Classification of fluorescence spectra from tumour images acquired in vivo

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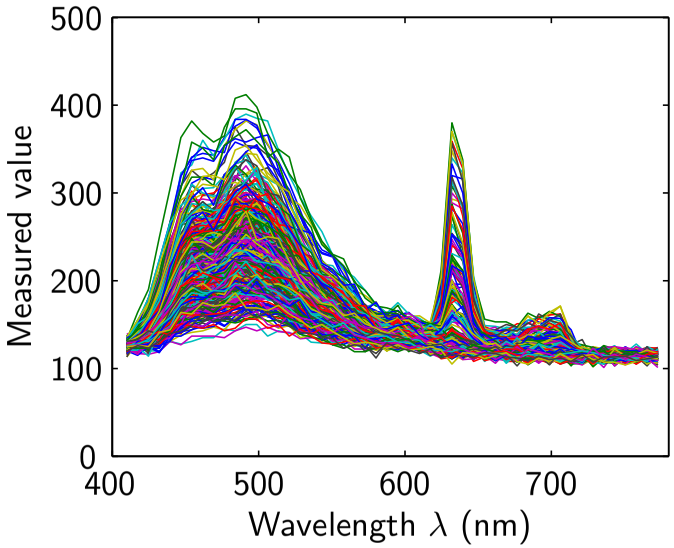
Outline

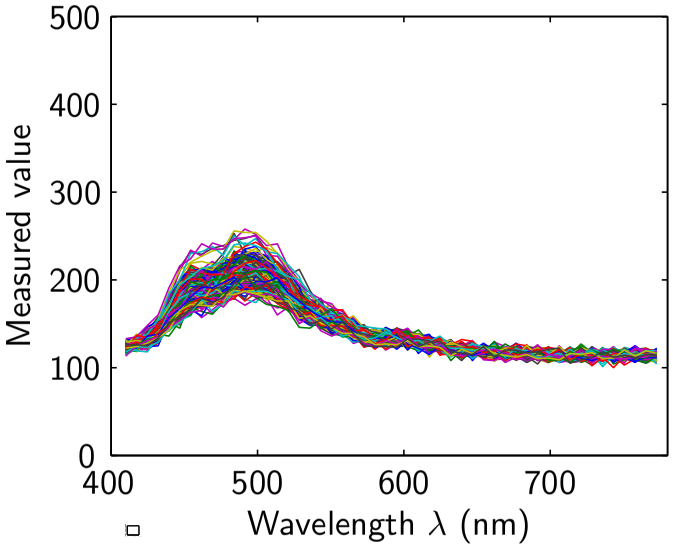
Laser induced fluorescence

- When substances (such as contents of human cells) are exposed to light from a Laser, they emit light.
- The spectral contents of the fluorescent light depends on the substance, making it possible to identify substances with known fluorescence spectrum.
- Since the contents of a normal cell may differ from a tumour cell, it may be possible to distinguish them via their fluorescence spectra.
- The natural spectrum differences are too small to be used by themselves, but by injecting a substance ("ALA"), with known spectrum, that gathers in the tumour, more information can be obtained.

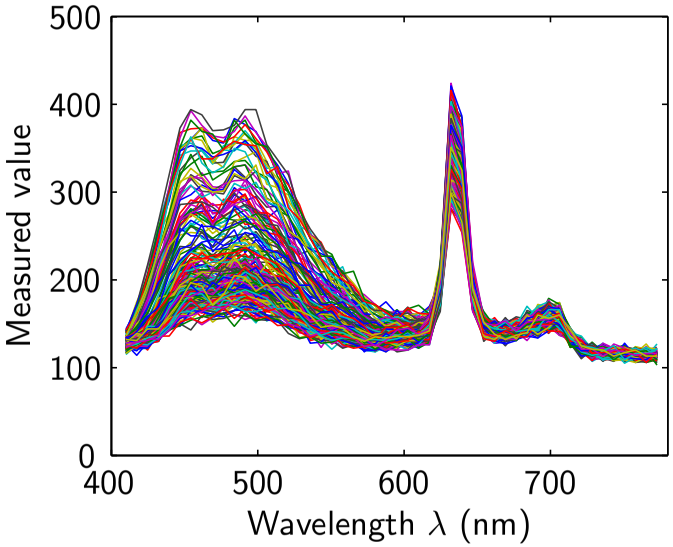
Any cells



Normal cells



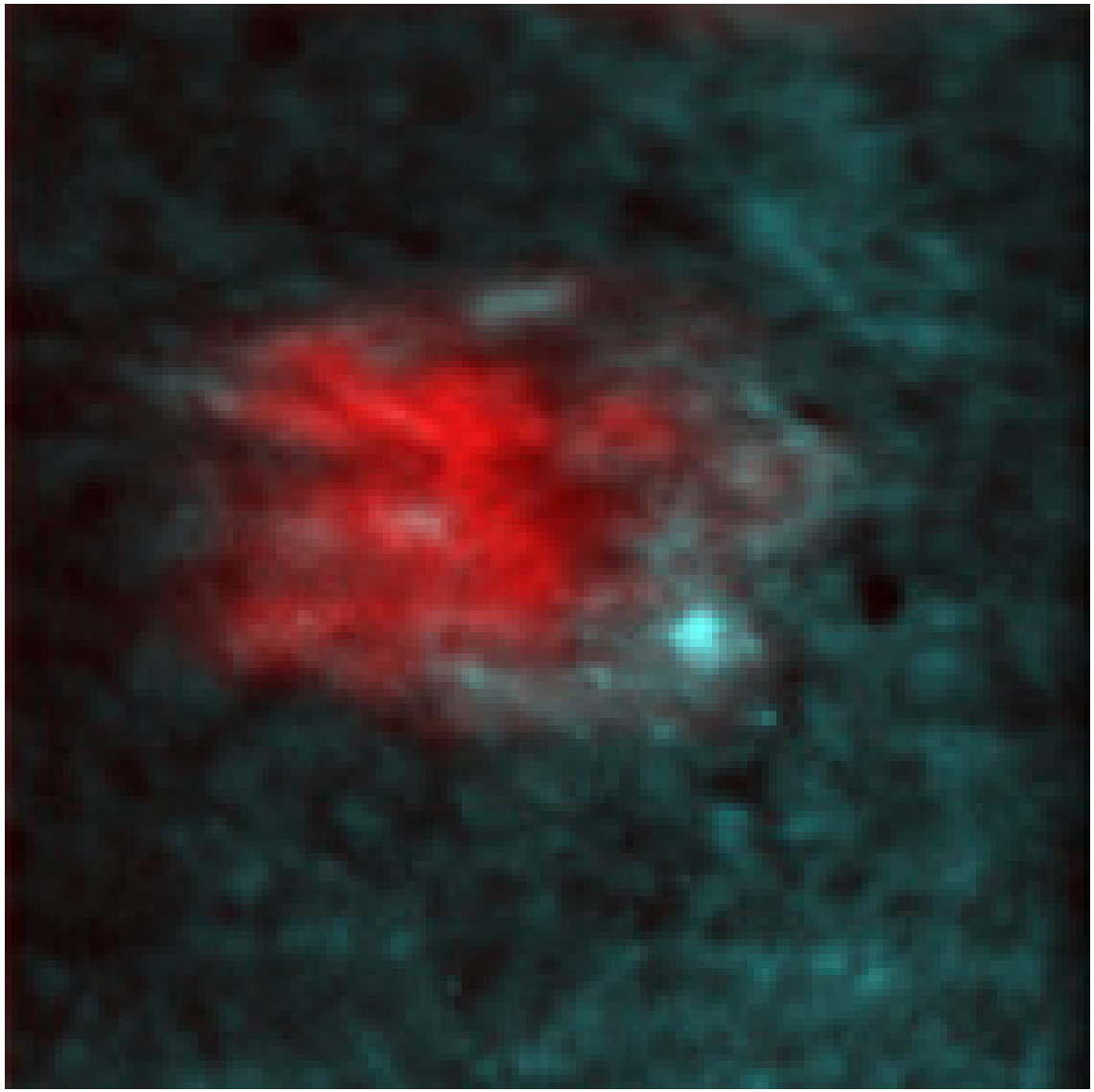
Tumour cells



Fluorescence imaging

- Previously, most work has been carried out by measuring the fluorescence in a single point at a time.
- Imaging equipment has previously been used to measure either the energy content of a single wavelength, or the mean spectrum over a region.
- Here, we measure a full spectrum (50 wavelengths) in each pixel of a 152 by 152 pixel image, across a square on the order of 2 by 2 centimetres.
- We want to answer the question "Where is the tumour?"

Pseudo-colour image from spectral image data



Data challenges

- Measurement offset
- The spectral components have unknown shape
- The measurement sensitivity is wavelength dependent
- Sensitivity may also depend on location

Previous approaches

- Calibrate the image for spatial and wavelength dependent variation before further analysis by normalising the spectra. Not good! The true spectral energy differs between locations, and the measurement noise must also be handled.
- Use PCA or PLS to find relevant components. Drawbacks:
  - Difficult to relate to actual physics.
  - No account is taken of known spectral properties, such as non-negativeness of the basic spectral components and the ALA-fluorescence.

Hierarchical statistical model

- Assume that each spectrum can be written as a linear combination of basic spectral components $m_0(\lambda) \geq 0$ (basic fluorescence), $m_\Delta(\lambda)$ (additional tumour fluorescence), and $m_A(\lambda) \geq 0$ (ALA): (u =location, λ =wavelength)

$$S(u, \lambda) = m_0(\lambda) + \alpha(u) m_\Delta(\lambda) + \beta(u) m_A(\lambda)$$

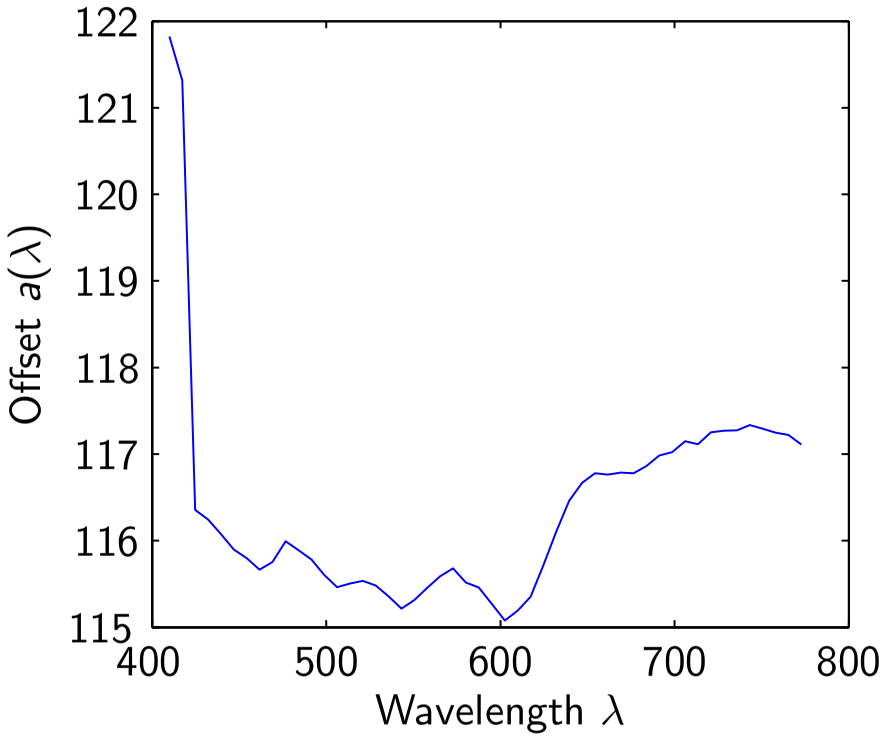
  with $0 \leq \alpha(u) \leq 1$ and $0 \leq \beta(u)$.
- A simple measurement model is given by

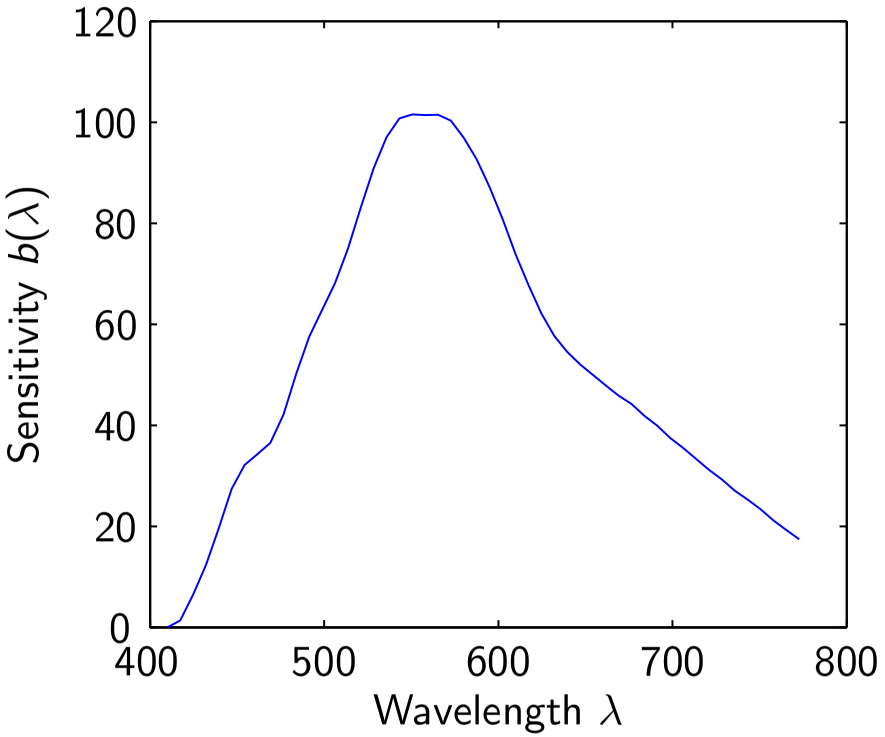
$$X(u, \lambda) = a(\lambda) + c(u) S(u, \lambda) b(\lambda) + \epsilon(u, \lambda)$$

  where $\epsilon(u, \lambda)$ is the measurement noise.
- Assume that the offset $a(\lambda)$ and sensitivity $b(\lambda)$ are independent of location.

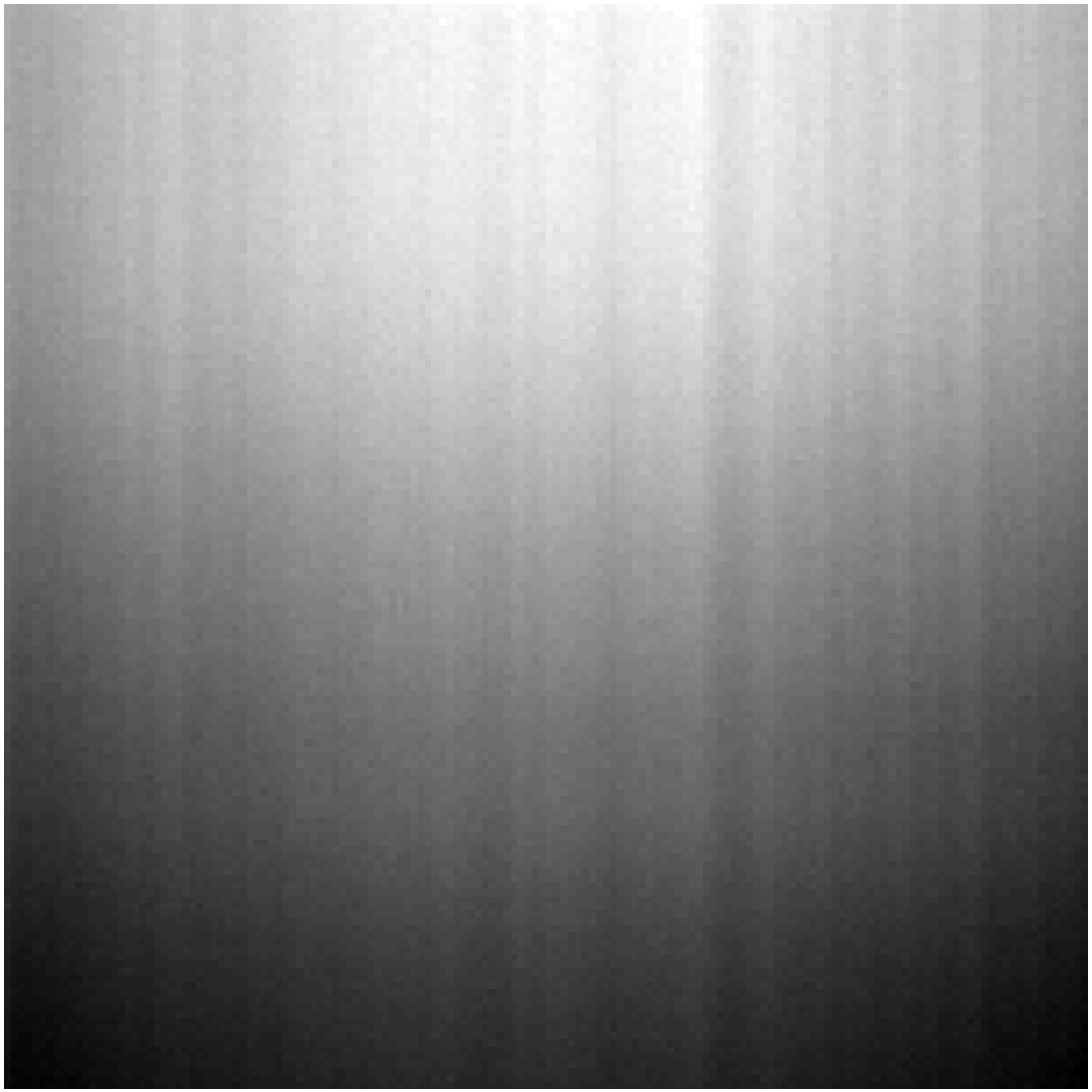
Measurement model estimation

The offset $a(\lambda)$ and sensitivity $b(\lambda)$ are estimated using images of "darkness" and a lamp with known spectrum.



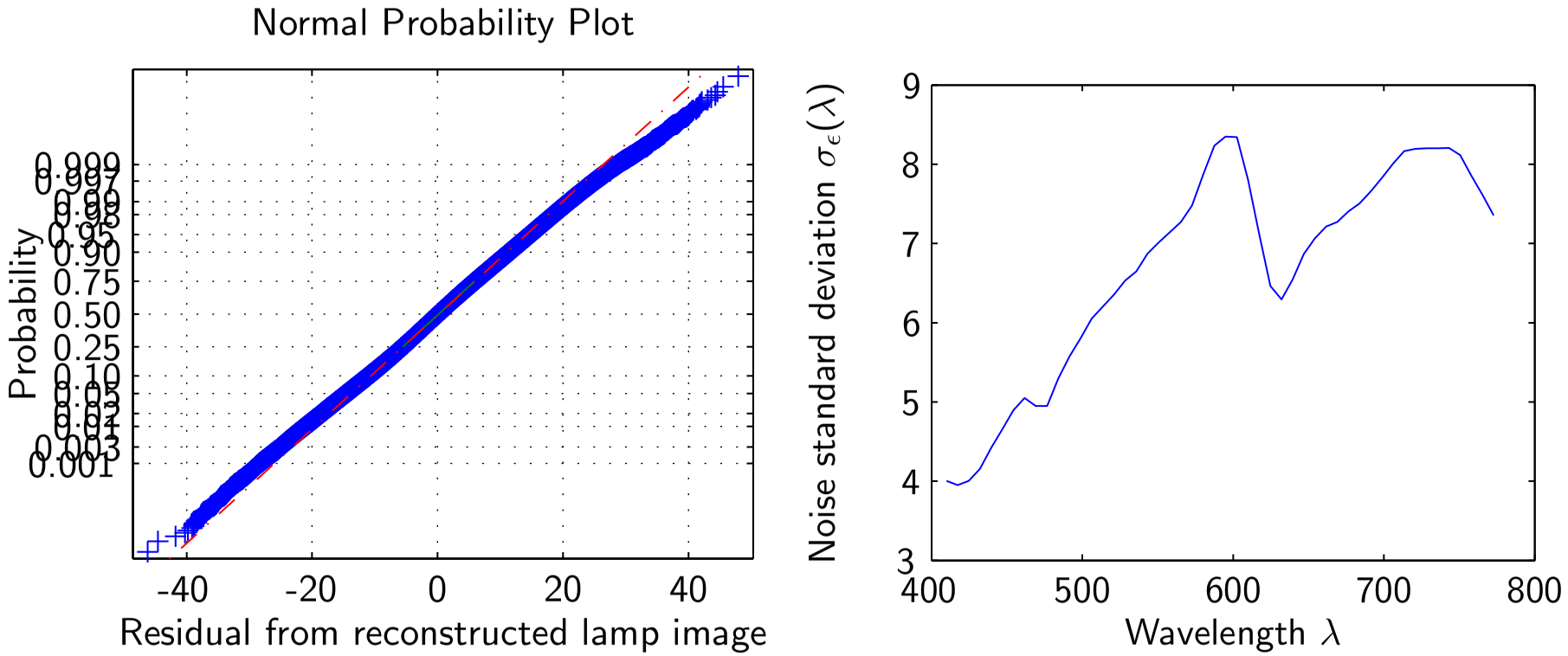


Spatially dependent scaling, $c(u)$



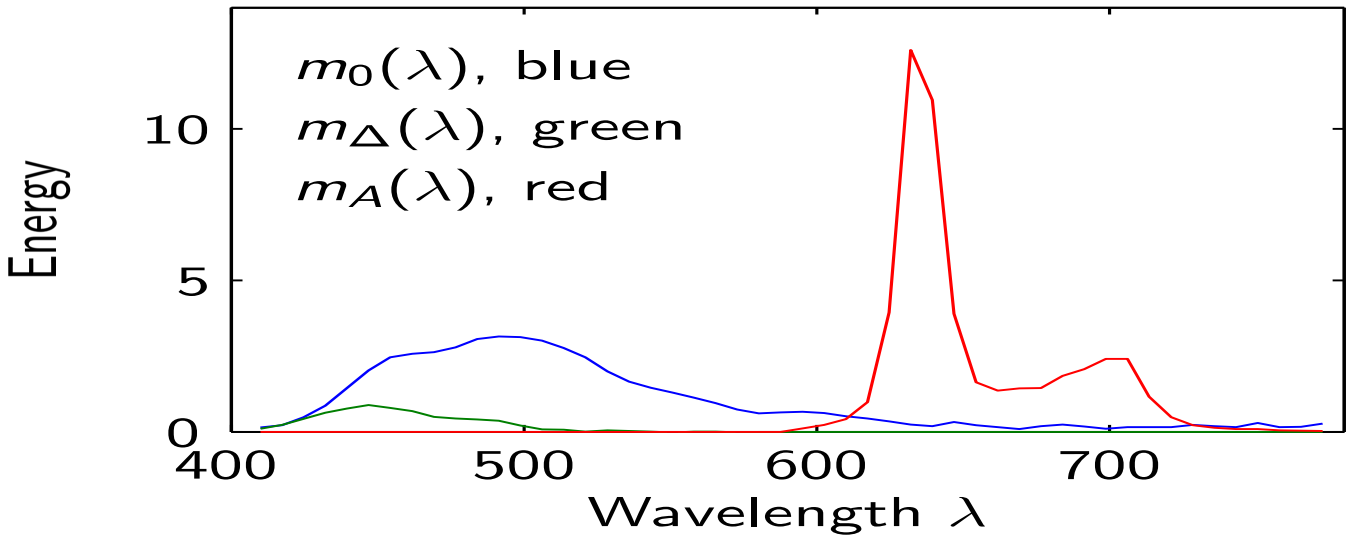
Noise parameters, $\sigma_\epsilon(\lambda)$

The residuals in the calibration data can be modelled as independent Gaussian variables, with variance depending on λ



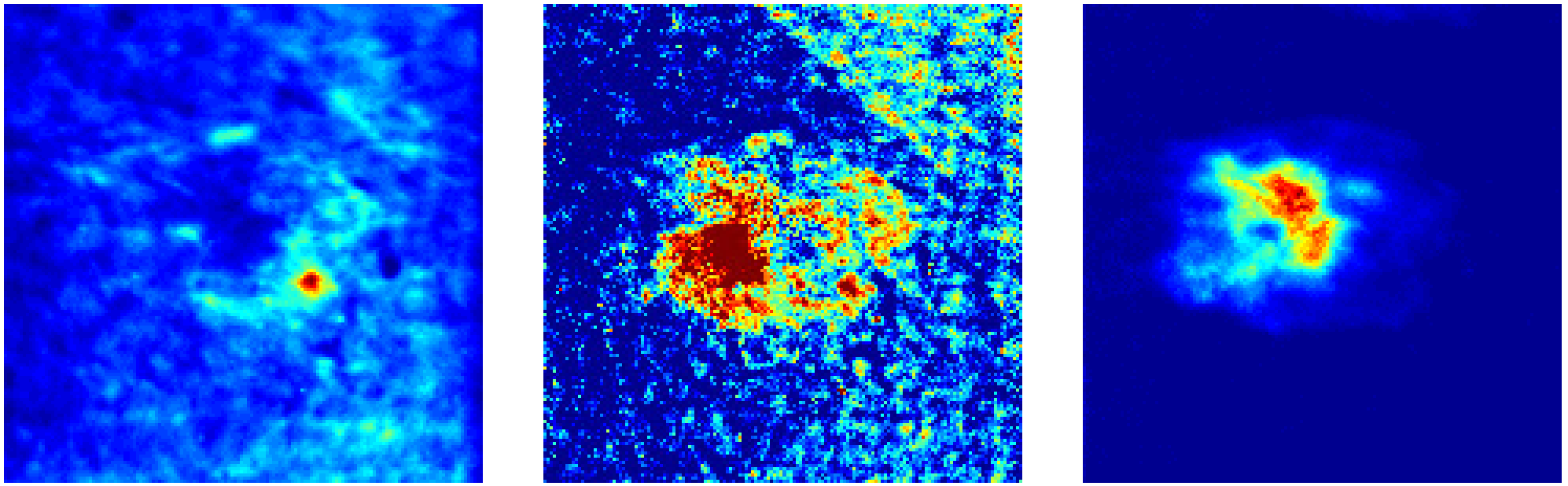
The spectral components

- Small training areas are marked in the image[1], $X(u, \lambda)$ for normal cells, $Y(v, \lambda)$ for tumour cells.
- Ideally, estimate everything simultaneously.
- Difficult to find the ML estimators of the spectral components, but an ad hoc method works well enough for now.
- The sensitivity factor $b(\lambda)$ is included in the estimated components, but can be compensated for later, if necessary.



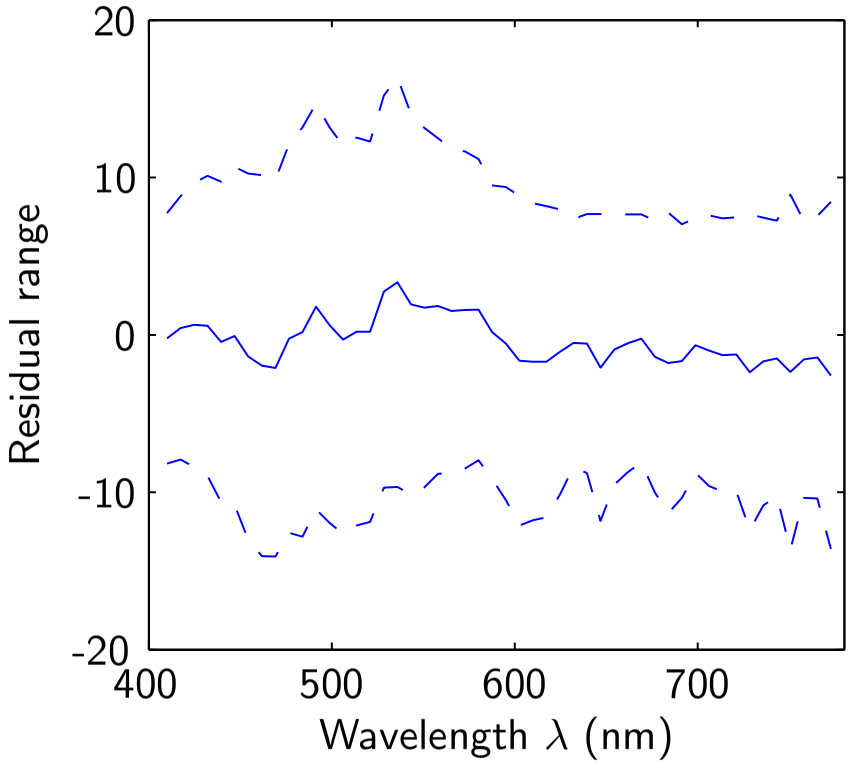
The regression parameters

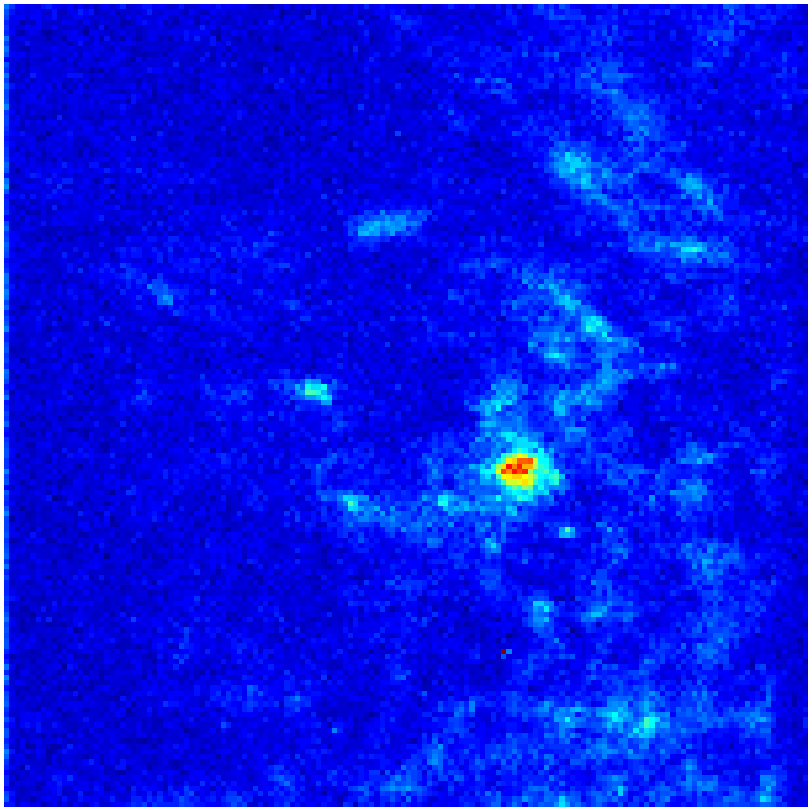
$c(u)$ $\alpha(u)$ $\beta(u)$



- $c(u)$ captures some biological effects, not only instrument calibration.
- $\alpha(u)$ indicates skin structure.
- $\beta(u)$ shows relative ALA-concentration.

Residuals

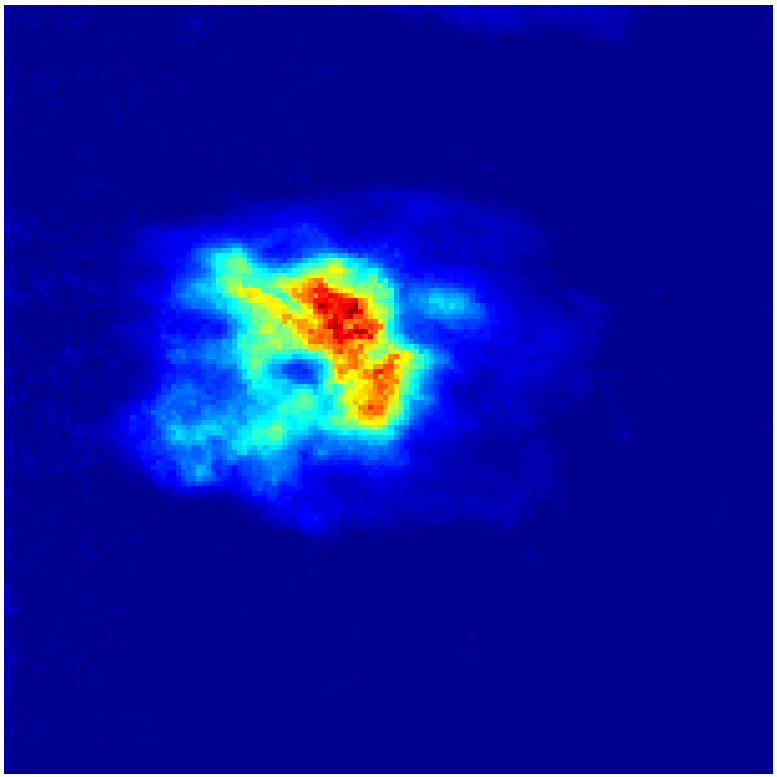




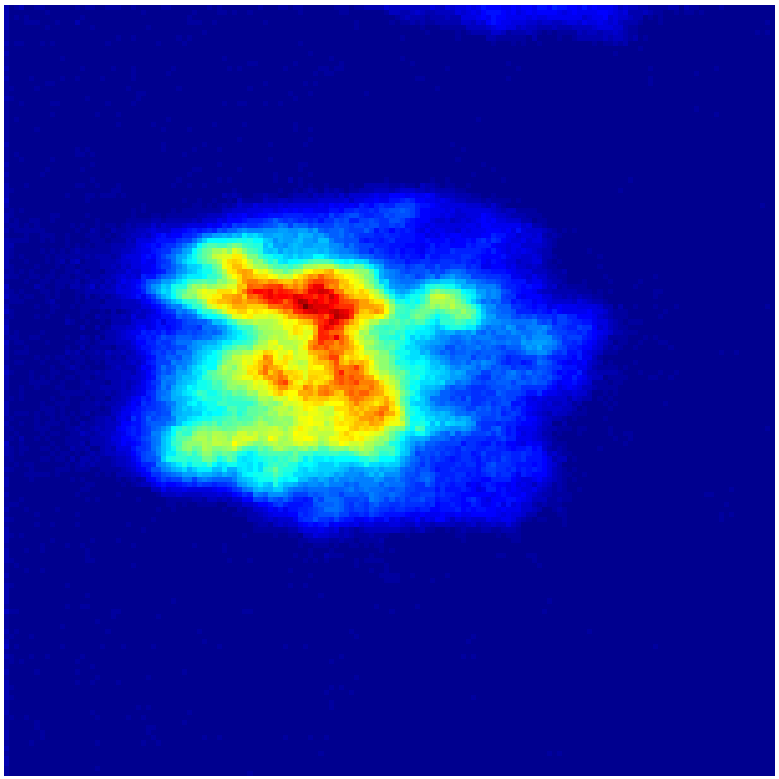
Scaling ambiguities

- What factors influence the scaling coefficients $c(u)$?
- Is the ALA fluorescence scaled the same as the natural fluorescence?
- More physics and biological input needed.

$\beta(u)$



$c(u)\beta(u)$



Conclusions and further work

- A hierarchical model for fluorescence spectrum images was developed.
- Fast estimation enables use in real-time clinical situations.
- No pre-calibration is needed, and measurement noise is handled well.

- Improved spectral component estimation.
- Include more statistics and physics in the spectral component estimates; mixed distributions, known spectral peaks etc.
- Determine what properties are the same in different images.
- Spatial dependence model for $\beta(u)$ for improved spatial accuracy.
- Construction of calibrated images, with $b(\lambda)$ taken into account, with attention to reducing variance due to errors in the offset and sensitivity estimation.
- Other types of tumours.