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In vivo fate of protein aggregates upon injection – the use of fluorescence optical *in vivo* imaging.

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07-02-2012

Utrecht Institute of Pharmaceutical Sciences (UIPS), Faculty of Science, University of Utrecht

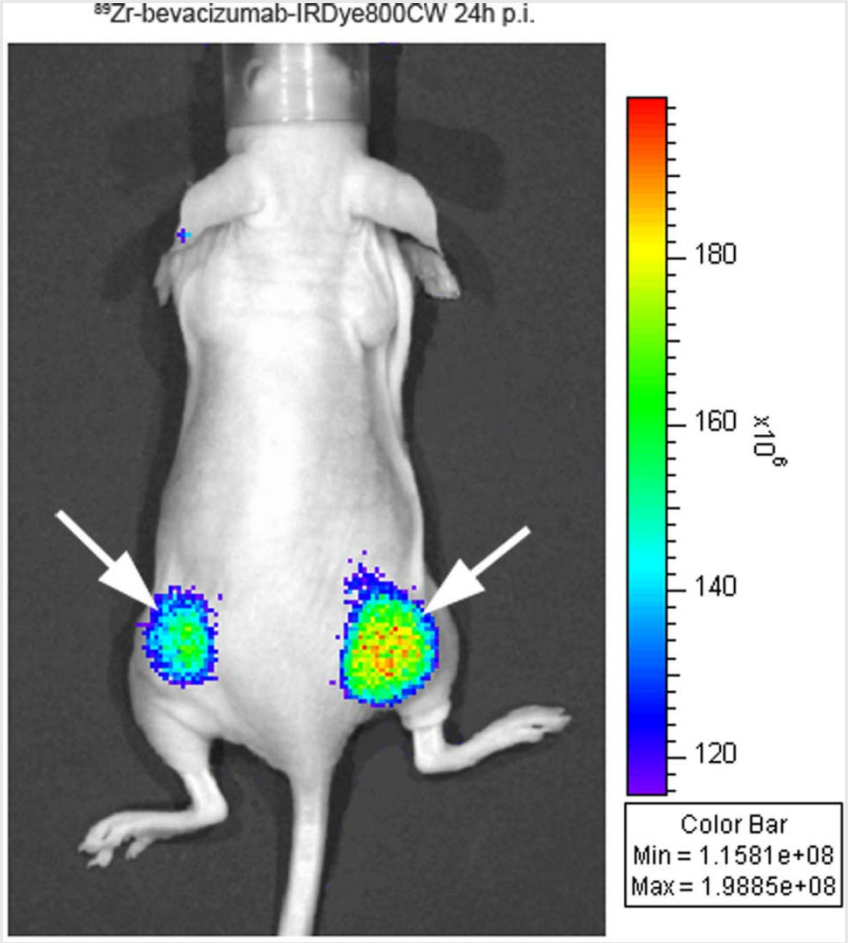
Aggregates – the major risk factor leading to immunogenicity.

- Factors influencing the immunogenic potential of aggregates:
  - Size, molecular weight (100kDa, >20 repetitive epitopes, 5-10nm spacing),
  - Amount,
  - "Nature": (native-like, denatured, rigidity)
  - solubility,
  - resemblance to microbial structures.

- What happens with aggregates upon injection:
  - Do they accumulate in injection spot/organs?
  - Are they cleared slower/faster than monomers?
  - Do they change in size?

In vivo Optical Imaging (applications).

- Gene expression.
- Stem cells.
- Infectious diseases.
- Neurosciences.
- **Oncology (e.g Mbs)**



Cohen et al. EJNMMI Research 2011, 1:31

In vivo optical imaging.

- Pro:
  - Safe for animals and people,
  - Fast,
  - Flexible (proteins, labels, number of administrations),
  - No special laboratories required (e.g. radiology),
  - Quantitative (?),
- Con:
  - Sensitivity,
  - "Nude" animals required (autofluorescence),
  - Labeled protein (change of characteristic?)

2. Aim of study.

Validation of fluorescence Optical Imaging technique for *in vivo* tracking of aggregates of therapeutic proteins.

Experimental design.

- Experimental setup:
  - 6 Tg and 6 non-Tg animals (n=1) from human IgG and IFNα transgenic mice,
  - hIFNα and hIgG,
  - doses: 10µg, 20µg and 50µg,
- Preparation of aggregates:
  - aggregates and monomers,
  - hIFNα -> metal oxidation (Hermeling, 2005)
  - hIgG -> 3 hours of shaking (Filipe, 2010)
  - duration: non-Tg IgG -> 2 weeks
    Tg IgG -> 1 week
    Tg/non-Tg IFNα -> 1 week
- Protein labeling:
  - Alexa® 700,
  - Probe/protein ratio [mol/mol]:
    - hIgG -> 4,
    - hIFNα -> 1

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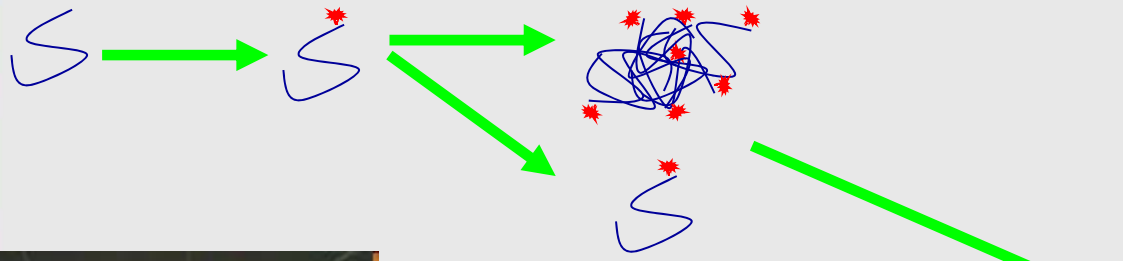
Experimental design.



Photon Imager™

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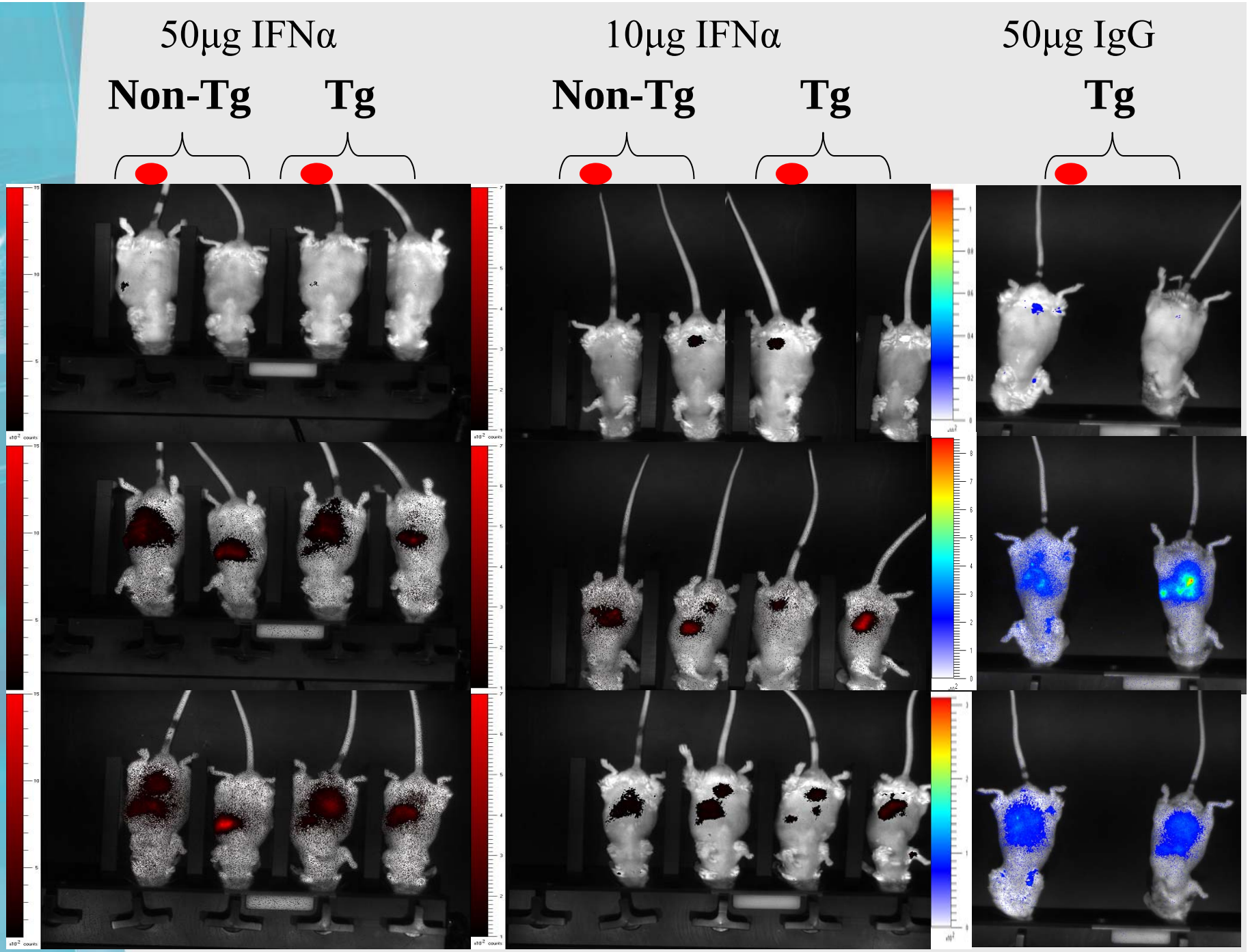
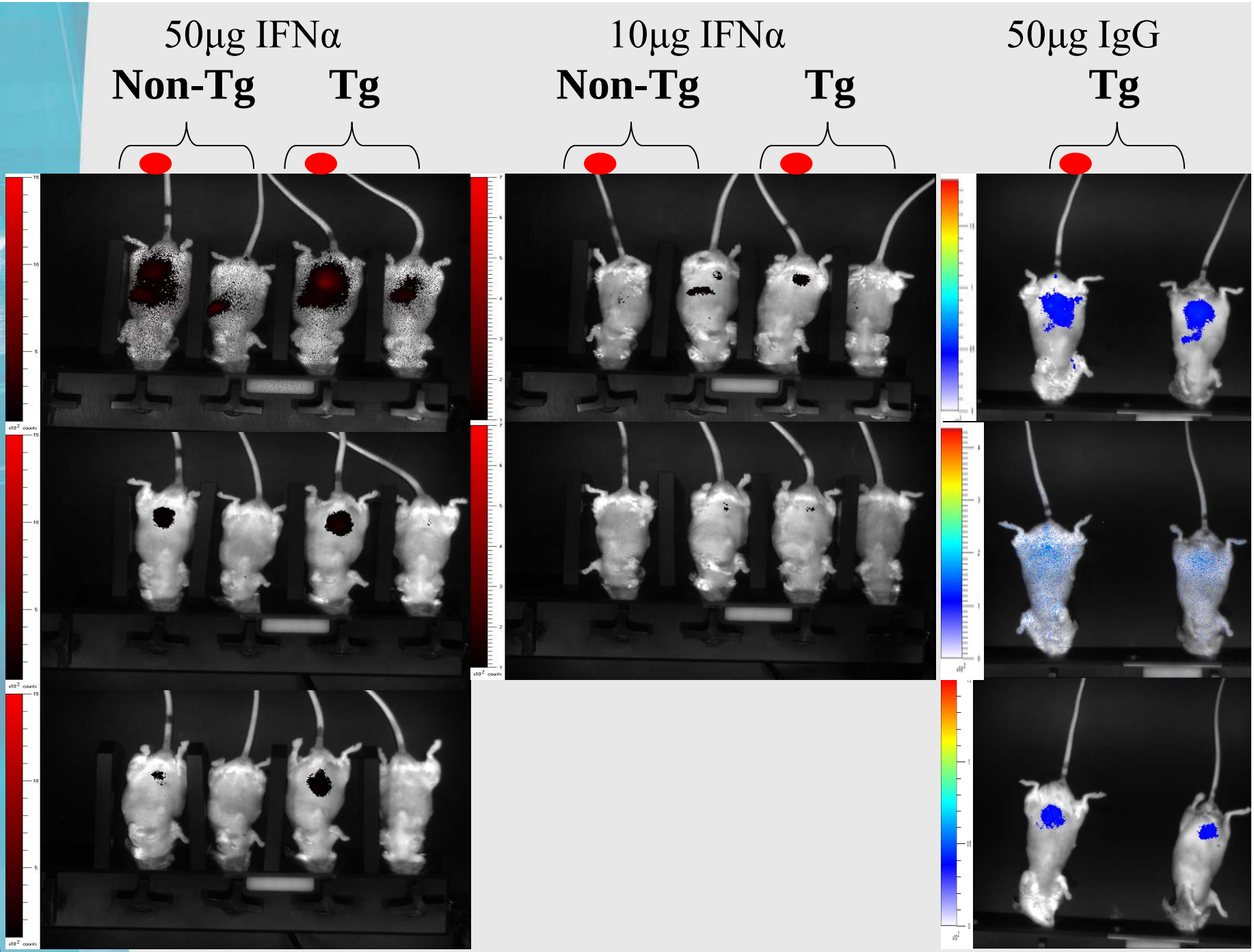
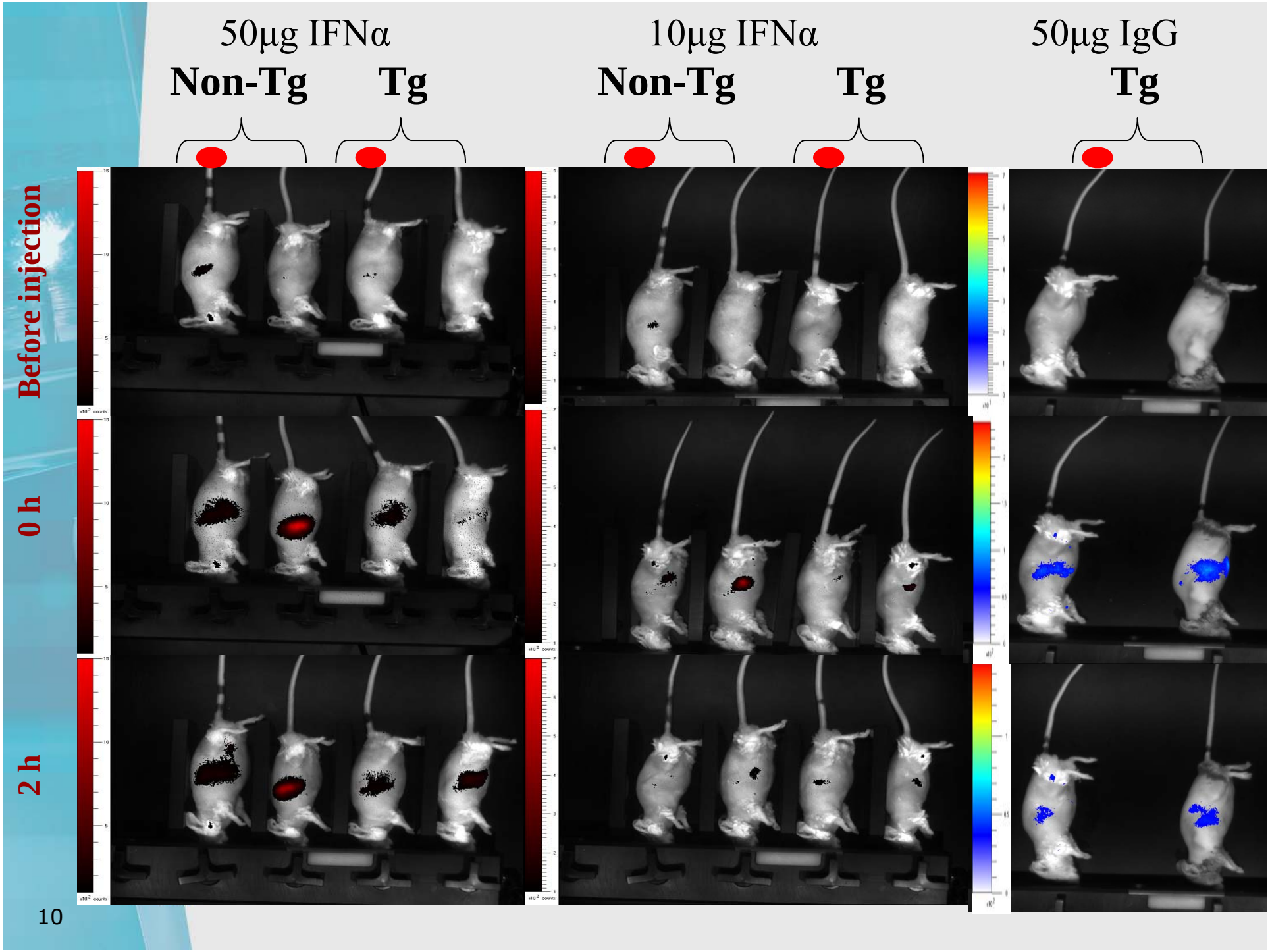
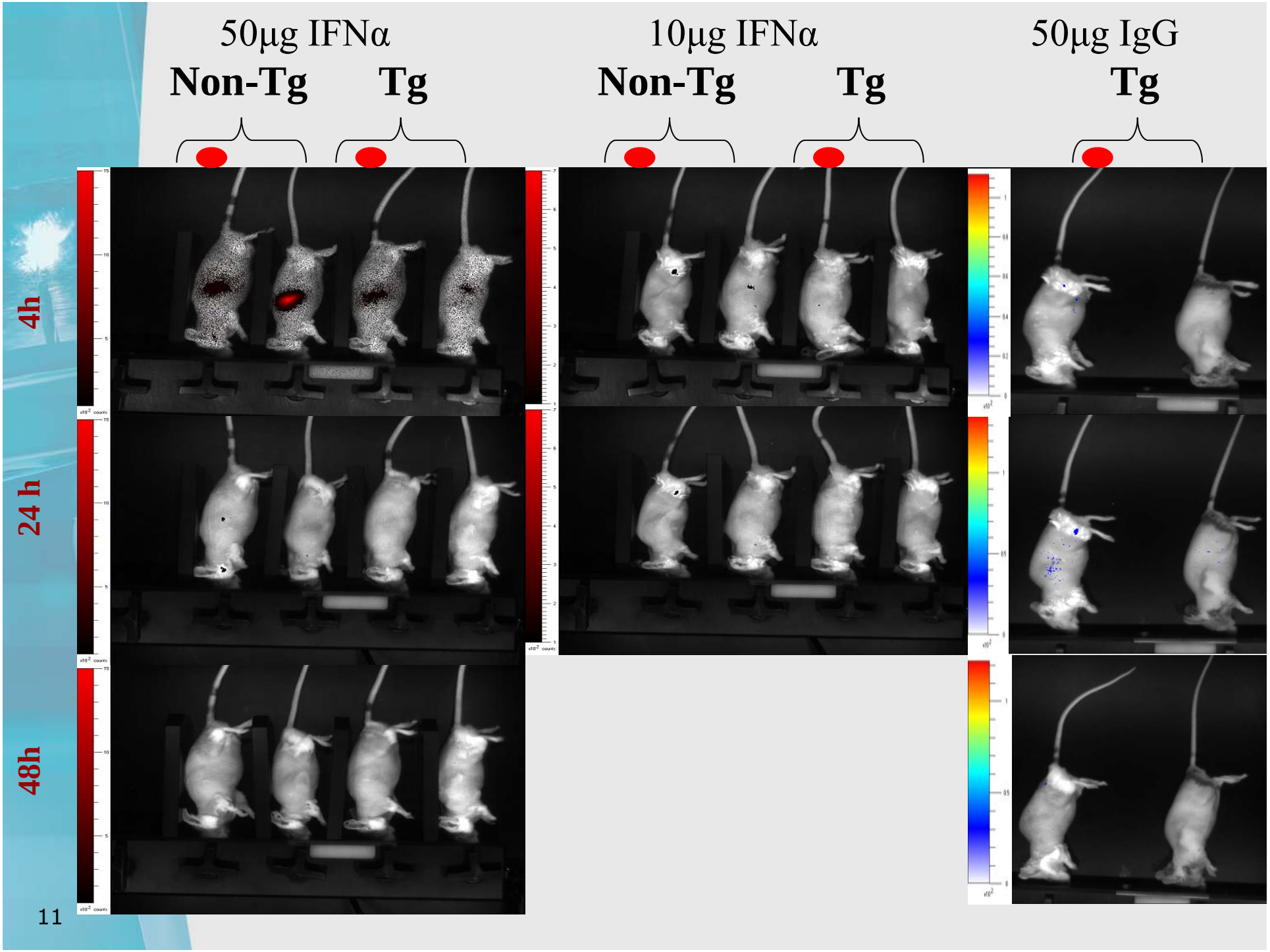
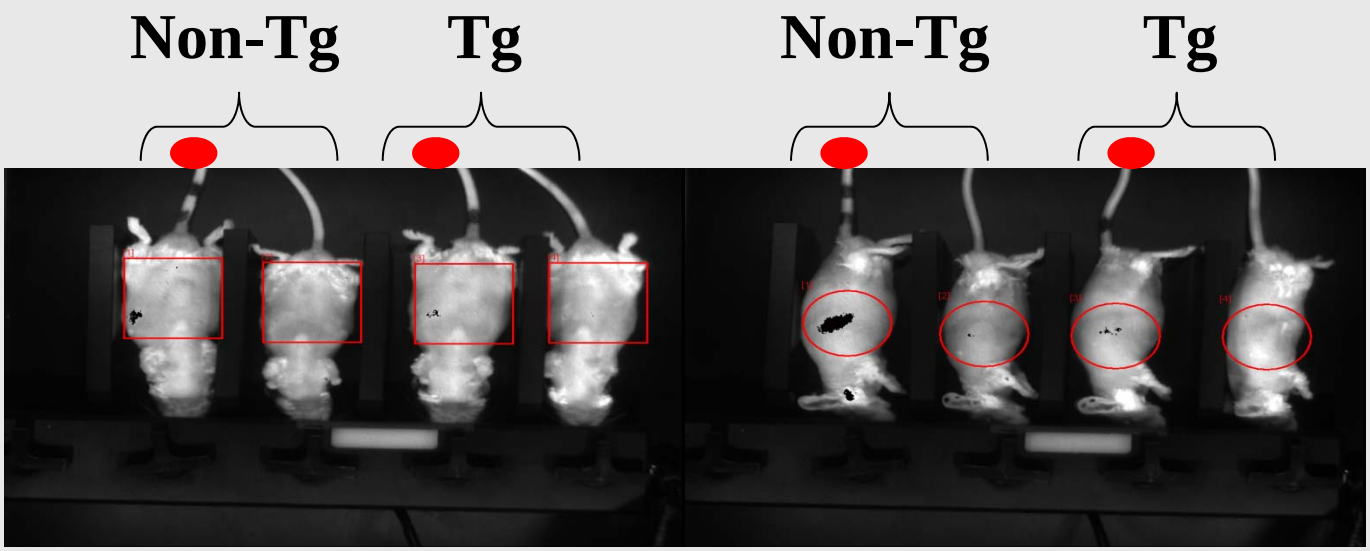
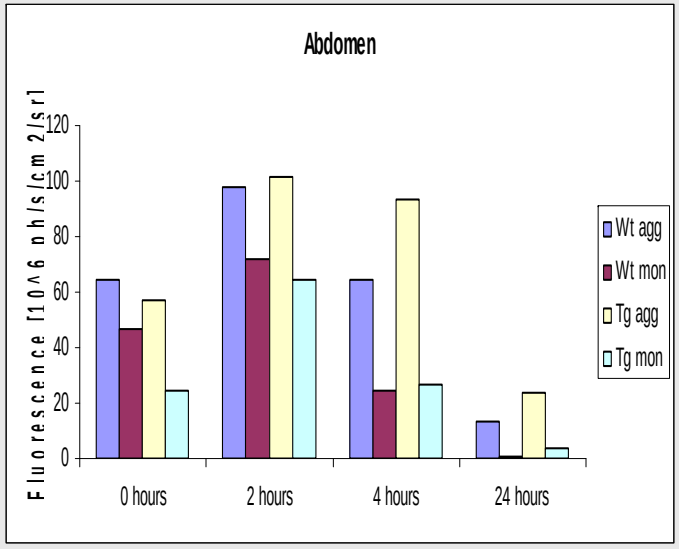

50μg IFNα
10μg IFNα
50μg IgG
Non-Tg
Tg
Non-Tg
Tg
Tg
Before injection
0 h
2 h

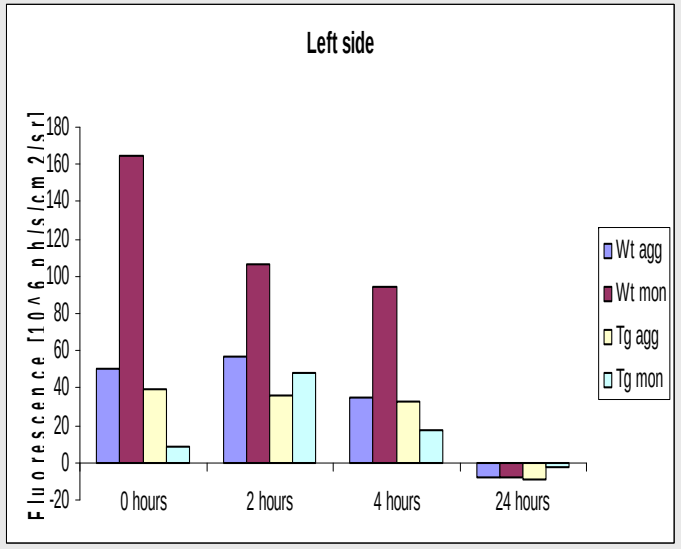

50μg IFNα
Non-Tg
Tg
10μg IFNα
Non-Tg
Tg
50μg IgG
Tg
4h
24 h
48h


50μg IFNα
Non-Tg
Tg
10μg IFNα
Non-Tg
Tg
50μg IgG
Tg
Before injection
0 h
2 h


50μg IFNα
Non-Tg
Tg
10μg IFNα
Non-Tg
Tg
50μg IgG
Tg
4h
24 h
48h


Non-Tg
Tg
Non-Tg
Tg


Abdomen
Fluorescence [10^6 nh/s/cm 2/sr]
120
100
80
60
40
20
0
0 hours
2 hours
4 hours
24 hours
Wt agg
Wt mon
Tg agg
Tg mon

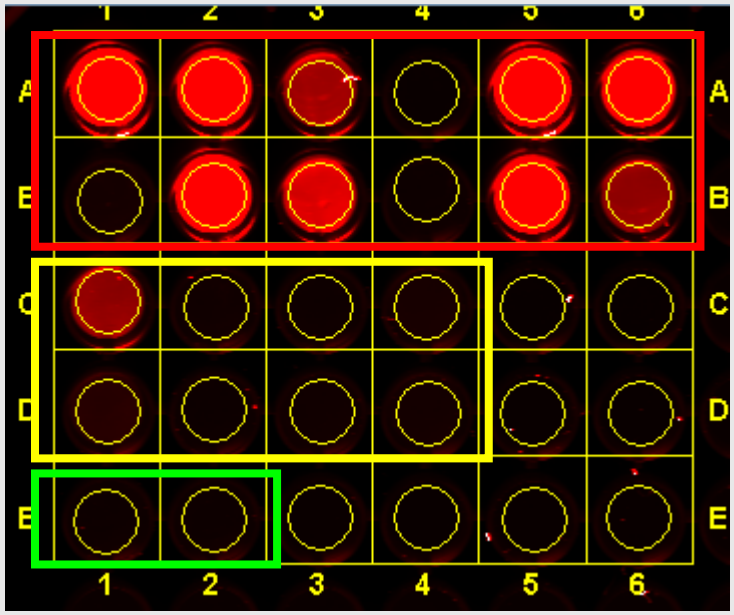

Left side
Fluorescence [10^6 nh/s/cm 2/sr]
180
160
140
120
100
80
60
40
20
0
-20
0 hours
2 hours
4 hours
24 hours
Wt agg
Wt mon
Tg agg
Tg mon

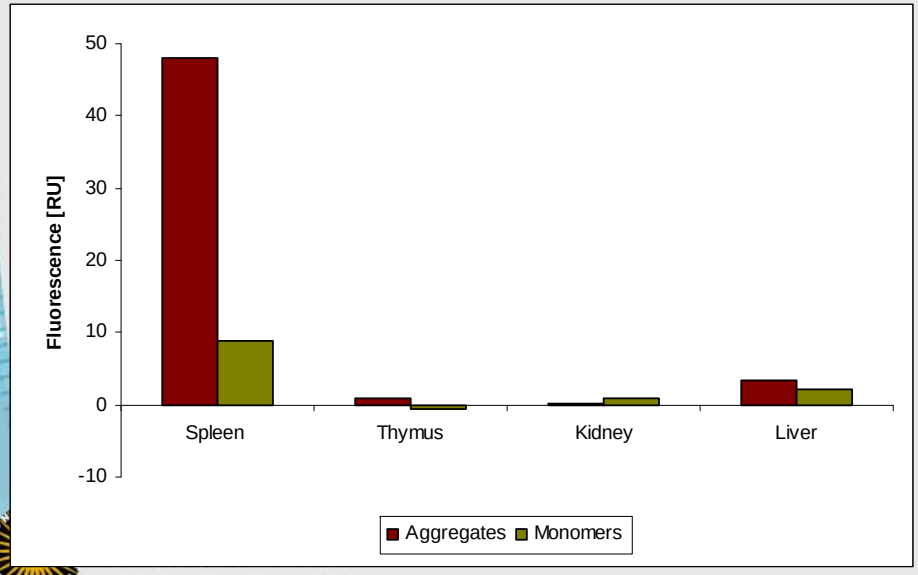
Infrared imager (IgG)

Blood → Tg / Non-Tg

Organs (Tg) →

Control →





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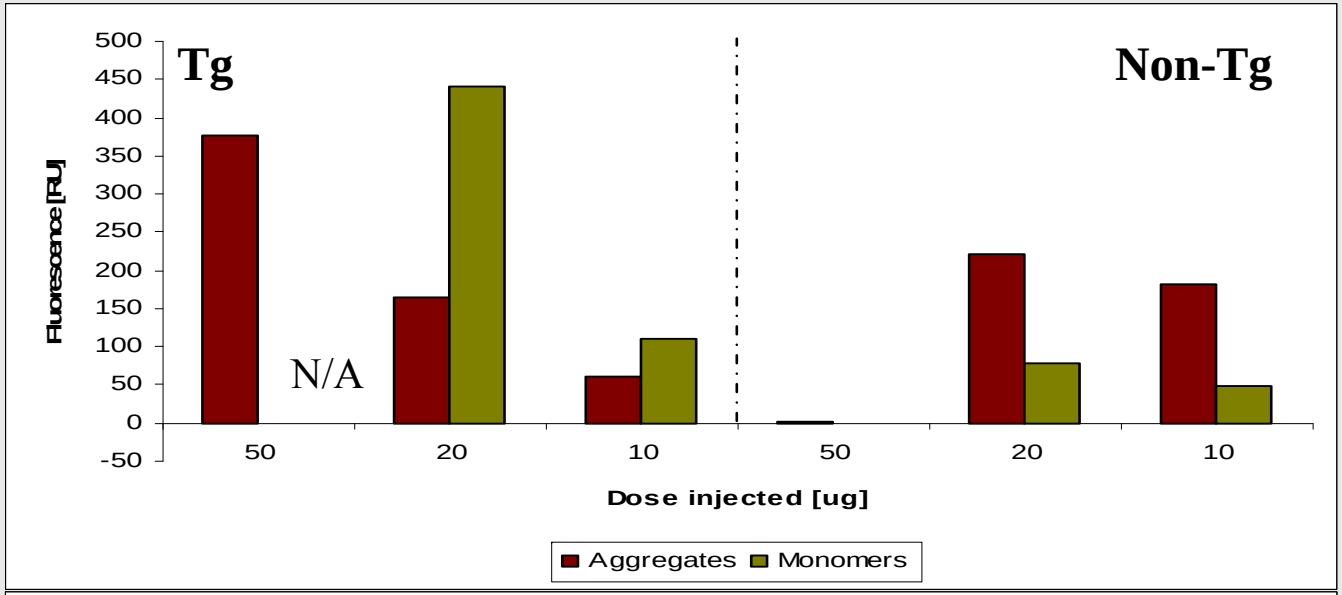
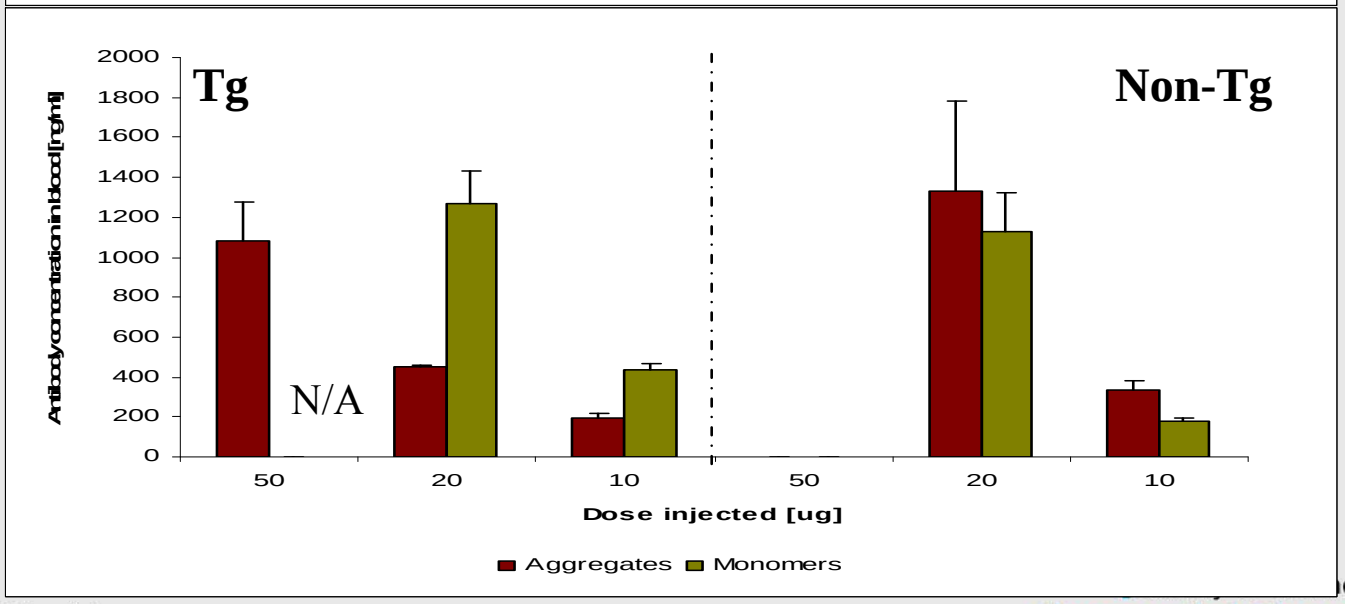
Infrared imager



ELISA



Conclusions.

- **Optical imaging can be used for study of aggregates fate in vivo.**

4. Future plans.

- Continuation of imaging study with mouse serum albumin (MSA) as a model protein:
  - different routes,
  - different immunizations (1 vs multiple injections),
  - time curve / bio distribution of aggregates.

5. Acknowledgements

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Thank you for attention!