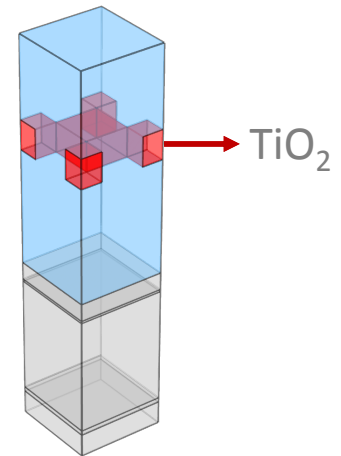
- Notch filter characteristics
- RRs over 10,000**



Absorbing vs. non-absorbing grating

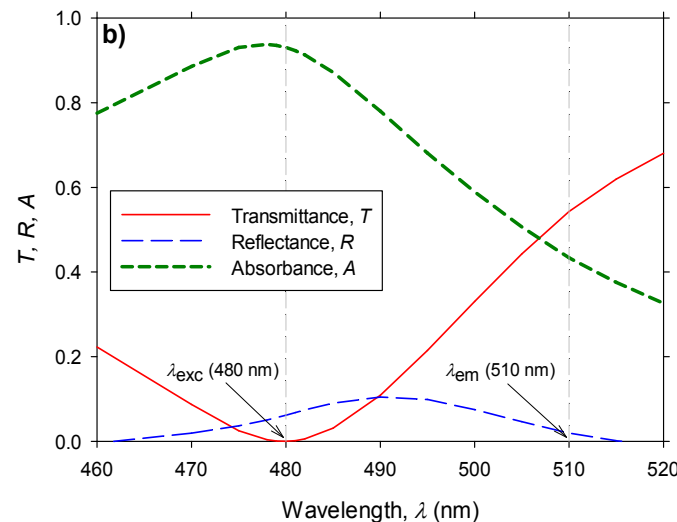
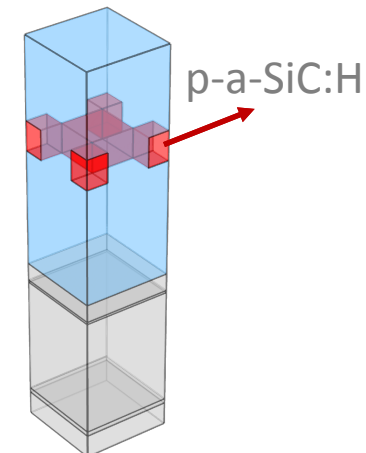
■ Non-absorbing grating

- › TiO_2
- › High reflectance
- › $T_{\text{em}} \approx 0.98$



■ Absorbing grating

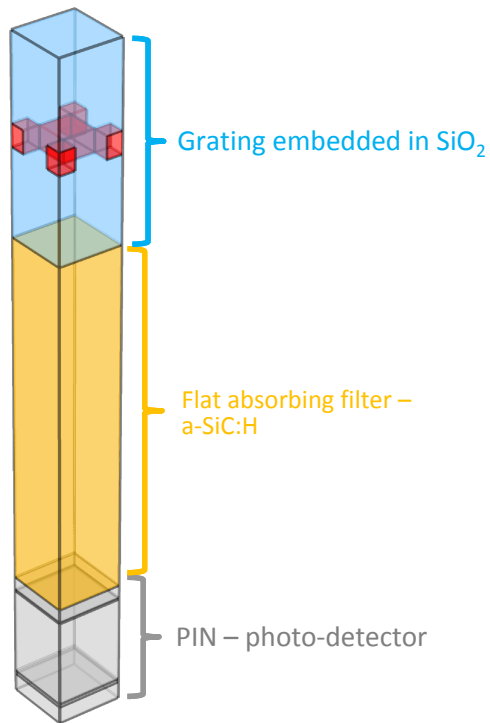
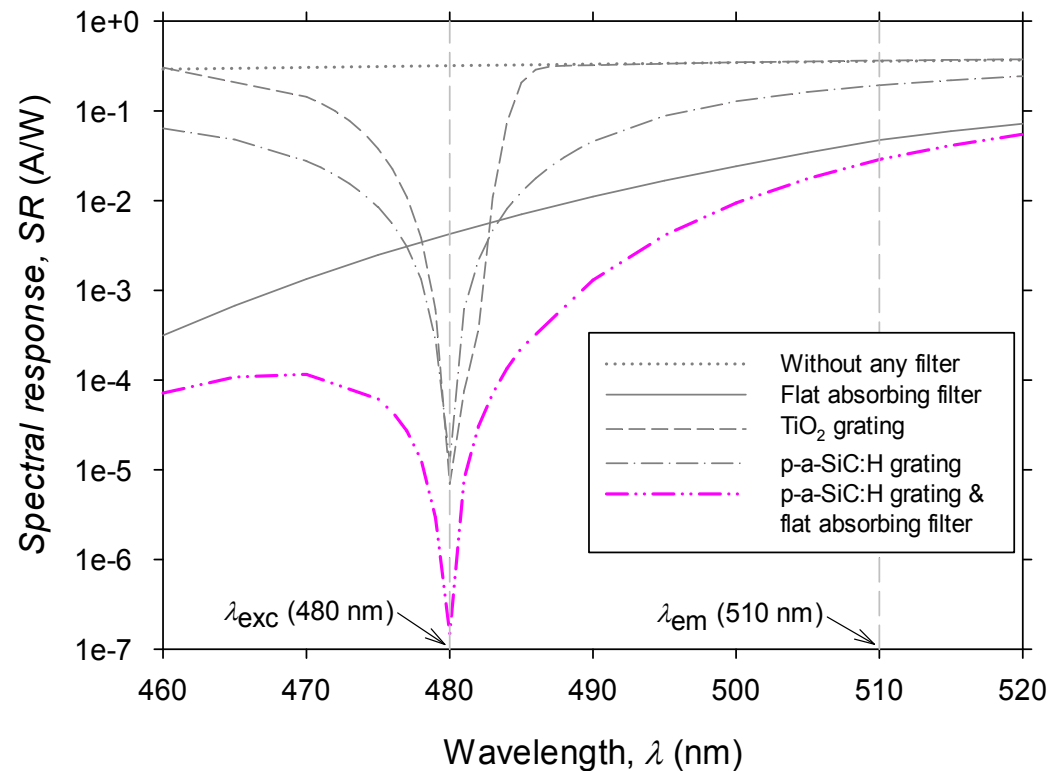
- › p-a-SiC:H
- › High absorption
- › $T_{\text{em}} \approx 0.54$



Results – combination of both solutions

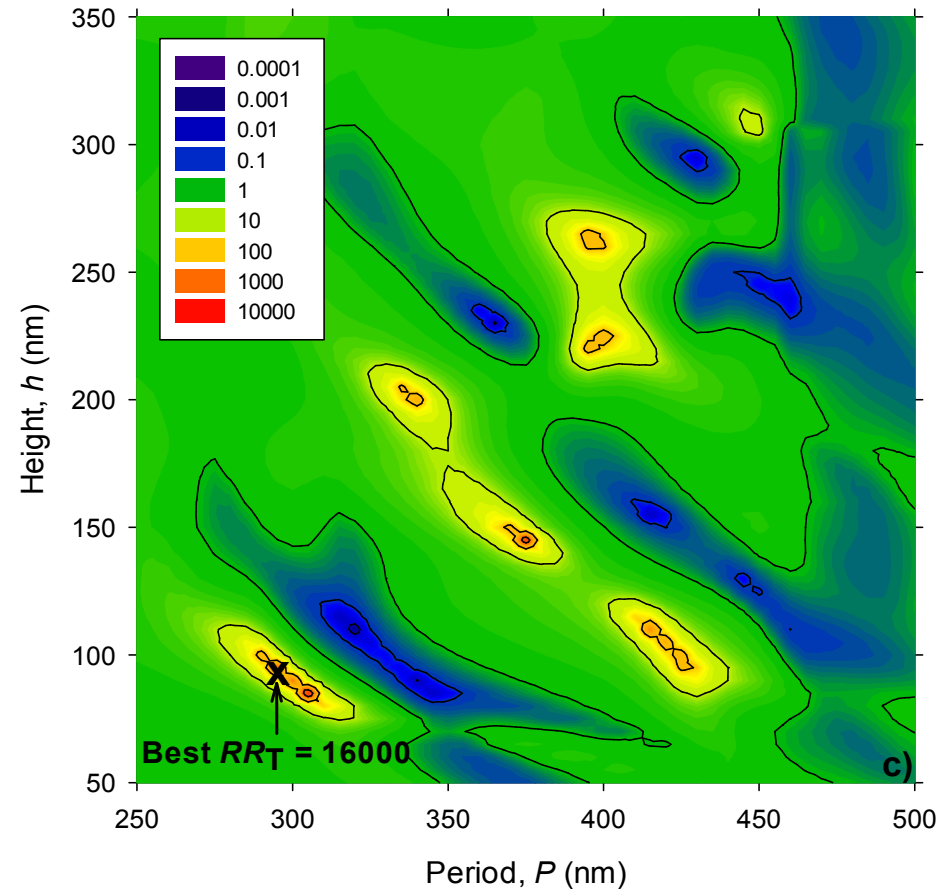
Best results:

› RRs over 100,000



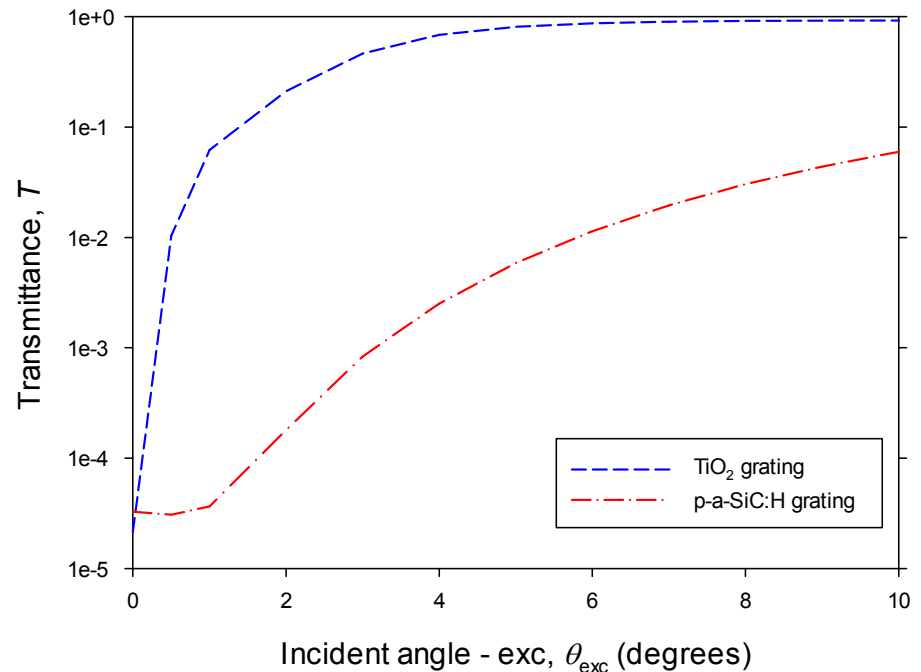
Properties - period & height dependance

- Only at specific P & h high RR
- Multiple areas with high & low RR
- Small deviation in P or h from optimal results in high change in RR



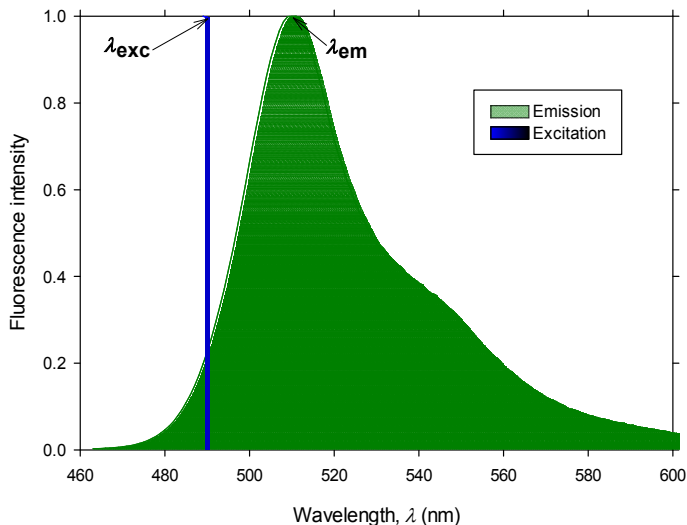
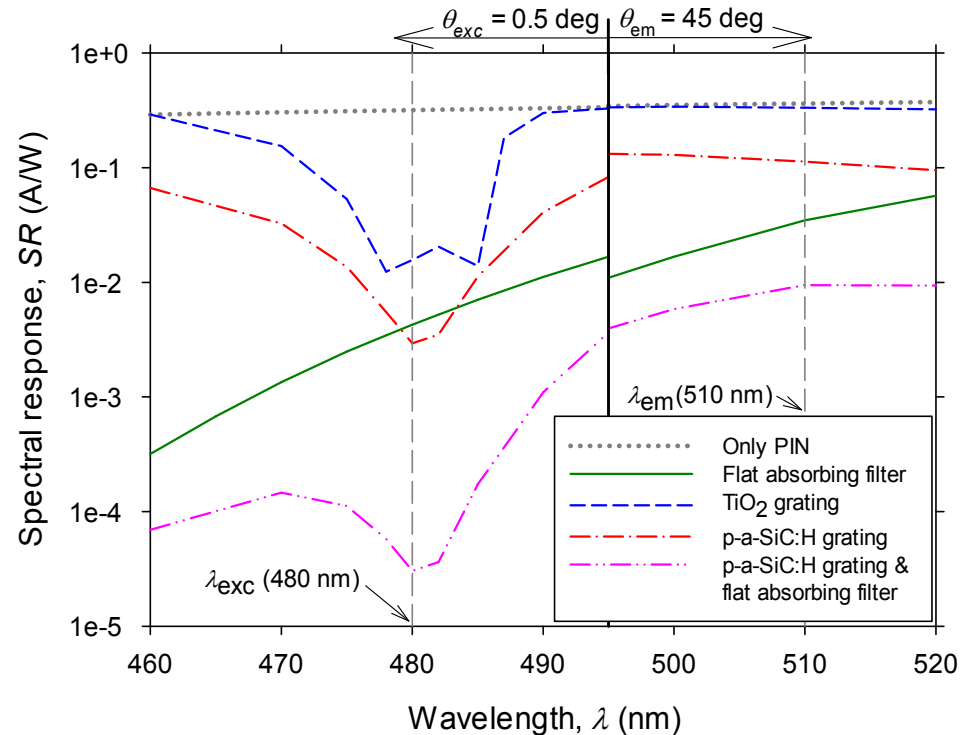
Incident angle dependence

- High dependence on excitation angle of light
 - › Laser light
- Low dependence on emission angle of light
 - › GFP – point sources



Realistic conditions

- $\pm 5\text{nm}$ inaccuracy
 - › In height & period
- Non-perpendicular light
 - › 0.5° for excitation light
 - › Average 45° for emission light
- Real GFP emission spectrum:



Best *RRs*:

- Flat absorbing filter: 18
- TiO₂ grating: 19
- p-a-SiC:H grating: 46
- absorbing & p-a-SiC:H grating: **1160**

Conclusions

- Fluorescent detection requires efficient optical filtering
- Diffraction grating as optical filters:
 - › Possibility to achieve very high RR s
 - › Versatile - applicable for other fluorophores, different grating shapes, possible combination with other technologies
 - › High dependence on manufacturing inaccuracies
 - Possible solution: modern manufacturing processes
 - › High dependence on incident angle of excitation light
 - Possible solution: laser light
 - › **Over 60 times improvement over flat absorbing filters**