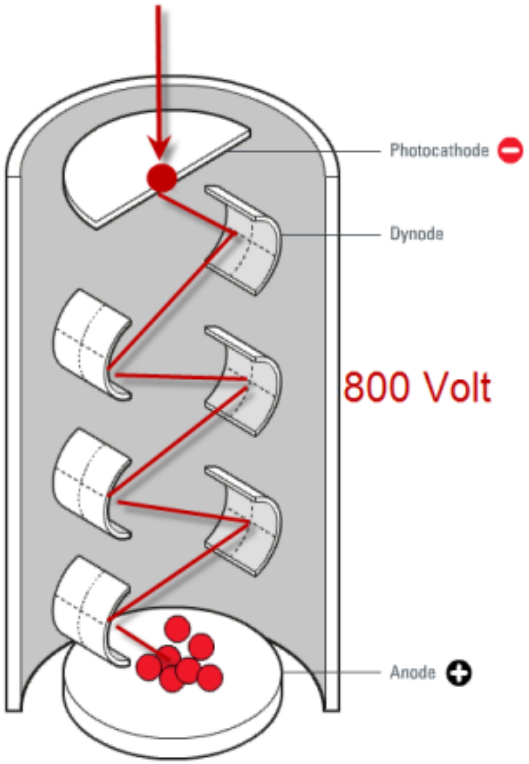
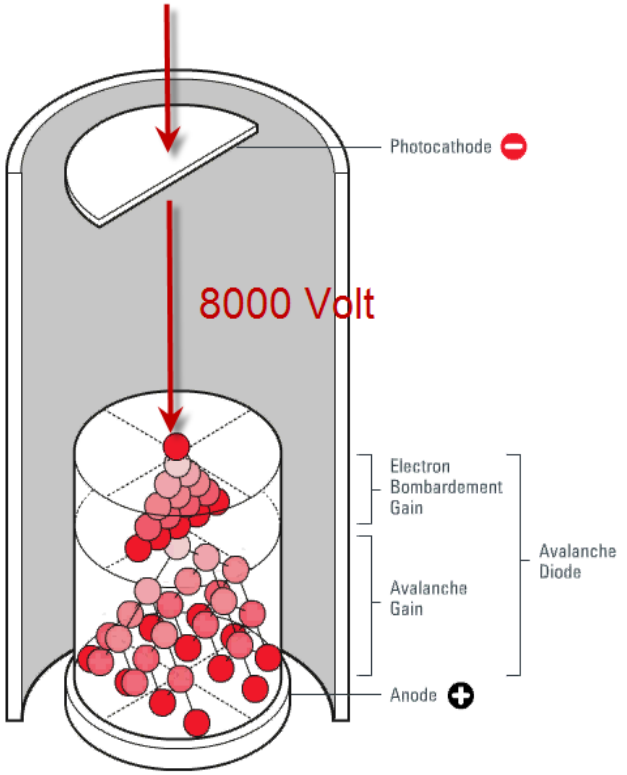
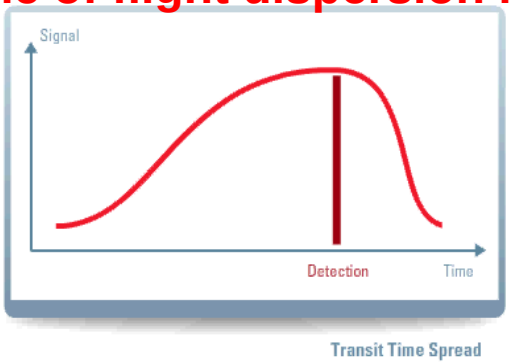
HyDs on the SP5

http://www.leica-microsystems.com/science-lab/step-by-step-guide-to-hybrid-detection-and-photon-counting/

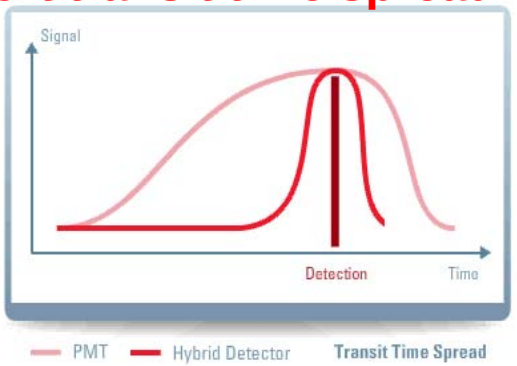




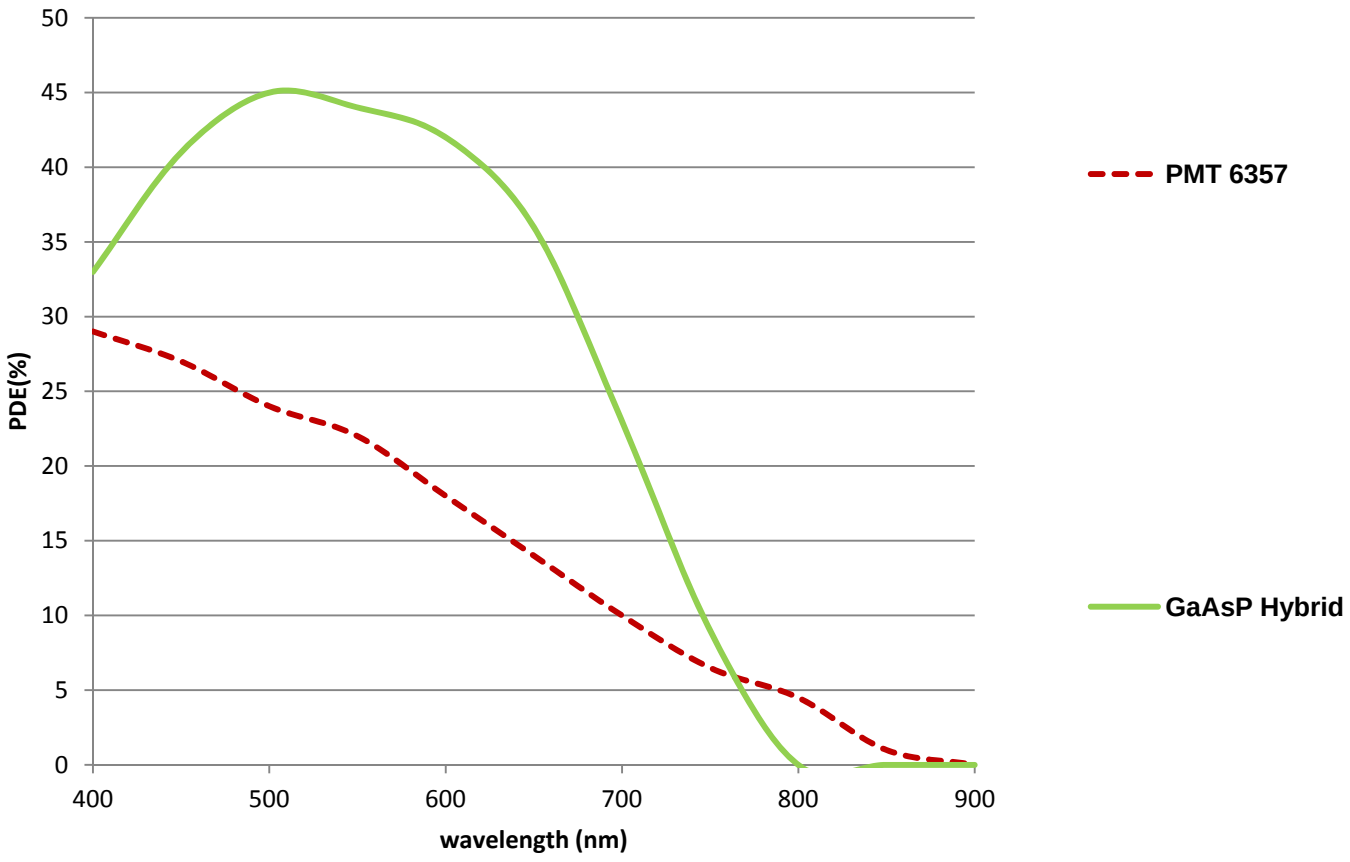
Time of flight dispersion in PMT's



Short transit time spread in HyD



High quantum efficiency



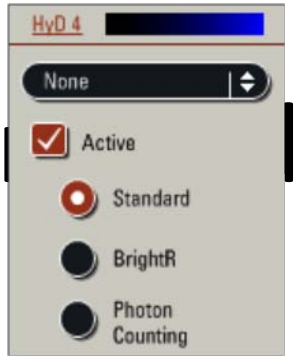
HyDs on our system

- **PMT2 and PMT4 were replaced by 2 HyDs,**
- **PMTs 1, 3 and 5 are still in place!!!!!**
- Higher sensitivity, less light is required
- Better signal-noise ratio (>> Huygens!!!!!)
- Standard functions (Setting „Standard“)
  - „badly stained samples“
  - more images (… z Stacks)
  - more line averages
  - faster imaging (… resonant scanner)
  - averageing>> accumulation (not good for PMTs due to noise)
- Advanced functions
  - „BrightR“: for high variation in sample intensity
  - „Photon counting“: special applications, image correlation, FRET,…

Specific HyD properties

- … have NO true GAIN and no OFFSET at all
- … „HyD gain“ is just a post-processing step of the computer
- …images should better be optimized using the laser intensity
- … produce a green signal in the background (i.e. zero background) when using the color table >> this is normal, not due to incorrect setting!
- **Can be overloaded/damaged by too much light**!

HyD LASAF operation modes



Standard

- **Operating mode for image acquisition.**
- **Can set the Gain of the detector as usual with PMTs**
- **Mapping absolute photon counts to a look-up table means one has to introduce a scaling factor**

BrightR

- **Specifically for dynamic samples with dark and bright in one image.**
- **In this operating mode, the Gain should be set to the lowest possible value.**

Photon Counting

- **Direct translation of photon counts into grey value**
- **No further amplification**
- **In this operating mode, the Gain of the light signals is set to a fixed value in order to ensure constant detection conditions for photon counting.**

DOs....

- DO start LASAF wait for first screen and select „Configuration“ > „machineHYD“ (only required once!)
- DO adjust laser settings: **1/10 to 1/5** th of your „normal“ levels
- DO set the „HyD gain“ to 100 %
- DO use the HyDs for the two weakest signals
- DO leave blue signals (DAPI) on the PMT: the improvement is the lowest (HyD vs PMT)

… AND DON‘Ts

- **DON‘T overload the HyDs with too much light**
  - Overload will result in a warning and will be counted by software („total number of overloads“)
- DON‘T use the HyDs for objective correction (you have enough light with PMTs)
- DON‘T use **mobile phones** (HyDs will not work properly); use the land line **EXT: 70296**
- DON‘T reload your old settings and press „live“

SPECIAL CASEs: FRAP....

1. **DANGEROUS: ROI-based FRAP with HyDs!!!**
   - **The bleach step will overload the HyDs!!**
   - the bleach process@high laser power will be monitored in the detectors.(HyD will get an overload!)
   - Very low intensity samples: … it can still work
2. **CRITICAL: Spot bleaching („beam park“ FRAP)**
   - …park the beam for 100 ms….
   - … no imaging during the bleach pulse….
   - …but HyD is still ON!
   - …for lower intensity samples OK!
   - … if the overall light not too much
2. **HIGH Intensity samples>>> use the PMTS!!!!!!**

…. AND FRET

1. **CRITICAL: FRET acceptor photobleaching**
   - **Possible** BUT use a **PMT for detection of the acceptor** (bleaching the acceptor causes high light intensities in the acceptor channel!!!!)
   - Use the **HyD for the donor** (anyway more important!!!)
2. **SAVE: FRET sensitized emission**
   - Okay: >> no bleaching step, **HyDs can (and should) be used for both donor and acceptor!**

Sequential scan

- … using line switch:
  - Example: blue=PMT1, green=HyD1
  - All detectors stay ON (even if they don‘t acquire)
  - HyD1 can be overloaded eg if
    - PMT1 is used for DAPI AND 405 nm at high intensity for exc. (saturation in the blue channel!!!)
    - Even though HyD1 is used for „green Channel“ it can get too much light from the „blue Channel“
  - „Normally“ sequential scans are safe, except if there is high saturation in one of the PMT channels…..!!!!