Application of Inverse Light Scattering to Single Red Blood Cells ...?

Workshop Inverse Problems for PDEs
Bremen 2016-04-01

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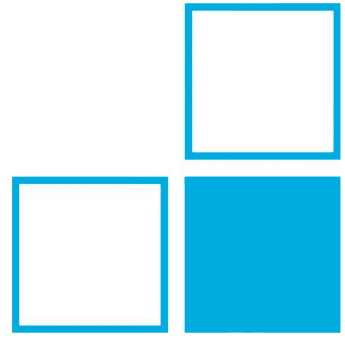
Group 8.41 – Modeling and Simulation



Outline of this talk

- Present a problem of biomedical optics, that we are working on (PTB's research groups 8.32 and 8.41).
- Model the problem mathematically.
- Discuss with you.

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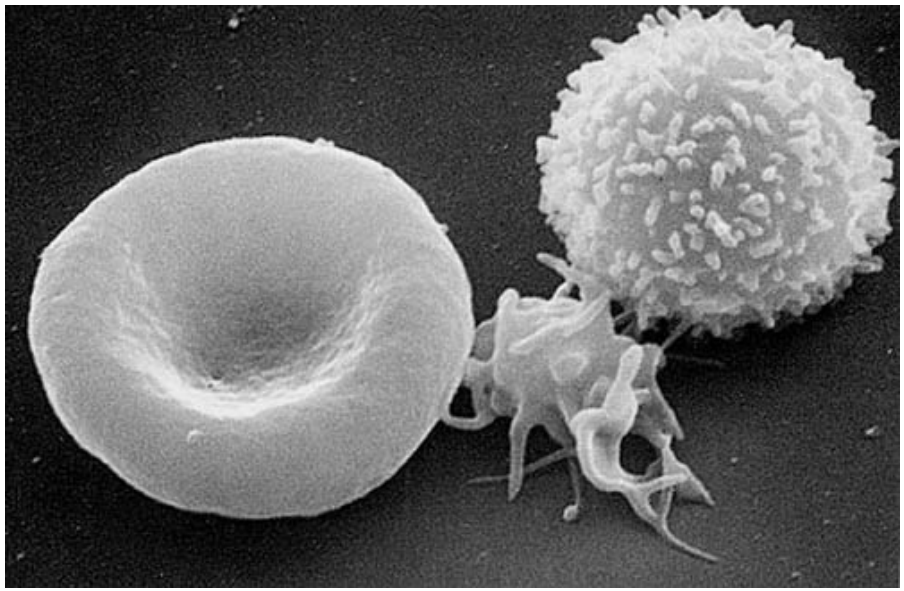
In case of questions or comments:
Please interupt at any time!

*Human **R**ed **B**lood **C**ells (RBCs), or Erythrocytes*



Scanning electron microscope (SEM) image of Erythrocyte, Thrombocyte and Leukocyte [https://commons.wikimedia.org/wiki/File:Red_White_Blood_cells.jpg]

Why RBCs are interesting

- $\approx 90\%$ of blood cells are red cells
- RBCs transport oxygen. They play an important role in the metabolism.

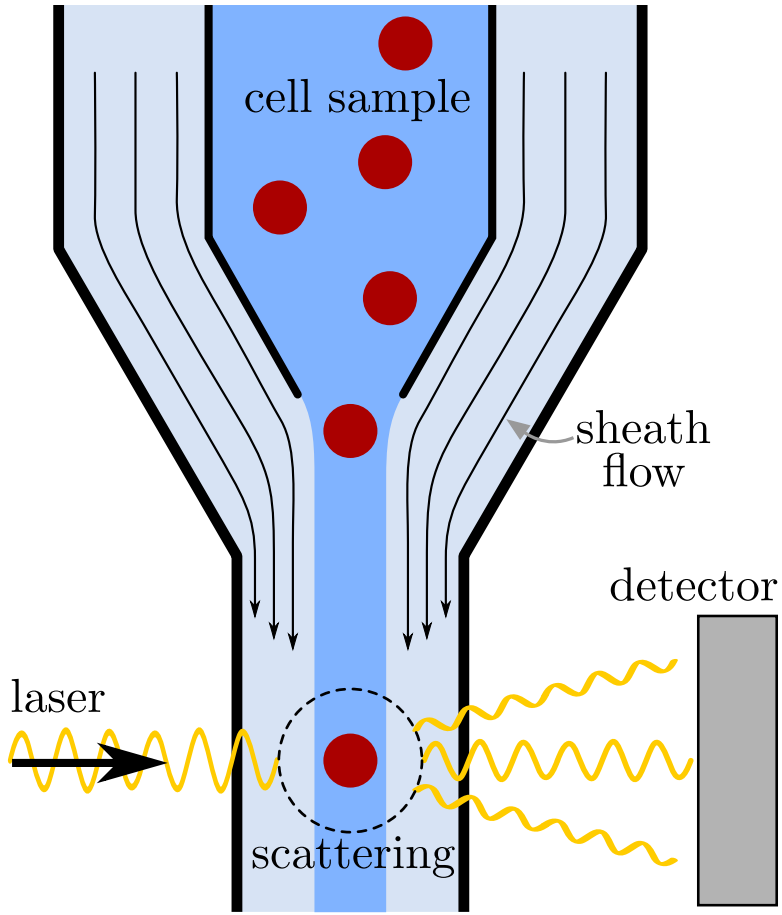
Standard procedure in medical laboratories: **whole blood count**.
Measured quantities (among others) for populations of $10^4 - 10^6$ cells:

- number concentrations of cells
- mean volume of RBCs
- width of RBC volume distribution
- mean RBC hemoglobin concentration

$\Longrightarrow$ diagnosis of diseases/blood disorders, e. g., anemia.

How can this be done?

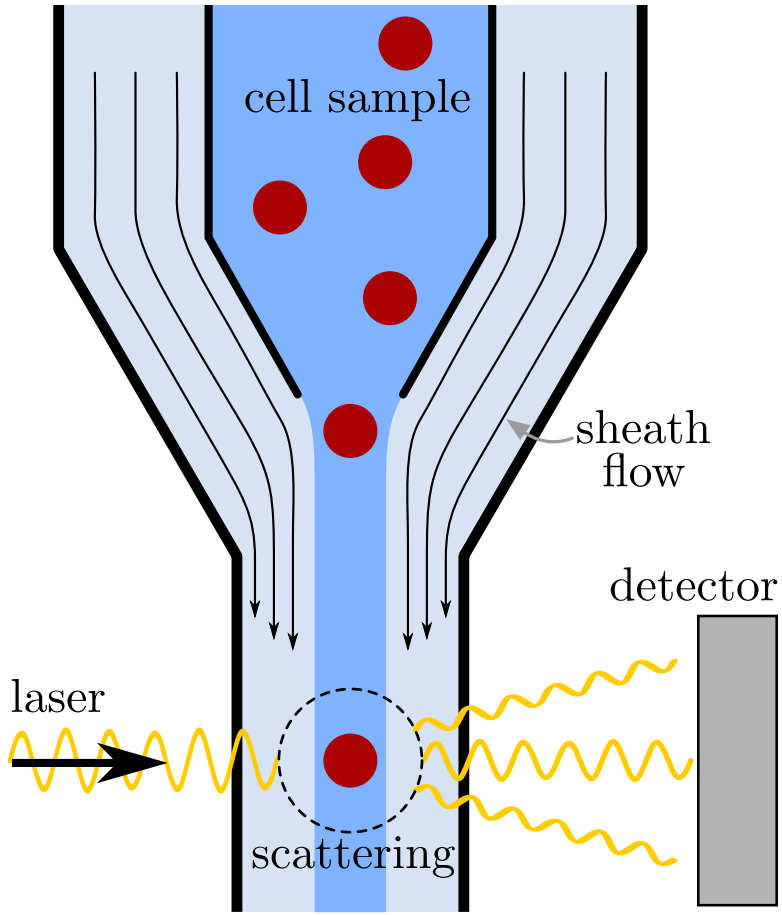
Using optical **flow cytometers**



How can this be done?

Using optical **flow cytometers**:

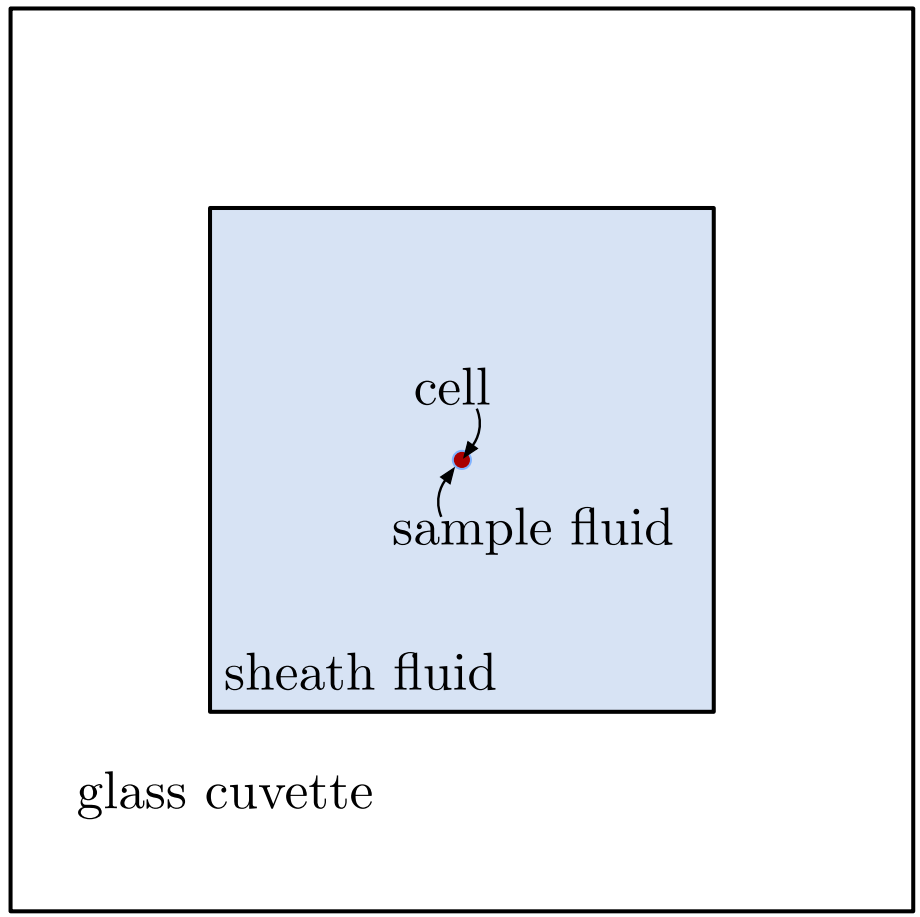
- High throughput, good statistics
- non-destructive
- differentiation of various cell types using empirical criteria or fluorescent dyes
- used in biology, materials research, cosmetics/food industry . . .



Drawback for RBCs: **Volume measurement** requires spherical cells or semi-empirical criteria $\Longrightarrow$ **not very accurate**.

Typical dimensions of flow cell and RBC:

- Laser light
  $\lambda = 0.35 \ldots 1.1\,\mu\mathrm{m}$
- (Red blood) cell $2 - 10\,\mu\mathrm{m}$
- Flow cell $250\,\mu\mathrm{m}$
- Glass cuvette: several mm
- Distance to detector:
  a few cm



Direct scattering problem

Mathematical formulation of scattering problem I

Maxwell equations for time-harmonic fields, source-free case:
Vector Helmholtz equation

$$-\Delta \boldsymbol{E} + k_0^2 N^2 \boldsymbol{E} = 0$$
$$\boldsymbol{H} = \frac{1}{\mathrm{i}\omega\mu} \nabla \times \boldsymbol{E}$$

with wavenumber $k_0 = 2\pi/\lambda_0 = \omega/c_0 \in \mathbb{R}$ and refractive index (RI)

$$N(\boldsymbol{x}) = \sqrt{\left(\varepsilon_r + \mathrm{i}\frac{\sigma}{\varepsilon_0 \omega}\right)\mu_r} = \begin{cases} n_{\mathrm{H_2O}} \in \mathbb{R} & \boldsymbol{x} \text{ outside cell} \\ n_{\mathrm{RBC}} \in \mathbb{C} & \boldsymbol{x} \text{ inside cell} \end{cases}$$

And $[\boldsymbol{\nu} \times \boldsymbol{E}] = 0 = [\boldsymbol{\nu} \times \boldsymbol{H}]$ on the cell boundary.

Mathematical formulation of scattering problem II

Incoming light (laser)

$$\boldsymbol{E}^i(t, \boldsymbol{x}) = \boldsymbol{E_0} \mathrm{e}^{\mathrm{i}(k\, z - \omega t)}, \quad \boldsymbol{E}_0 \perp \boldsymbol{e}_z,\ k = k_0\, n_{\mathrm{H_2O}}.$$

is a monochromatic plane wave. Also possible: Gaussian beam.

Seek for solution to Maxwell's equations in the form

$$\boldsymbol{E} = \boldsymbol{E}^i + \boldsymbol{E}^s,$$

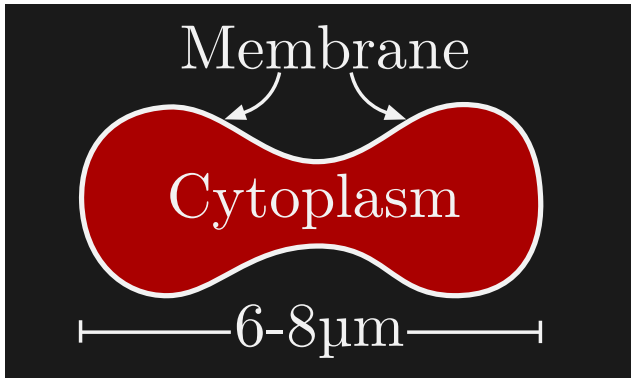
where $\boldsymbol{E}^s$ fulfills the Silver-Müller radiation condition

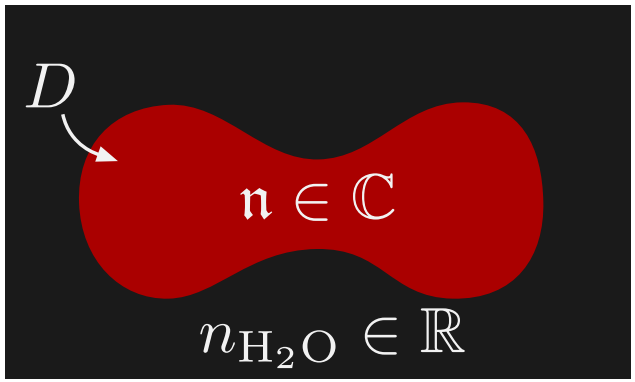
$$\lim_{r\to\infty} \left[\sqrt{\varepsilon}\boldsymbol{E} \times \boldsymbol{x} + \sqrt{\mu} r \boldsymbol{H}\right] = 0, \quad r = |\boldsymbol{x}|.$$

Anatomy of red blood cells

- Biconcave shape, very flexible
- Hardly any inner structure: No nucleus (DNA), no organelles
- RBC = membrane filled with aqueous hemoglobin (Hb) solution
- Cell membrane thin compared to visible wavelengths

$\Longrightarrow$ simplest cell model RBC = domain D with homogeneous optical properties (refractive index $\mathfrak{n}$)





Electric properties of RBCs I

Complex refractive index $\mathfrak{n} = n + \mathrm{i}\kappa = n_{\mathrm{H_2O}} + c_{\mathrm{Hb}}\, B$, where

$c_{\mathrm{Hb}} > 0$: mass concentration of hemoglobin, **unknown**

$B \in \mathbb{C}$: RI increment, complex, wavelength dependent, **known**.

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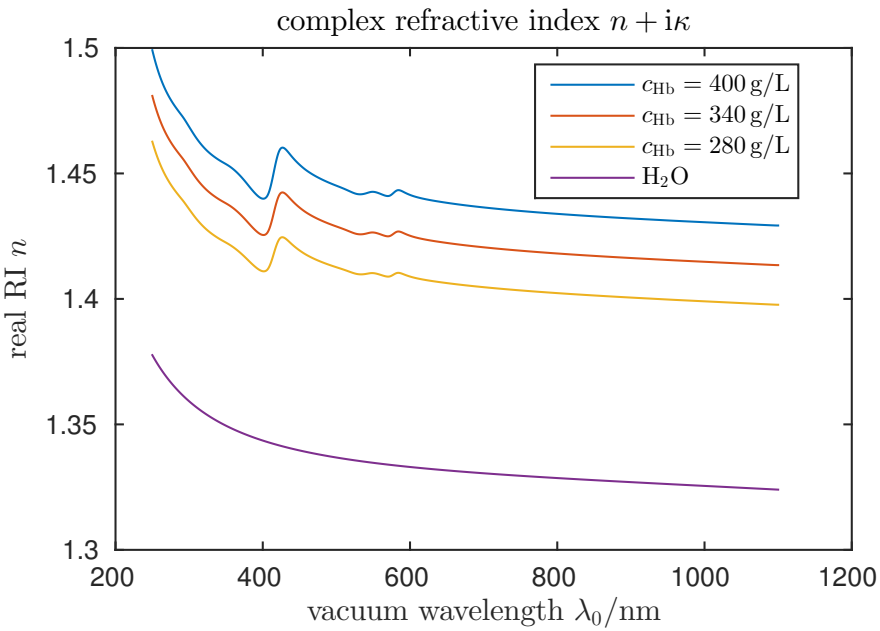
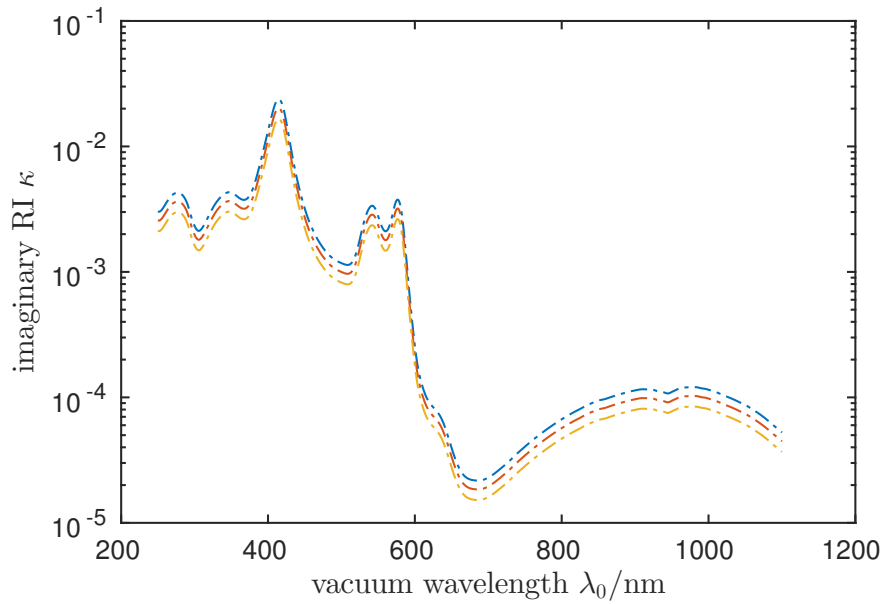
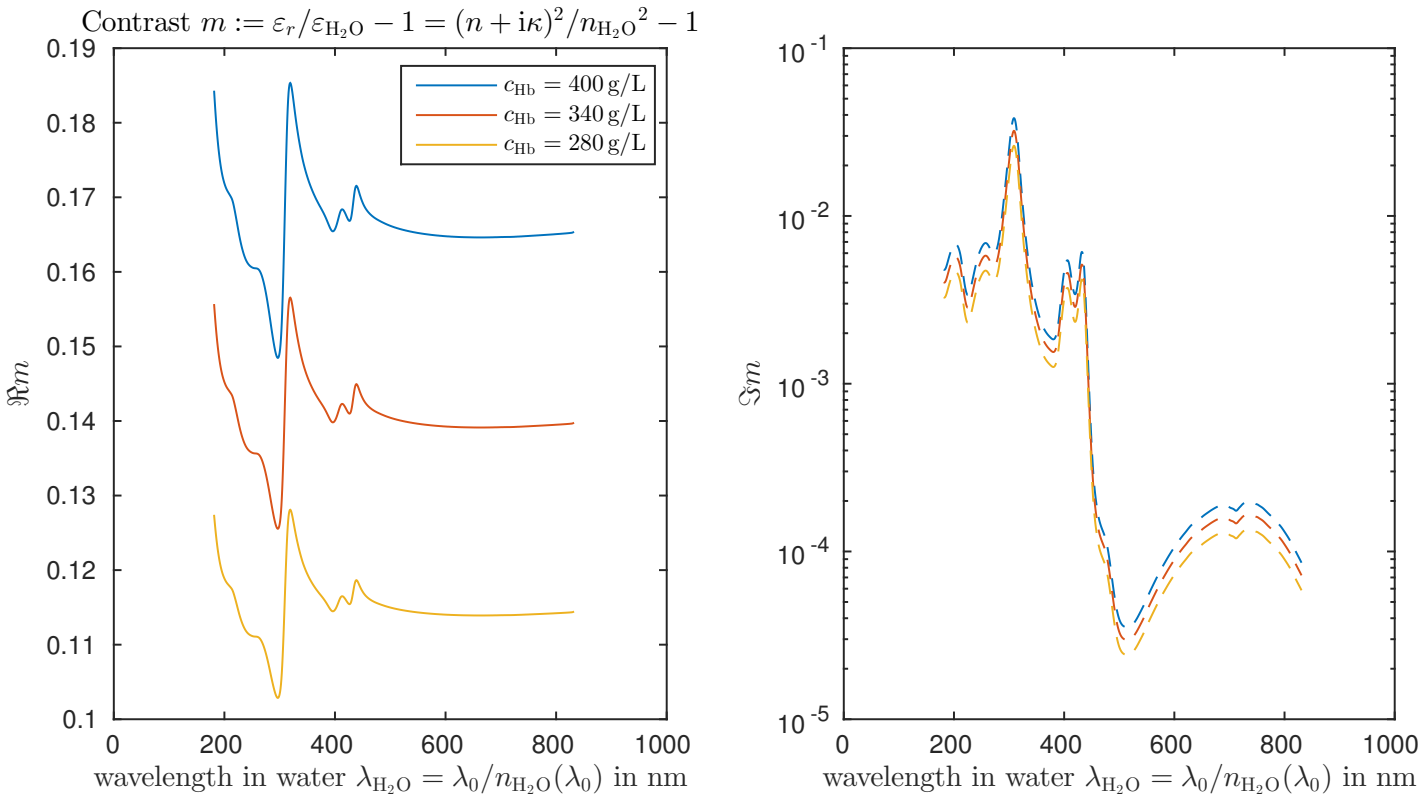




Electric properties of RBCs II

Electric contrast $m := \mathfrak{n}^2/n_{\mathrm{H_2O}}^2 - 1$



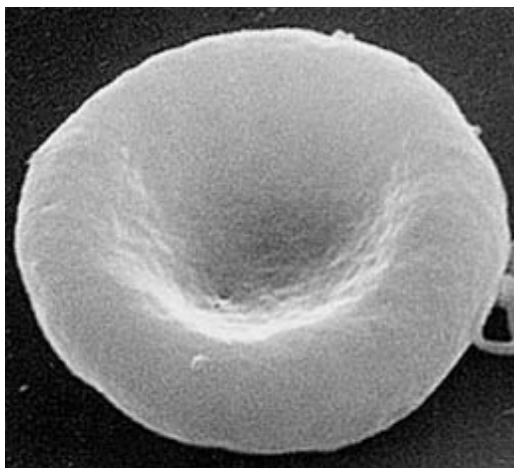
As far as is known: no magnetic contrast ($\mu_r = 1$), no optical activity.

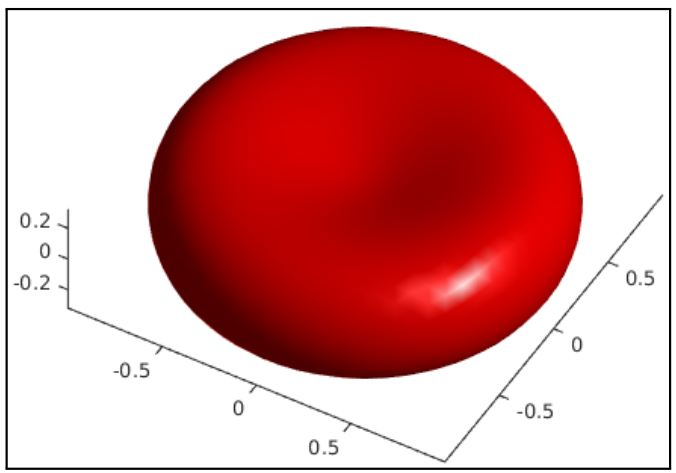
Shape models for RBCs I

Often one uses rotationally symmetric models like

$$D = \{(\rho, \varphi, z) \in \mathbb{R}^3 : |z| < f(\rho)\}$$

$$f(\rho) = \sqrt{R^2 - \rho^2} \sum_{n=0}^{N} a_n \left(\frac{\rho}{R}\right)^{2n}$$

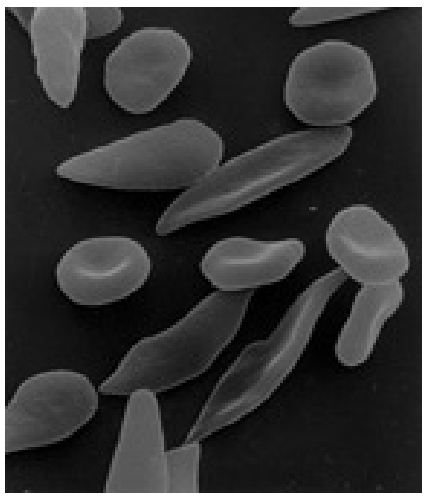




Shape models for RBCs II

Problem: Real RBCs do not have rotational symmetry. They may be deformed in flow. The membrane fluctuates thermally.
Diseased cells have other shapes (e. g., sickle cells).

$\Longrightarrow$ A more general way to describe the cell is required.



[https://commons.wikimedia.org/wiki/File:Sicklecells.jpg]

Shape models for RBCs III

Generic properties of domain D:

- simply connected
- non-convex

Additional properties in less pathological cases

- D is star-shaped
- some smoothness of the boundary ∂D can be assumed, e. g., C^k with $k \geq 2$

Shape models for RBCs IV

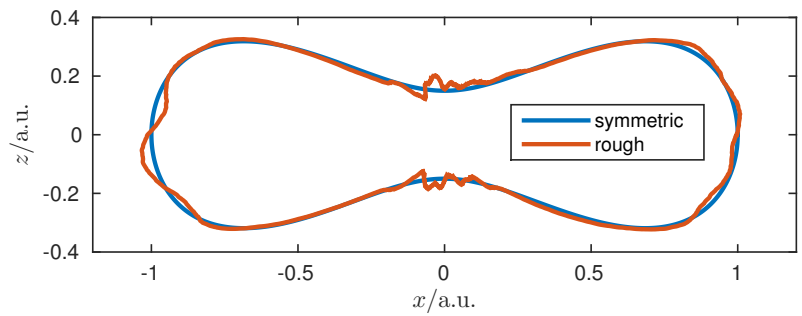
Ways for more general descriptions of cell shape:

1. Scalar contrast field $m : \mathbb{R}^3 \to \mathbb{C}$, whith (ideally) $\mathrm{supp}(m) = D$
   Given pointwise/voxelwise for volume discretization methods, such as DDA.

Shape models for RBCs IV

Ways for more general descriptions of cell shape:

1. Scalar contrast field $m : \mathbb{R}^3 \to \mathbb{C}$, whith (ideally) $\mathrm{supp}(m) = D$
   Given pointwise/voxelwise for volume discretization methods, such as DDA.
2. Parametrize boundary of simply connected domain D in a more general way:
   For star-shaped domains: $\boldsymbol{x} = r(\vartheta, \varphi)\, \boldsymbol{e}_r(\vartheta, \varphi)\ \forall \boldsymbol{x} \in \partial D$, modulo translation. Parametrize radius function $r : S^2 \to \mathbb{R}$, e. g., by values at discrete angles and interpolation.



Inverse scattering problem

What do we want to infer?

Interesting quantities:

- Information about RBC shape:
  for detecting sickle cell disease or measuring elastic properties
- cell volume (with smaller error than existing methods)
- refractive index $\widehat{=}$ Hb concentration

We don't care so much about:

- exact cell position
- orientation in space

What can be measured?

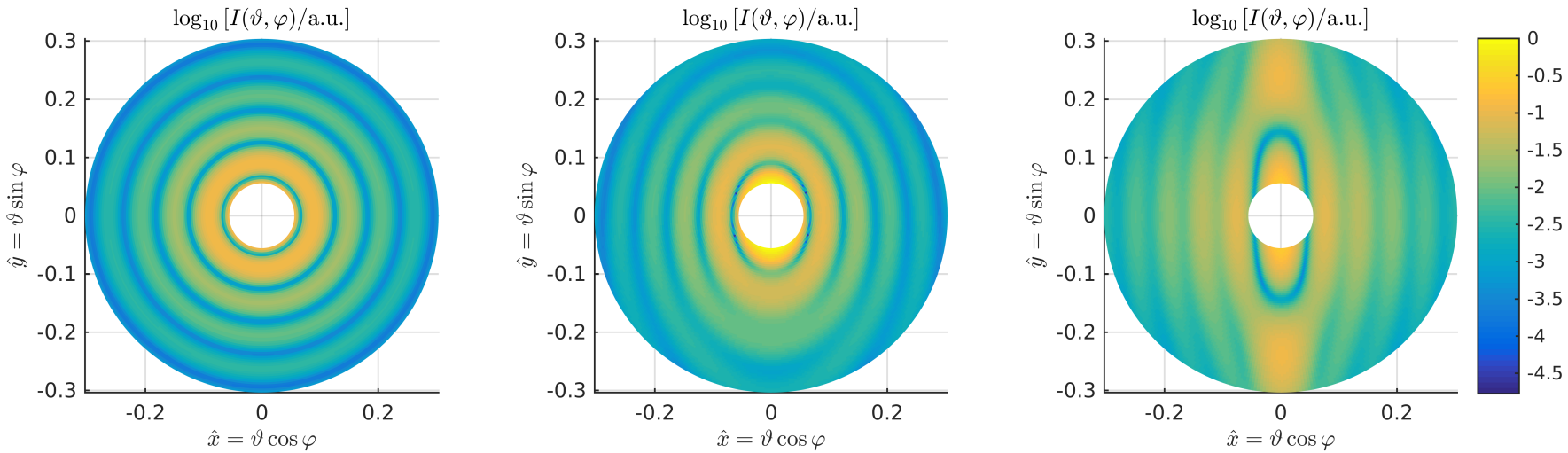
- The far field

$$\boldsymbol{E}^s(\boldsymbol{x}) = \frac{\mathrm{e}^{\mathrm{i}kr}}{r}\boldsymbol{E}^\infty(\boldsymbol{e}_r, \boldsymbol{d}) + \mathcal{O}(r^{-2}).$$

- Intensities/irradiances $I^\infty \propto \|\boldsymbol{E}^\infty\|^2$, i. e., phase information is not available.
  But: Light can be Fourier transformed using lenses, i. e., $\|\mathcal{F}[\boldsymbol{E}^\infty]\|^2$ is also measurable. Holographic techniques could also be used.
- Only one (or very few) directions of illumination $\boldsymbol{d} = \boldsymbol{k}/k$.
  Observation angle is limited to camera apertures of around $NA_{\max} \in [0.5, 0.6]$ or half-angles $\vartheta_{\max} \in [30°, 37°]$.
- Only one (or very few) laser wavelengths $\lambda = 2\pi/k$

How can data look like?

Numerical example: Rotationally symmetric shape model, $\lambda_0 = 488\,\mathrm{nm}$, $\mathfrak{n}/n_{\mathrm{H_2O}} = 1.060 + 0.0011\mathrm{i}$, diameter $2R = 6.7\,\mu\mathrm{m}$
Computed with **D**iscrete **D**ipole **A**pproximation (DDA), $\mathcal{O}(10^6)$ dipoles.
Angular domain: $(\vartheta, \varphi) \in [3.2°, 17.4°] \times [0, 360°]$.



$\beta = 0°$ (face on) $\beta = 46°$ (face on) $\beta = 90°$ (edge on)

Laser in z-direction, $x-$polarized; Rotation of RBC around x-axis.

Naive approach to the inverse problem

What we (as physicists) would try to do:
Select some shape model, e. g., rotationally symmetric model
$\Longrightarrow$ RBC characterized by parameters

$$\boldsymbol{p} = (\underbrace{c_{\mathrm{Hb}}}_{\text{hemoglobin concentration}}, \underbrace{R, a_0, \ldots, a_N}_{\text{shape parameters}}, \underbrace{\beta^T}_{\text{orientation}})^T.$$

For each $\boldsymbol{p}$ and wavelength λ, we can compute the far field pattern

$$I^\infty = F(\boldsymbol{p}; \lambda) \text{ for all pixels } j = 1, \ldots, N \text{ located at } (\vartheta_j, \varphi_j).$$

Solve (regularized) least-squares problem with Newton-type method:

$$\boldsymbol{p}^* = \arg\min \left[\sum_{j=1}^{N} w_j \left(I^\infty(\vartheta_j, \varphi_j) - I^*(\vartheta_j, \varphi_j) \right)^2 + \alpha \mathcal{R}(\boldsymbol{p}) \right]$$

Can it work?

For sphered cells, such a method has been used for 3 decades now.

[Tycko et al. "Flow-cytometric light scattering measurement of red blood cell volume and hemoglobin concentration" Appl. Opt. (1985)]

- Spherical cell has only 2 parameters: (1) diameter a,
  (2) hemoglobin concentration c_{Hb}
- Direct scattering problem solvable analytically ("Mie Theory")
- Measure 2 quantities:

$$x := \iint_{\vartheta\in[\alpha_1,\alpha_2]} I^{\infty}(\vartheta,\varphi)\mathrm{d}\Omega, \quad y := \iint_{\vartheta\in[\alpha_2,\alpha_3]} I^{\infty}(\vartheta,\varphi)\mathrm{d}\Omega$$

  For suitable angles $0 < \alpha_1 < \alpha_2 < \alpha_3 \leq \pi$.
- Compare measurements with Mie-solution (e. g., table lookup).

Is this a good approach in general?

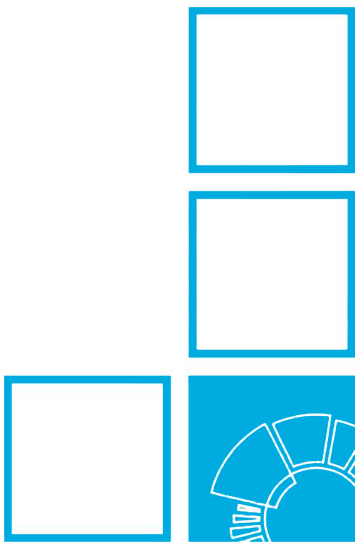
Maybe not:

- Requires solution of direct problem at each iteration
- Works only for simple, known shape models.
- What about well-posedness?

Any ideas how to tackle the problem?

Is it possible at all? Which kind of prior knowledge/constraints can lead to well-posedness? Regularization strategies? Literature? Algorithms? Software?

Thank you for your attention and your feedback!



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